Annual reports on
NMR Spectroscopy

Volume 58

ANNUAL REPORTS ON

NMR SPECTROSCOPY

This page is left intentionally blank

ANNUAL REPORTS ON

NMR SPECTROSCOPY

Edited by

G. A. WEBB

Royal Society of Chemistry, Burlington House, London, England

VOLUME 58

Amsterdam • Boston • Heidelberg • London • New York
Oxford • Paris • San Diego • San Francisco • Singapore
Sydney • Tokyo
Academic Press is an Imprint of Elsevier

Academic Press is an imprint of Elsevier
84 Theobald's Road, London WC1X 8RR, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

First edition 2006

ISBN-13: 978-0-12-505458-4
ISBN-10: 0-12-505458-0

ISSN: 0066-4103

For information on all Academic Press publications visit our website at books.elsevier.com

Printed and bound in the United Kingdom.

06 07 08 09 10 9 8 7 6 5 4 3 2 1

List of Contributors

I. Ando, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

T. Fujiwara, *Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita 565-0871, Japan*

B.P. Hills, *Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

W. Hu, *Immunology Division, BRI, City of Hope, Duarte Rd. 1500, Duarte, CA 91010, USA*

K. Kamiguchi, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

S. Kanesaka, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

S. Kim, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

S. Kuroki, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

M. Matsui, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

A. Ramamoorthy, *Biophysics Research Division and Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA*

L. Wang, *Computer Science Department, Dartmouth College, Hanover, NH 03755, USA*

A.G. Webb, *Department of Bioengineering, Pennsylvania State University, 315 Hallowell Building, University Park, PA 16802, USA*

Y. Yamane, *Department of Chemistry and Materials Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, Japan*

This page is left intentionally blank

Preface

It is a pleasure for me to introduce Volume 58 of Annual Reports on NMR, which consists of exciting contributions from five areas of NMR. The volume opens with a report on Advances in Probe Design for Protein NMR by A.G. Webb; following this is an account on Diffusion in Soft Polymer Systems as Approached by Field Gradient NMR by Y. Yamane, S. Kanesaksa, S. Kim, K. Kamiguchi, M. Matsui, S. Kuorki and I. Ando; the question How Far Can the Sensitivity of NMR be Increased? is addressed by T. Fujiwara and A. Ramamoorthy; B.P. Hills covers Applications of Low-Field NMR to Food Science; the final contribution is by W. Hu and L. Wang who report on Residual Dipolar Couplings: Measurements and Applications to Biomolecular Studies.

My sincere thanks go to all of these authors for their timely and interesting accounts.

Royal Society of Chemistry
Burlington House
Piccadilly
London, UK

G. A. WEBB
October 2005

This page is left intentionally blank

Contents

Applications of Low-Field NMR to Food Science
B.P. HILLS

Residual Dipolar Couplings: Measurements and Applications to Biomolecular Studies
WEIDONG HU AND LINCONG WANG

Advances in Probe Design for Protein NMR

A.G. WEBB

Department of Bioengineering, Pennsylvania State University, 315 Hallowell Building, University Park, PA 16802. USA

Advances in pulse sequences and increases in static magnetic fields have played major, and well-documented, roles in extending the experimental boundaries of NMR spectroscopy. An equally important component has been the improvement in NMR-probe technology, particularly in terms of sensitivity: this article summarizes some of these recent improvements. The most important breakthrough has been in cryogenic-probe technology, which has resulted in signal-to-noise improvements of up to a factor-of-four in biological samples. There has also been significant progress in NMR probes which can be used for mass-limited samples, and also for reducing sample heating in samples of high ionic concentration.

ANNUAL REPORTS ON NMR SPECTROSCOPY
VOLUME 58 ISSN 0066 4103
DOI 10.1016/S0066-4103(05)58001-3

1. INTRODUCTION

On the basis of the recent sequencing of a large number of genomes, several ambitious projects have been established, ranging in scope from determination of the three-dimensional (3D) structures of every protein from a single given organism, to the elucidation of the chemical structures of all major pharmaceutical targets in the human body. The success of a number of such genomic sequencing projects presents protein investigators with a substantial challenge in characterizing the large number of gene products that have already been identified, and this number is expected to increase considerably in the future.

