How enzyme economy shapes metabolic fluxes
Supplementary text

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Contents

S1 Basic notions of flux analysis

According to the three levels of feasibility – mass balance, energy balance, and benefit – we can classify flux distributions by three criteria: mass balance (productive or non-productive modes); thermodynamics (dissipative, non-dissipative, or anti-dissipative modes), and economics (beneficial, non-beneficial, or costly modes). This section summarises related concepts used in flux analysis. Notions for economic and thermodynamical flux analysis are explained in parallel.

Definitions for metabolic networks and flux distributions A metabolic network is defined by a stoichiometric matrix $\mathbf{N}^{\mathrm{tot}}$, which consists of submatrices $\mathbf{N}$ (for internal metabolites with levels c_i, described by differential equations) and $\mathbf{N}^{\mathrm{x}}$ (for external metabolites with predefined levels x_j). Flux distributions $\mathbf{v}$ are called *stationary* if they satisfy the stationarity condition $\mathbf{N}\,\mathbf{v} = 0$. Flux distributions without net production of metabolites (satisfying $\mathbf{N}^{\mathrm{tot}}\,\mathbf{v} = 0$) are called *non-productive* and flux distributions satisfying $\mathbf{N}^{\mathrm{z}}\,\mathbf{v} = \binom{\mathbf{z}^{\mathrm{v}\top}}{\mathbf{N}^{\mathrm{tot}}}\,\mathbf{v} = 0$ are called *non-beneficial*. Thus, stationary, non-productive, and non-beneficial flux distributions are kernel vectors of the stoichiometric matrices $\mathbf{N}, \mathbf{N}^{\mathrm{tot}}$, and $\mathbf{N}^{\mathrm{z}}$, and the right-kernel matrices $\mathbf{K}$, $\mathbf{K}^{\mathrm{tot}}$, and $\mathbf{K}^{\mathrm{z}}$ of these matrices (satisfying $\mathbf{N}\,\mathbf{K} = 0$, $\mathbf{N}^{\mathrm{tot}}\,\mathbf{K}^{\mathrm{tot}} = 0$, and $\mathbf{N}^{\mathrm{z}}\,\mathbf{K}^{\mathrm{z}} = 0$) contain stationary, non-productive, and non-beneficial modes as their columns. Non-productive modes are thermodynamically infeasible because they do not dissipate any Gibbs free energy, and in models with a production objectives, they are also non-beneficial.

Reactions with non-zero rates are called *active* and form the *active region* $\mathcal{A}(\mathbf{v})$ of a flux distribution. If all reactions are active, the flux distribution $\mathbf{v}$ is called *complete*. A stationary flux distribution $\mathbf{v}$ is called *elementary* if it does not contain any other stationary (non-productive, non-beneficial, costly) flux distribution $\mathbf{k}$ with a smaller active region $\mathcal{A}(\mathbf{k}) \subset \mathcal{A}(\mathbf{v})$. Analogous definitions hold for elementary non-productive, economically non-beneficial, or futile modes. The active reactions shared by two flux distributions $\mathbf{v}$ and $\mathbf{k}$ form the *shared active region* $\mathcal{A}(\mathbf{v}, \mathbf{k})$. If two flux distributions $\mathbf{v}$ and $\mathbf{k}$ share the same flux signs on their entire shared active region, they are called *sign-concordant*. If $\mathbf{k}$ is an non-productive flux distribution on the active region of $\mathbf{v}$ and if $\mathbf{v}$ and $\mathbf{k}$ are sign-concordant, then $\mathbf{v}$ is said to contain a flux mode (defined by the shared sign pattern).

Flux balance analysis Flux balance analysis is a method for predicting likely flux distributions in metabolic networks [1]. The fluxes are restricted by stationarity conditions and upper and lower bounds:

$$\begin{aligned} &\mathbf{N}\,\mathbf{v} = 0 \\ &v_l^{\min} \le v_l \le v_l^{\max}. \end{aligned} \tag{S1}$$

In models, such bounds can implement physiological flux directions, the availability of substrates, or maximal rates of certain enzymes, and may possibly stem from a previous thermodynamic or economic flux analysis. The conditions (S1) and (S1) define a convex polyhedron in flux space, the admissible region $\mathcal{P}_{\mathrm{FBA}}$. Given a flux weight vector $\mathbf{z}^{\mathrm{v}}$, a flux distribution $\mathbf{v}$ (or a surface of $\mathcal{P}_{\mathrm{FBA}}$) can be selected by maximising a linear benefit function

$$\max \stackrel{!}{=} \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}. \tag{S2}$$

Thermodynamic flux analysis Thermodynamic flux analysis, a variant of FBA, uses constraints to exclude flux distributions with flux cycles, which would preclude thermodynamic feasibility. Due to the second law of thermodynamics, any flux must run along the direction of the thermodynamic force $\Theta_l = -\Delta\mu_l/RT$, where the vector $\Delta\boldsymbol{\mu} = \mathbf{N}^{\mathrm{tot}\top}\boldsymbol{\mu}$ depends on the vector $\boldsymbol{\mu}$ of chemical potentials. There are two variants of this condition [2] According to the strong thermodynamic condition ("strictly feasible fluxes"), any positive force leads to a positive forward flux:

$$\mu_l \neq 0 \;\Rightarrow\quad v_l\,\Delta\mu_l < 0. \tag{S3}$$

According to the weak condition ("feasible fluxes"), fluxes may also vanish (e.g. because no active enzyme is present):

$$v_l \neq 0 \;\Rightarrow\; v_l\,\Delta\mu_l < 0. \tag{S4}$$

In flux analysis, one usually uses the weak sign condition. To determine feasible flux distributions, we can treat the fluxes v_l and chemical potentials μ_i as model variables: the sign constraint Eq. (S4), together with upper and lower limits for $\Delta\boldsymbol{\mu}$, read

$$\begin{aligned} v_l \neq 0 \;&\Rightarrow\; -v_l \sum_i N_{il}\,\mu_i > 0 \\ \Delta\mu_l^{\min} \leq \sum_i N_{il}\,\mu_i &\leq \Delta\mu_l^{\max}. \end{aligned} \tag{S5}$$

Instead, we can directly use the balances $\Delta\mu_l$ as model variables and obtain

$$\begin{aligned} &v_l \neq 0 \;\Rightarrow\; -v_l\,\Delta\mu_l > 0 \\ &\Delta\mu_l^{\min} \leq \Delta\mu_l \leq \Delta\mu_l^{\max} \\ &{\mathbf{K}^{\mathrm{tot}}}^{\top}\Delta\boldsymbol{\mu} = 0. \end{aligned} \tag{S6}$$

The Wegscheider condition (last equation) ensures that the vector $\Delta\boldsymbol{\mu}$ can be written in the form $\Delta\boldsymbol{\mu} = {\mathbf{N}^{\mathrm{tot}}}^{\top}\boldsymbol{\mu}$. $\mathbf{K}^{\mathrm{tot}}$ is a right-kernel matrix of $\mathbf{N}^{\mathrm{tot}}$.

Flux cycles For which kinds of flux distributions $\mathbf{v}$ will a feasible set of chemical potentials μ_i exist? A general algebraic criterion, the absence of flux cycles, was given by Beard et al. (Theorem 6.1 in [2]): to check a given flux distribution $\mathbf{v}$, one compares its sign pattern to elementary non-productive flux modes.

For the comparison, we define the notion of sign-orthogonality: two vectors $\mathbf{a}$ and $\mathbf{b}$ are sign-orthogonal if they either have no shared active region (i.e. for each l, either a_l or b_l vanishes) or if, within their shared active region, they have an element (index l) with identical signs ($\mathrm{sign}(a_l) = \mathrm{sign}(b_l)$) and another element (index j) with opposite signs ($\mathrm{sign}(a_j) = -\mathrm{sign}(b_j)$).

Given a flux distribution $\mathbf{v}$, we obtain two different criteria, depending on the condition used ((S3) or (S4)). The strong condition (S3) has a solution $\boldsymbol{\mu}$ if (and only if) $\mathbf{v}$ is sign-orthogonal on any non-productive flux distribution in the network. In other words, $\mathbf{v}$ may not be sign-concordant with any non-productive flux distribution.

For the weak thermodynamic constraint (S4), there is a similar criterion: $\mathbf{v}$ must satisfy

$$|\mathrm{sign}(\mathbf{v}) \cdot \mathrm{sign}(\mathbf{k})| < |\mathrm{sign}(\mathbf{k}) \cdot \mathrm{sign}(\mathbf{k})| \tag{S7}$$

for any cycle $\mathbf{k}$ [2]. This implies that $\mathbf{v}$ is sign-orthogonal on any flux cycle $\mathbf{k}$ within the active region of $\mathbf{v}$; in other words, there must be no cycle *within the active region of* $\mathbf{v}$ with the same signs as $\mathbf{v}$ on their shared active region. After constructing all elementary non-productive flux distributions, we can use them to detect local problems in flux distributions systematically.

Flux cost minimisation Flux solutions from FBA may contain futile cycles. Flux cost minimisation, in contrast, can exclude such solutions. Each flux vector is scored by a cost function, for instance, the sum of absolute fluxes. To choose a flux distribution, we prescribe a value of the flux objective, consider all feasible flux distributions that realise this value, and select the one (or the ones) that minimise the flux cost function. The biological idea is that large fluxes imply large enzyme levels and should be avoided unless they are vital for the metabolic objective. In practice, flux cost minimisation can be run as a second step after an FBA to reduce the set of solutions. The FBA constraints Eqs (S1) and (S1) will define a polytope $\mathcal{P}_{\mathrm{FBA}}$ of feasible flux distributions; by fixing the benefit at a certain value ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} = b$), we obtain the subset $\mathcal{P}_{\mathrm{FBA,b}}$, which is an n-dimensional surface of $\mathcal{P}_{\mathrm{FBA}}$. from this set, we can pick the flux distributions that minimise the sum of fluxes $|\mathbf{v}|_1$. Alternatively, we can minimise a weighted sum of fluxes or more general flux cost functions [3].

Metabolic economics The reaction balance Eq. (1) must hold for all kinetic models with in enzyme-optimal states [3]. It guarantees that the flux distribution is not only beneficial (i.e. provides a positive net benefit), but that all active enzymes contribute to the benefit (at least in one possible kinetic model). In the theory of metabolic economics, the return function $g(\mathbf{u}) = z(\mathbf{v}(\mathbf{u}), \mathbf{c}(\mathbf{u}))$ scores the stationary fluxes $\mathbf{v}$ and concentrations $\mathbf{c}$, while the enzyme levels u_l are penalised by an enzyme investment function $h(\mathbf{u})$. By choosing the enzyme profile with the maximal fitness $f(\mathbf{u}) - g(\mathbf{u}) - h(\mathbf{u})$, we obtain an enzyme-balanced state. In this state, we define

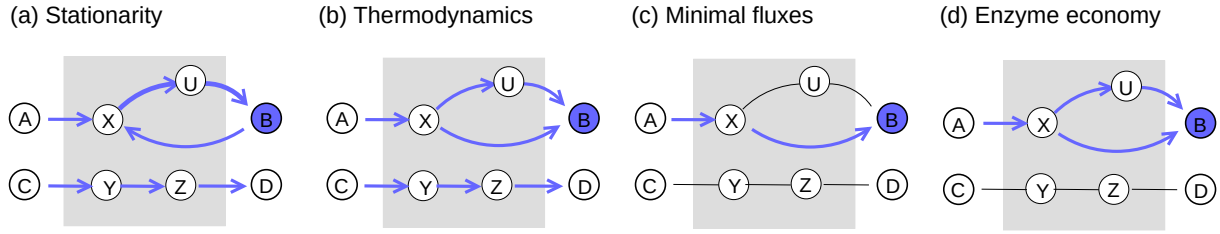

Figure S1: Flux prediction based on stationarity, thermodynamics, and economic constraints. (a) Example network (internal metabolites shown in box, fluxes shown as arrows). In the model, the production of B (marked in blue) is beneficial, the conversion from C to D is non-beneficial, and the rate of the reaction A $\to$ X is bounded. The non-productive cycle between X and B and the futile flux from C to D do not contribute any benefit. In an FBA solution, these fluxes are irrelevant and remain undetermined. (b) Thermodynamics excludes the flux cycle because it cannot be driven by chemical potential differences. (c) Flux minimisation excludes the longer pathway from X to B because of its higher flux costs, as well as the flux C $\to$ D. (d) The benefit principle used in EFA excludes both the cycle flux and the flux C $\to$ D because they are futile. Both pathway fluxes from X to B are feasible. A possible choice between them depends on assumptions about enzyme costs.

the flux gain vector $\mathbf{z}^{\mathrm{v}} = \partial z/\partial \mathbf{v}$ and the enzyme costs $y_l = \partial h/\partial \ln u_l$ (which are given by the enzyme prices $h_l^{\mathrm{u}} = \partial h/\partial u_l$, multiplied with the enzyme concentrations u_l). To satisfy the balance condition $\partial g/\partial u_l = \partial h/\partial u_l$, active reactions must have positive enzyme costs. After omitting all inactive reactions from the model, we obtain the flux gain condition

$$\mathbf{K}^\top \mathrm{Dg}(\mathbf{y})\, \mathbf{v}^{-\mathbf{1}} \quad = \quad \mathbf{K}^\top \mathbf{z}^{\mathrm{v}}, \tag{S8}$$

where $\mathbf{K}$ is a right-kernel matrix of $\mathbf{N}$ satisfying $\mathbf{N}\,\mathbf{K} = 0$. Flux distributions satisfying condition (S8) are called *economical* and will satisfy the reaction balance (1). In a basic version of metabolic economics, all reactions must be catalysed by specific enzymes; multifunctional enzymes and non-enzymatic reactions can be included, but require some changes in the formulae [3]. An economical flux distribution can be realised by a variety of kinetic models with different combinations of rate constants, metabolic returns, and investment functions [3]. To construct such models or to obtain a complete, consistent set of rate constants, further relations between enzyme costs and rate laws have to be taken into account. Uneconomical flux distributions cannot appear in kinetic models with optimal enzyme levels and may therefore be discarded in flux analysis. Flux distributions obtained by FCM are free of futile modes (flux cost minimisation theorem in [3]), and therefore economical.

