Enzyme economy in metabolic networks
Proofs and derivations

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April 17, 2014

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P1.1 Theorems of metabolic control theory for models with dilution

In growing cells, all compounds are constantly diluted. Dilution can be described by dilution fluxes $v_i^{\mathrm{dil}} = \kappa\, c_i$ proportional to concentrations c_i and growth rate κ. Formally, dilution resembles a non-enzymatic degradation. Thus, given the dilution fluxes in a flux distribution, the concentrations are known. In models with dilution, the Jacobian matrix contains an additional term $-\kappa\,\mathsf{I}$ and the control matrices read

$$\begin{aligned} \mathbf{C}^{\mathrm{S}} &= -\mathbf{L}\,(\mathbf{M} - \kappa\,\mathsf{I})^{-1}\,\mathbf{N}_{\mathrm{R}} \\ \mathbf{C}^{\mathrm{J}} &= \mathsf{I} + \bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}} \end{aligned} \tag{P1}$$

where $\mathbf{M} = \mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L}$ is the usual Jacobian for independent metabolites and $\mathbf{M}^{\mathrm{dil}} = \mathbf{M} - \kappa\,\mathsf{I}$. The control matrices with respect to independent supply fluxes are given by $\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}} = -\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1}$ and $\mathbf{C}^{\mathrm{J}}_{\varphi^{\mathrm{ind}}} = \bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}}$ (see SI P3.1). The summation and connectivity theorems in models with dilution read (proof see below)

$$\begin{aligned} \mathbf{C}^{\mathrm{S}}\,\mathbf{K} &= 0 \\ \mathbf{C}^{\mathrm{J}}\,\mathbf{K} &= \mathbf{K} \\ \mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}\,\mathbf{L} &= -\mathbf{L}\,(\mathsf{I} + \kappa\,\mathbf{M}^{\mathrm{dil}\,-1}) \\ \mathbf{C}^{\mathrm{J}}\,\bar{\mathbf{E}}\,\mathbf{L} &= -\kappa\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1} \end{aligned} \tag{P2}$$

These theorems cover both enzymatic and non-enzymatic reactions. With the unscaled parameter elasticity matrix $\bar{\mathbf{E}}_{\mathrm{P}}$, the response matrices read

$$\mathbf{R}^{\mathrm{S}} = \frac{\partial \mathbf{c}}{\partial \mathbf{p}} = \mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}_{\mathrm{P}} \quad \text{and} \quad \mathbf{R}^{\mathrm{J}} = \frac{\partial \mathbf{c}}{\partial \mathbf{p}} = \mathbf{C}^{\mathrm{J}}\,\bar{\mathbf{E}}_{\mathrm{P}} \tag{P3}$$

Moreover, if the cell growth rate κ is treated as a parameter, the corresponding response coefficient vectors read

$$\mathbf{R}^{\mathrm{S}}_{\kappa} = \frac{\partial \mathbf{c}}{\partial \kappa} = -\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{tot}}}\,\mathbf{c}, \qquad \mathbf{R}^{\mathrm{J}}_{\kappa} = \frac{\partial \mathbf{v}}{\partial \kappa} = -\mathbf{C}^{\mathrm{J}}_{\varphi^{\mathrm{tot}}}\,\mathbf{c} \tag{P4}$$

where $\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{tot}}} = \mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}}\mathbf{I}_{\mathrm{R}}$ and $\mathbf{C}^{\mathrm{J}}_{\varphi^{\mathrm{tot}}} = \mathbf{C}^{\mathrm{J}}_{\varphi^{\mathrm{ind}}}\mathbf{I}_{\mathrm{R}}$. The matrix $\mathbf{I}_{\mathrm{R}}$ acts as a projector from internal metabolites to independent internal metabolites. It is obtained from an identity matrix I (corresponding to the number of internal metabolites) by selecting only rows that correspond to *independent* internal metabolites.

Proof: Summation and connectivity theorems with dilution The summation and connectivity theorems (P2) can be derived as follows. To derive the summation theorems (P2), we right-multiply the control matrices Eq. (P1) by $\mathbf{K}$, noting that $\mathbf{N}_{\mathrm{R}}\,\mathbf{K} = 0$, and obtain

$$\begin{aligned} \mathbf{C}^{\mathrm{S}}\,\mathbf{K} &= 0 \\ \mathbf{C}^{\mathrm{J}}\,\mathbf{K} &= \mathbf{K}. \end{aligned} \tag{P5}$$

To derive the connectivity theorems, we first compute

$$\begin{aligned}\mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}\,\mathbf{L}-\kappa\,\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}} &= -\mathbf{L}\,(\mathbf{M}-\kappa\mathbf{I})^{-1}\mathbf{N}\,\bar{\mathbf{E}}\,\mathbf{L}+\kappa\,\mathbf{L}\,(\mathbf{M}-\kappa\mathbf{I})^{-1}\\ &= -\mathbf{L}\,(\mathbf{M}-\kappa\mathbf{I})^{-1}\mathbf{M}+\mathbf{L}\,(\mathbf{M}-\kappa\mathbf{I})^{-1}\kappa\,\mathbf{I}\\ &= -\mathbf{L}\,(\mathbf{M}-\kappa\mathbf{I})^{-1}(\mathbf{M}-\kappa\,\mathbf{I})=-\mathbf{L}\end{aligned} \tag{P6}$$

Therefore

$$\mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}\,\mathbf{L} = -\mathbf{L}+\kappa\,\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}} = -\mathbf{L}-\kappa\,\mathbf{L}\,(\mathbf{M}-\kappa\,\mathbf{I})^{-1} = -\mathbf{L}\,(\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}^{-1}}) \tag{P7}$$

$$\begin{aligned}\mathbf{C}^{\mathrm{J}}\,\bar{\mathbf{E}}\,\mathbf{L} &= (\mathbf{I}+\bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}})\,\bar{\mathbf{E}}\,\mathbf{L}=\bar{\mathbf{E}}\,\mathbf{L}+\bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}\,\mathbf{L}=\bar{\mathbf{E}}\,\mathbf{L}-\bar{\mathbf{E}}\,\mathbf{L}\left[\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}^{-1}}\right]\\ &= \bar{\mathbf{E}}\,\mathbf{L}\left[\mathbf{I}-\left[\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}^{-1}}\right]\right]=-\kappa\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{\mathrm{dil}^{-1}}\end{aligned} \tag{P8}$$

or briefly (with $\mathbf{M}^{\mathrm{dil}}=\mathbf{M}-\kappa\,\mathbf{I}$)

$$\begin{pmatrix}\mathbf{C}^{\mathrm{J}}\\ \mathbf{C}^{\mathrm{S}}\end{pmatrix}(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L}) = \begin{pmatrix}\mathbf{K} & -\kappa\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{\mathrm{dil}^{-1}}\\ 0 & -\mathbf{L}[\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}^{-1}}]\end{pmatrix}. \tag{P9}$$

P1.2 Demand conditions (Theorem 1)

The cost-benefit balance (3) is equivalent to the gain conditions (6) and (7). To show this, we rewrite Eq. (3) with the help of return control coefficients $\mathbf{g}^{\mathrm{v}\top}=\mathbf{C}^{\mathrm{g}}_{\mathrm{v}}$ as

$$\begin{aligned}\frac{\partial h}{\partial\mathbf{u}}=\frac{\partial g}{\partial\mathbf{u}} &= \mathrm{Dg}\left(\frac{\mathbf{v}}{\mathbf{u}}\right)\,\mathbf{g}^{\mathrm{v}\top}=\mathrm{Dg}\left(\frac{\mathbf{v}}{\mathbf{u}}\right)\,(\mathbf{z}^{\mathrm{v}\top}\,\mathbf{C}^{\mathrm{J}}+\mathbf{z}^{\mathrm{c}\top}\,\mathbf{C}^{\mathrm{S}})^{\top}\\ \Leftrightarrow\qquad \begin{pmatrix}\mathbf{C}^{\mathrm{J}}\\ \mathbf{C}^{\mathrm{S}}\end{pmatrix}^{\top}\begin{pmatrix}\mathbf{z}^{\mathrm{v}}\\ \mathbf{z}^{\mathrm{c}}\end{pmatrix} &= \mathrm{Dg}\left(\frac{\mathbf{u}}{\mathbf{v}}\right)\frac{\partial h}{\partial\mathbf{u}}.\end{aligned} \tag{P10}$$

Next, we use the summation and connectivity theorems of metabolic control theory in the form [1]

$$\begin{pmatrix}\mathbf{C}^{\mathrm{J}}\\ \mathbf{C}^{\mathrm{S}}\end{pmatrix}(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L}) = \begin{pmatrix}\mathbf{K} & 0\\ 0 & -\mathbf{L}\end{pmatrix} \tag{P11}$$

where the matrix $\bar{\mathbf{E}}$ contains the unscaled reaction elasticities with respect to internal metabolites. We left-multiply Eq. (P10) with $(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L})^{\top}$ and obtain

$$\begin{pmatrix}\mathbf{K}^{\top} & 0\\ 0 & -\mathbf{L}^{\top}\end{pmatrix}\begin{pmatrix}\mathbf{z}^{\mathrm{v}}\\ \mathbf{z}^{\mathrm{c}}\end{pmatrix} = \begin{pmatrix}\mathbf{K}^{\top}\\ \mathbf{L}^{\top}\,\bar{\mathbf{E}}^{\top}\end{pmatrix}\mathrm{Dg}\left(\frac{\mathbf{u}}{\mathbf{v}}\right)\,\frac{\partial h}{\partial\mathbf{u}}. \tag{P12}$$

These equations are the gain conditions (6) and (7). To see their equivalence to the cost-benefit balance (3), we note that the matrix $(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L})$ is quadratic and has full rank. The matrix is therefore invertible, and multiplying with it is an equivalence transformation. The fact that $(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L})$ is quadratic can be seen as follows: if the internal stoichiometric matrix $\mathbf{N}$ has the size $n_m\times n_r$ and rank r, then $\mathbf{K}$ has n_r-r columns, and $\mathbf{L}$ has r columns. Accordingly, $\mathbf{M}=(\mathbf{K}|\bar{\mathbf{E}}\mathbf{L})$ is an $n_r\times n_r$ matrix. To see that $(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L})$ has full rank, we left-multiply it with $\binom{\mathbf{K}}{\mathbf{N}_{\mathrm{R}}}$, which yields a matrix with full rank.

