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SEQUENTIAL RESONANCE ASSIGNMENTS AS A BASIS FOR
DETERMINATION OF SPATIAL PROTEIN STRUCTURES BY HIGH
RESOLUTION PROTON NUCLEAR MAGNETIC RESONANCE.

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Sequential Resonance Assignments as a Basis for Determination of Spatial Protein Structures by High Resolution Proton Nuclear Magnetic Resonance

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A general scheme is proposed for the determination of spatial protein structures by proton nuclear magnetic resonance. The scheme relies on experimental observation by two-dimensional nuclear magnetic resonance techniques of complete through-bond and through-space proton–proton connectivity maps. These are used to obtain sequential resonance assignments for the individual residues in the amino acid sequence and to characterize the spatial polypeptide structure by a tight network of semi-quantitative, intramolecular distance constraints.

1. Introduction

For over 20 years, single-crystal X-ray studies have set the standards for characterization of protein conformations (Schulz & Schirmer, 1979; Richardson, 1981). The highly sophisticated structural information obtained has long called for complementary techniques to obtain corresponding data for polypeptide chains in solution and in other non-crystalline environments. The potential of nuclear magnetic resonance for such studies has been indicated by various early experiments (see e.g. Roberts & Jardetzky, 1970; Wüthrich, 1976). However, as in n.m.r.[†] structure determinations for small molecules, the realization of the full potentialities of the method for structural studies of proteins depends on the identification of the individual resonance lines, and lack of reliable, generally applicable ways for obtaining individual assignments in polypeptide chains has so far been a severely limiting factor. With the use of high magnetic fields and two-dimensional spectroscopy techniques (Aue *et al.*, 1976; Freeman & Morris, 1979; Wüthrich *et al.*, 1979), individual assignments for almost all the resonance lines in protein ¹H n.m.r. spectra can now be obtained efficiently, as is described in detail in the following three papers (Billeter *et al.*, 1982; Wagner & Wüthrich, 1982;

† Abbreviations used: n.m.r., nuclear magnetic resonance; COSY, 2-dimensional correlated spectroscopy; SECSY, 2-dimensional spin echo correlated spectroscopy; NOE, nuclear Overhauser enhancement; NOESY, 2-dimensional NOE spectroscopy.

Wider et al., 1982). Of course, this provides a more solid basis for "conventional" applications of n.m.r.; for example, for delineation of active centers in enzymes, comparison of crystal and solution conformations from ring current calculations of the chemical shifts, or studies of internal mobility in globular proteins (see e.g. Roberts & Jardetzky, 1970; Wüthrich, 1976). Even more excitingly, experience from work with smaller sets of assigned resonances (Braun et al., 1981; Crippen et al., 1981) implies that with nearly complete individual assignments of the resonance lines in a protein ¹H n.m.r. spectrum, the three-dimensional structure could be determined entirely from the known amino acid sequence and a suitable set of n.m.r. data. This paper outlines a general scheme for ¹H n.m.r. determination of spatial protein structures, which relies on sequential individual resonance assignments.

2. Fundamental Considerations

During almost 25 years of n.m.r. studies with proteins, a variety of different ¹H n.m.r. manifestations of the spatial structures of polypeptide chains were described (for a survey, see e.g. Wüthrich, 1976). The dispersion of the chemical shifts that arises from interactions between nearby groups of atoms in interior parts of globular proteins (Fig. 1) is perhaps the most readily apparent, conformation-dependent n.m.r. spectral feature (McDonald & Phillips, 1967). Studies based on empirical comparison of chemical shifts yielded a wealth of interesting data on differences between protein conformations in different solvent media, internal flexibility of globular proteins, protein folding and various aspects of protein functions. However, one tackles a considerably more difficult problem when trying to determine the conformation of a polypeptide chain from the known amino acid sequence and n.m.r. data. While the dispersion of the chemical shifts (Fig. 1) obviously presents a criterion to discriminate between individual amino acid residues, and thus provides a basis for obtaining a many-parameter characterization of the spatial structure by n.m.r., it also leads to very complex, crowded protein ¹H n.m.r. spectra (compare traces A and B in Fig. 1). Furthermore, theoretical understanding of chemical shifts is too limited to allow determination of spatial structures based on measurements of this parameter. Therefore, a promising scheme for ¹H n.m.r. determination of protein conformations must include techniques able to resolve and assign the complex ¹H n.m.r. spectra, and to measure spectral parameters that can be directly correlated with the spatial structure.

