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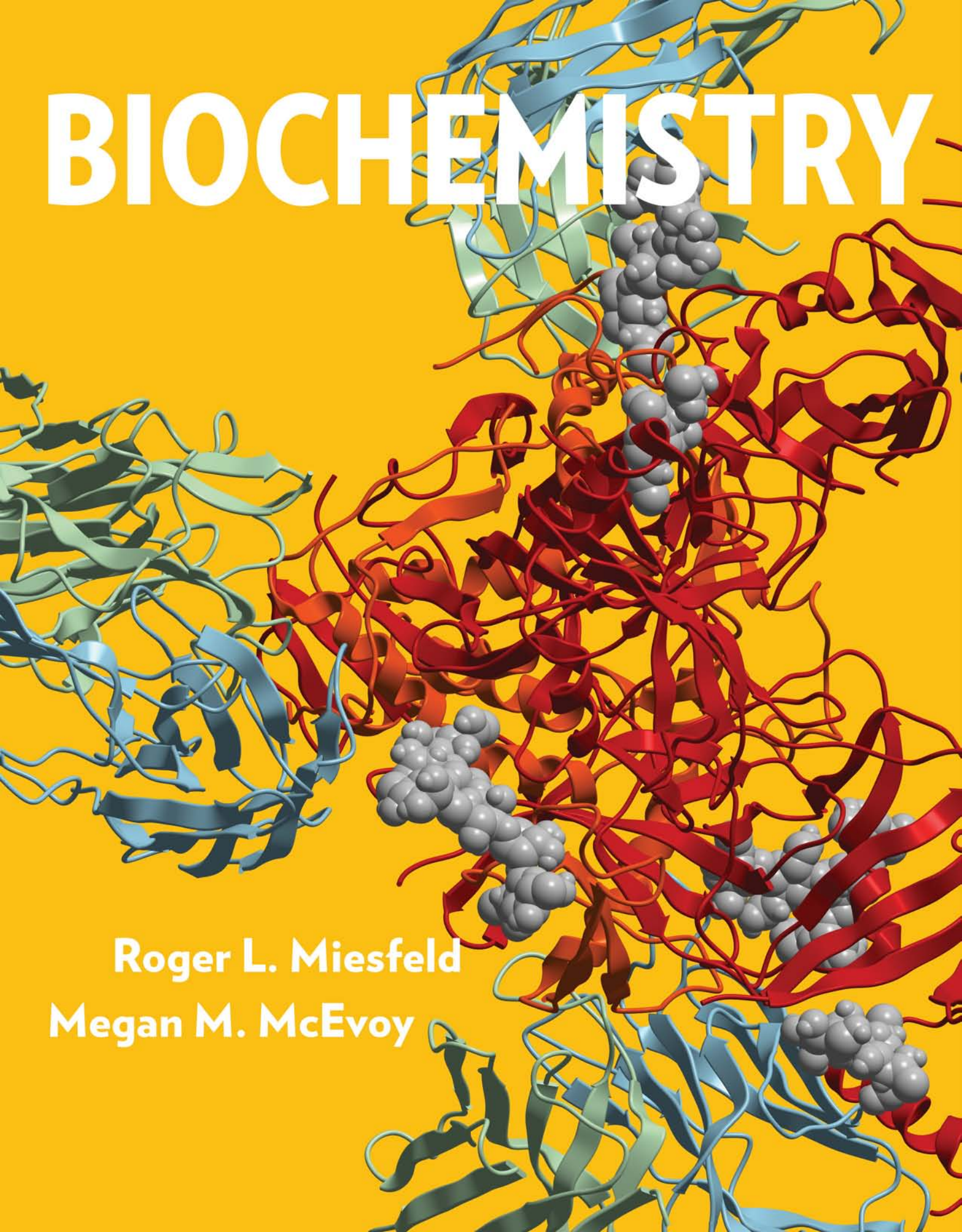

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BIOCHEMISTRY

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New York London

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To my academic mentors who taught me the importance of communicating science using clear and concise sentences—David C. Shepard, Norman Arnheim, Keith R. Yamamoto, and Michael A. Wells—and to my family for their patience and support.

—Roger L. Miesfeld

To the many people who have fostered my development as a scientist and educator, particularly my mentors Harry Noller, Kathy Triman, Jim Remington, and Rick Dahlquist, and to my family and friends who make every day a joy.

—Megan M. McEvoy

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Preface

This book was conceived more than 15 years ago when W. W. Norton editor Jack Repcheck popped his head into Roger Miesfeld's office one sunny afternoon in Tucson, Arizona. Jack had just seen Roger's new textbook on molecular genetics in the bookstore and had been impressed with the illustrations. He said, "Dr. Miesfeld, how would you like to author a full-color textbook that takes the same visual approach to biochemistry as you did for the topic of molecular genetics?" And with those fateful words began a conversation, and then the creation of a textbook that focuses on how biochemistry relates to the world around us without relying on rote memorization of facts by students. In 2011, Roger's colleague at the University of Arizona and next-door-office neighbor, Megan McEvoy, who is also an instructor of a large biochemistry service course, mentioned that she would be eager to work on a textbook that would improve pedagogy in the field. Thus, this project, which began years ago with a simple question, has resulted in the publication of the first truly new biochemistry textbook in decades.

Meanwhile, we (Roger and Megan) have been teaching biochemistry to undergraduate, graduate, and medical school students for nearly 40 years combined and have loved every minute of it—seriously. During this time, we noticed that many biochemistry textbooks seemed to sidestep a very basic question in the minds of most students: "Why do I need to learn biochemistry?" To answer this question in the classroom, we developed a number of story lines that revolve around a simple premise: how it works and why it matters. We used the assigned textbook to fill in the details for our students but used the in-class lectures to provide the context the students needed to see the big picture. During this same time, the Internet became much more accessible so that it was almost trivial to find the name of an enzyme in a metabolic reaction or the equation required for calculating changes in free energy.

But despite the ease with which "info-bytes" could be obtained, and often simply memorized, what still required thought was integration of these pieces of information to fully understand concepts such as allosteric regulation of an enzyme, rates of metabolic flux, or the importance of weak noncovalent interactions in assembling gene transcription complexes. We challenged the students in our classes to approach each biochemical process—especially those that are conceptually the most difficult—to answer the questions how does it work and why does it matter to me. The "it" could be a cancer drug that inhibits an enzyme, an external stimulus that activates a signaling pathway and controls blood sugar, or a biochemical assay that measures gene expression levels. We told them that to answer the how it works part, they would have to explain the biochemical process in clear and concise language, while the why it matters part required them to make it relevant to their own life experience.

As we collected more and more of these "how and why" examples over the years, it became clear to us that our biochemistry textbook should focus on presenting core concepts in a relatable way centered around three themes: (1) the interdependence of energy conversion processes, (2) the role of signal transduction in metabolic regulation, and (3) biochemical processes affecting human health and disease. The pedagogical foundation for each of these themes is that molecular structure determines chemical function. In developing the outline for the book, we ignored the urge to write it like an automobile owner's manual in which all of the parts are listed first (proteins, lipids, carbohydrates, nucleic acids), and then the function of the car (metabolic pathways) is described by assembling the parts in a systematic way (easy to memorize).

