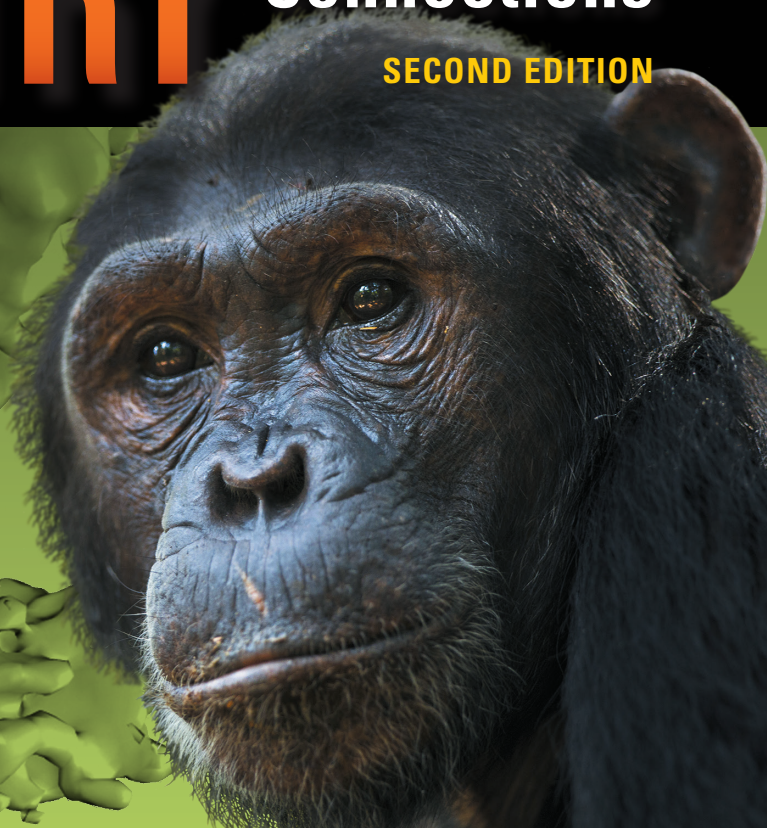


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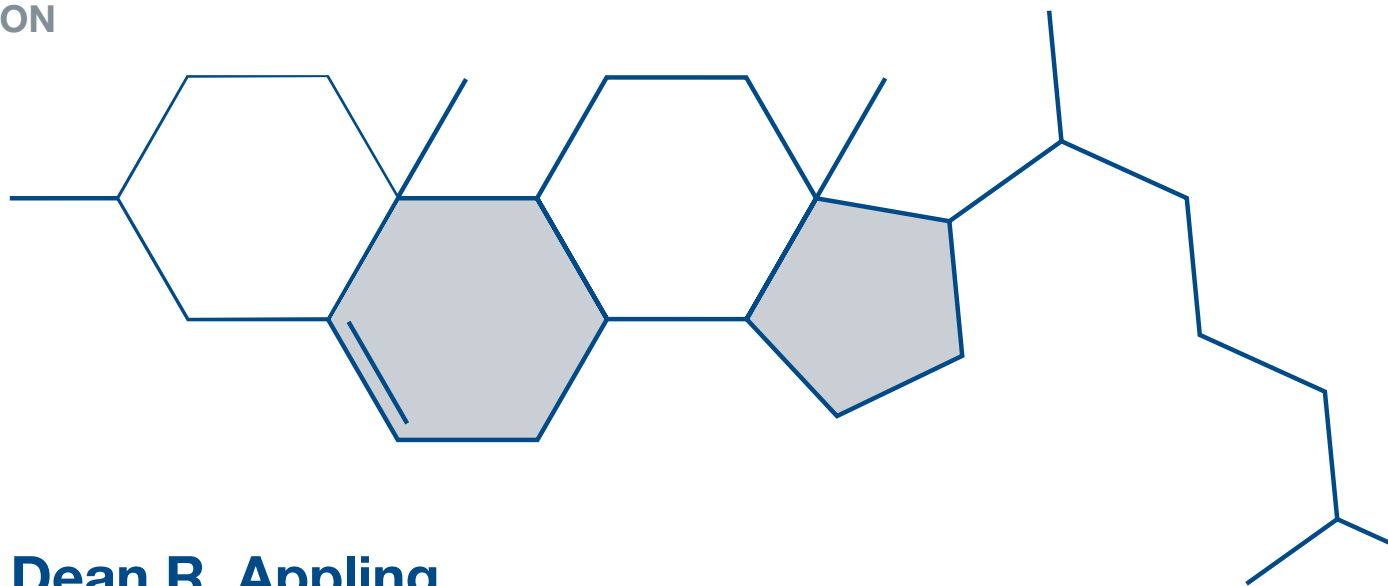
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Biochemistry

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 ΔG 57

ΔG versus ΔG° , 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

ΔG and Oxidation/Reduction Reactions in Cells 64

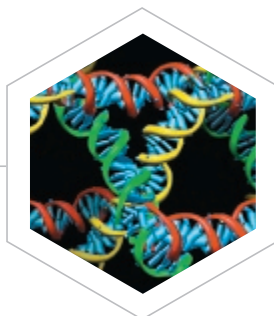
Quantification of Reducing Power: Standard Reduction Potential 64

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

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

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

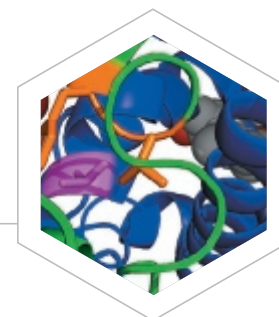
Transcription: DNA to RNA 95

Translation: RNA to Protein 95

TOOLS OF BIOCHEMISTRY 4A Manipulating DNA 99

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

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



5.1 Amino Acids 111

Structure of the α -Amino Acids 111

Stereochemistry of the α -Amino Acids 111

Properties of Amino Acid Side Chains: Classes of α -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

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

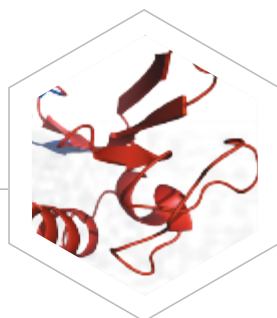
Symmetry in Multisubunit Proteins: Homotypic Protein–Protein Interactions 172

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 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

 α Helices and β 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

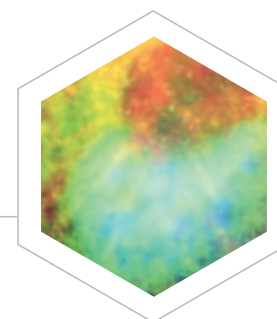
Internal Hydrogen Bonds 163

Van der Waals Interactions 163

The Hydrophobic Effect 163

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**

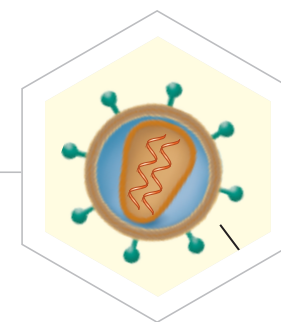
Shape and Charge Complementarity 196

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 α -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

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

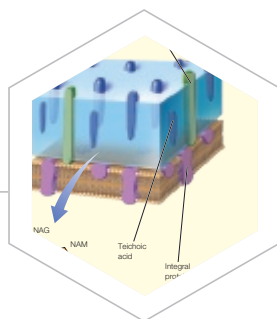
N-Linked Glycans 298

O-Linked Glycans 298

Blood Group Antigens 299

Erythropoetin: 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 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

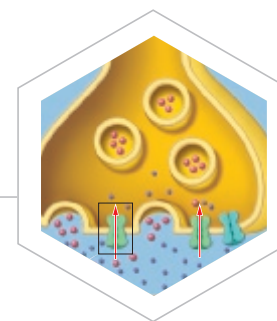
Alditols 288

Amino Sugars 288

Glycosides 288

9.3 Oligosaccharides 289

Oligosaccharide Structures 289

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

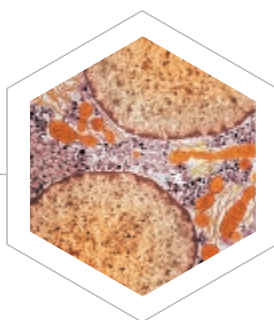
Motion in Biological Membranes 315

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 ΔG 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

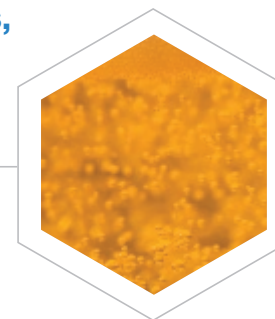
TOOLS OF BIOCHEMISTRY 11A Metabolomics 367

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

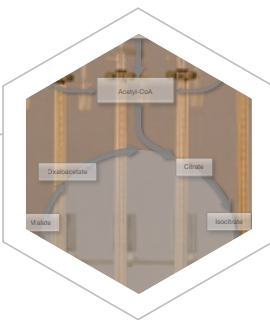
- Structure of Glycogen Phosphorylase 405
- Control of Phosphorylase Activity 406
- Proteins in the Glycogenolytic Cascade 406
 - Cyclic AMP-Dependent Protein Kinase 407
 - Phosphorylase *b* Kinase 407
 - Calmodulin 407
- Nonhormonal Control of Glycogenolysis 407
- Control of Glycogen Synthase Activity 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⁺) 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_1 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

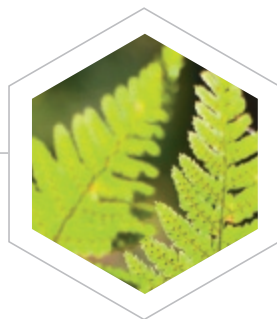
14.9 Oxygen as a Substrate for Other Metabolic Reactions 479

- 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_2 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_4 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 β -Oxidation Pathway 526

Reaction 1: The Initial Dehydrogenation 527

Reactions 2 and 3: Hydration and Dehydrogenation 527

Reaction 4: Thiolytic Cleavage 527

Mitochondrial β -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

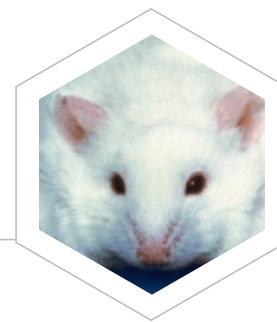
16.7 Steroid Metabolism 543

- 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

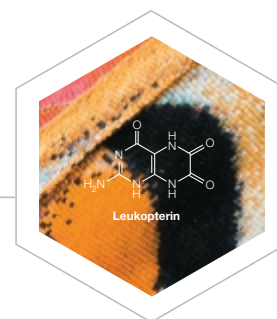
17.3 Responses to Metabolic Stress: Starvation, Diabetes 567

- 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

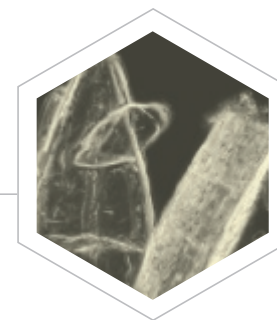
18.2 Utilization of Ammonia: Biogenesis of Organic Nitrogen 581

