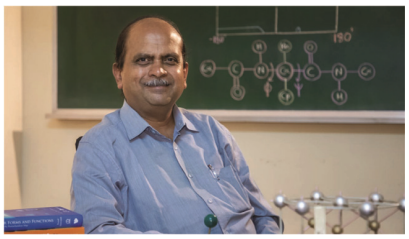
N. Srinivasan (1962–2021)

Narayanaswamy Srinivasan one of India's pre-eminent computational structural biologists passed away on 3 September 2021. Prof. Srinivasan developed many important computational methods in protein structural biology and applied them successfully to address fundamental questions on protein evolution, structure, interactions and function.

Proteins are biopolymers built from amino acid building blocks. There are 20 naturally occurring amino acids in proteins. These consist of conserved, so-called main-chain atoms, and sidechains that are unique to each amino acid. The main chain (backbone) atoms are connected by peptide bonds to form polypeptide chains that are typically 50–500 amino acids long. Most proteins form ordered, three-dimensional structures either in isolation or when bound to other proteins or ligands. These structures are intimately related to biological function and are largely determined by the amino acid sequence(s) involved. Protein sequences can be deduced from the corresponding gene sequences. Over the past decade, there has been a revolution in DNA sequencing technologies which has resulted in public availability of vast amounts of genomic sequence for many organisms, including humans. Deciphering the relationships between protein sequence, structure, interactions and biological function is thus an important and contemporary area of research.

Srinivasan was born in 1962 in Chennai to the late K. Narayanaswamy and N. Jayalakshmi, as the youngest in a family of eight children. He completed high school in Rajah Muthiah Higher Secondary School, Chennai in 1979, and subsequently earned his Bachelor's degree in physics (1979–82) at Jain College, Chennai. As a young boy, Srinivasan was sports-oriented and enjoyed playing cricket. As a young adult, he was known to spend long hours tutoring mathematics to younger students. For his Master's degree, he joined the Department of Biophysics, Madras University, Chennai obtaining his M.Sc. in 1984. The Madras School and Department of Biophysics was renowned; it is here that the late Prof. G. N. Ramachandran (GNR) carried out his seminal work on the Ramachandran map and structure of collagen. Subsequently, Srinivasan joined the Molecular Biophysics Unit (MBU) at the Indian Institute of Science, Bengaluru in 1984, for his Ph.D. under the supervision of the late Prof. C. Ramakrishnan (CR). GNR had founded MBU in 1971. CR was a former student of GNR, and was instrumental in performing the calculations for the Ramachandran map; Srinivasan continued in this distinguished tradition. The key feature of the Ramachandran map is that it predicted regions of conformational space that could be adopted by the protein backbone in terms of the φ and ψ main-chain dihedral angles.



This exceptionally compact and elegant representation of the protein structure was carried out without luxury of having coordinates for even a single experimentally determined protein structure available. Yet even today, after close to 200,000 structures being solved, this remains a gold standard for structural biologists to check the correctness of their structures. Srinivasan was productive during his Ph.D., a trend that would continue throughout his career. His 1991 two-volume thesis entitled 'Conformational studies on globular proteins: data analysis' weighed close to 3.5 kg and involved studies of the backbone conformational preferences of amino acids and studies of how helical regions in proteins are connected. The Acknowledgement contains the understated comment: 'Association with my friend Ms R. Sowdhamini proved valuable.'

Srinivasan was proud of his work on the Ramachandran plot and returned to it at various times in his career. In fact, some of his most recent work (see below) explores the effect of changes in bond lengths and bond angles on the allowed values of φ and ψ.

After his Ph.D., Srinivasan moved to the UK to work with Prof. Tom Blundell. During his seven-year stay there, he carried out a number of studies on modelling of protein structures and on protein assembly and evolution. In 1998, he returned to MBU as a faculty member, something that would have been difficult with the hiring constraints we have in place today.

At MBU, over the past 24 years, Srinivasan established a productive research group. He initially chose to focus on protein kinases, an extremely important class of enzymes that add phosphate groups to substrate proteins, thus modulating their function, as well as on the creation of databases relating protein sequences, structures and evolutionary relationships (phylogenies). Srinivasan's early work on human kinases, alongside similar work by Tony Hunter and co-workers, is internationally well recognized. His group performed genome-wide analyses of protein kinases for hundreds of organisms to obtain insights into how substrate specificity is achieved, and carried out an early analysis of kinases in the draft version of the human genome. Later work examined unusual domain combinations in human protein kinases, conservation of structural fluctuations in the family of protein kinases, characterization of their motions using molecular dynamics simulations, recognition of functional features of viral kinases, and significantly, recognition of regions of functional specialization in all known kinase families.

Structures of large proteins are typically composed of smaller units termed domains, interspersed with connecting linkers. While there are a vast number of protein sequences, they populate a relatively small number of domain folds, many of which are already known. While similar sequences with >30% identity to each other often adopt similar folds, in many cases, sequences with very low identity to each other can also adopt the same fold. Thus, the problem of protein structure prediction from sequence reduces to identifying the structural domains present as well as their relative orientation. Since many different sequences with low identity to each other can adopt the same fold, it is often challenging to predict the fold from sequence data alone. Srinivasan and his group made important contributions to this area, to detect structural homologues of proteins solely from sequence information. The approach involved generation and use of protein-like artificial sequences which were strategically designed to fill-in sparse regions in protein sequence space, leading to detection of remote homologues. Application of this approach enabled recognition of 3D structures and functions of thousands of proteins of previously unknown structure and function.

