

Kasturirangan said 'three generations of Indian Space Research Organizations' scientists and the leadership have played a very significant and crucial role on the Indian side'. He also cherished his association with several leading and distinguished French scientists and personalities. Finally, he said 'the success of any collaboration can never be complete without the political will. The Space Department has received the support of the political system in India, consistently, cutting across party lines, in forging a strong and enduring relationship with France'.

The Legion d'Honneur established on 19 May 1802 by Napoleon Bonaparte has five classes and is France's recognition for military and civil service. From its origins, the badge of the Legion d'Honneur has changed little, the badge is a five branch star with a central gold medallion and is worn from a red ribbon. France celebrates this year as the national bicentenary of the creation of the Legion d'Honneur.

Some Indo-French co-operation projects that are presently underway are, according to Kasturirangan, the following:

- Joint development of the satellite 'MEGHATROPIQUES' due for launch in 2005 for global climate studies.
- Co-operation between the Indian Institute for Remote Sensing, Dehradun and the French group for the Development of Space Teledetection, Toulouse.
- Joint programmes in the areas of tele-medicine, tele-education and natural disaster management that help social development.

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RESEARCH NEWS

NMR – Bigger, stronger, faster . . .

Siddhartha P. Sarma

Since the first demonstration of nuclear induction in bulk matter in 1946, by Purcell and Bloch at Harvard and Stanford respectively, nuclear magnetic resonance (NMR) has become unarguably the most important and widely used physical tool for investigating matter. Its range is astonishing, encompassing such diverse areas as imaging of tissues in living animals, organic and inorganic materials in the liquid, liquid crystal or solid phase to quantum computing. The growth of NMR into this pre-eminent state has been a long and arduous journey, punctuated with the award of the Nobel Prize to four scientists for their contributions to NMR. Purcell and Bloch shared the 1952 Nobel Prize in physics and Richard Ernst was awarded the 1991 Nobel Prize in Chemistry for his contributions to the development of the methodology of high resolution NMR spectroscopy. Only a few weeks ago the Nobel prize for 2002 in Chemistry was awarded to three scientists, one of whom is Kurt Wüthrich for his development of NMR spectroscopy for determining the three-dimensional structure of biological macromolecules in solution, and the other two being John Fenn and Koichi Tanaka for their development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules. The growth of NMR as a bioanalytical tool has been

aided by developments in other seemingly unrelated areas of science such as solid state physics and material science for the design and construction of exquisitely sensitive electronic components for the detection of very weak radio frequency signals and concurrently development of high field superconducting magnets that today range in field strength from 7.05 to 21.14 T (10,000 Gauss = 1 T, for comparison the earth's magnetic field is 0.5 Gauss) that increase the sensi-



Kurt Wüthrich

tivity of detection and resolution. Today NMR signals can be recorded on samples ranging in quantity from a few tenths of a milligram to a milligram (Purcell recorded his first NMR signal using a 1 kg block of paraffin wax as a sample). Last but not the least, bioanalytical NMR has benefited enormously from methodological improvements in genetic engineering and molecular biology for production of native proteins in quantities, usually milligrams, necessary for structural studies.

The impact of NMR in biology, particularly in structural biology and biochemistry is underscored by the fact that it is the only method currently available for determination of structures of biomacromolecules in solution. Pioneering efforts to establish NMR as an alternative and yet complementary method, to the older and better established single crystal X-ray diffraction method, for protein and nucleic acid structure determination were made by Kurt Wüthrich and coworkers in the late 1970s and early 1980s. Great success has been achieved in the study of proteins, but the techniques are not limited to proteins alone. In 1986 Wüthrich and coworkers determined the first novel structure of a globular protein (7.9 kDa) by NMR methods, that of Tendamistat¹ (Figure 1).

