## Contents

List of Figures ..... xix
List of Tables ..... xxix
List of Boxes ..... xxxi
Preface ..... xxxiii
Changes in Second Edition ..... xxxix
Acknowledgments ..... xli
Authors ..... xliii
Physical Quantities and Constants ..... xlv
List of Acronyms ..... xlvii
Chapter 1•Introduction ..... 1
1.1 IMPORTANCE OF PROTEINS IN LIVING ORGANISMS ..... 1
1.1.1 Life, proteins and mysterious forces ..... 1
1.1.2 Molecular organization of living organisms ..... 2
1.1.3 Proteins have numerous biological roles ..... 6
1.1.3.1 Catalysis of metabolic processes ..... 6
1.1.3.2 Energy transfer ..... 8
1.1.3.3 Gene expression ..... 11
1.1.3.4 Transport of solutes across biological membranes ..... 13
1.1.3.5 Cellular communication ..... 13
1.1.3.6 Molecular recognition ..... 16
1.1.3.7 Defense ..... 17
1.1.3.8 Forming intracellular and extracellular structures ..... 19
1.1.3.9 Cell- and tissue-specific functions ..... 20
1.1.4 Physiological and evolutionary importance of proteins ..... 22
1.1.5 Medical, industrial, and social importance of proteins ..... 22
1.1.5.1 Proteins as drug targets ..... 22
1.1.5.2 Proteins as toxin targets ..... 23
1.1.5.3 Industrial applications of proteins ..... 24
1.2 STRUCTURAL COMPLEXITY AND ITS EFFECT ON PROTEIN FUNCTION ..... 25
1.3 NONCOVALENT INTERACTIONS BETWEEN ATOMS IN BIOMOLECULES ..... 29
1.3.1 Electrostatic interactions ..... 31
1.3.1.1 Introduction ..... 31
1.3.1.2 Basic principles ..... 32
1.3.1.3 Hydrogen bonds ..... 44
1.3.1.4 Other types of electrostatic interactions ..... 45
1.3.2 Van der Waals interactions ..... 50
1.3.3 Nonpolar interactions and hydrophobic effect ..... 53
1.3.4 Conclusions ..... 55
1.4 SUMMARY ..... 56
1.5 ORGANIZATION OF BOOK ..... 56
EXERCISES ..... 57
REFERENCES ..... 57
Chapter 2•Protein Structure ..... 65
2.1 INTRODUCTION ..... 65
2.1.1 Hierarchy in protein structure ..... 65
2.1.2 Coenzymes and prosthetic groups ..... 66
2.2 PRIMARY STRUCTURE ..... 71
2.2.1 Amino acids and their properties ..... 72
2.2.1.1 Amino acid structure ..... 72
2.2.1.2 Configurations of amino acids ..... 77
2.2.1.3 Side chain properties ..... 79
2.2.1.4 Amino acid derivates in proteins ..... 104
2.2.2 Peptide bond ..... 110
2.3 SECONDARY STRUCTURE ..... 113
2.3.1 $\quad \alpha$-helix ..... 122
2.3.1.1 Geometry ..... 122
2.3.1.2 Intramolecular interactions ..... 122
2.3.1.3 Amphipathic $\alpha$-helices ..... 123
2.3.2 Non- $\alpha$-helices ..... 124
2.3.2.1 $\quad 3_{10}$-helix ..... 124
2.3.2.2 $\quad \pi$-helix ..... 125
2.3.2.3 Type II polyproline helix (PPII) ..... 126
2.3.3 $\quad \beta$ conformation ..... 129
2.3.4 Why helices and sheets? ..... 130
2.3.5 Reverse turns ..... 133
2.3.5.1 $\beta$-turn ..... 133
2.3.5.2 Loops ..... 134
2.3.6 Secondary structure preferences of amino acids ..... 135
2.3.6.1 $\alpha$-helix ..... 135
2.3.6.2 $\quad \beta$ conformation ..... 137
2.4 TERTIARY STRUCTURE ..... 139
2.4.1 Basic properties of tertiary structure ..... 141
2.4.1.1 Structural properties required for complex function ..... 141
2.4.1.2 Core versus surface ..... 141
2.4.1.3 Stabilizing forces ..... 143
2.4.2 Architecture of proteins ..... 143
2.4.2.1 Simple folding motifs ..... 143
2.4.2.2 Complex folds ..... 150
2.4.2.3 Domains ..... 161
2.4.2.4 Protein classification ..... 167
2.4.2.5 Knotted proteins ..... 173
2.4.3 Evolutionary conservation of structure and function in proteins ..... 174
