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Special Issue Preface

Round-Trip Journey of a Physical Chemist

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I was born and raised in Peking University (PKU), where my parents served on its faculty. PKU has possibly the most beautiful college campus in the world, where its views and vistas, each bit and every cranny, are the dearest to me. From PKU's affiliated kindergarten and primary and middle schools and then onward to college, most of my formative years took place on its picturesque grounds. At the awakening of China's economic reform, I went to the U.S. for graduate school and later became a Harvard professor. Riding the waves of technology revolution, I made my way from the cutting edge of experimental physical chemistry to new frontiers in biology and medicine, following the central theme of single-molecule and single-cell studies. My life took its shape from two different cultures—one that I inherited through my roots, the other where I spent most of my adulthood; and two universities—one that I grew up in and came back for, the other at which I enjoyed a fruitful career and my children were brought up. After spending more than three decades in the U.S., I chose to return to PKU, to pick up again my romance with this place: once the cradle of my beginnings, now the crest of my life's work.

FORMATIVE YEARS

My father, Youchang Xie, and my mother, Junying Yang, were then both teaching at PKU's department of chemistry. My childhood days were peaceful and idyllic until the "Cultural Revolution", when all normal activities were suspended at the university. I will never forget, in the dead of the night, when Red Guards marched into the houses of my neighbors to confiscate their property and abuse and insult them. As a young child I cowered in a corner trembling with fear, not understanding but knowing that everything had changed. Cut off from teaching and research, Father was deported for "reeducation through labor" camp hundreds of miles away from Beijing, where my mother, younger brother, and I had to stay behind.

Father returned home three years later. Having mastered the crafts of carpentry during "reeducation through labor", he made a spinning top specially for me. This exquisitely crafted, endlessly spinning toy sparked my curiosity. The first woodwork I crafted using Father's toolbox was a level scale—this was the first precision measurement tool I designed in my life! This hobby proved to be irresistible—I began making model airplanes, superheterodyne receivers, remote-controlled model ships, and a set of loudspeakers. My interest in experimental science thus began, and my calling in life was revealed to me—I would become a scientist.

Grandparents were sadly missing from my childhood. Father's parents passed away while I was very young. Mother's parents were educated in the U.S. and came back to China in the 1940s to teach at Sichuan University. After the founding of the People's Republic, they returned to America by way of Hong Kong, while Mother, then a patriotic undergraduate at Fudan University, stayed behind. Mother did not see her parents for more than 20 years, only until after Nixon's visit to China in 1972. Our family reunion was an emotional scene when my grandmother came to Beijing to visit us. It was then that I finally got to meet her for the first time.

By the time I entered PKU's Affiliated High School, life had returned to normal. Besides formal education, we also enjoyed colorful extracurricular activities. I was an ace spiker of our class volleyball team. I had also grown fond of classical music and became obsessed with inventing speakers with improved acoustics. My parents were finally able to return to their beloved teaching and research. Father wrote a college textbook on *Structural Chemistry*, while his work on monolayer dispersion eventually led to his life-long contributions to the commercialization of CO and O_2 absorbents.[1] Mother devoted herself to teaching and was loved and respected by her students. By their examples, I too felt the attraction in teaching and research.

The National College Entrance Examination was reinstated during my high school years. I was admitted to Peking University in 1980. PKU is a heavenly institution for learning with high scholarship. I immersed myself in absorbing all fields of knowledge. My major was chemistry, but I also audited classes from other departments, including classical mechanics, quantum mechanics, statistical mechanics and electrodynamics, electronics, and statistics. I would be richly rewarded for these efforts later on.

I was fortunate to do my thesis under Shengmin "Sunny" Cai, an electrochemist. A superb experimentalist as he was, Sunny had a wide range of interests and a great sense of humor and spoke fluent English. His academic guidance to me had a profound influence on my career. He was good at using vivid language to illustrate complex and abstract concepts. My thesis

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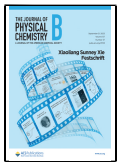

was on computer-controlled photoelectrochemical reactions, which involved lock-in amplifiers. I can still recall clearly how Sunny explained the working principle of lock-in amplifiers.

In the process of working on my thesis, I began to realize the importance of innovation in instrumentation to experimental science. This would be proven further in my own scientific career. My childhood, youth, and college years in PKU had nurtured in me the spirit of innovation and made science my life's true calling.

GRADUATE SCHOOL IN THE U.S.

Back in the 1980s, China was very much behind the rest of the world in science and technology; thus, I decided to go overseas for graduate school. I was admitted to the University of California at San Diego (UCSD). My generation was much more fortunate than that of my parents. In 1984, the year I graduated from college, PKU students held a banner that said "Hello, Xiaoping" during a parade on Tiananmen Square celebrating our 35th National Day. It was the salutation from the bottom of our hearts.

At the age of 23, I bid farewell to PKU for the first time and left for America, beginning a new chapter in my life. Coincidentally, my father was then a visiting professor in Gabor Somojai's lab at UC Berkeley. Before starting at UCSD, I stayed in the Bay Area with Father, my grandparents, and other relatives for a few months. I also met the flamboyant Somojai at his home party. Gabor left Bulgaria for the U.S. during the Cold War, rising from a graduate student to a world leader in surface chemistry at Berkeley. He said, "Welcome, young man, to the U.S.! There are many opportunities here! If you do well, you will have better opportunities, and if you continue to do well, you will have even better opportunities." He then winked and said, "There would never be an end." I remembered his words but only realized years later what they truly meant.

