Notes of a Protein Crystallographer - Our Unsung Heroes

For the new generations of protein crystallographers, beamlines dedicated to protein crystallography are taken for granted, the more the better. Few of the younger generation of crystallographers are familiar with the history of the technological developments that were necessary to have routine access to experimental stations permitting rapid and reliable data collection of excellent diffraction data from protein crystals. Moreover, the names and efforts of the people involved in this pioneering work are probably unknown to them. A recent essay (Cele Abad-Zapatero, *Structure* (2004), **12**: 523-527) gives the background and historical perspective to those scientific achievements with a personal point of reference. A brief excerpt from the essay (***Reprinted with permission from Elsevier)*** is printed below. Students and newcomers to the field are encouraged to read the entire article. The article is dedicated to the people who designed, built and currently work at synchrotron beamlines

In her delightful book *Longitude*(1), Dava Sobel details the troubles and tribulations that John Harrison (1693-1776) had to endure to claim the award money for having solved the most important technological problem of the 18th century- measuring longitude. And yet, the humble name and superb technological achievement of John Harrison is generally not recognized.

In history books, narration glorifies kings, presidents, generals, oligarchs, lawmakers and others but never the humble instrument makers, craftsmen who made possible dramatic societal advances with their quiet, unpretentious work. I fear that in the history of science we have the tendency to do the same. Are we going to tell the story of crystallography and structural biology the same way? Are we only going to recognize the principal investigators that solved the structure(s) of the largest supra-molecular assemblies? What about the people who built the instruments that made all those discoveries possible? What about the people who built the unique synchrotron beamlines that we take for granted? There are legions of such unsung heroes and, in this brief essay, I would like to focus on one who epitomizes these talented scientists, and extend my appreciation and modest homage to all.

After DESY in the early 70s (*Deutsches Elektronen Synchrotron*, 7.5 GeV), the storage ring DORIS (operating at 3-4 GeV) was about 1000-times brighter at 1.54 Å than a rotating anode source (2). Conceptually and experimentally, the achievement of the pioneers in the field was to bring such an intense but transient radiation from the emitted cone tangential to the trajectory of the moving electrons to an experimental sample a fraction of a millimeter in size, several tens of meters away. The radiation had to be extracted from the ring, monochromatized, focused, directed to the experimental 'bunker', shone on the minute sample and the resulting diffraction pattern had to be recorded, read and stored. Due to the high level of background radiation, all the manipulations had to be done by remote control.

What was the driving force behind these titanic efforts? The driving force was to understand structurally how muscle tissue worked. The goal was to be able to perform small-angle x-ray diffraction experiments with insect flight muscle and specially to be able to follow the changes in the diffraction pattern during the cyclic oscillations of muscle contraction. These were the interests of two pioneers of muscle research Hugh Huxley and Ken Holmes, who moved to Heidelberg in 1968.

Gerold Rosenbaum was born in Breslau, Germany, on August 22, 1942. He initiated his undergraduate studies in physics at the Freie Universität in Berlin but transferred to the Ludwig Maximilian Universität in Munich to continue his undergraduate degree, where he obtained first the *Vordiplom* (equivalent to B.S.,1965) and later the *Diplom* (equivalent to M.S.1968). Gerd's primary interest was never to build instruments for their own sake, but in the challenge of solving complex problems (first in physics, then in biology). After finishing his M.S., Gerd decided to leave physics and pursue a Ph.D. in biophysics, which he considered to be full of opportunities. He and Ken Holmes initiated a scientific collaboration in 1969 for the first use of synchrotron radiation for diffraction at low angles with biological samples.

Gerd Rosenbaum, John Barrington Leigh, and engineer Rolf Coors (right to left) celebrating the Richfest (German custom at the completion of the basic structure of a new building) of the EMBL bunker at DESY (ca. 1975).

Gerd, supported by Jean Witz designed, fabricated and put into action the necessary instrumentation to carry out the pioneering experiments on synchrotron radiation as a source for further x-ray diffraction. These test studies were performed at the F41 bunker at DESY. As part of these tests, the first ever x-ray diffraction pattern with synchrotron radiation was recorded. The preliminary results published in 1971 in *Nature* showed that, indeed, the measured radiation intensity emanating from DESY was consistent with previous calculations and amounted to about 300 times the one produced by the most powerful fine-focus x-ray tubes of the time(3).

