Evaluating proteome allocation of *Saccharomyces cerevisiae* phenotypes with resource balance analysis – Supplementary Text 2

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# 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 |

# 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. S2.1.** *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.

# Supplementary Figure for estimation of *in vivo* apparent kapp values

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**Fig. S2.2.** Distribution of estimated *in vivo* kapp ratios between perturbed and reference conditions. Distributions are visualized using standard box plots (i.e., box: interquartile range (IQR), whiskers: 1.5\*IQR, and dots: outliers). The value of “n” indicates the number of overlapping reactions of the respective two sets of reactions whose kapp can be estimated from available flux and protein measurements.

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