

Index

Page numbers in *italics* refer to entries in figures or tables.

96-well assay format 289, 290, 291–294
a
 A-349821 128, 129
 A-358239 128, 129
 ABC transporters 60, 82, 83
 acetylcholine receptors 52, 53, 156, 222, 236, 236
 adenosine A₁ receptor (A₁R) 54, 55, 222, 225
 adenosine A_{2A} receptor (A_{2A}R) 4, 221, 222, 223, 225
 – alanine scanning 290
 – *P. pastoris* 86, 87
 – *S. cerevisiae* 54, 55
 adenosine A_{2B} receptor (A_{2B}R) 54, 55
 adenosine AT_{1A} receptor 221
 adherent cell culture 140–142
 adrenal cytochrome 90, 91
 adrenergic α₁ receptor 257
 adrenergic α₂ receptor 257
 – GPCRs 223, 225, 230, 233
 – *S. cerevisiae* 54, 55
 adrenergic β₁ receptor 88, 89, 219, 223, 233, 290
 adrenergic β₂ receptor 4, 257, 222, 224, 233, 234, 236, 236
 – *P. pastoris* 86, 87, 95
 – *S. cerevisiae* 54, 55
 adrenergic β₃ receptor 54, 55
 affinity chromatography 190–192, 263, 304, 305, 306
 – dye-ligand 333
 – immobilized metal-affinity 397, 401
 – immunoaffinity and customized affinity 332
 – lectin-affinity 332, 333
 – metal-affinity 29, 50
 – recombinant proteins with engineered tags 330, 332

aggregation, protein 139, 319, 320, 328
 aggregation number 342–345, 320, 362, 363, 365, 398
 ÄKTA-FPLC™ 191–192
 alanine scanning 290, 291
 alcohol dehydrogenase 38
 α-helical membrane proteins 13, 14, 250, 251, 253
 – detergents used 260, 261
 – IR spectroscopy 338
 – and purification 327
 Alzheimer's disease 156
 amide bands 337, 338
 amino acid composition 252, 253
 ampicillin 23, 24, 121, 393
 amplicons 178, 179
 AmpR vectors 15, 23, 24
 analytical ultracentrifugation (AUC) 335, 336
 anhydrotetracycline (aTc) vectors 18, 20
 anionic detergents 321, 322, 347, 363
 antiapoptosis engineering 152–156
 antibiotic selection markers 40, 41
 antibody–antigen interaction 332
 antimalarial drug resistance protein 82, 83
AOX1 gene 80
 apolipoprotein 371
 apoptosis 152–154
 aquaglyceroporin 84, 85
 aquaporins 84, 85, 259
araBAD promoter 18, 19, 21
 l-arabinose 18, 19
 ASIC1 channel 257
 association motifs 253, 254
 ATP-binding cassette (ABC) transporters 60, 82, 83
 ATP synthase 61
Autographa californica nuclear polyhedrosis virus (AcMNPV) 110, 113

- autoinduction systems 24
 - photosynthetic bacteria 173, 174
- autonomous replicating sequences (ARSs) 40, 41
- auxotrophic selection markers 40, 41
- b**
- baby hamster kidney (BHK) 139, 224
- Bac to Bac method 113, 128
- bacmids 111, 113
- bacterial systems 13–31
 - chaperone overexpression 26, 27
 - clone stability 23, 24
 - fusion partners/membrane targeting peptides 25, 26
 - GPCR expression 220, 221, 226–228, 239
 - media types 24
 - post-translational modifications 148
 - potential expression yield 29, 30
 - protein instability 30
 - quality control proteases 27, 28
 - T7 expression system 20–22
 - tag selection 28, 29
 - vector/promoter types 15, 16–18, 19, 20
 - *see also Escherichia coli*; photosynthetic bacteria
- bacteriorhodopsin (bR) 236, 236, 237, 253, 254, 259
- baculovirus-infected insect cells 109–131, 152
 - histamine H₃ receptor 118–120, 121–124, 125–128
 - process of infection 115–118
 - recombinant baculovirus generation 113–115
 - recombinant protein production 111
 - vendors supplying reagents for 112
- baculoviruses 110
- basal activity 289
- batch culture 141, 142
- BCA assay 126, 400, 401
- Bcl-2 proteins 153–156
- β-barrel membrane proteins 13, 14, 250, 251, 260
 - IR spectroscopy 337
 - and purification 327
- β-lactamase 23, 393
- bicelles 300, 308, 309, 310
 - mixed detergent systems 365, 368–370
- Bio-beads 374
- biotherapeutics
 - mammalian cells 139, 157–159
 - *see also* drug targets
- BL21(DE3) strain 16, 17, 20–23, 391
- blood-brain barrier 158
- blotting, *see* colony filtration (CoFi) blot; electroblotting; immunoblotting; Western blot analysis
- blue-native electrophoresis 336
- BODIPY-neurotensin 281, 283
- bradykinin B2 86, 87, 221, 224, 225
- breast cancer resistance protein 82, 83
- Brij35 236, 237, 346
- budding yeast, *see* *Saccharomyces cerevisiae*
- c**
- ¹³C isotopic labeling 76
- C-terminus, modifications 256, 257
- C2 domain 204, 213, 214
- C12E8 342
- C41(DE3) strain 17, 22
- C43(DE3) strain 17, 22
- Ca²⁺-ATPase 37, 377, 378, 380
- 8-cadinene hydroxylase (CAH) 25
- calcitonin receptors 54, 55
- calcium-activated K⁺ channel 84, 85
- calcium phosphate transfection 143
- cancer cell lines 157, 158
- cannabinoid receptors 26, 257, 221, 223, 225, 227, 230, 232
 - *P. pastoris* 86–89
 - *S. cerevisiae* 54, 55
- carbenicillin 24
- carbohydrate transport protein 92, 93
- cardiolipin 45
- caspases 152, 153, 154, 155
- catabolite gene activator protein 15
- cationic detergents 322, 347, 363
- cationic liposomes 143, 146
- cationic polymer transfection 143
- caveolin 144
- CCR1 224
- CCR5 224, 367
- cell cultures
 - bacterial systems 24
 - costs 142, 160
 - mammalian 140–150
 - palmitoylation 212
 - photosynthetic bacteria 173, 174, 181, 182–185, 187, 188
- cell-free system 317
 - GPCR expression 235, 236, 236, 237–239, 302, 303
- cell lysis
 - baculovirus insect cells 120, 123, 124
 - *P. pastoris* 98
 - photosynthetic bacteria 188, 193
- cellubrevin 144

