Population FBA Predicts Metabolic Phenotypes in Yeast

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A1 Supplementary information

A1.1 Extended Methods: Metabolic Model and Experimental Data

The Yeast Metabolic Model 7.6 [1] was chosen for this study because it is the most complete and up-to-date model available, and because it was shown to have equal or better prediction power when compared to other yeast models [2] (for a comparison between biomass pseudo-reactions from recent models, see SI Table A8). Due to the presence of knockouts in the strain used in the SD medium experiments (namely the proteomics and growth rate distribution experiments on which this work relies [3,4]), when simulated under these conditions the model was changed accordingly. The strain in question is BY4741 (or ATCC 201388) with the following genotype: MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0. In our model, the genes YCL018W, YLR303W, YEL021W were inactivated, leading to zero flux being allowed through five reactions: 3-isopropylmalate dehydrogenase (r_0061), cysteine synthase (r_0312), O-acetylhomoserine (thiol)-lyase (r_0812, and r_0813) and orotidine-5-phosphate decarboxylase (r_0821). The histidine biosynthesis knockout is recovered when GFP is tagged to any protein, so the gene YOR202W was kept active.

The SD medium used contained 20 g/L glucose. The medium composition was taken from Sigma-Aldrich, Ref. #Y0626 and #Y1751. Uptake rates were obtained from the literature, searching conditions that closely matched the 30°C temperature and histidine dropout SD medium of the experiments [5]. Special attention was given to the glucose uptake rate, since there is extensive regulation of this cellular process which leads to different transporters being expressed and, consequently, different uptake rates depending on extra cellular glucose concentration. We set the uptake bound for glucose at 15 mmol gDwt⁻¹ hr⁻¹. For all other components of the medium, the uptake rate was set based on experimental values when available, or it was set to the highest experimental amino acid uptake rate. Table A1 shows the maximum uptake rate for all components available in the medium.

The turnover rates (kcat), for enzymes which have predicted copy numbers and that are found in the metabolic model yeast 7.6 were gathered from BRENDA database [6,7] using the SOAP-Python web interface or from literature (See Table A3). The highest
$k_{cat}$ was always selected, preferably from experiments expressing wild type *S. cerevisiae* proteins, but when one was not available, mutants and other species were allowed. Finally, for proteins which did not have a turnover rate in BRENDA, the highest available for a wild-type yeast enzyme, 38,000, was chosen so as to avoid over-constraining the model.

### A1.2 Noise Properties of Proteins in *S. cerevisiae*

Variability in protein copy number has been investigated previously in single cell fluorescence studies of yeast and *E. coli* [8,9]. Both studies observed two distinct regimes of noise behavior based on the mean copy number. Noise in low protein copy number proteins dropped hyperbolically with mean copy number; this noise has been described as intrinsic noise arising from the inherent stochastic nature of gene expression. Higher copy number proteins exhibit an approximately constant level of noise; this plateau is labeled associated with extrinsic sources arising from variability in common factors involved in gene expression like RNA polymerase or ribosomes. We observe similar behavior in noise measured by Dé fervaud et. al., (Fig A1). Proteins with mean copy numbers below approximately 1,000 show a decrease in their noise with mean copy number while proteins expressed at levels higher than approximately 1,000 have fairly constant noise.

### A1.3 Reliability of mRNA Microarray Correlation Data

We considered two ways of determining the reliability of the mRNA microarray-based correlation data we used. The first was to determine if the correlations exhibit discernible behavioral traits, such as anti-correlation between genes associated with fermentation and respiration. To see if this was reflected in the correlation data, we considered the glucose transporter HXT1. As seen in Fig A11, we found negative correlations between HXT1 and several genes associated with the TCA cycle and oxidative phosphorylation. We also see positive correlation between HXT1 and ethanol fermentation genes, as would be expected in a Crabtree-positive yeast strain. The second way we evaluated the reliability of our correlation data was by comparing it to experimentally established regulatory links in yeast [10]. To perform this comparison, we devised a distance metric (Equation A1):

$$\text{Distance} = \sum_{i,j} \text{Regs}_{i,j}(1 - |\rho_{i,j}|) + \delta(\text{Regs}_{i,j})|\rho_{i,j}|$$  \hspace{1cm} (A1)

where $\text{Regs}_{i,j}$ represents the number of common transcription factors regulating genes $i$ and $j$, and $\delta(x)$ represents the Kronecker delta function (1 if the argument is 0, and 0 otherwise)

This metric is based on the idea that if two genes share a common transcription factor, their expression should be correlated, either positively or negatively depending on the regulatory relationship between transcription factor and the genes. The first term penalizes gene pairs with large numbers of shared transcription factors but weak correlation, while the second penalizes gene pairs with large correlation but no shared transcription factors. If the correlations generally reflect the known regulatory links, the distance metric will be smaller. To see if this is true we compared the distance metric obtained for correlations from actual expression data [11] with the distance metric obtained for correlations from randomized expression data. As seen in Fig A12 top, the distance metric for the actual expression data is significantly smaller than the distribution of distance metrics (Fig A12 bottom) obtained by randomizing expression data.
A1.4 Extended Methodology: Genetic Algorithm for Constraint Selection