At UCSD, I chose to work under John Simon, studying chemical reactions with ultrafast lasers. John was a rising star in that field and eventually became President of Lehigh University. Being his second graduate student, I got to spend a lot of time with John in the lab. It was the early days of ultrafast lasers—he could always get quitting lasers back to work after hours of tweaking. I learned from him to build my confidence in the lab, a necessary trait for any experimental physical chemist. John is a prolific writer and coauthored two books during my time at UCSD. I did the proofreading for one of them, "*Physical Chemistry—A Molecular Approach*", coauthored with Donald McQuarrie. My first ever publication was a JPC paper,[2] and it happened like this: Following John's suggestion, I was studying flash photolysis of chromium carbonyl. One night, I got exciting results and left the data on John's chair before heading home in the early morning. When I came back after a short nap, John was waving a stack of papers at the end of the hallway: "Sunney, here is your first paper!" I insisted that John be the first author; after all, I owed him everything I learned from the beginning.

I had the pleasure of interacting with Kent Wilson, a distinguished physical chemist. Legendarily, he supported his large research group to conduct cutting-edge ultrafast study of molecular dynamics with money he earned in the stock market. For my qualifying exam, Kent picked a theoretical paper on wave packet dynamics by Rick Heller (who later became my colleague at Harvard). I worked hard to understand the paper. After the exam, Kent complimented my good comprehension of the material but cordially advised me to work on my communication skills. Apparently, my presentation was lost on the other two members of the committee. He told me, "The quality of your science, and even your life, is dependent on the quality of your communication." I took his advice to heart.

I later proposed and developed a novel technique for picosecond time-resolved circular dichroism (CD) to study conformational dynamics of biological macromolecules.[3] John was super supportive, for which I am forever grateful. I made use of the lock-in amplifier introduced to me by Sunny Cai. My way of convincing John to buy any equipment for the project was always to get a loaner first and then test it; if the experiment worked, he would have to pay for it. I applied time-resolved CD to study photodissociation of carbon monoxide from myoglobin[4] and the photosynthetic reaction center.[5] The latter was done in collaboration with George Feher, an esteemed biophysicist at UCSD, whose biophysics course I very much enjoyed. These projects eventually became my doctoral thesis. My thesis committee consisted of John Simon, Kent Wilson, George Feher, and Bruno Zimm, another renowned biophysicist I admired. Picosecond CD was my baby, so much so that I thought that I might not be able to do anything better. However, John did not think so. He said to me, "You must do something new as a postdoc to broaden your scope."

POSTDOCTORAL EXPERIENCE

Perhaps the picosecond CD work earned me a postdoctoral position with Graham Fleming, a world leader in chemical physics and ultrafast spectroscopy, then at the University of Chicago. In our first meeting, Graham asked me, "What do you want to do here?" Full of ideas about time-resolved CD but adhering to Simon's advice, I told Graham I wanted to learn sensitive time-resolved fluorescence measurements as well as nonlinear spectroscopy in his lab, which would lay the ground for my future work. In the end, the most important thing I learned from Graham is to seek quantitative understanding of chemical and biological phenomena.

At the University of Chicago, I developed a new technique for femtosecond time-resolved fluorescence measurements using a state-of-the-art mode-locked dye laser system constructed by Norbert Scherer and Tony Ruggiero, two fellow postdocs in the lab. We needed to spend most of our time tweaking the dye laser. Fortunately, I witnessed the emergence of passively mode-locked Ti:sapphire laser technology. I persuaded Graham to arrange a loaner laser from Coherent. I wasted no time on tweaking during the two-week loan, and we were able to measure primary electron transfer kinetics of the bacterial reaction center for the first time, in collaboration with Jim Norris.[6] I completed the work in weeks instead of years that it would have previously taken. As a young scientist, it was an eye-opening experience on how technological advances could transform the way we do science.

The challenge of the postdoctoral stage is to learn and master a new field, at the same time trying to figure out what to do next. Because I was learning photosynthesis from Graham and Laurens Mets, a plant biologist at University of Chicago, I followed Eric Betzig's development of near-field microscopy[7] and thought of using it to map the photosynthetic membrane, or to probe electron transfer in a single molecule of the reaction center.

I got an interview at Pacific Northwest National Laboratory (PNNL) situated at the Hanford Nuclear Reservation in

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Washington State, a site highly polluted from plutonium production during World War II and the Cold War era. U.S. Department of Energy (DOE) had invested $250 million to establish a new facility at PNNL, Environmental Molecule Science Laboratory (EMSL), and Steve Colson, a renowned physical chemist, left Yale to lead its building effort. He envisioned EMSL as a facility for integrative fundamental research and began the search for an ultrafast spectroscopist. Simon was their first choice, but he turned down the offer. During my interview, Colson asked me, "If you were given unlimited resources, what would you do to make a difference?" I proposed to use a near-field microscope to image single molecules. As a result, I was hired to build an ultrafast lab while attempting the single-molecule work on the side.

NATIONAL LAB CAREER

I arrived at PNNL in 1992. Bob Dunn joined me as my first postdoc—he was also Simon's graduate student at UCSD but three years behind me. Single-molecule spectroscopy had only been carried out at cryogenic temperatures by W. E. Moerner and Michel Orrit. It was a very exciting time as several labs around the world were racing to image single molecules at room temperature. Neither Bob nor I had any previous experience on vacuum techniques, which was needed to make the near-field probe, so we turned to Eric Betzig for help. Eric generously shared his know-how with us. Richland is a small and isolated town with few distractions. In fact, we never took a weekend off during our first year. Bob's optimism and sense of humor were very helpful to our later success. Each time our group had a paper, we would celebrate at a local bar three times, first for submission, then acceptance, and finally publication.

