Enzyme economy in metabolic networks

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Abstract

Metabolic systems are governed by a compromise between metabolic benefit and enzyme cost. This hypothesis and its consequences can be studied by kinetic models in which enzyme profiles are chosen by optimality principles. In enzyme-optimal states, active enzymes must provide benefits: a higher enzyme level must provide a metabolic benefit to justify the additional enzyme cost. This entails general relations between metabolic fluxes, reaction elasticities, and enzyme costs, the laws of metabolic economics. The laws can be formulated using economic potentials and loads, state variables that quantify how metabolites, reactions, and enzymes affect the metabolic performance in a steady state. Economic balance equations link them to fluxes, reaction elasticities, and enzyme levels locally in the network. Economically feasible fluxes must be free of futile cycles and must lead from lower to higher economic potentials, just like thermodynamics makes them lead from higher to lower chemical potentials. Metabolic economics provides algebraic conditions for economical fluxes, which are independent of the underlying kinetic models. It justifies and extends the principle of minimal fluxes and shows how to construct kinetic models in enzyme-optimal states, where all enzymes have a positive influence on the metabolic performance.

Keywords: Metabolic Control Analysis, Cost-benefit analysis, Futile cycle, Enzyme cost, Economic potential, Economic balance equation.

1 Introduction

Metabolic processes in cells are controlled and shaped by enzyme levels. Since protein production and maintenance are costly, enzyme profiles reflect a compromise between metabolic benefit and protein cost. This hypothesis raises various questions about metabolic strategies. How should enzyme investments be distributed between pathways, along pathways, and between reactions around a metabolite? If two ATP-producing pathways differ in their yields and enzyme investments, which of them will be preferable, considering that enzyme production itself consumes ATP? Should inefficient enzymes be expressed weakly (because they provide little benefit) or strongly (to compensate for their low efficiency)? How should metabolic fluxes be adjusted when enzyme costs are changing, e.g., at higher growth rates? To answer this, we need to look at metabolism from an economic pespective.

Optimal enzyme profiles can be predicted from kinetic models in which enzyme levels are chosen to maximise a metabolic benefit at a fixed total enzyme level [1] or at minimal enzyme costs [2, 3, 4]. Given a model, optimal static or dynamic enzyme profiles can be computed numerically [5, 6], but such results are anecdotical and do not provide general laws. To study optimal enzyme usage in general, one needs to know how enzyme levels (as control variables) act on the stationary fluxes and metabolite levels. For steady states under small perturbations, this relation is described by metabolic control coefficients [7, 8]. These coefficients, in turn, are related to optimal enzyme profiles. Klipp and Heinrich [1] studied a specific cost-benefit problem – flux maximisation at a fixed total enzyme level and unconstrained metabolite levels – and showed that the scaled control coefficients and enzyme levels in the optimised state are proportional, i.e., enzymes with a large relative influence on the flux are abundant.
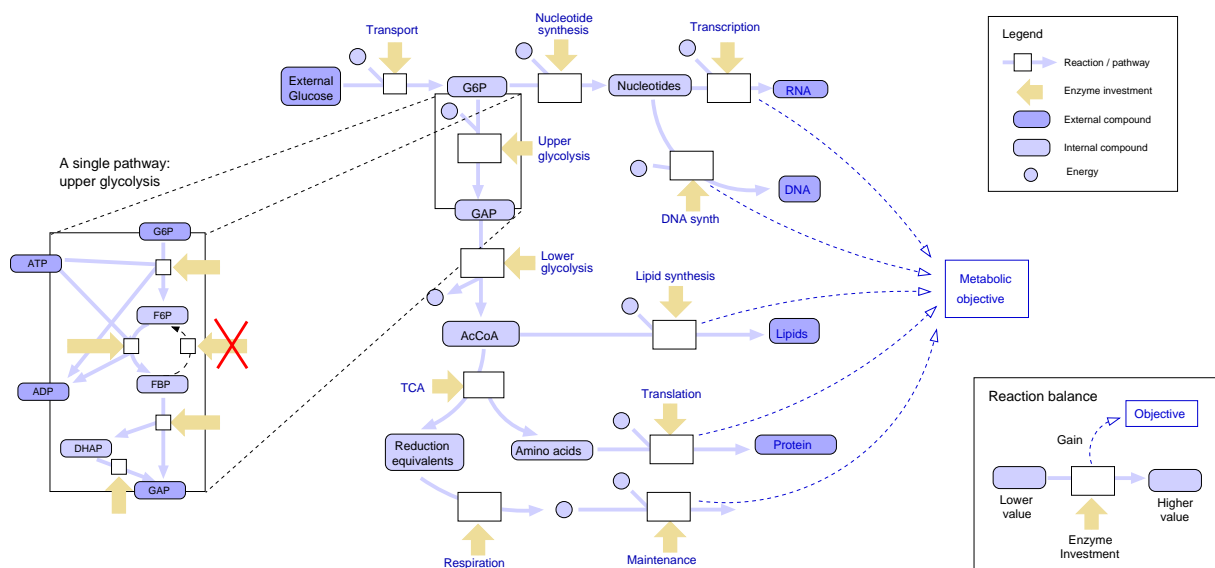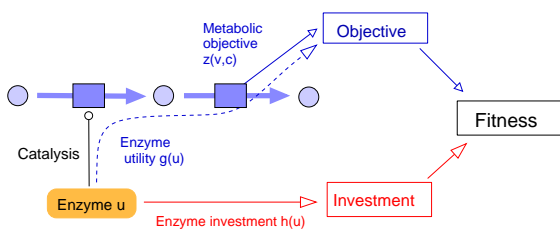
Figure 1: A cell's metabolic network resembles a chemical plant or a planned economy. Substance conversion by reactions or pathways must be catalysed by enzymes (yellow arrows). This entails costs, which need to be balanced by benefits. Metabolic economics assumes that enzyme levels are optimised for a maximal metabolic performance at minimal enzyme investments. The metabolic objective can depend on production rates, fluxes, and metabolite concentrations. As a general principle (bottom right), enzyme usage must be justified by a benefit, either a conversion from lower-valued to higher-valued metabolites or a direct contribution to the metabolic objective. Economic "values", called economic potentials, can be defined based on metabolic control theory. The same principles apply also to individual pathways (detailed model of upper glycolysis on the left, where a futile cycle is suppressed by repression of FBP). If the optimality problem is locally formulated for this pathway, metabolites on the pathway boundary are treated as external and their economic potentials define a local objective function.

Here, I address more general optimality problems for enzyme levels and introduce new concepts for their description. The problems could be solved numerically, yielding solutions for each particular case. However, I am interested in general principles, as exemplified by thermodynamics: thermodynamic notions like chemical potentials can greatly help understand the behaviour of kinetic models in general terms which hold for a vast range of systems. Similarly, I attempt to find general notions and laws for the economics of metabolic systems. Cell metabolism can be pictured as a chemical plant or as a planned economy (see Figure 1) with a predefined biological objective. The objective induces a demand for substances and fluxes, and optimally chosen enzyme levels will meet these demands.

The theory, called metabolic economics, can be formulated in terms of state variables called economic potentials and loads. Economic notions are formally derived from metabolic control analyis and allow one to characterise enzyme-optimal states in general and to realise such states systematically by particular kinetic models. The economic variables describe local demands for metabolite concentrations and production induced by the system's global objective function (see Figure 1) and help clarify the logic behind enzyme usage. With their help, the optimality conditions for enzymes can be written as local balance equations between neighbouring compounds and reactions. The equations make it possible to construct kinetic models in enzyme-optimal states with predefined fluxes. Such models are needed for cost-benefit studies, for instance, on enzyme adaptions to environmental changes [3] or on the benefit and cost of allosteric regulation [9].

The benefit principle – the fact that active enzymes must contribute to the metabolic benefit – is closely related to the principle of minimal fluxes [10], a variant of flux balance analysis. As a link between both

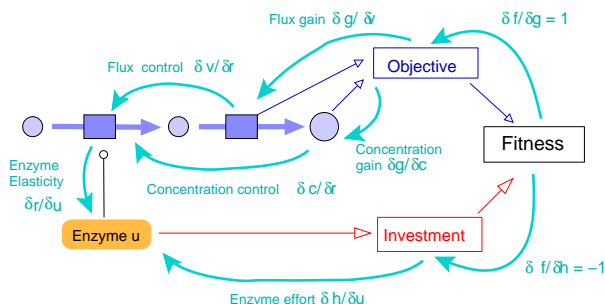(a) Fitness effects of an enzyme (b) Tracing back fitness effects to an enzyme

Figure 2: How metabolic network elements obtain their economic values. (a) Notions for enzyme-optimal states in kinetic models. An enzyme (level u) catalyses a reaction, affects the fluxes v and concentrations c, and contributes to the metabolic return $g(u) = z(v(u), c(u))$. The effort for maintaining the enzyme is described by an investment function $h(u)$, and the fitness function is the difference $f(u) = g(u) - h(u)$. A change in the enzyme level would affect the fitness in both ways. (b) Turning this around, we can characterise each enzyme by a *demands*, stating how its possible changes would affect the return. Using the chain rule, the total derivative can be split into local derivatives (here symbolically written as ratios of differentials $\frac{\delta g}{\delta u} = [\frac{\delta g}{\delta \mathbf{v}} \frac{\delta \mathbf{v}}{\delta r} + \frac{\delta g}{\delta \mathbf{c}} \frac{\delta \mathbf{c}}{\delta r}] \frac{\delta r}{\delta u}$). Similar chains of derivatives exist for external metabolites (not shown). In enzyme-balanced states, the demand $\delta g/\delta u$ for an enzyme is balanced with its *price* $\frac{\delta h}{\delta u}$. Metabolic economics extends this idea from model parameters (enzyme levels and external metabolites) to all metabolites and reactions in the network.

theories, I introduce the notion of economically feasible fluxes, metabolic flux distributions that have the right sign pattern to appear in enzyme-optimal states. In particular, economic fluxes must lead from lower to higher economic potentials. This constraint excludes economically futile modes in a similar way as thermodynamic constraints exclude non-productive cycles.

In this article, I introduce metabolic economics in its basic version and discuss possible extensions. Definitions and theorems are given in the appendix, proofs and other details in the Supplementary Information (SI) at www.metabolic-economics.de/metabolic-economics/. MATLAB code is freely available on github [11].

2   Kinetic models with optimal enzyme levels

In metabolic economics, we study kinetic metabolic models in which enzyme levels are control variables and chosen to maximise a fitness function (Figure 2 (a)). The fitness is given by the difference between a metabolic return g, the metabolic objective in steady state, and an enzyme investment h [2, 3, 4]. The same optimality condition can be mathematically derived from other optimality approaches (e.g., maximizing the growth rate at constrained compound concentrations). To describe states of optimal fitness, we need to understand how changes in single network elements would affect the fitness directly or indirectly. We can ask which elements cause a direct fitness change, what causes a change in those elements, and so on. This can be traced back by taking derivatives (schematically shown in Figure 2 (b)). Since we are concerned with steady state changes, this requires notions from Metabolic Control Analysis.

We first need some terminology for kinetic models (for details, see SI; mathematical symbols are listed in Tables 1 and 2). The metabolites in a kinetic model can be external (with fixed concentrations x_j) or internal (variable concentrations c_i) and reaction rates are given by rate laws $r_l(\mathbf{u}, \mathbf{c}, \mathbf{x}) = u_l\, r_l'(\mathbf{c}, \mathbf{x})$. The metabolic objective is given by a function $z(\mathbf{v}, \mathbf{c})$ of the fluxes v_l and internal concentrations c_i (which does not cover the cost of enzyme production). In whole-cell models, the objective function may rise with

the biomass production rate and decrease with the total metabolite level. In pathway models, it could score the consumption and production of substrates, products, and cofactors. The derivatives of the objective function with respect to fluxes and metabolite levels are called flux gains $z_l^{\mathrm{v}} = \partial z / \partial v_l$ and concentration gains $z_i^{\mathrm{c}} = \partial z / \partial c_i$. The gains are marginal quantities, describing how small variations of the state variables v_l and c_i affect the metabolic objective. If the objective depends on fluxes only (*flux objective* $z(\mathbf{v})$), the concentration gains vanish. The flux gain vector $\mathbf{z}^{\mathrm{v}}$ can be split in two parts

$$\mathbf{z}^{\mathrm{v}} = \mathbf{N}^{\mathrm{x}\top}\mathbf{z}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}} \tag{1}$$

where $\mathbf{N}^{\mathrm{x}}$ is the stoichiometric matrix for external metabolites, the production gain vector $\mathbf{z}^{\mathrm{x}}$ scores the production or consumption of external metabolites, and the remaining gains $\hat{z}_l^{\mathrm{v}}$ are directly attributed to reactions (e.g., scoring heat production). How $\mathbf{z}^{\mathrm{v}}$ is split precisely is a matter of choice[1]. A metabolic objective without direct flux gains is called *production objective*. Flux distributions $\mathbf{v}$ (i.e., stationary flux distributions) can be characterised by their flux benefit $b = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$. Those with a positive benefit are called *beneficial*, those with a negative benefit are called *costly*, and those with a vanishing benefit, satisfying $\mathbf{N}^{\mathrm{z}} \mathbf{v} = \left(\begin{smallmatrix}\mathbf{z}^{\mathrm{v}\top}\\\mathbf{N}\end{smallmatrix}\right) \mathbf{v} = 0$, are called *non-beneficial*. Non-beneficial and costly modes are also called *futile*. A flux distribution is *complete* if all reactions are active, i.e. carry non-zero fluxes.

The fitness function is defined by the difference between metabolic objective and enzyme investment[2]:

$$f(\mathbf{u}, \mathbf{x}) \quad = \quad z(\mathbf{J}(\mathbf{u}, \mathbf{x}), \mathbf{S}(\mathbf{u}, \mathbf{x})) - h(\mathbf{u}) = g(\mathbf{u}, \mathbf{x}) - h(\mathbf{u}). \tag{2}$$

The metabolic objective $g(\mathbf{u}) = z(\mathbf{J}(\mathbf{u}, \mathbf{x}), \mathbf{S}(\mathbf{u}, \mathbf{x}))$ in steady state, as a function of the enzyme levels u_l, is called *metabolic return function*. Its derivatives are called enzyme demand $g_l^{\mathrm{u}} = \partial g / \partial u_l$, or using the logarithmic derivative $b_l^{\mathrm{u}} = \partial g / \partial \ln u_l$, enzyme benefit. A beneficial flux distribution may still be wasteful for cells if it requires too much enzyme. To capture this in our models, the investment function $h(\mathbf{u})$ is subtracted from the metabolic return [3] (for details, see appendix). Its derivatives are called enzyme price $h_l^{\mathrm{u}} = \partial h / \partial u_l$ and enzyme cost $y_l = \partial h / \partial \ln u_l$. In particular, enzymes that do not contribute to the metabolic return should be inactive to save enzyme costs.

Metabolic economics is based on an optimality principle: the enzyme levels, as control variables, are chosen such that the fitness function reaches a local optimum. The enzyme-optimal state contain inactive enzymes and may thus be a boundary optimum[3]. Extensions of the optimality principle – including models with unspecific enzymes and non-enzymatic reactions, more constraints, and multi-objective optimisation – are discussed below. Our main aim will be to translate the optimality conditions for enzymes into economic laws for metabolic fluxes, which should remain valid even if the kinetic model, with its rate laws and investment function, is not precisely known.

The optimality condition for enzymes differs between active reactions ($v_l \neq 0$ and $u_l > 0$) and inactive reactions ($v_l = 0$ and $u_l = 0$). Inactive reactions with positive enzyme levels ($v_l = 0$ and $u_l > 0$) would be wasteful and can be ignored. For active reactions, the optimality condition reads $\partial f / \partial u_l = 0$ and implies a

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[1]On the one hand, we can set $\mathbf{z}^{\mathrm{x}} = 0$, and all flux gains z_l^{v} will be given as direct flux gains $\hat{z}_l^{\mathrm{v}}$. On the other hand, we could set all direct flux gains $\hat{z}_l^{\mathrm{v}}$ to zero and replace them by production gains z_j^{x} of virtual external metabolites (which are introduced only for this purpose).

[2]For fast growing microbes, we might consider the growth rate as an objective function to be maximized. Such a growth objective, under constraints on substrate levels to be treated with Lagrange multipliers, leads to fitness functions like Eq. (2).

