# Crystallography Times

Rigaku

**Protein Crystallography Newsletter**
Volume 2, No. 5, May 2010


**In this issue:**



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### Grant/Funding Preparation A Lab Roadmap

Times change and so do research bottlenecks. A first step, in advancing your laboratory's crystallographic capabilities, is a situation and needs analysis. Against this understanding, a variety of roadmaps can be laid out, relative to current technologies, that lead to fulfillment of new requirements.

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### Continuing Education Webinar

The webinar series continues in June.
Topic, date and time to be announced.

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### Software Webinars Available On-demand

Our Spring 2010 webinar program featured well known expert presenters in a mini-series devoted to commonly used data processing packages.

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## Crystallography in the news

May 18, 2010. National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH) granted University of Missouri researchers, led by Prof. Jianlin Cheng, $1.18 million to further develop and improve MULTICOM, a protein prediction software system. The tool will be used to understand protein structures in diseases for the purpose of improved drug design.

May 17, 2010. As part of the structure annotation process, wwPDB begins providing depositors with detailed Validation Reports that include the results of geometric and experimental data checking while keeping the coordinate file confidential.

May 14, 2010. Vanderbilt University investigators, led by Prof. Tina Iverson, determined the structure of a protein that resides in the outer membrane of the bacterium *Neisseria meningitidis*, a leading cause of bacterial meningitis. The findings suggest that immune system receptors initially recognize bacteria based on charged structural features in their surface proteins.

May 14, 2010. An international research team led by a Simon Fraser University cell biologist Michel Leroux is closer to piecing together a picture of what causes Bardet-Biedl syndrome (BBS). Using X-ray crystallography, they have uncovered the shape of protein BBS3 and discovered how mutations cause it to malfunction.

May 4, 2010. A research team, led by Prof. Hong Zhou, used *only* cryo-electron microscopy to image the structure of an aquareovirus at 3.3 Å. Dr. Zhou is faculty director of the Electron Imaging Center for Nanomachines (EICN) at UCLA's California NanoSystems Institute..

April 29, 2010. As announced by Galapagos® CEO Onno van de Stolpe, BioFocus® will perform drug-candidate screening and related services, including protein crystallography, for the CHDI Foundation's Huntington's disease drug development programs under a five-year collaboration agreement.

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## Managing all your protein crystallization data

Protein crystallization is a data intensive exercise, especially when viewed in the context of automation. Typically, once a protein has been successfully crystallized, one would want to know as much as possible about the hit: Screen information, growth conditions, incubation times, and much more. CrystalTrak™ is a software package designed to answer these questions; it is a database application built on Oracle that tracks, organizes, and makes searchable crucial sample and experiment data to ensure data is not lost and is easily retrieved.

CrystalTrak also simplifies setting up cumbersome and time consuming screen optimization tasks. Common optimization tasks such as optimizations around a single or multiple hits are done by "one-click" operations that automate experiment design, buffer interpolations and tedious stock management calculations.

Finally, when it comes to reviewing imaging results — currently one of the biggest bottlenecks in automated protein crystallization — CrystalTrak keeps track of previous inspection results and provides tools to remotely score and annotate images from any web browser within your intranet or over the internet.

Request more information on CrystalTrak.

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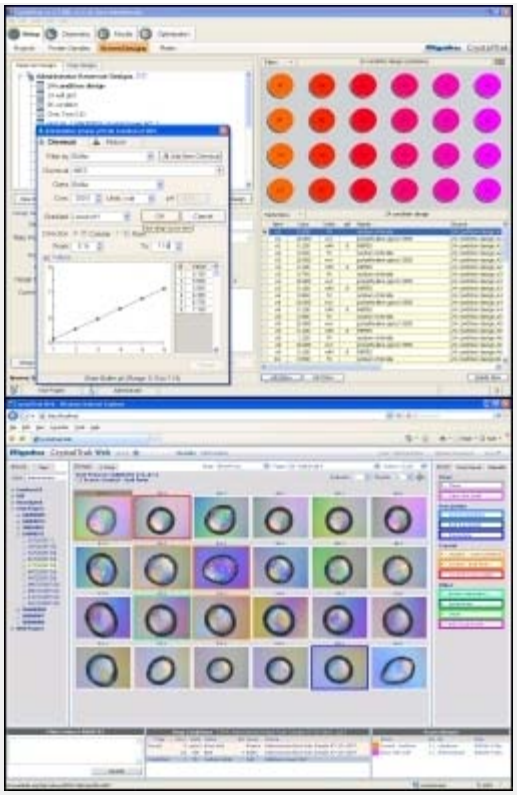
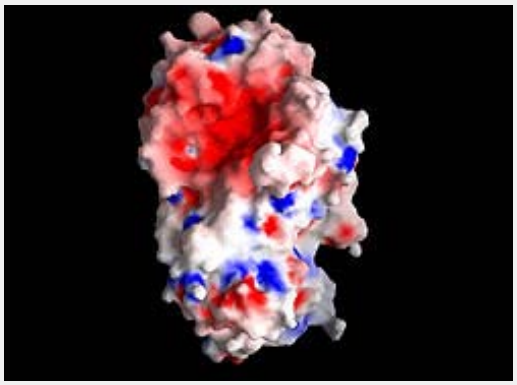


