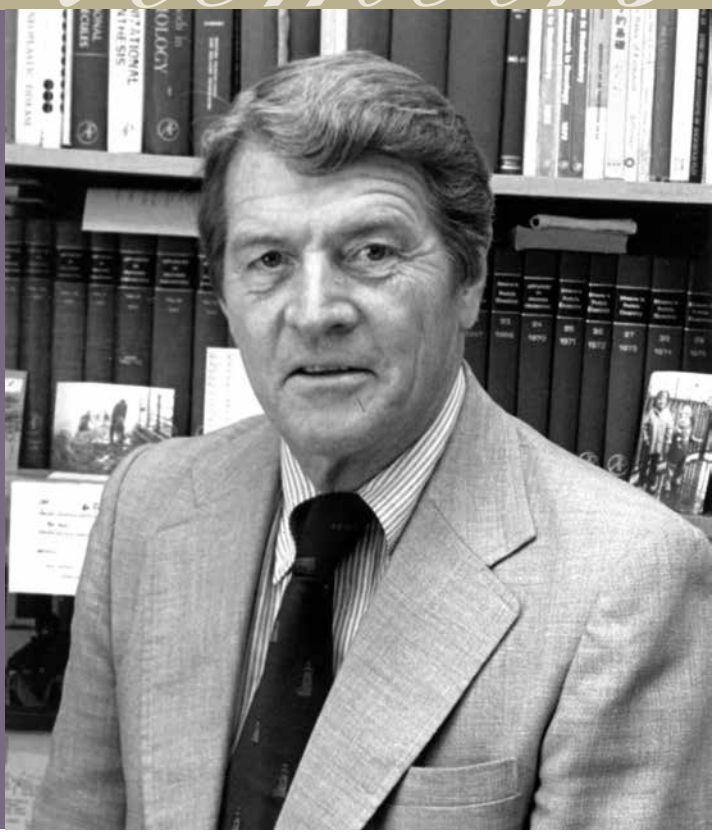
Christian B. Anfinsen

1916–1995

BIOGRAPHICAL *Memoirs*



A Biographical Memoir by
Alan N. Schechter

NATIONAL ACADEMY OF SCIENCES

CHRISTIAN BOEHMER **ANFINSEN**

March 26, 1916–May 14, 1995

Elected to the NAS, 1963

By Alan N. Schechter

In the spring of 1959 a little-known biochemist at the National Heart Institute sent an ambitious manuscript, titled "The Molecular Basis of Evolution" to John Wiley & Sons. The resulting book, published later that year, was the first rigorous attempt to integrate the newly developing field of protein chemistry with the classical concepts of genetics. More important, the book was based on the theme, expressed in the preface, that "Everyone in science must be interested in the evolutionary process as the central theme of biology." As a result, *The Molecular Basis of Evolution* helped set the stage for the phenomenal flowering of molecular biology—notably those aspects based on the chemical-sequence determinations of proteins and nucleic acids—in the decades that followed.

This memoir traces the background and accomplishments of that biochemist, Christian B. Anfinsen, employed by one of the institutes of the National Institutes of Health (NIH)—a U.S. government entity not yet renowned for making scientific or medical advances—when he wrote his book. At that time he also was involved in experimental studies of proteins that would lead to his sharing of the 1972 Nobel Prize in Chemistry "for his work on ribonuclease, especially concerning the connection between the amino acid sequence and the biologically active conformation" (as stated in the award citation).

Anfinsen was born in Monessen, Pennsylvania, the son of Norwegian immigrants from Bergen who had moved to the United States so that his father, a road-construction engineer, could work in that then-flourishing steel town. In the 1920s the family moved to the Philadelphia area, where he grew up and attended college—Swarthmore College, which was just then gaining recognition for its innovative curriculum as well as the seminars for select juniors and seniors based on the Oxford University model. However, Anfinsen later acknowledged that academic pursuits were not then his highest priorities; among other extracurricular activities, he played on this small college's football team.

Anfinsen earned a B.S. from Swarthmore in 1937, and he went on to study organic chemistry at the University of Pennsylvania, receiving an M.S. in 1939.

