Enzyme economy in metabolic networks
Supplementary text

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Contents

S1 Economic balance equations

The economic balance equations (11) and (14) can be illustrated by thought experiments. We assume that metabolite production, metabolite levels, and enzyme levels have an economic value for the cell and that their values are related between enzymes and their substrates and products. In metabolic economics, these values are represented by economic potentials, economic loads, and enzyme benefits. To establish relations between them, we consider hypothetical variations of supply fluxes (or metabolite levels) and enzyme levels, specifically chosen to leave the metabolic benefit unchanged.

S1.1 Reaction balance derived from thought experiment

The reaction balance (11) between fluxes, economic potentials, and flux gains can be derived from a thought experiment. We first consider a reaction without external reactants and direct flux gains ($\hat{z}_l^{\mathrm{v}} = 0$). In a compensated downregulation, the enzyme level is decreased from u_l to $u_l - \delta u_l$, but the effects of this change are compensated by virtual substrate outfluxes and product influxes that keep the net production of all metabolites unchanged (see Figure S1 (a)). Since δu is a differential, we can use a linear approximation: to compensate the flux decrease $\delta v_l = \bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l$, we choose supply fluxes $\delta\varphi_i^{\mathrm{tot}} = n_{il}\,\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l$ (note that they leave the conserved moieties unchanged). The lowered enzyme level reduces the cost by $h_l^{\mathrm{u}}\,\delta u_l$ (where h_l^{u} is the enzyme price in the unperturbed state). The supply fluxes $\delta\varphi_i^{\mathrm{tot}}$ come at a cost $w_i\,\delta\varphi_i^{\mathrm{tot}}$, where w_i, yet to be determined, is the economic potential of metabolite i. The net benefit from the supply fluxes reads

$$\sum_i w_i\,\delta\varphi_i^{\mathrm{tot}} = \sum_i w_i^{\mathrm{ind}}\,n_{il}^{\mathrm{ind}}\,\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l = \Delta w_l\,\frac{v_l}{u_l}\,\delta u_l. \tag{S1}$$

Now we assume that the compensated enzyme variation is cost-neutral, i.e., the saved cost $h_l^{\mathrm{u}}\,\delta u_l$ for the enzyme and the additional cost for the supply fluxes cancel each other. Equating the two and dividing by δu_l yields the balance equation

$$\Delta w_l = h_l^{\mathrm{u}}\,u_l/v_l. \tag{S2}$$

In a variant of the thought experiment, we assume that the reaction contains external metabolites. Again, we apply a compensated downregulation. Since external metabolites need not be balanced, no supply fluxes are needed for them. However, if the external metabolites have production gains (vector $\mathbf{w}^{\mathrm{x}}$), the altered flux will change the production benefit (Figure S1 (b)). With the definition $\Delta\mathbf{w} = \mathbf{N}^{\mathrm{c}\top}\mathbf{w}^{\mathrm{c}} + \mathbf{N}^{\mathrm{x}\top}\mathbf{w}^{\mathrm{x}}$, we obtain again Eq. (S2). Finally, we can assume that the reaction has a direct flux gain. In this case, the flux change $\delta v = \bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l$ will affect the metabolic benefit, changing the fitness by $\hat{z}_l^{\mathrm{v}}\,\delta v_l = \hat{z}_l^{\mathrm{v}}\,\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l$. Now the saved enzyme cost must be balanced with the cost for supply fluxes minus the benefit change due to δv_l:

$$y_l\,\delta u_l = \left[\Delta z_l^{\mathrm{v}}\,\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l\right] + \hat{z}_l^{\mathrm{v}}\,\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\delta u_l = \left[\Delta w_l + \hat{z}_l^{\mathrm{v}}\right]\,\frac{v_l}{u_l}\,\delta u_l. \tag{S3}$$

This is our general reaction balance. In the thought experiment, it followed from the fitness change of a compensated flux variations is given by the direct flux benefit. Since the compensation fluxes respect moiety conservation, the thought experiment also works for models with conserved moieties.

S1.2 Compound balance derived from thought experiment

The compound balance, which describes enzyme costs around a metabolite, can be explained by a similar thought experiment. If an external metabolite changes its concentration, this will directly affect the reactions in which it appears as a substrate, product, or regulator. For instance, the level of an imported nutrient directly affects its import rate. In a compensated variation, we increase the metabolite level by a small amount δx_j; this causes flux changes $\delta v_l^* = \bar{E}^{\mathrm{v_l}}_{\mathrm{x_j}}\,\delta x_j$ as a direct effect. These changes, in turn, are compensated by enzyme changes $\delta u_l = -u_l/v_l\,(\bar{E}^{\mathrm{v_l}}_{\mathrm{x_j}}\,\delta x_j)$ chosen to keep the fluxes constant, i.e.

$$\delta v_l \quad = \quad v_l/u_l\,\delta u_l + \bar{E}^{\mathrm{v_l}}_{\mathrm{x_j}}\,\delta x_j = 0. \tag{S4}$$

