

Index

- 38C2 antibody 221
 434 bacteriophage 371
 454 DNA sequencing technology 284
- α -helix structures 12–14, 91–94
 α/β hydrolase folds 12, 13
 α/β novel protein fold 372, 373
 α_3 D 73-residue protein 92, 94–96
 β -hairpin structures 91, 93, 94
 XAΔ4 protein 152, 153
 λ -repressor 100–102
- a**
- A-motif diversification 285
 accelerated degradation testing 135–137
Acetobacterium woodii 205
 acetylxytan esterase 12
Acidianus ambivalens 152
Acinetobacter sp. 129, 130
 aconitase enzymes (dehydratases) 195, 196
 actinol 198, 199
 active sites
 – buried 421–452
 – *de novo* enzymes 380–384
 – enzymes with one tunnel 436, 437
 – structural order requirement 15, 16
 – subtilisin, mutagenesis 254, 255
 activity of enzymes
 – *de novo* enzyme design 378, 380–384, 413, 414
 – tunnel engineering 442, 443
 acyl-phosphatase 100, 101
 acylation 9, 10, 244, 259, 263–265
 adenosine deaminase 383
 adenosine triphosphate (ATP) 169
Agrobacterium radiobacter 16
 alcohol dehydrogenase (ADH) 171, 172
 alcohol oxidase 1 gene promoter 51, 52, 55
 aldehydes 130, 131
 algorithms
 – Dead End elimination 369, 370
 – FASTER stochastic algorithm 369, 370
 – fixed-backbone protein design 368–371
 – phylogenetic tree construction 35
 – protein design 364–369, 372, 374, 382
 – SCHEMA algorithm 338
 alkaline tolerance 131, 132
Alkaliphilus transvaalensis 145
 alkenes 198–201
 O₆-alkylguanine-DNA alkyltransferase 78
 Amadori rearrangement reaction 14
 amino acids
 – *see also* sequences; variant libraries
 – Ancestral Mutation Method 31, 32
 – *de novo* folds/enzymes 387, 388
 – *DhaA* gene (*Rhodococcus rhodochrous*) 449
 – DNA shuffling 29, 37–39
 – fixed-backbone design 364–367
 – γ -humulene synthases 1, 2
 – natural diversity 29–44
 – non-natural 41, 42, 394
 – P450 BM3 fusion protein 335, 336
 – protein structure/folding 12, 13
 – REAP method 29, 30, 32–34, 36–38
 – ribozymal cofactors 163, 164
 – serine protease reaction mechanism 244
 – structure-promoting/dispromoting 14, 15
 – subtilisin 250, 252, 253, 268–271
 amphotericin B 308
 amphoteronolides 307, 308
 anaerobic biocatalysis 203–210
 Ancestral Mutation Method (AMM) 29, 31, 32
 Ancestral Sequence Reconstruction (ASR) 29–44

- biomedical applications 41
- drug discovery 41, 42
- experimental validation 43, 44
- industrial applications 40, 41
- paleobiology 42, 43
- practical steps to using 34–36
- REAP method 29, 32–34, 36–39
- synthetic biology 43
- technique 30, 31, 34–38
- Anchored Design software package 375
- anchors, organometallic 215, 216, 219–222
- anhydrous ionic liquid solvents 8
- anion-triggered proteases 255, 256, 267–269
- *see also* subtilisin
- antibodies/fragments 121, 122, 124, 125, 221
- Aquifex* 146
- Arabidopsis thaliana* 4
- Archaea, thermophilic 145–157
- η^6 -arene/*N*-tosylethylenediamine-Ru complexes 217, 228
- aromatic ketones 217
- aromatic side chains 223
- Arrhenius equation 135–137
- artemisinin 43
- artificial metalloenzymes 215–237
 - concept 215, 216
 - design 216–222
 - development goals 234–237
 - key developments 223
 - protocols 226–234
 - reactions catalyzed by 225
 - reasons for developing 223–226
- Arxula adeninivorans* 56, 64
- asymmetric catalysis, *see* enantioselectivity
- asymmetric nitroalkanes 198–200
- asymmetric transfer hydrogenation (ATH) 217
- autoxidation shunt 328, 329
- avidin 215–237

b

- B-FIT analysis/method 131
- Bacillus subtilis* 12, 131, 338–339
- baculovirus expression vector system (BEVS) 58–59
- Baeyer–Villiger monooxygenase (BVMO) 129
- Basic Local Alignment Search Tool (BLAST) 34
- Bayesian approaches 35, 36
- N*-benzyl nicotinamide derivatives 172, 173
- binders/anchors 77, 78, 215, 216, 219–222

- biotin-4-fluorescein 227, 228
- biotin–streptavidin technology 215–237
- 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl 216
- BM3 reductase domain (BMR) 349, 350
- Born approximation 367, 368
- BOSS/MCPRO simulation program 408
- bovid ribonuclease 30
- bovine pancreatic trypsin inhibitor 408, 409
- branched PdR-PdX-P450cam-fusion (bRXC) 352
- branching events 287
- bulk emulsions 75–78
- buried active sites 421–452
- Burkholderia cepacia* lipase 414
- butanol transesterification reactions 9

c

- C1 expression system 57–58
- Caldariomyces fumago* 58
- C α -C β vector distances 382
- Calvin–Benson–Bassham cycle 155
- camphor 332–334
- Candida* spp.
 - *C. antarctica* 8, 9, 408, 409, 411–413, 415
 - *C. rugosa* lipase 11, 411, 413, 414
- N*-carbamyl-D-amino acid amidohydrolase 130
- Carbohydrate-Active enZyme database 304
- carbonic anhydrase 219
- carbon–carbon bond cleavage 108
- catalytic promiscuity 21, 22
- catalytic selections 76, 77
- catch-and-release phage selection 265–267
- cells
 - cell-free expression systems 61–63, 152–154
 - cultures 48–56, 58–61, 64
 - eukaryotic expression systems 59, 60
 - hyperthermophiles 156, 157
 - signaling agents 122, 123
 - transport, riboflavin 180
- channels/tunnels distinction 423
- CHARMM MD force-field 367, 408
- chemical complementation 314
- chemical rescue 243–274
- chemotrypsin 411
- chimerogenesis 307–310
- chiral ligands 216
- chloramphenicol acetyl transferase (CAT) 347
- chloroperoxidase (CPO) 58

