Linear Response Theory of Evolved Metabolic Systems

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(Received 6 January 2023; accepted 6 June 2023; published 13 July 2023)

Predicting cellular metabolic states is a central problem in biophysics. Conventional approaches, however, sensitively depend on the microscopic details of individual metabolic systems. In this Letter, we derived a universal linear relationship between the metabolic responses against nutrient conditions and metabolic inhibition, with the aid of a microeconomic theory. The relationship holds in arbitrary metabolic systems as long as the law of mass conservation stands, as supported by extensive numerical calculations. It offers quantitative predictions without prior knowledge of systems.

DOI: 10.1103/PhysRevLett.131.028401

Metabolism is the physicochemical basis of life. Understanding its behavior has been a major goal of biophysics [1–4]. At the same time, the prediction of cellular metabolic states is a central problem in biology. In particular, prediction of the responses of metabolic systems against environmental variations or experimental operations is essential for manipulating metabolic systems to the desired states in both life sciences and in applications such as matter production in metabolic engineering [5] and the development of drugs targeting cellular metabolism [6–8].

Previous studies have mainly attempted to predict the metabolic responses by predicting the metabolic states before and after perturbations, and they require building an *ad hoc* model for each specific metabolic system. In systems biology, constraint-based modeling (CBM) has often been used to predict the cellular metabolic states [9–11]. In this method, the intracellular metabolic state is predicted by solving an optimization problem of models of metabolic systems, including a detailed description of each metabolic reaction. To construct the optimization problem, metabolic systems of cells are assumed to be optimized through (sometimes artificial) evolution for some objectives [9,10,12], e.g., maximization of the growth rate in reproducing cells such as cancer cells and microbes [13] and maximization of the production of some molecules in metabolically engineered cells [14]. Indeed, metabolic systems of reproducing cells exhibit certain ubiquitous phenomena across various species, and those phenomena can be explained as a result of optimization under physicochemical constraints [13]. Although the assumption of optimal metabolic regulation seems acceptable, knowing the true objective function of cells, which is essential to making a model for CBM, remains nearly impossible. Besides, even with remarkable progress in omics research, fully reconstructing metabolic network models for each individual species or cell of interest is still a challenge. Moreover, the numerical predictions are sensitive to the details of the concerned constraints and the objective functions selected [15–18]. Therefore, new methods independent of the details of metabolic systems are required.

Instead of metabolic states themselves, here, we focus on the responses of metabolic states to perturbations. At first glance, such a prediction is seemingly more difficult than predicting the cellular metabolic states because it seems to require information not only on the steady states but also on their neighborhoods. However, from another perspective, to predict only the metabolic responses, we may need to understand the structure of only a limited part of the state space of feasible metabolic states. In contrast, we must seek the whole space to predict the metabolic states themselves. If optimization through evolution and some physicochemical properties unique to metabolic systems constrain the behavior in the state space, there might be universal features in the responses of metabolic systems to perturbations, independent of system details, as in the linear response theory in statistical mechanics [19–21].

In this Letter, we demonstrate a universal property of intracellular metabolic responses in the optimized metabolic regulation, using a microeconomic theory [22–24]. By introducing a microeconomics-inspired formulation of metabolic systems, we can take advantage of tools and ideas from microeconomics such as the Slutsky equation that describes how consumer demands change in response to income and price. We thereby derive quantitative relations between the metabolic responses against nutrient abundance and those against metabolic inhibitions, such as the addition of metabolic inhibitors and leakage of intermediate metabolites; the former is easy to measure in experiments while the latter may not be. The relations universally hold independent of the details of metabolic systems as long as the law of mass conservation holds. Our theory is applicable to any metabolic system and will provide quantitative predictions on the intracellular metabolic responses without detailed prior knowledge of

