Haas, Abad-Zapatero and Petsko Highlight Technological Developments

In this issue of *RefleXions* David Haas and Cele Abad-Zapatero recount their Living History autobiographies. Both Cele and David worked with Michael Rossmann at one point in their career, David in 1962-1965 and Cele in the late 1970s and early 1980s. Both memoirs emphasize the important technological advances that enabled data collection and refinement of larger structures such as viruses and protein complexes.

David describes his experiments in the Rossmann lab, freezing lactate dehydrogenase crystals with sucrose cryoprotectant to extend crystal lifetime in the x-ray beam (1970). At the time, it seemed to him that there was very little interest in data collection at low temperature. In 1975 Greg Petsko extended these experiments to many other proteins by replacing the crystallization solution with high concentrations of organic solvents such as methylepentanediol or ethylene glycol. Nevertheless, in the early 1980s while he was in the Rossmann group Cele didn't use the freezing method; he ascribes the long delay in adopting this technique to the lack of devices to maintain frozen crystals reliably. It was not until the late 1980s that Håkon Hope successfully promoted this method, which is now routine. From the point of view of the history of science, the substantial gap between initial discovery and widespread application of low-temperature data collection illustrates our reliance on the instrument-makers.

In the early 1970s another instrumental development, the commercial oscillation camera with software to process the data, was a game-changing advance that Cele used both in graduate work with Marv Hackert and later in the Rossmann lab. Then during the 1970s synchrotron sources of x rays began to come online. The structure solution of rhinovirus by the Rossmann group (1985) was possible because of the rapid data collection and high resolution available at synchrotron sources. Cele also notes the advances in structure solution and refinement during the 1970s and early 1980s: non-crystallographic symmetry algorithms to solve virus structures and constrained-restrained refinement techniques by Joel Sussman (CORELS) and by Konnert and Hendrickson (PROLSQ) to refine proteins. Finally, Cele describes his pioneering application of computer graphics software FRODO (T. A. Jones) to refine the structure of lactate dehydrogenase.

Recent research, however, causes reevaluation of formerly groundbreaking technology. Although Greg Petsko was one of the early champions of low-temperature data collection, in his 2015 Buerger lecture ("Forty Years of Crystallographic Studies of Protein Structure, Function and Dynamics or Some Like It cold – But Should They?") he notes that temperatures below about 200-220 K result in significant changes in protein structure and dynamics. He calls this the "skeleton in the closet of structural biology" and he argues that by collecting data at low temperature we discard information we really want – flexibility of side chains, vibrational motion, protonation state, metal ion spin – the intricate variations in structure at room temperature that are responsible for binding, catalysis and product release. He suggests that the latest technological development, the x-ray free-electron laser, will provide the means to obtain room temperature structures for every unique protein in the PDB. If you missed his lecture in Philadelphia, you can view it online on YouTube from the ACA History website.

Virginia Pett

Memoirs: David J. Haas

David J. Haas's memoir gives a perspective on how scientific discoveries are made and recognized. He set out to test whether crosslinking a protein might stabilize the crystal and ended up discovering that freezing crystals vastly extends their lifetime in the x-ray beam. The value of this observation was not appreciated until much later, when freezing crystals at synchrotron sources became necessary.

I was born in Buffalo NY, but after WWII my family moved to south Texas, where I was raised in a farming community near the Mexican border. After attending several local colleges in Texas, I decided to transfer to the University of Buffalo where I still had relatives living in the Buffalo area. I graduated from the University of Buffalo (now SUNY at Buffalo) in 1962 with a physics degree, and then entered the Department of Biophysics in the Medical School. The department was closely associated with Roswell Park Memorial Cancer Institute where the Crystallographic Center had been established in 1960. This new center was created to house "The Protein Structure Project". David Harker was provided with a million dollars in 1949 for "The Protein Structure Project" by Irving Langmuir. Isidor Fankuchen apparently had sufficient interest, space and facilities for such an organization and invited David Harker to establish it there. The plan was to remain for about ten years and arrangements were made in 1960 to move the entire group to Buffalo. For the first few years in Buffalo, the crystallography group was situated in the basement of Roswell Park Research Building, and then in 1968 the Crystallographic Center was constructed on the Roswell Park Campus.

