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LETTER TO THE EDITOR

A tribute to Dr. Serge N. Timasheff, our mentor

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Abstract
Dr. Serge N. Timasheff, our mentor and friend, passed away in 2019. This article is a collection of tributes from his postdoctoral fellows, friends, and daughter, who all have been associated with or influenced by him or his research. Dr. Timasheff is a pioneer of research on thermodynamic linkage between ligand interaction and macromolecular reaction. We all learned a great deal from Dr. Timasheff, not only about science but also about life.

Introduction

Dr. Serge N. Timasheff passed away on February 25, 2019, after suffering pneumonia. He is a giant and pioneer in the field of physical biochemistry of protein chemistry. We all know his research achievements, which have been well documented in two comprehensive reviews, one written by himself [Timasheff 2004] and another by Schellman and Somero [Schellman and Somero 1996]. Thus, his research achievements will not be compiled here and therefore this article will not be an ordinary tribute/obituary. Instead, we have assembled our personal experiences, memories or recollections related to Dr. Timasheff's research and life and our personal interactions with him. Our goal is to describe, through our combined recollections, how Dr. Timasheff pursued science and student education - both in his lab and at home - at the highest level, and how his research and teaching have influenced our lives.

Introduction and Final Remarks were prepared by Marina Timasheff Charles and Tsutomu Arakawa, both of whom took initiative to organize this tribute collection.

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Kirk C. Aune: Tribute to Dr. Serge N. Timasheff, our mentor. A perspective from Kirk C. Aune

I chose my postdoctoral mentor in 1968 based on a recommendation from my PhD mentor, Charles Tanford (who is also a giant in physical chemistry of macromolecules and a close friend of Dr. Timasheff), written communications with Serge Timasheff, and a pre-decision visitation with Nathan Kaplan and William Jencks at Brandeis University. As many of you know, Professor Timasheff generally made annual summer trips to Europe, so he was not present that summer during my "interview/review" visit. My decision was made without having met the man.

I was fortunate to have the counsel of giants in physical protein chemistry, including Rufus Lumry (my undergraduate mentor), Charles Tanford, their respective laboratory colleagues, and a number of other Duke faculty to guide the process of my youthful career-building. All were most enthusiastic about the possibility.

My faith in those counselors was not to be challenged, for my experience with Serge N. Timasheff from August 1968 through July 1970 provided the seminal foundation for who I am today. Professor Timasheff provided me a fantastic forum for research and learning with the encouragement to follow my instincts (instincts that Dr. Timasheff always encouraged us to follow), express my thoughts, and publish my conclusions. I endeavored to do so, and with the twists and turns that life presents us all, I am still able to look back with no regrets for decisions made and courses taken, based mostly on the impact from my interactions with a quiet, gentle, brilliant man of life and science, Serge N. Timasheff.

As a new postdoctoral fellow, I knew I should migrate to research interests that departed from my thesis work but that it would be wise to draw upon the developed knowledge. The thermodynamic stability of protein structure, my PhD thesis work, was important to understanding the basics of protein function in biology, but it was becoming increasingly clear that the thermodynamics of interaction between those protein structures might be more incisive for understanding the details of protein function. Professor Timasheff's interests in solute/solvent impacts on macromolecular properties blended well with mine, so I sought out an interesting protein in the immediate environment and William Allison in Nathan Kaplan's laboratory graciously accommodated me by providing prepurified glyceraldehyde 3-Phosphate dehydrogenase from chicken heart. That protein was known to function in solution as a tetramer but exhibited possible disassociation tendencies under certain solvation conditions that required clarification.

Thus, before Professor Timasheff had returned to Brandeis that summer, I had formulated a goal and addressed both my interests in the thermodynamics of that putative behavior and what I thought would include Serge's interests in ionic solvation effects. To further pique my new advisor's attention, I capitalized on his strong interest in the use of light-scattering as a physical tool for measuring molecular size in solution. Moreover, his laboratory had a beautiful setup with a temperature controlled clean-room and up-to-date electronics, a facility currently unused by anyone else!

My excitement and self-imposed tension over a meeting with Professor Timasheff for the first time, whereby I could brief him on my proposed and on-going efforts, had to wait. A crisis in Boston Harbor, perhaps just less than that of the Boston Tea Party, had occurred upon his arrival. The Timasheffs' pride and joy—in the form of one sturdily-built and expensive Mercedes Benz automobile—had been dropped from a crane at a sufficient height coming off a ship, rendering it worthless! Subsequently, the laboratory personnel passed through a mourning and emotional calming process and the problem was ultimately resolved. That was my introduction to Drs. Serge and Marina Timasheff!

Professor Timasheff did finally express his satisfaction with my proposal so work continued. Before very long, however, dust, lint, filtration of protein solutions, electronic failures, and my impatience with the tedious procedure led me to turn to that familiar analytical ultracentrifuge standing nearby. Although Professor Timasheff had enjoyed the prospect of someone once again picking up the light-scattering tool, he seemed to forgive me, since the appropriate information came more facile out of analytical ultracentrifugation. In the end, our published works demonstrated that preferential hydration was at play rather than dissociation of the enzyme complex to explain molecular weight depression under certain conditions (Aune and Timasheff 1970).

Following that work, Professor Timasheff encouraged me to re-examine the association/dissociation of alpha-chymotrypsin in solution, for ionic considerations that affect protein-protein interaction may also need further clarification. Those works led to our two publications on the Dimerization of Alpha-Chymotrypsin (Aune and Timasheff 1971; Aune et al. 1971) before my departure.

Professor Timasheff was eager to provide the resources in the emerging computer technology, affording me an opportunity to address research in a manner not previously considered and opened interactions with other researchers in the environment. Those opportunities contributed greatly to my long-term career interests.

At that juncture in one's career, discussions such as on a proposition of a condition, eliminating illogical premises, proposals for new tests, and general considerations of emerging research are invaluable. Professor Timasheff provided that time with me, personally, and to a lesser extent in group chat sessions. I think he relished more the one-on-one interactions, and I shall always cherish those sessions.

It was in those private sessions that Professor Timasheff would share his personal admiration of scientists and our

profession in general. One day, we were talking about some of the ramifications of the analysis of solvent on macromolecules by analytical ultracentrifugation, and he dug out several pages of hand-written notes on yellow-lined paper authored by John Kirkwood, clutching them with reverence. He wanted to share them with me, and he encouraged me to make and take copies for my own inspiration; those I still have to this day.

I cherish the few hours that we talked during our drive back to Waltham from a busy week at a Gordon Research Conference. We chatted about our children—his daughter Marina getting bitten by a monkey at the zoo and my four-year-old daughter's thriving in a diverse preschool at Brandeis—and academic life, in general.

In another private moment, Professor Timasheff reminded me that all the great science I may explore and discover is like singing in the shower: No one will ever know whether you have talent or something to contribute unless you decide that you have developed a kernel of information, summarized it, and explained it to the world. No one could ever match his ability to assess and conclude a thought and move it to print. I was further astonished to learn of Professor Timasheff's linguistic abilities in at least seven languages. He wrote and spoke in all, sliding from one to another as the situation presented itself. We often witnessed him conversing effortlessly with lab visitors from France, Italy or elsewhere. During that same two-year time frame, he finished translating a physical chemistry book from original Russian to English without neglecting all other activities before him.

All good things must pass, and those two exciting and productive years came quickly to an end. On the occasion of my last day in the laboratory, my colleagues—with considerable participation from Professor Timasheff—arranged for a send-off party. We celebrated at an elegant level. First he presented us with wine and lobster (see Fig. 1).

Then, with that quiet yet surprising humor he sometimes expressed, plus a solemn sense of importance, Professor Timasheff staged a somewhat tongue-in-cheek but also dead-serious welcome into the Academe with my appointment to Assistant Professor at Ohio State University. He disappeared for a moment and returned, solemnly marching into the lab, regaled in pomp and circumstance, sword in hand. He stood before me, reading a ceremoniously-scrolled declaration and then suddenly—with a start, mind you—raised the sword high in the air, formally welcoming me to his world (see below Fig. 2)!

Time has its way of separating people, and that certainly happened with Professor Timasheff and me. My interests and health led me into academic administration and our opportunities for interaction faded. My last visit, now expressed in the familiar sense, with Serge and Marina was in their home for dinner in the 1980s, and it was a most enjoyable and memorable evening. I still found him to be gentle, kind, active, and intriguingly brilliant. I had feared he would view my leaving behind active academic research as a personal affront; instead, he gave me encouragement and understanding. We talked about interests, time, and travel. We mused about the common pride and privilege that we both had separately mentored V. Prakash through his productive postdoctoral experiences in our laboratories. It demonstrated to me that Serge's interest in the people who had passed through his laboratory was clearly of regular, thoughtful concern.

I was one of those most fortunate to have had Professor Serge N. Timasheff's mentorship during my formative career training years, and I will always appreciate the guidance, example, and encouragement he provided to me and the many others who passed through his laboratory.

Jim Lee: Serge N. Timasheff, a brilliant scientist, and a humble and devoted religious person

The groups of friends and former postdoctoral fellows, who submit testimony to the brilliance of Professor Serge N. Timasheff as an innovating scientist, caring mentor, and friend, are the very blessed ones. They are the ones who had the opportunity to know him personally. I am one of those who was accepted by Dr. Timasheff into his lab knowing how unprepared I was both scientifically and intellectually. I can frankly testify that the training I received provided me a career as a member of the society of biophysical biology. I would not venture to imagine the alternative.

I did not have a solid background for biophysical studies - no advance mathematics and physics - with only chemistry in college. Due to the war years (WWII and Liberation of China) frequently the family was moving between cities.

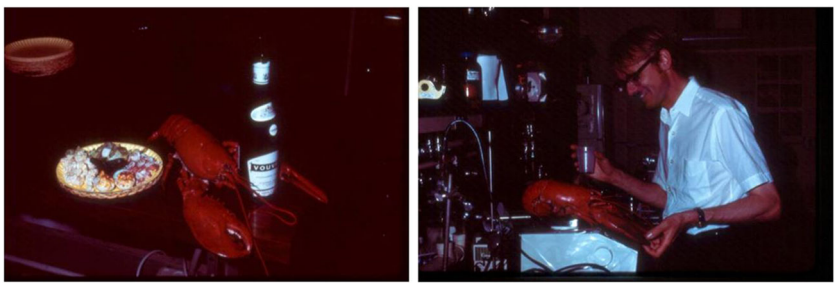

Fig. 1 Dr. Timasheff in front of famous New England lobster

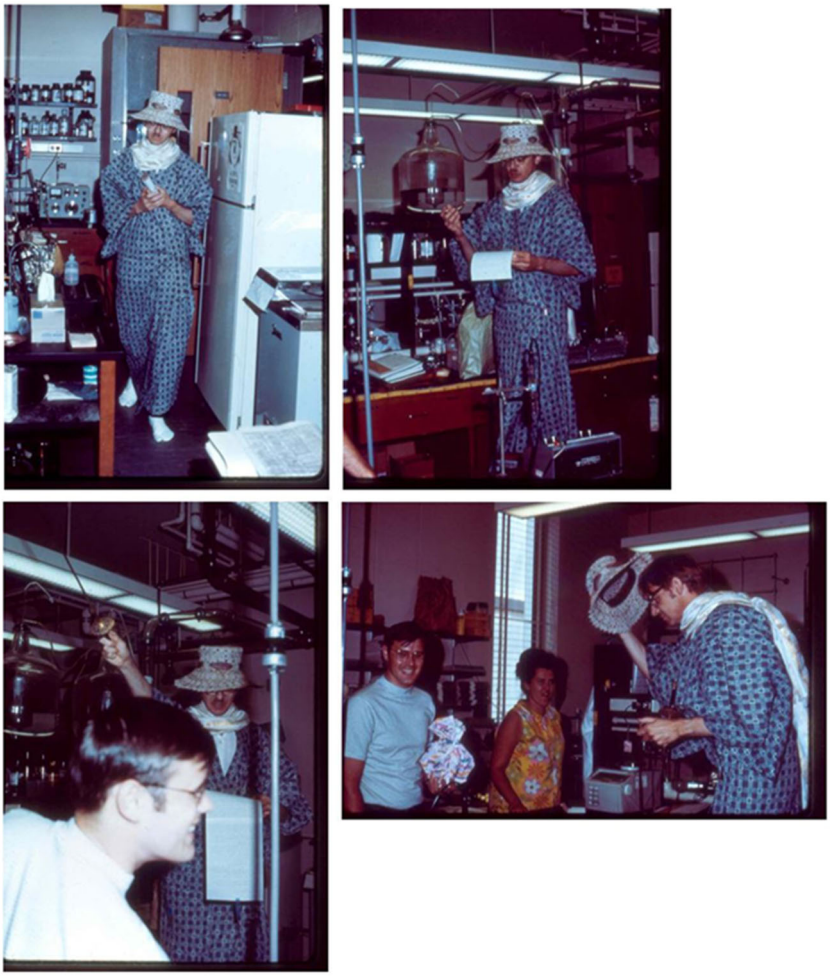

Fig. 2 Dr. Timasheff and Kirk Aune at Kirk's farewell party

Consequently, there were constant interruptions in my early education. To survive and move on, I learned what was required to handle issues one at a time. The only driving force is the desire to work hard and learn from others.

