

## Index

### **a**

acetaldehyde production, in *Streptococcus thermophilus* 574–575  
 acetate 472  
     acetate-to-acetyl-CoA process 750  
     production 749  
 acetoin 196, 432, 472, 492, 495, 558,  
     575–578  
 acetolactate synthase (ALS) 196, 354,  
     697, 706  
 acetone–butanol–ethanol (ABE)  
     metabolic route 611, 613  
     butanol production 698  
 acetyl-CoA 14–15, 738  
     cytoplasmic acetyl-CoA 713  
     cytosolic acetyl-CoA 746, 750, 753,  
     754  
 AcidifyME model 48  
 actinomycetes  
     CRISPR-based genome editing  
         techniques 663–665  
     generalized systems metabolic  
         engineering workflow for  
         672  
     genome editing of 663  
 actinomycetes synthetic biology  
     biological parts of 665, 667–669  
     biosensors 669–670  
     full pathway refactoring 671  
     riboswitches 670–671  
 activity-independent screening of target  
     molecule synthesis 492–493  
 acyl carrier proteins (ACPs) 354–356,  
     370, 377, 699–702

adaptive laboratory evolution (ALE)  
     50, 285, 343, 384, 493, 499,  
     534, 555, 566, 567–570, 694  
 adenosine triphosphate (ATP) 31, 102,  
     140, 147–148, 180, 188, 191,  
     194, 196, 213, 224, 284, 354,  
     355, 362, 424, 427, 434, 477,  
     487, 495–496, 522, 524,  
     528–529, 532, 555, 558, 585,  
     631, 693, 701, 704–705, 714,  
     740, 742, 744–746, 748–751,  
     775–777, 808–809  
 α-ketoglutarate (αKG) production 363,  
     420, 427, 703, 736, 738,  
     740–744, 750  
 alanine 117, 374, 416, 574  
 alanine (ALA<sub>2,3</sub>) 117  
 alarmones guanosine tetra- and  
     penta-phosphate 471  
 alcohol dehydrogenase (ADH) 283,  
     343, 354, 356, 359–360, 363,  
     432, 576–577, 583–585, 613,  
     692–693, 695, 697–698,  
     700–702, 704  
 algae biomass 829  
 algal biomass 415  
 alkaloids 367, 368, 370, 713, 775, 812,  
     817, 820, 824–825  
     production, in *S. cerevisiae*  
         710–712  
 alkane biosynthetic pathway 702  
 alleleome 33–34  
 Allelic Coupled Exchange (ACE)  
     couples 618, 703

- amino acids  
 aminovalerate 421–423  
 L-arginine 364  
 auxotrophic markers 745  
 cell factory 403  
 ectoine 425–426  
 L-glutamate 419–420  
 4-HIL production 427  
 L-lysine 420–421  
 L-pipecolic acid 426  
 L-theanine 427  
 L-threonine 364  
*trans*-4-hydroxyproline 426–427  
 L-valine 365  
 $\omega$ -amino acids  
 4ABA 357  
 5AVA 357–359  
 4-aminobutyric acid (4ABA) 357, 363  
 6-aminocaproic acid 357, 359, 363  
 aminovalerate 359, 421, 422, 430–431  
 5-aminovaleric acid (5AVA) 357, 359,  
     361, 362, 423  
 anaerobic reporter 631  
 anaplerosis 74, 101  
 anaplerotic glyoxylate pathway 472  
 anhydrotetracycline (aTc)-inducible  
     systems 627  
 anoxic *P. putida* chassis 531  
 anthocyanin 588, 709, 818–820,  
     834–835  
 antimicrobial activity (AA), of  
     bacteriocins 557, 582  
 anti-CRISPR proteins 620  
 antibiotic resistance genes 302, 479,  
     615, 868–869, 878  
 antibiotics 302, 313, 317, 365–366,  
     406, 496, 561, 581, 583, 653,  
     665–666, 673–676, 769,  
     780–781, 788–789, 865  
*Antirrhinum majus* 819  
 antiSMASH 658–660  
 approximative rate expression  
     159–160  
 aquatic cyanobacteria 806  
*Arabidopsis thaliana* 180, 356, 588,  
     632, 710, 806, 815  
 arabinose 408, 413–415, 420, 432, 523,  
     571–572, 495, 697  
 arabinoxylans 412, 415, 816  
 L-arginine 364, 403, 405, 419  
 aromatic amino acids 824  
     bacterial production of 200–202  
     bisynthesis, in *S. cerevisiae* 708  
     synthesis 824  
 aromatic compounds  
     mandelic acid 366  
     methyl anthranilate 366  
     S-styrene oxide 366, 367  
     L-tyrosine 365, 366  
 artemisinic acid production 369, 713,  
     715  
 Aspart 720  
 Aspartate transcarbamylase (ATCase)  
     188  
*Aspergillus niger* 765, 766, 776, 785,  
     789  
     enzyme set for plant polysaccharide  
         degradation 773  
     genom-scale metabolic models  
         770  
     glucoamylase 777  
     hyperbranching strain 786  
     metabolic and regulatory functions  
         771  
     X-ray microcomputed tomography  
         ( $\mu$ CT) 788  
*Aspergillus oryzae* 766, 778  
     enzyme set for plant polysaccharide  
         degradation 773  
*Aspergillus terreus* 430, 767, 779–780  
 asRNA knockdown method 625  
 atmospheric pressure chemical  
     ionization (APCI) 269  
 atom enumeration scheme 103–104  
 atom transition network 91, 92, 94–95,  
     104  
 atom transition network specification  
     103  
 atom transitions 91–92, 97, 99,  
     103–104  
 auto-scaling 275  
 azadirachtin 817

**b**

- Bacillus amyloliquefaciens* strains 472, 493  
*Bacillus subtilis* 469  
*B. subtilis* ATCC6051a 482  
*B. subtilis* BSK814 475  
*B. subtilis* MGB469 475  
*B. subtilis* MGB874 475  
CRISPR/Cas and related strains 481–486  
genome reduction projects 473–476  
*Bacillus licheniformis* 470  
*B. licheniformis* 2709 482  
isocitrate lyase (aceB) 472  
*Bacillus thuringiensis* cry3Bb gene 829  
*Bacillus* BioBrick Box 490  
*Bacillus* origin of replication (*ori*) 481  
bacitracin 486, 494, 496–497  
42.7 kb bacitracin synthase gene cluster *bacABC* 486  
backslopping process 566  
bacterial 3-dehydroshikimate dehydratase 816  
bacterial cellulose 379–380  
bacteriocins production, in LAB 581  
bacteriophage infections 565  
basic helix-loop-helix (bHLH) transcription factor 818–819  
batch cultures 109, 111, 112, 356, 530  
benzylisoquinoline alkaloids (BIAs) 370, 710–712  
bioactive natural products 653, 804  
biosynthetic pathways 655, 667, 668  
tools and strategies for discovery 653–655  
bioaugmentation 861–862  
biochemical databases 238, 240, 244, 251  
biochemical network 166, 240, 242–243, 249  
biochemical search space 238, 240  
Biochemical Systems Theory (BST) 173, 175  
biocondensates 803, 833–835  
bioenergetics 213–214  
bioethanol production  
first-generation 691–694  
second generation 694–697  
bioluminescent reporters 631  
biomass effluxes 102–103  
bioproducer strains 238  
bioproduction 243, 253–254, 285, 408, 415, 519, 530–531, 535, 780, 810–811, 817, 829–830, 873  
bioremediation 875  
challenges 859  
chemical emissions and parameters 862, 867  
definition 859  
transition from 2.0 to 3.0 862  
bioremediation 3.0 862–865, 867–869  
bioremediation 4.0 879–880  
bioremediation agent 521, 861, 862, 865, 870, 872, 878–879  
*P. putida* 873  
biosensors 13, 16, 383, 493, 525, 669, 670, 865  
biostimulation 861–862  
biosustainable industrial production platforms 806–808  
biosynthetic gene clusters (BGCs) 655, 657  
cloning and heterologous expression 660–663  
genome mining 657–660  
promoter engineering 667  
reporter-guided mutant selection (RGMS) 670  
biosynthetic pathways  
of natural products 655, 657  
search for 251  
block elasticities 186  
blood proteins 721  
*B. methanolicus* MGA3 482, 489  
bondomers 121  
bottromycin biosynthetic pathway 667  
branched chain amino acids (BCAA) 359, 365, 496, 552  
branched-chain alcohols 354  
BsubCyc 470