**Rigaku CrystalTrak**



Professors Gary Brayer, Natalie Strynadka and Filip Van Petegem (left to right) with the Department of Biochemistry and Molecular Biology at University of British Columbia.

### Rigaku Hardware Training Classes

Rigaku has scheduled our next training session, at our Texas facility, 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 and X-ray generators. Safety will be emphasized.

## Lab spotlight: University of British Columbia, Dept. of Biochemistry and Molecular Biology

For more than fifty years the UBC Department of Biochemistry & Molecular Biology has played an active and important role at UBC and in the greater scientific community. The Department includes 27 full-time faculty, some of whom are affiliated with research groups such as the BC Cancer Agency or the Centre for Blood Research. The department also has eleven associate faculty members.

Since its move to the new multidisciplinary Life Sciences Centre, the department has been brought together with the departments of Cellular & Physiological Sciences and Microbiology & Immunology, as well as independent researchers from Medical Genetics and Zoology. By working with members of other departments in this highly interactive environment, the UBC Department of Biochemistry & Molecular Biology continues to grow and develop in its role as one of the leaders in the field. Three of the faculty are X-ray crystallographers involved in structural biology.

The current research effort in Prof. Gary Brayer's group is focused on two proteins: the first involves the critical digestive enzyme human pancreatic alpha-amylase while the second system involves a unique hexameric form of the enzyme citrate synthase, which is only found in Gram-negative bacteria.

Professor Natalie Strynadka has, as a research goal, the structure-based design of novel, therapeutically useful antibiotics and inhibitors of antibiotic-resistance mechanisms. To achieve this goal, her group employs a combination of X-ray crystallography, molecular modelling, molecular docking and molecular biology in collaboration with medicinal chemistry to engineer drugs that specifically interact with and disable critical bacterial target proteins.

Assistant Professor Filip Van Petegem is engaged in research on ion channels that allow the selective passage of $Ca^{2+}$, how they interact with regulatory proteins, how they integrate various signals, and what their molecular architecture looks like. In particular, his group is investigating two classes of channels: voltage-gated calcium channels (CaVs) and ryanodine receptors (RyRs).

## Useful links for crystallography

BIOBAR is a powerful browsing and searching toolbar developed at EMBL-EBI's Protein Data Bank in Europe (PDBe). BIOBAR works inside Firefox browsers on all supported operating systems, and provides easy access to over 40 biological data sources, including Genomic, Proteomic, Functional, Literature, Taxonomic, Structural, Plant and Animal-specific databases. In addition to facilitating search and retrieval, BIOBAR also provides static links to important bioinformatics sites and services including those at the European Bioinformatics Institute (EBI), the National Center for Biotechnology Information (NCBI) and the DNA Data Bank of Japan (DDBJ). BIOBAR further provides one-stop access to major data-deposition sites for nucleotide, protein and 3D-structure data and links to many sequence and structure alignment and analysis tools.

MOVIES is a site, created by Prof. James Holton (UCSF), hosting a variety of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or cut-and-paste
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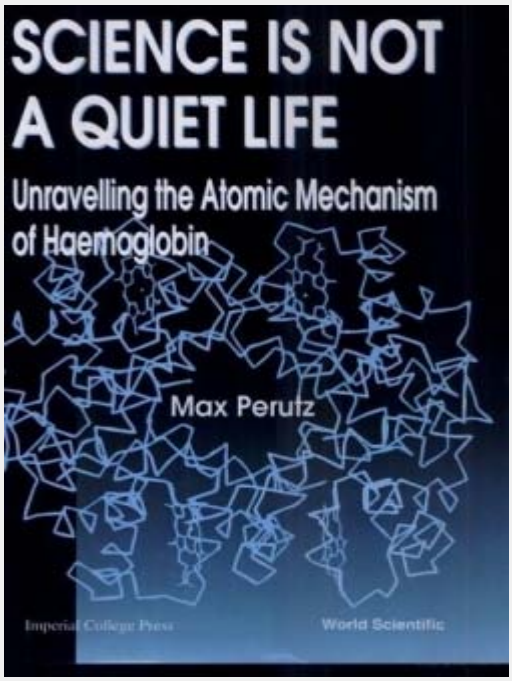