Comparison of flux analysis methods Cells can realise a variety of metabolic flux distributions with different product yields, consumption of cofactors, kinetic properties, or enzyme prices. An aim of flux analysis is to predict physically possible flux distributions. Different methods use different assumptions and data to delimit feasible fluxes or to pick one flux distribution. Figure S1 in the article compares economic flux analysis to other flux analysis methods. FBA requires a maximal yield, so flux cycles are excluded if they reduce the fitness by consuming metabolic resources such as ATP. Fluxes that have no effect on the yield – like the non-productive cycle between X and B or the flux from C to D – remain undetermined and can only be suppressed by ad hoc constraints. However, constraints can also be derived from general principles. Thermodynamic constraints, for instance, exclude the flux cycle because it does not dissipate any Gibbs free energy; the flux from C to D still remains undetermined because it possibly does. Economic constraints discard all fluxes that do not contribute to the benefit – as expressed by the reaction balance.

In contrast to FCM, where uneconomical fluxes simply disappear during optimisation, economic flux analysis forbids them explicitly via the economic constraints. These constraints can also be used in flux sampling [4] to implement enzyme optimality without actually running any numerical optimisation. In Figure S1, both fluxes between X and B – as well as positive linear combinations of them – are allowed; specific flux distributions could be chosen by additional heuristics. The principle of minimal fluxes, in contrast, will always pick shorter pathways; flux cost minimisation could prefer any of the pathways or a convex mixture, depending on the flux cost weights chosen. Thus, in flux cost minimisation we make stronger assumptions than economic flux analysis and use

additional information. Instead of feasible sign patterns, from which we can sample economical flux distributions, we obtain a single flux distribution, which depends on the flux cost weights chosen.

S2 Economic and thermodynamic constraints

S2.1 Analogy to electric circuits and thermodynamics

Electric circuits, chemical thermodynamics, and metabolic economics follow similar mathematical laws [10, 11]. Electrical circuits are governed by Kirchhoff's rules: (i) the node rule for currents (stationarity condition for circuits, guaranteeing charge conservation); and (ii) the loop rule for voltages (a Wegscheider condition for voltage differences, caused by the fact that voltages are differences of electric potentials). Furthermore, (iii) all currents must run from higher to lower electric potentials. Analogous rules apply to biochemical systems: there is (i) a stationarity condition for fluxes, (ii) the fact that thermodynamic forces $-\Delta\mu_l$, or indirect flux demands Δw_l, are derived from potentials and therefore satisfy loop rules, and (iii) a sign condition between fluxes and thermodynamic forces. The analogy between electricity and reaction thermodynamics is not surprising because electric and chemical potentials can be joined to a single electrochemical potential. The analogy to metabolic economics, is only formal, and striking.

Potentials Common to all three theories is that they are based on variational principles [9] and that potentials play an important role. Potentials are common in reaction thermodynamics [12], electricity, and even kinetic rate laws [13]. For instance, that fact that the electrostatic field is the gradient of an electric potential guarantees that this field is free of rotation and that the voltages in circuits satisfy the loop rule. Electric currents cannot flow in a circle unless there is some voltage source (battery, capacitor, or dynamic magnetic field). Similarly, the existence of economic potentials excludes futile cycles in flux distributions unless they are justified by direct flux gains. Accordingly, the power P consumed in electric circuits, the entropy production density σ in chemical reactions, and the cost y_l (or benefit) of enzymatic reactions are given by similar formulae

$$\begin{aligned} P &= I\,\Delta\varphi_{\mathrm{E}}^{\mathrm{tot}} \\ \sigma_l &= v_l\,[-\Delta\mu_l/T] \\ y_l &= v_l\,[\hat{z}_l^{\mathrm{v}} + \Delta w_l]. \end{aligned} \tag{S9}$$

In all three cases, a flux (electric current I or metabolic flux $\mathbf{v}$) is multiplied by a force derived from potentials (electric potentials $\varphi_{\mathrm{E}}^{\mathrm{tot}}$, chemical potentials μ_i, or economic potentials w_i).

Just like economic potentials refer to production gains, the economic loads refer to concentration gains. The compound balance equation shows that economic loads cause a jump between flux demands along a metabolic pathway, just like an electric point charge would cause a jump in the electric field. When comparing economics and electricity, the linear combination of flux demands or enzyme costs in the compound balance (see [3]) corresponds to the sum of outgoing electric currents, while the economic load corresponds to the electric charge at the node.

Gauge freedom As another analogy to electrodynamics, there is gauge freedom in choosing the economic potentials and loads. For instance, if ATP and ADP form a conserved moiety, increasing their economic potentials by the same number has no effect. For a given kinetic model, these quantities are uniquely defined. However, if we just aim at solving the balance equations, we can pick conserved moiety vectors ${\mathbf{g}_{\mathrm{w}}}^{\top}$ and ${\mathbf{g}_{\mathrm{q}}}^{\top}$ (left-kernel vectors of $\mathbf{N}$, satisfying ${\mathbf{g}_{\mathrm{w}}}^{\top}\mathbf{N} = {\mathbf{g}_{\mathrm{q}}}^{\top}\mathbf{N} = 0$) and replace the internal economic potential vector $\mathbf{w}^{\mathrm{c}}$ by $\mathbf{w}^{\mathrm{c}\prime} = \mathbf{w}^{\mathrm{c}} + \mathbf{g}_{\mathrm{w}}$ and the internal economic load vector $\mathbf{p}^{\mathrm{c}}$ by $\mathbf{p}^{\mathrm{c}\prime} = \mathbf{p}^{\mathrm{c}} + \mathbf{g}_{\mathrm{q}}$. This will change the potentials within conserved moieties, but without affecting the balances Δw_l. In networks without moiety conservation, there are no conserved moiety vectors, and no gauge freedom. However, all results of metabolic economics will remain unchanged if the functions $g(\mathbf{u})$ or $h(\mathbf{u})$ are shifted by constant values.

Differences between the theories We also note some differences between the theories:

1. In near-equilibrium thermodynamics and in Ohm's law for electric currents, the flux is proportional to the force. In metabolic economics, there is no such linear relationship.

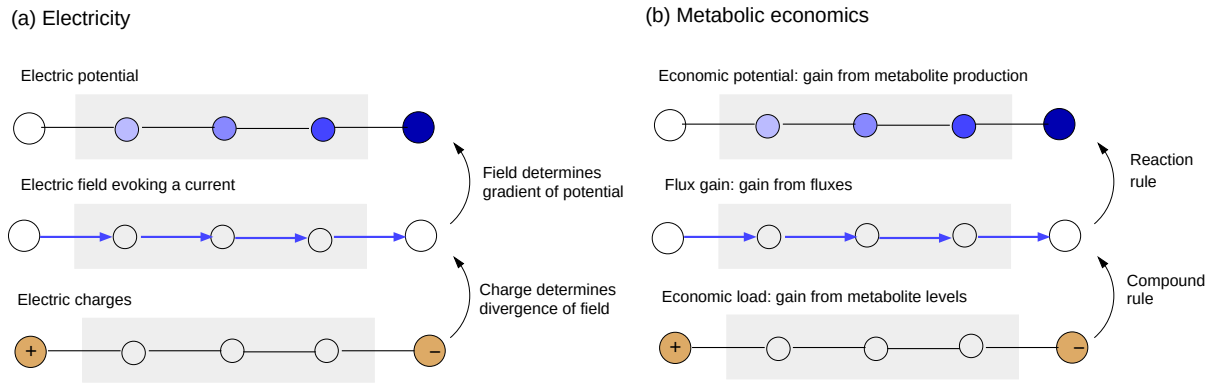

Figure S2: Analogy between metabolic economics and electric circuits. The economic loads, flux demands, and potentials can be compared to electric charges, fields, and potentials. (a) In electrostatics, charges are the sources (divergence terms) of fields, and fields represent the gradients of electric potential. (b) In metabolic economics, the corresponding relations are the compound balance (between loads and flux demands) and the reaction balance (between flux demands and potentials).

2. In metabolic economics, there is a direct flux demand $\hat{z}_l^{\mathrm{v}}$, with no counterpart in thermodynamics. In fact, this term is required to make the theory consistent. Remember that both $\mathbf{w}^{\mathrm{x}}$ and $\hat{\mathbf{z}}^{\mathrm{v}}$ stem from a (partially arbitrary) splitting of $\mathbf{z}^{\mathrm{v}}$ into $\mathbf{N}^{\mathrm{x}\top}\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}$. In a consistent theory, the formula for flux demands must be invariant against changes of this splitting, and this can only hold if the direct flux gain appears in this formula. Analogous terms in thermodynamics could account for extra forces, e.g. a proton gradients that drives ATP production in a model in which protons are not described as a molecule species.

S2.2 Analogies and differences between economic and thermodynamic constraints

Economic analysis resembles thermodynamic flux analysis Like many flux analysis methods [5, 2, 6, 7, 8, 9], economic flux analysis includes the weak thermodynamic constraint, ensuring that that every reaction can, at least potentially, dissipate Gibbs free energy (see SI S1). In a well-mixed chemical solution, each metabolite has a chemical potential $\mu_i = \partial G/\partial n_i$ describing its contribution to the system's Gibbs free energy G (in kJ/mol; n_i is the metabolite's mole number). According to the second law of thermodynamics, active reactions must dissipate Gibbs free energy, and to ensure this, non-zero fluxes v_l must run from higher to lower chemical potentials, that is, in the direction of the thermodynamic force $\Theta_l = -\Delta\mu_l/RT$. We obtain the weak sign constraint

$$v_l \neq 0 \quad \Rightarrow \quad \mathrm{sign}(v_l) = \mathrm{sign}(-\Delta\mu_l). \tag{S10}$$

Given a flux distribution $\mathbf{v}$, can this constraint be satisfied by some chemical potential vector $\boldsymbol{\mu}$? There is a simple criterion: if a flux distribution $\mathbf{v}$ and some non-productive test mode share all flux directions on their entire shared active region, the shared reactions, and therefore the flux distribution $\mathbf{v}$, are thermodynamically infeasible. The thermodynamic constraint (S10) resembles the economic constraint (2), allowing us to use the same mathematical methods (see Table S1). In both cases, fluxes may vanish no matter if $\Delta\mu$ or $\Delta w + z$ vanish or not. While the thermodynamic constraints apply to non-stationary states, the economic constraints are justified for stationarity states only. Thermodynamic and economic constraints should be used in combination. By studying how both constraints interact, we can see some of the difficult regulation problems that cells have to solve.

Analogies between thermodynamic and economic constraints The economic condition Eq. (2) and the thermodynamic condition Eq. (S10) are formally very similar: the flux directions are in both cases determined by potential differences[1]. Flux distributions that violate these conditions are thermodynamically infeasible or

[1]The economic constraints apply to active reactions only. They resemble the weak thermodynamic constraints, in which reaction rates can vanish despite a thermodynamic force (see S1). In the economic constraint, each enzyme species has a positive minimal

Thermodynamics		
Objective function	Dissipation of GFE:	$-\Delta\boldsymbol{\mu}^\top \mathbf{v}$
Potential	Chemical potential:	$\mu_i = \frac{\partial G}{\partial n_i} = \mu_i^{(0)} + RT \ln c_i$
Classification of flux distributions	Dissipative:	$-\Delta\boldsymbol{\mu} \cdot \mathbf{v} > 0$
	*Non-dissipative:	$-\Delta\boldsymbol{\mu} \cdot \mathbf{v} = 0$
	*Anti-dissipative:	$-\Delta\boldsymbol{\mu} \cdot \mathbf{v} < 0$
Matrix condition	Non-productive mode:	$\mathbf{N}^{\mathrm{tot}} \mathbf{v} = \binom{\mathbf{N}^{\mathrm{x}}}{\mathbf{N}} \mathbf{v} = 0$
Local sign constraint	Locally dissipative	$v_l \neq 0 \Rightarrow -\Delta\mu_l \, v_l > 0$

Enzyme economy		
Objective function	Flux gain:	$\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} = (\mathbf{N}^{\mathrm{x}\top} \mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}})^\top \mathbf{v}$
Potential	Economic potential:	$w_j^{\mathrm{x}} = \mathbf{z}^{\mathrm{x}}$; $w_i^{\mathrm{c}} = \frac{\partial g}{\partial \Phi_i}$
Classification of flux distributions	Beneficial:	$\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} > 0$
	*Non-beneficial:	$\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} = 0$
	*Costly:	$\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} < 0$
Matrix condition	Non-beneficial mode:	$\mathbf{N}^{\mathrm{z}} \mathbf{v} = \binom{\mathbf{z}^{\mathrm{v}\top}}{\mathbf{N}} \mathbf{v} = 0$
Local sign constraint	Economical	$v_l \neq 0 \Rightarrow [\hat{z}_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}}] \, v_l > 0$

Table S1: Thermodynamic and economic flux analysis. The table lists definitions and formulae from both theories. In terms of net metabolite production, modes can be productive or non-productive. Non-productive modes dissipate no Gibbs free energy, and in models with a production objective, they will be non-beneficial. Non-dissipative and anti-dissipative flux distributions are thermodynamically infeasible. In economic analysis, globally feasible modes are called economical and globally infeasible modes are called futile. Conditions for infeasible fluxes are marked by a star *. The symbol n_i denotes the mole number of substance i. GFE stands for Gibbs free energy.

uneconomic and may be excluded. In both cases, cycle fluxes would imply that the potentials increase in a circle, which is impossible. Such impossible cycles in a flux distribution can be detected by comparing a flux distribution to the infeasible test modes (elementary flux cycles in the case of thermodynamics, or elementary non-beneficial test modes in the case of economics). In both cases, the flux distribution $\mathbf{v}$ must be sign-orthogonal on all elementary test modes on $\mathbf{v}$'s own active region; this criterion reflects the weak thermodynamic sign constraint, and accordingly, the weak constraint stated by the reaction balance (fluxes require flux demands, but flux demands do not necessarily lead to fluxes). Further analogies between thermodynamic and economic constraints are listed in Table S1.

