Principles and Techniques of Biochemistry and Molecular Biology

Sixth edition

This is a new and expanded edition of the bestselling Principles and Techniques of Practical Biochemistry. It includes discussion of the theoretical principles, practical details and applications of the key experimental techniques that are routinely used in modern biochemistry and molecular biology, including those that have led to the emergence of the new disciplines of genomics, proteomics and bioinformatics.

All chapters have been updated and new sections added to cover the principles of experimental design, the statistical analysis of quantitative analytical data, and the principles and practice of clinical biochemistry. There are new chapters covering cell culture, microscopy and mass spectrometry. To enhance student understanding of each topic, in-text worked examples are included in most chapters. The textbook will be essential reading for all bioscience students and pre-clinical medical students.

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Contents

Preface to the sixth edition xi
List of contributors xiii
List of abbreviations xvi

1 Basic principles 1
K. Wilson (Section 1.7 in association with J. Fyffe)
1.1 Biochemical studies 1
1.2 Units of measurements 3
1.3 Weak electrolytes 10
1.4 Buffer solutions – their nature and preparation 15
1.5 pH and oxygen electrodes 18
1.6 Quantitative biochemical measurements 28
1.7 Principles of clinical biochemical analysis 48
1.8 Safety in the laboratory 68
1.9 Suggestions for further reading 69

2 Cell culture techniques 71
A. R. Baydoun
2.1 Introduction 71
2.2 The cell culture laboratory and equipment 72
2.3 Safety considerations in cell culture 76
2.4 Aseptic techniques and good cell culture practice 77
2.5 Types of animal cell and their characteristics in culture 81
2.6 Bacterial cell culture 93
2.7 Plant cell culture 97
2.8 Potential use of cell cultures 101
2.9 Suggestions for further reading 101
# 3 Centrifugation

K. Ohlendieck

- **3.1** Introduction 103
- **3.2** Basic principles of sedimentation 104
- **3.3** Types, care and safety aspects of centrifuges 109
- **3.4** Preparative centrifugation 117
- **3.5** Analytical centrifugation 124
- **3.6** Suggestions for further reading 130

# 4 Microscopy

S. W. Paddock

- **4.1** Introduction 131
- **4.2** The light microscope 133
- **4.3** Optical sectioning 146
- **4.4** Imaging living cells and tissues 151
- **4.5** The stereomicroscope 153
- **4.6** The electron microscope 154
- **4.7** Imaging and biochemistry 159
- **4.8** Specialised imaging techniques 160
- **4.9** Image archiving, presentation and further information 162
- **4.10** Suggestions for further reading 163

# 5 Molecular biology, bioinformatics and basic techniques

R. Rapley

- **5.1** Introduction 166
- **5.2** Structure of nucleic acids 167
- **5.3** Genes and genome complexity 174
- **5.4** Location and packaging of nucleic acids 178
- **5.5** Functions of nucleic acids 179
- **5.6** The manipulation of nucleic acids: basic tools and techniques 190
- **5.7** Isolation and separation of nucleic acids 191
- **5.8** Molecular biology and bioinformatics 198
- **5.9** Molecular analysis of nucleic acid sequences 200
- **5.10** The polymerase chain reaction 207
- **5.11** Nucleotide sequencing of DNA 216
- **5.12** Suggestions for further reading 224
6 Recombinant DNA and genetic analysis 225
   R. RAPLEY
   6.1 Introduction 225
   6.2 Constructing gene libraries 225
   6.3 Cloning vectors 235
   6.4 Hybridisation and gene probes 253
   6.5 Screening gene libraries 255
   6.6 Applications of gene cloning 259
   6.7 Expression of foreign genes 264
   6.8 Analysing genes and gene expression 270
   6.9 Analysing whole genomes 283
   6.10 Pharmacogenomics 288
   6.11 Molecular biotechnology and its applications 289
   6.12 Suggestions for further reading 291

7 Immunochemical techniques 292
   R. THORPE AND S. THORPE
   7.1 Introduction 292
   7.2 Production of antibodies 298
   7.3 Purification and fragmentation of immunoglobulins 308
   7.4 Immunoprecipitation 315
   7.5 Labelling antibodies 320
   7.6 Immunoblotting 328
   7.7 Immunoassays 331
   7.8 Immunohisto/cytochemistry 341
   7.9 Affinity and avidity 347
   7.10 Immunochemical use of surface plasmon resonance 347
   7.11 Suggestions for further reading 348

8 Protein structure, purification, characterisation and function analysis 349
   J. M. WALKER
   8.1 Ionic properties of amino acids and proteins 349
   8.2 Protein structure 353
   8.3 Protein purification 356
   8.4 Protein structure determination 378
   8.5 Proteomics and protein function 392
   8.6 Suggestions for further reading 404
## Mass spectrometric techniques