Also joining us as a postdoc was Lukas Novotny, with dual citizenship of the Czech Republic and Switzerland, whom I first met at a conference in Brno. Lukas had a background in electrical engineering; his understanding of electromagnetism was crucial to the problems we encountered in the early days of near-field microscopy.[8] I knew he would become a good experimentalist when Lukas brought along a fancy Geiger counter during apartment hunting at Hanford. He also graciously provided a classy Italian espresso machine, La Cimbali, to the lab for all of us to enjoy. Impressively, Lukas had already began writing in his spare time the now popular textbook "*Principles of Nano-Optics*".[9]

In October of 1993 at the Optical Society of America's annual meeting in Toronto, Betzig reported the first single-molecule fluorescence image at room temperature.[10] As developer of the near-field microscope, there was no one more deserving than him to win the race. I had lunch with Betzig and congratulated him. Then I called Bob—we had been working tirelessly toward the same goal, so obviously he was also disappointed. Steve Colson consoled us, "Why does it matter? It's only one technique and there will be others. What's important is what you do with the technique."

The next day at the same meeting I reported our result on single light-harvesting complexes with six chromophores instead of one.[11] We thought that six could be easier to detect. When Bob replaced with a single chromophore sample like Betzig's back at PNNL, he found instead that the signal was much stronger due to the lack of complicated photochemistry in the light-harvesting complexes. A few months after Betzig's milestone paper, our *Science* paper on probing single-molecule dynamics at room temperature was published.[12] Shortly after this work came out, Bob became a professor at the University of Kansas (where he is the current chair of its chemistry department). As Colson rightfully predicted, far-field techniques soon offered an easier way than near-field for single-molecule imaging at room temperature, opening the door to a broad range of applications in biology. Even super-resolution microscopy beyond the diffraction limit was eventually accomplished by far-field optics, pioneered by Stefan Hell, Eric Betzig, and Xiaowei Zhuang (she did the work at Harvard where she was my colleague and friend).

Peter Lu, another postdoc of mine at PNNL, and I embarked on single-molecule enzymology together with Luying Xun, a biochemist at Washington State University. The choice of studying flavin enzyme came to me when I was riding alongside Luying on a snowy mountain slope in the Pacific Northwest, where I picked up my lifelong passion for skiing. In 1998, we reported real-time monitoring of catalytical cycles of a single-enzyme molecule of cholesterol oxidase by fluorescence detection.[13] A chemical reaction of a single molecule occurs stochastically; i.e., the waiting time required for the reaction to complete is randomly distributed instead of predictably, as conventional experiments usually involve a large number of molecules. Nevertheless, the statistical properties of the waiting time are predictable, and we showed this experimentally for an enzymatic reaction.

This work was regarded as groundbreaking for single-molecule enzymology, offering the basis for commercialized single-molecule DNA sequencers, later developed by Steve Quake's, Watt Webb's, and Hagan Bayley's labs. In addition, we also discovered the dynamic disorder phenomenon of individual enzyme molecules, i.e., the enzymatic rate constant of a single molecule is actually not a constant but fluctuates over time, which has since been proven to be a general phenomenon. Because of this work, Peter later became an endowed professor.

Bearing in mind that I was hired by PNNL to build an ultrafast lab, we eventually developed a label-free Raman imaging technique. Our initial objective was to do a photon echo experiment in the chemically interesting infrared region (IR). Gary Holtom, a laser expert at PNNL, and my postdoc Rob Crowell were going to build a Ti:sapphire laser system with a 100 kHz Ti:sapphire regenerative amplifier for the project. While Gary was constructing the laser, Robin Hochstrasser's and Michael Feher's groups claimed IR echo. When Gary finished the laser, nice as it was,[14] I could not deliver the IR echo experiment because it did not provide high enough peak power. This gave me a lot of headaches as all investments were about to go in vain.

We ventured into nonlinear optical imaging by serendipity. When Andreas Zumbusch, a postdoc from Germany, focused two laser beams (ω_1 and ω_2) from the laser onto a piece of glass, we observed the glow of white light, which turned out to be the linear combinations of ω_1 and ω_2. We decided to use the signal $2\omega_1 - \omega_2$ as the contrast mechanism, which is the well-known coherent anti-Stokes Raman scattering with resonances occurring when molecular vibrational frequencies match $\omega_1 - \omega_2$. Conventional Raman spectroscopy was used to measure characteristic vibrational frequencies of molecules, but the signal is extremely weak and required a long signal averaging time for imaging. We reported for the first time that raster scanning the foci of two collinear laser beams made 3D nonlinear Raman imaging possible.[15]

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In retrospect, the national lab experience was very special to me. I do not think an academic setting would have enabled my lab to make the same technological achievements we did at PNNL. Technological advances often help young scientists to jump-start their careers. People began to ask whether I would consider a move to academia. Meanwhile, I was also looking for new challenges. In particular, I wanted to apply the single-molecule approach to biology in a more extensive way. At the age of 36, I was offered a full professorship by the Department of Chemistry and Chemical Biology (CCB) at Harvard University, becoming the first tenured professor recruited by Harvard among Chinese scholars who came to the United States since China's reform and opening-up in 1978.

Erik Sánchez, my first graduate student during my affiliated appointment at Portland State University, was the only one from PNNL to follow me to Harvard. Erik is a very interesting experimentalist. His relocation was more challenging than mine because he needed a storage place for the valuable equipment that he had been collecting for years. I asked him to be my postdoc, and he had only one condition—getting the same La Cimbali espresso machine as Lukas' at our Harvard lab. Of course, I said yes.

