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Moving Through Barriers in Science and Life

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Abstract

I thank the editors of the Annual Reviews of Biochemistry for the opportunity to look back over a long life and career in science. This first serious attempt at an autobiographical accounting has forced me to sit still long enough to compile my thoughts about a great number of events, some exciting and deeply satisfying and others bitter-sweet. I especially hope that my trajectory will be of interest and perhaps beneficial to much younger women who are just getting started in their careers. As the great writer Virginia Woolf commented at the beginning of the 20th century, “For most of history Anonymous was a Woman”. Fortunately, this quote was accompanied by “Nothing has really happened until it has been described” a harbinger of the enormous historical changes that were about to be recorded in science and other disciplines.

Keywords

quantum tunneling; origins of enzyme catalysis; oxygen activation; novel redox cofactors; autobiography

In composing this chapter, I have worked hard to integrate insights from my personal life with a description of the origins of my research interests and scientific discoveries. While some of the scientific details may be difficult to follow for biochemists with a less chemical perspective, my intention has been to showcase a scientific path that has been driven by curiosity and persistence – the latter becoming especially important as findings arose that differed from mainstream thinking. The final three sections are primarily personal, with reflections on the role of women in science, the importance that family has held for me as inspiration and stability and finally a summing up, “Looking Back, Looking Forward”.

Early Life:

I was born in 1941 in Philadelphia, eight months before the attack on Pearl Harbor that brought the United States into WWII. I have only a few memories from that time, consisting mainly of black velvet cloths being hung over our windows. As much as I can recall, war did not really infiltrate my awareness until many years later when I came across photographs of the American G.I.’s liberating Jews from concentration camps in Europe. These had been sent to us by the part of our family that, though Jewish, had somehow survived the war in Paris.

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The marriage between my mother and father was short lived, as my father, who was a union leader, returned from organizing farm workers in Gilroy California to announce that he had fallen in love with someone else. My mother thus found herself as a single Mom in the 1940s, a situation considered quite a disgrace to her immediate family. Her response to this onslaught of rejection was to sell off all of her belongings (including our home) and to move to a beach front residential hotel in Miami Beach. This was how my early, formative years unfolded: largely living as a carefree, fairly unsupervised “beach bum”.

The return to Philadelphia coincided with the end of our savings, at which point we went to live with my aunt and her family and my mother went to work. It was not very long before my mother met and married the man who was to become my step-father. At that point life changed dramatically, as we approximated the ideal/norm of that time, comprised of mom, step-dad, step-sister (almost one year my senior) and me (Figure 1). With the arrival of the 1950s, and the beginnings of an era of extreme conservatism that included McCarthyism (my uncle lost his job) and the Cold War (my sister and I identified where to hide in the event of a nuclear attack), this new family configuration offered me a much-needed blanket of security and conformity that lasted until I left home to go to university.

Turning Toward Science:

As a young girl, my original passion was ballet and incredibly, in the context of the excessive protectiveness of modern day parenting, I was allowed to take public transportation alone to a center city studio where I trained with the “maestro”. My heroine in those days was Anna Pavlova (rather than Marie Curie) and my exposure to science was nil until 9th grade. Although I attended a huge inner city school, whose hero was the to be famous basketball player Wilt “the Stilt” Chamberlin, there was a strong program for a cohort of about 30 of us labeled as academically gifted. My interest in ballet morphed first into a focus on French language and literature, then a life changing series of classes on physics and chemistry completely altered that plan. These science classes, taught by a dynamic, committed and curmudgeonly duo (of men), were truly the inspiration that led me to a lifetime of science. My experience at school contrasted with the pressure at home to be a “traditional woman”, though my step-dad’s training in engineering at Drexel University was an incredible asset when I encountered my first road blocks in physics and math.

Although our family was solidly middle class, my parents balked at sending me to the University of Pennsylvania, the far more expensive choice in relation to Temple University. Their agreement was that I could attend the University of Pennsylvania if I was offered a scholarship (I was) and if I commuted from home (I did). Later, seeing how hard the long commute was for me, my mother went back to work to provide me the funds so that I could live on campus.

The World Opens Up:

Attending the University of Pennsylvania was another fortunate turn of events. In 1958 I was officially admitted to the College for Women, where we were encouraged to convene for afternoon tea and all of its rituals. On the other hand, I gravitated toward an intoxicating mix

of new friends, the new stirrings of political activism on campus, and the opportunity to explore both the humanities and science. With a cohort of researchers interested in pursuing science at the interfaces of chemistry, biology and physics, U Penn turned out to be a perfect environment in which to hone my interests. My first “serious” research experience was as a lab tech at the Johnson Foundation, a magnet for world class researchers in biophysics. In spite of my proclivity to break pH electrodes and my general terror in the face of so many giants, I thrived there and was encouraged by senior scientists who included Britton Chance, Ron Estabrook and Helen Davies.

Finishing with an A.B. in chemistry in 1962, I moved to NYU downtown for my first year of graduate studies. I had been denied a teaching assistantship at Columbia University, my first choice, but in the end found the Bohemian environment of the Village to suit that time of my life. Although I only stayed one year, I am indebted to NYU for opening my eyes to the excitement and beauty of organic reaction mechanism.

Family and Travels:

Given the prodding from my nuclear family to lead a traditional life, as well as the social climate of the early 1960s, it is not surprising to me that I was married by the end of my first year of graduate school. My husband, Norm Klinman, and I were a perfect match for that time in our lives. He was a recent MD whose strong interest in science led to his enrolling in a Ph.D. program in immunology at the University of Pennsylvania and, later, a brilliant career in immunology that included the first preparation and characterization of monoclonal antibodies.(1) I turned back to my alma mater for further graduate training, continuing a relationship with the University of Pennsylvania that led to my Ph.D. in 1966 as well as an honorary degree in 2006. My choice of a Ph.D. advisor in chemistry was Edward Thornton, a wunderkind from the laboratory of Frank Westheimer. Many credit Westheimer for creating the field of mechanistic enzymology. Given a pre-determined, short time line, before my husband and I were scheduled to move on to postdoctoral studies, the next three years were spent intensely focused on solution studies of the role of imidazole in catalysis. Somehow, I managed to complete a thesis and to give birth to my first son during this time. I owe a great deal to my mother-in-law, Miriam, who stepped in to provide loving childcare as our deadline for departure approached. It didn’t hurt that I was only 24 years old at the time – a fountain of energy!