[3]In practice, finding realistic states by numerical enzyme optimisation can be difficult because of local maxima in the fitness. There may be a "locked state" at $\mathbf{u} = 0$, where enzyme levels, internal metabolite levels, and fluxes vanish. The locked state is a local optimum with an "activation barrier" for enzyme levels: only if enough enzymes are brought to sufficiently high levels, the system gets into the economic basin of attraction of a profitable enzyme-balanced state, and a further enzyme activation becomes beneficial. An alternative method for constructing enzyme-balanced states, which avoids this problem, is described below.

(a) Enzyme benefit and cost
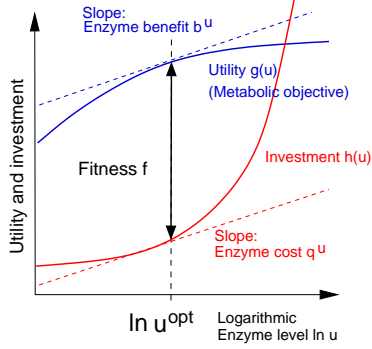
(b) Economic state variables

Derivative with respect to:

	log Enzyme level	Enzyme level	Flux (virtual derivative)
Fitness: Objective – investment	Economic imbalance y^Δ	Enzyme stress f^{u}	Flux stress f^{v}
Return function = Metabolic objective	Enzyme benefit b	Enzyme demand g^{u}	Flux demand g^{v}
Investment function	Enzyme cost y	Enzyme price h^{u}	Flux price h^{v}

Figure 3: (a) Cost-benefit balance. In an enzyme-optimal state, the slopes of investment h and return g as functions of $\ln u$ must be equal, i.e. enzyme benefit and cost must be balanced. Since enzyme costs are positive, the enzyme must have a positive benefit, i.e. a positive control over the metabolic return. (b) Fitness, metabolic return, investment, and their derivatives.

balance $g_l^{\mathrm{u}} = h_l^{\mathrm{u}}$ between enzyme demand and price (see Figures 3 and 10). Using logarithmic derivatives, we obtain the cost-benefit balance

$$v_l \neq 0 \quad \Rightarrow \quad \frac{\partial g}{\partial \ln u_l} = \frac{\partial h}{\partial \ln u_l} \tag{3}$$

between *enzyme benefit* $b_l = \frac{\partial g}{\partial \ln u_l} = g_l^{\mathrm{u}} u_l$ and *enzyme cost* $y_l = \frac{\partial h}{\partial \ln u_l} = h_l^{\mathrm{u}} u_l$. It must hold for all active enzymatic reactions to allow for an enzyme-optimal state[4]. If a flux distribution $\mathbf{v}$ can be realised by kinetic models satisfying Eq. (3), it is called *enzyme-balanced* or, if all reactions are active, *completely enzyme-balanced*. Eq. (3) has a simple and important consequence: since active enzymes have positive costs, they must also have positive benefits. This is called the *benefit principle*. For inactive reactions, the enzyme level vanishes and the optimality condition reads $\partial f/\partial u_l < 0$: the enzyme price h_l^{u} exceeds the enzyme demand g_l^{u}, and expressing the enzyme would decrease the fitness (see Figure 10).

3 Gain conditions and economical fluxes

Does the cost-benefit balance Eq. (3) say anything general about flux distributions, anything that does not depend on specific rate laws or investment functions? At first sight, this seems unlikely because the sensitivities g_l^{u} depend on the rate laws. However, we can directly link them to fluxes by metabolic control analysis (MCA) [7, 12]. In the language of MCA (see appendix), the enzyme demands g_l^{u} are response coefficients between the return g and the enzyme levels u_l. The corresponding control coefficients $g_l^{\mathrm{v}} = g_l^{\mathrm{u}}/\bar{E}_{\mathrm{u}_l}^{\mathrm{v}_l} = g_l^{\mathrm{u}} \frac{u_l}{v_l}$ are called *flux demands*. With their help, we can rewrite the enzyme demand as $g_l^{\mathrm{u}} = g_l^{\mathrm{v}} \frac{u_l}{v_l}$ and the enzyme benefit as $g_l^{\mathrm{u}} u_l = g_l^{\mathrm{v}} v_l$. The cost-benefit balance Eq. (3) now reads

$$g_l^{\mathrm{v}} v_l = h_l^{\mathrm{u}} u_l \tag{4}$$

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[4]The balance equation in its two versions is analogous to the relations found in [1], where a flux was maximised under a fixed sum of enzyme levels, corresponding to a uniform investment function for all enzymes (equal prices $h_l^{\mathrm{u}} = \partial h/\partial u_l$). The finding that all flux response coefficients are equal is an example of the price-demand balance $g_l^{\mathrm{u}} = h_l^{\mathrm{u}}$, and the proportionality between scaled flux control coefficients and enzyme levels is an example of the cost-benefit balance $g_l^{\mathrm{u}} u_l = h_l^{\mathrm{u}} u_l$.

with flux benefits $b_l = g_l^{\mathrm{v}} v_l$ and enzyme costs $y_l = h_l^{\mathrm{u}} u_l$, and the benefit principle can be written as $g_l^{\mathrm{v}} v_l > 0$. Dividing Eq. (4) by the flux, we obtain the balance

$$g_l^{\mathrm{v}} = h_l^{\mathrm{u}} \frac{u_l}{v_l} \tag{5}$$

between flux demand and the flux price $h_l^{\mathrm{v}} = h_l^{\mathrm{u}} \frac{u_l}{v_l}$ (i.e., the enzyme cost per flux). The flux demands g_l^{v} in Eqs (4) and (5) still depend on the model kinetics. However, since they are control coefficients, we can apply the summation and connectivity theorems of metabolic control theory [13] and rewrite Eq. (5) in the general form

$$\mathbf{K}^\top \mathrm{Dg}(\mathbf{y}) \, \mathbf{v}^{-1} \;\;=\;\; \mathbf{K}^\top \mathbf{z}^{\mathrm{v}} \tag{6}$$
$$(\bar{\mathbf{E}} \, \mathbf{L})^\top \mathrm{Dg}(\mathbf{y}) \, \mathbf{v}^{-1} \;\;=\;\; -\mathbf{L}^\top \mathbf{z}^{\mathrm{c}} \tag{7}$$

called *flux gain condition* and *concentration gain condition* (Theorem 1 in Appendix). The symbols are explained in the appendix. The vector $\mathbf{y}$ contains the enzyme costs $h_l^{\mathrm{u}} u_l$, the vector $\mathbf{v}^{-1}$ contains the inverse fluxes, and $\mathrm{Dg}(\mathbf{y}) \, \mathbf{v}^{-1}$ is the vector of flux prices $h_l^{\mathrm{v}} = y_l / v_l$. The equations hold for complete, fully enzymatic flux distributions. Inactive reactions must be omitted from the model, and in models with non-enzymatic reactions, the gain conditions must be modified (see SI P1.4). The gain conditions are our first main result: they relate the inverse fluxes to enzyme costs, flux gains z_l^{v}, and concentration gains z_i^{c}. Even if $\mathbf{y}$ and $\bar{\mathbf{E}}$ are unknown, the fact that $\mathbf{y}$ must be positive constrains the fluxes considerably. Together, both gain conditions completely determine the enzyme costs. For instance, for a simple metabolic pathway with flux objective $z(\mathbf{v})$, condition (6) relates the total enzyme cost to the total flux benefit[5] and condition (7) determines the ratios of enzyme costs along the chain[6].

If a metabolic state is enzyme-balanced with known enzyme costs, Eq. (6) and the stationarity condition determine the fluxes (see [14]). But even if the costs are unknown, their mere existence constrains the fluxes: flux distributions that can satisfy Eq. (6) with positive enzyme costs y_l are called economically feasible, or briefly *economical* (again, inactive reactions are assumed to be omitted, while flux distributions that vanish completely are defined to be uneconomical.). The notion of economical fluxes links fluxes and enzyme demands in a subtle way: economical flux distributions are not just beneficial ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} = 0$), but *locally beneficial*, i.e. each enzyme contributes to the benefit. Importantly, a flux distribution must be economical to be enzyme-balanced. Uneconomical flux distributions would entail a waste of enzyme resources, no matter which kinetic model we assume.

How can we check if a given flux distribution is economical? As a general criterion, it must be free of futile modes, which are mathematically defined by *test modes*: given a flux distribution $\mathbf{v}$, a non-vanishing flux distribution $\mathbf{k}$ on the active enzyme-catalysed subnetwork is called a test mode of $\mathbf{v}$. With the flux gain vector $\mathbf{z}^{\mathrm{v}}$, test modes can be classified as beneficial, non-beneficial, or costly. The active reactions shared by $\mathbf{v}$ and $\mathbf{k}$ form the *shared active region*, and if flux distributions $\mathbf{v}$ and $\mathbf{k}$ share all flux directions on their shared active region, they are *sign-concordant*. Now we can define futile modes in $\mathbf{v}$: if a test mode $\mathbf{k}$ is futile and sign-concordant with $\mathbf{v}$, the reactions on the shared active region, with their flux directions, are called a *futile mode* in $\mathbf{v}$ for $\mathbf{z}^{\mathrm{v}}$. If $\mathbf{v}$ is economical and $\mathbf{k}$ one of its test modes, the following holds (test mode theorem, 2, see Figure 4): if $\mathbf{k}$ is beneficial, it contains active reactions with the same flux direction as $\mathbf{v}$; if $\mathbf{k}$ is costly, it contains at least one flux with the opposite direction as $\mathbf{v}$; and if $\mathbf{k}$ is non-beneficial, both sorts of fluxes exist. The test mode theorem follows from Eq. (6) and from the fact that v_l and v_l^{-1} have equal signs. Like the gain conditions (6) and (7), it only applies if $\mathbf{v}$ is complete. Otherwise,

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[5]The kernel matrix $\mathbf{K}$ in Eq. (6) can be replaced by stationary flux distributions $\mathbf{k}$. If we replace it by $\mathbf{v}$ itself, we obtain the equality $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} = \sum_l y_l = \sum_l h_l^{\mathrm{u}} u_l$, so total flux benefit and enzyme cost must be equal. If we assume objective functions of the form $z(\mathbf{v}) = (\sum_l z_l^* v_l)^\beta$ and investment function $h(\mathbf{u}) = (\sum_l h_l^* u_l)^\gamma$, then Euler's theorem for homogeneous functions allows us to rewrite the optimality condition without derivatives: $\beta \, z(\mathbf{v}) = \gamma \, h(\mathbf{u})$.

[6]In models without moiety conservation, the link matrix $\mathbf{L}$ in Eq. (7) is an identity matrix $\mathbf{I}$. With equal fluxes through all reactions and with $\mathbf{z}^{\mathrm{c}} = 0$, we obtain a condition $\bar{E}_{c_i}^{v_l} y_l + \bar{E}_{c_i}^{v_{l+1}} y_{l+1} = 0$ for each metabolite i. The enzyme costs for producing a metabolite and for its consumption are inversely proportional to the elasticities $\bar{E}_{c_i}^{v_l} = \partial v_l / \partial c_i$.

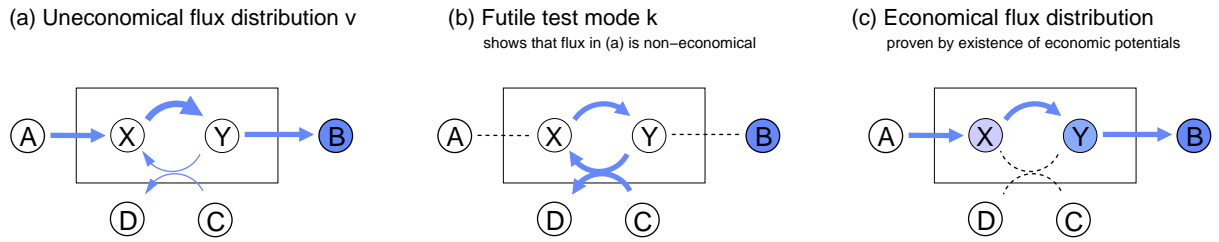| (a) Uneconomical flux distribution v	(b) Futile test mode k shows that flux in (a) is non–economical	(c) Economical flux distribution proven by existence of economic potentials

Figure 4: Economical flux distributions and test mode theorem. (a) Schematic pathway with the production of B as the metabolic objective. A flux distribution is economical if the flux gain condition can be satisfied. To show that this is not the case here, we consider a test mode k (shown in (b)), which is futile and sign-concordant with $\mathbf{v}$. According to the test mode theorem, the existence of $\mathbf{k}$ shows that $\mathbf{v}$ cannot be economical. We can see this from the flux gain condition (6): since $\mathbf{z}^{\mathrm{v}}$ scores only the production flux of B, we obtain the right-hand side $\mathbf{k} \cdot \mathbf{z}^{\mathrm{v}} = 0$, contradicting $\mathbf{k}^{\top} \mathrm{Dg}(\mathbf{y}) \mathbf{v}^{-1} \neq \mathbf{0}$ on the left. (c) Criterion for economical flux distributions. To prove that the fluxes are economical, we assign economic potentials (shades of blue) to all internal metabolites, increasing along the fluxes. The external economic potentials (of A, B, C, and D) are predefined by the metabolic objective. With the flux cycle in (a), and assuming that the economic potentials of C and D vanish, this would not be possible.

the inactive reactions must be omitted, and flux weights z_l^{y} and test modes $\mathbf{k}$ are defined on the active network.

The test mode theorem implies that economical flux distributions are free of futile modes. But also the opposite holds: according to the economical flux theorem (Theorem 3), flux distributions that are free of futile modes (which entails being beneficial) are economical. Thus, being economical depends only on the flux sign pattern and can be checked by a search for elementary futile modes[7]. Figure 4 shows an example: the pathway flux in (b), used as a test mode, are non-beneficial and sign-concordant with the one (a). Therefore, the pathway flux in (a) is uneconomical and cannot be realised by enzyme-optimal models. The fluxes in (c), in contrast, are economical.

4   Economic potentials and loads

The gain conditions (6) and (7) refer to inverse fluxes, which is unintuitive and difficult to handle in flux analysis. For a practical formalism, I now introduce economic state variables – called economic potentials and loads – which reflect the economic values of single metabolites (see Figure 5). The economic potentials refer to metabolite production, describing how virtual metabolite supplies would increase the metabolic return. The economic loads, in contrast, refer to metabolite levels, describing how virtual concentration changes would change the metabolic return. Both variables refer to a given metabolic state and describe indirect effects, that is, economic effects mediated by other reactions.

Let us see this in more detail. The economic potentials describe how an extra steady supply of metabolites would affect the metabolic return. For external metabolites, the economic potential w_j^{x} is given by the production gain z_j^{x}. For an internal metabolite i, we imagine a virtual supply flux φ_i, which changes the steady state. The metabolic return becomes a function $g(\mathbf{u}, \mathbf{x}, \varphi)$ of enzyme levels u_l, external levels x_j, and supply fluxes[8] φ_i, and the economic potential of metabolite i is defined[9] by $w_i^{\mathrm{c}} = \frac{\partial g}{\partial \varphi_i}$. If a model

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[7]One only needs to check for non-elementary modes because any flux distribution with a futile mode will also contain an elementary futile mode.

[8]The supply fluxes have no direct biological meaning. However, if we imagine that the cell realises the virtual fluxes by transporter proteins, the economic potential of a metabolite corresponds to the price of the transporter at the break-even point (where benefit and cost of the transporter cancel out).