Soon after, he secured a fellowship from the American-Scandinavian Foundation to study enzyme-based micro-methods at the Carlsberg Laboratory in Copenhagen, where Anfinsen came under the scientific and personal mentorship of the protein chemist Kaj Linderstrøm-Lang.[1] This interaction was cut short in 1940 by the spread of World War II to Scandinavia, whereupon Anfinsen returned home. But this visit, together with several other overseas fellowships later on, influenced Anfinsen's scientific outlook and experimental approaches throughout his lifetime. He often noted that scientific exchange (i.e. advances) readily occurs through such "exchanges of scientists" (e.g., visiting fellowships) and not through the formal delegations that were so prominent during the latter period of the Cold War or through the brief scientific meetings that are so abundant in this era of jet travel.

After returning to the United States, Anfinsen made two life-changing commitments: he entered the graduate program in biological chemistry at Harvard Medical School, where he pursued his interest in micro-enzymatic methods by studying retinal histochemistry with A. Baird Hastings; and in 1941 he married Florence ("Flossie") Kenenger—a marriage that produced three children and lasted until their divorce in 1978.

Anfinsen received his Ph.D. in 1943 after only three years, an outcome presumably catalyzed by the war effort. He immediately began working at Harvard on the malaria research program—via a contract with the federal office of Scientific Research and Development, then under the direction of Vannevar Bush—which resulted in Anfinsen's first publications. He was always particularly proud that one of his achievements during that period, his method of *in vitro* culture of malaria parasites at reduced oxygen concentrations, was an important step in the program's research, although it had to be rediscovered by others decades later.

Until 1950 Anfinsen was employed primarily by Harvard University, working in several different departments and tackling a variety of scientific problems. He returned to elegant micro-methods, using so-called Cartesian divers, and also began studies using proteolytic enzymes on protein preparations. In addition, he established the use of stable and radioactive isotopes—just then becoming available from the Manhattan Project—in

1 Among other American protein chemists who trained at Carlsberg (with A. Hvidt and M. Ottesen as well as K. Linderstrøm-Lang) were W. Harrington, W. Kauzmann, R. Lumry, F. Richards, and J. Schellman, many of whom remained close to Anfinsen throughout their lives.

This stay abroad, and others to come, reflected his appreciation of the need to constantly broaden one's scientific perspective, his need to indulge a wanderlust (with an emphasis on his Scandinavian roots), and his discomfort with the hierarchy of institutional bureaucracies.

the study of metabolic processes, including the biosynthesis of proteins. Much of this research was done with Arthur K. Solomon, then back from training in Cambridge, UK.

During part of this time Anfinsen was supported by Henry K. Beecher (who had also done research at Carlsberg) in the Department of Anesthesiology at Boston's Massachusetts General Hospital. Beecher was one of the pioneers in the integration of basic and clinical science, a concept that might have figured in Anfinsen's decision to move in 1950 to Bethesda, MD, and join a new hybrid research institution—the NIH—which had evolving plans to place laboratories and clinics on the same floors of a research hospital, then under construction.

Anfinsen's fellowship of a few years earlier, from 1947 to 1948, in Hugo Theorell's laboratory at the Medical Nobel Institute in Stockholm had marked the beginning of his focus on the new fields of enzyme purification and protein characterization. This stay abroad, and others to come, reflected his appreciation of the need to constantly broaden one's scientific perspective, his need to indulge a wanderlust (with an emphasis on his Scandinavian roots), and his discomfort with the hierarchy of institutional bureaucracies. The latter factor likely played into Anfinsen's decision to relinquish his position at Harvard, where he was already climbing the academic ladder, and become chief of the Laboratory of Cellular Physiology and Metabolism in the newly created National Heart Institute of the NIH.