The strategy for protein structure determination outlined in Figure 2 relies on very recent advances in n.m.r. techniques. Two-dimensional n.m.r. provides well-resolved protein ¹H n.m.r. spectra, and two different types of two-dimensional experiments are used to map two kinds of connectivities between the protons in the polypeptide chain. These are through-bond, scalar "J-connectivities" between hydrogen atoms linked via two or three bonds in the covalent polypeptide structure and through-space, dipolar "NOE-connectivities" between hydrogen atoms located at short distances in the spatial structure. J-connectivity maps of proteins have
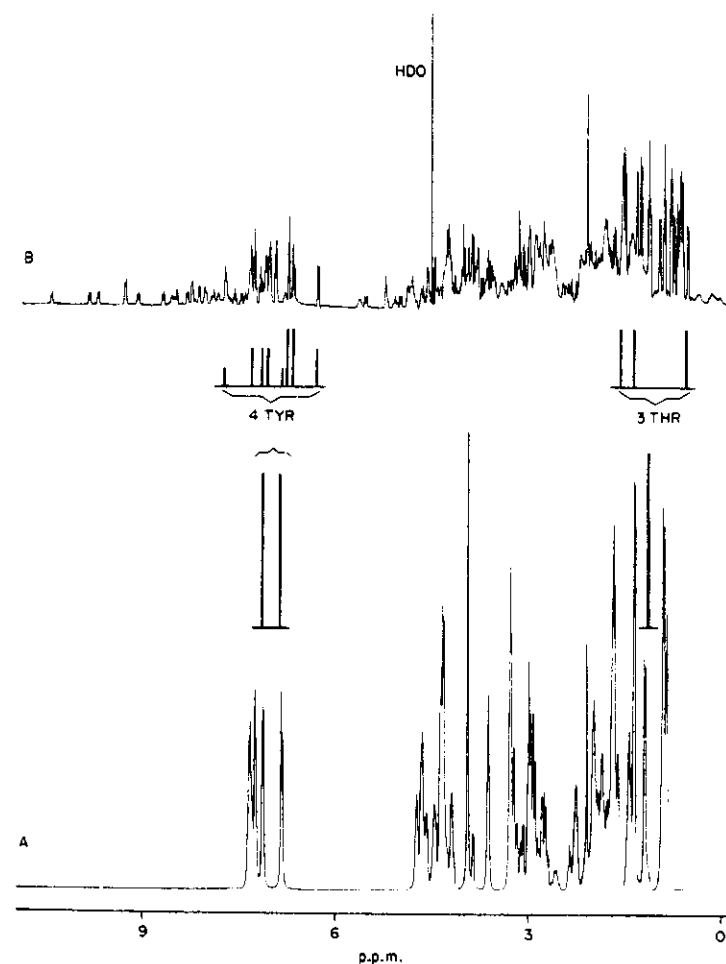

FIG. 1. Illustration of conformation-dependent ¹H n.m.r. chemical shifts in a globular protein. The lower trace (A) represents a hypothetical random coil spectrum for the polypeptide chain of the basic pancreatic trypsin inhibitor (BPTI) in ²H₂O solution, which was computed from the random coil parameters presented by Bundi & Wüthrich (1979a). Trace B is the 360 MHz ¹H n.m.r. spectrum of a freshly prepared solution of BPTI in ²H₂O (Wüthrich & Wagner, 1979). The stick diagrams below and above the letters 4 TYR and 3 THR in the centre of the Figure indicate chemical shifts and resonance intensities for the γ-methyl groups of the 3 threonine residues in positions 11, 32 and 54 and the aromatic protons of the 4 tyrosine residues in positions 10, 21, 23 and 35 in the spectra A and B, respectively. They show that in the random coil polypeptide, the peripheral side-chain hydrogen atoms of identical residues at different locations in the amino acid sequence have identical chemical shifts, whereas in the globular protein, one observes a dispersion of the chemical shifts due to the different microenvironments of the individual residues (McDonald & Phillips, 1967; Wüthrich, 1976). p.p.m., parts per million.

