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Dear David:

Re: our conversation in Boston (I think it was with you!) concerning Lisa Steiner's MD thesis at Yale from my lab, concerning Cys residues in RNase-A.

Lisa had most of her lab work done before Chris' first papers appeared, but the written thesis was not submitted until 1959. It's an amusing example of how hard it can be to see what is in front of your eyes!

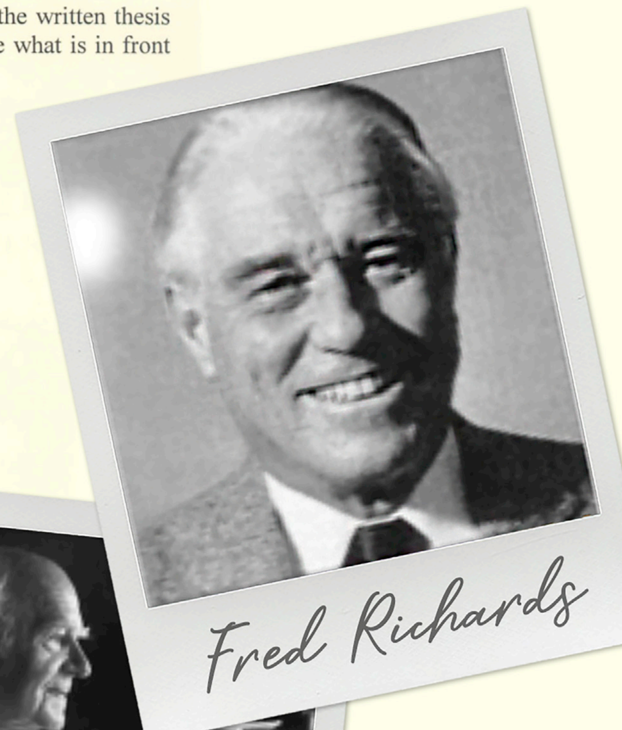
Best wishes,

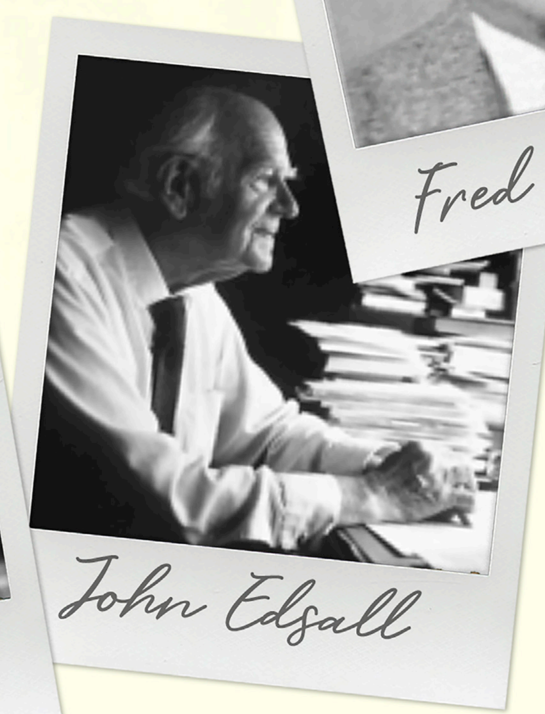
Fred

Frederic M. Richards
Senior Research Scientist
Sterling Professor Emeritus of
Molecular Biophysics & Biochemistry

FMR/jem

Enclosure: some papers from Steiner's thesis





How Hard It Is Seeing What Is in Front of Your Eyes

David S. Eisenberg

The central pillar of protein science is that amino acid sequence determines protein structure and function. This idea emerged from an experiment in the NIH laboratory of Christian Anfinsen in the late 1950s and early 1960s and led to the award of the Nobel Prize to Anfinsen in 1972 for “the connection between the amino acid sequence and the biologically active conformation.” In this experiment, the enzyme ribonuclease A was unfolded in urea and a thiol reagent, and when these agents were removed, the enzyme spontaneously refolded, recovering its structure and function. Astonishingly, essentially the same experiment had been performed earlier by a medical student at Yale, but neither her research supervisor nor her department chair thought it particularly significant, and her work was not published. Why did this transformative result lay hidden in her thesis? What does this tell us about the process of scientific discovery?

