Evaluating proteome allocation of *Saccharomyces cerevisiae* phenotypes with resource balance analysis – Supplementary Text 1

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# Materials and Methods

## RBA Model Reconstruction

The model *sc*RBA consists of (macro)molecules and reactions for metabolism and cellular machinery production linked through steady-state mass balance constraints as in FBA (48) (see Fig. 1a for a schematic representation). Here, we briefly explain the model elements linking (macro)molecules and reactions. The reconstruction method is described in detail in the Supplementary Text 2, with user instructions, formulation (adapted from Goelzer et al., 2011 (49)), and indexing. Metabolites and metabolic reactions are ported from *iSace*1144 (available at [https://github.com/maranasgroup/iSace\_GSM](https://github.com/maranasgroup/iSace_GSMd)). Blocked reactions identified by flux variability analysis (50) were excluded from *sc*RBA. Materials and energy required for macromolecules synthesis are encoded in the stoichiometric coefficients of protein translation, rRNA synthesis, ribosome synthesis, and enzyme formation reactions (see Supplementary Text 2). Separate requirements of nuclear and mitochondrial ribosomes in *S. cerevisiae* (51) to translate proteins encoded in the respective genomes are accounted for in the model. Biomass precursor producing reactions are included in *sc*RBA to inventory all enzymes, ribosomes, and all other macromolecules needed to form biomass. Precursors (and their relative compositions) of DNA, lipids, carbohydrates, metal ions, sulphate, phosphate, and cofactors are ported from the GSM model *iSace*1144. Based on experimental macromolecular measurements (36, 52–54) the mass fractions of most macromolecules are assumed to remain invariant except for protein, RNA, and carbohydrate fractions that change with increasing growth rate (see Supplementary Data 5). Instead of reconstructing multiple models with different biomass coefficients at different growth rates, the biomass reaction is recast as a set of precursor sink reactions whose fluxes are equal to the coefficients multiplied by the growth rate. We ensure that the biomass molecular weight is always 1 g mmol-1 so that growth yield and rate predictions are consistent (55).

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**Fig. 1.** (a) Schematic representation of the *sc*RBA model (macro)molecular participants and reactions. (b) Overview of bisection method and the RBA linear programming (RBA-LP) formulation that are solved iteratively to obtain the maximal growth rate. Flux variables are highlighted in red and the growth rate variable is highlighted in green. The topology of all *sc*RBA model captured variables are shown in Fig. 1a. Model parameters are briefly explained in the text and formulation details are available in the Supplementary Text 2.

The total amount of protein, enzyme, and ribosome produced is determined by the reaction‑enzyme and protein‑ribosome coupling constraints and limited by the protein and rRNA capacity constraints (see Fig. 1b and Supplementary Text 2). The kapp parameter values in the reaction‑enzyme coupling constraint are derived from experimental flux and proteomics data (see “Estimation of *in vivo* kapp” in Methods). In the protein-ribosome coupling constraint, the protein sequence length values () are taken from the SGD database (15) and the ribosome efficiency parameter (kribo) is fitted using growth phenotype data (37). From the experimentally derived (56) average value of 10.5 amino acids elongated per ribosome per second, we re‑parameterized the kribo value by successively increasing it from 10.5 in increments of 0.1 until the predicted growth rate matched the highest reported experimental value of 0.49 h‑1 (in rich media) (37). This was met for a slightly higher value of kribo of 13.2 amino acids per ribosome per second. In the *sc*RBA model, enzyme and ribosome production is limited by the experimentally measured protein and rRNA levels (36, 52–54) through capacity constraints. Molecular weights of protein and rRNA (i.e., and ) are used to convert molar amounts to grams which are set to less than the experimentally found limits (i.e., and ) in the capacity constraints. Production of proteins locating in mitochondria are also limited to a mass fraction (i.e., ) of the total proteome capacity. For glucose uptake conditions the reported mitochondria per cell volume/volume ratio of 5% (57) is used. This 5% threshold for mitochondrial proteins could be mechanistically explained as reflecting membrane surface area and inner volume limits for mitochondrial metabolic pathways (e.g., TCA cycle and electron transport chain) and ribosomes.

