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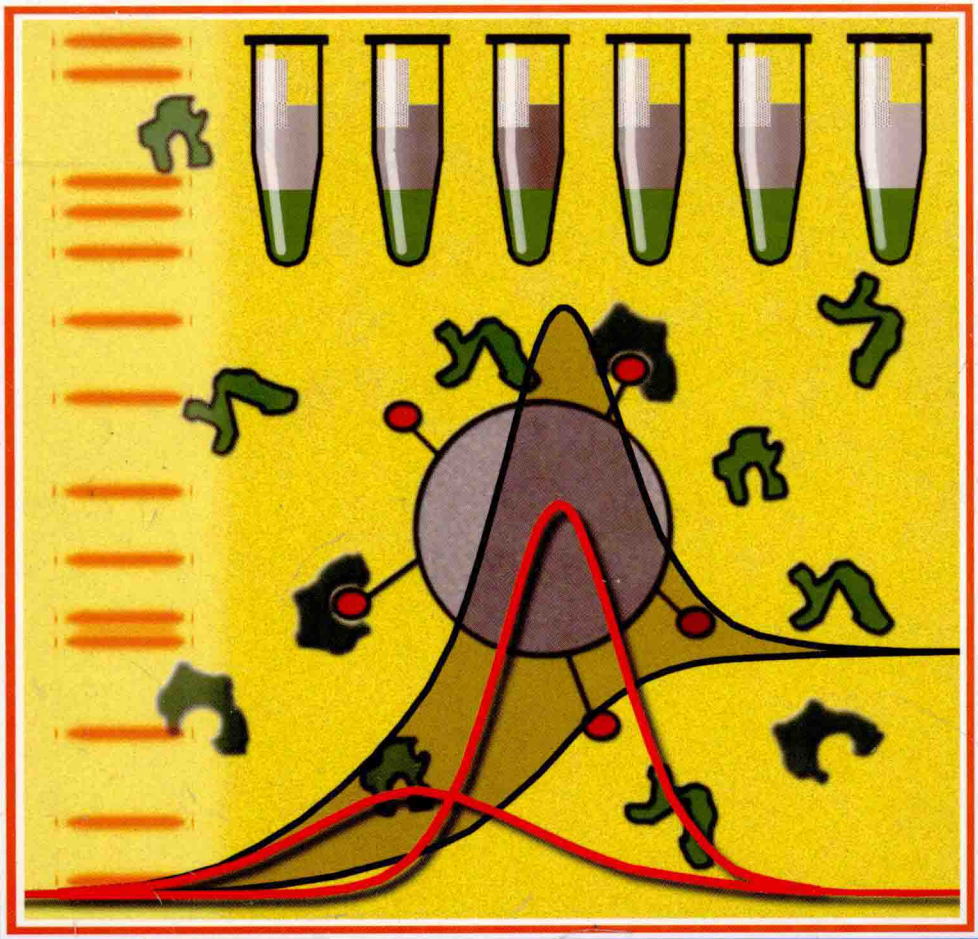
Biochemical Methods

A Concise Guide for Students and Researchers



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Preface to the English Edition

It is widely recognised that the biosciences are entering a post-genomic era, in which the focus of interest is moving away from genome sequences towards the proteins that they specify and the way that these interact to generate complexity in biological systems. One consequence of this shift in focus is that biochemistry is likely to re-assume a centre stage position in the biological world, having been eclipsed for many years by developments in molecular biology and cell biology, and more recently, by the prodigious efforts to obtain the sequences of whole genomes and to compare gene expression in different tissues by array technologies. The reasons for a resurgence of interest in the subject are not hard to discern. Powerful new methodologies, such as proteomics, are being used to study systems in a holistic manner, exploiting the information available in sequence and structural databases. These methodologies are mostly high throughput refinements and developments of existing techniques in biochemistry and molecular biology. The outcome of these studies are essentially descriptive, and although they can supply comprehensive information about biological systems, they cannot substitute for hypothesis-based investigations of how biological processes take place. Biochemistry provides the experimental foundation for mechanistic studies of such processes, and its power and scope are likely to be enhanced by the proper use of what have become termed the 'omic' sciences.