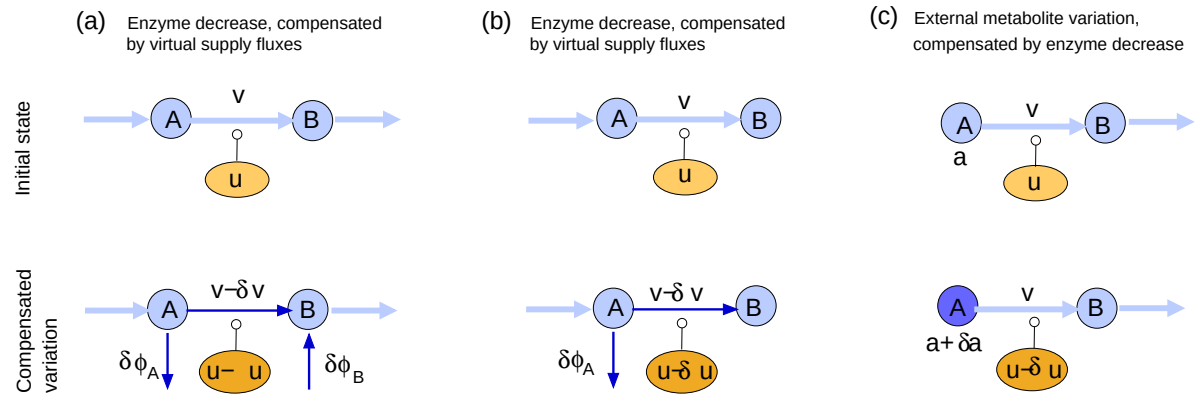

Figure S1: Thought experiments illustrating economic balances between enzyme costs, economic potentials, and economic loads. (a) Reaction balance. A reaction rate is decreased by decreasing the enzyme level; for compensation, virtual supply fluxes are added, keeping the metabolite net production (and thereby, their steady-state concentrations) unchanged. Since the fitness does not change, the cost of supply fluxes (given by $(w_\mathrm{B} - w_\mathrm{A})\,\mathrm{d}v = (w_\mathrm{B} - w_\mathrm{A})\frac{v}{u}\,\mathrm{d}u$) and the cost saving $h^\mathrm{u}\,\mathrm{d}u$ for the enzyme cancel out, yielding $(w_\mathrm{B} - w_\mathrm{A})\,\frac{v}{u} = h^\mathrm{u}$. (b) Reaction balance with an external metabolite: now the benefit loss due to decreased production of B appears in the balance (and no supply flux for B is needed). (c) Compound balance. An increase $\mathrm{d}a$ of the external concentration is compensated by a decrease $\mathrm{d}u = \frac{u}{v}\bar{E}_\mathrm{c}\,\mathrm{d}c$ of the enzyme level.

Together, the enzyme adaptions δu_l entail a cost

$$\delta h = \sum_l h_l^\mathrm{u}\,\delta u_l \quad = \quad -\sum_l h_l^\mathrm{u}\,\frac{u_l}{v_l}\,\bar{E}^{\mathrm{v}_l}_{\mathrm{x}_j}\,\delta x_j. \tag{S5}$$

In our thought experiment for the reaction balance, we imagined that cells can shift costs between enzyme levels and supply fluxes. Now we assume the same thing for enzyme levels and external concentrations: if the cell can increase the external concentration by δx, it can save enzyme costs worth $\frac{\partial g}{\partial x_j}\delta x$. The prefactor – the economic load of the external metabolite – is given by the benefit that would result from a non-compensated increase. Therefore, the benefit due to the changed metabolite concentration, $\delta f = \frac{\partial g}{\partial x_j}\delta x_j$ must be compensated by an enzyme cost. After dividing by δx, we obtain the local balance equation

$$\delta h/\delta x_j = -\sum_l \frac{u_l\,h_l^\mathrm{u}}{v_l}\,\bar{E}^{\mathrm{v}_l}_{\mathrm{x}_j} = -\frac{\partial g}{\partial x_j} \tag{S6}$$

for external metabolites. For internal metabolites, we consider a variant of this thought experiment. We imagine a variation of enzyme levels that specifically change one internal concentration, but leave all stationary fluxes constant: we consider the m^th independent internal metabolite; the m^th column of the link matrix $\mathbf{L}$ is called $\boldsymbol{\lambda}$, and the m^th column of the matrix product $\bar{\mathbf{E}}\,\mathbf{L}$ is called $\boldsymbol{\eta}$. From the connectivity theorem of metabolic control theory, we obtain the identities $\mathbf{C}^\mathrm{J}\,\boldsymbol{\eta} = 0$ and $\mathbf{C}^\mathrm{S}\,\boldsymbol{\eta} = -\boldsymbol{\lambda}$. We now consider enzyme changes to $\delta u_l = -\frac{u_l}{v_l}\,\eta_l\,\delta\omega$ with some small number $\delta\omega$. As a result, the stationary fluxes and concentrations change by

$$\begin{aligned} \delta\mathbf{v} \quad &= \quad -\mathbf{C}^\mathrm{J}\frac{v}{u}\,\delta\mathbf{u} = \mathbf{C}^\mathrm{J}\boldsymbol{\eta}\,\delta\omega = 0 \\ \delta\mathbf{c} \quad &= \quad -\mathbf{C}^\mathrm{S}\frac{v}{u}\,\delta\mathbf{u} = \mathbf{C}^\mathrm{J}\boldsymbol{\eta}\,\delta\omega = \boldsymbol{\lambda}\,\delta\omega \end{aligned} \tag{S7}$$

As intended, all fluxes remain constant and all metabolite levels remain unchanged except for our metabolite m and metabolites that depend on it via conservation relations. To make the compensated variation fitness-neutral, the benefit change

$$\delta g \quad = \quad \mathbf{z}^{\mathrm{c}\top}\delta\mathbf{c} = \mathbf{z}^{\mathrm{c}\top}\boldsymbol{\lambda}\,\delta\omega. \tag{S8}$$

and the enzyme investment change

$$\delta h \quad = \quad \mathbf{h}^{\mathrm{u}\top}\delta\mathbf{u} = -\mathbf{h}^{\mathrm{u}\top}\mathrm{Dg}\left(\frac{\mathbf{u}}{\mathbf{v}}\right)\boldsymbol{\eta}\,\delta\omega. \tag{S9}$$

must be balanced. By equating $\delta g = \delta h$ and dividing by $\delta\omega$, we obtain the compound balance for independent internal metabolites

$$\mathbf{z}^{\mathrm{c}\top}\boldsymbol{\lambda} = -\mathbf{h}^{\mathrm{u}\top}\mathrm{Dg}\left(\frac{\mathbf{u}}{\mathbf{v}}\right)\boldsymbol{\eta}. \tag{S10}$$

