Contents

Preface XIII
List of Contributors 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
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 Other Types of Molecular Motors 24
2.4 Biased Brownian Step Model 27
2.4.1 Asymmetric Potential 27
2.4.2 Comparison with Other Studies 29
2.4.3 Computer Simulation: from a Single Molecular Motor to Muscle 31
2.5 Conclusion for the Unique Mechanism of Biological Molecular Machines 33
References 35

3 Imaging and Molecular Motors 41
Yale E. Goldman
3.1 Introduction 41
3.2 Methods 42
3.2.1 Detection of Single Fluorophores 42
3.2.2 Sub-Diffraction Localization of Fluorescent Molecules 50
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

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
5.3 Association between EGF and EGFR and Formation of the Signaling 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
5.9 Stochastic Model of Transmembrane Signaling by Chemoattractant Receptors 111
5.10 Conclusions 115
References 115

6 Dynamics of Membrane Receptors: Single-molecule Tracking 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 Introduction 117
6.2 Single QD Imaging 118
6.3 Retrograde Transport of Activated EGFR Dimers 118
6.4 Single QD–EGF–EGFR Tracking 121
6.5 Programmable Array Microscopy 122
6.6 Concluding Remarks 125
Appendix 6.A: Materials and Methods 126
6.A.1 Reagents 126
6.A.2 Cell Lines 126
6.A.3 Cell Treatments 126
6.A.4 QD Conjugation to Epidermal Growth Factor 126
6.A.5 Wide-field Microscopy 126
6.A.6 PAM 127
6.A.7 Hyperspectral Imaging 127
Appendix 6.B: Software and Image Processing 128
6.B.1 Single Particle Tracking 128
6.B.2 Real Time Optically-sectioned Imaging with the PAM 128
References 129
7 Studying the Dynamics of Ligand–Receptor Complexes by Single-Molecule Techniques 131
Christophe Danezon 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 Forces of Ligand–Receptor Interactions in Living Cells 157
7.5.1 Principles of Single-molecule Dynamic Force Spectroscopy and Applications to Cell Surface Receptors 157
7.5.2 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
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 Subpopulations 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-Ångström-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
10 Two Rotary Motors of ATP Synthase 237
Ryota Uno 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 F₁ Driven by ATP Hydrolysis 240
10.2.1.1 Strategy for Visualization of Rotation 240
10.2.1.2 Large Torque Generated by F₁ 240
10.2.1.3 Steps in Rotation 241
10.2.1.4 A Model of Cooperative Chemo-mechanical Coupling in Rotating F₁ 243
10.2.2 Single-molecule Manipulation of F₁ Rotation 244
10.2.2.1 Mechanical Activation of Pausing F₁ 244
10.2.2.2 Highly Coupled ATP Synthesis by F₁ Forced to Rotate in the Reverse Direction 246
10.2.3 Rotation of F₀F₁ or F₀ 249
10.2.3.1 Steps in the Rotation of F₀F₁ driven by ATP hydrolysis 249
10.2.3.2 Ratchet versus Power Stroke as the Driving Force of F₀ Rotation 249
10.2.3.3 Rotation of F₀F₁ 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, Masaaki Taniguchi, Atsushi Miyagi, and Hayato Yamashita
12.1 Introduction 277
12.2 AFM Set-up and Operation 278
12.3 Imaging Rate and Feedback Bandwidth 279
12.4 Feedback Operation and Parachuting 280
12.5 Key Devices for High-Speed AFM 281
12.5.1 Small Cantilevers and Related Devices 282
12.5.2 Scanner 282
12.5.3 Dynamic PID Control 284
12.6 Bioimaging 284
12.7 Other Type of Imaging 289
12.7.1 Phase-contrast Imaging 289
12.7.2 Recognition Imaging 290
12.8 Substratum 290
12.9 Future Prospects 291
References 294

13 Force-clamp Spectroscopy of Single Proteins 297
Lorna Dougan, Jasna Brujic, and Julio M. Fernandez
13.1 Introduction 297
13.2 Single-protein AFM Techniques 298