Metabolic Control Analysis under Uncertainty: Framework Development and Case Studies

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SUPPLEMENTARY MATERIAL

(Log)linear MCA model

We start with the metabolite mass balance equations in the metabolic network with consideration of conserved moieties (see Table 1 in main text for nomenclatures):

$$\frac{d\boldsymbol{x}_i}{dt} = \boldsymbol{N}_R \boldsymbol{v}\left(\boldsymbol{x}_i, \boldsymbol{x}_d\left(\boldsymbol{x}_i, \boldsymbol{p}_m\right), \boldsymbol{p}_e, \boldsymbol{p}_s\right), \tag{S1}$$

and the steady state equations of the system:

$$\boldsymbol{N}_R \boldsymbol{v}\left(\boldsymbol{x}_i, \boldsymbol{x}_d\left(\boldsymbol{x}_i, \boldsymbol{p}_m\right), \boldsymbol{p}_e, \boldsymbol{p}_s\right) = 0\,. \tag{S2}$$

We can linearize the system around the steady state:

$$\boldsymbol{N}_R \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_i} d\boldsymbol{x}_i + \boldsymbol{N}_R \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_d} \frac{\partial \boldsymbol{x}_d}{\partial \boldsymbol{x}_i} d\boldsymbol{x}_i + \boldsymbol{N}_R \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_d} \frac{\partial \boldsymbol{x}_d}{\partial \boldsymbol{p}_m} d\boldsymbol{p}_m + \boldsymbol{N}_R \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{p}_e} d\boldsymbol{p}_e + \boldsymbol{N}_R \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{p}_s} d\boldsymbol{p}_s = 0\,. \tag{S3}$$

We then define the following diagonal matrices: steady state fluxes matrix, $\boldsymbol{V}$; independent metabolite concentrations matrix, $\boldsymbol{X}_i$; dependent metabolite concentration matrix, $\boldsymbol{X}_d$; conserved moiety concentrations matrix, $\boldsymbol{P}_m$; enzyme activity parameter matrix, $\boldsymbol{P}_e$; other system parameters matrix, $\boldsymbol{P}_s$. We next scale the system as follows:

$$\begin{aligned}&\boldsymbol{N}_R \boldsymbol{V}\boldsymbol{V}^{-1} \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_i} \boldsymbol{X}_i \boldsymbol{X}_i^{-1} d\boldsymbol{x}_i + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{V}^{-1} \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_d} \boldsymbol{X}_d \boldsymbol{X}_d^{-1} \frac{\partial \boldsymbol{x}_d}{\partial \boldsymbol{x}_i} \boldsymbol{X}_i \boldsymbol{X}_i^{-1} d\boldsymbol{x}_i + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{V}^{-1} \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{x}_d} \boldsymbol{X}_d \boldsymbol{X}_d^{-1} \frac{\partial \boldsymbol{x}_d}{\partial \boldsymbol{p}_m} \boldsymbol{P}_m \boldsymbol{P}_m^{-1} d\boldsymbol{p}_m \\ &+ \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{V}^{-1} \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{p}_e} \boldsymbol{P}_e \boldsymbol{P}_e^{-1} d\boldsymbol{p}_e + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{V}^{-1} \frac{\partial \boldsymbol{v}}{\partial \boldsymbol{p}_s} \boldsymbol{P}_s \boldsymbol{P}_s^{-1} d\boldsymbol{p}_s = 0\end{aligned}$$

. (S4)

Using the definitions of elasticities and weight matrices (see Table 1 in main text), Equation S4 can be transformed into the following equation:

$$\boldsymbol{N}_R \boldsymbol{V}\boldsymbol{E}_i d\ln \boldsymbol{x}_i + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{E}_d \boldsymbol{Q}_i d\ln \boldsymbol{x}_i + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{\Pi}_m d\ln \boldsymbol{p}_m + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{\Pi}_e d\ln \boldsymbol{p}_e + \boldsymbol{N}_R \boldsymbol{V}\boldsymbol{\Pi}_s d\ln \boldsymbol{p}_s = 0 \tag{S5}$$

where

$$\boldsymbol{\Pi}_m = \boldsymbol{E}_d \boldsymbol{Q}_m\,. \tag{S6}$$

The concentration control coefficient matrix can be derived as

$$\boldsymbol{C}_{\boldsymbol{p}}^{\boldsymbol{x}_i} = \frac{d\ ln\ \boldsymbol{x}_i}{d\ ln\ \boldsymbol{p}} = -\left(\boldsymbol{N}_{\boldsymbol{R}}\boldsymbol{V}\boldsymbol{E}_{\boldsymbol{i}} + \boldsymbol{N}_{\boldsymbol{R}}\boldsymbol{V}\boldsymbol{E}_{\boldsymbol{d}}\boldsymbol{Q}_{\boldsymbol{i}}\right)^{-1}\left[\boldsymbol{N}_{\boldsymbol{R}}\boldsymbol{V}\boldsymbol{\Pi}_{\boldsymbol{m}} \vdots \boldsymbol{N}_{\boldsymbol{R}}\boldsymbol{V}\boldsymbol{\Pi}_{\boldsymbol{e}} \vdots \boldsymbol{N}_{\boldsymbol{R}}\boldsymbol{V}\boldsymbol{\Pi}_{\boldsymbol{s}}\right]. \quad \text{(S7)}$$

