I decided to become a scientist rather early on—at the ripe age of four. My mother was a biochemist at the Nencki Institute of Experimental Biology in Warsaw, Poland. One day I offered to Professor Włodzimierz Niemierko, then director of the Institute, my future services subject to successful graduation from kindergarten, school, and university. Little did I know that I would actually accomplish that goal, but, to tell the truth, any thoughts about crystallography were definitely not on my mind then. Moreover, although those promising beginnings took place in Poland, almost my entire scientific career has been connected with the United States, a fact that I attribute to several circumstances.

When I was in high school, I was selected to become a member of the Polish delegation attending a meeting of the American Junior Red Cross on the hundredth anniversary of the establishment of the Red Cross in the United States. Luck was with me: English was not taught in my school, but my parents insisted early on that I study the language, so I did not have much competition in the selection process. That meeting in the summer of 1962 truly changed not only my own life but also the lives of many other participants. A visit to the White House hosted by President John F. Kennedy was truly inspiring (the arrow points to me). A visit to the United Nations headquarters in New York provided an impetus for another young meeting participant, Ban Ki-moon, to become a diplomat and later occupy the most important office in that same building for a decade. My own goal was also set: I would finish my high school and university studies and go to the United States for graduate studies.

When the time came to choose the direction of my university studies, I abandoned the idea of life sciences and decided to study physics. I followed that track for three years until 1966, when I had to select my specialization. That same year, Professor David Shugar started a completely new program in biophysics in Warsaw. I joined its very first class and decided to work on my master's thesis at none other than the Nencki Institute. However, rather than conducting experiments in physics, I dabbled in neurophysiology of vision but quickly convinced myself that torturing cats should definitely not become my future career.

At that time, many young people in Poland became very active politically and secret groups were discussing how to improve socialism to the point that it would actually deliver on its promises. Major political upheavals took place during and after March 1968, which strongly encouraged me to emigrate. Although I was already accepted to a graduate program in neurobiology at the University of Iowa, I also applied for doctoral studies at two universities in California, UCLA and Caltech. The major impetus was geography: I was a mountain climber, and realized in time that there were no mountains in Iowa. I later discovered that my application to Caltech was never considered, since I had been unable to send them the $10 application fee. However, I was not only accepted to the Molecular Biology program at UCLA but also awarded a stipend. Thus, it was not Iowa, but California, and not neurobiology, but molecular biology. The only problem was how to get there.

Due to some rather obscure American regulations, I could apply for a refugee U.S. visa only in Italy. Therefore, I went to Rome, filed visa applications, and waited. In a serendipitous development, I was hired as a completely unqualified technician in a laboratory in the Istituto Superiore di Sanita. The head of the laboratory was Rita Levi-Montalcini, who some years later became a Nobel laureate, and the area of study was a small protein called nerve growth factor (NGF). I became completely fascinated by this hormone that directs the growth of neurons. Of course, it was not at all clear to me that this would become important much later.

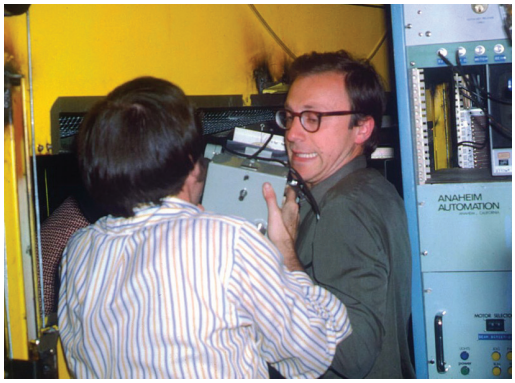
I came to Los Angeles in the summer of 1969 and started my graduate studies the day after my arrival. That same year, a young scientist named David Eisenberg moved from Caltech to UCLA to become an assistant professor. I became one of his first graduate students. David decided to establish at UCLA a new area of investigation, namely protein crystallography. It was barely a decade since the first protein structures had been determined by Max Perutz and John Kendrew, and only a few places in the world were engaged in such studies. I certainly did not plan on becoming a crystallographer when I started my graduate work, but I was very quickly converted and realized that this should be the field of my specialization.