[9]In the definition of economic potentials, the enzyme levels remain fixed. We could also assume that cells adapt their
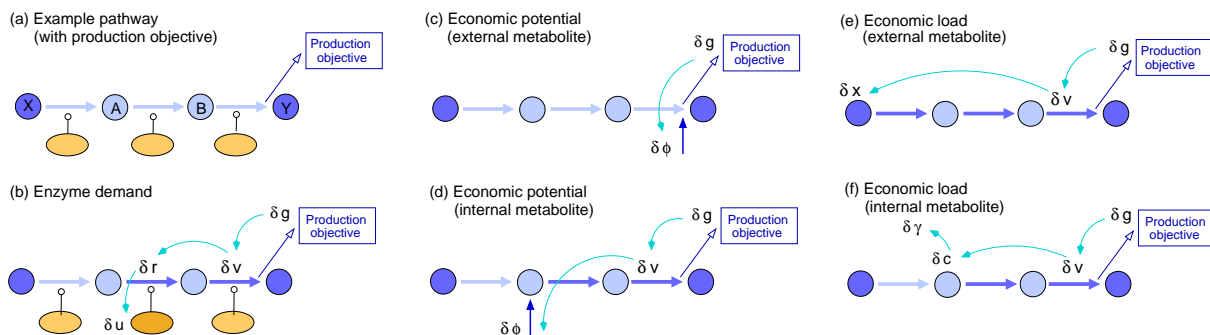
Figure 5: Economic potentials and loads. (a) Schematic example pathway with a production objective (scoring the production of Y). (b) The enzyme demand measures how the change of an enzyme level would affect the metabolic return. This influence can be broken down into derivatives (or ratios of differentials $\frac{\delta g}{\delta u} = \frac{\delta g}{\delta v} \frac{\delta v}{\delta r} \frac{\delta r}{\delta u}$) in the network. (c) Economic potentials of external metabolites are defined by effects of virtual production changes. (d) Economic potentials of internal metabolites are defined by effects of virtual supply fluxes. (e) Economic loads of external metabolites are defined by effects of virtual concentration changes. (f) Economic loads of internal metabolites are defined by effects of virtual concentration changes.

contains conserved moieties (e.g., if the reaction stoichiometries guarantee that the sum [ATP]+[ADP] is constant in time), some internal metabolite levels will depend linearly on others [15] and supply fluxes could destroy the steady state. Therefore, we only allow supply flux vectors $\varphi = \mathbf{L}\,\varphi^{\mathrm{ind}}$ (where the supply fluxes of independent metabolites in φ^{ind} can be freely chosen). By definition, the economic potentials of dependent metabolites vanish. However, we can also assign economic potentials to conserved moieties, which will shift the economic potentials of metabolites. This is a gauge transformation, which changes their numerical values, but without any impact on other quantities (see SI S2.3).

The economic loads describe the economic effects of metabolite levels or other quantities, like growth rate, compartment sizes, or the temperature. A metabolite's concentration demand describes how a virtual concentration change of metabolite i would affect the metabolic return. It consists of a direct demand – the metabolite gain – and an indirect demand, arising from its influence on the steady state. The indirect demand is called *economic load*. External parameters and metabolite levels x have no direct fitness effects, so their demands are given by their loads $p_j^{\mathrm{x}} = \frac{\partial g}{\partial x_j}$. For an internal metabolite, the load describes how virtual concentration variations would affect the return via changes of the adjacent reaction rates. In systems without moiety conservation, virtual variations would evoke a metabolic response that eliminates the original variation. Thus, the demand of an internal metabolite vanishes and the load $p_i^{\mathrm{c}} = -z_i^{\mathrm{c}}$ is given by the negative concentration gain. In systems with moiety conservation, the virtual variation will change the conserved moieties and cannot be eliminated, so the load contains additional terms. However, the equation $\mathbf{L}\,\mathbf{p}^{\mathrm{c}} = -\mathbf{L}\,\mathbf{z}^{\mathrm{c}}$ remains valid. Loads for other state variables are defined accordingly.

If a kinetic model is in an enzyme-optimal state, the economic variables satisfy general laws in the form of local balance equations. With these laws, we can characterise the economics of metabolic states simply and generally (see Figure 6 and the thought experiments in SI S1). The economic potentials play a key role because of the *reaction rule*: the total flux demand g_l^{v} of a reaction consists of two terms[10],

enzyme levels to the supply fluxes in order to increase the fitness. However, this is a second-order effect and therefore irrelevant for the (first-order) economic potentials, so enzyme adaption can be ignored in the definition (see SI P3.2).

[10]The reaction rule can be derived from a thought experiment (see Figure S1). Imagine a cell that can vary its enzyme levels and supply fluxes φ_i and performs a *compensated enzyme variation*. First, it decreases an enzyme level by a variation δu where the differential δa of a state variable a is defined as the vector of its derivatives with respect to all model parameters, for instance u_l and x_j). This variation would evoke an immediate rate variation $\delta v_l = \bar{E}_{u_l} \delta u_l$, which perturbs the mass balances of substrates and products. The cell restores the original state by adding supply fluxes $\delta\varphi_i = -n_{il}\,\delta v_l$. The metabolites remain mass-balanced, and the steady-state concentrations and fluxes remain unchanged except for the flux of the perturbed
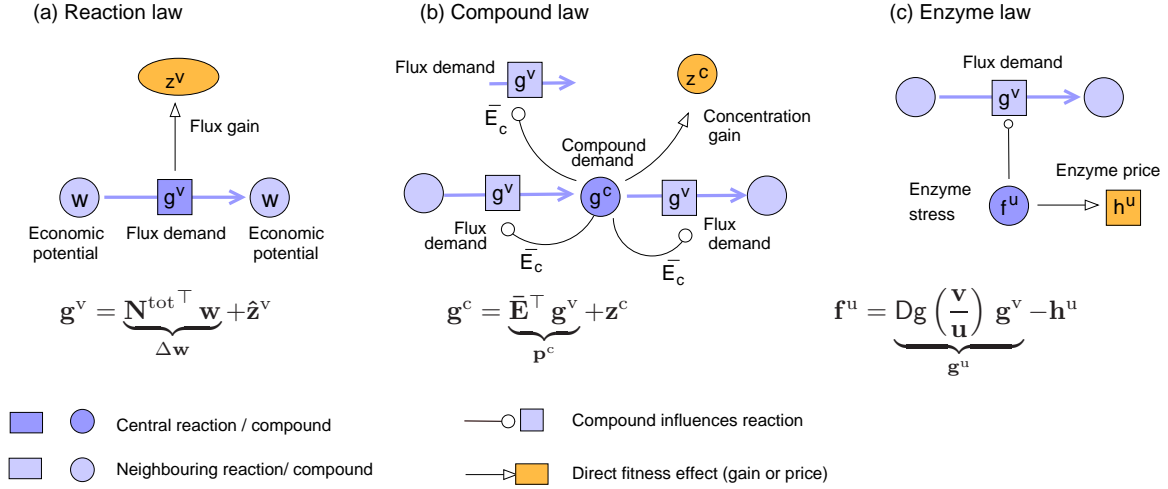
Figure 6: The economic state variables are linked by local laws. (a) Reaction law. A reaction's flux demand consists of an economic potential difference Δw^{c} ("indirect flux demand") and a direct flux gain $\hat{z}^{\mathrm{v}}$ ("direct flux demand"). (b) Compound law. A metabolite's concentration demand consists of the economic load ("indirect concentration demand") and the concentration gain z^{c} ("direct concentration demand"). In models without moiety conservation, the total concentration demand vanishes, so economic load and concentration gain have equal values and opposite signs. Similar laws hold for other quantities affecting the reaction rates like the temperature. (c) Enzyme law. The enzyme stress is the difference between enzyme demand $g^{\mathrm{u}} = g^{\mathrm{v}}\frac{v}{u}$ and enzyme price h^{u}. In optimal states, enzyme stresses vanish and enzyme demands and prices are equal.

$$g_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} + \Delta w_l, \tag{8}$$

the flux gain $\hat{z}_l^{\mathrm{v}}$ ("direct flux demand") and the balance $\Delta w_l = \sum_i n_{il}\, w_i$ of economic potentials along the reaction ("indirect flux demand") (see SI P3.1). This is important: even though the total flux demand is a systemic property, it can be expressed in terms of local variables (see SI P5.1).

The *compound rule* (proof SI P4.2)

$$p_i^{\mathrm{c}} = \sum_l g_l^{\mathrm{v}}\, \bar{E}_{\mathrm{c}i}^{\mathrm{v}_l} = \sum_l [\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, \bar{E}_{\mathrm{c}i}^{\mathrm{v}_l} \tag{9}$$

links a metabolite's load p_i^{c} to the flux demands g_l^{v} of the adjacent reactions (i.e. reactions whose rates are directly affected by it) and, via the reaction rule, to the economic potentials w_i in its neighbourhood. Reaction and compound rule do not require an optimal state, and the reactions need not be enzymatic. Given the elasticities, a metabolite's load can be computed from the demands for the fluxes affected by this metabolite, i.e., from the flux gains and economic potentials in its neighbourhood. The economic loads of external metabolites (or other model parameters that affect the reaction rates) satisfy rules similar to Eq. (19). Using the compound rule, we can compute the internal economic potentials from given gains and elasticities. In the general case (systems with moiety conservation and dilution), the internal economic

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reaction. The resulting fitness difference can be computed in two ways: (i) by the effect $z_l^{\mathrm{v}}\,\delta v_l$ exerted directly by the reaction, or (ii) by the systemic effect $g_l^{\mathrm{v}}\,\delta v$ of the uncompensated enzyme variation plus the systemic effect $\sum_i w_i^{\mathrm{c}}\,\delta\varphi_i = -\Delta w_l^{\mathrm{c}}\,\delta v_l$ of the compensating virtual fluxes. Equating both expressions and dividing by δv, we obtain the identity $g_l^{\mathrm{v}} = z_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}}$. Splitting the flux gain into $z_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} + \Delta w_l^{\mathrm{x}}$, we can rewrite the flux demand as $g_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} + \Delta w_l^{\mathrm{x}} + \Delta w_l^{\mathrm{c}}$, or as $g_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} + \Delta w_l$. One time, the term $\Delta\mathbf{w}^{\mathrm{x}}$ for external metabolites is included in $\mathbf{z}^{\mathrm{v}}$, the other time in $\Delta\mathbf{w}$ (for more details, see SI S1).
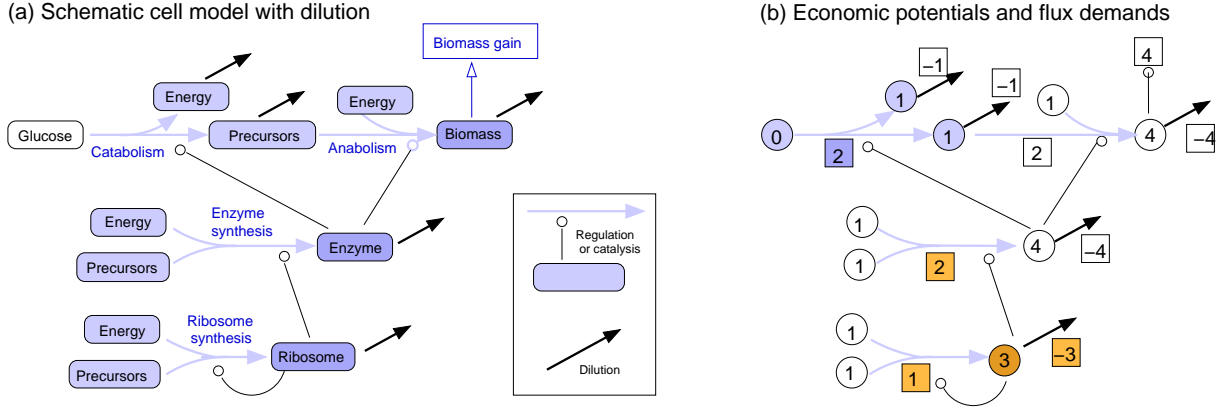
Figure 7: Economic balances in growing cells (schematic example). (a) Biomass is produced from glucose via precursor molecules; the reactions are catalysed by enzymes, which are produced by ribosomes. (b) Economic potentials and flux demands must satisfy two types of relations: (i) Reaction law: in each reaction, the flux demand (squares) equals the difference of economic potentials (circles). For instance, in catabolism (blue): $w^{\mathrm{v}} = w^{\mathrm{c}}_{\mathrm{Energy}} + w^{\mathrm{c}}_{\mathrm{Precursor}} - w^{\mathrm{c}}_{\mathrm{Glucose}} = 1 + 1 - 0 = 2$. (ii) Compound law: for each compound, the flux demands in the adjacent reactions, weighted with the elasticities, must vanish (e.g. ribosome (yellow): $1 \cdot w^{v}_{\mathrm{RibSynth}} + 1 \cdot w^{v}_{\mathrm{EnzSynth}} + 1 \cdot w^{v}_{\mathrm{RibDeg}} = 1 + 2 - 3 = 0$). The numbers are based on a simple choice of reaction elasticities.

potentials are given by (proof in SI P4.3)

$$\mathbf{w}^{\mathrm{c}} = -[\mathbf{L}\,\mathbf{M}^{\mathrm{dil}^{-1}}\,\mathbf{I}_{\mathrm{R}}]^{\top}\,\mathbf{z}^{\mathrm{c*}} \tag{10}$$

with the effective gain $\mathbf{z}^{\mathrm{c*}} = \bar{\mathbf{E}}^{\top}\mathbf{z}^{\mathrm{v}} + \mathbf{z}^{\mathrm{c}}$, the Jacobian matrix[11] $\mathbf{M}^{\mathrm{dil}} = \mathbf{N}_{\mathrm{R}}\,\bar{\mathbf{E}}\,\mathbf{L} - \kappa\,\mathbf{I}$, and $\mathbf{I}_{\mathrm{R}}$, the projector from internal to independent metabolites.

As an example, consider the schematic model of a growing cell in Figure 7. In the model, biomass is produced from external glucose via a pool of precursors. A catabolic reaction produces precursors and energy (high-energy phosphate groups in ATP) and an anabolic reaction converts them into biomass. Enzymes are not treated as control variables but as metabolites with a catalytic activity. Producing them requires energy and precursors, and the same holds for ribosome production. All cell compounds are diluted due to cell growth (with growth rate κ). The metabolic objective is represented by a concentration gain for biomass, equivalent to a demand for fast growth at a constrained biomass concentration. The economic potential for external glucose ($w_{\mathrm{glucose}} = 0$) and the biomass gain ($z^{\mathrm{c}}_{\mathrm{biomass}} = 4$) are fixed and given. From the reaction and compound rules, and with a simple choice of elasticities, we obtain internal economic potentials $\mathbf{w}^{\mathrm{c}} = (1, 1, 4, 3)^{\top}$ and flux demands $\mathbf{g}^{\mathrm{v}} = (2, 2, 2, 1)^{\top}$ (for details, see SI P6.3).

5 Economic balance equations

Reaction and compound rules show how economic potentials and loads are related to flux demands and reaction elasticities. To link them to enzyme costs, we need to include the optimality assumption. By inserting the cost-benefit balance Eq. (3) into the reaction rule Eq. (8), we obtain the reaction balance

$$[\hat{z}^{\mathrm{v}}_{l} + \Delta w_{l}]\,v_{l} = h^{\mathrm{u}}_{l}\,u_{l}, \tag{11}$$

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[11] The inverse Jacobian also appears in the formula for control coefficients [15].

(a) Reaction balance   (b) Compound balance

$$[\Delta w_l + \hat{z}^{\mathrm{v}}_l]\, v_l = y_l \qquad\qquad p^{\mathrm{c}}_i\, c_i = -z^{\mathrm{c}}_i\, c_i = \sum_l E^{\mathrm{v_l}}_{\mathrm{c}_i}\, y_l$$
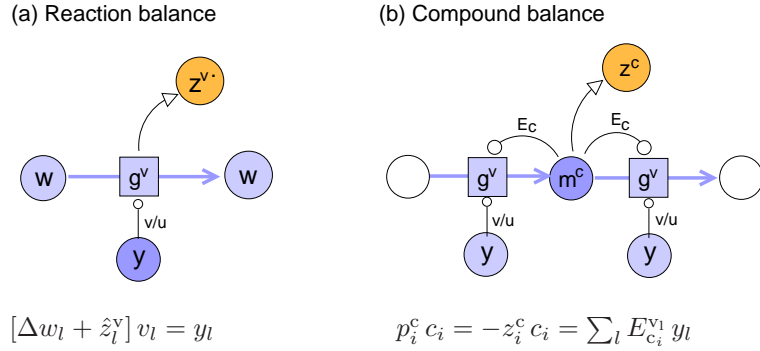
Figure 8: Local balances between enzyme costs, economic potentials, and economic loads. (a) A balance between flux benefit and enzyme cost ("reaction balance") follows from the optimality condition $f^{\mathrm{u}}_l = g^{\mathrm{u}}_l - h^{\mathrm{u}}_l = 0$ (compare Figure (6)) with economic potential difference Δw_l; flux v_l; direct flux gain $\hat{z}^{\mathrm{v}}_l$; enzyme cost $y_l = h^{\mathrm{u}}_l\, u_l$. Without direct flux gains, a forward flux requires that economic potentials increase between substrate and product. (b) Compound balance (economic load $p^{\mathrm{c}}_i = -z^{\mathrm{c}}_i$; reaction flux v_l; flux cost weight $h^{\mathrm{v}}_l = y_l/v_l$; scaled elasticity $E^{\mathrm{v_l}}_{\mathrm{c}_i}$). The enzyme costs in both reactions are balanced with the metabolite's economic benefit $p^{\mathrm{c}}_i\, c_i$, determined by the concentration gain z^{c}_i.

which must hold for all active enzymatic reactions. It states that the flux benefit $b_l = [\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l$ (that is, the local rate of benefit production) and the enzyme cost $y_l = h^{\mathrm{u}}_l\, u_l$ must be equal. Since enzyme costs are positive, flux demand $\Delta w_l + \hat{z}^{\mathrm{v}}_l$ and flux v_l must have the same signs. In reactions without direct flux gains $(\hat{z}^{\mathrm{v}}_l = 0)$, fluxes must lead from lower to higher economic potentials. In contrast, fluxes with direct gains $(\hat{z}^{\mathrm{v}}_l \neq 0)$ may run towards lower economic potentials, but this case can be excluded by reformulating the model with virtual external metabolites. Like the flux gain condition (6), the reaction balance can be useful even if the kinetic model is not known in all details. Given a complete flux distribution $\mathbf{v}$, it is always possible to find internal economic potentials w^{c}_i and positive enzyme costs y_l that satisfy the reaction balance? It is, whenever the flux distribution is economical (Theorems 2 and 3). For uneconomical flux distributions – i.e., flux distributions with futile cycles – no feasible set of potentials $\mathbf{w}^{\mathrm{c}}$ exists. A similar existence theorem holds for chemical potentials in thermodynamic flux analysis [16]. To obtain an analogous balance equation for flux gains and prices, we omit all inactive reactions, divide Eq. (11) by the flux v_l, and obtain

$$\hat{z}^{\mathrm{v}}_l + \Delta w^{\mathrm{c}}_l = h^{\mathrm{v}}_l = \frac{h^{\mathrm{u}}_l\, u_l}{v_l}. \tag{12}$$

The flux price on the right can also be written as $h^{\mathrm{v}}_l = \frac{y_l}{v_l}$ or $h^{\mathrm{v}}_l = \frac{h^{\mathrm{u}}_l}{r'_l}$. If the flux v_l is positive (which can be assumed without loss of generality), we obtain the inequality

$$\frac{h^{\mathrm{u}}_l\, u_l}{v_l} \geq \frac{h^{\mathrm{u,min}}_l}{k^{\mathrm{cat}}} = h^{\mathrm{v,min}}_l \tag{13}$$

where $h^{\mathrm{u,min}}_l$ is some lower bound on the enzyme price[12] and k^{cat} is the forward catalytic constant. Using estimates for these quantities, we obtain tight constraints on the flux demands.