- butanediol (BDO)-isomers 577  
 1,4-butanediol 57, 249, 363  
 2,3-butanediol 472, 494, 706  
 butanol 343, 697  
 production of 343, 588, 611–613,  
 698–699  
 butyrolactam 357, 363
- C**
- cadaverine 359, 361–362, 423  
 camalexin 833  
 cannabidiolic acid synthase 837  
*Cannabis sativa* 834, 837  
 cannibalism 374, 471, 478  
 capillary electrophoresis (CE) 267  
 capitulate-stalked trichome 834, 837  
 caprolactam 363, 533  
 Carbohydrate-Active enzyme database  
 (CAZy) 773  
 carbon atom transitions 91–92  
 carbon catabolite repressor 496  
 $\beta$ -carotene production 758, 759, 820  
 carotenoids 433, 524, 713, 718, 758,  
 817, 820, 826, 828–830  
 Cas-enzymes 308, 310, 312, 316, 320  
 Cas12a (Cpf1) enzyme 312, 663  
 Cas9 nickase (Cas9n) systems 482, 620  
 Cas9-Assisted Targeting of  
 CHromosome segments  
 (CATCH) 662  
 Cas9-mediated mutagenesis 564  
*Caulobacter crescentus* 379, 413, 473  
 CAZyme gene expression, molecular  
 mechanism of 773  
 cell type specific metabolic engineering  
 815  
 cell-free *in vitro* transcription systems  
 865  
 cell-free agents 860  
 cellular compartments 103, 282, 718,  
 834  
 cellular constraints 137–139  
 cellulase Egl-237 477  
*CeluStar CL* 783  
 central carbon intermediates 112  
 13CFLUX2 117  
 $^{13}\text{C}_1$  glucose 106
- chalcone isomerase (CHI) 369, 417,  
 418, 708, 709  
 chalcone synthase (CHS) 369, 417,  
 418, 708, 709  
*chassis* development 861  
*chassis* for bioremediation 873  
*chassis* metabolic model 246  
 chemical ionization (CI) 269  
 Chinese Hamster Ovary (CHO) 166,  
 188, 317–320  
 ChIPseq 61  
*Chlamydomonas reinhardtii* 806, 830  
 chloramphenicol acetyltransferase  
 (CAT) reporter 630  
 chlorophyll 822, 825–826, 828, 829  
 chloroplast stroma-localized ferredoxin  
 (*AtFedA*) 825  
 chloroplast thylakoid 809, 826  
 chloroplasts 805, 806, 810, 821–830,  
 834–835, 837  
 chromosome-less bacteria (SimCells)  
 860  
 cinnamic acid 367, 707, 708, 819  
 circular gRNA-containing plasmids  
 311  
*cis,cis*-muconate 428, 429  
*cis-cis*-muconic acid 417  
 in *P. putida* 533, 534  
 citrate production 739, 745–746  
*Y. lipolytica* 738  
 citrate synthase CitZ 496  
 citric acid production 765, 780, 788  
 in *A. niger* 776  
 $^{13}\text{C}$ -labeled substrate 77  
 Class II and Class I diterpenoid  
 synthases 822  
 cloning, of BGCs 661  
*Clostridium*  
 5'-UTRs & Riboswitches 634  
 genetic parts 626–627  
 promoters 627–630  
 reporters 630  
 enzymatic-based reporters  
 630–631  
 bioluminescent reporters 631  
 fluorescent reporters 631–632

- FbFP-based fluorescent reporters 632–633  
 FAST, HaloTag, and SNAP-tag fluorescent reporters 633  
 genome editing 614–615  
 ClosTron system 615  
 counter-selection markers 617–619  
 CRISPR systems 619–626  
 transposon-based random mutagenesis 615–617  
 terminators 633–634  
 ClosTron systems 615  
 cloud microbiology 879  
 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) 304, 308, 309, 619, 625  
 associated (Cas) genes 308  
 based metabolic engineering, in *T. thermophilus* 783  
 mediated gene manipulation systems, for filamentous actinomycetes 664  
 technology, for *C. glutamicum* 407  
<sup>13</sup>C metabolic flux analysis 76, 98, 162, 404, 406, 413, 415, 420, 739–740, 748  
 2-C-methyl-D-erythritol-4-phosphate pathway 496  
*codBA* system 479  
CO<sub>2</sub> fixation 15, 535, 783, 806, 811, 830  
coarse grained thermodynamic information 31  
coarse-grained integration of proteome constraints 139–144  
cobalamin (vitamin B12) production 578, 579  
cochlourine *N*-methyltransferase (CNMT) 370  
*Coleus forskohlii* 823, 834  
combinatorial biosynthesis 662, 667, 670  
combinatorial metabolic engineering 9, 12–14  
combinatorial supertransformation of transplastomic recipient lines (COSTREL) 828–829  
computational pathway design 238, 239, 252, 253  
computer-aided simulation 172  
concentration control coefficient 179–180, 185, 188  
confidence intervals (CI) 89–90, 229  
conjugative transposons 615  
connectivity matrix 31  
conserved metabolites 186  
constrained optimization 26  
COConstraint-Based Reconstruction and Analysis (COBRA) method 26–28, 30, 41, 51, 62, 240  
continuous culture 77, 83, 110–113, 174  
COPI vesicles 719–720  
COPII cargo proteins 720  
core phenylpropanoid biosynthesis 819  
CoReCo metabolic model reconstruction algorithm 770  
*Corynebacterium glutamicum* 191, 194, 403, 437, 473  
 aquatic sugars 415–416  
 amino acids 419–427  
 biopolymers 434–436  
 CRISPR technology 407  
 genome editing approaches 406  
 hemicellulosic biomass 408, 409, 412  
 industrial product production 419  
 natural products and active ingredients 433  
 industrial raw materials 407–408  
 lignin aromatics, valorization of 416–419  
 lignocellulosic sugars 408–415  
 L-lysine production 404  
 marker-free genome editing 407  
 metabolome analysis 405  
 organic acids and alcohols 427  
*cis-cis* muconate 428–429  
 3-hydroxypropionate 430–432

- Corynebacterium glutamicum* (*contd.*)
- glutarate production 429–430
  - itaconate 430
  - short-chain alcohols 432
  - plasmid-based expression 406
  - systems biology 404
  - systems metabolic engineering 404
  - Cosmid/fosmid library vectors 662
  - coumarins 817
    - biosynthesis 706, 708, 817
  - p*-coumaroyl-CoA 707, 816
  - counter-selection and markerless
    - genome editing 478–481
  - counter-selection markers 616, 617–619
  - coupling constraints 144–145, 147–150
  - Crabtree effect 141, 142, 150
  - $^{14}\text{C}$  radioisotope 80
  - Cre/loxP* system 315, 479, 561
  - CRISPR-BEST system 665, 666
  - CRISPR-Cas-technology 304, 313
    - based genome editing 314
    - in *B. subtilis* and related strains 481–486
    - LAB genome editing 563–565
  - CRISPR-Cas9
    - and Cas12a-based genome editing
      - methods 663
    - based approach 873
    - based genome editing 481, 489
  - CRISPR-dCas9
    - mediated base editing 488–489
    - mediated cytosine deaminase base editing system 489
    - mediated deaminase base editing 489
  - CRISPR interference (CRISPRi)
    - 487–490, 497, 564–565, 625–626
  - CRISPR interference and activation (CRISPRi/a) 487, 489
  - CRISPR/PmanPA-Cas9 vectors 486
  - CRISPR RNAs (crRNAs) 308, 624
  - CRISPR/SpCas9-based genome editing system 490
  - cross collisional section (CCS) 267
  - cross-over theorem 179
  - cucurbitacin synthesis 4
  - cumomers 121
  - cutin 813
  - cyanobacteria 356, 805–806, 808, 811, 822, 825–826, 829–831, 873
  - cyanogenic glucosides 817–818, 820, 824, 832–833
  - cyanophycin 380–381
  - cyborgization of bacterial strains 863
  - cyclodepsipeptides (CDPs) 784–785
  - cytochrome P450 (CYP) enzymes 369, 701, 707–708, 710, 714, 812, 816, 826
  - cytochrome P450 oxidoreductase 2 816
  - cytoplasmic acetyl-CoA 713, 716
  - cytosolic acetyl-CoA 705, 717, 736, 746–747, 750, 756, 757
  - availability 698, 753–754
- d**
- data analysis, in metabolomics
    - multivariate statistics 278
    - pathway analysis 278–279
    - univariate statistics 277–278
    - untargeted MS data processing 273–277
  - data dependent acquisition (DDA) 272
  - data-independent acquisition (DIA) 272
  - Daucus carota* 818
  - DcMYB6* 818
  - Del/Ros1* transgenic plant 819
  - $\Delta 9$  stearoyl-CoA desaturase (D9) 746, 747
  - de novo* triacylglycerols biosynthesis 745–746
  - dead' Cas9 (dCas9) 309
  - Debye–Hückel approximation 216, 221–223
  - deep alleleome 34
  - 2-dehydro-3-deoxy-phosphogluconate aldolase reaction 91–92
  - dehydrogenase 92
    - alcohol 283, 343, 354, 356, 359–360, 363, 432, 576–577, 583–585,