- chorismate mutase 16, 17
- chromium (Cr) 174, 175, 222
- Chrysosporium lucknowense* 51, 57
- chymotrypsin 11, 243, 245–247
- circular dichroism (CD) 90, 93, 98
- citrate synthase 139
- citronellal 200
- cleaning-in-place (CIP) procedures 131, 132
- Clostridium* spp. 200–202
- CO-difference spectra 328
- cobalt 103, 175, 176
- cocaine esterase (CE) 116, 127
- CoFactor database 166–168
- cofactors 163–181
 - *see also individual cofactors*
 - anions, protease specificity 266–269
 - binding strategies 219–222
 - chemical composition 164, 165
 - CYPs 329, 349–354
 - definition 163, 164
 - engineered subtilisins folding 252
 - flavin cofactor redesign 176–180
 - heme cofactor engineering 173–176
 - inorganic cofactors 165, 168
 - natural cofactors 164, 165
 - organic cofactors 166–169
 - overview 163, 164
 - redox cofactors 169–180
 - subtilisin binding site 267
- cognate sequence identification 255, 256
- coiled-coil interfaces 389
- colicin (DNAase) 389, 391
- Combinatorial Libraries Enhanced by Recombination in Yeast (CLERY) 50, 63
- combinatorial optimization 368, 369
- combined design methods 336–338, 387, 388
- compartmentalized self-replication (CSR) 76, 77, 155, 289–291
- competitive inhibition 5
- complicated folding pathways 91–94, 97–100
- computational modeling
 - *see also algorithms; databases*
 - combined methods 336–338, 387, 388
 - enzymes in organic solvents 407–417
 - power limitations 416
 - protein design
 - – DNA binding/specificity 394, 395
 - – enzymes 380–388
 - – force-fields 364–368
 - – key concepts 363–371
 - – named algorithms 364–369, 372, 374, 382
 - – protein core redesign 371–379
 - – protein–protein interface design 388–394
 - – protein folding/dynamics 89–111
 - – tunnel engineering software 444–447
- condition promiscuity 21, 22
- conformational changes
 - amino acid side chains 365–367, 369, 382
 - enzyme computational modeling 407–417
 - ionic liquid effects 7, 8
 - keyhole-lock-key model 421–452
 - organic solvents 5
 - proteases, difficulties 270–272
 - role in enzyme catalysis 438
 - solvent-induced 414–415
- coniferyl alcohol 4
- copper 175, 176
- core redesign 371–373
- coumarin 7-hydroxylation 328, 336
- coupled systems, cell-free 61, 62
- covalent bonds 178, 219–222
- covarian-type divergence 33
- [Cp*Rh(bpy)(H₂O)]²⁺ 218
- critical micelle concentration (CMC) 11
- cro* protein (bacteriophage 434) 371
- cross-reactivity, *see* promiscuous enzymes
- CstII sialyltransferase 311
- cutinase (*Fusarium solani*) 12
- cyclic amidohydrolases 13, 14
- cyclohexanone monooxygenase (CHMO) 129, 130
- CYPs, *see* cytochrome P450 monooxygenases
- cysteine 195, 196, 219–221, 223
- cysteine proteinases 197, 198
- cystine knot motif 138
- cytochrome P450 monooxygenases 327–355
 - catalytic cycle 328, 329
 - classes I/II definition 329, 330
 - CYP2B1, mutant 354
 - CYP2B6, human 343
 - CYP2B6dH, human 348
 - CYP2B11, canine 343
 - CYP2C9, human 331
 - CYP2D6, human 53
 - CYP105A3 348
 - CYP116B2 350, 351
 - CYP152A1 338, 339

- dirigent effects in biocatalysis 4
- engineering 330–354
 - cofactors 349–354
 - electron transport chain 349–354
 - enzyme activity improvement 332–340
 - molecular background 330, 331
 - recombinant expression 343–349
 - solubility 343–349
 - solvent stability 340–343
 - substrate selectivity 332–340
 - temperature stability 340–343
- overview 327, 328
- P450 BM3 fusion protein 333–337
 - 9-10A mutant 342–343
 - binding pocket structure 340
 - decoy molecules 339, 340
 - variant libraries 337
- P450cam 332–334
- redox partner proteins 329, 330
- *Thermus thermophilus* 351, 352

d

databases

- CAZy database 304
- CoFactor database 166–168
- homologous sequences 34, 35
- Metal-MaCiE database 165
- Protein Data Bank 396
- protein stability 124, 126
- tunnel engineering 445, 446
- dative anchoring 219–222
- de novo* design
 - diiron proteins 223
 - enzymes 378, 380–385, 390–392, 413, 414
 - Diels-Alderase 381, 384
 - folding of proteins 371–373, 375, 376, 385
 - one-sided protein interfaces 392
 - two-sided protein interfaces 390–392
- deacylation 244, 263–266
- Dead End elimination 369, 370
- decoy molecules 338–340
- Dehalospirillum multivorans* 203
- dehydratases (aconitases) 195, 196
- denatured state 7, 100
- deoxyribonucleic acid (DNA)
 - *see also* polymerases
 - binding/specificity computational redesign 394, 395
 - chiral scaffold 223
 - colicin (DNase) 389, 391
 - expression, microfluidic droplets 80
 - next-generation sequencing 284–288

- post-RNA evolution 163
- protein yield in microdroplets 83
- screening problems 37
- sequence space reduction 29
- shuffling 29, 37–39
- CYP enzymes 336, 337, 342
- substrates, catalytic selections 76, 77
- synthetic informational polymers 292, 294
- yeast protein engineering 50
- designer ionic liquid solvents 10
- DESIGNER software 364, 365, 368, 372, 384
- Desulfomonile tiedjei* st. DCB-1 203
- detergents/surfactants 10, 11, 148, 345
- deterministic combinatorial optimization algorithms 368, 369
- Dezymer software 369, 372, 380, 384
- DFtet-A/B peptides 92, 104, 105
- DhaA* gene 448–451
- diagnostic indicators 120, 121
- Diels-Alder reactions
 - *de novo* design 381, 384
 - myoglobin cofactor redesign 176, 177
 - organometallic chemistry 221–223
- dihydrofolate reductase (DHFR) 17–19
- diiron proteins 92, 102–105, 223
- dinucleotide cofactors 163, 166, 167, 169–173, 176–180
- dioctyl sodium sulfosuccinate 11
- direct product determination 208, 209
- direct screening of polymerases 289, 290
- directed evolution
 - computational enzyme design 385, 386, 393
 - CYPs 336–338, 342
 - glycosyltransferases 310–315
 - microdroplet-based 73–84
 - P450 BM3 fusion protein 334
 - proteases 243–274
 - protein stability 117–126
 - protein-protein interfaces 388, 389
- dirigent effects in biocatalysis 1–23
 - dirigent proteins 3, 4
 - overview 1–3
 - solvents 4–11
 - structure and folding 12–14
 - unconventional reaction media 4–11
- disqualifying interactions 248
- dNTP-ONH₂ reversible terminator 34
- double bulk emulsions 78, 79
- double displacement mechanism 306
- drug discovery 41, 42, 123, 124
- drug targeting 180