For the next 4 years, I tried to solve the crystal structure of rabbit muscle aldolase, but there was no structure by the time I was ready to write my thesis. However, it was still possible at that time to graduate without solving a protein crystal structure and by publishing only a single paper—thus, Ronald Reagan's signature was finally placed on my Ph.D. diploma (he was then the governor of California). While at UCLA, I tried to interest David in NGF, but he did not bite.

My next move was to look for a postdoctoral position. Brian Matthews at the University of Oregon must have learned that my Ph.D. thesis presented little experimental data, so he very politely turned me down. My luck somehow prevailed, however: I contacted Eric Shooter, a professor at Stanford University and one of the major players in the NGF field. Eric became interested and promised to support my quest for the structure of this protein, but since he did not have funds to support me, he made a deal

with Keith Hodgson, who at that time was starting a project to utilize synchrotron radiation as a source of X-rays for protein crystallography. Thus, I could work on both methods development and structure determination.

The summer of 1974 was the most successful period in my career as an experimental crystallographer. I crystallized not only NGF but also two other proteins, L-asparaginase and monellin. At that time, just crystallization of a protein alone was sufficient for a full publication, even in *Proceedings of the National Academy of Sciences*. However, the development of a synchrotron beamline as a source of X-rays was a much slower project, and I did not have any equipment to collect X-ray diffraction data at Stanford. I ended up flying regularly to Oregon, so Brian Matthews was stuck with me despite his earlier decision.



Nevertheless, my main project at Stanford was the development of the first synchrotron beamline for protein crystallography. That work was directed by Keith Hodgson, with further participation by Margueritte Yevitz Bernheim and a graduate student, James Phillips. We were joined by Julia Goodfellow (now Dame Julia) a year later. To say that our facilities were primitive is to overestimate the true state of affairs. Our only detector was an Enraf-Nonius precession camera that could be used with Polaroid films for alignment or with multiple packs of radiology films for "data collection." In the photo Keith Hodgson (with his back turned) and I were installing a precession camera in the hutch at the Stanford Synchrotron Radiation Lightsource in 1975.

We used this beamline to collect diffraction data for proteins such as NGF, L-asparaginase, azurin, and rubredoxin. Most crystals of these proteins were too small to provide measurable diffraction with standard laboratory X-ray tubes, so we considered the use of synchrotron radiation to be quite successful. Experiments involving rubredoxin, performed with Lyle Jensen and his colleagues at the University of Washington were particularly important, since we tuned the wavelength to match the absorption edge of iron, thus maximizing the anomalous signal. We were quite pleased to see even by the naked eye that there were differences between the intensities of Friedel mates (the central projection in the space group *R*3 is non-centrosymmetric). Those very early experiments clearly proved that the tunability and high intensity of the synchrotron X-ray beam would ultimately revolutionize protein crystallography.

Running experiments was exhausting, since the beam was dumped every two hours and it was necessary to adjust the camera after every fill. My longest single experiment took six nights and five days, with sleep possible in—at most—two-hour increments (on the floor, under a table). We felt pressure to get some positive results before others would beat us to it and, by mid-1976, we finally published our preliminary results in *Proceedings of the National Academy of Sciences*—just in time, since the results from Deutsches Elektronen-Synchrotron (DESY) in Hamburg came out soon thereafter, and another group in Novosibirsk was also developing a protein crystallography beamline.

My next move was in 1976, to the National Bureau of Standards (NBS, now the National Institute of Standards and Technology) in Maryland. My main project was to construct a neutron diffractometer capable of measuring data from protein crystals. To a large extent, progress was due to two colleagues with very extensive knowledge of neutron technology, Antonio Santoro and Ted Prince. They came up with the idea of building a flat-cone diffractometer utilizing a 1-meter-long linear detector and helped me with writing the operating software. I was later joined by my first postdoctoral fellow, Lennart Sjölin, who very successfully continued the process of software development. My predecessor, John Norvell, had already grown crystals of ribonuclease A (RNase A), the largest having a volume of 100 mm^3; thus, the course of action for the next eight years was set.

