In Memoriam: Professor G.N. Ramachandran (1922–2001)

Editor's note: Few scientists contribute an idea of such clarity and power that it appears in all the discipline's textbooks and bears the author's name. For the contribution to be relevant and universally employed almost forty years after it first appeared is even less common. Structural biology lost the author of such an idea with the death of G.N. Ramachandran, whose picture appears on the cover of this issue of *Protein Science.* His seminal contribution is described in remembrances of Professor Ramachandran's life and career by colleague and co-author of the 1963 paper, C. Ramakrishnan. A perspective by George D. Rose follows, which articulates the enduring impact of that work.

Remembrances of Professor G.N. Ramachandran (1922–2001)

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Professor Gopalasamudram Narayana Iyer Ramachandran, more popularly known as GNR among his colleagues and students, passed away at Chennai (erstwhile Madras) on April 7, 2001. Born in October 8, 1922 at Ernakulam, a town in Kerala state (the southwestern tip of India), he received a master's degree in physics from Madras University in 1942. He joined Indian Institute of Science, Bangalore, and carried out research under the able guidance of Nobel Laureate Sir C.V. Raman. He obtained a D.Sc. degree from Madras University and later a Ph.D. from Cambridge University. He was on the faculty of the Department of Physics, Indian Institute of Science, Bangalore, until about 1952 when he moved to Madras University, where a major portion of his research in crystallography and biophysics was performed.

In 1970 he returned to the Indian Institute of Science and founded the Molecular Biophysics Unit. It is to his credit that he was instrumental in putting the Molecular Biophysics Unit and the Department of Physics, University of Madras (later known as Centre of Advanced Study in Biophysics and Crystallography) on the international scientific map. While at Madras and Bangalore, he had the full support of Dr. Sir A. Lakshmanaswamy Mudaliar, Vice Chancellor of Madras University, and Professor Satish Dhawan, Director of the Indian Institute of Science.

Ramachandran's early research work at the Indian Institute of Science was largely in the fields of crystal physics and crystal optics. His interest in instrumentation enabled him to make a simple experimental device, an X-ray focusing mirror for the X-ray microscope. X-ray reflections recorded from a crystal plane (crystal topography) have found wide application in the areas of solid-state reactivity and crystal growth.

Ramachandran spent a few years at the Cavendish Laboratory, Cambridge, where his work with Professor Wooster first determined the elastic constants of cubic crystals from diffuse X-ray reflections. He remained a physicist throughout his career, and both physics and mathematics can be seen as an integral part of all the work with which he was involved. His major research can broadly be classified into two fields, namely, crystallography and biopolymer conformation, a subdivision of biophysics. He made extremely important contributions in the field of X-ray crystallography, in particular dealing with methodologies such as anomalous dispersion, new kinds of Fourier syntheses, and X-ray intensity statistics.

When Ramachandran moved to Madras University in 1952, though he continued his work on crystal physics, his interest shifted to the structure of biological macromolecules, which was the outcome of a visit by Professor J.D. Bernal to Madras and their subsequent scientific deliberations. He decided to work out the structure of the connective tissue protein, collagen, from available experimental X-ray data. Thus began his entry into the field of biophysics, a field of study he was to pursue for the rest of his career. He, along with Gopinath Kartha (who subsequently moved to Roswell Park Memorial Institute at Buffalo), proposed and published the triple helical structure of collagen. The structure was based on the observation that glycine, which forms one-third of amino acid residues in collagen, plays a crucial role in bringing about close packing and satisfactory hydrogen-bonding arrangements between chains. The model went

through many stages of refinement, the last being one where a role for hydroxyproline in its stability was proposed.

During one of his lectures, Professor Ramachandran mentioned that he got the idea for the coiled-coil model from astronomy: The moon, while it rotates, also revolves around the earth and always presents the same side to the earth because of their coordinated movements. This idea was incorporated into the collagen structure in which the glycyl residues always face the center of the triple helix.