- centromeric (CEN) plasmids 40, 41, 52, 56, 58
 ceramides 46
 cetyltrimethylammonium bromide (CTAB)
 344, 347
 chaperones
 – bacterial systems 26, 27
 – mammalian cells 156, 157
 characterization, IMPs 318, 319, 334–341
 charge, effects on purification 328, 329
 chemoattractants 56, 57
 chemoheterotrophic cultures 173, 174, 181
 chemokine receptors 56, 57, 227, 367
 – CCR1 221
 – CCR5 221, 367
 – CXCR2 298, 299
 – CXCR4 223, 367
 – *see also* CXCR1
 chimeric K⁺ channel 84, 85
 Chinese hamster ovary (CHO) 139, 155, 223
 chloride channel PAB2010 92, 93
 chloroquine resistance transporter 82, 83
 3-(3-cholamidopropyl)-dimethylammonio-2-
 hydroxy-1-propane sulfonate (CHAPSO)
 363, 368
 3-(3-cholamidopropyl)-
 dimethylammoniopropane sulfonate
 (CHAPS) 292, 293, 343, 345, 322, 348,
 363, 398
 cholates 344, 347, 362, 363, 371
 cholesterol 37, 46, 77, 139, 150, 326
 cholesteryl hemisuccinate (CHS) 292, 293
 chromatofocusing (CF) 328
 chromatography, *see* affinity chromatography;
 ion-exchange chromatography; liquid
 chromatography; size-exclusion
 chromatography (SEC)
 circular dichroism (CD) spectroscopy 192,
 336, 337
 – ligand binding 340, 341
 clone stability, bacterial systems 23, 24
 clorgyline-bound monoamine oxidase 90, 91
 cloud point 6, 342–345, 320
 CMC, *see* critical micelle concentration
 (CMC)
 colony filtration (CoFi) blot 286–288
 competition binding assays 126, 128, 308
 concanavalin A 333
 conjugal mating 180, 181
 constitutive promoters 38, 38
 consultate boundary 320
 contaminants 260
 Coomassie-stained gels 123, 265, 336
 copper transporter 82, 83, 145
 CorA overexpression 26
 corticotropin releasing factor 236
 costs, cell culture 142, 160
 cotranslational signal recognition particle
 (SRP) pathway 15
 covalent lipid anchor proteins 201, 202,
 204, 210–212
 critical micelle concentration (CMC) 190,
 319, 321, 342–345, 362, 363
 – detergents in water 365
 – and dialysis 374
 – in successful solubilization 324, 398
 critical solubilization concentration (CSC)
 324, 325
 cryopreservation 140
 crystal structures, *S. cerevisiae* expression 61
 crystallization 5, 6
 – current trends 260, 261
 – GPCRs 299
 – lipids and 258, 259
 – M2 transmembrane protein 266
 – rhodopsin 262, 264
 – RPE65 265
 crystallography
 – two-dimensional 373
 – X-ray crystallography 4, 5, 298, 299
 CUP1 gene 38, 39
 curvature elastic stress 378, 379, 380
 customized affinity 332
 CXCR1 298, 299
 – binding and activity 307, 308
 – cloning into pGEX2a 303
 – expression 304, 305
 – NMR samples 308, 309
 – NMR spectra 304, 309, 310
 – purification 305, 306
 CXCR2 298, 299
 CXCR4 223, 367
 cyclo-fos 322
 Cymal 322, 398
 cysteine scanning 289, 290
 cystic fibrosis 60, 61
 cystic fibrosis transmembrane conductance
 regulator 60, 61
 cytochrome *bc*₁ 61
 cytochrome *c* 153, 154, 259
 cytochrome *c*₂ 204, 205, 206
 cytochrome P450 90, 91, 145, 204
 cytomegalovirus 143
 cytoplasmic loop 3 (CL3) 4
- d**
- DDM, *see* *n*-dodecyl-β-D-maltopyranoside
 (DDM)
 DE3 prophage 16, 17, 20, 22