Due Ferro 1 (DF1) 92, 103–105
 dummy molecules 338–340
 dynamic knockout mutations 19

e

eEF1A protein 42
 EF-Tu protein 42
 EGAD software 368–370, 372
 elastases 246
 enantioselectivity
 – asymmetric nitroalkanes 198–200
 – chiral ligands in homogeneous catalysis 216
 – Diels-Alder reactions 176, 177
 – loop replacements 12
 – myoglobin heme substitution 175–177
 – P450 decoy molecules 339
 endo-1,4- β -xylanase 92
 endonucleases, homing 395
 ene-reductases 193–195, 198–200
 ENH-FSM1 (UVF) 92, 97
 EnHD structure 92
 enoate reductases 200–202
 ensemble protein structures 445, 447
 enthalpy/entropy 413
 environmental-sustainability 416
 enzyme membrane reactors (EMRs) 172
 enzyme-linked immunosorbent assay (ELISA) 313
 enzymes
 – *see also* cofactor redesign; enantioselectivity; *individual enzymes*
 – activity 332–340, 407
 – computational design 380–388
 – enzymatic fusion 352–354
 – folding/dynamics 106–110
 – identification 193–210
 – modeling in organic solvents 407–417
 – organic cofactor distribution 166, 167
 – protease specificity engineering 243–274
 – stability engineering examples 117–120
 – substrate specificity 383–386
 – thermophilic organisms 145–157
 – tunnels 421–452
 episomal plasmids 48
 error-prone PCR 336, 337, 341, 342
Escherichia coli host expression system 63
 – anaerobic HT screening 205–207
 – CYPs 343, 344, 348
 – dihydrofolate reductase 17–19
 – thermostable protein production 148, 149
 esterases, thermostability 148
 eukaryotic CYPs 53, 331, 343–348

eukaryotic expression systems 47–65
 – cell-free 61–63
 – filamentous fungi 56–58, 64
 – insect cells 58, 59, 64
 – mammalian cell cultures 59–61, 64
 – transgenic animals/plants 61
 – yeast expression platforms 48–56, 64
 evolution 42, 43, 100, 163, 164, 291, 437, 438
 – *see also* directed evolution; Reconstructing Evolutionary Adaptive Paths (REAP)
 experimental free energy change ($\Delta\Delta G$) 379
 expression systems 47–65, 315
 expression vectors 315
 extant protein sequences 29–44
 extremophiles
 – *see also* thermophile enzymes
 – types 145, 146

f

F-G loop modifications 346–348
 F87A P450 BM3 variant 341
 family A polymerases 33, 34
 fast folding 92, 94–97
 FASTER stochastic algorithm 369, 370
 fibroblast growth factor-1 (FGF-1) 127
 filamentous fungi 56–58, 64
 filamentous phage 289, 290
 finger domains 279, 280
 firefly luciferase (*Photinus pyralis*) 128
 first-generation DNA sequencing 282, 283
 Fischer, Emil 14
 fission yeast 56, 64
 fixed-backbone protein design
 – amino acids sequences 364–367
 – optimization algorithms 368–371
 – substrate specificity 383–385
 FKBP12 immunophilin 101, 102
 flavin cofactors 166, 176–180, 209, 329
 flavopapains 178
 flavoproteins 193–195, 198–200
 flexible backbone protein design 383–385
 fluorescence
 – binding sites determination 226, 227
 – energy transfer (FRET) 93
 – folding/dynamics 90
 – glycosyltransferases 311, 312
 – proteins, hypothetical for REAP analysis 37, 38
 – self-quenched peptides 265
 – single molecule sequencing approach 285

- fluorescence-activated cell sorting (FACS)
 - glycosyltransferases 311, 312
 - microdroplets 76–78
 - microfluidic droplets 82, 83
 - folding of proteins 89–111
 - complicated pathways 91–94, 97–100
 - computational fold alteration 373–379
 - *de novo* loop design 375, 376, 385
 - fold switching 376, 377
 - future aspects 377
 - loop grafting 374, 375
 - overview 373, 374
 - thermostability optimization 377–379
 - *de novo* fold design 371–373
 - dirigent effects 2, 3, 12–14
 - enzymes 106–110
 - engineered subtilisins 252, 253
 - glycosyltransferases 304, 305
 - Kemp elimination enzymes 108–110
 - retro-aldol enzyme 106–108
 - ligands 103–106
 - N/U/I model 115, 137, 138
 - overview 89, 90
 - peptide binding 105, 106
 - proof-of-principle protein designs 90–102
 - α_3 D 73-residue protein 92, 94–96
 - FSD-1 91–94
 - three-helix bundle proteins 92, 96, 97
 - Top7 92, 97–100
 - temperature effects 149
 - force-fields 364–368, 378, 407–417
 - Forsythia intermedia* 4
 - four-helix bundles 103–105
 - free energy calculations 364–368, 379
 - free-radical coupling 1
 - FSD-1 28-residue protein 91–94
 - functional design 102–110
 - functional divergence 32–34, 36–38
 - fungal expression systems 56–58, 64
 - Fusarium solani* cutinase 12
 - fusion DNA polymerases 281
 - fusion proteins
 - glycosyltransferases 307–310
 - P450 BM3 333–335, 337
 - subtilisin 255, 256, 263, 265, 266
- g**
- GB1 protein 390
 - GC-based biotransformation screening 208, 209
 - gene disruption, thermophiles 149–151
 - gene modification 150, 151
 - *see also* mutational studies
 - gene promoters 48, 51–53, 150, 152
 - Gene Site Saturation Mutagenesis (GSSM)
 - anaerobic robotic HT screening 205–209
 - F87A P450 BM3 variant 341
 - human β -glucuronidase enzyme 60
 - P450 BM3 fusion protein 334–337
 - tunnel residues 450
 - genetic algorithms 369–371
 - genome reconstruction 43
 - genotype–phenotype linkages 73, 74, 77, 78
 - global enzyme/solvent effects 410
 - global minimum energy conformation 369
 - glucokinases 20
 - glucose 9, 10, 20
 - Gly-to-Pro replacements 128, 129
 - glycosylation 49, 52
 - glycosyltransferases 303–318
 - engineering 307–315
 - overview 303, 304
 - screening practicalities 315–317
 - sequence/structure/mechanism 304–307
 - glycyl alkoxy radicals 197
 - green fluorescent protein (GFP)
 - eukaryotic CYPs solubility 347
 - stability engineering tool 128
 - subtilisin engineering 257, 258, 260–264
 - green supercomputing 416
 - GROMOS protein force-field 408
 - guanine deaminase 383, 385
- h**
- HaeIII* methyltransferase 76
 - half-inactivation temperature (T_{50}) 133
 - haloalkane dehalogenase 448–450
 - HEAT-repeat scaffold proteins 16
 - hemagglutinin (HA) 391–393
 - heme cofactors 173–177
 - heme domains 336
 - heparin 127
 - heterotachy 33
 - hexokinases 20
 - high-throughput screening
 - anaerobic 203–210
 - enoate reductases 200–202
 - Old Yellow Enzymes 198–200
 - overview 193, 194
 - oxygen-sensitive biocatalysts 194–198
 - protocol details 203–210
 - robotic 205–209
 - whole-cell fermentations 202, 203
 - microdroplet systems 73–84
 - P450 mutants 334
 - semi-anaerobic 204, 205

