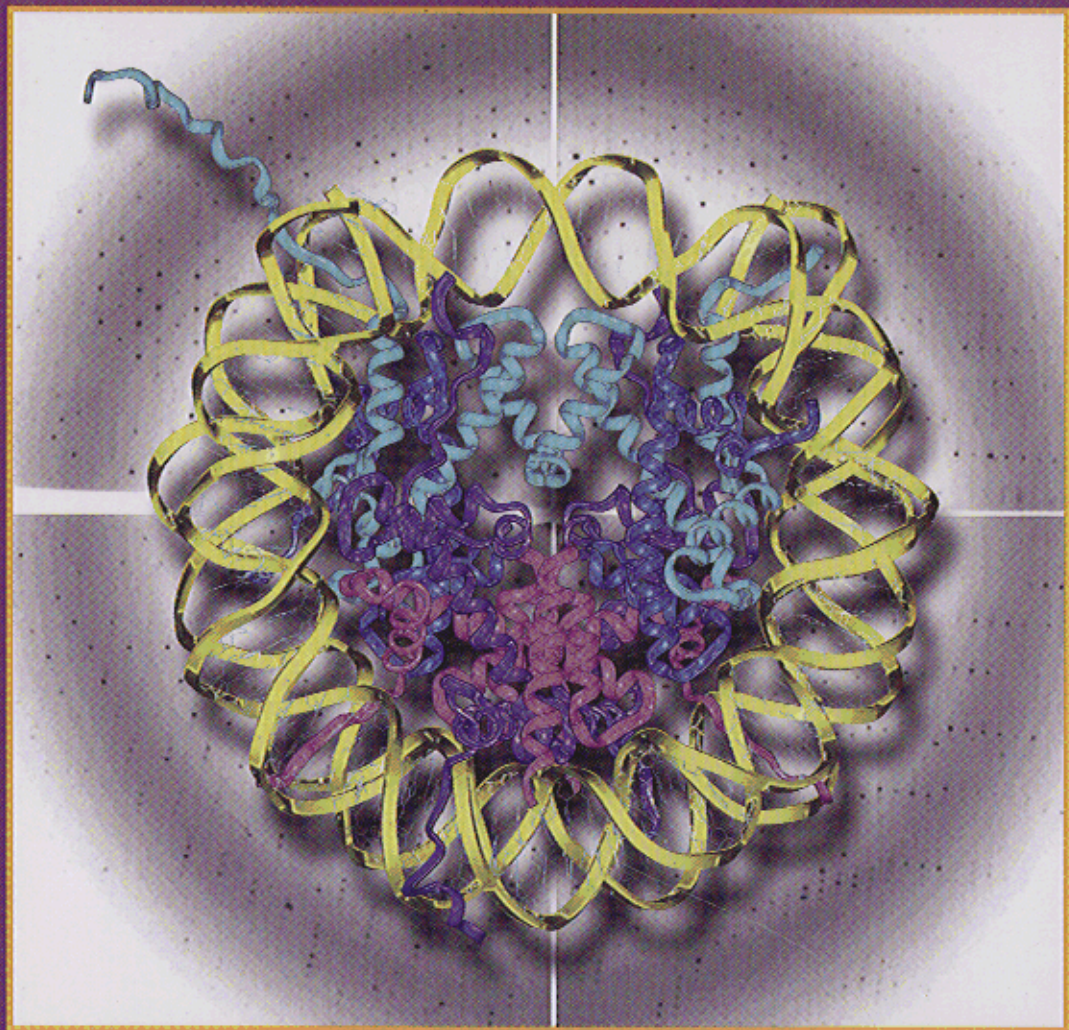


PRINCIPLES OF
Physical Biochemistry

SECOND EDITION



Kensal E. van Holde • W. Curtis Johnson • P. Shing Ho

Contents

Preface	xiii
Chapter 1 Biological Macromolecules	1
1.1 General Principles	1
1.1.1 Macromolecules	2
1.1.2 Configuration and Conformation	5
1.2 Molecular Interactions in Macromolecular Structures	8
1.2.1 Weak Interactions	8
1.3 The Environment in the Cell	10
1.3.1 Water Structure	11
1.3.2 The Interaction of Molecules with Water	15
1.3.3 Nonaqueous Environment of Biological Molecules	16
1.4 Symmetry Relationships of Molecules	19
1.4.1 Mirror Symmetry	21
1.4.2 Rotational Symmetry	22
1.4.3 Multiple Symmetry Relationships and Point Groups	25
1.4.4 Screw Symmetry	26
1.5 The Structure of Proteins	27
1.5.1 Amino Acids	27
1.5.2 The Unique Protein Sequence	31
Application 1.1: Musical Sequences	33
1.5.3 Secondary Structures of Proteins	34
Application 1.2: Engineering a New Fold	35
1.5.4 Helical Symmetry	36
1.5.5 Effect of the Peptide Bond on Protein Conformations	40
1.5.6 The Structure of Globular Proteins	42
1.6 The Structure of Nucleic Acids	52
1.6.1 Torsion Angles in the Polynucleotide Chain	54
1.6.2 The Helical Structures of Polynucleic Acids	55
1.6.3 Higher-Order Structures in Polynucleotides	61
Application 1.3: Embracing RNA Differences	64
Exercises	68
References	70

Chapter 2	Thermodynamics and Biochemistry	72
2.1	Heat, Work, and Energy—First Law of Thermodynamics	73
2.2	Molecular Interpretation of Thermodynamic Quantities	76
2.3	Entropy, Free Energy, and Equilibrium—Second Law of Thermodynamics	80
2.4	The Standard State	91
2.5	Experimental Thermochemistry	93
2.5.1	The van't Hoff Relationship	93
2.5.2	Calorimetry	94
	Application 2.1: Competition Is a Good Thing	102
	Exercises	104
	References	105
Chapter 3	Molecular Thermodynamics	107
3.1	Complexities in Modeling Macromolecular Structure	107
3.1.1	Simplifying Assumptions	108
3.2	Molecular Mechanics	109
3.2.1	Basic Principles	109
3.2.2	Molecular Potentials	111
3.2.3	Bonding Potentials	112
3.2.4	Nonbonding Potentials	115
3.2.5	Electrostatic Interactions	115
3.2.6	Dipole-Dipole Interactions	117
3.2.7	van der Waals Interactions	118
3.2.8	Hydrogen Bonds	120
3.3	Stabilizing Interactions in Macromolecules	124
3.3.1	Protein Structure	125
