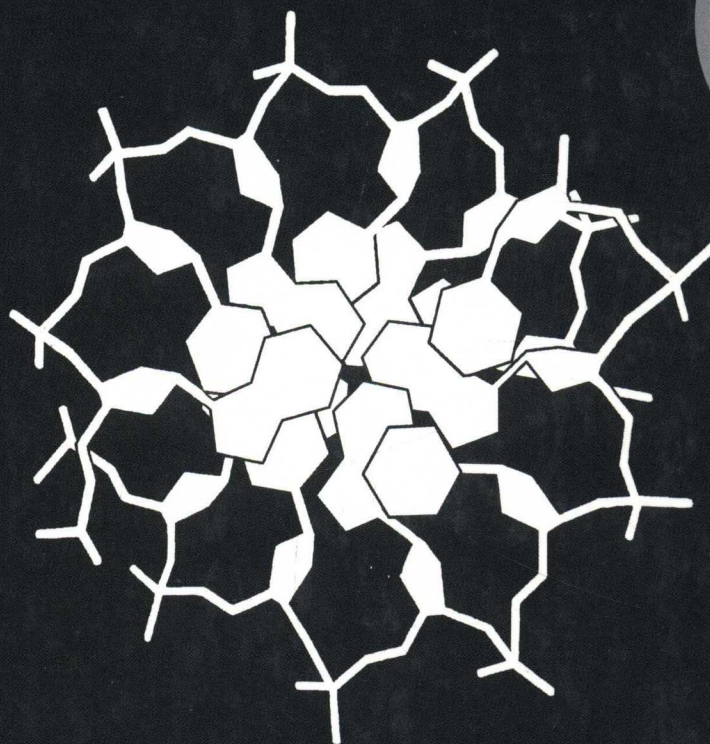


SEVENTH EDITION

Biochemistry

W. H. Freeman
Palgrave Macmillan

International
Edition

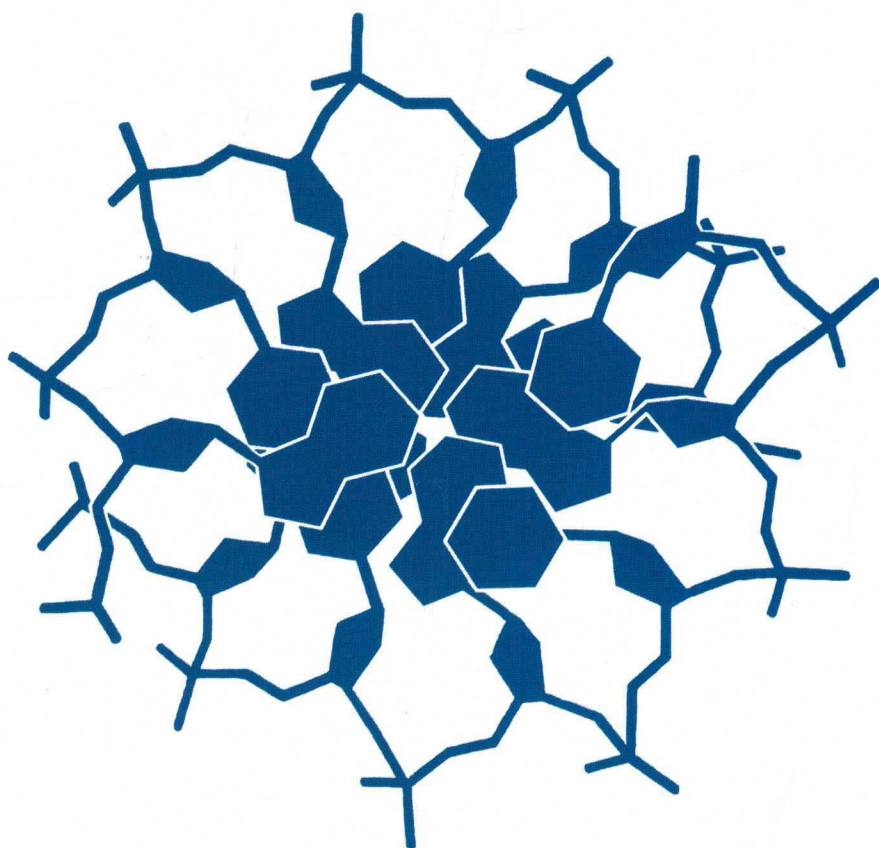


Jeremy M. Berg
John L. Tymoczko
Lubert Stryer

THIS EDITION IS FOR USE OUTSIDE THE USA AND CANADA

INTERNATIONAL SEVENTH EDITION

Biochemistry



Jeremy M. Berg

John L. Tymoczko

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with

Gregory J. Gatto, Jr.

