Preface

Ever since its discovery in the work of Rabi [1], Purcell [2], and Bloch [3], NMR, now into its eighth decade, has found widespread use in the determination of the structure, dynamics, and interactions of molecules their assemblies. The applications of modern NMR spectroscopy range from the study of biomolecules to the analyses of the properties of batteries; from the solution state, through the semi-solid to the solid state; from the structural analysis of an end product of a chemical synthesis comprising only a few atoms, to the determination of the structural organization of an intact viral capsid with tens of thousands of atoms. The path spanning more than five decades of protein NMR from the one-dimensional spectrum of ribonuclease by Saunders et al. in 1957 [4] to a detailed NMR analysis of gating in a 670 kDa 20S proteasomal core by Sprangers et al. in 2007 [5] required multiple layers of development, in terms of technology (60 MHz in 1957 to 800 MHz in 2007), in fundamental concepts (continuous wave, CW NMR in 1957 to multidimensional relaxation optimized Fourier spectroscopy in 2007), and in sample preparation (unlabeled protein bought from a commercial source in 1957 to bacterially expressed protein specifically ^{13}C, ^{1}H enriched on Ile, Leu, and Val methyl groups in an otherwise ^{12}C, ^{2}H background in 2007). The success of any NMR experiment depends on four basic stages of equal importance: (1) sample preparation, (2) spectroscopy, (3) data processing, and (4) analysis. Improper implementation of any of these steps has the potential to doom any NMR-based research project. Many approaches have emerged over the years to optimize each of these stages of an NMR experiment. These include advances in magnet/spectrometer/probe design, novel biochemical methodology to enable the production of proteins or their assemblies optimally labeled with NMR-active nuclei, new pulse sequences to manipulate spin dynamics, and new computational approaches for data collection and analysis. In the 20 chapters of this volume we will cover state-of-the-art approaches detailing many of these innovations with a focus on their application to proteins and protein complexes in solution and in the solid state.

As we embark on the eighth decade of NMR spectroscopy, the magnetic fields that are now available to NMR spectroscopists have increased by more than an order of magnitude enabling the discovery of new spin physics as well as the study of more complex systems. While—as recently as the 1980s—an NMR spectrometer operating at 60 MHz (1.4 T) was considered state of the art, chemistry departments even in the smallest research universities these days have access to NMR spectrometers 500 MHz (11.7 T) or higher. Spectrometers operating at 800 MHz (18.8 T) have become staples for most medium-to-large research universities; a 1000 MHz (23.5 T) spectrometer has been operational at Lyon since 2009 and several new 1200 MHz (28.2 T) spectrometers, which are currently being developed, are on order to be placed at multiple sites. A report detailing the development of a hybrid magnet that allows the performance of NMR experiments at 1500 MHz (35.2 T) in the solid-state has recently appeared in the literature [6]. Chapter 1 looks into the next decade providing an overview of emerging magnet technologies that enable the generation of homogenous magnetic fields higher than 23.5 T and the great advances that these ultra-high-field spectrometers are likely to provide for biomolecular NMR in solids and in the solution state.

It was recently realized that residual magnetization generated during the preparation phase of an NMR experiment but discarded during the detection phase, the so-called afterglow, could be further manipulated post acquisition and detected during subsequent periods. Thus, advances in modern spectrometer design have allowed the deployment of multiple acquisition periods during the course of a single experiment. Application of this approach in protein NMR in the solid state has been provided in Chapter 2 and a detailed protocol for the optimization of these afterglow experiments has been provided in Chapter 3.

Solid-state NMR provides the advantage of being applicable to large biomolecular assemblies such as intact viruses. Chapter 4 provides detailed approaches in optimizing intact virus particles for solid-state NMR studies. Chapter 5 provides a description of novel membranous assemblies based on inert nanoparticles that enable solid-state NMR studies of membrane proteins especially those whose conformations are sensitive to membrane curvature. Solid-state NMR spectra of large molecular assemblies are complicated and benefit from computational tools not only to simplify analyses but also to aid in the choice of optimal labeling schemes for spectral simplification. Chapter 6 provides an example of such a tool. Additionally, solid-state NMR studies of many large assemblies including viruses are generally hindered by sensitivity issues due to sample concentration and/or stability, thus preventing the application of several experiments in the NMR toolbox in an optimal fashion. Dynamic nuclear polarization (DNP) that utilizes electrons to polarize nuclear spins provides an excellent means to obtain large improvements in sensitivity, thus overcoming the drawbacks of low sample concentrations or speeding up the acquisition of data with optimal sensitivity in rapidly deteriorating samples. DNP has found application both in the solid state (Chapter 7) and in solution (so-called "dissolution" DNP, Chapter 8).

