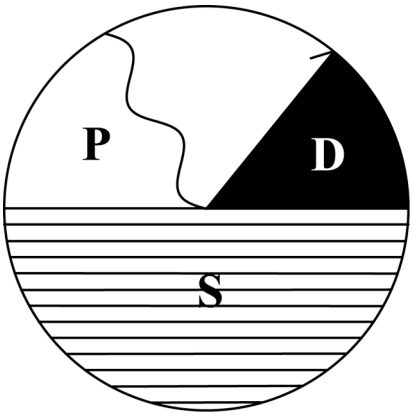


The 67th Annual Pittsburgh Diffraction Conference

University of Georgia
Athens, Georgia

29-31 October 2009

Program and Abstracts

Symposia

RNA Crystallography: Relating Form to Function

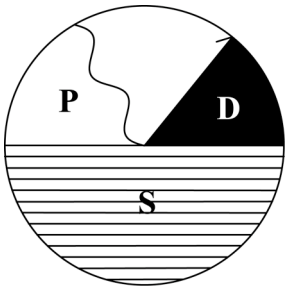
SAXS as applied to Biomolecules

Diffraction Studies of Materials

Crystallization of Hetero-Protein Complexes

Small Molecule Neutron Crystallography: Growing Up

Pittsburgh Diffraction Society Future Leaders Symposium



The 67th Annual Pittsburgh Diffraction Conference

Conference Chairman	John P. Rose
Symposium Organizers	Joseph Wedekind Jeff Urbauer Angus Wilkinson Joseph Ng Christine Hoffmann Bi-Cheng Wang
Program Chairman	M. Gary Newton
Local Arrangements Coordinators	Lili Li Lirong Chen

2009 Officers of the Pittsburgh Diffraction Society

President-Elect	John Rose
President	Aina Cohen
Past President	Robert Blessing
Treasurer	Charles Lake
Secretary	Joseph Wedekind
Board Member-at-Large	Charles Luke

Sponsors of
The 67th Annual Pittsburgh Diffraction Conference

www.artrobbinsinstruments.com

www.oxfordcryosystems.co.uk

blake4xray@worldnet.att.net

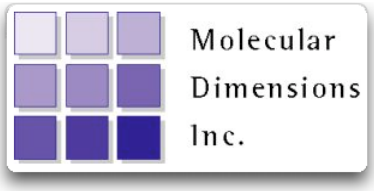
www.Rigaku.com

www.bruker-axs.com

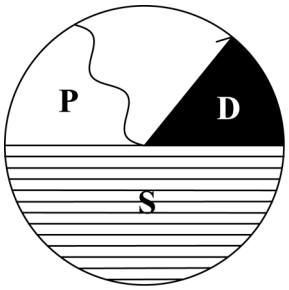
www.ttplabtech.com

www.hamptonresearch.com

www.bmb.uga.edu



www.moleculardimensions.com



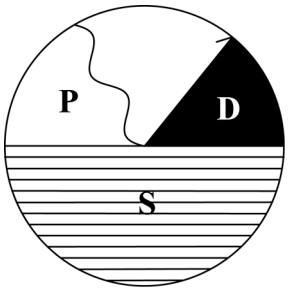
Sidhu Award

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

Note, the Sidhu award is given bi-annually when the conference takes place in Pittsburgh.

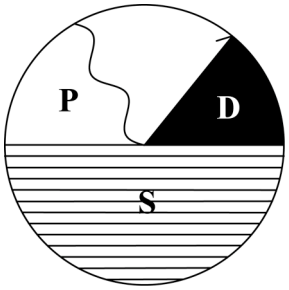


Chung Soo Yoo Award

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, 29th October 2009
Masters Hall
Georgia Center for Continuing Education

Morning Session A

RNA Crystallography: Relating Form to Function

Joseph Wedekind, University of Rochester, Chairman Organizer

08:45 **Welcome and Introduction**
John P. Rose, University of Georgia

09:00 **A.1. Chemical modification of RNA: crystallographic phasing and effects on stability and hydration**
Martin Egli, Vanderbilt University School of Medicine, *et al.*

09:30 **A.2. Quaternary self-assembly in prohead RNA of bacteriophage phi 29**
Ailong Ke, Cornell University, *et al.*

10:00 **A.3. Structural basis of translation regulation**
Christine M. Dunham, Emory University School of Medicine, *et al.*

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

11:00 **A.4. From orphan to structure: genetic control by a metal-sensing riboswitch**
Charles Dann III, Indiana University, *et al.*

11:30 **A.5. Antibiotic resistance ribosomal RNA methyltransferases: structures and target recognition**
Graeme Conn, Emory University, *et al.*

12:00 **A.6. How a ribozyme gets its kicks: relating non-coding RNA form to function with Raman crystallography**
Joseph Wedekind, University of Rochester School of Medicine & Dentistry, *et al.*

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

Afternoon Session B

SAXS as applied to Biomolecules

Jeff Urbauer, University of Georgia, Chairman Organizer

14:00 **B.1. Recent advances in biological small-angle X-ray scattering from solutions**
Dmitri Svergun, EMBL-Hamburg, *et al*.

14:45 **B.2. Applications of solution X-ray scattering on a proteomic scale and as an aid in crystallographic projects**
Greg Hura, Lawrence Berkeley National Laboratory, *et al*.

15:15 **B.3. How much can we learn about macromolecular structure from solution scattering data?**
Alexander Grishaev, National Institute of Diabetes & Digestive & Kidney Disease, *et al*.

15:45-16:15 **Afternoon Break**

16:15 **B.4. Probing conformational dynamics in the tRNA synthetase Gln4 using small angle X-ray scattering**
Thomas Grant, Hauptman-Woodward Medical Research Institute, *et al*.

16:45 **B.5. Structure of RNAs using a combined SAXS and NMR approach**
Sam Butcher, University of Wisconsin, *et al*.

17:15 **B.6. Incorporating SAXS into structural genomics and structural biology situations**
Jeff Habel, Lawrence Berkeley National Laboratory, *et al*.

17:50, Thursday evening, 29th October 2009

PDS General Membership Meeting
Masters Auditorium
Georgia Center for Continuing Education

19:00, Thursday evening, 29th October 2009

Poster Session and Conference Mixer
Georgia Center Atrium

M. Gary Newton, University of Georgia
Poster 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.

P.1. Move (-OH)ver; unraveling mechanisms. S.J. Polizzi and Z.A. Wood

P.2. Structural basis for substrate specificity of executioner caspases. Guoxing Fu, Alexander A. Chumanevich, Johnson Agniswamy, Bin Fang, Robert W. Harrison and Irene T. Weber

P.3. The solid-solution $Na_2(Zn, Co)SiO_4$. A possible dilute magnetic semiconductor material. Yuan Hu, Aaron Barnes, Gregory Kenning and Charles H. Lake

P.4. Structural studies of acid β-glucosidase in complex with potential pharmacological chaperones. Susan D. Orwig[1], Zhanqian Yu[2], Jeffery W. Kelly[2], Raquel L. Lieberman[1]

P.5. Evaluating the role of data reduction approach on the success rate on sulfur-SAD phasing for a moderately diffracting crystal. James Tucker Swindell II, John P. Rose and B.C. Wang

P.6. TBA

P.7 Sulfur-SAD Phasing: Historical Perspective and Progress at UGA and SER-CAT. Lirong Chen, Hao Xu, Hua

Zhang, Zheng-Qing Fu, James Swindell II, John Chrzas, Zhongmin Jin, John Rose and Bi-Cheng Wang

P.8. Taming of the Beast: methods for the expression and purification of the innate antiretroviral factor APOBEC3G in complex with the HIV-1 protein Vif. Geoffrey M. Lippa, Colleen Kellenberger, Jolanta Krucinska, Harold C. Smith, Joseph E. Wedekind

P.9. Structural Basis of Substrate Specificity of Caspase-3 at S4 and S5 Revealed by Crystallography and Kinetic Studies. Bin Fang, Guoxing Fu, Johnson Agniswamy, Robert W. Harrison, Irene T. Weber

P.10. Structural investigation of HBsAg: A progress report. Quentin Florence, Hao Xu, Jonny Yokosawa, James Laura, Yuri Khudyakov and John P. Rose

P.11. Structures of BenM DNA binding domain with its DNA promoter sites explain the common elements of promoter recognition used by lysr-type transcriptional regulators. Amer Alanazi, Ellen Neidle and Cory Momany

P.12. MacCHESS 2009. D.J. Schuller, R. Cerione, M. Cook, U. Englich, R.E. Gillilan, S.M. Gruner, C.U. Kim, I. Kriksunov, W. Miller, S. Smith, D.M. Szebenyi

P.13. Probing the effect of production platform on the immunogenicity of recombinant vaccine proteins. Dayong Zhou, Hao Xu, Frank Michel, Jeff Hogan, and John Rose

P.14. Crystallizing HIV-1 viral infectivity factor (vif) in complex with Elongin B and Elongin C. Jason D. Salter and Joseph E. Wedekind

P.15. The UGA Structural Biology Incubator: An Invitation to Georgia Researchers Needing Protein and/or Structure. Hao Xu, Lirong Chen, John P. Rose and Bi-Cheng Wang

P.16. Molecular mechanisms of drug resistance in HIV-1 protease. Chen-Hsiang Shen, Ying Zhang, Xiaxia Yu, Yuan-Fang Wang, Johnson Agniswamy, Robert W. Harrison, John M. Louis, Arun K. Ghosh, & Irene T. Weber

P.17. Variable temperature and pressure powder diffraction study of scandium fluoride. Benjamin K. Greve, Angus P. Wilkinson, and Karena Chapman

Friday, 30th October 2009
Masters Hall
Georgia Center for Continuing Education

Morning Session C

Diffraction Studies of Materials

Angus Wilkinson, Georgia Institute of Technology Chairman Organizer

09:30 **C.1. TBA**
Chris Tulk, Oak ridge National Laboratory

10:00 **C.2. Cocrystal design and packing analysis based on a family of crystal structures containing a common molecule using the *Materials* nodule of *Mercury CSD***
Scott Childs, Renovo Research

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

11:00 **C.3. Local structure studies of oxynitrides and ferroelectric perovskite oxides**
Katharine Page, Los Alamos National Laboratory

11:30 **C.4. Complex structure determination by high-resolution synchrotron powder diffraction**
Matthew Suchomel, APS, Argonne National Laboratory

12:00 **C.5. Local structure and its relationship to the physical properties of low and negative thermal expansion frameworks**
Angus Wilkinson, Georgia Institute of Technology

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

Afternoon Session D

Crystallization of Hetero-Protein Complexes

Joseph Ng, University of Alabama in Huntsville, Chairman Organizer

14:00 **D.1. Crystallization of protein-protein complexes**
Peter Sun, National Institute of Diabetes & Digestive & Kidney Disease

14:30 **D.2. Novel Membrane Protein Expression, Solubility/Stability and Crystallization Technologies**
Larry DeLucas, University of Alabama at Birmingham

15:00 **D.3. Fluorescence Applications to Protein Crystallization**
Marc Pusey, iXpressGenes Inc., Huntsville, AL

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

16:00 **D.4. Macromolecular Crystallography with Neutrons**
Leighton Coates, Oak Ridge National Laboratory

16:30 **D.5. Overcoming the protein crystallization bottleneck - Ways to ease the pain of optimization and get you back on the road to diffracting protein crystals.**
Jeanette R Hobbs, Ph.D., Molecular Dimensions Ltd, UK.

