OBITUARY

In Memoriam: Reflections on Charles Tanford (1921–2009)

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On October 1st, Jackie Reynolds notified me that Charles Tanford had died. I forwarded her message to the people on my email list who study proteins. The replies below were received the same day along with many others expressing similar thoughts.

“Sorry to hear this news. He was one of my scientific heroes. I named the position of the transition state on the reaction pathway from denaturant dependence as betaT in his honor, a name that has stuck. Farewell to a great protein scientist. His name will live on.”

—Alan Fersht

“Thank you for sharing the passing of one of the giants of our field, it is truly remarkable how much insight his classic experiments provided to our understanding of protein folding, experiments that still are carefully considered in the way we think about protein folding today.”

—Jeff Kelly

“It’s a very sad day. Charlie was truly a legendary biophysicist. I, for one, learned a huge amount from his work and writings, particularly his lucid books.”

—Ken Dill

“Very sorry to hear of Charles Tanford’s passing. A great scientist, an original thinker and a major impetus for the way that I and many others think about the folding problem.”

—Bob Matthews

“Thanks so much Nick for sharing this notice of the passing of a great historical figure in our field...his intellectual influence on me and others was enormous.”

—Paul Schimmel

“I’m really sad to hear that Nick. He was one of the giants of biophysical chemistry in the last century, and his work influenced me enormously.”

—Wayne Bolen

“Sad News. He was truly a great scientist.”

—Neville Kallenbach

“Thanks Nick. I guess we saw this coming. Fred, then Walter, now Charles ... a year of loss.”

—George Rose

"Oh my. Sad day it is. I was just thinking of him last night when I was with van Moudrianakis and Bertrand. They were reminiscing days of old."

—Carolyn Fitch

Carolyn Fitch received her Ph.D. from Johns Hopkins in 2002. I was the Outside Examiner on her dissertation committee. I was surprised to see that her thesis was dedicated to Charles Tanford. Carolyn said she never met him, but he had a great influence on her Ph.D. studies.

I was fortunate to be a graduate student in Charles Tanford's laboratory from 1962 to 1966. My dissertation was titled "The Reversible Denaturation of β -lactoglobulin A" and in the Acknowledgments I wrote: "I am indebted to Dr. Charles Tanford for providing advice, encouragement, criticism, a lab full of equipment and interesting people, and, perhaps most important, a very good example of how a very good scientist proceeds. For all of this, I am grateful."

All of these sentiments reflect the enormous impact that Charles Tanford had on those of us who study proteins.

Charles Tanford was born on 29 December 1921 in Halle, Germany. After the Nazi party did well in the elections in 1930, his parents, Max and Charlotte Tannenbaum, moved to England and changed their name to Tanford. Many of his relatives stayed behind in Germany and perished in the holocaust. In 1939, at the outbreak of the war in Europe, Charles moved to New York and lived with relatives. He earned a B.A. from NYU in 1943, then worked on the Manhattan Project at Oak Ridge for a year, and then earned a Ph.D. in chemistry from Princeton in 1947. He did postdoctoral work in protein chemistry in the laboratory of Edwin Cohn and John Edsall at Harvard University. Charles began his academic career at the University of Iowa in 1950 and moved to Duke University in 1960. In 1970, he was named the James B. Duke distinguished professor. Charles retired in 1988, moved to Easingwold, England, and began a second career writing about the history of science.

Charles married Lucia Brown while at Harvard and they had three children, Vicki, Alex, and Sarah. They were divorced in 1968, and soon thereafter Charles began a professional and personal relationship with Dr. Jacqueline (Jackie) Reynolds, a fellow biochemist, that lasted until his death.

Charles published over 200 articles during his scientific career. His first was an experimental study: "The Mercury-Sensitized Reaction between Hydrogen and Nitric Oxide" and it was published while he was an undergraduate at NYU.¹ At Princeton, Charles had planned to work with Henry Eyring, but he was required to work with R.N. Pease for his Ph.D. (Eyring was Tanford's favorite teacher at Princeton.) His Ph.D. work led to three theoretic-

cal papers on the combustion of gases.² Walter Kauzmann returned to Princeton in 1946 during Tanford's final year, and he had decided to become a protein physical chemist after reading the Cohn and Edsall treatise, *Proteins, Amino Acids, and Peptides*^{3,4} and other books on proteins in a cabin in the Colorado mountains. Kauzmann recalled⁴: "Tanford attended my informal lectures on proteins and we talked a lot about the subject. He decided that his future lay with proteins rather than with flames, and he went on to a postdoctoral position in the Cohn group at Harvard, and, of course, to a very distinguished career in protein chemistry. So, perhaps I can claim him as one of my most important discoveries."