My predoctoral training was not biophysical such as that practiced in the Timasheff lab. While looking for a potential lab for postdoctoral training, I was turned down by many labs. Many responses consisted of condemnation of the Vietnam war in siphoning all the federal funding to support postdoctoral fellows. One only offered a position with the "attractive" assignment to purify proteins. The Timasheff lab was the next one to visit. I was desperate because this was the lab I considered as the lab I wanted most to join. When I came for the interview, I met the distinguished professor, who appeared to be very formal, and then Kirk Aune, a senior postdoctoral fellow in the Timasheff lab. My legs lost their desire to move. My God, this is the quality of the postdoctoral fellows in this lab. I felt that there was no way that the Professor would accept me. At the end of the interview, I expressed my most sincere desire that I wanted to learn from him and colleagues in his lab.

A couple of weeks later I was in heaven that he had accepted me, but I had to write a proposal in solvent-protein interaction, a topic that had never been introduced in courses or research. Disaster! Sure enough, when he received my "proposal" he called my mentor to tell him that he did not appreciate receiving a proposal with scientific errors. Yet, he still accepted me to join his lab. Someone must have been acting as my protective angel.

Scientific expertise in the Timasheff lab

1. Solvent-macromolecule interactions

Dr. Timasheff was an insightful scientist who was leading the field in areas of solvent-macromolecule interaction, macromolecular recognition and self-assembly. When I reported to his lab on November 1, 1971, I was assigned to work on calf brain tubulin. What specific issue in tubulin do you want me to tackle? Acquire as much as possible of the fundamental knowledge of the system. Ron Frigon, a highly motivated and intelligent student, had just joined the lab and decided to work on the assembly process. I decided to take on the basics, e.g.,

molecular weight of tubulin subunit, partial specific volume, etc. At the same time, the High Precision Densimeter arrived from Anton Parr. Using the standard commercially available proteins, I decided to set up the instrument and establish the protocol to acquire accurate density and partial specific volume values in aqueous buffers and then tackle the nasty 6 M guanidine hydrochloride. Once I have developed some confidence in both my technique and configuration of the set up in the constant temperature room, I went on to my self-assigned project, i.e., determination of partial specific volume of these proteins under different conditions to acquire quantitative knowledge on guanidine hydrochloride-protein interaction. I analyzed the data and wrote a draft to send to Dr. Timasheff during his vacation in Europe. In about a few weeks, the manuscript came back. I was depressed when I found only one sentence of red ink at the end of the manuscript. He must think this is a bunch of nonsense and I have wasted his time and lab resources. But it said, "Remember, in your calculation your values are in terms of preferential interaction." The rest is history. I was just happy that Professor Timasheff deemed that work was good enough for him to submit for publication [Lee and Timasheff 1974]. I did something worth publishing!

While carrying out the assignment to establish the basic physical properties of tubulin, Ron Frigon and I found that pure tubulin is only stable enough for a couple days to conduct biophysical studies. We used sucrose to stabilize it, as many enzymologists did and still do. We were delighted to report to Dr. Timasheff the morning after that 'great time saving discovery'. At the end of the discussion, the question was raised. "Dr. Timasheff, what is the mechanism for stabilization of protein by sucrose?" "Why don't you find out?" That is a typical example of his mentoring philosophy. He was always willing to give sound advice and provide freedom to explore. That kept me busy for some time working on sucrose-protein interaction. As always, I learned the most when I provided him a rough draft of a manuscript and he would turn that 'water' to 'wine'. He came one morning all excited. "Jim, I got it. Surface tension." The work was published after I left his lab [Lee and Timasheff 1981]. In the meantime, Ron collected a wonderful set of data on self-assembly of tubulin induced by Mg^{++} employing the sedimentation velocity approach in analytical ultracentrifuge and published two outstanding papers on self-assembly of tubulin [Frigon and Timasheff 1975a, 1975b]. Ron found that tubulin forms a double ring structure which resembles the published micrographs of disassembled microtubules, the preparation of which contained other proteins known as MAPs (microtubule associated proteins). Dr. Timasheff came to me and urged me to test if our purified tubulin can form microtubules. His rationale was that the presence of the double-ring must be a common intermediate between the monomeric tubulin and filamentous microtubule. After the third enquiry I knew he wanted the result. I used the same condition reported in the literature by replacing sucrose with glycerol. When the turbidity of the solution turned cloudy at 37°C and returned to transparent at 5°C, I was overjoyed, but I was by myself at that time—no one would understand why I was whooping and laughing. Subsequent repeats led to similar results and, with longer incubation time, at 37°C turbidity slowly decreased. Not stable! I showed this to Dr. Timasheff next day. "Good news and bad news." He pulled out a book and turned to a paper of his from many years earlier, showing how the intensity of scattered light is related to a factor n of wavelength, where the n value becomes smaller with the aggregates transforming from long filaments to self-aligned bundles (more generally known as Mie theory). The decrease in turbidity that was observed for tubulin assembly reflected that transition of tubulin to microtubule to aligned microtubules. This is another example of his ingenuity and agility of thought process. That result proved that all the information is embodied in tubulin enabling the reversible conversion of tubulin ↔ microtubule. The MAPs are not essential for the assembly process. The role of glycerol is to shift the equilibrium in favor of assembly: namely, the role of glycerol is to replace the role that MAPs play. That began the years of study on glycerol by George Na and Kunihiko Gekko [Na and Timasheff 1981; Gekko and Timasheff 1981a, 1981b]. It was a very exciting time for me to be in this lab to witness the fact that the realization of stabilization solvent and denaturant basically is a continuum of the same principle. The differences in solution behavior of these solvents depend on how each solvent's components (i.e., water and such co-solvents as sucrose, glycerol and guanidine hydrochloride) interact with the macromolecule. This theory is most relevant to nature in the case of osmolytes and to pharmaceutical companies in term of formulation as exemplified below.

Dr. Timasheff could have benefited financially from the companies because they were looking for advisors with such expertise. He was invited to give lectures and asked to provide specific formula of solvent(s) for their needs. However, he was interested in developing principles so that others could use them for their applications. In this case, John Carpenter and Tsutomu Arakawa were the best ambassadors. Dr. Timasheff regularly commented to me how much he appreciated that John Carpenter had adopted him as a mentor in this scientific field. Furthermore, Dr. Timasheff was amazed by the efficiency of Dr. Arakawa in producing high quality information and his understanding of the concept of negative interactions.

2. Macromolecular assembly

The history of studies of macromolecular assembly in the Timasheff lab dated back to the 1950s and 1960s. I will cite two systems to illustrate the elegance of those studies. In the early days when Dr. Timasheff was with USDA, he studied

the assembly of β-lactoglobulin. There are two genetic species, A and B types, from bovine. A and B types differ only by one amino acid, but B is less capable of assembly into a tetramer. It was the first system that I am aware of to be studied by sedimentation velocity and analyzed by the Gilbert theory which described the patterns of self-associating system as a function of stoichiometry and association constants. Dr. Timasheff proved that he was a geneticist and demonstrated conclusively the potential impact of genetic variants on the physical properties of proteins. The combination of sedimentation velocity and Gilbert theory is still a very powerful and sensitive approach to analyzing complex assembly systems. Dr. Timasheff was able to apply the Gilbert theory to deduce a conclusion that a genetic sample was a mixture of A and B types decades before others started to appreciate the power of high resolution of these approaches. Upon isolating pure A and B types separately, it was proved that his initial analysis of the mixture was correct [Timasheff and Towend 1958]. This was followed by a series of seven publications in the same journal on detailed quantitation of association of β-lactoglobulin. The second system is the induced assembly of calf brain tubulin by Mg++ [Frigon and Timasheff 1975a, 1975b]. This is a complex system which initially undergoes isodesmic polymerization and the final product is the closing of the 26-mer ring. Ron Frigon obtained electron micrographs on the sample and observed the 26-mer ring. He then wrote the computer program to simulate the pattern which matched the experimental centrifuge pattern. I was blessed to witness the step-by-step development.

When George Na joined the lab, besides working on the glycerol issue, he was also involved in studying the assembly of tubulin induced by vinblastine, an anti-cancer drug. It was another beautifully and thoroughly conducted study [Na and Timasheff 1980a, 1980b]. These are classics which I used and encouraged others to use in my teaching. After Vish Prakash joined the group, he worked on the interaction between vinblastine and tubulin also. I did not have the pleasure of overlapping with V. Prakash. Please see the chapter by V. Prakash.

I surmise that these high-quality papers attracted other well trained biophysical young scientists to join the Timasheff lab from around the world. That included Jose Andreu, V. Prakash, Keith Shearwin and Larry Ward. I know of them because I heard their names being mentioned often in our phone conversations by Dr. Timasheff praising the good work they have accomplished.

3. Wyman linked function

Dr. Timasheff always reminded us of the wonderful theory of multiple linked reactions. This is the area in which the new group of young scientists did their excellent work on tubulin-drug interactions. Jose expended an extensive amount of effort in studying colchicine and dissected the contribution of each ring of colchicine in eliciting its effect. He combined essentially all the thermodynamic concepts in defining the mechanism of function of colchicine and its derivatives. This is the part that I benefited most from in my career, not because I have special affinity for the equations with partial derivatives but because of my interest in allosteric regulations. Hence, I am most indebted to Professor Timasheff's kindness for not sending me away for five years. He taught me everything. He was a gentle person who was slow in anger. However, I took his comments very seriously because he would make very simple but direct comments on the quality of work produced or the integrity of my actions.

Besides science, I enjoyed many conversations with him and Dr. Gorbunoff. We talked about religion, politics, and music, particularly about opera. He had a great sense of integrity and would not bend when he considered a concept was wrong. He did not like the change of the name of his beloved homeland, Russia. He never visited his homeland until it reverted to its original name. He did go back to visit, and I know that meant a lot to him. Lucy, my wife, and I remember vividly the wonderful friendship we shared with his daughter, Marina Timasheff. She was very young in primary school. She always demonstrated that she was intelligent and lively. Occasionally, I played catch with her, and the poor dog was running ragged between us.

Many of us still wish that we could contact him and have his wise comments. We all miss him tremendously as a person and mentor. Nevertheless, the 5 years in his lab were the best and most rewarding years of our lives. We shall always treasure them.

V. Prakash: The new vistas of Physical Biochemistry of Proteins and Solution thermodynamics laid by Serge Timasheff for the future generations.

It's a great loss to the family of physical biochemists and protein biochemists and solution thermodynamics scientists who are working in the area of macromolecules with the demise of Prof. S. N. Timasheff on February 25, 2019. I had a close association of working as a postdoctoral fellow in the late 70s and later as visiting research associate with him under the NSF Indo - USA program in the early 90s. The journey of reaching Brandeis for me not only was accidental but also changed my scientific career forever.

Let me start with my Ph.D programme at CFTRI, Mysore, India under the guidance of Prof. Pradip K. Nandi, a strong Physical Chemist. He did his postdoctoral on model peptides at Harvard Medical School, Boston with Prof. Dwight Robinson, who had a very close link with Brandies University. Prof. Nandi consistently directed me to the well-established journal series "Methods in Enzymology" edited

by Prof. Hirs and Prof. Timasheff for protein chemistry section. That was the first time I heard about these gigantic names. These names were ringing in my ears in 1972 as a graduate student. At that time, I was working on denaturation of proteins and association -dissociation of protein subunits in seed proteins [Prakash and Nandi 1977]. As I finished my graduate studies, it was very natural to think of the west for a postdoctoral position and I started writing to many places. I was not successful at first in procuring a position, as it was a change-over year with the election of a new US President and not many grants were foreseen. It struck a chord when I received an airmail letter from Prof. Kirk C. Aune that I was being given a postdoctoral position for a year in his laboratory. Prof. Kirk Aune, who was a graduate student of Dr. Charles Tanford (author of a textbook titled "Physical chemistry of macromolecules") in late 60s, was at that time in the Biochemistry Department at Baylor of Medicine, Houston, Texas. He was working on association -dissociation of ribosomal proteins and I accepted and moved on. Here I was inheriting two convergences. Prof. Aune was not only a student of Dr. Charles Tanford but also a postdoctoral fellow with Prof. Timasheff in the late 60s.

About a year after the publication of several papers with Kirk Aune [Prakash and Aune 1978], I realized that I had to return to India, as my Visa would expire unless I had a postdoctoral fellowship in the USA, as Kirk Aune was running out of grant funds, of which he warned me well ahead with a heavy heart. I was down, as I had a dream of building my career forward as a young protein chemist. Then came a turning point, as one day Prof. Kirk Aune called me into his room and asked me if I was ready to move to Brandeis University in Boston (actually Waltham, a suburban city of Boston) to work with Prof. Timasheff. The winters were scary in Boston for an Indian and that too from Houston! I took a day and answered yes, moved to Waltham and met Dr. Timasheff, a "tall person" who told to me to just settle down for a while, and we will talk of work later. That was his way of working. Diana (Timasheff's secretary) took me to all the postdoctoral fellows to introduce me and there I met Arakawa amidst George Na, Gay May Wu, Linda Grisham, Neil Tweedy, Steve Scheufele, Arthur Harvey and Jose Andreu, among others. For nearly a fortnight, I could not meet Dr. Timasheff as he was busy writing a grant, and I was left alone to fend for myself. I was supposed to work on tubulin and my passion was hydration and association-dissociation of proteins. After a fortnight, I met Dr. Timasheff and had a very long discussion, and I felt I was in the wrong place with so much physical chemistry of proteins! He described to me tubulin and other proteins and the clathrate structure of water and the hydration shell. It was all Greek and Latin to me! I knew the only way to understand Dr. Timasheff's mountainous knowledge was to read more and work hard and take his graduate course lectures. I started auditing the classes of Drs. Timasheff, Fasman, Jencks, Levine, Colin Steel, and Szent Georgi Jr., all wonderful physical chemists of great reputation. This exposed me to a wonderful teacher, Dr. Timasheff, a born gift that he had; however, one had to put extraordinary effort into preparing for his classes, which was demanding on me with my volume of work as a postdoctral fellow. I was in constant touch with Kirk Aune and Pradip Nandi during this period of settlement, like the early settlers at Plymouth, Massachusetts! Within no time I did settle down and got involved in tubulin work. (Steve Scheufele used to help me going to Hopkinton to get the calf brains.) Several papers stemmed from my tubulin work. I also picked up the fascinating world of protein hydration and even had a chapter in Methods in Enzymology, a series which I was introduced to during my graduate days, and here I am with a chapter in it on association and dissociation of proteins by ligand induction, and many other papers [Prakash and Timasheff 1985, 1986, 1992]. Prof. Timasheff, a fine gentleman, and Dr. Gorbunoff were excellent hosts and it was very pleasant to meet them in their residence over dinner many times.