16:30 **D.6. X-ray structure determination of DNA replication proteins derived from hyperthermophilic and pychrophilic archaea.**
Miranda Byrne-Steele, University of Alabama in Huntsville

18:30, Friday evening, 30th October 2009

Conference Banquet
Magnolia Ballroom
Georgia Center for Continuing Education

Saturday, 31th October 2009
Masters Hall
Georgia Center for Continuing Education

Morning Session E

Small Molecule Neutron Crystallography: Growing Up

Christina Hoffmann, Oak Ridge National Laboratory, Chairman Organizer

09 :30 **E.1. Single-crystal neutron diffraction at the Institut Laue-Langevin: facilities and science**
Garry McIntyre, Institute Laue-Langevin (ILL), Grenoble, France

10:00 **E.2. Disordered crystals – Diffuse scattering to the rescue**
Thomas Proffen, Lujan Neutron Scattering Center, Los Alamos National Laboratory

10:30-11:00 Morning Break

11:00 **E.3. Chemical Crystallography with neutrons - do the experiment!**
Alison Edwards, Bragg Institute, Australian Nuclear Science and Technology Organization

11:30 **E.4. Unambiguous determination of hydrogen atom positions - a quantitative comparison of X-ray, neutron, and joint refinements of rubredoxin**
Anna Gardberg, Center for Structural Molecular Biology, Oak Ridge National Laboratory

12:00 **E.5. Advances in neutron single crystal diffraction**
Christina Hoffmann, Neutron Scattering Sciences Division, Oak Ridge National Laboratory

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

Afternoon Session F

Pittsburgh Diffraction Society Future Leaders Symposium

Bi-Cheng Wang, University of Georgia, Chairman Organizer

14:00 **F.1. & P.1. Move (-OH)ver; unraveling mechanisms**
S. J. Polizzi & Z.A. Wood, University of Georgia

14:20 **F.2. & P.2. Structural basis for substrate specificity of executioner caspases**
Guoxing Fu *et al*., Georgia State University

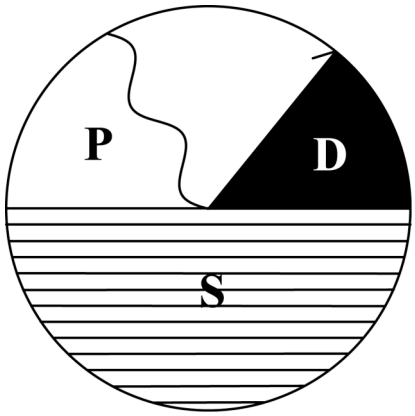
14:40 **F.3 & P.3 The solid-solution $Na_2(Zn, Co)SiO_4$. A possible dilute magnetic semiconductor material**
Yuan Hu, Aaron Barnes, Gregory Kenning & Charles H. Lake, Indiana University of Pennsylvania

15:00 **F.4. & P.4. Structural studies of acid β-glucosidase in complex with potential pharmacological chaperones**
Susan D. Orwig *et al.*, Georgia Institute of Technology

15:20-15:50 **Afternoon Break**

15:50 **F.5. & P.5. Evaluating the role of data reduction approach on the success rate on sulfur-SAS phasing for a moderately diffracting crystal**
James Tucker Swindell II, John P. Rose & Bi-Cheng Wang, University of Georgia

16:10 **F.6. & P.6. TBA**



LECTURE ABSTRACTS

RNA Crystallography: Relating Form to Function

Joseph Wedekind, University of Rochester, Chairman Organizer

A.1. Chemical modification of RNA: crystallographic phasing and effects on stability and hydration

Martin Egli

Department of Biochemistry, Vanderbilt University, Nashville, TN 37232

Selenium has been widely used for derivatization of proteins in the form of selenomethionine (Se-Met) that can be readily incorporated into proteins in place of methionine. The presence of selenium allows crystallographic phasing via the multi- or single-wavelength anomalous dispersion techniques (MAD or SAD, respectively). Selenoated nucleic acids are emerging as a powerful tool for crystallographic phasing. Compared to the well-known derivative preparation with halogenated pyrimidines, selenium can be incorporated at various sites (phosphate, sugar, or base). The resulting analogs are resistant to irradiation with light and intense X-rays and, in most cases, to oxidation. I will provide examples of structures determined by covalent Se modification and discuss the benefits and limitations of the approach. Biophysical and structural investigations of chemically modified nucleic acids can provide insights into the properties of native DNA and RNA that are beyond the reach of studies focusing on the latter alone. Using as an example the 2′-fluoro-2′-deoxyribonucleotide modification I will examine the role of the 2′-hydroxyl group in RNA pairing stability and hydration.

1. References: J. Sheng, Z. Huang (2008) Intl. J. Mol. Sci. 9, 258-271; M. Egli, P. S. Pallan (2007) Annu. Rev. Biophys. Biomol. Struct. 36, 281-305; P. S. Pallan, M. Egli (2007) Nature Protocols 2, 640-646; ibid, pp. 647-651; M. Egli et al. (2006) J. Am. Chem. Soc. 128, 10847-10856; C. Höbartner et al. (2005 J. Am. Chem. Soc. 127, 12035-12045; C. J. Wilds et al. (2002) J. Am. Chem. Soc. 124, 14910-14916; M. Teplova et al. (2002) Biochimie 84, 849-858; Q. Du et al. (2002) J. Am. Chem. Soc. 124, 24-25.

Financial support: Funding by the US NIH (R01 GM055237) is gratefully acknowledged.

A.2. Quaternary self-assembly in prohead RNA of bacteriophage phi 29

Ailong Ke[1], Fang Ding[1], Changrui Lu[1], Dwight Anderson[2], and Shelley Grimes[2]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, 14853; [2]Department of Diagnostic and Biological Sciences, University of Minnesota, Minneapolis, MN 55455

Prohead RNA (pRNA) of the *Bacillus subtilis* bacteriophage ø29 is an essential component of one of the most powerful molecular motors in biology. This RNA-protein, ATP-dependent motor packages the 19.3 kb dsDNA genome of ø29 into the precursor capsid (prohead). The 174-base ø29 pRNA molecules form an oligomeric ring by intermolecular base pairing, which binds to the dodecameric head-tail connector at a unique vertex of the prohead. ø29 pRNA is the only RNA described thus far that forms a higher multimer by intermolecular base pairing of identical molecules, and this multimer represents a novel structural motif in the RNA world. We determined the crystal structure of the oligomeric pRNA at 3.5 Å resolution. In the crystal lattice, pRNA molecules assemble, in a head-to-tail fashion, into a tetrameric ring structure. Oligomerization is mediated by intermolecular pseudoknot interactions involving four layers of Waston-Crick base pairs. The pRNA crystal structure fits nicely into the pentameric EM envelope, requiring only minor rigid-body adustment at its 3-way junction. A hexameric pRNA assembly, on the other hand, would be significantly larger in ring diameter. This resolves the long debate in the field about its oligomerization state on the phage prohead. EM fitting revealed that pRNA symmetry is assumed through surprising contacts with the pentamerically organized coat protein gp8. Towards the distal end, pRNA 'grabs', and therefore anchors, the packaging ATPase through an asymmetric internal loop. We conclude that pRNA plays a scaffolding role, similar to that found for the Signal Recognition Particle RNA, in the assembly of the protein components in the packaging motor. Mutagenesis and chemical probing are in progress to further characterize pRNA functions.

A.3. Structural basis of translation regulation

Christine M. Dunham[1], Crystal Fagan[1], Maria Selmer[2], Ann C. Kelley[3], Yong-Gui Gao[3], V. Ramakrishnan[3]

[1]Department of Biochemistry, Emory School of Medicine, Atlanta, GA; [2]Department of Cellular and Molecular Biology, Uppsala University, Uppsala, Sweden; [3]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Ribosomes are the complex, cellular machinery responsible for the synthesis of all proteins in every cell. This 2.5 million Dalton enzyme in bacteria contains three large RNAs and more than 50 proteins that form two asymmetric subunits to promote mRNA-directed translation of the genetic code. This essential process is highly regulated with a large portion of a cell's energy dedicated to maintaining accurate assembly of ribosomes and error-free translation. A key question in the translation field is how regulation is mediated by exogenous proteins and/or RNA ligands on a molecular level. I will discuss our recent high-resolution structures of functional ribosome complexes bound to suppressor tRNAs and toxin proteins that cause noncanonical reading of the mRNA.

A.4. From orphan to structure: genetic control by a metal-sensing riboswitch.

Charles E. Dann III[1], Catherine A. Wakeman[2], and Wade C. Winkler[2],

[1]*Department of Chemistry, Indiana University, Bloomington Indiana 47405-7102,* [2]*Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas Texas 75390-8816 USA.*

Interest in RNA has expanded tremendously over the last decade due to numerous discoveries of new roles for noncoding RNAs in cellular processes. Many *cis*-acting small regulatory RNAs that bind metabolites, termed riboswitches, have been identified in recent years. Riboswitches are widespread in bacterial transcripts and have also been identified in plants. The binding of a metabolite to riboswitches results directly in genetic regulation of its transcript through diverse mechanisms affecting transcription termination, translation initiation, mRNA stability and splicing. The M-box aptamer domain, previously referred to as the *ykoK* orphan riboswitch, has been shown through a convergence of many lines of biochemical, biophysical and structural data to act as a sensor for divalent cations. The mechanism of metal ion sensing based on our 2.6 Å crystallographic structure will be presented as well as supporting data for metal binding at key sites in the RNA. This work represents a substantial step forward in our thinking about potential ligands sensed by riboswitches and raises the possibility that additional metal-sensing RNAs could exist. Funding provided by the Searle Scholars Program, the UT Southwestern Medical Center Endowed Scholars Program, the Welch Foundation, the Sara and Frank McKnight Fund for Biochemical Research, Indiana University CoAS, and NIH Grant # 5R01GM081882-02 (WCW). Data was collected at APS SBC-CAT beamline 19ID.