David collecting diffraction data with the original Harker-Furnas Goniostat at Roswell Park Memorial Institute, 1964.

With David Harker (photo credit: AIP Emilio Segre Visual Archives, Physics Today Collection) as my advisor I specialized in x-ray crystallography at the Crystallographic Center, where I used the original goniostat units (Eulerian cradle) for collecting diffraction data and superficially participated in the ribonuclease protein work. My thesis project was to solve the crystal structures for six organic molecules which were known protein denaturants. Performing x-ray data collection on the GE goniostats required manual data collection. I spent hours isolated in the x-ray room dialing numbers into these units. All the data were punched into Hollerith cards, and I used one of two IBM 1620 computers which were at the Roswell Park Computer Center and the UB Engineering Department. There were boxes and boxes of punched cards to transport for the structure determination. All the software programs were supplied by Dr. Ahmed in Ottawa, Canada, which proved to be essential to my success. After solving the structures of the six organic molecules in a matter of months and providing some assistance to the ribonuclease group, each of the crystal structures was published as a paper in *Acta Crystallographica*, which presented me with much needed exercises in scientific writing and drawing electron density maps/structures by hand. I can say that Harker was surprised at how fast I determined these structures, and he instructed me to write my thesis, including all the data that I had sent for publication. I graduated in February 1965.

Harker was a marvelous instructor, and he was intimately involved with the various departments at the University of Buffalo. Besides the daily lunchtime discussions between the staff members (Doretta Norton, G. Kartha, Lena & Jake Bello and staff), the laboratory was frequently full of visitors including Max Perutz.

During this time I met my wife, and we married on June 10, 1962. Sandra and I have been partners ever since! She had unforeseen skills and talents which were demonstrated beyond her raising our three sons. Besides having her name on many patents, Sandra was Vice-President of Temtec, Inc. – the business that we operated for 21 years (1981-2002). She excelled as the HR and customer service manager.

Meanwhile, Harker had contacted several British protein crystallographers to locate a postdoctoral position for me. This was already five years after the structures of myoglobin and hemoglobin had been determined, and David C. Philips had just finished with the structure of lysozyme (March 1965). We knew it was an exciting time in Britain for protein crystallographers. Phillips responded that he would have a postdoc position available for me after September 1965. I immediately accepted. Harker suggested that in the intervening months I should learn as much as I could about the new direct methods that were just being perfected by the Karles in Washington, DC. Jerry Karle invited me to his lab for several months. Isabella and Jerry Karle were really wonderful, and I attempted to learn as much as I could about direct methods during this stay. I worked with a research assistant S.A. Brenner on collecting data on dimethylmalonic acid, and we published a paper using Symbolic Addition Methods to determine the structure. Isabella and Jerry were always available for discussions so my time with them was quite beneficial. Also, during the summer they frequently had barbeques for the laboratory staff and their spouses at their home in Falls Church. Great comraderie!

Sandra and I arrived in London September 1965. We spent a few days at David Phillip's home, getting oriented and learning the language! I spoke at length with my officemate – Charles Bunn who was a well-known industrial crystallographer. The real excitement at the Royal Institution had been the March Friday Evening Lecture by David Phillips on the structure and function of lysozyme, the first enzyme structure to be determined. (He was now working on the *Scientific American* article.)

It was suggested that I might investigate the effects and possible uses of crosslinking protein crystals, to increase mechanical stability and reduce radiation damage. No other experiments on reducing radiation damage had been successful. With an ample supply of surplus lysozyme crystals from Philips, I first tested crosslinking to different degrees, and lysozyme was an excellent test model as the crystals were very stable and rugged. Brief exposure to a dilute solution of glutaraldehyde produces only a "surface crosslinking". I found that the "surface-crosslinked" crystal would remain unchanged and basically normal. A measure of the degree of crosslinking was to denature the crystal and observe the volume of swelling. The less the crosslinking, the greater the swelling. The surface-crosslinked crystals, when denatured, swelled to enormous sizes, each crystal edge increasing more than three times. Most important, with denatured surface-crosslinked lysozyme crystals, slowly removing the denaturant and returning the crystal back to its original supernatant caused the crystal to shrink again and recover its x-ray diffraction pattern. This proved to be a remarkable renaturation property which shows that the protein molecules could actually recrystallize themselves. I presented several papers at European crystallographic meetings and published a short note in *Acta Crystallographica* in 1968.

