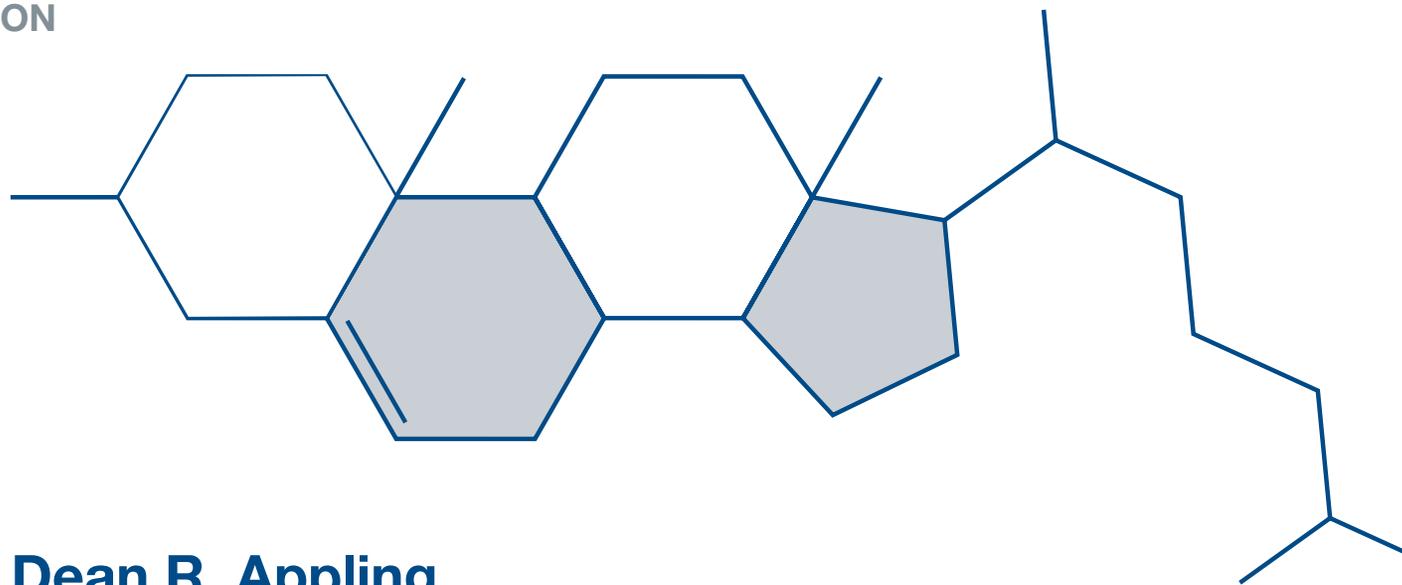


# Biochemistry

CONCEPTS AND CONNECTIONS

SECOND EDITION



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# Brief Contents

- 1** Biochemistry and the Language of Chemistry 2
- 2** The Chemical Foundation of Life: Weak Interactions in an Aqueous Environment 18
- 3** The Energetics of Life 48
- 4** Nucleic Acids 72
- 5** Introduction to Proteins: The Primary Level of Protein Structure 108
- 6** The Three-Dimensional Structure of Proteins 144
- 7** Protein Function and Evolution 190
- 8** Enzymes: Biological Catalysts 232
- 9** Carbohydrates: Sugars, Saccharides, Glycans 278
- 10** Lipids, Membranes, and Cellular Transport 304
- 11** Chemical Logic of Metabolism 340
- 12** Carbohydrate Metabolism: Glycolysis, Gluconeogenesis, Glycogen Metabolism, and the Pentose Phosphate Pathway 374
- 13** The Citric Acid Cycle 420
- 14** Electron Transport, Oxidative Phosphorylation, and Oxygen Metabolism 450
- 15** Photosynthesis 486
- 16** Lipid Metabolism 512
- 17** Interorgan and Intracellular Coordination of Energy Metabolism in Vertebrates 556
- 18** Amino Acid and Nitrogen Metabolism 576
- 19** Nucleotide Metabolism 610
- 20** Mechanisms of Signal Transduction 636
- 21** Genes, Genomes, and Chromosomes 664
- 22** DNA Replication 686
- 23** DNA Repair, Recombination, and Rearrangement 714
- 24** Transcription and Posttranscriptional Processing 742
- 25** Information Decoding: Translation and Posttranslational Protein Processing 766
- 26** Regulation of Gene Expression 796

APPENDIX I: ANSWERS TO SELECTED PROBLEMS A-1

APPENDIX II: REFERENCES A-20

CREDITS C-1

INDEX I-1



# Contents

## CHAPTER 1

### Biochemistry and the Language of Chemistry 2



- 1.1 The Science of Biochemistry 4**
  - The Origins of Biochemistry 4
  - The Tools of Biochemistry 6
  - Biochemistry as a Discipline and an Interdisciplinary Science 6
- 1.2 The Elements and Molecules of Living Systems 7**
  - The Chemical Elements of Cells and Organisms 7
  - The Origin of Biomolecules and Cells 8
  - The Complexity and Size of Biological Molecules 8
  - The Biopolymers: Proteins, Nucleic Acids, and Carbohydrates 9
  - Lipids and Membranes 11
- 1.3 Distinguishing Characteristics of Living Systems 11**
- 1.4 The Unit of Biological Organization: The Cell 13**
- 1.5 Biochemistry and the Information Explosion 14**

## CHAPTER 2

### The Chemical Foundation of Life: Weak Interactions in an Aqueous Environment 18



- 2.1 The Importance of Noncovalent Interactions in Biochemistry 20**
- 2.2 The Nature of Noncovalent Interactions 21**
  - Charge–Charge Interactions 22
  - Dipole and Induced Dipole Interactions 23
  - Van der Waals Interactions 23
  - Hydrogen Bonds 24
- 2.3 The Role of Water in Biological Processes 26**
  - The Structure and Properties of Water 26

- Water as a Solvent 27
- Ionic Compounds in Aqueous Solution 28
- Hydrophilic Molecules in Aqueous Solution 28
- Hydrophobic Molecules in Aqueous Solution 28
- Amphipathic Molecules in Aqueous Solution 29

- 2.4 Acid–Base Equilibria 29**
  - Acids and Bases: Proton Donors and Acceptors 30
  - Ionization of Water and the Ion Product 30
  - The pH Scale and the Physiological pH Range 31
  - Weak Acid and Base Equilibria:  $K_a$  and  $pK_a$  32
  - Titration of Weak Acids: The Henderson–Hasselbalch Equation 33
  - Buffer Solutions 34
  - Molecules with Multiple Ionizing Groups 35
- 2.5 Interactions Between Macroions in Solution 38**
  - Solubility of Macroions and pH 38
  - The Influence of Small Ions: Ionic Strength 40
- TOOLS OF BIOCHEMISTRY 2A** Electrophoresis and Isoelectric Focusing 44
- FOUNDATION FIGURE** Biomolecules: Structure and Function 46

## CHAPTER 3

### The Energetics of Life 48



- 3.1 Free Energy 50**
  - Thermodynamic Systems 50
  - The First Law of Thermodynamics and Enthalpy 50
  - The Driving Force for a Process 51
  - Entropy 52
  - The Second Law of Thermodynamics 53
- 3.2 Free Energy: The Second Law in Open Systems 53**
  - Free Energy Defined in Terms of Enthalpy and Entropy Changes in the System 53
  - An Example of the Interplay of Enthalpy and Entropy: The Transition Between Liquid Water and Ice 54
  - The Interplay of Enthalpy and Entropy: A Summary 54
  - Free Energy and Useful Work 56

### 3.3 The Relationships Between Free Energy, the Equilibrium State, and Nonequilibrium Concentrations of Reactants and Products 56

Equilibrium, Le Chatelier's Principle, and the Standard State 56

Changes in Concentration and  $\Delta G$  57

$\Delta G$  versus  $\Delta G^\circ$ ,  $Q$  versus  $K$ , and Homeostasis versus Equilibrium 57

Water,  $H^+$  in Buffered Solutions, and the "Biochemical Standard State" 59

### 3.4 Free Energy in Biological Systems 60

Organic Phosphate Compounds as Energy Transducers 60

Phosphoryl Group Transfer Potential 63

Free Energy and Concentration Gradients: A Close Look at Diffusion Through a Membrane 63

$\Delta G$  and Oxidation/Reduction Reactions in Cells 64

Quantification of Reducing Power: Standard Reduction Potential 64

Standard Free Energy Changes in Oxidation–Reduction Reactions 66

Calculating Free Energy Changes for Biological Oxidations under Nonequilibrium Conditions 67

A Brief Overview of Free Energy Changes in Cells 67

Alternative Nucleic Acid Structures: B and A Helices 84

DNA and RNA Molecules in Vivo 86

DNA Molecules 86

Circular DNA and Supercoiling 87

Single-Stranded Polynucleotides 88

### 4.4 Alternative Secondary Structures of DNA 90

Left-Handed DNA (Z-DNA) 90

Hairpins and Cruciforms 91

Triple Helices 91

G-Quadruplexes 92

### 4.5 The Helix-to-Random Coil Transition: Nucleic Acid Denaturation 93

### 4.6 The Biological Functions of Nucleic Acids: A Preview of Genetic Biochemistry 94

Genetic Information Storage: The Genome 94

Replication: DNA to DNA 94

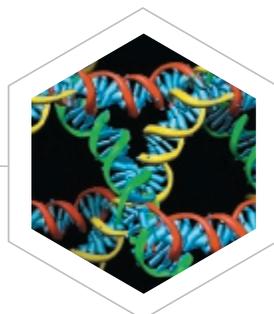
Transcription: DNA to RNA 95

Translation: RNA to Protein 95

**TOOLS OF BIOCHEMISTRY 4A** Manipulating DNA 99

**TOOLS OF BIOCHEMISTRY 4B** An Introduction to X-Ray Diffraction 104

## CHAPTER 4 Nucleic Acids 72



### 4.1 Nucleic Acids—Informational Macromolecules 74

The Two Types of Nucleic Acid: DNA and RNA 74

Properties of the Nucleotides 76

Stability and Formation of the Phosphodiester Linkage 77

### 4.2 Primary Structure of Nucleic Acids 79

The Nature and Significance of Primary Structure 79

DNA as the Genetic Substance: Early Evidence 80

### 4.3 Secondary and Tertiary Structures of Nucleic Acids 81

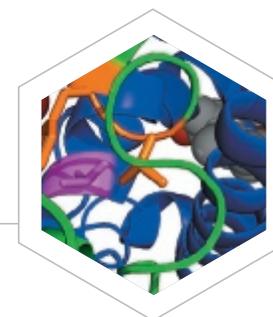
The DNA Double Helix 81

Data Leading Toward the Watson–Crick Double-Helix Model 81

X-Ray Analysis of DNA Fibers 81

Semiconservative Nature of DNA Replication 83

## CHAPTER 5 Introduction to Proteins: The Primary Level of Protein Structure 108



### 5.1 Amino Acids 111

Structure of the  $\alpha$ -Amino Acids 111

Stereochemistry of the  $\alpha$ -Amino Acids 111

Properties of Amino Acid Side Chains: Classes of  $\alpha$ -Amino Acids 115

Amino Acids with Nonpolar Aliphatic Side Chains 115

Amino Acids with Nonpolar Aromatic Side Chains 115

Amino Acids with Polar Side Chains 116

Amino Acids with Positively Charged (Basic) Side Chains 116

Amino Acids with Negatively Charged (Acidic) Side Chains 117

Rare Genetically Encoded Amino Acids 117

Modified Amino Acids 117

### 5.2 Peptides and the Peptide Bond 117

The Structure of the Peptide Bond 118

Stability and Formation of the Peptide Bond 119

Peptides 119

Polypeptides as Polyampholytes 120

**5.3 Proteins: Polypeptides of Defined Sequence 121****5.4 From Gene to Protein 123**

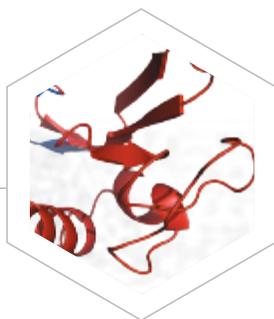
The Genetic Code 123

Posttranslational Processing of Proteins 124

**5.5 From Gene Sequence to Protein Function 125****5.6 Protein Sequence Homology 127****TOOLS OF BIOCHEMISTRY 5A** Protein Expression and Purification 131**TOOLS OF BIOCHEMISTRY 5B** Mass, Sequence, and Amino Acid Analyses of Purified Proteins 138

## CHAPTER 6

### The Three-Dimensional Structure of Proteins 144

**6.1 Secondary Structure: Regular Ways to Fold the Polypeptide Chain 146**

Theoretical Descriptions of Regular Polypeptide Structures 146

 $\alpha$  Helices and  $\beta$  Sheets 148

Describing the Structures: Helices and Sheets 148

Amphipathic Helices and Sheets 149

Ramachandran Plots 150

**6.2 Fibrous Proteins: Structural Materials of Cells and Tissues 152**

The Keratins 152

Fibroin 153

Collagen 154

**6.3 Globular Proteins: Tertiary Structure and Functional Diversity 156**

Different Folding for Different Functions 156

Different Modes of Display Aid Our Understanding of Protein Structure 156

Varieties of Globular Protein Structure: Patterns of Main-Chain Folding 157

**6.4 Factors Determining Secondary and Tertiary Structure 161**

The Information for Protein Folding 161

The Thermodynamics of Folding 162

Conformational Entropy 162

Charge–Charge Interactions 163

Internal Hydrogen Bonds 163

Van der Waals Interactions 163

The Hydrophobic Effect 163

Disulfide Bonds and Protein Stability 164

Prosthetic Groups, Ion-Binding, and Protein Stability 165

**6.5 Dynamics of Globular Protein Structure 166**

Kinetics of Protein Folding 166

The “Energy Landscape” Model of Protein Folding 167

Intermediate and Off-Pathway States in Protein Folding 168

Chaperones Facilitate Protein Folding in Vivo 168

Protein Misfolding and Disease 170

**6.6 Prediction of Protein Secondary and Tertiary Structure 171**

Prediction of Secondary Structure 171

Tertiary Structure Prediction: Computer Simulation of Folding 172

**6.7 Quaternary Structure of Proteins 172**

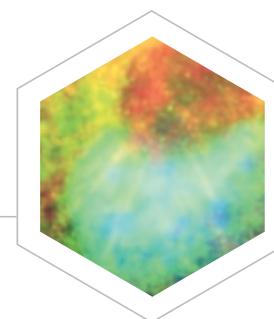
Symmetry in Multisubunit Proteins: Homotypic Protein–Protein Interactions 172

Heterotypic Protein–Protein Interactions 174

**TOOLS OF BIOCHEMISTRY 6A** Spectroscopic Methods for Studying Macromolecular Conformation in Solution 178**TOOLS OF BIOCHEMISTRY 6B** Determining Molecular Masses and the Number of Subunits in a Protein Molecule 185**FOUNDATION FIGURE** Protein Structure and Function 188

## CHAPTER 7

### Protein Function and Evolution 190

**7.1 Binding a Specific Target: Antibody Structure and Function 192****7.2 The Adaptive Immune Response 192****7.3 The Structure of Antibodies 193****7.4 Antibody:Antigen Interactions 195**