I left for India in 1980 and could not return back to the USA immediately, so I settled for a job in India. But later with an NSF Indo-USA grant with Dr. Timasheff, I came to his laboratory again, after 10 years, as a co-worker for three months a year for three years. And, every year, I used to meet Rajiv Bhat, Keith Shearwin, Octavio Monasterio and Trisha Murray. What changes in direction and events happened, when I look back and realize that there I was with Dr. Timasheff as a 5th generation person in the schematic flow of things from Drs. Jencks to Robinson to Nandi to Kirk Aune to Timasheff! And, en route, many other stalwarts whom perhaps I could never have met otherwise. All these scientists are a destiny linked with string of solution physical chemists in the necklace of my career, which I never dreamt of. My CV blossomed with so many publications in the area of tubulin and protein hydration with Prof. Timasheff, from which I was able to go on to build up a strong group of protein chemists in India, head the Department of Protein Chemistry in CFTRI, Mysore, India and finally end up as Director of the same Institute. I owe most of my success to my teachers and guides in this academic pursuit and to the key role that Dr. Timasheff played in shaping my new outlook of deep insight into solution chemistry. The thermodynamic insight was the unique knowledge that I could get in this process. Arakawa has created this wonderful opportunity to write a small tribute to Prof. Serge Nicholas Timasheff, a mountain by himself in solution physical chemistry and thermodynamics, and every paper of his has always an innovation with one more equation in Protein Chemistry. He was a simple and very humble and rather shy and silent professorial personality, who knew the world of science to its core as a voracious publisher of manuscripts and a deep thinker. We had informal discussions every Friday in his group (there used to be doughnuts, cheese

and crackers which Diana first and later Trisha used to organize) and Dr. Timasheff used to be always there even though for a short while (sometimes Marina, his daughter, used to join if she dropped in), but Dr. Timasheff's retraction from the group was in his characteristic way of moving backwards while saying bye (such was the humbleness he had), and now he has said goodbye to us forever.

In conclusion, I like what John Schellman and George Somero have in their concluding sentence of an excellent write up in Biophysical Journal, a living memoir to Prof Timasheff [Schellman and Somero 1996]. It says, "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." As his students, we must keep in touch with each other to share our thoughts to promote biophysical chemistry as Dr. Timasheff's extended family of large groups spread around the world. That's the best tribute we all can pay to Prof. Timasheff.

Rajiv Bhat: How my association with Prof. Serge Timasheff shaped future course of my research work

The year was 1985 and I got an opportunity as a graduate student working in the Indian Institute of Technology, Delhi to present part of my research work at the 2nd Biothermodynamics Conference, at an exquisite location called Schloss Seggau located on the top of a pristine hill in the small town of Graz in Austria. The castle, the venue of the conference, was mesmerizing and the Scientific gathering consisted of the who's who in the area of Biothermodynamics. I knew Prof. Serge Timasheff, to whom I had written a few months earlier about a possible postdoctoral position in his lab, was one of the prominent participants among the elite group of professors. Along with my poster presentation I also had a short oral presentation which generated some discussions. After that I sought out Prof. Timasheff during lunch break regarding the possibility of working with him. He seemed to be keen to know my academic strengths after having listened to my talk, and asked for a fresh cv and mentioned that he is likely to have an opening in a few months' time. From my conversation with him, I felt that he would consider me to join his lab provided he has a position to offer. My first impression of him was that he was a sophisticated soft-spoken gentleman, pleasant to talk to and quite humble in his conversation. Even though I was a young student in my mid-twenties, he listened to me with patience and answered all the curious and naïve questions I had about his work.

A few months later, I received an offer letter from him for the postdoctoral position, but he also needed a recommendation letter from my graduate supervisor before making a formal appointment. I landed in Boston in the first week of March 1986 and went straight to Brandeis University in Waltham where I stayed at Brandeis Guest house for more than a week's time before I could find my own place to live. The next day I went to Kosow Biochemistry Department lab to meet Prof. Timasheff, where I was warmly welcomed by him and two of his postdoctoral fellows; one was Prof. Hiroshi Doi from Japan and another Prof. Robert Seckler from Germany. On the same day, Prof. Timasheff handed over a bunch of papers from his lab to read and gave me a piece of paper mentioning that I could explore the effect of polyethylene glycols on the preferential solvation interactions and stability of a number of proteins as it was committed in his NIH grant. He was quick to add that it was necessary to do an initial exploration and expand the work further depending on the results obtained. He did not seem to be insisting that he already had a plan and I must follow it strictly. With my previous training, I was not used to be told what to do but explore on my own, and it gave me a sigh of relief that he was a person who would give you ample freedom to explore and to ask questions at every stage of your work and change course, if needed. I told him naively that in his lab I would have the opportunity to explore protein-solvent interactions and to work on proteins, which I had not worked on before as my graduate work was on amino acid and peptide solvation and calorimetry. He mentioned that in his lab I would have all the time I needed to play with proteins, and that made me happier. I asked him what made him select me, as I had found that most of the postdoctoral fellows that worked in his lab were either recommended by professors whom he knew personally or postdoctoral fellows who had worked with him, which was not the case for me. He had few postdoctoral fellows at a time in his lab and was very careful and cautious in selecting them. He told me that after going through my J. Phys Chem. Paper on heat capacities and partial specific volumes of amino acids and peptides in water using microcalorimetry and densimetry [Bhat and Auluwalia1985], he was convinced that I could get a head start in his lab quickly as I had prior experience in precision density measurements and the determination of preferential interaction parameters for proteins in aqueous solutions require meticulousness in measurements. At that time there was no one to teach me such measurements, as the other two postdoctoral fellows were biochemists and were working on Tubulin polymerization and its inhibition. Before me, Dr. Tsutomu Arakawa, who had spent a considerable time in his lab, had left to join Amgen. I was therefore left to fend for myself.

My problems started when I started the work trying to repeat the previous work done on protein-solvent interactions. The work required purifying the proteins, setting up dialysis equilibrium and the transfer of solutions into the density meter in a quantitative manner. I was a bit disappointed that the

DMA 02C density meter I was to use was quite an old one with display in the form of nixie tubes. I was wondering whether I would get precise results out of the machine having used a much more recent and modern version DMA 60/602 in my previous lab in Delhi. The water bath attached to it also had a second decimal accuracy in temperature, while I had used a set-up with third decimal accuracy in temperature earlier. But to my surprise there was a sixth decimal accuracy in density measurements with some fluctuations leading to sometimes fifth decimal accuracy. There was one difference in that the whole set-up was housed in a large temperature controlled walk-in room which I thought would compensate for the variations in measurements as all the work, including weighing of solutions, etc., was done in that room. However, despite my initial efforts, the results on apparent specific volumes that I obtained for RNase in buffer had greater uncertainty at the second decimal compared with third decimal reported by others from his group. I discussed this matter over phone with Dr. Arakawa and he also visited the lab at Brandeis later, but it took me more than a month to fix various issues and to realize how meticulous one has to be while carrying out such experiments. The catch was in determining the concentrations of the protein samples after their dilution following density measurements. Dilutions and transfers and cleaning of the cuvette and its drying had to be done in the most quantitative manner and required a third decimal accuracy in measuring absorbances. By this time I knew how to proceed in collecting good data on protein-polyethylene glycol (PEG) interactions [Bhat and Timasheff 1992].

I set out planning the work after discussing the plan with Prof. Timasheff. He was always available for discussions, but did not want us to disturb him unannounced as he could be writing papers or reading papers or discussing over the phone with a colleague about various scientific issues. I found him often discussing science over phone with experts in various areas while he was correcting manuscripts written by postdoctoral fellows. He would often come to the lab and ask if all was going well and most of us would nod in the affirmative. This was always an opportunity to discuss with him or to seek a suitable time if one needed a longer sitting to discuss academic issues. Once I was on track, I needed very little discussion with him except for when I had substantial data to discuss. Our long discussions would take place only after a month or so and never on a daily or weekly basis.

After a year into the work, I expressed my desire to visit my parents in India for a month and to present the work in a Protein Engineering Conference at the University of Oxford, probably the first one in the area. I was not sure if he would permit such a long leave, but I had a sigh of relief when he mentioned how happy he was with my work accomplished in a short span of time and also readily agreed to presentation of the work. This made me realize the humane values he had and the appreciation he would show of his colleagues' work. On another occasion, probably in 1988, he requested that I give a talk in the Friday Seminar Series in the Department of Biochemistry at Brandeis. This talk was done by stalwarts of science from outside as well as faculty members of the department. When I responded that he should be the one delivering the talk, he insisted that I give it and introduce the concepts of preferential interaction measurements to the general biochemistry audience. This made me quite nervous and I had to prepare hard not to let the lab down as I was not experienced enough to give such a talk. After it was over and generated quite a bit of interest and curiosity in the area, Prof. Timasheff walked over and thanked me for the nice talk and mentioned how happy he was as, unlike him, I was successful in conveying the nuances and complexities of the measurements and their applications to the faculty and students attending in a much simpler and effective way. This was quite a compliment for a young person like me and coming from a person of his stature was even more delighting.

After working monotonously measuring densities of solutions for 12 h a day, 6 days a week, I once requested him if I could learn aspects of the tubulin-related work going on in his lab. He welcomed me to learn as much as I could in areas other than those I was already working on. This enabled me to learn tubulin isolation and purification from calf brains which was in itself a very tedious one-week process as it was highly sensitive to denaturation and degradation and one had to keep the purified tubulin in liquid nitrogen for its long-term stability. I also learned the use of analytical ultracentrifugation in tubulin work and the use of HPLC in quantitatively analyzing GTP binding data and the use of other techniques to measure calcium binding to tubulin. This experience later helped me train other postdoctoral fellows after Hiroshi Doi and Robert Seckler had left the lab.

There came a time when Prof. Timasheff informed me that he would be going for a one-year sabbatical to Gif-Sur-Yvette in France and that I would have to take care of the lab. He told me that I need not worry in his absence as Trisha Murray, his trusted and long-term secretary, would take care of all logistics and I could always write him letters to which he promised to respond promptly, or I could call him over the phone, if required. During this period of little over one year, I corresponded with him frequently and have his hand-written letters with me even today. After going through them recently, one can clearly see his vision and clarity of thought and passion for doing excellent quality work without compromise. This is the reason that he was not a prolific publisher and used to take years to perfect the writing of a paper. Until he was satisfied, he would keep working on it. As a result, I found that he had a backlog of manuscripts written by earlier postdoctoral fellows lying with him in the filing cabinet and he would pick up one after the other. Nonetheless, most of his papers have remained highly cited.

There were no computers at Brandeis in those days for the faculty, and most of the typing work was done using electric

typewriters. Only in 1988 was he able to get a PC-XT for administrative work, which was an expensive gadget in those days and ran on an MS-DOS and WordPerfect typing software. I had found out that there was an LKB microcalorimeter in the department which had remained unused because either no one could use it properly or the interest was not there. I requested Prof. Timasheff if I could get it from the basement and try to activate it and use it in the lab as I had considerable experience in the area. This is the only time that he dissuaded me initially, but seeing my enthusiasm, allowed it to be brought to the lab. I realized soon after I struggled to get it going because many parts were missing, that he did not want me to get distracted and waste precious time. I soon gave up on the idea.

When he was present at Brandeis, we had some stalwarts in Protein Science visiting the lab, spending time with us and delivering general talks at Brandeis. Notable among them were Charles Tanford, Peter Privalov and Thomas Creighton. During the time I was working on protein-PEG interactions, Prof. Timasheff wanted to complete an earlier piece of work carried out by Tsutomu Arakawa on salting-in and salting-out effects on proteins in the presence of $MgCl_2$ and $MgSO_4$. This in his opinion required the measurements of solubilities of several proteins in their presence in order to evaluate the chemical potentials and then relate it to preferential binding or preferential hydration. This sounded like simple work and given my 'never say no' and 'learn as much as you can' policy, I accepted to do it. I asked how to do it without wasting too much protein, so he took me to one of his temperature-controlled rooms and showed me a small Beckman Airfuge (ultracentrifuge) with a mini rotor in which you can place 100 microliter tubes and spin them. Contrary to my earlier thinking, this sounded like quite a bit of a challenge to solubilize the proteins to saturation, centrifuge the samples and measure solubilities in the supernatant after ultracentrifugation. It took me almost a year to do the work, but considering the stringent requirements of accuracy and reproducibility, I managed to measure the values precisely. Later on, this work culminated in two papers with Tsutomu Arakawa which have been highly cited [Arakawa et al. 1990a, 1990b]. The best compliment I have ever heard from anyone was when Prof. Timasheff, while introducing me to Prof. Charles Tanford, mentioned to him that I had done work which no one was able to do before. When prompted by Tanford he explained about measuring solubilities of proteins in salts very precisely and getting reliable chemical potential values out of them.

