GLOBAL EDITION

Biochemistry

Concepts and Connections

Dean R. Appling • Spencer J. Anthony-Cahill • Christopher K. Mathews

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Concepts and Connections

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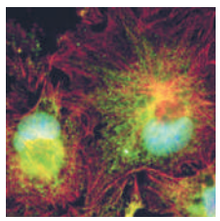

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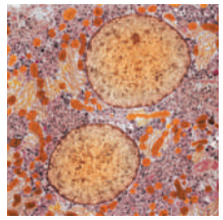

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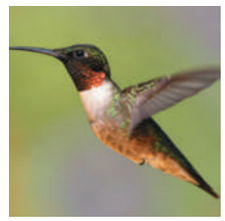

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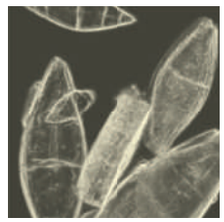

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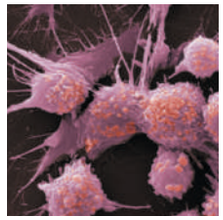

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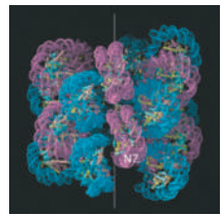

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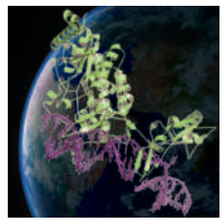

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PREFACE

Biochemistry: Concepts and Connections

As genomics and informatics revolutionize biomedical science and health care, we must prepare students for the challenges of the twenty-first century and ensure their ability to apply quantitative reasoning skills to the science most fundamental to medicine: biochemistry.

We have written *Biochemistry: Concepts and Connections* to provide students with a clear understanding of the chemical logic underlying the mechanisms, pathways, and processes in living cells. The title reinforces our vision for this book—twin emphases upon fundamental *concepts* at the expense of lengthy descriptive information, and upon *connections,* showing how biochemistry relates to all other life sciences and to practical applications in medicine, agricultural sciences, environmental sciences, and forensics.

Inspired by our experience as authors of the biochemistry majors' text, *Biochemistry, Fourth Edition,* and as teachers of biochemistry majors' and mixed-science-majors' courses, we believe there are several requirements that a textbook for the mixed-majors' course must address:

- The need for students to understand the structure and function of biological molecules before moving into metabolism and dynamic aspects of biochemistry.
- The need for students to understand that biochemical concepts derive from experimental evidence, meaning that the principles of biochemical techniques must be presented to the greatest extent possible.
- The need for students to encounter many and diverse real-world applications of biochemical concepts.
- The need for students to understand the quantitative basis for biochemical concepts. The Henderson–Hasselbalch equation, the quantitative expressions of thermodynamic laws, and the Michaelis–Menten equation, for example, are not equations to be memorized and forgotten when the course moves on. The basis for these and other quantitative statements must be understood and constantly repeated as biochemical concepts, such as mechanisms of enzyme action, are developed. They are essential to help students grasp the concepts.

In designing *Biochemistry: Concepts and Connections,* we have stayed with the organization that serves us well in our own classroom experience. The first 10 chapters cover structure and function of biological molecules, the next 10 deal with intermediary metabolism, and the final 6 with genetic biochemistry. Our emphasis on biochemistry as a quantitative science can be seen in Chapters 2 and 3, where we focus on water, the matrix of life, and bioenergetics. Chapter 4 introduces nucleic acid structure, with a brief introduction to nucleic acid and protein synthesis—topics covered in much more detail at the end of the book.

Chapters 11 through 20 deal primarily with intermediary metabolism. We cover the major topics in carbohydrate metabolism, lipid metabolism, and amino acid metabolism in one chapter each (12, 16, and 18, respectively). Our treatment of cell signaling is a bit unconventional, since it appears in Chapter 20, well after we present hormonal control of carbohydrate and lipid metabolism. However, this treatment allows more extended presentation of receptors, G proteins, oncogenes, and neurotransmission. In addition, because cancer often results from aberrant signaling processes, our placement of the signaling chapter leads fairly naturally into genetic biochemistry, which follows, beginning in Chapter 21.

With assistance from talented artists, we have built a compelling visual narrative from the ground up, composed of a wide range of graphic representations, from macromolecules to cellular structures as well as reaction mechanisms and metabolic pathways that highlights and reinforces overarching themes (chemical logic, regulation, interface between chemistry and biology). In addition, novel **Foundation Figures** integrate core chemical and biological connections visually, providing a way to organize the complex and detailed material intellectually, thus making relationships among key concepts clear and easier to study. **"Concept"** and **"Connection"** statements within the narrative highlight fundamental concepts and real-world applications of biochemistry.

In *Biochemistry: Concepts and Connections,* we emphasize our field as an experimental science by including 15 separate sections, called **Tools of Biochemistry,** that highlight the most important research techniques. We also provide students with end-of-chapter references (about 12 per chapter), choosing those that would be most appropriate for our target audience, such as links to Nobel Prize lectures.

We consider end-of-chapter problems to be an indispensable learning tool and have provided 15 to 25 problems for each chapter. About half of the problems have brief answers at the end of the book, with complete answers provided in a separate solutions manual. Additional tutorials in MasteringChemistry® will help students with some of the most basic concepts and operations.

Producing a book of this magnitude involves the efforts of dedicated editorial and production teams. We have not had the pleasure of meeting all of these talented individuals, but we consider them close friends nonetheless. First, of course, is Jeanne Zalesky, our sponsoring editor, now Editor-in-Chief, Physical Sciences, who always found a way to keep us focused on our goal. Coleen Morrison, Program Manager, kept us organized and on schedule, juggling disparate elements in this complex project. Jay McElroy, Art Development Editor, was our intermediary with the talented artists at Imagineering, Inc., and displayed considerable artistic and editorial gifts in his own right. Over the course of the project, we worked with three experienced development editors—Dan Schiller, John Murdzek, and Erica Pantages

Frost. Their edits, insights, and attention to detail were invaluable. Beth Sweeten, Senior Project Manager, coordinated the production of the main text and preparation of the Solutions Manual for the end-of-chapter problems. Gary Carlton provided great assistance with many of the illustrations. Chris Hess provided the inspiration for the US edition's cover illustration, and Stephen Merland helped us locate much excellent illustrative material. Once the book was in production, Francesca Monaco skillfully kept us all on a complex schedule.

