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Notes of a Protein Crystallographer: Our Unsung Heroes

Essay

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Dedicated to the people who designed, built, and currently work at synchrotron beamlines.

In her delightful book *Longitude*, Dava Sobel (1995) details the troubles and tribulations that John Harrison (1693–1776) endured to claim the award money for solving the most important technological problem of the 18th century—measuring longitude. John Harrison was born the son of a carpenter in Yorkshire, England. He learned the skills of carpentry and mechanics from his father but soon took this knowledge and advanced it to the limits of what was conceivable at the time. In 1726, he fabricated a clock with a pendulum that remained at the same length at any temperature by combining the different coefficients of expansion of the component metals. His craftsmanship and achievements as a horologist and instrument maker progressed through a series of superb timekeepers to the chronometer that won the prize of the Longitude Board. The Board of Longitude was created by the British government in 1714 and offered a prize of £20,000 (silver sterling pounds, a king's ransom in those times) for anyone who made an instrument or developed a method to determine the longitude at sea to an accuracy of half a degree of arc (30 min). Many other European nations used similar incentives to stimulate invention to make dangerous transoceanic navigation safe and reliable. It was clear that such a technology or device would give the winning nation control of the sea, plus the resulting increase in power and economic profitability. In my opinion, it was Harrison's fourth chronometer, accurate to within five seconds (one minute of longitude!) over the duration of two long voyages to the West Indies, which contributed the most to the domination of the British Navy during the ensuing years. As an anecdote, *H.M.S. Beagle*, the surveying ship that carried Charles R. Darwin around the world in 1831, carried 22 Harrison chronometers on board. And yet, the humble name and superb technological achievement of John Harrison is generally not recognized.

In history books kings, presidents, generals, oligarchs, lawmakers, and others are glorified but never the humble instrument makers, craftspeople who make dramatic societal advance possible with their quiet, unpretentious work. I fear that in the history of science we have the tendency to do the same. Who remembers the person(s) who built the first electron microscope that opened the window to the supramolecular structure of the cell? Who recalls the name of the person(s) who built the first NMR spectrometer? We honor the theoreticians who propose ideas and ignore the mechanic who created the instruments that enabled the critical measurements. Will we tell the story of crystallography and structural biology the same way, recognizing only the principal investigators that solved the structure(s) of the largest supramolecular assemblies? In our future textbooks, will we only mention the individuals that recognized the similarity of folds among different enzyme families or those that trapped a critical reaction intermediate in a crystal structure and ignore the people that built the instruments that are the workhorses of structural studies? For example, the individuals who built the unique synchrotron beamlines that we take for granted were superb scientists in their own right, and it is their creation that permit us to collect excellent data from minute and weak-diffracting crystals. 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.

Some background is needed for one to fully appreciate the accomplishment of the pioneers in the use of synchrotron radiation for structural studies. Electron synchrotrons were initially built and used by high-energy physicists to conduct experiments probing into subatomic elementary particles. Synchrotron radiation was an unwelcome byproduct of this procedure and was seen as a nuisance by the physicists. Nonetheless, certain scientists were beginning to use the available synchrotron radiation in a parasitic fashion; however, the first radiation available was extreme vacuum UV (VUV) and was used mainly for materials research.

The 7.5 GeV *Deutsches Elektronen Synchrotron* (DESY) that began to operate in the mid-60s was the first machine with high enough energy to emit a considerable flux of radiation near 1 Å. The synchrotron radiation group, F41, at DESY now used the more intense VUV radiation from DESY for solid-state physics. By 1970, when our subject joined the field, nobody had used the X-ray portion of the spectrum for experiments. Only Rupprecht Haensel, the leader of the F41 group, had measured the polarization of synchrotron light emitted between 10 and 30 KeV (0.3–1 Å), and other characteristics of the emitted radiation to verify the Schwinger equation that established the spectral and spatial distribution of the radiation (photon flux) emitted by a monoenergetic electron in a circular motion, as a function of time, unit of angle, and unit of wavelength (Schwinger, 1949).

