Metabolic Futile Cycles and Their Functions: A Systems Analysis of Energy and Control

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It has long been hypothesized that futile cycles in cellular metabolism are involved in the regulation of biochemical pathways. Following the work of Newsholme and Crabtree, we develop a quantitative theory for this idea based on open-system thermodynamics and metabolic control analysis. It is shown that the stoichiometric sensitivity of an intermediary metabolite concentration with respect to changes in steady-state flux is governed by the effective equilibrium constant of the intermediate formation, and the equilibrium can be regulated by a futile cycle. The direction of the shift in the effective equilibrium constant depends on the direction of operation of the futile cycle. High stoichiometric sensitivity corresponds to ultrasensitivity of an intermediate concentration to net flow through a pathway; low stoichiometric sensitivity corresponds to super-robustness of concentration with respect to changes in flux. Both cases potentially play important roles in metabolic regulation. Futile cycles actively shift the effective equilibrium by expending energy; the magnitude of changes in effective equilibria and sensitivities is a function of the amount of energy used by a futile cycle. This proposed mechanism for control by futile cycles works remarkably similarly to kinetic proofreading in biosynthesis. The sensitivity of the system is also intimately related to the rate of concentration fluctuations of intermediate metabolites. The possibly different roles of the two major mechanisms for cellular biochemical regulation, namely reversible chemical modifications via futile cycles and shifting equilibrium by macromolecular binding, are discussed.

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I. INTRODUCTION

Metabolic fluxes and intermediary metabolite concentrations are the essential currency of intracellular metabolism. The fluxes—or rates of turnover of the various reactions—determine the rate of production of required end products of a metabolic system, i.e., ATP synthesis by oxidative phosphorylation and lactate production via glycolysis. The intermediary metabolite concentrations are often key regulators in metabolic control. For example, fructose-1,6-bisphosphate is an allosteric regulator of pyruvate kinase [1]. One of the primary examples of futile cycles in metabolism is fructose-6-phosphate phosphorylation dephosphorylation, an extensively studied metabolic regulatory module [2,3,4,5]. In the present work, we study the quantitative relationship between a metabolic flux and the concentrations of its intermediary metabolites. In particular, we study the sensitivity of the intermediate concentration with respect to changes in the flux. Depending on the biological context, the sensitivity may be high for some metabolites (sensitive) while for others it may be low (robust) [5,5]. We show that the sensitivity is intimately related to the energetics, i.e. effective equilibrium constant, between the intermediate and the upstream substrate(s). Hence control of sensitivity has a thermodynamic interpretation.

The chemical equilibrium constant is an intrinsic property for a given chemical reaction and cannot be altered by enzyme activity. However, the effective equilibrium constant operating in a reaction in a network can be shifted via a futile cycle, as we describe below. This same idea is behind the concept of kinetic proofreading, in which a kinetic cycle involving GTP hydrolysis increases the effective binding affinity between a codon and its tRNA, improving the accuracy of protein biosynthesis [6,7,8]. At a deeper level, the kinetic proofread shares a same principle of nonequilibrium physics as the nuclear Overhauser effect in magnetic resonance and catalytic wheel [9].

The idea that futile cycles contribute to the regulation of metabolic functions has a long history [2,3,10,11]. In particular, Newsholme and Crabtree suggested that futile cycling may be important for effective regulation at low metabolic fluxes, allowing a pathway to be controlled with much smaller excursions in the concentrations of the allosteric regulators. However, there has not been a systematic mathematical theory substantiating this idea. Because of the lack of quantitative predictions, experimental verification of this important idea has been difficult. The present analysis provides strong support and a mechanistic quantification of the hypothesis of Newsholme & Crabtree on the biochemical function of futile cycles in metabolic systems [2,3]. The analysis focuses on the quantitative relation between the energy expendi-
ture, thermodynamics, and biochemical control.

II. METABOLIC FLUX, INTERMEDIATE CONCENTRATION, AND STOICHIOMETRIC SENSITIVITY

A. Sensitivity of Intermediate Concentration to Flux through a Metabolic Pathway

Consider a simple linear metabolic pathway with species 0, 1, and 2:

\[
0 \xrightleftharpoons[k_1^{-1}]{k_1} 1 \xrightleftharpoons[k_2^{-1}]{k_2} 2 \xrightarrow{J}
\]  

in which the concentration of the substrate, \(c_0\), is fixed. The constants \(k_1\) are either the mass-action rate constants for first-order chemical kinetics or the effective rate constants acting around a steady-state. (See Appendix A for a detailed analysis.) Note that in a metabolic network, there are different ways of maintaining the input substrate concentration. Two extreme cases are concentration clamping and constant flux injection [14]. While concentration clamping can be achieved experimentally with a large pool or buffer for the substrate, flux injection is closely related to the “flux-generating step” suggested in [13]. Both concentration clamping and flux injection provide thermochemical driving forces for open biochemical systems, analogous to batteries in electrical circuits.

Recall that there are two types of ideal batteries, those that provide constant voltage (voltage sources with zero internal resistance) and those that provide constant current (current sources with zero internal conductance). A real battery of course has a finite internal resistance and conductance. Metabolic concentration and flux play equally important roles in the steady-state of a biochemical system. They should be treated on equal footing in a complete theory of metabolic dynamics. Under an ideal setting, one can control the concentration(s) and let the fluxes change in response; Similarly, one can control the flux(s) and let the concentrations change in response. Controlling fluxes can, but not necessarily, be accomplished by changing enzyme activities.

Significant controversies exist in the literature on systems analysis of metabolic networks due to differences in implicit assumptions on how a system is sustained in a nonequilibrium steady-state. In other words, how a system’s steady-state responses to perturbations depends on how the steady-state is maintained. See [10] for an interesting case study. It is worth pointing out that almost all the existing work on metabolic control analysis (MCA) implicitly assumes concentration clamping [17]. In fact, we recently discovered that for systems driven by flux injections, the summation of flux control coefficients equals zero rather than unity (manuscript in preparation). Naturally, the realistic situation in a cell is likely to be mixed.

