# Christian B. Anfinsen

*The journey not the arrival matters* — Leonard Woolf, after Montaigne

1916–1995

CHRISTIAN BOEHMER ANFINSEN died suddenly on May 14, 1995 at the age of 79. At the time he was Professor of Biophysical Chemistry at the Johns Hopkins University, a position he had assumed after his retirement from the National Institutes of Health (NIH) in 1981. In 1972 he shared, with Stanford Moore and William H. Stein of Rockefeller University, the Nobel Prize for Chemistry. He had been cited by the Swedish Royal Academy of Sciences for his "studies on ribonuclease, in particular the relationship between the amino acid sequence and the biologically active conformation."

Chris, as he was known to even the most junior member of his laboratory, was the son of Norwegian immigrants from Bergen who had settled in western Pennsylvania. His father, a road construction engineer, later moved the family to the Philadelphia area where Chris graduated from Swarthmore College in 1937 and went on to study organic chemistry at the University of Pennsylvania. A fellowship from the American-Scandinavian Foundation sent him to the Carlsberg Laboratory in Copenhagen in 1939 to study enzyme-based micromethods. The outbreak of war forced his return to the United States in 1940, but not before he had the chance to see and understand the horrors then gripping Europe. His unusually deep and active sense of social responsibility certainly dated from that period, if not earlier.

Chris entered the renowned graduate program in Biological Chemistry at Harvard Medical School where he worked with A. Baird Hastings on a problem in retinal histochemistry. He was awarded his Ph.D. degree in 1943; by 1944 he was working at Harvard in the malaria research project of Vannevar Bush's Office of Scientific Research and Development. Thirty years later some of the observations Chris made then were used in developing the current methods of culturing malaria parasites.

He returned to the Harvard Biological Chemistry Department in 1946. During the next decade he worked on a variety of projects in what historically may now be seen as the transition phase of biochemistry from the study of intermediary metabolism to that of molecular and structural biology. He used micromethods ('Cartesian Divers') for measuring metabolic processes and, with A.K. Solomon, pioneered the use of stable and radioactive isotopes for tracing metabolic pathways, including the biosynthesis and degradation of proteins. A year (1947–1948) at the Medical Nobel Institute in Stockholm, in Hugo Theorell's laboratory, led to partial purification of aconitase, with Jack Buchanan, and further hints of his evolving interest in proteins.

In 1950, to the astonishment of his Boston colleagues, Chris did the unthinkable and gave up his position as Associate Professor at Harvard and moved to the pastures of Bethesda, Maryland to become Chief of the Laboratory of Cellular Physiology in the newly created National Heart Institute of the NIH. James Shannon had recently gone to Bethesda as scientific director of the new institute and was vigorously recruiting scientists to staff a partially renovated Building 3, built in 1938 largely as an animal facility when NIH moved from Washington, D.C. Among those who responded to Shannon's vision were Julius Axelrod, Robert Berliner, Robert Bowman, Bernard Brodie, Donald Fredrickson, Edward Korn, Earl and Thressa Stadtman, Daniel Steinberg, and Sidney Udenfriend. They joined Leon Heppel, Bernard Horecker, Herman Kalckar, Arthur Kornberg and others on the staff of the Experimental Biology and Medicine Institute. This ensemble of individuals, crowded into the very small building (but later dispersed on the NIH campus after the Clinical Center opened in 1953), was a crucial seed for the explosive growth of biomedical research at the NIH that followed.

During the next five years, a whole range of publications on plasma lipoprotein metabolism emerged from Chris' lab, as well as a continuing stream of papers on protein structure. While this lipoprotein work was undoubtedly related to the interests of his new employer, it was never fondly remembered by him, although the approaches he and his colleagues developed were very important in the subsequent clarification of the genetic bases of the lipoprotein diseases.

However, it was Fred Sanger's contemporaneous work on the amino acid sequence of insulin that really excited Chris and provided the theme for the remainder of his career. Surely the technique could be made to work to determine the primary structure of an enzyme and perhaps ultimately to synthesize the protein. The availability of a ready supply of bovine pancreatic ribonuclease (RNase) from the Armour Company, a by-product of its protein fractionation work, defined the enzyme of choice. In 1954, the first paper appeared from Chris's laboratory on the structure, cross-linkages and terminal sequences of RNase. Despite its apparent irrelevance to heart disease, this work

was the beginning of fifteen years of concentrated effort on RNase.

