The value structure of metabolic states

Mathematical details and proofs

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Contents

S1 Economic balance equations – derivations in comparison

The economic balance equations for various metabolic optimality problems can be derived in a few simple steps. Let us see this for the optimality problems shown in Figure **??**. First, to make the problems comparable, we write them all as *maximisation* problems in expanded form: all model variables are treated as free variables and all relations among them as explicit constraints. In the formulae below, similarities between optimality problems are highlighted by colours. For more detailed derivations, see SI section S2.

1. Flux cost minimisation In flux cost minimisation, we search for a stationary flux distribution $\mathbf{v}$ that minimises a flux cost function $a(\mathbf{v})$ at a given flux benefit $b(\mathbf{v})$. The optimality problem, the Lagrange function, and its optimality condition read:

Maximise$_v$	F	$=$	$-a(\mathbf{v})$	s.t. $\mathbf{N}_{\text{int}}\,\mathbf{v} = 0$	$b(\mathbf{v}) = b'$
	$\mathcal{L}$	$=$	$-a(\mathbf{v})$	$+{\mathbf{w}_{\text{r}}^{\text{int}}}^{\top}\,\mathbf{N}_{\text{int}}\,\mathbf{v}$	$+\alpha_{\text{b}}\,(b(\mathbf{v}) - b')$
$\partial_v\mathcal{L} = 0$:	0	$=$	$-\mathbf{a}_{\text{v}}$	$+\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}}$	$+\mathbf{b}_{\text{v}}$

The optimality condition contains the flux price vector $\mathbf{a}_{\text{v}} = \partial a/\partial\mathbf{v}$, Lagrange multipliers associated with stationarity constraints in a vector $\mathbf{w}_{\text{r}}^{\text{int}}$, and a vector $\mathbf{b}_{\text{v}} = \alpha_{\text{b}}\,\mathbf{b}_{\text{v}}$ (where α_{b} is the Lagrange multiplier for the flux benefit constraint and $\mathbf{b}_{\text{v}} = \partial b/\partial\mathbf{v}$). The resulting balance equation, with flux prices a_{v_l}, reads

$$\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} + \mathbf{b}_{\text{v}} \;=\; \mathbf{a}_{\text{v}}. \qquad \text{Reaction balance} \tag{S1}$$

2. Flux cost minimisation with enzymatic flux cost Now we consider a kinetic model and minimise an enzyme cost $h(\mathbf{e})$ at a fixed flux benefit $b(\mathbf{v})$; the fluxes must be stationary, the rate laws (between metabolite levels $\mathbf{c}$, enzyme levels $\mathbf{e}$, and fluxes $\mathbf{v}$) must be respected, and the metabolite levels must satisfy physiological bounds.

Maximise$_{v,c,e}$	$F =$	-h(e)	s.t. $\mathbf{N}_{\text{int}}\,\mathbf{v} = 0$	$\mathbf{v}(\mathbf{c},\mathbf{e}) = \mathbf{v}$	$b(\mathbf{v}) = b'$	$\mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	$-h(\mathbf{e})$	$+{\mathbf{w}_{\text{r}}^{\text{int}}}^{\top}\,\mathbf{N}_{\text{int}}\,\mathbf{v}$	$-\alpha_{\text{b}}\,(\mathbf{v} - \mathbf{v}(\mathbf{c},\mathbf{e}))$	$+{\mathbf{b}_{\text{v}}}^{\top}(b(\mathbf{v}) - b')$	$-{\mathbf{q}_{\text{c}^{\text{int}}}^{\min}}^{\top}(\mathbf{c} - \mathbf{c}_{\min})$ $+{\mathbf{q}_{\text{c}^{\text{int}}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{c})$
$\partial_v\mathcal{L} = 0$:	$0 =$	0	$+\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}}$	$-\mathbf{a}_{\text{v}}^{\text{kin}}$	$+\mathbf{b}_{\text{v}}$	
$\partial_c\mathcal{L} = 0$:	$0 =$	0	0	$\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}}$		$-\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}$
$\partial_e\mathcal{L} = 0$:	$0 =$	$-\mathbf{h}_e$	0	$\mathbf{E}_{\text{e}}\,\mathbf{a}_{\text{v}}^{\text{kin}}$		

Compared to the previous problem, we obtain the additional Lagrange multiplier vectors $\mathbf{a}_{\text{v}}^{\text{kin}}$ for rate law constraints (with signs chosen for convenience) and $-\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}} = \mathbf{q}_{\text{c}^{\text{int}}}^{\min} + \mathbf{q}_{\text{c}^{\text{int}}}^{\max}$ for metabolite bounds (which vanish for all inactive bounds). Note that a metabolite can have a non-zero entry in $\mathbf{q}_{\text{c}^{\text{int}}}^{\min}$ or $\mathbf{q}_{\text{c}^{\text{int}}}^{\max}$, but not in both of them. By solving the equations (in the order 3, 1, and 2), we obtain

$$\begin{aligned}
\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e \\
\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} + \mathbf{b}_{\text{v}} \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}.
\end{aligned}$$

Combining the equations yields the economic balance equations

$$\begin{aligned}
\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} + \mathbf{b}_{\text{v}} &= \mathbf{a}_{\text{v}}^{\text{kin}} && \text{Reaction balance} \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}} && \text{Load-potential balance}
\end{aligned} \tag{S2}$$

where the flux price $\mathbf{a}_{\text{v}}^{\text{kin}}$, originally a vector of Lagrange multipliers, can now be seen as a shortcut for $\mathbf{a}_{\text{v}}^{\text{kin}} = \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e$.

3. Enzyme benefit-cost optimisation In a kinetic model, we minimise the difference of a metabolic benefit $b(\mathbf{v},\mathbf{c})$ and an enzyme cost $h(\mathbf{e})$; fluxes must be stationary, rate laws must be respected, and metabolite levels must satisfy physiological bounds.

Maximise$_{v,c,e}$	$F =$	$b(\mathbf{v},\mathbf{c}) - h(\mathbf{e})$	s.t. $\mathbf{N}_{\text{int}}\mathbf{v} = 0$	$\mathbf{v}(\mathbf{c},\mathbf{e}) = \mathbf{v}$	$\mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	$b(\mathbf{v},\mathbf{c}) - h(\mathbf{e})$	$+{\mathbf{w}_{\text{r}}^{\text{int}}}^{\top}\mathbf{N}_{\text{int}}\,\mathbf{v}$	$-{\mathbf{a}_{\text{v}}^{\text{kin}}}^{\top}(\mathbf{v} - \mathbf{v}(\mathbf{c},\mathbf{e}))$	$-{\mathbf{q}_{\text{c}^{\text{int}}}^{\min}}^{\top}(\mathbf{c} - \mathbf{c}_{\min})$ $+{\mathbf{q}_{\text{c}^{\text{int}}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{c})$
$\partial_v\mathcal{L} = 0$:	$0 =$	$\mathbf{b}_{\text{v}}$	$+\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}}$	$-\mathbf{a}_{\text{v}}^{\text{kin}}$	
$\partial_c\mathcal{L} = 0$:	$0 =$	$-\mathbf{q}_{\text{c}^{\text{int}}}$	0	$\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	$-\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}$
$\partial_e\mathcal{L} = 0$:	$0 =$	$-\mathbf{h}_e$	0	$\mathbf{E}_{\text{e}}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	

By solving the equations (in the order 3, 1, and 2), we obtain

$$\begin{aligned}
\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e \\
\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} + \mathbf{b}_{\text{v}} \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{q}_{\text{c}^{\text{int}}} + \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}.
\end{aligned}$$

This yields the same economic balance equations as before (but now the terms have a different mathematical meaning)

$$\begin{aligned}
\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} + \mathbf{b}_{\text{v}} &= \mathbf{a}_{\text{v}}^{\text{kin}} && \text{Reaction balance (with kinetics-derived flux prices)} \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} && \text{Metabolite balance}
\end{aligned} \tag{S3}$$

with the apparent metabolite price $\mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} = \mathbf{q}_{\text{c}^{\text{int}}} + \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bound}}$ and again taking $\mathbf{a}_{\text{v}}^{\text{kin}} = \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e$ as a definition.

4. Cost minimisation in metabolite space at given fluxes In a kinetic model, we maximise the difference between metabolic benefit and enzyme cost; all fluxes are given (they must be thermodynamically feasible), the rate laws must be respected, and metabolite levels must satisfy physiological bounds.

Maximise $_{c,e}$	$F =$	$b(\mathbf{c}) - h(\mathbf{e})$	s.t. $\mathbf{v}(\mathbf{c},\mathbf{e}) = \mathbf{v}$	$\mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	$b(\mathbf{v}) - h(\mathbf{e})$	$-{\mathbf{a}_{\text{v}}^{\text{kin}}}^{\top}(\mathbf{v} - \mathbf{v}(\mathbf{c},\mathbf{e}))$	$-{\mathbf{q}_{\text{c}^{\text{int}}}^{\min}}^{\top}(\mathbf{c} - \mathbf{c}_{\min}) + {\mathbf{q}_{\text{c}^{\text{int}}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{c})$
$\partial_c\mathcal{L} = 0$:	$0 =$	$-\mathbf{q}_{\text{c}^{\text{int}}}$	$\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	$-\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}$
$\partial_e\mathcal{L} = 0$:	$0 =$	$-\mathbf{h}_e$	$\mathbf{E}_{\text{e}}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	

By solving the equations (in the order 2 and 1), we obtain

$$\begin{aligned}
\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{q}_{\text{c}^{\text{int}}} + \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}.
\end{aligned}$$

This yields the metabolite balance equation

$$\mathbf{E}_{\text{c}}^{\top}\,\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e = \mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} \qquad \text{Metabolite balance} \tag{S4}$$

with the effective metabolite price $\mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} = \mathbf{q}_{\text{c}^{\text{int}}} + \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}$.

5. Growth optimisation In a metabolic pathway model (with enzyme levels as control variables), we maximise the cell growth rate (a variable that affects dilution and enzyme cost); fluxes must be stationary; rate laws must be respected; metabolite levels must satisfy physiological bounds.

$\text{Maximise}_{\lambda,v,c,e}$	$F =$	$b(\lambda) - h(e,\lambda)$	s.t. $\mathbf{N}_{\text{int}}\mathbf{v} - \lambda\,\mathbf{c} = 0$	$\mathbf{v}(\mathbf{c},\mathbf{e}) = \mathbf{v}$	$\mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	$b(\lambda) - h(e,\lambda)$	${\mathbf{w}_{\text{r}}^{\text{int}}}^{\top}(\mathbf{N}_{\text{int}}\mathbf{v} - \lambda\,\mathbf{c})$	${-\mathbf{a}_{\text{v}}^{\text{kin}}}^{\top}(\mathbf{v} - \mathbf{v}(\mathbf{c},\mathbf{e}))$	${-\mathbf{q}_{\text{c}^{\text{int}}}^{\min}}^{\top}(\mathbf{c} - \mathbf{c}_{\min})$ $+\,{\mathbf{q}_{\text{c}^{\text{int}}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{c})$
$\partial_\lambda\mathcal{L} = 0$:	$0 =$	$b_\lambda - h_\lambda$	$-\mathbf{c}\,\mathbf{w}_{\text{r}}^{\text{int}}$		
$\partial_v\mathcal{L} = 0$:	$0 =$	0	$+\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}}$	$-\mathbf{a}_{\text{v}}^{\text{kin}}$	
$\partial_c\mathcal{L} = 0$:	$0 =$	0	$-\lambda\,\mathbf{w}_{\text{r}}^{\text{int}}$	$\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	$-\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}}$
$\partial_e\mathcal{L} = 0$:	$0 =$	$-\mathbf{h}_e$	0	$\mathbf{E}_{\text{e}}\,\mathbf{a}_{\text{v}}^{\text{kin}}$	

By solving the equations (in the order 4, 3, 2, and 1), we obtain

$$\begin{aligned}
\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e \\
\lambda\,\mathbf{w}_{\text{r}}^{\text{int}} &= \mathbf{E}_{\text{c}}^{\top}\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e - \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}} \\
\mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e &= \mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} \\
b_\lambda - h_\lambda &= \mathbf{c}\cdot\mathbf{w}_{\text{r}}^{\text{int}}.
\end{aligned}$$

This yields the economic balance equations

$$\begin{aligned}
\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}} &= \mathbf{a}_{\text{v}}^{\text{kin}} && \text{Reaction balance} \\
\mathbf{E}_{\text{c}}^{\top}\,\mathbf{a}_{\text{v}}^{\text{kin}} &= \mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} && \text{Metabolite balance} \\
b_\lambda - h_\lambda &= \mathbf{c}\cdot\mathbf{w}_{\text{r}}^{\text{int}} && \text{Growth balance equation}
\end{aligned} \tag{S5}$$

where $\mathbf{a}_{\text{v}}^{\text{kin}} = \mathbf{E}_{\text{e}}^{-1}\,\mathbf{h}_e$ and, this time, $\mathbf{q}_{\text{c}^{\text{int}}}^{\text{app}} = \mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}} + \lambda\,\mathbf{w}_{\text{r}}^{\text{int}}$. This time we have no term $\mathbf{b}_{\text{v}}$. Note that third equation determines a scaling of all economic variables; if the scaling does not matter to us, we can ignore this equation, and $\mathbf{c}$ need not be known. In the metabolite balance, we find the effective metabolite price $\mathbf{q}_{\text{c}^{\text{int}}}^{\text{bnd}} + \lambda\,\mathbf{w}_{\text{r}}^{\text{int}}$. The five different problems shown lead to very similar balance equations. However, these are only a few examples. By exchanging constraints and optimality criteria or by considering multi-criteria optimisation, we can construct many more variants – but the balance equations remain the same.

