Serge Timasheff: The Man with a Genius for Solutions in Biology

To summarize adequately the accomplishments of Serge Timasheff in the space of a few short pages is an impossible feat. His achievements include so many seminal advances in theory, methodology, and basic discovery that even the full set of papers that follows in this special volume cannot adequately document all that he has contributed to the biological sciences. However, one short sentence can summarize what seems to us to be the most noteworthy aspect of Serge's achievements: to our knowledge, no other physical biochemist has made contributions that can truly be said to impact significantly every discipline in biology from biophysics to protein and nucleic acid biochemistry, to renal physiology, to molecular evolution, and most recently even to applied work in the areas of cell preservation and cryobiology. To buttress this claim, it is appropriate to trace the evolution of Serge's ideas, an ontogeny that is characterized by an ability to seize important questions, develop clever new technical approaches toward them, and then provide answers that long eluded less creative spirits.

Born in Paris in 1926, Serge became a naturalized U.S. citizen in 1944. He received his higher degrees (B.S. (1946), M.S. (1947), Ph.D. (1951)) at Fordham University. Following receipt of his Ph.D., he first was a research fellow at the California Institute of Technology and then a postdoctoral fellow at Yale University. For the next 11 years, with the exception of one year as a National Science Foundation postdoc in Strasbourg, France, Serge was a physical chemist with the U.S. Department of Agriculture. He joined Brandeis University in 1966, where, for the most part, he has developed his extraordinary career.

Dates and addresses do not, of course, tell an intellectual history. The key events marking Serge's intellectual development include a significant period in the laboratory of Professor J.G. Kirkwood in the 1950s. At this time, Kirkwood was a major figure in the field of application of statistical mechanics to chemistry. Serge, coming from a biochemical background and with considerable talent in experimentation, proved to be exactly the type of associate that Kirkwood needed. He supplied expertise in areas outside the competence of the laboratory, and the result was unusually close contact with Kirkwood, who was normally somewhat shy and noncommunicative. They exchanged ideas on an almost daily basis. It is not certain how much Kirkwood learned from Serge during this collaboration, but much of Serge's later work shows that he had acquired his mentor's fundamental, analytical approach to biophysical problems.

An especially significant development in Serge's career that occurred in Kirkwood's laboratory was his introduction to the study of the thermodynamics of three component systems, especially solutions of macromolecules in water containing another ingredient—a cosolvent. Kirkwood's interest in these phenomena had begun shortly before Serge joined his group. Together with Goldberg, Kirkwood had developed a theory of light-scattering in which they showed that the presence of a third component could perturb the scattering and give misleading results. Kirkwood also showed how the scattering could be corrected for the presence of the third component. Serge applied the same type of theoretical idea to the scattering of x rays from solutions. This work put the interpretation of the x-ray scattering of nucleic acids on a firm theoretical basis and eliminated systematic errors that were affecting the credibility of the method.

Although the theory of light scattering from mixed solvents is rather sophisticated, the mechanistic explanation of the phenomenon is relatively simple. It involves the concept of preferential interaction, sometimes called preferential hydration, which is a special case of great importance. Much of Serge's work, especially that related to physiology and biotechnology, cannot be appreciated without an understanding of this concept, so it is worth a few lines of explanation. If a substance is dissolved in a mixture of two solvents, generally one of them will form more favorable interactions with the substance than the other. The result is that the solvent layer in immediate contact with the molecule tends to be richer in one of the two components, such that the solvent composition of the nearby solution bathing the molecule differs from that of the bulk solution. For

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biological molecules the preferred solvent is most often water, hence the term preferential hydration. This phenomenon affects light scattering or x-ray scattering in the following way: when a macromolecule diffuses into the scattering volume, it carries with it a solvent of different composition from the bulk solvent, and the excess scattering power of this layer relative to the bulk solution is part of the total scattering power.