Biochemistry is an experimental science characterised by an analytical and quantitative approach, and these themes underlie our presentation of the subject in this book, which is a revised and updated version of the German Edition published in 1997. The objective of the book remains the same: to offer students of biochemistry and molecular biology a concise introduction to the current techniques and methods of the subject. The plan of the book mirrors in some respects a quintessential programme of biochemical investigation: first, getting to grips with the literature on the subject, then the basic laboratory procedures and handling of the biological sample material, followed by processes of separation, purification and analysis, and finally the various investigative techniques that can be applied, and the mathematical analysis of the findings. However, as in the first edition, the book is not intended to be a practical handbook; our aim is to enable the reader to judge what information can be derived from particular experimental approaches, and to assist in the outline planning of experiments.

Many of the revisions in this edition deal with advances in methods for analysing and characterising proteins, such as 2D-gel electrophoresis and the use of mass spectrometry in protein analysis. We have also included new sections on microcalorimetry and biomolecular interaction analysis, reflecting the increasing interest in molecular interactions, particularly protein-protein interactions, in biological systems. There has been extensive updating of other subjects covered in the first edition. A major change is the inclusion of a new chapter (Chapter 9) on quantitative analysis of biochemical data, together with a supporting compact disk. We recognise that this is a subject where many students of biochemistry may feel that they lack the necessary mathematical and computational skills. We hope that hands-on experience of relevant methods will help develop greater familiarity and confidence.

Biochemistry appears to have a bright future, and students entering the subject can do so with confidence that there is every prospect that their science will flourish. We shall be pleased if this book helps them with the important task of gaining experience of the experimental methods that underpin the subject.

Alfred Pingoud, Claus Urbanke, Jim Hoggett and Albert Jeltsch

Giessen, Hannover and York
June 2002

Preface to the German Edition

Biochemistry is essentially an experimental science supported by an array of methodologies which are now being applied across the whole range of life sciences. This transfer of methodologies has been a reciprocal one, and many techniques have been assimilated into biochemistry from neighbouring disciplines. This is particularly the case with modern methods of molecular and cell biology, microbiology and immunology, but also important input has been derived from physical and analytical chemistry.

This book is the result of our joint efforts to present students of biochemistry with an understanding of the current technologies of the subject in concise form. The theoretical knowledge conveyed in lectures, and skills developed in practical classes require a supporting text which, we hope, would assist students in rounding off and deepening their understanding of the subject. We have prepared this book for students who are biochemists or molecular biologists in the broadest sense of the words, and who are working, or propose to work in the area, and are seeking to gain an overview of its basic technologies.

Despite its limited size, this book has a fairly broad scope: it starts with access to the literature of biochemistry, sets out the main features of the organisation of a biochemical laboratory, discusses a range of laboratory methods, both general and more specialised and concludes with coverage of some advanced instrumental techniques. It is not a practical handbook in the sense that experiments are described, or experimental protocols are presented in detail, rather it contains the information necessary for the reader to plan experiments and to be in a position to appreciate what conclusions could in principle be drawn from particular experimental approaches. It is also one of our aims to stimulate the reader to use methods that they may not be currently very familiar with. For reasons of space, we have been able to describe methods in detail in only a limited number of cases, and for this we have selected core techniques, which find wide application. Elsewhere, we have had to rely on the literature to take matters further; in doing this, we have given preference to widely accessible journals, reviews and more recent monographs.

We are aware that a book of this kind can by no means be comprehensive. We have, therefore, emphasised methods that we believe young scientists who wish to work in biochemistry should have in their repertoire of skills, and also methods that they should be aware of so that they can, if necessary, find out more from an expert.

A comment about the units which are used in this book: the use of international SI units is prescribed by all scientific authorities and the most important SI units are given in an Appendix. Other systems of units are, however, in common use in laboratory practice. In our experience this leads to constant confusion. We have, therefore, attempted to use SI units rigorously and where other systems of units are in common use we have given the corresponding conversions.

We hope that this book will prove useful to students of the subject, and that it will also be found of value to colleagues in our area.

