



Frederic M. Richards

1925–2009

BIOGRAPHICAL

Memoirs

*A Biographical Memoir by
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and George D. Rose*

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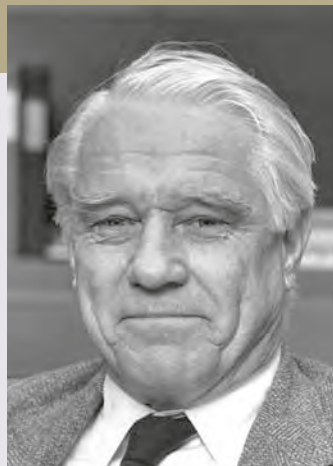
NATIONAL ACADEMY OF SCIENCES

FREDERIC MIDDLEBROOK RICHARDS

August 19, 1925–January 11, 2009

Elected to the NAS, 1971

Frederic M. Richards (who preferred to be called Fred) is remembered as a guiding influence in 20th century protein chemistry and structural biology. In a landmark 1957 experiment, he discovered that the proteolytic enzyme subtilisin cuts the protein ribonuclease into two inactive fragments which, when mixed, associate spontaneously and restore activity completely. This single experiment at once established the field of protein molecular recognition, showing, for example, how peptide hormones might activate their protein receptors. Later, Fred pioneered computational approaches for analyzing and interpreting protein structures that remain in widespread use today. He was the founding chair of the Department of Molecular Biophysics and Biochemistry at Yale, which became the world's preeminent department of molecular biophysics soon after its inception. He was an outspoken critic against the social forces that erode time and trivialize achievement. He served the scientific community at large in many influential roles, and his work garnered many of the most prestigious awards.



Fred Richards

By Robert L. Baldwin
and George D. Rose

Fred was a natural leader with a charismatic, fun-loving personality. He cherished life and reveled in ideas, conversation, the sheer joy of doing, and, especially, sailing. For Fred, a quintessential part of the full life was to spend at least 4 to 6 weeks a year sailing. At this he was no amateur; he and a small crew crossed the Atlantic in a 40-foot sailboat—twice.

Early years

Fred was born August 19, 1925, in New York City, the youngest of three children. His parents, George H. Richards and Marianna Middlebrook Richards, were from old Connecticut families dating back to 17th century America. His father was a lawyer who enjoyed sailing. A sister, Marianna, 14 years his senior, was a biochemist. His other sister, Sarah, 11 years older, and her husband, Gifford Pinchot—son and namesake of

Heeding advice from Chris Anfinsen, Fred spent a postdoctoral year with Kaj Linderstrøm-Lang at the Carlsberg Laboratory in Copenhagen, which was, thanks to Lang (as everyone called him), a world-famous mecca for research on protein folding.

the famed conservationist—were ardent sailors. In retrospect, these early influences in biochemistry and sailing were prophetic.

Fred acquired his first chemistry set at age eight, and he supplemented it incrementally with glassware and chemicals provided by Marianna, who was then pursuing her Ph.D. at Columbia. Fred attended secondary school at Phillips Exeter Academy in New Hampshire, where, among other characteristic pursuits, he memorized the RCA vacuum-tube catalog and also devised an ingenious apparatus to measure the gravitational constant—a pendulum with a sensitive optical system to detect

changes in position when juxtaposing two 97-kilogram cannonballs. Graduation arrived, and the project was never finished, but Fred's inclinations were already apparent. In a 1997 account of his scientific life and work,¹ he credited his school years at Phillips Exeter with interesting him in science, especially in physics and in building instruments.

At the urging of older sister Marianna, Fred broke with family tradition and matriculated at MIT instead of Yale. His undergraduate education was interrupted by two years of military service, which he described as “uneventful.” Graduating with a B.S. in 1948, he moved on to E. J. Cohn's department at Harvard for graduate training in biochemistry and biophysics. Cohn's department was unusual in being centered on protein physical chemistry in relation to medicine and public health. Although the department was large, with about 100 professional scientists, there were only about five Ph.D. students.

