Crystallography Times

Rigaku

Protein Crystallography Newsletter
Volume 2, No. 9, September 2010

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Continuing Education Webinar
Scientific inquiry, inference and critical reasoning in the macromolecular crystallography curriculum
Presenter: Dr. Benard Rupp
October 21, 2010
1PM EDT (18:00 GMT)

Dr. Rupp will expand on his recent *Journal of Applied Crystallography* article that discusses higher education curricula in the context of scientific analysis. Bernhard analyzes recent cases of high profile structure retractions and argues that "With the great power of modern crystallography comes great responsibility for its appropriate use."

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.

Crystallography in the news

September 28, 2010. Collaboration between Trinity Dublin College (TDC) and Oxford University used X-ray crystallography to discover how two proteins interact to guide connections between brain cells. As decribed by Dr Kevin Mitchell, a senior lecturer at the Smurfit Institute of Genetics and the Institute of Neuroscience at TCD, the research examined the interaction between a "semaphorin" protein (known as Sema6A) and another protein, PlexinA2.

September 27, 2010. The National Institutes of Health (NIH) has awarded a prestigious EUREKA award to Prof. Robert Woods, at the University of Georgia Complex Carbohydrate Research Center, to develop a new method (diagnostic antibody specificity) for understanding how antibodies interact with large molecules known as glycans that are a major component of all cell surfaces. The EUREKA award, given to support "Exceptional, Unconventional Research Enabling Knowledge Acceleration," will provide Woods with approximately $200,000 per year for four years.

September 27, 2010. Proteros biostructures GmbH (Proteros) announced today that it has entered into an agreement with Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (J&JPRD), under which Proteros will perform research to discover small molecule lead compounds against a specific target for delivery to J&JPRD.

September 24, 2010. Prof. Edward Yu and his colleagues at Iowa State University, in conjunction with Ames Laboratory researchers, have discovered the X-ray crystal structures of pumps that remove heavy metal toxins from bacteria, making them resistant to antibiotics. What these pumps do is "recognize and actively export these substances out of bacterial cells, thereby allowing the bugs to survive in extremely toxic conditions."

September 23, 2010. The University of Arkansas Center for Protein Structure and Function, which includes the X-ray crystallography facility headed by Prof. Joshua Sakon, will receive more than $5.4 million over the next five years from the National Institutes of Health (NIH) to continue biomedical research in cancer, heart disease, osteoporosis, flu and other diseases and conditions.

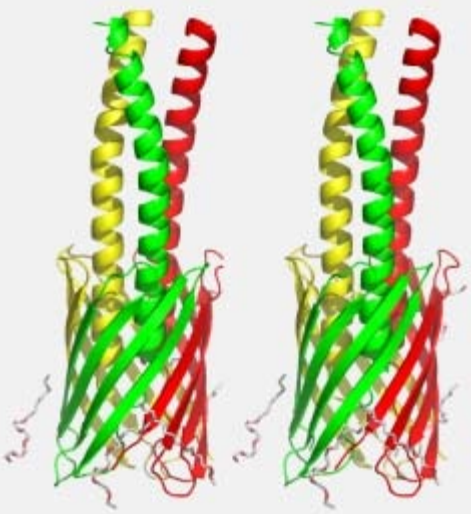
September 20, 2010. Scientists at Rutgers, led by Prof. Eddy Arnold, employed X-ray crystallography to discover how HIV-1, the virus that causes AIDS, resists AZT, a drug widely used to treat the deadly disease. The results describe in atomic detail how the AZT-resistance mutations allow reverse transcriptase to recruit ATP to remove the AZT.

September 16, 2010. The Donald Danforth Plant Science Center in St. Louis, Missouri (USA) will be hosting its 12th annual Fall Symposium from September 29th to October 1st, 2010. This year's symposium includes a wide range of topics with a focus, in part, on using X-ray crystallography to elucidate important biological problems, including virus structures, drug discovery, plant hormone receptors, photosynthetic complexes, engineering of plant metabolic pathways, membrane proteins, circadian rhythms, and DNA repair.