P1.3 Demand conditions with dilution

The gain conditions for models with dilution (growth rate κ, Jacobian $\mathbf{M}^{\mathrm{dil}}=\mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L}-\kappa\,\mathbf{I}$, all reactions enzymatic) read

$$\begin{aligned}\mathbf{K}^{\top}\,\mathbf{z}^{\mathrm{v}} &= \mathbf{K}^{\top}\,\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}\\ -\mathbf{L}^{\top}\,\mathbf{z}^{\mathrm{c}}-\kappa\,(\mathbf{M}^{\mathrm{dil}^{-1}})^{\top}\,\mathbf{L}^{\top}\,\mathbf{z}^{\mathrm{c}*} &= \mathbf{L}^{\top}\,\bar{\mathbf{E}}^{\top}\,\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}.\end{aligned} \tag{P13}$$

with $\mathbf{z}^{c*} = \bar{\mathbf{E}}^\top \mathbf{z}^v + \mathbf{z}^c$

Proof The proof works as the one before. Instead of the usual summation and connectivity theorems Eq. (P11), we use the form

$$\begin{pmatrix}\mathbf{C}^J\\ \mathbf{C}^S\end{pmatrix}(\mathbf{K}|\bar{\mathbf{E}}\,\mathbf{L}) \;=\; \begin{pmatrix}\mathbf{K} & -\kappa\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1}\\ 0 & -\mathbf{L}[\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}\,-1}]\end{pmatrix}. \tag{P14}$$

(see P1.1). We continue as before and obtain

$$\begin{pmatrix}\mathbf{K}^\top & 0\\ (-\kappa\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{\mathrm{dil}\,-1})^\top & (-\mathbf{L}[\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}\,-1}])^\top\end{pmatrix}\begin{pmatrix}\mathbf{z}^v\\ \mathbf{z}^c\end{pmatrix} \;=\; \begin{pmatrix}\mathbf{K}^\top\\ (\bar{\mathbf{E}}\,\mathbf{L})^\top\end{pmatrix}\mathrm{Dg}(\mathbf{y})\,\mathbf{v}^{-\mathbf{1}}, \tag{P15}$$

which can be written as

$$\begin{aligned}\mathbf{K}^\top\mathbf{z}^v &= \mathbf{K}^\top\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}\\ -\kappa\,(\mathbf{M}^{\mathrm{dil}\,-1})^\top\mathbf{L}^\top\bar{\mathbf{E}}^\top\mathbf{z}^v - [\mathbf{I}+\kappa\,\mathbf{M}^{\mathrm{dil}\,-1}]^\top\mathbf{L}^\top\mathbf{z}^c &= (\bar{\mathbf{E}}\,\mathbf{L})^\top\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}.\end{aligned} \tag{P16}$$

The last equation can be rewritten as

$$-\mathbf{L}^\top\mathbf{z}^c - \kappa\,{\mathbf{M}^{\mathrm{dil}\,-1}}^\top\,\mathbf{L}^\top[\bar{\mathbf{E}}^\top\mathbf{z}^v + \mathbf{z}^c] \;=\; (\bar{\mathbf{E}}\,\mathbf{L})^\top\mathrm{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}}. \tag{P17}$$

Thus, the flux gain condition is unchanged by dilution, while the concentration gain condition contains an additional term $-\kappa\,{\mathbf{M}^{\mathrm{dil}\,-1}}^\top\,\mathbf{L}^\top\mathbf{z}^{c*}$, where $\mathbf{z}^{c*} = [\bar{\mathbf{E}}^\top\mathbf{z}^v + \mathbf{z}^c]$.

P1.4 Demand conditions involving inactive or non-enzymatic reactions

To derive gain conditions accounting for non-enzymatic reactions, we split the flux distribution $\mathbf{v}$ into subvectors $\mathbf{v}_{\mathrm{enz}}$ (active enzymatic reactions) and $\mathbf{v}_{\mathrm{non}}$ (inactive or non-enzymatic reactions). The resulting flux and concentration gain conditions read

$$\mathbf{k}\cdot\mathbf{z}^v - \mathbf{k}_{\mathrm{non}}\cdot\mathbf{g}^v_{\mathrm{non}} \;=\; \mathbf{k}^\top_{\mathrm{enz}}\mathrm{Dg}(\mathbf{y}_{\mathrm{enz}})\,\mathbf{v}^{-\mathbf{1}}_{\mathrm{enz}}. \tag{P18}$$

$$-\mathbf{L}^\top\mathbf{z}^c - (\bar{\mathbf{E}}\,\mathbf{L})^\top_{\mathrm{non}}\,\mathbf{g}^v_{\mathrm{non}} \;=\; ((\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{enz}})^\top\mathrm{Dg}(\mathbf{y}_{\mathrm{enz}})\mathbf{v}^{-\mathbf{1}}_{\mathrm{enz}}. \tag{P19}$$

where $\mathbf{g}^v_{\mathrm{non}} = \Delta\mathbf{w}_{\mathrm{non}} + \hat{\mathbf{z}}^v_{\mathrm{non}}$ as usually. In the case of dilution reactions (where $k_i = \kappa\,c_i$, $\Delta w_i^{\mathrm{dil}} = -w_i$, $\hat{\mathbf{z}}^v_i = 0$ and $\bar{E}^{\mathrm{dil}} = \kappa$), the extra terms would read

$$-\mathbf{k}_{\mathrm{non}}\cdot\mathbf{g}^v_{\mathrm{non}} \;=\; \sum_i k_i\,g^v_i = \sum_i \kappa\,c_i(-w_i) = -\kappa\sum_i c_i\,w_i \tag{P20}$$

$$-(\bar{\mathbf{E}}\,\mathbf{L})^\top_{\mathrm{non},i}\,\mathbf{g}^v_{\mathrm{non},i} \;=\; -\kappa\,-w_i = \kappa\,w_i. \tag{P21}$$

Proof The cost-benefit balance for active enzymatic reactions reads (compare Eq. P10)

$$\mathbf{h}^u_{\mathrm{enz}} = \mathbf{g}^u_{\mathrm{enz}} = \mathrm{Dg}\left(\frac{\mathbf{v}_{\mathrm{enz}}}{\mathbf{u}_{\mathrm{enz}}}\right)\mathbf{g}^v_{\mathrm{enz}} \qquad \Rightarrow \qquad \mathbf{g}^v_{\mathrm{enz}} = \mathrm{Dg}(\mathbf{u}_{\mathrm{enz}})\mathrm{Dg}(\mathbf{h}^u_{\mathrm{enz}})\,\mathbf{v}^{-1}_{\mathrm{enz}} \tag{P22}$$

Now let $\mathbf{k}$ be a test mode, i.e, a stationary mode on the active subnetwork of $\mathbf{v}$. We can split the product $\mathbf{g}^v\cdot\mathbf{k}$ into

$$\mathbf{k}\cdot\mathbf{g}^v = \mathbf{k}_{\mathrm{enz}}\cdot\mathbf{g}^v_{\mathrm{enz}} + \mathbf{k}_{\mathrm{non}}\cdot\mathbf{g}^v_{\mathrm{non}}. \tag{P23}$$

Since $\mathbf{g}^v = \Delta\mathbf{w}^c + \mathbf{z}^v$ and $\Delta\mathbf{w}^c\cdot\mathbf{k} = 0$, obtain

$$\mathbf{k}\cdot\mathbf{z}^v = \mathbf{k}_{\mathrm{enz}}\cdot\mathbf{g}^v_{\mathrm{enz}}. \tag{P24}$$

After equating the last equations and rearranging, we obtain the flux gain condition

$$\mathbf{k}\cdot\mathbf{z}^{\mathrm{v}} - \mathbf{k}_{\mathrm{non}}\cdot\mathbf{g}^{\mathrm{v}}_{\mathrm{non}} \quad = \quad \mathbf{k}^{\top}_{\mathrm{enz}}\mathsf{Dg}(\mathbf{h}^{\mathrm{u}}{}_{\mathrm{enz}})\,\mathsf{Dg}(\mathbf{u}_{\mathrm{enz}})\,\mathbf{v}^{-1}_{\mathrm{enz}}. \tag{P25}$$

To derive the concentration gain condition, we start from

$$\mathbf{g}^{\mathrm{v}\top}\bar{\mathbf{E}}\,\mathbf{L} = [\mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}} + \mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}}]\,\bar{\mathbf{E}}\,\mathbf{L} = -\mathbf{z}^{\mathrm{c}\top}\,\mathbf{L}. \tag{P26}$$

where we used the connectivity theorems. The left-hand side can be split into

$$\mathbf{g}^{\mathrm{v}\top}\bar{\mathbf{E}}\,\mathbf{L} = \mathbf{g}^{\mathrm{v}}_{\mathrm{enz}}{}^{\top}\,(\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{enz}} + \mathbf{g}^{\mathrm{v}}_{\mathrm{non}}{}^{\top}\,(\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{non}}. \tag{P27}$$

By equating the equations, we obtain

$$-\mathbf{z}^{\mathrm{c}\top}\,\mathbf{L} = \mathbf{g}^{\mathrm{v}}_{\mathrm{enz}}{}^{\top}\,(\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{enz}} + \mathbf{g}^{\mathrm{v}}_{\mathrm{non}}{}^{\top}\,(\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{non}}. \tag{P28}$$

By inserting Eq. (P22) and taking the tranpose, we obtain the concentration gain condition

$$-\mathbf{L}^{\top}\,\mathbf{z}^{\mathrm{c}} \quad = \quad ((\bar{\mathbf{E}}\,\mathbf{L})_{\mathrm{enz}})^{\top}\mathsf{Dg}(\mathbf{h}^{\mathrm{u}}{}_{\mathrm{enz}})\mathsf{Dg}(\mathbf{u}_{\mathrm{enz}})\mathbf{v}^{-1}_{\mathrm{enz}} + (\bar{\mathbf{E}}\,\mathbf{L})^{\top}_{\mathrm{non}}\,\mathbf{g}^{\mathrm{v}}_{\mathrm{non}}. \tag{P29}$$

P1.5 Total cost and benefit for homogeneous objective and investment functions

If the enzyme investment $h(\mathbf{u}$ is a homogeneous function (satifying $h(\lambda\,\mathbf{u}) = \lambda^{\gamma}\,h(\mathbf{u})$ with real exponent γ), we can use Euler's theorem: $\sum_l \frac{\partial h}{\partial \ln u_l} = \gamma\,h$. For cost functions, we may use exponents $1 \leq \gamma_u < 2$. In particular, for a linear cost function (where $\gamma = 1$), we obtain $h = \sum_l \frac{\partial h}{\partial \ln u_l} = \sum_l y_l$. In analogy, we can use homogeneous flux cost functions $\bar{H}(\mathbf{v}) = (\sum_l \bar{H}^{\mathrm{v}}_l\,|v_l|)^{\alpha}$ or flux objective functions $z(\mathbf{v}) = (\sum_l z^*_l\,v_l)^{\beta}$. These sum formulae can be used to state balances between total cost and benefit. With such choices, from $\mathbf{z}^{\mathrm{v}}\cdot\mathbf{v} = \mathbf{y}\cdot\mathbf{u}$, the total cost-benefit balance reads $\gamma_z\,z = \gamma_u\,h$.

P2 Economical flux distributions

P2.1 Test mode theorem (Theorem 2)

Let $\mathbf{v}$ be a complete flux distribution and $\mathbf{k}$ be a non-vanishing, non-beneficial test mode (satisfying $\mathbf{z}^{\mathrm{v}\top}\,\mathbf{k} = 0$). To be economical, $\mathbf{v}$ must satisfy the flux gain condition Eq. (6) with a positive enzyme cost vector $\mathbf{y}$ satisfying

$$\mathbf{k}^{\top}\mathsf{Dg}(\mathbf{y})\mathbf{v}^{-\mathbf{1}} = \mathbf{0}. \tag{P30}$$

All elements of $\mathbf{y}$ are positive, none of the elements v_l is zero, and at least some of the fluxes k_l are non-zero,, Therefore, Eq. (P30) can only hold if $k_l\,v_l > 0$ for some index l, and $k_j\,v_j < 0$ for some other index j.

P2.2 Non-beneficial modes and the existence of economic potentials

For use in Theorem 3, we need the following lemma:

Lemma. (a) If a complete flux distribution $\mathbf{v}$ satisfies the reaction balance (11) with positive enzyme costs y_l, it is free of non-beneficial modes. (b) If a complete flux distribution $\mathbf{v}$ is free of non-beneficial modes, it satisfies the reaction balance Eq. (11) with positive enzyme costs y_l.