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To our teachers and our students

ABOUT THE AUTHORS

JEREMY M. BERG received his B.S. and M.S. degrees in Chemistry from Stanford (where he did research with Keith Hodgson and Lubert Stryer) and his Ph.D. in Chemistry from Harvard with Richard Holm. He then completed a postdoctoral fellowship with Carl Pabo in Biophysics at Johns Hopkins University School of Medicine. He was an Assistant Professor in the Department of Chemistry at Johns Hopkins from 1986 to 1990. He then moved to Johns Hopkins University School of Medicine as Professor and Director of the Department of Biophysics and Biophysical Chemistry, where he remained until 2003. He then became Director of the National Institute of General Medical Sciences at the National Institutes of Health. He is an elected Fellow of the American Association for the Advancement of Science and an elected member of the Institute of Medicine of the National Academy of Sciences. He received the American Chemical Society Award in Pure Chemistry (1994) and the Eli Lilly Award for Fundamental Research in Biological Chemistry (1995), was named Maryland Outstanding Young Scientist of the Year (1995), received the Harrison Howe Award (1997), the Distinguished Service Award from the Biophysical Society (2009), and the Howard K. Schachman Public Service Award from the American Society for Biochemistry and Molecular Biology (2011). He also received numerous teaching awards, including the W. Barry Wood Teaching Award (selected by medical students), the Graduate Student Teaching Award, and the Professor's Teaching Award for the Preclinical Sciences. He is coauthor, with Stephen J. Lippard, of the textbook *Principles of Bioinorganic Chemistry*.

JOHN L. TYMOCZKO is Towsley Professor of Biology at Carleton College, where he has taught since 1976. He currently teaches Biochemistry, Biochemistry Laboratory, Oncogenes and the Molecular Biology of Cancer, and Exercise Biochemistry and coteaches an introductory course, Energy Flow in Biological Systems. Professor

Tymoczko received his B.A. from the University of Chicago in 1970 and his Ph.D. in Biochemistry from the University of Chicago with Shutsung Liao at the Ben May Institute for Cancer Research. He then had a postdoctoral position with Hewson Swift of the Department of Biology at the University of Chicago. The focus of his research has been on steroid receptors, ribonucleoprotein particles, and proteolytic processing enzymes.

LUBERT STRYER is Winzer Professor of Cell Biology, Emeritus, in the School of Medicine and Professor of Neurobiology, Emeritus, at Stanford University, where he has been on the faculty since 1976. He received his M.D. from Harvard Medical School. Professor Stryer has received many awards for his research on the interplay of light and life, including the Eli Lilly Award for Fundamental Research in Biological Chemistry, the Distinguished Inventors Award of the Intellectual Property Owners' Association, and election to the National Academy of Sciences and the American Philosophical Society. He was awarded the National Medal of Science in 2006. The publication of his first edition of *Biochemistry* in 1975 transformed the teaching of biochemistry.

GREGORY J. GATTO, JR., received his A.B. degree in Chemistry from Princeton University, where he worked with Martin F. Semmelhack and was awarded the Everett S. Wallis Prize in Organic Chemistry. In 2003, he received his M.D. and Ph.D. degrees from the Johns Hopkins University School of Medicine, where he studied the structural biology of peroxisomal targeting signal recognition with Jeremy M. Berg and received the Michael A. Shanoff Young Investigator Research Award. He then completed a postdoctoral fellowship in 2006 with Christopher T. Walsh at Harvard Medical School, where he studied the biosynthesis of the macrolide immunosuppressants. He is currently an Investigator in the Heart Failure Discovery Performance Unit at GlaxoSmithKline Pharmaceuticals.

PREFACE

In writing this seventh edition of *Biochemistry*, we have balanced the desire to present up-to-the minute advances with the need to make biochemistry as clear and engaging as possible for the student approaching the subject for the first time. Instructors and students have long relied on *Biochemistry* for:

- **Clear writing** The language of biochemistry is made as accessible as possible. A straightforward and logical organization leads the reader through processes and helps navigate complex pathways and mechanisms.
- **Single-concept illustrations** Illustrations in this book address one point at a time so that each illustration clearly tells the story of a mechanism, pathway, or process without the distraction of excess detail.
- **Physiological relevance** Biochemistry is the study of life on the smallest scale, and it has always been our goal to help students connect biochemistry to their own lives. Pathways and processes are presented in a physiological context so that the reader can see how biochemistry works in different parts of the body and under different environmental and hormonal conditions.
- **Clinical insights** Wherever appropriate, pathways and mechanisms are applied to health and disease. These applications show students how biochemistry is relevant to them while reinforcing the concepts that they have just learned. (For a full list, see p. xi.)
- **Evolutionary perspective** Evolution is evident in the structures and pathways of biochemistry and is woven into the narrative of the textbook. (For a full list, see p. x.)

