Report: Nobel Prize in Chemistry, 2002

Using NMR To Determine Protein Structure in Solution

by Silvia Cavagnero

Half of the 2002 Nobel Prize for Chemistry was awarded to Kurt Wüthrich for the development of methods leading to the structure determination of proteins in solution by nuclear magnetic resonance (NMR) spectroscopy (see Figures 1 and 2). This is an important achievement that has enabled scientists all over the world to obtain very high resolution three-dimensional images of several molecules that lie behind the most intimate mysteries of life. Without a high resolution picture detailing the arrangement of all the relevant atoms and their dynamic behavior, it would be virtually impossible to learn how these molecules work and how they are able to support a variety of chemical and biochemical processes of key importance to all living cells. Moreover, obtaining this information in aqueous solution, as opposed to within a crystal, gives a "true report" of the biologically active conformation under physiologically relevant conditions.

Molecular Structures in Aqueous Solution

The development of these new methodologies has opened diverse avenues to the study of dynamic and structural properties of biomolecules in water under different sets of environmental conditions (pH, temperature, ionic strength, native or denaturing conditions). A whole new branch of structural biology has also emerged as a result of the ability to get structural information in water by NMR, that is, the study of ligand–receptor and protein–protein interactions at atomic resolution. This field includes the precise identification of binding sites and contact interfaces, the study of ligand binding-driven dynamic processes, and the analysis of macromolecular assembly phenomena involving multiple subunits.

A large portion of the complex and fascinating events that take place inside living cells involves peptides and proteins. These function either as enzymes (catalysts for biological reactions), ion and biomolecule transporters, or signaling/transduction mediators. Proteins are composed of a variable combination of amino acids (20 different kinds, depending on their individual side chain structure). These are joined together to form a long chain just like an array of beads in a string. This string of amino acids usually folds back onto itself in three dimensions to form an exquisitely specific and organized structure. Due to the many interactions between amino acid side chains, most protein structures are well defined and stable under physiological conditions in aqueous solution.

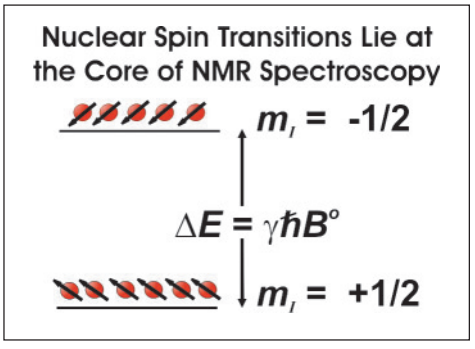

Figure 1. Illustration of the basic principles behind the NMR phenomenon for the simple case of a one-spin system. The two different spin states are denoted by the values of the nuclear spin quantum numbers m_I. Radiofrequency pulses cause transitions from the m_I = + ˚ to the m_I = -1/2 spin state by providing the necessary energy ΔE.

Kurt Wüthrich

Kurt Wüthrich's scientific journey and career spans continents and has taken some unexpected turns over the years. He did not fall in love with biomolecules and their structure determination from the start, as he himself recalls *(1)*. Wüthrich actually initiated his studies as an inorganic chemist and got his Ph.D. degree with Silvio Fallab at the University of Basel (Switzerland). He subsequently did a postdoc at the University of California, Berkeley with Robert Connick where he worked on relaxation processes in small metal complexes by both NMR and electron spin resonance (ESR) spectroscopies. He then spent some time at the Bell Telephone Laboratories in Murray Hill, New Jersey, with Robert Shulman. There he started concentrating his efforts on the NMR of metal-containing biomolecules. He finally returned to Europe in 1969 to take an academic position at the Eidgenössiche Technische Hochschule (ETH) in Zürich, still working on inorganic molecules. It was not until 1975 that his primary interest changed towards the high resolution structure determination of biomolecules by NMR. This change in focus was triggered by his reflections on the status of the field as he was writing a review article on the early years of macromolecular NMR *(2)*.