Instead, we chose to organize the book using five core blocks (collections of chapters, or parts) that consist of modules (individual chapters) made up of concept-based submodules (numbered chapter sections) with limited, focused, unnumbered subsections. The five core blocks we chose are "Part 1: Principles of Biochemistry" (Chapters 1–3), "Part 2: Protein Biochemistry" (Chapters 4–8), "Part 3: Energy Conversion Pathways" (Chapters 9–12), "Part 4: Metabolic Regulation" (Chapters 13–19), and "Part 5: Genomic Regulation" (Chapters 20–23). This organization provides the student with an opportunity to work through related concepts before moving on to new ones. For example, what is needed to understand protein structure and function is presented in Part 2, including how proteins function as enzymes or as relay partners in a signal transduction pathway. In Part 4, carbohydrate structure and function (Chapter 13) and carbohydrate metabolism (Chapter 14) are paired together, as are lipid structure and function (Chapter 15) and lipid metabolism (Chapter 16),

while the structure of nitrogen-based biomolecules and their metabolism are presented together in Chapters 17 (amino acids) and 18 (nucleotides).

The figures in our book have been paramount since the very beginning; indeed, it was a commitment by W. W. Norton to a modern art program that hooked Roger in the first place. So we created each chapter starting with a collection of 30–40 hand-drawn illustrations or Web images that were complemented with molecular renderings based on Protein Data Bank (PDB) files and with photographs of people, places, or things. At the beginning of each chapter section, the topic is presented broadly, and then the reader is led into the themed concepts. With regularity, examples of everyday biochemistry are woven into the story line to provide an opportunity to step back for a moment and see the relevance of the topic to life around us. In our classes, we tell the students to use the everyday biochemistry examples as a way to make it personal, rather than as more info-bytes to memorize. The point of these examples is to generate excitement about biochemistry so that the student can get through the more difficult concepts knowing there is a good reason to push ahead—it is likely to be relevant.

Instructors may engage students more fully in the beauty of the world's biological diversity using this book's chemical framework, which frequently rises into the cellular level. One could follow our sequence through Parts 1–5 as we do in our classes or mix and match using a sequence that works best for the instructor. Students can likewise use our book as a biochemistry reference and read sections individually without having to read the book cover to cover. There are plenty of online materials and ancillary tools that have been developed for instructors and students, and we urge you to take full advantage of them.

Finally, we encourage you to look for new examples of everyday biochemistry and send the details to us so that we can add them to the collection for future editions.

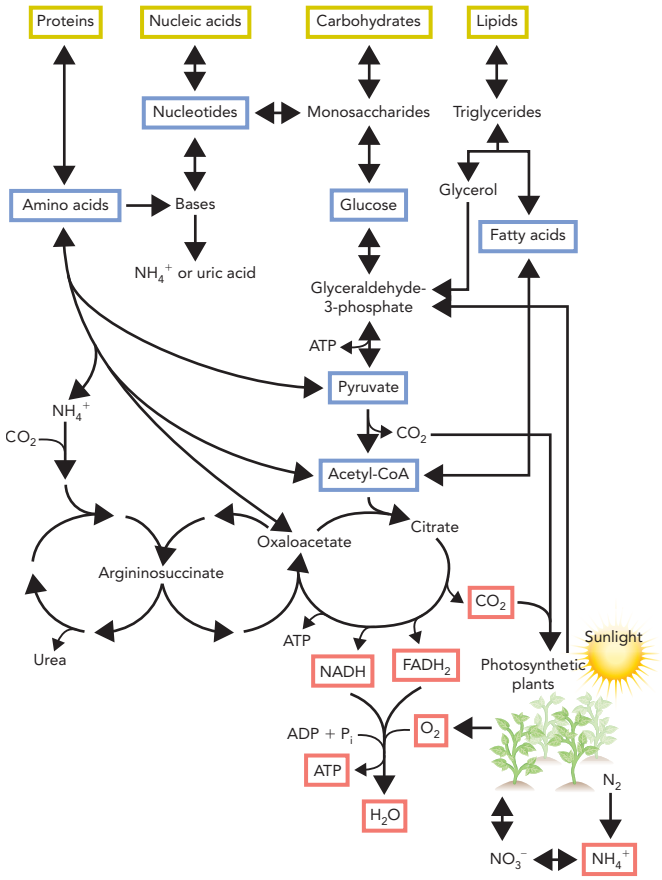
Roger L. Miesfeld
Megan M. McEvoy

Authors' Tour of the Book Features

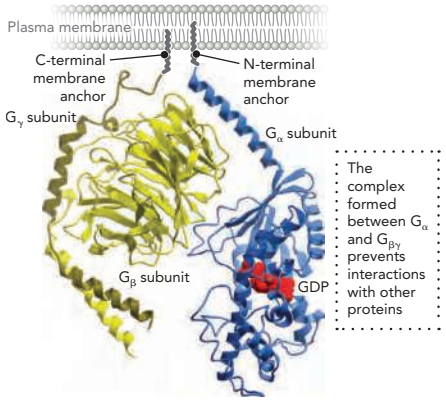
The Only Textbook That Makes Visuals the Foundation of Every Chapter

Every figure in this textbook originated in our biochemistry lectures, and our preparation of each chapter involved creating the figures we wanted to include *first* and then writing the text of the chapter to fit those figures. The result is a book in which the figures and the text are inseparable from one another; they are one learning tool that strengthens students' understanding of how biochemical processes and structures work. Specifically:

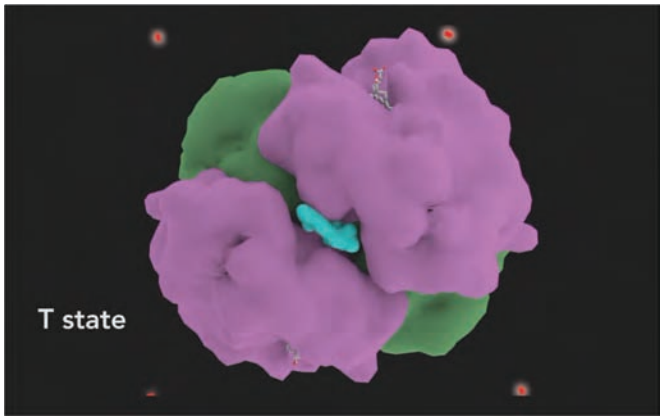
- We've made sure that key chapter figures help students see how biochemistry functions in context. For example, Figure 9.3 in Chapter 9 provides a basic metabolic map that emphasizes the major biomolecules in cells and the interdependence of pathways. On the basis of this detailed figure, Figure 9.4 and similar figures in subsequent chapters of Parts 3 and 4 present simplified, iconic metabolic maps that clearly divide pathways into two discrete groups: those linked to energy conversion (red) and those linked to metabolite synthesis and degradation pathways (blue).



