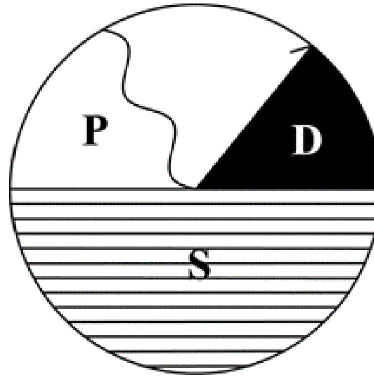
The 66th Annual Pittsburgh Diffraction Conference



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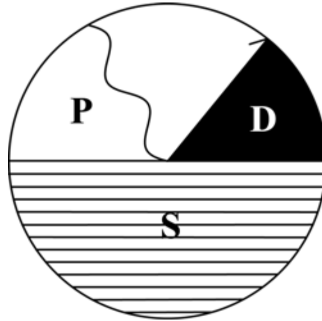

Sidhu Award

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

The award recognizes an outstanding contribution to crystallographic or diffraction research by an investigator whose doctoral degree was conferred within six years before the award date. The award carries a cash prize of $2000. The award winner will present the Sidhu Award Lecture on November 1st.

Previous winners of the award are:

1967 A.I. Bienenstock
1968 R.M. Nicklow
1969 T.O. Baldwin
1970 S.-H. Kim
1971 L.K. Walford
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1988 Q. Shen
1989 M. Luo
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1993 M. Pressprich & T. Yeates
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1996 M.J. Regan
1999 C. Ban & M. Wahl
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2005 C.-Y. Ruan
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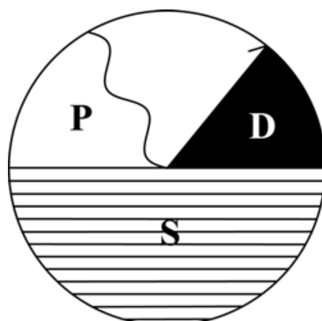
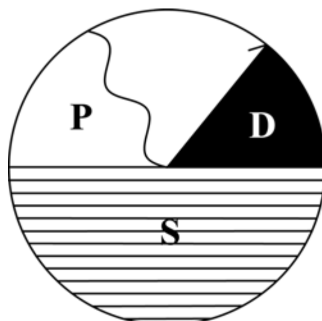


Chung Soo Yoo Award

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 awarded to the graduate student presenting the best poster at the annual Pittsburgh Diffraction Conference.



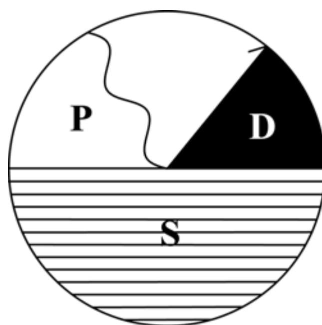
The PDS Award Funds

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



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Lifetime membership in the Pittsburgh Diffraction Society requires a payment of $100 or more.

Program

Thursday, 30th October 2008

7:45 Continental Breakfast / Registration

8:15 Opening Remarks

Morning Session A

Macromolecule Structure Refinement and Validation

Ana Gonzalez (Stanford Synchrotron Radiation Laboratory)
Paul Adams (Lawrence Berkeley National Laboratory)

8:30 **The Theoretical Limits of Macromolecular Crystallography**
James Holton (UCSF, LBNL)

9:00 **Macromolecular Model Building with Resolve and the PHENIX AutoBuild Wizard**
Li-Wei Hung (LBNL)

9:30 **Structure Refinement with phenix.refine**
Pavel Afonine (LBNL)

10:00 Coffee Break

10:20 **Ultra High Resolution Protein Crystallography and Charge Density Analysis**
Christian Jelsch (LCM3B, Nancy University)

10:55 **New Algorithms for Improving Structures of Supramolecular Complexes and Membrane Proteins at Lower Resolutions of X-ray Diffraction**
Jianpeng Ma (Baylor College of Medicine)

Afternoon Session B

SAD, Why do three times the work?

Julian Adams (Australian Synchrotron)

11:30 **SAD Phasing Basics:**
How and Why it Works, and When it's Likely to Fail
William Furey (University of Pittsburgh)

12:00 Lunch

13:30 **SAD Data Collection and Experimental Setup**
Kanagalaghatta Rajashankar (NE-CAT, Advanced Photon Source)

14:00 **High-Energy Sulfur SAD at the Australian Synchrotron**
Tom Caradoc-Davies (Australian Synchrotron)

14:30 **25 Years of SAS Phasing**
John Rose (University of Georgia)

15:00 Afternoon Break

15:20 **SAD Data Collection Using a Home Source**
Angela R. Criswell (Rigaku Americas Corporation)

Afternoon Session C

Seeing the World and Making it Better using Diffraction
(part 1)

Steve Geib (University of Pittsburgh)
Apurva Mehta (Stanford Synchrotron Radiation Laboratory)

15:55 **Insights Obtained on Micromechanical Deformation of Biomedical Devices and Biomaterials from 2D X-ray Diffraction**
Apurva Mehta (SSRL)

16:30 **Characterizing Compaction-Induced Disordering of Active Pharmaceutical Ingredients using Powder X-ray Diffraction**
Michael D. Moore (Duquesne University)

7:00-10:00 PM, Thursday, 30th October 2008

Poster Session and Reception

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. Posters must be removed by 3:00 PM, the close of the conference on Saturday.

P.1. Protein crystallography at the Australian Synchrotron
Julian Adams *(Australian Synchrotron)*

P.2. Metal-organic frameworks constructed from biomolecular building blocks: Synthesis, characterization, and emerging properties
Jihyun An (University of Pittsburgh)

P.3. Macromolecular Crystallography Beam Line Facilities at the Stanford Synchrotron Radiation Laboratory
Aina Cohen (SSRL)

P.4. Structure flexibilty allows sequence diversity
Frédérique Favier (LCM3B, Nancy University)

P.5. Preliminary Model for a CAP Class-I and UP-element Transcription Activation Complex Subassembly
Samuel Lara-Gonzalez (Rutgers University)

P.6. A potential macromolecular test crystal system for assurance of beamline quality
Chuck Luke (Molecular Dimensions)

P.7. Crystal structure of M. tuberculosis ribokinase in complex with ribose and dinucleotide
Eugene Masters (Southern Research Institute)

P.8. **Solution and crystal structures of a sugar binding site mutant of Cyanovirin-N: no evidence of domain-swapping**
Elena Matei (University of Pittsburgh)

P.9. **Synthesis, Structural and Physicochemical Characterization of the Diamond-Like Semiconductor Ag_2CdGeS_4**
William Minsterman (Duquesne University)

P.10. **Alkali Chalcogenide Flux Synthesis and Characterization of the Quaternary Diamond-Like Semiconductor, Li_2CdSnS_4**
Meghann A. Moreau (Duquesne University)

P.11. **Non-Stick Collagen: Crystal Structures of Fluorinated Collagens**
Kenneth A. Satyshur (University of Wisconsin-Madison)

P.12. **Direct space and simultaneous direct-reciprocal space integer optimization models for phasing crystal structures**
Alex B. Smith (Carnegie Mellon University)

P.13. **The Crystal Structures of Enterococcus Aminoglycoside (2") Phosphotransferase variants Ib and Ic, Enzymes Implicated in Antibiotic Resistance**
Clyde Smith (SSRL)

P.14. **A Single Atom Controls the Catalytic Fitness of an RNA Enzyme**
Robert C. Spitale (University of Rochester)

P.15. **Are X-rays damaging to structural biology? A case study with xylose isomerase.**
Kristin Wunsch (Hauptman-Woodward Institute)

P.16. **Automated Deposition Tools for Depositors**
Huanwang Yang (Rutgers University)

P.17. **Studies of Site Occupation and Chemical Composition in Mn-Doped $CuInSe_2$ Chalcopyrites**
Jinlei Yao (Duquesne University)

Friday, 31st October 2008

Morning Session D

Seeing the World and Making it Better using Diffraction
(part 2)

Steve Geib (University of Pittsburgh)
Apurva Mehta (Stanford Synchrotron Radiation Laboratory)

8:30 **Measurement of Strain in Silicon Thin Films by X-ray Diffraction**
Matthew Bibee (SSRL)

9:00 **Bio-MOFs: Synthesis, Characterization, and Application**
Nathaniel Rosi (University of Pittsburgh)

9:30 **Advanced Crystallographic Program in ChemMatCARS**
Yu-Sheng Chen (ChemMatCARS, APS)

10:00 Coffee Break

10:20 **Structural Studies of Hydrogen Storage Materials**
Hui Wu (NIST)

10:55 **Crystal Truncation Rod (CTR) Surface Diffraction in Geochemistry**
Glenn Waychunas (LBNL)

11:30 **Photonic Crystal Materials Derived from Crystalline Colloidal Arrays**
Sanford Asher (University of Pittsburgh)

12:00 Lunch

Afternoon Session E

Before the Crystal and Further than the Crystal

Edward Snell (Hauptman-Woodward Institute)
James Conway (University of Pittsburgh)

13:30 **Bridging the gap between structure and dynamics with elastic network models**
Tim Lezon (University of Pittsburgh)

14:00 TBA
Angela Gronenborn (University of Pittsburgh)

14:30 **High-resolution Solution X-ray Scattering and Envelope Based Phasing**
Xinguo Hong (MacCHESS, Cornell University)

15:00 Afternoon Break

15:20 **Virus capsid structures revealed by cryo-electron microscopy combined with subunit atomic models**
James Conway (University of Pittsburgh)

15:55 **Using Raman to Follow Chemistry and Chemical Reactions in Macromolecular Crystals**
Paul Carey (Case Western Reserve University)

16:30 **XAS and XES techniques for studying the structure and mechanism of the photosynthetic water-splitting catalyst**
Junko Yano (LBNL)

17:00 Break

17:05 **A novel microfluidic device for protein crystallization condition finding**
Michael Biros (SpinX Technologies)