TENURE AT HARVARD

The CCB department sparkled with the luster of brilliant minds, yet its atmosphere is very collegial. During my interview, Jim Anderson, then Chair of the Department, and Charles Lieber were my hosts, and later my close friends. Jim is known for his experimental proof of the mechanism by which chlorofluorcarbons create the polar ozone holes, and Charlie is a world leader in nanoscience and technology. E. J. Corey, Nobel laureate in organic chemistry, listened attentively to my job talk. He praised our work but kindly pointed out that I made a minor mistake about the structure of cholesterol. Jeremy Knowles, a prominent enzymologist and then Dean of Harvard's Faculty of Arts and Sciences, was very keen on our single-enzyme study. A Harvard senior faculty offer comes with a decent personal package, a brand-new laboratory, and a generous start-up package. Of course, expectations also ran high. I asked Jim Anderson how much research funding each CCB faculty member raised on average. The answer was $1.3 million. I was intimidated by the figure as I never had to apply for a grant at PNNL, and I did not want to fall below the average. I told Charlie Lieber about this. He kindly consoled me that he had the same fear when he first moved from Columbia. It turned out my fear was indeed unnecessary. I wrote six proposals upon joining Harvard, and luckily, I was awarded all six—my preliminary results at PNNL were most helpful.

CCB rarely gave tenure to its assistant professors at that time. I was fortunate to start as a full professor, skipping the grinding tenure process. To Jim, the department chair, faculty recruiting is probably the hardest part of his job, with low odds of success, especially when other top universities were also in fierce competition. I was Jim's first hire. Jim's legacy was his successful recruitment of many junior faculty members who would go on to become superstars, including Xiaowei Zhuang, David Liu, Hungkun Park, David Reichman, and Alán Aspuru-Guzik. I helped Jim to recruit Xiaowei Zhuang, and subsequently, Xiaowei and I helped to recruit Adam Cohen. They all became leaders of their own fields and received tenure because of their original contributions, which were distinctly different from their mentors. At CCB, it was an unwritten rule that assistant professors should not remain in their mentors' previous paths. Had I stayed in the ultrafast field, I certainly would not have been recruited by Harvard.

Another of Jim's accomplishment as our chair was space renovation for CCB. During the process, Dudley Herschbach, a Nobel laureate in physical chemistry, kindly offered his lab space to me. Dudley told me that he "inherited" the space from his mentor E. Bright Wilson, a student of the great Linus Pauling. Dudley pioneered molecular beam study of gas phase chemical reactions, for which he and his former postdoc Yuan T. Lee received the Nobel Prize in Chemistry. Their molecular-beam machine "Hope" was built in the exact location that became my Harvard office, with a ceiling full of signatures left by students at moments of exciting discoveries. I made a plaque of "Hope" in the hallway to honor Dudley's achievements. For my 20 years in that office, I felt the innovative spirit permeating through its walls.

The first project I launched was single-molecule enzymology. Postdoc Haw Yang developed a single-molecule electron transfer experiment, similar to what I originally envisioned. He observed conformational fluctuations on a broad range of time scales,[16] which give rise to the dynamic disorder phenomenon on single enzymes. As such, my graduate students Wei Min and Brian English, together with collaborators, realized that the fundamental Michaelis–Menten equation still holds at the single-molecule level and proved experimentally the validity of the single-molecule Michaelis–Menten equation.[17] In collaboration with my friend Hong Qian at the University of Washington, we realized that, different from conventional chemical kinetics, our single-molecule enzymatic turnover experiments are in the nonequilibrium steady condition[18] with a constant free energy supply and consumption, which mimics the condition in a live cell.

Single-molecule enzymology, like enzymology in general, is critically dependent on assay designs. Postdocs Antoine van Oijen and Peng Chen and graduate students Paul Blainey, Sangjin Kim, Xiaolin Nan, Shasha Chong, and Chongyi Chen contributed to many of our new single-molecule assays, both *in vitro* and *in vivo*, among which two led to fundamental biological discoveries—one was allostery through DNA: two DNA binding proteins nearby can affect each other's binding affinity in a surprising way;[19] the other was a ubiquitous phenomenon of transcription, which exhibits transcriptional bursts under induced conditions and is associated with DNA supercoiling in looped DNA.[20]

While at Harvard, I had the great pleasure to collaborate and interact with many stimulating theoreticians, among them Martin Karplus, Peter Wolynes, Shaul Mukamel, Yossi Klafter, Hong Qian, Attila Szabo, Biman Bachi, Eugene Shakhnovich, James Hynes, Binny Cherayil, Jun Liu, Sam Kou, Jianshu Cao, Hao Ge, and Yiqin Gao. I can still recall that Martin Karplus was shuttling between Harvard and Strasbourg (France), and he would stop by to find out our new progress whenever he was in town. He continued to do so even after receiving the Nobel Prize in Chemistry in 2013.

When I first arrived at Harvard, I had a hunch that the single-molecule approach would make an impact on molecular biology, but first I needed to learn the subject myself. Together with my students, I audited a molecular biology course at Harvard taught by Richard Losick. Rich showed us cartoon movies on mechanisms of RNA polymerase and ribosomes, the players in the central dogma of molecular biology—the production of proteins according to genetic information

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encoded in DNA. As I listened to his lively lectures, my mind was planning how to monitor gene expression in a live cell in real time. In a living cell, DNA exists in the form of single molecules; therefore, gene expression must occur stochastically, just like a single-enzyme molecule in nonequilibrium steady state.

