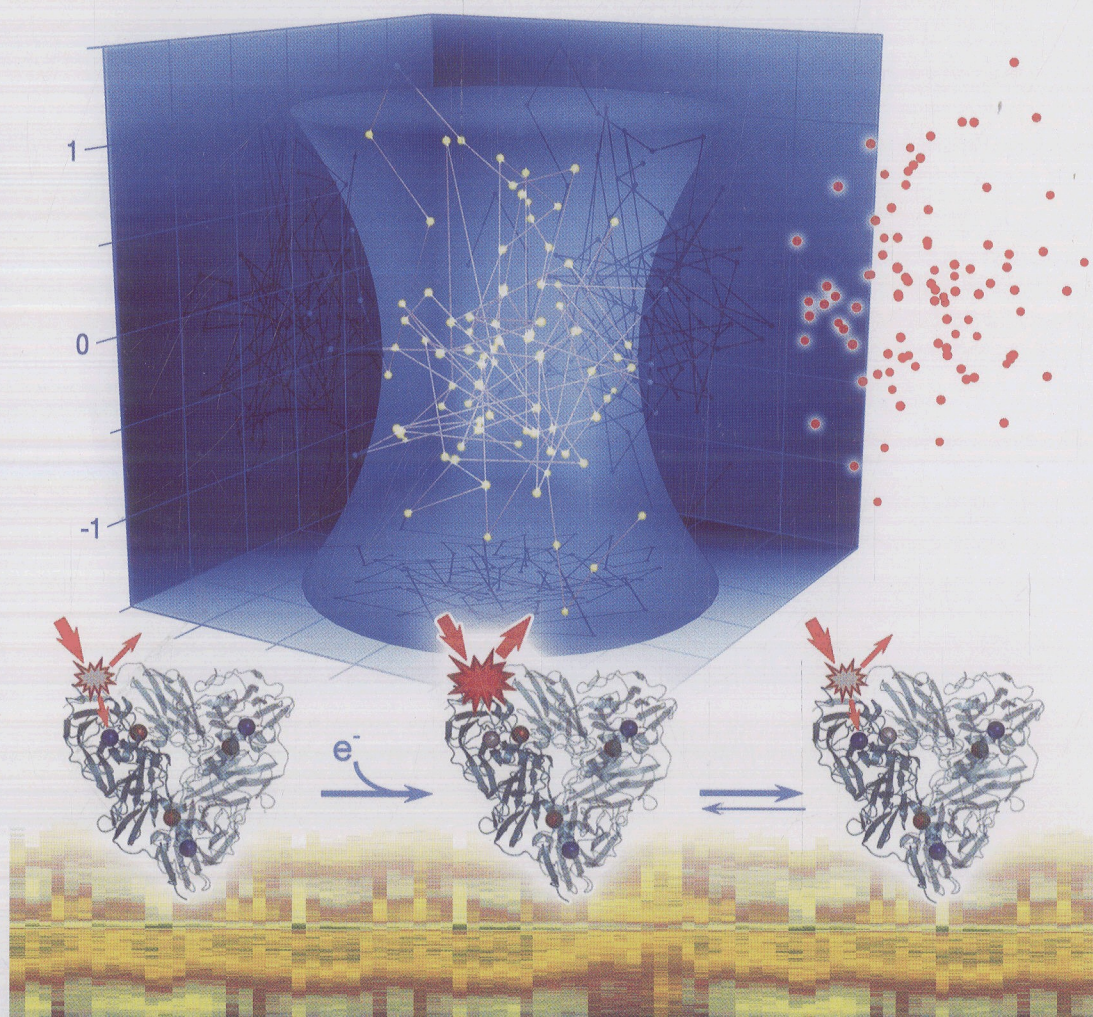


Edited by Christoph Bräuchle,
Don C. Lamb, and Jens Michaelis

 WILEY-VCH

Single Particle Tracking and Single Molecule Energy Transfer



Contents

Preface *XI*

List of Contributors *XIII*

Part I Single-Particle Imaging and Tracking *1*

1 Three-Dimensional Particle Tracking in a Laser Scanning Fluorescence Microscope *3*

Valeria Levi and Enrico Gratton

- 1.1 Introduction *3*
- 1.2 Image-Based Single-Particle Tracking Methods *5*
- 1.3 Advanced Fluorescence Microscopy Techniques for Single-Particle Tracking *7*
- 1.4 Two-Photon Excitation Microscopy *9*
- 1.5 3-D Tracking in Image-Based SPT Approaches *10*
- 1.6 3-D Tracking in Laser Scanning Microscopes *12*
- 1.7 Instrumentation *14*
- 1.8 Background Noise *15*
- 1.9 Simultaneous Two-Particle Tracking *15*
- 1.10 Application: Chromatin Dynamics in Interphase Cells *16*
- 1.11 Conclusions *18*
- References *20*

2 The Tracking of Individual Molecules in Cells and Tissues *25*

Laurent Holtzer and Thomas Schmidt

- 2.1 Introduction *25*
- 2.2 Single-Molecule and Single-Particle Localization *26*
- 2.3 Positional Accuracy *30*
- 2.4 Tracking *32*
- 2.5 Trajectory Analysis *33*
- 2.6 Applications *35*
- 2.7 Conclusions *40*
- References *40*

3	Messenger RNA Trafficking in Living Cells	43
	<i>Ulrich Kubitscheck, Roman Veith, Jörg Ritter, and Jan-Peter Siebrasse</i>	
3.1	Intranuclear Structure and Dynamics	43
3.2	FCS and FRAP Studies of Nuclear mRNP Mobility	44
3.3	Single-Particle Tracking of mRNA Molecules	44
3.3.1	The Aim and Purpose of Intranuclear Single mRNA Tracking	44
3.3.2	Single-Particle Tracking of “Designer” mRNPs	45
3.4	Single-Particle Tracking of Specific, Native mRNPs	47