Giessen and Hanover, February 1997 Alfred Pingoud and Claus Urbanke

Contents

1
Biochemical literature

In this chapter we outline the sources of information about biochemistry and molecular biology that are available in the literature. We start with textbooks, of which there are many, with different emphases, before moving on to more specialised monographs and then the primary literature in scientific journals. The subject is well served by reference books, practical handbooks, and periodicals which summarise methodological aspects, and these are considered next. There then follows a brief discussion of computer-based literature searches. The chapter concludes with advice about the use of protocols in biochemical and molecular biological experiments and keeping proper records of experimental work in laboratory books.

Accessing the literature is an integral part of experimental work and of particular importance in biochemistry and molecular biology, which are characterised by having a very broad methodological base. In this chapter, we describe the nature of the biochemical literature, and give advice about how to make an entry into the literature on a particular topic, and how to use it effectively. An important objective of scientific work is that the findings should be communicated. To do this, it is essential that experimental work should be properly documented; we give advice about how this should be done.

We have quite deliberately placed this chapter at the beginning of our book on "Biochemical Methods". There are two reasons for this: first, because it is essential that the relevant literature should be consulted before the practical work is initiated, and second, because, as mentioned above, it is important that the work should be properly documented as it is carried out.

1.1
Accessing the biochemical literature

Any practising biochemist will testify that an hour spent in the library can save days or even weeks in the laboratory. The literature is an essential tool which every biochemist needs to become familiar with. Different aspects of the literature will need to be consulted at different phases of the scientific work. Initially, it is important to gain an overview of the area, which is best done using the most recent editions of

comprehensive textbooks of biochemistry, molecular biology and cell biology. This should be followed by consulting specialist up-to-date reviews of the relevant primary literature. In the course of a scientific project, it is vital to keep up with progress in the field by following the primary literature of the subject. It may also be necessary to develop new methodological skills, which can be done by consulting monographs and journals dedicated to specialist techniques. Throughout the course of the work, it will often be necessary to consult dictionaries of the subject and books of collected tables for information on technical terms, chemical structures, or the magnitude of physical constants.

1.1.1 Textbooks of biochemistry

The following comprehensive textbooks are suitable as introductions into biochemistry, and they allow a graded approach to more detailed levels of coverage.

Reginald H. Garrett and Charles M. Grisham (1999) *Biochemistry.* Saunders College Publishing, Fort Worth, FL.

Albert L. Lehninger, David L. Nelson and Michael L. Cox (2000) *Principles of Biochemistry* 3rd Edition. Worth Publishers, New York.

Christopher K. Mathews, K.E. van Holde and Kevin G. Ahern (2000) *Biochemistry* 3rd Edition. Addison-Wesley, San Francisco, CA.

Donald Voet, Judith G. Voet and Charlotte Pratt (1999) *Fundamentals of Biochemistry.* John Wiley & Sons, New York.

Jeremy M. Berg, John L. Tymoczko and Lubert Stryer (2002) *Biochemistry* 5th Edition. W.H. Freeman, New York.

Similar texts, but oriented towards molecular cell biology are:

Harvey Lodish, Arnold Berk, S. Lawrence Zipursky, Paul Matsudaira, David Baltimore and James Darnell (2000) *Molecular Cell Biology* 4th Edition. W.H. Freeman, New York.

Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walker (2002) *Molecular Biology of the Cell* 4th Edition. Garland Publishing, New York.

The above textbooks of biochemistry and molecular and cell biology deal in only a superficial way with important concepts and methods in biophysical chemistry. The following volumes are important sources of additional information in this area:

Kensal E. van Holde, W. Curtis Johnson and P. Shing Ho (1998) *Principles of Physical Biochemistry.* Prentice-Hall, Englewood Cliffs, NJ.

David Sheehan (2000) *Physical Biochemistry: Principles and Applications.* John Wiley & Sons, Chichester.

Charles R.Cantor and Paul R Schimmel (1980) *Biophysical Chemistry.* W.H. Freeman, San Francisco, CA.

The latter book, in three volumes, has become a classic in biophysical chemistry, providing comprehensive coverage of the whole subject.