For the proof, we recall Gordan's theorem [2]: Let $\mathbf{A}$ be a real $m\times n$ matrix and $\mathbf{1} = (1,1,...,1)^{\top} \in \mathbb{R}^m$. Gordan's theorem states that either the inequality system $\mathbf{A}\,\mathbf{x} > 0$ has a solution $\mathbf{x} \in \mathbb{R}^n$ or the system $\mathbf{A}^{\top}\,\mathbf{y} = 0, \mathbf{y} \geq 0$, $\mathbf{1}^{\top}\mathbf{y} > 0$ has a solution $\mathbf{y} \in \mathbb{R}^m$, but never both.

Proof direction (a): If $\mathbf{v}$ satisfies the reaction balance with gain vector $\mathbf{z}^{\mathrm{v}}$ and positive enzyme costs y_l, $\mathbf{v}$ is free of non-beneficial modes. We consider a complete flux distribution $\mathbf{v}$ and define the reaction directions

such that all fluxes v_l are positive. If $\mathbf{v}$ contains a non-beneficial mode, there is a non-beneficial test mode $\mathbf{k}$ that shares with $\mathbf{v}$ all flux directions on their overlap, so $\binom{\mathbf{z}^{\mathrm{v}\top}}{\mathbf{N}}\mathbf{k} = 0$, $\mathbf{k} \geq 0$, and $\mathbf{1}^\top\mathbf{k} > 0$. On the contrary, if $\mathbf{v}$ satisfies the reaction balance

$$\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}} \quad = \quad q_l/v_l > 0,$$

the inequality $\binom{(\mathbf{z}^{\mathrm{v}}|}{\mathbf{N}^\top)\binom{1}{\mathbf{w}^{\mathrm{c}}}>0}$ has a solution $\binom{1}{\mathbf{w}^{\mathrm{c}}}$. According to Gordan's theorem, only one of the two statements can hold, so if the reaction balance holds, no non-beneficial modes can exist.

Proof direction (b): If $\mathbf{v}$ is free of non-beneficial modes with respect to $\mathbf{z}^{\mathrm{v}}$, it satisfies a reaction balance with flux gain vector $\mathbf{z}^{\mathrm{v}}$ and positive enzyme costs. If $\mathbf{v}$ is free of non-beneficial modes, the inequality system $\binom{\mathbf{z}^{\mathrm{v}\top}}{\mathbf{N}}\mathbf{k} = 0, \mathbf{k} \geq 0, \mathbf{1}^\top\mathbf{k} > 0$ has no solution $\mathbf{k}$. Thus, according to Gordan's theorem, there must be a solution to the inequality system $\binom{\mathbf{z}^{\mathrm{v}\top}}{\mathbf{N}}^\top \mathbf{x} > 0$. Without loss of generality, we can assume that the first element of $\mathbf{x}$ is -1, 0, or 1, and can identify the rest of the vector with $\mathbf{w}^{\mathrm{c}}$. Therefore, one of the following inequalities must have a solution (for all l):

$$\begin{aligned} \Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}} &> 0 \\ \Delta w_l^{\mathrm{c}} &> 0 \\ \Delta w_l^{\mathrm{c}} - z_l^{\mathrm{v}} &> 0. \end{aligned}$$

If we multiply with the fluxes v_l and sum over all reactions, the term $\sum_l v_l \Delta w_l^{\mathrm{c}}$ drops out and we obtain, respectively, three alternative inequalities

$$\begin{aligned} \mathbf{z}^{\mathrm{v}\top}\mathbf{v} &> 0 \\ 0 &> 0 \\ -\mathbf{z}^{\mathrm{v}\top}\mathbf{v} &> 0. \end{aligned}$$

Since $\mathbf{v}$ is beneficial by assumption ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} > 0$), the first inequality must hold. Therefore $\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}} > 0$ must hold, and consistent enzyme costs are given by $y_l = [\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}}]\, v_l$.

P2.3 Criteria for economical flux distributions (Theorem 3)

Proposition For complete flux distributions $\mathbf{v}$ (with $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} > 0$), the following statements imply each other: (i) $\mathbf{v}$ is economical; (ii) $\mathbf{v}$ satisfies the reaction balance Eq. (11) for some choice of internal economic potentials w_i^{c} and positive enzyme costs y_l; (iii) $\mathbf{v}$ is free of non-beneficial modes.

Proof: To prove that the conditions are equivalent, we show that each of them implies the next one in a circle: (a) a complete, beneficial flux distribution that is free of futile modes; (b) a complete flux distribution that is beneficial and free of non-beneficial modes solves the reaction balance; (c) a complete flux distribution that solves the reaction balance is economical.

(a) Economical modes are free of futile modes. The absence of futile modes follows directly from the test mode theorem and from the definition of futile modes.

(b) Complete flux distributions that are free of non-beneficial modes solve the reaction balance This has been shown in the previous section P2.2.

(c) The reaction balance implies the flux gain condition. We start from the reaction balance in the form $[\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}}] = y_l/v_l$, multiply from the left by a test mode $\mathbf{k}^\top$, and obtain

$$\begin{aligned} \Rightarrow \mathbf{k}^\top\,[\mathbf{N}^\top\mathbf{w}^{\mathrm{c}} + \mathbf{z}^{\mathrm{v}}] \quad &= \quad \mathbf{k}^\top\,\mathrm{Dg}(\mathbf{v})^{-1}\,\mathbf{y} \\ \Rightarrow \mathbf{k}^\top\,\mathbf{z}^{\mathrm{v}} \quad &= \quad \mathbf{k}^\top\,\mathrm{Dg}(\mathbf{y})\,\mathbf{v^{-1}} \end{aligned} \tag{P31}$$

because $\mathbf{k}^\top\,\mathbf{N} = 0$. If this holds for any stationary flux mode $\mathbf{k}$, it also holds for the entire kernel matrix $\mathbf{K}$.

P2.4 Flux cost minimisation and economic flux modes (Theorem 4)

Proposition Let $\mathbf{v}$ be the solution of a non-flux-enforcing flux cost minimisation problem

$$\min \stackrel{!}{=} \bar{H}(\mathbf{v}') \qquad \text{such that} \qquad \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}' = b, \qquad \mathbf{N}\,\mathbf{v}' = 0 \tag{P32}$$

Then $\mathbf{v}$ is economical and satisfies the balance equation

$$[z_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}}]\, v_l = y_l.$$

for all active reactions (where $v_l \neq 0$), with positive values y_l and with w_l^{c} being proportional to the Lagrange multipliers with respect to the stationarity condition. It also holds that $\sum_l z_l^{\mathrm{v}}\, v_l = \sum_l y_l = b$.

Proof: The weighted flux minimisation is convex and has therefore at least one solution, which satisfies

$$0 = \frac{\partial \bar{H}}{\partial v_l} - \alpha\, z_l^{\mathrm{v}} - \sum_i \beta_i\, n_{il} \tag{P33}$$

with Lagrange multipliers α (for the benefit constraint) and β_i (for the stationarity constraints). For all reactions l with non-zero fluxes v_l, the derivative $\frac{\partial \bar{H}}{\partial v_l}$ must be positive. Now we take the scalar product between Eq. (P37) and $\mathbf{v}$ and we obtain:

$$0 = \sum_l \left(\frac{\partial \bar{H}}{\partial v_l'}\, v_l - \alpha\, z_l^{\mathrm{v}}\, v_l - \sum_i \beta_i\, n_{il}\, v_l \right) = \sum_l \left(\frac{\partial \bar{H}}{\partial v_l'}\, v_l - \alpha\, z_l^{\mathrm{v}} v_l \right). \tag{P34}$$

The term with n_{il} vanishes. Thus, we see that

$$\sum_l \frac{\partial \bar{H}}{\partial v_l'}\, v_l = \sum_l \alpha\, z_l^{\mathrm{v}} v_l = \alpha\, b. \tag{P35}$$

We set $y_l = \frac{1}{\alpha} \frac{\partial \bar{H}}{\partial v_l'}\, v_l$ and obtain

$$\sum_l y_l = \sum_l z_l^{\mathrm{v}} v_l = b. \tag{P36}$$

Inserting this into (P37) again, we obtain

$$y_l = z_l^{\mathrm{v}}\, v_l + \sum_i \frac{\beta_i\, n_{il}}{\alpha}\, v_l \tag{P37}$$

which is equivalent to the reaction balance we want to show.

Alternative proof: solutions of FCM problems are free of non-beneficial modes As an alternative proof, we show that a complete flux distribution solving Eq. (P38) is free of non-beneficial modes. The proof consists of three parts:

1. If $\mathbf{v}$ has a non-beneficial mode (defined by the test mode $\mathbf{k}$), a linear combination $\mathbf{v} - \varepsilon\,\mathbf{k}$ (for small $\varepsilon > 0$) violates the FBA inequality constraints. **Proof:** Since $\mathbf{N}^\top \mathbf{k} = 0$ and $\mathbf{z}^{\mathrm{v}\top} \mathbf{k} = 0$, $\mathbf{v} - \varepsilon\mathbf{k}$ is stationary and has the same FBA benefit as $\mathbf{v}$ itself. In addition, it satisfies $|\mathbf{v} - \varepsilon\mathbf{k}|_1 < |\mathbf{v}|_1$ (for a non-weighted flux minimisation problem) or $\mathbf{h}^{\mathrm{v}}(\mathbf{v} - \varepsilon\mathbf{k}) < \mathbf{h}^{\mathrm{v}}(\mathbf{v})$ (for any FCM problem, where $\mathbf{h}^{\mathrm{v}}(\cdot)$ is the flux cost function). Therefore, $\mathbf{v}$ cannot solve the FCM problem unless $\mathbf{v} - \varepsilon\mathbf{k}$ violates the inequality constraints.

2. If $\mathbf{v} - \varepsilon\mathbf{k}$ violates the inequality constraints for arbitrarily small $\varepsilon > 0$, the inequality constraints must contain some bounds $v_l^{\min} > 0$ or $v_l^{\max} < 0$.

   Proof: Assume that $\mathbf{v} - \varepsilon\mathbf{k}$ (for any small $\varepsilon > 0$) violates the lower flux bound for the l^{th} reaction ($v_l - \varepsilon k_l < v_l^{\min}$). Since $\mathbf{v}$ does not, this must be an active constraint, i.e. $v_l = v_l^{\min}$, and $v_l^{\min}$ and k_l share the same signs. Since subtracting εk_l decreases the flux, this sign must be positive, so $v_l^{\min} > 0$. A similar argument holds for $v_l^{\max} < 0$.

3. Given that $v_l^{\min} \leq 0$ and $v_l^{\min} \geq 0$ by assumption, the solution $\mathbf{v}$ of our FCM problem cannot contain any non-beneficial modes. This argument can be extended to incomplete flux distributions: if an incomplete flux distribution $\mathbf{v}$ solves a non-flux-enforcing FCM problem, the subvector of active fluxes $\mathbf{v}_{\mathrm{act}}$ will solve a corresponding FCM problem on the active region. Therefore, the active flux distribution, and also $\mathbf{v}$ itself, must be free of non-beneficial modes.