In standard MCA, one defines a flux control coefficient as the sensitivity of a flux \(J\) to an enzyme concentration [18, 19, 20]. This work, however, focuses on a different aspect of network control: the steady-state sensitivity of the concentrations of the intermediates, \(c_1\) and \(c_2\), to the flux \(J\), at fixed enzyme activities. This stoichiometric sensitivity is also different from the elasticity coefficient, which is a property of a single enzyme—an elasticity coefficient (also called local [17] or intrinsic [15], or immediate [21] control coefficient) is determined from the rate law of a single enzymatic reaction in isolation. Our stoichiometric sensitivity is related to the co-response coefficients introduced by Rohwer and coworkers [22, 23]. The co-response coefficient emphasizes the concomitantly changes in a flux and a concentration in response to a perturbation in a given enzyme. While changing enzyme is one of the possible means to perturb kinetics, there are other means to perturb a flux. Hence the stoichiometric sensitivity is a more general kinetic concept than the co-response coefficient in MCA. In fact, the present analysis focuses on sensitivity of network concentrations to steady-state network fluxes. Even though both metabolites and enzymes are chemical species in a biochemical reaction system, their roles in metabolic kinetics and thermodynamics are very different. A change in the enzyme amounts to a change in both forward and backward effective rate constants for the catalyzed reaction without altering their ratio (Haldane’s equation).

With given \(c_0\) and \(J\) as input and output in a steady-state, the intermediate concentrations in the reaction of Eq. 1 are

\[
c_1 = \frac{k_1}{k_{-1}} c_0 - \frac{1}{k_{-1}} J \\
c_2 = \frac{k_1 k_2}{k_{-1} k_{-2}} c_0 - \frac{k_2 + k_{-1}}{k_{-1} k_{-2}} J.
\]

(See Appendix A.) The stoichiometric sensitivity coefficients are defined as

\[
\eta_1 = \frac{\partial \ln c_1}{\partial \ln J} = \frac{J}{k_{-1} c_1} \quad \text{(4)} \\
\eta_2 = \frac{\partial \ln c_2}{\partial \ln J} = \frac{(k_2 + k_{-1}) J}{k_{-1} k_{-2} c_2} \quad \text{(5)}
\]

While the steady-state concentrations do not necessarily increase or decrease in the order from input to output along the pathway, the sensitivity increases for intermediates as one moves from “upstream” to “downstream” (\(\eta_2 > \eta_1\) since \(k_2 c_1 - k_{-2} c_2 > 0\)). This observation generally holds true. For a sequence of reaction of arbitrary length

\[
0 \xrightleftharpoons[k_{-1}^{-1}]{k_1} 1 \xrightleftharpoons[k_{-2}^{-1}]{k_2} 2 \cdots \xrightleftharpoons[k_{-(i+1)}^{-1}]{k_{i}} \cdots \xrightleftharpoons[k_{-n}^{-1}]{k_{n}} n \xrightarrow{J},
\]

the stoichiometric sensitivity for the \(i\)th intermediate is

\[
\eta_i = \frac{1 + \frac{k_i}{k_{-(i-1)} k_{-i}^{-1}}} {k_{-i} c_i} J.
\]
This expression is related to the well-known exit probability and mean first passage time out of state $i$ [24], as is shown in [25]. The Gibbs free energies of the metabolites, $G_i = G_i^o + RT \ln c_i$, decreases along the pathway following the direction of the flux. As we shall see, this correlation between the sensitivity and energetics is not just a coincidence.

The sensitivity $\eta_i$ can also be expressed as $J/J_\to$, where $J_\to$ is the backward flux from state 1 to state 0, and the net flux $J$ is equal to the forward flux minus the backward flux $J = J_+ - J_\to$. $\frac{\partial \ln J}{\partial \ln c_i} = J/J_\to$ is an elegant result that was known to Newsholm and Crabtree [12]. The flux ratio is a measure of whether a reaction operates near equilibrium ($J/J_\to \ll 1$) or far from equilibrium ($J/J_\to \gg 1$). The expression $\eta_i = J/J_\to$ applies to an intermediate at an arbitrary position in the reaction sequence when all the upstream reactions are in rapid equilibrium. For example, if $k_{-1} \gg k_1$, then the reaction between state 0 and state 1 is maintained near equilibrium, and $\eta_2 = J/J_\to$ for the reaction between state 1 and state 2.

Quantities such as $\frac{\partial \ln J}{\partial \ln c_i}$ have been discussed in the context of allosteric regulation, by a metabolite $c_i$ of some enzyme which in turn regulates the $J$ [17]. Brand and his coworkers have extensively used an empirical, “top-down” elasticity analysis in assessing the fractional changes in the fluxes of metabolic reactions in response to a change in the concentration of an effector [20]. These studies, which emphasize the interactions between metabolites and enzymes, are different from the above direct sensitivity of $J$ with respect to an intermediate concentration due solely to the nature of stoichiometric networks.