For his advisor, Fred chose Barbara Low, a recently appointed X-ray crystallographer who had worked with Dorothy Crowfoot Hodgkin on the X-ray crystal structure of penicillin. At that time, the phase problem had yet to be solved, and the first protein X-ray structure² was still in the offing. For his thesis research, Fred designed and built a micro-balance³ for measuring the density and solvent content of a protein crystal, and he used these values to determine the protein's molecular weight from its unit cell dimensions. After completing his Ph.D. in 1952, Fred remained an additional year as Cohn's personal assistant; he later commented wryly that this experience taught him how to deal with a domineering personality.⁴

A formative postdoctoral year in Copenhagen

Heeding advice from Chris Anfinsen, Fred spent a postdoctoral year with Kaj Linderstrøm-Lang at the Carlsberg Laboratory in Copenhagen, which was, thanks to Lang (as everyone called him), a world-famous mecca for research on protein folding. Anfinsen and Walter Kauzmann were regular visitors in the early 1950s. Other visitors during Fred's year there included John and Charlotte Schellman, Bill Harrington, and Harold Scheraga, a group of all-stars who, like Fred, were intent on learning the mechanism of protein folding.

On arrival, Fred asked Lang for a research problem. Lang suggested a challenge that he sometimes gave to visitors: to see whether the proteolytic enzyme subtilisin does something interesting to one of the handful of standard proteins. In those days, the primary standard proteins were derived from milk, egg-white, blood plasma, or pancreatic juice. The motivation for Lang's suggestion was a chance discovery by Martin Ottesen, Lang's chief assistant. A few years earlier Ottesen had found, quite by accident, that subtilisin clips a short peptide from ovalbumin and converts it to a new form, plakalbumin, that crystallizes in thin plates.

The specific protein that Lang suggested to Fred was ribonuclease A (RNase A) from bovine pancreas, an especially popular standard because Armour Laboratories supplied the crystalline protein in abundance and at little cost. The action of subtilisin on RNase A had already been investigated by Sumner Kalman, a visitor to the Carlsberg Lab who was departing as Fred arrived. It was clear from Kalman's results that subtilisin does cleave RNase A and that the outcome could be interesting—though it might instead turn out to be a can of worms. Fred set to work on purifying the products of the action of subtilisin on RNase A using column chromatography, a newly available tool, and his results were published together with Kalman's.⁵

By the end of his postdoctoral year Fred had purified a subtilisin-modified derivative of RNase A and found evidence that its enzymatic activity was altered.⁶ His analysis relied on the fact that the enzyme cleaves RNA in two separable steps. First, it cleaves the phosphodiester bond between the 5'-ribose of the nucleotide and the phosphate group attached to the 3'-ribose of an adjacent pyrimidine nucleotide, forming a 2'-3'-cyclic phosphate. Then, the cyclic phosphate is hydrolyzed to the 3'-nucleoside phosphate. Lang gave Fred a limited quantity, 20 milligrams, of a chemically synthesized cyclic phosphate (uridine cyclic 2', 3' phosphate), a substrate for the second step. Kalman had already measured the diesterase activity of subtilisin-modified RNase A, the first

step of the cleavage reaction. By combining activity data for both steps, Fred found that subtilisin modification had altered the enzymatic properties of RNase A, although the step-wise details were ambiguous because enzymatic activity alone was insufficient to determine the amount of native enzyme.

It was a pivotal year for Fred. When the time came to leave Carlsberg in the summer of 1955, he was able to give Lang a substantive report of his research, and, topping it off, to return 10 milligrams of the precious RNase A substrate. Lang's suggestion had been transformed from an enlightened hunch into a promising program for future research—indeed, far more promising than either of them could have realized at the time. Anxiously, Fred asked Lang for permission to continue working on the problem when he returned to the United States, where he was joining the faculty of the Yale Biochemistry Department. Fortunately, Lang said yes; Fred commented that he did not know what he would have done had Lang said no.