Proposition: Let $\mathbf{v}$ be a complete economical flux distribution solving the reaction balance

$$[z_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}}]\, v_l = y_l.$$

Then, $\mathbf{v}$ is a solution of a weighted flux minimisation problem

$$\min \stackrel{!}{=} \bar{H}(\mathbf{v}') \qquad \text{such that} \qquad \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}' = b, \qquad \mathbf{N}\,\mathbf{v}' = 0 \tag{P38}$$

where $\bar{H}(\mathbf{v}') = \sum_l \frac{y_l}{v_l} |v_l'|$. Moreover, the Lagrange multipliers with respect to the stationarity condition are given by the economic potentials w_l^{c} (except for a possiible regauging of conserved moieties), and it must hold that $b = \sum_l z_l^{\mathrm{v}}\, v_l = \sum_l y_l$.

Proof: Since the FCM problem is convex, any flux distribution that satisfies the constraints as well as the optimality condition (P37) must be an optimum. Thus, we need to find suitable values of the Lagrange multipliers α and β_i such that Eq. (P37) is satisfied for all reactions with $v_l \neq 0$. To do this, we set $\alpha = 1$ and $\beta_i = w_i^{\mathrm{c}}$. With this choice, the optimality condition now reads

$$0 \stackrel{!}{=} y_l / v_l - z_l^{\mathrm{v}} - \sum_i \beta_i\, n_{il} \tag{P39}$$

This is equivalent to the assumed balance equation and must therefore hold true.

P3 Economic potentials

P3.1 Economic potentials and flux demand (reaction rule Eq. (8))

Control coefficients with respect to supply fluxes The effect of small supply fluxes φ_m^{ind} on the steady state concentrations $c_i = S_i(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})$ and fluxes $v_l = J_l(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})$ is described by control matrices $\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} = \partial\mathbf{c}/\partial\boldsymbol{\varphi}^{\mathrm{ind}}$ and $\mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} = \partial\mathbf{v}/\partial\boldsymbol{\varphi}^{\mathrm{ind}}$, which are computed as follows. Consider a network with n_{int} internal metabolites. If there exist conserved moieties, we select a number of independent metabolites and split the stoichiometric matrix, into a product $\mathbf{N} = \mathbf{L}\,\mathbf{N}_{\mathrm{R}}$ with an $n_{\mathrm{int}} \times n_{\mathrm{ind}}$ link matrix $\mathbf{L}$ and a reduced stoichiometric matrix $\mathbf{N}_{\mathrm{R}}$, whose rows correspond to independent metabolites. We can write $\mathbf{N}_{\mathrm{R}} = \mathbf{l}_{\mathrm{R}}\mathbf{N}$, where the matrix $\mathbf{l}_{\mathrm{R}}$ is obtained from an $n_{\mathrm{int}} \times n_{\mathrm{int}}$ identity matrix by selecting the rows corresponding to independent metabolites; in addition, we obtain $\mathbf{l}_{\mathrm{R}}\,\mathbf{L} = \mathbf{I}$. The stationarity condition for independent metabolites in the presence of supply fluxes $\boldsymbol{\varphi}^{\mathrm{ind}}$ reads

$$0 \quad = \quad \mathbf{N}_{\mathrm{R}}\mathbf{v} + \boldsymbol{\varphi}^{\mathrm{ind}} \tag{P40}$$

and the derivative with respect to $\boldsymbol{\varphi}^{\mathrm{ind}}$ yields

$$0 \quad = \quad \mathbf{N}_{\mathrm{R}} \frac{\partial \mathbf{v}}{\partial \mathbf{c}} \frac{\partial \mathbf{S}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} + \mathbf{I} = \mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{C}^{\mathrm{S,ind}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} + \mathbf{I} \tag{P41}$$

where $\mathbf{v}(\mathbf{c})$ describes the rate laws, $\mathbf{S}(\boldsymbol{\varphi}^{\mathrm{ind}})$ describes how the independent metabolite concentrations in steady state depend on the supply fluxes, and the control matrix $\mathbf{C}^{\mathrm{S,ind}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}$ contains the corresponding derivatives. The matrix product $\mathbf{M} = \mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L}$ is the Jacobian matrix for the independent internal metabolites. Solving Eq. (P41) for $\mathbf{C}^{\mathrm{S,ind}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}$, we obtain

$$\mathbf{C}^{\mathrm{S,ind}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} = -\mathbf{M}^{-1} \tag{P42}$$

and from this

$$\begin{aligned}\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} &= \mathbf{L}\,\mathbf{C}^{\mathrm{S,ind}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} = -\mathbf{L}\,\mathbf{M}^{-1} \\ \mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} &= \bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}} = -\bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{-1}.\end{aligned} \tag{P43}$$

For convenience, we define the matrices

$$\begin{aligned}\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}} &= \mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}\,\mathbf{I}_{\mathrm{R}} \\ \mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{tot}}} &= \mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}\,\mathbf{I}_{\mathrm{R}}.\end{aligned} \tag{P44}$$

A comparison to the unscaled control matrices [3]

$$\begin{aligned}\mathbf{C}^{\mathrm{S}} &= -\mathbf{L}\,\mathbf{M}^{-1}\mathbf{N}_{\mathrm{R}} \\ \mathbf{C}^{\mathrm{J}} &= \mathbf{I} - \bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{-1}\mathbf{N}_{\mathrm{R}},\end{aligned} \tag{P45}$$

shows that these can be written in terms of $\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}$ as

$$\begin{aligned}\mathbf{C}^{\mathrm{S}} &= \mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}\,\mathbf{N} \\ \mathbf{C}^{\mathrm{J}} &= \mathbf{I} + \mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}\,\mathbf{N}.\end{aligned} \tag{P46}$$

Economic potentials Now we consider the internal economic potentials $\mathbf{w}^{\mathrm{c}} = \mathbf{I}_{\mathrm{R}}^{\top}\,\partial g/\partial\boldsymbol{\varphi}^{\mathrm{ind}}$ and the flux demand $\mathbf{g}^{\mathrm{v}} = \mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}$. With the chain rule and Eq. (P46), we can write them as

$$\begin{aligned}\mathbf{w}^{\mathrm{c}} &= \mathbf{I}_{\mathrm{R}}^{\top}\,\frac{\partial g}{\partial\boldsymbol{\varphi}^{\mathrm{ind}}} = [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}\,\mathbf{I}_{\mathrm{R}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{ind}}}\,\mathbf{I}_{\mathrm{R}}]^{\top} = [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{tot}}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}]^{\top} \\ \Rightarrow \Delta\mathbf{w}^{\mathrm{c}} &= \mathbf{N}^{\top}\,\mathbf{w}^{\mathrm{c}} = [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}\,\mathbf{N} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}\,\mathbf{N}]^{\top} \\ &= [\mathbf{z}^{\mathrm{v}\top}(\mathbf{C}^{\mathrm{J}} - \mathbf{I}) + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}]^{\top} = \mathbf{g}^{\mathrm{v}} - \mathbf{z}^{\mathrm{v}}.\end{aligned} \tag{P47}$$

We therefore obtain

$$\mathbf{g}^{\mathrm{v}} = \Delta\mathbf{w}^{\mathrm{c}} + \mathbf{z}^{\mathrm{v}} = \Delta\mathbf{w}^{\mathrm{c}} + \Delta\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}} = \Delta\mathbf{w} + \hat{\mathbf{z}}^{\mathrm{v}}. \tag{P48}$$

By inserting Eq. (P44) into Eq. (P47), we obtain the explicit formula

$$\mathbf{w}^{\mathrm{c}} = -\left[[\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top}]\,\mathbf{L}\,\mathbf{M}^{-1}\mathbf{I}_{\mathrm{R}}\right]^{\top} = \left[[\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top}]\,\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}\right]^{\top} = \mathbf{z}^{\mathrm{c}*\top}\,\mathbf{C}^{\mathrm{S}}_{\boldsymbol{\varphi}^{\mathrm{tot}}}. \tag{P49}$$

These formulae also hold in the presence of non-enzymatic reactions and dilution. Given a kinetic steady-state model and a metabolic objective $z(\mathbf{v}, \mathbf{c})$, the economic potentials and their balances within reactions read

$$\begin{aligned}\mathbf{w}^{\mathrm{c}\top} &= -(\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top})\mathbf{L}(\mathbf{N}_{\mathrm{R}}\bar{\mathbf{E}}\,\mathbf{L})^{-1}\,\mathbf{I}_{\mathrm{R}} \\ \Delta\mathbf{w}^{\top} &= \mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}} - \mathbf{z}^{\mathrm{v}\top} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}.\end{aligned} \tag{P50}$$

P3.2 Enzyme adaption can be neglected in the definition of economic potentials and loads

Economic potentials describe how small supply fluxes would affect the steady-state benefit. In the definition, the enzyme levels are kept fixed. If they were adapted to the supply fluxes, this could increase the benefit. With such an adaption, we would obtain an alternative definition of "adaptive" economic potentials. However, since the additional fitness benefit is a second-order effect, adaptive and non-adaptive economic potentials are identical. This is shown now. The steady-state fluxes v_l and the internal concentrations c_m form a state variable vector $\mathbf{s} = \binom{\mathbf{c}}{\mathbf{v}}^{\top}$. We assume that this vector is a differentiable function $\mathbf{s}(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})$ of the enzyme vector $\mathbf{u}$, the

external concentration vector $\mathbf{x}$, and the supply flux vector $\boldsymbol{\varphi}^{\mathrm{ind}}$ for independent metabolites. We now consider a fitness function

$$f(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) = g(\mathbf{s}(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})) - h(\mathbf{u}). \tag{P51}$$

Given external concentrations x_j and virtual supply fluxes φ^{ind} for independent metabolites, an optimal enzyme profile is defined as

$$\mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) = \mathsf{argmax}_{\mathbf{u}}\, f(\mathbf{u}, \mathbf{s}(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})) \tag{P52}$$

and the optimal fitness $g^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})$ is defined as the fitness at optimal enzyme levels,

$$\begin{aligned} g^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) &= f(\mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}), \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})) \\ &= z(\mathbf{s}(\mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})), \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}},) - h(\mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}})). \end{aligned} \tag{P53}$$

We now consider a state without supply fluxes ($\boldsymbol{\varphi}^{\mathrm{ind}} = 0$). The usual, non-adaptive external economic loads and internal economic potentials in this state are defined by

$$p_j^{\mathrm{x}} = \frac{\partial g}{\partial x_j}|_{\boldsymbol{\varphi}^{\mathrm{ind}}=0}, \qquad w_m^{\mathrm{ind}} = \frac{\partial g}{\partial \varphi_m^{\mathrm{ind}}}|_{\boldsymbol{\varphi}^{\mathrm{ind}}=0}, \tag{P54}$$

at constant enzyme levels $\mathbf{u}^{\mathrm{opt}}$. The adaptive quantities, in contrast, are defined by

$$p_j^{\mathrm{x\,adaptive}} = \frac{\partial g^{\mathrm{opt}}}{\partial x_j}|_{\boldsymbol{\varphi}^{\mathrm{ind}}=0}, \qquad w_m^{\mathrm{ind\,adaptive}} = \frac{\partial g^{\mathrm{opt}}}{\partial \varphi_m^{\mathrm{ind}}}|_{\boldsymbol{\varphi}^{\mathrm{ind}}=0}. \tag{P55}$$

Despite the different definitions, their values are identical because enzyme adaption is a second-order effect and does not affect the first derivatives (Proof in section P3.2).