been recorded with two-dimensional correlated spectroscopy or spin echo correlated spectroscopy (Aue *et al.*, 1976; Nagayama *et al.*, 1979,1980; Anil Kumar *et al.*, 1980*b*; Wagner *et al.*, 1981). A COSY or SECSY spectrum provides, with a single instrument setting, a complete map of all ^{1}H–^{1}H J-connectivities in a polypeptide chain. From this, the spin systems of the different types of amino acid residues (Wüthrich, 1976) can be identified. NOE connectivity maps have been
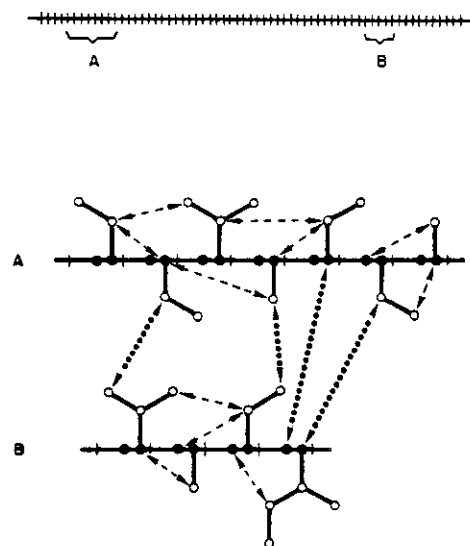

FIG. 2. Schematic representation of the proposed strategy for determination of spatial protein structures by ^{1}H n.m.r. At the top of the Figure, the horizontal line intersected by vertical dashes represents a polypeptide chain. In the larger scale drawings of segments A and B, short vertical dashes separate neighbouring residues, filled circles represent CH and NH groups of the backbone, and open circles represent CH groups of the amino acid side-chains. The first step in the structure determination is the sequential assignment of the resonances to the individual amino acid residues (Dubs *et al.*, 1979; Wagner *et al.*, 1981; Billeter *et al.*, 1982; Wagner & Wüthrich, 1982; Wider *et al.*, 1982). From distance constraints between distinct hydrogen atoms in the amino acid sequence, which are manifested in NOE connectivity maps, the spatial structure is then evaluated (Braun *et al.*, 1981). For example, distance constraints between backbone amide, C^α and C^β hydrogen atoms of nearby residues in the amino acid sequence (◀ – ~ – – ▶ in A and B) characterize the secondary structure, and long-range distance constraints (with respect to the locations of the residues in the amino acid sequence) between backbone and amino acid side-chain hydrogen atoms (◀ ● ● ● ● ▶) define the supersecondary and tertiary structures.

obtained by two-dimensional nuclear Overhauser enhancement spectroscopy (Jeener *et al.*, 1979; Anil Kumar *et al.*, 1980*a,b*; Wagner *et al.*, 1981). As the spectral manifestations of NOE connectivities develop slowly during a period of several hundred milliseconds, different NOESY spectra can be obtained by variation of one of the instrument settings, the so-called "mixing time". Semi-quantitative measurements of non-bonding distances in the range 2 to 5 Å can thus be obtained

(Anil Kumar *et al.*, 1981)[†]. These distance constraints mapped in the NOESY spectra can then be correlated directly with the protein conformation (Braun *et al.*, 1981).

3. Sequential Resonance Assignments

Individual assignments of a large proportion of the hundreds or even thousands of resonance lines (Fig. 1, trace B) to particular residues in the amino acid sequence represent the core of the presently described scheme for ^{1}H n.m.r. determination of protein conformations. This can be appreciated readily if one considers that intramolecular distance constraints (arrows in Fig. 2) or other potentially suitable parameters for structure determination can be correlated with the protein conformation only when they have been assigned to particular hydrogen atoms in the polypeptide chain.

