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[1]Focal contributions to molecular biophysics and structural biology: a personal view. Part III[☆,1]

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1. Introduction

The purpose of this venture is to write a personal history of the biophysical sciences based on focal contributions from approximately the beginning of the 20th century, as seen at the end of maybe the most evolutionary, or rather revolutionary, period in the development of the natural sciences. By personal history I emphasize that it is not a complete history as produced by a historian, but rather a live presentation of a selection of scientists, laboratories and events as encountered in the course of a scientific career extending from approximately the middle of the 20th century to the present day [1]. Thus, part of the story is based on personal encounters and recollections, and part on events and personalities close to my own existence, but in contact only through their work or through common personal connectivities. I will, in Part I, give a detailed reason for describing these focal contributions to modern biology. Professional physicists who until recently carefully avoided biological research have now become engaged in this activity in an intensive way. However, for reasons not clear to many of us, they practically disregard biophysical work performed before their time, which I plan to describe in this work. I recall Hans Frauenfelder quoting Stanislaw Ulam, when asked for his opinion about biology and physics: 'Ask not what physics can do for biology, but rather what biology can do for physics' [2].

I acknowledge a large number of written recollective contributions, quoted in this work, from which I have culled material for my efforts within the framework, albeit incomplete, of presenting the drama of discovery from various points of view, in a hopefully unified way. I also acknowledge valuable criticism from many highly qualified friends and colleagues who have experienced the same thrill as myself, maybe from a slightly different point of view. I first conceived writing these lines in 1994 while I was a visitor in the Dipartimento di Scienze Biochimiche 'A. Rossi Fanelli', Universita di Roma 'La Sapienza', invited by my friend, Milina Chiancone. Maurizio Brunori is now the Director of the Department, some of whose seminal contributions to modern science, in which he participated, will be described below. During my stay in Rome we tried and succeeded to put into operation the latest state-of-the art commercial ana-

☆ Dedicated to Maurizio Brunori on his 65th birthday. This work is in three parts with age of dedication decreasing with increasing numbering. Parts I and II are in preparation.

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[1] While this manuscript was in press, Max Perutz died on 6th February 2002, at age 87. His seminal contributions to science and human values will not be lost.

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Fig. 1. La Cura Conference in Caprarola, 1980, Participants names are from left to right; (a) Rodney Biltonen, Gary Ackers, Giovanni Rialdi, Milina Chiancone (in front), Eraldo Antonini, Bill Eaton; (b) Eraldo Antonini, Joseph Bonaventura, Celia Bonaventura, Heini Eisenberg, Ted Traylor, Giovanni Rialdi, Takashi Yonetani; (c) Bill Eaton, Maurizio Brunori; (d) Celia Bonaventura, Jeffries Wyman, Bill Eaton (he seems to be almost everywhere).

lytical ultracentrifuge, beautiful in its advanced conception and execution and, in those days, still unmatched in the complexity of its day-to-day performance.

History plays strange tricks, and it is appropriate here to mention the heroic efforts of The Svedberg in Uppsala in the second and third decades of the 20th century in creating from scratch and from his dreams a series of the first analytical ultracentrifuges, confirming the nature and size of protein macromolecules. I have discussed the 'Birth of the Macromolecule' [3] in dedication to the memory of Bill Harrington, and recommend study of the recent presentation of Charles Tanford and Jacqueline Reynolds [4] relating to the colloid/macromolecule debate.

My association with Maurizio extends over a broad range of personal and scientific relations over a large number of years. I was privileged to attend in 1980 the last La Cura conference in Caprarola, north of Rome, devoted to hemoglobin and to the celebration of Jeffries Wyman's upcoming 80th birthday [5]. The monks in the monastery had asked us to drink all the wine in the cellar, as they needed space for the new wine to be bottled the current year. We therefore were never thirsty.

