THE JOURNAL OF PHYSICAL CHEMISTRY B
A JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/JPCB

Special Issue Preface

Autobiography of Steven G. Boxer

Published as part of The Journal of Physical Chemistry virtual special issue "Steven G. Boxer Festschrift".

Cite This: *J. Phys. Chem. B* 2023, 127, 8711−8716

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I am honored to be asked to write this summary of my career. Let me start by thanking Professors Lauren Webb, Sayan Bagchi, Neal Woodbury, and Paul Cremer, four terrific former postdocs, for making this happen and to friends and colleagues who have contributed to this Festschrift. Getting old is inevitable, but an occasion like this makes it worthwhile.

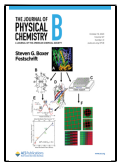
I grew up in New Jersey. My parents were immigrants from Vienna in the late 1930s. Both came from Jewish families, secular and assimilated into the fin de siècle world of early 20th century Vienna. My father, George E. Boxer, was a medical student at the University of Vienna, forced to wear a yellow star of David, and part way into his studies escaped as Austria was invaded. Through friends he found his way to the University of Cambridge and, joined by his then fiancé Lily, came to the US with essentially nothing. They were supported by Catholic charities (a story in itself). My mother worked as a maid and secretary. My father applied to the Ph.D. program in Biochemistry at Columbia University and was admitted after an on-the-spot oral exam. His Ph.D. thesis, "Disturbances in Lipid Metabolism studied with the Aid of Isotopes", was completed in 1943 under the guidance of DeWitt Stetten, later the founding Dean of the Rutgers Medical School and several high positions at the NIH. They had access to stable isotopes through the Manhattan Project, and quite remarkably, I have also used stable isotopes for imaging mass spectrometry of lipids over the last 10 years. My father was recruited to Merck in Rahway, New Jersey, by Max Tishler. During his career, cut short by lung cancer in 1968, my father rose to become Director of the Merck Institute for Therapeutic Research where he did important work on metabolic pathways relevant to cancer.

As I grew up, I was quite athletic, was interested in music and stamp collecting, and had a laboratory in the basement, inside an old coal bin. This was my private world of experiments, some chemistry but mostly biology using my father's old Zeiss microscope, which was a beautiful instrument. Many truly dangerous things happened in that lab; I was left alone and never burned the place down! My father brought home quite a collection of chemicals and even mice. I was an OK student. I did not get into any Ivy League schools but was admitted to Johns Hopkins and Tufts. Max Tishler, by then the President of the Merck Sharp and Dohme Laboratories and a legendary scientist and administrator, who was a Tufts alumnus, took me to dinner and convinced me to go to Tufts.

Tufts proved to be a great choice. I was vaguely interested in biology but also many other subjects including the humanities. That year (1965) Tufts obtained a grant from the Ford Foundation to create an integrated 2-year science course as part of Tufts Experimental College. I took a chance and signed up; this course had a huge influence on me. The first semester was MWF math, TThS (yes Saturday) physics, with two biology laboratories using principles from physics. There were 20 students and a group of faculty from all fields who attended each other's lectures. It was a fantastic experience and continued with this level of integration for 2 years. Chemistry was initially taught by Professor Gordon Evans, an organic chemist, whose intellectual style had a big influence on me. I have advocated for this type of integrated introductory science course ever since; however, it is very expensive and only a few institutions have tried it. I ended up taking math courses all 8 semesters and many physics courses, and by the summer of my junior year, I had become primarily interested in theory. I worked with a young faculty member in Chemistry, Mel Feinberg, who got his Ph.D. from Klaus Ruedenberg, and with him did quantum chemical calculations on H_2^+ using early computers and approaches based on the Hellman−Feynman theorem. This was also the time of much turmoil on campus due to the Vietnam war, and I was very involved in protests. Many of my closest friends came from the humanities, and it was a very exciting time to be a student with no idea what he wanted to do.