6. Resource balance analysis (maximal growth) To obtain a general linear RBA model, we slightly modify the previous optimality problem. Now enzymes are not mentioned explicitly, but included in the compound vector $\mathbf{c}$. Again, we maximise the growth rate, where fluxes must be stationary ("mass balance constraint"); linear capacity constraints must be satisfied ("capacity constraint"); weighted sums of metabolite levels must be bounded ("density constraint")

$\text{Maximise}_{\lambda,v,c}$	$F =$	λ	s.t. $\mathbf{N}_{\text{int}}\mathbf{v} - \lambda\,\mathbf{c} = 0$	$-\mathbf{E}_{\text{r}}\,\mathbf{c} \le \mathbf{v} \le \mathbf{E}_{\text{f}}\,\mathbf{c}$	$\mathbf{c}_{\min} \le \mathbf{D}\,\mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	λ	${\mathbf{w}_{\text{r}}^{\text{int}}}^{\top}(\mathbf{N}_{\text{int}}\mathbf{v} - \lambda\,\mathbf{c})$	${-\mathbf{a}_{\text{v}}^{\text{r}}}^{\top}(\mathbf{v} + \mathbf{E}_{\text{r}}\,\mathbf{c})$ $+{\mathbf{a}_{\text{v}}^{\text{f}}}^{\top}(\mathbf{E}_{\text{f}}\,\mathbf{c} - \mathbf{v})$	${-\mathbf{p}_{\text{d}}^{\min}}^{\top}(\mathbf{D}\,\mathbf{c} - \mathbf{c}_{\min})$ $+{\mathbf{p}_{\text{d}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{D}\,\mathbf{c})$
$\partial_\lambda\mathcal{L} = 0$:	$0 =$	1	$-\mathbf{c}\,\mathbf{w}_{\text{r}}^{\text{int}}$		
$\partial_v\mathcal{L} = 0$:	$0 =$	0	$+\mathbf{N}_{\text{int}}^{\top}\,\mathbf{w}_{\text{r}}^{\text{int}}$	$-\mathbf{a}_{\text{v}}$	
$\partial_c\mathcal{L} = 0$:	$0 =$	0	$-\lambda\,\mathbf{w}_{\text{r}}^{\text{int}}$	$\mathbf{E}^{\top}\,\mathbf{a}_{\text{v}}$	$-\mathbf{D}^{\top}\,\mathbf{p}_{\text{d}}$

where $\mathbf{a}_{\text{v}} = \mathbf{a}_{\text{v}}^{\text{f}} + \mathbf{a}_{\text{v}}^{\text{r}}$, $\mathbf{p}_{\text{d}} = \mathbf{p}_{\text{d}}^{\min} + \mathbf{p}_{\text{d}}^{\max}$, and where the state-specific matrix $\mathbf{E}$ contains the elements of $\mathbf{E}^{\text{f}}$ for reactions that run in forward direction, and the elements of $-\mathbf{E}^{\text{r}}$ for reactions that run in backward reaction (and unspecified values for inactive reactions). Again we have no term $\mathbf{b}_{\text{v}}$. Note that for each reaction l, $a_{v_l}^{\text{f}}$ or $a_{v_l}^{\text{r}}$ can be non-zero, but not both at the same time. Moreover, as shown in , every active reaction will hit its capacity constraint, so we obtain $\text{sign}(\mathbf{a}_{\text{v}}) = \text{sign}(\mathbf{v})$ (both for active and inactive reactions). By solving the equations

(in the order 2,3,1), we obtain the economic balance equations

$$\begin{aligned} \mathbf{N}_{\mathrm{int}}^{\top}\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} &= \mathbf{a}_{\mathrm{v}} && \text{Reaction balance} \\ \mathbf{E}^{\top}\,\mathbf{a}_{\mathrm{v}} &= \mathbf{D}^{\top}\,\mathbf{p}_{\mathrm{d}} + \lambda\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} && \text{Metabolite balance} \\ \mathbf{c}\cdot\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} &= 1 && \text{Scaling of economic variables} \end{aligned} \tag{S6}$$

In operator notation, the equations read

$$\begin{aligned} \mathbf{w}_{\mathrm{r}}^{\mathrm{int}}\,\square_{\mathrm{v}} &= \mathbf{a}_{\mathrm{v}} && \text{Reaction balance} \\ \mathbf{a}_{\mathrm{v}}\,\bigcirc_{\mathrm{c}} &= \mathbf{p}_{\mathrm{d}}\,\triangleleft_{\mathrm{c}} + \lambda\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} && \text{Metabolite balance} \\ \mathbf{w}_{\mathrm{r}}^{\mathrm{int}}\cdot\mathbf{c} &= 1 && \text{Scaling of economic variables} \end{aligned} \tag{S7}$$

Given a solution of our optimality problem (with known flux directions and active density constraints), we may use these equations to determine the economic variables (see SI S2.7).

7. Resource balance analysis (fixed growth) We can also consider RBA problems at a fixed growth rate, and with an optimisation of some linear objective $\mathbf{b}_{\mathrm{v}}\cdot\mathbf{v} - \mathbf{q}_{\mathrm{c}}\cdot\mathbf{c}$:

Maximise$_{\lambda,v,c}$	$F =$	$\mathbf{b}_{\mathrm{v}}\cdot\mathbf{v} - \mathbf{q}_{\mathrm{c}}\cdot\mathbf{c}$	s.t. $\mathbf{N}_{\mathrm{int}}\mathbf{v} - \lambda\,\mathbf{c} = 0$	$-\mathbf{E}_{\mathrm{r}}\,\mathbf{c} \le \mathbf{v} \le \mathbf{E}_{\mathrm{f}}\,\mathbf{c}$	$\mathbf{c}_{\min} \le \mathbf{D}\,\mathbf{c} \le \mathbf{c}_{\max}$
	$\mathcal{L} =$	$\mathbf{b}_{\mathrm{v}}\cdot\mathbf{v} - \mathbf{q}_{\mathrm{c}}\cdot\mathbf{c}$	${\mathbf{w}_{\mathrm{r}}^{\mathrm{int}}}^{\top}(\mathbf{N}_{\mathrm{int}}\mathbf{v} - \lambda\,\mathbf{c})$	${\mathbf{a}_{\mathrm{v}}^{\mathrm{r}}}^{\top}(\mathbf{v} + \mathbf{E}_{\mathrm{r}}\,\mathbf{c})$ ${\mathbf{a}_{\mathrm{v}}^{\mathrm{f}}}^{\top}(\mathbf{E}_{\mathrm{f}}\,\mathbf{c} - \mathbf{v})$	${\mathbf{p}_{\mathrm{d}}^{\min}}^{\top}(\mathbf{D}\,\mathbf{c} - \mathbf{c}_{\min})$ ${\mathbf{p}_{\mathrm{d}}^{\max}}^{\top}(\mathbf{c}_{\max} - \mathbf{D}\,\mathbf{c})$
$\partial_v\mathcal{L} = 0$:	$0 =$	$\mathbf{b}_{\mathrm{v}}$	$+\mathbf{N}_{\mathrm{int}}^{\top}\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}}$	$-\mathbf{a}_{\mathrm{v}}$	
$\partial_c\mathcal{L} = 0$:	$0 =$	$-\mathbf{q}_{\mathrm{c}}$	$-\lambda\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}}$	$\mathbf{E}^{\top}\,\mathbf{a}_{\mathrm{v}}$	$-\mathbf{D}^{\top}\,\mathbf{p}_{\mathrm{d}}$

where $\mathbf{a}_{\mathrm{v}} = \mathbf{a}_{\mathrm{v}}^{\mathrm{f}} + \mathbf{a}_{\mathrm{v}}^{\mathrm{r}}$ and $\mathbf{p}_{\mathrm{d}} = \mathbf{p}_{\mathrm{d}}^{\min} + \mathbf{p}_{\mathrm{d}}^{\max}$ as before. Again, by solving the equations (in the order 2,3,1), we obtain the economic balance equations

$$\begin{aligned} \mathbf{N}_{\mathrm{int}}^{\top}\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} + \mathbf{b}_{\mathrm{v}} &= \mathbf{a}_{\mathrm{v}} && \text{Reaction balance} \\ \mathbf{E}^{\top}\,\mathbf{a}_{\mathrm{v}} &= \mathbf{q}_{\mathrm{c}^{\mathrm{int}}}^{\mathrm{app}} && \text{Metabolite balance} \end{aligned} \tag{S8}$$

where, this time, $\mathbf{q}_{\mathrm{c}^{\mathrm{int}}}^{\mathrm{app}} = \mathbf{q}_{\mathrm{c}} + \mathbf{D}^{\top}\,\mathbf{p}_{\mathrm{d}} + \lambda\,\mathbf{w}_{\mathrm{r}}^{\mathrm{int}}$.

S2 Metabolic optimality problems and economic balance equations

To maximise the metabolic fitness under the constraints of a kinetic model, we can use, as the free variables, either enzyme levels or metabolite levels and fluxes. In this section, I explain the resulting optimality problems and discuss some variants. For each problem, the optimality conditions are derived and translated into economic balance equations.

S2.1 Flux optimisation

In flux optimisation, one may minimise a (flux-dependent) benefit-cost difference, maximise benefit at a fixed cost (as in FBA with molecular crowding [1] or in classical FBA with a constant cost term), or minimise cost at a fixed benefit (e.g. by minimal-flux FBA, or flux cost minimisation as in section S2.1). Here, we consider the latter option. We use the metabolic fluxes as free variables, constrain them to be stationary and to reach a given benefit value ($\mathbf{z}\cdot\mathbf{v} \ge b'$ or, for simplicity, $\mathbf{z}\cdot = b'$), and minimise a flux cost function (see Figure S1). The flux

cost may be a linear or nonlinear function of the fluxes, and its derivatives $a_{v_l} = \partial a/\partial v_l$ must have the same signs as the fluxes v_l, i.e. $a_{v_l}\, v_l > 0$ whenever $v_l \neq 0$. At $v_l = 0$, the cost function has a kink and the derivative a_{v_l} is undefined. Flux cost minimisation has been described in the main article. Let us consider three variants:

1. **Minimising a weighted sum of fluxes** As a simple form of FCM, some variants of FBA use the minimisation of a weighted sum of fluxes $a = \sum_l a'_{v_l} |v_l|$ (with constant weights a'_{v_l}). Assuming positive fluxes (assuming that the flux directions are known, and orienting the reactions accordingly in our model), we obtain the derivatives $\partial a/\partial v_l = a'_{v_l}$, and the economic reaction balance reads

$$a'_{v_l} = b_{v_l} + \Delta w_l \tag{S9}$$

   for all active fluxes, where $b_{v_l} = \omega_\mathrm{b}\, z_l$ and ω_b is a Lagrange multiplier.

2. **Minimising enzyme cost as a function of fluxes** The kinetic flux cost is an apparent flux cost function (see Figure S1, bottom) that describes the minimal metabolite and enzyme costs a cell needs to invest to realise the fluxes. It can be defined based on a kinetic model, and be computed by a cost minimisation in metabolite space (section S2.3). The kinetic model defines a relation between enzyme levels, metabolite levels, and fluxes. Our aim is to find a flux distribution that minimises the total enzyme cost under the given flux constraints. We first consider an optimisation at predefined metabolite levels. In this case, the flux cost reads $a(\mathbf{v}) = \sum_l h_{e_l}\, e_l = \sum_l h_{e_l}\, \frac{v_l}{k_l}$, and its derivatives, with the predefined metabolite profile, read $\partial a/\partial v_l = \frac{h_{e_l}}{k_l}$. We obtain the same reaction balance as before, but with $\frac{h_{e_l}}{k_l}$ replacing . If we optimise the metabolite levels as part of the optimality problem, we obtain the same formula; we just need to insert the optimal metabolite levels (to be computed by ECM).

3. **Thermodynamic constraints on flux directions derived from a flux cost minimisation problem** Economic balance equations and energetic balance equations can be derived from the same variational principle. In a chemical reaction system, all active reactions produce entropy. This fact implies a relation between flux directions and chemical potential differences (or "thermodynamic driving forces"): fluxes must lead from higher to lower chemical potentials. This constraint, which is used in some variants of FBA, follows from a principle of minimal entropy production (or, in systems at given pressure and temperature, of dissipation of Gibbs free energy). Interestingly, this principle is formally equivalent to a flux cost minimisation problem. We just need to use Rayleigh's dissipation function, describing heat production in a chemical system, as an objective to be minimised, while constraining the fluxes to a given external production of Gibbs free energy. The optimality condition states that there must be chemical potentials μ_i satisfying $\mathrm{sign}(v) = -\mathrm{sign}(\Delta_r \mu)$ in all active reactions. The existence of these chemical potentials excludes certain flux cycles and put constraints on the flux directions.

S2.2 Enzyme optimisation

In enzyme optimisation as described by Eq. (**??**) (i.e. cost-benefit optimisation in enzyme space), the enzyme levels are treated as control variables, and the aim is to maximise a fitness function given by the difference between metabolic benefit (scoring fluxes and metabolite levels) and enzyme cost. Enzyme optimisation can be used to design optimal enzyme profiles in metabolic engineering or to better understand enzyme usage in naturally evolved metabolic pathways. For examples in the literature, see [2, 3, 4, 5]. The optimisation includes constraints: the rate laws must be satisfied, the metabolic fluxes must be stationary, and the enzyme levels, metabolite levels or fluxes may be constrained (e.g. to avoid negative values or to impose upper and lower bounds). In models with moiety conservation, the concentrations of conserved moieties can also be predefined. Each single constraint will give rise to an economic variable, which will appear in the balance equations. Let us see this in detail.

