A brief look back at 45 years of research in physical biochemistry

Allen P. Minton

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I have been invited to contribute a short essay based upon consideration of my career as a research scientist. Short is difficult. Don't expect distilled wisdom.

As a young, enthusiastic physical chemist, freshly minted in 1968, I was certain that knowledge of fundamental principles of physics and chemistry would help me to provide the answers to at least some of the great remaining puzzles of biology. After all, wasn't biology just a potentially comprehensible application of these principles? Suffice it to say that subsequent experience has been humbling. However, I wasn't completely wrong. We—the scientific community—have been extraordinarily successful in devising ingenious means for efficient collection of ever-increasing amounts of data, but comprehension and the resulting ability to predict biological phenomena remain limited to rather special cases. Progress toward the goal of “understanding” biology remains incremental, but in the words of Lao-tzu, “A journey of 1000 miles begins with a single step.” Hopefully my first step will be remembered as a contribution toward the understanding of how a complex environment can affect biochemical equilibria and kinetics.

It has been my privilege to have studied with and worked under three mentors who taught me how to conduct scientific research: my graduate advisor Willard F. Libby at UCLA (1964–68), my first postdoctoral advisor Henryk (Heini) Eisenberg at the Weizmann Institute of Science (1968–1970), and my second postdoctoral advisor and subsequent Laboratory Chief Harry A. Saroff at the NIH (1971–75). Each provided me with instruction and insight, the freedom to pursue independent thought, and an indelible commitment to scientific integrity. A large debt of gratitude is owed to them.

My initial postdoctoral efforts during the late 1960s and early 1970s were aimed at elucidating some of the interesting and unique properties of liquid water. A byproduct of that period was one of the first publications casting doubt on claims for the existence of a super-strong hydrogen bond between water molecules leading to the formation of a controversial substance called polywater. Younger readers can Google this term to learn about a curious episode that has been the subject of sociological study.

I next turned my attention to elucidating the mechanism underlying the cooperative binding of oxygen (and other gases) to hemoglobin, a problem that I had previously encountered during my graduate studies. At that time—late 1960s to mid-1970s—the hemoglobinologists were divided into two semi-hostile factions, one advocating a two-state allosteric model similar to that originally proposed by Monod, Wyman and Changeux, and a sequential binding model frequently attributed to Koshland, Nemethy and Filmer, but which was essentially a generalization of ideas originally developed by Pauling and Coryell. My basic take on the situation, as expressed in several publications, was that the data available at that time could be interpreted equally well in the context of both models, and that the true explanation would prove to be more subtle and complex.

Not wanting to get involved in factional infighting, I redirected my efforts toward a different but related subject. As a result of my interest in hemoglobin, I became interested in the mechanism of the polymerization of deoxygenated hemoglobin S, a phenomenon underlying the pathology of sickle-cell disease. Pauling and Itano had discovered that a single mutation on the human beta chain led to a marked increase in the propensity of the hemoglobin to aggregate when deoxygenated, leading to the formation of rodlike hemoglobin polymers and subsequent gelation of the contents of a deoxygenated erythrocyte. During the mid- to late 1970s we proposed thermodynamic models for the dependence of the propensity to undergo gelation, quantified as an effective

A. P. Minton (✉)

Laboratory of Biochemistry and Genetics (LBG), Physical Biochemistry Section, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health Building 8, Room 226 8 Center Dr., Bethesda, MD 20814, USA
e-mail: minton@helix.nih.gov

solubility, upon the composition of the solution, including mixtures of hemoglobin S and other hemoglobins, the presence of chemical additives that could bind to hemoglobin, and the degree of oxygenation. I tested these models against data in the literature and measurements made in my laboratory by my first postdoctoral fellow, John Pumphrey, and myself.

