Contents

Preface	XIII	
List of C	ontributors	XV

1	A Road Map to Single Molecule Dynamics 1	
	Yoshiharu Ishii	
1.1	Visualization of Single Molecules 1	
1.2	Single Molecule Position Tracking 1	
1.3	Single Molecules in Live Cells 2	
1.4	Fluorescence Spectroscopy and Biomolecular Dynamics 3	
1.5	Single Molecule Manipulation and Molecular Motors 4	
1.6	Mechano-Chemical Coupling of Molecular Motors 5	
1.7	DNA-Based Motors 5	
1.8	Imaging with AFM and Force Measurements 6	
	References 6	
2	Single Molecule Study for Elucidating the Mechanism Used by	
	Biosystems to Utilize Thermal Fluctuations 11	
	Toshio Yanagida	
2.1	Introduction 11	
2.1.1	Differences between Man-Made and Biological Molecular Machines 11	
2.1.2	Single Molecule Imaging and Nano-Detection 13	
2.2	Simultaneous Measurements of Individual ATP Hydrolysis Cycles	
	and Mechanical Events by a Myosin Motor 14	
2.2.1	ATP Hydrolysis Cycles 14	
2.2.2	Mechanical Events 16	
2.2.3	Simultaneous Measurements 16	
2.3	Resolving the Process of a Displacement by Scanning Probe	
	Nanometry 16	
2.3.1	Observation and Manipulation of a Single Myosin Motor 18	
2.3.2	Displacements 18	

Contents	
2.3.3	Sub-steps within a Displacement 20
2.3.4	Nature of Sub-steps 22
2.3.5	Comparing the Actions of Individual Myosin Motors with those of Muscle 22
2.3.6	
2.3.6	Other Types of Molecular Motors 24 Biased Brownian Step Model 27
2.4.1	Asymmetric Potential 27
2.4.1	Comparison with Other Studies 29
2.4.2	Computer Simulation: from a Single Molecular Motor to Muscle 31
2.4.3	Conclusion for the Unique Mechanism of Biological Molecular
2.3	Machines 33
	References 35
	References 33
3	Imaging and Molecular Motors 41
2.1	Yale E. Goldman
3.1	Introduction 41
3.2	Methods 42
3.2.1	Detection of Single Fluorophores 42 Sub-Diffraction Localization of Fluorescent Molecules 50
3.2.2 3.2.3	
	Darkfield Imaging with One Nanometer Accuracy (DIONA) 53
3.2.4	Single-molecule High Resolution Imaging with Photobleaching (SHRImP) 53
3.2.5	Single Molecule Fluorescence Resonance Energy Transfer
	(smFRET) 53
3.2.6	Orientation of Single Molecules 54
3.2.7	Polarized Total Internal Reflection Fluorescence Microscopy
	(polTIRF) 55
3.2.8	Defocused Orientational and Positional Imaging (DOPI) 57
3.3	Molecular Motors 58
3.3.1	Myosin V 60
3.3.2	Myosin II 65
3.3.3	Myosin VI 66
3.3.4	Conventional Kinesin 68
3.3.5	Other Kinesins 69
3.3.6	Dyneins 71
3.3.7	Single Molecule Intracellular Imaging 73
3.4	Conclusions 75
	References 76
4	Ion Channels 87
	Toru Ide, Minako Hirano, and Yuko Takeuchi
4.1	Introduction 87
4.2	Artificial Bilayers 88
4.2.1	Solid Supported Bilayers 88
4.2.2	Self-Standing Bilayers 89