- Glutamate Dehydrogenase: Reductive Amination of α -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
- B₁₂ Coenzymes 589
- B₁₂ 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
- α -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



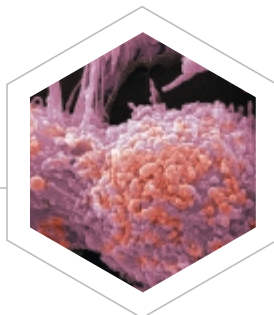
- 19.1 Outlines of Pathways in Nucleotide Metabolism 612**
- Biosynthetic Routes:
- De Novo and Salvage Pathways 612
- Nucleic Acid Degradation and the Importance of Nucleotide Salvage 613
- PRPP, a Central Metabolite in De Novo and Salvage Pathways 613
- 19.2 De Novo Biosynthesis of Purine Ribonucleotides 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

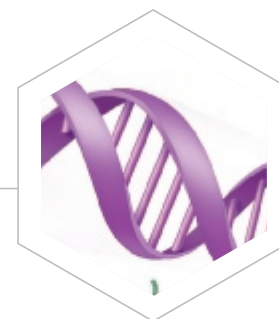
- Viral and Cellular Oncogenes 653
- Oncogenes in Human Tumors 654
- The Cancer Genome Mutational Landscape 655

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

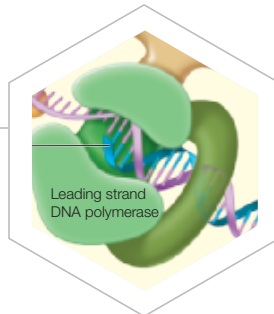
- Restriction and Modification 675
- Properties of Restriction and Modification Enzymes 676
- Determining Genome Nucleotide Sequences 677

- 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

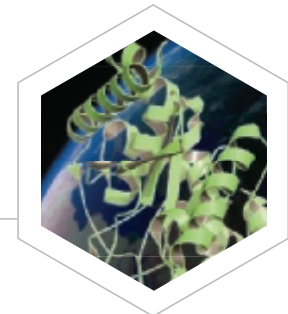


- 22.5 Initiation of DNA Replication 704**
 - Initiation of *E. coli* DNA Replication at *ori*^c 704
 - Initiation of Eukaryotic Replication 705
- 22.6 Replication of Linear Genomes 705**
 - Linear Virus Genome Replication 705
 - Telomerase 706
- 22.7 Fidelity of DNA Replication 707**
 - 3' Exonucleolytic 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
 - O⁶-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 λ : 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. Mastering Chemistry for <i>Biochemistry: Concepts and Connections</i>, 2/e 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	<i>Biochemistry: Concepts and Connections</i> 2/e now offers Pearson eText, optimized for mobile , 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"> • Offline access on most iOS and Android phones/tablets. • Accessibility (screen-reader ready) • Configurable reading settings, including resizable type and night reading mode • Instructor and student note-taking, highlighting, bookmarking, and search tools • Embedded videos for a more interactive learning experience
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 www.pearson.com
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 www.pearson.com

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

Reviewers

The following reviewers provided valuable feedback on the manuscript at various stages throughout the writing process:

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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-

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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!



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** (μ), 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:

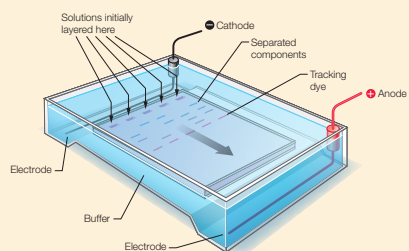
$$\mu = \frac{Ze}{f} \quad (2A.1)^3$$

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.³ Because ions and macromolecules differ in both respects, electrophoresis provides a powerful way of separating them.

Gel Electrophoresis

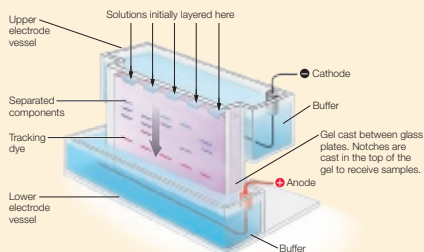
In **gel electrophoresis**, a gel containing the appropriate buffer solution is cast in a mold (for agarose gel electrophoresis, shown in **Figure 2A.1**) or as a thin slab between glass plates (for polyacrylamide gel electrophoresis, shown in **Figure 2A.2**). The gel is placed between electrode compartments, 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

³Equation 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.

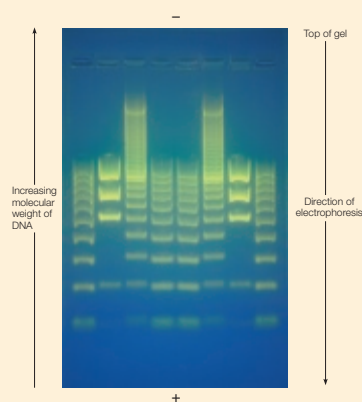


▲ FIGURE 2A.1 Electrophoresis. A molecule with a net positive charge will migrate toward the cathode, whereas a molecule with a net negative charge will migrate toward the anode.

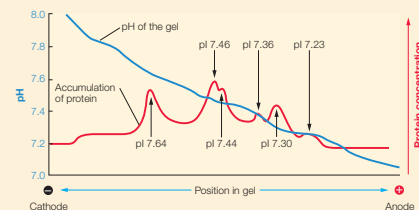
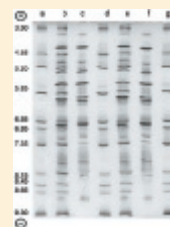
or nucleic acid mixture was applied as a narrow band in the well of the gel, components migrating with different electrophoretic mobilities appear as separated bands on the gel. **FIGURE 2A.3** shows an example of separation of DNA fragments by this method using an agarose gel. An example of the electrophoretic 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 (Z) 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 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 polyelectrolytes; 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

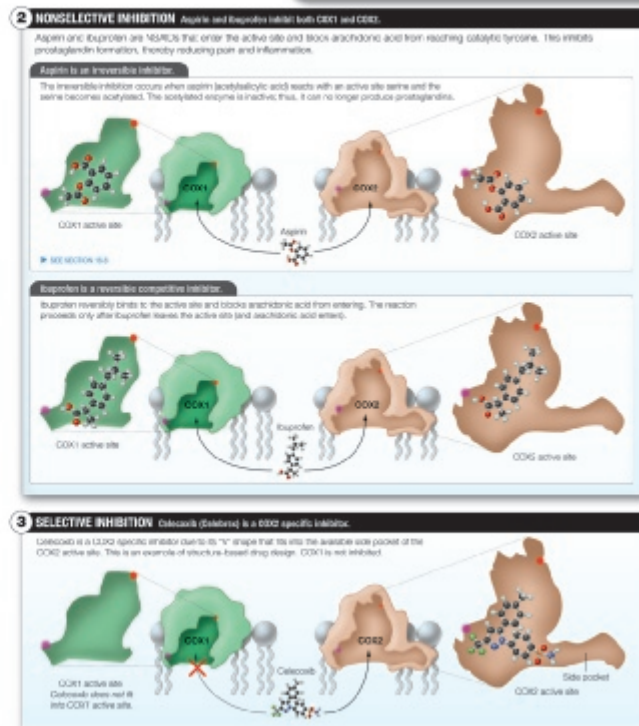
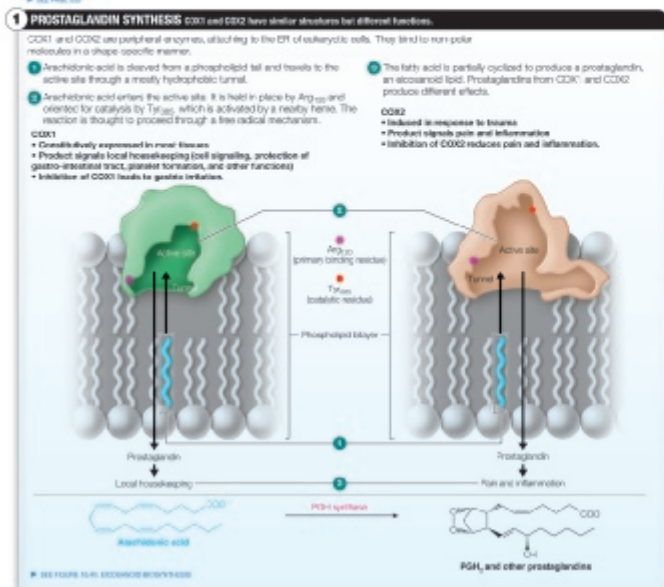
Targeting Pain and Inflammation through Drug Design

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 H2 synthase (PGH synthase) (aka 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.

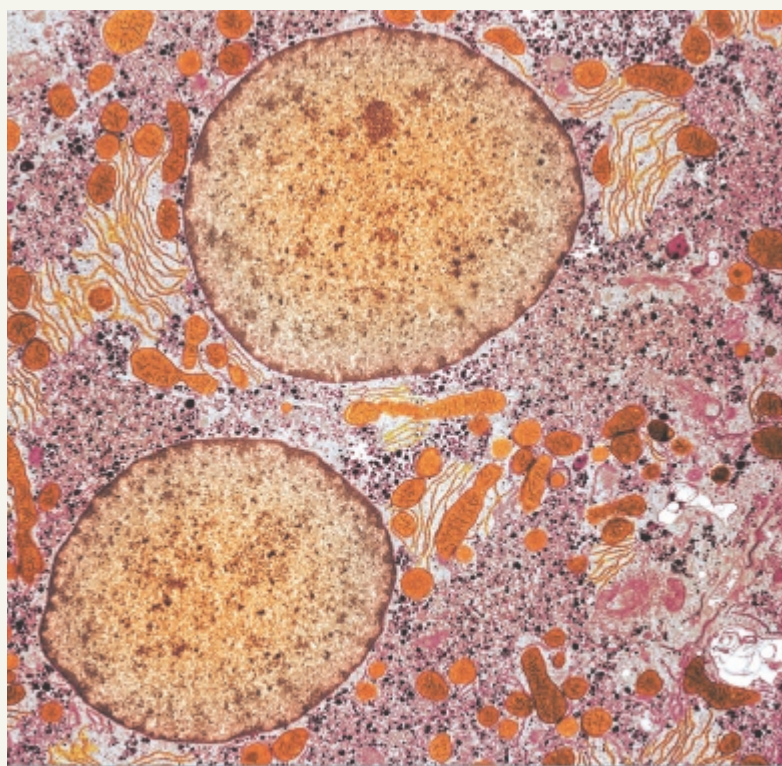
Aspirin and ibuprofen, two molecules with similar shapes and polarities, both bind to COX1 and COX2. Aspirin is an irreversible inhibitor, while ibuprofen is reversible and competitive. COX1 is constitutively expressed and its prostaglandin product is induced in response to trauma and its product signals 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

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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

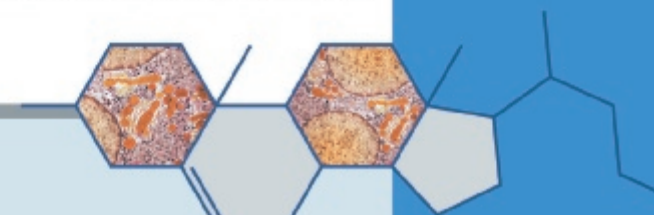
A CHEMIST CARRYING out an organic synthesis rarely runs more than one reaction in a single-reaction vessel at any one time. This strategy is essential to prevent unwanted by-products and to optimize the yield of the desired product. Yet a living cell carries out thousands of reactions simultaneously, 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

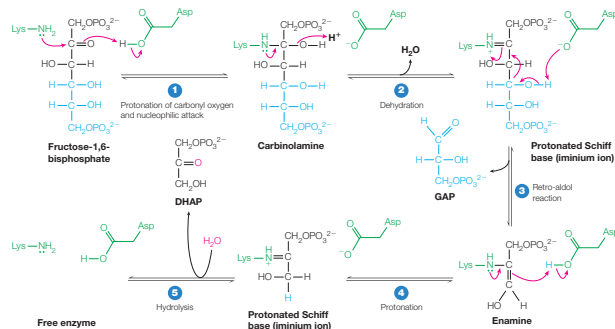
- 11.1 A First Look at Metabolism
 - 11.2 Freeways on the Metabolic Road Map
 - 11.3 Biochemical Reaction Types
 - 11.4 Bioenergetics of Metabolic Pathways
 - 11.5 Major Metabolic Control Mechanisms
 - 11.6 Experimental Analysis of Metabolism
- Tools of Biochemistry**
- 11A Metabolomics
 - 11B Radioactive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



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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.

that the reaction proceeds as written in vivo. Reaction 4 demonstrates the importance of considering the conditions *in the cell* (ΔG) rather than standard state conditions (ΔG°) 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 ϵ -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.