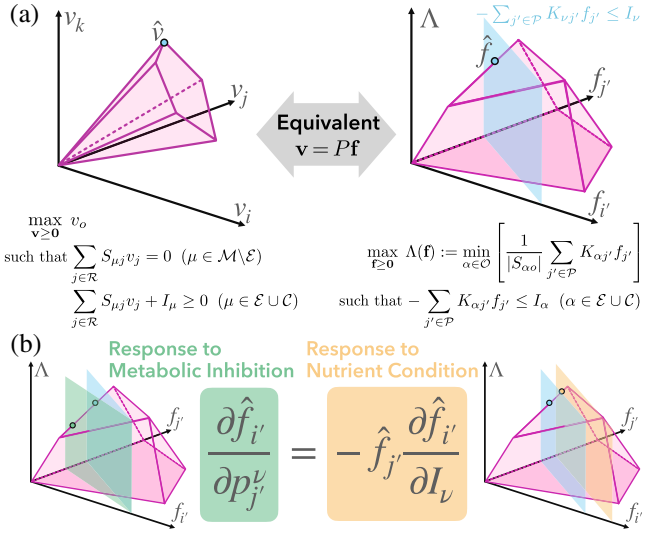

FIG. 1. Schematic illustration. (a) (left) Metabolic CBM formulation with reaction fluxes $\mathbf{v}$ as variables. The solution subspace (convex set of possible allocations), called the flux cone, is shown in pink. (right) Microeconomic formulation with pathway fluxes $\mathbf{f}$ as variables and an objective flux Λ. The pink area in the $\mathbf{f}$ plane (bottom surface) represents the solution subspace, whereas the blue plane vertical to the $\mathbf{f}$ plane is the budget constraint for a component ν. The blue points $\hat{\mathbf{v}}$ and $\hat{\mathbf{f}}$ represent the optimized fluxes of reactions and pathways, respectively. Given $\mathbf{v} = P\mathbf{f}$ with pathway matrix P, both formulations are equivalent optimization problems (see the Supplemental Material [25], Sec. S1 for details and Sec. S2 and Fig. S1 for a simple example). (b) Liner relation between the metabolic responses against changes in nutrient conditions (yellow) and those against metabolic inhibitions (green) [Eq. (3)].

microscopic molecular mechanisms and cellular objective functions.

Microeconomic formulation of metabolic regulation.—We first provide a microeconomic formulation of optimized metabolic regulation, which is equivalent to linear programming problems in CBM (Fig. 1).

We denote the set of all chemical species (metabolites) and that of all constraints by $\mathcal{M}$ and $\mathcal{C}$, respectively. $\mathcal{C}$ can reflect every type of constraint such as the allocation of proteins [29,30], intracellular space [31], membrane surfaces [32], and Gibbs energy dissipation [18] as well as the bounds of reaction fluxes.

In the microeconomic formulation, variables to be optimized are the fluxes of metabolic pathways, whereas they are fluxes of reactions in usual CBM approaches; a metabolic pathway is a linked series of reactions and thus comprises multiple reactions. The sets of reactions and pathways are denoted by $\mathcal{R}$ and $\mathcal{P}$, respectively. Let us then consider two stoichiometry matrices for reactions and pathways, S and K, respectively (see also the Supplemental Material [25], Table S1). For chemical species $\alpha(\in \mathcal{M})$, $|S_{\alpha i}|$ represents the number of units of species α produced if $S_{\alpha i} > 0$ and consumed if $S_{\alpha i} < 0$ in reaction i; whereas if α denotes a constraint ($\alpha \in \mathcal{C}$), $S_{\alpha i}$ is usually negative and $|S_{\alpha i}|$ represents the number of units of constraint α required for reaction i. The stoichiometry matrix K for metabolic pathways $\mathcal{P}$ is also defined similarly. Throughout the Letter, we use indices with primes such as i' to denote pathways and those without primes such as i to denote reactions, and $|S_{\alpha i}|$ and $|K_{\alpha i'}|$ are called input (output) stoichiometric coefficients of reaction i and pathway i', respectively, if $S_{\alpha i}$ and $K_{\alpha i'}$ are negative (positive).