Here the generalized parameter set include all system parameters:

$$\boldsymbol{p}^{\boldsymbol{T}} = \left[\boldsymbol{p}_{\boldsymbol{m}}^{\boldsymbol{T}} \vdots \boldsymbol{p}_{\boldsymbol{e}}^{\boldsymbol{T}} \vdots \boldsymbol{p}_{\boldsymbol{s}}^{\boldsymbol{T}}\right]. \quad \text{(S8)}$$

Similarly, the flux control coefficient matrix can be derived as:

$$\boldsymbol{C}_{\boldsymbol{p}}^{\boldsymbol{v}} = \frac{d\ ln\ \boldsymbol{v}}{d\ ln\ \boldsymbol{p}} = \left(\boldsymbol{E}_{\boldsymbol{i}} + \boldsymbol{E}_{\boldsymbol{d}}\boldsymbol{Q}_{\boldsymbol{i}}\right)\boldsymbol{C}_{\boldsymbol{p}}^{\boldsymbol{x}_i} + \left[\boldsymbol{\Pi}_{\boldsymbol{m}} \vdots \boldsymbol{\Pi}_{\boldsymbol{e}} \vdots \boldsymbol{\Pi}_{\boldsymbol{s}}\right]. \quad \text{(S9)}$$

Anaerobic glycolytic pathway model of nongrowing yeast, *Saccharomyces cerevisiae*

Our analysis is based on an established kinetics model (Teusink et al., 2000) (see Figure 7 in main text). The set of ordinary differential equations that describes the mass balances of the metabolic intermediates is listed below.

The following abbreviations are used: Chemical species: G^{out}, extracellular glucose; G^{in}, intracellular glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FdP, fructose 1,6-diphosphate; GAP, glyceraldehydes-3-phosphate; DHAP, dihydroxy acetone phosphate; BPG, bisphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate kinase; AcAld, acetaldehyde; ETOH, ethanol; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NADH, nicotinamide adenine dinucleotide.

Pathway steps and enzymes: *trans*, glucose cross-membrane transport; *HK*, hexokinase; *PGI*, phosphoglucose isomerase; *PFK*, phosphofructokinase; *ALD*, fructose 1,6-diphosphate aldolase; *TPI*, triose phosphate isomerase; *GAPDH*, glyceraldehydes-3-

phosphate dehydrogenase; *PGK*, phosphoglycerate kinase; *PGM*, phosphoglycerate mutase; *ENO*, enolase; *PYK*, pyruvate kinase; *PDC*, pyruvate decarboxylase; ADH, alcohol dehydrogenase; ATPase, net ATP consumption; AK, adenylate kinase.

$$\frac{d[G_{in}]}{dt} = v_{trans,f} - v_{trans,b} - v_{HK,f} + v_{HK,b} \quad \text{(S10)}$$

$$\frac{d[G6P]}{dt} = v_{HK,f} - v_{HK,b} - v_{PGI,f} + v_{PGI,b} - 2v_{trehalose} - v_{glycogen} \quad \text{(S11)}$$

$$\frac{d[F6P]}{dt} = v_{PGI,f} - v_{PGI,b} - v_{PFK} \quad \text{(S12)}$$

$$\frac{d[FdP]}{dt} = v_{PFK} - v_{ALD,f} + v_{ALD,b} \quad \text{(S13)}$$

$$\frac{d[GAP]}{dt} = v_{ALD,f} - v_{ALD,b} + v_{TPI,f} - v_{TPI,b} - v_{GAPDH,f} + v_{GAPDH,b} \quad \text{(S14)}$$

$$\frac{d[DHAP]}{dt} = v_{ALD,f} - v_{ALD,b} - v_{TPI,f} + v_{TPI,b} - v_{glycerol} \quad \text{(S15)}$$

$$\frac{d[BPG]}{dt} = v_{GAPDH,f} - v_{GAPDH,b} - v_{PGK,f} + v_{PGK,b} \quad \text{(S16)}$$

$$\frac{d[3PG]}{dt} = v_{PGK,f} - v_{PGK,b} - v_{PGM,f} + v_{PGM,b} \quad \text{(S17)}$$

$$\frac{d[2PG]}{dt} = v_{PGM,f} - v_{PGM,b} - v_{ENO,f} + v_{ENO,b} \quad \text{(S18)}$$

$$\frac{d[PEP]}{dt} = v_{ENO,f} - v_{ENO,b} - v_{PYK,f} + v_{PYK,b} \quad \text{(S19)}$$

$$\frac{d[PYR]}{dt} = v_{PYK,f} - v_{PYK,b} - v_{PDC} \quad \text{(S20)}$$

$$\frac{d[AcAld]}{dt} = v_{PDC} - v_{ADH,f} + v_{ADH,b} - 2v_{succinate} \quad \text{(S21)}$$

$$\frac{d[NADH]}{dt} = v_{GAPDH,f} - v_{GAPDH,b} - v_{glycerol} - v_{ADH,f} + v_{ADH,b} + 3v_{succinate} \quad \text{(S22)}$$

$$\frac{d[NAD]}{dt} = -v_{GAPDH,f} + v_{GAPDH,b} + v_{glycerol} + v_{ADH,f} - v_{ADH,b} - 3v_{succinate} \quad \text{(S23)}$$