hinge variability experiments 14
 His-tag positioning 345
 historical diversity 29–44
 – *see also* Ancestral...
 holoenzymes 164
 homing endonucleases 395
 homologous recombination 51
 homologous sequences 34, 35
 horseradish peroxidase (HRP) 50, 83, 129
 hot-spots, interfacial 390, 391, 393
 Hoveyda-Grubbs-type catalysts 225
 humans
 – β -glucuronidase (h β G) enzyme 60
 – computational input requirement 378
 – cytochrome P450 monooxygenases 53, 331, 341–343, 348
 – pancreatic lipase 11
 γ -humulene synthases 1, 2
 D-hydantoinase 130
 hydantoinases 13, 14
 hydration, enzymes 6
 hydrogen bond characteristics 7, 8
 hydrogen peroxide 134, 135, 329, 338
 hydrogenases 156, 157, 171, 172, 218
 hydrogenation, asymmetric transfer (ATH) 217, 228
 α/β hydrolase fold 12, 13
 hydrolases
 – cofactors 169
 – engineering in microdroplets 78
 – metal cofactors 165, 169
 – protein stability engineering 118–120, 122, 125, 126
 – thermostability 148
 – tunnel-possessing 429–431, 434
 – – engineered 440–441
 hydrophilic residues 146
 hydrophobicity 5, 8, 90, 146, 148, 412
 4-hydroxy-4-(6-methoxy-2-naphthyl)-2-butanone 108
 hydroxynitrile lyase 53
 Hyh-I/II cystolic hydrogenase 156
 hyperbranching 287
 hyperglycosylation 49, 52
 hyperthermophiles 148–154, 156, 157

i

I-AniI homing endonuclease 395
 Illumina DNA sequencing 285
 imaging techniques 11, 15
 – *see also* green fluorescent protein (GFP)
 – circular dichroism 11, 90, 93, 98
 – intrinsically-disordered proteins 15

 – ionic liquid effects 8
 – NMR 15, 89, 95, 98, 314
 – X-ray crystal structures 15, 330, 331
 immobilization, enzyme stabilization 145
 immunophilin FKBP12 100–102
in vitro compartmentalization (IVC) 73–84
in vitro systems 61–63, 152–154
 – *see also* cell-free...
in vivo assembly of mutant libraries 51
in vivo complementation 289, 290
in vivo overlap extension (IVOE) 51, 53, 54
 inactivation, thermal 133, 134
 indirect kinetic assay method 208, 209
 induced-fit model 14, 421, 422
 industrial applications
 – Ancestral Sequence Reconstruction 40, 41
 – *Aspergillus/Trichoderma* fermentation 57
 – mutagenic expression cassettes 53
 – oxygen-sensitive enzymes 198, 199, 202
 industrial-scale anaerobic screening 193–210
 influenza hemagglutinin (HA) 391–393
 inorganic cofactors 164, 165, 168
 insect cell expression systems 58, 59, 64
 interfaces, protein–protein 388–394
 International Union of Pure and Applied Chemistry (IUPAC) 164
 intrinsically disordered proteins 3, 14–16
 inverting glycosyltransferase 305, 306
 Ion Semiconductor Sequencing (ISS) 288
 ionic liquid (IL) solvents 8–10, 148
 iridium piano-stool complexes 228–233
 iron
 – cofactors 165, 168–170
 – – myoglobin redesign 175, 176
 – diiron proteins 92, 102–105, 223
 – iron–sulfur-containing proteins
 – – clostridial 200–202
 – – clusters, cofactors 168
 – – enoate reductases 201
 – – oxygen sensitivity 195–197
 isoalloxazine ring systems 177, 178
 isomerases 165, 169, 432, 435, 442
 isozymes 3, 19, 20

k

kanamycin nucleotidyltransferase (KNT) 155
 Kemp eliminases 108–110, 381–384
 ketones 198, 217
 keyhole-lock-key model 421–452
 kinases 20, 169, 392

kinetics

- measurement 132–137
- microfluidic droplets 80
- proteases 244, 271, 272
- robotic anaerobic screening 208, 209
- stability testing 135–137
- subtilisin 251, 263–266

