
Index

A

- ABP-280, G protein-coupled receptor regulation, 27
- Actin, phospholipase D1 interactions, coimmunoprecipitation, 291, 292 co-sedimentation assay, 292, 293 GTP γ S induction, 290, 291, 296
- ADAMs, crosstalk in signaling, 29
- Adenylate cyclase, G protein regulation, 12
- β -Adrenergic receptor, protein–protein interactions in signaling, 80
- Adrenoceptor agonists, receptor crosstalk in signaling, 61
- AKAP, G protein-coupled receptor regulation, 26
- Angiotensin II, epidermal growth factor receptor transactivation, 55–57 insulin-like growth factor-I receptor activation, 58 platelet-derived growth factor receptor transactivation, 57, 58 signaling effectors, 54, 55
- Ankyrin repeats, protein–protein interactions in signaling, 89 structure, 88
- ARFs, G protein-coupled receptor endocytosis role, 20
- β -Arrestin, G protein-coupled receptor negative regulation, 13, 16, 160 posttranslational modification, 18, 19 receptor classification by interaction, 17, 18 signaling scaffolds for mitogen-activated protein kinase activation, 31–33 Src activation, 33–35 types, 16, 17
- ATRAP, G protein-coupled receptor regulation, 26, 27

B

- Bioluminescence resonance energy transfer (BRET), cell culture studies, adherent cells, 202, 204 suspensions, 201 cell fraction studies, crude membrane preparations, 200 sucrose density gradient centrifugation fractions, 200, 201, 204 donor saturation experiments, 202–207 expression constructs, 198 flow cytometry, 150, 155 materials, 198 measurements, 199, 203 partially purified receptors, 199, 200, 204 permeabilized cell studies, 201 principles, 196, 197 transfection, 199, 203
- Bone marrow, genetic reconstitution for ex vivo signal transduction studies, high-titer retrovirus generation, 335, 336, 340 materials, 333, 334 MIGR1 retroviral vector construction, 334, 335 overview of retroviral transduction system, 331, 332 retroviral transduction of murine bone marrow, donor bone marrow preparation, 337 flow cytometry, 338–341 5-fluorouracil treatment of donors, 336, 337, 340 graft-vs-host disease, 336 markers, 336 reconstitution of donor mice, 338, 340, 341 spin infection, 337, 338, 340

- Bradykinin, receptor crosstalk in signaling, 59, 60
- BRET, *see* Bioluminescence resonance energy transfer
- Bromodomain,
 - histone code hypothesis, 84
 - structure, 84, 85
- C**
- Calcitonin receptor-like receptor (CRLR),
 - receptor activity-modifying protein complex, 23
- Calcium flux, dimerization of G protein-coupled receptors, 153, 155
- CAM, *see* Constitutively active mutant
- Caveolae,
 - isolation,
 - detergent isolation, 185, 188
 - immunoisolation, 187, 188
 - materials, 184
 - plasma membrane preparations, 185, 186
 - sodium carbonate isolation, 185
 - sucrose density gradient centrifugation, 186–188
 - signaling functions, 182–184
- Caveolins, lipid raft markers, 174
- Cdc42, *see* Rho GTPases
- Chemoattractant receptors, *see* DNA microarray
- Chromodomain,
 - histone code hypothesis, 84
 - structure, 85
- Clustering assay, *see* PDZ domain
- Coimmunoprecipitation,
 - actin–phospholipase D1 interactions, 291, 292
 - dimerization of G protein-coupled receptors, 147, 154
 - in vivo* relevance, 91
 - PDZ domain interactions,
 - cell lysate preparation, 241
 - immunoprecipitation, 241–243
 - materials, 234
 - transfection, 240–243
- Confocal scanning laser microscopy,
 - clustering assay, 249
 - CXCR4 subcellular localization, 133, 135–138
 - lipid raft protein visualization and localization, 174–177
- Constitutively active mutant (CAM),
 - CXCR4 expression in yeast,
 - antagonist screening, 124, 125
 - applications, 125, 126
 - culture, 120, 126
 - expression vector construction, 119
 - materials, 116–118
 - reporter assays,
 - β -galactosidase activity, 121, 122
 - histidine-independent growth, 120, 121, 126
 - selection for constitutional active mutants, 122, 123, 126
 - strains and reporter plasmids, 118
 - transformation, 119, 120
 - diseases, 130
 - G protein-coupled receptor
 - phosphorylation assay, 131–135
 - GTP γ S binding assay of CXCR4
 - constitutive activity, 131–134, 137
 - properties, 130, 131- CRLR, *see* Calcitonin receptor-like receptor
- CXCR4,
 - GTP γ S binding assay of constitutive activity, 131–134, 137
 - hematopoietic cell isolation for signaling studies,
 - cobblestone area-forming cell assay, 109, 111
 - colony-forming unit tests for differentiated progenitor cell evaluation, 109–111
 - DELTA assay, 109, 111
 - expansion of derived cells, 111, 112
 - long-term culture-initiating cell assay, 108, 109
 - materials, 105, 106
 - mononuclear cells,
 - culture, 107
 - isolation with immunomagnetic beads, 106, 107, 111
 - overview, 104, 105
 - stromal-derived factor-1 as ligand, 103, 104, 116
 - subcellular localization, 133, 135–138
 - yeast constitutively active mutant expression,
 - antagonist screening, 124, 125