Our next stop in 1966 was Israel, for a 14 month visit to the Weizmann Institute in Rehovot. Israel was a young, idealistic and pioneering country, with a primary goal to put food on the table, and a lifestyle free of televisions and telephones. Weizmann had been a chemist in Manchester and a Zionist who was to become the founder of a research institute destined to be world class. I arrived in Israel pregnant with my second son and ready to begin work with David Samuel, who had promised access to ca. 100 % oxygen-18 enriched water for mechanistic studies. The next period was filled with the birth of my second son, new friendships, discovery, and unanticipated drama (that included the Six Day War of 1967). Scientifically, I designed and performed a series of experiments aimed at understanding the role of metal ions in the hydrolysis of high energy acyl phosphates using the labeled water promised by David Samuel. This was a timely topic, as Mitchell’s chemiosmotic theory of

ATP production was not yet proven or generally accepted, and researchers were still focused on the possible role of a chemical intermediate (i.e., the elusive X~P). These studies ignited in me an interest regarding the role of metal ions in enzyme catalysis, a subject I have continued to pursue until the present day. The younger scientists who are reading this account may also be surprised to learn that it was during this time that the first X-ray structure of an enzyme (lysozyme) was reported. I heard about it first hand, when David Phillips visited the Weizmann Institute from the University of Oxford to present a seminar on his spectacular advance.

Although we left Israel in January of 1968, we would not return until the following summer to the United States, where my husband had a faculty position waiting for him at the University of Pennsylvania Medical School. The interim period was spent living in London, where I was able to wangle a non-paying apprenticeship at University College in the laboratory of Charles Vernon, ironically one of the scientists involved in dispelling the notion of a high energy chemical intermediate in oxidative phosphorylation. It was during this time that I began to delve seriously into biochemistry, in preparation for joining the laboratory of Irwin Rose at the Fox Chase Cancer Research Institute upon our return to Philadelphia.

Home Again:

The late 1960s and 1970s were a convulsive time, both for U.S. politics and for my personal life. Upon our immediate return to Philadelphia in 1968, we settled into a high-rise apartment in center city and I began an association with The Institute for Cancer Research (a part of the Fox Chase Cancer Center) where I was to remain for a decade. The initial years as a postdoctoral associate with Irwin (Ernie) Rose were incredibly challenging and satisfying. Those days, though circumscribed by a very tight commuting schedule between the downtown and outskirts of Philadelphia, were my initiation into experimental biochemistry - and I loved every minute of the experience. Despite the designation as a center for cancer research, there was a strong commitment to basic research. Much to my delight and future training, ICR had become a world magnet for researchers interested in the study of enzyme mechanism and, in addition to Ernie Rose’s laboratory, there were quite a few related research groups, for example the labs of Larry Loeb, pursuing the mechanism of DNA repair, of Jenny Glusker, studying protein X-ray crystallography and of Al Mildvan, who had established a center for nuclear magnetic resonance mapping of metal centers in enzymes.

After much discussion with Ernie, I chose a project that would use my previous training in chemistry to study an enzyme catalyzed isomerization of trans-to cis-aconitate, where the former is a polyanion that accumulates in plants and the latter is an intermediate in the conversion of citrate to isocitrate in the citric acid cycle. Using a series of isotope probes (deuterium, tritium and carbon 14), we could show that the isomerization did not require any bond rotations at all and was achieved by a simple 1,3 prototropic, suprafacile proton shift within a suitably aligned substrate via the participation of a single base on the enzyme (2). A bonus of this study was the ability to use this newly characterized property of aconitate isomerase to analyze the stereo-outcomes of two enzymes that catalyze aldol cleavages/

condensations involving chiral methyl groups (3). This was the first instance in which Ernie and I seriously matched wits, each of us going home with the newly collected data to determine whether the new bonds were related by a retention or inversion of configuration. The beauty of biological stereochemistry is that there are generally only two possible answers, with a 50:50 chance of being correct. While the fact that we had different answers to the problem, and mine was correct, may have been fortuitous, this felt like a monumental step in the direction of forging my own identity as a scientist. Many years later, near the end of his life, Ernie confessed to me that he had initially been quite uncertain about my future in science.

Moving Toward Scientific Independence:

By 1970, I was itching to start my own research lab and looking for projects that would be both original and forward-looking. This was an era when kinetic probes were undergoing heavy development for the study of the chemical mechanisms of enzyme reactions. One issue that troubled me was a lack of knowledge about rate limiting steps, raising serious issues of interpretation. Could I use my graduate school training, in the use of solvent kinetic isotope effects (KIEs) to study acid-base catalysis of small molecule reactivity, to good effect? Though solvent KIEs were beginning to be applied to enzyme reactions, I wanted a probe that would permit a more straight-forward interpretation. The $NAD(P)^+$ cofactors looked like a good place to start, with a growing number of enzymes dependent on these cofactors undergoing structural and kinetic characterization. Importantly, the reduced form of $NAD(P)^+$ had been synthesized with non-exchangeable isotopes in their reactive positions. I chose yeast alcohol dehydrogenase as a test system and studied its reaction in the forward and reverse directions using either labeled cofactor or a series of ring substituted benzyl alcohols (with protium or deuterium in their reactive positions). This approach provided a combined analysis of isotope effects and structure reactivity correlations, where the KIEs could be used to show conditions whether hydride transfer was rate limiting and the substituents on the alcohol ring provided insight into charge distribution at the enzymatic transition state (4, 5). This approach, toward the application of a combination of physical-organic probes to the study of enzyme mechanism, is now a mainstay in the field. Looking back, I am indebted to Ernie, who both provided funds from his own research grant for these studies and was fully in support of my early sole author publications.

A Laboratory of My Own.

By 1972, in part catalyzed by the offer of an Assistant Professorship from UT Southwestern, ICR appointed me to an equivalent research position, and I moved into the ranks of the staff scientists. By this time, my two sons were 5 and 7 years old, and at 31 years old myself, I was filled with excitement for my personal and professional future! Alas, my life has shown itself to be a long winding path, with repeated lessons of change and unexpected consequences.

My first independent laboratory was quite small with space for at most 4 researchers. I did not feel deprived and had free access to equipment in both Ernie’s adjacent lab and also that of other researchers at ICR. There was a strong cohort of young scientists in my age group

and we became good friends and colleagues. This group included Ruth Angeletti, Helen Berman and Byron Rubin. Both Helen and Byron were trained in X-ray crystallography and Ruth in protein chemistry with a strong interest in the biology of chromaffin vesicles. Ruth was influential in my second choice of a long-standing research project, pointing out the fascinating copper enzyme, dopamine beta-monooxygenase (DβM) that is found within the storage vesicles of adrenal glands and within the sympathetic nervous system. It would be many decades before a suitable protein expression system would become available for biochemical studies of DβM and related enzymes. I remember well the days spent dissecting out the medullae of bovine adrenals as a starting point for the preparation of pure enzyme. I would cover the lab bench tops with padded paper and the entire crew of friends would show up to aid in the process, some of us using a device invented by Byron to speed the dissection. What is so astonishing in the context of the pace of research today, is the many friends who were willing and happy to contribute their time to this tedious procedure.