X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are two of the main experimental techniques used to obtain protein structure information at the atomic level. These methods are particularly powerful since they can be used to determine the 3D shape of the protein, which is known to encode protein function.[1] Major efforts are underway to identify, express and purify, as rapidly and efficiently as possible, proteins that are amenable to NMR spectroscopy, X-ray crystallography and other biophysical techniques.[2,3] The process of structure determination by X-ray crystallography is already relatively streamlined, incorporating robotics for optimizing crystallization conditions, parallel data acquisition from synchrotons, and semi-automated analysis software.[4,5] Although structure determination by NMR spectroscopy is, in comparison, relatively time-consuming and requires ^{15}N, ^{13}C and, for larger proteins ^{2}H, isotopic enrichment, NMR spectroscopy is one of the very few experimental methods that provides detailed insights into both the structure and dynamics of unfolded and partially folded states of proteins.[6–8] High-throughput analyses of protein NMR spectra are an area of intense interest, as described in detail in a recent review.[9]

NMR studies of proteins can be performed in the solution-state (in which the protein is dissolved in an aqueous buffer), solid-state (with the protein sample in powder or micro-/nanocrystalline form), or as samples which are partially aligned (either mechanically aligned by placement between glass plates or *via* preparation in some form of gel). Solution-state NMR spectroscopy has become a standard method for the characterization of the 3D structure and dynamics of proteins that undergo fast molecular reorientations in solution.[10–17] The advantages of solution-state NMR include being able to investigate protein structure in a state closely resembling physiological conditions, to monitor binding processes, to extract dynamic properties from relaxation and chemical exchange data, and to provide important information for crystallization-resistant targets. Although many methodological advances have been made for NMR studies of large proteins and protein complexes using deuterium labeling,[18] transverse relaxation optimized spectroscopy (TROSY)-based pulse sequences,[19] and very high magnetic field strengths,[20] NMR methods are applied mostly to proteins under ~20 kDa molecular weight to avoid problems associated with spectral overlap and broad spectral linewidths. Approaches to higher-molecular-weight proteins include the cloning, expression, and purification of individual constituent domains of the full protein.

Membrane proteins are also increasingly being studied using solution-state NMR, although presenting considerable challenges.[21–27] It should also be noted that solution-state NMR is widely used for the investigation of complex-binding interactions within proteins, as well as for large-scale screening by the pharmaceutical industry of molecular libraries *via* their interaction with proteins.[28–31]

Solid-state NMR spectroscopy is used in cases where proteins do not form crystals for X-ray studies, or are not amenable to solution-state NMR techniques. Examples include many membrane proteins, protein aggregates, and other fibrous proteins such as amyloids.[32] Either magic angle spinning (MAS) experiments[33,34] or static solid-state NMR techniques can be used in such studies. MAS and strong proton decoupling are both necessary to achieve linewidths <1 part-per-million (ppm). Homonuclear couplings can also be removed by selective isotopic labeling.[35–37] Certain membrane proteins have also been studied using techniques involving various methods of fully or partially orienting the sample with respect to magnetic field.[38–42] For example, lipid bilayers can be aligned on glass plates,[38–40] and are prepared either by fusion of unilamellar-reconstituted lipid vesicles with the glass plates, or *via* deposition from organic solvents followed by evaporation and lipid hydration. The membranes form planar bilayers whose normal axis is parallel to the glass plate.[39,40,43–49] Because of the alignment of the membranes and the magnetic field direction, measurements of anisotropic NMR chemical shifts and dipolar couplings can provide information about the peptide orientation relative to the membrane.[50–52]