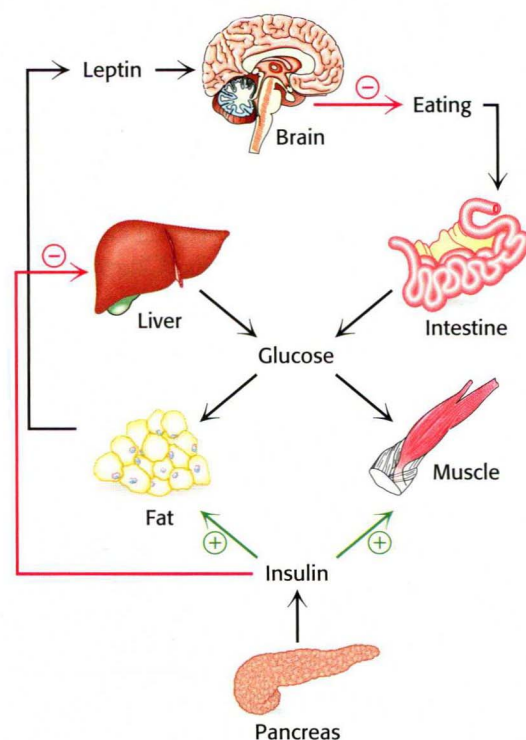
New to This Edition

Researchers are making new discoveries in biochemistry every day. The seventh edition takes into account the discoveries that have changed how we think about the fundamental concepts in biochemistry and human health. New aspects of the book include:

- **Metabolism integrated in a new context** New information about the role of leptins in hunger and satiety has greatly influenced how we think about obesity and the growing epidemic of diabetes. In this edition, we cover the integration of metabolism in the context of diet and obesity.
- **New chapters on gene regulation** To relate to the rapidly growing understanding of the biochemical aspect of eukaryotic gene regulation,

we have greatly expanded our discussion of regulation and have split the chapter in the preceding editions into two: Chapter 31, “The Control of Gene Expression in Prokaryotes,” and Chapter 32, “The Control of Gene Expression in Eukaryotes.” These chapters address recent discoveries such as quorum sensing in prokaryotes, induced pluripotent stem cells, and the role of microRNAs in regulating gene expression.

- **Experimental techniques updated and clarified** We have revised Chapters 3 (“Exploring Proteins and Proteomes”), 5 (“Exploring Genes and Genomes”), and 6 (“Exploring Evolution and Bioinformatics”) to give students a practical understanding of the benefits and limitations of the techniques that they will be using in the laboratory. We have expanded explanations of mass spectrometry and x-ray crystallography, for instance, and made them even clearer for the first-time student. We explain new techniques such as next-generation sequencing and real-time PCR in the context of their importance to modern research in biochemistry. (For a full list, see p. xii.)



Chapter 27 A schematic representation illustrates a few of the many metabolic pathways that must be coordinated to meet the demands of living.

Recent Advances

Some of the exciting advances and new topics that we present in the seventh edition include:

- Osteogenesis imperfecta, or brittle bone disease (Chapter 2)
- Intrinsically unstructured proteins and metamorphic proteins (Chapter 2)
- Recent updates in protein-misfolding diseases (Chapter 2)
- The use of recombinant DNA technology in protein purification (Chapter 3)
- Expanded discussion of mass spectrometry and x-ray crystallography (Chapter 3)
- Next-generation sequencing methods (Chapter 5)
- Real-time PCR (Chapter 5)
- DNA microarrays (Chapter 5)
- Carbon monoxide poisoning (Chapter 7)
- Single-molecule studies of enzyme kinetics (Chapter 8)
- Myosins as a model of a catalytic strategy for ATP hydrolysis (Chapter 9)
- Glycobiology and glycomics (Chapter 11)
- Hurler disease (Chapter 11)
- Avian influenza H5N1 (Chapter 11)
- Lipid rafts (Chapter 12)
- Transferrin as an example of receptor-mediated endocytosis (Chapter 12)
- Long QT syndrome and arrhythmia caused by the inhibition of potassium channels (Chapter 13)
- Defects in the citric acid cycle and the development of cancer (Chapter 17)
- Synthesizing a more efficient rubisco (Chapter 20)
- The structure of mammalian fatty acid synthetase (Chapter 22)
- Pyrimidine salvage pathways (Chapter 25)
- Physical association of enzymes in metabolic pathways (Chapter 25)
- Phosphatidic acid phosphatase in the regulation of lipid metabolism (Chapter 26)
- The regulation of SCAP-SREBP movement in cholesterol metabolism (Chapter 26)
- Mutations in the LDL receptor (Chapter 26)
- The role of HDL in protecting against arteriosclerosis (Chapter 26)

