

Contents

Preface XV

List of Contributors XIX

Part One Background, Theory, and Concepts 1

1 How I Remember Theodor Förster 3

Herbert Dreeskamp

2 Remembering Robert Clegg and Elizabeth Jares-Erijman and Their Contributions to FRET 9

Thomas M. Jovin

2.1 Biographical Sketch of Bob Clegg 10

2.2 Biographical Sketch of Eli Jares-Erijman 11

2.3 The Pervasive Influence of Gregorio Weber 12

2.4 Contributions by Bob Clegg to FRET 12

2.5 Contributions by Eli Jares-Erijman to FRET 16

2.6 A Final Thought 18

References 19

3 Förster Theory 23

B. Wieb van der Meer

3.1 Introduction 23

3.2 Pre-Förster 23

3.3 Bottom Line 25

3.4 9000-Form, 9-Form, and Practical Expressions of the R_0^6 Equation 26

3.5 Overlap Integral 28

3.6 Zones 31

3.7 Transfer Mechanisms 33

3.8 Kappa-Squared Basics 34

3.9 Ideal Dipole Approximation 35

3.10 Resonance as an All-or-Nothing Effect 36

3.11 Details About the All-or-Nothing Approximation of Resonance 39

3.12	Classical Theory Completed	41
3.13	Oscillator Strength–Emission Spectrum Relation for the Donor	42
3.14	Oscillator Strength–Absorption Spectrum Relation for the Acceptor	43
3.15	Quantum Mechanical Theory	44
3.16	Transfer in a Random System	47
3.17	Details for Transfer in a Random System	48
3.18	Concentration Depolarization	51
3.19	FRET Theory 1965–2012	52
	References	59
4	Optimizing the Orientation Factor Kappa-Squared for More Accurate FRET Measurements	63
	<i>B. Wieb van der Meer, Daniel M. van der Meer, and Steven S. Vogel</i>	
4.1	Two-Thirds or Not Two-Thirds?	63
4.2	Relevant Questions	65
4.3	How to Visualize Kappa-Squared?	65
4.4	Kappa-Squared Can Be Measured in At Least One Case	68
4.5	Averaging Regimes	70
4.6	Dynamic Averaging Regime	72
4.7	What Is the Most Probable Value for Kappa-Squared in the Dynamic Regime?	76
4.8	Optimistic, Conservative, and Practical Approaches	83
4.9	Comparison with Experimental Results	85
4.10	Smart Simulations Are Superior	90
4.11	Static Kappa-Squared	92
4.12	Beyond Regimes	101
4.13	Conclusions	102
	References	103
5	How to Apply FRET: From Experimental Design to Data Analysis	105
	<i>Niko Hildebrandt</i>	
5.1	Introduction: FRET – More Than a Four-Letter Word!	105
5.2	FRET: Let’s get started!	106
5.3	FRET: The Basic Concept	107
5.4	FRET: Inevitable Mathematics	112
5.4.1	Förster Distance (or Förster Radius)	112
5.4.2	FRET Efficiency	113
5.4.2.1	Determination by Donor Quenching	113
5.4.2.2	Determination by Acceptor Sensitization	113
5.4.2.3	Determination by Donor Quenching and Acceptor Sensitization	114
5.4.2.4	Determination by Donor Photobleaching	115
5.4.2.5	Determination by Acceptor Photobleaching	115
5.4.3	FRET with Multiple Donors and/or Acceptors	116

5.5	FRET: The Experiment	118
5.5.1	The Donor–Acceptor FRET Pair	118
5.5.2	Förster Distance Determination	119
5.5.3	The Main FRET Experiment	122
5.5.3.1	Steady-State FRET Measurements	123
5.5.3.2	Time-Resolved FRET Measurements	130
5.5.3.3	Interpretation of Time-Resolved FRET Data	133
5.6	FRET beyond Förster	139
5.6.1	Time-Resolved FRET with Lanthanide-Based Donors	140
5.6.1.1	Terbium to Quantum Dot FRET Using Time-Resolved Donor Quenching and Acceptor Sensitization Analysis	141
5.6.2	BRET and CRET	147
5.6.3	Energy Transfer to Metal Nanoparticles (FRET, NSET, DMPET, NPILM, etc.)	148
5.6.4	Other Transfer Mechanisms	150
5.6.4.1	Electron Exchange Energy Transfer (Dexter Transfer)	151
5.6.4.2	Charge Transfer (Marcus Theory)	152
5.6.4.3	Plasmon Coupling	153
5.6.4.4	Singlet Oxygen Diffusion	154
5.7	Summary and Outlook	155
	References	156
6	Materials for FRET Analysis: Beyond Traditional Dye–Dye Combinations	165
	<i>Kim E. Sapsford, Bridget Wildt, Angela Mariani, Andrew B. Yeatts, and Igor Medintz</i>	
6.1	Introduction	165
6.2	Bioconjugation	166
6.3	Organic Materials	171
6.3.1	Ultraviolet, Visible, and Near-Infrared Emitting Dyes	171
6.3.2	Quencher Molecules	173
6.3.3	Environmentally Sensitive Fluorophores	175
6.3.4	Dye-Modified Microspheres/Nanomaterials	179
6.3.5	Dendrimers and Polymer Macromolecules	180
6.3.6	Photochromic Dyes	182
6.3.7	Carbon Nanomaterials	186
6.4	Biological Materials	188
6.4.1	Natural Fluorophores	188
6.4.2	Nonnatural Amino Acids	190
6.4.3	Green Fluorescent Protein and Derivatives	192
6.4.4	Light-Harvesting Proteins	200
6.4.5	DNA-Based Macrostructures/Nanotechnology	201
6.4.6	Enzyme-Generated Bioluminescence	201
6.4.7	Enzyme-Generated Chemiluminescence	209