James A. Shannon, who was a leader of the New York University Medical School's branch of the wartime malaria project, had become scientific director of the National Heart Institute—one of four disease-category institutes at the NIH (which had previously been the National Institute [singular] of Health). Within a few short years, Shannon packed an amazing array of distinguished scientists into his warren of cramped but ambitious laboratories.[2] By 1953, Anfinsen was able to move his gradually expanding laboratory

2 During this period Shannon was also able to squeeze J. Axelrod, R. Berliner, R. Bowman, B. Brodie, D. Fredrickson, E. Korn, E. Stadtman, T. Stadtman, D. Steinberg, and S. Udenfriend, among others, into a former animal facility, "Building 3."

into more spacious and functional quarters in the newly opened Clinical Center, said to be the largest brick building (with some 7,000,000 bricks) in the world. Shannon and another physician, Robert Berliner, were Anfinsen's "bosses" (from 1950 until his temporary departure in 1962) in the informal National Heart Institute hierarchy, but having recognized his scientific potential from the beginning they were also his strong supporters.

In addition to his work on protein structure, Anfinsen initiated a number of projects at NIH apparently related to the interests of his new employer, as he had done at Harvard—a lesson sometimes overlooked in the present oft-stated quest of some scientists for total "scientific freedom." These projects included the study of biological oxidations, lipoprotein metabolism, and atherosclerosis. Indeed, he continued to publish in the area of lipids until 1959 and trained many of the subsequent leaders of the field, including Daniel Steinberg, Martha Vaughan, and Donald Fredrickson.

Later, when a separate Laboratory of Metabolism was split off, Anfinsen's unit became the Laboratory of Cellular Physiology. He was always willing to allow such subdividing and never let his own administrative responsibility grow larger than three independent sections. In that way, he was able to keep his focus on his own research yet also have regular interactions with other scientists pertaining to their projects.

Anfinsen's nascent interest in protein structure clearly was sparked by Fred Sanger's contemporaneous work in the early 1950s, at the Medical Research Council Laboratory in Cambridge, UK, on determining the amino acid sequence of insulin. As often told, Chicago's Armour Co., a meat processor, had a large supply of bovine pancreatic ribonuclease (RNAse), presumably a byproduct of insulin extraction for medical uses, which was available to Anfinsen. By 1954, he had published his first report on the general properties of this small disulfide-linked protein and had begun studies with proteolytic enzymes to obtain peptides for amino acid sequence determination.

With NIH being quite generous then in allowing stays abroad, Anfinsen spent time at the Carlsberg Laboratory again to work with Linderstrøm-Lang and others on the physical biochemistry of RNAse. On his return to NIH a year later, it became clear that the well-established protein chemistry laboratory of William H. Stein and Stanford Moore at the Rockefeller Institute was going to achieve the sequence determination of RNAse first. But the loss of this "race"—which concerned some other scientists more than Anfinsen, who during his career tended to keep the big picture, as opposed to isolated competitions, in mind—ultimately led him to frame and answer a question of

more general importance than the specific sequence of RNAse. This question, regarding the ability of proteins to spontaneously fold, arose from his study of the breakage and formation of disulfide bonds from the pairing of the eight cysteine residues in RNAse.

Stein and Moore had used irreversible oxidation methods to cleave the disulfide bonds for sequencing, while Anfinsen had used other reagents, some of which—iodoacetic acid and mercaptoethanol, for example—allowed for reversibility. In both cases, enzymatic activity was lost with full disulfide cleavage, but the reversible reagents also required use of denaturing conditions, such as 8M urea, to achieve full reduction. From about 1957 to 1960, these studies were pursued in light of previous results, which suggested that peptide active centers of enzymes could be isolated from larger globular proteins and which were interpreted in terms of essential and nonessential disulfide bonds. But Anfinsen gradually abandoned this hypothesis.

Anfinsen and his colleagues[3] had noted that removal both of the denaturing agent and the reducing agent allowed return of some enzymatic activity under oxidizing conditions, such as exposure to room air. He came to realize that this trace of activity, which most scientists would probably have dismissed as uninteresting, suggested a different hypothesis—that in solution, without other macromolecules (as potential templates) or folding enzymes present, an unfolded and even reversibly modified protein could refold to its chemical active form.