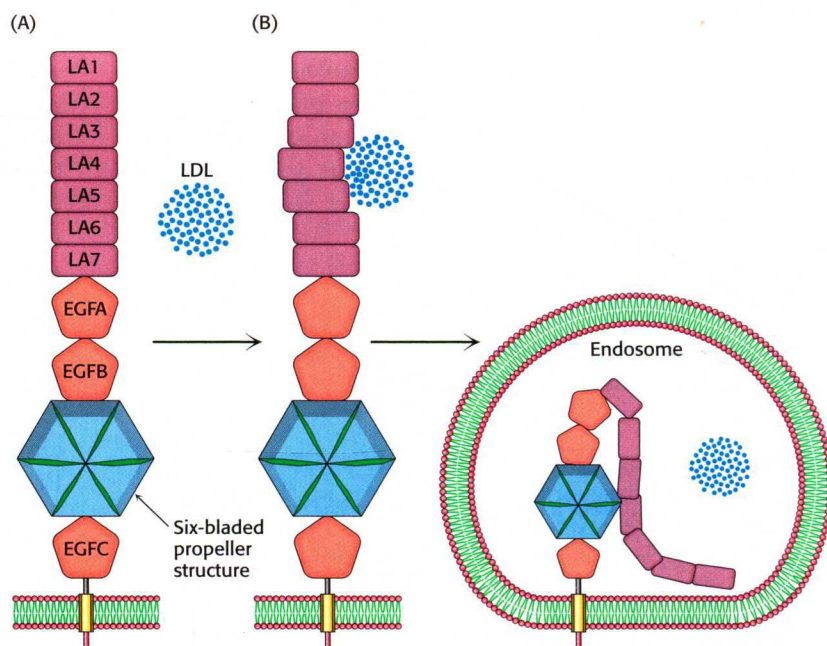


Figure 26.24 LDL receptor releases LDL in the endosomes. [After I. D. Campbell, *Biochem. Soc. Trans.* 31:1107–1114, 2003, Fig 1A.]

- Aromatase inhibitors in the treatment of breast and ovarian cancer (Chapter 26)
- The role of leptin in long-term caloric homeostasis (Chapter 27)
- Obesity and diabetes (Chapter 27)
- Exercise and its effects on cellular biochemistry (Chapter 27)
- Updated detailed mechanism of helicase's action (Chapter 28)
- Updated detailed mechanism of topoisomerase's action (Chapter 28)
- Riboswitches (Chapter 29)
- The production of small regulatory RNAs (Chapter 29)
- Vanishing white matter disease (Chapter 30)
- Quorum sensing (Chapter 31)
- Biofilms (Chapter 31)
- Induced pluripotent stem cells (Chapter 32)
- The role of microRNAs in gene regulation (Chapter 32)
- How vaccines work (Chapter 34)
- The structure of myosin head domains (Chapter 35)

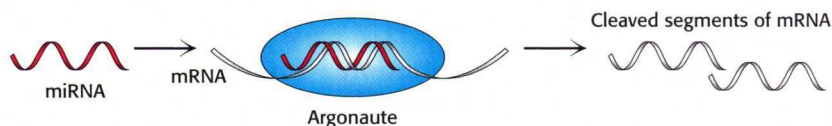


Figure 32.27 MicroRNA action.

New End-of-Chapter Problems

Biochemistry is best learned by practicing it. In addition to many traditional problems that test biochemical knowledge and the ability to use this knowledge, we have three categories of problems to address specific problem-solving skills.

- **Mechanism problems** ask students to suggest or elaborate a chemical mechanism.
- **Data interpretation problems** ask questions about a set of data provided in tabulated or graphic form. These problems give students a sense of how scientific conclusions are reached.
- **Chapter integration problems** require students to use information from several chapters to reach a solution. These problems reinforce a student's awareness of the interconnectedness of the different aspects of biochemistry.

Brief answers to these problems are presented at the end of the book; expanded solutions are available in the accompanying *Student Companion*.

Visualizing Molecular Structure

All molecular structures have been selected and rendered by Jeremy Berg and Gregory Gatto. To help students read and understand these structures, we include the following tools:

- A **molecular-model “primer”** explains the different types of protein models and examines their strengths and weaknesses (see appendices to Chapters 1 and 2).
- **Figure legends** direct students explicitly to the key features of a model.