Main Methods: A new procedure for filtering overly-constraining turnover rates based on the Micro Genetic Algorithm (GA) formalism was developed [12]. This method utilizes an entire growth distribution as a target for optimizing the selection of experimental constraints. Micro Genetic Algorithm was chosen instead of a “regular” Genetic Algorithm solely for computational cost concerns. In a “regular” GA algorithm in dozens to hundreds of genomes would have to be simulated at each generation, and several hundred generations could need to be evaluated to reach the same results. The computational cost would be extremely higher as compare to our GA implementation. In our attempt to reduce the size of search space we have restricted GA variables to binary values representing whether to use a particular \( k_{\text{cat}} \) or 38,000 s\(^{-1}\) rather than more flexible values \( k_{\text{cat}} \) can take in the doubling procedure. Briefly, a population of 10 “genomes” was simulated, each one composed of a list of “genes” that indicated if a protein’s \( k_{\text{cat}} \) would be kept at its original value, or if it would be raised to 38,000 s\(^{-1}\). The original \( k_{\text{cat}} \) values are either obtained from BRENDA or from literature (See Table A3 and SI File S1). The genomes were allowed to evolve by exchanging information, and each new generation was created by a random selection of solutions biased by their fitness, while always taking the best solution to the next generation (see SI Section Extended Methodology: Genetic Algorithm for Constraint Selection for details). The fitness of each genome was determined by simulating a cell population based in its \( k_{\text{cat}} \) selection, and then calculating the goodness-of-fit between the resulting growth rate distribution and the observed distribution [4].

Extended Methods: Each genome was composed of 368 “Boolean genes”, one for each protein that had a \( k_{\text{cat}} \) available. The value of the binary gene indicated whether a original \( k_{\text{cat}} \) would be used with its respective protein count to calculate a \( v_{\text{max}} \), or if the maximum \( k_{\text{cat}} \) of 38,000 s\(^{-1}\) would be used for the \( v_{\text{max}} \) calculation. Since the micro GA applies a uniform cross-over operator, where each gene in an offspring is randomly selected from one of two parent genomes, no mutation operator was used. Moreover, a tournament selection strategy was applied with a sample size of 4, while also applying elitism for the fittest genome. This guarantees high variability and a fast convergence, while preserving optimal results. Population convergence was determined by comparing each genome to the fittest genome in a generation, and counting the number of different gene states between them. When all genomes had less than 5 different gene values when compared with the fittest genome, we determined the population had converged, in which case the fittest genome was kept and all other 9 genomes were re-set to random states. The fitness was calculated by simulating a 4800 cell population from each genome, and then calculating the Watson variation of the Cramér-von-Misses goodness of fit test, comparing the simulated and the experimentally observed growth rate distribution [4] for the entire population. The fitness function was defined as the inverse of the test statistic. The genetic algorithm took anywhere from 2.5 to 15.5 hours to achieve a good goodness-of-fit between simulated and observed growth distribution using 320 CPUs.

A1.5 Proteins with Significant Mean Copy Number But Zero Flux Predicted

Metabolic models are mappings between the genotype of an organism and the reactions that can be catalyzed by their gene products. Given a growth medium and knowledge of the strain (specifically the existence of gene knock-outs, etc.), these models can predict which reactions can carry flux and which cannot. Flux variability analysis with
zero optimal growth requirement was used to determine the minimum and maximum possible flux through each reaction. So called “dead ends”—reactions that can never carry flux due to incompleteness of the model—were not considered. We observed several proteins (Table A7) that were measured in significant copy number despite the reactions they catalyze being unable to carry any flux. This inconsistency may be due to two reasons. The first is that the function of the protein might be out of scope of the metabolic model. Some proteins could be involved in both metabolic and non-metabolic functions within the cell; the expression of such a protein might be predominantly associated with its “moonlighting” function. Another possibility is that certain proteins are highly expressed due to transcriptional regulation which is not accounted for by the metabolic model. For example URA1 is expressed at a copy number of around 18,000 even though there is a deletion in URA3, a gene downstream of URA1 in the strain being used. Other proteins in uracil biosynthesis like URA4 and URA5 (Fig A13) are also expressed in high copy number. This might be due to the inducing activity of dihydroorotic acid [13] which is a metabolic intermediate in uracil biosynthesis and is known to up-regulate expression of URA1 and URA4. The deletion of URA3 could result in the accumulation of dihydroorotic acid as cells might not have adapted to the deletion of this key biosynthetic enzyme. Similarly in the case of leucine biosynthesis, the LEU2 deletion might be causing buildup of α-Isopropylmalate, also a metabolic intermediate, which regulates expression of LEU1 [14].

A1.6 Metabolic Map

Metabolic maps are extensively applied by the systems biology community as a tool to both explore and understand metabolic activity in a cell. They allow an easy way of visualizing the flux distribution throughout the simulated metabolic pathways. Despite their importance, they suffer from a problem which afflicts most efforts to combine large scale biological information: conflicting naming conventions. Maps built for previously developed metabolic models can rarely be reused because metabolites and reaction identifiers constantly change from version to version, and between organisms and data sources (such as fluxomics, transcriptomics and proteomics). The latest versions of the yeast metabolic models tried to unify nomenclature and create a consistent pattern for metabolites and reaction names, but a new map was not created to make use of such developments. In this work, a comprehensive map representation for Yeast 7.6 was built using Escher [15], and it is presented and made available to the community.
Fig. A1. Behavior of noise in protein copy number. Noise as function of protein mean copy number for *E. coli* data from Taniguchi et al., [9] and for *S. cerevisiae* from Denervaud et al., [3].
Fig. A2. Flux distribution using FBA without proteomics constraints
Compared to the flux distribution in $^{13}$C growth medium calculated without using protein constraints (red), the Flux distribution calculated using population FBA with protein constraints (black) agrees very well with experimentally determined fluxes (green).
Table A1. Growth medium composition for glucose minimal medium used for $^{13}$C fluxomics experiments [16] and glucose synthetic defined medium (SD) for proteomics experiments [3]