A.5. Antibiotic resistance ribosomal RNA methyltransferases: structures and target recognition

Graeme L. Conn[1], Mark S. Dunstan[2], Rachel Macmaster[1,2] and Miloje Savic[2]

[1]Emory University, Dept. of Biochemistry, 1510 Clifton Road NE, Atlanta GA.
[2]Unversity of Manchester, Manchester Interdisciplinary Biocentre, Faculty of Life Sciences, 131 Princess Street, Manchester, M1 7DN, UK.

Antibiotics that bind the bacterial ribosome typically target highly conserved functional regions of ribosomal RNA (rRNA) in either the 30S or 50S subunit. Among the possible mechanisms of resistance to these antibiotics, specific chemical modification of a nucleoside in the drug binding pocket by RNA methyltransferase enzymes can confer high level and often broad resistance. Such rRNA modification is commonly found in antibiotic-producing Actinomycetes, but is now being increasingly identified as a cause of resistance in clinical bacterial isolates. This presentation will focus on our recent structural-function studies of one 23S rRNA methyltransferase enzyme and members of two closely related families of 16S rRNA methyltransferases. In particular, initial insights into mechanisms of methyltransferase-target RNA recognition from X-ray crystal structures, in vitro binding and activity assays and modeling will be discussed. One longer term goal will be to determine whether specific features of target recognition can be exploited to help combat the rise of resistance to clinically useful antibiotics such as the 16S rRNA-binding aminoglycosides.

A.6. How a ribozyme gets its kicks: relating non-coding RNA form to function with Raman crystallography

Joseph E. Wedekind,[1,*] Man Guo,[2] Rosaria Volpini,[3] Jolanta Krucinska,[1] Gloria Cristalli,[3] and Paul R. Carey[2,*]

[1]Department of Biochemistry & Biophysics and Center for RNA Biology, 601 Elmwood Avenue Box 712, Rochester New York 14642 USA, [2]Department of Biochemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106 USA, [3]Department of Chemical Sciences, University of Camerino, Via S. Agostino 1, 62032 Camerino, Italy. (*email: joseph.wedekind@rochester.edu or prc5@case.edu)

Catalytic RNA molecules can achieve rate acceleration by shifting base pK_a values toward neutrality. Prior evidence suggested that base Ade38 of the hairpin ribozyme plays an important role in phosphoryl transfer, possibly functioning as a general acid, or by orienting a specific water molecule for proton transfer. To address the role of Ad38, we used Raman crystallography to measure the pK_a of the N1-imino moiety in the context of crystals representative of 'pre-catalytic' and 'transition-state' conformations, respectively. The results revealed that the pK_a of Ade38 is shifted to 5.46 ± 0.05 in the pre-catalytic state, and 6.29 ± 0.06 in the transition state. These values are significant when compared to a solution of the nucleotide AMP, whose pK_a was 3.68 ± 0.06. The elevated microscopic pK_a of Ade38 agrees well with the first titration point of the macroscopic pH-rate profile of the hairpin ribozyme in solution. Changes in the scissile-bond geometry and nearby chemical groups modulate the Ade38 pK_a directly. Overall, the results establish a cogent structure-function paradigm that expands our understanding of how RNA structure can enhance nucleobase reactivity despite a limited chemical repertoire relative to protein catalysts.

References: Guo *et al*. & Wedekind (2009) Direct Raman measurement of an elevated base pK_a in the active site of a small ribozyme in a precatalytic conformation. *J. Am. Chem. Soc.* **131**, 12908-9; Spitale *et al.* & Wedekind (2009) Identification of an imino group indispensable for cleavage by a small ribozyme. *J. Am. Chem. Soc.* **131**, 6093-5; MacElrevey *et al*. Wedekind (2008) Structural effects of nucleobase variations at key active site residue Ade38 in the hairpin ribozyme. *RNA* **14**, 1600-16;

Supported by NIH R01 GM063162 to JEW & NIH R01 GM084120 to PRC.

SAXS as applied to Biomolecules

Jeff Urbauer, University of Georgia, Chairman Organizer

B.1. Recent advances in biological small-angle X-ray scattering from solutions

Maxim V. Petoukhov and Dmitri I. Svergun

European Molecular Biology Laboratory, Hamburg Outstation c/o DESY, Notkestr. 85, D22603 Hamburg, Germany

Small-angle X-ray scattering (SAXS) experiences a renaissance in the studies of macromolecular solutions allowing one to study the structure of native particles and to rapidly analyze structural changes in response to variations in external conditions. Novel data analysis methods [1] significantly enhanced resolution and reliability of structural models provided by the technique. Emerging automation of the experiment, data processing and interpretation make solution SAXS a streamline tool for large scale structural studies in molecular biology. The method provides low resolution macromolecular shapes *ab initio* and is readily combined with other structural and biochemical techniques in multidisciplinary studies. In particular, rapid validation of predicted or experimentally obtained high resolution models in solution, identification of biologically active oligomers and addition of missing fragments to high resolution models are possible. For macromolecular complexes, quaternary structure is analyzed by rigid body movements/rotations of individual subunits. Recent developments made it possible also to quantitatively characterize flexible macromolecular systems, including intrinsically unfolded proteins. The novel methods will be illustrated by advanced SAXS applications to solutions of biological macromolecules.

[1]. Petoukhov, M.V. & Svergun, D. I. (2007) *Curr Opin Struct Biol*. **17**, 562-571.

B.2. Applications of solution X-ray scattering on a proteomic scale and as an aid in crystallographic projects.

Greg L. Hura

Advanced Light Source , Physical Bioscience Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

High throughput solution structural analyses by small angle X-ray scattering efficiently enables the characterization of shape and assembly for nearly any purified protein. Crystallography has provided a deep and broad survey of macromolecular structure. Shape and assembly from SAXS in combination with available structures is often enough to answer critical mechanistic questions both enhancing the value of a structure and obviating larger crystallographic projects. Moreover, SAXS is a solution based technique, sample requirement are modest and compatible
with many other biophysical methods. Here we present our high throughput SAXS data collection and analysis pipeline as applied to structural genomics targets, and metabolic pathways. Our goals of metabolic engineering and understanding protein mediated reactions rely on knowing the shape and assembly state of reactive complexes under an array of conditions. Given the number of gene products involved in metabolic networks, S AXS will play an important role in characterizing the structure of each individually, in complex with partners, and in various contexts. SAXS is well positioned to efficiently bridge the rapid output of bioinformatics and the relatively slow output of high resolution structural techniques.

B.3. How much can we learn about macromolecular structure from solution scattering data?

Alexander Grishaev

Laboratory of Chemical Physics, National Institute of Diabetes & Digestive & Kidney Diseases, National Institutes of Health

Solution scattering has been traditionally considered a low data content technique due to loss of information in a spherically averaged scattering pattern. This notion is currently being challenged due to a dramatic increase of the precision of the SAXS data and expansion of the experimentally attainable resolution range, both possible with synchrotron sources. Ease of sample preparation and high speed of data collection and analysis have greatly contributed to the popularity of Bio-SAXS as an emerging method of structural investigation. Determination of low-resolution molecular envelopes from SAXS data alone is a vivid illustration of method's capabilities. Structural studies gain significantly when scattering data are combined with prior constraints, such as when geometries of the individual domain are known while multi-domain constructs or protein/protein complexes are investigated. On the other hand, combination of solution scattering with other experimental techniques that provide site-specific high-resolution information can be very advantageous as well. A joint application of SAXS and solution NMR is especially attractive, often resulting in a significant improvement of model quality for multi-domain protein and oligonucleotide systems. SAXS data are also very useful when studying macromolecular complexes in solution. In those cases, however, structural refinement against solution NMR and X-ray scattering data does not always result in a unique model. In these circumstances, neutron scattering data can provide a powerful non-redundant restraint which can drive the structure calculation to a unique solution. A particularly powerful approach utilizes 1H/2H contrast variation and neutron scattering in combination with selective 1H/2H isotopic labeling of the individual components within a complex. The information content of such dataset is thus increased, allowing to establish separate self- and cross-scattering functions for the individual components within a complex. We will discuss applications of such hybrid approaches to several systems illustrating the advantages and limitations of these methods.

B.4. Probing conformational dynamics in the tRNA synthetase Gln4 using small angle X-ray scattering

Thomas D. Grant[1,2], Joseph R. Luft[1,2], Jennifer Wolfley[1], Hiro Tsuruta[3], Stephanie Corretore[4], Erin Quartely[4], Eric M. Phizicky[4,5], Elizabeth J. Grayhack[4,5], and Edward H. Snell[1,2]

[1]Hauptman-Woodward Medical Research Institute; [2]Department of Structural and Computational Biology, SUNY at Buffalo; [3]Stanford Synchrotron Radiation Lightsource; [4]Department of Pediatrics, University of Rochester Medical Center; [5]Department of Biochemistry and Biophysics, University of Rochester Medical Center

Gln4 is a yeast glutaminyl tRNA synthetase that covalently couples glutamine to the 2'-OH of $tRNA^{GLN}$ for its essential role in translation of decoding glutamine codons in mRNA and adding glutamine residues to the growing peptide chain during protein synthesis. Many eukaryotic tRNA synthetases like Gln4 differ from their prokaryotic homologs by the attachment of an additional domain appended to their N- or C-terminus, but it is unknown how these domains contribute to tRNA synthetase function, and why they are not found in prokaryotes. We have solved the crystal structure of Gln4 and determined that the N-terminal domain is disordered; 95% of it, over 200 residues, is missing in the structure. We have confirmed that these residues are present in the crystal. The packing diagram shows large water channels, which could accommodate this domain in the lattice. Using X-ray crystallography and Small Angle X-ray Scattering (SAXS), we have determined that this N-terminal domain adopts multiple conformations in solution that may be explained by a single functional pathway.