One of my fondest memories about working at the Royal Institution was the daily 4 p.m. tea gathering. Most of the staff appeared for the daily discussions (Gareth Mair, Colin Blake, Louise Johnson, Tony North, Ragupathy Sarma) including Sir William Lawrence Bragg. I recently read in Georgina Ferry's

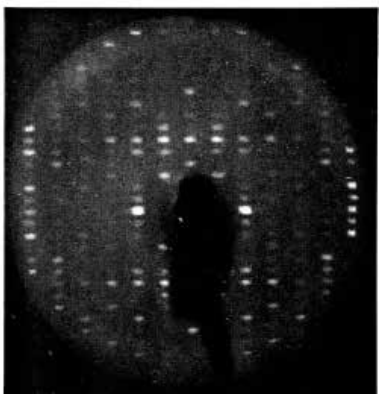
book on Max Perutz that these tea breaks were begun by Sir William Henry Bragg when he first took over management of the Royal Institution laboratory in 1923.

In the fall of 1966 David Philips informed me that he was moving the group to Oxford University and that I would need to find another laboratory to continue my NIH Fellowship. He

Sandra and I arrive in Israel from England at the Weizmann Institute of Science with our son Stuart.

suggested the Weizmann Institute in Israel and arranged for me to work in the Crystallography Group of the Chemistry Department with Wolfie Traub and Gerhard Schmidt. Until recently I had no idea why he made this suggestion, but it appears that he attended a meeting in Israel earlier in 1966 and was impressed with the quality of the people and work at the Weizmann Institute of Science. Wolfie Traub indicated that we should just take a ship to Haifa, and they would be ready for us at any time. As my NIH Fellowship was not transferable, Wolfie said the Weizmann Institute would also provide a Weizmann Fellowship which included housing and a stipend. (Thank you Mr. and Mrs. Van Leer for your Fellowship.) The Weizmann Institute proved to be a remarkable scientific institution, and what an experience for both Sandra and me.

Taking all my lysozyme materials and chemicals, I continued work on the effects of crosslinking protein crystals. I determined that crosslinking had little or no effect on reducing radiation damage by exposing various crystals for days in the full-power x-ray beam and then taking precession photographs of a standard pattern. This was quite disappointing! Fortunately, there was an unused crystal-freezing apparatus setup on one of the Philips x-ray generators. Considering whether crosslinked crystals would remain intact when frozen, I determined that crosslinked crystals fractured from ice crystals in exactly the same manner as native (uncrosslinked) crystals. Then I discovered that crosslinked crystals could be put into almost any solution of salts or organic solvents (cryoprotectant) because the crosslinking prevented the crystal from dissolving in the supernatant (as well as providing structural stability). With surface crosslinked lysozyme crystals in different concentrations of sucrose, frozen crystals gave excellent diffraction patterns above a certain sucrose concentration (sufficient to prevent ice formation). I confirmed that freezing in sucrose did not damage the crosslinked crystal by taking standard diffraction patterns repeatedly before and after diluting out the cryoprotectant.

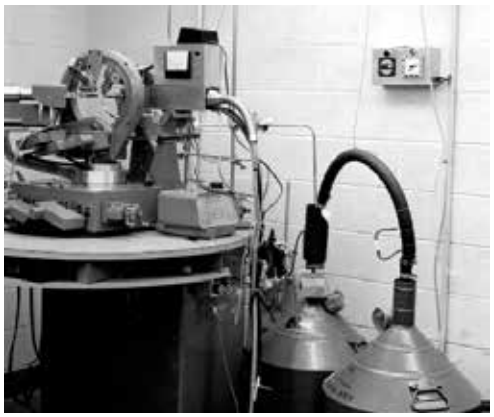


Precession photograph of the first successful frozen protein crystal, crosslinked lysozyme at -50°C.

