

Wilson and Walker's
Principles and Techniques of
BIOCHEMISTRY AND MOLECULAR BIOLOGY

Bringing this best-selling textbook right up-to-date, the new edition uniquely integrates the theories and methods that drive the fields of biology, biotechnology and medicine, comprehensively covering both the techniques students will encounter in lab classes and those that underpin current key advances and discoveries. The contents have been updated to include both traditional and cutting-edge techniques most commonly used in current life science research. Emphasis is placed on understanding the theory behind the techniques, as well as analysis of the resulting data. New chapters cover proteomics, genomics, metabolomics and bioinformatics, as well as data analysis and visualisation. Using accessible language to describe concepts and methods, and with a wealth of new in-text worked examples to challenge students' understanding, this textbook provides an essential guide to the key techniques used in current bioscience research.

ASSOCIATE PROFESSOR ANDREAS HOFMANN is the Structural Chemistry Program Leader at Griffith University's Eskitis Institute and an Honorary Senior Research Fellow at the Faculty of Veterinary and Agricultural Sciences at The University of Melbourne. He is also Fellow of the Higher Education Academy (UK). Professor Hofmann's research lab focusses on the structure and function of proteins in infectious and neurodegenerative diseases, and also develops computational tools. He is the author of *Methods of Molecular Analysis in the Life Sciences* (2014) and *Essential Physical Chemistry* (2018).

DR SAMUEL CLOKIE is the Principal Clinical Bioinformatician at the Birmingham Women's and Children's Hospital, UK, where he leads the implementation of bioinformatic techniques to decipher next-generation sequencing data in clinical diagnostics. He is also Honorary Senior Research Fellow at the Institute of Cancer and Genomic Sciences at The University of Birmingham and before that was a research fellow at the National Institutes of Health in Bethesda, USA.

Cambridge University Press & Assessment

978-1-316-61476-1 — Wilson and Walker's Principles and Techniques of Biochemistry and Molecular Biology

Edited by Andreas Hofmann , Samuel Clokie

Frontmatter

[More Information](#)

Wilson and Walker's
Principles and Techniques of
**Biochemistry and
Molecular Biology**

Edited by

ANDREAS HOFMANN

Griffith University, Queensland

SAMUEL CLOKIE

Birmingham Women's and Children's Hospital



CAMBRIDGE
UNIVERSITY PRESS

Cambridge University Press & Assessment

978-1-316-61476-1 — Wilson and Walker's Principles and Techniques of Biochemistry and Molecular Biology

Edited by Andreas Hofmann, Samuel Clokie

Frontmatter

[More Information](#)



CAMBRIDGE
UNIVERSITY PRESS

Shaftesbury Road, Cambridge CB2 8EA, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi – 110025, India

103 Penang Road, #05–06/07, Visioncrest Commercial, Singapore 238467

Cambridge University Press is part of Cambridge University Press & Assessment,
a department of the University of Cambridge.

We share the University's mission to contribute to society through the pursuit of
education, learning and research at the highest international levels of excellence.

www.cambridge.org

Information on this title: www.cambridge.org/9781316614761

DOI: 10.1017/9781316677056

© Andreas Hofmann and Samuel Clokie 2018

This publication is in copyright. Subject to statutory exception and to the provisions
of relevant collective licensing agreements, no reproduction of any part may take
place without the written permission of Cambridge University Press & Assessment.

First published 2018

A catalogue record for this publication is available from the British Library

Library of Congress Cataloging-in-Publication data

Names: Wilson, Keith, 1936– editor. | Walker, John M., 1948– editor. | Hofmann, Andreas, editor.
| Clokie, Samuel, editor.

Title: Wilson and Walker's principles and techniques of biochemistry and molecular biology /
edited by Andreas Hofmann, Griffith University, Queensland, Samuel Clokie, Birmingham
Women's and Children's Hospital.

Other titles: Principles and techniques of biochemistry and molecular biology.

Description: [2018 edition]. | Cambridge : Cambridge University Press, 2018. | Includes
bibliographical
references and index.

Identifiers: LCCN 2017033452 | ISBN 9781107162273 (alk. paper)

Subjects: LCSH: Biochemistry – Textbooks. | Molecular biology – Textbooks.

Classification: LCC QP519.7 .P75 2018 | DDC 572.8–dc23

LC record available at <https://lcn.loc.gov/2017033452>

ISBN 978-1-107-16227-3 Hardback

ISBN 978-1-316-61476-1 Paperback

Additional resources for this publication at www.cambridge.org/hofmann

Cambridge University Press & Assessment has no responsibility for the persistence
or accuracy of URLs for external or third-party internet websites referred to in this
publication and does not guarantee that any content on such websites is, or will
remain, accurate or appropriate.