1.1.2 Current reviews of the biochemical literature

The following series focus on specific areas of biochemistry:

Accounts of Chemical Research
Advances in Cancer Research
Advances in Carbohydrate Chemistry
Advances in Cell Biology
Advances in Comparative Physiology and Biochemistry
Advances in Enzyme Regulation
Advances in Enzymology and Related Areas of Molecular Biology
Advances in Immunology
Advances in Lipid Research
Advances in Pharmacology and Chemotherapy
Advances in Protein Chemistry
Annual Review of Biochemistry
Annual Review of Biophysics and Biomolecular Structure
Annual Review of Cell and Developmental Biology
Annual Review of Genetics
Annual Review of Nutrition
Annual Review of Neuroscience
Annual Review of Microbiology
Annual Review of Pharmacology and Toxicology
Annual Review of Physiology
Annual Review of Plant Physiology and Plant Molecular Biology
Bacteriological Reviews
BBA Reviews on Cancer
BBA Reviews on Biomembranes
Biological Reviews
Chemical Reviews
Cold Spring Harbor Symposia on Quantitative Biology
Critical Reviews in Biochemistry and Molecular Biology
Critical Reviews in Plant Sciences
Current Biology
Current Opinion in Biotechnology
Current Opinion in Cell Biology
Current Opinion in Genetics and Development
Current Opinion in Immunology
Current Opinion in Neurobiology
Current Opinion in Structural Biology
EJB Reviews
Essays in Biochemistry
Harvey Lectures
International Review of Cytology

Nature Reviews
Physiological Reviews
Progress in Biophysics and Biophysical Chemistry
Progress in Nucleic Acid Research and Molecular Biology
Quarterly Review of Biology
Quarterly Reviews of Biophysics
Quarterly Review of the Chemical Society
Trends in Biochemical Sciences
Trends in Biotechnology
Trends in Cell Biology
Trends in Endocrinology and Metabolism
Trends in Genetics
Trends in Neurosciences
Trends in Pharmacological Sciences
Trends in Plant Science

Reviews in the *Current Opinion* and the *Trends* series offer concise and up-to-date overviews on various subjects. In addition, the following journals contain regular short reviews or mini-reviews:

Biological Chemistry
Cell
European Journal of Biochemistry
FASEB Journal
Journal of Biological Chemistry

1.1.3
The primary biochemical literature: scientific journals

Scientific journals are the most important medium for communicating new findings, and they are therefore the most fruitful source of information about current research. There are so many journals with biochemical content that it is not practicable to list them all here; it is also the case that most university libraries can subscribe to only a fraction of them. The more common biochemical journals that one would expect to find in the libraries of most universities or biochemical institutes are listed below:

Archives of Biochemistry and Biophysics
Biochemical and Biophysical Research Communications
Biochemical Journal
Biochemical Journal (Tokyo)
Biochemistry
Biochemistry (Moscow)
Biochemistry and Molecular Biology International
Biochimica et Biophysica Acta
Biochimie

Biological Chemistry (earlier known as Biological Chemistry Hoppe-Seyler)
European Journal of Biochemistry
FEBS Letters
Journal of Biological Chemistry

In addition, the following periodicals cover all aspects of science and for that reason regularly have articles with biochemical content:

Proceedings of the National Academy of Science USA
Nature
Science

Periodicals that are dedicated to specific biochemical themes include:

Biophysical Chemistry
Biopolymers
Chemistry and Biology
Cell
EMBO Journal
EMBO Reports
FASEB Journal
Gene
Genes and Development
Journal of the American Chemical Society
Journal of Bacteriology
Journal of Biotechnology
Journal of Molecular Biology
Journal of Protein Chemistry
Molecular and Cellular Biology
Molecular and General Genetics
Nature Biotechnology
Nature Cell Biology
Nature Genetics
Nature Immunology
Nature Medicine
Nature Neuroscience
Nature Structural Biology
Nucleic Acids Research
Oncogene
Protein Engineering
Protein Science
Protein Structure, Function and Genetics
RNA
Structure with Folding and Design