I applied to graduate school, largely to avoid the draft, thinking I might go into theoretical chemistry. The GRE's were a big hurdle since I had largely forgotten chemical chemistry, so I randomly checked the answers, left after a few minutes, and joined a protest on campus. Fortunately, a few places did not require (or maybe ignored) the GRE's, including the University of Chicago, a mecca of theory, and miraculously I got in. The University of Chicago is a serious place. All the physical/theoretical first year graduate students were required to take Gerhard Closs's physical organic course and Jack Halpern's inorganic course—I loved the former and really disliked (and flunked) the latter. At the end of the first year, we had to take qualifying exams, incredibly difficult and long written exams, as well as present an analysis of an assigned paper from the recent literature (a paper from Brad Moore on

Published: October 19, 2023



IVR in CO_2 lasers) and a research proposal. This was a terrifying experience as something like 40% flunked out, but that week I knew more physical chemistry than ever since and I passed. I still thought I was headed for theory, but my closest friends were so obviously better (and have since gone on to distinguished careers in theory) that I started to waver. However, then the lottery for the draft ended exemptions for graduate students. A group of us gathered to watch the drawing, and before the drinks were poured, my number came up, #5. Not sure what to do, I contacted my mother in New Jersey who put me in touch with a Quaker group that counseled draft avoidance options. I was grilled for 6 h and came away convinced that I was a conscientious objector. With coaching from this advisor (and a haircut) I appeared before my draft board to argue my case, a situation not so dissimilar from the oral qualifying exams at the University of Chicago (except the pictures on the walls were not Mulliken, Urey, and others but rather Nixon and General Hershey), and to my surprise, I got CO status. That meant 2 years of civilian alternative service, and I started looking at opportunities at the University of Chicago hospital.

I ended up as a technician in the lab of Dr. Angelo Scanu, a well-known cardiologist, who studied serum lipoproteins. My job was isolating lipoprotein fractions, HDL, LDL, VLDL, doing protein hydrolysis, and running the amino acid analyzer that was used to sequence the proteins. Scanu had developed a process to isolate the proteins by "de-lipidation", essentially extraction with ether. He was interested in physical methods and gave me the opportunity to pursue my own research project on the side since running the amino acid analyzer (a monster with roughly a mile of plumbing) only took a few hours each day. I was particularly interested in spin labels, a new technique developed by Harden McConnell, that I thought could be used to characterize the interactions between the lipid and protein components of HDL as it was possible to exchange lipids or reconstitute with lipids. I have notebooks filled with data, but nothing was published. One of the people in the Scanu lab told me about an evening informal seminar in biophysics where I first met Jim Norris, then at Argonne National Lab, and I began to see magnetic resonance as an interesting area to pursue.

After my 2 years as a CO, I returned across the street to the Ph.D. program, completely uncertain what direction to pursue, but now more an experimentalist than a theorist. I met with Gerhard Closs whose physical organic course had made a strong impression, and he told me that, if I could find support, I could join his lab. I mentioned Jim Norris, and Closs said I should talk with Joe Katz at Argonne, then Jim's boss. Katz was well-known for having fully deuterated simple living organisms like bacteria and algae. He mostly worked on chlorophylls and focused on aggregation states of chlorophylls which he believed were the form taken in photosynthetic organisms as he did not think proteins played an important role. This had led him to use NMR—Closs was an expert on NMR and, as a postdoc with R. B. Woodward, had participated in the synthesis of chlorophyll. Together they published the assignment of the proton NMR spectrum of chlorophyll *a* and characterized self-assembly in different solvents by evaluating ring current shifts in the NMR spectrum. Katz had amazing sources of stable isotopes and was producing ^{13}C and ^{15}N labeled pigments, so it looked like the then newly developed Fourier transform methods could be used to study their NMR spectra. Katz got me an Atomic Energy Commission fellowship, so Closs took me on as a Ph.D. student. I had been warned by many people that he was a tough guy to work for, and indeed he was—his standards were incredibly high and he did not hesitate to tell you what he thought, but we developed a wonderful and productive relationship.

I was certainly not an organic chemist, but Closs bridged between organic chemistry and physical chemistry at a level that is rare. It was entirely unclear how to assign the ^{13}C spectrum of chlorophyll (this was before 2D methods), so we put together a simple setup to sit on an assigned proton peak and scan the range of ^{13}C chemical shifts using ^{13}C enriched chlorophyll looking for coupled resonances, an INDOR experiment. Coupled peaks were detected using a primitive signal averager, and a giant puzzle emerged to match the ^{13}C peaks with the coupled protons allowing full assignment of all but 1 of the 54 carbons. The ^{15}N spectrum was simpler because splittings from ^{15}N to assigned protons were evident and could be assigned by decoupling. This was a lot of data, but Closs sought a deeper understanding and what came out was a nice interpretation of the shifts, and my first full paper published in *JACS*.