Preferential interaction (or preferential hydration) is far more than an artifact of certain types of measurements; it is rather the fundamental thermodynamic expression of the interaction of molecules with a complex solvent environment. As Serge's work has demonstrated for over three decades, preferential hydration is a decisive factor in the determination of the stability and conformation of proteins and nucleic acids. Indeed, the concept provides the necessary theoretical context for answering one of the most fundamental questions in evolutionary biology: why has natural selection favored the evolution of the particular mixtures of solutes found in cells? We will see how Serge's evolution of the concept of preferential interaction has provided an answer to this question, and how this answer has major heuristic value for studies in physiology, medicine, and, most recently, biotechnology.

His first attempts at measuring preferential interaction were only moderately successful because of the extremely demanding nature of these experiments. One is typically dealing with a macromolecule at a concentration of 10^{-6} to 10^{-4} M and a cosolvent, which may be present at concentrations ranging from a few tenths molar to several molar. The detection of a few dozen extra molecules associated with each macromolecule is a very challenging experiment, indeed. Casassa and Eisenberg had given rigorous thermodynamic formulas that related the refractive increment and density to the preferential interaction, but these formulas had been used mainly to correct scattering experiments. The extension of this formulation to experiment and its development into a broad intellectual construct with far-reaching implications for all fields of biology awaited the full application of Timasheff's genius.

His interests in overcoming the difficulties entailed in analyzing preferential hydration were in part a result of his studies of tubulin, which confronted him with a particularly annoying experimental difficulty.

Solutions of tubulin that had been prepared in the morning deteriorated completely by the afternoon. This bothersome result led him to explore the effects of different solutes on the stability of tubulin, work that began rather empirically and as novel findings emerged took on an elegant, quantitative dimension. These experiments did succeed in solving the immediate experimental conundrum of keeping tubulin stable; the molecule could now be kept in the native state for up to a year. This achievement was important in facilitating the pathbreaking studies conducted by Serge and his co-investigators, notably, R.P. Frigon, G.C. Na, and J.M. Andreu, on tubulin self-association and ligand-mediated protein assembly involving association of tubulin with vinblastin and colchicine. These studies of tubulin represent one of the most important events in the elucidation of the mechanisms by which multimeric proteins are stabilized. However, the success of the work with solutes that were capable of stabilizing tubulin as well as other proteins, coupled with the important addition of a new apparatus, led his group to focus increasingly on an in-depth analysis of questions about protein-water-cosolvent interactions, work that succeeded in answering long-standing questions about the effects of common inorganic salts and organic osmotic solutes (osmolytes) on proteins.

Preferential hydration can be measured directly by studying osmotic equilibrium. The explanation of preferential hydration is that the addition of a macromolecule to one side of a dialysis membrane will cause some of the preferred solvent to pass through the membrane to go into the solvation layer around the molecule. To study this phenomenon, a method is needed that will measure concentrations precisely and consume relatively small quantities of protein. The electronic densimeter of Kratky and Porod became available just as Serge needed such a device for refining and extending his measurements of preferential hydration. This instrument gives almost instantaneous, nondestructive, high precision measurements of density on small samples. With his background and experience, Serge was able to recognize the instrument's potential for his work. Even with the electronic densimeter, however, measurements of preferential hydration remain extremely difficult and Serge's laboratory is the only one that has been able to use this method with consistent success.

Thus began over two decades of important measurements of preferential hydration. By studying preferential hydration as a function of concentration, it is possible to obtain the change in molar free energy of a macromolecule induced by the addition of cosolvents such as sucrose, a stabilizer, or urea, a destabilizer of proteins. If one is dealing with a molecule that can exist in two forms, native and unfolded, then the effect of an added cosolvent will be the stabilization of the native protein if the cosolvent is preferentially excluded, relative to water, from the protein surface. Destabilizing cosolvents like urea will preferentially interact with the unfolded protein and favor its unfolding. This is a modified form of Le Chalelier's principle. The measurements provide quantitative descriptions of enhanced or diminished stability, and permit the a priori determination of the conditions of maximal stabilization.