$$\frac{d[ATP]}{dt} = -v_{HK,f} + v_{HK,b} - v_{trehalose} - v_{glycogen} - v_{PFK} + v_{PGK,f} - v_{PGK,b} + v_{PYK,f} - v_{PYK,b} - 4v_{succinate} - v_{ATPase} + v_{AK,f} - v_{AK,b} \quad \text{(S24)}$$

$$\frac{d[ADP]}{dt} = -v_{HK,f} + v_{HK,b} - v_{trehalose} - v_{glycogen} - v_{PFK} + v_{PGK,f} - v_{PGK,b} + v_{PYK,f} - v_{PYK,b} - 4v_{succinate} - v_{ATPase} - 2v_{AK,f} + 2v_{AK,b} \quad \text{(S25)}$$

$$\frac{d[AMP]}{dt} = v_{AK,f} - v_{AK,b} \quad \text{(S26)}$$

where subscripts "*f*" and "*b*" denote forward and backward fluxes, respectively.

At the steady state, a net metabolic flux distribution can be calculated by MFA with available information of products formation rates scaled by glucose uptake rate (Teusink et al., 2000) (Figure S1, some values have been adjusted to obtain the feasible steady state).

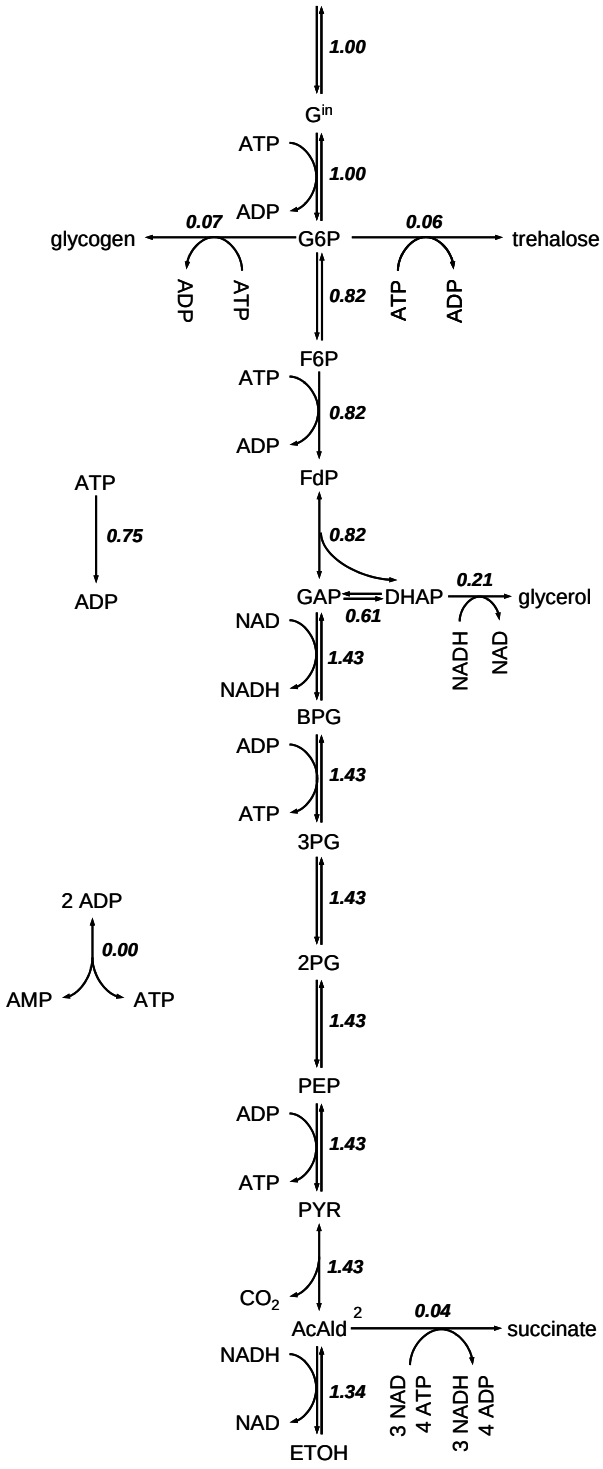

Figure S1. Steady state flux distributions of anaerobic glycolytic pathway model of nongrowing yeast, *Saccharomyces cerevisiae*, with glucose as the sole carbon source.

Forward and backward flux rates for reversible reactions were estimated using the equilibrium coefficients, ρ, according to the information provided in the model (Table S1).

Metabolic Flux	ρ
trans	10000
HK	10000
trehalose production	+∞
glycogen production	+∞
PGI	1.23
PFK	∞
ALD	12.8
TPI	1.02
glycerol production	+∞
GAPDH	1.14

Metabolic Flux	ρ
PGK	1.14
PGM	1.43
ENO	11.5
PYK	128
PDC	+∞
ADH	1.89
succinate production	+∞
ATPase	+∞
AK	1.00

Table S1. Equilibrium coefficients for the fluxes in glycolytic pathway model.

Glycolytic enzyme kinetics and elasticity expressions

For the kinetic mechanisms of the enzymes catalyzing glycolytic fluxes, we follow the published kinetic model (Teusink et al., 2000), and apply the Michaelis-Menten kinetics when information are not available (Segel, 1975).