In 2006, after three years of hard work, our two "debuts" in the field of molecular biology were published simultaneously in *Science* and *Nature*. The papers reported for the first time real-time observation of protein production one molecule at a time in a bacterial cell and described at a quantitative level the central dogma of molecular biology.[21,22] Long Cai, a Harvard undergraduate working with Dudley Herschbach, was my first graduate student to head fearlessly in this direction. He and Nir Friedman, an Israeli postdoc, developed an elegant theory for the copy number distribution of a particular protein,[23] which they experimentally verified simultaneously with postdocs Jie Xiao and Yu Ji by two different experiments.[21,22] In a different direction, Swedish postdoc Johan Elf and graduate student Gene-wei Li achieved the live cell study of facilitated diffusion of transcription factors,[24] which was further investigated by graduate student Paul Blainey (jointly supervised with Greg Verdine) and Professor Biman Bachi, then on sabbatical with us.[25] Postdoc Yuichi Taniguchi and graduate students Paul Choi and Huiyi Chen carried out system-wide studies by constructing a genome-wide YFP library.[26] The significance of our work was evident when Rich Losick began using our real-time movies to explain gene expression in his class, according to new students who attended the class—I was most delighted.

One week after the publication of our two papers in 2006, I received a call from the Gates Foundation inviting me to apply for a grant, as they hoped our new technology might be useful in understanding why a small subgroup of tuberculosis-causing bacteria cells was showing drug resistance—tuberculosis was claiming the lives of millions of children in Africa each year. In the following year, Bill Gates, "the most successful college dropout", was awarded an honorary doctorate by Harvard and gave a most moving commencement address. When Gates visited my lab, I was impressed by his knowledge on molecular biology—I reckoned he too must have learned it on his own. What was unexpected was his familiarity with the ultrafast lasers used in our experiments. Though we have yet to solve the problem of drug resistance in tuberculosis, the Gates Foundation experience made me wonder: could the advances we make in fundamental science be used to benefit society?

The completion of the Human Genome Project in 2001 is a milestone in mankind's history. After 2006, I witnessed a technological revolution, this time of a new generation of sequencers reducing the cost of DNA sequencing at a rate faster than exponential decay. Former Harvard postdoc Hongye Sun, who was leading the single-molecule sequencing effort at Applied BioSystem (ABI), invited me to consult for them, and I later joined ABI's scientific advisory board (SAB). This was an eye-opening experience for me. The tremendous potential of next generation sequencing (NGS) for personalized medicine was so obvious and exciting yet well in line with our forte. Incidentally, when ABI was sold to Life Technologies, its SAB was dismissed by the management. Freed from SAB's restrictions, my group thus began to make the transition to genomics.

The biggest challenge for any researcher is to develop and maintain their ability to innovate. Any research field that was once active may eventually become saturated, or even outdated. As a result, a scientist usually has to explore new fields during his or her career. When it comes to an experimental physical chemist, the difficulty of a field change would be having enough resources to set up new laboratory equipment. I was very fortunate to have been funded twice by the National Institutes of Health (NIH) Director's Pioneer Award, which supports high risk research projects with the potential of high return. This allowed me to buy three DNA sequencers from different manufacturers within a period of two years, each of them going obsolete within six months after purchase.

After much trial and error, graduate student Peter Sims, postdocs Will Greenleaf and Haifeng Duan, and I finally invented a new chemistry of DNA sequencing, "fluorogenic sequencing", and reported a self-made DNA sequencer in 2011.[27] With Illumina's monopoly on NGS, America did not seem to need another sequencer. Peter and Will became professors at Columbia and Stanford, respectively, and Haifeng Duan later joined my PKU colleague Yanyi Huang's lab. The fluorogenic DNA sequencer was further developed and eventually commercialized by Yanyi and his students, particularly Zhitian Chen.[28] Over time, the advantages of fluorogenic sequencing have become more and more tantalizing.

Mainly because of NGS, another technological revolution, single-cell genomics, was emerging on the horizon. In 2009, Jim Eberwine of University of Pennsylvania and I co-organized the symposium "Single Cell Analyses" at Cold Spring Harbor. Jim began to embark on single-cell transcriptomes before NGS even became available. Also joining the meeting was Kaiqin Lao of ABI, a friend I met when we were both at the University of Chicago. Kaiqin told me about his work on single-cell transcriptomes in collaboration with Fuchou Tang, then a postdoctoral fellow in the lab of Azim Surani at Cambridge University.[29] My former student Paul Blainey, at the time a postdoc working in Steve Quake's lab, also gave a talk about genome sequencing of single bacterial cells. I was curious and began pondering on the possibility of sequencing a single human cell, and I realized that the challenge lay in the single-cell whole genome amplification (scWGA). At that time, the Nobel winning PCR technique was an exponential amplification that had single copy sensitivity but strong sequence-dependent bias, yielding a whole genome coverage of only ~5%.

As soon as I returned to Harvard, I launched a new project of developing scWGA. New postdoc Chenghang Zong, a former graduate student of Peter Wolynes, immediately took on the challenge. In 2012, with the help from my graduate students Sijia Lu and Alec Chapman, Chenghang developed a quasi-linear (hence more even) scWGA method called MALBAC (Multiple Annealing and Looping-Based Amplification Cycles), which allowed for more accurate DNA sequencing of individual human cells.[30] The name was a play on the red wine *Malbec*.

I then realized the potential of a newly available TN5 transposase for single-cell genomics. In 2017, my graduate students Chongyi Chen and Longzhi Tan and postdoc Dong Xing made use of it to develop a truly linear and even more accurate scWGA method, which we christened Linear Amplification with Transposase Insertion (LIANTI),[31] named after another red wine. Using a similar method, Longzhi and Dong successfully determined the 3D structure of

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the six-billion bases of DNA in a single human cell in 2018,[32] 17 years after the 1D human genome sequence was first determined. This was not done via a conventional imaging technique but by a WGA method and an algorithm to impute paternal and maternal chromosomes in a human diploid cell. The 3D structure of the genome is important because it determines cell functions. Longzhi's Ph.D. thesis won *Science* magazine's annual Science and SciLifeLab Prize for Young Scientists, the Grand Prize for Genomics, Proteomics and Systems Biology in 2019. Meanwhile, Yanyi and collaborators used TN5 transposase to develop SHERRY for single-cell transcriptome, and my graduate student Yunlong Cao used it to develop CABERNET for single-cell methylome, adding to our toolbox (and wine cellar) for single-cell genomics.