Shape and Charge Complementarity 196

Generation of Antibody Diversity 197

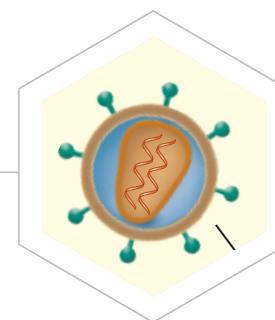
**7.5 The Immunoglobulin Superfamily 198****7.6 The Challenge of Developing an AIDS Vaccine 198****7.7 Antibodies and Immunoconjugates as Potential Cancer Treatments 199**

- 7.8 Oxygen Transport from Lungs to Tissues: Protein Conformational Change Enhances Function** 200
- 7.9 The Oxygen-Binding Sites in Myoglobin and Hemoglobin** 201  
Analysis of Oxygen Binding by Myoglobin 203
- 7.10 The Role of Conformational Change in Oxygen Transport** 204  
Cooperative Binding and Allostery 204  
Models for the Allosteric Change in Hemoglobin 206  
Changes in Hemoglobin Structure Accompanying Oxygen Binding 206  
A Closer Look at the Allosteric Change in Hemoglobin 208
- 7.11 Allosteric Effectors of Hemoglobin Promote Efficient Oxygen Delivery to Tissues** 211  
Response to pH Changes: The Bohr Effect 211  
Carbon Dioxide Transport 212  
Response to Chloride Ion at the  $\alpha$ -Globin N-Terminus 212  
2,3-Bisphosphoglycerate 213
- 7.12 Myoglobin and Hemoglobin as Examples of the Evolution of Protein Function** 214  
The Structure of Eukaryotic Genes: Exons and Introns 214
- 7.13 Mechanisms of Protein Mutation** 215  
Substitution of DNA Nucleotides 215  
Nucleotide Deletions or Insertions 216  
Gene Duplications and Rearrangements 216  
Evolution of the Myoglobin–Hemoglobin Family of Proteins 216
- 7.14 Hemoglobin Variants and Their Inheritance: Genetic Diseases** 218  
Pathological Effects of Variant Hemoglobins 218
- 7.15 Protein Function Requiring Large Conformational Changes: Muscle Contraction** 220
- 7.16 Actin and Myosin** 221  
Actin 221  
Myosin 221
- 7.17 The Structure of Muscle** 223
- 7.18 The Mechanism of Contraction** 223  
Regulation of Contraction: The Role of Calcium 226

**TOOLS OF BIOCHEMISTRY 7A** Immunological Methods 230

## CHAPTER 8

### Enzymes: Biological Catalysts 232



- 8.1 Enzymes As Biological Catalysts** 234
- 8.2 The Diversity of Enzyme Function** 234
- 8.3 Chemical Reaction Rates and the Effects of Catalysts** 235  
Reaction Rates, Rate Constants, and Reaction Order 235  
First-Order Reactions 235  
Second-Order Reactions 237  
Transition States and Reaction Rates 237  
Transition State Theory Applied to Enzymatic Catalysis 239
- 8.4 How Enzymes Act as Catalysts: Principles and Examples** 240  
Models for Substrate Binding and Catalysis 241  
Mechanisms for Achieving Rate Acceleration 241  
Case Study #1: Lysozyme 243  
Case Study #2: Chymotrypsin, a Serine Protease 245
- 8.5 Coenzymes, Vitamins, and Essential Metals** 248  
Coenzyme Function in Catalysis 248  
Metal Ions in Enzymes 249
- 8.6 The Kinetics of Enzymatic Catalysis** 250  
Reaction Rate for a Simple Enzyme-Catalyzed Reaction: Michaelis–Menten Kinetics 250  
Interpreting  $K_M$ ,  $k_{cat}$ , and  $k_{cat}/K_M$  252  
Enzyme Mutants May Affect  $k_{cat}$  and  $K_M$  Differently 253  
Analysis of Kinetic Data: Testing the Michaelis–Menten Model 253
- 8.7 Enzyme Inhibition** 254  
Reversible Inhibition 254  
Competitive Inhibition 254  
Uncompetitive Inhibition 256  
Mixed Inhibition 258  
Irreversible Inhibition 259  
Multisubstrate Reactions 260  
Random Substrate Binding 260  
Ordered Substrate Binding 260  
The Ping-Pong Mechanism 260  
Qualitative Interpretation of  $K_M$  and  $V_{max}$ : Application to Multisubstrate Reaction Mechanisms 260

**8.8 The Regulation of Enzyme Activity 262**

- Substrate-Level Control 262
- Feedback Control 262
- Allosteric Enzymes 263
  - Homoallostery 263
  - Heteroallostery 264
- Aspartate Carbamoyltransferase: An Example of an Allosteric Enzyme 264

**8.9 Covalent Modifications Used to Regulate Enzyme Activity 266**

- Pancreatic Proteases: Activation by Irreversible Protein Backbone Cleavage 267

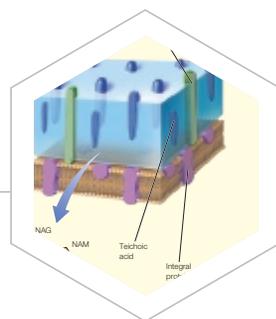
**8.10 Nonprotein Biocatalysts: Catalytic Nucleic Acids 268**

**TOOLS OF BIOCHEMISTRY 8A** How to Measure the Rates of Enzyme-Catalyzed Reactions 273

**FOUNDATION FIGURE** Regulation of Enzyme Activity 276

## CHAPTER 9

### Carbohydrates: Sugars, Saccharides, Glycans 278

**9.1 Monosaccharides 281**

- Aldoses and Ketoses 281
- Enantiomers 281
  - Alternative Designations for Enantiomers: D-L and R-S 281
- Monosaccharide Enantiomers in Nature 282
- Diastereomers 282
  - Tetrose Diastereomers 282
  - Pentose Diastereomers 283
  - Hexose Diastereomers 283
- Aldose Ring Structures 283
  - Pentose Rings 283
  - Hexose Rings 285
- Sugars with More Than Six Carbons 287

**9.2 Derivatives of the Monosaccharides 287**

- Phosphate Esters 287
- Lactones and Acids 288
- Alditols 288
- Amino Sugars 288
- Glycosides 288

**9.3 Oligosaccharides 289**

- Oligosaccharide Structures 289

Distinguishing Features of Different Disaccharides 289

Writing the Structure of Disaccharides 290

Stability and Formation of the Glycosidic Bond 291

**9.4 Polysaccharides 292**

- Storage Polysaccharides 293
- Structural Polysaccharides 294
  - Cellulose 294
  - Chitin 295
- Glycosaminoglycans 296
  - The Proteoglycan Complex 296
- Nonstructural Roles of Glycosaminoglycans 296
- Bacterial Cell Wall Polysaccharides; Peptidoglycan 297

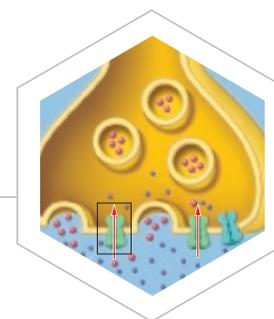
**9.5 Glycoproteins 298**

- N-Linked and O-Linked Glycoproteins 298
  - N-Linked Glycans 298
  - O-Linked Glycans 298
- Blood Group Antigens 299
- Erythropoietin: A Glycoprotein with Both O- and N-Linked Oligosaccharides 300
- Influenza Neuraminidase, a Target for Antiviral Drugs 300

**TOOLS OF BIOCHEMISTRY 9A** The Emerging Field of Glycomics 303

## CHAPTER 10

### Lipids, Membranes, and Cellular Transport 304

**10.1 The Molecular Structure and Behavior of Lipids 306**

- Fatty Acids 306
- Triacylglycerols: Fats 308
- Soaps and Detergents 309
- Waxes 309

**10.2 The Lipid Constituents of Biological Membranes 309**

- Glycerophospholipids 310
- Sphingolipids and Glycosphingolipids 311
- Glycoglycerolipids 312
- Cholesterol 312

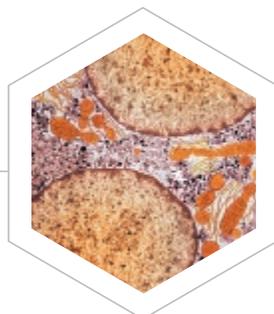
**10.3 The Structure and Properties of Membranes and Membrane Proteins 313**

- Motion in Membranes 314
  - Motion in Synthetic Membranes 314
  - Motion in Biological Membranes 315
- The Asymmetry of Membranes 315

- Characteristics of Membrane Proteins 316
- Insertion of Proteins into Membranes 317
- Evolution of the Fluid Mosaic Model of Membrane Structure 319
- 10.4 Transport Across Membranes 321**
  - The Thermodynamics of Transport 321
  - Nonmediated Transport: Diffusion 322
  - Facilitated Transport: Accelerated Diffusion 323
    - Carriers 323
    - Permeases 324
    - Pore-Facilitated Transport 325
    - Ion Selectivity and Gating 326
  - Active Transport: Transport Against a Concentration Gradient 328
- 10.5 Ion Pumps: Direct Coupling of ATP Hydrolysis to Transport 328**
- 10.6 Ion Transporters and Disease 330**
- 10.7 Cotransport Systems 331**
- 10.8 Excitable Membranes, Action Potentials, and Neurotransmission 332**
  - The Resting Potential 332
  - The Action Potential 333
  - Toxins and Neurotransmission 334
- FOUNDATION FIGURE** Targeting Pain and Inflammation through Drug Design 338

## CHAPTER 11

### Chemical Logic of Metabolism 340



- 11.1 A First Look at Metabolism 342**
- 11.2 Freeways on the Metabolic Road Map 343**
  - Central Pathways of Energy Metabolism 343
  - Distinct Pathways for Biosynthesis and Degradation 346
- 11.3 Biochemical Reaction Types 347**
  - Nucleophilic Substitutions 347
  - Nucleophilic Additions 348
  - Carbonyl Condensations 348
  - Eliminations 350
  - Oxidations and Reductions 350
- 11.4 Bioenergetics of Metabolic Pathways 350**
  - Oxidation as a Metabolic Energy Source 350
  - Biological Oxidations: Energy Release in Small Increments 351

- Energy Yields, Respiratory Quotients, and Reducing Equivalents 351
- ATP as a Free Energy Currency 352
- Metabolite Concentrations and Solvent Capacity 354
- Thermodynamic Properties of ATP 355
- The Important Differences Between  $\Delta G$  and  $\Delta G^\circ'$  356
- Kinetic Control of Substrate Cycles 356
- Other High-Energy Phosphate Compounds 357
- Other High-Energy Nucleotides 358
- Adenylate Energy Charge 358

- 11.5 Major Metabolic Control Mechanisms 358**
  - Control of Enzyme Levels 358
  - Control of Enzyme Activity 359
  - Compartmentation 359
  - Hormonal Regulation 360
  - Distributive Control of Metabolism 361

- 11.6 Experimental Analysis of Metabolism 362**
  - Goals of the Study of Metabolism 362
  - Levels of Organization at Which Metabolism Is Studied 362
    - Whole Organisms 362
    - Isolated or Perfused Organs 362
    - Whole Cells 362
    - Cell-Free Systems 363
    - Purified Components 363
    - Systems Level 363
  - Metabolic Probes 363

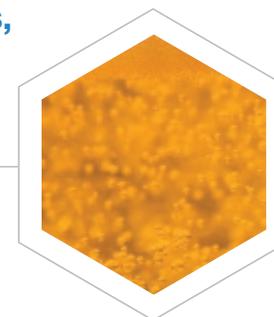
**TOOLS OF BIOCHEMISTRY 11A** Metabolomics 367

**TOOLS OF BIOCHEMISTRY 11B** Radioactive and Stable Isotopes 370

**FOUNDATION FIGURE** Enzyme Kinetics and Drug Action 372

## CHAPTER 12

### Carbohydrate Metabolism: Glycolysis, Gluconeogenesis, Glycogen Metabolism, and the Pentose Phosphate Pathway 374



- 12.1 An Overview of Glycolysis 377**
  - Relation of Glycolysis to Other Pathways 377
  - Anaerobic and Aerobic Glycolysis 377
  - Chemical Strategy of Glycolysis 379

**12.2 Reactions of Glycolysis 379**

- Reactions 1–5: The Energy Investment Phase 379
- Reaction 1: The First ATP Investment 379
- Reaction 2: Isomerization of Glucose-6-Phosphate 381
- Reaction 3: The Second Investment of ATP 381
- Reaction 4: Cleavage to Two Triose Phosphates 381
- Reaction 5: Isomerization of Dihydroxyacetone Phosphate 382
- Reactions 6–10: The Energy Generation Phase 383
- Reaction 6: Generation of the First Energy-Rich Compound 383
- Reaction 7: The First Substrate-Level Phosphorylation 383
- Reaction 8: Preparing for Synthesis of the Next High-Energy Compound 384
- Reaction 9: Synthesis of the Second High-Energy Compound 385
- Reaction 10: The Second Substrate-Level Phosphorylation 385

**12.3 Metabolic Fates of Pyruvate 386**

- Lactate Metabolism 386
- Isozymes of Lactate Dehydrogenase 388
- Ethanol Metabolism 388

**12.4 Energy and Electron Balance Sheets 389****12.5 Gluconeogenesis 390**

- Physiological Need for Glucose Synthesis in Animals 390
- Enzymatic Relationship of Gluconeogenesis to Glycolysis 391
- Bypass 1: Conversion of Pyruvate to Phosphoenolpyruvate 391
- Bypass 2: Conversion of Fructose-1,6-bisphosphate to Fructose-6-phosphate 392
- Bypass 3: Conversion of Glucose-6-phosphate to Glucose 392
- Stoichiometry and Energy Balance of Gluconeogenesis 393
- Gluconeogenesis 393
- Reversal of Glycolysis 393
- Substrates for Gluconeogenesis 393
- Lactate 393
- Amino Acids 394
- Ethanol Consumption and Gluconeogenesis 394

**12.6 Coordinated Regulation of Glycolysis and Gluconeogenesis 394**

- The Pasteur Effect 394
- Reciprocal Regulation of Glycolysis and Gluconeogenesis 395
- Regulation at the Phosphofructokinase/ Fructose-1,6-Bisphosphatase Substrate Cycle 396

Fructose-2,6-bisphosphate and the Control of Glycolysis and Gluconeogenesis 396

Regulation at the Pyruvate Kinase/Pyruvate Carboxylase + PEPCK Substrate Cycle 399

Regulation at the Hexokinase/Glucose-6-Phosphatase Substrate Cycle 399

**12.7 Entry of Other Sugars into the Glycolytic Pathway 400**

- Monosaccharide Metabolism 400
- Galactose Utilization 400
- Fructose Utilization 400
- Disaccharide Metabolism 400
- Glycerol Metabolism 401
- Polysaccharide Metabolism 401
- Hydrolytic and Phosphorolytic Cleavages 401
- Starch and Glycogen Digestion 402