The PEG work I had carried out was written as a set of two papers, one for lower molecular weight PEGs and another one for higher molecular weight PEGs, as the work was quite exhaustive, including five proteins, different pH conditions and eight PEGs of different molecular sizes ranging from 200 to 8000. This was written for Biochemistry and I was certain of its publication. Before it could be scrutinized by him and sent, he informed me that he received a call from Prof. Hans Neurath, who would be the Editor-in-Chief of the newly established journal Protein Science and would want our best work to be published in the inaugural issue. It seems that Prof. Timasheff could not deny his long-term friend and associate Prof. Hans Neurath and had already agreed. Upon hearing his decision, I was quite disappointed and conveyed to him that the new journal was not known at all and might not stand tall in years ahead, but when he mentioned that with Hans at the helm of affairs and a strong Editorial Board it would become as good as Biochemistry in future, I relented. He was right and the paper, though squeezed to one paper from two upon reviewers' suggestions, became a highly cited work [Bhat and Timasheff 1992].

Prof. Timasheff wanted to carry out preferential interaction work at higher temperatures and asked me whether it would be possible for me to do the work. He never insisted or ordered me to do work but always asked for willingness to do it and simply expressed his desire. I agreed but found the work too difficult to do for technical reasons, as the dialysis equilibrium experiments needed to be done at higher temperatures of 50 or 60 degrees. This was difficult as there were considerable evaporation losses creating large uncertainties in density measurements. I had little time left as I had got a faculty position in India, but toward the end of my time, I had trained a postdoctoral fellow from China, Gui-Fu Xie, who was willing to work hard and learn with great care. Many years later, she was able to accomplish the very difficult work, which Prof. Timasheff did mention in his biography as being extremely challenging work. Overall, my little over three years in his lab were highly productive and allowed me to carry out cutting edge research in the area, learn new vistas, make great friends both American and foreign, many of whom are faculty in leading universities of the US and the world or at very senior positions in the biotech industry or having founded their own companies. One of them, Rod Mackinon, who won the Nobel Prize in Physiology and Medicine in 2010, was a postdoctoral fellow in Chris Miller's lab working on electrophysiological aspects and his Nobel winning work was certainly inspired by his earlier work at Brandeis. Prof. Timasheff's benevolent approach toward his postdoctoral fellows and colleagues allowed me to explore the US in its breadth and width. Many Americans colleagues whom I met at Brandeis felt jealous that I had known and explored more of the US than they had even after living there for so many decades.

After returning to India in August 1989, I joined the Centre for Biotechnology at Jawaharlal Nehru University as Assistant Professor, after a brief stint at Delhi University. I had carried a few proteins from the US to be used in my research and wanted to explore the stability of proteins in various stabilizers, calculate the free energies of stabilization upon thermal denaturation and eventually relate it to preferential interactions to further authenticate and reconfirm the role of

preferential interaction effect to protein stabilization over a wider range of proteins and varying condition. This work led to a number of highly cited papers and reconfirmed the importance of the hypotheses proposed by Prof. Timasheff. Our work on the effect of trehalose and other polyols on protein stability remain the most cited papers from our group. Currently, we are also exploring the applications of the preferential interaction hypothesis towards the effect of protein structure stabilizing molecules, which had been explored for globular proteins, on intrinsically disordered proteins of the synuclein family, especially human brain alpha-synuclein. The generalizations of the effects observed in Prof. Timasheff's lab with his vision and foresight are having a lasting impact on the field of protein-solvent interactions and protein stabilization with applications ranging from physiology (e.g., tributes by Crowe or Somero) to industrial biotechnology (e.g., tributes by Carpenter or Arakawa). What I learned from Prof. Timasheff was that it is not the machines but the ideas behind the work that make all the difference.

Jose Andreu: Life at the Timasheff lab circa 1980

During my PhD work on the quaternary structure of bacterial F_1-ATPase, I became progressively interested in protein assembly systems. Tubulin, the subunit of spindle, cytoplasmic and flagellar microtubules, had been discovered not long before and was the target of such antimitotic drugs as colchicine and vinblastine. Two seminal papers by Lee and Timasheff, published in Biochemistry [Lee and Timasheff 1975, 1977], showed how microtubules could be reconstituted from purified tubulin in the absence of other macromolecules, with magnesium, GTP and the co-solvent glycerol. I found extremely attractive their discovery that the tubulin molecule contained basically all the information required to self-assemble forming microtubules, following an Oosawa nucleated polymerization mechanism. When I wrote to Dr. Serge N. Timasheff (SNT) applying for a postdoctoral position, I was carefully interviewed by means of several air-mail letters during the next months. There was no internet at that time, telephone talks through transoceanic cable had a disturbing delay time, and a Madrid-Boston round trip would have been quite expensive for an interview. Thus, without having ever met in person, building on mutual trust, I joined the Timasheff lab November 1978, with a fellowship from the US-Spain cooperation program that was followed by a NIH Fogarty fellowship. The three years that I spent at Brandeis shaped the rest of my scientific career during the coming forty years.

Shortly after arriving at New England, close to Thanksgiving in the middle of snow, which I recall SNT considered "a mild winter day," I remember my wife and me renting an apartment in Waltham (SNT: "go and find an apartment") and buying a car (SNT: "you will find that living in the USA without a car is almost impossible"). I ventured into North Cambridge to buy a reasonably used car from a black man, who answering my questioning about the state of the large vehicle said: "I wouldn´t lie to you, brother."

I was assigned a bench and desk back-to-back with Linda Grisham, possibly one of the few black scientists in all Brandeis at that time, from whom I learned how to purify my own tubulin, around half gram at a time, as we became good friends. "The prep" started at the slaughterhouse and was a kind of tour de force, dealing with several liters of calf brain homogenate in the cold room, simultaneously running centrifuges and performing large-scale chromatographic procedures, while the yield decreased with the time passed before stabilizing the protein; this has changed little since then. The other postdoctoral fellows in the lab at that time included George Na, V. Prakash, Gay-May Wu and Arthur Harvey, followed by Octavio Monasterio, and Neil Tweedy who was the predoctoral. Each of them studied the interactions of tubulin with different antitumor drugs or ligands, whereas protein-solvent interactions were the realm of tireless Arakawa. The influence of the outstanding work by Ron Frigon and Jim Lee could still be felt in the lab.

Each of us worked independently and was helped by the others as required. I found myself, a fresh postdoctoral fellow, basically on my own. But I could go to and request a talk with SNT at his office, if he was not travelling around the world, or he would come to me at the lab, always careful not to interrupt any experiments. There was no need of endless talking. He was a deep thinker who had the sharp mind and the right words to help you realize what the important problems were and how to tackle them, if you were receptive and prepared to work. I learned from him the importance of rigorous reasoning and experiments, as well as to let results lead me into further research. His elegant data analysis, frequently employing Wyman linked functions and thermodynamic boxes, rounded off the papers. When at some point I urged him to have my papers published, his calm answer was "José, young people need good papers." I attended Professor Timasheff´s course on Physical Biochemistry at the Graduate Department of Biochemistry, which behind a somewhat dull appearance proved to be a real source of inspiration for our research. I still treasure my notes from that course, as well as notes from Professor Don Caspar`s classes on Biological Assembly at the Rosenstiel Research Center penthouse.

Following some initial exploratory experiments with podophyllotoxin, Dr. Timasheff asked me to sort out the interaction of tubulin with colchicine, which was hardly amenable to equilibrium binding measurements, due to the very slow binding kinetics and the extremely slow dissociation of the tubulin-colchicine complex. We decomposed colchicine into two rapidly binding single-ring fragments, whose specific weak interactions properly added account for the large free

energy change of colchicine binding [Andreu and Timasheff 1982a]. We also found that the tubulin-colchicine complex self-assembles into abnormal polymers, which helped to explain the inhibition of microtubule assembly by colchicine [Andreu and Timasheff 1982b]. Following my return home I started my own lab at CSIC-Madrid with the initial help of a USA-Spain joint grant with SNT. I came back a couple of times for short work periods at Brandeis. We collaborated on the design of high-affinity, reversibly binding simplified colchicine analogs, which were synthesized by Dr. Marina Gorbunoff, and further deepened into the mechanism of microtubule inhibition by colchicine. Our last work was published 1998 in Biochemistry (Andreu et al. 1998), completing a series of fifteen papers together. Thus, my postdoctoral fellowship with Dr. Timasheff spanned twenty years. He first was my mentor and later an example to follow, while my research interests spread across the tubulin superfamily of proteins. Timasheff´s studies on tubulin and on protein stabilization by co-solvents continued being a source of inspiration for my own work, as for the tubulin structural assembly switch and, recently, the osmolyte-induced refolding of bacterial cell division protein FtsZ. Nowadays, more than two decades after his retirement, the Timasheff papers are still being heavily cited, underscoring the wide biological impact of his colossal work on protein thermodynamics.

On a personal note, I would say that Serge N. Timasheff remained a European in the States, because of his White Russian parents and having grown up in France. It took me some time at Brandeis to realize that he kept the Orthodox Christian faith. He was gentle but had a strong and constant character. He enjoyed arts and history, conversation, good reading, food and wine. He liked different cultures and visiting many places, even small Romanesque churches in the Pyrenees. I did not see a television set while at his home for dinner. We developed a long-lasting friendship, with mutual visits in Boston, Madrid and Paris. Sadly, we last met his wife Marina Gorbunoff and him in the summer of 2012, months after he had suffered a physically disabling stroke. They still are on my mind.

Octavio Monasterio: Tribute to Professor Sergei N. Timasheff

My first thought when writing these lines about Professor Timasheff is that of the figure of a great man worthy of admiration who has made a mark not only in science, with his contribution from the area of protein chemical physics towards the understanding of the behavior of solvents to understand processes inside the cell, but also in the training of researchers who in one way or another have set the course for the advancement of knowledge by laying the molecular bases of complex cellular functioning. Regarding the human contribution to the development of science, with his example, his teachings, and his exemplary life, he contributed to the integration of working groups of several of his disciples through mutual collaborations. It is important to highlight his way of approaching the common thread of his research lines, always showing a balance between his interests and the interests of others who participated in his laboratory. This "freedom" of work, very unusual in competitive groups, is a faithful reflection of the development of his scientific life. This can be appreciated from the reading of his memoirs, which begin with the name of a classic opera "La Forza del Destino" by Giuseppe Verdi. He published this in a biography [Timasheff 2004]. After this brief profile about Dr. Timasheff, I will refer to my stay in his laboratory, especially with anecdotes and a brief description of the scientific work that I carried out there.

I had the opportunity to meet him for three years of my life in the early eighties. The first thing that caught my attention was his human quality. I remember that when I arrived at the laboratory after arriving in Boston, the first thing he asked me was "Have you found a place to live?" and after giving me several tips, he told me "take your time and once you are installed come to the laboratory." It was like meeting someone from my family. Already in the laboratory, he proposed to me to work with FRET (Fluorescence Energy Transfer) to resolve distances in the non-exchangeable magnesium binding site using terbium as a metal. The existence of fluorescent probes at that time was still precarious, so I began searching in the literature, and in the experimental part, I began my training in the purification of tubulin, from calf brains that we acquired in a Cambridge slaughterhouse. To arrive at a pure, good quality, and stable protein took me some time. Fortunately, the yields were good, between 500 and 600 mg from 10 or 12 calf brains. The challenge was to lower the critical concentration of polymerization that was already established in the laboratory for a given magnesium concentration. I remember showing him the results and his response was "it is as usual." This, instead of discouraging me, prompted me to continue working and to have more and better results, because, given the freedom in the laboratory, you were responsible for your work. With the challenge of the location of the non-exchangeable metal and the proximity of the nuclear magnetic resonance instruments in the department, I decided on the task of measuring the distance from the metal to the GTP analog, with fluorine in the gamma phosphate, which probably was bound to the exchangeable site, and I used manganese instead of magnesium.

