Crystallography Times
Rigaku

Protein Crystallography Newsletter
Volume 2, No. 6, June 2010

In this issue:

Continuing Education Webinar

d*TREK & HKL-2000:
Scaling and Statistics Revealed

Presenter: Dr. Jim Pflugrath
July 15th at 11AM EDT
(14:00 GMT)

This GoToMeeting webinar spends more time on the scaling of Bragg reflections derived from diffraction images processed with d*TREK and other packages.

Examples of how to merge, scale, and average data sets from the same crystal and/or from multiple crystals will be explained. Also shown will be how to use d*TREK to calculate "Table 1" for publication, even if you use other packages to process and scale your data. The differences in the equations used by d*TREK and HKL-2000 will be highlighted and explained.

The final output can be used with the CCP4 or other packages to produce structural models.

Grant/Funding Preparation

Times change and so do research bottlenecks. A first step, in advancing your lab's crystallographic capabilities, is a situation and needs analysis.

Crystallography in the news

June 25, 2010. During the 132nd annual Spring Convocation of the University of Manitoba, Prof. Michael N. G. James was awarded an honorary degree. Dr. James has been one of the preeminent leaders in protein crystallography in Canada for 40 years, establishing in 1968 the first laboratory in Canada devoted to the study of proteins by crystallography. In 1974, James determined the first high-resolution structure of a protein in Canada.

June 23, 2010. Using the World Community Grid at the Help Conquer Cancer Project, scientists led by Prof. Igor Jurisica, at the Ontario Cancer Institute, together with colleagues at the Hauptman-Woodward Medical Research Institute, have found a way to automate and speed up protein crystallography through the use of a novel protein crystal image analysis algorithm.

June 22, 2010. Scientists led by Prof. David Price, at the University of Iowa Carver College of Medicine, and Prof. Tahir Tahirov, at the University of Nebraska Eppley Institute, have used X-ray crystallography to create a three-dimensional representation of the interaction of the Tat protein from HIV, the AIDS virus, and the human protein called P-TEFb. The Tat protein plays a key role in the reproduction of HIV.

June 15, 2010. Employing special techniques to analyze structure at high resolution using cryo-EM, researchers led by Dr. Saori Maki-Yonekura and Dr. Koji Yonekura, from the RIKEN SPring-8 Center, and Prof. Keiichi Namba, of Osaka University, have revealed the mechanism for transitioning between different conformational changes of a constituent protein of the propeller-like flagellum. The researchers hope that their research will help in the development of new drugs against pathogenic bacteria, and eventually lead to an artificial nano-screw.

June 8, 2010. A team of investigators from the University of California in Los Angeles (UCLA), led by researcher Huaidong Jiang and Prof. John Miao, managed to obtain the first-ever 3D images of the entire structure of cells. The impressive achievement was made possible only through the use of an advanced observations instrument called an X-ray diffraction microscope. The new technique, which employs a phenomenon known as lensless imaging, is capable of producing images at resolutions as low as 50 to 60 nanometers.

June 3, 2010. Working for Prof. Birthe B. Kragelund at the University of Copenhagen, crystallographer Dr. Johan Olsen is the lead singer for Magtens Korridorer, one of the country's most popular rock bands. In 2006, they won the Danish equivalent of a Grammy for new band of the year and have performed with headliners like Prince and Patti Smith. Magtens Korridorer is in the middle of a 30-concert tour across Denmark, promoting their fourth album, Milan Allé (YouTube video).

A new instrument for crystal screening

Designed to complement the way you work, ScreenMachine™ is proudly introduced by Rigaku. As synchrotron beamlines have become more prevalent for research in structural biology, many home lab X-ray systems are used mainly for screening crystals in advance of synchrotron data collection. To meet the needs of this type of work flow, Rigaku has developed the ScreenMachine, a self contained unit that is optimized for safe crystal mounting and recovery, optimized for evaluating small crystals, designed for minimal maintenance, and, best of all, collects amazingly high quality data.

Easily and simply screen crystals ...
Test new crystallization hits for diffraction, ensure that proper cryo-conditions are selected, and optimize your beamtime schedule by ensuring you have the best samples.

The ScreenMachine

Train and teach your students **...**
Having a ScreenMachine in a structural biology lab ensures that students and other researchers will have the opportunity to learn proper crystal mounting and handling techniques with the instant feedback that can only come from having an in-house X-ray source.