Cells are assumed to maximize the flux of some objective reaction $o(\in \mathcal{R})$ such as biomass synthesis in reproducing cells and ethanol or adenosine triphosphate (ATP) synthesis in metabolically engineered cells. We define the set of the species consumed in and the components required for reaction o as objective components $\mathcal{O}(\subset \mathcal{M}\cup\mathcal{C})$, and thus $S_{\alpha o}$ for each objective component $\alpha(\in \mathcal{O})$ is negative. Because the reactants of a reaction cannot be compensated for each other due to the law of mass conservation [24,33,34], the flux of objective reaction o, i.e., the objective function, is limited by the minimum available amount of objective components $\mathcal{O}$ as follows:

$$\Lambda(\mathbf{f}) := \min_{\alpha\in\mathcal{O}}\left[\frac{1}{-S_{\alpha o}}\left(\sum_{j'\in\mathcal{P}} K_{\alpha j'} f_{j'} + I_\alpha\right)\right], \tag{1}$$

where $\mathbf{f} = \{f_{i'}\}_{i'\in\mathcal{P}}$ represents the fluxes of metabolic pathways. The arguments of the above min function represent biologically different quantities: if α is a species ($\alpha \in \mathcal{M}$), I_α is its intake flux and $\sum_{j'} K_{\alpha j'} f_{j'}$ represents its total production rate, while if α is a constraint ($\alpha \in \mathcal{C}$), I_α is the total capacity for constraint α and $\sum_{j'} K_{\alpha j'} f_{j'} + I_\alpha$ is the amount of α that can be allocated to the objective reaction.

The optimized solution $\hat{\mathbf{f}}$ is determined as a function of K and $\mathbf{I}$ with the following constraints for the available pathway fluxes $\mathbf{f}$:

$$-\sum_{j'\in\mathcal{P}} K_{\alpha j'} f_{j'} \leq I_\alpha. \quad (\alpha \in \mathcal{E}\cup\mathcal{C}) \tag{2}$$

Here, $\mathcal{E}(\subset \mathcal{M})$ denotes the set of exchangeable species that are transported through the cellular membrane. That is, the above constraints reflect that the total consumption of species cannot exceed their intakes. If species α is produced by objective reaction o, the intake effectively increases, and $S_{\alpha o}\Lambda$ is added to the right-hand side of Eq. (2), although this is not the case for most species.

This optimization problem [Eqs. (1) and (2)] can be interpreted as a microeconomic problem in the theory of consumer choice [22–24], considering $\Lambda(\mathbf{f})$ as the utility function. By focusing on an arbitrary component ν, one of the inequalities in Eq. (2) serves as the budget constraint for ν if $K_{\nu j'} \leq 0$ for all pathways j', while the remaining inequalities in Eq. (2) then determine the solution space [Fig. 1(a)]: for example, if we choose glucose as ν, the

corresponding inequality in Eq. (2) represents carbon allocation. Here, the maximal intake I_ν of ν corresponds to the income, and the input stoichiometric coefficient for each pathway, $p^\nu_{j'} := -K_{\nu j'}$, serves as the price of pathway j' in terms of ν.

Relation between responses of pathway fluxes to nutrient abundance and metabolic inhibition.—Because Eqs. (1) and (2) can be interpreted as a microeconomic optimization problem, we can apply and generalize the Slutsky equation in the theory of consumer choice [22]. The equation shows the relationship between changes in the optimized demands for goods in response to income and price. In metabolism, it corresponds to the relationship between the responses of optimal pathway fluxes $\hat{\mathbf{f}}$ (see the Supplemental Material [25], Sec. S4 for derivation):

$$\frac{\partial \hat{f}_{i'}(K, \mathbf{I})}{\partial p^\nu_{j'}} = -\hat{f}_{j'}(K, \mathbf{I}) \frac{\partial \hat{f}_{i'}(K, \mathbf{I})}{\partial I_\nu}. \tag{3}$$

The right-hand side represents the responses of pathway i' against increases in I_ν, whereas the left-hand side represents those against metabolic inhibitions in pathway j' because the metabolic price $p^\nu_{j'} = -K_{\nu j'}$ quantifies the inefficiency of conversion from substrate ν to end products in pathway j' [24].

