

Brief Contents

Preface	xvii
Acknowledgments	xxiii
About the Authors	xxv

PART 1 Principles of Biochemistry

- 1 Principles of Biochemistry 2
- 2 Physical Biochemistry: Energy Conversion, Water, and Membranes 38
- 3 Nucleic Acid Structure and Function 90

PART 2 Protein Biochemistry

- 4 Protein Structure 146
- 5 Methods in Protein Biochemistry 210
- 6 Protein Function 250
- 7 Enzyme Mechanisms 308
- 8 Cell Signaling Systems 370

PART 3 Energy Conversion Pathways

- 9 Glycolysis: A Paradigm of Metabolic Regulation 428
- 10 The Citrate Cycle 480
- 11 Oxidative Phosphorylation 524
- 12 Photosynthesis 578

PART 4 Metabolic Regulation

- 13 Carbohydrate Structure and Function 632
- 14 Carbohydrate Metabolism 678
- 15 Lipid Structure and Function 728
- 16 Lipid Metabolism 774
- 17 Amino Acid Metabolism 834
- 18 Nucleotide Metabolism 898
- 19 Metabolic Integration 942

PART 5 Genomic Regulation

- 20 DNA Replication, Repair, and Recombination 998
- 21 RNA Synthesis, Processing, and Gene Silencing 1054
- 22 Protein Synthesis, Posttranslational Modification, and Transport 1102
- 23 Gene Regulation 1142

Answers	A-1
Glossary	G-1
Index	I-1

Protein Structure-Function Relationships
Can Reveal Molecular Mechanisms 33

Physical Biochemistry:
Energy Conversion, Water,
and Membranes 38

Energy Conversion in
Biological Systems 40

Sunlight Is the Source of Energy on Earth 41

The Laws of Thermodynamics Apply
to Biological Processes 43

Exergonic and Endergonic Reactions
Are Coupled in Metabolism 50

The Adenylate System Manages Short-
Term Energy Needs 53

2.2 Water Is Critical for
Life Processes 56

Hydrogen Bonding Is Responsible for
the Unique Properties of Water 57

Weak Noncovalent Interactions in
Biomolecules Are Required for Life 60

Effects of Osmolarity on Cellular
Structure and Function 67

The Ionization of Water 71

2.3 Cell Membranes Function as
Selective Hydrophobic Barriers 79

Chemical and Physical Properties of
Cell Membranes 80

Organization of Prokaryotic and
Eukaryotic Cell Membranes 83

Contents

Preface	xvii
Acknowledgments	xxiii
About the Authors	xxv

PART 1 Principles of Biochemistry

1

Principles of Biochemistry 2

1.1 What Is Biochemistry?	5
1.2 The Chemical Basis of Life: A Hierarchical Perspective	7
Elements and Chemical Groups Commonly Found in Nature	8
Four Major Classes of Small Biomolecules Are Present in Living Cells	11
Macromolecules Can Be Polymeric Structures	13
Metabolic Pathways Consist of Linked Biochemical Reactions	15
Structure and Function of a Living Cell	17
Multicellular Organisms Use Signal Transduction for Cell–Cell Communication	20
The Biochemistry of Ecosystems	21
1.3 Storage and Processing of Genetic Information	23
Genetic Information Is Stored in DNA as Nucleotide Base Pairs	24
Information Transfer between DNA, RNA, and Protein	25
1.4 Determinants of Biomolecular Structure and Function	28
Evolutionary Processes Govern Biomolecular Structure and Function	29

2

Protein Structure–Function Relationships Can Reveal Molecular Mechanisms	33
---	----

Physical Biochemistry: Energy Conversion, Water, and Membranes 38

2.1 Energy Conversion in Biological Systems	40
Sunlight Is the Source of Energy on Earth	41
The Laws of Thermodynamics Apply to Biological Processes	43
Exergonic and Endergonic Reactions Are Coupled in Metabolism	50
The Adenylate System Manages Short- Term Energy Needs	53
2.2 Water Is Critical for Life Processes	56
Hydrogen Bonding Is Responsible for the Unique Properties of Water	57
Weak Noncovalent Interactions in Biomolecules Are Required for Life	60
Effects of Osmolarity on Cellular Structure and Function	67
The Ionization of Water	71
2.3 Cell Membranes Function as Selective Hydrophobic Barriers	79
Chemical and Physical Properties of Cell Membranes	80
Organization of Prokaryotic and Eukaryotic Cell Membranes	83