Our second balance equation, the compound balance, relates a metabolite level to the enzyme costs in the surrounding reactions (i.e. reactions in which the metabolite appears as a reactant, catalyst, or allosteric regulator). To derive it, we assume that the state is enzyme-balanced and that all reactions are enzymatic[13] and insert the reaction balance $y_l = [\hat{z}^{\mathrm{v}}_l + \Delta w_l]\, v_l$ into the compound rule (19). For external metabolites

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[12]With Eq. (17), enzyme prices can be estimated from relative sizes and effective degradation rates of enzymes.
[13]For a general compound balance, including non-enzymatic reactions, see Eq. (20)

(levels x_j and loads p_j^{x}) or internal metabolites (levels c_i and loads p_i^{c}), it reads, respectively (see SI P5.2),

$$
\begin{aligned}
p_j^{\mathrm{x}}\, x_j &= \sum_l h_l^{\mathrm{u}}\, u_l\, E_{\mathrm{x}_j}^{\mathrm{v}_1} \\
p_i^{\mathrm{c}}\, c_i &= \sum_l h_l^{\mathrm{u}}\, u_l\, E_{\mathrm{c}_m}^{\mathrm{v}_1}.
\end{aligned}
\tag{14}
$$

Similar balance equations hold for other state variables and parameters, including parameters like the temperature that affect several reactions. Dividing by the concentration, we obtain compound balances in the form

$$
p_j^{\mathrm{x}} = \sum_l h_l^{\mathrm{v}}\, \bar{E}_{\mathrm{x}_j}^{\mathrm{v}_1}, \qquad p_i^{\mathrm{c}} = \sum_l h_l^{\mathrm{v}}\, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_1}.
\tag{15}
$$

A compound balance shows which combinations of enzyme costs around a metabolite are economically feasible. The enzyme costs are weighted by the reaction elasticities $E_{\mathrm{c}_i}^{\mathrm{v}_1}$, which can be positive or negative, and their weighted sum must be balanced with the metabolite's concentration gain. If a metabolite has a vanishing level ($c_i = 0$) or load ($p_i^{\mathrm{c}} = 0$, e.g., an internal metabolite that does not appear in the metabolic objective), the sum in Eq. (14) must vanish.

As an example, consider a linear pathway with flux objective ($\mathbf{z}^{\mathrm{c}} = 0$) and a positive flux: for each internal metabolite, the costs of neighbouring enzymes are inversely proportional to their scaled elasticities: $y_l/y_{l+1} = |E_{c_l}^{v_l}/E_{c_l}^{v_{l+1}}|$. The same relation follows from the concentration gain condition. On the contrary, if a low internal metabolite level is penalised, the metabolite has a positive gain and thus a negative load $p_i^{\mathrm{c}} > 0$. Then, the elasticity-weighted enzyme costs for producing reactions must exceed the costs for consuming reactions. Since producing reactions tend to have smaller elasticities [17], the flux prices of the producing reactions will be higher. If an external nutrient has a positive influence on the return, its load is positive and cells should invest in its import. In contrast, if an external metabolite has a vanishing load or concentration, the transporter would not be profitable.

6 Constructing kinetic models in enzyme-optimal states

Knowing that fluxes, economic potentials, and loads from enzyme-optimal kinetic models satisfy the balance equations, we may wonder if the opposite holds as well – if such variables, once they satisfy the balance equations, can always be realised by kinetic models, and if such models can be systematically constructed. This is in fact possible. Given the gain vectors $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$, we can use balance equations to realise economical flux distributions by enzyme-balanced kinetic models (for details, see SI S3). There is one limitation: some concentration gain vectors $\mathbf{z}^{\mathrm{c}}$ may not comply with our choice of economic potentials: in this case, the algorithm will adjust them to enforce a solution. By sampling the reaction elasticities within feasible ranges and solving for the kinetic constants, we can construct kinetic models that realise our flux distribution. Inactive reactions can be ignored (that is, justified by assuming large enzyme prices or low catalytic constants). Aside from its practical use, the model construction shows that any economical flux distribution can be realised by enzyme-balanced models, and is therefore enzyme-balanced.

Figure 9 shows a model of yeast central metabolism constructed in this way. Some flux directions were predefined, ATP production was used as the metabolic objective, and feasible fluxes were determined by flux minimisation. The flux directions were then used to define linear constraints on the chemical and economic potentials. Chemical potentials were chosen within predefined bounds, and economic potentials were computed by fitting the enzyme costs to mass-weighted proteome data[14]. Then, economic loads

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[14]Different heuristic principles can be used in choosing the economic potentials. The principle of uniform costs states that enzyme costs (or equivalently, enzyme benefits) should be similar between enzymes. As another heuristics, one may predefine plausible enzyme costs (or benefits) and approximate them by the model. Predefined flux demands or prices can be treated
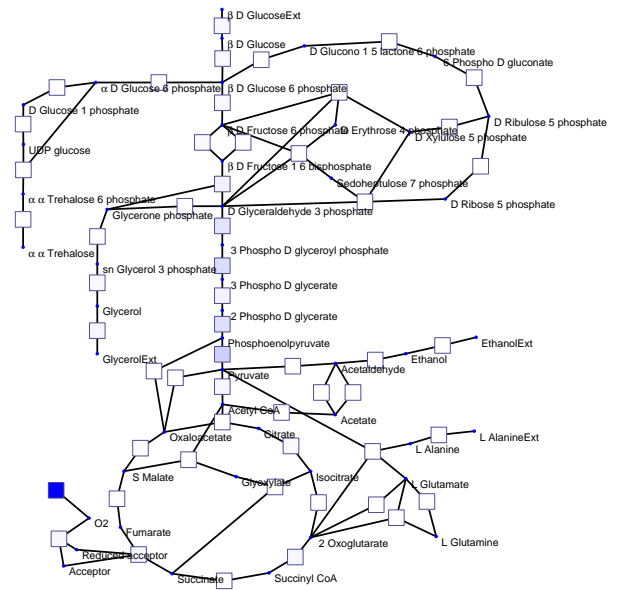
(a) Economic potentials
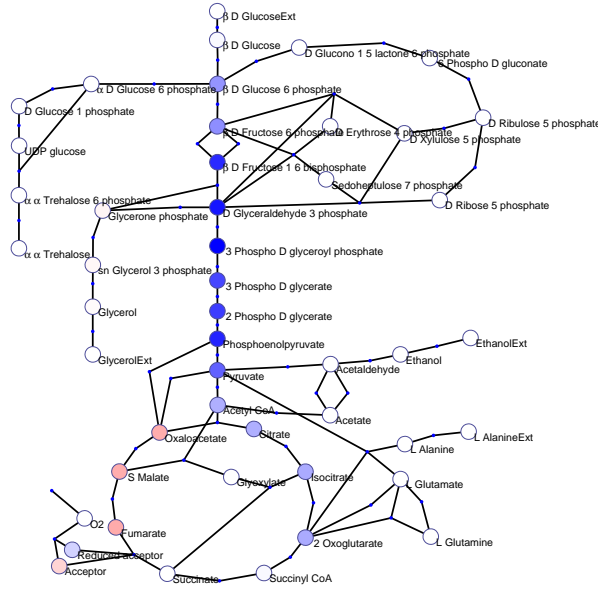
(b) Enzyme costs

Figure 9: Kinetic model in an enzyme-balanced state. (a) Reconstructed model of yeast central metabolism. The model has economical, thermodynamically feasible fluxes which point towards higher economic potentials (circle colours: pink: negative; white: zero; blue: positive; cofactors not shown). (b) Enzyme demands in the reconstructed model. A set of enzyme demands (estimated from enzyme levels) was predefined and approximated by the model by a unique set of economic potentials. Another possibility would be the principle of uniform costs, assuming that all enyzme costs (and accordingly, all enzyme benefits) should be as similar as possible.

and elasticities were chosen, corresponding rate constants were determined, and the full kinetic model was constructed.

If we reconstruct a kinetic model in this way, will its enzyme levels really be optimal? The cost-benefit balance Eq. (3) is a necessary, but not a sufficient condition for optimality. Additionally, the state must be dynamically and economically stable: any enzyme variation must decrease the fitness. In a second-order approximation, we can require that the fitness curvature matrix $\mathbf{F}_{uu} = \partial^2 f/(\partial u_l \, \partial u_k)$ for active enzymatic reactions be negative definite. Since each enzyme level must be economically stable, the fitness curvatures $\partial^2 f/\partial u_l^2$ of active enzymes must be negative. If an enzyme-balanced kinetic model has been constructed as described above, will it be economically stable? For models with linear flux objectives and sufficiently curved investment functions, this is the case. In metabolic pathways, the upregulation of a single enzyme will typically reduce its flux control, so a flux objective, as a function of single enzyme levels, will be negatively curved. Since enzyme prices increase more than linearly with protein levels [18], the stability condition will be satisfied for each single enzyme. However, whether the entire enzyme profile is economically stable (i.e. whether the curvature matrix as a whole is negative definite) is a more difficult

similarly [14]. Flux prices $h_l^{\mathrm{v}} = y_l/v_l$, estimated from known k_{cat} values and enzyme sizes, can be used in flux analysis or in the reconstruction of enzyme-balanced kinetic models. With Eqs (6) and (7), they can be adjusted to make them fit into enzyme-optimal states.

question[15]. In any case, strongly curved investment functions can guarantee economic stability[16]. Our model construction algorithm produces states that are enzyme-balanced, but maybe not enzyme-optimal. To obtain enzyme-optimal states, we need to sample enzyme-balanced models and select the ones with dynamically and economically stable states (for details, see SI S3).

7   Discussion

How should cells allocate enzyme investments to metabolic pathways, and how should this choice depend on the interplay of pathway fluxes and on the specific costs and efficiencies of enzymes? Economics, which studies compromises between opposing needs, can help answer such questions. Metabolic economics cannot be used to prove or disprove enzyme optimality – which would be epistemologically impossible. However, as a theory, it addresses optimality assumptions that biologists tend to make, but often do not spell out. Making these assumptions explicit and testing their consequences by models can help understand how organisms function and under which selection pressures and constraints they evolve.

A main difficulty in kinetic models is how tightly all processes are linked. For instance, if biomass production is the metabolic objective, enzymes will obtain their economic value by contributing to biomass production. However, translating the value of biomass into values of individual enzymes is difficult, not only because we need to trace all causal effects in the network, but also because enzyme levels are, as we assume, optimised and therefore adapted to each other. Flux demands, economic potentials, and enzyme costs, the key variables in metabolic economics, describe the values of fluxes, metabolite production, and enzymes induced by the fitness function. Like metabolic control coefficients, and unlike simple molecule properties like rate constants or molecular weights, they emerge from the enzyme's role in cellular networks and from the economic compromises between them. Importantly, they are marginal quantities (e.g., describing the effect of such enzyme changes, not the absolute fluxes catalysed by enzymes in the present state). This resembles the way in which enzyme costs and benefits are defined in experimental studies [4, 18].

Given a fitness function, the enzyme levels in kinetic models can be optimised numerically. Like in other numerics-based methods such as FBA, this works only on a case-by-case basis. Metabolic economics, in contrast, provides general laws based on notions like enzyme-balanced states, economical flux distributions, or futile modes. It provides ways to compute economical flux distributions and shows that these, and no others, can appear in enzyme-balanced states – that is, states in which all active enzymes have a positive effect on the metabolic return. This can be an important criterion for constructing realistic models. Unlike usual flux analysis, metabolic economics accounts for the complex relations between enzyme levels and stationary fluxes, for allosteric regulation, and for concentration-dependent fitness functions. A number of extensions of the theory are described in appendix E. Metabolic economics highlights the *economic state* of a system, as described by economic potentials and loads, which complements the metabolic state given by fluxes and concentrations. The economic variables form a new layer of description, linking the economic notions between kinetic and stoichiometric models. On the one hand, they allow us to analyse enzyme-balanced states without fully specifying the rate laws or the enzyme cost functions. On the other, once a feasible economic state has been constructed from balance equations, it can be realised by enzyme-balanced kinetic models, which can be constructed systematically.

Although fluxes and metabolite levels are closely coupled, their flux gains and concentration gains appear

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[15]If a model contains only enzyme-catalysed reactions, a proportional scaling of enzyme levels, at constant external metabolite levels, leads to a proportional scaling of fluxes. If the objective function is linear in the fluxes, the return function $g(\mathbf{u}, \mathbf{x})$ will be linear with respect to the overall scaling, so the curvature matrix will have a vanishing eigenvalue (with the eigenvector $\mathbf{u}$). To obtain an economically stable state in this case, the investment function needs to be positively curved in the same direction (given by $\mathbf{u}$).

[16]If all enzymes have equal prices, the gradient and curvature matrix of the investment function have the forms $\mathbf{h}^{\mathrm{u}} = \alpha(\mathbf{u})\mathbf{1}$ and $\mathbf{H}_{\mathrm{uu}} = \beta(\mathbf{u})\mathbf{1}\mathbf{1}^{\top}$ with convex increasing functions α and β. The enzyme demand vector $\partial g / \partial \mathbf{u}$ in an enzyme-balanced state must have the form $\mathbf{g}^{\mathrm{u}} = \alpha\mathbf{1}$, implying that $\mathbf{g}^{\mathrm{v}} \mathrm{Dg}(\mathbf{v}) \sim \mathbf{u}$. This resembles the proportionality $C_l^J \sim u_l$ between scaled flux control coefficients and enzyme levels shown in [1].

in separate equations. On the one hand, there is an economics of metabolite production, described by flux gains and economic potentials and subject to flux gain condition and reaction balance. On the other, there is an economics of metabolite levels, described by concentration gains and economic loads and governed by concentration gain condition and compound balance. Both sets of equations describe the same metabolic state, but from different angles. If we start from a kinetic model, we can optimise the enzyme levels and obtain the economic potentials by taking derivatives. Treating them as state variables helps us focus on the different ways in which this network can be operated economically. Just like metabolic states are described by fluxes and concentrations and thermodynamic states are described by driving forces and chemical potentials, the economic state is described by economic potentials, loads, and enzyme costs. Using the economic variables, we can explore the space of enzyme-balanced states, realised by different kinetic models of the same network.