The proposed structure of collagen was not without controversy. The main objection was raised by Alexander Rich and Francis Crick who had earlier proposed a structure that was slightly different and had only one hydrogen bond per three residues, in contrast to two in Ramachandran's structure. Rich and Crick believed that the latter's structure contained atoms that were too close, which would cause steric hindrance based on van der Waal's radii of atoms. This was easily countered by V. Sasisekharan in an examination of the crystal structures of amino acids and peptides, which showed that shorter interatomic distances do exist, and hence the structure could be considered quite acceptable. But more interesting things were yet to follow. Ramachandran, a scientist who wanted to tackle problems at the basic level, decided to use this information to examine the various polypeptide conformations then known and also to develop a good yardstick that could be used for examining and assessing any structure in general, but peptides in particular. When this was taking place in 1960, I had the good luck to join Ramachandran as a doctoral student at Madras and was glad to be associated with such a fundamental problem. The rest is history. The outcome of his idea was the evolution of the now-famous Ramachandran Map. When Ramachandran decided to work out the details, he wanted to do it from the very first step. Fortunately, the *trans*-peptide unit and its dimensions (as postulated by Linus Pauling) were well established, and what remained was to pick a suitable basic system on which further work could be performed. An obvious choice for such a system was a pair of *trans*-peptide units linked at an α-carbon atom.

At a time when computers were unknown in India, marathon calculations had to be performed using electronic desk calculators. Ramachandran maintained enough patience for the calculations to be completed (although patience was not one of his virtues). The result which emerged from these calculations in 1962, now commonly known as the Ramachandran Map, was published in the *Journal of Molecular Biology* in 1963 and has become a household name in the field of protein conformation. It is worth remembering that at the time of its publication, the crystal structure of any protein was not available, and the map was expected to be valuable for studies of peptide and polypeptide structures.

It is appropriate to recall a few instances that give glimpses of Ramachandran's open-minded approach to problems. In about 1964, Ramachandran received from H.C. Watson of the MRC, Cambridge, the plot of the conformations of residues in the nonhelical regions of the protein, myoglobin, solved by Professor John Kendrew and his group. Except for two residues, the rest were well within the allowed regions. Ramachandran examined these conformations against the corresponding map for glycyl residues, which was then available, and found them to lie within the allowed regions. He came to the conclusion that if the map were to be correct, these residues should be glycyl and only glycyl. On communicating with Watson, he found that these were indeed glycyl residues, and his joy knew no bounds.

A second instance illustrates his meticulous approach to work. The structures of myoglobin and lysozyme showed clusters in the disallowed region between the extended and α-helical regions of the original Ramachandran Map. Instead of merely connecting the two regions based on the observation, he wanted a detailed investigation of the contact distances of conformations in this region to be made. It turned out that the steric hindrances, which disallow the conformations, were marginal, and hence it would be reasonable to have connectivity. He called this the "bridge region" (the region bridging α-helical and β-sheet conformations). Later results proved this to be correct as evidenced by the data now available on the large number of protein structures in which conformations regularly occur in this region.

After 1965, Ramachandran turned his attention to many topics related to the conformation of peptides and also to the formation of potential energy functions for hydrogen bonds. In particular, he was instrumental in expanding the work on different aspects related to peptides, including types of β-turns, conformation of prolyl residues, *cis*-peptide units, occurrence and need for non-planarity of the peptides, NMR coupling constants, peptides containing L and D residues, and others. The list is almost endless. The application of the Ramachandran Map and its uses slowly began to be felt in the sixties and seventies as the number of protein structures solved steadily increased. In the initial stages, these were used to test the correctness and robustness of the map. Protein crystallographers also used it as a tool for examining their structures, even at a preliminary stage of structure determination. For biophysicists and biologists, the representation and understanding of the various regular and irregular structural regions in a protein was made easy, particularly in view of the simplicity of the map, which can represent complex three-dimensional folding in a two-dimensional plane. Another aspect to note is that Ramachandran angles (ϕ,ψ) serve as a convenient tool for communication, representation, and various kinds of data analysis.

When Ramachandran moved from Madras to Bangalore, his main ambition was to supplement the various facets of his theoretical work with support from the experimental side in the field of biopolymer conformation. This he could achieve by promoting different components, such as peptide

synthesis, X-ray crystallography, NMR and other optical studies, and physico-chemical experimentation, all under one roof, namely, in the Molecular Biophysics Unit.