However, one night, following the evening lectures, we got very hungry. At midnight, led by Maurizio, we broke into the kitchen and Maurizio prepared the most delicious pasta for the believers in allostery and hemoglobin, that we have ever eaten (Fig. 1). With Maurizio I share membership at EMBO, the European Molecular Biology Organization, and in 1981 and 1986 we organized with Sandro Coda two exciting EMBO biophysics schools in Pavia. We were also shown then the bench in the lecture hall at the Pavia University where Napoleon sat listening to Alessandro Volta lecturing. With Maurizio I shared successful joint activities in the council of IUPAB, the International Union of Pure and Applied Biophysics, in the organization with Israel Pecht of the outstanding 9th International Biophysics Congress in August 1987 in Jerusalem. In 1990 Maurizio beat me in his election to the Presidency of IUPAB and I had to content myself with the Vice-Presidency. Italy, where I spent two years in the British Army in World War II, is my land of choice, and in 1982 with my wife Nutzi and our then 8-year-old grandson Ido, we stayed in the Federica and Maurizio Brunori home in the Gianicolo hills overlooking Rome, before driving south to Sicily for a summer school in Erice, near Palermo.

2. MRC Laboratory of Molecular Biology, Cambridge

In 1928 Hermann Mark and Kurt H. Meyer proposed a tentative structure of silk fibroin, the first protein structure obtained by X-ray diffraction [6]. A few years later Mark became professor of physical chemistry in Vienna and Max Perutz was an undergraduate there. In 1935 Perutz had asked Mark going on a trip to Cambridge to arrange a place for him in the biochemical laboratory of Gowland Hopkins, however, Mark got so excited by the X-ray diffraction results of John Desmond Bernal and Dorothy Crowfoot (later Hodgkin) of crystalline pepsin, suspended in its mother liquor, that he arranged for Perutz to work with Bernal in the Cavendish physics laboratory. Perutz arrived in Cambridge in 1936. He was taught useful crystallography by the American crystallographer Isidore Fankuchen in the lab of the ‘sage’, Bernal, as he was called by his collaborators. Perutz was looking for an appropriate protein to study. In 1937 he was advised by Felix Haurowitz, husband of one of his Prague cousins, to tackle hemoglobin. The Cambridge physiologist Gilbert Adair taught Perutz how to grow hemoglobin crystals. In 1924 Adair had been the first to correctly determine the 67 000 g/mole mass of the hemoglobin tetramer and that of other proteins, by using osmotic pressure. This was confirmed by Svedberg in 1925 in his early analytical ultracentrifuge studies [3]. David Keilin, professor of biology, offered Perutz bench space in the Molteno Institute in Downing street, to start his studies.

Hemoglobin conveys oxygen from the lungs to the tissues and promotes the return of carbon dioxide from the tissues back to the lungs. A hemoglobin molecule consists of four polypeptide chains, two α chains of 141 amino acid residues each and two β chains of 146 residues each. Each of the four chains harbors one heme, which gives blood its red color. The heme consists of a ring of carbon, nitrogen and hydrogen atoms called porphyrin, with an atom of iron at its center. A single polypeptide chain with a single heme is a subunit, or monomer of hemoglobin; in the complete hemoglobin four subunits are joined to form a tetramer.

In 1937 William Lawrence Bragg was appointed to lead the Cavendish Laboratory following the death of Ernest Rutherford. When Bernal moved to Birkbeck College in London in 1938 Perutz did not follow him because of lack of money. However, a few weeks later Bragg got excited by being shown the initial Perutz X-ray pictures of horse ferric hemoglobin, and realized the future significance of X-ray analysis of the large molecules of the living cell. He appointed Perutz as his research assistant with a grant received from the Rockefeller Foundation.

In March of 1940 Perutz obtained his Ph.D. degree, however, a strange interlude in his life and scientific career occurred in May of 1940, after the outbreak of World War II. He and many Jews and other refugees from Nazi Germany were transported to Canada under difficult conditions as enemy aliens [7], many killed by German U-boat action. Perutz returned to Cambridge in January 1941, still registered as an enemy alien. He and Hermann

Mark then took part in the tragicomic production of reinforced ice, the 'Habakkuk' project originated by Geoffrey Pyke to create huge seafaring ice-surfaces for accelerating victory in the war [7]. Eventually, late in 1943, after the 'Habakkuk' demise, Perutz could return to his scientific work at the Cavendish. He was joined in the autumn of 1945 by a young smartly dressed officer, John Kendrew, keen to do a Ph.D. in protein crystallography, following his release from army service. Kendrew choose sperm whale ferric myoglobin for this research.