*sc*RBA directly accounts for only proteins participating in metabolism and translation elongation. Proteins involved in other processes such as protein folding chaperone and cellular maintenance are not functionally part of *sc*RBA. We assumed that the modeled proteome including metabolic and ribosomal proteins is 55% of the total proteome (26). The cost of producing the remaining 45% is accounted for in an aggregate manner assuming average amino acid composition (53). In resource allocation models this is formulated by adding a reaction producing a non-functional so-called “dummy” protein (26, 58). The model accounts explicitly the six rRNA species that are part of ribosomes (see Supplementary Text 2 for details) which constitute as much as 80% of total RNA (59). For computational efficiency, mRNA and tRNA demand (i.e., reserving 5% and 15% of total RNA, respectively) are accounted for in an aggregate manner assuming average composition (22). Similar to the proteome, a reaction producing a non-functional RNA is added to the model. *S. cerevisiae* maintains reserved ribosome (56) and enzyme (60) capacity which makes up the difference between experimentally observed and required amounts (estimated under nitrogen limited conditions (56, 60)). The reserved proteome capacity is maintained to prepare cells for changes in growth conditions in the same manner that reserved ribosome capacity enables faster growth immediately upon nutrient availability upshift (56, 60). In model, the amounts of non-functional protein and RNA representing reserved capacity are treated as fitted variables so as to recapitulate reserved capacity being present or exhausted depending on growth conditions.

## Estimation of ATP Maintenance Rates

ATP maintenance rates are parameters used in both FBA and RBA model to account for the energy cost of replicating cells. Growth-associated ATP maintenance (GAM) (in mmol gDW-1) rate captures the energy demand per unit of produced biomass. Non-growth associated ATP maintenance (NGAM) (in mmol gDW-1 h-1) rate captures the energy demand associated with cellular processes such as repair and maintenance (61). GAMFBA (i.e., GAM in FBA model) and NGAM parameters were regressed from growth phenotype datasets recorded at different growth rates using the FBA model *iSace*1144. For every dataset, ATP maintenance rate was estimated by maximizing flux through the ATP hydrolysis reaction (i.e., ) subject to experimentally measured extracellular fluxes and growth rate (see Supplementary Data 1). NGAM parameter was equal to the maximal ATP hydrolysis flux estimated from growth-arrested data (52, 62, 63). GAMFBA is the slope of a linear regression of maximal ATP hydrolysis flux vs. growth rate values whereas the intercept is the NGAM value. GAMRBA (i.e., GAM in RBA model) is estimated by subtracting from GAMFBA value the portion equivalent to protein translation elongation’s energy cost, which is approximately 2 mmol of ATP per mmol of amino acid (26). The subtracted amount is 7.6 mmol ATP gDW‑1, derived from experimental amino acid measurements (53). The NGAM parameter is estimated from growth-arrested data where neither biomass nor protein synthesis is underway and thus the parameter is the same for both FBA and RBA. Different GAMFBA and NGAM parameter sets were regressed from datasets under the following growth conditions: (i) (nutrient-abundant) batch and anaerobic or microaerobic, (ii) C-limited chemostats and anaerobic or microaerobic, (iii) batch or C‑limited chemostats and aerobic, (iv) N-limited chemostats and aerobic. Experimental flux inputs and calculated results are recorded in Supplementary Data 2.

## Estimation of in vivo kapp

kapp was calculated by dividing estimated intracellular metabolic fluxes by experimental enzyme concentrations (64) (see Supplementary Text 2 for the workflow and Supplementary Data 2 for details). From literature-reported data (36–39), different kapp parameter sets were determined for growth conditions under (i) (nutrient-abundant) batch/glucose, (ii) batch/galactose, (iii) batch/maltose, (iv) batch/trehalose, (v) C-limited chemostats/glucose D = 0.1 h-1 and (vi) D = 0.3 h-1, and (vii) N-limited chemostats/glucose D = 0.1 h-1.

## Growth Maximization and Predicted Yield using scRBA and FBA

An overview of the RBA optimization model that identifies the maximal growth rate is provided in Fig. 1b. By fixing the growth rate the *sc*RBA model is converted into a linear programming formulation (i.e., RBA-LP) which can be efficiently solved. An iterative method is employed to converge the upper (infeasible) and lower (feasible) bounds on growth rate within a tolerance criterion of 10-5 h-1.