**12.8 Glycogen Metabolism in Muscle and Liver 402**

- Glycogen Breakdown 402
- Glycogen Biosynthesis 403
- Biosynthesis of UDP-Glucose 403
- The Glycogen Synthase Reaction 404
- Formation of Branches 405

**12.9 Coordinated Regulation of Glycogen Metabolism 405**

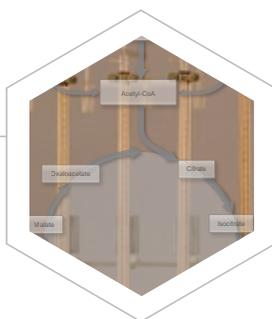
- Structure of Glycogen Phosphorylase 405
- Control of Phosphorylase Activity 406
- Proteins in the Glycogenolytic Cascade 406
- Cyclic AMP-Dependent Protein Kinase 407
- Phosphorylase *b* Kinase 407
- Calmodulin 407
- Nonhormonal Control of Glycogenolysis 407
- Control of Glycogen Synthase Activity 408
- Congenital Defects of Glycogen Metabolism in Humans 409

**12.10 A Biosynthetic Pathway That Oxidizes Glucose: The Pentose Phosphate Pathway 410**

- The Oxidative Phase: Generating Reducing Power as NADPH 411
- The Nonoxidative Phase: Alternative Fates of Pentose Phosphates 411
- Production of Six-Carbon and Three-Carbon Sugar Phosphates 411
- Tailoring the Pentose Phosphate Pathway to Specific Needs 413
- Regulation of the Pentose Phosphate Pathway 414
- Human Genetic Disorders Involving Pentose Phosphate Pathway Enzymes 415

## CHAPTER 13

### The Citric Acid Cycle 420



- 13.1 Overview of Pyruvate Oxidation and the Citric Acid Cycle 423**  
 The Three Stages of Respiration 423  
 Chemical Strategy of the Citric Acid Cycle 424  
 Discovery of the Citric Acid Cycle 426
- 13.2 Pyruvate Oxidation: A Major Entry Route for Carbon into the Citric Acid Cycle 426**  
 Overview of Pyruvate Oxidation and the Pyruvate Dehydrogenase Complex 426  
 Coenzymes Involved in Pyruvate Oxidation and the Citric Acid Cycle 427  
 Thiamine Pyrophosphate (TPP) 428  
 Lipoic Acid (Lipoamide) 428  
 Coenzyme A: Activation of Acyl Groups 428  
 Flavin Adenine Dinucleotide (FAD) 429  
 Nicotinamide Adenine Dinucleotide (NAD<sup>+</sup>) 431  
 Action of the Pyruvate Dehydrogenase Complex 431
- 13.3 The Citric Acid Cycle 433**  
 Step 1: Introduction of Two Carbon Atoms as Acetyl-CoA 433  
 Step 2: Isomerization of Citrate 434  
 Step 3: Conservation of the Energy Released by an Oxidative Decarboxylation in the Reduced Electron Carrier NADH 435  
 Step 4: Conservation of Energy in NADH by a Second Oxidative Decarboxylation 435  
 Step 5: A Substrate-Level Phosphorylation 436  
 Step 6: A Flavin-Dependent Dehydrogenation 437  
 Step 7: Hydration of a Carbon–Carbon Double Bond 437  
 Step 8: An Oxidation that Regenerates Oxaloacetate 437
- 13.4 Stoichiometry and Energetics of the Citric Acid Cycle 438**
- 13.5 Regulation of Pyruvate Dehydrogenase and the Citric Acid Cycle 438**  
 Control of Pyruvate Oxidation 439  
 Control of the Citric Acid Cycle 440
- 13.6 Organization and Evolution of the Citric Acid Cycle 440**
- 13.7 Citric Acid Cycle Malfunction as a Cause of Human Disease 441**

### 13.8 Anaplerotic Sequences: The Need to Replace Cycle Intermediates 441

- Reactions that Replenish Oxaloacetate 442  
 The Malic Enzyme 442  
 Reactions Involving Amino Acids 442

### 13.9 The Glyoxylate Cycle: An Anabolic Variant of the Citric Acid Cycle 443

- TOOLS OF BIOCHEMISTRY 13A** Detecting and Analyzing Protein–Protein Interactions 448

## CHAPTER 14

### Electron Transport, Oxidative Phosphorylation, and Oxygen Metabolism 450



- 14.1 The Mitochondrion: Scene of the Action 453**
- 14.2 Free Energy Changes in Biological Oxidations 453**
- 14.3 Electron Transport 456**  
 Electron Carriers in the Respiratory Chain 456  
 Flavoproteins 456  
 Iron–Sulfur Proteins 456  
 Coenzyme Q 456  
 Cytochromes 457  
 Respiratory Complexes 458  
 NADH–Coenzyme Q Reductase (Complex I) 458  
 Succinate–Coenzyme Q Reductase (Complex II; Succinate Dehydrogenase) 460  
 Coenzyme Q: Cytochrome *c* Oxidoreductase (Complex III) 461  
 Cytochrome *c* Oxidase (Complex IV) 462
- 14.4 Oxidative Phosphorylation 463**  
 The P/O Ratio: Energetics of Oxidative Phosphorylation 463  
 Oxidative Reactions That Drive ATP Synthesis 464  
 Mechanism of Oxidative Phosphorylation: Chemiosmotic Coupling 465  
 A Closer Look at Chemiosmotic Coupling: The Experimental Evidence 466  
 Membranes Can Establish Proton Gradients 466  
 An Intact Inner Membrane Is Required for Oxidative Phosphorylation 466  
 Key Electron Transport Proteins Span the Inner Membrane 467  
 Uncouplers Act by Dissipating the Proton Gradient 467

Generation of a Proton Gradient Permits ATP Synthesis  
Without Electron Transport 467

**Complex V: The Enzyme System for ATP Synthesis** 467

Discovery and Reconstitution of ATP Synthase 467

Structure of the Mitochondrial F<sub>1</sub>ATP Synthase Complex 469

Mechanism of ATP Synthesis 469

### 14.5 Respiratory States and Respiratory Control 472

### 14.6 Mitochondrial Transport Systems 475

Transport of Substrates and Products into and out  
of Mitochondria 475

Shuttling Cytoplasmic Reducing Equivalents  
into Mitochondria 476

### 14.7 Energy Yields from Oxidative Metabolism 477

### 14.8 The Mitochondrial Genome, Evolution, and Disease 477

### 14.9 Oxygen as a Substrate for Other Metabolic Reactions 479

Oxidases and Oxygenases 479

Cytochrome P450 Monooxygenase 479

Reactive Oxygen Species, Antioxidant Defenses,  
and Human Disease 480

Formation of Reactive Oxygen Species 480

Dealing with Oxidative Stress 480

**FOUNDATION FIGURE** Intermediary  
Metabolism 484

## CHAPTER 15

### Photosynthesis 486



### 15.1 The Basic Processes of Photosynthesis 490

### 15.2 The Chloroplast 491

### 15.3 The Light Reactions 492

Absorption of Light: The Light-Harvesting  
System 492

The Energy of Light 492

The Light-Absorbing Pigments 492

The Light-Gathering Structures 493

**Photochemistry in Plants and Algae:**  
Two Photosystems in Series 495

Photosystem II: The Splitting of Water 497

Photosystem I: Production of NADPH 499

Summation of the Two Systems: The Overall Reaction  
and NADPH and ATP Generation 500

An Alternative Light Reaction Mechanism:  
Cyclic Electron Flow 502

Reaction Center Complexes in Photosynthetic  
Bacteria 502

Evolution of Photosynthesis 502

### 15.4 The Carbon Reactions: The Calvin Cycle 503

Stage I: Carbon Dioxide Fixation  
and Sugar Production 504

Incorporation of CO<sub>2</sub> into a Three-Carbon Sugar 504

Formation of Hexose Sugars 505

Stage II: Regeneration of the Acceptor 505

### 15.5 A Summary of the Light and Carbon Reactions in Two-System Photosynthesis 506

The Overall Reaction and the Efficiency  
of Photosynthesis 506

Regulation of Photosynthesis 506

### 15.6 Photorespiration and the C<sub>4</sub> Cycle 507

## CHAPTER 16

### Lipid Metabolism 512



### Part I: Bioenergetic Aspects of Lipid Metabolism 515

### 16.1 Utilization and Transport of Fat and Cholesterol 515

Fats as Energy Reserves 515

Fat Digestion and Absorption 515

Transport of Fat to Tissues: Lipoproteins 517

Classification and Functions of Lipoproteins 517

Transport and Utilization of Lipoproteins 518

**Cholesterol Transport and Utilization**  
in Animals 519

The LDL Receptor and Cholesterol Homeostasis 520

Cholesterol, LDL, and Atherosclerosis 522

Mobilization of Stored Fat for Energy Generation 523

### 16.2 Fatty Acid Oxidation 523

Early Experiments 523

Fatty Acid Activation and Transport  
into Mitochondria 525

The  $\beta$ -Oxidation Pathway 526

Reaction 1: The Initial Dehydrogenation 527

Reactions 2 and 3: Hydration and Dehydrogenation 527

Reaction 4: Thiolitic Cleavage 527

**Mitochondrial  $\beta$ -Oxidation Involves Multiple Isozymes** 528

- Energy Yield from Fatty Acid Oxidation 528
- Oxidation of Unsaturated Fatty Acids 529
- Oxidation of Fatty Acids with Odd-Numbered Carbon Chains 530
- Control of Fatty Acid Oxidation 530
- Ketogenesis 531

### 16.3 Fatty Acid Biosynthesis 532

- Relationship of Fatty Acid Synthesis to Carbohydrate Metabolism 532
- Early Studies of Fatty Acid Synthesis 533
- Biosynthesis of Palmitate from Acetyl-CoA 533
- Synthesis of Malonyl-CoA 533
- Malonyl-CoA to Palmitate 534
- Multifunctional Proteins in Fatty Acid Synthesis 536
- Transport of Acetyl Units and Reducing Equivalents into the Cytosol 537
- Elongation of Fatty Acid Chains 538
- Fatty Acid Desaturation 538
- Control of Fatty Acid Synthesis 539

### 16.4 Biosynthesis of Triacylglycerols 540

## Part II: Metabolism of Membrane Lipids, Steroids, and Other Complex Lipids 541

### 16.5 Glycerophospholipids 541

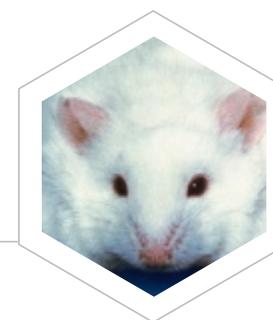
### 16.6 Sphingolipids 542

### 16.7 Steroid Metabolism 543

- Steroids: Some Structural Considerations 543
- Biosynthesis of Cholesterol 544
- Early Studies of Cholesterol Biosynthesis 544
- Stage 1: Formation of Mevalonate 545
- Stage 2: Synthesis of Squalene from Mevalonate 545
- Stage 3: Cyclization of Squalene to Lanosterol and Its Conversion to Cholesterol 545
- Control of Cholesterol Biosynthesis 546
- Cholesterol Derivatives: Bile Acids, Steroid Hormones, and Vitamin D 548
- Bile Acids 548
- Steroid Hormones 548
- Vitamin D 548
- Lipid-Soluble Vitamins 550
- Vitamin A 550
- Vitamin E 551
- Vitamin K 551

### 16.8 Eicosanoids: Prostaglandins, Thromboxanes, and Leukotrienes 551

## CHAPTER 17 Interorgan and Intracellular Coordination of Energy Metabolism in Vertebrates 556



### 17.1 Interdependence of the Major Organs in Vertebrate Fuel Metabolism 558

- Fuel Inputs and Outputs 558
- Metabolic Division of Labor Among the Major Organs 558
- Brain 558
- Muscle 560
- Heart 560
- Adipose Tissue 560
- Liver 560
- Blood 560

### 17.2 Hormonal Regulation of Fuel Metabolism 561

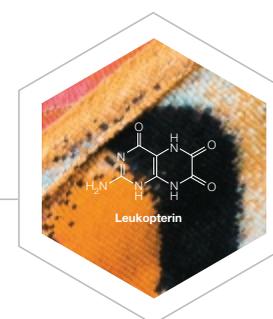
- Actions of the Major Hormones 561
- Insulin 562
- Glucagon 562
- Epinephrine 563
- Coordination of Energy Homeostasis 563
- AMP-Activated Protein Kinase (AMPK) 563
- Mammalian Target of Rapamycin (mTOR) 564
- Sirtuins 565
- Endocrine Regulation of Energy Homeostasis 566

### 17.3 Responses to Metabolic Stress: Starvation, Diabetes 567

- Starvation 568
- Diabetes 569

**FOUNDATION FIGURE** Energy Regulation 574

## CHAPTER 18 Amino Acid and Nitrogen Metabolism 576



### 18.1 Utilization of Inorganic Nitrogen: The Nitrogen Cycle 579

- Biological Nitrogen Fixation 579
- Nitrate Utilization 581

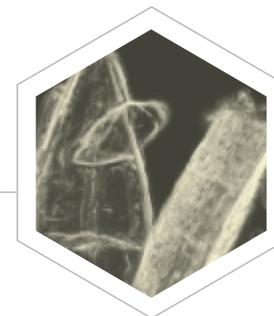
### 18.2 Utilization of Ammonia: Biogenesis of Organic Nitrogen 581

- Glutamate Dehydrogenase: Reductive Amination of  $\alpha$ -Ketoglutarate 581
- Glutamine Synthetase: Generation of Biologically Active Amide Nitrogen 582
- Carbamoyl Phosphate Synthetase: Generation of an Intermediate for Arginine and Pyrimidine Synthesis 582
- 18.3 The Nitrogen Economy and Protein Turnover 582**
- Metabolic Consequences of the Absence of Nitrogen Storage Compounds 582
- Protein Turnover 583
- Intracellular Proteases and Sites of Turnover 583
- Chemical Signals for Turnover—Ubiquitination 584
- 18.4 Coenzymes Involved in Nitrogen Metabolism 584**
- Pyridoxal Phosphate 584
- Folic Acid Coenzymes and One-Carbon Metabolism 586
- Discovery and Chemistry of Folic Acid 586
- Conversion of Folic Acid to Tetrahydrofolate 587
- Tetrahydrofolate in the Metabolism of One-Carbon Units 587
- Folic Acid in the Prevention of Heart Disease and Birth Defects 589
- B<sub>12</sub> Coenzymes 589
- B<sub>12</sub> Coenzymes and Pernicious Anemia 590
- 18.5 Amino Acid Degradation and Metabolism of Nitrogenous End Products 590**
- Transamination Reactions 590
- Detoxification and Excretion of Ammonia 591
- Transport of Ammonia to the Liver 591
- The Krebs–Henseleit Urea Cycle 592
- 18.6 Pathways of Amino Acid Degradation 594**
- Pyruvate Family of Glucogenic Amino Acids 594
- Oxaloacetate Family of Glucogenic Amino Acids 595
- $\alpha$ -Ketoglutarate Family of Glucogenic Amino Acids 595
- Succinyl-CoA Family of Glucogenic Amino Acids 596
- Acetoacetate/Acetyl-CoA Family of Ketogenic Amino Acids 596
- Phenylalanine and Tyrosine Degradation 598
- 18.7 Amino Acid Biosynthesis 599**
- Biosynthetic Capacities of Organisms 599
- Amino Acid Biosynthetic Pathways 600
- Synthesis of Glutamate, Aspartate, Alanine, Glutamine, and Asparagine 600
- Synthesis of Serine and Glycine from 3-Phosphoglycerate 600
- Synthesis of Valine, Leucine, and Isoleucine from Pyruvate 601