CONTENTS

<i>Foreword to the Eighth Edition</i>	page xiii
<i>Preface</i>	xv
<i>List of Contributors</i>	xvii
0 Tables and Resources	xxi
1 Biochemical and Molecular Biological Methods in Life Sciences Studies	1
<i>Samuel Clokie and Andreas Hofmann</i>	
1.1 From Biochemistry and Molecular Biology to the Life Sciences	1
1.2 The Education of Life Scientists	2
1.3 Aims of Life Science Studies	3
1.4 Personal Qualities and Scientific Conduct	5
1.5 Suggestions for Further Reading	7
2 Basic Principles	8
<i>Parisa Amani and Andreas Hofmann</i>	
2.1 Biologically Important Molecules	8
2.2 The Importance of Structure	9
2.3 Parameters of Biological Samples	18
2.4 Measurement of the pH: The pH Electrode	24
2.5 Buffers	26
2.6 Ionisation Properties of Amino Acids	30
2.7 Quantitative Biochemical Measurements	32
2.8 Experiment Design and Research Conduct	33
2.9 Suggestions for Further Reading	38
3 Cell Culture Techniques	40
<i>Anwar R. Baydoun</i>	
3.1 Introduction	40
3.2 The Cell Culture Laboratory and Equipment	41
3.3 Safety Considerations in Cell Culture	46
3.4 Aseptic Techniques and Good Cell Culture Practice	46
3.5 Types of Animal Cells, Characteristics and Maintenance in Culture	51
3.6 Stem Cell Culture	61
3.7 Bacterial Cell Culture	67
3.8 Potential Use of Cell Cultures	70

3.9	Acknowledgements	71
3.10	Suggestions for Further Reading	71
4	Recombinant DNA Techniques and Molecular Cloning	73
	<i>Ralph Rapley</i>	
4.1	Introduction	73
4.2	Structure of Nucleic Acids	74
4.3	Genes and Genome Complexity	80
4.4	Location and Packaging of Nucleic Acids	83
4.5	Functions of Nucleic Acids	85
4.6	The Manipulation of Nucleic Acids: Basic Tools and Techniques	96
4.7	Isolation and Separation of Nucleic Acids	98
4.8	Automated Analysis of Nucleic Acid Fragments	103
4.9	Molecular Analysis of Nucleic Acid Sequences	104
4.10	The Polymerase Chain Reaction (PCR)	110
4.11	Constructing Gene Libraries	118
4.12	Cloning Vectors	128
4.13	Hybridisation and Gene Probes	145
4.14	Screening Gene Libraries	146
4.15	Applications of Gene Cloning	148
4.16	Expression of Foreign Genes	153
4.17	Analysing Genes and Gene Expression	157
4.18	Analysing Genetic Mutations and Polymorphisms	167
4.19	Molecular Biotechnology and Applications	173
4.20	Pharmacogenomics	175
4.21	Suggestions for Further Reading	176
5	Preparative Protein Biochemistry	179
	<i>Samuel Clokie</i>	
5.1	Introduction	179
5.2	Determination of Protein Concentrations	180
5.3	Engineering Proteins for Purification	184
5.4	Producing Recombinant Protein	188
5.5	Cell-Disruption Methods	191
5.6	Preliminary Purification Steps	195
5.7	Principles of Liquid Chromatography	196
5.8	Chromatographic Methods for Protein Purification	206
5.9	Other Methods of Protein Purification	210
5.10	Monitoring Protein Purification	213
5.11	Storage	217
5.12	Suggestions for Further Reading	218
6	Electrophoretic Techniques	219
	<i>Ralph Rapley</i>	
6.1	General Principles	219

