CONTENTS

Preface xi

1 INTRODUCTION 1
   1.1 A Brief History of Enzymology 2
   1.2 Goal of Enzymology: The Elucidation of Mechanism 11
   1.3 The Emergence of Mechanism from Data 13

2 KINETICS OF SINGLE-SUBSTRATE ENZYMATIC REACTIONS 19
   2.1 The Dependence of Initial Velocity on Substrate Concentration and the Requirement for an E:S Complex 19
   2.2 Derivation of the Rate Equation for a Single-Substrate Reaction with the Assumption of Rapid Equilibrium 21
   2.3 Derivation of Rate Equations Using the Steady-State Assumption 24
   2.4 Methods of Enzyme Assay 26
   2.5 Enzyme Kinetics Practicum: Assay Development, Experimental Design, Data Collection, and Data Analysis 32

3 KINETICS OF SINGLE-SUBSTRATE ENZYMATIC REACTIONS: SPECIAL TOPICS 41
   3.1 Transition State Theory and Free Energy Diagrams 41
   3.2 Kinetic Consequences of an Enzyme:Substrate Complex 46
   3.3 Reactions with More Than One Intermediary Complex 51
   3.4 Deviations from Michaelis–Menten Kinetics 53
   3.5 Kinetics of Enzymatic Action on Substrates with Multiple Reactive Centers 65

4 ENZYME INHIBITION: THE PHENOMENON AND MECHANISM-INDEPENDENT ANALYSIS 73
   4.1 Enzyme Inhibition: The Phenomenon 74
   4.2 Enzyme Inhibition: The First Quantitative Steps 76
4.3 Enzyme-Inhibitor Systems Misbehaving 77
4.4 Case Studies 83

5 KINETIC MECHANISM OF INHIBITION OF ONE-SUBSTRATE ENZYMATIC REACTIONS 89
5.1 Importance in Drug Discovery 89
5.2 Theoretical Considerations 90
5.3 Analysis of Initial Velocity Data for Enzyme Inhibition 96
5.4 Inhibition of One-Substrate, Two-Intermediate Reactions 108
5.5 Inhibition by Depletion of Substrate 111

6 TIGHT-BINDING, SLOW-BINDING, AND IRREVERSIBLE INHIBITION 115
6.1 Importance in Drug Discovery 115
6.2 Tight-Binding Inhibition 116
6.3 Slow-Binding Inhibition 122
6.4 Irreversible Inhibition 137

7 KINETICS OF TWO-SUBSTRATE ENZYMATIC REACTIONS 141
7.1 Importance in Drug Discovery 141
7.2 Basic Mechanisms 142
7.3 Conceptual Understanding of Sequential Mechanisms 143
7.4 Derivation of Rate Equations for Sequential Mechanisms 147
7.5 Ping-Pong Mechanisms 153
7.6 Determining the Kinetic Mechanism for Two-Substrate Reactions 156
7.7 A Conceptual Understanding of the Shapes of Secondary Plots 163
7.8 Mistaken Identity: Rapid Equilibrium Random Versus Steady-State Ordered 168

8 KINETIC MECHANISM OF INHIBITION OF TWO-SUBSTRATE ENZYMATIC REACTIONS 169
8.1 Importance in Drug Discovery 170
8.2 Mechanisms of Inhibition of Two-Substrate Reactions 170
8.3 Inhibition by Substrate Analogs 185
8.4 Analysis of Sequential Reactions in which Inhibitor Binds to Enzyme: Product Complexes 191
8.5 Driving SAR Programs for Two-Substrate Enzymatic Reactions 196
9 ALLOSTERIC MODULATION OF ENZYME ACTIVITY \hspace{1cm} 199
  9.1 Mechanisms of Enzyme Modulation \hspace{1cm} 201
  9.2 Kinetics of Allosteric Modulation \hspace{1cm} 202
  9.3 Meaning of $\beta$ and $\gamma$ \hspace{1cm} 208
  9.4 Case Studies: Dependence of Allosteric Modulation on Structural Features of the Substrate \hspace{1cm} 212

10 KINETICS-BASED PROBES OF MECHANISM \hspace{1cm} 219
  10.1 pH Dependence of Enzymatic Reactions \hspace{1cm} 220
  10.2 Temperature Dependence of Enzymatic Reactions \hspace{1cm} 229
  10.3 Viscosity Dependence of Enzymatic Reactions \hspace{1cm} 235
  10.4 Kinetic Isotope Effects on Enzyme-Catalyzed Reactions \hspace{1cm} 239

APPENDIX A BASIC PRINCIPLES OF CHEMICAL KINETICS \hspace{1cm} 251
  A.1 One-Step, Irreversible, Unimolecular Reactions \hspace{1cm} 252
  A.2 One-Step, Irreversible, Bimolecular Reactions \hspace{1cm} 253
  A.3 One-Step, Reversible Reactions \hspace{1cm} 254
  A.4 Two-Step, Irreversible Reactions \hspace{1cm} 257
  A.5 Two-Step Reaction, with Reversible First Step \hspace{1cm} 259

APPENDIX B TRANSITION STATE THEORY AND ENZYMOLOGY: ENZYME CATALYTIC POWER AND INHIBITOR DESIGN \hspace{1cm} 263
  B.1 Catalytic Power of Enzymes \hspace{1cm} 263
  B.2 Transition State Analog Inhibition \hspace{1cm} 268

APPENDIX C SELECTING SUBSTRATE CONCENTRATIONS FOR HIGH-THROUGHPUT SCREENS \hspace{1cm} 275
  C.1 Balancing the Steady State for One-Substrate Reactions \hspace{1cm} 276
  C.2 Balancing the Steady State for Two-Substrate, Rapid Equilibrium-Ordered Enzymatic Reactions \hspace{1cm} 276
  C.3 Balancing the Steady State for Two-Substrate, Rapid Equilibrium Random Enzymatic Reactions \hspace{1cm} 279
  C.4 Balancing the Steady State for Nonequilibrium Enzymatic Reactions Involving a Second Steady-State Intermediate \hspace{1cm} 282
  C.5 Balancing the Steady State for Two-Substrate, Ping-Pong Enzymatic Reactions \hspace{1cm} 283

Index \hspace{1cm} 287