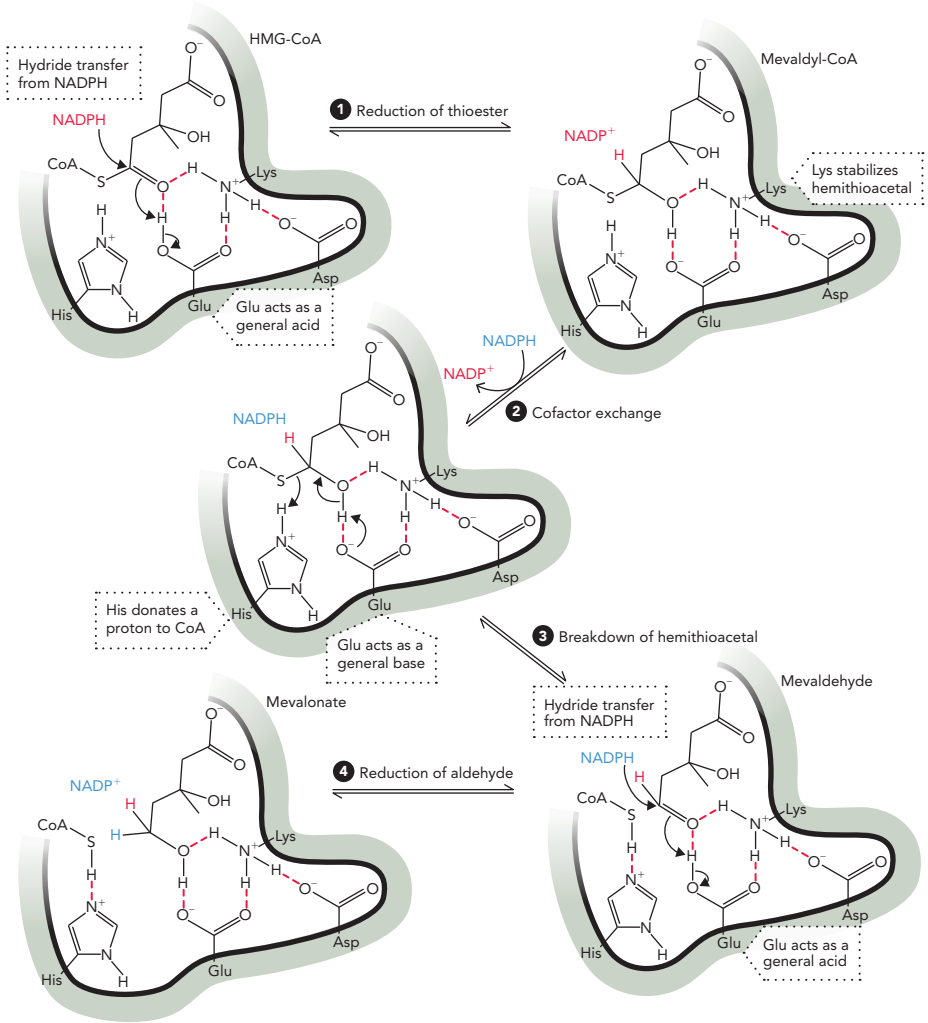
- We've included hundreds of vibrant, precise, and information-rich molecular representations. These figures in the text are paired with state-of-the-art 3D interactive versions in the online homework.



- In the digital resources available to instructors, we are making available cutting-edge process animations—many reflecting state-of-the-art 3D technology—that will strengthen students' understanding of challenging biochemical processes.



- We've added abundant in-figure text boxes, numbered steps, and icons to help students navigate the most complex biochemical processes. Figure 7.35 provides a good example of our art program's pedagogical value: It clearly illustrates a complex four-step reaction through numbered steps, descriptive captions, and a thorough complementary explanation in the text.



Clear Explanations and a Distinctive Chapter Sequence Help Students Make Connections between Concepts

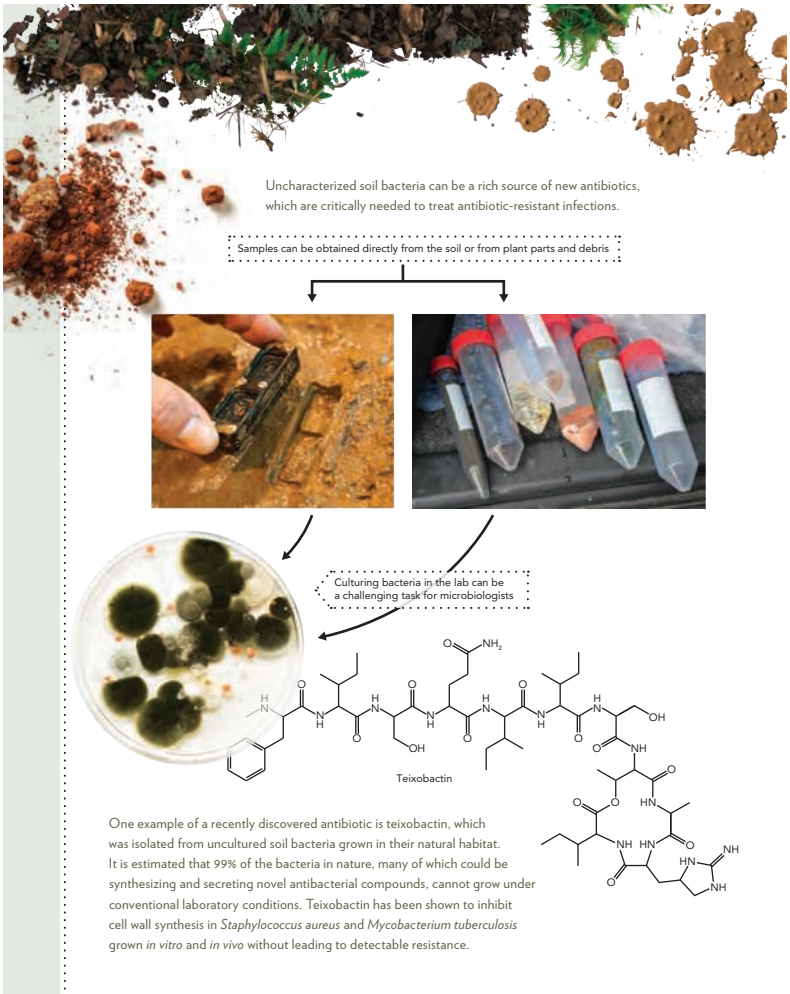
Our distinctive chapter sequence highlights connections between key biochemical processes, encouraging students to move beyond mere memorization to consider *how* biochemistry works.

- In Part 1, we introduce essential, unifying concepts that are interwoven throughout the chapters that follow: hierarchical organization of biochemical complexity; energy conversion in biological systems; the chemical role of water in life processes; the function of cell membranes as hydrophobic barriers; and the central dogma of molecular biology from a biochemical perspective.
- As a capstone to the chapters on protein structure and function (Part 2), we present signal transduction (Chapter 8) as the prototypical example of how proteins work to mediate cellular processes.
- The topical sequence in Parts 3 and 4 underscores the importance of energy conversion as the foundation for all other metabolic pathways, introducing enzyme regulation of metabolic flux as a central theme. In Part 3, we present the pathways involved in energy conversion processes before presenting degradative and biosynthetic pathways in Part 4. This helps students see complex processes and connections between concepts more clearly.
- We present the biomolecular structure and function of carbohydrates, lipids, amino acids, and nucleotides in Part 4 in the context of their metabolic pathways. This integrated approach encourages students to associate biochemical structure with cellular function in a way that promotes deeper understanding.
- Rather than an encyclopedic list of individual reactions that can obscure students' understanding of the important concepts, in Parts 3 and 4 we emphasize the regulation of 10 major (and broadly representative) metabolic pathways, with a special emphasis on the human diseases associated with these pathways.

Unmatched Emphasis on Applications and Biomedical Examples Motivates Learning by Helping Students Connect the Material to both Their Majors and Their Everyday Experience

We know from our teaching that students can be equally engaged by biomedical examples and examples of biochemistry in the world around them. So throughout this book we've reinforced key biochemical concepts with applied examples that show why biochemistry matters.

- Each chapter-opening vignette provides an introduction to a biochemical application connected to the chapter's central topic. Later, we ask students to reexamine the application in light of their newly acquired knowledge of the biochemistry behind it. For example, the opening vignette for Chapter 22 examines how an ingenious laboratory method enabled study of soil bacteria that were previously impossible to culture in the lab, which led to discovery of a new antibiotic. Another example is the opening vignette for Chapter 13, which visually presents the biochemistry behind the commercial product Beano.