Compound law with moiety conservation In models with conserved moieties, the variation of a metabolite concentration will not be fully neutralised by the system response. A concentration variation can be split into a "controllable" contribution that can be neutralised by the system, and a "non-controllable" contribution that shift the conserved moieties. With this additional effect, the thought experiment looks as follows: We consider a local variation of metabolite levels, described by a variation vector $\delta\boldsymbol{\gamma}$. The direct effect on adjacent reaction rates is $\delta\mathbf{v} = \bar{\mathbf{E}}\,\delta\boldsymbol{\gamma}$, causing a benefit variation $\mathbf{g}^{\mathrm{v}\top}\bar{\mathbf{E}}\,\delta\boldsymbol{\gamma}$ where $\mathbf{g}^{\mathrm{v}} = \Delta\mathbf{w} + \hat{\mathbf{z}}^{\mathrm{v}}$. Thus, the local fitness balance reads

$$\delta\, g = \mathbf{z}^{\mathrm{c}\top}\,\delta\boldsymbol{\gamma} + \mathbf{z}^{\mathrm{v}\top}\,\bar{\mathbf{E}}\,\delta\boldsymbol{\gamma}. \tag{S11}$$

Alternatively, the fitness balance can be written in terms of systemic effects

$$\delta\, g = \mathbf{z}^{\mathrm{c}\top}\,C^{\mathrm{S}}_{\boldsymbol{\gamma}}\,\delta\boldsymbol{\gamma} + \mathbf{z}^{\mathrm{v}\top}\,C^{\mathrm{J}}_{\boldsymbol{\gamma}}\,\delta\boldsymbol{\gamma}. \tag{S12}$$

where $C^{\mathrm{S}}_{\boldsymbol{\gamma}}$ and $C^{\mathrm{J}}_{\boldsymbol{\gamma}}$ are the control matrices with respect to virtual concentration variations $\delta\boldsymbol{\gamma}$. By equating both expressions and omitting $\delta\boldsymbol{\gamma}$, we obtain

$$[\mathbf{z}^{\mathrm{c}\top}\,C^{\mathrm{S}}_{\boldsymbol{\gamma}} + \mathbf{z}^{\mathrm{v}\top}\,C^{\mathrm{J}}_{\boldsymbol{\gamma}}] - \mathbf{z}^{\mathrm{c}\top} = \mathbf{g}^{\mathrm{v}\top}\,\bar{\mathbf{E}}. \tag{S13}$$

This is the compound rule

$$\mathbf{p}^{\mathrm{c}\top} = \mathbf{g}^{\mathrm{v}\top}\,\bar{\mathbf{E}}. \tag{S14}$$

S1.3 Metabolic economics requires reversible rate laws

If a model contains irreversible rate laws, there may be enzymes that catalyse a flux but have no control over it. This is a paradoxical situation, and in metabolic economics, it could lead to paradoxical results. As an example, consider a linear pathway with a flux objective (Figure S2). If the first rate law is irreversible (and not allosterically regulated), the other enzymes have no flux control and their enzyme benefits vanish. A virtual supply of intermediates could not slow down the first reaction and would directly add to the pathway flux, so all intermediates have the same economic potential as the end product. This is surprising, but agrees with the vanishing enzyme benefits. Since enzyme benefits and costs must be balanced, all enzyme levels except for the first must vanish, so there cannot even be a flux: in brief, the model has no enzyme-optimal steady state[1]. In models with reversible rate laws only, this would not occur: each enzyme would have some flux control, and supply fluxes of the downstream metabolites would contribute more strongly to the production objective, so the economic potentials would increase along the pathway. To ensure this, models in metabolic economics should only contain reversible rate laws.

S2 Extensions of metabolic economics

Metabolic economics as described in the article makes some simplifying assumptions that limit its use. We shall now drop some of them to extend the theory.

[1] If we optimise the enzyme levels numerically, all enzyme levels except for the first will decrease until a flux imbalance makes the steady state break down. In any case, the steady state will be disrupted before costs and benefits can become balanced.

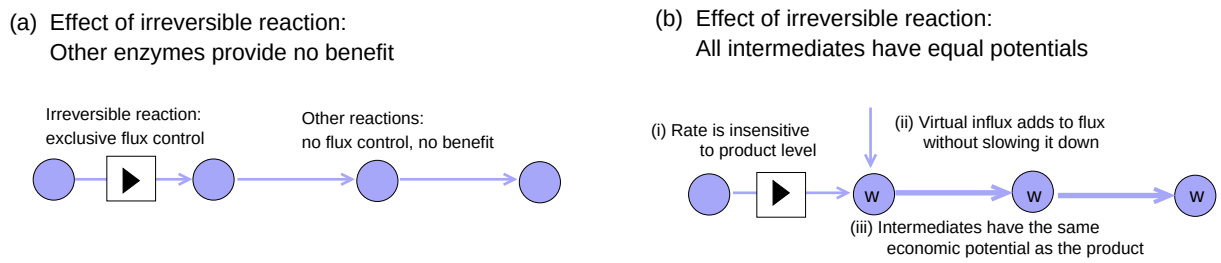

Figure S2: Kinetic models in metabolic economics must have thermodynamically feasible, reversible rate laws. The example shows why. (a) Simple pathway with flux objective. If the first rate law is irreversible, the other enzymes have no flux control and therefore provide no benefit. In enzyme-optimal states, their levels would have to vanish, which would make the flux break down. (b) Also caused by the irreversible reaction, supply fluxes would directly add to the pathway flux (instead of slowing down the upstream reactions, as usually). All intermediates have to same economic potential as the end product, which agrees with the vanishing enzyme demands between them. This paradoxical effect can be avoided by banning irreversible rate laws.

