How enzyme economy shapes metabolic fluxes

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Abstract

Metabolic fluxes are governed by physical and economic principles. Stationarity constrains them to a subspace in flux space and thermodynamics makes them lead from higher to lower chemical potentials. At the same time, fluxes in cells represent a compromise between metabolic performance and enzyme cost. To capture this, some flux prediction methods penalise larger fluxes by heuristic cost terms. Economic flux analysis, in contrast, postulates a balance between enzyme costs and metabolic benefits as a necessary condition for fluxes to be realised by kinetic models with optimal enzyme levels. The constraints are formulated using economic potentials, state variables that capture the enzyme labour embodied in metabolites. Generally, fluxes must lead from lower to higher economic potentials. This principle, which resembles thermodynamic constraints, can complement stationarity and thermodynamic constraints in flux analysis. Futile modes, which would be incompatible with economic potentials, are defined algebraically and can be systematically removed from flux distributions. Enzymes that participate in potential futile modes are likely targets of regulation. Economic flux analysis can predict high-yield and low-yield strategies, and captures preemptive expression, multi-objective optimisation, and flux distributions across several cells living in symbiosis. Inspired by labour value theories in economics, it justifies and extends the principle of minimal fluxes and provides an intuitive framework to model the complex interplay of fluxes, metabolic control, and enzyme costs in cells.

Keywords: Flux analysis, Enzyme cost, Benefit-cost analysis, Thermodynamic flux analysis, Futile cycle, Principle of minimal fluxes, Labour value.

Abbreviations: PFK: phosphofructokinase; FBP: fructose bisphosphatase; ATP: adenosine triphosphate; ADP: adenosine diphosphate; F6P: fructose 6-phosphate; F16BP: fructose 1,6-bisphosphate; P: phosphate.

1 Introduction

Cells invest a considerable fraction of their proteome in metabolic enzymes. A metabolic network can support various flux distributions, but only some of them are physically and biologically plausible. Knowing the underlying principles could help engineer microbes and better understand phenomena like the Warburg effect in cancer [1]. Flux balance analysis (FBA) [2] and its many variants predict fluxes from assumptions about stationarity, thermodynamics, metabolic yield, and principles of flux minimisation. A known flux mode – i.e., active reactions and flux directions – can help restrict the possible flux distributions. Flux directions can either be constrained ad hoc or based on general principles: thermodynamic laws, for instance, restrict fluxes to run from higher to lower chemical potentials [3, 4, 5, 6, 7, 8, 9], which make certain cycle fluxes impossible. Within these physical limits, various flux distributions remain possible and cells can choose between them, at least partially, by their enzyme abundances. By repressing some enzymes, cells can disrupt futile modes and choose between pathways that allow for fast growth or for high yield [10, 11]. Enzymes compete for available space [12], and their production or presence in cells can lead to growth defects [13, 14]. Therefore, cells must prioritise the usage of metabolic pathways and balance their fluxes according to supply and demand, and the choice of metabolic fluxes will reflect a compromise between metabolic tasks – e.g. production of biomass components – and a limited enzyme capacity.

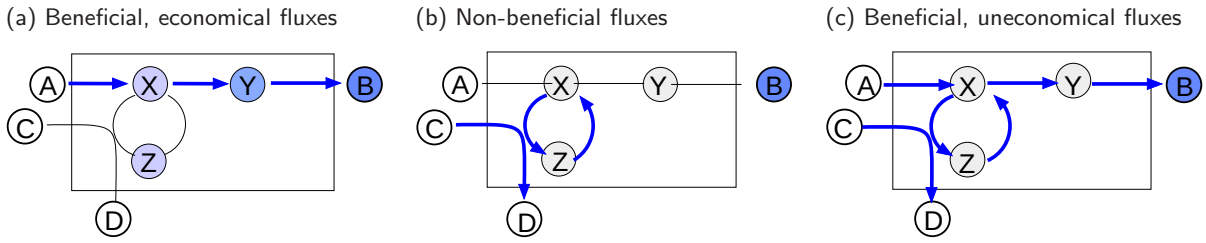

Figure 1: Economical fluxes, economic potentials, and a futile cycle. (a) Schematic metabolic pathway with a production objective (production of metabolite B). In steady state, the production and consumption of internal metabolites (within box) are balanced. In a kinetic model, an optimisation of enzyme levels would lead to a steady flux from A to B (unless enzyme expression is too costly to make this flux profitable), while the other two enzymes are not used. The flux is not only beneficial (providing a positive benefit as a whole), but also economical (all active enzymes provide individual benefits). We can choose economic potentials (shades of blue; white represents zero values) such that fluxes leads from lower to higher potentials. (b) The flux cycle between X and Z provides no benefit and is futile, even though it is thermodynamically feasible. The flux distribution in (c) – the sum of both flux distributions from (a) and (b) – is beneficial, but uneconomical because enzyme is wasted for driving the cycle. In (b) and (c), no consistent economic potentials can be chosen, confirming that the fluxes are not economical.

How can such compromises be described mathematically? In kinetic models [15, 16, 17, 13, 11], optimal enzyme profiles can be predicted from cost-benefit optimisation: enzyme profiles are scored by metabolic objective functions – which depend on metabolic fluxes and concentrations – and are penalised by cost functions [15, 16, 13] or constrained to a maximal sum of enzyme levels [16]. The numerical optimisation can be difficult and requires detailed kinetic models, which are hard to obtain for large metabolic networks. To date, only constraint-based models allow for flux prediction on a genomic scale. In some methods, the metabolic objective (a linear function of the fluxes) is traded against heuristic flux costs. According to the principle of minimal fluxes [18, 19], a flux distribution must minimise a heuristic cost function while achieving a predefined value of the metabolic objective. Flux minimisation avoids futile cycles (what counts as futile depends on the benefit and cost functions chosen) and can prefer low yield/low cost flux distributions over high yield/high cost strategies. In comparative studies of metabolic objectives [20, 21], different objective functions seemed to be suitable for different growth conditions, and flux costs were an important factor for flux prediction.

As a main drawback, flux analysis cannot capture the precise relationship between enzyme levels and stationary fluxes. In penalising fluxes instead of enzyme levels, it implies a simple proportionality between the two, which would only hold at fixed reactant levels. In reality, the stationary fluxes depend on enzyme levels in complicated ways and the empirical correlations between them are rather low [22]. If this is true, how can flux costs or the principle of minimal fluxes be justified by theory? For instance, can certain flux modes (sets of active reactions and their signs) be ruled out because they would imply a waste of enzyme in any underlying kinetic model?

Figure 1 shows an example: in a kinetic model with optimised enzyme levels, the cycle flux should be suppressed no matter which rate laws or enzyme investment function are used. Metabolic economics, a theory of enzyme-optimal states in kinetic models, provides criteria for economically feasible fluxes [23]. As a central concept, it introduces the economic potentials, state variables representing a demand for individual metabolites induced by the global objective. In enzyme-optimal states, all active enzymes must have a positive control over the metabolic return to balance their costs. To be economical, fluxes must lead from lower to higher economic potentials. In fact, the potentials play a similar role as the chemical potentials in thermodynamics [24]. In Figure 1 (a), this is clearly visible: economic potentials increase along the pathway flux, showing that the flux distribution is economical. In the futile cycle in (b), such an increase is impossible and any pathway flux with this cycle, like in Figure 1 (c), is uneconomical – even

it provides a positive overall benefit. Mathematically, the economic constraints resemble thermodynamic constraints, but hold independently of them: if metabolite C has a high chemical potential, the cycle in Figure 1 can be thermodynamically feasible, but will still remain futile.

