
CONTRIBUTED PAPERS

THE PROTEIN STRUCTURE PROJECT, 1950-1959: FIRST CONCERTED EFFORT OF A PROTEIN STRUCTURE DETERMINATION IN THE U.S.*

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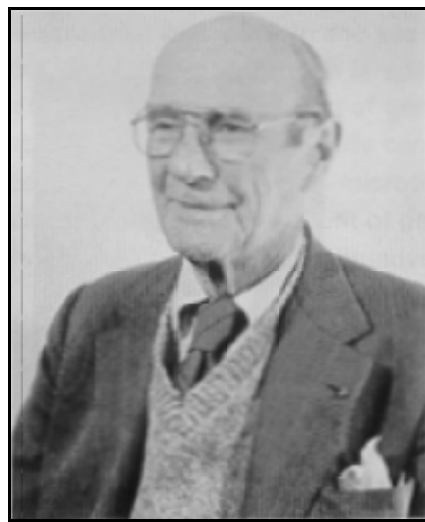
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A scientific discipline usually develops in a small number of different laboratories and grows from there and this is more or less true of protein crystallography. In this country, however, protein crystallography started in an organized, big-bang way in 1950 with the formation of The Protein Structure Project (PSP) at the Polytechnic Institute of Brooklyn. The aim of The Project, led by David Harker, was to solve the structure of a protein molecule in ten years because no protein structure had yet been determined. The following account describes how the PSP began and proceeded through the years. It also underscores some of the monumental problems the PSP and other protein crystallographers faced and how many of them were first solved and overcome. The target protein of the PSP was ribonuclease; its structure was published by Dave Harker some seventeen years after the work started [1].

Amorphous or fiber X-ray diffraction patterns of myosin and feather keratin were originally reported by W. T. Astbury during 1931-1933. The first single crystal pattern of a soluble, globular, wet protein crystal was that of pepsin [2] recorded a year later at Cambridge University by John D. Bernal and Dorothy Crowfoot Hodgkin¹. The work was a tactical breakthrough because it showed that such crystals are better examined in the wet state. Hodgkin went to Oxford University in 1934 where she continued her protein

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¹ The pepsin crystals grew while a Dr. John Philpot in Svedberg's laboratory at Uppsala was on a skiing holiday, they were received by Bernal and Crowfoot four weeks before submittal of their communication, which was published about a week later on May 26, 1934.



David Harker: 1906-1991

work on insulin and lactoglobulin [3]. The next year, Max Perutz came to Bernal from Herman Mark's laboratory in Vienna, where the structure determination of gas molecules by electron diffraction began in 1929, and he started his studies on hemoglobin that were to become classic. The initial work led to a report by Bernal, Isidore Fankuchen and Perutz in 1938 of diffraction by single crystals of chymotrypsin and horse methemoglobin corroborating the importance of the use of wet crystals and offering the prophetic suggestion that a direct Fourier analysis of molecular structure may be possible once complete sets of reflections were available from different states of hydration of crystals [4]. The former has now been realized in a most astonishing way although not by the

application of the latter method. Thus was protein crystallography born.

The growth of single crystal protein crystallography continued in Great Britain with investigations of spherical viruses [5-7] and Bernal and Fankuchen's hallmark discovery that tobacco mosaic virus was composed of a regular substructure [8]. However, further progress was soon to be adversely affected by World War II. Meanwhile in this country, pre-war successes in crystal structure studies of amino acids and peptides resulted from a program initiated by Linus Pauling at the California Institute of Technology and implemented by Robert Corey, Eddie Hughes and co-workers. The work eventually led to Pauling and Corey's monumental prediction in 1950 of the α - and γ -helical configurations that were possible for a polypeptide chain [9, 10] and that they were an important part of the structure of both fibrous and globular proteins. The war years of the forties, for good reasons, were otherwise a generally subdued time for protein crystallography both here and abroad.