molecular graphics movies that illustrate a variety of points: from the importance of resolution to the importance of phase and data completeness ... and more. The movies were created with MOVIEFY, a program intended for summarizing X-ray image graphics as movies.

CSAR is the Community Structure-Activity Resource page, hosted by the University of Michigan, that aims to improve docking and scoring through participation of the entire scientific community. CSAR disseminates experimental datasets of crystal structures and binding affinities for diverse protein-ligand complexes. Some datasets are generated in house at University of Michigan, while others will be collected from the literature or deposited by academic labs, national centers, and the pharmaceutical industry.

## Selected recent crystallographic papers

Structure of the bacterial teichoic acid polymerase TagF provides insights into membrane association and catalysis. A.L. Lovering, L. Y-C Lin, E.W. Sewell, T. Spreter, E.D. Brown and N.C.J. Strynadka. *Nature Structural & Molecular Biology* 2010; **17:** 582-589.

Migrastatin analogues target fascin to block tumour metastasis. L. Chen, S. Yang, J. Jakoncic, J.J. Zhang and X-Y Huang. *Nature* 2010; **464:** 1062-1066.

Super-resolution biomolecular crystallography with low-resolution data. G.F. Schröder, M. Levitt and A.T. Brunger. *Nature* 2010; **464**: 1218-1222.

Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. R. Xu, D.C. Ekiert, J.C. Krause, R. Hai, J.E. Crowe and I.A. Wilson. *Science* 2010; **328** (5976): 357-360.

Structural basis for receptor recognition by New World hemorrhagic fever arenaviruses. J. Abraham, K.D. Corbett, M. Farzan, H. Choe and S.C. Harrison. *Nature Structural & Molecular Biology* 2010; **17**: 438-444.

Diversity in DNA recognition by p53 revealed by crystal structures with Hoogsteen base pairs. M. Kitayner, H. Rozenberg, R. Rohs, O. Suad, D. Rabinovich, B. Honig and Z. Shakked. *Nature Structural & Molecular Biology* 2010; **17**: 423-429.

Conformational change of flagellin for polymorphic supercoiling of the flagellar filament. S. Maki-Yonekura, K. Yonekura and K. Namba. *Nature Structural & Molecular Biology* 2010; **17**: 417-422.

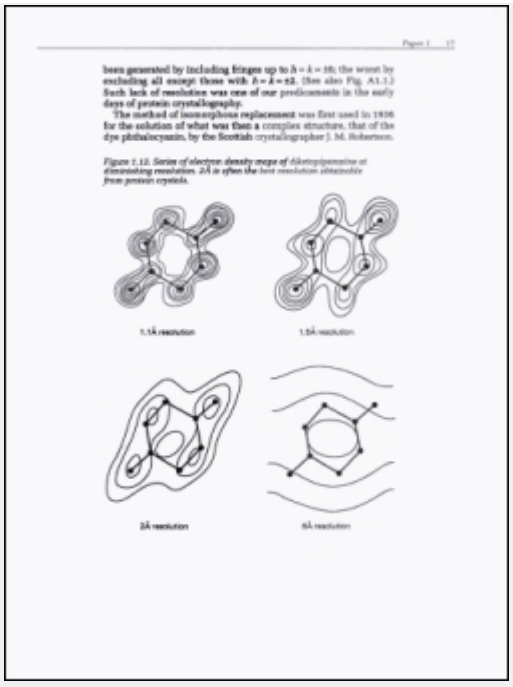
'Broken symmetries' in macromolecular crystallography: phasing from unmerged data. M. Schiltz and G. Bricogne. *Acta Cryst.* 2010; **D66**: 447-457.

Isothermal compressibility of macromolecular crystals and macromolecules derived from high-pressure X-ray crystallography. I. Ascone, R. Kahn, E. Girard, T. Prangé, A.C. Dhaussy, M. Mezouar, N. Ponikwicki and R. Fourme. *J. Appl. Cryst.* 2010; **43**: 407-416.