September 6, 2010. In two closely related studies, two teams of Scripps Research Institute scientists, led by Professors Wendy Havran and Ian Wilson, have discovered the underlying mechanisms that activate a type of immune cell in the skin and other organs. Together, the new research sheds light on γd ("gamma delta") T cells, an immune cell found within epithelial tissues—the thin layer of cells that makes up the outermost layer of skin and organs like the intestines and lungs. The research identified a junctional adhesion molecule, JAML, as a new costimulatory receptor for γd T cells that binds to the ligand CAR (coxsackie and adenovirus receptor) expressed on keratinocytes.

Desktop Alchemist™

Rigaku Desktop Alchemist can make optimization screens directly into Linbro and Nextal plates, in addition to Deep Well Blocks for 96-well SBS plate preparation.



From the Waksman lab at the Institute of Structural Molecular Biology, Birkbeck and University College London: structure of the translocator domain of the Hia trimeric autotransporter.

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

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optimization directly into Linbro and Nextal plates

The **NEW** Desktop Alchemist™ liquid handling system for protein crystallization optimization is a cost effective laboratory automation tool, allowing complex fine screens to be setup in under 3 minutes, with an entire screen completed in under 25 minutes. Based on the unique Rigaku BirdFeeder technology,Alchemist provides one aspirate with multiple dispenses – in a volume range of 1 µl to 10 ml – directly into VDX, Linbro or Nextal plate formats.

Rigaku Desktop Alchemist can make *optimization screens directly into Linbro and Nextal plates*, in addition to Deep Well Blocks for 96-well SBS plate preparation. This capability provides full flexibility and compatibility with all major formats. To make most efficient use of Deep Well Blocks, the powerful CrystalTrak™ software allows for a simple drag and drop of four 24-well optimizations into one 96-well block. While a fine screen of a single set of conditions across 96-wells is perhaps unnecessarily fine, one can boost productivity by having four slightly coarser and different optimizations per block.

Solving the stock solution storage and management problem, the Desktop Alchemist's CrystalTrak database holds thousands of stock chemicals that can be added or removed from the 26 position deck in a matter of seconds using barcoded BirdFeeder assemblies. The CrystalTrak relational database is easily searched.

Request more information on Rigaku Alchemist liquid handling systems.

Lab spotlight: Waksman Lab @ Birkbeck College

Gabriel Waksman's Lab at the Institute of Structural Molecular Biology, Birkbeck and University College London is focused on gaining insight into the structural and molecular basis of secretion in Gram-negative bacterial pathogens. Dr. Waksman and his group of 8 postdocs and 3 graduate students are particularly interested in unraveling the structures of large, multicomponent assembly systems acting as nanopumps.

Specifically, work is focused on the structural biology of bacterial secretion systems, biophysical studies of SH2 domains, and structural and functional studies of DNA Polymerase I enzymes.

Credentials for Gabriel Waksman, PhD, FMedSci include:

- Courtauld Professor of Biochemistry at UCL
- Professor of Structural Molecular Biology at UCL and Birkbeck
- Wolfson - Royal Society Research Merit Award 2003 – EMBO Member 2007 – Academy of Medical Sciences 2008
- Director, Institute of Structural Molecular Biology
- Head, School of Crystallography, Birkbeck College
- Head, Department of Biochemistry and Molecular Biology, University College London.

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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.

Survey Question of the Month

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

- Screen the crystal "in situ" in the crystallization plate
- Loop and flash-cool the crystal and mail it to an available beamline.
- Loop and flash-cool the crystal travel to an available beamline.
- Loop and flash-cool the crystal, screen for diffraction at home, then do 1 or 2 above.
- Loop and flash-cool the crystal and collect as much data at home as you can.
- Don't touch the crystal until you have reproduced it or used a fine screen to try and optimize the conditions.
- Other (please specify)

Take Survey
or cut-and-paste
http://www.surveymonkey.com/s/9ZL8NPP
into your browser.

August Survey Results

In which journal would you prefer to publish your next structure and have it featured on the cover?

Useful links for crystallography

ACMI - Automatic Crystallographic Map Interpreter uses a probabilistic approach, known as a Markov field, to automate tracing of the protein backbone in the electron density image. Testing of this method has shown that an accurate backbone model could be traced even with lower-resolution and poor-quality density maps. Principal author: George Phillips, University of Wisconsin.