During those early days, David Harker was one of Pauling's students when in 1936 he developed the theory of sections and projections of Patterson diagrams [11], which are today still called Harker sections. Shortly after, he discovered with Jose Donnay a new law of crystal morphology extending Bravais' Law, to become known as the Donnay-Harker Law [12], which is said to rank with the original work of Bravais for its lasting and pervasive influence [13]. Subsequently, Harker went to The General Electric Research and Development Center at Schenectady, NY. While there, he and John Kasper derived inequalities relating modified structure factors to the intensities of other X-ray reflections that became known as Harker-Kasper inequalities [14] and served for the development of modern direct methods of Hauptman and Karle relegating 'small molecule' structure determination to a routine exercise.

Legend has it that in the late forties Irving Langmuir, also then at GE, asked Dave Harker what he would do if he had a million dollars. Dave said he would take a ten-year leave and solve the structure of a protein molecule. Langmuir raised the million for the ten year period. The money came from The Dean Langmuir Foundation, The Rockefeller Foundation, The Damon Runyon Memorial Fund, The New York Foundation and The American Cancer Society. Thus, in late 1950, David Harker accepted the responsibility of establishing a research group that would seriously

and concertedly attack the problem of finding the atomic arrangement in a protein molecule. The next thing to be done was to negotiate laboratory space and administrative assistance at a consenting institution.

The end of the war brought rapid development of punched-card techniques of computing and soon Fourier syntheses giving electron density maps and other crystallographic calculations could be done in three-dimensions with relative ease (30-40 man hours per calculation for non-protein problems). Harker recognized the importance of having suitable computational support for the protein problem. Larger computing devices, however, had not yet become commonplace in university or business settings and the Korean War tied-up many for military use. Perceiving that IBM Corp. punched-card electronic calculators would be the wave of the future, Dave arranged a favorable relationship with Thomas J. Watson whereby computations connected with the work on protein structure could be carried out on IBM computers, but by his own people, at IBM's Watson Computing Laboratory on 116th Street in New York near Columbia University. Therefore, the research group had to be located in or near New York City, which proved to be a surprising problem because most institutions in the area were unsympathetic and unenthusiastic about providing space for establishing a full-fledged X-ray protein crystallography laboratory [15]. This was not the case for the Polytechnic Institute of Brooklyn, which was already an important world center for X-ray crystallography with an outstanding contingent of crystallographers in the Department of Physics: Paul R Ewald (Chairman), who developed the dynamical theory of diffraction as a student with Arnold Sommerfeld in Munich, Herman F. Mark (Director of Polymer Research), one of the early pioneers of X-ray crystallography and ever present for advice, Isidore (Fan) Fankuchen of early protein crystallography fame, Rudolph Brill, a research professor diffraction physicist and Ben Post, a then young, but all-around superb X-ray crystallographer. It was Fan who called the President of Brooklyn Poly and persuaded him to provide Harker space and then closed the deal with Dave to come to Poly [15]. The new research group was called The Protein Structure Project and it was assigned about half of the 4th floor of 55 Johnson St. near downtown Brooklyn and the Brooklyn Bridge directly across the street from the Brooklyn Post Office.

Harker gathered a small group to tackle the then known fearsome aspects of protein structure

determination (crystallization, intensity data collection, computation). The research space consisted of Harker's office shared with his secretary and wife Katherine, two other shared offices, a general work space, a chemical and crystallization laboratory and a X-ray diffraction room adjoining a dark room. The initial staff of postdoctorals were Beatrice S. Magdoff, to carry out crystallographic analyses, Thomas C. Furnas, Jr., an X-ray diffraction physicist to design and construct new diffraction equipment and Murray Vernon King, a chemist to grow crystals and do chemistry on proteins. Another very important, and in ways indispensable, member of the original team was William (Bridgie) Weber, instrument maker *par excellence*. Bridgie was at GE Schenectady as a master machinist and had previously worked for Dave when Dave helped design a commercial X-ray powder diffractometer for GE X-ray Corp. in Milwaukee. Dave convinced Bridgie to come to Brooklyn because he knew that new diffraction equipment would have to be invented and built to cope with the protein data collection problem. Bridgie retired from GE, went to and remained with the PSP throughout its stay in Brooklyn sharing an apartment with Furnas until Tom was married and even moved with The Project to Buffalo in 1959. Beatrice Magdoff left about 1955 and went to the Boyce Thompson Institute for Plant Research in Yonkers to start a program on southern bean mosaic virus while Furnas and King remained until near the end of the decade when the PSP moved and continued at The Roswell Park Memorial Institute in Buffalo, NY.