3.4.1	Properties of <i>Chironomus tentans</i> Salivary Gland Cells	47
3.4.2	Measurement of Nuclear Viscosity	49
3.4.3	Mobility Measurements by Photobleaching	49
3.4.4	Analysis of Mobility by Single-Particle Tracking	50
3.4.5	Tracer Particles in Cell Nuclei	52
3.5	<i>In Vivo</i> Labeling of Native BR2 mRNPs	52
3.5.1	Mobility Analysis of BR2 mRNPs	53
3.5.2	BR mRNPs Diffuse in a Discontinuous Fashion	56
3.5.3	Verification of Particle Identity and High mRNP Mobility	57
3.5.4	A Discussion of mRNP Mobility	59
3.6	Outlook: Light Sheet-Based Single-Molecule Microscopy	61
	References	63
4	Quantum Dots: Inorganic Fluorescent Probes for Single-Molecule Tracking Experiments in Live Cells	67
	<i>Maxime Dahan, Paul Alivisatos, and Wolfgang J. Parak</i>	
4.1	Introduction	67
4.2	Fluorescent Labels for Single-Molecule Tracking in Cells	68
4.2.1	Organic Fluorophores	68
4.2.2	Fluorescent Proteins	68
4.2.3	Fluorescent Microspheres	70
4.2.4	Colloidal Quantum Dots	70
4.3	Optical Properties of Colloidal Quantum Dots	72
4.3.1	Absorption and Emission Properties	72
4.3.2	QDs as Fluorescent Biological Probes	73
4.3.3	Single Quantum Dot Detection	74
4.3.4	Fluorescence Intermittency of Individual Quantum Dots	75
4.4	Synthesis of Colloidal Fluorescent Quantum Dots	75
4.5	Surface Chemistry for the Water-Solubilization of Quantum Dots	77
4.5.1	General Strategies for Water-Solubilization	78
4.5.2	Solubilization with Ligand Exchange	79
4.5.3	Surface Coating with Amphiphilic Molecules	80
4.6	Interfacing Quantum Dots with Biology	81
4.6.1	Conjugation of QDs to Biomolecules	81
4.6.2	Cytotoxicity of Semiconductor QDs	83
4.7	Single Quantum Dot Tracking Experiments in Live Cells	83
4.7.1	Why Experiments at the Single Molecule Level?	83