The three of us give special thanks to friends and colleagues who provided unpublished material for us to use as illustrations. These contributors include John S. Olson (Rice University), Jack Benner (New England BioLabs), Andrew Karplus (Oregon State University), Scott Delbecq and Rachel Klevit (University of Washington), William Horton (Oregon Health and Science University), Cory Hamada (Western Washington University), Nadrian C. Seaman (New York University), P. Shing Ho (Colorado State University), Catherine Drennan and Edward Brignole (MIT), John G. Tesmer (University of Michigan), Katsuhiko Murakami (Penn State University), Alan Cheung (University College London), Joyce Hamlin (University of Virginia), Erik Johansson (Umeå University), Stefano Tiziani, Edward Marcotte, David Hoffman, and Robin Gutell (University of Texas at Austin), Andreas Martin and Gabriel Lander (University of California, Berkeley), Dean Sherry and Craig Malloy (University of Texas-Southwestern Medical Center), and Stephen C. Kowalczykowski (University of California, Davis).

We are also grateful to the numerous talented biochemists retained by our editors to review our outline, prospectus, chapter drafts, and solutions to our end-of-chapter problems. Their names and affiliations are listed separately.

Our team—authors and editors—put forth great effort to detect and root out errors and ambiguities. We undertook an arduous process of editing and revising several drafts of each chapter in manuscript stage, as well as copyediting, proofreading, and accuracy reviewing multiple rounds of page proofs in an effort to ensure the highest level of quality control.

Throughout this process, as in our previous writing, we have been most grateful for the patience, good judgment, and emotional support provided by our wives—Maureen Appling, Yvonne Anthony-Cahill, and Kate Mathews. We expect them to be as relieved as we are to see this project draw to a close, and hope that they can share our pleasure at the completed product.

Dean R. Appling
Spencer J. Anthony-Cahill
Christopher K. Mathews

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As much fun as writing a textbook might be, Dean would rather be outdoors. He is an avid fisherman and hiker. Recently, Dean and his wife, Maureen, have become entranced by the birds on the Texas coast. They were introduced to bird-watching by coauthor Chris Mathews and his wife Kate—an unintended consequence of writing textbooks!

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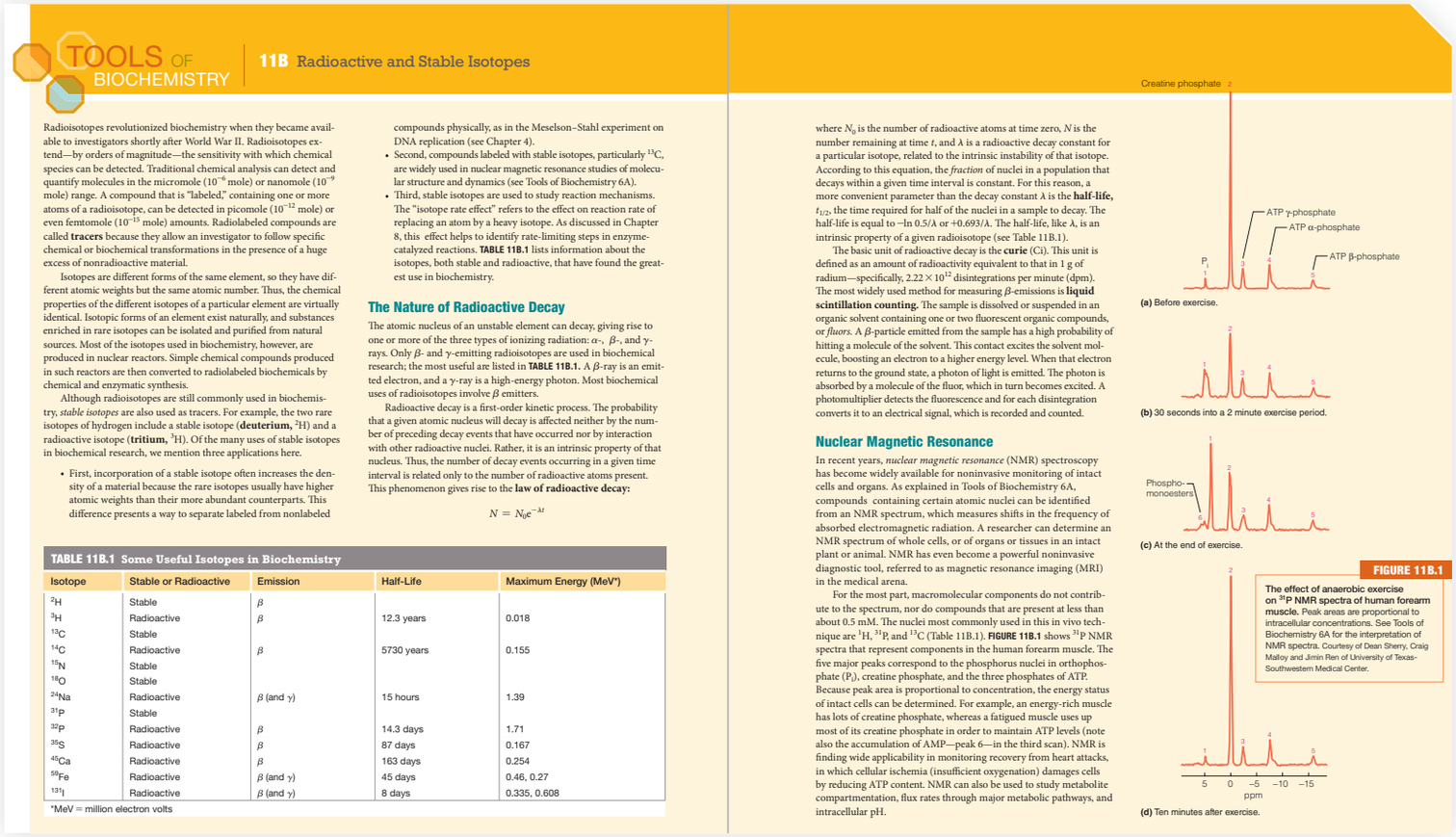
He has backpacked and floated the mountains and rivers, respectively, of Oregon and the Northwest. As an enthusiastic birder he has served as President of the Audubon Society of Corvallis and is President of the Great Basin Society, which operates the Malheur Field Station in eastern Oregon.

