

Contents

Contributors	xiii
Preface	xxi
CHAPTER 1 Spatial Intensity Distribution Analysis (SpIDA): A New Tool for Receptor Tyrosine Kinase Activation and Transactivation Quantification	1
Annie Barbeau, Jody L. Swift, Antoine G. Godin, Yves De Koninck, Paul W. Wiseman, Jean-Martin Beaulieu	
Introduction	2
1.1 Theory of Spatial Intensity Distribution Analysis.....	4
1.2 SpIDA: Examples of Application to RTK	4
1.3 Procedure for SpIDA	6
1.4 Discussion	17
Acknowledgments	18
References.....	18
CHAPTER 2 Dimerization of Nuclear Receptors	21
Pierre Germain, William Bourguet	
Introduction	22
2.1 Methods.....	24
Acknowledgments	39
References.....	39
CHAPTER 3 Network Analysis to Uncover the Structural Communication in GPCRs	43
Francesca Fanelli, Angelo Felling, Francesco Raimondi	
Introduction	44
3.1 Materials.....	45
3.2 Methods.....	45
3.3 Discussion	50
Summary	56
Acknowledgments	59
References.....	59
CHAPTER 4 Simulating G Protein-Coupled Receptors in Native-Like Membranes: From Monomers to Oligomers	63
Ramon Guixà-González, Juan Manuel Ramírez-Anguita, Agnieszka A. Kaczor, Jana Selent	
Introduction	64

4.1	Membranes	65
4.2	GPCR Monomers in Membranes.....	72
4.3	GPCR Dimers and Oligomers in Membranes	80
	References.....	83
 CHAPTER 5 Structure-Based Molecular Modeling Approaches to GPCR Oligomerization		
	Agnieszka A. Kaczor, Jana Selent, Antti Poso	
	Introduction	92
5.1	Protein-Protein Docking.....	93
5.2	MD Simulation.....	96
5.3	Normal Mode Analysis	99
5.4	Electrostatics Studies	100
	Acknowledgments	101
	References.....	102
 CHAPTER 6 Biochemical and Imaging Methods to Study Receptor Membrane Organization and Association with Lipid Rafts		
	Bruno M. Castro, Juan A. Torreno-Piña, Thomas S. van Zanten, Maria F. Gracia-Parajo	
	Introduction and Rationale.....	106
6.1	Methods to Determine Protein Association with Lipid Rafts.....	107
6.2	Optical Techniques to Study Receptor Membrane Organization and Association with Lipid Rafts	110
	Concluding Remarks	118
	Acknowledgments	119
	References.....	119
 CHAPTER 7 Serotonin Type 4 Receptor Dimers		
	Sylvie Claeysen, Romain Donneger, Patrizia Giannoni, Florence Gaven, Lucie P. Pellissier	
	Introduction	124
7.1	Materials.....	125
7.2	Methods	127
7.3	Discussion	136
	Summary	137
	Acknowledgments	137
	References.....	138

CHAPTER 8	Bioluminescence Resonance Energy Transfer Methods to Study G Protein-Coupled Receptor–Receptor Tyrosine Kinase Heteroreceptor Complexes	141
	Dasiel O. Borroto-Escuela, Marc Flajolet, Luigi F. Agnati, Paul Greengard, Kjell Fuxe	
	Introduction	142
8.1	The Method Principle	143
8.2	Setting Up a BRET Assay	146
8.3	Methods and Detailed Protocols	148
8.4	Discussion and Note on Critical Parameters	159
	Acknowledgments	161
	References	162
CHAPTER 9	A Simple Method to Detect Allostery in GPCR Dimers	165
	Eugénie Goupil, Stéphane A. Laporte, Terence E. Hébert	
	Introduction and Rationale	166
9.1	Materials	169
9.2	Methods	170
9.3	Discussion	174
	Summary	176
	Acknowledgments	176
	References	176
CHAPTER 10	Fluorescence Correlation Spectroscopy and Photon-Counting Histogram Analysis of Receptor–Receptor Interactions	181
	Katharine Herrick-Davis, Joseph E. Mazurkiewicz	
	Introduction	182
10.1	Materials	184
10.2	Methods	185
10.3	Discussion	194
	Summary	195
	Acknowledgments	195
	References	195
CHAPTER 11	Monitoring Receptor Oligomerization by Line-Scan Fluorescence Cross-Correlation Spectroscopy	197
	Mark A. Hink, Marten Postma	
	Introduction	198

11.1	Materials.....	199
11.2	Methods.....	202
11.3	Discussion.....	209
	Acknowledgments.....	210
	References.....	210
CHAPTER 12	Biochemical Assay of G Protein-Coupled Receptor Oligomerization: Adenosine A₁ and Thromboxane A₂ Receptors Form the Novel Functional Hetero-oligomer.....	213
	Natsumi Mizuno, Tokiko Suzuki, Yu Kishimoto, Noriyasu Hirasawa	
	Introduction.....	214
12.1	Detection of GPCR Oligomerization.....	216
12.2	Measurement of the Signaling Pathway.....	220
12.3	Materials and Methods.....	223
	Conclusion.....	224
	References.....	224
CHAPTER 13	Oligomerization of Sweet and Bitter Taste Receptors.....	229
	Christina Kuhn, Wolfgang Meyerhof	
	Introduction and Rationale.....	230
13.1	Materials.....	232
13.2	Methods.....	233
13.3	Discussion.....	240
	Summary.....	241
	Acknowledgments.....	241
	References.....	241
CHAPTER 14	Analysis of Receptor-Receptor Interaction by Combined Application of FRET and Microscopy.....	243
	Sonal Prasad, Andre Zeug, Evgeni Ponimaskin	
	Introduction and Rationale.....	244
14.1	Theory.....	245
14.2	Materials / Experimental Setups.....	249
14.3	Methods (Protocols and Procedure).....	253
14.4	Results and Discussion.....	263
	Acknowledgments.....	264
	References.....	264