- 18.8 Amino Acids as Biosynthetic Precursors 602**
- S-Adenosylmethionine and Biological Methylation 602
- Precursor Functions of Glutamate 604
- Arginine Is the Precursor for Nitric Oxide and Creatine Phosphate 604
- Tryptophan and Tyrosine Are Precursors of Neurotransmitters and Biological Regulators 604

## CHAPTER 19

### Nucleotide Metabolism 610



- 19.1 Outlines of Pathways in Nucleotide Metabolism 612**
- Biosynthetic Routes:
- De Novo and Salvage Pathways 612
- Nucleic Acid Degradation and the Importance of Nucleotide Salvage 613
- PRPP, a Central Metabolite in De Novo and Salvage Pathways 613
- 19.2 De Novo Biosynthesis of Purine Ribonucleotides 614**
- Synthesis of the Purine Ring 614
- Enzyme Organization in the Purine Biosynthetic Pathway 616
- Synthesis of ATP and GTP from Inosine Monophosphate 616
- 19.3 Purine Catabolism and Its Medical Significance 617**
- Uric Acid, a Primary End Product 617
- Medical Abnormalities of Purine Catabolism 618
- Gout 618
- Lesch–Nyhan Syndrome 619
- Severe Combined Immunodeficiency Disease 619
- 19.4 Pyrimidine Ribonucleotide Metabolism 620**
- De Novo Biosynthesis of UTP and CTP 620
- Glutamine-Dependent Amidotransferases 621
- Multifunctional Enzymes in Eukaryotic Pyrimidine Metabolism 622
- 19.5 Deoxyribonucleotide Metabolism 622**
- Reduction of Ribonucleotides to Deoxyribonucleotides 622
- RNR Structure and Mechanism 623
- Source of Electrons for Ribonucleotide Reduction 623
- Regulation of Ribonucleotide Reductase Activity 623

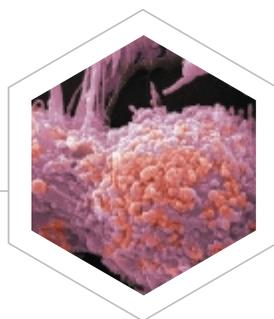
- Regulation of dNTP Pools by Selective dNTP Degradation 626
- Biosynthesis of Thymine Deoxyribonucleotides 626
- Salvage Routes to Deoxyribonucleotides 627
- Thymidylate Synthase: A Target Enzyme for Chemotherapy 629

### 19.6 Virus-Directed Alterations of Nucleotide Metabolism 631

### 19.7 Other Medically Useful Analogs 632

## CHAPTER 20

### Mechanisms of Signal Transduction 636



#### 20.1 An Overview of Hormone Action 638

- Chemical Nature of Hormones and Other Signaling Agents 639
- Hierarchical Nature of Hormonal Control 639
- Hormone Biosynthesis 640

#### 20.2 Modular Nature of Signal Transduction Systems: G Protein-Coupled Signaling 640

- Receptors 640
- Receptors as Defined by Interactions with Drugs 640
- Receptors and Adenylate Cyclase as Distinct Components of Signal Transduction Systems 640
- Structural Analysis of G Protein-Coupled Receptors 641
- Transducers: G Proteins 642
- Actions of G Proteins 642
- Structure of G Proteins 643
- Consequences of Blocking GTPase 643
- The Versatility of G Proteins 643
- Interaction of GPCRs with G Proteins 644
- G Proteins in the Visual Process 644
- Effectors 644
- Second Messengers 645
- Cyclic AMP 645
- Cyclic GMP and Nitric Oxide 645
- Phosphoinositides 646

#### 20.3 Receptor Tyrosine Kinases and Insulin Signaling 648

#### 20.4 Hormones and Gene Expression: Nuclear Receptors 650

#### 20.5 Signal Transduction, Growth Control, and Cancer 653

- Viral and Cellular Oncogenes 653
- Oncogenes in Human Tumors 654
- The Cancer Genome Mutational Landscape 655

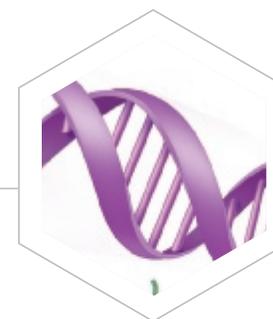
#### 20.6 Neurotransmission 656

- The Cholinergic Synapse 656
- Fast and Slow Synaptic Transmission 657
- Actions of Specific Neurotransmitters 658
- Drugs That Act in the Synaptic Cleft 659
- Peptide Neurotransmitters and Neurohormones 659

**FOUNDATION FIGURE** Cell Signaling and Protein Regulation 662

## CHAPTER 21

### Genes, Genomes, and Chromosomes 664



#### 21.1 Bacterial and Viral Genomes 666

- Viral Genomes 666
- Bacterial Genomes—The Nucleoid 666

#### 21.2 Eukaryotic Genomes 667

- Genome Sizes 667
- Repetitive Sequences 668
- Satellite DNA 668
- Duplications of Functional Genes 669
- Alu Elements 669
- Introns 669
- Gene Families 670
- Multiple Variants of a Gene 670
- Pseudogenes 670
- The ENCODE Project and the Concept of “Junk DNA” 670

#### 21.3 Physical Organization of Eukaryotic Genes: Chromosomes and Chromatin 670

- The Nucleus 670
- Chromatin 671
- Histones and Nonhistone Chromosomal Proteins 672
- The Nucleosome 672
- Higher-order Chromatin Structure in the Nucleus 674

#### 21.4 Nucleotide Sequence Analysis of Genomes 674

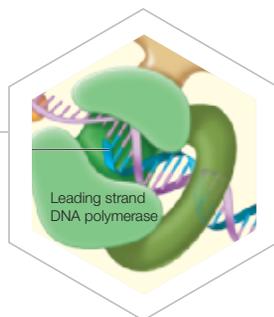
- Restriction and Modification 675
- Properties of Restriction and Modification Enzymes 676
- Determining Genome Nucleotide Sequences 677

- Mapping Large Genomes 678
- Generating Physical Maps 678
- The Principle of Southern Analysis 678
- Southern Transfer and DNA Fingerprinting 680
- Locating Genes on the Human Genome 680
- Sequence Analysis Using Artificial Chromosomes 681
- Size of the Human Genome 681
- TOOLS OF BIOCHEMISTRY 21A** Polymerase Chain Reaction 684

## CHAPTER 22

### DNA Replication 686

- 22.1 Early Insights into DNA Replication 688**
- 22.2 DNA Polymerases: Enzymes Catalyzing Polynucleotide Chain Elongation 689**
  - Structure and Activities of DNA Polymerase I 690
  - DNA Substrates for the Polymerase Reaction 690
  - Multiple Activities in a Single Polypeptide Chain 690
  - Structure of DNA Polymerase I 690
  - Discovery of Additional DNA Polymerases 691
  - Structure and Mechanism of DNA Polymerases 691
- 22.3 Other Proteins at the Replication Fork 692**
  - Genetic Maps of *E. coli* and Bacteriophage T4 692
  - Replication Proteins in Addition to DNA Polymerase 693
  - Discontinuous DNA Synthesis 693
  - RNA Primers 695
  - Proteins at the Replication Fork 695
  - The DNA Polymerase III Holoenzyme 696
  - Sliding Clamp 697
  - Clamp Loading Complex 697
  - Single-Stranded DNA-Binding Proteins: Maintaining Optimal Template Conformation 697
  - Helicases: Unwinding DNA Ahead of the Fork 698
  - Topoisomerases: Relieving Torsional Stress 699
  - Actions of Type I and Type II Topoisomerases 699
  - The Four Topoisomerases of *E. coli* 701
  - A Model of the Replisome 701
- 22.4 Eukaryotic DNA Replication 702**
  - DNA Polymerases 702
  - Other Eukaryotic Replication Proteins 702
  - Replication of Chromatin 703

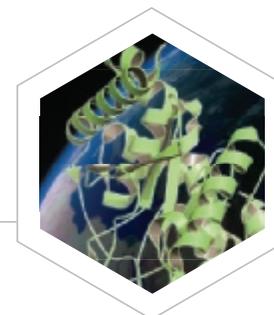


- 22.5 Initiation of DNA Replication 704**
  - Initiation of *E. coli* DNA Replication at *ori*<sup>c</sup> 704
  - Initiation of Eukaryotic Replication 705
- 22.6 Replication of Linear Genomes 705**
  - Linear Virus Genome Replication 705
  - Telomerase 706
- 22.7 Fidelity of DNA Replication 707**
  - 3' Exonucleolytic Proofreading 707
  - Polymerase Insertion Specificity 708
  - DNA Precursor Metabolism and Genomic Stability 709
  - Ribonucleotide Incorporation and Genomic Stability 709
- 22.8 RNA Viruses: The Replication of RNA Genomes 710**
  - RNA-Dependent RNA Replicases 710
  - Replication of Retroviral Genomes 710

## CHAPTER 23

### DNA Repair, Recombination, and Rearrangement 714

- 23.1 DNA Repair 716**
  - Types and Consequences of DNA Damage 716
  - Direct Repair of Damaged DNA Bases: Photoreactivation and Alkyltransferases 718
  - Photoreactivation 718
  - O<sup>6</sup>-Alkylguanine Alkyltransferase 718
  - Nucleotide Excision Repair: Excinucleases 719
  - Base Excision Repair: DNA *N*-Glycosylases 721
  - Replacement of Uracil in DNA by BER 721
  - Repair of Oxidative Damage to DNA 722
  - Mismatch Repair 722
  - Double-Strand Break Repair 724
  - Daughter-Strand Gap Repair 725
  - Translesion Synthesis and the DNA Damage Response 725
- 23.2 Recombination 726**
  - Site-Specific Recombination 726
  - Homologous Recombination 727
  - Breaking and Joining of Chromosomes 727
  - Models for Recombination 727
  - Proteins Involved in Homologous Recombination 728
- 23.3 Gene Rearrangements 730**
  - Immunoglobulin Synthesis: Generating Antibody Diversity 730



- Transposable Genetic Elements 732
- Retroviruses 733
- Gene Amplification 734
- TOOLS OF BIOCHEMISTRY 23A** Manipulating the Genome 738
- FOUNDATION FIGURE** Antibody Diversity and Use as Therapeutics 740

## CHAPTER 24

### Transcription and Posttranscriptional Processing 742



- 24.1 DNA as the Template for RNA Synthesis 744**
  - The Predicted Existence of Messenger RNA 744
  - T2 Bacteriophage and the Demonstration of Messenger RNA 745
  - RNA Dynamics in Uninfected Cells 746
- 24.2 Enzymology of RNA Synthesis: RNA Polymerase 747**
  - Biological Role of RNA Polymerase 747
  - Structure of RNA Polymerase 748
- 24.3 Mechanism of Transcription in Bacteria 749**
  - Initiation of Transcription: Interactions with Promoters 749
  - Initiation and Elongation: Incorporation of Ribonucleotides 750
  - Punctuation of Transcription: Termination 751
    - Factor-Independent Termination 752
    - Factor-Dependent Termination 753
- 24.4 Transcription in Eukaryotic Cells 753**
  - RNA Polymerase I: Transcription of the Major Ribosomal RNA Genes 754
  - RNA Polymerase III: Transcription of Small RNA Genes 754
  - RNA Polymerase II: Transcription of Structural Genes 755
  - Chromatin Structure and Transcription 756
  - Transcriptional Elongation 757
  - Termination of Transcription 757
- 24.5 Posttranscriptional Processing 757**
  - Bacterial mRNA Turnover 757
  - Posttranscriptional Processing in the Synthesis of Bacterial rRNAs and tRNAs 758
  - rRNA Processing 758

- tRNA Processing 758
- Processing of Eukaryotic mRNA 759
  - Capping 759
  - Splicing 759
  - Alternative Splicing 761
- TOOLS OF BIOCHEMISTRY 24A** Analyzing the Transcriptome 764
- TOOLS OF BIOCHEMISTRY 24B** Chromatin Immunoprecipitation 765

## CHAPTER 25

### Information Decoding: Translation and Posttranslational Protein Processing 766



- 25.1 An Overview of Translation 768**
- 25.2 The Genetic Code 769**
  - How the Code Was Deciphered 769
  - Features of the Code 770
  - Deviations from the Genetic Code 771
  - The Wobble Hypothesis 771
  - tRNA Abundance and Codon Bias 772
  - Punctuation: Stopping and Starting 772
- 25.3 The Major Participants in Translation: mRNA, tRNA, and Ribosomes 773**
  - Messenger RNA 773
  - Transfer RNA 773
  - Aminoacyl-tRNA Synthetases:
    - The First Step in Protein Synthesis 775
    - The Ribosome and Its Associated Factors 777
    - Soluble Protein Factors in Translation 778
    - Components of Ribosomes 778
    - Ribosomal RNA Structure 779
    - Internal Structure of the Ribosome 779
- 25.4 Mechanism of Translation 782**
  - Initiation 782
  - Elongation 783
  - Termination 785
  - Suppression of Nonsense Mutations 786
- 25.5 Inhibition of Translation by Antibiotics 787**
- 25.6 Translation in Eukaryotes 788**
- 25.7 Rate of Translation; Polyribosomes 789**

**25.8 The Final Stages in Protein Synthesis: Folding and Covalent Modification 789**

Chain Folding 790

Covalent Modification 790

**25.9 Protein Targeting in Eukaryotes 791**

Proteins Synthesized in the Cytoplasm 791

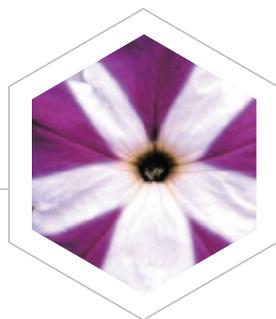
Proteins Synthesized on the Rough

Endoplasmic Reticulum 793

Role of the Golgi Complex 793

## CHAPTER 26

### Regulation of Gene Expression 796

**26.1 Regulation of Transcription in Bacteria 798**

The Lactose Operon—Earliest Insights into Transcriptional Regulation 798

Isolation and Properties of the Lactose Repressor 800

The Repressor Binding Site 800

Regulation of the *lac* Operon by Glucose:

A Positive Control System 802

The CRP–DNA Complex 802

Some Other Bacterial Transcriptional Regulatory Systems: Variations on a Theme 803

Bacteriophage  $\lambda$ : Multiple Operators, Dual Repressors, Interspersed Promoters and Operators 803

The SOS Regulon: Activation of Multiple Operons by a Common Set of Environmental Signals 805

Biosynthetic Operons: Ligand-Activated Repressors and Attenuation 806

Applicability of the Operon Model—Variations on a Theme 808

**26.2 Transcriptional Regulation in Eukaryotes 808**

Chromatin and Transcription 808

Transcriptional Control Sites and Genes 809

Nucleosome Remodeling Complexes 810

Transcription Initiation 811

Regulation of the Elongation Cycle by RNA Polymerase Phosphorylation 811

**26.3 DNA Methylation, Gene Silencing, and Epigenetics 812**

DNA Methylation in Eukaryotes 812

DNA Methylation and Gene Silencing 813

Genomic Distribution of Methylated Cytosines 813

Other Proposed Epigenetic Phenomena 814

5-Hydroxymethylcytosine 814

Chromatin Histone Modifications 814

**26.4 Regulation of Translation 814**

Regulation of Bacterial Translation 814

Regulation of Eukaryotic Translation 815

Phosphorylation of Eukaryotic Initiation Factors 815

Long Noncoding RNAs 816

**26.5 RNA Interference 816**

MicroRNAs 816

Small Interfering RNAs 817

**26.6 Riboswitches 817****26.7 RNA Editing 818****FOUNDATION FIGURE** Information Flow in Biological Systems 822

APPENDIX I: ANSWERS TO SELECTED PROBLEMS A-1

APPENDIX II: REFERENCES A-20

CREDITS C-1

INDEX I-1

# 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 the first edition of this book, 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 highlight and reinforce overarching themes (chemical logic, regulation, interface between chemistry and biology). In addition, we have added two new **Foundation Figures** to the Second Edition, bringing the total number to 10. These 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. The “**CONCEPT**” and “**CONNECTION**” statements within the narrative, which highlight fundamental concepts and real-world applications of biochemistry, have been reviewed and revised for the Second Edition.