I was also fortunate in having a handful of excellent co-workers in the lab: Kate Welsh, Judy Voet, Hope Humphries, Don Creighton, Radmila Markovic and Michael Summers. The use of isotopes remained a prominent theme in these early studies, with Kate and Don continuing to pursue deuterium isotope effects in dehydrogenase reactions, and Judy and Hope studying tritium isotope effects in DβM. It had been assumed, prior to studies of DβM that reactions catalyzed by O_2-dependent enzymes would need to bind their substrate(s) prior to the creation of a site for O_2; however Judy and Hope's results showed very clearly that the addition of substrate and O_2 was random, implying a mode of interaction of protein with molecular oxygen that was independent of the presence of substrate (6). At the suggestion of Michael Summers, a postdoc initially in Ernie Rose's lab, I also became interested in a second class of copper proteins, the copper amine oxidases (CAOs). Although Michael was primarily interested in the stereochemistry of these enzymes (7), he pointed out to me the confusion and uncertainty regarding the active site cofactor, a problem we would finally resolve many years later. In retrospect, it is clear that many of my scientific values and interests were forged in these early years at ICR.

Westward Ho.

By 1975 my marriage was failing and a messy and painful personal transition to a new kind of independence was taking root. As it became increasingly evident that divorce was inevitable, I needed to step back and take a hard look at both my options and heart desires. The protracted US-Vietnam War was just coming to an end. The accompanying breakdown in societal norms that had resulted from extended social protest of this war had taken its toll at many levels including the personal lives of many people of my generation. The West Coast had emerged as a growing progressive place, with outstanding public education at the University level. I "screwed up my courage" and wrote letters to two University of California campuses: UCLA and UC Berkeley to inquire about possible positions. The Department of Chemistry at UCLA wrote back saying they were not interested while the Chemistry Department at UC Berkeley wrote to invite me for an interview. After many interactions, negotiations, and (with a great deal of intimidation) I accepted their offer of an Associate Professorship. I did not realize at the time that this appointment was as the first woman not only in chemistry (Figure 2) but in all of the physical sciences at Cal.

Importantly, the biophysicists within the Department were quite welcoming and I was assigned a large laboratory space in Hildebrand Hall that had been occupied by Jim Wong prior to his relocation to Harvard University. In the interim between Philadelphia and Berkeley and after I had sold our home in Mount Airy, my two sons and I went to live with Helen Berman and her husband Peter. Together with a visiting scientist from New Zealand, we created our own version of a 1970's hippie family that was nurturing, fun and I think a great shock to my in-laws when they would come to visit their grandsons.

Early Years at Cal.

By the time I arrived in Berkeley in 1978, my two sons were 11 and 13, on the cusp of full blown adolescence and I had never taught more than 2 or 3 classroom lectures a year. The new responsibilities, especially as a single parent in a new city, were overwhelming. Two important components critical to success were the strength and character of my sons Andrew and Douglas (a well justified prejudice of Mom) and my decision to vigorously prioritize my family and research/teaching, with very little time or energy left over for coffee klatching with colleagues and administrative duties. After some sampling of different teaching responsibilities, I began a cycle of alternating between lecturing in a second year organic chemistry class tailored to biologists and a graduate course on enzyme mechanism that had been originally developed by Jack Kirsch and which we proceeded to teach jointly over many decades. At the same time, I attracted a small and highly capable core of researchers that included Sue Miller, now Prof at UCSF, and Natalie Ahn, now Prof at U. of Colorado, Boulder as my first two graduate students, Monica Palcic, the first postdoc to join my Berkeley laboratory (from Canada), and Matt Krueger, an unassuming and highly efficient lab technician. The early research projects were, in part, an adaptation of the approach I had initiated at the Institute for Cancer Research on the dehydrogenases, with Sue using the combined interrogation of isotope effects and structure reactivity relationships to show that DβM functions via a hydrogen atom abstraction mechanism (8) and Natalie combining isotope effects with pH and the DβM effector fumarate to understand the roles of acid base catalysis and anion activation (9). Natalie bravely took on a second project that examined the activity of DβM within its *in vivo* sub-cellular compartment, the norepinephrine storage vesicles of the adrenal gland that we had spent many years throwing away! Though this project attracted very little attention at the time, it was an important effort at extending the *in vitro* characterization of an enzyme to its more biologically relevant contexts (10). During this time Monica initiated our first forays into the kinetic and chemical mechanism of the copper amine oxidase from bovine serum (11), bringing our focus more and more in the direction of metallo-biochemistry. The growing theme, of linking bioinorganic, bioorganic and biophysical approaches to enzyme studies, grew more robust with the next wave of graduate students and postdocs leading to many mechanistic insights into the copper-containing enzymes DβM and the CAOs. An exciting collaboration between Dr. Dale Edmondson at Emory University and Mitch Brenner in my laboratory led to the important discovery that DβM requires *two* uncoupled copper ions per active site to function (12); this was followed by single turnover, rapid mixing experiments to show that both of the (pre-reduced) copper centers undergo rapid reoxidation in a single phase that correlates with the time constant for the formation of norepinephrine from dopamine (13). The presence of two,

uncoupled copper centers was later confirmed by X-ray crystallography (14), introducing the new challenge of understanding how spatially distant copper centers separated by solvent water molecules are capable of supporting a highly-controlled oxygen-insertion into dopamine (15, 16), in the absence of any of the uncoupling reactions (17) commonly seen in monooxygenases. Our most recent proposal of a long range proton coupled electron transfer (PCET) (16) is relevant to many biological processes, and has emerged as a highly active research area at the interface of theory and experiment

Second Decade at Cal and the Discovery of Quino-Enzymes.

An unexpected and remarkable intersection of metallo-biochemistry and cofactor chemistry emerged in the mid- to late 1980s, with the appearance of reports from the Netherlands that a significant number of eukaryotic proteins required a low molecular weight quinone cofactor, pyrroloquinoline quinone (PQQ), for function (18). This captured our interest for several reasons. To start with, PQQ had been assigned the role of a freely dissociable cofactor in a range of bacterial enzymes and had, until that time, no associated role in eukaroytes. Further, two of the enzyme systems concluded to require PQQ were DβM and the CAOs, and these enzymes had already been subjected to extensive study in my laboratory. In no instance had we observed a requirement for low molecular weight, dissociable cofactors other than copper ion, and importantly, the available kinetic and mechanistic properties seemed to be well rationalized in the absence of such factors. That said, there remained the continuing enigma of the non-dissociable cryptic cofactor in the CAOs, which had been ascribed to an aldehyde that either resembled or was derived from pyridoxal phosphate (19).