3

Nucleic Acid Structure and Function 90

3.1 Structure of DNA and RNA 92

Double-Helical Structure of DNA 93

DNA Denaturation and Renaturation 99

DNA Supercoiling and Topoisomerase Enzymes 101

Structural Differences between DNA and RNA 107

Nucleic Acid Binding Proteins 112

3.2 Genomics: The Study of Genomes 116

Genome Organization in Prokaryotes and Eukaryotes 116

Genes Are Units of Genetic Information 118

Computational Methods in Genomics 121

3.3 Methods in Nucleic Acid Biochemistry 128

Plasmid-Based Gene Cloning 128

High-Throughput DNA Sequencing 134

Polymerase Chain Reaction 135

Transcriptome Analysis 139

PART 2 Protein Biochemistry

4

Protein Structure 146

4.1 Proteins Are Polymers of Amino Acids 149

Chemical Properties of Amino Acids 150

Peptide Bonds Link Amino Acids Together to Form a Polypeptide Chain 162

Predicting the Amino Acid Sequence of a Protein Using the Genetic Code 166

4.2 Hierarchical Organization of Protein Structure 168

Proteins Contain Three Major Types of Secondary Structure 171

Tertiary Structure Describes the Positions of All Atoms in a Protein 180

Quaternary Structure of Multi-subunit Protein Complexes 186

4.3 Protein Folding 193

Protein-Folding Mechanisms Can Be Studied *In Vitro* 196

Chaperone Proteins Aid in Protein Folding *In Vivo* 198

Protein Misfolding Can Lead to Disease 201

5

Methods in Protein Biochemistry 210

5.1 The Art and Science of Protein Purification 212

Cell Fractionation 213

Column Chromatography 217

Gel Electrophoresis 221

5.2 Working with Oligopeptides: Sequencing and Synthesis 227

Edman Degradation 227

Mass Spectrometry 229

Solid-Phase Peptide Synthesis 230

5.3 Protein Structure Determination 232

X-ray Crystallography 234

NMR Spectroscopy 236

5.4 Protein-Specific Antibodies Are Versatile Biochemical Reagents 237

Generation of Polyclonal and Monoclonal Antibodies 239

Western Blotting 240

Immunofluorescence 242

Enzyme-Linked Immunosorbent Assay 242

Immunoprecipitation 244

6

Protein Function 250

6.1 The Five Major Functional Classes of Proteins 252

Metabolic Enzymes 252

Structural Proteins 253

Transport Proteins 255

Cell Signaling Proteins 256

Genomic Caretaker Proteins 257

6.2 Globular Transport Proteins:

Transporting Oxygen 259

Structure of Myoglobin and Hemoglobin 259

Function and Mechanism of Oxygen

Binding to Heme Proteins 262

Allosteric Control of Oxygen Transport
by Hemoglobin 268

Evolution of the Globin Gene Family 272

6.3 Membrane Transport Proteins:

Controlling Cellular Homeostasis 276

Membrane Transport Mechanisms 277

Structure and Function of Passive

Membrane Transport Proteins 280

Active Membrane Transport Proteins

Require Energy Input 284

6.4 Structural Proteins:

The Actin–Myosin Motor 295

Structure of Muscle Cells 296

The Sliding Filament Model 297

7

Enzyme Mechanisms 308

7.1 Overview of Enzymes 310

Enzymes Are Chemical Catalysts 313

Cofactors and Coenzymes 315

Enzyme Nomenclature 317

7.2 Enzyme Structure and Function 319

Physical and Chemical Properties of

Enzyme Active Sites 319

Enzymes Perform Work in the Cell 327

7.3 Enzyme Reaction Mechanisms 332

Chymotrypsin Uses Both Acid–Base

Catalysis and Covalent Catalysis 333

Enolase Uses Metal Ions in the Catalytic
Mechanism 336

The Mechanism of HMG–CoA Reductase
Involves NADPH Cofactors 338

