



ACA Living History Project Update



Virginia Pett
pett@wooster.edu

After consultation with Council and Kristin Stevens, webmaster Vanessa Reitz has created an attractive ACA History home page that fits the online style of the ACA pages now online with MemberClicks, the new ACA website management system. Vanessa also made a user-friendly history navigation menu. However, while she was reconstructing broken links, Vanessa discovered that a large proportion of ACA History pages are missing from the new site. Fortunately, Kristin had retained our relationship with the old website management system, and all the History pages are backed up. It will take some time to resolve these and other difficulties.

In this issue of ACA RefleXions Ron Stenkamp presents his Living History. After his graduate research with Lyle Jensen at the University of Washington in the 1970s, Ron spent the bulk of his career at the UofW determining both small-molecule and protein structures. He and his colleagues investigated oxygen-binding proteins (such as hemerythrin and rubredoxin) and drug-detoxification enzymes (such as glutathione S-transferase and cytochrome P450) by mutation and ligand-binding studies. In 2000 he and colleagues published the structure of rhodopsin, the light-absorbing molecule in the retina of the eye. This was the first high-resolution structure of a G-protein coupled receptor.

Ronald E. Stenkamp Living History



I attended my first scientific meeting as a junior in high school in 1964. NASA came to Bend, Oregon, to see how astronauts could walk on lava fields in their space suits. Two of us locals listened to several talks concerning the source of the moon's craters, i.e., volcanos or meteorites. I didn't understand much of it, but I thought it was boring, and if that's what scientists did for a living, maybe I'd do something else.

I was already thinking science wasn't very inviting. In response to Sputnik in 1957, science educators had generated special programs and tools to get kids interested in science. In 6th grade, I learned all about hypothesis generation and testing, and I was not impressed. I still have trouble with hypothesis-based science. Making observations and asking questions is what I enjoy doing. Posing hypotheses isn't.

I did well in school, and without planning it, I'd taken and enjoyed all sorts of classes. I greatly enjoyed mechanical drawing, and I'd out-run Mr. Lively's curriculum in Advanced Mechanical Drawing. He generated new projects for me, and engineering looked like a possible field for me, but I wanted a liberal arts education, so when it came time to pick a college, I chose the University of Oregon (UofO) instead of Oregon State University. Besides, the UofO's school colors (green and gold) matched those of my grade school.

The UofO has an Honors College (HC) which provides smaller courses focused on a core curriculum in the humanities and liberal arts. I applied to the program and was accepted. There was also a special center that provided a place to work with the other HC students, and I made many friends there, including my future wife, Larilyn.

Before joining the HC, I had chosen chemistry as my major, based largely on enjoying my high school chemistry class with Mrs. Cruickshank. When I started talking with other HC students about what we wanted to do with our lives, two of

them expressed interest in molecular biology. I'd never heard of that. This was 1966, and Kendrew, Perutz, Watson, Crick, and Wilkins had gotten their Nobel Prizes just four years earlier (1962). (Along with Steinbeck (Literature) and Pauling (Peace)). Molecular Biology was a brand-new field, but the UofO already had an Institute of Molecular Biology organized for interdisciplinary studies.

In the fall of 1966, my college days started with the honors section of freshman chemistry. In the second quarter laboratory class, I had an accident that foreshadowed much of my chemical career. One of our first tasks was to produce a dichromate/sulfuric acid cleaning solution. I made the solution OK, but a few days later, when I lifted the bottle up from my cabinet, I didn't lift it quite enough, and it clipped the stone counter top, about ¼ inch above the bottom of bottle. The bottle broke all the way around, and a liter of cleaning solution hit the benchtop, spilled down the front of the cabinet, and splashed a little on me. The TA quickly got the spill under control, but I spent the rest of the lab period cleaning up the mess.

I had a fair amount of growing-up to do in college. I came from Bend thinking I was a pretty smart guy. It turns out a lot of bright people go off to college, and many of them were a lot brighter and harder-working than I was. It was harder for me to get good grades in college, and this was a big blow to my ego. I grumbled a lot about the lousy teachers in college. Clearly, if they knew how to teach, I would have been doing better academically. Prof. Donald Swinehart was our instructor for freshman chemistry lab, and I talked a lot with him about teaching and learning. He wasn't very sympathetic. He told me about his experience in college where he decided to master a class in spite of the professor. I thought that was outrageous, but several years later, I ended up thanking him for that. In addition to listening to my complaints, Swinehart was willing to let me take reading courses with him where I could learn about special topics I found interesting.