In the early 70s, DESY in Hamburg, Germany was still the highest energy synchrotron of the time. As mentioned, it operated at up to 7.5 GeV at an average current of 10 mA and had a spectral luminosity 150 times greater than a rotating anode of the time (an Elliot, GX-6) (Rosenbaum et al., 1971). The spectral luminosity (or brilliance) is the number of photons per second radiated per unit

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area, solid angle and wavelength interval, measured in the narrow cone of emitted radiation ($\sim 3 \times 10^{11}$ photons/(s mm^2 mrad^2 0.1% bandwidth) for DESY at 7 GeV in 1970). At DESY, bursts of 50 pulses of 10 ms each were injected per second ($\sim 6 \times 10^{10}$ electrons). The electrons were accelerated to the final 7.5 GeV in 10 ms and most of the radiation was emitted during the last 3 ms of each pulse. Consequently, little radiation was produced at the lower energies, and thus the average radiation intensity at the nominal wavelength of copper K radiation (1.5 Å) was only 20% of the peak value.

The next development, the storage ring, DORIS (operating at 3–4 GeV) was about 1000 times brighter at 1.54 Å than a rotating anode source (a brilliance of 5×10^{12} units as above) (Barrington Leigh and Rosenbaum, 1976). It is interesting to compare these initial values with the numbers available from third generation storage rings such as ESRF, APS, or Spring8 (a brilliance of $\sim 1 \times 10^{20}$ operating at 6–8 GeV, respectively, units as above). Conceptually and experimentally, the achievement with this feature 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 recorded, read, and stored. In addition, the high level of background radiation present meant that all the manipulations had to be done by remote control from outside the bunker that housed the experiment.

What was the impetus behind these titanic efforts? Did these visionaries simply want to show it was possible to build those exquisite pieces of hardware for the sole purpose of exhibiting their mechanical wizardry? Certainly, not! The driving force was to understand how muscle tissue worked from a structural perspective. Their goal was to perform small-angle X-ray diffraction experiments with insect flight muscle and to specially follow the changes in the diffraction pattern during the cyclic oscillations of muscle contraction/relaxation. Researchers intended to use nucleotide analogs to stop the actin-myosin machine at different stages of its cycle and correlate the biochemical, mechanical, and structural changes observed in the muscle at these time points.

For some time, it had appeared that the high-powered rotating anode X-ray generators would produce the high intensities needed to tackle diffraction studies on muscle tissue. These were the GX13 anodes, the so-called "big wheels" manufactured by Elliot Automation, Ltd in England, that were used in the early 70s in several European laboratories. Its design and production was initiated in 1969. At this juncture, our hero entered the world of structural biology by joining the laboratory of Ken Holmes, a well-known pioneer of muscle structure and function.

Dr. Gerold Rosenbaum, Gerd for the remaining of the narration, was born in Breslau, Germany, on August 22, 1942. He began his undergraduate studies in physics at the Freie Universität in Berlin in the Spring of 1962. After three semesters, Gerd transferred to the Ludwig Maximilian Universität in Munich to continue his degree, from where he obtained first the *Vordiplom* (equivalent to B.S., 1965) and later the *Diplom* (equivalent to M.S., 1968). According to his own recollection, his interest in synchrotron radiation originated in early 1966 when he saw a notice offering a summer studentship at DESY. He applied and was accepted. Fortunately for the field and, dare I say, for Gerd, he was assigned to the F41 group of DESY, and within this group to researchers from the University of Munich. He returned to F41 in 1967 to do his diploma thesis work, when he built a new polarizer for VUV radiation to measure the degree of polarization of the synchrotron radiation exiting a monochromator.

Another thread of this story suggests that, even in the earliest years of protein crystallography, Kenneth Holmes and Hugh Huxley were dreaming about using intense radiation sources for biological X-ray diffraction. Hugh Huxley had been measuring small angle diffraction in muscle since the early 50s (ca. 1952). He remained in the UK and has continued to pursue this work to this day. In contrast, Ken Holmes moved to the Max Plank Institut für Medizinische Forschung (Department of Biophysics) in Heidelberg in 1968 and made structural studies on muscle a rather significant part of his research program. Both Holmes and Huxley commissioned the initial GX-13 "big wheel" rotation anode that was intended to generate the X-ray intensity necessary to handle the muscle work.