7.4 Enzyme Kinetics 341

Relationship between $\Delta G^\ddagger$ and the
Rate Constant k 341

Michaelis–Menten Kinetics 342

Enzymes Have Different Kinetic Properties 347

7.5 Regulation of Enzyme Activity 350

Mechanisms of Enzyme Inhibition 351

Allosteric Regulation of Catalytic Activity 356

Covalent Modification of Enzymes 359

Enzymes Can Be Activated by Proteolysis 362

8

Cell Signaling Systems 370

8.1 Components of Signaling Pathways 372

Small Biomolecules Function as Diffusible Signals 375

Receptor Proteins Are the Information
Gatekeepers of the Cell 381

8.2 G Protein–Coupled Receptor Signaling 384

GPCRs Activate Heterotrimeric G Proteins 387

GPCR-Mediated Signaling in Metabolism 389

Termination of GPCR-Mediated Signaling 394

8.3 Receptor Tyrosine Kinase Signaling 397

Epidermal Growth Factor Receptor Signaling 397

Defects in Growth Factor Receptor
Signaling Are Linked to Cancer 401

Insulin Receptor Signaling Controls
Two Major Downstream Pathways 404

8.4 Tumor Necrosis Factor Receptor Signaling 409

TNF Receptors Signal through Cytosolic
Adaptor Complexes 410

TNF Receptor Signaling Regulates
Programmed Cell Death 411

8.5 Nuclear Receptor Signaling 415

Nuclear Receptors Bind as Dimers to Repeat
DNA Sequences in Target Genes 416

Glucocorticoid Receptor Signaling Induces
an Anti-inflammatory Response 418

PART 3 Energy Conversion Pathways

9

Glycolysis: A Paradigm of Metabolic Regulation 428

9.1 Overview of Metabolism 430

The 10 Major Catabolic and Anabolic Pathways in Plants and Animals 431

Metabolite Concentrations Directly Affect Metabolic Flux 433

9.2 Structures of Simple Sugars 438

Monosaccharides 440

Disaccharides 444

9.3 Glycolysis Generates ATP under Anaerobic Conditions 447

The Glycolytic Pathway Consists of 10 Enzymatic Reactions 448

Stage 1 of the Glycolytic Pathway: ATP Investment 451

Stage 2 of the Glycolytic Pathway: ATP Earnings 456

9.4 Regulation of the Glycolytic Pathway 463

Glucokinase Is a Molecular Sensor of High Glucose Levels 464

Allosteric Control of Phosphofructokinase-1 Activity 465

Supply and Demand of Glycolytic Intermediates 467

9.5 Metabolic Fate of Pyruvate 473

10

The Citrate Cycle 480

10.1 The Citrate Cycle Captures Energy Using Redox Reactions 483

Overview of the Citrate Cycle 483

Redox Reactions Involve the Loss and Gain of Electrons 486

Free Energy Changes Can Be Calculated from Reduction Potential Differences 487

10.2 Pyruvate Dehydrogenase Converts Pyruvate to Acetyl-CoA 491

Five Coenzymes Are Required for the Pyruvate Dehydrogenase Reaction 491

The Pyruvate Dehydrogenase Complex Is a Metabolic Machine 497

Pyruvate Dehydrogenase Activity Is Regulated by Allosteric and Phosphorylation 502

10.3 Enzymatic Reactions of the Citrate Cycle 504

The Eight Reactions of the Citrate Cycle 506

10.4 Regulation of the Citrate Cycle 514

10.5 Metabolism of Citrate Cycle Intermediates 517

Citrate Cycle Intermediates Are Shared by Other Pathways 517

Pyruvate Carboxylase Catalyzes the Primary Anaplerotic Reaction 518

11

Oxidative Phosphorylation 524

11.1 The Chemiosmotic Theory 526

Redox Energy Drives Mitochondrial ATP Synthesis 527

Peter Mitchell and the Ox Phos Wars 532

11.2 The Mitochondrial Electron Transport System 535

The Mitochondrial Electron Transport System Is a Series of Coupled Redox Reactions 535

