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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Principles and Techniques of Biochemistry and Molecular Biology

Edited by Keith Wilson and John Walker

Sixth edition



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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 NEOAS. 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

Preface to the sixth edition

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

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Abbreviations

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

ADP adenosine 5'-diphosphate ATP adenosine 5'-triphosphate	
1 I	
has been using	
bp base-pairs	
cAMP cyclic AMP	
CHAPS 3-[(3-chloroamidopropyl)dimethylamino]-1-propan	esulphonic acid
c.p.m. counts per minute	
CTP cytidine triphosphate	
DDT 2,2-bis-(<i>p</i> -chlorophenyl)-1,1,1-trichloroethane	
DMSO dimethylsulphoxide	
DNA deoxyribonucleic acid	
e ⁻ electron	
EDTA ethylenediaminotetra-acetate	
FAD flavin adenine dinucleotide (oxidised)	
FADH ₂ flavin adenine dinucleotide (reduced)	
FMN flavin mononucleotide (oxidised)	
FMNH ₂ flavin mononucleotide (reduced)	
GTP guanosine triphosphate	
HAT hypoxanthine, aminopterin, thymidine medium	
Hepes 4(2-hydroxyethyl)-1-piperazine-ethanesulphonic ac	cid
kb kilobase-pairs	
<i>M</i> _r relative molecular mass	
min minute	
NAD ⁺ nicotinamide adenine dinucleotide (oxidised)	
NADH nicotinamide adenine dinucleotide (reduced)	
NADP ⁺ nicotinamide adenine dinucleotide phosphate (oxid	lised)
NADPH nicotinamide adenine dinucleotide phosphate (redu	iced)
Pipes 1,4-piperazinebis(ethanesulphonic acid)	
r · · · · · · · · · · · · · · · · · · ·	
P_i inorganic phosphate	

xvii Abbreviations

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