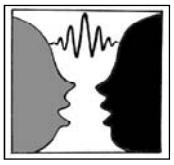
Face to Face



This section features conversations with personalities related to science, highlighting the factors and circumstances that guided them in making the career choice to be a scientist.

Viewing Life Through Numbers

C Ramakrishnan talks to Sujata Varadarajan

Students of molecular biology are familiar with the name of G N Ramachandran, which appears in their textbooks alongside an apparently simple looking map. This map, which allows the definition of a polypeptide chain through the torsion angles ϕ and ψ (*Figure* 1), has gained universal acceptance due to its general applicability to the study of protein structure and conformation. This work has changed the way people view protein secondary structure, and is now used to test the accuracy of coordinates determined from protein X-ray diffraction data. It was first conceived and carried out by Prof. G N Ramachandran and his group in the University of Madras in the 1960s. It is often regarded as the single most important contribution of Indian science to the field of modern biology (*Figure* 2).

Was the work as simple as it appears? How was the idea conceived, and equally importantly, how was it executed? We discover the details from Prof C Ramakrishnan, who was the young student to be entrusted with the actual calculations of the Ramachandran Map. This also serves as an introduction to a different way of thinking about biological molecules – one that entirely uses numbers to define aspects of structure and stability. Prof C Ramakrishnan (or CR as he is generally called by his colleagues and students) also touches upon related issues: his interactions with Prof G N Ramachandran (referred to as GNR in the remaining part of this article) and the path his own research has taken over the years. The interview that follows gives a glimpse of certain personable qualities of CR – his dogged determination in pursuing a problem, his drive for precision and his desire to work for the sake of working.

While it is a sobering thought that work on the Ramachandran Map did not receive a Nobel Prize (and another reminder to us as to the strange importance we attach to awards), this is not an aspect that CR dwells on at any stage. As he says during the course of the conversation – "I need not be answerable to anyone but I must be answerable to myself."

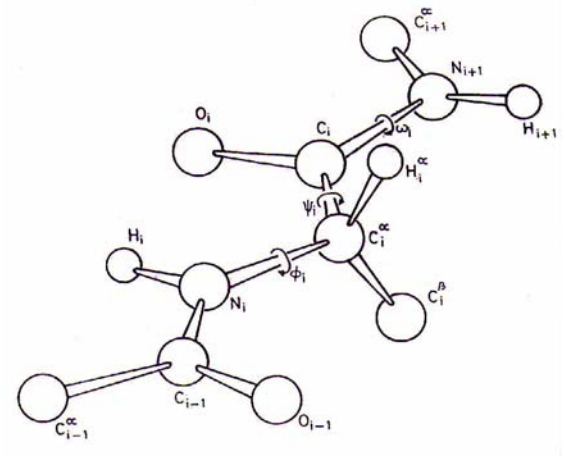
CR My experience was that many people knew of a scientist called Ramachandran. They knew only one thing about him, namely that he was almost close to getting a Nobel Prize. But what more did they know about him? Everybody said "Oh, he has worked with collagen." But what is GNR's contribution in the field of protein structure, apart from the discovery of collagen structure?

Proteins are complicated molecules, but they need not be studied in a complicated way. There is a simple way to understand the various arrangements that proteins can take.

Everybody had the impression that the Ramachandran Map is the one which can be used for the prediction of protein structures. I want to dispel that idea from their minds. Ramachandran Map is a tool which can be used for testing a structure – this is now being done extensively. It is not one which can be used for predicting whether a protein is going to take up a helical or β sheet structure.

SV How did you decide to enter research?

CR Going back to my college days, I graduated through a course called BSc(Hons), in Physics, (while the normal degree course (BSc,) was of two years' duration) at St.