S2.1 Isoenzymes

So far, we assumed that each reaction is catalysed by a single specific enzyme. In reality, several proteins may catalyse one reaction (isoenzymes) and a single enzyme may catalyse several reactions (unspecific enzyme). In models, isoenzymes can be described by separate reactions showing the same sum formula and direct flux demand. Summing over the reaction balances (indices l for isoenzymes), we obtain the joint reaction balance (index L)

$$[z_L^{\mathrm{v}} + \Delta w_L]\, v_L = \sum_{l \in L} h_l^{\mathrm{u}}\, u_l \tag{S15}$$

If the enzymes are not involved in any other reactions, we can replace them by a single enzyme with level $u_L = \sum u_l$ and enzyme price $h_L^u = \frac{\sum_l h_l^{\mathrm{u}}\, u_l}{\sum_l u_l}$.

S2.2 Unspecific enzymes

Unspecific (or multifunctional) enzymes require a change in the reaction balance equation. Consider an enzyme (index l) that catalyses several reactions (indices $j \in J(l)$). In the reaction balance, we sum over all enzyme benefits arising from the different reactions:

$$\sum_{j \in J(l)} [\Delta w_j + z_{\mathrm{v}_j}]\, v_j = h_{u_l} u_l. \tag{S16}$$

The engagement in several reactions has two effects. First, if all reactions $j \in J$ show positive enzyme benefits, the enzyme cost will be higher than for an enzyme catalysing only a single reaction. This is plausible: since more substrates compete for the enzyme, a higher total enzyme level is needed to catalyse all reactions, which leads to a higher cost. Second, some of the reactions may have a negative benefit, but can still be active if the same enzyme catalyses other reactions with a strong positive benefit.

S2.3 Conserved moieties and gauging of economic potentials

Some metabolic models contain conserved moieties. For instance, if ADP and ATP are interconverted, but not individually produced or consumed by any reaction, the sum of their concentrations will be constant in time. Such conserved moieties are associated with left-kernel vectors of the internal stoichiometric matrix $\mathbf{N}$.

Metabolites forming in a conserved moiety have dependent concentrations. To account for this in MCA and metabolic economics, we pick a set of independent metabolites (concentration vector $\mathbf{c}^{\mathrm{ind}}$), which uniquely

determine all metabolite concentrations. The concentration changes of all metabolites can then be written as $\mathrm{d}\mathbf{c}/\mathrm{d}t = \mathbf{L}\,\mathrm{d}\mathbf{c}^{\mathrm{ind}}/\mathrm{d}t$, with a link matrix $\mathbf{L}$ satisfying $\mathbf{N} = \mathbf{L}\,\mathbf{N}_{\mathrm{R}}$.

In metabolic economics, we consider conservation relations involving not only internal, but also external metabolites. Therefore, we define conserved moiety vectors with respect to the full stoichiometric matrix $\mathbf{N}^{\mathrm{tot}}$, satisfying $\mathfrak{g}^\top \mathbf{N}^{\mathrm{tot}} = 0$. For metabolic economics, conserved moieties have several consequences:

1. The economic potentials of internal metabolites are defined by virtual supply fluxes. If a metabolic model contains linear conservation relations, these influxes must not affect the conserved moieties (otherwise they the state would become non-stationary). A consistent definition of economic potentials for this case is as follows: The supply fluxes need to have the form $\boldsymbol{\varphi}^{\mathrm{tot}} = \mathbf{L}\,\boldsymbol{\varphi}^{\mathrm{ind}}$ with supply fluxes $\boldsymbol{\varphi}^{\mathrm{ind}}$ for independent metabolites. In the definition, this yields the economic potentials for independent internal metabolites, while the dependent internal metabolites have vanishing economic potentials.

2. When we define economic potentials in kinetic models, the definition is unique but refers to a specific choice of independent metabolites. However, there remains some gauge freedom: in the reaction balance, economic potentials do not appear separately, but only in the form of differences along reactions. If metabolites form a conserved moiety, their economic potentials can be gauged without affecting the balances $\Delta\mathbf{w}_l$, and therefore the balance equation. Adding a conserved moiety vector $\mathfrak{g}$ to the economic potential vector ($\mathbf{w} \to \mathbf{w} + \mathfrak{g}$), does not change the economic potential balances because ${\mathbf{N}^{\mathrm{tot}}}^\top[\mathbf{w} + \mathfrak{g}] = {\mathbf{N}^{\mathrm{tot}}}^\top\mathbf{w}$. If economic potentials are obtained "experimentally", it is important to note that this gauging freedom exists, and that the "absolute" economic potentials within conserved moieties have no influence on enzyme usage.

There is also a gauge freedom for metabolites that do not participate in active reactions, and in cases where the flux distribution consists of disconnected parts (not connected by any internal nor external metabolites).

S2.4 Enzyme optimisation under constraints

The fact that enzyme levels cannot be negative is implemented in metabolic economics as a constraint $u_l \geq 0$. However, we may consider more constraints, for instance that metabolites like ATP must stay above some minimal level. Minimal values for fluxes, ranges for concentrations, and bounds on the total enzyme level or on certain enzyme fractions (e.g., enzymes in mitochondrial membranes [12]) can be written as linear inequalities

$$\mathbf{M}_{\mathrm{v}}\,\mathbf{v} \geq \mathbf{b}_{\mathrm{v}}, \qquad \mathbf{M}_{\mathrm{c}}\,\mathbf{c} \geq \mathbf{b}_{\mathrm{c}}, \qquad \mathbf{M}_{\mathrm{u}}\,\mathbf{u} \geq \mathbf{b}_{\mathrm{u}}. \tag{S17}$$