I spent some of my undergraduate years in the library, looking at history of science books and others indicating what science was all about. I was greatly impressed by Pauling's "The Architecture of Molecules", and Roger Hayward's accompanying artwork of molecules looked like mechanical drawings of molecules. Maybe looking at molecular structures could combine my science and mechanical drawing interests?

One feature of the Honors College core classes was that after taking them for three quarters, you had to pass a comprehensive exam to remain in good standing. However, another option was to "challenge" a class by just taking the comprehensive exam. If you succeeded, you could get credit for the class without actually taking it. The exams were given in the spring and in the fall.

The history comprehensive exam was feared by most students who took the course, but I'd always enjoyed reading and thinking about history, so I figured I could challenge the history exam. So, for my summer "fun" project, I did all the readings for the HC history class. That meant I needed to do daily history reading assignments through the summer to cover the material discussed in class during the previous nine months. I borrowed the books and notes from a friend who'd passed the course that year, and I managed to read and study nearly all the assignments that summer. I did well with the fall comprehensive exam, and it promoted my confidence in doing independent study.

My sophomore year was very challenging and caused a lot of self-assessment. My basic problem that year was organic chemistry. The first two quarters were about reactions (which I've never understood), but the third quarter had more physical organic content, so my understanding and grades went up for that. But lab was awful. One of my two worst grades was for third quarter organic lab. My lab skills (and luck) were not compatible with qualitative organic. I had one unknown that I could not make derivatives of. Even the prof couldn't do it, and the unknown was a natural product he'd pulled off his shelf. But in a concession to justice, he gave me half credit. Half credit! I had trouble with that class in addition to the intellectual content. And I'm not at all bitter, 50 years later. Further complicating my sophomore year were physics and a second year of math. Overall, by the end of the year, I figured anything had to make the next year better than this one.

After a summer working on a surveying crew (which paid enough to cover half of my school expenses), I returned for my junior year. Physical chemistry was a lot better for me. I could understand much of it, but it wasn't terribly exciting. By the end of the year, I was thinking I still needed to find some chemical field that was exciting and inspirational.

Then a wonderful thing happened. Brian Matthews joined the faculty to do something called protein crystallography. I had decided to stay in Eugene

for summer school, and I arranged with Brian to take a reading class where I could learn about crystallography.

When school started in the fall, I asked if I could do my senior Honors College thesis with him. He agreed and set me to solving a small molecule structure. Bill Simpson's lab was interested in organic compounds with metal-like spectroscopic properties, and they wanted to know what the crystal structure was of one of their compounds. Brian's X-ray lab was just setting up and had an Enraf-Nonius Weissenberg camera, so he had me collect diffraction data for 3-bis(dimethylamino)-trimethinium perchlorate using that camera. There was no film scanner available, so I eye-estimated the intensities on those films. I generated an intensity scale by exposing a single reflection for various lengths of time on a film. This gave a spot with the same shape and extent as those on my data frames. And then I spent several months over a light box determining the relative intensities of the reflections.

At that point, the school year was finishing, and I had to write my undergraduate thesis. Peter Colman joined the lab as a post-doc, and he ended up solving the structure. Soon after, a diffractometer arrived, and a higher quality data set was obtained for refinement. My efforts on this structure got me co-authorship with Peter and Brian and started my publication list.

Of course, while this project was important for my future career developments, other important things went on that year. First, Larilyn and I had to make wedding plans. Second, we needed to figure out what to do with our lives. The main thing we were reasonably skilled at was being students. And it seemed the natural consequence of that was to keep going and get our Ph.D. degrees. To prepare for that, we took three quarters of biochemistry and finally started seeing what molecular biology was about. In addition, we took a fantastic statistical mechanics class (mainly filled with graduate students), and several computer programming classes (assembly language and FORTRAN).

But we still needed to decide on a graduate school. We considered three schools, and Verner Schomaker, the chair of Chemistry at the University of Washington, let us know that protein crystallography was being done in Lyle Jensen's lab, and interdisciplinary research would be OK with Verner. That sounded terrific, and in the spring of 1970, Lyle came to Eugene to give a seminar about his group's refinement of rubredoxin. This was exciting, since it was the first protein to be successfully refined crystallographically. What was more exciting was that after talking with him, he said I should come to Seattle, "and we'll have some fun." I was convinced.

So, in late August of 1970, we got married, went to San Francisco for our honeymoon, drove back north to visit with family in Oregon, and moved to Seattle.