| Chemical          | $^{13}$C Minimal Medium | SD Medium          |
|-------------------|-------------------------|--------------------|
|                   | Concentration mM        | Uptake Bound mmol gDwt$^{-1}$ hr$^{-1}$ | Concentration mM | Uptake Bound mmol gDwt$^{-1}$ hr$^{-1}$ |
| Carbon source     |                         |                    |                  |                                      |
| D-glucose         | 55.56                   | 20                 | 111.11           | 15                                    |
| Salts             |                         |                    |                  |                                      |
| Ammonium          | 37.84                   | 1000               | 37.84            | 1000                                  |
| Iron(3+)          | 0.01                    | 1000               | 0.01             | 1000                                  |
| Iron(2+)          | 22.04                   | 1000               | 7.34             | 1000                                  |
| Potassium         | 22.04                   | 1000               | 7.34             | 1000                                  |
| Sodium            | 0                       | 1000               | 1.71             | 1000                                  |
| Sulphate          | 39.87                   | 1000               | 39.84            | 1000                                  |
| Magnesium         | 2.03                    | 1000               | 2.03             | 1000                                  |
| Calcium           | 0.031                   | 1000               | 0.68             | 1000                                  |
| Chloride          | 0.061                   | 1000               | 3.07             | 1000                                  |
| Vitamins          |                         |                    |                  |                                      |
| (R)-pantothenate  | 0.0042                  | 0.78               | 0.0016           | 0.78                                  |
| 4-aminobenzoate   | 0.0015                  | 0.78               | 0.047            | 0.78                                  |
| biotin            | 0.00020                 | 0.78               | 0.00000082       | 0.78                                  |
| Folic acid        | 0                       | 0                  | 0.00000045       | 0.78                                  |
| myo-Inositol      | 0.14                    | 0.78               | 0.0111           | 0.78                                  |
| Nicotinate        | 0.0081                  | 0.78               | 0.0033           | 0.78                                  |
| Pyridoxine        | 0.0059                  | 0.78               | 0.0019           | 0.78                                  |
| Riboflavin        | 0                       | 0                  | 0.00053          | 0.78                                  |
| Thiamine(1+)      | 0.0030                  | 0.78               | 0.00012          | 0.78                                  |
| Other nutrients   |                         |                    |                  |                                      |
| Adenine           | 0                       | 0                  | 0.098            | 0.78                                  |
| Citrate(3-)       | 0                       | 0                  | 1.67             | 0.78                                  |
| Uracil            | 0                       | 0                  | 0.678            | 0.78                                  |
| Amino acids       |                         |                    |                  |                                      |
| Glycine           | 0                       | 0                  | 1.01             | 0.78                                  |
| L-alanine         | 0                       | 0                  | 0.85             | 0.1                                   |
| L-arginine        | 0                       | 0                  | 0.36             | 0.31                                  |
| L-asparagine      | 0                       | 0                  | 0.51             | 0.36                                  |
| L-aspartate       | 0                       | 0                  | 0.57             | 0.72                                  |
| L-cysteine        | 0                       | 0                  | 0.43             | 0.78                                  |
| L-glutamate       | 0                       | 0                  | 0.41             | 0.6                                   |
| L-glutamine       | 0                       | 0                  | 0.52             | 0.23                                  |
| L-isoleucine      | 0                       | 0                  | 0.58             | 0.78                                  |
| L-leucine         | 0                       | 0                  | 2.90             | 0.78                                  |
| L-lysine          | 0                       | 0                  | 0.42             | 0.78                                  |
| L-methionine      | 0                       | 0                  | 0.51             | 0.78                                  |
| L-phenylalanine   | 0                       | 0                  | 0.46             | 0.78                                  |
| L-proline         | 0                       | 0                  | 0.66             | 0.78                                  |
| L-serine          | 0                       | 0                  | 0.72             | 0.47                                  |
| L-threonine       | 0                       | 0                  | 0.64             | 0.78                                  |
| L-tryptophan      | 0                       | 0                  | 0.37             | 0.78                                  |
| L-tyrosine        | 0                       | 0                  | 0.34             | 0.13                                  |
| L-Valine          | 0                       | 0                  | 0.65             | 0.78                                  |
Table A2. Ratios of mRNA expressed by cells growing in $^{13}$C and SD media. The ten most down- and up-regulated values are shown. Complete list of ratios is in SI File S2.

| Genes     | Names                                      | Ratios ($^{13}$C/SD) |
|-----------|--------------------------------------------|----------------------|
|           | Downregulated in $^{13}$C medium vs. SD medium |                      |
| YMR169C   | Cytoplasmic aldehyde dehydrogenase         | 0.06                 |
| YLR038C   | Subunit VIb of cytochrome c oxidase        | 0.08                 |
| YLR044C   | Major of three pyruvate decarboxylase isozymes | 0.10                 |
| YDL400W   | Uridine nucleosidase (uridine-cytidine N-ribohydrolase) | 0.10                 |
| YMR278W   | Phosphoribomutase                          | 0.12                 |
| YER178W   | E1 alpha subunit of the pyruvate dehydrogenase (PDH) complex | 0.13                 |
| YDL185W   | Subunit A of the V1 peripheral membrane domain of V-ATPase | 0.13                 |
| YGR260W   | High affinity nicotinic acid plasma membrane permease | 0.16                 |
| YDR380W   | Phenylpyruvate decarboxylase               | 0.19                 |
| YOR128C   | Phosphoribosylaminomimidazole carboxylase  | 0.30                 |
|           | Upregulated in $^{13}$C medium vs. SD medium |                      |
| YJR105W   | Adenosine kinase                           | 13.18                |
| YLR231C   | Kynureninase                               | 9.99                 |
| YNL220W   | Adenylosuccinate synthase                  | 8.28                 |
| YNL241C   | Glucose-6-phosphate dehydrogenase (G6PD)   | 7.26                 |
| YMR208W   | Mevalonate kinase                          | 6.36                 |
| YLR028C   | Enzyme of de novo purine biosynthesis      | 5.74                 |
| YEL024W   | Ubiquinol-cytochrome-c reductase           | 5.66                 |
| YMR062C   | Mitochondrial ornithine acetyltransferase  | 5.62                 |
| YMR000C   | Phosphoribosylpyrophosphate amidotransferase (PRPPAT) | 5.31                 |
| YHR163W   | 6-phosphogluconolactonase                  | 5.17                 |