3.3.2	Dipole Interactions	129
3.3.3	Side Chain Interactions	131
3.3.4	Electrostatic Interactions	131
3.3.5	Nucleic Acid Structure	133
3.3.6	Base-Pairing	137
3.3.7	Base-Stacking	139
3.3.8	Electrostatic Interactions	141
3.4	Simulating Macromolecular Structure	145
3.4.1	Energy Minimization	146
3.4.2	Molecular Dynamics	147
3.4.3	Entropy	149
3.4.4	Hydration and the Hydrophobic Effect	153
3.4.5	Free Energy Methods	159
	Exercises	161
	References	163

Chapter 4	Statistical Thermodynamics	166
4.1	General Principles	166
4.1.1	Statistical Weights and the Partition Function	167
4.1.2	Models for Structural Transitions in Biopolymers	169
4.2	Structural Transitions in Polypeptides and Proteins	175
4.2.1	Coil-Helix Transitions	175
4.2.2	Statistical Methods for Predicting Protein Secondary Structures	181
4.3	Structural Transitions in Polynucleic Acids and DNA	184
4.3.1	Melting and Annealing of Polynucleotide Duplexes	184
4.3.2	Helical Transitions in Double-Stranded DNA	189
4.3.3	Supercoil-Dependent DNA Transitions	190
4.3.4	Predicting Helical Structures in Genomic DNA	197
4.4	Nonregular Structures	198
4.4.1	Random Walk	199
4.4.2	Average Linear Dimension of a Biopolymer	201
	Application 4.1: LINUS: A Hierarchic Procedure to Predict the Fold of a Protein	202
4.4.3	Simple Exact Models for Compact Structures	204
	Application 4.2: Folding Funnels: Focusing Down to the Essentials	208
	Exercises	209
	References	211
Chapter 5	Methods for the Separation and Characterization of Macromolecules	213
5.1	General Principles	213
5.2	Diffusion	214
5.2.1	Description of Diffusion	215
5.2.2	The Diffusion Coefficient and the Frictional Coefficient	220
5.2.3	Diffusion Within Cells	221
	Application 5.1: Measuring Diffusion of Small DNA Molecules in Cells	222
5.3	Sedimentation	223
5.3.1	Moving Boundary Sedimentation	225
5.3.2	Zonal Sedimentation	237
5.3.3	Sedimentation Equilibrium	241
5.3.4	Sedimentation Equilibrium in a Density Gradient	246
5.4	Electrophoresis and Isoelectric Focusing	248
5.4.1	Electrophoresis: General Principles	249
5.4.2	Electrophoresis of Nucleic Acids	253
	Application 5.2: Locating Bends in DNA by Gel Electrophoresis	257
5.4.3	SDS-Gel Electrophoresis of Proteins	259
5.4.4	Methods for Detecting and Analyzing Components on Gels	264