After that, I largely shifted my base of operations to the University though maintaining close connections at Argonne. Closs and his students along with Rob Kaptein in The Netherlands had pioneered the technique of chemically induced dynamic nuclear polarization (CIDNP). I thought this might be useful for looking at electron transfer reactions, e.g., between photoexcited chlorophylls and quinones, since electron transfer generates a radical pair, required for CIDNP. Many interesting observations were made, most not published to this day (CIDNP from reversible reactions was one; Closs published this in a book without my name). I noticed differential line broadening of NMR peaks even in the absence of an electron acceptor, and after studying simpler aromatic molecules such as anthracene and naphthalene, we concluded that the line broadening was due to photoexcited triplets exchanging upon collision with molecules in the ground state, analogous to well-known effects with radicals. Although not a very useful method, this led to my second paper. Finally, Closs and I thought it might be interesting to covalently connect two chlorophyll molecules and see how they self-assembled. Work from Norris and physicist George Feher had shown that the primary electron donor in photosynthetic bacteria was a "special pair" of chlorophylls. What emerged was a structural model that has similarity to what was observed much later by X-ray crystallography, though different in important ways. The "Boxer−Closs" dimer was an early example of the use of covalent linkages to assemble molecules in interesting ways. I owe much to Dr. Hugo Scheer who was a postdoc at Argonne and helped with the chlorophyll chemistry.

One day Closs walked into the lab and said I should look for a job. I was not thinking about this at all, as I was having too much fun doing research and had a blossoming relationship with a pretty M.D./Ph.D. student one floor up in Tom Kaiser's lab. He told me to apply to a few places, and very naively I did. I had three proposals: prepare and characterize electron transfer in covalently connected donor−acceptor systems (this was motivated by a seminar given by Henry Taube on his beautiful bridged mixed valence systems; Closs had been hired by Henry at the University of Chicago, and I met with him during his seminar visit where he told me about an opening at Stanford); the second was to study radical pair dynamics in 2-dimensional systems, stimulated by a theoretical paper by John

https://doi.org/10.1021/acs.jpcb.3c06223
J. Phys. Chem. B 2023, 127, 8711−8716

Deutsch; and third, an open ended study of the photochemistry of the pigment stentorian from unicellular organisms that I learned about from my brother Peter. I interviewed at Harvard as an organic chemist, while I interviewed at Stanford as a physical chemist. My primary reason for interviewing at Stanford was to get access to Carl Djerassi's CD machine to look at chiral exciton interactions in my synthetic chlorophyll dimer. The interview at Stanford was terrifying because Harden McConnell was in the audience, the pioneer of line broadening effects due to electron exchange in free radicals and inorganic systems (among many important contributions). He interrupted my presentation with a question on spin exchange, throwing me completely off balance. I got the CD spectrum, and to my surprise, both places offered me jobs. Jeremy Knowles was particularly effective in recruiting at Harvard despite the well-known fate of assistant professors which he promised would (and did) improve under his leadership. He also saw far into my future, predicting what I would be working on 10 years hence, completely different from my immediate plans, and he was right. Harvard decided I could also be a physical chemist, but the fate of assistant professors and the great weather made Stanford very attractive. My relationship with Linda, who later became my wife, was a big factor. She came along on a second visit to Stanford, creating all sorts of problems because we were not married (managed gracefully as my "consort"). She found a good lab to work in and, taking a big chance, agreed to come along.

I had little idea what I wanted to work on. In contrast to starting faculty now, I had not thought about a start-up package, though I vaguely wanted to continue shining light into NMR spectrometers, something that horrified the person running the NMR lab. In any case there was no lab, so I was put in an office in a basement near the Djerassi group. For a year, I mulled over what I wanted to do. The idea of linking molecules to create new properties seemed interesting, so linking donors and acceptors or more complicated chlorophyll assemblies is where we started with first year student Rod Bucks, once we had a lab. Photosynthesis always fascinated me, and I thought we might isolate our own bacterial reaction centers (RCs). I had no experience growing anything, let alone isolating membrane proteins, though I'd learned quite a bit during those 2 years as a technician in the Scanu lab. Several undergraduates tried growing photosynthetic bacteria but mostly grew mold. One day a woman came into my office who attended Foothill College. She was taking microbiology classes but was also a sculptress, a very interesting person, and she offered to get us going as an intern. This really moved things forward as she successfully grew photosynthetic bacteria, and she also helped attract a first-year graduate student, Chris Chidsey, into our small group (they later married; Chris, after becoming an electrochemist and doing elegant work on electron transfer at electrode interfaces at Bell Laboratories, joined the faculty in our Department).