Unambiguous assignments for a small number of resonances have been obtained in previous work with selective modification or substitution of individual residues (e.g. Snyder *et al.*, 1975). This will undoubtedly be a suitable approach also for future work that relies on the observation of a small number of resonance lines, but it appears unrealistic to use selective modification of individual residues by chemical or biological techniques for assignments of the bulk of a protein ^{1}H n.m.r. spectrum.

Most of the resonance assignments described so far have relied in part or entirely on reference to the crystal structure of the protein, or on observations linked with the functional properties. While this may have been an appropriate procedure in

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† In principle, it should be possible to relate proton–proton distances shorter than ~5·0 Å quantitatively with suitably executed NOE measurements (see e.g. Noggle & Schirmer, 1971; Gordon & Wüthrich, 1978; Wagner & Wüthrich, 1979; Bothner-By & Noggle, 1979; Poulsen *et al.*, 1980; Anil Kumar *et al.*, 1981). In practice, however, a variety of different factors tend to mask the quantitative distance information contained in NOE data. Thus, distance measurements with the use of NOE experiments can be complicated by fundamental physical properties of the systems studied. These may include spin relaxation by other mechanisms than proton–proton dipolar coupling, for example by ^{14}N–^{1}H dipolar coupling or by paramagnetic impurities (Wüthrich, 1976), flexibility of the molecular structure (Braun *et al.*, 1981), and adverse effects of spin diffusion by cross relaxation (Kalk & Berendsen, 1976; Gordon & Wüthrich, 1978; Bothner-By & Noggle, 1979; Wagner & Wüthrich, 1979; Anil Kumar *et al.*, 1981). Practical experience (Dubs *et al.*, 1979; Wagner *et al.*, 1981; Braun *et al.*, 1981; Wagner & Wüthrich, 1982; Wider *et al.*, 1982) has shown that these fundamental complications do not, in general, preclude the use of NOE experiments for obtaining the semi-quantitative distance constraints needed for the presently discussed sequential resonance assignments and spatial structure determinations in globular proteins. It remains to be seen to what extent these effects could, in specified systems, be properly accounted for in order to obtain quantitative distance measurements from NOE studies. In addition, there are also various technical complications involved in the quantitative evaluation of NOE peak intensities in the NOESY spectra. One of these problems has been solved recently, i.e. the complete separation of the contributions from incoherent and coherent magnetization transfer to the peak intensities in NOESY spectra recorded with short mixing times (Macura *et al.*, 1981,1982). Other problems currently under investigation include, for example, the influence of a variety of filtering functions on the relative intensities of different peaks in NOESY spectra and the use of absolute value spectra *versus* absorption mode spectra for quantitative NOE measurements. This short and incomplete list of complications that can arise in the quantitative analysis of NOESY spectra illustrates that there is a lot of room for further advances. It should also explain why we are so pleased that the presently described approach for structure determination relies exclusively on qualitative analysis of the peak intensities in the NOESY spectra.

some of the studies concerned, it is not acceptable for the present scheme, which should provide a basis for meaningful, detailed comparisons of protein conformations in single crystals and non-crystalline environments, and for structural interpretations of functional properties of proteins in non-crystalline environments.

A straightforward concept for obtaining individual resonance assignments in a polypeptide chain is to identify the resonance lines of the individual residues step by step along the amino acid sequence. In practice, combined use of J-connectivity maps obtained from COSY or SECSY experiments and NOE connectivity maps obtained from NOESY experiments with short mixing times presents an efficient, reliable and generally applicable method for obtaining sequential resonance assignments. This is described in detail in the following three papers (Billeter *et al.*, 1982; Wagner & Wüthrich, 1982; Wider *et al.*, 1982). In this procedure, the connectivities between neighbouring residues are established *via* the amide protons. It is therefore important that COSY, SECSY and NOESY spectra can be recorded with comparable ease in ^{2}H$_2$O and H$_2$O solutions of proteins (Anil Kumar *et al.*, 1980b; Wagner *et al.*, 1981), because some or all of those critical amide protons may be exchanged too rapidly with the solvent to be observed in ^{2}H$_2$O (Wüthrich, 1976). Since the sequential assignments depend on ^{1}H–^{1}H distance constraints between neighbouring residues, one obtains simultaneously a first, qualitative outline of the secondary structure, which can be specified by those same distances (Dubs *et al.*, 1979; Wagner *et al.*, 1981; Billeter *et al.*, 1982).

