

Lehninger
**PRINCIPLES OF
BIOCHEMISTRY**

fourth edition



**DAVID L. NELSON
MICHAEL M. COX**

Lehninger

PRINCIPLES OF BIOCHEMISTRY

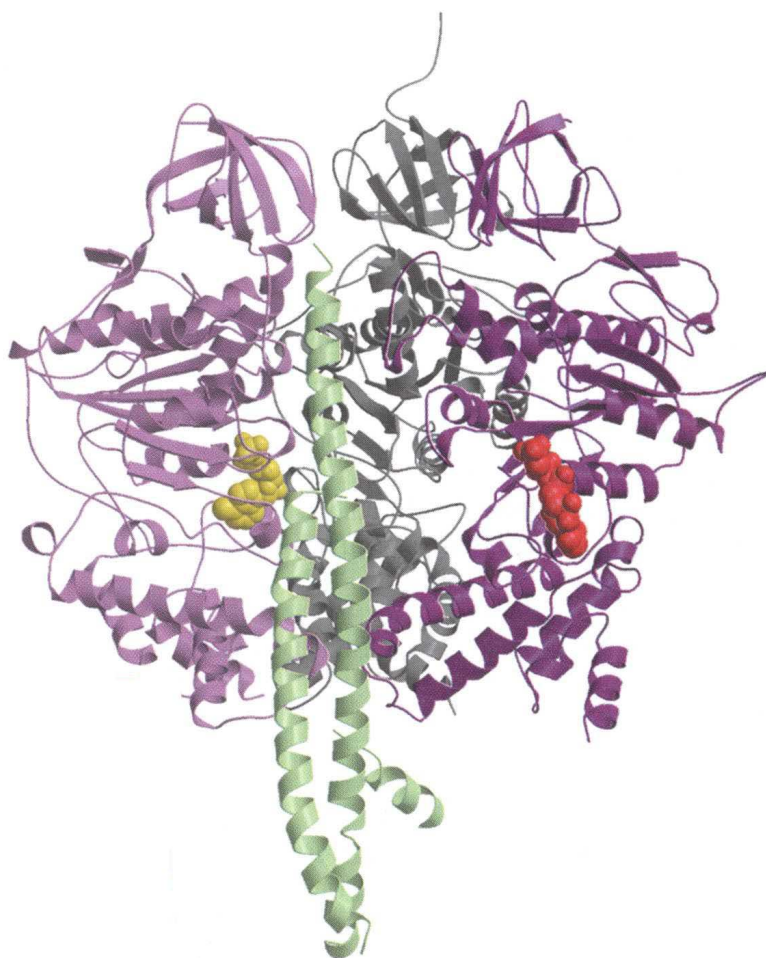
fourth edition

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On the cover: The F_1 ATPase, part of a complex responsible for ATP synthesis in eukaryotic mitochondria. See Chapter 19.

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Synthesizing an Oligonucleotide Array
Screening an Oligonucleotide Array for Patterns
of Gene Expression
Yeast Two-Hybrid Systems
Creating a Transgenic Mouse

Chapter 11 Biological Membranes and Transport

Living Graphs:
Free-Energy Change for Transport
(Equation 11-2)
Free-Energy Change for Transport of an Ion
(Equation 11-3)

Chapter 12 Biosignaling

3D Structure Tutorial: Trimeric G Proteins—
Molecular On/Off Switches

Chapter 13 Principles of Bioenergetics

Living Graphs:
Free-Energy Change (Equation 13-3)
Free-Energy of Hydrolysis of ATP (Box 13-1)

Chapter 14 Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway

Mechanism Animations:
Phosphohexose Isomerase Mechanism
(Figure 14-4)
Alcohol Dehydrogenase Mechanism
(Figure 14-12)
Thiamine Pyrophosphate Mechanism
(Figure 14-13)

Chapter 16 The Citric Acid Cycle

Mechanism Animation: Citrate Synthase
Mechanism (Figure 16-9)

Chapter 17 Fatty Acid Catabolism

Mechanism Animation: Fatty Acyl-CoA
Synthetase Mechanism (Figure 17-5)

Chapter 18 Amino Acid Oxidation and the Production of Urea

Mechanism Animations:
Pyridoxal Phosphate Reaction Mechanism
(Figure 18-6)

Carbamoyl Phosphate Synthetase I Mechanism
(Figure 18-11a)

Argininosuccinate Synthetase Mechanism
(Figure 18-11b)

Chapter 19 Oxidative Phosphorylation and Photophosphorylation

Living Graph: Free-Energy Change for
Transport of an Ion (Equation 19-8)
3D Structure Tutorial: Bacteriorhodopsin

Chapter 20 Carbohydrate Biosynthesis in Plants and Bacteria

Mechanism Animation: Rubisco Mechanism
(Figure 20-7)

Chapter 22 Biosynthesis of Amino Acids, Nucleotides, and Related Molecules

Mechanism Animations:
Tryptophan Synthase Mechanism
(Figure 22-18)
Thymidylate Synthase Mechanism
(Figure 22-50)

Chapter 24 Genes and Chromosomes

Animation: Three-Dimensional Packaging of
Nuclear Chromosomes

Chapter 25 DNA Metabolism

3D Structure Tutorial: Restriction
Endonucleases
Animation:
Nucleotide Polymerization by DNA Polymerase
DNA Synthesis

Chapter 26 RNA Metabolism

Animation: mRNA Splicing
3D Structure Tutorial: Hammerhead Ribozyme
Animation: Life Cycle of an mRNA