2.4.3.1 Interests of individual versus those of species ..... 174
2.4.3.2 Structure conservation: evolutionary mechanisms ..... 176
2.4.3.3 Evolution of function ..... 179
2.4.4 Water molecules inside proteins ..... 180
2.5 QUATERNARY STRUCTURE ..... 182
2.5.1 Introduction ..... 182
2.5.2 Characteristics ..... 183
2.5.2.1 Dimensions and complexity ..... 183
2.5.2.2 Symmetry ..... 183
2.5.2.3 Subunit interactions ..... 185
2.5.3 Advantages of quaternary structure ..... 186
2.6 POST-TRANSLATIONAL MODIFICATIONS ..... 188
2.6.1 Introduction ..... 188
2.6.2 Phosphorylation ..... 191
2.6.3 Glycosylation ..... 193
2.6.4 Acylation ..... 195
2.6.4.1 $\quad \varepsilon$ - $N$-acetylation ..... 195
2.6.4.2 $\quad N^{\prime}$-myristoylation and $S$-palmitoylation ..... 196
2.6.4.3 Ubiquitination and SUMOylation ..... 197
2.6.5 Alkylation ..... 198
2.6.5.1 Methylation ..... 198
2.6.5.2 $S$-prenylation ..... 199
2.6.5.3 Adenylation ..... 199
2.6.6 Hydroxylation and oxidation ..... 199
2.6.7 Proteolysis ..... 200
2.6.8 Amidation ..... 200
2.6.9 Addition of metal ions ..... 200
2.6.9.1 Stabilization of protein structure ..... 201
2.6.9.2 Ligand binding ..... 201
2.6.9.3 Electron transport ..... 201
2.6.9.4 Enzymatic catalysis ..... 202
2.6.10 Mixed modifications ..... 204
2.6.11 Pathological aspects of post-translational modifications ..... 205
2.6.11.1 Cancer ..... 205
2.6.11.2 Age-related illnesses ..... 207
2.6.12 Identifying post-translational modifications ..... 208
2.7 FIBROUS PROTEINS ..... 209
2.7.1 Fiber-based structures inside and outside cells ..... 209
2.7.1.1 Mechanical support ..... 209
2.7.1.2 Tissue organization and cell-environment communication ..... 214
2.7.1.3 Motion ..... 216
2.7.1.4 External structures ..... 218
2.7.1.5 Other roles ..... 219
2.7.2 Fiber-forming versus fibrous proteins ..... 221
2.7.3 Structural differences between globular and fibrous proteins ..... 221
2.7.4 Structure-function relationships in helical proteins $\alpha$-keratin and collagen ..... 223
2.7.4.1 $\alpha$-Keratin ..... 223
2.7.4.2 Collagen ..... 224
2.8 SUMMARY ..... 232
EXERCISES ..... 233
REFERENCES ..... 235
Chapter $3 \cdot$ Methods of Structure Determination and Prediction ..... 259
3.1 INTRODUCTION ..... 259
3.2 DIFFRACTION AND SCATTERING METHODS ..... 260
3.2.1 $\quad$ X-ray diffraction and scattering ..... 261
3.2.1.1 Principles ..... 261
3.2.1.2 Steps of procedure ..... 262
3.2.1.3 Information obtained from crystallography ..... 263
3.2.1.4 Problems of method ..... 266
3.2.1.5 $\quad$ X-ray scattering ..... 267
3.2.2 Neutron scattering ..... 270
3.2.2.1 Principles ..... 270
3.2.2.2 Advantages and shortcomings ..... 272
3.2.3 Electron microscopy (EM) ..... 273
3.2.3.1 Principles ..... 273
3.2.3.2 Advantages and shortcomings ..... 277
3.3 SPECTROSCOPIC METHODS ..... 278
3.3.1 Nuclear magnetic resonance (NMR) spectroscopy ..... 278
3.3.1.1 Principles ..... 278
3.3.1.2 Steps in protein structure determination by NMR spectroscopy ..... 280
3.3.1.3 Advantages and shortcomings ..... 282
3.3.2 Electron paramagnetic resonance (EPR) spectroscopy ..... 283
3.3.3 Information derived from other methods ..... 284
3.3.3.1 Fluorescent spectroscopy ..... 284
3.3.3.2 Circular dichroism spectroscopy ..... 285
3.3.3.3 Mass spectrometry ..... 286
3.4 COMPUTATIONAL METHODS FOR STRUCTURE PREDICTION ..... 291
3.4.1 Introduction ..... 291