- A great variety of types of molecular structures are represented, including clearer renderings of membrane proteins.
- For most molecular models, the **PDB number** at the end of the figure legend gives the reader easy access to the file used in generating the structure from the Protein Data Bank Web site (www.pdb.org). At this site, a variety of tools for visualizing and analyzing the structure are available.
- **Living figures** for most molecular structures now appear on the Web site in Jmol to allow students to **rotate three-dimensional molecules** and view alternative renderings online.

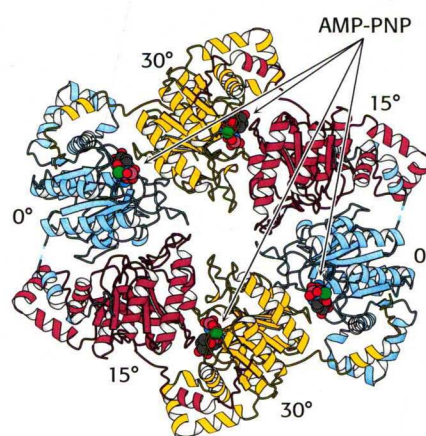


Figure 28.12 Helicase asymmetry. Notice that only four of the subunits, those shown in blue and yellow, bind AMP-PNP. [Drawn from 1EOK.pdb.]

Media and Supplements

A full package of media resources and supplements provides instructors and students with innovative tools to support a variety of teaching and learning approaches.

Companion Web Site

www.whfreeman.com/berg7e

For students

- **Living figures** allow students to explore protein structure in 3-D. Students can zoom and rotate the “live” structures to get a better understanding of their three-dimensional nature and can experiment with different display styles (space-filling, ball-and-stick, ribbon, backbone) by means of a user-friendly interface.
- **Concept-based tutorials** by Neil D. Clarke help students build an intuitive understanding of some of the more difficult concepts covered in the textbook.
- **Animated techniques** help students grasp experimental techniques used for exploring genes and proteins.
- The **self-assessment tool** helps students evaluate their progress. Students can test their understanding by taking an online multiple-choice quiz provided for each chapter, as well as a general chemistry review.
- The **glossary** of key terms.
- **Web links** connect students with the world of biochemistry beyond the classroom.

For Instructors

All of the student resources plus:

- All **illustrations and tables** from the textbook, in jpeg and PowerPoint formats optimized for classroom projection.
- The **Assessment Bank** offers more than 1500 questions in editable Microsoft Word format.

Instructor's Resource DVD

[1-4292-8411-0]

The CD includes all the instructor's resources from the Web site.

Overhead Transparencies

[1-4292-8412-9]

200 full-color illustrations from the textbook, optimized for classroom projection

Student Companion

[1-4292-8735-7]

For each chapter of the textbook, the *Student Companion* includes:

- Chapter Learning Objectives and Summary
- Self-Assessment Problems, including multiple-choice, short-answer, matching questions, and challenge problems, and their answers
- Expanded Solutions to end-of-chapter problems in the textbook

Molecular Evolution



This icon signals the start of the many discussions that highlight protein commonalities or other molecular evolutionary insights.

