Crystallography Times

Rigaku

Protein Crystallography Newsletter
Volume 2, No. 10, October 2010

In this issue:

Continuing Education Webinar
Crystallization Strategies for Macromolecules
Presenter: Dr. Janet Newman
November 18, 2010
6PM EST (23:00 GMT)

Coming to you live from Australia, Dr. Newman will expand upon the most current and successful strategies employed to crystallize difficult proteins.

Macromolecular Crystallography Training Class

March 23-25, 2011
October 26-28, 2011
The Woodlands, Texas

This class is tailored towards the needs of macromolecular crystallographers and their staff. Course format will be a series of short lectures on theory followed by hands-on activities with detectors, X-ray generators, and software. Class will also feature a training session on processing data with HKL.

Reader Input on S-SAD Phasing

We are accumulating success stories for Cu S-SAD phasing and thus invite our readers to share their success stories with us. We would love to hear from you.

Share Your Success Story

Crystallography in the news

October 28, 2010. Brookhaven National Laboratory researchers, led by Columbia University scientist Wayne Hendrickson, have uncovered the structure of a protein responsible for closing the "mouths," or stomata, of plants. The protein in question is an anion channel, which moves chloride ions across the cell membrane to reduce the plant's water pressure. Low pressure causes the guard cells to go limp, and subsequently, the stomata to close.

October 22, 2010. A multi-institutional consortium led by The Scripps Research Institute scientists, the Joint Center for Structural Genomics (JCSG) is the sole focus of a special issue of the journal Acta Crystallographica Section F. This is the first time in the history of the monthly journal, which publishes peer-reviewed crystallography and structural biology articles, that an entire issue is devoted to the works of a single scientific center.

October 20, 2010. Researchers at the Protein Crystallography and Crystallogenesis Laboratory at the J.P. Ebel Institute of Structural Biology, together with Metallic Chemistry and Biology Laboratory (CEA/CNRS/Joseph Fourier University) and the Life Sciences and Technologies Research Institute (IRTSV), have developed a new approach combining protein crystallography and biomimetic chemistry for observing they key steps of a process essential to life: oxygen activation. This was achieved by creating a complex artificial metalloenzyme composed of a chemical catalyst and a protein, and observing it via X-ray crystallography at the European Synchrotron Radiation Facility (ESRF).

October 20, 2010. University of Missouri Chemistry Professor and crystallographer John Tanner has joined forces with Pablo Sobrado, an assistant professor of biochemistry at Virginia Tech, to study a unique enzyme in *Aspergillus fumigatus*, a fungus of the genus *Aspergillus* and one of the most common Aspergillus species to cause pulmonary disease in immuno-compromised individuals.

October 19, 2010. In a lecture at Mahatma Gandhi University on "Beauty and purpose in the building blocks of life — the architecture of protein," Prof. Robert Huber - winner of the Nobel Prize in Chemistry in 1988 - discussed how protein crystallography has transformed the world of science. The lecture was the first in a week-long lecture series devoted to protein crystallography.

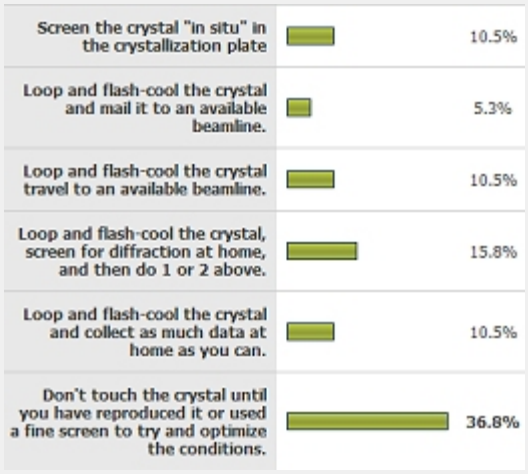
October 13, 2010. Crystallographer Pamela Björkman, the Max Delbruck Professor of Biology at the California Institute of Technology, will receive an honorary degree during Memorial University's (Newfoundland, Canada) fall convocation. Dr. Björkman's work has led to several previous honours, including the Gairdner Award in 1994, election to membership in the U.S. National Academy in Science in 2001 and the L'Oreal-UNESCO Women in Science Award in 2006.

