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Constraints on Reciprocal Flux Sensitivities in Biochemical Reaction Networks

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ABSTRACT We identify a connection between the structural features of mass-action networks and the robustness of their steady-state fluxes against rate constant variations. We find that in all positive steady states of so-called injective networks—networks that arise, for example, in metabolic and gene regulation contexts—there are certain firm bounds on the flux control coefficients. In particular, the control coefficient of the flux of a reaction, with respect to variation in its own rate constant, is delimited in a precise way. Moreover, for each pair of reactions, the flux of at least one of them must have a precisely delimited control coefficient with respect to variation in the rate constant of the other. The derived bounds can, however, be violated in noninjective networks, so for them a more pronounced lack of robustness could be exhibited. These results, which indicate a mechanism by which some degree of robustness is induced in the injective setting, also shed light on how robustness might evolve.

INTRODUCTION

Phenotypes of biochemical reaction networks are often characterized by the steady-state concentrations of their constituent biochemical species and the steady-state rates of (or fluxes through) their constituent reactions. The identification of phenotypes with steady-state fluxes has proven to be particularly useful in the study of metabolic reaction networks, where metabolite fluxes through enzyme catalyzed reactions are often of primary interest.

Yet the characterization of phenotypes solely by means of steady-state fluxes (and concentrations) leaves something to be desired, because in vivo biochemical networks are subject to ever-present fluctuations in parameters such as pH, temperature, and total building-block concentrations.

Parameter fluctuations can arise from both environmental and genetic sources. For example, the rate constants employed in mass-action models might depend on physical quantities such as temperature, and might also have incorporated in them the concentrations of chemical species such as ATP or H^+. Thus, changes in the physical properties or the chemical make-up of the ambient cellular environment will generally cause the rate constants employed in models to shift. Mutations might also cause rate constants to vary as a result of modifications to the mutual affinity of reactants. Therefore, an understanding of phenotypes entails not only that we know the fluxes (concentrations) themselves, but also that we characterize the sensitivity of each flux (concentration) with respect to changes in each of the parameters. Such knowledge of the flux sensitivities also provides valuable information about which parameters should be known with higher accuracy in the formulation of mathematical models.

One approach for studying the robustness of fluxes (concentrations) is by means of sensitivity analysis. This approach, which is common in systems engineering, was introduced to biology by Higgins (1) in the 1960s. Subsequently, it became an important tool in *biochemical systems theory*, pioneered by Savageau (2), and *metabolic control analysis*, pioneered by Kacser and Burns (3) and Heinrich and Rapoport (4,5). Nowadays the use of sensitivity analysis is commonplace in biology.

The main mathematical object employed in sensitivity analysis is the *sensitivity matrix*. Loosely speaking, the sensitivity matrix is the array whose elements are the fractional derivatives of the fluxes (concentrations) with respect to the parameters, evaluated at a particular steady state. Each entry in, for example, the flux sensitivity matrix, indicates the fractional change in one of the steady-state fluxes with respect to the fractional change in one of the parameters.

Consider, for example, the simple mass-action network

$$2A \underset{\leftarrow}{\rightarrow} B, \tag{1}$$

which describes, say, a protein dimerization process. Suppose that, for the rate constant values $k^*_{2A\to B}$ and $k^*_{B\to 2A}$, c^*_A and c^*_B are steady-state concentrations and that the corresponding steady-state fluxes are $J^*_{2A\to B}$ and $J^*_{B\to 2A}$. Suppose also that we fix the total protein concentration $T = c^*_A + 2c^*_B$, and we let the rate constants fluctuate about their nominal values. It can then be shown that there are smooth functions $J_{2A\to B}(\cdot,\cdot)$ and $J_{B\to 2A}(\cdot,\cdot)$ that map each choice of rate constants in a neighborhood of $(k^*_{2A\to B},\ k^*_{B\to 2A})$ to a unique steady-state flux, with $J^*_{2A\to B} = J_{2A\to B}(k^*_{2A\to B}, k^*_{B\to 2A})$ and $J^*_{B\to 2A} = J_{B\to 2A}(k^*_{2A\to B}, k^*_{B\to 2A})$. With this in mind, the flux sensitivity matrix corresponding to the network of Eq. 1, evaluated at $k^* \equiv (k^*_{2A\to B}, k^*_{B\to 2A})$, is the array