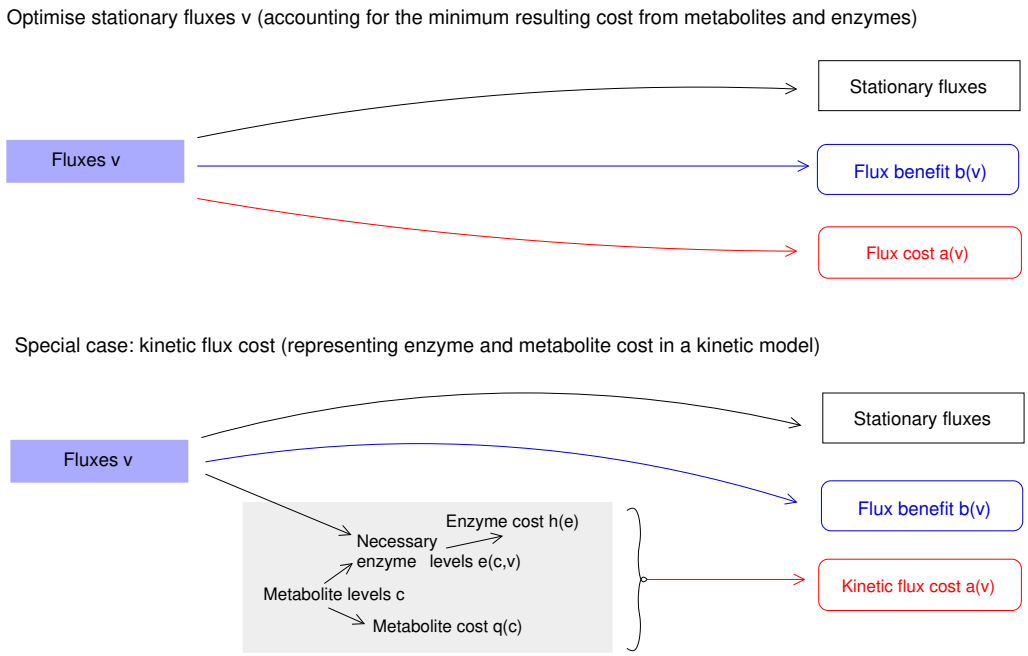


Figure S1: Flux cost minimisation. Top: flux cost minimisation at a fixed flux benefit and with stationarity constraint for fluxes. Bottom: A kinetic model can be used to define an apparent, kinetics-based flux cost function that represents enzyme and metabolite costs in. The calculation of a flux cost entails an optimisation of metabolite and enzyme levels given the chosen fluxes. The grey box represents the optimisation shown in Figure S3.

1. Optimality problem In enzyme optimisation (see Figure S2), we consider a kinetic model with rate laws $v_l = v_l(\mathbf{c}, \mathbf{u})$, control variables u_l, a metabolic objective[1] $b(\mathbf{v}, \mathbf{c})$, and a cost function $h(\mathbf{u})$. The vector $\mathbf{u}$ may contain enzyme levels or other kinds of control variables, such as mRNA levels; however, the control variables must appear as prefactors in rate laws. We refer here to enzyme levels as the paradigmatic example. For generality, we assume that our model contains conserved moieties. This means to things: the values of the conserved moieties need to be fixed, and the metabolic dynamics concerns only a subset of the internal metabolites, called independent internal metabolites[2]. The stationarity condition for these metabolites reads $\mathbf{N}_{\mathrm{ind}}\,\mathbf{v} = 0$, and moiety conservation is formulated as $\mathbf{G}\,\mathbf{c} = \mathbf{c}'_{\mathrm{cm}}$ (with a predefined vector $\mathbf{c}'_{\mathrm{cm}}$ of conserved moiety concentrations). The optimality problem reads

$$\begin{aligned} &\text{Maximise} \quad b(\mathbf{v}, \mathbf{c}) - h(\mathbf{u}) \quad \text{with respect to } \mathbf{v}, \mathbf{c}, \mathbf{u} \\ &\text{subject to} \quad \mathbf{N}_{\mathrm{ind}}\,\mathbf{v} = 0, \quad \mathbf{v}(\mathbf{c}, \mathbf{u}) = \mathbf{v}, \quad \mathbf{c}'_{\mathrm{cm}} = \mathbf{G}\,\mathbf{c}. \end{aligned} \tag{S10}$$

2. Optimality conditions Using Lagrange multipliers (in vectors $\boldsymbol{\omega}_\varphi$, $\boldsymbol{\omega}_{\mathrm{v}}$, and $\boldsymbol{\omega}_{\mathrm{cm}}$), we can rewrite the optimality problem as

$$\begin{aligned} \text{Maximise} \quad f^* = b(\mathbf{v}, \mathbf{c}) - h(\mathbf{u}) + \boldsymbol{\omega}_\varphi^\top \mathbf{N}_{\mathrm{ind}}\,\mathbf{v} + \boldsymbol{\omega}_{\mathrm{v}}^\top [\mathbf{v}(\mathbf{c}, \mathbf{u}) - \mathbf{v}] + \boldsymbol{\omega}_{\mathrm{cm}}^\top [\mathbf{c}'_{\mathrm{cm}} - \mathbf{G}\,\mathbf{c}] \\ \text{with respect to } \mathbf{v}, \mathbf{c}, \mathbf{u}. \end{aligned} \tag{S11}$$

To obtain necessary optimality conditions, we take the derivatives with respect to v_l, c_i, and u_l and set them to

[1] This combined metabolic objective may represent a difference $b(\mathbf{v}) - q(\mathbf{c})$ of a flux benefit and a metabolite cost.

[2] In models with conserved moieties, the economic potentials of dependent metabolites can be set to zero. Since the choice of dependent metabolites depends on the model formulation, the is some aribtrariness in this choice, related to a kind of gauge symmetry. This also concerns other optimality problems, including flux cost minimisation. However, to keep things simple, this complication is only considered here.

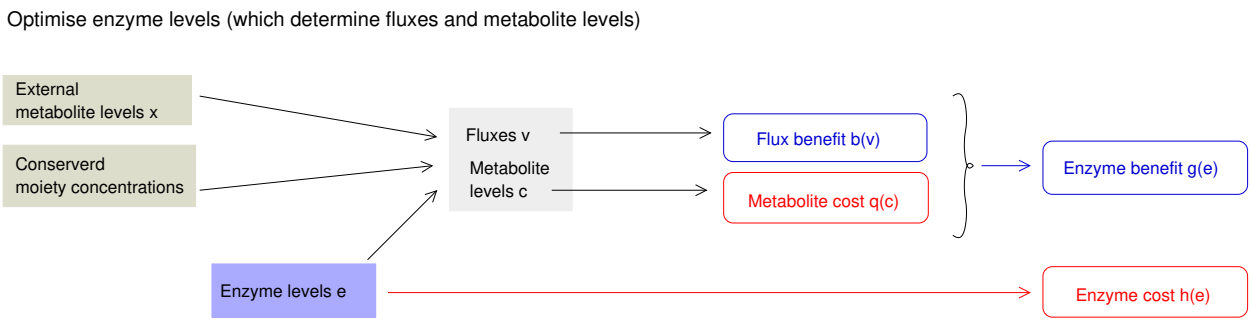


Figure S2: Enzyme optimisation. In enzyme optimisation(see section S2.2), we maximise the benefit-cost difference as a function of enzyme levels. Alternatively, one may maximise flux (as a benefit function) at a fixed total enzyme level (as a cost) [6], or to minimise enzyme cost at fixed fluxes [7].

zero. With the abbreviations

$$b^\star_{v_l} = \frac{\partial b}{\partial v_l}, \quad b^{(c)}_{c_i} = \frac{\partial b}{\partial c_i}, \quad E^{v_l}_{c_i} = \frac{\partial v_l}{\partial c_i}, \quad E^{v_l}_{u_l} = \frac{v_l}{u_l}, \quad h_{u_l} = \frac{\partial h}{\partial u_l}, \tag{S12}$$

we obtain the optimality conditions (for derivatives in row vectors)

$$\begin{aligned} 0 &= \frac{\partial f^*}{\partial \mathbf{v}} = \mathbf{b}^{\star\top}_{\mathrm{v}} + \boldsymbol{\omega}^\top_\varphi \mathbf{N}_{\mathrm{ind}} - \boldsymbol{\omega}^\top_{\mathrm{v}} \\ 0 &= \frac{\partial f^*}{\partial \mathbf{c}} = \mathbf{b}^\top_{\mathrm{c}} + \boldsymbol{\omega}^\top_{\mathrm{v}} \mathbf{E}_{\mathrm{c}} - \boldsymbol{\omega}^\top_{\mathrm{cm}} \mathbf{G} \\ 0 &= \frac{\partial f^*}{\partial \mathbf{u}} = -\mathbf{h}^\top_{\mathrm{u}} + \boldsymbol{\omega}^\top_{\mathrm{v}} \mathbf{E}_{\mathrm{u}}. \end{aligned} \tag{S13}$$

3. Symbols for the economic variables To write the optimality conditions (S13) as economic balance equations, we define the economic variables b_{v_l} (direct flux values), w_{r_i} (economic potentials), and $y^{\mathrm{int}}_{c_i}$ (economic loads). We first define the new names $\mathbf{w}_{\mathrm{r,ind}} = \boldsymbol{\omega}_\varphi; \mathbf{w}_{\mathrm{v}} = \boldsymbol{\omega}_{\mathrm{v}}; \mathbf{y}_{\mathrm{cm}} = \boldsymbol{\omega}_{\mathrm{cm}}$ and then define:

1. The rows of $\mathbf{N}_{\mathrm{ind}}$ and the elements of $\mathbf{w}_{\mathrm{r,ind}}$ refer to the *independent* internal metabolites: we define the vector $\mathbf{w}^{\mathrm{int}}_{\mathrm{r}}$ for all internal metabolites. Its components for the independent metabolites stem from $\mathbf{w}_{\mathrm{r,ind}}$, while the component for dependent metabolites vanish. Using this vector, we can now rewrite ${\mathbf{w}_{\mathrm{r,ind}}}^\top \mathbf{N}_{\mathrm{ind}} = {\mathbf{w}^{\mathrm{int}}_{\mathrm{r}}}^\top \mathbf{N}_{\mathrm{int}}$.

2. The vector $\mathbf{b}^\star_{\mathrm{v}}$ is split into a sum $\mathbf{b}^\star_{\mathrm{v}} = \mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{ext}}\, \mathbf{w}^{\mathrm{ext}}_{\mathrm{r}}$, with direct flux values b_{v_l} and external economic potentials $w^{\mathrm{ext}}_{r_i}$. Merging $\mathbf{w}^{\mathrm{int}}_{\mathrm{r}}$ and $\mathbf{w}^{\mathrm{ext}}_{\mathrm{r}}$ into a vector $\mathbf{w}_{\mathrm{r}}$, referring to all metabolites, we have different ways to split the flux values into different terms:

$$\mathbf{b}^\star_{\mathrm{v}} + \mathbf{N}^\top_{\mathrm{ind}}\, \mathbf{w}_{\mathrm{r,ind}} = \mathbf{b}_{\mathrm{v}} + \mathbf{N}^\top_{\mathrm{ext}}\, \mathbf{w}^{\mathrm{ext}}_{\mathrm{r}} + \mathbf{N}^\top_{\mathrm{int}}\, \mathbf{w}^{\mathrm{int}}_{\mathrm{r}} = \mathbf{b}_{\mathrm{v}} + \mathbf{N}^\top_{\mathrm{all}}\, \mathbf{w}_{\mathrm{r}} = \mathbf{b}_{\mathrm{v}} + \Delta\, \mathbf{w}_{\mathrm{r}}. \tag{S14}$$

3. To capture the constraints for conserved moieties, we define the concentration value vector $\mathbf{w}_{\mathrm{c}} = \mathbf{G}^\top\, \mathbf{y}_{\mathrm{cm}}$, and for models without conserved moieties, we obtain $\mathbf{w}_{\mathrm{c}} = 0$.

4. Economic rules We insert the new variables into Eq. (S13), transpose and rearrange the equations, and obtain the economic rules

$$\mathbf{w}_{\mathrm{v}} = \mathbf{N}^\top_{\mathrm{all}}\, \mathbf{w}_{\mathrm{r}} + \mathbf{b}_{\mathrm{v}} \tag{S15}$$

$$\mathbf{w}_{\mathrm{c}} = \mathbf{E}^\top_{\mathrm{c}}\, \mathbf{w}_{\mathrm{v}} + \mathbf{b}_{\mathrm{c}} \tag{S16}$$

$$\mathbf{h}_{\mathrm{u}} = \mathbf{E}^\top_{\mathrm{u}}\, \mathbf{w}_{\mathrm{v}}. \tag{S17}$$

For reasons of elegance, these equations (and also the following ones) can be written in a form that resembles field equations from physics. We define the network operators $\square_{\mathrm{v}} = \mathbf{N}_{\mathrm{int}}$ (for reactions), $\bigcirc_{\mathrm{c}} = \mathbf{E}_{\mathrm{c}}$ (for metabolites), and $\bigcirc_{\mathrm{e}} = \mathbf{E}_{\mathrm{u}}$ (for enzymes), which act on row vectors on their left. They are called operators because they translate the fitness values between production rates and reaction rates and between reaction rates and metabolite or enzyme concentrations. Using these operators, we obtain the economic rules in the simple form

$$\begin{aligned} \mathbf{w}_{\mathrm{v}} &= \square_{\mathrm{v}}\, \mathbf{w}_{\mathrm{r}} + \mathbf{b}_{\mathrm{v}} \\ \mathbf{w}_{\mathrm{c}} &= \bigcirc_{\mathrm{c}}\, \mathbf{w}_{\mathrm{v}} + \mathbf{b}_{\mathrm{c}} \\ \mathbf{h}_{\mathrm{u}} &= \bigcirc_{\mathrm{e}}\, \mathbf{w}_{\mathrm{v}}. \end{aligned} \tag{S18}$$

The first rule relates the flux values $\mathbf{w}_{\mathrm{v}}$ to the flux gain $\mathbf{b}_{\mathrm{v}}$ and to the economic potentials (production values) $\mathbf{w}_{\mathrm{r}}$ of adjacent metabolites, where metabolites and reaction are linked by stoichiometric coefficients (compare Eq. (S14)). The second rule relates the concentration value $\mathbf{w}_{\mathrm{c}}$ to the concentration gain $\mathbf{b}_{\mathrm{c}}$ and to the flux values $\mathbf{w}_{\mathrm{v}}$ of adjacent reactions, where reactions and metabolite are linked by reactant elasticities. The economic loads in the vector $\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{w}_{\mathrm{c}} - \mathbf{b}_{\mathrm{c}}$ describe the metabolites' indirect influences on the metabolite's indirect value (i.e. the metabolic objective). According to rule (S16), the loads are given by $\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{E}_{\mathrm{c}}^{\top}\, \mathbf{w}_{\mathrm{v}}$. The third rule relates the flux values $\mathbf{w}_{\mathrm{v}}$ to the enzyme prices of adjacent enzymes (linked by enzyme elasticities $E_{\mathrm{u_l}}^{\mathrm{v_l}} = \frac{v_l}{u_l}$). The term $a_{v_l}^{\mathrm{kin}} = h_{u_l}\, u_l / v_l$ on the right has the role of a flux price.