In *Biochemistry: Concepts and Connections*, we emphasize our field as an experimental science by including 17 separate sections, called **Tools of Biochemistry**, that highlight the most important research techniques. We also provide students with 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 provide 15 to 25 problems for each chapter. (In the Second Edition we have added 3 to 4 new end-of-chapter problems to 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 Mastering Chemistry will help students with some of the most basic concepts and operations. See the table of Instructor and Student Resources on the following page.

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 colleagues 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. Susan Malloy, Program Manager, kept us organized and on schedule, juggling disparate elements in this complex project—later replaced by Anastasia Slesareva. 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. We also worked with an experienced development editor, Matt Walker. His suggested 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 our cover illustration, and Mo Spuhler helped us locate much excellent illustrative material. Once the book was in production, Mary Tindle skillfully kept us all on a complex schedule.

## Instructor and Student Resources

Resource	Instructor or Student Resource	Description
Solutions Manual ISBN: 0134814800	Instructor	Prepared by Dean Appling, Spencer Anthony-Cahill, and Christopher Mathews, the solutions manual includes worked-out answers and solutions for problems in the text.
Mastering™ Chemistry pearson.com/mastering/chemistry ISBN: 0134787250	Student & Instructor	Mastering™ Chemistry is the leading online homework, tutorial, and assessment platform, designed to improve results by engaging students with powerful content. Instructors 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. Learn more about Mastering Chemistry. <b>Mastering Chemistry for Biochemistry: Concepts and Connections, 2/e</b> now has hundreds of more biochemistry-specific assets to help students tackle threshold concepts, connect course materials to real world applications, and build the problem solving skills they need to succeed in future courses and careers.
Pearson eText ISBN: 0134763025	Student	<b>Biochemistry: Concepts and Connections 2/e</b> now offers <b>Pearson eText, optimized for mobile</b> , which seamlessly integrates videos and other rich media with the text and gives students access to their textbook anytime, anywhere. Pearson eText is available with Mastering Chemistry when packaged with new books, or as an upgrade students can purchase online. The Pearson eText mobile app offers: <ul style="list-style-type: none"> <li>• Offline access on most iOS and Android phones/tablets.</li> <li>• Accessibility (screen-reader ready)</li> <li>• Configurable reading settings, including resizable type and night reading mode</li> <li>• Instructor and student note-taking, highlighting, bookmarking, and search tools</li> <li>• Embedded videos for a more interactive learning experience</li> </ul>
TestGen Test Bank ISBN: 0134814827	Instructor	This resource includes more than 2000 questions in multiple-choice answer format. Test bank problems are linked to textbook-specific learning outcomes as well as MCAT-associated outcomes. Available for download on the Pearson catalog page for <i>Biochemistry: Concepts and Connections</i> at <a href="http://www.pearson.com">www.pearson.com</a>
Instructor Resource Materials ISBN: 0134814843 ISBN: 0134814835	Instructor	Includes all the art, photos, and tables from the book in JPEG format, as well as Lecture Powerpoint slides, for use in classroom projection or when creating study materials and tests. Available for download on the Pearson catalog page for <i>Biochemistry: Concepts and Connections</i> at <a href="http://www.pearson.com">www.pearson.com</a>

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), Stefano Tiziani, Edward Marcotte, David Hoffman, and Robin Gutell (University of Texas at Austin), Dean Sherry and Craig Malloy (University of Texas-Southwestern Medical Center), and Stephen C. Kowalczykowski (University of California, Davis). The cover image, representing in part the structure of the human spliceosome, was kindly provided by Karl Bertram (University of Göttingen, Germany).

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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**Dean R. Appling** is the Lester J. Reed Professor of Biochemistry and the Associate Dean for Research and Facilities for the College of Natural Sciences at the University of Texas at Austin, where he has taught and done research for the past 32 years. Dean earned his B.S. in Biology from Texas A&M Uni-

versity (1977) and his Ph.D. in Biochemistry from Vanderbilt University (1981). The Appling laboratory studies the organization and regulation of metabolic pathways in eukaryotes, focusing on folate-mediated one-carbon metabolism. The lab is particularly interested in understanding how one-carbon metabolism is organized in mitochondria, as these organelles are central players in many human diseases. In addition to coauthoring *Biochemistry, Fourth Edition*, a textbook for majors and graduate students, Dean has published over 65 scientific papers and book chapters.

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!



**Spencer J. Anthony-Cahill** is a Professor and chair of the Department of Chemistry at Western Washington University (WWU), Bellingham, WA. Spencer earned his B.A. in chemistry from Whitman College and his Ph.D. in bioorganic chemistry from the University of California, Berkeley. His graduate work, in the laboratory of Peter Schultz, focused on the biosynthetic incorporation of unnatural amino acids into proteins. Spencer was an NIH postdoctoral

fellow in the laboratory of Bill DeGrado (then at DuPont Central Research), where he worked on *de novo* peptide design and the prediction of the tertiary structure of the HLH DNA-binding motif. He then worked for five years as a research scientist in the biotechnology industry, developing recombinant hemoglobin as a treatment for acute blood loss. In 1997, Spencer decided to pursue his long-standing interest in teaching and moved to WWU, where he is today.

In 2012, Spencer was recognized by WWU with the Peter J. Elich Award for Excellence in Teaching.

Research in the Anthony-Cahill laboratory is directed at the protein engineering and structural biology of oxygen-binding proteins. The primary focus is on the design of polymeric human hemoglobins with desirable therapeutic properties as a blood replacement.

Outside the classroom and laboratory, Spencer is a great fan of the outdoors—especially the North Cascades and southeastern Utah, where he has often backpacked, camped, climbed, and mountain biked. He also plays electric bass (poorly) in a local blues-rock band and teaches Aikido in Bellingham.



**Christopher K. Mathews** is Distinguished Professor Emeritus of Biochemistry at Oregon State University. He earned his B.A. in chemistry from Reed College (1958) and his Ph.D. in biochemistry from the University of Washington (1962). He served on the faculties of Yale University and the University of Arizona from 1963 until 1978, when he moved to Oregon State University as

Chair of the Department of Biochemistry and Biophysics, a position he held until 2002. His major research interests are the enzymology and regulation of DNA precursor metabolism and the intracellular coordination between deoxyribonucleotide synthesis and DNA replication. From 1984 to 1985, Dr. Mathews was an Eleanor Roosevelt International Cancer Fellow at the Karolinska Institute in Stockholm, and in 1994–1995, he held the Tage Erlander Guest Professorship at Stockholm University. Dr. Mathews has published about 190 research papers, book chapters, and reviews dealing with molecular virology, metabolic regulation, nucleotide enzymology, and biochemical genetics. From 1964 until 2012, he was principal investigator on grants from the National Institutes of Health, the National Science Foundation, and the Army Research Office. He is the author of *Bacteriophage Biochemistry* (1971) and coeditor of *Bacteriophage T4* (1983) and *Structural and Organizational Aspects of Metabolic Regulation* (1990). He was lead author of four editions of *Biochemistry*, a textbook for majors and graduate students. His teaching experience includes undergraduate, graduate, and medical school biochemistry courses.

He has backpacked and floated the mountains and rivers, respectively, of Oregon and the Northwest. As an enthusiastic birder, he is serving as President of the Audubon Society of Corvallis.

# Tools of Biochemistry

## TOOLS OF BIOCHEMISTRY

### 2A Electrophoresis and Isoelectric Focusing

When an electric field is applied to a solution, solute molecules with a net positive charge migrate toward the cathode, and molecules with a net negative charge move toward the anode. This migration is called **electrophoresis**. Although electrophoresis can be carried out free in solution, it is more convenient to use some kind of *supporting medium* through which the charged molecules move. The supporting medium could be paper or, most typically, a gel composed of the polysaccharide agarose (commonly used to separate nucleic acids; see **FIGURE 2A.1**) or crosslinked polyacrylamide (commonly used to separate proteins).

The velocity, or **electrophoretic mobility** ( $\mu$ ), of the molecule in the field is defined as the ratio between two opposing factors: the force exerted by the electric field on the charged particle, and the frictional force exerted on the particle by the medium:

$$\mu = \frac{Ze}{f} \quad (2A.1)^{\dagger}$$

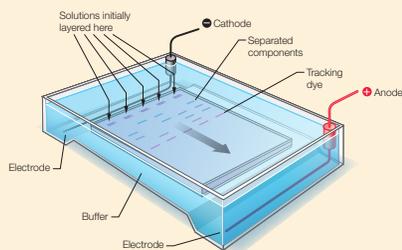
The numerator equals the product of the negative (or positive) charge ( $e$ ) times the number of unit charges,  $Z$  (a positive or negative integer). The greater the overall charge on the molecule, the greater the force it experiences in the electric field.

The denominator  $f$  is the **frictional coefficient**, which depends on the size and shape of the molecule. Large or asymmetric molecules encounter more frictional resistance than small or compact ones and consequently have larger frictional coefficients. Equation 2A.1 tells us that the mobility of a molecule depends on its charge and on its molecular dimensions.<sup>†</sup> Because ions and macromolecules differ in both respects, electrophoresis provides a powerful way of separating them.

#### Gel Electrophoresis

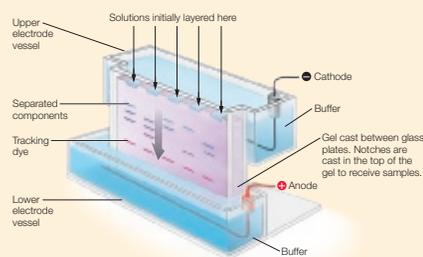
In **gel electrophoresis**, a gel containing the appropriate buffer solution is cast in a mold (for agarose gel electrophoresis, shown in **Figure 2A.1**) or as a thin slab between glass plates (for polyacrylamide gel electrophoresis, shown in **Figure 2A.2**). The gel is placed between electrode compartments, and the samples to be analyzed are carefully pipetted into precast notches in the gel, called wells. Usually, glycerol and a water-soluble anionic "tracking" dye (such as bromophenol blue) are added to the samples. The glycerol makes the sample solution dense, so that it sinks into the well and does not mix into the buffer solution. The dye migrates faster than most macromolecules, so the experimenter is able to follow the progress of the experiment. The current is turned on until the tracking dye band is near the side of the gel opposite the wells. The gel is then removed from the apparatus and is usually stained with a dye that binds to proteins or nucleic acids. Because the protein

<sup>†</sup>Equation 2A.1 is an approximation which neglects the effects of the ion atmosphere. See van Holde, Johnson, and Ho in Appendix II for more detail.

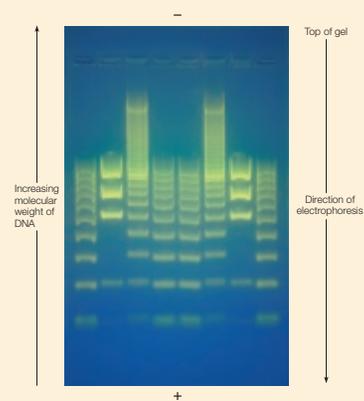


**▲ FIGURE 2A.1** Electrophoresis. A molecule with a net positive charge will migrate toward the cathode, whereas a molecule with a net negative charge will migrate toward the anode.

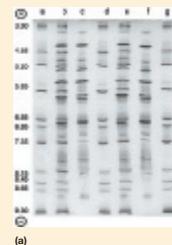
or nucleic acid mixture was applied as a narrow band in the well of the gel, components migrating with different electrophoretic mobilities appear as separated bands on the gel. **FIGURE 2A.3** shows an example of separation of DNA fragments by this method using an agarose gel. An example of the electrophoretic separation of proteins using a polyacrylamide gel is shown in Chapter 5 (see **Figure 5A.9**).



**▲ FIGURE 2A.2** Gel electrophoresis. An apparatus for polyacrylamide gel electrophoresis is shown schematically. The gel is cast between plates. The gel is in contact with buffer in the upper (cathode) and lower (anode) reservoirs. A sample is loaded into one or more wells cast into the top of the gel, and then current is applied to achieve separation of the components in the sample.



**▲ FIGURE 2A.3** Gel showing separation of DNA fragments. Following electrophoretic separation of the different-length DNA molecules, the gel is mixed with a fluorescent dye that binds DNA. The unbound dye is then washed off, and the stained DNA molecules are visualized under ultraviolet light.



**▲ FIGURE 2A.4** Isoelectric focusing of proteins. (a) An isoelectric focusing gel with a pH gradient from 3.50 (anode end) to 9.30 (cathode end). (b) A schematic showing where proteins of the indicated pIs would accumulate (peaks shown in red) in a pH gradient gel.

Polyelectrolytes like DNA or polylysine have one unit charge on each residue, so each molecule has a charge ( $Ze$ ) proportional to its molecular length. But the frictional coefficient ( $f$ ) also increases with molecular length, so to a first approximation, a macromolecule whose charge is proportional to its length has an electrophoretic mobility almost independent of its size. However, gel electrophoresis introduces additional frictional forces that allow the separation of molecules based on size. For linear molecules like the nucleic acid fragments in **Figure 2A.3**, the relative mobility in an agarose gel is a approximately a linear function of the logarithm of the molecular weight. Usually, standards of known molecular weight are electrophoresed in one or more lanes on the gel. The molecular weight of the sample can then be estimated by comparing its migration in the gel to those of the standards. For proteins, a similar separation in a polyacrylamide gel is achieved by coating the denatured protein molecule with the anionic detergent sodium dodecylsulfate (SDS) before electrophoresis. This important technique is discussed further in Chapter 5.

