

PREFACE

Nuclear magnetic resonance spectroscopy of biological molecules continues to be a central tool in biology, biochemistry, and biophysics. Since its first applications to problems in chemistry and biology, NMR spectroscopy has shown continual evolution and episodic advancements in its capabilities and applications. Much has revolved around optimization of samples to facilitate full exploitation of the physics of NMR in the context of macromolecules such as proteins. In this Part A of a two-part series on biological NMR spectroscopy, recent advancements in isotopic enrichment of proteins are presented and protocols described. Three chapters center on methyl-labeling strategies. A contribution by Gardner and colleagues describes recent advances in the production of methyl-labeled membrane proteins in yeast, while Lingel describes the strategies for methyl labeling of proteins recombinantly expressed in *E. coli*. A chapter by Weininger focuses on recent advances in optimal isotopic labeling of aromatic rings in proteins for detailed studies of dynamics by NMR. A chapter by Dötsch describes the production of membrane proteins in various membrane mimetics, while Kalodimos and colleagues provide strategies for using auxotrophs for the production of methyl labeling of large soluble proteins. Over the past decade the role of posttranslational modifications in the control of protein activity has become ever clearer and central to studies of eukaryotic biology. Three chapters of this Part A present strategies and protocols for the preparation of biophysical quantities of phosphorylated, lipidated, and glycosylated proteins by Peti, Distefano, Barb, and their colleagues, respectively. A chapter by Glover and Tommos examines an often-overlooked potential artifact arising from the commonly used purification by tags based on affinity for divalent cations such as nickel.

Continual improvements in analytical and computational strategies have accompanied these advances in sample preparation. In their chapter, Wagner and colleagues describe the recent advances in nonuniform data acquisition and processing. Kalodimos and colleagues present protocols for an elegant algorithm for obtaining methyl assignments. Allain and coworkers carry the protein-nucleic acid torch by showing how NMR-based structural restraints can be combined with molecular dynamics simulations to characterize protein-RNA complexes. Also highlighted in this volume are chapters illustrating the use of knowledge-based approaches in conjunction

with more traditional structural restraints for the determination of structural models of proteins and their complexes. Nerli and Sgourakis present detailed protocols for the use of the CS-Rosetta structure determination software package, while Montelione and colleagues describe how knowledge of "evolutionary couplings" can be combined with sparse NMR data sampling to yield structural models. Finally, taking NMR deep into the territory of experimental biophysics, Royer and colleagues present the issues and strategies for employing high pressure in the context of solution NMR spectroscopy.

Part B in the series turns to more specialized topics. Sharp provides an overview of how direct connections between NMR observables and molecular dynamics simulations can be made. Chapters by Stetz and colleagues and Iwahara and colleagues center on recent developments in NMR relaxation studies of ps–ns motion of methyl-bearing and basic side chains in proteins, respectively. The former contribution also presents protocols for the use of the recently established NMR-based dynamical proxy for conformational entropy. Palmer and Koss contribute a comprehensive chapter on the use of chemical exchange phenomena manifested in NMR observables to characterize motions in the μ s–ms time regime. There are three chapters describing various routes to characterization of protein hydration, which has resisted a comprehensive experimental description for many decades. Prosser and colleagues present an overview of the use of ^{19}F NMR to characterize the protein structural ensemble including its hydration. Jorge and coworkers utilize the advantageous physical and chemical properties of the nanoscale water core of reverse micelles to probe protein hydration dynamics using the classical NOE and ROE approach. As a companion chapter, Fuglestad and colleagues provide protocols and strategies for optimal encapsulation of single protein molecules within reverse micelles. Franck and Han introduce the Overhauser dynamic nuclear polarization (DNP) technique for the characterization of the hydration shell of macromolecules. To represent the dramatic advances of the general DNP approach to sensitivity enhancement in solid-state NMR, Frederick shows how DNP can be used to probe the structure of biomolecules in their native cellular environments. Amplifying the recent advances in solid-state NMR, Carlomagno and colleagues describe various approaches to the structural characterization of RNA. The volume then turns to small molecule characterization and drug discovery by NMR. Br uschweiler and coworkers show how a hybrid approach using NMR and mass spectrometry can enable resolution of the complex mixtures of

metabolomics. Kim and Hilty further illustrate the power of DNP in the search for small molecule binders to proteins. Huang and Leung and Angulo and colleagues present the recent advances in utilizing water and small molecule magnetization transfer to proteins in drug discovery. Finally, Montelione and colleagues describe an intriguing strategy for identifying the ligand–receptor pairs that underlie complex eukaryotic biology and will be the ever-increasing focus of structural biology by NMR in the future.

On behalf of the contributing authors and myself I thank you for your interest in these volumes and hope that you find them useful.

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