In an enzyme-optimal state, some of these inequalities will be active and can be treated like equality constraints. With Lagrange multipliers (in vectors $\boldsymbol{\lambda}_{\mathbf{v}}$, $\boldsymbol{\lambda}_c$, and $\boldsymbol{\lambda}_{\mathbf{u}}$), the constrained optimisation problem reads

$$\min \stackrel{!}{=} f(\mathbf{u}) + \boldsymbol{\lambda}_{\mathrm{v}}^\top \mathbf{M}_{\mathrm{v}}\,\mathbf{J}(\mathbf{u}) + \boldsymbol{\lambda}_{\mathrm{c}}^\top \mathbf{M}_{\mathrm{c}}\,\mathbf{S}(\mathbf{u}) - \boldsymbol{\lambda}_{\mathrm{u}}^\top \mathbf{M}_{\mathrm{u}}\,\mathbf{u}. \tag{S18}$$

The resulting cost-benefit balance resembles Eq. (3), but with gain vectors $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$ replaced by effective gain vectors $\mathbf{z}^{\mathrm{v}\prime} = \mathbf{z}^{\mathrm{v}} + \mathbf{M}_{\mathrm{v}}^\top \boldsymbol{\lambda}_{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}\prime} = \mathbf{z}^{\mathrm{c}} + \mathbf{M}_{\mathrm{c}}^\top \boldsymbol{\lambda}_{\mathrm{c}}$, and with the enzyme price vector $\mathbf{h}^{\mathrm{u}}$ replaced by an effective vector $\mathbf{h}^{\mathrm{u}\prime} = \mathbf{h}^{\mathrm{u}} + \mathbf{M}_{\mathrm{u}}^\top \boldsymbol{\lambda}_{\mathrm{u}}$. The effective vectors do not only represent actual gains and prices, but also effective gains and prices caused by the constraints.

S2.5 Inactive enzymes

How do inactive reactions fit into metabolic economics? In an enzyme-optimal state, reactions are inactive if expressing the enzyme would not pay off, i.e. if already at small expression levels, the enzyme cost exceeds the benefit. The case in which an enzymes are inactive, but their costs and benefits are exactly balanced, is unlikely and can be ignored. Instead of a cost-benefit balance (3), inactive reactions ($v_l = 0$) satisfy an inequality $\frac{\partial g}{\partial u_l} < \frac{\partial h}{\partial u_l}$. The difference $\frac{\partial g}{\partial u_l} - \frac{\partial h}{\partial u_l}$ is called *economic stress*. Since $g_l^{\mathrm{u}} = g_l^{\mathrm{v}}\,\frac{\partial v_l}{\partial u_l}$, we obtain an economic imbalance $g_l^{\mathrm{v}} < h_l^{\mathrm{u}}(\frac{\partial v_l}{\partial u_l})^{-1}$. Inactive reactions in an enzyme-optimal state will remain inactive under infinitesimal

perturbations (e.g. virtual enzyme changes). Thus for most practical purposes, inactive reactions can simply be omitted from the models. The gain conditions (6) and (7) (which underly most formulae in metabolic economics) do not apply if some of the reaction fluxes vanish. To make them applicable, we omit the inactive reactions from the network and study only the active region. The central formulae – the gain conditions and economic balance equations – hold only for complete flux distributions, but can be applied to incomplete flux distributions after the inactive reactions have been omitted. In the formulae, matrices like $\mathbf{N}, \mathbf{K}, \mathbf{L}, \bar{\mathbf{E}}$ and the test modes $\mathbf{k}$ have to refer to the active region. To justify why reactions are inactive in enzyme-optimal states, we can assume that their enzymes are very costly.

S2.6 Leaky enzyme expression

There is another way to model inactive reactions: we assume that enzyme levels cannot be shut down completely. Due to leaky enzyme expression, there remains a small enzyme level ε, whose complete suppression would be difficult. In a model, this is expressed by an extra cost term $h^{\mathrm{leak}}(\mathbf{u})$, which rises as the enzyme level becomes very small. For instance, we may set $h^{\mathrm{leak}}(\mathbf{u}) = \sum_l \varepsilon^2/u_l$ with the gradient $h^{\mathrm{leak}}_{u_l} = -(\varepsilon/u_l)^2$. The new fitness function, including this cost term, diverges for $u_l \to 0$ and has no boundary optima, but an internal optimum with positive (possibly very small) enzyme levels. From the optimality condition $\max \stackrel{!}{=} g(\mathbf{u}) - h(\mathbf{u}) - h^{\mathrm{leak}}(\mathbf{u})$, we obtain the cost-benefit balance

$$g_{u_l} - h^{\mathrm{u}}_l = h^{\mathrm{leak}}_{u_l} = -(\varepsilon/u_l)^2. \tag{S19}$$

The right-hand side approaches zero if $u_l \gg \varepsilon$ and negative otherwise (corresponding to boundary optima in usual metabolic economics). The resulting reaction balance

$$[\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l = h^{\mathrm{u}}_l\, u_l - \varepsilon^2/u_l \tag{S20}$$

contains a second cost term, which replaces the Lagrange multiplier associated with the hard constraint $u_l > 0$. For active enzymes, the normal cost term dominates, but for inactive enzymes the stress term becomes important.

S2.7 Non-optimal states and economic imbalance

In the reaction balance Eq. (11), we assume that costs and benefits of active enzymes are balanced. We can also model non-optimal states, i.e. states in which the reaction balance is violated. For example, if an enzyme (index l) has been knocked down, its level will be below the optimal value, even if other enzyme levels are adjusted to buffer the price. In other cases, enzymes may show non-optimal levels directly after an external change. The mismatch between optimal and actual enzyme costs is called *economic stress*. To describe non-optimal states, we assume that non-optimal enzymes satisfy an inequality

$$[\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l \neq h^{\mathrm{u}}_l\, u_l. \tag{S21}$$

It states that a change in regulation – if it were possible – would be profitable. We can formally replace this inequality by a modified reaction balance

$$[\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l = h^{\mathrm{u}}_l\, u_l + y^{\Delta}_l \tag{S22}$$

with the economic stress $y^{\Delta}_l = \partial f/\partial u_l = [\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l - h^{\mathrm{u}}_l\, u_l$. Here h^{u}_l is the actual cost of the enzyme; the economic stress tells us how strongly the reaction balance is violated. If the enzyme level is too low, the economic stress will be positive; if the level is too high, it is negative.