Myoglobin is similar in constitution to the β subunit of hemoglobin but consists of only one polypeptide chain and one heme. Myoglobin combines with the oxygen released by red blood cells, stores it and carries it to the subcellular organelles, mitochondria, where chemical energy is produced by the combustion of glucose to carbon dioxide and water. In principle the determination of the X-ray structure of myoglobin should be simpler than that of hemoglobin, however, providing basic information towards an understanding of hemoglobin structure and function.

Excited by the remote chance of success of the hemoglobin and myoglobin work, Bragg succeeded in convincing the Medical Research Council (MRC) to establish on 1 October 1947 'The MRC Unit for the Study of the Molecular Structure of Biological Systems', simplified by Perutz 9 years later to 'Laboratory of Molecular Biology'. In due course Perutz and Kendrew were joined in 1948 by the physicist Francis Crick who in 1953 solved the DNA double helix structure with Jim Watson [8,9] who came in 1950 from the USA, using data from Rosalind Franklin's studies. Sidney Brenner joined the unit in 1957 collaborating with Crick in the discovery of messenger RNA and the triplet nature of the genetic code. Hugh Huxley joined Kendrew in 1949 as a research student, and eventually solved the sliding filament model of muscle [10].

In 1953 Perutz found a way of solving the phase problem in X-ray diffraction patterns of crystalline proteins using heavy atom derivatives, the isomorphous replacement method [11]. This led in 1960 to the solution of the structures of myoglobin by Kendrew and collaborators to 2.0 Å and of hemoglobin by Perutz and collaborators to 5.5 Å resolution. Perutz and collaborators extended the resolution of the hemoglobin structure to 2.8 Å in 1968 and to 1.74 Å in 1984 [12]. In 1970 Perutz presented a detailed atomic model for the stereochemical basis of cooperative oxygen binding [13,14].

Realizing the potential of molecular biology achievements the MRC decided to exploit these developments by constructing a new building to accommodate the exciting work in progress. The Laboratory became operative on Hills Road in 1962, allowing for continuous outstanding operation under more appropriate and pleasant conditions. Perutz was the Director of the Laboratory until 1979 and 'Gisela, Max's marvelous wife', in the words of the late Paul Sigler [15], 'made sure that the canteen, the intellectual hub of the lab, was an inviting place with good food—a job made easier by the prevailing English standard. There were no locks on the office and laboratory doors at the LMB and, I suspect for the same reason, there was never someone called Sir Max'.

Fred Sanger, who provided the sequencing methodologies for both proteins and nucleic acids, moved to the new MRC-LMB laboratory in 1962 [16]. He was joined in 1963 by Cesar Milstein, creator with Georges Köhler of monoclonal antibodies, introducing immunology into the ranks of the molecular sciences [17]. Aaron Klug, started work on biological structures in Bernal's department at Birkbeck College in London 'along the road of virus structure' with Rosalind Franklin, who died at an early age by cancer in 1958. Collaborating with Ken Holmes Klug moved into the LMB laboratory in 1962 [18]. He became Director of the laboratory in 1986 and the present Director is Richard Henderson, active in the successful study of membrane structure by electron microscopy. Klug continued to work on the structure of viruses and on the assembly of tobacco mosaic virus, TMV. Study of electron micrographs of viruses led to quantitative methods for their analysis and the calculation of three-dimensional maps. Work on the structure of DNA and of RNA was included. The crystal structure of t-RNA was established in 1974 and more recently a hammerhead ribozyme RNA was solved. Analysis of the nucleosome core particle, defined by Roger Kornberg

on a working visit to the LMB, and higher order structures, led to a better understanding of DNA packing in chromosomes. Studies of transcription factor binding to DNA with Daniela Rhodes, led to the discovery of the zinc finger domain.

3. The 'Alessandro Rossi Fanelli' Department of Biochemical Sciences in Rome

The study of hemoglobin goes back to the 19th century and hemoglobin was first crystallized in 1840 [4]. A plausible molar mass of 16 700 was determined by analysis of the low but fixed iron content of hemoglobin from many different species. The Adair osmotic pressure and Svedberg analytical ultracentrifugation studies established the tetramer nature of the protein.

