

In Memoriam: Frederic M. Richards (1925-2009)

I first met Fred Richards in 1976 when he came to the University of Delaware for a seminar. He was already a defining presence in the field, and I was an over-aged, self-conscious postdoc starting a second career. He arrived from Yale by train, and I was assigned to fetch Dr. Richards from the Wilmington station (in my third-hand car) and drive him to the university. By the time we traveled the 15 miles to Newark, he had become Fred, and we began a conversation that continued for the next 30 years. It was a characteristic introduction to Fred.

Fred entered the scene at a heady time. Following completion of his PhD at Harvard in 1952 and a brief stint as a research fellow, he made the pilgrimage to the Carlsberg Lab in Copenhagen in 1954, a Valhalla of protein chemistry under Kaj Linderstrøm-Lang<sup>1</sup>. The following year was spent at Cambridge in the UK, after which he joined the Yale faculty in 1955, where he remained for the duration.

More or less contemporaneous with Fred's personal timetable, Pauling's model of the  $\alpha$ -helix <sup>2</sup> was published and immediately validated by Perutz <sup>3</sup>, the Watson-Crick DNA structure appeared <sup>4</sup>, and both the first protein X-ray structure <sup>5</sup> and DNA polymerase <sup>6</sup> were in the offing. Also, the Armour Company had provided the field with a kilogram of crystalline ribonuclease from cow pancreas, this at a time when some still doubted that proteins were macromolecules.

Most biophysical chemists can be categorized as either experimentalists or theoreticians, but Fred's integrated thinking defied such a discrete classification. His question was direct and undifferentiated: what can ribonuclease teach us about proteins?

Thus began a remarkable record of discovery and transformative insights. Fred had shown that limited subtilisin digestion of ribonuclease cuts the molecule reproducibly into two fragments, splitting it between residues 20-21. Neither fragment alone showed activity on an RNA assay <sup>7</sup>. As he recounted the story, on December 7, 1957 at about 7PM, alone in the lab, he titrated one fragment with the other while monitoring activity on his Beckman-DU spectrophotometer. As he watched, the needle climbed steadily, then stopped at a value corresponding to 100% activity when a 1:1 complex was attained. No covalent bonds were formed. In essence, the resultant complex was a dimer for which association



between the two fragments resulted in complete restoration of enzymic activity<sup>8</sup>. Any lingering doubt that proteins were authentic macromolecules was dispelled on that December night, and the field of protein molecular recognition was duly launched.

Three months later, the X-ray elucidated structure of myoglobin was published in Nature <sup>5</sup>, the culmination of a 25-year quest to interpret the three-dimensional structure of a protein from the crystal's diffraction pattern <sup>9</sup>. In due course, Harold Wyckoff, who had been involved in solving the myoglobin structure, joined the Yale department; Fred described him as a "complete diffractionist". In 1967, Wyckoff, Richards and their colleagues published the three-dimensional structure of Ribonuclease S <sup>10</sup>, a tie for the third new protein structure to be solved by X-ray

crystallography (counting myoglobin <sup>5</sup>/hemoglobin <sup>11</sup> as first, lysozyme <sup>12</sup> as second, and a third place tie between ribonuclease and chymotrypsin <sup>13</sup>).

Fred had a passion for equipment, and he devised a clever way to fit an atomic model to electron density using a half-silvered mirror <sup>14</sup>. B. K. Lee recalls:

"One thing I remember about him is that he liked to do things himself, in the old Yankee engineering tradition. Not long after I joined his group as a fresh post-doc, he invited me and my wife to his house for a dinner. The most impressive thing I remember about his house was this 'Fred's Fix-it shop' in his backyard, with all kinds of fascinating gadgets. After seeing this, I understood how he had built the 'Fred's box' for constructing the atomic model of a protein from its electron density map. Once the ribonuclease S structure was solved, Fred spent most of his time doing theoretical work and thinking about the structure, with able assistance from Master Technician Johnny Mouning and various post-doctoral fellows. Yet, throughout those years, he kept a small wet lab where he would do experiments with his own hands. He was always direct and a lot of fun to be around."

