

Contents

Preface to the first edition	xv
Preface to the second edition.....	xvii
Acknowledgments to the first edition	xix
Acknowledgments to the second edition.....	xxi
Authors	xxii

PART I THERMODYNAMICS

Chapter 1	Systems, state functions, and the laws of thermodynamics	3
1.1	Systems and their surroundings	3
1.2	State variables and state functions	4
1.3	The internal energy U and the first law of thermodynamics	6
1.3.1	Internal energy, heat, and work	6
1.3.2	The first law of thermodynamics.....	7
1.3.3	The ideal gas: a convenient system to understand thermodynamic principles.....	8
1.3.4	Changes in the state of an ideal gas.....	11
1.3.5	Thermodynamic cycles: back and forth or round and round	20
1.3.6	The temperature dependence of the internal energy U	24
1.4	The enthalpy H	25
1.5	The entropy S and the second law of thermodynamics.....	28
1.5.1	Predicting spontaneity of processes: dissipation of heat and matter	28
1.5.2	Entropy and heat	29
1.5.3	Temperature dependence of the entropy.....	32
1.5.4	The third law of thermodynamics and absolute entropy	33
1.5.5	Entropy and order: the statistic interpretation	34
1.6	The free energy G : combining system and surroundings.....	37
1.6.1	Entropy- and enthalpy-driven reactions	39
1.6.2	Pressure and temperature dependence of the free energy	41
1.6.3	Standard states	43
1.6.4	Relation of free energy, enthalpy, and entropy to molecular properties.....	44
1.7	The chemical potential μ	46
1.7.1	The chemical potential as a driving force for chemical reactions.....	47
1.7.2	The chemical potential and stable states: phase diagrams	48

1.7.3	Pressure and temperature dependence of the chemical potential	51
1.7.4	The chemical potential as a partial molar property	51
1.7.5	The chemical potential of compounds in mixtures	53
1.7.6	The chemical potential of solutions	55
1.7.7	Colligative properties	57
	Questions	64
	References	67
Chapter 2	Energetics and chemical equilibria	68
2.1	The free energy change and the equilibrium constant.....	68
2.1.1	Temperature dependence of the equilibrium constant	70
2.1.2	The principle of Le Chatelier	71
2.2	Binding and dissociation equilibria and affinity.....	72
2.3	Protolysis equilibria: the dissociation of acids and bases in water	75
2.4	Thermodynamic cycles, linked functions, and apparent equilibrium constants	76
	Questions	84
	References	86
Chapter 3	Statistical thermodynamics	87
3.1	Probabilities, statistical weights, energy, and the Boltzmann distribution	87
3.2	Sum of states: the partition function	90
3.2.1	General form of the partition function	90
3.2.2	The partition function for systems with degenerate states	92
3.2.3	From the molecular partition function to the partition function of ensembles.....	93
3.3	Statistical thermodynamics and structural changes in biomolecules.....	95
3.3.1	General description of structural transitions.....	95
3.3.2	Applications of the zipper model to structural transitions of biomolecules	99
3.4	Statistical thermodynamics and binding equilibria	100
3.4.1	Multiple equivalent, non-interacting binding sites.....	101
3.4.2	Multiple classes of non-equivalent, non-interacting binding sites.....	104
3.4.3	Cooperative binding sites	105
	Questions	107
	References	109
Chapter 4	Thermodynamics of transport processes.....	110
4.1	Diffusion.....	110
4.2	The chemiosmotic hypothesis.....	116
4.3	Active and passive transport	118
4.4	Directed movement by the Brownian ratchet.....	120
	Questions	124
	References	125
Chapter 5	Electrochemistry.....	126
5.1	Redox reactions and electrochemical cells.....	126
5.2	Types of half-cells.....	129
5.3	Standard electrode potentials	130
5.4	The Nernst equation.....	131
5.5	Measuring pH values	133
5.6	Redox reactions in biology	134
5.6.1	Cysteines and disulfide bonds	134
5.6.2	The respiratory chain	134
5.6.3	The light reaction in photosynthesis	135
5.7	The electrochemical potential and membrane potentials.....	136