Glucose transport

The glucose transport across cell membrane follows a symmetric kinetics

$$v_f = v_{\max,f} \frac{g_o}{1 + g_o + g_i + g'_o g_i}, \tag{S27}$$

$$v_b = v_{max,b} \frac{g_i}{1 + g_o + g_i + g_o' g_i}, \tag{S28}$$

where g_o and g_o' are the scaled concentrations of extracellular glucose with respect to two different enzymatic constants, g_i is the scaled concentrations of intracellular glucose. The elasticities can be expressed in the following forms

$$\varepsilon_{f,G^{out}} = \frac{1 + g_i}{1 + g_o + g_i + g_o' g_i}, \tag{S29}$$

$$\varepsilon_{f,G^{in}} = \frac{-g_i - g_o' g_i}{1 + g_o + g_i + g_o' g_i}, \tag{S30}$$

$$\varepsilon_{b,G^{out}} = \frac{-g_o - g_o' g_i}{1 + g_o + g_i + g_o' g_i}, \tag{S31}$$

$$\varepsilon_{b,G^{in}} = \frac{1 + g_o}{1 + g_o + g_i + g_o' g_i}. \tag{S32}$$

According to the Monte Carlo sampling procedure described in the main text, we uniformly sample the degree of enzyme active site saturation, and therefore generate random independent samples of scaled metabolite concentrations, g_o, g_o', and g_i. These samples are used to calculate the elasticities. Figure S2 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of g_o, g_o', and g_i.

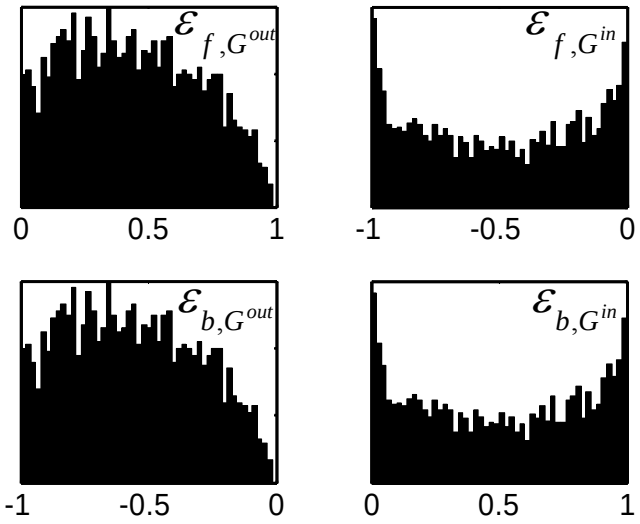

Figure S2. PDFs of the randomized elasticities of *trans*

HK, GAPDH, PGK, and PYK

The kinetics of these four enzymes follows

$$v_f = v_{\max,f} \frac{s_1 s_2}{(1+s_1+p_1)(1+b_2+p_2)}, \tag{S33}$$

$$v_b = v_{\max,b} \frac{p_1 p_2}{(1+s_1+p_1)(1+s_2+p_2)}. \tag{S34}$$

where s_1 and s_2 are the scaled concentrations of substrates (G^{in} and ATP for *HK*, GAP and NAD for *GAPDH*, BPG and ADP for *PGK*, PEP and ADP for *PYK*), p_1 and p_2 are the scaled concentrations of products (G6P and ADP for *HK*, BPG and NADH for *GAPDH*, 3PG and ATP for *PGK*, PYR and ATP for *PYK*). The elasticities can be expressed as

$$\varepsilon_{f,s_1} = \frac{1+p_1}{1+s_1+p_1}, \tag{S35}$$

$$\varepsilon_{f,s_2} = \frac{1+p_2}{1+s_2+p_2}, \tag{S36}$$

$$\varepsilon_{f,p_1} = \frac{-p_1}{1+s_1+p_1}, \tag{S37}$$

$$\varepsilon_{f,p_2} = \frac{-p_2}{1+s_2+p_2}, \tag{S38}$$

$$\varepsilon_{b,s_1} = \frac{-s_1}{1+s_1+p_1}, \tag{S39}$$

$$\varepsilon_{b,s_2} = \frac{-s_2}{1+s_2+p_2}, \tag{S40}$$

$$\varepsilon_{b,p_1} = \frac{1+s_1}{1+s_1+p_1}, \tag{S41}$$

$$\varepsilon_{b,p_2} = \frac{1+s_2}{1+s_2+p_2}. \tag{S42}$$

Similarly, we generate random independent samples of scaled metabolite concentrations, s_1, s_2, p_1 and p_2. These samples are used to calculate the elasticities. Figure S3 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