Maximal compound production rate was identified by maximizing the corresponding (sink) exchange reaction flux variable subject to a glucose uptake of 13.2 mmol gDW-1 h-1 (37) and growth rate set at a minimum of 0.1 h-1. Hexadecanoic acid and hexadecanol (i.e., C16) were used as proxies in model for *in vivo* mixture of free fatty acids and fatty alcohols of different chain lengths, respectively. Heterologous pathways for the synthesis of tested compounds were reconstructed based on previous studies (see “Information on Synthesis for 28 Products” section for details), as necessary. The RBA-predicted maximal production yield (i.e., , in g g-Glucose-1) was calculated using the following equation:

|  |  |
| --- | --- |
|  | (Eq. 1) |

where is the RBA-predicted maximal production rate, is the product molecular weight, is the RBA-predicted glucose uptake rate, and is the molecular weight of glucose. FBA-calculated maximal production yield (i.e., ) was determined using Eq. 1 but with FBA-predicted rather than RBA-predicted quantities. Fluxes are in mmol gDW-1 h-1 and molecular weights are in g mmol-1.

## Software Implementation

COBRApy (65) with IBM ILOG CPLEX solver (version 12.10.0.0) were used for FBA model optimization. The FBA model *iSace*1144 is available at <https://github.com/maranasgroup/iSace_GSM> (66). General Algebraic Modeling System (GAMS) programming language (version 39.1.0, GAMS Development Corporation) with Soplex solver (version 6.0) (67) was used for RBA model kapp parameterization and optimization. Input files as excel spreadsheets were used to build the RBA model in GAMS format. Python 3.6 was used as the central platform to run all mentioned processes. All scripts and input and output files are available in the GitHub repository <https://github.com/maranasgroup/scRBA>.

# List of Carbon-limited and Batch Conditions Datasets in the Fig. 5 of the Main Text

Datasets are from various studies (1–15).

|  |  |  |  |
| --- | --- | --- | --- |
| **References** | **Glucose**  (mmol gDW‑1 h-1) | **Growth**  (h-1) | **Ethanol**  (mmol gDW-1 h-1) |
| Lahtvee et al., 2017 (15) | 1.2305 | 0.1 | 0 |
| Yu et al., 2020 (3) | 2.439 | 0.2 | 0 |
| Yu et al., 2021 (1) | 1.254 | 0.1 | 0 |
| Elsemman et al., 2022 (5) | 2.40625 | 0.199 | 0 |
| Elsemman et al., 2022 (5) | 2.78498 | 0.227 | 0 |
| Elsemman et al., 2022 (5) | 3.338685 | 0.2705 | 0 |
| Elsemman et al., 2022 (5) | 4.8804 | 0.3012 | 2.2441 |
| Bjorkeroth et al., 2020 (2) | 13.2000 | 0.4236 | 15.98 |
| Elsemman et al., 2022 (5) | 13.4617 | 0.371 | 18.12 |
| Postma et al., 1989 (9) | 2.775 | 0.25 | 0 |
| Postma et al., 1989 (9) | 3.33 | 0.3 | 0 |
| Postma et al., 1989 (9) | 3.552 | 0.32 | 0 |
| Postma et al., 1989 (9) | 3.738 | 0.33 | 0 |
| Verduyn et al., 1990 (10) | 16 | 0.47 | 16.5 |
| Bakker et al., 2000 (11) | 15.7 | 0.38 | 22.3 |
| Boer et al., 2003 (12) | 1.1 | 0.1 | 0 |
| Vemuri et al., 2007 (13) | 1.12 | 0.1 | 0 |
| Vemuri et al., 2007 (13) | 3.27 | 0.27 | 0 |
| Jewett et al., 2013 (14) | 0.604 | 0.0515 | 0 |
| Kumar et al., 2021 (4) | 1.5542 | 0.12 | 0 |
| Kumar et al., 2021 (4) | 3.2749 | 0.26 | 0 |
| Kummel et al., 2010 (7) | 22.43 | 0.46 | 36.09 |
| Heyland et al., 2009 (6) | 20.2 | 0.4 | 30 |
| Vos et al., 2016 (8) | 0.039 | 0 | 0 |