In terms of the stoichiometric sensitivity, we now ask a typical engineering question. How can one reduce (or increase) the sensitivity of $c_1$ with respect to $J$, with given substrate source $c_0$, flux $J$, intermediary metabolite concentration $c_1$? We assume here that $c_1$ is a regulator and/or control agent for some other parts of the cell, the specific nature of which is not of our concern. To change $\eta_i$ according to Eq. 1 while maintaining $c_1$ at its fixed value, the effective $k_1$ and $k_{-1}$ must be changed simultaneously so that

$$\delta c_1 = \delta k_1 \left( \frac{\partial c_1}{\partial k_1} \right) + \delta k_{-1} \left( \frac{\partial c_1}{\partial k_{-1}} \right) = 0,$$

which leads to

$$\frac{\delta k_1}{k_1} = \frac{\delta k_{-1}}{k_{-1}} \frac{c_1 k_{-1}}{c_0 k_1}.$$  

However, by altering enzyme activity, the effective $k_1$ and $k_{-1}$ can be changed only according to $\delta k_1/k_1 = \delta k_{-1}/k_{-1}$. Thus the sensitivity around a set-point flux and intermediate concentration cannot be effectively adjusted by increasing or reducing the enzyme activity for the reaction $c_0 = c_1$. To adjust $k_1$ and $k_{-1}$ independently of one another it is necessary to change the effective $\Delta G^o = RT \ln(k_{-1}/k_1)$ for reaction $0 \rightleftharpoons 1$. (Doing this is known as impact control in [27]). From Eqs. 1 and 1 it can be shown that $J = k_1 c_0 - k_{-1} c_1 = J_\to (k_1 c_0/k_{-1} c_1 - 1)$, hence

$$\eta_i = \frac{c_0}{c_1} e^{-\Delta G^o/RT} - 1.$$

Eq. 10 is our first key result. It shows that the sensitivity of an intermediary metabolite concentration with respect to the steady-state flux is governed by the $\Delta G^o$, the Gibbs free energy of the intermediate formation. Fig. 1 shows how $\eta_i$ changes as a function of the $\Delta G^o$. Qualitatively, this can be understood without the mathematics: There are two extreme cases of a steady state of the pathway in 1. Case I (far from equilibrium) is that $J = k_1 c_0 - k_{-1} c_1 \gg k_{-1} c_1$. In this case $\Delta G^o/RT \ll \ln(c_0/c_1)$ and $\eta_i \gg 1$. Case II (near equilibrium) is that the $k_{-1} c_1 \gg J$. In the case where the reaction $0 \rightleftharpoons 1$ is maintained near equilibrium, there is little sensitivity of $c_1$ to flux $J$. High sensitivity occurs when $k_1 \gg k_{-1}$; all other things being equal, the smaller the value of $k_{-1}$, the smaller the value of $\Delta G^o$, and the greater the sensitivity. Note that $\eta_i$ goes to zero when $c_0$ and $c_1$ are in equilibrium; $\eta_i$ computed by Eq. 10 remains positive for all cases for which the reaction proceeds in the positive direction.

![FIG. 1: Relation between Gibbs free energy, $\Delta G^o$, and the stoichiometric sensitivity $\eta_i$. For reaction $0 \rightleftharpoons 1 \rightarrow$, with $c_0/c_1 = 8$. Smaller the $\Delta G^o$, greater the sensitivity ($\eta_i$) of $c_1$ in response to output flux (Eq. 10). Everything else being equal, greater $\Delta G^o$ means greater backward flux from $c_1$ to $c_0$. When it is significantly greater than the net flux $J$, the level of $c_1$ will be insensitive to changes in $J$.](https://example.com/figure1)

### B. Impact of Futile Cycles on Stoichiometric Sensitivity

For isolated chemical reactions, modifying $\Delta G^o$ can be accomplished only through modifying the solvent conditions. Such a mechanism is clearly not of primary importance for cellular biochemical reactions. Structural modifications change the nature of the chemical reactions,
leading to different values of $\Delta G^o$. This mechanism represents a possible biological strategy from an evolutionary standpoint, but it is not useful as a mechanism for dynamic regulation of the sensitivity in a cell.

\[ \theta \xrightleftharpoons[k_{-1}]{k_1} 1 \]

FIG. 2: Shown in (a), a biochemical reaction between species 0 and 1 in isolated system reaches its equilibrium with concentrations $c_0^o/c_1^o = k_1/k_{-1} = e^{-\Delta G^o/RT}$. Enzyme can change the rate constants, but not the free energy difference $\Delta G^o$. However, if this reaction is coupled to other reactions in an open biochemical network as shown in (b), a futile cycle is able to shift the population ratio $c_1/c_0$ to be greater (or less) than the equilibrium value $k_1/k_{-1}$. In (b), the additional reactions involve species D and E. There is now a futile cycle involving species 0 and 1. The equilibrium between $D$ and $E$ is $c_D^o/c_E^o = c_0^o k_3/(c_1^o k_{-3}) = k_3 k_3/(k_{-3} k_{-3})$. If the concentrations of $D$ and $E$ are not at their equilibrium, then $\ln(c_D k_3/(c_D k_{-3})) = \Delta G_{DE} \neq 0$, which is the active energy source (e.g., nucleotide hydrolysis) that pumps the futile cycle. In a steady state this energy is dissipated as heat. The same mechanism is behind the nuclear Overhauser effect in magnetic resonance, kinetic proofreading in biosynthesis, and catalytic wheel.

Since the free energy of formation of an intermediary metabolite (at a given temperature, pressure, and solvent condition) cannot be altered, is there a solution to reducing (increasing) the sensitivity in a reaction network? One of the possible mechanisms for increasing or reducing $\eta_1$ is a coupling between the reaction and a futile cycle. Fig. 2 shows a futile cycle attached to the reaction $0 \rightleftharpoons 1$, where if $\frac{c_D k_3}{c_E k_{-3}} \neq 1$, then the apparent free energy difference between species 0 and 1, $\Delta G^o$ is

\[ e^{\Delta G^o/RT} = \frac{k_{-1} + \hat{k}_{3}}{k_1 + \hat{k}_{-3}} e^{\Delta G^o/RT} \left( \frac{1 + \sigma e^{\Delta G_{DE}/RT}}{1 + \sigma} \right) \]