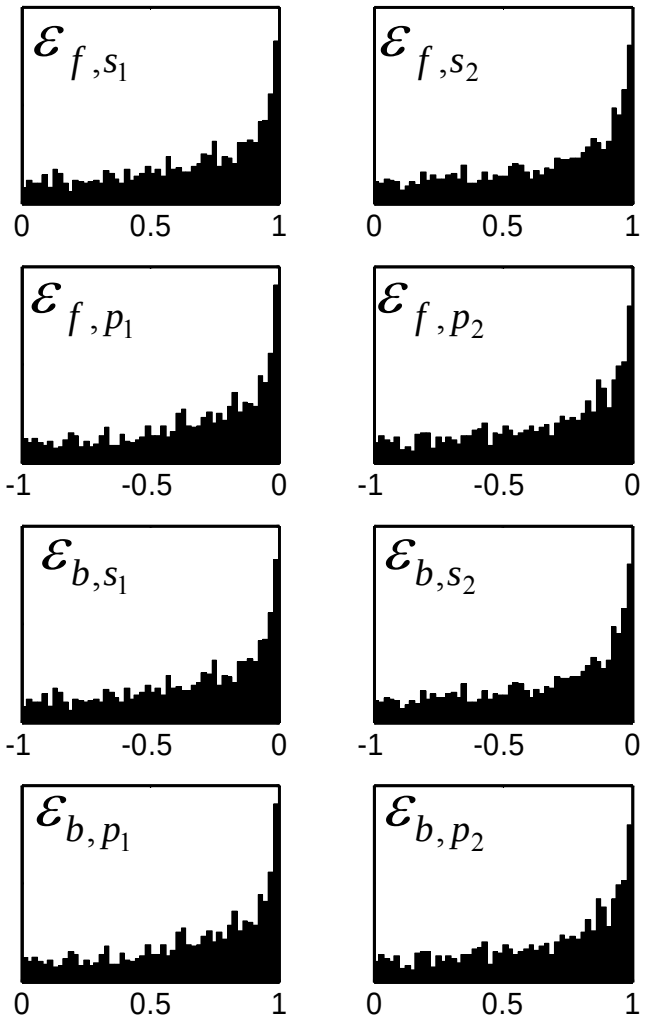

Figure S3. PDFs of the randomized elasticities of *HK*, *GAPDH*, *PGK*, and *PYK*

Trehalose production

The irreversible reaction of trehalose production follows the kinetics

$$v_f = v_{\max,f} \frac{s_1^{\,2} s_2}{1 + s_1^{\,2} + s_2 + s_1^{\,2} s_2'}. \tag{S43}$$

where s_1 is the scaled concentration of G6P, s_2 and s_2' are the scaled ATP concentrations with respect to two enzymatic constants. The corresponding elasticities are

$$\varepsilon_{f,G6P} = \frac{2(1+s_2)}{1+s_1^{\,2}+s_2+s_1^{\,2}s_2'}, \tag{S44}$$

$$\varepsilon_{f,ATP} = \frac{1+s_1^{\,2}}{1+s_1^{\,2}+s_2+s_1^{\,2}s_2'}. \tag{S45}$$

Similarly, we generate random independent samples of scaled metabolite concentrations, s_1, s_2 and s_2'. These samples are used to calculate the elasticities. Figure S4 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

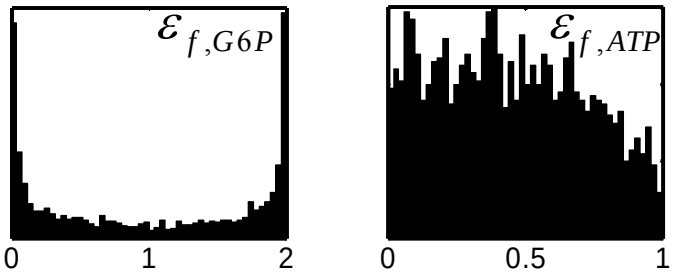

Figure S4. PDFs of the randomized elasticities of trehalose production

Glycogen and glycerol production

These two fluxes follow the irreversible symmetric kinetics,

$$v_f = v_{\max,f}\frac{s_1 s_2}{1+s_1+s_2+s_1's_2}. \tag{S46}$$

where s_1 and s_1' are the scaled concentration of G6P, for glycogen production, and DHAP, for glycerol production, with respect to two enzymatic constants. s_2 is the scaled

concentrations of ATP, for glycogen production, and NADH, for glycerol production. The corresponding elasticities are

$$\varepsilon_{f,G6P/DHAP} = \frac{1+s_2}{1+s_1+s_2+s_1's_2}, \tag{S47}$$

$$\varepsilon_{f,ATP/NADH} = \frac{1+s_1}{1+s_1+s_2+s_1's_2}. \tag{S48}$$

We generate random independent samples of scaled metabolite concentrations, s_1, s_1', and s_2. These samples are used to calculate the elasticities. Figure S5 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

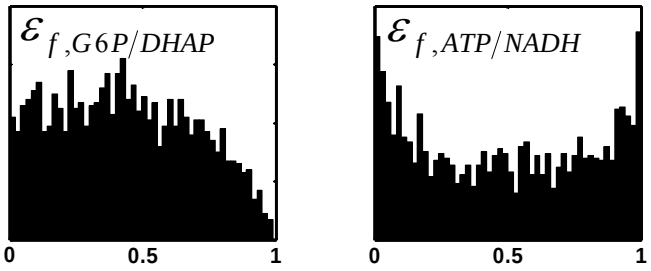

Figure S5. PDFs of the randomized elasticities of glycogen and glycerol production