# Information on Synthesis Pathways of 28 products

**Table 1.** FBA-predicted, RBA-predicted, and literature-reported experimental yields for 28 products

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Product** | **Precursors** |  | **\*\*** | | ## | **Pathway & yield reference** |
| **w/o MP** | **w/ MP** |
| Free fatty acid | Fatty acyl-CoA | 0.30 | 0.26 | 0.26 | 0.1 | (16) |
| Fatty alcohol | Fatty acyl-CoA | 0.27 | 0.24 | 0.24 | 0.005 | (17) |
| Triacylglycerol++ | Triacylglycerol | 0.29 | 0.26 | 0.26 | 0.14 | (18) |
| Citramalate | Acetyl-CoA and pyruvate | 0.77 | 0.77 | 0.77 | N/A | (19) |
| n-Butanol | (Acetyl-CoA and pyruvate) or l-threonine | 0.35 | 0.32 | 0.32 | 0.04 | (19) |
| Polyhydroxybutyrate | Acetoacetyl-CoA | 0.54 | 0.52 | 0.52 | 0.13 | (20) |
| Artermisinic acid | Farnesyl diphosphate | 0.28 | 0.23 | 0.23 | N/A | (21) |
| Sesquiterpene | Farnesyl diphosphate | 0.31 | 0.26 | 0.26 | 0.003 | (22, 23) |
| Ethanol++ | Ethanol | 0.49 | 0.48 | 0.48 | 0.44 | (24) |
| l-Lactate | Pyruvate | 0.91 | 0.90 | 0.90 | 0.69 | (25) |
| (2R,3R)-Butanediol | Pyruvate | 0.49 | 0.49 | 0.49 | 0.11 | (26) |
| **Isobutanol** | **Isobutanol** | **0.39** | **0.34** | **0.05** | **0.06** | (27) |
| 3-Hydroxypropionic acid | Malonyl-CoA | 0.76 | 0.69 | 0.69 | 0.13 | (28) |
| **3-Hydroxypropionic acid** | **l-Alanine** | **0.84** | **0.81** | **0.49** | **0.14** | (29) |
| Succinate++ | Succinate | 0.93 | 0.20 | 0.20 | 0.11 | (30, 31) |
| **Malate++** | **Malate** | **1.18** | **1.16** | **1.07** | **0.31** | (32) |
| Shikimate++ | Shikimate | 0.71 | 0.70 | 0.70 | N/A | (33) |
| cis,cis-Muconate | 3-Dehydroshikimate | 0.61 | 0.60 | 0.60 | 0.07 | (34) |
| **p-Hydroxybenzoate** | **Chorismate** | **0.52** | **0.51** | **0.50** | **0.07** | (35) |
| **p-Aminobenzoate++** | **p-Aminobenzoate** | **0.50** | **0.42** | **0.41** | **0.01** | (36) |
| 2-Phenylethanol | l-Phenylalanine | 0.36 | 0.35 | 0.35 | 0.08 | (37) |
| Styrene | l-Phenylalanine | 0.30 | 0.29 | 0.29 | 0.001 | (38) |
| p-Coumaric acid | l-Tyrosine | 0.49 | 0.48 | 0.48 | 0.15 | (39) |
| Reticuline | l-Tyrosine | 0.35 | 0.24 | 0.24 | N/A | (40) |
| (2S)-Naringenin | l-Tyrosine and malonyl-CoA | 0.45 | 0.43 | 0.43 | N/A | (41) |
| Resveratrol | l-Tyrosine and malonyl-CoA | 0.38 | 0.36 | 0.36 | 0.0002 | (42) |
| Glycerol++ | Glycerol | 0.77 | 0.75 | 0.75 | 0.5 | (43) |
| **1,3-Propanediol** | **Glycerol** | **0.57** | **0.49** | **0.47** | **N/A** | (44) |

Optimization constraints: growth rate = 0.1 h-1, glucose uptake rate’s upper bound = 13.2 mmol gDW-1 h-1 (referencing experimental value(2)). and (in g g‑Glucose-1): FBA- and RBA-calculated maximal theoretical yields. : literature-reported experimental yields.