Produce research grade data ...
Coupled with the microfocus sealed tube X-ray source is a CCD detector that has been designed to provide good diffraction resolution coverage for both screening and data collection. Equipped with an Oxford Cryostream 700, the ScreenMachine allows for the collection of full data sets for: crystals that are hard to obtain, time sensitive projects, crystals that do not travel well, and samples that do not require a synchrotron for appropriate diffraction.

Request more information on the all new ScreenMachine.

Course spotlight: Deep Saturation Mutagenesis
R. Bryan Sutton, Ph.D.

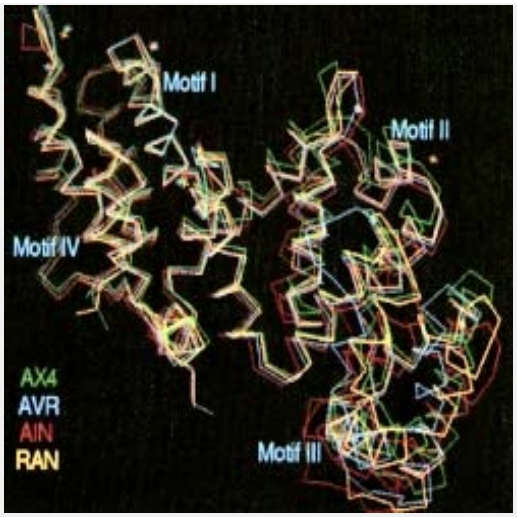
Prof. Bryan Sutton has organized a unique course, for the Biotechnology Program at Texas Tech University and Department of Cell Physiology and Molecular Biophysics at Texas Tech University Health Sciences Center, to introduce students of all types to some of the principles of structural biology. The fundamental concept of this course is to involve students in real-life science, while still learning some of the basic concepts of protein structure and structural biology. The course is entitled, **Deep Saturation Mutagenesis** (DSM); a concept borrowed from the Hubble Telescope Deep Field (HDF) project.

In the HDF, a very small region of the sky was exposed to the Hubble Telescope's cameras for a period of 10 days. The resulting image was a highly detailed view of deep space. DSM utilizes similar ideas, but for protein structure. Over the lifetime of the course, they will introduce all possible point mutations at all locations in a single enzyme, and measure the resulting effects on enzymatic activity and protein structure. Similar saturation type mutagenesis has been attempted for other proteins, but not to this degree.

Their target protein is glutaredoxin from *Synechocystis sp.*, as this small enzyme possesses only 89 amino acids; further, related proteins are already well characterized from a crystallographic perspective. At the beginning of the course, the students will select a single point mutation, at random; there are 1690 possible point mutations required to achieve complete saturation of this protein.

The students will learn mutagenesis, protein purification and protein crystallization. They will then collect their X-ray data on the Rigaku ScreenMachine located at TTUHSC. After solving the X-ray structure, and learning some basic X-ray refinement principles, students will learn how to interpret their results. Of course, all mutations will not be tractable; however, they consider that valid data.

All of the data obtained over the lifetime of the course will be maintained by a Laboratory Information Management System (LIMS), so the students will also become familiar with a more rigorous style of data management. This course is intended to become viral; and, thus they will eventually make this course available to other Universities and Colleges. Prof. Sutton would also like



Professor Sutton's research interest is in the biophysics of C2 domains. C2 domains are membrane interacting domains that are common to a wide-range of proteins. His group's present focus is on the C2 domains of synaptotagmin and the C2 domains of human dysferlin. Synaptotagmin is the major Ca^{2+} sensor in neuron and mutations in dysferlin have been implicated in Limb-Girdle muscular dystrophy in humans.

Rigaku Hardware Training Courses

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.

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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to conduct abbreviated summer sessions, where High School science teachers could learn up-to-date biochemical techniques and contribute to the distributed effort of this experiment.

Their DSM course is not intended to train professional protein X-ray crystallographers. However, with the advent of newer technologies such as the ScreenMachine, it is intended to introduce students from a wide-variety of backgrounds to principles to which they would not normally be exposed.

Useful links for crystallography

Protein Geometry Database: a new version of the PGD is now available. The PGD allows you to explore either protein conformation or protein covalent geometry or the correlations between protein conformation and bond angles and lengths.

Mustang-MR Structural Sieving Server: Useful for producing multiple alternative search models for molecular replacement calculations.

PDBeMotif is an extremely fast and powerful search tool that facilitates exploration of the Protein Data Bank (PDB) by combining protein sequence, chemical structure and 3D data in a single search. It can be used to examine the characteristics of the binding sites of single proteins, or classes of proteins such as kinases, and the conserved structural features of their immediate environments - either within the same species or across different species.

