Edited by **Keith Wilson & John Walker**

Principles and Techniques of Practical Biochemistry

Fifth edition

实用生物化学的原理与技术

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Preface to the fifth edition

Teachers of genetics, cell biology, biochemistry and physiology are acutely aware of the rapid expansion of the knowledge base of their subjects that has taken place in the recent past. Each weekly batch of journals provides new discoveries for inclusion in an already crowded curriculum. Much of this expansion is the direct result of developments in molecular genetics, in particular of protocols for gene cloning and expression, which have resulted in routine procedures for the identification, cloning, sequencing and expression of genes for proteins ranging in function from metabolic enzymes and structural proteins to membrane receptors and regulatory proteins. The advent of such routine procedures has revolutionised our understanding of biological processes at the molecular level and has resulted in the coalescing of previously disparate disciplines. At the same time, this new knowledge of the molecular nature of biological processes has been exploited to medical and commercial advantages. Even the layperson has been made aware of application of molecular biology to areas as divergent as archaeology, plant and animal breeding, diagnostic tests for a wide range of inherited conditions, and new approaches to the diagnosis and treatment of chronic illnesses, particularly cancer. The decoding of the genome of many unicellular organisms and rapid progress on the Human Genome Project, which is now projected to be completed in the early years of the new millennium, promises even more spectacular applications in the future.

These advances in molecular biology have been paralleled, and in some cases made possible, by equally fundamental developments in immunology, cell culture, protein analysis and techniques such as chromatography, electrophoresis, mass spectrometry and various forms of spectroscopy. In planning this new edition of our book, our challenge has been to incorporate details of these developments whilst retaining our original aim, namely to concentrate on those techniques and principles which underlie practical exercises that undergraduates in all the biological sciences can expect to encounter in their practical classes and to cover in less detail the more sophisticated techniques that have made possible the advances they will learn about in their lectures and associated reading. In accordance with this aim, we have decided to cover techniques in molecular biology in greater detail than in earlier editions. There are now two chapters devoted to this area. Chapter 2 deals with the basic theoretical and practical details and Chapter 3

concentrates on their applications. Both chapters have been written by Dr Ralph Rapley, a new contributor to our book. Chapter 4, on immunological techniques, has also been completely rewritten by Susan and Robin Thorpe, from the National Institute for Biological Standards and Control, also new contributors to the book. A new chapter on protein purification has been introduced to emphasise its central importance in modern practical biology (Chapter 6). Whilst previous editions have devoted a chapter to enzyme techniques, no opportunity has previously been taken to emphasise similarities between enzyme–substrate (or inhibitor) binding and the binding of ligands to membrane receptors and membrane transporters. In view of the fundamental and physiologically important advances that have been made in our understanding of cell–cell interactions and the associated signal transduction and amplification processes, a new chapter has been introduced covering these important topics (Chapter 8).

The two chapters on spectroscopic and spectrometric techniques have been revised so that they are now presented in three chapters. Chapters 9 and 10 deal with those methods that are based on quantum principles and cover such important techniques as visible and ultraviolet spectroscopy, fluorimetry, luminescent spectroscopy, circular dichroism, turbidimetry, nephelometry and atomic absorption (Chapter 9), and infrared spectroscopy, electron spin resonance spectroscopy and nuclear magnetic resonance spectroscopy (including magnetic resonance imaging) (Chapter 10). Chapter 11 gives a detailed account of the various forms of mass spectrometry and includes a discussion of its use in protein structure determination, an application unthought of only a few years ago. Throughout the three chapters, opportunities have been taken to stress the complementary nature of spectroscopic and spectrometric data by considering the applications of the various techniques to the molecule phenacetin.

The chapters on centrifugation techniques (Chapter 5), electrophoresis (Chapter 12), chromatography (Chapter 13), radioisotope techniques (Chapter 14), and electrochemical techniques (Chapter 15) have all been updated. Throughout the book, emphasis has been placed on the quantitative nature of practical biochemistry. Nearly all chapters, therefore, now include a set of calculations, with answers, to enable students to test their understanding of the principles being covered. To further help students identify the key topics, each chapter also includes a section of 'key terms' to understand, together with another on suggestions for further reading. Inevitably, many of the chapters deal with common topics and every effort has been made to cross-reference to other chapters and minimise unnecessary duplication. However, a small amount of duplication between chapters has deliberately been retained, particularly in places where the slightly different approaches adopted by the authors were felt to add to the overall understanding and presentation. Many chapters make reference to common thermodynamic principles and equations. To strengthen, and thereby emphasise, the importance of thermodynamics to biochemical principles, a new section on the subject has been introduced in Chapter 1. This chapter now also includes worked numerical examples, some of a very fundamental, practical kind, which are truly basic to all practical work, but which many undergraduates initially have

difficulty in handling. Examples include the difference between concentration and amount, calculation of pH and various thermodynamic values. We hope the innovation will prove helpful to our readers. In producing the new edition we have attempted to incorporate the many helpful suggestions made by readers of the fourth edition. We continue to welcome comments from all those who use the book as part of their studies and wish to express our gratitude to the many authors who have granted us permission to reproduce their copyright figures.