For the synthesis of the compound, my first steps were to consult the German chemistry literature. Due to my ignorance of the German language, I received a lot of help from Dr. Timasheff's wife, Dr. Marina Gorbunoff, who was an expert in chemistry. This was a purely chemical job and luckily I had a high yield of GTP fluoride-labeled gamma phosphate and beta labeled phosphate of GDP. The procedure set up in the

laboratory, on one hand, allowed me to count on a sufficient quantity for all my work, and, on the other hand, had the advantage that it was not a commercial product, so I had almost no competition. For the characterization of these compounds, I used phosphate and fluorine nuclear magnetic resonance and I was able to verify their presence without any doubt. During these studies, I met Dr. Alfred Redfield, Professor of Physics and Biochemistry, who was a genius on the development of NMR and I was able to gain his trust as he allowed me to manipulate the spectrometers that he had built. With this, all was ready to start the distance measurements. The next step was to demonstrate that the GTP analog was bound to the exchangeable site and to determine its effect on microtubule assembly. The compound turned out to be an inhibitor of the polymerization of both the dimeric tubulin and the elongating microtubules, since when they were added to the polymerization solution, they produced an arrest of the increment in turbidity. To understand what was happening, I tested its effect on GTPase activity induced by polymerization of tubulin and it turned out to be a competitive inhibitor. To explain the inhibition, I recall having used the steady-state kinetic theory, dependent on protein concentration. When Dr. Timasheff saw the equations that explained the phenomenon, he was greatly interested in finding an alternative way to test the robustness of the interpretation, and we had interesting discussions on the subject. I remember a word that he used with great emphasis, "carefully." This showed the rigor of him and that he demanded of those who worked with him. These results were published in the journal Biochemistry [Monasterio and Timasheff 1987]. The work with NMR, specifically with the measurements of the longitudinal and transverse relaxation times, was developed during my last year in his laboratory. For this, I had to make use of the two pieces of equipment that Dr. Redfield had, and this meant, on more than one occasion, getting up early to put them back in the conditions used by the rest of the users. Our results showed that the metal was not found in the exchangeable site attached to the nucleotide [Monasterio 1987], which had been previously proposed [Jemiolo and Grisham 1982]. Later this was confirmed in an elegant work by Correia's group [Correia et al. 1988]. I want to highlight here the generosity of Dr. Timasheff who allowed me to explore other areas of knowledge within the "freedom" that I always felt in his laboratory.

Before concluding with this brief story, I wish to show another of the facets of Dr. Timasheff, his preoccupation with the members of his laboratory. At the end of the first year of my stay, I remember that he approached my desk and asked me if I was going to take a vacation. I told him that I had not really thought about it and that I had to talk with my wife, who worked at Harvard Medical School. When I gave him the answer a few days later, telling him that we planned a trip to Europe, he reminded me that he would take a sabbatical in Paris in the laboratory of Dr. Pantaloni. We made plans to visit Italy, where my wife's family is from, and France and then Spain, where we would visit José Manuel Andreu and greet him on the birth of his first son. When we visited Paris, we contacted Dr. Timasheff and his wife. I especially remember a visit with them to the Palace of Versailles (Fig. 3), where he told us about his childhood, since he lived very close to this palace, which was on the way to his school. I remember that both of them very kindly accompanied us to the train when we left Paris. This, as I said, was a reflection of his human values.

I remember that on more than one occasion they invited us for dinner at his house, which was a place with a cultural air with European style. In his living room there was a grand piano and all the conversations were about current affairs in counterpoint with great knowledge of history, as he relates in his Comprehensive Biochemistry article [Timasheff 2004]. It also much caught my attention that he practiced culinary art, with exquisite dishes that always had an associated history.

Our friendship and I dare say it this way lasted until the day he left this world. In the late 1980s and in the 1990s, I had the opportunity to invite him twice to Chile. Once was to participate in the Annual Meeting of the Biochemistry and Molecular Biology Chilean Society and the other was to the Annual Meeting of the Society of Biology. He gave magnificent lectures on the effect of solvents on the stability of proteins and about dynamics of cytoskeleton. Before the meetings, he met with students and professors from our Faculty of Sciences to discuss science. On the last occasion, he also had the opportunity to travel through the South of our country and Argentina, and he ended his trip at the impressive Iguazu Falls. I have no doubt that I have omitted many things to show the greatness of this scientist who has left us a legacy that has been key, at least for me, in my scientific work. Thank you very much Dr. Timasheff.

Fig. 3 Visit to the Palace of Versailles in October 1982. In the picture from left to right, Dr. Rosalba Lagos, Dr. Marina Gourbunoff and Dr. Timasheff. At the back the Palace

Bernardo Perez-Ramirez: Professor Timasheff a towering figure in solution thermodynamics and a gentleman of his time.

I first encountered Dr. Timasheff as an undergraduate in biochemistry in 1981 at the Universidad Austral in Valdivia, Chile. I spent time at the library reading from the large collection of the "Methods of Enzymology" book series, of which Dr. Timasheff was one of the editors . However, it was not until 1990 that we met for the first time. While I was completing my PhD in protein chemistry at the University of Missouri under the direction of Dr. Marino Martinez-Carrion, working on the problem of the topology of the acetylcholine receptor, it became clear that understanding the physical biochemistry of protein interactions was important in order to expand my knowledge of protein-structure and function. With my PhD dissertation thesis almost complete, my advisor asked me about my plans for postdoctoral work. I told him that I had applied to two positions—one of which was at Brandeis University with Dr. Timasheff. Immediately, my advisor discouraged me from going to Dr. Timasheff's laboratory, letting me know that he had not prepared me for that complex kind of work, and that I was grossly unprepared. The same message was conveyed by my advisor to Dr.Timasheff and is summarized by Dr.Timasheff in his Recollections [Timasheff 2004]. Nevertheless, in March 1990 the telephone rang in Dr. Martinez-Carrion's lab, and it was Dr. Timasheff who wanted to speak with me. He told me that there might be an opening in his laboratory to do postdoctoral work on the problem of the colchicine induced GTPase activity of tubulin. He asked me to think whether I was interested in that project. He also wanted to meet with me, as soon as possible, before his annual trip to France. It was April 1990 when I arrived at the Department of Biochemistry at Brandeis University to meet Dr. Timasheff; I confess that I was scared, because of what my advisor had told me about my unpreparedness. Trisha Murray, his faithful secretary, greeted me, knocked on the door of Dr. Timasheff's office and from inside a voice said, "come in." We entered the office and Dr. Timasheff was sitting at his desk facing the large window, with his back to the main door. I think he chose to set up his desk that way, because the view of the trees was magnificent and during the fall there was a festival of red, orange, and yellow colors from the leaves. He turned around, and we were introduced by Trisha Murray, before she left. He asked about my PhD thesis project, and explained the potential postdoctoral project on the activation of the tubulin GTPase. He was soft spoken, dressed in a white shirt and a matching tie, and had the demeanor of a true gentleman. He asked me why I wanted to go to his lab, and I told him that I wanted to learn [Timasheff 2004]. To my surprise, after my response, he immediately asked me when I could start.

I joined Dr. Timasheff's laboratory in September 1990. There were already three other postdoctoral fellows: Keith Shearwin from Australia, Gui-Fu Xie from China, and Tiao Yin Lin from Taiwan. My appointment was for a period of two years, but he allowed me to stay in his laboratory for 6 years and I turned out to be the last postdoctoral fellow when I left his laboratory in March 1996. I returned later that year to help him dismantle the laboratory when he retired.

When I joined Dr. Timasheff's laboratory, he was in France, so Keith Shearwin showed me all the procedures on how to prepare tubulin and trained me in how to operate the old model E analytical ultracentrifuge. Keith and I worked on the different tubulin projects together, became good friends, and established extensive collaborations (Timasheff 2004).

Dr. Timasheff gave me total freedom to plan my experiments and do independent research. When he wanted to discuss my projects' progress, he asked "can we talk"? He then sat close to my desk in the lab on the second floor of the Kosow building and listened carefully to my debriefing on the project. Those were the moments when he provided his input or offered another way to analyze the data. However, when I wrote the manuscripts and put them in triple space for his review, as he requested, that is where Dr. Timasheff's true genius for thermodynamic solutions came out. Usually, he worked on the manuscripts for a long time. There was no rush on his part to accelerate publication. Rather, he was very concerned about not introducing errors in the literature, as he often said. Keith Shearwin and I used to joke that the manuscripts were in a black hole, because he kept them for a long time under his review. Nevertheless, when Dr.Timasheff returned the manuscripts with his corrections, I was amazed by the depth of the analysis and the different complex variables that he mathematically simplified to monitor few parameters in the laboratory as confirmatory experiments. The editing of the papers was a long process, where I had the opportunity to work directly with him and learn his approach to science. We went from several drafts in triple space to double space until he said: "after you complete these corrections, give it to Trisha." That was the signal that the manuscript was ready for formatting to be submitted to the journal. That is the way he taught me, and that is the way I learned from him. One of his greatest strengths was to be able to see in the data other ways to analyze the results, free from any dogmatic principles or scientific beliefs. He used to say, "I did not join the mainstream, the mainstream came to me."

My first project as a postdoctoral fellow in his laboratory was to work on the mechanism of the induction of the GTPase activity in tubulin by colchicine binding, following the work previously done by Jose Manuel Andreu (Andreu and Timasheff 1981). We discovered that preferentially excluded solvents increased the rate of hydrolysis of the colchicine-induced GTPase activity of tubulin [Pérez-Ramírez et al. 1994]. The problem was addressed in terms of the point of action of the co-solvent: 1) physical drug binding, 2) transition from the inactive to the active form of tubulin, 3) the enzymatic cleavage reaction. Detailed

kinetic analysis showed that the increase in the activity was due to k_{cat} as K_m remained unchanged. The increase in k_{cat}/K_m showed no correlation with solvent viscosity, nor did the solvents induce any change in the state of association of tubulin. Using the reversible binding analog of colchicine ALLO, similar increases in activity were observed. However, the binding affinity was not affected in the presence of the cosolvents. From these observations, it was concluded that the solvents i) did not affect the kinetics or thermodynamics of the binding of the drug to tubulin, ii) did not affect the binding of the substrate (E-site GTP) to the active protein (K_m constant). They did affect the measured k_{cat} that led to the discovery of a previously undetected conformational transition of tubulin-colchicine and its control by cosolvents [Pérez-Ramírez and Timasheff 1994]. The other problem that I worked on with Dr. Timasheff was the question of the substoichiometric vs. stoichiometric inhibition of microtubule assembly by colchicine and its analogs, in collaboration with Jose Manuel Andreu [Pérez-Ramírez et al. 1996]. The problem was scrutinized in terms of a competition equilibrium between the growth of microtubules and their arrest by the binding to tubulin of an inhibiting drug. This was described by a simple model that led to the conclusion that substoichiometric inhibition requires a carbonyl (keto) group on ring C (C') of colchicine [Pérez-Ramírez et al. 1998; Andreu et al. 1998].

When we weren't discussing science, my conversations with Dr. Timasheff focused on art, wine, politics, and the human aspect of the other great scientists of his time. He knew that I also liked to paint with oils and pastels, so it was easy to connect on that topic. Sometimes he would come to the lab and say, "I am going to Chicago to see my favorite Seurat" (A Sunday Afternoon on the Island of La Grande Jatte). On other occasions, he would visit the Museum of Fine Arts in Boston on Wednesdays during their extended evening hours to avoid the large crowds. I mentioned to him on one occasion that maybe now was the time to visit Russia, after Perestroika—knowing that he had never been in Russia. He looked at me with a melancholic face and said, "The Russia of my father does not exist anymore." I was happy to learn that, after his retirement, he indeed was able to travel to Russia.

Every year around the month of April, he went to Paris with his wife Dr. Marina Gorbunoff (a talented organic chemist who synthesized the colchicine analogs used in our studies). They wouldreturn to Boston only months later, in October. During that period, our work in the laboratory continued and everybody knew what to do in his absence. Dr. Timasheff would contact us mainly by sending an individual handwritten fax to each postdoctoral fellow from time to time, providing additional suggestions to explore in the respective projects or requesting the status of the project, that needed to be provided via fax. Trisha Murray called me and said, *"you have a fax from Dr. Timasheff."* After reading the first paragraph of the fax, I entered into total relaxation: it was his custom during the first paragraph of the fax to describe the weather and the beauty of the area he was visiting—sometimes he described lunch walks with Jeffries Wyman, his conversations with him or visits to museums. I believe he admired beauty and he found beauty in art and science. The day I left his laboratory in 1996, he gave me two copies of the book *Methods in Enzymology* with a small dedication. I have kept those books in my office as a special treasure. At that time, I also invited him to give lectures at Genetics Institute (now Pfizer) where I was a formulation scientist. Large groups attended his seminars and round table discussions. We remained in contact through email, mainly through Trisha Murray who wrote to me on behalf of Dr. Timasheff until around the year 2003, when we lost contact. He shared with me pre-prints of his latest manuscripts and was mainly concerned with clarifying misinterpretations of the preferential exclusion mechanisms done by certain groups, where they said that all can be explained by an osmotic effect—ignoring that the surface of a protein is not inert to the cosolvents and the mechanism of exclusion is different for each cosolvent.

His approach to binding and linkage constitutes a powerful education that he instilled in me and which allowed me to explain the behavior of biological macromolecules in solution. Moreover, when I am confronted with technical information that has been collected using rigorous means, but does not fit the established path, I ask myself what Dr Timasheff would do under those circumstances. He certainly would not be discouraged. He would think deeply and come up with clever solutions to advance our knowledge as he did all throughout his career.

Keith Shearwin: from Brisbane to Boston

Whilst in the final year of my PhD at the University of Queensland in Brisbane, Australia, I asked my PhD supervisor, Prof. Don Winzor, about where he would recommend I consider for a postdoctoral position. Don was always very keen to send his students out into the wider scientific world, and his short list of recommendations included Professor Serge Timasheff at Brandeis University. Knowing little about Boston and even less about Brandeis, I did some background reading and decided that I would enquire if Prof. Timasheff had any positions available for a newly minted PhD graduate who had some (limited) experience with a Beckman Model E ultracentrifuge (a huge analytical machine used before development of the current generation of Optima analytical ultracentrifuges from the same vendor). Being late 1989, this was before email, and so, after various handwritten airmail exchanges over several months, I was accepted into Prof. Timasheff's lab, with the accompanying offer/condition that I would housesit for him and Dr. Gorbunoff while they went to Europe for the summer of 1990. How could I refuse?

