Crystallography Times

Rigaku

Protein Crystallography Newsletter
Volume 2, No. 8, August 2010

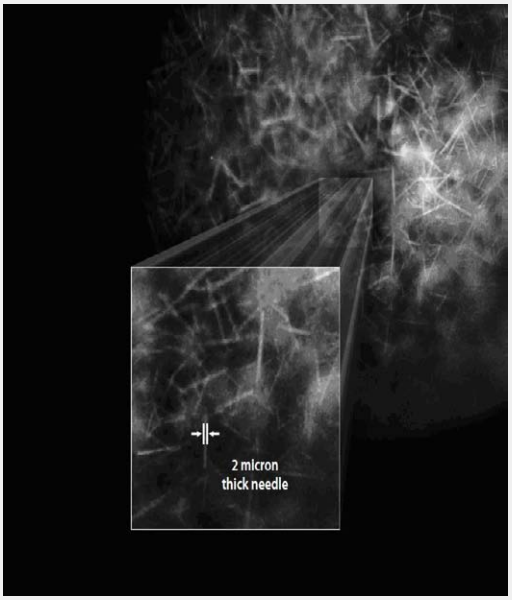
In this issue:

Macromolecular Training Class

October 27 - 29, 2010
The Woodlands, Texas

Register

Rigaku will hold a training session, at our Texas facility, on October 27 - 29, 2010. 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. October's class will also feature a training session on processing data with HKL taught by Dr. Wladek Minor (pictured above).



Results of our UV resolution test showing a fully resolved 2 micron diameter needle.

Crystallography in the news

August 27, 2010. After more than a decade of research, Scripps Research Institute scientists, led by Professors Glen Nemerow and Vijay Reddy, have pieced together the structure of a human adenovirus — the largest complex ever determined at atomic resolution.

August 25, 2010. Using X-ray crystallography, Prof. Song Tan and his colleagues at Penn State University were, for the first time, able to visualize the binding of a protein to nucleosomes in three dimensions.

August 20, 2010. Researchers at Loyola University, under the direction of Edward M. Campbell, have employed a wide-field "deconvolution" microscope to identify the key components of a protein (TRIM5a) that destroys HIV in rhesus monkeys.

August 16, 2010. The Linac Coherent Light Source at the SLAC National Accelerator Laboratory was officially dedicated with a visit from U.S. Energy Secretary Steven Chu. The only one of its kind in the world, the laser makes ultrabright, ultrafast X-ray pulses.

August 15, 2010. Researchers at the RIKEN Advanced Science Institute, led by Dr. Hiroyuki Osada, used X-ray crystallography to determined how a small molecule (TPh A) binds to human pirin protein and thus inhibit the migration of melanoma cells by reducing expression of the tumor mobility protein SNAI2.

August 12, 2010. Researchers at the RIKEN Systems and Structural Biology Center and the University of Tokyo, led by Dr. Shigeyuki Yokoyama, have clarified the structural basis for the biosynthesis of selenocysteine, an amino acid whose encoding mechanism offers clues about the origins of the genetic alphabet.

August 8, 2010. New findings by cancer researchers at UC Santa Cruz, led by Prof. Seth Rubin, reveal the molecular mechanisms involved in the gate-like functioning of the retinoblastoma tumor suppressor protein, which is involved in many types of cancer.

August 6, 2010. Scientists from the US Department of Energy's Lawrence Berkeley National Laboratory, led by Dr. Bing Jap, have obtained the closest look yet of how a gargantuan molecular machine (tripeptidyl peptidase II) breaks down unwanted proteins in cells, a critical housekeeping chore that helps prevent diseases such as cancer.

August 4, 2010. An international team of molecular biologists, led by Prof. Robert Robinson at the A*STAR Institute of Molecular and Cell Biology in Singapore, has uncovered a mechanism for regulating the construction of actin filaments, which are the major component of the cellular skeleton and integral to many cellular processes.

ACA 2010: imaging the future with the Minstrel HT UV

The ACA 2010 meeting was a great opportunity for us to present the new Rigaku Minstrel HT UV to a larger audience. This high throughput protein drop imager had already attracted a lot of interest beforehand because of its exceptional resolution capabilities, both under UV and visible light: When tested under realistic conditions, namely a drop in which we grew a shower of needles, the Minstrel HT UV could resolve needle diameters of 2 microns or less, both under UV and visible light.

But superior optics is not the only thing that sets the new Minstrel HT UV

The new Minstrel HT UV system.