The Richards box (aka Fred's folly) was a useful tool for protein crystallographers for more than a decade, but it was eventually superseded by computer graphics, and Fred turned his full attention to the computer analysis of proteins.

At the end of Fred's life, there were 55,000 molecules in the protein data bank <sup>15</sup>, but when he joined the Yale faculty there were none. In the late 1950s, structural information about proteins was limited: experimental methods like sedimentation could provide accurate molecular weights and an indication of overall shape (i.e. axial ratios), but little else. At best, protein molecules were represented in illustrations by sketches of cigars or saucers (i.e. prolate or oblate ellipsoids of revolution), information that could be specified completely by three numbers. With the advent of protein crystallography, a molecule like ribonuclease, with 1000 heavy atoms, gave rise to 1000 x,y,z-coordinates, specified by 3000 numbers – a thousand-fold increase in the available experimental data.

X-ray crystallography opened an urgent and unprecedented new frontier in protein science. Most crystallographers responded by redoubling their efforts to solve crystal structures. Fred responded by asking what can be learned from crystal structures.

In particular, do native proteins have recognizable structural features? What are the conspicuous differences between the native structure and its denatured counterpart? Could those differences account for the forces involved in stabilizing the native form? What is the relationship between the stability of the native form and its structure? In other words, how do proteins solve the folding problem?

How does one analyze a protein structure with such questions in mind? The answer is far from self-evident. Fully detailed scale models (e.g. Kendrew models) successfully capture the positions of atoms and, implicitly, bond lengths and angles, but one is hard-pressed to distinguish even the most familiar elements of structure, like an  $\alpha$ -helix, within the congested jungle-gym of metal parts.

Is the interior of a protein like a liquid? A gel? A solid? It's virtually impossible to answer such a question by inspection of a model; a different approach is needed. For his PhD thesis with Barbara Low, Fred had devised a method to determine protein crystal densities <sup>16</sup>. Now, with crystal structures in hand, he calculated protein packing densities, a ratio of the summed atom volumes to the molecular volume <sup>17</sup>. On average, the packing density of proteins is <0.75>, equivalent to the packing density of aliphatic solids, which are themselves at the close-packing limit for spheres of uniform size. In other words, the interiors of proteins are well-packed indeed, more like a solid than a liquid. There are few voids, if any, large enough to accommodate a carbon atom, and interior water molecules are a rarity.

Consequently, there must be substantial interactions between and among the sidechains that constitute the molecular interior, this despite the fact that residue sidechains have idiosyncratic shapes that lack any obvious complementarity. To quantify this inference, B.K. Lee and Fred developed a method to calculate the solvent-accessible surface area of a protein from its atomic coordinates <sup>18</sup>. Conceptually, the protein is probed exhaustively using a water-sized sphere, and the sphere-accessible surface is mapped. Intramolecular interactions bury surface, sequestering the interacting sites from solvent access. Assuming maximum exposure of the polypeptide chain under denaturing conditions, the difference in solvent-accessible surface area between denatured and native forms measures the interactions that accompany folding. That measure, an area, scales linearly with the experimentally-determined free energy of transfer of residues from water to organic solvent <sup>19</sup>. Though not without controversy or qualification, this relationship links changes in free energy to changes in structure.

Fred also implemented the method of Bernal and Finney <sup>20,21</sup> to calculate residue volumes from atomic coordinates using irregular polyhedra (Voronoi polyhedra) <sup>17</sup>. In essence, each atom is enclosed in a cage formed by its nearest spatial neighbors, and the volume of that cage is taken to be the atom's volume. The average volume of each residue type in the folded protein, calculated from the Voronoi sums, was then compared with its corresponding crystal volume, to determine the volume change on folding.