The major deterrent to identification of the implied organic cofactor in the CAOs was its inability to be isolated in a reproducible form following protein hydrolysis into peptides. Working on the premise that the cofactor contained a carbonyl functional group, but in a form that was chemically reactive, we proceeded to derivatize enzyme with the carbonyl reagent phenylhydrazine (20). This was intended both to produce a stabilized structure from the native carbonyl and to impart a UV/Vis absorbance band that could be easily detected outside of the background absorbance from the remaining protein-derived peptides. These studies were performed prior to the availability of a recombinant form of CAO and the enzyme used, bovine serum amine oxidase (BSAO), was obtained the old-fashioned way from bovine blood collected at a slaughterhouse! I was fortunate to have a truly gifted graduate student, Susan Janes, who excelled at everything the project required – from protein purification, derivatization and hydrolysis, to peptide isolation in high enough yield to enable both a mass spectrometric and an NMR analysis of the resulting peptide product. In this manner, the structure of the CAO cofactor was identified as 6-hydroxydopa (named topa quinone or TPQ), previously known to be present *in vivo* as a hydrolysis product of dopa with demonstrated toxic properties (20). We were quite amazed that Nature would have evolved an enzymatic cofactor that, when sequestered within the confines of an enzyme active site, could be used to promote the selective oxidative deamination of a range of biological amines.

The identification of TPQ set off a flurry of research activity around the world, with many new protein-derived quinone cofactors emerging over the following decade that included our own discovery of LTQ in bovine aorta lysyl oxidase.(21) These cofactors are all structured around an aromatic side chain (Tyr or Trp), that subsequently undergoes hydrolysis (TPQ) or covalent attachment to a second amino acid such as Lys (LTQ), Trp (TTQ) and Cys (CTQ). In all instances, the enzymes containing these cofactors function via two sequential steps with the substrate amine first undergoing oxidation and hydrolysis to release ammonia, an aldehyde product and the reduced, quinol form of the cofactor. A mechanistic divergence occurs in the recycling of the reduced cofactor, and this occurs either via reduction of O_2 (for the oxidases that contain TPQ and LTQ) or electron transfer to another protein (for the dehydrogenases associated with TTQ and CTQ). The four cofactors also segregate with regard to the mechanisms of production of the protein-bound cofactors (22). In subsequent years, a major focus in my lab was on the eukaryotic-derived oxidases that contain TPQ and LTQ, with experiments demonstrating that biogenesis occurs as an autocatalytic process that depends solely on active site Cu^{2+} and molecular oxygen (23, 24). Once the mechanistic aspects of this process were uncovered, we could step back and marvel at how a single enzyme active site could have evolved to perform both O_2-dependent hydroxylation (as required for biogenesis) and O_2-dependent oxidation (as occurs during amine oxidation) (25). Many talented students and postdoctoral associates contributed to these studies, including Minae Mure, Sophie Wang, Jen DuBois, Ben Schwartz, Joanne Dove, Danying Cai, David Mu, Julie Plastino, Joanie Hevel and Steve Mills. A network of collaborators including structural biologists (Scott Matthews and Carey Wilmot) and spectroscopists (Joann Sanders-Loehr) also aided immensely (both professionally and personally) to progress in this area. In parallel studies, other laboratories elaborated the quite different strategies for the production of TTQ and CTQ, prokaryotic cofactors whose formation is dependent on a suite of additional enzyme activities encoded within specifically tailored operons (26, 27). While the mechanistic aspects of catalysis and biogenesis in this field are fairly well established, much remains unknown regarding the biological role of the TPQ-containing enzymes in eukaryotes, where these are found to be located ectopically in the vasculature and in adipocytes. In the former case, there is evidence of a role for the enzyme in immune cell recruitment (28), while adipocytes may also use the TPQ-dependent copper amine oxidase to initiate an inflammatory response (29). This aspect of the quino-enzyme field is wide open, and ripe for new discoveries that relate to amine-based peroxide signaling. There is also the growing implication of a redox role for the bacterially produced PQQ in mammalian physiology (30, 31).

The Discovery of Room Temperature Quantum Tunneling in Enzymatic C-H Cleavage Reactions.

The Gordon Research Conference on Isotopes, initiated after WWII and continuing until 2014, played a key role in a second, major research direction within my laboratory at Cal. This biennial meeting brought together a seemingly disparate group of chemists, geologists, environmental and atmospheric scientists and biologists, with a shared interest in using isotopes to study their individual disciples. At the core of this recurring in-gathering was a curiosity and openness to new ideas and disciplines. For a long time the meeting was reigned

over by Jacob Bigeleisen, who had worked at the Manhattan Project on the extraction of uranium-235 from uranium ore and later, co-authored (with Maria Goeppert-Mayer) the classic paper on the use of statistical mechanics to formalize the impact of isotopic substitution on rate and equilibrium processes (32). I began to attend this conference with regularity in the 1970's and was thrilled (and terrified) when I was asked to talk about our early development of isotope effects for the study of enzyme mechanism. With time, the name of this GRC evolved from Isotopes in Chemistry and Physics to Isotopes in the Biological and Chemical Sciences, to represent the growing presence of biochemists contributing to the meetings.

While the use of statistical mechanics and transition state theory continued to dominate the theoretical framework of isotope effects for many years, new perspectives were welcomed (such as variational transition state theory developed by Don Truhlar (33)) and other observations outside of the main stream (such as mass-independent isotope effects in atmospheric chemistry demonstrated by Mark Thiemens at UCSD (34) and the role of nuclear quantum effects in biological hydrogen transfer reactions implied from the early work of Mo Cleland at the University of Wisconsin and ourselves (35, 36)) While hydrogen tunneling had been recognized earlier at the Isotopes conference, it had never been fully embraced for fear that the experimental designs and observations were flawed, especially in the context of free radical reactions which can be notoriously difficult to study in solution. Enzymology was to play an important role in changing opinions about the importance of tunneling, in particular because of the much more controlled reaction pathways within the confines of enzyme active sites.

