

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

Professor Gopalasamudram Narayana Iyer Ramachandran, more popularly known as GNR among his colleagues and students, passed away at Chennai (erstwhile Madras) on 7 April 2001. Born in 8 October 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 DSc degree from Madras University and later a PhD 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 Prof. 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 Prof. 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 Prof. 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, Prof. 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 glycol 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  $\alpha$ -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*

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*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 Prof. 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 glycylic 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 glycylic and only glycylic. On communicating with Watson, he found that these were indeed glycylic 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  $\alpha$ -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  $\alpha$ -helical and  $\beta$ -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  $\beta$ -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 ( $\phi$ ,  $\psi$ ) 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.

Prof. 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.

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.

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