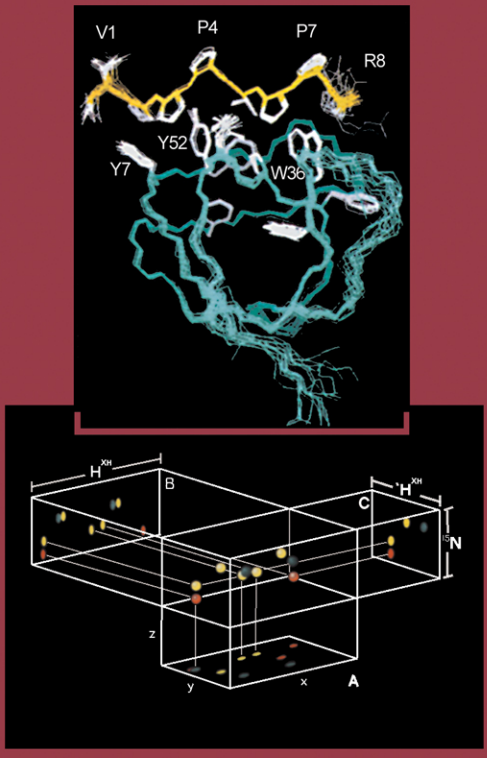
NMR SPECTROSCOPY AND ITS APPLICATION TO BIOMEDICAL RESEARCH

Edited by

SUSANTA K. SARKAR



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1996
ELSEVIER
AMSTERDAM – LAUSANNE – OXFORD – NEW YORK – SHANNON – TOKYO

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

ISBN 0-444-89410-1

This book is printed on acid-free paper.

Printed in The Netherlands

To:
my wife, Indrani
and
our daughter, Saiontoni
— for their constant encouragement, support and love

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Preface

NMR has become the most diverse spectroscopic tool available to date in biomedical research. It is now routinely used to study biomolecular structure and dynamics particularly as a result of the recent developments of a cascade of highly sophisticated multidimensional pulse sequences, and of advances in genetic engineering to produce biomolecules, uniformly or selectively enriched with ^{13}C, ^{15}N and ^{2}H. This book, written by acknowledged experts, provides an up-to-date treatment of the current status of multi-dimensional NMR, including the basic aspects, and its application to problems of biomedical interest.

William Westler, in Chapter 1, provides a practical introduction to two-dimensional NMR through coherent flow network. This description makes it easy to conceptualize the pulse sequence details and provides a basis for understanding further complicated sequences. In Chapter 2, Luciano Mueller and N. Vasant Kumar describe the current status of multi-dimensional NMR and its utility in structure determination of proteins and nucleic acids. In addition to a brief description of different classes of multidimensional experiments they also summarize the spectrometer requirements for implementing these sequences.

Because of the recent developments in molecular biology techniques for preparing labeled samples, it is now possible to perform NMR studies on large macromolecules. An overview of different procedures for isotopic enrichment of proteins by labeled amino acids is given by Brian Stockman in Chapter 3. A critical discussion of the advantages and disadvantages of different procedures is also provided. Chapter 4, by Paul Weber, is a critical survey of a number of methods used for structure calculation of proteins from NMR data and provides the user with an insight into the process. This chapter also provides a list of different options available for structure calculation.

In order to understand protein function, it is essential to consider the internal mobility of proteins, which can be studied by recently introduced methods to measure heteronuclear relaxation. Linda Nicholson, Lewis Kay and Dennis Torchia give an introduction to relaxation theory including the recently developed pulse sequences to measure relaxation parameters in Chapter 5. A discussion of the data processing steps and a critical analysis of the motional parameters are also included. The

entire process is demonstrated with examples of detailed dynamic studies on staphylococcal nuclease.

Chapters 6 and 7 review the basic NMR methods and their modifications, particularly useful for resonance assignments in nucleic acids and carbohydrates. David Wemmer, in Chapter 6, discusses the steps necessary for building solution structure of nucleic acids from the available NMR data and the NMR methods for analyzing dynamics of nucleic acids. Specific examples are given to illustrate these approaches and a critical analysis is given about the specific issues for structural studies of DNA, RNA and their complexes with drugs and proteins. In Chapter 7, Laura Lerner describes the NMR methods and their application to structural analysis of oligosaccharides and their interactions with receptors.

Solid state NMR provides a powerful tool for structural and dynamic studies of many biological molecules not amenable to (a) solution NMR studies because of their size or (b) X-ray diffraction because of the unavailability of single crystal samples. Alexandra Simmons, Susanta Sarkar and Lynn Jelinski, in Chapter 8, outline the differences between nuclear interactions in solution and solid state and review the techniques commonly used in solid state NMR to obtain high resolution spectra from solid samples. Selected examples from the literature are used to demonstrate the application of solid state NMR studies to questions of biomedical interest.