I

lab-on-a-chip, microdroplets 73–84

Lactobacillus plantarum 103

LanGT2 scaffolds 309, 310

Last Universal Common Ancestor (LUCA)
40, 41

leakage, microfluidic droplets 81, 82

Lego approach 349

Lennard–Jones interactions 409

levodione reductase, actinol production 199

libraries, *see* variant libraries

lids, open/closed 414, 415

ligands

- chiral organometallic 216
- folding/dynamics 103–106
- ligand/substrate specificity 41
- metal-binding four-helix bundles 103–105
- Noyori-type ligands 216, 228
- protein–ligand complex ensembles 447

ligases

- cofactors 169
- metal-dependent, cofactors 165, 169
- tunnel-possessing 433, 435
- – engineered 442

lignin biosynthesis 1

linear expression cassettes 53

lipases

- *Bacillus subtilis* 12, 131
- computational modeling 407, 414, 415
- enantioselectivity 413, 414
- α/β hydrolase fold 12, 13
- pancreatic human lipase 11
- reversed micelle system effects 11
- *Rhizomucor miehei* 414
- *Rhizopus oryzae* lipase 130
- thermostability 148

local enzyme/solvent interactions 410

lock-and-key model 14, 19, 421, 422

log *P* value 5, 8, 148, 412

loop design, computational 373–379, 385

loop-swapping experiments 12–14

low-throughput glycosyltransferase assays
314, 315

luciferases 128

Luciola cruciata luciferase 128

lyases 165, 169, 432, 435, 441, 442

m

M13 phage 263

mammalian cell cultures 59–61, 64

manganese catalase 92, 103–105, 149

manganese (Mn) 174, 175, 222, 224

Markov Chain Monte Carlo (MCMC)

method 369

Massively Parallel Signature Sequencing
284

mechanism-based selection 259–266

medium engineering 5

membrane-bound eukaryotic CYPs 331,
343–348

messenger RNA (mRNA) 62, 63, 292

metals

- *see also* artificial metalloenzymes
- cobalt 103, 175, 176
- copper 175, 176
- diiron proteins 92, 103–105, 223
- heme cofactors 173–177
- heme domains 336
- iridium piano-stools 228–233
- iron cofactors 165, 168–170, 175, 176
- iron–sulfur proteins 168, 195–197,
200–202
- magnesium 165
- manganese 174, 175, 222, 224
- metal-binding four-helix bundles
103–105
- metal-dependent ligase cofactors 165,
169
- Metal-MaCiE database 165
- organometallic complexes 215–237
- as redox catalysts 165
- Methanococcus jannaschii* 16, 17
- methanol oxidase (MOX) gene 54, 55
- 7-methoxycoumarin O-demethylation 328,
336
- 7-methoxyresorufin O-demethylation 328,
336
- methyl parathion hydrolase 128, 129
- (*R*)-methyl-3-hydroxy-2-methylpropionate
200
- α -methyl dihydrocinnamaldehyde 199,
200
- methylotrophic yeasts 51–55, 64
- Metropolis–Hastings Monte Carlo scheme
369
- micelles, reversed systems 10, 11
- Michaelis–Menten kinetics 20, 380
- microdroplets 73–84
- formats 75–83
- future perspectives 83, 84
- leakage 81, 82

- microfluidic droplets 79–83
 - overview 73–75
 - unit operations 79, 81
 - microemulsions 10, 11
 - microorganisms 145–157, 193–210
 - *see also individual microorganisms*
 - microplate screens 312
 - modified nicotinamide cofactors (mNADs) 170–173
 - molecular dynamics (MD) simulation 22
 - α_3 D 73-residue protein 95
 - dirigent effects in biocatalysis 6
 - force-fields 364, 366, 408–410, 415, 417
 - Kemp elimination enzymes 108
 - protein folding 99
 - proteins/solvents 408–410
 - replica exchange 93, 94
 - tunnel engineering 445, 447
 - molecular Lego approach 349
 - molecular recognition elements 15
 - monoclonal microdroplet compartments 74, 75
 - monolignol coupling reactions 3
 - mononucleotide cofactors 166, 177–179
 - monooxygenases 128, 129
 - *see also cytochrome P450 monooxygenases*
 - Monte Carlo methods 369, 409
 - moonlighting proteins 3, 19–22
 - MSD-FASTER multi-state design package 370
 - Multigraft software package 375
 - multi-objective computational design 370, 371
 - multiple sequence alignments (MSAs) 34–37
 - multiple tunnels 433, 436
 - multistate modeling 370, 379, 387
 - mutagenic expression cassettes 53
 - mutational studies
 - *see also Gene Site Saturation Mutagenesis (GSSM); site-directed mutagenesis (SDM)*
 - Ancestral Mutation Method 31, 32
 - dihydrofolate reductases 18
 - engineered tunnels 439–442
 - protein stability engineering examples 117–126
 - protein structure/folding 12, 13
 - subtilisin 254, 255
 - Myceliophthora thermophila* 51, 57
 - myoglobin (Mb) 174, 175, 222, 224
- n**
- N-terminal domains 304, 305, 344–346
 - N/U/I model of protein inactivation 115
 - natural diversity-guided protein engineering 29–44
 - approaches 29–34
 - future applications 38–44
 - protocols 34–38
 - natural state/processes
 - cofactors 164, 165
 - denatured state evolution 100
 - enzyme folding/dynamics 100, 103
 - protease characteristics 243
 - protein–protein interface redesign 389
 - NC0 engineered protein 96, 97
 - NC3-Ncap engineered protein 96, 97
 - next-generation DNA sequencing 284–288
 - nicotinamide adenine dinucleotide (NAD)
 - enoate reductases 200–202
 - Old Yellow Enzyme reactions 194
 - redesign 163, 167, 169–173
 - nicotinamide adenine dinucleotide (phosphate) (NAD(P))
 - $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ 218
 - cytochrome P450 monooxygenases 329
 - dihydrofolate reductases 17, 18
 - enzymatic synthesis of mNADs 171
 - Old Yellow Enzyme-catalyzed alkene reduction 208
 - oxidoreductases 169
 - pig brain NADase 170, 171
 - redox chemistry 171
 - nitric oxide reductase mimics 223, 224
 - nitroalkanes, asymmetric 198–200
 - nitroaromatics 202
 - p*-nitrophenol ester hydrolysis 11
 - nitroreduction 197, 202
 - non-natural amino acids 41, 42, 394
 - non-natural decoy molecules 338–340
 - non-thermal stability gains 115–136
 - Noyori-type ligands 216, 228
 - nuclear magnetic resonance (NMR) 15, 89, 95, 98, 314
 - nucleic acids
 - *see also deoxyribonucleic acid (DNA)*
 - microdroplets 76, 77
 - ribonucleic acid 62, 63, 163, 292, 294
 - synthetic informational polymers 291–294
 - α -L-threofuranosyl nucleic acids 294
 - nucleotide diphosphate (NDP)-donor substrates 315, 316
- o**
- occluded conformations 17, 18
 - Old Yellow Enzyme (OYE) 193–195, 198–200
 - oleandomycin glucosyltransferase 312, 313