B.5. Structure of RNAs using a combined SAXS and NMR approach

Samuel E. Butcher

Department of Biochemistry, University of Wisconsin-Madison

Size limitation has been a longstanding problem for NMR structure determination of biological macromolecules. Most molecular structures determined by NMR are below 40 kDa molecular weight. The size limitation of NMR originates from an increase in resonance line-widths that scales with the overall correlation time of the molecule, which increases with molecular weight. This increase in line-width, combined with the increase in the number of proton resonances in larger molecules, results in severe spectral overlap that becomes difficult or impossible to interpret. RNAs in particular suffer from this problem, since their chemical makeup is limited to 4 highly similar nucleotides and therefore their associated chemical shifts are intrinsically even less disperse than proteins. New approaches are therefore required in order to deal with this problem. One such approach is a combined NMR-SAXS method. SAXS works particularly well for RNA, which strongly scatters x-rays due to the electron dense phosphate backbone. SAXS is also highly complimentary to NMR, in that it measures the overall molecular dimensions that cannot be obtained by NMR. Furthermore, SAXS can be performed directly on a fraction of an NMR sample. The incorporation of SAXS data during NMR structure refinement of large RNAs significantly improves the accuracy of the structure models [Zuo et al., J. Am. Chem. Soc. 2008, 130(11):3292]. The NMR-SAXS approach will be illustrated using examples of a 30 kDa tetraloop receptor complex and a 40 kDa U2-U6 RNA complex.

B.6. Incorporating SAXS into structural genomics and structural biology situations

Jeff Habel and Li-Wei Hung

Lawrence Berkeley National Lab, Berkeley, CA 94720

Small-angle X-ray scattering (SAXS) is a quick and easy way to gather important structural information on small amounts of protein. One of the most crucial tasks today is to incorporate and expand the amount of information we can garner from the SAXS experiment. The ability to see flexibility, folding state, and aggregation information on solution samples of proteins allows us to make important decisions affecting construct design. In addition, SAXS information can help guide the process of choosing affective and potentially useful additives and molecular replacement models for structure solution. By combining SAXS with other structural genomic information, we can have a positive affect on the solution of structural genomics targets. Here we present examples of experimental setup and processing in order to incorporate this new structural information into the ISFI and TBSGC initiatives.

Diffraction Studies of Materials

Angus Wilkinson, Georgia Institute of Technology Chairman Organizer

C.1. CHRIS TULK

TBA

Chris Tulk, Oak ridge National Laboratory

C.2. Cocrystal design and packing analysis based on a family of crystal structures containing a common molecule using the *Materials* nodule of *Mercury CSD*

Scott L. Childs

President and CSO, Renovo Research, 1256 Briarcliff Rd. NE, Atlanta, GA 30306 Tel. (404)377-7876 [scott@rre.net] [www.renovoresearch.com] and Visiting Scholar, Emory University, Department of Chemistry, Atlanta, GA

Crystal Engineering studies can be credited with giving rise to the recent interest in cocrystals (molecular complexes) of pharmaceuticals as a means of improving the physical properties of pharmaceutical dosage forms. Hydrogen bonds have been the traditional tool used for cocrystal design as well for analysis of crystal structures. The fact that hydrogen bonds can be observed in crystal structures and visualised easily does not necessarily mean that they are 'structure-directing' and other, less directional, interactions may be energetically competitive. Topics will include the importance of dispersion-dominated packing interactions in cocrystals compared to that of hydrogen bonds as well as investigating packing similarity, polymorphism, pseudo-isostructurality, and the occurrence of common 1D channel structures within a family of related cocrystals containing a common active pharmaceutical ingredient (API).

Although these concepts will be demonstrated using a set of small molecule single crystal structures, the *Mercury CSD* application from the Cambridge Crystallographic Data Centre and the analysis techniques can be applied to a wider range of structural systems – from biological to inoragnic. A goal of this talk is to illustrate the use of the *Mercury CSD* application in a way that will allow others to apply the concepts to their own work.

C.3. Local structure studies of oxynitrides and ferroelectric perovskite oxides

Katharine Page and Thomas Proffen

Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM

Ferroelectric materials play an important role in science and engineering, and efforts to understand, characterize, design and engineer them at the nanoscale are driven, for example, by a continued demand for smaller and more robust electronics and by the search for multifunctional materials. It is widely known that the properties of ferroelectrics are intricately tied to their crystal structures. At the same time, there is a growing awareness that nanoscale inhomogeneities, either compositional or electronic, play a critical role. Such features are not fully described by traditional crystallographic approaches as, by definition, they possess limited translational periodicity. With sensitivity to intermediate range order over nanometer length scales and local atomic ordering in nearest neighbor pair-pair correlations, Pair Distribution Function (PDF) methods are well-suited to contribute to our understanding of these systems. We present our work with high resolution neutron and X-ray total scattering towards average (crystallographic) and local (nanoscale) structure determination in bulk and nanoparticle ferroelectric perovskite oxides and related oxynitrides. We will describe the case of organically-capped 5 nm $BaTiO_3$ particles and compare the nature and length scale of metal-oxygen correlations to those found in bulk $BaTiO_3$, commenting on their distinct ferroelectric behavior. We will also give several examples of nanoscale structural features and related phenomena that emerge in bulk mixed metal oxides and oxynitrides. For example, we will reveal how local off-centering in otherwise cubic Nb-substituted $BaTiO_3$ give rise to insulating behavior while retained local symmetry in Nb-substituted $SrTiO_3$ render it metallic. This work provides unique insight into atomic configurations that influence properties in these functional materials and highlights the advantages of total scattering approaches for nanomaterials and nanoscale structural features.

C.4. Complex structure determination by high-resolution synchrotron powder diffraction

Matthew R. Suchomel[1,2], Brian H. Toby[1], Lynn Ribaud[1], John B. Claridge[2], Mathieu Allix[2], Matthew J. Rosseinsky[2]

1. Advanced Photon Source (APS), Argonne National Laboratory, Argonne, IL USA; 2. Dept. of Chemistry, University of Liverpool, Liverpool, United Kingdom

Synchrotrons have revolutionized powder diffraction. They make possible the rapid collection of data with tremendous resolution and sensitivity, and have thus become a key research tool for structure determination and for parametric structural studies of variables such as temperature or pressure. The flux and energy of a synchrotron source affords numerous advantages over traditional x-ray powder diffraction instruments, including increased observations and reduced sample absorption.

This presentation will highlight recent progress using synchrotron powder diffraction to characterize the complex incommensurate structures observed in novel polar and magnetically ordered Bi-based bulk perovskite oxide compounds synthesized by high pressure, high temperature solid state methods. Our interest in Bi-based perovskite oxides has been motivated by intriguing predictions of multiferroic coupling in Bi-based oxide systems and by the possibility of using Bi-based materials to replace current environmentally unfriendly Pb-containing ferroelectric and piezoelectric materials. The "lone-pair" $6s^2$ electronic configuration of the Bi^{3+} cation promotes polar structural distortions; while the flexibility of the trivalent B site in the perovskite structure permits the substitution of a wide range of complex combinations of transition metal cations that may promote magnetic and/or ferroelectric responses.

The talk will conclude by introducing potential users to the high-resolution 11-BM powder diffractometer at the APS and to its convenient mail-in program. The performance and capabilities of the beamline will be discussed, and the simple steps required of would-be users to obtain access and the potential benefits to their research from the synchrotron powder diffraction program at 11-BM will be discussed.

C.5. Local structure and its relationship to the physical properties of low and negative thermal expansion frameworks

Angus P. Wilkinson,[1] Benjamin K. Greve,[1] Andrew C. Jupe,[1] Chad J. Ruschman,[1] Mehmet Cetinkol,[1] Kenneth L. Martin,[2] Karena W. Chapman,[3] Peter J. Chupas[3] and Peter L. Lee[3]

(1) School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, (2) Chemistry Dept., Berry College, P. O. Box 5016, Mount Berry, GA 30149-5016, (3) X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Argonne, Illinois 60439

The cubic "ReO_3" framework structure is simple, and yet it has all the structural features necessary for negative thermal expansion (NTE) due to the transverse thermal motion of bridging anions. In practice, materials with this structure display a wide variety of properties, ranging from pronounced NTE through to strong positive thermal expansion. We will discuss the thermal expansion and compressibility of several fluorides and oxyfluorides with this structure, and examine the role that O/F disorder plays in determining their properties.
Many negative thermal expansion materials undergo pressure induced amorphization at modest pressures. An analysis of the changes in local structure accompanying the amorphization of $ZrMo_2O_8$ will be presented.

Crystallization of Hetero-Protein Complexes

Joseph Ng, University of Alabama in Huntsville, Chairman Organizer

D.1. Crystallization of protein-protein complexes

Peter Sun

National Institute of Diabetes & Digestive & Kidney Disease

A survey of crystallization conditions was carried out for 650 published protein-protein complexes in the Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB). This resulted in the establishment of a Protein Complex Crystallization Database (PCCD) and a set of configuration space boundaries for protein complex crystallizations. Overall, polyethylene glycol (PEG)-based conditions accounted for 70-80% of all crystallizations with PEG 3000-4000, 5000-6000, and 8000 being the most frequently used. The median values of PEG concentrations were between 10-20% and inversely correlated with their molecular weights. Ammonium sulfate remained the most favorable salt precipitant with a median concentration of 1.6 M. The crystallization pH for the vast majority of protein complexes was between 5.0 and 8.0. Overall, the boundaries for the crystallization configuration space of protein complexes appear to be more restricted than those of soluble proteins. This may reflect the limited stability and solubility of protein-protein complexes. Based on the statistical analysis of the database, a sparse-matrix and a systematic buffer/pH screens were formulated to best represent the crystallization of protein complexes.

D.2. Novel Membrane Protein Expression, Solubility/Stability and Crystallization Technologies

Larry DeLucas*, John Kappes*, David Johnson*, Tom Lewis* and W.W. Wilson[#]

**University of Alabama at Birmingham, Birmingham, Al; [#]Mississippi State University, Starkville, Ms*

Membrane biology represents one of the most important yet difficult challenges facing research scientists today. Although aqueous proteins are being crystallized and structures determined at a rapid pace, less than 100 membrane protein structures have been determined with less than 10% of these from eukaryotic sources. Innovative technologies that have the potential to significantly enhance capabilities to express, purify, crystallize, and thus, to solve the structure of integral membrane proteins will be described. We have developed an innovative protein expression system by integrating technologies that appear to overcome the major limitations for the manufacturing of mg quantities of recombinant protein, including IMPs, in mammalian cells. This includes the cloning, packaging, transduction and expression of target genes via highly efficient lentiviral vectors, engineered inducible mammalian cell lines capable of high recombinant protein expression and minimal leakiness, continuous, stable culture of transduced cell lines in the non-induced state, and scale-up mg-quantity production of IMPs. This is combined with a novel diagnostic technology, high-throughput self-interaction chromatography (HT-SIC) that allows rapid determination of the ideal solution conditions for maximum protein solubility and physical stability throughout the purification process. We have developed a second technology, high-throughput self-interaction chromatography (SIC), to rapidly measure the second virial coefficient of different proteins in a variety of co-solvents. The use of SIC provides a rapid diagnostic for determining the optimum solution conditions that promote increased protein stability, and minimizing unwanted non-specific aggregation. In addition, SIC allows us to select specific solution conditions (including detergent type and concentration) for subsequent crystallization and crystal optimization experiments.