TOOLS OF BIOCHEMISTRY



TOOLS OF BIOCHEMISTRY | **11B** Radioactive and Stable Isotopes

Radioisotopes revolutionized biochemistry when they became available to investigators shortly after World War II. Radioisotopes extend—by orders of magnitude—the sensitivity with which chemical species can be detected. Traditional chemical analysis can detect and quantify molecules in the micromole (10^{-6} mole) or nanomole (10^{-9} mole) range. A compound that is "labeled," containing one or more atoms of a radioisotope, can be detected in picomole (10^{-12} mole) or even femtomole (10^{-15} mole) amounts. Radiolabeled compounds are called **tracers** because they allow an investigator to follow specific chemical or biochemical transformations in the presence of a huge excess of nonradioactive material.

Isotopes are different forms of the same element, so they have different atomic weights but the same atomic number. Thus, the chemical properties of the different isotopes of a particular element are virtually identical. Isotopic forms of an element exist naturally, and substances enriched in rare isotopes can be isolated and purified from natural sources. Most of the isotopes used in biochemistry, however, are produced in nuclear reactors. Simple chemical compounds produced in such reactors are then converted to radiolabeled biochemicals by chemical and enzymatic synthesis.

Although radioisotopes are still commonly used in biochemistry, *stable isotopes* are also used as tracers. For example, the two rare isotopes of hydrogen include a stable isotope (**deuterium,** ^{2}H) and a radioactive isotope (**tritium,** ^{3}H). Of the many uses of stable isotopes in biochemical research, we mention three applications here.

- First, incorporation of a stable isotope often increases the density of a material because the rare isotopes usually have higher atomic weights than their more abundant counterparts. This difference presents a way to separate labeled from nonlabeled compounds physically, as in the Meselson–Stahl experiment on DNA replication (see Chapter 4).
- Second, compounds labeled with stable isotopes, particularly ^{13}C, are widely used in nuclear magnetic resonance studies of molecular structure and dynamics (see Tools of Biochemistry 6A).
- Third, stable isotopes are used to study reaction mechanisms. The "isotope rate effect" refers to the effect on reaction rate of replacing an atom by a heavy isotope. As discussed in Chapter 8, this effect helps to identify rate-limiting steps in enzyme-catalyzed reactions. **TABLE 11B.1** lists information about the isotopes, both stable and radioactive, that have found the greatest use in biochemistry.

The Nature of Radioactive Decay

The atomic nucleus of an unstable element can decay, giving rise to one or more of the three types of ionizing radiation: α-, β-, and γ-rays. Only β- and γ-emitting radioisotopes are used in biochemical research; the most useful are listed in **TABLE 11B.1.** A β-ray is an emitted electron, and a γ-ray is a high-energy photon. Most biochemical uses of radioisotopes involve β emitters.

Radioactive decay is a first-order kinetic process. The probability that a given atomic nucleus will decay is affected neither by the number of preceding decay events that have occurred nor by interaction with other radioactive nuclei. Rather, it is an intrinsic property of that nucleus. Thus, the number of decay events occurring in a given time interval is related only to the number of radioactive atoms present. This phenomenon gives rise to the **law of radioactive decay:**

$$N = N_0 e^{-\lambda t}$$

TABLE 11B.1 Some Useful Isotopes in Biochemistry

Isotope	Stable or Radioactive	Emission	Half-Life	Maximum Energy (MeV*)
^{2}H	Stable			
^{3}H	Radioactive	β	12.3 years	0.018
^{13}C	Stable			
^{14}C	Radioactive	β	5730 years	0.155
^{15}N	Stable			
^{18}O	Stable			
^{24}Na	Radioactive	β (and γ)	15 hours	1.39
^{31}P	Stable			
^{32}P	Radioactive	β	14.3 days	1.71
^{35}S	Radioactive	β	87 days	0.167
^{45}Ca	Radioactive	β	163 days	0.254
^{59}Fe	Radioactive	β (and γ)	45 days	0.46, 0.27
^{131}I	Radioactive	β (and γ)	8 days	0.335, 0.608

*MeV = million electron volts

where N_0 is the number of radioactive atoms at time zero, N is the number remaining at time t, and λ is a radioactive decay constant for a particular isotope, related to the intrinsic instability of that isotope. According to this equation, the *fraction* of nuclei in a population that decays within a given time interval is constant. For this reason, a more convenient parameter than the decay constant λ is the **half-life,** $t_{1/2}$, the time required for half of the nuclei in a sample to decay. The half-life is equal to $-\ln 0.5/\lambda$ or $+0.693/\lambda$. The half-life, like λ, is an intrinsic property of a given radioisotope (see Table 11B.1).

The basic unit of radioactive decay is the **curie** (Ci). This unit is defined as an amount of radioactivity equivalent to that in 1 g of radium—specifically, 2.22×10^{12} disintegrations per minute (dpm). The most widely used method for measuring β-emissions is **liquid scintillation counting.** The sample is dissolved or suspended in an organic solvent containing one or two fluorescent organic compounds, or *fluors*. A β-particle emitted from the sample has a high probability of hitting a molecule of the solvent. This contact excites the solvent molecule, boosting an electron to a higher energy level. When that electron returns to the ground state, a photon of light is emitted. The photon is absorbed by a molecule of the fluor, which in turn becomes excited. A photomultiplier detects the fluorescence and for each disintegration converts it to an electrical signal, which is recorded and counted.

Nuclear Magnetic Resonance

In recent years, *nuclear magnetic resonance* (NMR) spectroscopy has become widely available for noninvasive monitoring of intact cells and organs. As explained in Tools of Biochemistry 6A, compounds containing certain atomic nuclei can be identified from an NMR spectrum, which measures shifts in the frequency of absorbed electromagnetic radiation. A researcher can determine an NMR spectrum of whole cells, or of organs or tissues in an intact plant or animal. NMR has even become a powerful noninvasive diagnostic tool, referred to as magnetic resonance imaging (MRI) in the medical arena.

For the most part, macromolecular components do not contribute to the spectrum, nor do compounds that are present at less than about 0.5 mM. The nuclei most commonly used in this in vivo technique are ^{1}H, ^{31}P, and ^{13}C (Table 11B.1). **FIGURE 11B.1** shows ^{31}P NMR spectra that represent components in the human forearm muscle. The five major peaks correspond to the phosphorus nuclei in orthophosphate (P_i), creatine phosphate, and the three phosphates of ATP. Because peak area is proportional to concentration, the energy status of intact cells can be determined. For example, an energy-rich muscle has lots of creatine phosphate, whereas a fatigued muscle uses up most of its creatine phosphate in order to maintain ATP levels (note also the accumulation of AMP—peak 6—in the third scan). NMR is finding wide applicability in monitoring recovery from heart attacks, in which cellular ischemia (insufficient oxygenation) damages cells by reducing ATP content. NMR can also be used to study metabolite compartmentation, flux rates through major metabolic pathways, and intracellular pH.