By including the economic constraints into flux analysis, we obtain a physically, biochemically, and economically sound theory of metabolic fluxes, called economic flux analysis (EFA). With this framework, we can address general questions about metabolic strategies. For instance, the metaphor of "currency metabolites" is often used for metabolites that carry energy or chemical groups between biochemical processes. Here we assign actual values to such metabolites, and to all other substances in the cell. In reactions or pathways, the costs for maintaining a flux can be broken down into costs for substrates, cofactors, side products, and enzymes. In comprehensive cell models, the enzyme costs could further be split into costs for amino acid production, ATP, ribosomes, mRNA, and so on. Knowing the costs, we can study whether substances should be imported or synthesised by the cell (which will depend on the protein costs of transporter and pathway and on the value of substrates, cofactors, and energy), and we can claim that substances that embody large enzyme investments should be used more efficiently (i.e., downstream of high-cost transporters or pathways, high-yield strategies should be preferred).

This article describes economic flux analysis and some of its applications. I compare and combine the economic constraints with thermodynamic constraints and discuss how economical fluxes relate to futile cycles, their biochemical regulation, and choices between alternative pathways. Details are given in the supplementary information (SI) at www.metabolic-economics.de/metabolic-economics. MATLAB code is freely available on github [25].

2 Economic flux analysis

Flux balance analysis (FBA) is a method for predicting flux distributions in metabolic networks. The fluxes need to be stationary and satisfy upper and lower flux bounds. To ensure thermodynamically feasible fluxes, one may additionally require that metabolites have plausible chemical potentials, whose differences along reactions predefine the flux directions. With these constraints, the feasible flux distributions form a non-convex polytope in flux space. Economic flux analysis adds another constraint, related to an economic usage of enzyme. Like in FBA, we consider a linear benefit function $z(\mathbf{v}) = \mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$. Flux distributions can be classified as beneficial (positive benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} > 0$), non-beneficial (zero benefit, satisfying $\mathbf{N}^{\mathrm{z}}\,\mathbf{v} = \binom{\mathbf{z}^{\mathrm{v}}\cdot}{\mathbf{N}}\,\mathbf{v} = 0$), or costly (negative benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v} < 0$). Non-beneficial and costly modes are called *futile*. For convenience, the flux gain vector is split into $\mathbf{z}^{\mathrm{v}} = \mathbf{N}^{\mathrm{x}\top}\mathbf{w}^{\mathrm{x}} + \hat{\mathbf{z}}^{\mathrm{v}}$. One term scores the net production of external metabolites ($\mathbf{N}^{\mathrm{x}}$ is the part of the stoichiometric matrix referring to these metabolites), the other one scores fluxes directly. The w_j^{x} are called economic potentials (of the external metabolites) and the $\hat{z}_l^{\mathrm{v}}$ are called *direct flux gains*. The splitting of $\mathbf{z}^{\mathrm{v}}$ can be chosen at will. Setting $\mathbf{z}^{\mathrm{v}} = \hat{\mathbf{z}}^{\mathrm{v}}$ and $\mathbf{w}^{\mathrm{x}} = 0$, we can attribute the flux gains directly to reactions. In contrast, we may attribute all the benefit to external metabolite production (possibly introducing virtual metabolites whose potentials w_j^{x} are proxies for direct flux gains).

In EFA, we do not use the flux objective for a direct optimisation like in FBA, but postulate that all active enzymatic reactions must satisfy a reaction balance

$$[\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, v_l = y_l \tag{1}$$

with positive enzyme costs y_l. The term in brackets, called *flux demand*, consists of the direct flux gain $\hat{z}_l^{\mathrm{v}}$ and an indirect flux demand Δw_l, that is, an effective demand for metabolite conversion. As the symbol $\Delta w_l = \sum_i n_{il}\, w_i$ suggests, it depends on the economic potentials w_i of reactants. While direct flux gain $\hat{z}_l^{\mathrm{v}}$ and external economic potentials w_j^{x} are predefined by the benefit function, the internal potentials w_m^{c} and enzyme costs y_l are variables to be determined. Since enzyme costs must be positive, fluxes and flux demands must have the same signs, whenever $\hat{z}_l^{\mathrm{v}} = 0$, fluxes lead from lower to higher potentials.

(a) Mass balance $0 = \frac{\mathrm{d}c_i}{\mathrm{d}t} = \sum_l n_{il}\, v_l$

(b) Thermodynamics $0 < \sigma_l = -\frac{v_l\, \Delta\mu_l}{T}$

(c) Enzyme economy $0 < u_l\, h_l^{\mathrm{u}} = [\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, v_l$

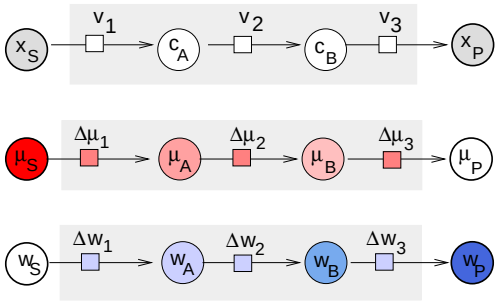

Figure 2: In economic flux analysis, metabolic fluxes are governed by three principles: stationarity, thermodynamics, and enzyme economy. (a) In stationary state, production and consumption of internal metabolites must be balanced: in the linear pathway shown, all fluxes v_l must be equal. (b) Reaction fluxes must dissipate Gibbs free energy. To ensure a positive entropy production σ_l per volume, fluxes must run from higher to lower chemical potentials μ_i, i.e., in the direction of the thermodynamic force $\Theta_l = -\Delta\mu_l/RT$. (c) In enzyme-optimal states, fluxes and flux demands $\hat{z}_l^{\mathrm{v}} + \Delta w_l$ must have equal signs, allowing for positive enzyme cost $h_l^{\mathrm{u}}\, u_l$. Without direct flux gains $\hat{z}_l^{\mathrm{v}}$, fluxes run from lower to higher economic potentials w_i.

Thus, economic potentials tend to increase along the production chain and, in analogy to labour values in economics, describe the enzyme labour embodied in a metabolite.

In EFA, the reaction balance Eq. (1) appears as a postulate. Why should we assume it? The balance equation can be justified in two ways, starting from different theories. One justification is based on flux cost minimisation (FCM), a generalised form of the principle of minimal fluxes [18]). We consider FCM problems with increasing cost functions $\bar{H}(\mathbf{v})$ (i.e., $\mathrm{sign}(\partial\bar{H}/\partial v_l) = \mathrm{sign}(v_l)$ whenever $v_l \neq 0$) and excluding any flux constraints that, by themselves, would enforce non-zero fluxes. Any solution to such a problem will satisfy a reaction balance Eq. (1) with $y_l \sim (\partial\bar{H}/\partial v_l)\, v_l$ and Lagrange multipliers w_l associated with the stationarity constraint.

A second, alternative justification comes from kinetic models with optimal enzyme levels as studied in metabolic economics [23] and SI S1. In such models, the enzyme levels u_l are control variables, chosen to maximise the difference between a metabolic return $g(\mathbf{u})$ and an enzyme investment $h(\mathbf{u})$. As a condition for optimal enzyme allocation, the flux benefit $\partial g/\partial \ln u_l = [\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, v_l$ and the cost $\partial h/\partial \ln u_l = y_l$ of each enzyme must be balanced, where $\hat{z}_l^{\mathrm{v}}$, w_l, and y_l are defined by derivatives of the return and investment functions in steady state. In flux analysis, we do not specify a kinetic model, but we may still require that predicted flux distributions should be realisable by *some* kinetic model in an enzyme-optimal state. The reaction balance (1) is a necessary condition for this and guarantees that flux distributions are not only globally beneficial, but beneficial in every reaction. In this respect, it resembles the flux sign constraint from thermodynamic analysis, whereby metabolic systems must dissipate Gibbs free energy not only in total, but in every single reaction.