Since the two constraints are so similar, is it necessary to use both of them? In fact, there is a number of reasons:

1. **Different assumptions** Economic and thermodynamic constraints represent different and independent assumptions about cells. The thermodynamic constraint Eq. (S10) follows from physical laws and holds for all chemical reactions, both in test tubes and in living cells. Exceptions can occur due to simplifying model assumptions, for instance if cofactors are not mentioned in the model. The economic constraint, in contrast, only applies if enzyme levels – in cells or technical systems – are assumed to be optimal.

2. **Chemical and economic potentials** The economic potentials are not molecule properties, but effective gains from metabolites in the state of the cell in question. They are defined by systemic sensitivities, and are therefore state variables. The same holds, in principle, for chemical potentials. However, the chemical potentials can also be directly related to metabolite concentrations. For economic potentials, this is not the case.

cost per molecule. For a non-zero flux, the flux demand must exceed some positive value. Therefore, even a positive flux demand may still be too small to justify a non-zero flux.

3. **Independent choices of external potentials** The chemical potentials μ_i depend on metabolite levels and on Gibbs free energies of formation. The economic potentials w_i, in contrast, arise from the flux gain vector, so their numerical values carry different kinds of information. For instance, if biomass production is the metabolic objective, we can assign an economic potential of 1 to biomass, and vanishing potentials to all extracellular metabolites. The chemical potentials, in contrast, depend on metabolite concentrations and on standard Gibbs free energies. Since the chemical and economic potentials differ, they constrain the fluxes (and potentials of internal metabolites) in different ways.

4. **Dependence on stationarity** The thermodynamic constraint holds for any biochemical reactions, whereas the metabolic economics – and therefore the reaction balance – is restricted to steady states.

5. **How local constraints arise** The fact that reactions must dissipate Gibbs free energy is a local condition which holds for every reaction. The reason is that the system's Gibbs free energy can be split into Gibbs free energies of the metabolites, which directly depend on the metabolite concentrations. The economic constraint, in contrast, represents the systemic influence of enzymes on the metabolic objective in steady state. This influence is mediated by various pathways, involves changes of the global steady state, and is therefore non-local. Only by using a trick – rewriting the control coefficients in terms of economic potentials – we can represnt the constraint as a local balance condition.

6. **Sign-orthogonality** To be able to dissipate Gibbs free energy in every reaction, flux distributions must not contain any flux cycles, i.e., they must be sign-orthogonal on all non-productive modes (which satisfy $\mathbf{N}^{\mathrm{tot}}\,\mathbf{v} = 0$). The criterion for economical flux distributions is very similar: they must not contain any non-beneficial modes, i.e., they must be sign-orthogonal on all non-beneficial flux distributions (satisfying $\mathbf{N}^{\mathrm{z}}\,\mathbf{v} = 0$).

7. **Direct flux demand** In reactions with a direct flux benefit, the reaction balance contains a term $\hat{z}_l^{\mathrm{v}}$, which has no analogon in the thermodynamic constraint. However, this is not a fundamental difference. On the one hand, models in which cofactors are missing may require such terms even in the thermodynamic constraint (S10). On the other hand, direct flux demands can be omitted from model and be represented by the economic potentials of virtual substrates.

S2.3 Combining economic and thermodynamic constraints

If the metabolic fluxes must satisfy thermodynamic and economic constraints together, there will be dependencies between chemical and economic potentials. Since fluxes run towards smaller chemical and larger economic potentials, economic potentials and chemical potentials should be anti-correlated. In particular, the potentials must satisfy the condition $v_l \neq 0 \Rightarrow (\hat{z}_l^{\mathrm{v}} + \Delta w_l) \cdot \mu_l \leq 0$. We can see this, for instance, in Figure 4 in the article.

In some models, thermodynamic and economic constraints entail each other directly. In models with a production objective ($\hat{\mathbf{z}}^{\mathrm{v}} = 0$), economical flux distributions are free of non-productive flux cycles[2]. Thus, by eliminating all futile modes – for instance, by applying flux cost minimisation – we automatically obtain flux distributions that are economically and thermodynamically feasible. The opposite statement – thermodynamically feasible modes are economical – holds only under some circumstances (proof in SI S2.4): again, our model must has a production objective; the network structure must allow for beneficial flux distributions; and the external production rates must show fixed ratios in all steady states (this holds, for instance, if a model has no more than two external metabolites; or if a network produces biomass from extracellular substrates that need to be used in fixed stoichiometries). In this case – and if there are no further constraints on the chemical or economic potentials – thermodynamics and economics will constrain the fluxes in exactly the same way and will exclude the same kinds of cycles.

[2]Proof: In such systems, any non-productive flux distribution $\mathbf{k}$ (satisfying $\mathbf{N}^{\mathrm{tot}}\,\mathbf{k} = 0$) is also non-beneficial (satisfying $\mathbf{N}^{\mathrm{z}}\,\mathbf{k} = 0$). Thus, a flux distribution containing a flux cycle will also contain a futile mode. In contrast, an economical flux distribution – a flux distribution without futile modes – must be free of flux cycles.

S2.4 Economic and thermodynamic constraints can be equivalent

Proposition Consider a model with the following properties: the objective depends on net production; the network structure allows for beneficial flux distributions; and for stoichiometric reasonse, the external production rates show the same ratios in all steady states. If there are no further constraints on the chemical or economic potentials, thermodynamics and metabolic economics constrain the fluxes in exactly the same ways and exclude the same kinds of cycles (see section S2).

Remark The condition that external production rates show the same ratios in all steady states can be written as $\mathrm{rank}(\mathbf{N}^{\mathrm{x}}\,\mathbf{K}) = 1$. It will be satisfied, for instance, if a model produces one main product (e.g., a pathway product for a pathway model; or biomass in a cell model), and produces it from a fixed composition of substrates, and with a fixed composition of side products (e.g., CO2).

Proof The relation between economic and thermodynamic flux distributions is shown in four steps:

1. If a production objective is assumed ($z(\mathbf{v}) = \mathbf{w}^{\mathrm{x}\top}\,\mathbf{N}^{\mathrm{x}}\,\mathbf{v}$), non-productive flux distributions are non-beneficial.
   Proof: Any non-productive flux distribution $\mathbf{v}$ (satisfying $\mathbf{N}^{\mathrm{tot}}\,\mathbf{v} = \binom{\mathbf{N}^{\mathrm{x}}}{\mathbf{N}}\,\mathbf{v} = 0$) satisfies $\mathbf{N}^{\mathrm{z}}\,\mathbf{v} = \binom{\mathbf{w}^{\mathrm{x}\top}\mathbf{N}^{\mathrm{x}}}{\mathbf{N}}\,\mathbf{v} = 0$ and is therefore non-beneficial.

2. If a production objective is assumed, flux distributions that contain flux cycles are not economical.
   Proof: If for a flux distribution $\mathbf{v}$, there are no μ_i that satisfy the sign condition Eq. (S10), $\mathbf{v}$ contains a flux cycle defined by an non-productive flux mode $\mathbf{k}$. This cycle, as a test mode, is economically non-beneficial, and thus defines a futile mode. Therefore, $\mathbf{v}$ is not economical.

3. If a production objective is assumed and the network structure is such that external production and consumption rates show fixed proportionalities in all steady states, then all non-beneficial modes are non-productive.
   Proof: We assume two things: (i) $\mathrm{rank}(\mathbf{N}^{\mathrm{x}}) = 1$, i.e., the external production and consumption rates come in fixed ratios across all steady states; (ii) for the given flux gain vector (which defines the external economic potentials), there exists at least one beneficial flux distribution. Then, a zero benefit can only be realised by vanishing production rates: in such systems, the equation $\mathbf{w}^{\mathrm{x}\top}\mathbf{N}^{\mathrm{x}}\,\mathbf{v} = 0$ implies $\mathbf{N}^{\mathrm{x}}\,\mathbf{v} = 0$ (non-beneficial), and accordingly, $\mathbf{N}^{\mathrm{z}}\,\mathbf{v} = 0$ implies $\mathbf{N}^{\mathrm{tot}}\,\mathbf{v} = 0$ (non-productive).

Under the assumptions made, we can therefore obtain all elementary non-beneficial modes by enumerating the elementary flux cycles. As a consequence, all test modes defining non-beneficial modes are non-productive; thus, if a flux distribution contains non-beneficial modes, it is also thermodynamically infeasible.

S2.5 Economic and thermodoynamic constraints derived from flux cost minimisation

Thermodynamic and economic constraints are not only similar, but can be derived from one common principle, general flux cost minimisation. As a first observation pointing in this direction, the thermodynamic feasibility constraints can be expressed as economic constraints for a specifically chosen objective function.

With entropy production as metabolic objective, economic and thermodynamic constraints are identical. Can we construct a metabolic objective that makes the chemical and economic potentials (and therefore, the economic and thermodynamic constraints) coincide? Rayleigh's dissipation function $T\,\sigma = -\sum_l v_l\,\Delta\mu_l$ is such an objective. It describes the heat production in chemical systems (where σ is the entropy production per volume). This function, used as the objective, can be written as $z(\mathbf{v}) = -\boldsymbol{\mu}_{\mathrm{ext}}^{\top}\,\mathbf{N}^{\mathrm{x}}\,\mathbf{v}$. With the flux gain vector $\mathbf{z}^{\mathrm{v}} = -\mathbf{N}^{\mathrm{x}\top}\,\boldsymbol{\mu}_{\mathrm{ext}}$, the external economic potentials are given by the negative chemical potentials, and there are no direct flux demands. To solve the resulting reaction balance equation, we can equate the internal economic potentials to the negative chemical potentials; the enzyme costs y_l will be given by Rayleigh's dissipation function $T\,\sigma_l = -v_l\,\Delta\mu_l$ in each reaction. Thus, if we assume a maximal dissipation of Gibbs free energy as an objective, the economic and thermodynamic constraints become identical.

Economic and thermodynamic balances can both be derived from flux cost minimisation The thermodynamic and the economic constraints can both be derived from a common variational principle, general flux cost

minimisation. In both cases, we minimise a function of the fluxes (internal entropy production, or a flux cost or enzyme investment function) under a fixed linear target (external entropy production, or a linear metabolic objective) and under the assumption of stationarity. This shows clearly that the thermodynamic constraints are a special case of the economic ones, with entropy production replacing the fitness function.

1. **Thermodynamic constraints** Consider metabolites with concentrations c_i in a volume Ω. The entropy production density (permutation entropy change per time and volume) reads[3] $R(\ln(\mathbf{c}/x))^\top \mathbf{N}\,\mathbf{v}$ where $x = 1/(\Omega\, N_A)$. We assume fixed concentrations c_i and use this as a flux cost function. According to Prigogine's principle of minimal entropy production in steady states, we minimise this function at a fixed external entropy production $\mu_{\text{ext}}^\top \mathbf{N}^{\text{x}}\,\mathbf{v}$ and assuming stationarity $\mathbf{N}\,\mathbf{v} = 0$. This yields the optimality condition

$$\min \stackrel{!}{=} RT(\ln(\mathbf{c}/x))^\top \mathbf{N}\,\mathbf{v} - \mu_{\text{ext}}^\top \mathbf{N}^{\text{x}}\,\mathbf{v} - \mu_{\text{int}}^\top \mathbf{N}\,\mathbf{v} \tag{S11}$$

where T and the elements of μ_{int} are Lagrange multipliers. By taking the derivative with respect to v_l, setting it to zero, and taking again the scalar product with $\mathbf{v}$, we obtain

$$\underbrace{RT(\ln(\mathbf{c}/x))^\top \mathbf{N}\,\mathbf{v}}_{\sigma\, T} = \underbrace{\mu_{\text{ext}}^\top \mathbf{N}^{\text{x}}\,\mathbf{v} + \mu_{\text{int}}^\top \mathbf{N}\,\mathbf{v}}_{\Delta\mu\cdot\mathbf{v}} \tag{S12}$$

We can interpret μ as the vector of chemical potentials and T as the absolute temperature, and the entropy production density is given by $\sigma = \frac{\Delta\mu\cdot\mathbf{v}}{T}$.