Metabolic economics relies on kinetic models, but some of its main results – the futile mode theorem and the reaction balance – are directly applicable to flux analysis, where we want fluxes to be realisable by enzyme-optimal kinetic models, but without specifying these models in detail. In an FBA problem with flux objective $z_{\mathrm{FBA}}(\mathbf{v}) = \mathbf{z}^{\mathrm{v}}_{\mathrm{FBA}} \cdot \mathbf{v}$, we may interpret $\mathbf{z}^{\mathrm{v}}_{\mathrm{FBA}}$ as the flux gain vector $\mathbf{z}^{\mathrm{v}} = \partial z/\partial \mathbf{v}$ of a kinetic model whose economic potentials we attempt to find. The internal economic potentials w_i^{c} must satisfy the benefit principle $[z_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}}] v_l > 0$, which can be included into FBA in the same way as thermodynamic flux constraints [19]. Geometrically, the benefit principle restricts flux distributions to feasible segments in flux space (orthants and their surfaces). *Economic flux analysis* – constraint-based flux prediction under thermodynamic and economic constraints – rules out flux modes that are incompatible with thermodynamics or enzyme optimality. The economic and thermodynamic constraints are strikingly similar and can be derived from a common variational principle (general flux cost minimisation) and treated with the same mathematical methods [14].

Metabolic economics is closely related to flux cost minimisation, which penalises fluxes by cost functions like the weighted sum of fluxes [10, 20]. Both methods avoid excessive costs and exclude futile cycles, but do so in different ways. In flux cost minimisation, fluxes are optimised for low costs at a fixed metabolic benefit. Numerical optimisation yields a specific flux distribution, which depends on the cost function chosen. In metabolic economics, costs are not assigned to fluxes, but to enzyme levels. Nevertheless, the benefit principle leads to sign constraints for the fluxes, which can be used in flux analysis without reference to a specific kinetic model. The metabolic objective can depend on metabolite levels, but the concentration gains do not appear in the reaction balance (11) and are only reflected in the numerical values of the economic potentials. Despite their different assumptions, metabolic economics and FCM lead to essentially the same constraints on flux directions (flux cost minimisation theorem, Theorem 4). Therefore, metabolic economics justifies flux cost minimisation as a method for computing economical flux distributions. If a production objective is used, flux distributions obtained by FCM will satisfy the thermodynamic and economic constraints.

To find biologically plausible economic potentials, heuristic principles like the principle of uniform costs can be used, which are based on assumptions and additional data and do not require a full kinetic model. Thus, given a flux distribution, we can estimate economic potentials and enzyme costs, possibly based on proteome and protein size data, and realise the economic state by a kinetic model. Introducing optimality considerations into kinetic model construction helps integrate various data – metabolite levels, fluxes, and kinetic constants on the one hand, metabolic efficiency and protein costs on the other.

If the enzyme levels in a network are optimised, the enzyme levels will depend on each other, and modelling this state pathway by pathway may seem difficult. Yet, metabolic economics is suited for this type of modular modelling: it translates the global metabolic objective into a production of value, described by local balance equations for each reaction and pathway, where pathway borders can be freely chosen. This agrees with the principle of individual optimality: if the enzyme profile is optimised as a whole, each enzyme level will be optimal *given* the rest of the system. In such modular models, pathways must still be able to "negotiate" to balance the enzyme activities between them. The pathways are linked by communicating metabolites at their boundaries, the "negotiation" happens through the economic potentials of these

metabolites, and possibly through nonlinear enzyme investment functions. Thus, the economic potentials can serve as connecting variables aside from fluxes, concentrations, or chemical potentials. If pathway models are prepared with consistent concentrations and economic potentials on their boundaries, they can directly be combined and the resulting model will show a consistent enzyme-balanced state by construction. Since metabolic economics is a local theory, we can focus on a single pathway: the demands in this pathway, induced by gains in the surrounding network, can effectively be represented by the economic potentials on the pathway boundary.

A modular picture of cells (like in Figure 1) shows how demands are propagated between pathways. For instance, the production cost of a substance can be broken down into a cost for substrates and a costs for the enzyme, whose production entails costs for amino acids and energy, costs of ribosomes and mRNA, and so on, and this is not just a verbal description, but equations between mathematically defined quantities. Thinking in terms of modules can help understand regulation problems in metabolic networks. If regulation functions are optimally chosen, they should map metabolic states (to be achieved) to optimal enzyme levels (necessary to achieve them), and are therefore an "inverted model" of cell metabolism. If there is a "modular regulation task" behind optimal enzyme levels (because the expression of pathway enzymes depends on a few boundary metabolites), this may be reflected in modular systems for enzyme regulation.

Real cells do not seem to behave optimally in all cases. For wild-type cells, which are not yet adapted to laboratory conditions and treatments like gene knockouts, this is not surprising . There are different ways to reconcile metabolic economics with non-optimality: on the one hand, we may assume that cells behave optimally, but with more complex objectives or constraints than considered in our models. Phenomena like preemptive expression or inhomogeneous expression in populations, like in bacterial persistence, show how cells can adapt to complex environments with changing nutrient supplies or rare severe challenges by antibiotics. Enzyme profiles that look wasteful (if we just consider the current environment) may be economical if we consider an adaption to uncertain future challenges – in the sense of bet-hatching strategies – in the optimality problem. On the other hand, we may simply state that some enzyme profiles are not optimal – for the presumable objective chosen – and may quantify their deviations from optimality by economic stresses. By doing this for various objective functions, we may learn which of them come closest to explaining the real behaviour of cells.

Metabolic economics extends the relation between control coefficients and enzyme levels, stated by Klipp and Heinrich, far beyond its original scope. Flux- and concentration-dependent objectives and general enzyme cost functions can be used and general conclusions about flux distributions are drawn. The benefit principle – enzymes need to provide positive benefits to match their positive costs – could be applied to systems beyond metabolism such as protein production and degradation, usage of structural proteins and membranes, or cell signalling. Like in metabolism, the benefits of molecule species or various quantities in cells can be defined by control coefficients, and balance equations follow from thought experiments with compensated variations. Metabolic economics could even be extended to dynamic processes (controlled by temporal enzyme profiles), to spatial distributions of metabolites, and to uncertainties and variability of cellular variables (considering the value of information [21] embodied in the time profiles of signalling molecules).

Acknowledgements

References

[1] E. Klipp and R. Heinrich. Competition for enzymes in metabolic pathways: implications for optimal distributions of enzyme concentrations and for the distribution of flux control. *BioSystems*, 54:1–14, 1999.

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

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

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

[5] E. Klipp, R. Heinrich, and H.G. Holzhütter. Prediction of temporal gene expression. Metabolic optimization by re-distribution of enzyme activities. *Eur. J. Biochem.*, 269:1–8, 2002.

[6] A. Zaslaver, A.E. Mayo, R. Rosenberg, P. Bashkin, H. Sberro, M. Tsalyuk, M.G. Surette, and U. Alon. Just-in-time transcription program in metabolic pathways. *Nature Genetics*, 36:486–491, 2004.

[7] R. Heinrich and S. Schuster. *The Regulation of Cellular Systems*. Chapman & Hall, 1996.

[8] J.-H.S. Hofmeyr and A. Cornish-Bowden. Regulating the cellular economy of supply and demand. *FEBS Letters*, 476(1-2):47–51, 2000.

[9] J. Uhlendorf. Prediction of optimal enzymatic regulation architectures. Master's thesis, Department of Mathematics and Computer Science, Free University Berlin, 2009.

[10] H. Holzhütter. The principle of flux minimization and its application to estimate stationary fluxes in metabolic networks. *Eur. J. Biochem.*, 271(14):2905–2922, 2004.

[11] www.github.com/liebermeister/metabolic-economics.

[12] J.-H.S. Hofmeyr. Metabolic control analysis in a nutshell. In *ICSB 2001 Online Proceedings, http://www.icsb2001.org/toc.html*, 2001.

[13] R. Heinrich and S. Schuster. The modelling of metabolic systems. Structure, control, and optimality. *BioSystems*, 47:61–77, 1998.

[14] W. Liebermeister. How enzyme economy shapes metabolic fluxes. *Preprint on arxiv.org*.

[15] C. Reder. Metabolic control theory: a structural approach. *J. Theor. Biol.*, 135:175–201, 1988.

[16] H. Qian and D.A. Beard. Thermodynamics of stoichiometric biochemical networks in living systems far from equilibrium. *Biophysical Chemistry*, 114(2-3):213–220, 2005.

[17] W. Liebermeister, J. Uhlendorf, and E. Klipp. Modular rate laws for enzymatic reactions: thermodynamics, elasticities, and implementation. *Bioinformatics*, 26(12):1528–1534, 2010.

[18] I. Shachrai, A. Zaslaver, U. Alon, and E. Dekel. Cost of unneeded proteins in E. coli is reduced after several generations in exponential growth. *Molecular Cell*, 38:1–10, 2010.

[19] A. Hoppe, S. Hoffmann, and H. Holzhütter. Including metabolite concentrations into flux-balance analysis: Thermodynamic realizability as a constraint on flux distributions in metabolic networks. *BMC Syst. Biol*, 1(1):23, 2007.

[20] H. Holzhütter. The generalized flux-minimization method and its application to metabolic networks affected by enzyme deficiencies. *BioSystems*, 83:98–107, 2006.

[21] O. Rivoire and S. Leibler. The value of information for populations in varying environments. *Journal of Statistical Physics*, 142(6):1124–1166, 2011.

[22] D.A. Beard, E. Babson, E. Curtis, and H. Qian. Thermodynamic constraints for biochemical networks. *J. Theor. Biol.*, 228(3):327–333, 2004.

[23] R. Heinrich and HG. Holzhütter. Efficiency and design of simple metabolic systems. *Biomed Biochim Acta*, 44(6):959–969, 1985.

[24] A. Bar-Even, E. Noor, Y. Savir, W. Liebermeister, D. Davidi, D.S. Tawfik, and R. Milo. The moderately efficient enzyme: evolutionary and physicochemical trends shaping enzyme parameters. *Biochemistry*, 21:4402–4410, 2011.

[25] E. Kussell and S. Leibler. Phenotypic diversity, population growth, and information in fluctuating environments. *Science*, 309(5743):2075 – 2078, 2005.

[26] R. Schuetz, N. Zamboni, M. Zampieri, M. Heinemann, and U. Sauer. Multidimensional optimality of microbial metabolism. *Science*, 336(6081):601–604, 2012.

A Mathematical symbols

Kinetics		
c_i	mM	Internal metabolite concentration
x_j	mM	External metabolite concentration
u_l	mM	Enzyme level
v_l	mM/s	Reaction rate
$r_l(\mathbf{c}, \mathbf{x})$	mM/s	Rate law $r_l(\mathbf{c}, \mathbf{x}) = u_l \, r_l'(\mathbf{c}, \mathbf{x})$
$r_l'(\mathbf{c}, \mathbf{x}) = r_l/u_l$	1/s	Enzyme-specific rate
κ	1/s	Cell growth rate
κ_l	1/s	Rate constant for degradation of enzyme l

Flux modes		
$\mathbf{N}^{\mathrm{tot}}, \mathbf{N}, \mathbf{N}^{\mathrm{x}}$	1	Stoichiometric matrix (total n_{il}^{tot}, internal n_{il}, external n_{jl}^{x})
$\mathbf{K}, \mathbf{K}^{\mathrm{tot}}$	1	Right-kernel (stationary flux) matrices (satisfying $\mathbf{N}\,\mathbf{K} = 0$, $\mathbf{N}^{\mathrm{tot}}\,\mathbf{K}^{\mathrm{tot}} = 0$)
$\mathbf{G}$	1	Left-kernel (conserved moiety) matrix (satisfying $\mathbf{G}\,\mathbf{N} = 0$)
$\mathbf{v}$	mM/s	Stationary flux distribution
$\mathbf{k}$	mM/s	Test mode (stationary on active region)
$\mathcal{P}_{\mathrm{FBA}}$		Stationary flux polytope in FBA problem: $\mathbf{N}\,\mathbf{v} = 0$ and $\mathbf{v}^{\mathrm{min}} \leq \mathbf{v} \leq \mathbf{v}^{\mathrm{max}}$
$\mathcal{P}_{\mathrm{FBA,b}}$		Objective-flux polytope, additional condition $b = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$

Thermodynamics		
μ_i	kJ/mol	Chemical potential
Θ_l	1	Thermodynamic driving force $\Theta_l = -\Delta G_l/RT = -\sum_i n_{il}\,\mu_i/RT$
σ_l	kJ/(s m^3)	Entropy production per volume $\sigma_l = R\,(\Theta_l \cdot v_l)$

Metabolic control		
$\mathbf{L}$	1	Link matrix
$E_{\mathrm{c}_i}^{\mathrm{v}_l}$	1	Scaled metabolite elasticity (internal $E_{\mathrm{c}_i}^{\mathrm{v}_l} = \frac{c_i}{v_l}\frac{\partial v_l}{\partial c_i}$, external $E_{\mathrm{x}_j}^{\mathrm{v}_l} = \frac{x_j}{v_l}\frac{\partial v_l}{\partial x_j}$)
$\bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l}$	1/s	Unscaled metabolite elasticity (internal $\bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l} = \frac{\partial v_l}{\partial c_i}$, external $\bar{E}_{\mathrm{x}_j}^{\mathrm{v}_l} = \frac{\partial v_l}{\partial x_j}$)
$\bar{E}_{\mathrm{u}_l}^{\mathrm{v}_l}$	1/s	Unscaled enzyme elasticity $\bar{E}_{\mathrm{u}_l}^{\mathrm{v}_l} = \frac{v_l}{u_l} = r_l'$
$S_i(\mathbf{u}, \mathbf{x})$	mM	Steady-state internal metabolite concentration
$J_l(\mathbf{u}, \mathbf{x})$	mM/s	Steady-state flux
$R_{il}^{\mathrm{S}}, R_{rl}^{\mathrm{J}}$		Unscaled response coefficient (internal conc. i and flux r) for enzyme l.
$C_{il}^{\mathrm{S}}, C_{rl}^{\mathrm{J}}$	1, s	Unscaled control coefficient (internal conc. i and flux r) for reaction l.
$C_{\varphi_i^{\mathrm{ind}}}^{\mathrm{S}}, C_{\varphi_i^{\mathrm{ind}}}^{\mathrm{J}}$	1, s	Unscaled control coefficient w.r.t independent supply fluxes

Flux cost minimisation		
$b(\mathbf{v}) = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$	D	Flux benefit function
$\bar{H}(\mathbf{v})$	D	Flux cost function, e.g. weighted flux sum $\bar{H}(\mathbf{v}) = \sum_l \bar{H}_l^{\mathrm{v}*}|v_l|$
$\bar{H}_l^{\mathrm{v}} = |\partial \bar{H}/\partial v_l|$	D s/mM	Flux price
$y_l^{\mathrm{v}} = \bar{H}_l^{\mathrm{v}}|v_l|$	D	Flux cost

Table 1: Notions and symbols for kinetic models. Reaction rates and enzyme levels are given in units of mM/s and mM. Other units (mol/s and mol) could be used instead. Indices denote metabolites in general (i), external metabolites (j), and reactions (l).