- Only L amino acids make up proteins (p. 27)
- Why this set of 20 amino acids? (p. 33)
- Additional human globin genes (p. 219)
- Fetal hemoglobin (p. 221)
- Catalytic triads in hydrolytic enzymes (p. 268)
- Major classes of peptide-cleaving enzymes (p. 271)
- Zinc-based active sites in carbonic anhydrases (p. 279)
- Common catalytic core in type II restriction enzymes (p. 286)
- P-loop NTPase domains (p. 291)
- Conserved catalytic core in protein kinases (p. 312)
- Why might human blood types differ? (p. 345)
- Archaeal membranes (p. 362)
- Ion pumps (p. 386)
- P-type ATPases (p. 390)
- ATP-binding cassettes (p. 390)
- Sequence comparisons of Na⁺ and Ca⁺ channels (p. 398)
- Small G proteins (p. 424)
- Metabolism in the RNA world (p. 463)
- Why is glucose a prominent fuel? (p. 471)
- NAD⁺ binding sites in dehydrogenases (p. 485)
- The major facilitator superfamily of transporters (p. 493)
- Isozymic forms of lactate dehydrogenase (p. 506)
- Evolution of glycolysis and gluconeogenesis (p. 507)
- The α -ketoglutarate dehydrogenase complex (p. 525)
- Domains of succinyl CoA synthase (p. 527)
- Evolution of the citric acid cycle (p. 536)
- Mitochondria evolution (p. 545)
- Conserved structure of cytochrome c (p. 561)
- Common features of ATP synthase and G proteins (p. 568)
- Related uncoupling proteins (p. 575)
- Chloroplast evolution (p. 588)
- Evolutionary origins of photosynthesis (p. 604)
- Evolution of the C₄ pathway (p. 620)
- The coordination of the Calvin cycle and the pentose phosphate pathway (p. 629)
- Evolution of glycogen phosphorylase (p. 649)
- Increasing sophistication of glycogen phosphorylase regulation (p. 650)
- The α -amylase family (p. 651)
- A recurring motif in the activation of carboxyl groups (p. 669)
- Prokaryotic counterparts of the ubiquitin pathway and the proteasome (p. 701)
- A family of pyridoxal-dependent enzymes (p. 708)
- Evolution of the urea cycle (p. 712)
- The P-loop NTPase domain in nitrogenase (p. 732)
- Similar transaminases determine amino acid chirality (p. 737)
- Feedback inhibition (p. 748)
- Recurring steps in purine ring synthesis (p. 767)
- Ribonucleotide reductases (p. 773)
- Increase in urate levels during primate evolution (p. 780)
- The cytochrome P450 superfamily (p. 811)
- DNA polymerases (p. 851)
- Thymine and the fidelity of the genetic message (p. 871)
- Sigma factors in bacterial transcription (p. 890)
- Similarities in transcription between archaea and eukaryotes (p. 901)
- Evolution of spliceosome-catalyzed splicing (p. 913)
- Classes of aminoacyl-tRNA synthetases (p. 931)
- Composition of the primordial ribosome (p. 934)
- Homologous G proteins (p. 937)
- A family of proteins with common ligand-binding domains (p. 962)
- The independent evolution of DNA-binding sites of regulatory proteins (p. 963)
- Regulation by attenuator sites (p. 968)
- CpG islands (p. 982)
- Iron-response elements (p. 988)
- miRNAs in gene evolution (p. 990)
- The odorant-receptor family (p. 997)
- Photoreceptor evolution (p. 1007)
- The immunoglobulin fold (p. 1024)
- Relationship of actin to hexokinase and prokaryotic proteins (p. 1061)

Clinical Applications



This icon signals the start of a clinical application in the text. Additional, briefer clinical correlations appear in the text as appropriate.