Then I discovered that lysozyme crystals could have their supernatant slowly changed to the appropriate compound concentration "without crosslinking" simply by selecting the appropriate compound (at room temperature and taking more precession x-ray patterns). These frozen *native crystals in sucrose cryoprotectant* gave excellent diffraction patterns, and I was quite impressed with their stability. (Note: The term cryoprotectant used here had not been created at this time.) Exposing several crystals to many days of x-rays at full power while taking repeat precession patterns demonstrated that the frozen lysozyme crystals showed substantially less radiation damage than native lysozyme crystals at room temperature. Hence, a solution to the radiation problem may be at hand! I was unable to continue this work at the Weizmann because the Six Day War began on June 5, 1967, and I had sent my wife and newborn son back to London for safety. I had already taken sufficient precession patterns for publication to demonstrate that radiation damage is absolutely reduced by freezing protein crystals! Fortunately, I had already arranged for a position with Michael Rossmann at Purdue when I returned to the United States.

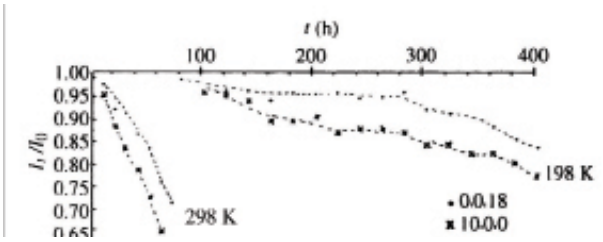
The Rossmann laboratory was a beehive of activity with M. J. Adams, A. J. Wonacott and A. McPherson all working on the lactate dehydrogenase (LDH) project. Michael had the latest equipment and the group was always willing to help me learn the new methods. Within the first months, I wrote the short paper describing my findings of reduced radiation damage at low temperatures and presented my finding to the Rossmann crystallography group. They were skeptical that the benefits of extending the useful life of their crystals would be worth the "perceived" complexity and difficulty of building, installing and operating cooling apparatus. I believe that everyone expected the radiation damage reduction to be only a few hours, not the hundreds of hours that finally came to be with low-temperature x-ray data collection. And certainly, no one could even imagine the importance of freezing crystals with synchrotron radiation sources! Again, I would not have had a convincing argument for reduced radiation damage without the lysozyme precession photographs from the Weizmann Institute. The general notion at the time was that freezing protein crystals was no different from freezing food – I do not believe anyone in the laboratory knew the Birds Eye frozen food story, and it appeared that freezing anything, in general, was a bad idea!

After extended deliberations, Michael Rossmann agreed to loan me one of the Picker Automated diffractometers, and he would fund the purchase and construction of the most primitive freezing apparatus, as it probably would only be used once for my project. (Thank you Michael!) The glass laboratory fabricated a co-axial gas delivery jet for directing the nitrogen gas onto the



Freezing apparatus in the Rossmann lab for collecting the first data from frozen LDH crystals.

crystal with a dry air cylindrical barrier jet surrounding the nitrogen stream. The crystal would be mounted in a Lindemann glass vial in the conventional manner along with a small amount of mother liquor. The work would be performed on LDH as this was the protein being worked on by Michael Rossmann and sufficient crystals were available. The same sucrose cryoprotectant was successfully used as demonstrated previously.



Cryoprotected LDH crystals show less radiation damage at 198 K than unprotected crystals at 298 K.

I assembled all the equipment and fabricated simple control circuits, purchased a suitable air dryer and several bathroom scales for tracking the weight on the nitrogen-filled Dewars. After determining the pounds/hour usage of the liquid nitrogen, I would calculate when the "feed" Dewar had to be manually refilled from the storage Dewar. Of course, this was typically in the middle of the night! I scheduled regular monitoring of two reference reflections so as to measure the actual x-ray deterioration during the hundreds of hours of exposure. The results of this first x-ray data collection from a frozen crystal were published and I also presented a paper at the next ACA meeting in 1969. I can only say that there was basically "no interest" in this work whatsoever! Furthermore, I never heard a word about freezing protein crystal for the next 30 years until 1999 when I again revisited Michael Rossmann at Purdue. He said, "What a wonderful technique cryocrystallography had become". Actually, I could hardly remember the work, so Michael gave me a copy of our 1970 paper to read!