Determination of this structure was preceded by the development of several

methodologies² which included multipulse techniques for acquisition of multi-dimensional NMR data for separating specific interactions between pairs of protons in two dimensions, systematic methods for assignment of resonance signals in these two-dimensional spectra to individual protons or groups of protons (~ 530 proton signals in Tendamistat) within the protein and development of mathematical methods to compute structural models using NMR-based structural restraints, i.e. distance information between pairs of interacting protons from NOESY (Nuclear Overhauser Enhancement Spectroscopy) data and angular constraints from proton-proton scalar coupling constants measured across three bonds.

The early successes were met with much skepticism with the general conclusion that NMR-based methods could yield structures of peptides and small proteins. Indeed the upper size limit was placed at 7–8 kDa. Since then NMR spectroscopists have been developing methods to study larger and larger proteins and it is here that genetic engineering and molecular biology have played a pivotal role. A study of larger molecules by NMR is accompanied by certain intrinsic problems. The larger the molecule the greater the number of resonances and hence greater the spectral overlap. Additionally, as the size of the molecule increases, the intrinsic line width increases resulting in poorer resolution and sensitivity. To overcome

these problems several innovative NMR methods were introduced in the late 1980s and early 1990s. These include development of multipulse data acquisition techniques that separate interactions between protons, between protons and heteronuclei (nuclei other than protons such as carbon-13 and nitrogen-15) and between heteronuclei in two, three and even four dimensions by using the enormous chemical shift dispersion of carbon-13 and nitrogen-15 nuclei present in the protein backbone and side-chains. These experiments are more efficient when the protein samples are enriched to > 95–98% in carbon-13 and nitrogen-15 stable isotopes. This is easily achieved in cases where the protein of interest is cloned and over-expressed in bacterial cultures and the culture medium contains uniformly enriched ¹³C₆-glucose as the sole source of carbon and ¹⁵NH₄Cl as the sole source of nitrogen. The increase in resolution afforded by these so-called 'isotope edited' NMR methods has greatly speeded up the 'assignment' process in NMR and in fact has reduced the duration from a couple of months for analysis of proton-proton spectra down to a few days³. These methods have enabled the determination of structures of proteins in the size range of 15–25 kDa molecular mass and in favourable cases structures of proteins with a molecular mass of ~ 30 kDa. Introduction of deuterium labelling along with ¹³C and ¹⁵N isotope enrichment has

enabled even larger monomeric proteins such as the maltose-binding protein⁴ (~ 42 kDa) and MurB⁵ (38.5 kDa) to be assigned and their solution secondary structure to be determined. In the case of deuterium labelled samples, all non-exchangeable protons are replaced with deuterons. This leads to significant improvements in sensitivity and resolution in the case of larger proteins. Such samples have been used to map interactions in protein DNA-complexes as large as 64 kDa. The development of TROSY⁶ (Transverse Relaxation Optimized Spectroscopy) by Wüthrich and coworkers in 1997 held the promise for much larger proteins to be studied. Initial studies demonstrated that high quality spectra could be obtained for proteins as large as 110 kDa (ref. 7) (an octamer of a 13.75 kDa protein). Protein-protein interactions in a 51 kDa complex⁸ were fully mapped using TROSY-based methods, thus bringing into fruition the long-held promise that solution NMR were ideal for studying intermolecular interactions of biological macromolecules. Much like the legendary pole-vaulter Sergei Bubka did during his illustrious career (he set the indoor and outdoor world records a total of 35 times!), NMR spectroscopists too have been raising the limit on the size of molecules that can be studied. In the recent past, two reports, one in *Nature* and the other in the *Journal of the American Chemical Society* have shown that this time too the bar has been raised and not by any small amount. The report in *Nature*, by Wüthrich and coworkers describes the NMR study of an ~ 900 kDa complex formed between the homoheptameric co-chaperonin GroES (*M_r* 72 kDa) and the homotetradecameric chaperonin GroEL (*M_r* 800 kDa)⁹. Chemical shifts of the resonances in the co-chaperonin were monitored to identify those regions of the protein directly involved in protein-protein contacts. It is truly remarkable that a spectrum of such high quality can be recorded of such a large complex! The report in *JACS* is a stunning example of the type and size of proteins that can be studied by NMR spectroscopy. Kay and coworkers describe the near complete chemical shift and secondary structure assignment of malate synthase G¹⁰ (a 723 residue, *M_r* 81.4 kDa, single chain protein). Shown in Figure 2 is a region of the proton-nitrogen correlation spectrum of malate synthase G recorded at 18.79 T. The region shown contains about a twen-