- Osteogenesis imperfecta (p. 45)
- Protein-misfolding diseases (p. 55)
- Protein modification and scurvy (p. 55)
- Antigen detection with ELISA (p. 90)
- Synthetic peptides as drugs (p. 98)
- Gene therapy (p. 173)
- Functional magnetic resonance imaging (p. 205)
- Carbon monoxide poisoning (p. 221)
- Sickle-cell anemia (p. 217)
- Thalassemia (p. 218)
- Aldehyde dehydrogenase deficiency (p. 240)
- Action of penicillin (p. 252)
- Protease inhibitors (p. 272)
- Carbonic anhydrase and osteoporosis (p. 274)
- Isozymes as a sign of tissue damage (p. 307)
- Emphysema (p. 316)
- Vitamin K (p. 320)
- Hemophilia (p. 321)
- Tissue-type plasminogen activator (p. 322)
- Monitoring changes in glycosylated hemoglobin (p. 335)
- Erythropoietin (p. 340)
- Hurler disease (p. 341)
- Blood groups (p. 345)
- I-cell disease (p. 346)
- Influenza virus binding (p. 349)
- Clinical applications of liposomes (p. 366)
- Aspirin and ibuprofen (p. 370)
- Digitalis and congenital heart failure (p. 389)
- Multidrug resistance (p. 390)
- Long QT syndrome (p. 404)
- Signal-transduction pathways and cancer (p. 434)
- Monoclonal antibodies as anticancer drugs (p. 435)
- Protein kinase inhibitors as anticancer drugs (p. 435)
- Vitamins (p. 457)
- Lactose intolerance (p. 487)
- Galactosemia (p. 488)
- Exercise and cancer (p. 494)
- Phosphatase deficiency (p. 532)
- Defects in the citric acid cycle and the development of cancer (p. 533)
- Beriberi and mercury poisoning (p. 535)
- Mitochondrial diseases (p. 576)
- Hemolytic anemia (p. 629)
- Glucose 6-phosphate deficiency (p. 631)
- Glycogen-storage diseases (p. 656)
- Carnitine deficiency (p. 670)
- Zellweger syndrome (p. 676)
- Diabetic ketosis (p. 679)
- The use of fatty acid synthase inhibitors as drugs (p. 687)
- Effects of aspirin on signaling pathways (p. 689)
- Diseases resulting from defects in E3 proteins (p. 700)
- Diseases of altered ubiquitination (p. 702)
- Using proteasome inhibitors to treat tuberculosis (p. 703)
- Inherited defects of the urea cycle (hyperammonemia) (p. 712)
- Alcaptonuria, maple syrup urine disease, and phenylketonuria (p. 721)
- High homocysteine levels and vascular disease (p. 743)
- Inherited disorders of porphyrin metabolism (p. 754)
- Anticancer drugs that block the synthesis of thymidylate (p. 775)
- Adenosine deaminase and severe combined immunodeficiency (p. 778)
- Gout (p. 779)
- Lesch-Nyhan syndrome (p. 780)
- Folic acid and spina bifida (p. 781)
- Second messengers derived from sphingolipids and diabetes (p. 793)
- Respiratory distress syndrome and Tay-Sachs disease (p. 793)
- Diagnostic use of blood-cholesterol levels (p. 802)
- Hypercholesterolemia and atherosclerosis (p. 804)
- Mutations in the LDL receptor (p. 805)
- The role of HDL in protecting against arteriosclerosis (p. 806)
- Clinical management of cholesterol levels (p. 807)
- Aromatase inhibitors in the treatment of breast and ovarian cancer (p. 813)
- Rickets and vitamin D (p. 814)
- Antibiotics that target DNA gyrase (p. 861)
- Blocking telomerase to treat cancer (p. 867)
- Huntington disease (p. 872)
- Defective repair of DNA and cancer (p. 872)
- Detection of carcinogens (Ames test) (p. 873)
- Antibiotic inhibitors of transcription (p. 893)
- Burkitt lymphoma and B-cell leukemia (p. 901)
- Diseases of defective RNA splicing (p. 909)
- Vanishing white matter disease (p. 942)
- Antibiotics that inhibit protein synthesis (p. 943)
- Diphtheria (p. 944)
- Ricin, a lethal protein-synthesis inhibitor (p. 945)
- Induced pluripotent stem cells (p. 980)
- Anabolic steroids (p. 984)
- Color blindness (p. 1008)
- The use of capsaicin in pain management (p. 1012)
- Immune-system suppressants (p. 1030)
- MHC and transplantation rejection (p. 1038)
- AIDS vaccine (p. 1039)
- Autoimmune diseases (p. 1041)
- Immune system and cancer (p. 1041)
- Vaccines (p. 1042)
- Charcot-Marie-Tooth disease (p. 1058)
- Taxol (p. 1061)

Tools and Techniques

The seventh edition of *Biochemistry* offers three chapters that present the tools and techniques of biochemistry: “Exploring Proteins and Proteomes” (Chapter 3), “Exploring Genes and Genomes” (Chapter 5), and “Exploring Evolution and Bioinformatics” (Chapter 6). Additional experimental techniques are presented throughout the book, as appropriate.

Exploring Proteins and Proteomes (Chapter 3)

Protein purification (p. 68)
Differential centrifugation (p. 69)
Salting out (p. 70)
Dialysis (p. 71)
Gel-filtration chromatography (p. 71)
Ion-exchange chromatography (p. 71)
Affinity chromatography (p. 72)
High-pressure liquid chromatography (p. 73)
Gel electrophoresis (p. 73)
Isoelectric focusing (p. 75)
Two-dimensional electrophoresis (p. 76)
Qualitative and quantitative evaluation of protein purification (p. 77)
Ultracentrifugation (p. 78)
Edman degradation (p. 82)
Protein sequencing (p. 84)
Production of polyclonal antibodies (p. 88)
Production of monoclonal antibodies (p. 88)
Enzyme-linked immunoabsorbent assay (ELISA) (p. 90)
Western blotting (p. 91)
Fluorescence microscopy (p. 91)
Green fluorescent protein as a marker (p. 91)
Immunoelectron microscopy (p. 93)
MALDI-TOF mass spectrometry (p. 93)
Tandem mass spectrometry (p. 95)
Proteomic analysis by mass spectrometry (p. 96)
Automated solid-phase peptide synthesis (p. 97)
X-ray crystallography (p. 100)
Nuclear magnetic resonance spectroscopy (p. 103)
NOESY spectroscopy (p. 104)

Exploring Proteins (other chapters)

Basis of fluorescence in green fluorescent protein (p. 58)
Using irreversible inhibitors to map the active site (p. 249)
Enzyme studies with catalytic antibodies (p. 251)
Single-molecule studies (p. 254)