Lennart and I initially concentrated on the determination of the crystal structure of RNase A based on neutron data alone, but we quickly realized that this might not be the best way of proceeding. However, discussions with Wayne Hendrickson, with whom I would meet quite regularly during the Washington Crystal Colloquia—organized by no less than a future Nobel laureate, Jerome Karle—led us to adapt Konnert and Hendrickson's program PROLSQ for joint X-ray and neutron refinement. This approach allowed us to publish quite significant data on the protonation states of residues such as histidine and on amide hydrogen exchange. Subsequently, we decided to investigate another small protein, bovine pancreatic trypsin inhibitor (BPTI). In retrospect, our BPTI work was much more important than our RNase A work, as BPTI became a prototype for the development of macromolecular NMR and for computational methods interpreting the folding, structure, and dynamics of proteins. Since BPTI was originally studied in Munich, I established a very fruitful collaboration with the future Nobel laureates Robert Huber and Hans Deisenhofer. X-ray data personally collected by Robert, merged with our neutron data, were used for joint refinement, leading to the first truly atomic-resolution (1 Å) protein structure to be deposited in the Protein Data Bank (PDB). The structure of the even-smaller protein crambin was refined earlier but deposited later by Martha Teeter.

A few years after my move to the NBS, I attended the 1978 Congress of the International Union of Crystallography in Warsaw. There I met Tom Blundell, one of the top British crystallographers of the second generation. Tom and I discussed the NGF stalemate in considerable detail and came to an understanding: his laboratory would take over the project, but I would be kept in a supporting role. That agreement held for the next 13 years—that was how long it took to finally determine the structure of this very small protein. The results were worth it, though: the structure, published in *Nature* in 1991, elucidated a newly discovered fold

that included a cystine knot, later found in many other important proteins. Tom held his part of the bargain and I was included as a co-author of that paper, even though by then we were more competitors than collaborators.

Another important event in my career that could be traced to attending a scientific meeting took place in 1986. I participated in a Congress of the European Crystallographic Association in Wroclaw, Poland, where I met a distinguished Polish crystallographer, Professor Zofia Kosturkiewicz, who suggested that I accept her former student Mariusz Jaskólski as a visitor to my laboratory. Indeed, Mariusz came to the U.S. a year later, and we then started our very successful collaboration that continues until today and that has resulted so far in more than 40 joint publications. In the 2011 photo above, Mariusz and I are at a Multi-Pole conference in Warsaw, Poland.

My move to the National Cancer Institute (NCI) was an indirect result of Joel Sussman's sabbatical at the NIH. Joel worked closely with us on structural investigations of DNA duplexes containing unpaired bases and spent lots of time in our laboratory. In 1986, he told me about a plan to start a structural biology laboratory at the NCI in Frederick, Maryland, and encouraged me to apply for a group leader position (he himself applied for the position of a lab chief). However, Joel ultimately decided to accept the position of the director of the PDB (then at Brookhaven), and George Vande Woude, the head of the Frederick program, offered me the lab chief position. I accepted and moved to Frederick in 1987, with Irene Weber assuming a group leader position, and with Ron Rubin joining us as a group leader a little later.

The moment of transition between the NBS and NCI happened when my laboratory became engaged in a new and exciting research area, namely structural investigations of retroviral proteases (PRs). Inactivation of HIV PR was shown to prevent viral particles from maturing into their infective form, thus making HIV PR a potential target for antiviral drugs. However, genuine proteins from HIV-1 were very difficult to come by at that time. As is often the case, the start of the project was quite fortuitous—through my introduction to Jonathan Leis, who at that time worked at the Case Western Reserve University in Cleveland, Ohio. Jonathan had been working for a long time on biochemical characterization of various retroviral proteins and had successfully purified milligram quantities of PR from Rous sarcoma virus (RSV, now usually called avian sarcoma virus, or ASV). We immediately decided to investigate its three-dimensional structure as a stand-in for the structure of the much more medically important enzyme encoded by human immunodeficiency virus type 1 (HIV-1).