Prof. John Kuriyan, Chancellor's Professor, Department of Molecular and Cell Biology & Department of Chemistry, University of California, Berkeley; Investigator, Howard Hughes Medical Institute.

Continuing Education Webinar

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

Introducing the Rigaku Automation Blog

The new Rigaku Automation blog is an additional way to communicate with you in a timely manner and to provide background information that is otherwise difficult to incorporate in traditional web pages. For example, this week's post is about optical resolution for drop imaging.

The idea, in short, is that protein drops are three-dimensional objects. Traditional methods used to measure optical resolution are not well suited for drop imagers as apart from its predecessor, the Minstrel HT. The new Minstrel HT UV also received revised control software that greatly simplifies using this instrument, both in automatic and manual mode (the Minstrel HT UV can be used as a walk-up inspection unit, very much like a conventional microscope). Changes most notably include an algorithm for managing inspection schedules and a redesigned interface that simplifies the administration and use of this system.

Request more information on the new Minstrel HT UV.

Lab spotlight: Kuriyan Lab, UC Berkeley

John Kuriyan's Laboratory at UC Berkeley is interested in the structure and mechanism of the enzymes and molecular switches that carry out cellular signal transduction and DNA replication. They use X-ray crystallography to determine the three-dimensional structures of proteins involved in signaling and replication, as well as biochemical, biophysical, and computational analyses to figure out how they work. A major focus in their laboratory is understanding the allosteric mechanisms that enable proteins to be exquisitely sensitive to input signals

Cell Signaling: The major class of signaling molecules that they study are the protein kinases, a large family of closely related enzymes that catalyze the addition of phosphate to serine, threonine, and tyrosine residues in proteins.

Processive DNA Replication: DNA polymerases that replicate chromosomes achieve high speed by utilizing specialized proteins that allow the polymerase to move rapidly along DNA without letting go. These proteins include the "sliding DNA clamp" and the clamp loader complex that couples ATP binding and hydrolysis to the opening of the beta clamp and its loading onto DNA.

Useful links for crystallography

Ringer 1.0. The Alber lab at UC Berkeley is pleased to release of the code for Ringer version 1.0. Ringer is a program to detect molecular motions by systematic X-ray electron-density sampling.

The aim of Ringer is to go beyond static structural snapshots of proteins by uncovering structural ensembles in X-ray electron density. This information can reveal not only which parts of proteins are flexible and which parts are rigid, but it also can define alternate conformations that may be important for function. Alternate conformations of binding sites also may afford additional targets for drug design.

The Ringer method is described by Lang et al. in *Protein Sci.* 2010 Jul; **19**(7): 1420-31. An application of Ringer, determining the structural underpinnings of the side chain dynamics critical for the function of the enzyme proline isomerase, was published by Fraser et al. in *Nature.* 2009 Dec 3;**462**(7273): 669-73. Ringer is freely available to academics.

Selected recent crystallographic papers

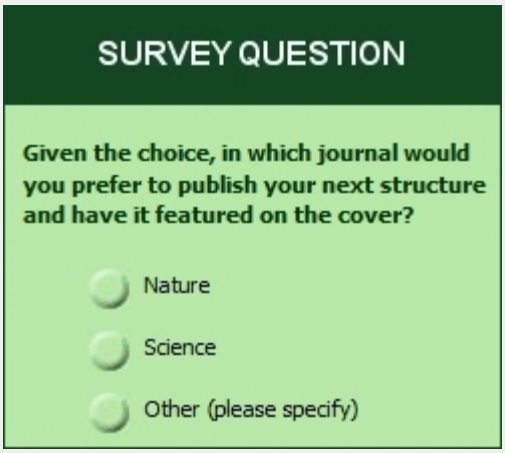
Structure of FocB - a member of a family of transcription factors regulating fimbrial adhesin expression in uropathogenic *Escherichia coli*. Hultdin, U.W.; Lindberg, S.; Grundström, C.; Huang, S.; Uhlin, B.E. and Sauer-Eriksson, A.E. *FEBS Journal*. Aug2010, 277(16): 3368-3381.

Raman-assisted X-ray biocrystallography. Vergara, A.; Merlino, A.; Balsamo, A.; Sica, F. and Mazzarella, L. *AIP Conference Proceedings.* 8/6/2010,

they are based on two-dimensional test patterns. This understanding is fundamental when it comes to designing a protein drop imager. The post then continues with how Rigaku measures resolution and why the new Minstrel HT UV produces protein drop images with such astonishing quality.