- decyl maltoside (DM) 292, 293, 343, 346, 364, 398
 - deglycosylation 255
 - denaturation, protein 363, 366–381
 - Deriphat 189, 190, 195
 - detergents 6
 - advantages and drawbacks 342–345
 - α -helical membrane proteins 260, 261
 - classification 321, 322
 - detergent-free bilayer systems 370–372
 - general characteristics 362–365
 - GFP systems 398, 399
 - GPCRs 236, 237, 238, 289
 - IMPs 288, 289
 - mixed detergent systems 365, 366–370
 - phospholipids 364, 365, 367–370
 - physicochemical characteristics 319, 320
 - in protein purification 118, 119, 125, 126, 189, 190
 - protein–detergent complex (PDC) 6, 322, 323, 324–326
 - reconstitution of proteoliposomes 372–375
 - saturation 375
 - solubilization 322–325
 - solubilization vs stability 361–366
 - *see also* protein–detergent complex (PDC)
 - DH10Bac cells 111, 113
 - diacylglycerol kinase (DGK) 289, 290, 368, 377, 380, 381
 - dialysis 363, 364, 374, 375
 - digitonin 342
 - 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (di(C6 : 0)PC) 364, 365, 368, 369
 - dihydrofolate reductase (DHFR) 22, 143
 - dilution method 373
 - dimethyl sulfoxide (DMSO) 97
 - directed protein evolution 4, 279
 - GPCR 280–286
 - disintegrations per minute (DPM) 125
 - disulfide bond formation 44, 148, 202
 - “divide-and-conquer” approach 253
 - DM, *see* decyl maltoside (DM)
 - DnaK overexpression 27
 - n*-dodecyl- β -D-maltopyranoside (DDM) 122, 125, 127, 292, 293, 346
 - advantages and drawbacks 343
 - characteristics 364
 - GFP-fusion 398
 - protein yield 126
 - dopamine receptors 56, 57, 86, 87
 - dot-blot detection 230, 286–288
 - Drosophila melanogaster* 225, 234
 - drug targets
 - GPCRs 109, 219, 261, 297
 - membrane proteins 249
 - dye-ligand affinity chromatography 333
- e**
- EDG1 receptor 86, 87
 - electroblotting 185, 186
 - electrophoresis, *see* blue-native
 - electrophoresis; sodium dodecyl sulfate–polyacrylamide
 - gel electrophoresis (SDS–PAGE)
 - electroporation 143
 - electrostatic proteins 201, 202, 204, 204–207
 - elongation factor 1 α 143
 - elution profiles 127
 - endoplasmic reticulum (ER)
 - glycosylation 43
 - lipids in 44
 - signal sequences 47
 - UPR activation 49
 - endothelin B receptor 47, 86, 87, 222, 225–236
 - enzymes, from *P. pastoris* expression 88–92
 - epidermal growth factor receptors (ErbB)
 - 92, 93, 144, 155, 157
 - ergosterol 37, 46, 47
 - error-prone PCR (epPCR) 281, 282, 293
 - Escherichia coli*
 - β -barrel proteins 13, 14
 - cell-free systems 236, 237
 - colony filtration (CoFi) blot 287
 - DH10Bac 111, 113
 - GFPs 391, 393
 - GPCRs 2, 221, 226, 301
 - lipid composition 376
 - *M. tuberculosis* 2, 3
 - multidrug transporter 236
 - NTR1 281
 - peripheral membrane proteins 202, 204, 208, 211
 - photosynthetic bacteria 174, 179–181
 - RPE65 264
 - strains 16, 17, 19
 - uncontrolled expression 14
 - *see also* bacterial systems
 - eukaryotic membrane proteins 2, 7, 37, 167
 - expression yield 29, 30
 - using *Pichia pastoris* 75
 - EXP strains 17, 22
 - expression 2–4
 - CXCR1 304, 305
 - scaling-up 396–398
 - *see also* heterologous expression; higher expression; overexpression; T7 expression
 - expression systems

- bacterial 13–31
- baculovirus-infected insect cells 109–131
- GPCRs 301–303
- mammalian 139–161
- optimization of conditions 96–98
- *P. pastoris* 75–99
- photosynthetic bacteria 167–197
- *S. cerevisiae* 37–62
- expression vectors
 - GFPs 393, 394
 - mammalian 143, 144, 145
 - *P. pastoris* 79, 82–92
 - photosynthetic bacteria 170–172, 175, 176, 179
- expression yield
 - bacterial systems 29, 30
 - GFPs 394–398
 - *S. cerevisiae* 53, 55, 57, 59
- extremophiles 278
- extrinsic fluorescence 340

f

- F1 ATPase 61
- farnesylation of proteins 210
- fast protein liquid chromatography (FPLC)
 - 191, 192, 304, 305, 306
- Fe³⁺ ABC protein 92, 93
- fed-batch culture 141, 142
- fetal bovine serum 116, 142
- fibroblast growth factor 144
- filter-binding assays 119, 125, 127, 130
- FLAG tag 29, 232, 331
- flow cytometry 147
- fluorescence 340
 - immunofluorescence 147
 - mean fluorescence intensity 282, 283
 - whole cell and in-gel 391, 394–396, 403, 404
- fluorescence-activated cell sorting (FACS)
 - 257, 281–283, 284, 285, 292, 293
- fluorescence correlation spectroscopy 340
- fluorescence-detection size-exclusion chromatography 398, 399
- fluorescent ligands 340
 - GPCRs 285, 286
- folding
 - membrane proteins 249, 250
 - *see also* refolding
- formate-nitrite transporter 84, 85
- formyl peptides 56, 57
- Förster resonance energy transfer (FRET)
 - 340
- fos-choline 10 (F-10) 348
- fos-choline 12 (F-12) 125, 322, 348, 364

- protein yield 126
- structure 365
- Fourier self-deconvolution 338
- Fps1p production 42
- FtsH protease 26, 27
- fusion partners
 - bacterial systems 25, 26
 - N-terminal 25, 226
 - *S. cerevisiae* 52, 54, 56, 58
- fusion proteins 301, 302, 305
- p8-derived 14, 15
- peptides 52, 54, 56, 58
- production 330, 331

g

- G-protein-coupled receptors (GPCRs) 3, 4, 219–239, 297, 298
- baculovirus-infected insect cells 109–131
- directed protein evolution 280–286
- engineering higher stability 288, 289
- expression systems 301–303
- heterologous expression 221–225
- lipid-detergent mixtures 367
- mammalian cell expression 151, 219, 302
- numbers of 2
- oligomerization 254
- *P. pastoris* expression 86–89, 94–98, 302
- pharmaceutical role 109, 219, 261, 297
- post-translational modifications 255, 256
- *R. sphaeroides* 174
- *S. cerevisiae* 47, 50, 52–59, 302
- schematic structure 220
- sequence modification 257
- solubilization and purification 238, 324
- stabilizing
 - alanine-scanning and single-clone screening 290, 291
 - random mutagenesis and 96-well screening 291–294
- structures 299
- *see also* CXCR1; rhodopsin; adenosine A1; adenosine A2aR; adrenergic beta1; adrenergic b2; cannabinoid G418 selection marker 40, 41
- GAL gene family 38, 39
- galactokinase 39
- galactose 38
- gel filtration 125, 127, 306
 - *see also* size-exclusion chromatography (SEC)
- gene delivery, mammalian systems 143, 144, 145, 146, 147
- genetic selection 280
- gentamicin 121
- glucagon-like peptide-1 receptor 47

