

METHODS IN MOLECULAR BIOPHYSICS

Second Edition

Current techniques for studying biological macromolecules and their interactions are based on the application of physical methods, ranging from classical thermodynamics to more recently developed techniques for the detection and manipulation of single molecules. Reflecting the advances made in biophysics research over the past decade, and now including a new section on medical imaging, this new edition describes the physical methods used in modern biology.

All the key techniques are covered, including mass spectrometry, hydrodynamics, microscopy and imaging, diffraction and spectroscopy, electron microscopy, molecular dynamics simulations, and nuclear magnetic resonance. Each method is explained in detail using examples of real-world applications. Short asides are provided throughout to ensure that explanations are accessible to life scientists, physicists, and those with medical backgrounds.

The book remains an unparalleled and comprehensive resource for graduate students of biophysics and medical physics in science and medical schools, as well as for research scientists looking for an introduction to techniques from across this interdisciplinary field.

Nathan R. Zaccai is a Research Associate at the Cambridge Institute for Medical Research, University of Cambridge. His current research focuses on the molecular and thermodynamic basis of the transport and presentation at cell surfaces of proteins involved in pathogen evasion and host immunity.

Igor N. Serdyuk (1939–2012) was Professor of Molecular Biology and Head of the Laboratory of Nucleoprotein Physics at the Institute of Protein Research, Pushchino, Russia.

Joseph Zaccai is Directeur de Recherche Emeritus at the Centre Nationale de la Recherche Scientifique and Visiting Scientist at the Institut Laue-Langevin and Institut de Biologie Structurale, Grenoble. His current research interests include the exploration of the role of dynamics and physical chemical limits for life. He has many years of experience in teaching biophysics to biologists, medical students, and physicists.

REVIEWS FROM THE FIRST EDITION

I first asked what methods in molecular biophysics I would expect to use as a biochemist and structural biologist. This text book provides an introduction to the physics of each of [the techniques used by my own group] as well as a review of the applications. . . . [It] will be in demand by third year undergraduates in the many courses run by physicists to introduce them to biological themes. It would also be used by the many post-graduate students doing . . . research degrees as well as post-doctorals in chemical biology, biochemistry, cell biology and structural biology research groups. . . . In summary, this is a valuable contribution to the field. . . . this is an area which has advanced tremendously and the major texts in biophysical methods are now simply out of date. The text covers the methods that young researchers and some undergraduates will wish to learn. I am sure that it will find itself on the shelves of many laboratories throughout the world. There is nothing quite like it at the moment.

***Sir Tom Blundell FRS, FMedSci, Professor and Head, Department of Biochemistry,
University of Cambridge***

Thank you very much for giving me the opportunity to preview this wonderful text book. It has outstanding breadth while maintaining sufficient depth to follow modern experiments or initiate a deeper understanding of a new subject area. I love the 'Physicist's' and 'Biologist's Boxes' to address specific subjects for researchers with different backgrounds. This is one of the most comprehensive and highly relevant texts on biophysics that I have encountered in the last 10 years, clearly written and up-to-date. It is a must-have for biophysicists working in all lines of research, and certainly for me.

Nikolaus Grigorieff, Professor of Biochemistry, Brandeis University

[This is] a wonderful up-to-date treatise on the many and diverse methods used . . . in the fields of molecular biophysics, physical biochemistry, molecular biology, biological physics and the new and emerging field of quantum nanobiology. The wide range of methods available . . . in these multidisciplinary fields has been overwhelming for most researchers, students and scientists [who fail] to fully appreciate the utility and usefulness of the methods [other than their own]. [In many cases, this has] created disagreements and . . . controversy. The only way to understand and appreciate fully the problems in quantum nanobiology and their complexity is to utilize and fully understand the many diverse methods covered by the authors in this very fine treatise . . . [It] should be in the library of any serious researcher in the many diverse multidisciplinary fields working on problems in quantum nanobiology. . . . They will be greatly rewarded by an ability to see and view the problems and their complexity through different perspectives, aspects and points of view, . . .

***Karl J. Jalkanen, Associate Professor of Biophysics, Quantum Protein Centre,
Technological University of Denmark***

This most welcome text provides an up-to-date introduction to the vast field of biophysical methods. Written in an accessible style with an eye to a broad audience, it will appeal to biologists who wish to understand how to determine how macromolecules function and to scientists with a physics or physical chemistry background who wish to know how measurement of the physical world can impact our understanding of biological problems. The book succeeds in unifying disparate approaches under the aegis of developing an understanding of how macromolecules work.

Importantly, the text also provides the relevant historical background, an invaluable guide that will aid in the appreciation of what has gone before and should serve to orient them towards the future and what may be possible. It is a valuable resource for novice and seasoned biophysicists alike.

Dan Minor, California Institute for Quantitative Biomedical Research University of California, San Francisco

Methods in Molecular Biophysics is now the book I consult first when faced with an unfamiliar experimental technique. Both classic analytical techniques and the latest single-molecule methods appear in this single comprehensive reference.

Philip Nelson, Department of Physics, University of Pennsylvania, and author of Biological Physics

The authors provide an overview of many of the major recent accomplishments in the use of physical tools to investigate biological structure. There are interesting historical and biographical comments that lead the reader into understanding contemporary concepts and results. The book will be valuable both for students and research scientists.

Michael G. Rossmann, Hanley Professor of Biological Sciences, Purdue University

The melding of physics, chemistry and biology in modern science has changed our view of the natural world and opened avenues for detailed understanding of the origin of biological regulation. *Methods in Molecular Biophysics* provides an up-to-date view of classical biophysics, theory and practice of modern chemical biology and represents an essential text for the interdisciplinary scientist of the 21st Century. A great achievement and presentation awaits the student who reads this book, along with an excellent reference for the seasoned practitioner of biophysical chemistry.

Milton H Werner, Laboratory of Molecular Biophysics, The Rockefeller University

The methods, concepts, and discoveries of molecular biophysics have penetrated deeply into the fabric of modern biology. Physical methods that were once seemingly arcane are now commonplace in modern cell biology laboratories. This well written, thorough, and elegantly illustrated book provides the connections between molecular biophysics and biology that every aspiring young biologist needs. At the same time, it will serve physical scientists as a guide to the key ideas of modern biology.

Stephen H. White, Professor, Department of Physiology and Biophysics, University of California at Irvine

Methods in Molecular Biophysics offers a well-written, modern and comprehensive coverage of the properties of biological macromolecules and the techniques used to elucidate these properties. The authors have done a great service to the biophysics community in providing a long-needed update and expansion of previous texts on analysis of biological macromolecules. The choice and organization of material is especially well done. This book will be of considerable value not only to students, but also, due to the scope and breadth of coverage, to experienced researchers. I enthusiastically recommend *Methods in Molecular Biophysics* to anyone who wishes to know more about the techniques by which the properties of biological macromolecules are determined.

