

# Contents

Preface — 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 — 5
    - 1.1.4 Binding of oligonucleotides and thermodynamics — 10
    - 1.1.5 Synthetic DNA as a biochemical tool — 16
  - 1.2 Introduction to proteins — 19
    - 1.2.1 Amino acids — 21
    - 1.2.2 Zwitterionic character,  $pK$  and  $pI$  — 22
    - 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 — 33
  - 1.3 Enzymes — 34
    - 1.3.1 Substrate specificity — 36
    - 1.3.2 Active sites, coenzymes and cofactors — 37
    - 1.3.3 Oxidoreductases — 40
    - 1.3.4 Kinetics of enzymatic reactions — 43
  - 1.4 Antibodies and antigens — 47
    - 1.4.1 Methods to produce antibodies — 47
    - 1.4.2 Antibody structure — 49
    - 1.4.3 Classification of immunoglobulins — 52
    - 1.4.4 Antigen–antibody interaction — 53
    - 1.4.5 Factors influencing antigen–antibody interactions — 55
    - 1.4.6 Quantitative evaluation of antibody properties — 56
    - Further readings — 59
  
- 2 Introduction to bioanalytical assays and biosensors — 61**
  - 2.1 Molecular biorecognition and analytical assays — 61
    - 2.1.1 Example: bioassays for lactate — 63

- 2.2 Principles of kinetic analytical methods — **66**
- 2.3 Introduction to biosensors — **71**
- 2.4 Classification of biosensors according to the receptor — **72**
  - 2.4.1 Catalytic biosensors — **72**
  - 2.4.2 Affinity biosensors — **73**
- 2.5 Classification of biosensors on the basis of the transducer — **74**
- 2.6 Immobilisation of biomolecules — **75**
  - 2.6.1 Physical entrapping within dialysis membranes — **77**
  - 2.6.2 Physical adsorption of biomacromolecules — **78**
    - 2.6.3 Electrostatic adsorption — **79**
    - 2.6.4 Physical entrapment within a polymeric gel — **79**
    - 2.6.5 Cross-linking with polyfunctional reagents — **80**
    - 2.6.6 Non-specific covalent bonds — **81**
    - 2.6.7 Specific covalent bonds — **81**
    - 2.6.8 Biospecific adsorption — **82**
  - 2.7 Immobilisation of biomolecules in practice — **83**
    - 2.7.1 Entrapment within dialysis membranes — **83**
    - 2.7.2 Entrapment within polymeric matrices — **84**
    - 2.7.3 Encapsulation in bilayer lipid membranes — **89**
    - 2.7.4 Cross-linking — **90**
    - 2.7.5 Covalent bonding — **92**
- 2.8 Functionalisation of transducer surfaces — **96**
  - 2.8.1 Self-assembled monolayers — **96**
  - 2.8.2 Silanisation — **98**
  - 2.8.3 Functionalisation of surfaces with diazonium salts — **100**
    - Further readings — **101**
- 3 Enzymatic biosensors — 103**
  - 3.1 Properties of immobilised enzymes — **103**
    - 3.1.1 Effects of the thickness of the enzymatic layer — **105**
  - 3.2 Electrochemical biosensors — **105**
    - 3.2.1 Potentiometric biosensors — **106**
    - 3.2.2 Principles of dynamic electrochemical techniques — **115**
    - 3.2.3 Electron transfer between enzymes and electrodes — **128**
    - 3.2.4 Amperometric biosensors — **136**
  - 3.3 Optical biosensors — **144**
    - 3.3.1 Optical fibres — **145**
    - 3.3.2 Light source and detector — **148**
    - 3.3.3 Optical phenomena employed in biosensors — **149**
    - 3.3.4 Principles of the most common optical sensors — **152**
    - 3.3.5 Enzymatic optodes — **156**
      - Further readings — **161**