Crystallography was a big deal at the UW. The senior crystallographer was Ed Lingafelter. He'd joined the Chemistry faculty in the late-1930s, straight from being a graduate student at UC Berkeley. He was a physical chemist, and in 1938 or 39, he was joined by his "best" graduate student, Lyle Jensen (one year younger than Ed). Lyle was from Stanwood, a small town about 40 miles north of Seattle. He'd attended Walla Walla College and come to the UW to get his Ph.D. For his thesis, he determined unit cell parameters for a series of long-chain organic compounds. Once he obtained his Ph.D., Lyle joined the Manhattan Project in Chicago and worked with plutonium compounds. He left that position to teach at a church-sponsored college before going to The Ohio State University to work on the physical chemistry of liquid hydrogen. Soon after that, the UW needed additional instructors to deal with the large number of returning GIs, so he came back west and took up an instructorship in Chemistry. In 1948, just after the UW Medical School opened, he talked with the chair of Anatomy who offered him a junior faculty position the next day. Stan Bennett had a very broad view of "anatomy" and thought crystallography and electron microscopy would eventually be important for studying biological structures. Imagine that!

The third senior crystallographer was Verner Schomaker. Verner was a Cal Tech product where he did a lot of electron diffraction of compounds of interest to Pauling. There are many footnotes in Pauling's "The Nature of the Chemical Bond" referring to "V. Schomaker, unpublished results". The story I heard was that Pauling would get interested in some M-X bond and get Verner to use electron diffraction to obtain the M-X interatomic distance. In the mid-1960s, after years at Cal Tech and Union Carbide, Verner became chair of the Chemistry Department at the UW.

By 1970, other crystallography faculty at the UW included G.H. Stout in Chemistry, Jon Herriott in Biochemistry, and Art Camerman in Neurology. Subrata Ghose joined Geology sometime in the 70s. There were many graduate students, post-docs and research associates associated with these faculty, so there were people solving enough structures to support a weekly X-ray seminar. It was a wonderful environment for learning the fundamentals and

cutting-edge techniques in crystallography.

Our first year at the UW was filled with normal graduate school issues. We had coursework to manage, teaching assistantships to master, research groups to join, etc. Larilyn joined Ernest Davidson's quantum mechanical group, and after a bit of negotiation with a new chair of Chemistry, I could work in Jensen's group. (Lingafelter was my official



Ed Lingafelter and Verner Schomaker.

advisor, but Lyle was in charge and made any decisions a supervisor had to make. This qualified me as Ling's "easiest" grad student.)

Lyle wanted me to learn small molecule techniques that I could then apply to macromolecular structures. Accordingly, my first projects in the lab were crystal structure determinations of dipeptides. The first molecule I tried to solve was a chloromethyl ketone of acetyl-leucyl-phenylalanine. I determined the unit cell and space group using Weissenberg and precession photos, and then we (mainly Larry Sieker) used a Picker FACS-1 (driven by a PDP-8, with paper tape output) to collect a diffraction data set. The structure suffers from super-symmetry, but a bigger problem was that the crystal was greatly radiation damaged before we put it on the diffractometer. I didn't solve that structure, but it taught me a lot about crystallographic computing.

Following that, I grew crystals of two more dipeptides and solved their structures. I also worked on two small computational projects having to do with less-than reflections and resolution. Crystallography's appeal for me was (and is) tied up with the idea that solving or refining a structural model is just a big puzzle, and the big question is to see if I understand my craft enough to solve the puzzle. My research efforts have focused on using crystallographic techniques, and not so much on the molecules being studied or the biochemical questions being asked.

While the research work was of great importance,

equally so were the social interactions in Lyle's research group and in the crystallographic community at the UW. Lyle's been recognized by many as a gracious, dignified leader. And those characteristics pertained in day-to-day life in his group. He delegated responsibilities well and made us feel like our projects were ours. He would occasionally come by our offices to see how we were progressing, but almost every day, I went to his office to ask questions and just talk about stuff. I still consider him my "boss", but he was really a friend.

And the supportive environment carried over with the other members of his group. Larry Sieker was the main crystallizer/data collector and for me, the lowly graduate student, he was the number two person in the group. He knew how to get things done and he was as dedicated to doing good science as Lyle was. That was true of everyone in the group, a dedication to doing good work. While several people came through Lyle's group as post-docs and visitors, the major post-docs who educated me about crystallography and computing were Keith Watenpaugh, Ellie Adman, and Jonathan Hanson. I can't express how grateful I am for the things I learned from them and their continued friendship.