One example of a recently discovered antibiotic is teixobactin, which was isolated from uncultured soil bacteria grown in their natural habitat. It is estimated that 99% of the bacteria in nature, many of which could be synthesizing and secreting novel antibacterial compounds, cannot grow under conventional laboratory conditions. Teixobactin has been shown to inhibit cell wall synthesis in *Staphylococcus aureus* and *Mycobacterium tuberculosis* grown *in vitro* and *in vivo* without leading to detectable resistance.

- Real-life examples from nature help students understand how structure (of a protein, lipid, carbohydrate, or nucleic acid) affects function, an important takeaway insight we stress in our biochemistry courses. A great example is the discussion in Chapter 2 concerning antifreeze proteins in fish and insects that live in extreme cold. Threonine amino acids in these proteins line up perfectly with ice crystals and thus prevents them from growing within the animals.
- We distributed human health examples, particularly discussions of human disease, throughout the text. These are especially relevant for the many students planning to pursue careers in medicine or other health-related professions. A prominent example occurs in Chapter 21—the description of a degenerative disease of the retina called retinitis pigmentosa, which is caused by defects in the RNA splicing machinery. This is a surprise to students, who expect that most human disease is the result of enzyme defects.

Thoughtful Pedagogy and Assessment Promotes Mastery of Biochemical Concepts

We feel strongly that myriad boxes and sidebars in textbooks distract from the content of the chapters and are rarely read by students. As a result, this book has a design that is clean and uncluttered.

- A Concept Integration question and its answer occurs at the end of each numbered chapter section. This feature prompts students to think critically about what they're reading and to synthesize concepts in a meaningful way.

concept integration 5.1

A frog species was found to contain a cytosolic liver protein that bound a pharmaceutical drug present at high levels in effluent from a wastewater facility. Describe how this protein could be purified.

The first step in purifying an uncharacterized protein is to develop a method to detect it specifically, such as an enzyme activity assay or binding assay. In this case, the protein is known to bind to a small molecule (pharmaceutical drug), and this binding activity can be used to develop a protein detection assay. The assay could be based on protein binding to the drug that has been radioactively labeled or it might be possible to develop a fluorescently labeled version of the drug that has an altered absorption or emission spectrum as a function of specific protein binding. The next step would be to use cell fractionation, centrifugation, and a combination of gel filtration and ion-exchange column chromatography to enrich for drug binding activity relative to total protein in the frog liver extract. A final step would be to develop an affinity column that contains the drug covalently linked to a solid matrix and use this column to bind specifically, and then elute, the high-affinity binding protein. The purity of the protein would be assessed by SDS-PAGE at several steps within the purification protocol.

concept integration 14.3

Why does it make physiologic sense for muscle glycogen phosphorylase activity to be regulated by both metabolite allosteric control and hormone-dependent phosphorylation?

Muscle glycogen phosphorylase is allosterically activated by AMP, which signals low energy charge in the cell. High AMP levels also indicate a need for glycogen degradation and release of glucose substrate for ATP generation to support muscle contraction. Both ATP and glucose-6-P are allosteric inhibitors of muscle glycogen phosphorylase activity and signal a ready supply of chemical energy without the need for glycogen degradation. Both types of allosteric regulation occur rapidly on a timescale of seconds in response to sudden changes in AMP, ATP, and glucose-6-P levels. Allosteric control by metabolites provides a highly efficient means to control rates of glycogen degradation in response to the immediate energy needs of muscle cells. In contrast, hormonal regulation of muscle glycogen phosphorylase activity by glucagon and epinephrine is a delayed response (occurring on a timescale of hours), resulting in phosphorylation and activation of the enzyme after neuronal and physiologic inputs at the organismal level. Similarly, insulin signaling, which inhibits muscle glycogen phosphorylase activity through dephosphorylation, is also a delayed response at the organismal level and depends on multiple physiologic inputs. Taken together, allosteric regulation of muscle glycogen phosphorylase activity provides a rapid-response control mechanism to modulate muscle glucose levels, whereas hormonal signaling requires input from multiple stimuli at the organismal level and provides a longer-term effect on enzyme activity through covalent modifications.

- We know the quality and quantity of end-of-chapter problems is an important litmus test for many instructors when reviewing textbooks. Our end-of-chapter material includes a plentiful, balanced mix of basic Chapter Review questions and thought-provoking Challenge Problems.
- Online homework is becoming a more and more powerful learning tool for biochemistry courses. Norton's Smartwork5 online homework platform offers book-specific assessment through a wide array of exercises: art-based interactive questions, critical-thinking questions, application questions, process animation questions, and chemistry drawing questions, as well as all of the book's end-of-chapter questions. We are particularly excited to be the first to offer interactive 3D molecular visualization questions within the homework platform. Everything the student needs to interrogate a molecular structure is embedded in Smartwork5 using Molsoft's ICM Browser application.

Resources for Instructors and Students

Smartwork5

This dynamic and powerful online assessment resource uses answer-specific feedback, a variety of engaging question types, the integration of the stunning book art, 3D molecular animations, and process animations to help students visualize and master the important course concepts. Smartwork5 also integrates easily with your campus learning management system and features a simple, intuitive interface, making it an easy-to-use online homework system for both instructors and students.

3D Molecular Animations

Eleven photorealistic 3D molecular animations based on PDB files were created by renowned molecular animator Dr. Janet Iwasa from the Department of Biochemistry at the University of Utah College of Medicine. Janet brings some of the most difficult concepts in biochemistry to life in stunning detail. These animations are available to students in coursepack assessments and through the ebook and are available with associated assessments for instructors to assign in the Smartwork5 homework system. Links to the animations are available to instructors at wwnorton.com/instructors.

Process Animations

Twenty process animations showcase the complex topics that students find most challenging. The animations are available to students in mobile-compatible format in the coursepack and the ebook, as well as online. Assessments written specifically for the animations are included in Smartwork5. Links to the animations are available to instructors at wwnorton.com/instructors.

Ultimate Guide to Teaching with Biochemistry

This enhanced instructor's manual will help any professor enrich his or her course with active learning. Each chapter includes sample lectures, descriptions of the molecular animations with discussion questions and suggestions for classroom use, multimedia suggestions with discussion questions, an active learning activity, a think–pair–share style of activity, book-specific learning objectives, and full solutions. A list of other resources (animations, coursepack resources, and so forth) will also be listed for each chapter to ensure instructors are aware of the many instructor-provided materials available to them. Activity handouts will be available for download at wwnorton.com/instructors for easy printing and distribution.

Coursepacks

Available at no cost to professors or students, Norton Coursepacks for online or hybrid courses are available in a variety of formats, including Blackboard, Desire2Learn (D2L), and Canvas. With just a simple download from the instructor's website, instructors can bring high-quality Norton digital media into a new or existing online course. Content is fully customizable and includes chapter-based assignments with high-quality visual assessments, perfect for distance learning classes or assignments between classes. The coursepack for *Biochemistry* also features the full suite of animations, vocabulary flashcards, and assignments based on 3D animations as well as art from the book—everything students need for a great out-of-the-classroom experience.

PowerPoint Presentations and Figures

PowerPoint slide options meet the needs of every instructor and include lecture PowerPoint slides providing an overview of each chapter, five clicker questions per chapter, and links to animations. There is also a separate set of art PowerPoint slides featuring every photograph and drawn figure from the text. In addition, the PDB files used as the basis for many of the molecular structures in the book are available for download.

Test Bank

The Test Bank for *Biochemistry* is designed to help instructors prepare exams quickly and effectively. Questions are tagged according to Bloom's taxonomy, and each chapter includes approximately 75 multiple-choice and 25 essay questions. Five to ten questions per chapter use art taken directly from the book. In addition to tagging with Bloom's, each question is tagged with metadata that places it in the context of the chapter and assigns it a difficulty level, enabling instructors to easily construct tests that are meaningful and diagnostic.