Coherent Raman microscopy also flourished at Harvard and has become a field of its own, thanks to generations of my students, postdocs, and collaborators, including Ji-xin Cheng, Andreas Volkmer, Eric Potma, Conor Evans, Wei Min, Chris Freudiger, Brian Saar, Peijun Cong, Dan Fu, Dan Orringer, Mingbiao Ji, Frank Lu, and Wenlong Yang. Unlike the previous situation for near-field microscopy, we were able to quantitatively design the experiment based on our understanding of coherent Raman theory. Two technological highlights were the development of stimulated Raman scattering microscopy[33] and the realization of video rate imaging.[34] For seven consecutive years, we organized hands-on coherent Raman summer workshops open to the entire world, which were extremely well received. I am delighted that coherent Raman microscopy is now used in brain surgery to distinguish tumor margins in a less time-consuming and labor-intensive way than conventional H&E staining cryosection.[35] Our coherent Raman imaging work produced 5 Harvard Ph.D. theses and helped 10 Xie group members to start their faculty careers around the world. I recall that, when I first came to Harvard, a new graduate student asked me if she could take on coherent Raman imaging as her thesis project. Afraid of uncertainty, I advised her against it and gave the project to more experienced postdocs instead. People sometimes think I am bold when moving to new fields; looking back, I only wish I was even bolder.

NEW LIFE AT PKU

After settling down in America, each time I traveled back to China I was always amazed and moved by its tremendous transformation. Thanks to the Economic Reform and Opening Up policy, China has experienced unparalleled growth and development in the past 40 years. When I returned to Beijing to watch the 2008 Olympics with my kids, I was heartened by the Chinese national team winning the most gold medals. However, I knew China still had a long way to claiming gold in the scientific arena.

I became a visiting professor at Peking University in 2001. Two former PKU presidents, Jianhua Lin and Enge Wang, together with Yi Rao, then Dean of the PKU School of Life Sciences, persuaded me to open a lab at PKU. In 2010, along with Professors Xiaodong Su and Yanyi Huang, both returnees and PKU alumni, I proposed to our alma mater to build the Biodynamic Optical Imaging Center (BIOPIC). Although we did not know each other before, our shared dream for BIOPIC brought us together. BIOPIC was inaugurated in December 2010 with the vision of establishing a biomedical research institution empowered by technological innovations and cross-integration of multiple disciplines. It was renamed to Biomedical Pioneering Innovation Center in 2018, during the PKU presidency of Ping Hao. BIOPIC's scientific achievements have led to the birth of seven start-up companies and significant impact to the betterment of human health.

BIOPIC's not so humble beginning was a prefab building nicknamed the "Little White House", elegantly designed by Yanyi Huang. Yanyi organized the eighth coherent Raman summer workshops there, an absolute blast. BIOPIC's SAB was chaired by Tom Maniatis, a renowned molecular biologist, my colleague and friend who left Harvard for Columbia and is currently the Director of NY Genome Center. BIOPIC has attracted an assemblage of outstanding talents from abroad. Fuchou Tang was the first scholar I recruited from Cambridge University. He has since become an authority figure in the field of single-cell genomics. Zemin Zhang joined BIOPIC from Genentech of the San Francisco Bay Area. A leading cancer immunologist, he eventually succeeded me as BIOPIC's Director. Wensheng Wei, a molecular biologist, is now a star in gene editing. Yiqin Gao, a theoretical chemist returnee who had two Nobel laureates as mentors, Martin Karplus and Rudy Marcus, returned to PKU after his professorship at Texas A&M and is now a leading expert on combining machine learning with molecular dynamics. Hao Ge, BIOPIC's sole mathematician, joined us after his Harvard sabbatical with me.

In the first three years, in order to set a standard for high quality work at BIOPIC, I collaborated on a major paper with each BIOPIC PI and also my two co-PIs at the time, Fan Bai and Yujie Sun. Perhaps the most intellectually stimulating collaboration was among five PIs on studying allostery through DNA,[19] which earned six commentaries on Faculty of 1000. Yiqin Gao's participation was quite unusual. After hearing of our paper being accepted by *Science*, Yiqin pointed out to me that my interpretation of the phenomenon might be problematic. He proposed to do a molecular dynamics simulation to confirm. I called the *Science* editor to delay the publication, and Yiqin's hunch proved to be correct.

The experience of running a research center was quite different from running a lab. In a BIOPIC SAB meeting, Tom Maniatis, with his incomparable scientific and administrative wisdom, quipped that I was "too scary" running everyone else's groups instead of driving my own science. He advised me to focus on building up my own PKU group instead. I did so painstakingly after Fan Bai and Yujie Sun became independent BIOPIC PIs, and it was indeed a rewarding process.

Back-to-back with our MALBAC paper in *Science* was my first collaborative work with BIOPIC colleagues, sequencing of individual sperm cells.[36] Sijia Lu, the first author, was a mischievous Harvard graduate student—he was more interested than his advisor in finding out whether his advisor's sperm was healthy. For any normal male regardless of age, 5% of his total sperm count shows abnormal copy number variation that may lead to genetic diseases or reproductive disorder. The probability of such genomic abnormality is much higher in the female counterpart, oocyte, and increases drastically with age. On the other hand, there are ~6000 known monogenic diseases. The disease-causing mutation has a 50% chance of being passed to the next generation, which used to be left entirely up to fate!