- 613, 692–693, 695, 697–698, 700–702, 704
- GAP 428, 432, 748–749
- lactate 196, 362, 377, 423–424, 434–435, 554, 571–573, 577, 587–588
- 3-dehydroshikimate 428–429, 816
- deoxyviolacein 433
- desorption electrospray ionization (DESI) 269
- dhurrin synthesis 835
- diacetyl production 574
- diamines 360–361
- 1,3-diaminopropane 360
  - cadaverine 361
  - putrescine 361
- diaminopimelate 92, 359, 416, 421, 422, 431
- 1,3-diaminopropane 360–361
- dicarboxylic acids 361–362
- glutaric acid 361, 362
  - succinic acid 362
- dihydrofolate reductase (DHFR) 317–318
- L-3,4-dihydroxyphenylalanine (L-DOPA) 370
- dimethylallyl diphosphate (DMAPP) 355, 367, 369, 713–716, 757–758, 822
- diols
- 1,4-butanediol 363
  - production 706
  - 1,3-propanediol 362–363
- “direct” omics approach 100
- diterpenoid forskolin 834–835
- diterpenoids 821, 830
- DNA double-strand break (DSB) 489
- DNA double-strand breaks (DSB)
- 302–303, 305, 307–314, 317–318, 407, 489, 563–565, 619–620, 624, 626, 663–665
- DNA microarray analysis 722
- DNA microarrays 405
- docosanol 702
- L-DOPA decarboxylase (DODC) 370
- double crossover 615–619
- double-gene knockout (DKO) predictions 34
- double-helix model 320
- double-stranded breaks (DSBs) 302
- Drosophila* DNA-binding Ubx homeodomain 305
- dSpyCas9* 625
- dual plasmid approaches 482
- dwarf-type mutants 814
- D-xylose 695, 697
- dynamic degradation of TAG' (ddTAG) strategy 673
- dynamic mass balances 23–25, 28
- dynamic metabolons 831
- e**
- ecModels 140, 142, 144–145, 147, 150–151
- ectoine production 425–426
- EDEMP cycle 524–525
- Ehrlich degradation pathway 354
- eicosapentaenoic acid (EPA) 755–756, 760, 830
- electron impact (EI) ionization 269
- electrospray ionization (ESI) 268–269, 280–281
- Elementary Metabolite Units (EMU) 121
- Embden–Meyerhof–Parnas pathway 524
- Embryophyta 806
- emodepside 785
- enantiopure L-lactic acid production 572
- endogenous chloroplast-localized NADP-malic enzyme 810
- endometabolomics 260
- endonucleases
- CRISPR 308–310
  - TALEs 306–308
  - ZFN 304–306
- endoplasmic reticulum (ER) 8, 701, 745–746, 750, 778, 818
- endoplasmic reticulum-associated protein degradation (ERAD) 719
- endoxylanases 436

- energy-demanding sporulation process 471  
 energy-rich adenosine triphosphate (ATP) 808  
 Entner–Doudoroff pathway 92, 101, 282, 283, 524  
 environmental biocatalysts, development of 870  
 environmental Galenics 861, 877–*Environmental Galenic Science* 877  
 environmental microbiology 865  
 environmental microbiomes, fortification of 879  
 environmental pollution 859, 865, 867, 874  
 microbiological agents for bioremediation 860  
 enzymatic rate equations 25  
 enzyme abundance 155–156, 163  
 enzyme bottom-up approach 186  
 enzyme kinetics factors affecting intracellular enzyme kinetics 155–156  
 Michaelis–Menten formula 153–154  
 enzyme mechanisms 91, 174  
 enzyme prediction for orphan and novel reactions 244–246, 251, 257  
 enzyme properties 153, 155, 172, 180–186  
 equality constraints 107  
*ERG9* 714–715, 717  
 erythrose-4-phosphate (E4P) 14, 201, 428, 709  
*Escherichia coli* (*E. coli*) 36, 473, 556  
 bulk chemicals  $\omega$ -amino acids 357–359  
 diamines 360–361  
 dicarboxylic acids 361–362  
 diols 362–363  
 hydroxy acids 359–360  
 lactams 363  
 definition 341  
 fuels production non-native biofuel producers 343  
 non-natural biofuels 343  
 renewable biofuels 342  
 microbial biopolymers non-protein poly(amino acid)s 380–381  
 PHAs 374–379  
 polysaccharides 379–380  
 nanomaterials (NMs) 381–383  
 natural products 367–371  
 recombinant protein production 371, 372  
 membrane proteins 372, 373  
 protein-based materials 374  
 therapeutic proteins 371, 373  
 specialty chemicals aromatic compounds 365–367  
 L-amino acids 364–365  
*Escherichia coli* K-12 MG1655 23, 36  
 essential genes 32, 437, 470, 473–475, 478, 488, 497, 499, 565, 626, 824  
 ethanol production 229, 233, 284, 343, 575, 611, 691–695, 697–698, 703–706  
 eukaryotic microalgae 806  
 evolutionary engineering 341, 571, 697  
 exometabolomics 260  
 exopolysaccharide 379–380  
 expression cassette 302, 310, 318, 479, 482, 737, 741, 747, 755, 760, 769  
 extended Debye–Hückel limiting law 221, 222  
 external constraints 138–139  
 external metabolites 174  
 extra-cytoplasmic function sigma factors (ECF) 493  
 extracellular alkaline cellulase Egl-237 477  
 extracellular fluxes 6, 78–79, 83–85, 91, 93, 98, 106, 111–113  
 extracellular reactions 78, 103
- f**
- FAIMS 267  
 Faraday constant 223, 227  
 $\beta$ -farnesene production 713, 716

- fatty acid *de novo* biosynthesis 355, 375, 377  
 fatty acid alkyl esters (FAAEs) 355  
 fatty acid ethyl esters (FAEEs) 354–356  
 fatty acid methyl esters (FAMEs) 354–356  
 fatty acid pathway 355–356  
 feedback inhibition simulation 205–207  
 fermentative pathway 343, 354  
 ferulic acids 588, 816, 835  
 filamentous actinomycetes genome-scale metabolic models 674–675  
 multi-omics studies 671–674  
 filamentous fungi 313, 767, 784  
*A. niger* 765, 776–778  
*A. oryzae* 778–779  
*A. terreus* 779–780  
 carbon catabolism 775  
 cell factories and products 766  
 CRISPR genome editing protocols for 768, 769  
 genetic and genome tool development 768–769  
 improved substrate utilization 773–775  
 industrially exploited 768  
 macromorphology of 785–788  
 metabolic and regulatory models 770–772  
 morphologies 767  
 natural metabolic capacities of 765  
*P. chrysogenum* 780–781  
 protein production 788  
 secondary metabolites from 784  
*T. reesei* 781–783  
*T. thermophilus* 783  
 fine-tuned integration of proteome constraints coupling constraints 148, 150 pcModels 144, 145, 147 ribosome assembly reaction 148 TPI enzyme 146  
 first-generation bioethanol production 691–694  
 five-carbon containing prenyl isomers 822  
 FK506 biosynthesis 674  
 flavonoids 817, 819 biosynthesis 708, 818 in *S. cerevisiae* 709  
 flavonols 418–419, 708–710, 819  
 fluorescence-activating and absorption-shifting tag (FAST) protein 633  
 fluorescent reporters 631–633  
 5-fluoro-dUMP 479  
 5-fluorouracil (5-FU) 479  
 flux balance analysis (FBA) 6, 7, 162, 172, 215, 251 additional constraints 27, 29 analogy to deriving enzymatic rate equations 25 constrained optimization 26 dynamic mass balances 23–25 flux-concentration duality 28 genome-scale 25, 26  
 flux-concentration duality 28  
 flux connectivity theorem 184  
 flux constraints 107  
 flux control coefficient 175–180, 184, 186–191, 195–198, 201–202, 204–206, 283  
 flux quotient 96  
 flux summation theorem 178–179, 199  
 flux vector 25  
 flux–enzyme relationship 176–178  
 fluxomics 50, 75, 77–79, 167, 406, 864  
 FMN-based fluorescent proteins (FbFPs) 632  
 FokI-cleavage domain 305  
 folate production, in LAB 579  
 forskolin 822–823, 834–835, 837  
 forward simulation 84–85, 98, 121, 123  
 Fourier-transform ion cyclotron resonance (FT-ICR) 271  
 fractional labeling enrichment (FLE) 81, 82, 106, 116  
 free amino acids 112, 115, 116, 735, 755

- free fatty acids (FFAs) 354, 355, 701, 735, 755  
 free/independent fluxes 84  
 fructose 6-phosphate 31, 140, 434, 481, 580  
 fruit-specific *E8* promoter 819  
 fumaric acid 783  
 functional CRISPR/Cas9-sgRNA complex formation 490  
 functional kinetic model 157–158, 163, 165–166  
 fungal biotechnology 766, 775, 788–789  
 fusafungine 785
- g**  
 $\gamma$ -aminobutyrate (GABA) 587  
 GAP dehydrogenase (GapA) 428, 432, 748–749  
 gas chromatography (GC) 114, 266  
 GE-free synthetic biology 865  
 GEM with enzymatic constraints using kinetics and omics data (GECKO) 139–142, 144  
 gene expression knockdowns 625  
 gene expression, in lactic acid bacteria 557  
 gene/genetically modified organisms (GMOs) 555, 565–566, 574, 579, 590, 829, 867, 872, 873, 877  
 gene-protein-reaction (GPR) 32–33, 674  
 generalized mass action (GMA) 159–160  
 genome annotation 7, 32, 36, 157, 168, 470, 522  
 genome editing  
     definition of 301  
     of industrially relevant eukaryotes  
         CHO 317–320  
         filamentous fungi 313, 315–316  
         yeast 310–313  
     principles of 301–304  
 genome editing tools  
     counter-selection and markerless genome editing 478–481  
 CRISPR/Cas in *B. subtilis* and related strains 481–486  
 genome mining 581, 671, 673, 675–676, 784  
     for biosynthetic gene clusters 657–660  
 genome mining tools 658, 660  
     limitations 658  
 genome reduction projects in *B. subtilis* 473–477, 481  
 genome-reduced strain MGB874 476–477  
 genome-reduced variants, of *P. putida* 528–529  
 genome-scale kinetic models 7, 156, 168  
 genome-scale metabolic models (GEMs) 213, 215, 674, 770  
 internal constraints  
     membrane economics 139  
     molecular crowding 139  
     proteome constraints 139  
 genome scale models (GSM) 7, 23–63, 137–151, 156, 240  
 genome-scale models (GEMs) 7, 23  
     *E. coli* 36–42  
     developments 50–55  
     from metabolism to the proteome 42–49  
     perspectives 56–59  
 genome-wide CRISPRi 488  
 genome-wide transcriptome analysis 690  
*Geobacillus thermodenitrificans* 490  
 geranylgeranyl diphosphate (GGDP) 758, 822–824, 828  
 geranylgeranyl pyrophosphate (GGPP) 496, 713, 714, 717–718, 758  
 geranylgeranyl pyrophosphate synthase (GGPPS) 496  
 gibberellins 817, 826  
 Gibbs free energy 214–218, 220–223, 225–228, 233, 247  
     of pseudoisomer 222  
 global environmental problems, by  
     urban and industrial activities 866