Saturday, 1st November 2008

8:00 Introduction

8:10

Sidhu Award Lecture

Structural Studies of Integral Membrane Proteins involved in G-protein Signaling
Michael Hanson (The Scripps Research Institute)

9:00 Coffee Break

Session F

Pushing the Boundaries of Structural Biology

Mark Macbeth (Carnegie Mellon University)
Andy VanDemark (University of Pittsburgh)

09:20 **Allosteric regulation of glucocorticoid receptor activity by DNA binding sequence**
Miles Pufall (University of California, San Francisco)

09:55 **Solution NMR Characterization of Important Enzyme Motions**
Patrick Loria (Yale University)

10:30 **Modeling Bacteriophage HK97 capsid maturation**
Robert Duda (University of Pittsburgh)

11:00 Coffee Break

11:20 **The History of the Pittsburgh Diffraction Society**
Bryan Craven (Indiana University of Pennsylvania)

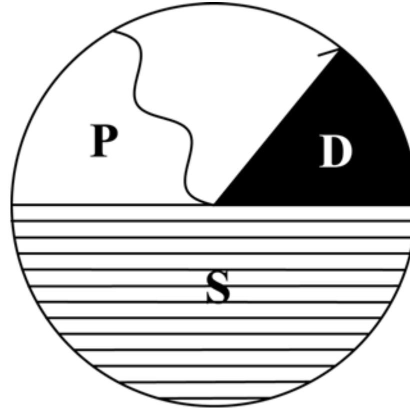
11:50 **PDS Business Meeting**

12:10 Lunch

13:30 **Structural Studies of Transcription**
Guillermo Calero (University of Pittsburgh)

14:00 **Using unnatural amino acids to probe the structure-function relationship in human GSTs.**
Kaustibh Sinha (Carnegie Mellon University)

14:30 **The Superstructure of an Antimicrobial Peptide, Alamethicin, in Lipid Membranes**
Jianjun Pan (Carnegie Mellon University)



LECTURE ABSTRACTS

A.1. The Theoretical Limits of Macromolecular Crystallography

James M. Holton[1,2]

[1]Assistant Adjunct Professor of Biochemistry and Biophysics, University of California, San Francisco, CA 94107
[2]Lawrence Berkeley National Laboratory, Berkeley, CA 94720

James M. Holton: JMHolton@lbl.gov

In general, a large, high-quality crystal will yield sufficient data to solve a protein structure long before significant radiation damage is done, and a small or poor quality crystal will suffer serious damage long before enough data can be collected. In practice, many crystals fall between these extreme cases, and one must strike a balance between data quality and dose. Devising such a data collection strategy requires the answer to several critical questions: How much exposure is required to solve the structure? When is a crystal so small or so poor in quality that data collection will be a waste of time? Dividing the total exposure over more images allows for radiation damage to be evaluated after data collection, but at what point does higher redundancy with shorter exposures add “too much” read-out noise? What about a better detector? What about a perfect detector? Answering these questions requires that damage, noise and signal be placed on a common, absolute scale. To this end, a quantitative simulator of the entire diffraction experiment was created and called "MLFSOM" (MOSFLM in reverse). The input to the simulator is a protein data bank (PDB) file and parameters such as photon flux, crystal size and detector performance characteristics entered in conventional units such as photons/s and millimeters. MLFSOM produced images in SMV format that were subsequently processed with ELVES. The general result of these trials was that one and only one of the many sources of noise in the diffraction experiment will dominate a given experiment, but the read-out noise of a modern detector cannot have a significant impact on anomalous data. The optimal strategy for MAD/SAD data collection was many very short exposures. How short depends on beam stability and shutter performance, but not the detector.

A.2. Macromolecular Model Building with Resolve and the PHENIX AutoBuild Wizard

Li-Wei Hung[1], Ralf W. Grosse-Kunstleve[2], Paval V. Afonine[2], Nigel W. Moriarty[2], Peter H. Zwart[2], Randy J. Read[3], Paul D. Adams[2], and Thomas C. Terwilliger[1]

[1]Los Alamos National Laboratory, Los Alamos, NM 87545, USA,
[2]Lawrence Berkeley National Laboratory, One Cyclotron Road, Berkeley, CA 94720, USA,
[3]Department of Haematology, University of Cambridge, Cambridge CB2 0XY, England

Li-Wei Hung: lwhung@lbl.gov

The *RESOLVE* model-building is based on template matching and iterative fragment extension algorithms. These methods have been shown to work effectively even for data at medium to low resolution. Enhanced automation and functionality has been implemented in the *AutoBuild* Wizard in the *PHENIX* software suite. The *PHENIX AutoBuild* wizard is a highly automated tool for iterative model building, structure refinement and density modification using *RESOLVE* model building, *RESOLVE* statistical density modification and *phenix.refine* structure refinement. Recent advances in the *AutoBuild* wizard and *phenix.refine* include automated detection and application of NCS from models as they are built, extensive model-completion algorithms and automated solvent-molecule picking. We have found that the *AutoBuild* wizard can yield highly complete and well refined models for structures with resolutions at as low as 3.2A.

References:

1. Iterative model building, structure refinement and density modification with the PHENIX AutoBuild wizard. T. C. Terwilliger, R. W. Grosse-Kunstleve, P. V. Afonine, N. W. Moriarty, P. H. Zwart, L.-W. Hung, R. J. Read, and P. D. Adams Acta Cryst. D64, 61-69 (2008)
2. Automated side-chain model building and sequence assignment by template matching. T.C. Terwilliger. Acta Cryst. D59, 45-49 (2003)
3. Automated main-chain model building by template matching and iterative fragment extension. T.C. Terwilliger. Acta Cryst. D59, 38-44 (2003)

A.3. Structure refinement with phenix.refine

Pavel Afonine, Ralf Grosse-Kunstleve, Peter Zwart, Thomas Terwilliger, Nigel Moriarty, Nathaniel Echols and Paul Adams

Lawrence Berkeley National Laboratory, Berkeley, CA

Pavel Afonine: PAfonine@lbl.gov

phenix.refine state-of-the-art refinement module of *PHENIX* is an example of how a combination of highly efficient programming tools and new or improved crystallographic algorithms provides a very high level of automation and robustness in crystallographic structure refinement. phenix.refine possesses a complete set of tools that cover most of refinement needs and scenarios, such as:

- Automatic handling of most experimental data formats (CNS, SHELX, MTZ, etc)
- Individual coordinates refinement using minimization or simulated annealing
- Highly optimized and automated rigid body refinement (multiple-zones algorithm)
- ADP refinement: individual isotropic or anisotropic, grouped, TLS or any mixture
- Occupancy refinement (grouped, individual, constrained for alternative conformations or any mixture)
- Automatic NCS detection and use in refinement as restraints
- Automatic twinning detection and use in refinement
- Various refinement targets (maximum-likelihood, maximum-likelihood with experimental phase information, and amplitude least-squares)
- Robust mask-based bulk-solvent correction and anisotropic scaling
- A-weighted map output with any user-defined coefficients
- Refinement using X-ray data, neutron data or joint refinement with both
- Refinement at ultra-high resolution using novel Inter Atomic Scatterers model
- Use of H atoms in refinement at any resolution
- Straightforward inclusion of novel ligands
- Runs on most of platforms (Mac, Linux, Windows)

More tools are under active development and to appear in future.

phenix.refine allows multi-step complex refinement jobs, for example, containing: rigid body refinement, Simulated Annealing, individual or grouped B-factors combined with TLS refinement, constrained occupancy refinement, automatic water picking to be performed in one run. The flexibility of phenix.refine allows any combination of refinement strategies to be applied to any selected part of a model.

phenix.refine is tightly integrated with other PHENIX components making structure solution, building and refinement a one step process (Automr, Autobuild wizards).

phenix.refine is periodically tested by automatic re-refinement of all models in the PDB for which unambiguous experimental data and cross validation flags are available.

The PHENIX package, complete documentation and references to relevant publications are available from www.phenix-online.org.

A.4. Ultra High Resolution Protein Crystallography & Charge Density Analysis

Christian Jelsch, Slawomir Domagala, Benoit Guillot, Bertrand Fournier, Virginie Pichon, Claude Lecomte

Laboratoire de Cristallographie et Modelisation des Materiaux Mineraux et Biologiques, Nancy University, Vandoeuvre les Nancy, France

Christian Jelsch: christian.jelsch@uhp.uhp-nancy.fr

Experimental charge-density analyses are commonly applied to small molecules. With an increasing number of biomacromolecular crystal structures being measured at ultra-high resolution, it has become possible to extend these methods to large systems. Charge density analyses of small molecules require subatomic resolutions, typically 0.5 Angstrom. The deformation of the electron density due to covalent bonding and intermolecular interactions can be observed and refined. The Hansen & Coppens [1] multipolar pseudo-atom is generally used to model the molecular electron density and derive electrostatic potential distributions.

A library of average multipole populations describing the electron density of chemical groups in all 20 amino acids found in proteins was built [2]. The library values are obtained from several small peptide or amino acid crystal structures refined against ultra-high-resolution X-ray diffraction data. The library transfer can be applied automatically in the MoPro software suite [3] to peptide and protein structures measured at atomic resolution. The transferred multipolar parameters are kept fixed while the positional and thermal parameters of the macromolecule are refined. A new library including more atom types is under construction. The atom types are defined on the basis of their chemical environment, local symmetry and properly defined local coordinate system. This new database is constructed from the electron density refinement of ultra high resolution X-ray diffraction data for ~40 peptides and organic compounds. These diffraction data were collected previously in the laboratory, downloaded from crystallographic journals websites or obtained directly from authors of articles. The introduction of constraints allows reducing the number of needed multipolar parameters for the description of the molecular electron density. The constraints on the charge density originate from:

- the local symmetry of an atom which applies consequently to the multipoles
- the chemical equivalence of atoms: several atoms share the same valence and multipoles populations.