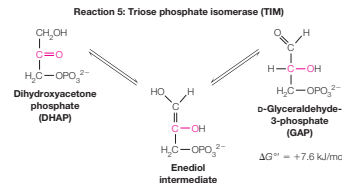
The Schiff base intermediate is advantageous in this reaction because it can delocalize electrons. The positively charged iminium ion is thus a better electron acceptor than a ketone carbonyl, facilitating retro-aldol reactions like this one and, as we shall see, many other biological

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.

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Reaction 5: Isomerization of Dihydroxyacetone Phosphate

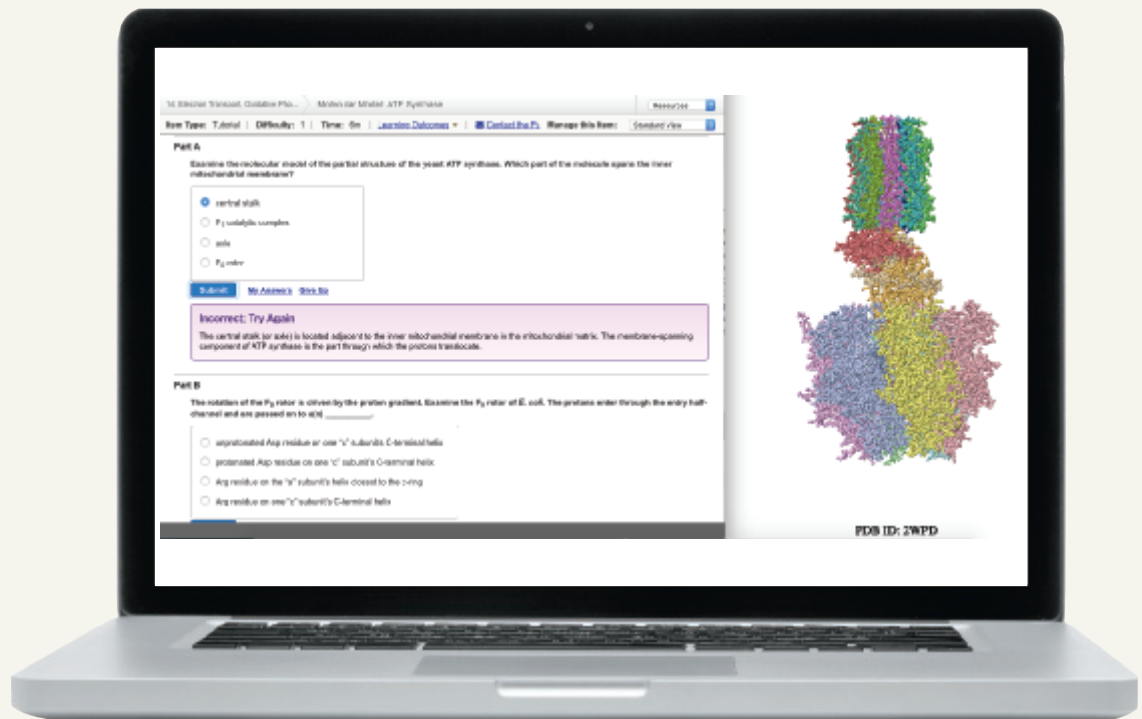
In reaction 5, triose phosphate isomerase (TIM) catalyzes the isomerization of dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (GAP) via an enediol intermediate.



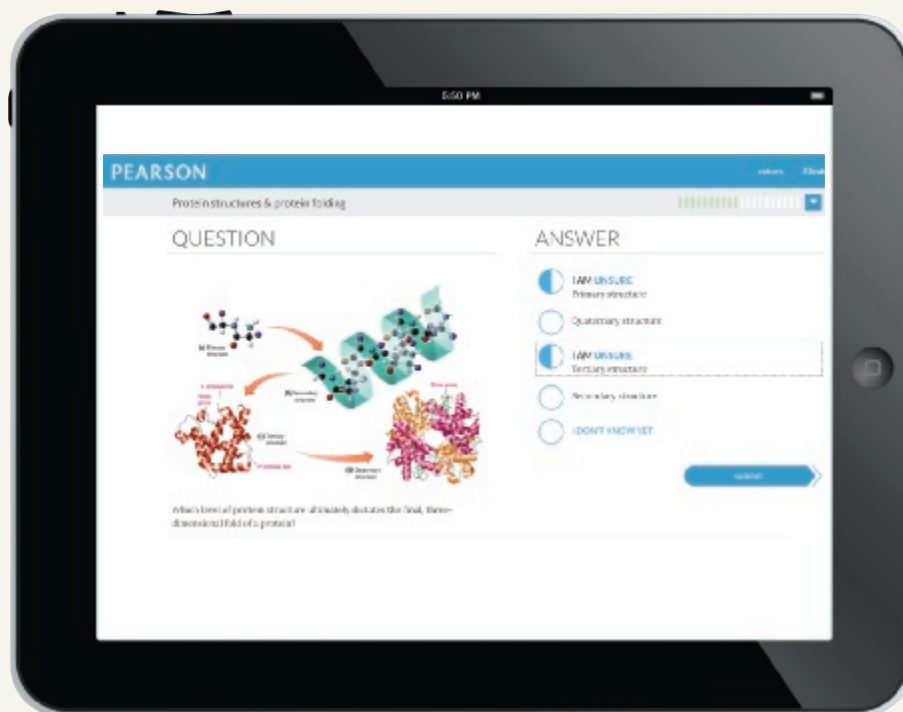
Like reaction 4, reaction 5 is weakly endergonic under standard conditions, but the intracellular concentration of GAP is low because it is consumed in subsequent reactions. Thus, reaction 5 is drawn toward the right.

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.

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and understand what's happening on the cellular level

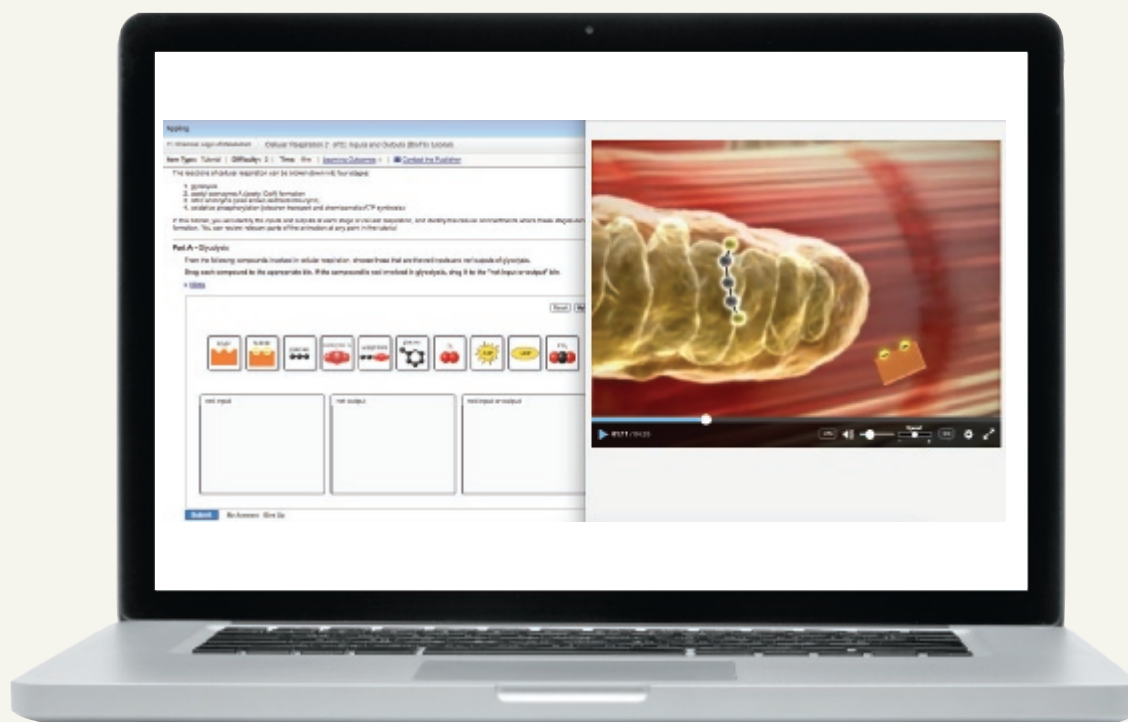


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Synthesis of information is simplified through features

FOUNDATION FIGURE | Protein Structure and Function

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.

The function of a protein is determined by its structure. The polypeptide chain synthesized on the ribosome folds from an initial unstructured state to a functional, more structured state.

- This folded structure typically represents a state of minimal free energy. Thus, the folding process is thermodynamically favorable.
- Proteins can also misfold and form highly stable aggregates. Protein misfolding is associated with several diseases.
- Cells have evolved to include folding accessory proteins called chaperones, which bind to partially folded proteins and prevent them from aggregating, thereby allowing them to adopt their native (functional) structures.

1 FOLDING Proteins fold to functional structures, which represent low free energy conformations.

When proteins fold correctly...

Primary (1°) structure: Amino acid sequence (primary structure) determines secondary and tertiary structures.

Secondary (2°) structure: Some parts of the primary sequence adopt a local regular repeating structure ("2° structure").

Tertiary (3°) structure: Several 2° structure elements associate along their hydrophobic surfaces to give a stable folded structure ("3° structure").

When proteins misfold...

Misfolded intermediates: GroEL/GDG is one example of a molecular chaperone. Chaperones play a critical role in helping partially folded proteins on the pathway to proper folding, and thereby preventing aggregation which can lead to disease.