#### Isoelectric Focusing

Proteins are polyanions; thus, a protein will migrate in an electric field like other ions if it has a net positive or negative charge. At its isoelectric point, however, its net charge is zero, and it is attracted to neither the anode nor the cathode. If we use a gel with a stable pH gradient covering a wide pH range, each protein molecule in a complex mixture of proteins migrates to the position of its isoelectric point and accumulates there. This method of separation, called **isoelectric focusing**, produces distinct bands of accumulated proteins and can separate proteins with very small differences in the isoelectric point (**Figure 2A.4**). Since the pH of each portion of the gel is known, isoelectric focusing can also be used to determine experimentally the isoelectric point of a particular protein.

What we have presented here is only a brief overview of a widely applied technique. Additional information on electrophoresis and isoelectric focusing can be found in Appendix II.

**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 44
- 4A** Manipulating DNA 99
- 4B** An Introduction to X-Ray Diffraction 104
- 5A** Protein Expression and Purification 131
- 5B** Mass, Sequence, and Amino Acid Analyses of Purified Proteins 138
- 6A** Spectroscopic Methods for Studying Macromolecular Conformation in Solution 178
- 6B** Determining Molecular Masses and the Number of Subunits in a Protein Molecule 185
- 7A** Immunological Methods 230

- 8A** How to Measure the Rates of Enzyme-Catalyzed Reactions 273
- 9A** The Emerging Field of Glycomics 303
- 11A** Metabolomics 367
- 11B** Radioactive and Stable Isotopes 370
- 13A** Detecting and Analyzing Protein-Protein Interactions 448
- 21A** Polymerase Chain Reaction 684
- 23A** Manipulating the Genome 738
- 24A** Analyzing the Transcriptome 764
- 24B** Chromatin Immunoprecipitation 765

# Foundation Figures

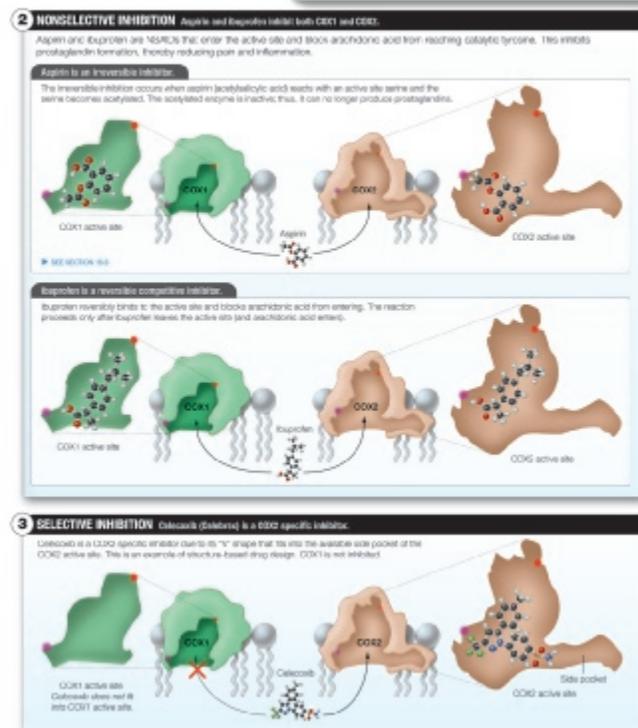
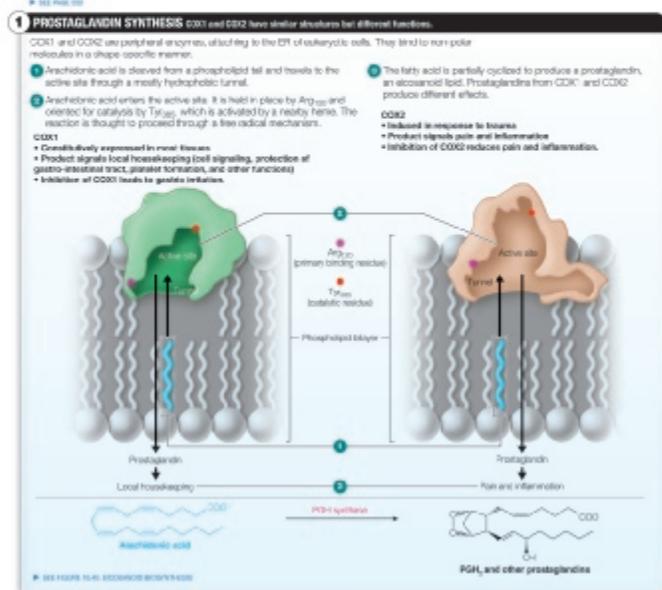
## FOUNDATION FIGURE | Targeting Pain and Inflammation through Drug Design

### Mastering Chemistry for Biochemistry

Mastering Chemistry 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.

**Pain and inflammation:** Non-steroid anti-inflammatory drugs (NSAIDs) like ibuprofen and aspirin target the two main isoforms of Prostaglandin H<sub>2</sub> synthase (COX synthase) also COX1 and COX2, by binding to the active site and inhibiting the synthesis of prostaglandins (local signaling molecules). The COX abbreviation refers to the first part of a two step process in prostaglandin synthesis, with cyclooxygenase activity being the first and peroxidase being second. These two enzyme activities are carried out within a single enzyme, "COX synthase", which is commonly referred to as the COX enzyme. These peripheral enzymes bind to non-polar molecules in a shape-specific manner.

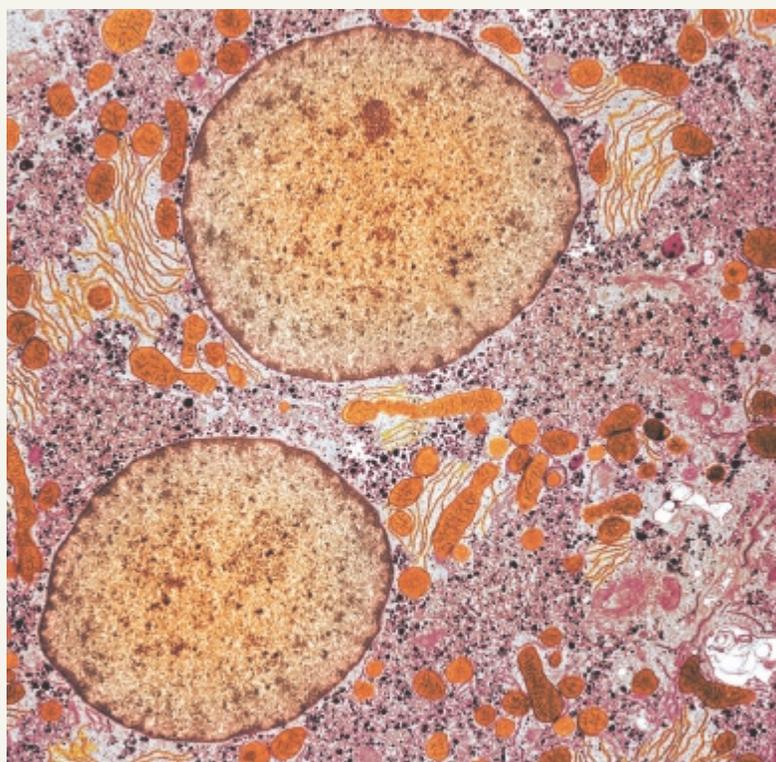
Aspirin and ibuprofen, two molecules with similar shapes and polarities, both bind to COX1 and COX2. Aspirin is an irreversible inhibitor, while ibuprofen is reversible and competitive. COX1 is constitutively expressed and its prostaglandin product is responsible for local "housekeeping" signaling, whereas COX2 is induced in response to trauma and its prostaglandin product is responsible for pain and inflammation. Thus, selective targeting of COX2 would block pain and inflammation and not the essential housekeeping functions of COX1. Understanding the structure of the active sites of COX1 and COX2 allowed the development of selective inhibitors.



**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.

- Chapter 2** Biomolecules: Structure and Function 46
- Chapter 6** Protein Structure and Function 188
- Chapter 8** Regulation of Enzyme Activity 276
- Chapter 10** Targeting Pain and Inflammation through Drug Design 338
- Chapter 11** Enzyme Kinetics and Drug Action 372
- Chapter 14** Intermediary Metabolism 484
- Chapter 17** Energy Regulation 574
- Chapter 20** Cell Signaling and Protein Regulation 662
- Chapter 23** Antibody Diversity and Use as Therapeutics 740
- Chapter 26** Information Flow in Biological Systems 822

# Enhanced art and media programs engage students



A living cell, such as this liver cell, carries out thousands of reactions simultaneously. How are these metabolic pathways organized and controlled within such an intricate architecture?

## Chemical Logic of Metabolism

A CHEMIST CARRYING out an organic synthesis rarely runs more than one reaction in a single-reaction vessel at any one time. This strategy is essential to prevent unwanted by-products and to optimize the yield of the desired product. Yet a living cell carries out thousands of reactions simultaneously, with each reaction sequence controlled so that unwanted accumulations or deficiencies of intermediates and products do not occur. Reactions of great mechanistic complexity and stereochemical selectivity proceed smoothly under mild conditions—1 atm pressure, moderate temperature, and osmotic pressure, and a pH near neutrality. How then, do cells avoid metabolic chaos? A goal of the next several chapters is to understand how cells carry out and regulate these complex reaction sequences and, in so doing, control their internal environment.

In Chapter 8 we discussed the properties of individual enzymes and the control mechanisms that affect their activity. In this chapter, we now consider how individual biochemical reactions combine to form metabolic pathways, a series of chemical reactions whereby the products of one reaction are the substrates for the next reaction,

11

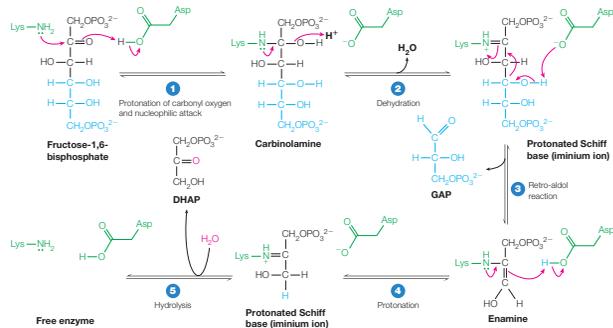
### Chapter 11

- 11.1 A First Look at Metabolism
  - 11.2 Freeways on the Metabolic Road Map
  - 11.3 Biochemical Reaction Types
  - 11.4 Bioenergetics of Metabolic Pathways
  - 11.5 Major Metabolic Control Mechanisms
  - 11.6 Experimental Analysis of Metabolism
- Tools of Biochemistry**
- 11A Metabolomics
  - 11B Radioactive and Stable Isotopes
- Foundation Figure**  
Enzyme Kinetics and Drug Action

**UPDATED & REVISED!** The second edition of Appling, Mathews, & Anthony-Cahill's **Biochemistry: Concepts and Connections** builds student understanding even more with an enhanced art program and a deeper, more robust integration with Mastering Chemistry. This renowned author team's content engages students with visualization, synthesis of complex topics, and connections to the real world resulting in a seamlessly integrated experience.

# Best-in-class visualization tools help students to see

10 | CHAPTER 12 Carbohydrate Metabolism: Glycolysis, Gluconeogenesis, Glycogen Metabolism, and the Pentose Phosphate Pathway



**▲ FIGURE 12.5** Reaction mechanism for fructose-1,6-bisphosphate aldolase. The figure shows the protonated Schiff base intermediate (iminium ion) between the substrate and an active site lysine residue. An aspartate residue facilitates the reaction via general acid-base catalysis.

**● CONCEPT** Aldolase cleaves fructose-1,6-bisphosphate under intracellular conditions, even though the equilibrium lies far toward fructose-1,6-bisphosphate under standard conditions.

Reaction 4 is so strongly endergonic under standard conditions that the formation of fructose-1,6-bisphosphate is highly favored. However, from the actual intracellular concentrations of the reactant and products,  $\Delta G$  is estimated to be approximately  $-1.3$  kJ/mol, consistent with the observation that the reaction proceeds as written *in vivo*. Reaction 4 demonstrates the importance of considering the conditions *in the cell* ( $\Delta G$ ) rather than standard state conditions ( $\Delta G^\circ$ ) when deciding in which direction a reaction is favored.

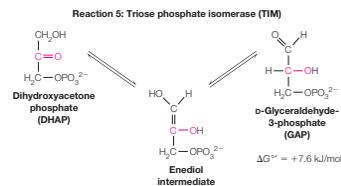
Aldolase activates the substrate for cleavage by nucleophilic attack on the keto carbon at position 2 with a lysine  $\epsilon$ -amino group in the active site, as shown in **FIGURE 12.5**. This is facilitated by protonation of the carbonyl oxygen by an active site acid (aspartate) **1**. The resulting carbinolamine undergoes dehydration to give an iminium ion, or protonated **Schiff base** **2**. A Schiff base is a nucleophilic addition product between an amino group and a carbonyl group. A retro-aldol reaction then cleaves the protonated Schiff base into an enamine plus GAP **3**. The enamine is protonated to give another iminium ion (protonated Schiff base) **4**, which is then hydrolyzed off the enzyme to give the second product, DHAP **5**.

The Schiff base intermediate is advantageous in this reaction because it can delocalize electrons. The positively charged iminium ion is thus a better electron acceptor than a ketone carbonyl, facilitating retro-aldol reactions like this one and, as we shall see, many other biological

conversions. This mechanism also demonstrates why it was important to isomerize G6P to F6P in reaction 2. If glucose had not been isomerized to fructose (moving the carbonyl from C-1 to C-2), then the aldolase reaction would have given two- and four-carbon fragments, instead of the metabolically equivalent three-carbon fragments.

#### Reaction 5: Isomerization of Dihydroxyacetone Phosphate

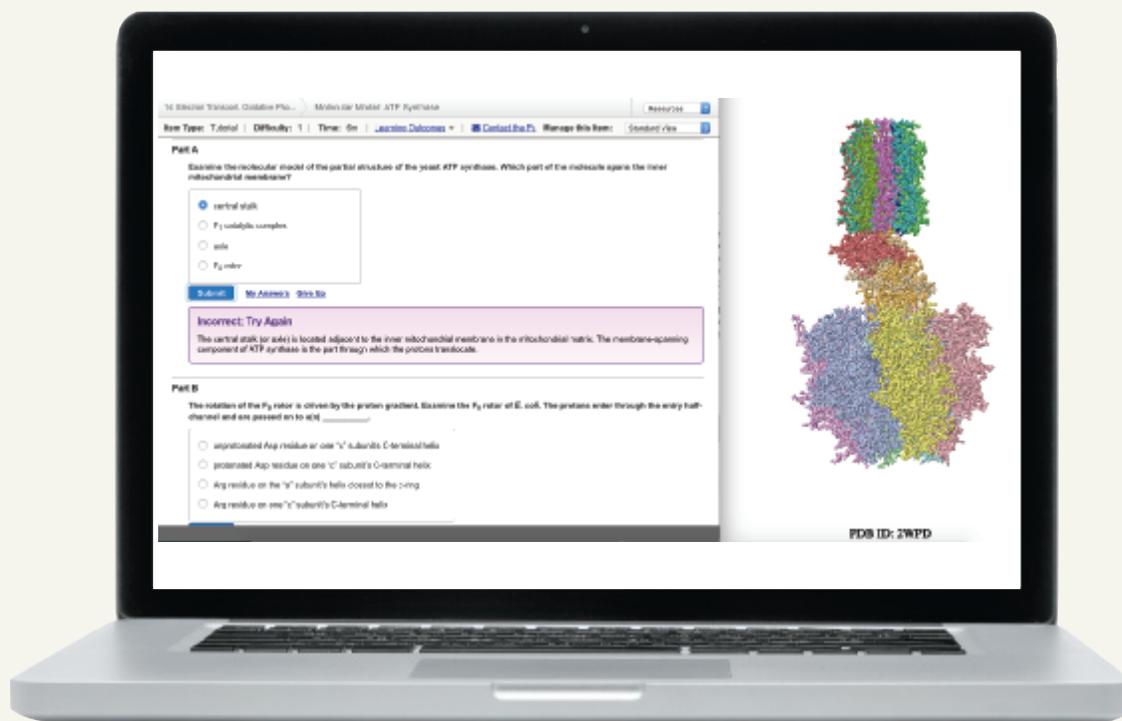
In reaction 5, **triose phosphate isomerase (TIM)** catalyzes the isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (GAP) via an enediol intermediate.