\*\*: Separate RBA yield values were calculated with mitochondrial proteome capacity constraint included and excluded (i.e., labeled with “w/ MP” and “w/o MP”, respectively). Rows are in bold-face if calculated yields with mitochondrial proteome constraint are different than the ones without.

++: These metabolites are produced with native pathways

##: “N/A”, or not available, is designated to metabolites with experimental studies where only titers were reported. Citramalate and shikimate were intermediates for other terminal metabolite productions and thus their yields were not reported. A higher yield of 0.43 g-succinate g-glucose-1 was reported for a laboratory-evolved strain.

# Effect of Protein Capacity Limit on Metabolic Fluxes

## Methods

In analogy to flux variability analysis (FVA) (45), lower and upper bounds of reaction fluxes can be calculated using *sc*RBA and FBA models by updating the objective function of the model to the minimization or maximization of the flux in question and imposing the experimental glucose uptake rate of 13.2 mmol gDW-1 h-1 and growth rate of 0.42 h-1 (2). Experimental (absolute) glucose uptake and growth rates were used in the simulations to be consistent with model parameters derived from absolute flux and concentration measurements. Flux ranges under FBA and RBA are contrasted to elucidate the role of capacity constraints on the flux allocation flexibility.

## Results

Enzyme(s) availability bottlenecks can add additional barriers to reaching FBA calculated maximum theoretical limits. Identifying these yield-limiting enzymes is important so as to guide specific gene overexpression strategies remedying these shortcomings without wasting resources on enzymes that are not limiting. To this end, we contrasted the calculated flux bounds (i.e., FVA analysis) using model *sc*RBA (with kapp parameters for batch aerobic conditions typically used in compound production) and model *iSace*1144 using FBA. RBA/FBA absolute upper bound flux ratios were calculated for 800 flux-carrying metabolic reactions under glucose uptake conditions. We performed RBA runs with and without the mitochondrial proteome capacity constraint (i.e., limiting to 5% of total proteome capacity) and contrasted results to pinpoint mitochondrially originated metabolic limitations (see Fig. 1 for a summary and see Supplementary Data 6 for details).

Diagram

Description automatically generated with medium confidence

**Fig. 1.** *sc*RBA model’s metabolic flux variability analysis accounting for protein capacity limit. (a) Histogram of RBA/FBA flux upper bound ratio values. (b) RBA- (white and striped bars) and FBA-calculated (black bars) flux ranges for reactions in central metabolism subject to experimental glucose uptake and growth rates (2). Reaction IDs are in BiGG format (46) and reaction details are available in the *sc*RBA github repository. (c) Central metabolism network (drawn by the Escher software (47)) with overlayed reaction IDs and corresponding RBA/FBA flux upper bound ratio values (annotated as colors of arrows) for RBA runs with mitochondrial proteome constraint. The figure inset shows much higher flux upper bounds for TCA cycle reactions from RBA runs without the mitochondrial proteome constraint.

Under only total proteome and rRNA capacity limitations, RBA/FBA ratios are less than 20% for as many as 516 out of 800 flux-carrying reactions (see Fig. 1a). This indicates that catalytic resource limitation as encoding in model *sc*RBA are propagated to most reactions in the metabolic network. In central metabolism, FBA (through FVA analysis) allows for maximal glycolysis and pentose phosphate pathway (PPP) fluxes that are up to an order of magnitude larger than the glucose uptake rate (i.e., 13.2 mmol gDW-1 h-1) (see Fig. 1b). These very high fluxes are caused by activating ATP-consuming cycles. For example, the FBA-calculated maximal flux of phosphofructosekinase (ID: PFK\_c) reaction in glycolysis is 225 mmol gDW-1 h‑1 which contains an ATP-consuming cycle (i.e., 95% of total flux) with fructose bisphosphate phosphatase reaction in gluconeogenesis. These cycles are retained in FBA because without any additional constraints extra glucose can be used to produce ATP at a yield of up to 25.6 mol ATP / mol glucose. Imposing the total proteome capacity constraint in RBA greatly reduce the extent of ATP-consuming cycles (see Fig. 1b and 6c). For example, the RBA-calculated PFK\_c maximal flux is 27.6 mmol gDW-1 h-1 which is an order of magnitude smaller than the FBA-calculated one.