The role of Italian biochemistry in hemoprotein studies goes back to 1882 and has been described recently [19]. Alessandro Rossi Fanelli entered this research field in Naples in the late 1930s and produced the first experimental evidence that hemoglobin and myoglobin both had the same prosthetic group, meaning that the physico-chemical and functional differences between the two molecules had to be traced to differences in the globins [19]. Rossi Fanelli moved in 1945 to Pavia where he switched from horse to human myoglobin, and assumed in 1949 leadership of the La Sapienza University of Rome Department of Biochemical Sciences, which now carries his name.

Following partial destruction of the university building in Rome during the war, lack of adequate equipment and absence of technical and research personnel in the post-war years, recreation of productive circumstances became a major objective, successfully achieved by Rossi Fanelli. Doriano Cavallini returned from a long period of stay at the Du Vigneaud laboratory in Cornell University, New York. Eraldo Antonini, Carlo de Marco and Bruno Mondavi joined the laboratory to complete their studies. The building was reconstructed and equipment obtained through the Marshall Plan and the Rockefeller Foundation. The Italian Biochemical Society was founded in Rome in 1951 and Maurizio Brunori and Emilia Chiancone joined Antonini in collaborative research. Research in myoglobin was continued, the discovery of the transheme reaction, the exchange of myoglobin and hemoglobin hemes under physiological conditions became a much cited achievement, probing the role of the chemical nature of the prosthetic groups in controlling the phenomenon of heme–heme interaction and the Bohr effect.

Jeffries Wyman arrived in Rome in the spring of 1960, 'captivated by Eraldo Antonini's engaging personality' [20]. Following this visit Wyman, at age 60 at that time, stayed in Rome for 25 years, collaborating with the Rome group in understanding the structural and functional properties of hemoglobin and hemoglobin sub-units at a time when the three-dimensional structure was determined by Perutz, increasing in resolution with time. Arguments centered around discussions whether the induced fit model of Koshland, Némethy and Filmer was more or less appropriate than the Monod, Wyman and Changeux model to describe the behavior of hemoglobin in biological action. Discussions in Rome and at the La Cura Conferences in the vicinity of Rome were attended by outstanding worldwide scientists in terms of hemoglobin behavior and with the broad picture of protein chemistry in mind. In 1983 the scientific community in Rome was deeply shaken by the death of Antonini at only 53 years of age [21]. This also led to the eventual departure of Wyman from Rome. One should also mention briefly successful work started in 1963 by Rossi Fanelli and Mondovi in a work group including biochemists, oncologists and anesthetists on necrosis of cancerous tissue by the application of heat to blood, with excellent clinical results when applied to man [19].

Functional and structural aspects of hemoglobin in fast moving research achievements were summarized by Antonini and Brunori in 1971 [22] and in a symposium dedicated to Perutz in 1982 [23]. Brunori, more recently, reflects on hemoglobin as an honorary enzyme [24], dedicated to Jeffreys Wyman and his achievements in his stay in Rome from 1960 to 1986, when he moved back to Paris and died in 1995. In this last period of his life Wyman collaborated with Stanley Gill, who also died at a relatively young age, to write a comprehensive book [25] on binding and linkage of proteins and nucleic acids with a variety of low molar mass molecules of biological interest. In his article

[24] Brunori discusses the broad activities of the Rome school following the newly available three-dimensional Perutz X-ray structures of ligand bound hemoglobin and deoxyhemoglobin. Wyman, whose interest in linked functions in hemoglobin research went back to 1948 [26], collaborated with his Paris friends in the creation of the Monod–Wyman–Changeux model [27] explaining allosteric effects in hemoglobin and in enzyme systems. The concept of allostery was applied to heme–heme interactions and the classical Bohr effect, based on the existence of two quaternary states in the tetramer containing four heme groups separated by a large distance. An exciting period started with agreement and opposition to the MWC model, in distinction to a 1935 model developed by Linus Pauling and expanded by Koshland, Némethy and Filmer (KNF), known as the induced-fit model. I refer to the Brunori article [24] and to the recent review [28] by Bill Eaton, Eric Henry, Jim Hofrichter and Andrea Mozzarelli, on the preferred validity of the two-state MWC allosteric mechanism over the KNF induced-fit model, supported by novel physical experiments on a fast time scale, and rigorous quantitative analyses by a number of authors.