6.2	Support Media and Buffers	223
6.3	Electrophoresis of Proteins	226
6.4	Electrophoresis of Nucleic Acids	240
6.5	Capillary Electrophoresis	246
6.6	Microchip Electrophoresis	250
6.7	Suggestions for Further Reading	252
7	Immunochemical Techniques	253
	<i>Katja Fischer</i>	
7.1	Introduction	253
7.2	Antibody Preparation	261
7.3	Immunoassay Formats	271
7.4	Immuno Microscopy	277
7.5	Lateral Flow Devices	278
7.6	Epitope Mapping	279
7.7	Immunoblotting	279
7.8	Fluorescence-Activated Cell Sorting (FACS)	280
7.9	Cell and Tissue Staining Techniques	281
7.10	Immunocapture Polymerase Chain Reaction (PCR)	281
7.11	Immunoaffinity Chromatography	282
7.12	Antibody-Based Biosensors	282
7.13	Luminex® Technology	283
7.14	Therapeutic Antibodies	283
7.15	Suggestions for Further Reading	285
8	Flow Cytometry	287
	<i>John Grainger and Joanne Konkel</i>	
8.1	Introduction	287
8.2	Instrumentation	289
8.3	Fluorescence-Activated Cell Sorting (FACS)	294
8.4	Fluorescence Labels	294
8.5	Practical Considerations	298
8.6	Applications	305
8.7	Suggestions for Further Reading	312
9	Radioisotope Techniques	313
	<i>Robert J. Slater</i>	
9.1	Why Use a Radioisotope?	313
9.2	The Nature of Radioactivity	314
9.3	Detection and Measurement of Radioactivity	322
9.4	Other Practical Aspects of Counting Radioactivity and Analysis of Data	334
9.5	Safety Aspects	340
9.6	Suggestions for Further Reading	344

10	Principles of Clinical Biochemistry	346
	<i>Gill Rumsby</i>	
10.1	Principles of Clinical Biochemical Analysis	346
10.2	Clinical Measurements and Quality Control	350
10.3	Examples of Biochemical Aids to Clinical Diagnosis	361
10.4	Suggestions for Further Reading	380
11	Microscopy	381
	<i>Stephen W. Paddock</i>	
11.1	Introduction	381
11.2	The Light Microscope	384
11.3	Optical Sectioning	396
11.4	Imaging Live Cells and Tissues	403
11.5	Measuring Cellular Dynamics	407
11.6	The Electron Microscope	411
11.7	Image Management	417
11.8	Suggestions for Further Reading	420
12	Centrifugation and Ultracentrifugation	424
	<i>Kay Ohlendieck and Stephen E. Harding</i>	
12.1	Introduction	424
12.2	Basic Principles of Sedimentation	425
12.3	Types, Care and Safety Aspects of Centrifuges	430
12.4	Preparative Centrifugation	438
12.5	Analytical Ultracentrifugation	446
12.6	Suggestions for Further Reading	452
13	Spectroscopic Techniques	454
	<i>Anne Simon and Andreas Hofmann</i>	
13.1	Introduction	454
13.2	Ultraviolet and Visible Light Spectroscopy	460
13.3	Circular Dichroism Spectroscopy	471
13.4	Infrared and Raman Spectroscopy	476
13.5	Fluorescence Spectroscopy	479
13.6	Luminometry	492
13.7	Atomic Spectroscopy	494
13.8	Rapid Mixing Techniques for Kinetics	497
13.9	Suggestions for Further Reading	498
14	Basic Techniques Probing Molecular Structure and Interactions	500
	<i>Anne Simon and Joanne Macdonald</i>	
14.1	Introduction	500
14.2	Isothermal Titration Calorimetry	501
14.3	Techniques to Investigate the Three-Dimensional Structure	502
14.4	Switch Techniques	521

14.5	Solid-Phase Binding Techniques with Washing Steps	524
14.6	Solid-Phase Binding Techniques Combined with Flow	526
14.7	Suggestions for Further Reading	532
15	Mass Spectrometric Techniques	535
	<i>Sonja Hess and James I. MacRae</i>	
15.1	Introduction	535
15.2	Ionisation	537
15.3	Mass Analysers	549
15.4	Detectors	556
15.5	Other Components	557
15.6	Suggestions for Further Reading	557
16	Fundamentals of Bioinformatics	559
	<i>Cinzia Cantacessi and Anna V. Protasio</i>	
16.1	Introduction	559
16.2	Biological Databases	560
16.3	Biological Data Formats	563
16.4	Sequence Alignment and Tools	569
16.5	Annotation of Predicted Peptides	576
16.6	Principles of Phylogenetics	584
16.7	Suggestions for Further Reading	596
17	Fundamentals of Chemoinformatics	599
	<i>Paul Taylor</i>	
17.1	Introduction	599
17.2	Computer Representations of Chemical Structure	602
17.3	Calculation of Compound Properties	614
17.4	Molecular Mechanics	616
17.5	Databases	626
17.6	Suggestions for Further Reading	627
18	The Python Programming Language	631
	<i>Tim J. Stevens</i>	
18.1	Introduction	631
18.2	Getting Started	632
18.3	Examples	661
18.4	Suggestions for Further Reading	676
19	Data Analysis	677
	<i>Jean-Baptiste Cazier</i>	
19.1	Introduction	677
19.2	Data Representations	679
19.3	Data Analysis	702
19.4	Conclusion	730
19.5	Suggestions for Further Reading	730