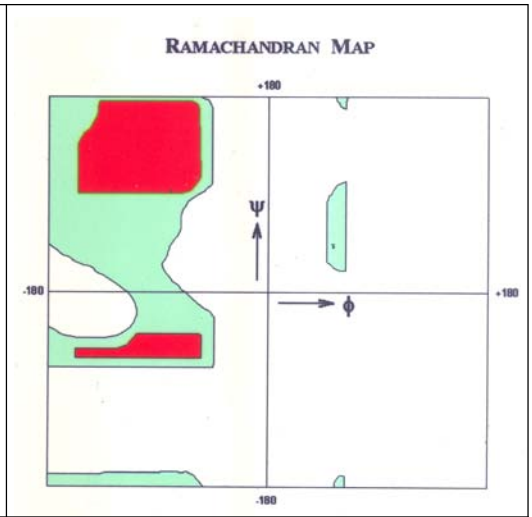


Figure 1 (left). Two linked planar* trans *peptide units. (ϕ_i, ψ_i) are the Ramachandran angles at the central a-carbon atom C^{α}_i.

Figure 2 (right). The Ramachandran Map. The red, green and white regions represent fully allowed, partially allowed and disallowed conformations of a polypeptide chain.

A detailed description is given in Ramachandran and his Map, *Resonance*, October 2001.

Joseph's college, Trichy. This course was presumed to be equivalent to (but not the same as) a Master's degree. I did not have any background in biology except in the school days, which was more descriptive in nature and hence was not much to my liking. I did not have much exposure to chemistry either because that was not a subject in Hons. course.

The Madras University Physics department had a course called 'one year M.Sc. (by examination)', with specialization in X-rays and crystallography. There were just 5 or 6 seats available for that course. That was conducted at the University department of Physics, for which GNR was the head.

When I wanted to join the MSc course, the only condition GNR put for me was that I must continue research after the course. So, he was clear that this course is not for those who want to get just a Masters degree but for students who want to pursue research.

Though honestly, I was not having any idea of research either in University or laboratories, nor had I been exposed to research in my earlier days, I said "Yes", with the idea of joining the course, because had I said "No", I may not be in the MSc course. In that one year, in the department, I was exposed to a research atmosphere, which made me understand what research actually means. When the course came to an end, GNR said "Now that it's over, are you ready to join research? I'll recommend you for a Government of India scholarship". The scholarship amount was Rs 200 per month, which was a large amount in those days. Being familiar with the ongoing research activities in the department, I inherently felt "Why not, I can also do research".

After I joined, GNR asked me a tough question "Which branch do you want to do research in Crystallography or Biophysics?" I replied that I would like to work in Biophysics and definitely not in Cyrstallography. Normally, any other person would have, out of curiosity, asked the question as to why I didn't want to work in Crystallography. This would definitely have put me in a little embarrassing situation, since the department had a large population of crystallographers. But GNR didn't ask anything. He said "I am glad. Now you can begin." That is how my whole future was set for me….

My fascination has always been with mathematics. So when I joined the department the first thing which attracted my attention was an electrical desk calculator, 'Marchant' make of which could do such wonderful things as addition, subtraction, multiplication and division.

SV When you began work with collagen, what was the approach?

CR When I joined the department, in June 1960, it was buzzing with activity on collagen because there was going to be a symposium at the Central Leather Research Institute (CLRI) in the following December. Many were working on the structural and X-ray aspects. The amino acid composition of collagen was known but not the sequence. Well known scientists Alexander Rich and Francis Crick had proposed an arrangement, with one hydrogen bond for three residues which was consistent with the repeat sequence Glycine- Proline- Hydroxy-proline. GNR had proposed another with two hydrogen-bonds for three residues (wherever Proline occurs, one hydrogen bond will break). *

My first assignment was to do a Fourier transform of both structures and to find out the intensity distribution pattern for both. This involves calculating the places where the X-ray intensities will occur, and how intense (dark) they will be. While the answer to the question 'Where' was easy, the answer to the second was difficult and involved a lot of calculations. In the end, X-ray diffraction patterns agreed with both structures though there were some places where the two-bonded structure showed better agreement.

But collagen gave birth to the Ramachandran Map. Crick's objection to GNR's structure was that when you have close packing, some of the van der Waal's distances will be violated. This question was not only approached from the collagen point of view by GNR, but from a generalized point of view. He realized that "this is not just an isolated problem with collagen, but one which is going to be involved with any molecule which has peptides and amino acids."

