

## Frederic M Richards 1925–2009

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Frederic Middlebrook Richards, Sterling Professor Emeritus of Molecular Biophysics and Biochemistry at Yale University, died on 11 January 2009 at the age of 83 (Fig. 1). A founding father of the modern field of structural biology and a member of the US National Academy of Sciences, Richards grappled for decades with understanding how proteins fold and, in the process, broadly influenced how we determine, analyze and interpret molecular structures today. A master of seeing the forest from the trees, Fred's steady hand helped steer the nascent field forward with logic, common sense, creativity and a preternatural clarity of vision.

Inspired by a chemist older sister, the young Richards was quickly drawn to the blossoming world of mid-twentieth century protein chemistry. After obtaining his undergraduate degree in chemistry at the Massachusetts Institute of Technology in 1948, he entered graduate school in E. J. Cohn's Department of Physical Chemistry at Harvard Medical School, where he studied the density and solvent content of protein crystals during his thesis with Barbara Low. In 1954, Richards set off for a short postdoctoral stint with Linderstrøm-Lang at the Carlsberg Laboratory in Copenhagen, where he was to initiate his classic work on the enzyme RNase.

At that time, bovine pancreatic RNase A (RNase A) was the system of choice for protein chemists, as the Armour Meat Packing Company had purified a whopping 1 kg of the protein and offered samples to scientists. At the Carlsberg Laboratory, Richards experimented with treating RNase A with limiting amounts of the protease subtilisin. He discovered that he could covalently cleave the protein to create a new form, called RNase S, that still retained enzymatic activity. When Richards returned to the United States, now as a faculty member at Yale, he showed in 1957 that RNase S could be chromatographically separated into two fragments (referred to as S-protein and S-peptide), each of which lacked any enzymatic activity. But, to the surprise of the protein chemistry community, Richards observed that when he remixed aliquots of the two fragments complete RNase activity was instantly restored<sup>1,2</sup>. This demonstration that the information encoding the protein's structure and function was robust enough to defy covalent cleavage and physical separation was a landmark finding in the young field of protein folding, and it helped set the stage for Anfinsen's classic experiments demonstrating that RNase A could spontaneously refold to its native state even after chemical denaturation and scrambling of its disulfide linkages. Richards' RNase S work also illustrated the remarkable specificity of protein-protein interactions and how they can regulate protein activity, concepts central to modern cell biology.

Desperately curious to know what these protein structures looked like, Richards played an important part in the beginning days of protein

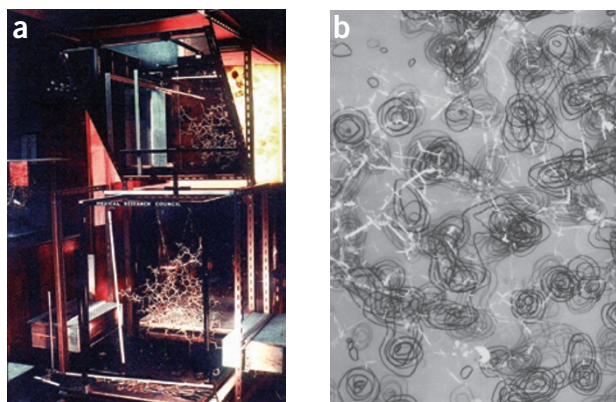


**Figure 1** Fred Richards with his wife, Sally, aboard their sailboat Hekla in 1994. A dedicated sailor since childhood, Fred almost always took a month off each summer to captain a major sailing excursion, returning to lab afterwards refreshed and ready to work. His sailing adventures included several transatlantic voyages. He was also an avid ice hockey player (photo by F. Schley).

crystallography. In 1967, he and Hal Wykoff solved the structure of RNase S<sup>3</sup>, which was the third protein structure determined (Harker and colleagues also determined the structure of RNase A that year). While on sabbatical in David Phillip's lab at Oxford, Richards also devised a crucial new method to fit atomic models into electron density maps<sup>4</sup>. Before this point, Kendrew had used colored clips, placed on a forest of metal rods, to represent electron density. The brass atomic models of the protein were then built within the forest of rods, although this cumbersome setup severely limited the ability to see and to adjust the atomic model. Never one to sit around when presented with an important challenge, Fred set about devising a solution that was typically ingenious in its simplicity. His invention—the optical comparator (also known as the Richards Box or 'Fred's Folly')—used a half-silvered mirror placed in the right location relative to a stack of electron density contours and an atomic model (Fig. 2). By viewing the device from the proper angle, the user could simultaneously see both the model and the electron density map and could easily build and adjust the brass atomic pieces so that they fit the density. The Richards Box remained an indispensable tool used by crystallography labs around the world for about 10 years, when it was finally replaced by computer graphics systems. The earliest computer systems were referred to as 'electronic Richards Boxes', and today's programs still use the same basic superposition of electron density maps and atomic models for structure building.

