

Ewald Award to Professor G.N. Ramachandran

Acceptance and Lecture by Professor Vijayan



PROFESSOR G.N. RAMACHANDRAN

Early career

G.N. Ramachandran was born and brought up and had his early education in the southern Indian state of Kerala. He took his first degree in Physics from the University of Madras in 1942. Then he joined the Indian Institute of Science, Bangalore, to do his Masters degree in Electrical Engineering. At that time, Professor C.V. Raman, the Nobel Laureate of the Raman Effect fame, was the Professor of Physics at the Institute. Ramachandran came into contact with Raman. Ramachandran now wanted to shift to Physics to do research under Raman. In turn, Raman was also highly impressed by Ramachandran. He used several polite arguments with the Professor of Electrical Engineering to let Ramachandran go, but without any avail. Eventually Raman appears to have lost his patience and is rumoured to have told the Professor of Electrical Engineering that Ramachandran was simply too bright to be an electrical engineer. Ramachandran then moved to Physics, worked under the supervision of Raman and earned his M.Sc. and D.Sc. degrees. During this period his work was primarily concerned with optics. He also worked on the X-ray topographs of diamond. In 1947, he went to Cambridge on an 1851 Exhibition Scholarship to work in the Cavendish Laboratory, then headed by Sir Lawrence Bragg. At Cavendish he worked under the supervision of W.A. Wooster along with Andrew Lang. Ramachandran's work at Cambridge was primarily concerned with the measurement of elastic constants using diffuse X-ray scattering. He took a second doctorate from Cambridge and returned to Bangalore as Assistant Professor in the Department of Physics where he established an X-ray crystallography laboratory, along with Gopinath Kartha of ribonuclease A fame, whom many of us remember with great fondness.

The Tale of Two Cities

In 1952, Ramachandran moved from Bangalore to the University of Madras, one of the three oldest modern Universities in India, to establish a Department of Physics there. That marked the beginning of the golden era in Ramachandran's career. He was to remain at Madras for nearly 20 years. He moved back to Bangalore in 1971 to establish the Molecular Biophysics Unit at the Indian Institute of Science

where he continued the work started at Madras. In fact, Ramachandran spent most of his adult life in these two cities, first at Bangalore, then at Madras and again at Bangalore. He established leading research centres of crystallography and structural biology at both the places. As Ramachandran himself is reported to have remarked, his story may be called the Tale of Two Cities.

Collagen

When he moved to Madras, he was not entirely certain what major problem he should start working on. By then, Linus Pauling had already proposed the α -helical and the β -sheet models of the polypeptide chain. The momentous discovery of the double-helical structure of DNA was only a year away. Ramachandran was helped to make up his mind by a visit of the legendary J.D. Bernal to Madras during 1952-53. Bernal felt that the structure of collagen was a major unresolved problem at that time and suggested that Ramachandran might examine it. Ramachandran quickly followed up this suggestion and started by taking a fibre diffraction photograph of collagen at the newly established X-ray laboratory at Madras.

Fibre patterns of course do not provide detailed information. Using the fibre pattern and the available biochemical and physico-chemical information, Ramachandran and Kartha published the first approximation to their model in Nature in 1954. It was known at that time that a third of the residues in collagen are glycine. It also contained a large proportion of proline and hydroxy proline. The first approximate model built by Ramachandran and Kartha essentially consisted of three left-handed 3-fold helices arranged at the apices of an equilateral triangle. They assumed every third residue to be a glycine. Glycine is the simplest amino acid with no side chain and only this residue can be accommodated at the interface of the three helices. The model contained no intra-chain hydrogen bonds. The hydrogen bonds were all between the chains.

A detailed examination showed that the first model was not entirely compatible with the fibre pattern. The fit between the model and the pattern became perfect, when the three helices were made to coil around a common axis. Now each of the three helices had 3.3 residues per turn and they had a right-handed coil around the common axis. This is the well accepted coiled-coil structure of collagen. The modified structure was published in 1955, again in Nature.

