

Principles and Techniques of  
**Biochemistry** and  
**Molecular Biology**

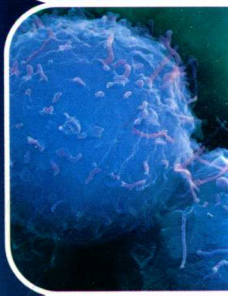
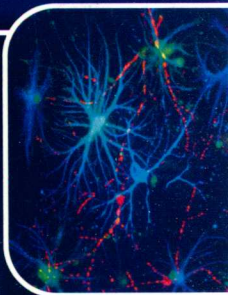
SEVENTH EDITION

Edited by

**Keith Wilson**

and

**John Walker**





Principles and Techniques of

# Biochemistry and Molecular Biology

Seventh edition

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KEITH WILSON AND JOHN WALKER

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# Principles and Techniques of Biochemistry and Molecular Biology

Seventh edition

**EDITED BY KEITH WILSON AND JOHN WALKER**

This new edition of the bestselling textbook integrates the theoretical principles and experimental techniques common to all undergraduate courses in the bio- and medical sciences. Three of the 16 chapters have new authors and have been totally rewritten. The others have been updated and extended to reflect developments in their field exemplified by a new section on stem cells. Two new chapters have been added. One on clinical biochemistry discusses the principles underlying the diagnosis and management of common biochemical disorders. The second one on drug discovery and development illustrates how the principles and techniques covered in the book are fundamental to the design and development of new drugs. In-text worked examples are again used to enhance student understanding of each topic and case studies are selectively used to illustrate important examples. Experimental design, quality assurance and the statistical analysis of quantitative data are emphasised throughout the book.

- Motivates students by including cutting-edge topics and techniques, such as drug discovery, as well as the methods they will encounter in their own lab classes
- Promotes problem solving by setting students a challenge and then guiding them through the solution
- Integrates theory and practise to ensure students understand why and how each technique is used.

**KEITH WILSON** is Professor Emeritus of Pharmacological Biochemistry and former Head of the Department of Biosciences, Dean of the Faculty of Natural Sciences, and Director of Research at the University of Hertfordshire.

**JOHN WALKER** is Professor Emeritus and former Head of the School of Life Sciences at the University of Hertfordshire.

## Cover illustration

**Main image** Electrophoresis gel showing recombinant protein. Photographer: J.C. Revy. Courtesy of Science Photo Library.

**Top inset** Transcription factor and DNA molecule. Courtesy of: Laguna Design/Science Photo Library.

**Second inset** Microtubes, pipettor (pipette) tip & DNA sequence. Courtesy of Tek Image/Science Photo Library.

**Third inset** Stem cell culture, light micrograph. Photographer: Philippe Plailly. Courtesy of Science Photo Library.

**Fourth inset** Embryonic stem cells. Courtesy of Science Photo Library.

**Bottom inset** Herceptin breast cancer drug, molecular model. Photographer: Tim Evans. Courtesy of Science Photo Library.

## PREFACE TO THE SEVENTH EDITION

In designing the content of this latest edition we continued our previous policy of placing emphasis on the recommendations we have received from colleagues and academics outside our university. Above all, we have attempted to respond to the invaluable feedback from student users of our book both in the UK and abroad. In this seventh edition we have retained all 16 chapters from the previous edition. All have been appropriately updated to reflect recent developments in their fields, as exemplified by the inclusion of a section on stem cells in the cell culture chapter. Three of these chapters have new authors and have been completely rewritten. Robert Burns, Scottish Agricultural Science Agency, Edinburgh has written the chapter on immunochemical techniques, and Andreas Hofmann, Eskitis Institute of Molecular Therapies, Griffith University, Brisbane, Australia has written the two chapters on spectroscopic techniques. We are delighted to welcome both authors to our team of contributors.

In addition to these changes of authors, two new chapters have been added to the book. Our decision taken for the sixth edition to include a section on the biochemical principles underlying clinical biochemistry has been well received and so we have extended our coverage of the subject and have devoted a whole chapter (16) to this subject. Written in collaboration with Dr John Fyffe, Consultant Biochemist, Royal Hospital for Sick Children, Yorkhill, Glasgow, new topics that are discussed in the chapter include the diagnosis and management of kidney disease, diabetes, endocrine disorders including thyroid dysfunction, conditions of the hypothalamus-pituitary-adrenal axis such as pregnancy, and pathologies of plasma proteins such as myeloma. Case studies are included to illustrate how the principles discussed apply to the diagnosis and treatment of individual patients with the conditions.