Given a metabolic network and a flux gain vector $\mathbf{z}^{\mathrm{v}}$, how can we determine economic pathway fluxes? To allow for positive enzyme costs y_l, fluxes and flux demands must have equal signs. If the economic potentials w_i were known, they would define the directions of all active fluxes, and the flux signs could be used as bounds in FBA. On the contrary, to test whether a given flux distribution $\mathbf{v}$ is economical, we can search for internal potentials w_m^{c} such that

$$\mathrm{sign}(\hat{z}_l^{\mathrm{v}} + \Delta w_l) = \mathrm{sign}(v_l) \tag{2}$$

holds for all active enzymatic reactions. Condition (2), the *enzyme benefit principle* for fluxes, can be used in flux analysis as a third constraint aside from stationarity and thermodynamic feasibility (see Figure 2). With the thermodynamic driving force $\Theta_l = -\Delta\mu_l = -\Delta\mu_l^{(0)} - RT\sum_i n_{il}\, \ln c_i$ and the flux demand

$g_l^{\mathrm{v}} = \hat{z}_l^{\mathrm{v}} - \Delta w_l$, and assuming that all reactions are enzyme-catalysed, economic flux analysis simply requires that $\mathrm{sign}(v_l) = \mathrm{sign}(\Theta_l)$ for all active reactions and $\mathrm{sign}(v_l) = \mathrm{sign}(g_l^{\mathrm{v}})$ for all active enzymatic reactions. For an even stricter condition, we may put a lower bound ${y_l}^{\min}$ on the flux benefit

$$[\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, v_l > {y_l}^{\min} \tag{3}$$

for all active enzymatic reactions[1]. Fluxes as well as chemical and economic potentials satisfying the constraints can be computed by mixed-integer programming [27]. In contrast to chemical potentials, economic potentials are not directly related to metabolite concentrations and can therefore appear as separate variables.

Under which conditions can Eq. (2) be solved, i.e. for which flux distributions do feasible economic potentials w_i^{c} exist? As a necessary and sufficient condition, the flux distribution must be free of futile modes. A futile mode, as defined in [23], is a subset of active reactions in $\mathbf{v}$ that can support stationary futile fluxes with the same flux directions[2]. Like the enzyme benefit principle, this definition of futile modes depends only on flux directions, not on quantitative fluxes. Economical flux distributions, economic potentials, and futile modes are closely related. Figure 1 shows an example: the flux distribution in (a) is economical because economic potentials can increase along the flux. The flux cycle in (b) and (c) would require economic potentials that increase around a circle, which is impossible, so the flux cycle must be uneconomical. A main advantage of the flux cycle criterion is that it is local: flux distributions can be discarded based on small futile modes (even if the entire flux distribution is not fully known).

Economic flux analysis must comprise all three conditions (stationarity, thermodynamics, and economics). To be realisable in enzyme-optimal kinetic models, flux distributions must be economical; since the economic potentials are defined for steady states, the fluxes must be stationary, and thermodynamic correctness is necessary to ensure that all enzymes have some flux control[3]. EFA is closely related to FBA with flux minimisation (SI S1). However, instead of yielding a single flux distribution, it can be used for sampling of economical flux distributions (see Figure S1). In FBA with thermodynamic and economic constraints, the chemical and economic potentials appear as variables, and constraints between them and the fluxes define feasible flux modes (i.e. patterns of active fluxes and their signs).

EFA selects flux distributions with economically feasible sign patterns. What does this mean geometrically? In FBA, the nullvectors of the stoichiometric matrix $\mathbf{N}$ form a subspace in flux space. The feasible flux distributions, as points in this space, form a convex polytope, the intersection between the subspace and a box representing upper and lower flux bounds. An example is shown in Figure 3. Thermodynamically feasible flux distributions must show feasible sign patterns and these patterns correspond to segments of flux space, that is, orthants or their surfaces. By excluding infeasible segments, the constraints restrict the FBA polytope to a polytope of feasible flux distributions, which can be non-convex. Sampling and linear optimisation on this polytope is much harder than on the convex polytope used in FBA. If economic constraints are imposed (Eq. (2)), more segments may be excluded (see Figure 3). In some cases, both conditions may coincide. In particular, *non-productive* flux distributions, which do not produce or consume

[1] If all fluxes are positive (which we can assume without loss of generality), we obtain the inequalities

$$\hat{z}_l^{\mathrm{v}} + \Delta w_l^{\mathrm{c}} = \frac{h_l^{\mathrm{u}}}{r_l} \geq \frac{{h_l}^{\mathrm{u,min}}}{k_l^{\mathrm{cat}}\,(1 - \mathrm{e}^{\Theta_l}} \geq \frac{{h_l}^{\mathrm{u,min}}}{k_l^{\mathrm{cat}}} \tag{4}$$

where r_l is the flux per enzyme level, ${h_l}^{\mathrm{u}\,\min}$ is a lower estimate of the enzyme price, k^{cat} is the forward catalytic constant, and $1 - \mathrm{e}^{\Delta G/RT}$ is the thermodynamic efficiency (i.e., the ratio between net flux and partial forward flux [26]). With estimates for these quantities, we obtain tighter constraints for the flux demand $\hat{z}_l^{\mathrm{v}} + \Delta w_l$ and for the economic potentials w_i therein.

[2] For a formal definition, we consider stationary flux distributions $\mathbf{k}$ (called *test modes*) on the active region of $\mathbf{v}$. If $\mathbf{k}$ is futile with respect to $\mathbf{z}^{\mathrm{v}}$ and sign-concordant with $\mathbf{v}$ (i.e. fluxes in $\mathbf{k}$ and $\mathbf{v}$ have the same directions on their common active reactions), the flux mode $\mathrm{sign}(\mathbf{k})$ is a *futile mode in* $\mathbf{v}$ with respect to $\mathbf{z}^{\mathrm{v}}$.

[3] If a metabolic pathway contains an irreversible reaction – whose rate does not depend on the product level at all – the downstream reactions have no control over the pathway flux. This creates a paradoxical situation: on the one hand, their enzymes provide no benefit (because the flux does not depend on them). Without the enzymes, on the other hand, the flux breaks down. In a numerical optimisation, there would be no feasible optimum for the enzyme levels. To prevent such situations, reversible, thermodynamically feasible rate laws are mandatory in metabolic economics [28].

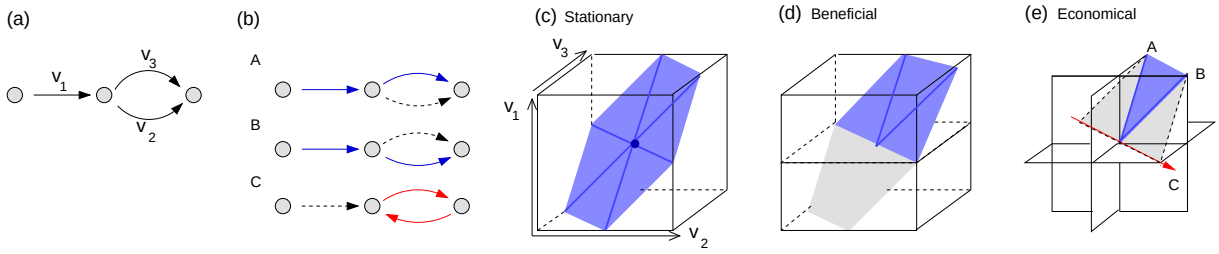

Figure 3: Economical flux distributions as points in flux space. (a) Example pathway with flux bounds $-1 < v_l < 1$. (b) Elementary beneficial (blue) and futile (red) flux modes. (c) Since each reaction flux can have three possible signs (1, 0, or -1), there are $3^3 = 27$ possible sign patterns, corresponding to segments of flux space. Only 13 of them can be realised by stationary fluxes (six triangles, six lines, and central dot representing $\mathbf{v} = (0, 0, 0)trans$). The other segments are not intersected by the plane of stationary fluxes. (d) A linear FBA objective (here, the rate of reaction 1) defines a flux gain vector $\hat{\mathbf{z}}^{\mathrm{v}} = (1,\, 0,\, 0)^{\top}$ for economic flux analysis. Only five modes (those for which $v_1 > 0$) yield positive benefits (condition for being free of flux modes) while all others are excluded. (e) Only three of these (blue triangle and edges A and B) are sign-orthogonal on the futile cycle (red arrow) and therefore economical. Points from the feasible region can be sampled by weighted flux minimisation with random cost weights.

any (internal or external) metabolites, are thermodynamically infeasible, and if a production objective is assumed, they are also uneconomical. For such objectives, the economic constraints make thermodynamic constraints without concentration bounds obsolete. The analogies and differences between economic and thermodynamic constraints, the analogies to Kirchhoff's laws for electric networks [29, 30], and cases in which both constraints coincide, are discussed in SI S2.