The derivation of Eq. (3) relies solely on the law of mass conservation, i.e., the reactants of a reaction cannot be compensated for each other. Because the law of mass conservation stands in every chemical reaction, the relation of the two measurable quantities must hold in arbitrary metabolic systems as long as their metabolic regulation is optimized for a certain objective. In particular, the case $i' = j'$ will be useful: it indicates that measuring the responses of a pathway flux to changes in the nutrient environment provides quantitative predictions of the pathway's responses to metabolic inhibition or activation, and vice versa.

To confirm the validity of Eq. (3), we numerically solved the optimization problems [Eqs. (1) and (2)] with pathway fluxes $\mathbf{f}$ as variables using the *E. coli* core model [9,35] and randomly chosen stoichiometric coefficients for the single constraint (Fig. 2). In this numerical calculation, metabolic pathways from exchangeable species to objective components are chosen as linear combinations of extreme pathways or elementary flux modes [36] for stoichiometry without objective reaction o [Fig. 2(b)], although the above arguments do not depend on the specific choices of metabolic pathways (see the Supplemental Material [25], Sec. S3 for details). As shown in Fig. 2(a), the linear relation between metabolic responses is indeed satisfied. Notably, it is satisfied regardless of the number and type of constraint(s) $\mathcal{C}$, whereas the metabolic states themselves can sensitively depend on the concerned constraints and environmental conditions.

Relation between responses of reaction fluxes.—Although Eq. (3) generally holds for arbitrary metabolic pathways, it may be experimentally easier to manipulate a single metabolic reaction. Manipulation of a single reaction can affect multiple pathways because they are often tangled via a common reaction in the metabolic network. Thus, we should consider the contributions of multiple pathways. The simplest way to do this is to sum up Eq. (3) for all the pathways that include the perturbed reaction i. However, to precisely conduct this summation, we need to know the whole stoichiometry matrix or metabolic network. Hence, another relation closed only for the reaction fluxes $\mathbf{v}$ is required for application without the need to know the details of the metabolic systems.

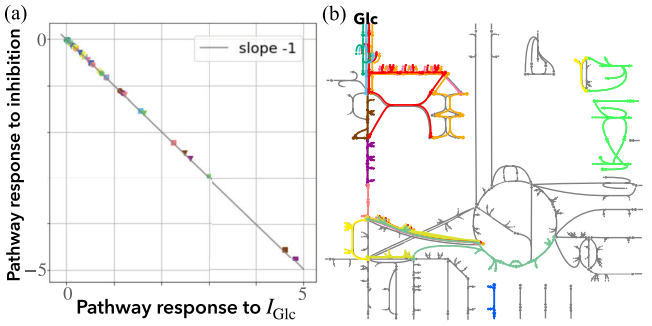

FIG. 2. Responses of the optimized pathway fluxes $\hat{\mathbf{f}}$. (a) Responses to metabolic inhibitions, $\Delta\hat{f}_{i'}(\Delta K_{\mathrm{Glc},j'})/\Delta p^{\mathrm{Glc}}_{j'}$, are plotted against the nutrient responses, $\hat{f}_{j'}\Delta\hat{f}_{i'}(\Delta I_{\mathrm{Glc}})/\Delta I_{\mathrm{Glc}}$. All different shapes and colors of markers represent different i' and j', respectively. $I_{\mathrm{Glc}} = 5$ [mmol/gDW/h]. (b) Thirteen active extreme pathways, computed using efmtool [37], are shown. Colors correspond to those of the markers for manipulated pathways j' in panel (a). The whole metabolic network of the *E. coli* core model is shown in gray.