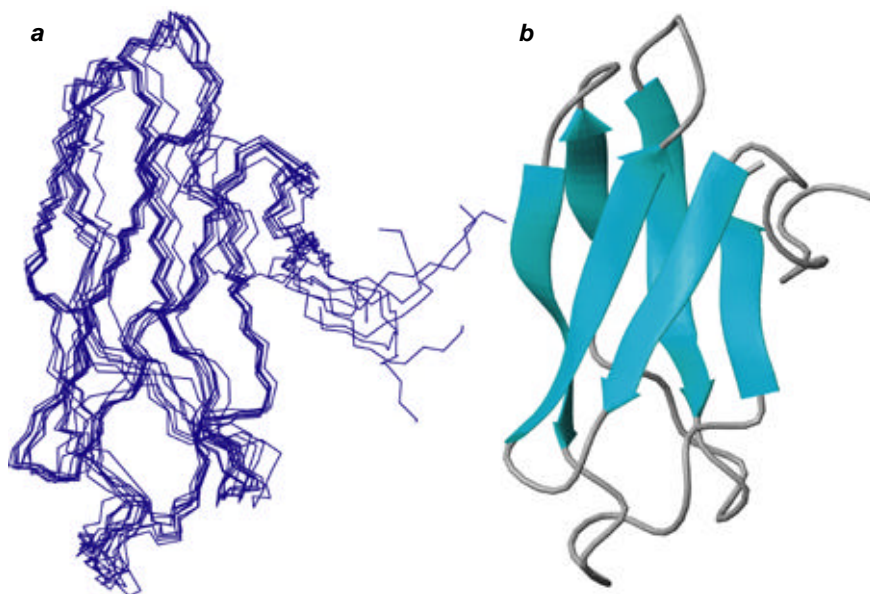


Figure 1. **a**, Ensemble of NMR structures of Tendamistat; **b**, Ribbon diagram of a representative NMR structure of Tendamistat. The Tendamistat coordinates are taken from the Protein Data Bank, PDB code 2ait.