In the laboratory of Cohn and Edsall at Harvard Medical School, Tanford began his career as a protein chemist. His research led to his first paper on proteins,⁵ a careful experimental study of the hydrogen ion titration of human serum albumin, and a theoretical analysis of the results based on the model of Linderstrom-Lang.⁶ In the Acknowledgments, Tanford "... expresses his gratitude to Dr. E.J. Cohn for suggesting this problem, and to Drs. J.T. Edsall, J.L. Oncley, George Scatchard, and W.L. Hughes, Jr., for many invaluable discussions." This experience and the lectures by Kauzmann left Tanford well equipped to begin his work on proteins.

At Iowa, Tanford continued his studies of the hydrogen ion equilibria of proteins, and related topics. Titration curves were determined for bovine serum albumin, insulin, lysozyme, and ribonuclease. The study of bovine serum albumin in 1955 was cited over 500 times,⁷ and the figures used in several textbooks. In 1956/1957 he did a sabbatical with J. G. Kirkwood at Yale to improve the theoretical treatment of the acid-base properties of proteins. The older Linderstrom-Lang model represented the protein molecule as a sphere with a continuous and uniform distribution of charge on its surface.⁶ In the new model, discrete charges were placed at fixed positions on the surface of the protein.⁸ The Abstract ends with: "General equations are obtained which express the titration curve as a function of the locations of ionizable sites and of their intrinsic properties. It is concluded that the intrinsic properties may themselves be quite sensitive to the location of the dissociable site with respect to the surface of the protein molecule." This paper triggered an interest in the factors that determine pK values of the ionizable groups of proteins that continues to the present day. The work from this period is summarized in a review published in 1962.⁹

Tanford taught a course on the physical chemistry of polymers at Iowa and decided to write a textbook. His 10 years of work led to the publication in 1961 of *Physical Chemistry of Macromolecules*.¹⁰ He has written an interesting "Recollections" article

about the book.¹¹ He noted: “There were two reviewers and their criticism was scathing; I had got it all wrong, they said, and the book was declared unpublishable.” When he met with the publishers he told them “... that I had every confidence in what I had written and would not change a word.” The book was a great success and has now sold over 25,000 copies, and has been republished, unaltered. For many of us, it was an essential reference book for our teaching and research.

Tanford moved to Duke in 1960 and he expanded his research into new areas. When I arrived in 1962, he had a group studying antibody structure, and another group studying various aspects of protein folding. Laboratory meetings were held at 3 pm on Friday afternoons and generally went to at least 6 pm. This was when we learned from Tanford. (Tanford and Bob Hill taught an excellent course on proteins and enzymes that was also a great learning experience.) The laboratory was crowded and my desk also served as my lab bench. We had two Beckman Model E ultracentrifuges (one inherited from Hans Neurath) and, shortly after I arrived, Cary 60 spectropolarimeter #3. (The first two had gone to Elkan Blout and Henry Eyring.) It was an exciting time to be in the laboratory. The experiments that led to the characterization of the denatured state were underway, and Yas Nozaki was overseeing solubility measurements on amino acids and peptides that led to the ΔG_{tr} values for urea and GdnHCl used to understand how these compounds unfold proteins. The studies of protein folding were reviewed by Tanford in a 1968 article¹² that has been cited over 2200 times, his most cited paper, and in a continuation article.¹³ These articles paved the way for the explosion of research in protein folding that occurred when site directed mutagenesis became available.

In the late 1960s, Tanford began his long, productive collaboration with Jackie Reynolds. With Tanford's interest in the hydrophobic effect and Reynold's background in protein-lipid interactions, the laboratory moved into the area of membranes and membrane proteins with great success. The two papers they published together in 1970 were cited over 1500 times, and revolutionized the study of membrane proteins.^{14,15} The following year, Nozaki and Tanford published the first hydrophobicity scale.¹⁶ In 1973, Tanford published his second book *The Hydrophobic Effect: Formation of Micelles and Biological Membranes* and it was also a great success.¹⁷ Tanford had a long and very successful academic career. He published 14 articles that were each cited over 500 times. Tanford remained at Duke until 1988 when he retired, and began writing about the history of science and related topics.

