

## Contents

Foreword to the Second Edition *XIII*

Introduction *1*

What is Biophysical Chemistry? – An Example from Drug  
Screening *1*

### Part One Basic Methods in Biophysical Chemistry *11*

**1 Basic Optical Principles** *13*

1.1 Introduction *13*

1.2 What Does the Electronic Structure of Molecules Look Like?  
Orbitals, Wave Functions and Bonding Interactions *15*

1.3 How Does Light Interact with Molecules? Transition Densities  
and the Transition Dipole Moment *20*

1.4 Absorption Spectra of Molecules in Liquid Environments.  
Vibrational Excitation and the Franck–Condon Principle *24*

1.5 What Happens After Molecules have Absorbed Light? Fluorescence,  
Nonradiative Transitions and the Triplet State *27*

1.6 Quantitative Description of all Processes: Quantum Efficiencies,  
Kinetics of Excited State Populations and the Jablonski Diagram *33*  
Problems *38*

Bibliography *39*

**2 Optical Properties of Biomolecules** *41*

2.1 Introduction *41*

2.2 Experimental Determination of Absorption and Fluorescence  
Spectra *41*

2.3 Optical Properties of Proteins and DNA *45*

2.3.1 Intrinsic Absorption and Fluorescence of Amino Acids,  
Peptides and Proteins *45*

2.3.2 Intrinsic Absorption of Nucleotides, DNA and RNA *47*

2.4 Optical Properties of Important Cofactors *49*

2.4.1	Haem	49
2.4.2	Nicotinamide Adenine Dinucleotides	52
2.4.3	Flavins	53
2.4.4	Chlorophylls	54
2.4.5	Carotenoids	56
	Problems	58
	Bibliography	58
<b>3</b>	<b>Basic Fluorescence Techniques</b>	<b>61</b>
3.1	Introduction	61
3.2	Fluorescent Labelling and Linking Techniques	61
3.2.1	Primary Amino Group Reactive Labels	63
3.2.2	Thiol Group Reactive Labels	64
3.2.3	Avidin–Biotin Techniques	65
3.2.4	His-Tag	66
3.2.5	Thiolinkers and Gold Surfaces	67
3.2.6	Fluorescent Proteins	67
3.3	Fluorescence Detection Techniques	68
3.4	Fluorescence Polarization Anisotropy	70
3.4.1	Principles and Theoretical Background	70
3.4.2	Application Example: Receptor–Ligand Interactions	78
3.4.3	Application Example: Estimation of Molecular Mass	79
3.4.4	Application Example: Enzyme Function and Kinetics	80
3.4.5	Application Example: Enzyme Inhibition, Activation and Regulation	83
3.5	Förster Resonance Energy Transfer	84
3.5.1	Principles and Theoretical Background	84
3.5.2	Application Examples	90
3.6	Fluorescence Kinetics	93
3.7	Fluorescence Recovery after Photobleaching	98
3.8	Biochemiluminescence	99
	Problems	100
	Bibliography	103
<b>4</b>	<b>Chiroptical and Scattering Methods</b>	<b>105</b>
4.1	Chiroptical Methods	105
4.1.1	Circular Dichroism (CD)	105
4.1.2	Optical Rotatory Dispersion	107
4.2	Light Scattering	109
4.2.1	Scattering of Light at Molecules Smaller than the Optical Wavelength	110
4.2.2	Scattering of Light at Particles Equal to or Larger than the Optical Wavelength	112
4.2.3	Dynamic Light Scattering	115
4.3	Vibrational Spectra of Biomolecules	115

Problems 118  
Bibliography 119

<b>5</b>	<b>Magnetic Resonance Techniques</b>	121
5.1	Nuclear Magnetic Resonance of Biomolecules	121
5.1.1	Principles	121
5.1.2	Theoretical Framework	123
5.1.3	Primary Information Deduced from NMR Spectra	125
5.1.4	Pulsed NMR Spectroscopy	126
5.1.5	Two-Dimensional NMR Spectroscopy	130
5.1.6	Correlated Spectroscopy (COSY)	130
5.1.7	Nuclear Overhauser Effect and NOESY Spectra	135
5.1.8	NMR-Based Structural Analysis of Biomolecules	138
5.2	Electron Paramagnetic Resonance	141
	Problems	145
	Bibliography	147

<b>6</b>	<b>Mass Spectrometry</b>	149
6.1	Introduction	149
6.2	MALDI-TOF	149
6.2.1	Ionization	149
6.2.2	Analyser	152
6.2.3	Detector	153
6.2.4	Signals and Signal Improvements	154
6.3	ESI-MS	156
6.3.1	Ionization	156
6.3.2	Analyser and Detection	158
6.3.3	Signals and Signal Improvements	161
6.4	Structural and Sequence Analysis Using Mass Spectrometry	163
	Problems	164
	Bibliography	165

## **Part Two Advanced Methods in Biophysical Chemistry** 167

<b>7</b>	<b>Fluorescence Microscopy</b>	169
7.1	Introduction	169
7.2	Conventional Fluorescence Microscopy	169
7.2.1	Confocal Fluorescence Microscopy	169
7.2.2	Laser Scanning Microscopy	174
7.2.3	Wide-Field Fluorescence Microscopy	174
7.3	Total Internal Reflection Fluorescence Microscopy	176
7.4	Light-Sheet Microscopy	178
	Problems	180
	Bibliography	181

