

Contents

	Preface	XI
	List of Contributors	XIII
1	Amperometric Biosensors	1
	<i>Sabine Borgmann, Albert Schulte, Sebastian Neugebauer, and Wolfgang Schuhmann</i>	
1.1	Introduction	1
1.1.1	Definition of the Term "Biosensor"	3
1.1.2	Milestones and Achievements Relevant to Biosensor Research and Development	7
1.1.3	"First-Generation" Biosensors	7
1.1.4	"Second-Generation" Biosensors	7
1.1.5	"Third-Generation" Biosensors	13
1.1.6	Reagentless Biosensor Architectures	15
1.1.7	Parameters with a Major Impact on Overall Biosensor Response	18
1.1.8	Application Areas of Biosensors	22
1.2	Criteria for "Good" Biosensor Research	23
1.3	Defining a Standard for Characterizing Biosensor Performances	25
1.4	Success Stories in Biosensor Research	28
1.4.1	Direct ET Employed for Biosensors and Biofuel Cells	29
1.4.2	Direct ET with Glucose Oxidase	32
1.4.3	Mediated ET Employed for Biosensors and Biofuel Cells	36
1.4.4	Nanomaterials and Biosensors	38
1.4.4.1	Modification of Macroscopic Transducers with Nanomaterials	39
1.4.4.2	Nanometric Transducers	41
1.4.4.3	Modification of Biomolecules with Nanomaterials	42
1.4.5	Implanted Biosensors for Medical Research and Health Check Applications	42
1.4.6	Nucleic Acid-Based Biosensors: Nucleic Acid Chips, Arrays, and Microarrays	48
1.4.7	Immunosensors	52
1.4.7.1	Labeled Approaches	53
1.4.7.2	Nonlabeled Approaches	54

1.5	Conclusion	55
	Acknowledgments	56
	Abbreviations	57
	Glossary	57
	References	61
2	Imaging of Single Biomolecules by Scanning Tunneling Microscopy	85
	<i>Jingdong Zhang, Qijin Chi, Palle Skovhus Jensen, and Jens Ulstrup</i>	
2.1	Introduction	85
2.2	Interfacial Electron Transfer in Molecular and Protein Film Voltammetry	87
2.2.1	Theoretical Notions of Interfacial Chemical and Bioelectrochemical Electron Transfer	88
2.2.2	Nuclear Reorganization Free Energy	90
2.2.3	Electronic Tunneling Factor in Long-Range Interfacial (Bio)electrochemical Electron Transfer	90
2.3	Theoretical Notions in Bioelectrochemistry towards the Single-Molecule Level	92
2.3.1	Biomolecules in Nanoscale Electrochemical Environment	92
2.3.2	Theoretical Frameworks and Interfacial Electron Transfer Phenomena	92
2.3.2.1	Redox (Bio)molecules in Electrochemical STM and Other Nanogap Configurations	93
2.3.2.2	New Interfacial (Bio)electrochemical Electron Transfer Phenomena	95
2.4	<i>In Situ</i> Imaging of Bio-related Molecules and Linker Molecules for Protein Voltammetry with Single-Molecule and Sub-molecular Resolution	97
2.4.1	Imaging of Nucleobases and Electronic Conductivity of Short Oligonucleotides	97
2.4.2	Functionalized Alkanethiols and the Amino Acids Cysteine and Homocysteine	98
2.4.2.1	Functionalized Alkanethiols as Linkers in Metalloprotein Film Voltammetry	100
2.4.2.2	<i>In Situ</i> STM of Cysteine and Homocysteine	102
2.4.2.3	Theoretical Computations and STM Image Simulations	104
2.4.3	Single-Molecule Imaging of Bio-related Small Redox Molecules	105
2.5	Imaging of Intermediate-Size Biological Structures: Lipid Membranes and Insulin	107
2.5.1	Biomimetic Mono- and Bilayer Membranes on Au(111) Electrode Surfaces	107
2.5.2	Monolayers of Human Insulin on Different Low-Index Au Electrode Surfaces Mapped to Single-Molecule Resolution by <i>In Situ</i> STM	109
2.6	Interfacial Electrochemistry and <i>In Situ</i> Imaging of Redox Metalloproteins and Metalloenzymes at the Single-Molecule Level	112
2.6.1	Metalloprotein Voltammetry at Bare and Modified Electrodes	112