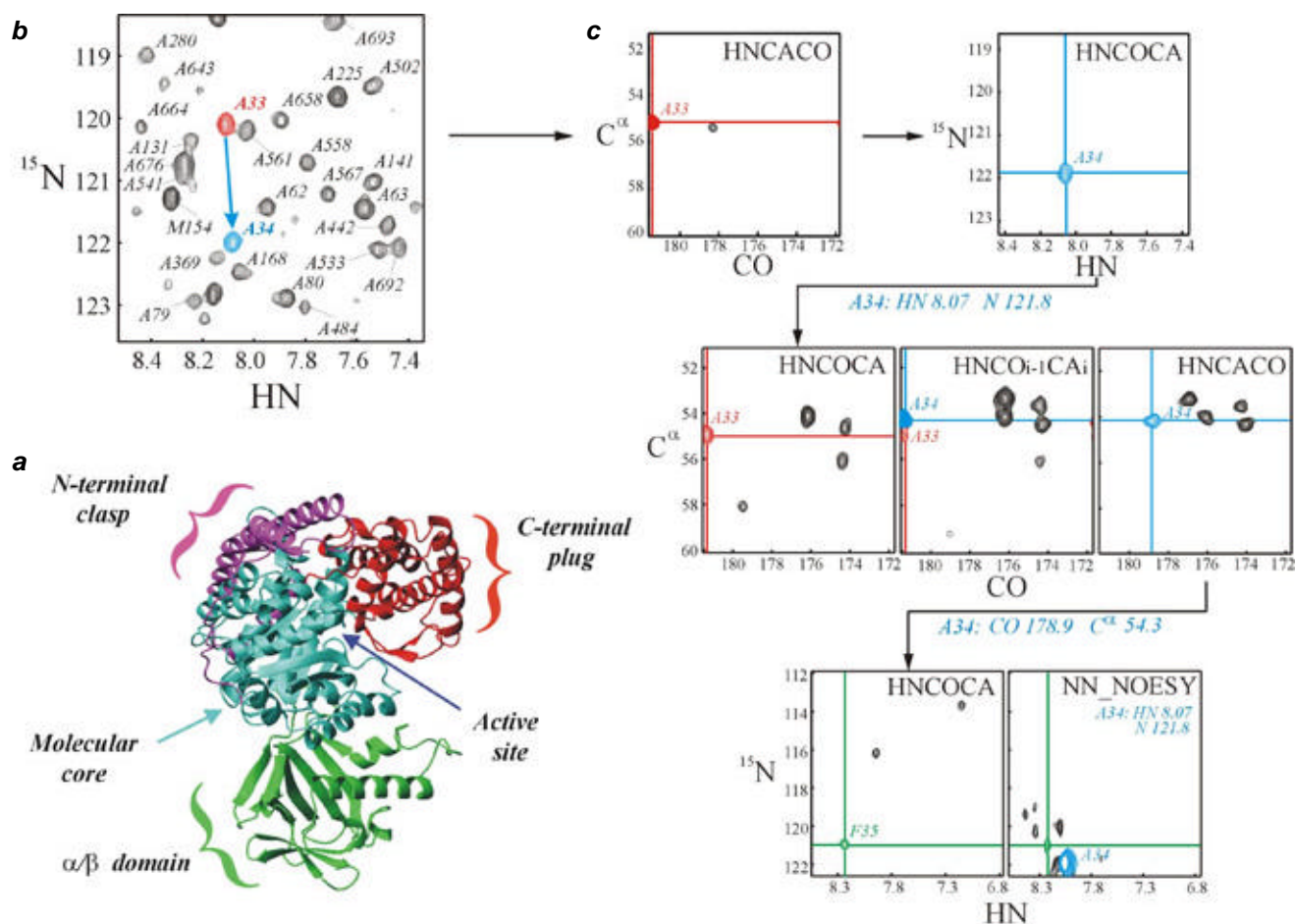


Figure 2. **a**, Ribbon diagram of MSG illustrating the four main domains of the molecule. The MSG coordinates are taken from the Protein Data Bank, PDB code 1d8c.22; **b**, Portion of an 800 MHz (18.79 T) ^{15}N - ^1H TROSY-HSQC spectrum of malate synthase G recorded at 37°C; **c**, Sequential backbone assignments for residues A33–F35 of MSG starting from the amide correlation of A33. Alpha-carbon and carbonyl-carbon and amide nitrogen–amide proton planes of the 4-D spectra used in the assignment process. The assignment procedure illustrated begins with the amide nitrogen–amide proton of alanine 33. (Figure 2 was kindly provided by Professor Lewis Kay, University of Toronto, Canada)

tieth of the resonances present in the protein. Also shown in the figure are the sequential assignments for three of the backbone amide proton–nitrogen pairs using isotope-edited three- and four-dimensional TROSY-based NMR spectroscopic methods. Strategies shown in the figure were used for making near complete sequence-specific resonance assignments. As world records go this is a significant one, for it lays to rest all speculation as to the size of single chain proteins that will be studied in the future. Wüthrich's Nobel Prize recognizes his seminal contributions to macromolecular NMR spectroscopy

and Lewis Kay's record work demonstrates that molecular size limits, as far as NMR is concerned, are a thing of the past.

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