Selected recent crystallographic papers

Cooperative binding of MgATP and MgADP in the trimeric P(II) protein GlnK2 from *Archaeoglobus fulgidus*. Helfmann, Sarah; Lü, Wei; Litz, Claudia; Andrade, Susana L.A. *Journal of Molecular Biology*, Sep2010, Vol. 402 Issue 1, p165-177; DOI: 10.1016/j.jmb.2010.07.020.

High-yield bacterial expression and structural characterization of recombinant human insulin-like growth factor binding protein-2. Swain, Monalisa; Slomiany, Mark G.; Rosenzweig, Steven A.; Atreya, Hanudatta S.. *Archives of Biochemistry & Biophysics*, Sep2010, Vol. 501 Issue 2, p195-200; DOI: 10.1016/j.abb.2010.06.006.

Transport mechanisms in the ammonium transporter family. Lamoureux, G.; Javelle, A.; Baday, S.; Wang, S.; Bernèche, S. *Transfusion Clinique et Biologique*, Sep2010, Vol. 17 Issue 3, p168-175; DOI: 10.1016/j.tracli.2010.06.004.

Structural and functional views of salt-bridge interactions of λ integrase in the higher order recombinogenic complexes visualized by genetic method. Lee, Sang Yeol. *Biochemical & Biophysical Research Communications*, Sep2010, Vol. 400 Issue 1, p1-6; DOI: 10.1016/j.bbrc.2010.08.030.

Single particle reconstruction of membrane proteins: A tool for understanding the 3D structure of disease-related macromolecules. Mio, Kazuhiro; Maruyama, Yuusuke; Ogura, Toshihiko; Kawata, Masaaki; Moriya, Toshio; Mio, Muneyo; Sato, Chikara. *Progress in Biophysics & Molecular Biology*, Sep2010, Vol. 103 Issue 1, p122-130; DOI: 10.1016/j.pbiomolbio.2010.03.001.

H-NS forms a superhelical protein scaffold for DNA condensation. Arold, Stefan T.; Leonard, Paul G.; Parkinson, Gary N.; Ladbury, John E.. *Proceedings of the National Academy of Sciences of the United States of America*, 9/7/2010, Vol. 107 Issue 36, p15728-15732; DOI: 10.1073/pnas.1006966107.

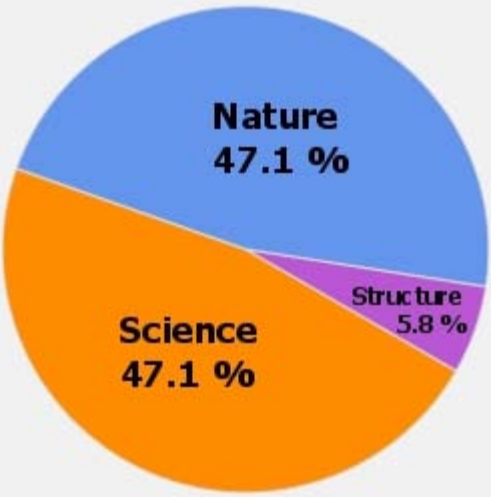
The structure of the FnIII tandem A77-A78 points to a periodically conserved architecture in the myosin-binding region of titin. Bucher, Rainer M.; Svergun, Dmitri I.; Muhle-Goll, Claudia; Mayans, Olga. *Journal of Molecular Biology*, Sep2010, Vol. 401 Issue 5, p843-853; DOI: 10.1016/j.jmb.2010.06.011.

Acetylcholinesterase: From 3D structure to function. Dvir, Hay; Silman, Israel; Harel, Michal; Rosenberry, Terrone L.; Sussman, Joel L. *Chemico-Biological Interactions*, Sep2010, Vol. 187 Issue 1-3, p10-22; DOI: 10.1016/j.cbi.2010.01.042.

Disulfide bond stabilization of the hexameric capsomer of human immunodeficiency virus. Pornillos, Owen; Ganser-Pornillos, Barbie K.; Banumathi, Sankaran; Hua, Yuanzi; Yeager, Mark. *Journal of Molecular Biology*, Sep2010, Vol. 401 Issue 5, p985-995,; DOI: 10.1016/j.jmb.2010.06.042.