Fig. A3. Growth rate distribution predicted in $^{13}$C growth medium. Growth rate distribution predicted in $^{13}$C growth medium using Populations FBA with rescaled protein distributions.
Table A3. List of $k_{cat}$ values found manually from Literature. Corresponding doublings both for $^{13}$C and SD medium are also listed. Final $v_{\text{max}}$ was calculated for each protein using its mean copy, corresponding $k_{cat}$, doublings and unit conversion factor of $3.0 \times 10^{-7}$ to convert units from $s^{-1}$ to mmol.gDwt$^{-1}$ hr$^{-1}$.

| ID       | Short Name | Full Name | Literature | $k_{\text{cat}}$ (s$^{-1}$) | $^{13}$C Literature | $^{13}$C SD | Doublings   | $^{13}$C Literature | $^{13}$C SD | Mean Protein (SD) |
|----------|------------|-----------|------------|-----------------|----------------------|--------------|-------------|----------------------|--------------|-------------------|
| YLR258W  | GSY2       | Glycogen synthase | 0.0185     | 604.17 | 19 | 5 | 19 | 2 | 4.91 | 2.84 | 4.91 | 14.2 | 1304.98 |
| YOL140W  | ARG8       | Acetylornithine aminotransferase | 1.55 | 2.45 | 15 | 14 | 13 | 0 | 10 | 2.11 | 1.67 | 0.00 | 0.21 | 276.87 |
| YGL055Y  | OLE1       | Delta-[9] fatty acid desaturase | 0.3 | 0.705 | 12 | 11 | 11 | 10 | 10 | 0.10 | 0.14 | 0.05 | 0.07 | 321.49 |
| YGL148Y  | AR2        | Bifunctional chorismate synthase and flavin reductase | 0.87 | 33.34 | 11 | 6 | 0 | 0 | 0.65 | 0.77 | 0.00 | 0.01 | 2360.06 |
| YOR184W  | SER1       | 3-phosphoerine aminotransferase | 1.75 | 2.49 | 15 | 9 | 9 | 7 | 5 | 1.49 | 2.12 | 0.37 | 0.14 | 5538.01 |
| YGR091W  | VAS1       | Mitochondrial and cytoplasmic valyl-tRNA synthetase | 0.2 | 3.2 | 12 | 9 | 5 | 10 | 6 | 0.21 | 0.21 | 0.43 | 0.43 | 6978.33 |
| YGL253W  | IXK2       | Hexokinase isoenzyme 2 | 1.67 | 200.34 | 9 | 2 | 8 | 2 | 27.03 | 25.3 | 15.5 | 25.3 | 105287.26 |
| YNL104C  | LEU4       | Alphaisopropylmalate synthase (2-isopropylmalate synthase) | 2.17 | 13.79 | 24 | 9 | 6 | 0 | 0 | 1.21 | 0.76 | 0.00 | 0.01 | 2897.71 |
| YDR154W  | THP4       | Anthranilate phosphoribosyl transferase | 2.9 | 69.67 | 4 | 9 | 4 | 0 | 0 | 0.20 | 0.15 | 0.00 | 0.01 | 446.59 |
| YGL062W  | PYC1       | Pyruvate carboxylase isoenzyme | 60 | 89.79 | 26 | 7 | 5 | 7 | 0 | 4.97 | 7.45 | 0.04 | 0.06 | 2153.01 |
| YCI404W  | GLK1       | Glucose kinase | 1.66 | 1492.96 | 7 | 0 | 7 | 0 | 19.44 | 13.7 | 19.42 | 1.37 | 3046.59 |
| YML120C  | ND1        | NADH ubiquinone oxidoreductase | 0.031 | 550.78 | 5 | 3 | 20 | 5 | 0.00 | 1.58 | 11.64 | 6.31 | 1190.41 |
| YFL018C  | LPD1       | Dihydrolipoamide dehydrogenase | 6.49 | 899.36 | 5 | 5 | 5 | 5 | 24.64 | 34.14 | 24.61 | 34.14 | 3950.66 |
| YLR075W  | TSH1       | Threonyl-tRNA synthetase | 0.05 | 3.24 | 21 | 5 | 6 | 11 | 5 | 0.00 | 0.44 | 0.21 | 0.22 | 6862.29 |
| YNL277W  | MET2       | L-homocysteine-cysteine synthase | 0.98 | 122.31 | 5 | 5 | 5 | 0 | 0.00 | 0.44 | 0.22 | 0.22 | 548.34 |
| YGL245W  | GUS1       | Glutaryl-tRNA synthetase (GlbS) | 0.016 | 4.44 | 12 | 4 | 5 | 12 | 4 | 0.00 | 0.53 | 0.25 | 0.27 | 12937.66 |
| YGR064C  | ADE6       | Formylglycinamidine-ribonucleotide (FGAM)-synthetase | 0.05 | 5 | 34 | 4 | 4 | 0 | 0 | 0.00 | 0.24 | 0.00 | 0.01 | 9802.89 |
| YNL037C  | IDH1       | Submit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase | 30 | 124.54 | 3 | 2 | 9 | 8 | 0.19 | 0.38 | 11.87 | 24.55 | 2574.91 |
| YOR136W  | IDH2       | Submit of mitochondrial NAD(+)-dependent isocitrate dehydrogenase | 30 | 124.34 | 3 | 2 | 9 | 8 | 0.12 | 0.24 | 7.56 | 15.65 | 1641.49 |
| YLR041C  | SDH2       | Iron-sulfur protein submit of succinate dehydrogenase | 60 | 658.54 | 2 | 3 | 6 | 2 | 0.04 | 0.85 | 0.62 | 0.42 | 536.45 |
| YNR014C  | CIT1       | Citrate synthase | 167 | 2100.96 | 2 | 2 | 0 | 0 | 0 | 0.53 | 1.65 | 0.13 | 1.65 | 2023.29 |
| YLR044C  | PDC1       | Major of three pyruvate decarboxylase isoenzymes | 12.42 | 37 | 9 | NA | 4 | NA | 10033.33 | NA | 50.10 | 839441.26 |
| YDR403C  | HXK1       | Hexokinase isoenzyme 1 | 1.67 | 200.69 | 0 | 0 | 0 | 0 | 0.04 | 4.75 | 0.04 | 4.75 | 79143.12 |
| YLR450W  | HMG2       | HMG-CoA reductase | 0.023 | 3800.77 | 0 | 0 | 0 | 16 | 0.00 | 6.60 | 0.26 | 6.60 | 578.88 |
| YOR108Y  | LEU9       | Alphaisopropylmalate synthase II (2-isopropylmalate synthase) | 2.717 | 7.28 | 15 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 1482.78 |
| YDR248W  | ADK1       | Adenylate kinase, required for purine metabolism | 0.0063 | 8784.58 | 0 | 0 | 0 | 0 | 0.00 | 5.46 | 5.12 | 5.46 | 20667.6 |
| YGR087C  | PDC5       | Minor isomer of pyruvate decarboxylase | 0.921 | 37 | 9 | NA | 0 | NA | 0.00 | 0.00 | NA | 0.00 | 940.76 |
| YLR134W  | PDC5       | Minor isomer of pyruvate decarboxylase | 10.32 | 37 | 9 | NA | 0 | NA | 0.00 | 0.00 | NA | 0.00 | 1510.85 |
| YPR081C  | GHS2       | Glycine-tRNA synthetase | 0.73 | 15.39 | 0 | 0 | 0 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 471.63 |
| YKR086W  | MTD1       | 5,10-methylenetetrahydrofolate dehydrogenase (NAD) | 1.63 | 1643 | 39 | 0 | 0 | 12 | 2 | 1.63 | 1.63 | 6676 | 6572 | 2707 |