Although there are many review articles on various aspects of protein NMR, including pulse sequences, data processing, and sample preparation,[53] discussions of the developments in NMR-probe technology, which have contributed significantly to the expansion of the field, are relatively rare. A number of excellent general reviews on NMR-probe design have been published.[54–58] This review article aims to provide information specifically relevant to the design and construction of, as well as to recent developments in, probes for NMR spectroscopy of proteins. Section 2 reviews typical pulse sequences used for solution- and solid-state NMR protein studies, and outlines the requirements imposed by these sequences on the design of the NMR probe. Section 3 describes the basics of radio frequency (RF) coil design in terms of optimizing the B_1 and B_0 homogeneity over the sample, and electrical circuit optimization for multiple-frequency operation. Sections 4–6 concentrate on important recent developments in probe design for protein NMR: Section 4 describes the development and applications of cooled-NMR probes, often referred to as ''cryoprobes'', Section 5 on new designs for coils designed to reduced sample heating during high-power decoupling by minimizing the electric field present in the sample, and Section 6 on small-coil NMR for mass-limited proteins.

2. PROTEIN NMR EXPERIMENTS

In order to design NMR probes for protein studies, it is necessary to consider the requirements of specific pulse sequences in terms of, for example, the required B_1

homogeneity over the sample, decoupling field strength and power-handling requirements, and efficient multiple-frequency channel operation. The following sections review briefly typical pulse sequences used in solution and solid-state protein NMR studies.

2.1. Solution-state pulse sequences

For NMR experiments a protein is typically either single labeled with ^{15}N or double-labeled with ^{13}C and ^{15}N, and can in addition be partially or fully deuterated if the protein is particularly large. The protein is normally dissolved in 95% H_2O/5% D_2O, with the appropriate amount of NaCl (10–200 mM) and a buffer, usually phosphate-based, added to bring the solution to the desired pH value.

A typical set of experiments to obtain structural information on a protein might include an initial two-dimensional (2D) HSQC, followed by 3D HNCO,[59,60] HNCA,[59,60] and/or HN(CO)CA[60,61] sequences to assign backbone resonances, and then assignment of aliphatic side-chain resonances using HCCH–COSY[62–64] and HCCH-TOCSY[65,66] or aromatic side-chain resonances using TOCSY–HCH–COSY. Finally, assignment of nuclear overhauser effect (NOE) cross-peaks and measurement of residual dipolar couplings may be performed in order to improve the local geometry calculations.[67–71] More specialized sequences such as HCA(CO)N or CBCANH may also be used to determine specific structural characteristics. For NMR-based screening of small molecule binding to proteins, experiments are usually based on detecting changes in chemical shifts from rapidly acquired HSQC spectra,[28,72–80] although proton only[31,81–89] and ^{19}F-based variations[90–92] also exist.

In terms of NMR-probe design, experiments such as ^{1}H–^{15}N HSQC require short, simultaneous RF pulses on both proton and nitrogen channels, with nitrogen decoupling applied during data acquisition on the proton channel. Variants such as constant-time ^{13}C HSQC incorporate additional nitrogen-decoupling during the evolution period. Three-dimensional sequences such as HSQC–NOESYand HMQC–NOESY–HMQC are identical in probe requirements to ^{1}H–{^{15}N} HSQC. Sequences such as HSQC–TOCSY require proton spin-locking pulses, whereas HNCA, HNCO, and HN(CO)CA require simultaneous pulsing on ^{1}H, ^{13}C, and ^{15}N channels, with ^{15}N decoupling during data acquisition. Constant-time H(CA)NH requires simultaneous pulsing on ^{1}H, ^{15}N, and ^{13}C channels, with carbon decoupling and proton spin-locking during the sequence, and nitrogen decoupling during acquisition of the proton signal. HCA(CO)N requires simultaneous pulsing on ^{1}H, ^{15}N, and ^{13}C channels with carbon decoupling during acquisition on the proton channel. Sequences such as CBCA(CO)NH[93] and CBCANH[93] require pulsing on ^{1}H, ^{15}N, and ^{13}C channels, with simultaneous nitrogen and proton decoupling during the sequence and nitrogen decoupling during signal acquisition, as shown in Fig. 1(a). Some of the most challenging sequences for probe design are those such as four-dimensional TROSY triple resonance spectroscopy,[94,95] which requires pulsing on ^{1}H, ^{15}N, ^{13}C, and ^{2}H with ^{2}H decoupling during the carbon pulses, as shown in Fig. 1(b).

