# PREFACE

Biomolecules labeled with stable isotopes (mainly $^{2}H$, $^{13}C$, $^{15}N$) are often used to obtain structural information using techniques such as small-angle neutron scattering, neutron reflectometry, and nuclear magnetic resonance (NMR). Stable isotope labeling of biomolecules can be classified into five categories: uniform labeling, in which all atoms in the molecules are labeled; fractional labeling, in which not all atoms are labeled but the labeling is distributive along the biomolecule; site-specific labeling, in which a specific amino acid or specific atom (e.g., a specific carbon on the sugar ring) is labeled; selective labeling, in which one type of amino acid or nucleotide is labeled throughout the protein, peptide, or nucleic acid; and segmental labeling, in which only a part of the molecule (e.g., a domain within a protein) is labeled.

The aim of this volume of *Methods in Enzymology* is to provide comprehensive methodologies for the production and purification of labeled biomolecules of various types and from different sources. The accompanying volume describes different experimental techniques that use labeled biomolecules.

The first step is to produce the biomolecule in the presence of a labeled chemical, precursor, or amino acid. The most commonly used system to label protein is heterologous expression in *Escherichia coli* in the presence of media containing $^{2}H$, $^{13}C$, and/or $^{15}N$. Two chapters provide detailed descriptions of uniform and fractional protein labeling in bacteria using fermenters (Chapter 1 by Duff and coworkers) or shaking flasks (Chapter 2 by Hoopes et al.). In Chapter 3, Lin et al. describe the use of *E. coli* auxotroph host strains for amino acid-selective labeling of protein. Sharaf and Gronenborn describe two methods for $^{19}F$-labeling of protein in *E. coli*: one for selective amino acid incorporation and a second for site-specific incorporation of $^{19}F$-modified amino acids (Chapter 4).

In addition to proteins, bacteria are also used to label other biomolecules. In Chapter 5, Russell and coworkers describe the use of bacteria to produce deuterated polyhydroxyalkanoate biopolyesters and cellulose, and in Chapter 6 O'Neill et al. describe a method for the deuteration of bacterial cellulose. Proteins can also be labeled in archaea as described by Cleveland and Kelman in Chapter 7 on the labeling of proteins in *Halobacterium salinarum*. One approach to achieve selective amino acid labeling is via

amino acid-selective unlabeling, as described by the laboratory of Hanudatta Atreya in Chapter 8.

Many proteins, in particular eukaryotic proteins, cannot be expressed in bacteria in an active, folded, form. In addition, when posttranslational modifications (such as glycosylation) are required for proper folding or activity, bacteria cannot be used for expression. Therefore, methods have been developed to express proteins in eukaryotic cells. Fan et al. describe the use of yeast to label membrane proteins (Chapter 9), and Peter Holden's laboratory describes the use of yeast to make the polysaccharide chitosan (Chapter 5). Evans and Shah describe approaches for deuterium incorporation in plants (Chapter 10). Skora et al. describe the use of insect cells for uniform labeling of proteins or selective labeled amino acid incorporation (Chapter 11). In Chapter 12, Sastry et al. describe the use of an adenovirus-based system for two types of protein labeling in mammalian cells: uniform labeling with $^{13}C$ and/or $^{15}N$, or selective labeling using an amino acid.

Biomolecule labeling can also be performed *in vitro*, and several chapters in the volume provide protocols. Terada and Yokoyama (Chapter 13) and Xian and coworkers (Chapter 14) describe two approaches for the labeling of mammalian proteins using an *E. coli* cell-free system, while LaGuerre et al. provide a protocol for the labeling of membrane proteins with a cell-free system (Chapter 15). A procedure for segmental labeling of an individual domain in a multidomain protein is described by Michel and Allain (Chapter 16). Zhang et al. describe *in vitro* chemical methods to introduce stable isotopes into monosaccharides (Chapter 17).

Labeled nucleotides aid in structural analysis of nucleic acids by NMR. The contribution by Wunderlich et al. describes chemoenzymatic methods for site-specific labeling of RNA (Chapter 18). The contribution from the laboratory of Kwaku Dayie describes methods for uniform $^{15}N$ and site-specific $^{13}C$ labeling of RNA in *E. coli* (Chapter 19). Duss et al. describe protocols for making very short labeled RNAs as well as RNA fragments of any desired size (Chapter 20).

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.

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Edited by Zvi Kelman