As a consequence of reduced number of variables, more meaningful results and stable charge density refinement is gained. Optimal new local axes definitions have been developed and this approach might be useful for the description of transition metal complexes. An automatic generation of the local axes systems and charge density refinement strategy is proposed by the MoPro software.

Crystals of human aldose reductase (hAR) in complex with NADPH and the Idd594 inhibitor diffract up to 0.66Å resolution [4]. hAR is a 6700 atoms NADPH dependent enzyme involved in diabetes complications. Such subatomic resolution X-ray data allows, after a restrained refinement using a spherical atom model, the observation of significant residual deformation electron density on the covalent bonds [5]. The residual electron density peaks are the deviation from the spherical atom approximation of the atomic electron clouds, due to chemical bonding.
After transfer from our charge density parameters database, a constrained multipolar refinement of hAR was performed with the MoPro software. The multipolar refinement leads to decreased atomic thermal motion amplitudes and better stereochemistry [6]. The charge density analysis focusing on hAR active site will be described. Deformation electron densities of the catalytic chemical groups have been modelled using the multipolar formalism. A topological analysis was performed in order to characterize the inhibitor binding and catalytic residues interactions.

Reference:

1. Hansen N.K. & Coppens P. Acta Cryst. A34, 1978. 909-921. Testing aspherical atom refinements on small-molecule data sets.
2. Zarychta, B., Pichon-Pesme, V., Guillot, B., Lecomte, C. & Jelsch, C. (2007). Acta Cryst. A63, 108-125. On the application of an experimental multipolar pseudo-atom library for accurate refinement of small-molecule and protein crystal structures.
3. Jelsch, C., Guillot, B., Lagoutte, A. & Lecomte, C. (2005). J. Appl. Cryst. 38, 38-54. Advances in Proteins and Small Molecules. Charge Density Refinement Methods using software MoPro.
4. E. Howard, R. Sanishvili, R.E. Cachau, A. Mitschler, B. Chevrier, P. Barth, V. Lamour, M. Van Zandt, E. Sibley, C. Bon, D. Moras, T. R. Schneider, A. Joachimiak & A. Podjarny. Proteins: Struct Funct Genet.55, 792-804 ,2004. Ultra-high resolution drug design I: Human aldose reductase inhibitor complex at 0.66 Å shows experimentally protonation states and atomic interactions which have implications for the inhibition mechanism
5. Muzet M, Guillot B, Jelsch C, Howard E. & Lecomte C. Proc. Natl. Acad. Sci. USA (2003), 100: 8742-8747. On the Electrostatic Complementarity in a Human Aldose Reductase / NADP+ / Inhibitor Complex as derived from First Principles Calculations and Ultra-High Resolution Crystallography.
6. Guillot, B., Jelsch, C., Podjarny, A. & Lecomte, C. (2008). Acta Cryst. D64, 567-588. Charge-density analysis of a protein structure at subatomic resolution: the human aldose reductase case.

A.5. New Algorithms for Improving Structures of Supramolecular Complexes and Membrane Proteins at lower Resolutions of X-ray Diffration

Jianpeng Ma

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We report a novel X-ray crystallographic refinement protocol for modeling anisotropic thermal parameters of supramolecular complexes and membrane proteins. With this protocol, a very small set of low-frequency normal modes (e.g., 25 ~ 50 modes) can be used to reconstruct the thermal motions in X-ray diffraction. The method was applied on a series of supramolecular complexes and membrane proteins, all of which structures were solved at moderate resolutions. Examples are FTCD complex (3.4 Å, PDB code: 1TT9) (1), potassium channel KcsA (3.2 Å, PDB code: 1BL8) (2), voltage dependent potassium channel Shaker Kv1.2 (2.9 Å, PDB code: 2A79), and mechanosensitive channels MscL (3.5 Å, PDB code: 2OAR) and MscS (3.7 Å, PDB code: 2OAU). The results universally showed that the R_{free} values of the normal-mode-refined models were lower than the original isotropically refined models. Most importantly, the refinement resulted in improvement in electron density maps that allowed for building of a substantial amount of missing atoms. In one case, channel Kv1.2, we were able to rebuild almost all missing atoms that constitutes 1/3 of total molecular mass. The distribution of anisotropic thermal ellipsoids also revealed structure flexibility that is important for molecular mechanisms. We believe that the new protocol will help to significantly improve the structures of many highly-flexible supramolecular complexes and membrane proteins, for which further refinement is beyond any currently available methods. The figure shows typical density map improvement after the normal-mode-based refinement. Left panel is the original map of a flexible loop region, and the right panel is the map of the same region after refinement. The example was taken from a segment of Kv1.2 channel (to be published).

References:

1. Poon, B. K., Chen, X., Lu, M., Vyas, N. K., Quiocho, F. A., Wang, Q., and Ma, J., Normal mode refinement of anisotropic thermal parameters for a supramolecular complex at 3.42-A crystallographic resolution, Proc Natl Acad Sci U S A, 104, 7869 (2007).
2. Chen, X., Poon, B. K., Dousis, A., Wang, Q., and Ma, J., Normal-mode refinement of anisotropic thermal parameters for potassium channel KcsA at 3.2 Å crystallographic resolution, Structure, 15, 955 (2007).

B.1. SAD Phasing Basics: How and Why it Works, and When it's Likely to Fail

William Furey

VA Medical Center and Dept. of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

William Furey: fureyw@pitt.edu

The procedure for phasing macromolecular structures from a data set containing independent measurements of Freidel or Bijvoet mates collected at a single wavelength, currently called SAD phasing, is becoming an increasingly important component in the crystallographer's arsenal for structure determination. While the general method had been outlined over twenty years ago, to a large extent the procedures have become highly automated such that many crystallographers, particularly current post-docs and other newcomers to the field, have little understanding of the underlying principles that can dictate the likelihood of success or failure in a given case. This presentation is intended to explain the basic theory and principles behind SAD phasing, provide some information regarding current practices, and highlight strengths and weaknesses to aid potential users in assessing the methods applicability to any particular problem at hand.

B.2. SAD Data Collection and Experimental Setup

Kanagalaghatta Rajashankar

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Success of any SAD experiment depends upon the data quality. Data quality, in turn, depends upon how optimal is the experimental setup and how optimized is the data collection strategy for a particular SAD experiment. Beam line components can be optimized for SAD experiments in terms of the purity of the incident radiation (bandpass, hormonic rejection, divergence, positional and chromatic stability etc.). Sample stability is also an issue that needs to be taken into account while carrying out X-ray diffraction experiments in general and SAD experiments in particular. To accurately measure the anomalous differences for a SAD experiment, one needs to collect a redundant data. Luckily, unlike MAD, there is only one energy to worry about. However, collection of highly-redundant datasets requires many more frames, meaning higher total X-ray dose on the sample. Such data sets can suffer from moderate to serious radiation damage. Hence one has to strike a compromise between resolution, redundancy and radiation damage. At low energies absorption by the sample and the sample holder (loop) contributes to systematic errors, thereby shadowing the signal to some extent. Empirical absorption corrections can be applied to improve the data quality at lower energies (say lower than 8000eV). This is found to be useful, in particular for weak anomalous scatterers like sulfurs. In summary success of a SAD experiment depends upon an intelligent decision in terms of choice of energy, data quality, data resolution, completeness, redundancy, radiation damage and diffraction strength. During the presentation these topics will be described with specific examples.

B.3. High-energy sulfur SAD at the Australian Synchrotron

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Single wavelength anomalous dispersion (SAD) is a powerful technique for solving macromolecular structures. As only a single wavelength of data is required SAD experiments can be faster than traditional MAD experiments and SAD phasing has found favor in high-throughput techniques such as structural genomics. The use of the intrinsic sulfur signal for SAD phasing of proteins is well established but technical challenges remain due the requirement to correct the data for absorption of the diffracted beam. The magnitude of absorption of the diffracted beam (by the crystal itself, cryo-solution, loop and air path) increases with the cube of the wavelength. At the longer wavelengths usually used for sulfur SAD (>2Å) the systematic error introduced by absorption can easily dwarf the anomalous signal unless it is appropriately corrected for. While the anomalous scattering of sulfur steadily reduces at shorter wavelengths the potential errors introduced by absorption almost vanish. Therefore, while the anomalous signal is reduced the anomalous signal/noise of the dataset may be significantly improved.

In this study redundant datasets were collected at relatively high energies (13keV) on cubic insulin crystals where the intrinsic sulfur of six cysteine residues is the only anomalous scatterer. The f" for sulfur at 13keV is only 0.22 electrons. The structure was solved using both SHELX and AutoSHARP to 1.35Å resolution and demonstrates that very low anomalous signals can be accurately measured at beamline 3BM-1 of the Australian Synchrotron. The use of shorter wavelength X-rays for S-SAD may allow the successful determination of structures that have proved challenging using traditional wavelengths and experiments to test this hypothesis are underway at the AS.

B.4. 25 Years of SAS Phasing

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It has been over 25 years since B.C. Wang first carried out simulation studies showing that the sulfur anomalous scattering signal from a signal disulfide, measured with Cu Kα X-rays, could be used to phase the 114 residue Bence Jones protein Rhe (provided that the data could be measured accurately). The simulation clearly demonstrated the potential of using single wavelength anomalous scattering (SAS) data for protein structure determination however limitations in X-ray optics, crystal cooling, detectors and data reduction algorithms at that time limited its application to real data. Since then, improved X-ray sources, detectors, cryogenic data collection and better data reduction algorithms have made SAS structure determination the most commonly used approach for de novo structure determination.

My talk will focus on what we have learned over the years about collecting useful SAS data and SAS structure determination in general. Examples include: the structure determination of bovine neurophysin II using the anomalous scattering signal provided by a non-covalently bound iodinated hormone mimic, the structure determination of human ferrochelatase using the iron anomalous scattering signal provided by an iron-sulfur cluster and the structure determination of the photoprotein obelin using the sulfur anomalous scattering signal provided by the native protein alone. The talk will conclude with an update on soft X-ray SAS data collection and structure determination at SER-CAT (www.ser-cat.org).