Fred loved his time at the Carlsberg Lab. His 1992 reminiscence⁴ paints a vivid, heart-warming picture of Linderstrøm-Lang and his remarkable laboratory. He described Lang as “a delightful individual, full of fun and jokes as well as science.”

Yale

Recruited by Joseph Fruton, Fred moved to Yale in 1955 and remained there for the rest of his working life. Three years later he was the sole author of a paper on subtilisin-modified RNase A⁷ that shook the entire protein community.

Progress in science usually comes in fits and starts. Single, clear-cut experiments that lift the veil of ignorance to reveal a transformative truth are rare and therefore memorable. Elucidation of the double-stranded DNA structure is one of history's most extreme examples. In the 1958 paper, Fred reported such an experiment.⁷

Fred expected that subtilisin cleavage would result in breakage of at least one of the four S-S bonds that cross-link RNase A, allowing cleaved peptide to separate from the remaining protein. To his surprise, when he precipitated the subtilisin-modified RNase A with trichloroacetic acid (a standard procedure in protein chemistry), a single peptide (residues 1-20) remained behind, and the results were reproducible. He named the peptide S-pep and the protein moiety S-prot (residues 21-124).

As Fred recounted the story, at about 7 p.m. on December 7, 1957, 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 percent activity when a 1:1 complex was attained. No covalent bonds were formed. Isolated S-pep is largely unfolded and S-prot is only weakly folded, yet the two fragments recognized each other and, as Fred showed, they associated stoichiometrically and restored enzymic activity completely. Fred named the complex RNase S (for subtilisin-cleaved RNase). Any lingering doubt that proteins are authentic macromolecules was dispelled.

Life as we know it is based on molecular recognition by proteins. Fred's telling experiment launched our current approach to studying this molecular phenomenon, showing that discrimination can be realized by purified proteins in buffer and can be analyzed using physical/chemical methods. Fred's breakthrough result came at about the time that Chris Anfinsen found that the free energy change upon folding RNase A drives its folding process.⁸ The restoration of RNase from association of its subtilisin-cleaved fragments⁷ and the refolding of denatured RNase⁷ are highly related processes; each occurs spontaneously with exacting specificity and requires only purified reactants in buffer. Molecular recognition in Fred's experiment is analogous to self-recognition in Anfinsen's experiment. A detailed mechanistic understanding of both processes remains in active study today.

Linderstrøm-Lang was still alive in 1958 when Fred published his paper. He lived to see that the problem he gave Fred in 1954 was in fact transformative and a gold mine, not at all a can of worms.

Within a few months of that memorable December night, the first protein X-ray structure was solved, initiating the era of structural biology. Now Fred was determined to get the X-ray structure of RNase S. His first postdoctoral fellow, Paul Vithayathil, had succeeded in crystallizing it as a side project while using chemical modification of S-peptide to dissect the roles of individual amino-acid residues in the enzymatic functions of RNase S.⁹ With crystals in hand, Fred, who was by then chair of his department, recruited Hal Wyckoff to Yale, and together they solved the structure of RNase S in 1967,^{10, 11} in a tie for the third protein X-ray structure (with ribonuclease A,¹² carboxypeptidase,¹³ and chymotrypsin.¹⁴) Richards and Wyckoff, together with Norma Allewell,¹⁵ quickly went on to analyze the mechanism of specific binding of phosphate-containing ligands.

In 1967, Fred, who had been married since 1959 to his second wife, Sarah "Sally" Wheatland Richards, spent a sabbatical year in David Phillips' laboratory for structural

biology at Oxford. At the time, trans-Atlantic travel by commercial ocean liner was far more common than it is today. But commercial ocean liners were not for Fred, who captained his own sailboat across the Atlantic with Sally and two crew members.

During those early days of protein crystallography, protein X-ray structures were still built by hand, a complicated procedure involving thousands of precisely aligned atoms. While on sabbatical and with his talent for designing instruments, Fred devised an apparatus—known colloquially as the Richards Box or “Fred’s Folly”—that used a half-silvered mirror to let the investigator build the structure directly into an image of the electron density map.¹⁶ Today, Fred’s procedue has been automated by using a computer to do the fitting.