- applications, 125, 126
- culture, 120, 126
- expression vector construction, 119
- materials, 116–118
- reporter assays,
 - β -galactosidase activity, 121, 122
 - histidine-independent growth, 120, 121, 126
- selection for constitutional active mutants, 122, 123, 126
- strains and reporter plasmids, 118
- transformation, 119, 120

D

- Desensitization, mechanism, 14, 16
- Dimerization, G protein-coupled receptors,
 - antibody fluorescent labeling, 149
 - cell cycle analysis, 145
 - crosslinking, 146, 154
 - electrophoretic mobility shift assay, 148, 149
 - flow cytometry,
 - immunostaining, 145
 - receptor number, 145, 146, 154
 - resonance energy transfer, 150, 155
 - fluorescence lifetime imaging microscopy, 151
 - fluorescence resonance energy transfer microscopy, 151, 155
 - fluorescent protein tagging, 149, 155
 - functional assays,
 - calcium flux, 153, 155
 - overview, 152
 - transwell migration, 153
 - materials for study, 143, 144, 153
 - overview, 21–23, 141–143
 - phosphatidylinositol 3-kinase assay, 147
 - pull-down assays, 148, 155
 - tagged receptors and
 - coimmunoprecipitation, 147, 154
 - two-color fluorescence microscopy, 150
 - Western blot, 146, 153, 154
- DNA microarray, formyl-Leu-Met-Phe gene response studies,
 - Affymetrix technology, 314, 327
 - biotin-labeled cRNA preparation, 321, 322, 327
 - data analysis,
 - detection and normalization, 324, 325, 328
 - gene filtering and clustering, 325–327

- double-stranded cDNA synthesis,
 - first strand, 319, 320, 327
 - second strand, 320, 321, 327
- GeneChip,
 - hybridization, 323, 328
 - scanning, 323, 324, 328
 - washing and staining, 323, 328
- materials, 314–316, 327
- overview, 314, 316, 327
- RNA isolation, 318, 319, 327
- DRIP78, G protein-coupled receptor regulation, 26

E

- EGFR, *see* Epidermal growth factor receptor
- Electrophoretic mobility shift assay (EMSA),
 - dimerization of G protein-coupled receptors, 148, 149
- EMSA, *see* Electrophoretic mobility shift assay
- Endothelin, receptor crosstalk in signaling, 59
- Epidermal growth factor receptor (EGFR),
 - angiotensin II transactivation, 55–57
 - crosstalk in signaling, 61