I learned of this situation in July 1995, at the annual lunch meeting of the editors of the review series Advances in Protein Chemistry. Our senior editor at 92 years of age was John T. Edsall, still interested in new developments of the field that he had helped to shape decades before. I was seated next to Fred Richards, the next most senior editor. This was our first annual meeting without our charismatic co-editor Chris Anfinsen, who had died two months earlier. Anfinsen was on our minds, and I was eager to learn more about his famous experiment. At the end of our business lunch, while Edsall was otherwise occupied, I took the opportunity to ask Fred Richards, “What did you think at the time of Anfinsen’s great experiment?” Richards responded, “I didn’t think much of it. My student had done the same experiment a couple of years before Chris.”

Why did this transformative result lay hidden in her thesis?

I didn’t know how to respond to this startling statement. Awkwardly I asked, “Where did you publish it?” Richards looked down at the table and said, “Didn’t. Didn’t think it was worth it.”

Now I really didn’t know what to say, but rescuing us from further awkward exchanges, Edsall stood up to leave; lunch was over. Walking out of the restaurant, we encountered the nearly unbearable heat and humidity of Boston at its most uncomfortable. I was concerned about Edsall’s health, wearing his traditional blue woolen suit and planning to return to his apartment in Cambridge by the way he arrived, on the underground. “What about a taxi,” I suggested and pointed to the cab stand down the block. Edsall gave his agreement, and as we strolled, I asked, “Did you hear what Fred Richards said about the Anfinsen experiment at the end of lunch?” Edsall said he had not, and I repeated the brief conversation. “How could that be?” I asked. “Fred is so creative, so articulate, so scientifically ambitious. Why would he have failed to publish that result?” From John Edsall, the contemplative scholar, I did not expect a snap answer. As he slowly folded his tall frame into the cab, he said, “I don’t know, David. I’ll think about it and get back to you.”

Edsall did so, six weeks later, but just one week following our meeting, I received a letter from Fred Richards:

> Dear David:
> Re: our conversation in Boston (I think it was with you!) concerning Lisa Steiner’s MD thesis at Yale from my lab, concerning Cys residues in RNase-A.
> Lisa had most of her lab work done before Chris’ first papers appeared, but the written thesis was not submitted until 1959. It’s an amusing example of how hard it can be to see what is in front of your eyes!
> Best wishes, Fred

Richards enclosed several pages from the thesis with the critical data and its conclusion: “Air oxidation of fully or partially inactivated protein resulted, in some cases, in the recovery of up to

July 1995 letter from Prof. Fred Richards to the author noting “how hard it can be to see what is in front of your eyes.” Superimposed are photos of Lisa Steiner, Fred Richards, Chris Anfinsen, and John Edsall.

40 per cent of the enzyme activity which had been lost as a result of reduction." The letter reinforced what Richards had told me in the restaurant, but it did nothing to clear up the puzzle of why a leading researcher had not seen what was in front of his eyes. The letter also disclosed the name of the student researcher. A few minutes on the internet revealed that Lisa Steiner was then a full professor at MIT. Apparently, the lack of this early publication had not been ruinous to her scientific career.

Seven years later, in his 100th year, John Edsall passed away. He had been a pioneer of modern protein chemistry, and in different generations of his long career, he had been a mentor in one way or another to Chris Anfinsen, Fred Richards, and me, among many others. In the receiving line at his memorial gathering in Cambridge, Massachusetts, I found myself standing next to a woman of sufficient maturity to have been a med student 45 years before, with the name tag Lisa Steiner. I braved a self-introduction, and Prof. Steiner said she would tell me more about her thesis on my next trip to Boston.