Our second major innovation for this new edition is the introduction of a new chapter on drug discovery and development. The strategic approaches to the discovery of new drugs has been revolutionised by developments in molecular biology. Pharmaceutical companies now rely on many of the principles and experimental techniques discussed in the chapters throughout the book to identify potential drug targets, screen chemical libraries and to evaluate the safety and efficacy of selected candidate drugs. The new chapter illustrates the principles of target selection by reference to current drugs used in the treatment of atherosclerosis and HIV/AIDS, emphasises the strategic decisions to be taken during the various stages of drug discovery and

development and discusses the issues involved in clinical trials and the registration of new drugs.

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.

KEITH WILSON AND JOHN WALKER

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## ABBREVIATIONS

The following abbreviations have been used throughout this book.

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-( <i>p</i> -chlorophenyl)-1,1,1-trichloroethane
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
e <sup>-</sup>	electron
EDTA	ethylenediaminetetra-acetate
ELISA	enzyme-linked immunosorbent assay
FAD	flavin adenine dinucleotide (oxidised)
FADH <sub>2</sub>	flavin adenine dinucleotide (reduced)
FMN	flavin mononucleotide (oxidised)
FMNH <sub>2</sub>	flavin mononucleotide (reduced)
GC	gas chromatography
GTP	guanosine triphosphate
HAT	hypoxanthine, aminopterin, thymidine medium
Hepes	4(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
HPLC	high-performance liquid chromatography
kb	kilobase-pairs
M <sub>r</sub>	relative molecular mass
min	minute
NAD <sup>+</sup>	nicotinamide adenine dinucleotide (oxidised)
NADH	nicotinamide adenine dinucleotide (reduced)
NADP <sup>+</sup>	nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
Pipes	1,4-piperazinebis(ethanesulphonic acid)

- P<sub>i</sub> inorganic phosphate
- p.p.m. parts per million
- p.p.b. parts per billion
- PP<sub>i</sub> inorganic pyrophosphate
- RNA ribonucleic acid
- r.p.m. revolutions per minute
- SDS sodium dodecyl sulphate
- Tris 2-amino-2-hydroxymethylpropane-1,3-diol

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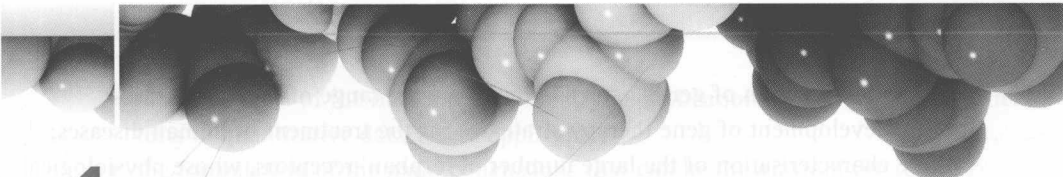
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# 1 Basic principles

K. WILSON

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- 1.2 Units of measurement
- 1.3 Weak electrolytes
- 1.4 Quantitative biochemical measurements
- 1.5 Safety in the laboratory
- 1.6 Suggestions for further reading

## 1.1 BIOCHEMICAL AND MOLECULAR BIOLOGY STUDIES

### 1.1.1 Aims of laboratory investigations

Biochemistry involves the study of the chemical processes that occur in living organisms with the ultimate aim of understanding the nature of life in molecular terms. Biochemical studies rely on the availability of appropriate analytical techniques and on the application of these techniques to the advancement of knowledge of the nature of, and relationships between, biological molecules, especially proteins and nucleic acids, and cellular function. In recent years huge advances have been made in our understanding of gene structure and expression and in the application of techniques such as mass spectrometry to the study of protein structure and function. The Human Genome Project in particular has been the stimulus for major developments in our understanding of many human diseases especially cancer and for the identification of strategies that might be used to combat these diseases. The discipline of molecular biology overlaps with that of biochemistry and in many respects the aims of the two disciplines complement each other. Molecular biology is focussed on the molecular understanding of the processes of replication, transcription and translation of genetic material whereas biochemistry exploits the techniques and findings of molecular biology to advance our understanding of such cellular processes as cell signalling and apoptosis. The result is that the two disciplines now have the opportunity to address issues such as:

- the structure and function of the total protein component of the cell (*proteomics*) and of all the small molecules in the cell (*metabolomics*);
- the mechanisms involved in the control of gene expression;