20	Fundamentals of Genome Sequencing and Annotation	732
	<i>Pasi K. Korhonen and Robin B. Gasser</i>	
20.1	Introduction	732
20.2	Genomic Sequencing	733
20.3	Assembly of Genomic Information	737
20.4	Prediction of Genes	742
20.5	Functional Annotation	745
20.6	Post-Genomic Analyses	752
20.7	Factors Affecting the Sequencing, Annotation and Assembly of Eukaryotic Genomes, and Subsequent Analyses	754
20.8	Concluding Remarks	755
20.9	Suggestions For Further Reading	755
21	Fundamentals of Proteomics	758
	<i>Sonja Hess and Michael Weiss</i>	
21.1	Introduction: From Edman Sequencing to Mass Spectrometry	758
21.2	Digestion	759
21.3	Tandem Mass Spectrometry	759
21.4	The Importance of Isotopes for Finding the Charge State of a Peptide	765
21.5	Sample Preparation and Handling	767
21.6	Post-Translational Modification of Proteins	769
21.7	Analysing Protein Complexes	772
21.8	Computing and Database Analysis	776
21.9	Suggestions for Further Reading	777
22	Fundamentals of Metabolomics	779
	<i>James I. MacRae</i>	
22.1	Introduction: What is Metabolomics?	779
22.2	Sample Preparation	780
22.3	Data Acquisition	785
22.4	Untargeted and Targeted Metabolomics	787
22.5	Chemometrics and Data Analysis	797
22.6	Further Metabolomics Techniques and Terminology	802
22.7	Suggestions for Further Reading	807
23	Enzymes and Receptors	809
	<i>Megan Cross and Andreas Hofmann</i>	
23.1	Definition and Classification of Enzymes	809
23.2	Enzyme Kinetics	813
23.3	Analytical Methods to Investigate Enzyme Kinetics	831
23.4	Molecular Mechanisms of Enzymes	838
23.5	Regulation of Enzyme Activity	840
23.6	Receptors	844
23.7	Characterisation of Receptor–Ligand Binding	845
23.8	Suggestions for Further Reading	862

24 Drug Discovery and Development	864
<i>David Camp</i>	
24.1 Introduction	864
24.2 Molecular Libraries and Drug-Discovery Strategies	865
24.3 Assembling a Molecular Library	881
24.4 Compound Management	887
24.5 Screening Strategies Used in Hit Discovery	889
24.6 Active-to-Hit Phase	895
24.7 Hit-to-Lead Phase	895
24.8 ADMET	896
24.9 Lead Optimisation	903
24.10 Suggestions for Further Reading	903
<i>Index</i>	906

FOREWORD TO THE EIGHTH EDITION

When the first edition of our book was published in 1975, biochemistry was emerging as a new discipline that had the potential to unify the previously divergent -ologies that constituted the biological sciences. It placed emphasis on the understanding of the structure, function and expression of individual proteins and their related genes, and relied on the application of analytical techniques such as electrophoresis, chromatography and various forms of spectrometry. In the ensuing 40 years the completion of the Human Genome Project has confirmed the central role of DNA in all of the activities of individual cells and the emergence of molecular biology as the means of understanding complex biological processes. This in turn has led to the new disciplines of bioinformatics, chemoinformatics, proteomics and metabolomics. The succeeding six editions of our book have attempted to reflect this evolution of biochemistry as a unifying discipline. All editions have placed emphasis on the experimental techniques that undergraduates can expect to encounter during the course of their university studies and to this end we have been grateful for the excellent feedback we have received from the users of the book.

The point has now been reached where we believed that it was appropriate for us to hand over the direction and academic balance of future editions to a new editorial team and to this end we are delighted that Andreas Hofmann and Samuel Clokie have agreed to take on the role. We wish them well and look forward to the continuing success of the book.

KEITH WILSON AND JOHN WALKER

PREFACE

It has been a tremendous honour being asked by Keith Wilson, John Walker and Cambridge University Press to take on the role of editorship for this eighth edition of *Principles and Techniques*. In designing the content, we extend the long and successful tradition of this text to introduce relevant methodologies in the biochemical sciences by uniquely integrating the theories and practices that drive the fields of biology, biotechnology and medicine. Methodologies have improved tremendously over the past ten years and new strategies and protocols that once were just applied by a few pioneering laboratories are now applied routinely, accompanied by ever more fluent boundaries between core disciplines – leading to what is now called the life sciences.