My interest in the possibility of detecting hydrogen tunneling in enzyme reactions had been brewing for quite a while, leading in the mid-1980s to a summer sabbatical in the laboratory of Pierre Douzou, an experimental physical chemist at the Institut Pierre and Marie Curie who had developed cryo-solvents to study enzyme behavior at low temperature (37). During this time, my focus was on low temperature studies, since I thought this would be the only way to detect significant tunneling in enzyme systems. Pierre Douzou and his associates were charming and gracious hosts; however, my attention was split between the laboratory and the allure of Paris. The latter was shared with my mother as well as my older son and some of his friends, who descended on (or rather ascended to) a 5^{th} floor walk-up apartment near the Arc de Triomphe.

As it turned out, studies at low temperature for the observation of tunneling proved unnecessary, as emerging data and discussions revealed that hydrogen tunneling could be inferred at room temperature from deviations in the magnitude of secondary kinetic isotope effects measured with substrates that contained specific patterns of protium, deuterium and tritium. Following the laborious work of synthesizing the appropriately labelled substrates, together with the development of appropriate enzyme assay methodologies, we succeeded in publishing our first study implicating room temperature tunneling in the hydride transfer catalyzed by yeast alcohol dehydrogenase in 1989 (38), Over the next decade, the available tool box for detecting tunneling expanded, to include the size and temperature dependence of primary kinetic isotope effects using assays that allowed kinetic isolation and analysis of the hydrogen transfer step (e.g., (39–41)). As this field developed, it initially encountered

considerable resistance with claims stating that our data must be wrong and, when that was shown not to be true, that the findings were likely irrelevant to biology. Now, from the vantage point of 2018, and as a consequence of tremendous research efforts from many different laboratories throughout the world over a period of more than three decades, the role of quantum tunneling for all major classes of enzymatic C-H cleavage reaction is accepted, while the implications for catalysis have changed the way we thing about enzyme function (discussed below).

New Ways of Looking at Enzyme Catalysis.

A number of developments occurred in my laboratory during the 1990's that would propel studies of tunneling into new domains of relevance first, to physical models for the origins of enzyme catalysis and second, to the development of theories for proton coupled electron transfer processes at room temperature. One experimental advance was a use of site directed mutagenesis to examine the contribution of bulky hydrophobic residues to catalytic efficiency in enzymes. This meant moving away from a dominant focus of mutagenesis on charged and hydrogen bonding protein side chains implicated in acid/base and electrostatic catalysis and toward the participation of large hydrophobic side chains in "tuning" active site reactivity. An early collaboration between Jodie Chin, a graduate student in my laboratory and two X-ray crystallographers, first Barry Goldstein and later Brian Bahnson, would lead to the demonstration that reduction in the size of a single active site hydrophobic side chain could alter, concomitantly, the degree of deviation of secondary kinetic isotope effects from their semi-classical limits (our earliest experimental reporter of hydrogen tunneling) and the distance between the reactive carbon centers of bound cofactor and a substrate (42). This first structural intimation of the importance of "active site close packing" in enzyme catalysis had its genesis in a mini-sabbatical to the University of Rochester. I had arrived to work with Bill Saunders, an expert in computer-based analysis of secondary isotope effects and an early proponent of the contribution of tunneling effects in room temperature solution reactions (43). Byron Rubin, a good friend from earlier days at the Institute for Cancer Research and now affiliated with the University of Rochester, offered me a room in his home and later introduced me to Barry Goldstein and his wife Andrea Barrett (who was immersed in writing her novel "Ship Fever" which would go on to win the National Book Award). Within this mixture of deep friendship, exciting cultural tendrils and cold enough weather to send us out ice skating together, Barry and I initiated our structure/function collaboration. So began the recognition of the importance of donor-acceptor distances in the manifestation of the properties of active site hydrogen tunneling and, ultimately in enzyme reactions in general.

Serendipity has always played a role in the emergence of new research directions in my laboratory and that was certainly the case when Michael Glickman took up the subject of hydrogen transfer in the soybean lipoxygenase for his Ph.D. thesis. Given the ability of kinetic isotope effects (KIEs) to sort out detailed enzyme kinetics, Michael began a series of careful studies comparing the reactivity of protio- and perdeuterio- labelled linoleic acid substrates. When his first measurements produced a kinetic isotope effect of 80, I sent him back to the laboratory. (Up to that point the upper limit for k_H/k_D near room temperature was predicted to be around 7, though values as high as 10–12 had been seen and rationalized

by the contribution of a small tunneling correction). Several years and reams of controls later, Michael stood fast (44, 45) and I reported his results at a Gordon Conference - to an audience that was both skeptical but also intrigued. After my talk, Dexter Northrup commented that he remembered a graduate student from the University of Wisconsin who had seen a huge KIE with lipoxygenase, but no one believed his finding and it laid buried in an unread Ph.D. thesis. Quickly, theoreticians entered the fray in an effort to explain the experimental observations, with an important new perspective put forth in 1999; this emanated from a group of Russian electrochemists who had been postulating for quite a few years that quantum effects would dominate hydrogen transfer near room temperature (46) Over the next two decades, and with the help of many talented graduate students and postdoctoral researchers (Thor Jonsson, Michael Knapp, Matt Meyer, Keith Rickert, Sudhir Sharma, Adam Offenbacher, and Shenshen Hu) and expert collaborators (Sharon Hammes-Schiffer in theory (e.g. (47, 48)) and Brian Hoffman in spectroscopy (e.g. (49)), lipoxygenase emerged as a work horse and prototype of enzymatic hydrogen tunneling. Ironically, the originally controversial kinetic isotope effect of 80 is now seen to be “small” in relation to a room temperature kinetic isotope of 661 ± 27 observed for a variant of SLO in which two bulky hydrophobic side chains have been pared down to alanine (48). These studies emphasize the need for revision of earlier dominant theories regarding the origin of primary kinetic isotope effects as arising solely from changes in vibrational frequencies (50).

Perhaps most importantly the inherent properties of quantum mechanical tunneling in lipoxygenase along with many other systems have necessitated a reexamination of the origins of enzyme catalysis, showing how any description of enzymatic reaction coordinates must take into account a temporal and spatial hierarchy of protein motions (e.g., (51, 52)). In very recent experiments, designed to obtain a spatial resolution of such motions in SLO, Adam and Shenshen employed time, temperature and mutation-dependent hydrogen-deuterium exchange to identify a protein dynamical network that communicates between the protein/solvent interface and the active site, 15 to 30 Å away (53). The role of protein dynamics and their link to specific protein networks has emerged as an important new direction in enzymology, not only for understanding function, but also as a basis for the future design of new catalysts that can function as well as enzymes.