4.7.2	Tracking Single Membrane Receptor Molecules	85
4.7.3	Visualizing Internalization Pathways	88
4.7.4	Tracking Intracellular Motor Molecules	89
4.7.5	From Single Molecules to Populations	90
4.8	Conclusions and Perspectives	91
	References	92

Part II Energy Transfer on the Nanoscale 97

5 Single-Pair FRET: An Overview with Recent Applications and Future Perspectives 99

Don C. Lamb

5.1	Introduction	99
5.2	Principles of FRET	100
5.3	spFRET in Solution	102
5.3.1	Experimental Considerations	103
5.3.2	Protein-Folding Kinetics	104
5.3.3	Chaperon-Assisted Protein Folding	106
5.3.4	Photon Distribution Analysis (PDA)	109
5.4	spFRET on Immobilized Molecules	111
5.4.1	Immobilization Methods	112
5.4.2	Mechanism of DNA Unwinding by the NS3 Helicase	115
5.4.3	Dynamics of the TBP-NC2-DNA Complex	116
5.4.4	Hidden Markov Modeling	119
5.5	Future Prospects	120
5.5.1	Three-Color FRET	121
5.5.2	FRET of Tracked Particles	123
	Abbreviations	124
	References	124

6 Alternating-Laser Excitation and Pulsed-Interleaved Excitation of Single Molecules 131

Seamus J. Holden and Achilles N. Kapanidis

6.1	Introduction	131
6.2	ALEX: The Principles of Operation	132
6.3	μ sALEX	137
6.3.1	Accurate FRET Using ALEX	138
6.3.2	Applications of μ sALEX	139
6.4	Nanosecond-ALEX/Pulsed Interleaved Excitation (PIE)	145
6.4.1	Applications of nsALEX/PIE	148
6.5	msALEX	151
6.5.1	Applications of msALEX	152
6.6	Three-Color ALEX	156
6.7	Conclusions and Outlook	157
	References	158

7	Unraveling the Dynamics Bridging Protein Structure and Function One Molecule at a Time	163
	<i>Jeffery A. Hanson, Yan-Wen Tan, and Haw Yang</i>	
7.1	Introduction	163
7.1.1	Rough Energy Landscape	165
7.1.2	Functional Roles of Conformational Dynamics	167
7.2	Converting Chemical Energy to Mechanical Work: Molecular Motors	168
7.2.1	F ₀ F ₁ ATP Synthase	169
7.2.2	Kinesin	169
7.3	Allostery in Proteins	173
7.3.1	Shift in Conformational Population	173
7.3.2	Ligand Binding and Recognition: Calmodulin	174
7.4	Enzyme Catalysis	177
7.4.1	Slowly-Varying Catalytic Rate: Single-Molecule Enzymology	177
7.4.2	Direct Measurement of Enzyme Conformational Dynamics	178
7.4.3	Mechanistic Roles of Enzyme Conformational Dynamics: Adenylate Kinase	181
7.5	Conclusions	186
	References	186
8	Quantitative Distance and Position Measurement Using Single-Molecule FRET	191
	<i>Jens Michaelis</i>	
8.1	Introduction	191
8.2	Fundamentals of FRET	192
8.3	FRET as a Spectroscopic Ruler: Initial Experiments and Limitations	194
8.4	Measuring the Quantum Yield	195
8.5	The Orientation of Donor and Acceptor Molecules	198
8.6	Accurate FRET Measurements Using Fluorescence Correlation Spectroscopy	201
8.7	FRET-Based Triangulation and the Nanopositioning System	203
8.8	Conclusions and Outlook	210
	References	211
	Part III Single Molecules in Nanosystems	215
9	Coherent and Incoherent Coupling Between a Single Dipolar Emitter and Its Nanoenvironment	217
	<i>Vahid Sandoghdar</i>	
9.1	Introduction	217
9.2	Systems	218
9.2.1	Single Molecules	219