The initial reports (around 1961) of the return of enzymatic activity were not well received by all in the enzymology community, and there were many who, reflecting J. B. Haldane's famous four-stage characterization of the resistance to new scientific ideas,[4] questioned the validity or importance of these results. In response, for the next several years Anfinsen and his colleagues worked out the conditions to optimize the rate and extent of return of activity, and they showed that parameters of protein tertiary structure (and some measures of secondary structure, including the disulfide bonds themselves) had been restored and that the phenomenon could be demonstrated for a number of other proteins. Gradually, the Anfinsen team's experimental results and their implications were accepted. Perhaps the team's paper, "Genetic Control of Tertiary

3 Scientists who worked on ribonuclease with Anfinsen, generally at the postdoctoral-fellowship level, included R. Redfield, W. Carroll, M. Sela, F. White, Jr., E. Haber, J. Potts, C. Epstein, R. Goldberger, D. Givol, F. De Lorenzo, and I. Kato.

4 "The four stages of acceptance: 1. This is worthless nonsense. 2. This is an interesting, but perverse, point of view. 3. This is true but quite unimportant. 4. I always said so."

Protein Structure: Studies with Model Systems" (Epstein, Goldberger, and Anfinsen 1963), was the tipping point for this new thermodynamic hypothesis of protein folding.

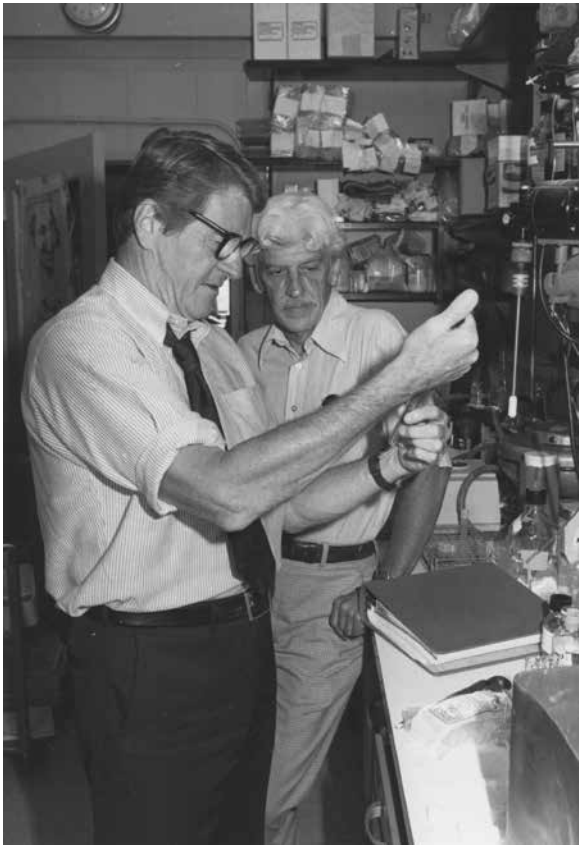
The hypothesis is stated in Anfinsen's 1973 *Science* paper based on his Nobel lecture:

> *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.…In terms of natural selection through the 'design' of macromolecules during evolution, this idea emphasize[s] the fact that a protein molecule only makes stable, structural sense when it exists under conditions similar to those for which it was selected—the so-called physiological state. (Anfinsen 1973)*

This concept quickly became a fundamental extension of the 1950s paradigm of molecular biology, the "central dogma" that DNA→RNA→protein. Ironically, as often occurs in science, many of the naysayers of the validity of the thermodynamic hypothesis were succeeded by those who said it was almost trivial and self-evident. In addition to its elegance and theoretical importance, the thermodynamic hypothesis helped jumpstart the field of biotechnology; as Anfinsen realized, it implied that chemical- or DNA/RNA-directed synthesis of proteins should be feasible. Such in vitro systems would not be expected to require templates of any kind to actuate the linear sequence if that sequence were properly constructed. Note also Anfinsen's continued attention to the importance of natural selection and evolution.

