

---

# Contents

<i>Preface</i> . . . . .	<i>v</i>
<i>Contributors</i> . . . . .	<i>ix</i>

## PART I DEVELOPING MODELS FOR STUDYING GPCRS

1 Inducible Expression of G Protein-Coupled Receptors in Transfected Cells . . . . .	3
<i>Beryl Koener and Emmanuel Hermans</i>	
2 Using the Flp-In™ T-Rex™ System to Regulate GPCR Expression . . . . .	21
<i>Richard J. Ward, Elisa Alvarez-Curto, and Graeme Milligan</i>	
3 Viral Infection for GPCR Expression in Eukaryotic Cells . . . . .	39
<i>Antonio Porcellini, Luisa Iacovelli, and Antonio De Blasi</i>	
4 Generation of Epitope-Tagged GPCRs . . . . .	53
<i>Yan Huang and Gary B. Willars</i>	
5 The Use of Site-Directed Mutagenesis to Study GPCRs . . . . .	85
<i>Alex C. Conner, James Barwell, David R. Poyner, and Mark Wheatley</i>	
6 Approaches to Study GPCR Regulation in Native Systems . . . . .	99
<i>Jonathon M. Willets</i>	
7 Heterologous Expression of GPCRs in Fission Yeast . . . . .	113
<i>John Davey and Graham Ladds</i>	

## PART II EXAMINING GPCR EXPRESSION AND AGONIST-INDUCED COVALENT MODIFICATIONS

8 Radioligand Binding Methods for Membrane Preparations and Intact Cells. . . . .	135
<i>David B. Bylund and Myron L. Toews</i>	
9 Quantification of GPCR mRNA Using Real-Time RT-PCR . . . . .	165
<i>Trond Brattelid and Finn Olav Levy</i>	
10 Determining Allosteric Modulator Mechanism of Action: Integration of Radioligand Binding and Functional Assay Data . . . . .	195
<i>Christopher J. Langmead</i>	
11 Design and Use of Fluorescent Ligands to Study Ligand–Receptor Interactions in Single Living Cells. . . . .	211
<i>Stephen J. Briddon, Barrie Kellam, and Stephen J. Hill</i>	
12 Examining Site-Specific GPCR Phosphorylation . . . . .	237
<i>Adrian J. Butcher, Andrew B. Tobin, and Kok Choi Kong</i>	
13 Ubiquitination of GPCRs . . . . .	251
<i>Adriana Caballero and Adriano Marchese</i>	

## PART III EXAMINING EARLY EVENTS IN GPCR SIGNALING

- 14 [<sup>35</sup>S]GTPγS Binding as an Index of Total G-Protein  
and Gα-Subtype-Specific Activation by GPCRs. . . . . 263  
*Rajendra Mistry, Mark R. Dowling, and R.A. John Challiss*
- 15 Using Calcium Imaging as a Readout of GPCR Activation . . . . . 277  
*Martin D. Bootman and H. Llewelyn Roderick*
- 16 Measuring Spatiotemporal Dynamics of Cyclic AMP Signaling  
in Real-Time Using FRET-Based Biosensors. . . . . 297  
*Frank Gesellchen, Alessandra Stangherlin, Nicoletta Surdo,  
Anna Terrin, Anna Zoccarato, and Manuela Zaccolo*
- 17 Determining the Activation of Rho as an Index of Receptor  
Coupling to G<sub>12/13</sub> Proteins. . . . . 317  
*Michio Nakaya, Mina Ohba, Motohiro Nishida, and Hitoshi Kurose*
- 18 The Use of Translocating Fluorescent Biosensors for Real-Time  
Monitoring of GPCR-Mediated Signaling Events . . . . . 329  
*Carl P. Nelson and R.A. John Challiss*

## PART IV RECEPTOR–RECEPTOR AND RECEPTOR–PROTEIN INTERACTIONS

- 19 Study of GPCR–Protein Interactions Using Gel Overlay Assays  
and Glutathione-S-Transferase-Fusion Protein Pull-Downs . . . . . 347  
*Ashley E. Brady, Yunjia Chen, Lee E. Limbird, and Qin Wang*
- 20 Study of GPCR–Protein Interactions by BRET. . . . . 357  
*Martina Kocan and Kevin D.G. Pfleger*
- 21 Time Resolved FRET Strategy with Fluorescent Ligands  
to Analyze Receptor Interactions in Native Tissues:  
Application to GPCR Oligomerization . . . . . 373  
*Martin Cottet, Laura Albizu, Laetitia Comps-Agrar, Eric Trinquet,  
Jean-Philippe Pin, Bernard Mouillac, and Thierry Durroneux*
- 22 Peptide Affinity Purification for the Isolation and Identification  
of GPCR-Associated Protein Complexes. . . . . 389  
*Pascal Maurice, Avaïs M. Daulat, and Ralf Jockers*
- 23 Tandem Affinity Purification and Identification of GPCR-Associated  
Protein Complexes . . . . . 399  
*Avaïs M. Daulat, Pascal Maurice, and Ralf Jockers*
- 24 Identification of GPCR Localization in Detergent Resistant Membranes . . . . . 411  
*Ranju Kumari and Anna Francesconi*
- 25 Analysis of GPCR Localization and Trafficking. . . . . 425  
*James N. Hislop and Mark von Zastrow*

## PART V STATISTICAL METHODS

- 26 Statistical Methods in Research. . . . . 443  
*Domenico Spina*
- Index* . . . . . 473



<http://www.springer.com/978-1-61779-125-3>

Receptor Signal Transduction Protocols

Third Edition

Willars, G.B.; Challiss, R.A.J. (Eds.)

2011, XI, 479 p., Hardcover

ISBN: 978-1-61779-125-3

A product of Humana Press