Chapter 28 Regulation of Gene Expression

3D Structure Tutorial: Lac Repressor

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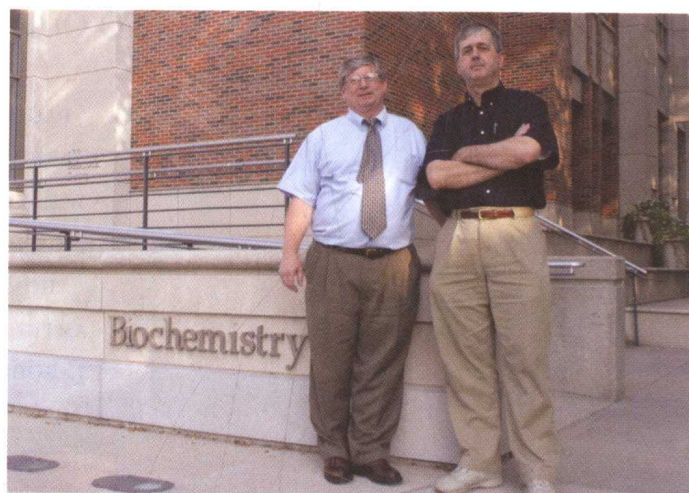
About the Authors

David L. Nelson, born in Fairmont, Minnesota, received his BS in Chemistry and Biology from St. Olaf College in 1964 and earned his PhD in Biochemistry at Stanford Medical School under Arthur Kornberg. He was a postdoctoral fellow at the Harvard Medical School with Eugene P. Kennedy, who was one of Albert Lehninger's first graduate students. Nelson went to the University of Wisconsin–Madison in 1971 and became a full professor of biochemistry in 1982.

Nelson's thesis research at Stanford was on the intermediary metabolism of sporulating and germinating bacteria. At Harvard he studied the energetics, genetics, and biochemistry of ion transport in *E. coli*. At Wisconsin his research has focused on the signal transductions that regulate ciliary motion and exocytosis in the protozoan *Paramecium*. The enzymes of signal transductions, including a variety of protein kinases, are primary targets of study. His research group uses enzyme purification, immunological techniques, electron microscopy, genetics, molecular biology, and electrophysiology to study these processes.

Dave Nelson has a distinguished record as a lecturer and research supervisor. For 32 years he has taught an intensive survey of biochemistry for advanced biochemistry undergraduates and graduate students in the life sciences (using Lehninger's *Biochemistry* and *Principles of Biochemistry* for much of that time). He has also taught a survey of biochemistry for nursing students, a graduate course on membrane structure and function, and a graduate seminar on membranes and sensory transductions. He has sponsored numerous PhD, MS, and undergraduate honors theses, and has received awards for his outstanding teaching, including the Dreyfus Teacher–Scholar Award and the Atwood Distinguished Professorship. In 1991–1992 he was a visiting professor of chemistry and biology at Spelman College. In 2002 he was appointed Director of the University of Wisconsin Center for Biology Education.

Michael M. Cox was born in Wilmington, Delaware. In his first biochemistry course, Lehninger's *Biochemistry* was a major influence in refocusing his fascination with biology and inspiring him to pursue a



David L. Nelson (left) and Michael M. Cox

career in biochemistry. After graduating from the University of Delaware in 1974, Cox went to Brandeis University to do his doctoral work with William Jencks, and then to Stanford in 1979 for postdoctoral study with I. Robert Lehman. He moved to the University of Wisconsin–Madison in 1983, and became a full professor of biochemistry in 1992.

His doctoral research was on general acid and base catalysis as a model for enzyme-catalyzed reactions. At Stanford, Cox began work on the enzymes involved in genetic recombination. The work focused particularly on the RecA protein, designing purification and assay methods that are still in use, and illuminating the process of DNA branch migration. Exploration of the enzymes of genetic recombination has remained the central theme of his research.

Mike Cox has coordinated a large and active research team at Wisconsin, investigating the enzymology, topology, and energetics of genetic recombination. A primary focus has been the mechanism of RecA protein-mediated DNA strand exchange and the role of ATP in the RecA system. More recently, part of the research program has focused more generally on recombinational DNA repair processes in *E. coli* and *Deinococcus radiodurans*. For the past 20 years he has taught (with Dave Nelson) the survey of biochemistry to undergraduates and has lectured in graduate courses on DNA structure and topology, protein-DNA interactions, and the biochemistry of recombination. He has received awards for both his teaching and his research, including the Dreyfus Teacher–Scholar Award and the 1989 Eli Lilly Award in Biological Chemistry. His hobbies include gardening, wine collecting, and assisting in the design of laboratory buildings.

PREFACE

Lehninger Principles of Biochemistry, Fourth Edition, is an introduction to our favorite subject—and our attempt to make it yours. In the four years since the publication of the third edition, both the pace and the scope of discovery in biochemistry have been breathtaking. We are learning not only what occurs within a cell but where and when it occurs, what determines location, and what controls timing. From the draft sequence of the human genome to elucidation of the high-resolution structure of the ribosome, major recent advances deepening our understanding of biochemistry fill the pages of this book.

While including these exciting new developments, we have also tried to remain focused on our mission: to communicate to students the fundamentals of biochemistry in a way that reflects the field as it is understood today. As always, our efforts to integrate new discoveries are balanced by our efforts to improve the accuracy and pedagogy of the core material of the field.

Coverage of Major Recent Advances in Biochemistry

Every chapter has been thoroughly revised and updated. Some of the major recent advances incorporated into the Fourth Edition include:

- A revised mechanism for the enzyme lysozyme (Chapter 6)
- Functions of heparan sulfates in extracellular matrix (Chapter 7)
- The human genome (Chapter 9)
- The structure and roles of membrane rafts and caveolae (Chapter 11)