At that time, everything had to be developed *ab initio*. Sasisekharan (a colleague of GNR at the time) had done the literature search of available peptide structures to develop the criteria for minimum contact distances which could address the problem of steric hindrances with collagen structure.

Regarding my work, some easy geometrical methods existed which were used to

* Collagen is the main component of our body framework (including bones, tendons, skin, ligaments, blood vessels, membranous tissues), hence its study is of tremendous significance. GNR was the first to propose the correct structure of collagen- a coiled coil comprising of three left-handed polypeptide chains. There is a glycine residue at every third position allowing the helices to pack closely, rigidity is imparted by the frequently occuring proline residues and hydrogen bonding occurs through the glycine N-H as well as the hydroxy-proline O-H groups, stabilizing the structure. Sadly, GNR was not given due credit for this even after the controversy raised by Crick and Rich (over the close packing of the helices) subsided.

calculate the helical parameters of a polypeptide chain. Peptides being planar were known from Corey and Pauling's analysis of crystal structures. Pauling had also given all the peptide dimensions. Pauling focussed more on the á helix, but GNR focussed on one level below that. The initial idea was: If you take a pair of peptide units, how do you mathematically develop a helix out of it? Then came the question of defining the orientation. It can be angles between the planes, it can be angles between the two bonds, or it can be anything. In this case the most pertinent parameters are the angles of rotation. The alphabet for the conformational study can be a simple system of 4 atoms linked to each other, where you can have a rotation about the middle bond.

When the angles had to be defined there was no starting point. GNR said "We will take the fully extended chain as the starting point."

Torsion angles were known in chemistry but they were used to describe preferred arrangements; to go from one conformation to the other was also known. But to take two planar units and go from one conformation to another was not easy to tackle from a mathematical point of view.

Each peptide unit constituting the protein structure is a rigid unit, but the totality of the picture viz. protein/peptide is not a rigid body. So actually it was very difficult at that time to imagine an angle of rotation without having a defined initial position. What was needed was to apply the principles (of physics and mathematics) to the actual case of a peptide, and to transform it in a way that you can work with. Having done this, it was only a matter of time to formulate and carry out the necessary mathematical (numerical) calculations involving a lot of matrices.

SV How long did it take to complete the calculations?

CR About one and a half years. From January 1961, it took about 3 – 4 months to get the ideas crystallized. Journals were not easily accessible. There was no computer. There was no book which gave the matrix explicitly for numerical (hand) calculations. For about two months I had to search book after book for this. Finally one of my colleagues, S T Rao told me "You check up the book on Classical Mechanics by so- and- so, for the matrix". I rushed but that book was not available in the library. Then I had to find out who has taken the book, find out where he is and then, finally get the book. There the matrix was available. It took time because the libraries were situated in two campuses (A C College and Marina) and buses were not very frequent. After getting the matrix I knew I was at home because matrix is something connected with mathematics and physics and that is my home ground.

We had only two electric desk calculators in the department and one was mostly occupied by the crystallographers. There was only one person who could service these calculators in the city, and if something went wrong, somehow we had to find him. Telephones in those days were not a common sight everywhere. I mention all these to highlight the practical problems. One consoling factor was that there was a similar calculator in the adjacent institution, Central Leather Research Institute (CLRI) and people were kind enough to allow me to use it for a short time.

SV The amount of information that has been represented in a simple, two-dimensional way is impressive.

CR Anyone with mathematics background naturally thinks of representing the results through any graphical way, for easy understanding by others. This case is no exception. When you have two parameters variable, it becomes an X-Y plot. It is easy to perceive and to communicate, but if you are going to work with it you must remember that the two ends are the same; the top and the bottom are the same. It is akin to latitudes and longitudes which go from 0^o to 180^o each way. In essence, it is something like a globe.

SV Were the calculations tried beyond the C^β atom?

CR No, because the moment you go beyond the C^β, there will be rotations and it won't be a 2-D problem. It will become a 3-D or 4-D problem. But the main question was whether nature will tolerate steric hindrance of the backbone in order that the side groups are accomodated or will the side groups adjust themselves (after the backbone adopts a conformation). To answer this question, one has to wait for more and more structures to be solved. As a matter of fact, there are still some who think that the map has nothing to do with proteins because it was done with two peptide units. Had it not been essentially correct, the first 50 or 100 structures which were solved without the help of the Ramachandran Map would have shown that the map was incorrect. The points would have fallen outside the allowed regions of the map.