David Worcester, Department of Biological Sciences, University of Missouri – Columbia

METHODS IN MOLECULAR BIOPHYSICS

Structure, Dynamics, Function for Biology and Medicine
Second Edition

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To Ol’ga, Brinda, Missy

CONTENTS IN BRIEF

Contents	xi	E3 Raman Scattering Spectroscopy	288
Preface to the First Edition	xxi	E4 Optical Activity and Circular Dichroism	306
Preface to the Second Edition	xxiii		
<hr/>			
Introduction: Molecular Biophysics at the Beginning of the Twenty-First Century: From Ensemble Measurements to Single-Molecule Detection	1	Part F Optical Microscopy	323
<hr/>			
Part A Biological Macromolecules and Physical Tools	11	F1 Light Microscopy	325
A1 Macromolecules in Their Environment	13	F2 Single Molecule Manipulation and Atomic Force Microscopy	335
A2 Macromolecules as Physical Particles	25	F3 Fluorescence Microscopy	384
A3 Understanding Macromolecular Structures	43	<hr/>	
<hr/>			
Part B Mass Spectrometry	69	Part G X-ray and Neutron Diffraction	405
B1 Mass and Charge	71	G1 The Macromolecule as a Radiation Scattering Particle	407
B2 Structure Function Studies	85	G2 Small-Angle Scattering and Reflectometry	423
<hr/>			
Part C Thermodynamics	113	G3 X-ray and Neutron Macromolecular Crystallography	456
C1 Thermodynamic Stability and Interactions	115	<hr/>	
C2 Differential Scanning Calorimetry	126	Part H Electron Diffraction	487
C3 Isothermal Titration Calorimetry	141	H1 Electron Microscopy	489
C4 Surface Plasmon Resonance and Interferometry-Based Biosensors	149	H2 Three-Dimensional Reconstruction from Two-Dimensional Images	502
<hr/>			
Part D Hydrodynamics	159	Part I Molecular Dynamics	519
D1 Biological Macromolecules as Hydrodynamic Particles	161	I1 Energy and Time Calculations	521
D2 Analytical Ultracentrifugation	184	I2 Neutron Spectroscopy	531
D3 Fluorescence Depolarization	215	<hr/>	
D4 Dynamic Light Scattering and Fluorescence Correlation Spectroscopy	229	Part J Nuclear Magnetic Resonance	543
<hr/>			
Part E Optical Spectroscopy	251	J1 Distances and Angles from Frequencies	545
E1 Visible and IR Absorption Spectroscopy	253	J2 Experimental Techniques	566
E2 Two-Dimensional IR Spectroscopy	281	J3 Structure and Dynamics Studies	594
<hr/>			
		Part K Medical Imaging	627
		K1 Radiology and Positron Emission Tomography	629
		K2 Ultrasound Imaging	639
		K3 Magnetic Resonance Imaging	648

CONTENTS

<i>Preface to the First Edition</i>	xxi	A2.2 Biological Molecules and the Flow of Genetic Information	26
<i>Preface to the Second Edition</i>	xxiii	A2.3 Proteins	28
		A2.3.1 Chemical Composition and Primary Structure	28
		A2.3.2 Structures of Higher Order	29
Introduction: Molecular Biophysics at the Beginning of the Twenty-First Century: From Ensemble Measurements to Single-Molecule Detection	1	A2.4 Nucleic Acids	33
		A2.4.1 Chemical Composition and Primary Structure	33
1 A Brief History and Perspectives	1	A2.4.2 Structures of Higher Order	33
2 Languages and Tools	3	A2.5 Carbohydrates	34
3 Length and Timescales in Biology	4	A2.5.1 Chemical Composition and Primary Structure	36
4 The Structure–Function Hypothesis	4	A2.5.2 Higher-Order Structures	37
5 Complementarity of Physical Methods	5	A2.6 Lipids	38
6 Thermodynamics	6	A2.6.1 Chemical Composition	38
7 Hydrodynamics	6	A2.6.2 Higher-Order Structures	40
8 Radiation Scattering	7	A2.6.3 Lipids and Membrane Proteins	41
9 Spectroscopy	7	A2.7 Checklist of Key Ideas	41
10 Single-Molecule Detection	8	Suggestions for Further Reading	42
11 Biophysics and Medicine	10		
 PART A BIOLOGICAL MACROMOLECULES AND PHYSICAL TOOLS	 11	 A3 Understanding Macromolecular Structures	 43
 A1 Macromolecules in Their Environment	 13	A3.1 Historical Review	43
A1.1 Historical Review	13	A3.2 Basic Physics and Mathematical Tools	44
A1.2 Macromolecular Solutions	13	A3.2.1 Waves	44
A1.2.1 Concentration	13	A3.2.2 Simple Harmonic Motion	48
A1.2.2 Partial Volume	14	A3.2.3 Fourier Analysis	50
A1.2.3 Colligative Properties	14	A3.2.4 Quantum Mechanics	54
A1.2.4 Chemical Potential and Activity	15	A3.2.5 Measurement Space, Mathematical Functions, and Straight Lines	58
A1.2.5 Temperature	16	A3.3 Dynamics and Structure, Kinetics, Kinematics, Relaxation	58
A1.2.6 Osmotic Pressure	16	A3.3.1 Macromolecular Stabilization Forces	58
A1.2.7 Virial Coefficients	16	A3.3.2 Length and Timescales in Macromolecular Dynamics	59
A1.3 Macromolecules, Water, and Salt	18	A3.3.3 A Physical Model for Protein Dynamics	59
A1.3.1 Ionic Strength and Debye–Hückel Theory	19	A3.4 Checklist of Key Ideas	67
A1.3.2 Polyelectrolytes and the Donnan Effect	19	Suggestions for Further Reading	68
A1.3.3 Macromolecule–Solvent Interactions	20		
A1.3.4 Water, Salt and the Hydrophobic Effect	20	 PART B MASS SPECTROMETRY	 69
A1.4 Checklist of Key Ideas	23	 B1 Mass and Charge	 71
Suggestions for Further Reading	24	B1.1 Historical Review	71
 A2 Macromolecules as Physical Particles	 25	B1.2 Introduction to Biological Applications	72
A2.1 Historical Review and Biological Applications	25	B1.3 Ions in Electric and Magnetic Fields	73