4. NIH: from two to five

In January 1993 Building 2 personnel on the National Institutes of Health (NIH) campus in Bethesda moved into the renovated Building 5 in the musical chairs game devised for the physical renewal of the antiquated NIH buildings. My own formal association with the Building 2 goes back to 1965, but I had been in its ‘proximity’ much earlier [29].

In the summer of 1952, while a post-doc with Ray Fuoss in the Yale Chemistry Department, I came to visit Washington and made an appointment with Terrell Hill, then at the Naval Medical Research Institute on Rockville Pike in Bethesda. Terrell was well known to us in the Polymer Department at the Weizmann Institute for his work on statistical mechanics of charged macromolecules, polyelectrolytes, which we were studying in Rehovot with our teacher, Aharon Katchalsky, as models of biological macromolecules. Note that this was before Watson and Crick.

I took the trolley to Chevy Chase Circle and noticed that there were separate seatings for Blacks and Whites, fortunately a thing of the past now. From Chevy Chase Circle I boarded the bus to Rockville Pike for my meeting with Terrell. I did not know at that time that NIH existed just across the Pike. Of course there was no Building 10 or any of the other ‘large’ buildings on campus.

Incidentally, Terrell [30] left the Navy to set up strong physical biology Departments in Eugene, Oregon, and then in Santa Cruz, California, before coming back to Bethesda years later, this time to the Laboratory of Molecular Biology (LMB) in Building 2, for an extended fruitful stay. By public acclaim he also became the tennis champion of Building 2, as duly recorded by Bill Eaton. We also played tennis with Ira Pastan, a distinguished molecular biologist from the National Cancer Institute at the NIH, whose son Peter decided to go into Italian cooking. He opened a fancy restaurant in Washington, however, additionally, in his down-to-earth Pizzeria Paradiso, he created the best pizza and Italian delicacies in world-wide context (you can find it on the internet!). Terrell is now retired in his favored Santa Cruz on the Pacific Ocean.

My first visit to the NIH, Building 10 to be exact, was in the summer of 1959 when I was invited by Chris Anfinsen to give a talk on my work. Michael Sela had come to work with Chris in 1956 and I had met Chris on his visit to the Weizmann Institute one year later. As I was planning to spend 1958 and 1959 at the Mellon Institute in Pittsburgh, under the direction of Paul Flory, Chris invited me to visit NIH during my stay there. With my wife Nutzi and our two boys we drove our old Chevy to Bethesda, leaving the Pennsylvania Turnpike for smaller country roads, until reaching the narrow two-lane Old Georgetown road in Bethesda, our destination.

Getting to know NIH even in that short visit was an exciting experience. Lasting friendships were made with Herb Sober, Bill Harrington and Bill Carroll. Bill Carroll came with his family to Rehovot for a year, to work on devising ways and means to produce and study uniquely sized and charged polystyrenesulfonic acids as models for

biological macromolecules. This exemplified the strongly evolving connectivities between the Weizmann Institute and the NIH.

In Pittsburgh we were fortunate to meet Gary Felsenfeld, who had left the NIH and was now in the Biophysics Department, headed by Max Lauffer, at Pittsburgh University. Gary had been advised as a Harvard undergraduate by John Edsall, his doctoral studies were with Linus Pauling at Caltech, and his post-doctoral work with Charles Coulson, in Oxford (wow!). While at the NIH, he and David Davies, with Alex Rich, first reported poly-ribonucleotide three-stranded structures [31]. Our strong relationship started in discussions ranging over nucleic acids, polynucleotides and, later, chromatin. Gary returned to the NIH and in 1965 I came for a year-long stay to work in Building 4 with Bill Carroll on synthetic polyelectrolytes and in Building 2 with Gary on Poly A solution structure. My major work at the Mellon Institute in 1958–1960 was the thermodynamic analysis of multicomponent solutions with Ed Casassa, first summarized in 1964 [32].

When I arrived at the NIH Gary had told me to introduce myself to the Laboratory of Molecular Biology (LMB) lab chief Gordon Tomkins. Expecting a formal engagement with a high-ranking official I dressed carefully with jacket and tie. Imagine my surprise when Gordie, wearing an open shirt, turned out to be one of the most attractive intellectual and social personalities I had met in a lifetime. We became close friends, enjoyed his science, talk, ideas, music, art and jazz he was playing himself and with professional players. Though this chapter is essentially a personal story relating to Building 2, certainly not an official history, I cannot avoid mentioning the close relationships woven inside and outside the NIH campus.