From the brief description above, it is clear that the NMR probes must operate simultaneously at a number of different frequencies with excellent electrical

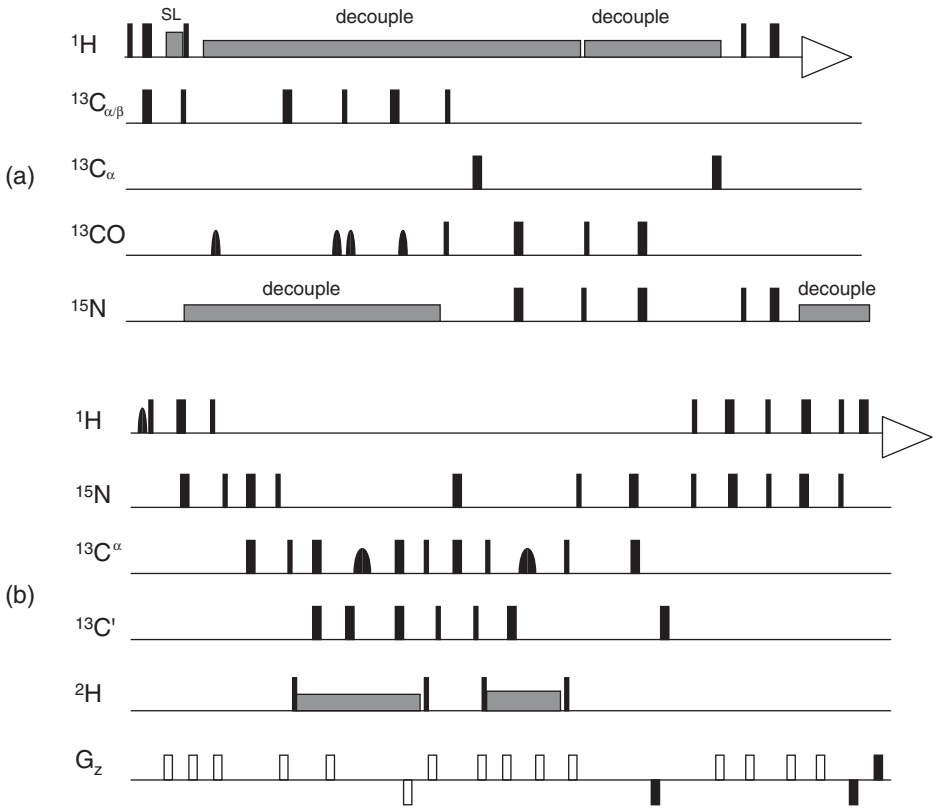

Fig. 1. Two pulse sequences for solution-state NMR that represent significant challenges to probe design: (a) 3D CBCA(CO)NH sequence and (b) four-dimensional HNCACO TROSY sequence. Thin-rectangular lines represent 90° pulses, thick-rectangular lines 180° pulses, and thin/thick hemispherical shapes frequency-selective 90° and 180° pulses, respectively. For simplicity incremental delays t_1, t_3, and t_4 are not shown.

isolation between individual channels. For example, if electrical isolation is not very high, there will be significant noise added to the detected proton signal from simultaneous nitrogen decoupling in the CBCA(CO)NH sequence.