Adaptive and non-adaptive economic potentials Adaptive and non-adaptive economic potentials are defined differently, but their values are identical. The proof is as follows. The non-adaptive economic potentials for internal metabolites read

$$\mathbf{w}^{\mathrm{ind}} = \frac{\partial g}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} = \frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} \tag{P56}$$

where the state vector $\mathbf{s}(\mathbf{u}, \mathbf{x}, \mathbf{L}\,\boldsymbol{\varphi}^{\mathrm{ind}}) = \binom{\mathbf{J}(\mathbf{u}, \mathbf{x}, \mathbf{L}\,\boldsymbol{\varphi}^{\mathrm{ind}})}{\mathbf{S}(\mathbf{u}, \mathbf{x}, \mathbf{L}\,\boldsymbol{\varphi}^{\mathrm{ind}})}$, $z(\mathbf{s})$ is the metabolic objective function, and $g(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) = z(\mathbf{s}(\mathbf{u}, \mathbf{x}, \mathbf{L}\,\boldsymbol{\varphi}^{\mathrm{ind}}))$ is the metabolic return function. For the adaptive economic potentials, we define the adapted enzyme levels $\mathbf{u}^{\mathrm{opt}}$ and the adaptive benefit function

$$\begin{aligned} \mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) &= \mathsf{argmax}_{\mathbf{u}} \left[g(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) - h(\mathbf{u}) \right] \\ g^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}) &= g(\mathbf{u}^{\mathrm{opt}}(\mathbf{x}, \boldsymbol{\varphi}^{\mathrm{tot}}), \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}). \end{aligned} \tag{P57}$$

The adaptive economic potentials for internal metabolites read:

$$\mathbf{w}^{\mathrm{ind,opt}} = \frac{\partial g^{\mathrm{opt}}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} = \frac{\partial z}{\partial \mathbf{s}} \left[\frac{\partial \mathbf{s}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} + \frac{\partial \mathbf{s}}{\partial \mathbf{u}} \frac{\partial \mathbf{u}^{\mathrm{opt}}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} \right] = \frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}} + \left[\frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \mathbf{u}} \right] \frac{\partial \mathbf{u}^{\mathrm{opt}}}{\partial \boldsymbol{\varphi}^{\mathrm{ind}}}. \tag{P58}$$

The term in brackets is $\partial g / \partial \mathbf{u}$, the total derivative of the return with respect to the enzyme levels. If the metabolic state is completely enzyme-balanced (i.e. all reactions are active and satisfy the cost-benefit balance), this term vanishes and Eq. (P58) yields the same result as Eq. (P56). A similar argument holds for the external economic loads: if we differentiate g^{opt} by $\mathbf{x}$ instead of $\boldsymbol{\varphi}^{\mathrm{ind}}$, we obtain

$$p^{\mathrm{x,opt}} = \frac{\partial g^{\mathrm{opt}}}{\partial \mathbf{x}} = \frac{\partial z}{\partial \mathbf{s}} \left[\frac{\partial \mathbf{s}}{\partial \mathbf{x}} + \frac{\partial \mathbf{s}}{\partial \mathbf{u}} \frac{\partial \mathbf{u}^{\mathrm{opt}}}{\partial \mathbf{x}} \right] = \frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \mathbf{x}} + \left[\frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \mathbf{u}} \right] \frac{\partial \mathbf{u}^{\mathrm{opt}}}{\partial \mathbf{x}} = \frac{\partial z}{\partial \mathbf{s}} \frac{\partial \mathbf{s}}{\partial \mathbf{x}} = p^{\mathrm{x}}. \tag{P59}$$

To summarise: the first-order adaptive and non-adaptive economic potentials are identical, and the same holds for the economic loads. Since we use only first-order quantities, the distinction between adaptive and non-adaptive quantities does not play a role.

P4 Economic loads and compound balance

P4.1 The economic load

The total demand g_i^{c} of an internal metabolite (i.e., the marginal effect $g_i^{\mathrm{c}} = \frac{\partial g}{\partial \gamma_i}$ of a virtual concentration change $\delta\gamma_i$ on the metabolic return g) is the sum

$$g_i^{\mathrm{c}} = z_i^{\mathrm{c}} + \sum_l C_l^{\mathrm{g}} \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l} \tag{P60}$$

of a direct concentration gain z_i^{c} and an indirect concentration demand, which I call the economic load p_i^{c}. The load is an effective concentration gain induced by the system, i.e., the fact that the metabolite's concentration has an influence on the metabolic return via changes of the steady state. The vector of internal loads reads

$$\mathbf{p}^{\mathrm{c}} = [\mathbf{C}^{\mathrm{g}}\,\bar{\mathbf{E}}]^\top = [\mathbf{z}^{\mathrm{v}\top}\,\mathbf{C}^{\mathrm{J}}\,\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top}\,\mathbf{C}^{\mathrm{S}}\,\bar{\mathbf{E}}]^\top \tag{P61}$$

By summing the economic loads over conserved moieties and applying the connectivity theorem we obtain the simple equality

$$\mathbf{L}^\top\,\mathbf{p}^{\mathrm{c}} = [[\mathbf{z}^{\mathrm{v}\top}\,\mathbf{C}^{\mathrm{J}} + \mathbf{z}^{\mathrm{c}\top}\,\mathbf{C}^{\mathrm{S}}]\,\bar{\mathbf{E}}\,\mathbf{L}]^\top = -\mathbf{L}^\top\,\mathbf{z}^{\mathrm{c}}. \tag{P62}$$

where we used the connectivity theorems of metabolic control analysis.

P4.2 Compound law Eq. (19)

Demonstration by thought experiment The compound rule Eq. (19) between economic loads and potentials can be shown by a thought experiment. Consider a compensated local variation, consisting of the metabolite variation $\mathrm{d}c$ and compensating enzyme variations $\mathrm{d}u_l$ in all reactions directly affected by $\mathrm{d}c$. For exact compensation, the enzyme levels must satisfy

$$0 = \mathrm{d}v_l = \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}\,\mathrm{d}c_i + \frac{v_l}{u_l}\,\mathrm{d}u_l \quad \Rightarrow \quad \mathrm{d}u_l = -\frac{u_l}{v_l}\,\bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}\,\mathrm{d}c_i. \tag{P63}$$

The concentration change, by itself, would yield a direct fitness change $z_i^{\mathrm{c}}\,\mathrm{d}c_i$ (by changing the metabolite level directly) and an indirect fitness change $p_i^{\mathrm{c}}\,\mathrm{d}c_i$ (by affecting the rest of the system, which includes compensating effects on the metabolite level in question). The enzyme variations would cause direct fitness changes $-h_l^{\mathrm{u}}\,\mathrm{d}u_l$ and indirect fitness changes $g_l^{\mathrm{v}}\,\frac{v_l}{u_l}\,\mathrm{d}u_l$. Therefore, the total fitness change reads

$$\mathrm{d}f = z_i^{\mathrm{c}}\,\mathrm{d}c_i + p_i^{\mathrm{c}}\,\mathrm{d}c_i - h_l^{\mathrm{u}}\,\mathrm{d}u_l + g_l^{\mathrm{v}}\,\frac{v_l}{u_l}\,\mathrm{d}u_l. \tag{P64}$$

Since the compensated variation causes no change in the surrounding network, the fitness change can also be written as a sum of direct effects

$$\mathrm{d}f = z_i^{\mathrm{c}}\,\mathrm{d}c_i - \sum_l h_l^{\mathrm{u}}\,\mathrm{d}u_l. \tag{P65}$$

A comparison between Eqs (P64) and (P65) shows that the indirect effects must cancel out:

$$p_i^{\mathrm{c}}\,\mathrm{d}c_i = -\sum_l g_l^{\mathrm{v}}\,\frac{v_l}{u_l}\,\mathrm{d}u_l \tag{P66}$$

This holds for any compensated local variation: the indirect fitness changes cause by the different local variations must add up to zero. By inserting $\mathrm{d}u_l$ from Eq. (P63), we obtain the compound rule

$$p_i^{\mathrm{c}}\,\mathrm{d}c_i = \sum_l g_l^{\mathrm{v}}\,\bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}\,\mathrm{d}c_i \quad \Rightarrow \quad p_i^{\mathrm{c}} = \sum_l g_l^{\mathrm{v}}\,\bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}. \tag{P67}$$

In fact, our thought experiment would also work with non-enzymatic reactions (in this case, we could consider virtual changes of rate constants). Therefore, the result holds generally for networks with non-enzymatic reactions or non-specific enzymes.

Direct proof of compound rule Eq. (19) The internal loads on the left, written as a row vector, read

$$\mathbf{p}^{\mathrm{c}\top} = [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}]\,\bar{\mathbf{E}}. \tag{P68}$$

The right-hand side reads

$$\mathbf{g}^{\mathrm{v}\top}\bar{\mathbf{E}} = [\hat{\mathbf{z}}^{\mathrm{v}} + \Delta\mathbf{w}]^{\top}\bar{\mathbf{E}} = [\mathbf{z}^{\mathrm{v}} + \mathbf{N}^{\top}\mathbf{w}^{\mathrm{c}}]^{\top}\bar{\mathbf{E}} = \mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{w}^{\mathrm{c}\top}\mathbf{N}\bar{\mathbf{E}} \tag{P69}$$

We insert

$$\mathbf{w}^{\mathrm{c}} = \mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}}_{\varphi^{\mathrm{ind}}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}} = [\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top}]\,\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}} \tag{P70}$$

and obtain

$$\begin{aligned}\mathbf{g}^{\mathrm{v}\top}\bar{\mathbf{E}} &= \mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + [\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}\top}]\mathbf{C}^{\mathrm{S}}_{\varphi^{\mathrm{ind}}}\mathbf{N}\bar{\mathbf{E}} = [\mathbf{z}^{\mathrm{v}\top}[\mathbf{I} + \bar{\mathbf{E}}\,\mathbf{C}^{\mathrm{S}}] + \mathbf{z}^{\mathrm{c}\top}\,\mathbf{C}^{\mathrm{S}}]\,\bar{\mathbf{E}}\\ &= [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}} + \mathbf{z}^{\mathrm{c}\top}\,\mathbf{C}^{\mathrm{S}}]\,\bar{\mathbf{E}},\end{aligned} \tag{P71}$$

the same result as for the left-hand side.