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$$\begin{pmatrix} C_{2A\to B}^{2A\to B} & C_{B\to 2A}^{2A\to B} \\ C_{2A\to B}^{B\to 2A} & C_{B\to 2A}^{B\to 2A} \end{pmatrix}_{k^*} = \begin{pmatrix} \frac{k^*_{2A\to B}}{J^*_{2A\to B}}\frac{\partial J_{2A\to B}}{\partial k_{2A\to B}} & \frac{k^*_{B\to 2A}}{J^*_{2A\to B}}\frac{\partial J_{2A\to B}}{\partial k_{B\to 2A}} \\ \frac{k^*_{2A\to B}}{J^*_{B\to 2A}}\frac{\partial J_{B\to 2A}}{\partial k_{2A\to B}} & \frac{k^*_{B\to 2A}}{J^*_{B\to 2A}}\frac{\partial J_{B\to 2A}}{\partial k_{B\to 2A}} \end{pmatrix}_{k^*}, \quad (2)$$

the elements of which are called the *flux control coefficients*. The flux control coefficient $C_{B\to 2A}^{2A\to B}(k^*)$, for example, indicates the fractional change in the flux through the reaction $2A \to B$ with respect to variations in the rate constant of the reaction $B \to 2A$, evaluated at k^*.

Generally, examination of the flux sensitivity matrix will indicate which fluxes are robust to which parameters (small flux control coefficients) and which fluxes are sensitive to which parameters (large flux control coefficients). In fact, experimental study and theoretical analysis of specific metabolic networks has revealed that flux control coefficients tend to be small (6), indicating overall robustness in the network. This observation motivates the following question: Are the magnitudes of flux control coefficients inherently constrained, and, if so, what are the mechanistic sources of these constraints?

Here, we focus on mass-action networks, and we study the sensitivity of their steady-state fluxes with respect to variations in rate constant values. (Related investigations of the concentration sensitivities with respect to the total building block concentrations are presented in Shinar et al. (7) and Shinar and Feinberg (8).) In particular, we deal with the class of mass-action networks that possess the property of *injectivity*. This class, which we call *the injective class*, contains a variety of biologically relevant models, including the set of linear and branched chains of Michaelis-Menten reactions (see later in Fig. 2 and Section S3 in the Supporting Material), some classic enzyme-catalysis mechanisms (9), and gene regulation models (10).

Later in this article, we will define injectivity and discuss in detail how to determine whether a network is injective. For now, it suffices to note that injectivity is a subtle network property, which often manifests itself in an unintuitive way. As an example for this, consider the networks in Table 1: Note that of the otherwise-similar Entries 5 and 6, both of which realize catalytic mechanisms for the two-substrate reaction $S1 + S2 \to P$, only one possesses injectivity. Another example: the competitive inhibition and uncompetitive inhibition Michaelis-Menten networks in Entries 2 and 3, respectively, both of which are injective, combine to give the mixed-inhibition Michaelis-Menten network in Entry 4, which fails to be injective. The point that we wish to emphasize, and to which the examples in Table 1 attest, is this: At present, injectivity is not easily discerned from more familiar or intuitive biological or biochemical notions, and ascertaining injectivity requires specially developed mathematics. This mathematics, however, is readily available, as is a freely accessible computer program (11) for determining the injectivity of a mass-action network.