- ompA* signal peptide 345
 - one-sided *de novo* protein interface design 391–393
 - open/closed sub-states 16
 - OPLS protein force-field 408
 - OptGraft software package 382
 - optimized potentials for liquid simulations (OPLS) 409
 - ORBIT/CPDS software 366, 368, 369, 372, 380, 384
 - organic cofactors 163–169, 173–180
 - organic solvents 5, 8–10, 407–417
 - organometallic complexes 215–237
 - concept 215, 216
 - design 216–222
 - development 223–226, 234–237
 - protocols 226–234
 - reactions catalyzed by 225
 - OSPREY software package 366, 369, 370, 372
 - overexpression, ancestral proteins 35, 36
 - overlap extension PCR (OE-PCR) 53, 54
 - oxidase shunts 328, 329
 - oxidative damage mechanisms 196
 - oxidative stability measurement 134, 135
 - oxidoreductases
 - cofactors 169
 - high-throughput anaerobic screening 193–210
 - metal cofactors 165, 169
 - protein stability engineering 117, 120, 125
 - pyruvate:ferredoxin oxidoreductase 157
 - tunnel-possessing 425–428, 434
 - – engineered 439, 440
 - oxygen, cofactors 168
 - oxygen-sensitivity 193–210
- P**
- P' fixed backbone problem 368
 - P1' problem 364
 - P1 specificity 246, 247
 - P4 pocket of subtilisin 250, 253–256, 268–270
 - p21-activated kinase (PAK1) 392
 - P450 enzymes, *see* cytochrome P450 monooxygenases
 - pairwise decomposability 366, 367
 - paleobiology 42, 43
 - palm domain, DNA polymerases 279, 280
 - pancreatic lipase, human 11
 - papain 220, 221
 - paraoxon hydrolysis 16
 - paraoxonases 32
 - Parsimony approaches 34, 35
 - partitioning coefficient 5
 - penicillin G amidase (PGA) 138
 - Penicillium purpurogenum* 12
 - pentaerythritol tetranitrate (PETN) 195, 199, 205–207
 - peptide binding 105, 106
 - peptide hydrolysis 243–274
 - peptide nucleic acid pentamers 292, 293
 - perfume production 199, 200
 - perimycin 308
 - permanent genotype–phenotype linkages 77, 78
 - 19-*O*-perosaminyl-amphoteronolide 308
 - peroxide shunts 328, 329, 338
 - Pfu* DNA polymerase 281
 - pH indicator assays 314
 - pH memory effect 5
 - phage display
 - polymerases 289, 290
 - subtilisin 255, 256, 263, 265–268
 - phage-assisted continuous evolution 291
 - (E)-2-phenyl-1-nitropropene 199
 - N*-(5'-phosphoribosyl) anthranilate (PRA) 14
 - phosphoribosylpyrophosphate (PRPP) 436, 437
 - phosphoribosyltransferase (PRTase) 436
 - phosphotriesterases 16
 - Photinus pyralis* luciferase 128
 - phylogenetic trees 35–37
 - piano-stool complexes 228–233
 - Pichia* spp. 51–55, 64
 - Picrophilus torridus* 145
 - pig brain NADase 170, 171
 - (+)-pinoselinol 4
 - Poisson–Boltzmann equation 367
 - polarity
 - polarizable protein models 409, 415
 - solubility 5
 - solvents
 - – additives 412
 - – enzyme activity 407
 - polydispersity noise 75, 79
 - polymerases
 - chain reaction 53, 54, 281, 336, 337
 - engineering 279–294
 - – sequencing 281–288
 - – strategies 288–291
 - REAP method 33, 34
 - thermophiles 154–156
 - post-translational modifications 49, 52, 223, 303–318
 - PP2a phosphatase 16

- PR65 HEAT-repeat scaffold 16
 procarboxypeptidase 100–102
 PRODA-MATCH software 382
 prodomains, subtilisin 255, 256, 258–260
 proliferating cell nuclear antigen 353, 354
 proline-rich CYP regions 345
 promiscuous enzymes 3
 – catalytic promiscuity 21, 22
 – condition promiscuity 21, 22
 – dirigent effects in biocatalysis 19–22
 – glycosyltransferases 303–318
 – γ -humulene synthases 1, 2
 – oleandomycin glucosyltransferases 312, 313
 – substrate promiscuity 21, 22
 promoters 48, 51–53, 150, 152
 proofreading-deficient polymerases 287
 proteases, *see* serine proteases; subtilisin
 Protein Engineering Analysis Tool (PEAT) 139
 protein tunnels 421–451
 – channels distinction 423
 – examples 425–433, 439–442
 – haloalkane dehalogenase 448–450
 – keyhole-lock-key model
 – – definition 422–424
 – – engineering implications 438–444
 – – robustness/applicability 424–437
 – multiple tunnels 433, 436
 – one tunnel 424, 433
 – – two distinct active sites 436, 437
 – software tools 444–447
 proteins
 – *see also* computational protein design; *individual proteins*; protein tunnels
 – amino acid set expansion 42
 – interfaces, computational design 388–394
 – molecular dynamics simulations 408–410
 – organometallic complex scaffolds 215–237
 – production, hyperthermophiles 148–154
 – protein A 131
 – protein G 390
 – protein L 100–102
 – PT modifications 49, 52, 223, 303–318
 protein–ligand complex ensembles 447
 protein–protein interfaces 388–394
Prunus amygdalus 53
Pseudomonas spp. 11, 16
 PUPPET system 353, 354
 purification proteases 258, 259
 purified enzyme HT screening 207, 208
 putidaredoxin (Pdx) 352, 353
 putidaredoxin reductase (Pdr) 352, 353
 putrescine oxidase (PuO) 178, 179
pyrF gene 150, 151
Pyrobaculum spp. 139, 147
Pyrococcus spp. 149, 156, 157, 391
Pyrolobus fumarii 145
 pyruvate:ferredoxin oxidoreductase (POR) 157
 pyruvate formate lyase (PFL) 197
- q**
 quadruple mutants 341
 quantitative assays 79
 quantum mechanics 109
- r**
 RA22 retro-aldol enzyme 108
 random mutagenesis 267, 341, 385, 449, 450
 rapid-folding pathways 92, 94–97
 rational design
 – CYPs selectivity/activity 332–336
 – folding/dynamics 89–111
 – glycosyltransferases 307–310
 – protein stability 117–126
 – protein tunnels 421–451
 – serine proteases 243–274
 recombinant expression 343–349
 Reconstructing Evolutionary Adaptive Paths (REAP) 29, 32–34, 37
 redox activity
 – *see also* Old Yellow Enzyme (OYE); oxidoreductases
 – cofactors 169
 – – flavins 177, 178
 – – inorganic 165, 168
 – – nicotinamide ring systems 163, 167, 169–173
 – CYP redox partner proteins 329, 330, 349
 – redox centers mode of action 196
 reductase domains 342, 349, 350
 remove and regenerate strategy 251, 252
 replica exchange molecular dynamics 93, 94
 restriction endonucleases 243
 restriction proteases 272, 273
 restriction/modification systems 76
 retro-aldol (RA) enzyme 106–108
 reverse glycosylation reactions 312, 313
 reverse transcriptase (RT) activity 154–156
 reversed micelle systems 10, 11
 RhFRed CYP116B2 reductase domain 350, 351
Rhizomucor miehei lipase 414
Rhizopus oryzae lipase (ROL) 130