Richards' contributions to protein crystallography also included early experiments that helped the young science weather the skepticism of biochemists who would frequently ask, "Why should there be any relation between the structure of a protein in a crystal and in its biologically relevant form in solution?"<sup>5</sup>. With Marilyn Doscher and

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**Figure 2** The Richards Box: an optical comparator for fitting atomic models to electron density maps. (a) The original Richards Box, which was assembled in front of David Phillips' office at Oxford while Richards was on sabbatical there in 1967–1968. The half-silvered mirror for viewing is in the upper left, the atomic model is below and the electron density map (contours printed on acetates) are in the back (photo from E. Martz). (b) The superimposed image of model and map that the user would see when using the Richards Box (photo from W. Lim).

Flo Quijcho, Richards performed decisive experiments demonstrating that enzymes are still active in their crystalline state, thus effectively silencing the nagging doubts and providing support for the crystallographer's leap of faith<sup>6,7</sup>. Never tied down to one technique, Fred also made key contributions to the use of NMR, photoaffinity labels and proton exchange to probe molecular structure<sup>8–11</sup>. In the later stages of his career, Fred helped shepherd the field as a leader in the movement to require public deposition of published structure coordinates.

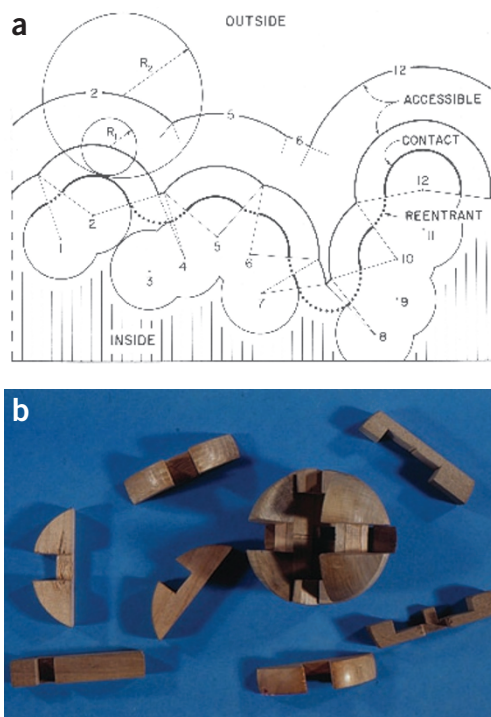
Perhaps Richards' longest-lasting impact has been on how we think about and interpret structures—developing the concepts of solvent-accessible surface area and internal packing<sup>12</sup>. The earliest protein structures yielded complex, disorganized masses that defied the elegant and obvious functional logic of the DNA double helix. Cutting through this complexity, Richards saw the need for simple geometric ways to understand protein structure and function, and in 1971, with B. K. Lee, he developed the concept of solvent-accessible surface area—the surface defined by rolling a sphere the size of solvent over the molecule<sup>13</sup> (Fig. 3). This systematic way to determine and quantify the properties of a protein's 'inside' and 'outside', as well as the stereochemical properties of the surface that it presents to other potential interaction partners, has played a central part in nearly all studies of protein folding, protein interactions and the forces that underlie them. Because of the clarity with which molecular surfaces illustrate functional characteristics of a protein, nearly every structural paper today has a molecular surface depiction determined in this basic way.

Richards also quantitatively analyzed the packing within proteins and showed that their interiors are as densely packed as small-molecule crystals<sup>12</sup>. His thinking on how proteins fold in a manner that solves the puzzle-like problem of close packing in their interiors<sup>14</sup>—allowing both maximal occlusion of hydrophobic surface area and optimization of van der Waals interactions—is central to modern methods in structure prediction and protein design. Current drug-design algorithms also trace their origins to ideas of optimizing surface complementarity.