4.3	Simultaneous Optical and Electrical Recording of the Single BK-Channels 92
4.4	Detection of Channel Conformational Change 95
4.5	"Optical Patch-Clamping" 95
4.6	Conclusion 96
	References 96
F	Signal Transduction across the Plasma Membrane 99
5	Signal Transduction across the Plasma Membrane 99 Masahiro Ueda, Tatsuo Shibata, and Yasushi Sako
5.1	Introduction 99
5.2	Signal Transduction Mediated by Receptor Tyrosine Kinase 99
5.2	Association between EGF and EGFR and Formation of the Signaling
3.3	Dimers of EGFR 100
5.4	Amplification and Propagation of EGFR Activation 104
5.5	Dynamics of the NGF/NGFR Complex 105
5.6	Stochastic Signal Processing and Transduction in Living Cells 108
5.7	Chemotactic Signaling System of Eukaryotic Cells 109
5.8	Stochastic Nature of Chemotactic Signaling Molecules 109
5.8 5.9	Stochastic Model of Transmembrane Signaling by Chemoattractant
3.9	Receptors 111
5.10	Conclusions 115
3.10	References 115
	References 113
6	Dynamics of Membrane Receptors: Single-molecule Tracking
6	Dynamics of Membrane Receptors: Single-molecule Tracking of Quantum Dot Liganded Epidermal Growth Factor 117
6	• • •
6	of Quantum Dot Liganded Epidermal Growth Factor 117
6 6.1	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke,
	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin
6.1	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117
6.1 6.2	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118
6.1 6.2 6.3	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118
6.1 6.2 6.3 6.4	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121
6.1 6.2 6.3 6.4 6.5	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122
6.1 6.2 6.3 6.4 6.5	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125
6.1 6.2 6.3 6.4 6.5 6.6	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126
6.1 6.2 6.3 6.4 6.5 6.6	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD-EGF-EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126
6.1 6.2 6.3 6.4 6.5 6.6	of Quantum Dot Liganded Epidermal Growth Factor 117 Guγ M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Treatments 126
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3 6.A.4	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Treatments 126 QD Conjugation to Epidermal Growth Factor 126
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3 6.A.4 6.A.5	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Treatments 126 QD Conjugation to Epidermal Growth Factor 126 Wide-field Microscopy 126
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3 6.A.4 6.A.5 6.A.6	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Lines 126 QD Conjugation to Epidermal Growth Factor 126 Wide-field Microscopy 126 PAM 127
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3 6.A.4 6.A.5 6.A.6	of Quantum Dot Liganded Epidermal Growth Factor 117 Guy M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD-EGF-EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Lines 126 QD Conjugation to Epidermal Growth Factor 126 Wide-field Microscopy 126 PAM 127 Hyperspectral Imaging 127
6.1 6.2 6.3 6.4 6.5 6.6 6.A.1 6.A.2 6.A.3 6.A.4 6.A.5 6.A.6 6.A.7	of Quantum Dot Liganded Epidermal Growth Factor 117 Guγ M. Hagen, Keith A. Lidke, Bernd Rieger, Diane S. Lidke, Wouter Caarls, Donna J. Arndt-Jovin, and Thomas M. Jovin Introduction 117 Single QD Imaging 118 Retrograde Transport of Activated EGFR Dimers 118 Single QD–EGF–EGFR Tracking 121 Programmable Array Microscopy 122 Concluding Remarks 125 Appendix 6.A: Materials and Methods 126 Reagents 126 Cell Lines 126 Cell Lines 126 QD Conjugation to Epidermal Growth Factor 126 Wide-field Microscopy 126 PAM 127 Hyperspectral Imaging 127 Appendix 6.B: Software and Image Processing 128

7	Studying the Dynamics of Ligand–Receptor Complexes by Single-Molecule Techniques 131
	Christophe Danelon and Horst Vogel
7.1	Introduction 131
7.2	Labeling Methods for Cell Surface Receptors 132
7.2.1	General Considerations 132
7.2.2	Suppressor tRNA Technology 134
7.2.3	O6-Alkylguanine–DNA Alkyltransferase (AGT) 134
7.2.4	Acyl-carrier Protein (ACP) 135
7.2.5	Nitrilotriacetate (NTA) 135
7.2.6	Reversible Sequential Labeling (ReSeq) 136
7.3	Functional Mobility of Receptors in Cell Membranes 136
7.3.1	Organization and Dynamics of Cell Membranes 136
7.3.2	Techniques 137
7.4	Investigating Kinetics and Thermodynamics of Ligand–Receptor Interactions by FCS 138
7.4.1	Principles 138
7.4.2	FCS at High Fluorophore Concentrations 150
7.5	Modulation of Ion Channel Current by Ligand Binding 151
7.5.1	Ligand-activated Ion Channels: Decoupling Ligand Binding and Channel Gating with Single-molecule Patch-clamp 151
7.5.2	The Nicotinic Acetylcholine Receptor as a Prototypical Example 151
7.5.3	Chemical Gating by Specific Ligand Binding inside Ion Channels 153
7.5.4	Facilitated Translocation of Sugars through Bacterial Porins 154
7.5.5	Combined Electrical and Fluorescence Measurements 157
7.6	Forces of Ligand–Receptor Interactions in Living Cells 157
7.6.1	Principles of Single-molecule Dynamic Force Spectroscopy and Applications to Cell Surface Receptors 157
7.6.2	Novel AFM-based Techniques 159
	References 166
8	RNA in cells 171
	Valeria de Turris and Robert H. Singer
8.1	Why Study RNA? 171
8.2	RNA Visualization inside Cells 172
8.2.1	Techniques to Label RNA 172
8.2.2	Advancements in Imaging Technologies 175
8.3	RNA Dynamics in the Nucleus 175
8.3.1	Dynamics in Transcription 176
8.3.2	A Journey from the Transcription Site to the Nuclear Envelope 177
8.3.3	Transport through the Nuclear Pore Complex 179
8.4	RNA Dynamics in the Cytoplasm 181
8.4.1	Non-localizing RNA 181
8.4.2	RNA Localization 182