Crystals of RSV PR were grown by Maria Miller within a month of receiving the protein. Derivatization of the crystals with a uranyl compound, an excellent anomalous scatterer of CuKα radiation, yielded a single-site derivative (which marked, as it later turned out, the active site) that enabled the proper choice of the space group enantiomorph and helped in setting some additional derivatives in common origin and handedness. J. K. Mohana Rao and Mariusz Jaskólski were crucial participants in that phase of the project. The electron density map, based on multiple isomorphous replacement phases from the four best derivatives, allowed us to trace the main chain of the dimeric molecule, and the atomic model of RSV PR was complete in October 1988.

As soon as the first RSV PR model was complete, Irene Weber built a homology model of the HIV-1 enzyme. The model looked very plausible: it had all the features of the template, with differences limited to the loop regions. The structure of RSV PR was published in *Nature* in early February 1989. A week later, in the same journal, the crystal structure of HIV-1 PR was unveiled by Manuel Navia, Paula Fitzgerald, and co-workers from Merck Sharp & Dohme, and that same week, Irene's model was published in *Science*. After the first burst of joy, suddenly there was consternation because the crystal structures of the RSV and HIV-1 PRs, while similar in the basic features, also showed some perplexing differences, especially in the C-terminal region of the molecules. Whereas the RSV PR model had a clear α-helix, the HIV-1 PR structure had a straight β-strand, and the topology of the dimer interface was completely different. Instead of the interlaced termini with three inter-subunit β-sheet connections found in the RSV PR, the HIV-1 PR crystal structure had a hairpin with only one area of inter-subunit contact, and a disordered N terminus. The latter difference was not trivial; rather, it had profound consequences for the dimer stability and for the PR's ability to excise itself from the viral Gag-Pol fusion polyprotein synthesized in the infected cell. Moreover, the question about the correct features of retroviral PR was not purely academic, because an accurate HIV-1 PR model was badly needed for a structure-guided design of inhibitors that might be developed into AIDS drugs.

The dilemma of which HIV-1 PR model could be resolved only by experiment, but the main question was how to obtain the protein. Help came from Stephen Kent, then at the Caltech, who was pioneering the methodology of protein synthesis using a purely chemical process. He and Jens Schneider quickly sent us 0.2 mg of chemically synthesized HIV-1 PR, enough to grow a few crystals. Our molecular replacement calculations had to rely on Irene's model of HIV-1 PR, as the coordinates of the Merck structure were not made available. However, more material was needed to produce heavy-atom derivatives, because it was critical to obtain phase information experimentally, to avoid model bias, and to produce an independent model of the protein. More protein was also needed for cocrystallization trials with inhibitors. The Kent group set a precedent by producing for us, within a period

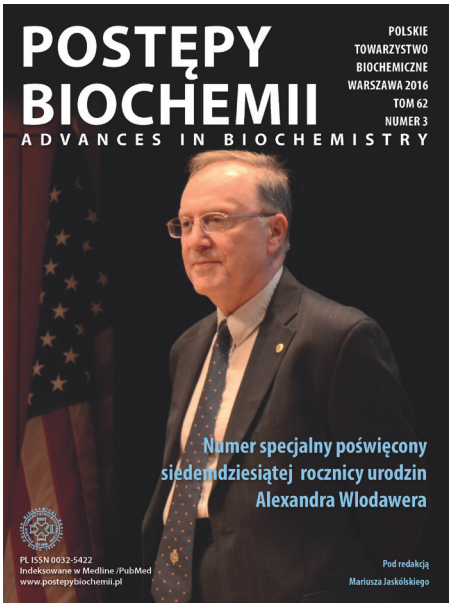
of just two weeks, milligram quantities of HPLC-purified enzyme for successful crystallographic studies. The definitive structure of the HIV-1 PR apoenzyme, showing its agreement with the RSV PR-derived model, was published in *Science* in August 1989.