- glucose transporters 82–85
- glucosidases 149
- glutamate receptors 234, 332
 - metabotropic 222, 225, 234
 - vesicular 84, 85
- glutamine synthetase 143
- glutathione S-transferase (GST) 287, 288, 303–305
- glycan processing 148, 149
- glyceraldehyde-3-phosphate dehydrogenase 39
- glycerol-3-phosphate transporter 258
- glycerophospholipids 45, 46
- glycophorin A 253
- P-glycoproteins 82–85, 371
- glycosyl phosphatidylinositol 210
- glycosylation
 - mammalian cells 148, 149
 - *P. pastoris* 76, 77
 - rhodopsin 255, 256
 - *S. cerevisiae* 43, 44
- glycosylphosphatidylinositol (GPI) anchors 150
- Golgi apparatus, glycosylation 43
- gonadotropin releasing hormone type I receptor 145
- GPCRs, *see* G-protein-coupled receptors (GPCRs)
- Grace's supplemented medium (TNM-FH medium) 116
- gramicidin 260
- Green Fluorescent Protein (GFP) 4, 48, 139, 203, 229, 257, 331
 - GFP-His₈ 396, 403, 44
 - optimization of membrane protein production 391–405
- green monkey kidney (COS-1) 139, 222
- Group IB secreted phospholipase A₂ 204, 205–207
- Group V secreted phospholipase A₂ 204
- Group X secreted phospholipase A₂ 204, 207
- growth factors
 - ErbB 92, 93, 144, 155, 157
 - fibroblast 144
 - immobilization 159
 - insulin-like 158
- growth hormone-releasing hormone 56, 57
- GST, *see* glutathione S-transferase (GST)
- GTP-binding proteins 3
- GTPγS binding 120, 122

- h***
- heat-shock response, *S. cerevisiae* 49
- hemagglutinin 331
- hemifluorinated surfactants 322
- heterologous expression
 - GPCRs 221–225
 - membrane proteins 1, 2, 167, 168
 - photosynthetic bacteria 174
 - in *S. cerevisiae* 37
- heteronuclear single-quantum correlation (HSQC) spectra 309, 310
- high-copy plasmids, *S. cerevisiae* 40, 41
- High Five cell line 115, 116, 120, 122–124, 126, 233
- high-throughput (HTP) screening 4, 327, 328
- His tag 331, 332
- histamine H₁ receptor 223, 232, 237, 238
- histamine H₂ receptor 56, 57
- histamine H₃ receptor 118–120, 121–124, 125–128
- homolog screening 278
- homologous recombination
 - recombinant baculovirus 113
 - *S. cerevisiae* 37, 40–42
- homology (comparative) modeling 203
- horseradish peroxidase 186
- host strains
 - *E. coli* 16, 17, 19
 - *P. pastoris* 78, 79, 82–92, 94
 - *R. sphaeroides* 172, 173
 - *S. cerevisiae* 38–40, 52, 54, 56, 58
 - *see also individual strains*
- human calcium receptor 145
- human embryonic kidney (HEK) cells 47, 139, 222, 234
- human proteins, palmitoylation 212
- human serum paraoxonase 1 (PON1) 204, 210
- hydrophobic absorption 374, 375
- hydrophobic mismatch 376–378
- hydrophobic patch proteins 201, 202, 203, 204, 207–210
- hydrophobic thickness 376, 377
- hydrophobicity, protein 328
- hyperglycosylation 43, 44

- i*
- imetit 128, 129
- imidazole 191, 332, 400, 401
- immobilization, growth factors 159
- immobilized metal-affinity chromatography (IMAC) 397, 401
- immunoaffinity 332
- immunoblotting 181, 182, 184, 185, 193
 - development 186, 187
 - rhodopsin 256
- immunofluorescence 147
- immunotoxin Cyt2Aa1 90, 91

- IMPs, *see* integral membrane proteins (IMPs)
in cubo methods 6
 in-gel fluorescence 391, 394–396, 403, 404
 “In Membrane” 3
in meso methods 6
 inclusion bodies 13, 319
 – CXCR1 304, 305, 306
 – GPCR expression 228, 229
 inducible promoters 38, 39
 induction
 – autoinduction systems 24, 173, 174
 – in bacterial systems 18, 19–21
 – IPTG 18, 19, 21, 391, 392, 394
 influenza A virus 265–267
 infrared (IR) spectroscopy 337, 338
 – ligand binding 341
 inositol-1,4,5-triphosphate (IP_3) 213, 214
 insect cells
 – baculovirus infection of 115–118
 – GPCR expression 222, 223, 231–233, 239, 302
 – post-translational modifications 148
 – *see also* baculovirus-infected insect cells
 insulin-like growth factor-binding protein (IGFBP) 158
 integral membrane proteins (IMPs)
 – characterization 318, 319, 334–341
 – directed evolution of a GPCR for higher expression 280–286
 – engineering higher stability 288–294
 – purification 288, 317, 318, 331, 325–334
 – random mutagenesis and dot-blot screening 286–288
 – solubilization 317, 318, 319–325
 integrated membrane yeast two-hybrid (iMYTH) 60
 integrating plasmids 41, 42, 54
 interleukin-8 (IL-8) 298, 299
 intestinal peptide transporter 82, 83
 intracytoplasmic membrane (ICM) 168, 169, 170, 172, 173, 174, 189, 193
 intrinsic fluorescence 340
 inward-rectified K^+ channel 84, 85
 ion channels 156, 174
 – chloride channel PAB2010 92, 93
 – K^+ channels 84, 85
 – voltage-dependent anion channel (VDAC-1) 372
 ion-exchange chromatography 328
 ionic detergents 321, 322, 342, 344, 346
 – characteristics 363
 – *see also* nonionic detergents; zwitterionic detergents
 isatin-bound monoamine oxidase 88, 89
 isoelectric focusing 328
 isoelectric point (pI) 328, 329
 isopropyl- β -D-thiogalactopyranoside (IPTG)
 – induction 18, 19, 21, 391, 392, 394
 isothermal titration calorimetry 339
- k**
 kanamycin 393, 394
 Klett-Summerson colorimeter 183
Komagataella pastoris 76
Komagataella phaffii 76
 Krafft point 321
 KRX strain 17, 21
 KT3 tag 331
 K_v1.1 protein 144
- l**
lac promoter 15, 16–18, 19
 – *lacUV5* 15, 18, 19, 20, 391, 392
 LacI repressor protein 15, 16–18, 19, 20
 lactose permease (LacY) 258, 259, 376
 lauryldimethylamine-*N*-oxide (LDAO) 125, 345, 363, 398
 – protein yield 126
 – structure 365
 lectin affinity 332, 333
 lectin-like oxLDL receptor 1 90, 91
Lemo21(DE3) strain 16, 21, 22, 23, 391–405
 leukotriene B₄ receptors 88, 89, 221, 228, 229
 leukotriene C₄ synthase 90, 91
 lidocaine 157
 ligand binding assays 118, 119, 125
 – CXCR1 307, 308
 – IMPs 339–341
 – radioligand 284, 291, 292
 ligation-independent cloning (LIC) 170, 171, 172, 175–179
 light-harvesting antennae (LHI) 170, 171, 174, 254
 light scattering 335
 lipid anchor proteins, covalent 201, 202, 204, 210–212
 lipid-binding-domain proteins 201, 202, 204, 212–214
 lipid requirements
 – mammalian systems 148, 149, 150
 – *P. pastoris* 77
 – *S. cerevisiae* 44–47
 – in solubilization 325
 lipids
 – composition 375–381
 – detergent-free 370–372
 – lipid/detergent ratio 365, 368, 370, 371
 – mesophase crystallization 6