Selected recent crystallographic papers

Crystal structure of HIV-1 Tat complexed with human P-TEFb. Tahirov TH, Babayeva ND, Varzavand K, Cooper JJ, Sedore SC and Price DH. *Nature.* 2010; **465** (7299): 747-51.

A novel and unified two-metal mechanism for DNA cleavage by type II and IA topoisomerases. Schmidt BH, Burgin AB, Deweese JE, Osheroff N and Berger JM. *Nature.* 2010; **465** (7298): 641-4.

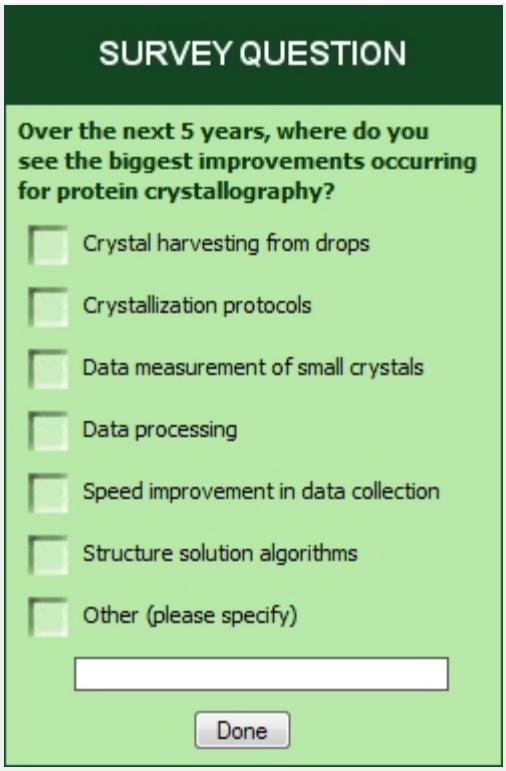
De-icing: recovery of diffraction intensities in the presence of ice rings. Chapman MS and Somasundaram T. *Acta Crystallogr D Biol Crystallogr*. 2010; **66** (Pt 6): 741-4.

A crystallization screen based on alternative polymeric precipitants. Grimm C, Chari A, Reuter K and Fischer U. *Acta Crystallogr D Biol Crystallogr*. 2010; **66** (Pt 6): 685-97.

Acoustic matrix microseeding: improving protein crystal growth with minimal chemical bias. Villaseñor AG, Wong A, Shao A, Garg A, Kuglstatter A and Harris SF. *Acta Crystallogr D Biol Crystallogr*. 2010; **66** (Pt 5): 568-76.

Selenium derivatization of nucleic acids for x-ray crystal-structure and function studies. Sheng J and Huang Z. *Chemistry & Biodiversity*. 2010; **7** (4): 753-785.

Growth of large protein crystals by a large-scale hanging-drop method. Kakinouchi K, Nakamura T, Tamada T, Adachi H, Sugiyama S, Maruyama M, Takahashi Y, Takano K, Murakami S, Inoue T, Kuroki R, Mori Y and



Click to take survey
or cut-and-paste
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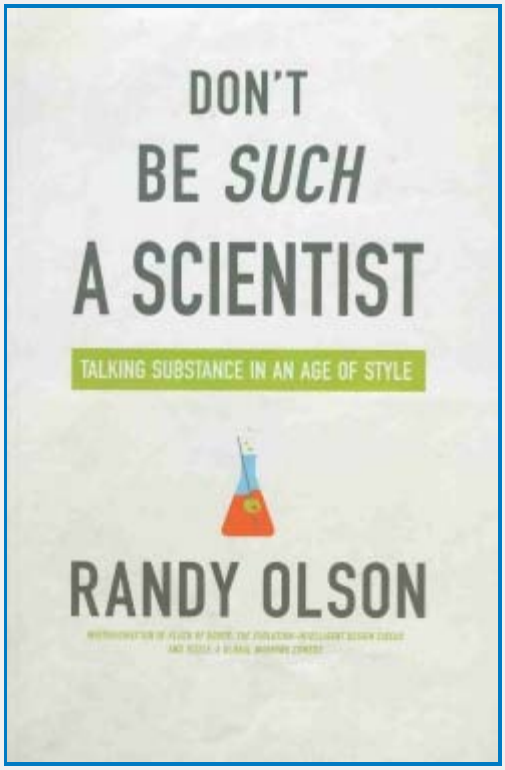
into your browser.



Matsumura H. J. *Appl. Cryst.* 2010; **43**.