The target protein of the PSP was the enzyme bovine pancreatic ribonuclease: it was available in pure form from Armour Laboratories in Chicago, it was relatively small (MW= 13.7 kD) and it had been crystallized by Moses Kunitz at Rockefeller Institute (but with no diffraction studies). However, Vernon King was unable to grow ribonuclease crystals during the first year of the PSP. Late in 1951 a delegation from The Project visited Kunitz and conferred with him about his crystallization procedure. Nothing different could be found from the methods being used at the PSP so the group was understandingly discouraged. Not long after to everyone's delight, ribonuclease crystals appeared in amorphous precipitates of several of King's crystallization tubes and the PSP dodged a potentially devastating obstacle². Thereafter, thirteen other different crystal forms of ribonuclease were prepared and characterized by X-ray diffraction [16, 17]. To the very end, Harker

suspected that during the Kunitz visit they had picked up seed crystals and carried them back to Brooklyn where they transformed incipient crystallization into a reality.

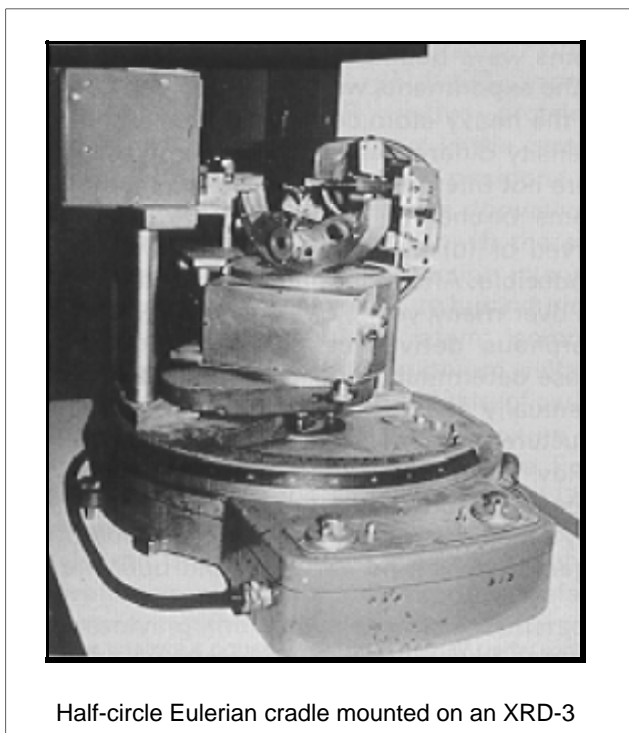
One of the overwhelming problems that faced protein structure determination was the measurement of diffraction intensities. While the number of three-dimensional reflections of small molecules were generally in the low thousands (2,500 for vitamin B₁₂), this number was 10-20 times greater for smaller proteins. Precession photographs were the favored measurement method at the time, which gave undistorted representations of the reciprocal lattice ("diffraction space"). Protein X-ray exposures typically required 15-20 hours and since crystals suffered X-ray damage, only 1-2 exposures could be obtained from a crystal. If 30-40 layers of three-dimensional data had to be recorded, many crystals were required to complete the measurements. Add to this the time required to align each crystal, the many screen checks of crystal alignment that had to be made, the layers that had to be remeasured because crystals slipped in their wet mounts during exposure and crystals mounted in other directions to record blind regions of reciprocal space, not to mention estimating and processing all the intensities from the photographs, six months of very hard and continuous work along with some luck might possibly produce a threedimensional data set. In practice, application of Murphy's constant π to such estimates gave more realistic times.

Harker was acutely aware of these difficulties, which were partially the reason for recruiting Tom Furnas who had just completed his Ph.D. at MIT working for Richard Bear, Martin Buerger and Bert Warren. Tom and Dave decided to measure intensities of reflections by depending solely and exclusively on calculated coordinates of reciprocal lattice points of reflections and to measure reflections only in the