5.4.5	Capillary Electrophoresis	266
5.4.6	Isoelectric Focusing	266
	Exercises	270
	References	274
Chapter 6	X-Ray Diffraction	276
6.1	Structures at Atomic Resolution	277
6.2	Crystals	279
6.2.1	What Is a Crystal?	279
6.2.2	Growing Crystals	285
6.2.3	Conditions for Macromolecular Crystallization	286
	Application 6.1: Crystals in Space!	289
6.3	Theory of X-Ray Diffraction	290
6.3.1	Bragg's Law	292
6.3.2	von Laue Conditions for Diffraction	294
6.3.3	Reciprocal Space and Diffraction Patterns	299
6.4	Determining the Crystal Morphology	304
6.5	Solving Macromolecular Structures by X-Ray Diffraction	308
6.5.1	The Structure Factor	309
6.5.2	The Phase Problem	317
	Application 6.2: The Crystal Structure of an Old and Distinguished Enzyme	327
6.5.3	Resolution in X-Ray Diffraction	334
6.6	Fiber Diffraction	338
6.6.1	The Fiber Unit Cell	338
6.6.2	Fiber Diffraction of Continuous Helices	340
6.6.3	Fiber Diffraction of Discontinuous Helices	343
	Exercises	347
	References	349
Chapter 7	Scattering from Solutions of Macromolecules	351
7.1	Light Scattering	351
7.1.1	Fundamental Concepts	351
7.1.2	Scattering from a Number of Small Particles: Rayleigh Scattering	355
7.1.3	Scattering from Particles That Are Not Small Compared to Wavelength of Radiation	358
7.2	Dynamic Light Scattering: Measurements of Diffusion	363
7.3	Small-Angle X-Ray Scattering	365
7.4	Small-Angle Neutron Scattering	370
	Application 7.1: Using a Combination of Physical Methods to Determine the Conformation of the Nucleosome	372
7.5	Summary	376

Exercises	376
References	379
Chapter 8 Quantum Mechanics and Spectroscopy	380
8.1 Light and Transitions	381
8.2 Postulate Approach to Quantum Mechanics	382
8.3 Transition Energies	386
8.3.1 The Quantum Mechanics of Simple Systems	386
8.3.2 Approximating Solutions to Quantum Chemistry Problems	392
8.3.3 The Hydrogen Molecule as the Model for a Bond	400
8.4 Transition Intensities	408
8.5 Transition Dipole Directions	415
Exercises	418
References	419
Chapter 9 Absorption Spectroscopy	421
9.1 Electronic Absorption	421
9.1.1 Energy of Electronic Absorption Bands	422
9.1.2 Transition Dipoles	433
9.1.3 Proteins	435
9.1.4 Nucleic Acids	443
9.1.5 Applications of Electronic Absorption Spectroscopy	447
9.2 Vibrational Absorption	449
9.2.1 Energy of Vibrational Absorption Bands	450
9.2.2 Transition Dipoles	451
9.2.3 Instrumentation for Vibrational Spectroscopy	453
9.2.4 Applications to Biological Molecules	453
Application 9.1: Analyzing IR Spectra of Proteins for Secondary Structure	456
9.3 Raman Scattering	457
Application 9.2: Using Resonance Raman Spectroscopy to Determine the Mode of Oxygen Binding to Oxygen-Transport Proteins	461
Exercises	463
References	464
Chapter 10 Linear and Circular Dichroism	465
10.1 Linear Dichroism of Biological Polymers	466
Application 10.1 Measuring the Base Inclinations in dAdT Polynucleotides	471
10.2 Circular Dichroism of Biological Molecules	471
10.2.1 Electronic CD of Nucleic Acids	476
Application 10.2: The First Observation of Z-form DNA Was by Use of CD	478