Protein Components of the Electron Transport System 538

Bioenergetics of Proton-Motive Force 548

11.3 Structure and Function of the ATP Synthase Complex 551

Structural Organization of the ATP Synthase Complex 551

Proton Flow through F_0 Alters the Conformation of F_1 Subunits 554

11.4 Transport Systems in Mitochondria 558

Transport of ATP, ADP, and P_i across the Mitochondrial Membrane 559

Cytosolic NADH Transfers Electrons to the Matrix via Shuttle Systems 561

Net Yields of ATP from Glucose Oxidation in Liver and Muscle Cells 562

11.5 Regulation of Oxidative Phosphorylation 565

Inhibitors of the Electron Transport System and ATP Synthesis 565

Uncoupling Proteins Mediate Biochemical Thermogenesis 569

Inherited Mitochondrial Diseases in Humans 570

12

Photosynthesis 578

12.1 Plants Harvest Energy from Sunlight 580

Overview of Photosynthesis and Carbon Fixation 581

Structure and Function of Chloroplasts 585

12.2 Energy Conversion by Photosystems I and II 588

Chlorophyll Molecules Convert Light Energy to Redox Energy 588

The Z Scheme of Photosynthetic Electron Transport 594

Protein Components of the Photosynthetic Electron Transport System 596

12.3 Photophosphorylation Generates ATP 605

Proton-Motive Force Provides Energy for Photophosphorylation 605

Cyclic Photophosphorylation Controls ATP-to-NADPH Ratios 606

12.4 Carbohydrate Biosynthesis in Plants 608

Carbon Fixation by the Calvin Cycle 609

The Activity of Calvin Cycle Enzymes Is Controlled by Light 617

The C₄ and CAM Pathways Reduce Photorespiration in Hot Climates 619

12.5 The Glyoxylate Cycle Converts Lipids into Carbohydrates 625

PART 4 Metabolic Regulation

13

Carbohydrate Structure and Function 632

13.1 Carbohydrates: The Most Abundant Biomolecules in Nature 634

Glycobiology: Study of Glycan Structure and Function 635

Related Oligosaccharides Are Derived from the Same Disaccharide 638

Cellulose and Chitin Are Structural Carbohydrates 640

Starch and Glycogen Are Storage Forms of Glucose 644

13.2 Important Biological Functions of Glycoconjugates 648

Glycoconjugates Function in Cell Signaling and Immunity 649

ABO Human Blood Types Are Determined by Variant Glycosyltransferases 651

Proteoglycans Contain Glycosaminoglycans Attached to Core Proteins 656

β -Lactam Antibiotics Target Peptidoglycan Synthesis 657

13.3 Biochemical Methods in Glycobiology 665

Glycan Determination by Chromatography and Mass Spectrometry 666

Use of High-Throughput Arrays for Glycoconjugate Analysis 669

14

Carbohydrate Metabolism 678

14.1 The Pentose Phosphate Pathway 680

Enzymatic Reactions in the Oxidative Phase 683

Enzymatic Reactions in the Nonoxidative Phase 684

Glucose-6-Phosphate Dehydrogenase Deficiency in Humans 687

14.2 Gluconeogenesis 690

Gluconeogenesis Uses Noncarbohydrate Sources to Synthesize Glucose 691

Gluconeogenic Enzymes Bypass Three Exergonic Reactions in Glycolysis 693

Reciprocal Regulation of Gluconeogenesis and Glycolysis by Allosteric Effectors 698