5.8	Electrophysiology: patch-clamp methods to measure ion flux through ion channels	139
	Questions	141
	References	142

PART II KINETICS

Chapter 6	Reaction velocities and rate laws	145
	Questions	149
Chapter 7	Integrated rate laws for uni- and bimolecular reactions	150
	Questions	161
Chapter 8	Reaction types	162
	8.1 Reversible reactions	162
	8.2 Parallel reactions	166
	8.3 Consecutive reactions	168
	Questions	174
	References	174
Chapter 9	Deriving integrated rate laws by solving sets of differential equations with matrix algebra	175
	9.1 Reversible reactions	175
	9.2 Consecutive, irreversible reactions	180
	9.3 Reversible reaction followed by an irreversible second step	183
	Questions	188
	References	188
Chapter 10	Rate-limiting steps	189
	Questions	193
	References	193
Chapter 11	Binding reactions: One-step and two-step binding	194
	Questions	202
	References	202
Chapter 12	Single-molecule kinetics	203
	12.1 Integrated rate laws, dwell times, dwell-time and cumulative dwell-time distributions	203
	12.2 Single-molecule kinetics and the principle of ergodicity	204
	12.3 Single-molecule kinetics of reversible reactions	206
	12.4 The limits of dwell-time histograms: potential pitfalls	209
	12.5 Double-exponential dwell-time histograms	210
	Questions	212
	References	212
Chapter 13	Steady-state (enzyme) kinetics	213
	13.1 Rapid equilibrium (Michaelis-Menten formalism)	214
	13.2 Steady-state approximation (Briggs-Haldane formalism)	216
	13.3 pH dependence	220
	13.4 Two or more non-interacting active sites	224
	13.5 Two or more interacting active sites: cooperativity and the Hill equation	228
	13.6 Inhibition of enzyme activity	232
	13.6.1 Product inhibition in reversible reactions	232
	13.6.2 Competitive inhibition	235

13.6.3	Non-competitive inhibition	236
13.6.4	Mixed inhibition	238
13.6.5	Quantifying enzyme inhibition: inhibition constants <i>versus</i> IC_{50} values.	240
13.6.6	Time-dependent inhibition	242
	Questions	251
	References	254
Chapter 14	Complex reaction schemes and their analysis	255
14.1	Binding of two substrates	255
14.1.1	Random binding	255
14.1.2	Ordered binding	257
14.2	Ping-pong mechanism	260
14.3	Net rate constants and transit times	261
	Questions	264
	References	265
Chapter 15	Temperature dependence of rate constants	266
15.1	The Arrhenius equation	266
15.2	Transition state theory	266
15.3	Collision theory	269
15.4	Kinetic and thermodynamic control of reactions	270
	Questions	271
Chapter 16	Principles of catalysis	273
16.1	Heterogeneous catalysis: the Langmuir-Hinshelwood formalism for surface-catalyzed reactions	274
16.2	Homogeneous catalysis by enzymes	276
16.2.1	Acid-base catalysis	278
16.2.2	Electrostatic and covalent catalysis	281
16.2.3	Intramolecular catalysis and effective concentrations	282
	Questions	283
	References	283

PART III MOLECULAR STRUCTURE AND STABILITY

Chapter 17	Molecular structure and interactions	287
17.1	Isomers: configuration and conformation	287
17.1.1	Configurations	288
17.1.2	Conformations	290
17.2	The configurational and conformational energy of molecules	290
17.2.1	Covalent interactions	290
17.2.2	Non-covalent interactions	295
17.3	Energy landscapes and the most stable conformation	305
	Questions	306
	References	307
Chapter 18	Proteins	308
18.1	Amino acids and the peptide bond	308
18.1.1	Properties of the twenty canonical amino acids	308
18.1.2	The peptide bond	311
18.1.3	Side-chain rotamers	313

18.1.4	Post-translational modifications.....	313
18.1.5	Expansion of the genetic code	320
18.2	Protein sequence.....	322
18.2.1	Analysis of individual sequences for global and local properties	323
18.2.2	Sequence alignment.....	325
18.3	Protein structure	327
18.3.1	Helical secondary structure elements	329
18.3.2	β -strands and their super-secondary structures (β -sheets)	334
18.3.3	Reverse turns.....	337
18.3.4	Protein domains and tertiary structure	341
18.3.5	Quaternary structure.....	344
18.3.6	Protein-protein interfaces	347
18.3.7	Protein-ligand interactions	351
18.4	Membrane proteins and their lipid environment	354
18.4.1	Biological roles of lipids and membranes	354
18.4.2	Types of lipids	355
18.4.3	Super-structures formed by amphiphiles	356
18.4.4	Properties and structure of membrane proteins.....	364
18.4.5	Solubilization of membrane proteins.....	367
18.5	Folding and stability	369
18.5.1	Driving forces for protein folding.....	369
18.5.2	First folding experiments and the Levinthal paradox.....	372
18.5.3	Energy landscapes for protein folding	373
18.5.4	Mathematical description of the two-state model.....	374
18.5.5	Folding pathways and mechanisms of protein folding	380
18.5.6	Protein folding diseases	388
	Questions	389
	References	392
Chapter 19	Nucleic acids	395
19.1	Nucleobases, nucleosides, and nucleotides.....	396
19.1.1	Hydrogen bonds between nucleobases – base pairs	397
19.1.2	Non-standard nucleobases in DNA.....	399
19.1.3	Non-standard nucleobases in RNA	400
19.2	Ribose and nucleobase conformations	401
19.2.1	Sugar pucker	401
19.2.2	Syn- and anti- conformations	402
19.3	Primary structure of nucleic acids.....	403
19.3.1	Nucleic acid sequences and codon usage	403
19.3.2	Backbone conformation	405
19.4	Base-pair geometry and stacking	406
19.4.1	Importance of base-pair stacking for double helix formation.....	407
19.4.2	Base-pair geometries	408
19.5	DNA structures and conformations	409
19.5.1	DNA double helical structures	409
19.5.2	Triple and quadruple DNA helices.....	412
19.5.3	Higher-order DNA structures	414
19.5.4	DNA interactions with proteins and ligands.....	422
19.6	RNA structure	428
19.6.1	RNA secondary structure and their prediction	429
19.6.2	RNA tertiary structure.....	431
19.6.3	Ribozymes, riboswitches, and small molecule binding to RNA.....	434

19.6.4	RNA folding	436
19.6.5	Ribonucleoprotein (RNP) complexes	437
	Questions	439
	References	441
Chapter 20	Macromolecular modeling	443
20.1	Molecular mechanics and dynamics	443
20.1.1	Force fields	444
20.1.2	Energy minimization	445
20.1.3	Simulated annealing	447
20.1.4	Coarse-grained modeling	448
20.1.5	Molecular mechanics and dynamics	449
20.2	Principles of neural networks and machine learning	454
20.3	Simulating protein folding	456
20.3.1	Homology modeling	456
20.3.2	Sequence-based structure prediction	458
20.3.3	Quality assessment of predicted three-dimensional protein models	461
20.3.4	Biophysical value and limitations of predicted structural models	463
	Questions	465
	References	466
	Online resources	466

PART IV METHODS

Chapter 21	Optical spectroscopy	471
21.1	Interaction of light and matter	471
21.1.1	Light as an electromagnetic wave	471
21.1.2	Principles of spectroscopy: transitions in two-state systems	473
21.2	Absorption	475
21.2.1	Electronic, vibronic, and rotational energy levels	475
21.2.2	Transitions and transition dipoles	476
21.2.3	The Lambert-Beer law	478
21.2.4	Solvent effects and influence of the local environment	479
21.2.5	Instrumentation	480
21.2.6	Biological chromophores	481
21.2.7	Applications	485
21.2.8	Potential pitfalls	492
21.3	Linear and circular dichroism	493
21.3.1	Linearly polarized light and linear dichroism	493
21.3.2	Circularly polarized light and circular dichroism	496
21.3.3	Instrumentation	500
21.3.4	Biological chromophores that show circular dichroism	501
21.3.5	Applications	502
21.3.6	Potential pitfalls	503
21.4	Infrared spectroscopy	504
21.4.1	Bond vibrations: the harmonic oscillator	504
21.4.2	Molecule geometry, degrees of freedom, and vibrational modes	506
21.4.3	Instrumentation	508
21.4.4	Applications	509
21.5	Fluorescence	510
21.5.1	General considerations	510
21.5.2	Instrumentation	512

21.5.3	Quantum yield and lifetime	513
21.5.4	Fluorophores and fluorescent labeling	514
21.5.5	Applications.....	523
21.5.6	Potential pitfalls	531
21.5.7	Fluorescence quenching	535
21.5.8	Fluorescence anisotropy	537
21.5.9	Time-resolved fluorescence	542
21.5.10	Förster Resonance Energy Transfer	550
	Questions	561
	References	565
Chapter 22	Magnetic resonance	570
22.1	Nuclear magnetic resonance	570
22.1.1	Nuclear spins and the Zeeman effect.....	570
22.1.2	A one-dimensional NMR spectrum: Larmor frequency, chemical shift, <i>J</i> -coupling, and multiplicity.....	572
22.1.3	The nuclear Overhauser effect: distance information	577
22.1.4	Magnetization and its relaxation to equilibrium: Fourier transform-NMR and the free induction decay	580
22.1.5	Two-dimensional FT-NMR: COSY and NOESY.....	585
22.1.6	Extending NMR to structure determination of large molecules	592
22.1.7	NMR and dynamics	594
22.1.8	Solid-state NMR and biology	597
22.1.9	NMR and imaging	598
22.2	Electron paramagnetic resonance	599
22.2.1	Principle of electron paramagnetic resonance	599
22.2.2	Spin-spin interactions: hyperfine coupling of unpaired electrons with nuclei....	601
22.2.3	EPR probes and spin labeling	601
22.2.4	EPR as a probe for mobility and dynamics	603
22.2.5	EPR as a probe for accessibility.....	605
22.2.6	Measuring spin-spin distances	606
22.2.7	Distance determination by pulsed EPR: PELDOR/DEER.....	606
	Questions	609
	References	611
Chapter 23	Solution scattering.....	615
23.1	Light scattering	615
23.1.1	Static light scattering	615
23.1.2	Dynamic light scattering	620
23.1.3	Raman scattering.....	622
23.2	Small angle scattering	624
23.2.1	Scattering of X-rays and neutrons.....	624
23.2.2	SAS intensity distribution.....	627
23.2.3	Distance distribution function	631
23.2.4	Small angle X-ray scattering	632
23.2.5	Small angle neutron scattering	635
	Questions	637
	References	638
Chapter 24	Crystallography.....	640
24.1	Phase problem and requirement for crystals	640
24.2	Crystallization of macromolecules	641
24.3	Symmetry and space groups	647

24.4	X-ray crystallography	652
24.4.1	Generation of X-rays	653
24.4.2	X-ray diffraction from crystals	657
24.4.3	Diffraction data collection and analysis	661
24.4.4	Phasing methods	665
24.4.5	Electron density and model building	673
24.4.6	Model refinement and validation	675
24.5	Time-resolved crystallography	679
24.6	Serial crystallography and X-ray free electron lasers	680
24.7	Neutron crystallography	682
24.8	Electron crystallography	682
	Questions	683
	References	685
	Online resources	686
Chapter 25	Fluorescence imaging and microscopy	687
25.1	Optical principles of microscopy	687
25.1.1	Focusing and collecting light by optical lenses	688
25.1.2	Microscopes: how to achieve magnification with optical lenses	690
25.1.3	The diffraction limit of optical resolution	691
25.2	Wide-field fluorescence microscopy	693
25.3	Confocal scanning microscopy	695
25.4	Total internal reflection microscopy	697
25.5	Fluorescence lifetime imaging microscopy	700
25.6	Fluorescence (cross-)correlation spectroscopy	701
25.6.1	Fluorescence correlation spectroscopy	701
25.6.2	FCS to monitor binding events	703
25.6.3	Fluorescence cross-correlation spectroscopy	707
25.7	Single-molecule fluorescence microscopy	708
25.7.1	Principles of single-molecule microscopy	708
25.7.2	Why single molecules?	711
25.7.3	Localization and tracking of single molecules	712
25.7.4	Kinetic information from single-molecule microscopy	714
25.7.5	Colocalization of molecules	716
25.7.6	Single-molecule FRET	718
25.8	Super-resolution microscopy	723
	Questions	727
	References	728
Chapter 26	Electron microscopy	731
26.1	Outline of an electron microscope	733
26.1.1	Generation of electron beams	734
26.1.2	Electron energy and resolution limit of EM	734
26.1.3	Electromagnetic lenses	736
26.1.4	Electron detection and detector noise	739
26.2	Electron-matter interactions	741
26.2.1	Elastic scattering of electrons	741
26.2.2	Inelastic scattering of electrons	742
26.2.3	Radiation damage	742
26.3	Sample preparation for EM	743
26.3.1	EM grids and sample supports	744
26.3.2	Metal shadowing for SEM	744
26.3.3	Negative staining for TEM	745

26.3.4	Native samples in vitreous ice	745
26.4	TEM imaging and diffraction	746
26.4.1	Plane waves, defocus, and phase shifts	748
26.4.2	Image contrast, amplitude contrast, and phase contrast	749
26.4.3	Contrast transfer function	751
26.5	Three-dimensional electron microscopy	756
26.5.1	Cryo-electron tomography	756
26.5.2	Single-particle cryo-EM	759
26.5.3	Resolution and interpretation of cryo-EM potential maps	762
	Questions	764
	References	765
Chapter 27	Scanning probe microscopy and force measurements	766
27.1	Scanning probe microscopy: scanning tunneling, scanning force, and atomic force microscopy	766
27.2	Force measurements	768
27.2.1	Effect of forces on the energetics and kinetics of macromolecules	768
27.2.2	Force spectroscopy by AFM	770
27.2.3	Optical tweezers	776
27.2.4	Magnetic tweezers	782
	Questions	786
	References	786
Chapter 28	Transient kinetic methods	791
28.1	Stopped flow	791
28.2	Quench flow	794
28.3	Laser flash photolysis	795
28.4	Relaxation kinetics: pressure- and temperature-jump	797
	Questions	799
	References	799
Chapter 29	Molecular mass, size, and shape	801
29.1	Mass spectrometry	801
29.1.1	Ionization	802
29.1.2	Ion storage and manipulation	804
29.1.3	Detection	811
29.1.4	Mass spectra	813
29.1.5	Applications	813
29.2	Analytical ultracentrifugation	820
29.2.1	Instrumentation and detection systems	820
29.2.2	Behavior of a molecule in a gravitational field	822
29.2.3	Sedimentation velocity	826
29.2.4	Sedimentation equilibrium	831
29.2.5	Zonal, band, or isopycnic centrifugation	834
29.3	Surface plasmon resonance	837
29.3.1	Physical background of SPR	838
29.3.2	Principle and information content of an SPR experiment	839
29.3.3	Mass transport limitation	841
29.3.4	Receptor immobilization on the sensor surface	842
29.3.5	Stoichiometry of binding in an SPR experiment	845
29.3.6	Specificity of binding in an SPR experiment	845
	Questions	846
	References	850

Online resources.....	852
Chapter 30 Calorimetry.....	853
30.1 Isothermal titration calorimetry.....	853
30.1.1 General principle.....	853
30.1.2 ITC data analysis.....	855
30.1.3 Origin of enthalpic changes.....	857
30.1.4 Practical considerations.....	858
30.1.5 Measuring high affinities with ITC by competition.....	861
30.1.6 Measuring Michaelis-Menten enzyme kinetics with ITC.....	861
30.2 Differential scanning calorimetry.....	864
30.2.1 General principle.....	864
30.2.2 Two-state unfolding of macromolecules.....	867
30.2.3 Two-state unfolding with subunit dissociation.....	869
Questions.....	870
References.....	872

APPENDIX

Chapter 31 Mathematical concepts used in this book.....	875
31.1 Sums and products.....	875
31.2 Quadratic equation.....	876
31.3 Binomial coefficients.....	876
31.4 Trigonometry.....	877
31.5 Logarithms and exponentials.....	878
31.6 Differentiation and integration.....	880
31.6.1 Implicit derivatives.....	881
31.6.2 Partial and total derivatives.....	881
31.6.3 Integration.....	882
31.6.4 Differential equations.....	883
31.7 Partial fractions.....	885
31.8 l'Hôpital's rule.....	886
31.9 Vectors.....	886
31.10 Matrices.....	888
31.10.1 Basic operations on matrices.....	889
31.10.2 Solving systems of linear equations by Gaussian elimination.....	890
31.10.3 Eigenvalues and Eigenvectors to solve systems of linearly independent equations.....	891
31.11 Complex numbers.....	893
31.12 Basic elements of statistics.....	895
31.13 Error propagation.....	897
31.14 Series expansion.....	899
31.14.1 Taylor series.....	899
31.14.2 Fourier series.....	900
31.15 Fourier transformation.....	901
31.16 Convolution.....	903
Index.....	905