Work is supported in part with funds from the National Institutes of Health, Veterans Administration, The University of Pittsburgh, The University of Georgia Research Foundation, The Georgia Research Alliance and The Southeast Regional Collaborative Access Team.

B.5. SAD Data Collection Using a Home Source

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Single-wavelength anomalous diffraction (SAD) phasing has become increasing popular and many examples of S-SAD and Se-SAD phasing have been reported with diffraction data collected with copper radiation (1.54 Å) and at the selenium K absorption edge (0.98 Å). More recently, a number of successful SAD experiments using Cr radiation (2.29 Å) has been published by several groups. Cr radiation is well suited for phasing structures because the signal is doubled from weak anomalous scatterers such as sulfur, selenium, calcium and many other intrinsic atoms in protein crystals. With the addition of Cr radiation to the crystallographer's toolkit, in-house X-ray sources routinely provide softer wavelength options to improve the efficiency of macromolecular structure solution. This report discusses the results of both SAD experiments with Cr radiation and dual-wavelength anomalous diffraction phasing experiments using combined diffraction data collected on both Cu and Cr radiation sources.

C.1. Insights Obtained on Micromechanical Deformation of Biomedical Devices and Biomaterials from 2D X-ray Diffraction

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Biomaterials, such as nacre, bone and wood, are synthesized at near ambient temperatures from very common materials but are architecturally very complex nanoscale composites. The complex architectural arrangements give them mechanical properties that are far superior than those of any of the components. It is one of the major challenges of our times to understand how from the interaction of these common components in the nanoscale architecture emerges the very different global behavior. On the other hand, technology for biomedical devices and inserts has advanced tremendously over the last decade. Not only is the design of some of the advance devices influenced by biological architecture, but many of these devices are now made from advance and often "smart" materials. One of the prominent smart materials for biomedical devices, from vascular stents to orthopedic implants, is Nitinol which because of its superelasticity is able to recover from a large deformation. But the micromechanical behavior of many of these smart materials, including Nitinol, is still poorly understood, especially under the application of large and several million deformation cycles it sees over its life time *in vivo*.

Understanding how a nanoscale biocomposite distributes an externally applied force among its components or what makes an advance material smart requires not only completely determination of the three-dimensional response at a local scale for each of its components, but also the knowledge of the mode or the mechanism by which each component deforms. Traditional strain measurement methods cannot measure the full 3D response or directly discriminate among the different micromechanical modes of deformation, and therefore, fall short of yielding deeper understanding of micromechanical behavior of materials.

Probing mechanical response at such a depth can be only achieved through a diffraction based method. In spite of this, diffraction based methods are still not as commonly employed for strain measurements because they are perceived as very time intensive and non-intuitive. In here, we introduce the concept of strain ellipsoid, and show how it's shape, thickness orientation represents the complete deformation state in a very convenient and powerfully visual and intuitive way. Further, we will show how using a large 2D detector at a synchrotron based diffractometer reduces the data collection time by two orders of magnitude and yields a much fuller understanding of the micromechanical properties of biomedical devices and complex biomaterials.

C.2. Characterizing Compaction-Induced Disordering of Active Pharmaceutical Ingredients using Powder X-ray Diffraction

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Solid-state phase changes resulting from shear-intensive, high-energy processing pose a significant challenge to the development and manufacturing of acceptable pharmaceutical dosage forms. Quality control criteria are established to satisfy product reproducibility demands of worldwide regulatory bodies, and do not allow for the variability in dosage form attributes (i.e., *in vivo* performance, product stability, etc.) that can be caused by inadvertent conversions of the drug to metastable solid forms during processing. A relatively new concept that has captured significant attention in this area involves systems in which regions of disorder are generated in materials that were historically assumed to retain full crystallinity throughout manufacturing. Changes to the crystallinity of drug materials during compaction is believed to be the source of significantly different physical and mechanical properties observed at the time of end-product testing, which owing to their thermodynamic potential to recrystallize during further storage, may result in highly unpredictable drug products. Powder X-ray diffraction (PXRD) analysis was used to assess intact, consolidated binary powder mixtures. Compacts were formed by combining powders of theophylline, an active pharmaceutical ingredient (API) known to undergo compaction-induced disordering, with excipients (non-bioactive components) having distinctly different deformation mechanisms. Powder X-ray diffraction patterns obtained from binary compacts were separated into individual patterns of each constituent, using a chemometric-based multivariate multiplicative preprocessing technique. Isolated diffraction patterns of the API were analyzed for indications of compaction-induced disordering using pair distribution functions (PDF) in conjunction with principal components analysis (PCA). Results indicate that variations in PDF transforms attributable to compaction-induced disordering of anhydrous theophylline were successfully detected in the presence of another material. Further, the use of PCA for decomposing matrices of PDF transforms provides an additional tool to the weighted agreement factor commonly used to compare PDF transforms. This work ultimately demonstrates the potential for yielding PDF information on individual constituents through transformation of accurately mathematically-separated multi-component PXRD patterns without prior physical separation or manipulation.

D.1. Measurement of Strain in Silicon Thin Films by X-ray Diffraction

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Strained Si on insulator (sSOI) is a promising substrate for future generations of CMOS technology. In sSOI, a thin surface layer of biaxially strained Si allows improved transistor performance through increased carrier mobility. An embedded SiO_2 layer provides insulation for overlying devices, allowing increased device operating speeds and decreased electrical power loss. Using epitaxy and wafer bonding technology, strained Si on insulator (sSOI) wafers can be reliably fabricated with large diameters (up to 12 inches). Unlike most alternative substrates, sSOI is easily incorporated into standard silicon device fabrication processes. However, these processes often involve several moderate to high temperature annealing sequences, which create opportunities for the strain in the thin (< 1 μm) Si layer to relax through formation of threading dislocations and other defects. These defects tend to lower the carrier mobility through scattering processes. Thus, an understanding of the mechanisms for the early stages of strain relaxation (specifically during heating) and the resultant dislocation nucleation is of great importance to widespread applicability of sSOI technology for fast devices.

We present a study of the strain and defect pattern in sSOI wafers using synchrotron x-ray techniques. Annealing at temperatures up to 1175°C was performed using tube and RTA furnaces. X-ray reflectivity measurements allowed characterization of the sSOI wafer structure, and high resolution x-ray diffraction (XRD) was used to map the average strain and measure misalignment (due to wafer bonding) of the strained Si layer. The strained Si layer exhibited compressive strain normal to the sample surface and tensile strain in-plane and was found to be aligned within a fraction of a degree to the crystal lattice of the bulk wafer substrate. XRD also provides information about defect patterns through its sensitivity to domain size and strain gradients. These properties were measured as a function of annealing time and temperature. Comparing these findings with AFM measurements of surface topology, we present a possible model for strain relaxation in sSOI.

D.2. Bio-MOFs: Synthesis, Characterization, and Application

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Simple biologically relevant small molecules such as amino acids, nucleobases, and nucleosides have rich coordination chemistries and can exhibit a variety of metal-coordination modes.

Exploration of this coordination chemistry and enumeration of the various possible coordination modes of these molecules in the context of synthesizing periodic polyhedra and metal-organic frameworks represents one of the first steps toward developing a class of biologically compatible porous materials that can be systematically designed for specific applications. Toward this end, we have prepared a family of porous frameworks constructed using metal ions and small biomolecules. This talk addresses the synthesis, structure, porosity, and host-guest properties of these materials, with a particular focus on their drug delivery capabilities.

D.3. Advanced Crystallographic Program in ChemMatCARS

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ChemMatCARS has established itself as a unique and relevant high-brilliance national synchrotron x-ray resource at the Advanced Photon Source (APS) at Argonne National Laboratory. ChemMatCARS is dedicated primarily to forefront research in static and dynamic condensed matter chemistry and materials science using x-rays from a third generation undulator source. The experimental infrastructure and facilities developed and in place at the resource on APS Sector 15 enable cutting edge research on solids, liquids and interfaces on atomic, molecular and mesoscopic length scales with high spatial and energy resolution. A major upgrade of the beamline optics and x-ray delivery is underway and will maintain the facility at the forefront of science in the areas it serves. The ChemMatCARS forefront facilities in surface science and single crystal diffraction are unique on the APS experimental floor and nationally.

Current and on going crystallographic research projects will be presented

D.4. Structural Studies of Novel Hydrogen Storage Materials

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Successful development of hydrogen as a primary fuel will simultaneously reduce the dependence on fossil fuel and emissions of greenhouse gases and pollutants. This is especially critical in this century with increasing concerns on energy security and global warming. Regarding hydrogen storage, one of the major challenges to widespread use of hydrogen is the lack of suitable hydrogen storage materials with the on-board operating storage capabilities for fuel-cell vehicular applications. Current hydrogen storage materials being intensively investigated include metal hydrides, chemical hydrides and high surface area adsorbents, which can chemically or physically store hydrogen in the form of atoms, ions or molecules. In this talk, I will present some of our recent work on light-weight metal hydrides (e.g., a mixed hydrides/amide system [1]), chemical hydrides (e.g., alkali- and alkaline-earth metal amidoboranes [2]), and metal organic frameworks (e.g., zeolitic imidazolate framework-8 [3]). I will focus on the structural aspects of these novel materials, investigated using x-ray or neutron powder diffraction method. The crystal chemistry derived from the structural analysis and their implications for hydrogen storage will also be discussed.

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D.5. Crystal Truncation Rod (CTR) Surface Diffraction in Geochemistry

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Crystal Truncation Rod (CTR) surface diffraction is an excellent tool for the structural characterization of mineral surfaces in equilibrium with aqueous solutions, as well as the sorption topology of surface complexes. Initial studies featured the isostructural sapphire (0001) and hematite (0001) surfaces, and demonstrated that there was considerable relaxation from the bulk structure in both, and a specific type of more reactive functional group on the hematite surface. Later work showed nonstoichiometry on the hematite and sapphire (10-12) surfaces, and an apparent sensitivity to the degree of surface protonation. Most recent work on goethite surfaces, taking advantage of a decade of advances in data collection and analysis, was able to define surface protonations, termination domains, and detect several layers of three-dimensionally ordered water on the (100) surface.