2. **Economic constraints** We consider a flux cost function $\bar{H}(\mathbf{v})$ whose slopes $\partial\bar{H}_l^{\text{v}}/\partial v_l$ must have the same signs as the fluxes v_l. If we minimise this cost, at a given metabolic performance $\mathbf{z}^{\text{v}}\cdot\mathbf{v} = b$ and under the stationarity condition $\mathbf{N}\,\mathbf{v} =$, we obtain the optimality condition

$$0 = \mathcal{L}\,\frac{\partial\bar{H}_l^{\text{v}}}{\partial v_l} - z_l^{\text{v}} - \sum_i w_i^{\text{c}} n_{il} \tag{S13}$$

with Lagrange multipliers $\mathcal{L}$ and w_i^{c}, and thus

$$\underbrace{\mathcal{L}\,\frac{\partial\bar{H}_l^{\text{v}}}{\partial v_l} v_l}_{y_l} = \left[z_l^{\text{v}} + \sum_i w_i^{\text{c}} n_{il}\right] v_l. \tag{S14}$$

where y_l is positive. The flux cost y_l in this formula represents a scaled derivative of the flux cost function with respect to fluxes. However, we can also re-interpret it: we assume that each reaction rate is given by $v_l = u_l\, r_l(\mathbf{c})$ and that the enzyme levels u_l are scored by an investment function $h(\mathbf{u})$. For fixed metabolite concentrations, we define the flux cost function by the corresponding enzyme investment: $\bar{H}(\mathbf{v}) = h(\mathbf{u}(\mathbf{v})) = h(\mathbf{v}/r(\mathbf{c}))$. Therefore, we can replace

$$\frac{\partial\bar{H}_l^{\text{v}}}{\partial v_l} v_l = \frac{\partial h}{\partial u_l}\frac{1}{r_l(\mathbf{c})}\, v_l = \frac{\partial h}{\partial u_l} u_l. \tag{S15}$$

Costs and benefits in flux analysis represent marginal quantities It may seem obvious that flux costs in FCM and enzyme costs in EFA are equivalent. However, enzyme costs and flux costs in the two methods represent very different assumptions. Enzyme levels and stationary fluxes in kinetic models are not proportional and the relation between them is complex. For instance, non-uniform enzyme changes along a pathway will cause a uniform stationary flux change. Therefore, it is not obvious how enzyme costs could be translated into flux costs. However, as shown in [3], flux costs and enzyme investments can be related to each other if we

[3] The permutation entropy, for particle number n, is given by $S = k\,\ln n! \approx k\,n((\ln n) - 1)$. With $n = c\,\Omega\,N_A$ (with volume Ω and Avogadro's constant N_A, we obtain $S \approx R\,c\,\Omega(\ln(c\,\Omega\,N_A) - 1)$. The permutation entropy for metabolite i thus reads $S_i = R\,c_i[(\ln c_i/x) - 1]$ where $x = 1/(\Omega\,N_A)$. The permutation entropy production (per volume and time) reads therefore $\sigma = \partial_t \sum_i S_i = \sum_i R\,c_i\,(\ln(c_i\,\Omega\,N_A) - 1) = R\sum_i \dot{c}_i\,(\ln(c_i\,\Omega\,N_A) - 1) + c_i\frac{1}{c_i\,\Omega\,N_A}\Omega\,N_A\dot{c}_i = R\sum_i \dot{c}_i(\ln(c_i\,\Omega\,N_A)) = R\sum_{il}(\ln(c_i\,\Omega\,N_A))n_{il}v_l$.

treat the same model by FCM and metabolic economics and choose the cost functions such that the same flux distribution is optimal in both cases. Then, the flux cost weights $\bar{H}_l^{\mathrm{v}} = |\partial \mathbf{h}^{\mathrm{v}}/\partial v_l|$ in FCM and the specific flux costs $h_l^{\mathrm{v}} = h_l^{\mathrm{u}}/\frac{v_l}{u_l}$ in metabolic economics corresponds to each other, differing only by a scaling factor. In general, given a kinetic metabolic model in an enzyme-optimal state, we can reconstruct this state by FCM, using a flux cost function $\sum_l \bar{H}_l^{\mathrm{v}}|v_l| = \gamma \sum_l h_l^{\mathrm{u}}\, u_l$ with some constant scaling factor γ. In the kinetic model, h_l^{u} may depend on the state, but if h_l^{u} is constant (i.e., $h(\mathbf{u})$ rises linearly with each enzyme level u_l), flux costs and enzyme costs are equivalent. The comparison also shows that the flux benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$ in FBA does not correspond to the absolute objective z in a kinetic model, but to the sum of logarithmic derivatives $\sum_l \frac{\partial z}{\partial \ln |v_l|} = \sum_l \frac{\partial z}{\partial v_l}\, v_l$. Maybe surprisingly, it is a marginal quantity. However,for linear objective functions $z(\mathbf{v}) = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$ both functions are identical. Similarly, flux costs $\sum_l \bar{H}_l^{\mathrm{v}}\, v_l$ correspond to the total enzyme cost $\sum_l h_l^{\mathrm{u}}\, u_l = \sum_l \frac{\partial h}{\partial \ln u_l}$ (another marginal quantity), not to the enzyme investment function $h(\mathbf{u})$. This interpretation of flux costs implies positive scaled derivatives $(\partial a/\partial v_l)\, v_l$, which implies a minimum at $v_l = 0$. With a positive lower bound on $\partial a/\partial v_l$, there must be a kink at $v_l = 0$, which excludes, for instance, a sum of quadratic fluxes as a flux cost function.

S2.6 Economic flux analysis and economic theory

Labour value theories The economic potentials can be compared to the prices of commodities. In EFA, enzyme prices are "embodied" in the economic potentials of metabolites. In this respect, EFA resembles the labour theories of value in economics, which relate the values of goods to the human labour needed for their production. The concept has been suggested by Aristotle and was put forward by David Ricardo[4]. In Marxian economics, labour is expressed in units of time, and the "natural price" of a commodity is defined as the socially necessary labour time invested into its production [15]. When a product is manufactured, its value gradually increases by the amounts of work put into it. If the workers' productivity is increased by the use of machines, the investment in machines needs to be accounted for as well. In any case, labour time is the common conversion unit between materials, commodities, and work. In the analogy between economics and metabolism, the commodities correspond to metabolites, labour time to enzyme cost, and labour values to economic potentials. Importantly, the enzyme costs do not correspond to labour values in a strict sense (the average time investments per worker), but to a marginal form of labour values (the extra time investment that would be needed to increase the production). Like their counterparts in economics, the economic potentials play a double role: as labour values, they embody the enzyme costs that arise when continuously producing the metabolite, and as values in use, they represent the metabolites' marginal contribution to the metabolic objective.

Calculation of economic potentials But why can we assume that supply and demand, or labour values and values in use, will be balanced? In planned economies, this is exactly a problem: prices of commodities and materials have to be determined, ideally in a way that later supply and demand will match. This can be difficult and requires input-output analyses of production and consumption in the entire economy. The methods are based on linear algebra and resemble those that we use for computing economic potentials in EFA. Since the economic potentials are not easily measurable, we need to determine reasonable values from models by solving the reaction balance. In economic flux analysis, the enzyme demands and labours have to be equal for a simple reason: mismatches would indicate a non-optimal usage of enzymes and therefore contradict our optimality postulate. Of course, real cells and real economies will not work optimally – there is always some waste of material, energy, or effort. Nevertheless, economic considerations may help, in both cases, to see how demands (for commodities or for biomass components) and mechanisms (of industrial production or biosynthesis) lead to stationary or dynamic behaviour.

S3 How economics shapes metabolism

S3.1 Under which conditions should enzymes or pathways be used?

[4]Ricardo defines in [14] (Chapter 1, Section 1.1): "The value of a commodity, or the quantity of any other commodity for which it will exchange, depends on the relative quantity of labour which is necessary for its production, and not on the greater or less compensation which is paid for that labour."

The critical flux demand The choice between enzymes or pathways depends on their flux prices, i.e., the enzyme costs per flux. Each enzyme molecule has a minimal cost and a maximal activity, and the ratio between the two defines a minimal enzyme price for this specific enzyme. A usage of the enzyme can only be economical if the flux demand in the reaction exceeds this minimal price. We can see this as follows. If we divide the reaction balance (1) by the flux v_l, we obtain the equality

$$\hat{z}_l^{\mathrm{v}} + \Delta w_l = \frac{h_l^{\mathrm{u}}}{v_l/u_l} \tag{S16}$$

between flux demand and flux cost. The latter depends on the enzyme cost h_l^{u} and on the catalytic efficiency v_l/u_l of the enzyme. Assume that the flux, and thus the ration on the right, is positive. On the one hand, h_l^{u} must exceed $h_l^{\mathrm{u}\min}$, the cost of this enzyme at small expression levels; on the other, the catalytic efficiency v_l/u_l most be below the turnover number $k^{\mathrm{cat}} = (v_l/u_l)^{\max}$ for this flux direction. Therefore, the ratio must be larger than $h_{l\,\min}^{\mathrm{v}} = h_l^{\mathrm{u}\min}/k^{\mathrm{cat}}$, a minimal flux price, which sets a lower bound on the flux demand.

This argument can also be extended from reactions to pathways. For simplicity, we assume a linear pathway with a production objective and (enzyme-dependent) minimal enzyme costs $h_l^{\mathrm{u}\min}$. We sum over all the reaction balances and obtain

$$\Delta w_{\mathrm{pathway}} = \sum \frac{h_l^{\mathrm{u}}\, u_l}{v_l} \geq \sum_l \frac{h_l^{\mathrm{u}\min}}{k_l^{\mathrm{cat}+}}. \tag{S17}$$

The minimal enzyme price on the right can be estimated from the enzyme properties $h_l^{\mathrm{u}\min}$ and $k_l^{\mathrm{cat}+}$. If the flux demand $\Delta w_{\mathrm{pathway}}$ is below this bound, the pathway flux is not economical. However, this is not a tight bound: even higher demands may still not suffice for a flux.

For calculations of Δw_l, estimates of u_l/v_l can be obtained from experimental proteome and flux data, while marginal flux costs can be estimated from enzyme chain lengths, catalytic constants, and life times [16] and from measured enzyme prices (see SI S5.5) [17]. Of course, k^{cat} is only a rough approximation of v_l/u_l; for tighter bounds, one may also consider the thermodynamic forces [18]. Furthermore, upper bounds for flux demands can be obtained from estimates on maximal enzyme prices and minimal enzyme-specific rates.

Choices between isoenzymes or alternative pathways Isoenzymes, which catalyse the same reaction, also share the same flux demand $\mathbf{g}^{\mathrm{v}} = \Delta\mathbf{w} + \mathbf{z}^{\mathrm{v}}$. By considering reaction balances for both reactions and taking the ratio, we obtain

$$\frac{(\Delta w + z_{\mathrm{v}})\, v_1}{(\Delta w + z_{\mathrm{v}})\, v_2} = \frac{h_{u_1}\, u_1}{h_2^{\mathrm{u}}\, u_2} \quad \Rightarrow \quad \frac{h_1^{\mathrm{u}}\, u_1}{v_1} = \frac{h_2^{\mathrm{u}}\, u_2}{v_2} \quad \Rightarrow \quad h^{\mathrm{v}}{}_1 = h^{\mathrm{v}}{}_2. \tag{S18}$$

Thus, isoenzymes can only be active together (in an optimal state) if the flux prices (i.e., enzyme costs per flux) are equal. If we consider a kinetic model and optimise the enzyme levels, it is unlikely Eq. (S18) will be exactly satisfied. The theory predicts that only one the enzymes, the cheaper one, is expressed.

Again, a similar argument holds for entire pathways: if two pathways have the same net flux stoichiometries (and a single stationary flux distribution each), they are called *isopathways*. If the metabolic objective function does not score any concentrations or fluxes inside the pathways, both isopathways will be scored by the same flux demand; to be active at the same time, they would have to satisfy

$$\frac{v_1}{v_2} = \frac{\sum_{l\in 1} h_l^{\mathrm{u}}\, u_l}{\sum_{l\in 2} h_l^{\mathrm{u}}\, u_l} \quad \Rightarrow \quad \frac{v_1/u_1^{\mathrm{tot}}}{h_1^{\mathrm{u,tot}}} = \frac{v_2/u_2^{\mathrm{tot}}}{h_2^{\mathrm{u,tot}}} \quad \Rightarrow \quad h^{\mathrm{v}\mathrm{tot}}{}_1 = h^{\mathrm{v}\mathrm{tot}}{}_2 \tag{S19}$$

where u_L^{tot} is the total protein level in pathway L, and the ratio u_L^{tot}/v_L is the pathway enzyme gain per unit flux [19]. The fluxes v_L must be comparable between pathways, that is, pathways with equal fluxes $v_{L_1} = v_{L_2}$ must show the same net conversion. The apparent pathway cost $h_L^{\mathrm{u,tot}}$ is defined as above. Again, it is unlikely that two isopathways in a kinetic model will show exactly the same net flux costs, so one of them will be preferred.

Choice or combination of pathways? Cells may accomplish the same task (e.g. ATP production) by different pathways, either switching between them (see Figure S3) or using them in combination. For instance, bacteria often repress the uptake of other carbon sources as long as glucose is present, with some exceptions (*B. subtilis* uses glucose and malate simultaneously). If flux cost minimisation is used for prediction, it selects one of the pathways, and the choice depends on the flux costs weights. Only if the costs of both fluxes are identical, combinations of both are allowed. Economic flux analysis allows for combinations because any combination of pathway fluxes may be optimal in some kinetic model. EFA offers criteria for choosing between flux distributions, but they rely on details of kinetics and enzyme investment function, which require additional assumptions like the principle of even enzyme costs, or estimated flux costs. Above, I argued that isoenzymes and isopathways should be used exclusively rather than in combination: If we sample kinetic models (by randomly assigning kinetic constants) and solve for their enzyme-optimal flux distributions. In a given kinetic model, it is unlikely that different pathways will have precisely the same flux costs. Therefore, pure pathway fluxes are much more likely to appear. However, there are also arguments against this prediction:

1. Assume that two flux distributions, as well as their convex combinations, are economical. When sampling such flux distributions, one would obtain combined flux distributions with a probability of 1. This contradicts the above argument. However, it well possible that different ways of sampling (in the space of kinetic models, or in flux space) lead to different results.