The first step in EFA is to choose a metabolic objective or, equivalently, external economic potentials and direct flux gains. The rate of biomass production, chosen as an objective, yields a positive economic potential for biomass while the economic potentials of other external metabolites vanish[4]. Given an objective function, economical flux distributions can be computed in various ways: by FBA with economic and thermodynamic constraints, by flux cost minimisation, or by choosing stationary fluxes and removing all futile modes (SI).

If a flux distribution is given, it will constrain the economic potentials and enzyme costs, but will not determine them precisely: many choices of economic potentials may satisfy the reaction balance. Each choice corresponds to possible kinetic models with specific rate laws and investment functions [23]. For a unique choice of economic variables, further assumptions and data – for instance, bounds on enzyme prices, estimated for single enzymes – can be used. There are different possibilities (for details, see SI S5). If an economical flux distribution is given, we can use the flux directions, and possibly bounds on the *flux prices* $h_l^{\mathrm{v}} = y_l / v_l$, to obtain linear constraints for the internal economic potentials w_m^{c}. Lower bounds on flux prices may be obtained from estimated minimal enzyme prices h_l^{u} and catalytic constant k_l^{cat}. With the constraints Eq. (3), economic potentials can be sampled, optimised, or chosen at will, and the enzyme costs can be computed from the reaction balance. As a heuristic rule for choosing the economic potentials (see SI S5.4), we may assume that all enzymes have similar costs ("principle of uniform enzyme costs") or, alternatively, flux prices. The two assumptions are equivalent to assuming uniform enzyme benefits $[\hat{z}_l^{\mathrm{v}} + \Delta w_l]\, v_l$ or flux demands $\hat{z}_l^{\mathrm{v}} + \Delta w_l$. If estimated enzyme costs are given, we can also choose economic potentials that, by the resulting flux benefits, approximate these values. The calculation is similar to a calculation with uniform enzyme costs. Finally, if the fluxes are unknown and feasible enzyme costs y_l are precisely given, the fluxes can be derived from the flux gain condition (see SI S5.6). Feasible sets of

[4] In such objective functions, external metabolites (e.g., nutrients from the growth medium) are treated as "free gifts of nature", like natural resources in some economic theories. With such objectives, there will be no incentive for sustainable, resource-saving behaviour.

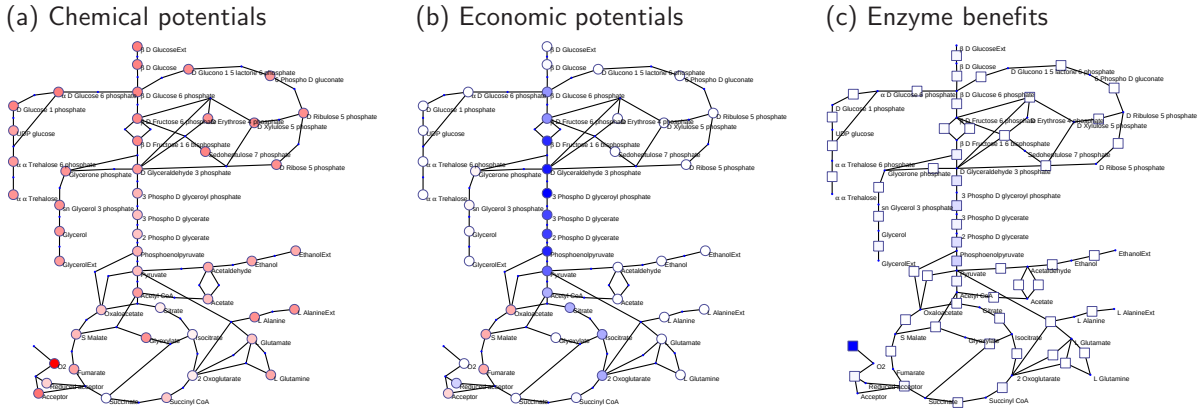

Figure 4: Economic flux analysis applied to central metabolism in *S. cerevisiae*. (a) Network model comprising glycolysis, the TCA cycle, the pentose phosphate pathway, and some side branches. Cofactors like ATP or NADH are part of the model, but not shown. ATP production is used as the metabolic objective. Thermodynamically and economically feasible fluxes (arrows) were determined by flux cost minimisation. Chemical potentials (white: low, red: high) were determined within constraints. (b) Economic potentials (blue: high. white: low. pink: negative), computed based on measured enzyme abundances (used as a proxy for enzyme costs). (c) Enzyme costs. The highest cost and benefit appears in oxidative phosphorylation (bottom left).

enzyme costs can be obtained by projections [23]. The enzyme costs predetermine a set of active fluxes, and condition (S8), together with the stationarity condition, can be solved numerically for the fluxes and the economic potentials w_m^c. Since the flux directions are not predefined, but emerge from the calculation, solutions may be thermodynamically infeasible. An example is shown in Figure S6 in the SI. Using these methods, one can compute realistic economic potentials (and possibly flux distributions) under constraints for enzyme levels, catalytic activities, enzyme costs, and flux prices. Bounds on flux demands $\Delta \mathbf{w}^c$ can be obtained from experimental data [31] (see SI S5.5).

Economic flux distributions obtained by EFA can be realised by kinetic models with enzyme-balanced states and specific rate laws and investment functions. Such models can be systematically constructed. Figure 4 shows an example, a network model of central metabolism in yeast, where ATP production was used as metabolic objective. Starting from an economical flux distribution (determined by flux minimisation), chemical and economical potentials are constructed based on heuristic assumptions [23]. In the calculation, knowledge about enzyme levels, protein sizes, and catalytic constants can be employed. Given the economic potentials, a variety of kinetic models is still possible. To obtain specific models, economic loads and reaction elasticities are chosen in agreement with the compound balance, and rate constants are computed from them (see [23] and SI S3.4). The resulting models are kinetically and economically plausible and can serve as starting points for studies on optimal metabolic behaviour, e.g., enzyme adaption to external perturbations [17].

Methods like FBA and flux cost minimisation (FCM) assume that the metabolic objective – e.g. the rate of biomass production – is a function of fluxes only. In reality, a cell's fitness will also depend on metabolite levels. This can create crucial trade-offs: high levels of intermediates may be required for large fluxes, but may also entail a high osmolarity and a high loss by dilution [32], and some compounds may be toxic at high concentrations. Thus, an economic theory of metabolism should include concentration benefits and show how they affect the flux distributions. Since FBA deals only with fluxes, it cannot capture such trade-offs. Metabolic economics, in contrast, considers flux and concentration gains and their coupling in kinetic models, so it fully accounts for these trade-offs. Nevertheless, fluxes and concentrations (represented by

economic potentials or loads) are governed by different gain conditions and balance equations and can therefore be studied separately.

In EFA, we focus on fluxes, so the relevant variables are economic potentials and flux demands. Since compound gains $z_i^{\mathrm{c}} = \partial z / \partial c_i$ do not explicitly appear in the reaction balance, they can be ignored in EFA (but are still captured, implicitly, by the values of economic potentials). Since EFA and FCM are equivalent, this shows that FCM, even if its fitness function ignores metabolite concentrations, is consistent with models that consider such effects. In models with dilution, however, concentration gains can explicitly appear in EFA: in such models, intermediates are diluted at a rate proportional to their own concentrations, and this requires a positive production (instead of a zero balance, the usual stationarity condition). Since dilution fluxes and concentrations are directly coupled, concentration objectives can be formulated as production objectives and vice versa, and concentration gains z_i^{c} can be rewritten as flux gains z_l^{v} for usage in FBA.