S2.8 Non-enzymatic reactions

Kinetic models with non-enzymatic reactions and dilution So far we assumed that all chemical reactions were catalysed by enzymes, which makes them directly controllable. In reality, some reactions happen spontaneously, ranging from fast protonation and deprotonation of acids to the damage of proteins or DNA by free

radicals. Moreover, all metabolites in growing cells are diluted: if metabolites are described by concentrations, dilution acts as an effective degradation, with a rate constant κ given by the cell growth rate. In metabolic economics, non-enzymatic processes require a change in the formulae: for instance, we saw that economic potentials are typically rising along synthesis pathways. If the end product is diluted, the dilution reaction has a negative balance of economic potentials, so the flux distribution looks futile. However, the flux may be needed to keep the end product at a constant concentration (to meet the concentration gain).

If spontaneous reactions or dilution exist, fluxes and concentrations, economic potentials and loads become coupled. To describe this, some of the equations of metabolic economics need to be modified. We consider models with non-enzymatic reactions and dilution (where dilution fluxes are not part of the flux vector $\mathbf{v}$). The system equations

$$\mathrm{d}\mathbf{c}/\mathrm{d}t = \mathbf{N}^{\mathrm{enz}}\,\mathbf{v}^{\mathrm{enz}} + \mathbf{N}^{\mathrm{non}}\,\mathbf{v}^{\mathrm{non}} - \kappa\,\mathbf{c} \tag{S23}$$

contain terms for enzymatic and non-enzymatic reactions as well as dilution. The flux vector $\mathbf{v} = \binom{\mathbf{v}^{\mathrm{enz}}}{\mathbf{v}^{\mathrm{non}}}$, the stoichiometric matrix $\mathbf{N} = (\mathbf{N}^{\mathrm{enz}}|\mathbf{N}^{\mathrm{non}})$, and the internal elasticity matrix $\bar{\mathbf{E}} = \binom{\bar{\mathbf{E}}^{\mathrm{enz}}}{\bar{\mathbf{E}}^{\mathrm{non}}}$ are split accordingly. The Jacobian matrix with dilution can be split into three terms: $\mathbf{M}^{\mathrm{dil}} = \mathbf{M}^{\mathrm{enz}} + \mathbf{M}^{\mathrm{non}} - \kappa\,\mathbf{I}$. It contains the terms $\mathbf{M}^{\mathrm{enz}} = \mathbf{N}_{\mathrm{R}}^{\mathrm{enz}}\,\bar{\mathbf{E}}^{\mathrm{enz}}\,\mathbf{L}$ (for enzymatic reactions), $\mathbf{M}^{\mathrm{non}} = \mathbf{N}_{\mathrm{R}}^{\mathrm{non}}\,\bar{\mathbf{E}}_{\mathrm{c}}^{\mathrm{non}}\,\mathbf{L}$ (for non-enzymatic reactions) and $-\kappa\,\mathbf{I}$ (for dilution). Metabolic control coefficients for such systems, as well as their summation and elasticity theorems are given in SI P1.1.

Demand conditions with dilution The gain conditions for models with dilution read (proof see P1.3)

$$\begin{aligned} \mathbf{K}^{\top}\,\mathbf{z}^{\mathrm{v}} &= \mathbf{K}^{\top}\,\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}} \\ -\mathbf{L}^{\top}\,\mathbf{z}^{\mathrm{c}} - \kappa\,(\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1})^{\top}\,\mathbf{z}^{\mathrm{c}*} &= (\bar{\mathbf{E}}\,\mathbf{L})^{\top}\,\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}. \end{aligned} \tag{S24}$$

where $\mathbf{z}^{\mathrm{c}*} = \bar{\mathbf{E}}^{\top}\,\mathbf{z}^{\mathrm{v}} + \mathbf{z}^{\mathrm{c}}$. The flux gain condition remains unchanged, while the concentration gain condition contains the extra term $-\kappa\,(\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1})^{\top}\,\mathbf{z}^{\mathrm{c}*}$.

Demand conditions with non-enzymatic reactions For the gain conditions as shown in the paper, all reactions in a flux distribution must be active and enzyme-catalysed. Non-enzymatic reactions cause no direct enzyme costs, but they also cannot be blocked even if they degrade valuable metabolites, and may therefore require costly enzyme investments in other places. This must be captured somehow by the gain conditions. Non-enzymatic reactions give rise to an extra term in the flux gain condition. For simplicity, let the list of reactions be ordered (enzymatic reactions first, then non-enzymatic reactions). Thus, flux vectors can be split into subvectors $\mathbf{v} = \binom{\mathbf{v}_{\mathrm{enz}}}{\mathbf{v}_{\mathrm{non}}}$. We require optimal enzyme levels, consider the cost-benefit balance (3), and obtain flux and concentration gain conditions for the active enzymatic reactions (Proof in section P1.4):

$$\mathbf{k}\cdot\mathbf{z}^{\mathrm{v}} - \mathbf{k}_{\mathrm{non}}^{\top}\mathbf{g}_{\mathrm{non}}^{\mathrm{v}} = \mathbf{k}_{\mathrm{enz}}^{\top}\mathrm{Dg}(\mathbf{y})\mathbf{v}_{\mathrm{enz}}^{-\mathbf{1}} \tag{S25}$$

$$-(\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{non}}^{\top}\,\mathbf{g}_{\mathrm{non}}^{\mathrm{v}} = ((\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{enz}})^{\top}\mathrm{Dg}(y_{\mathrm{enz}})\mathbf{v}_{\mathrm{enz}}^{-1}. \tag{S26}$$