A. Aitken

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1 Introduction</td>
<td>405</td>
</tr>
<tr>
<td>9.2 Ionisation</td>
<td>407</td>
</tr>
<tr>
<td>9.3 Mass analysers</td>
<td>413</td>
</tr>
<tr>
<td>9.4 Detectors</td>
<td>430</td>
</tr>
<tr>
<td>9.5 Structural information by tandem mass spectrometry</td>
<td>430</td>
</tr>
<tr>
<td>9.6 Analysing protein complexes</td>
<td>443</td>
</tr>
<tr>
<td>9.7 Computing and database analysis</td>
<td>444</td>
</tr>
<tr>
<td>9.8 Suggestions for further reading</td>
<td>447</td>
</tr>
</tbody>
</table>

## Electrophoretic techniques

J. M. Walker

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1 General principles</td>
<td>449</td>
</tr>
<tr>
<td>10.2 Support media</td>
<td>453</td>
</tr>
<tr>
<td>10.3 Electrophoresis of proteins</td>
<td>457</td>
</tr>
<tr>
<td>10.4 Electrophoresis of nucleic acids</td>
<td>473</td>
</tr>
<tr>
<td>10.5 Capillary electrophoresis</td>
<td>478</td>
</tr>
<tr>
<td>10.6 Microchip electrophoresis</td>
<td>483</td>
</tr>
<tr>
<td>10.7 Suggestions for further reading</td>
<td>484</td>
</tr>
</tbody>
</table>

## Chromatographic techniques

K. Wilson

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.1 Principles of chromatography</td>
<td>485</td>
</tr>
<tr>
<td>11.2 Chromatographic performance parameters</td>
<td>489</td>
</tr>
<tr>
<td>11.3 Liquid chromatography (LPLC and HPLC)</td>
<td>500</td>
</tr>
<tr>
<td>11.4 Adsorption chromatography</td>
<td>515</td>
</tr>
<tr>
<td>11.5 Partition chromatography</td>
<td>518</td>
</tr>
<tr>
<td>11.6 Ion-exchange chromatography</td>
<td>524</td>
</tr>
<tr>
<td>11.7 Molecular exclusion (gel filtration) chromatography</td>
<td>529</td>
</tr>
<tr>
<td>11.8 Affinity chromatography</td>
<td>533</td>
</tr>
<tr>
<td>11.9 Gas–liquid chromatography</td>
<td>541</td>
</tr>
<tr>
<td>11.10 Thin-layer (planar) chromatography</td>
<td>546</td>
</tr>
<tr>
<td>11.11 Selection of a chromatographic system</td>
<td>549</td>
</tr>
<tr>
<td>11.12 Suggestions for further reading</td>
<td>550</td>
</tr>
</tbody>
</table>
12 Spectroscopic techniques: I Atomic and molecular electronic spectroscopy

D. B. GORDON

12.1 Introduction 551
12.2 γ-Ray spectroscopy and γ-ray resonance spectroscopy 554
12.3 X-ray spectroscopy 556
12.4 Ultraviolet and visible light spectroscopy 557
12.5 Spectrofluorimetry 571
12.6 Circular dichroism spectroscopy 579
12.7 Turbidimetry and nephelometry 583
12.8 Luminometry 584
12.9 Atomic spectroscopy 586
12.10 Lasers 591
12.11 Suggestions for further reading 592

13 Spectroscopic techniques: II Vibrational spectroscopy and electron spin orientation in magnetic fields

D. B. GORDON

13.1 Introduction 593
13.2 Infrared and Raman spectroscopy 594
13.3 Electron spin resonance spectroscopy 596
13.4 Nuclear magnetic resonance spectroscopy 603
13.5 Suggestions for further reading 620

14 Radioisotope techniques

R. J. SLATER

14.1 The nature of radioactivity 621
14.2 Detection and measurement of radioactivity 628
14.3 Other practical aspects of counting radioactivity and analysis of data 651
14.4 Inherent advantages and restrictions of radiotracer experiments 655
14.5 Safety aspects 656
14.6 Applications of radioisotopes in the biological sciences 659
14.7 Suggestions for further reading 664
15 Enzymes 665
K. Wilson
15.1 Characteristics and nomenclature 665
15.2 Analytical methods for the study of enzyme reactions 668
15.3 Enzyme steady-state kinetics 679
15.4 Enzyme active sites and catalytic mechanisms 702
15.5 Control of enzyme activity 709
15.6 Suggestions for further reading 718

16 Cell membrane receptors 719
K. Wilson
16.1 Receptors for cell signalling 719
16.2 Quantitative aspects of receptor–ligand binding 720
16.3 Techniques for the study of receptor–ligand binding 729
16.4 Molecular structure of receptors 746
16.5 Mechanisms of signal transduction 751
16.6 Receptor desensitisation and trafficking 763
16.7 Suggestions for further reading 767