(11)

in which $\sigma = \frac{\hat{k}_{-3}}{\hat{k}_{1}}$, $\hat{k}_3 = k_3 c_E$ and $\hat{k}_{-3} = k_{-3} c_D$ are pseudo-first order rate constants, and $\Delta G_{DE} = RT \ln \frac{k_{-1} k_3}{k_{-3} k_1}$ is the chemical driving force in the futile cycle in Fig. 2. By apparent free energy, we mean that we would treat the nonequilibrium steady-state concentration ratio between 0 and 1 as they were in an equilibrium: $c_1/c_0 = e^{-\Delta G^o/RT}$. In Eq. 10 the sensitivity $\eta_1$ is expressed as a function of $\Delta G^o$. With the reaction coupled to the futile cycle illustrated in Fig. 2, $\Delta G^o \rightarrow \Delta G^o$, and $\eta_1$ is expressed as a function of $\Delta G^o$

\[ \eta_1 = \frac{c_0 e^{-\Delta G^o/RT}}{c_1} - 1. \]

(12)

Eq. 12 can be expressed

\[ \eta_1 = (\eta_1^o + 1) \left( \frac{1 + \sigma}{1 + \sigma e^{\Delta G_{DE}/RT}} \right) - 1 \]

(13)

where $\eta_1^o$ is equal to $\eta_1$ in the absence of the futile cycle ($\Delta G_{DE} = 0$). This is our second key result. It shows that the change in sensitivity $\eta_1$ can be controlled through the amount of available energy, i.e. the driving force $\Delta G_{DE}$ (also see Appendix B). The amount of energy consumed by each turn of the futile cycle is $|\Delta G_{DE}|$. Fig. 3 shows how the sensitivity $\eta_1$ varies as functions of the energy expenditure $\Delta G_{DE}$ for different $\sigma$, the relative rates of the two steps in the loop.

\[ (\eta_1^o + 1) \]

\[ \Delta G_{DE}/RT \]

FIG. 3: Stoichiometric sensitivity ($\eta_1$) as a function of the driving force in the futile cycle ($\Delta G_{DE}$) given in Eq. 13, with different values for the parameter $\sigma$: (a) $\sigma = 10$, (b) $\sigma = 1$, and (c) $\sigma = 0.01$. $\eta_1^o$ is the corresponding $\eta_1$ when $\Delta G_{DE} = 0$, i.e., without the regulation from the futile cycle. Note from Fig. 2, when the $\Delta G_{DE} < 0$ the flux in the futile cycle goes clockwise and when $\Delta G_{DE} > 0$, it is counter clockwise; They correspond to $\eta_1 < \eta_1^o$ and $\eta_1 > \eta_1^o$, respectively.

A third key result follows from Eq. 13 which allows us to diagnose the putative role of a futile cycle in a biological system. When $\Delta G_{DE} < 0$, then the futile cycle of Fig. 2 is driven in the clockwise direction, moving the reaction (at fixed $c_0$ and $c_1$) away from equilibrium, and
increasing the sensitivity of \( c_1 \) to changes in flux. If the futile cycle is thermodynamically driven in the counterclockwise direction, i.e., when \( \Delta G < 0 \), the intermediate concentration is made robust to changes in flux. In both cases there is an energy expenditure and heat dissipation. The “high grade” chemical energy is transformed into “low grade” heat, but is not wasted from the standpoint of information regulation.

### C. Sensitivity of Intermediate Concentration to Input Concentration

To investigate the potential impact of a futile cycle on the sensitivity of intermediate concentration to the input concentration, we define \( \zeta_1 \) as the sensitivity of intermediate concentration \( c_1 \) to changes in input concentration \( c_0 \), at a given steady state flux \( J \),

\[
\zeta_1 = \frac{\partial \ln c_1}{\partial \ln c_0}.
\]

From the equation \( c_1 = e^{-\Delta G^o/RT}(1-J/(k_1c_0))c_0 \), it is straightforward to show that

\[
\zeta_1^o = \left( \frac{c_0}{c_1} \right) e^{-\Delta G^o/RT},
\]

when there is no futile cycle acting on the reaction. For the case when the futile cycle of Fig. 2 is present,

\[
\zeta_1 = \left( \frac{c_0}{c_1} \right) e^{-\Delta G^o/RT} = \zeta_1^o \left( \frac{1 + \sigma}{1 + \sigma e^{\Delta G_{DE}}/RT} \right).
\]

Equation (10) shows that the futile cycle has qualitatively the same impact on intermediate concentration sensitivity to input concentration as on intermediate concentration sensitivity to flux.

### III. Optimal Intermediary Metabolite Sensitivity and Robustness

The previous discussion was based on the example of Eq. 11. We now present a more general theory for stochiometric sensitivity in biochemical networks. For a given reaction \( r \rightarrow p \) in a biochemical network, its kinetics, according to the law of mass actions, and thermodynamics are determined by its forward and backward fluxes \( J_+ \) and \( J_- \):

\[
J = J_+ - J_-, \quad \Delta G = RT \ln \frac{J_+}{J_-}.
\]

If we perturb concentrations and flux around a steady-state, we have

\[
\delta J = \delta J_+ - \delta J_-, \quad \delta \Delta G = RT \left( \frac{\delta J_+}{J_+} - \frac{\delta J_-}{J_-} \right).
\]

Solving \( \delta J_+ \) and \( \delta J_- \) in terms of \( \delta J \) and \( \delta \Delta G \), we have equations that relate changes in the concentrations of the reactant and the product, \( c_r \) and \( c_p \) change to change in the metabolic flux and free energy:

\[
\begin{align*}
\frac{\delta c_r}{c_r} &= \frac{\delta J_+}{J_+} - \frac{\delta J_-}{J_-} = \frac{\delta J}{J} \left( \frac{\delta \Delta G}{RT} \right), \\
\frac{\delta c_p}{c_p} &= \frac{\delta J_+}{J_+} - \frac{\delta J_-}{J_-} = \frac{\delta J}{J} \left( \frac{\delta \Delta G}{RT} \right).
\end{align*}
\]