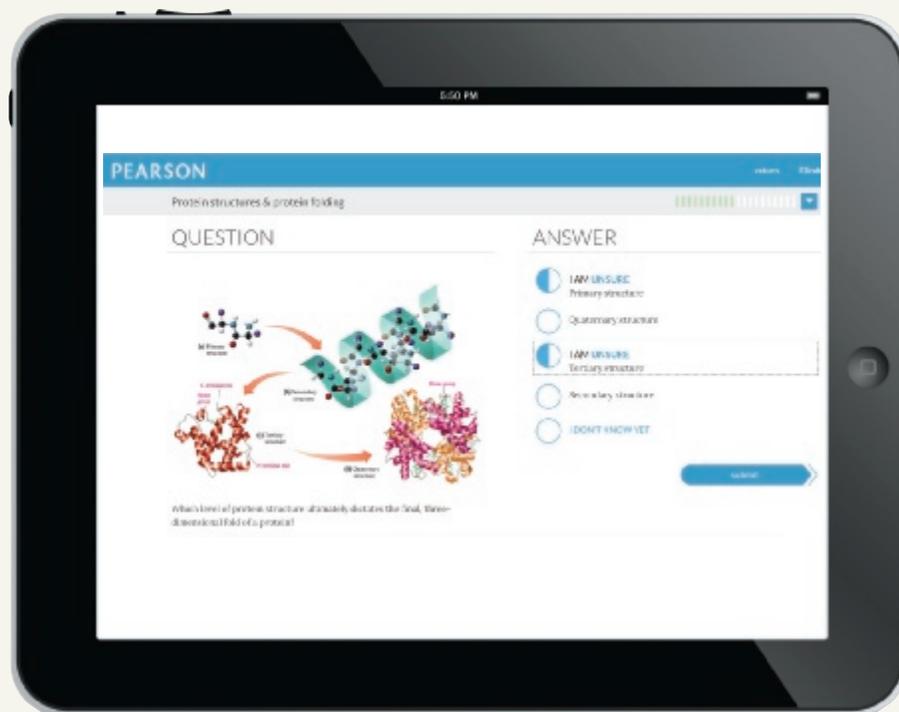
Like reaction 4, reaction 5 is weakly endergonic under standard conditions, but the intracellular concentration of GAP is low because it is consumed in subsequent reactions. Thus, reaction 5 is drawn toward the right.

**NEW!** Color-coded and numbered process steps from **Figures** have been added to the narrative to improve students' ability to quickly track between the discussion and the related art.

Students can explore interactive **3D molecular models** while related follow-up questions provide answer-specific feedback.



# and understand what's happening on the cellular level

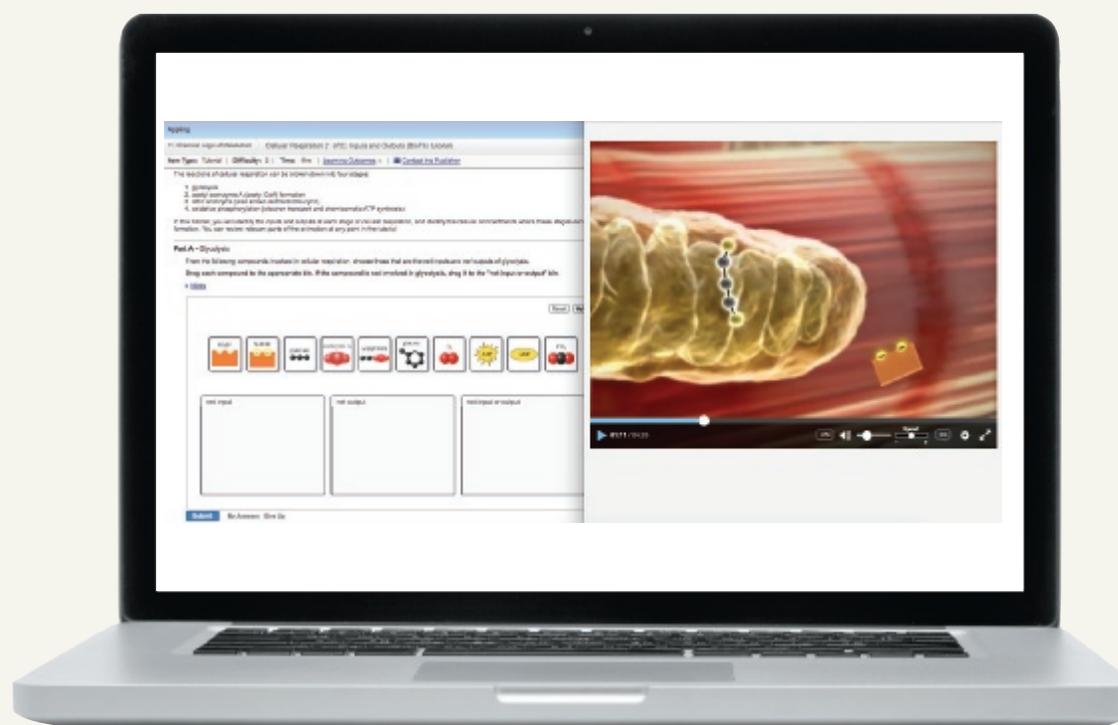


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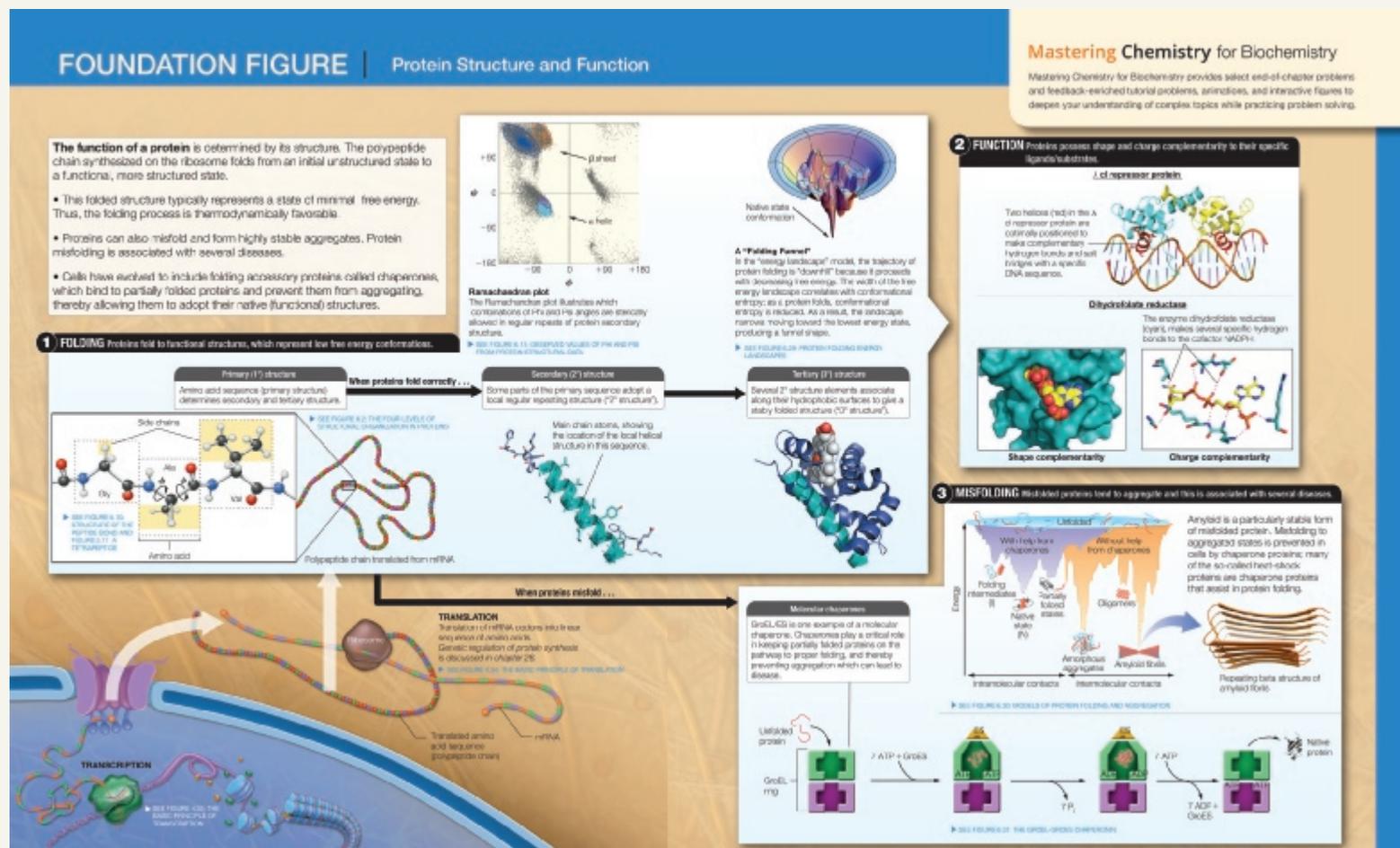
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# Synthesis of information is simplified through features



**UPDATED & REVISED! Foundation Figures** integrate core chemical and biological connections visually and provide a way to organize the complex and detailed material intellectually, making relationships among key concepts clear and easier to study. The second edition includes two new foundation figures as well as updated layouts based on learning design principles. Foundation Figures are assignable in Mastering Chemistry and are embedded in the Pearson eText as an interactive part of the narrative.

# that help students connect complex concepts

Note that we have expressed concentrations of all solutes in units of molarity, then divided by the proper standard state concentration (also in units of molarity). These steps ensure that the terms in  $Q$  are of the proper magnitude and stripped of units:

$$\Delta G = -32.2 \frac{\text{kJ}}{\text{mol}} + \left( 2.478 \frac{\text{kJ}}{\text{mol}} \right) \ln \left( \frac{(0.0001)(0.035)(0.398)}{(0.005)} \right) \quad (3.31b)$$

or

$$\Delta G = -32.2 \frac{\text{kJ}}{\text{mol}} + -20.3 \frac{\text{kJ}}{\text{mol}} = -52.5 \frac{\text{kJ}}{\text{mol}} \quad (3.31c)$$

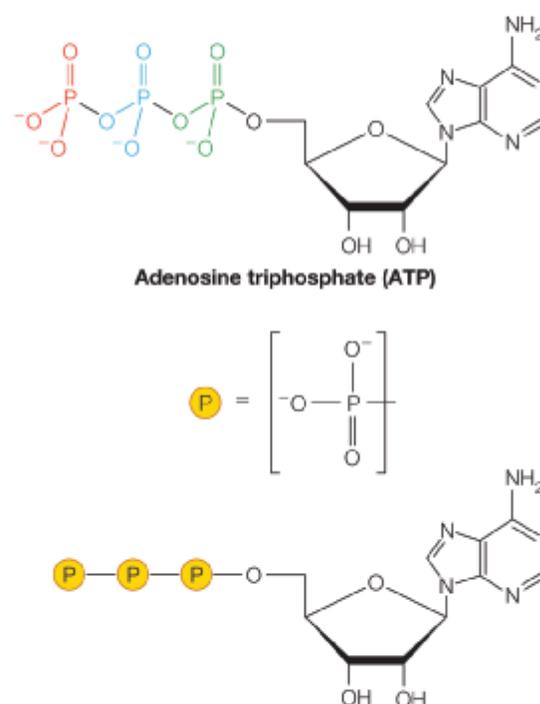
Note that the value calculated for  $\Delta G$  is much more negative (i.e., more favorable) than the standard free energy change  $\Delta G^{\circ'}$ . This last point underscores the fact that it is  $\Delta G$  and not  $\Delta G^{\circ'}$  that determines the driving force for a reaction. However, to evaluate  $\Delta G$  using Equation 3.19, we must be given, or be able to calculate,  $\Delta G^{\circ'}$  for the reaction of interest. Recall that  $\Delta G^{\circ'}$  can be calculated from  $K$  using Equation 3.22. In the remaining pages of this chapter, we will use examples relevant to biochemistry to illustrate two alternative methods for calculating  $\Delta G^{\circ'}$ .

## 3.4 Free Energy in Biological Systems

Understanding the central role of free energy changes in determining the favorable directions for chemical reactions is important in the study of biochemistry because every biochemical process (such as protein folding, metabolic reactions, DNA replication, or muscle contraction) must, overall, be a thermodynamically favorable process. Very often, a particular reaction or process that is necessary for life is in itself endergonic. Such intrinsically unfavorable processes can be made thermodynamically favorable by *coupling* them to strongly favorable reactions. Suppose, for example, we have a reaction  $A \rightarrow B$  that is part of an essential pathway but is endergonic under standard conditions:



C in our previous example) that can undergo reactions with large negative free energy changes. Such substances can be thought of as energy transducers in the cell. Many of these energy-transducing compounds are organic phosphates such as ATP (FIGURE 3.5), which can transfer a phosphoryl group ( $-\text{PO}_3^{2-}$ ) to an acceptor molecule. You will see many examples of phosphoryl group transfer reactions in this text. As shown in Figure 3.5, we will use a common shorthand notation,  $\textcircled{\text{P}}$ , to represent the phosphoryl group when describing these processes.