3.4.2 Ab initio (physical) approach ..... 292
3.4.2.1 Calculating total potential energy of system ..... 292
3.4.2.2 Sampling configurational space of system ..... 294
3.4.2.3 Limitations and partial solutions ..... 297
3.4.3 Template-based (comparative) approach ..... 307
3.4.3.1 Introduction ..... 307
3.4.3.2 Homology modeling ..... 308
3.4.3.3 Fold recognition via threading ..... 315
3.4.4 Integrative and fragment-based methods ..... 317
3.4.5 Prediction assessment and verification ..... 324
3.5 EXPERIMENTALLY GUIDED COMPUTATIONAL PREDICTION ..... 325
3.5.1 Introduction ..... 325
3.5.2 Applications and tools ..... 326
3.6 CONCLUSIONS ..... 329
3.7 PROTEIN DATA BANK (PDB) ..... 329
3.8 SUMMARY ..... 333
EXERCISES ..... 334
REFERENCES ..... 335
Chapter 4 Energetics and Protein Stability ..... 355
4.1 BASIC PRINCIPLES OF THERMODYNAMICS ..... 355
4.1.1 Introduction ..... 355
4.1.2 Free energy and spontaneous processes ..... 356
4.1.3 Enthalpy, entropy, and molecular thermodynamics ..... 358
4.1.3.1 Enthalpy ..... 358
4.1.3.2 Entropy ..... 363
4.1.3.3 Computational approaches focus on individual interactions ..... 364
4.1.4 Thermodynamics and protein structure ..... 365
4.2 PROTEIN STABILITY AND FORCES INVOLVED ..... 365
4.2.1 How stable are proteins? ..... 365
4.2.2 Dominant driving forces ..... 366
4.2.2.1 $\quad$ Nonpolar interactions ( $\Delta G_{n p}$ ) ..... 367
4.2.2.2 Configurational entropy effect $\left(-T \Delta S_{\text {con }}\right)$ ..... 369
4.2.3 Electrostatic interactions $\left(\Delta G_{e l e c}\right)$ ..... 371
4.2.4 van der Waals interactions $\left(\Delta G_{v d W}\right)$ ..... 375
4.2.5 Summary and conclusions ..... 375
4.3 PROTEIN DENATURATION AND ADAPTATION TO EXTREME CONDITIONS ..... 377
4.3.1 Denaturation as experimental tool ..... 377
4.3.1.1 Temperature-dependent denaturation ..... 378
4.3.1.2 $\quad \mathrm{pH}$-dependent denaturation ..... 379
4.3.1.3 Pressure-induced denaturation ..... 379
4.3.1.4 Chemical denaturation ..... 379
4.3.2 Adaptation of proteins to extreme environments ..... 380
4.3.3 Conclusions ..... 382
4.4 STABILITY ENHANCEMENT OF INDUSTRIAL ENZYMES USING PROTEIN ENGINEERING ..... 383
4.4.1 Enzymes in industry ..... 383
4.4.2 Enzyme engineering ..... 384
4.4.3 Rational engineering of enzymes for increased stability ..... 3844.5 SUMMARY387
EXERCISES ..... 388
REFERENCES ..... 388
Chapter 5•Protein Dynamics ..... 397
5.1 INTRODUCTION ..... 397
5.2 PROTEIN FOLDING ..... 400
5.2.1 Kinetic aspects ..... 400
5.2.1.1 Levinthal's paradox and energy landscape theory ..... 400
5.2.1.2 Folding models and mechanisms ..... 403
5.2.2 In vivo folding ..... 405
5.2.2.1 In vivo factors that complicate folding ..... 405
5.2.2.2 Assisted folding ..... 416
5.3 FOLDED STATE DYNAMICS ..... 425
5.3.1 Spontaneous dynamics ..... 426
5.3.1.1 Proteins are conformational ensembles ..... 426
5.3.1.2 Statistical-thermodynamic view of protein dynamics ..... 426
5.3.1.3 Dynamics of disordered proteins ..... 428
5.3.1.4 Biological significance of thermally induced conformational changes ..... 428
5.3.1.5 Effects of solvents on protein dynamics ..... 433
5.3.2 External effects on protein dynamics ..... 434
5.3.2.1 Ligand-induced dynamics and allostery ..... 434
5.3.2.2 Dynamics induced by environmental changes ..... 456
5.3.2.3 Enzyme-mediated protein dynamics ..... 456
5.4 METHODS FOR STUDYING PROTEIN DYNAMICS ..... 457