The later birth of the field of chaperones, which catalyze the folding or refolding of proteins within cells, has led some to question the importance or even the validity of the thermodynamic hypothesis. But I believe that these concerns represent a fundamental confusion about the alternate ways—thermodynamic and kinetic—of looking at biochemical processes. Although Anfinsen and his colleagues (including this author) did a variety of studies on the kinetics of refolding, their fundamental interest was always in the final biologically active structure(s) of the globular proteins. Thus from its beginning the concept was deemed a *thermodynamic* hypothesis. It was this structure, or limited range of structures, that was crucial for the enzymatic activity or function and its modulation or allosteric control. Anfinsen and his colleagues did not concentrate on the details

of the pathways by which these structures were obtained in vitro or in various in vivo systems, including synthesis in cells. Indeed interpreting kinetic studies has proven of great difficulty to this day, with slow progress in modeling. There even remains uncertainty as to whether most proteins follow one or a few pathways of folding or if folding can occur by very large, almost random, numbers of pathways. Further the characterization in Anfinsen's laboratory of the protein, later known as protein disulfide isomerase, from liver tissue, was part of a search for ways to catalyze the refolding of disulfide-linked proteins and rectify incorrectly-linked proteins. The relevant publication in 1964 may well be considered the beginning of the important discoveries of the roles of chaperones in catalyzing the folding or refolding of proteins within cells.



C. B. Anfinsen and J. E. Rall in the lab, the usual forum for scientific or administrative discussion with Anfinsen. Rall, who was scientific director of the NIAMD, recruited Anfinsen back from Harvard Medical School in 1963, after one year in Boston and remained a close friend and scientific colleague. (Picture from NIH files)

In 1962 Anfinsen returned to Harvard Medical School, where he was said to be in line for chairing the well-respected Department of Biological Chemistry. But he found some aspects of the school's overall atmosphere to be no better than during his previous employment there. Within a year, Anfinsen was back at the NIH, recruited to the National Institute of Arthritis and Metabolic Diseases (NIAMD)[5] by its new scientific director, J. Edward Rall, who became a close friend and major supporter over the next several decades. Anfinsen's main role during this time was to lead the NIAMD's Laboratory of Chemical

5 The NIAMD is now known as the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Biology, located two floors above his old National Heart Institute space in the Clinical Center.

Here Anfinsen changed his research focus, as he tended to do every 10 years or so, from the study of RNAse as a model protein to Staphylococcal nuclease instead. The latter is a relatively small, soluble, and stable protein—as is RNAse—but, unlike RNAse, Staphylococcal nuclease lacks reactive cysteine residues, which make total chemical synthesis of this protein simpler. Bruce Merrifield at Rockefeller Institute had initiated studies of solid-phase protein synthesis a few years before, and in 1963 he published the first major paper on the method. Anfinsen began studies simultaneously on the isolation and characterization of Staphylococcal nuclease[6] and of methods to apply and optimize the solid-phase synthetic methods.

Anfinsen at his desk in the Laboratory of Chemical Biology. He spent brief periods during the day here, with his trusty Royal typewriter and a limited range of mementos. The picture was probably taken just before his retirement in 1981, as the lower shelf of the bookcase usually held a complete set of *Advances in Protein Chemistry*, which likely had been shipped to Israel.
(Picture from NIH files)

Anfinsen's courage in returning to methodologies of classical chemistry, from which he had been largely removed for almost 25 years, and his courage in other methodological changes, should be noted as basic traits of his research career. Interestingly, as X-ray diffraction-determined structures of globular proteins were solved (including of the nuclease); he eagerly incorporated this information into his approaches, unlike many other protein chemists.

Unfortunately, the development of peptide synthetic methods, whether in solution or in solid phase, proved inadequate for the robust synthesis of peptides/proteins of more than a few dozen residues, and the goal of total synthesis was never accomplished. Rather, complementation of protein fragments was used to study structure-function relations.

6 Scientists who worked on Staphylococcal nuclease with Anfinsen included H. Taniuchi, S. Fuchs, P. Cuatrecasas, D. Ontjes, M. Ohno, G. Omenn, A. Schechter, L. Moravek, M. Wilchek, I. Chaiken, H. Epstein, I. Parikh, D. Sachs, B. Dunn, and B. Furie.