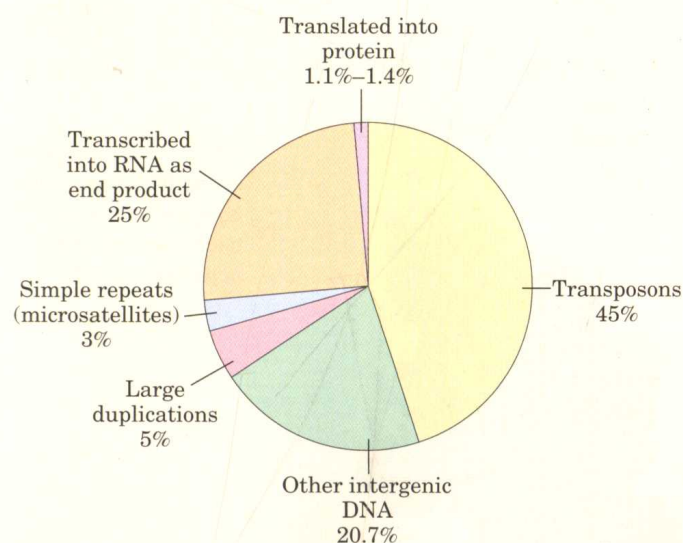


Figure 9–19 Snapshot of the human genome. p. 324

- The structure and function of ABC transporters (Chapter 11)
- Assembly of signaling complexes by the interaction of protein domains with phosphoserine and phosphotyrosine residues in partner proteins (Chapter 12)
- A two-component signaling system in bacteria and plants (Chapter 12)
- Receptorlike protein kinases in plant signaling (Chapter 12)
- Nonclassical actions of steroid hormones at plasma membrane receptors (Chapter 12)
- New developments in metabolic regulation and in the structure of enzymes and enzyme complexes, and new examples of human diseases that result from defective metabolism (throughout Part II)
- Inorganic polyphosphate metabolism (Chapter 13)
- Mechanisms by which metabolism of carbohydrates and fats is integrated in mammalian tissues (Chapters 14, 16, 17, and 23)
- Prevention of ATP hydrolysis by ATP synthase inhibitory protein during ischemia (Chapter 19)
- The role of mitochondria in apoptosis and oxidative stress (Chapter 19)
- The glycine decarboxylase complex and photorespiration (Chapter 20)
- A proposed lipid primer in cellulose synthesis (Chapter 20)
- A proposed new mechanism for starch synthesis from its reducing end (Chapter 20)
- The pathway for glyceroneogenesis, discussed in the context of its role in type II diabetes (Chapter 21)
- Hormonal regulation of body weight and the medical problems posed by the current epidemic of obesity in industrialized countries (Chapter 23)
- The role of cohesins and condensins in chromosome structure (Chapter 24)
- Coordination of multiple replication forks in replication factories (Chapter 25)
- New information on the mechanism and structure of bacterial RNA polymerase (Chapter 26)
- The high-resolution structure of the bacterial ribosome (Chapter 27)
- RNA interference (Chapter 28)

New Methodologies

As scientists and as teachers, we are interested in new technology not just for its own sake but for what

it can teach us—how it can provide us with clues to the biochemical mysteries around us. The following list includes just a few of the new experimental methodologies and their applications that are introduced or expanded in this edition:

- **High throughput methodologies** for determining protein structure, which have revealed many new structures that give us insight into the function of proteins and their interactions with each other and with ligands/substrates (Chapter 5)
- An introduction to modern **genomics** and **proteomics** (Chapter 9)
- Improvements in **DNA microarrays** that allow the detailed and comprehensive study of changes in gene expression caused by hormones and drugs and by mutations that lead to defects in function, including human diseases (Chapter 9)
- Visualization at the level of subcellular organelles of proteins fused genetically with green fluorescent protein (**GFP**) (Chapters 9, 12), providing information on cellular location and function
- **Atomic force microscopy**, used to visualize protein complexes and membrane surfaces (Chapter 11)
- Use of **fluorescence** for following the movement of a single lipid molecule in the plasma membrane (Chapter 11)
- Fluorescence resonance energy transfer (**FRET**), used to measure transient changes in second messenger or protein kinase activity in living cells (Chapter 12)
- Improved methods for **purifying and crystallizing membrane proteins**, which have provided a wealth of information about the details of such processes as oxidative phosphorylation and photophosphorylation (Chapter 19)
- RNA interference (**RNAi**), used to manipulate specific gene products in living cells, and the technology for creating knockout mice; these methods provide windows into the function of specific gene products in a whole organism. One

strain of knockout mice has opened up a large field of research aimed at understanding the control of body weight—a subject of great interest, given the increasing incidence of obesity and its many medical consequences (Chapter 28).

More Molecular Graphics

Our understanding of molecular structure has advanced significantly in the past few years, as has the interest on the part of students and instructors in working with this new structural information. As in the last edition, all the molecular structures you will find here are unique to this book, the result of close collaboration with our colleague Jean-Yves Sgro, biophysicist and master of molecular graphics. Jean-Yves has produced more than 70 new images for this edition. We have also added the Protein Data Bank identification code (PDB ID) to the legend of every figure where a structure appears. Students can use the PDB ID to access data about the molecule from the Protein Data Bank website and follow links to a variety of other resources. Structures new to this edition include:

- Aquaporin and voltage-gated K^+ channels—structures revealed by 2003 Nobel Prize-winning research (Chapter 11)
- The lactose permease of *E. coli* (Chapter 11)
- Extraordinary views of photosystem II and the cytochrome b_6f complex (Chapter 19)
- Bacterial RNA polymerase (Chapter 26)
- Multiple images of the bacterial ribosome in all its detail (Chapter 27)

Redesigned and Expanded Treatment of Enzyme Reaction Mechanisms

In response to instructor feedback, we have expanded our coverage of reaction mechanisms. We have also