Ebook

Available for students to purchase online at any time, the *Biochemistry* ebook offers students a great low price, exceptional functionality, and access to the full suite of accompanying resources.

Acknowledgments

This book was a very long time in the making, and it would not have been possible without the hard work, dedication, and care of dozens of people. To begin with, we would like to thank our editors at Norton, the late Jack Repcheck, Vanessa Drake-Johnson, Michael Wright, and last but certainly not least, Betsy Twitchell. Your combination of vision, patience, and persistence kept us going even when the going was rough. Our deepest gratitude to project editor Carla Talmadge, the "master of the schedule," for keeping the innumerable moving parts of our book organized and in forward motion. Our developmental editor, David Chelton, is, simply put, a rock star, and we were so lucky to work with him through the many years that it took to find the perfect balance of chemistry, biology, and everyday biochemistry examples that make this book so remarkable. It can't be easy to copyedit a book this big, but Christopher Curioli brought a level of skill and expertise that was truly remarkable. We owe a huge debt of gratitude to Elyse Rieder, who miraculously tracked down every photograph our hearts desired, and to Ted Szczepanski for being with her every step of the way. We were very fortunate to work with incredibly talented designer Anne DeMarinis on the book design, chapter openers, and cover. It is through Anne's vision that our thousands of pages of manuscript became the beautiful book you're holding in your hands. We must thank the unsung heroes of this project, editorial assistants Taylere Peterson, Katie Callahan, Courtney Shaw, Cait Callahan, Callinda Tayler, and the many who came before them for their hours spent posting files, making copies, mailing proofs, and countless other essential tasks. Production manager Ben Reynolds adeptly managed the process of translating our raw material into the polished final product; for that he has our deepest thanks. The amazing folks at Imagineeringart.com Inc. deserve medals for living up to our high standards for every figure and every page in our book regardless of how many times we sent the artwork back for just one more tweak until we considered it perfect. Thank you to Wynne Au Yeung, Alicia Elliott, and the rest of the Imagineering team.

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The original figures we developed for this book, and the end of chapter review questions and challenge problems, have been used in our classes at the University of Arizona for well over a decade, which means we have had the benefit of constructive feedback from literally thousands of students. We truly appreciate each and every one of these comments as they helped guide the book's development.

We thank our three contributing authors for helping us draft the final chapters in our book—Kelly Johanson, Scott Lefler, and John W. Little. Your effort was the x-factor that got us over the finish line, and for that you have our eternal gratitude. We also want to acknowledge the late Professor Michael A. Wells of the University of Arizona who provided W. W. Norton with the first set of PDB files for homework questions that were similar in many ways to the current set of Smartwork5/Molsoft questions we have today. In addition, we thank Dr. Andrew Orry at Molsoft, LLC (La Jolla, California), who provided personal guidance on how best to use Molsoft's ICM Browser Pro rendering program to create the stunning molecular images we have included in the book and the online materials.

Finally, we thank each and every one of the biochemists who reviewed chapters in our text throughout the years. Your feedback—sometimes positive, sometimes not—has been absolutely invaluable to the development of this book. We are deeply grateful for your willingness to give us your time so that we can benefit from your experience.

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About the Authors

Roger L. Miesfeld is a professor and department head in the Department of Chemistry and Biochemistry at the University of Arizona in Tucson. Dr. Miesfeld's research focus for the past 30 years has been on regulatory mechanisms governing signal transduction in eukaryotic cells. For much of this time, his lab investigated steroid hormone signaling in human disease models, primarily cancer (leukemia and prostate cancer) and asthma. More recently, his research group has been studying metabolic regulation of blood meal metabolism in vector mosquitoes that transmit the dengue and Zika viruses (*Aedes aegypti*). Their current efforts are aimed at identifying mosquito-selective and bio-safe small-molecule inhibitors of processes regulating mosquito eggshell synthesis. Dr. Miesfeld has taught a variety of undergraduate, graduate, and medical school biochemistry courses over the years and now teaches the largest undergraduate biochemistry courses at the University of Arizona. He has authored two other textbooks, *Applied Molecular Genetics* and *Biochemistry: A Short Course*, and was the recipient of the University of Arizona Honors College Faculty Excellence Award.

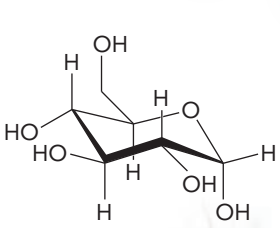
Dr. Miesfeld received his BS and MS degrees in cell biology from San Diego State University, and his PhD in biochemistry from Stony Brook University. He was a Jane Coffin Childs Postdoctoral Fellow in the Department of Biochemistry and Biophysics at the University of California, in San Francisco, before becoming a faculty member at the University of Arizona in 1987.

Megan M. McEvoy is broadly trained as a protein biochemist and structural biologist, and her research work is primarily concerned with how metal ions are handled in microbial systems. She is interested in the general area of how metal ions are acquired when needed or eliminated when in excess. Her work focuses on studies of protein–protein interactions and conformational changes and how metal ions are specifically recognized by proteins. Dr. McEvoy has taught numerous undergraduate biochemistry courses, including courses for majors, nonmajors, and honors students. Along with Dr. Miesfeld, she taught the nonmajors biochemistry courses at the University of Arizona for many years.

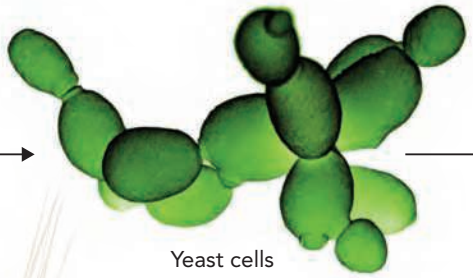
Dr. McEvoy received her BS degree in biochemistry and molecular biology from the University of California, Santa Cruz, and her PhD in chemistry from the University of Oregon. She started her career at the University of Arizona as an assistant professor in the Department of Biochemistry and Molecular Biophysics, then became an associate professor in the Department of Chemistry and Biochemistry. She is now a professor in the Department of Microbiology, Immunology, and Molecular Genetics at the University of California, Los Angeles.

BIOCHEMISTRY

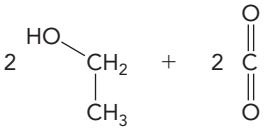
Grapes are fermented by yeast to yield wine



Glucose



Yeast cells



Ethanol Carbon dioxide

Barley is fermented by yeast to yield beer

Grapes and barley are the sources of sugar and natural flavors that are metabolized by live yeast cells to produce alcoholic wine and beer, respectively.

1

Principles of Biochemistry

◀ In the late 1800s, chemists in Europe sought to uncover the chemical basis for alcoholic fermentation in hopes of improving the quantity and quality of beer and wine production. In 1897, the German chemist Eduard Buchner discovered that an extract of yeast cells could be used *in vitro* (outside a living cell) to convert glucose to carbon dioxide and ethanol under anaerobic conditions. The discovery that some yeast proteins could function as chemical catalysts in the fermentation reaction ushered in the modern era of biochemistry.

CHAPTER OUTLINE

The birth of modern biochemistry can be traced to the end of the 19th century, when chemists discovered that cell extracts of brewer's yeast contained everything necessary for alcoholic fermentation. That is, processes associated with living organisms could actually be understood in terms of fundamental chemistry. The reductionist approach of breaking open cells and isolating their components for use in *in vitro* chemical reactions continued for most of the 20th century. During this time, scientists made numerous discoveries in cellular biochemistry that transformed our understanding of the chemical basis of life. These advances included describing the chemical structure and function of the major classes of biomolecules: nucleic acids, proteins, carbohydrates, and lipids. Moreover, thousands of metabolic reactions that direct molecular synthesis and degradation in cells were characterized in bacteria, yeast, plants, and animals. Knowledge gained from these biochemical studies has been used to develop pharmaceutical drugs, medical diagnostic tests, microbial-based industrial processes, and herbicide-resistant plant crops, among other things.