Adding the mitochondrial proteome capacity constraint significantly reduces the RBA/FBA flux upper bound ratios of the TCA cycle reactions from 80% to 6% (see Fig. 1c) and of electron transport chain (ETC) from 99% to 24%. FBA-predicted TCA and ETC fluxes are much higher than RBA-predicted values because of the readily available precursors acetyl-CoA and NADH (respectively) synthesized from the glucose uptake surplus. Reduced mitochondrial protein capacity for respiration leads to lowered ATP availability and ultimately constrains ATP-consuming flux cycles reflected in the significantly lower RBA/FBA flux upper bound ratios for many reactions across the metabolic network (see Fig. 1a). Overall, the RBA framework implementing total proteome capacity constraint provides flux predictions with large reductions for fluxes that can participate in futile ATP-consuming cycles. Implementing mitochondrial proteome capacity constraints has a direct and dramatic impact on the maximal fluxes of mitochondrial enzymes and an indirect effect on non-mitochondrial enzymes constrained by ATP availability.

Fluxes of the six non-cycling glycolysis reactions are well resolved through FBA as they are fully coupled to the pre-specified glucose uptake (see Fig. 1c). Counterintuitively, their upper bounds are slightly higher using RBA (with or without the mitochondrial proteome capacity constraint) than using FBA by about 1.5 – 1.8% (see Fig. 1c). This is because a slightly higher glycolysis/pentose phosphate pathway (PPP) split ratio for a given glucose uptake is predicted by optimizing NADPH usage in the *sc*RBA model. The lower flux through the NADPH-generating PPP is due to the fact that the actual NADPH needs for amino acid synthesis (accounting in detail by RBA) is slightly less than the lumped amount of the stoichiometric description used in FBA.

## Discussions

We found that a significant fraction of *S. cerevisiae* reactions has an upper bound that is set correctly at a much lower value by *sc*RBA due to the mitochondrial and total proteome allocation limits. Despite this *sc*RBA-predicted product yield calculations are generally only marginally lower than the FBA-based theoretical limit. This is primarily due to the fact that most predicted upper bound excursions by FBA are associated with reactions that can participate in ATP-consuming cycles. While this ATP overhead is tolerated in FBA calculations by draining from a large glucose surplus, the associated increased proteome allotments are severely curtailed in RBA calculations. Because by design product synthesis pathways do not involve futile cycles, reductions in the predicted maximum yields under RBA are not significant.

# References

1. R. Yu, E. Vorontsov, C. Sihlbom, J. Nielsen, Quantifying absolute gene expression profiles reveals distinct regulation of central carbon metabolism genes in yeast. *Elife* **10** (2021).

2. J. Björkeroth, *et al.*, Proteome reallocation from amino acid biosynthesis to ribosomes enables yeast to grow faster in rich media. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 21804–21812 (2020).

3. R. Yu, *et al.*, Nitrogen limitation reveals large reserves in metabolic and translational capacities of yeast. *Nat. Commun. 2020 111* **11**, 1–12 (2020).

4. K. Kumar, V. Venkatraman, P. Bruheim, Adaptation of central metabolite pools to variations in growth rate and cultivation conditions in *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **20** (2021).

5. I. E. Elsemman, *et al.*, Whole-cell modeling in yeast predicts compartment-specific proteome constraints that drive metabolic strategies. *Nat. Commun. 2022 131* **13**, 1–12 (2022).

6. J. Heyland, J. Fu, L. M. Blank, Correlation between TCA cycle flux and glucose uptake rate during respiro-fermentative growth of *Saccharomyces cerevisiae*. *Microbiology* **155**, 3827–3837 (2009).

7. A. Kümmel, *et al.*, Differential glucose repression in common yeast strains in response to HXK2 deletion. *FEMS Yeast Res.* **10**, 322–332 (2010).

8. T. Vos, *et al.*, Maintenance-energy requirements and robustness of *Saccharomyces cerevisiae* at aerobic near-zero specific growth rates. *Microb. Cell Fact.* **15** (2016).

9. E. Postma, C. Verduyn, W. A. Scheffers, J. P. Van Dijken, Enzymic analysis of the crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **55**, 468–477 (1989).