D.3. Fluorescence Applications to Protein Crystallization

Marc Pusey

iXpressGenes Inc., 601 Genome Way Huntsville, AL 35806

It is widely recognized that the crystallization of new proteins is the bottleneck process in the gene to structure pathway. The determination of crystallization conditions is typically carried out by an empirical trial-based process, Considerable effort has gone into the development of methods to facilitate this screening process, such as a plethora of random screening cocktails to use, the development of robotic systems for rapidly setting up screens at reduced volumes, followed by additional robotic systems for imaging and evaluating the outcomes of those screens. Current practice then reduces to throwing robots at the problem, to cover more variables using less protein.

We are currently developing two fluorescence-based approaches to the protein crystallization screening process which could readily be extended for use in the crystallization of complexes. The first is the use of trace fluorescently labeled protein to facilitate finding protein crystals in a given screening condition. Trace labeling is defined as < 1%, and typically <0.1 %, of the protein molecules having a fluorescent probe covalently attached. Fluorescence intensity is proportional to the probe density. Crystalline protein would be the most densely packed protein form, and when trace fluorescently labeled will have the brightest fluorescence emission intensity. We have previously shown[1] using model proteins that the presence of the covalently bound probe, at up to 5-10% labeling, does not affect the X-ray data quality obtained. We subsequently showed that intensity-based analysis of non-crystalline screening outcomes could be used to determine crystallization conditions[2]. We are now working on the automation of this process; preliminary tests have shown that image analysis software that finds fluorescent crystals can carry out the requisite process in < 1 second. As labeled crystals can 'shine through' otherwise obscuring precipitate, this is a considerable advantage over methods that rely on edge detection.

The second approach is the use of fluorescence anisotropy to screen for likely crystallization conditions, again using fluorescently labeled protein.Traditional crystallization screening outcomes take days to months to obtain and most often result in non-crystalline precipitate or clear solutions, which are routinely dropped from further consideration when in fact they may actually be close to crystallization conditions. Fluorescence anisotropy measures the rotational rate (and thus size) of the labeled analyte. The data is obtained as a curve of anisotropy vs. protein concentration. For crystallization conditions increasing concentrations result in a progressive increase in the anisotropy. The FACTs (Fluorescence-based Analytical Crystallization Technologies) approach has been tested with model proteins (where plate screen data is available) and experiments with test proteins (no plate data and have not been previously crystallized) are now being performed. The model protein anisotropy data resulted in the optimization screening of a number of conditions which were previously scored as clear solutions or precipitate in traditional crystallization trials, with a number of these being subsequently found to yield crystals. Current efforts are focusing on the data analysis process; the model protein data indicates that fluorescence intensity may also be a significant diagnostic indicator. A near term goal of the FACTs development process is to reduce the solution assay volume to 10-20 nL, down from the current volume of 3.0 mL, enabling a complete 96 condition screen to be carried out with < 20 mg of protein.

References:Forsythe, E.L., Achari, A., and Pusey, Marc L. (2006), Acta Cryst. D62, 339-346; Pusey, Marc L., Forsythe, E., and Achari, A. (2007), *Methods in Molecular Biology, Vol. 426: Structural Proteomics: High-Throughput Methods*; B. Kobe, M. Guss, and T. Huber, Eds.; Humana Press, pp 377-386.

D.4. Macromolecular Crystallography with Neutrons

Leighton Coates

Oak Ridge National Laboratory, Neutron Scattering Science Division, 1 Bethel Valley Rd, Oak Ridge, TN, 37831, USA.

Knowing exactly where hydrogen atoms are and how they are transferred between macromolecules, solvent molecules, and substrates is important for understanding many processes. Neutron macromoleular crystallography is a powerful technique for locating hydrogen atoms and is used to provide information on the protonation states of molecules and the nature of bonds involving hydrogen. The ability of neutron diffraction to determine the protonation states of amino acids with and without ligands makes it invaluable in the study of catalytic mechanisms of various molecules. These assets will be of great use in the overall project to help investigate the function of large molecules and assemblies.
Three instruments are currently being constructed at Oak Ridge National Laboratory that can collect macromolecular neutron diffraction an outline of each instrument will be given. Some recent case studies that have used neutrons will also be presented.
Oak Ridge National Laboratory (ORNL) is managed by UT-Battelle LLC for the US Department of Energy under Contract No. DE-AC05-00OR22725

D.5. Overcoming the protein crystallization bottleneck - Ways to ease the pain of optimization and get you back on the road to diffracting protein crystals.

Jeanette R Hobbs, Ph.D.,

Molecular Dimensions Ltd, Newmarket, Suffolk, UK.

The main problem in protein crystallization lies in either getting no crystals at all or, even more frustrating, getting crystals that are not of high enough quality to enable structure determination. With the first step, what do you do with those drops that remain clear? With the latter, how do you optimize conditions to obtain better diffracting protein crystals?
The ultimate means in obtaining good diffracting crystals is to control their conception stage (i.e. the nucleation stage), which is the first step that determines the entire crystallization process-no easy task! Hence the Holy Grail is to find a 'universal' nucleant – a substrate that would induce crystallization of any protein.
And if you have no crystals, what then? One applied method and technique is dynamic light scattering (DLS), which has found use in the detection of aggregation and nucleation in crystallization droplets. The data can provide information to understand the process of crystal initiation and growth, which in turn allows the experimenter to assess the probability of obtaining macromolecular crystals a long time before crystallization actually takes place.
In this talk you will get an illustration of the technique of using nucleation grains to aid in optimization of 'tricky' proteins and how the use of DLS combined with the use of a combined white/UV illumination source (to enable identification of proteins and biomolecules) can improve your chances of obtaining diffraction quality protein crystals.

D.6. X-ray structure determination of DNA replication proteins derived from hyperthermophilic and pychrophilic archaea.

Miranda Byrne-Steele and Joseph D. Ng,

University of Alabama in Huntsville, Huntsville, AL 35816.

The process of DNA replication in all organisms is complex and requires the concerted action of multiple proteins in order to achieve faithful replication of the genomic template. Since DNA replication is essential for the reproduction of an organism at a given temperature, proteins involved in this process likely have considerable selective pressure to adapt to their respective environments. We have determined the crystallographic structure of two classes of proteins central to the DNA replication machinery from a hyperthermophilic marine archaeon *Thermococcus thioreducens* and a psychrophilic archaeon *Methanococcoides burtonii* DSM 6242. The crystal structure of a family B DNA polymerase from *T. thioreducens* (TtPolB) has been solved to 2.0 Å, and the structure of the homotrimeric proliferating cell nuclear antigen (PCNA) from this organism (TtPCNA) has been solved to 1.9 Å. In addition, the structure of the PCNA from the psychrophilic archaeon *M. burtonii* DSM 6242 (MbPCNA) has been determined to 2.4 Å, and the DNA polymerase from this organism (MbPolB) has been cloned and overexpressed. The two PCNA structures are placed in the context of known sliding clamp structures from all domains of life, indicating high topological similarity despite low sequence identity. In contradiction to available literature, we demonstrate that the interfacial interactions responsible for maintaining a toroidal structure are not correlated with the temperature environment of the host organism, but rather are highly influenced by phylogeny. Our analysis indicates that the euryarchaeal PCNAs can be divided into two groups on the basis of interfacial interactions: one that relies on both charge-shape complementarity and one that is primarily stabilized by charge-charge complementarity. In addition, we demonstrate that the PCNA from the cold-adapted genome maintains its secondary structure well beyond the temperature associated with the optimal growth temperature of the organism, indicating the presence of stable proteins in cold-adapted genomes. In contrast, the DNA polymerase from this organism demonstrates significant instability as it precipitates at temperatures extending into the mesophilic range. Structure-based sequence comparisons with TtPolB are performed to assess differences that may be related to their relative stabilities.

Byrne-Steele, M. L. and Ng, J. D. (2009). Expression, purification, and preliminary X-ray analysis of Proliferating Cell Nuclear Antigen from the archaeon *Thermococcus thioreducens*. Acta Crystallogr F65, 906-909.

Byrne-Steele, M. L., Hughes R.C., and Ng, J. D. (2009). Recombinant production, crystallization, and preliminary X-ray analysis of PCNA from the psychrophilic archaeon *Methanococcoides burtonii* DSM 6242. Acta Crystallogr F. *In Press.*

Small Molecule Neutron Crystallography: Growing Up

Christina Hoffmann, Oak Ridge National Laboratory, Chairman Organizer

E.1. Single-crystal neutron diffraction at the Institut Laue-Langevin: facilities and science

G.J. McIntyre

Institut Laue-Langevin, BP156, 38042 Grenoble Cedex 9, France

A survey of single-crystal proposals over the last 15 years at the Institut Laue-Langevin, the high-flux research reactor in Grenoble, France, reveals a strong preference for studies of magnetism, for single-reflection experiments as a function of external influence, and for exotic sample environment. With a choice of eight, soon nine, dedicated single-crystal diffractometers - in stark contrast to the maximum of two at spallation sources – researchers can select the optimum instrument for their particular experiments, according to the mean wavelength, monochromatic versus polychromatic, solid angle of detection versus low background, and the option of polarisation. This variety and specialisation is illustrated by typical recent experiments: the first observation of long-range ferromagnetism in the single-molecule magnet Mn_{12} acetate [1]; the identification of exchange pathways in an organic molecular magnet by polarised-neutron diffraction [2]; and precise characterisation of hydrogen-metal bonding in organo-metallic complexes [e.g. 3].

The last experiments, on uncommonly small crystals, were only possible on the image-plate thermal-neutron Laue diffractometer, VIVALDI [4], which in its first years of operation has produced spectacular diffraction patterns and exciting new science at a furious rate, with gains in data collection rate over conventional diffractometers of up to 100 fold. The Laue technique has also proven to be very well suited to rapid chemical crystallography, reciprocal-space surveys, and studies of structural and magnetic transitions.

[1] F. Luis, J. Campo, J. Gómez, G.J. McIntyre, J. Luzón & D. Ruiz-Molina, Phys. Rev. Lett. **95** (2005) 227202.
[2] J. Luzón, J. Campo, J.M. Rawson, R.J. Less, C.M. Pask, A. Alberola, G.J. McIntyre, R.D. Farley, D.M. Murphy, A.E. Goeta & F. Palacio, In preparation.
[3] M. Yousufuddin, M.J. Gutmann, J. Baldamus, O. Tardif, Z. Hou, S.A. Mason, G.J.McIntyre & R. Bau, J. Am. Chem. Soc. **130** (2008) 3888-3891.
[4] G.J. McIntyre, M.-H. Lemée-Cailleau & C. Wilkinson, Physica B **385-386** (2006) 1055-1058.