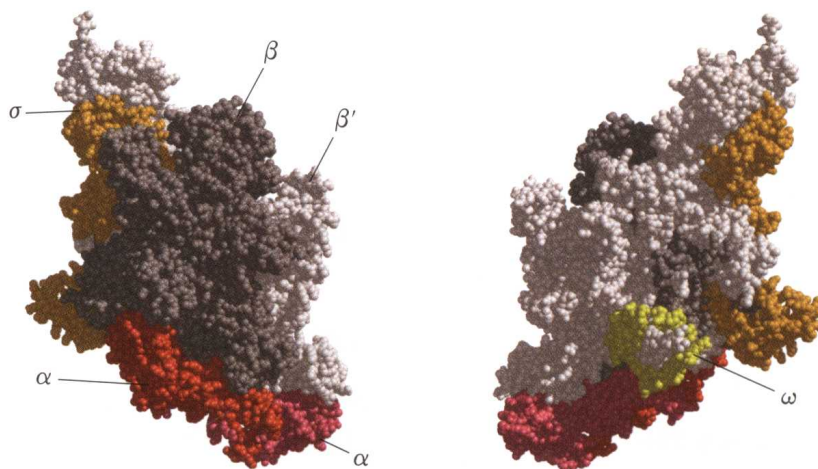


Figure 26-4 Structure of the RNA polymerase holoenzyme of the bacterium *Thermus aquaticus*. p. 999

worked to address the concern that students often enter biochemistry without a solid grasp of how to read reaction mechanisms. So, new in this edition:

- An expansive, two-page figure on the mechanism of chymotrypsin (the first reaction mechanism treated in the book), which serves as the framework for an illuminating discussion of how to read and extract information from mechanism diagrams (Fig. 6–21). Conventions are presented and explained.
- Building on the information communicated in the chymotrypsin figure, we've designed new **Mechanism Figures** that walk students through complex mechanisms step by step.
- Many new mechanisms; every major coenzyme is represented, with a discussion of its characteristic chemical role. Additional mechanisms highlight key classes of metabolic transformations and illuminate the chemical logic of reaction sequences in pathways.

Detailed Mechanisms in the Fourth Edition

Alcohol dehydrogenase (Chapter 14) **NEW**

Aminoacyl-tRNA synthetase (Chapter 27)

Bacterial topoisomerase (Chapter 24) **NEW**

Chymotrypsin (Chapter 6) **SIGNIFICANTLY REVISED**

Citrate synthase (Chapter 16) **NEW**

Class I aldolase (Chapter 14) **NEW**

Coenzyme B₁₂ reactions (Chapter 17)

DNA photolyase (Chapter 25) **SIGNIFICANTLY REVISED**

DNA polymerases (Chapter 25) **SIGNIFICANTLY REVISED**

Enolase (Chapter 6)

Fatty acyl-CoA synthetase (Chapter 17)

Glutamine amidotransferases (Chapter 22) **NEW**

Glyceraldehyde 3-phosphate dehydrogenase

(Chapter 14) **SIGNIFICANTLY REVISED**

Isocitrate dehydrogenase (Chapter 16) **NEW**

Lysozyme (Chapter 6) **NEW**

Phosphohexose isomerase (Chapter 14) **NEW**

Pyridoxal phosphate (PLP) reactions

(Chapter 18) **NEW**

Pyruvate carboxylase (Chapter 16) **NEW**

Ribonucleotide reductase (Chapter 22)

SIGNIFICANTLY REVISED

RNA polymerase (Chapter 26) **SIGNIFICANTLY REVISED**

Rubisco (Chapter 20) **SIGNIFICANTLY REVISED**

Thiamine pyrophosphate (TPP) reactions

(Chapters 14, 16)

Thymidylate synthase (Chapter 22)

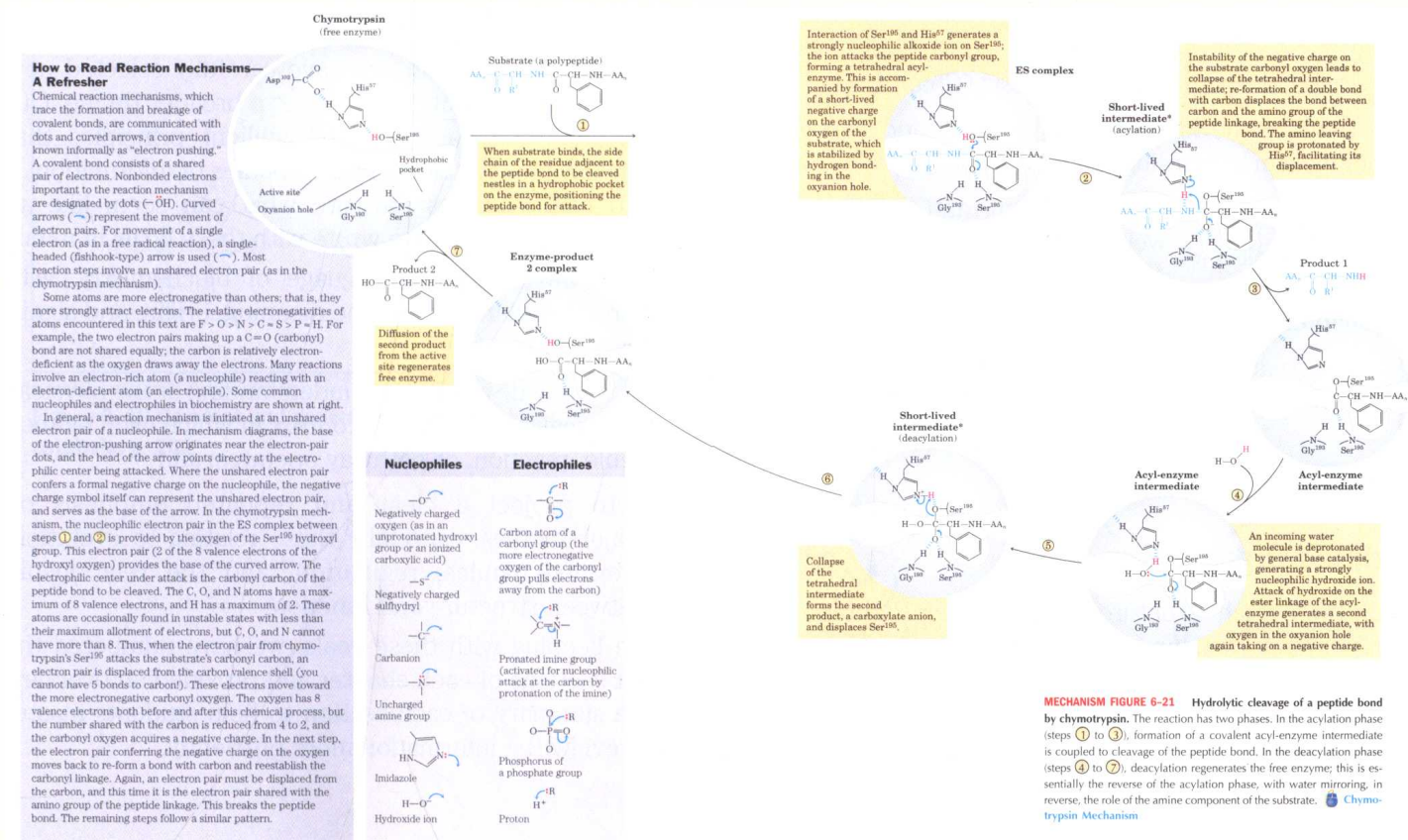
Tryptophan synthase (Chapter 22)