Sequential assignments can, in principle, also be obtained with combined use of homonuclear and heteronuclear J-connectivities between ^{1}H, ^{13}C and ^{15}N (Llinás *et al.*, 1977; Okhanov *et al.*, 1980). At present, these experiments appear to be too laborious to be of practical use even for small proteins. However, any approach that depends entirely on through-bond J-connectivities would be appealing, and it is very possible that with future improvements in n.m.r. methodology, these experiments will become a viable alternative to those used at present.

4. Determination of Spatial Polypeptide Structures *via* Intramolecular Distance Constraints

As an alternative to the more familiar listings of atomic co-ordinates or dihedral angles (Schulz & Schirmer, 1979), spatial protein structures may also be characterized by a list of intramolecular distances between specified pairs of atoms (Crippen, 1979; Havel *et al.*, 1979), provided the chirality of the structure is independently specified (Cohen & Sternberg, 1980; Braun *et al.*, 1981). An extension of Crippen's distance geometry algorithm for use with the distance constraints obtained from NOE connectivity maps was described recently (Braun *et al.*, 1981) and applied to determine spatial structures for short segments of the polypeptide chains of lipid-bound glucagon (Braun *et al.*, 1981) and melittin (Brown *et al.*, 1982). While these structure determinations were limited by the small number of resonance assignments available at the time, they indicated a general feature that should have important consequences also when working with complete sets of assigned resonances. The implication is that it will be more

effective to obtain a large number of relatively inaccurate distance constraints; for example, stating that the distances separating particular pairs of hydrogen atoms are $\lesssim 5.0$ Å, rather than a small number of accurate interatomic distances. This further supports the suggestion that the NOE connectivity maps in NOESY spectra present suitable data sets for spatial structure determination, since they provide numerous semi-quantitative distance constraints (Anil Kumar *et al.*, 1980a,b;1981).

On the basis of the sequential resonance assignments, the NOE connectivities in the NOESY spectra recorded with different mixing times (Anil Kumar *et al.*, 1981) can be correlated with distance constraints between distinct hydrogen atoms in the amino acid sequence†. The distance constraints can then be grouped into different classes, depending on the hierarchic level of structure (Schulz & Schirmer, 1979) with which they are correlated (Fig. 2). Thus, distance constraints between backbone amide, C$^\alpha$ and C$^\beta$ hydrogen atoms in residues that are closely spaced in the amino acid sequence would be used to characterize the secondary structure. Distance constraints among backbone and C$^\beta$ hydrogen atoms of residues that are further apart in the sequence (e.g. between residues located in segments A and B in Fig. 2) provide the most reliable data on supersecondary structures, such as the combination of extended polypeptide segments into β-sheets (Wagner *et al.*, 1981) or the packing of α-helices. Finally, the connectivities with peripheral side-chain hydrogen atoms would be used to orient the amino acid side-chains, and thus obtain a characterization of the tertiary structure. It remains to be seen to what extent the structural analysis of the experimental distance constraints will be done by global evaluation of the data for the entire protein; for example, with the use of a distance geometry algorithm (Braun *et al.*, 1981), or by more intuitive methods, such as interactive use of a computer graphics system (Billeter, 1980).

5. Concluding Remarks

The general scheme for structure determination outlined in Figure 2 and sections 3 and 4 above should be applicable to obtain essentially complete descriptions of the conformations of small proteins, and also for partial structure elucidations in high molecular weight species. For example, it is conceivable that the resonances of more flexible polypeptide segments in large proteins could, on the basis of the different relaxation times (Wüthrich, 1976), be separated from those of the more rigid molecular regions, and thus become accessible for detailed studies. Similarly, the resonances of polypeptide chains bound to ordered lipid structures (Braun *et al.*, 1981; Brown *et al.*, 1982; Wider *et al.*, 1982) or to high molecular weight nucleic acids might be singled out for detailed investigation.