B1.4	Mass Resolution and Mass Accuracy	73	PART C THERMODYNAMICS	113
B1.4.1	Mass Resolution	73		
B1.4.2	Molecular Mass Accuracy	74		
B1.5	Ionization Technique	74	C1 Thermodynamic Stability and Interactions	115
B1.5.1	From Ions in Solution to Ions in the Gas Phase	74	C1.1	Historical Overview and Biological Applications 115
B1.5.2	Laser Desorption, Matrix-Assisted Laser Desorption Ionization, and Photodissociation MS	76	C1.2	The Laws of Thermodynamics 116
B1.5.3	Electrospray Ionization (ESI)	77	C1.2.1	Fundamental Definitions and the Zeroth Law 117
B1.6	Instrumentation and Innovative Techniques	77	C1.2.2	The First Law and Energy 117
B1.6.1	Quadrupole Mass Filter	78	C1.2.3	The Second Law and Entropy 118
B1.6.2	Quadrupole Ion Trap	78	C1.2.4	The Third Law and Absolute Zero 119
B1.6.3	Ion Cyclotron Resonance Mass Spectrometry (ICR-MS)	78	C1.3	Useful Concepts and Equations 119
B1.6.4	Orbitrap Analyzer	80	C1.3.1	Free Energy and Allied Concepts 120
B1.6.5	TOF Mass Spectrometer	80	C1.3.2	Binding Studies 122
B1.6.6	Fourier Transform Mass Spectrometry (FT-MS)	81	C1.3.3	Calorimetry and Binding 123
B1.6.7	Tandem Mass Spectrometry (MS-MS)	82	C1.3.4	Activation Thermodynamics 124
B1.7	Checklist of Key Ideas	83	C1.4	Checklist of Key Ideas 124
	Suggestions for Further Reading	83		Suggestions for Further Reading 125
B2	Structure Function Studies	85	C2 Differential Scanning Calorimetry	126
B2.1	Proteins	85	C2.1	Historical Overview 126
B2.1.1	Mass Determination	85	C2.2	Basic Theory 126
B2.1.2	Proteomics	85	C2.3	Experimental Considerations 126
B2.1.3	Protein Sequencing	88	C2.3.1	Instrument Specifications 126
B2.1.4	Protein Folding and Dynamics	90	C2.3.2	Sensitivity of Heat Capacity Measurements 126
B2.2	Non-Covalent Complexes and Native (Top-Down) MS	93	C2.3.3	Sample Requirements 127
B2.2.1	Protein Complexes	93	C2.4	The Heat Capacity of Proteins 127
B2.2.2	Ribosomes, Ribosomal Subunits and Ribosomal Proteins	94	C2.4.1	The Heat Capacity Versus Temperature Curve 127
B2.3	Nucleic Acids	95	C2.4.2	Partition Function Analysis of the Heat Capacity Curve 128
B2.3.1	Oligonucleotide Mixture Analysis	99	C2.4.3	Two-State Transition: Calorimetric and Van't Hoff Enthalpies are Equal 128
B2.3.2	Non-Covalent Complexes	99	C2.4.4	Calorimetric and Van't Hoff Enthalpies are Not Equal: Cooperative Domains 129
B2.3.3	Large and Very Large Nucleic Acids	99	C2.4.5	Folding Intermediates and Effects of Mutations 129
B2.3.4	DNA Sequencing	100	C2.4.6	Complex Proteins 130
B2.3.5	Mass Spectrometry of RNA	101	C2.4.7	Solvent Effects on the Transition and the Absolute Partial Heat Capacity Difference Between Folded and Unfolded States of a Macromolecule 132
B2.4	Complex Carbohydrates	102	C2.4.8	Heat Capacity Calculations from Structural Data 132
B2.4.1	Oligosaccharides	102	C2.4.9	Protein Stabilization Forces 136
B2.4.2	Glycopeptides	102	C2.5	Nucleic Acids and Lipids 139
B2.5	Lipidomics and Membrane Protein Interactions	104	C2.6	Checklist of Key Ideas 139
B2.6	Mass Spectrometry in Medicine	104		Suggestions for Further Reading 140
B2.7	Bacteria and Bacterial Taxonomy	106	C3 Isothermal Titration Calorimetry	141
B2.8	Imaging Mass Spectrometry	107	C3.1	Historical Review 141
B2.8.1	Single-Cell Level	107	C3.2	Experimental Aspects and Equations 141
B2.8.2	Mammalian Tissue Level	108		
B2.9	Checklist of Key Ideas	109		
	Suggestions for Further Reading	110		

Contents

C3.2.1	Measuring Protocol and Samples	141	D1.5.3	Macroscopic Theory of Diffusion and Fick's Equations	169
C3.2.2	Binding Enthalpy and Heat Capacity	142	D1.5.4	Solutions to Fick's Equations	170
C3.2.3	Affinity Constants	142	D1.5.5	Experimental Methods for Directly Determining Diffusion Coefficients	171
C3.3	Applications	144	D1.6	Translational Friction and Diffusion Coefficients	172
C3.3.1	Entropic Versus Enthalpic Optimization	144	D1.6.1	Einstein–Smoluchowski Relation	172
C3.3.2	Relating Binding Energy and Structure	144	D1.6.2	Diffusion Coefficients of Biological Macromolecules	174
C3.3.3	Combining ITC and Other Biophysical Methods	144	D1.6.3	Dependence of the Diffusion Coefficient on the Molecular Mass of Globular Proteins	175
C3.4	Checklist of Key Ideas	148	D1.6.4	Dependence of the Diffusion Coefficient on the Molecular Mass of DNA	176
	Suggestions for Further Reading	148	D1.6.5	The Limits to Stokes' Law	176
C4	Surface Plasmon Resonance and Interferometry-Based Biosensors	149	D1.7	Hydrodynamic Experiments	176
C4.1	Historical Overview and Introduction to Biological Problems	149	D1.7.1	Measurement of Translational Frictional Coefficients	178
C4.2	Measuring Surface Binding	149	D1.7.2	Measurement of Rotational Frictional Coefficients	179
C4.2.1	Layout of a Biosensor	149	D1.7.3	Measurement of Viscosity	180
C4.2.2	SPR Biosensor	150	D1.7.4	Prediction of Hydrodynamic Properties	181
C4.2.3	Interferometers as Biosensors	151	D1.8	Checklist of Key Ideas	182
C4.2.4	Other Types of Biosensor	151		Suggestions for Further Reading	183
C4.2.5	Coupling Ligands to a Surface	152	D2	Analytical Ultracentrifugation	184
C4.3	Binding Between a Soluble Molecule and a Surface	152	D2.1	Historical Review	184
C4.3.1	Thermodynamics of Surface Interactions	152	D2.2	Instrumentation and Innovative Technique	185
C4.3.2	Measurement of the Equilibrium Constant	152	D2.2.1	Rotors and Cells	186
C4.3.3	The Determination of the k_{off} and k_{on} of an Interaction	153	D2.2.2	Optical Detection Systems	187
C4.4	Experimental Analysis	155	D2.2.3	Data Acquisition	188
C4.4.1	Scope of Analytes	155	D2.3	The Lamm Equation	189
C4.4.2	Experimental Controls and Pitfalls	155	D2.4	Solutions of the Lamm Equation for Different Boundary Conditions	190
C4.4.3	Cell–Cell Interactions	155	D2.4.1	Exact Solutions	190
C4.4.4	SPR and Mass Spectrometry	155	D2.4.2	Analytical Solutions	190
C4.5	Checklist of Key Ideas	156	D2.4.3	Numerical Solutions	192
	Suggestions for Further Reading	157	D2.5	Sedimentation Velocity	193
PART D	HYDRODYNAMICS	159	D2.5.1	Macromolecules in a Strong Gravitational Field	193
D1	Biological Macromolecules as Hydrodynamic Particles	161	D2.5.2	Determination of Sedimentation and Diffusion Coefficients from the Moving Boundary	195
D1.1	History and Introduction to Biological Problems	161	D2.5.3	Highly Heterogeneous Systems	196
D1.2	Hydrodynamics at a Low Reynolds Number	163	D2.5.4	Sedimentation Coefficients of Biological Macromolecules	198
D1.2.1	Reynolds Number	163	D2.5.5	Differential Sedimentation for Measuring Small Changes in Sedimentation Coefficients	198
D1.2.2	Movement at Low Reynolds Number	163	D2.6	Molecular Mass from Sedimentation and Diffusion Data	200
D1.3	Hydration	163	D2.7	Sedimentation Equilibrium	201
D1.4	Friction	165	D2.7.1	Molecular Mass	201
D1.4.1	“Stick” and “Slip” Boundary Conditions	165			
D1.4.2	Hydrodynamic Quantities	165			
D1.5	Diffusion	168			
D1.5.1	Translational Diffusion Coefficients	168			
D1.5.2	Microscopic Theory of Diffusion	169			