P4.3 Economic potentials obtained from compound rule Eq. (10)

To derive Eq. (10), we first write the compound rule as $\mathbf{p}^{\mathrm{c}} = \bar{\mathbf{E}}^{\top}[\mathbf{z}^{\mathrm{v}} + \Delta\mathbf{w}^{\mathrm{c}}]$ (splitting the flux demand into total flux gain $\mathbf{z}^{\mathrm{v}}$ and the internal economic potential balance $\mathbf{w}^{\mathrm{c}}$). In models without moiety conservation, we can replace $\mathbf{p}^{\mathrm{c}} = -\mathbf{z}^{\mathrm{c}}$ and obtain $-\mathbf{z}^{\mathrm{c}} = \bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \bar{\mathbf{E}}^{\top}\mathbf{N}^{\top}\mathbf{w}^{\mathrm{c}}$ and thus

$$\mathbf{w}^{\mathrm{c}} = -((\mathbf{N}\,\bar{\mathbf{E}})^{\top})^{-1}\,[\bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \mathbf{z}^{\mathrm{c}}] = -(\mathbf{M}^{\top})^{-1}\mathbf{z}^{\mathrm{c}*}.$$

In models with moiety conservation, we left-multiply the compound rule by $\mathbf{L}^{\top}$ and obtain $\mathbf{L}^{\top}\mathbf{p}^{\mathrm{c}} = \mathbf{L}^{\top}\bar{\mathbf{E}}^{\top}[\mathbf{z}^{\mathrm{v}} + \mathbf{N}_{\mathrm{R}}^{\top}\mathbf{L}^{\top}\mathbf{w}^{\mathrm{c}}]$. We can now replace $\mathbf{L}^{\top}\mathbf{p}^{\mathrm{c}} = -\mathbf{L}^{\top}\mathbf{z}^{\mathrm{c}}$ and obtain $-\mathbf{L}^{\top}\mathbf{z}^{\mathrm{c}} = \mathbf{L}^{\top}\bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \mathbf{L}^{\top}\bar{\mathbf{E}}^{\top}\mathbf{N}_{\mathrm{R}}^{\top}\mathbf{L}^{\top}\mathbf{w}^{\mathrm{c}}$. Rearranging yields $0 = \mathbf{L}^{\top}[\mathbf{z}^{\mathrm{c}} + \bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}}] + (\mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L})^{\top}\mathbf{L}^{\top}\mathbf{w}^{\mathrm{c}}$ and thus $\mathbf{L}^{\top}\mathbf{w}^{\mathrm{c}} = -((\mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L})^{\top})^{-1}\mathbf{L}^{\top}\,[\bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \mathbf{z}^{\mathrm{c}}]$. With the standard defintion of economic potentials (where dependent metabolites have vanishing economic potentials), we finally obtain

$$\mathbf{w}^{\mathrm{c}} = -\mathbf{I}_{\mathrm{R}}^{\top}\,((\mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L})^{\top})^{-1}\mathbf{L}^{\top}\,[\bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \mathbf{z}^{\mathrm{c}}] = -\mathbf{I}_{\mathrm{R}}^{\top}\,(\mathbf{M}^{\top})^{-1}\mathbf{L}^{\top}\,\mathbf{z}^{\mathrm{c}*}.$$

P5 Balance equations

P5.1 Reaction balance (Theorem 6)

To derive the reaction balance Eq. (11), we start from the cost-benefit balance $g_l^{\mathrm{v}}\,v_l = h_l^{\mathrm{u}}\,u_l$ and insert Eq. (**??**) for the flux demands. Above, we defined the economic potentials as the demand with respect to supply fluxes and showed that they satisfy the reaction balance. Instead, we can also derive the reaction balance directly and interpret the quantities w_i appearing in the equation as potentials. From the cost-benefit balance (3), we obtain

$$\begin{aligned}\frac{\partial h}{\partial \mathbf{u}} = \frac{\partial g}{\partial \mathbf{u}} &= \mathbf{C}^{\mathrm{g}}\,\mathrm{Dg}(\bar{\mathbf{E}}_{\mathrm{u}}) = [\mathbf{z}^{\mathrm{v}\top}\mathbf{C}^{\mathrm{J}} + \mathbf{z}^{\mathrm{c}\top}\mathbf{C}^{\mathrm{S}}]\,\mathrm{Dg}(\mathbf{v}/\mathbf{u})\\ &= [\mathbf{z}^{\mathrm{v}\top}(\mathbf{I} - \bar{\mathbf{E}}\,\mathbf{L}\,\mathbf{M}^{-1}\mathbf{N}_{\mathrm{R}}) - \mathbf{z}^{\mathrm{c}\top}\mathbf{L}\,\mathbf{M}^{-1}\mathbf{N}_{\mathrm{R}}]\,\mathrm{Dg}(\mathbf{v}/\mathbf{u})\\ &= [\mathbf{z}^{\mathrm{v}\top} - \underbrace{(\mathbf{z}^{\mathrm{v}\top}\bar{\mathbf{E}} + \mathbf{z}^{\mathrm{c}})\,\mathbf{L}\,\mathbf{M}^{-1}\mathbf{I}_{\mathrm{R}}}_{\hat{\mathbf{w}}^{\mathrm{c}\top}}\,\mathbf{N}]\,\mathrm{Dg}(\mathbf{v}/\mathbf{u})\\ &= [\mathbf{z}^{\mathrm{v}\top} + \hat{\mathbf{w}}^{\mathrm{c}\top}\,\mathbf{N}]\,\mathrm{Dg}(\mathbf{v}/\mathbf{u}).\end{aligned} \tag{P72}$$

The vector $\hat{\mathbf{w}}^{\mathrm{c}}$ is exactly the vector of economic potentials defined in Eq. (P49). This trick – rewriting the control coefficients in terms of local balances – works not only for our metabolic objective $z(\mathbf{v}, \mathbf{c})$, but for any differentiable

function $a(\mathbf{v}, \mathbf{c})$ of the state variables – including likelihood functions, relating a model to experimental data. In general, we can write its control coefficients as

$$\begin{aligned} \mathbf{C}_v^{\mathrm{a}} &= (a_c^\top \mathbf{C}^{\mathrm{S}} + a_v^\top \mathbf{C}^{\mathrm{J}}) = a_v + (a_c^\top + a_v^\top \bar{\mathbf{E}})\mathbf{C}^{\mathrm{S}} = a_v + (a_c^\top + a_v^\top \bar{\mathbf{E}})(-\mathbf{L})(\mathbf{N}\bar{\mathbf{E}}\mathbf{L})^{-1}\mathbf{N}_{\mathrm{R}} \\ &= a_v + \boldsymbol{\alpha}^\top \mathbf{N}_{\mathrm{R}} \end{aligned} \tag{P73}$$

with the abbreviations $a_{v_l} = \partial a/\partial v_l$ (direct flux effect on a) and $\boldsymbol{\alpha} := (a_c + a_v\bar{\mathbf{E}})(-\mathbf{L})(\mathbf{N}\,\bar{\mathbf{E}}\,\mathbf{L})^{-1}$ (potential of internal metabolites representing the indirect flux effects on a).

P5.2 Compound balance (Theorem 6)

To prove the compound balance Eq. (14), we think of a metabolite that participates in enzymatic reactions only.

External metabolites. The economic load p_j^{x} of an external metabolite is defined as $p_j^{\mathrm{x}} = \partial g/\partial x_j$, where $g(\mathbf{u}, \mathbf{x})$ is the metabolic return in steady state. The load can be written as

$$p_j^{\mathrm{x}} = \sum_l g_l^{\mathrm{v}}\, \bar{E}_{\mathrm{x}_j}^{\mathrm{v}_l} \tag{P74}$$

with unscaled elasticities $\bar{E}_{\mathrm{x}_j}^{\mathrm{v}_l}$. According to the cost-benefit balance (3), the flux demand g_l^{v} must be balanced with the flux price $h_l^{\mathrm{u}}\, u_l/v_l$. By inserting this into Eq. (P74) and multiplying with the external metabolite level x_j, we obtain

$$x_j\, p_j^{\mathrm{x}} = \sum_l h_l^{\mathrm{u}} u_l \frac{x_j}{v_l}\, \bar{E}_{\mathrm{x}_j}^{\mathrm{v}_l} = \sum_l h_l^{\mathrm{u}}\, u_l\, E_{\mathrm{x}_j}^{\mathrm{v}_l} \tag{P75}$$

with scaled elasticities $E_{\mathrm{x}_j}^{\mathrm{v}_l}$.

External parameters For other parameters p_n affecting the rates, we can define economic loads $q_{p_n} = \partial g/\partial p_n$ and obtain an analogous formula

$$p_n\, q_{p_n} = \sum_l h_l^{\mathrm{u}} u_l \frac{p_n}{v_l} \bar{E}_{\mathrm{p}_n}^{\mathrm{v}_l}. = \sum h_l^{\mathrm{u}}\, u_l\, E_{\mathrm{p}_n}^{\mathrm{v}_l}. \tag{P76}$$

P5.3 Compound balance with non-enzymatic reactions

External metabolite Compound balances can comprise non-enzymatic reactions. We first consider the equation for an external metabolite. If we sum separately over enzymatic and non-enzymatic reactions, Eq. (P75) becomes

$$x_j\, p_j^{\mathrm{x}} = \sum_{l\in\mathrm{enz}} h_l^{\mathrm{u}}\, u_l\, E_{\mathrm{x}_j}^{\mathrm{v}_l} + \sum_{l\in\mathrm{non}} g_l^{\mathrm{v}}\, E_{\mathrm{x}_j}^{\mathrm{v}_l}\, v_l. \tag{P77}$$

With the definitions of flux benefits $b_l^{\mathrm{v}} = g_l^{\mathrm{v}}\, v_l$ and enzyme costs $y_l = h_l^{\mathrm{u}}\, u_l$, this reads

$$x_j\, p_j^{\mathrm{x}} = \sum_{l\in\mathrm{enz}} y_l\, E_{\mathrm{x}_j}^{\mathrm{v}_l} + \sum_{l\in\mathrm{non}} b_l^{\mathrm{v}}\, g_l^{\mathrm{v}}\, E_{\mathrm{x}_j}^{\mathrm{v}_l}. \tag{P78}$$

Internal metabolite For internal metabolites, we split the compound rule into

$$p_i^{\mathrm{c}} = \sum_{l\in\mathrm{enz},i} h_l^{\mathrm{v}}\, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l} + \sum_{l\in\mathrm{non}} g_l^{\mathrm{v}}\, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}, \tag{P79}$$

and the final compound balance with non-enzymatic reactions reads

$$p_i^{\mathrm{c}*}\, c_i = \sum_{l\in\mathrm{enz},i} y_l\, E_{\mathrm{c}_i}^{\mathrm{v}_l} + \sum_{l\in\mathrm{non}} b_l^{\mathrm{v}}\, E_{\mathrm{c}_i}^{\mathrm{v}_l}. \tag{P80}$$

Compound balance with dilution fluxes Dilution in growing cells can be described by virtual dilution reactions with rates $v_i^{\mathrm{dil}} = \kappa\, c_i$. The economic potential differences for these reactions $\Delta w_i^{\mathrm{c}} = -w_i^{\mathrm{c}}$, and with the unscaled elasticities read $\bar{E}_{ii}^{\mathrm{dil}} = \mathrm{d}v_i^{\mathrm{dil}}/\mathrm{d}c_i = \kappa$, we obtain an additional term $-w_i^{\mathrm{c}}\,\kappa\, c_i$ on the right. If we bring it to the left, we obtain the effective load $p_i^{\mathrm{c}} + w_m^{\mathrm{c}}\,\kappa\, c_i$.

P6 Reconstruction of enzyme-optimal metabolic models

P6.1 Reconstruction algorithm

A flux distribution is called enzyme-optimal if it appears in a kinetic model in a stable steady state with optimal enzyme levels. Thus, the model must enzyme-balanced and economically stable. To prove that a flux distribution is enzyme-optimal, we need to construct such a model. To do so, one can first determine a flux distribution, then all other steady-state variables, and finally the rate constants. Some model quantities can be predefined (e.g., based on experimental data) in agreement with the relevant constraints. As rate laws, we choose the simultaneous binding modular rate law [4]. By construction, the model is thermodynamically correct and satisfies the reaction balance and the compound balance. The construction consists of two phases.