5.4.1 Tools for studying slow (ms-sec) to intermediate ( $\mathrm{ns}-\mu \mathrm{s}$ ) motions ..... 458
5.4.1.1 Tools for studying rapid motions (fs-ps) ..... 460
5.5 SUMMARY ..... 461
EXERCISES ..... 462
REFERENCES ..... 463
Chapter 6-Intrinsically Unstructured Proteins ..... 477
6.1 INTRODUCTION ..... 477
6.1.1 Molecular recognition ..... 479
6.1.2 Entropic chain activity ..... 482
6.2 SEQUENCE AND STRUCTURAL ORGANIZATION OF IUPs AND IDRs487
6.3 STRUCTURE-FUNCTION RELATIONSHIP ..... 489
6.3.1 IUP binding to target proteins ..... 489
6.3.1.1 IUPs are designed for fast protein binding and release ..... 489
6.3.1.2 Mechanism and kinetics of binding-folding coupling in IUPs ..... 492
6.3.1.3 Significance of PPII helix in IUPs ..... 493
6.3.1.4 Disorder can be used for regulation ..... 494
6.3.2 Entropy assistance-related roles ..... 494
6.4 IUPs IN VIVO ..... 495
6.5 SUMMARY ..... 495
EXERCISES ..... 496
REFERENCES ..... 496
Chapter $7 \cdot$ Membrane-Bound Proteins ..... 503
7.1 INTRODUCTION ..... 503
7.2 STRUCTURE AND ORGANIZATION OF BIOLOGICAL MEMBRANES ..... 506
7.2.1 General structure and properties ..... 506
7.2.2 Composition of lipid bilayer ..... 508
7.2.2.1 Glycerophospholipids ..... 508
7.2.2.2 Sphingolipids ..... 508
7.2.2.3 Sterols ..... 511
7.2.2.4 Ethers ..... 511
7.2.2.5 Variability ..... 511
7.2.3 Lipid property effects on membranes ..... 514
7.2.3.1 Amphipathicity ..... 514
7.2.3.2 Asymmetry ..... 514
7.2.3.3 Degree of order and thickness ..... 515
7.2.3.4 Curvature ..... 516
7.3 PRINCIPLES OF MEMBRANE PROTEIN STRUCTURE ..... 518
7.3.1 Overview ..... 518
7.3.2 Structures of integral membrane proteins ..... 519
7.3.2.1 Primary structure ..... 521
7.3.2.2 Secondary structure ..... 530
7.3.2.3 Tertiary structure ..... 532
7.3.3 Peripheral membrane proteins ..... 552
7.4 PROTEIN-MEMBRANE INTERACTION ..... 553
7.4.1 Lipid bilayer effects on membrane proteins ..... 553
7.4.1.1 Effects of general bilayer properties ..... 553
7.4.1.2 Effects of specific bilayer lipids ..... 558
7.4.2 Effects of membrane proteins on lipid bilayer properties ..... 565
7.4.2.1 Decrease in mobility ..... 565
7.4.2.2 Deformation and curvature changes ..... 565
7.5 G PROTEIN-COUPLED RECEPTORS ..... 568
7.5.1 Introduction ..... 568
7.5.2 GPCR signaling ..... 569
7.5.2.1 General view ..... 569
7.5.2.2 G-protein mechanisms and regulation ..... 572
7.5.3 GPCR structure ..... 575
7.5.3.1 General features ..... 575
7.5.3.2 Structural variations among GPCRs ..... 578
7.5.4 GPCR and G-protein activation ..... 588
7.5.4.1 Structural changes in GPCRs upon activation ..... 589
7.5.4.2 Agonist effect and G-protein activation ..... 593
7.5.5 GPCR desensitization ..... 600
7.5.6 GPCRs of other classes ..... 601
7.5.6.1 Class B GPCRs ..... 601
7.5.6.2 Class C GPCRs ..... 607
7.5.6.3 Class F GPCRs ..... 608
7.5.7 GPCR-targeting drugs ..... 609
7.6 SUMMARY ..... 613
EXERCISES ..... 614
REFERENCES ..... 616
Chapter 8 • Protein-Ligand Interactions ..... 637
8.1 INTRODUCTION ..... 637
8.2 THEORIES ON PROTEIN-LIGAND BINDING AND DYNAMICS ..... 638
8.3 PROTEIN-LIGAND BINDING ENERGETICS ..... 641
8.3.1 Total binding free energy ..... 641
8.3.1.1 Protein-ligand binding displays diverse affinities ..... 641
8.3.1.2 Calculating absolute binding free energy ..... 643
8.3.1.3 Calculating relative binding energies ..... 647
8.3.2 Thermodynamic determinants of binding energy ..... 648