How can I use the blog?
A blog has some distinct advantages over a traditional web site as it allows for a more interactive experience. For example, you can comment on posts (and we encourage you to do so) or you can send us suggestions on topics you'd like us to cover. Or, if there is something on your mind you'd like to share, you could even become a guest contributor!

How can I stay updated?
Of course you can simply bookmark http://rigakublog.com/ and check back from time to time, but if you want to make sure you don't miss any new content, you can subscribe via email or syndicate the content via RSS. Just use the top item in the sidebar area.



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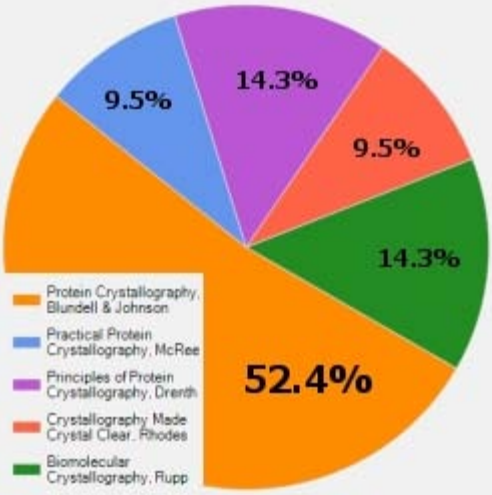
into your browser.

July Survey Results

Which do you consider to be the most influential book in macromolecular crystallography?



1267(1): 866-866.

The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. Stewart, M.L.; Fire, E.; Keating, A.E. and Walensky, L.D. *Nature Chemical Biology*. Aug2010, 6(8): 595-601.

X-ray crystallographic and MD simulation studies on the mechanism of interfacial activation of a family I.3 lipase with two lids. Angkawidjaja, C.; Matsumura, H.; Koga, Y.; Takano, K. and Kanaya, S. *Journal of Molecular Biology*. Jul2010, 400(1): 82-95.

Crystallization of the membrane protein hVDAC1 produced in cell-free system. Deniaud, A.; Liguori, L.; Blesneac, I.; Lenormand, J.L. and Pebay-Peyroula, E. *BBA - Biomembranes*. Aug2010, 1798(8): 1540-1546.

Diversity of function-related conformational changes in proteins: Coordinate Uncertainty, Fragment Rigidity, and Stability. Rashin, A.A.; Rashin, A.H.L. and Jernigan, R.L. *Biochemistry*. 7/13/2010, 49(27): 5683-5704.

Solution structure of the N-terminal transactivation domain of ERM modified by SUMO-1. Lens, Z.; Dewitte, F.; Monté, D.; Baert, J.; Bompard, C.; Sénéchal, M.; Van Lint, C.; de Launoit, Y.; Villeret, V. and Verger, A. *Biochemical & Biophysical Research Communications*. Aug2010, 399(1): 104-110.

About the albumin structure in solution and related electro-spinnability issues. Regev, O.; Khalfin, R.; Zussman, E. and Cohen, Y. International *Journal of Biological Macromolecules*. Aug2010, 47(2): 261-265.

Insights into eukaryotic DNA priming from the structure and functional interactions of the 4Fe-4S cluster domain of human DNA primase. Vaithiyalingam, S.; Warren, E.M.; Eichman, B.F. and Chazin, W.J. *Proceedings of the National Academy of Sciences*. 8/3/2010, 107(31): 13684-13689.

Structure and mechanism in membrane trafficking. Hughson F.M. and Reinisch K.M. *Current Opinion in Cell Biology*. 2010 Aug, 22(4):454-60.

Thermodynamics and structure of a salmon cold active goose-type lysozyme. Kyomuhendo P.; Myrnes B.; Brandsdal B.O.; Smalås A.O.; Nilsen I.W. and Helland R. *Comparative Biochemistry & Physiology Part B*. Aug2010, 156(4): 254-63.

Role of a PAS sensor domain in the Mycobacterium tuberculosis transcription regulator Rv1364c. Jaiswal, R.K.; Manjeera, G. and Gopal, B. *Biochemical & Biophysical Research Communications*. Jul2010, 398(3): 342-349.