To derive such a relation, we consider effective changes in the stoichiometric coefficients $S_{\alpha i}$ for reaction i as metabolic inhibitions: e.g., inhibition of enzymes, administration of metabolite analogs, leakage of metabolites, and inefficiency in the allocation of some resource. We then obtain an equality on the optimized reaction fluxes $\hat{\mathbf{v}}$, formally similar to Eq. (3) (see the Supplemental Material [25], Sec. S4 for derivation),

$$\frac{\partial \hat{v}_i(S, \mathbf{I})}{\partial q^\nu_i} = -\hat{v}_i(S, \mathbf{I}) \frac{\partial \hat{v}_i(S, \mathbf{I})}{\partial I_\nu}, \tag{4}$$

by defining the metabolic price q^ν_i of reaction i in terms of ν as a function of S, instead of the metabolic price $p^\nu_{i'}$ of pathway i' as a function of K,

$$q^\nu_i := \sum_{\alpha \in \mathcal{M} \cup \mathcal{C}} -S_{\alpha i} \frac{\partial \hat{v}_i}{\partial I_\alpha} \Big/ \frac{\partial \hat{v}_i}{\partial I_\nu}. \tag{5}$$

The coefficient $(\partial\hat{v}_i/\partial I_\alpha)/(\partial\hat{v}_i/\partial I_\nu) =: c^\nu_\alpha(i)$ quantifies the number of units of component ν that can compensate for one unit of α in reaction i and is experimentally measurable. For example, if ν is glucose and α is another metabolite

such as an amino acid, c_α^ν indicates how many units of glucose are required to compensate for one unit of the amino acid, similar to the "glucose cost" in previous studies [38].

For the linear response relation [Eq. (4)], it is sufficient to calculate only the change in metabolic price (not the metabolic price itself), which depends on the type of manipulations of concern: (I) manipulations leading to the loss of a single component and (II) those leading to the loss of multiple components.

If experimental manipulation causes the loss of a single component $\alpha(\in \mathcal{M} \cup \mathcal{C})$ in reaction i, $S_{\alpha i}$ effectively changes only for that α [Fig. 3(a)]. In such a case, the metabolic price change is just given by $\Delta q_i^\alpha = \Delta S_{\alpha i}$. An example of such experimental manipulations is the administration of an analog to a reactant of a multibody reaction: if α and β react [see Fig. 3(a)], the metabolic analog of β can produce incorrect metabolite(s) with α, leading to the loss of α, and thus, reaction i requires more α to produce the same number of products, causing effective increases in the input stoichiometric coefficient $|S_{\alpha i}|$. Another example is the changes in the total capacity and effective stoichiometry for a constraint: for example, the mitochondrial volume capacity will work as such a constraint and can be genetically manipulated [39–41]. Equation (4) for case (I) is numerically confirmed in Fig. 3(a).

If metabolic inhibition of multiple reactant species of a reaction i is simultaneously caused, the stoichiometric coefficients $S_{\mu i}$ for multiple reactants μ of reaction i will effectively change [Fig. 3(b)]. Accordingly, the reaction price changes by $\Delta q_i^\nu = \sum_\mu \Delta S_{\mu i} c_\mu^\nu(i)$. In experiments, such cases would correspond to the inhibition of enzymes, leakage of the intermediate complex of reaction i, and so forth. Even in this case (II), the linear relation [Eq. (4)] is verified by numerically calculating the price changes of reaction j defined in Eq. (5) with the *E. coli* core model including 77 reactions [Fig. 3(b)] as well as a larger-scale metabolic model including 931 reactions [42] (Supplemental Material [25], Fig. S2). Here, although the precise calculation of the coefficients $c_\mu^\nu(i)$ requires information regarding not only the responses of $\hat{v}_i$ to I_ν but also those to I_μ, they can be approximated in ways easier and independent of reaction i. For example, under extreme situations in which only the carbon sources limit the objective reaction, c_μ^ν should be the ratios of the carbon numbers of species μ and ν; alternatively, the simplest approximation could be just taking c_μ^ν as unities. Even with these approximations, the relation [Eq. (4)] appears to hold well (Supplemental Material [25], Fig. S3), and thus such approximations will be useful for qualitatively predicting whether metabolic inhibition promotes or suppresses the reaction of interest.