5. Balance equations By combining the rules in pairs, we obtain three balance equations:

1. By inserting rule (S15) into rule (S16), in the form $\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{w}_{\mathrm{v}}^{\top}\, \mathbf{E}_{\mathrm{c}}$, we obtain the relationship

$$\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{E}_{\mathrm{c}}^{\top}\, [\mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{all}}^{\top}\, \mathbf{w}_{\mathrm{r}}] \tag{S19}$$

   between economic loads and potentials

2. By equating rules (S15) and (S17), we obtain the reaction balance

$$\mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{all}}^{\top}\, \mathbf{w}_{\mathrm{r}} = \mathbf{a}_{\mathrm{v}}^{\mathrm{kin}}, \tag{S20}$$

   with kinetic flux price vector $\mathbf{a}_{\mathrm{v}}^{\mathrm{kin}} = \mathbf{u}/\mathbf{v} \circ \mathbf{h}_{\mathrm{u}}$.

3. By combining rules (S16) and (S17) and using the identity $\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{E}_{\mathrm{c}}^{\top}\, \mathbf{w}_{\mathrm{v}}$, we obtain the metabolite balance

$$\mathbf{y}_{\mathrm{c}}^{\mathrm{int}} = \mathbf{E}_{\mathrm{c}}^{\top}\, \mathbf{a}_{\mathrm{v}}^{\mathrm{kin}}. \tag{S21}$$

6. Optimality conditions in scaled form We have obatined the economic balance equations in "value" form. To obtain balance equations in benefit form, we multiply the rules (S15) and (S17) by the fluxes v_l, and rule (S16) by the concentrations c_i. Introducing the abbreviations $z_{v_l} = w_{v_l}\, v_l$ ("local flux benefit"), $z_{c_i} = w_{\mathrm{c_i}}^{\mathrm{int}}\, c_i$, and $\underline{h}_{u_l} = h_{u_l}\, u_l$ (" partial enzyme cost"), and noting that $\mathrm{Dg}(\mathbf{c})\, \mathbf{E}_{\mathrm{c}}{}^{\top}\, \mathrm{Dg}(\mathbf{v}) = \mathcal{E}_{\mathrm{c}}^{\mathrm{v}}$, we obtain the equations

$$\begin{aligned} \underline{\mathbf{b}}_{\mathrm{v}}^{\star} &= \mathbf{v} \circ [\mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{all}}^{\top}\, \mathbf{w}_{\mathrm{r}}] \\ \underline{\mathbf{g}}_{\mathrm{c}} &= \mathbf{c} \circ [\mathbf{b}_{\mathrm{v}} + \mathbf{E}_{\mathrm{c}}\, \mathbf{w}_{\mathrm{v}}] \\ \underline{\mathbf{b}}_{\mathrm{v}}^{\star} &= \mathbf{u} \circ \mathbf{h}_{\mathrm{u}}. \end{aligned} \tag{S22}$$

These equations represent the reaction and compound rules in scaled form, as well as the equality between enzyme partial benefits and partial enzyme costs. By combining them in pairs as above, we obtain the scaled balance

equations.

$$\begin{aligned}
\mathbf{c} \circ \mathbf{y}_{\mathrm{c}}^{\mathrm{int}} &= \mathcal{E}_{\mathrm{c}}^{\mathrm{v}\top}\, \mathrm{Dg}(\mathbf{v})\, [\mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{all}}^{\top}\, \mathbf{w}_{\mathrm{r}}] \\
\mathbf{u} \circ \mathbf{h}_{\mathrm{u}} &= \mathbf{v} \circ [\mathbf{b}_{\mathrm{v}} + \mathbf{N}_{\mathrm{all}}^{\top}\, \mathbf{w}_{\mathrm{r}}] \\
\mathbf{c} \circ \mathbf{y}_{\mathrm{c}}^{\mathrm{int}} &= \mathcal{E}_{\mathrm{c}}^{\mathrm{v}\top}\, \underline{\mathbf{h}}_{u}.
\end{aligned} \tag{S23}$$

7. Additional constraints on enzyme levels, fluxes, concentrations, and production rates What if more constraints are added to the optimality problem. With additional equality constraints on enzyme levels ($\mathbf{M}_{\mathrm{u}}\, \mathbf{u} = \mathbf{u}^{\mathrm{fix}}$), fluxes ($\mathbf{M}_{\mathrm{v}}\, \mathbf{v} = \mathbf{v}^{\mathrm{fix}}$), concentrations ($\mathbf{M}_{\mathrm{c}}\, \mathbf{c} = \mathbf{c}^{\mathrm{fix}}$), and external production rates ($\mathbf{M}_{\mathrm{x}}\, \mathbf{N}_{\mathrm{ext}}\, \mathbf{v} = \mathbf{v}^{\mathrm{prod,fix}}$), we obtain the optimality problem

$$\begin{aligned}
\text{Maximise} \quad & b^{(\mathrm{c,v})}(\mathbf{v}, \mathbf{c}) - h(\mathbf{u}) + \boldsymbol{\omega}_{\varphi}^{\top}[\mathbf{N}_{\mathrm{ind}}\, \mathbf{v} - 0] + \boldsymbol{\omega}_{\mathrm{v}}^{\top}[\mathbf{r}(\mathbf{c}, \mathbf{u}) - \mathbf{v}] + \boldsymbol{\omega}_{\mathrm{cm}}{}^{\top}\, [\mathbf{c}'_{\mathrm{cm}} - \mathbf{G}\, \mathbf{c}] \\
& + \boldsymbol{\beta}_{\mathrm{u}}^{\top}[\mathbf{M}_{\mathrm{u}}\, \mathbf{u} - \mathbf{u}^{\mathrm{fix}}] + \boldsymbol{\beta}_{\mathrm{v}}^{\top}[\mathbf{M}_{\mathrm{v}}\, \mathbf{v} - \mathbf{v}^{\mathrm{fix}}] + \boldsymbol{\beta}_{\mathrm{c}}^{\top}[\mathbf{M}_{\mathrm{c}}\, \mathbf{c} - \mathbf{c}^{\mathrm{fix}}] + \boldsymbol{\beta}_{\psi}^{\top}[\mathbf{M}_{\mathrm{x}}\, \mathbf{N}_{\mathrm{ext}}\, \mathbf{v} - \mathbf{v}^{\mathrm{prod,fix}}]
\end{aligned} \tag{S24}$$

with respect to $\mathbf{v}$, $\mathbf{c}$, and $\mathbf{u}$. By taking derivatives with respect to $\mathbf{v}$, $\mathbf{c}$, and $\mathbf{u}$, and setting them to zero, we obtain

$$\begin{aligned}
0 &= \mathbf{b}_{\mathrm{v}}^{\star\top} + \boldsymbol{\omega}_{\varphi}^{\top}\, \mathbf{N}_{\mathrm{ind}} - \boldsymbol{\omega}_{\mathrm{v}}^{\top} + \boldsymbol{\beta}_{\mathrm{v}}^{\top}\, \mathbf{M}_{\mathrm{v}} + \boldsymbol{\beta}_{\psi}^{\top}\, \mathbf{M}_{\mathrm{x}}\, \mathbf{N}_{\mathrm{ext}} \\
0 &= \mathbf{b}_{\mathrm{c}}^{\top} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\, \mathbf{E}_{\mathrm{c}} + \boldsymbol{\omega}_{\mathrm{cm}}{}^{\top}\, \mathbf{G} + \boldsymbol{\beta}_{\mathrm{c}}^{\top}\, \mathbf{M}_{\mathrm{c}} \\
0 &= -\mathbf{h}_{\mathrm{u}}^{\top} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\, \mathbf{E}_{\mathrm{u}} + \boldsymbol{\beta}_{\mathrm{u}}^{\top}\, \mathbf{M}_{\mathrm{u}}.
\end{aligned} \tag{S25}$$

To account for the new constraints, we introduce economic variables as above, define the burden and gain terms

$$\begin{aligned}
\mathbf{h}_{\mathrm{u,con}} &= -\mathbf{M}_{\mathrm{u}}{}^{\top} \boldsymbol{\beta}_{\mathrm{u}} \\
\mathbf{b}_{\mathrm{v,con}}^{\star} &= \mathbf{M}_{\mathrm{v}}{}^{\top} \boldsymbol{\beta}_{\mathrm{v}} \\
\mathbf{b}_{\mathrm{c,con}} &= \mathbf{M}_{\mathrm{c}}{}^{\top} \boldsymbol{\beta}_{\mathrm{c}} \\
\mathbf{w}_{\mathrm{r,con}}^{\mathrm{ext}} &= \mathbf{M}_{\mathrm{x}}{}^{\top}\, \boldsymbol{\beta}_{\psi},
\end{aligned} \tag{S26}$$

and obtain

$$\begin{aligned}
0 &= \mathbf{b}_{\mathrm{v}}^{\star\top} + \mathbf{b}_{\mathrm{v,con}}^{\star}{}^{\top} + \mathbf{w}_{\mathrm{r,ind}}{}^{\top}\, \mathbf{N}_{\mathrm{ind}} + \mathbf{w}_{\mathrm{r,con}}^{\mathrm{ext}}{}^{\top}\, \mathbf{N}_{\mathrm{ext}} - \mathbf{w}_{\mathrm{v}}^{\top} \\
0 &= \mathbf{b}_{\mathrm{c}}^{\top} + \mathbf{b}_{\mathrm{c,con}}^{\top} + \mathbf{w}_{\mathrm{v}}^{\top}\, \mathbf{E}_{\mathrm{c}} + \mathbf{y}_{\mathrm{cm}}^{\top}\, \mathbf{G} \\
0 &= -\mathbf{h}_{\mathrm{u}}^{\top} - \mathbf{h}_{\mathrm{u,con}}^{\top} + \mathbf{w}_{\mathrm{v}}^{\top}\, \mathbf{E}_{\mathrm{u}}.
\end{aligned} \tag{S27}$$

By incorporating the new terms into the economical variables[3], we obtain simple equations of the form (S13). The same extra terms will also appear in models with lower and upper bounds instead of equality constraints. In this case, their signs depend on the type of bound hit (positive for lower bounds, negative for upper bounds, and the elements for inactive bounds will vanish) (see SI section S3.1). For example, the gain vectors $\mathbf{b}_{\mathrm{v,con}}^{\star}$ or $\mathbf{b}_{\mathrm{c,con}}$ contain negative values where the corresponding variables (e.g. a flux, for a flux gain) hit an upper bound. An upper bound resembles an additional cost function, which excludes high values. Therefore, an active upper bound has the same effect as a side objective that punishes high values: both of them keep the variable low. Similarly, an active lower bound resmebles a benefit function: it keeps the variable high.

[3] To ensure that the additional terms have unique values and a clear interpretation, redundant constraints in the optimality problems must be avoided. For example, if there is an upper bound on an export flux, there should not be a second bound on the production rate of the external substance. Redundant bounds are no problem for solving the optimality problem, but the values of the Lagrange multipliers related to these bounds will be underdetermined, which makes it hard to interpret them. Mathematically, an optimality problem with redundant constraints does not satisfy the conditions for applying the Karush-Kuhn-Tucker optimality conditions (see SI section S3.1).

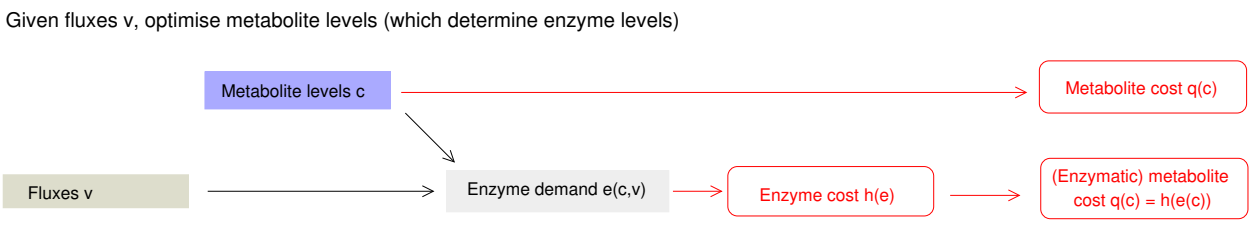


Figure S3: Cost minimisation in metabolite space with given fluxes to be realised. The metabolite levels are scored by a cost function $q(\mathbf{c})$ comprising the a direct metabolite cost and an indirect enzymatic cost. The enzymatic cost in metabolite space is defined as follows. At the desired fluxes, each metabolite profile defines a corresponding enzyme profile and therefore an enzyme cost. The enzyme costs can be written as a function of metabolite level. It is mathematically convenient to run the optimisation in log-metabolite space (see section S2.3).