F

- Far-Western blot, principles, 92
- FGF, *see* Fibroblast growth factor
- Fibroblast growth factor (FGF), receptor crosstalk in signaling, 62
- FLIM, *see* Fluorescence lifetime imaging microscopy
- Flow cytometry,
 - bone marrow *ex vivo* signal transduction studies, 338–341
 - dimerization of G protein-coupled receptors,
 - immunostaining, 145
 - receptor number, 145, 146, 154
 - resonance energy transfer, 150, 155
- Fluorescence lifetime imaging microscopy (FLIM), dimerization of G protein-coupled receptors, 151
- Fluorescence resonance energy transfer (FRET),
 - dimerization of G protein-coupled receptors, 151, 155
 - flow cytometry, 150, 155
- fMLP, *see* formyl-Leu-Met-Phe

Focal adhesion, crosstalk in signaling, 30
 formyl-Leu-Met-Phe (fMLP), *see* DNA
 microarray
 14-3-3
 G protein-coupled receptor regulation, 27
 signaling, 84
 FRET, *see* Fluorescence resonance energy
 transfer
 Frizzled, signaling, 35
 Functional Interaction Trap, principles, 93, 94

G

GeneChip, *see* DNA microarray
 Glutathione *S*-transferase pull-down assay,
 see Pull-down assay
 GPCR, *see* G protein-coupled receptor
 G protein,
 desensitization, 14, 16, 20, 160
 effector mode of signaling, 4, 5, 12, 13,
 159, 160
 recycling, 19, 20
 resensitization, 19
 sequestration, 17–19
 structure–function relationships, 11, 12
 taxonomy, 10, 11
 G protein-coupled receptor (GPCR), *see*
 also specific receptors,
 abundance, 3, 4, 115, 313
 architecture, 6, 8, 52, 129
 constitutively active mutants, *see*
 Constitutively active mutant
 crosstalk,
 adrenoceptor agonists, 61
 angiotensin II, 54–58
 bradykinin, 59, 60
 endothelin, 59
 epidermal growth factor, 61
 fibroblast growth factor, 62
 insulin-like growth factor-I, 63
 integrins, 63, 64
 lysophosphatidic acid, 58, 59
 mechanisms, 27–30, 52, 53
 nerve growth factor, 62
 platelet-derived growth factor, 61, 62
 sphingosine 1-phosphate, 60
 thrombin, 60
 vascular endothelial growth factor,
 62, 63

dimerization, *see* Dimerization, G
 protein-coupled receptors
 live cell imaging, *see* Live cell video
 microscopy, G protein-coupled
 receptors
 negative regulation of signaling, 13, 14
 PDZ domain protein interactions in
 regulation, 24, 25
 receptor activity-modifying proteins, 21,
 23, 24
 signaling scaffolds,
 binding proteins as signaling
 scaffolds, 31–35
 overview, 30, 31
 structure–function relationships, 9, 10
 taxonomy, 8
 transmembrane domain interactions in
 activation, 130
 GRKs,
 G protein-coupled receptor desensitization
 role, 17
 G protein regulation, 13
 membrane targeting, 16
 posttranslational modification, 16
 types, 16

H

HB-EGF, *see* Heparin-binding epidermal
 growth factor
 Hematopoietic stem cell, *see* CXCR4
 Heparin-binding epidermal growth factor
 (HB-EGF), angiotensin II and
 receptor crosstalk, 57
 Homer proteins, G protein-coupled receptor
 regulation, 25, 26
 HP1, chromodomain, 85

I

IGF-I, *see* Insulin-like growth factor-I
 Immunofluorescence microscopy,
 CXCR4 subcellular localization, 133,
 135–138
 lipid raft protein visualization and
 localization, 174–177
 Insulin-like growth factor-I (IGF-I)
 receptor,
 angiotensin II activation, 58
 crosstalk in signaling, 63