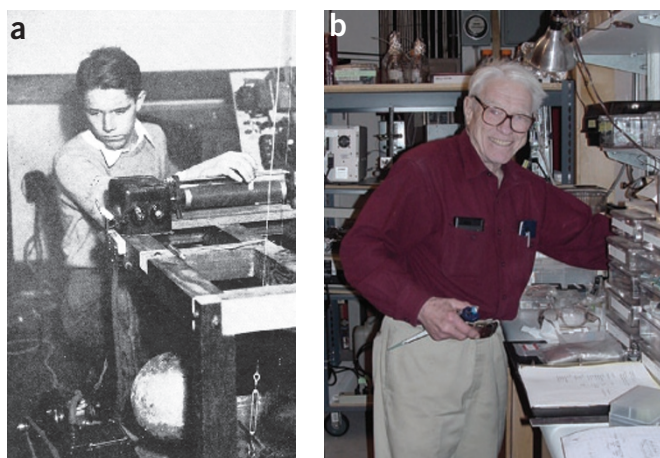
Fred deeply influenced many scientists, beyond his own students and postdocs. At Yale, Fred was the rock upon which the Department

of Molecular Biophysics and Biochemistry was built (he was the founding chair in 1967). This department went on to become one of the premier groups in modern structural biology, with seven of the faculty he hired later becoming members of the National Academy of Sciences. Fred, who was often referred to as 'the Chief', led the department with respect and dignity for all. A New England blueblood who could trace his lineage back to the pilgrims, Fred still spoke to everyone with the same square-jawed directness, respect and humor—whether you were a Nobel laureate, a wide-eyed first-year graduate student or a loyal technician. Despite a sometimes curmudgeonly veneer, it never took long before Fred would break out his famous grin. His renowned balanced thinking and sage advice led to his prominent role on diverse scientific advisory committees and as the president of several major scientific societies.

Fred's uncanny ability to home in on the heart of a problem was a key attribute that allowed him to make so many pioneering discoveries and inventions in diverse areas of structural biology. His keen abilities of perception were evident to the generations of Yale students who can recount similar stories: while attending a seminar on a distantly related topic, Fred seemingly dozes off mid-seminar, but awakens as the speaker concludes, only to ask a question that goes straight to the key issue in the field and outlines directions that the field should move in over the next 10 years. Because of his clarity of thought, Fred was not often swayed by the distracting forces of dogma and intellectual fashion. Thus, his ideas and opinions always seemed remarkably modern and never outdated.



**Figure 3** Analyzing protein structures. (a) Richards (with B. K. Lee) developed the concept of solvent-accessible surface area. These surfaces were defined by effectively rolling a sphere the size of a water molecule across the protein. This simple geometrical concept allowed a systematic and quantitative analysis of the properties of the 'inside' and 'outside' of protein structures (image from ref. 12). (b) Richards' analysis of dense packing within the interior of proteins led to his comparison of protein folding to solving a three-dimensional puzzle (slide from F. Richards).



**Figure 4** Still having fun. (a) While in high school at Philips Exeter Academy (1943), Richards and his roommate, John King, attempted to replicate the 1797 Cavendish experiment to measure the Earth's mass and gravitational constant, using a home-built apparatus (photo courtesy of D. Engelman and D. Eisenberg). (b) Richards, still working at the bench in 2004 at the age of 79 (photo by K. Fleming).

Perhaps what Fred would have wanted to be remembered for the most was the sheer joy he had for the practice of science. I recall Fred once telling me about how, as a young boy, one of his greatest pleasures was convincing his mother to take him to the chemical shops of lower Manhattan, where he would delight in searching for chemicals that

might cause pleasing explosions and such. During my postdoc with Fred in the mid-1990s, I witnessed this 70-year-old emeritus professor coming into work each day, giddy with excitement as he sweated at the hood trying to develop a new method to chemically footprint exposed surfaces on proteins<sup>15</sup> (Fig. 4). The delight that he took in pursuing his latest creative endeavor was evident. After all of those decades, little had changed—Fred Richards always knew how to have fun.

*Note: Wendell Lim was a postdoctoral fellow in Fred Richards' lab from 1992 to 1996. Much of this material was adapted from an autobiographical review by Richards<sup>4</sup>. The author wishes to thank the many admirers of Fred Richards who passed on stories and comments, with special thanks to David Eisenberg (from whom the descriptor "square-jawed directness" is taken), Bob Sauer, Tack Kuntz, Ron Raines, Gerry Olack, Karen Fleming, Melody Lan and Raghavan Varadarajan. Thanks to Eric Martz for providing pictures of the original Richards optical comparator.*

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