Being on a student budget, and never having travelled further than a 2-h flight to New Zealand, I had booked the cheapest flights I could find, which departed Brisbane, and travelled via

Hawaii, Los Angeles and Cleveland, finally arriving in Boston some two and a half days later. The combined effects of such a lengthy trip, at a time when smoking was still allowed on board international flights, with jet lag, the reversal of seasons and some sort of cold picked up along the way, meant I was not at my sharpest upon arrival. Fortunately, Prof. Timasheff had arranged for his wonderful secretary, Trisha Murray, to collect me, help me buy some supplies and deliver me to my temporary accommodation near Brandeis. Even better, I was given an extra day to recover before reporting to the lab.

Though I had read his many tubulin papers carefully, I don't recall having even seen a picture of Prof. Timasheff before our first meeting, and we had certainly never spoken over the phone, so these unknowns had conspired to make me rather nervous. To find that he was softly spoken, with a gentle, encouraging manner was quite a relief. Rather than giving me a defined project, he handed me his most recent grant application and said "Keith, please read this and in a few days, tell me what you would like to work on." This freedom to choose my own project was a fantastic gift, and something I try to encourage with my own students. One of the first tasks to learn was to purify tubulin, which had to be done from (very) fresh calf brains obtained from what I believe was the only abattoir in Massachusetts. It was as surreal experience arriving at the slaughterhouse at six in the morning and mingling with other scientists from across Boston, casually chatting about what body parts they were waiting to collect.

The Timasheff lab, including Dr. Gorbunoff's chemistry lab across the corridor, were so different in many ways from the more typical super-competitive US style labs that I had heard of, and in fact similar to the somewhat more relaxed Winzor lab I had been accustomed to. There was no rush to publish, and indeed Dr. Timasheff seemed surprised when I provided him with a draft manuscript when I thought I had finished a particular project on the effect of colchicine binding on tubulin double ring formation. After providing me with a hand-written series of equations over several pages from one of his famous yellow legal notepads, I realized how much more could be extracted, with the right thermodynamic analysis, from the data I had collected. Many hours were spent in the lab running the Model E ultracentrifuge, and perhaps even more hours spent laboriously measuring the resulting film records containing the schlieren patterns from sedimentation velocity experiments of tubulin. These days, of course, with the newly developed Beckman analytical ultracentrifuge optics and computer software, the data are collected and analyzed almost instantaneously.

My time in the lab from 1990 until early 1993 was a wonderful time of new experiences, both in the lab and outside. There were four postdoctoral fellows (and famously no PhD students, even though we were in the Graduate Department of Biochemistry)[1]. Two of us (Bernardo Perez-Ramirez from Chile and myself from Australia) worked on tubulin and two (Gui-Fu Xia from China and Tiao-Yin Lin from Taiwan) on preferential hydration (Fig. 4). We all became very good friends over that time. Outside of the lab, Bernardo and I used to meet up at Brandeis for tennis on Saturday mornings (I remember having to scrape the snow off the court on occasions, a novel experience for someone from sub-tropical Brisbane), and we learnt the basics of sailing on the Charles River on Sunday mornings. I also remember fondly dinners at Gui-Fu's house, where she would confide how helpful Prof. Timasheff had been at quietly facilitating her, and then gradually over time, her husband and then her children to obtain visas to move to the US. Very enjoyable also were the occasional return visits by former Timasheff lab members, including Jim Lee, Dr. Prakash from India, and Octavio Monasterio from Chile. Even though I was meeting them for the first time, it felt like I already knew them well, having read their papers so many times (and having inherited some of their named tube racks!).

Dr. Timasheff and Dr. Gorbunoff (Fig. 5) were tremendously hospitable during my time at Brandeis, frequently inviting me for dinner where we would talk about all manner of topics, including many questions about Australia, where they had never visited. Knowing their love of fine wine, I had told them about the various wine producing regions in Australia, and attempted to find an example of an Australian wine in the Boston area. Knowing little about wine myself, the one and only product I could locate was more of the type produced for quantity, rather than quality. While typically very diplomatic, I suspect my efforts to convince Prof. Timasheff of the merits of the Australian wine industry were counterproductive.

Recently reading Dr. Timasheff's autobiographical article [Timasheff 2004], filled in many gaps about his early life that he had only hinted at on rare occasions. I had of course heard him and Dr. Gorbunoff conversing in what I could recognise as Russian, but I was unaware that he could speak fluently in so many other languages. Their faith was central to their life together, and it was so pleasing to discover that they were able to spend many happy years travelling together in their

Fig. 4 The Timasheff lab postdoctoral fellows 1993. From left, Tiao Yin Lin, Bernardo Perez Ramirez, Keith Shearwin, Gui-Fu Xie

[1] There was a graduate student from 1977 to 1982.

Fig. 5 Prof Timasheff and Dr Gorbunoff in Prof. Timasheff's office (1993)

retirement, visiting remote locations to visit early churches and study their mosaics and iconography.

Tsutomu Arakawa: Journey from protein-solvent interaction study to biopharmaceutical development

In the fall of 1977, I stepped into Dr. Timasheff's lab as a postdoctoral fellow and was assigned binding (interaction) measurement of protein with such co-solvents as glucose and lactose in aqueous solution. As shown below (see photo, Fig. 6, of one of Dr. Timasheff's four lab rooms), I was given a bench across the Dr. Gorbunoff's bench. After struggling for about 6 months to obtain reproducible data, I was finally able to confirm the previously observed results on sucrose and glycerol [Lee and Timasheff 1981; Gekko and Timasheff 1981a, b] (Note that sadly Dr. Kunihiko Gekko, a friend of mine and the co-author of the above glycerol paper, passed away in 2020) . During a difficult time as I perfected experimental procedure, Dr. Timasheff was patient and did not try pushing me in getting the data, as he wanted me to be fully confident in the meticulous technology that was required for the measurement. The results with glucose and lactose were as expected, i.e., negative binding [Arakawa and Timasheff 1982]. Namely, these sugars were excluded from proteins, which was used to explain the stabilization effects of these sugars on proteins. This is one of the Dr. Timasheff's pioneering achievements, "Exclusion is linked to protein stabilization." It was then obvious to us that this exclusion principle might explain another principle which had been unexplained for many decades, so-called "Hofmeister series." In 1888, Hofmeister published a universal correlation in the effect of salts on protein solubility [Hofmeister 1888]. The order of salts in decreasing protein solubility followed SO_4 > Cl > SCN for anions and NH_4 > Na > Mg > Guanidinum for cations. Interaction measurements on several proteins showed greater exclusion of salts on the left side of the equation [Arakawa and Timasheff 1984]. Thus, it was evident to us that exclusion correlates with the effects of co-solvents on protein stability and solubility. Just before leaving Dr. Timasheff's lab in 1982, I was able to complete two studies, one on osmolytes and another on amino acids. Both topics had lasting impacts on both of us.

Fig. 6 Tsutomu Arakawa preparing samples across Dr. Gorbunoff's bench

Osmolytes are cell metabolites that accumulate in the cell on water stress under high environmental salt concentration. They increase the osmotic pressure inside the cell according to increasing external salt concentration. There are a number of organisms, including sea creatures and bacteria used in salty fermented foods, that can survive or even thrive in high salt concentration. These osmolytes are essential for the survival of these organisms. Interaction measurements showed that they are excluded from proteins and thus do not interfere with the macromolecular functions [Arakawa and Timasheff 1985a, b]. On top of that, they do stabilize proteins in the cell by the same principle as is used to explain the effects of sugars and glycerol. This brought us to a long-lasting interaction with formulation scientists, who develop solution formula to prolong shelf-life of pharmaceutical proteins in biotech industries.

Another study was on amino acids. Several amino acids have been used to stabilize proteins. Interaction measurements showed these amino acids to be excluded from the protein surface [Arakawa and Timasheff 1983]. Arginine, also one of the amino acids, was different from the above stabilizing amino acids. It showed binding depending on the solution condition [Kita et al. 1994]. This binding caused us to think about its application in biotechnology 20 years after the finding: namely, the work completion was in 1982, publication in 1994 and application research in 2002 (described below).

In 1984, this experience in Dr. Timasheff's lab led me to find a job in the then startup biotech company, Amgen, which is currently one of the largest biotech companies in the world. At that time, biotech companies needed a large quantity of recombinant proteins for biological and clinical evaluation. Unlike many other biotech companies, Amgen's strategy was to produce recombinant proteins in bacterial cells, which in most cases resulted in the unavoidable consequence that the produced proteins were denatured and precipitated. Solubilization and renaturation necessitated manipulation of solvent condition and hence an understanding of protein-solvent interaction. Such application work was somewhat unanticipated, as Dr. Timasheff once told me that protein-solvent interaction study was too basic to gain a high score in NIH grant applications. My colleagues at Amgen expressed a strong interest in inviting him for a seminar. He visited Amgen in 1985 (or 1986) and answered many questions from young scientists among my colleagues. When I showed my setup in the lab consisting mainly of columns and electrophoresis apparatus, Prof. Timasheff told me that they were all I needed for basic biochemical studies. This turned out to be true then and even now, as I am currently investigating ion exchange behavior of uncharged solutes and protein gel electrophoresis based on agarose.

Amgen undertook a major reorganization in 1997, making me consider a career change. Dr. Timasheff encouraged me to look over different options. After a 1-year search, I decided with my friend, John Philo, to found a company, Alliance Protein Laboratories. Two years later, Dr. Timasheff came to Ventura (the county where I lived) for a conference. My wife and I got together with him for dinner. (By the way, Dr. Timasheff had kindly offered my wife a one-year postdoctoral position during my last year in his lab.) We constructed a hand-made tensiometer for surface tension measurement of amino acid solution. This study led us to the 1994 paper based on amino acid interactions [Kita et al. 1994]. Among many things we talked about at the dinner, he told me that I should put some effort into doing research since I was now, post-Amgen, free from any employment-associated restrictions. This pushed me to do research on amino acids. As amino acids are natural products and non-toxic, they are perfectly fine for use in biopharmaceutical products. Our work 20 years earlier on interaction with amino acids had caused us to realize that arginine was different as described above. Arginine, unlike other amino acids, did not stabilize proteins, but enhanced protein solubilities, a totally unexpected observation [Arakawa and Kita 2014]. We had published our final findings in 1994 [Kita et al. 1994]. As I turned back to research in 2002, this observation regarding arginine brought me a 20-year long collaboration with Ajinomoto, an amino acid-based chemical company in Japan. The collaborative study with Ajinomoto on arginine resulted in our final publication [Arakawa et al. 2007]. Here, again, as with the osmolyte study, what had seemed a basic study became application science. Arginine was found to be useful in various cases of column chromatography.

I have so many positive memories of Dr. Timasheff. The first thing that comes to my mind is his patience and persistence in ensuring completeness of all work. He wanted to spend as much time as possible to make any publication complete, rather than worrying about beating the competition. He once told me that the analyses or data acquisition that may seem trivial at the time of acquisition could become significant later, meaning that we often do not know, until some time in the future, the significance of the results we obtained. Dr. Timasheff gave me a lot of freedom in my studies during my postdoctoral time. I never felt forced to do things he wanted to pursue. Instead, he always gave me timely advice. For example, in the analysis of Hofmeister series, he suggested that I should try a much higher salt concentration than I was using as I was afraid of protein precipitation. Lastly, he never lost patience with my English. I must admit that it took nearly 5 years before I became comfortable in English, which was about the time of my departure from his lab. For all publications, he wrote the English edition and corrections embedded in the hand-written manuscript draft with many scientific suggestions, which of course requires an understanding of English writing and scientific content. I used his hand-written edit on the manuscript draft as my textbook for English writing. This helped me write reports at Amgen and for clients of Alliance Protein Laboratories. I cannot say enough about my great experience and memories with Dr. Timasheff from the time I joined his lab in the fall of 1977 through over four decades that followed. He is my great mentor and I was honored to be able to work for and learn from him.

John Carpenter: formulation development for therapeutic proteins

Professor Timasheff did not learn until late in his career that his work on protein-solvent interactions was critically important for mechanistic understanding—and proper development—of stable formulations of therapeutic proteins. The revolution in medicine due to recombinant protein drug products started with the launch of a human insulin product in 1982. Since then, there have been hundreds of protein drug products approved, which provide unique treatments for hundreds of millions of people around the world. All of these products must be sufficiently stable for a shelf life of two years of more. Meeting this goal in a rational, efficient manner is dependent on understanding the "Timasheff" mechanism for protein stabilization in aqueous solution by co-solvents (aka stabilizing excipients). The mechanism also applies to protein

stabilization during freezing, so the Timasheff mechanism is important for freeze-dried protein formulations as well.

Many scientists working in the early biotechnology companies were trained in fundamental protein biophysical chemistry, and at least some of them knew of the Timasheff mechanism for protein stabilization. Others had to gain understanding from reading Prof. Timasheff's many papers. Fortunately, some of us working in the field were personally tutored by the Professor, when he graciously accepted invitations to give lectures at conferences on cryobiology and pharmaceutical biotechnology, and to present departmental seminars. He provided clear, concise explanations of the mechanism for protein stabilization by co-solvents and patiently answered questions. He greatly enjoyed participating in the conversations after the lectures and he loved learning about the applications of the fundamental insights about protein-cosolvent interactions to solving practical protein stability problems for the then new class of recombinant protein medicines.