2. In reality, isoenzymes (e.g., transporters with different binding affinities) are often expressed together. While this rules out an enzyme level optimisation for maximal cost efficiency, it could be a case of preemptive expression, in which cells use a mixed strategy to anticipate potential changes of their metabolic objective.

S3.2 The choice between high-yield and low-yield strategies

Enzyme cost terms help to predict low-yield strategies Classical FBA maximises the rate of biomass production at limited nutrient uptake rates. Since flux distributions predicted by FBA scale proportionally with the flux bounds used, FBA maximises the yield (biomass production per nutrient influx) rather than the absolute rate of biomass production (production per time) [20]. Other methods overcome this limitation by penalising or limiting large fluxes. Limiting the sum of fluxes creates trade-offs between fluxes in different pathways, and minimising this sum can lead to low-yield solutions. Costs and benefits can be combined in different ways: FBA with molecular crowding [24] optimises the metabolic benefit under a non-linear constraint on the fluxes, supposed to reflect a bound on the total enzyme level. Flux cost minimisation, on the contrary, fixes the FBA benefit and minimises a flux cost. In both cases, numerical optimisation leads to a single flux distribution. This flux distribution depends on the quantitative parameters assumed in the enzyme investment function; for instance, catalytic constants and enzyme prices can help to obtain realistic flux predictions [16]. In these models, there is no need to compare cost and benefits directly (and thus, to express them in common units) because one of them is always kept fixed. EFA resembles these approaches, but relies on underlying kinetic models in which the free variables are not fluxes, but enzyme levels, and in which a difference of enzyme benefit and cost is optimised. Different kinetic models will yield different optimal fluxes. Accordingly, the flux benefit principle does not specify a unique flux distribution, but a whole range, encompassing both high-yield and low-yield strategies.

S3.3 Economic imbalance as a signal for optimal regulation

How can optimal enzyme profiles be realised biochemically? Metabolic economics states optimality conditions for enzyme profiles, but it does not explain how enzyme profiles are realised by biochemical regulation. The enzyme levels in cells are set by transcriptional and post-transcriptional regulation, which is linked to metabolism in a feedback loop. Which feedback mechanisms – i.e., which metabolic signals and regulation functions for transcription and translation – could produce optimal profiles?

Let us consider an analogous problem in biomechanics: how the structure of bones is dynamically adapted to mechanical tasks. Struts in bones, oriented along directions of mechanical stress, improve the bones' mechanical stability [27]. Such stress-adapted structures can develop and be maintained by feedback control: osteoblasts and

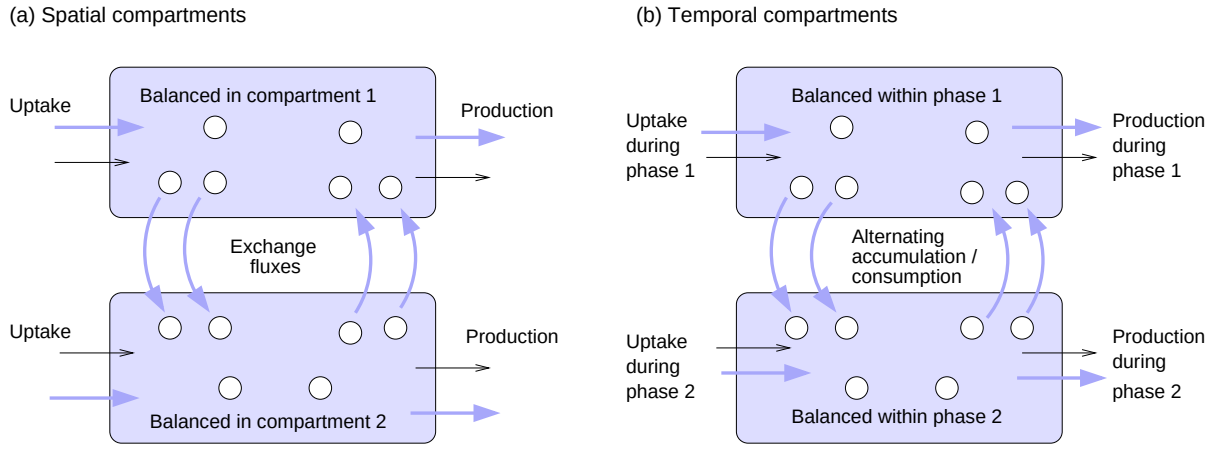

Figure S3: Economic flux analysis can be applied to fluxes with functional (spatial or temporal) compartments. (a) Compartment model (cell organelles or body organs): the same compound in different compartments can have different concentrations, chemical potentials, and economic potentials. Fluxes and flux demands can also differ. Communicating metabolites can be exchanged between compartments by (possibly enzyme-catalysed) fluxes satisfying the sign constraints. (b) Temporal compartments, achieving some net production on average; some internal metabolites must be balanced in every phase, some only on average (these are accumulated and consumed later, or depleted and replenished later; in the network scheme, they are "exchanged" between the temporal compartments). If such exchange metabolites are strongly buffered (or stored in macromolecules, where they do not cause thermodynamic forces), there will be *no sign constraints* in the "exchange" fluxes; only the mass balance in the exchange fluxes needs to be satisfied. Both scenarios shown work also for more than two compartments.

osteoclasts respond to physical stresses, re-enforce the structure where stresses are high, and remove material where stresses are low. In this way, bone structure is adjusted to typical external stresses and adapted while bones are growing. The mechanostat model [28] describes this remodelling process as a feedback control. Also the normal development of bone structure is guided by mechanical tasks: muscle movements during embryonic development lead to stresses that trigger the progress of ossification [29]. Thus, instead of being genetically encoded, bone structures unfold in the embryo guided by training.

Let us take the structural adaption of bones as a metaphor for enzyme adaption. While the main function of bones is to provide stability against typical external stresses, metabolic enzymes need to support metabolic fluxes and concentrations that meet the changing supply and demand. A maladapted metabolic system will face enzyme stresses, and an adjustment of enzyme levels can reduce the economic stress and bring the metabolic state closer to its optimum. Finally, like bone structures, enzyme profiles are not encoded explicitly in the genes, but emerge from regulation. For instance, an adaption can be achieved by transcription factors whose activation depends on metabolite levels. The resulting feedback loop between enzyme levels, key metabolite levels, transcription factor activities, and enzyme production can adjust enzyme levels, and metabolic state to changing external challenges. Its dynamics depends on molecular interactions, and in particular on transcription factors' binding affinities to metabolites and DNA. With a suitable choice of these affinities, feedback systems could, at least approximately, realise the optimal states postulated by metabolic economics.

Economic potentials as hypothetical input signals for gene regulation The remodelling of a piece of bone is guided by local physical stresses. Likewise, enzyme levels are controlled by local inputs to the gene regulation functions. How can such a regulation lead to optimal enzyme levels? In principle, gene regulation functions could be chosen to match optimal metabolite profiles to optimal enzyme profiles (as predicted by metabolic economics). This would allow the system to show the optimal state. But would the state be robust? Robustness could be achieved by a feedback from economic stresses to enzyme levels, which cancels economic stresses as they appear. In the example of bones, the local stress emerges from biomechanics: since it is good proxy for the demand to

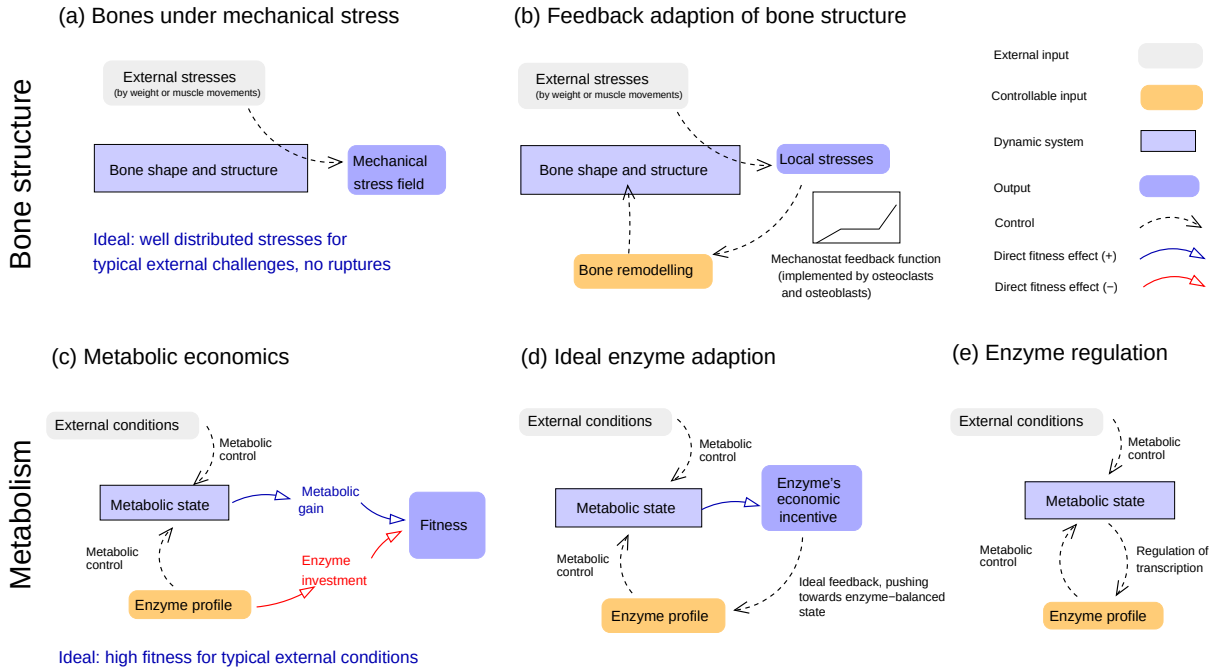

Figure S4: Feedback regulation and optimal control. An analogy between regulation of bone structures and of metabolic states. (a) External mechanical stresses in bones evoke a stress field which depends on the bone's shape and structure. (b) According to the mechanostat model [28], bones adapt themselves to their typical stresses. High local stresses induce a local enforcement ("modelling"), low local stresses induce a local removal of material ("remodelling"). (c) In cost-benefit models of metabolism, the enzyme profile determines the fitness by evoking a certain metabolic state. (d) The local economic stress (mismatch between the enzyme's benefit and cost) would be an ideal input signal for enzyme regulation. (e) In reality, enzyme adjustments are achieved by transcriptional regulation. To yield optimal enzyme profiles, the feedback function should realise the optimal regulation function predicted by (d).

enforce the bone, it is a useful signal that comes "for free". The systemic demand for enzymes, in contrast, is not directly represented by existing biochemical signals, but needs to be established "computationally" by the gene regulation network. In a "demand-driven" system for enzyme regulation, economic potentials would be helpful input signals.

Even though they cannot be physically sensed, they may still be statistically correlated to metabolite levels, which could serve as biochemical proxies. If economic potentials decrease with the metabolite levels, a transcriptional activation by pathway substrates (or inhibition by pathway products) tends to stabilise the flux demand. Such regulation patterns could also work for entire pathways. Under simplifying assumptions (given a flux distribution and assuming the principle of uniform enzyme costs), the economic potentials within a pathway can be determined from economic potentials on the pathway boundary. Thus, if a metabolic network consists of interconnected pathways and if the connecting metabolite levels (cofactors or key intermediates) are correlated with their economic potentials, the metabolite levels carry information about the economic potentials of many metabolites. This would make them useful input signals for gene expression.

Enzyme stresses may not only represent a need for transcriptional regulation, but also selection pressures acting on average enzyme levels during evolution. If an enzyme level is too high or too low on average, the average enzyme stress $\langle y_l^{\triangle} \rangle \neq 0$ represents a selection pressure on the enzyme level or on the gene regulation function behind it. If an enzyme does not exist in an organism, but would be useful, the (still inactive) reaction will have a positive flux demand, i.e., an enzyme stress. If this stress exceeds the evolutionary cost of the enzyme – which measures the probability that an enzyme will emerge by a series of mutations [30, 31] – the enzyme can be expected to evolve. On the contrary, if an enzyme's flux demand never exceeds the minimal flux price $h^{\mathrm{v},\mathrm{min}}$ in Eq. (4), the enzyme should be constantly repressed and may disappear by mutations.

S3.4 Economic potentials in pathways and networks

Global reaction balance for pathways and networks The balance between enzyme benefit and cost holds not only for individual enzymes, but for entire metabolic pathways and networks. By summing the reaction balance Eq. (1) over some active enzyme-catalysed reactions, we obtain the pathway balance equation

$$\sum_l \Delta w_l \, v_l + \sum_l \hat{z}_l^{\mathrm{v}} \, v_l = \sum_l h_l^{\mathrm{u}} \, u_l. \tag{S20}$$

The first term, the production of economic potential (i.e., the enzyme benefit), depends only on metabolites at the pathway boundary because the contributions from metabolites inside the pathway cancel out. The balance equation Eq. (S20), applied to a linear pathway L with identical fluxes v in all reactions, can be written as

$$\left[\sum_{l\in L} \Delta w_l^{\mathrm{c}} + \sum_{l\in L} z_l^{\mathrm{v}}\right] v = h_L^{\mathrm{u}} \sum_{l\in L} u_l \tag{S21}$$

where $h_L^{\mathrm{u}} = (\sum_l u_l \, h_l^{\mathrm{u}})/(\sum_l u_l)$ is the apparent pathway cost. Since Eq. (S21) resembles the balance equation for single reactions, metabolic economics allowing for a lumping of reactions in metabolic models.