3 Futile cycles and enzyme regulation

If all metabolic enzymes of a cell were expressed together, futile cycles could easily arise. To prevent this, potential futile modes must be blocked by repressing some of the enzymes. As an example, consider the enzymes phosphofructokinase (PFK) and fructose bisphosphatase (FBP) in upper glycolysis (Figures 4 and 5). PFK transfers phosphate from ATP to fructose 6-phosphate (F6P), converting it into fructose 1,6-bisphosphate (F16BP). FBP catalyses the reverse reaction, but instead of producing ATP it releases inorganic phosphate. Together, the enzymes would drive a substrate cycle that effectively splits ATP into ADP and phosphate and produces heat (see Figure 5). Cells normally avoid this by repressing one of the enzymes (see, for instance, [33]).

Unlike previous verbal or topological definitions, the algebraic definition of futile cycles by futile test modes leads to clear mathematical statements: a flux distribution with futile modes is uneconomical and cannot appear in enzyme-optimal states. Futile modes need not be cycles, but may also be linear pathways that have no valuable product. Futile cycles can cause two different problems: wasting enzyme resources without metabolic advantage, or even bringing a metabolic disadvantage like the futile PFK-FBP loop which degrades ATP. In EFA, the two problems appear in the form of non-beneficial and wasteful cycles. In reality, cells may accept a waste of enzyme resources (e.g., expressing FBP preemptively, as a preparation for possible flux reversals), but avoid the waste of cofactors (in this case, by inhibiting FBP allosterically).

In economic flux analysis, the contrary regulation of PFK and FBP is directly explained by a need to avoid the futile cycle. Let us study the possible flux distributions (see Figure 5). If ATP (plus water) has a higher economic potential than ADP (plus phosphate) and if there are no direct flux gains, the PFK-FBP cycle is futile and all flux distributions containing it are uneconomical. Since all fitness demands are subsumed in the economic potentials, it does not matter if ATP, ADP, and phosphate are modelled as external or internal, and the surrounding network need not even be known. Running in reverse direction, the cycle would produce ATP. However, this would require an unphysiological drop in chemical potential between F16BP and F6P. Taken together, thermodynamic and economic constraints imply that only one of the reactions can be active at a time.

Non-beneficial flux distributions are important in EFA because, used as test modes, they define futile modes. However, enumerating them can be hard because of their large number: for instance, any two flux distributions can be linearly combined to yield a non-beneficial flux distribution. However, to rule out futile modes, we can restrict ourselves to *elementary* non-beneficial test modes, i.e., non-beneficial modes that contain no smaller non-beneficial modes. This definition resembles the definitions of elementary modes [34] and elementary flux modes [5]. Elementary non-beneficial modes, costly modes, and multi-objective modes (which must satisfy $\binom{\mathbf{A}}{\mathbf{N}} \mathbf{k} = 0$ with some matrix $\mathbf{A}$) are defined accordingly. For small networks, it is possible to enumerate all elementary futile test modes, so potential futile modes can be detected systematically. The model of yeast central metabolism shown in Figure 6 contains 303894 elementary

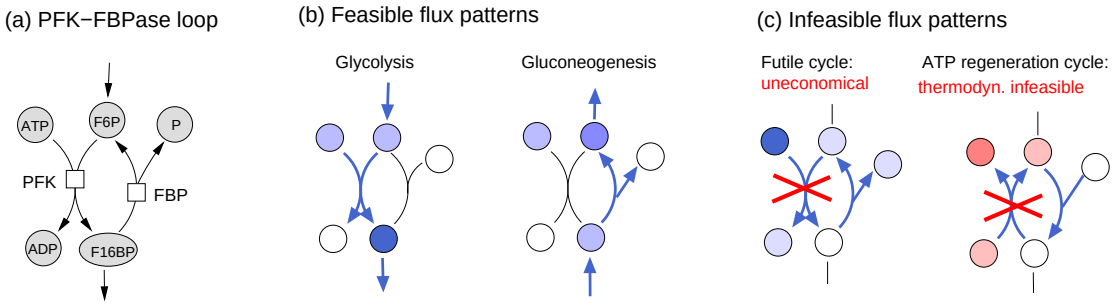

Figure 5: A futile cycle in upper glycolysis can be excluded by economic and thermodynamic constraints. (a) The enzymes phosphofructokinase (PFK) and fructose bisphosphatase (FBP) can form a futile cycle. (b) Feasible flux patterns. PFK is only used in glycolysis, FBP only in gluconeogenesis. Both flux modes are economical and thermodynamically feasible, and consistent economic potentials $w_{\mathrm{ATP}} > w_{\mathrm{ADP}} + w_{\mathrm{phosphate}}$ (shades of blue) can be assigned. (c) Infeasible flux patterns. The first cycle degrades ATP and is usually suppressed. If the economic potentials decrease from ATP to ADP and if there is no direct flux gain (e.g., for heat production), the cycle is futile. The cycle in reverse direction would regenerate ATP, but it is thermodynamically infeasible at physiological reactant levels (chemical potentials shown in red).

futile modes. Many of them are large, comprising up to 42 reactions (calculation using efmtool [35, 36]). Enzymes that participate in many futile modes are plausible targets for regulation because their repression will disrupt many modes at once (see Figure 6 (d)). In the yeast model, PFK and FBP rank among the top candidates for transcriptional regulation.

Futile modes show which reactions make a flux distribution uneconomical. Once a futile mode has been found in a flux distribution, it can be eliminated by subtracting the corresponding test mode $\mathbf{k}$, yielding the new flux distribution $\mathbf{v} - \lambda\mathbf{k}$. The prefactor λ is chosen such that one flux in the mode vanishes, but no flux changes its direction: this breaks the mode and keeps the flux distribution thermodynamically feasible. As an example, consider the flux distribution in Figure 1 (c). To remove the mode, we subtract the elementary flux distribution shown in (b) and obtain the mode shown in (a). The flux distribution changes only slightly, remains thermodynamically feasible, and becomes economical. Several futile modes in a flux distribution could be removed one after the other (see SI 2) and thermodynamically infeasible modes can be removed in a similar way [37].

Although substrate cycles waste resources – this is why we call them futile – they exist in cells. An example is autophagy, during which the same proteins may be degraded and produced. Futile cycles in metabolism are often explained by beneficial side effects, like heat production or a preparation for future flux reversals. Whether a flux distribution appears as futile depends on the fitness function assumed. For instance, whenever a production objective is assumed (e.g., biomass production as the only objective), any non-productive test mode $\mathbf{k}$ (satisfying $\mathbf{w}^{\mathrm{x}\top}\,\mathbf{N}^{\mathrm{x}}\,\mathbf{k} = 0$) will define a futile mode. However, if we count heat production as a beneficial side effect (e.g., a higher temperature in a compost pile inhabited by bacteria can speed up biochemical processes), and include it in our objective, each reaction obtains an additional flux gain $-\gamma\,\Delta\mu_l\,v_l$ (where γ scores the relative benefit of heat production), and previously futile modes may become beneficial.

Some modes that look futile, but are actually not, are caused by non-enzymatic reactions. If valuable compounds are non-enzymatically degraded, enzymes need to produce them constantly to keep them at desirable levels. Thus, even if a metabolite acts only as a catalyst, it must constantly be produced. If we observed this flux pattern, not knowing that the degradation is non-enzymatic, the simultaneous production and consumption would appear futile. However, if the degradation cannot be avoided, the flux distribution will be economical. The definition of futile modes and economical fluxes accounts for such cases, and pseudo-futile modes are correctly classified as economical (see [23]).