² These crystals were monoclinic, space group P2₁ (modification II) [16] and they were a different form from Kunitz's crystals. Discussing the crystallization procedure more carefully with Kunitz, King noted that the beef pancreases of cows used for isolating ribonuclease were collected at a slaughter house in galvanized buckets. Vernon suspected that metal ions may have been leached from the pail so he tried crystallization setups in the presence of Zn⁺² ion and various other transition metal ions like Fe⁺², Fe⁺³ and Ni⁺². It is a credit to Vernon's insight that he discovered the orthorhombic crystal form of ribonuclease grows only in the presence of Ni⁺² ion, which are Kunitz crystals.

equatorial plane defined by the X-ray source, the crystal and the detector [18]. Very importantly, all possible reflections could be measured by this procedure with only one orientation or mounting of the crystal. All that was required was a device capable of reorientating a crystal so that every diffracted beam could be made to occur in the equatorial plane. This instrument was designed based on a half-circle orienter mounted on a GE XRD-3 powder diffractometer with a scintillation counter detector and built by Bridgie Weber in the first few years of the PSP and the half-circle was known as an Eulerian cradle [19, 20]. Tom later designed a quarter circle instrument that was marketed commercially by GE X-ray under the name Single Crystal Orienter. He also wrote an excellent instruction manual [21], both theory and practice, to accompany the Orienter

The Eulerian cradle was manually operated by setting three angles at pre-computed positions, measuring the intensity of a reflection and background and hard copying the results. As many as 75-100 reflections (10 second count time) could be measured in an hour, total exposure times were reduced to about 50-60 hours for about 10,000 reflections, the number of crystals to complete a data set was very small and a three-dimensional data collection could be completed in about 2 weeks. Thus, the Eulerian cradle reduced protein data collection to manageable proportions. A second full-circle instrument using Eulerian geometry



was under construction at the PSP by 1954 to incorporate complete automation through computer control by IBM punched-cards. Tom Furnas left the PSP in 1958 to go to the Picker X-ray Corp. in Cleveland where he went on to invent a whole new array of diffraction equipment, the foremost of which was the FACS I system, the first directly-coupled computer controlled single crystal X-ray diffractometer. It revolutionized intensity data collection and its offspring are still the instrument of choice thirty years (early 1990's) later for small molecule structure determination.

Harker strengthened his group during the first five years with additional technical personnel in Dalia David, who helped with punched-card calculations on IBM plug-board machines like the 602A, 607 and 409 printer, Edith Pignataro, who was primarily concerned with measurements using the Eulerian cradle and Myra Edelman, a Ph.D. graduate student. Illustrious visitors Vittorio Luzzati from Paris and Francis Crick from Cambridge University were also in residence during this time. Crick and Luzzati collaborated with Magdoff (1953-54) on a number of different problems ranging from shrinkage stages of ribonuclease [22] and the calculation of its three-dimensional Patterson function [23] to theoretical aspects of isomorphous replacement in protein crystals [24].

By the mid-fifties, it had become clear that study of shrinkage stages of protein crystals was not going to lead to structure. However, the new work of Max Perutz and his colleagues in England with heavy atom isomorphous replacement was showing great promise. Using p-mercuribenzoate, about 100 phase angles of centrosymmetric (h0l) reflections (\pm signs) of hemoglobin had been determined [25]. In principle, three isomorphous crystals are a necessary and sufficient condition to determine all the phases of a non-centrosymmetrical protein crystal. The derivation and mathematics of the multiple isomorphous replacement method was first described completely by Dave Harker in a classic paper appearing as the first article, of the first issue, of *Acta Crystallographica* in 1956 [26]. All the early protein structures were solved by this method (myoglobin, hemoglobin, lysozyme, ribonuclease, chymotrypsin, carboxypeptidase, cytochrome c, etc.).

I came to the PSP as a new post-doc in September 1955 from Princeton University after obtaining a degree with John G. White who was working on the "Princeton structure" of vitamin B₁₂. John's work

impressed me deeply and inspired me to also go on to big structures. Little did I know what would be in store during the next 40 years. My first assignment at the PSP was to extend multiple isomorphous replacement to include anomalous scattering because Dave had recognized its potential power and many advantages. My most important contributions to The Project, however, involved writing crystallographic computer programs and computations related to structure analysis.