6.5	Inorganic Materials	211
6.5.1	Luminescent Lanthanide Complexes and Doped Nano-/Microparticles	212
6.5.2	Luminescent Transition Metal Complexes	217
6.5.3	Noble Metal Nanomaterials (Gold, Silver, and Copper)	219
6.5.4	Silicon-Based Materials	222
6.5.5	Semiconductor Nanocrystals	223
6.6	Multi-FRET Systems	231
6.7	Summary and Outlook	236
	References	236

Part Two Common FRET Techniques/Applications 269

7	<i>In Vitro</i> FRET Sensing, Diagnostics, and Personalized Medicine	271
	<i>Samantha Spindel, Jessica Granek, and Kim E. Sapsford</i>	
7.1	Introduction	271
7.2	Small Organic Molecules and Synthetic Organic Polymers	272
7.3	Carbohydrate–Lipid	273
7.4	The Biotin–Avidin Interaction	273
7.5	Proteins and Peptides	275
7.5.1	Binding Proteins	275
7.5.2	Antigens and Epitope-Based Peptide Sequences	277
7.5.3	Peptide Sequences for Enzymatic Sensing	279
7.6	Antibodies	282
7.7	Nucleic Acid (DNA/RNA)	287
7.7.1	Molecular Beacons	288
7.7.2	Polymerase Chain Reaction and FRET	289
7.7.2.1	FRET Hybridization Probes	290
7.7.2.2	TaqMan	291
7.7.2.3	Scorpion Assay	292
7.7.2.4	Others	294
7.7.3	Isothermal Amplification Reactions and FRET	294
7.7.4	Clinical Applications of Nucleic Acid Detection Using FRET	295
7.7.4.1	Detection of Pathogens	295
7.7.4.2	Prognostic and Diagnostic Applications	296
7.7.4.3	Pharmacogenomics and Personalized Medicine	298
7.8	Aptamers	299
7.9	High-Throughput and Point-of-Care Devices	302
7.9.1	PoC Technology Advances	302
7.9.2	PoC Material Advances	304
7.10	Conclusions	305
	References	305

8	Single-Molecule Applications	323
	<i>Thomas Pons</i>	
8.1	Introduction	323
8.2	Single-Molecule FRET of Immobilized Molecules	324
8.2.1	Experimental Setup	324
8.2.1.1	Molecule Immobilization	324
8.2.1.2	Fluorophore Photostability	325
8.2.1.3	Optical Setup	326
8.2.2	Data Analysis	326
8.2.3	Applications	329
8.2.4	Analyzing Complex FRET Trajectories	334
8.3	Single-Molecule FRET of Freely Diffusing Molecules	336
8.3.1	Experimental Setup	336
8.3.2	Applications	337
8.3.3	Advanced Solution smFRET Methods	343
8.3.3.1	Alternate Laser Excitation	343
8.3.3.2	Multiparameter Fluorescence Detection	344
8.4	Single-Molecule FRET Studies Involving Multiple FRET Partners	346
8.4.1	Multistep FRET	347
8.4.2	Multi-Acceptor and Multi-Donor Systems	348
8.5	Conclusions and Perspectives	351
	References	353
9	Implementation of FRET Technologies for Studying the Folding and Conformational Changes in Biological Structures	357
	<i>Philip J. Robinson and Cheryl A. Woolhead</i>	
9.1	Introduction to Using FRET in Biological Systems	357
9.2	Förster Formalism in the Determination of Biological Structures	358
9.3	FRET Experiments in Complex Biological Systems	360
9.3.1	The Importance of Experimental Design	360
9.3.2	Site-Specific Labeling and Choosing the Most Effective FRET Pair	361
9.4	Biological Model System 1: The Ribosome	362
9.4.1	Intersubunit Rotation within the Ribosome	363
9.4.2	Dynamic Intrасubunit Movement Within the Ribosome	365
9.5	Biological System 2: Nascent Polypeptide Structure	365
9.6	Biological System 3: Chaperone-Mediated Protein Folding	368
9.6.1	Signal Recognition Particle	368
9.6.2	Trigger Factor	369
9.7	Biological System 4: Mature Protein Folding Intermediates	371
9.7.1	Unfolding Kinetics of Monellin	372
9.7.2	Intermediate Folding State of the Src Homology 3 Domain	374
9.8	Biological System 5: Intersubunit Distance in Multimeric Protein Complexes	375
9.9	Biological System 6: Protein–Protein Interactions in the Assembly of Protein Polymers	378