In this eighth edition, all core methodologies covered in the previous edition have been kept and appropriately updated and consolidated. New chapters have been added to address the requirements of today's students and scientists, who operate in a much broader area than did the typical biochemists one or two decades ago. The contents of this text are thus structured into six areas, all of which play pivotal roles in current research: basic principles, biochemistry and molecular biology, biophysical methods, information technology, -omics methods and chemical biology. Of course, due to space restraints that help to keep this text accessible, the addition of new material required us to consolidate and carefully select content to be kept from the previous edition. These decisions have been guided by the over-arching theme of this text, namely to present principles and techniques. Importantly, our experience as teachers has always been that many undergraduates are challenged by quantitative calculations based on these principles, and hence we have continued the tradition of this text by including relevant mathematical and numerical tools as well as examples.

Indeed, new chapters on data processing, visualisation and Python have example applications that can be accessed from the CUP website to aid understanding.

Sadly, two authors of previous editions have passed away: John Fyffe died shortly after the seventh edition was launched and Alastair Aitken passed away in 2014. Also, Keith Wilson, John Walker and Robert Burns decided to retire, and we thus invited several new authors to contribute to this eighth edition.

We are grateful to the authors and publishers who have granted us permission to reproduce and adapt their figures. We further wish to express our gratitude to Sonja Biberacher, Madeleine Dallaston, Emma Klepzig, Hannah Leeson and Megan Cross who have helped tremendously with preparing figures and photographs. The manuscript and figures for this book have been compiled entirely with open-source and academic software under Linux, and we would like to acknowledge the efforts of software developers and programmers who make their products freely available.

Finally, our sincere thanks also extend to Katrina Halliday and her team at Cambridge University Press whose invaluable support has made it possible to produce this new edition.

We welcome constructive comments from all students who use this book within their studies, and from teachers and academics who adopt the book to complement their courses.

ANDREAS HOFMANN AND SAMUEL CLOKIE

CONTRIBUTORS

PARISA AMANI

Structural Chemistry Program, Eskitis Institute for Drug Discovery,
Griffith University,
Nathan, Queensland, Australia

ANWAR R. BAYDOUN

School of Life Sciences, University of Hertfordshire,
Hatfield, Hertfordshire, UK

DAVID CAMP

School of Environment, Griffith University,
Nathan, Queensland, Australia

CINZIA CANTACESSI

Cambridge Veterinary School,
University of Cambridge,
Cambridge, UK

JEAN-BAPTISTE CAZIER

Centre for Computational Biology,
University of Birmingham,
Edgbaston, Birmingham, UK

SAMUEL CLOKIE

Bioinformatics Department,
West Midlands Regional Genetics Laboratory
Birmingham Women's and Children's NHS Foundation Trust,
Birmingham, UK

MEGAN CROSS

Structural Chemistry Program, Eskitis Institute for Drug Discovery,
Griffith University,
Nathan, Queensland, Australia

KATJA FISCHER

Scabies Laboratory, QIMR Berghofer,
Locked Bag 2000, Royal Brisbane Hospital,
Herston, Queensland, Australia

ROBIN B. GASSER

Faculty of Veterinary and Agricultural Sciences,
The University of Melbourne,
Parkville, Victoria, Australia

JOHN GRAINGER

Faculty of Biology, Medicine and Health,
University of Manchester,
Manchester, UK

STEPHEN E. HARDING

School of Biosciences,
University of Nottingham,
Sutton Bonington, UK

SONJA HESS

Proteome Exploration Laboratory, Beckman Institute,
California Institute of Technology,
Pasadena, USA

ANDREAS HOFMANN

Structural Chemistry Program, Eskitis Institute for Drug Discovery,
Griffith University,
Nathan, Queensland, Australia
and

Faculty of Veterinary and Agricultural Sciences,
The University of Melbourne,
Parkville, Victoria, Australia

JOANNE KONKEL

Faculty of Biology, Medicine and Health,
University of Manchester,
Manchester, UK

PASI K. KORHONEN

Faculty of Veterinary and Agricultural Sciences,
The University of Melbourne,
Parkville, Victoria, Australia

JOANNE MACDONALD

School of Science and Engineering,
University of the Sunshine Coast,
Maroochydore DC, Queensland, Australia
and

Department of Medicine,
Columbia University,
New York, NY, USA

JAMES I. MACRAE
Metabolomics Unit,
The Francis Crick Institute,
London, UK

KAY OHLENDIECK
Department of Biology,
National University of Ireland, Maynooth,
Co. Kildare, Ireland