October 8, 2010. A team of researchers at the University of Cambridge, led by Canadian crystallographer Prof. Randy Read, has found the missing first step in the mechanism that raises blood pressure in pre-eclampsia, a potentially deadly condition that occurs in pregnancy. The switch involves the making and breaking of a bond between two sulfur atoms that hold the key portion of the source protein for the hormone angiotensins.

October 7, 2010. Scripps research scientists reveal the first structure of a class of proteins that sniff out signals guiding blood cell movement. The structure offers a detailed view of how cell surface receptor CXCR4 interacts with molecules outside the cell and has implications for developing new drugs for hematopoietic stem cell transplantation, a therapeutic path to treat

September Survey Results

You have been trying to crystallize a protein for two years and finally obtain one crystal. What would you most likely do next?



Screen Maker — Desktop Alchemist™
Create complex fine screens quickly and accurately. Make optimization screens directly into Limbro/Nextal plates, in addition to Deep Well Blocks for 96-well SBS plate preparation.

Crystal Analysis — Desktop Minstrel™ UV
UV imaging simplifies detecting protein crystals under difficult conditions.

Plate Storage — Gallery™ 160 Plate Hotel
Automated inspection of up to 160 plates.

Crystallization Plate Setup
Configured with any of the popular plate setup robots, including the Art Robbins Phoenix™ or Gryphon™, as well as the TTP Mosquito™.

X-ray Diffraction — ScreenMachine™
Fully contained laboratory X-ray system for screening and testing cryo-conditions, teaching structural biology, and complete data collection for structure solution when necessary.

Crystallization Automation - CrystalTrak™
Data management system ties all of Rigaku's protein crystallization tools together and keeps track of the data generated by these instruments, from screen information to imaging results.

Automated X-ray Software -
Integrating data collection, data reduction, phasing and model building to significantly accelerate the process of structure determination while minimizing the number of data sets and the amount of synchrotron time required for structure solution. The system, when run in semiautomatic mode, provides the experimenter the ability to check the most important parameters defining the quality of the

cancer, immune disorders and HIV infection. Scripps Research Professor Raymond C. Stevens, who was senior author of the collaborative study, credits the perseverance and creativity of Research Associate Beili Wu, Scientific Associate Ellen Chien, and Assistant Professor Vadim Cherezov for noteworthy breakthroughs. The work was done in close collaboration with colleagues Alexei Brooun, Chris Bi, and Peter Wells at Pfizer La Jolla and Professors Tracy Handel and Ruben Abagyan at the University of California, San Diego.

October 5, 2010. Evotec AG (Frankfurt:EVT) announced that it has entered into a multiple target drug discovery collaboration with Shionogi & Co Ltd. (Tokyo and Osaka stock exchanges: 4507) to identify small molecule modulators of various protein-protein interaction targets. Evotec will apply its proprietary and integrated fragment-based drug discovery platform, EVOlution™ to the program, combining biochemical and biophysical techniques including nuclear magnetic resonance (NMR), surface plasmon resonance (SPR) and X-ray crystallography.

Starting a new lab? Protein Structure Workbench

Whether one is starting a new structural biology lab based around crystallography or adding crystallography as a technique to an existing lab, the challenges a PI faces are similar. First and foremost is the need to create an environment in which researchers can quickly and efficiently produce results that will support the underlying biological research. And results must be reproducible — there are too many examples of projects that have stalled because crystals could not be reproduced or people have failed to keep accurate lab notebooks.

Synergy between crystallization automation and X-ray
With technologies now available, it is possible to design a work environment from the ground up that will ensure compliance with record-keeping through use of embedded databases in both the crystallization phase and the crystallography phase. A PI can easily monitor the progress of crystallization experiments using a web-based interface or track the progress of a structure refinement, because all information is stored in an accessible format.