The basic technique methodology will be presented and important results placed in a context of surface crystal chemistry and electrical double layer formalism. The use of non-linear optical spectroscopy in concert with CTR analysis to characterize surface water structure will be described. Finally, new experiments that are developing ultrafast CTR measurement capabilities for the imaging of interfacial chemical reactions will be briefly discussed.

D.6. Photonic Crystal Materials Derived from Crystalline Colloidal Arrays

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Monodisperse colloidal particles with numerous strong surface acid groups ionize in aqueous solution to form highly charged colloidal particle macroions. Concentrated dispersions of these particles show strong interparticle repulsive interactions. For low solution ionic strengths the particles will self assemble into fcc or bcc cubic arrays which have spacings such that the arrays Bragg diffract light in the visible to near IR spectral region, depending upon the array spacing. The Bragg diffraction efficiency of these photonic crystal materials is very high and these systems can be used as passive high efficiency narrow band optical filters. We developed methods to solidify these arrays in hydrogel matrices and to further solidify these arrays into rigid materials. These materials can alter their lattice spacing and resulting diffraction wavelengths. They can be fabricated into chemical sensing materials as well as materials that dramatically change volume in response to temperature changes. We will discuss these applications as well as other photonic crystal phenomena.

E.1. Bridging the gap between structure and dynamics with elastic network models

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Elastic network models (ENMs) enable the prediction of large-scale protein dynamics from structural data alone, and their predictions have repeatedly been shown to correlate well with experimentally measured dynamical quantities. The reciprocal nature of structure and dynamics can be exploited through ENMs both to infer dynamics and function from structure and to refine structural data. Here I outline the basic theory of ENMs and provide specific examples of their utility in calculating both dynamics and structure. I further discuss the relationship between ENM predictions, X-ray data and NMR data, and I explore what these models can tell us about experimental techniques.

E.3. High-resolution Solution X-ray Scattering and Envelope Based Phasing

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X-ray scattering from biological macromolecules in solution is an increasingly important technique that yields low-resolution structural information on the molecules, and can be used to monitor their shape and conformational changes, including molecular associations 1, 2, 3.

However, in contrast to numerous successful applications of small angle X-ray scattering (SAXS), e.g. ref. 4-6, practical use of wide-angle X-ray scattering (WAXS) data has been limited due to the weak protein scattering despite of the higher-resolution structural information of disordered and partially ordered systems. Recently, we have succeeded in precisely measuring WAXS data on protein solutions at a standard macromolecular diffraction station[7]. The SAXS and WAXS data were combined to give a full scattering curve out to 2.5 Å resolution. Both indirect and direct Fourier transforms of the full scattering pattern exhibit that some high resolution aspects of the structural hierarchy and function of a protein can be investigated in solution.

In addition, we have employed the high resolution scattering curve to solve the phase problem, which remains central to crystallographic structure determination. The higher-resolution envelope was produced by combining SAXS and WAXS data. A 6-dimensional search method of molecular replacement (FSEARCH) was used to locate a low-resolution molecular envelope determined from small angle X-ray scattering (SAXS) within the crystallographic unit cell. We tested the method on horse hemoglobin, using the most probable model selected from a set of a dozen bead models constructed from SAXS/WAXS data using the program GASBOR at 5 Å resolution (qmax=1.25 Å-1) to phase a set of single-crystal diffraction data. We find that inclusion of wide-angle X-ray scattering data is essential for correctly locating the molecular envelope in the crystal unit cell, as well as for locating heavy atom sites 8. The initial phases can be used as a starting point for a variety of phase-extension techniques; successful application of which will result in complete phasing of a crystallographic data set and determination of a macromolecule's internal structure to atomic resolution.

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E.5. Using Raman to Follow Chemistry and Chemical Reactions in Macromolecular Crystals

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What is this Raman stuff you have been hearing about anyway? Well, using a modified optical microscope you can obtain the Raman spectrum of a protein or nucleic acid single crystal in a hanging drop in tens of seconds. By changing the contents of the hanging drop you can change the conditions inside the crystal, eg pH, or soak in/soak out ligands, or substrates. Then you use the Raman (difference) spectrum to follow the resultant changes occurring within the crystal. This can give unprecedented insight into chemical changes in the crystal and forms a powerful adjunct to X-ray analysis. Occasionally, even giving the crystallographers information they may not wish to know. Examples will be given of kinetic crystallography – detailing intermediates on the beta-lactamase reaction pathway, and, separately, defining metal binding sites in RNA crystals.

E.6. XAS techniques for studying the structure and mechanism of the photosynthetic water-splitting catalyst

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The water-oxidation center is a metallo-enzyme complex that is closely associated with the Photosystem II (PS II) reaction center in thylakoid membranes in plants and cyanobacteria. The metal cluster contains 4 Mn and 1 Ca atoms. Single crystals of PS II isolated from thermophilic cyanobacteria have been studied by X-ray diffraction, and analyses at resolutions between 3.0 and 3.8 Å have been published (Ferreira et al. 2004, Loll et al. 2005). These studies have localized electron density associated with the Mn_4Ca cluster within the large complex of PS II proteins, but the limited resolution is short of what is needed to place individual metal atoms precisely in the cluster. Additionally, the Mn_4Ca is proven to be significantly susceptible to radiation damage caused under the condition of current crystallography. Alternative methods**,** spectroscopic techniques such as EPR, IR and X-ray Absorption spectroscopy have also been used to derive information about the cluster arrangement and its structural changes during the oxygen-evolving reaction.

Recent studies using X-ray absorption spectroscopy have revealed several important aspects regarding the Mn_4Ca structure. The following topics will be presented: (1) Radiation damage to the Mn_4Ca cluster occur at quite low X-ray dose, and the damage is significantly temperature dependent. (2) Single crystal X-ray absorption spectroscopy of PS II single crystals from cyanobacteria show pronounced dichroism in the X-ray absorption spectra depending on crystal orientation relative to the electric vector of the polarized X-rays. We have used the X-ray dichroism to evaluate the Mn cluster geometry based on the published crystallographic data (Yano et al. 2006). (3) Range extended EXAFS study using a high-resolution spectrometer shows structural details of Mn_4Ca cluster with an improved distance resolution of 0.1Å. (4) Also, we have used Sr-substituted PS II instead of native Ca PS II, and observed a critical structural role for Sr (Ca) during the oxygen-evolving reaction.

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E.7. A novel microfluidic device for protein crystallization condition finding

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SpinX Technologies has developed a novel microfluidic liquid handling and detection system suitable for protein crystallization condition finding. The user's only physical contact with the system is the addition of concentrated samples and stock reagents to reservoirs in a 384-well format; the use of pipettes and microplates has been eliminated by using a fully integrated lab-on-a-chip device. The system automatically dilutes and mixes tens-of-nanoliters of reagents, with a total assay volume of 200 nanoliters. The integrated detection system images each sealed reaction chamber, enabling time-course monitoring of crystal formation. Microbatch and modified vapor-mediated diffusion results will be presented.

F.1. Allosteric regulation of glucocorticoid receptor activity by DNA binding sequence

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Nuclear hormone receptors integrate inter and extracellular signals to initiate specific gene programs. I am exploring the role of DNA sequence as a signal affecting glucocorticoid receptor (GR) activity. GR binds directly to a 15bp sequence composed of two inverted 6bp half sites separated by a 3bp spacer. Using a simple reporter assay, we demonstrate that the sequence of the half sites as well as the spacer affect GR activity. Further, sequence appears to direct use of distant functional surfaces including activation function 1 (AF1), activation function 2 (AF2) and the dimerization interface. Using X-ray crystallography we show that DNA sequence directs changes in the lever arm – a loop region connecting the recognition helix to the dimerization interface of the DNA binding domain. Subtle changes in packing of the DNA binding domain allow the lever arm to adopt different conformations through interactions specific to GR. Importantly, disruption of these interactions by mutation as well as in a natural splice variant, GRγ, affect the activity of the receptor resulting in a scrambled gene program. We therefore propose that binding sequences not only recruit transcription factors to genes, but are also allosteric signals that direct activity. However, several links in the chain between reading DNA sequence and differential functional surface usage remain to be elucidated. Progress on establishing some of these links using NMR techniques will be discussed.

F.2. Solution NMR Characterization of Important Enzyme Motions

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Solution NMR spectroscopy was used to study catalytically important motions by monitoring the effects of this motion on the transverse spin relaxation rates for the backbone positions. The opening/closing motion of the active site loop (loop 6) in triosephosphate isomerase (TIM) is investigated in solution by TROSY NMR spin-relaxation experiments and mutagenesis. The data show evidence for motion with an exchange rate constant (kex) = 9000 s-1, consistent with this motion as partially rate-limiting to catalytic throughput.

Similar rate constants are observed for residues in both the N- and C-terminal hinges of loop 6 suggesting motional coupling of these two regions. Mutation of tyrosine 208 to a phenylalanine (Y208F) eliminates a hydrogen bond in the closed loop conformation. Similar studies with Y208F indicate an increase in the population of the open conformer and concomitant increase in the opening rate constant and decrease in the rate of loop closure. The destabilization of the closed conformer is similar in magnitude to the decrease in affinity of Y208F for ligand.

Mutations of conserved loops in chicken TIM were made to partially resemble those loops in thermophilic TIM enzymes. Significant alterations in motion and function are observed and are placed in perspective on the enzymatic reaction coordinate. Solution NMR experiments on the 66 kDa tRNase Z enzyme reveal significant conformational rearrangements that must occur for substrate binding and activity.