The plug-board IBM punched-card electronic calculators of the time (about 35 memory locations and 50 operational steps maximum) were horrendously tedious and prone to error due to the massive card handling required in even fairly simple computations. For example, when the three-dimensional Patterson map of ribonuclease was calculated it was “believed to contain only minor errors, say less than $1.0 \times 10^{-2} \text{ \AA}^{-3}$ at any point” [23]. This was not the expected error due to errors in the intensities of reflections but rather, the expected reproducibility of the calculation if it were done again. All eventually became history in 1956 with the introduction and advent of the IBM 650 computer having a 2000, ten digit word magnetic drum memory with millisecond access times. It was the first of the modern day programmed computers where instructions were entered as coded numbers. Harker sent me to IBM school for the 650 at Watson Laboratory. Thereafter, programs were written for most of the computations required to solve a protein structure. Many of these were shared with Barbara Low's group (Jan Drenth, Ralph Einstein, Wolfie Traub) at Columbia University working on the structure of insulin and also using Watson Laboratory for computations.

During the final five years of the PSP (1955-59) computing underwent a tremendous transformation with the appearance of IBM 700 machines (701, 704, 709, 7090, 7094)³. Random access magnetic core memory was introduced along with microsecond cycle times and massive memory storage devices like magnetic tape drives with data channels and large capacity magnetic drums and disks making computational problems of protein crystallography feasible to accomplish within reasonable time frames. The crystallization, intensity data collection and computational aspects of the PSP were now in place. All that

³ It was difficult keeping up with the turn-around because each new machine generally had new and quite different programmable features.

remained was to prepare several suitable heavy atom isomorphous derivatives of ribonuclease crystals to solve the protein phase angles and compute an electron density map that would reveal the structure at the atomic level.

Heavy atom isomorphous searches were a very new area of investigation and not much was known about them at the time, so success did not come easily (still somewhat true today). Heavy atom searches, in fact, rank a close second to protein crystallization as an art form. Vernon King began searching for isomorphous crystals by preparing ribonuclease derivatives attaching heavy atoms to side chains. Most of these approaches failed to produce crystals or produced crystals that were not isostructural with the native enzyme. Another approach was co-crystallizing ribonuclease with heavy atom chelate complexes or dyes⁴ but with similar results. Since the expected diameter of ribonuclease was about 30 \AA , many of the trials probably did not succeed because this enzyme was small and the resultant changes were fairly large. The smallness would therefore also apply to the interstitial space in crystals filled with mother liquor. With time, the most expedient way of making heavy atom isomorphous derivatives proved to be by diffusing *small molecule* heavy atom containing compounds, preferably neutral or anionic, into crystals from days to weeks and examining X-ray patterns for changes in diffraction intensities to ascertain whether heavy atoms were bound in a systematic way. Many of the experiments were disappointing because: (a) the heavy atom compound did not bind, (b) intensity differences led to Patterson maps that were not interpretable because too many heavy atoms bound, (c) isomorphism was not preserved or (d) derivative formation was not reproducible. After a large number of different trials over many years, 6-7 good heavy atom isomorphous derivatives of varying degrees of phase determining quality and usefulness were eventually found that permitted the molecular structure of ribonuclease to be determined (1).

Roy Worthington joined the PSP from Adelaide, Australia about the same time as I did. Roy's thesis was on low angle diffraction of collagen for which he designed and built the first rotating anode X-ray tube.

⁴ Once when Vernon King was wearing a sweater with loud colors, Dave pointed out wool was a protein and dyes contained heavy atoms, which led to the use of the latter in heavy atom derivative searches at the PSP.

He later went on to Kings College (London) continuing low angle diffraction studies of muscle and membranes and is now at Carnegie-Mellon University. Jake Bello and Gopinath Kartha came shortly after, went to Roswell Park when The Project moved, and were the prime movers in the ultimate determination of the structure of ribonuclease [1]. Jake came from Eastman Kodak Co., Rochester, and assumed the chemistry aspects of the work when Vernon King went to Childrens Hospital in Boston to work on glucagon (now at NY State Department of Health, Albany) while Gopi Kartha, a student of G.N. Ramachandran at Madras and later a postdoc at the National Research Council in Ottawa, overlapped and shared responsibilities with me until I left for a teaching and research position at Yale University in 1959. Erik von Sydow of the University of Uppsala in Sweden was also a member of the PSP about this time as a visiting scientist well-known for his crystallographic work on long chain fatty compounds.