- ‘–’ means not found in BRENDA, '*' means wrongly listed in BRENDA hence set to highest value known for yeast.
Table A4. Mapping Between Reaction Code, Abbreviation and Full Name.

| Code  | Abbreviation | Full Name                                      |
|-------|--------------|------------------------------------------------|
| r.0534 | HK           | hexokinase (D-glucose:ATP)                     |
| r.0467 | PGI          | glucose-6-phosphate isomerase                  |
| r.0886 | PFK          | phosphofructokinase                            |
| r.0450 | FBA          | fructose-bisphosphate aldolase                 |
| r.1054 | TIM          | triose-phosphate isomerase                     |
| r.0486 | GAPDH        | glyceraldehyde-3-phosphate dehydrogenase      |
| r.0962 | PGK          | phosphoglycerate kinase                        |
| r.0933 | PGM          | phosphoglycerate mutase                        |
| r.0366 | ENO          | enolase                                        |
| r.1054 | TIM          | triose-phosphate isomerase                     |

TCA Cycle

| Code  | Abbreviation | Full Name                                      |
|-------|--------------|------------------------------------------------|
| r.2034 | PYRt         | pyruvate transport                             |
| r.0961 | PDH          | pyruvate dehydrogenase                         |
| r.0300 | CS           | citrate synthase                               |
| r.0300 | ACONTa       | citrate to cis-aconitate                       |
| r.0300 | ACONTb       | cis-aconitate to isocitrate                    |
| r.0961 | PDH          | pyruvate dehydrogenase                         |
| r.0658 | ICDH         | isocitrate dehydrogenase (NAD+)                |
| r.0832 | AKGDa        | oxoglutarate dehydrogenase (lipoamide)        |
| r.0831 | AKGDb        | oxoglutarate dehydrogenase (dihydrolipoamide S-succinyltransferase) |
| r.1022 | SUCOAS       | succinate-CoA ligase (ADP-forming)             |
| r.1021 | SUCD         | succinate dehydrogenase (ubiquinone-6)         |
| r.0451 | FUM          | fumarase                                       |
| r.0713 | MDH          | malate dehydrogenase                           |

Electron Transport Chain

| Code  | Abbreviation | Full Name                                      |
|-------|--------------|------------------------------------------------|
| r.0770 | NADH2c       | NADH dehydrogenase cytosolic/mitochondrial     |
| r.0773 | NADHD        | NADH:ubiquinone oxidoreductase mitochondrial   |
| r.0439 | CYOR         | ferrocytochrome-c:oxygen oxidoreductase        |
| r.0438 | CYOO         | ferrocytochrome-c:oxygen oxidoreductase (O2)   |
| r.0226 | ATPS         | ATP synthase                                   |

Ethanol Production

| Code  | Abbreviation | Full Name                                      |
|-------|--------------|------------------------------------------------|
| r.0959 | PYRDC        | pyruvate decarboxylase                         |
| r.2115 | ALCD         | alcohol dehydrogenase (acetaldehyde to ethanol) |

All reaction codes reflect the yeast model 7 naming convention.

