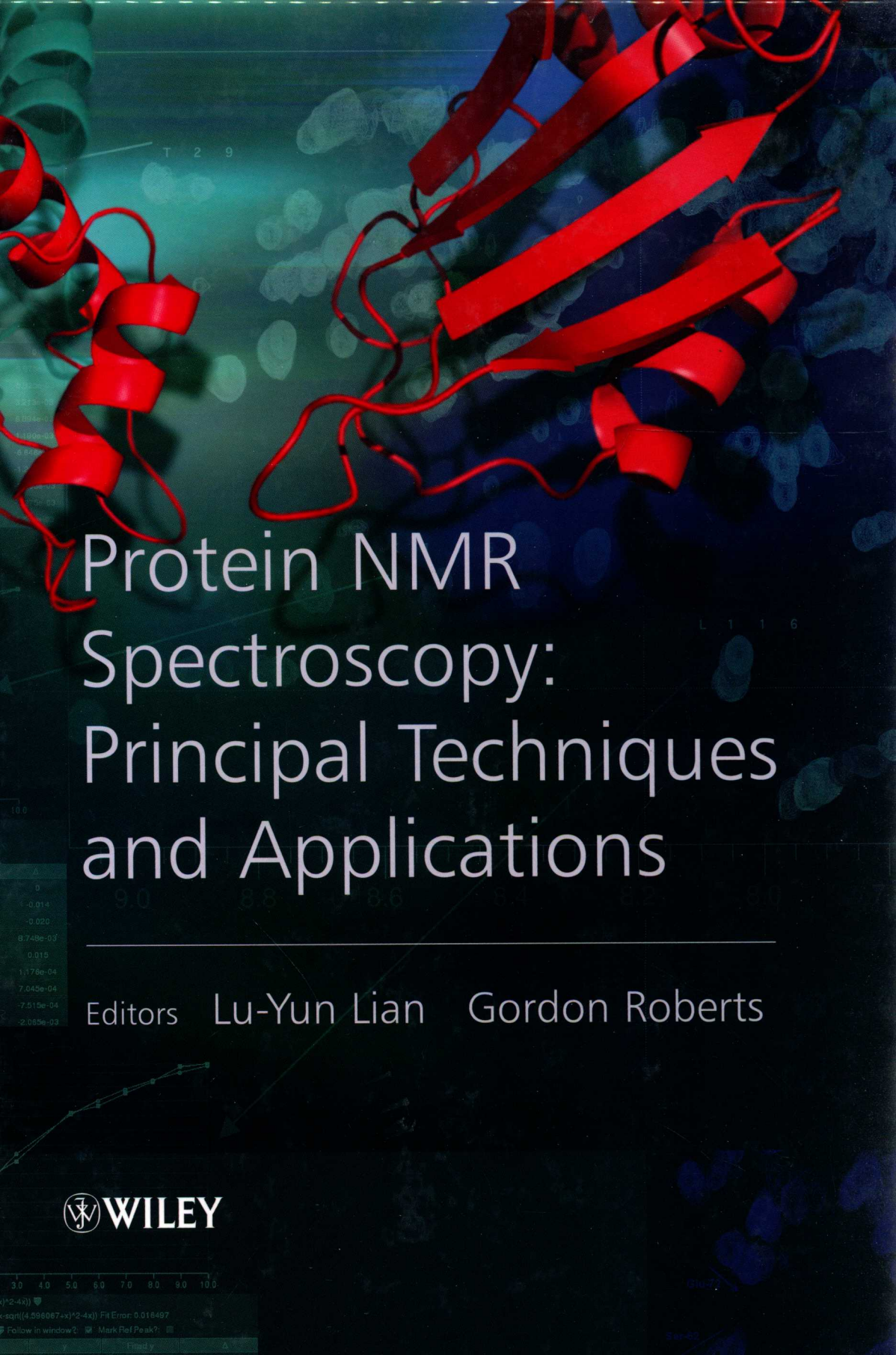

Protein NMR Spectroscopy: Principal Techniques and Applications

Editors Lu-Yun Lian Gordon Roberts

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Protein NMR Spectroscopy: Practical Techniques and Applications