- Insulin receptor, bioluminescence resonance energy transfer of partially purified receptors, 199, 200
- Integrins, receptor crosstalk in signaling, 63, 64
- Isoelectric focusing, *see* Neutrophil proteomics
- J, L**
- JAK-STAT pathway, G protein-coupled receptor signaling, 31
- Lipid rafts, isolation,
 - detergents, 171, 176, 185, 188
 - Jurkat cell membrane rafts on toxin association, 172, 173, 176, 177
 - plasma membrane preparations, 185, 186
 - signaling complexes from detergent-resistant membranes, 172
 - sodium carbonate isolation, 185
 - sucrose density gradient centrifugation, 173, 174, 186–188
- markers, 174, 177
- materials for study, 170, 171, 176, 184
- protein visualization and localization, 174–177
- signaling functions, 169, 170, 181–184
- Live cell video microscopy, G protein-coupled receptors,
 - cell culture, 161, 162
 - image processing, 163, 165
 - imaging, 162–165
 - materials, 160, 161
 - overview, 159, 160
 - transfection, 162, 163
- Lysophosphatidic acid, receptor crosstalk in signaling, 58, 59
- M**
- MAPKs, *see* Mitogen-activated protein kinases
- Mass spectrometry, *see* Neutrophil proteomics
- Membrane microdomains, *see* Lipid rafts
- Microinjection studies,
 - principles, 257–259, 264
 - resources, 264
- Rho studies,
 - cell fixation and staining, 263, 264, 266
 - cell preparation, 261, 264, 265
 - DNA injection, 262, 263, 265
 - materials, 259, 260, 264
 - overview, 257–259, 264
 - protein injection, 261, 262, 264, 265
- Mitogen-activated protein kinases (MAPKs),
 - β -arrestins as signaling scaffolds for activation, 31–33
 - crosstalk in signaling, 27–29
- N**
- Nerve growth factor (NGF), receptor crosstalk in signaling, 62
- Neutrophil proteomics,
 - expression studies, 351, 352
 - mass spectrometry,
 - peptide fingerprint analysis, 351, 355
 - sample preparation, 350, 351, 354, 355
 - materials, 344, 345
 - overview, 343, 344
 - protein extraction, 345–347, 354
 - signaling studies, 352, 353
 - two-dimensional gel electrophoresis,
 - denaturing gel electrophoresis, 349, 350, 354
 - in-gel digestion, 350, 354
 - isoelectric focusing, 349, 354
 - limitations, 353
 - sample preparation,
 - desalting precipitation, 348, 354
 - detergent lysis buffer, 347, 354
 - ultrafiltration, 348, 349, 354
- NGF, *see* Nerve growth factor
- P**
- PDE, *see* Phosphodiesterase
- PDGFR, *see* Platelet-derived growth factor receptor
- PDZ domain,
 - binding sites, 87
 - clustering assay,
 - cover slips,
 - cleaning, 247, 248
 - coating, 248
 - imaging, 249, 250
 - immunocytochemistry, 248, 249
 - materials, 247, 249
 - overview, 246
 - transfection, 248, 249