Urea production: origin of amino groups

(multiple reactions) (Chapter 18) **NEW**

Many more mechanisms are provided in abbreviated form.

Mechanism Figure 6–21 Hydrolytic cleavage of a peptide bond by chymotrypsin. pp. 216–217



More Medically Relevant Examples



This icon is used throughout the book to denote material of special medical interest.

We have added many new and fascinating examples relating biochemistry to medicine to help students see the relevance of biochemistry to their lives. Some of the medical examples we have added in this edition:

- Scurvy (Chapter 4)
- Carbon monoxide poisoning (Chapter 5)
- Role of ABC transporters in multidrug resistance (Chapter 11)
- Adverse physiological consequences of defective ion channels (Chapter 11)
- Defect in the PTEN gene that causes tumors (Chapter 12)
- Treatment of erectile dysfunction with sildenafil (Viagra) (Chapter 12)
- Lactose intolerance (Chapter 14)
- Role of the pentose phosphate pathway in prevention of oxidative damage (Chapter 14)
- Exacerbation of Wernicke-Korsakoff syndrome by a defect in transketolase (Chapter 14)
- Glycogen storage diseases (Chapter 15)
- Disease caused by genetic defects in fatty acyl-CoA dehydrogenases (Chapter 17)
- Zellweger syndrome and X-linked adrenoleukodystrophy (Chapter 17)
- Refsum's disease (Chapter 17)
- Kidney stones (Chapter 18)
- Diabetes and glyceroneogenesis (Chapter 21)
- Heme as the source of bile pigments and its colorful metabolism in bruising (Chapter 22)
- Obesity as a medical problem, and the hormonal regulation of body weight (Chapter 23)
- Adiponectin and insulin resistance in mice (Chapter 23)
- RNAi and its potential uses in medicine (Chapter 28)

Updated Organization

While the overall structure of *Lehninger Principles of Biochemistry* remains consistent with previous editions, we have made a few key changes in the organization of the fourth edition, in consultation with many teachers and students.

- Earlier placement of the thoroughly revised chapter on **DNA-based information technologies** (Chapter 9). This includes recent advances in genomics and proteomics and introduces the human and other genomes. Providing this material early in the text allows us to refer to important findings from molecular genetics throughout subsequent chapters.

- **Glycolysis and gluconeogenesis** presented in a single chapter (Chapter 14); the parallels between these pathways are thus more easily seen.

- A new chapter on the **regulation of metabolism** (Chapter 15). Using the pathways of glycolysis, gluconeogenesis, glycogenolysis, and glycogenesis to illustrate general principles, we discuss the various mechanisms by which cells and organisms regulate their metabolic affairs. We also introduce the principles of **metabolic control analysis**, a powerful way to determine which enzymes limit the flux through a pathway, and say goodbye to the concept of a single limiting step.

- Reorganization of the introductory material previously presented in three chapters and its condensation into one chapter, in which we describe the cellular, chemical, physical, genetic, and evolutionary **foundations of biochemistry** (Chapter 1). For students who would benefit from more background information on biology and chemistry, third edition Chapters 1 to 3 are available on the book's website, www.whfreeman.com/lehninger.

Focus on Fundamental Principles

As in the third edition, we have carefully selected new material that illustrates or clarifies a broader theme. Our major objective has been to highlight principles that rationalize and systematize large bodies of information.

To help students navigate what can seem like an ocean of information, we wrote with these goals in mind:

- to introduce the language of biochemistry, with careful explanations of the meaning, origin, and significance of terms;
- to provide a balanced understanding of the physical, chemical, and biological context in which each biomolecule, reaction, or pathway operates; and
- to project a clear and repeated emphasis on major themes, especially those relating to evolution, thermodynamics, regulation, and the relationship between structure and function.

In keeping with these goals, we have numbered the major sections of each chapter and provided an overview and a summary of each section to help students master and review the information more efficiently.

Supplements

Instructor's Resource CD-ROM with Test Bank; 0-7167-5953-5

To help instructors create their own websites and orchestrate dynamic lectures, the two-disc IRCD contains:

- **Animated enzyme mechanisms**, which show key mechanisms step-by-step, in Flash format
- **Living Graphs**, which bring to life important equations from the text by allowing the user to see the graphic result of changing the parameters
- **Test Bank**, organized by chapter in both PDF files and editable Word files
- **All the figures** and tables from the text in JPEG and PowerPoint formats, **optimized for projection** with enhanced colors, higher resolution, and enlarged fonts for easy reading in the lecture hall

Overhead Transparency Set; 0-7167-5956-X

The full-color transparency set contains 150 key illustrations from the text that have been **optimized** with enlarged labels, bolder lines, and more vivid colors that project more clearly for lecture hall presentation.

