

CONTENTS

1 Protein Structure Prediction

Patrice Koehl

1.1.	Introduction	1
1.2.	Basic Principles of Protein Structure.....	3
1.3.	The Energetics of Protein Structure.....	8
1.4.	Homology Modeling	10
1.5.	Ab-Initio Protein Structure Prediction.....	20
1.6.	The CASP Experiment	25
1.7.	Conclusions	26

2 Molecular Modeling of Biomembranes: A How-to Approach

Allison N. Dickey and Roland Faller

2.1.	Introduction to Molecular Dynamics.....	35
2.2.	Specifics of the Molecular Modeling of Biomembranes	37
2.3.	Force Fields: Simulation Models.....	38
2.4.	Degree of Detail: Atomistic versus Coarse-Grained	39
2.5.	Visualizations	41
2.6.	Area per Molecule and Thickness, Comparison to X-Ray Data.....	41
2.7.	Order Parameters and Other Single-Lipid Properties	42
2.8.	Radial Distribution Functions.....	44
2.9.	Hydrogen Bonding and Advanced Static Analysis.....	44
2.10.	Pressure and Pressure Profiles.....	46
2.11.	Two-Dimensional Diffusion.....	46
2.12.	Reorientations and NMR.....	48
2.13.	Dynamics of Individual Molecules: Correlations of Distribution Functions.....	49
2.14.	Interactions with Small Molecules	49
2.15.	Summary	51

3 Introduction to Electron Paramagnetic Resonance Spectroscopy

Marcin Brynda

3.1.	Introduction	59
3.2.	Historical Background.....	60
3.3.	Basic Equations	61
3.4.	Spin Hamiltonian.....	63
3.5.	Continuous Wave (CW) versus Pulsed EPR Techniques	70
3.6.	EPR Instrumentation	75
3.7.	EPR Measurements in Solution and Solid State.....	79
3.8.	Time-Resolved EPR	82
3.9.	Dynamic Processes Observed by EPR	83
3.10.	Multifrequency Experiments	85
3.11.	Quantum Chemistry Calculations.....	88
3.12.	EPR in Biology and in Bioinorganic Chemistry.....	91

4 Theory and Applications of Biomolecular NMR Spectroscopy

James B. Ames

4.1.	Introduction	99
4.2.	Nuclear and Electronic Spin	100
4.3.	Quantum Description of Nuclear Spin	100
4.4.	Spin-State Populations in Ensembles	101
4.5.	Nuclear Shielding and Chemical Shift	103
4.6.	NMR Scalar Coupling	103
4.7.	Dipolar Coupling.....	104
4.8.	NMR Relaxation and Dynamics.....	105
4.9.	Neuronal Calcium Sensor Proteins.....	108
4.10.	NMR Structural Analysis of Ca ²⁺ -Myristoyl Switch Proteins	110
4.11.	Summary	114

5 FRET and Its Biological Application as a Molecular Ruler

Jie Zheng

5.1.	Introduction	119
5.2.	Distance Dependence of FRET Efficiency.....	121

5.3.	Spectra Overlap between FRET Pairs	122
5.4.	Orientation Factor.....	122
5.5.	Advantages of FRET for Biological Applications.....	123
5.6.	Donor Dequenching.....	125
5.7.	Enhanced Acceptor Emission.....	125
5.8.	Three-Cube FRET	126
5.9.	Spectra FRET	126
5.10.	Fluorescence Intensity Ratio between Donor and Acceptor	126
5.11.	Lifetime Measurements	127
5.12.	FRET Quantification through Photobleaching Rate Measurements	127
5.13.	Protein Complex Formation	128
5.14.	Subunit Stoichiometry of Protein Complex.....	129
5.15.	Binding of Ligands or Modulatory Molecules	129
5.16.	Conformational Rearrangements.....	130
5.17.	Intracellular Event Indicators	131

6 Introduction to Modern Techniques in Mass Spectrometry

Caroline S. Chu and Carlito B. Lebrilla

6.1.	Introduction	137
6.2.	Ionization Techniques (Ion Sources).....	138
6.3.	Electron Impact	138
6.4.	Chemical Ionization.....	139
6.5.	Electrospray Ionization.....	139
6.6.	Matrix-Assisted Laser Desorption/Ionization.....	141
6.7.	Fourier Transform Ion Cyclotron Resonance	143
6.8.	Time of Flight.....	146
6.9.	Tandem Mass Spectrometry	148
6.10.	Collision-Induced Dissociation	148
6.11.	Infrared Multiphoton Dissociation	149
6.12.	Electron Capture Dissociation	150
6.13.	Electron Transfer Dissociation	150
6.14.	Summary	150

7 Transmission Electron Microscopy and Computer-Aided Image Processing for 3D Structural Analysis of Macromolecules

Dominik J. Green and R. Holland Cheng

7.1.	The Transmission Electron Microscope	155
7.2.	Phase-Contrast Imaging.....	160
7.3.	The Contrast Transfer Function.....	161
7.4.	The Projection Theorem and Single-Particle Reconstruction.....	164
7.5.	Advantages of SPR over X-Ray Crystallography.....	166
7.6.	Sample Preparation.....	167
7.7.	Imaging Conditions	168
7.8.	Data Validation.....	168
7.9.	Data Selection and Preparation.....	169
7.10.	Multivariate Statistical Analysis.....	169
7.11.	Classification.....	172
7.12.	Angular Reconstitution.....	174
7.13.	Reference-Based Refinement	174
7.14.	Initial Model Generation	176
7.15.	Reconstruction Techniques	179
7.16.	Reliability Assessment	179
7.17.	Analysis of Heterogeneity	180
7.18.	Summary	181

8 Raman Spectroscopy of Living Cells

Tyler Weeks and Thomas Huser

8.1.	Introduction	185
8.2.	Raman Scattering: Inelastic Light Scattering by Molecular Bonds.....	186
8.2.1.	The Induced Dipole Moment.....	188
8.2.2.	Polarizability and Raman Scattering.....	189
8.2.3.	Spectroscopy.....	192
8.2.4.	Experimental Implementation of Micro-Raman Spectroscopy.....	196
8.2.5.	Signal Calibration.....	200
8.2.6.	Sample Preparation.....	201
8.2.7.	Advanced Forms of Raman Spectroscopy.....	202
8.3.	Summary	207

Problem Solutions.....	211
-------------------------------	------------

Index.....	231
-------------------	------------