As a side note, during 1969, I developed an instrument for setting dihedral angles on metal atomic models that made it very easy to accurately fabricate the backbone for known protein structures. A manufacturer became interested in the "Dihedral Angle Dialer" and manufactured it for several years. What is interesting is that I sent a request to the Purdue University Intellectual Property Committee for permission to apply for a patent, but they just had no interest in scientific patents. And now I can note that if I had applied for a patent on the process for freezing protein crystals for radiation damage reduction, they surely would also have refused to consider it! Scientific patents were just for science, not commercialization! How the world has changed!

In 1970, I entered industry by joining Philips Electronic Instruments in Mt. Vernon NY as Principal X-ray Scientist and was immediately appointed Radiation Safety Officer. (I now know that being the RSO was considered by the managers as an awful waste of time!) The Mt. Vernon facility was the original Philips plant set up in the United States by N. V. Philips in 1935. The facility manufactured analytical x-ray equipment and sold industrial x-ray systems as well as other Philips instrumentation. I remained on the ANSI Committee for X-Ray Analytical Instruments for many years, but 1977 was the most valuable as we published the revised X-ray Safety Standard N43.2-1977.

My first project was to complete the software for the Automatic Powder Diffractometer System which I believe was the very first commercial computer-controlled x-ray diffractometer system. It used the Philips Digital Controller (4k with optional 4k for analytics) and would scan 35 consecutive powder samples without human intervention. The automatic sample changer and strip chart recorder/paper tape data output would keep an industrial laboratory fully operational on a continuous QC and analysis basis. After completing the software, I wrote the instruction manual and provided technical expertise for the salesmen and at trade shows. As a high-speed automated instrument, it was very advanced for the early 1970s and many were sold to industry worldwide.

About 1975, I participated in making the analytical x-ray safety film for the Bureau of Radiological Health ("The Double-Edged Sword"). The film provided safety advice and precautions for individuals who work with analytical and x-ray spectroscopic equipment. The film appears to have been successful, and I was told that it has continued to be shown into the 21st century. (I recently saw it on YouTube!)

In 1973, I was asked to assist in designing the first Philips "Dynafluor" Airport X-ray Security System because on January 5, 1973, the FAA had instituted mandatory passenger security screening: walk-through metal detectors for people and low-dose x-ray for baggage and hand-carried articles. (Several engineers at Philips Government Systems, Mahwah NJ, were the inventors of the original low-dose security system in 1970.) During 1973 and 1974, the Philips Industrial Group initially built a lead-lined moving-tunnel unit and then supplied a large conveyorized low-dose x-ray unit to several airlines (Dynafluor IV) that proved to be a "dinosaur". I initiated a stealth project to create a lightweight, mobile, low profile system that provided a successful second-generation system for many airlines (Dynafluor VI). In addition to selling hundreds of units worldwide, the Dynafluor VI provided a modern Electronic Security Screening System similar to the current airport units with low profile, short tunnels, high speed and easy-to-use. So during the 1970s, Philips became a major supplier for aviation security equipment. About a dozen patents were issued for the various Dynafluor Security Systems but Philips terminated the business before enforcing any of their patents.

Philips Electronic Instruments moved to Mahwah, NJ in 1976.

I remained with them until 1983 and was mostly involved in low-dose x-ray security matters. One surprising item was that until 1976 neither the FAA nor any airline had actually provided a visual training program (slides or movie) for security personnel (guards) who operated the metal detectors or low-dose x-ray systems at the airports. Philips decided that this was important (as well as an excellent marketing tool) so we arranged to rent a truck, place a low-dose x-ray unit with an electric generator inside so that we could photograph the actual x-ray images of guns, explosives and bombs. I did this on an Army firing range in Virginia with a substantial variety of explosives and weapons. These high-quality photographic images of actual explosives provided the FAA with an excellent slide-training program for aviation security personnel. The slide set was used for years by airlines security managers all around the world and probably saved many lives because it provided actual x-ray images of guns, bombs, and other hazardous items.