And he became a close friend and supportive mentor to many of us who did not "grow up in the protein biophysical chemistry field." And some of us surely did not want to try to follow all of those equations relating to the Timasheff mechanism. I fondly recall many conversations with Prof. Timasheff over dinners, at conferences or during hikes when he visited Colorado. One stands out the most. It occurred, of all places, when he and I stopped at a gas station across the street from my campus, on our way to hike in the Colorado mountains. At the time I was trying to boil down the elegant Timasheff mechanism into a simple description based only on words and without the need to follow the rationale via equations. Of course, he joined me outside the car as I was pumping the gas, so we could continue the conversation we had started before I pulled into the gas station. I asked, "Can we just ignore water when we talk about the consequences of preferential exclusion for protein stabilization." He answered, "Of course, in that context water is just an innocent bystander." Whew, I now could ignore a whole bunch of equations. Then, I asked, "So is all that matters is that a preferentially excluded co-solvent (excipient, osmolyte, nonspecific stabilizer) increases the protein chemical potential and the magnitude of this effect is greater for the unfolded than for the native state? And, therefore, the system reduces this chemical potential perturbation by shifting the equilibrium toward the native state; the native state is stabilized?" Prof. Timasheff answered, "Of course. That is the mechanism." I said, "Then why don't you write the description this way and leave out the water and all of the equations?". And my patient, but motivating, mentor said, "Why don't you write it that way?"

So my collaborators and I have written the mechanistic description this way in research papers and reviews. Because of Prof. Timasheff's kindness, patience and enthusiastic interest in all areas of science to which he could make contributions, he helped me and many other pharmaceutical scientists learn important lessons for our research and development on protein-based medicines.

But more importantly, this old-world gentleman taught us to live life fearlessly ("no helmets for bike riding") with kindness, generosity and graciousness; to find joy in hard work and scientific accomplishments; and to enjoy and revere family, friends, travel, exploration, great wine and food, walks through the woods and all of the adventures and relationships that make a full and meaningful life. He was a dear friend and inspiring mentor to whom I will always be grateful.

John and Lois Crowe

We first met Professor Timasheff in the late 1980s. By that time, we had been studying freezing and freeze drying liposomes for some time, and we were eager to hear directly from him his thoughts on how his preferential exclusion mechanism for stabilizing proteins in aqueous solution might influence our thinking about phospholipid bilayers. One of our first discussions centered around where the solute resides in relation to the surface of the protein. He gave us his patented sweet smile and said, "I don't have to know that; I'm a thermodynamicist, and all I have to know is the thermodynamic consequences."

As experimental biologists (and mostly self-trained biophysicists), we were not entirely happy with that advice, but with time we came to appreciate what could be learned from the thermodynamics, even without understanding the details of how the solute and macromolecular assemblages (i.e., proteins and bilayers) interact. We stayed in contact with Professor Timasheff both through scientific meetings and correspondence over the next decades, and he made insightful contributions repeatedly to our work. Here are examples of his thinking:

1. Solute effects on bilayers in solution. Early on he suggested we examine effects of the sugars (trehalose in particular) we had been studying on physical properties of bilayers in water. Much to our surprise, we found that at low concentrations trehalose increased the gel to liquid crystalline phase transition temperature (Tm), thus increasing the order, in apparent agreement with his findings for proteins. But at higher concentrations the solute either had no effect or decreased Tm. We found that at high concentrations trehalose appeared to bind to the bilayer. He immediately provided a possible thermodynamic explanation for the binding. The polar head groups have water molecules hydrogen bonded around them. Suppose, he suggested, the binding of trehalose displaces those water molecules. Thus, the decreased entropy represented by the binding of the sugar might be balanced by the increased entropy from the water displacement. So, you

see, we found early in our talks that he was not really honest when he said he did not need to know about the molecular details. But when the thermodynamic measurements were not yet made he could devise a possible thermodynamic explanation based on the likely behavior of the participating molecules. That liquidity in thinking was refreshing.

2. Solute effects on dry bilayers. Tm in dry phospholipids increases by tens of degrees, dominated by van der Waals interactions among the acyl chains. Removal of the water hydrogen bonded to the polar headgroups decreases their spacing, leading to increased interactions between the acyl chains, and thus to increased Tm. But when the lipids are dried with trehalose Tm is depressed by as much as 100 °C, often well below Tm for the lipid in water. This remarkable effect dominated our thinking about the consequences of drying bilayers, even with extrapolation to biological membranes. There was, and is, no question about the existence of this huge decrease in Tm, but how it works was a little mysterious until we established by studies with spectroscopy, solid state nmr, and direct measurement of binding that the sugar was hydrogen bonded to the polar headgroups, relaxing the opportunities for van der Waals interactions among the acyl chains, and thus decreasing Tm. One question that came up repeatedly about this interaction is its thermodynamic driving force; after all, binding of the sugar to the polar headgroups would decrease entropy, which would seem to be unfavorable. Professor Timasheff had a ready explanation: that decrease in entropy could be balanced by the increased entropy from the fluidization of the acyl chains. The interaction would be entropically driven—like most things in the physical world.

To this day, we are still not entirely comfortable with putting entropy at the center our thinking, but if our friend Serge said it makes sense we would believe him. He taught us that what is possible can be evaluated through thermodynamics, without knowing the molecular details. We miss him, and, indeed, the world of biophysics mourned his passing.

George Somero: Organic osmolytes and the evolution of biological solutions

I cannot, regrettably, list myself among that fortunate group within the authors of these essays that enjoyed many years of close contact with Professor Timasheff and thereby directly experienced his wonderful mentorship skills. That regret being expressed, however, there is probably no scientist other than my postdoctoral advisor, Peter Hochachka, that has had a more profound impact on my studies in the field in which I've worked for six decades: biochemical adaptation. The central goal of research in this field is to explain at a biochemical level how organisms manage to survive and thrive so well in such a remarkable range of habitats, notably ones that differ widely in temperature, hydrostatic pressure, and osmolality. Over the years, it's become increasingly clear that the success that all types of organisms have enjoyed in adapting to the challenges posed by the abiotic environment stem from two fundamental types of biochemical adaptations: 1) Macromolecular adaptations—alterations in the intrinsic structural and functional properties of macromolecules like proteins, and 2) Micromolecular adaptations—modulation of macromolecular stability and function through adjusting the composition of the solutions in which macromolecules do their work [Somero et al. 2017]. The term "micromolecules" refers to the complex sets of inorganic ions and small organic molecules that occur in cells. The latter are commonly termed "organic osmolytes," and include polyhydric alcohols like trehalose and glycerol, free amino acids, methylammonium and methylsulfonium compounds, and urea [Yancey et al. 1982]. The cooperative adaptive effort between macromolecules and micromolecules has been of pivotal importance in allowing organisms to thrive over a temperature range of over 150°C, pressures up to 1100 atmospheres in the deepest regions of the seas, and salinities that likewise span a very wide range.

Most studies (not to mention funding!) of molecular adaptation to abiotic stressors have focused on macromolecules, especially proteins. The other evolutionary path, micromolecular evolution, is still a relative frontier. Gaining a vision of this frontier and discovering how to explore it most effectively required climbing onto "the shoulders of a giant," Professor Serge Timasheff. Below, I'll briefly explain how his ideas have formed a roadmap for study of micromolecular evolution and why those of us who work in this field owe such a debt to this great man.

The phenomenon of micromolecular adaptation came to my attention in the early 1970s, during my first years as a faculty member at the University of California's Scripps Institution of Oceanography. Two of my first Ph.D students, Paul Yancey and David Bowlus, were curious about the osmotic relationships of marine animals, certain of which had osmolalities equal to that of seawater. Whereas in many cases, the osmotic composition of the extracellular fluids was much like seawater and consisted primarily of Na^+ and Cl^-, the intracellular fluids of these osmotically concentrated animals were typically not much saltier than in non-marine species, but contained high concentrations of small organic osmolytes rather than inorganic salts. This reliance on organic osmolytes raised a number of questions. Thus, why did sharks and their cartilaginous relatives (skates, rays, etc.) have in their blood and cellular fluids such a bizarre "soup" of osmolytes: urea (at a whopping concentration near 0.5 M) plus two methylammonium osmolytes, trimethylamine-N-oxide (TMAO) and glycine betaine? Urea is a strong protein

denaturant and seems poorly suited for a role as an osmolyte. How sharks and their kin survived at these seemingly toxic levels of urea was a long-running mystery in physiology. Invertebrate body fluids posed additional questions. In their cells, one finds a different "soup" from that found in sharks: high concentrations of free amino acids (glycine, glutamate, proline, etc.). Why "waste" these otherwise useful molecules by employing them for osmotic regulation?

These questions have prompted a career-long effort to develop an inclusive analysis of biochemical adaptation, one that recognizes the important roles played by both macromolecules and micromolecules. When we initiated our studies in the 1970s, micromolecular evolution was a terra incognita, so our initial phase of exploration focused more on the literature of physical biochemistry than on evolution per se. As is almost always the case when one initiates a new line of study, a great deal of time with new literature is required in order to create the proverbial "prepared mind" needed for success. In my case, the key papers that led to essentially all of our work on micromolecular evolution came from the work of Professor Timasheff and his close colleagues. Serge Timasheff thus became an important mentor decades before we met in person at a symposium in Switzerland—an occasion that afforded me the opportunity—at long last—to give him thanks for all that he did for my career.

Perhaps the best way to summarize the intellectual impact that Dr. Timasheff's work had on those of us who study molecular adaptation is to say that he enabled the pieces to all come together into an integrated whole, where the pieces in question included macromolecules, organic osmolytes, and—importantly—water. I can vividly recall my first exposure to the concept of preferential interaction (or preferential hydration) and its critical role in governing the interactions between small solutes ("cosolvents") and proteins. With three new graduate students (Phillip S. Low plus Paul and Dave) we carefully studied papers from the Timasheff team that detailed the remarkable experimental work and theoretical developments that underlay the concept of preferential interaction. As we slowly assimilated that key information, the proverbial "scales fell from our eyes." We soon initiated several lines of work to learn how the organic osmolytes of marine animals affected protein structure and function. With the Timasheff model as our guide, we learned why sharks tolerate high urea concentrations: the disruptive effects of urea on proteins were fully counteracted by the methylammonium solutes that were present at approximately one-half the concentration of urea. We termed this biochemical balancing act the "counteracting solutes strategy" [Yancey et al. 1982]. We also learned why marine invertebrates have taken their particular evolutionary route in dealing with osmotic issues. These animals use weakly stabilizing osmolytes like glycine, glutamate and proline, known collectively as compatible solutes [Yancey et al. 1982]. The concentrations of free amino acids were shown to be non-perturbing of protein structure and function, which facilitated their use in the face of changes in external salinity that led to changes in osmolalities of the animals' body fluids.

In the following years, the insights provided by Professor Timasheff's work led to a diversity of discoveries in micromolecular evolution by a growing number of laboratories. The counteracting solute strategy was not just an invention of marine cartilaginous fishes, but also was employed in the mammalian kidney where urea concentrations can be very high in the inner medulla region (Burg 1992). Biotechnological exploitation of stabilizing organic osmolytes also has become important, as contributors to this set of essays relate. One of my favorite stories about micromolecular adaptation is the relatively recent finding by Paul Yancey and his colleagues that deep-living animals, both fishes and invertebrates, accumulate increasing amounts of trimethylamine-N-oxide (TMAO) as they move to greater depths [Yancey et al. 2014]. By increasing the concentration of this strong protein stabilizer, the perturbing effects of increased hydrostatic pressure are offset, much as TMAO offsets the effects of urea.

Many other examples of this cooperativity between macro- and micromolecular evolution have been discovered throughout all phyla (for review, see Somero et al. 2017). Professor Timasheff's deep understanding of the thermodynamics of three component systems, as so clearly presented in many of his publications [e.g., Timasheff 1992], has thus been foundational for all these discoveries.

In addition to the studies of organic osmolytes in marine organisms that I've just discussed, another line of study during my early years at Scripps was founded on studies and theoretical developments of Professor Timasheff, namely his analyses of the water-solute interactions that underlie the fascinating Hofmeister Series of ions [Hofmeister 1888]. As most readers will be familiar, inorganic anions and cations affect macromolecules in distinct ways; some, like the ammonium ion, are strong stabilizers, whereas others, like the iodide ion, are denaturants (for a classic review, see von Hippel and Schleich's article [von Hipple and Schleich 1969] in the volume edited by Timasheff and Fasman 1969). My graduate student Philip S. Low and I wondered how these ions might differentially affect the activities of enzymes. The basis of our hypothesizing about Hofmeister Series effects was the fact that during the catalytic process there are rapid changes in enzyme conformation that involve alterations in exposure to solvent of protein side-chains and peptide backbone linkages. Some Hofmeister salts would facilitate protein-water interactions; other salts would reduce these interactions. To address this question, we measured the volume changes that occurred during the activation event of catalysis, using a high-pressure cell. We reasoned that changes in water organization around an enzyme would lead to a change in system (protein plus solvent) volume whose size (or sign) might depend on the ions present in the solution. Indeed, this was the case; we

found a remarkably consistent agreement between the size of the activation volume and the rankings of ions in the Hofmeister series [Low and Somero 1975a, 1975b]. Thus, we obtained at least indirect evidence for differential hydration of the protein surface in the presence of different salts. Moreover, the size (or sign) of the volume change correlated with the salts' effects on the rate of the enzyme's activity. Taken together, these data on salt effects on activation volume and catalytic rate led us to postulate that rapid and reversible changes in protein surface hydration play important roles in setting the activation free energies of enzyme reactions. In these studies, too, our debt to the insights of Professor Timasheff is immense.