The 1990s also led to a fortuitous meeting with Amnon Kohen while attending a chemistry conference in the UK. Amnon was about to complete his Ph.D. studies at the Technion in Haifa and was looking for a laboratory where he could expand his skills in enzyme kinetics. After hearing me talk on the role hydrogen tunneling in enzyme catalysis, his enthusiasm seemed unbridled and a year later he arrived at UC Berkeley for postdoctoral training. During this time there was increasing discussion of how families of prokaryotic proteins are able to sustain their function across a wide temperature range, especially when the 3-D structures of such protein often appear to be almost identical. My interest in this area was further stimulated by a fascinating sabbatical visit to the University of Waikato in New Zealand, to work with Roy Daniel, one of the world experts in the isolation and characterization of high temperature proteins. A thermophilic homolog of the yeast alcohol dehydrogenase had just been isolated and partially characterized (54), and Amnon and I entered into a collaboration with these researchers, with the goal of relating protein activity to thermal stability. Remarkably a kinetic break in behavior was seen at 30 ° C with this

thermophilic protein (designated ht-ADH) when measuring either the enthalpy of activation controlling hydride transfer or the isotope effect on this step (55). The behavior of the latter was initially very puzzling to us, indicating temperature independence of the kinetic isotope effect (KIE) in the physiologically relevant temperature range of ht-ADH, that became quite temperature dependent below the breakpoint. This change in behavior of the KIE for ht-ADH with temperature - both fascinating and difficult to fully understand until several years later - is now understood to be a result of a critical link between the maintenance of overall protein flexibility and the creation of tightly packed active site structures as a prerequisite for efficient catalysis (56–58).

Many researchers in my lab carried these findings on prokaryotic ADHs in important new directions. A new postdoctoral associate, Zhao-Xun Liang in collaboration with Natalie Ahn at the University of Colorado, carried out the first hydrogen deuterium exchange linked to mass spectrometry in our laboratory, showing that catalytically relevant motions in ht-ADH (and subsequently a psychrophilic variant, ps-ADH) are restricted to selected regions of protein (59, 60). Zac Nagel introduced mutation of bulky hydrophobic side chains within the active site of ht-ADH, showing how the combined impact of site specific mutagenesis further exacerbated the impairment of ht-ADH at low temperature. By way of comparison of ht-ADH to ps-ADH, Zac also uncovered a pathway for dynamical communication between a subunit interface in ht-ADH and the bound substrate over a distance of ca. 17Å (61–63). Corey Meadows introduced fluorescence spectroscopy using engineered forms of ht-ADH with single tryptophans (64, 65). This has been extended by Jianyu Zhang to include a T-jump FRET study of ht-ADH (in collaboration with co-workers in the laboratories of Tom Spiro and Brian Dyer). In an exciting turn of events, a microsecond FRET has been detected that displays an activation energy above 30 °C that is almost identical to the previously measured enthalpic barrier for (millisecond) catalysis. These studies directly link a local protein motion to the enthalpic barrier for active site tunneling (66). With feasibility established, it should be possible to systematically extend such studies to a wide range of dehydrogenases, ultimately testing the generality of the findings with ht-ADH.

While this type of approach to experimental biology– the placement, one by one, of many “solid bricks of data”– requires time and patience, with luck and persistence a robust structure for understanding Nature can emerge. This is exactly what has happened as a result of studies of SLO and ht-ADH, which when combined with decades of studies of other enzyme systems, have given way to a new framework for our understanding of the physical origins of catalysis (52).

Oxygen Activation and More Isotope Effects:

From an evolutionary point of view, the complexity of current life on Earth is, simply put, remarkable - directly attributable to the emergence of aerobic life and the use of molecular oxygen (O_2) as the terminal electron acceptor in respiration. There is also a “dark side” to life in the presence of atmospheric O_2, a result of the series of one electron steps whereby O_2 normally undergoes reduction to water, generating free radical intermediates capable of carrying out damaging oxidative side reactions.

Looking back, the focus on O_2 activation in my laboratory was a natural outcome of our focus on C-H activation, given the large number of enzyme systems that directly couple O_2 reduction to subsequent C-H cleavage. Some of the mechanistic questions that captured our attention over the years were: (i) Can O_2 bind to an enzyme prior to the formation of the enzyme-substrate complex (6)? (ii) Is the interaction of O_2 with a protein bound transition metal a prerequisite for O_2 binding? Or (iii) Are discrete binding pockets, generated by hydrophobic side chains, capable of sequestering active site O_2 for subsequent reaction (67, 68). And finally (iv) Can proteins create efficient networks to guide the movement of O_2 from solvent toward the reactive carbon(s) of a bound substrate (69–71)? Studies of this kind have continued to attract the attention of researchers in a wide range of enzyme systems, recently, for example in the nitrogenase reaction where it becomes critical to distinguish substrate N_2 from inhibitory O_2 (72).

Additionally, and, perhaps, most critically throughout many decades of research on oxygenase and oxidase mechanism, has been the question of the nature of O_2-derived intermediates that give rise to substrate oxidation (expected to be critically dependent on the energetic demands of the reaction being catalyzed as well as the availability of enzyme active site features for stabilization and sequestration of reactive intermediates). As with hydrogen and its three isotopes (H, D and T), oxygen also offers three isotopes (O-16, O-17 and O-18) as mechanistic probes. While our primary focus was, over many years, the comparison of O-16 to O-18, an early study made use of all three oxygen isotopes in the context of looking for alternative explanations to the huge (non-classical) kinetic isotope effects first seen in the lipoxygenase reaction.

In 1994, a proposal was put forth by Charles Grissom at the University of Utah that the inflated hydrogen effects in lipoxygenase might be the result of a differential magnetic coupling between the high spin active site iron center of lipoxygenases and protio- vs deuterio- labelled substrates (73). To test this hypothesis, he and his co-workers applied an external magnetic field to the lipoxygenase reaction mixture and measured its impact on the size of the experimental k_H/k_D. While no impact was detected, it remained possible that an internal, active site iron-induced magnetic field could be operative. Michael Glickman, the student who initiated hydrogen isotope effect studies on lipoxygenase, reasoned that such a magnetic effect might become apparent in the rate of combination of the enzyme bound, substrate-derived radical intermediate with molecular oxygen to yield the lipid hydroperoxide product. Setting up a collaboration with Mark Thiemens at UCSD, they proceeded to compare $k_{(O\text{-}16)}/k_{(O\text{-}18)}$ to $k_{(O\text{-}17)}/k_{(O\text{-}18)}$ in the second partial reaction of lipoxygenase, where O_2 traps the intermediate linoleyl radical (74). Alas, no deviation from mass-dependent effects was seen with molecular oxygen, clearing the path for a full quantum mechanical interpretation of the non-classical deuterium isotope effects in SLO.