9.9.1	FtsZ Assembly and Subunit Exchange	379
9.9.2	Defining the Molecular Link in Serpin Polymers	380
9.10	Biological System 7: FRET in Nucleic Acid Systems	385
9.10.1	Determining the Structure and Configuration of DNA Junctions	386
9.10.2	Measuring the Opening and Closing of a Nanoscale DNA Box	388
9.10.3	FRET Between a DNA Polymerase and Its Substrate	390
	References	392

10 FRET-Based Cellular Sensing with Genetically Encoded Fluorescent Indicators 397

Jonathan C. Claussen, Niko Hildebrandt, and Igor Medintz

10.1	Introduction	397
10.2	Enzymes	399
10.2.1	Kinase Activity/Phosphorylation	399
10.2.2	Protease Activity	403
10.3	Metabolites	407
10.3.1	Sugars	407
10.3.2	Glutamate	410
10.4	Second Messengers	412
10.4.1	cAMP	412
10.4.2	cGMP	415
10.4.3	Nitric Oxide	417
10.4.4	Calcium	419
10.5	Conclusions	421
	References	423

Part Three FRET with Recently Developed Materials 431

11 FRET with Fluorescent Proteins 433

Hiofan Hoi, Yidan Ding, and Robert E. Campbell

11.1	Introduction to FPs	433
11.1.1	Wild-Type FPs	433
11.1.1.1	Natural Sources	433
11.1.1.2	Structure	434
11.1.1.3	Chromophore Formation	436
11.1.2	Engineered FPs for FRET Applications	438
11.1.2.1	Overview	438
11.1.2.2	Blue–Green FRET Pairs	440
11.1.2.3	Cyan–Yellow FRET Pairs	441
11.1.2.4	FRET with Orange, Red, and Far-Red FPs	443
11.1.2.5	Atypical FPs Useful for FRET Applications	445

11.1.3	Why Use FPs for FRET?	446
11.2	Using FPs for FRET Imaging	446
11.2.1	Photophysical Properties and Typical Förster Radii	446
11.2.1.1	Overview	446
11.2.1.2	Spectral Overlap	447
11.2.1.3	Orientation Factors	449
11.2.2	Potential Sources of Artifacts During FRET Imaging	450
11.2.2.1	Photobleaching	450
11.2.2.2	Photoconversion	451
11.2.2.3	pH Dependence	452
11.2.3	Biochemical and Structural Considerations	453
11.2.3.1	General Considerations when Labeling Proteins with FPs	453
11.2.3.2	Labeling Proteins for Intermolecular FRET Experiments	454
11.2.3.3	Labeling Proteins for Intramolecular FRET Experiments	454
11.2.3.4	FP Oligomerization and FRET Efficiency	455
11.2.4	Applications and Examples	458
11.2.4.1	Overview	458
11.2.4.2	FRET Biosensor Case Study	459
11.2.4.3	FRET between FPs and Other Donor or Acceptor Materials	460
11.3	Conclusions	462
	References	463
12	Semiconductor Quantum Dots and FRET	475
	<i>W. Russ Algar, Melissa Massey, and Ulrich J. Krull</i>	
12.1	Introduction	475
12.2	A Quick Review of FRET	476
12.3	Quantum Dots	477
12.3.1	A Brief History	478
12.3.2	The Structure of Quantum Dots: The Core	478
12.3.3	The Optical Properties of Quantum Dots	480
12.3.4	Overcoming the Limitations of Molecular Fluorophores	482
12.3.5	The Structure of Quantum Dots: The Shell	483
12.3.6	Quantum Confinement	485
12.3.7	Quantum Dot Photophysics	488
12.3.8	Quantum Dot Synthesis	491
12.3.9	Quantum Dot Coatings	493
12.3.10	Quantum Dot Bioconjugation	496
12.3.11	Quantum Dot Nomenclature in This Chapter	499
12.4	Quantum Dots and FRET	499
12.4.1	Quantum Dots as Donors	499
12.4.2	Applicability of the Förster Formalism	502
12.4.3	QDs as Acceptors	504
12.4.4	The Importance of Bioconjugate Chemistry	506
12.5	Quantum Dots as Donors in Biological Applications	508
12.5.1	Association and Dissociation to Modulate QD-FRET	508