Chris made the first covalently connected donor−acceptor system. We saw the fluorescence from the donor was quenched but never pursued this further, though there have since been hundreds of variations on this theme. Instead, I had a crazy idea to look at electron-spin/electron-spin interactions in radical pairs, and we started to build a pulsed EPR spectrometer from scratch. Thinking about the mechanism of initial electron transfer in RCs, I thought we might be able to use the microwave amplifier to modulate the yield of charge separation (a technique I later learned was well-developed, reaction yield detected magnetic resonance, RYDMR); thus, we positioned a Si detector behind the microwave cavity to detect the effect. By chance we observed a substantial effect of the applied magnetic field from the EPR instrument (in the absence of microwaves) on the yield of an intermediate. Several groups were looking at the effects of small magnetic fields on the yield of triplets formed by radical recombination in the RC. They showed that the yield dropped upon application of a small field, but we observed a large increase in the yield as the field got larger. Around this time, Klaus Schulten visited, having just spent time with Bill Parson at the University of Washington. Klaus told us that Parson and his postdoc Bob Blankenship found that deuteration of RCs did not alter the low-field magnetic field effect. This did not make sense for a normal radical pair mechanism where the origin of the effect at low field is hyperfine induced singlet−triplet mixing. Our effect at higher field, on the other hand, could fit with a difference in g-factors of the two spins in the radical pair. The pulsed EPR spectrometer was abandoned, and Chidsey and another graduate student, Mark Roelofs, studied the effects of large magnetic fields on the triplet yield. Norris and Closs did get the RYDMR experiment to work; our pulsed EPR spectrometer was used by Gary Brudvig, then a graduate student in Sunny Chan's lab at Caltech, now at Yale, and the parts went to Mel Klein's group at UC Berkeley.

The effects of applied magnetic fields, both large and small, kept us very busy for several years. The theory at high field is much simpler and more intuitive than that at low field. Perhaps the most important result was Chidsey's observation that the special pair triplet state decay rate depends on a magnetic field, in a sense mirroring the magnetic field effect on its formation. This suggested that the triplet state decays in part by reversing to reform the radical pair from which it was born. The activation energy for this process, combined with the triplet state phosphorescence energy measured a few years later in our lab by Larry Takiff, provided important information on the driving force for primary charge separation in photosynthesis.

Although Katz thought chlorophyll was a lipid-like molecule and associated with membranes, there was growing evidence that most photosynthetic pigments were associated with proteins. At that time there was little information on how proteins might affect the physical or spectroscopic properties of bound prosthetic groups like chlorophylls. I thought it might be interesting to create an artificial chlorophyll−protein complex. Working with a talented graduate student, Karen Wright, we replaced the heme in myoglobin with a chlorophyll derivative creating "chloroglobin". This was crystallized and used to determine the transition dipole moment direction for chlorophyll. Karen died tragically near the end of her Ph.D. Later, Atsuo Kuki replaced the hemes in hemoglobin with a chlorophyll derivative, either in the α or β chains or in all 4. This was used to study energy transfer and characterize the orientation dependence of the Förster energy transfer mechanism in a well-defined multi-chromophore system. The work on magnetic field effects and chloroglobin, along with CIDNP experiments probing protein tertiary structure, were apparently sufficient to get me tenure. It was also clear that labels like physical and organic were too constraining. Biophysical chemistry was a better identity, then not so common but now a home to many physical chemists. Prodded by McConnell and Buzz Baldwin in Biochemistry, I revitalized the Stanford graduate biophysics program, wrote the first NIH training grant proposal and two renewals, and ran the program

https://doi.org/10.1021/acs.jpcb.3c06223
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for 11 years. This program has expanded and attracts some of the best graduate students from the physical sciences to work on biological problems.