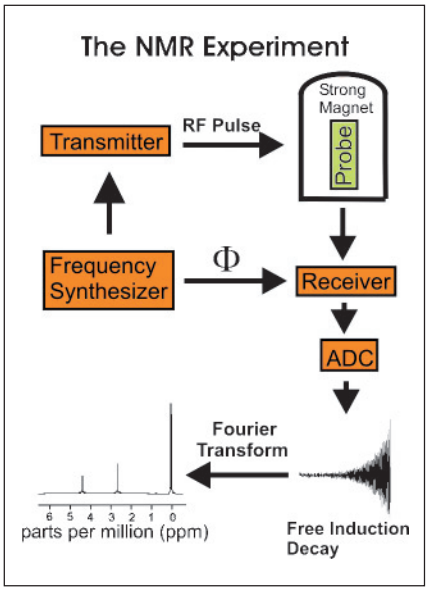

Figure 2. Schematic representation of the setup for a typical NMR experiment. Modern spectrometers utilize superconducting magnets capable of reaching proton resonance frequencies as high as 900 MHz.

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As a result of many years of efforts in this area, Wüthrich and coworkers attained a number of milestone achievements, including the first protein structure determination in solution by NMR in 1985 *(3),* the high resolution study of protein–DNA complex hydration *(4),* the determination of residual structure in an unfolded protein *(5),* and more recently, the development of transverse relaxation optimized spectroscopy (TROSY, ref *6*), which expands the useful molecular weight range of liquid-state NMR and enables the structural analysis of very large biomolecules, including the GroEL–GroES chaperone complex *(7).*

Structure Determination by NMR

The methods for structure determination in solution developed by Wüthrich and coworkers are based on the detection of a very large number (up to thousands) of intramolecular distances and their subsequent computational manipulation to yield a low-energy, well defined conformation *(8).* These methods are largely based on a well known effect in nuclear magnetic resonance known as the nuclear Overhauser effect (NOE). This effect is due to the through-space dipolar coupling among different NMR-active nuclei and it yields peaks whose intensities are proportional to $1/r^6$, where r is the internuclear distance of individual nuclear spin pairs. It is through the determination of these individual NOEs that multiple internuclear distances are obtained. These distances provide definite constraints to the allowed conformations of a polypeptide or protein in solution. The computational manipulation of these distances goes through appropriate "distance geometry" and "molecular dynamics" sophisticated calculations whose algorithms have been developed in the Wüthrich laboratory. Each typical NOE experiment on a protein yields hundreds of peaks due to the presence of many nearby nuclei. These peaks are so numerous and close in resonance frequency that they cannot be handled properly in typical simple, one-dimensional experiments. Two-dimensional and three-dimensional experiments are necessary in order to properly spread these peaks in multiple dimensions and correctly identify them. The multidimensional NMR techniques that made this possible have been a seminal contribution in NMR spectroscopy. One of its main developers, who followed early inspiring intuitions by the Belgian spectroscopist Jean Jeener, is Richard Ernst. He was recognized for this achievement (as well as for the development of Fourier Transform NMR) by the Nobel Prize for Chemistry in 1991.

Applications to Biological Systems

In addition to developing the methodologies that allowed protein structure determination in solution, Wüthrich and coworkers have been able to extensively apply these tools to a number of biological systems and were able to gain important structure-based insights into their function. The very first high resolution structure in solution was that of the bull seminal protease inhibitor (BUSI) *(3).* Shortly thereafter, the α-amylase inhibitor tendamistat *(9)* and rat metallothionein *(10)* structures were solved. These initial efforts were met with a considerable dose of skepticism by the scientific community

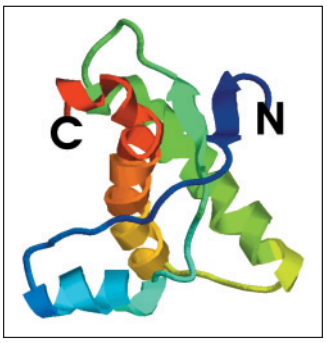

Figure 3. Three-dimensional image of the murine 121-231 prion protein domain. The structure was determined in solution by NMR spectroscopy *(14).* N and C denote the N- and C-termini of the polypeptide chain, respectively. [Structure from PDB database, code 1AG2; *http://www.rcsb.org/pdb* (accessed Dec 2002), using RasMol.]

especially in the case of the latter protein, whose structure had concurrently been solved by X-ray crystallography *(11)* and looked significantly different from the one determined in the Wüthrich laboratory. The X-ray structure was later proved to be incorrect *(12)* and, partially as a result of this controversy, the NMR method started gaining more and more momentum in both the spectroscopy and biology communities *(13).*