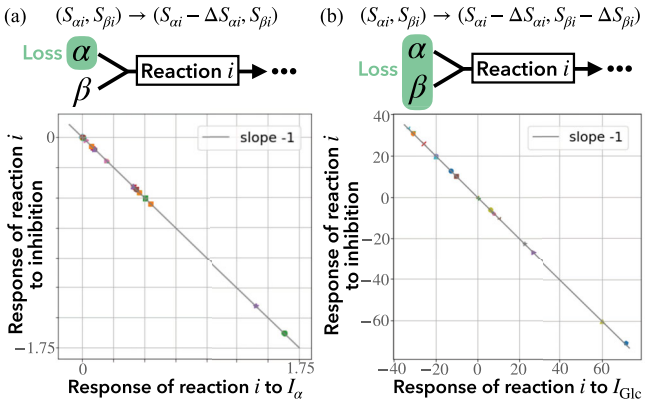

FIG. 3. Responses of the optimized reaction fluxes $\hat{\mathbf{v}}$ against metabolic inhibitions of reaction i. As the simplest example, a two-body reaction of components α and β is illustrated in the upper panels. (a) Metabolic inhibition of a single component (α in upper panel): case (I). The horizontal axis shows the responses to the available amount of a constraint $\alpha(\in \mathcal{C})$, $\hat{v}_i \Delta \hat{v}_i(\Delta I_\alpha)/\Delta I_\alpha$, and the vertical axis shows the responses to metabolic inhibitions, $\Delta \hat{v}_i(\Delta S_{\alpha i})/\Delta q_i^\alpha$. (b) Metabolic inhibition of multiple components (α and β in upper panel): case (II). Responses of the reaction flux $\hat{v}_i$ to effective changes in the input stoichiometric coefficients $\{S_{\mu i}\}_{\mu \in \mathcal{M}}$ for reaction i, $\Delta \hat{v}_i(\{\Delta S_{\mu i}\}_{\mu \in \mathcal{M}})/\Delta q_i^{\mathrm{Glc}}$, are plotted against those to intake changes, $\hat{v}_i \Delta \hat{v}_i(\Delta I_{\mathrm{Glc}})/\Delta I_{\mathrm{Glc}}$. $I_{\mathrm{Glc}} = 8.05$ [mmol/gDW/h]. Each marker denotes a different reaction i.

Remarkably, our above argument does not depend on specific choices of objective reaction o, whereas we have utilized the biomass synthesis reaction as o (Figs. 2 and 3). To highlight the independence of the relation [Eq. (4)] from cellular objective functions, we also numerically confirmed that it is satisfied even when objective reaction o is set as a reaction for matter production, such as ethanol or ATP synthesis (Supplemental Material [25], Fig. S4). These synthesis reactions are often considered as the objectives for metabolically engineered cells [5,43,44].

In the present study, we showed that the metabolic responses against resource availability and those against metabolic inhibitions are negatively proportional. The quantitative relations we found should be universally satisfied with arbitrary reaction networks, constraints, and objective functions of cells. In particular, although the predicted optimal metabolic states can drastically depend on the assumed objective function, the relations of the responses should be always satisfied independent of it (see also the Supplemental Material [25], Fig. S4). Even though we can never know the true objective function of cells, we can still predict the metabolic responses.

In the linear relations, the metabolic responses against different perturbations are linked because both are determined from the identical objective function and constraints (see also Fig. 1). It is similar to the linear response theories in statistical mechanics: they are derived from the fact that different thermodynamic quantities are given as the derivatives of an identical thermodynamic potential [45]. Note here that the thermodynamic potential works as an

objective function: e.g., entropy is maximized at the thermal equilibrium.