TABLE 1 Examples of injective and noninjective networks (adapted from Craciun et al. (9))

Entry	Network	Remark	Injectivity
1	$E + S \rightleftarrows ES \to E + P$	Simple Michaelis-Menten kinetics: $S \to P$	Yes
2	$E + S \rightleftarrows ES \to E + P$ $E + I \rightleftarrows EI$	Michaelis-Menten kinetics with competitive inhibition: $S \to P$	Yes
3	$E + S \rightleftarrows ES \to E + P$ $ES + I \rightleftarrows ESI$	Michaelis-Menten kinetics with uncompetitive inhibition: $S \to P$	Yes
4	$E + S \rightleftarrows ES \to E + P$ $E + I \rightleftarrows EI$ $ES + I \rightleftarrows ESI \rightleftarrows EI + S$	Michaelis-Menten kinetics with mixed inhibition: $S \to P$	No
5	$E + S1 \rightleftarrows ES1$ $S2 + ES1 \rightleftarrows ES1S2 \to E + P$	Two-substrate enzyme catalysis with sequential (ordered) substrate binding: $S1 + S2 \to P$	Yes
6	$E + S1 \rightleftarrows ES1 \quad E + S2 \rightleftarrows ES2$ $S2 + ES1 \rightleftarrows ES1S2 \rightleftarrows S1 + ES2$ $\downarrow$ P	Two-substrate enzyme catalysis with random (unordered) substrate binding: $S1 + S2 \to P$	No

All species in each network are assumed to undergo degradation reactions of the type $s \to 0$, which are not explicitly shown. The examples demonstrate that injectivity is a subtle network property: Otherwise-similar networks can differ with respect to injectivity (compare, for example, Entries 5 and 6). Moreover, mechanisms that possess injectivity in isolation can lack it when combined (compare, for example, Entries 2 and 3 with Entry 4).

Our main result is a theorem that, for each positive steady state of any injective mass-action network, gives bounds on the diagonal elements of the corresponding flux sensitivity matrix, and also gives constraints on the values that any pair of reciprocal off-diagonal elements may take. More precisely, we find that in each positive steady state of any injective mass-action network the diagonal flux control coefficients are constrained to lie between 0 and 1, and in each pair of reciprocal off-diagonal flux control coefficients at least one coefficient is constrained to lie between -1 and 1. These sensitivity bounds can, however, be violated in noninjective networks, so for them a more pronounced lack of robustness could be exhibited. Thus, we identify a constraint on the flux control coefficients in injective mass-action networks, a constraint that has its roots in network structure alone.

The remainder of this article is organized as follows: First, we will introduce some concepts from *chemical reaction network theory*, with emphasis on the concept of injectivity. These concepts are required for stating our main result. Second, to place our results in perspective, we will briefly discuss the summation theorem of metabolic control analysis in the context of mass-action networks. Third, we will state our main result, a reciprocity theorem that provides constraints on flux control coefficients in the injective setting. We will illustrate the theorem's usage with (simplified) mass-action models of metabolic and gene

regulation networks, and with a classic enzyme catalysis mechanism. We will also point out how the conclusion of our theorem might relate to classical results in metabolic control analysis. Finally, we will offer concluding remarks, with focus on a possible connection between our results and the evolution of robustness.

Some concepts from chemical reaction network theory

Here we provide concepts and definitions from chemical reaction network theory, which are required for stating our main result. (For a general introduction to chemical reaction network theory, see Feinberg (12,13); an introduction for mathematicians can be found in Feinberg (14).) Our presentation will be somewhat informal. For a proper formal presentation of the concepts, see Section S2 in the Supporting Material.

A *chemical reaction network* is a collection of three sets: The first is the set of *species*, denoted $\mathcal{S}$, which in Eq. 1 is simply $\{A, B\}$. The second is the set of *complexes*, denoted $\mathcal{C}$, whose members are the objects that appear before and after the reaction arrows. In Eq. 1, $\mathcal{C} = \{2A, B\}$. The third set is the set of *reactions*, denoted $\mathcal{R}$. In Eq. 1, $\mathcal{R} = \{2A \rightarrow B, B \rightarrow 2A\}$.