Our ability to compare the relative reactivity of O-16, O-17, and O-18 was a result of Thieman's developed methodology for the direct detection of the isotopic composition of unreacted O_2. More generally, oxygen isotope effect measurements have followed a protocol developed by Joe Berry at the Carnegie Institute in Palo Alto that involves separation of unreacted O_2 from N_2, *combustion* of the O_2 to CO_2, and analysis of the resulting CO_2 by mass spectrometry (75). Use of this latter technique in my laboratory was initiated in 1992

by Gaochao Tian and proved to be quite adequate for several decades of measurement of k_{O-16}/k_{O-18} in a range of enzyme systems.

Gaochao was a brilliant postdoc, newly arrived from mainland China, who made the decision to first measure the magnitude of O-18 isotope effects in well understood Fe and Cu systems that act as reversible O_2 carriers, thereby allowing the creation of a "ruler" for the equilibrium conversion of free O_2 to bound species with well-defined structures (76). This was followed by the determination of kinetic oxygen isotope effects by Gaochao (77) and, later, many others (e.g., (78–81)). While such kinetic measurements can become complicated due to the presence of multiple rate determining steps as well as compensatory transition state fractionations, the ensuing work was able to distinguish patterns that could be correlated with the type of chemistry catalyzed. Perhaps most important, was the use of a double isotope effect method, introduced by Mo Cleland at the University of Wisconsin in the context of other types of enzyme reactions (82). In the case of both Fe and Cu-dependent oxygenases, we were able to show that a measured O-18 isotope effect can depend on deuterium labelling of substrate at the reactive (cleaved) hydrogen (77, 81). This property, arising when the reactive oxidizing species is formed reversibly prior to C-H activation, has implicated metal-superoxo species in numerous C-H activation reactions, overcoming the long held expectation that reactive, O_2-derived species would need to be more reduced (e.g., as seen in P-450 chemistry (83)) to carry out their catalytic functions.

A description of our multi-decade pursuit of O_2 chemistry in biology would not be complete without a short summary of the work by Justine Roth on the flavo-enzyme glucose oxidase. Justine broke new ground at many levels in a seemingly straight forward and well understood enzyme system. Her work showed that glucose oxidase reduces O_2 via an outer sphere electron transfer mechanism that is dependent on a single active site, protonated histidine to reduce the size of the environmental reorganization barrier (84). Perhaps even more revealing, a subsequent study of O-18 isotope effects as a function of reaction driving force led to the unanticipated but powerful conclusion that the heavy atoms of O_2 must also behave quantum mechanically, undergoing tunneling in the process of preparing the reaction coordinate for electron transfer (85). Looking to the future, it will be fascinating to see the extent to which the now well accepted quantum effects in biological electron and hydrogen transfer will be extended to include the movement of larger masses (cf.). We have come a long way from the early assumptions that the phenomenon of tunneling would be unimportant under the warm conditions of biology.

Back to the Beginning (of Quino-cofactors): Unraveling the Pathway for Pyrroloquinolone Quinone (PQQ) Production.

As I write this Perspective I am *ca.* 3 years away from a targeted retirement date. One nagging and unfinished aspect of our research trajectory has been a complete description of the multi-step biosynthetic pathway for production of the bacterial cofactor PQQ. Conserved prokaryotic operons for PQQ production indicate a role for five (six in some cases) open reading frames with gene products of unique properties and mechanisms.(86) As I write, we can describe four of the essential components in reasonable detail. PQQ has been identified

as a cofactor in ca. 15% of aerobic bacteria, many of which are opportunistic pathogens, and we have been able to draw on our years of experience of working with O_2 and C-H activation in unraveling the pathway (86). That said, there have been many surprises that include (i) the role for a Radical SAM enzyme as the first catalyst in the modification of a ribosomally produced peptide (87–89), (ii) the requirement for a previously unknown peptide chaperone (90, 91), and (iii) a final catalyst in the pathway that carries out an 8-electron oxidation within a single enzyme active site (92–96). Once again, an amazing cohort of graduate students and postdocs have contributed to these findings (Olafur Magnusson, Steve Wecksler, Jordan RoseFigura, Florence Bonnot, John Latham and Ian Barr) as well as numerous good-natured and talented collaborators with essential skills and interest in this tough project (Kimmen Sjolander, Carrie Wilmot, Steve Almo, David Britt). With some luck, the remaining years will be enough to get close to a full description of this series of biochemical transformations. With few precedents in eukaryotes, it is possible that one or more of the identified reactions on the PQQ pathway will offer up new directions for future antibiotic development, an area of urgent need with the increasing development of antibiotic resistance.

On Being a Woman in Science.

When I began my studies and later research in science, I never expected to be a professor, let alone at UC Berkeley. In fact, I never expected to be paid for my work. This may (and should) seem incomprehensible to most women scientists today, but in the 1960s I was simply grateful to have the opportunity to follow my strong interests and curiosity and to have an acceptable outlet for creativity that was distinct from my mother's pursuits as an artist! Luck has also played an important role in my career. The women's movement was emerging during this time as a powerful force, giving many of us a community and context from which to advocate for a footing equal to our male colleagues. The Fox Chase Cancer Research Center was, in retrospect, a "virtual hotbed" for women activists and during the 10 years I was there we advocated for and received equal pay and succeeded in overseeing the creation of a child care center. Many of the mainstays of women in science grew out of activities at Fox Chase, including an early chapter of the organization the Association for Women in Science (AWIS) and a training ground for future leaders in higher education (for example, Shirley Tilghman who would go on to become president of Princeton). I was very fortunate during this time to also have associates at the University of Pennsylvania, including Phoebe Leboy, an early and informed voice for women's rights, and Mildred Cohen, a brilliant and strong scientific colleague who would go on to become a mentor and friend.

With this background of forward thinking and colleagueship in Philadelphia, I was quite shocked at the extent to which UC Berkeley was a bastion of male privilege! When I first arrived at the University California I was asked to join a group of senior women who had been fighting their own battles, that included climbing through the windows of the campus faculty club since they were welcome there as wives but not as independent faculty members (hence, their strong support of a second, Women's Faculty Club on the UCB campus). One of, my main responses to the position I found myself on arriving at Cal in 1978 was to hunker down and focus on science and family. The main effect of this behavior was

considerable isolation and loneliness. Once again, the times produced what I very much needed – a group of women peers who had gathered together to hear and help each other in their professional and personal lives. Starting in 1980, I have gathered with them twice a month, and in the interim we have celebrated our successes, cried together over our losses and watched each other grow old. There is a book written about our group, “Every Other Thursday” published by Yale University Press in 2006 and authored by Ellen Daniell (97), one of the founding members. Since the book was published, many young women (and men) have thanked us for offering a blueprint for navigating the challenging waters of scientific careers.