Steady-state phase In the steady-state phase, we determine a thermodynamically and economically feasible steady state:

1. **Feasible stationary flux distribution (x_j, c_m, v_l)** Fluxes and concentrations are determined by economic flux analysis. The economic balance equation is used as a condition aside from stationarity and thermodynamic constraints. We first compute consistent fluxes; concentrations; equilibrium constants; from the latter, the chemical potentials and thermodynamic driving forces; and finally economic potentials and enzyme costs. These quantities must satisfy the thermodynamic sign constraints and the reaction balance (with predefined gains $\mathbf{z}^{\mathrm{v}}$).

2. **Concentrations $\mathbf{c}$ and equilibrium constants $\mathbf{k}^{\mathrm{eq}}$** together determine the thermodynamic driving forces (vector $\boldsymbol{\Theta} = -\Delta\mathbf{G}/RT = \ln \mathbf{k}^{\mathrm{eq}} - \mathbf{N}^{\mathrm{tot}\top} \log \mathbf{c}$). The equilibrium constants must satisfy the Wegscheider conditions ($\mathbf{N}^{\top} \ln \mathbf{k}^{\mathrm{eq}} = 0$), and thermodynamic forces and fluxes must share the same directions ($v_l \neq 0 \Rightarrow \mathsf{sign}(\Theta_l) = \mathsf{sign}(v_l)$). The reaction affinities Θ_l are bounded to avoid extreme kinetic properties later in the algorithm.

3. **Reaction balance: compute or refine economic potentials and enzyme costs** If the flux distribution $\mathbf{v}$ is incomplete, we restrict our model to the active region. The inactive reactions can always be justified by large enzyme prices or low catalytic constants. In the rest of the algorithm, the flux distribution $\mathbf{v}$ can be assumed to be complete.

Kinetic phase In the kinetic phase of the algorithm, we construct kinetic rate laws in agreement with the predefined steady state. We use the simultaneous binding modular rate law [4] with complete allosteric activation or inhibition. The aim is to find rate constants such that the resulting elasticities satisfy the compound balance. In practice, we predefine economic loads, solve the compound balance for the reaction elasticities, and then solve for the rate constants. To ensure that our elasticities are consistent with the fluxes and thermodynamical laws, we express them by saturation values [4], which are thermodynamically independent. In detail, the kinetic phase of the algorithm looks as follows:

1. **Linear dependencies for saturation values** To satisfy the compound balance, we need to determine saturation levels $\beta_{li}^{\mathrm{M}}, \beta_{li}^{\mathrm{A}}, \beta_{li}^{\mathrm{I}}$ and consistent values of the economic loads. For the rate laws chosen (simultaneous binding modular rate laws with complete allosteric regulation), the scaled elasticities can be written as

$$E_{\mathrm{c}i}^{\mathrm{v}_l} = \underbrace{\frac{\zeta_l m_{li}^{+} - m_{li}^{-}}{\zeta_l - 1}}_{E_{li}^{\Theta}} - \beta_{li}^{\mathrm{M}} \left(m_{li}^{+} + m_{li}^{-}\right) + \alpha_{li}^{\mathrm{A}}\, m_{li}^{\mathrm{A}} - \beta_{li}^{\mathrm{I}}\, m_{li}^{\mathrm{I}} \tag{P81}$$

   where $\zeta_l = \mathrm{e}^{-h_l\, \Delta\mu_l/RT}$ and m_{li}^{+} and m_{li}^{-} are the molecularities for substrates and products. The coefficients m_{li}^{A} and m_{li}^{I} indicate allosteric regulation. The saturation values β_{li}^{M}, β_{li}^{A}, and β_{li}^{I} describe the binding of reactants, allosteric activators, and allosteric inhibitors to the enzyme. Formula (P81) stems from Eqs (33), (34), (35), and (37) in [4]. From the compound balance, we obtain conditions for the saturation values.

For external metabolites with index j, we obtain

$$\sum_l y_l \, E^{\Theta}_{lj} \;=\; p^{\mathrm{x}}_j \, x_j + \sum_l (y_l \, m_{lj}) \, \beta^{\mathrm{M}}_{lj} - \sum_l (y_l \, m^{\mathrm{A}}_{lj}) \, \alpha^{\mathrm{A}}_l + \sum_l (y_l \, m^{\mathrm{I}}_{lj}) \, \beta^{\mathrm{I}}_{lj} \tag{P82}$$

and for independent internal metabolites (with index i)

$$\sum_l y_l \, E^{\Theta}_{li} \;=\; \mathbf{p}^{\mathrm{c}} \, c + \sum_l (y_l \, m_{li}) \, \beta^{\mathrm{M}}_{li} - (y_l \, m^{\mathrm{A}}_{li}) \, \alpha^{\mathrm{A}}_{li} + (y_l \, m^{\mathrm{I}}_{li}) \, \beta^{\mathrm{I}}_{li}. \tag{P83}$$

For details, see P6.2.

2. **Linear constraints for saturation values** With their left-hand sides being predetermined by earlier steps of the algorithm, Eqs (P82) and (P83) can be used as linear constraints for the saturation values. In addition, we set lower and upper bounds (e.g., restrict saturation values to the range [0.05, 9.95] to avoid full saturation); metabolite loads can be predefined, but it may not be possible to realise them precisely (in which case they are approximated). Thus, we obtain a set of linear inequalities for the saturation values and for the metabolite loads: to obtain specific solutions, we can sample parameter sets under these constraints, or we choose an optimal parameter set within the feasible region. In practice, we first search for a solution using a predefined concentration gain vector $\mathbf{z}^{\mathrm{c}}$. If no solution exists, we repeat the search, but allow for changes of $\mathbf{z}^{\mathrm{c}}$, which we also minimise.

3. **Solving for the rate constants** Before we determine the rate constants, we choose enzyme levels u_l in a physiologically sensible range (based on proteome data or proportional to the enzyme costs y_l). Then we compute the K_{M} values and activation and inhibition constants from the saturation values and the metabolite level. For each enzyme, there remains one unknown parameter k^{V}_l, the geometric mean of forward and reverse catalytic constants. It needs to be chosen such that the reaction rates $r_l(\mathbf{c}, \mathbf{u})$ from the rate laws match the predefined fluxes v_l. Given all other kinetic constants, equilibrium constants, metabolite concentrations, enzyme levels, and fluxes v_l, we can directly solve for the the constants k^{V}_l.

The resulting model has an enzyme-balanced steady state. If it is dynamically and economically stable (which can be tested by inspecting the Jacobian and the fitness curvature matrix), the flux distribution is enzyme-optimal. We can use this test as a filter for sampled models: if the Jacobian or the fitness curvature matrix has a positive eigenvalue, the sampled model is discarded. .

P6.2 Solving the compound balance equations for reaction elasticities

In the construction of enzyme-balanced kinetic models, there is a point where fluxes v_l, concentrations c_i, and chemical potential differences $\Delta\mu_l$ have been fixed, the model has been restricted to active reactions, and enzyme costs y_l and economic potentials w_i have been determined in accordance with the reaction balance. Now elasticities and economic loads need to be chosen in agreement with the load balance equation. The simultaneous binding modular rate laws [4] (which are used in the model) are saturable and their scaled elasticities can be written in terms of saturation values, which we treat here as free variables. The compound balance defines linear constraints for the saturation values; we can then determine them by quadratic programming (to find the maximum of some Gaussian probability distribution) or by uniform sampling. These are the relevant constraints:

1. Compound balance for scaled elasticities $\bar{E}^{\mathrm{v_l}}_{\mathrm{x}_j}$ and economic loads, for external metabolites

$$p^{\mathrm{x}}_j \, x_j = \sum_{li} y_l \, E^{\mathrm{v_l}}_{\mathrm{x}_j} \tag{P84}$$

and for internal metabolites

$$p^{\mathrm{c}}_i \, c_i = \sum_l y_l \, E^{\mathrm{v_l}}_{\mathrm{c}_i}. \tag{P85}$$

Given the values of x_j, c_i, and y_l, these equations can be used as linear constraints for p^{c}_i and $E^{\mathrm{v_l}}_{\mathrm{c}_i}$.

2. The internal economic loads p_i^{c} satisfy the equality $\mathbf{L}\,\mathbf{p}^{\mathrm{c}} = -\mathbf{L}\,\mathbf{z}^{\mathrm{c}}$. For external metabolites, we can assume that metabolites being consumed have positive loads and metabolites being produced have negative loads. This yields the sign constraints (for all external loads p_j^{x})

$$\mathsf{sign}\left(\sum_l n_{jl}\, v_l\right) = -\mathsf{sign}(p_j^{\mathrm{x}}). \tag{P86}$$

These sign constraints are not mandatory, but only rules of thumb. For the case that uptake and production fluxes of a metabolite cancel out, the sign of p_j^{x} remains unconstrained.

3. In the modular rate laws, the saturation values β_{li}^{M}, β_{li}^{A}, and β_{li}^{I}, describe the binding of reactants, allosteric activators, and allosteric inhibitors in a metabolic state in question. For convenience, we define $\alpha_{li}^{\mathrm{A}} = 1 - \beta_{li}^{\mathrm{A}}$. The scaled elasticities of the simultaneous binding modular rate law with complete allosteric regulation [4] can be written in terms of the saturation values as

$$E_{\mathrm{c}_i}^{\mathrm{v}_l} = E_{li}^{\Theta} - \beta_{li}^{\mathrm{M}}\left(m_{li}^{+} + m_{li}^{-}\right) + \alpha_{li}^{\mathrm{A}}\, m_{li}^{\mathrm{A}} - \beta_{li}^{\mathrm{I}}\, m_{li}^{\mathrm{I}} \tag{P87}$$

where $E_{li}^{\Theta} = \frac{\zeta_l m_{li}^{+} - m_{li}^{-}}{\zeta_l - 1}$, $\zeta_l = \mathrm{e}^{-h_l\,\Delta\mu_l/RT}$, and m_{li}^{+} and m_{li}^{-} are the molecularities of substrates and products. The coefficients m_{li}^{A} and m_{li}^{I} indicate allosteric regulation. Formula (P81) stems from Eqs (33), (34), (35), and (37) in [4]. With predefined values ζ_l, $m_{li}^{\pm}$, and $w_{li}^{\pm}$, the elasticity $E_{\mathrm{c}_i}^{\mathrm{v}_l}$ depends linearly on the saturation values.

4. All saturation values are constrained by $0 \le \beta_{li} \le 1$ or tighter constraints (e.g., between 0.05 and 0.95). Of course, only relevant saturation values (corresponding to non-zero values of m_{li}^{+}, m_{li}^{-}, m_{li}^{A}, or m_{li}^{I}) need to be considered.