8.4.2.1	Some Examples of Localization in Mammalian Cells and	
	Drosophila 183	
8.5	Conclusion 185	
	References 185	
9	Protein Dynamics and Interactions 191	
	Ted A. Laurence and Shimon Weiss	
9.1	Introduction 191	
9.1.1	The Single-molecule Approach to Protein Dynamics and Interactions 191	
9.1.1.1	Distributions of Subpoupulations 192	
9.1.1.2	Dynamics of Unsynchronized Trajectories 192	
9.1.1.3	Order of Events/States 192	
9.1.2	Example Biological Systems 193	
9.2	Fluorescence Spectroscopy as a Tool for Dynamic Measurements of Molecular Conformation and Interactions 194	
9.2.1	Jablonski Diagram (Intensity, Spectrum, Lifetime, Polarization) 194	
9.2.2	Point Emission-Localization Measurements 196	
9.2.3	Fluorescence Polarization-Measures Rotational Movement and Freedom	
	of Movement 197	
9.2.4	Fluorescence Resonance Energy Transfer-nm-scale Ruler 197	
9.2.5	Single-molecule Electron Transfer-Ångstrøm-scale Ruler 199	
9.3	Single-molecule Data Acquisition and Analysis Methods 200	
9.3.1	Choosing a Labeling Configuration: What is the	
	Observable? 200	
9.3.2	Should a Freely-diffusing or Immobilized Format be used? 202	
9.3.3	What Excitation/Optical Isolation Format should be used? 203	
9.3.3.1	Optical Isolation of a Single Point 205	
9.3.3.2	Multiple Points 207	
9.3.3.3	How many Excitation Lasers? 207	
9.3.3.4	Pulsed Laser Excitation 209	
9.3.4	What Detection Format should be used? 209	
9.3.5	Data Reduction and Analysis Methods 210	
9.3.5.1	Photon Streams and Films 210	
9.3.5.2	Time Traces 211	
9.3.5.3	Single-molecule Identification 211	
9.3.5.4	Histogram-based Analysis (Including Correlation Analysis) 212	
9.3.5.5	Analysis of Histograms of Single Molecules 213	
9.3.5.6	Single-molecule Sorting 213	
9.3.5.7	Trajectory Analysis of Single Molecules 214	
9.3.6	Modeling and Simulations of Single-molecule Experiments 214	
9.4	Examples 214	
9.4.1	Single-molecule Fluorescence Studies of Protein Folding and Conformations 215	
9.4.1.1	Observables for Protein Folding 215	

х	Contents	
-	9.4.1.2	Labeling Schemes for Protein Folding 215
	9.4.1.3	Equilibrium Unfolding Studies on Simple Model Two-state Folders 217
	9.4.1.4	Single-molecule Protein Folding under Non-equilibrium Conditions 219
	9.4.1.5	Monitoring Conformational Dynamics using Fluorescence Lifetime 220
	9.4.1.6	Single-pair FRET Studies on Immobilized Proteins 222
	9.4.1.7	Probing Biomolecular Dynamics via Fluorescence Quenching and Electron Transfer 223
	9.4.2	Single-molecule Measurements of DNA-processing Enzymes 225
	9.4.2.1	RNAP – Retention of Sigma 227
	9.4.2.2	RNAP – Abortive Initiation 229
	9.4.2.3	Future Directions 230
	9.5	Conclusion 231
		References 231
	10	Two Rotary Motors of ATP Synthase 237 Ryota lino and Hiroyuki Noji
	10.1	Introduction 237
	10.1.1	ATP Synthase: a Significant and Ubiquitous Enzyme in the Cell 237
	10.1.2	Boyer's Proposal and Walker's Crystal Structure 238
	10.2	Rotation of ATP Synthase 240
	10.2.1	Single-molecule Imaging of Rotation of F ₁ Driven by ATP Hydrolysis 240
	10.2.1.1	Strategy for Visualization of Rotation 240
	10.2.1.2	Large Torque Generated by F ₁ 240
	10.2.1.3	Steps in Rotation 241
	10.2.1.4	A Model of Cooperative Chemo-mechanical Coupling in Rotating F_1 243
	10.2.2	Single-molecule Manipulation of F ₁ Rotation 244
	10.2.2.1	Mechanical Activation of Pausing F ₁ 244
	10.2.2.2	Highly Coupled ATP Synthesis by F ₁ Forced to Rotate in the Reverse Direction 246
	10.2.3	Rotation of F_0F_1 or F_0 249
	10.2.3.1	Steps in the Rotation of F _o F ₁ driven by ATP hydrolysis 249
	10.2.3.2	Ratchet versus Power Stroke as the Driving Force of F _o Rotation 249
	10.2.3.3	Rotation of F _o F ₁ Driven by the Proton Motive Force 250
	10.3	Perspectives 251
		References 251
	11	Single-molecule FRET Studies of Helicases and Holliday
		Junctions 257
		Taekjip Ha
	11.1	Introduction 257

