



Biosensors

Second Edition

Edited by

**Jon Cooper
and Tony Cass**

**PRACTICAL
APPROACH**

Contents

Protocol list xi

Abbreviations xiii

Contributors xv

Redox hydrogel-based electrochemical biosensors 1

Adam Heller

- 1 Electron conducting redox polymers in biosensors 1
 - 1.1 Relationship to mediator-based sensors 1
 - 1.2 The electrocatalytic activity of redox hydrogels and the "wiring" of enzymes 2
 - 1.3 Dependence of the diffusivity of electrons on crosslinking and the advantage of composite electrodes 2
 - 1.4 The redox polymers and their electrochemistry 3
 - 1.5 Crosslinkers and crosslinking 4
- 2 Enzyme electrodes 4
 - 2.1 Electron transfer between enzyme and polymer redox centers 4
 - 2.2 Microscopic homogeneity and salt effects in the redox polymer-enzyme system 6
 - 2.3 Optimal compositions 6
 - 2.4 Special matrices 7
- 3 Specific sensor examples 7
 - 3.1 Amperometric and potentiometric biosensors of substrates of "wired" redox enzymes 7
 - 3.2 Sensor measuring the turnover rate of hydrolytic and other non-redox enzymes 8
 - 3.3 *In vivo* glucose sensors 10
 - 3.4 Affinity sensors 10

Hybridization at oligonucleotide sensitive electrodes 19

Daren J. Caruana

- 1 Introduction 19
- 2 Function of oligonucleotide sensitive electrodes 20
- 3 Hybridization efficiency and sensitivity 21

- 4 Probe oligonucleotide structure and dynamics 22
 - 4.1 Surface concentration 22
 - 4.2 Probe length and orientation 23
 - 4.3 Attachment of probe 25
- 5 Hybridization conditions 30
 - 5.1 Temperature 31
 - 5.2 Ionic strength 32
 - 5.3 Base mismatch 33
 - 5.4 Mass transport 34
 - 5.5 Nonspecific adsorption 34
 - 5.6 Other factors 36
- 6 Hybridization kinetics 36
- 7. Summary 38

Screen-printing methods for biosensor production 41

Xian-En Zhang

- 1 Introduction 41
- 2 Screen-printing technology 42
 - 2.1 Materials and methods 42
 - 2.2 Apparatus 47
 - 2.3 Printing patterns 48
 - 2.4 Printing process 49
- 3 Applications 51
 - 3.1 Clinical diagnosis 51
 - 3.2 Food analysis bioprocess control 52
 - 3.3 Environmental monitoring 52
 - 3.4 Other approaches 54
- 4 Conclusion 55

Kinetic modeling for biosensors 59

Philip Bartlett and Chee-Seng Toh

- 1 Introduction 59
 - 1.1 The purpose and practice of modeling 59
 - 1.2 Enzyme kinetics 60
 - 1.3 Basic electrochemistry 63
- 2 Modeling 69
 - 2.1 The flux diagram for the membrane|enzyme|electrode 70
 - 2.2 Simplifying assumptions 70
 - 2.3 The flux equations 71
 - 2.4 Solution of flux equations 73
 - 2.5 Deriving a complete kinetic model 78
 - 2.6 Experimental verification of approximate analytical kinetic models 81
 - 2.7 Numerical simulation methods 82
- 3 Applications 89
- 4 Kinetic modeling in other types of biosensors 89
 - 4.1 Potentiometric enzyme electrodes 90
 - 4.2 Optical and photometric biosensors 90
 - 4.3 Immunosensors 91

5 Conclusions 92

List of symbols 92

Bio-, chemi-, and electrochemiluminescence for fiber-optic biosensors 97*Loïc J. Blum and Pierre R. Coulet*

1 Introduction 97

2 Design of the biosensor 97

2.1 Optical waveguide 97

2.2 Setup 98

2.3 Light-emitting reactions 100

2.4 Preparation of the sensing layer 101

3 Examples of determinations with the luminescence sensors 105

3.1 ATP determination 105

3.2 NADH determination 105

3.3 Extension to other analytes using dehydrogenases as auxiliary enzymes 105

3.4 H_2O_2 determination 1063.5 Extension to other analytes involving H_2O_2 detection 107

4 Concluding remarks 108

Determination of metal ions by fluorescence anisotropy: A practical biosensing approach 109*Richard Thompson, Badri Maliwal, Hui Hui Zeng, and Michele Loetz Cramer*

1 Introduction and rationale 109

1.1 Why fluorescence anisotropy to determine metal ions? 109

2 Theory of anisotropy-based determination of metal ions 111

2.1 "Reagent" approaches 111

2.2 "Reagentless" approach 112

3 Fluorescent aryl sulfonamides for zinc(II) determination 114

4 Removal of zinc from carbonic anhydrase (CA) 115

5 Avoidance of metal ion contamination 117

6 Determination of Zn using a reagent approach 119

7 Determination of Cu and other ions by using a reagentless approach 123

8 Calibration of anisotropy 124

Fluorescence-based fiber-optic biosensors 131*David R. Walt, Caroline L. Schauer, Shannon E. Stitzel, Michael S. Fleming, and Jason R. Epstein*

1 Introduction 131

1.1 Fiber polishing 133

2 Single-analyte detection using an enzymatic sensing layer 134

2.1 Enzymatic sensing layer 134

2.2 PAN gel immobilization 135

3	Multi-analyte arrays	136
3.1	Immobilization via polymer photodeposition	136
3.2	Microwell array platform preparation	138
3.3	Live-cell array fabrication	146
4	Conclusions	151
	Functional analysis of ion channels: Planar patch clamp and impedance spectroscopy of tethered lipid membranes	153
	<i>Michael Mayer, Samuel Terretaz, Laurent Giovangrandi, Thierry Stora, and Horst Vogel</i>	
1	Introduction	153
2	Planar patch clamp	154
2.1	Concept of patch clamp on a chip	154
2.2	Formation of planar bilayers on a chip	157
2.3	Chip-based planar bilayers: single-channel measurements of alamethicin pores	165
3	Impedance spectroscopy of tethered lipid membranes	168
3.1	Basics of impedance spectroscopy	168
3.2	Measuring technique and electrochemical cell	170
3.3	Hybrid lipid layer	170
3.4	Tethered lipid bilayers	174
3.5	Lipid bilayer tethered via surface-attached proteins	177
3.6	Highly insulating tethered lipid bilayers for single-channel experiments	179
	Protein engineering for biosensors	185
	<i>Gianfranco Gilardi</i>	
1	Introduction	185
2	Rational protein engineering	187
2.1	Modeling and calculations on protein structures	188
2.2	Site-directed mutagenesis	199
3	Directed evolution	222
3.1	Random mutagenesis: error prone PCR	224
3.2	Recombination: DNA shuffling	226
3.3	Functional screening of the library	230
4	Functional characterization of the mutants	234
5	Other aspects of protein engineering	234
6	Concluding remarks	238
	<i>Index</i>	241