<b>8</b>	<b>Super-Resolution Fluorescence Microscopy</b>	<b>183</b>
8.1	Stimulated Emission Depletion (STED) Microscopy	184
8.2	Photoactivated Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM)	187
8.3	3D Super-Resolution Fluorescence Microscopy	190
8.3.1	3D-STED	190
8.3.2	3D-PALM/STORM	191
8.4	Imaging of Live Cells	191
8.4.1	Observation Duration	192
8.4.2	Irradiation Intensity	193
8.4.3	Imaging Depths	194
8.4.4	Labelling Conditions	194
8.5	Multicolour Super-Resolution Fluorescence Microscopy	195
8.6	Structured Illumination Microscopy	195
8.7	SOFI	197
8.8	Final Comparison	199
	Problems	201
	Bibliography	202
<b>9</b>	<b>Single-Biomolecule Techniques</b>	<b>203</b>
9.1	Introduction	203
9.2	Optical Single-Molecule Detection	203
9.2.1	Application Example 1: Observation of the Rotation of Single ATPase Complexes	206
9.2.2	Application Example 2: Single-Molecule Observation of the Elementary Steps of Biomolecular Motors	209
9.3	Fluorescence Correlation Spectroscopy	213
9.3.1	Autocorrelation Analysis and Observable Key Parameters	214
9.3.2	Autocorrelation Analysis, Mathematical Background	220
9.3.3	Quantitative Determination of Important Parameters from Autocorrelation Curves	221
9.3.3.1	Relationship Between Volume and Molecular Mass of a Protein and its Diffusion Time	222
9.3.3.2	Two-Dimensional Diffusion and Active Transport	222
9.3.3.3	Mixtures of Fluorescing Particles	223
9.3.4	Further Correlation Effects	224
9.3.5	Cross-Correlation Analysis	226
9.4	Optical Tweezers	230
9.4.1	Theoretical Background	230
9.4.2	Application Examples	236
9.4.2.1	Unfolding of RNA and DNA Hairpins	236
9.4.2.2	RNA Polymerase	238
9.4.2.3	DNA-Polymerase	239
9.5	Atomic Force Microscopy of Biomolecules	240
9.5.1	Principle of an AFM	241

9.5.2	Application Examples	242
9.5.2.1	Unfolding of DNA Hairpins	242
9.5.2.2	Receptor–Ligand Binding Forces	244
9.5.2.3	Protein Unfolding	244
9.6	Patch Clamping	245
9.6.1	Ion Channels	245
9.6.2	Patch Clamp Configurations	246
	Problems	250
	Bibliography	254
<b>10</b>	<b>Ultrafast- and Nonlinear Spectroscopy</b>	<b>257</b>
10.1	Introduction	257
10.2	Nonlinear Microscopy and Spectroscopy	258
10.2.1	Multiphoton Excitation	258
10.2.2	Advantages and Disadvantages of Two-Photon Excitation in Fluorescence Microscopy	259
10.2.3	How are Nonlinear Optical Signals Observed From Biological Samples?	262
10.2.4	Further Distinct Properties and Advantages of Two-Photon Excitation	263
10.2.5	Wavemixing and Other Nonlinear Optical Techniques	266
10.3	Ultrafast Spectroscopy	270
10.3.1	Pump–Probe Spectroscopy	270
10.3.2	Application Example: Ultrafast Light-Harvesting and Energy Conversion in Photosynthesis	274
10.3.2.1	Chlorophyll <i>b</i> → Chlorophyll <i>a</i> → Reaction Centre Energy Flow	276
10.3.2.2	Carotenoid → Chlorophyll Energy Flow	278
	Problems	280
	Bibliography	282
<b>11</b>	<b>DNA Sequencing and Next-Generation Sequencing Methods</b>	<b>285</b>
11.1	Sanger Method	285
11.2	Next-Generation Sequencing Methods	287
11.2.1	Dye Sequencing (Approach I)	289
11.2.2	Sequencing by Ligation, Pyrosequencing and Ion Semiconductor Sequencing (Approaches II to IV)	292
11.2.2.1	Emulsion PCR	292
11.2.2.2	Sequencing by Ligation (Approach II)	294
11.2.2.3	Pyrosequencing (Approach III)	296
11.2.2.4	Ion Semiconductor Sequencing (Approach IV)	297
11.2.3	Single-Molecule Real-Time Sequencing (Approach V)	299
	Problems	300
	Bibliography	301
<b>12</b>	<b>Special Techniques</b>	<b>303</b>
12.1	Introduction	303

12.2	Fluorescing Nanoparticles	303
12.3	Surface Plasmon Resonance Detection	308
12.4	DNA Origami	310
12.5	DNA Microarrays	314
12.6	Flow Cytometry	317
12.7	Fluorescence <i>In Situ</i> Hybridization	319
12.8	Microspheres and Nanospheres	320
	Problems	321
	Bibliography	321
<b>13</b>	<b>Assay Development, Readers and High-Throughput Screening</b>	<b>323</b>
13.1	Introduction	323
13.2	Assay Development and Assay Quality	323
13.3	Microtitre Plates and Fluorescence Readers	326
13.4	Application Example: Drug Discovery and High-Throughput Screening	332
	Problems	336
	Bibliography	337
	<b>Index</b>	<b>339</b>