In summary, it is hoped that this book would be useful to NMR spectroscopists, chemists, biochemists, and to molecular biologists interested in the use of NMR techniques for solving biological problems. The intended audience is NMR spectroscopists who are interested in biological problems and biologists who would like to use NMR.

I would like to thank all the authors for their contributions, which reflect the opportunities and the challenges of NMR spectroscopy and its application to biomedical research. I would also like to thank Mrs. Marjorie J. Krog for her help in preparing this book.

Susanta K. Sarkar
King of Prussia, PA
October 1996

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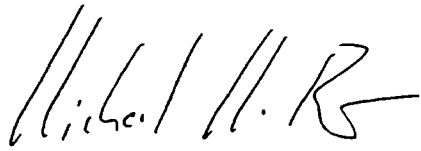
Chapter 3. Preparation of ^{2}H, ^{13}C and ^{15}N Isotopically-enriched Proteins for NMR Spectroscopic Investigations
by B.J. Stockman

Chapter 4. Protein Structure Determination from NMR Data
by P.L. Weber

Foreword

What would be molecular biology without NMR, and what would be NMR without its applications to molecular biology? — The world of science would miss a fruitful and exciting field. Next to clinical medicine, molecular biology has become the field most dependent on the recent progress in NMR technology. A powerful arsenal of versatile tools is available today for studying biomolecular structure and intramolecular dynamics. In particular, multi-dimensional spectroscopy has expanded in an unprecedented manner the possibilities of gaining insight into biological macromolecules.

This volume provides an up-to-date treatment of NMR methodology in view of biomedical research. Basic aspects as well as most refined modern pulse techniques are covered by a group of leading NMR spectroscopists. The book will undoubtedly prove useful in the hands of practising spectroscopists and biochemists who intend to apply modern NMR techniques.



Richard R. Ernst
Zürich
October 1996

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Chapter 1

Two-Dimensional NMR Spectroscopy: A Graphical, "Top-Down" Description

WILLIAM M. WESTLER

ABSTRACT

An introduction to the principles and uses of homonuclear and heteronuclear two-dimensional NMR spectroscopy is presented. The pulse sequences and phase cycling procedures for a few of the most common experiments are analyzed in detail. The experiments are described by using a visual description of spin system evolution and coherence transfer processes. This method of description allows for the discussion of any multidimensional, multinuclear pulse sequence by a "top-down" approach.

1. INTRODUCTION

Two-dimensional NMR spectroscopy has become a mainstay experiment in many disciplines [1]. The use of two- and higher- dimensional NMR has greatly advanced the applications of NMR to biochemical systems. Before the introduction of two-dimensional spectroscopy, researchers were generally limited to assigning the few resonances that are resolved in one-dimensional spectra or to use specific ^{13}C or ^{15}N labels. Over the past decade, techniques have been developed for the assignment of most, if not all, of the NMR resonances in a macromolecule, while advances in the field of molecular biology have led to the production of the necessary isotopically labeled macromolecules. The assignment of individual resonances to particular nuclei in the molecules

provides a multitude of probes with which to interrogate the molecular system. With the complete assignment of the proton network, the nuclear Overhauser effect yields distances between protons in macromolecules and, by a variety of methods, three-dimensional structures of macromolecules in solution can be obtained.

The concept of using more than one dimension in NMR spectroscopy was first introduced by Jeener [2] and developed by Ernst and coworkers in the late 1970s [3]. Two-dimensional NMR takes advantage of the non-linear properties of the nuclear spin system by passing frequency, amplitude, and phase information from one nucleus to another. The transferred information is observed indirectly as a modulation of the detected nuclei. The mechanisms of transfer can be classified as incoherent or coherent. The incoherent mechanism of information transfer uses either the dipolar interaction or physical chemical exchange, whereas the coherent mechanism relies on the information being passed through the scalar coupling interaction. Most multidimensional experiments are of the coherence transfer type. The information that is gained from these experiments is used to connect nuclei that are part of a scalar-coupled network of spins. Since the scalar coupling interaction occurs between nuclei that are one to a few chemical bonds apart, these experiments are used to obtain information about the primary structure of the molecule. The coupling constant information can be used to determine various dihedral angles within the scalar-coupled network and lends information about molecular secondary structure. While the number of experiments that use incoherent transfer of magnetization is small, these experiments hold a very important role in the determination of molecular structure. From the dipolar interaction, through-space distance information is obtained. The measurable distances are generally less than about 5 Å; since the information does not rely on the presence of a chemical bond between the interacting nuclei, primary, secondary and tertiary structural information is available.

A number of books [1,4] and review articles [5–7] have been published on the principles and uses of 2D NMR spectroscopy and I will not repeat the many references contained within those articles. My goal here is to present a practical introduction to the principles and uses of a few common 2D experiments. As an introduction to two-dimensional NMR methods, a simple, although naive, experiment is described that shows the fundamentals of multidimensional NMR by describing the behavior of nuclei undergoing exchange from one site to another in an idealized molecule. An introduction to heteronuclear coherence transfer