- global gene expression analyses 470  
 global scale 6, 404, 803, 821, 838, 880  
 global Transcriptional Machinery  
     Engineering (gTME) method 12, 366, 373, 492  
*glpX* gene 495  
 glucoamylase 776–778, 786  
 glucose 6-phosphate (G6P) generation 31–32, 165, 180, 224, 359, 413, 422, 431, 434–435, 495, 497, 524–525, 554, 750  
 glucose-6-phosphate dehydrogenase 495, 497, 554  
 glucose-6-phosphate isomerase (PGI) 32, 142, 162, 165, 554  
 $\beta$ -glucosidase 436–437, 530, 571, 697, 774  
 glucosinolates 817, 820  
 L-glutamate 364, 383, 403, 405, 412, 419–420, 427, 435–436, 587  
 glutamate (GLU<sub>1–5</sub>) 117  
 glutamate dehydrogenase RocG 476  
 glutamate racemases 497  
 Glutamine Synthase (GS) 318  
 glutarate production 431, 741, 742  
 glutaric acid 357, 361, 362  
 glyceraldehyde 3-phosphate (GAP)  
     dehydrogenase approach 749  
     dehydrogenase-encoding gene 496  
 glycerate 809  
 glycerol kinase (GlpK) 38, 495  
 glycerol metabolism, in LAB 585  
 glycerol transport facilitator (GlpF) 495  
 glycine betaine 829  
 glycolysis 14, 31–32, 101, 112, 140, 142, 145, 156, 161, 165, 188, 192, 205, 224, 229, 233, 497, 524–525, 553, 674, 704–705, 738, 740, 743, 745, 778  
 glycosylated proteins 373, 720  
 glyoxylate shunt 101, 360, 472, 703, 742, 750, 779  
 Golden Rice 820  
 Golgi apparatus 718–720, 821  
 Golgi-derived secretory vesicles 720  
 gram cell dry weight (gCDW) 8, 144, 149  
 graph-based search 243–244  
 green fluorescent protein (GFP) 318, 492–493, 631–632, 667  
 guide RNA (gRNA) 308–309, 620, 663  
 guilt-by-association approach 771
- h***  
*Haemophilus influenzae* 23  
 “hard” constraints 138–139  
 heat stress tolerance 567–569  
 height equivalent of a theoretical plate (HETP) 264  
 heme biosynthesis 721  
 hemicellulosic polysaccharides 813  
 hemoglobin 495, 721  
 hepatitis B surface antigen (HBsAg) 722  
 hepatitis B vaccines 722  
 high resolution mass spectrometry 270, 280  
 high-resolution fluxomics 406  
 high-throughput metabolomics 279–280  
 Hill coefficient 184  
 Hill equation 159, 206  
 homogalacturonan 813  
 homogentisic acid 826  
 homologous recombination (HR)  
     mechanism 301, 303, 406, 559–560, 564, 616–620, 626, 662–663, 737, 769, 807, 826, 830, 831  
 horizontal gene transfer (HGT) 861  
     based, large-scale bioremediation 878  
     events 868  
 HR-mediated technique for genome editing 302, 304  
 human insulin 720–721  
 human papillomavirus (HPV) vaccines 722  
 Hunter’s syndrome 306  
 hyaluronan 379–380  
 hyaluronic acid (HA) production 435  
     in *C. glutamicum* 434, 435

- hydrocortisone 717  
 hydrophilic interaction liquid chromatography (HILIC) 265, 276  
 hydroxy acids 3HP 360  
 4HB 360  
 4-hydroxybutyric acid (4HB) 359–360  
 4-hydroxycinnamic acid (p-HCA) 707–709  
 3-hydroxydecanoate (3HD) 375  
 3-hydroxydodecanoate (3HDD) 375, 377  
 3-hydroxyhexanoate (3HHx) 375  
 4-hydroxyisoleucine (4-HIL) production 403, 427  
 3-hydroxyoctanoate (3HO) 375  
 3-hydroxypropionate (3-HP) 375, 377, 430, 432  
 3-hydroxypropionic acid (3-HP) 359–360, 585, 703, 705  
 hygromycin B 315, 737, 745, 747
- i**  
*i*Bsu1103 470  
*i*Chip 654  
 illumina sequencing 760  
*i*Modulons 52–55, 57, 62  
 in-depth global transcriptome 470  
 inactivated whole cells 860  
 INCA 117  
 independent component analysis (ICA) 51  
 inducible promoters 620, 627  
 inequality constraints 107  
 input labeling composition 104  
 input labeling design 93  
*in silico* cell 33  
*in silico* experimental ILE design 108  
 INST-<sup>13</sup>C-MFA 83, 123  
 integrative analysis of omics data 166  
 intermediates, toxicity of 247  
 internal constraints 138–139  
 internal metabolites 174, 179, 184, 200  
 intracellular compartments 8  
 intracellular enzyme kinetics 155–156  
 intracellular fluxes 46, 75, 77–79, 82–84, 91, 93, 99–100, 103, 107, 112, 260  
 intracellular metabolite pool size 78, 95, 106, 123  
 inverse problem 85, 98, 121  
*in vitro* enzyme assays 161  
*in vivo* RBS-selector 667  
 ion chromatography (IC) 264, 267  
 ion mobility 267  
 ion-pairing RPLC (IP-RPLC) 265–266, 283  
 ionization techniques 268–269, 280  
 irregular xylem (IRX) mutants 814  
 IRX7 deficient plants 814  
 isobutanol production 698–699  
 isocitrate dehydrogenase Icd 496  
 isoflavonoids 708, 710, 817  
 isopentenyl diphosphate 354–355, 434, 757–758, 822  
 isopentenyl pyrophosphate (IPP) 14, 354–355, 367–369, 661, 713–717, 757–758  
 isoprenoid pathway 343, 355  
 isopropanol 263, 265, 343, 354, 613  
 isotope labeling experiment 100  
     atom transition network specification 103–104  
     input labeling composition 104–106  
     metabolic network specification 101–103  
     modeling and simulation 101  
 isotopic labeling 78–80, 96, 98, 115  
 isotopic steady state (ISS) 77, 90, 123  
     approximation 115–116  
 isotopic tracers 4, 6, 77, 79–80, 99  
 isotopic tracing 8–9  
 isotopically labelled tracer 8  
 isotopologues 97, 261, 274  
 isotopomer fractions 94, 96–97, 103  
 isotopomers 91–98, 106, 114, 116  
 itaconate 428, 430, 777, 779, 780  
*A. niger* 777

**k**

- keto acid decarboxylase (KDC) 354, 697  
 2-keto-acid route 698  
 $\alpha$ -ketoglutarate production 741–742  
 ketones bioreduction 584–585  
 keyicin production 673  
 kinetic feasibility 246, 247  
 kinetic model  
   applications 166–167  
   approximative rate expression 159–160  
   description 156  
   functional 157–158  
   mechanistic rate expressions 158–159  
   perspectives 167–168  
   rate expressions 160–166  
   scope 156–157  
   toy example 164–165  
   toy model 164–165  
   yeast 165–166  
 kinetome 51, 168  
 kirromycin biosynthesis 656, 657  
 knowledge bases 24, 35, 57, 62, 404, 838, 862  
 kojic acid  
   *A. oryzae* 778, 779  
 k-OptForce 41