PGI, PGM, ENO, and TPI

PGI, *PGM*, *ENO* and *TPI* follow one-substrate one-product reversible Michaelis-Menten kinetics,

$$v_f = v_{\max,f}\frac{s}{1+s+p}, \tag{S49}$$

$$v_b = v_{\max,b}\frac{p}{1+s+p}. \tag{S50}$$

where s is the scaled concentrations of the substrate (G6P for *PGI*, 3PG for *PGM*, 2PG for *ENO*, DHAP for *TPI*), p is the scaled concentrations of the product (F6P for *PGI*, 2PG for *PGM*, PEP for *ENO*, GAP for *TPI*). And the elasticities have the forms

$$\varepsilon_{f,s} = \frac{1+p}{1+s+p}, \tag{S51}$$

$$\varepsilon_{f,p} = \frac{-p}{1+s+p}, \tag{S52}$$

$$\varepsilon_{b,s} = \frac{-s}{1+s+p}, \tag{S53}$$

$$\varepsilon_{b,p} = \frac{1+s}{1+s+p}. \tag{S54}$$

We generate random independent samples of scaled metabolite concentrations, s and p. These samples are used to calculate the elasticities. Figure S6 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

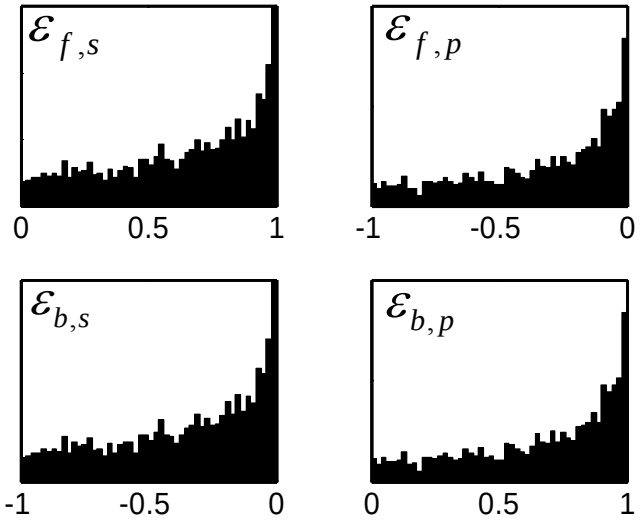

Figure S6. PDFs of the randomized elasticities of *PGI*, *PGM*, *ENO*, and *TPI*

PFK

A simplified kinetics of *PFK* has the form

$$v_f = v_{\max,f} \frac{Rst}{R^2 + LT^2}, \tag{S55}$$

with

$$R = 1 + k_1 st, \tag{S56}$$

$$T = 1 + k_2 t, \tag{S57}$$

$$L = L_m \left(\frac{1 + k_\alpha t}{1 + \alpha t} \right)^2 \left(\frac{1 + k_\beta m}{1 + \beta m} \right)^2, \tag{S58}$$

where s, t, and m are the scaled concentrations of F6P, ATP, and AMP, respectively. After deriving the elasticity expressions and sampling metabolite concentrations with respect to different enzymatic constants, we obtain the PDFs of the elasticities as shown in Figure S7 for 2000 random sets of samples of the scaled concentrations..

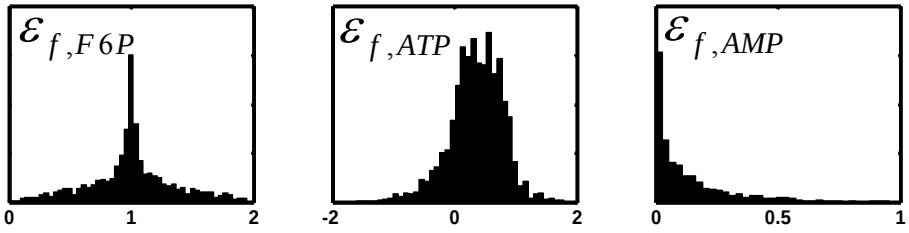

Figure S7. PDFs of the randomized elasticities of *PFK*

ALD

ALD follows the reversible kinetics as

$$v_f = v_{\max,f} \frac{s}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S59}$$

$$v_b = v_{\max,b} \frac{p_1 p_2}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S60}$$

where s and s' are the scaled concentrations of FdP with respect to two different enzymatic constants, p_1 and p_2 are the scaled concentrations of DHAP and GAP. The elasticities are calculated as

$$\varepsilon_{f,FdP} = \frac{1 + p_1 + p_2 + p_1 p_2}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S61}$$

$$\varepsilon_{f,DHAP} = \frac{-p_1 - p_1 p_2}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S62}$$

$$\varepsilon_{f,GAP} = \frac{-\left(p_2 + p_1 p_2 + s' p_2\right)}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S63}$$

$$\varepsilon_{b,FdP} = \frac{-\left(s + s' p_2\right)}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S64}$$

$$\varepsilon_{b,DHAP} = \frac{1 + s + p_2 + s' p_2}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}, \tag{S65}$$

$$\varepsilon_{b,GAP} = \frac{1 + s + p_1}{1 + s + p_1 + p_2 + p_1 p_2 + s' p_2}. \tag{S66}$$

We generate random independent samples of scaled metabolite concentrations, s, s', p_1 and p_2. These samples are used to calculate the elasticities. Figure S8 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