- clustering of signal response proteins, 88
- coimmunoprecipitation of PDZ domain interactions,
 - cell lysate preparation, 241
 - immunoprecipitation, 241–243
 - materials, 234
 - transfection, 240–243
- protein–protein interactions in G protein-coupled receptor regulation, 24, 25
- PSD-95 and clustering, 245, 246
- species distribution, 87
- structure, 233, 245
- yeast two-hybrid assay of PDZ domain interactions,
 - bait and prey construct generation, 237, 238, 242
 - materials, 234
 - screening, 240, 242
 - transformation, 239
 - vectors, 235–237
- Peptide mass fingerprinting, *see* Neutrophil proteomics
- Phosphatidylinositol 3-kinase (PI3K),
 - dimerization of G protein-coupled receptor assay, 147
- Phosphodiesterase (PDE), G protein-coupled receptor negative regulation, 13
- Phospholipase C (PLC), G protein regulation, 12, 13
- Phospholipase D (PLD),
 - assay in cell-free systems,
 - actin interaction with PLD1,
 - coimmunoprecipitation, 291, 292
 - co-sedimentation assay, 292, 293
 - GTP γ S induction, 290, 291, 296
 - cell culture,
 - macrophages derived from monocytes, 284
 - monocyte cell lines, 284
 - cell disruption with nitrogen cavitation, 285, 294, 295
 - detergent-insoluble cytosolic fraction assay,
 - detergent concentration effects on activity, 288, 289
 - fractionation, 286, 287
 - phospholipid and protein content determination, 287, 288
 - stimulation studies, 289, 290
 - isoform-specific activity assay, 293, 294, 296
 - materials, 282, 283, 294, 295
 - membrane assay, 285, 286
 - overview, 282
 - separation of membrane and cytosol fractions, 285, 295
 - functions, 281, 282, 300
 - isoforms, 300
 - regulation studies using cytosol-depleted cells,
 - assay using tritiated choline depletion, 305, 307
 - materials, 303, 304
 - overview, 299–303
 - reconstitution of GTP γ S-stimulated activity, 308
 - refractory state induction, 302, 307–309
- PI3K, *see* Phosphatidylinositol 3-kinase
- Platelet-derived growth factor receptor (PDGFR),
 - angiotensin II transactivation, 57, 58
 - crossstalk in signaling, 61, 62
- PLC, *see* Phospholipase C
- PLD, *see* Phospholipase D
- Propidium iodide, cell cycle analysis, 145
- Protein–protein interactions,
 - actin–phospholipase D1 interactions, *see* Phospholipase D
 - ankyrin repeats, 88, 89
 - clustering assay, *see* PDZ domain
 - dimerization, *see* Dimerization, G protein-coupled receptors
 - genetics analysis, 93
 - histone code and bromodomains/ chromodomains, 84, 85
 - PDZ domains, 87, 88
 - prospects for study, 94
 - resonance energy transfer, *see* Bioluminescence resonance energy transfer; Fluorescence resonance energy transfer
 - SH2 domains, 82–84
 - SH3 domains, 86, 87
 - specificity, 89–91
 - validation of in vitro studies, 90–94
 - yeast two-hybrid system, *see* Yeast two-hybrid screen
- Proteomics, *see* Neutrophil proteomics