When discussing an arbitrary reaction in a network we shall use the symbol $y \rightarrow y'$ to indicate the reaction. The symbol y indicates the *reactant complex* of the reaction, and the symbol y' indicates the *product complex* of the reaction. Thus, in the reaction $2A \rightarrow B$, $y = 2A$ is the reactant complex, and $y' = B$ is the product complex.

When we have a particular reaction network in mind, an instantaneous *composition* is a specification of a nonnegative molar concentration for each species. Thus, for the network in Eq. 1, a composition amounts to a specification of values for the molar concentrations c_A and c_B. In this case, it will be convenient to represent the composition in the form $c = c_A A + c_B B$—that is, as a linear combination of the species, with each species multiplied by its corresponding molar concentration. When there are more species, representation of compositions in this form would proceed in the same way. (Formally, the compositions reside in the vector space of all linear combinations of species with real number coefficients. This vector space is analogous to the more familiar Euclidean space R^n, but it has the technical advantage that no order is preimposed on the species. In our example, each composition $c = c_A A + c_B B$ will be analogous to the vector $c = (c_A, c_B) = c_A(1,0) + c_B(0,1)$ in R^2.)

A *mass-action system* is a reaction network taken together with an assignment of a positive *rate constant* to each reaction in the network. We designate the assignment of rate constants to reactions by the symbol k, and we indicate the rate constant of a particular reaction by giving k the appropriate reaction subscript. Thus, in Eq. 1, the rate constant assigned to the reaction $2A \rightarrow B$ is denoted $k_{2A\rightarrow B}$, and the rate constant assigned to $B \rightarrow 2A$ is denoted $k_{B\rightarrow 2A}$.

Consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. Of particular importance in the study presented here is the *mass-action rate function* of the network, denoted $v(\cdot,\cdot)$, which assigns to each choice of composition c and to each assignment of rate constants k the instantaneous rate of formation of each species,

$$v(c,k) = \sum_{y\rightarrow y'\in\mathcal{R}} k_{y\rightarrow y'} c^y (y' - y), \tag{3}$$

where the symbol c^y indicates the product of the molar concentrations appearing in composition c, each raised to the corresponding (stoichiometric) coefficient in the reactant complex y:

$$c^y = \prod_{s\in\mathcal{S}} (c_s)^{y_s}. \tag{4}$$

Thus, in the case of Eq. 1, the mass-action rate function takes the form

$$\begin{aligned} v(c,k) &= k_{2A\rightarrow B}(c_A)^2(c_B)^0(B-2A) \\ &\quad + k_{B\rightarrow 2A}(c_A)^0(c_B)^1(2A-B) \\ &= \left[2k_{B\rightarrow 2A}c_B - 2k_{2A\rightarrow B}(c_A)^2\right]A \\ &\quad + \left[k_{2A\rightarrow B}(c_A)^2 - k_{B\rightarrow 2A}c_B\right]B. \end{aligned} \tag{5}$$

The coefficient of A (B) on the right-hand side of Eq. 5 is the instantaneous rate of increase in the molar concentration of species A (B).

Next, consider the mass-action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$ obtained by taking together the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ with a fixed assignment k of rate constants. The differential equation governing the time rate of change of the composition is given by

$$\dot{c} = v(c,k). \tag{6}$$

Thus, from Eqs. 5 and 6, we see that the differential equation corresponding to Eq. 1 takes the component form

$$\begin{aligned} \dot{c}_A &= 2k_{B\rightarrow 2A}c_B - 2k_{2A\rightarrow B}(c_A)^2, \\ \dot{c}_B &= k_{2A\rightarrow B}(c_A)^2 - k_{B\rightarrow 2A}c_B. \end{aligned} \tag{7}$$

A composition c, for which the right-hand side of Eq. 6 is zero, is called a *steady state* of the mass-action system.

Once again, let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a reaction network. A composition c, together with an assignment of (positive) rate constants k, is called a *network steady-state* if $v(c, k) = 0$. A network steady state is *positive* whenever all the components of c are strictly positive. Thus, the *positive steady-state set* of the network is exactly the set of all pairs (c, k) for which c is a positive steady state of the mass-action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$. In biological applications, such as metabolism, signaling, and gene expression, the steady states encountered will often be strictly positive.