S2.3 Metabolite optimisation

In cost minimisation in metabolite space (e.g. enzyme cost minimisation, ECM [7]), we predefine a flux vector $\mathbf{v}$ and search for metabolite and enzyme levels that realise these fluxes in a cost-optimal way (see Figure S3). The logarithmic metabolite levels (in a vector $\ln \mathbf{c}$) are treated as free variables, and since the fluxes are given, the enzyme levels can be easily computed by inverting the rate laws. In the optimality problem, we minimise the sum of direct and enzymatic metabolite cost:

$$\text{Maximise} \quad -q(\mathbf{c}) - q^{\text{enz}}(\mathbf{c},\mathbf{v}) \text{ with respect to } \mathbf{c} \text{ subject to } \mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max} \tag{S28}$$

The enzymatic cost $q^{\text{enz}}(\mathbf{c},\mathbf{v}) = h(\mathbf{e}(\mathbf{c},\mathbf{v}))$ describes the enzyme cost $h(\mathbf{e})$ caused by the enzyme demand $\mathbf{e}(\mathbf{c},\mathbf{v})$ that arises from a choice of metabolite levels. To ensure a solution (while assuming thermodynamically feasible rate laws), we require that the fluxes must be free of thermodynamically infeasible cycles. There are different ways to formulate this problem. In appendix S1, I treated the enzyme levels as independent variables and described the relation between fluxes, metabolite levels, and enzyme levels by explicit constraints. We shall now see a different derivation in which we consider a given effective cost, as a function of $\mathbf{c}$ and $\mathbf{v}$. With this cost function $q(\mathbf{c},\mathbf{v})$, the optimality problem for $\mathbf{c}$ reads

$$\text{Minimise } q(\mathbf{c},\mathbf{v}) \text{ with respect to } \mathbf{c} \text{ subject to } \mathbf{c}_{\min} \le \mathbf{c} \le \mathbf{c}_{\max} \tag{S29}$$

Using Lagrange multipliers (in a vector $\mathbf{b}_{\text{c}}^{\text{bnd}}$), we can write the optimality problem as

$$\text{Minimise } q(\mathbf{c},\mathbf{v}) + \mathbf{b}_{\text{c}}^{\text{lb}} \cdot [\mathbf{c}_{\min} - \mathbf{c}] + \mathbf{b}_{\text{c}}^{\text{ub}} \cdot [\mathbf{c} - \mathbf{c}_{\max}] \text{ with respect to } \mathbf{c}, \tag{S30}$$

and (after joining $\mathbf{b}_{\text{c}}^{\text{lb}}$ and $\mathbf{b}_{\text{c}}^{\text{ub}}$ into one vector), the optimality conditions for $\mathbf{c}$ become

$$\mathbf{q}_{\text{c}} \quad = \quad \mathbf{b}_{\text{c}}^{\text{bnd}}. \tag{S31}$$

where $\mathbf{q}_{\text{c}} = \frac{\partial q}{\partial \mathbf{c}}$. For an enzymatic metabolite cost $q(\mathbf{c},\mathbf{v}) = \sum_l h_{e_l} e_l(\mathbf{c},\mathbf{v}) = \sum_l \frac{h_{e_l} v_l}{k_l(\mathbf{c})}$, we can compute this derivative:

$$\frac{\partial q}{\partial c_i} = \frac{\partial}{\partial c_i} \sum_l \frac{h_{e_l} v_l}{k_l(\mathbf{c})} = \sum_l h_{e_l} v_l \frac{\partial}{\partial c_i} \frac{1}{k_l(\mathbf{c})} = \sum_l h_{e_l} v_l \frac{-1}{(k_l)^2} \frac{\partial k_l}{\partial c_i} = -\sum_l \frac{h_{e_l} v_l}{(k_l)^2 e_l} \frac{\partial v_l}{\partial c_i} = -\sum_l \frac{h_{e_l} e_l}{v_l} E_{c_i}^{v_l}. \tag{S32}$$

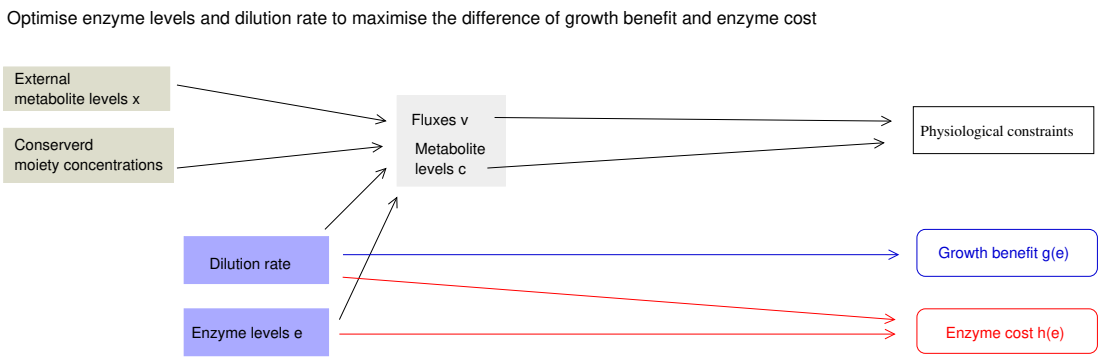


Figure S4: Growth maximisation, with a cost term for enzyme usage.

By inserting this expression into Eq. (S31) and defining the kinetic flux price $a^{\mathrm{kin}}_{v_l} = \frac{h_{e_l}\, e_l}{v_l}$, we obtain the metabolite balance

$$-\sum_l a^{\mathrm{kin}}_{v_l} E^{v_l}_{c_i} \quad = \quad b^{\mathrm{bnd}}_{c_i}. \tag{S33}$$

The effective metabolite gains $b^{\mathrm{bnd}}_{c_i}$ arise from the Lagrange multiplier associated with lower and upper bounds. If the cost function $q(\mathbf{c}, \mathbf{v})$ contains a direct metabolite cost q^{met}, the gradient $\mathbf{q}^{\mathrm{met}}_{\mathrm{c}}$ will appear as an extra term on the left. Usually, metabolite benefits are considered instead of metabolite costs: if we consider $b^{(\mathrm{c})}_{c_i} = -q^{\mathrm{met}}_{c_i}$ and rearrange the equation, we obtain the metabolite balance

$$-b^{\mathrm{bnd}}_{c_i} - b^{(\mathrm{c})}_{c_i} \quad = \quad \sum_l a^{\mathrm{kin}}_{v_l} E^{v_l}_{c_i}. \tag{S34}$$

Since Eq. (S28) contains no constraints on conserved moieties, the left-hand side represents the effective economic load $y^{\mathrm{int}}_{c_i}$.

S2.4 Growth optimisation

The economic balance equations describing metabolism can be derived by assuming that cells need to grow fast while keeping metabolism in a functional state, despite the dilution of compounds. The model is shown in Figure S4. In a growing cell, the state of a metabolic pathway (fluxes and metabolite levels) depends on enzyme levels e_l and dilution rate λ. Instead of varying the growth rate as a parameter, we score it as an optimality objective. We assume that fitness is a function $b(\lambda)$ of the dilution rate λ, minus a cost term for enzyme production, (e.g. $h = \lambda \sum_l e_l$)[4]. As shown in the appendix, we obtain the usual balance equations, as well as an extra equation for the dilution rate:

$$b_\lambda \quad = \quad h_\lambda + \mathbf{w}^{\mathrm{int}}_{\mathrm{r}} \cdot \mathbf{c}, \tag{S35}$$

where $h_\lambda = \partial h / \partial \lambda$. The balance equation (S35) shows how the direct gain b_λ from higher growth is balanced with growth-related losses of enzyme and with metabolism. Interestingly, we obtain, again, the same reaction balanceand metabolite balanceas in the case of simple metabolic obectives. As shown in SI section **??**, this fact can be used to replace growth objective of a whole-cell model, effectively, by apparent metabolic objectives for models of pathways within that cell. These objectives represent inequality constraints from the original model. If

[4]The enzyme cost terms can describe the fact that enzymes are produced and maintained by the cell, which reduces growth. Instead of describing these effects explicitly (as in whole-cell models), we summarise them in an effective cost term to keep our present model simple.

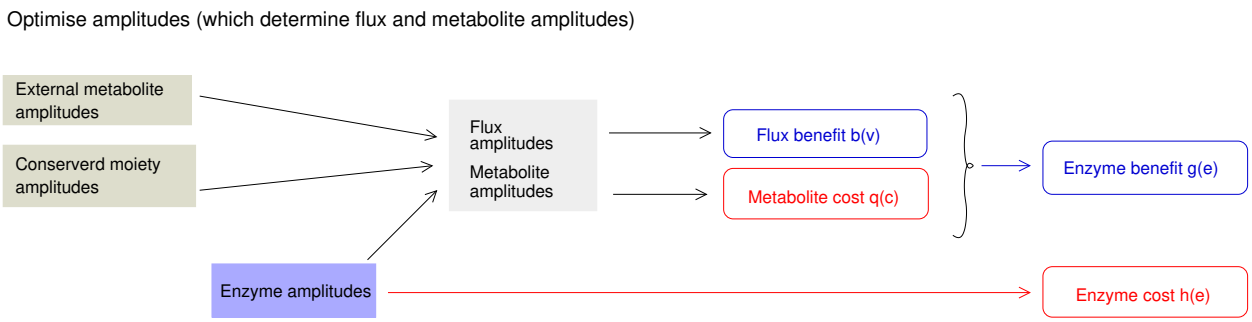


Figure S5: Optimisation of enzyme rhythms.

we replace the growing cell model by a simple metabolic pathway model, we can re-interpret these vectors as the derivatives $\mathbf{b}_{\mathrm{v}}^{\star} = \partial b/\partial \mathbf{v}$ and $\mathbf{b}_{\mathrm{c}} = \partial b/\partial \mathbf{c}$ of some hypothetical objective $b(\mathbf{v}, \mathbf{c})$.

We now derive a formula for the total enzyme partial cost in the metabolic network of a growing cell. Noting that $\mathbf{c} = \frac{1}{\lambda}\,\mathbf{v}^{\mathrm{dil}}$, the term $\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \cdot \mathbf{c}$ can be written as

$$\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \cdot \mathbf{c} \quad = \quad \frac{1}{\lambda}\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \cdot \mathbf{v}^{\mathrm{dil}} = \frac{1}{\lambda}\mathbf{w}_{\mathrm{r}}^{\mathrm{int}\,\top}\,\mathbf{N}_{\mathrm{int}}\,\mathbf{v} = \frac{1}{\lambda}\Delta\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \cdot \mathbf{v}. \tag{S36}$$

Inserting this into Eq. (S35) and summing over enzymatic ("enz") and non-enzymatic ("non") reactions separately, we can split the marginal growth benefit b_λ into four contributions:

$$b_\lambda \quad = \quad h_\lambda + \underbrace{\frac{1}{\lambda}\sum_{l\in\mathrm{enz}}\left[\Delta w_{\mathrm{r}_l}^{\mathrm{int}} + b_{v_l}^{\star}\right] v_l}_{\underline{h}_u} + \frac{1}{\lambda}\sum_{l\in\mathrm{non}}\left[\Delta w_{\mathrm{r}_l}^{\mathrm{int}} + b_{v_l}^{\star}\right] v_l - \frac{1}{\lambda}\sum_{l} b_{v_l}^{\star}\, v_l, \tag{S37}$$

. The first sum yields the usual enzyme point cost $\underline{h}_u = \mathbf{h}_{\mathrm{u}} \cdot \mathbf{e}$ of the metabolic network, divided by λ. Multiplying Eq. (S37) by λ and solving for $\underline{h}_u$, we obtain the formula for the total partial enzyme cost in a growing cell:

$$\underline{h}_u \quad = \quad \underbrace{\lambda(b_\lambda - h_\lambda)}_{\text{growth point fitness}} \quad + \quad \underbrace{\mathbf{b}_{\mathrm{v}}^{\star} \cdot \mathbf{v}}_{\text{flux benefit}} \quad - \quad \underbrace{\left[\Delta\mathbf{w}_{\mathrm{r}}{}^{\mathrm{non}} + \mathbf{b}_{\mathrm{v,non}}\right] \cdot \mathbf{v}^{\mathrm{non}}}_{\text{flux partial cost of non-enzymatic reactions}} \quad . \tag{S38}$$

Without non-enzymatic reactions, the total partial enzyme cost is balanced by the growth point fitness. However, if the non-enzymatic reactions will typically have a negative partial benefit (if they destroy valuable compounds and do not produce more valuable ones), and so the last term will further increase the partial enzyme cost.