Printed Test Bank, Fourth Edition, by Terry Platt and Eugene Barber (University of Rochester Medical Center) and David L. Nelson and Brook Chase Soltvedt (University of Wisconsin–Madison); 0-7167-5952-7

The new *Test Bank* contains many new multiple-choice and short-answer problems and solutions—approximately 50 problems and solutions per chapter. Each problem is keyed to the corresponding chapter of the text and rated by level of difficulty. The *Test Bank* is also available on the IRCD, organized by chapter, as PDF and editable Word files.

Website at www.whfreeman.com/lehninger

Created with both instructors and students in mind, the website features:

- **All the figures** and tables from the book **optimized for projection**, available in PowerPoint and JPEG format; also available on the IRCD
- **Animated enzyme mechanisms**, showing key mechanisms step-by-step, in Flash format
- **Living Graphs**, dynamically illustrating equations and graphed material featured in the text

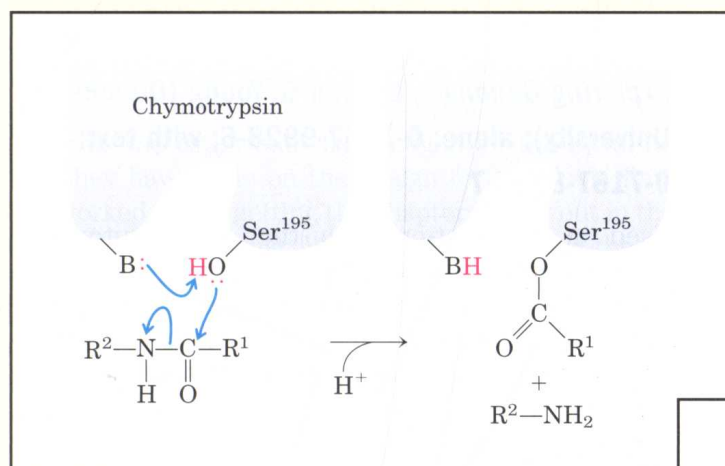
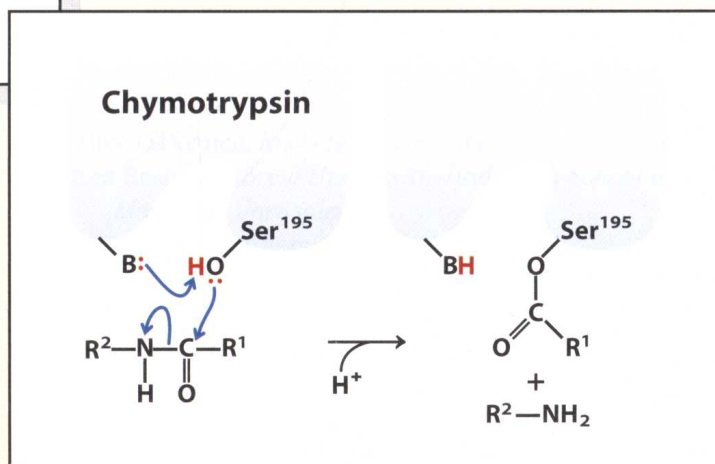


Figure 6-10 Covalent and general acid-base catalysis. p. 202

Figure 6-10 Optimized for projection



- **Biochemistry in 3D molecular structure tutorials**, self-paced interactive tutorials based on the Chemscape Chime molecular visualization browser plug-in; allow students to explore the basic and advanced topics covered in the textbook
- **Online quizzing** for each chapter, a new way for students to review material and prepare for exams
- **Flashcards** on key terms from the text
- Bonus material from *Lehninger Principles of Biochemistry*, Third Edition, fundamental Chapters 1, 2, and 3, which instructors find useful for students requiring a basis for the study of biochemistry



Throughout the text, this icon alerts the reader to a website connection

***The Absolute, Ultimate Guide to Lehninger Principles of Biochemistry, Fourth Edition, Study Guide and Solutions Manual*, by Marcy Osgood (University of New Mexico School of Medicine) and Karen Ocorr (University of California, San Diego); 0-7167-5955-1**

The Absolute, Ultimate Guide combines an innovative study guide with a reliable solutions manual in one convenient volume. Thoroughly class-tested, it includes for each chapter:

- **Major Concepts:** a roadmap through the chapter
- **What to Review:** questions that recap key points from previous chapters
- **Discussion Questions:** provided for each section; designed for individual review, study groups, or classroom discussions
- **A Self-Test:** “Do you know the terms?”; cross-word puzzles; multiple-choice, fact-driven questions; and questions that ask students to apply their new knowledge in new directions—plus answers!

- **Biochemistry on the Internet problems:** provided for many chapters, to encourage students to use the Web to solve problems
- **Complete and Detailed Solutions:** solutions for all end-of-chapter problems

A poster-size **Cellular Metabolic Map** is packaged with the Guide, on which students can draw the reactions and pathways of metabolism in their proper cellular compartments. To order the Cell Map separately, use ISBN 0-7167-9950-2.

Lecture Notebook; 0-7167-5954-3

For students who find that they are too busy copying drawings to keep up with the lecture, the *Notebook* is an indispensable classroom companion, offering

- **Illustrations** in the order in which they appear in the textbook, with plenty of room to take notes
- **Essential reaction equations** and **mathematical equations** with identifying labels
- Complete **pathway diagrams** and **individual reaction diagrams** for all metabolic pathways in the book
- **References** that key the material in the text to the book's website.
- **Three-hole-punched, perforated pages** so that students can reorganize pages in any order necessary to follow instructor's lectures, and can insert instructor handouts

***Exploring Genomes*, by Paul G. Young (Queens University); alone: 0-7167-9928-6; with text: 0-7167-8995-7**

Used in conjunction with the online tutorials at www.whfreeman.com/young, *Exploring Genomes* guides students through live searches and analyses on the most commonly used National Center for Biotechnology Information (NCBI) databases.