New directions

The availability of both protein crystal structures and fast, affordable digital computers (though not by today’s standards) opened new vistas. Now, with crystal structures in hand, it would be possible to find previously unattainable answers to critical questions.

How are the observable changes in structural parameters upon folding linked to the thermodynamic forces involved in stabilizing and selecting the native form? The problem is clear enough, but the way to tackle it is far from obvious. In this regard, Fred is rightly credited with important development of methods—computer algorithms to determine surface area, volume, and packing density from three-dimensional coordinates—although he wasn’t the first to do so.¹⁷⁻¹⁹ However, more than others, Fred’s comprehensive approach to the overall question shaped current views, so that today such calculations are performed almost by reflex.

Harking back to his Ph.D. thesis, Fred had devised a method to determine protein crystal densities.³ Now, with crystal structures in hand, he calculated protein packing densities, a ratio of the summed atom volumes to the molecular volume.²⁰ On average, the packing density of proteins is $\langle 0.75 \rangle$, 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 rare.

Consequently, there must be substantial interactions between and among the side chains that constitute the molecular interior, this despite the fact that residue side chains have idiosyncratic shapes that lack any obvious complementarity. To quantify this subject,

B.K. Lee and Fred developed an algorithm to calculate the solvent-accessible surface area of a protein from its atomic coordinates.²¹ 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.

Fred also implemented the method of Bernal and Finney^{19, 22} to calculate residue volumes from atomic coordinates using irregular polyhedra (Voronoi polyhedra).²⁰ 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, could then be compared with its corresponding crystal volume. Fred was his own programmer for the Voronoi volume calculation; he debugged the program while serving as an alternate on a jury, running back to the computer center at lunch time to submit his Fortran code in those bygone days of card decks and batch processing.

Applying these algorithms, Fred observed that there is little change in the individual residue volumes upon folding. In contrast, solvent accessible surface areas of residues change dramatically, by a factor of about three, as interacting surfaces coalesce, expel interior water, and engender the close-packed interior. All of this is beautifully described in his classic review on these topics.²³ These area and volume relationships are related to free energy changes upon folding, and today, half a century later, they remain active areas of research.

Protein folders can be divided into “minimizers” and “packers”. The former seek to minimize the interaction energy among atoms or groups of atoms, whereas the latter approximate energy with 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. His approach has historical roots in theories of excluded volume effects in polymers, and, more specifically, in Ramachandran plots²⁴ and Pauling's space-filling CPK models.²⁵ Under any feasible physical conditions, two atoms cannot occupy the same space at the same time, as quantified by the steeply repulsive, albeit somewhat arbitrary, term in a Lennard-Jones potential.²⁶ This principle can be implemented by approximating atoms as hard spheres.

Of course, atoms are neither totally incompressible nor entirely spherical, but quoting from the 1977 review.²³

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.

Similarly, a geometric representation of hydrogen bond energy is often viewed with skepticism, but quoting from the detailed analysis of Hagler and Lifson:

*...is there such a thing as a hydrogen bond energy of the system C=O...H-N?...It is more appropriate to consider the hydrogen bond as the sum of all interactions which make the system C=O - H-N particularly stable. In this connection it is worth noting that the most general and useful definitions of hydrogen bonds are geometric.*²⁷

Today, the field has focused largely on the organizing role of attractive forces that enrich energetically favored conformers in the thermodynamic population. However, excluding forces – steric clashes and unsatisfied hydrogen bonds – also impose organization by depleting energetically disfavored conformers from the thermodynamic population. Excluding forces can be captured effectively using distances and angles, and Fred thought that they are major factors in attaining the folded structure:

*In summary it seems reasonable that there is a generalized driving force with no particular specificity produced by the tendency of non-polar groups to “escape” from aqueous contact. Permissible folded structures are then the result of the relative absence of two energetically unfavorable factors: (a) the lack of pairing of buried polar groups, and (b) poor geometrical packing of internal groups both polar and non-polar. The combination of these latter two restrictions is perhaps more severe than might be imagined and should markedly reduce the number of possible native structures even without further knowledge of the interaction parameters.*¹⁵