Over the years, many different solutes have been examined in Timasheff's laboratory. Initially, these solutes were common laboratory reagents often used to stabilize or denature proteins. His work with inorganic salts provided a thermodynamic basis for the ranking of inorganic ions in the Hofmeister series. Since the empirical work of Hofmeister in the 1880s, it has been known that different salts show consistent relative effects as protein stabilizers or destabilizers. For example, ammonium and sulfate ions stabilize proteins, whereas bromide, iodide, and thiocyanate, in increasing order of effect, disrupt protein structure. The rank-

ing of ions in the Hofmeister series is virtually independent of the protein studied, indicating that a fundamental property of the solution chemistry of proteins is involved in Hofmeister series effects. Serge's work showed this property to arise from solute-dependent differences in preferential interactions between proteins, solvent, and cosolvent.

Having solved a mystery that had puzzled biochemists for almost a century, Timasheff and his colleagues extended their studies to phenomena that involved events that took place between three and four billion years ago at the dawn of cellular evolution. By shifting their experimental focus to the small organic osmolytes found in osmotically concentrated salt-water organisms and in selected tissues of mammals, they showed that the process of cellular evolution is marked by the exploitation of organic solutes that resemble, in their actions as cosolvents, the favorable ions of the Hofmeister series. The rationale for occurrence of organic osmolytes such as methylammonium (e.g., glycine betaine, trimethylamine-*N*-oxide, and glycerophosphorylcholine) and methylsulfonium (β-dimethylsulfoniopropionate) compounds, polyhydroxy alcohols (e.g., sorbitol and glycerol), and free amino acids was shown to be based on the same principles that are involved in explaining the differential effects of simple inorganic ions on proteins. In essence, natural selection has shown itself to be a wise protein chemist through favoring the evolution of an intracellular milieu that is hospitable for protein function and structure. With the notable exception of urea—whose perturbing effects are counteracted by certain methylammonium compounds which also are found in urea-rich elasmobranch fishes (sharks and their relatives) and in the fluids of the inner medulla of the mammalian kidney—organic osmolytes exhibit the preferential exclusion from protein surfaces noted for stabilization by inorganic ions. At the concentrations used in cells, these compatible organic osmolytes can be employed at high and widely varying concentrations without perturbing protein activity or structure. Thus, adaptation to osmotic stress is generally achieved not through modification of amino acid sequences to offset effects of salts—a strategy that seems to occur only in halophilic archaea—but instead through the regulation of intracellular concentrations of small organic molecules that balance external salinity while keeping proteins in their native functional states.

By explaining the mechanistic basis for this evolutionary pattern, Timasheff and colleagues, including T. Arakawa, K. Gekko, V. Prakash, and J.C. Lee, not only answered an important question in evolutionary biology, but they also provided key insights into important issues in medicine and biotechnology. The group's investigations of cosolvent effects have provided avenues for the rational development of in vitro media for preservation of cells, tissues, and proteins at low temperatures and in the dried state. In a sense, Serge's career has come full circle. His appointment, following postdoctoral work, to a position in an applied science laboratory run by the Department of Agriculture for study of milk proteins, quickly led to major theoretical insights into mechanisms of protein structure and stability. The theories he subsequently developed in almost four decades of basic research are now paying great practical dividends in applied science.

A final accomplishment of Serge Timasheff is perhaps implicit in what has been said above: he not only does great science, but he communicates it effectively and makes it accessible to a wide readership. In addition to the remarkable set of original research papers that has come from work in his lab, Timasheff and colleagues have produced definitive reviews that stand as classics in the literature. In addition, he has played major editorial roles, for example, as a co-editor with G. D. Fasman in the Biological Macromolecules Series, and as an editorial board member for virtually all of the major journals in biochemistry.

We believe that even this brief account of the achievements of Serge Timasheff fully supports our point that his work has messages of both theoretical and practical importance for scientists across the full expanse of the biological science spectrum, including protein chemists, for whom his studies on destabilizing agents have contributed strongly to the interpretation of protein unfolding and to the important reverse process, the mechanism of protein folding; renal physiologists, who have been provided with vital insights into how nitrogenous waste solutes are dealt with in the kidney; biotechnologists, whose goal of stabilizing proteins has been given a firm theoretical foundation; and students of molecular evolution, who ask basic questions about why things evolve as they do. It is fortunate for scientists working in all of these diverse fields that the career of this remarkable man, Serge Timasheff, evolved as it did to provide for so many of us critically important new ways of viewing the world and doing our science.

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