The Cori Cycle Provides Glucose to Muscle Cells during Exercise 701

14.3 Glycogen Degradation and Synthesis 702

Enzymatic Reactions in Glycogen Degradation 705

Enzymatic Reactions in Glycogen Synthesis 711

Hormonal Regulation of Glycogen Metabolism 715

Human Glycogen Storage Diseases 719

15**Lipid Structure and Function 728**

15.1 Many Lipids Are Made from Fatty Acids 730

Structures of the Most Common Fatty Acids 731

Biological Waxes Have a Variety of Functions 737

Structure and Nonmetabolic Uses of Triacylglycerols 738

15.2 Triacylglycerols Are Energy Storage Lipids 742

Dietary Triacylglycerols Are Transported by Chylomicrons 743

Triacylglycerols Synthesized in the Liver Are Packaged in VLDL Particles 746

Adipocytes Cleave Stored Triacylglycerols and Release Free Fatty Acids 746

15.3 Cell Membranes Contain Three Major Types of Lipids 749

Cell Membranes Have Distinct Lipid and Protein Compositions 751

Glycerophospholipids Are the Most Abundant Membrane Lipids 753

Sphingolipids Contain One Fatty Acid Linked to Sphingosine 754

Cholesterol Is a Rigid, Four-Ring Molecule in Plasma Membranes 756

15.4 Lipids Function in Cell Signaling 758

Cholesterol Derivatives Regulate the Activity of Nuclear Receptor Proteins 758

Eicosanoids Are Derived from Arachidonate 763

16**Lipid Metabolism 774**

16.1 Fatty Acid Oxidation and Ketogenesis 776

The Fatty Acid β -Oxidation Pathway in Mitochondria 777

Auxiliary Pathways for Fatty Acid Oxidation 784

Ketogenesis Is a Salvage Pathway for Acetyl-CoA 788

16.2 Synthesis of Fatty Acids and Triacylglycerols 791

Fatty Acid Synthase Is a Multifunctional Enzyme 793

Elongation and Desaturation of Palmitate 800

Synthesis of Triacylglycerol and Membrane Lipids 801

The Citrate Shuttle Exports Acetyl-CoA from Matrix to Cytosol 804

Metabolic and Hormonal Control of Fatty Acid Synthesis 805

16.3 Cholesterol Synthesis and Metabolism 810

Cholesterol Is Synthesized from Acetyl-CoA 810

Cholesterol Metabolism and Cardiovascular Disease 816

Sterol Regulatory Element Binding Proteins 824

17**Amino Acid Metabolism 834**

17.1 Nitrogen Fixation and Assimilation 837

Nitrogen Fixation Reduces N_2 to form NH_3 838

Assimilation of Ammonia into Glutamate and Glutamine 843

Metabolite Regulation of Glutamine Synthetase Activity 844

Aminotransferase Enzymes Play a Key Role
in Amino Acid Metabolism 846

17.2 Amino Acid Degradation 850

Dietary and Cellular Proteins Are
Degraded into Amino Acids 851

The Urea Cycle Removes Toxic
Ammonia from the Body 857

Degradation of Glucogenic and
Ketogenic Amino Acids 866

17.3 Amino Acid Biosynthesis 873

Amino Acids Are Derived from Common
Metabolic Intermediates 873

Nine Amino Acids Are Synthesized
from Pyruvate and Oxaloacetate 876

Chorismate Is the Precursor to Tryptophan,
Tyrosine, and Phenylalanine 878

17.4 Biosynthesis of Amino Acid Derivatives 881

Heme Nitrogen Is Derived from Glycine 882

Tyrosine Is the Precursor to a Variety of Biomolecules 884

Nitric Oxide Synthase Generates Nitric
Oxide from Arginine 888

18

Nucleotide Metabolism 898

18.1 Structure and Function of Nucleotides 900

Cellular Roles of Nucleotides 900

Nucleotide Salvage Pathways 903

18.2 Purine Metabolism 904

The Purine Biosynthetic Pathway Generates IMP 905

Feedback Inhibition of Purine Biosynthesis 912

Uric Acid Is the Product of Purine Degradation 912

Metabolic Diseases of Purine Metabolism 914

18.3 Pyrimidine Metabolism 918

The Pyrimidine Biosynthetic Pathway Generates UMP 918

Allosteric Regulation of Pyrimidine Biosynthesis 920

Pyrimidines Are Degraded by a Common Pathway 921

18.4 Deoxyribonucleotide Metabolism 924

Generation of Deoxyribonucleotides by
Ribonucleotide Reductase 924

Metabolism of Thymine Deoxyribonucleotides 930

Inhibitors of Thymidylate Synthase Are
Effective Anticancer Drugs 931

19

Metabolic Integration 942

19.1 Metabolic Integration at the Physiologic Level 944

Specialized Metabolic Functions of
Major Tissues and Organs 945

Metabolite Flux between Tissues Optimizes