S2.5 Optimal enzyme rhythms

The economics of periodic metabolic behaviour can be described by complex economic variables (e.g. enzyme demands and metabolites' economic potentials), which satisfy their own balance equations. These variables and balance equations can be derived by posing an optimality problem for periodic enzyme amplitudes with the help of Lagrange multipliers. The optimisation (see Figure S5) resembles an optimisation of static enzyme levels. We consider a kinetic model under periodic perturbations, described by periodic profiles

$$\begin{aligned} \mathbf{x}(t) &= \mathbf{x}_{\mathrm{ref}} + \mathrm{Re}(\tilde{\mathbf{x}}\,\mathrm{e}^{i\,\omega\,t}) \\ \mathbf{u}(t) &= \mathbf{u}_{\mathrm{ref}} + \mathrm{Re}(\tilde{\mathbf{u}}\,\mathrm{e}^{i\,\omega\,t}) \end{aligned} \tag{S39}$$

of external enzyme and metabolite levels. With these profiles as inputs, the system equations

$$\begin{aligned} \frac{\partial \mathbf{c}(t)}{\partial t} &= \mathbf{N}_{\rm int}\,\mathbf{v}(t) \\ \mathbf{v}(t) &= \mathbf{v}(\mathbf{c}(t),\mathbf{x}(t),\mathbf{u}(t)) \end{aligned} \tag{S40}$$

lead to periodic concentration and flux profiles, which can be approximated by

$$\begin{aligned} \mathbf{c}(t) &\approx \mathbf{c}_{\rm ref} + \mathrm{Re}(\tilde{\mathbf{c}}\,\mathrm{e}^{i\,\omega\,t}) \\ \mathbf{v}(t) &\approx \mathbf{v}_{\rm ref} + \mathrm{Re}(\tilde{\mathbf{v}}\,\mathrm{e}^{i\,\omega\,t}). \end{aligned} \tag{S41}$$

To comply with the system equations, the amplitude vectors $\tilde{\mathbf{c}}$ and $\tilde{\mathbf{v}}$ must satisfy

$$\begin{aligned} \tilde{\mathbf{c}} &= i\,\omega\,\mathbf{N}_{\rm int}\,\tilde{\mathbf{v}} \\ \tilde{\mathbf{v}} &\approx \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{c}}\,\tilde{\mathbf{c}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{x}}\,\tilde{\mathbf{x}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{u}}\,\tilde{\mathbf{u}}, \end{aligned} \tag{S42}$$

with effective periodic elasticity matrices $\mathring{\mathbf{E}}^{\tilde{v}}_{\cdot}$ referring to the periodic metabolic state around which we expand (see SI of [8]). With complex amplitude vectors $\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{x}}$, and $\tilde{\mathbf{u}}$ as physical variables, we now introduce the corresponding economic variables and derive their economic balance equations. First, we define the fitness function $f(\mathbf{c},\mathbf{v},\mathbf{x},\mathbf{u}) = b(\mathbf{c},\mathbf{v},\mathbf{x}) - h(\mathbf{u})$ and consider the optimality problem[5]

$$\begin{aligned} &\text{Maximize } b(\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{x}}) - h(\tilde{\mathbf{u}}) \quad \text{with respect to } \tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{u}} \\ &\text{subject to} \quad \mathbf{N}_{\rm int}\,\tilde{\mathbf{v}} = i\,\omega\,\tilde{\mathbf{c}}, \quad \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{c}}\,\tilde{\mathbf{c}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{x}}\,\tilde{\mathbf{x}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{u}}\,\tilde{\mathbf{u}} = \tilde{\mathbf{v}}, \quad 0 = \mathbf{G}\,\tilde{\mathbf{c}} \end{aligned} \tag{S43}$$

with a predefined amplitude vector $\tilde{\mathbf{x}}$ describing the external perturbations. For the three constraints, we introduce the vectors of complex-valued Lagrange multipliers[6] $\tilde{\boldsymbol{\omega}}_\varphi, \tilde{\boldsymbol{\omega}}_{\rm v}$, and $\tilde{\boldsymbol{\omega}}_{\rm cm}$. We thus need to maximise the Lagrangian

$$b(\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{x}}) - h(\tilde{\mathbf{u}}) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_\varphi \cdot [\mathbf{N}_{\rm int}\,\tilde{\mathbf{v}} - i\,\omega\,\tilde{\mathbf{c}}]) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm v} \cdot [-\tilde{\mathbf{v}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{c}}\,\tilde{\mathbf{c}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{x}}\,\tilde{\mathbf{x}} + \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{u}}\,\tilde{\mathbf{u}}]) - \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm cm} \cdot \mathbf{G}\,\tilde{\mathbf{c}}) \tag{S44}$$

with respect to the vectors $\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{u}}$ and with a suitable choice of $\tilde{\boldsymbol{\omega}}_\varphi, \tilde{\boldsymbol{\omega}}_{\rm v}, \tilde{\boldsymbol{\omega}}_{\rm cm}$. To do so, we take the derivatives with respect to $\tilde{\mathbf{c}},\tilde{\mathbf{v}}$, and $\tilde{\mathbf{u}}$ and set them to zero; and obtain

$$\begin{aligned} 0 &= \frac{\partial}{\partial \tilde{\mathbf{v}}}\left[b(\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{x}}) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_\varphi \cdot \mathbf{N}_{\rm int}\,\tilde{\mathbf{v}}) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm v} \cdot [-\tilde{\mathbf{v}}])\right] \\ 0 &= \frac{\partial}{\partial \tilde{\mathbf{c}}}\left[b(\tilde{\mathbf{c}},\tilde{\mathbf{v}},\tilde{\mathbf{x}}) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_\varphi \cdot [-i\,\omega\,\tilde{\mathbf{c}}]) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm v} \cdot \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{c}}\,\tilde{\mathbf{c}}) - \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm cm} \cdot \mathbf{G}\,\tilde{\mathbf{c}})\right] \\ 0 &= \frac{\partial}{\partial_{\tilde{u}}}\left[-h(\tilde{\mathbf{u}}) + \mathrm{Re}(\tilde{\boldsymbol{\omega}}_{\rm v} \cdot \mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{u}}\,\tilde{\mathbf{u}})\right], \end{aligned} \tag{S45}$$

where irrelevant terms have been omitted. Since $\tilde{\boldsymbol{\omega}}_\varphi \cdot [-i\,\omega\,\tilde{\mathbf{c}}]) = \omega\,[i\,\tilde{\boldsymbol{\omega}}_\varphi] \cdot \tilde{\mathbf{c}})$, this yields

$$\begin{aligned} 0 &= \frac{\partial b}{\partial \tilde{\mathbf{v}}} + \mathbf{N}_{\rm int}^\top\,\tilde{\boldsymbol{\omega}}_\varphi - \tilde{\boldsymbol{\omega}}_{\rm v} \\ 0 &= \frac{\partial b}{\partial \tilde{\mathbf{c}}} + (\mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{c}})^\dagger \tilde{\boldsymbol{\omega}}_{\rm v} + i\,\omega\,\tilde{\boldsymbol{\omega}}_\varphi - \mathbf{G}^\top\,\tilde{\boldsymbol{\omega}}_{\rm cm} \\ 0 &= -\frac{\partial h}{\partial_{\tilde{u}}} + (\mathring{\mathbf{E}}^{\tilde{v}}_{\tilde{u}})^\dagger\,\tilde{\boldsymbol{\omega}}_{\rm v}. \end{aligned} \tag{S46}$$

[5] In this optimality problem, we already consider the linearised reaction rates instead of the actual (unknown) function $\tilde{\mathbf{v}}(\tilde{\mathbf{c}},\tilde{\mathbf{x}},\tilde{\mathbf{u}})$. However, since derivatives are taken in the following step, this does not change the end result.

[6] Complex-valued Lagrange multipliers can be defined as follows. The Lagrange term for complex-valued control variables in a vector $\mathbf{z}$ and for constraints in a vector $\mathbf{f}(\mathbf{z})$ can written separately for real and imaginary parts. It reads $\boldsymbol{\lambda}^\top \frac{\partial \mathbf{f}}{\partial \mathrm{Re}(\mathbf{z})} + \boldsymbol{\lambda}'^\top \frac{\partial \mathbf{f}}{\partial \mathrm{Im}(\mathbf{z})}$ with real-valued Lagrange multiplier vectors $\boldsymbol{\lambda}$ and $\boldsymbol{\lambda}'$. By joining these vectors into one complex-valued Lagrange vector $\boldsymbol{\omega}_\varphi = \boldsymbol{\lambda} + i\boldsymbol{\lambda}'$, also the Lagrange terms can be merged into one term $\mathrm{Re}(\boldsymbol{\omega}_\varphi \cdot \frac{\partial \mathbf{f}}{\partial \mathbf{z}})$.

Now we define the complex-values economic variables $\mathbf{b}_{\tilde{\mathrm{v}}} = \frac{\partial b}{\partial \mathbf{v}}$, $\mathbf{b}_{\tilde{\mathrm{c}}} = \frac{\partial b}{\partial \mathbf{c}}$, $\mathbf{w}_{\tilde{\mathrm{c}}} = \tilde{\boldsymbol{\omega}}_{\varphi}$, $\Delta\,\mathbf{w}_{\tilde{\mathrm{c}}} = \mathbf{N}_{\mathrm{int}}^{\top}\,\tilde{\boldsymbol{\omega}}_{\varphi}$, $\mathbf{y}_{\tilde{\mathrm{c}}}^{\mathrm{cm}} = \tilde{\boldsymbol{\omega}}_{\mathrm{cm}}$, $\mathbf{g}_{\tilde{\mathrm{c}}} = \mathbf{G}^{\top}\,\tilde{\boldsymbol{\omega}}_{\mathrm{cm}}$, $\mathbf{y}_{\tilde{\mathrm{c}}} = \mathbf{g}_{\tilde{\mathrm{c}}} - \mathbf{b}_{\tilde{\mathrm{c}}}$, and $\mathbf{w}_{\tilde{\mathrm{v}}} = \tilde{\boldsymbol{\omega}}_{\mathrm{v}}$. Inserting them, we obtain the balance equations

$$\begin{aligned} \mathbf{w}_{\tilde{\mathrm{v}}} &= \mathbf{b}_{\tilde{\mathrm{v}}} + \Delta\mathbf{w}_{\tilde{\mathrm{c}}} \\ \mathbf{y}_{\tilde{\mathrm{c}}} - i\,\omega\,\mathbf{w}_{\tilde{\mathrm{c}}} &= (\mathring{\mathbf{E}}_{\tilde{c}}^{\tilde{v}})^{\dagger}\mathbf{w}_{\tilde{\mathrm{v}}} \\ \mathbf{h}_{\tilde{\mathrm{u}}} &= (\mathring{\mathbf{E}}_{\tilde{u}}^{\tilde{v}})^{\dagger}\,\mathbf{w}_{\tilde{\mathrm{v}}}. \end{aligned} \tag{S47}$$

These equations resemble our balance equations for optimal steady states, with a number of small differences. The economic variables are now complex-valued, because they do not refer to variations of static values but to variations of complex amplitudes. Accordingly, the elasticities used are not the usual elasticities derived from the rate laws, but "periodic" elasticities defined for oscillatory metabolic state. Finally, there is a frequency-dependent extra term $i\,\omega\,\mathbf{w}_{\tilde{\mathrm{c}}}$. Interestingly, a similar term $-\lambda\,w_{c_i}$ appears in the economic balance equations for metabolic models with dilution (e.g. models of steadily growing cells with cell growth rate λ). We can obtain this dilution term from Eq. (S47) by choosing an imaginary frequency $\omega = i\,\lambda$, i.e. replacing the oscillations $\mathrm{e}^{i\,\omega\,t}$ by decreasing exponentials $\mathrm{e}^{-\lambda\,t}$.

S2.6 Models of growing cells

Finally, we consider detailed whole-cell models, formulated as ordinary differential equations[7]. The models may be coarse-grained or fine-grained, the compounds can represent metabolites and macromolecules, and the model can contain other dynamic variables describing, for example, compartment sizes, pH values, or electrostatic potentials. Let us consider a general form of such models, which can cover kinetic metabolic models, constraint-based metabolic models, and whole-cell models as special cases [9, 10]. Even if our model is kinetic, constraint-based whole-cell models can be formulated similarly. A kinetic models in its steady state, written with explicit constraints, yield the same constraints as a constraint-based model (and some more). This is why some of the optimality conditions derived here will readily apply to constraint-based whole-cell models such as in Resource Balance Analysis [11].

As dynamical variables, we consider the model contains compound concentrations $\mathbf{c}$, metabolic fluxes $\mathbf{v}$, fixed external variables $\mathbf{x}$, variables $\mathbf{p}$ determined by algebraic equations, variables $\mathbf{q}$ determined by differential equations. Moreover, there are control variables $\mathbf{u}$, for example, kinetic constants describing allosteric or transcriptional enzyme regulation. The growth rate λ, which also determines dilution, can appear as a dynamic variable, control variable, or fixed parameter (e.g. in a chemostat). The model dynamics is given by the system equations

$$\begin{aligned} \mathbf{v} &= \boldsymbol{\eta}_v(\mathbf{c},\mathbf{p},\mathbf{q},\mathbf{x},\mathbf{u}) \\ \mathbf{p} &= \boldsymbol{\eta}_p(\mathbf{c},\mathbf{p},\mathbf{q},\mathbf{x},\mathbf{u}) \\ \frac{\mathrm{d}\mathbf{q}}{\mathrm{d}t} = \boldsymbol{\psi} &= \boldsymbol{\eta}_q(\mathbf{c},\mathbf{p},\mathbf{q},\mathbf{x},\mathbf{u}) \\ \frac{\mathrm{d}\mathbf{c}}{\mathrm{d}t} = \boldsymbol{\varphi} &= \mathbf{N}\,\mathbf{v} - \lambda\,\mathbf{c}. \end{aligned} \tag{S48}$$

To define an optimality problem, we score the cell state by a fitness function f, which can depend on all variables. Given external conditions $\mathbf{x}$, the aim is to find a control vector $\mathbf{u}$ and a growth rate λ that allow for a steady state with a (locally) maximal fitness.

We formulate this as an optimality problem (see Figure S6). Given the external conditions $\mathbf{x}$, maximise $f(\mathbf{v},\mathbf{c},\mathbf{p},\mathbf{q},\mathbf{x},\mathbf{u},\lambda)$

[7] There are still more general types of models, e.g. partial differential equation systems, delay differential equations, or stochastic processes.