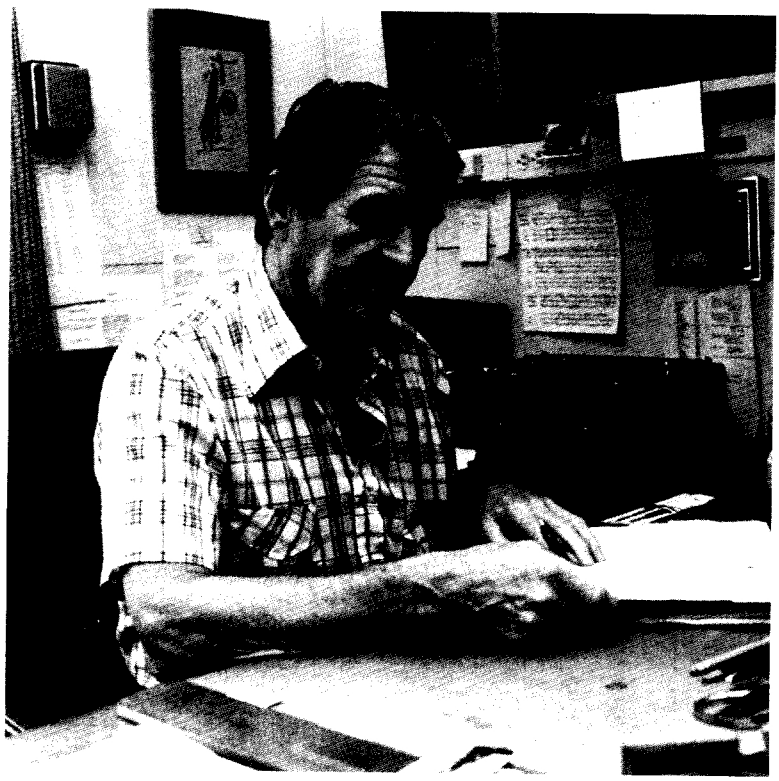
In 1954, Chris returned to the Carlsberg Laboratory on a one-year Rockefeller fellowship, to work with Kai Linderstrøm-Lang, using physical chemistry to study RNase. He joined a remarkable group of scientists, including Aase Hvidt, Martin Ottesen, Bill Harrington and John Schellman who, over the next decades, contributed greatly to defining the properties of globular proteins. Although, ironically, the main conclusion of the combined efforts of this group on RNase that "the data further support the possibility that a considerable part of the enzyme structure may be superfluous from the catalytic standpoint" did not stand up to later work, the structural concepts that Chris added to his chemical and enzymological background were of major importance in the next few years.

Upon returning to the NIH it became apparent to him that the Moore and Stein team at Rockefeller would likely complete the amino acid sequence of RNase first. Instead of an all-out effort to compete on the sequence, he chose to focus on the difficult problem of defining the disulphide cross-links among the eight cysteine residues in RNase. In 1956, during work to develop reversible cleavage techniques for the four cystine cross-links, he noted serendipitously that the disulphide bonds could reform with restoration of enzymatic activity, under certain conditions. It was at this moment that Chris' genius for getting to the essence of research problems fully manifested itself. He quickly realized that the unexpected reversibility of this process, which later included the demonstration of the restoration of secondary and tertiary structure as well as the disulphide bonds, implied that the information for the folding of a protein is contained in the amino-acid sequence. The result was also of interest to him in that it implied that the synthesis of functional proteins would only require the sequential polymerization of the appropriate amino acids.

The efforts over the next six years of Chris and his colleagues—especially Fred White, Michael Sela, Ed Haber, Charles Epstein and Robert Goldberger—supplied the detailed experimental analyses of the refolding of RNase and other proteins to lead to the full 'thermodynamic hypothesis'. This hypothesis stated that 'the three-dimensional structure of a native protein in its normal physiological milieu (solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature, and others) is the one in which the Gibbs free energy of the whole system is lowest; that is, that the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment"[1].

This simple and elegant principle has, over the last third-of-a-century, become part of the fundamental paradigm of molecular biology, as well as the basis of vast biotechnology efforts. The enormous skepticism at the time among the scientific establishment to both the experimental results and their interpretation has now been largely forgotten. Indeed, to some the concepts were considered—consistent with J.B.S. Haldane's quip about the response to all new ideas—to have been self-evident. On the other hand, in recent years work on chaperones has been interpreted by some to contradict Chris' hypothesis. This confusion between thermodynamic and kinetic analyses, common in many other areas of science, misses the fundamental nature of the contribution. In fact, Chris and his colleagues—Franco deLorenzo, David Givol and Sara Fuchs—were the first to identify and characterize a chaperone, the protein-disulphide isomerase. Although Chris participated actively in both kinetic and equilibrium experiments (including with this author), at heart he favoured a thermodynamic approach. He was more interested in finding out what existed in nature or what one could synthesize, then the details of the process being studied.

From 1962 to 1963, Chris returned for a year to Harvard as Professor of Biological Chemistry, but was recruited back to the NIH in 1963 by J.E. Rall to head a newly created Laboratory of Chemical Biology in the National Institute of Arthritis and Metabolic Diseases. At this point a new model protein, staphylococcal nuclease, was added to his



Chris photographed in his office at the National Institutes of Health in the late 1970s with the typewriter (background) that he used to write all his notes and letters.