After finishing his M.S., Gerd decided to leave physics and pursue a Ph.D. in biophysics, which he considered to be at the frontier of new science opportunities. Joining a group that investigated neuronal function was initially appealing but also somewhat nebulous at the time. In the end, it was the study of muscle tissue and the possibility of time-resolved studies of muscle fibers with intense X-rays radiation that polarized his decision. Gerd's primary interest was never to build instruments for its own sake, but in the challenge of solving complex problems (first in physics, then in biology). This forced him to explore new opportunities and to design and build new instruments and improve on existing ones. Through this combination of interests, Gerd Rosenbaum and Ken Holmes initiated a scientific collaboration in 1969 that resulted in the first use of synchrotron radiation for diffraction at low angle with biological samples.

First, the question of how intense an X-ray source would be needed to study muscle fibers had to be answered. The preliminary designs of the large diameter rotation anodes showed promise but had yet to be optimized and fully tested. As part of his thesis work, Gerd solved the equations of the heat flow and evaluated the stress in the anode caused by the large centrifugal forces. In the process, he realized that the GX13 was only within a factor of five of the theoretical limit of any "big wheel" design. This limitation was set by the ultimate strength of material and thus it became clear that large rotation anodes would never provide the factor of a hundred increase in brilliance required for the time-resolved diffraction studies in muscle (Rosenbaum, 1979).

In parallel, Gerd, supported by Jean Witz who had

Figure 1.

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

worked on X-ray optics during his stay at the MRC in Cambridge, designed, fabricated, and put into action the instrumentation necessary to begin the groundbreaking experiments on using synchrotron radiation as a source for X-ray diffraction. These studies were performed at the F41 bunker at DESY and successfully generated the first X-ray diffraction pattern with synchrotron radiation (Figure 3 in Rosenbaum et al., 1971). The initial optics consisted only of a focusing X-ray quartz monochromator housed inside a vacuum chamber with a simple slit assembly and a beryllium window. Preliminary results published in 1971 in *Nature* (Rosenbaum et al., 1971) showed that, as predicted, the measured radiation intensity emanating from DESY was consistent with previous calculations and amounted to about 300 times the intensity produced by the most powerful fine-focus X-ray tubes of the time (Rosenbaum et al., 1971).

Based on these findings, the director of DESY, Willibald Jentschke, encouraged Holmes and Rosenbaum to build a bunker to perform X-ray diffraction experiments on biological samples. The long-term plan was to set up a full X-ray laboratory on the future storage ring DORIS, which would be at least 100 times brighter. However, DORIS was still being planned and was not expected to emerge until possibly a few years down the road. Time was of the essence! During the major shutdown in 1971, a basic X-ray laboratory was built onto DESY that would be known as "Bunker 2." For this project, Gerd was assisted by John Barrington Leigh. Gerd designed the bunker layout and designed the first beamline and experimental station at DESY. This beamline had more elaborate optics, including two adjustable bent mirrors and a quartz monochromator, all of which were operated by remote control.

Subsequently, the development of Bunker 2 became linked with the history of the European Molecular Biology Laboratory (EMBL). Sir John Kendrew, then head of the EMBO (European Molecular Biology Organization) committee for the EMBL "project," realized that using synchrotron radiation sources for structural biology studies was an ideal focus for a translational laboratory. Thus, EMBL and DESY entered a formal agreement in 1975 to set up what was to be known as the EMBL outstation in Hamburg (Holmes and Rosenbaum, 1998). An ambitious and pioneering project championed by its director Sir John Kendrew and Ken Holmes that was followed by many similar projects at other synchrotrons all over the world.