In collaboration with Jie Qiao's group at Peking University Third Hospital, Fuchou Tang's group in BIOPIC, Sijia Lu of Yikon Genomics, and our group (led by Lei Huang) demonstrated the use of MALBAC to prevent transmission of monogenic diseases to offspring and to simultaneously avoid

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abnormal chromosome numbers through preimplantation genetic screening, in a much more precise way than previous methods.[37]

In early 2013, a man in Beijing wrote to me and asked if we could help him and his wife have a healthy baby of their own. After being turned down repeatedly by medical institutes around the world, he learned of our latest technology from a friend. Suffering from bone tumors on his joints, he had to undergo surgery every three to five years during his childhood. His own NGS result showed he is a carrier of a known hereditary disease called hereditary multiple exostoses (HME), because of a point mutation at the EXT1 gene inherited from his father. Meanwhile, his wife, though in good health, was 36 years old, with increased risks of chromosome abnormality. Touched by his fighting spirit and impressed by his thorough homework, we immediately admitted the couple as Case 1 of Jie Qiao's clinical trial.

China's first successful IVF baby was delivered by Dr. Lizhu Zhang, Jie Qaio's mentor, ten years after the birth of the world's first IVF baby Louise Brown. This time, Jie Qiao was well ahead of the world on preimplantation genetic screening through combination of NGS and scWGA. I will never forget how thrilled I was on September 19, 2014, when we went to see the first "MALBAC baby". Healthy as she was, she did not cry at all but smiled at me. The satisfaction was beyond words when I recently received a handicraft from her on Teacher's Day. She is now 9 years old.

This achievement epitomized precision medicine in action. To date, Yikon Genomics, led by Sijia Lu, has successfully helped more than 4000 couples in China avoid passing their monogenic diseases to their children. In recent years, we have succeeded in making the technique noninvasive—sequencing embryonic DNA leaked into the spent culture of each embryo and hence selecting viable embryos without the risks of biopsy.[38,39]

After 20 years at Harvard, including eight years of shuttling between PKU and Harvard with one or two trips per month, I relocated to PKU full-time in July of 2018, bridging my past with my future. Many people asked me why. It was by no means an easy decision but involved many considerations, such as my love for my parents, my alma mater, the motherland, and so forth. What really tipped the balance, in the end, was science. The research opportunities for me were simply better in Beijing, which is a true reflection of what China has achieved during my years spent in America.

I am forever indebted to many of my CCB colleagues for their support. In my 20 years there, I looked up to the highest standards set by them, and they nurtured and inspired my growth both as a scientist and a person. Needless to say, such inclusiveness was indispensable to America's current dominance in science and technology. One only wishes for this American character to prevail in the future.

Not long after my returning and settling down in Beijing, the COVID-19 pandemic broke out. When Wuhan was locked down on January 2020, I was attending the World Economic Forum in Davos, Switzerland. With a hunch that single-cell genomics might be useful in fighting against the pandemic, I took an earlier returning flight back to Beijing, landing on the third day of the Chinese New Year.

Arriving on campus at the same time was Yunlong Cao, while the rest of my PKU lab was quarantined at home. Yunlong was one of my final three Harvard Ph.D.'s and the only Harvard lab member to join my PKU group. We talked to many experts and learned that each B cell from convalescent patients' peripheral blood contains a specific DNA sequence for producing a unique antibody. I came to the realization that our expertise in single-cell genomics would allow us to quickly identify potent neutralizing antibodies (nAbs) against the SARS-CoV-2 virus and mass produce them *in vitro* based on their sequences. On February 2, 2020, I visited YouAn Hospital, the designated COVID-19 hospital in Beijing, and its head Dr. Ronghua Jin immediately agreed to collaborate and provide blood samples of convalescent patients. The following months were most exciting and memorable. Suffice to say that I bore witness to the sense of duty and dedication of young scientists in China. Within three months, our team was able to identify highly potent SARS-CoV-2 nAbs.[40]

Like all the other commercial nAb drugs, ours were soon escaped by new SARS-CoV-2 variants (meaning they could no longer recognize the virus), especially Omicron.[41] As such, Yunlong, along with my graduate students including Fanchong Jian and Ayijiang Yisimayi, as well as our collaborators first reported that the Omicron variant could escape 85% of humoral antibodies from previous variants.[42] The sad fact that all our investment went in vain roused us to develop a high-throughput yeast display-based deep mutational scanning (DMS) platform to study how a particular antibody can be escaped by each of ∼4000 possible single amino acid mutations of the virus' receptor binding domain, and we now have done so for ∼6000 SARS-CoV-2 nAbs. This allowed us to understand and classify the antibodies. Our goal was to find "broad spectrum" nAbs which are difficult to be escaped.

All hope is not lost, as we identified a broad spectrum nAb SA55 from SARS recovered patients who had received SARS-CoV-2 vaccines,[43] which can neutralize all SARS-CoV-2 variants to date. The SA55 nAb has been licensed to Sinovac and is undergoing clinical trial. During the outbreak in Beijing at the end of 2022, SA55 effectively served thousands of people by compassionate use for both prevention and treatment and saved many lives. SA55 has proven to be difficult to escape by future variants. At the time of finalizing this autobiography, the latest EG.5 and BA.2.86 variants once again escaped big pharmas' newly produced "broad spectrum" antibody drugs, while SA55 again survived.

All this happened as I was stepping into a new role as Director of Changping Laboratory, a newly founded institution focusing on fundamental and translational biomedical research that addresses national and global challenges, while maintaining my professorship at PKU. Our top priority was of course fighting against COVID. I took pride in the formidable team of scientists I assembled, including Yunlong Cao, Ronghua Jin, Youchun Wang (viologist), Chuan Qin (animal trial expert), and Junyu Xiao (structural biologist), among many others. Yunlong established a high-throughput DMS platform at Changping Laboratory, and the team was able to give successful and repetitive predictions on which variants would become dominant in the next worldwide infection wave.[44] Because of this accomplishment, Yunlong was named one of *Nature*'s Top Ten people who shaped science in 2022, as the "COVID predictor". He has just become an assistant professor, also at BIOPIC.