(a) Before exercise.
(b) 30 seconds into a 2 minute exercise period.
(c) At the end of exercise.
(d) Ten minutes after exercise.

FIGURE 11B.1
The effect of anaerobic exercise on ^{31}P NMR spectra of human forearm muscle. Peak areas are proportional to intracellular concentrations. See Tools of Biochemistry 6A for the interpretation of NMR spectra. Courtesy of Dean Sherry, Craig Malloy and Jimin Ren of University of Texas-Southwestern Medical Center.

Tools of Biochemistry emphasize our field as an experimental science and highlight the most important research techniques relevant to students today.

2A Electrophoresis and Isoelectric Focusing 76

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5A Protein Expression and Purification 163

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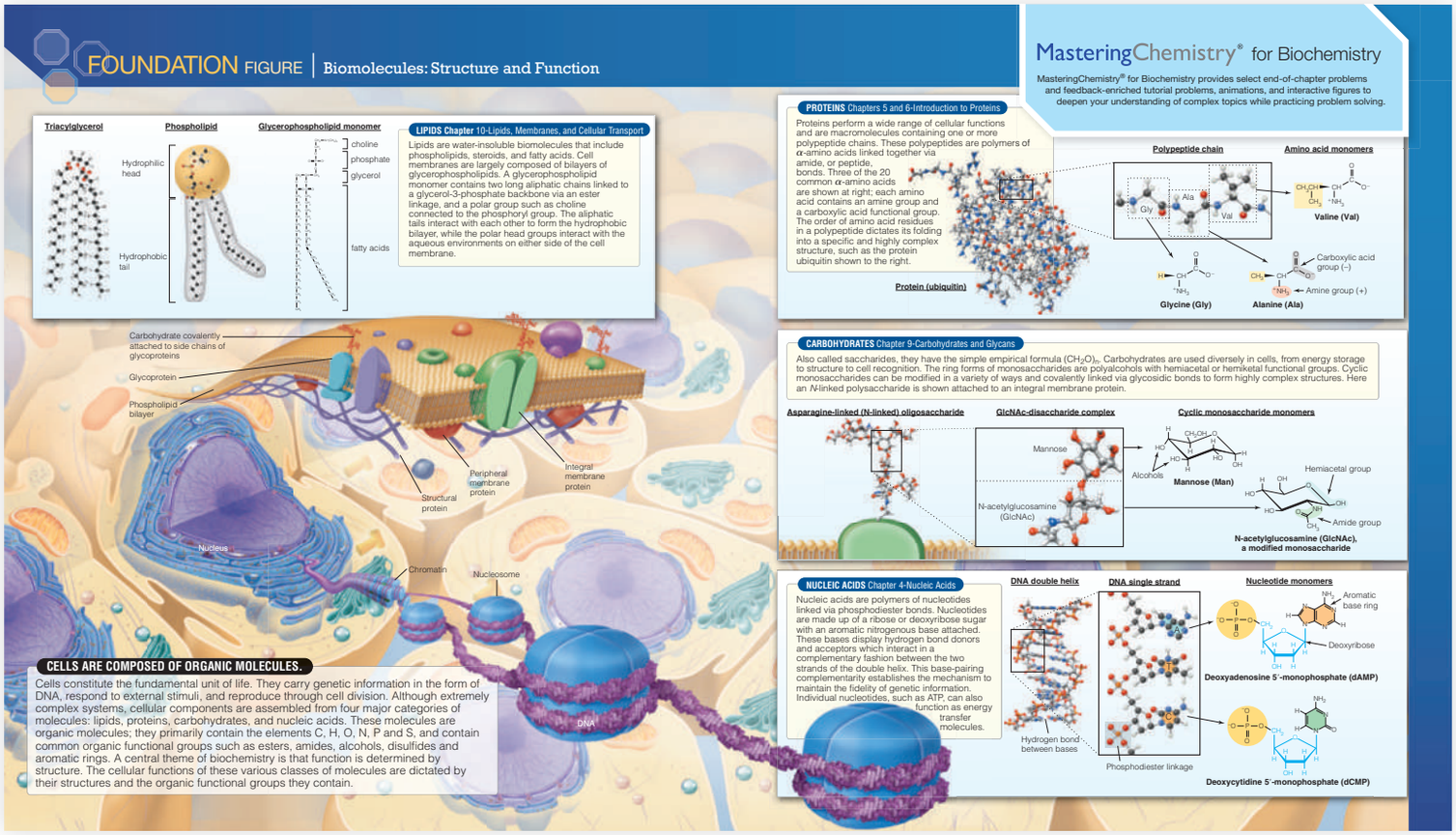
FOUNDATION FIGURES



Foundation Figures integrate core chemical and biological connections visually and provide a way to organize the complex and detailed material intellectually, thus making relationships among key concepts clear and easier to study.

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2 Protein Structure and Function 220

3 Regulation of Enzyme Activity 304

4 Enzyme Kinetics and Drug Action 398

5 Intermediary Metabolism 510

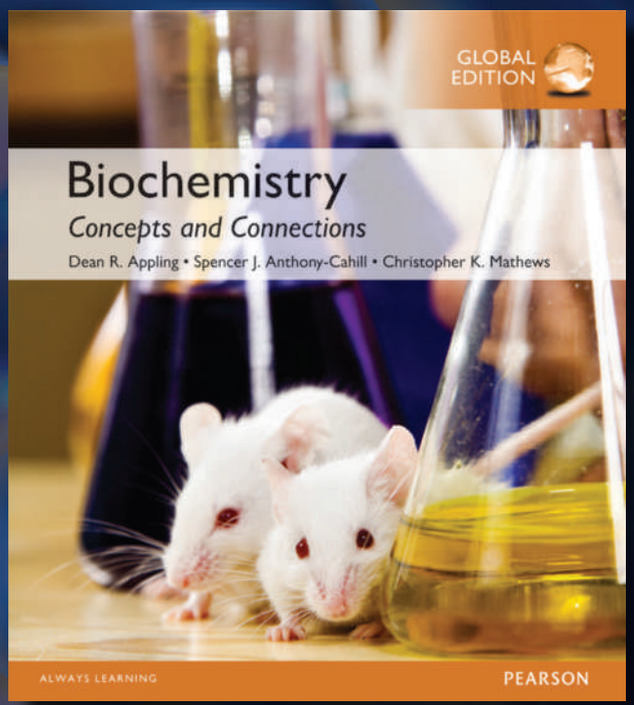
6 Cell Signaling and Protein Regulation 682

7 Antibody Diversity and Use as Therapeutics 760

8 Information Flow in Biological Systems 844

Making Connections

BIOCHEMISTRY: CONCEPTS AND CONNECTIONS engages students in the rapidly evolving field of biochemistry, better preparing them for the challenges of 21st century science through quantitative reasoning skills and a rich, chemical perspective on biological processes.