Table A5. Percentage variance accounted by first three PCA components accounting for maximum variance in the data for population simulated in $^{13}$C fluxomics experiment and proteomics experiment

| SD       | % Var | Pathway                                    | $^{13}$C | % Var |
|----------|-------|--------------------------------------------|----------|-------|
| Fermentation vs. Respiration | 71.07 | Fermentation vs. Respiration | 92.10 |
| Glycine-Serine Cycle | 14.40 | Glucose uptake vs. ATP synthase | 2.98 |
| Mitochondrial Ethanol production | 4.67 | Glycerol production vs. pyruvate efflux | 1.37 |
Fig. A4. Analysis of metabolic fluxes from yeast 7.6 simulations in $^{13}$C medium. (A) Glucose uptake, oxygen uptake and ethanol efflux in glucose minimal medium. (B) First PCA component showing fast growing cells performing respiration along with fermentation. (C) Second PCA component showing rise in ATP synthase usage as glucose uptake gets limited in fast growing cells. (D) Third PCA component showing few slower growing cells secreting pyruvate while few cells with intermediate growth rate secreting glycerol.
Fig. A5. All non-zero transport reactions in (negative) and out (positive) of the cell for population growing on glucose minimal medium used for $^{13}$C fluxomics experiments.
Fig. A6. Reactions constrained most often in $^{13}$C growth medium. Blue dots are actual fluxes and Orange circles are upper bounds on those reactions.
Fig. A7. Impact of correlated sampling of protein counts on simulated metabolic fluxes. Mean flux values through central metabolism for simulations that either imposed (blue) or did not impose (black) correlations in protein sampling. Values between brackets were taken from experimental measurements [16]. All simulations used yeast 7.6 and $^{13}$C media conditions.
Fig. A8. Active transport reactions for yeast 7.6 model in SD medium. Negative and positive fluxes indicate secretion and uptake respectively of metabolites from the cell.
Fig. A9. Reactions constrained most often in SD growth medium. Blue dots are actual fluxes and Orange circles are upper bounds on those reactions.
Table A6. 51 genes consistently filtered by all 10 GA optimization runs in SD medium

| Gene      | Starting Kcat | Doublings | Mean Copy |
|-----------|---------------|-----------|-----------|
| YAL038W   | 232           | 5         | 52704.83  |
| YBR011C   | 200           | 5         | 3465.71   |
| YBR265W   | 1.07          | 8         | 264.36    |
| YDL067C   | 1500          | 4         | 141.60    |
| YEL024W   | 1500          | 4         | 162.02    |
| YGL055W   | 0.705         | 10        | 171.07    |
| YGL245W   | 4.4           | 4         | 12375.27  |
| YGR094W   | 3.2           | 6         | 6209.55   |
| YGR175C   | 0.076         | 7         | 3071.99   |
| YGR185C   | 6.08          | 3         | 5268.56   |
| YGR240C   | 62            | 5         | 76573.72  |
| YHR042W   | 6.47          | 3         | 593.74    |
| YHR190W   | 3.3           | 8         | 123.54    |
| YIL020C   | 32            | 6         | 226.16    |
| YIL078W   | 3.32          | 5         | 6074.67   |
| YIL116W   | 4.1           | 5         | 2231.67   |
| YJR121W   | 539           | 6         | 1974.70   |
| YKL067W   | 13.3          | 9         | 1819.59   |
| YKL152C   | 490           | 2         | 136288.75 |
| YLM008C   | 16.9          | 4         | 4267.74   |
| YLR100W   | 1.2           | 5         | 846.81    |
| YMR205C   | 0.01083       | 9         | 4823.98   |
| YMR220W   | 62            | 5         | 62358.96  |
| YNR043W   | 4.9           | 5         | 1920.91   |
| YOR074C   | 1.2           | 4         | 1081.38   |
| YPL160W   | 5             | 3         | 23765.41  |
| YPR039C   | 40            | 2         | 2190.87   |
| YPR183W   | 70.9          | 6         | 1030.60   |
| YKL094W   | 2.66          | 0         | 760.17    |
| YOR236W   | 11.5          | 1         | 564.86    |
| YPR081C   | 0.15          | 0         | 277.52    |
| YFL030W   | 45            | 9         | 261.77    |
| YDL141W   | 30.1          | 6         | 691.07    |
| YER099C   | 60.68         | 4         | 626.48    |
| YMR267W   | 200           | 7         | 614.65    |
| YBL099W   | 539           | 6         | 4342.78   |
| YBR039W   | 539           | 6         | 1884.31   |
| YDR377W   | 539           | 6         | 163.70    |
| YKL016C   | 539           | 6         | 263.25    |
| YLR295C   | 539           | 6         | 6067.36   |
| YML081C-A | 539           | 6         | 260.84    |
| YPL271W   | 539           | 6         | 6758.82   |
| YBL045C   | 1500          | 4         | 242.32    |
| YFR033C   | 1500          | 4         | 753.44    |
| YGR183C   | 1500          | 4         | 354.91    |
| YHR001W-A | 1500          | 4         | 311.99    |
| YJR166W   | 1500          | 4         | 116.73    |
| YJR048W   | 1500          | 4         | 263.19    |
| YOR065W   | 1500          | 4         | 1030.83   |
| YLR044C   | 12.42         | 4         | 1713243.78|
Fig. A10. Comparison of flux distributions between two independent GA optimizations. Bimodality in amino acid uptake can be observed in either slow growing cells (A) or fast growing cells (B) and was linked to the degree with which glycolysis is constrained (see Results Sections SD media: Bimodality in Amino Acid Utilization and Degeneracy of Constraint Selection). Black dots indicate individual cells, red squares and bars indicate mean and square deviation.
Fig. A11. Correlations between reactions in Central metabolism and glucose transporter HXT1. Correlations of reactions were calculated by processing the correlations of individual genes involved through Gene-Protein-Reaction (GPR) relationships from the metabolic model. Minimum correlation coefficient was taken in case of AND relationships and sum of correlation coefficients in case of OR relationship. Reactions with positive correlations were marked in blue and the ones with negative correlations in red.
Fig. A12. Analysis of correlations in protein expression. Top: Distance metric of correlations from actual expression data as compared to randomized correlation data. Bottom: Distribution of distance metric obtained from randomizing expression data.
**Fig. A13. Uracil biosynthesis pathway in yeast.** *URA3* was deleted in this strain. Copy number of *URA1*, *URA4* and *URA5* were observed to be significant in spite of the deletion possibly due to upregulation by dihydroorotate (encircled).