Two compositions are called *stoichiometrically compatible* relative to a given reaction network if a change from one composition to another would not violate any conservation conditions intrinsic to the network. Thus, in the network

of Eq. 1, two compositions c and c' are stoichiometrically compatible only if the total protein concentration in both is the same: $c_A + 2c_B = c'_A + 2c'_B$. The notion of stoichiometric compatibility is made more precise in Section S2 in the Supporting Material.

Injective networks

We will now briefly review the concept of injectivity, which plays a crucial role in this work. (The theory of injective mass-action networks is described in Craciun and Feinberg (15–18). Related theory about networks that are not necessarily mass-action can be found in Banaji et al. (19) and Banaji and Craciun (20,21).)

A mass-action network is called *injective* if, for each assignment of rate constants, the following is true for any pair of distinct but stoichiometrically compatible positive compositions: There is at least one species whose net production rate (as determined by the species formation rate function v) evaluated at the first composition is different from its net production rate evaluated at the second composition. In other words, no matter what the rate constants are, two different stoichiometrically compatible compositions cannot give rise to production rates that, species-by-species, are completely identical. (This is made more precise in Section S3 in the Supporting Material.) An injective network is thus precluded, for any assignment of rate constants, from displaying multiple stoichiometrically compatible positive steady states.

We draw the reader's attention to Table 1 to emphasize the following point: Injectivity is a subtle network property that is not easily ascertained from coarse-grained biochemical or biological considerations. Table 1 shows networks that are otherwise similar in their structure, but some of their operative catalytic mechanisms may nevertheless differ as to whether or not they possess injectivity.

Although ascertaining injectivity in a network is not straightforward, there exist both computational (11,15) and graph-theoretic (16–18) criteria for determining injectivity in large classes of networks. Here, we shall present a brief account of the graph theoretic criterion. To keep the presentation concise, we will focus on *fully open* networks, in which each species s is subject to degradation (or dilution) reactions of the form $s \to 0$. An extension of the graph theoretic method to the more general case is provided in Craciun and Feinberg (18) and is described in Section S3 in the Supporting Material. Software for ascertaining injectivity is readily available for download from Ji et al. (11).

To facilitate our discussion of injectivity, consider the mass-action network displayed in Fig. 1 *A*. This network describes a metabolic bifurcation point, symbolized by

$$P1 \xleftarrow{E1} S0 \xrightarrow{E2} P2,$$

where two Michaelis enzymes, $E1$ and $E2$, compete for the same substrate $S0$, and convert it, respectively, to products $P1$ and $P2$. We assume that all species in the network are subject to degradation and that $S0$, $E1$, and $E2$ are synthesized at constant rates, as indicated by the reactions $0 \to S0$, $0 \to E1$, and $0 \to E2$. The network of Fig. 1 *A*, as we will show in what follows, belongs to the injective class. Other examples of biologically relevant injective reaction networks include the class of branched Michaelis-Menten chains (exemplified in Fig. 2 and analyzed in Section S3 in the Supporting Material), which are often used in modeling metabolic pathways, and gene expression models, such as the autoinhibitory module (10) of Fig. 3 (analyzed in Section S7 in the Supporting Material).

One way to check for injectivity in a fully open network is by examining its *species-reaction (SR) graph*. The SR graph for a fully open network is constructed from the network's *true reactions*, that is, the reactions for which neither the reactant complex nor the product complex is 0. In Fig. 1 *A*, the true reactions correspond to solid arrows.

The nodes of the SR graph are either species or reactions: there is one node for each true reaction (a pair of true reversible reactions corresponds to a single node), and one node for each species appearing in the true reactions.