Making the most of your synchrotron time
We believe that the most efficient way to generate research grade crystals for synchrotron data collection is to have crystallization automation and in-house X-ray capabilities working together. In-house X-ray screening provides instant feedback of crystal quality, and the results can immediately lead to a new direction in crystallization. In support of this concept, Rigaku offers a complete solution: the Protein Structure Workbench.

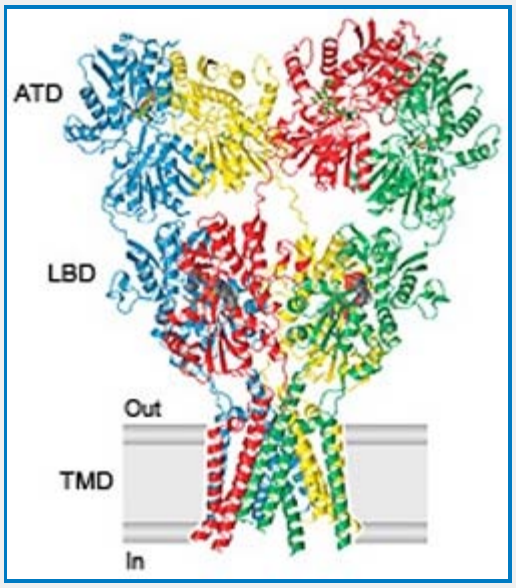
Request more information on the Rigaku Protein Structure Workbench.

Lab spotlight: Gouaux Lab @ Oregon H&S University

Members of Dr. Eric Gouaux's laboratory study the molecular principles underpinning the structure and function of chemical synapses. These specialized junctions are the primary sites of communication between neurons and are fundamental to the development and function of the peripheral and central nervous systems. Disruption of signal transduction at chemical synapses is implicated in a broad range of diseases, disorders and injuries and thus their studies not only provide fundamental insights into a crucial element of the nervous system but they also can be exploited for the development of new therapeutic agents.

Work includes studies of ionotropic glutamate receptors, ATP-gated P2X receptors and acid sensitive ion channels, as well as sodium-coupled glutamate, glycine, GABA and biogenic amine transporters. The group employs multiple biochemical, biophysical and electrophysiological methods to investigate the structure and biological functions of these molecules, with a particular emphasis on X-ray diffraction methods.

diffraction data and gives insight into the particular steps of the structure elucidation process. In most cases, using the default settings of the program results in a highly complete model of a macromolecule.



From the Gouaux Lab at Oregon Health & Science University: the AMPA-sensitive GluA2 ionotropic glutamate receptor (Sobolevsky et al., 2009).

Survey Question of the Month

SAXS is becoming a popular technique in structural biology labs for studying proteins in solution. Which of the following results do you consider to be the most important contribution from the SAXS experiment to a protein crystallographer (choose one)?

- Determination of whether the protein is unfolded, flexible, or aggregated.
- Determination of molecular weight or radius of gyration
- *ab initio* shape determination; modeling missing residues in high resolution structures
- Conformational changes in solution, such as those that occur on binding ligands
- Other (please specify)

Take Survey
or cut-and-paste
http://www.surveymonkey.com/s/oct_survey

J. Eric Gouaux, Ph.D., senior scientist at the Vollum Institute and Howard Hughes Medical Institute Investigator, was elected to the National Academy of Sciences in 2010. As part of the NAS, Dr. Gouaux serves as scientific adviser to the U.S. government.

The Gouaux Lab currently has sixteen members, including 9 postdocs and 3 graduate students. If you are interested in joining the lab, please contact Prof. Gouaux at the address listed on the lab home page.

Useful links for crystallography

HOMSTRAD (HOMologous STRucture Alignment Database) is a curated database of structure-based alignments for homologous protein families. All known protein structure are clustered into homologous families (i.e., common ancestry), and the sequences of representative members of each family are aligned on the basis of their three dimensional (3D) structures. These structure-based alignments are annotated and examined individually. Further, the database provides annotated structural alignments in various formats, superimposed structures and links to other databases.