E.2. Disordered crystals – Diffuse scattering to the rescue.

Thomas Proffen

Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM 87545

Structural characterization is mainly based on the measurement of *Bragg intensities* and yields the *average* structure of the crystalline material. However, this approach ignores any defects or local structural deviations that manifest themselves as *diffuse scattering*. Recent developments in instrumentation and modeling software make the analysis of diffuse scattering more accessible. In this presentation examples based on the analysis of single crystal diffuse scattering as well as the atomic pair distribution function obtained from powders will be discussed.

E.3. Chemical Crystallography with neutrons - do the experiment!

Alison J. Edwards

Bragg Institute, Australian Nuclear Science and Technology Organization

The advent of nuclear reactors (and subsequently spallation sources) as sources of neutron beams facilitated the investigation of some materials of chemical and physical importance by means of single crystal neutron Bragg Diffraction methods. Early sources and detection methods meant that such experiments were considered to be extremely difficult, not least because the size of crystal required could literally exceed the amount of material in existence!

The resurrection of the Laue method for synchrotron X-ray structure analysis in the 1980's and the construction of the neutron Laue instruments SXD@ISIS, SCD@IPNS (now located at the Lujan Centre) and VIVALDI@ILL has potentially opened up the feasibility of neutron diffraction for chemical crystallography and provided the prospect of increasing application of neutron diffraction to chemical crystallography problems. When ANSTO was considering the construction of neutron beam instruments, the instrument commissioned, KOALA, was a Laue diffractometer with a neutron image-plate detector system closely aligned to the VIVALDI instrument. A significant factor in this choice was that the size of crystal for which data may be collected is considerably smaller than for Bragg diffraction methods from the same source. Inherent in the image-plate detection system is the requirement that the wavelength of the diffraction spot be derived from the experiment and the Laue method is of lower precision than Bragg diffraction.

In refining structures from Laue data, careful consideration of the parameterization of the model is required in order to optimize the utility of the final refined structure. Routine, brute-force and push-button approaches can lead to unsatisfactory models from which little chemical information is available. With careful modelling it is clear that the major advantages of neutron diffraction: unequivocal location of hydrogen positions (or exclusion of the presence of hydrogen), discrimination between elements of similar X-ray scattering power and the location of light atoms in the presence of heavy atoms can be achieved with a high degree of certainty. Examples of recent studies and the modelling approach employed will be discussed.

E.4. Unambiguous determination of hydrogen atom positions - a quantitative comparison of X-ray, neutron, and joint refinements of rubredoxin

Anna Gardberg

Center for Structural Molecular Biology, Oak Ridge National Laboratory, P.O.Box 2008, Oak Ridge TN 37831-6142

The locations of hydrogen atoms in biological structures can be difficult to determine with X-ray diffraction methods. Neutron diffraction offers relatively greater scattering magnitude from hydrogen and deuterium atoms. Here we compare the visibility of deuterium atoms in maps computed from neutron (1.65 Å at room temperature) and X-ray (1.1 Å at 100 K, 1.1 and 1.6 Å at RT) crystal structures for the small, iron-containing protein rubredoxin (MW = 6 kDa). The neutron resolution was achieved by complete deuteration of the protein and subsequent crystallization in D2O-based phosphate buffer. Crystals used for neutron work were ~4 mm3 in volume. Both neutron and high-resolution X-ray studies enable the determination of some D atom positions, with a 7-fold advantage for 1.65 Å resolution neutron data at 295 K over X-ray structures determined to 1.1 Å resolution at 100 K.

E.5. Advances in neutron single crystal diffraction

Christina Hoffmann and Xiaoping Wang

Oak Ridge National Laboratory, P.O.Box 2008, Oak Ridge, TN 37831, USA.

High flux neutron sources have moved neutron single crystal diffraction into an exciting new era. There are a number of neutron single crystal diffractometers in various stages of development in Oak Ridge National Laboratory. In addition to a four-circle diffractometer that is operational, a large area, quasi-Laue diffractometer (IMAGINE) has just been approved for funding at the HFIR research reactor. The Spallation Neutron Source (SNS) is housing an operating high-pressure diffractometer (SNAP). A macromolecular crystallography diffractometer (MaNDi) is under construction. The multi-purpose single crystal diffractometer (TOPAZ) under commissioning is capable of measuring complicated crystal structure of a large unit cell with the sample in sub-millimeter sizes, comparable with that used in conventional X-ray analysis. We will show science highlights that emphasize the individual strengths of each instrument and present recent results from the commissioning of the new TOPAZ single crystal diffractometer.

This research is supported by UT Battelle, LLC under Contract No. DE-AC05-00OR22725 for the U.S. Department of Energy, Office of Science

Pittsburgh Diffraction Society Future Leaders Symposium

Bi-Cheng Wang, University of Georgia, Chairman Organizer

F.1. & P.1. Move (-OH)ver; unraveling mechanisms

S.J. Polizzi and Z.A. Wood

Department of Biochemisty and Molecular Biology, University of Georgia, Athens, GA

UDP-xylose is the sugar donor for the first committed step in the biosynthesis of extracellular matrix (ECM) proteoglycans. UDP-xylose synthase (UXS) catalyzes the stepwise conversion of UDP-xylose from UDP-glucuronic acid via a short-lived UDP-4-keto-xylose intermediate. We have identified a novel zebrafish mutation with a UXS defect that prevents skeletal development and has been named the *man of war* (*mow*) mutation for its jellyfish-like morphology. We report the preliminary 2.7 A crystal structure and enzymatic characterization of *mow* UXS. The *mow* defect is due to an R236H point mutation that results in partial unfolding in the dimer interface and unraveling of a helix containing a catalytic tyrosine (Y231). Analytical ultracentrifugation studies show that despite the disruption, the dimer can still form in solution, but exists with a slower sedimenting form consistent with a looser structure. Using capillary zone electrophoresis, we show R236H and a related R236A mutation catalyze the decarboxylation of UDP-glucuronic acid but cannot reduce the UDP-4-keto-xylose intermediate in the second half of the reaction. Mutating Y231 to phenylalanine removes the tyrosine hydroxyl from the active site and results in the accumulation of the UDP-4-keto-xylose intermediate. Completion of the first catalytic step without Y231 suggests UXS and other nucleotide-sugar decarboxylases deviate from the classical model of catalysis proposed for other members of the short chain dehydrogenase/reductase family.

F.2. & P.2. Structural basis for substrate specificity of executioner caspases

Guoxing Fu[1], Alexander A. Chumanevich[1], Johnson Agniswamy[1], Bin Fang[1], Robert W. Harrison[2] and Irene T. Weber[1]

[1]Department of Biology and [2]Department of Computer Science, Molecular Basis of Disease Program, Georgia State University, Atlanta, Georgia 30303

Caspase-3, -6 and -7 are executioner caspases and cleave many proteins at specific sites to induce apoptosis, which is disrupted in many diseases including cancer, heart disease, and neurodegenerative diseases. Their recognition of the P5 position in substrates has been investigated by kinetics, modeling and crystallography. Caspase-3 and -6 recognize P5 in pentapeptides as shown by enzyme activity data and interactions observed in the crystal structure of caspase-3/LDESD and in a model for caspase-6/LDESD. In caspase-3 the P5 main-chain was anchored by interactions with Ser209 in loop-3 and the P5 Leu side-chain interacted with Phe250 and Phe252 in loop-4 consistent with 50% increased hydrolysis of LDEVD relative to DEVD. Caspase-6 formed similar interactions and showed a preference for polar P5 in QDEVD likely due to interactions with polar Lys265 and hydrophobic Phe263 in loop-4. Caspase-7 exhibited no preference for P5 residue in agreement with the absence of P5 interactions in the caspase-7/LDESD crystal structure. Initiator caspase-8, with Pro in the P5-anchoring position and no loop-4, had only 20% activity on tested pentapeptides relative to DEVD. Therefore, caspases-3 and -6 bind P5 using critical loop-3 anchoring Ser/Thr and loop-4 side-chain interactions, while caspase-7 and -8 lack the P5-binding residues. These discoveries will be valuable for the future design of novel inhibitors that are more specific for target caspase members. The distinct preferences observed for P5 residue in substrates will help define the particular cellular signaling pathways associated with each executioner caspase.

F.3 & P.3. The solid-solution $Na_2(Zn, Co)SiO_4$. A possible dilute magnetic semiconductor material

Yuan Hu, Aaron Barnes, Gregory Kenning and Charles H. Lake

Indiana University of Pennsylvania, Indiana, PA 15705

Dilute Magnetic Semiconductor (DMS) materials incorporate novel magnetic as well as conventional semiconductor properties. So far, research in the field has examined mostly binary and the ternary materials which have demonstrated limited applications due to their low Curie temperatures and low magnetic moment before anti-ferromagnetic behavior occurs.

The solid-solution $Na_2(Zn,Co)SiO_4$ is a possible DMS material. The nonmagnetic host structure Na_2ZnSiO_4 was published by Parthe.[1] Theoretical studies by Ohno have predicted that quaternary tetrahedral compounds could have a high Currie Temperatures and the electrostatic valance principle predicts that magnetic super-exchange will have little effect on these types of compounds therefore, they should possess larger overall magnetic moments anti-ferromagnetic interchange occurs.[2]

A crystalline powder of $Na_2(Zn_{1-x},Co_x)SiO_4$ (x = 0.50) was prepared by high temperature ceramic methods. The band gap was measured to be approximately 1.7 eV, which is in the semiconductor region. X-ray powder diffraction data were collected with a Rigaku Miniflex II diffractometer with Copper $K\alpha$ radiation. Data were collected from 5˚ to 90˚ in two-theta. Examination of the resulting data verified that the Co^{2+} and Zn^{2+} ions were randomly distributed in divalent sites forming a single Zn-Co phase. Parthe's Na_2ZnSiO_4 structure was used as the starting model in a Rietveld analysis. Soft restraints were placed on all bond distances. The model was refined to convergence with χ^2 of 1.894 and R = 2.49 %. All atoms possess tetrahedral environments, but those associated with the Sodium ions are distorted. Initial magnetic studies reveal that the compound to be paramagnetic at 80 K although, analysis based upon Curie-Weiss and Langevin functions indicate possible antiferromagnetic interactions.

[1] E. Pathe, et al. J. Sol. Stat. Chem., 1969, 1: 1-5.