11.2	Single-molecule FRET 258
11.2.1	Non-perturbative Immobilization: BSA and PEG Surfaces, and
	Vesicle Encapsulation 258
11.3	smFRET Studies of Rep Helicase 259
11.3.1	Helicase: Essential Motor Proteins on the Nucleic Acid Highway 260
11.3.2	Single-molecule Techniques Applied to Helicase Studies 261
11.3.3	Different Types of smFRET Approaches to Probe Helicase
	Mechanisms 262
11.3.3.1	DNA–DNA FRET 262
11.3.3.2	Protein–DNA FRET 263
11.3.3.3	Repetitive Shuttling 263
11.3.3.4	ssDNA Flexibility 264
11.4	SmFRET Studies of Holliday Junction 265
11.4.1	Structure and Function of HJ 265
11.4.2	Conformer Transitions of Non-migratable HJ 266
11.4.2.1	Single-molecule Three-color FRET on HJ 268
11.4.3	Spontaneous Branch Migration Observed with a Single Step
	Resolution 269
11.5	Outlook 271
	References 271
12	High-speed Atomic Force Microscopy for Nano-visualization
	of Biomolecular Processes 277
	Toshio Ando, Takayuki Uchihashi, Noriyuki Kodera, Daisuke Yamamoto,
	Toshio Ando, Takayuki Uchihashi, Noriyuki Kodera, Daisuke Yamamoto, Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita
12.1	
12.1 12.2	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita
	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277
12.2	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278
12.2 12.3	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279
12.2 12.3 12.4	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280
12.2 12.3 12.4 12.5	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281
12.2 12.3 12.4 12.5 12.5.1	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282
12.2 12.3 12.4 12.5 12.5.1 12.5.2	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2 12.8	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290 Substratum 290
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2 12.8 12.9	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290 Substratum 290 Future Prospects 291 References 294
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2 12.8	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290 Substratum 290 Future Prospects 291 References 294 Force-clamp Spectroscopy of Single Proteins 297
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2 12.8 12.9	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290 Substratum 290 Future Prospects 291 References 294 Force-clamp Spectroscopy of Single Proteins 297 Lorna Dougan, Jasna Brujic, and Julio M. Fernandez
12.2 12.3 12.4 12.5 12.5.1 12.5.2 12.5.3 12.6 12.7 12.7.1 12.7.2 12.8 12.9	Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita Introduction 277 AFM Set-up and Operation 278 Imaging Rate and Feedback Bandwidth 279 Feedback Operation and Parachuting 280 Key Devices for High-Speed AFM 281 Small Cantilevers and Related Devices 282 Scanner 282 Dynamic PID Control 284 Bioimaging 284 Other Type of Imaging 289 Phase-contrast Imaging 289 Recognition Imaging 290 Substratum 290 Future Prospects 291 References 294 Force-clamp Spectroscopy of Single Proteins 297

XII Contents	
13.2.1	Force-extension Spectroscopy 299
13.2.2	Force-clamp Spectroscopy 300
13.3	Order Statistics in Unfolding 303
13.4	Disordered Free Energy Landscape 305
13.5	Protein Folding 306
13.5.1	The Force Quench Experiment 307
13.5.2	Developing a Model for Protein Folding 309
13.6	Force as a Probe of Protein Chemistry 313
13.7	Conclusions 315
	References 315

Index 323