The edges of the SR graph connect its nodes in the following way: A species and a reaction will be joined by an edge if the species appears in one of the complexes of the reaction. In addition, the edge will be labeled by the complex in which the species occurs. (If the species occurs

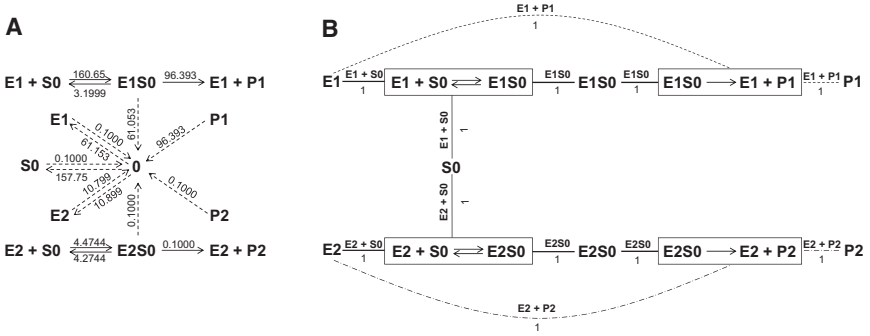

FIGURE 1 Mass-action model of the metabolic bifurcation point $P1 \xleftarrow{E1} S0 \xrightarrow{E2} P2$. (*A*) The underlying reaction network with an indicated choice of rate constants. (*Solid arrows*) True reactions. (*Dashed arrows*) Reactions containing the 0 complex. (*B*) The SR graph of the network. (*Dashed edges*) The c-pairs. The complex label of each edge appears above the edge. The stoichiometric coefficient of each edge appears below the edge.

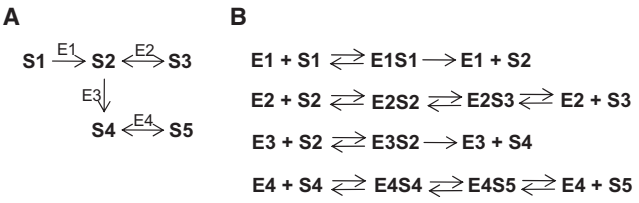

FIGURE 2 Branched Michaelis-Menten chains. (*A*) An example Michaelis-Menten network diagram. $S1,\ldots,S5$ denote substrates, and $E1,\ldots,E4$ denote simple Michaelis-Menten enzymes. Directed edges of the form $S \xrightarrow{E} S'$ indicate that the Michaelis-Menten enzyme E catalyzes the conversion of substrate S to substrate S'. Undirected edges of the form $S \xleftrightarrow{E} S'$ indicate that the Michaelis-Menten enzyme E catalyzes the conversion of S to S', and also the reverse conversion of S' to S. Here, and in the general case (see Section S3 in the Supporting Material), isolated substrates, pairs of edges carrying the same enzyme label, and edges that join a substrate to itself, are precluded. A Michaelis-Menten network qualifies as a branched Michaelis-Menten chain whenever it has no cycles, as in the present example. (*B*) The mass-action reaction network corresponding to panel *A*. All reactions of the form $Si \rightarrow 0$ ($i = 1,\ldots,5$) are assumed present but not shown. Reactions of the form $0 \rightarrow S$ can also be present. All mass-action networks corresponding to branched Michaelis-Menten chains are injective (see Section S3 in the Supporting Material).

in both the reactant and the product complex then two edges will connect the species and the reaction nodes, with one edge labeled by the reactant complex and the other edge labeled by the product complex.)

A pair of edges that meet in a reaction node is called a *complex-pair (c-pair)* if the two edges carry the same complex label. Two cycles in the SR graph are said to *split a c-pair* if each of the cycles contains at least one edge of the c-pair and one of the cycles contains just one edge of the c-pair. A cycle in the SR graph is called an *odd-cycle* if it contains an odd number of c-pairs; otherwise, the cycle is called an *even-cycle*. The *stoichiometric coefficient of an edge* is the coefficient of the species adjacent to the edge in the complex label of the edge. A cycle in the SR graph is called a *stoichiometric-cycle (s-cycle)* if we obtain the result of one by alternately multiplying and dividing the stoichiometric coefficients of the edges as we traverse the cycle.

