PREFACE

A number of structural biology techniques such as nuclear magnetic resonance (NMR), neutron reflectometry (NR), and small-angle neutron scattering (SANS) can be performed on biomolecules with nuclei at natural isotope abundance. However, the use of biomolecules labeled with stable isotopes (mainly ^{2}H, ^{13}C, or ^{15}N) allows for additional experimental approaches. Labeled biomolecules are also important in quantitative mass spectrometry (MS), and deuteration of exchangeable protons is required for hydrogen–deuterium exchange mass spectrometry (HDX-MS).

The aim of this volume of *Methods in Enzymology* is to provide methodologies for the use of labeled biomolecules in different structural and biophysical applications. The accompanying volume describes the production and purification of labeled biomolecules.

Structural characterization of biomolecules by NMR has utilized stable isotopic enrichment to allow for the use of the NMR active spin ½ of ^{13}C and ^{15}N. Correlations to these heteronuclei alleviate ^{1}H resonance overlap. And perdeuteration of biomolecules increases the accessibility of large proteins for NMR analysis. In Chapter 1, Arbogast and coworkers describe the use of 2D spectra collected at natural isotopic abundance of ^{13}C and ^{15}N in the structural analysis of protein therapeutics. Chapter 2, from the laboratory of Gianluigi Veglia, describes an approach to assign the resonances of labeled methyl group side chains in large proteins.

Fluorine (^{19}F) nuclei, which have 100% natural abundance, are spin ½, have a high gyromagnetic ratio, and provide NMR signals that can serve as sensitive and specific reporters of the structural and chemical environment around the site of substitution since ^{19}F is absent from all naturally occurring biomolecules. Two chapters describe the use of ^{19}F-labeled nucleotides for structural analysis by NMR. Scott and Hennig describe the use of ^{19}F-labeled nucleotides for nucleic acid structural analysis (Chapter 3), while Brinson and coworkers describe the use of these molecules in the structural and dynamic analysis of homopyrimidine/homopurine tracts (Chapter 4).

In addition to NMR, deuterated proteins are used in structural determination by scattering techniques. One of the advantages of using deuterated material is the ability to perform contrast variation experiments, in which molecules containing hydrogen and those containing deuterium can be matched out against the isotopic composition of the solvent. Chapter 5, from the

laboratory of Trevor Forsyth, provides an overview of the methods used for deuteration of biomolecules, the type of labeling (perdeuterated, fractional, etc.), and the assays that use deuterated material. Zaccai et al. describe the use of deuterium labeling and SANS contrast variation in the study of protein–protein interactions (Chapter 6). In Chapter 7, Heinrich provides general applications of deuterated material in biological NR studies, while Le Brun and coworkers describe the use of NR and deuterium labeling to study the bacterial outer membrane (Chapter 8). Callaway and Bu describe the strategies and general considerations for analyzing protein dynamics by neutron spin echo spectroscopy in Chapter 9.

Stable isotopes are also commonly used in MS as standards for protein quantitation. Kilpatrick describes a computer program to calculate the percentage of ^{15}N incorporation in peptides and proteins (Chapter 10). In Chapter 11, Scott et al. describe the design, expression, and preparation of labeled concatenated peptides (QconCAT) and their use as internal standards for absolute or relative protein quantification. Reddy et al. (Chapter 12) describe the use of ^{15}N-labeled DNA repair proteins as internal standards for MS.

HDX-MS is used to study protein structure and dynamics, protein–protein interactions, and protein–ligand interactions. In Chapter 13, Mayne discusses the principle behind HDX-MS and the considerations needed when using this technique. Gallagher and Hudgens describe the use of HDX-MS in the study of protein–ligand interactions (Chapter 14), while Guttman and Lee describe the use of the technique for the study of viruses (Chapter 15).

I hope that this and the accompanying volume prove useful for the scientific community by providing descriptions of techniques for biomolecule labeling and their use in structural analysis.

I would like to acknowledge the help of Dr. Lori M. Kelman during the course of editing these volumes.

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