Instead of describing pathways by their net reactions, one may study a pathway in detail and omit the network around it. However, the economic effects of the network must be preserved in the model. A pathway is linked to the surrounding network by communicating metabolites; their economic potentials w_i represent a benefit that stems from reactions or metabolites outside the pathway, but is now considered in the pathway model. For modular modelling, we need to construct effective models of isolated pathways which, nevertheless, behaves as if the pathway were embedded in the cellular network. The economic potentials of the communicating metabolites retain their values from the whole-network model, but are reinterpreted as external economic potentials, thereby defining an effective objective function for the isolated pathway.

The enzyme costs of biochemical pathways The reaction balance (1) holds not only for individual reactions, but also for pathways and metabolic networks (see SI S3.4). By summing over the reactions in a pathway L, we obtain the pathway's enzyme cost and benefit, which of course will be equal. The enzyme cost $y_L = \sum_{l\in L} h_l^{\mathrm{u}} \, u_l$ can be written as $h_L^{\mathrm{u}} \, u_{\mathrm{L}}$, where u_{L} is the sum of enzyme levels and h_L^{u} is the average enzyme price in the pathway. The benefit is determined by the boundary metabolites, while the internal metabolites drop out from the sum. If we normalise the pathway costs by the total enzyme cost in the network, we obtain the fractional pathway costs $y_L / \sum_L y_L$. If the network consists of non-overlapping pathways, their fractional costs sum to 1, and if all enzyme molecules are equally costly, the fractions correspond to the relative protein abundances in the pathways.

The metabolic benefit of pathways can be expressed by economic potentials on the pathway boundaries To study the economics of individual pathways, we need effective pathway objectives. Ideally, they should effectively describe how the pathway contributes to the overall benefit, as determined in a larger network model. Thus, how does a benefit function for the larger network, for instance biomass production, induce benefit functions for specific pathways? Pathway products may not directly contribute to biomass, but may affect biomass production indirectly. These effects should be captured by an effective objective function.

Pathway objectives with this property can be constructed from the economic potentials on the pathway boundary. Imagine two models of the pathway: one model of the isolated pathway ("local model") and one in which the pathway is part of a larger network with a benefit depending on biomass production ("system model"). The system model (which may remain unspecified in detail) is assumed to be in an enzyme-optimal state. All variables in the pathway must coincide between local model and system model: not only fluxes and concentrations, but also economic potentials and enzyme costs have to be identical between the models. Then, the reactions satisfy the same reaction balances in both models, and the pathway in the local model will be subject to the same optimality requirements as in the systemic model. The potentials at the pathway boundaries arise from the optimised metabolic state. In the small model, they define a local objective function for the pathway. Even if a direct comparison between models is not possible in practice, the though experiment shows how local benefit functions for pathway can reflect global benefits in a larger network.

Economic potentials as connecting variables between metabolic pathways Metabolic networks can be split into pathways, which may be linked by cofactors (such as ATP or NADH) or by highly connected metabolites [32]. If these metabolites are seen as external (fixed concentrations, no mass balance required), the pathways become uncoupled and can be modelled separately. To merge the models, the communicating variables need to agree between models. In modular response analysis, used to study metabolic control in modular networks [33], concentrations and production or consumption fluxes are relevant communicating variables. If we consider thermodynamics, the chemical potentials will be relevant, and if we consider enzyme economics, the economic potentials are exactly the communicating variables that need to be matched.

How do the potentials at the boundary of a pathway affect the potentials within that pathway? If the boundary potentials rise, the internal potentials are likely to rise as well; if their spread becomes larger (e.g. if the total flux demand of a pathway (the potential difference between products and substrates) increases), this spread will be distributed over the pathway, and the individual enzyme costs will increase. This certainly holds if we employ heuristic principles like the principle of uniform enzyme costs (see SI S5.4): in this case, and for fixed fluxes, the economic potentials within a pathway follow directly from those at the boundary. Thus, the economic potentials of key metabolites, such as imported nutrients, cofactors, and biomass precursors, carry crucial information for metabolic economics. If their values change – and if the fluxes are seen as constant – the local economic adaptions can be studied separately for each pathway, without the need for an economic model of the entire network.

Analogy to parts of electric circuits Pathways that form a metabolic network resemble the parts of electric circuits. In a resistor, there will be a current when an external voltage (i.e., potential difference) is applied. Likewise, in a metabolic pathway there will be a flux when there is a flux demand, i.e., an economic potential difference. The relations $v(\mathbf{w}^{\mathrm{x}})_L$ between flux demands and fluxes may be complicated, but by linearising them around some operating point – the metabolic state in question – we may obtain an economic "Ohm's law" for reactions or pathways. An "economic resistance" can, for instance, be estimated from the principle of uniform enzyme costs. Likewise, pathways can be seen as "voltage sources" that establish economic potential differences. The enzymes of glycolysis, for instance, have a certain minimal cost per flux, which defines a lower bound on the flux demand (the "voltage"), which can then "clamp" the economic potential difference between the substrates (glucose, ADP, and phosphate) and products (pyruvate and ATP) (see Eq. (S16)). Whenever glycolysis is active in an enzyme-optimal state, the glycolytic enzymes set a lower bound on the potential difference, comparable to a battery. Like the voltage in a battery, the flux cost of a pathway is not exactly constant, but affected by the gain.

S4 Multiple objectives

S4.1 Mixed objectives and preemptive expression

Mixed objectives Economic flux analysis assumes a single metabolic objective. In reality, cells or metabolic pathways may have multiple objectives and need to realise a compromise between them. Central metabolism produces both ATP and biosynthesis precursors; both tasks are important and their relative importance may depend on the situation. Another trade-off arises between needs for large fluxes and low intermediate concentrations. Moreover, cells may not be optimally adapted to their environment, but may anticipate environmental changes. In economic flux analysis, multi-objective problems can be treated by by translating them into single-objective problems. A number of biologically relevant multi-objective problems can be captured by convex combinations of benefit functions:

1. **Trade-offs between objectives** Different objective functions $z_n(\mathbf{v}, \mathbf{c})$ may be combined in a single objective $z(\mathbf{v}, \mathbf{c}) = \mathcal{Z}(z_1(\mathbf{v}, \mathbf{c}), z_2(\mathbf{v}, \mathbf{c}), ...)$. As a practical choice, we can consider convex combinations $z(\mathbf{v}, \mathbf{c}) = \sum_n \lambda_n z^{(n)}(\mathbf{v}, \mathbf{c})$ with positive weights λ_n, where $\sum_n \lambda_n = 1$. The gains $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$ will be convex combinations as well.
2. **Adaption to time-averaged objective** Changing an enzyme profile takes time. If the cell's objective changes much faster than enzyme levels can be adapted, the cell may choose a static enzyme profiles that is optimal *on average*: if the objective function alternates between distinct objectives, the

average benefit of an enzyme profile is given by a convex combination of objective functions, where the prefactors represent the relative durations. If the enzyme prices remain constant, the fitness function $f(\mathbf{u},\mathbf{x}) = \sum \lambda_n \, z_n(\mathbf{v}(\mathbf{u},\mathbf{x}),\mathbf{c}(\mathbf{u},\mathbf{x})) - h(\mathbf{u})$ can be written as $f(\mathbf{u},\mathbf{x}) = \sum \lambda_n \, f_n(\mathbf{u},\mathbf{x})$ where $f_n(\mathbf{u},\mathbf{x}) = z_n(\mathbf{v}(\mathbf{u},\mathbf{x}),\mathbf{c}(\mathbf{u},\mathbf{x})) - h(\mathbf{u})$, so it does not matter if convex combinations are applied to benefit or fitness functions.

3. **Adaption to expected objective** In other cases, convex combinations may not describe time averages, but expectation values of objective functions. Cells must choose their enzyme profiles ahead of time: when a protein molecule is produced, it is still uncertain which objectives will prevail during its lifetime. Considering different possible objective functions with different probabilities, the cell may maximise its *expected benefit*. Again, the effective fitness function is a convex combinations, and the coefficients λ_n describe the probabilities of possible benefit functions in the typical environment. Anticipation may play a role even in relatively constant environments: in this case, the current benefit function should have a large weight in the convex combination, and possible future benefit functions should be weighted less.

When modelling such mixed strategies, there is a fundamental difference between changing tasks (e.g., the need to produce energy) and changing environmental conditions (e.g., the presence of nutrients). Let us consider a given kinetic model. In the first case, one choice of enzyme levels will always yield the same fluxes, and we simply average over the different objectives. In the second case, the same enzyme profile will give rise to different flux distributions and flux gain vectors. This can only be modelled directly, based on the kinetic model.

S4.2 Trade-offs and Pareto optimality

Pareto optimality Compromises between opposing objectives can be described by Pareto optimality [34]. Consider a number of metabolic objectives $z_n(\mathbf{v},\mathbf{c})$ and an enzyme investment function $h(\mathbf{u})$, yielding the fitness functions $f_n(\mathbf{u})$. A state in which none of them can be improved without compromising the others is called *Pareto-optimal*. Importantly, none of the objectives needs to be fully optimal. Typically, there is not a single Pareto optimum, but a whole spectrum of Pareto-optimal solutions called the Pareto front. A Pareto-optimal point satisfies a simple criterion: there are positive numbers λ_n (satisfying $\sum_n \lambda_n = 1$) such that the equation

$$\lambda_1 \frac{\partial f_1}{\partial \mathbf{u}} + \lambda_2 \frac{\partial f_2}{\partial \mathbf{u}} + ... = 0 \tag{S22}$$

or equivalently

$$\lambda_1 \frac{\partial g_1}{\partial \mathbf{u}} + \lambda_2 \frac{\partial g_2}{\partial \mathbf{u}} + ... = \frac{\partial h}{\partial \mathbf{u}} \tag{S23}$$

is satisfied[5]. This is a cost-benefit balance for a convex combination of the benefit functions. Any Pareto-optimal point is also the solution of some problem with a single mixed objective, e.g., the expected fitness under varying or uncertain conditions. To sample solutions from the Pareto front, we may sample vectors $\boldsymbol{\lambda}$, construct the mixed gain vectors $\mathbf{z}^{\mathrm{v}} = \sum_n \lambda_n \, \mathbf{z}^{\mathrm{v}}_{(n)}$, and solve the resulting single-objective problems. The Pareto approach can capture compromises between different metabolic objectives, between objectives and enzyme investments, between investments in different enzymes, or between any other additive terms in the fitness function.

Simultaneous objectives and symbiosis Can we also require that a cell optimises several objective functions fully and at the same time? Each objective would define a set of economic potentials, and the flux distribution would have to satisfy reaction balances for all the objectives. However, simultaneous objectives may apply to cells that live in a symbiosis: the cells pursue different objectives, but support each other by exchanging

[5]For instance, we may think of fitness functions $f_{(n)}(\mathbf{u}) = g_{(n)}(\mathbf{u}) - h(\mathbf{u})$ obtained from multiple return functions $g_{(n)}(\mathbf{u})$ and one cost function $h(\mathbf{u})$. A mixture of such fitness functions will correspond to a mixture of return functions with the same prefactors:

$$\sum_n \lambda_n f_{(n)}(\mathbf{u}) = \sum_n \lambda_n [g_{(n)}(\mathbf{u}) - h(\mathbf{u})] = \left[\sum_n \lambda_n \, g_{(n)}(\mathbf{u})\right] - h(\mathbf{u}). \tag{S24}$$

metabolites (see Figure 9). Here we are interested in flux distributions that span both cells, are profitable for each of them, and in fact so profitable that neither of the cells could further improve it. From the point of view of cell A, each enzymatic reaction within cell A must satisfy a reaction balance, with economic potentials referring to A's biomass production. The same holds, *mutatis mutandis*, for cell B, but regarding its own enzymatic reactions and a second set of economic potentials representing biomass production in cell B. A flux distribution that satisfies both reaction balances describes, potentially, a situation that is profitable for both cells, i.e., an evolutionarily stable strategy.

Multiple objectives in biotechnology In biotechnology, compromises between opposing objectives are a critical point: if microbes are engineered to produce a desired chemical, this will impair their growth, and there there may be a selection advantage for low product yields. To avoid this, one might engineer microbial strains in which product formation is compatible with growth, even necessary for it. This would give good producers a selection advantage. Economic flux analysis may help to spot conflicts between objectives and to assign them to specific parts of the network. This may help to find genetic modifications to circumvent the problem.

S5 Algorithms

S5.1 Calculation of economical fluxes and economic potentials

Computing the fluxes and economic potentials in a network step by step The flux benefit principle states that active enzymes must have positive control over the metabolic return. To use it in flux analysis, we introduce the economic potentials as variables and require that they satisfy the reaction balance.

Given a metabolic network with flux gain vector $\mathbf{z}^{\mathrm{v}}$, the reaction balance (1) and the thermodynamic constraints can be solved for stationary fluxes v_l, chemical potentials μ_i, and economical potentials w_m^{c} in a single step by mixed-integer linear programming. This can be numerically costly. However, if we just manage to find feasible flux directions, we can compute all other variables step by step:

1. **Flux directions** First, we determine an economical flux distribution with realistic flux directions. Thermodynamically and economically feasible flux distributions can be obtained by flux cost minimisation (details see below); by varying the cost weights, we can obtain flux distributions with different sign patterns. Alternatively, we can start from a (non-economical) flux distribution and remove all flux cycles and futile modes (see S5.3).

2. **Flux values** From one economical flux distribution, we can construct others with the same feasible sign pattern, but more realistic flux values. Such modes can be obtained, for instance, by FBA or by sampling under linear constraints.