It is an important asset of the presently proposed strategy for protein structure determination by n.m.r. that it relies on qualitative or, at most, semi-quantitative interpretation of NOE connectivity maps†. The available experience indicates that a tight network of qualitative distance constraints, providing the data needed for a structure determination, can quite generally be obtained for the interior regions of

† See footnote to p. 315.

globular proteins and for polypeptide segments that are otherwise restricted in their local mobility; for example, by binding to an ordered lipid surface or to other macromolecules (Braun *et al.*, 1981). Additional structure refinements might then be obtained for these molecular regions by quantitating certain NOE measurements (Noggle & Schirmer, 1971; Kalk & Berendsen, 1976; Gordon & Wüthrich, 1978; Wagner & Wüthrich, 1979; Anil Kumar *et al.*, 1981; Macura *et al.*, 1981,1982). Furthermore, theoretical methods, for example energy refinement (Levitt, 1974), might profitably be employed to further improve the structures. In contrast, meaningful distance constraints from NOE-connectivities cannot generally be expected for highly flexible surface areas (Noggle & Schirmer, 1971), for example the chain termini and peripheral fragments of long amino acid side-chains. Therefore, in the final stages of the structure determination, different n.m.r. experiments should be used for obtaining additional information needed to characterize the protein surface. These may include measurements of spin-spin coupling constants by J-resolved two-dimensional spectroscopy (Nagayama *et al.*, 1977; Nagayama & Wüthrich, 1981), studies of pH titration connectivities between different groups (Brown *et al.*, 1976,1978; Bundi & Wüthrich, 1979b), use of paramagnetic shift and relaxation reagents (Dwek, 1973) and many other experiments that have hitherto profitably been used in n.m.r. studies of various aspects of protein structure and functions (see e.g. Dwek, 1973; Wüthrich, 1976). Combined with the individual resonance assignments and with the data on the core of the protein structure obtained by the new techniques outlined in this paper, such "conventional" n.m.r. experiments should be able to provide additional precise structural data, and thus contribute towards a rather complete determination of protein conformations in non-crystalline environments.

REFERENCES

Anil Kumar, Ernst, R. R. & Wüthrich, K. (1980a). *Biochem. Biophys. Res. Commun.* **95**, 1-6.
Anil Kumar, Wagner, G., Ernst, R. R. & Wüthrich, K. (1980b). *Biochem. Biophys. Res. Commun.* **96**, 1156-1163.
Anil Kumar, Wagner, G., Ernst, R. R. & Wüthrich, K. (1981). *J. Amer. Chem. Soc.* **103**, 3654-3658.
Aue, W. P., Bartholdi, E. & Ernst, R. R. (1976). *J. Chem. Phys.* **64**, 2229-2246.
Billeter, M. (1980). Diploma thesis, ETH-Zürich.
Billeter, M., Braun, W. & Wüthrich, K. (1982). *J.Mol. Biol.* **155**, 321-346.
Bothner-By, A. A. & Noggle, J. A. (1979). *J. Amer. Chem. Soc.* **101**, 5152-5155.
Braun, W., Bösch, C., Brown, L. R., Gō, N. & Wüthrich, K. (1981). *Biochim. Biophys. Acta*, **667**, 377-396.
Brown, L. R., De Marco, A., Wagner, G. & Wüthrich, K. (1976). *Eur. J. Biochem.* **62**, 103-107.