Metabolic objective

$z(\mathbf{v}, \mathbf{c})$	D	Metabolic objective	
$z_i^{\mathrm{c}} = \partial z/\partial c_i$	D/mM	Compound gain	
$z_l^{\mathrm{v}} = \partial z/\partial v_l$	D s/mM	Total flux gain	
z_j^{x}	D s/mM	Production gain	From decomposition $\mathbf{z}^{\mathrm{v}} = \mathbf{N}^{\mathrm{x}\top}\, \mathbf{z}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}$
$\hat{z}_l^{\mathrm{v}}$	D s/mM	Direct flux gain	From decomposition $\mathbf{z}^{\mathrm{v}} = \mathbf{N}^{\mathrm{x}\top}\, \mathbf{z}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}$
$z_i^{\mathrm{c}*}$	D/mM	Effective concentration gain	$z_i^{\mathrm{c}*} = z_i^{\mathrm{c}} + \sum_l \bar{E}_{\mathrm{c}i}^{\mathrm{v}l}\, z_l^{\mathrm{v}}$
$\mathbf{N}^{\mathrm{z}} = \begin{pmatrix} \mathbf{z}^{\mathrm{v}\top} \\ \mathbf{N} \end{pmatrix}$		Benefit-stoichiometric matrix	Right-kernel matrix is called $\mathbf{K}^{\mathrm{z}}$

Enzyme economy

$f(\mathbf{u}, \mathbf{x}) = g(\mathbf{u}, \mathbf{x}) - h(\mathbf{u})$	D	Fitness function	
$g(\mathbf{u}, \mathbf{x}) = z(\mathbf{J}(\mathbf{u}, \mathbf{x}), \mathbf{S}(\mathbf{u}, \mathbf{x}))$	D	Metabolic return	
$h(\mathbf{u})$	D	Enzyme investment	
$g_l^{\mathrm{u}} = \partial g/\partial u_l$	D/mM	Enzyme demand	= Load of enzyme level
$g_j^{\mathrm{x}} = \partial g/\partial x_j$	D/mM	External metabolite demand	= Load of external metabolite level
$g_r^{\mathrm{q}} = \partial g/\partial p_r$		Demand for quantity q_r	= Load of quantity q_r
$h_l^{\mathrm{u}} = \partial h/\partial u_l$	D	Enzyme price	Direct (= total) enzyme price
φ_i^{ind}	mM/s	Virtual supply flux	Perturbation parameter in $g(\mathbf{u}, \mathbf{x}, \mathbf{L}\, \boldsymbol{\varphi}^{\mathrm{ind}})$
γ_i	mM/s	Virtual concentration change	Perturbation parameter in $g(\mathbf{u}, \mathbf{x}, \boldsymbol{\varphi}^{\mathrm{ind}}, \boldsymbol{\gamma})$
$g_i^{\varphi} = \partial g/\partial \varphi_i^{\mathrm{ind}}$	D s/mM	Demand for virtual supply flux	(for independent metabolites i; otherwise 0)
$g_i^{\mathrm{c}} = \partial g/\partial \gamma_i$	D/mM	Concentration demand	Vanishes unless there are conserved moieties

Economic state variables

w_i	D/(mM/s)	Economic potential	Internal: $w_i^{\mathrm{c}} = g_i^{\varphi}$; external: $w_j^{\mathrm{x}} = z_j^{\mathrm{x}}$
Δw_l	D s/mM	Conversion demand	$\Delta \mathbf{w} = \mathbf{N}^{\mathrm{tot}\top}\, \mathbf{w}$
p_i^{c}	D/mM	Metabolite load	Internal: $p_i^{\mathrm{c}} = g_i^{\mathrm{c}} - z_i^{\mathrm{c}}$; external $p_j^{\mathrm{x}} = g_j^{\mathrm{x}}$
$p_r^q = g_r^{\mathrm{q}}$		Load of quantity q_r	
$g_l^{\mathrm{v}} = g_l^{\mathrm{u}}\, u_l/v_l$	D s/mM	Flux demand	$g_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} + \Delta w_l$
$\hat{g}_l^{\mathrm{v}}$	D s/mM	Direct flux demand	$\hat{g}_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}}$

Benefit and cost

$b_l^{\mathrm{u}} = \partial g/\partial \ln u_l$	D	Enzyme benefit	Scaled enzyme demand, $= g_l^{\mathrm{u}}\, u_l$
$b_l^{\mathrm{v}} = g_l^{\mathrm{v}}\, v_l$	D	Flux benefit	$b_l^{\mathrm{v}} = [\Delta g_l^{\mathrm{v}} + \hat{g}_l^{\mathrm{v}}]\, v_l$
$b_i^{\mathrm{c}}, b_i^{\mathrm{x}}$	D	Metabolite benefit	Scaled values $b_i^{\mathrm{c}} = p_i^{\mathrm{c}}\, c_i$, $b_j^{\mathrm{x}} = p_j^{\mathrm{x}}\, x_j$
b_r^{q}	D	Benefit of quantity q_r	Scaled values $b_i^{\mathrm{p}} = p_r^p\, p_r$
$y_l = \partial h/\partial \ln u_l = h_l^{\mathrm{u}}\, u_l$	D	Enzyme cost	
$h_l^{\mathrm{v}} = y_l/v_l$	D s/mM	Flux price	Enzyme cost/flux = enzyme price/spec. act.
$f_l^{\mathrm{u}} = \partial f/\partial u_l$	D/mM	Enzyme stress	$g_l^{\mathrm{u}} - h_l^{\mathrm{u}} = [\Delta w_l + \hat{z}_l^{\mathrm{v}}] \frac{v_l}{u_l} - h_l^{\mathrm{u}}$
$y_l^{\Delta} = \partial f/\partial \ln u_l$	D	Economic imbalance	$f_l^{\mathrm{u}}\, u_l = [\Delta w_l + \hat{z}_l^{\mathrm{v}}]\, v_l - y_l$
$f_l^{\mathrm{v}} = (\partial f/\partial \ln u_l)/v_l$	D s/mM	Flux stress	$f_l^{\mathrm{u}}\, u_l/v_l = [\Delta w_l + \hat{z}_l^{\mathrm{v}}] - h_l^{\mathrm{v}}$

Table 2: Mathematical symbols for economic state variables. Fitness, metabolic objective, and investment are expressed in a hypothetical unit called Darwin (D). For instance, if the fitness of microbes is measured in terms of logarithmic growth rates, one Darwin corresponds to an e-fold increase of the growth rate.
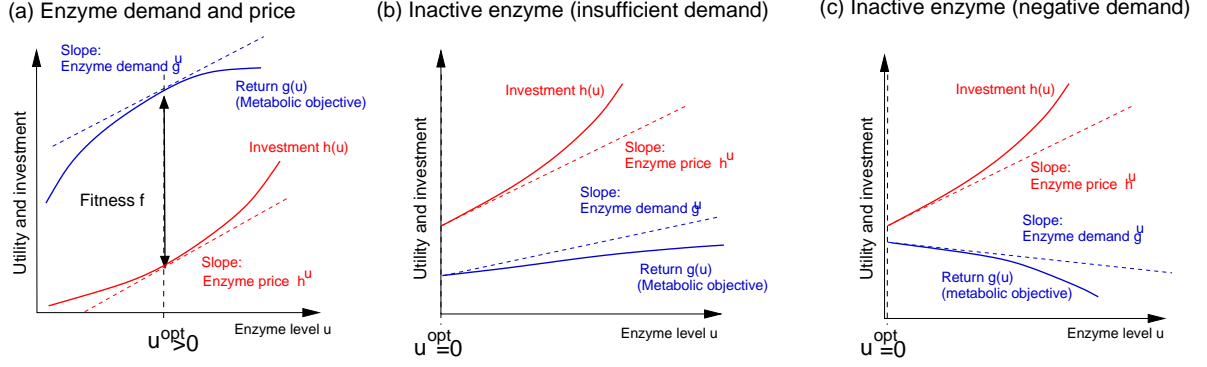
Figure 10: Enzyme demand and price. (a) The slopes of return and investment function – called enzyme demand and price – must be balanced (compare cost-benefit balance in Figure 3). (b) If the demand is always lower than the price, the enzyme in the optimal state must be inactive. (c) If the demand is always negative, the enzyme must be inactive as well.

B   Definitions

Definition 1 (Kinetic model) *We consider kinetic models with reversible rate laws Eq. (16) and thermo-dynamically feasible rate constants [17]. Reaction rates are given by rate laws*

$$v_l = r_l(\mathbf{u}, \mathbf{c}, \mathbf{x}) = u_l\, r_l'(\mathbf{c}, \mathbf{x}). \tag{16}$$

Each reaction is catalysed by a specific enzyme with level u_l which appears as a prefactor[17]. The rate laws – e.g. mass-action or Michaelis-Menten kinetics – must be reversible and thermodynamically feasible (see SI S1.3) and the rate constants must satisfy Wegscheider conditions and Haldane relations [17]. Allosteric regulation by metabolites is included in the rate laws while covalent enzyme modifications (e.g. phosphory-lation that changes the enzyme activity) may be captured by the variables u_l. Rate laws and stoichiometric matrix $\mathbf{N}$ (referring to internal metabolites) define a kinetic model. We consider models with a stable steady state. The steady-state concentrations $c_i = S_i(\mathbf{u}, \mathbf{x})$ and fluxes $v_l = J_l(\mathbf{u}, \mathbf{x}) = r_l(\mathbf{S}(\mathbf{u}, \mathbf{x}), \mathbf{x})$ depend on the model parameters, i.e. enzyme levels u_l and external concentrations x_j.

The internal stoichiometric matrix $\mathbf{N}$ contains general structural information. The rows of its left-kernel matrix $\mathbf{G}$ describe conserved moieties, and the columns of the right-kernel matrix $\mathbf{K}$ describe stationary fluxes. In models with moiety conservation, $\mathbf{N}$ can be split into a product $\mathbf{L}\,\mathbf{N}_{\mathrm{R}}$, where the rows of $\mathbf{N}_{\mathrm{R}}$ (reduced stoichiometric matrix) belong to independent metabolites and are linearly independent. $\mathbf{L}$ is called link matrix. The splitting helps to describe moiety conservation, which make some metabolite levels linearly dependent (e.g., via a constant sum of concentrations [ATP] + [ADP] + [AMP]).

The matrix $\bar{\mathbf{E}} = \partial \mathbf{r}/\partial \mathbf{c}$ contains the unscaled elasticities for internal metabolites, and $\bar{E}_{u_l}^{v_l} = v_l/u_l$ denotes the unscaled enzyme elasticities. Scaled elasticities for internal metabolites are defined by $E_{c_i}^{v_l} = \frac{\partial \ln |v_l|}{\partial \ln c_i} = \frac{c_i}{v_l}\frac{\partial v_l}{\partial c_i}$. In the definition of elasticities, reactant and enzyme levels are controllable parameters, i.e. the reactions are considered in isolation. The response and control coefficients, in contrast, are derivatives of the global steady-state concentrations S_i and fluxes J_l. For a list of variables, see Table 1.

Definition 2 Investment function *The investment function $h(\mathbf{u})$ represents costs in enzyme production and maintenance, ribosome production, or molecular crowding – processes that do not explicitly appear in*

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[17]General symbols u_l and x_j are used instead of E_l and c^{ext} to emphasise their roles as controllable and non-controllable model parameters. The vectors $\mathbf{u}$ and $\mathbf{x}$ can also comprise other quantities: for instance, the growth rate as a controllable parameter u_l, or the temperature as a non-controllable parameter x_j.

the metabolic model. Simple investment functions can be obtained as follows. An enzyme's translation rate (enzyme index l) is proportional to enzyme level u_l, protein chain length L_l (number of amino acids), and effective degradation rate $(\kappa_l + \kappa)$, where κ_l is the rate constant for protein degradation and κ is the cell growth rate. This yields the total translation rate $\sum_l (\kappa_l + \kappa) L_l u_l$ for all enzymes. A faster protein production would require more ribosomes, which need to be produced: to account for this, the protein translation rate is multiplied by a ribosome overhead factor $\lambda(\kappa)$, which increases with the growth rate κ (see SI P7). In experiments, the protein investment – measured by growth impairments after an induction of idle protein – increases more than linearly with the protein level [4, 18]. Assuming such nonlinear relationships, we obtain enzyme investment functions

$$h(\mathbf{u}) = H\left(\lambda(\kappa) \sum_l (\kappa_l + \kappa) L_l u_l\right) = H\left(\sum_l h_l^* u_l\right). \tag{17}$$

The enzyme prices $h_l^{\mathrm{u}} = \partial h / \partial u_l$ (i.e. marginal enzyme investments) are given by $h_l^{\mathrm{u}} = H' h_l^*$, where H' is the derivative of the nonlinear function H. Therefore, the gradient $\mathbf{h}^{\mathrm{u}}$ is proportional to $\mathbf{h}^*$ (with a prefactor depending on $\mathbf{u}$) and the curvature matrix $\mathbf{H}_{\mathrm{uu}}$ is proportional to $\mathbf{h}^* \cdot \mathbf{h}^{*\top}$. If $H(\cdot)$ is a power-law function $H(x) = x^\gamma$ (e.g., with $1 \leq \gamma < 2$), we can use Euler's theorem for homogeneous functions and obtain the total cost $\sum_l y_l = \sum_l \frac{\partial h}{\partial \ln u_l} = \gamma h(\mathbf{u})$ (see SI P1.5).

Remark: The enzyme investment function can be justified by models in which protein production is included in the metabolic network (at a given growth rate, or under an optimisation for growth).

Definition 3 (Enzyme-balanced kinetic model) *A kinetic model in stable steady state and with a fitness function Eq. (2) is called* enzyme-balanced *if all active enzymatic reactions satisfy the cost-benefit balance Eq. (3). If all reactions are enzymatic and active, it is* completely enzyme-balanced*. Economic state variables like the flux and concentration gains z_l^{v} and z_i^{c}, economic potentials w_i, economic loads p_i, enzyme prices h_l^{u}, and enzyme costs y_l are listed in Table 2.*

Definition 4 (Economical flux distribution) *Consider a metabolic network containing only enzymatic reactions and a flux gain vector $\mathbf{z}^{\mathrm{v}}$. A flux distribution $\mathbf{v}$ is called* economical *if, after reducing the model to the active subnetwork of $\mathbf{v}$, there is a positive vector $\mathbf{y}$ satisfying the flux gain condition (6).*

Remark: Models with non-enzymatic reactions are discussed in SI S2.8.

Definition 5 (Test modes and futile modes) *If $\mathbf{v}$ is a flux distribution, all non-vanishing flux distributions $\mathbf{k}$ on the active region are called* test modes *of $\mathbf{v}$. Let $\mathbf{z}^{\mathrm{v}}$ be a flux gain vector. If $\mathbf{v}$ and a non-beneficial test mode $\mathbf{k}$ are sign-concordant (i.e., if all fluxes on the shared active region have the same directions), the shared sign pattern is called a* non-beneficial mode *in $\mathbf{v}$ for $\mathbf{z}^{\mathrm{v}}$. If $\mathbf{v}$ and a costly test mode $\mathbf{k}$ are sign-concordant, the shared sign pattern is called a* costly mode*. Non-beneficial or costly modes are also called* futile*.*

Definition 6 (Flux cost minimisation) *FBA problems are linear programming problems of the form $\mathbf{v} = argmax_{\mathbf{v}'} \, \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}'$ with the constraints $\mathbf{N} \mathbf{v}' = 0$ and $\mathbf{v}^{\min} \leq \mathbf{v}' \leq \mathbf{v}^{\max}$. A flux cost minimisation (FCM) problem is a non-linear optimisation problem of the form*

$$\mathbf{v} = argmin_{\mathbf{v}'} \bar{H}(\mathbf{v}') \quad \text{such that} \quad \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}' = b, \qquad \mathbf{N} \mathbf{v}' = 0, \quad \text{and} \quad \mathbf{v}^{\min} \leq \mathbf{v}' \leq \mathbf{v}^{\max}. \tag{18}$$

The flux cost function $\bar{H}(\mathbf{v})$ must have a positive scaled derivative $(\partial \bar{H}(\mathbf{v}) / \partial v_l) \, v_l$ whenever $v_l \neq 0$. This implies a single minimum at $v_l = 0$. FCM problems with cost functions $\mathbf{h}^{\mathrm{v}}(v_l) = \sum_l \bar{H}_l^{\mathrm{v}*} |v_l|$ and positive flux costs $\bar{H}_l^{\mathrm{v}}$ are called weighted flux minimisation *problems. An FCM problem is called* flux-enforcing *if it contains flux bounds $v_l^{\min} > 0$ or $v_l^{\max} < 0$, i.e., if the bounds exclude the thermodynamic equilibrium state.*

Definition 7 (Economic potential) *Consider an enzyme-balanced model with metabolic return function* $g(\mathbf{u}, \mathbf{x}, \mathbf{L}\,\varphi^{\mathrm{ind}})$. *The vector* φ^{ind} *refers to independent metabolites and defines a virtual supply flux vector* $\varphi = \mathbf{L}\,\varphi^{\mathrm{ind}}$ *for all internal metabolites. The economic potentials are defined by the production gains* $w_j^{\mathrm{x}} = z_j^{\mathrm{x}}$ *(for external metabolites), by* $w_i^{\mathrm{c}} = \frac{\partial g}{\partial \varphi_i^{\mathrm{ind}}}$ *(for independent internal metabolites) and by* $w_i^{\mathrm{c}} = 0$ *(for dependent internal metabolites).*

Remark. The definition yields economic potentials in standard gauging (referring to a given choice of independent metabolites). The economic potentials can be regauged by replacing $\mathbf{w}^{\mathrm{c}} \to \mathbf{w}^{\mathrm{c}} + \mathbf{G}^{\top}\,\mathbf{w}^{\mathrm{CM}}$, where $\mathbf{G}$ is a left-kernel matrix of $\mathbf{N}$ and $\mathbf{w}^{\mathrm{CM}}$, the economic potential vector of the conserved moieties, can be freely chosen.