D2.7.2	Binding Constants	202	D4.4.1	Particles that are Small Compared to the Wavelength of the Incoming Light	236
D2.8	The Partial Specific Volume	204	D4.4.2	Rigid Particles of Dimension Comparable to the Wavelength of Light	237
D2.9	Density Gradient Sedimentation	204	D4.4.3	Flexible Macromolecules: DNA	238
D2.9.1	Velocity Zonal Method	204	D4.4.4	Macromolecules in Uniform Motion: Electrophoretic Light Scattering	239
D2.9.2	Equilibrium Sedimentation in a Density Gradient	206	D4.5	DLS under Non-Gaussian Statistics	241
D2.10	Molecular Shape from Sedimentation Data	207	D4.5.1	Scattering of a Small Number of Particles (Number Fluctuations)	241
D2.10.1	Homologous Series of Quasi-Spherical Particles: Globular Proteins in Water	207	D4.5.2	Cross-Correlation (Method of Two Detectors)	242
D2.10.2	Homologous Series of Random Coils: Proteins in Guanidine Hydrochloride	209	D4.5.3	Scattering of Single Particles	242
D2.10.3	From Slightly Flexible Rod to Nearly Perfect Random Coil: DNA	209	D4.6	Fluorescence Correlation Spectroscopy	243
D2.10.4	Ribosomal RNAs, Ribosomal Particles, and RNP Complexes	210	D4.6.1	General Principles of FCS	243
D2.11	Checklist of Key Ideas	213	D4.6.2	Basics and Applications	244
	Suggestions for Further Reading	214	D4.6.3	Dual-Color Fluorescence Cross-Correlation Spectroscopy	248
			D4.7	Checklist of Key Ideas	248
				Suggestions for Further Reading	249
D3	Fluorescence Depolarization	215			
D3.1	Historical Review	215	PART E OPTICAL SPECTROSCOPY	251	
D3.2	Introduction to Biological Problems	215			
D3.3	Theory of Fluorescence Depolarization	216	E1	Visible and IR Absorption Spectroscopy	253
D3.3.1	Fluorescence as a Physical Phenomenon	216	E1.1	Brief Historical Review and Biological Applications	253
D3.3.2	Lifetime of Fluorophore and Rotational Correlation Time	217	E1.2	Brief Theoretical Outline	254
D3.3.3	Steady-State Fluorescence Depolarization	217	E1.2.1	The Extinction Coefficient and Absorbance	255
D3.3.4	Time-Resolved Fluorescence Depolarization	219	E1.3	The UV-Visible Spectral Range	255
D3.4	Instrumentation	219	E1.3.1	UV-Visible Spectrophotometers and Measurement Strategies	257
D3.5	Depolarized Fluorescence and Brownian Motion	221	E1.3.2	UV Absorption Spectra of Proteins	258
D3.5.1	Steady-State or Static Polarization	221	E1.3.3	Visible Absorption Spectra of Protein-Associated Groups	260
D3.5.2	Fluorescence Anisotropy Decay Time	221	E1.3.4	UV Absorption Spectra of Nucleic Acids	265
D3.5.3	Rotational Correlation Time of Globular Proteins	222	E1.4	IR Absorption Spectroscopy	266
D3.6	Depolarized Fluorescence and Molecular Interactions	225	E1.4.1	IR Spectrometers	266
D3.7	Checklist of Key Ideas	227	E1.4.2	Molecular Vibrations	267
	Suggestions for Further Reading	228	E1.4.3	IR-Active and IR-Inactive Modes	268
			E1.4.4	Quantum Mechanical Treatment of Vibrations	268
			E1.4.5	Vibrational Modes of Polyatomic Molecules	270
D4	Dynamic Light Scattering and Fluorescence Correlation Spectroscopy	229	E1.4.6	Resolution Enhanced FTIR Spectra	271
D4.1	Historical Review	229	E1.4.7	From Amide Bands to Protein Secondary Structure	273
D4.2	Introduction to Biological Problems	230	E1.4.8	IR Difference Spectroscopy	274
D4.3	Dynamic Light Scattering as a Spectroscopy of Very High Resolution	231	E1.4.9	Time-Resolved IR Spectroscopy	276
D4.3.1	Fluctuations and Time-Correlation Functions	232	E1.4.10	DNA Conformation	277
D4.3.2	Measurements of the Dynamic Part of Scattered Light	233	E1.5	Checklist of Key Ideas	279
D4.3.3	Diffusion Coefficients from DLS	235		Suggestions for Further Reading	280
D4.4	Dynamic Light Scattering Under Gaussian Statistics	236	E2	Two-Dimensional IR Spectroscopy	281
			E2.1	Historical Review and Introduction to Biological Problems	281