The field of biochemistry enjoyed tremendous growth in the 1970s, when techniques were developed to manipulate deoxyribonucleic acid (DNA) based on an experimental approach that became known as recombinant DNA technology. This achievement led to the creation of the first biotechnology company in 1977, which later went on to use recombinant DNA technology to produce human insulin in bacteria. The following 20 years were an explosive time for biochemical research. In addition to the development of more sophisticated biochemical tools, scientists achieved vast improvements in protein purification and structure determination as a result of new instrumentation and computational power.

Modern biochemistry encompasses both organic chemistry and physical chemistry, as well as areas of microbiology, genetics, molecular biology, cell biology, physiology, and computational biology. In this introductory chapter, we first present an overview of modern biochemistry. We then describe three biochemical principles that together provide a framework for understanding life at the molecular level:

1. The hierarchical organization of biochemical processes within cells, organisms, and ecosystems underlies the chemical basis for life on Earth.
2. DNA is the chemical basis for heredity and encodes the structural information for RNA and protein molecules, which mediate biochemical processes in cells.
3. The function of a biomolecule is determined by its molecular structure, which is fine-tuned by evolution through random DNA mutations and natural selection.

In Chapter 2, we describe three additional biochemical principles:

4. Biological processes follow the same universal laws and thermodynamic principles that govern physical processes.
5. Life depends on water because of its distinctive chemical properties and its central role in biochemical reactions.
6. Biological membranes are selective hydrophobic barriers that define aqueous compartments in which biochemical reactions take place.

1.1 What Is Biochemistry?

Biochemistry aims to explain biological processes at the molecular and cellular levels. As its name implies, biochemistry is at the interface of biology and chemistry. It is a hands-on experimental science that relies heavily on quantitative analysis of data. Biochemists are interested in understanding the structure and function of biological molecules. Biochemical research often involves mechanistic studies that focus on hypothesis-driven experiments designed to answer specific biological questions. Examples include determining how a group of proteins catalyze the synthesis of a complex biomolecule or why biological membranes have different physical properties depending on their chemical composition.

One of the first biochemical processes to be investigated was **fermentation**: the conversion of rotting fruit or grain into solutions of alcohol through the action of yeast. The Egyptians knew as early as 2000 B.C. that crushed dates produce both an intoxicating substance (ethanol) and a caustic acid (acetic acid). The Greeks used "zyme" (yeast) to produce gas (carbon dioxide) in bread and turn grapes into wine. Through the 17th and 18th centuries, great scientific debates centered around the question whether fermentation was the result of an ethereal "vital life force" present in living cells or instead was based only on the fundamental laws of chemistry and physics that govern the physical world. Some scientists reasoned that if fermentation could be shown to occur outside of a living cell, it would provide evidence that a vital life force was not required for this chemical process.

Numerous attempts by Louis Pasteur and others to prepare cell-free extracts from yeast cells failed, which some interpreted to mean that a vital life force was indeed required for fermentation. The turning point came in 1897, when the German chemist Eduard Buchner (**Figure 1.1**) demonstrated that carbon dioxide and ethyl alcohol could in fact be produced from sugar using brewer's yeast extracts in an *in vitro* reaction. Buchner published his observations and proposed that fermentation required the "ferments of zyme," now known as **enzymes**, which function as catalysts to drive the *in vitro* reactions. Buchner's work set a foundation for the field of biochemistry, where *in vitro* studies are the cornerstone for numerous advances in medical science.

As is often the case in an experimental science such as biochemistry, several arbitrary decisions led to the success of Buchner's extracts. First, where Pasteur had used glass to grind up yeast and release the fermentation "juices," Buchner chose to use quartz mixed with diatomaceous earth (*kieselguhr*) to prepare the extract. This choice was a good one because it avoided making the extract alkaline and inactive, which occurs when yeast proteins come in contact with glass. Second, after trying a variety of preservatives to prevent coagulation, Buchner decided to use a 40% sucrose solution, not realizing at the time that this would provide the necessary glucose for alcoholic fermentation. Lastly, Buchner used a strain of yeast called *Saccharomyces cerevisiae*, provided by the local brewery in Munich, to prepare an undiluted cell-free extract. This strain of yeast turned out to work much better than yeast strains available in Paris, where Pasteur had done his experiments years earlier. Although it might appear from this that Buchner's accomplishment of *in vitro* alcoholic fermentation was the result of luck, his optimized protocol was developed only after many failed attempts. Indeed, Buchner's systematic approach to solving the problem of inactive cell-free extracts is a classic example of experimental biochemistry.

As we shall see shortly, all living cells contain enzymes. These biomolecules, either protein or ribonucleic acid (RNA), function as reaction catalysts to increase the rates

Figure 1.1 Biochemical reactions are often studied or used in *in vitro* systems. Eduard Buchner (1860–1917) was the first to demonstrate that cell-free yeast extracts could accomplish *in vitro* fermentation of sugar into alcohol and carbon dioxide, a discovery that led to the birth of modern biochemistry. Buchner was awarded the 1907 Nobel Prize in Chemistry for his groundbreaking research on *in vitro* fermentation.
HULTON ARCHIVE/GETTY IMAGES.

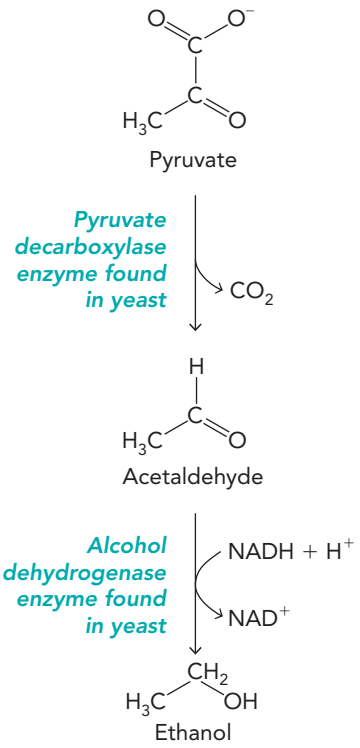


Figure 1.2 The yeast enzymes pyruvate decarboxylase and alcohol dehydrogenase are responsible for converting pyruvate, a product of glucose metabolism, into alcohol and carbon dioxide.

of biochemical reactions dramatically. Enzymes are responsible for aerobic respiration, fermentation, nitrogen metabolism, energy conversion, and even programmed cell death. Two key enzymes are required for the fermentation of glucose by yeast. The first is pyruvate decarboxylase, which converts pyruvate, a breakdown product of glucose, into acetaldehyde and carbon dioxide (CO_2). The second is alcohol dehydrogenase, an enzyme that reduces acetaldehyde to form ethanol (**Figure 1.2**).

Following the lead of Buchner and others, biochemists throughout much of the 20th century focused on systematically dismantling each of the chemical reactions required for cellular life. Almost half of this book describes the biochemical reactions and metabolic pathways (functionally related chemical reactions in cells) elucidated by early biochemists (Chapters 9–19). The rest of the book is devoted to biochemical discoveries made primarily since the 1970s, focusing on the structure and function of proteins (Chapters 4–8) and the biochemistry of genetic inheritance (Chapters 20–23). Both of these modern advances in biochemistry can be traced to the *Eureka!* moment in 1953 when James Watson and Francis Crick solved the molecular structure of DNA.