The enzyme cost vector $\mathbf{y} = \mathrm{Dg}(\mathbf{u})\,\mathbf{h}^{\mathrm{u}}$ refers to the enzymatic reactions, and $\mathbf{g}_{\mathrm{non}}^{\mathrm{v}} = [\Delta\mathbf{w}^{\mathrm{c}}{}_{\mathrm{non}} + \mathbf{z}_{\mathrm{non}}^{\mathrm{v}}]$. Let us focus on the flux gain condition (S27). The active non-enzymatic reactions contribute an additional term. With $\mathbf{k}\cdot\mathbf{z}^{\mathrm{v}} = \mathbf{k}_{\mathrm{enz}}\cdot\mathbf{z}^{\mathrm{v}}{}_{\mathrm{enz}} + \mathbf{k}_{\mathrm{non}}\cdot\mathbf{z}^{\mathrm{v}}{}_{\mathrm{non}}$, we can rewrite the equation as

$$\begin{aligned} \mathbf{k}_{\mathrm{enz}}\cdot\mathbf{z}_{\mathrm{enz}}^{\mathrm{v}} - \mathbf{k}_{\mathrm{non}}\cdot\Delta\mathbf{w}^{\mathrm{c}}{}_{\mathrm{non}} &= \mathbf{k}_{\mathrm{enz}}^{\top}\mathrm{Dg}(\mathbf{y})\mathbf{v}_{\mathrm{enz}}^{-\mathbf{1}} \\ \Rightarrow \mathbf{k}\cdot\begin{pmatrix}\mathbf{z}_{\mathrm{enz}}^{\mathrm{v}} \\ -\Delta\mathbf{w}^{\mathrm{c}}{}_{\mathrm{non}}\end{pmatrix} &= \mathbf{k}_{\mathrm{enz}}^{\top}\mathrm{Dg}(\mathbf{y})\mathbf{v}_{\mathrm{enz}}^{-\mathbf{1}}. \end{aligned} \tag{S27}$$

Equations (S27) and (S29) differ from the usual flux gain condition in two ways: aside from the term for enzyme-catalysed reactions, there is a term describing how non-enzymatic reactions contribute to the benefit. To see what it does, we consider two special cases: (i) For test modes $\mathbf{k}$ containing only enzymatic reactions, we reobtain the original flux gain condition. (ii) After inserting a mode $\mathbf{v}$ as its own test mode, Eq. (S27) leads to the balance

$$\sum_l y_l = \mathbf{v}\cdot\mathbf{z}^{\mathrm{v}} - \mathbf{v}_{\mathrm{non}}\cdot[\Delta\mathbf{w}_{\mathrm{non}} + \mathbf{z}_{\mathrm{non}}^{\mathrm{v}}]^{\top}. \tag{S28}$$

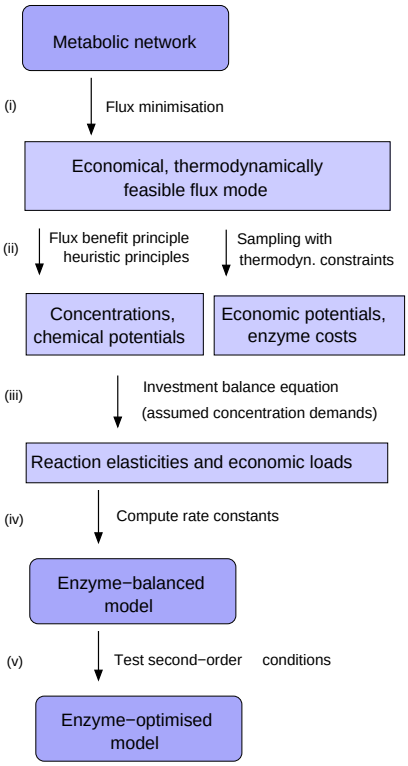

Figure S3: Algorithm for model construction: (i) Given the metabolic network, an economical, thermodynamically feasible flux distribution is determined (e.g. by flux cost minimisation). (ii) Consistent economic potentials and enzyme costs (satisfying the reaction balance) and (iii) reaction elasticities and economic loads (satisfying the compound balance), are computed as described in SI S3. (iv) The rate constants are computed from the reaction elasticities. (v) Dynamic and economic stability (the second-order conditions) have to be checked separately.

Thus, the total enzyme cost equals the total flux benefit minus a flux benefit caused by non-enzymatic reactions. If this term is positive, enzyme costs can be saved; if it is negative, the enzyme costs will increase. Dilution reactions can be treated similarly as part of the vector $\mathbf{v}_{\mathrm{non}}$.

S3 Enzyme-balanced kinetic models reconstructed from economical fluxes

Enzyme-optimal models and enzyme-optimal states In a kinetic model with optimal enzyme levels, the state variables v_l and c_i, the economic potentials w_i and loads p_i^{c}, and the gain vectors $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$ will satisfy conditions like the cost-benefit balance, gain conditions, absence of futile modes, and the economic balance equations. In practice, optimising the enzyme levels in kinetic models may be difficult, and the state obtained fofrom a model may show implausible metabolite concentrations and fluxes. To construct, instead, enzyme-optimal states matching given flux data, we would have to fit the model parameters, which would drastically increase the numerical effort. As a practical alternative, we may determine a consistent set of state variables (v_l, c_i, μ_i, $E_{\mathrm{c}_i}^{\mathrm{v}_l}$, z_l^{v}, z_i^{c}, and w_i^{c}), and then construct a kinetic model that realises this state. In this construction, the state variables need to satisfy all economic constraints. Once all state variables have been determined, solving for the necessary rate constants is relatively easy.