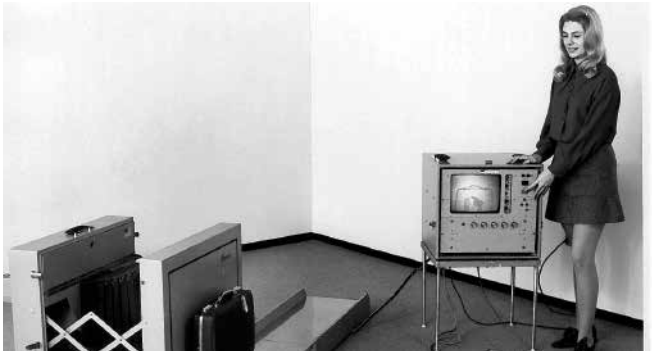
During the late 1970s, I developed an idea for a new type of security ID badge which would prevent the reuse of temporary badges and parking permits for visitors, contractors, etc. After several years of laboratory work at home, Sandra and I introduced the self-expiring Visitor Badge, which changed color a day after being issued to prevent reuse. Our first badge was a coated paper badge with a photochemical that turned blue when the individual left the facility and entered daylight. It was sensitive only to short UV light and was an effective daytime temporary ID. However, our second self-expiring badge changed color by dye migration and was a one-day Time Expiring Badge. Between 1981-2002, more than 20% of all visitor badges issued in the United States were TEMPbadge badges. The business was sold in 2002.

We have returned to the Weizmann Institute of Science several times and supported them with a graduate student fellowship, exactly the same kind that I received in 1966. We were very pleased when Ada Yonath received the Nobel Prize in 2009 for the ribosome structure, using cryocrystallographic techniques. As it turns out, she was a graduate student in the Weizmann Institute's Crystallography Department while I was there in 1966.

One of my desires was to become involved in historical artifacts before they reached the museums, so between 1995 and 2005, I volunteered for many archeological digs and research projects in Israel, Great Britain and Canada. These have provided an ongoing excitement for Sandra and me, particularly when we visit one of the dig sites, and I can show people the inch or so in the "square" that I dug through!

During 2006, I became involved in a short research project for the Smithsonian Institution on the official biography of James Smithson. The author, Heather Ewing, had begun researching this book in 2000 and because of a publication deadline of June 2007, was unable to research the archives of the Royal Institution of Great Britain on Albemarle Street (the Institution where the Braggs had been directors, the Institution in which James Smithson had been a founding member, and the Institution where I spent 18 months of postdoctoral work in 1966 with David Phillips). With a letter of introduction from the "Historian of the Smithsonian Institution", I spent three week in the Royal Institution's vault looking through all the original minutes and notes from 1799 to 1829, the year that James Smithson passed away. My notes and digital photos of the relevant pages were passed on to Heather Ewing who published the book in late 2007 (*The Lost World of James Smithson* by Heather Ewing, 2007).

Beginning in late 2005 with a book project, I spent three years of archival research to determine the people instrumental in developing secure identification credentials and, in particular, who invented the original photo ID badge. During 2007 another project of interest was the invention of the first low-dose security x-ray system, and how the United States Government (and the public) were convinced to pass laws requiring 100% mandatory physical screening of aviation passengers. The first low-dose x-ray system was developed by an engineering group at Philips Government Systems in Mahwah NJ in 1970 by George W. Shepherd Jr. (deceased) and Neal Diepeveen. The Philips group worked "as an unauthorized project" for three years and demonstrated their system to government and airline officials on Sept 25, 1970 in a hanger at Washington National Airport. This was sufficiently convincing for the airlines and the US Government



The original "Saferay", the first low-dose x-ray system developed at Philips 1968-1970.

to institute and pass laws for mandatory security screening of aviation passengers. With 100% mandatory physical screening begun on January 5,1973, the passenger aviation industry was basically saved from worldwide terrorism and criminals. And two new industries were created: metal and explosives detectors for people and low-dose x-ray for baggage and hand-carried articles. Shepherd and Diepeveen were nominated in 2015 for the National Medal of Technology and Innovation.

In 1999, after becoming aware of the success of biological crystal structure determination, I have once again begun reading, watching and attending crystallographic lectures as well as ACA meetings. The entire field of cryocrystallography is new to me, but Elspeth Garman provided me with a number of technical articles on the subject. WOW! In 2006, I attended the cryocrystallography session held at the ACA meeting in Chicago and have been watching many historical lectures on the internet (like the ACA historical videos). I am amazed by the enormous technical growth in biological structures determination in the past 40 years. Crystallography has really become an extraordinary research and industry tool for society!