Contents

E2.2	Linear and Multidimensional Spectroscopy	281	E4.3	Instruments	311
E2.3	Principles of 2D-IR Spectroscopy	281	E4.4	CD of Proteins	311
E2.3.1	Pump Probe Experiments	281	E4.4.1	Circular Dichroism of Protein Secondary Structures	311
E2.3.2	Selection Rules for Two-Dimensional Spectroscopy	282	E4.4.2	Near-UV CD and Protein Tertiary Structure	314
E2.3.3	NMR and 2D-IR Spectroscopy: Similarity and Difference	283	E4.4.3	Protein Folding	315
E2.4	From Amide Bands to Protein Tertiary Structure	283	E4.5	Nucleic Acids and Protein–Nucleic Acid Interactions	317
E2.4.1	Simulations of 2D-IR Spectroscopy	284	E4.5.1	RNA	317
E2.4.2	Determination of Peptide Structures	284	E4.5.2	DNA	318
E2.5	Checklist of Key Ideas	286	E4.5.3	Protein–Nucleic Acid Interactions	318
	Suggestions for Further Reading	287	E4.6	Carbohydrates	319
			E4.7	CD from IR Radiation to X-rays	320
			E4.8	Checklist of Key Ideas	320
				Suggestion for Further Reading	321
E3	Raman Scattering Spectroscopy	288			
E3.1	Historical Review and Introduction to Biological Problems	288	PART F	OPTICAL MICROSCOPY	323
E3.2	Classical Raman Spectroscopy	288			
E3.2.1	Raman Spectra	288	F1	Light Microscopy	325
E3.2.2	Frequency, Intensity, and Polarization	289	F1.1	Historical Review	325
E3.2.3	Raman Spectrometers and Raman Microscopes	290	F1.2	Light Microscopy Inside the Classical Limit	326
E3.2.4	Protein Secondary Structure from Raman Spectra	291	F1.2.1	The Standard Light Microscope	326
E3.2.5	Protein Conformational Dynamics in Solution and in Crystals	293	F1.2.2	The Problem of Contrast	328
E3.2.6	Conformation of DNA	293	F1.3	Subwavelength Resolution within the Restrictions of Geometrical Optics	329
E3.3	Resonance Raman Spectroscopy (RRS)	295	F1.3.1	Confocal Microscopy	329
E3.4	Surface Enhanced Raman Spectroscopy (SERS)	295	F1.4	Lensless Microscopy	331
E3.5	Vibrational Raman Optical Activity	296	F1.5	Checklist of Key Ideas	333
E3.5.1	Vibrational Circular Dichroism (VCD)	296		Suggestions for Further Reading	333
E3.5.2	Raman Optical Activity (ROA)	296			
E3.6	Differential Raman Spectroscopy	300	F2	Single Molecule Manipulation and Atomic Force Microscopy	335
E3.7	Time-Resolved Resonance Raman Spectroscopy	301	F2.1	Historical Review	335
E3.7.1	Light-Initiated Methods	301	F2.2	Nanoscale Manipulation in Biology	336
E3.7.2	Rapid Mixing Methods	303	F2.2.1	Optical Traps (Laser Tweezers)	336
E3.8	Checklist of Key Ideas	304	F2.2.2	Magnetic Traps (Magnetic Tweezers)	338
	Suggestions for Further Reading	304	F2.2.3	Cantilever in the Force-Measuring Mode of the AFM	339
			F2.2.4	Glass Microneedles	340
E4	Optical Activity and Circular Dichroism	306	F2.3	General Principles of AFM	340
E4.1	Historical Review and Introduction to Biological Problems	306	F2.3.1	The Tip: A Key Element of Scanning Force Microscopy	342
E4.2	Brief Theoretical Outline	307	F2.3.2	Imaging Modes	342
E4.2.1	Plane, Circularly, and Elliptically Polarized Light	307	F2.4	Imaging Biological Structures	344
E4.2.2	CD, Ellipticity, and ORD	308	F2.4.1	Imaging DNA	344
E4.2.3	Electronic Transitions, Dipole, and Rotational Strengths	309	F2.4.2	Imaging Proteins	345
E4.2.4	Rotational Strength and Structural Organization	310	F2.4.3	Biological Macromolecules at Work: High-Speed AFM	346
			F2.4.4	The AFM Probe as a Nanoscalpel	348

F2.4.5	Study of Crystal Growth	348
F2.5	Combination of NSOM and AFM	349
F2.6	Macromolecular Mechanics: Nanometer Steps and Piconewton Forces	350
F2.6.1	Linear Molecular Motors	351
F2.6.2	Rotary Molecular Motors	360
F2.6.3	The Bacteriophage ϕ 29 DNA Packaging Motor	363
F2.6.4	Molecular Motors and Brownian Motion	365
F2.6.5	Molecular Motors and the Second Law of Thermodynamics	366
F2.6.6	DNA Mechanics	366
F2.6.7	RNA Mechanics	373
F2.6.8	Protein Mechanics	375
F2.6.9	Deformation of Polysaccharides	379
F2.7	Checklist of Key Ideas	381
	Suggestions for Further Reading	382
F3	Fluorescence Microscopy	384
F3.1	Historical Review	384
F3.2	Fluorescence Microscopy Inside the Classical Limit	386
F3.2.1	The Standard Wide-Field Fluorescence Microscope	386
F3.2.2	Two-Photon Excited Microscopy	386
F3.2.3	Total Internal Reflectance Fluorescence Microscopy (TIRFM)	388
F3.3	Fluorescence Spectroscopy of Single Molecules	389
F3.3.1	Laser-Induced Fluorescence	389
F3.3.2	Labeling Schemes and Observable Values	390
F3.4	Increasing the Resolution of Fluorescence Microscopy	392
F3.4.1	4Pi-Confocal Microscopy	392
F3.4.2	Stimulated Emission Depletion Microscopy	393
F3.4.3	Standing-Wave Illumination Fluorescence Microscopy (SWFM)	394
F3.5	Fluorescence Resonance Energy Transfer	395
F3.5.1	FRET as a Spectroscopic Ruler in Static and Dynamic Regimes	395
F3.6	Green Fluorescent Protein	397
F3.6.1	GFP as a Conformational Sensor	399
F3.6.2	GFP as a Cellular Reporter	399
F3.7	Fluorescence Lifetime Imaging Microscopy (FLIM): Seeing the Machinery of Live Cells	400
F3.8	Photo-Activated Localization Microscopy and Stochastic Optical Reconstruction Microscopy	402
F3.9	Checklist of Key Ideas	402
	Suggestions for Further Reading	403

PART G	X-RAY AND NEUTRON DIFFRACTION	405
G1	The Macromolecule as a Radiation Scattering Particle	407
G1.1	Historical Review and Introduction to Biological Applications	407
G1.2	Radiation and Matter	408
G1.2.1	X-ray and Neutron Scattering	409
G1.2.2	Absorption	409
G1.2.3	Energy Momentum and Wavelength	409
G1.3	Scattering by a Single Atom (the Geometric View)	410
G1.3.1	Point Scattering: Scattering Length	411
G1.3.2	Cross-Sections and Sample Size	412
G1.4	Scattering Vector and Resolution	413
G1.5	Scattering by an Assembly of Atoms	414
G1.5.1	Coherent and Incoherent Scattering	414
G1.5.2	Elastic and Inelastic Scattering	414
G1.5.3	Summing Waves, Fourier Transformation, and Reciprocal Space	415
G1.5.4	The Phase Problem	416
G1.6	Solutions and Crystals	416
G1.6.1	One-Dimensional Crystals	416
G1.6.2	Two- and Three-Dimensional Crystals	417
G1.6.3	Disordered Systems	417
G1.7	Resolution and Contrast	418
G1.8	The Practice of X-ray and Neutron Diffraction	419
G1.8.1	Complementarity	419
G1.8.2	Sources and Instruments	419
G1.9	Checklist of Key Ideas	421
G2	Small-Angle Scattering and Reflectometry	423
G2.1	Theory of Small-Angle Scattering from Particles in Solution	423
G2.1.1	Dilute Solutions of Identical Particles	423
G2.1.2	The Scattering Curve at Small Q Values: The Guinier Approximation, the Forward Scattered Intensity, and Radius of Gyration	425
G2.1.3	Asymptotic Behavior of the Scattering Curve at Large Q Values: The Porod Relation	426
G2.1.4	The Full Scattering Curve: The Distance Distribution Function	427
G2.1.5	The Information Content in $p(r)$ and $I(Q)$ for a Monodisperse Solution of a Particle with a Well-Defined Envelope	428
G2.1.6	Polydisperse Solutions	430