During his research career, Ramachandran spent most of his time in India. He was a visiting professor at the University of Michigan from 1965 to 1966 and was associated with the University of Chicago from 1967 to 1977. During that time, he did some exciting work on three-dimensional image reconstruction from radiographs and electron micrographs, which became applicable to computer-aided tomography.

Professor Ramachandran authored many reviews and organized two international symposia at Madras, one in January 1963 and the other in January 1967, both well attended by eminent scientists in the field of biopolymer structure and conformation. Attendees included Professors Linus Pauling, Severo Ochoa, David Phillips, Maurice Wilkins, Dorothy Hodgkin, Stanford Moore, Harold Scheraga, Elkan Blout, Murray Goodman, John Schellman, Paul Flory, Tatsuo Miyazawa, and many others. The proceedings from these symposia were published as four volumes and were edited by Ramachandran. In addition, he published many review articles on collagen and conformation and with colleague R. Srinivasan, wrote a book entitled, *Fourier Methods in Crystallography,* which has been very useful to students of crystallography. The review, "Conformation of Polypeptides and Proteins," written with V. Sasisekharan, which appeared in *Advances in Protein Chemistry,* likewise proved to be a handy reference tool for those learning or working on the basics and principles of protein conformation. Ramachandran deservedly received many awards and honors, most notably the Shanti Swarup Bhatnagar Award for Physics in India and the Fellowship of the Royal Society of London. Very recently, The International Union of Crystallography honored him with its prestigious Ewald Prize for his outstanding contributions to crystallography.

Professor Ramachandran always set high goals and would never compromise those goals with mediocrity. He was receptive to new ideas from anyone and did not hesitate to share his thoughts with others. He was easily accessible, and those who came to him for discussions were sure to depart with new ideas. He was an able research guide and an excellent lecturer, possessing great clarity of thought and expression. During the last few years of his life, he was affected by a stroke (which resulted in a slight slurring of speech) and by Parkinsonism, but he retained a sharp mind until the end. There is no doubt that he was a great source of inspiration for all those who were connected with him in his different walks of life. His intuitive and logical approach to the postulation of the structure of collagen and his elegant, systematic approach to the basic conformational problem of peptides and proteins are outstanding examples of his scientific excellence. Although Ramachandran is no longer with us, he left indelible footprints on the field of protein structure and conformational analysis through the development of the Ramachandran Map and the use of the Ramachandran angles, enduring symbols of his scientific excellence.

Perspective

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No biochemistry textbook is complete without a ϕ,ψ-plot of the alanine dipeptide, or more precisely, the compound Cα-CO-NH–CαHR–CO-NH-Cα, which has two degrees of backbone freedom like a dipeptide (Ramachandran et al. 1963). This plot ranks alongside the double helix and the α-helix among fundamentals of structural biochemistry. The plot is a compact and accessible representation of a profound idea, one that has thoroughly conditioned our thinking about the structure of proteins.

Sadly, G.N. Ramachandran (GNR, as he was known in India) died on April 7 at the age of 79. His long-time colleague and friend, C. Ramakrishnan, has written an obituary for *Protein Science.* Interested readers should also see the recent perspective by Richard Lavery (2000) who was a postdoc with Ramachandran in the mid 1970s. My remarks here are limited to the ϕ,ψ-plot and its implications.

The early 1950s were an exciting time in structural biochemistry. In 1952, J.D. Bernal visited India and urged Ramachandran to work on the structure of collagen (see Sarma 1998 for an account of this meeting). The Pauling-Corey-Branson model of the α-helix (Pauling et al. 1951) had just been published and was followed almost immediately by Perutz's dramatic experimental confirmation (Perutz 1951).

Ramachandran pursued Bernal's suggestion enthusiastically, and less than two years later, the paper describing the Ramachandran-Kartha triple-stranded, coiled-coil structure

was published (Ramachandran and Kartha 1954). The structure of DNA had just appeared the preceding year (Watson and Crick 1953). Certain nonbonded distances were too short in the Ramachandran-Kartha collagen structure, as noted by Rich and Crick (1955), but given the model-building facilities available to Ramachandran and Kartha at the time, this is hardly surprising. As Sarma (1998) points out,

"Computers had not arrived in Indian science, and they even lacked sophisticated model-building facilities in Madras. In fact, Kartha measured the bond distances in their crude models using pieces of string or the ribs of coconut leaves!"