Ramachandran's coiled-coil structure of collagen contained two interchain hydrogen bonds. Two British groups, particularly Crick and Rich, maintained that there could only be one interchain hydrogen bond. The formation of the second hydrogen bond would involve unacceptable steric contacts. In fact, the controversy involving the one hydrogen bonded structure and the two hydrogen bonded structure raged for a time. But in retrospect, as it often happens, this controversy appears somewhat meaningless. It turns out that in addition to the one interchain hydrogen bond everybody agreed on, there could be a water-bridge connecting two chains. In a related development, Ramachandran and his student Manju Bansal worked in the seventies on the role of hydroxyproline on collagen. Its main role appears to be the formation of a water bridge between adjacent chains. Therefore, it was not a straight choice between one or two interchain hydrogen bonds. The real situation appeared to involve a direct interchain hydrogen bond and a water bridge which often involved a hydroxyproline.

Much water has flowed down the bridge since Ramachandran last worked on collagen. In recent years, Helen Berman, Barbara Brodsky and others have solved the crystal structures of oligopeptides incorporating collagen-like and indeed natural collagen sequences. These structures confirm the Ramachandran model of collagen, including the water bridges, often involving hydroxyproline.

The Ramachandran plot

I now come to probably the most widely cited contribution of Ramachandran, the Ramachandran

plot. The work leading to the plot had its origin in his work on collagen. The controversy involving the one hydrogen bonded and the two hydrogen bonded models of collagen hinged on the minimum non-bonded distance between atoms. Ramachandran and his then student V. Sasisekharan undertook in the late fifties a thorough survey of the non-bonded contacts in the crystal structures of amino acids and related compounds. They found that non-bonded atoms usually came much closer than the sum of their respective van der Waals radii. From the data, they prescribed two limiting distances for each type of non-bonded-distances, the normal limit within which the distances usually fell and the extreme limit which is sometimes possible. In 1960, C. Ramakrishnan joined Ramachandran as a graduate student and from then on Ramachandran, Sasisekharan and Ramakrishnan together worked on the problem. They realised that, with planar peptide units, the flexibility of the polypeptide chain involved only rotations about the two single bonds hinged at C^α , which they then called ϕ and ϕ^1 ; we now call them ϕ and Ψ . They then delineated the sterically possible values of ϕ and Ψ for an alanyl dipeptide, using the table of normal and extreme limits of non-bonded distances derived from crystal structure data. That of course led to the Ramachandran plot. We must realise that the work involved tremendous calculations. These were essentially pre-computer days, at least in India. All these calculations, spanning several months, were carried out by Ramakrishnan on an electric desk top calculator. In fact these calculations formed part of his Ph.D. thesis. It is worth remembering that it was only during the period when the work was being carried out that the first high resolution structure of a globular protein, that of myoglobin, became available.

Soon after the Ramachandran map was devised, the late Herman Watson plotted all the ϕ , Ψ values of myoglobin on the map. A majority of them fell in the allowed regions. But a substantial number of them did not. It turned out that most of them corresponded to glycyl residues. As all of us know, glycine does not have a side chain and therefore, both the halves of the Ramachandran map are allowed for it.

Carbohydrates, nucleic acids.

Although Ramachandran's major effort in conformational analysis was concerned with proteins and peptides, he initiated work on carbohydrates and nucleic acids as well. In fact he published a paper on chitin in 1962 along with Ramakrishnan and another in 1963 setting out the rules that govern the conformation of polysaccharides. Subsequently, the work on polysaccharides was taken over and continued by V.S.R. Rao. Similarly, his first paper on nucleic acid conformation was published in 1967. The work on nucleic acids was later carried forward by Sasisekharan and still later by Manju Bansal.

Crystallography.