Contents

G2.1.7	Interactions Between the Particles	430	G3.3.3	Crystallization Methods	461
G2.2	Models and Simulations	431	G3.3.4	Identifying Crystals and Precipitates: Crystal Shapes and Sizes	462
G2.2.1	From Structure to Scattering Curve	431	G3.3.5	Cryo-Crystallography and Cryo-Protectants	464
G2.2.2	From the Scattering Curve to a Set of Structures	435	G3.3.6	Crystal Mounting	464
G2.3	General Contrast Variation: Particles in Different Solvents “Seen” by X-rays and “Seen” by Neutrons	436	G3.3.7	Labeling	465
G2.3.1	Two-Component Particles and the Parallel Axes Theorem	436	G3.4	From Intensity Data to Structure Factor Amplitudes	466
G2.3.2	The Stuhrmann Analysis of Contrast Variation	438	G3.4.1	Data Collection and Processing	466
G2.3.3	Deuterium Labeling and Triangulation	440	G3.4.2	Indexing Bragg Reflections	466
G2.4	The Thermodynamics Approach in SAS	441	G3.4.3	Scaling the Reflection Intensities	467
G2.4.1	Fluctuations in Hydrodynamics and Scattering	441	G3.4.4	Twinning	467
G2.4.2	Relating the Thermodynamics and Particle Approaches	443	G3.4.5	Radiation Damage	467
G2.5	Interactions, Molecular Machines, and Membrane Proteins	443	G3.4.6	Determination of the Unit Cell Dimensions	467
G2.5.1	Aminoacyl tRNA Synthetase Interactions with tRNA	443	G3.4.7	Determination of the Space Group	468
G2.5.2	ATP, Solvent- and Temperature-Induced Structural Changes of the Thermosome	444	G3.4.8	Redundancy and Statistics	468
G2.5.3	Membrane Proteins	444	G3.4.9	Molecular Packing in the Unit Cell and the Patterson Function	469
G2.6	SAS Combined with Other Methods for a Global Structural Study	446	G3.5	Finding a Model to Fit the Data	470
G2.7	Size-Exclusion Chromatography		G3.5.1	The Model	470
G2.8	Reflectometry (or Reflectivity)	448	G3.5.2	Assessing Agreement Between the Model and the Data	470
G2.8.1	Background	448	G3.5.3	Assessing Agreement Between the Model and Chemistry	472
G2.8.2	Instrumental Set-up	451	G3.6	From the Data to the Electron Density Distribution: Initial Phase Estimate	473
G2.8.3	Examples	452	G3.6.1	Argand Diagram	473
G2.9	Checklist of Key Ideas	454	G3.6.2	Molecular Replacement	473
	Suggestions for Further Reading	455	G3.6.3	Direct Methods	474
			G3.6.4	Single and Multiple Isomorphous Replacement (SIR, MIR)	475
			G3.6.5	Single and Multiple Anomalous Dispersion (SAD, MAD)	475
			G3.7	From the Electron Density to the Atomic Model: Refinement of the Model – Phase Improvement	477
G3	X-ray and Neutron Macromolecular Crystallography	456	G3.7.1	Fitting to the Electron Density by Eye and by Hand	477
G3.1	Historical Review	456	G3.7.2	Minimization of a Target Function (Maximum Likelihood)	477
G3.2	From Crystal to Model	457	G3.7.3	Crystallographic Refinement Restraints	478
G3.2.1	Reciprocal Lattice, Ewald Sphere, and Structure Factors	457	G3.7.4	Refinement Procedures	479
G3.2.2	Space Group Symmetry	458	G3.7.5	Final Assessment of the Structure	482
G3.2.3	Electron Density	459	G3.7.6	Structural Genomics	482
G3.2.4	Technical Challenges and the Crystallographic Model	460	G3.8	Kinetic Crystallography	483
G3.3	Crystal Growth: General Principles Involved in the Transfer of a Macromolecule from Solution to a Crystal Form	460	G3.8.1	Trapping of Intermediate States	483
G3.3.1	Purity and Homogeneity	460	G3.8.2	Laue Diffraction and Time-Resolved Crystallography	483
G3.3.2	Crystallization Screens	461	G3.9	Neutron Crystallography	484
			G3.10	Checklist of Key Ideas	485
				Suggestions for Further Reading	486

PART H ELECTRON DIFFRACTION 487

H1 Electron Microscopy 489

H1.1	Historical Review	489
H1.2	Introduction to Biological Problems	490
H1.2.1	The Electron Microscope Image	490
H1.2.2	Applications of EM	490
H1.2.3	Techniques Covered	490
H1.3	Principles of Electron Diffraction and Imaging	490
H1.3.1	Properties of Electrons	490
H1.3.2	Electromagnetic Lens	491
H1.3.3	The Image Recorded by an Electron Microscope	491
H1.3.4	Transfer of Information from Sample to Image	492
H1.4	Electron Microscopes	494
H1.4.1	Electron Beam Generation	494
H1.4.2	Transmission and Scanning Electron Microscopes	494
H1.4.3	Electron Images	495
H1.4.4	Image Recording System	495
H1.5	Techniques in Specimen Preparation	496
H1.5.1	Specimen Support	496
H1.5.2	Negative Staining	497
H1.5.3	Freezing of the Sample	497
H1.6	Data Collection	498
H1.6.1	Factors to Consider during Data Collection	498
H1.6.2	Data from Single Particles	498
H1.6.3	Imaging Crystals and Helical Molecules	498
H1.6.4	Tomography	499
H1.7	Immunochemistry	500
H1.8	Checklist of Key Ideas	500
	Suggestions for Further Reading	501

H2 Three-Dimensional Reconstruction from Two-Dimensional Images 502

H2.1	EM in Biology	502
H2.1.1	Structural Biology with EM	502
H2.1.2	Examples of Electron Cryo-Microscopy Reconstructions	502
H2.2	EM Data Preparation	503
H2.2.1	Preliminary Analysis of the Image	503
H2.2.2	Particle Selection	503
H2.2.3	Correction for the Contrast Transfer Function	503
H2.3	Single-Particle Reconstruction Procedures	503
H2.3.1	Coordinate System	503
H2.3.2	Reconstruction from Projections	503
H2.3.3	Iteration Procedure: Reprojection Method	505

H2.3.4	Common Lines Reconstruction Procedure	505
H2.3.5	Polar Fourier Transform Reconstruction	505
H2.3.6	Real-Space Reconstruction Procedure	506
H2.3.7	Focal and Tilt Pairs	506
H2.4	Reconstruction Procedures for One- and Two-Dimensional Crystals	506
H2.4.1	Helical Reconstruction	506
H2.4.2	Electron Crystallography	507
H2.5	Classification Procedures	508
H2.5.1	Statistical Analysis	508
H2.5.2	Multivariate Statistical Analysis	508
H2.6	Determination of the Resolution	508
H2.6.1	Number of Images Required for a Reconstruction	508
H2.6.2	Definition of Resolution	509
H2.6.3	Over-Fitting and Validation of the EM Reconstruction	510
H2.7	Map Enhancement	510
H2.7.1	Symmetry	510
H2.7.2	Weighting	511
H2.7.3	Use of Other Structural Information	511
H2.8	Applications and Examples	512
H2.8.1	The Ribosome	512
H2.8.2	Icosahedral Viruses	512
H2.8.3	Microtubules	514
H2.8.4	Integral Membrane Proteins	514
H2.9	Checklist of Key Ideas	516
	Suggestions for Further Reading	517