On a rainy morning several months later, in a coffee shop on Beacon Hill, I asked Steiner how she became interested in science and entered Richards's lab. She related that she was born in Vienna, where after the country's 1938 annexation by Nazi Germany, her mother was wise enough to see that Austria was not a good place to raise a Jewish family. They moved in several steps to Kew Gardens in Queens, New York, where Lisa attended Forest Hills High School. She played the violin, was on the math team, and was one of the 40 finalists in the Westinghouse Science Talent Search. On a scholarship to Swarthmore, she majored in math and minored in biology, graduating with highest honors. For graduate school in math she chose Harvard, aware that Princeton did not accept women. But during her initial year, she yearned for an occupation more socially relevant. Deciding to become a physician, she chose Yale partly because Yale required a research thesis. There she ran into Maxine Singer, whom she had known at Swarthmore. Singer suggested Lisa might join the lab of a new assistant professor of biochemistry, Fred Richards. Richards suggested the project of denaturing ribonuclease A and attempting to renature it, on which she started lab work in the summer of 1956.

When I asked Steiner how she felt when Anfinsen's papers appeared, she said, "They made me feel good because they found the same result as I did." When I asked how she felt about the adulation and awards that Anfinsen received for finding what she had found, she replied: "That was only right, David. He understood the significance. We did not." Her manner disclosed no hint of self-pity or bitterness. When I asked "Was there any discussion of publication," she replied, "No discussion." And then, in case I might get the impression that she was criticizing Richards, she immediately added, "I want to emphasize that Fred Richards was a wonderful supervisor. He showed me so much about science and even sailing, and he set me on the path of my career."

Science is not like the TV quiz show Jeopardy!*, in which bright contestants come up with the right question, given the answer.*

It was John Edsall's letter and packet of Xeroxed research papers sent to me in August 1995 that helped to explain why intelligent scientists might not see what was in front of their eyes. Edsall noted that the period of Lisa Steiner's research at Yale (1956–1959) was one of intense discovery of how biological information passes from the genome to proteins. The ribosome was discovered in 1955, tRNA in 1956, Crick propounded his central dogma in 1958, and the genetic code was deciphered in 1961. Edsall wrote:

> When Chris [Anfinsen] started his work on protein refolding, tremendous new discoveries about biosynthesis and the genetic code had been made. It became apparent that the finished polypeptide, with its sequence prescribed according to the code, is released from the ribosome as a wiggly, twisting polymer; in other words it is a denatured protein. It has to find its way into the proper 3-dimensional structure, with or without the help of chaperones. These findings necessarily sharpened attention on the protein folding problem, since the problem of how nature reproduces the correct sequence had essentially been solved.

In other words, by the early 1960s, but not by the mid-1950s, the scientific context posed the question of how the 1D information of DNA determines the 3D structure of proteins. The answer to this question required a new idea. That idea, which Anfinsen termed the "thermodynamic hypothesis," was developed gradually by him and his coworkers in a series of papers and stated in his Nobel Prize acceptance speech:

> the three-dimensional structure of a native protein in its normal physiological milieu . . . 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 short, sequence determines 3D structure and function, as Lisa Steiner's experiment showed. She had the answer to a hugely important question, but that question had not yet been posed. Science is not like the TV quiz show *Jeopardy!*, in which bright contestants come up with the right question, given the answer. We scientists make significant discoveries only when our observations can be linked by scientific context to significant questions. When Lisa Steiner was renaturing ribonuclease A, the scientific context had not yet posed the question of how the wiggly protein chain assumes its 3D structure. That question was before its time.

Further evidence that this question was before its time came from the reaction of Steiner's department chair to her thesis. He was Joseph Fruton, co-author of the most authoritative textbook of biochemistry at that period. On reading Steiner's thesis, describing an experiment that later led to a Nobel Prize, his only comment was on the spelling of ''thioglycolic acid.''

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Perhaps the lesson from this little history is that we scientists must struggle to interpret our observations and calculations in their broadest context, to find the most significant question they answer, but this will not always be possible.