I found the LMB and Building 2 to be a most unique place of research and human interactions. Much of it I ascribe to the personality of Gordie whom one could not pass in the corridor or near the coffee pot without a fruitful scientific exchange, or a humorous but profound comment on a current political, social or cultural event. Scientists most often stand at their bench or sit at their desk and talk little to other scientists in close-by laboratories or offices, who are doing the same. Here, on the other hand, communication and human relationship became a major contributing factor to the scientific work. Further exchanges were conducted in a continuous stream of outstanding lectures, group meetings and journal clubs. I am not sure whether Gordie was responsible for creating this atmosphere or whether it already started in a period with which I was not familiar. After all, in an earlier period Arthur Kornberg roamed in and out Building 2 and left a lasting impression. It has certainly maintained itself strongly after Gordie left in 1969 to go to the University of California in San Francisco. The outstanding scientists and Section Chiefs in Building 2 at that time, Gary Felsenfeld, David Davies, Marty Gellert, Bob Martin and Todd Miles decided they would rotate in the position of Lab Chief, vacated by the departure of Gordie, a procedure maintained for many years. Gordie unfortunately died in 1977 at a young age, following unsuccessful surgery.

Much of the smoothness and success with which LMB was run was due to Ed Rall, Director of the Institute's intramural research, who carried the whole administrative burden and provided strong encouragement for new and ongoing scientific programs. To hire a new worker or to order a new piece of expensive machinery, all that was necessary at the time for the LMB group leaders to do was to pick up the phone and talk to Ed who would give each justifiable request due consideration and quick action. The scientists could therefore devote their full attention to their work and many outstanding contributions have emerged from the LMB in Building 2 such as the studies by Marty Gellert and collaborators of topoisomerases, the gyrase responsible for DNA supercoiling, the study of 'reverse gyrase' from a thermophilic archaebacterium, which supercoils DNA in the opposite direction from a normal gyrase, and the study of the rearrangement of immunoglobulin and T-cell receptor genes, known as V(D)J recombination [33]. Fundamental X-ray studies by David Davies and collaborators of HIV integrase [34], and studies of DNA and chromatin organization by Gary and collaborators [35,36], leading to a better understanding of transcription and gene expression in higher organisms.

I was happy during my stay in LMB in 1965 to complete a study on Poly A configuration with Gary in which I used the Model E analytical ultracentrifuge and the SOFICA light scattering machine which I set up in the attic. Both these instruments have now been replaced by ultramodern Brookhaven laser light scattering and Beckman Optima XLA analytical ultracentrifuge instruments, run with great gusto by Rodolfo Ghirlando. We could show that the Poly A in solution could be made to follow the rules devised by Paul Flory for synthetic polymers [37], a result which prodded Flory to go into nucleic acid research with his newly arrived student, Wilma Olson.

One day, still in 1965, Gordie got hold of me in the corridor and said could I spend an afternoon to solve the molar mass and subunit structure of bovine liver glutamate dehydrogenase, a highly controversial topic at that time. It took more than an afternoon but we established [38] the then considered outrageous hexamer structure of the enzyme monomer, contradicting Monod's symmetry dogma; the monomer associates to form long rods. We have continued for a number of years [39] to work in Rehovot on this interesting system and it eventually led us to the study of dehydrogenases from extreme halophilic bacteria from the Dead Sea [40,41]. I have been coming back continuously to Building 2 since these early days, collaborating with Gary and colleagues on chromatin and β-globin structure. Being there continued to be a source of non-ending delight.

Many well-known scientists have spent longer and shorter periods in the LMB in Building 2. When I was there in 1965 Bruce Ames among many others was part of the crew, and I mention him in particular because, to the end he still haunted the upper floors. On the benches and in the cold rooms one still ran across desiccators, flasks, stirrers, etc., clearly marked with his name. Did the younger generations notice this and were they aware that this was the guy who so often appeared in the pages of Science?