Around this time, I heard about a postdoc at Stanford, Tom Beatty, who was working with two legends of molecular biology, Charlie Yanofsky and Stan Cohen. Tom (now at the University of British Columbia) gave a group meeting in which he described his efforts to characterize the protein involved in oxygen sensing in facultative photosynthetic organisms. This was all new to me, but it was becoming clear that proteins could be manipulated using the tools of molecular biology and this could be useful for our work. I was fortunate to get a Presidential Young Investigator award and persuaded a new graduate student, Raghavan Varadarajan (aka Rags), to join the lab to clone and express myoglobin, with the idea that we would insert chlorophyll derivatives and perturb their spectra by making mutations near the pigment. This was crazy as very few eukaryotic proteins had been expressed in *E. coli* at that time, nearly all in the nascent biotech industry. Because I knew nothing about this, I agreed with Raghavan that I would provide whatever equipment and supplies were needed and take courses with him and talk with experts to learn this new area. Raghavan managed to isolate the gene for human myoglobin from a skeletal muscle cDNA library (obtained from Larry Kedes' lab where my wife was a postdoc) and after many failures was able to express and purify the protein. This was a heroic effort, something we would now do routinely, but in the early to mid 1980s there were only a handful of commercially available restriction enzymes, routine mass spectrometry of proteins did not exist, we had to run huge and unreliable gels for DNA sequencing, and synthetic DNA was hard to come by (Jamie Williamson, then a graduate student in the lab, built a DNA synthesizer, the "oligomatic", to make probes and for his studies of DNA hairpins by NMR). Raghavan then used the newly developed, but still painfully primitive, method of site-directed mutagenesis to replace a buried hydrophobic amino acid in the heme pocket of human myoglobin with potentially charged amino acids. This was the first example of this concept and began our work on protein electrostatics that continues to this day. Working with Harry Gray's group at Caltech, he measured the effects of buried charges on the redox potentials of the heme iron. Spectral effects on chlorophyll also proved to be revealing and gave the first information on the free energy of burying a charge inside a protein. Raghavan also organized joint group meetings with Peter Kim, Fred Hughson, Sue Marqusee, and other graduate students in Buzz Baldwin's lab in the Biochemistry Department where I learned a lot. These were heady days at Stanford as so much of modern molecular biology was being developed all around us. Raghavan has gone on to a distinguished career at IISC in Bangalore. His work completely transformed the direction and capabilities of our lab and made it clear that physical chemists could exploit these approaches.

There was much excitement in the mid 1980s in the photosynthesis community when the X-ray structure of the bacterial RC was published by Michel, Deisenhofer, and Huber. This structure and the possibility of using molecular biology to manipulate the RC had a huge impact on this field. The high point for me was sitting in the dark in front of an Evans & Sutherland display with George Feher and Hans Deisenhofer in Martinsried, while the structure was still being refined, and seeing for the first time the structural basis for all that was known at the time about the RC. The following year, an abstract from George Feher's lab at UCSD was published in the annual meeting proceedings of the Biophysical Society suggesting that there was an unusual effect of an applied electric field on the absorption spectrum of the RC special pair. Dave Lockhart, a graduate student who had been working on holeburning in RCs and chloroglobin, picked up on this and, with help from Rich Mathies at UC Berkeley, whose Ph.D. had been on Stark spectroscopy of simple aromatic molecules and later rhodopsin, we followed up on Feher's work. The Stark effect, or effect of an external electric field on a spectrum, is a well-known experiment in the gas phase but much less common in condensed phases, let alone proteins. There were many technical challenges, but Lockhart got this to work and focused on both the magnitude and direction of the change in dipole moment for the lowest energy electronic absorption of the special pair. As had been hinted at by Feher's abstract, the effect was large, much larger than that for an isolated bacteriochlorophyll molecule, and surprisingly, the direction of charge transfer suggested that photoexcitation of the special pair breaks the symmetry of the RC, one of the biggest surprises of the RC X-ray structure. This suggested that charge separation begins at the moment of excitation. In related work, Dave Gottfried discovered a huge electronic Stark effect for the nearly inversion symmetric carotenoid in LHII antenna complexes, suggesting that the field from the organized protein environment could induce a large dipole moment in a polarizable chromophore. The notion that fields within proteins can be large and have functional consequences would prove to be an ongoing theme of our work.