- nanodisks 370–372
- *see also* phospholipids
- Lipofectamine™ 2000 146
- lipofection 143
- lipopeptide detergents 322
- liposomes 372
 - cationic 143, 146
 - detergent saturation 375
 - reconstitution of proteoliposomes 372–375
- liquid chromatography
 - FPLC 191, 192, 304, 305, 306
 - RP-HPLC 266
- Lon protease 28
- low-affinity cation transporter 82, 83
- low-copy plasmids, *S. cerevisiae* 40, 41
- LYS2 selection marker 40, 41
- lysophosphatidic acid 56, 57
- lysozyme 397

- m**
- M2 protein 265–267
- magic angle spinning 5, 300
- magnetic beads 291, 292, 333, 334
- maltose binding protein (MBP) 25, 226, 227
- mammalian cells 139–161
 - antiapoptosis engineering 152–156
 - biotherapeutics 139, 157–159
 - cancer cell lines 157, 158
 - cell culture types and media optimization 140–150
 - chaperones 156, 157
 - expression by virus vectors 150–152
 - GPCR expression 219, 223, 224, 234, 239, 302
 - membrane protein case studies 144, 145
 - post-translational modifications 148
- mannose 148
- mannose-binding protein 331
- mannosidases 43, 149
- MAPAS method 208
- mean fluorescence intensity 282, 283
- media optimization
 - mammalian cells 141, 142
 - *see also* cell cultures
- melatonin 56, 57, 224, 236
- melittin signal sequence 120, 121
- membrane filtration 330
- membrane insertion 251, 252
- membrane isolation
 - Lemo21(DE3) 396–398
 - photosynthetic bacteria 188
- membrane scaffold protein (MSP) 370, 371
- metabotropic glutamate receptor 224, 225, 234
- metal-affinity chromatography 29, 50
- immobilized 397, 401
- metal-dependent hydrolase 92, 93
- metallothionein 38, 39
- methanol utilization 75–78
- methionine sulfoximide 143
- methotrexate 143
- (R)- α -methylhistamine 128, 129
- [3 H]-(*N*)- α -methylhistamine 120, 122, 125, 126, 127, 130
- MG1 cell line 115
- micelles 319, 320
 - detergent saturation 375
 - formation 362
 - mixed detergents 365, 366–368
 - in solubilization process 323
 - *see also* critical micelle concentration (CMC)
- microsomes, phospholipids in 45
- Mistic fusion proteins 14
- mitochondria, phospholipids in 45
- mitochondrial ADP/ATP carrier 338
- mixed detergent systems 365, 366–370
- molecular weight 252
- monoamine oxidase 61, 88–91
- monotopic-integral proteins 201
- MPEX 48
- 2 μ -based plasmids 40, 41, 50, 52, 54, 56, 58
- μ -opioid receptor 86–89, 144, 224, 230, 238
- multidrug resistance protein 82, 83
- multiplicity of infection (MOI) 116
- mutagenesis, *see* random mutagenesis; site-directed mutagenesis
- mutants in mannan biosynthesis (*mnn*) 43
- Myc tag 331
- Mycobacterium tuberculosis* 2, 3
- myristylation of proteins 149, 150, 202, 211

- n**
- 15 N isotopic labeling 76
- N-methyl-D-aspartate receptors (NMDA) 145
- n*-octyl- β -D-glucopyranoside (NOG) 125, 126, 398
- N-terminus
 - fusion partners 25, 227
 - modifications 256, 257
- Na/K-ATPase 88–91, 378
- nanodisks, lipid 370–372
- native sources 1, 249, 256, 264, 265
 - drawbacks 337
- Na, 1.8 channel 157
- NEB Express I^q strain 16, 19
- necrosis 152
- neurokinin NK₁ 221