Even if domain structures or folds can be successfully identified, predicting their relative orientation and dynamic properties in large assemblies is difficult. Srinivasan combined information from experiments, including low resolution cryo-EM data, bioinformatics analysis, evolutionary studies and simulations to predict complex molecular structures such as those of spliceosome components and binding of large RNA segments to the human ribosome. In many macromolecular systems, binding of a ligand at one location results in significant structural and functional effects some distance away. While these so-called allosteric interactions are widespread, a molecular-level understanding is in many cases, elusive. Srinivasan examined how the simple act of tethering domains together to form multi-domain proteins results in allosteric effects, altering the flexibility and dynamics of functional residues. Other work combined normal mode analyses with molecular dynamics simulations to understand how allosteric effects modulate the binding of multiple ligands to the same structure at non-overlapping sites.

Living in India with its large burden of infectious diseases, Srinivasan was also actively involved in using computational methods to facilitate the understanding of various pathogens. His group was one of the earliest to demonstrate the power of computational approaches in recognizing protein–protein interactions across a host and a pathogen in the context of the malarial parasite. This approach was subsequently extended to several other pathogens, including *Mycobacterium tuberculosis* (Mtb), hepatitis C virus, *Helicobacter pylori* and *Salmonella typhimurium*, to name a few. Toxin–antitoxin systems are ubiquitous in bacteria and comprise a toxin component which causes cell death or slow growth along with an antitoxin which serves as an antidote for the toxin, with both components encoded in a single operon. Mtb contains over 80 such systems, most with unknown function. Srinivasan carried out detailed bioinformatic studies to predict interactions between Mtb toxins and cognate as well non-cognate antitoxins, some of which were subsequently demonstrated by laboratory experiments. He also proposed and applied a computational framework for repurposing FDA-approved drugs against proteins from pathogens. In several cases predictions were confirmed by laboratory and clinical studies.

Macromolecular structures provide remarkable insights into molecular function. Many macromolecular crystal structures have bound small-molecule ligands. These structures are particularly important for drug-discovery efforts. The process of structure solution by X-ray crystallography involves fitting molecular models into electron-density maps derived from experimental diffraction data. Higher resolution structures have sharper electron density maps; however, resolution as a single number hides the wide variation in electron density in different regions of the structure. In a recent study, Srinivasan and his students came to the surprising and disturbing conclusion that over 10% of macromolecular crystal structures with small-molecule ligands, including some at high and medium resolution, lack properly interpretable density for the ligand, with several cases of ligands being fit into virtually non-existent density. In a remarkable display of forbearance, the results were presented only in aggregate, without explicitly listing the errant structures and the associated authors.

Next year marks the birth centenary of GNR. Very recently and most appropriately, in association with his former Ph.D. supervisor, CR, Srinivasan applied the powerful principle of avoidance of steric overlap using the contact criteria worked out by Ramachandran, Ramakrishnan and Sasisekharan, over 50 years ago, to the small peptide structures available in the Cambridge Structural Database. This resulted in a repertoire of approximately 2000 Ramachandran maps using bond parameter values observed in small peptide crystal structures, with many of them quite different from the classical Ramachandran map. This work brings in new insights into the acceptability of the φ, ψ values which lie in the disallowed regions in the classical Ramachandran map. A paper with three academic generations of authors (CR, Srinivasan and his student Ashraya Ravikumar) with a direct link to their academic progenitor, GNR himself, was published in 2019 in *Structure*; this was work that was close to his heart.

Srinivasan was one of the most productive faculty members in the Biological Sciences Division at IISc with over 350 publications and more than 10,000 citations (https://scholar.google.com/citations?user=dxiu7GQAAAAJ&hl=en&oi=ao). A few years ago, in a survey conducted by DST, he was listed as being amongst the top-five most productive researchers in the subject areas of biochemistry, genetics and molecular biology. Srinivasan was an excellent teacher and mentor, with 28 students receiving their Ph.Ds under his supervision. Several more were working with him at the time of his unfortunate and all-too-early demise. Srinivasan received many awards nationally and internationally for his research contributions and scholarship. These included the International Senior Fellow of the Wellcome Trust, UK (1999–2004); the National Bioscience Award of DBT in 2005; the Shanti Swarup Bhatnagar Prize in 2007; the J. C. Bose Fellowship since 2014, and in 2020, the Rustum Choksi Award for Excellence in Research, IISc. In addition, Srinivasan was an elected Fellow of the three major science academies in the country. He served on the Editorial Boards of many journals, and during the course of his career, was a visiting Professor at Reunion University, University of Nantes, and a Senior Fellow at Manchester University. He was a sought-after collaborator both within and outside his MBU. Most important, he was a gentle, kind and ever courteous person, always interested in discussions, scientific or otherwise. Notwithstanding his high research output, Srinivasan always had time for students, colleagues and visitors, faithfully attending all seminars in MBU and setting an example for all of us to follow. For the past few years, in addition to his other responsibilities, he was also the Departmental Chair, a job he handled meticulously and cheerfully, especially looking out for and supporting younger colleagues in MBU. Throughout his long illness and extended hospital stays since December 2020, he remained upbeat and engaged in animated conversation with all around, and was more productive than many of us in good health.

Srinivasan passed away all too soon. Tragically, like many others, this was a death that could have been avoided, had annual health check ups, so prevalent in the corporate world but largely absent in Indian academia, been in place.

He is missed by many and survived by his closest and dearest collaborator, his wife Sowdhamini, a Professor at NCBS, Bengaluru, and their daughter Jayashree.

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