Acknowledgments

Our writing has been supported by many people whose advice and encouragement were critical throughout the years of revision, and we are indebted to all of them. At W. H. Freeman and Company, the organization of the project was overseen by Kate Ahr and Sara Tenney and developed by Morgan Ryan. Kate, Sara, and Morgan were part of our immediate production team. Their creative input and constructive challenges inspired many of the improvements in this edition. The final manuscript benefited from the superb copyediting of Linda Strange (who has been involved in the writing of every edition—this is number 6—of Lehninger's biochemistry texts!). Our project editor, Jane O'Neill, has kept us all on track if not always on schedule, showing inordinate patience. Our indexer, Ellen Brennan, has compiled a great index on a very tight schedule. We thank our production coordinator, Paul Rohloff, for helping us to produce the book on time. In Madison, we relied heavily on Brook Soltvedt for her invaluable editorial and organizational input, involving every phase of the project. Brook helped the two authors find a similar voice and avoid redundancies, while keeping the countless pieces of the project moving. Shelley Lusetti read and expertly critiqued the page proofs for every chapter. It was a great privilege to work with so talented and energetic a production team, and they have all had much to do with the quality of the book before you.

The new art for this edition includes work from Fine Line Illustrations, coordinated by Shawn Churchman. We have been impressed and gratified to witness the process by which our often indecipherable scribbles have been transformed into the elegant figures that grace this book. We thank Rae Grant and Blake Logan for the beautiful work they have done on the design for the book. Paul Lacy worked magic getting the chapters to lay out in the new two-column format. We have already noted the invaluable contribution of Jean-Yves Sgro, who prepared all the molecular graphics. The photographs used in this edition were carefully tracked down by Vikii Wong. Melanie Mays and Nick Tymoczko arranged for the many reviewers of the text and, with Jeff Ciprioni, coordinated the development of the supplemental materials—the website, *Test Bank*, *Lecture Notebook*, overhead transparencies, PowerPoint files, and *Absolute, Ultimate Guide*. Marcy Osgood of the University of New Mexico School of Medicine and Karen Ocorr of the University of California, San Diego, made helpful comments on the textbook manuscript as they revised their study guide for this edition. Five of the boxes in the book were written by Lou Pech of Carroll College.

One of the special advantages we have had is our access to the extraordinary community of researchers at the University of Wisconsin–Madison. More often than not, the expertise we needed to tie down a loose end or develop a new topic was right at hand. Our colleagues provided us with timely data, critiqued sections

of the text, helped us develop figures, answered our questions, and helped in innumerable other ways. Space precludes a detailing of every contribution, but we are especially grateful to the following colleagues at the University of Wisconsin–Madison:

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The book has benefited enormously from the combined wisdom and experience of countless reviewers. Every

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Contents in Brief

Preface v

1 The Foundations of Biochemistry 1

I STRUCTURE AND CATALYSIS 45

- 2 Water 47
- 3 Amino Acids, Peptides, and Proteins 75
- 4 The Three-Dimensional Structure of Proteins 116
- 5 Protein Function 157
- 6 Enzymes 190
- 7 Carbohydrates and Glycobiology 238
- 8 Nucleotides and Nucleic Acids 273
- 9 DNA-Based Information Technologies 306
- 10 Lipids 343
- 11 Biological Membranes and Transport 369
- 12 Biosignaling 421

II BIOENERGETICS AND METABOLISM 481

- 13 Principles of Bioenergetics 489
- 14 Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway 521
- 15 Principles of Metabolic Regulation: Glucose and Glycogen 560
- 16 The Citric Acid Cycle 601
- 17 Fatty Acid Catabolism 631
- 18 Amino Acid Oxidation and the Production of Urea 656
- 19 Oxidative Phosphorylation and Photophosphorylation 690
- 20 Carbohydrate Biosynthesis in Plants and Bacteria 751
- 21 Lipid Biosynthesis 787
- 22 Biosynthesis of Amino Acids, Nucleotides, and Related Molecules 833
- 23 Hormonal Regulation and Integration of Mammalian Metabolism 881

III INFORMATION PATHWAYS 921

- 24 Genes and Chromosomes 923
- 24 DNA Metabolism 948
- 26 RNA Metabolism 995
- 27 Protein Metabolism 1034
- 28 Regulation of Gene Expression 1081

Appendix A Common Abbreviations in the Biochemical Research Literature A-1

Appendix B Abbreviated Solutions to Problems AS-1

Glossary G-1

Credits C-1

Index I-1

CONTENTS

1 The Foundations of Biochemistry 1

1.1 Cellular Foundations 3

- Cells Are the Structural and Functional Units of All Living Organisms 3
- Cellular Dimensions Are Limited by Oxygen Diffusion 4
- There Are Three Distinct Domains of Life 4
- Escherichia coli* Is the Most-Studied Prokaryotic Cell 5
- Eukaryotic Cells Have a Variety of Membranous Organelles, Which Can Be Isolated for Study 6
- The Cytoplasm Is Organized by the Cytoskeleton and Is Highly Dynamic 9
- Cells Build Supramolecular Structures 10
- In Vitro Studies May Overlook Important Interactions among Molecules 11

1.2 Chemical Foundations 12

- Biomolecules Are Compounds of Carbon with a Variety of Functional Groups 13
- Cells Contain a Universal Set of Small Molecules 14
- Macromolecules Are the Major Constituents of Cells 15
- Box 1-1 Molecular Weight, Molecular Mass, and Their Correct Units 15**
- Three-Dimensional Structure Is Described by Configuration and Conformation 16
- Box 1-2 Louis Pasteur and Optical Activity: *In Vino, Veritas* 19**
- Interactions between Biomolecules Are Stereospecific 20

1.3 Physical Foundations 21

- Living Organisms Exist in a Dynamic Steady State, Never at Equilibrium with Their Surroundings 21
- Organisms Transform Energy and Matter from Their Surroundings 22
- The Flow of Electrons Provides Energy for Organisms 22
- Creating and Maintaining Order Requires Work and Energy 23
- Energy Coupling Links Reactions in Biology 23
- Box 1-3 Entropy: The Advantages of Being Disorganized 24**
- K_{eq} and ΔG Are Measures of a Reaction's Tendency to Proceed Spontaneously 26
- Enzymes Promote Sequences of Chemical Reactions 26
- Metabolism Is Regulated to Achieve Balance and Economy 27