10. C. Verduyn, E. Postma, W. A. Scheffers, J. P. Van Dijken, Physiology of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures. *J. Gen. Microbiol.* **136**, 395–403 (1990).

11. B. M. Bakker, *et al.*, The mitochondrial alcohol dehydrogenase Adh3p is involved in a redox shuttle in *Saccharomyces cerevisiae*. *J. Bacteriol.* **182**, 4730–4737 (2000).

12. V. M. Boer, J. H. De Winde, J. T. Pronk, M. D. W. Piper, The genome-wide transcriptional responses of *Saccharomyces cerevisiae* grown on glucose in aerobic chemostat cultures limited for carbon, nitrogen, phosphorus, or sulfur. *J. Biol. Chem.* **278**, 3265–3274 (2003).

13. G. N. Vemuri, M. A. Eiteman, J. E. McEwen, L. Olsson, J. Nielsen, Increasing NADH oxidation reduces overflow metabolism in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 2402–2407 (2007).

14. M. C. Jewett, *et al.*, Mapping condition-dependent regulation of lipid metabolism in *Saccharomyces cerevisiae*. *G3 Genes, Genomes, Genet.* **3**, 1979–1995 (2013).

15. P. J. Lahtvee, *et al.*, Absolute quantification of protein and mRNA abundances demonstrate variability in gene-specific translation efficiency in yeast. *Cell Syst.* **4**, 495-504.e5 (2017).

16. T. Yu, *et al.*, Reprogramming yeast metabolism from alcoholic fermentation to lipogenesis. *Cell* **174**, 1549-1558.e14 (2018).

17. Y. Hu, Z. Zhu, J. Nielsen, V. Siewers, Engineering *Saccharomyces cerevisiae* cells for production of fatty acid-derived biofuels and chemicals. *Open Biol.* **9** (2019).

18. S. Arhar, *et al.*, Engineering of *Saccharomyces cerevisiae* for the accumulation of high amounts of triacylglycerol. *Microb. Cell Fact.* **20**, 1–15 (2021).

19. S. Shi, *et al.*, Metabolic engineering of a synergistic pathway for n-butanol production in *Saccharomyces cerevisiae*. *Sci. Reports 2016 61* **6**, 1–10 (2016).

20. K. Kocharin, Y. Chen, V. Siewers, J. Nielsen, Engineering of acetyl-CoA metabolism for the improved production of polyhydroxybutyrate in *Saccharomyces cerevisiae*. *AMB Express* **2**, 1–11 (2012).

21. D. K. Ro, *et al.*, Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* **440**, 940–943 (2006).

22. C. L. Liu, *et al.*, Metabolic engineering strategies for sesquiterpene production in microorganism. *Crit. Rev. Biotechnol.* **42** (2022).

23. G. Scalcinati, *et al.*, Combined metabolic engineering of precursor and co-factor supply to increase α-santalene production by *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **11**, 1–16 (2012).

24. J. Ruchala, O. O. Kurylenko, K. V. Dmytruk, A. A. Sibirny, Construction of advanced producers of first- and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (Scheffersomyces stipitis, Ogataea polymorpha). *J. Ind. Microbiol. Biotechnol.* **47**, 109–132 (2020).

25. V. Novy, B. Brunner, G. Müller, B. Nidetzky, Toward “homolactic” fermentation of glucose and xylose by engineered *Saccharomyces cerevisiae* harboring a kinetically efficient l-lactate dehydrogenase within pdc1-pdc5 deletion background. *Biotechnol. Bioeng.* **114**, 163–171 (2017).

26. C. Y. Ng, M. Y. Jung, J. Lee, M. K. Oh, Production of 2,3-butanediol in *Saccharomyces cerevisiae* by *in silico* aided metabolic engineering. *Microb. Cell Fact.* **11** (2012).

27. J. Wess, M. Brinek, E. Boles, Improving isobutanol production with the yeast *Saccharomyces cerevisiae* by successively blocking competing metabolic pathways as well as ethanol and glycerol formation. *Biotechnol. Biofuels* **12** (2019).