For solution-state NMR experiments, commercial vendors offer a number of different types of probes. For standard heteronuclear experiments triple resonance, commonly called TXI (triple resonance/X-nucleus/inverse detection), probes enable pulsing on proton, deuterium, carbon, and nitrogen channels. The usual configuration, described in more detail later, is to have two RF coils, one inside the other, with the inner coil operating at the ^{1}H and ^{2}H frequencies, and the outer one at the ^{13}C and ^{15}N frequencies. The inner coil, having a higher filling factor, has a higher sensitivity than the outer coil. The term inverse detection refers to the fact that the signal is acquired on the proton channel, and thus in this configuration the proton channel is the inner coil, which also provides deuterium decoupling and detects the lock signal. For nucleic acid

applications, the outer coil is usually tuned to ^{13}C and ^{31}P. Some vendors offer a "combined probe" in which the inner coil is triple-tuned to ^{1}H, ^{2}H, and ^{31}P, with the outer coil tuned to ^{13}C and ^{15}N frequencies to allow both protein and nucleic acid studies. If triple-resonance experiments are not required, as in for example protein screening using ^{1}H–^{15}N HSQC sequences, then a simple indirect detection probe can be used, in which the inner coil is now tunable to either ^{1}H or ^{19}F frequencies, as well as ^{2}H, and the outer coil is resonant at only one frequency, but this resonance can be single-tuned over a range of frequencies, typically covering ^{15}N, ^{13}C, and ^{31}P.

One other factor, as shown in Figs. 1(a) and 1(b), is the requirement for pulsed magnetic field gradients in the NMR probe. These are used primarily for coherence selection within the sequences, and have largely replaced phase cycling as a way to minimize artifacts in NMR spectroscopy.[96,97] Although not discussed in detail here, the gradients must be rapidly switchable, and can be designed as magic angle,[98–101] single- or triple-axis configurations. Many vendors have also introduced "automatic tuning" into their probe design, such that long-term drifts in probe tuning can be minimized and probes can also be retuned rapidly and automatically retuned if the dielectric and conductivity properties of successive samples vary significantly. Although information on exact probe configurations is proprietary, a good discussion of the issues involved in automatic tuning is covered in the paper by Hwang and Hoult.[102]

2.2. Solid-state pulse sequences

As outlined previously, solid-state NMR is used in cases where proteins do not form crystals easily, are too disordered for X-ray crystallography, or are not amenable to solution-state techniques owing to limited solubility. For solid-state NMR the protein is usually selectively or fully isotopically labeled with either ^{13}C, ^{15}N, or a combination of the two: labeling strategies and sample preparation have been reviewed by Straus.[103] The NMR spectrum of randomly oriented solid-phase molecules is extensively broadened by anisotropic interactions, with the linewidth (LW) generally being dominated by homonuclear scalar and dipolar ^{13}C coupling in uniformly labeled samples.[104,105] Rapid spinning at the magic angle, high-power proton decoupling during acquisition, and high magnetic fields can improve the spectral resolution considerably. Alternatively, homonuclear couplings can be minimized by selective isotopic labeling.[35–37] If the protein is uniformly ^{13}C and ^{15}N labeled typical experiments involve multi-dimensional ^{13}C–^{13}C and ^{13}C–^{15}N correlation sequences.[106,107] Low γ-nuclei have smaller dipolar couplings and larger chemical shift dispersions than protons, and so spectral resolution is greater for these nuclei. The primary structure of the protein can be determined starting with intra-residue resonance assignment *via* homonuclear ^{13}C–^{13}C correlation experiments.[108] Pulse sequences using selective polarization transfer from backbone ^{15}N to $^{13}C_{\alpha}$ atoms, followed by ^{13}C–^{13}C correlation can be used to determine ^{15}N–^{13}C connectivities. More recently, high sensitivity indirect detection experiments such as ^{13}C–^{1}H or ^{15}N–^{1}H HSQC spectroscopy, have been reported,[109] in which the authors obtained high-quality $^{15}N/^{1}H$ HSQC spectra with 500 nmol of perdeuterated