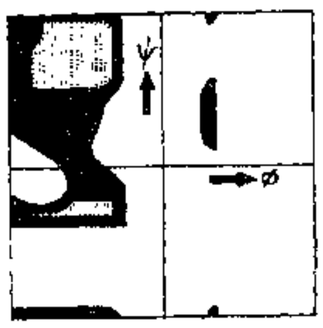

This issue

SPECTACULAR advances over the last four decades in X-ray crystallography, computational methods and nuclear magnetic resonance have contributed enormously to the growth of structural biology. There has been an explosive upsurge of information relating molecular structure to biological function. Molecular reductionism is central to our understanding of life processes. The practitioners of biology today include in their ranks many whose major preoccupations are in the area of biomolecular structures. The origins of structural biology in India can be traced to G. N. Ramachandran's laboratory in the Physics Department (now the Department of Crystallography and Biophysics) at the University of Madras. Here, in the span of a little over fifteen years, several fundamental researches were carried out on the structure of biopolymers.

In the early 1950s X-ray fibre diffraction studies were to lead Ramachandran and Kartha to propose the triple-helical (coiled-coil) structure of collagen in 1954, following closely on the heels of Pauling's α-helix for polypeptides and the Watson–Crick double helix for DNA. This led logically to an analysis of the general principles of polypeptide chain folding, resulting in the development of methods for assessing the 'stereochemical allowedness' of peptide conformations. The 1963 paper of Ramachandran, Ramakrishnan and Sasisekharan (reproduced in this issue) marks the beginning of the modern era of biopolymer conformational analysis. The Ramachandran plot, today, is one of the most widely used stereochemical representations in the literature of biophysics and structural biology.

This issue of *Current Science* seeks to focus attention on Ramachandran's outstanding accomplishments in the area of polypeptide and protein conformations and presents a collection of articles intended to provide a flavour of the field.

The Ramachandran plot and the structure and folding of proteins

Proteins exhibit a remarkable range of three-dimensional structures and established dogma dictates that one-dimensional sequences determine final architecture. One of the, as yet, unsolved problems of molecular biology is the understanding of the 'folding code', which relates primary sequence to final form. Protein interiors are densely packed, apparently irregular arrays of largely apolar side-chains. Fred Richards and his collaborators from Yale University examine (page 819) the side-chain packing problem in protein interiors and attempt to develop an approach which will permit the effects of specific substitutions on the energetics of the folding process. Their experimental system, the S-peptide–ribonuclease S complex provides a convenient means of probing specific internal interactions in proteins. There is still a long way to go, but, as the authors note, '...the underlying principles on which the Ramachandran map was based are still widely used and without serious challenge'.

Nuclear magnetic resonance (NMR) has rapidly developed into an amazingly powerful technique for determining three-dimensional structures of biological macromolecules in solution. Central to this approach has been the development of the methods of two-dimensional spectroscopy (more recently extended to three and even four dimensions), coupled with the computational devices of distance-geometry algorithms and molecular dynamics calculations. Kurt Wüthrich of the Eidgenossische Technische Hochschule, Zürich, one of the leaders of this NMR revolution and the pioneer in the determination of protein structures in solution, analyses (page 825) the role played by the Ramachandran plot in developing general strategies for sequential assignments of NMR resonances and for the identification of regular secondary structures. Wüthrich also recalls his visit to Ramachandran's laboratory at Bangalore sixteen years ago, at a time when these NMR methods were yet to be conceived.

The Ramachandran (ϕ,ψ) plot is an enormously useful device for representing and assessing the quality

of protein structures determined by X-ray crystallography or NMR spectroscopy. Brian Matthews and his group from the University of Oregon illustrate (page 833) the utility of the Ramachandran map in their study of mutant T4 lysozymes. The appearance of chiral L-residues in left-handed helical conformations (ironically on the right side of the Ramachandran map) is a relative rarity in proteins. These authors choose two such residues and engineer their replacement by the achiral glycine residue and analyse the mutant structures. They also present an interesting account of the use of the Ramachandran map in structure evaluation—a matter of some concern, with the increasing number of incorrect structures.

Glycine residues are also the subject of an exhaustive analysis by C. Ramakrishnan and N. Srinivasan, Indian Institute of Science, Bangalore, who examine (page 851) the conformational preferences of this amino acid, the smallest of all the protein constituents. The wealth of protein structural information available today lends itself to stereochemical analysis and the Ramachandran angles prove to be most useful parameters.

Collagen

X-ray diffraction data led Ramachandran and Kartha to the triple-helical structure of collagen, which today is found in textbooks of biochemistry and molecular biology. But have the X-ray photographs been completely and satisfactorily interpreted? V. Sasisekharan and M. Bansal of the Indian Institute of Science, Bangalore, examine (page 863) the problem in the light of the X-ray diffraction patterns yielded by collagen fibrils. They invoke the principle of self-similarity in describing the assembly of rod-like collagen molecules. These authors also point to the role of the discussions on the collagen structure in catalysing the developments of the methods for assessing structures on the basis of 'non-bonded contacts' between atoms, leading eventually to the Ramachandran map.

Predicting and analysing protein structures

A reliable method to predict unambiguously the three-dimensional structure of proteins from the primary amino acid sequence remains the Holy Grail of biophysics. The pioneering approach to structure prediction was presented in 1974 by Peter Chou and Gerald Fasman of Brandeis University. On page 839 of this issue Fasman returns to this original theme and asks whether such predictions are 'fact or fiction'. Considered in the context of the structural information available today and that which will become known in the near future, prediction methods offer a powerful tool in studying protein structure and function. Fasman illustrates these methods with some examples and argues for a pragmatic approach.

Nature provides many puzzles for protein folding enthusiasts. Functionally related proteins sometimes display widely different sequences but nevertheless adopt similar tertiary structures. 'Structural plasticity' is a popular term to describe the remarkable tolerance of many proteins to the numerous amino acid substitutions made by 'protein engineers'. However, surveys of evolutionarily related families of proteins reveal that some key residues are invariably conserved, implying that these are probably crucial for catalytic function or in maintaining the integrity of the folding pattern. Tom Blundell and his group from Birbeck College, London, identify (page 867) key residues in homologous proteins of known three-dimensional structure, using amino acid substitution tables. They hold out the possibility of identifying residue patterns in proteins of unknown structure, characteristic of domains, supersecondary structures or distinct structural motifs.

Synthetic peptide and protein design

Can synthetic proteins, designed from first principles, be assembled in the laboratory? Several attempts at *de novo* design have already been reported in the literature. On page 875 Isabella Karle of the Naval Research Laboratory, Washington DC and P. Balaram of the Indian Institute of Science, Bangalore, and their collaborators describe an approach based on the modular assembly of stereochemically rigid secondary structure units. This strategy rests on the ability of certain non-protein amino acids, specifically α-aminoisobutyric acid, to direct polypeptide chain folding, a consequence of the limited 'allowed regions' of Ramachandran ϕ, ψ space. These authors demonstrate the construction of a rigid α-helical module and envisage a 'Meccano set' approach to building up larger structures. They conclude that a desirable goal would be to enhance 'the repertoire of amino acids with strong preferences for specific regions of the Ramachandran map'.