In those early years of protein crystallography, we at the PSP (and also other protein crystallographers) worried about some seemingly insurmountable problems that many today do not know were ever a concern. Some of these included: (a) using ammonium sulfate or alcohols for crystallization, (b) allowable changes in unit cell dimensions on heavy atom derivative formation, (c) measuring mosaic spreads of X-ray reflections to choose the best unique region of reciprocal space to measure and use, (d) calculating non-centrosymmetric difference Pattersons with coefficients $(|F|_{p+h}-|F|_p)^2$ ($|F|_{p+h}$ = protein plus heavy atom, $|F|_p$ =native protein), which are not even strictly correct in the centro case, (e) referring the heavy atom positions of two or more different isomorphous derivatives to the same crystallographic origin, (f) the absolute scattering scale of the diffraction intensities and much more. These kept us busy during the seemingly endless heavy atom isomorphous derivative searches. Ammonium sulfate and alcohols were known to be capable of causing structural changes so a protein structure of such grown crystals could be questionable. Furnas considers (c) thoroughly in his Manual [21] while Harker addressed points (b), (e) and (f) at great length in his original paper on multiple isomorphous replacement [26] without satisfactorily resolving the difficulties. The Patterson difference coefficients were eventually accepted on the faith that most of the time the heavy atom vector maps were correct. The origin problem was definitively solved by Michael Rossmann

calculating the difference Pattersons between heavy atom isomorphs [27]. The absolute scale, which eventually proved to be a non-issue, led Harker to commission Roy Worthington and myself to accurately solve the structure of basic beryllium acetate to be later used to experimentally place protein diffraction on an absolute scale [28-30]. An unexpected byproduct of the work was the very early, if not the first, observation of bonding electrons and the general acceptance of beryllium acetate crystals as an intensity standard.

The initial crystallography of ribonuclease concentrated on the orthorhombic, $P2_12_12_1$ (modification I) crystal form [16]. The three centrosymmetric projections and the three Harker sections at 1/2 of this space group were distinct advantages; the crystals, however, only diffracted X-rays moderately to about 2.5-2.8Å resolution. Many potential heavy atom derivatives were measured and analyzed with high hopes over a 2-3 year period only to spawn discouragement as few appeared to be useful. When John Kendrew visited the PSP in 1958 and was shown some of the complicated difference Patterson maps, he told Kartha and me "these are the kind we discard at Cavendish". He was the person to know because their derivatives produced the structure of myoglobin [31]. Most of the crystallographers of Poly's Department of Physics were also there in attendance, including Lindo Patterson from The Institute of Cancer Research in Philadelphia, examining the 6Å resolution map and model of myoglobin. All of us were somewhat surprised, if not amazed, by the unexpected irregular intestine-like folding of the myoglobin molecule and some even wondered if it were correct. Not long after, the doubts disappeared when the 2.0Å resolution map and structure of myoglobin was published [32].

Kartha and I kept trying to interpret unruly, complicated heavy atom difference Patterson maps applying many different strategies including Patterson superposition methods and minimum function principles. These led Gopi to perceive the utility of a double phased Fourier or a weighted single isomorphous replacement method to obtain structure with only one heavy atom isomorphous derivative in the non-centrosymmetrical case. The idea was to assign both the ambiguous phases (the correct phase and the incorrect one) calculated from a single isomorphous derivative to the observed amplitude and compute a double phased Fourier synthesis of the electron density [33]. It was simple in its conception:

the correct phases produce the structure; the incorrect ones do not systematically synthesize structure through the Fourier series and generally only contribute randomly to the background. We tested the idea on a hypothetical structure and showed that it worked satisfactorily [33]. Subsequently, it was tested with a real case, the then large 50 atom (nonhydrogen) unknown antibiotic structure of isoquinocycline A that was also affected by observational errors. An unwanted complication was the 18 replaceable electrons were located at a special position introducing a spurious mirror plane of symmetry in the resulting map [34]. The method worked fine regardless of the problems. Rossmann and Blow developed a similar treatment about the same time [35] (the PSP work was completed by late 1958) and even showed it could be combined with anomalous scattering data. Since then it has been applied many times in protein structure determination with B.C. Wang's solvent flattening procedure judiciously adjusting the weight of the two phase solutions.