F.3. Modeling Bacteriophage HK97 Capsid Maturation

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The 420 subunits of the bacteriophage HK97 capsid undergo very large covalent and conformational changes during assembly and maturation of the icosahedrally symmetric capsid or head. The resulting changes in the capsid include an increase in particle diameter that doubles the enclosed volume and the self catalyzed formation of covalent cross-links that stabilize the final structure. Intermediates have been identified in this process, some of which accumulate in mutants which affect the process, some which we identified by "freezing" the maturation reaction. We have studied these variants biochemically and structurally using x-ray crystallography and cryo-EM. Multiple X-ray and cryo-EM structures were combined to produce movies of the expansion process. Mutational analysis of a "catalytic" residue in the capsid protein, inspired by the structures, suggest that this residue acts as a proton acceptor in a mechanism that appears to be conserved widely in phage capsids, and separately, in the pili of many pathogenic bacteria.

F.4. Structural Studies of Transcription

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Transcription of DNA to generate messenger RNA involves promoter and start site recognition, DNA melting and unwinding, polymerization of a DNA-RNA hybrid and transcript proofreading during elongation. Assemblies of up to forty proteins carry out these highly intricate tasks in a timely manner. Crystal structures of transcriptional complexes will allow us to understand the molecular mechanisms of gene expression and regulation.

F.5. Using unnatural amino acids to probe the structure-function relationships in human GSTs.

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Glutathione transferases (GSTs) are a group of detoxification enzymes that catalyze the conjugation between glutathione (GSH) and a wide variety of hydrophobic compounds which can be endogenous or exogenous. They are instrumental in detoxification of carcinogens, pesticides, herbicides and reactive products generated under oxidative stress, such as α-β unsaturated carbonyls, quinines and hydroperoxides. As a consequence of their catalytic function, GSTs have been implicated in the resistance of tumors towards various alkylating, electrophilic anti-cancer drugs.

Human GSTA4-4 differs from other enzymes of the alpha class in both its choice of substrates and the behavior of its C-terminus. Unlike other enzymes in its class, it exhibits high catalytic activity with long hydrocarbon chain lipid peroxidation products such as 4-hydroxynon-2 enal (HNE). Also, unlike other alpha-class enzymes, it possesses an ordered C-terminal α-helix even in the absence of any ligand. Residue Tyr212 is an important residue in the C-terminus of hGSTA4-4. It has been suggested that Tyr212 contributes to the greater structural stability of the C-terminal helix by tightly anchoring it to the hydrophobic core of the protein. It is also considered to be important in catalyzing the reaction of glutathione with its natural substrate, 4-hydroxynon-2-enal by hydrogen-bonding to the carbonyl on HNE and polarizing it for a nucleophilic attack by the sulfur of the glutathione.

Site-specific incorporation of the unnatural amino acids p-F-Phe and O-Me-Tyr is a great way of decoupling the hydrogen-donor property of Tyr while still having an electronegative atom in the side chain. We mutated the Y212 codon to the amber codon and then used an orthogonal tRNA/tRNA-synthetase pair to site-specifically incorporate the unnatural amino acids at position 212. The mutant enzymes had very low activities when catalyzing the reaction of GSH with trans-2-nonenal but behaved like the wildtype when using CDNB as the substrate. The Km and kcat values for GSH indicate that the structure of the mutants is similar to that of the wildtype A4-4. Based on some preliminary molecular dynamic simulations, we propose that the long carbon chain of nonenal allows its aldehydic oxygen to be within hydrogen bonding distance of the hydroxyl group of Tyr212 which facilitates the reaction to be catalyzed. An important advantage of incorporating these unnatural amino acids is that NMR (F19 or C13) can be used to further study the properties of the enzyme using simple NMR experiments.

F.6. The Superstructure of an Antimicrobial Peptide, Alamethicin, in Lipid Membranes

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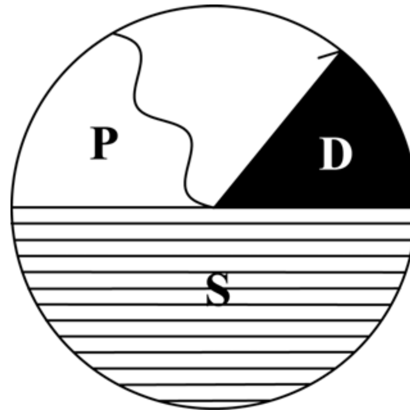
Alamethicin (Alm) is one of the most extensively studied antimicrobial peptides that affect the plasma membrane, and several models that involve a channel structure formed by monomer aggregation have been proposed. Alamethicin channel structure has been observed experimentally by x-ray and neutron in-plane scattering and channel size has been estimated. The barrel stave model suggests that there are 6-8 Alm monomers per channel.

In this work we investigate the effect of membrane hydration and hydrophobic mismatch on the Alm channel superstructure in an oriented multilayer sample by x-ray scattering. Wide angle x-ray (WAXS) scattering near 1.5 $Å^{-1}$ indicates that the lipid chain region is not much perturbed by the incorporation of up to 10 mole percent Alm. Low angle x-ray scattering (LAXS) indicates that when the sample is very dry, which promotes interactions between neighboring bilayers, a body centered tetragonal crystal packing of Alm channels is formed. As the hydration level increases closer to biological conditions, the separation between bilayers increases, the interbilayer interactions weaken, and the crystalline order disappears while considerable diffuse scattering remains. The effect of hydrophobic mismatch is examined for two mono-unsaturated lipids, diC18:1PC and diC22:1PC, that have different bilayer thicknesses. There is also additional in-plane scattering at medium q of 0.7 $Å^{-1}$ that our analysis suggests may not be from the Alm channel structure.



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 $400 prize will be presented at the Conference Banquet on Friday evening. Posters must be removed by 3:00 PM, the close of the conference on Saturday.

P.1. Protein crystallography at the Australian Synchrotron

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The Australian Synchrotron is a new 3rd generation synchrotron source. The initial funding covered the construction of the machine and nine beamlines of which two are dedicated to Protein Crystallography. Protein crystallography is the largest single science user of synchrotrons world wide. Details of the commissioning and user programs of the two protein crystallography beamlines will be presented.

P.2. Metal-organic Frameworks Constructed from Biomolecular Building Blocks: Synthesis, Characterization, and Emerging Properties

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Porous materials constructed from bio-compatible and environmentally-friendly constituents could potentially be useful for a variety of applications in areas such as environmental clean-up, greenhouse gas sequestration, the food industry, and medicine. We are developing a coordination-chemistry based approach for designing and constructing crystalline porous materials using biocompatible metal-ions and biomolecules. Here, we describe a new class of metal-organic framework materials that consist of biologically compatible components, including nucleic acid constituents. We examine their porosity through gas sorption studies, and we show that their intrinsic porosity leads to new and unusual properties that will render these materials useful for a range of biological and environmental applications.

P.3. Macromolecular Crystallography Beam Line Facilities at the Stanford Synchrotron Radiation Laboratory

Aina Cohen, Ana Gonzalez, Michael Hollenbeck, Irimpan Mathews and Clyde Smith representing the entire SSRL SMB team.

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The SSRL Structural Molecular Biology group operates six crystallography beam lines, BL1-5, BL7-1, BL9-1, BL9-2, BL11-1, and BL12-2. BL7-1 has been recently upgraded to fully utilize the SPEAR3 source including a new wiggler and x-ray optics. BL12-2, a new undulator station optimized for data collection with very small crystals, is currently undergoing commissioning. All of the crystallography beam lines are fully automated, with samples being mounted using the Stanford Automated Mounting system (SAM) and controlled with the Blu-Ice/DCS software system. Data collected during sample screening are automatically analyzed and the results, including the number of spots, Bravais lattice, unit cell, estimated mosacity and resolution, are visible through Blu-Ice and Web-Ice. Users of these beam lines have the option to conduct experiments while remaining at their home institution and in 2007 over 75% of the experiments were conducted remotely.

P.4. Structure of Flexibilty Allows Sequence Diversity

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Highly reactive oxygen or nitrogen species (ROS & NOS) generated in cells through various natural mechanisms or during a peculiar stress are responsible for a wide range of damages which affect all the cell components. Organisms have different levels of defence to protect them from ageing and early death: small molecules (ascorbic acid, glutathione) and enzymes (superoxide dismutase, peroxiredoxin, glutathione peroxidase) trap ROS and NOS prior they affect macromolecules; nucleotides and proteins that are modified nevertheless are repaired by enzymes (BER and NER systems, methionine sulfoxide reductases); and systems such as proteasome 20S, protease Lon degrade the non repairable biomaterials.

Several enzyme families involved in ROS trapping and protein regeneration have been studied in our group. Present in a wide range of organisms, each of them display some variability in its structure/function relationship. One case will be presented. Peptide methionine sulfoxide reductases (Msr) are dedicated to regeneration of proteins that are oxidized on their methionine residues despite the first defence barriers in the cell. They reduce their substrate thanks to a catalytic cysteine residue that evolves to a sulfenic acid, then reduced by a second cysteine to form an internal disulfide bridge later reduced by an external reducing system like thioredoxin/thioredoxin reductase/NADPH. The position of the second cysteine displays a large variation in the sequence through organisms. In some cases, distances larger than 15 Å are observed in the corresponding structures. A strong conformational change has to occur in order to approach the cysteine residues so that they could react together. The comparison between the reduced and oxidized forms of Msrs from different organisms reveal that a considerable flexibility of there small proteins probably allows their observed diversity despite a common catalytic mechanism.