STEPHEN W. PADDOCK
Howard Hughes Medical Institute,
Department of Molecular Biology,
University of Wisconsin,
Madison, Wisconsin, USA

ANNA V. PROTASIO
Wellcome Trust Sanger Institute,
University of Cambridge,
Hinxton, Cambridge, UK

RALPH RAPLEY
Department of Biosciences,
University of Hertfordshire,
Hatfield, Hertfordshire, UK

GILL RUMSBY
Clinical Biochemistry,
HSL Analytics LLP,
London, UK

ANNE SIMON
Université Claude Bernard Lyon 1,
Bâtiment Curien, Villeurbanne,
France
and
Laboratoire Chimie et Biologie des Membranes et des Nanoobjets,
Université de Bordeaux, Pessac,
France

ROBERT J. SLATER
Royal Society of Biology,
Charles Darwin House,
London, UK

TIM J. STEVENS
MRC Laboratory of Molecular Biology,
University of Cambridge,
Cambridge, UK

PAUL TAYLOR
School of Biological Sciences,
University of Edinburgh,
Edinburgh, Scotland, UK

MICHAEL WEISS
Phase I Pilot Consortium,
Children's Oncology Group,
Monrovia, CA, USA



0 Tables and Resources

Table 0.1 **Abbreviations**

ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-monophosphate
ATP	adenosine 5′-triphosphate
bp	base pairs
cAMP	cyclic AMP
CAPS	<i>N</i> -cyclohexyl-3-aminopropanesulfonic acid
CHAPS	3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonic acid
cpm	counts per minute
CTP	cytidine triphosphate
DDT	2,2-bis-(<i>p</i> -chlorophenyl)-1,1,1-trichloroethane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
e [−]	electron
EDTA	ethylenediaminetetra-acetate
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorting
FAD	flavin adenine dinucleotide (oxidised)
FADH ₂	flavin adenine dinucleotide (reduced)
FMN	flavin mononucleotide (oxidised)
FMNH ₂	flavin mononucleotide (reduced)
GC	gas chromatography
GTP	guanosine triphosphate
HAT	hypoxanthine, aminopterin, thymidine medium
HEPES	4(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
HPLC	high-performance liquid chromatography
IMS	industrial methylated spirit
kb	kilobase pairs

Table 0.1 (cont.)	
M_r	relative molecular mass
MES	4-morpholine-ethanesulfonic acid
min	minute
MOPS	4-morpholine-propanesulfonic acid
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(ethanesulfonic acid)
ppb	parts per billion
ppm	parts per million
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulfate
TAPS	<i>N</i> -[tris-(hydroxymethyl)]-3-aminopropanesulfonic acid
TRIS	2-amino-2-hydroxymethylpropane-1,3-diol
v/v	volume per volume
w/v	weight per volume

Table 0.2 Biochemical constants		
Unit	Symbol	SI equivalent
Atomic mass unit	u	1.661×10^{-27} kg
Avogadro constant	L or N_A	6.022×10^{23} mol ⁻¹
Faraday constant	F	9.648×10^4 C mol ⁻¹
Planck constant	h	6.626×10^{-34} J s
Universal or molar gas constant	R	8.314 J K ⁻¹ mol ⁻¹
Molar volume of an ideal gas at standard conditions ^a	V_m	22.41 dm ³ mol ⁻¹
Velocity of light in a vacuum	c	2.997×10^8 m s ⁻¹

Note: ^aStandard conditions: $p^0 = 1$ bar, $\theta_{\text{normal}} = 25$ °C.

Table 0.3 **SI units: basic and derived units**

Quantity	SI unit	Symbol (basic SI units)	Definition of SI unit	Equivalent in SI units
Basic units				
Length	metre	m		
Mass	kilogram	kg		
Time	second	s		
Electric current	ampere	A		
Temperature	kelvin	K		
Luminous intensity	candela	cd		
Amount of substance	mole	mol		
Derived units				
Area	square metre	m ²		
Concentration	mole per cubic metre	mol m ⁻³		
Density	kilogram per cubic metre	kg m ⁻³		
Electric charge	coulomb	C	1 A s	1 J V ⁻¹
Electric potential difference	volt	V	1 kg m ² s ⁻³ A ⁻¹	1 J C ⁻¹
Electric resistance	ohm	Ω	1 kg m ² s ⁻³ A ⁻²	1 V A ⁻¹
Energy, work, heat	joule	J	1 kg m ² s ⁻²	1 N m
Enzymatic activity	katal	katal	1 mol s ⁻¹	60 mol min ⁻¹
Force	newton	N	1 kg m s ⁻²	1 J m ⁻¹
Frequency	hertz	Hz	1 s ⁻¹	
Magnetic flux density	tesla	T	1 kg s ⁻² A ⁻¹	1 V s m ⁻²
Molecular mass	atomic mass units (dalton)	u	1 u = 1 Da	
Molar mass	gram per mole	g mol ⁻¹	1 g mol ⁻¹	
Power, radiant flux	watt	W	1 kg m ² s ⁻³	1 J s ⁻¹
Pressure	pascal	Pa	1 kg m ¹ s ⁻²	1 N m ⁻²
Volume	cubic metre	m ³		