In Tanford's second career, his first book was titled: *Ben Franklin Stilled the Waves: an Informal*

History of Pouring Oil on Water with Reflections on the Ups and Downs of Scientific Life in General.¹⁸ Next Tanford and Reynolds wrote two travel guides for scientists. *The Scientific Traveler*¹⁹ is a great resource for anyone who wants to visit the sites in Europe where important scientific discoveries were made. A related book, *A Travel Guide to Scientific Sites of the British Isles*,²⁰ was published 3 years later. Their last book, *Nature's Robots: A History of Proteins* was published in 2001.²¹ The last line of Henryk Eisenberg's review in *Nature*²² was “... anyone interested in proteins will find *Nature's Robots* an absorbing and often exciting story, as well as a major contribution to scholarship.” In addition to these books, Tanford and Reynolds wrote many always interesting book reviews for *Nature*.

I will conclude with a few personal observations on Charles Tanford during my time in his laboratory. Tanford suggested three possible projects for my Ph.D. research. One caught my interest. Kauzmann's seminal review showed convincingly that hydrophobic bonds stabilize proteins, and the model compound data showed that they become stronger as the temperature goes up.²³ Tanford pointed out that this did not make sense because everyone knows that proteins unfold at higher temperatures. My project was to figure this out. He suggested that I work on β -lactoglobulin because we had 12+ g in the freezer that had been given to him by Serge Timasheff and Bob Townend. This was a fortunate choice. It turned out that β -lactoglobulin is most stable at 35°C and unfolds at both higher and lower temperatures.²⁴ Consequently, we were the first to observe the cold denaturation of a protein and show that proteins can be unfolded by either lowering or raising the temperature. ΔH for unfolding was strongly temperature dependent varying from -40 to $+40$ kcal/mol between 10°C and 50°C. This was a reflection of the large change in heat capacity, ΔC_p , that accompanies protein unfolding,²⁴ as shown earlier by Brandts.²⁵ This research benefitted others in ways we had not suspected. Efraim Racker wrote me a nice note to thank us because they began purifying their protein at room temperature rather than in the cold room and got a better yield.

Tanford and Kazuo Kawahara published a paper in 1966 showing that aldolase is a tetramer with a molecular weight of 158,000.²⁶ (Kazuo took the nice picture of Tanford that accompanies this article.) Three earlier papers in *Biochemistry* had suggested that that aldolase was a trimer with a lower molecular weight. Later that year at Federation Meetings in Atlantic City, a talk by a graduate student presented overwhelming evidence that aldolase was in fact a trimer. I wondered how Tanford could possibly respond. Tanford had broken his leg earlier and was on crutches. He hobbled to the microphone and said something like: we have measured the molecular

weights of proteins A, B, C, etc. and got them all exactly right and I am absolutely certain that aldolase is a tetramer. Tanford was right, as usual.

Robinson and Jencks had used peptide models to measure the ΔG_{tr} of a peptide group from water to urea and GdnHCl solutions.²⁷ Tanford was interested in this because they had used different peptide models for the same purpose and the results did not agree. (This question was only recently resolved by Auton and Bolen.²⁸) Consequently, Tanford invited Bill Jencks to Duke to give a seminar. In the introduction, Tanford said that despite the fact that Jencks did not understand thermodynamics at all, he had some interesting experimental results to present.

I was fortunate to have two long car rides with Tanford; they were a chance to learn about things other than proteins. In one, we were riding from Atlantic City to Durham and he explained to me why he enjoyed bird watching and classical music, two things I knew little about. Later, he would loan me records to take home and play with the hope that I would develop an appreciation of classical music. Tanford did not succeed in all of his ventures.

Tanford called me into his office to set me straight on two occasions. The first was when I suggested that Philip Handler did not deserve to be elected to membership in the National Academy of Science. (Handler later served two terms as President.) Tanford explained to me that Handler had done more than anyone to gain support for scientific research in Congress, and he certainly deserved to be a member. (Like many of us, Tanford was mightily impressed by Handler, as he has described.²⁹) The second was when I had a disagreement with a faculty member and used some inappropriate language. In this case, he began the conversation with "I hope you are still in graduate school."

Finally, near the end of my post doc with Gordon Hammes at Cornell, I began looking for a job in industry. I interviewed at four companies and at each they asked me who this fellow Tanford was. It turns out that in my letter of reference he said I was good, much too good for industry. I guess it was a good strategy, they all offered me a job.

We celebrated Tanford's retirement on Cape Cod in 1988. At the time, Tanford suggested that his success resulted from all of the good experimental data gathered by his students. He was just being nice to us. More important was Tanford's ability to take the experimental results and write the great papers that helped so many of us gain a better understanding of proteins. Few if any made a greater contribution to protein science than Charles Tanford. (For more information on Tanford's origins and his thoughts on his research and his career, see his article "Fifty Years in the World of Proteins" published in 2003.²⁹)

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