CHAPTER 15	Site-Specific Labeling of Genetically Encoded Azido Groups for Multicolor, Single-Molecule Fluorescence Imaging of GPCRs	267
	He Tian, Thomas P. Sakmar, Thomas Huber	
15.1	Purpose	270
15.2	Theory	270
15.3	Protocol 1: Genetically Encoded Azido Groups for Bioorthogonal Conjugation	273
15.4	Protocol 2: Labeling with Fluorescent Probes Using Cyclooctynes	276
15.5	Protocol 3: Preparation of Biotinylated Antibodies	282
15.6	Protocol 4: Preparation of Detergent-Solubilized Lipids	285
15.7	Protocol 5: Single-Molecule Immunoprecipitation on Glass-Bottom Microplates	287
15.8	Protocol 6: Automated Multicolor, Single-Molecule TIRF Microscopy	293
	Acknowledgments	302
	References	302
CHAPTER 16	Analysis of EGF Receptor Oligomerization by Homo-FRET	305
	Cecilia de Heus, Nivard Kagie, Raimond Heukers, Paul M.P. van Bergen en Henegouwen, Hans C. Gerritsen	
	Introduction	306
16.1	Theory Homo-FRET Quantification	309
16.2	Materials	313
16.3	Methods	316
16.4	Discussion	317
	Acknowledgments	319
	References	320
CHAPTER 17	Detection of G Protein-Coupled Receptor (GPCR) Dimerization by Coimmunoprecipitation	323
	Kamila Skieterska, Jolien Duchou, Béatrice Lintermans, Kathleen Van Craenenbroeck	
	Introduction	324
17.1	Materials	326
17.2	Methods	327
17.3	Discussion	338
	Acknowledgments	339
	References	339

CHAPTER 18	Lipid-Dependent GPCR Dimerization	341
	Alan D. Goddard, Patricia M. Dijkman, Roslin J. Adamson, Anthony Watts	
	Introduction	342
18.1	Materials and Method	343
18.2	FRET Measurements and Analysis	351
18.3	Interpreting Results	354
	Summary	355
	References	355
CHAPTER 19	Monitoring Peripheral Protein Oligomerization on Biological Membranes	359
	Robert V. Stahelin	
	Introduction	360
19.1	Materials and Methods	362
19.2	Considerations	365
	Summary and Conclusion	366
	Acknowledgments	369
	References	369
CHAPTER 20	Single-Molecule Imaging of Receptor-Receptor Interactions	373
	Kenichi G.N. Suzuki, Rinshi S. Kasai, Takahiro K. Fujiwara, Akihiro Kusumi	
	Introduction	374
20.1	Receptor Expression and Fluorescent Labeling	376
20.2	Single-Molecule Imaging	380
20.3	Data Analysis	382
	Acknowledgments	387
	References	387
CHAPTER 21	Visualization of TCR Nanoclusters via Immunogold Labeling, Freeze-Etching, and Surface Replication	391
	Gina J. Fiala, María Teresa Rejas, Wolfgang W. Schamel, Hisse M. van Santen	
	Introduction	393
21.1	Materials	394
21.2	Equipment	395
21.3	Methods	395
21.4	Analysis	405
21.5	Considerations	405

	Acknowledgments	408
	References.....	408
CHAPTER 22	Identification of Multimolecular Complexes and Supercomplexes in Compartment-Selective Membrane Microdomains.....	411
	Panagiotis Mitsopoulos, Joaquín Madrenas	
	Introduction and Rationale.....	413
22.1	Detergent-Insoluble Glycosphingolipid (DIG) Microdomain Isolation	415
22.2	Plasma Membrane Isolation.....	418
22.3	Cardiolipin-Enriched Mitochondrial Membrane Microdomain Isolation	421
22.4	Mitochondrial Supercomplex Identification by Blue-Native Gel Electrophoresis	423
22.5	Discussion	428
	Summary	429
	Acknowledgments	429
	References.....	430
CHAPTER 23	G Protein-Coupled Receptor Transactivation: From Molecules to Mice.....	433
	Kim C. Jonas, Adolfo Rivero-Müller, Ilpo T. Huhtaniemi, Aylin C. Hanyaloglu	
	Introduction	434
23.1	Materials.....	435
23.2	Methods.....	436
23.3	Discussion	447
	Summary	448
	Acknowledgments	448
	References.....	448
CHAPTER 24	Crystallization of G Protein-Coupled Receptors	451
	David Salom, Pius S. Padayatti, Krzysztof Palczewski	
	Introduction	452
24.1	Crystallization of Bovine Rhodopsin	453
24.2	Crystallization of β 2-AR	460
24.3	Discussion	465
	Acknowledgments	465
	References.....	466
	Index.....	469