[2] T. Dietl, H. Ohno, F. Matsukura, Phys. Rev. B, 2001, 63: 195205.

F.4. & P.4. Structural studies of acid β-glucosidase in complex with potential pharmacological chaperones

Susan D. Orwig[1], Zhanqian Yu[2], Jeffery W. Kelly[2], Raquel L. Lieberman[1]

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA[1]; The Scripps Research Institute, La Jolla, CA[2]

Lysosomal storage disorders are 'loss of function' disorders, as the result of deficient lysosomal glycolipid hydrolase activity. Gaucher disease, the most common lysosomal storage disorder, is caused by inherited point mutations in acid-β-glucosidase (GCase), the enzyme that hydrolyzes glucosylceramide (GlcCer) in the lysosome. These point mutations cause overall thermal destabilization and result in its degradation in the endoplasmic reticulum (ER). The lysosomal concentration of GCase is subsequently decreased, and these events lead to an accumulation of GlcCer in the lysosome. Missense mutations of GCase are not localized to the active site. Thus, while mutations affect the stability and cellular trafficking, they do not abolish enzymatic activity of GCase. We are involved in understanding how ER permeable small molecules, called pharmacological chaperones, can bind to endogenous mutant GCase and stabilize the native fold in the ER, which subsequently enables cellular trafficking of the complex to the lysosome. In order for mutated acid β-glucosidase to escape ER associated degradation, we hypothesize that the enzyme needs to adopt the native fold, which is likely a substrate ready conformation. We are working to ascertain the structural determinants for an effective pharmacological chaperone by solving co-crystal structures of GCase with these compounds and exploring the plasticity of the GCase active site.

F.5. & P.5. Evaluating the role of data reduction approach on the success rate on sulfur-SAD phasing for a moderately diffracting crystal

James Tucker Swindell II, John P. Rose and B.C. Wang

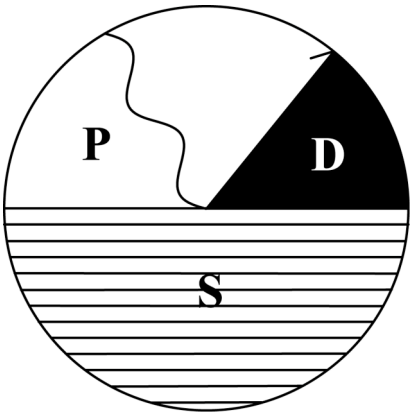
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602

The inherent difficulty of solving the phase problem in macromolecular crystallography has been somewhat alleviated in recent years due to advances in all areas of the diffraction experiment including protein preparation, crystallization, X-ray sources, cryo-crystallography, detection technologies, data reduction and anomalous scattering based phasing methods such as MAD or SAD.

A phasing approach, sulfur-SAD phasing, aimed removing the generic necessity of either including selenium via protein engineering or derivatization using heavy atoms was hypothesized in 1985 by B.C. Wang and is, albeit gradually, increasing in popularity.

We have recently determined the structure of AF1982 a small 95-residue protein encoded by *Archaeoglobus fulgidus* by sulfur-SAD phasing using data collected on a moderately (2.65Å) diffracting crystal. Two 360° data sets, processed with HKL2000 and scaled together using SCALEPACK, were required to provide an interpretable electron density map.

We then asked the question "Can we solve the structure using only single data set processed with an alternate data reduction program?" Herein we report a comparative analysis of data reduction programs (HKL2000, d*TREK, XDS, Proteum2) on the success rate of sulfur-SAD phasing for data collected on a moderately diffracting crystal using 1.9Å SER-CAT (22ID) X-rays.



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.6. Sulfur-SAD Phasing: Historical Perspective and Progress at UGA and SER-CAT.

Lirong Chen[1], Hao Xu[1], Hua Zhang[1], Zheng-Qing Fu[1,2], James Swindell II[1], John Chrzas[1,2], Zhongmin Jin[1,2], John Rose[1,2] and Bi-Cheng Wang[1,2]

[1]Dept. of Biochemistry and Molecular Biology, The University of Georgia, Athens, GA [2]SER-CAT, Advanced Photon Source, Argonne National Laboratory

Sulfur-SAD phasing, as part of the direct approach to structure determination by crystallography (direct crystallography), was introduced more than 25 years ago. Over the past decade, interest from the crystallographic community worldwide in sulfur-SAD phase determination has grown. More than 40 structures have been reported in the PDB as being solved by S-SAD phasing since year 2000.
Researchers at the University of Georgia (UGA) have a long history and practical interest in sulfur-SAD methodology development. In the past few years, more than 10 protein crystal structures have been determined by sulfur-SAD phasing on home X-ray source at UGA and by soft X-rays at SER-CAT beamlines. Sulfur-SAD phasing, including the use of advanced computing pipelines developed at UGA and SER-CAT, and analyses of those structures, will be summarized and reviewed. In addition, data collection and data reduction strategies aimed at enhancing the anomalous scattering signal, such as "Multiple-Data-Set" Data Collection and Zero-dose extrapolation of diffraction data, will be discussed.

P.7. Taming of the Beast: methods for the expression and purification of the innate antiretroviral factor APOBEC3G in complex with the HIV-1 protein Vif

Geoffrey M. Lippa, Colleen Kellenberger, Jolanta Krucinska, Harold C. Smith, Joseph E. Wedekind

Department of Biochemistry & Biophysics, University of Rochester, Rochester, NY 14642

APOBEC3G (A3G) is part of a family of deaminases that target DNA or RNA substrates. APOBEC3G restricts the replication of human immunodeficiency virus-1 (HIV-1) through the deamination of multiple cytidines present in the negative strand of the HIV-1 virus during reverse transcription. This leads to dG-to-dA hypermutations in the positive, coding strand of the proviral DNA that impair HIV-1 infectivity.

A3G comprises two homologous cytidine deaminase (CD) domains on a single polypeptide that arose from gene duplication. The C-terminal domain possesses dC-to-dU catalytic activity. Conversely, the N-terminal domain has RNA binding activity, and is the binding site for the HIV-1 viral infectivity factor (Vif). Interaction with Vif causes A3G to be targeted for degradation by the host cell's own proteasome. At present, there is limited structural data on the mode by which A3G interacts with Vif. High-resolution structures of the A3G-Vif complexes would elucidate critical sequence and stereochemical information sought for structure-guided drug-design.

The focus of my work has been on the engineering of protein complexes comprising A3G and Vif for the purpose of producing milligram amounts of material for structural analyses. Herein, I describe the use of an *E. coli* expression system that features a novel, fusion-protein approach for expression. The results are compared to other constructs in which the proteins are expressed in *trans*. Progress on this challenging project is described with the goal of describing new approaches to protein complex formation when conventional methods fall short.

P.8. Structural Basis of Substrate Specificity of Caspase-3 at S4 and S5 Revealed by Crystallography and Kinetic Studies

Bin Fang, Guoxing Fu, Johnson Agniswamy, Robert W. Harrison, Irene T. Weber

Department of Biology, Molecular Basis of Disease, Georgia State University, Atlanta, Georgia 30303

The molecular basis of substrate specificity for human caspase-3 at S4 substrate-binding pocket has been investigated using peptide analog inhibitors that vary at P4 positions. Caspase-3 complexes with seven substrate analog inhibitors were solved at resolutions of 1.6~2.6 Å. Structural analysis well correlated with the experimental Ki of seven inhibitors. Trp206 and Trp214 of caspase-3 in S4 were observed to interact with nonpolar P4 side chains. Phe250 and 252 were shown to interact with P4 Trp and nonpolar P5. In addition, enzyme kinetic, structural modeling and crystallography studies were performed on caspase-3, -6, and -7 in order to extend the investigation of their P5 recognitions. Favorable interactions between P5 residues and S5 binding site were observed in crystal structure of caspase-3/LDESD (1.6 Å) and structural model of caspase-6/LDESD. Consistent with structural evidences, our kinetic data suggested that caspase-3 prefers hydrophobic P5 residues since P5 Leu of substrate Ac-LDEVD-pNA increased the catalytic efficiency for 50% compared with the canonical substrate Ac-DEVD-pNA. In contrast, caspase-6 showed the preference for polar P5 residues whereas caspase-7 showed no preference for the P5 residues.

P.9. Structural investigation of HBsAg: A progress report

Quentin Florence[1], Hao Xu[1], Jonny Yokosawa[2], James Laura[2], Yuri Khudyakov[2] and John P. Rose[1]

[1]*Dept. of Biochemistry and Molecular Biology, The University of Georgia, Athens, GA USA and* [2]*Centers for Disease Control and Prevention, Atlanta, GA USA.*

Hepatitis B virus (HBV) is a blood-born-associated virus that has infected over 2 billion people worldwide. It is also one of the major causes of liver cancer. The overall goal of this study is to provide structural insight of the HBsAg protein, the antigenic component of the currently licensed vaccine for HBV. Despite its worldwide usage and its application as a carrier for epitope presentation, the tertiary structure of this important protein is still unknown. In addition, the lack of a HBsAg structure has (1) prevented us from understanding the effects of mutations on polymerase drug-resistance and on the antigenic properties of the HBsAg major neutralizing antigenic epitope and (2) impeded the development of bivalent vaccines where HBsAg is used as a carrier for foreign antigenic epitopes.

In summary, the results of the proposed studies will provide the basis for understanding how various mutations and fusion of foreign epitopes may affect the tertiary structure of the HBsAg neutralizing epitope. In addition, the studies should provide sufficient understanding of molecular recognition between the immunogenic site ('a'-determinant region) of HBsAg and antibodies. Such knowledge is necessary for the development of improved HBV vaccines, bivalent vaccines and understanding the role of drug-resistant mutations in HBV immune escape.

Our initial structural studies have been focused on conducting Small Angle X-ray Scattering (SAXS) experiments and the production of diffraction quality crystals for X-ray diffraction experiments. Results from SAXS experiments and initial crystallization screens will be presented.

Work is supported with funds from the Georgia Research Alliance (GRA.VAC09.G/GRA.VAC10.G) and the University of Georgia Research Foundation.