Table 0.4 Conversion factors for non-SI units		
Unit	Symbol	SI equivalent
Energy		
calorie	cal	4.184 J
erg	erg	10 ⁻⁷ J
electron volt	eV	1.602 × 10 ⁻¹⁹ J
Pressure		
atmosphere	atm	1.013 × 10 ⁵ Pa
bar	bar	10 ⁵ Pa
millimetres of mercury	mm Hg (Torr)	133.322 Pa
pounds per square inch	psi	6.895 × 10 ⁴ Pa
Temperature		
degree Celsius	°C	$\left[\frac{\theta}{1^{\circ}\text{C}} + 273.15 \right] \text{K}$
degree Fahrenheit	°F	$\left[\left(\frac{t}{1^{\circ}\text{F}} - 32 \right) \times \frac{5}{9} + 273.15 \right] \text{K}$
Length		
ångström	Å	10 ⁻¹⁰ m
inch	in	0.0254 m
Mass		
pound	lb	0.4536 kg

Table 0.5 Common unit prefixes associated with quantitative terms					
Multiple	Prefix	Symbol	Multiple	Prefix	Symbol
10 ²⁴	yotta	Y	10 ⁻¹	deci	d
10 ²¹	zetta	Z	10 ⁻²	centi	c
10 ¹⁸	exa	E	10 ⁻³	milli	m
10 ¹⁵	peta	P	10 ⁻⁶	micro	μ
10 ¹²	tera	T	10 ⁻⁹	nano	n
10 ⁹	giga	G	10 ⁻¹²	pico	p
10 ⁶	mega	M	10 ⁻¹⁵	femto	f
10 ³	kilo	k	10 ⁻¹⁸	atto	a
10 ²	hecto	h	10 ⁻²¹	zepto	z
10 ¹	deca	da	10 ⁻²⁴	yocto	y

Table 0.6 **Interconversion of non-SI and SI units of volume**

Non-SI unit		Non-SI subunit		SI subunit		SI unit
1 litre (l)	=	10 ³ ml	=	1 dm ³	=	10 ⁻³ m ³
1 millilitre (ml)	=	1 ml	=	1 cm ³	=	10 ⁻⁶ m ³
1 microlitre (µl)	=	10 ⁻³ ml	=	1 mm ³	=	10 ⁻⁹ m ³
1 nanolitre (nl)	=	10 ⁻⁶ ml	=	1 nm ³	=	10 ⁻¹² m ³

Table 0.7 **Interconversion of mol, mmol and µmol in different volumes to give different concentrations**

Molar (M)		Millimolar (mM)		Micromolar (µM)
1 mol dm ⁻³	=	10 ³ mmol dm ⁻³	=	10 ⁶ µmol dm ⁻³
=		=		=
1 mmol cm ⁻³	=	10 ³ µmol cm ⁻³	=	10 ⁶ nmol cm ⁻³
=		=		=
1 µmol mm ⁻³	=	10 ³ nmol mm ⁻³	=	10 ⁶ pmol mm ⁻³

Table 0.8 **pK_a values of some acids and bases that are commonly used as buffer solutions**

Acid or base	pK _a
Acetic acid	4.8
Barbituric acid	4.0
Carbonic acid	6.1, 10.2
CAPS ^a	10.4
Citric acid	3.1, 4.8, 6.4
Glycylglycine	3.1, 8.1
HEPES ^a	7.5
MES ^a	6.1
MOPS ^a	7.1
Phosphoric acid	2.0, 7.1, 12.3
Phthalic acid	2.8, 5.5
PIPES ^a	6.8
Succinic acid	4.2, 5.6
TAPS ^a	8.4
Tartaric acid	3.0, 4.2
TRIS ^a	8.1

Note: ^aSee list of abbreviations (Table 0.1).