In 1959, work at the PSP was winding down in preparation for the move to Buffalo and the Roswell Park Memorial Institute. It was easy on my part to tell Gopi as I was leaving that he should abandon the orthorhombic crystals in favor of the original monoclinic variety (modification II) [16], which diffracted X-rays to about 1.8 Å resolution. It was probably much more difficult on his part to make the decision because of all the work invested in the orthorhombic form. No matter, Gopi made the monoclinic choice so he and Jake Bello had to backtrack to produce monoclinic crystals, measure a complete set of native monoclinic intensities, rewrite computer programs (because some were space group specific for speed of computation) and begin heavy atom isomorphous searches of this crystal form. The effort received a significant boost in 1963 when Smyth, Stein and Moore determined the sequence of ribonuclease and the National Science Foundation and the National Institutes of Health provided financial support. Things went fairly smoothly thereafter, the monoclinic choice was the right one, and the structure was solved at 2.0 Å resolution about six years after leaving Brooklyn and rebuilding the laboratory.⁵ A sad note of the time was that Dave Harker's long time dear friend Bridgie Weber (and

mine also) passed away with a stroke in 1963 after they had dinner and were walking home together. Gopi Kartha stayed on at Roswell to begin structural studies of gramicidin that were cut short by his untimely death in 1984. Jake Bello also remained at Roswell and retired a few years ago. And by the early 1960s, the U.S. was well-represented in protein crystallography with many laboratories at various stages of growth and development. Among the earliest were those of Bill Lipscomb, Harvard and Lyle Jensen at the University of Washington, both also renowned for their small molecule crystallography, other labs that started were: Michael Rossmann, Purdue; Dick Dickerson and Larry Steinrauf, Illinois; Al Cotton, MIT, Hal Wyckoff and Fred Richards, Yale; Joe Kraut, LaJolla; David Davies, NIH; Len Banazak and Scott Mathews, Washington University and myself at Michigan State.⁶ Most Of these crystallographers were postdocs of Kendrew and Perutz coming off the triumphant structure determinations of myoglobin and hemoglobin in Great Britain. All went on to solve protein structures and leave positive and lasting marks on the field.

It can be said safely that the tremendous progress and growth of protein crystallography worldwide over the last 30-40 years is probably stunning even to those with whom it originated. The development of ever faster computers, their time-sharing capabilities and miniaturization has proceeded at an unprecedented rate with no end in sight at corresponding lower costs so no computation is any longer "too big". However, interactive computer graphics might well be the one most crucial and important development of protein crystallography because it serves as the channel through which results of protein crystallography are passed on to the remainder of the scientific community. Early reports about myoglobin, lysozyme and hemoglobin appeared in Scientific American primarily because the graphical artwork was in place [36-38]. Add to all this, three-dimensional intensity data collecting times reduced to hours by area detectors, high intensity mirror-focused rotating anode X-ray tubes and synchrotron X-ray sources and charged-coupled detectors on the horizon capable of making measurements an order of magnitude faster, protein crystallography is indeed

⁵ The structure of the orthorhombic form has also been recently completed in R. Parthasarathy's laboratory at Roswell.

⁶ Memories are fallible. This compilation is from memory. If I overlooked anyone, I'm sorry.

wondrous and is attracting the finest intellectual talent in the world. There is reason to expect the next 40 years to be even more so. Given the opportunity, however I would still not trade the experiences of my marvelous past for all the wonders that most certainly lie ahead.

David Harker became Research Professor Emeritus of the Medical Foundation of Buffalo in 1976. He was one of the most notable scientists of this century and at the Medical Foundation he continued working on his life-long passion of symmetry, studying colored space groups and infinite two-dimensional polyhedral sets. His final paper describing the symmetry of a new class of polyhedral sets appeared in the Proceedings of the National Academy of Sciences about a month before he passed away. Dave was a warm, friendly and unpretentious person always delighted with and concerned about others and above-all, always helpful. His joy for science and his humble demeanor made him a great teacher of many different things, not only crystallography. Herb Hauptmann put it aptly when he said “he (Dave Harker) was a tireless seeker of the truth, wherever he could find it, and in this quest he succeeded as few others have.”

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