Reference: Mizuguchi K, Deane CM, Blundell TL, Overington JP. (1998) HOMSTRAD: a database of protein structure alignments for homologous families. Protein Science 7:2469-2471

Selected recent crystallographic papers

Structural Characterization of Protein-Protein Complexes by Integrating Computational Docking with Small-angle Scattering Data. Pons, Carles; D'Abramo, Marco; Svergun, Dmitri I.; Orozco, Modesto; Bernadó, Pau; Fernández-Recio, Juan. *Journal of Molecular Biology*, Oct2010, **403**(2): 217-230. DOI: 10.1016/j.jmb.2010. 08.029.

Structural Analysis of HMGD–DNA Complexes Reveals Influence of Intercalation on Sequence Selectivity and DNA Bending. Churchill, Mair E.A.; Klass, Janet; Zoetewey, David L. *Journal of Molecular Biology*, Oct2010, **403**(1): 88-102. DOI: 10.1016/j.jmb.2010. 08.031.

SAXS and X-ray Crystallography Suggest an Unfolding Model for the GDP/GTP Conformational Switch of the Small GTPase Arf6. Biou, Valérie; Aizel, Kaheina; Roblin, Pierre; Thureau, Aurélien; Jacquet, Eric; Hansson, Sebastian; Guibert, Bernard; Guittet, Eric; van Heijenoort, Carine; Zeghouf, Mahel; Perez, Javier; Cherfils, Jacqueline. *Journal of Molecular Biology*, Oct2010, **402**(4): 696-707. DOI: 10.1016/j.jmb.2010. 08.002.

Structural characterization of proteins and complexes using small-angle X-ray solution scattering. Mertens, Haydyn D.T.; Svergun, Dmitri I. *Journal of Structural Biology*, Oct2010, **172**(1): 128-141. DOI: 10.1016/j.jsb.2010. 06.012.

Baculovirus production of fully-active phosphoinositide 3-kinase alpha as a p85a–p110a fusion for X-ray crystallographic analysis with ATP competitive enzyme inhibitors. Sinnamon, Robert H.; McDevitt, Patrick; Pietrak, Beth L.; Leydon, Vaughan R.; Xue, Yu; Lehr, Ruth; Qi, Hongwei; Burns, Matthew; Elkins, Patricia; Ward, Paris; Vincentini, Giorgia; Fisher, Donald; Grimes, Maggie; Brandt, Martin; Auger, Kurt R.; Ho, Thau; Johanson, Kyung; Jones, Christopher S.; Schwartz, Benjamin; Sweitzer, Thomas D. *Protein Expression & Purification*, Oct2010, **73**(2): 167-176. DOI: 10.1016/j.pep.2010. 05.002.

A structural overview of the PDI family of proteins. Kozlov, Guennadi; Määttänen, Pekka; Thomas, David Y.; Gehring, Kalle. *FEBS Journal*, Oct2010,

into your browser.

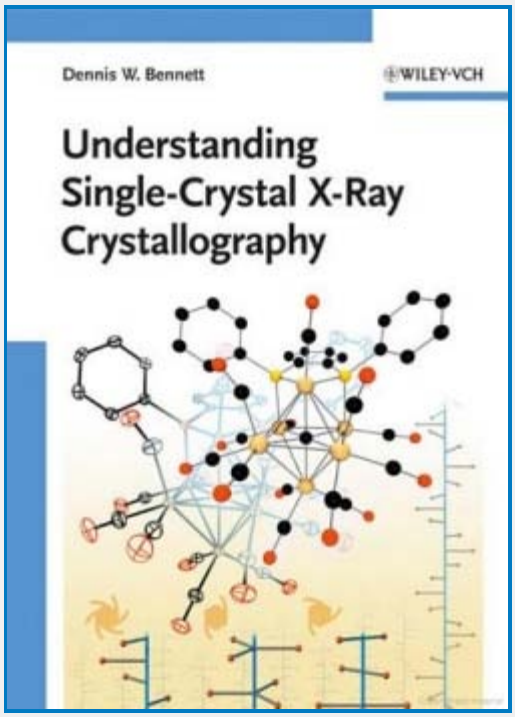
Introducing the "Quick Puck Loader"

The Quick Puck Loader, invented at Structural Genomics Consortium at Toronto, is designed to load crystal mounted pins into the Rigaku style puck quickly while reducing the potential for mis-seating. This tool was designed to be used with both a Rigaku Puck and Puck Tong.