Biochemistry, like genetics and cell biology, is a core discipline in the life sciences. Biochemistry provides the underlying chemical principles guiding discoveries in medicine, agriculture, and pharmaceuticals. A molecular understanding of chemical reactions in living cells and of how cells communicate to one another in a multicellular organism has led to a dramatic increase in expected human life spans through improved health care, food production, and environmental science. Biochemistry is also a powerful applied science that uses advanced experimental methods to develop *in vitro* conditions for exploiting cellular processes and enzymatic reactions. Examples include the development of new pharmaceutical drugs based on the knowledge of biochemical processes under pathologic conditions, as well as diagnostic tests that detect these abnormalities (**Figure 1.3**). Improved detergents based on enzymatic reactions and the faster ripening of fruits and vegetables using ethylene gas are other examples of applied biochemistry. Moreover, environmental science has benefited from advances in biochemistry through the development of quantitative field tests that can provide vital information about changes in fragile ecosystems due to industrial or biological contamination.

It is an exciting time to be learning biochemistry! Indeed, in this current "Age of Biology," no field is more centrally positioned to exploit this new era. Technological advances in microanalytical chemical methods such as mass spectrometry and enhanced techniques to render high-resolution images of biomolecular structures provide immense opportunity for new discoveries in biochemistry. Chemists, life scientists, and health-field professionals with a firm understanding of the role that biochemistry plays in the chemical nature of life are certain to have a distinct advantage in applying biological discoveries made during the next 50 years.

concept integration 1.1

How did *in vitro* alcoholic fermentation provide evidence for the "chemistry of life"?

Eduard Buchner's *in vitro* experiment in 1897 used a yeast cell-free extract to convert glucose into ethanol and CO_2, thereby providing the first compelling evidence that a "vital force" was not required for alcoholic fermentation. Moreover, this landmark biochemical experiment suggested that conventional chemical reactions were likely to be the molecular basis for life itself and stimulated 50 years of research to prove it.

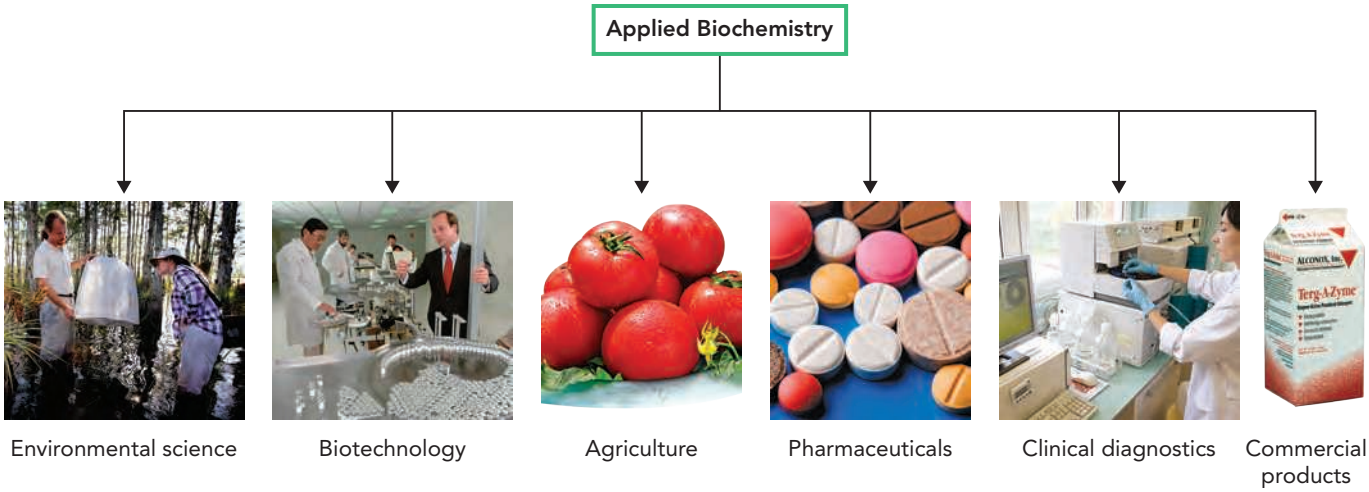


Figure 1.3 Applied biochemistry uses a basic understanding of biochemical principles to guide advances in agriculture, medicine, and industry. ENVIRONMENTAL SCIENCE: EMILY MICHOT/MIAMI HERALD/MCT VIA GETTY IMAGES; BIOTECHNOLOGY: ROGER RESSMEYER/CORBIS; AGRICULTURE: TOHRU MINOWA/A.COLLECTIONRF/GETTY IMAGES; PHARMACEUTICALS: DIMA SOBKO/SHUTTERSTOCK; CLINICAL DIAGNOSTICS: JAVIER LARREA/AGEFOTOSTOCK; COMMERCIAL PRODUCTS: ©ALCONOX, INC.

1.2 The Chemical Basis of Life: A Hierarchical Perspective

We have seen that biochemistry is an interdisciplinary science that brings together many concepts from chemistry, cell biology, and physiology. This integrated approach to molecular life science makes biochemistry very important, but it also means that the student needs to master many terms and definitions. In this section, we review seven levels of biochemical hierarchy—or levels of organizational complexity—that encompass the chemistry of life and use terminology that you will encounter throughout the book.

The foundation of this hierarchy is chemical elements and functional groups (**Figure 1.4**). Next, chemical groups are organized into **biomolecules**, of which there are four major types in nature: amino acids, nucleotides, simple sugars, and fatty acids. Then, higher-order structures of biomolecules form **macromolecules**, which can be chemical polymers such as proteins (polymers of amino acids), nucleic acids (polymers of nucleotides), or polysaccharides such as cellulose, amylose, and glycogen (polymers of the carbohydrate glucose).

Organization of macromolecules and enzymes into **metabolic pathways** is the next hierarchical level. These pathways enable cells to coordinate and control complex biochemical processes in response to available energy. Examples of metabolic pathways include glucose metabolism (glycolysis and gluconeogenesis), energy conversion (citrate cycle), and fatty acid metabolism (fatty acid oxidation and biosynthesis). Metabolic pathways function within membrane-bound cells. The membranes create aqueous microenvironments within the cells for biochemical reactions involving metabolites and macromolecules.

Cell specialization, the next level of organizational complexity, allows multicellular organisms to exploit their environment through **signal transduction** mechanisms that facilitate communication between cells. Organisms represent the subsequent level, as they consist of large numbers of specialized cells, allowing multicellular organisms to respond to environmental changes. One way multicellular organisms