Beginning in 1990, the National Academy of Sciences, which had also been a long-time male bastion, was beginning to elect an increasing number of women to its ranks. Some of the first women to be honored in this cohort were Christine Guthrie and Carol Gross, both members of our women’s group, and in the next year myself and a few years after Mimi Koehl. While I had barely heard of the NAS up to that point, within a short period of time half of the members of group were members. My own election led to an out-pouring of congratulations from friends and colleagues, a true highlight in my long journey through science, as well as an offer to move from the Department of Chemistry at UC Berkeley to MIT. This was a wonderful opportunity that I took very seriously, though the process unearthed a fact that was painful to confront: when asked to submit my current salary at UCB to MIT, so that a formal offer could be completed, I learned that my salary was much too low, in fact at or near the bottom of the entire chemistry faculty. Here was an important issue I had neglected, which was to ensure that I was being compensated in an appropriate and fair way.

In the end, after much soul searching I made the decision to remain at Cal, going on to become the first woman chair of a major chemistry department in 2000. Although I only served as chair for 3 years, I am very proud of the progress we made during that period, including the creation of an undergraduate major in chemical biology and the hiring of 8 outstanding new faculty members who span experimental physical chemistry, organic chemistry and biochemistry. My main regret is that none of the n faculty hired during my tenure were women, something I lament to this day. However, the gauntlet was successfully taken on by several of the subsequent chairs, one of whom, Michael Marletta, had been hired during my tenure.

As I continue to travel to scientific meetings, both in the US and internationally, it is common for women to come to me and to talk about their fears and the difficulties they encounter. Though so much progress has been made here and abroad, there are still many barriers, not the least of which is how to manage family creation at the same time as receiving tenure in an academic department. I try not be angry that it is still so hard and to be grateful for the progress in evidence as I write this chapter.

Notes to my Family.

I feel very fortunate that my life has led to an expansive family – and with lines between scientific (Figure 3) and personal family (Figure 4) often blurred. Many of the students and

postdocs who have graced my laboratory are mentioned above, with apologies to those who are not specifically named. Perhaps, the most important consequence of my willingness to take on the role of “first woman in physical sciences here at UCB” has been the chance to work with so many gifted, brilliant and courageous young people.

At the beginning of graduate school, my entire focus was on training for a career in science. My early first marriage and relocation from NYC back to U Penn seemed consistent with this trajectory, but this would change quickly when I discovered I was pregnant during my second year of graduate school. I had read “The Good Earth” by Pearl Buck and thought – if the women in China can give birth and go right back to their work in the fields – I could (somehow) combine my graduate school training with being a young mother. This cavalier philosophy enabled me to work up to a week before my first son (Andrew) was born and to return to work shortly after. Two and a half years after the birth of my first son, while pursuing postdoctoral studies at the Weizmann Institute, Andrew’s brother Douglas was born in Israel and, once again I returned quickly to work. Alas, from the perspective of being 76 years old, I ask myself, “What was I thinking?” The one major regret in my life is that I did not take enough time after my sons were born to enjoy the incredible sweetness and challenges of the early baby years. Of course, this is easier to say now than to accomplish in 1965–1967. There were no contingencies for women in graduate school and the strong bias was that it was better not to train women since they would leave the work force as soon they became pregnant. I had something to prove, but at a cost. In her book “Do Babies Matter: Gender and Family in the Ivory Tower” (98), Mary Ann Mason makes it clear how hard academic women with families must work and how often this does interfere with getting tenure. Published in 2013, this seminal work highlights the central importance of making science adaptable enough to accommodate women who choose to combine family formation with the pursuit of a high-powered research-focused career.

Had divorce not intervened in 1978, my family formation would have been complete, I would likely have lived the rest of my life in Philadelphia, and much of the narrative in this Perspective would not have taken place. The actual trajectory of my life tells a different story, showing that second (and third) chances really do happen in life. In 1980, I met Mordechai Mitnick, a grass roots organizer who later established a much sought-after psychotherapy practice in Oakland. After a fair degree of struggle and uncertainty, he and I made the leap to raise four children together (Alexandra, Joshua, Andrew and Douglas), each of whom has gone on to create meaningful lives and families of their own. This proliferation of family (currently eight grandchildren), at many times overwhelming, has been a bulwark supporting me throughout my almost 40 years of research and teaching at UC Berkeley. Some of my fondest memories from those early years include the line-up of lunch bags in the mornings, lovingly prepared by Mordechai for each of the kids and me, as well as the evenings around the kitchen table in which delicious vegetarian food was the backdrop for our family’s recounting of the challenges, successes and failures that we encountered each day. This “second chance life” that Mordechai and I were able to stitch together has also been enriched by our long-term commitment to the integration of meditative practices into our daily lives and a shared perspective on politics, literature and the arts.

Looking Back, Looking Forward.

From the vantage point of more than half a century since receiving my Ph.D., I am struck by how privileged the last 50 years have been for basic scientists. The end of WWII, combined with the spur to US science that came from the Soviet Union's launch of Sputnik, brought forth an extraordinary era of well-funded, curiosity driven research. I am grateful to have benefited from this largesse and an unbroken stream of federal funding for my scientific explorations. The 21st century's growing emphasis on applied science and engineering is a natural outcome of growing societal needs linked to rapid climate change and loss of species diversification. However, my early support of curiosity driven research, while cavalier and, in part, self-serving, has only grown stronger with the years: there really is no predicting where the next important shifts in understanding will occur that will alter the course and well-being of planet Earth. My own contributions to shifts in scientific paradigms, while acknowledged and awarded in my lifetime (99) (Figure 4), will likely be altered and surpassed by the next generation of scientists. But, for this to happen, there must be a societal commitment to continued scientific discovery. The full participation of women in science is a vital part of such forward thinking. In this area, I am actually encouraged by the emergence of a cadre of self-confident and full voiced women who are already changing both the face and content of science.

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Figure 1:
My sister and I on the street outside our home in post-WWII Philadelphia, 1948.

Figure 2:
The members of the Department of Chemistry at the University of California, Berkeley, shortly after I joined the faculty in 1978.

Figure 3:

Photograph of a lab reunion in Berkeley, on the occasion of my 70th birthday, April 16–17, 2011. My assistant, Mae Tulfo, 4th from the right in the first row, contributed immeasurably to this enterprise over a long period of time that extended from the 1990s to her retirement in 2014.

Figure 4:
Receipt of the National Medal of Science in 2014 from President Obama. Mordechai and I are flanked by our four children.