**I**

- L- and/or D-glutamic acid monomers 495  
 L-arabinose 283, 695, 697  
 labeled substrate 77, 80–81, 99, 104, 108, 110–113, 116  
 labeling enrichments 81–83, 85, 90–91, 100, 103, 106, 109, 113, 115–117, 121, 123  
 lactams 357, 363  
 lactate dehydrogenase (LDH) 196, 362, 377, 423–424, 434–435, 554, 571–573, 577, 587–588  
 lactic acid 195, 572, 573, 613, 705  
 lactic acid bacteria (LAB) 555  
   as biocatalysts 583–589  
   bacteriocins 581–583

biotransformations 587–588

- bulk chemical production  
   acetoin 575–577  
   butanediol-isomers 577–578  
   ethanol 575  
   butanol production 588  
 CRISPR-Cas-technology for genome editing 563–565  
 features and phylogeny of 552, 553  
 fermentation 552  
 food ingredients  
   acetaldehyde 574–575  
   alanine 574  
   diacetyl 574  
   lactic acid 572, 573  
 gene expression 557–559  
 genetic engineering of 559–565  
 glycerol conversion 585–587  
 heat tolerance  
   acid tolerance 569  
   oxygen tolerance 570–571  
 host for plant metabolite production 588  
 ketones bioreduction 584–585  
 metabolism of 553–555  
 multistress-resistance 567  
 plasmid integration by homologous recombination 559–560  
 plasmid integration using phage attachment sites 560–561  
 polyols production 579–580  
 preservative effect 551  
 recombineering based genetic engineering 561–563  
 roles and functions 551  
 shuttle vectors 556–557  
 starter cultures for food fermentation 565–567  
 stress tolerance  
   heat tolerance 567–569  
 substrate utilization routes 571–572  
 therapeutic proteins production 580–581  
 thermotolerance 568  
 transformation of 556–557  
 vitamins production  
   cobalamin (vitamin B12) 579

- lactic acid bacteria (LAB) (*contd.*)  
 folate (vitamin B9) 579  
 riboflavin (vitamin B2) 578  
 lactic acid production 569, 572, 587,  
   705  
*Lactobacillus casei* 556, 561, 563–564,  
   568–569, 577, 580, 582, 584,  
   587  
*Lactobacillus reuteri* 561–562, 579,  
   584, 586  
*Lactococcus lactis* 473, 556–558, 560,  
   570  
 for bulk chemicals production 576  
 as drug delivery vehicle (DDV) 580  
 for over-producing flavor compounds  
   573  
 lanthipeptides 581–582  
*ligD* positive strains 487  
 lignans 817  
 lignocellulose 530, 694, 782  
 lignocellulosic biofuel production 694  
 limiting rate 172, 181, 183  
 lin-log modeling approach 160  
 linear ion traps (LITs) 270  
 linear plus linear homologous  
   recombination (LLHR) 662  
 linearized statistics 89–90  
 lipid synthesis 747, 833  
 lipid-rich yeast 753  
 lipolase 313  
 lipopeptide surfactin 497  
*Lisianthius nigrescens* 834–835  
 liquid chromatography (LC) 114,  
   264–266, 669  
 liquid–liquid extraction (LLE) 263  
 lithium acetate method 737  
 lovastatin 653, 779–780  
 low resolution mass spectrometry  
   269–270  
*Lpd1* 740  
 lycopene, in *S. avermitilis* 667  
 L-lysine 110, 357, 359, 361, 364,  
   403–405, 413, 415–416,  
   419–423, 425–426  
 lysophosphatidate acyltransferase  
   (LPAAT) 204–205
- m**
- machine learning approaches 241, 242,  
   249, 660  
 maize 806, 821  
 malate synthase 472, 697, 810  
 malate–pyruvate–oxaloacetate cycle  
   approach 749  
 malic acid production 703–704  
*A. oryzae* 779  
 maltose consumption 477  
 mandelic acid 366, 378–379  
*Manihot esculenta* 832  
 mannitol 420–421, 579–580  
   utilization 415–416  
 mannose 414  
 mannose phosphoenolpyruvate-  
   dependent phosphotransferase  
   system 481  
 mannose-specific transporter *ManP*  
   481  
*manPA* alleles 481  
 manual pathway design 238  
 Mariner-transposable *Himar1*-based  
   systems 617  
 markerless-deletion system 619  
 Markov chain Monte Carlo (MCMC)  
   approach 89  
   sampling 26  
 mass isotopomers 97–98, 114,  
   116–117  
 mass spectrometry (MS) 268  
   acquisition modes  
     for targeted metabolomics  
       271–272  
     for targeted MS 272  
     for untargeted metabolomics 272  
   features 268  
   high resolution 270–271  
   ionization techniques 268–269  
   low resolution 269–270  
 Mathieu equations 270  
 matrix effect 263, 268–269, 279  
 matrix-assisted laser desorption  
   ionization (MALDI)  
   269–270, 280  
 max-min driving force (MDF) of  
   pathway 282

- Maximum Likelihood Estimator (MLE) 163  
maximum reaction velocity 25  
mCherry-reporter cassette 319  
ME models 42–49, 57  
mechanistic rate expressions 158–160  
medium chain length PHA (MCL-PHA) 375, 378  
meganucleases 302–304, 320  
membrane bound cytochrome P450 (P450) enzymes 812  
metabolic control analysis (MCA) 6, 153, 171, 173, 283  
aromatic amino acids, bacterial production of 200–202  
based methods 166  
concentration control coefficient 179–180  
demand for product 194–195  
flux control coefficient 175–176  
flux summation theorem 178–179  
flux–enzyme relationship 176–178  
inhibition of competing pathways 195–196  
limitation of 171, 188  
linking control coefficients to enzyme properties 181  
alterations of enzyme activity 188–190  
block elasticities 186  
control coefficients and elasticities 184–186  
elasticity coefficient 181–184  
enzyme rate equations 181–184  
feedback inhibition 186–188  
feedback inhibition, abolishing 191–194  
top–down analysis 186  
metabolic steady state 174–175  
Universal Method 199–200  
yeast tryptophan synthesis 197–198  
yield impacts 203–205  
metabolic engineering  
  bioenergetics in life 213–214  
  of *Bacillus*  
activity-independent screening of target molecule synthesis 492–493  
biotechnological application 493–497  
generally regarded as safe 470  
minimal cell concept 472–478  
optimization, standardization, and modularity in gene expression 490–492  
physiological traits and circuits 470–472  
quality presumption of safety 470  
tools for genome editing 478–490  
cellular metabolism and physiology  
‘omics’ technologies 7–9  
FBA 6, 7  
isotopic tracing 8, 9  
MCA 6  
MFA 6, 7  
combinatorial metabolic engineering 12–14  
*Escherichia coli* 341–384  
goal of 171  
history and overview of 3  
host organism selection 15  
industrial biotechnology 17  
rational metabolic engineering 10–11  
strain development process 17, 18  
substrates 15–16  
synthetic biology 16  
systems metabolic engineering 14  
Metabolic Engineering: Principles and Methodologies 3  
metabolic flux analysis (MFA) 6, 7  
<sup>13</sup>C-MFA 97–99, 124–125  
<sup>13</sup>C-MFA variants 76–77  
bidirectional reaction steps 95–96  
biotechnologically relevant organisms 108  
carbon atom transitions 91–93  
extracellular fluxes 111–112  
flux constraints 107  
fluxomics 77–79  
from the data to the intracellular fluxes 82–83

- metabolic flux analysis (MFA) (*contd.*)
- in silico* experimental ILE design 108
  - input labeling design 93–94
  - INST-<sup>13</sup>C-MFA 83–84, 123–124
  - introduction 73–77
  - isotope labeling experiment 100–108
  - isotopic labeling 79–81
  - isotopic steady state approximation 115–116
  - isotopomer fractions 96–97
  - isotopomers 94–95
  - labeled substrate 112–113
  - measurement specification 106–107
  - metabolic and isotopic stationarity 110–111
  - metabolic and isotopic stationary state 90–91
  - metabolomics 113–115
  - natural isotope abundance 116–117
  - parameter fitting 84–86
  - simulation of labeling data and flux estimation 117–123
  - statistical analysis 86, 89–90
  - statistical evaluation and optimal experimental design 99–100
- metabolic pathway design 237–238
- metabolic pseudo-steady state approaches 76, 109
- metabolic steady state (MSS) 77, 173–175, 182, 188, 281
- assumption 77
- metabolism 153
- of LAB 553–555
  - metabolism 523
  - sugar 554
- metabolite extraction 8, 262
- metabolomics 8, 113, 259
- analytical techniques
    - sample preparation 262–264
    - separation techniques 264–267
  - applications 281–285
  - calibration curves 261
  - challenges 259
  - data analysis 272–277
- and interpretation 277–279
  - engineer medium composition 285
  - experimental design 260
  - improving stress tolerance 284–285
  - internal standards 261
  - pathway design by thermodynamic analysis 281–283
  - reduction of side products and metabolite damage 284
  - sequences and standards 261–262
  - targeted and untargeted 260–261
- metabolons 825, 831–833
- MetaCyc 101
- metallo-proteome 43–45, 55
- meta-organismal catalysts 875
- methotrexate (MTX) inhibits 317
- methyl anthranilate 366, 404
- 2-methyl-D-erythritol 4-phosphate (MEP) pathway 822
- methylerythritol-4-phosphate (MEP) 355
- mevalonate (MVA) pathway 354, 355, 822
- in *S. cerevisiae* 714
- mevalonate diphosphate (MVAPP) 758
- mevalonate pathway 588–589, 757–759
- Michaelis constant 159, 181
- Michaelis–Menten constant 25
- Michaelis–Menten formula 153–154
- enzyme kinetics 154
  - formulation 159
- Michaelis–Menten kinetics 7, 154, 164
- Michaelis–Menten reaction mechanism 25
- microalgae 803, 806, 811, 829–831
- microbial activities, multi-scale propagation of 869
- microbial biocatalysts 4
- microbial biopolymers
- non-protein poly(amino acid)s 380
  - PHAs 374–379
  - polysaccharides 379–380
- microbial consortia 874, 879
- microbiological agents for bioremediation 860