- PSD-95, function and clustering, 245, 246
- PTB domain, signaling, 83
- Pull-down assay,
 dimerization of G protein-coupled receptors, 148, 155
 principles, 92
- Rho GTPase activation assays,
 overview, 269, 270
- Rac/Cdc42 and Pak1 PBD assay,
 cytosol incubation with
 glutathione beads, 272, 273
 extract preparation, 274, 278
 gel electrophoresis, 273, 278
 glutathione *S*-transferase–PBD fusion
 protein preparation, 272, 278
 materials, 271
 nucleotide loading of lysates, 274
- RhoA and Rhotekin Rho-binding
 domain assay,
 aluminum fluoride stimulation,
 277, 278
 extract preparation, 276–278
 gel electrophoresis, 276
 glutathione *S*-transferase–binding
 domain fusion protein
 preparation, 275, 276
 materials, 271
- R**
- Rab, G protein-coupled receptor
 endocytosis role, 19, 20
- Rac, *see* Rho GTPases
- Rafts, *see* Lipid rafts
- RAMPs, *see* Receptor activity-modifying
 proteins
- Reactive oxygen species (ROS), angiotensin
 II and receptor crosstalk, 56, 57
- Receptor activity-modifying proteins (RAMPs),
 calcitonin receptor-like receptor
 complex, 23
 G protein-coupled receptor modulation,
 21, 23, 24
- Receptor tyrosine kinases (RTKs),
 activation, 52
 crosstalk,
 adrenoceptor agonists, 61
 angiotensin II, 54–58
 bradykinin, 59, 60
 endothelin, 59
 epidermal growth factor, 61
 fibroblast growth factor, 62
 insulin-like growth factor-I, 63
 integrins, 63, 64
 inter-receptor tyrosine kinase
 signaling, 63
 lysophosphatidic acid, 58, 59
 mechanisms, 29, 52–54
 nerve growth factor, 62
 platelet-derived growth factor, 61, 62
 sphingosine 1-phosphate, 60
 thrombin, 60
 vascular endothelial growth factor,
 62, 63
 signaling scaffolds, 52
- Rho GTPases,
 activation assays using pull-down,
 overview, 269, 270
 Rac/Cdc42 and Pak1 PBD assay,
 cytosol incubation with
 glutathione beads, 272, 273
 extract preparation, 274, 278
 gel electrophoresis, 273, 278
 glutathione *S*-transferase–PBD fusion
 protein preparation, 272, 278
 materials, 271
 nucleotide loading of lysates, 274
- RhoA and Rhotekin Rho-binding
 domain assay,
 aluminum fluoride stimulation,
 277, 278
 extract preparation, 276–278
 gel electrophoresis, 276
 glutathione *S*-transferase–binding
 domain fusion protein
 preparation, 275, 276
 materials, 271
- domains, 269, 270
- microinjection studies,
 cell fixation and staining, 263, 264, 266
 cell preparation, 261, 264, 265
 DNA injection, 262, 263, 265
 materials, 259, 260, 264
 overview and principles, 257–259, 264
 protein injection, 261, 262, 264, 265
 resources, 264
- ROS, *see* Reactive oxygen species
- RTKs, Receptor tyrosine kinases

S

- SDF-1, *see* Stromal-derived factor-1
- Sequestration, mechanism, 17–19
- SH2 domain,
 - protein–protein interactions in signal transduction, 81, 82
 - tyrosine phosphorylation, 82–84
- SH3 domain,
 - protein–protein interactions in signal transduction, 81, 82, 86
 - Sos recruitment, 86, 87
- Specificity, protein–protein interactions, 89, 90
- Sphingosine 1-phosphate, receptor crosstalk in signaling, 60
- Src, β -arrestin activation, 33–35
- Stromal-derived factor-1 (SDF-1),
 - function, 116
 - hematopoietic cell signaling, 104
 - knockout mouse, 104
 - receptor, *see* CXCR4

T

- Tandem affinity purification, principles, 92
- Thrombin, receptor crosstalk in signaling, 60
- Two-dimensional gel electrophoresis, *see* Neutrophil proteomics

U, V, W

- Ubiquitination, β -arrestin, 18
- Vascular endothelial growth factor (VEGF),
 - receptor crosstalk in signaling, 62, 63
- VEGF, *see* Vascular endothelial growth factor
- Western blot, dimerization of G protein-coupled receptors, 146, 153, 154

Y

- Yeast two-hybrid screen,
 - bait plasmid selection, 216, 218, 219, 228, 229
 - complementary DNA library and prey plasmid, 219, 220
 - false-positive elimination, 227, 228
 - β -galactosidase assays,
 - filter assay, 225
 - liquid assay, 225, 226
 - overview, 224, 225
 - materials, 213–215
 - PDZ domain interactions,
 - bait and prey construct generation, 237, 238, 242
 - materials, 234
 - screening, 240, 242
 - transformation, 239
 - vectors, 235–237
 - plasmid isolation, 226, 227, 229, 230
 - plating on selective media, 223
 - positive clone screening, 223, 224
 - principles, 211–213
 - replating on selective media, 224
 - transformation,
 - electroporation, 222, 223, 229
 - large-scale transformation by lithium acetate, 221, 222
 - small-scale transformation by lithium acetate, 220, 221, 229
 - validation and analysis of positive interactions, 228, 230