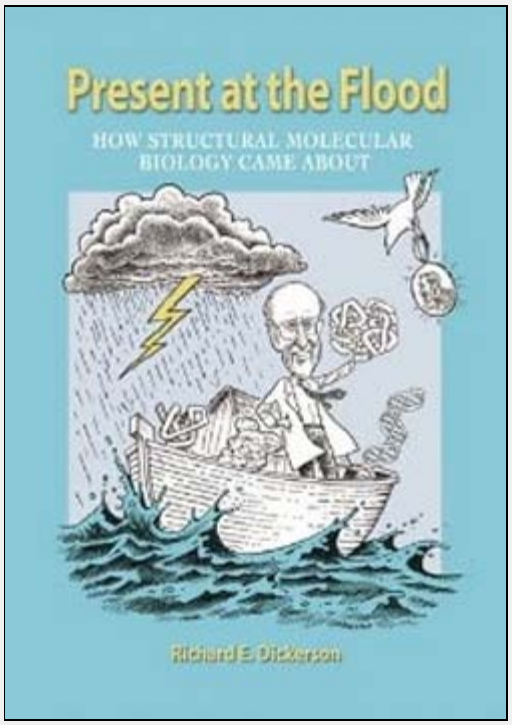
Design and structure of an equilibrium protein folding intermediate: A hint into dynamical regions of proteins. Ayuso-Tejedor, S.; Angarica, V.E.; Bueno, M.; Campos, L.A.; Abián, O.; Bernad&oacutel;, P.; Sancho, J. and Jiménez, M.A. *Journal of Molecular Biology*. Jul2010, 400(4): 922-934.

Book reviews:

Present at the Flood:
How Structural Molecular Biology Came About
by Richard Earl Dickerson, Sinauer Associates, 2005, ISBN: 978-0878931682

Present at the Flood (PATF) is a retrospective that provides a detailed overview of the personalities and work that is at the origin of structural biology. Each chapter has an introduction and then a series of papers that elucidate a particular step in the process. For brevity, some of the papers are abridged. However, a number of those papers also appeared in their entirety in *Science is not a Quiet Life* by Max Perutz, reviewed here previously. Each chapter is well referenced and has a set of study questions at the end (with answers at the end of the book).

Dickerson begins with an introduction to the problems of protein and DNA structure that he will elucidate in later chapters. In Chapter 2, he covers the work of Astbury and Wood and introduces the reader to fiber diffraction. Then he reviews the models for chains of amino acids that evolved through



Richard Earl Dickerson

the work of D.W. Wrinch, who mistakenly generated the cyclol model, then the work of Pauling and others.

In chapter 4 we see how the model for the alpha helix is developed by Pauling. Interestingly, Pauling used a rolled up tube of paper to visualize this model. Perutz had missed an important datum because all the fiber diffraction patterns, collected to date, were collected on perfectly oriented samples. The 1.5 Å reflection needed to confirm such a model was outside the diffraction conditions. Once Perutz tilted a fiber sample he saw the reflection and confirmed Pauling's model. This left the Cavendish lab on edge, so when the structures for hemoglobin, myoglobin and DNA were ready there was no delay in publication. The phrase, "I wish I had made you angry earlier," summarizes the conversation between Bragg and Perutz on the observation of that reflection.

In chapter 5, Dickerson recapitulates Perutz' concept of crystallography without mathematics, which provides a good introduction to many concepts for novices. This provides a direct path to understanding the diffraction pattern for a helix with points of electrons along the path. Next Dickerson reviews the dead ends associated with the determination of the helix model and the final, correct structure. Of course, discussion of the history surrounding the structure of DNA would not be complete without mentioning the tragedy of Rosalind Franklin. Dickerson suggests that one should read the first 8 books in the bibliography and come to their own conclusion, a valid suggestion.

Chapter 6 reviews Perutz' breakthrough in discovering multiple isomorphous replacement, which allowed Kendrew to solve the structure of myoglobin and Perutz the structure of hemoglobin. Dickerson takes the reader through the solution of the phase problem and the slow increase in the understanding of these structures. It is very clear that 2D projections were not enough and full 3D models were needed to visualize these structures. I want to point out that the first paper reprinted is actually a parody on crystallography, the solution of the structure of the fictitious protein globglobin. I thoroughly enjoyed it.

In chapter 7, we see the transition from the low resolution structure of myoglobin, 6 Å, to the high resolution 2 Å structure through modern computational methods. We learn also that the placement of the heme in the original myoglobin structure was wrong but corrected at 2 Å. The 5.5 Å hemoglobin structure is discussed, and the cyclol model is revisited and buried forever. Chapters 8 reviews the state of affairs after the initial structures were solved and the exponential growth of the PDB. The epilogue, Chapter 9, finishes the story with "a whatever happened to" the main characters in the book.

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