This concise first edition teaches mixed-science majors the chemical logic underlying the mechanisms, pathways, and processes in living cells through ground-breaking biochemical art and a clear narrative which illustrates biochemistry's relation to all other life sciences. Integration of biochemistry's experimental underpinnings alongside modern techniques, encourages students to consider how their understanding of biochemistry can, and will, contribute to solving problems in medicine, agricultural sciences, environmental sciences, and forensics.

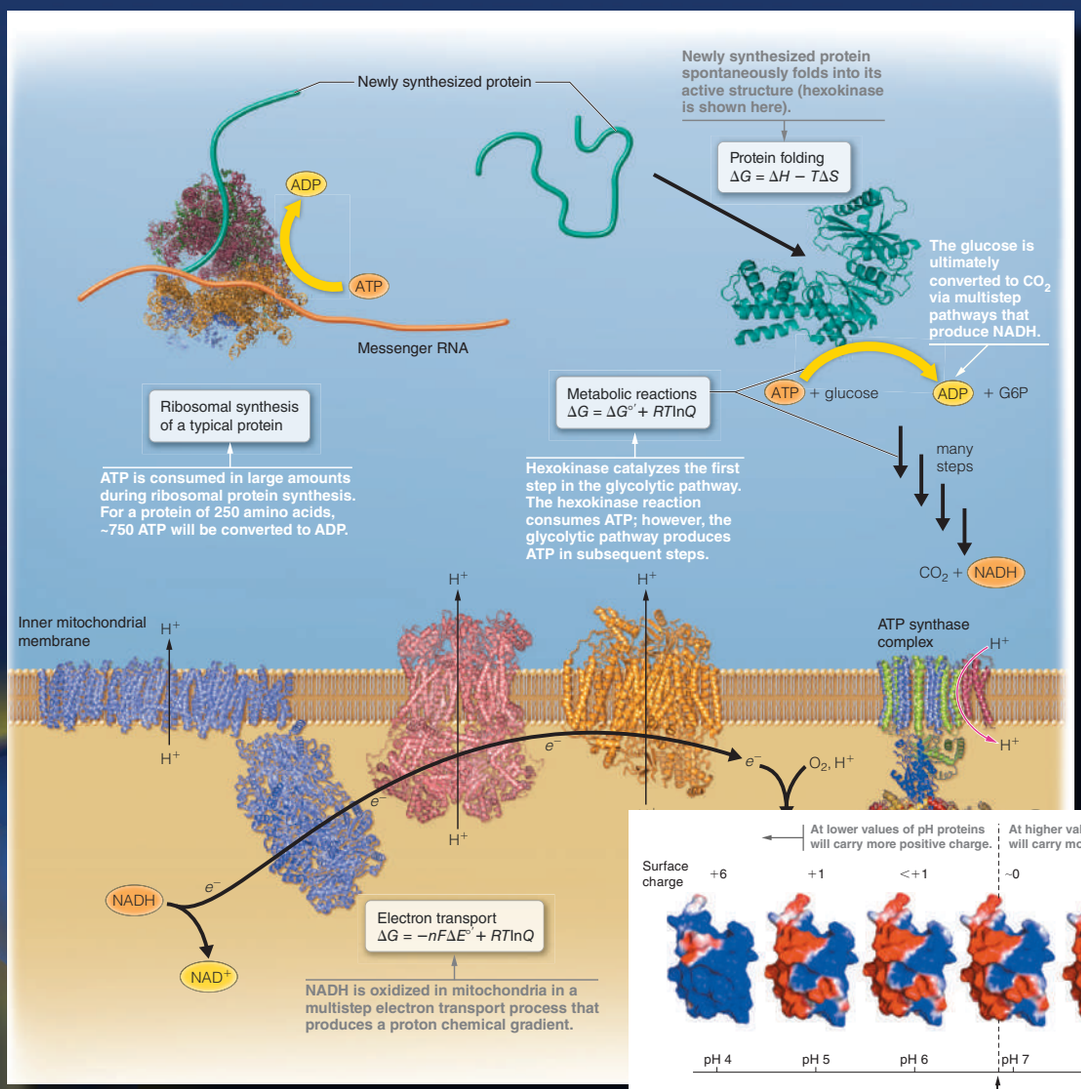
The text is fully integrated with MasteringChemistry® to provide support for students before, during, and after class. Highlights include interactive animations and tutorials based on the textbook's biochemical art program.

Visually compelling chapter openers show the relevancy of the material to draw students into biochemistry at every turn.

Groundbreaking Biochemical Art

AN INNOVATIVE VISUAL NARRATIVE TEACHES BIOCHEMICAL DETAILS while reinforcing over-arching themes of chemical logic, regulation, and the interface between chemistry and biology to help students see the bigger picture.

▼ Figure 3.10: Bioenergetic calculations mapped to three-dimensional structures create a visual and mathematical overview of selected cellular processes. Integrated text explains specifics of how the equations are linked to physical elements within a cell.



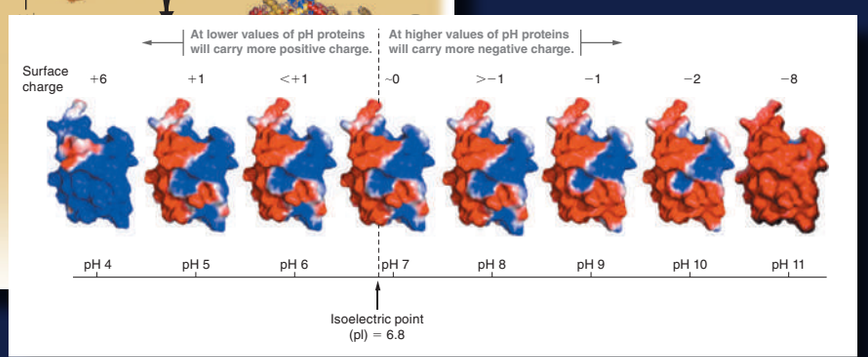


Figure 2.18: Several layers of information (surface charge, pH, and how the charges are distributed across a three-dimensional protein) are combined in an easy to follow format to explain the effect of pH on overall surface charge. Annotations reinforce the major concepts. ▲

