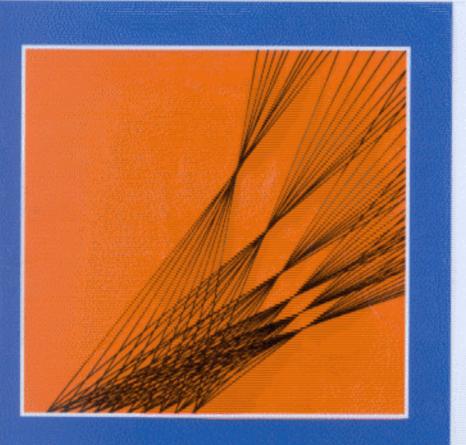


Hans Bisswanger

## **Enzyme Kinetics**

Principles and Methods





## **Contents**

Symbols and Abbreviations XIII					
Introduction 1					
1	Multiple	e Equilibria 5			
	1.1	Diffusion 5			
	1.2 1.2.1 1.2.2	Interaction of Ligands and Macromolecules 10 Binding Constants 10 Derivation of the Binding Equation 11			
	1.3 1.3.1 1.3.2 1.3.3	Macromolecules with Identical Independent Binding Sites 11 General Binding Equation 11 Graphic Representation of the General Binding Equation 17 Binding of Various Ligands, Competition 22			
	1.4	Macromolecules with Non-Identical, Independent Binding Sites 26			
	1.5	Macromolecules with Identical, Interacting Binding Sites, Cooperativity 28			
	1.5.1	The Hill Equation 28			
	1.5.2	The Adair Equation 30			
	1.5.3	The Pauling Model 32			
	1.5.4	Allosteric Enzymes 32			
	1.5.5	The Symmetry Model 33			
	1.5.6	The Sequential Model and Negative Cooperativity 38			
	1.5.7	Physiological Aspects of Cooperativity 41			
	1.5.8	Analysis of Cooperativity 44			
	1.5.9	Examples of Allosteric Enzymes 45			
	1.6	Non-Identical, Interacting Binding Sites 48			
	1.7	References 49			
2	Enzyme Kinetics 51				
	2.1	Reaction Order 51			
	2.1.1	First Order Reactions 51			
	2.1.2	Second Order Reactions 53			
	2.1.3	Zero Order Reactions 54			

<b>2.2</b> 2.2.1	Steady-State Kinetics and the Michaelis-Menten Equation 55 Derivation of the Michaelis-Menten Equation 55
2.3 2.3.1 2.3.2	Analysis of Enzyme Kinetic Data 58 Graphical Representations of the Michaelis-Menten Equation 58 Determination of the Reaction Rate 71
<b>2.4</b> 2.4.1 2.4.2 2.4.3	Reversible Enzyme Reactions 75 Rate Equation for Reversible Enzyme Reactions 75 The Haldane Equation 77 Product Inhibition 78
2.5 2.5.1 2.5.2 2.5.3	Enzyme Inhibition 80 Reversible Enzyme Inhibition 81 Irreversible Enzyme Inhibition 103 Enzyme Reactions with Two Competing Substrates 106
2.6 2.6.1 2.6.2 2.6.3 2.6.4 2.6.5 2.6.6 2.6.7	Multi-Substrate Reactions 108  Nomenclature 108  Random Mechanism 109  Ordered Mechanism 113  Ping-Pong Mechanism 115  Haldane Relationships in Multi-Substrate Reactions 117  Mechanisms with More than Two Substrates 118  Other Notations for Multi-Substrate Reactions 120
2.7.1 2.7.2 2.7.3	Derivation of Rate Equations of Complex Enzyme Mechanisms 120 King-Altman Method 120 Simplified Derivations According to the Graph Theory 126 Combination of Equilibrium and Steady-State Assumptions 127
2.8 2.8.1 2.8.2	Kinetic Treatment of Allosteric Enzymes 129 Hysteretic Enzymes 129 Kinetic Cooperativity, the Slow Transition Model 130
<b>2.9</b> 2.9.1 2.9.2	Special Enzyme Mechanisms 131 Kinetics of Immobilised Enzymes 131 Polymer Substrates 138
2.10 2.10.1 2.10.2 2.10.3 2.10.4	pH and Temperature Dependence of Enzymes 139 pH Optimum Curve and Determination of pK Values 139 pH Stability of Enzymes 141 Thermal Stability of Enzymes 142 Temperature Dependence of Enzyme Reactions 143
<b>2.11</b> 2.11.1 2.11.2	Isotope Exchange 146 Isotope Exchange Kinetics 146 Isotope Effects 150

	2.12	Application of Statistical Methods in Enzyme Kinetics 153
	2.12.1	General Remarks 153
	2.12.2	Statistical Terms Used in Enzyme Kinetics 156
	2.13	References 158
3	Methods	s 161
	3.1	Methods for the Investigation of Multiple Equilibria 161
	3.1.1	Equilibrium Dialysis and General Aspects of Binding Measurements 162
	3.1.2	Continuous Equilibrium Dialysis 168
	3.1.3	Ultrafiltration 170
	3.1.4	Gel Filtration 172
	3.1.5	Ultracentrifugation Methods 175
	3.2	Electrochemical Methods 180
	3.2.1	The Oxygen Electrode 181
	3.2.2	The CO <sub>2</sub> Electrode 183
	3.2.3	Potentiometry, Oxidation-Reduction Potentials 183
	3.2.4	The pH-Stat 184
	3.2.5	Polarography 185
	3.3	Calorimetry 186
	3.4	Spectroscopic Methods 188
	3.4.1	Absorption Spectroscopy 190
	3.4.2	Bioluminescence 201
	3.4.3	Fluorescence 201
	3.4.4	Circular Dichroism and Optical Rotation Dispersion 212
	3.4.5	Infrared and Raman Spectroscopy 217
	3.4.6	Electron Spin Resonance Spectroscopy 219
	3.5	Measurement of Fast Reactions 222
	3.5.1	Flow Methods 223
	3.5.2	Relaxation Methods 231
	3.5.3	Flash Photolysis, Pico- and Femtoseconds Spectroscopy 236
	3.5.4	Evaluation of Rapid Kinetic Reactions (Transient Kinetics) 238
	3.6	References 241

Index 247