2.6.2	Single-Molecule Imaging of Functional Electron Transfer Metalloproteins by <i>In Situ</i> STM	112
2.6.2.1	Small Redox Metalloproteins: Blue Copper, Heme, and Iron-Sulfur Proteins	114
2.6.2.2	Single-Molecule Tunneling Spectroscopy of Wild-Type and Cys Mutant Cytochrome b_{562}	114
2.6.2.3	Cytochrome c_4 : A Prototype for Microscopic Electronic Mapping of Multicenter Redox Metalloproteins	116
2.6.2.4	Redox Metalloenzymes in Electrocatalytic Action Imaged at the Single-Molecule Level: Multicopper and Multiheme Nitrite Reductases	119
2.6.2.5	Au-Nanoparticle Hybrids of Horse Heart Cytochrome c and <i>P. aeruginosa</i> Azurin	120
2.7	Some Concluding Observations and Outlooks	123
	Acknowledgments	126
	References	126

3 Applications of Neutron Reflectivity in Bioelectrochemistry 143

Ian J. Burgess

3.1	Introduction	143
3.2	Theoretical Aspects of Neutron Scattering	144
3.2.1	Why Use Neutrons?	144
3.2.2	Scattering from a Single Nucleus	145
3.2.2.1	The Fermi Pseudo Potential	147
3.2.3	Scattering from a Collection of Nuclei	147
3.2.3.1	Neutron Scattering Cross Sections	147
3.2.3.2	Coherent and Incoherent Scattering	148
3.2.3.3	Effective Potential and Scattering Length Density	148
3.2.4	Theoretical Expressions for Specular Reflectivity	149
3.2.4.1	The Continuum Limit	149
3.2.4.2	The Kinematic Approach	151
3.3	Experimental Aspects	154
3.3.1	Experimental Aspects of Reflectometer Operation	154
3.3.2	Substrate Preparation and Characterization	157
3.3.3	Cell Design and Assembly	160
3.3.4	Data Acquisition and Analysis	162
3.4	Selected Examples	168
3.4.1	Supported Proteins, Peptides, and Membranes without Potential Control	168
3.4.1.1	Quartz- and Silicon-Supported Bilayers	168
3.4.1.2	Hybrid Bilayers on Solid Supports	170
3.4.1.3	Protein Adsorption and DNA Monolayers	173
3.4.2	Electric Field-Driven Transformations in Supported Model Membranes	175
3.5	Summary and Future Aspects	182

	Acknowledgments	184
	References	185
4	Model Lipid Bilayers at Electrode Surfaces	189
	<i>Rolando Guidelli and Lucia Becucci</i>	
4.1	Introduction	189
4.2	Biomimetic Membranes: Scope and Requirements	189
4.3	Electrochemical Impedance Spectroscopy	192
4.4	Formation of Lipid Films in Biomimetic Membranes	194
4.4.1	Vesicle Fusion	196
4.4.2	Langmuir–Blodgett and Langmuir–Schaefer Transfer	198
4.4.3	Rapid Solvent Exchange	200
4.4.4	Fluidity in Biomimetic Membranes	201
4.5	Various Types of Biomimetic Membranes	201
4.5.1	Solid-Supported Bilayer Lipid Membranes	201
4.5.2	Tethered Bilayer Lipid Membranes	203
4.5.2.1	Spacer-Based tBLMs	204
4.5.2.2	Thiolipid-Based tBLMs	205
4.5.2.3	Thiolipid–Spacer-Based tBLMs	215
4.5.3	Polymer-Cushioned Bilayer Lipid Membranes	216
4.5.4	S-Layer Stabilized Bilayer Lipid Membranes	218
4.5.5	Protein-Tethered Bilayer Lipid Membranes	220
4.6	Conclusions	222
	Acknowledgments	223
	References	223
5	Enzymatic Fuel Cells	229
	<i>Paul Kavanagh and Dónal Leech</i>	
5.1	Introduction	229
5.1.1	Enzymatic Fuel Cell Design	231
5.1.2	Enzyme Electron Transfer	231
5.2	Bioanodes for Glucose Oxidation	235
5.3	Biocathodes	243
5.4	Assembled Biofuel Cells	255
5.5	Conclusions and Future Outlook	259
	Acknowledgments	261
	References	262
6	Raman Spectroscopy of Biomolecules at Electrode Surfaces	269
	<i>Philip Bartlett and Sumeet Mahajan</i>	
6.1	Introduction	269
6.2	Raman Spectroscopy	270
6.3	SERS and Surface-Enhanced Resonant Raman Spectroscopy	272
6.4	Comparison of SE(R)RS and Fluorescence for Biological Studies	276