Crystal structure of the ligand-binding domain of the promiscuous EphA4 receptor reveals two distinct conformations. Singla, Nikhil; Goldgur, Yehuda; Xu, Kai; Paavilainen, Sari; Nikolov, Dimitar B.; Himanen, Juha P. *Biochemical & Biophysical Research Communications*, Sep2010, Vol. 399 Issue 4, p555-559; DOI: 10.1016/j.bbrc.2010.07.109.

Crystal structure of a metal ion-bound oxoiron(IV) complex and implications for biological electron transfer. Fukuzumi, Shunichi; Morimoto, Yuma; Kotani, Hiroaki; Naumov, Pance; Yong-Min Lee; Wonwoo Nam. *Nature Chemistry,*



2010 Carl Brändén Award
The Protein Society
Nobuhiro Go, Ph.D.
RIKEN Harima Institute, Japan

The Carl Brändén Award, sponsored by Rigaku Corporation, was presented to Dr. Nobuhiro Go for his scientific achievements, including the establishment of a lattice model of proteins, now well-known as the Go model. Dr. Go has also contributed markedly to the NMR distance geometry analysis as well as the analysis of protein dynamics. His current work in protein biophysics, protein physical chemistry and computer science has brought important physical principles to the field.

Sep2010, Vol. 2 Issue 9, p756-759; DOI: 10.1038/nchem.731.

A paralog of lysyl-tRNA synthetase aminoacylates a conserved lysine residue in translation elongation factor P. Yanagisawa, Tatsuo; Sumida, Tomomi; Ishii, Ryohei; Takemoto, Chie; Yokoyama, Shigeyuki. *Nature Structural & Molecular Biology*, Sep2010, Vol. 17 Issue 9, p1136-1143; DOI: 10.1038/nsmb.1889.

X-ray crystal structure of JNK2 complexed with the p38a inhibitor BIRB796: Insights into the rational design of DFG-out binding MAP kinase inhibitors. Kuglstatter, Andreas; Ghate, Manjiri; Tsing, Stan; Villaseñor, Armando G.; Shaw, David; Barnett, Jim W.; Browner, Michelle F.. *Bioorganic & Medicinal Chemistry Letters*, Sep2010, Vol. 20 Issue 17, p5217-5220; DOI: 10.1016/j.bmcl.2010.06.157.

Recombinant formate dehydrogenase from *Arabidopsis thaliana*: Preparation, crystal growth in microgravity, and preliminary X-ray diffraction study. Shabalin, I.; Serov, A.; Skirgello, O.; Timofeev, V.; Samygina, V.; Popov, V.; Tishkov, V.; Kuranova, I.. *Crystallography Reports*, Sep2010, Vol. 55 Issue 5, p806-810; DOI: 10.1134/S1063774510050159.

Structural biology in fragment-based drug design. Murray CW and Blundell TL. *Current Opinion in Structural Biology*, 2010 Aug; 20(4): 497-507. DIO: 10.1016/j.sbi.2010.04.003.

Growth and excitement in membrane protein structural biology. Tate CG and Stevens RC. *Current Opinion in Structural Biology*, 2010 Aug;20(4): 399-400. DOI: 10.1016/j.sbi.2010.07.002.

Is too 'creative' language acceptable in crystallography? Alexander Wlodawer, Jacek Lubkowski, Wladek Minor, and Mariusz Jaskolski.
Acta Crystallogr D Biol Crystallogr. 2010 September 1; 66(Pt 9): 1041–1042. DOI: 10.1107/S090744491002799X.