- microfluidics 13, 280–281, 483, 654, 784
- microhomology-mediated end-joining (MMEJ) 301, 302, 319
- minGenome 41
- MiniBacillus* 494
- MiniBacillus* framework 475
- minimal cell concept
- genome reduction projects in *B. subtilis* 473–476
  - minimal genomes 472–473
  - productivity of genome-reduced strains 477–478
- minimal genomes 61, 472–473, 499, 789
- missing values 85, 274
- mixed-integer linear program (MILP) 38, 215, 227–228
- mixed-integer nonlinear program (MINLP) 41
- monoclonal antibodies (mAbs) 371, 373
- monoterpene indole alkaloids (MIAs) 370, 710–712
- monoterpenes, in *S. cerevisiae* 716
- Monte Carlo based approach 163
- MPD pipeline 787–788
- M. thermoacetica* gas fermentation 751
- multi-strain reconstructions 33
- multiple reaction monitoring (MRM) method 271
- multiplex automated genome editing/engineering (MAGE) 342, 526, 863
- multiplex genome editing 486–487
- multivariate statistics 278
- Mummichog algorithm 279
- mutagenic ssDNA recombineering 870
- mutS* mutations 569
- Mycoplasma mycoides* JCVI-syn3.0 strain 473
- mycosporine-like amino acid (MAA) 423
- n**
- NADH oxidase (NOX) of *Lactococcus lactis* 196
- NADPH-P450 oxidoreductase *SbPOR2b* 825
- NADPH-P450 oxidoreductases (PORs) 817
- Nannochloropsis oceanica* 806, 830
- nanomaterials (NMs) 381–383
- nanopore sequencing technology 655
- nanopore sequencing, in *Y. lipolytica* genome 760
- nanostructure-initiator mass spectrometry (NIMS) 280
- natural abundance (NA) 80
- correction 116–117
- NAtural Deep Eutectic Solvent (NADES) 834–835, 837
- natural isotope abundance 116–117
- natural isotope enrichments 105
- natural products
- alkaloids 368, 370
  - phenylpropanoids 368–370
  - polyketides 368, 370–371
  - terpenoids 367–369
- naturally labeled <sup>12</sup>C glucose 80
- N-containing bioactive natural products 820–821
- network data representation 242
- network reconstruction
- availability of GEMs 35
  - basic principles 30–32
  - computational queries 32–33
  - curation 32
  - genomic basis 32
  - knowledge bases 35
  - scope expansion 33–35
- Nicotiana benthamiana* 807
- nicotinamide adenine dinucleotide phosphate (NADPH) 714, 808, 809
- Nisin controlled gene expression (NICE) system, in LAB 557, 558, 565
- nisin Z 582
- nisK* 557, 558
- nisR* 557, 558

- non-homologous end joining (NHEJ)  
 mechanism 301, 302, 313,  
 314, 318, 489, 619, 626, 663,  
 737, 741, 745, 747, 760
- non-native biofuel producers  
 fatty acids pathway 355–356  
 fermentative pathway 343–354  
 isoprenoid pathway 355  
 keto acid pathway 354–355
- non-natural biofuels 343
- non-oxidative pentose phosphate  
 pathway (PPP) 96, 101, 284,  
 285, 495, 553, 554, 748, 750,  
 751, 753, 754
- non-protein poly(amino acid)s  
 380–381
- nonribosomal peptide synthetases  
 (NRPSs) 371, 496, 534, 657,  
 667
- R,S*-norlaudanosoline 370
- normal-phase liquid chromatography  
 (NPLC) 265
- novel reactions 244–245, 251, 257, 583
- nuclear localization signal (NLS) 306,  
 308
- O**
- Obiwarp method 274
- off-target effects 305, 307, 309, 316,  
 319
- oleochemicals 699
- oligo-annealed promoter shuffling  
 (OAPS) 491
- oligosaccharides 412, 414–415, 773
- omega-3 fatty acid 818
- 4'-*O*-methyltransferase (4'OMT) 368,  
 370
- omics' technologies 7–9
- OPENFLUX 117
- OptForce 38, 41
- optimal experimental design (OED)  
 89, 99–100, 101, 108, 109,  
 123
- OptKnock 38
- OptStrain 38
- orbital trap 270
- Orbitrap instruments 270, 271
- organic acids production  
*S. cerevisiae* 702
- orotate transporter (*oroP*) 559, 560
- Oryza sativa* 806
- oxidative pentose phosphate pathway  
 553, 748
- oxidative TCA cycle 703, 704
- OxidizeME 43, 46, 48
- 2-oxoglutarate 419, 476, 711
- oxygen tolerance 570–571
- P**
- paclitaxel 367, 717, 818
- pangenome 41, 61, 522
- paralogous proteins 473
- parameter covariance matrix 89
- parameter fitting 84–86, 96, 98, 107,  
 116, 121, 123, 163
- parametric Monte Carlo bootstrap 89
- pathway design 238  
 available tools for 247–249  
 practical example of 249  
 successful applications of 249
- pathway feasibility 246–247
- pathway search 237, 242–243, 244, 249  
 algorithm 244
- PCR-generated sgRNA expression 487
- P450-dependent biosynthetic pathway  
 824, 825
- peak grouping 274
- peak picking 273–274
- pectin matrix 813
- penicillin 202–203, 420, 653, 769, 780,  
 781
- penicillin-producing fungi 313
- Penicillium chrysogenum* 767, 780  
 enzyme set for plant polysaccharide  
 degradation 773
- GEMs for 770
- secondary metabolite production  
 774
- X-ray microcomputed tomography  
 ( $\mu$ CT) 787–788
- pentose fermentation rate 695
- pentose phosphate pathway (PPP) 96,  
 101, 156, 229, 284, 285, 495,

- 524, 553, 554, 674, 695, 748,  
750, 753, 754
- pentose sugars 413, 553, 695, 697
- $\gamma$ -PGA biosynthesis 495
- pG<sup>+</sup>host system 560
- phage-assisted continuous evolution  
(PACE) 13
- phenylalanine ammonia lyase (PAL)  
359, 367, 707, 708, 819, 820
- 3-phenyllactic acid (PLA) 375, 378,  
587, 588
- phenylpropanoid biosynthesis 819
- phenylpropanoid pathway 707, 819,  
833
- phenylpropanoids 367, 368–370,  
706–710, 813, 817, 819
- phosphite oxidoreductase (PtxD) 829
- phosphoenolpyruvate carboxykinase  
gene 416, 422, 431, 496, 744
- phosphofructokinase (PFK1) 31, 180,  
188, 191, 414, 432, 497, 498,  
554, 674, 692, 776
- 6-phosphofructokinase 497, 498, 674  
butanol production 698
- phosphoglucose isomerase (PGI) 31,  
32, 33, 142, 165, 692
- 3-phosphoglycerate 165, 692, 809
- phosphoglycerate mutase (PGM) 165,  
692
- phosphotransferase systems (PTS)  
201, 414, 415, 428, 522, 523,  
554, 555, 580
- photorespiration 809, 810
- photosynthetic cells  
biosustainable industrial production  
platforms 806–808
- plants 803–805  
solar radiation 805–806
- photosynthetic organisms  
cyanobacteria 808  
metabolic engineering strategies  
810
- photorespiration 809
- ribulose-1,5-bisphosphate 809
- Rubisco 808
- thylakoids 808
- phylloquinone 826
- physiological effects 8, 499
- L-pipecolic acid 403, 426
- plant bioactive natural products  
phenylpropanoids 817  
synthesis and regulation of 817–818
- terpenoids 817
- upregulated anthocyanin synthesis  
818–819
- plant cell wall 530, 803, 814, 816, 813
- plant metabolic engineering 803, 804,  
820, 838
- plant metabolites 588–589, 807, 822
- plant-derived taxadiene synthase 495
- plants 803–808
- plasmid copy number effect 477
- plasmid-based CRISPR/Cas9 systems  
482
- plastoquinols 826
- plastoquinone 809, 826, 829
- pollutant-responding whole cell  
biosensors 865
- poly(diaminobutyric acid) 380
- poly(diaminopropionic acid) 380
- poly(glutamic acid) 380, 381
- poly(lactate-*co*-glycolate) (PLGA) 377,  
378, 379
- poly(lysine) 380
- poly(PhLA-*co*-3HB) production 379
- poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA) 494, 495
- polyglutamate (PGA) 435–436, 494,  
495
- polyglutamate synthesis 495
- polyhydroxyalkanoates (PHAs)  
energy and redox storage material  
374
- MCL-PHA 375, 378
- PHA (SCL-PHA) 375
- PhaCs 378
- PLGA 379
- poly(3HB) 375, 377
- poly(PhLA-*co*-3HB) production 379
- polyketides 14, 367, 368, 370–371,  
534, 581, 713, 754, 775
- polymerase chain reaction (PCR) 12,  
311, 478, 481, 482, 487, 527,  
558, 662, 663, 736