2 FUNCTION Proteins possess shape and charge complementarity to their specific ligands/substrates.

L. of repressor protein: Two helices (red in the A of repressor protein are critically positioned to make complementary hydrogen bonds a short distance from a specific DNA sequence.

Dihydrolate reductase: The enzyme dihydrolate reductase (DHAP) makes several specific hydrogen bonds to the collector NADPH.

Shape complementarity and **Charge complementarity**

3 MISFOLDING Misfolded proteins tend to aggregate and this is associated with several diseases.

Unfolded protein: Will rely on chaperones. Folding intermediates. Native state (N). Partially folded states. Aggregates. Amyloid fibrils.

Energy: Intermolecular contacts, Intermolecular contacts, Repeating beta structure of amyloid fibrils.

Native protein: GroEL (17) + ATP + GroES → GroEL-GroES complex → GroEL-GroES complex + TP_i → GroEL-GroES complex + T ADP + GroES → Native protein.

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

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

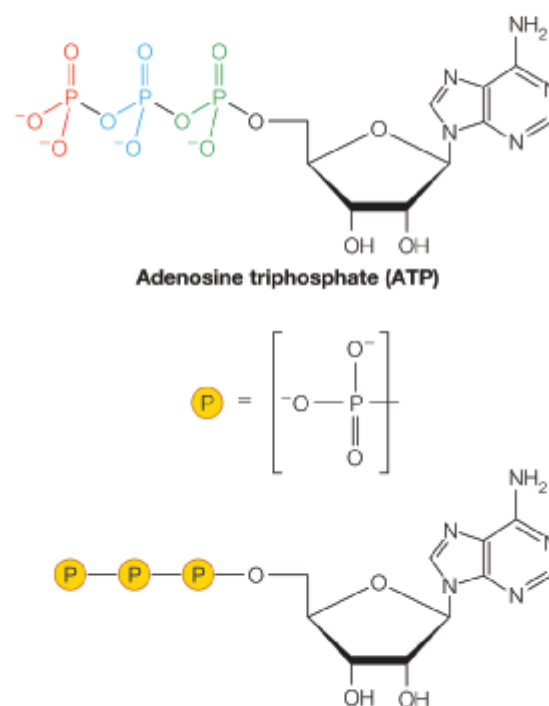
Note that the value calculated for ΔG is much more negative (i.e., more favorable) than the standard free energy change $\Delta G^{\circ'}$. This last point underscores the fact that it is ΔG and not $\Delta G^{\circ'}$ that determines the driving force for a reaction. However, to evaluate ΔG using Equation 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.

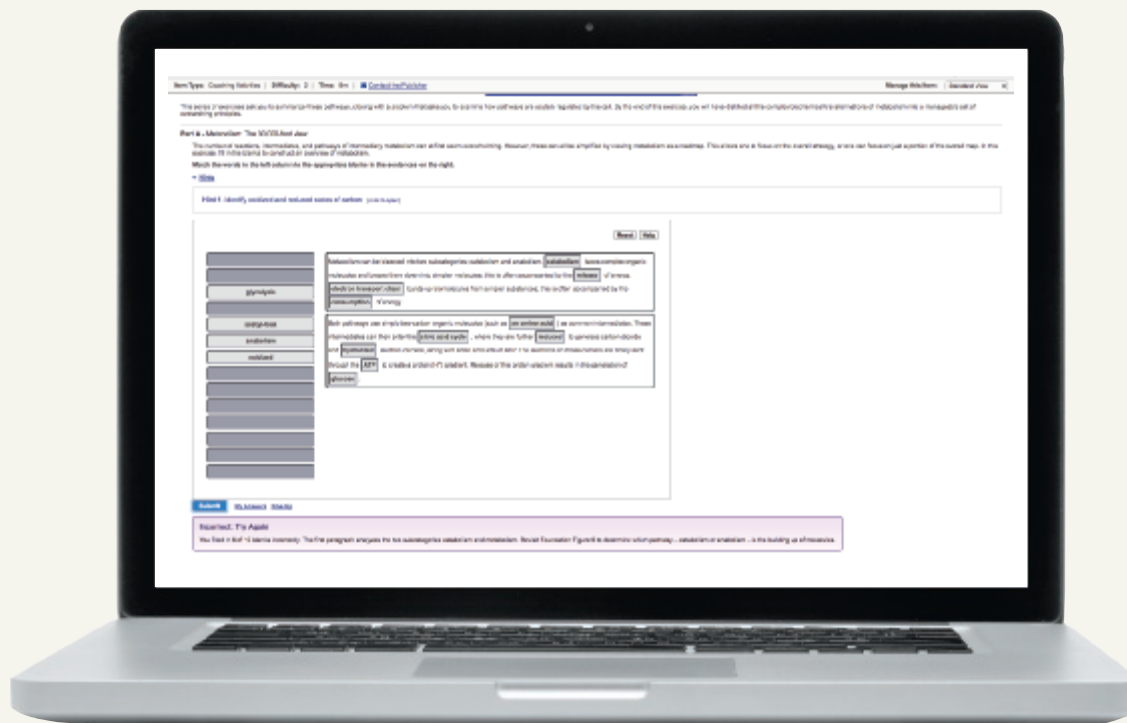


▲ FIGURE 3.5 The phosphoryl groups in ATP. Top: The three phosphoryl groups in ATP are shown in red, blue, and green. Middle: A commonly used shorthand for a phosphoryl group is the symbol $\textcircled{\text{P}}$. Bottom: The three phosphoryl groups in ATP are represented by this symbol.

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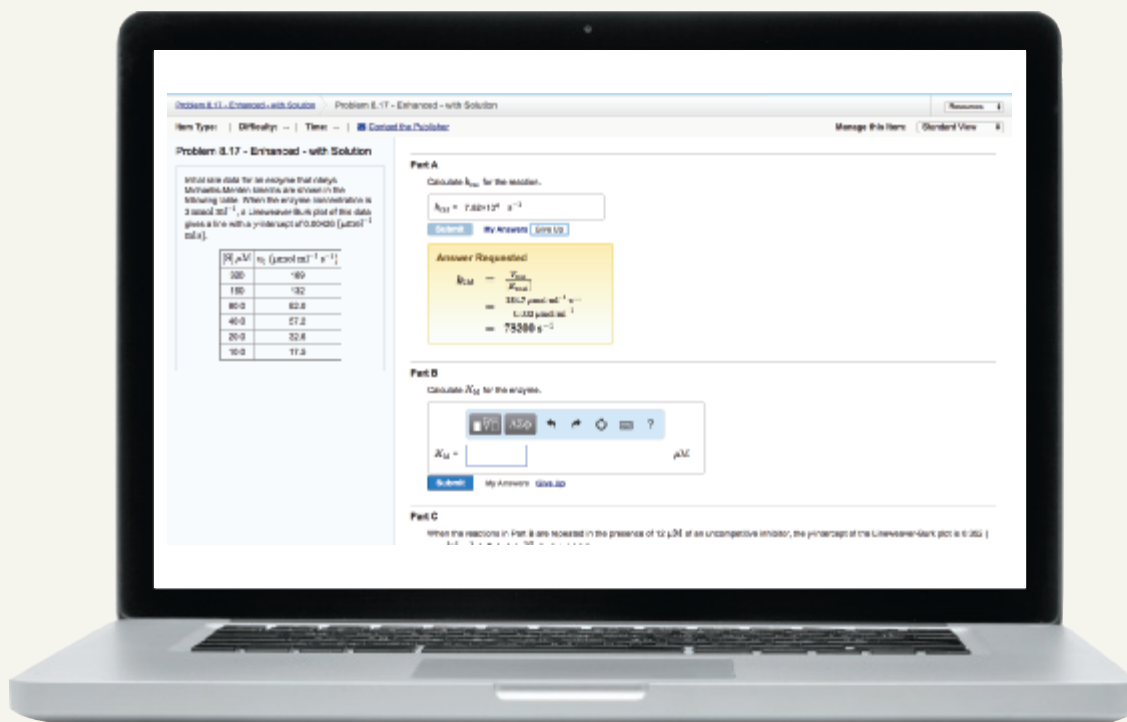
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The hallmark Hints and Feedback offer instruction similar to what students would experience in an office hour, allowing them to learn from their mistakes without being given the answer.

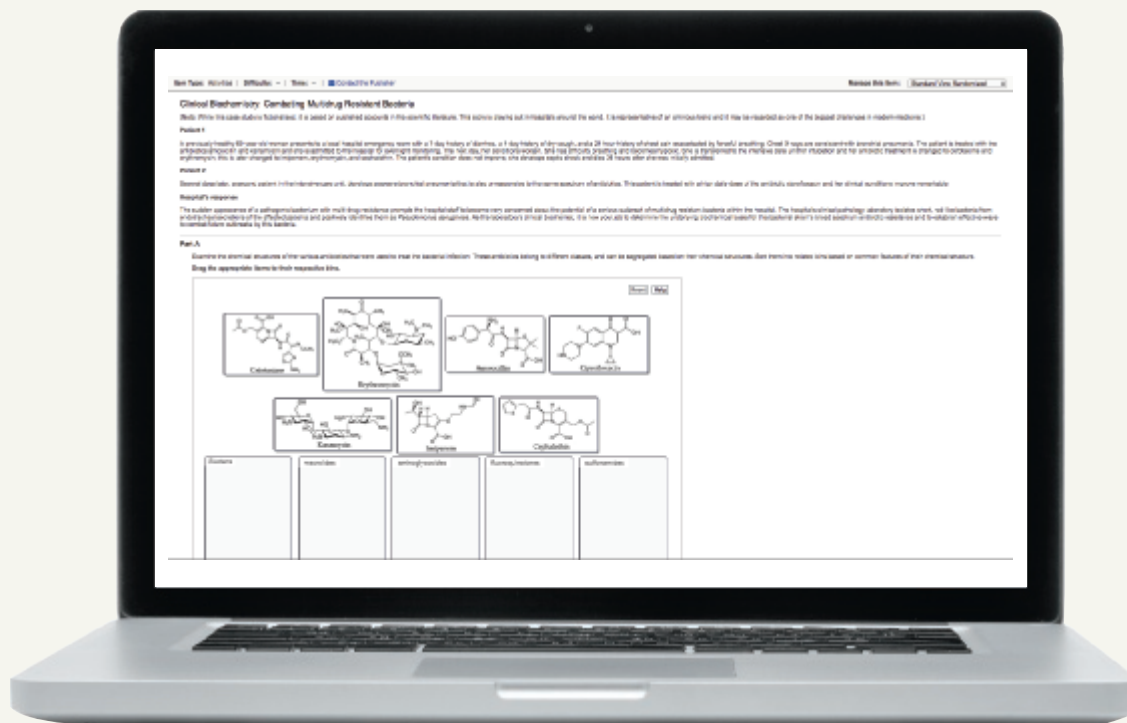
Extended coverage of biochemistry topics and new real-world applications such as non-glucose metabolism have been added to the second edition.