If the flux \( J \) changes as the metabolic network moves from one steady state to another, the concentrations \( c_r \) and \( c_p \) change according to Eq. 19. The total relative change in reactant and product concentrations associated with the reaction can be calculated as,

\[
\begin{align*}
\left( \frac{\delta c_r}{c_r} \right)^2 + \left( \frac{\delta c_p}{c_p} \right)^2 &= 2 \left( \frac{\delta J}{J} \right)^2 + 2 \left( \frac{J_+ + J_-}{J_+} \right), \\
\left( \frac{\delta J}{J} \right) \left( \frac{\delta \Delta G}{RT} \right) + \frac{J_+^2 + J_-^2}{J^2} \left( \frac{\delta \Delta G}{RT} \right)^2.
\end{align*}
\]

The total relative change in reactant and product concentrations associated with the reaction can be calculated as,

\[
\eta = \sqrt{\left( \frac{\partial \ln c_r}{\partial \ln J} \right)^2 + \left( \frac{\partial \ln c_p}{\partial \ln J} \right)^2}
\]

\[
= \sqrt{\left( 1 - J_- \right)^2 + \left( 1 - J_+ \right)^2},
\]
where \( \theta = -\frac{1}{J} \left( \Delta G / \Delta J \right) \) is the steady-state change in the \( \Delta G \) in response to a change in \( J \). It can be thought of as the nonlinear biochemical resistance of the reaction. With given \( J_+ \) and \( J_- \), \( \eta \) reaches its minimum \( \eta^* = \left( \frac{J_+^2 + J_-^2}{J_+ J_-} \right)^{1/2} \). This is the least sensitive, maximal robustness condition for the reaction, irrespective of how the reaction is situated in a network. This result suggests that in maintaining certain reactions in metabolic pathways near equilibrium, i.e., \( J \ll J_+, J_- \), biological systems may tend to minimize the sensitivities of concentrations of certain key species to perturbations in flux. This insight may be used as a lead for identifying regulatory sites in metabolic systems.

This results allows us to associate chemical thermodynamics with robustness in a biochemical network. However, as we have seen in the previous section, it is not possible to increase or decrease robustness by increasing or decreasing the enzyme activity for a given reaction \( r = p \). By increasing enzyme activity, \( J_+ \), \( J_- \), and \( J \) will all increase in the same proportion. Hence the minimum value of \( \eta \) is not affected. A futile cycle, however, is capable of regulating the minimal \( \eta \).

Several special cases are important.

(i) If the reaction is near equilibrium, \( J_+, J_- \gg J \), then we have the approximate relationship between \( \Delta G \) and \( J \)

\[
\Delta G = RT \ln \left( 1 - \frac{J}{J_+} \right) \approx -RT \left( \frac{J}{J_+} + \frac{1}{2} \left( \frac{J}{J_+} \right)^2 \right)
\]

Hence, \( \theta \approx \frac{1}{J_+} \left( 1 + \frac{1}{J_+} \right) \). Similarly, we have \( \theta \approx \frac{1}{J_-} \left( 1 - \frac{1}{J_-} \right) \).

Substitute these into Eq. 41 we have \( \eta \approx \frac{\sqrt{\Delta G}}{RT} \). In this regime, the total sensitivity of the concentrations to the flux, \( \eta \), is simply proportional to the driving force \( \Delta G \). It is clear that the sensitivity is related to how far the system is away from equilibrium.

(ii) If the concentration \( c_p \) is clamped, then \( \delta J_+ = 0 \) in Eq. 18 and \( \delta \Delta G = -RT J_+ \delta J_- \). Hence \( \theta = \frac{1}{J_+} \), and \( \eta = \frac{1}{J_-} \). The ratio \( \frac{1}{J_-} \) is known as irreversibility of the reaction; for an irreversible reaction it is unity, and it approaches zero as the reaction approaches equilibrium. This result was first obtained in 12.

(iii) By a similar argument, if the \( c_r \) is clamped then \( \theta = \frac{1}{J_-} \), and \( \eta = \frac{1}{J_+} \). This is our Eq. 41.

(iv) If the reaction is a control point for the flux, then the crossover theorem for unbranched reaction pathway 28 indicates that for \( \delta J > 0 \), one has \( \delta c_r \geq 0 \) and \( \delta c_p \leq 0 \). This yields \( \frac{1}{J_-} \leq \theta \leq \frac{1}{J_+} \). Substituting this into Eq. 41, one can easily show that \( \eta \) has upper and lower bounds

\[
\frac{J}{\sqrt{J_+^2 + J_-^2}} \leq \eta \leq \frac{J}{J_-}.
\]
In addition, the costs of binding regulation and the futile cycles regulation are different. The former requires significant amount of biosynthesis of effectors in advance, while the latter requires only a small amount of enzymes for the hydrolysis reaction. The latter consumes energy during the regulation while the former pays in advance in the biosynthesis. In engineering terms, this is an issue of overhead versus operational costs.

V. REGULATORY SENSITIVITIES AND STATISTICAL THERMODYNAMICS

The present work suggests an important relation between the regulatory sensitivity and robustness of metabolic systems in cell and the thermodynamics of biochemical reactions. This rather unexpected quantitative relationship deserves further investigation, especially from a systems biology perspective. In this section, we provide some initial discussions for the subject.

A. Reversibility of biochemical reactions

All chemical reactions are reversible, although in some cases the backward rate may be negligibly small. Approximating such cases as irreversible is often acceptable in kinetic analysis, but is problematic for a thermodynamic analysis as illustrated in [32]. In the biochemical literature a reaction usually is operationally considered irreversible if \( J_+ / J_- > 5 \) [23]. When \( J_+ / J_- \approx 1 \), i.e., \( J_+ \approx J_+ - J_- \), a reaction is considered to be near equilibrium and there is a linear relation between its flux and the chemical potential difference \( \Delta G = RT \ln(J_+ / J_-) \approx RT J_+ / J_+ \approx RT J / J^0 \). Thus the equilibrium forward and reverse flux \( J^0 \approx J^0 / (RT) \) is the conductance of a biochemical reaction operating near equilibrium [10].

