PREFACE TO THE FIRST EDITION

Concomitant developments of modern molecular biology and multidimensional nuclear magnetic resonance (NMR) spectroscopy have increased explosively the use of NMR spectroscopy for generating structural and dynamical information on small to medium-sized biological macromolecules. Efficient molecular biological techniques for incorporation of the stable, NMR active, ^{13}C and ^{15}N isotopes into overexpressed proteins have resulted in dramatic advances in the design and implementation of multidimensional heteronuclear NMR spectroscopic techniques. Consequently, the maximum size protein amenable to complete structural investigation has increased from ~10 kDa using ^{1}H homonuclear NMR spectroscopy to ~30 kDa using ^{13}C and ^{15}N heteronuclear NMR spectroscopy and perhaps to ~40 or ~50 kDa using ^{13}C and ^{15}N heteronuclear NMR spectroscopy combined with fractional ^{2}H enrichment. Most recently, *in vitro* transcription techniques have expanded the application of ^{13}C and ^{15}N heteronuclear NMR spectroscopy to RNA molecules. Research programs for isotopically enriching DNA and carbohydrate molecules promise to further extend the reach of these powerful NMR techniques.

The maturation of the field of structural biology has made the study of structure-function relationships of biological macromolecules by NMR spectroscopy an integral part of diverse chemical and biological research efforts. As an indication of the success of the technique, NMR

spectroscopy increasingly is being utilized by chemical and biological scientists not specifically trained as NMR spectroscopists. At the same time, a bewildering number of complex ^{13}C and ^{15}N heteronuclear NMR experiments that make increasingly sophisticated use of the quantum mechanics of nuclear spin systems have been developed (for example, compare the two ^{1}H radiofrequency pulses utilized in the COSY experiment with the 27 radiofrequency pulses applied at five different frequencies and four extended decoupling sequences utilized in the CBCA(CO)NH experiment). These developments have occurred largely after the publication of the seminal texts *NMR of proteins and nucleic acids*, by K. Wüthrich in 1986 and *Principles of nuclear magnetic resonance in one and two dimensions*, by R. R. Ernst, G. Bodenhausen and A. Wokaun in 1987.

In our view, a definite need exists for a graduate-level textbook that not only describes the practical aspects of state-of-the-art techniques in biomolecular NMR spectroscopy, but also presents the fundamental principles used to develop these techniques. Only a thorough understanding of the unifying principles of NMR spectroscopy empowers a student or researcher to evaluate, implement and optimize new techniques that continue to emerge at a dizzying pace. In this spirit, *Protein NMR Spectroscopy: Principles and Practice* systematically explicates NMR spectroscopy from the basic theoretical and experimental principles, to powerful theoretical formulations of the quantum mechanics of nuclear spin systems, and ultimately to optimal experimental methods for biomolecular investigations. Although the text concentrates on applications of NMR spectroscopy to proteins, all of the theory and most of the experiments are equally relevant to nucleic acids, carbohydrates and small organic molecules. The text focuses on the NMR spectroscopy of diamagnetic molecules (without unpaired electron spins); issues germane specifically to paramagnetic molecules (with unpaired electron spins) are discussed in other sources (see Suggested Reading). This text will serve a wide audience of students and researchers reflective of the variety of disciplines that employ NMR spectroscopy, including biochemistry, biology, chemistry, and physics.

Protein NMR Spectroscopy: Principles and Practice provides a comprehensive treatment of the principles and practice of biomolecular NMR spectroscopy. The theoretical basis of NMR spectroscopy is described in Chapters 1, 2, 4 and 5. Classical NMR spectroscopy of isolated spins is introduced through the Bloch equations in Chapter 1. The density matrix and product operator theoretical formalisms of NMR spectroscopy of coupled multi-spin systems are presented in Chapter 2. The major principles of multidimensional NMR

spectroscopy, including frequency labeling of coherences, coherence transfer and mixing, and coherence pathway selection, are described in Chapter 4. The principles of nuclear spin relaxation and chemical exchange are developed by using the Bloch, Solomon and semiclassical theoretical descriptions in Chapter 5. The experimental techniques used in modern multidimensional NMR spectroscopy of biological macromolecules in solution are described in Chapters 3, 6, and 7. Theoretical and practical aspects of experimental NMR spectroscopy, including data acquisition and data processing, are introduced in Chapter 3. Widely used spectroscopic techniques, such as spin decoupling, water suppression, composite pulses, selective pulses and one-dimensional NMR spectroscopy, also are presented in Chapter 3. Multidimensional ^{1}H homonuclear NMR spectroscopy is described theoretically and illustrated with experimental examples in Chapter 6. Multidimensional $^{13}C/^{15}N$ heteronuclear NMR spectroscopy is described theoretically and illustrated with experimental examples in Chapter 7. Both Chapter 6 and 7 present the principal experimental techniques used to obtain resonance assignments, to measure internuclear distances, and to determine scalar coupling constants. Methods for the interpretation of NMR spectra, including resonance assignment strategies and protocols for structure calculations, are summarized in Chapter 8. These aspects of biomolecular NMR spectroscopy are evolving rapidly and detailed discussions could constitute an entire additional book. Consequently, Chapter 8 is intended to provide an overview of the subject and an entry into the primary literature.

In order to provide continuity and consistency throughout the text, a single protein, ubiquitin (76 amino acid residues, $M_r = 8{,}565$ Da), is used to demonstrate the experimental aspects of NMR spectroscopy. Unlabeled bovine ubiquitin was purchased from Sigma Chemical Company (product number U6253, St. Louis, MO). ^{15}N-labeled and $^{13}C/^{15}N$-double-labeled human ubiquitin were purchased from VLI Research (Southeastern, PA). The human and bovine amino acid sequences are identical. NMR spectroscopy was performed using Bruker 500- and 600-MHz NMR spectrometers at a temperature of 300 K. Sample concentrations were 2.0 mM for unlabeled ubiquitin and 1.25 mM for labeled ubiquitin. Samples were prepared in aqueous (95% H_2O/5% D_2O or 100% D_2O) 50 mM potassium phosphate buffer at pH 5.8. NMR samples in 100% D_2O solutions were prepared from samples in 95% H_2O/5% D_2O by performing four cycles of lyophilizing and dissolving in D_2O (99.999 atom%) in the NMR tube.

A common lament of the scientist who wishes to understand a new discipline is "What books should I read?" We hope that *Protein NMR*

Spectroscopy: Principles and Practice provides an answer for students and researchers with an interest in biomolecular NMR spectroscopy.

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