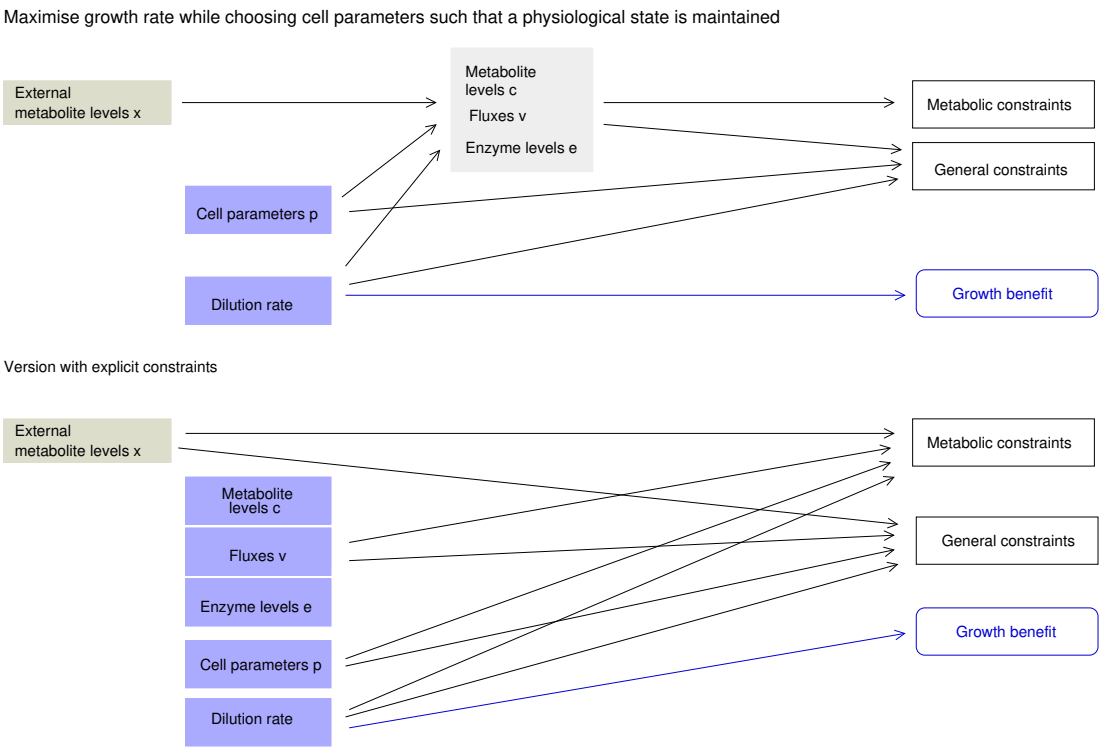


Figure S6: Growth optimisation in kinetic whole-cell models. Top: In the original model, the control variables are biochemical paramaters (e.g. kinetic constants in gene regulation systems). Stationary fluxes, metabolite levels, and enzyme levels are state variables determined by the model dynamics. Bottom: the optimality problem after its reformulation. Now all variables are treated as control variables, and their physical relationships are encoded as explicit constraints.

with respect to $\mathbf{u}$ and λ such that

$$\begin{aligned} \exists\, \mathbf{v}, \mathbf{c}, \mathbf{p}, \mathbf{q}: \quad \mathbf{v} &= \boldsymbol{\eta}_v(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}) \\ \mathbf{p} &= \boldsymbol{\eta}_p(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}) \\ 0 &= \boldsymbol{\eta}_q(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}) \\ 0 &= \mathbf{N}\,\mathbf{v} - \lambda\,\mathbf{c}. \end{aligned} \tag{S49}$$

In addition, there may be lower and upper bounds on $\mathbf{v}, \mathbf{c}, \mathbf{p}$, and $\mathbf{q}$, but for simplicity, we shall not consider such bounds. To write the problem in expanded form, we treat all variables $\mathbf{v}, \mathbf{c}, \mathbf{p}, \mathbf{q}$, $\mathbf{u}$, and λ as free variables and formulate all dependencies as explicit constraints (see Figure S6 bottom):

Given external conditions $\mathbf{x}$, find Lagrange multipliers (in vectors $\boldsymbol{\omega}_\mathrm{v}, \boldsymbol{\omega}_\mathrm{p}, \boldsymbol{\omega}_\psi, \boldsymbol{\omega}_\varphi$) such that

$$\begin{aligned} f(\mathbf{v}, \mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}, \lambda) \quad + \quad & \underbrace{\boldsymbol{\omega}_\mathrm{v}^\top \left[\boldsymbol{\eta}_v(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}) - \mathbf{v}\right]}_{\text{from rate equation}} + \underbrace{\boldsymbol{\omega}_\mathrm{p}^\top \left[\boldsymbol{\eta}_p(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u}) - \mathbf{p}\right]}_{\text{from algebraic variables}} \\ + \quad & \underbrace{\boldsymbol{\omega}_\psi^\top\, \boldsymbol{\eta}_q(\mathbf{c}, \mathbf{p}, \mathbf{q}, \mathbf{x}, \mathbf{u})}_{\text{from variable stationarity}} \quad + \quad \underbrace{\boldsymbol{\omega}_\varphi^\top \left[\mathbf{N}\,\mathbf{v} - \lambda\,\mathbf{c}\right]}_{\text{from metabolite stationarity}} \end{aligned} \tag{S50}$$

is locally maximal with respect to $\mathbf{u}, \mathbf{v}, \mathbf{c}, \mathbf{p}, \mathbf{q}$, and λ. To obtain the necessary optimality conditions, we differen-

tiate Eq. (S50) by $\mathbf{v}, \mathbf{c}, \mathbf{p}, \mathbf{q}$, $\mathbf{u}$, λ and set the resulting derivatives (given in row vectors) to zero:

$$\begin{aligned}
0 &= \frac{\partial f}{\partial \mathbf{v}} - \boldsymbol{\omega}_{\mathrm{v}}^{\top} + \boldsymbol{\omega}_{\varphi}^{\top}\mathbf{N} \\
0 &= \frac{\partial f}{\partial \mathbf{c}} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\frac{\partial \boldsymbol{\eta}_v}{\partial \mathbf{c}} + \boldsymbol{\omega}_{\mathrm{p}}^{\top}\frac{\partial \boldsymbol{\eta}_p}{\partial \mathbf{c}} + \boldsymbol{\omega}_{\psi}^{\top}\frac{\partial \boldsymbol{\eta}_q}{\partial \mathbf{c}} - \boldsymbol{\omega}_{\varphi}^{\top}\lambda \\
0 &= \frac{\partial f}{\partial \mathbf{p}} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\frac{\partial \boldsymbol{\eta}_v}{\partial \mathbf{p}} + \boldsymbol{\omega}_{\mathrm{p}}^{\top}\frac{\partial \boldsymbol{\eta}_p}{\partial \mathbf{p}} - \boldsymbol{\omega}_{\mathrm{p}}^{\top} + \boldsymbol{\omega}_{\psi}^{\top}\frac{\partial \boldsymbol{\eta}_q}{\partial \mathbf{p}} \\
0 &= \frac{\partial f}{\partial \mathbf{q}} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\frac{\partial \boldsymbol{\eta}_v}{\partial \mathbf{q}} + \boldsymbol{\omega}_{\mathrm{p}}^{\top}\frac{\partial \boldsymbol{\eta}_p}{\partial \mathbf{q}} + \boldsymbol{\omega}_{\psi}^{\top}\frac{\partial \boldsymbol{\eta}_q}{\partial \mathbf{q}} \\
0 &= \frac{\partial f}{\partial \mathbf{u}} + \boldsymbol{\omega}_{\mathrm{v}}^{\top}\frac{\partial \boldsymbol{\eta}_v}{\partial \mathbf{u}} + \boldsymbol{\omega}_{\mathrm{p}}^{\top}\frac{\partial \boldsymbol{\eta}_p}{\partial \mathbf{u}} + \boldsymbol{\omega}_{\psi}^{\top}\frac{\partial \boldsymbol{\eta}_q}{\partial \mathbf{u}}.
\end{aligned} \tag{S51}$$

All derivatives in these formulae are fitness derivatives (e.g. $\partial f/\partial \mathbf{v}$) or coefficients that link neighbouring model elements (e.g. reaction elasticities $\mathbf{E}_{\mathrm{c}} = \partial \mathbf{v}/\partial \mathbf{c}$ between concentrations and reaction rates). The Lagrange multipliers (in the vectors $\boldsymbol{\omega}_{\mathrm{v}}, \boldsymbol{\omega}_{\mathrm{p}}, \boldsymbol{\omega}_{\psi}, \boldsymbol{\omega}_{\varphi}$) can be seen as economic variables, associated with the physical variables $\mathbf{v}, \mathbf{p}, \boldsymbol{\psi}$, and φ.

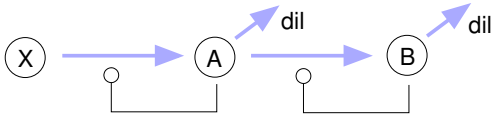
S2.7 Resource balance analysis

After solving an RBA problem (either maximising the growth rate, or optimisin a linear objective at given growth), we can use the economic balance equations to compute the economic variables, i.e., the Lagrange multipliers corresponding to our objective function. Let us focus on the first case, optimisation of growth. Knowing the model structure, the optimal growth rate λ, and the flux pattern (active fluxes and flux directions in the optimal state), we can solve the balance equations for $\mathbf{w}_{\mathrm{r}}^{\mathrm{int}}$, $\mathbf{a}_{\mathrm{v}}$, and $\mathbf{p}_{\mathrm{d}}$. The concentrations $\mathbf{c}$, in the last equation, are only needed for scaling the entire set of economic variables. If the absolute scaling of economic variables does not matter, we can ignore $\mathbf{c}$ and omit this last equation, solve only the first two equations, and obtain arbitrarily scaled economic variables. IN any case, the flux pattern needs to be known to obtain the capacity matrix $\mathbf{E}_{\mathrm{cap}}$ and the signs of $\mathbf{a}_{\mathrm{v}}$. Furthermore, we need to know which density constraints are active, in order to require positive values in $\mathbf{p}_{\mathrm{d}}$ for compounds involved in these density constraints. To solve the equations, we write them as

$$\begin{pmatrix} \mathbf{N}_{\mathrm{int}}^{\top} & -\mathbf{I} & 0 \\ -\lambda\mathbf{I} & \mathbf{E}_{\mathrm{cap}}{}^{\top} & -\mathbf{D}^{\top} \\ \mathbf{c}^{\top} & 0 & 0 \end{pmatrix} \begin{pmatrix} \mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \\ \mathbf{a}_{\mathrm{v}} \\ \mathbf{p}_{\mathrm{d}} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \tag{S52}$$

and consider the constraints $\mathrm{sign}(a_{v_l}) = \mathrm{sign}(v_l)$ for all active reactions (and $a_{v_l} = 0$ for all inactive reactions), and $p_{d_j} > 0$ for all active density constraints (and $p_{d_j} = 0$ for all inactive ones). These are linear constraints on our economic variables. Will there be a solution, and will it be unique? To check this, we remove all inactive reactions $v_l = 0$, irrelevant metabolites $c_i = 0$, and inactive density constraints. Then, to yield a solution, the remaining matrix on the left must have full column rank; and to yield a unique solution, it must be invertible. This implies that the number of economic variables (columns) and equalities (rows) must be equal. The number of variables is given by the total number of intracellular compounds, reactions, and active density constraints. The number of rows in our matrix is the total number of reactions and (intracellularl) compound, plus 1. Thus, if more than one constraint is active, the economic variables will for sure be underdetermined.

Let us see an example, one in which the economic variables can be uniquely determined. We consider a simple cell model with reactions $X \to A \to B$, where X is an external compound, the compounds A and B are internal and diluted. A acts as the catalyst of reaction 1 and B acts as the catalyst of reaction 2.



For the optimal solution, we assume that the two reactions show positive fluxes and that A and B hit the density constraint $d_1 [A] + d_2 [B] = d_{\mathrm{tot}}$. We obtain the matrices

$$\mathbf{N}_{\mathrm{int}} = \begin{pmatrix} 1 & -1 \\ 0 & 1 \end{pmatrix}, \qquad \mathbf{E}_{\mathrm{cap}} = \begin{pmatrix} k_1 & 0 \\ 0 & k_2 \end{pmatrix}, \qquad \mathbf{D} = (d_1, d_2). \tag{S53}$$

Thus, our balance equations read

$$\begin{aligned} \begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix} \mathbf{w}_{\mathrm{r}}^{\mathrm{int}} &= \mathbf{a}_{\mathrm{v}} \\ \begin{pmatrix} k_1 & 0 \\ 0 & k_2 \end{pmatrix} \mathbf{a}_{\mathrm{v}} &= \begin{pmatrix} d_1 \\ d_2 \end{pmatrix} \mathbf{p}_{\mathrm{d}} + \lambda\, \mathbf{w}_{\mathrm{r}}^{\mathrm{int}} \\ (c_1, c_2)\, \mathbf{w}_{\mathrm{r}}^{\mathrm{int}} &= 1 \end{aligned} \tag{S54}$$

or, together,

$$\begin{pmatrix} 1 & 0 & -1 & 0 & 0 \\ -1 & 1 & 0 & -1 & 0 \\ -\lambda & 0 & k_1 & 0 & -d_1 \\ 0 & -\lambda & 0 & k_2 & -d_2 \\ c_1 & c_2 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} w_1 \\ w_2 \\ b_1 \\ b_2 \\ p_d \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}, \tag{S55}$$

This matrix is typically invertible (unless some parameters vanish), and the economic variables can be directly determined by matrix inversion.

S3 Proofs and derivations

S3.1 Signs of the Lagrange multipliers

Inequality constraints in optimality problems can be handled with Lagrange multipliers, whose signs reflect the type of constraint (upper or lower bound) and which vanish when constraints are inactive. In optimality problems with equality and inequality constraints, the solution must satisfy the following Karush-Kuhn-Tucker optimality conditions [12]. We consider an optimality problem

Minimise $F(\mathbf{x})$ with respect to $\mathbf{x} \in \mathbb{R}^n$ subject to $G_i(\mathbf{x}) \leq G'$ and $H_j(\mathbf{x}) = H'$, where $i \in \{1, .., m\}, j \in \{1, .., n\}$

We assume that the gradients of the active inequality constraints $G_i(\mathbf{x})$ and the gradients of the equality constraints $H_j(\mathbf{x})$ are linearly independent at the optimal point[8] The optimality conditions (for an optimal point $\mathbf{x}^*$)

[8] In metabolic economics, inequality constraints are typically used to put bounds on the model variables; these constraints are mutually independent and independent of equality constaints representing stationarity or rate laws. Redundant bounds or duplicate rate laws could cause problems, leading to non-unique values of the Lagrange multipliers.

then read

$$0 = \nabla F + \sum_i \mu_i \nabla G_i + \sum_j \lambda_j \nabla H_j. \tag{S56}$$

Primal feasibility requires that $g_i(\mathbf{x}^*) \leq 0$ and $h_j(\mathbf{x}^*) = 0$ (i.e. the constraints must be satisfied for all i and j). Dual feasibility requires that $\mu_i \geq 0$ and $\mu_i\, g_i(\mathbf{x}^*) = 0$ (complementary slackness condition) for all i. Let us consider, as a special case, a variable with an upper bound $x_l \leq x_l^{\max}$, i.e. $x_l - x_l^{\max} \leq 0$. When this bound is active, it is associated with a positive Lagrange multiplier. Accordingly, an active lower bound will be associated with a negative Lagrange multiplier.