P.5. Preliminary Model for a CAP Class-I and UP-element Transcription Activation Complex Subassembly

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In all cellular organisms, RNA is synthesized by a DNA-dependent RNA polymerase (RNAP). At many promoters in *E. coli*, the catabolite activator protein (CAP) activates transcription through direct interactions with *E. coli* RNAP.[1] A current focus of our laboratory is to obtain accurate structural information for transcription activation complexes involving CAP. At class I CAP-dependent promoters like *lac*, the CAP site is centered at position -61.5 relative to the transcription start. The interaction between CAP and the RNAP α subunit C-terminal domain (αCTD) places αCTD adjacent to the RNAP σ^{70} subunit, and permits functional protein-protein interaction between αCTD and σ^{70}.[2] αCTD interacts with CAP through the "287 determinant" region, and with DNA through the "265 determinant." It has been proposed that the interaction between αCTD and σ^{70} is mediated by the "261 determinant" of αCTD and residues 593-604 of σ^{70} region 4 (σR4).[2] αCTD also plays roles in promoter recognition in the absence of CAP.[2,3] In these cases, αCTD binds to an "UP-element subsite" comprising a 6 base-pair A/T-rich sequence centered at position -42. In these cases too, the "261 determinant" of αCTD has been proposed to interact with residues 593-604 of σR4.[2,3] Here we will present recent results of crystallization screening and preliminary diffraction experiments of a ternary complex of αCTD, σR4 and a 21 base-pair duplex DNA. Two different constructs of αCTD, three different constructs of σR4, and three DNA duplex designs have been used in several combinations for screening. A sparse matrix screening approach using the hanging drop vapor diffusion method yielded several crystallization conditions. Based on a dataset collected at NSLS-X25 to 3.8 Å resolution from one crystal, we have been able to determine the structure of the complex by molecular replacement, using selected coordinates from the CAP-αCTD-DNA complex (pdb id 1lb2)[4] and the σR4-DNA complex (pdb id 1ku7)[5] as search models. The current model, after rigid body refinement, has $R = 0.3610$ and $R_{free} = 0.3650$. We are working to improve crystal diffraction quality to better visualize the αCTD-σR4 interface, and to validate the model with a 5-bromodeoxyuridine-DNA derivative.

This work is funded by NIH grants GM21589 to C.L.L. and GM41376 and a Howard Hughes Medical Institute Investigatorship to R.H.E.

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P.6. A potential macromolecular test crystal system for assurance of beamline quality

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The aim of this study is to develop a suite of suitable macromolecular crystal samples, which can be used for the validation of a synchrotron beamline or an in-house-X-ray source in a quick and automated manner. One of the limiting factors is that macromolecular crystals do not exhibit infinite lifetime in an X-ray beam. Even at liquid nitrogen temperature, the radiation reduces the quality of the diffraction data. Thus one of the tasks is to find some means of stabilizing crystals in order to develop reproducible protocols for pre-testing them. Potential candidate proteins should meet certain requirements. They should be readily available and easily and reproducibly crystallise in a high symmetry space group with low mosaic spread and provide diffraction to sufficiently high resolution. We have chosen tetragonal lysozyme [1]. The crystals were cross-linked with glutardialdehyde [2]. Those crystals are no longer dissolvable in water, glycerol, styrene or paraffin oil. They are stable for several months, stored at room temperature, mounted in nylon loops and covered with polystyrene or nail polish. They can be frozen prior to data collection and thawed after that for several times.

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P.7. Crystal structure of M. tuberculosis ribokinase in complex with ribose and dinucleotide

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Ribokinase (RK) catalyzes the phosphorylation of ribose to ribose-5-phosphate using ATP and functions as a first step in ribose metabolism. RK belongs to the PfkB family of carbohydrate kinases which also include the structurally similar adenosine kinases.

The Mycobacterium tuberculosis (MTb) gene Rv2436 has been tentatively annotated as a ribokinase based on its sequence similarity with other ribokinases. In order to confirm the identity of Rv2436 as a ribokinase and to better understand ribose metabolism within MTb we have determined the crystal structure of Rv2436 in complex with ATP analog (AMP-PNP) and ribose to 2.0 Å. The structure contains two molecules within the asymmetric unit. Each monomer in turn forms a dimer around a crystallographic axis through the interaction of the N-terminal domains. This N-terminal domain forms a lid over the substrate binding cleft of the catalytic body of the enzyme. The MTb RK structure exhibits high overall structural overlap with other previously determined ribokinases and also conservation of catalytically important residues within the binding cleft. Additionally, biochemical studies confirm the preference for ribose as a substrate and the influence of inorganic phosphate on activity, consist with what is seen for RK from other organisms. One notable difference between previously solved RK structures and the MTb RK structure has to do with the conformation of the lid domains with respect to the catalytic body. Previously solved RK structures consist of either Apo forms in which both lids of the dimer are in an open arrangement or substrate bound complexes in which both lids of the dimer cover the substrate binding cleft in a closed conformation. Within the MTb RK structure, ribose is bound into one monomer with the lid domain tightly closed over the substrate binding cleft while in the other monomer the lid is in an open conformation with AMP-PNP bound into the binding cleft. This previously unseen conformation with one catalytic site closed and one site open suggests some cooperation between monomers may be important for the catalytic cycle.

P.8. Solution and crystal structures of a sugar binding site mutant of Cyanovirin-N: no evidence of domain-swapping

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Cyanovirin-N (CV-N) is a cyanobacterial lectin that exhibits antiviral activity against HIV at low nanomolar concentration by interacting with high-mannose oligosaccharides on the virus surface envelope glycoprotein gp120. Three dimensional structures of wild type CV-N revealed either a monomer in solution or a domain-swapped dimer in the crystal, with the monomer comprising two independent carbohydrate binding sites that individually bind with micromolar affinity to di- and tri-mannoses. In the mutant CVNmutDB, the binding site on domain B was abolished and the protein was found to be completely inactive against HIV. Here, we determined the solution NMR and crystal structures of this variant and characterized its sugar binding properties. In solution and the crystal, CVNmutDB is a monomer and no domain-swapping was observed. The protein binds to Man-3 and Man-9 with similar dissociation constants (~4 uM). This confirms that the nanomolar activity of wild type CV-N is related to the multi-site nature of the protein carbohydrate interaction.

P.9. Synthesis, Structural and Physicochemical Characterization of the Diamond-Like Semiconductor Ag_2CdGeS_4

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Diamond-like semiconductors are materials with structures related to that of diamond. These compounds may possess interesting optical or magnetic properties. Diamond-like semiconductors may be useful in fields such as non-linear optics and spin-based electronics. Our research has focused on synthesizing quaternary diamond-like materials, specifically those of the formula I2-II-IV-VI4. Ag_2CdGeS_4 has been synthesized from a stoichiometric combination of the elements via a high temperature solid state reaction at 800° C. Orange crystals with a needle-like morphology were obtained and analyzed through single crystal X-ray diffraction. The compound was determined to crystallize in the orthorhombic space group Pna21 with lattice parameters a = 13.7353(3) Å, b = 8.0129(2) Å and c = 6.5828(2) Å. The final refinement yielded R1 = 0.0276 and wR2 = 0.0806 for $I>2\sigma(I)$. In this structure, each metal ion is coordinated to four sulfur ions in a tetrahedral arrangement in order to form a 3-dimensional honey-comb network. Interestingly, a literature report describes the structure of Ag_2CdGeS_4 as crystallizing in the space group Pmn21 with different ordering of the cations. An analysis of these two structures combined with thermal analysis data suggests that two polymorphs of this material may exist. UV/Vis/NIR diffuse reflectance spectroscopy was used to determine a band gap energy of 2.29 eV for this semiconductor.

P.10. Alkali Chalcogenide Flux Synthesis and Characterization of the Quaternary Diamond-Like Semiconductor, Li_2CdSnS_4

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Diamond-like semiconductors are normal valance compounds that adopt the cubic or hexagonal diamond structures. Diamond-like semiconductors are of interest because they have shown potential uses in nonlinear optics, light emitting diodes and spin-based electronics. The goal of our research is to synthesize and study diamond-like semiconductors of the I_2-II-IV-VI_4 class. Li_2CdSnS_4 has been prepared as orange, needle-like single crystals from a lithium polysulfide flux at 650°C. This phase is water and air stable. The compound crystallizes in the orthorhombic space group $Pmn2_1$ with unit cell parameters of a = 7.9555(3) Å, b = 6.9684(3) Å and c = 6.4886(3) Å. Final R indices of the single crystal refinement were R1 = 0.0247 and wR2 = 0.1081 for all data. Lithium, cadmium and tin cations are tetrahedrally coordinated by sulfur to form a three-dimensional distorted honey-comb structure. However, if the Li-S bonds are considered to be more ionic than covalent, the structure consists of $[CdSnS_4]^{-2}$ layers sandwiched between lithium cations. Additionally, the band gap of Li_2CdSnS_4 was determined to be 3.1 eV and powder X-ray diffraction was used to identify a small CdS impurity in the product.

P.11. Non-Stick Collagen: Crystal Structures of Fluorinated Collagens

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Collagen is ubiquitous in nature; well-known as the major component of connective tissues in animals, more than 100 collagen-like proteins have also been identified in eubacteria and viruses. Collagen polypeptides form right-handed triple helices of repeating strands of the amino acids Xaa:Yaa:Gly, where Xaa and Yaa are often proline. In animals, prolines in the Yaa position are nearly always post-translationally hydroxylated to form (2*S*,4*R*)-4-hydroxyproline (Hyp)(1). This hydroxylation at the γ position enhances the stability of the triple helix. Replacing the hydroxyl group of Hyp with the highly electronegative fluorine atom leads to the most thermodynamically stable collagen of its size yet reported (2). We have found that additional stability is provided when a (4R)-methyl is added to the Xaa position proline residue.

In order to better understand the structural determinants of the hyper stability of these modified collagen triple helices, we have crystallized two forms of fluorinated, methylated collagen triple helices: Type A, $[(\text{flpMepGly})_7]_3$ (Mep = (2*S*,4*S*)-4-methylproline and flp = (2*S*,4*S*)-4-fluoroproline) with a melting temperature T_m = 51 C, and the more thermodynamically stable type B, $[(\text{mepFlpGly})_7]_3$ (mep = (2*S*,4*R*)-4-methylproline and Flp = (2*S*,4*R*)-4-fluoroproline) (T_m = 55 C). Type A forms hexagonal plates and half plates, which belong to a hexagonal point group with cell edges 29.1 Å x 179.9 Å. Type B forms monoclinic crystals with cell constants 26.4 Å x 25.31 Å x 61.9 Å, β = 90.05°. Both crystal forms grow with defects along the long axis, high mosaicitity, and a few very intense reflections. Type A has not been solved, but Type B was solved and refined to 1.2 Å resolution. The structure of this most stable collagen reveals well-ordered residues in the core of the collagen triple helix, but considerable disorder on the ends. The ring puckers are the preferred Cγ-endo for mep and Cγ-exo for Flp, resulting in a 3.3 Å packing interaction between the fluorine atoms and methyl groups of neighboring strands. Well-ordered carbonate molecules are also found within the lattice.