3. **Economic potentials and enzyme costs** Finally, given a feasible sign pattern, we can determine the chemical and economic potentials, possibly accounting for (known or estimated) enzyme properties like enzyme sizes or catalytic constants. To do so, we can use linear programming or sampling under constraints on the demands as described above; the enzyme costs y_l follow automatically from the reaction balance. Sampled solutions – although being feasible – may contain unrealistic values, for instance large investments into relatively unimportant enzymes. To obtain realistic results, we can adjust the economic potentials to other data, for instance, known or guessed enzyme levels. If the flux directions are known, such adjustments are easy: we may keep the economic potentials w_i^{c} fixed and vary fluxes and enzyme costs proportionally (the fluxes need to remain stationary and their signs are kept fixed), or we keep the fluxes fixed and vary flux demands $\hat{z}_l^{\mathrm{v}} + \Delta w_l$ and costs y_l.

S5.2 Tests for economical flux distributions

Criteria for economical flux distributions By definition, an economical flux distribution must be stationary and

satisfy the flux gain condition Eq. (S8) with fluxes and flux costs $h_l^{\mathrm{v}} = h_l^{\mathrm{u}}\, u_l/v_l$ of identical signs. Inactive reactions are irrelevant or can complicate the analysis, so we should discard these reactions from the very beginning[6]. Economical flux distributions must be beneficial and free of futile modes (see [3]):

1. **Economical flux distributions must be beneficial.** All economical flux distributions $\mathbf{v}$ have a positive flux benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} > 0$. To show this, we insert $\mathbf{k} = \mathbf{v}$ into the flux gain condition Eq. (S8) and obtain the equality $\sum_l y_l = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$. Since all y_l are positive, the benefit must be positive.

2. **Futile modes** The test mode theorem [3] provides a practical criterion for economical fluxes. Consider an economical flux distribution $\mathbf{v}$ and a test mode $\mathbf{k}$ (i.e., a stationary flux distribution on the active region of $\mathbf{v}$). (i) If $\mathbf{k}$ is beneficial ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} > 0$), $\mathbf{v}$ and $\mathbf{k}$ share at least one active reaction with the same flux direction. (ii) If $\mathbf{k}$ is costly ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} < 0$), they share at least one active reaction with opposite flux directions. (ii) If $\mathbf{k}$ is non-beneficial ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} = 0$), they share at least one reaction of each sort. Therefore, economical flux distributions must be free of futile modes (defined as the flux signs on the shared active region between the flux distribution and a non-beneficial or wasteful test mode). For practical tests, only elementary modes need to be considered as test modes. In models with production objective, any elementary flux cycle will make a flux distribution uneconomical.

Finding economical flux distributions can be difficult because the thermodynamic and economic flux constraints are non-linear. Mixed-integer programming can be used, but it is numerically more expensive than linear FBA. However, if a flux distribution $\mathbf{v}$ is given, the sign constraints can be checked by linear programming. If a flux distributions is given, there are different ways to test it for being economical:

1. **Demand condition.** To satisfy the flux gain condition Eq. (S8), there must be a flux cost vector $\mathbf{h}^{\mathrm{v}}$ (representing y_l/v_l) such that

$$\begin{aligned} \mathbf{K}^\top \mathbf{h}^{\mathrm{v}} &= \mathbf{K}^\top \mathbf{z}^{\mathrm{v}} \\ \mathrm{Dg}(\mathbf{v})\, \mathbf{h}^{\mathrm{v}} &> 0, \end{aligned} \tag{S25}$$

   where $\mathbf{K}$ is a right-kernel matrix of $\mathbf{N}$. The inequality forces $\mathbf{h}^{\mathrm{v}}$ to show the same sign pattern as $\mathbf{v}$. In practic, the matrix $\mathbf{K}$ may either contain a minimal number independent flux distributions or a complete set of elementary modes; the latter can help to find small sets of reactions that make $\mathbf{v}$ uneconomical.

   Reaction balance with consistent economic potentials. A complete flux distribution $\mathbf{v}$ is economical if it satisfies the reaction balance with suitable vectors $\Delta\mathbf{w}^{\mathrm{c}}$ and $\mathbf{y} > 0$. To show this, we need to determine as a vector $\mathbf{w}^{\mathrm{c}}$ of economic potentials satisfying the reaction balance

$$\mathrm{Dg}(\mathbf{v})\, (\mathbf{N}^\top \mathbf{w}^{\mathrm{c}} + \mathbf{z}^{\mathrm{v}}) > 0 \tag{S26}$$

   or a difference vector $\Delta\mathbf{w}^{\mathrm{c}}$ satisfying

$$\begin{aligned} \mathrm{Dg}(\mathbf{v})\, (\Delta\mathbf{w}^{\mathrm{c}} + \mathbf{z}^{\mathrm{v}}) &> 0 \\ \mathbf{K}_{\mathrm{R}}^\top\, \Delta\mathbf{w}^{\mathrm{c}} &= 0. \end{aligned} \tag{S27}$$

   The equation at the bottom is a Wegscheider condition [36, 37] for $\Delta\mathbf{w}^{\mathrm{c}}$ arising from the definition $\Delta\mathbf{w}^{\mathrm{c}} = \mathbf{N}^\top \mathbf{w}^{\mathrm{c}}$.

3. **Inequalities on production weights.** Conclusions about the flux directions can be drawn from inequalities $\mathbf{A}\, \mathbf{w}^{\mathrm{x}} > \mathbf{b}$ for the production gains $\mathbf{w}^{\mathrm{x}}$. To show that a flux distribution $\mathbf{v}$ is economical, we need to find

[6] Boundary optima for all reactions with a vanishing flux, can be achieved by a proper choice of the cost function, namely by choosing values $h_l^{\mathrm{u}} > g_{x_l}$. With a linear cost function $h(\mathbf{u}) = \sum_l \alpha_l\, u_l$, for instance, this could always be achieved by an appropriate choice of the values α_l.

a vector $\binom{\mathbf{w}^{\mathrm{x}}}{\mathbf{y}}$ such that

$$\begin{aligned} \mathbf{K}^{\top}\left(\mathbf{N}^{\mathrm{x}\top}, -\mathrm{Dg}(\mathbf{v})^{-1}\right)\begin{pmatrix}\mathbf{w}^{\mathrm{x}}\\ \mathbf{y}\end{pmatrix} &= 0 \\ \begin{pmatrix}\mathbf{A} & 0\\ 0 & \mathbf{I}\end{pmatrix}\begin{pmatrix}\mathbf{w}^{\mathrm{x}}\\ \mathbf{y}\end{pmatrix} &> \begin{pmatrix}\mathbf{b}\\ 0\end{pmatrix}. \end{aligned} \tag{S28}$$

The criteria (S25) and (S28) apply to complete flux distributions only. In incomplete flux distributions, the inactive reactions must be omitted. The criteria Eq. (S26) and (S27) can also be applied to individual active reactions within incomplete flux distributions. Like for the inactive reactions, no balance equation needs to be satisfied, and their being inactive is sufficiently justified by assuming high flux costs for these enzymes.

S5.3 Detecting and removing flux cycles from a given flux distribution

Finding cycle-free flux distributions in large networks can be numerically expensive. However, flux cycles can be detected and removed relatively easily if the elementary modes of the network are known. Consider a metabolic network with stoichiometric matrix $\mathbf{N}^{\mathrm{tot}}$; all elementary non-productive modes have been computed (for instance, by efmtool [35]) and collected in the columns of a matrix $\mathbf{C}$. Let $\mathbf{v}$ be a flux distribution whose flux cycles we want to find and remove. Depending on the type of thermodynamic feasibility criterion required (weak or strong) there are two different algorithms:

1. **Criterion for weak thermodynamic feasibility** In a feasible flux distribution, all non-zero fluxes must be driven by thermodynamic forces in the flux direction. As a necessary condition, the flux distribution must be free of flux cycles. To find violations of this rule, we consider non-productive test modes $\mathbf{k}$ and check if the elements v_r and k_r have the same signs on the entire active region of $\mathbf{k}$. If we find such a test mode, the sign condition is violated. For correction the flux distribution, we choose from the elements found the one with the smallest absolute value (called ξ) and replace $\mathbf{v}$ by $\mathbf{v} - \xi\,\mathrm{sign}(\mathbf{k})$. The updated flux vector has a zero value on the active region of $\mathbf{k}$, and therefore no flux cycle defined by this test mode.

2. **Criterion for strong thermodynamic feasibility** In a strictly feasible flux distribution, any non-zero force must lead to a reaction flux of the same sign. The test resembles the one for weak thermodynamic feasibility. We check for each non-productive mode $\mathbf{k}$ (column of $\mathbf{C}$) if all elements v_r and k_r have the same signs on the shared active region of $\mathbf{v}$ and $\mathbf{k}$. If we find such a mode $\mathbf{k}$, the sign condition is violated. Again, to correct the flux distribution, we choose the mean value of these elements (called ξ) and replace $\mathbf{v}$ by $\mathbf{v} - \xi\,\mathrm{sign}(\mathbf{k})$. The new flux vector will either have no overlap with $\mathbf{k}$ or at least some negative and some positive value on the shared active region and can therefore not be ruled out by $\mathbf{k}$ anymore.

In both cases, all non-productive elementary modes $\mathbf{k}$ are tested and flux cycles are removed until all no constraint violations are left. The same method can be used to futile modes. However, the number of elementary futile test modes can be much higher and the calculations may become expensive.

S5.4 Computing the economic potentials with heuristic principles

A given economical flux distribution $\mathbf{v}$ restricts the economic potentials w_i^{c}, but does not determine them precisely. Further assumptions about the enzyme costs y_l may lead to unique solutions for $\mathbf{w}^{\mathrm{c}}$ and $\mathbf{y}$. I shall discuss two possibilities: we can assume that all enzymes have similar costs ("principle of uniform enzyme costs"), or that their costs are close to some predefined values. In both cases, a complete economical flux distribution $\mathbf{v}$ with flux gain vector $\mathbf{z}^{\mathrm{v}}$ must be given; our aim is to find internal economic potentials $\mathbf{w}^{\mathrm{c}}$ and enzyme costs $\mathbf{y}$ that satisfy the reaction balance and agree with our heuristic assumptions. If the network contains conserved moieties, there remains some gauge freedom in the choice of $\mathbf{w}^{\mathrm{c}}$ (right-kernel vector of $\mathbf{N}^{\top}$ can be freely added), and a unique solution can be enforced by regularisation.

- **Estimating enzyme costs from measured enzyme levels** Enzyme costs for a metabolic state can be roughly estimated from proteome data. We may assume that the enzyme costs in a certain metabolic state are proportional to measured enzyme levels u_l^{data}, possibly weighted by protein masses, turnover rates, etc. To fit such such enzyme costs into a model, we normalise them such that their sum yields the total flux benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$. We obtain presumable enzyme costs ${y_l}^{\mathrm{data}} = (\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v})/(\sum_j u_j^{\mathrm{data}})\, u_l^{\mathrm{data}}$ for our model.

- **Economic potentials realising uniform enzyme costs.** To determine internal economic potentials $\mathbf{w}^{\mathrm{c}}$, we can heuristically assume that all enzymes should have similar costs. If we sum all reaction balances (all reactions must be enzymatic), we obtain

$$\sum_l y_l = \sum_l \partial h_l^{\mathrm{u}}\, u_l = \sum_l [\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}}]\, v_l = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} \tag{S29}$$

  Therefore, the flux distribution predetermines the sum of all enzyme costs y_l. If we simply minimise the sum of squared enzyme costs $\sum_l (y_l)^2$ under the constraint $\sum_l y_l = q_{\mathrm{tot}}$, we can employ a Lagrange multiplier λ and obtain the solution $y_l = -\lambda/2 = \mathrm{const.}$, i.e. all enzyme costs are equal. Using the reaction balance as a second constraint, the minimisation of squared enzyme costs yields

$$\begin{aligned} \min \quad &\stackrel{!}{=} \quad \sum (y_l)^2 = \sum_l \left(v_l\, (\mathbf{N}^\top\, \mathbf{w}^{\mathrm{c}})_l + v_l\, z_l^{\mathrm{v}}]\right)^2 \qquad \text{w.r.t.} \quad \mathbf{w}^{\mathrm{c}} \\ \Rightarrow \min \quad &\stackrel{!}{=} \quad \mathbf{w}^{\mathrm{c}\top}\, \mathbf{N}\, \mathrm{Dg}(\mathbf{v})^2 \mathbf{N}^\top\, \mathbf{w}^{\mathrm{c}} + 2\, \mathbf{w}^{\mathrm{c}\top}\, \mathbf{N}\, \mathrm{Dg}(\mathbf{v})^2\, \mathbf{z}^{\mathrm{v}}. \end{aligned} \tag{S30}$$

  Finally, all enzyme costs must be positive, i.e., larger than some positive minimal cost ${y_l}^{\mathrm{min}}$:

$$\begin{aligned} \forall l: \qquad & q^{\mathrm{min}} \le y_l = v_l[\Delta w_l^{\mathrm{c}} + z_l^{\mathrm{v}}] \\ \Rightarrow \qquad & q^{\mathrm{min}} \cdot \hat{\mathbf{1}} - \mathrm{Dg}(\mathbf{v})\, \mathbf{z}^{\mathrm{v}} \le \mathrm{Dg}(\mathbf{v})\, \mathbf{N}^\top \mathbf{w}^{\mathrm{c}} \end{aligned} \tag{S31}$$

  where $\hat{\mathbf{1}} = (1, 1, ..)^\top$. Equation (S30) with constraints (S31) can be solved for $\mathbf{w}^{\mathrm{c}}$ by quadratic programming. If the matrix $\mathbf{N}\, \mathrm{Dg}(\mathbf{v})^2 \mathbf{N}^\top$ is not invertible, the solution is not unique. This happens, for instance, when models contain conserved moieties. To obtain a unique solution, we can add a regularisation term, e.g. $\alpha ||\mathbf{w}^{\mathrm{c}}||^2$ with a small weight α. Again, the calculation works only for complete flux distribution; if $\mathbf{v}$ contains inactive reactions, they must be omitted from the model.

