

Contents

Preface to the first edition — V

Preface to the second expanded edition — VII

About the authors — XIII

Abbreviation list — XV

Symbol list — XXI

1	Biomacromolecules in analytical chemistry — 1
1.1	Nucleic acids — 1
1.1.1	Structure of nucleotides — 1
1.1.2	Structure of oligonucleotides and duplexes — 3
1.1.3	DNA and genetic information: the central dogma of molecular biology — 6
1.1.4	Binding of oligonucleotides and thermodynamics — 10
1.1.5	Synthetic DNA as a biochemical tool — 15
1.2	Introduction to proteins — 20
1.2.1	Amino acids — 21
1.2.2	Zwitterionic character, pK and pI — 21
1.2.3	The peptide bond — 24
1.2.4	The structure of proteins — 26
1.2.5	Protein folding and denaturation — 32
1.2.6	The biological function of proteins — 32
1.3	Enzymes — 33
1.3.1	Substrate specificity — 35
1.3.2	Active sites, coenzymes and cofactors — 36
1.3.3	Oxidoreductases — 40
1.3.4	Kinetics of enzymatic reactions — 43
1.4	Antibodies and antigens — 46
1.4.1	Methods to produce antibodies — 47
1.4.2	Antibody structure — 49
1.4.3	Classification of immunoglobulins — 51
1.4.4	Antigen–antibody interaction — 53
1.4.5	Factors influencing antigen–antibody interactions — 54
1.4.6	Quantitative evaluation of antibody properties — 55
	Further readings — 59

2	Introduction to bioanalytical assays and biosensors — 60
2.1	Molecular biorecognition and analytical assays — 60
2.1.1	Example: bioassays for lactate — 62
2.2	Principles of kinetic analytical methods — 65
2.3	Introduction to biosensors — 70
2.4	Classification of biosensors according to the receptor — 71
2.4.1	Catalytic biosensors — 71
2.4.2	Affinity biosensors — 72
2.5	Classification of biosensors on the basis of the transducer — 73
2.6	Immobilisation of biomolecules — 74
2.6.1	Physical entrapping within dialysis membranes — 76
2.6.2	Physical adsorption of biomacromolecules — 77
2.6.3	Electrostatic adsorption — 77
2.6.4	Physical entrapment within a polymeric gel — 78
2.6.5	Cross-linking with polyfunctional reagents — 79
2.6.6	Non-specific covalent bonds — 79
2.6.7	Specific covalent bonds — 80
2.6.8	Biospecific adsorption — 80
2.7	Immobilisation of biomolecules in practice — 81
2.7.1	Entrapment within dialysis membranes — 81
2.7.2	Entrapment within polymeric matrices — 83
2.7.3	Encapsulation in bilayer lipid membranes — 88
2.7.4	Cross-linking — 89
2.7.5	Covalent bonding — 90
2.8	Functionalisation of transducer surfaces — 94
2.8.1	Self-assembled monolayers — 95
2.8.2	Silanisation — 97
2.8.3	Functionalisation of surfaces with diazonium salts — 97
	Further readings — 100
3	Enzymatic biosensors — 101
3.1	Properties of immobilised enzymes — 101
3.1.1	Effects of the thickness of the enzymatic layer — 103
3.2	Electrochemical biosensors — 103
3.2.1	Potentiometric biosensors — 104
3.2.2	Principles of dynamic electrochemical techniques — 113
3.2.3	Electron transfer between enzymes and electrodes — 126
3.2.4	Amperometric biosensors — 134
3.3	Optical biosensors — 142
3.3.1	Optical fibres — 143
3.3.2	Light source and detector — 146
3.3.3	Optical phenomena employed in biosensors — 147

- 3.3.4 Principles of the most common optical sensors — **150**
- 3.3.5 Enzymatic optodes — **154**
- Further readings — **159**