A great strength of NMR spectroscopy that sets it apart from a variety of other structural techniques is its ability to quantify the dynamics of proteins on timescales that range from the fast (picoseconds to nanoseconds) to the slow (microsecond to millisecond) to the very slow (seconds and slower). Over the years, many methods have emerged to study these dynamics using the measurement of NMR spin-relaxation rates. Generally, in order to accurately quantify motional modes in the fast regime, relaxation rates have to be measured at multiple static field strengths in order to accurately interpret the dynamics in a site-specific fashion. In Chapter 9, the authors describe an approach that utilizes the fringe field of a standard high-field NMR magnet together with some novel engineering to measure relaxation rates that extend over three orders of magnitude in magnetic field strength. Chapter 10 provides detailed protocols on measuring dynamics that occur on the slow regime and generally involve large-scale conformational changes. These conformational changes involve activation barriers that lead to sampling of so-called "excited states." These states, which often have relevance to folding and function, are sparsely populated (often less than 1%) and are thus invisible to conventional techniques. Chapter 11 describes how the structures of these excited states may be accessed using specialized relaxation measurements. Additional effects such as the enhancement of relaxation rates due to close albeit transient encounters with a paramagnetic center (paramagnetic relaxation enhancement, PRE) may also provide a means to probe these lowly populated states. This approach is described in Chapter 12.

An essential part of modern NMR spectroscopy is the processing of raw data obtained from the spectrometer using a variety of mathematical operations, e.g., digital filtering of

solvent, apodization etc. prior to Fourier transformation, and the subsequent analysis of the processed data, e.g., resonance assignment and often the extraction of constraints for the generation of atomic models. Chapters 13 and 14 describe various aspects of data analysis and structure calculation, respectively, using two of the most widely used software platforms. While most common NMR experiments still utilize the Fourier transform that requires digital sampling of the time-domain signal in linear fashion, i.e., in equally spaced intervals, it has been realized that nonlinear sampling often provides significant advantages in some cases. Many functional aspects of nonlinear sampling approaches are described in Chapter 15. Chapter 16 describes a novel computational means that relies on specific correlations obtained in protein NMR spectra to simplify the resonance assignment procedure in large proteins with a significant amount of spectral crowding where conventional approaches fail to resolve ambiguities.

As NMR studies are extended to larger systems, the complexity of NMR spectra, the suboptimal performance of many experiments, and loss of information due to the use of certain essential labeling schemes, e.g., replacement of the information-rich ^{1}H nuclei by ^{2}H, lead to incomplete or sparse data that are insufficient to determine structures de novo. In such cases NMR data has to be integrated with that from orthogonal techniques, e.g., X-Ray or neutron scattering techniques (SAXS, SANS), to obtain structural information using a hybrid strategy. Chapter 17 describes a computational methodology that integrates NMR data with those obtained from SAXS to obtain atomic detail information on macromolecular complexes.

Ever since its discovery in the 1950s from the work of Proctor and Yu [7], Dickinson [8], Hahn [9], and Arnold [10], the chemical shift has played a central role in chemical, and indeed in biomolecular NMR as an excellent indicator of local structural environment and its changes upon molecular interaction. It was recently realized that a survey of correlated chemical shift changes in response to discrete perturbations, e.g., binding of specific ligands, provides insight into pathways of connectivity and/or allosteric networks in proteins. A detailed method to perform such an analysis is described in Chapter 18.

The final two chapters of this volume deal with two major areas of the recent application of biomolecular NMR spectroscopy—Chapter 19 provides a step-by-step guide to the expression, purification, and preparation of highly homogenous samples of a G-protein coupled receptor (GPCR) for studies using ^{19}F NMR. GPCRs are of immense medical importance and NMR provides an avenue to probe their regulation by agonists and antagonists given that many relevant conformational states are dynamic and/or transient and therefore difficult to characterize by other means. Traditionally, most biomolecular NMR has relied on proteins or protein complexes purified to homogeneity and studied in a suitable buffer. This scenario represents a significant departure from the heterogeneous cellular milieu which may have a substantial influence on physiochemical properties of the protein or protein complex in question by altering its "quinary" structure. Chapter 20 describes recent efforts to extend NMR studies to a more physiological setting using in-cell methodology.

Given the broad applications of modern biomolecular NMR spectroscopy of proteins, it is not possible to compile an exhaustive compendium of all approaches and methodologies. However, based on the selection here, I have attempted to provide the reader a flavor of various aspects of modern protein NMR spectroscopy. I expect that the detailed step-by-step protocols provided will be of great use to specialists and also to nonspecialists who would like to add NMR spectroscopy to their repertoire of experimental tools.

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New York, NY, USA *Ranajeet Ghose*

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