Indeed, except for short peptides, the development of recombinant DNA methods for expressing proteins in various cellular systems largely supplanted the decade of focus on chemical synthesis.

However, the focus on nuclease continued for almost a decade, and Anfinsen and his colleagues helped develop other concepts and methods in the study of globular proteins in solution. Among them were techniques for labeling active sites; affinity chromatography based on immobilization of specific ligands; discovery of the immunogenicity of peptide fragments; and the idea that while proteins ordinarily flicker among closely related conformations, the binding of ligands or substrates limits such flickering and tends to stabilize a conformation.

The 1972 Nobel Prize in Chemistry, shared by Anfinsen and the team of Stein and Moore, was both a culmination of and an end to 20 years of study of model proteins for the purpose of deriving insights into the relationships among the primary, secondary, and tertiary levels of structure (Linderstrøm-Lang's terminology). Examining Anfinsen's 100 or so primary publications of this period, one is struck by the very broad palette of techniques—including new isolation methods, enzymology, amino acid sequencing, chemical synthesis, immunochemistry, physical biochemistry, and others—that he used to obtain as complete a view as possible of the protein being studied.

After the awarding of the Nobel Prize, and the attendant year or more of resulting distractions, Anfinsen again opted for a new area of research. He initiated a program to isolate interferon, then considered a potential therapy for various cancers as well as an antiviral agent. Moderate progress occurred, but again the development of DNA methodologies would scoop the classical protein chemistry approach.

In 1981 Anfinsen retired from NIH, largely in order to move to Israel, where he had gained many friends and a deep concern about the country's struggles. He had been appointed head of a biotechnology company to be spun off from the Weizmann Institute and based in Rehovot. But the position did not materialize—the potential of this new field was not yet apparent to investors—and probably would not have suited his laissez-faire style of management and avoidance of administrative burdens. He returned to the United States after one year to a home in a Baltimore suburb—and to his modest bayside vacation home, with a dock, in Annapolis—and he became a professor of biophysical chemistry at the Johns Hopkins University. Anfinsen remained professionally active for the rest of his life, adequately funded to study extremely thermostable enzymes

both for understanding the chemistry of their resistance to heat denaturation and for their potential applications.

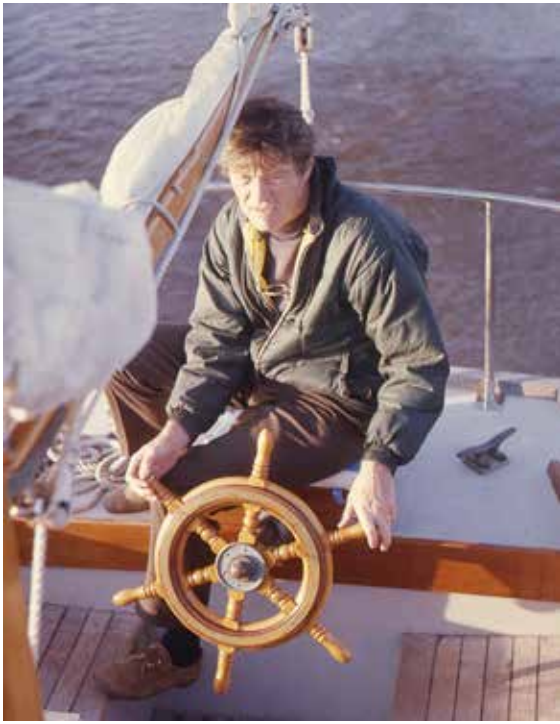
Anfinsen was blessed with an adventuresome personality, but I believe that much of his courage in delving into diverse scientific problems—over an active career of more than 50 years—also stemmed from his several years abroad on fellowships. During those sojourns he was able to learn new approaches from the laboratories he visited and to make numerous contacts among other visiting scientists (as well as among local scientists), many of whom became close friends. In addition to his several stays in Scandinavia, Anfinsen spent periods not only at the Weizmann Institute but also at the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK.