- Rhodococcus* spp. 116, 127, 448–451
- riboflavin 180
- ribonucleic acid (RNA) 62, 63, 163, 292, 294
- ribosomal S6 100–102
- ribosome display 62
- ribosome-mediated protein translation 42
- ribozymes 163, 164
- ribulose-1,5-bisphosphate carboxylase 20, 155, 156
- robotic HT anaerobic screening 205–210
- Roche ester 200
- rolling circle amplification 287
- root-mean-square deviation (RMSD) 91, 110
- Rosetta software
 - *de novo* enzyme design 381, 384
 - description 372
 - DNA binding/specificity 395
 - fold switching 376
 - folding/dynamics 90–102
 - force fields 367
 - loop grafting 374
 - one-sided *de novo* protein interface design 392
 - optimization algorithms 370, 386
 - pre-screened variant libraries 385
 - substrate specificity of enzymes 383
- rotamers 365–367, 369, 382, 388
- rubredoxin 195, 196
- S**
- S1 pockets 246–249
- S4 site of subtilisin 250, 268
- Saccharomyces cerevisiae* 43, 48–51, 64
- salen complexes 174, 175, 222
- salting-out effect 7
- Sanger sequencing 283
- scaffolds 215–237, 387, 388
- SCHEMA algorithm 338
- Schiff bases 106, 107
- Schizosaccharomyces pombe* 56, 64
- second coordination sphere 218, 234
- secondary structures 92
 - *see also* folding of proteins
- selected-fit model 421, 422
- selection markers 60
- selenocysteine 42
- self-assembling oligomers 352–354
- semi-rational design 332–336
- sequences
 - *see also* Ancestral Sequence Reconstruction (ASR); site-directed mutagenesis (SDM)
 - aromatic side chain modification 223
 - glycosyltransferases 304–307
 - homologous 34, 35
 - polymerase engineering 281–288
 - sequencing-by-synthesis 283, 284
 - signature functional divergence 32–34, 36–38
 - single molecule 285–288
 - space reduction, DNA shuffling 29
- serine 219, 221
- serine proteases
 - binding interactions 245, 246
 - design challenges 270, 271
 - design of mechanism-based selection system 259–266
 - evolving protease specificity with anion cofactors 266–269
 - reaction mechanism 244
 - restriction proteases quest 272, 273
 - specificity engineering 243–274
- serum albumins (SAs) 175–177
- sesquiterpene synthases 1, 2
- SHARPEN/CHOMP software package 370, 372
- SHI cystolic hydrogenase 156, 157
- short-patch CSR (spCSR) 155, 291
- sialyltransferase CstII 311
- signal sequences 49
- simulated annealing 369
- single molecule fluorescent sequencing 285
- single molecule real-time (SMRT) sequencing 286–288
- single nucleotide polymorphism (SNP) analysis 289
- single protein tunnel structures 445
- site-directed mutagenesis (SDM)
 - haloalkane dehalogenase protein tunnels 448
 - human CYP2B1 solvent stability 341, 342
 - P450 BM3 fusion protein 335–337
 - P450cam 333
 - polymerases 286
 - sequence space 39
- sludge digestion 202, 203
- small polypeptides 223
- small-angle X-ray scattering (SAXS) 11
- S_N1-like mechanisms 306, 307
- SNAP-display 76–78
- software packages 363, 364, 372, 444–447
 - *see also* individual software packages
- Solexa DNA sequencing 284, 285
- Solfolobus solfataricus* 353
- solubility 5, 343–349

- solvation models 368, 393
- solvents 2
 - dirigent effects 4–11
 - enzymes
 - – binding 6
 - – organic solvents 407–417
 - – stability 148
 - – CYPs 340–343
 - log *P* value 5, 8, 148, 412
 - molecular dynamics simulations 408–410
 - stability engineering against 130, 131
- somatic hypermutation (SHM) 60
- spacers 215, 216, 219, 224, 225
- specificity
 - *see also* enantioselectivity
 - dirigent effects in biocatalysis 19–22
 - DNA binding, computational design 394, 395
 - ligand/substrate, biomedical uses 41
 - proteases engineering 243–274
 - – measurement 244
 - tunnel engineering 443
- Sphingobium japonicum* UT26 448, 449
- Spodoptera frugiperda* 58
- src SH3 folding/dynamics 100–102
- stability engineering 115–136
 - aldehydes tolerance 130, 131
 - alkaline tolerance 131, 132
 - aspects/approaches 116
 - cytochrome P450 monooxygenases 340–343
 - developments 137–139
 - examples 117–126
 - immobilization 145
 - ionic liquid solvents 8
 - kinetic stability 132–137
 - oxidative stability 134, 135
 - power/scope of techniques 116–132
 - protein folding/dynamics 89–111
 - solvents 130, 131
 - subtilisins 252, 253
 - thermal stability 116, 127–129, 132–134
 - tunnels 443, 444
- stereochemistry gate loops (SGLs) 13, 14
- stereoselectivity 3, 4, 443
 - *see also* enantioselectivity
- stiffening proteins 128, 129
- stochastic combinatorial optimization 368, 369
- streptavidin (Sav) 215–237
- Streptomyces* spp. 348, 352
- structural proteins 124
- structure-guided engineering 131, 308
- structure-promoting/dispromoting amino acids 14, 15
- structure(s)
 - biocatalytic dirigent effects 12–14
 - DNA polymerases 279, 280
 - enzyme computational modeling 407–417
 - glycosyltransferases 304–307
 - human CYP2C9 331
 - isalloxazine ring systems 177, 178
 - nicotinamide ring systems 171
 - P450 BM3 fusion protein binding pocket 340
 - P450cam 332
 - tunnel-possessing enzymes 434, 435
 - X-ray crystal structures 330, 331
- substituted heme derivatives 174
- substrates
 - binding, CYPs 331
 - keyhole-lock-key model definition 422–424
 - modifications, biomedical uses 41
 - selectivity
 - – computational redesign 383–386
 - – CYPs engineering 332–340
 - substrate promiscuity 21, 22
 - subtilisins 251
- subtilisin
 - active site 246
 - α -chymotrypsin-catalyzed peptide synthesis 21
 - organometallic chemistry 221
 - P4 pocket 250, 253–255, 268–270
 - phage display 255, 256, 263, 265–268
 - prodomains 255, 256, 258–260
 - S1 pocket 246–249
 - specificity engineering 243–274
 - subtilisin Carlsberg 411, 414
 - ternary complexes 259–263
- sugar binding 305
- sugar nucleotides 312
- Sulfolobus* spp. 150, 152
- sulfotransferases 32
- sulfoxidation 176
- sulfur oxygenase gene 152
- superactivity 11
- supertalented enzymes 3, 19–22
- supramolecular anchoring strategy 215, 216, 219, 220, 222
- surface display 49, 50, 54, 62, 63, 73–84
- surfactants/detergents 10, 11, 148, 345
- switches, substrate-specific 383–385
- synthetic informational polymers 291–294