While LMB occupied floors 2 and 3 and the attic, floor 1 and the lower depths of the basement in Building 2 were occupied by the outstanding Laboratory of Chemical Physics (LCP), led for many years by Ted Becker, an early leader in nuclear magnetic resonance research [42]. I have in particular enjoyed close relations and discussions with Karl Sollner, the well-known colloid chemist, and Elliot Charney [43], on optical spectroscopy and the dynamics of biological macromolecules. Bill Eaton, present chief of the LCP lab, was not there in 1965, he came later, belonging to a younger generation assembled by him in the laboratory, doing outstanding research in dynamic laser spectroscopy of hemoglobin. Additional groups are devoted to multidimensional NMR of proteins and modern theory. Eaton, Jim Hofrichter and younger collaborators study the dynamics of protein folding and conformational changes using pulsed laser methods and computer simulations, kinetics and thermodynamics of sickle cell hemoglobin polymerization and therapy of sickle cell disease [44]. They have strong collaborations with outstanding experimentalists and theoreticians in this field, and in particular with Italian scientists and students from and in Parma, the city of Gian Luigi Rossi, and Rome. Maurizio Brunori spent fruitful time in Building 2 in the pursuance of common interests with Bill and colleagues [45]. The theoreticians Attila Szabo [46], also active in hemoglobin performance work, and the somewhat older Bob Zwanzig [47], whom I had met at Yale in 1952, are names known to all. Philip Anfinrud [48,49], who more recently joined the LCP performs extensive ultrafast laser spectroscopy of hemoglobin, bacteriorhodopsin, and more proteins.

In the NMR field Ad Bax [50,51] developed and applied advanced multidimensional techniques and developed a method for weakly aligning biological macromolecules with respect to the magnetic field by using a nematic liquid crystalline solution of large particles. This novel method holds promise for improving the accuracy of NMR structure determination, and for extending the size limit of proteins and nucleic acids that can be studied by NMR. Angela Gronenborn and Marius Clore [52] are well known for their extensive contributions in the field of multi-dimensional NMR of protein solutions. Robert Tycko [53] provides distinguished contributions to solid state and optically pumped NMR in the process towards the solution of protein structures. In summary, we are dealing with one of the world-wide top NMR groups in a

field that has achieved considerable significance in modern research, approaching X-ray diffraction in complementary fashion, in a number of still evolving ways.

I would also like to mention Allen Minton, in Building 8, across the road from Building 5, with whom I have had a long association. Allen completed his Ph.D. at the UCLA with Willard F. Libby on the attempt to measure microwave dielectric properties of heme-heme interactions in hemoglobin in 1968. In 1969 Allen joined our group in Rehovot and produced significant contributions to water structure and in the demolishment of the polywater dogma. Allen then joined the NIH and contributed to the development of analytical ultracentrifugation in various exciting forms. However, his most interesting contribution is in the development of the notion of 'crowded systems', relating to the fact that biological cellular systems differ from common test-tube experiments. In the biological cell crowding is achieved by the fact that in addition to active nucleic acids and proteins cellular space is filled with additional variably sized and interacting or just space-filling molecules. These ideas, shared with Steven Zimmerman of the LMB, provide an additional dimension to biophysical biological research [54,55].

The atmosphere in the LCP was as stimulating as in the LMB on the upper floors. I attended their seminars and journal clubs, played tennis with some of them, the intellectual game which brings scientists together for an exchange of balls and of impressions of a deeper nature, and I was also grateful for being invited to their Xmas party when I was in town. Relations between the two laboratories were excellent and it was a pleasure to enjoy their complementarity in outlook and scientific problematics. They moved together to Building 5 in 1993, and continue on a steady path of progress.

Moving into another more modern and comfortable building, better designed and executed, was not meant to affect the subtle interactions which, in my belief, are an essential ingredient in the creation of great science. Doors to offices and labs should remain open in a real and a figurative sense, even if the design strives towards increasing isolation. The human values of Building 2 should not be lost in the process of cold modernization. At the time of the move I suggested to my friends that the appellation Building 2 should be maintained and moved into the new surroundings. This was apparently not approved and the move was to Building 5. So, in conclusion, I suggested, *take five*, but *remember two*, and proceed on the path which should continue to produce great science, while maintaining the humanity which makes it all worthwhile.

I have just become aware of the recently published book by Charles Tanford and Jacqueline Reynolds on the history of protein research [56]. As it most likely meets the quality and excitement of the classical Tanford physical chemistry of macromolecules text [57], an order should be placed immediately.

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