Fred observed that there is little change in the individual residue volumes upon folding. In contrast, residue solvent-accessible surface areas change dramatically, by a factor of approximately three for a protein with the molecular weight of ribonuclease, as interacting surfaces coalesce, squeeze out interior water, and engender the close-packed interior. All of this is beautifully described in Fred's classic review on these topics <sup>22</sup>.

Nowadays, it is a routine matter to distinguish those residues that constitute the outside of a protein of known structure, to delineate its core, or to identify an atypical landmark within the interior. But it was not always so. Fred's viewpoint has become our viewpoint.

Protein folders can be divided into "minimizers" and "packers". The former seek to minimize the interaction energy among atoms or groups of atoms, while the latter concentrate on probable geometry, guided by both excluded volume limitations and structural motifs seen in proteins of known structure. Fred was a founding father of the packers, influenced in large part by his observations on packing density, areas and volumes. This line of inquiry has historical roots in theories of excluded volume effects in polymers, and, more specifically, in Ramachandran plots <sup>23</sup> and Pauling's space-filling CPK models <sup>24</sup>. Under any feasible physical conditions, two atoms cannot occupy the same space at the same time, as quantified by the steeply repulsive term in a Lennard-Jones potential <sup>25</sup>. This principle can be implemented simply by approximating atoms as hard spheres. Of course, atoms are neither totally incompressible nor entirely spherical, but quoting from the 1977 review <sup>22</sup>:

"For chemically bonded atoms the distribution is not spherically symmetric nor are the properties of such atoms isotropic. In spite of all this, the use of the hard sphere model has a venerable history and an enviable record in explaining a variety of different observable properties. As applied specifically to proteins, the work of G.N. Ramachandran and his colleagues has provided much of our present thinking about permissible peptide chain conformations."

To what extent does excluded volume limit the permissible conformations of a folded protein? This is an important question in polymer theory. Fred's way of pursuing it, motivated in part by earlier work of Ptitsyn and Rashin<sup>26</sup>, was to perform a computer experiment, using myoglobin as a test case. In classic Richards style, Fred Cohen, Timothy Richmond and Fred<sup>27</sup> enumerated all structures that can be generated by packing preformed myoglobin helices together and determined the fraction of these conformers eliminated by excluded volume constraints. In greater detail, conformations were generated systematically by pairing geometrically-simplified helices at potential interaction sites<sup>28</sup>, subject to the constraint that every helix was paired at least once, resulting in a total of 3 x 10<sup>8</sup> structures. This set was then winnowed using two steric filters: (1) non-paired helices were not allowed to clash, and (2) the length of the polypeptide chain was sufficient to span the distance between the C-terminus of one helix and the N-terminus of the next helix. Upon application of these plausible steric filters, only 121 structures survived. With slightly tighter constraints, that number was further reduced to 20 survivors, the native structure among them. The result is dramatic: excluded volume effects reduce the folded population by a factor of 0.000000067.

Despite subsequent work by others, the 1979 approach did not lead to a break through, and the paper is rarely cited today. However, in recent years, the field has begun to recognize that the relevant thermodynamic population under conditions of interest is substantially less diverse than previously realized. Both bioinformatics- and physics-based approaches have witnessed a newfound emphasis on systematically eliminating improbable conformers. Viewed in retrospect, Fred's approach of implementing excluded volume constraints in a protein-specific algorithm was ahead of its time.

It is beyond the scope of this piece to provide a complete list of Fred's research triumphs. Time and again, his work explained something important that no one realized before; taken together, these insights transformed our thinking about proteins.