- polysaccharides 374, 379–380, 408, 530, 773, 775, 782, 813, 814, 815, 816
- pOri plasmid 560
- practical non-identifiability 100
- pravastatin 781
- precursor compound 237, 242, 243, 253
- P43 reference promoter 491
- prenyl transferases 822
- principal component analysis 55, 278
- pristinamycin 663, 664
- pro-vitamin A 820
- productivity of genome-reduced strains 477–478
- profile likelihoods 89
- proinsulin 720, 721
- promoters 627–630
- promoters, in actinomycetes 667
- 1,2-propanediol 428, 432, 706
- 1,3-propanediol (1,3-PDO) 360, 362, 363, 375, 377, 428, 430, 432, 519, 585, 586
- protein precipitation 262, 263
- protein sequence 47, 244, 246
- protein structures 35, 47, 50, 55, 57, 632, 782
- protein-based biopharmaceuticals 371
- proteinogenic amino acids 112, 114, 115, 116, 403, 406, 419, 655, 657
- proteome constraints
- coarse-grained integration of 139–144
  - fine-tuned integration of 144–150
- proteome-constrained models
- (pcModel) 144, 145, 147, 150, 168
- protospacer adjacent motif (PAM) sequence 308, 309, 407, 620
- Prunus dulcis* 818
- Pseudomonas putida* 473, 528
- EDEMP cycle 524
  - in silico* analysis 522
  - in silico* metabolic potential 522
  - aromatic molecules, production of 532–534
- biosynthesis of natural products 534
- carbon substrate range, expansion of 529–530
- characteristics of 521–522
- CRISPR/Cas-based gene editing techniques 526
- features 529
- genome-reduced variants 528–529
- glucose uptake 523
- glycerol metabolism 523
- high-efficiency multiple genomic site engineering (HEMSE) of 526
- nutritional landscape 530
- organic acids, production of 533
- oxygen-dependent lifestyle engineering 530–532
- solvent-tolerant strains 521
- substrate utilization and core metabolism 522–524
- synthetic biology tools for 524–528
- taxonomic credentials 521
- xylose catabolism, pathways for 523
- Pseudomonas putida* KT2440 357, 519
- ptb* 619, 627
- putative BCAA permease 496
- putrescine 359, 360, 361, 412, 423
- pyrazines 433
- pyruvate 741
- acetaldehyde-acetate-AcCoA route 756
  - and  $\alpha$ -ketoglutarate production 740
- pyruvate decarboxylase negative strain (PDC) 704, 705
- q**
- quadrupoles 269–271
- quantum mechanical/molecular dynamics (QM/MD) 216
- quasi-steady state assumption (QSSA) 25, 174
- quenching 110, 114, 262, 281, 811, 830
- quorum sensing systems 876
- r**
- random mutagenesis 12, 304, 378, 419, 491, 532, 566, 567, 615–617, 670, 781

- rate expressions 153, 156–166, 163, 164, 167, 168  
 rate-limiting steps 173, 365, 721  
 rational metabolic engineering 9, 10–11, 613  
 reaction directionality 214, 242  
 reaction prediction 237, 240–241, 242  
 reaction reversibility 214  
**Recombinant DNA Biotechnology III + The Integration of Biological and Engineering Science** 3  
 recombinant DNA technologies 3, 862  
 recombinant proteins 371, 372, 404, 436, 718–723, 828, 830  
 recombinant protein production  
   membrane proteins 371, 372, 373  
   protein-based materials 374  
   therapeutic proteins 371–373  
 recombinant RNA 437  
**Recombinase-Mediated Cassette Exchange (RMCE)** 319, 320  
 recombineering based genetic engineering, in LAB 561–562  
 reduced-genome variants, of *P. taiwanensis* VLB120 529  
 RelA/SpoT homologues 471  
 renewable biofuels 342  
 Repeat Variable Diresidues (RVDs) 306, 307  
 reporter-guided mutant selection (RGMS) 670  
 repression under secretion stress (RESS) 778, 782  
*rep60*-specific sgRNA 487  
 resveratrol 369, 418, 818  
 retention time 264, 266, 272, 274, 276  
 (*S*)-reticuline derived morphine type alkaloids 824  
 retrobiosynthesis 241–242, 243, 251  
 retrobiosynthetic approach 115, 243  
 reverse catabolite repression strategy 523  
 reverse-phase liquid chromatography (RPLC) 265, 266, 268, 276, 283  
 reversible Michaelis–Menten equation 181, 182  
 rhamnogalacturonan 813  
 Ribo-seq 61  
 ribosomal binding sites (RBSs) 433, 667, 668, 871  
 ribosomal proteins 147, 473, 476  
 riboswitches 493, 634, 670, 865  
 ribozymes 316, 312  
 ribulose-1,5-bisphosphate 808, 809  
   carboxylase-oxygenase (Rubisco) 808  
 rice 571, 778, 806, 809, 816, 818, 820  
 RNA sequencing 48, 51, 53, 61, 405  
 roseoflavin 578  
 RPLC 265, 266, 268, 276, 283  
 rRNAs 473  
 16s rRNA-based unrooted phylogenetic tree, of LAB 552–553
- S**
- SABIO-RK13 161–162  
*Saccharomyces cerevisiae*  
   alkaloid compounds production 710  
   aromatic amino acids 708  
   commodity chemicals production  
     diols 706  
     organic acids 702–705  
   cytosolic fatty acid synthesis 699  
   fatty acid synthesis 699–700  
   fatty acids synthesis 699–700  
   first-generation bioethanol production 691–694  
   flavonoids biosynthesis 710  
   higher alcohol production 695–697  
   history 689  
   insulin analogue expression 721  
   mevalonate and sterol pathway 713–714  
   monoterpene, triterpenes, and isoprenoids production 716–718  
   phenylpropanoids production 706–710  
   protein secretory pathway 718–719  
   recombinant proteins production 718–723

- Saccharomyces cerevisiae* (contd.)
- second generation bioethanol 694–697
  - timeline 690
  - virus like particles in 721–723
- S-adenosylmethionine (SAM) synthesis 356, 366, 493
- Salinispore* genomes 672
- SAM-dependent methyltransferase (AAMT1) 366
- sample batches 275
- sample-specific variations, correction of 275
- S. cerevisiae* GEM 140, 157
- scrambling reactions 102, 104, 107
- second generation bioethanol 694–697
- second law of thermodynamics 213–215, 227
- Secondary Metabolite Bioinformatics Portal 658
- secondary metabolites 653, 655, 665, 673
- biosynthesis 660–671
  - BGC cloning for heterologous expression 661–663
  - genome mining, software for 658, 659
  - host strain selection, for heterologous expression 660–661
  - genome mining, software for 658
  - related bioinformatics tools 658
- sedoheptulose-1,7-bisphosphatase 830
- selected reaction monitoring (SRM) 271
- sequencing-based approaches 655
- sesquiterpenoid artemisinic acid 829
- sgRNA-guided Cas9 488
- shallow' alleleome 34
- shikimate *p*-coumaroyl transferase 816
- shinorine production, in *C. glutamicum* 423, 424
- short chain length PHA (SCL-PHA) 375
- short-chain alcohols 432, 534, 697
- SigB-controlled general stress response 471
- SigB-controlled regulon 471
- sigma factor SigB 471
- signal-recognition particle (SRP) 718
- simultaneous saccharification and fermentation (SSF) 694
- single cell metabolomics 280–281
- single-gene knockout (SKO) 33, 34
- single-strain multi-step process *vs.* consortium-based distributed catalysis 874–875
- site-directed mutagenesis 357, 582, 711
- SNPeffect 41
- Solanum lycopersicum* 819
- solar radiation 805–806, 825
- solid-phase extraction (SPE) 263, 264
- Sophora japonica* 834
- sorbitol 579, 580, 694
- space charging 270
- SpCas9-mediated dsDNA cleavage 490
- specialized metabolites 653, 804, 805
- spectinabilin gene cluster 671
- SporeWeb 470
- SpyCas9* 624, 625
- squalene 714, 715, 717
- S-reticuline by an epimerase (STORR) 368, 370
- Standard European Vector Architecture (SEVA) 525, 527
- statistical analysis 86–90, 122, 161, 163–166
- sterol pathway, in *S. cerevisiae* 714
- stilbenes 418, 817
- stoichiometric coefficient 25, 27, 31, 138, 139, 140, 143, 215, 754
- stoichiometric feasibility 246
- stoichiometric matrix-based search 243
- stoichiometric pathway evaluation 249, 251
- strain development methods 566
- strain development process 17, 342
- strand invasion 302

