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MILESTONE 16

# Solution for solution structures

> NMR has become a powerful technique for protein structure determination.

In the mid-1970s, major advances in solution nuclear magnetic resonance (NMR) spectroscopy set the stage for a revolutionary new application: solving the three-dimensional (3D) structures of proteins in the solution state. At the time, X-ray crystallography was already a well-established tool for the determination of protein crystal structures. Unlike crystallography, NMR does not require proteins to form diffracting crystals and this broadens the range of proteins that can be investigated. Furthermore, most proteins exist naturally in a solution state, or in contact with fluids, so knowledge of their properties in their native environment has physiological relevance.

A unique strength of NMR is its ability to supplement molecular structures with information on dynamic processes, which may be influenced, for example, by ligand binding in solution: even before structure determination by NMR became feasible, the technique was used to obtain information about protein dynamics. In 1971, Adam Allerhand and colleagues demonstrated the existence of sub-nanosecond segmental motions in proteins, and by the middle of the decade, the groups of Brian Sykes, Robert J. P. Williams and Kurt Wüthrich presented evidence for lower-frequency motions in globular proteins.

Two fundamental advances in the late 1970s set the scene for the development of NMR into a method for determining previously unknown protein structures (rather than refining incomplete structures). First, Richard Ernst, building on a breakthrough idea by Jean Jeener, demonstrated the principle of two-dimensional (2D) NMR spectroscopy. This technique, which also applies to other spectroscopies, allowed researchers to record not only chemical shifts (Milestone 10) but also the interactions between pairs of nuclear spins — it later won Ernst the 1991 Nobel Prize in Chemistry. Second, Wüthrich discovered that the nuclear Overhauser effect could be exploited in NMR experiments with proteins, allowing the mapping of networks of near-by atom pairs that are not connected through covalent bonds. Beginning in 1976, Wüthrich and Ernst joined forces, and with Kuniaki Nagayama and Anil Kumar they developed a number of 2D NMR experiments, which became the basis for solving protein structures.

In 1982, Gerhard Wagner and Wüthrich published the sequence-specific assignments for a small protein, basic pancreatic trypsin inhibitor. Meanwhile, Werner Braun and Timothy Havel in the Wüthrich group were developing algorithms and software capable of calculating protein structures from NMR data. In 1985, Michael Williamson, Havel and Wüthrich reported the first solution-state protein structure — that of proteinase inhibitor IIA from bull seminal plasma. The results were met with disbelief. It was not until several structures solved initially using NMR were solved again using crystallography that the NMR technique was accepted. In 2002, Wüthrich was rewarded with the Nobel Prize in Chemistry.

NMR has become a powerful technique for protein structure determination. Numerous advances made during the past two decades — including the development of three- and four-dimensional spectroscopy, isotope labelling methods, and increases in magnetic field strength — have raised the limit on the size of proteins that can be investigated. Currently, about 10% of structures being deposited in the Protein Data Bank are solved using NMR. Perhaps the most exciting frontier is the application of NMR to investigate protein dynamics, especially for large molecular machines, which will undoubtedly lead to new insights in biology.

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**ORIGINAL RESEARCH PAPERS** Allerhand, A. *et al.* Conformation and segmental motion of native and denatured ribonuclease A in solution. Application of natural-abundance carbon-13 partially relaxed Fourier transform nuclear magnetic resonance. *J. Am. Chem. Soc.* **93**, 544–546 (1971) | Wüthrich, K. & Wagner, G. NMR investigations of the dynamics of the aromatic amino acid residues in the basic pancreatic trypsin inhibitor. *FEBS Lett.* **50**, 265–268 (1975) | Dobson, C. M., Moore, G. R. & Williams, R. J. P. Assignment of aromatic amino acid PMR resonances of horse ferricytochrome c. *FEBS Lett.* **51**, 60–65 (1975) | Snyder, G. H., Rowan, R. & Sykes, B. D. Complete tyrosine assignments in the high-field proton nuclear magnetic resonance spectrum of bovine pancreatic trypsin inhibitor selectively reduced and carboxamidomethylated at cystine 14-38. *Biochemistry* **15**, 2275–2283 (1976) | Aue, W. P., Bartholdi, E. & Ernst, R. R. Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J. Chem. Phys.* **64**, 2229–2246 (1976) | Nagayama, K., Wüthrich, K., Bachmann, P. & Ernst, R.R. Two-dimensional J-resolved $^1$H NMR spectroscopy for studies of biological macromolecules. *Biochem. Biophys. Res. Commun.* **78**, 99–105 (1977) | Kumar, A., Ernst, R. R. & Wüthrich, K. A two-dimensional nuclear Overhauser enhancement (2D NOE) experiment for the elucidation of complete proton–proton cross-relaxation networks in biological macromolecules. *Biochem. Biophys. Res. Commun.* **95**, 1–6 (1980) | Wagner, G. & Wüthrich, K. Sequential resonance assignments in protein $^1$H nuclear magnetic resonance spectra: basic pancreatic trypsin inhibitor. *J. Mol. Biol.* **155**, 347–366 (1982) | Williamson, M. P., Havel, T. F. & Wüthrich, K. Solution conformation of proteinase inhibitor IIA from bull seminal plasma by $^1$H nuclear magnetic resonance and distance geometry. *J. Mol. Biol.* **182**, 295–315 (1985)