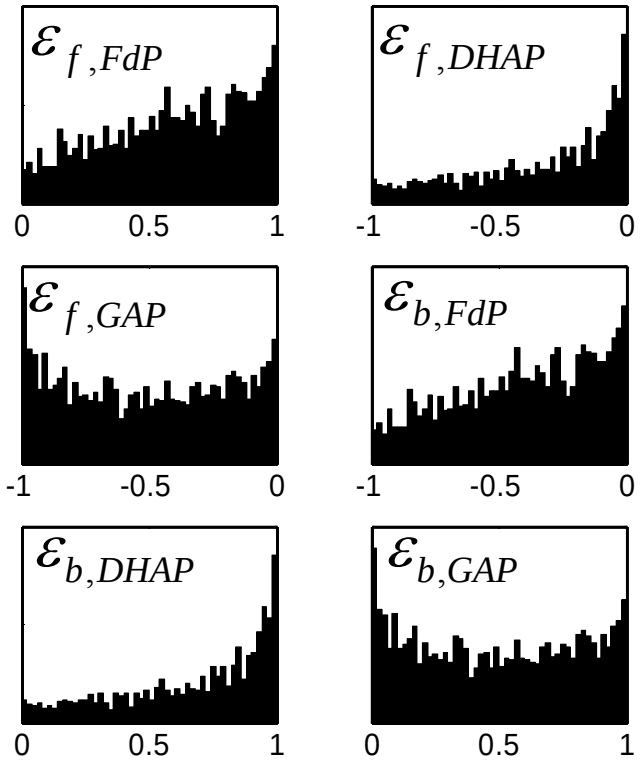

Figure S8. PDFs of the randomized elasticities of *ALD*

PDC

PDC follows irreversible Hill kinetics with a Hill coefficient of 2.

$$v_f = v_{\max,f} \frac{s^2}{1+s^2}, \tag{S67}$$

where s is the scaled PYR concentration. The elasticity has the form

$$\varepsilon_{f,PYR} = \frac{2}{1+s^2}. \tag{S68}$$

We generate random independent samples of scaled metabolite concentrations, s. These samples are used to calculate the elasticities. Figure S9 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

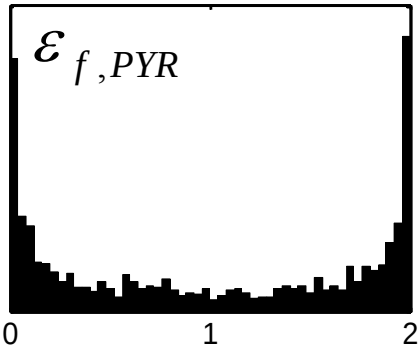

Figure S9. PDF of the randomized elasticities of *PDC*

ADH

ADH follows ordered *bi-bi* kinetics, with cofactor binding first,

$$v_f = v_{\max,f} \frac{s_1 s_2}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S69)}$$

$$v_b = v_{\max,b} \frac{p_1 p_2}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S70)}$$

where s_1 and s_1' are the scaled concentration of AcAld with respect to two different enzymatic constants, s_2 and s_2' are the scaled concentration of NADH with respect to two different enzymatic constants, p_1 and p_1' are the scaled concentration of ETOH with respect to two different enzymatic constants, p_2 and p_2' are the scaled concentration of NAD with respect to two different enzymatic constants. The elasticities can be expressed as

$$\varepsilon_{f,AcAld} = \frac{1 + s_2' + p_1' + p_2 + s_2' p_2 + p_1 p_2 + s_2 p_1 p_2'}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S71)}$$

$$\varepsilon_{f,NADH} = \frac{1 + s_1 + p_1' + p_2 + s_1 p_1' + p_1 p_2}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S72)}$$

$$\varepsilon_{f,ETOH} = \frac{-\left(p_1' + s_1 p_1' + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'\right)}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S73)}$$

$$\varepsilon_{f,NAD} = \frac{-\left(p_2 + s_2' p_2 + p_1 p_2 + s_2 p_1 p'\right)_2}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S74)}$$

$$\varepsilon_{b,AcAld} = \frac{-\left(s_1 + s_1 s_2 + s_1 p_1' + s_1' s_2 p_1\right)}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S75)}$$

$$\varepsilon_{b,NADH} = \frac{-\left(s_2' + s_1 s_2 + s_2' p_2 + s_1' s_2 p_1 + s_2 p_1 p'\right)}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S76)}$$

$$\varepsilon_{b,ETOH} = \frac{1 + s_1 + s_2' + p_2 + s_1 s_2 + s_2' p_2}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}, \quad \text{(S77)}$$

$$\varepsilon_{b,NAD} = \frac{1 + s_1 + s_2' + p_1' + s_1 s_2 + s_1 p_1' + s_1' s_2 p_1}{1 + s_1 + s_2' + p_1' + p_2 + s_1 s_2 + s_1 p_1' + s_2' p_2 + p_1 p_2 + s_1' s_2 p_1 + s_2 p_1 p_2'}. \quad \text{(S78)}$$

We generate random independent samples of scaled metabolite concentrations, s_1, s_1', s_2, s_2', p_1, p_1', p_2, p_2'. These samples are used to calculate the elasticities. Figure S10 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