12.5.1.1	Bioanalysis of Carbohydrates	509
12.5.1.2	Homogeneous Immunoassays	510
12.5.1.3	Hybridization Assays	511
12.5.1.4	Bioanalyses Using Aptamers and DNazymes	516
12.5.1.5	Bioanalysis of Hydrolytic Enzymes	519
12.5.1.6	Gene Delivery	524
12.5.2	Changes in Distance to Modulate QD-FRET	524
12.5.3	Conformational Insights from QD-FRET	528
12.5.4	Dynamic Modulation of the Spectral Overlap Integral and QD-FRET	530
12.5.5	Single-Pair QD-FRET	534
12.5.6	Solid-Phase QD-FRET	540
12.5.6.1	Biomolecular Surface Tethers	542
12.5.6.2	Chemical Conjugation to an Interface	544
12.5.6.3	Interfacial Ligand Exchange	545
12.5.6.4	Electrostatic Immobilization	547
12.5.6.5	Advantages of Immobilized QDs	548
12.5.7	Photodynamic Therapy	549
12.6	Quantum Dots as Acceptors in Biological Applications	552
12.6.1	Chemiluminescence Resonance Energy Transfer (CRET)	553
12.6.2	Bioluminescence Resonance Energy Transfer (BRET)	555
12.6.3	Lanthanide Donors	560
12.6.4	Quantum Dot Donors (for Quantum Dot Acceptors)	565
12.7	Energy Transfer between Quantum Dots and Other Nanomaterials	569
12.7.1	Gold Nanoparticles	569
12.7.2	Carbon Nanomaterials	575
12.7.2.1	Graphene and Graphene Oxide	575
12.7.2.2	Carbon Nanotubes	577
12.8	Nonbiological Applications of Quantum Dots and FRET	578
12.8.1	Photovoltaic Cells	580
12.8.2	Light-Emitting Diodes (LEDs)	582
12.9	Summary	583
	References	584

13 Multistep FRET and Nanotechnology 607

Bo Albinsson and Jonas K. Hannestad

13.1	Introduction	607
13.2	Fundamentals of Multistep FRET	608
13.2.1	Hetero-FRET	609
13.2.2	Multicolor FRET and Alternating-Laser Excitation	611
13.2.3	Homo-FRET	612
13.3	Energy Transfer in Photosynthesis	615
13.4	Photonic Wires and Multistep FRET in Nanotechnology	617
13.4.1	Photonic Wires	617
13.4.2	Beyond Wires	628

13.4.3	Light Harvesting	632
13.4.4	Functional Control	638
13.4.5	Quantum Dots in Multistep FRET	641
13.4.6	Potential Outputs and Uses for Channeled Excitation Energy	643
13.5	Summary	647
13.6	Note Added in Proof	648
	References	648

Part Four Supporting Information and Conclusions 655

14	Data	657
	<i>Alice G. Byrne, Matthew M. Byrne, George Coker III, Kelly B. Gemmill, Christopher Spillmann, Igor Medintz, Seth L. Sloan, and B. Wieb van der Meer</i>	
14.1	Tables before 1987	658
14.2	Introduction to the Table of Traditional Chromophores	658
14.3	Förster Distances and Other FRET Data before 1994	703
14.4	Förster Distances for Traditional Probes More Recent Than 1993	703
14.5	FRET Data on Fluorescent Proteins	703
14.6	FRET Data on Quantum Dots	742
14.7	Donor–Acceptor Pairs with a Förster Distance in a Given Range	742
14.8	Table–Reference Directory	744
	References	745
15	Outlook on FRET: The Future of Resonance Energy Transfer	757
15.1	A Rosy Crystal Ball View of FRET	757
	<i>Thomas M. Jovin</i>	
15.2	Do Not Ask What FRET Can Do for You, Ask What You Can Do for FRET	757
	<i>B. Wieb van der Meer</i>	
15.3	FRET: Future Research with an Exciting Technology	758
	<i>Niko Hildebrandt</i>	
15.4	Future of FRET	760
	<i>Kim E. Sapsford</i>	
15.5	Outlook on Single-Molecule FRET	760
	<i>Thomas Pons</i>	
15.6	Outlook on FRET with fluorescent proteins	761
	<i>Robert E. Campbell</i>	
15.7	Luminescent Nanoparticles: Scaffolds for Assembling “Smarter” FRET Probes	762
	<i>W. Russ Algar</i>	
	References	764
	Index	767