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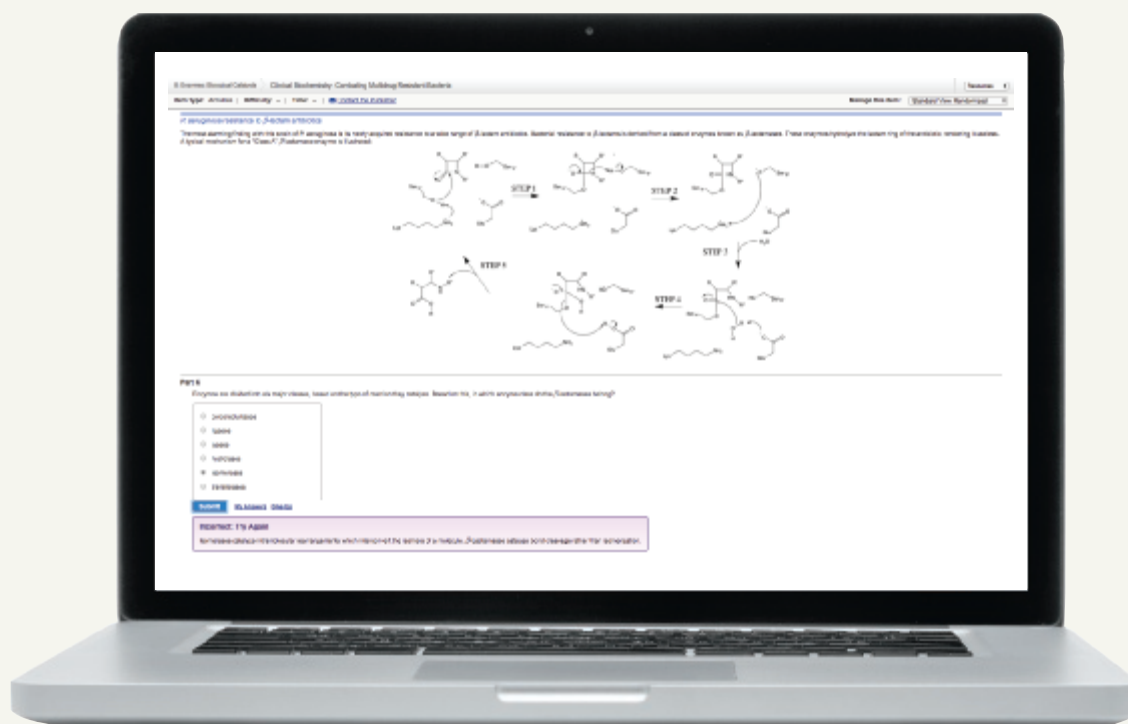
from the textbook are assignable within Mastering Chemistry and help students prepare for the types of questions that might appear on an exam. All end-of-chapter problems are automatically graded and include author-written solutions.



to help students connect course materials to real world applications



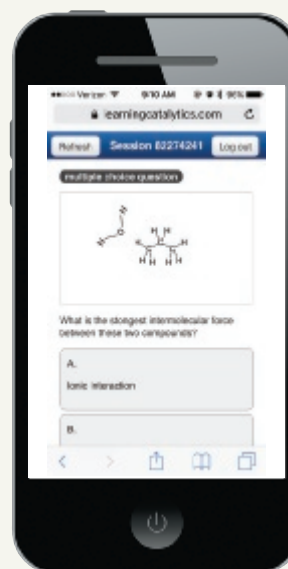
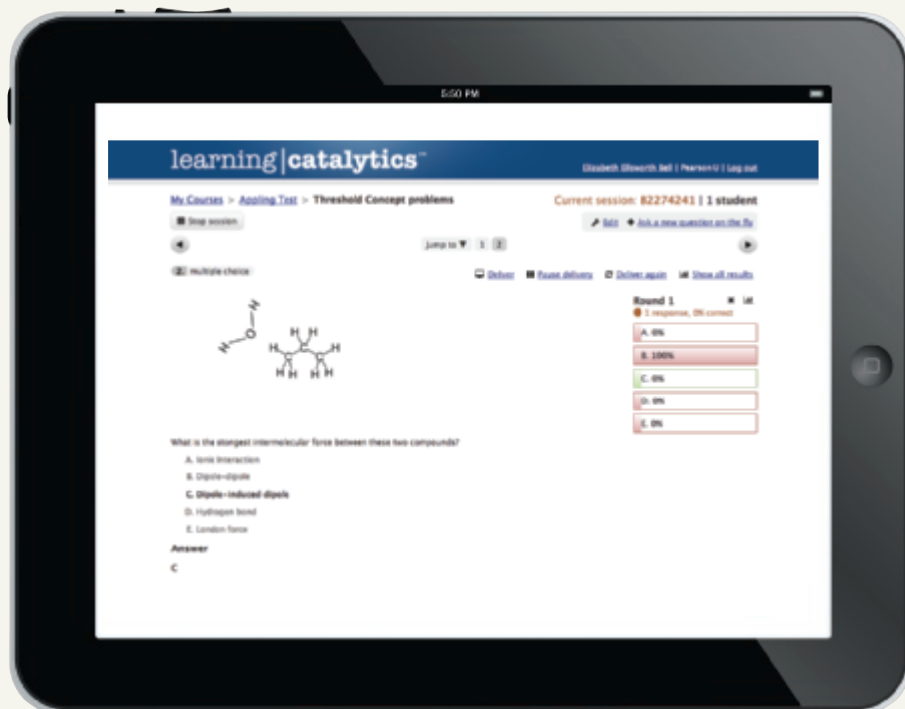
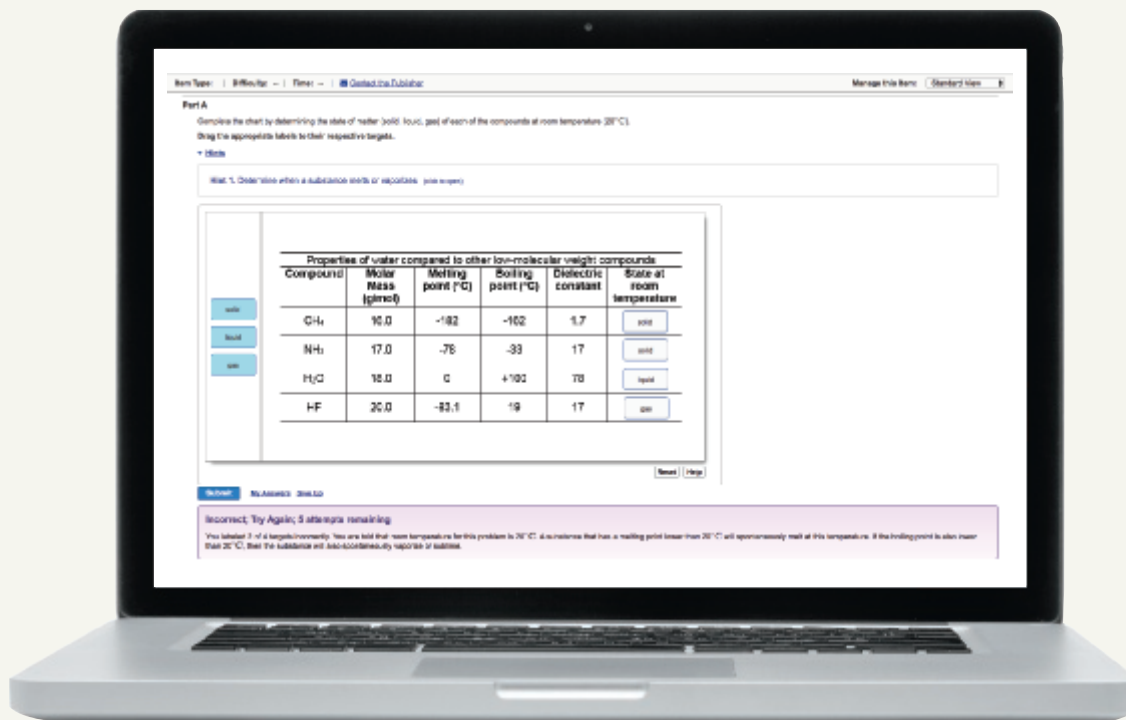
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NEW! Threshold Concept Tutorials prepare students for success in biochemistry. Much of biochemistry requires foundational knowledge from earlier courses. Unfortunately, many students begin the course either never having truly grasped the important concepts or having forgotten them since they last took the prerequisite.

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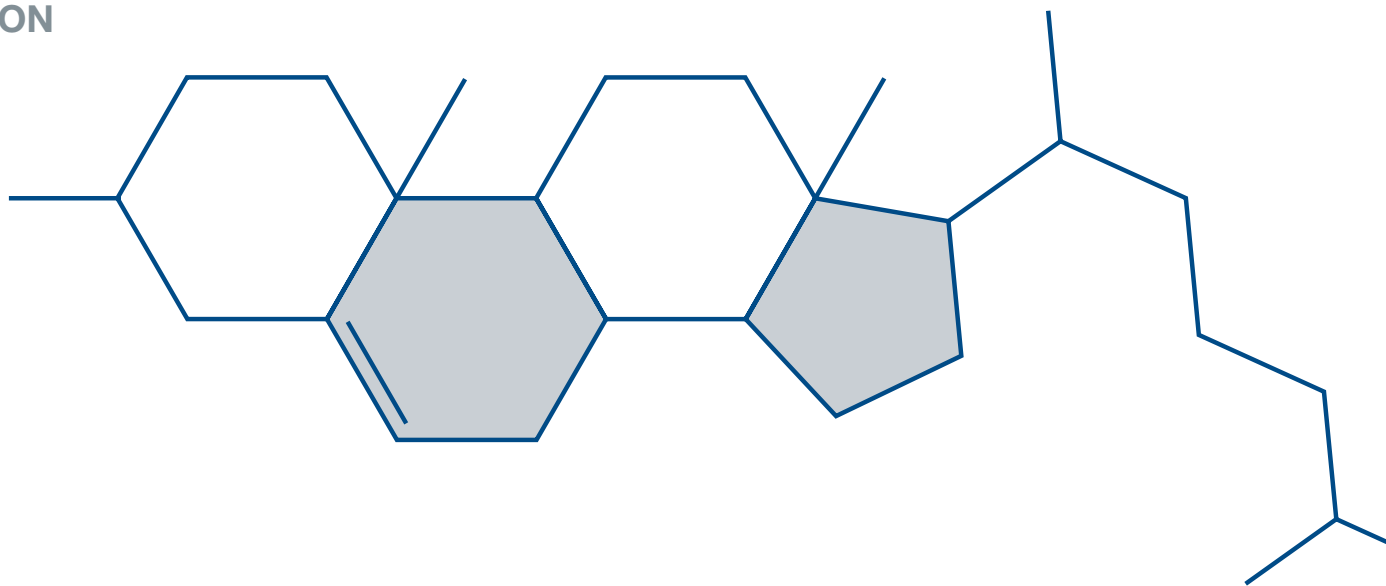
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Discovering new medicines requires comprehension of the structure and function of the drug target, whether that be an enzyme, a gene, or a signaling molecule. Success in drug discovery requires deep understanding of biochemistry and its allied disciplines.