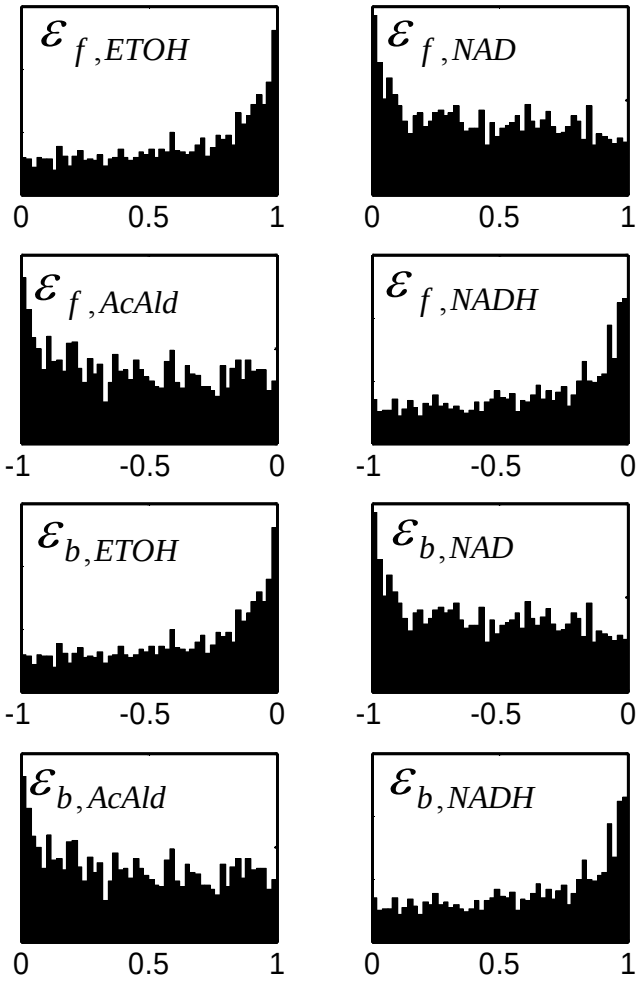

Figure S10. PDF of the randomized elasticities of *ADH*

ATPase and succinate production

These two irreversible processes follow linear kinetics,

$$v_f = ks, \tag{S79}$$

where s is the scaled concentration of the substrate (ATP for *ATPase*, AcAld for succinate production). The elasticities for first order kinetics are always equal to 1:

$$\varepsilon_{f,s} = 1. \tag{S80}$$

AK

AK follows reversible Michaelis-Menten type kinetics as

$$v_f = v_{\max,f} \frac{d^2}{1+d^2+m+t}, \tag{S81}$$

$$v_b = v_{\max,b} \frac{mt}{1+d^2+m+t}, \tag{S82}$$

where d, m, and t represents the scaled concentrations of ADP, AMP, and ATP, respectively. Their elasticities are expressed in the following equations:

$$\varepsilon_{f,ADP} = \frac{2(1+m+t)}{1+d^2+m+t}, \tag{S83}$$

$$\varepsilon_{f,ATP} = \frac{-t}{1+d^2+m+t}, \tag{S84}$$

$$\varepsilon_{f,AMP} = \frac{-m}{1+d^2+m+t}, \tag{S85}$$

$$\varepsilon_{b,ADP} = \frac{-2d^2}{1+d^2+m+t}, \tag{S86}$$

$$\varepsilon_{b,ATP} = \frac{1+d^2+m}{1+d^2+m+t}, \tag{S87}$$

$$\varepsilon_{b,AMP} = \frac{1+d^2+t}{1+d^2+m+t}. \tag{S88}$$

Figure S11 illustrates the PDFs of the randomized elasticities for 2000 random sets of samples of the scaled concentrations.

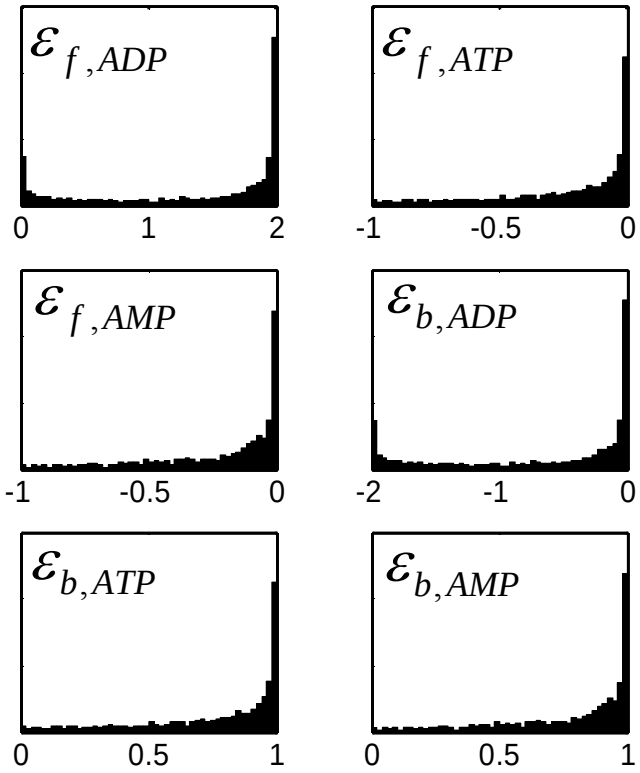

Figure S11. PDF of the randomized elasticities of *AK*

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