In a computer experiment, Fred tested the extent to which excluding forces limit permissible conformations, using myoglobin as a test case. Motivated in part by earlier work of Ptitsyn and Rashin,²⁸ Fred Cohen, Timothy Richmond, and Fred 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.²⁹

In greater detail, conformations were generated systematically by pairing geometrically simplified helices at potential interaction sites, subject to the constraint that every helix was paired at least once, resulting in a total of 3×10^8 structures. This set was then winnowed using two straightforward filters: (1) nonpaired helices were not allowed to clash and (2) the polypeptide chain segment had to be long enough 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 0.00000067.

Extending the idea that excluded volume constraints can impose substantial organization on the folding polypeptide, Ponder and Richards turned to the inverse folding problem: enumeration of all sequences compatible with a given fold.³⁰ In their approach, key assumptions included maintenance of a rigid backbone and tightly packed core. A crucial reduction in combinatorics was achieved by using a pre-compiled rotamer library of favorable side chain conformations, an original idea that is now in common use. Similar to the earlier calculation of assembled myoglobin helices, there was an enormous reduction in the number of sequences compatible with these plausible constraints.

Despite early excitement, these computational experiments did not lead to a breakthrough understanding of the folding problem. The protein backbone is more flexible than the rigid geometry used in these calculations and side chains can repack efficiently, rather like a collection of nuts and bolts in a jar. Nevertheless, Fred had ideas that were way ahead of his time concerning how to analyze and interpret protein structures, and his ideas have shaped the future development of protein structural and folding work. Although the specific computational experiments did not culminate in a revealing flash of illumination, like the 1958 subtilisin-modified RNase experiment, the type of thinking that motivated these experiments framed future directions that continue to guide the field, and his methods have become durable staples in today's arsenal of computational tools.

Founding chair of molecular biology and biophysics (MB&B)

In 1963 Yale President Kingman Brewster asked Fred to take over the Department of Biophysics, which, like similar departments in other universities, had been slanted toward radiation biology. Fred served a term (1963-1967) as chair before leaving for his 1967-1968 sabbatical with David Phillips. Meanwhile, upon advice from Sydney Brenner, Brewster decided to create a radically new department, Molecular Biophysics and Biochemistry (MB&B), merging two existing departments, Biochemistry in the Medical School and Biophysics in Arts and Sciences. Fred, age 44, was enlisted to chair it. The rest, as they say, is history.

Fred was given ample resources and many slots for new faculty. Already in place were Dieter Söll, Lubert Stryer, and other well-known scientists. In rapid succession Fred hired an exciting group of young faculty: John Cronan, Don Engelman, Alan Garen, Peter Lengyel, Peter Moore, Tom and Joan Steitz, and David Ward. Recruited largely from the best British labs in structural biology, they brought with them the British spirit of informal interactions and lively conversation. Fred cultivated an atmosphere in which this group became fast friends— socializing over wine, hiking, collaborating.

Fred was the principal investigator of an NIH program project grant that included himself, Hal Wyckoff, Don Engelman, Peter Moore, and Tom Steitz (nicknamed the WERMS group). They established a world-class center for structural work, where X-ray equipment, computers, and a library of useful programs were consolidated in a central core facility, available for use by everyone. The facility was also a place for shared knowledge, where one person's technical problem could often be overcome because another person had encountered it previously and knew a solution. Soon Yale became the place to go if you had crystals of a protein and wanted to solve its X-ray structure.

Under Fred's guidance MB&B quickly rose to become the world's preeminent department of molecular biophysics, and it remains one of the top few today. Eight of the faculty mentioned above are now members of the National Academy, and, so far, one of them, Tom Steitz, is a Nobel laureate.