Biochemistry and the Language of Chemistry

“**MUCH OF LIFE** can be understood in rational terms if expressed in the language of chemistry. It is an international language, a language for all of time, and a language that explains where we came from, what we are, and where the physical world will allow us to go.” These words were written in 1987 by Arthur Kornberg (1918–2007), one of the greatest biochemists of the twentieth century, and they provide a backdrop for our study of biochemistry. Because it seeks to understand the chemical basis for all life processes, biochemistry is at once a biological science and a chemical science. Indeed, all of the traditional disciplines within biology—including physiology, genetics, evolution, and ecology, to name a few—now use the language and techniques of chemistry. Many of you who are using this book are planning careers in life sciences—in teaching, basic research, health sciences, science journalism, drug discovery, environmental science, bioengineering, agriculture, science policy, and more. You will find biochemistry at the heart of all fields within the biological sciences.

1

Chapter 1

- 1.1** The Science of Biochemistry
- 1.2** The Elements and Molecules of Living Systems
- 1.3** Distinguishing Characteristics of Living Systems
- 1.4** The Unit of Biological Organization: The Cell
- 1.5** Biochemistry and the Information Explosion



As we proceed through our study of biochemistry, think about “the language of chemistry.” To understand a language, we must become familiar with the words and how to incorporate them into sentences. In this text we will be faced with numerous chemical names and structures that must be learned, such as the amino acids in proteins or the sugars in starch or cellulose. These are the *words* in the biochemical language, and learning them will occupy much of the first several chapters of this book. Next, we begin putting these words into *sentences*—chemical reactions—and *paragraphs*—metabolic pathways, which are made up of linked sequences of two or more individual reactions. Reading the sentences and paragraphs will require that we learn about enzymes and catalysis of biochemical reactions. Later we move from paragraphs to pages and chapters, as we explore how metabolic processes in different tissues interrelate to explain, for example, the adaptation of an animal to starvation, or the possible effects of calorie restriction on life-span extension. We will also learn what regulates expression of the biochemical language when we explore chromosomes, genomes, and genes—and how the controlled expression of genes dictates which sentences will be printed and in which cells, and how instructions in the language are transmitted from generation to generation.

As we discuss the biochemical language and its expression, three themes will dominate our

discussion—*metabolism, energy, and regulation*. What are the chemical reactions? How is metabolic work done? How is expression of the language controlled?

● **CONCEPT** All of the life sciences require an understanding of the language of chemistry.

In order to apply the language of chemistry to learning biochemistry, you will need

to recall much of what you learned in organic chemistry—the structures and properties of the principal functional groups, for example. Chapter 2 provides a brief review of the major functional groups, and Chapter 11 describes those reaction mechanisms most directly involved in biochemistry.

Because most of you are learning the biochemical language for the first time, our initial emphasis must be on individual reactions and pathways, operating to some extent in isolation. Be aware, however, that plucking individual reactions out of a cell for investigation is artificial and that a chemical reaction within a cell is but one in a coordinated system of hundreds or thousands of individual reactions, all occurring in the same time and space. In the past two decades, techniques have been developed that allow analysis of a true *systems biology*—chemical reactions as they occur within a complex system rather than in isolation. In time, we will discuss these techniques and what they teach us, but the emphasis in a first course in biochemistry is on elements and expression of the biochemical language.

1.1 The Science of Biochemistry

Humankind has harvested the fruits of biochemistry for thousands of years, perhaps beginning some 8000 years ago with the fermentation of grapes into wine. **FIGURE 1.1** illustrates winemaking as it was carried out in Egypt in about 1500 B.C. However, the science behind winemaking and many other biochemical applications, such as medicinal folk remedies or the tanning of leather, remained obscure until the past three centuries or so, with the birth of biochemistry as a science. With respect to winemaking, see Chapter 12 for a presentation of **glycolysis**, the fundamental process for the breakdown of sugars, which in yeast and other microorganisms converts the sugar to ethanol.

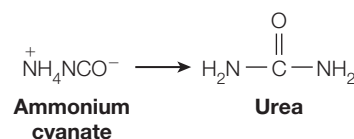
The Origins of Biochemistry

Biochemistry as a science can be said to have originated early in the nineteenth century, with the pioneering work of Friedrich Wöhler

(1800–1882) in Germany. Prior to Wöhler’s time, it was believed that the substances in living cells and organisms were somehow qualitatively different from those in nonliving matter and did not behave according to the known laws of physics and chemistry. In 1828 Wöhler showed that urea, a substance of biological origin, could be synthesized in the laboratory from the inorganic compound, ammonium cyanate. As Wöhler phrased it in a letter to a colleague, “I must tell you that I can prepare urea without requiring a kidney or an animal, either man or dog.” This was a shocking statement in its time, for it breached the presumed barrier between the living and nonliving.

Another landmark in the history of biochemistry occurred in the mid-nineteenth century when the great French chemist Louis

Wöhler’s synthesis of urea from ammonium cyanate:





▲ **FIGURE 1.1** An ancient application of biochemistry. Manufacture of wine in Egypt, around 1500 B.C.

Pasteur (1822–1895) turned his attention to fermentation in order to help the French wine industry. Pasteur recognized that wine could be spoiled by the accidental introduction of bacteria during the fermentation process and that yeast cells alone possess the ability to convert the sugars in grapes to ethanol in wine. Following this discovery, he devised ways to exclude bacteria from fermentation mixtures.

● **CONCEPT** Early biochemists had to overcome the doctrine of vitalism, which claimed that living matter and nonliving matter were fundamentally different.

Although Pasteur demonstrated that yeast cells in culture could ferment sugar to alcohol, he adhered to the prevailing view known as *vitalism*, which held that biological reactions took place only through the action of a mysterious “life force” rather than physical or chemical processes. In other words, the fermentation of sugar into ethanol could occur only in whole, living cells.

The vitalist dogma was shattered in 1897 when two German brothers, Eduard (1860–1917) and Hans Buchner (1850–1902), found that extracts from broken and thoroughly dead yeast cells could carry out the entire process of fermentation of sugar into ethanol. This discovery opened the door to analysis of biochemical reactions and processes **in vitro** (Latin, “in glass”), meaning in a test tube—or, more generally, outside of a living organism or cell, rather than **in vivo**, in living cells or organisms. In the following decades, other metabolic reactions and reaction pathways were reproduced *in vitro*, allowing identification of reactants and products and of the biological catalysts, known as **enzymes**, that promoted each biochemical reaction. The name “enzyme,” coined in 1878, comes from the Greek *en zyme* (meaning “in yeast”), reflecting the fact that the chemical nature of

Although Pasteur demonstrated that yeast cells in culture could ferment sugar to alcohol, he adhered to the prevailing view known as *vitalism*, which held that biological reactions took place

only through the action of a mysterious “life force” rather than physical or chemical processes. In other words, the fermentation of sugar into ethanol could occur only in whole, living cells.

question, we must understand expression of **genes**, which control synthesis of the enzymes involved. The idea of the gene, a unit of hereditary information, was first proposed in the mid-nineteenth century by Gregor Mendel (1822–1894), an Austrian monk, from his studies on the genetics of pea plants. By about 1900, cell biologists realized that genes must be found in chromosomes, which are composed of proteins and nucleic acids. Subsequently, the new science of genetics provided increasingly detailed knowledge of patterns of inheritance and development. However, until the mid-twentieth century no one had isolated a gene or determined its chemical composition. Nucleic acids had been recognized as cellular constituents since their discovery in 1869 by Friedrich Miescher (1844–1895). But their chemical structures were poorly understood, and in the early 1900s nucleic acids were thought to be simple substances, fit only for structural roles in the cell. Most biochemists believed that only proteins were sufficiently complex to carry genetic information.

That belief turned out to be incorrect. Experiments in the 1940s and early 1950s proved conclusively that **deoxyribonucleic acid (DNA)** is the primary bearer of genetic information (**ribonucleic acid, RNA**, is also an informational molecule). The year 1953 was a landmark year, when James Watson (1928–) and Francis Crick (1916–2004) described

● **CONCEPT** Biology was transformed in 1953, when Watson and Crick proposed the double-helical model for DNA structure.

the double-helical structure of DNA. This concept immediately suggested ways in which information could be encoded in the structure of molecules and transmitted intact from

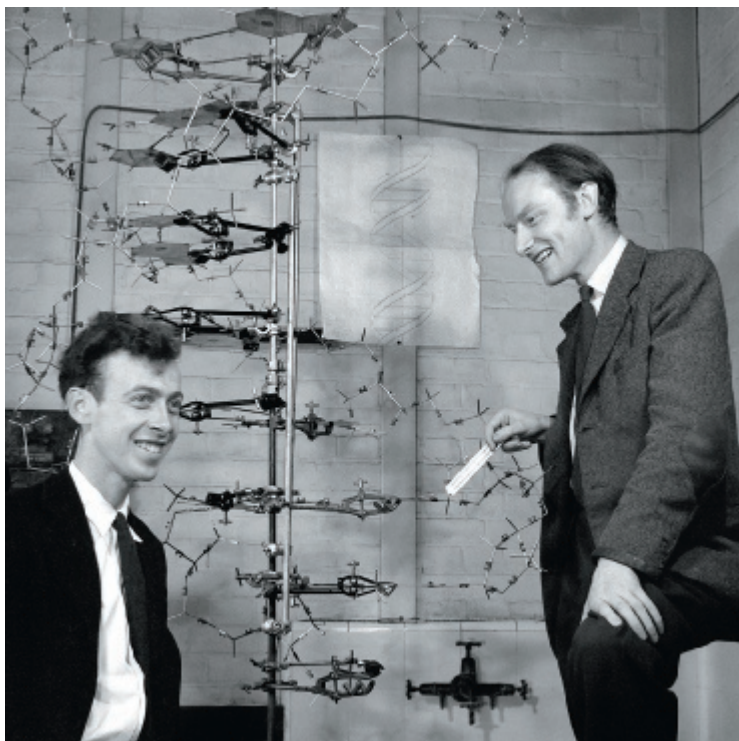
one generation to the next. The discovery of DNA structure, which we describe more fully in Chapter 4, represents one of the most important scientific developments of the twentieth century (**FIGURE 1.2**).

these catalysts did not become known until some time later, as described below.

The nature of biological catalysis remained the last refuge of the vitalists, who held that the structures of enzymes were far too complex to be described in chemical terms. But in 1926, James B. Sumner (1887–1955) showed that an enzyme from jack beans, called **urease**, could be crystallized like any organic compound and that it consisted entirely of protein. Although proteins have large and complex structures, they are just organic compounds, and their structures can be determined by the methods of chemistry and physics. This discovery marked the final fall of vitalism.

Although developments in the first half of the twentieth century revealed in broad outline the chemical structures of biological materials, identified the reactions in many metabolic pathways, and localized these reactions within the cell, biochemistry remained an incomplete science. We knew that the uniqueness of an organism is determined by the totality of its chemical reactions. However, we had little understanding of how those reactions are controlled in living tissue or of how the information that regulates those reactions is stored, transmitted when cells divide, and processed when cells differentiate.

What factors determine why yeast cells might ferment sugars to ethanol, while bacteria contaminating a wine culture might convert the sugars to acetic acid and turn the wine culture to vinegar? To answer this



▲ **FIGURE 1.2** James Watson and Francis Crick with their hand-assembled wire model of the structure of DNA.