28. K. R. Kildegaard, *et al.*, Engineering and systems-level analysis of *Saccharomyces cerevisiae* for production of 3-hydroxypropionic acid via malonyl-CoA reductase-dependent pathway. *Microb. Cell Fact.* **15**, 1–13 (2016).

29. I. Borodina, *et al.*, Establishing a synthetic pathway for high-level production of 3-hydroxypropionic acid in *Saccharomyces cerevisiae* via β-alanine. *Metab. Eng.* **27**, 57–64 (2015).

30. A. M. Raab, G. Gebhardt, N. Bolotina, D. Weuster-Botz, C. Lang, Metabolic engineering of *Saccharomyces cerevisiae* for the biotechnological production of succinic acid. *Metab. Eng.* **12**, 518–525 (2010).

31. J. M. Otero, *et al.*, Industrial Systems Biology of *Saccharomyces cerevisiae* Enables Novel Succinic Acid Cell Factory. *PLoS One* **8**, e54144 (2013).

32. R. M. Zelle, *et al.*, Malic acid production by *Saccharomyces cerevisiae*: Engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. *Appl. Environ. Microbiol.* **74**, 2766–2777 (2008).

33. N. J. H. Averesch, J. O. Krömer, Metabolic engineering of the shikimate pathway for production of aromatics and derived compounds-Present and future strain construction strategies. *Front. Bioeng. Biotechnol.* **6**, 32 (2018).

34. G. Wang, *et al.*, Improvement of cis, cis-Muconic Acid Production in *Saccharomyces cerevisiae* through Biosensor-Aided Genome Engineering. *ACS Synth. Biol.* **9**, 634–646 (2020).

35. N. J. H. Averesch, A. Prima, J. O. Krömer, Enhanced production of para-hydroxybenzoic acid by genetically engineered *Saccharomyces cerevisiae*. *Bioprocess Biosyst. Eng.* **40**, 1283–1289 (2017).

36. N. J. H. Averesch, G. Winter, J. O. Krömer, Production of para-aminobenzoic acid from different carbon-sources in engineered *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **15**, 1–16 (2016).

37. E. J. Hassing, P. A. de Groot, V. R. Marquenie, J. T. Pronk, J. M. G. Daran, Connecting central carbon and aromatic amino acid metabolisms to improve de novo 2-phenylethanol production in *Saccharomyces cerevisiae*. *Metab. Eng.* **56**, 165–180 (2019).

38. R. McKenna, B. Thompson, S. Pugh, D. R. Nielsen, Rational and combinatorial approaches to engineering styrene production by *Saccharomyces cerevisiae*. *Microb. Cell Fact.* **13** (2014).

39. Q. Liu, *et al.*, Rewiring carbon metabolism in yeast for high level production of aromatic chemicals. *Nat. Commun. 2019 101* **10**, 1–13 (2019).

40. W. C. Deloache, *et al.*, An enzyme-coupled biosensor enables (S)-reticuline production in yeast from glucose. *Nat. Chem. Biol.* **11**, 465–471 (2015).

41. S. Gao, *et al.*, Efficient biosynthesis of (2S)-naringenin from p-coumaric acid in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **68**, 1015–1021 (2020).

42. M. Li, *et al.*, De novo production of resveratrol from glucose or ethanol by engineered *Saccharomyces cerevisiae*. *Metab. Eng.* **32**, 1–11 (2015).

43. K. M. Overkamp, *et al.*, Metabolic engineering of glycerol production in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **68**, 2814–2821 (2002).

44. Z. Rao, *et al.*, Engineered *Saccharomyces cerevisiae* that produces 1,3-propanediol from D-glucose. *J. Appl. Microbiol.* **105**, 1768–1776 (2008).

45. R. Mahadevan, C. H. H. Schilling, The effects of alternate optimal solutions in constraint-based genome-scale metabolic models. *Metab. Eng.* **5**, 264–276 (2003).

46. Z. A. King, *et al.*, BiGG Models: A platform for integrating, standardizing and sharing genome-scale models. *Nucleic Acids Res.* **44** (2016).

47. Z. A. King, *et al.*, Escher: A Web Application for Building, Sharing, and Embedding Data-Rich Visualizations of Biological Pathways. *PLOS Comput. Biol.* **11**, e1004321 (2015).