In metabolic economics, we often deal with *maximisation* instead of minimisation problems. To maximise of F, we can minimise $-F$, which leads to the optimality condition

$$0 = \nabla F - \sum_i \mu_i \nabla G_i - \sum_j \lambda_j \nabla H_j, \tag{S57}$$

or, equivalently, to the original optimality condition

$$0 = \nabla F + \sum_i \bar{\mu}_i \nabla G_i + \sum_j \bar{\lambda}_j \nabla H_j, \tag{S58}$$

where the signs of all Lagrange multipliers are switched: $\bar{\mu}_i = -\mu_i$, $\bar{\lambda}_j = -\lambda_j$, . Therefore, in maximisation problems, lower bounds (of the form $G_i(\mathbf{x}) \geq G_i'$) lead to positive Lagrange multipliers, while upper bounds (of the form $G_i(\mathbf{x}) \leq G_i'$) lead to negative Lagrange multipliers.

	Minimisation problem for F	Maximisation problem for F
Optimality condition	$0 = \nabla F + \sum_i \mu_i \nabla g_i + ..$	$0 = \nabla F + \sum_i \bar{\mu}_i \nabla g_i + ..$
Active lower bound $G(\mathbf{x}) \geq G'$	$\mu < 0$	$\bar{\mu} > 0$
Active upper bound $G(\mathbf{x}) \leq G'$	$\bar{\mu} > 0$	$\bar{\mu} < 0$
Inactive bound	$\mu = 0$	$\bar{\mu} = 0$

S3.2 Partial flux costs of enzymatic flux cost functions

Given a flux cost function $a(\mathbf{v})$, the flux partial cost is defined as the logarithmic derivative $\underline{h}_{u_l} = \partial a / \partial \ln v_l$. The enzymatic flux cost a^{enz}, for example, represents the optimal enzyme cost in a kinetic model. With this cost function, the flux partial cost is identical to the enzyme point cost $\underline{h}_{u_l} = \partial h / \partial \ln u_l$ in enzyme cost-benefit optimisation. We can see this as follows. We assume that our fluxes $\mathbf{v}$ are realised by a given kinetic model, with reaction rates given by $v_l = u_l\, k_l(\mathbf{c})$ and enzyme levels u_l, and an cost function $h(\mathbf{u})$. For fixed metabolite concentrations $\mathbf{c}$, we define a flux cost $a(\mathbf{v}|\mathbf{c})$ by the enzyme cost needed to realise the fluxes: $a(\mathbf{v}|\mathbf{c}) = h(\mathbf{u}(\mathbf{v})) = h(\mathbf{v}/\mathbf{k}(\mathbf{c}))$, where vectors are divided componentwise. For this flux cost function, we obtain the flux partial cost

$$\underline{h}_{u_l} \;=\; \frac{\partial a^{\mathrm{enz}}}{\partial v_l} v_l = \frac{\partial h}{\partial u_l} \frac{1}{k_l(\mathbf{c})}\, v_l = \frac{\partial h}{\partial u_l} u_l. \tag{S59}$$

The equality holds for any metabolite levels chosen, and therefore also if metabolite levels are not predetermined but to be optimised.

S3.3 Economic balances in a growing cell

We consider a simple model of a growing cell (with growth rate λ) as shown in Figure **??**. Since we maximise growth at a limited biomass concentration, we can safely assume a positive concentration demand for biomass (but not for the other compounds). Let us compute the economic variables for our example model. 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). We assume that all stoichiometric coefficients have numerical values of 1 and that there are no direct flux gains. Then the flux demands are given by the reaction rules

$$\begin{aligned} w_C^{\mathrm{v}} &= w_e^{\mathrm{int}} + w_i^{\mathrm{int}} - w_{\mathrm{glc}}^{\mathrm{ext}} \\ w_A^{\mathrm{v}} &= w_b^{\mathrm{int}} - w_e^{\mathrm{int}} - w_i^{\mathrm{int}} \\ w_E^{\mathrm{v}} &= w_e^{\mathrm{int}} - w_p^{\mathrm{int}} - w_i^{\mathrm{int}} \\ w_R^{\mathrm{v}} &= w_r^{\mathrm{int}} - w_p^{\mathrm{int}} - w_i^{\mathrm{int}}, \end{aligned} \tag{S60}$$

where glc stands for glucose.The compound rules, on the contrary, read

$$\begin{aligned} 0 &= E_C^p\, w_C^{\mathrm{v}} + E_A^p\, w_A^{\mathrm{v}} + E_E^p\, w_E^{\mathrm{v}} + E_R^p\, w_R^{\mathrm{v}} - \lambda\, w_p^{\mathrm{int}} \\ 0 &= E_C^i\, w_C^{\mathrm{v}} + E_A^i\, w_A^{\mathrm{v}} + E_E^i\, w_E^{\mathrm{v}} + E_R^i\, w_R^{\mathrm{v}} - \lambda\, w_i^{\mathrm{int}} \\ 0 &= E_C^e\, w_C^{\mathrm{v}} + E_A^e\, w_A^{\mathrm{v}} + E_E^e\, w_E^{\mathrm{v}} + E_R^e\, w_R^{\mathrm{v}} - \lambda\, w_e^{\mathrm{int}} \\ 0 &= E_C^r\, w_C^{\mathrm{v}} + E_A^r\, w_A^{\mathrm{v}} + E_E^r\, w_E^{\mathrm{v}} + E_R^r\, w_R^{\mathrm{v}} - \lambda\, w_r^{\mathrm{int}} \\ h_{\mathrm{BM}}^{\mathrm{c}} &= E_C^b\, w_C^{\mathrm{v}} + E_A^b\, w_A^{\mathrm{v}} + E_E^b\, w_E^{\mathrm{v}} + E_R^b\, w_R^{\mathrm{v}} - \lambda\, w_b^{\mathrm{int}}. \end{aligned}$$

We can write these two equations in matrix form:

$$\begin{pmatrix} w_C^{\mathrm{v}} \\ w_A^{\mathrm{v}} \\ w_E^{\mathrm{v}} \\ w_R^{\mathrm{v}} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \\ -1 & -1 & 0 & 0 & 1 \\ -1 & -1 & 1 & 0 & 0 \\ -1 & -1 & 0 & 1 & 0 \end{pmatrix} \begin{pmatrix} w_p^{\mathrm{int}} \\ w_i^{\mathrm{int}} \\ w_e^{\mathrm{int}} \\ w_r^{\mathrm{int}} \\ w_b^{\mathrm{int}} \end{pmatrix} + \begin{pmatrix} -1 \\ 0 \\ 0 \\ 0 \end{pmatrix} w_{\mathrm{glc}}^{\mathrm{ext}}$$

$$\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ h_{\mathrm{BM}}^{\mathrm{c}} \end{pmatrix} = \begin{pmatrix} E_C^p & E_A^p & E_E^p & E_R^p \\ E_C^i & E_A^i & E_E^i & E_R^i \\ E_C^e & E_A^e & E_E^e & E_R^e \\ E_C^r & E_A^r & E_E^r & E_R^r \\ E_C^b & E_A^b & E_E^b & E_B^r \end{pmatrix} \begin{pmatrix} w_C^{\mathrm{v}} \\ w_A^{\mathrm{v}} \\ w_E^{\mathrm{v}} \\ w_R^{\mathrm{v}} \end{pmatrix} - \lambda \begin{pmatrix} w_p^{\mathrm{int}} \\ w_i^{\mathrm{int}} \\ w_e^{\mathrm{int}} \\ w_r^{\mathrm{int}} \\ w_b^{\mathrm{int}} \end{pmatrix},$$

or briefly

$$\begin{aligned} \mathbf{w}_{\mathrm{v}} &= \mathbf{N}_{\mathrm{int}}^{\top}\, \mathbf{w}_{\mathrm{r}}^{\mathrm{int}} + \mathbf{N}_{\mathrm{ext}}^{\top}\, \mathbf{w}_{\mathrm{r}}^{\mathrm{ext}} \\ \mathbf{q}_{\mathrm{c}^{\mathrm{int}}} &= \mathbf{E}_{\mathrm{c}}^{\top}\, \mathbf{w}_{\mathrm{v}} - \lambda\, \mathbf{w}_{\mathrm{r}}^{\mathrm{int}}, \end{aligned}$$

which we can solve for

$$\mathbf{w}_{\mathrm{r}}^{\mathrm{int}} = -([\mathbf{N}_{\mathrm{int}}\, \mathbf{E}_{\mathrm{c}} - \lambda\, \mathbf{I}]^{\top})^{-1}[-\mathbf{q}_{\mathrm{c}^{\mathrm{int}}} + \mathbf{E}_{\mathrm{x}}^{\top} \mathbf{N}_{\mathrm{ext}}^{\top}\, \mathbf{w}_{\mathrm{r}}^{\mathrm{ext}}].$$

Altogether, we have obtained nine linear equations for our flux values and five economic potentials. To solve them, we need to assign numbers to the elasticities and to the external economic potentials (in this case, the potential of glucose). For an illustrative example, we make 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 our choice of concentrations and stoichiometric coefficients, the steady-state fluxes must be proportional to $(3, 1, 1, 1)^\top$. When choosing 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. The enzyme e and the ribosome r would normally exert scaled elasticities of 1, but since each of them catalyses two reactions, the elasticities are multiplied by a factor of 1/2. By multiplying the scaled elasticities by the fluxes (and assuming concentrations of 1), we obtain the (transposed) unscaled elasticity matrix

$$\mathbf{E}_\mathrm{c} = \begin{pmatrix} E_C^p & E_A^p & E_E^p & E_R^p \\ E_C^i & E_A^i & E_E^i & E_R^i \\ E_C^e & E_A^e & E_E^e & E_R^e \\ E_C^r & E_A^r & E_E^r & E_R^r \\ E_C^b & E_A^b & E_E^b & E_B^r \end{pmatrix}^\top = \begin{pmatrix} -1 & -1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 0 & 0 \\ 1/3 & 1/3 & 0 & 1 & 0 \\ 1/3 & 1/3 & 0 & 1 & 0 \end{pmatrix}$$

Assuming a dilution rate $\lambda = 1$ and an external economic potential of 4 for the biomass (instead of 1, as above), we obtain from Eq. (S61)

$$\begin{aligned} \mathbf{w}_\mathrm{r}^\mathrm{int} &= -\left[\begin{pmatrix} -8/3 & -8/3 & 1/3 & 1/3 & 1 \\ -8/3 & -8/3 & 1/3 & 1/3 & 1 \\ 0 & 0 & 0 & 0 & 1 \\ -2 & -2 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & \end{pmatrix}^\top - \mathbf{I}\right]^{-1} \left[\begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 4 \end{pmatrix} + 0 \cdot \begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}\right] \\ &= -\begin{pmatrix} -1/2 & 1/2 & -1/4 & -1/4 & -1/4 \\ 1/2 & -1/2 & -1/4 & -1/4 & -1/4 \\ 0 & 0 & -1 & 0 & -1 \\ 3/2 & 3/2 & -15/4 & -19/4 & -3/4 \\ 0 & 0 & 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 6 \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 4 \\ 3 \\ 4 \end{pmatrix}. \end{aligned} \tag{S61}$$

The resulting flux demands read $\mathbf{w}_\mathrm{v} = (2, 2, 2, 1)^\top$. These numbers are plausible: all flux demands are positive, and all internal potentials are between 0 and the biomass potential (where actually, the enzymes have the same potential as the biomass).

References

[1] Q.K. Beg, A. Vazquez, J. Ernst, M.A. de Menezes, Z. Bar-Joseph, A.-L. Barabási, and Z.N. Oltvai. Intracellular crowding defines the mode and sequence of substrate uptake by Escherichia coli and constrains its metabolic activity. *PNAS*, 104(31):12663–12668, 2007.

[2] J.G. Reich. Zur Ökonomie im Proteinhaushalt der lebenden Zelle. *Biomed. Biochim. Acta*, 42(7/8):839–848, 1983.

[3] W. Liebermeister, E. Klipp, S. Schuster, and R. Heinrich. A theory of optimal differential gene expression. *BioSystems*, 76:261–278, 2004.

[4] E. Dekel and U. Alon. Optimality and evolutionary tuning of the expression level of a protein. *Nature*, 436:588–692, 2005.

[5] W. Liebermeister. Enzyme economy in metabolic networks. *Preprint on arxiv.org: arXiv:1404.5252*, 2014.

[6] R. Heinrich and E. Klipp. Control analysis of unbranched enzymatic chains in states of maximal activity. *J. Theor. Biol.*, 182(3):243–252, 1996.

[7] E. Noor, A. Flamholz, A. Bar-Even, D. Davidi, R. Milo, and W. Liebermeister. The protein cost of metabolic fluxes: prediction from enzymatic rate laws and cost minimization. *PLoS Comput Biol*, 12(10):e1005167, 2016. doi:10.1371/journal.pcbi.1005167.

[8] W. Liebermeister. The economic basis of periodic enzyme dynamics. *Preprint on arxiv.org: arxiv.org/abs/1602.05167*, 2016.

[9] D. Molenaar, R. van Berlo, D. de Ridder, and B. Teusink. Shifts in growth strategies reflect tradeoffs in cellular economics. *Molecular Systems Biology*, 5:323, 2009.

[10] A.Y. Weiße, D.A. Oyarzún, V. Danos, and P.S. Swain. Mechanistic links between cellular trade-offs, gene expression, and growth. *PNAS*, 112(9):E1038–E1047, 2015.

[11] A. Goelzer and V. Fromion. Bacterial growth rate reflects a bottleneck in resource allocation. *Biochim Biophys Acta*, 1810(10):978–988, 2011.

[12] H.W. Kuhn and A.W. Tucker. Nonlinear programming. In *Proceedings of 2nd Berkeley Symposium. Berkeley: University of California Press*, pages 481–492, 1951.