Although Watson and Crick made their landmark discovery over six decades ago, the revolution ushered in by that discovery is still underway, as seen by some of the major advances that have occurred since 1953. By the early 1960s, we knew much about the functions of RNA in gene expression, and the genetic code had been deciphered (see Chapters 24 and 25). By the early 1970s, the first recombinant DNA molecules were produced in the laboratory (see Chapter 4), opening the door, as no other discovery had done, to practical applications of biological information in health, agriculture, forensics, and environmental science. By the next decade, scientists had learned how to amplify minute amounts of DNA (see Chapter 21) so that any gene could be isolated by cloning (Chapter 4), allowing any desired change to be made in the structure of a gene. After another decade, by the early 1990s, scientists had learned not only how to introduce new genes into the germ line of plants and animals, but also how to disrupt or delete any gene, allowing analysis of the biochemical function of any gene product (see Chapter 23). A decade later, the nearly complete nucleotide sequence of the human genome was announced— 2.9×10^9 base pairs of DNA, representing more than 20,000 different genes. At about the same time came discoveries regarding noncoding properties of RNA, in catalysis and gene regulation (Chapters 7, 25, and 26). The 20-teens saw development of CRISPR (**clustered regularly interspersed short palindromic repeats**) technology, which allowed unprecedented opportunities for editing genes in living organisms (Chapter 23). The wealth of information from genomic sequence analysis and gene regulation by RNA continues to transform the biochemical landscape well into the twenty-first century.

The Tools of Biochemistry

The advances in biochemistry discussed in the previous section and described throughout this book would not have been possible without the

development of new technologies for studying biological molecules and processes. Biochemistry is an experimental science—more so, for example, than physics, with its large theoretical component. To understand the key biochemical concepts and processes, we must have some understanding of the experiments that helped us elucidate them. We will describe the experimental basis for much of our understanding of biochemistry in this book. In some cases, the description of experimental techniques will be set apart in end-of-chapter segments called “Tools of Biochemistry.”

In the case of DNA structural analysis, the needed technology came from X-ray diffraction. Physicists and chemists had learned that the molecular structures of small crystals could be determined by analyzing patterns showing how X-rays are deflected upon striking atoms in a crystal. Stretched DNA fibers yield comparable data, and these patterns (obtained by Rosalind Franklin, 1920–1958; see Chapter 4), along with the chemical structures of the individual nucleotide units in DNA, led Watson and Crick to their leap of intuition.

FIGURE 1.3 shows a timeline for introduction of methods related to biochemistry beginning at the end of World War II (1945) with the introduction of radioisotopes; these are used to tag biomolecules so that they can be followed through reactions and pathways. Other notable developments include gel electrophoresis (early 1960s), which allows separation and analysis of nucleic acids and proteins. By the early

● **CONCEPT** Powerful new chemical and physical techniques have accelerated the pace at which biological processes have become understood in molecular terms.

1970s, restriction enzymes (Chapter 21) had been shown to cut DNA strands at particular sequences in DNA molecules; this finding opened the door to isolating individual genes by recombinant DNA technol-

ogy. Polymerase chain reaction (Chapter 21) allowed the amplification of selected DNA sequences from minute tissue samples. CRISPR technology (Chapter 23), introduced in 2013, allowed unprecedented opportunities for genome editing in living cells. Throughout this book we will be describing these and other benchmark technologies, and you may wish to refer back to this figure.

Biochemistry as a Discipline and an Interdisciplinary Science

In trying to define biochemistry, we must consider it both as an interdisciplinary field and as a distinct discipline. Biochemistry shares its major concepts and techniques with many disciplines—with organic chemistry, which describes the properties and reactions of carbon-containing molecules; with physical chemistry, which describes thermodynamics, reaction kinetics, and electrical parameters of oxidation–reduction reactions; with biophysics, which applies the techniques of physics to study the structures of biomolecules; with medical science, which increasingly seeks to understand disease states in molecular terms; with nutrition, which has illuminated metabolism by describing the dietary requirements for maintenance of health; with microbiology, which has shown that single-celled organisms and viruses are ideally suited for the elucidation of many metabolic pathways and regulatory mechanisms; with physiology, which investigates life processes at the tissue and organism levels; with cell biology, which describes the metabolic and mechanical division of labor within a cell; and with genetics, which analyzes mechanisms that give a particular cell or organism its biochemical identity. Biochemistry draws strength from all of these disciplines, and it nourishes them in return; it is truly an interdisciplinary science.

2015	<ul style="list-style-type: none"> • Cryo-electron microscopy • CRISPR-Cas9 technology
2010	<ul style="list-style-type: none"> • Synthetic biology • RNA-sequence analysis • Chromatin immunoprecipitation/sequencing • Induced pluripotent cells • Second generation
2005	<ul style="list-style-type: none"> • DNA sequence analysis • Proteomic analysis with mass spectrometry
2000	<ul style="list-style-type: none"> • Genetic code expansion • Gene analysis on microchips • Single-molecule dynamics
1995	<ul style="list-style-type: none"> • Targeted gene disruption • In vivo NMR • Atomic force microscopy
1990	<ul style="list-style-type: none"> • Scanning tunneling microscopy
1985	<ul style="list-style-type: none"> • Pulsed field electrophoresis • Transgenic animals • Amplification of DNA: polymerase chain reaction • Automated oligonucleotide synthesis
1980	<ul style="list-style-type: none"> • Site-directed mutagenesis of cloned genes • Automated micro-scale protein sequencing • Rapid DNA sequence determination • Monoclonal antibodies
1975	<ul style="list-style-type: none"> • Southern blotting • Two-dimensional gel electrophoresis • Gene cloning
1970	<ul style="list-style-type: none"> • Restriction cleavage mapping of DNA molecules • Rapid methods for enzyme kinetics
1965	<ul style="list-style-type: none"> • High-performance liquid chromatography • Polyacrylamide gel electrophoresis • Solution hybridization of nucleic acids
1960	<ul style="list-style-type: none"> • X-ray crystallographic protein structure determination • Zone sedimentation velocity centrifugation • Equilibrium gradient centrifugation • Liquid scintillation counting
1955	<ul style="list-style-type: none"> • First determination of the amino acid sequence of a protein • X-ray diffraction of DNA fibers
1950	
1945	<ul style="list-style-type: none"> • Radioisotopic tracers used to elucidate reactions

◀ **FIGURE 1.3** The recent history of biochemistry as shown by the introduction of new research techniques. The timeline begins with the introduction of radioisotopes as biochemical reagents, immediately following World War II.

You may wonder about the distinction between biochemistry and *molecular biology*, because both fields take as their ultimate aim the complete definition of life in molecular terms. The term *molecular biology* is often used in a narrower sense to denote the study of nucleic acid structure and function and the genetic aspects of biochemistry—an area we might more properly call *molecular genetics* or *genetic biochemistry*.

Regardless of uncertainty in terminology, biochemistry is a distinct discipline, with its own identity. It is distinctive in its emphasis on the structures and reactions of biomolecules, particularly on enzymes and biological catalysis and on the elucidation of metabolic pathways and their control. As you read this book, keep in mind both the uniqueness of biochemistry as a separate discipline and the absolute interdependence of biochemistry and other physical and life sciences.

1.2 The Elements and Molecules of Living Systems

All forms of life, from the smallest bacterial cell to a human being, are constructed from the same chemical elements, which in turn make up the same types of molecules. The chemistry of living systems is similar throughout the biological world; the reactions and pathways that will concern us involve fewer than 200 different molecules. Undoubtedly, this continuity in biochemical processes reflects the common evolutionary ancestry of all cells and organisms. Let us begin to examine the composition of living systems, starting with the chemical elements and then moving to biological molecules.

The Chemical Elements of Cells and Organisms

Life is a phenomenon of the second generation of stars. This rather strange-sounding statement is based on the fact that life, as we conceive it, could come into being only when certain elements—carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur (C, H, O, N, P, and S)—were abundant (**FIGURE 1.4**). The primordial universe was made up almost entirely of hydrogen (H) and helium (He), for only these simplest elements were produced in the condensation of matter following the primeval explosion, or “big bang,” which we think created the universe. The first generation of stars contained no heavier elements from which to form planets. As these early stars matured over the next seven to eight billion years, they burned their hydrogen and helium in thermonuclear reactions. These reactions produced heavier elements—first carbon, nitrogen, and oxygen, and eventually all the other members of the periodic table. As large stars matured, they became unstable and exploded as novae and supernovae, spreading the heavier elements through the cosmic surroundings. This matter condensed again to form

1																	He																						
H																	He																						
1																	He																						
3	4															5	6	7	8	9	10																		
Li	Be															B	C	N	O	F	Ne																		
11	12															13	14	15	16	17	18																		
Na	Mg															Al	Si	P	S	Cl	Ar																		
23	24															27	28	29	30	31	32	33	34	35	36														
K	Ca	Sc	Ti	23	24	25	26	27	28	29	30	31	32	33	34	35	36																						
39	40			51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe																						
87	88			96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn																						
137	138			184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	
Fr	Ra	Ac	Rf	Ha																																			

▲ **FIGURE 1.4** Periodic table pertinent to biochemistry. The four tiers of chemical elements, grouped in order of their abundance in living systems, are highlighted in separate colors.

● **CONCEPT** Life depends primarily on a few elements (C, H, O, N, S, and P), although many others have essential functions as well.

second-generation stars, at least some of which (like our sun) have planetary systems incorporating these heavier elements. Our universe, which is now rich in second-generation stars, has an elemental composition compatible with life as we know it.

Relatively few elements are involved in the creation of living systems. Living creatures on Earth are composed primarily of just four elements—carbon, hydrogen, oxygen, and nitrogen. These are also the most abundant elements in the universe, along with helium and neon. Helium and neon, inert gases, are not equipped for a role in life processes; they do not form stable compounds, and they are readily lost from planetary atmospheres.

The abundance of oxygen and hydrogen in organisms is explained partly by the major role of water in life on Earth. We live in a highly aqueous world, and, as we will see in Chapter 2, the solvent properties of water are indispensable in biochemical processes. The human body, in fact, is about 70% water. The elements C, H, O, and N are important to life because of their strong tendencies to form covalent bonds. In particular, the stability of carbon–carbon bonds and the possibility of forming single, double, or triple bonds give carbon the versatility to be part of an enormous diversity of chemical compounds.

But life is not built on these four elements alone. Many other elements are necessary for organisms on Earth, as you can see in Figure 1.4. A “second tier” of essential elements includes sulfur and phosphorus, which form covalent bonds, and the ions Na^+ , K^+ , Mg^{2+} , Ca^{2+} , and Cl^- . Sulfur is a constituent of nearly all proteins, and phosphorus plays essential roles in energy metabolism and the structure of nucleic acids. Beyond the first two tiers of elements (which correspond roughly to the most abundant elements in the first two rows of the periodic table), we come to those that play quantitatively minor—but often indispensable—roles. As Figure 1.4 shows, most of these third- and fourth-tier elements are metals, some of which serve as aids to the catalysis of biochemical reactions. In succeeding chapters we shall encounter many examples of the importance of these trace elements to life. Molybdenum, for example, is essential in nitrogen fixation—the reduction of nitrogen gas in the atmosphere to ammonia, for synthesis of nucleic acids and proteins (see Chapter 18).