The rest is history. The review published by John Barrington Leigh and Gerd Rosenbaum(2) in 1976 presented the progress that had been achieved by then in the different sources available at the time, which included the German storage ring DORIS, SPEAR (at Stanford) and DCI in the U.K. It is important to realize that even at that time the most significant advances had been made in low angle x-ray diffraction. There was a small section dedicated to 'other applications' where the initial results on Small-Angle Scattering obtained by the biological group at the SPEAR ring at Stanford were discussed. Within the same section, it was reported that a group at the chemistry department at Stanford had succeeded in taking single crystal precession photographs of different biological macromolecules (6 times faster than a rotating anode!), using a camera at an unfocused beam in one of the SPEAR beam lines(4). More modest improvements were observed at about the same time by a German group using the radiation from DESY(5). Furthermore, the same group at Stanford had also investigated the anomalous scattering in crystals of rubredoxin. Precession photographs taken with wavelengths just below (1.78 Å) and just above (1.74 Å) the iron K-edge (1.7435 Å or 7.1111 KeV), had shown changes in the average intensities of the Friedel pairs ranging from 4-2% (6). The authors of the review finished the section on protein crystallography with the open-ended sentence 'How far one can apply this method in solving the phase problem in crystallography using synchrotron radiation is not yet clear'. The answer began to emerge a few years later (references 6-8), and now synchrotron experiments tuned to optimize anomalous scattering dominate in *de novo* protein structure determination.

Aren't these beamlines comparable to unique sculptural, architectural or artistic masterpieces? Aren't our current synchrotron beamlines technological icons of our time comparable to the timekeepers that earned Mr. Harrison the prize of the Board of Longitude? And in a different vein, aren't these beamlines the reflection of a creative mind comparable to the best artistic minds of all times? Indeed, each component part has a specific design and purpose within the operation of the whole, but isn't the conceptual design, fabrication and execution comparable to the conception, development and harmonization of the score for an entire symphony? Currently, our work depends on the skills, creativity and dedication of anonymous instrument makers. The number of structures published, refined or deposited at the Protein Data Bank does not measure directly their ingenuity and achievements. Rather, their accomplishments are part of our latest storage rings, the optical components of our newest beamlines, and the elements of our most sophisticated experimental hutches. Our most recent and more spectacular structures may be part of the news and bring honor and fame to many members of the crystallographic community and to crystallography as a field of research. However, we should never forget our unsung heroes who built the storage rings and experimental stations and who made those amazing achievements possible.

Notes

(1) Longitude. "The True Story of a Lone Genius who solved the Greatest Scientific Problem of His Time. Dava Sobel" Walker and Company: New York, 1995.

(2) Barrington Leigh, J. & Rosenbaum, G. (1976). "Synchrotron X-Ray Sources: A New Tool in Biological Structural and Kinetic Analysis" *Ann. Rev. of Biophys. Bioeng*. 5: 239-270.

(3) Rosenbaum, G., Holmes, K. C. and Witz, J. (1971) "Synchrotron Radiation as a Source for X-ray Diffraction" *Nature* 230: 434-437.

(4) Phillips, J. C., Wlodawer, A., Yevitz, M.M. and Hodgson, K.O. (1976) "Applications of synchrotron radiation to protein crystallography: preliminary results" *PNAS* 73: 128-32.

(5) Harmsen, A., Leberman, R. and Schulz, G. E. (1976). "Comparison of protein crystal diffraction patterns and absolute intensities from synchrotron and conventional x-ray sources" *J. Mol.Biol*. 104:311-314.

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(8) Kahn, R., Fourme, R., Bosshard, R., Chiadmi, M., Risler, J.L., Dideberg, O. and Wery J. P. (1985). "Crystal Structure Study of Opsanus tau Parvalbumin by Multiwavelength Anomalous Diffraction" *FEBS Lett*. 179: 133-137.

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Cele Abad-Zapatero

Editor's Note: This article is especially timely in light of Nguyen-Huu Xuong being awarded the first-ever Charles Supper Award at ACA Chicago.