- **Economic potentials realising approximately known enzyme costs.** For another heuristics, we predefine enzyme costs ${y_l}^{\mathrm{data}}$ and try to approximate them by costs y_l in the model. For consistency reasons, the predefined values must be scaled to satisfy Eq. (S29). To compute the economic potentials, we try to make the ratio $y_l/{y_l}^{\mathrm{data}}$ similar between enzymes and implement this by

$$\min \stackrel{!}{=} \sum \frac{(y_l)^2}{{y_l}^{\mathrm{data}}} \qquad \text{s.t.} \quad \sum y_l = \sum_l {y_l}^{\mathrm{data}} \tag{S32}$$

  (principle of uniform scaled costs). Again, we obtain a quadratic optimality problem for $\mathbf{w}^{\mathrm{c}}$

$$\min \quad \stackrel{!}{=} \quad \mathbf{w}^{\mathrm{c}\top}\, \mathbf{N}\, \mathrm{Dg}(\mathbf{v})^2\, \mathrm{Dg}(\mathbf{y}^{\mathrm{data}})^{-\mathbf{1}} \mathbf{N}^\top\, \mathbf{w}^{\mathrm{c}} + \mathbf{2}\, \mathbf{w}^{\mathrm{c}\top}\, \mathbf{N}\, \mathrm{Dg}(\mathbf{v})^{\mathbf{2}}\, \mathrm{Dg}(\mathbf{y}^{\mathrm{data}})^{-\mathbf{1}}\, \mathbf{z}^{\mathrm{v}}. \tag{S33}$$

  The constraints Eq. (S31) remain, and for models with moiety conservation, regularisation may again be needed to obtain a unique solution. By inserting the solution $\mathbf{w}^{\mathrm{c}}$ into the reaction balance, we obtain enzyme costs $\mathbf{y}$, which will approximate the predefined cost $\mathbf{y}^{\mathrm{data}}$. For ensemble modelling, this algorithm can be combined with a random sampling of potential positive enzyme costs.

The equations (S31) and (S33) show that, for a given flux distribution and assuming uniform or given enzyme costs, the internal economic potentials depend linearly on external economic potentials and on direct flux demands. In fact, the formulae show how each internal economic potential will depend on the external economic potentials. This holds both for pathways and for the entire metabolic network.

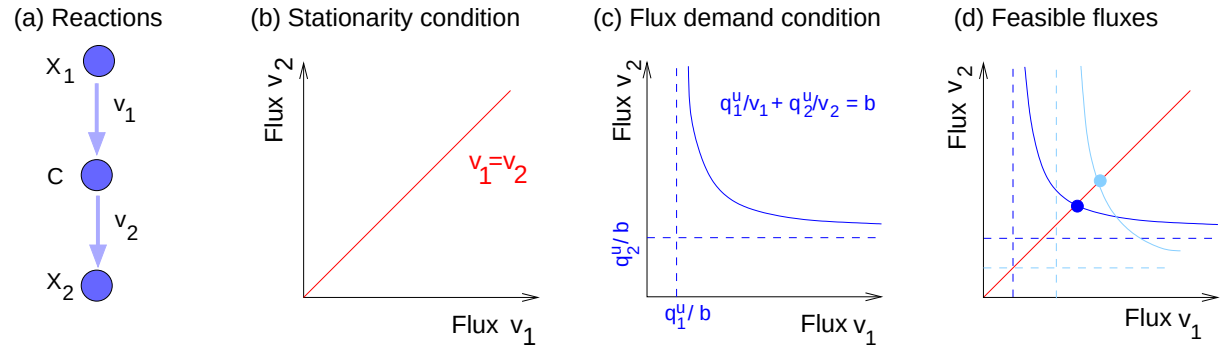

Figure S5: Computing the metabolic fluxes from enzyme costs and flux benefit. (a) Example model with internal metabolite C. (b) In steady state, both reactions must have the same flux, $v_1 = v_2$. (c) The flux gain condition with $\mathbf{K} = (1, 1)^\top$ (unitless) and $\mathbf{z}^{\mathrm{v}} = (0, b)^\top$, i.e. a demand for the production of X_2, reads $y_1/v_1 + y_2/v_2 = b$. The solutions $\mathbf{v}$ form a hyperbola with the asymptotic fluxes $\mathbf{v} = (y_1/b, \infty)^\top$ and $\mathbf{v} = (\infty, y_2/b)^\top$. (d) Both conditions together determine the flux. Two example cases with different assumptions about enzyme costs y_l (dark and light blue) are shown. The calculation for longer pathways is similar: the flux gain condition with the flux distribution $\mathbf{k} = (1, 1, ..., 1)^\top$ leads to the sum rule $\sum_l y_l/v_l = b$. Since all fluxes must be equal, we obtain $\mathbf{v} = (\sum_l y_l)/b \cdot (1, 1, ..., 1)^\top$.

S5.5 Estimating enzyme costs from enzyme masses and rate constants

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Estimating flux demands from enzyme parameters An economical flux distribution must satisfy the reaction balance (1) for a choice of internal economic potentials w_i^{c} and positive enzyme costs y_l. If $\mathbf{v}$ is economical, then other flux distributions with the same signs will be economical as well. Such modes can be easily found by FBA, and compatible economic potentials can be found as described above. But which of these solutions are biologically plausible? In the underlying kinetic model, economic potentials w_i and enzyme prices h_l^{u} are related to enzyme parameters. To guide our search for realistic solutions (in particular, for the flux demands $\Delta\mathbf{w} + \hat{\mathbf{z}}^{\mathrm{v}}$), we can employ additional information about rate constants. The rate of a single reaction is given by $v = u\,r$, where $-k^{\mathrm{cat}-} \le r \le k^{\mathrm{cat}+}$. For the enzyme prices, across all enzymes in a metabolic state, we can approximate

$$h^{\mathrm{u}} \sim L_{\mathrm{u}}\,(\kappa + \kappa_{\mathrm{u}}) \tag{S34}$$

with enzyme size L_{u} (number of amino acids), cell growth rate κ, and enzyme degradation rate κ_{u}. Inserting this into the reaction balance and dividing by $v = u\,r$, we obtain

$$\Delta w + z_{\mathrm{v}} = h^{\mathrm{u}}\,\frac{u}{v} \sim \frac{L_{\mathrm{u}}\,(\kappa + \kappa_{\mathrm{u}})}{r} \tag{S35}$$

If all enzymes operate at full speed with known specific rates $r_l = k_+^{\mathrm{cat}}$ (or $r_l = -k_-^{\mathrm{cat}}$ for negative fluxes), Eq. (S35) yields estimates of the flux demands $\hat{z}_l^{\mathrm{v}} + \Delta w_l$. To obtain the proportionality constant, the sum $\sum_l v_l[\hat{z}_l^{\mathrm{v}} + \Delta w_l]$ can be equated to a (presumably known) overall cost.

Adjusting economic potentials to uniform or predefined flux demands If flux demands $\hat{z}_l^{\mathrm{v}} + \Delta w_l$ are approximately known (or assumed to be uniformly distributed), we may search for economic potentials that agree with these values. In practice, we may compute the assumed enzyme cost $q_l^{\mathrm{data}} = [\Delta {w_l}^{\mathrm{data}} + \hat{z}_l^{\mathrm{v}}]\,v_l$ and proceed as in section S5.4. Given the flux directions and bounds $y_l \ge q_l^{\mathrm{min}} > 0$, we have to satisfy the constraint

$$q_l^{\mathrm{min}} \quad < \quad \mathrm{sign}(v_l)\,[\Delta w_l + \hat{z}_l^{\mathrm{v}}]. \tag{S36}$$

Adjusting given flux demands or enzyme costs to the reaction balance To satisfy the reaction balance, enzyme costs $h_l^{\mathrm{u}}\, u_l$, fluxes v_l, and flux gains z_l^{v} must satisfy the equation

$$\mathsf{Dg}(\mathbf{h}^{\mathrm{u}})\frac{\mathbf{u}}{\mathbf{v}} - \mathbf{z}^{\mathrm{v}} = \mathbf{N}^{\top}\mathbf{w}^{\mathrm{c}\,\top} \in \mathsf{Span}(\mathbf{N}^{\top}). \tag{S37}$$

If measured or guessed enzyme costs are used, this equation may be violated. If values for h_l^{u}, u_l and v_l are approximately known, the enzyme cost values can be adjusted by projecting the result of the left-hand side onto the hyperplane spanned by the columns of $\mathbf{N}^{\top}$.

S5.6 Computing the economical fluxes from enzyme costs

If the enzyme costs in a model are precisely known, they may even determine the quantitative fluxes. Consider a vector $\mathbf{y}$ of enzyme costs, which must be feasible, stem from a kinetic model in an enzyme-balanced state.

We aim at reconstructing the flux distribution $\mathbf{v}$ in this state (where the flux gain vector $\mathbf{z}^{\mathrm{v}}$ is supposed to be known). First, we remove all inactive reactions (which follow from the zeros in $\mathbf{y}$). In the remaining active region, we compute the flux distribution from the stationarity condition and the flux gain condition Eq. (S8). From the stationarity condition, we obtain n_{ind} linear equations for $\mathbf{v}$ (where n_{ind} is the number of independent metabolites), and from the flux gain condition, n_{stat} linear equations for $\mathbf{v}^{-1}$ (where n_{stat} is the number of independent stationary flux distributions). Together, we obtain $n_{\mathrm{ind}} + n_{\mathrm{stat}}$ equations, matching the number of reactions in the system (the number of columns of the stoichiometric matrix equals the rank n_{ind} of the matrix plus the dimension n_{stat} of the right null space). If the equations are non-redundant, they determine the flux vector $\mathbf{v}$. Simple examples, which can be solved analytically, are shown in Figures S5 and S6. For larger networks, $\mathbf{v}$ can be computed numerically: after omitting all inactive reactions, we write the flux gain condition in the form:

$$\mathbf{K}^{\top}\mathbf{z}^{\mathrm{v}} = \mathbf{K}^{\top}\mathsf{Dg}(\mathbf{y})\frac{\mathbf{1}}{\mathbf{v}} \tag{S38}$$

where the vector $\frac{1}{\mathbf{v}}$ contains the inverse components of $\mathbf{v}$. To satisfy the stationarity condition, we represent $\mathbf{v}$ by $\mathbf{v} = \mathbf{K}\,\hat{\mathbf{v}}$ and solve Eq. (S38) numerically by minimising the mismatch between both sides,

$$\hat{\mathbf{v}} = \mathsf{argmin}_{\hat{\mathbf{v}}'} \sum_i \left(a_i - \sum_l \frac{A_{il}}{\sum_j K_{lj}\,\hat{v}'_j} \right)^2 \tag{S39}$$

with $\mathbf{a} = \mathbf{K}^{\top}\mathbf{z}^{\mathrm{v}}$ and $\mathbf{A} = \mathbf{K}\,\mathbf{y}$. From the solution, we obtain $\mathbf{v}$. If the predefined enzyme costs are infeasible (e.g., if positive enzyme costs are assigned to pathways that cannot contribute to the metabolic benefit, or if futile modes are active according to $\mathbf{y}$), the enzyme costs will not be realisable by economical flux distributions. In this case, Eq. S39 will yield a non-zero mismatch.

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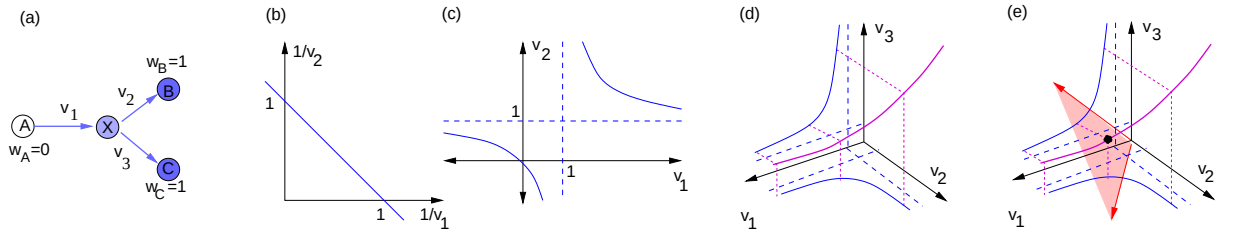

Figure S6: Metabolic fluxes computed from known enzyme costs. (a) Schematic branch point network. (b) The flux gain condition Eq. (S8), $\mathbf{k}^\top \mathrm{Dg}(\mathbf{y})\,\mathbf{v}^{-\mathbf{1}} = \mathbf{k}\cdot\mathbf{z}^{\mathrm{v}}$, states a linear relationship between the inverse fluxes v_1^{-1} and v_2^{-1}. (c) This implies a hyperbolic relationship between the (non-inverse) fluxes. (d) For the branch point, there are two such relationships (projections on the v_1/v_2 and v_1/v_3 planes shown in blue, common constraint curve shown in purple). (e) Intersecting this curve with the plane of stationary fluxes (red), we obtain a unique flux distribution (black dot). As an example, we assume external metabolite demands $w_{\mathrm{A}} = 0$, $w_{\mathrm{B}} = w_{\mathrm{C}} = 1$ and enzyme costs $\mathbf{y} = (\mathbf{1},\mathbf{1},\mathbf{1})^\top$. With the test modes $\mathbf{k}^{(1)} = (1,1,0)^\top$ and $\mathbf{k}^{(2)} = (1,0,1)^\top$, the flux gain conditions read $1/v_1 + 1/v_2 = 1$ and $1/v_1 + 1/v_3 = 1$. Together with the stationarity condition $v_1 = v_2 + v_3$, we obtain the flux vector $\mathbf{v} = (3, 3/2, 3/2)^\top$.

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