B. Sensitivity and concentration fluctuations

Because of the thermal agitations and the stochastic nature of molecular reactions, the concentrations of a biochemical species fluctuate, in a test tube reactions and in cells [25]. Rigorously, the concentrations (such as \( c_1 \) and \( c_2 \)) discussed so far represent the mean values of the concentrations of these species. If one is able to measure the fluctuating concentration of a species as a function of time, say \( c_1(t) \) for species 1, then one may calculate the magnitude of the concentration fluctuation by \( \int_0^\infty \langle \Delta c_1(0) \Delta c_1(t) \rangle dt \) where \( \Delta c_1(t) = c_1(t) - \langle c_1 \rangle \) is the deviation of \( c_1(t) \) from its mean value. The notation \( \langle \cdots \rangle \) denotes average of the quantity in the brackets, and \( \langle \Delta c_1(0) \Delta c_1(t) \rangle \) is known as time-correlation function of fluctuating \( c_1 \) [33].

One might imagine that a biochemical species with high sensitivity of concentration will display similarly large concentration fluctuations. This is indeed the case. The sensitivities introduced in Eqs. (1) and (3) are intimately related to the steady-state concentration fluctuations in the intermediary metabolites [30]. To see this, we note that from Eqs. (1) and (3), \( \eta_1 = J \left( c_0 k_1 - J \right)^{-1} \) and \( \eta_2 = J \left( c_0 k_1 k_2 - J \right)^{-1} \), respectively.

It is shown in Appendix C (Eqs. (14) and (15) and the discussion there) that the \( c_0 k_1 \) and \( c_0 k_1 k_2 / (k_2 + k_{-1}) \) can be expressed as:

\[
(c_0 k_1)^{-1} = \int_0^\infty \frac{\langle \Delta c_1(0) \Delta c_1(t) \rangle}{\langle c_1 \rangle^2} dt, \quad (24)
\]

and

\[
\left( \frac{c_0 k_1 k_2}{k_2 + k_{-1}} \right)^{-1} = \int_0^\infty \frac{\langle \Delta c_2(0) \Delta c_2(t) \rangle}{\langle c_2 \rangle^2} dt. \quad (25)
\]

The right-hand-sides of Eqs. (21) and (25) are the concentration fluctuations. Thus, we see that smaller the fluctuations, smaller the sensitivities. Elf et. al. have reached a similar conclusion based on a linear approximation of a nonlinear, stochastic biochemical kinetics [41].

C. Futile cycle and heat dissipation

While the major function of futile cycles in signal transduction seems to be improving the performance of information processing against noise, the functional roles of futile cycles in metabolic systems potentially include improving sensitivity or robustness of metabolite concentrations. In addition to affecting sensitivity, it is possible that futile cycles play an important role in generating heat and regulating temperature [12]. Hence it is important to analyze metabolic regulations with a systems perspective. In particular the intriguing suggestion [12] that the futile cycles are important components in obesity, in weight loss, and even in the so-called Atkins’ diet [43, 44, 45], deserves further investigation. It is timely to rethink the issues of nutrition, thermogenesis [46], and futile cycles with a molecular as well as modern systems biology and metabolic engineering approach [47, 48, 49]. The present work provides a thermodynamic basis for studying futile cycles, which is likely to be essential in such studies.

VI. SUMMARY

Spending energy to gain control is not a foreign concept in engineering. Since this strategy is hallmark of control engineering, it should not come as surprise that biological cells use energy in controlling metabolism, transcription, and translation. Thus futile cycles which utilize biochemical energy are not necessarily “futile”; they likely serve as mechanisms of biochemical regulation.
It has become increasingly clear from our recent work that futile cycles play a unique and essential role in cellular regulation and signal transduction in the form of protein phosphorylation dephosphorylation (and GTPase). While the phosphate group serves as a structural signal in enzyme activation, the phosphorylation reaction also provides a source of energy. The energy expenditure in fact increases the accuracy, sensitivity, specificity, and robustness of the cellular information processing, overcoming cellular internal noises from thermal fluctuations, small copy numbers, and limited affinities.

However to date, the role of futile cycles in metabolic regulation has been less quantitatively understood. In this work we have shown that metabolic futile cycles shift the effective equilibrium constants for biochemical reactions, modulating the sensitivity and robustness of intermediate concentrations to changes in flux. By shifting the effective equilibrium so that a reaction is moved away from equilibrium, the stoichiometric sensitivity is increased. Shifting it in the other direction reduces the sensitivity and enhances robustness. The direction of the shift, and hence a putative physiological role for a given futile cycle, can be diagnosed from the direction of operation of the futile cycle. When a futile cycle drives a reaction in the forward direction (clockwise in Figs. 2b and 4a), then the sensitivity of the concentration of intermediate species 1 to the steady-state flux is enhanced. When a futile cycle drives the reaction in the direction opposite the net flux (counterclockwise in Figs. 2b and 4a), then the sensitivity is reduced and the intermediate concentration is made robust to changes in the flux.

VII. ACKNOWLEDGMENTS

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A: Sensitivity with different output flux controls

Consider the reaction

\[ 0 \xrightarrow{k_1 \, \text{in}} 1 \xrightarrow{k_2 \, \text{out}} 2 \xrightarrow{J} \]

(26)

With \( c_0 \) fixed, there are several ways to control the output flux \( J \), either (i) directly controlling flux \( J \), or (ii) by controlling the rate constant \( k_3 \) in the downstream reaction, 2 \( \xrightarrow{k_3 \, \text{in}} \) 3, or (iii) by controlling the concentration of downstream species, \( c_3 \) in 2 \( \xrightarrow{k_3 \, \text{out}} \) 3, or the enzyme for the reaction. We show here that all these three cases yield identical expressions for the stoichiometric sensitivity.