SV Were you under any pressure while doing fundamental research?

CR From the point of view of pressure, both GNR and myself were in resonance that we must do the work for work's sake. It was not a question of whether the work will be useful. So, from that point of view, I didn't have that pressure but I had the pressure of time, because GNR will say "Go and do it" and I have to do it fast because I really don't know, from the next day onwards, when he is going to call me and ask for the results.

SV What was GNR like, as a Professor and as a person?

CR I can say only a few things – his sincerity to the work in the department was extraordinary. Whatever he wanted to do he would put his whole body and soul into it. But the negative point was that he was very impatient. He would think that everything should be done very fast and correctly, in the right way. Though he appeared very strict, he had a very good heart. He would give due credit to the person/s who has done the work, however insignificant it may be.

He had a knack of seeing things correctly, and was very imaginative. Though there may be people like him, I have not come across any.

SV When did you realize the impact of the Ramachandran Map?

CR For me, it was a continuous process because right from the beginning I have been using it. Whenever I had a problem about structure or conformation I could immediately use it. It was my day to day tool. At the same time one cannot exactly say how much it can be used. It all depends upon the person (using it).

Now we have come far away from the Ramachandran Map. As a tool it has served its purpose and to that extent it was an amazing learning experience. The main lesson was "don't think in the beginning itself, whether it will be useful or not, whether it will be really earthshaking or not, whether you can publish it in this journal or that, but start work, plan it carefully, execute it correctly and present it in such a way that other people can understand".

SV What did you do subsequently?

CR I was a lecturer in Madras University when I submitted my thesis. At this time I started work on cyclic peptides. I went to Chicago for a year, in 1967, on GNR's scheme, and continued this work. When I returned, I was promoted to a Reader. In 1970, GNR moved to Bangalore and in 1972 he asked if I would be willing to join his department at IISc.

Talking of cyclic peptides, the Ramachandran Map becomes very useful because the structure can be represented as a polygon on Ramachandran plane and here you have a useful method of comparing conformations, studying the hydrogen bonding, etc.

If you want to cyclize a molecule, what kind of comfomational angles must you have in order that you may be able to bring the ends together? You can test if these angles satisfy the Ramachandran Map. It is useful when you want to synthesize peptide mimics.

In Madras University, I was also working on hydrogen bonding. I asked my first student to do an analysis of NH-O hydrogen bonds. The idea I had was that parameters involving lone pair electrons at the acceptor end should also be involved in characterization and study of the bond (along with those at the donor end).

After I came to Bangalore, I concentrated on the OH-O hydrogen bond to see if the results I had compared with those of the NH-O bonds. Meanwhile I was also studying cyclic peptides from the conformational point of view. I did studies on cyclic tripeptides, tetrapeptides, pentapeptides and hexapeptides and generated a cyclic peptide library. It gave some ideas as to how cyclization could be done from the geometric aspect, involving hydrogen bonds for stabilization. So my hydrogen bonding concept could be extended to the cyclic peptide work. It is by chance that I entered the field of proteins and along with my students, started developing algorithmic methods for protein structure determination. I am also doing modelling of disulphides in proteins (MODIP), which has the potential to be explored further.

SV Were there many changes over time?

CR Yes, quite a lot. The students are of the same type except their exposure to science is very large now-a-days compared to the earlier times. In one sense it is good because they come to know a lot of things. In another sense it is not because they get confused so much. So, it is necessary for a person who joins research to be properly guided in order to know what exactly is meant by research, because whatever you read in literature is the final outcome. It does not reveal how much work and effort has gone in. Nor does it record how much failure and disappointment the person has to undergo before success is achieved.

Now-a-days peer pressure is so much. In my days I didn't even think of my PhD when I started my research. I didn't even know when I will submit my thesis and what my thesis will be at the end of four or five years.

SV What are your current research interests?