PART I MOLECULAR DYNAMICS 519

I1 Energy and Time Calculations 521

I1.1	Historical Review of Biological Applications	521
I1.2	Dynamics, Kinetics, Kinematics, and Molecular Stabilization Forces	522
I1.3	Length and Timescales in Macromolecular Dynamics	522
I1.4	Normal Mode Analysis	522
I1.5	Molecular Dynamics Simulations	523
I1.5.1	Force Field	523
I1.5.2	Parameterization of the Force Field	523
I1.5.3	Potential Energy Surface and Energy Minimization	523
I1.5.4	Modeling the Solvent	524
I1.5.5	Typical Molecular Mechanics Simulation Protocol	525
I1.5.6	Analysis of Results	525
I1.6	Application Examples	526
I1.6.1	BPTI and Lysozyme	526
I1.6.2	Protein Folding	527
I1.6.3	Structure Refinement	527
I1.6.4	ATP Synthase: A Molecular Machine	527

Contents

I1.7	Checklist of Key Ideas	529	J2.5	Two-Dimensional NMR	579
	Suggestions for Further Reading	530	J2.5.1	Correlation Spectroscopy	580
			J2.5.2	Nuclear Overhauser Enhanced Spectroscopy	582
I2	Neutron Spectroscopy	531	J2.5.3	Transverse Relaxation-Optimized Spectroscopy	583
I2.1	Historical Overview and Introduction to Biological Applications	531	J2.6	Multi-Dimensional, Homo- and Heteronuclear NMR	585
I2.2	Theory	532	J2.7	Sterically Induced Alignment	586
I2.2.1	Momentum and Energy, Distance Traveled, and Time	532	J2.7.1	Residual Dipolar Couplings	586
I2.2.2	The Dynamic Structure Factor, Intermediate Scattering Function and Correlation Function	533	J2.7.2	Chemical Shift Anisotropy (CSA)	589
I2.2.3	Coherent and Incoherent Cross-Sections	533	J2.8	Isotope Labeling of Proteins and Nucleic Acids	590
I2.3	Applications	534	J2.8.1	Labeling Strategies for Proteins	590
I2.3.1	Energy and Time Resolution	534	J2.8.2	Labeling Strategies for RNA	592
I2.3.2	Space-Time Window	534	J2.9	Checklist of Key Ideas	592
I2.3.3	Q Dependence of the Elastic Intensity	535		Suggestions for Further Reading	592
I2.3.4	Quasi-Elastic Scattering and Diffusion	537	J3	Structure and Dynamics Studies	594
I2.3.5	Inelastic Scattering and Vibrations	537	J3.1	Structure Calculation Strategies from NMR Data	594
I2.4	Samples and Instruments	538	J3.2	Three-Dimensional Structure of Biological Macromolecules	595
I2.5	Checklist of Key Ideas	541	J3.2.1	Proteins	595
	Suggestions for Further Reading	541	J3.2.2	Nucleic Acids	598
			J3.2.3	Carbohydrates	600
			J3.3	Dynamics of Biological Macromolecules	602
			J3.3.1	Protein Dynamics from Relaxation Measurements	602
			J3.3.2	Protein Dynamics from Amide Proton Exchange	606
			J3.3.3	Dynamics of Slow Events: Translational Diffusion	606
			J3.4	Solid-State NMR	608
			J3.4.1	Solution and Solid-State NMR: Comparative Analysis	608
			J3.4.2	Solving Three-Dimensional Structures in Solid-State NMR	609
			J3.4.3	Atomic Structure of the Injection Needle Used by Pathogenic Bacteria	611
			J3.4.4	Rapid Proton-Detected NMR Assignment for Proteins with Fast MAS	612
			J3.5	NMR, X-ray Crystallography, Small-Angle X-ray and Neutron Scattering (SAXS and SANS)	613
			J3.5.1	Structure of Macromolecules in Crystal and in Solution: Comparative Studies	615
			J3.5.2	NMR in Structural Genomics and for Intrinsically Disordered Proteins	616
			J3.5.3	NMR Combined with SAXS and SANS	619
			J3.6	In-Cell NMR	620
			J3.7	Checklist of Key Ideas	624
				Suggestions for Further Reading	625

PART J NUCLEAR MAGNETIC RESONANCE **543**

J1 Distances and Angles from Frequencies **545**

J1.1	Historical Review	545
J1.2	Fundamental Concepts	547
J1.2.1	Quantum Mechanical Description	547
J1.2.2	Classical Mechanical Description	551
J1.2.3	Nuclear Environment Effects on NMR	555
J1.3	Checklist of Key Ideas	564
	Suggestions for Further Reading	564

J2 Experimental Techniques **566**

J2.1	Fourier Transform NMR Spectroscopy	566
J2.1.1	Principles	566
J2.1.2	The Fourier Transform NMR Spectrometer	568
J2.2	Single-Pulse Experiments	569
J2.2.1	Data Acquisition and Processing	569
J2.2.2	Free Induction Decay	570
J2.3	Multiple-Pulse Experiments	570
J2.3.1	The Inversion Recovery Method to Measure Spin-Lattice Relaxation Time T_1	572
J2.3.2	The Spin-Echo Effect to Measure T_2	572
J2.3.3	Polarization Transfer	575
J2.4	Nuclear Overhauser Enhancement	577

PART K MEDICAL IMAGING	627		
K1 Radiology and Positron Emission Tomography	629		
K1.1 Historical Review	629	K2.2 Ultrasound Waves	639
K1.2 Health Physics	630	K2.3 Health Physics, Absorption, and Attenuation of Ultrasound Waves in Biological Tissue	642
K1.2.1 Simple Rules to Protect the Medical Practitioner	630	K2.3.1 Effects of Ultrasound	642
K1.2.2 Ionizing Radiation Dose	631	K2.3.2 Attenuation	642
K1.2.3 Dosimetry	631	K2.4 Principles of Ultrasound Imaging	643
K1.2.4 Practice to Reduce Effective Dose in the Patient	632	K2.4.1 Imaging Mode	643
K1.3 Interaction of X-rays and γ-rays with Matter	632	K2.5 Checklist of Key Ideas	646
K1.3.1 Processes of X-ray and γ -ray Absorption in Matter	633	Suggestion for Further Reading	647
K1.3.2 Mass Attenuation Coefficient	634	K3 Magnetic Resonance Imaging	648
K1.4 Production of X-rays	634	K3.1 Introduction to MRI and Historical Review	648
K1.5 Detection of X-rays	634	K3.2 Principles of Nuclear Magnetic Resonance	649
K1.6 Principles of the CT Scan	635	K3.3 Principles of MRI	651
K1.7 Positron Emission Tomography	637	K3.3.1 Health Physics	652
K1.7.1 Principles of PET	637	K3.3.2 T_1 and T_2 Image Formation, Signal Localization, and Pulse Sequences	652
K1.8 Checklist of Key Ideas	638	K3.4 Brain Water, Functional MRI, and PET	656
Suggestion for Further Reading	638	K3.5 Checklist of Key Ideas	656
		Suggestions for Further Reading	657
K2 Ultrasound Imaging	639	<i>References</i>	658
K2.1 Historical Review	639	<i>Author Index</i>	672
		<i>Subject index</i>	676