Definition 8 (Economic load) *Consider an enzyme-balanced model with a metabolic return function* $g(\mathbf{u}, \mathbf{x}, \varphi^{\mathrm{ind}}, \gamma)$ *depends on a virtual concentration perturbations* γ_i. *In a virtual perturbation, we replace* $c_i \to c_i + \gamma_i$ *wherever* c_i *appears in rate laws or in the objective function. With a* $\gamma_i > 0$, *the metabolite level appears larger, and the system responds by decreasing the actual concentration* c_i *until* $c_i + \gamma_i$ *reaches the original value of* c_i. *If the metabolite is involved in conserved moieties, this may not possible, and a net deviation will remain. The economic loads are defined by* $p_j^{\mathrm{x}} = \frac{\partial g}{\partial x_j}$ *(for external metabolites) and by* $\mathbf{p}^{\mathrm{c}} = \frac{\partial g}{\partial \gamma} - \frac{\partial z}{\partial \mathbf{c}}$ *(for internal metabolites), with the concentration gains* $\partial z / \partial c_i = z_m^{\mathrm{c}}$.

C Theorems of metabolic economics

Theorem 1 (Gain conditions for inverse fluxes) *If a kinetic model is completely enzyme-balanced, the cost-benefit balance Eq. (3) is equivalent to the gain conditions (6) and (7) (Proof in SI P1.2):*

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

Remark: In states with inactive reactions, the model must be restricted to the active region. Gain conditions accounting for non-enzymatic reactions are given in SI S2.8.

Theorem 2 (Test mode theorem) *If* $\mathbf{v}$ *is an economical flux distribution and* $\mathbf{k}$ *is a test mode, the following holds: if* $\mathbf{k}$ *is beneficial* $(\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} > 0)$, *then* $\mathbf{v}$ *and* $\mathbf{k}$ *share active reactions with the same flux signs; if* $\mathbf{k}$ *is costly* $(\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} < 0)$, *they share active reactions with opposite flux signs; and if* $\mathbf{k}$ *is non-beneficial* $(\mathbf{z}^{\mathrm{v}} \cdot \mathbf{k} = 0)$, *they share active reactions with the same flux signs and others with opposite signs.*

Remark: The proof (see SI P2.2) is based on the flux gain condition (6). Without loss of generality, we assume that $\mathbf{v}$ is complete. If $\mathbf{v}$ is economical, some positive vector $\mathbf{y}$ must satisfy the condition $\mathbf{k}^{\top} Dg(\mathbf{y})\,\mathbf{v}^{-1} = \mathbf{k} \cdot \mathbf{z}^{\mathrm{v}}$ for any test mode $\mathbf{k}$. If a test mode $\mathbf{k}$ is non-beneficial, the right-hand side will vanish and to obtain a zero value on the left, positive and negative summands must cancel out. Therefore, $\mathbf{k}$ and $\mathbf{v}^{-1}$ (or equivalently $\mathbf{v}$) must contain reactions with equal signs and others with opposite signs. Similar arguments hold for beneficial test modes (positive right-hand side) and costly test modes (negative right-hand side). In the language of oriented matroids (used by Beard et al. in their analysis of thermodynamic constraints), this can be stated as follows: the sign pattern of economical flux distributions $\mathbf{v}$ and the sign patterns of their non-beneficial test modes must be orthogonal [22].

Theorem 3 (Criteria for economical flux distributions) *If all reactions in a flux distribution* $\mathbf{v}$ *are enzyme-catalysed, the following statements are equivalent: (i)* $\mathbf{v}$ *is economical (i.e., satisfies the flux gain condition with positive enzyme costs); (ii)* $\mathbf{v}$ *satisfies the reaction balance (11) with some internal economic potentials* w_i^{c} *and positive enzyme costs* y_l; *(iii)* $\mathbf{v}$ *is free of futile modes (Proof SI P2.2 and P2.3).*

Remark (1) Flux distributions without futile modes are always beneficial. This is easy to see: if $\mathbf{v}$ is not beneficial ($\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} \leq 0$), it can be used *itself* as a futile test mode, defining a futile mode. (2) Another criterion for economical flux distributions is as follows: $\mathbf{v}$ and $\mathbf{z}^{\mathrm{v}}$ can be realised by a kinetic model in an enzyme-balanced state (with an appropriate choice of $\mathbf{z}^{\mathrm{c}}$). This follows from the algorithm for model reconstruction (section S3 in SI).

Theorem 4 (Flux cost minimisation and economic modes) *(a) If a flux mode $\mathbf{v}$ solves a non-flux-enforcing FCM problem with benefit function $b(\mathbf{v}) = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$, it is economical with respect to $\mathbf{z}^{\mathrm{v}}$. The opposite holds as well: (b) If a flux distribution is complete and economical for $\mathbf{z}^{\mathrm{v}}$, it solves a weighted FCM problem with benefit function $b(\mathbf{v}) = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$ and flux prices $\bar{H}_l^{\mathrm{v}} = \frac{h_l^{\mathrm{u}} u_l}{v_l}$. Moreover, it will solve all FCM problems with the same benefit function and the same cost slopes (Proof SI P2.4). The Lagrange multipliers (related to the stationarity constraint in an FCM problem) can be seen as economic potentials.*

Theorem 5 (Compound law) *Consider a kinetic model with a given metabolic objective. A metabolite's load and the flux demands of the surrounding reactions (i.e., reactions directly influenced by it) are linked by the compound rule*

$$p_i^{\mathrm{c}} = \sum_l g_l^{\mathrm{v}} \, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}} = \sum_l [\hat{z}_l^{\mathrm{v}} + \Delta w_l] \, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}}. \tag{19}$$

The reactions need not be enzymatic and may comprise dilution reactions. A similar rule holds for the loads of other quantities that directly affect the reaction rates (e.g., the temperature) .

Theorem 6 (Economic balance equations) *In enzyme-balanced kinetic models, all active enzymatic reactions satisfy a reaction balance (11) (Proof in SI P5.1)*

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

between the economic potentials w_i and the positive enzyme prices h_l^{u} and enzyme levels u_l. Furthermore, all internal metabolites satisfy a compound balance (Proof SI P5.2)

$$p_i^{\mathrm{c}} c_i \;\; = \;\; \sum_l y_l \, E_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}}$$

with economic loads p_i^{c} and scaled reaction elasticities $E_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}}$. Similar balance equations hold for external metabolites and for other quantities that directly affect the reaction rates.

Remark (1) Models with non-enzymatic reactions satisfy the compound balances

$$p_i^{\mathrm{c}} c_i \;\; = \;\; \sum_{l \in \mathrm{enz}} y_l \, E_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}} + \sum_{l \in \mathrm{non}} y_l^{\Delta} \, E_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}} \tag{20}$$

with set "enz" and "non" of enzymatic and non-enzymatic reactions. The enzyme stress $y_l^{\Delta} = [\hat{z}_l^{\mathrm{v}} + \Delta w_l] \, v_l$ can also be seen as the benefit of a hypothetical enzyme with a level $u_l = 1$ (proof section P5.3). (2) Reaction balance and compound balance can be written in terms of prices and demands (instead of costs and benefits):

$$\hat{z}_l^{\mathrm{v}} + \Delta w_l \;\; = \;\; h_l^{\mathrm{u}} \, u_l / v_l$$
$$p_i^{\mathrm{c}} \;\; = \;\; \sum_l h_l^{\mathrm{v}} \, \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_\mathrm{l}}.$$
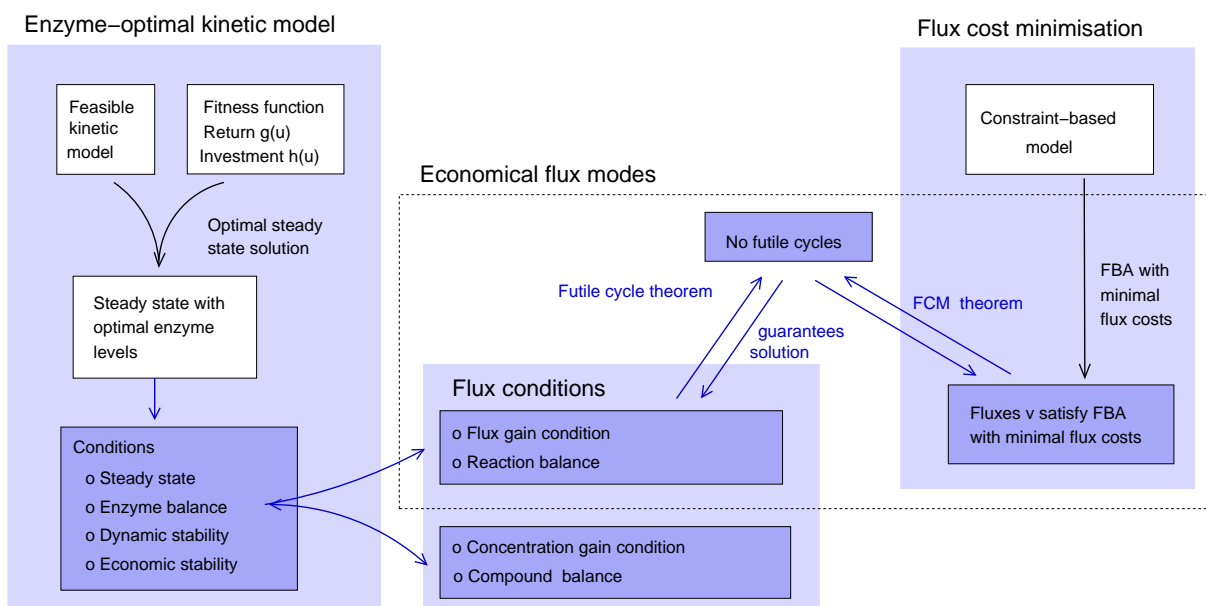
Figure 11: Conditions for models in enzyme-optimal states. Kinetic models in enzyme-optimal states (left box) satisfy first-order conditions (stationary state and cost-benefit balance Eq. (3), i.e. a fitness extremum) and second-order conditions (dynamic stability, i.e. a negative definite Jacobian; and economic stability, i.e. a negative definite curvature matrix $\mathbf{F}_{uu}$ ensuring a fitness maximum). To satisfy the cost-benefit balance, active enzymes must exert a positive control on the metabolic return ("benefit principle"). This is equivalent to the gain conditions (6) and (7) and implies the economic balance equations (11) and (14). Economical flux distributions (dashed box) satisfy flux gain condition and reaction balance, are free of futile modes, and are solutions of flux cost minimisation problems (right box).

D   Optimality conditions and invariance properties

The conditions for enzyme-optimal states can be formulated in several ways. Figure 11 gives an overview. The cost-benefit balance Eq. (3) must hold for all active reactions. As a consequence, a model must satisfy the gain conditions (6) and (7) on its active region, and equivalently the balance equations (11) and (14). All these equations are necessary conditions for enzyme-optimal states (proof in SI P1.2). Notably, the conditions consist of two groups: the flux gain condition, on the one hand, stems from the summation theorem and is related to flux gains $\mathbf{z}^{v}$ and the reaction balance; the concentration gain condition, on the other, stems from the connectivity theorem and is related to concentration gains $\mathbf{z}^{c}$ and the compound balance. Both conditions complement each other, and one may use the reaction balance while disregarding the concentration-dependence of the metabolic objective. The flux gain condition plays a central role: it excludes futile modes and links metabolic economics to flux cost minimisation. All balance equations can equally be applied to reactions, pathways, and the entire metabolic network.

It is interesting to compare the benefit principle to other optimality criteria used in flux analysis. In methods like FBA with minimal fluxes, fluxes are treated as free variables without any account of kinetics, and trade-offs between metabolic pathways are modelled by heuristic flux cost functions [10, 20]. Flux costs are a less plausible assumption than enzyme costs, but they can be computed without kinetic information, which makes them suitable for flux analysis. The principle of minimal fluxes [10] postulates that fluxes must satisfy the FBA constraints (stationarity and flux bounds), yield a fixed metabolic benefit, and minimise the sum of absolute fluxes. This cost function can also be generalised: by weighting the fluxes differently, we obtain flux cost functions $\bar{H}(\mathbf{v}) = \sum \bar{H}_l^{v*}|v_l|$ ("weighted flux minimisation"). In *flux cost minimisation*

(FCM), the cost function $\bar{H}$ can be general continuous functions: the only restriction is that the derivatives $\partial \bar{H}/\partial v_l$ and fluxes v_l must have equal signs. An FCM problem is called *flux-enforcing* if it contains positive lower or negative upper bounds, i.e. if the equilibrium state $\mathbf{v} = 0$ is excluded by the constraints[18]. FCM and metabolic economics rely on different assumptions, but constrain the flux directions in similar ways. The flux cost minimisation theorem (Theorem 4) shows that solutions of non-flux-enforcing FCM problems are economical, that all economical flux distributions are solutions of FCM problems, and that the economic potentials are related to Lagrange multipliers in the FCM problems. Accordingly, given an enzyme-balanced state with flux gain vector $\mathbf{z}^{\mathrm{v}}$, we can construct various FCM problems with the same flux objective $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$ and different cost functions $\bar{H}$. All these FCM problems will yield economical flux distributions and any economical flux distribution can be generated in this way. Flux-enforcing FCM problems need to be excluded because the flux bounds could enforce futile modes. The equivalence between flux and enzyme optimality holds for FCM in general and for weighted flux minimisation in particular, and weighted flux minimisation is a good way to obtain economical flux distributions in practice. Each choice of the flux cost function yields a different solution and by sampling them, all possible economical flux distributions could be obtained.

Metabolic economics, as a theory, shows some general invariance properties: models may be formulated in different ways, but describe the same reality and make the same predictions. For instance, an shift or a joint rescaling of return and investment functions has no effects because their derivatives, and not their absolute values appear in the balance equation. Likewise, if models contain conserved moieties, the economic potentials $\mathbf{w}^{\mathrm{c}}$ can be gauged while leaving the differences $\Delta \mathbf{w}$ unchanged (see SI S2.3).

An important decision in modelling concerns the reactions included in the network and the external metabolites marking its boundaries. A metabolic pathway may either be described in isolation, with external metabolites on its boundary (model A), or embedded in a larger network with the boundary metabolites being internal (model B). In the two cases, the same metabolic objective must be formulated differently: in model A, the objective function can only score metabolites and reactions within the pathway; in model B, it may score variables in other regions of the network. Nevertheless, both models describe the same reality and should therefore show the same metabolic and economic state. Thus, there must be different fitness functions for which the same enzyme levels and the same metabolic state can be optimal. We can achieve this by matching the metabolic states and economic potentials between both models. The economic potentials of the boundary metabolites in model B can be transferred to model A, thus defining an effective objective function for this model.

As another example, consider two models of a chemostat culture: model A comprises the cell and treats substance in the growth medium as external (with fixed concentrations). Model B describes them dynamically and their concentrations are governed by the chemostat equation (comprising in- and outfluxes of the chemostat and cellular uptake or secretion rates). In model A, the extracellular substances will affect the cells' fitness by their concentrations as described by economic loads $p_i^{\mathrm{c}} = \partial g/\partial x_i$. In model B, they affect the fitness by uptake rates as described by economic potentials $w_i^{\mathrm{c}} = \partial g/\partial \varphi_i^{\mathrm{tot}}$. Again, both models describe the same situation. We consider a concentration variation δx_i in model A, and to achieve the same variation in model B, we assume a supply flux $\delta \varphi_i^{\mathrm{ind}} = \sum_l \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l} \delta x_i$ (summing over all reactions that produce or consume the metabolite). Accordingly, the economic load in model A and the economic potential w_i^{c} in model B are related by $p_i^{\mathrm{c}} = \sum_l \bar{E}_{\mathrm{c}_i}^{\mathrm{v}_l} w_i^{\mathrm{c}}$.

E   Extending the theory

Metabolic economics, as developed so far, pictures cells in a very simple way. Reactions are catalysed by specific enzymes, a static enzyme profile leads to a steady stationary state, the metabolic return depends

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[18]Flux-enforcing bounds may be used to model a baseline consumption of ATP in maintenance reactions. In metabolic economics, flux bounds are implemented by constraints. The resulting Lagrange multipliers can appear as effective gains into the vector $\mathbf{z}^{\mathrm{v}}$ (see SI S2.4), based on which futile modes are defined. When comparing metabolic economics to FCM problems, these effective gains reappear in the vector $\mathbf{z}^{\mathrm{v}}$ used in the FCM problem.

only on fluxes and concentrations, and the enzyme levels are optimised. Real cells, in contrast, show dynamic behaviour, operate in uncertain environments, and may show non-optimal states. Metabolic economics can be extended to address this (for details, see SI S2).