9.2.2	Plasmonic Nanoparticles as Classical Atoms	219
9.3	Coupling of Two Oscillating Dipoles	221
9.3.1	Two Single Molecules	221
9.3.2	Two Plasmonic Nanoparticles	225
9.4	A Dipole Close to a Surface	228
9.4.1	A Molecule Close to a Metallic Surface	228
9.4.2	A Metallic Nanoparticle Close to a Dielectric Surface	230
9.4.3	A Dipole Senses its Nanoenvironment via the Modification of its Resonance Spectrum	231
9.5	A Single Molecule and a Single Nanoparticle	232
9.5.1	A Molecule Close to a Dielectric Nanoparticle	233
9.5.2	A Molecule Close to a Metallic Nanoparticle	235
9.6	Modification of the Spontaneous Emission and Quantum Efficiency by Nanoantennae	238
9.7	Conclusions	240
	References	240

10 Energy Transfer in Single Conjugated Polymer Chains 243

Manfred J. Walter and John M. Lupton

10.1	Introduction	243
10.2	Why Single Chain Spectroscopy?	245
10.3	Experimental Approach and Material Systems	247
10.4	Photophysics of Single Conjugated Polymer Chains	251
10.4.1	Single Chromophores	251
10.4.2	Interchromophoric Coupling: Energy Transfer	253
10.4.3	Blinking and Spectral Diffusion	255
10.4.3.1	Blinking	255
10.4.3.2	Spectral Diffusion	256
10.4.4	The Role of Chromophore Shape	257
10.5	Energy Transfer in Single Chains	258
10.5.1	Blinking	258
10.5.2	Polarization Anisotropy	261
10.5.3	Steady-State Spectroscopy	263
10.5.4	Time Domain Spectroscopy	265
10.5.5	Combined Fluorescence and Raman Scattering	265
10.6	Influence of Initial Excitation Energy on Energy Transfer	270
10.7	Conclusions	274
	References	275

11	Reactions at the Single-Molecule Level	281
	<i>Maarten B. J. Roelffaers, Gert De Cremer, Bert F. Sels, Dirk E. De Vos, and Johan Hofkens</i>	
11.1	Introduction	281
11.2	Biocatalysis at the Single-Molecule Level	281
11.2.1	Kinetics of Single Biocatalysts	282
11.2.1.1	Single Enzyme Studies	284
11.2.1.2	Individual Enzymatic Turnover Studies	286
11.2.2	Unraveling the Modes of Action of Individual Enzymes	294
11.2.2.1	Phospholipase A1 on Phospholipid Bilayers	294
11.3	Chemocatalysis at the Single-Molecule Level	295
11.3.1	Spatial Resolution	296
11.3.2	Sorption and Diffusion Studies on Chemocatalytic Materials	297
11.3.2.1	Intercrystalline Diffusion	297
11.3.2.2	Intracrystalline Diffusion at the Individual Particle Level	297
11.3.2.3	Intracrystalline Diffusion at the Single-Molecule Level	299
11.3.3	Chemical Transformation at the Single-Molecule Level	299
11.3.4	Conclusions	304
	References	305
12	Visualizing Single-Molecule Diffusion in Nanochannel Systems	309
	<i>Christophe Jung and Christoph Bräuchle</i>	
12.1	Introduction	309
12.2	Correlation of Structural and Dynamic Properties Using TEM and SMT	310
12.3	Phase Mixture	314
12.4	Heterogeneous Dynamics of a Single Molecule	316
12.5	Oriented Single Molecules with Switchable Mobility in Long Unidimensional Nanochannels	318
12.6	High Localization Accuracy of Single Molecules Down to the Single Channel Limit	320
12.7	Probing Chemical Interactions in Silica Thin Films Using Fluorescence Correlation Spectroscopy (FCS)	322
12.8	Functionalized Mesoporous Silica Structures	325
12.9	Single-Molecule Studies of Mesoporous Silica Structures for Drug-Delivery Applications	327
12.10	Conclusions and Outlook	329
	References	331