Index 769

The colour figure section is between pp. 142 and 143.
Preface to the sixth edition

In the preface to previous editions of our book we set ourselves the task of producing an undergraduate text that covered the theoretical principles and practical details of the experimental techniques that are basic to an understanding of, and that support advances in, biochemistry. In the 30 years that have elapsed since the first edition was launched in 1975, there have been dramatic advances in our understanding of the biochemical processes that characterise living cells. Such advances are typified by the recent completion of the Human Genome Project and the emergence of numerous allied fields of study such as bioinformatics and proteomics. The new generic discipline of molecular biology embraces many of these areas of research and so we have felt it appropriate to broaden the title of the book to include molecular biology, as it clearly falls within our original objective. In the process of taking a decision on the content of this sixth edition of our book, we have also attempted to respond to the extremely constructive and encouraging feedback we have received to the survey we conducted of the many academic departments in UK and overseas universities and other institutions that routinely use our book and recommend it to their students. The outcome is that we have broadened the topics covered within the book by including two new chapters, one on cell culture, the other on microscopy. In addition we have considered it appropriate to include major new sections on the principles and practice of clinical biochemistry, including diagnostic enzymology and the statistical considerations underlying the assessment of the quality of quantitative analytical biochemical data and the role and operation of external quality assessment schemes such as the UK NEQAS. We have also taken the decision to modify our original aim of concentrating on those experimental techniques that undergraduates are most likely to encounter in their practical classes and, instead, to discuss all the techniques that now contribute to the rapid advances in our understanding of cellular function. Two specific examples of this new policy are, first, that we have felt it appropriate to place the emphasis of the chapter on mass spectrometry on its indispensable role in protein chemistry and proteomics and, secondly, within the chapter on membrane receptors to discuss in some detail the analytical techniques, such as plasmon-coupled resonance spectroscopy, that are central to modern approaches to the understanding of receptor function and cell signalling. Continuing chapters have been updated to cover recent developments and most
include integrated text examples to support the principles discussed in the main text.

We welcome five new contributors: Alastair Aitken (mass spectrometry), Anwar Baydoun (cell culture), John Fyffe (clinical biochemistry), Kay Ohlendieck (centrifugation) and Stephen Paddock (microscopy). We would like to express our sincere thanks to all our contributors for their cooperation in producing this new edition. Sadly we must record the untimely death of Derek Gordon, the author of two chapters on spectroscopic techniques. Derek was an enthusiastic, dedicated and respected teacher of biochemistry, keen to emphasise to his students the chemical principles underlying many analytical techniques central to practical biochemistry.

We continue to welcome constructive comments from all students who use our book as part of their studies and academics who adopt the book to complement their teaching. Finally, we wish to express our gratitude to the authors and publishers who have granted us permission to reproduce their copyright figures and our thanks to Katrina Halliday and her colleagues at Cambridge University Press who have been so supportive in the production of this new edition.

John Walker and Keith Wilson
November 2004

(Continued from p. iv)

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Abbreviations

The following abbreviations have been used throughout this book without definition.

- AMP  adenosine 5’-monophosphate
- ADP  adenosine 5’-diphosphate
- ATP  adenosine 5’-triphosphate
- bp   base-pairs
- cAMP cyclic AMP
- CHAPS 3-(3-chloroamidopropyl)dimethylamino-1-propanesulphonic acid
- c.p.m. counts per minute
- CTP  cytidine triphosphate
- DDT 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane
- DMSO dimethylsulphoxide
- DNA  deoxyribonucleic acid
- e−  electron
- EDTA ethylenediaminetetra-acetate
- FAD  flavin adenine dinucleotide (oxidised)
- FADH₂ flavin adenine dinucleotide (reduced)
- FMN  flavin mononucleotide (oxidised)
- FMNH₂ flavin mononucleotide (reduced)
- GTP  guanosine triphosphate
- HAT  hypoxanthine, aminopterin, thymidine medium
- Hepes 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
- kb  kilobase-pairs
- M₀  relative molecular mass
- min  minute
- NAD⁺ nicotinamide adenine dinucleotide (oxidised)
- NADH nicotinamide adenine dinucleotide (reduced)
- NADP⁺ nicotinamide adenine dinucleotide phosphate (oxidised)
- NADPH nicotinamide adenine dinucleotide phosphate (reduced)
- Pipes 1,4-piperazinebis(ethanesulphonic acid)
- Pi  inorganic phosphate
- p.p.m. parts per million
Abbreviations

p.p.b. parts per billion
PPi inorganic pyrophosphate
RNA ribonucleic acid
r.p.m. revolutions per minute
SDS sodium dodecyl sulphate
Tris 2-amino-2-hydroxymethylpropane-1,3-diol