- 4 Immunochemical assays and immunosensors — 163**
  - 4.1 Introduction — **163**
  - 4.2 Immunoprecipitation and radioimmunoassay — **164**
  - 4.3 Enzyme immunoassays (EIA and ELISA) — **167**
  - 4.4 Lateral flow immunoassays — **172**
  - 4.4.1 Pregnancy test — **174**
  - 4.4.2 Strip tests for antibodies and antigens related to SARS-CoV-2 — **175**
  - 4.5 Western blotting — **177**
  - 4.5.1 Electrophoretic separation — **177**
  - 4.5.2 Transfer to solid membrane — **178**
  - 4.5.3 Blocking unspecific binding — **179**
  - 4.5.4 Incubation with antibodies — **179**
  - 4.5.5 Detection — **180**
  - 4.6 Enzyme label immunosensors — **181**
  - 4.7 Microbead-based immunoassays — **183**
  - 4.7.1 Multiplexed microbead immunoassays based on flow cytometry — **184**
  - 4.7.2 Magnetic beads electrochemiluminescence assays — **186**
  - 4.7.3 Mechanism of electrochemiluminescence — **188**
  - 4.8 Label-free immunosensors: SPR and QCM — **189**
  - 4.8.1 Surface plasmon resonance immunosensors — **190**
  - 4.8.2 Quartz crystal microbalance: immunosensors based on piezoelectric effect — **193**
    - Further readings — **197**
  
- 5 Analysis of nucleic acids — 199**
  - 5.1 DNA extraction — **199**
  - 5.2 Southern blotting — **199**
  - 5.3 Amplification and detection of specific DNA sequences: PCR — **201**
  - 5.3.1 The polymerase chain reaction — **201**
  - 5.3.2 Analytical PCR: quantitative PCR or real-time PCR — **203**
  - 5.3.3 Signal generation in PCR — **206**
  - 5.3.4 PCR in action: the coronavirus disease (COVID-19) — **210**
  - 5.3.5 Digital PCR — **213**
  - 5.4 DNA microarrays — **216**
  - 5.5 DNA sequencing — **219**
  - 5.5.1 Sanger sequencing — **219**
  - 5.5.2 Illumina sequencing: an example of short-read next-generation sequencing — **220**

5.5.3	Pacific Biosciences and Oxford Nanopore Technologies sequencing: examples of long-read third-generation sequencing — <b>230</b>
	Further readings — <b>234</b>
<b>6</b>	<b>Nanotechnologies for bioanalysis — 237</b>
6.1	Introduction — <b>237</b>
6.2	Classification of nanomaterials — <b>237</b>
6.2.1	Zero-dimensional nanomaterials — <b>237</b>
6.2.2	One-dimensional nanomaterials — <b>238</b>
6.2.3	Two-dimensional nanomaterials — <b>241</b>
6.2.4	Three-dimensional nanomaterials — <b>243</b>
6.3	Synthesis of nanomaterials — <b>244</b>
6.3.1	Approaches based on physical changes — <b>245</b>
6.3.2	Approaches based on chemical transformations — <b>249</b>
6.4	Functionalisation of nanomaterials — <b>260</b>
6.5	Analytical techniques for nanomaterial characterisation — <b>262</b>
6.6	Bioanalytical applications of nanomaterials — <b>262</b>
6.6.1	Optical properties of metal nanoparticles — <b>262</b>
6.6.2	DNA–AuNP sensors — <b>264</b>
6.6.3	Colorimetric detection of proteins with nanoparticles — <b>267</b>
6.6.4	SERS assay for DNA denaturation — <b>268</b>
6.7	Electrochemical nano-biosensors — <b>271</b>
6.7.1	Voltammetry with nanoelectrode arrays — <b>272</b>
6.7.2	Bioelectroanalysis with nanoelectrode arrays — <b>276</b>
6.8	Final remarks — <b>278</b>
	Further readings — <b>278</b>
<b>Index</b>	<b>— 281</b>