Linderstrøm-Lang's caricature poster of Chris Anfinsen exhibited at the Carlsberg Laboratory Christmas party, 1954 (Courtesy of Carlsberg Foundation Picture Archives, Copenhagen).

experimental program and over the next decade it became the centre of his research interest. Its lack of disulphide bonds and its great stability made it a better experimental system than RNase to study the process of protein folding by synthetically altering individual amino-acid residues. During this period Chris, and a very large number of young colleagues, used organic synthetic, chemical, enzymological, biophysical, immunological and genetic approaches to study the properties of this protein and its folding. A folding mechanism incorporating 'flickering' secondary structure nucleation sites and subsequent condensation of tertiary structure was evolved that is not much different from today's concepts. By the mid-1970's Chris' group, with the help of a crystal structure determination by F.A. Cotton and his associates, had added 'staph' nuclease to the small number of globular proteins, including hemoglobin, myoglobin, lysozyme as well as RNase, which had been most thoroughly characterized. The goal of synthesis of nuclease and its analogues was partially achieved with 'semi-synthesis', by condensation of fragments made by the solid-phase method. Recombinant DNA technologies ultimately made this goal fully practical.

Along this reseach path Chris—with Iku Kato, Meir Wilchek and Pedro Cuatrecasas—was also key in establishing affinity chromatography as a major tool for biochemistry. In the years following these studies and the award of the Nobel Prize, Chris contributed significantly to the purification of human interferon and later to the study of highly thermostable enzymes. Indeed at the time of his death he was funded by the National Science Foundation for developing thermostable enzymes for the remediation of environmental contamination.

This summary of Chris' scientific accomplishments would be incomplete without mention of several other aspects of his career. In 1959 he published the monograph *The Molecular Basis of Evolution*[2]. Appearing at the time of the Darwin centennial, this book was important in initiating the current era of the use of macromolecular structure information in evolutionary analyses. It was highly influential in shaping the perspective of many of us who entered science in the following decade. His editorship, since 1957, of *Advances in Protein Chemistry*, most recently with John Edsall, Fred Richards, and David Eisenberg, is considered by many to have been equally influential in defining the field of protein chemistry.

Chris was also instrumental in making the NIH much more like a university than a typical government laboratory. He helped create The Foundation for Advanced Education in the Sciences at the NIH and other teaching programs—modelled after the Swarthmore honors seminars—which made the NIH an unusually excellent learning experience for the many young scientists, especially physicians, who came during his three decades there.

Chris' laboratory was an international mecca for scientists. As he said, the best way to promote scientific exchange among countries is to exchange scientists. Since his 1957 visit to Israel, at the invitation of Michael Sela and Ephraim and Aaron Katchalski, Chris maintained a deep interest in that country and its science. He frequently visited and served for almost four decades on the Board of Governors of the Weizmann Institute of Science in Rehovot. This deep interest later extended to the Hebrew language and the Jewish religion.

Chris' sensitivity to political issues was manifest, among other activities, in his work leading up to the 1963 treaty banning atmospheric nuclear testing, in his opposition to the US involvement in Vietnam, and in his human rights activism, especially with regard to scientists in the USSR and in Latin America. For a number of years he chaired the human rights committee of the US National Academy of Sciences.

Playing the piano or the viola and, most of all, sailing provided him with limited escapes from science. In sailing, as in all things, he was always the optimist; his casualness in his sailboat made these voyages only for the intrepid. However, when crises occurred on the water, everyone knew that Chris would solve the problem, and he always did.

But above all of his professional activities was Chris' impeccable standards of personal behaviour, whether he was your teacher, colleague, collaborator, competitor, boss or friend. The roles melded completely into each other; all individuals, from the stock clerk to the director of the Institutes, were treated as fellow crew members in the quest to understand nature.

Chris never looked back. Even the Nobel lecture had to have new, unpublished data. Only death has finally stopped a research journey which began with his trip to Copenhagen fifty-six years ago.

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Christian Boehmer Anfinsen, biochemist; born Monessen, Pennsylvania, 26 March 1916; B.A., Swarthmore College 1937; M.S., University of Pennsylvania, 1939; Ph.D., Harvard University Medical School; professor, Harvard 1946-1950, 1962-1963; laboratory chief, National Institutes of Health, 1950-1962, 1963-1981; professor, Weizmann Institute 1981-1982; professor, Johns Hopkins, 1982-1995; Nobel Prize in Chemistry, 1972; marriage to Florence Kenenger, 1941-1978 (one son, two daughters); married Libby Shulman Ely 1979; died Pikesville, Maryland, 14 May 1995.

**1.** Anfinsen, C.B. Principles that govern the folding of protein chains. (Nobel Lecture) *Science* **181**, 223–230 (1973).
**2.** Anfinsen, C.B. The Molecular Basis of Evolution (John Wiley, New York; 1959).