Book reviews:

Don't Be Such a Scientist: Talking Substance in an Age of Style
by Randy Olson, Island Press, 2009, ISBN: 978-1-59726-563-8

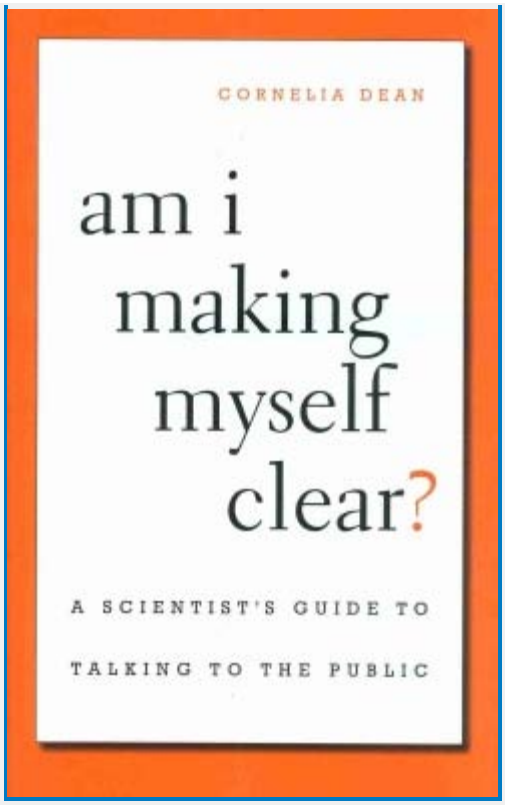
Am I Making Myself Clear? A Scientist's Guide to Talking to the Public
by Cornelia Dean, Harvard University Press, 2009, ISBN: 978-0-674-03635-2

I saw these books reviewed in *Science* and was intrigued. I have read both and have learned, or perhaps relearned, how to communicate better. While it is important to be able communicate amongst ourselves as scientists, what these books enforce is that it is also important, perhaps more important, to be able to communicate to the non-scientists with which we share the planet.

Randy Olson was a marine biologist who became a screen writer and director in his late thirties. The title comes from the result of his first acting lesson in which the teacher screamed at him "Don't be such a scientist!" for being too analytical in class. Some of Olson's film credits include *Flock of Dodos: The Evolution-Intelligent Design Circus* and *Sizzle: a Global Warming Comedy*. Olson's book outlines the differences between the way scientists think and communicate amongst ourselves and with the general public. Olson explains how to express our ideas to the general public with clarity and respect with the following four rules: don't be so cerebral, don't be so literal minded, don't be such a poor storyteller and don't be so unlikable. The underlying method he describes is the arouse-and-fulfill strategy.

Olson uses the movies *An Inconvenient Truth* and *Too Hot Not To Handle*, both produced by Laurie David, as examples of how to communicate to the general public. *Too Hot Not To Handle* uses the conventional format for a science documentary; lots of facts and interviews with prominent scientists. *An Inconvenient Truth* has many fewer facts, some personal stories and a few errors that do not change the general trends that show the earth is warming. Olson is not recommending that errors be allowed but stresses that what is important to the general audience is the trend not the fine details. We all know the result: *An Inconvenient Truth* was a phenomenon; *Too Hot Not To Handle*, well at least I can watch it on Google video.

Cornelia Dean is a writer for the *New York Times'* Science Times. Her book comes from the perspective of journalist who has learned to cover science. There is a lot of overlap in the concepts between this book and *Don't Be Such a Scientist*, but there are enough differences that you should read both books. Dean's book is a detailed how-to guide on the subject of communicating science to non-scientists. Dean covers the topics of knowing your audience, journalism and journalists and discusses science, scientists as sources of information, using public relations effectively, working with modern media including radio and TV, hardcopy and online documents, and the web. Dean also provides good hints for writing op-ed pieces, being a witness and dealing with lawyers. In the last section of the book, she explores the obligation we have to help make policy and work with the general public.



I had one complaint about *Am I Making Myself Clear?* The type is quite small and was hard to for me to read. It turns out that is by design. Dean suggests small type forces the reader to pay attention longer. I found it annoying. On a positive note, I did find one tidbit interesting: she says that English is a Germanic language and we should use words of German origin, rather than Latin or Greek, in constructing sentences for general reading. For someone who spent four years learning Latin and only one learning German, this was a revelation.

Olson's book also convinced me to rent *Flock of Dodos*. This movie has a number of elements for which Olson is criticized by scientists: too much humor, not enough facts and short sound bites. I watched it and walked away very worried. Remember that dodos did not adapt to a rapidly changing environment; watch the movie, think carefully about who the "dodos" in the movie really are, then read the two books above, if you haven't already.

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