As I mentioned earlier, my mentorship by Dr. Timasheff was carried out anonymously over a continent-wide distance for the first two decades of my career. Finally, in 1990 in Crans-sur-Sierre, Switzerland, I was able to introduce myself to this great scholar. I was a bit apprehensive about this initial meeting because of his stature and my inherent shyness and feeling of insecurity about my ability to adequately express appreciation for all that he has meant to my career. As things turned out—and as those who know what an open and warm-hearted individual Dr. Timasheff was—our interactions at the meeting went extremely well. I not only managed to express my gratitude, but I learned even more about the interactions among macromolecules, organic osmolytes, and water. His talk at that meeting was masterful [Timasheff 1992]. During part of the talk, he summarized our own work on organic osmolytes in marine animals in such a clear and synthetic manner that I recall thinking, "I wish I could present my own work as well as he is able to!" Perhaps the memory from that Swiss meeting that I most cherish is the long conversation on the thermodynamics of biological solutions that we had during our trip in a gondola car up to the top of the mountain of the ski resort. Reflecting on this gondola ride, I wonder what the other, non-scientifically inclined passengers aboard the gondola were thinking. I suspect that Dr. Timasheff's soft, warm, yet attention-commanding voice fully held their attention, much as it held mine.

Several years after this meeting, I was asked by Professor John Schellman of the University of Oregon to join him in writing a tribute to Professor Timasheff. This short scientific biography was a deep pleasure to write; it taught me even more about this great scientist whom we honor in the present essays [Schellman and Somero 1996]. I'll end my tribute with a quote from our article that, I think, encapsulates Professor Timasheff's wide and deep contributions: …"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." The truth of this short summary of Dr. Timasheff's contributions rings out from the statements made by the other authors of the present commemorative article. We all owe an enormous debt to this great man. Where would our careers have gone without his guidance?

Pete Gagnon: The unforeseen but long happy marriage of preferential interactions with chromatography

I remember with perfect clarity the bright cold January morning in 1983 when Timasheff's world-view found me; mid-morning break from the lab, steaming mug of black coffee in hand, absently browsing the library for something new, and there was the latest issue of Biochemistry: Preferential interactions of proteins with salts [Arakawa and Timasheff 1982]. I was a few years into what was to become my lifelong fascination with chromatography, impelled by my counterpoint perpetual frustration with never being quite able to figure out how to make it do what I wanted it to do. Hydrophobic interaction chromatography was a particular interest of the moment. With that article, I sensed that I was holding the Holy Grail.

Indeed I was, but it became quickly apparent that it was written in a language far above my experience. I nevertheless captured enough to understand that I had encountered the organizational principle to unlock chromatography in a way I had never conceived. Truthfully, it took years for me to fully appreciate the subtle experimental designs and transform the exquisitely precise data into an intuitive framework that I could explore creatively. Now four decades later, I still find new understanding in that publication, and in its siblings before and after, but my first step was taken on that winter morning in 1983.

The key insight for chromatography was understanding that solvents of all sorts interact preferentially with chromatography media in the same ways they interact with proteins and other biologics. Then came the realization that the overwhelming surface area of chromatography media dominated these systems. For excluded salts, that meant proteins tended to associate more with the solid phase than with each other. I remember the light going on as I suddenly understood a series of early papers where investigators bound proteins to non-hydrophobic chromatography columns, even to ion exchangers, at high concentrations of excluded salts but where the proteins were still completely soluble [Mevarech et al. 1976; Leicht and Pundak 1981; Arakawa et al. 2008]. None of those papers had made any sense from the perspective of hydrophobic interaction dogma at the time, but they made perfect sense in a world where solute-solid phase interactions were driven by preferential exclusion. Their linkage to Timasheff's work was overlooked for many years, which was

unfortunate and ironic, since it revealed that most of what has become codified as hydrophobic interaction chromatography is not. It is preferential exclusion chromatography where hydrophobicity of the solid phase is exploited as a relatively minor selectivity modifier. Performing the technique on size exclusion chromatography media produces virtually the same order of elution as C4–C6, benzyl, and phenyl media. Indeed, there have been studies showing that preferential exclusion chromatography on non-hydrophobic media is preferable in some instances because it conserves the conformational stabilization effects of excluded salts while suspending the risk of denaturing large complex structures like lipid-enveloped viruses at strongly hydrophobic surfaces [Burden et al. 2012].

Arakawa and Timasheff published their work on polyethylene glycol (PEG) in 1985 [Arakawa and Timasheff 1985b]. This established the foundation for a method now known as Steric Exclusion Chromatography, which uses PEG to promote binding on hydroxylated chromatography surfaces [Lee et al. 2012; Gagnon et al. 2014]. The solute size dependency effects noted by Arakawa and Timasheff meanwhile inspired inclusion of PEG in ion exchange and hydroxyapatite buffers to promote separation of aggregates from non-aggregated antibodies [Milby et al. 1989; Gagnon et al. 1996; Yoshimoto et al. 2015; Kluters et al. 2015a, 2015b, 2015c; Zhou et al. 2011; Gagnon 2008; Snyder et al. 2009]. Hydroxyapatite, of course, was also a subject of interest for Professor Timasheff, who contributed to Marina Gorbunoff's still-classic publications on its retention mechanisms [Gorbunoff 1984a, 1984b; Gorbunoff and Timasheff 1984]. Preferential exclusion and steric exclusion were also exploited to accelerate binding kinetics and dynamic binding capacity with weak bioaffinity chromatography media [Gagnon 1996; Arakawa and Gagnon 2018].

Where do chromatography and preferential interactions go from here? At this point, most of the obvious linkages have been established. As in the field of chromatography generally, the future lies in development of specific applications, especially for very large solutes like virus particles, extracellular vesicles, plasmid DNA, and mRNA. Each of these solute classes integrates solubility, conformational variability, stability, and fractionation-from-contaminant challenges that represent nightmares for people lacking a deep background in chromatography. Process developers well versed with Professor Timasheff's perspective find themselves instead with exactly the toolbox they need to rationally and systematically develop solutions that work. This will take some time. It always does. But getting it right, as with Professor Timasheff's work will produce the bedrock foundation needed to support the generations that follow after. I gratefully acknowledge that Professor Timasheff's pioneering concepts reside at the core of all of my work.

Marina Timasheff Charles: Growing up with my father, a man of faith, scientific method and joy

From early childhood, I knew that my father was from a family of deeply thinking intellectuals who lived lives of purpose. I was inspired by his accounts of his father, a professor of law in St. Petersburg before the Bolshevik revolution and, following emigration to the U.S., a professor of sociology and one of the founders of the discipline of sociology of law; and his grandfather, who served as Minister of Commerce and Industry under Tsar Nicholas II. Serge Timasheff's parents fled the Bolshevik revolution in the 1920s, then the Nazi wave 15 years later. From them and the Russian émigré community of his childhood he learned that no matter what twists and turns life takes – what obstacles arise, one finds a way forward, taking each step that arises, without worrying, trusting that the steps lead somewhere.

His Russian Orthodox faith was his anchor. He attended service every Sunday, every religious holiday, and many Saturday evenings. Each day began and ended with prayers said quietly in his room. He had a deep love of and connection to his faith. It was the source of his gentle, patient strength and peace. It was by this faith that he lived a life of enthusiasm, joy and purpose.

He was able, more than most people I know, to accept life as it presented itself and to engage wholeheartedly in life's endeavors. As a result, he was a happy scholar who was in his element in the academic life. He loved his work, was proud of his achievements, of the scientific breakthroughs of which he was a part, and treasured his relationships with those with whom he shared his endeavors.

The people he worked with were as important as the scientific purpose on which they engaged. His students and colleagues were not just part of a professional setting. They were the people with whom he shared his life's work. If you were someone who shared the spark of inspiration that makes for complete absorption in scientific discovery, then he could relate to you. If you were someone who enjoyed sharing that enthusiasm with colleagues and collaborators, then you were a valued friend. My father loved his interactions with the people with whom he shared his world of research. At the dinner table he enjoyed relating conversations with colleagues (not all about science – they could be about family history, personal or professional plans, a shared love of travel, music or art). That is how I got to know his students and colleagues.

His capacity for simple joy was the core of his success as a human being, and the biggest lesson I took from him. It was a quiet joy, because he was a quiet person, but it burned brightly inside him. He knew worries and struggles, but he handled them without outward agitation because he was too much of a gentleman to share them with those around him. And, somehow, he knew that those were passing moments, to be handled

… so he could then settle back into the content pattern of pursuit of the life that had been placed before him, that he felt fortunate to have.

A family friend related to me an incident from shortly before he retired, which somehow summed it all up. During a visit to her home in Alsace, France, he described the reactions he received to a recent lecture series he had completed in Paris. He said, in a tone of slightly surprised reflection, "I think I am becoming well-known." It was because he was humble that he continued to feel to the very end of his professional career that spirit of joyful surprise, of adventure, about his work and about his times with those with whom he shared his journey.

He had several hobbies, all of which he pursued with a simultaneous sense of adventure and scientific approach. His love of classical music led, for instance, to evenings spent comparing the conducting styles of three great twentieth century exponents of Beethoven symphonies. Sunday afternoons were dedicated to pursuit of French cooking: comparing multiple conflicting recipes for a challenging dish; selecting the best sauce pots and sauté pans; evaluating progress at taste-testing points along the way. The results were invariably delicious, and his eyes lit up and he smiled his warmest smile as we all enjoyed.

He was responsible for our unique foreign adventures (often reflecting my mother's latest art history reading), planned over months. (In an age of no internet and no cell phones, all was done by letter.) Our travels were deep dives into foreign culture or ancient civilization. Whether in Mexico, Morocco, Guatemala or Egypt, each stop, each hotel was carefully selected, daily itineraries worked out on maps ordered in advance. That is how, in 1982, before driving across the Sinai Peninsula to the Red Sea, we stayed in Aswan, Egypt, not in one of the many hotels in the middle of town, but in the nineteenth century Old Cataract Hotel. Our room's large windows looked out directly on the Nile River. The dark waters flowed slowly past; the silhouettes of ancient ruins rose up directly ahead of us on Elephantine Island; and the Aga Khan's memorial, on the rise of the opposing bank of the river, glowed in the sunset.

His meticulous, detailed planning was also responsible for a 1980 trip to Guatemala that included traveling dirt roads by jeep across a mountain range and remote frontier from Guatemala into Honduras to visit the famous ruins of Copan, and a domestic flight from Guatemala City to Tikal via what looked like DC 3 aircraft. Tikal's accommodations were then limited to a small airstrip with a lean-to shelter and the Jungle Lodge. The latter consisted of a long dormitory-style building and three bungalows with private bath. Dinner was at communal tables. Dinner guests included not only arrivals from Guatemala City, but people who mysteriously walked in from the jungle. Electricity was provided by a power generator that turned off by midnight. Under military police patrol, no one was allowed to leave the hotel grounds after dark. It never crossed my father's mind that any of these complicated journeys might not be possible. If he was able to plan it, it could be done.

One of the greatest gifts he gave me was France. Born outside of Paris, for my father our trips to France were a return home; over the years, I came to share his love of the country. Thanks to him, I hiked the Alps, the Pyrenees, the Vosges, and the Massif Central; walked the countryside of Provence, Alsace, Burgundy, Poitou and Languedoc; and explored the coastal cliffs of Brittany and Corsica. I felt the serenity of the Romanesque churches of Brittany, and read Dickens and Austen on the front stoop of a small rented farmhouse in the rural south.

So, here's to my father, the unassuming yet dignified, intelligent, thoughtful old-style European gentleman, the happy scholar, the man of staunch faith who taught me what it means to be gracious in one's dealings, to be patient and thoughtful, and to accept life as it comes, seeking joy in the gift of each day, and celebrating the adventures that come your way, and that, with an open heart and mind, one never stops learning.

Final remarks

Are there any lessons from the life of Dr. Timasheff? We believe many. One of them is naturally in the world of science. He did not worry about competition. Indeed, most of his post-doctoral fellows saw their last publications with him in print only long after their final days at his lab. He pursued perfection in publications, so that they did not introduce errors in the literature, but conveyed clearly the messages of the observations and provided the principles behind the observations. He gave us freedom in both scientific and daily activities. After giving us the starting point for our research, he allowed us to expand on it freely. He encouraged us to follow our instincts in developing the research. He gave us the freedom to work in flex time, never forced us to work on a fixed time schedule and encouraged us to take time off and vacations.

But what underlay his approach to life in the scientific world was a deeper lesson: In everything he did, and in the ways in which he encouraged and interacted with us, the message of his life was to approach everything one does with both mind and heart completely and purposefully engaged. That applied as much to the specifics of the research and choices of research direction, as it did to interactions with us and his other colleagues and friends, as it did to his vibrant pursuit of his many and varied interests outside of work. And the enduring success of his life-approach is obvious: these tributes were written by fifteen different authors, many of whom are still friends or collaborators in research so many years later, all of whom felt called to express their enduring gratitude, affection

and respect for the inspiration and guiding force in their lives that was Professor Serge N. Timasheff.

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