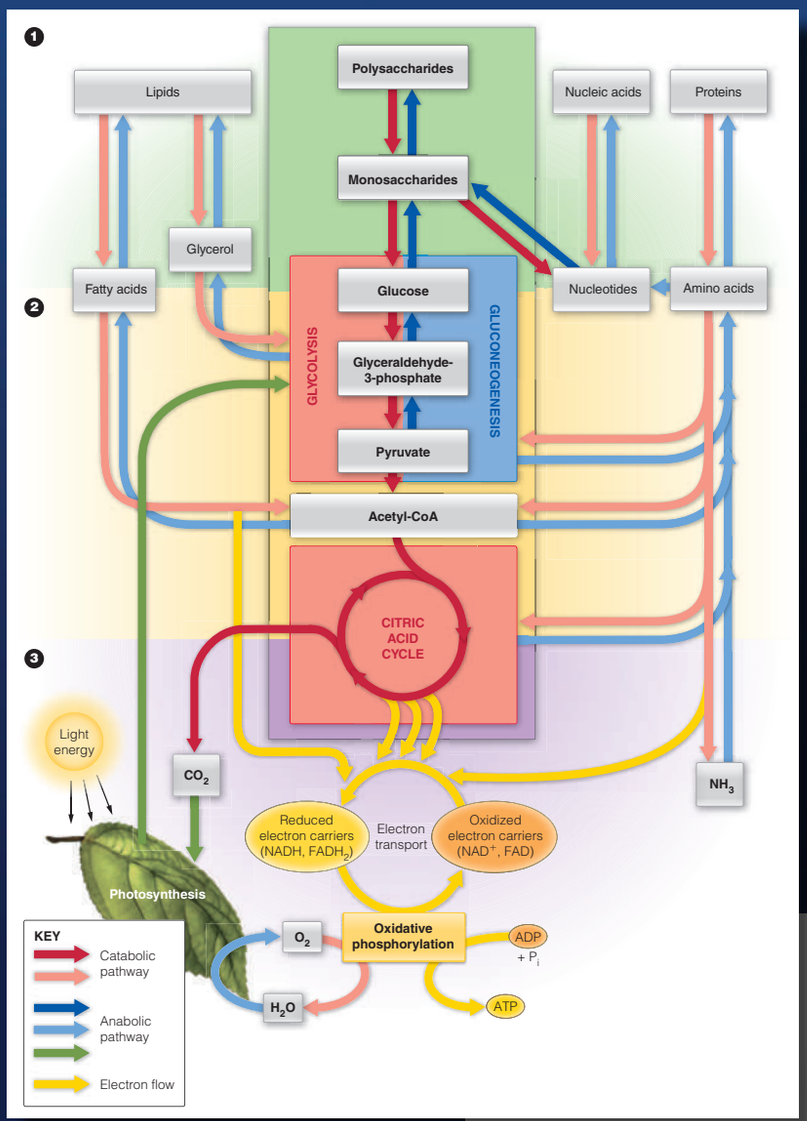
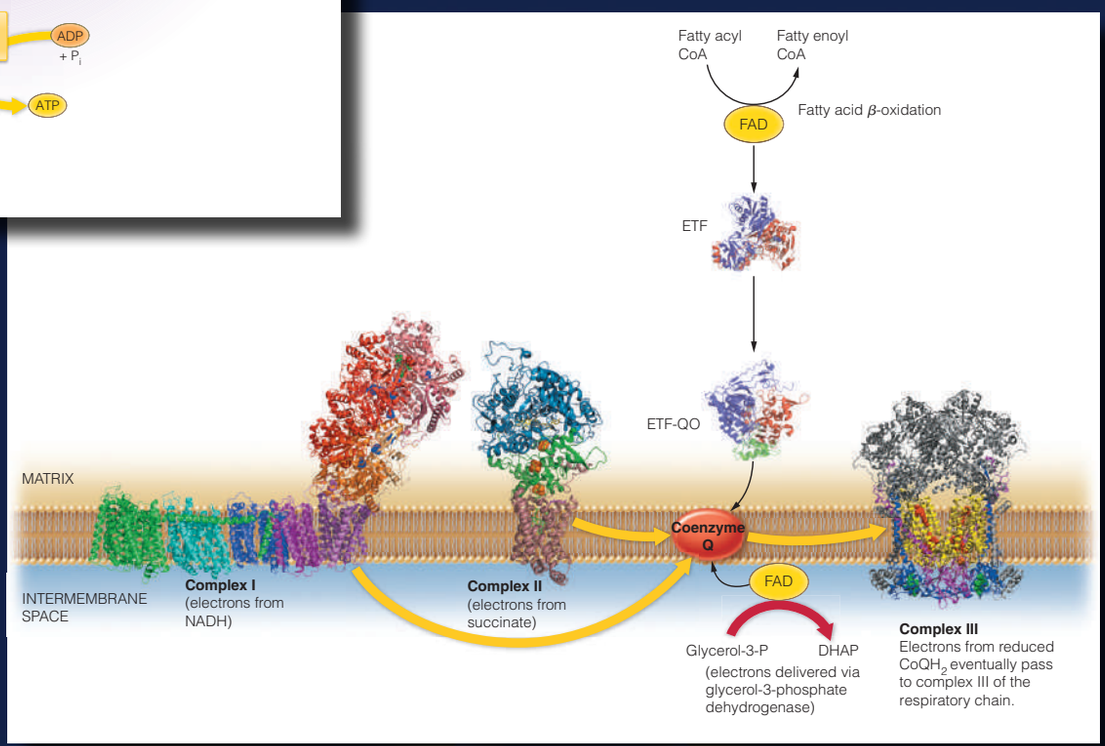