KEITH WILSON
JOHN WALKER

Contributors

Professor D. B. Gordon
Department of Biological Sciences
Metropolitan University of Manchester
Chester Street
All Saints
Manchester M15 6BH, UK

Dr A. Griffiths
Department of Biological Sciences
Oxford Brookes University
Headington
Oxford OX3 0BP, UK

Dr R. Rapley
Department of Biosciences
University of Hertfordshire
Hatfield Campus
College Lane
Hatfield
Herts AL10 9AB, UK

Dr P. K. Robinson
Department of Applied Biology
University of Central Lancashire
Preston
Lancs PR1 2HE, UK

Dr I. Simpkins
Department of Biosciences
University of Hertfordshire
Hatfield Campus
College Lane
Hatfield
Herts AL10 9AB, UK

Professor R. J. Slater
Department of Biosciences
University of Hertfordshire
Hatfield Campus
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
Department of Biosciences
University of Hertfordshire
Hatfield Campus
College Lane
Hatfield
Herts AL10 9AB, UK

Professor K. Wilson
Department of Biosciences
University of Hertfordshire
Hatfield Campus
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
CHAPS	3-[(3-chloramidopropyl)dimethylamino]-1-propanesulphonic acid
c.p.m.	counts per minute
DDT	2,2-bis-(*p*-chlorophenyl)-1,1,1-trichlorethane
DNA	deoxyribonucleic acid
d.p.m.	disintegrations per minute
e^-	electron
EDTA	ethylenediaminetetra-acetate
EGTA	[ethylenebis (oxonitrilo)] tetra-acetic acid
e.m.f.	electromotive force
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
HAT	hypoxanthine, aminopterin, thymidine medium
Hepes	4(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
kb	kilobase-pairs
log	logarithm to the base 10
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-piperazinediethanesulphonic acid
P_i	inorganic phosphate
PP_i	inorganic pyrophosphate
p.p.m.	parts per million
RNA	ribonucleic acid
s.t.p.	standard temperature and pressure
Tris	2-amino-2-hydroxymethylpropane-1,3-diol

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Chapter 1

General principles of biochemical investigations

1.1 THE NATURE OF BIOCHEMISTRY

Biochemistry is an interdisciplinary science that integrates systematically the principles of mathematics, physics and chemistry to attempt to explain the distinctive characteristics of life processes in terms of structure–function correlations. Advances in biochemistry have therefore largely exploited principles and techniques first applied in the physical sciences. Biochemistry is no longer simply an academic discipline but an applied science in which scientific discovery is expected to lead to material benefits. In recent years the fusion of biochemistry, cell biology and microbiology to form molecular biology has led to spectacular advances in the understanding and control of biological processes in medicine, agriculture, pharmaceutics and the food and drinks industry. This justifies to many the widely held belief that biotechnology will become the pre-eminent industry of the new millennium.

Biochemistry is quintessentially both analytical and quantitative in using model biological systems of different physiological complexities to explain cause-and-effect relationships in molecular terms. Analysis means literally 'getting to the bottom of things', i.e. taking to pieces. Analysis is useful only, however, when combined with synthesis, the piecing together, through interpretation and extrapolation of observations made on the disassembled parts, into the working whole. Analysis and synthesis therefore in combination define the boundaries between systems and surroundings, i.e. what components are part of the system and consequently affect the system directly in contrast to external factors that affect it only indirectly. Repeated investigations lead in many cases to the definition of a system.

In analytical biochemistry, for example, experimental models are subjected first to qualitative analysis, in which predominantly heterogeneous biological material is subjected to disruption techniques and the constituent parts separated, concentrated and identified. Qualitative analytical biochemistry is concerned with identifications mainly at the molecular level but sometimes at the electronic level. Quantitative analysis is concerned with measuring amounts and/or concentrations (amount per unit of volume) of constituents identified by qualitative analysis. The technique relies heavily on assay methods and instruments measuring the values of biochemical samples, representative of the whole population of