The Origin of Biomolecules and Cells

Once the chemical elements had formed, during cooling of the second-generation stars, how did the complex molecules that we associate with living systems come into being on Earth? An educated guess is that they arose as part of a “primordial soup” within the oceans. Because the strong oxidant, oxygen, was absent from Earth’s atmosphere, scientists hypothesize that a highly reducing environment prevailed within the primordial atmosphere, a condition that tends to promote joining reactions of atoms and molecules. Moreover, high-energy discharges were thought to occur through lightning or volcanic eruptions, providing sufficient energy to drive atoms and small molecules together.

In 1953, Stanley Miller tested this hypothesis by simulating the presumed primordial environment. Miller mixed ammonia, methane, water, and hydrogen in a closed system subject to continuous electric discharge. After several days, the system was analyzed and shown to contain several amino acids, as well as other simple compounds, including carbon monoxide, carbon dioxide, and hydrogen cyanide. Thus, it was established that biological compounds could have been produced abiotically (without living

systems). Refinements of the Miller experiment have shown that much more complex organic molecules can also arise under similar conditions.

How we went from the primordial soup, rich in potential biomolecules, to primitive living systems is still a matter of conjecture. Many biochemists believe that the earliest primitive systems, capable of self-replication and some form of metabolism, were based on ribonucleic acid (RNA). RNA is a more versatile molecule than DNA, as we discuss in Chapters 4 and 8, and it is capable of catalyzing chemical reactions as well as storing information. Thus, biochemists speak of an ancient “RNA world,” in which simple self-replicating cellular structures, surrounded by crude, lipid-rich membranes, might have existed. Eventually, because DNA is more stable than RNA, this presumed chemical evolution would have led to processes by which RNA or its component nucleotides could give rise to DNA-based life forms.

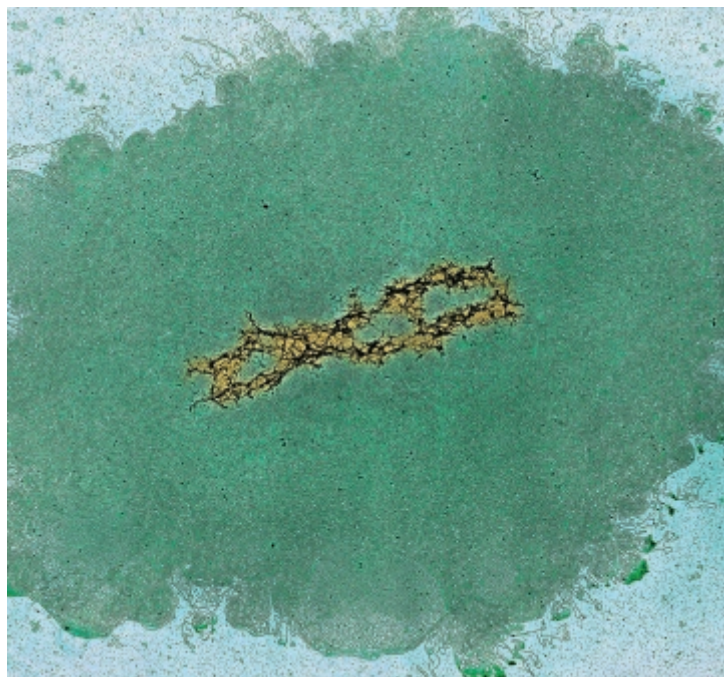
The earliest living systems would almost certainly have been anaerobic because of the absence of oxygen in the atmosphere. Energy was probably obtained from coupled oxidation–reduction reactions involving inorganic compounds of sulfur and iron. Over time, photosynthetic capability would have arisen, as some organisms evolved the ability to harness light energy from the sun to drive the reduction of inorganic compounds, notably CO_2 , to reduced organic compounds. Eventually, organisms would have developed the ability to use water as an electron donor, thereby creating enough oxygen over time to enrich the atmosphere with oxygen. Because much more energy can be derived through complete oxidation of organic compounds than from anaerobic processes (see Chapter 11), aerobic organisms would have had a large evolutionary advantage.

As primitive bacteria underwent the numerous changes leading to characteristic features of eukaryotic cells—condensation of genes into chromosomes, development of intracellular membranous structures—some eukaryotic cells acquired new metabolic capabilities through infection with aerobic bacteria or photosynthetic bacteria. Over time, the intracellular organisms living in this symbiotic relationship underwent their own evolution, eventually becoming what we now recognize as mitochondria and chloroplasts in present-day cells.

How long might this process have taken? Geologists tell us that Earth was formed about 4.6 billion years ago. Rocks containing carbon of likely biological origin have been dated to more than 3.5 billion years ago. Evidence for aerobic bacteria and an oxygen-rich atmosphere dates to about 2.5 billion years ago, with the first eukaryotic microorganisms following about one billion years later. The earliest multicellular eukaryotes are 400 to 500 million years old. Although we understand the forces that have shaped life since it arose—and these will be described as we proceed through our study of biochemistry—our understanding of the origin of life is conjectural. Although the spontaneous generation of self-replicating entities seems highly improbable, the enormous amount of time during which this could have occurred changes the almost impossible to highly likely, and perhaps inevitable.

The Complexity and Size of Biological Molecules

The complexity of life processes requires that many of the molecules governing them be enormous. Consider, for instance, the DNA molecules released from one human chromosome, as shown in **FIGURE 1.5**. The long, looped thread you see corresponds to a small part of a huge molecule, with a molecular mass of about 20 billion daltons. (A dalton, Da, is 1/12 the mass of a carbon-12 atom, 1.66×10^{-24} g.) Even a simple organism such as the single-celled bacterium *Escherichia coli* contains a DNA molecule with a molecular mass of about 2 billion Da—more than one millimeter long. Protein molecules are generally much smaller than DNA molecules,



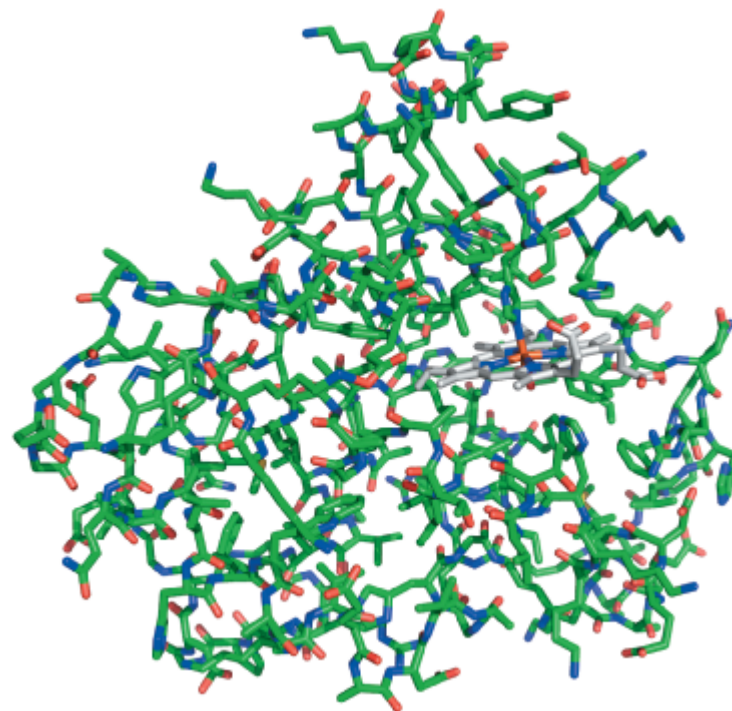
▲ **FIGURE 1.5** Part of the DNA from a single human chromosome. Most of the chromosomal proteins have been removed in this color-enhanced electron micrograph, leaving only a protein “skeleton” from which enormous loops of DNA emerge.

but they are still large, with molecular masses ranging from about 10,000 to one million Da. The complexity of these molecules is seen from the three-dimensional structure of even a fairly small protein. **FIGURE 1.6** illustrates the structure of myoglobin, an oxygen-carrying protein of muscle, which has a molecular mass of about 17,000 Da.

Biological **macromolecules** are giant molecules made up of smaller organic molecule subunits. In living organisms, there are four major classes of macromolecules, all essential to the structure and function of cells: proteins, nucleic acids, carbohydrates, and lipids. As we shall see throughout this text, there are good reasons for some biological materials to be so large. DNA molecules, for example, can be thought of as tapes from which genetic information is read out in a linear fashion. Because the amount of information needed to specify the structure of a multicellular organism is enormous, these tapes must be extremely long. In fact, if the DNA molecules in a single human cell were stretched end to end, they would reach a length of about 2 meters. As revealed in the early twenty-first century through the Human Genome Project, the information encoded in this DNA is sufficient to encode about 100,000 proteins, although the actual number of genes is far smaller.

The Biopolymers: Proteins, Nucleic Acids, and Carbohydrates

The synthesis of such large molecules poses an interesting challenge to the cell. If the cell functioned like an organic chemist carrying out a complex laboratory synthesis bit by bit, millions of different types of reactions would be involved, and thousands of intermediates would accumulate. Instead, cells use a modular approach for constructing large polymeric molecules. These **biopolymers** are made by joining together prefabricated units, or **monomers**. Of the four classes of macromolecules, three of them are biopolymers: proteins, nucleic acids, and carbohydrates. Lipids, the fourth class of macromolecule, are not considered polymers and are discussed in the next section.



▲ **FIGURE 1.6** The three-dimensional structure of myoglobin. This computer-generated stick model portrays sperm whale myoglobin, the first protein whose structure was deduced by X-ray diffraction. It depicts, therefore, our first indication of the complexity and specificity of the three-dimensional structure of proteins. PDB ID: 1mbn.

The monomers of a given type of macromolecule are of limited diversity and are linked together, or **polymerized**, by identical mechanisms. Each process involves **condensation**, or removal of a molecule of water in the joining reaction. A simple example is the carbohydrate **cellulose** (**FIGURE 1.7(a)**), a major constituent of the cell walls of plants. Cellulose is a polymer made by joining thousands of molecules of glucose, a simple sugar. In this polymer, all of the chemical linkages between the monomers are identical. Covalent links between glucose units are formed by removing a water molecule between two adjoining glucose molecules; the portion of each glucose molecule remaining in the chain is called a glucose **residue**. Because cellulose is a polymer of a simple sugar, or **saccharide**, it is called a **polysaccharide**. This particular polymer is constructed from identical monomeric units, so it is called a **homopolymer**. In contrast, many polysaccharides—and all nucleic acids and proteins—are **heteropolymers**, polymers constructed from a number of different kinds of monomer units.

Nucleic acids (Figure 1.7(b)) are polymers made up of four **nucleotides**, so nucleic acids are also called **polynucleotides**. Similarly, proteins (Figure 1.7(c)) are assembled from combinations of

● **CONCEPT** Cells use a modular approach for constructing large molecules.

20 different **amino acids**. Protein chains are called **polypeptides**, a term derived from the **peptide bond** that joins two amino acids together.

Polymers form much of the structural and functional machinery of the cell. Polysaccharides serve both as structural components, such as cellulose, and as reserves of biological energy, such as **starch**, another type of glucose polymer found in plants. The nucleic acids, DNA and RNA,