With many technical advances, electronic Stark spectroscopy became a routine tool in our lab. Dennis Oh, an M.D./Ph.D. student, used this to probe metal-to-ligand charge transfer transitions in seemingly symmetric molecules like $Ru(bpy)_3^{2+}$ but found that the transition is localized between the metal and the ligand, helping to settle a long-standing debate. Dennis measured Stark spectra of the intervalence charge transfer bands in mixed valence systems to settle whether these transitions were localized or delocalized in different complexes. These experiments brought us in contact with Noel Hush and his close collaborator Jeff Reimers at the University of Sydney. Hush's treatment of electron transfer and intervalence charge transfer transitions such as in the Creutz−Taube ion had a big impact on our thinking; I had close and inspiring contact with Hush until his death in 2019.

Dennis wondered whether we could look directly at the Stark effect on the vibrational transitions of bridging ligands. There were essentially no reports of vibrational Stark effect spectra, the assumption being that the movement of charge from one vibrational level to another should be small, as for a harmonic oscillator, but of course, real potentials are anharmonic. A new postdoc in the lab, Arun Chattapadhyay, took up the challenge and was able to measure the vibrational Stark spectrum of the C≡N stretch in anisonitrile in 1995. This opened up a major new direction for our lab. Steve Andrews did detailed studies of nitriles, followed by extensions to other vibrational modes such as carbonyl groups by Ian Suydam. Around that time, the myoglobin part of the lab was using IR to measure CO stretches in CO-myoglobin mutants. We realized that these spectral shifts in different mutants could be interpreted as differences in the electric field projected on the C═O bond axis. This turned around the use of vibrational Stark spectroscopy, which became a calibration method for vibrational probes whose spectral shifts in different environ-

https://doi.org/10.1021/acs.jpcb.3c06223
J. Phys. Chem. B 2023, 127, 8711−8716

ments could be interpreted as changes in electric fields, now not from external fields but from the fields inside matter. This shifted our focus to mapping electric fields in proteins mostly using nitrile probes, e.g., work by Ian, Lauren Webb, and Nick Levinson mapping fields at the active sites of several important enzymes.

A more recent development was an experiment by graduate student Stephen Fried and postdoc Sayan Bagchi to probe the functionally important electric field at the active site of the enzyme keto-steroid isomerase. As the name implies, this enzyme engages a keto-carbonyl group on a steroid and through a proton transfer mechanism shifts a double bond. The questions were how large is the electric field projected on the carbonyl group where charge separates going to the transition state and does the field, interpreted through the vibrational Stark effect, correlate with the activation barrier for catalysis? Remarkably, they found a direct correlation and could estimate what the activation barrier would be in the absence of electrostatics, thereby establishing quantitatively the large electrostatic contribution to catalysis for this enzyme, a concept promoted theoretically by Arieh Warshel. Although met initially with considerable resistance from the mechanistic enzymology community, this result and follow-on experimental and theoretical work in our lab and others has confirmed this result. This approach has now been extended to several other enzymes and has stimulated a great deal of work in many laboratories. This is currently an active part of the lab.

Several other new directions emerged in 1995−1996. I had always been puzzled by how the RC copes with the huge charge-separated dipoles generated by electron transfer without substantial reorganization. If such a reaction occurred in water, it would be limited by solvent reorganization, but electron transfer in the RC is essentially activationless. Going back to the early work on electrostatic interactions in myoglobin, I wondered whether dynamic Stokes shifts, a standard method for observing solvation dynamics in simple solvents, could be applied to proteins. Using the dye DANCA, provided by Gregorio Weber, and a newly built fluorescence upconversion setup, Dan Pierce found that that the "solvent" response of DANCA-myoglobin occurred over many time scales, very different from simple solvents. The dilemma was that the structure of DANCA-myoglobin was not known. Then, in 1994, Marty Chalfie's paper appeared in *Science* showing that green fluorescent protein (GFP) could be expressed in many organisms, even *C. elegans*. Although the structure of GFP was not yet known, I thought this would be a well-defined chromophore−protein complex for studying the solvent response.

