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.

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 and Head of the School of Life Sciences and also Head of the Science Training Centre at the University of Hertfordshire.

Principles and Techniques of Biochemistry and Molecular Biology

Edited by Keith Wilson and John Walker

Sixth edition

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo, Delhi

Cambridge University Press
32 Avenue of the Americas, New York, NY 10013-2473, USA

www.cambridge.org
Information on this title: www.cambridge.org/9780521535816

First published by Edward Arnold 1975 as *A biologist's guide to principles and techniques of practical biochemistry*
Second edition 1981; Third edition 1986
Third edition first published by Cambridge University Press 1992; Reprinted 1993
Fourth edition published by Cambridge University Press 1994 as *Principles and techniques of practical biochemistry*; Reprinted 1995, 1997; Fifth edition 2000
Sixth edition first published by Cambridge University Press 2005
as *Principles and techniques of biochemistry and molecular biology*
7th printing 2009

Printed in the United States of America

A catalog record for this publication is available from the British Library.

Library of Congress Cataloging in Publication Data

Principles and techniques of biochemistry and molecular biology/edited by Keith Wilson, John Walker. – 6th ed.
p. cm.
New & expanded ed. of: Principles and techniques of practical biochemistry. 5th ed. c2000.
Includes bibliographical references and index.
ISBN 0-521-82889-9 (alk. paper) – ISBN 0-521-53581-6 (pbk.: alk. paper)
1. Biochemistry – Methodology. 2. Molecular biology – Methodology. I. Wilson, Keith, 1936– . II. Walker, John M., 1948– . III. Principles and techniques of practical biochemistry.
QP519.7.P75 2005
572–dc22 2004054541
CIP

ISBN 978-0-521-82889-5 hardback
ISBN 978-0-521-53581-6 paperback

(Continued on p. xii)

Contents

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*)

Contributors

Professor A. Aitken
Division of Biomedical & Clinical Laboratory Sciences
University of Edinburgh
George Square
Edinburgh EH8 9XD
Scotland, UK

Dr A. R. Baydoun
School of Life Sciences
University of Hertfordshire
College Lane
Hatfield
Herts AL10 9AB, UK

Dr J. Fyffe
Consultant Clinical Biochemist
Department of Clinical Biochemistry
Royal Hospital for Sick Children
Yorkhill
Glasgow G3 8SF
Scotland, UK

Professor D. B. Gordon (Deceased)
Formerly
Department of Biological Sciences
Metropolitan University of Manchester
Chester Street
Manchester M15 6BH, UK

Professor K. Ohlendieck
Department of Biology
National University of Ireland
Maynooth
Co. Kildare
Ireland

Dr S. W. Paddock
Howard Hughes Medical Institute
Department of Molecular Biology
University of Wisconsin
1525 Linden Drive
Madison, WI 53706
USA

Dr R. Rapley
School of Life Sciences
University of Hertfordshire
College Lane
Hatfield
Herts AL10 9AB, UK

Professor R. J. Slater
School of Life Sciences
University of Hertfordshire
College Lane
Hatfield
Herts AL10 9AB, UK

Professor R. Thorpe
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Potters Bar
Herts EN6 3QG, UK

Dr S. Thorpe
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Potters Bar
Herts EN6 3QG, UK

Professor J. M. Walker
School of Life Sciences
University of Hertfordshire
College Lane
Hatfield
Herts AL10 9AB, UK

Professor K. Wilson
Emeritus Professor of Pharmacological Biochemistry
School of Life Sciences
University of Hertfordshire
College Lane
Hatfield
Herts AL10 9AB, UK

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	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 acid
kb	kilobase-pairs
M_r	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)
P_i	inorganic phosphate
p.p.m.	parts per million

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