I also benefitted from interactions with the other crystallographers on campus through the weekly X-ray seminars. For the presentations of new structures, and there were many of them, there was usually a figure showing the bond lengths and angles for the molecules. What was especially fun was to watch the senior faculty (usually Ling, Verner and Lyle) get interested in comparing the bond lengths and angles to see what the bonding was like. If the presenter was particularly successful, he or she could get the old guys talking and arguing and end up using a substantial portion of the seminar time. I don't remember ever manipulating my talk to succeed at this, but it happened accidentally enough to make it a career goal.

Ling and Verner also were great influences on how I approach problems, but in addition, they were important for my academic progress. The Chemistry Department expected its graduate students to pass cumulative exams to ensure they had a broad understanding of whichever branch of chemistry they were studying. These exams were given twice each quarter on Saturday mornings. If you could pass four of the first six that you took, you became a PhD candidate (and got a pay raise). Or you could pass five of 12 or six of 18. The faculty alternated in producing questions for the exams, so Verner put in

a question for the physical chemistry exam about the structure of diamond. None of us did very well with the question, and he pretty much read me the riot act about it. Since exam-taking is very much a game, we figured we were safe when it came to diamonds and didn't study the structure. Of course, on the next exam, here came another diamond question. This time there was no riot act, but Verner just shook his head at me when we next passed in the hall. After sufficient time, he seemed to get back to thinking I was OK.

Jensen's lab attracted many visitors, especially those interested in crystallographic refinement of macromolecules. Seattle was on the way to Japan and Asia, so we often had people come by for short visits during their travels. I tended to be shy when the famous visitors showed up. I found it hard to talk with these people who were my great heroes. I'm especially irritated that I never worked up the courage to spend more time talking with Max Perutz or Dorothy Hodgkin. Lyle had spent a sabbatical in Cambridge, so he knew Max from that time. And Lyle and Dorothy had competed on at least one small molecule structure. When Max and Dorothy came to visit (probably more than once each), they stayed at Lyle's house. Lyle and his wife, Mildred, held wonderful picnics in their backyard for the visitors and the rest of us. I wish I had an opportunity to re-live those get-togethers. I would work a lot harder at talking with the guests.

While completing my small molecule projects and writing them up, Lyle and I considered several protein problems for my thesis research. The problem that became my PhD project was hemerythrin. This is an octameric, non-heme iron, oxygen-binding protein with a molecular weight of 108,000, found in a few marine invertebrates. It fit with the Jensen group's overall interest in redox- and metalloproteins. Joann Sanders Loehr at Portland State University collaborated with Lyle and Larry and provided protein for a structure determination.

Data collection in Jensen's group was on the FACS-1 diffractometer. Larry oversaw the equipment, and his dedication to keeping the green machine aligned and in good condition, and Lyle's emphasis on precision, were important reasons I managed to solve the structure. The asymmetric unit for our crystal form contained four subunits from two hemerythrin octamers. The structure was solved at 5 Ångstrom resolution using a single mercury iodide derivative and its anomalous scattering signal. There are about 7500 reflections out to that resolution, and the quick

step-scan data collection protocol (developed largely by Jonathan Hanson and Keith Watenpaugh) resulted in our collecting 1500 reflections per day.

We lost a large part of our data when the paper tape output messed up. There wasn't time to re-collect it. We still had a printout, so I ended up key-punching about 2000 reflections onto computer cards so I could keep the project going.

Then it was time to use the campus' CDC6400 to generate a difference Patterson map. Ellie Adman had solved a multi-site Patterson in working out the structure of ferredoxin, so she'd set an example for working your way through a Patterson. Still, the major thing I remember from solving the six-site mercury derivative was the sense of desperation that came with computing in the evenings and trying to make sense of the vector map. (Desperation can be a major driving force in research. Sometimes, you just have to get things done.) Subsequently, Lyle complimented me on being able to work through that noisy Patterson map, so I suppose I must have shown a bit of skill in doing it.

In 1975, a low-resolution structure of a protein was a significant result, and because the hemerythrin subunit is a four-alpha-helical bundle, there was a lot to be said about the molecule at low resolution. It was time to write my dissertation.

Once again, I benefitted from having Ling, Verner and Lyle on my thesis committee. Without any special effort on my part, the three of them disagreed on what I should include in my thesis. Should I just staple my small molecule structure papers together and call it a thesis? Should I just write up the hemerythrin structure? Should I combine all of those in a larger volume? When they disagreed, I had room to negotiate with Lyle. I ended up writing up just my hemerythrin work, about a year's worth of my PhD research.