Following a presentation of my work in 1975 I was asked how my models would be affected by the fact that the polymerization of sickle hemoglobin was taking place in a solution containing over 300 g/l of protein. I gave a hand-waving answer that I subsequently recognized as inadequate, and undertook to understand how polymerization reactions might be affected by the large extent of thermodynamic nonideality in such a concentrated solution. It turned out that a realistic treatment of the nonideal behavior in concentrated hemoglobin solutions developed with my colleague Philip Ross greatly simplified and clarified the interpretation of many observations that had previously required rather complex and ad hoc explanations.

I then realized that the influence of solution nonideality in concentrated protein solutions had ramifications far beyond application to the polymerization of sickle hemoglobin. In the early 1980s I developed and published general treatments of the effect of excluded volume in highly volume-occupied solutions upon generic macromolecular associations and isomerization. The theoretical work was accompanied by parallel experimental investigations aimed at testing and verifying specific predictions of the general models, and providing data that would enable the models to be refined and extended. Both theoretical and experimental investigations of the phenomena collectively referred to as “macromolecular crowding” have continued in our laboratory until present, and represent the work of numerous postdoctoral collaborators: Jacob Wilf, Arun K. Attri, Ron Chatelier, Nobuhiro Muramatsu, Germán Rivas, Damien Hall, Kenji Sasahara, Begoña Monterroso, Cristina Fernández, Adedayo Fodeke, and most recently Di Wu, Asaf Grupi, and Travis Hoppe.

Realizing that excluded volume interactions with cosolutes are not the only factor influencing macromolecular behavior in complex media, we also have proposed models for effects upon chemical reactivity due to confinement of macromolecules in small elements of volume of varying shape and dimensionality, and physical adsorption to surfaces. Recently we have begun to explore the contributions of attractive interactions, in addition to steric repulsion, to the behavior of macromolecules in crowded media.

Another enduring interest has been the development of new instrumentation and techniques for the detection and quantitative characterization of protein–protein (and other macromolecular) interactions in dilute as well as crowded

solutions. I have always felt that new technology can lead to new science. Our work has resulted in innovations in the instrumentation, measurement, and analysis of sedimentation equilibrium, static light scattering, and viscosity. Postdoctoral fellows participating in this general effort include Arun K. Attri, Peter Schuck, Carol Hsu, Saleh Darawshe, Germán Rivas, Susan Lakatos, Amanda Nourse, Cristina Fernández, and most recently Asaf Grupi. During the 1980s I was privileged to work with the team at Spinco Division of Beckman Instruments developing the new generation analytical ultracentrifuge, and provided algorithms for simulation and data analysis software and firmware. It is a source of pride to have contributed in a small way to the renaissance of analytical ultracentrifugation.

During the last 2 years my colleague Peter McPhie and I have been studying how specific ligands, metal ions, and “inert” cosolutes affect the structure and function of a ribonucleic acid sequence called a riboswitch. The study of nucleic acids is a novel endeavor for us lifelong protein researchers, and I think that the pleasure we have derived from making new discoveries in this area goes far to refute the common notion that “you can’t teach an old dog new tricks.”

I would be remiss if I failed to mention the extended collaboration that I have enjoyed for over 20 years with Germán Rivas and his colleagues at the Center for Biological Research in Madrid. Over this period we have explored various aspects of the self-assembly of proteins in general, and more recently, the self-assembly of the bacterial septation protein FtsZ.

I have always believed that the pursuit of knowledge about the natural world via the scientific method was a vocation or calling, not just a profession. It has been my great good fortune to have enjoyed the opportunity to undertake this pursuit with the blessing of generous and far-sighted supervisors, in the company of dedicated and talented postdoctoral fellows and collegial peers. I have attempted to share my enthusiasm for scientific investigation with my junior colleagues throughout our collaborative endeavors, and with other interested individuals by teaching extension courses on various subjects related to physical biochemistry at the NIH Foundation for Advanced Education in the Sciences for over 30 years.

I will never forget that most of my scientific accomplishments would not have been possible without the generous support of the United States government by way of the National Institutes of Health, and, indirectly, the American taxpayer. For that support I am most sincerely grateful.

Allen P. Minton
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