Brown, L. R., De Marco, A., Richarz, R., Wagner, G. & Wüthrich, K. (1978). *Eur. J. Biochem.* **88**, 87-95.
Brown, L. R., Braun, W., Anil Kumar & Wüthrich, K. (1982). *Biophys. J.* **37**, Nr. 1.
Bundi, A. & Wüthrich, K. (1979a). *Biopolymers*, **18**, 285-298.
Bundi, A. & Wüthrich, K. (1979b). *Biopolymers*, **18**, 299-312.
Cohen, F. E. & Sternberg, M. J. E. (1980). *J. Mol. Biol.* **138**, 321-333.
Crippen, G. M. (1979). *Int. J. Pept. Protein Res.* **13**, 320-326.
Crippen, G. M., Oppenheimer, N. J. & Connolly, M. L. (1981). *Int. J. Pept. Protein Res.* **17**, 156-169.
Dubs, A., Wagner, G. & Wüthrich, K. (1979). *Biochim. Biophys. Acta*, **577**, 177-194.
Dwek, R. A. (1973). *Nuclear Magnetic Resonance (NMR) in Biochemistry*, Clarendon Press, Oxford.
Freeman, R. & Morris, G. A. (1979). *Bull. Magn. Reson.* **1**, 5-26.
Gordon, S. L. & Wüthrich, K. (1978). *J. Amer. Chem. Soc.* **100**, 7094-7096.
Havel, T. F., Crippen, G. M. & Kuntz, I. D. (1979). *Biopolymers*, **18**, 73-81.
Jeener, J., Meier, B. H., Bachmann, P. & Ernst, R. R. (1979). *J. Chem. Phys.* **71**, 4546-4553.
Kalk, A. & Berendsen, H. J. C. (1976). *J. Magn. Reson.* **24**, 343-366.
Levitt, M. (1974). *J. Mol. Biol.* **82**, 393-420.
Llinás, M., Wilson, D. M. & Klein, M. P. (1977). *J. Amer. Chem. Soc.* **99**, 6846-6850.
Macura, S., Huang, Y., Suter, D. & Ernst, R. R. (1981). *J. Magn. Reson.* **43**, 259-281.
Macura, S., Wüthrich, K. & Ernst, R. R. (1982). *J. Magn. Reson.* **46**.
McDonald, C. C. & Phillips, W. D. (1967). *J. Amer. Chem. Soc.* **89**, 6332-6344.
Nagayama, K. & Wüthrich, K. (1981). *Eur. J. Biochem.* **114**, 365-374.
Nagayama, K., Wüthrich, K., Bachmann, P. & Ernst, R. R. (1977). *Biochem. Biophys. Res. Commun.* **78**, 99-105.
Nagayama, K., Wüthrich, K. & Ernst, R. R. (1979). *Biochem. Biophys. Res. Commun.* **90**, 305-311.
Nagayama, K., Anil Kumar, Wüthrich, K. & Ernst, R. R. (1980). *J. Magn. Reson.* **40**, 321-334.
Noggle, J. H. & Schirmer, R. E. (1971). *The Nuclear Overhauser Effect*, Academic Press, New York.
Okhanov, V. V., Afanas'ev, V. A. & Bystrov, V. F. (1980). *J. Magn. Reson.* **40**, 191-195.
Poulsen, F. M., Hoch, J. C. & Dobson, C. M. (1980). *Biochemistry*, **19**, 2597-2607.
Richardson, J. S. (1981). *Advan. Protein Chem.* **34**, 167-339.
Roberts, G. C. K. & Jardetzky, O. (1970). *Advan. Protein Chem.* **24**, 447-545.
Schulz, G. E. & Schirmer, R. H. (1979). *Principles of Protein Structure*, Springer, New York.
Snyder, G. H., Rowan III, R., Karplus, S. & Sykes, B. D. (1975). *Biochemistry*, **14**, 3765-3777.
Wagner, G. & Wüthrich, K. (1979). *J. Magn. Reson.* **33**, 675-680.
Wagner, G. & Wüthrich, K. (1982). *J. Mol. Biol.* **155**, 347-366.
Wagner, G., Anil Kumar & Wüthrich, K. (1981). *Eur. J. Biochem.* **114**, 375-384.
Wider, G., Lee, K. H. & Wüthrich, K. (1982). *J. Mol. Biol.* **155**, 367-388.
Wüthrich, K. (1976). *NMR in Biological Research: Peptides and Proteins*, North-Holland Publishing Company, Amsterdam.
Wüthrich, K. & Wagner, G. (1979). *J. Mol. Biol.* **130**, 1-18.
Wüthrich, K., Nagayama, K. & Ernst, R. R. (1979). *Trends Biochem. Sci.* **4**, N178-N181.

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