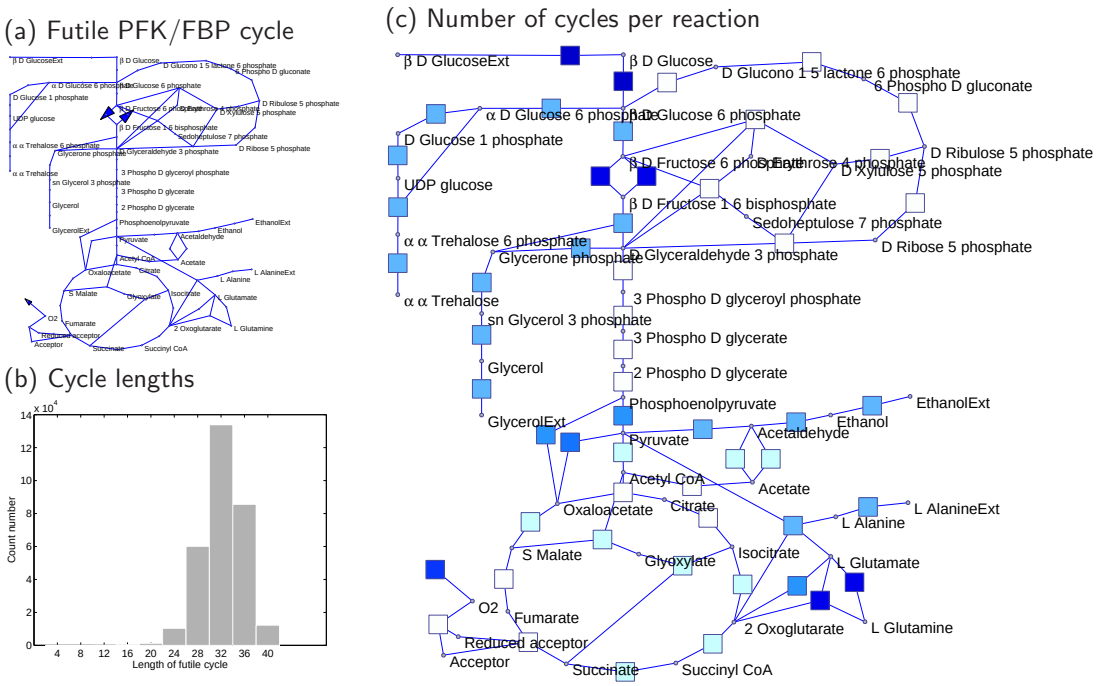

Figure 6: Futile cycles in central metabolism of the yeast *S. cerevisiae* (model as in Figure 4). The network contains two elementary non-productive cycles (indicators of thermodynamically infeasible flux distributions) and 303894 elementary futile cycles (all of them are thermodynamically feasible). (a) An elementary futile cycle (shown by arrows) produces ATP in oxidative phosphorylation (bottom left) and consumes it in the PFK/FBP cycle (see Figure 5). (b) Length distribution of elementary futile cycles. (c) A reaction participates in a certain number of elementary futile cycles. These numbers are shown by shades of blue (white: 0; dark blue: 10). For clarity, only cycles up to length 10 are considered. Reactions with large cycle count numbers (dark blue) are plausible targets of regulation.

Let us assume that all fitness effects and non-enzymatic reactions are considered in a model. Among the futile modes, some will be thermodynamically infeasible, but all others need to be disrupted by enzyme repression. If an enzyme participates in many possible modes, it will be a plausible target of transcriptional or posttranscriptional enzyme regulation. Such enzymes can be found by screening the network for elementary futile test modes. If a futile mode contained only one disruptible enzyme, this enzyme would have to be repressed constantly. Therefore, potential futile modes should contain at least two disruptible enzymes, and these should show mutually exclusive expression like in the PFK-FBP system. When cells switch between two flux distributions (e.g., from glycolysis to gluconeogenesis in the PFK-FBP system), there exists an intermediate range of enzyme levels where a futile mode could arise. This can be partially avoided if the enzymes are controlled by a bistable switch (in their transcriptional or post-transcriptional regulation).

Futile modes may not only be suppressed by regulation, but also selected against during evolution. In evolved metabolic networks, we can expect an avoidance of futile, thermodynamically feasible flux modes. Only enzymes and pathways involved in at least one beneficial flux mode should exist. A systematic detection of futile modes can be helpful for automatic metabolic network reconstruction.

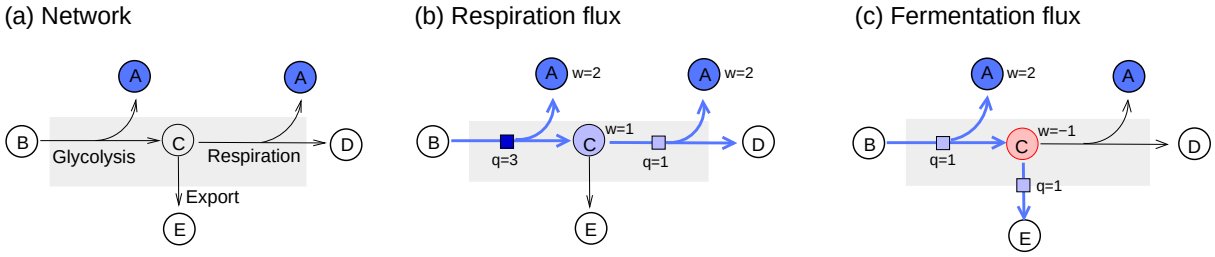

Figure 7: Choice between metabolic strategies. (a) Schematic model of central metabolism comprising glycolysis, respiration, and an export of incompletely oxidised metabolites (e.g. lactate or ethanol). The metabolic objective scores the production of A (representing ATP). (b) FBA favours the respiration pathway because of its higher yield (i.e. the amount of A produced per amount of B). Enzyme costs y_l and economic potentials w_i are shown as numbers and by shades of blue. (c) Low-yield fermentation flux. The intermediate C has a negative economic potential (pink as in Figure 4), and the zero potential of metabolite E is reached by a positive investment in the export. If both flux distributions are compared at equal ATP production rates (with a two-fold influx in (c)), their total enzyme benefit (and total cost) will be equal. A numerical enzyme optimisation in kinetic models would yield one of the flux distributions shown, or some linear combination.

4 Flux costs and choices between flux distributions

According to EFA and related flux analysis methods [18, 38, 21, 39], the choice between alternative pathways depends on enzyme costs. In contrast to FBA and FCM, kinetic models relate fluxes to rate laws, enzyme profiles, and reactant levels and can account for enzyme saturation and allosteric regulation. Taken as a biological assumption, minimisation of enzyme levels is more plausible than minimisation of fluxes, and allows us to make explicit assumptions about enzyme prices, kinetic mechanisms and metabolic objectives. By capturing the compromises between pathways, between benefit and cost, and between different objectives, EFA can address questions like the following: If the activity of an enzyme is perturbed (e.g., by mutation or knock-down), should the rest of the pathway be upregulated (to keep the flux constant) or downregulated (and compensated by other pathways)? What determines the choice between yield-maximising and rate-maximising strategies (see Figure 7)? How does this choice depend on previous investments in the substrate (see Figure 8)? Other methods would assess this on a case-by-case basis. Economic flux analysis explains more generally how enzyme costs, flux demands, and the choice of fluxes affect each other.

However, the different approaches are tightly related. Both economic flux analysis and flux cost minimisation (FCM) [18, 19] predict metabolic flux distributions (and thus, the usage of pathways) and trade metabolic benefit against enzyme cost. Despite their different concepts and assumptions, EFA and FCM constrain flux distributions in very similar ways [23]. Using FCM with variable cost functions, we can generate many flux distributions for a given network. If no fluxes are enforced by flux bounds, all these flux distributions will be economical, and the Lagrange multipliers arising from the stationarity constraints can play the role of economic potentials. On the contrary, any economical flux distribution can be obtained by a flux cost minimisation with appropriate flux cost functions. From a geometrical point of view, the benefit principle defines a set of economically feasible segments in flux space; intersecting them with the FBA-feasible flux polytope $\mathcal{P}_{\mathrm{FBA}}$ yields a non-convex polytope $\mathcal{P}_{\mathrm{EFA}}$ of economical flux distributions. The solutions of FCM fall into this polytope and fill it completely. Since EFA and flux cost minimisation (with variable cost weights) are equivalent, their advantages can be combined. EFA provides a theoretical justification for FCM, and FCM (with randomly sampled cost weights and without flux-enforcing constraints) can be used to generate economical, thermodynamically feasible flux distributions. The advantage of FCM is that it uses convex optimisation, which is easier than solving the reaction balance for $\mathbf{v}$ and $\mathbf{w}$ (by mixed-integer linear programming) or enumerating the flux cycles in large networks. Once a feasible flux