- neurological disorders 158
 neuromedin U receptor 88, 89
 neuropeptide Y2 221
 neuropeptide Y4 236
 neuropeptin 281–284, 290, 292, 293
 – BODIPY-neuropeptin 281, 283
 – GPCRs 221, 226, 231, 234, 236
 – *S. cerevisiae* 56, 57
 α_7 nicotinic acetylcholine receptor (nAChR) 156
 NIH Protein Structure Initiative 202
 nonaethylene glycol monodecyl ether C12E9 346
 nonionic detergents 320, 321, 325, 342
 – characteristics 364
 nonspecific electrostatic proteins 201, 202, 204, 204–207
 NR vectors 18
 NTR1 281
 nuclear magnetic resonance (NMR) 5
 – bicelle/membrane protein 370
 – CXCR1 304, 308–310
 – fos-choline 12, 364
 – GPCRs 220, 226, 227, 233, 298, 300, 301
 – IMPs 337, 338
 – ligand binding 341
 – nanodisks 372
 – proteoliposomes 373
 – recoverin 211
 nucleotide-binding domain 60
- o**
 octyl- β -D-glucopyranoside (OG) 342, 348, 364, 381
 OD₆₀₀ 394–396
 odorant receptor 236, 237
 olfactory receptor 226, 238
 oligomeric state, protein 334–336
 oligomerization, membrane proteins 253, 254
 oligonucleotide primers, design 177, 178
 OmpA 252
 OmpF porin 5
 OmpT protease 28
 opioids 56, 57, 224
 – see also μ -opioid receptor
 opsin 224
 OPTI-MEM® I 146
 Orientations of Proteins in Membranes (OPM) database 203, 204, 205, 208
 oriented sample (OS) solid-state NMR 300, 301, 308, 309
 overexpression
 – chaperone 26, 27
- UPR activation 49
 Overnight Express™ autoinduction system 24
 oxidosqualene cyclase 88, 89
- p**
 p8-derived fusion proteins 14, 15
 PAFAH-II 204, 209
 palmitoylation 149, 212, 255, 256
 P_{AOX1} promoter 79, 93
 paraoxonase 1 (PON1) 204, 210
 parathyroid hormone 224
 Parkinson's disease 156
 pASK75 vector 18, 20
 pBAD vectors 17, 18, 19, 24
 PCR, *see* polymerase chain reaction (PCR)
 PDC, *see* protein–detergent complex (PDC)
 pDEST™8 destination vector 121
 Penta-His HRP (horseradish peroxidase) 186
 peptides 253
 – formyl peptides 56, 57
 – fusion peptides 52, 54, 56, 58
 – glucagon-like 47
 – histamine H₃ receptor 120, 121, 124, 125
 – intestinal transporter 82, 83
 – lipopeptide 322
 – membrane targeting 25, 26
 – neuropeptides 221, 236
 perfusion culture 141, 142
 peripheral membrane proteins 3, 201–215
 – classes 201, 202
 – definition 201
 – *protein data bank* entries 203, 204
 permeability transition pore (PTP) 153, 154
 peroxisomal membrane protein 92, 93
 peroxisomes 77, 78, 144
 – phospholipids in 45
 pET vectors 16–18, 20, 23, 24
 pGEX2a 303, 305
 pH gradient 329
 phase diagrams 320
 phase separation 342–345, 320, 321, 334
 phosphatidate lipids 378, 379
 phosphatidylcholine 45, 46
 phosphatidylethanolamine 45, 46
 phosphatidylserine 45
 phosphocholine lipids 378, 379, 380
 phosphoethanolamine lipids 376, 378, 379
 phosphoinositides 45
 phospholeman 90, 91
 phospholipase
 – phospholipase C 204
 – secreted phospholipase A₂ (sPLA₂) 204, 205–207

- phospholipids 258, 259, 308
 - detergent combinations 367–370
 - detergent-like 364, 365
 - *S. cerevisiae* 44–46
- phosphorylation 148
- phosphotidylinositol 45
- photosynthetic bacteria 167–197
 - autoinduction 173, 174
 - detergent solubilization and chromatographic purification 189–192
 - expression constructs preparation 175–179
 - expression strategies 170–174
 - host strains 172, 173
 - large-scale culture 187, 188
 - physiology 168, 169, 170
 - protein identification and purity assessment 192
 - *see also Rhodobacter sphaeroides*
- photosystem II/light harvesting complex II 254
- physical selection 280
- Pichia pastoris* 75–99
 - biotechnological tool 76, 77
 - expression conditions and culturing formats 80, 81
 - GPCR expression 2, 86–89, 94–98, 225, 230, 231, 302
 - host strains and plasmids 78, 79, 82–92, 94
 - methanol utilization 75–78
 - recombinant membrane proteins from 81, 82–92
 - transformation and clone selection 80
- pK_i, histaminergic ligands 129
- plaque-forming unit 116
- plasma membrane
 - phospholipids in 45
 - Pma1p role in 51, 60
- plasma platelet-activating factor
 - acetylhydrolase (pPAFAH) 204, 208–210
- plasmids
 - bacterial systems 16, 17, 21, 23
 - *P. pastoris* 78, 79, 82–92
 - photosynthetic bacteria 170–172, 175, 176, 179–181
 - *R. sphaeroides* 180, 181
 - *S. cerevisiae* 40–42, 50, 52, 54, 56, 58
- platelet-activating factor acetylhydrolase Type II (PAFAH-II) 204, 212
- pleckstrin homology (PH) domain 204, 213
- pLemo plasmid 16
- pLysS plasmid 16, 21
- Pma1p 50, 51, 60
- polyacrylamide gel electrophoresis (PAGE),
 - *see* sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)
- polydispersity 13
- polyhedrin promoters 114
- polyhistidine tag 29, 232
- polymerase chain reaction (PCR)
 - epPCR 281–283, 293
 - photosynthetic bacteria 178, 179
 - *S. cerevisiae* 42
- polymorphonuclear neutrophils 298, 299
- polyoxyethylenes 364
- poly(vinylidene fluoride) membrane 185, 186
- porins
 - aquaglyceroporin 84, 85
 - aquaporins 84, 85, 259
 - crystallization 5, 6
- porous bead volume 329
- post-translational modifications
 - mammalian cells 139, 147–150
 - membrane proteins 254–256
 - peripheral membrane proteins 202, 203
 - *S. cerevisiae* 43, 44
 - *see also* glycosylation; palmitoylation
- pQE vectors 18, 19
- prediction methods, membrane proteins 251
- prenylation of proteins 149, 150
- pRKPLHT1/pRKLICHT1 170, 172, 174–177
- PrlF antitoxin 28
- proapoptosis proteins 153, 154
- prokaryotic proteins 278
 - expression yield 29
 - higher stability 289, 290
- promoters
 - bacterial systems 15, 16–18, 19, 20
 - baculovirus-infected insect cells 114
 - inducible and constitutive 38, 39
 - mammalian cells 143, 144, 145
 - *P. pastoris* 79, 93
 - *Saccharomyces cerevisiae* 38, 39, 52, 54, 56, 58
 - *see also individual promoters*
- proteases 152, 319
 - quality control 27, 28
 - TEV protease 400
- protein denaturation 363
 - mitigating 366–381
- protein–detergent complex (PDC) 6, 322, 323, 324
 - stability 325, 326
- protein engineering
 - antiapoptosis 152–156