Just load pins directly into the Quick Puck Loader, and then fit the Rigaku Puck right overtop ... and use Puck tongs to firmly grasp and flip. Pins are perfectly seated. No more losing crystals. The device is currently used in labs from University Health Network (Canada), York University and the Structural Genomics Consortium.

The Quick Puck Loader is made of durable aluminum and comes with a 1-year warranty on the part. Colors available include red, blue and gold. Mixed colors are also available.

View more information on the new Quick Puck Loader.



277(19): 3924-3936. DOI: 10.1111/j.1742-4658.2010. 07793.x.

Merging structural biology with chemical biology: Structural Chemistry at Eskitis. Hofmann, Andreas; Wang, Conan K.; Osman, Asiah; Camp, David. *Structural Chemistry*, Oct2010, **21**(5): 1117-1129. DOI: 10.1007/s11224-010-9654-2.

Use of the spliceosomal protein U1A to facilitate crystallization and structure determination of complex RNAs. Ferré-D'Amaré, Adrian R. *Methods*, Oct2010,**52**(2): 159-167. DOI: 10.1016/j.ymeth.2010. 06.008.

Rapid global structure determination of large RNA and RNA complexes using NMR and small-angle X-ray scattering. Wang, Yun-Xing; Zuo, Xiaobing; Wang, Jinbu; Yu, Ping; Butcher, Samuel E. *Methods*, Oct2010, **52**(2): 180-191. DOI: 10.1016/j.ymeth.2010. 06.009.

Identification and Characterization of a Misfolded Monomeric Serpin Formed at Physiological Temperature. Pearce, M.C.; Powers, G.A.; Feil, S.C.; Hansen, G.; Parker, M.W.; Bottomley, S.P. *Journal of Molecular Biology*, Oct2010, **403**(3): 459-467. DOI: 10.1016/j.jmb.2010. 09.007.

Development of Bimetallic Titanocene-Ruthenium-Arene Complexes As Anticancer Agents: Relationships between Structural and Biological Properties. Frédéric Pelletier; Virginie Comte; Alexandre Massard; Margot Wenzel; Stéphanie Toulot; Philippe Richard; Michel Picquet; Pierre Le Gendre; Olivier Zava; Fabio Edafe; Angela Casini; Paul J. Dyson. *Journal of Medicinal Chemistry*, Oct2010, **53**(19): 6923-6933.

Metals in protein structures: a review of their principal features. Harding, Marjorie M.; Nowicki, Matthew W.; Walkinshaw, Malcolm D. *Crystallography Reviews*, Oct2010,**16**(4): 247-302. DOI: 10.1080/0889311X.2010. 485616.

The Protein Circular Dichroism Data Bank, A Web-Based Site for Access to Circular Dichroism Spectroscopic Data. Whitmore, Lee; Woollett, Benjamin; Miles, Andrew J.; Janes, Robert W.; Wallace, B.A. *Structure*, Oct2010, **18**(10): 1267-1269. DOI: 10.1016/j.str.2010. 08.008.

Book review:

Understanding Single Crystal X-ray Crystallography
by Dennis W. Bennett, Wiley-VCH, 2010, ISBN: 978-3-527-32677-8

I came across this book while browsing the "stacks" at Amazon. I remembered Professor Bennett from the traveling salesman algorithm he published in the late 1980s for 4-circle diffractometers, and I met him while he was on sabbatical at the Medical College of Wisconsin, so I ordered a copy. With the wisdom gained by the 25 years that have elapsed since I last took a crystallography class, I can honestly say I wish I had this book when I was in school.