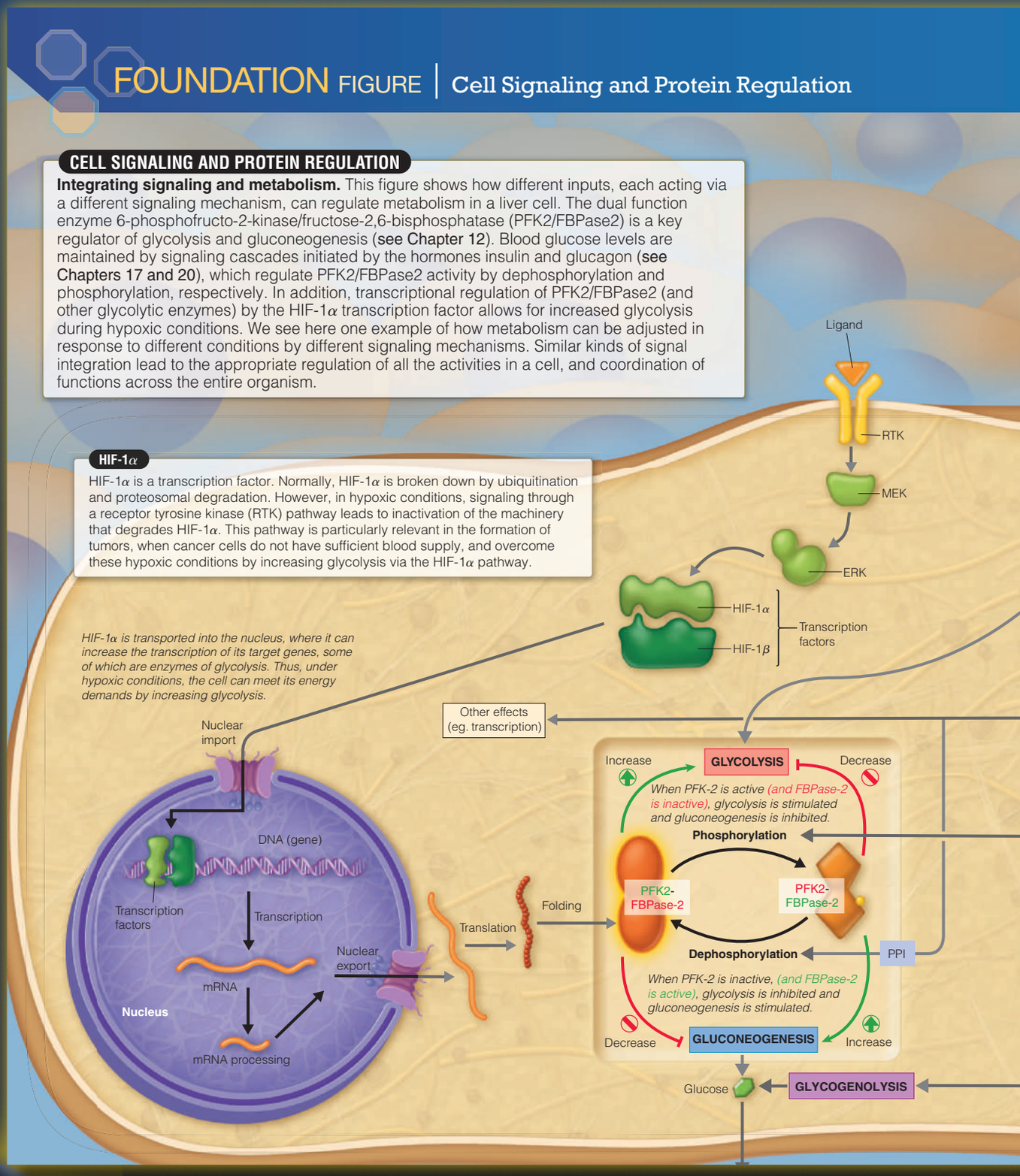
Figure 11.2: Major biochemical themes such as intermediary metabolism are presented as carefully designed reference charts connecting relevant concepts from multiple chapters. These flow charts enable students to visualize the big picture and think about relationships while referring to chapter text for detailed descriptions.

Figure 14.12: Detailed molecular models lend interest and realism to microscopic processes. Here, proteins in the inner mitochondrial membrane give context to electron flow in a portion of the respiratory chain. Vibrant color-coded arrows make multiple pathways clear and understandable.



Interactive Foundation Figures

INTERACTIVE FOUNDATION FIGURES integrate core chemical and biological connections visually and provide a way to organize highly complex and detailed material, making biochemistry more manageable, understandable, and easier to synthesize. These figures will have dedicated questions for use in class via Learning Catalytics™ and will also be assignable in MasteringChemistry® as step-wise animations with follow-up assessment.

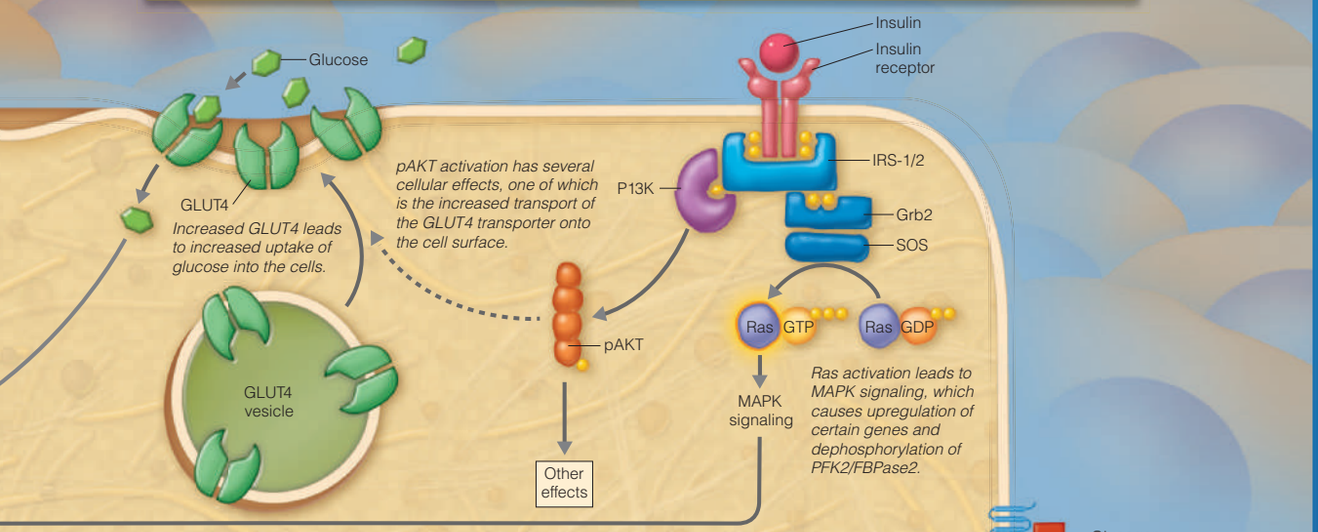


MasteringChemistry® for Biochemistry

MasteringChemistry® for Biochemistry provides select end-of-chapter problems and feedback-enriched tutorial problems, animations, and interactive figures to deepen your understanding of complex topics while practicing problem solving.