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Introduction

Lu-Yun Lian and Gordon Roberts

The nuclear magnetic resonance (NMR) method is one of the principal techniques used to obtain physical, chemical, electronic and three-dimensional structural information about molecules in solution, whether small molecules, proteins, nucleic acids, or carbohydrates. NMR is a physical phenomenon based upon the magnetic properties of certain atomic nuclei. When exposed to a very strong magnetic field (2–21.1 Tesla) these nuclei align with this field. During an NMR experiment, the alignment is perturbed using a radiofrequency signal (typically a few hundred megahertz). When the radio transmitter is turned off, the nucleus returns to equilibrium and in the process re-emits radio waves. The usefulness of this technique in biochemistry results largely from the fact that nuclei of the same element in different chemical and magnetic environments give rise to distinct spectral lines. This means that each NMR-active atom in a large molecule such as a protein can be observed and can provide information on structure, conformation, ionisation state, pK_a, and dynamics. The nuclei which are most relevant to the study of biological macromolecules are shown in Table 1. The proton (^1H) is the most sensitive nucleus for NMR detection. For biological studies, ^{13}C and ^{15}N are now just as important, although enrichment with these stable isotopes is necessary.

The first published NMR spectrum of a biological macromolecule was the 40 MHz ^1H spectrum of pancreatic ribonuclease reported in 1957. Since then, the significant milestones for NMR include:

- Fourier Transform NMR in the late 1960s;
- the development of two-dimensional NMR in the early 1970s;
- the development of INEPT/HMQC pulse sequences in the late 1970s;

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Table 1 *Properties of nuclei of interest in NMR studies of proteins*

Isotope	Spin	Frequency (MHz) at 14.0954T	Natural Abundance	Relative Sensitivity[a]
^{1}H	$^{1}/_{2}$	600.13	99.99	1.00
^{2}H	1	92.124	0.0115	9.65×10^{-3}
^{3}H	$^{1}/_{2}$	640.123	0	1.21
^{13}C	$^{1}/_{2}$	150.903	1.108	1.59×10^{-2}
^{14}N	1	43.367	99.636	1.01×10^{-3}
^{15}N	$^{1}/_{2}$	60.834	0.37	1.04×10^{-3}
^{17}O	$^{5}/_{2}$	81.356	0.038	2.91×10^{-2}
^{19}F	$^{1}/_{2}$	546.686	100	0.83
^{31}P	$^{1}/_{2}$	242.937	100	6.63×10^{-2}
111Cd	$^{1}/_{2}$	127.32	12.80	9.66×10^{-2}
113Cd	$^{1}/_{2}$	133.188	12.22	1.11×10^{-2}

[a]For equivalent numbers of nuclei (i.e. 100 % isotope).

- the application of NMR to solve the full three-dimensional structure of a protein in solution in the early 1980s;
- the introduction, in the late 1980s, of three- and four- multidimensional heteronuclear experiments for use with $^{13}C/^{15}N$ isotopically labelled proteins, followed in the late 1990s by the TROSY experiments (which require protein deuteration in addition).

Each stage of these developments has been accompanied by improvements in the spectrometer hardware. In particular, the increases in magnetic field strengths, improved probeheads such as cryogenically-cooled probeheads and better electronics have together led to very substantial improvements in resolution and sensitivity. In addition, continuous advances in molecular biology and sample preparation have allowed these NMR-based improvements to be exploited, particularly in the speed with which samples of significant quantities can be produced in a cost-effective way and the ease with which stable isotope enrichment can be accomplished. Finally, the data analysis is now more streamlined and in the case of very high-quality data, the structure determination process, from resonance assignment to structure calculation, can be automated. These developments are important in order for NMR to remain a mainstream technique for high-resolution structure determination and to make significant contributions in structural biology.