**Table A7.** List of proteins with significant mean protein count but catalyze reactions which can’t carry any flux in glucose SD medium growth conditions according to metabolic model for auxotrophic strain used in proteomics study based on Yeast 7.6 model.

| Systematic Name | Protein Name | Mean Copy | Name | Subsystem                      |
|-----------------|--------------|-----------|------|--------------------------------|
| YKL216W         | URA1         | 18179.54  | Dihydroorotate dehydrogenase | Pyrimidine biosynthesis          |
| YGL009C         | LEU1         | 16243.73  | Isopropylmalate isomerase    | Leucine biosynthesis             |
| YLR420W         | URA4         | 2345.43   | Dihydroorotate               | Pyrimidine biosynthesis          |
| YGR260W         | TNA1         | 2052.47   | Nicotinate permease           | Permeases                        |
| YML106W         | URA5         | 1945.02   | orotate phosphoribosyltransferase isozyme | Pyrimidine biosynthesis          |
| YMR113W         | FOL3         | 1543.35   | Dihydrofolate synthetase     | Folic acid biosynthesis          |
| YDL100C         | GET3         | 1085.58   | Guanine nucleotide exchange factor | Cofactor biosynthesis            |
| YGR255C         | COQ6         | 1090.97   | Flavin-dependent monoxygenase | Ubiquinone biosynthesis          |
| YPL059W         | GRX5         | 830.09    | Glutathione-dependent oxidoreductase | Iron sulfur center assembly      |
| YGR010W         | NMA2         | 800.00    | Nicotinic acid mononucleotide adenylyltransferase | NAD+ biosynthesis               |
Fig. A14. Growth rate distributions predicted using 50% of the available protein distributions in red (268 out of 535) and 33% of the available protein distributions in blue (179 out of 535) in SD medium. Also shown is experimental distribution in bars and growth rate distribution obtained using all 535 protein distributions in dashed line.

Fig. A15. Growth rate distributions predicted by replacing gamma distributions for all 535 proteins with uniform (red) or normal (blue) distributions keeping the mean protein copy number intact. Also shown is experimental growth rate distribution in bars and growth rate distribution obtained using gamma distributions in gray dashed line. Several artifacts emerge when using normally-distributed protein counts. Because normal distributions can take negative values, all sampled protein counts from the negative tails were changed to 2.87 (see Methods section “Conversion of Fluorescence to Protein Copy Numbers” in the main manuscript). This led to several “spikes” at low growth rates in our normally-distributed protein count population.
Fig. A16. Decrease in flux through serine glycine cycle because of irreversibility of three reactions involved. Average flux from 1,000 cells generated using population FBA in SD medium and metabolic model with reversibilities for three reactions (marked with red star) from older version of the metabolic model yeast 7.11. Average flux obtained using latest version of the metabolic model yeast 7.6 are in parentheses. Directionality and thickness of reaction arrows are based on the flux distribution as predicted using yeast 7.6.
FVA with 100 % Growth and 100% Total flux

Fig. A17. Flux variability of reactions in serine glycine cycle among fast growing cells with 100 % optimality for growth and total flux Minimum and maximum fluxes through reactions in the serine glycine cycle according to flux variability analysis while maintaining same growth rate and total flux as calculated using pFBA. Fluxes are calculated for 605 fast growing cells with growth rate $> 0.35$ hr$^{-1}$. Little variation is exhibited except in the reactions involving methylenetetrahydrofolate dehydrogenase (MTD) which are alternate pathways for NADH and NADPH production.
Fig. A18. Flux variability of reactions in serine glycine cycle among fast growing cells with 90% optimality for growth and 100% for total flux