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P.12. Direct space and simultaneous direct-reciprocal space integer optimization models for phasing crystal structures

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The phase problem has recently been approached via combinatorial optimization techniques and the resulting Sieve method has been demonstrated to be effective for phasing centrosymmetric structures [1, 2]. The purpose of the current work is to develop a more robust model for accurate phasing in the presence of odd triplets, and more important, to provide a combinatorial optimization approach to phasing non-centrosymmetric crystals. Two mixed-integer linear programming models for phasing are proposed; both of which include the introduction of specific direct space constraints with one additionally operating in reciprocal space. Direct space is constrained through sampling of electron density on a grid. Structure factors are calculated at these points in terms of the integer variables, which describe the phases. Bounds are then formulated using experimental data. Computational results are presented for a variety of structures.

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P.14. The Crystal Structures of Enterococcus Aminoglycoside (2") Phosphotransferase variants Ib and Ic, Enzymes Implicated in Antibiotic Resistance

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The emergence of bacteria resistant to important classes of antibiotics has become a major clinical problem over the last few years, and now almost all antibacterial compounds in use today have associated examples of resistant bacterial isolates. In the United States, Enterococci are among one of the most common resistant bacteria isolated in nosocomial infections. The synergistic use of ampicillin or vancomycin with an aminoglycoside (kanamycin or gentamicin), has long been the optimal therapy for enterococcal infections, but many previously susceptible strains have since acquired resistance to the aminoglycosides. Resistance arises by gene transfer from other bacteria, these genes coding for enzymes that deactivate the antibiotics by chemically altering specific groups on the drug. We have determined the structures of two aminoglycoside phosphotransferases, APH(2")-Ib and APH(2")-Ic.

We have crystallized APH (2")-Ib as the gentamicin complex, the AMP complex and a ternary AMPPNP/streptomycin complex. The APH(2")-Ic enzyme, although related to the Ib enzyme, has a 400-fold preference for GTP over ATP, and we have solved the structure of the GDP complex of the enzyme. The APH(2") enzymes have a two-domain fold very similar to the protein kinases. Gentamicin binds in a cleft in the C-domain between a conserved central core and a variable helical subdomain. Streptomycin (an inhibitor) also binds in the same site but in a conformation which precludes phosphorylation. Comparison of the gentamicin and streptomycin complexes shows how the enzyme is able to distinguish between the 4,6-disubstituted aminoglycosides (substrates) and the 4,5-disubstituted molecules (inhibitors).

P.15. A Single Atom Controls the Catalytic Fitness of an RNA Enzyme

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A major challenge in structural enzymology is to observe an enzyme at different stages of its reaction coordinate. Visualization of the reaction pathway is a necessary process in fully elucidating the molecular basis for chemical catalysis. Ribozymes are ubiquitous in nature, have potential use in gene therapy, and represent anti-microbial drug targets. The hairpin ribozyme is employed by tobacco ringspot virus satellite RNA to cleave genomic RNA during rolling circle replication. Although a great deal of biochemical information is known for the hairpin mechanism, the mode by which this small ribozyme utilizes active-site functional groups for rate acceleration remains elusive. The reaction proceeds without need of a metal co-factor, implicating direct nucleobase involvement. An adenosine residue harbored in the active site has been hypothesized to function as a general acid in cleavage (**AH** in **Figure 1**). Previous work in our lab demonstrated that base changes at this key adenine resulted in disruption of the local fold, making it difficult to assign chemical function to specific functional groups. To better elucidate the hairpin ribozyme's chemico-structural basis for activity, we synthesized N1-deazaadenosine, a conservative inert isostere of adenosine. Here, we present crystal structures of the hairpin ribozyme harboring the adenosine analog in the context of variants previously demonstrated to represent pre-cleavage and reaction-intermediate conformations. An overview of the organic synthesis of the modified nucleoside, and a kinetic analysis of the ribozyme variants will be presented as well. The results directly implicate the N1 moiety of adenosine in rate acceleration by promoting a conformation that facilitates rate enhancement via electrostatic and functional group interactions. The chemical insight gained provides a functional 'window' with which to view the activities of more complex ribocatalysts essential for cellular activities such as protein translation, intron splicing and tRNA processing.

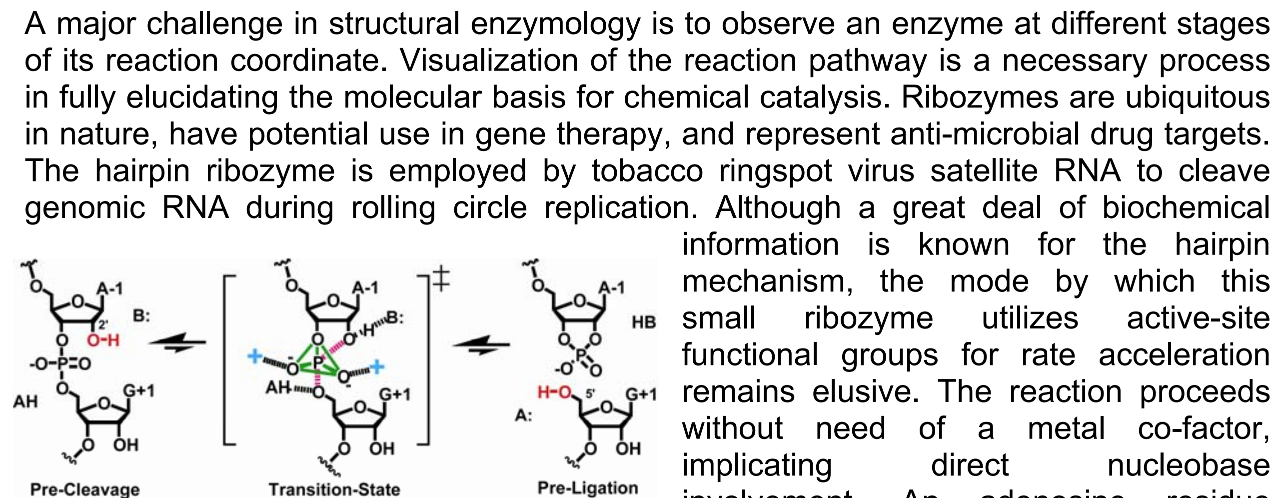

Figure 1: Reaction Coordinate of the Hairpin Ribozyme

P.16. Are X-rays damaging to structural biology? A case study with xylose isomerase.

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

P.17. Automated Deposition Tools for Depositors

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The RCSB Protein Data Bank (PDB) has developed software tools designed to assemble, validate, and deposit macromolecular structure data entries. **pdb_extract** (http://pdb-extract.rcsb.org/) takes key information about data reduction, phasing, molecular replacement, density modification, and refinement from the output files produced by many X-ray crystallographic applications. It merges these data into macromolecular Crystallographic Information File (mmCIF) data files that can be directly entered into **ADIT** (the AutoDep Input Tool: http://deposit.rcsb.org/adit/) to perform validation and deposition. **SF-Tool** (http://pdb-extract.rcsb.org/auto-check/index-ext.html/) converts structure factors from one format to another for single or multiple data sets. It also can be used to validate structure factors. **Ligand Expo** (http://ligand-expo.rcsb.org/) is a new tool to visualize, and build reports about all residue and small molecule components found in PDB entries. It can also be used to prepare a file for deposition. Collectively, these software tools reduce the human effort required to assemble very complete and validated protein structure entries ready for PDB deposition.

The RCSB PDB is supported by funds from the NSF, NIGMS, DOE, NLM, NCI, NCRR, NIBIB, NINDS, and NIDDK. It is a member of the Worldwide Protein Data Bank (wwPDB).

P.18. Studies of Site Occupation and Chemical Composition in Mn-Doped $CuInSe_2$ Chalcopyrites

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Chalcopyrite ternaries, such as $CuInSe_2$ and $CuInS_2$, have been investigated intensely because of their potential application in solar cell technology. Recently theoretical and experimental work suggests that the Mn-doped chalcopyrites exhibit ferromagnetic properties around room temperature, leading to a new series of dilute magnetic semiconductors. In this study, the I III VI_2-type chalcopyrite, $CuInSe_2$, was chosen as the parent compound, and the crystal structures and phase composition of Mn-doped samples were studied by energy dispersive X-ray spectroscopy and the Rietveld refinement of powder X-ray diffraction patterns. The powder patterns show that single-phase samples with chalcopyrite structure persist up to 10% and 20% manganese doping for $CuIn_{1-x}Mn_xSe_2$ and $Cu_{1-y}In_{1-y}Mn_{2y}Se_2$, respectively. Attempts to introduce greater manganese content result in phase segregation. X-ray mapping shows that all of the elements, Cu, In, Mn and Se, distribute homogenously in the single-phase samples. In the single phase region, the lattice parameters increase linearly with increasing manganese concentration, and thus obey Vegard's law. The refinement shows that single-phase $CuIn_{1-x}Mn_xSe_2$ compounds are Cu-poor and Se-rich. Moreover, the manganese doping on the indium site pushes the Se anion closer to Cu cations, leading to shorter Cu-Se bonds and more distorted tetrahedra.

Additionally, a two-phase model was envisaged to clarify the manganese occupation on the indium and copper sites. This analysis indicates that a small portion of the manganese ions may occupy the copper site, however, a large amount is still on the indium site in $CuIn_{1-x}Mn_xSe_2$.