The public good

Fred's vision and integrity led to many honors and responsibilities. He was elected President of both the Biophysical Society and the American Society of Biological Chemists (now ASBMB). Together with John Edsall and Chris Anfinsen, his service as Editor of *Advances in Protein Chemistry* ushered in the publication of an exceptional

series of important reviews. Fred had a broad knowledge of biophysics and knew who was doing exciting work. Appropriately, when the Burroughs-Wellcome Foundation launched its innovative Interfaces Program to bridge the gap separating biology from mathematics and physics, he was asked to chair the first awards committee. He served on many editorial boards and advisory committees, including the first Council of the Protein Society, where later he continued to serve on selected committees, such as the one entrusted with choosing the new editor of *Protein Science*. He chaired a special committee to advise the NIH director on “the Gallo/Montagnier debacle” (his phrase). A pointed description of this experience is given in his autobiographical memoir¹ entitled characteristically: “Whatever happened to the fun?” From 1976 to 1991 Fred was also director of the Jane Coffin Childs Fund, a committee assignment he especially enjoyed. The fund’s purpose is to identify and support outstanding postdoctoral fellows; more than 800 fellows had been selected at the time of Fred’s 1997 memoir.

Sailing

Summer was sailing season for Fred and Sally. Apparently he inherited a sailing gene that remained constitutive throughout his life, and his students, postdocs, and colleagues enjoyed memorable times on his sailboat, *Hekla*.

In sailing as in other activities, Fred was one of those people who always knew the right thing to do. Don Engelman described this adventure with Fred:

Fred, Sally and I set out from the Maine coast aboard Hekla, their Hinckley Bermuda 40, one of the finest cruising sailboats ever built. The day was iffy, but we made it across the Bay of Fundy to an anchorage near Yarmouth by 4:00 and passed a pleasant evening with stories, adult beverages and cribbage. The next day looked worse. We debated whether to set out, and unwisely chose to go for it. The weather deteriorated, and became extreme—gusts of 50 knots, pounding rain, no visibility. The sea was white, with huge swells. The LORAN navigation system, which was the state of the art in the 80’s, quit, and we found ourselves in distress—sailing with only the storm jib, heeled over so far that the radar read only the sky and the sea, and losing our way. What to do? Fred used a chart, the depth gauge and the compass to locate our position and to follow our course based on observing the features of the bottom. We eventually made it to a somewhat sheltered inlet, where we managed to anchor

after three tries, and sat out the rest of the storm, soothed by an even more generous apportionment of the adult beverages. Our adventure encapsulates Fred's approach—clear thinking, use of available resources, problem solving.

Fred retired from the active faculty as Sterling Professor of Molecular Biophysics and Biochemistry, becoming emeritus in 1991. During his lifetime, he was recognized with many of the most prestigious awards and honors. By no means an exhaustive list, he received the Pfizer—Paul-Lewis Award in Enzyme Chemistry (1965), the Kaj Linderstrøm-Lang Prize in Protein Chemistry (1978), the Stein and Moore Award of the Protein Society (1988), the Merck Award, the American Society for Biochemistry and Molecular Biology Award (1988), and the Connecticut Medal of Science (1995). He was a Guggenheim Fellow (1967-1968), a Fellow of the American Academy of Arts and Sciences (from 1968), and a Member of the National Academy of Sciences (from 1971) and of the American Philosophical Society (from 1992).

Fred died of natural causes on January 11, 2009. He predeceased his wife, sailing partner, and best friend, Sally, who passed away two years later, on April 3, 2011. He was survived by his three children: Sarah O. Richards, Ruth G. Richards, and George H. Richards, and their respective partners, Rick Blank, Lee Blackwell, and Sally B. Richards, as well as by his four grandchildren, Benjamin H. Lillie, George H. Richards III, William S. Richards, and Kate E. Richards.

We are grateful to Don Engelman for his account of weathering a storm with Fred and for information about the beginnings of MB&B. A few paragraphs in this biography were taken almost verbatim and with permission from the authors' In Memoriam tributes, published earlier in *Protein Science* (RLB) and *Proteins* (GDR).

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