- Streptococcus thermophilus* 308, 555, 565
- Streptomyces*
- antibiotics producers 673
  - biosensor 669
  - metabolic engineering 654
  - species 489, 661
  - strains 473
- Streptomyces coelicolor* 659, 660, 663, 666, 670, 673, 675
- genome-scale metabolic models 674–675
- Streptomyces tsukubaensis* 673
- genome-scale metabolic models 674–675
- streptomycin 527, 665
- streptothricin 665
- stressME model 43, 46
- structural non-identifiability 100
- structural systems biology 35, 55, 56
- S-styrene oxide 366, 367
- suberin 813
- substrate mixture 94, 104, 105
- substrate utilization routes, LAB 571
- subtilisin-like alkaline protease M 477
- SubtiWiki 470
- succinate dehydrogenase (Sdh) complex 360, 704, 743, 744
- succinate production 343, 412, 743, 744
- succinic acid 361, 362, 690, 703, 704, 743
- succinylase 92, 359
- sugar metabolism, of lactic acid bacteria 554
- supercritical fluid chromatography (SFC) 264, 266, 267, 268
- supervised methods, in untargeted metabolomics 278
- surfactin 494, 497
- surfactin biosynthesis 497
- switchgrass 816
- synthetic biology 862
- metabolic engineering 16
- synthetic biology agents (SBAs) 872, 873, 874, 877
- synthetic biology-based technologies for large-scale bioremediation 868–869
- synthetic morphologies 875
- synthetic promotor libraries (SPL) 557–558
- synthetically lethal gene pairs 32
- systems biology 11, 14, 28, 35, 36, 41, 55, 56, 63, 73, 181, 341, 404, 438, 519, 520, 614, 627, 654, 672
- systems metabolic engineering 5, 9, 14, 73, 341, 342, 383, 384, 404–407, 420, 424, 429, 435, 671–675, 776

**t**

- target compound 237, 238, 240, 241, 242, 243, 246, 249, 250, 251, 660, 663, 674, 817, 818, 831, 861, 864, 865
- targeted metabolomics 260–261, 262, 268, 272, 273, 274, 275, 276, 277, 278, 284, 285
- target gene *egl-237* 477
- TATA-box 312
- taxa-4,11-diene 496
- taxadiene 368, 369, 495, 496, 717, 830
- Taylor cone 268
- TCA cycle 229
- in cancer cells 4
- temperature-sensitive derivative pE194ts 481
- temperature-sensitive pSG5 *ori* 481
- temporal control 11
- temporal drifts 275
- terminators 436, 612, 633, 634, 667
- terminators, in actinomycetes 667
- terpenes 367, 712–714, 775, 812
- terpenoid production 713, 718, 812
- terpenoids 367–369, 433, 534, 712, 713, 714, 716, 717, 718, 817, 821, 822, 824, 825, 830, 837
- Terraforming Earth' approach 868
- terrestrial charophycean green algae 805

- tert*-butyl dimethylsilyl (TBDMS) 117, 118
- tetLM* riboswitch 486
- tetracycline 486, 581, 627, 653, 673
- tetrahydro-cannabinolic acid synthase 837
- L-theanine production 427
- theophylline responsive riboswitch 634, 670
- therapeutic proteins 371–373, 552, 580–583
- thermodynamic feasibility 214, 232, 237, 246–247, 251, 253, 282
- thermodynamic flux analysis (TFA) 215, 227, 228, 229, 232, 247, 251, 252, 253
- thermodynamic pathway evaluation 249, 251–257
- thermodynamics-based flux analysis (TFA) workflow 215
- characterizing feasible concentration space 229–233
- compartment-specific ionic strength and pH 220–221
- constraining flux space with metabolomics data 228–229, 232
- estimation of standard free energies of formation 216–221
- free energy of formation for isomer distributions 221–223
- mathematical formulation 227–228
- model curation 215–216
- transformed free energies of reaction 223–227
- thermostable cellulases 782
- thermostable ThermoCas9 490
- Thermothelomyces thermophilus* 766–767, 774, 783
- L-threonine 359, 364, 365, 431
- thylakoids 808, 821, 831, 837
- thymidylate synthase 479, 581
- time-course simulations of dynamic processes 166
- time-of-flight (TOF) MS 114, 270, 271, 272, 273, 274, 405
- time-resolved metabolomic measurements 281
- tocochromanols 826, 828
- tocopherols 818, 826, 828
- tocotrienols 826, 828
- top-down control analysis 186, 204
- toxic pyrimidine analogue 5-fluorouracil (5-FU) 479, 618
- toxin-antitoxin systems 479, 619
- toy model 142, 145, 148, 164
- TP901-1 integration system has 561
- trans-activating RNA (tracrRNA) 308, 309, 482
- transcription factor (TF)-based biosensors 493
- Transcription-Activator Like Effectors (TALEs) 306
- Transcription-Activator Like Effector Nucleases (TALENs) 304, 306–308, 310, 315, 318
- transcriptional regulatory networks (TRNs) 51
- transcriptomics 8, 259, 405, 413, 470, 589, 672, 673, 675, 690, 769, 771, 778, 837
- transformation-associated recombination (TAR) 662
- trans*-4-hydroxyproline (4-HYP) production 403, 426–427
- triacetic acid lactone (TAL) production 756–757
- triacylglycerols (TAGs) 673, 735, 736, 745, 747, 748, 751, 753, 754, 755, 756
- triacylglycerols production
- Y. lipolytica*
  - cytosolic acetyl-CoA availability 753–754
  - desaturation of fatty acyl chains 747–748
  - lower raw substrate cost 749–753
  - pathway yield through balancing redox cofactors 748–749
  - push-and-pull strategy 747
- Triticum aestivum* 806
- tricarboxylic acid (TCA) cycle 156, 472, 522, 703

- tricarboxylic citric acid cycle (TCA) 4, 101, 110, 124, 156, 229, 233, 362, 407, 413, 419, 421, 426, 427, 472, 403, 406, 674, 703, 704, 705, 739, 742, 743, 750, 751
- Trichoderma reesei* 767, 771, 781  
enzyme set for plant polysaccharide degradation 773  
growth on cellulose 774  
overexpression of hybrid transcription factor in 774  
2,4,5-trimethyl-1,3-dioxolane (TMDX) 284
- triple-quadrupole (QQQ) instruments 271
- Triticum aestivum* 806
- tRNAs 147, 473, 712
- type I-B CRISPR systems 624
- type II CRISPR system 620, 624
- tyrosinase (TYR) 370, 382, 383, 778
- L-tyrosine 365, 366, 369, 370, 379, 419, 707–710
- tyrosine-derived cyanogenic glucoside dhurrin (D-glucopyranosyl-oxy-(S)-*p*-hydroxymandelonitrile) 824–825
- u**
- ubiquinone 829
- UDP-Glucose 380, 435, 498, 588, 824, 825
- UDP-glucosyltransferase mediated de-toxification 824
- UDP-N-acetyl-glucosamine 497
- UDPG-glucosyltransferase *SbUGT85B1* 825
- unfolded protein response (UPR) 719
- univariate intervals 89
- Universal Method 199, 200, 564
- unlabeled isotopomer 94
- unsteady FBA (uFBA) 27
- untargeted metabolomics 260–261, 268, 272–278, 284, 285
- untargeted MS data processing  
annotation 276–277  
missing values 274
- normalization approach 275
- peak alignment and retention time correction 274
- peak grouping 274
- peak picking 273–274
- preprocessing step 273
- upper glycolysis in *S. cerevisiae* 139–140, 142
- upregulated anthocyanin synthesis 818–819
- uracil-phosphoribosyltransferase (UPRTase) gene *upp* 479, 560
- uridine diphosphate-glucuronic acid 497, 498
- v**
- valerolactam 357, 363, 534
- L-valine 364, 365, 405, 419, 431
- vanilla orchid 824, 834, 835
- Vanilla planifolia* 824, 834
- vanillin 417, 588, 835, 837
- vegetative SigA-type promoter 477
- very-long-chain fatty acid (VLCFA)-derived chemicals 702
- violacein 404, 423, 433
- Viridiplantae 806
- virus like particles (VLPs), in *S. cerevisiae* 721
- vitamin E 818, 825, 826, 828
- w**
- waste-free circular economy 867
- weighted sum of squared residual (SSR) 86, 89, 121, 527
- wheat 571, 806, 809
- whole-cell biocatalysis 519, 520, 524, 589
- x**
- XlnR transcriptional regulator 778
- xylan production 814–816
- xylo-oligosaccharides (XOSs) 412, 414, 415
- xylose 408  
inducible *PxylA* 486  
inducible promoter *PxylA* 488, 627

**y**

*Yarrowia lipolytica* 15, 285  
 applications 736  
 $\beta$ -carotene production 757–759  
*cytosolic acetyl-CoA* 753–754  
 eicosapentaenoic acid production 755–756  
 genetic engineering tools for 737–738  
 genotypic features 735  
 homologous recombination mechanism 737  
 lactose as carbon source 752  
 NHEJ mechanism 737  
 opportunities and challenges 759–760  
 phenotypic features 735  
 proteases secretion 735  
 short-chain organic acid production 738  
 citrate 738–740  
 pyruvate and  $\alpha$ -ketoglutarate 740–741  
 succinate 743–745

triacetic acid lactone (TAL) production 756–757  
 triacylglycerol production 746, 747–748, 750  
*de novo* biosynthesis 745–746  
 cytosolic acetyl-CoA availability 753–754  
 desaturation of fatty acyl chains 747–748  
 lower raw substrate cost 749–753  
 pathway yield through balancing redox cofactors 748–749  
 push-and-pull strategy 747  
 yeast 310–313  
*S. cerevisiae* 689  
*Y. lipolytica* 735  
 yeast tryptophan synthesis 197–198  
 $\gamma$ -genes 36  
 yellow fluorescent protein (YFP) 632

**z**

*Zea mays* 806  
 zinc-finger nucleases (ZFNs) 304–306, 307, 308, 310, 318, 320



