The next goal was to determine the structure of HIV-1 PR in complex with inhibitors. The first such inhibitor, MVT-101, was provided to us by Garland Marshall (Washington University). Cocrystals with the synthetic enzyme grew overnight, and we were able to complete and publish the structure of the complex four months after the publication of the structure of the apoenzyme. It is worth stressing that the coordinates of the synthetic HIV-1 PR:MVT-101 complex were deposited in the PDB in April 1990 and, for the two most critical years, were the only ones freely available to all researchers worldwide who were working on the design of specific retroviral PR inhibitors, although we were not directly involved in such efforts. The first HIV-1 PR inhibitor to become a drug, Saquinavir (Ro-8959), was developed by Roche and approved for clinical use in December 1995. It is generally recognized that determining the structure of HIV-1 PR has been the springboard for the development of successful rational drug design strategies not only in that particular case but also for other pharmacological targets, taking the idea from a flimsy dream to practical reality.

With very significant participation by Alla Gustchina, who had studied the structure of pepsin in Moscow and was thus well acquainted with aspartic proteases, we continued to study retroviral PRs from other sources, such as FIV, EIAV, XTLV, and XMRV. Our focus was on models of potential resistance to anti-HIV drugs, as well as on the function of these enzymes in carcinogenic viruses.

My involvement with atomic-resolution protein structures also happened through serendipity. In the mid-1990s, Fred Dyda and I convinced the NIH management to create a facility for our institution at beamline X9B at the Brookhaven synchrotron. However, having access to the beamline was clearly not enough; we needed someone who could professionally operate it. Here, luck was with us: Zbyszek Dauter, who for almost 10 years had worked at the EMBL DORIS beamlines in DESY Hamburg, was persuaded in 1997 to move to Brookhaven. This was a significant loss for the European crystallographic community but a clear win for us, since Zbyszek has many talents and has been known for years as not only a great crystallographer but also a superb collaborator. Since he excels at working at the resolution of 1 Å and beyond, engaging him in projects that involved studies at atomic resolution benefited us tremendously. Many such projects have been completed through our collaboration during the last 20 years.

It is not a coincidence that many names mentioned in this memoir are Polish. Although I never explicitly tried to find collaborators in that country, my contacts in Poland resulted in several scientists visiting my laboratory, and some staying for many years. Additionally, many distinguished crystallographers with Polish roots are very active and successful around the world. Thus, the "Polish Crystallographic Mafia" came into existence—in addition to my colleagues mentioned above, it also includes many others. Wladek Minor, well known as a co-author of the HKL3000 package, has been particularly involved in our efforts to maintain and enhance the quality standards of macromolecular structures deposited in the PDB. Years ago, I was involved in the first crusade to make deposition of atomic coordinates of published structures mandatory—it is hard to believe today that, at that time, many prominent protein crystallographers were fervidly opposed to such a policy. However, it became the law of the land, followed some years later by the requirement to deposit experimental structure factors as well. In the last few years, we have become, together with some other colleagues who do not claim any Polish roots, self-appointed policemen monitoring the PDB, plucking rotten apples and rectifying less-severe errors of selected structures. I think that these efforts may ultimately turn out to be quite important, since the presence of bad apples in the PDB bushel is guaranteed to cause serious problems in meta-analyses, in particular by biasing projects that might lead to the creation of new drugs. Other efforts of the Mafia included the organization of meetings entitled Multi-Pole Approach to Structural Science, as well as editing the latest textbook of protein crystallography. One indication of our success was that Mariusz Jaskólski and I received in 2015 the first-ever Polish-American Scientific Collaboration Award given by the Foundation for Polish Science and the American Association for the Advancement of Science. We were very proud of being selected in a highly competitive contest encompassing all fields of science. My photo taken during the award ceremony was used for the cover of a special edition of the Polish journal *Postępy Biochemii (Advances in Biochemistry)*, for an issue celebrating my 70th birthday. That issue included reviews and primary research articles by my mentors, students, and collaborators.



I would like to think that being invited to write this memoir does not indicate that my scientific life is over—I certainly hope this is not the case, since quite a few projects are still a long way from being completed. I have been blessed with having excellent mentors, with being able to work in well-equipped laboratories, and, most importantly, with having superb collaborators, who were principally responsible for whatever successes my laboratory could claim. I am very grateful to all those already identified, and to the many individuals whose names I did not have a chance to mention. Thank you all!