As a boy, I heard my father saying that teaching is beneficial for research. I taught a biophysics course at Harvard for many years. A handful of my research ideas were formulated during the teaching process. I have been writing my book, "*Single-Molecule Biophysical Chemistry: Underpinnings of Life's*

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Processes", for quite a long time. Each time I taught the course, I felt compelled to finish the book, yet burdens from miscellaneous responsibilities had always prevented me from reaching this goal. During my last trip to the U.S. in 2019, I visited James Watson, Nobel laureate who co-discovered the structure of DNA at his home at Cold Spring Harbor Lab (CSHL). I had pored over his book, "*Double Helix*", as a freshman at PKU. Jim gave me a signed leatherbound copy, a very precious gift that I treasure. Learning about my returning to PKU, he said to me "China needs you!" A few months prior to my visit, I had the honor of speaking after him at the opening ceremony of CSHL Asia Academic Center in Suzhou. He said to me afterward, "I talked about biology's past; you talked about biology's future"—I know it was only his kind encouragement yet given with touching sincerity.

Jim is an experienced textbook writer; I read his popular molecular biology textbook when auditing Losick's course. He also urged me to finish my own book, using the great Linus Pauling as inspiration, telling me that "*The Nature of the Chemical Bond*", published in 1939, was essential to Pauling's influence and legacy. Jim's advice further strengthened my determination. Sadly, COVID took away another three years of my time. I now teach the same course at PKU. The students in my class remind me how courteous the PKU students are—they always applaud after I finish my lecture. I am finally completing my book now, even if just for them. It has a problem-solving book coauthored with Winston, and is being translated to Chinese by Fanchong.

I am grateful to my research groups over the years. My greatest joy has always been pursuing scientific innovation, working side by side, day and night with my students, postdocs, and collaborators. Many among them were luckier than me—they had made important contributions during their Ph.D. and postdoctoral periods. I am gratified to see many of them in faculty positions at top universities around the world, being recognized as experts or leaders in their own fields. We had a reunion at my 50th birthday at Harvard and a small gathering at the 2015 ACS meeting when I received the Peter Debye Award in Physical Chemistry.

When Wei Min, Shaul Mukamel, and Yiqin Gao first approached me about the *JPC* special issue on the occasion of my 60th birthday, I hesitated. On the one hand, I felt it was too early for me to write an autobiography. On the other hand, as I am no longer publishing *JPC* papers as often as before (~29 *JPC* papers including three Festschrifts), I felt obligated to share with young physical chemists my own experience that our background is highly valuable for life science, as it is evolving from a data-poor science to a data-rich, technology-driven, quantitative science.

As individuals, our lives are often shaped by the fate of our motherlands. Fortunately, my life journey as a scientist has been mostly shaped by my own interests, scientific and technological challenges, as well as societal needs, albeit some unforeseeable forces. I am grateful to the people who have influenced me throughout my life. At the beginning, I used to write papers in highly specialized fields that only a few people in the world would take interest. When we started to do single-molecule enzymology, live-cell and noninvasive imaging, I found that more people were following our work; after we turned to single-cell genomics, MALBAC babies, and broad-spectrum neutralizing antibody drugs, even more people followed. Gradually, our work began to make an impact in the real world. This has brought a lot of satisfaction to my students, postdocs, and myself.

I recall Gabor Somojai's remark: "There would never be an end." In retrospect, at that time, I did not expect that the never-ending opportunities would one day be available in China, for me and for Chinese young scientists as well. I am truly fortunate to be leading a creative life here with my students and colleagues.

On the personal side, I was first married to Dr. Song, my PKU classmate, when we were both graduate students at UCSD. Our son Kevin was born when we were at PNNL and identical twin daughters Kristin and Kara shortly after I moved to Harvard. She devoted herself to raising our children, whom we are both very proud of. I was grateful for her support not only at home but also to my lab. Over time, however, we grew apart—my leaving Harvard for PKU was not helpful. We did not formally separate until Kristin was about to enter NYU and Kara was about to enter Harvard, like her brother Kevin. Kevin recently earned his MBA from Wharton business school in Philadelphia. Kristin and Kara have also graduated from college, the three of them now all working in New York City.

My current wife, Dr. Bao, and I were introduced to each other by friends during the pandemic, my busiest time ever, and she has been most supportive of me and my work. She earned her Ph.D in film studies. Our affection for each other is mutual. My brother, Brian, also a chemist, lives with his family in the Bay Area. Meanwhile, my parents, who are very happy that I am back with them in China, are preparing for a celebration of their 90th birthdays. In the summer of 2019, Kevin, Kristin, and Kara came to Beijing to visit me. I emotionally recalled my mother's reunion with Grandma, hoping that history would never go backwards.

My special thanks to many of you who have contributed to this special issue, for which I am immensely grateful. Also, my sincere appreciation goes to Wei Min, Shaul Mukamel, and Yiqin Gao, who suggested the idea of this special issue to me and made it happen. I also thank Drs. Yeqin Ma and Patty Purcell for their help in preparing this autobiography.

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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.3c05597.

Curriculum vitae of Xiaoliang Sunney Xie (PDF)

Colleagues of Xiaoliang Sunney Xie (PDF)

Publications of Xiaoliang Sunney Xie (PDF)

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Notes

Views expressed in this preface are those of the author and not necessarily the views of the ACS.

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