4 Immunoassays and affinity sensors — 161

- 4.1 Introduction — **161**
- 4.2 Immunoprecipitation and radioimmunoassay — **162**
- 4.3 Enzyme immunoassays (EIA and ELISA) — **165**
- 4.4 Lateral flow immunoassays — **170**
 - 4.4.1 Performance evaluation of qualitative bioanalytical tests — **172**
 - 4.4.2 Pregnancy test — **173**
 - 4.4.3 LFIA for virus detection — **174**
- 4.5 Western blotting — **180**
 - 4.5.1 Electrophoretic separation — **180**
 - 4.5.2 Transfer to solid membrane — **181**
 - 4.5.3 Blocking unspecific binding — **182**
 - 4.5.4 Incubation with antibodies — **182**
 - 4.5.5 Detection — **182**
- 4.6 Enzyme-labelled immunosensors — **183**
- 4.7 Microbead-based immunoassays — **186**
 - 4.7.1 Multiplexed microbead immunoassays based on flow cytometry — **187**
 - 4.7.2 Magnetic beads' electrochemiluminescence assays — **189**
 - 4.7.3 Mechanism of electrochemiluminescence — **191**
- 4.8 Label-free affinity sensors — **192**
 - 4.8.1 Surface plasmon resonance immunosensors — **193**
 - 4.8.2 Quartz crystal microbalance: immunosensors based on piezoelectric effect — **196**
 - 4.8.3 Affinity sensors based on electrochemical impedance spectroscopy — **200**
- 4.9 Synthetic receptors — **205**
 - 4.9.1 Molecularly imprinted polymers (MIPs) — **206**
 - 4.9.2 Aptamers — **215**
- Further readings — **221**

5 Analysis of nucleic acids — 223

- 5.1 DNA extraction — **223**
- 5.2 Southern blotting — **223**
- 5.3 Amplification and detection of specific DNA sequences — **225**
 - 5.3.1 The polymerase chain reaction (PCR) — **225**
 - 5.3.2 Analytical PCR: quantitative PCR or real-time PCR — **227**
 - 5.3.3 Signal generation in PCR — **230**
 - 5.3.4 PCR in action: the coronavirus disease (COVID-19) — **234**

5.3.5	Digital PCR —	236
5.3.6	Other amplification techniques —	240
5.4	DNA microarrays —	244
5.5	CRISPR-based biosensors —	246
5.5.1	What is CRISPR-Cas? —	246
5.5.2	CRISPR-Cas biosensors —	249
5.6	DNA sequencing —	253
5.6.1	Sanger sequencing —	253
5.6.2	Illumina sequencing: an example of short-read next-generation sequencing —	255
5.6.3	Pacific Biosciences and Oxford Nanopore Technologies sequencing: examples of long-read third-generation sequencing —	265
	Further readings —	268
6	Nanotechnologies for bioanalysis —	270
6.1	Introduction —	270
6.2	Classification of nanomaterials —	270
6.2.1	Zero-dimensional nanomaterials —	270
6.2.2	One-dimensional nanomaterials —	271
6.2.3	Two-dimensional nanomaterials —	274
6.2.4	Three-dimensional nanomaterials —	276
6.3	Synthesis of nanomaterials —	277
6.3.1	Approaches based on physical changes —	278
6.3.2	Approaches based on chemical transformations —	282
6.4	Functionalisation of nanomaterials —	293
6.5	Analytical techniques for nanomaterial characterisation —	294
6.6	Bioanalytical applications of nanomaterials —	295
6.6.1	Optical properties of metal nanoparticles —	295
6.6.2	DNA–AuNP sensors —	297
6.6.3	Colorimetric detection of proteins with nanoparticles —	300
6.7	SERS for bioanalytical chemistry —	301
6.7.1	Introduction to Raman spectroscopy —	301
6.7.2	Motivation for SERS —	303
6.7.3	SERS analytical methodologies —	304
6.7.4	SERS assay for DNA denaturation —	307
6.8	Electrochemical nano-biosensors —	310
6.8.1	Voltammetry with nanoelectrode arrays —	312
6.8.2	Bioelectroanalysis with nanoelectrode arrays —	315
6.9	Final remarks —	317
	Further readings —	318