The power of the book is that Bennett begins each topic from first principles and derives the detailed concepts with full mathematical rigor. If I were in a position to teach a course in small molecule crystallography, this is the book I would use. I disagree with Bennett's use of Δf' and Δf" instead of f' and f" to represent the real and imaginary contributions to the scattering factor, but I am nitpicking. There are some typos in the book, and I would like to see the community do for *Understanding Single Crystal X-ray Crystallography* what it has done for Rupp's *Biomolecular Crystallography*.

The book is divided into sections on crystal lattices and symmetry, diffraction theory and experiment, structure solution, and structure refinement, and features 9 appendices. Each chapter includes a set of problems for the student to solve. Bennett develops the concept of the crystal lattice through vector and matrix mathematics. Next he uses these tools to develop point groups and space groups. Much attention is paid to interpreting the space group tables found in the *International Tables for Crystallography*.

In chapter 3, the author develops the theory of X-ray diffraction from first principles, ending with the equations for calculating structure factors and

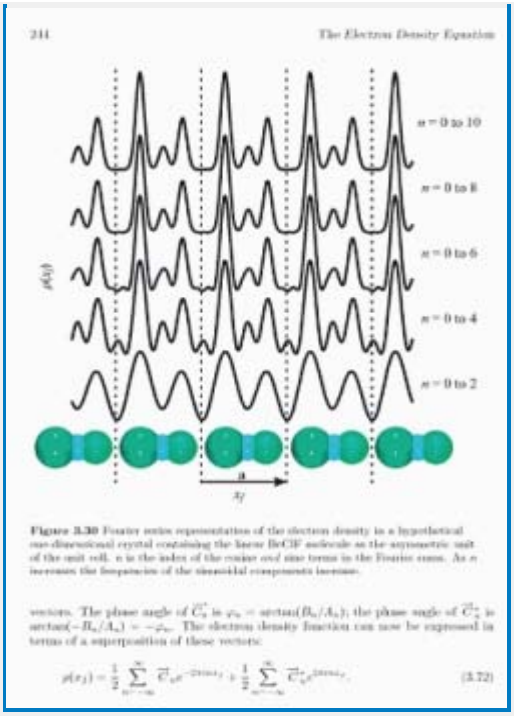


Figure 3.30 Fourier series representation of the electron density in a hypothetical one-dimensional crystal containing the linear BrClF molecule as the asymmetric unit of the unit cell. n is the index of the cosine *and* sine terms in the Fourier sums. As n increases the frequencies of the sinusoidal components increase.

vectors. The phase angle of $\vec{C}_n^+$ is $\varphi_n = \arctan(B_n/A_n)$; the phase angle of $\vec{C}_n^-$ is $\arctan(-B_n/A_n) = -\varphi_n$. The electron density function can now be expressed in terms of a superposition of these vectors:

$$\rho(x_j) = \frac{1}{a}\sum_{n=-n_0}^{n_0} \vec{C}_n e^{-(2\pi i n x_j/a)} = \frac{1}{a}\sum_{n=-n_0}^{n_0} \vec{C}_n^* e^{(2\pi i n x_j/a)} \qquad (3.72)$$

Understanding Single Crystal X-ray Crystallography
by Dennis W. Bennett.

electron density. Chapter 4 covers the experimental aspects of data collection, including classical film and serial diffractometer methods and modern area detector methods. Chapter 5 is devoted to the discussion of random and systematic experimental errors. There is an excellent description of statistics for crystallography.

The next two chapters are devoted to structure solution, with Chapter 6 discussing Patterson, heavy atom (isomorphous replacement and anomalous scattering) and Fourier methods. Chapter 7 discusses direct and probability methods for structure solution.

The final chapter reviews all aspects of structure refinement, including linear and non-linear least squares, weighting, constraints and restraints. The basics of macromolecular structure refinement are provided, but not in enough detail for a macromolecular crystallography course. If I were to teach macromolecular crystallography I would use this book to supplement Rupp's excellent book.

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