6.5	Surfaces for SERS	278
6.6	Plasmonic Surfaces	280
6.7	SERS Surfaces for Electrochemistry	281
6.8	Tip-Enhanced Raman Spectroscopy	291
6.9	SE(R)RS of Biomolecules	292
6.9.1	DNA Bases, Nucleotides, and Their Derivatives	292
6.9.2	DNA and Nucleic Acids	296
6.9.3	Amino Acids and Peptides	299
6.9.4	Proteins and Enzymes	303
6.9.4.1	Redox Proteins	303
6.9.4.2	Other Proteins	307
6.9.4.3	Enzymes	308
6.9.5	Membranes, Lipids, and Fatty Acids	310
6.9.6	Metabolites and Other Small Molecules	311
6.9.6.1	Neurotransmitters	311
6.9.6.2	Nicotinamide Adenine Dinucleotide	312
6.9.6.3	Flavin Adenine Dinucleotide	313
6.9.6.4	Bilirubin	315
6.9.6.5	Glucose	315
6.10	Conclusion	315
	References	316
7	Membrane Electroporation in High Electric Fields	335
	<i>Rumiana Dimova</i>	
7.1	Introduction	335
7.1.1	Giant Vesicles as Model Membrane Systems	335
7.1.2	Mechanical and Rheological Properties of Lipid Bilayers	337
7.2	Electrodeformation and Electroporation of Membranes in the Fluid Phase	338
7.3	Response of Gel-Phase Membranes	342
7.4	Effects of Membrane Inclusions and Media on the Response and Stability of Fluid Vesicles in Electric Fields	345
7.4.1	Vesicles in Salt Solutions	345
7.4.2	Vesicles with Cholesterol-Doped Membranes	347
7.4.3	Membranes with Charged Lipids	349
7.5	Application of Vesicle Electroporation	350
7.5.1	Measuring Membrane Edge Tension from Vesicle Electroporation	350
7.5.2	Vesicle Electrofusion	353
7.5.2.1	Fusing Vesicles with Identical or Different Membrane Composition	353
7.5.2.2	Vesicle Electrofusion: Employing Vesicles as Microreactors	355
7.6	Conclusions and Outlook	357
	Acknowledgments	358
	References	358

8	Electroporation for Medical Use in Drug and Gene Electrotransfer	369
	<i>Julie Gehl</i>	
8.1	Introduction	369
8.2	A List of Definitions	370
8.3	How We Understand Permeabilization at the Cellular and Tissue Level	371
8.4	Basic Aspects of Electroporation that are of Particular Importance for Medical Use	374
8.4.1	Delivery of Drugs	374
8.4.2	Delivery of DNA	375
8.4.3	Delivery of Other Molecules	376
8.4.4	Delivery of Electric Pulses	376
8.4.5	End of the Permeabilized State	376
8.4.6	The Vascular Lock	377
8.5	How to Deliver Electric Pulses in Patient Treatment	377
8.5.1	Pulse Generators and Electrodes	377
8.5.2	Anesthesia	377
8.6	Treatment and Post-treatment Management	378
8.7	Clinical Results with Electrochemotherapy	378
8.7.1	Tumors Up to Three Centimeters in Size	378
8.7.2	Larger Tumors	380
8.8	Use in Internal Organs	380
8.8.1	Endoscopic Use	381
8.8.2	Bone Metastases	381
8.8.3	Brain Metastases, Brain Tumors, and Other Tumors in Soft Tissues	381
8.8.4	Liver Metastases	381
8.9	Gene Electrotransfer	381
8.9.1	Gene Electrotransfer to Muscle	383
8.9.2	Gene Electrotransfer to Skin	383
8.9.3	Gene Electrotransfer to Tumors	384
8.9.4	Gene Electrotransfer to Other Tissues	385
8.10	Conclusions	386
	References	386

Index	389
--------------	------------