For structural biology, NMR is unique in that it can be used for studies of macromolecules in both the solution and solid states and it is, furthermore, the only method that can provide information on dynamics at the atomic level. This book focuses on the use of NMR to study protein structure and interactions in solution, with the aim of providing a practical guide to users of the method. The book attempts to deal with methods, approaches and issues commonly encountered in the everyday use of NMR in structural biology. No attempt is made to provide a description of the fundamental physics of NMR, but in some chapters it is necessary to detail the theoretical aspects of the methodology in order that the methods can be appropriately applied. A full discussion of the fundamental basis of the wide range of solution NMR experiments used in structural biology can be found in [1] and other valuable introductions to modern NMR spectroscopy include [2,3].

The success of any application of NMR depends on the correct sample preparation, the appropriate use of parameters for data acquisition and processing; these are covered in Chapter 1. Once initial data has been collected to assess if a protein system is suitable for NMR studies, the next step will depend upon whether the objective is a determination of the three-dimensional structure of a protein or its complex, or a more limited specific objective such as screening for ligand binding or the determination of pK_a values. For all but the smallest proteins, isotope-labelling will be required (Chapter 2), and to go beyond purely qualitative experiments resonance assignments (Chapter 3) will be essential. Structure determination involves the acquisition and treatment of structural restraints (Chapter 4) and the use of these to obtain structural ensembles (Chapter 5). Chapter 6 describes the additional information on protein structure or complex formation which can be obtained when the protein contains a paramagnetic species – either naturally occurring or introduced specifically for the purpose. Chapter 7 describes different approaches to the study of the binding of small molecules, ranging from screening to full structure determination of the complex; this requires an understanding of the theoretical and practical aspects of the effects of chemical exchange in NMR, which is also important in many other areas of biological NMR. Chapter 8 provides a comprehensive description of the use of NMR to study macromolecular complexes; this is a challenging area and the chapter outlines the problems and approaches which can be taken to overcome these challenges. Chapter 9 focuses on the structural studies of intrinsically disordered proteins. The widespread existence and significance of these proteins are becoming increasingly recognised and NMR is currently the best method to provide detailed information on their conformational distributions.

NMR is uniquely suited for the characterisation of biomolecular dynamics. Since so many nuclei can be detected simultaneously, NMR can provide a comprehensive description of the internal motions and conformational fluctuations at atomic resolution, and NMR methods have been developed to quantify motions that occur at a wide range of timescales, from picoseconds to days and months. At the same time, consideration of dynamics and the averaging processes to which they lead is an essential part of the use of NMR to obtain structural information. As a result, several chapters in this book deal with methods for obtaining dynamic information from NMR. For additional information the reader is also directed to the following reviews [4–7].

Over the last few years there have been significant developments in the application of solid-state NMR techniques as a tool for determining the high-resolution structures of proteins, ranging from microcrystalline soluble proteins to protein fibrils and membrane proteins. It is now possible to assign the spectra of proteins larger than 100 amino acids using ^{13}C,^{15}N –labelling [8]. However, as yet this remains an area for the expert and it is not covered in detail in this book (although some of the methods for isotope-labelling described in Chapter 2 will also be relevant to solid-state studies). For useful reviews, the reader is directed to [9,10].

Note Added in Proof

Several valuable relevant reviews have appeared while this book was in production. In particular, two useful qualitative introductions to biomacromolecular NMR for the newcomer to the field would serve as valuable initial reading [11,12]. Clore [13] has

reviewed the use of relaxation methods (see Chapters 6 and 7) to observe species with low population, Wishart [14] has reviewed the use of chemical shifts in structure determination (see Chapters 4 and 5), and Dominguez *et al.* [15] have reviewed the use of NMR in the study of protein-RNA complexes (see Chapter 8).

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