PREFACE

Over 100 years ago, two lines of corroborating evidence led to the discovery of isotopes: identification of the decomposition products of radioactive elements and the detection by primordial mass spectrometry of two types of neon (Aston, 1920; Rutherford & Soddy, 1903; Thomson, 1912). In the 1920s, isotopes of many elements were identified ending with the isolation of deuterium in 1932 (Urey, Brickwedde, & Murphy, 1932). Almost immediately these isotopes were conscripted into the service of biology as tracers of metabolism. Not until the 1950s were the effects of isotopes on the rates of reaction given a theoretical basis of interpretation by Bigeleisen (Bigeleisen & Mayer, 1947).

The application of kinetic isotope effects to the study of enzyme reactions adopted this theoretical approach. Volumes devoted to the study of kinetic isotope effects have appeared with some regularity starting with Melander's text in 1960 (Melander, 1960). In the middle of the 1970s, two volumes inspired renewed interest in the application of kinetic isotope effects to the study of enzyme reactions: The *Sixth Steenbock Symposium on Isotope Effects on Enzyme Reactions* (Cleland, O'Leary, & Northrop, 1977) and Gandour and Schowen's *Transition States of Biochemical Processes* (Gandour & Schowen, 1978). Methodological and theoretical advances focusing on using isotope effects to understand biological catalysis were highlighted in Cook's *Enzyme Mechanism from Isotope Effects* (Cook, 1991). Isotopes and isotope effects continue to see broad application in chemical and geochemical research as reviewed in *Isotope Effects in the Chemical, Geological, and Bio Sciences* edited by Wolfsberg and colleagues (Wolfsberg, Van Hook, Paneth, & Rebelo, 2009). The most recent review volume published in 2006 was edited by Limbach and Kohen, *Isotope Effects in Chemistry and Biology* (Kohen & Limbach, 2005).

The historic background of isotope effect studies continues to inform the research advances described in the contributions to this volume. The application of both radioactive isotopes as described in the chapters by Stratton et al., and of mass spectrometry remains the major analytical approaches used to achieve the necessary level of precision necessary for quantitative measurement of kinetic isotope effects. Originally only applicable to gases, the development of ionization methodologies applicable to both highly charged and macromolecular substrates opens up new horizons in isotope

studies. The new higher resolution spectrometers and innovative methods of introducing analytes into isotope ratio mass spectrometers have dramatically expanded the scope of isotope effect studies. Some of these methods are reflected in contributions of chapters by Szatkowski et al., Harris et al., Pati et al., Tea & Tcherkez, and Kluger. Scintillation counting methods have been refined and developed for application to multiplexed studies as described by Schramm. In the middle of the 20th century the different nuclear spin properties of isotopes became a feature of NMR analyses. The demands of high quantitative precision required of kinetic isotope effect studies initially limited the application of NMR methods. In this volume, several new applications that have been developed relying on the higher resolution and stability of modern NMR spectrometers are highlighted in the chapters by Bennet, Perrin, and Robins. In the case of large deuterium effects, direct determination of rate constants is possible and the chapter by Hay illustrates progress curve analysis useful which can be very powerful in many cases and applicable to a broad range of enzyme assays.

The inclusion of computational methods to model kinetic isotope effects (KIEs) would require a similar sized volume to survey the advances that have been made in quantum chemistry methods of modeling transition states (or modifications to transition state theory to accommodate tunneling, etc.). Nonetheless, theoretical models are so integral to isotope effect studies they do appear in some of the contributions, notably in Ruszczycky and Liu's contribution on measuring kinetic isotope effects on intermediate distributions and their interpretation as effects on net rate constants. The coordination between theory and experiment is also important to description of RNA 2′-O-transphosphorylation transition states by Harris and colleagues. The contribution by Stratton et al. on binding isotope effects (BIEs) summarizes the range of enzyme BIEs and interpretations based on computation. Indeed, the importance of combined KIE analysis with computation to test mechanistic predictions is illustrated very clearly by the overview of theory and experiment by Kohen and colleagues aimed at understanding heavy enzyme isotope effects, and by Allemann describing chemical ligation methods to experimentally locate these effects.

Similarly, it would be intractable to comprehensively review the impact that isotope effect measurement and analysis has had on revealing the chemical details of enzyme catalysis. The long history of deriving enzyme mechanism from isotope effects would itself provide material for an independent volume. The broad applicability of isotope effects is nonetheless illustrated

by the range of systems examined in this volume including orotidine 5′-monophosphate decarboxylase (Richard), *N*-acetylpolyamine oxidase (Fitzpatrick), ribonuclease (Harris), dehalogenase (Dybala-Defratyka), fatty acid hydroxylation (Robins), enzymes of steroid biosynthesis (Piper), dioxygenase (Hofstetter), and the radical SAM enzyme DesII (Ruszczycky). Application of isotope effect analyses to probe new reactions is illustrated by the contribution by Richard aimed at reactions in which hydron is incorporated into the reaction product from solvent water. The kind of insights possible for specific systems are expertly surveyed by Guengerich for cytochrome P450 catalysis, Lipscomb and colleagues for diiron oxygenases, and Mueller for pseudouridine synthase. For each of the enzymes used in the methods described herein unique challenges had to be overcome that together can provide a collection of useful guidelines for exploration of many other systems. The field continues to be advanced by clever adaptation of technologies to measure isotope effects as shown in the chapter by Fitzpatrick showing how continuous flow mass spectrometry can resolve effects inaccessible by any other means.

We hope that this volume will serve as a guide to new developments that take advantage of the increased instrumental capabilities that have become available in recent years. This volume also reflects the expansion of kinetic isotope effects into the environmental and drug metabolism fields where we hope they will find greater interest and application. While we can already anticipate newer methods from higher resolution spectrometers, we believe this volume can help expand the interest and application of isotope effect studies to a greater understanding of the biological world.

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