Fred's influence on biophysics and structural biology extended well beyond his own research. In 1963, Yale president Kingman Brewster asked him to switch departments and become chair of Biophysics, which was then renamed the Department of Molecular Biophysics and Biochemistry. During his tenure as chair, Fred hired eight faculty, seven of whom were to became members of the National Academy<sup>29</sup>, and the department quickly gained pre-eminent stature. Robert ("Buzz") L. Baldwin of Stanford said it best:

"Fred built the Department of Molecular Biophysics and Biochemistry at Yale into a world-class center for the determination of protein structures by X-ray crystallography, and by example he showed other institutions in the U.S. how this could be done. He insisted that X-ray crystallographers must not take personal advantages from their protein structures while withholding the details (structural coordinates) and, against powerful opposition, he won this struggle, especially because of the personal respect in which he is held by X-ray crystallographers."

Fred was a substantial contributor to the *National Good* in other professional activities as well. He served as president of both the American Society of Biochemistry and Molecular Biology and the Biophysical Society, on the councils of the Protein Society and the International Union of Pure and Applied Biophysics and on numerous editorial boards. Together with John Edsall and Chris Anfinsen, his service as Editor of *Advances in Protein Chemistry* ushered in the publication of an exceptional stream of famous reviews. His conspicuous wisdom and absolute integrity were always in demand for high-level committees and sensitive investigative bodies. His two decades of service to the Jane Coffin Childs Memorial Fund for Medical Research, first as Director and then as board member, identified and launched an all-star cast of young scientists. This is by no means an exhaustive list.

Fred's contributions did not go unrecognized. He was honored with an impressive array of prestigious international awards and was a member of the National Academy of Sciences, the American Philosophical Society, and a Fellow of the American Academy of Arts and Sciences.

Fred cherished life. He lived a full life himself and raged against the social forces that erode time and trivialize activity. No one described this crime against creativity better than Fred, and his prefatory chapter, "Whatever happened to the fun? An autobiographical investigation" is a must-read for any young academic <sup>30</sup>. Like all of Fred's writing, one can hear his voice when reading his words.

Fred liked to have fun: with ideas, with talk, with the sheer joy of doing. And, of course, with sailing. For Fred, a quintessential part of the full life was to spend at least 4-6 weeks a year away from science, and in his case that meant sailing. Summer was the sailing season for Fred and his wife Sally. He came from a family of sailors, and his students, post-docs and colleagues have fine sea-stories to tell. Of course, there were also the stories told by Fred himself, who sailed his boat across the Atlantic to a sabbatical in England in 1967. In this, as in all his activities, Fred's interests pushed the envelope. Pat Fleming, now at Hopkins, was Fred's Yale colleague for many years, and he tells this story:

"Many people know that Fred was an accomplished sailor. But in recent years he and Sally probably spent more time on "Sally's barge" than on their sailboat, Hekla. Sally's barge was a utility boat with derrick, outboard motors and a fearsome open deck and steering station. They used the barge on Long Island Sound in all kinds of weather for various marine related activities. In typical fashion Fred built the barge from plans drawn by Ron Noe, a local naval architect and fellow sailor. Friends and other scientists were invited to help with the final fiberglassing – a day long party that added to the lore of Fred's extracurricular projects."



Fred aboard Chris Anfinsen's boat, under sail to Bermuda (left to right: Fred, Anfinsen, Ashwell and Fisher).

I last saw Fred when he came to Hopkins in 2006, invited by the graduate students to present the inaugural Pioneers in Biology Anfinsen Lecture. It was a celebration. Fred's seminar ranged over his childhood, research, major influences, and sailing trips, all set against a historical backdrop of protein chemistry. His description of the ribonuclease experiment on that December night in 1957 raised goosebumps in the audience. Afterward, there was a party at Blake Hill's house where our students had the opportunity to meet Fred. For some, it was a memorable introduction, but their conversations, unlike mine, were destined to be short-lived.



Fred surrounded by the student organizers Pioneers in Biology inaugural Christian B. Anfinsen Lecture Johns Hopkins University, 1 June 2006

Fred died of natural causes on January 11. He is survived by his family and friends, former students and current colleagues, a field he did much to transform – and by the many for whom his life made a meaningful difference.

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