10.2.2	Electronic CD of Proteins	481
10.2.3	Singular Value Decomposition and Analyzing the CD of Proteins for Secondary Structure	485
10.2.4	Vibrational CD	496
	Exercises	498
	References	499
Chapter 11	Emission Spectroscopy	501
11.1	The Phenomenon	501
11.2	Emission Lifetime	502
11.3	Fluorescence Spectroscopy	504
11.4	Fluorescence Instrumentation	506
11.5	Analytical Applications	507
11.6	Solvent Effects	509
11.7	Fluorescence Decay	513
11.8	Fluorescence Resonance Energy Transfer	516
11.9	Linear Polarization of Fluorescence	517
	Application 11.1: Visualizing c-AMP with Fluorescence	517
11.10	Fluorescence Applied to Protein	524
	Application 11.2: Investigation of the Polymerization of G-Actin	528
11.11	Fluorescence Applied to Nucleic Acids	530
	Application 11.3: The Helical Geometry of Double-Stranded DNA in Solution	532
	Exercises	533
	References	534
Chapter 12	Nuclear Magnetic Resonance Spectroscopy	535
12.1	The Phenomenon	535
12.2	The Measurable	537
12.3	Spin-Spin Interaction	540
12.4	Relaxation and the Nuclear Overhauser Effect	542
12.5	Measuring the Spectrum	544
12.6	One-Dimensional NMR of Macromolecules	549
	Application 12.1: Investigating Base Stacking with NMR	553
12.7	Two-Dimensional Fourier Transform NMR	555
12.8	Two-Dimensional FT NMR Applied to Macromolecules	560
	Exercises	575
	References	577
Chapter 13	Macromolecules in Solution: Thermodynamics and Equilibria	579
13.1	Some Fundamentals of Solution Thermodynamics	580
13.1.1	Partial Molar Quantities: The Chemical Potential	580

13.1.2	The Chemical Potential and Concentration: Ideal and Nonideal Solutions	584
13.2	Applications of the Chemical Potential to Physical Equilibria	589
13.2.1	Membrane Equilibria	589
13.2.2	Sedimentation Equilibrium	597
13.2.3	Steady-State Electrophoresis	598
	Exercises	600
	References	603
Chapter 14	Chemical Equilibria Involving Macromolecules	605
14.1	Thermodynamics of Chemical Reactions in Solution: A Review	605
14.2	Interactions Between Macromolecules	610
14.3	Binding of Small Ligands by Macromolecules	615
14.3.1	General Principles and Methods	615
14.3.2	Multiple Equilibria	622
	Application 14.1: Thermodynamic Analysis of the Binding of Oxygen by Hemoglobin	641
14.3.3	Ion Binding to Macromolecules	644
14.4	Binding to Nucleic Acids	648
14.4.1	General Principles	648
14.4.2	Special Aspects of Nonspecific Binding	648
14.4.3	Electrostatic Effects on Binding to Nucleic Acids	651
	Exercises	654
	References	658
Chapter 15	Mass Spectrometry of Macromolecules	660
15.1	General Principles: The Problem	661
15.2	Resolving Molecular Weights by Mass Spectrometry	664
15.3	Determining Molecular Weights of Biomolecules	670
15.4	Identification of Biomolecules by Molecular Weights	673
15.5	Sequencing by Mass Spectrometry	676
15.6	Probing Three-Dimensional Structure by Mass Spectrometry	684
	Application 15.1: Finding Disorder in Order	686
	Application 15.2: When a Crystal Structure Is Not Enough	687
	Exercises	690
	References	691
Chapter 16	Single-Molecule Methods	693
16.1	Why Study Single Molecules?	693
	Application 16.1: RNA Folding and Unfolding Observed at the Single-Molecule Level	694
16.2	Observation of Single Macromolecules by Fluorescence	695

16.3	Atomic Force Microscopy	699
	Application 16.2: Single-Molecule Studies of Active Transcription by RNA Polymerase	701
16.4	Optical Tweezers	703
16.5	Magnetic Beads	707
	Exercises	708
	References	709
Answers to Odd-Numbered Problems		A-1
Index		I-1