- higher expression 278–280
- higher stability 288–294
- protein kinase C 204
- protein–protein interaction 332
- proteoliposomes, reconstitution 372–375
- proteolytic processing 148
- puc* operons 170, 171, 173
- pUC* vectors 15, 18
- puf* operons 170, 171, 173, 174
- purification 5, 118, 119
 - and contaminants 260
 - CXCR1 305, 306
 - detergents and 118, 119, 125, 126, 189, 190
 - GPCRs 238
 - IMPs 288, 317, 318, 331, 325–334
 - membrane protein GFP-fusion 333–334
 - new approaches 327–333
 - process 335–339
 - rhodopsin 261–264
 - RPE65 264, 265
- purinergic receptors 58, 59
- putative membrane protein 92, 93
- Pyrococcus horikoshii* 278

q

- quality control proteases, bacterial systems 27, 28

r

- radioligand binding assay 284, 291, 292
- radioligands 118, 19, 126, 130
- Ralstonia metallidurans* 278
- random mutagenesis 279–281, 286–288
 - GPCR stabilization 291–294
 - prokaryotic IMPs 289, 290
 - stable proteins 381
- receptor smoothened HuMOR 86, 87
- receptor tyrosine kinases (RTKs) 155, 157
- recombinant protein production 334
 - from affinity chromatography 331, 332
 - from baculovirus-infected insect cells 111, 113–115
 - from *Pichia pastoris* 81, 82–92
 - *S. cerevisiae* 37, 40–42
- reconstitution
 - CXCR1 304, 305, 307
 - proteoliposomes 372–375
- recoverin 204, 211
- refolding
 - CXCR1 304, 305–307
 - inclusion bodies 228, 229
- restriction enzyme digestion 178
- retina rod cells, *see* rod cells

- retinal pigmented epithelium-specific protein 65 (RPE65) 260, 264, 265
- reverse-phase high-performance liquid chromatography (RP-HPLC) 266
- rhaBAD* promoter 18, 19, 21
- L-rhamnose 18, 19, 22, 23, 391, 392, 394, 395
- Rhodobacter sphaeroides* 169, 170, 171, 174
 - cytochrome *c*₂ from 205
 - detergent solubilization 189
 - GPCRs 221, 228
 - ligation-dependent cloning 177, 178
 - plasmid DNA transfer to 180, 181
 - preparation of specialized membranes 192–194
 - small-scale screening of target protein 181–187
 - strains 172, 173
- Rhodopseudomonas viridis* 1
 - photosynthetic reaction centre 6
 - rhodopsin 4, 58, 59, 219, 222, 224, 227
 - glycosylation 255, 256
 - hydrophobic mismatch 377
 - oligomerization 254
 - test case protocols 261–264
 - ribosomal binding sites (RBSSs) 15
 - *Rhodobacter* 177
 - RNA polymerase 18, 19
 - T7 16, 17, 20–23, 391, 392
 - rod cells
 - GPCR expression 234, 235, 239, 257
 - rhodopsin 255, 256
 - rod outer segments (ROS) 261–263
 - Rosetta 17
 - Rous sarcoma virus 143
 - RPE65 (retinal pigmented epithelium-specific protein 65) 260, 264, 265

s

- Saccharomyces cerevisiae* 37–62
 - case studies 49–61
 - cellular responses to protein expression 49
 - crystal structures from expression 61
 - expression conditions 42
 - GPCR expression 225, 229, 278, 302
 - host strains 39, 40, 52, 54, 56, 58
 - lipid requirements 44–47
 - plasmids and homologous recombination 40–42, 50, 52, 54, 56, 58
 - post-translational modifications 43, 44
 - promoters 38, 39, 52, 54, 56, 58
 - selection markers 40, 41
 - signal sequences 47
 - topology determination 47, 48