t

- T-cell receptors 124
- T7 RNA polymerase 291
- Taq DNA polymerase 283, 289–291
- temperature 147, 149, 340–343
 - *see also* therm...
- template circularization 287
- tenascin 100–102
- ternary complexes, subtilisin 259–263
- terpenoids 199, 200
- therapeutic proteins
 - Ancestral Sequence Reconstruction 41
 - computational protein design 394
 - engineered glycosyltransferases 303, 309
 - mNAD probes/agents 172
 - riboflavin carrier protein 180
 - thermal stabilizations 116, 121–123, 127–129
 - transgenic animal/plant expression systems 61
 - yeast expression systems 53
- thermal inactivation measurement 133, 134
- thermal profile measurement 132, 133
- thermal stability engineering 116, 127–129, 132–134
- Thermococcus kodakarensis* 150–154, 156
- thermodynamic water activity (a_w) 6
- Thermomyces lanuginosa* lipase 415
- thermophile enzymes 145–157
 - future perspectives 157
 - gene modification methods 150, 151
 - hyperthermophiles 146
 - cell engineering 156, 157
 - protein production 148–154
 - overview 145, 146
 - reactions catalyzed by 146–148
 - thermophilic protein engineering 154–156
- thermostability
 - Ancestral Mutation Method 31, 32
 - *Arxula adeninivorans* 56, 64
 - computational fold alteration 377–379
 - cytochrome P450 monooxygenases 342, 343
 - industrial applications 40, 41
 - Taq DNA polymerase 283, 289–291
 - thermophile enzymes 145–157
- Thermotoga* 146, 154
- Thermus* alcohol dehydrogenase (TADH) 218
- Thermus* spp. 146, 149, 154, 218, 351, 352
- thioanisole sulfoxidation 6, 7
- thioredoxin (Trx) 30, 31, 380, 384
- three-helix bundles 92, 96, 97
- α -L-threofuranosyl nucleic acids (TNAs) 294
- thumb domains 279, 280
- timescale of simulations 415, 416
- tissue plasminogen activator (tPA) 59
- Top7 92, 97–100
- toxicity of engineered proteases 272
- transferases 123, 169, 428, 429, 434, 440
- transgenic expression systems 61
- transglutaminase (TGase) method 352, 353
- transition state analogs (TSAs) 16, 17
- transition states 16, 17, 380, 381
- 1,2,3-trichloropropane (TCP) 450, 451
- Trichoderma reesei* 57
- Trichoplasia ni* (TN-368 BTI-TN-5B1-4) 58
- triggered catalysis 255, 256, 258, 259, 261, 267–270
- triosephosphate isomerase (TIM) barrels 108
- trypsin binding interactions 245–247
- tumor necrosis alpha (TNF- α) 52
- tunability, subtilisins 257, 258
- tunnels, *see* protein tunnels
- two-phase systems 5
- two-sided *de novo* protein interfaces 390–392
- type I/II functional divergence 33

u

- U1A RNA-binder 100–102
- ubiquitin 412, 413
- unconventional reaction media 2, 4–11
- uncoupling reactions 329
- unfolding of proteins 7, 115, 138
 - *see also* folding of proteins
- unstructured domains 14–19
- URA3 gene 150
- urdamycin glycosyltransferases 307, 309
- UVF (ENH-FSM1) 92, 97

v

- validation 43, 44
- variant libraries
 - computationally pre-screened/random comparison 385, 386
 - glycosyltransferases 310
 - HRP recombinant proteins 50, 83
 - HT anaerobic screening 193–210
 - microdroplet compartment-directed evolution 73–84

- P450 BM3 fusion protein 337
- PETN reductase 205–209
- REAP method 37, 38
- rotamers for computational protein design 366
- subtilisin anion site 267
- vdW potential 366
- viscosity of ionic liquid solvents 8

W

- water
 - activity coefficient (γ_i) 6
 - enzyme modeling in organic solvents 407–409
 - organometallic catalysis 215–237
 - water pool (W_0) 11
 - water-stripping effect 6
- weighted sum method (WSM) 371
- whole-cell systems 202, 203, 207, 208

X

- X-ray crystal structures 15, 330, 331
- xenobiotics 202, 203
- XNA synthesis 294
- xylanase scaffolds 92, 386

Y

- Yarrowia lipolytica* 55, 56, 64
- yeast expression platforms 48–56, 64
 - *Arxula adeninivorans* 56, 64
 - *Pichia* spp. 51–55, 64
 - *Saccharomyces cerevisiae* 48–51, 64
 - *Schizosaccharomyces pombe* 56, 64
 - *Yarrowia lipolytica* 55, 56, 64
- YqjM PETN reductase mutant 207, 208

Z

- zinc cofactors 165, 170
- zinc finger folds 90–93