Exploring Genes and Genomes (Chapter 5)

Restriction-enzyme analysis (p. 147)
Southern and northern blotting techniques (p. 148)
Sanger dideoxy method of DNA sequencing (p. 149)
Solid-phase synthesis of nucleic acids (p. 150)
Polymerase chain reaction (PCR) (p. 151)
Recombinant DNA technology (p. 154)
DNA cloning in bacteria (p. 155)
Creating cDNA libraries (p. 160)

Mutagenesis techniques (p. 162)
Next-generation sequencing (p. 166)
Quantitative PCR (p. 167)
Examining expression levels (DNA microarrays) (p. 168)
Introducing genes into eukaryotes (p. 169)
Transgenic animals (p. 170)
Gene disruption (p. 170)
Gene disruption by RNA interference (p. 171)
Tumor-inducing plasmids (p. 172)

Exploring Genes (other chapters)

Density-gradient equilibrium sedimentation (p. 123)
Chromatin immunoprecipitation (ChIP) (p. 981)

Exploring Evolution and Bioinformatics (Chapter 6)

Sequence-comparison methods (p. 182)
Sequence-alignment methods (p. 184)
Estimating the statistical significance of alignments (by shuffling) (p. 185)
Substitution matrices (p. 186)
Performing a BLAST database search (p. 189)
Sequence templates (p. 192)
Detecting repeated motifs (p. 192)
Mapping secondary structures through RNA sequence comparisons (p. 194)
Construction of evolutionary trees (p. 195)
Combinatorial chemistry (p. 196)
Molecular evolution in the laboratory (p. 197)

Other Techniques

Functional magnetic resonance imaging (fMRI) (p. 205)
Sequencing of carbohydrates by using MALDI-TOF mass spectroscopy (p. 346)
The use of liposomes to investigate membrane permeability (p. 365)
The use of hydropathy plots to locate transmembrane helices (p. 372)
Fluorescence recovery after photobleaching (FRAP) for measuring lateral diffusion in membranes (p. 373)
Patch-clamp technique for measuring channel activity (p. 395)
Measurement of redox potential (p. 546)

▶ Animated Techniques

Animated explanations of experimental techniques used for exploring genes and proteins are available at www.whfreeman.com/berg7e.

Acknowledgments

Thanks go first and foremost to our students. Not a word was written or an illustration constructed without the knowledge that bright, engaged students would immediately detect vagueness and ambiguity. We also thank our colleagues who supported, advised, instructed, and simply bore with us during this arduous task. We are also grateful to our colleagues throughout the world who patiently answered our questions and shared their insights into recent developments.

Fareed Aboul-El
Louisiana State University

Paul Adams
University of Arkansas, Fayetteville

Kevin Ahern
Oregon State University

Edward Behrman
Ohio State University

Donald Beitz
Iowa State University

Sanford Bernstein
San Diego State University

Martin Brock
Eastern Kentucky University

W. Malcom Byrnes
Howard University College of Medicine

C. Britt Carlson
Brookdale Community College

Graham Carpenter
Vanderbilt University

Jun Chung
Louisiana State University

Michael Cusanovich
University of Arizona

David Daleke
Indiana University

Margaret Daugherty
Colorado College

Dan Davis
University of Arkansas, Fayetteville

Mary Farwell
East Carolina University

Brent Feske
Armstrong Atlantic University

Wilson Francisco
Arizona State University

Masaya Fujita
University of Houston, University Park

Peter Gegenheimer
University of Kansas

John Goers
California Polytechnic University, San Luis Obispo

Neena Grover
Colorado College

Paul Hager
East Carolina University

Frans Huijing
University of Miami

Nitin Jain
University of Tennessee

Gerwald Jogl
Brown University

Kelly Johanson
Xavier University of Louisiana

Todd Johnson
Weber State University

Michael Kalafatis
Cleveland State University

Mark Kearly
Florida State University

Sung-Kun Kim
Baylor University

Roger Koeppe
University of Arkansas, Fayetteville

Dmitry Kolpashchikov
University of Central Florida

John Koontz
University of Tennessee

Glen Legge
University of Houston, University Park

John Stephen Lodmell
University of Montana

Timothy Logan
Florida State University

Michael Massiah
Oklahoma State University

Diana McGill
Northern Kentucky University

Michael Mendenhall
University of Kentucky

David Merkle
University of South Florida

Gary Merrill
Oregon State University

Debra Moriarity
University of Alabama, Huntsville

Patricia Moroney
Louisiana State University

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University of Michigan, Dearborn

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Ohio University
Laura Zapanta
University of Pittsburgh

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