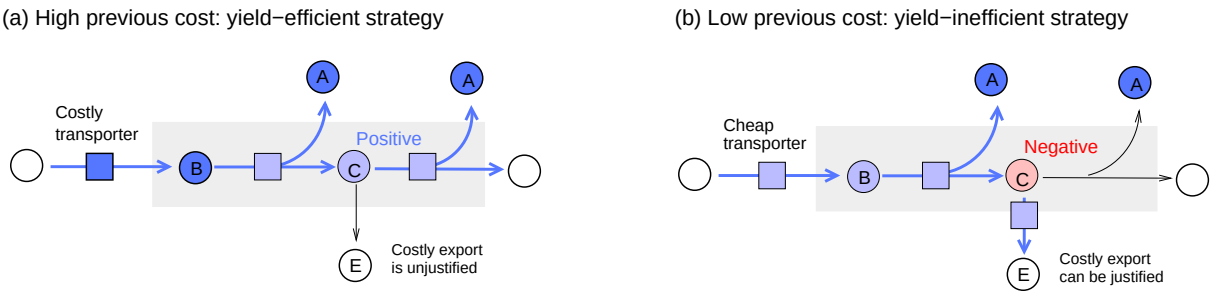

Figure 8: The choice between high-yield and low-yield strategies depends on enzyme investment embodied in metabolites. (a) Pathway from Figure 7, with an additional transport reaction. Due to previous investments, the intermediate C has a positive economic potential and exporting it would be wasteful. Only a yield-efficient strategy can be economical. (b) With a cheaper transporter, the potential of C can be negative, and a (yield-inefficient) export strategy can be economical. The transporter's price could be estimated from molecule properties like catalytic constant and protein size. The underlying principle – valuable metabolites should be used efficiently – may hold very generally.

distribution has been obtained, others with the same signs can be easily sampled.

According to EFA, the usage of enzymes and pathways and the choices between them depend crucially on the *enzyme demands*, and thus on the economic potentials of substrates and products. For instance, since isoenzymes catalyse one reaction, they share the same flux demand. If strict optimality is required, isoenzymes can only be active if their flux prices $\sum_l h_l^{\mathrm{u}} u_l / v_l$ are equal. If we pick a kinetic model randomly and optimise its enzyme levels, this is unlikely (unless both isoenzymes have identical kinetic properties). Thus, if cells behave strictly optimally, they must choose between isoenzymes instead of using them in combination. If a cost-efficient enzyme is expressed, it will fix the flux demand at a low value and thus suppress the usage of less cost-efficient isoenzymes. However, if the first enzyme disappears (e.g., by a knock-out), the flux demand can rise, allowing other isoenzymes to be used. The same holds for "isopathways", i.e. pathways with the same overall stoichiometries. It is clear that cells are not bound to follow these rules and that other demands (e.g., being prepared for unforseen changes) can override a strict cost-optimality.

Pathway fluxes with low yields may entail larger thermodynamic forces, and thus lower enzyme demands, effectively leading to larger production rates than yield-efficient modes. FBA, by construction, favours high-yield fluxes. Flux optimisation, with its predefined flux cost function, tends to favour short pathways, which may even have a lower yield. Usage of individual cost weights can lead to other – high-yield or low-yield – solutions, exactly those flux distributions that satisfy the reaction balance. Figure 7 shows an example. The scheme of central metabolism represents glycolysis, respiration, and the export of an incompletely oxidised metabolite like pyruvate, lactate, or ethanol. FBA would favour respiration because of its higher ATP yield per glucose molecule. EFA can allow for respiration, but also for the fermentation flux, which may have larger ATP production per enzyme investment, as well as for convex combinations of both. In case (b), metabolite C has a positive economic potential and can still be beneficially converted into the more valuable A. In (c), its economic potential must be negative because its export is costly and leads to a zero economic potential.

Flux analysis can be applied to multi-objective problems. In flux analysis, Pareto optimality has been used to describe trade-offs between precursor production, ATP production, and small fluxes in central metabolism [21]. Multi-objective problems can be also be addressed in economic flux analysis. Mixed objective functions can join several terms including individual enzyme costs, effective gains arising from hard constraints or economic imbalance terms used to describe non-optimal enzyme levels. If objectives are uncertain or rapidly changing, it can be an optimal control strategy to adapt the enzyme levels to the expected or time-averaged objective. We just have to assume a flux gain $\mathbf{z}^{\mathrm{v}} = \sum_n p_n \, \mathbf{z}^{\mathrm{v}}_{(n)}$, where the p_n

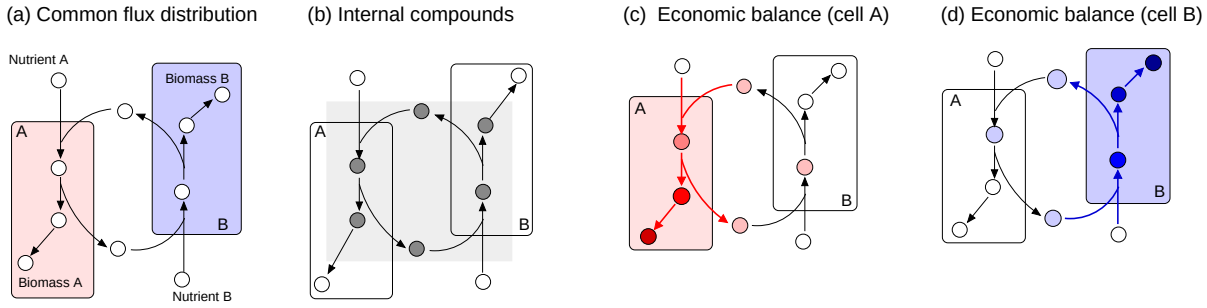

Figure 9: Flux distribution between two cells living in symbiosis (schematic model). (a) Cells A and B exchange metabolites needed for their biomass production. (b) Internal metabolites in the model (shaded) must be balanced. (c) Biomass production in cell A, seen as a metabolic objective, defines a set of economic potentials (shades of red). All reactions within cell A (red arrows) must satisfy the economic balance equation for these potentials. (d) The same holds for cell B, and the economic potentials for its own biomass production (shades of blue). In the resulting state, none of the cells would profit from small variations of its enzyme levels: the flux distribution is evolutionarily stable.

are probabilities or relative durations of different objectives, and the $\mathbf{z}^{\mathrm{v}}_{(n)}$ are the different flux gain vectors. Compromises between opposing objectives can be described by Pareto optimality [21]. Any Pareto-optimal flux distribution is also an optimum for some convex mixture of the objectives – which can be treated by EFA.

A joint optimisation of several objectives – comparable to simultaneous compliance with thermodynamic and economic constraints – may be impossible. However, it may be suitable to describe social behaviour like symbiosis. In Figure 9, two cells with different objective functions share a flux distribution that, within each cell, complies with the cell's own objective. As a condition for such evolutionarily stable fluxes, the economic balance equations must hold within each cell for the respective objective. EFA can also be extended to describe a "division of labour" between different flux distributions in one cell, whereby the same reactions in different spatial or temporal compartents can show different fluxes (see Figure S3 in SI). Such a specialisation of flux distributions can make metabolism more efficient, because compartments can establish different conditions (chemical and economic potentials) allowing for different flux directions or different cost-efficient uses of enzymes. Although all economic variables are derived from one metabolic objective, we can analyse each compartment separately. In each compartment, the economic potentials on the boundary define an objective locally, for this compartment.