1.4 Genetic Foundations 28

- Genetic Continuity Is Vested in Single DNA Molecules 29
- The Structure of DNA Allows for Its Replication and Repair with Near-Perfect Fidelity 29
- The Linear Sequence in DNA Encodes Proteins with Three-Dimensional Structures 29

1.5 Evolutionary Foundations 31

- Changes in the Hereditary Instructions Allow Evolution 31
- Biomolecules First Arose by Chemical Evolution 32
- Chemical Evolution Can Be Simulated in the Laboratory 32
- RNA or Related Precursors May Have Been the First Genes and Catalysts 32
- Biological Evolution Began More Than Three and a Half Billion Years Ago 34
- The First Cell Was Probably a Chemoheterotroph 34
- Eukaryotic Cells Evolved from Prokaryotes in Several Stages 34
- Molecular Anatomy Reveals Evolutionary Relationships 36

- Functional Genomics Shows the Allocations of Genes to Specific Cellular Processes 38
- Genomic Comparisons Will Have Increasing Importance in Human Biology and Medicine 38

I STRUCTURE AND CATALYSIS 45

2 Water 47

2.1 Weak Interactions in Aqueous Systems 47

- Hydrogen Bonding Gives Water Its Unusual Properties 47
- Water Forms Hydrogen Bonds with Polar Solutes 49
- Water Interacts Electrostatically with Charged Solutes 50
- Entropy Increases as Crystalline Substances Dissolve 51
- Nonpolar Gases Are Poorly Soluble in Water 52
- Nonpolar Compounds Force Energetically Unfavorable Changes in the Structure of Water 52
- van der Waals Interactions Are Weak Interatomic Attractions 54
- Weak Interactions Are Crucial to Macromolecular Structure and Function 54
- Solutes Affect the Colligative Properties of Aqueous Solutions 56

Box 2-1 Touch Response in Plants: An Osmotic Event 59

2.2 Ionization of Water, Weak Acids, and Weak Bases 60

- Pure Water Is Slightly Ionized 60
- The Ionization of Water Is Expressed by an Equilibrium Constant 61
- The pH Scale Designates the H^+ and OH^- Concentrations 61

Box 2-2 The Ion Product of Water: Two Illustrative Problems 62

- Weak Acids and Bases Have Characteristic Dissociation Constants 63
- Titration Curves Reveal the pK_a of Weak Acids 64

2.3 Buffering against pH Changes in Biological Systems 65

- Buffers Are Mixtures of Weak Acids and Their Conjugate Bases 66
- A Simple Expression Relates pH, pK_a , and Buffer Concentration 66
- Weak Acids or Bases Buffer Cells and Tissues against pH Changes 67

Box 2-3 Solving Problems Using the Henderson-Hasselbalch Equation 67

Box 2-4 Blood, Lungs, and Buffer: The Bicarbonate Buffer System 69

2.4 Water as a Reactant 69

2.5 The Fitness of the Aqueous Environment for Living Organisms 70

3 Amino Acids, Peptides, and Proteins 75

3.1 Amino Acids 75

- Amino Acids Share Common Structural Features 76
- The Amino Acid Residues in Proteins Are L Stereoisomers 77
- Amino Acids Can Be Classified by R Group 78
- Uncommon Amino Acids Also Have Important Functions 80
- Amino Acids Can Act as Acids and Bases 81

Box 3-1 Absorption of Light by Molecules: The Lambert-Beer Law 82

- Amino Acids Have Characteristic Titration Curves 82
- Titration Curves Predict the Electric Charge of Amino Acids 84
- Amino Acids Differ in Their Acid-Base Properties 84

3.2 Peptides and Proteins 85

- Peptides Are Chains of Amino Acids 85
- Peptides Can Be Distinguished by Their Ionization Behavior 86
- Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes 86
- Polypeptides Have Characteristic Amino Acid Compositions 87
- Some Proteins Contain Chemical Groups Other Than Amino Acids 88
- There Are Several Levels of Protein Structure 88

3.3 Working with Proteins 89

- Proteins Can Be Separated and Purified 89
- Proteins Can Be Separated and Characterized by Electrophoresis 92
- Unseparated Proteins Can Be Quantified 94

3.4 The Covalent Structure of Proteins 96

- The Function of a Protein Depends on Its Amino Acid Sequence 96
- The Amino Acid Sequences of Millions of Proteins Have Been Determined 96
- Short Polypeptides Are Sequenced Using Automated Procedures 97
- Large Proteins Must Be Sequenced in Smaller Segments 99
- Amino Acid Sequences Can Also Be Deduced by Other Methods 100

Box 3-2 Investigating Proteins with Mass Spectrometry 102

- Small Peptides and Proteins Can Be Chemically Synthesized 104
- Amino Acid Sequences Provide Important Biochemical Information 106

3.5 Protein Sequences and Evolution 106

- Protein Sequences Can Elucidate the History of Life on Earth 107

4 The Three-Dimensional Structure of Proteins 116

4.1 Overview of Protein Structure 116

- A Protein's Conformation Is Stabilized Largely by Weak Interactions 117
- The Peptide Bond Is Rigid and Planar 118

4.2 Protein Secondary Structure 120

- The α Helix Is a Common Protein Secondary Structure 120
- Amino Acid Sequence Affects α Helix Stability 121

Box 4-1 Knowing the Right Hand from the Left 122

- The β Conformation Organizes Polypeptide Chains into Sheets 123

- β Turns Are Common in Proteins 123

- Common Secondary Structures Have Characteristic Bond Angles and Amino Acid Content 124

4.3 Protein Tertiary and Quaternary Structures 125

- Fibrous Proteins Are Adapted for a Structural Function 126