During the 50's and the 60's, only part of his work was concerned with conformational analysis. The other part dealt with crystallography. He worked on several aspects of crystallography, in collaboration with R. Srinivasan, who was to succeed him as Professor of Physics at Madras, Parthasarathy and many others. The first major contribution to emanate from him, that was in 1956, was concerned with anomalous dispersion. As Bijvoet had earlier shown, in the presence of anomalous dispersion, the Friedel equivalents have unequal intensities. Ramachandran along with S. Raman derived the correct formula for calculating phase angles using Bijvoet differences. This formula has been used for solving several structures. Notable among them in the early years was that of a vitamin B₁₂ derivative called Factor V1A by Venkatesan in Dorothy Hodgkin's laboratory. Since 1956, Ramachandran, Srinivasan and their colleagues carried out extensive studies on the use of anomalous dispersion and the work has indeed been monumental.

Another area in which Ramachandran's contributions have been outstanding, is concerned with Fourier transforms in crystallography. He published several papers in the area and also wrote a book on Fourier Methods in Crystallography, along with Srinivasan. His ideas were essentially simple. He took different quantities in the reciprocal space, such as F^2 , structure factor amplitude and phase angle, and then sought their Fourier transforms in real space. He then used different types of combinations of these

quantities to derive additional information. Specifically, the situation one often comes across is one in which part of the structure is known and we need to determine the unknown part of the structure. He devised several syntheses for doing so. In addition to its practical utility, Ramachandran's work illuminates the mind and takes us to the very foundations of crystallography.

Ramachandran worked in many other areas of crystallography, including crystallographic statistics, but in my opinion the work on anomalous dispersion and Fourier transforms stands out among them.

Other contributions.

I briefly touched upon three major areas of Ramachandran's contributions. He was a many splendoured scientist and worked in many more. For example, in the early seventies, he, along with Lakshminarayanan, devised a new method involving convolutions for image reconstruction. I understand that this method has since been extensively used. He, along with Chandrasekharan, worked out the conformational features of peptides containing L and D residues. This work turned out to be of considerable significance in relation to peptide antibiotics. During the early seventies, he was concerned about the non-planarity of the peptide group. The non-planarity results not just from an ω rotation, but also from the slight pyramidal nature of the amide nitrogen. C-H...O hydrogen bonds are extensively discussed today. Ramachandran invoked them in as early as 1966 in his model of polyglycine. I can go on and on with his other contributions.

In the late seventies he more or less stopped working in structural biology and crystallography. He then turned his attention to mathematical philosophy and logic. But came back he did to crystallography. In a significant publication in 1990 in *Acta Crystallographica* he proposed a new method of structure analysis.

Ramachandran was very keen on initiating experimental macromolecular crystallography in India. For a variety of reasons, mainly to do with inadequate financial resources, regular macromolecular crystallographic work got off the ground in India only after Ramachandran's active days in structural biology were over. However, the Molecular Biophysics Unit at the Indian Institute of Science, Bangalore, one of the two schools established by him, played a major role in nucleating and leading the macromolecular crystallography effort in India. To those of us who have been involved in this effort, Ramachandran has been a great source of inspiration. As Ramachandran wished, we now have a reasonable level of macromolecular crystallographic activity in India, distributed over several centres, although we are yet to scale the heights similar to those Ramachandran conquered in his chosen areas of endeavour a generation ago.

Concluding remarks.

To sum up, G.N. Ramachandran is among the most outstanding crystallographers and structural biologists of our times. The model of collagen developed by him has stood the test of time and has contributed greatly in understanding the role of this important fibrous protein. His pioneering contributions in crystallography, particularly in relation to methods of structure analysis using Fourier techniques and anomalous dispersion, are well recognised. A somewhat less widely recognised contribution of his is concerned with three-dimensional image reconstruction. Much of the foundation of the currently thriving field of molecular modelling was laid by him. The Ramachandran plot remains the simplest and the most commonly used descriptor and tool for the validation of protein structures.

Ramachandran established a great scientific tradition. That tradition, the Ramachandran tradition, lives on and thrives in the world, in India and in the two research schools he founded.

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