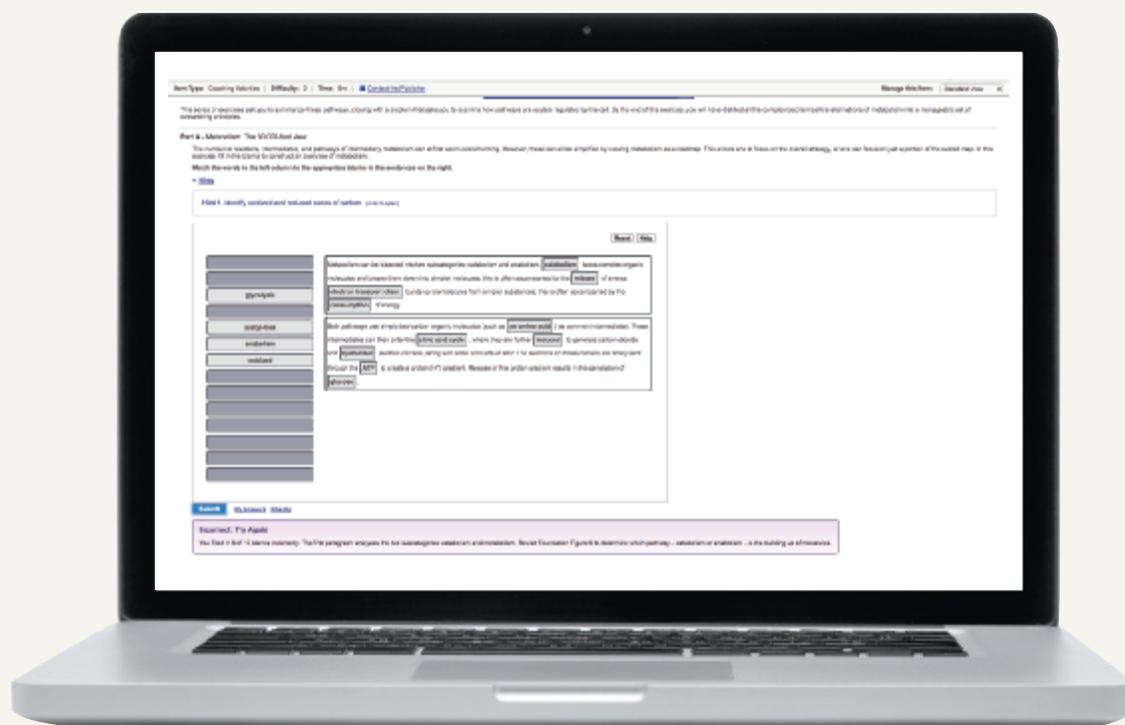


▲ FIGURE 3.5 The phosphoryl groups in ATP. Top: The three phosphoryl groups in ATP are shown in red, blue, and green. Middle: A commonly used shorthand for a phosphoryl group is the symbol  $\textcircled{\text{P}}$ . Bottom: The three phosphoryl groups in ATP are represented by this symbol.

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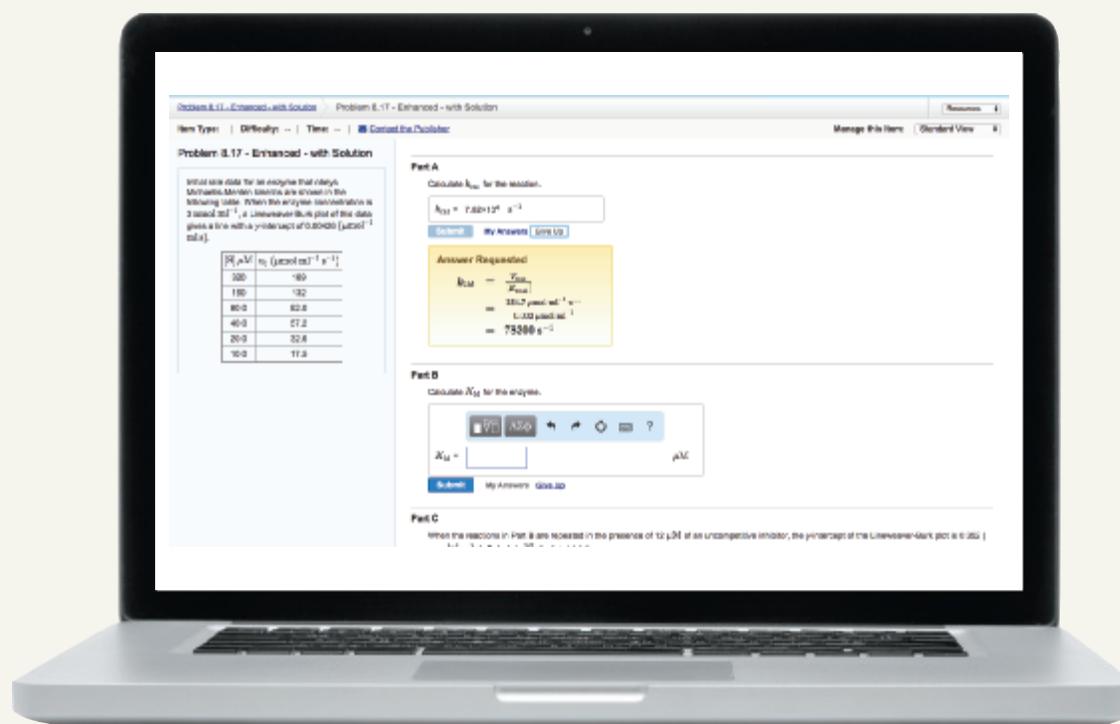
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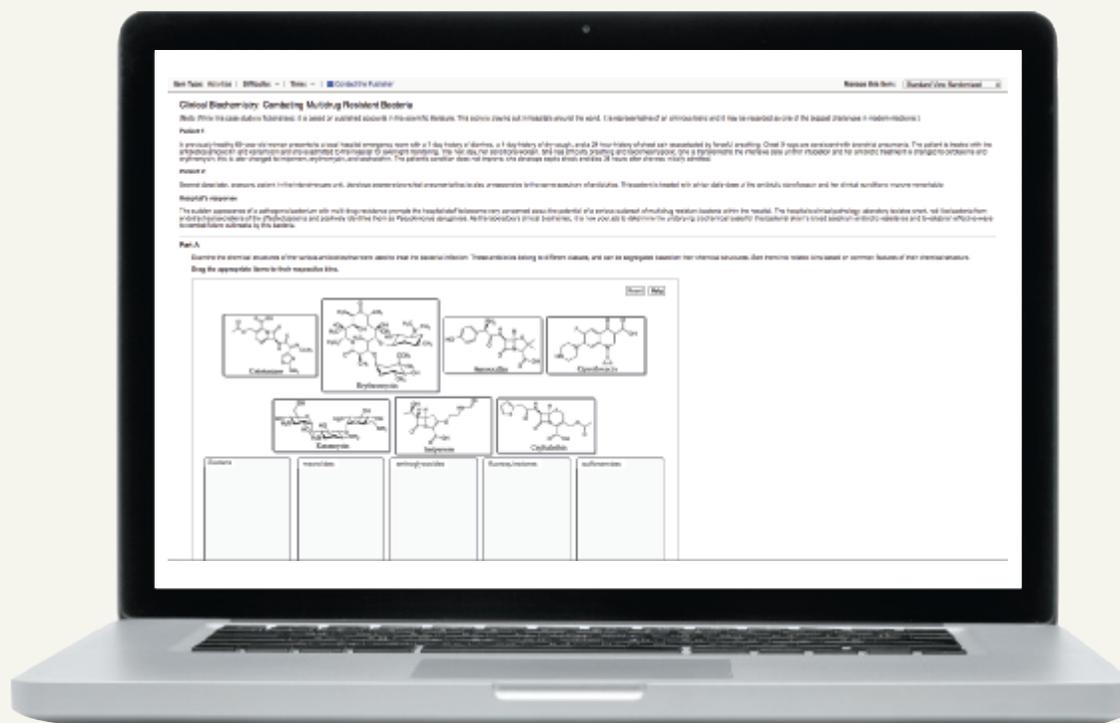
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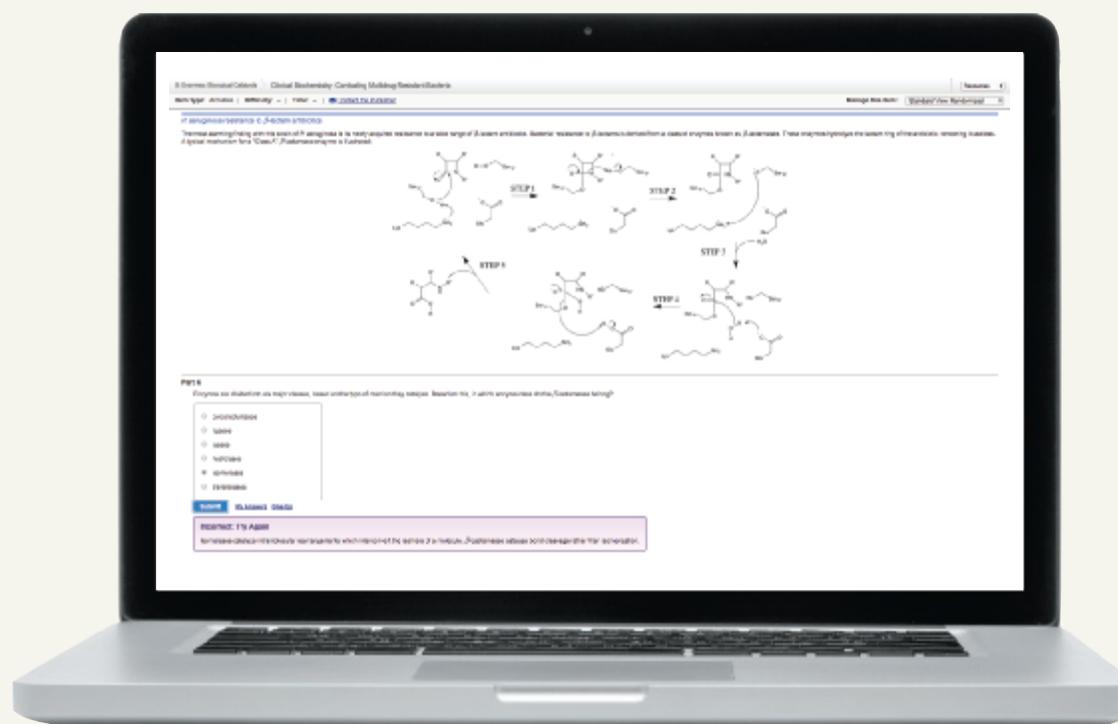
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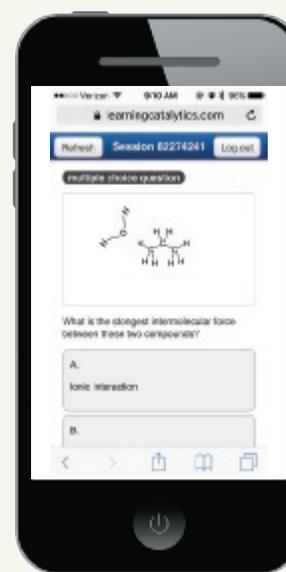
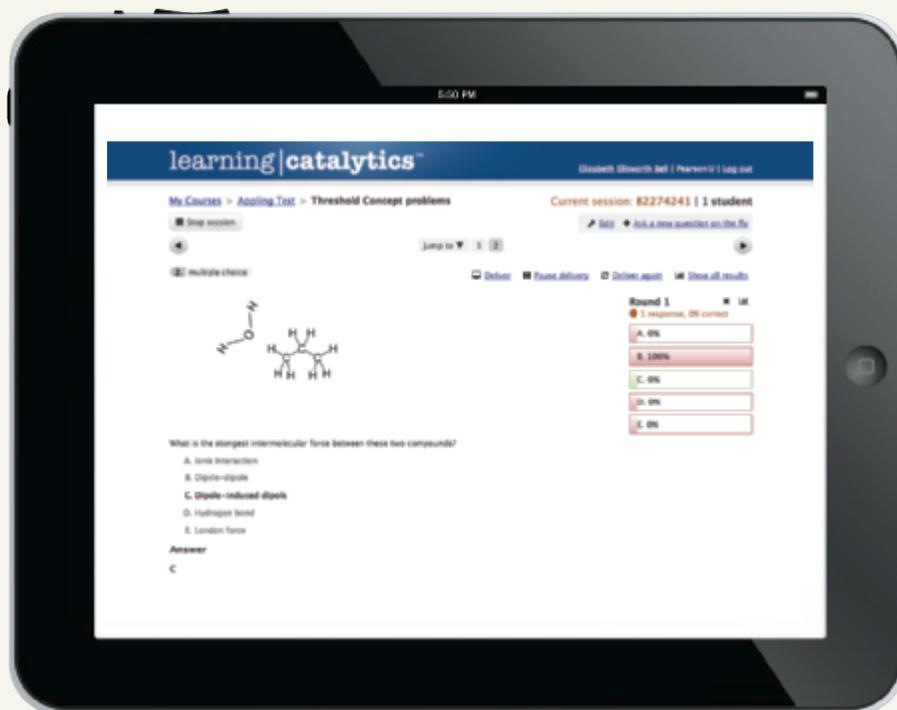
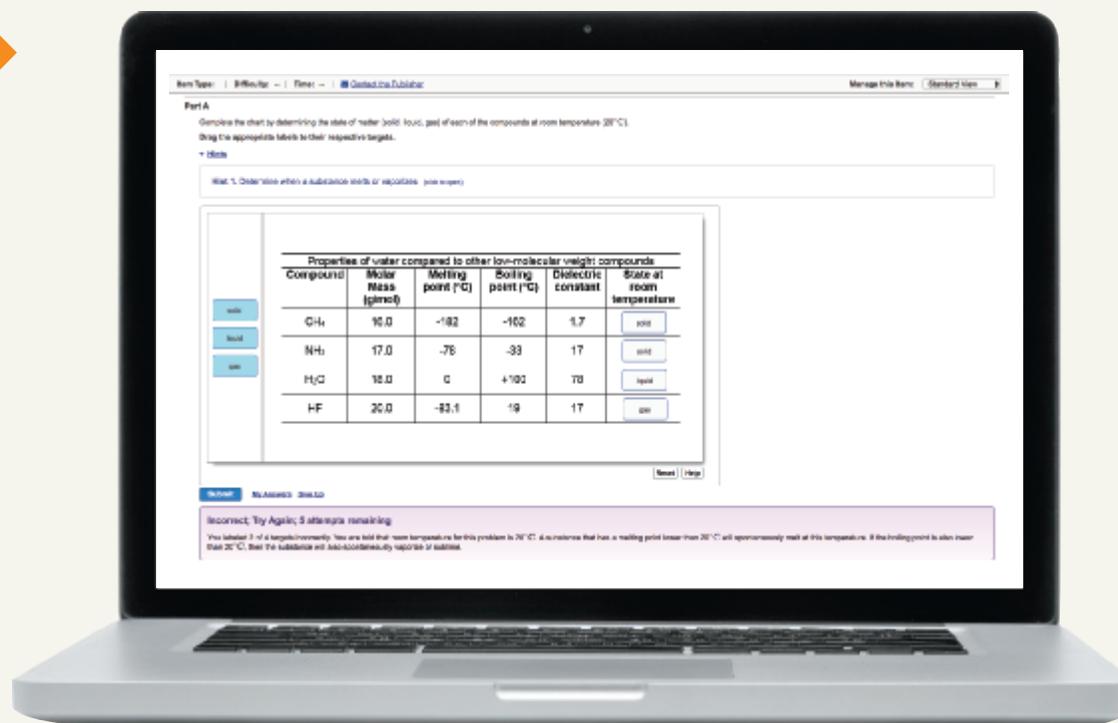


# Students get the threshold concept coverage they need to succeed

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