Book reviews:

Cooking for Geeks

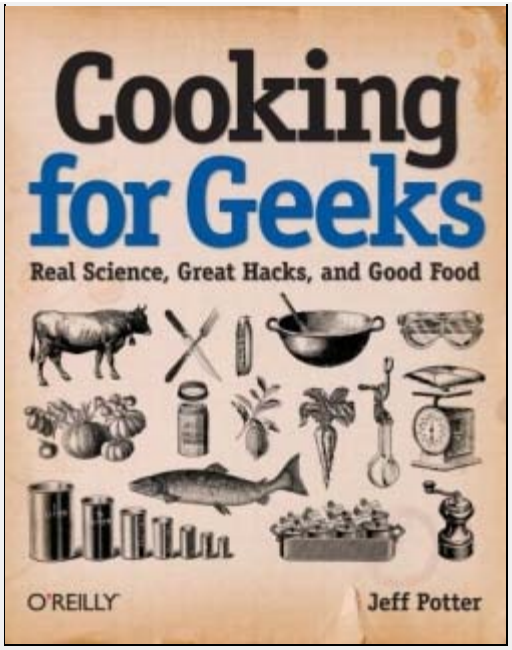
by Jeff Potter, O'Reilly Media, ISBN-13: 978-0596805883

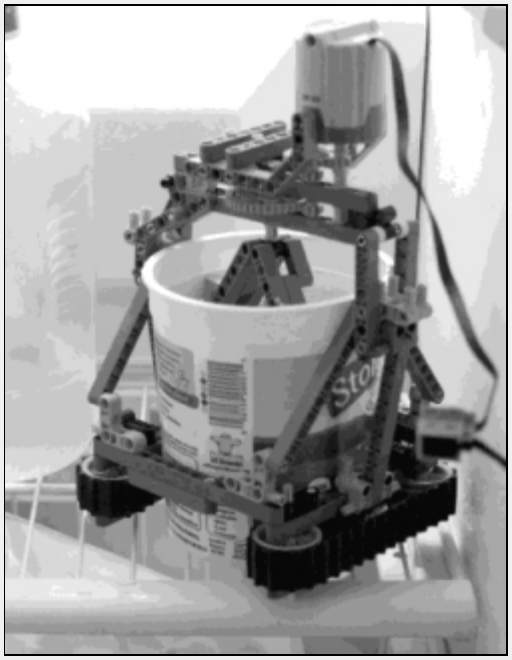
I heard an interview with Mr. Potter on NPR's Science Friday a few weeks ago and immediately bought a copy of this book for myself. The author is a software developer who likes to cook. The book is published by O'Reilly Media, the company that has brought out so many software cookbooks. The "Geeks" in the title are software developers, but let's face it, most of us crystallographers are geeks too and a lot of us like to cook. My last argument for reviewing *Cooking for Geeks* is that cooking is really just applied chemistry.

The book pays due homage to *Mastering the Art of French Cooking* by Julia Child and On Food and Cooking by Harold McGee. The author treats cooking like running a program. Potter starts with a discussion on initializing your kitchen and calibrating your tools. Much attention is paid to heat and time and the interchangeability (to a degree) of the two. His favorite tool is an infrared thermometer for temperature calibration but he also describes the use of sugar to calibrate ovens to 365°F.

There are some useful discussions about the tasty products of chemical reactions that occur during the cooking process: Mallaird reactions, caramelization and protein denaturation. Although the description of the last reaction was a little weak and the description of using alcohol to make aroma molecules lighter is obviously wrong, I found the rest enlightening. For example, I really like the idea of replacing the glass window in my oven with Pyroceram® to make a proper 850°F pizza oven. All I have to do is convince my wife nothing will happen to the house.

The other "hack" I am interested in trying is sous vide (under vacuum) cooking. In this method the food item is placed in a sealed plastic bag brought to an exact temperature, 140° F for example, in a water bath and allowed to reach thermal equilibrium. The results are supposed to produce delicious and tender meat and fish. I am sure there are enough low temp controllers and pumps around here that I can cobble something together in a





Lego® ice cream maker as described in *Cooking for Geeks* by Jeff Potter.

few hours. Speaking of fish, did you know that if you brine salmon for 20 minutes in 5-10% saline solution and rinse, the albumin will set and won't leach out during grilling? This works for mahi-mahi too.

Another interesting trick is to create a recipe by averaging a number of existing recipes for the desired product. However, I am sure it is the outliers that make for the interesting results. Throughout the book you will find sidebars with interviews from experts and recipes that demonstrate the topics being covered.

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