## Book review: *Science Is Not a Quiet Life*
### by Max Perutz

I have to thank Angela Criswell for bringing this book to my attention. We thought "Science Is Not a Quiet Life: Unraveling the Atomic Mechanism of Haemoglobin" would complement well Michael Rossmann's recent webinar title "From Haemoglobin to West Nile Virus". I was so enthralled by the book that I gave Angela's copy to a student just starting her career in the study of hemoglobin ... only to find that it is actually quite hard to find. Anyway, I did find a replacement copy and the book is worth reading, especially if your library or supervisor has a copy.

The book is divided into 12 chapters. The first chapter provides a non-crystallographer's introduction to crystallography titled "Diffraction Without Tears: A Pictorial Introduction to X-ray Analysis of Crystal Structures" that first appeared in his 1992 book Protein Structure: New Approaches to Disease. This chapter does what it is says, both in schematic pictures and state-of-the-art graphics. State-of-the-art graphics from the 1940s to the 1980s, that is. Those of you with 3D monitors should appreciate how difficult crystallography was when Fourier transforms were calculated by hand.



been generated by including fringes up to $h = k = 16$; the worst by excluding all except those with $h = k = \pm 2$. (See also Fig. A1.1.) Such lack of resolution was one of our predicaments in the early days of protein crystallography.

The method of isomorphous replacement was first used in 1936 for the solution of what was then a complex structure, that of the dye phthalocyanin, by the Scottish crystallographer J. M. Robertson.

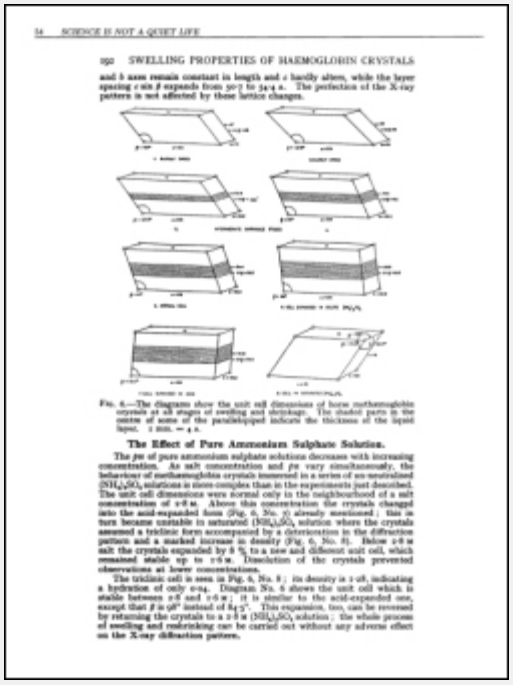
*Figure 1.12. Series of electron density maps of diketopiperazine at diminishing resolution. 2Å is often the best resolution obtainable from protein crystals.*

1.1Å resolution

1.5Å resolution

2Å resolution

6Å resolution



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192 SWELLING PROPERTIES OF HAEMOGLOBIN CRYSTALS

and $b$ axes remain constant in length and $c$ hardly alters, while the layer spacing $c \sin \beta$ expands from 30·7 to 34·4 Å. The perfection of the X-ray pattern is not affected by these lattice changes.

FIG. 6.—The diagrams show the unit cell dimensions of horse methæmoglobin crystals at all stages of swelling and shrinkage. The shaded parts in the centre of some of the parallelepiped indicate the thickness of the liquid layer. 1 mm. = 4 Å.

**The Effect of Pure Ammonium Sulphate Solution.**

The *pH* of pure ammonium sulphate solutions decreases with increasing concentration. As salt concentration and *pH* vary simultaneously, the behaviour of methæmoglobin crystals immersed in a series of un-neutralized $(NH_4)_2SO_4$ solutions is more complex than in the experiments just described. The unit cell dimensions were normal only in the neighbourhood of a salt concentration of 1·6 M. Above this concentration the crystals changed into the acid-expanded form (Fig. 6, No. 7) already mentioned; this in turn became unstable in saturated $(NH_4)_2SO_4$ solution where the crystals assumed a triclinic form accompanied by a deterioration in the diffraction pattern and a marked increase in density (Fig. 6, No. 8). Below 1·6 M salt the crystals expanded by 8 % to a new and different unit cell, which remained stable up to 1·0 M. Dissolution of the crystals prevented observations at lower concentrations.