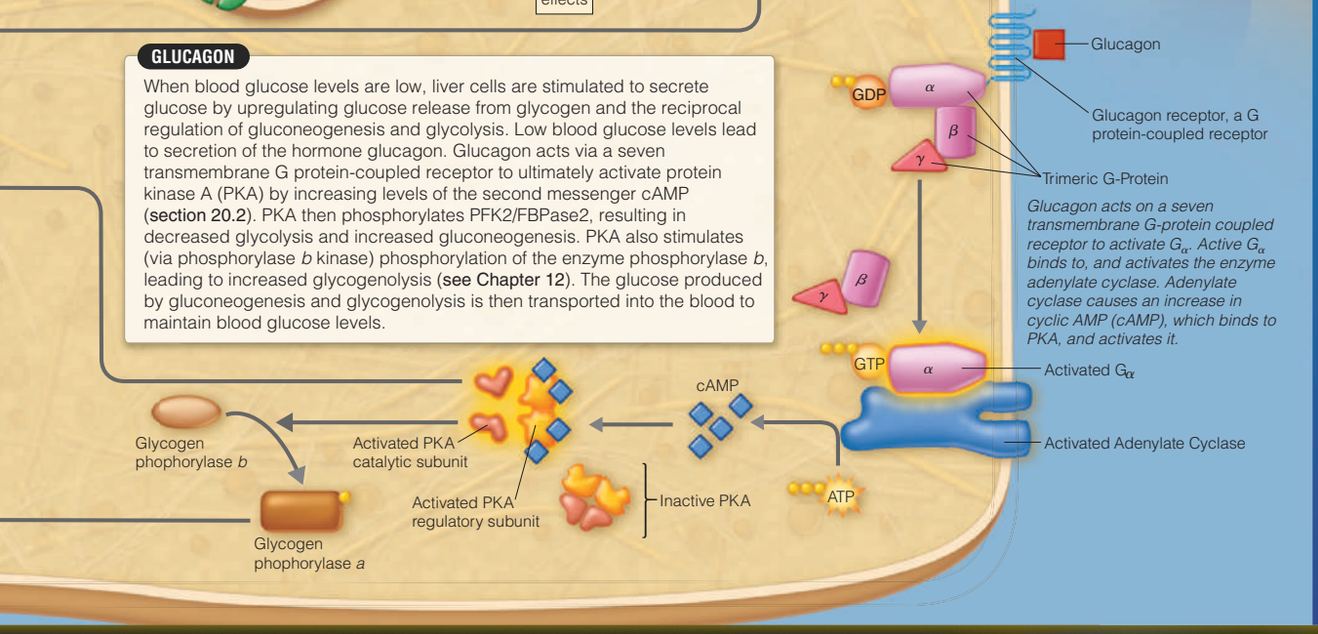
INSULIN

Under conditions of high blood glucose, the pancreatic β-cells secrete the hormone insulin. Insulin binds to its receptor on the liver cell, causing autophosphorylation and activation of the receptor. Activation of the insulin signaling pathway (**section 20.3**) leads to activation of two main proteins– pAKT, and Ras. Dephosphorylated PFK2/FBPase2 increases glycolysis while decreasing gluconeogenesis (**see Chapter 12**). These two activities of insulin (increased glucose transport into cells, and increased glucose utilization) result in an overall decrease in the levels of blood glucose.



GLUCAGON

When blood glucose levels are low, liver cells are stimulated to secrete glucose by upregulating glucose release from glycogen and the reciprocal regulation of gluconeogenesis and glycolysis. Low blood glucose levels lead to secretion of the hormone glucagon. Glucagon acts via a seven transmembrane G protein-coupled receptor to ultimately activate protein kinase A (PKA) by increasing levels of the second messenger cAMP (**section 20.2**). PKA then phosphorylates PFK2/FBPase2, resulting in decreased glycolysis and increased gluconeogenesis. PKA also stimulates (via phosphorylase *b* kinase) phosphorylation of the enzyme phosphorylase *b*, leading to increased glycogenolysis (**see Chapter 12**). The glucose produced by gluconeogenesis and glycogenolysis is then transported into the blood to maintain blood glucose levels.



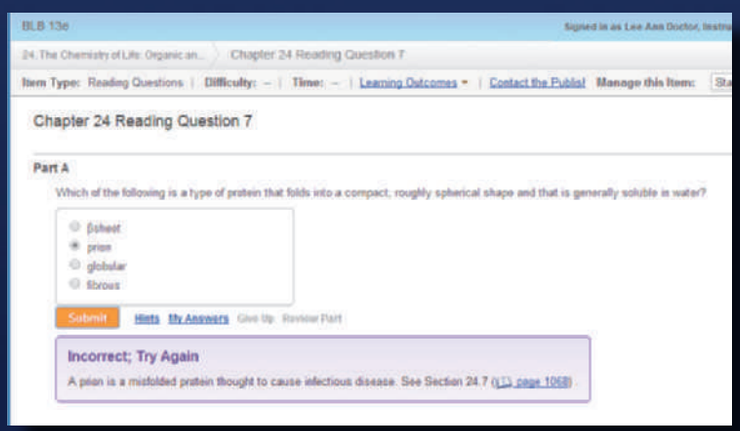
MasteringChemistry®

New MasteringChemistry for Biochemistry provides interactive animations and tutorials based on the textbook's biochemical art program and Foundation Figures helping students visualize complex processes, test conceptual understanding, apply what they have learned to novel scenarios, and practice quantitative reasoning.

Ensure students arrive ready to learn by assigning educationally effective content before class, and encourage critical thinking and retention with in-class resources such as Learning Catalytics. Students can further master concepts after class through traditional homework assignments that provide hints and answer-specific feedback. The Mastering gradebook records scores for all automatically graded assignments while diagnostic tools give instructors access to rich data to assess student understanding and misconceptions.

Mastering brings learning full circle by continuously adapting to each student and making learning more personal than ever—before, during, and after class.

BEFORE CLASS



READING QUIZZES

READING QUIZZES give instructors the opportunity to assign reading and test students on their comprehension of chapter content.

DYNAMIC STUDY MODULES

DYNAMIC STUDY MODULES (DSMs) enable your students to study the required organic chemistry and fundamental biochemistry concepts effectively on their own in order to be better prepared for higher-order learning in class. These modules can be completed on smartphones, tablets, or computers and assignments will automatically be synced to the MasteringChemistry Gradebook.