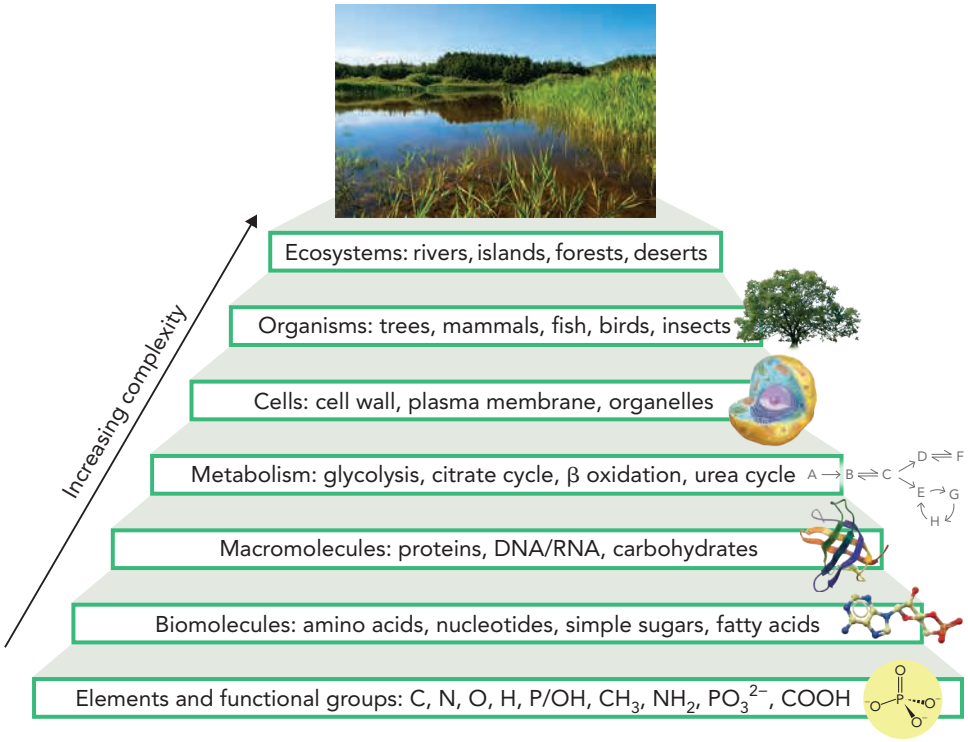


Figure 1.4 A summary of the hierarchical organization and chemical complexity of living systems, including the seven hierarchical levels, along with examples of organizational complexities within these levels.
ECOSYSTEM: JACOBH/ISTOCK/360/GETTY IMAGES; TREE: VISUALL2/SHUTTERSTOCK.

are able to adapt to change is through signal transduction mechanisms that facilitate cell–cell communication. Finally, cohabitation of different organisms in the same environmental niche creates a balanced **ecosystem**, characterized by shared use of resources and waste management. As you will see, the field of biochemistry incorporates the study of chemical life at all levels of this hierarchy.

Elements and Chemical Groups Commonly Found in Nature

Almost 100 chemical elements are found in nature, and chemists have organized them into the periodic table according to their atomic properties. The distribution of these elements in living systems is very different from that in the physical world. In particular, more than 97% of the weight of most organisms consists of just six elements: hydrogen, oxygen, carbon, nitrogen, phosphorus, and sulfur (**Table 1.1**). The vast majority of this mass comes from hydrogen and oxygen, most of which is present as H_2O (the human body is 70% water). In addition to the six most abundant elements, trace elements such as zinc, iron, manganese, copper, and cobalt are required for life, primarily as cofactors in proteins. Essential ions include calcium, chloride, magnesium, potassium, and sodium, many of which play key roles in cell signaling and neurophysiology. The amount of carbon in living organisms is disproportionately high, being 100 times more abundant in the human body than in Earth's crust.

Although the abundance of elements in biological systems is quite different from the abundance of elements in Earth, biochemical reactions are no different from other chemical reactions with regard to bond properties and reaction mechanisms. As you learned in introductory chemistry, covalent bonds form when two atoms share unpaired electrons in their outer shells. The strength of a covalent bond depends on the relative affinities of the two atoms for electrons, the distance between the bonding electrons and the nucleus of each atom, and the nuclear charge of each atom. For example, water, ammonia, carbon dioxide, and carbonic acid are formed by covalent bonds between

Table 1.1 ELEMENTAL COMPOSITION OF THE HUMAN BODY AS A PERCENTAGE OF DRY WEIGHT

			Additional trace elements (<0.1%)	
Element	Symbol	Percent dry weight (%)	Element	Symbol
Carbon	C	62	Manganese	Mn
Nitrogen	N	11	Iron	Fe
Oxygen	O	9	Cobalt	Co
Hydrogen	H	6	Copper	Cu
Calcium	Ca	5	Zinc	Zn
Phosphorus	P	3	Selenium	Se
Potassium	K	1	Molybdenum	Mo
Sulfur	S	1	Iodine	I
Chlorine	Cl	<1	Fluorine	F
Sodium	Na	<1	Chromium	Cr
Magnesium	Mg	<1	Tin	Sn

Note: These values exclude the contribution of oxygen and hydrogen to the large amount of water in the human body (70% by weight).

H, O, N, and C (**Figure 1.5**). Hydrogen requires two electrons to complete its outer shell, whereas O, N, and C each require eight electrons. Ions such as hydronium ion, H_3O^+, ammonium ion, NH_4^+, and bicarbonate ion, HCO_3^- are formed by the gain of a proton and loss of an electron (or vice versa), so as to maintain a complete outer shell. Double bonds are stronger than single bonds, as more energy is required to break a double bond (**Table 1.2**).

The chemical nature of life on Earth is based on the element carbon (Figure 1.5). Molecules containing carbon are called organic molecules, and organic chemistry is the study of carbon-based compounds. Indeed, early biochemists were often organic chemists who became interested in "biological" chemistry. Carbon has a unique ability

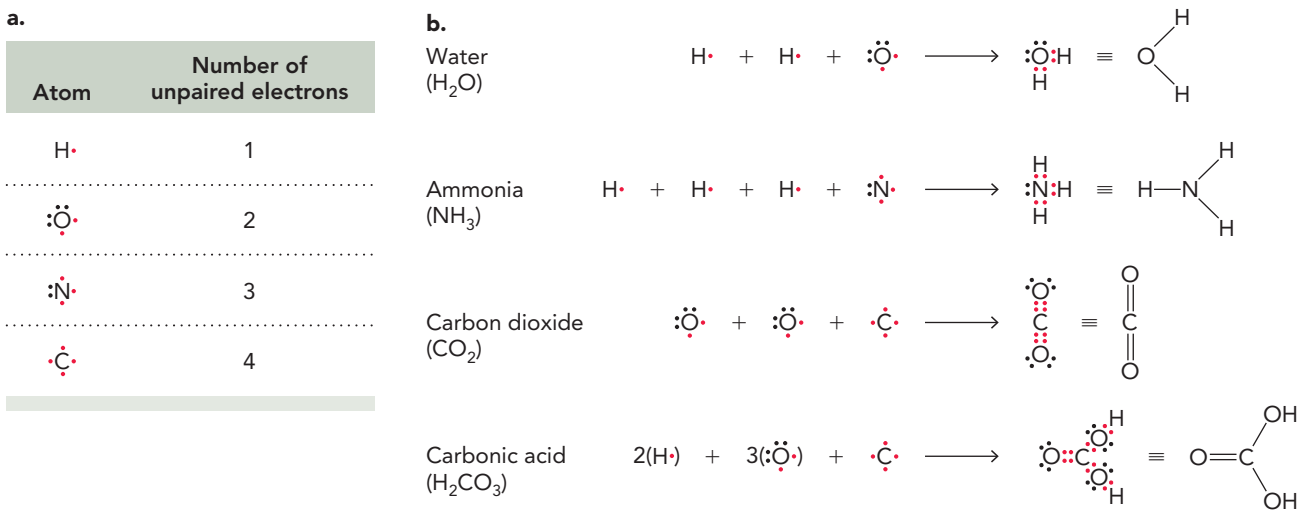


Figure 1.5 Covalent bonds result from sharing of an electron pair between two atoms. **a.** H, O, N, and C all have unpaired electrons in their outer shell that can participate in bond formation. Unpaired electrons are shown as red dots and paired electrons as black dots. **b.** The arrangement of electron sharing for some common biomolecules. Covalent bonds occur when unpaired electrons in each of two atoms interact, forming an electron pair that is shared between the atoms.