Table 0.9 **Abbreviations for amino acids and average molecular masses, free and within peptides (residue mass)**

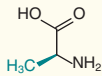
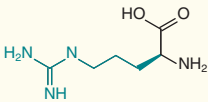
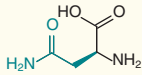
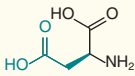
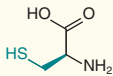
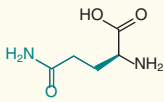
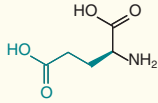
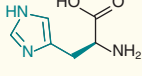
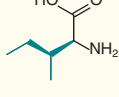
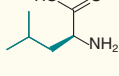
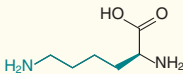
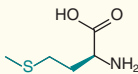
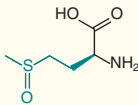
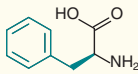
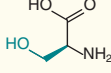
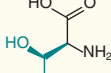
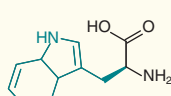
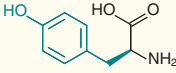
Amino acid	Three-letter code	One-letter code	Molecular mass <i>M</i>	Residue mass <i>M</i> - <i>M</i> (H ₂ O)		Side Chain
			Nominal mass (Da)	Average mass (Da)	Monoisotopic mass (Da)	
Alanine	Ala	A	89	71.08	71.037114	
Arginine	Arg	R	174	156.19	156.10111	
Asparagine	Asn	N	132	114.10	114.04293	
Aspartic acid	Asp	D	133	115.09	115.02694	
Asparagine or aspartic acid	Asx	B				
Cysteine	Cys	C	121	103.15	103.00919	
Glutamine	Gln	Q	146	128.13	128.05858	
Glutamic acid	Glu	E	147	129.12	129.04259	
Glutamine or glutamic acid	Glx	Z				
Glycine	Gly	G	75	57.05	57.021464	
Histidine	His	H	155	137.14	137.05891	
Isoleucine	Ile	I	131	113.16	113.08406	
Leucine	Leu	L	131	113.16	113.08406	

Table 0.9 (cont.)

Amino acid	Three-letter code	One-letter code	Molecular mass <i>M</i>	Residue mass <i>M</i> – <i>M</i> (H ₂ O)		Side Chain
			Nominal mass (Da)	Average mass (Da)	Monoisotopic mass (Da)	
Lysine	Lys	K	146	128.17	128.09496	
Methionine	Met	M	149	131.20	131.04048	
Met-sulfoxide ^a	MetSO	MSO	165	147.20	147.03540	
Phenylalanine	Phe	F	165	147.18	147.06841	
Proline	Pro	P	115	97.12	97.052764	
Serine	Ser	S	105	87.08	87.032029	
Threonine	Thr	T	119	101.11	101.04768	
Tryptophan	Trp	W	204	186.21	186.07931	
Tyrosine	Tyr	Y	181	163.18	163.06333	
Valine	Val	V	117	99.13	99.068414	

Notes: Although some amino acids are similar in mass, they can be distinguished by modern high-resolution mass spectrometry. ^aThis is a frequently found modification in mass spectrometric investigation of proteins and peptides.

Table 0.10 Ionisable groups found in amino acids				
Amino acid	p <i>K</i> _{a1}		p <i>K</i> _{a2}	
	α-carboxyl group		α-ammonium ion	
	side-chain group		p <i>K</i> _{a3}	
Alanine	2.3	9.7	-	6.0
Arginine	2.2	9.0	12.5	10.8
Asparagine	2.0	8.8	-	5.41
Aspartic acid	1.9	9.6	3.7	2.8
Cysteine	2.0	8.2	8.4	5.1
Glutamine	2.2	9.1	-	5.7
Glutamic acid	2.2	9.7	4.3	3.2

Glycine	2.3	9.6	-	6.0		6.0
Histidine	1.8	9.2	-	7.6		6.0
Isoleucine	2.4	9.6	-	6.0		6.0
Leucine	2.4	9.6	-	6.0		6.0
Lysine	2.2	9.0	-	9.7		10.5
Methionine	2.3	9.2	-	5.7		5.7
Phenylalanine	1.8	9.1	-	5.5		5.5
Proline	2.0	10.6	-	6.3		6.3
Serine	2.2	9.2	-	5.7		5.7
Threonine	2.1	9.1	-	5.6		5.6
Tryptophan	2.8	9.4	-	5.9		5.9
Tyrosine	2.2	9.1	-	5.7		10.1
Valine	2.3	9.6	-	6.0		6.0

Table 0.11 The genetic code: triplet codons and their corresponding amino acids								
	U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly