BIOPHYSICAL CHEMISTRY
Part II: Techniques for the study of biological structure and function

Cantor and Schimmel

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PART

II

TECHNIQUES FOR THE STUDY OF BIOLOGICAL STRUCTURE AND FUNCTION

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Contents in brief of Parts I and III

Preface

Biophysical Chemistry is concerned with biological macromolecules and complexes or arrays of macromolecules. The work deals with the conformation, shape, structure, conformational changes, dynamics, and interactions of such systems. Our goal is to convey the major principles and concepts that are at the heart of the field. These principles and concepts are drawn from physics, chemistry, and biology.

We have aimed at creating a multilevel textbook in three separately bound parts. The material covers a broad range of sophistication so that the text can be used in both undergraduate and graduate courses. It also should be of value to general scientific readers who simply wish to become familiar with the field, as well as to experienced research scientists in the biophysical area. For example, perhaps half of the material requires only the background provided by a one-semester undergraduate course in physical chemistry. A somewhat smaller fraction necessitates the use of concepts and mathematical techniques generally associated with a more sophisticated background, such as elementary statistical thermodynamics and quantum mechanics.

Biophysical Chemistry is organized into three parts. The first part deals with the structure of biological macromolecules and the forces that determine this structure. Chapter 1 introduces the fundamental questions of interest to biophysical chemists, Chapters 2–4 summarize the known structures of proteins, nucleic acids, and other biopolymers, and Chapters 5–6 treat noncovalent forces and conformational analysis.

Part II summarizes some of the techniques used in studying biological structure and function. The emphasis is on a detailed discussion of a few techniques rather than an attempt to describe every known technique. Chapters 7–9 cover spectroscopic methods, Chapters 10–12 deal with hydrodynamic methods, and Chapters 13–14 discuss x-ray and other scattering and diffraction techniques.

Part III demonstrates how techniques and principles are used in concert to gain an understanding of the behavior and properties of biological macromolecules. The focus is on the thermodynamics and kinetics of conformational changes and ligand interactions. New techniques are introduced as needed, and a few selected case

histories or systems are discussed in considerable detail. The topics include ligand interactions (Chapters 15–17), the special theories and techniques used to study molecules that are statistical chains rather than definite folded conformations (Chapters 18–19), protein conformational changes (Chapters 20–21), nucleic acid conformational changes (Chapters 22–24), and membranes (Chapter 25).

We have made every effort to keep the chapters as independent as possible, so that the reader has a wide choice of both the material to be covered and the order in which it is to be treated. Extensive cross-references to various chapters are included to help the reader find necessary background material if the parts are not read in sequence. Where possible, examples are taken repeatedly from a small number of systems, so that the reader can have the experience of contrasting information gained about the same protein or nucleic acid from a variety of different approaches.

Within each chapter, we have attempted to maintain a uniform level of rigor or sophistication. Short digressions from this level are segregated into boxes; longer digressions are indicated by a bullet (•) preceding the section or subsection heading. Readers with a less rigorous background in physics, mathematics, and physical chemistry should find helpful the many boxes that review elementary material and make the text fairly self-contained; Appendix A provides a basic review of principles of matrix algebra. Other boxes and special subsections are aimed at advanced readers; in many cases, these discussions attempt to illuminate points that we ourselves found confusing.

In different sections, the level of mathematical sophistication varies quite significantly. We have tried to use the simplest mathematical formulation that permits a clear presentation of each subject. For example, hydrodynamic properties are treated in one dimension only. The form of a number of the fundamental equations is extracted by dimensional analysis rather than through lengthy (and not particularly instructive) solutions of hydrodynamic boundary-value problems. On the other hand, x-ray and other scattering phenomena are treated by Fourier transforms, and many problems in statistical mechanics are treated with matrix methods. These advanced mathematical techniques are used in only a few chapters, and numerous boxes are provided to assist the reader with no previous exposure to such methods. The remaining sections and chapters are self-contained and can be understood completely without this advanced mathematical formalism.

Some techniques and systems are not covered in any fair detail. This represents a biased choice by the authors, not necessarily of which techniques we feel are important, but simply of which are instructive for the beginning student in this field.

Each chapter concludes with a summary of the major ideas covered. In addition, each chapter is heavily illustrated, including some special drawings by Irving Geis. Certainly, much can be learned simply by reading the chapter summaries and by studying the illustrations. Also, we believe the illustrations convey some of the excitement of the field.

Problems are provided at the end of each chapter. These vary in difficulty from relatively simple to a few where the full answer is not known, at least to the authors. Answers to problems are provided in Appendix B.

Detailed literature citations are not included, except to acknowledge the source of published material reproduced or adapted here. However, a list of critical references for each chapter is included. In virtually all cases, these articles will provide an immediate entrée to the original papers needed for more detailed study.

The problem of notation and abbreviations in this field is a difficult one. In drawing together material from so many different types of research, we have had to adapt the notation to achieve consistency and to avoid confusion among similar symbols. Wherever possible, we have followed the recommendations of the American Chemical Society, but inevitably we have had to develop some conventions of our own. A glossary of some of the more frequently used symbols is provided.

At MIT some of this material has been used in an undergraduate course in biophysical chemistry. The course was designed to meet the needs of students wishing a second course in physical chemistry, but developed in a biochemical framework. The idea was to construct a course that covered much of the same material with the same rigor as a parallel, more traditional course. The only preparation required was a one-semester course in undergraduate physical chemistry, which at MIT is largely concerned with chemical thermodynamics.

Over the years graduate courses in biophysical chemistry at MIT and at Columbia have made use of much of the material presented here. In addition, a special-topics course in protein structure has used some of the material. Because a broad range of subjects is covered, its usefulness as a text will hopefully meet a variety of individual teaching tastes and preferences, as well as enable instructors to vary content as needs develop and change.

It is obvious that a work of this complexity cannot represent solely the efforts of its two authors. As we sought to master and explain the wide range of topics represented in biophysical chemistry, we learned why so few books have been written in this field in the past two decades. We owe a great debt to many who helped us in ways ranging from sharing their understanding to providing original research data.

We give special thanks to Irving Geis, for his effort on a number of complex illustrations and for his helpful advice on numerous other drawings; to Wilma Olson, for reading a major portion of the entire manuscript; to Robert Alberty and Gordon Hammes, for their influence, through teaching and discussions, on the material on biochemical equilibria and kinetics; to Richard Dickerson, for providing material and advice that were essential for the preparation of Chapter 13; to Paul Flory, for inspiring our treatment of conformational energies and configurational statistics of macromolecules; to Howard Schachman, whose course at Berkeley inspired parts of several chapters; to R. Wayne Oler, for bringing the authors together for this undertaking, and to Bruce Armbruster, for sealing the commitment; to the helpful people at W. H. Freeman and Company, including Ruth Allen, Arthur Bartlett, Robert Ishi, Larry McCombs, and Pearl Vapnek; to Kim Engel, Karen Haynes, Marie Ludwig, Joanne Meshna, Peggy Nelson, Cathy Putland, and Judy Schimmel, for typing and related work associated with the manuscript; and to Cassandra Smith and to Judy, Kathy, and Kirsten Schimmel, for their patience with the intrusion this work has made on the authors' lives.

This glossary includes some of the symbols used extensively throughout the text. In many cases, the same or very similar symbols are used in certain contexts with other meanings; the meaning of a symbol is explained in the text where it is introduced.

Symbol	Meaning
A	Absorbance.
A_{ij}	Amplitude of kinetic decay.
Å	Angstroms.
a	Hyperfine splitting constant. Long semi-axis of ellipse. Persistence length.
a	Unit cell basis vector.
a*	Reciprocal cell basis vector.
a_{ij}	Parameters composed of rate constants.
a_s	Exponent relating sedimentation to chain length.
a_η	Exponent relating viscosity to chain length.
b	Short semiaxis of ellipse.
b	Unit cell basis vector.
b*	Reciprocal cell basis vector.
C	Molar concentration.
C_n	Rotational symmetry group element. Characteristic ratio.
C_∞	Limiting characteristic ratio.
ΔC_p^0	Standard constant pressure heat capacity change per mole.
c	Velocity of light in vacuum. Ratio of k_R/k_T. Weight concentration.

Symbol	Meaning
c_p	Plateau weight concentration.
$\hat{c}_i$	Weight concentration of ith species or component.
c	Unit cell basis vector.
c*	Reciprocal cell basis vector.
D	Debye.
D	Translational diffusion constant.
D_n	Dihedral symmetry group element.
D_{rot}	Rotational diffusion constant.
$D_{20,w}$	D extrapolated to 20° C, water.
E_a	Activation energy.
E_d	Interaction energy between two dipoles.
E_{kl}	Nonbonded pair interaction potential.
E_{tor}	Torsional potential energy.
$E(\Phi_i, \Psi_i),$ E_i	Total rotational potential for residue i.
E	Electric field.
e	Exponential function. Unit of charge on electron.
F	Frictional coefficient ratio.
$F(S)$	Structure factor.
$F_H(S)$	Structure factor, heavy-atom contribution.

Symbol	Meaning	Symbol	Meaning
$F_{Tot}(S)$	Structure factor for an array.	H_{res}	Magnetic field at which resonance occurs.
$F_m(S)$	Molecular structure factor.		
F	Force.	$\underset{\sim}{H}$	Hamiltonian operator.
$\mathscr{F}$	The Faraday.	ΔH_{loc}	Magnetic field generated by local environment.
f	Translational frictional coefficient.		
f_{app}	Apparent fractional denaturation.	h	Planck's constant.
f_D	Fraction in denatured state.	$\hbar$	$h/2\pi$
f_N	Fraction in native state.	I	Intensity of radiation. Nuclear spin quantum number. Ionic strength.
f_{min}	Translational friction coefficient of anhydrous sphere.	$I(S)$	Scattering intensity relative to a single electron at the origin.
$\hat{f}_{rot}$	Rotational friction coefficient for sphere.		
f_{sph}	Translational friction coefficient for sphere.	i	$\sqrt{-1}$
		$\hat{\imath}$	Cartesian unit vector.
f_a, f_b	Rotational friction coefficient around a, b axis of ellipse.	J	NMR coupling constant.
G	Gibbs free energy.	J_2	Solute flux.
ΔG^0	Standard Gibbs free energy change per mole.	$\underset{\sim}{\hat{\jmath}}$	Cartesian unit vector.
		K_D	True equilibrium constant for conversion from fully native to fully denatured state.
$\Delta \bar{G}^0$	Intrinsic standard free energy change (with statistical component removed).		
		K_p	Michaelis constant for product.
$\Delta G_{I,ij}$	Free energy of interaction between two ligands.	K_s	Michaelis constant for substrate.
		K_η	Coefficient relating viscosity to chain length.
ΔG_r	ΔG per residue.		
ΔG_{Tot}	Total free energy change per mole.	K_s	Coefficient relating sedimentation to chain length.
ΔG_{el}	Change in electrostatic free energy.		
ΔG_T	Total free energy of formation of configuration.	K_{app}	Apparent equilibrium constant for conversion from fully native to fully denatured state.
$\Delta \Delta G_T$	Difference in ΔG_T between two configurations.	K_i	Macroscopic equilibrium constant. Equilibrium constant for forming ith configuration. Equilibrium constant for transition from native state to intermediate state i.
$\Delta \bar{G}_{gr}$	Average helix growth free energy change per residue pair.		
g	g value for free electron, 2.00232.		
g_x, etc.	Component of g-factor tensor.	$\tilde{K}_i$	Apparent dissociation constant, one-ligand system.
H	Enthalpy.		
H_{xy}	Magnetic field in xy plane.	$\tilde{K}_{ij}$	Apparent dissociation constant, two-ligand system.
ΔH	Enthalpy change per mole.		
ΔH^0	Standard enthalpy change per mole.	k	Boltzmann's constant. Microscopic equilibrium dissociation constant.
ΔH_r	ΔH per residue.		
ΔH_D	Enthalpy change for conversion from fully native to fully denatured state.	k_R	Microscopic dissociation constant for R state.
ΔH_{app}	Apparent enthalpy change for conversion from fully native to fully denatured state.	k_T	Microscopic dissociation constant for T state.
		k_i	Microscopic equilibrium constant.
H	Magnetic field.	$\hat{k}$	Cartesian unit vector.

Symbol	Meaning
L_c	Contour length.
L, L'	Equilibrium constant for R_0 T_0.
$\mathbf{L}$	Angular momentum.
l	Length of one polymer bond.
l_e	Length of statistical segment.
M	Molecular weight.
$\bar{M}_n$	Number-average molecular weight.
$\bar{M}_w$	Weight-average molecular weight.
$\bar{M}_i$	Molecular weight of ith macromolecular species.
M_{ij}	Species with i bound L_1 and j bound L_2.
$M^{(j)}$	Set of all species with j bound L_2.
$\mathbf{M}$	Magnetization.
$\mathbf{M}_{xy}$	Magnetization in xy plane.
$\underline{M}$	Statistical weight matrix.
m	Colligative molality. Mass of object.
m_e	Mass of electron.
m_i	Molality of ith species.
m_s	Quantum number of electron spin along z axis.
m_I	Quantum number of nuclear spin along z axis.
m'	Total molality.
$\mathbf{m}$	Magnetic dipole operator.
N_0	Avogadro's number.
N_C	Number of carbons in amphiphile R chain.
N'_C	Number of carbons in amphiphile that are imbedded in hydrocarbon core of micelle.
N_e	Number of statistical segments.
N_{Ch}	Number of chains in micelle.
N_{hg}	Number of head groups in micelle.
n	Refractive index. Number of sites. Number of bonds in polymer.
n_i	Number of moles of component i. Number of sites of certain type.
n_w	Weight-average degree of polymerization.
P	Pitch of helix. Pressure. Patterson function.
P_0	Solvent vapor pressure.
P_v	Solvent vapor pressure in presence of solute.

Symbol	Meaning
P_r	Axial ratio.
pK_a	$-\log_{10} K_a$
pO_2	Partial pressure of oxygen.
$(pO_2)_{1/2}$	Partial pressure of oxygen at half saturation.
$\mathbf{P}$	Momentum operator.
Q	Configurational partition function.
R	Gas constant.
R_G	Radius of gyration.
$\langle R_G^2 \rangle_0$	Unperturbed mean square radius of gyration.
$\bar{R}$	Fraction of molecules in R state.
$\mathbf{R}$	Nuclear position operator.
$\underline{R}(\alpha,\beta)$	Coordinate transformation matrix.
r	Distance of separation.
r_D	Donnan ratio.
r_e	Radius of equivalent sphere.
$\langle r^2 \rangle_0$	Unperturbed mean square end-to-end distance.
$\mathbf{r}$	Polymer end-to-end vector.
$\underline{r}$	Electron position operator.
S	Svedberg (unit of sedimentation coefficient).
S_A	Partial molal entropy.
S'_A	Unitary part of S_A.
ΔS_r	ΔS per residue.
ΔS^0	Standard entropy change.
ΔS_u^0	Unitary standard entropy change.
$\mathbf{S}$	Scattering vector.
s	Sedimentation coefficient. Statistical weight. Equilibrium constant for helix growth. Equilibrium constant for base-pair formation.
$s_{20,w}$	Sedimentation coefficient corrected to $20°$ C, water
$\hat{s}$	Unit vector along scattered radiation.
$\hat{s}_0$	Unit vector along incident radiation.
T	Temperature (in degrees Kelvin usually).
T_m	Melting temperature.
T_1	Longitudinal relaxation time.
T_2	Transverse relaxation time.
$\underline{T}_i$	Transformation matrix.
t	Time.

Symbol	Meaning
U^0_{mic}	Attractive part of μ^0_{mic}.
u	Component of M_{xy} in phase with H_{xy}. Electrophoretic mobility.
V	Volume.
V_h	Hydrated volume.
$\bar{V}_i$	Partial specific volume of component i.
V_p	Maximum reaction velocity in reverse direction.
V_s	Maximum reaction velocity in forward direction.
v	Speed (also called velocity). Component of M_{xy} out of phase with H_{xy}.
v_i	Initial reaction velocity.
$\langle v_2 \rangle$	Effective average solute velocity.
$\bar{v}$	Partial molar volume.
$\bar{v}_s$	Partial molar volume of pure solvent.
$\mathbf{v}$	Velocity.
$W(r)$	Radial distribution function of end-to-end distance.
$W(x,y,z)$	End-to-end distance distribution function.
W^0_{mic}	Repulsive part of μ^0_{mic}.
$(\bar{X}_i)$	Equilibrium concentration.
$\Delta(X_i)$	Difference between temporal and equilibrium concentration.
x_b	Bottom of cell.
x_m	Meniscus position.
y	General physical property.
y_D	Physical property of denatured state.
y_N	Physical property of native state.
$\bar{y}$	Fractional saturation of site.
$\bar{y}_F$	Fractional saturation with ligand F.
z	Charge on macromolecule or ion in units of e.
z_i	Ionic valence of ith ion.
α	Degree of association. Dimensionless binding parameter like $(F)/k_R$.
α_H	Hill constant.
β	Dimensionless binding parameter.
β_e	Bohr magneton.
β_n	Nuclear magneton.
β_s	Mandelkern–Flory–Scheraga parameter.
β'	Scheraga–Mandelkern parameter.