The last six years of Kurt Wüthrich's career have been particularly productive and have yielded extremely insightful results. In 1996, the solution structure of the murine prion protein appeared *(14).* This protein is the mouse version of the protein that causes bovine spongiform encephalopathy, better known as BSE or mad cow disease *(15).* This mysterious disease caused several deaths in England in 1996 among people who had eaten meat contaminated by the "scrapie" form of the prion protein. Since then, occasional deadly cases have occurred throughout the world, yet no firm cause–effect relationships for this lethal disorder have been established to date. Recent work suggests that the prion protein can be found both in a "cellular" non-harmful conformation and in an alternative deadly conformation known as the "scrapie" form. While the biomedical and biochemical aspects of this disease and the prion protein have actively been pursued by Stanley Prusiner (who won the 1997 Physiology and Medicine Nobel Prize for his discovery of the prion protein) and others, it was only as a result of the NMR structure solved in the Wüthrich lab in 1996 that people started gaining rational insights into what specific conformational changes may affect the onset of the disease and possibly the switch between the cellular and scrapie forms. For instance, the structure solved by Wüthrich and coworkers has an unusual, totally unstructured N-terminal portion and a compact, mixed β-sheet/α-helical C-terminal region. The structure of the C-terminal region is shown in Figure 3. The unstructured region may be involved in triggering a conformational transition. Additionally, a specific loop in the structured region (with variable dynamic behavior across species) may serve as a nucleation site for the transition to the deadly conformation. While none of these hypotheses has been confirmed yet and the issue is still far from being solved to date, it is clear that no detailed understanding of the conformational origins of the prion-related diseases would be possible in the absence of structural and dynamic information on this intriguing protein.

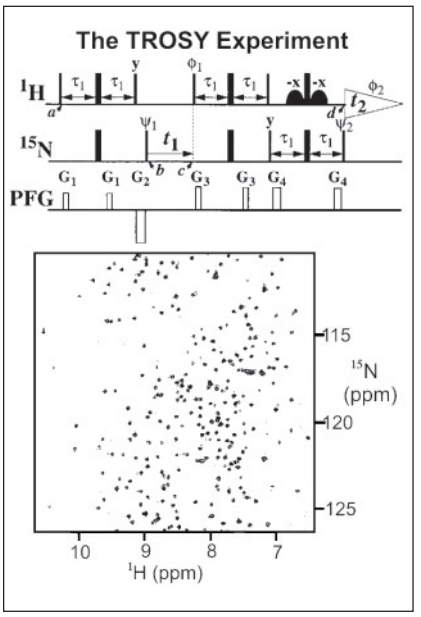

Figure 4. NMR Pulse sequence for the TROSY experiment (above) and 2D TROSY-type [^{1}H,^{15}N]-correlation spectrum of uniformly ^{15}N- and ^{2}H-labeled gyrase-45 (a 45 kiloDalton protein) from *Staphylococcus aureus* in water at 25 °C (below). [Pulse sequence from ref *6*, copyright © 1997 National Academy of Sciences, U.S.A. Spectrum reprinted from ref *17*, copyright © 1999 with permission from Elsevier Science.]

Transverse Relaxation Optimized Spectroscopy

A final important recent contribution from the Wüthrich lab is the development of transverse relaxation optimized spectroscopy (TROSY) and its related implementations CRINEPT and CRIPT *(6, 16, 17)*. These novel pulse sequences directly address, and improve upon, one of the limitations of NMR spectroscopy, that is, the inability to obtain high quality data for globular proteins larger than about 20 kDa. This is due to the extensive line broadening that takes place for peaks belonging to high molecular weight species. The TROSY sequence and its above-mentioned close relatives ingeniously take advantage of the destructive interference between dipole–dipole and chemical shift anisotropy relaxation mechanisms for specific NMR resonances at high applied magnetic fields. As a result of judiciously exploiting this interference, peaks are obtained that are considerably sharper than expected. When combined with protein perdeuteration, the TROSY-based techniques enable tackling solution structures much larger than previously possible (see Figure 4). This considerably extends the current capabilities of the NMR technique. Two eloquent examples of the power of this approach are the recent conformational studies on the gigantic GroEL–GroES chaperone complex (900 kDa) *(7)* and the all β-sheet membrane protein OmpX reconstituted in dihexanoyl phosphatidyl choline (DHPC) micelles *(18, 19)*. All these spectacular achievements speak towards the scientific excellence of Kurt Wüthrich's work and hold promise for even more exciting developments in the area of biomolecular NMR spectroscopy for the years to come.

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