Perhaps just as important, Anfinsen was always willing to collaborate and learn from others, no matter how junior they might be. Also, I remember him pushing vats of cell-growth media across the NIH campus, from the Clinical Center to a unit designed for large-scale cell culture, well after he had won the Nobel Prize—another indication of his style. Concerns about authorship or credit were never apparent with him, if indeed they even existed. The difference between that mode of doing science and the current emphasis on competition—in grants, publications, and now patents—seems obvious to this author, although others tell me there was more such competition in those days than I've realized.

Anfinsen influenced the development of modern macromolecular chemistry not only through his diverse research but also as a result of his extensive outreach efforts. In addition to writing the seminal book *The Molecular Basis of Evolution*, he served for decades as coeditor of *Advances in Protein Chemistry,* and he authored many influential review articles, including his Nobel lecture.

Two other professional activities characterized Anfinsen's NIH years. First, he was instrumental in the 1959 creation of the in-house Foundation for Advanced Education, tasked with helping to make NIH more like a university than was true at most other government agencies. The Foundation offered a wide range of courses and other academic activities, such as a graduate degree program, a bookstore, and a music series. He also helped devise a seminar learning program at NIH—modeled on Oxford and Swarthmore—that was aimed at the large number of physicians coming for research and clinical training there in the era before the general availability of M.D./Ph.D. programs.

During the 1960s, Anfinsen and David Davies led an evening seminar on protein structure. Each week, one participant presented a review of a particular topic, and throughout the year the group constructed models of various proteins as their atomic coordinates were being solved. These seminars helped populate many medical schools with leaders who understood both basic and clinical research and who were insightful about the training required for such careers. Indeed, on the whole Anfinsen was probably more successful in training physicians than in training other basic scientists.



Anfinsen on the water at the helm of his 31-foot ketch "Good Girl." (Picture courtesy Bruce Furie.)

Second and quite unusual for a government scientist, in the late 1950s Anfinsen was one of the founders of the Federation of American Scientists, whose efforts helped bring about the 1963 treaty that banned the atmospheric testing of nuclear weapons. Subsequently he became active in the anti-Vietnam war movement and in activities to enhance the freedom of scientists, especially the dissidents in the Soviet Union and Latin America. He also participated energetically in the human rights committee of the U.S. National Academy of Sciences and was almost always willing to sign diverse petitions on behalf of the scientific community.

As noted earlier, Anfinsen had an especially strong connection to Israel. His 1957 visit to the Weizmann Institute of Science, following Michael Sela's stay at NIH, had a profound effect both on his science and his personal life. He became close friends with Ephraim and Aharon Katchalski-Katzir and other leaders of Israeli science, and he was acquainted with many of the country's political leaders. Anfinsen collaborated on numerous projects with the chemistry and immunochemistry groups at the Weizmann Institute and was a

longtime member of its advisory committee. In addition, he became very interested in the Hebrew language and Jewish history and religion. At the time of his second marriage, to Libby Shulman Ely in 1979, Anfinsen formally converted to Judaism and afterward followed many of its religious practices.

Those fortunate enough to know Anfinsen well were also aware of his musical interests—he played the piano and viola—and of his love of sailing, even under conditions when only the most intrepid would dare to venture forth. Crew members, including those with vast sailing experience, were often concerned about but invariably impressed with his optimism and bravery on the water—traits that also were key to his research successes.

But for all his professional and personal accomplishments, one most remembers the immense respect he commanded for his high standards, modesty, and high regard for all human beings; stock clerks and visiting Grand Poobahs were treated equally and with grace. He suffered from occasional periods of depression, but they were not evident to most of his colleagues. To paraphrase Leonard Woolf,[7] for Anfinsen it was "the journey, not the arrival, that mattered," and that journey was still going full steam even on the day of his death.[8]

7 Quoted previously in A. N. Schechter. 1995. Christian B. Anfinsen. *Nature Structural Biology* 2:621–623.

8 Anfinsen's papers are available at the National Librabry of Medicine (http://oculus.nlm.nih.gov/anfinsen) and further biographical information is available at NLM's *Profiles in Science* series (http://profiles.nlm.nih.gov/ps/retrieve/Collection/CID/KK).

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