The triclinic cell is seen in Fig. 6, No. 8; its density is 1·26, indicating a hydration of only 0·24. Diagram No. 6 shows the unit cell which is stable between 1·6 and 1·0 M; it is similar to the acid-expanded one, except that $\beta$ is 98° instead of 84·5°. This expansion, too, can be reversed by returning the crystals to a 2·0 M $(NH_4)_2SO_4$ solution; the whole process of swelling and reshrinking can be carried out without any adverse effect on the X-ray diffraction pattern.

Each subsequent chapter consists of a short introduction by Perutz followed by a series of papers associated with the period of study. The papers within in each chapter are in chronological order, but across chapters they are not. It might be interesting to go back and read the papers in strict chronological order to get the historical critical perspective.

In chapter 2, "Early Studies", we see the inception of protein crystallography in the 30s with J. D. Bernal. In this chapter is also a paper titled "The Composition and Swelling Properties of Haemoglobin Crystals" — this is the beginning of the use of humidity control to systematically change the properties of protein crystals. The next chapter, "Solution of the Phase Problem" discusses the heroic efforts Perutz and his collaborators, including Lawrence Bragg and Michael Rossmann, used to determine the structure at 5.5 Å. The development of isomorphous replacement, first using different salt concentrations, then heavy atom derivatives, unfolds before us in the papers presented.

Chapter 4, titled "From the First Molecular Structure Model to the Allosteric Mechanism" covers the evolution of the structural model, from 5.5 Å to 2.8 Å, with the elucidation of the allosteric effect. The paper "Structure of Myoglobin: A Three-Dimensional Fourier Synthesis at 2 Å Resolution" by Kendrew et al. is provided for historical perspective. In chapter 5, "The Haemoglobin Battles" describes how Perutz used synchrotron data collected in 1980 at LURE to increase the resolution to 1.74 Å, and used other methods to show how the binding of oxygen changed the structure and induced the allosteric effect. Perutz then turns his efforts to understanding the "Molecular Pathology of Human Haemoglobin" starting with hemoglobin in sickle-cell anemia, and then other less common conditions.

Chapter 7 covers "Haemoglobin as a Drug Receptor" while chapter 8 presents the case for evolution via "Species Adaptation in Haemoglobin". Chapters 9 and 11 cover the topics "Early Shots at the Folding and Unfolding Problems" and "Haemoglobin as an Oxygen Sensor That Regulates Expression of Nitrogenase Genes" concluding the work on hemoglobin.

I skipped chapter 10 above because here Perutz goes into a different direction with "Present Work: Polar Zippers and Neurodegenerative Disease" where glutamine repeats are the polar zippers. The final chapter, "Glaciers" is quite interesting. This has nothing to do with cryo-crystallography, and tells us about Perutz' effort during World War II to test the hypothesis that large icebergs could be converted into unsinkable aircraft carriers. Research required field work in the Alps to gather data to test creep in glaciers. This activity provided much enjoyment since Perutz enjoyed skiing—a skill needed to reach the glaciers he was studying.

Joseph D. Ferrara, Ph.D.

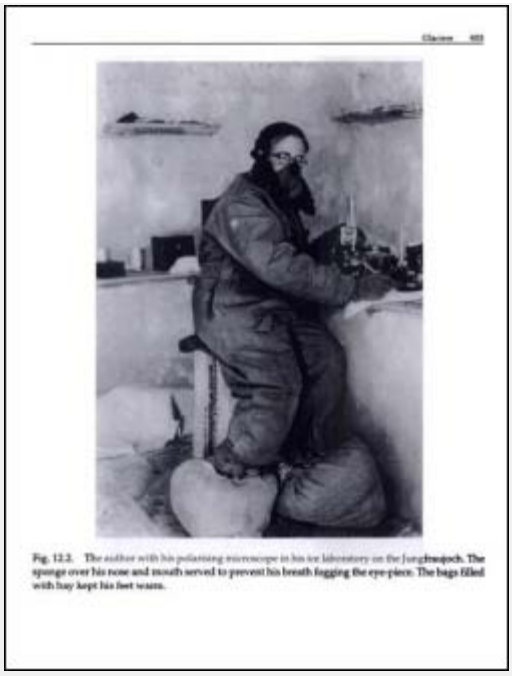

Fig. 12.2. The author with his polarizing microscope in his ice laboratory on the Jungfraujoch. The sponge over his nose and mouth served to prevent his breath fogging the eye-piece. The bags filled with hay kept his feet warm.