Title

METHODS IN ENZYMOLOGY Metabolic Analysis Using Stable Isotopes PREFACE

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PREFACE

Biochemical reactions are the driving force for virtually all living systems. In the last century, biochemists have painstakingly mapped the diverse set of chemical reactions catalyzed in cells (often one enzyme at a time). Though far from complete, we now have comprehensive maps and databases outlining the substrates, products, cofactors, and enzymes that comprise *metabolism*.

Concomitant with the description of metabolic pathways that occurred over the last ~100 years, we have also seen technological advances that now enable precise characterization and quantitation of chemical processes. Chromatographic separation and detection using high-mass resolution mass spectrometers or alternatively nuclear magnetic resonance are emerging as powerful tools for metabolic analyses, particularly when combined with the application of chemically synthesized stable isotope tracers. Finally, computational modeling and simulation allows more reliable interpretation of the data generated using such technologies. These advances offer amazing opportunities for metabolic biochemists but also create challenges in terms of learning the technical details, which often require diverse skills that range from animal surgery to analytical chemistry to programming. In this volume of *Methods in Enzymology*, I have brought together experts who commonly apply stable isotope tracers to study metabolism in mammalian systems. Although the details of each protocol are available in a number of formats, I have attempted to include descriptive methods in a more integrated manner that makes the protocols more accessible to newcomers and young scientists.

Chapters 1 and 2 of this volume provide comprehensive protocols for the use of hyperpolarized metabolic tracers in tissues, cells, and isolated enzymes. The enhanced sensitivity afforded by hyperpolarization enables real-time metabolic imaging via magnetic resonance spectroscopy. In Chapter 1, Chaumeil et al. outline the theory, methodologies, and important considerations for application of dynamic nuclear polarization (DNP) to *in vitro* and *in vivo* systems in great detail. In Chapter 2, Lumata et al. provide additional insights into DNP methods with a particular focus on its application to perfused organs (i.e., liver and heart). Chapters 3 and 4 focus on the analysis of intact tissues using mass spectrometry-based mass isotopomer (or isotopologue) analysis, outlining approaches to study metabolism in the heart and retina, respectively. Ruiz et al. detail methods for the surgical preparation, setup, perfusion, and analysis of *ex vivo* hearts in both the

Langendorff and working modes. Next, Du et al. describe methods for probing metabolism in the retina, another tissue that exhibits high metabolic activity as well as unique compartmentalization and architecture.

Chapters 5 and 6 address the analysis of metabolism in cultured cells using mass spectrometry-based approaches. MacKay et al. first provide detailed methods and considerations on the application of liquid chromatography coupled to mass spectrometry (LC-MS) and variations therein (i.e., tandem mass spectrometry) to cultured cells. Tumanov et al. next specify details on the application of LC-MS as well as gas chromatography-mass spectrometry to fatty acid metabolism.

In Chapter 7, Holmes et al. provide a comprehensive protocol and discussion on the application of ^2H_2O (heavy water) to study proteome dynamics *in vivo*. Here, proteins are metabolically labeled via amino acid metabolism, and LC-MS/MS-based detection is applied to quantify isotope incorporation in peptides. Mass isotopomer distribution analysis is subsequently applied to estimate protein turnover. In Chapter 8, Weindl et al. outline an experimental approach and data analysis pipeline that enable detection of tracer fates in a nontargeted manner. Theoretical considerations of this method are described in great detail. Kelleher and Nickol then describe the early application of nonlinear modeling in metabolic research as embodied by isotopomer spectral analysis in Chapter 9, illustrating specific findings that were ascertained using this model-based approach. Finally, in Chapter 10 Previs et al. outline a model and associated calculations that can be employed to estimate how error propagates during the *in vivo* application of ^2H_2O for quantitation of lipid and protein metabolism.

The methods outlined herein only scratch the surface of technologies that can be applied to study metabolic pathways. Chromatography, mass spectrometry, and NMR will continue to improve rapidly such that larger and more comprehensive datasets are becoming available. As these data become available, researchers are beginning to apply quantitative, genome-scale models of metabolism based on flux balancing or metabolic tracing data to both microbial and mammalian systems. Detailed methods for such modeling approaches are available elsewhere, though I sincerely apologize to the many excellent scientists whose contributions could not be included here. There are much too many individuals who have directly or indirectly influenced my work in metabolism in the last decade to list. I hope the protocols included here will facilitate the recruitment of more highly skilled researchers in the area of metabolic research.

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