The independence from cellular objective functions is derived from the microeconomic formulation for metabolic regulation and application of the Slutsky equation in economics. Note that the Slutsky equation basically requires detailed information regarding the objective functions (utility functions in economics) because it includes a term for the so-called substitution effect that quantifies the substitutability of goods and depends on the objective functions (see also the Supplemental Material [25], Sec. S4). However, the term disappears when applied to metabolism because the law of mass conservation implies the nonsubstitutability of reactants.

Although the linear relations [Eqs. (3) and (4)] are general due to the generality of the law of mass conservation, there are also some limitations. First, since our results rely on the assumption of optimal metabolic regulation, they will not hold in suboptimal metabolic responses; conversely, any observed deviation from the relations [Eqs. (3) and (4)] will indicate the suboptimality in the regulation of the real metabolic system in question. Second, our linear relations work only for continuous metabolic responses. Third, the approximation of coefficients c_μ^ν in Eqs. (4) and (5) could be prohibitive when the coefficients become negative, e.g., in the case that the fluxes from different nutrient sources must be balanced for a metabolic reaction of interest and an increase in one source promotes the reaction while an increase in another source inhibits it.

Because our results are valid regardless of how abstract the concerned model is, from coarse-grained toy models to genome-scale metabolic networks, they would be important both for quantitative predictions and for discovering qualitatively novel phenomena. The Warburg effect or overflow metabolism is a prominent example of the latter. In the Warburg effect, as the amount of the carbon source taken up by a cell increases, the cell decreases the flux of the respiration pathway and utilizes fermentation or aerobic glycolysis instead [13,46]. From the relation [Eq. (3)], one can immediately predict that the inhibition of respiration (e.g., administration of uncouplers of respiration [47]) will counterintuitively increase the respiration flux. Such an increase in the respiration flux was observed in a coarse-grained model, which was termed the drug-induced reverse Warburg effect [24]. This phenomenon has been indeed reported in several published experiments [47–50].

Likewise, for controlling cellular metabolic states, e.g., for metabolic engineering and medicine, some counterintuitive manipulations can promote pathway or reaction fluxes. Although metabolic inefficiency is considered to suppress the flux in general, when an increase in the intake of a substrate suppresses a pathway or reaction flux, making the metabolic pathway or reaction less efficient will counterintuitively promote the flux (see also the Supplemental Material [25], Sec. S2 and Fig. S1 for an example of coarse-grained models). In experimental application, the intake or total capacity can be altered by shifts in environmental conditions, genetic manipulations, and so forth. Changes in the metabolic price can be also implemented in various ways: e.g., administration of a metabolite analog, leakage of a metabolite, addition of an alternative pathway or reaction through metabolic engineering manipulations, and inhibition of some enzyme that will lead to the accumulation of the reactants and possibly promote their excretion or conversion to other chemicals. They cause a loss of reactants, and thus, the corresponding reaction(s) require more metabolites to produce the same number of products.

The relations [Eqs. (3) and (4)] allow us to predict the responses of an arbitrary reaction or pathway flux to metabolic inhibitions only by measuring its fluxes in several different nutrient conditions, and vice versa. The predictions do not require detailed information regarding the concerned intracellular reaction networks, and they are valid even when the precise estimation of effective changes in the stoichiometric coefficients is difficult, at least qualitatively (Supplemental Material [25], Fig. S3). Therefore, they will be useful as quantitative and qualitative guidelines to operate the metabolic states toward the desirable directions in various fields such as microbiology, metabolic engineering, and medicine.

The supporting data for this Letter, including the associated PYTHON code and data to reproduce figures in this work, are openly available from Ref. [51].

We would like to acknowledge Wolfram Liebermeister, Chikara Furusawa, Yasushi Okada, Kunihiko Kaneko, and Takuma Ōnishi for helpful discussions and useful comments. This work was partially supported by JSPS KAKENHI Grants No. JP21J22920 and No. JP21K15048 and the Masason Foundation.

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