The rest is history. A review article written by John Barrington Leigh and Gerd Rosenbaum (Barrington Leigh and Rosenbaum, 1976) presented the achievements that had been made to date in developing different X-ray sources, including the German storage ring DORIS, SPEAR (at Stanford), and DCI in the U.K. It is important to realize that at that time the most significant advances had been made in low angle X-ray diffraction. In this review, a small section was dedicated to discussing "other applications" and focused on the initial results on Small-Angle Scattering obtained by the biological group at the SPEAR ring at Stanford. Alongside, 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 beamlines (Phillips et al., 1976). At around the same time, a German group made more modest improvements in the technology using the radiation from DESY (Harmsen et al., 1976). Furthermore, the Stanford-based researchers 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% (Barrington Leigh and Rosenbaum, 1976). 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 (e.g., see Phillips et al., 1977; Hendrickson, 1985; Kahn et al., 1985), and now synchrotron experiments tuned to optimize anomalous scattering are predominant in the de novo protein structure determination.

Following these auspicious beginnings, Gerd went on

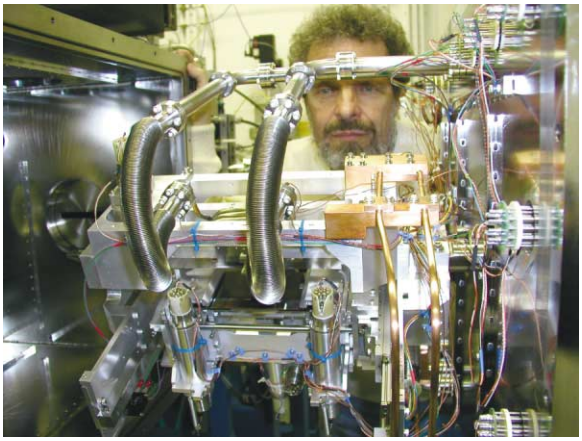

Figure 2.

Gerd Rosenbaum looking into the heart of the monochromator of 22-BM (SER-CAT) at the APS in 2003.

to design the experimental station at the DORIS storage ring and can now claim 34 years of experience in designing and construction of synchrotron beamlines and related instrumentation for biological applications. His career took him to the NSLS at Brookhaven to build a beamline dedicated to biological problems. Among his masterpieces, I can highlight the design and construction of the BM and ID beamlines of sector 19 (Structural Biology Center: SBC-CAT, 1993-1997) at the Advanced Photon Source (APS) and the next generation of beamlines as implemented at SER-CAT (Sector 22). The undulator line at 19-ID facilitated solution of high-resolution structure of the ribosomes and many other breakthroughs in structural biology (Ban et al., 2000; Schluenzen et al., 2000). The accompanying photos show Gerd Rosenbaum and colleagues celebrating the completion of the basic structure of the EMBL bunker (Figure 1, ca. 1975) and, more recently, in action looking into the heart of the monochromator at the BM line of sector 22 (SER-CAT) (Figure 2).

Not being a mechanically inclined person myself, I stand in awe at the achievements of our scientific colleague Gerd Rosenbaum and his coworkers. I ask myself, are these beamlines not comparable to unique sculptural, architectural, or artistic masterpieces? Are our current synchrotron beamlines not the 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 that is 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?

We may wonder what unique waves or electrical impulses combined in Gerd's brain to produce the ideas that allowed for such an amazing richness, intricacy, beauty, and efficiency of design. I certainly cannot answer this question and fear that future generations of structural biologist might not have an answer either, even using the most sophisticated X-ray sources of the future. However, it is obvious that Gerd and so many other designers of scientific instruments are unique individuals who possess outstanding creative imaginations and—at the same time—superior analytical skills that allow them to see the critical features of an instrumental design and discern minor errors from basic flaws. In his own words, Gerd has indicated to me that "it is the ability of being able to discern the overall forest and also to understand and visualize the minute details of each individual tree." Only by the rare combination of these two abilities can these scientists be so creative and effective at what they do.

Currently, our work depends on the skills, creativity, and dedication of anonymous instrument makers, and 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.

Acknowledgments

I have felt honored to be Gerd's friend for sometime now. I wish to thank him for the time he devoted to flesh out this article with the factual information about his life accomplishments and for the accompanying photographs. W.A. Hendrickson's insightful comments and suggestions on the manuscript are greatly appreciated.

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