Minimum and maximum fluxes through reactions in the serine glycine cycle according to flux variability analysis while maintaining 90% of the optimal growth rate and 100% of optimal total flux as calculated using pFBA. Optimal total flux is calculated at 100% optimal growth. Fluxes are calculated for 605 fast growing cells with growth rate > 0.35 hr⁻¹. Little variation is exhibited except in the reactions involving methylenetetrahydrofolate dehydrogenase (MTD) which are alternate pathways for NADH and NADPH production. Lower fluxes are observed than the case when 100% optimality is enforced both for growth rate and total flux because lower growth requirement reduces the energy requirement and hence the flux through the cycle.
Fig. A19. **Flux variability in threonine uptake among selected cells** Flux variability in threonine uptake of over 400 cells selected randomly from cells growing slower than 0.4 hr$^{-1}$ as shown in the left panel. Right panel shows histogram with no variability in threonine uptake while growth rate and total flux is fixed at its optimal value as determined using pFBA.
Table A8. Comparison of Biomass Pseudo-Reactions Among the Latest Yeast Metabolic Models.

| Metabolite | Coefficients | Metabolite | Coefficients |
|------------|--------------|------------|--------------|
| (1;3)-beta-D-glucan | 1.1348 | (1;6)-beta-D-glucan | 1.1348 |
| chitin | 0.000001 | | 0.000001 |
| glycogen | 0.5185 | 0.519 | 0.5185 | 0.5185 |
| mannan | 0.8079 | 0.821 | 0.8079 | 0.8079 |
| trehalose | 0.0234 | 0.0234 | 0.0234 | 0.0234 |
| lipids | 1 | 1 | 1 | 1 |
| AMP | 0.046 | 0.051 | 0.046 | 0.046 |
| ATP | 59.276 | 59.3 | 59.276 | 59.276 |
| CMP | 0.0447 | 0.05 | 0.0447 | 0.0447 |
| GMP | 0.046 | 0.051 | 0.046 | 0.046 |
| UMP | 0.0599 | 0.067 | 0.0599 | 0.0599 |
| dAMP | 0.0036 | 0.000359 | 0.0036 | 0.0036 |
| dCMP | 0.0024 | 0.00243 | 0.0024 | 0.0024 |
| dGMP | 0.0024 | 0.00243 | 0.0024 | 0.0024 |
| dTMP | 0.0036 | 0.000359 | 0.0036 | 0.0036 |
| H2O | 59.276 | 59.3 | 59.276 | 59.276 |
| sulphate | 0.02 | 0.02 | 0.02 | 0.02 |
| riboflavin | 0.00099 | 0.0009 | 0.00099 | 0.00099 |
| heme a | 0.000001 | | |
| L-alanine | 0.4588 | 0.357 | Ala-tRNA(Ala) | 0.4588 | 0.4588 |
| L-arginine | 0.1607 | 0.136 | Arg-tRNA(Arg) | 0.1607 | 0.1607 |
| L-asparagine | 0.1017 | 0.172 | Asn-tRNA(Asn) | 0.1017 | 0.1017 |
| L-aspartate | 0.2975 | 0.172 | Asp-tRNA(Asp) | 0.2975 | 0.2975 |
| L-cysteine | 0.0066 | 0.0429 | Cys-tRNA(Cys) | 0.0066 | 0.0666 |
| L-glutamate | 0.1054 | 0.268 | Glu-tRNA(Glu) | 0.1054 | 0.1054 |
| L-glutamine | 0.3018 | 0.268 | Glu-tRNA(Glu) | 0.3018 | 0.3018 |
| L-glycine | 0.2904 | 0.325 | Gly-tRNA(Gly) | 0.2904 | 0.2904 |
| L-histidine | 0.0663 | 0.075 | His-tRNA(His) | 0.0663 | 0.0663 |
| L-isoleucine | 0.1927 | 0.172 | Ile-tRNA(Ile) | 0.1927 | 0.1927 |
| L-leucine | 0.2964 | 0.25 | Leu-tRNA(Leu) | 0.2964 | 0.2964 |
| L-lysine | 0.2802 | 0.239 | Lys-tRNA(Lys) | 0.2802 | 0.2802 |
| L-methionine | 0.0507 | 0.05 | Met-tRNA(Met) | 0.0507 | 0.0507 |
| L-phenylalanine | 0.1339 | 0.114 | Phe-tRNA(Phe) | 0.1339 | 0.1339 |
| L-proline | 0.1647 | 0.129 | Pro-tRNA(Pro) | 0.1647 | 0.1647 |
| L-serine | 0.1854 | 0.254 | Ser-tRNA(Ser) | 0.1854 | 0.1854 |
| L-threonine | 0.1914 | 0.197 | Thr-tRNA(Thr) | 0.1914 | 0.1914 |
| L-tryptophan | 0.0284 | 0.028 | Trp-tRNA(Trp) | 0.0284 | 0.0284 |
| L-tyrosine | 0.102 | 0.0965 | Tyr-tRNA(Tyr) | 0.102 | 0.102 |
| L-valine | 0.2646 | 0.257 | Val-tRNA(Val) | 0.2646 | 0.2646 |
| cAMP | 0.000001 | CoA | 0.000001 |
| Ergst | 0.0007 | | 0.000001 |
| Glutathione reduced | 0.000001 | FAD | 0.000001 |
| Phosphatidate | 0.000006 | | 0.000001 |
| Phosphatidylethanolamine | 0.0000045 | | 0.000001 |
| Phosphatidyl-1D-myo-inositol | 0.000053 | | 0.0000017 |
| Zymosterol | 0.0015 | | |
| Triglyceride | 0.000066 | | |
| Protoheme | 0.000001 | | 0.000001 |

Aminoacylation reactions are not present in all models, therefore their biomass pseudo-reaction uses either amino acids (iMM904 and Yeast 5), or charged tRNAs (Yeast 6 and Yeast 7). All yeast consensus models were taken from [yeast.sourceforge.net](http://yeast.sourceforge.net).
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