The kinetic equations for reaction system in (26) are

\[
\frac{dc_1}{dt} = k_1c_0 - (k_{-1} + k_2)c_1 + k_{-2}c_2, \\
\frac{dc_2}{dt} = k_2c_1 - k_{-2}c_2 - \begin{cases} J & \text{if case (i)} \\ k_3c_2 & \text{if case (ii)} \\ k_3c_2 + k_{-3}c_3 & \text{if case (iii)} \end{cases}
\]

(27, 28)

where the last term in Eq. (28) represents the three cases above.

VIII. APPENDICES
Consider the dynamic equation for \( c_2 \) with the general expression,
\[
\frac{dc_1}{dt} = k_1c_0 - (k_{-1} + k_2)c_1 + k_{-2}c_2,
\]
\[
\frac{dc_2}{dt} = k_2c_1 - k_{-2}c_2 - J(c_2, c_3, k_3, k_{-3}).
\]
In steady-state, we have
\[
k_1c_0 - (k_{-1} + k_2)c_1 + k_{-2}c_2 = 0, \quad (31)
k_2c_1 - k_{-2}c_2 - J(c_2, c_3, k_3, k_{-3}) = 0. \quad (32)
\]
Therefore, we have
\[
-(k_{-1} + k_2)(\frac{\partial c_1}{\partial J}) + k_{-2}(\frac{\partial c_2}{\partial J}) = 0, \quad (33)
k_2(\frac{\partial c_1}{\partial J}) - k_{-2}(\frac{\partial c_2}{\partial J}) = 1. \quad (36)
\]
Solving this pair of algebraic equations yields
\[
\frac{\partial c_1}{\partial J} = -\frac{1}{k_{-1}}, \quad \frac{\partial c_2}{\partial J} = -\frac{k_2 + k_{-1}}{k_{-1}k_{-2}}. \quad (37)
\]

\section{B. B: A simple example of how futile cycle drives concentration ratio away from equilibrium}

This example is motivated by the classic work on kinetic proofreading. Let’s consider a 3-state kinetic system with \( A, B \) and \( C \) shown in Fig. 5. The equilibrium constant \( K_{12} = \frac{[B]^*}{[A]^*} \), and reaction between \( B \) and \( C \) is coupled to an energy source \( \Delta G_{DE} = RT \ln \frac{[E][k_{-1}c]}{[B][k_{-2}c,k_{-3}]} \). We shall denote pseudo-first order rate constants \( k_2 = k_{2}^{[E]}[D] \) and \( k_{-2} = k_{-2}^{[E]}[E] \), and \( \gamma = \frac{k_{2}^{[E]}[k_{-2}]}{k_{-2}^{[E]}k_{-3}} = e^{-\Delta G_{DE}/RT} \). Then we have
\[
\frac{[C]}{[A]} = (1 + K_{12}) \frac{[C]}{([A] + [B])} = \frac{k_{-3}}{k_3} \frac{(k_{-2} + k_3)}{(k_{-2} + k_2)} \quad (38)
\]
where \( RT \ln \gamma = -\Delta G_{DE} \) is the amount of energy pumped into the reaction. \( \gamma = 1 \) for a closed system in equilibrium. For large \( \gamma \), i.e., the kinetic cycle goes clockwise, it is possible to have \( \gamma k_{-2} \gg k_3 \gg k_{-2} \) and,
\[
\frac{[C]}{[A]} \approx \frac{k_{-3}}{k_3} \frac{(k_{-2} + k_3)}{(k_{-2} + k_2)} \gg \frac{k_{-3}}{k_3}. \quad (39)
\]
the expected equilibrium ratio between \( B \) and \( A_1 \). On the other hand, for small \( \gamma \), i.e., the energy pumping is counter-clockwise, it is possible to have \( \gamma k_{-2} \ll k_3 \ll k_{-2} \), and
\[
\frac{[C]}{[A]} \approx \frac{k_{-3}}{k_3} \frac{k_3}{(k_{-2})} \ll \frac{k_{-3}}{k_3}. \quad (40)
\]