Use of Stored Energy 952

Control of Metabolic Homeostasis
by Signal Transduction 955

Mobilization of Metabolic Fuel during Starvation 964

19.2 Metabolic Energy Balance 967

The Role of Genes and Environment
in Energy Balance 968

Control of Energy Balance by Hormone
Signaling in the Brain 971

The Metabolic Link between Obesity and Diabetes 975

19.3 Nutrition and Exercise 982

Biochemistry of Macronutrition and Dieting 982

Metabolic Effects of Physical Exercise 987

AMPK and PPAR γ Coactivator-1 α
Signaling in Skeletal Muscle 988

PART 5 Genomic Regulation

20

DNA Replication, Repair, and Recombination 998

20.1 DNA Replication 1000

Overview of Genome Duplication 1000

Structure and Function of DNA Polymerases 1003

Structure and Function of Replication Fork Proteins 1009

Initiation and Termination of DNA Replication 1016

20.2 DNA Damage and Repair 1027

Unrepaired DNA Damage Leads to Genetic Mutations 1027

Biological and Chemical Causes of DNA Damage 1030

DNA Repair Mechanisms 1033

20.3 DNA Recombination 1041

Homologous Recombination during Meiosis 1041

Integration and Transposition of Viral Genomes 1043

Rearrangement of Immunoglobulin Genes 1048

21

RNA Synthesis, Processing, and Gene Silencing 1054

21.1 Structure and Function of RNA 1056

RNA Is a Biochemical Polymer with Functional Diversity 1057

Protein-Synthesizing RNA Molecules: mRNA, tRNA, rRNA 1058

Noncoding RNA Serves Important Functions in Eukaryotes 1066

21.2 Biochemistry of RNA Synthesis 1065

RNA Polymerase Is Recruited to Gene Promoter Sequences 1066

Proteins Required for RNA Synthesis in Prokaryotes 1069

Proteins Required for RNA Synthesis in Eukaryotes 1072

21.3 Eukaryotic RNA Processing 1074

Ribozymes Mediate RNA Cleavage and Splicing Reactions 1074

Structure and Function of Spliceosomes 1077

Processing of Eukaryotic tRNA and rRNA Transcripts 1081

RNA Polymerase II Coordinates Processing of Precursor mRNA 1084

Messenger RNA Decay Is Mediated by 3' Deadenylation and 5' Decapping 1086

A Single Gene Can Give Rise to Many Different mRNA Transcripts 1088

21.4 RNA-Mediated Gene Silencing 1091

The Discovery of RNA Interference 1091

Biogenesis and Function of miRNA 1094

Applications of RNA-Mediated Gene Silencing 1096

22

Protein Synthesis, Posttranslational Modification, and Transport 1102

22.1 Deciphering the Genetic Code 1104

The Molecular Adaptor Required for Protein Synthesis Is tRNA 1104

Solving the Genetic Code Using Experimental Biochemistry 1105

The tRNA Wobble Position Explains Redundancy in the Genetic Code 1108

22.2 Biochemistry of mRNA Translation 1111

Transfer RNA Synthetases Provide a Second Genetic Code 1111

Ribosomes Are Protein Synthesis Machines 1114

Polypeptide Synthesis: Initiation, Elongation, Termination 1116

Some Antibiotics Target Bacterial Protein Synthesis 1122

22.3 Posttranslational Modification of Proteins 1126

Covalent Attachment of Functional Groups to Proteins 1126

Ran-Mediated Nuclear Import and Export of Eukaryotic Proteins 1127

Co-translational Modification of Proteins in the Endoplasmic Reticulum 1129

Vesicle Transport Systems in Eukaryotic Cells 1136

23

Gene Regulation 1142**23.1 Principles of Gene Regulation 1145****Specificity of Gene Regulation 1146****Basic Mechanisms of Gene Regulation 1153****Biochemical Applications That Exploit
Gene Regulatory Processes 1158****23.2 Mechanisms of Prokaryotic
Gene Regulation 1161****Regulation of the *E. coli lac* Operon 1161****Regulation of the *E. coli* SOS Regulon 1166****Regulation of an Epigenetic Switch
in Bacteriophage λ 1169****Regulatory Mechanisms Governing the *trp* Operon 1170****23.3 Mechanisms of Eukaryotic****Gene Regulation 1174****Eukaryotic Gene Regulation Is Most Often
Transcriptional Activation 1174****Regulation of Galactose Metabolism in Yeast 1183****Gene Expression Patterns in Developing
Drosophila Embryos 1185****Reprogramming Gene Expression: Induced
Pluripotent Stem Cells 1186****Answers A-1****Glossary G-1****Index I-1**