
Preface

The field of protein NMR spectroscopy has rapidly expanded into new areas of biochemistry, molecular biology, and cell biology research that were impossible to study as recently as 10 years ago. The potential to study macromolecular systems that were once considered too large or too transient or too complex by using NMR spectroscopy is now being realized with the development of innovative technologies. Standard NMR technologies are also getting a facelift in part due to the pervasive nature of high-throughput approaches in biochemical and biomedical research. These advances warrant a new edition of *Protein NMR Techniques* that includes an authoritative but down-to-earth description of new methodologies. This edition consists of 24 chapters divided into four major categories: NMR sample preparation, solution NMR methodologies, solid-state NMR methodologies, and data processing. The material presented contains enough detail for use not only in specialized NMR laboratories, but in biochemical, molecular, and cell biology research labs that have access to high-field NMR spectrometers.

Preparing proteins for NMR spectroscopy can be a time-consuming process that may take longer than data collection and analysis combined. Expression in bacterial cells still remains one of the most popular ways of preparing NMR samples. *Chapter 1* discusses new methods for optimizing and increasing the production of isotope-labeled protein in bacteria. However, some proteins are difficult to express in bacteria, in these cases an alternative approach involves using yeast cells. *Chapter 2* describes a methodology for producing proteins in yeast that are usually secreted into the growth medium. This technique is proving to be as robust and economic as bacterial production. One drawback to using proteins secreted by yeast is that they may exhibit altered patterns of glycosylation and phosphorylation. To avoid this problem and achieve proper posttranslational modifications, proteins are best produced in insect or mammalian cells. Advances in the use of these cells for producing NMR samples are detailed in *Chapters 3* and *4*. Cell-free expression of proteins has become a method of choice for high-throughput protein production especially in cases, where the yields from in vivo overexpression are very low. Cell-free systems allow for the selective incorporation of any isotope-labeled amino acid into a target protein with minimal scrambling. *Chapters 5* and *6* describe the cell-free production of proteins for solution and solid-state NMR, respectively. Finally, although well-expressed in bacterial cells, some soluble proteins do not fold properly in sufficient quantity to permit analyses of structure, dynamics, and interactions. *Chapter 7* presents a methodology for expressing and purifying such proteins in a cost-efficient manner.

The chapters on solution NMR methodologies range from the study of individual proteins, large multidomain proteins, protein–ligand and protein–nucleic acid complexes in vitro, to the study of proteins inside living cells. A strategy for studying supramolecular systems, which has become possible due to advances in isotope labeling and NMR pulse sequences, is described in *Chapter 8*. *Chapter 9* presents basic protocols and the latest improvements for measuring relaxation rates and analyzing protein dynamics. Methods to help overcome difficulties in applying solution NMR to the study of membrane proteins are

detailed in *Chapter 10*. Structurally characterizing multi-domain proteins can be challenging due to the inherent flexibility present in these systems and requires special approaches outlined in *Chapter 11*. To regulate biological activity, proteins engage in interactions with other macromolecules present in the cell. A description of methods used to prepare protein–RNA, protein–DNA, and protein–ligand complexes suitable for study by using NMR spectroscopy are presented in *Chapters 12, 13, and 14*. Lastly, *Chapter 15* describes in-cell NMR spectroscopy, a relatively new area of NMR research that affords atomic resolution information about isotope-labeled proteins inside living cells.

Solid-state NMR spectroscopy presents a complementary approach to studying proteins, especially since the method is not limited by the molecular size constraints that hamper solution NMR. With the availability of high-field NMR spectrometers, solid-state NMR has become a viable technique for acquiring unique information about protein systems that are difficult to characterize by using solution NMR. *Chapter 16* reviews the use of magic angle spinning solid-state NMR to study the structure and dynamics of perdeuterated proteins. The preparation and characterization of protein complexes for solid-state NMR and methodologies to analyze the structures and dynamics of protein complexes are presented in *Chapter 17*. The area of membrane protein expression has seen extensive advances of late, spurred by intense interest in signaling pathways, but impeded by difficulties in preparing samples in sufficient quantity for NMR spectroscopy. *Chapter 18* details methods for producing membrane proteins suitable for study by using solid-state NMR.

Processing and analyzing NMR data has historically been an extremely laborious part of NMR research, requiring skillful NMR spectroscopists to assign chemical shifts and to determine atomic resolution structures of proteins. With the advent of high-throughput assignment protocols, this task has become largely manageable by a trained graduate student. Nevertheless, there are difficult cases for which there is no substitution for the experienced spectroscopist. For example, characterization of eukaryotic kinases by NMR spectroscopy is complicated by the extensive dynamics and large size exhibited by these proteins. *Chapter 19* describes the procedures used to assign backbone resonances for ERK2. The reactivity of solvent-exposed backbone amides varies by a factor of at least a billion-fold because of electrostatic interactions at the protein surface. The use of electrostatic analysis of hydrogen exchange rates to analyze protein flexibility is reviewed in *Chapter 20*. *Chapter 21* presents a strategy for assigning the backbone resonances of small- to medium-sized globular proteins in a few hours by using a highly automated program, BATCH, to acquire, process, and analyze NMR data. A versatile protocol, UNIO, that provides nearly fully automated structure determination is described in *Chapter 22*. In UNIO, user-intervention is encouraged and facilitated by graphical tools for preparing, analyzing, validating, and presenting the NMR structure. *Chapter 23* details the use of the ARIA software, which incorporates both solution and solid-state NMR structural constraints to perform structure calculations. The final chapter, *Chapter 24*, introduces the software DYNAMICS for analyzing relaxation rates that characterize the overall tumbling and local dynamics of a protein.

This book presents a comprehensive description of the latest innovations in the field of protein NMR. It focuses on the importance of biochemistry, molecular biology, and cell biology to NMR spectroscopy while avoiding excessive repetition of existing material, which is readily available through a number of excellent texts and reviews that cover topics

relevant to studying proteins by using NMR. Rather than reiterating the fundamental principles behind NMR methodologies, we have emphasized the practical aspects of experimental design combined with practical advice and examples. We hope that this book will provide both experienced NMR spectroscopists and biochemists, who are new to the field of NMR, with enough background to successfully apply these techniques to their research.

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