Isoenzymes and unspecific enzymes First, we can discard the premise "one reaction, one enzyme". Biochemical reactions can be catalysed by several enzymes (*isoenzymes*), and enzymes may catalyse multiple reactions (*unspecific* or *multifunctional* enzymes). In models, isoenzymes can be described by separate reactions, and unspecific enzymes can be captured by a modified reaction balance (see SI S2.2). The compound balance remains unchanged, but the reaction elasticities will have smaller values because enzymes split their activity between several reactions.

Constraints on state variables In the optimisation of enzyme profiles, we may put constraints on metabolite levels, fluxes, or enzyme levels. For instance, certain enzyme fractions may be limited by space restrictions, and cells may need some minimal ATP level to survive. In the optimisation problem, this is described by inequality constraints, and the corresponding Lagrange multipliers will appear as effective gains or prices in $\mathbf{z}^{\mathrm{v}}$, $\mathbf{z}^{\mathrm{c}}$, and $\mathbf{h}^{\mathrm{u}}$ (SI S2.4). As an example, consider the positivity constraint for enzyme levels: when an enzyme level hits the constraint at $u_l = 0$, the corresponding Lagrange multiplier can be treated as an effective price $h_l^{\mathrm{u}*}$ and the cost-benefit imbalance $g_l^{\mathrm{u}} < h_l^{\mathrm{u}}$ becomes an equality $g_l^{\mathrm{u}} = h_l^{\mathrm{u}} - h_l^{\mathrm{u}*}$. Likewise, constraints on fluxes or metabolite levels can give rise to effective gains. For instance, assume that the flux of a maintenance reaction is kept above some lower bound by a flux constraint. If the flux hits the bound, the Lagrange multiplier, as an effective gain $z_l^{\mathrm{v}*}$, adds to the direct flux gain (and thus, to the flux demand) of that reaction. In the reaction balance, this term can justify a flux even if no valuable compounds are produced. Similarly, upper bounds on enzyme levels could be used to limit the enzyme abundance in cells, in compartments, or on membranes (e.g. the photosystem or ATP synthases in energy metabolism).

Soft constraints on enzyme levels Instead of limiting the metabolite levels, fluxes, or enzyme levels by hard constraints, we may penalise them by cost terms. As an example, consider the positivity constraint $u_l \geq 0$ for enzyme levels. Instead of using the constraint, we may penalise small enzyme levels by a penalty term that increases strongly as u_l goes to zero. This term may be justified by assuming leaky protein expression whose suppression would consume additional resources. With a penalty term instead of hard constraints, the "inactive enzymes" in an optimal state will have small, positive levels. The penalty cost corresponds to the effective cost that would arise from the hard constraints (see SI S2.4).

The balance equations (11) and (14) describe two basic constellations: a reaction with its reactants, and a metabolite with reactions around it. Balance equations for other network elements, such as allosteric effectors, can be derived. For reactions affected by several parameters, we can sum over their equations and obtain a balance between flux demand and the parameter's weighted average price $g_l^{\mathrm{v}} = \langle h_n^{\mathrm{p}}/\bar{E}_{\mathrm{p}_n}^{\mathrm{v}_l} \rangle$ (proof: from $\sum_p g_l^{\mathrm{v}} v_l = \sum_p h_n^{\mathrm{p}} p_n/E_{\mathrm{p}_n}^{\mathrm{v}_l}$ follows $g_l^{\mathrm{v}} v_l = \frac{1}{N} \sum_p h_n^{\mathrm{p}} p_n/E_{\mathrm{p}_n}^{\mathrm{v}_l}$). If several reactions are affected by one parameter, we can sum over the reaction balances and obtain a balance between the elasticity-weighted average flux demand and the parameter price $\sum_l g_l^{\mathrm{v}} \bar{E}_{\mathrm{p}_n}^{\mathrm{v}_l} = h_n^{\mathrm{p}}$ (proof: $\sum_l g_l^{\mathrm{v}} v_l E_{\mathrm{p}_n}^{\mathrm{v}_l} = h_n^{\mathrm{p}} p_n$). In fact, the reaction balance is a special case of the compound balance, treating the enzyme as an external compound with elasticities $\bar{E}_{\mathrm{x}_j}^{\mathrm{v}_l} = \frac{v_l}{u_l}$ and a load p_j^{x} given by the enzyme price h_l^{u}.

Non-optimal states Enzyme optimality can be a helpful hypothesis, but may not hold for real cells. Even if cells tended to optimise their enzyme levels, and even if our fitness function captured all relevant fitness requirements, enzyme levels could still be maladapted, for instance after sudden perturbations. Knock–downs or artificial enzyme expression can enforce non-optimal enzyme levels, and leaky transcription can cause an enzyme expression where reactions should be inactive. When enzyme benefit and cost do not match, we obtain an *economic imbalance* $y_l^{\Delta} = \frac{\partial f}{\partial \ln u_l}$. With this term, we obtain the reaction imbalance

$$[\Delta w_l + \hat{z}_l^{\mathrm{v}}] v_l \quad = \quad y_l + y_l^{\Delta} \tag{21}$$

for non-optimal states (see SI S2.7). Likewise, we obtain balances between enzyme demand and price,

$$[\Delta w_l + \hat{z}_l^{\mathrm{v}}] \frac{v_l}{u_l} = h_l^{\mathrm{u}} + f_l^{\mathrm{u}} \tag{22}$$

with the enzyme stress $f_l^{\mathrm{u}} = \frac{\partial f}{\partial u_l}$, or between flux demand and flux price

$$\Delta w_l + \hat{z}_l^{\mathrm{v}} = h_l^{\mathrm{v}} + f_l^{\mathrm{v}} \tag{23}$$

with the flux stress $f_l^{\mathrm{v}} = \frac{1}{v_l} \frac{\partial f}{\partial \ln u_l}$. Positive economic stresses (or flux stresses whose signs match the flux direction) show that an enzyme upregulation would be profitable, i.e., the enzyme's marginal benefit exceeds its cost. If non-optimality has been enforced by constraints (e.g., if an enzyme knock-down is modelled by fixing the enzyme level at a lower value), the effective gain caused by the constraint will appear as a flux stress. Situations in which organisms are missing an enzyme (but may evolve it) can be described similarly: the selection pressure for an enzyme with flux demand $g_l^{\mathrm{v}} = \Delta w_l + \hat{z}_l^{\mathrm{v}}$ is given by Eq. (23). If it exceeds the evolutionary cost [23, 24], the enzyme could be expected to evolve.

Non-enzymatic reactions Also non-enzymatic reactions can play a role in metabolic models. Such reactions can degrade valuable metabolites or produce toxic metabolites, which would compromise the metabolic performance. Although these reactions are not directly regulated by enzymes, they may be compensated or controlled by enzymatic reactions. This affects the optimal choice of enzyme levels. For instance, if a metabolite is only needed as a catalyst, but not as a pathway intermediate, it should just exist at a constant concentration and not be produced nor consumed. However, if the metabolite is degraded non-enzymatically, enzymes need to produce it continuously to keep it at the right level. The resulting flux will look futile because economic potential is constantly produced and destroyed. In fact, it *would* be futile if all reactions were enzymatic. However, if the degradation reaction is non-enzymatic, such a *pseudo-futile mode* may be the best the cell can achieve, and the flux distribution must be described as economical by our theory.

To ensure this, we need to modify some details of metabolic economics: (i) Test modes (used to define futile modes) can only contain enzymatic reactions. (ii) The gain conditions (6) and (7) must contain terms for non-enzymatic reactions (details see SI P1.4). (iii) The reaction balance holds only for enzymatic reactions. To obtain reaction balances for non-enzymatic reactions, we can assume that they are catalysed by hypothetical enzymes with non-optimal levels (see SI S2.7), entailing an economic imbalance $y_l^{\Delta} = \partial f / \ln u_l$ on the right-hand side of the reaction balance (21). A positive imbalance y_l^{Δ} describes which enzyme costs would be economical if the reaction were enzymatic. A negative imbalance, in contrast, describes a loss (negative benefit) in a non-enzymatic reaction. (iv) In the compound balance, there are additional terms related to non-enzymatic production, degradation, or dilution of the metabolite. These terms do not represent enzyme costs, but (possibly negative) flux benefits. (v) The definition of economic potentials and loads, as well as the compound rule, remain unchanged.

The dilution in growing cells can effectively be described as a non-enzymatic degradation of all metabolites. This will not only affect the stationary fluxes and the model dynamics, but also its economics, e.g.., the metabolite and enzyme demands. To adapt our formulae (e.g., the gain conditions and economic balance equations), we describe the dilution of each metabolite i as a first-order non-enzymatic degradation with rate κc_i (where κ is the cell growth rate). This leads to an extra term $-\kappa\mathbf{I}$ in the Jacobian matrix and changes the summation and connectivity theorems for control coefficients (P2). Gain conditions and balance equations also obtain new terms (see SI P1.2). To see this, consider the reaction balance in an unbranched metabolic pathway with production objective: if the internal metabolites are diluted, the fluxes v_l decrease along the chain. Since $\mathbf{z}^{\mathrm{c}} = 0$, the sum rule $\sum_l y_l = \sum_l z_l^{\mathrm{v}} v_l$ must still hold. If we compare this model to a model without dilution and the same flux benefit, the sum of marginal costs $\sum_l y_l = \sum_l h_l^{\mathrm{u}} u_l$ must be higher in the presence of dilution.

Multiple objectives and preemptive expression In metabolic economics, the metabolic objective can depend on all fluxes and metabolite levels. Which of these variables are relevant in reality, and in which

(a) Alternating objectives

Objective A
Objective B
Objective C

Time

(b) Uncertain objectives

? A
? B
? C

(c) Opposing simultaneous objectives

Objective C
Pareto surface
Infeasible region
Objective B
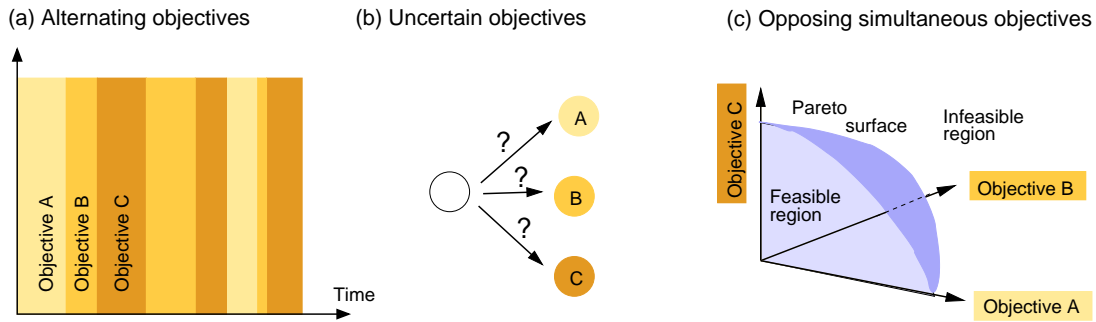Feasible region
Objective A

Figure 12: Metabolic economics with mixed objectives. (a) Alternating metabolic objectives. Different metabolic objectives $z_{(n)}(\mathbf{v}, \mathbf{c})$ (represented by colours) occur with average durations $\tau_{(n)}$. (b) Metabolic objectives can be uncertain, either because the cell has vague information about its environment or because it needs to "guess" future requirements appearing with probabilities p_n. In both cases, enzyme levels can be optimised for convex combinations of the objectives with weights p_n. (c) Multi-objective optimisation. Pareto-optimal states are states in which an improvement in one objective entails losses in other objectives. The tangent vector of the Pareto surface must have at least one negative component. The condition for Pareto-optimal states is equivalent to an optimality condition for a mixed objective (i.e., a convex combination of the objectives).

combinations? The metabolic objective in a given state is characterised by gain vectors $\mathbf{z}^{\mathrm{v}}$ and $\mathbf{z}^{\mathrm{c}}$. The non-zero elements, signs, and values in gain vectors show which state variables should be high or low. In addition, there may be "effective gains", Lagrange multipliers arising from flux or concentration constraints and indicating which state variables hit upper or lower bounds. A pure metabolic objective depends on one state variable only, and its gain vectors contain a single entry (e.g., related to one product to be produced). General objectives score several variables and their gain vectors have several non-zero elements. However, cells may not just have a single objective, but varying or opposing objectives. If the external conditions are fixed and only the objectives are changing, we can replace such multi-objective problems by problems with a single mixed objective. Given a number of objective functions $g_{(n)}(\mathbf{u})$, mixed objectives are convex combinations $g(\mathbf{u}) = \sum_n \lambda_n g_{(n)}(\mathbf{u})$ with positive weights $\lambda > 0$ normalised to 1, and the mixed gain vectors read $\mathbf{z}^{\mathrm{v}} = \sum_n \lambda_n \mathbf{z}^{\mathrm{v}}_{(n)}$ and $\mathbf{z}^{\mathrm{c}} = \sum_n \lambda_n \mathbf{z}^{\mathrm{c}}_{(n)}$. Using mixed objectives, we can describe different types of optimality problems (see Figure 12):

1. **Adaption to rapidly changing requirements** Assume that a cell's objective alternates between different objective functions with average durations τ_n. If these changes are much faster than enzyme levels can be adapted and if the environmental conditions (e.g., the external concentrations) remain fixed, cells can optimise their average fitness by an adaption to the average objective $\langle \mathbf{z}^{\mathrm{v}} \rangle = \sum_n p_n \mathbf{z}^{\mathrm{v}}_{(n)}$, where $p_n = \tau_n / \sum_m \tau_m$.

2. **Uncertain objectives** Uncertainties about the objective can arise for different reasons: because cell signals contain too little information about the cell's current situation or because cells need to "anticipate" future demands (proteins are always produced ahead of time, with a delay between production and the last usage given by the production time plus the effective protein lifetime). To be prepared for different objectives (with flux gain vectors $\mathbf{z}^{\mathrm{v}}_{(n)}$ and probabilities p_n), cells may maximise their expected fitness, with an average gain vector $\langle \mathbf{z}^{\mathrm{v}} \rangle$. Such "bet-hatching" strategies are not only important in completely uncertain situations, but also in stable and certain environments with a small chance for deviating objectives [25, 21]. An optimisation with mixed objectives yields mixed enzyme profiles. This may explain preemptive enzyme expression observed in real cells.

3. **Compromise between opposing objectives** Usually, one enzyme profile cannot optimise several objectives. However, we can consider compromise strategies in which none of the objectives is fully

optimised, but any possible improvement in one objective would compromise the others. Vilfredo Pareto used this criterion to describe compromises between social agents with opposing interests. Following the use in flux analysis [26], we can use his criterion to describe trade-offs between opposing objectives in metabolic systems. The Pareto-optimality problem can be reduced to an optimisation with mixed objectives: for each Pareto-optimal state, there is a mixed objective $g = \sum_n \lambda_n g_{(n)}$ whose optimality condition is equivalent to Pareto's optimality condition. The weights λ_n must be positive and sum to 1 like probabilities. To determine potential Pareto-optimal states, we can sample such weights, determine mixed gain vectors $\langle \mathbf{z}^{\mathrm{v}} \rangle = \sum_n \lambda_n \mathbf{z}^{\mathrm{v}}_{(n)}$ and $\langle \mathbf{z}^{\mathrm{c}} \rangle = \sum_n \lambda_n \mathbf{z}^{\mathrm{c}}_{(n)}$, and use them in metabolic economics. This will yield *all* solutions to the multi-objective problem, and maybe others that are not on the Pareto front for a specific kinetic model.

Other control variables In metabolic economics, enzyme levels are not explained mechanistically (by processes like transcription, translation, and protein degradation), but determined from optimality principles. Why are enzyme levels, and not for instance mRNA levels, described as control variables? This choice is not fixed, but depends on the modeller's questions: if our system of interest is the metabolic network and if enzyme levels appear as its parameters, it is natural to treat them as control variables. If enzyme production is included in the metabolic network, other quantities like enzyme transcription rates or mRNA levels could be control variables. In other cases, even the dilution rate (and thus, growth), could be a controllable parameter to be optimised. There may be cases in which several quantities affect the same reaction or one quantity affects several reactions. For a control parameter p_n affecting a single reaction (with parameter price h^{p}_n and elasticity $\bar{E}^{\mathrm{v}}_{p_n}$), we obtain the balance equation $g^{\mathrm{y}}_l v_l = h^{\mathrm{p}}_n p_n / E^{\mathrm{v}_l}_{\mathrm{p}_n}$. Whatever control variables are chosen, they must be penalised by a investment function, or the fitness function must depend on them in contrary ways that create a trade-off between costly and beneficial effects.