- tractable targets 4
- sample homogeneity, protein 334–336
- saturation, detergent 375
- screening method 279, 280, 285, 286–288
 - 96-well assay format 289, 290, 291–294
 - dot-blot detection 230, 286–288
 - single-clone screening 290, 291
- SDS, *see* sodium dodecyl sulfate (SDS)
- SDS-PAGE, *see* sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)
- SEC, *see* size-exclusion chromatography (SEC)
- Sec translocase 14, 25, 26
- secreted phospholipase A₂ (sPLA₂) 204, 205–207
- secretin 222
- sedimentation equilibrium 335, 336
- sedimentation velocity 335, 336
- selection markers
 - *P. pastoris* 79
 - *S. cerevisiae* 40, 41
- selection method 280, 282, 285, 286
- Semliki Forest virus 151, 152
- sequence modifications 256–258
- serotonin 58, 59
- serotonin 5-HT 221, 226
- serotonin transporter 82, 83
- serum
 - baculovirus-infected insect cells 115, 116
 - mammalian cell culture 142, 146, 158
 - paraoxonase 1 (PON1) 204, 210
- Sf9 cell line 2, 115–117, 120, 222, 223, 232, 233
- RPE65 expression 264
- Sf21 cell line 115, 116, 223, 233
- Shaker B protein 144
- signal recognition particle (SRP) 15, 25, 28
- signal sequences
 - melittin 120, 121
 - *S. cerevisiae* 47
- simian virus 40 143
- Sindbus virus 151
- single-clone screening 290, 291
- Single Protein Production (SPP) System 22
- single-spanning membrane proteins 13
- Single Step strain 17
- site-directed mutagenesis 43, 50, 51
- size-exclusion chromatography (SEC) 252, 257, 306, 307
 - fluorescence-detection 398, 399
 - IMP characterization 334, 335
 - IMP purification 328, 329
 - *see also* gel filtration
- sodium cholate 344, 362
- sodium deoxycholate 344, 347
- sodium dodecyl sulfate (SDS) 120, 123, 124, 344, 347
 - characteristics 362, 363
 - sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) 29, 252
 - CXCR1 purification 306
 - GFP fluorescence 391
 - photosynthetic bacteria 181, 182, 183, 184–186, 192
 - RPE65 purification 265
- sodium/glucose cotransporter 82, 83
- solid-state NMR 5, 300, 301, 308, 309, 310, 373
- solubilization 5
 - CMC in 324, 398
 - GPCRs 238, 324
 - histamine H₃ receptor 118, 119, 125, 126
 - IMPs 317, 318, 319–326
 - photosynthetic bacteria 189, 190
 - process 323, 324, 365, 366
 - vs stability of detergents 361–366
- soluble proteins, numbers 2
- solution NMR 5, 300, 308, 310
- somatostatin 58, 59
- Sonic Hedgehog 150
- spectroscopy, *see* circular dichroism (CD)
 - spectroscopy; fluorescence correlation spectroscopy; infrared (IR) spectroscopy; transverse relaxation optimized spectroscopy (TROSY)
- spheroplasts 193, 194
- sphingolipids 46
- sPLA₂ 207
- SPOCTOPUS 48
- Spodoptera frugiperda* 114, 233
- ST8Sia protein 144
- stability
 - engineering higher stability 288–294
 - protein–detergent complex 325, 326
 - vs solubilization of detergents 361–366
- stability index 292, 294
- stable isotope-labeled proteins 235
- stable proteins, making/selection 381
- static light scattering 335
- Ste2p 50, 224
- 11-OH steroid dehydrogenases 88, 89
- sterols
 - *S. cerevisiae* 37, 46, 47
 - *see also* cholesterol
- Stoke's radius of soluble proteins 335, 340
- Strep tag 331
- streptavidin-coated magnetic beads 291, 292, 333

- structural analysis 5–7
 – membrane proteins 249–261
 subcellular fractionation 174, 327
 sucrose density gradient centrifugation 193
 sucrose monododecanoate 125, 126
 surface plasmon resonance 119, 339
 suspension cell culture 141, 142
 synaptic adhesion-like molecule 145
- t**
 T4 DNA polymerase 172
 T5 promoter 18, 19
 T7 expression 16–18, 19–25
 T7 lysozyme (lysY) 16–18, 20–22
 T7 RNA polymerase 16, 17, 20–23, 391, 392
tac promoter 15, 18, 19
 tachykinin 223
 tags
 – bacterial systems 28, 29
 – IMP purification 330, 331, 332
 – *S. cerevisiae* 53, 55, 57, 59
 – *see also individual tags*
 Terrific Broth (TB) 24
tetA promoter 18, 20
 tetraspanin 92, 93
 TEV protease site 121, 125
Thermotoga maritima 367
Thermus thermophilus 278
 thioperamide 128, 129
 “three-detector method” 335
 thrombin 29
 thromboplastin, tissue factor 90, 91
 thromboxane 224
 thyroid-stimulating hormone 221
 titerless infected cell preservation and scale-up (TIPS) 115
 TM0026 367
 tobacco etch virus (TEV) 29, 120
 – protease 400
 TOP10 strain 17, 22
 TOPCONS 48
 topology
 – membrane proteins 392–394
 tractable targets 4
 transfection, mammalian cell cultures 143, 146, 147
 transient receptor potential melastatin, or channel 145
 translocons 252
 transmembrane helices
 – association motifs 253, 254
 – prediction 251
 transmembrane regions 13
 – M2 protein from influenza A virus 265–267
 transporter proteins
 – ABC transporters 60, 82, 83
 – copper transporter 82, 83, 145
 – from *E. coli* 236
 – glycerol-3-phosphate transporter (GlpT) 258
 – from *P. pastoris* expression 82–85
 transverse relaxation optimized spectroscopy (TROSY) 5, 339, 341
trc promoter 15, 18, 19
Trichoplusia ni 115, 223, 233
 tripod amphiphiles 322
 Triton X-100 342, 346, 364, 398
 Triton X-114 342, 364
 Trypan blue dye exclusion 116
 trypsin digestion 120, 140
 – histamine H₃ receptor 123, 124
 tryptophan 340
 tunable T7 expression systems 21, 22
 Tuner strain 17, 21
 turbidity 375
 “two-detector method” 335
- u**
n-undecyl-β-D-maltopyranoside (UDM) 398
 unfolded protein response (UPR), *S. cerevisiae* 49
 URA3 selection marker 40, 41
- v**
 vacuolar protein sorting (VPS) genes 51
 vacuole, phospholipids in 45
 vasopressin 58, 59, 236
 vectors
 – bacterial systems 15, 16–18, 19, 20
 – virus vectors 150–152
 – *see also expression vectors; individual vectors*
 Venezuelan equine encephalitis virus 151
 vesicular glutamate transporter 84, 85
 viral titers 115
 virus vectors
 – mammalian cells 150–152
 – *see also baculovirus-infected insect cells*
 voltage-dependent anion channel (VDAC-1) 372
 voltage-dependent K⁺ channel 84, 85
 voltage-sensitive K⁺ channel 84, 85
- w**
 water, in membrane proteins 259
 water channel proteins, from *P. pastoris* expression 84, 85

- Western blot analysis 29, 118, 120, 123, 147, 155
whole-cell fluorescence 391, 394–396, 403, 404
- x**
X-ray crystallography 5, 298, 299
– GPCRs 4
Xenopus laevis 225, 235, 257
- y**
yeast
– post-translational modifications 148
– *see also Pichia pastoris; Saccharomyces cerevisiae*
YidC protein 14, 23, 27
- z**
zwitterionic detergents 320, 345, 347, 348
– characteristics 363, 364