A friend in the medical school who was part of the worm community gave us the clone, and postdoc Mita Chattoraj produced wild-type GFP. GFP has two transitions in the visible, a strong one at around 400 nm and a much weaker one at 470 nm, with the green fluorescence around 520 nm. I thought the 470 nm peak was an impurity (foolishly, as it had been reported to be in the fluorescence excitation spectrum of the green fluorescence) and thus thought there was a huge Stokes shift from 400 to 520 nm that we could study. To our surprise, excitation at 400 nm generated very short-lived (ps) blue fluorescence at around 460 nm, fluorescence that had not been seen in steady-state spectra. Furthermore, the green fluorescence at 520 nm rose with the same time constant as the decay of the blue fluorescence, so clearly the two absorption bands were connected. Then, on a whim, I suggested that we exchange the buffer for a deuterated buffer, and to our further surprise, we observed a large kinetic isotope effect on the decay of the blue fluorescence and rise of the green fluorescence. This led to the suggestion that excited state proton transfer connected the two states and that the band at 400 nm is associated with the protonated form of the chromophore and the band at 470 nm is the deprotonated form of the chromophore. I named the former the A state and the latter the B state, and these names have stuck. The excited state dynamics are more complicated, but these results have stood the test of time. I asked Michael Kasha, whom I'd met at a conference, to sponsor our paper at *PNAS*. The reviewers found the work well done but "not of broad interest", and it was rejected twice. Kasha went to bat and managed to get the paper published in 1996, nearly 18 months after submission. This paper is the most cited paper from our group—so much for predicting what will or will not be of broad interest! GFP continues to be a testbed for our ideas about chromophore−protein interactions, color and quantum yield tuning, electrostatic control of photoisomerization pathways, and "split GFP" which we have elaborated as an optogenetic tool.

Around this same time, I suggested a nonlinear optics experiment on RCs. This required a uniaxially oriented sample. There were reports in the literature of attaching proteins to surfaces using engineered surface cysteine residues, but this failed miserably with the RC (and most proteins) which denatured on the surface. The biophysics graduate student working on this project, Jay Groves, spent a lot of time in Harden McConnell's lab where he learned about supported lipid bilayers. Working with another graduate student, Josh Salafsky, they showed that functional RCs can be assembled in supported bilayers on glass surfaces. Jay was interested in the lipid component and was measuring lateral mobility using fluorescently labeled lipids. By chance he scratched the surface with a pair of tweezers and noticed that lipids did not cross the scratch. That was the birth of membrane patterning, which Jay and Nic Ulman, a postdoc from electrical engineering, turned into a technology for controlling the organization of lipid bilayers on surfaces. Paul Cremer studied these scratches and supported bilayer formation in depth. Using these well-defined corrals, Jay developed the method of membrane electrophoresis in which charged molecules move in two dimensions, subject to confinement, in an applied electric field. The resulting gradients, analyzed in collaboration with Harden McConnell, provide a wealth of information on lipid interactions and critical phenomena in membranes. Alexander van Oudenaarden, a postdoc in the lab, used sophisticated patterning methods and membrane electrophoresis to build the first Brownian rachet, effectively separating molecules using noise and biased diffusion. Jay, now on the faculty at UC Berkeley, has done beautiful work exploiting patterned membranes to study many membrane proteins and to manipulate the spatial organization of receptors on cell surfaces. Work in my lab continues on membrane−membrane interactions, membrane fusion using DNA−lipid conjugates we developed (covalent linkages again), viral membrane fusion, and the development of advanced imaging methods.

Looking back over the evolution of the work in my lab, there have been many twists and turns. Most new directions grew out of work related to my first love, the photosynthetic RC. We focus more on problems than methods and find or develop methods to solve problems. This is only possible with a group of super talented and open-minded co-workers, some of whom

https://doi.org/10.1021/acs.jpcb.3c06223
J. Phys. Chem. B 2023, 127, 8711−8716

I've mentioned here, but there are many others. All have gone on to distinguished and highly diverse careers in academia and in industry. The environment at Stanford, with basic science departments only a few steps from the Medical School and the School of Engineering, has proven to be an ideal environment for my style of science. Gerhard Closs inspired me to use physical methods but to care about the molecules, Clyde Hutchison taught me rigor in spectroscopy, and I've benefited greatly from interactions with Harden McConnell, Buzz Baldwin, Henry Taube, Jim Collman, Noel Hush, John Brauman, and many others. I have also been blessed with reasonably good health and a long and happy marriage to Linda, the Stanley McCormick Memorial Professor in the Stanford School of Medicine, where she is now Vice Dean. Our two grown children, Lisa, a neuroscientist who recently started her own lab at the NIH, and George, a number theorist who just started on the faculty in pure mathematics at Imperial College, carry on the family tradition.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.3c06223.

Publications of Steven G. Boxer (PDF)
Curriculum vitae of Steven G. Boxer (PDF)
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Notes

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https://doi.org/10.1021/acs.jpcb.3c06223
J. Phys. Chem. B 2023, 127, 8711−8716