P6.3 Example: enzyme and ribosome production in growing cells

Let us now consider the simple example of a growing cell in Figure 7, with a choice of elasticities that allows for a solution. To effectively model growth maximisation at a constrained biomass concentration, we assume a positive concentration demand for biomass (but not for the other compounds). Feasible economic potentials can be computed from the compound rules, which entail the flux demands g_l^{v}. In the general case, the reaction rule (P92) and the compound rule (P91) become

$$\begin{aligned} \mathbf{g}^{\mathrm{v}} &= \mathbf{N}^{\top}\mathbf{w}^{\mathrm{c}} + \mathbf{N}^{\mathrm{x}\top}\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}} \\ \mathbf{z}^{\mathrm{c}} &= \bar{\mathbf{E}}^{\top}\mathbf{g}^{\mathrm{v}} - \kappa\,\mathbf{w}^{\mathrm{c}}. \end{aligned} \tag{P88}$$

This yields

$$\begin{aligned} 0 &= \mathbf{z}^{\mathrm{c}} + \bar{\mathbf{E}}^{\top}[\mathbf{N}^{\top}\mathbf{w}^{\mathrm{c}} + \mathbf{N}^{\mathrm{x}\top}\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}] - \kappa\,\mathbf{w}^{\mathrm{c}} \\ \Rightarrow\quad \mathbf{w}^{\mathrm{c}} &= -((\mathbf{N}\bar{\mathbf{E}} - \kappa\,\mathbf{I})^{\top})^{-1}[\mathbf{z}^{\mathrm{c}} + \bar{\mathbf{E}}^{\top}\left[\mathbf{N}^{\mathrm{x}\top}\,\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}\right]] \\ &= -((\mathbf{N}\bar{\mathbf{E}} - \kappa\,\mathbf{I})^{\top})^{-1}[\mathbf{z}^{\mathrm{c}} + \bar{\mathbf{E}}^{\top}\,\mathbf{z}^{\mathrm{v}}]. \end{aligned} \tag{P89}$$

The matrix in brackets is the Jacobian, and if the system is dynamically stable, it is invertible. This means that, given the external economic potentials and the growth rate κ, we can solve for the internal economic potentials.

Now let us apply this to our example. We use subscripts for compounds (energy p, intermediate i, enzyme e, ribosome r, and biomass b) and reactions (catabolism C, anabolism A, enzyme production E, ribosome production R). Assuming that all stoichiometric coefficients are 1, and that there are no direct flux gains, the flux demands are given by the reaction rules

$$\begin{aligned} g_C^{\mathrm{v}} &= w_e^{\mathrm{c}} + w_i^{\mathrm{c}} - w_{glucose}^{\mathrm{c}} \\ g_A^{\mathrm{v}} &= w_b^{\mathrm{c}} - w_e^{\mathrm{c}} - w_i^{\mathrm{c}} \\ g_E^{\mathrm{v}} &= w_e^{\mathrm{c}} - w_p^{\mathrm{c}} - w_i^{\mathrm{c}} \\ g_R^{\mathrm{v}} &= w_r^{\mathrm{c}} - w_p^{\mathrm{c}} - w_i^{\mathrm{c}} \end{aligned} \tag{P90}$$

or, in matrix form,

$$\begin{pmatrix} g_C^{\mathrm{v}} \\ g_A^{\mathrm{v}} \\ g_E^{\mathrm{v}} \\ g_R^{\mathrm{v}} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \\ -1 & -1 & 0 & 0 & 1 \\ -1 & -1 & 1 & 0 & 0 \\ -1 & -1 & 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} w_p^{\mathrm{c}} \\ w_i^{\mathrm{c}} \\ w_e^{\mathrm{c}} \\ w_r^{\mathrm{c}} \\ w_b^{\mathrm{c}} \end{pmatrix} + \begin{pmatrix} -1 \\ 0 \\ 0 \\ 0 \end{pmatrix} w_{glucose}^{\mathrm{c}}. \tag{P91}$$

where the first matrix is $\mathbf{N}^\top$. The compound rules, on the contrary, read

$$\begin{aligned} 0 &= \bar{E}_p^C\, g_C^{\mathrm{v}} + \bar{E}_p^A\, g_A^{\mathrm{v}} + \bar{E}_p^E\, g_E^{\mathrm{v}} + \bar{E}_p^R\, g_R^{\mathrm{v}} - \kappa\, w_p^{\mathrm{c}} \\ 0 &= \bar{E}_i^C\, g_C^{\mathrm{v}} + \bar{E}_i^A\, g_A^{\mathrm{v}} + \bar{E}_i^E\, g_E^{\mathrm{v}} + \bar{E}_i^R\, g_R^{\mathrm{v}} - \kappa\, w_i^{\mathrm{c}} \\ 0 &= \bar{E}_e^C\, g_C^{\mathrm{v}} + \bar{E}_e^A\, g_A^{\mathrm{v}} + \bar{E}_e^E\, g_E^{\mathrm{v}} + \bar{E}_e^R\, g_R^{\mathrm{v}} - \kappa\, w_e^{\mathrm{c}} \\ 0 &= \bar{E}_r^C\, g_C^{\mathrm{v}} + \bar{E}_r^A\, g_A^{\mathrm{v}} + \bar{E}_r^E\, g_E^{\mathrm{v}} + \bar{E}_r^R\, g_R^{\mathrm{v}} - \kappa\, w_r^{\mathrm{c}} \\ -z_{\mathrm{BM}}^{\mathrm{c}} &= \bar{E}_b^C\, g_C^{\mathrm{v}} + \bar{E}_b^A\, g_A^{\mathrm{v}} + \bar{E}_b^E\, g_E^{\mathrm{v}} + \bar{E}_b^R\, g_R^{\mathrm{v}} - \kappa\, w_b^{\mathrm{c}} \end{aligned}$$

or, in matrix form,

$$\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ -z_{\mathrm{BM}}^{\mathrm{c}} \end{pmatrix} = \begin{pmatrix} \bar{E}_p^C & \bar{E}_p^A & \bar{E}_p^E & \bar{E}_p^R \\ \bar{E}_i^C & \bar{E}_i^A & \bar{E}_i^E & \bar{E}_i^R \\ \bar{E}_e^C & \bar{E}_e^A & \bar{E}_e^E & \bar{E}_e^R \\ \bar{E}_r^C & \bar{E}_r^A & \bar{E}_r^E & \bar{E}_r^R \\ \bar{E}_b^C & \bar{E}_b^A & \bar{E}_b^E & \bar{E}_r^B \end{pmatrix} \begin{pmatrix} g_C^{\mathrm{v}} \\ g_A^{\mathrm{v}} \\ g_E^{\mathrm{v}} \\ g_R^{\mathrm{v}} \end{pmatrix} - \kappa \begin{pmatrix} w_p^{\mathrm{c}} \\ w_i^{\mathrm{c}} \\ w_e^{\mathrm{c}} \\ w_r^{\mathrm{c}} \\ w_b^{\mathrm{c}} \end{pmatrix}.$$

Altogether, we obtain four linear equations for the four internal economic potentials. To solve them, we need to assign numbers to the external economic potentials and to the elasticities. We make some simple assumptions. First, we assume that high-energy phosphates (in moles), as well as intermediates, enzymes, and ribosomes (in carbon moles) come in equal amounts, which we set to 1. Due to dilution, and with the concentrations and stoichiometric coefficients chosen, a steady state requires that the fluxes show the proportions $(3, 1, 1, 1)^\top$. For the elasticities, we assume that: p and i exert scaled elasticities of 1/2 (partial saturation) if they appear as a substrate or as a product; e and r would normally exert scaled elasticities of 1, but since each of them catalyses two reactions, the elasticities are (factor of 1/2 for each reaction). By multiplying this with the fluxes (and assuming that all concentrations have values of 1), we obtain the (transposed) unscaled elasticity matrix

$$\bar{\mathbf{E}} = \begin{pmatrix} \bar{E}_p^C & \bar{E}_p^A & \bar{E}_p^E & \bar{E}_p^R \\ \bar{E}_i^C & \bar{E}_i^A & \bar{E}_i^E & \bar{E}_i^R \\ \bar{E}_e^C & \bar{E}_e^A & \bar{E}_e^E & \bar{E}_e^R \\ \bar{E}_r^C & \bar{E}_r^A & \bar{E}_r^E & \bar{E}_r^R \\ \bar{E}_b^C & \bar{E}_b^A & \bar{E}_b^E & \bar{E}_r^B \end{pmatrix}^\top = \begin{pmatrix} -1 & -1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 \\ 1/3 & 1/3 & 0 & 1 & 0 \\ 1/3 & 1/3 & 0 & 1 & 0 \end{pmatrix}$$

Assuming, in addition, a dilution rate $\kappa = 1$ and an external economic potential of 4 (instead of 1, as above) for the biomass, we obtain

$$\begin{aligned} \mathbf{w}^{\mathrm{c}} &= -([\mathbf{N}\,\bar{\mathbf{E}} - \kappa\,\mathbf{I}]^\top)^{-1}[\mathbf{z}^{\mathrm{c}} + \bar{\mathbf{E}}_{\mathrm{x}}^\top \mathbf{N}^{\mathrm{x}\top}\,\mathbf{w}^{\mathrm{x}}] \\ &= -\left[\begin{pmatrix} -8/3 & -8/3 & 1/3 & 1/3 & 1 \\ -8/3 & -8/3 & 1/3 & 1/3 & 1 \\ 0 & 0 & 0 & 0 & 1 \\ -2 & -2 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & \end{pmatrix}^\top - \mathbf{I}\right]^{-1} \left[\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 4 \end{pmatrix} + 0 \cdot \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}\right] \\ &= -\begin{pmatrix} -1/2 & 1/2 & -1/4 & -1/4 & -1/4 \\ 1/2 & -1/2 & -1/4 & -1/4 & -1/4 \\ 0 & 0 & -1 & 0 & -1 \\ 3/2 & 3/2 & -15/4 & -19/4 & -3/4 \\ 0 & 0 & 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 6 \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 4 \\ 3 \\ 4 \end{pmatrix} \end{aligned} \tag{P92}$$

The vector of flux demands reads $\mathbf{w}^{\mathrm{v}} = (2,\, 2,\, 2,\, 1)^\top$.

P7 Ribosome overhead cost

A ribosome overhead factor $f_{\mathrm{rib}}(\kappa)$ can be computed as follows. We assume ribosomes with concentration r (in mM) and size L_{rib} (amino acids per ribosome) and non-ribosomal proteins with concentrations p_l and sizes L_l. We further assume that all ribosomes are stable, that they operate at full speed, and that a ribosomes processes ρ amino acid molecules per second. With a cell growth rate κ and specific degradation rates κ_l and κ_{rib}, the effective degradation rates (in amino acid units per second) are given by

$$v_{\mathrm{rib}} = (\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}\, r, \quad v_{\mathrm{prot}} = \sum_l (\kappa\ + \kappa_l)\, L_l\, p_l. \tag{P93}$$

The sum of these rates must be balanced with the total protein production rate, given by $v_{\mathrm{tot}} = \rho\, r$. We obtain the equality

$$\rho\, r = v_{\mathrm{rib}} + v_{\mathrm{prot}} = (\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}\, r + v_{\mathrm{prot}}. \tag{P94}$$

Solving this for the ribosome level, we obtain

$$r = \frac{v_{\mathrm{prot}}}{\rho - (\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}}. \tag{P95}$$

Thus, the effective ribosome degradation rate yields

$$v_{\mathrm{rib}} = (\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}\, r = \frac{(\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}}{\rho - (\kappa + \kappa_{\mathrm{rib}})\, L_{\mathrm{rib}}} v_{\mathrm{prot}}. \tag{P96}$$

Assuming that ribosomes are not degraded (thus setting $\kappa_{\mathrm{rib}} = 0$), we obtain

$$v_{\mathrm{tot}} = v_{\mathrm{prot}} + v_{\mathrm{rib}} = \left(1 + \frac{\kappa\, L_{\mathrm{rib}}}{\rho - \kappa\, L_{\mathrm{rib}}}\right) v_{\mathrm{prot}} = \left(\frac{\rho}{\rho - \kappa\, L_{\mathrm{rib}}}\right) v_{\mathrm{prot}}. \tag{P97}$$

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