Ramachandran appears to have taken these criticisms much to heart, and his response was a testament to scientific creativity of the first order. What started in criticism emerged, some years later, as an exhaustive representation of dipeptide stereochemistry—the famous ϕ,ψ-plot. The full impact of the plot was not immediately apparent. It soon became so after John Edsall invited Ramachandran to contribute a review to *Advances in Protein Chemistry* (Ramachandran and Sasisekharan 1968). In this remarkable review, Ramachandran and Sasisekharan anticipated many directions the field would take for years to come. The fact that the ϕ,ψ-plot was based only on the hard sphere (i.e., the repulsive part of the Lennard-Jones potential) had led some to underestimate the generality of this work. In this regard, Fred Richards (1977) commented dryly,

"For chemically bonded atoms the distribution is not spherically symmetric nor are the properties of such atoms isotropic. In spite of all this, the use of the hard sphere model has a venerable history and an enviable record in explaining a variety of different observable properties. As applied specifically to proteins, the work of G.N. Ramachandran and his colleagues has provided much of our present thinking about permissible peptide chain conformations."

It is worth noting that similar stereochemical ideas can also be applied to the analysis of RNA conformation, despite the fact that the monomer unit (i.e., a mononucleotide) in this case has greater backbone freedom than a dipeptide. Pioneering work of Sasisekharan and Lakshminarayanan (1969) and Sundaralingam (1969) has been pursued by several groups, most recently by Duarte and Pyle (1998) and Murthy et al. (1999).

The ϕ,ψ-plot is a model of physical reality, and its validity needed to be tested by experiment. That test was passed with flying colors as an increasing number of experimentally determined protein structures was solved. Now, of course, it is theory that is used to validate experiments, not the reverse, in programs like PROCHECK (Laskowski et al. 1993). Like Kepler's laws, the theory accounts for the data satisfactorily so that is has become synonymous with reality.

Use of the ϕ,ψ-plot for validation of experimental structures is so commonplace that it tends to overshadow some of the deeper implications of the plot. For residues other than glycine or proline, sterically allowed conformers fall almost exclusively within two discrete islands, one near $\phi,\psi = -60°, -40°$, the other near $\phi,\psi = -120°, +130°$. Repetition of the backbone dihedral angles from the first island results in an α-helix, whereas repetition of values from the other island results in a β-strand. At the level of the dipeptide, protein structure is essentially digital, because the two islands are discrete.

It has been thought that the conformation of each ϕ,ψ-pair in a polyalanine peptide is independent of its neighbors (Flory 1969). If so, a chain of length N, in which each residue can occupy either of two islands, can visit 2^N conformers. While formally true within a persistence length, most mixed conformers are scarcely populated, because the chain tends to clash with itself whenever it adopts them (Pappu et al. 2000). Consequently, almost all segments are either extended or helical; sterics inhibit structural hybrids. This conclusion is borne out by the familiar observation that known protein structures consist of isodirectional segments—either helices or strands—interconnected by turns and loops. Chimeric segments fashioned partly from helix and partly from strand are seldom seen. At root, this dichotomy originates in sterics, and it is built into the covalent backbone structure at the level of the alanine dipeptide.

The steric dichotomy between helix and strand populations in proteins is one of nature's deep organizing principles. Many biological phenomena at all levels are discrete, ranging from the hydrophobic effect (e.g., oil vs. water) to genetic phenotypes (brown eyes vs. blue eyes). Such examples of digital assortment, in which phenomena self-classify into distinct, unmixed states, are fundamental to reliable recognition, both microscopic and macroscopic. Perhaps the most far-reaching implication of the Watson-Crick structure (Watson and Crick 1953) is the realization that DNA is a digital molecule. Protein molecules are digital too: Helix-favoring conditions are necessarily strand-disfavoring, and conversely. Above all, we owe this discovery to G.N. Ramachandran and the stereochemical analysis of the alanine dipeptide.

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