\section{C. C: Concentration fluctuations in open systems}

Here we consider concentration fluctuations in the kinetic pathway with constant source \( c_0 \) and sink \( c_3 = 0 \):
\[
0 \overset{k_1}{\underset{k_{-1}}{\Rightarrow}} 1 \overset{k_{-2}}{\underset{k_2}{\Rightarrow}} 2 \overset{k_3, k_{-3}}{\Rightarrow} 3. \quad (41)
\]
For a general theory on open, linear biochemical networks see [22]. In stochastic terms, the probability of the numbers of species 1 and 2 being \( m \) and \( n \) at time \( t \), \( P(m,n,t) \) satisfies the chemical master equation [50, 51]
\[
\frac{dp(m,n,t)}{dt} = -(k_1c_0 + m(k_{-1} + k_2) + (k_{-2} + k_3)n)p(m,n) \quad + k_1c_0p(m-1,n) + (m+1)k_{-1}p(m+1,n) + (m+1)k_2p(m+1,n-1)
\]
\[
+(n+1)k_{-2}p(m-1,n+1) + (n+1)k_3p(m,n+1) \quad (42)
\]
From Eq. (12) it is easy to show that the mean values of \( m \) and \( n \) follow the standard deterministic kinetic equations
\[
\frac{d}{dt}\langle m \rangle = k_1c_0 - (k_{-1} + k_2)\langle m \rangle + k_{-2}\langle n \rangle \quad (43)
\]
\[
\frac{d}{dt}\langle n \rangle = k_2\langle m \rangle - (k_{-2} + k_3)\langle n \rangle \quad (44)
\]
and furthermore their variances and covariance,
\[
\frac{d}{dt}\langle (\Delta m)^2 \rangle = k_1c_0 + (k_{-1} + k_2)\langle m \rangle + k_{-2}\langle n \rangle - 2(k_{-1} + k_2)\langle (\Delta m)^2 \rangle + 2k_{-2}\langle \Delta m \Delta n \rangle, \quad (45)
\]
\[
\frac{d}{dt}\langle (\Delta n)^2 \rangle = k_2\langle m \rangle + (k_{-2} + k_3)\langle n \rangle + 2k_2\langle \Delta m \Delta n \rangle - 2(k_{-2} + k_3)\langle (\Delta n)^2 \rangle, \quad (46)
\]
\[
\frac{d}{dt}\langle \Delta m \Delta n \rangle = -k_2\langle m \rangle - k_{-2}\langle n \rangle + k_2\langle (\Delta m)^2 \rangle + k_{-2}\langle (\Delta n)^2 \rangle - (k_{-1} + k_2 + k_{-2} + k_3)\langle \Delta m \Delta n \rangle, \quad (47)
\]
where \( \Delta m = m - \langle m \rangle \) and \( \Delta n = n - \langle n \rangle \). The steady state mean values
\[
\langle m \rangle = \frac{k_1k_{-2}c_0}{k_{-1}(k_{-2} + k_3) + k_2k_{-3}}, \quad \langle n \rangle = \frac{k_1k_2c_0}{k_{-1}(k_{-2} + k_3) + k_2k_{-3}} \quad (48)
\]
which agree with Eqs. (2) and (3) if we substitute \( J = \langle m \rangle \), and more,
\[
\langle (\Delta m)^2 \rangle = \langle m \rangle, \quad \langle (\Delta n)^2 \rangle = \langle n \rangle, \quad \langle \Delta m \Delta n \rangle = 0. \quad (49)
\]
In fact, one can verify that the stationary probability distribution for \( m \) and \( n \) is Poissonian [23], \( \langle m \rangle^{ss}e^{-\langle m \rangle^{ss}} \langle n \rangle^{ss}e^{-\langle n \rangle^{ss}} \), the stationary distribution for Eq. (42). However, while Eq. (10) indicates that the steady-state
fluctuations for \(m\) and \(n\) are independent. It does not mean that \(m\) and \(n\) are completely independent. In fact, we have \(\langle \Delta m(0) \Delta m(t) \rangle \neq 0\) for \(t \neq 0\):

\[
\langle \Delta m(0) \Delta n(t) \rangle = \frac{\langle m \rangle^{ss}}{\lambda_1 - \lambda_2} (e^{\lambda_1 t} - e^{\lambda_2 t})
\]

(47)

\[
\langle \Delta n(0) \Delta m(t) \rangle = \frac{\langle n \rangle^{ss}}{\lambda_1 - \lambda_2} (e^{\lambda_1 t} - e^{\lambda_2 t})
\]

(48)

\[
\langle \Delta m(0) \Delta n(t) \rangle = \frac{k_2 \langle m \rangle^{ss}}{\lambda_1 - \lambda_2} (e^{\lambda_1 t} - e^{\lambda_2 t})
\]

(49)

\[
\langle \Delta n(0) \Delta m(t) \rangle = \frac{k_2 \langle n \rangle^{ss}}{\lambda_1 - \lambda_2} (e^{\lambda_1 t} - e^{\lambda_2 t})
\]

(50)

where \(\lambda_1\) and \(\lambda_2\) are the two eigenvalues of the matrix

\[
F = \begin{pmatrix}
-k_{-1} - k_2 & k_{-2} \\
-2k_2 & k_{-3} - k_3
\end{pmatrix}
\]

(51)

Note that the concentration \(c_1\) \((c_2)\) and number of molecules \(m\) \((n)\) differ by the volume of the system. Hence, dentifying \(\langle c_1(0) c_1(t) \rangle = \langle m(0)m(t) \rangle \) and \(\langle c_2(0)c_2(t) \rangle = \langle n(0)n(t) \rangle \), and integrating Eqs. 47 and 48 we arrive at Eqs. 24 and 25.

We shall also point out that the stochastic dynamics on a mesoscopic scale can be described by a Fokker-Planck equation 50 51

\[
\frac{\partial}{\partial t} P(m,n,t) = \nabla \cdot \left[ \frac{\Gamma}{2} \nabla P - F \left( \frac{\Delta m}{\Delta n} \right) P \right]
\]

(52)

in which

\[
\Gamma(m,n) =
\]

\[
\begin{pmatrix}
k_1c_0 + (k_{-1} + k_2)m + k_{-2}n & -k_2m - k_{-2}n \\
-k_2m - k_{-2}n & k_2m + (k_{-2} + k_3)n
\end{pmatrix}
\]

(53)

and \(F\) is given in Eq. 51. We see that \(\Gamma\) can be expressed as \(\frac{1}{2} S (J^+ + J^-) S^T\) where the stoichiometric matrix 14

\[
S = \begin{pmatrix}
-1 & +1 & 0 \\
0 & -1 & +1
\end{pmatrix}
\]

(54)

representing two species and three reactions in Eq. 1. According to the Fokker-Planck equation, the stationary distribution \(P^{ss}(m,n)\) is Gaussian centered around \(\langle m^{ss}, n^{ss} \rangle\) with covariant matrix \(\sigma\) satisfying 51 52 53

\[
F \sigma + \sigma F^T = -\Gamma \left( \langle m^{ss}, n^{ss} \rangle \right).
\]

(55)

It can be easily verified via matrix multiplication that the solution to the Eq. 55 is precisely Eq. 10. We should point out that the fluctuation covariance is not \(FF^T\) as recently suggested 54. The information on the concentration fluctuations is not contained in the relaxation rate \(F\) alone; it has to depend on \(\Gamma\) which represents the rate of fluctuations 14.