CR My work mainly deals with looking for patterns that could be picked out from protein structures. In view of expanding (concepts of) science, if I can give some input from my area to someone else's work and if it can be useful, I would love it. Using what I know, trying to find something that is hidden in the protein data and catalogue it, so that it can be used by other people for future studies. In some cases you are able to get an answer to an observation, in some cases you are not. For the latter, one must continue

one's search from various other angles and see if the answer is obtainable. Till that time it will be a question. It's a never-ending process.

SV What are the aspects of your work that you particularly enjoy?

CR Right from my early days, I have had a fascination for teaching. After I finished my BSc(Hons), my idea was to teach. I joined MSc to facilitate this, but during the one year when I was in GNR's lab, things changed.

I enjoy teaching in the (classical) classroom style, with a blackboard and chalk, and models to show protein conformation, rather than using power point slides. I like to motivate students to ask questions.

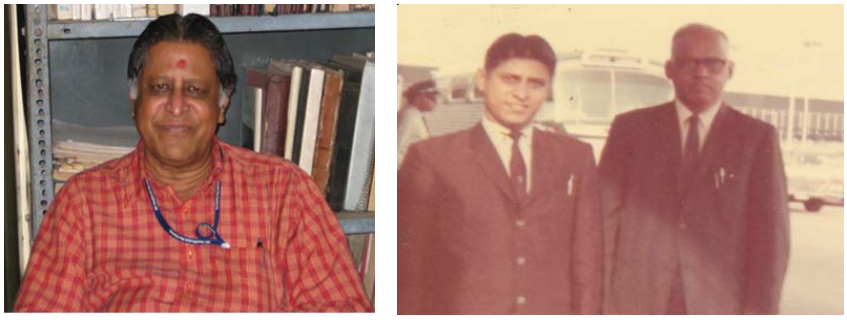
Another thing I enjoy is computer programming. My addiction to calculators later on turned into an addiction to computers and I started writing programs from 1965 onwards, in Fortran. I started programming when I had just finished my Ph.D. The first computer (IBM 1620) came to the Government Engineering College in Madras around that time. The program which one could run on it was FORGO and FORTOGO. In 1967, by the time I went to Chicago, I was completely familiar with programming, and began to use it for my work on cyclic peptides.

Although I had learnt programming on my own, I felt that it would be better to have an organized course to teach programming to students of Biology or Biophysics. I taught such a course in IISc for almost ten years. At that time computers were not as common as they are now, and many students who learnt programming then, I understand, found it useful later on.

I find that it is such a good tool that helps you think logically, step-by-step. So, the flowcharting which is used in programming has almost become a part of my day-to-day life. Before I start doing anything, I make a mental flowchart.

SV What are your other interests?

CR One of the things is my liking for Carnatic music. I began liking it initially because of the science. I tried to relate it with the diatonic scale and tempered scale of music, tried to judge the sound in terms of quality and frequency. Later, I started developing an artistic taste for music. My interest initially was in the rhythm rather than in the melody. So, I learned how to play the mridangam for 8 – 10 years and began to appreciate the mathematical complexity and nuances involved in the tala structures.



(Left) Prof C Ramakrishnan in his office, Molecular Biophysics Unit, IISc, 2007. (Right) GNR and CR, Chicago airport, 1967.

The second thing which I enjoy is the mathematical calculations involved in cricket scores and statistics. I have tried to write programs on how to code the cricket scores and have compiled my own data base. The enjoyment part of it for me comes through my (love for) programming and science.

Due to my intrinsic faith in God, I also spend time in religious activities – going to the temple, prayers, etc. – which keeps me mentally alert and gives me a lot of peace.

★★★★★★★★★★

Sujata Varadarajan obtained her PhD from the National Centre for Biological Sciences, Bangalore. She has worked on a range of research projects, including NMR spectroscopy of globular proteins, viral RNA protein complexes, DNA protein interactions in chromatin, the cell biology of viral superinfection exclusion and the genetic mechanisms of wing development in the fruit fly. She had a brief stint in the Ayurvedic Medical College, Bangalore, studying the basic principles of Ayurveda. She is currently involved in freelance writing and learning Yoga.

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