Symbol	Meaning
Γ	Parameter affecting relaxation amplitudes.
γ	Magnetogyric ratio. $(A)/K_{AR}$ binding parameter. Velocity gradient dv_x/dz.
λ_1, λ_2	Parameters composed of rate constants.
δ	Chemical shift parameter. Phase shift.
$\delta(x)$	Dirac delta function of argument x.
δ_1	Hydration (in grams per gram).
δ_{ij}	Kronecker delta.
ε	Dielectric constant. Molar decadic or residue extinction coefficient.
$\Delta\varepsilon$	Circular dichroism ($\varepsilon_L - \varepsilon_R$).
η	Solution viscosity.
η_0	Solvent viscosity.
η_{rel}	Relative viscosity.
η_{sp}	Specific viscosity.
$[\eta]$	Intrinsic viscosity.
Θ_i	Fractional saturation of ith site.
θ	Scattering angle. Fractional helicity.
$[\theta]$	Molar ellipticity.
$\underset{\sim}{\Lambda}$	Matrix of λ_i's.
λ	Eigenvalue. Wavelength. Kinetic decay time.
λ_j	jth kinetic decay time of jth eigenvalue.
μ_i	Chemical potential per mole.
μ^0_i	Standard chemical potential per mole.
$\hat{\mu}_i$	Chemical potential per gram.
$\hat{\mu}^0_i$	Standard chemical potential per gram.
μ^0_{mic}	Standard chemical potential of amphiphile in micelle.
μ^0_w	Standard chemical potential of amphiphile in aqueous phase.
μ_m	Magnetic moment.
$\underset{\sim}{\mu}$	Electric dipole moment operator.
v	Frequency. Simha factor in viscosity. Moles of ligand bound per mole of macromolecule.
v_N	Saturation density for lattice with N units.
π	Osmotic pressure.
ρ	Mass density (in grams per cm^3).
$\rho(\mathbf{r})$	Electron density.
σ	Nucleation constant.

Symbol	Meaning
σ_h	Superhelix density.
τ	Number of supercoils.
τ_F	Fluorescence decay time.
τ_a, τ_b	Rotational relaxation time for a-, b-axis orientation.
τ_c	Rotational correlation time.
τ_r	Rotational relaxation time of sphere.
τ, τ_j	Reaction relaxation times.
Φ	Electrical potential. Voltage difference.
Φ_c	Universal constant for random coils $2 \cdot 1 \times 10^{23}$.
ϕ	N–C′ torsional angle. Phase of complex number.
$\phi_{1a}, \phi_{20},$ etc	Monomer wave functions.
ϕ_F	Fluorescence quantum yield.
ϕ_p	Practical osmotic coefficient.
ϕ', ϕ''	Nucleic acid backbone torsional angles.
$[\phi]$	Molar rotation per residue.
χ	Mole fraction of all solute species.
χ_i	Mole fraction of ith component.
χ_A	Mole fraction of Ath component.
χ_{gc}	Mole fraction G + C.
χ	Glycosidic bond torsional angle.
ψ	C′–C torsional angle.
ψ', ψ''	Nucleic acid backbone torsional angles.
Ω_{jk}	Number of ways of putting k helical units into j separated sequences.
Ω_k	$(n - k + 1)$ number of ways of placing k helical units in one sequence within chain of n residues.
$\Omega_{n,i}$	Number of ways of assorting i items (ligands) in n boxes (sites).

Symbol	Meaning
ω	Circular frequency or angular velocity.
ω_0	Larmor frequency.
ω', ω''	Nucleic acid backbone torsional angles.
$\Delta\omega_{1/2}$	Line width.
ω	Angular velocity.
imag	Imaginary part of.
$\langle \rangle$	Average.
$\langle | \rangle$	Overlap integral.
$\langle \| \rangle$	Expectation value integral.
$*$	Superscript, complex conjugate, as in $F*$
$\|$	Amplitude of complex number or length of vector, as in $|F|$.
∇	Vector differential.
$(\ )$	Molar concentration, as in (A).
†	Superscript, transpose of matrix, as in $A^\dagger$.
⌒	Superscript, convolution product, as in $\widehat{AB}$.

General Rules

K	Macroscopic equilibrium constant.
k	Microscopic equilibrium constant or rate constant.
C	Molar concentration.
c	Weight concentration.
$\mathbf{M}$	All matrices and operators.
$\hat{\imath}$	All unit vectors.
R_G	Radius of gyration.
χ	Mole fraction.
Φ	Voltage or electrical potential.