5 Discussion

Biological structures like the shapes of trees and bones (see SI S3.3), the structure and dynamics of biochemical networks, and even chemical properties of biomolecules can be studied as functional adaptions. If enzyme levels in metabolic networks are optimised, this should shape the fluxes in specific ways. Metabolic economics [23] helps us explore this hypothesis under realistic assumptions about reaction kinetics, metabolic objectives, and enzyme costs. The need for metabolites and enzymes (that is: their beneficial effect for the cell) is subsumed in economic potentials and enzyme demands. Economic flux analysis uses these concepts for flux prediction. It relies on three principles: stationarity, dissipation of Gibbs free energy, and economically chosen enzyme levels. Interestingly, thermodynamic and economic constraints show many analogies: both can be derived from a common variational principle, they constrain flux directions by potential differences, exclude flux cycles, and in all this, resemble Kirchhoff's laws for electric circuits [29]. Similarities and dependencies between economic and thermodynamic constraints are

discussed in detail in SI S2.

Flux analysis methods like flux minimisation and FBA with molecular crowding describe compromises between enzyme investments by hypothetical flux costs. Why should one care about the reaction balance equation, which contains additional variables and is harder to solve? Unlike flux minimisation, the reaction balance is firmly based on kinetic models and its terms can be derived from metabolic control analysis. The economic potentials, which subsume rate laws and metabolic objective, provide a new layer of abstraction between kinetic models and flux analysis, linking the optimality concepts between both approaches. EFA captures common assumptions about flux costs and benefits, enzyme costs, and numerical enzyme optimisation, and provides general principles for economical fluxes. Futile cycles are not just suppressed during numerical optimisation (like in flux cost minimisation), but can be systematically detected and removed, like the cycles in thermodynamic flux analysis [40, 41]. This can be useful in metabolic network reconstruction. Since flux cost minimisation (with variable cost functions) is equivalent to EFA, it is proven to be consistent with an optimal use of enzymes. The comparison also shows that metabolic objective and flux costs in FCM correspond to marginal quantities in kinetic models, the benefits and enzyme costs (see SI S2.5).

If we think a cell's entire metabolic network and assume that biomass needs to be produced, all enzymes should, at least indirectly, contribute to this objective. To compute optimal enzyme profiles on this scale, we would need kinetic models much larger than existing models today. However, if all enzyme levels in a network are optimised, this must also hold for every pathway and every reaction within the network. Accordingly, if a flux distribution is economical, it must be locally beneficial in every reaction. The reaction balance can be summed over several reactions and thus be extended to pathways with arbitrarily drawn borders. This allows us to focus on individual pathways, keeping in mind that they are embedded in a larger network. The fact that global benefits can be written in terms of local economic potentials is very practical. In particular, the metabolic objectives of the larger network can be subsumed in the economic potentials at the boundaries of pathways. With their help, an enzyme's overall benefit $\partial g/\partial u_l$ effects can be represented as a local production of value within the pathways. Together, the flux benefits of all reactions add up to the overall benefit $\mathbf{z}^{\mathrm{v}} \cdot \mathbf{v}$. Relations between fluxes and economic potentials in larger networks is discussed at length in SI S3.4: that flux demands must exceed a critical value (defined by molecular properties of the enzymes, and how this shapes the usage of enzymes); how the laws for enzyme investment can be extended from reactions to pathways; and how metabolic objectives for individual pathways can be established.

In each pathway, the internal economic state will be strongly determined by the ecnomic potentials on its boundaries. Like boundary conditions in a partial differential equation, these potentials represent a pathway's interaction with the outside world, i.e., the rest of the network. This can help us dissect networks into pathways linked by "communicating" metabolites, whose concentrations, production rates, and economic potentials are the connecting variables. If we prepare pathway models such that fluxes, chemical potentials, and economic potentials at their boundaries match , they can be directly combined. Thus, like in modular response analysis [42], we can study pathways separately, knowing how they will interact in the larger network.

The usage of economic potentials as connecting variables is not only helpful for modelling, but also to understand biological regulation. The usage of enzymes or pathways can be assumed to depend on the economic potentials of some key metabolites, e.g. cofactors and precursor molecules. If this is true, these potentials will be ideal input signals for controlling the enzymes' activities. For instance, a hypothetical gene regulation function could respond to the economic potentials of substrates and products, accounting for the enzyme's momentary demand: an enzyme could be repressed if its flux demand falls below the critical value set by the enzyme's minimal price. Of course, economic potentials cannot be sensed biochemically – but metabolite levels, could represent them as biochemical signals. Similarly, permanent mismatches between flux benefit and enzyme cost ("economic imbalance") may lead to selection pressures on the enzyme's gene regulation function.

For a comprehensive view of enzyme investments in a cell, one may hierarchically classify the enzymes into

pathways and pathways into larger categories, which together form the metabolic network. Since enzyme costs are positive and additive, they can be treated like amounts. In the branches of the hierarchy, a fraction is devoted to metabolism, a smaller fraction of this to glycolysis, and finally to each glycolytic enzyme. Normalised to a sum of 1, the costs form a measure in the mathematical sense on the enzyme hierarchy. Such hierarchical enzyme costs may be visualised similar to protein abundances by treemaps (see www.protemaps.net), picturing the enzyme economy of a cell in a comprehensive and detailed way. Under the optimality assumption, the same picture will also describe the *enzyme benefits*, i.e., the scaled control that each enzyme exerts over the metabolic objective. The fact that the benefits can be also displayed as amounts may sound surprising (because they are actually response coefficients $\partial g/\partial \ln u_l$, which generally can be negative). However, in enzyme-optimal states, the benefit principle ensures their positivity.

Since resources like nutrients, energy, and space are limited, cells cannot run all biochemical processes at full speed, but need to prioritise and balance. Not surprisingly, EFA borrows concepts like cost, benefit, or Pareto optimality from economics, which is typically concerned with opposing needs. According to the reaction balance, a metabolite's economic potential embodies the enzyme investment that is needed for its continuous production. This resembles the concept of labour value, the necessary labour invested in the production of a commodity (see SI S2.6). Since demands and prices in metabolic economics are defined as marginal quantities (i.e. assuming small changes of the current state), enzyme costs do not correspond to labour values in the classical sense (the average time investment needed for an item produced), but to a marginal form of labour values (the extra time investment needed to increase the production by one item). The cost-benefit balance for enzyme levels resembles a marginal decision rule, stating that goods or services should be consumed at quantities at which marginal benefits and costs match [43]. The global metabolic objective function is locally represented by economic potentials, which therefore resemble the prices of goods and services in neoclassical value theory. On the contrary, the calculation of consistent economic potentials in metabolic networks resembles the calculation of consistent prices for planned economies, and similar mathematical methods could be used in both cases.

EFA could be experimentally tested on metabolic pathways under strong selection pressure. As a testable prediction, substances in which much has been invested (e.g., by means of costly transporters for small molecules, or costly machinery for macromolecule synthesis) should be used efficiently. Quantitatively, one could measure the economic potentials of substances by finding the break-even point for transporter costs, at which transporters start to be used. Differences of economic potentials along pathways, defined in this way, should be balanced with the prices of enzymes within the pathways. All this requires precise measurements of enzyme costs, and since we are concerned with optimal states, a previous evolution in the lab [13, 44]. Enzyme prices could be measured by assessing artificial protein expression [13, 14] or the effects of increased protein costs (by nitrogen depletion or inhibitors of translation). Economic cell models would generally profit from precise measurements of enzyme levels, catalytic constants, and enzyme costs at different growth rates. Furthermore, it would be interesting to relate calculated economic imbalances in enzyme levels to the rate of evolutionary changes in these levels.

Metabolic economics could be extended from metabolism of small molecules to all reactions in growing cells, including macromolecule synthesis. Explicit investment functions would become obsolete because costs of protein and ribosome production would automatically arise from their demand for precursor molecules. Finally, to account for changing environments and anticipation of future events [23], one might consider complex fitness functions comprising not just the current metabolic task, but probabilistic mixtures of future tasks. The resulting bet-hatching strategies [45, 46] could explain observed behaviour like preemptive expression of (allosterically inhibited) enzymes, or the production of storage compounds like starch or glycogen. Experimental studies of such processes would require an artificial evolution in variable environments.

Acknowledgements

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