P.10. Structures of BenM DNA binding domain with its DNA promoter sites explain the common elements of promoter recognition used by lysr-type transcriptional regulators

Amer Alanazi[1], Ellen Neidle[2] and Cory Momany[1]

[1]Pharmaceutical and Biomedical Sciences Department, University of Georgia, Athens; [2]Microbiology Department, University of Georgia, Athens, GA

BenM is a LysR-type transcriptional regulator (LTTR) involved in controlling benzoate degradation in the soli bacterium *Acinetobacter.baylyi*. The high-resolution structure of the unbound BenM DNA binding domain (BenM DBD) revealed that the BenM DNA binding domain forms a compact globular domain composed of three helices with a helix-turn-helix motif (α2-α3) and longer helix resembling that of the DBD of winged helix proteins. Based on the structures of BenM DBD with *benA* and *catB* promotor sites, BenM DBD dimers span a large region of bent DNA where both recognition helices of one dimer bind into two consecutive DNA major grooves. The specific DNA major groove interactions that make up the LTTR conserved recognition motif (T-N_{11}-A) are defined by the interaction of two proline residues at the N-terminal end of the recognition helices with the methyl group of the thymine base and a glutamine residue hydrogen bonds to the adenine. The wing of the winged HTH motif interacts mainly with the phosphate backbone of the DNA minor groove and provides structural stability and proper positioning of the N-terminal end of the helix.

P.11. MacCHESS 2009

D.J. Schuller, R. Cerione, M. Cook, U. Englich, R.E. Gillilan, S.M. Gruner, C.U. Kim, I. Kriksunov, W. Miller, S. Smith, D.M. Szebenyi

MacCHESS, Cornell Univ., Ithaca, NY 14853 USA.

MacCHESS ("Macromolecular diffraction at CHESS") is an NIH/NCRR funded resource at the Cornell High Energy Synchrotron Source. Beam time is open to the structural biology community; our next run is scheduled to start in January 2010.

Inquire or apply for time through our web site: http://www.macchess.cornell.edu.

MacCHESS facilities and services include:

- A1, a high flux monochromatic beamline for crystallography, tuned to the selenium edge, with an ADSC Quantum 210 detector.
- F1, a high flux monochromatic beamline for crystallography, tuned to the bromine edge, with an ADSC Quantum 270 detector, an ALS automounter, and BL-2 biohazard qualification.
- F2, a MAD beamline with an ADSC Quantum 210 detector.
- F3, a part-time soft X-ray beamline for SAD, with an ADSC Quantum 4 detector.
- SAXS collection at various beamlines.
- Microcrystallography with capillary focusing at various beamlines.
- High pressure cryo-cooling.

In addition to offering routine data collection, MacCHESS engages in research to advance structural biology. Current projects include: Automation, Microcrystallography, high pressure cryo-cooling, SAXS and low resolution phasing, and soft X-ray anomalous phasing. This poster will highlight some recent research accomplishments by MacCHESS users and MacCHESS staff, including successful incorporation of the XREC auto-centering routine into the MacCHESS crystal centering interface, plans for a second generation ALS-style automounter at beamline A1, and some notes on cryo-SAXS.

P.12. Probing the effect of production platform on the immunogenicity of recombinant vaccine proteins

Dayong Zhou[1], Hao Xu[1], Frank Michel[2], Jeff Hogan[2], and John Rose[1]

[1]Department of Biochemistry and Molecular Biology, Franklin College and
[2]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 USA.

Ebola virus infection of humans and non-human primates results in a severe disease characterized by fever, macropapular rash, liver and kidney dysfunction, shock, and both internal and external bleeding. Its high mortality rate, approaching 80% in humans, the lack of treatment options and the ability to infect both humans and non-human primates, via aerosolization, make Ebola virus a potential biowarfare agent.

Extensive efforts over the last 30 years have been made to develop vaccines against Ebola virus with little success. Of the progress that has been made, the most promising vaccine candidates share a common theme: induction of a host immune response against the Ebola virus glycoprotein (GP).

One approach for rational vaccine development, which is gaining considerable interest in the community, uses knowledge of the target's 3-dimensional structure coupled with functional and other data to design improved immunogens. However, protein production and post-translational modifications in prokaryotic and eukaryotic systems differ greatly. Thus, we asked the question "Does the source of the recombinant protein used to produce the vaccine affect the protein structure and subsequent antigen-specific immune response?"

To answer this question we are studying the antigen-specific immune response to the Ebola virus small glycoprotein sGP produced using a variety (bacterial-, yeast-, insect- and kidney-cell) of expression systems. We have currently expressed and purified the 295-residue sGP (sGP) in *E. coli*, insect and mammalian cell lines in preparation for antigenicity, immunogenicity protective efficacy characterization and crystallization trials.

The results of these studies will have direct applicability to vaccine production and aid in our long-term goal of using structural biology to aid in the design of more effective vaccines. Details of this work will be presented.

This work was funded in part by the Department of Defense Congressionally Directed Medical Research Program (CDMRP) award W81XWH-04-1-0920 to J. H. and the University of Georgia Research Foundation to J.R.

P.13. Crystallizing HIV-1 viral infectivity factor (vif) in complex with Elongin B and Elongin C

Jason D. Salter and Joseph E. Wedekind

*Department of Biochemistry & Biophysics, 601 Elmwood Avenue Box 712, Rochester, New York 14642 USA. (*email: Jason_Salter@urmc.rochester.edu)*

$CD4^+$ T cells, the primary target for HIV-1 infectivity, express the protein APOBEC3G, a cytidine deaminase that possesses an innate anti-retroviral activity. The enzyme catalyzes sequence-specific dC-to-dU deamination on negative-polarity single-stranded HIV-1 reverse transcripts. This reaction produces G-to-A mutations of the positive polarity (coding) strand of HIV-1 genomic RNA and coincides subsequently with diminished viral infectivity.

HIV-1 suppresses the innate antiretroviral activity of A3G with its protein vif (viral infectivity factor), a diminutive 23 kD essential accessory protein. Vif functions by directly binding A3G and recruiting it to a Cullin-RING E3 ubiquitin ligase (CRL) for ubiquitination and subsequent proteasomal degradation. This CRL comprises the cellular proteins Cullin5 (Cul5), ElonginB, ElonginC (EloB/C), and Rbx2. Acting as a substrate receptor for the CRL, vif binds A3G with N-terminal residues and makes critical contacts with EloB/C, and Cul5 through conserved C-terminal motifs. Essentially, vif tricks the cell into destroying its own antiretroviral protein just when it is needed most.

At present, several truncated forms of vif have been designed and recombinantly co-expressed with EloB/C to form highly purified tripartite complexes. These complexes are capable of binding recombinantly expressed Cul5, suggesting they possess biological functionality and are thus good candidates for crystallization. Several small crystals of these EloB/C-vif complexes have been grown that display Bragg diffraction upon exposure to x-rays. Here, I present these data and current efforts to produce superior crystals suitable for structure determination.

Supported by NIH R21 AI076085 to JEW. JDS is supported by NIH T32 AI049815.

P.14. The UGA Structural Biology Incubator: An Invitation to Georgia Researchers Needing Protein and/or Structure

Hao Xu, Lirong Chen, John P. Rose and Bi-Cheng Wang

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602 USA

There is growing interest in the community in structure assisted therapeutic development where structural information, at the atomic level, is used in the design of new drugs and vaccines. A world-class facility has been built on the University of Georgia campus for carrying out atomic-level structural investigations of macromolecules by X-ray Crystallography. Using the gene as the starting point, the protein can be expressed, purified, crystallized and its structure determined. The incubator serves as a one-stop-shop for researchers, crystallographers and non-crystallographers alike who need protein and/or structure for their research.

Established in 1995 and leveraging significant investment from the Georgia Research Alliance, the University of Georgia and the National Institutes of Health, this facility is capable of carrying out high throughput (HT) crystallographic studies for research and development. Facility capabilities include:

- Protein production (cloning, expression and purification
- Robotic screening of crystallization space
- Automated data collection for *de novo* structure solution or binding studies
- Access to the SER-CAT beamlines at the Advanced Photon Source
- A powerful cluster based structure determination pipeline
- A highly experienced technical staff

Access to the facility can be arranged either by collaboration or on a cost recovery basis.

Work supported by funds form the NIH (GM62407), IBM Life Sciences, the Georgia Research Alliance and the University of Georgia Research Foundation.

P.15. Molecular mechanisms of drug resistance in HIV-1 protease

Chen-Hsiang Shen[1], Ying Zhang[2], Xiaxia Yu[1], Yuan-Fang Wang[1], Johnson Agniswamy[1,4], Robert W. Harrison[3], John M. Louis[5], Arun K. Ghosh[6], & Irene T. Weber[1,2,4]

[1]Departments of Biology, [2]Chemistry, and [3]Computer Science, [4]Molecular Basis of Disease Program, Georgia State University, Atlanta, GA, USA; [5]Laboratory of Chemical Physics, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA.; [6]Departments of Chemistry and Medicinal Chemistry, Purdue University, West Lafayette, IN, USA.

A major challenge in long term AIDS therapy arises when HIV mutates and gains resistance to antiviral drugs. Our group studies the molecular mechanisms of resistance to antiviral inhibitors of HIV protease and uses structural information to design new inhibitors providing enhanced interactions with conserved regions of the protease. Crystal structures of antiviral inhibitors with mutant protease were solved at high to atomic resolutions (0.84-1.5Å) to demonstrate structural alterations caused by drug resistance mutations. We have analyzed HIV-1 protease with diverse single mutations and selected combinations to reveal the molecular mechanisms of drug resistance. Three distinct mechanisms have been observed for drug resistance mutations: 1) mutations in the inhibitor binding cavity directly alter inhibitor binding; 2) mutations at the dimer interface alter protease stability; 3) other mutations have indirect effects on protease activity and inhibition by altering the unliganded protease or the interactions with reaction intermediates. Our strategy of novel inhibitor design is to introduce new polar interactions between protease and inhibitor based on early studies of the conserved hydrogen bond interactions. Darunavir, which was approved in 2006 for AIDS therapy, and a series of novel antiviral inhibitors demonstrate the success of this structure-guided strategy to overcome HIV drug resistance. The new knowledge of the mechanisms of drug resistance and successful strategies for drug design are important for development of the next generation of antiviral inhibitors to combat drug resistance.

Acknowledgements:

The research was supported in part by the Georgia State University Molecular Basis of Disease Program, the Georgia Research Alliance, and the National Institute of Health grants GM062920 and GM053386.

P.16. Variable temperature and pressure powder diffraction study of scandium fluoride

Benjamin K. Greve[1], Angus P. Wilkinson[1], and Karena Chapman[2]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology; Atlanta, GA and [2]Advanced Photon Source, Argonne National Laboratory; Argonne, IL

Scandium fluoride (ScF_3) adopts the cubic ReO_3 framework under ambient conditions. The thermal expansion of this material was characterized using both synchrotron x-ray and neutron powder diffraction. Subsequently, the effects of pressure on the coefficient of thermal expansion were examined using both a gas pressure cell and diamond anvil cells. A first order cubic to rhombohedral phase transition was observed under compression, and a preliminary pressure-temperature phase diagram is presented.