PREFACE TO THE FIRST EDITION

André Guinier, whose fundamental discoveries contributed to the X-ray diffraction methods that are the basis of modern structural molecular biology, died in Paris at the beginning of July 2000, only a few weeks after it was announced in the press that a human genome had been sequenced. The sad coincidence serves as a reminder of the intimate connection between physical methods and progress in biology. Not long after, Max Perutz, Francis Crick and then David Blow, the youngest of the early protein crystallographers, passed away. The period marked the gradual closing of the era in which molecular biology was born and the opening of a new era. In what has been called the post-genome sequencing era, physical methods are now increasingly being called upon to play an essential role for the understanding of biological function at the molecular and cellular levels.

Molecular biophysics classical text books published in the previous decades have been overtaken not only by significant developments in existing methods such as those brought about by the advent of synchrotrons for X-ray crystallography or higher magnetic fields in NMR, but also by totally new methods with respect to biological applications, such as mass spectrometry and single molecule detection and manipulation. Our ambition in attempting this book was to be as up-to-date and exhaustive as possible. In their respective parts, we covered classical and advanced methods based on mass spectrometry, thermodynamics, hydrodynamics, spectroscopy, microscopy, radiation scattering, electron microscopy, molecular dynamics and NMR. But rapid progress in the field (we couldn't very well ask the biophysics community to stop working during the few years it takes to write and prepare a book!), and the requirement to keep the book to a manageable size meant that certain methods are either omitted or not perfectly up-to-date.

The key-word in molecular biophysics is *complementarity*. The Indian story of the six blind men and the elephant (see Frontispiece) is an appropriate metaphor for the field. Each of the blind men touched a different part of the elephant, and concluded on its nature: a big snake said the man who touched the trunk, the tusks were spears, its side a great wall, the tail a paint brush, the ears huge fans, the legs were tree trunks. We could add a seventh very short-sighted man to the story who can see the whole elephant but as a blurred grey cloud to illustrate diffraction methods. As we wrote in the Introduction, the ideal molecular biophysics method does not exist. It would be capable of observing not only the positions of atoms in molecules *in vivo*, but also the atomic motions and

conformational changes that occur as the molecules are involved in the chemical and physical reactions associated with their biological function, regardless of the timescale involved. No single experimental technique is capable of yielding this information. Each provides us with a partial field of view with its clear regions and areas in deep shadow. In the 21st century, physical methods have to cope with very complicated biological problems, whose solution will depend on the ability to transfer structural and functional knowledge from the operation of a single molecule to the cellular level, and then to the whole organism. The splendor and complexity of the task is humbling, but the challenge will be met.

We are deeply obliged to Professor Don Engelman of Yale University, USA, and Professor Pierre Joliot of the Institut de Biologie Physico Chimique, France, who agreed to write forewords for the book. Outstanding scientists and teachers, each is both major actor and observer in biophysical research and the development of modern biology. We are very grateful to Brinda Muthusamy who painted the frontispiece. Grateful thanks also to expert colleagues for critical discussions on the different methods: Martin Blackledge and the members of the NMR laboratory, Christine Ebel, Dick Wade, Hugues Lortat-Jacob, Patricia Amara, the members of the laboratory of mass spectrometry, all of the Institut de Biologie Structurale, France, Regine Willumeit of the GKSS, Forschungszentrum Geesthacht, Germany, Victor Aksenov of the Joint Institute of Nuclear Research, Russia, Lesley Greene, Christina Redfield, Guillaume Stewart-Jones, Yvonne Jones and David Stuart of the University of Oxford, UK, Jonathan Ruprecht and Richard Henderson of the Laboratory of Molecular Biology, UK, Simon Hanslip and Robert Falconer of the University of Cambridge, UK. We gratefully acknowledge support from the Radulf Oberthuer Foundation, Germany, the Institut de Biologie Structurale and the Institut Laue Langevin, France, and the Cyril Serdyuk Company, Ukraine. We are indebted to Genadiy Yenin of the Institute of Protein Research, Russia for drawing figures and scientific illustrations, and to Aleksandr Timchenko, Margarita Shelestova, Margarita Ivanova, Tatyana Kuvshinkina, and Albina Ovchinnikova (Institute of Protein Research, Russia) for technical assistance. And finally, we would like to thank all our colleagues, friends and families, and the staff of Cambridge University Press, who supported us with much patience, understanding and encouragement.

Igor N. Serdyuk, Nathan R. Zaccai, Joseph Zaccai
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PREFACE TO THE SECOND EDITION

As we wrote in the preface to the first edition, our ambition in attempting *Methods in Molecular Biophysics* was to be as up-to-date and exhaustive as possible, considering the rapid progress in the field. Judging by broad readers' responses, the book usefully fulfilled its mission. The historical introduction to each method and "physicist" and "biologist" boxes were especially appreciated. Criticism focused on the inclusion of methods which even if once important are no longer topical, and relative inattention to emerging methods that were subsequently proven to be very powerful. Scientific predictions are, of course, particularly difficult to make, especially as progress may come from difficult to foreknow technical breakthroughs. The development of new detector systems, which now permit approaching atomic resolution in cryo-electron microscopy, comes to mind. The unwieldy size and weight of the first edition also invited justified criticism (it is interesting to note that the Russian edition is in two tomes). In this second edition, we have chosen a different book format that we hope will be easier and more pleasant to handle. We have carefully gone through the text to reorganize, bring up-to-date, and prune each of the chapters. We have added a new section on medical imaging so that the book now includes the range of topics covered in most medical school biophysics courses.

To the list of colleagues gratefully acknowledged in the first edition preface, we would like to add Frank Gabel,

Institut de Biologie Structurale, Grenoble, for his critical reading of the first edition to suggest corrections and improvements, and expert colleagues who checked the updates, revisions, and additions in the second edition: Elisabetta Boeri Erba, Martin Blackledge, Dimitrios Skoufias of the Institut de Biologie Structurale, Grenoble; Harriet Crawley-Snowdon, James Edgar, Antoni Wrobel, of the Cambridge Institute for Medical Research, University of Cambridge; Antony Fitzpatrick, Laboratory of Molecular Biology, Cambridge; Massimo Antognozzi, School of Physics, University of Bristol; Lotte Stubkjaer Fog, Medical Physicist, Section for Radiotherapy, Oncology Clinic, Rigshospitalet, Copenhagen; Alberto Bravin, Bio-medical Beam Line, European Synchrotron Radiation Facility, Grenoble; Jeremy Smith, Governor's Chair and Director, University of Tennessee/Oak Ridge National Laboratory Center for Molecular Biophysics.

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It is with sadness that we recall the memory of Igor Serdyuk, our co-author, who died suddenly in Spring 2012.

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