Writing a dissertation in 1975 was considerably different from the current process. First, you had to find a typist who was careful and skilled enough to meet the formatting standards of the Graduate School. The margins were checked with a steel ruler, and edits had to fit within the margins, so re-typing had to be minimized, especially since we paid by the page. Of course, typographical errors had to be avoided, and spell-checking was a human-based process, not a button in a word-processing program.

Since it was such an involved process, Larilyn and I decided I should go first, so three months before she finished, I wrote my thesis and got it typed up. It was

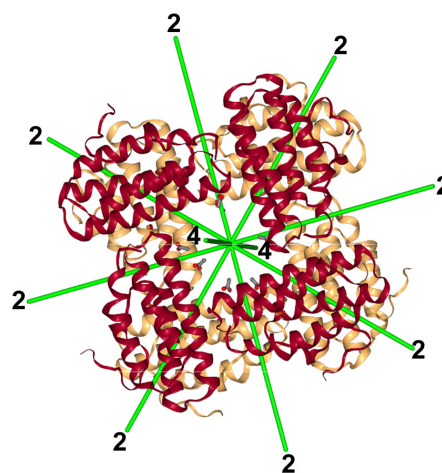
71 pages long, and 25 of those were Calcomp plots of the hemerythrin electron density map. We had to submit our theses for approval before scheduling our thesis presentation, and during the two-week waiting period, I built a balsa wood model of the low-resolution electron density map. (See photo below of Lyle holding the model.)

When the big day arrived for my presentation, I was a little nervous. And there was still Verner.

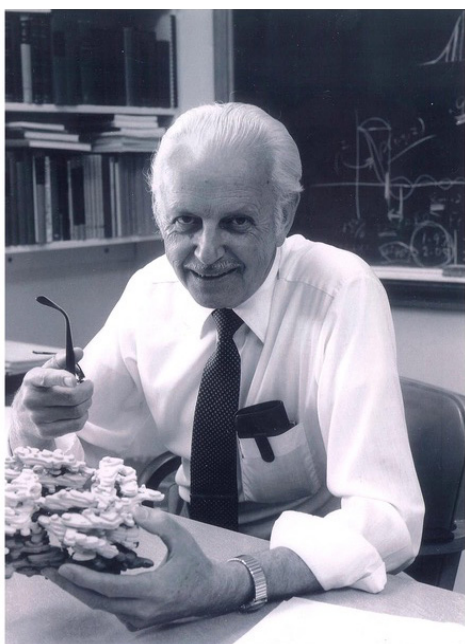
Hemerythrin's octamer has 422 symmetry relating the subunits. I'd built a couple models of the possible subunit arrangement using Styrofoam balls for the subunits. At some point in the presentation, I held up one of the models and stated the symmetry was 422. Verner immediately asked, "What's the symmetry of that model?" Gasp... I quickly answered that the symmetry was higher than 422 due to the spherical balls, and I said I didn't have time right now to figure out the actual point group. And I moved on with my talk. And amazingly, Verner let me go. (Diagram of hemerythrin (PDB 2HMQ) shown at right.)

I've thought of that moment often over the years, and I still think of it as a sort of personal triumph. It signified a time when I was the expert in the room. No one knew as much about my structure as I did. I was invincible (that day). The next day, I started on my path to being more and more confused by things, both scientific and not. I've heard many people, especially Lyle, Ling and Verner, talk about how they just didn't understand this or that thing. With the

exuberance and certainty of youth, I thought they must be crazy. They obviously knew more about those things than any of the rest of us did. I now understand what they meant. I will always have more questions to answer. It's part of what makes us scholars and scientists.



Hemerythrin.

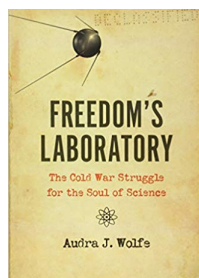


Lyle Jensen with molecular model.

Book Reviews

Freedom's Laboratory: The Cold War Struggle for the Soul of Science

Audra J. Wolfe (ISBN 9781421426730)



Freedom's Laboratory provides a detailed history of science and its role in society during The Cold War. Wolfe takes a deep dive into the role the United States government played in scientific inquiry and discovery around the world in the years following World War II. The Space Race was merely one facet of the heightened, science-related tensions between the capitalist United States and the communist Soviet Union.