8.4 LIGAND-BINDING SITES ..... 650
8.4.1 Overview ..... 650
8.4.2 Geometric complementarity ..... 650
8.4.3 Electrostatic complementarity ..... 652
8.4.4 Binding specificity and promiscuity ..... 654
8.5 PROTEIN-PROTEIN INTERACTIONS ..... 665
8.5.1 Overview ..... 665
8.5.2 Protein-protein binding domains ..... 666
8.5.3 Structure-function relationships ..... 667
8.5.3.1 Protein-protein interface ..... 667
8.5.3.2 PPII helices in protein-protein interactions ..... 676
8.5.4 Effect of molecular crowding on protein-protein interactions ..... 677
8.6 PROTEIN-LIGAND INTERACTIONS IN DRUG ACTION AND DESIGN ..... 679
8.6.1 Involvement of proteins in disease ..... 679
8.6.2 How pharmaceutical drugs work ..... 680
8.6.2.1 Principal modes of action ..... 680
8.6.2.2 Selectivity and side effects ..... 684
8.6.3 Drug development and design ..... 685
8.6.3.1 General sources of pharmaceutical drugs ..... 685
8.6.3.2 Drug development process ..... 686
8.6.3.3 Principal steps in rational drug design ..... 687
8.6.3.4 Rational drug design case study: ACE inhibitors ..... 700
8.7 SUMMARY ..... 713
EXERCISES ..... 714
REFERENCES ..... 715
Chapter 9 • Enzymatic Catalysis ..... 729
9.1 INTRODUCTION ..... 729
9.1.1 Metabolic needs of cells ..... 729
9.1.2 Cellular processes must be catalyzed in order to sustain life ..... 729
9.1.3 Why were enzymes selected as biocatalysts? ..... 737
9.1.4 Why is it important to understand enzyme action? ..... 739
9.1.5 Enzyme classification ..... 739
9.1.5.1 Oxidoreductases (EC 1) ..... 744
9.1.5.2 Transferases (EC 2) ..... 753
9.1.5.3 Hydrolases (EC 3) ..... 766
9.1.5.4 Lyases (EC 4) ..... 774
9.1.5.5 Isomerases (EC 5) ..... 778
9.1.5.6 Ligases (EC 6) ..... 779
9.1.5.7 Catalytic promiscuity ..... 781
9.2 ENZYME KINETICS ..... 783
9.2.1 Basic concepts ..... 784
9.2.2 Michaelis-Menten model ..... 786
9.2.3 Use of Michaelis-Menten kinetic parameters for enzyme analysis ..... 791
9.2.3.1 Enzyme-substrate affinity ..... 791
9.2.3.2 Enzyme efficiency and specificity ..... 792
9.2.3.3 Enzyme proficiency ..... 794
9.2.4 Limitations of M-M formalism ..... 794
9.3 HOW DO ENZYMES CATALYZE REACTIONS? ..... 795
9.3.1 Overview ..... 795
9.3.2 Binding specificity and selectivity ..... 796
9.3.3 Catalysis ..... 799
9.3.3.1 Substrate confinement ..... 802
9.3.3.2 Electrostatic preorganization and noncovalent stabilization of transition state ..... 803
9.3.3.3 Covalent catalysis and electronic polarization of substrate bonds ..... 807
9.3.3.4 Metal ion catalysis ..... 814
9.3.3.5 General acid-base catalysis ..... 817
9.3.3.6 Mechanisms related to protein dynamics ..... 821
9.4 ENZYME COFACTORS ..... 824
9.4.1 Overview ..... 824
9.4.2 Chemical characteristics of organic cofactors ..... 830
9.4.3 Functional characteristics ..... 833
9.5 ENZYME INHIBITION ..... 833
9.5.1 Overview ..... 833
9.5.2 Modes of enzyme inhibition ..... 834
9.5.2.1 Reversible inhibition ..... 835
9.5.2.2 Irreversible inhibition ..... 845
9.6 INDUSTRIAL USES OF ENZYMES ..... 848
9.6.1 Medical uses of enzymes ..... 848
9.6.1.1 Drugs and drug targets ..... 848
9.6.1.2 Diagnostic roles ..... 849
9.6.2 Use of enzymes as industrial catalysts ..... 850
9.6.3 Limitations and solutions ..... 853
9.7 SUMMARY ..... 855
EXERCISES ..... 856
REFERENCES ..... 868
APPENDIX: ENZYME NOMENCLATURE RECOMMENDATIONS OF THE NC-IUBMB ..... 881
Index ..... 891