Necessary and sufficient conditions for enzyme-optimal states To construct enzyme-balanced models with predefined steady states, let us revisit the various conditions for economic enzyme usage. Some conditions entail

each other; some are necessary, and some sufficient for enzyme-optimal states. To distinguish different cases, we call flux distributions enzyme-optimal, enzyme-balanced, or economical.

1. **Enzyme-optimal** A flux distribution $\mathbf{v}$ is called *enzyme-optimal* if it appears in an enzyme-optimal metabolic model satisfying four conditions: (i) the rate laws are reversible and the kinetic constants satisfy Wegscheider conditions and Haldane relations [11]; (ii) the model has a steady state with flux distribution $\mathbf{v}$; (iii) this steady state is stable, i.e. all eigenvalues of the Jacobian matrix (for independent metabolites) have negative real parts; (iv) the state is economically stable, that is, the enzyme levels must represent a local maximum of the fitness function: in the subnetwork of active reactions, the fitness gradient $\mathbf{f}^{\mathrm{u}}$ has to vanish and the fitness curvature matrix $\mathbf{F}_{\mathrm{uu}}$ must be negative definite.

2. **Enzyme-balanced** A flux distribution is called *enzyme-balanced* if it appears in a kinetic model satisfying conditions (i) and (ii), as well as the cost-benefit balance (3). The steady state may be unstable and the enzyme levels may represent any extremum of the fitness function (where $\mathbf{f}_{\mathrm{u}} = 0$), not necessarily an optimum (e.g. $\mathbf{G}_{\mathrm{uu}}$ can have positive eigenvalues). Enzyme-balanced flux modes satisfy the conditions (6) and (7) and the economic balance equations (11) and (14).

3. **Economical** A flux distribution is called *economical* if it satisfies the flux gain condition (6) on its active region with a positive cost vector $\mathbf{y}$. According to theorems 3 and 4, this is equivalent to any of the following conditions: (i) $\mathbf{v}$ satisfies the reaction balance (11) for some economic potentials w_i^{c} and positive enzyme costs y_l; (ii) $\mathbf{v}$ is free of non-beneficial modes; (iii) $\mathbf{v}$ is the solution of a non-flux-enforcing flux cost minimisation problem.

Constructing kinetic models in enzyme-balanced states A kinetic model with optimal enzyme levels satisfies all criteria stated above. However, does also the opposite hold? Can any set of state variables that satisfy these criteria be realised by an enzyme-optimal metabolic model? If this were true, we could choose steady state variables – metabolic fluxes, economic potentials, and so on – and directly construct and screen models that comply with them. A construction of enzyme-balanced model is possible and shows under which conditions economical flux distributions can be kinetically realised.

A method for model construction is shown in Figure S3. In the algorithm, $\mathbf{K}$ and $\mathbf{L}$ are defined by the network and gain vectors $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$ are also predefined. The steady-state variables (fluxes, concentrations, economic potentials, enzyme costs, economic loads, and reaction elasticities) are chosen one after the other, in agreement with the ones chosen before. Altogether, the following constraints have to be satisfied: $\mathbf{v}$ must be stationary, thermodynamically feasible and complete, $\bar{\mathbf{E}}$ must be consistent with $\mathbf{v}$ and the chemical potentials [11], y_l must be positive, and both economic balance equations must be satisfied. In practice, we proceed as follows:

1. We use flux analysis to compute an economical flux distribution $\mathbf{v}$, metabolite levels c_i, and economic potentials w_i, to satisfying the thermodynamic constraints and the reaction balance. With a production objective (defined by the vector $\mathbf{z}^{\mathrm{v}}$), the flux distribution must be free of non-productive and non-beneficial modes; we can find such flux distributions, for instance, by flux cost minimisation. Linear constraints for feasible logarithmic concentrations and economic potentials are obtained from the previously chosen fluxes. This yields concentrations c_i, standard chemical potentials $\mu_i^{(0)}$, economic potentials $\mathbf{w}^{\mathrm{c}}$, and enzyme costs $\mathbf{y}$ that satisfy the reaction balance.

2. In the second phase, we need to determine the rate laws. To ensure that the model will satisfy the compound balance (14), we choose a vector $\mathbf{z}^{\mathrm{c}}$ and determine reaction elasticities satisfying the compound balance. To ensure thermodynamically feasible reaction elasticities, we represent the elasticities by saturation values as described in [11]. To avoid full enzyme saturation, we limit the saturation values, for instance to a range $\beta_{li} < 0.9$. It may not be possible to satisfy the compound balance for a predefined vector $\mathbf{z}^{\mathrm{c}}$. However, if there is no solution, we may allow deviations from our initial vector $\mathbf{z}^{\mathrm{c}}$, which we penalise by quadratic costs. Then, a solution with $\mathbf{z}^{\mathrm{c}}$ as close as possible to the predefined values can be obtained from a quadratic programming problem. Finally, from the saturation values we can compute the rate constants and obtain a consistent model.

Constructing enzyme-optimal models To show that a flux distribution is enzyme-optimal (and not only enzyme-balanced), we need to realise it by an enzyme-optimal kinetice model. The model will have to satisfy the second-order conditions, i.e. the steady state must be stable (i.e., the Jacobian matrix for independent metabolites must be negative definite) and economically stable (the fitness curvature matrix must be negative definite). The first criterion, stability of the steady state, is not guaranteed by our construction, but we can use it as an additional criterion when choosing the reaction elasticities. The second criterion, negative fitness curvatures, can be satisfied by assuming a strongly curved investment function $h(\mathbf{u})$, giving rise to a strongly negative curvature matrix $\mathbf{H}_{\mathrm{uu}}$; however, large curvatures may not be justifiable biologically. By running the second phase of the algorithm with sampled elasticities, we obtain an ensemble of enzyme-balanced models realising one set of state variables (see [11]). If one of these models satisfies the second-order conditions, the flux distribution is enzyme-optimal, otherwise the question remains open.

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