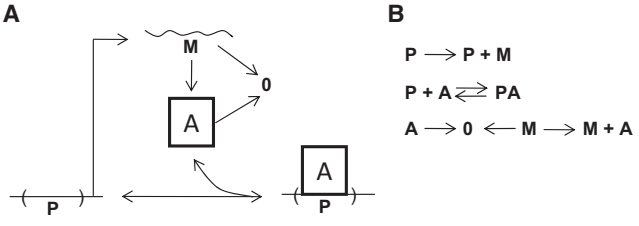

FIGURE 3 A model of a gene autoinhibitory module (10). (*A*) Schematic diagram of the model. Unbound promoter *P* is transcribed to messenger RNA *M*, which is subsequently translated to protein *A*. Protein *A* inhibits its own production by binding *P*, thereby inhibiting the expression of *M*. Both *M* and *A* are degraded by subsequent biological processes. (*B*) The mass-action network corresponding to panel *A*. This network is injective (see Section S7 in the Supporting Material).

We are now ready to state the graph-theoretic criterion for injectivity in fully open networks from Craciun and Feinberg (15,17):

A fully open network is injective if all cycles in its SR graph are either odd-cycles or s-cycles, and no two even-cycles split a c-pair.

Consider the SR graph in Fig. 1 *B*, which corresponds to the network of Fig. 1 *A*. The stoichiometric coefficients of all of the edges in the SR graph are equal to 1. Therefore, both cycles in the SR graph are s-cycles. Each of the two cycles in the SR graph is even, because neither cycle contains a c-pair. The two cycles do not share any edge, and as a result the two cycles cannot (and do not) split a c-pair. From the graph theoretic criterion for injectivity, we therefore have that the network of Fig. 1 *A* is injective.

To highlight yet again the subtle nature of injectivity, consider the networks displayed as Entries 5 and 6 in Table 1. Both networks describe classic enzyme catalysis mechanisms whereby substrates $S1$ and $S2$ are joined by enzyme E to form a product P. This is often symbolized by

$$S1 + S2 \xrightarrow{E} P.$$

The networks differ only in the substrate-binding mechanism: Entry 5 describes a sequential binding mechanism, whereas Entry 6 describes a random binding mechanism. Yet this subtle difference is sufficient to make the sequential binding mechanism of Entry 5 injective (9) and the random binding mechanism of Entry 6 noninjective (9).

A note on the summation theorem of metabolic control analysis

To put the main result of this article in perspective, we briefly discuss here the *summation theorem* (22) of metabolic control analysis, restricted to the case of mass-action networks.

Consider a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ and suppose that (c^*, k^*) is a positive network steady state. Suppose also that there exists a unique function $\hat{c}(\cdot)$ that maps each assignment of rate constants in a neighborhood of k^* to a steady-state composition that is stoichiometrically compatible with c^*. Moreover, assume that $c^* = \hat{c}(k^*)$. (We provide a sufficient condition for the existence of such a function $\hat{c}(\cdot)$ in Section S4 in the Supporting Material.) Then the steady-state flux through each reaction $y \rightarrow y'$ of the (mass-action) network is given by the function

$$J_{y\rightarrow y'}(\,\cdot\,) = k_{y\rightarrow y'}\left[\hat{c}(\,\cdot\,)\right]^{y},$$

with $J_{y\rightarrow y'}(k^*) \equiv J^* = k_{y\rightarrow y'}(c^*)^{y}$.

With the steady-state flux functions $J_{y\rightarrow y'}(\cdot)$ at our disposal, the flux sensitivity matrix is well defined, and its elements, the flux control coefficients evaluated at k^*, are given for each ordered pair of reactions $\hat{y} \rightarrow \hat{y}'$ and $y \rightarrow y'$ by

$$C^{\hat{y}\to\hat{y}'}_{y\to y'}(k^*) = \frac{k^*_{y\to y'}}{J^*_{\hat{y}\to\hat{y}'}}\left(\frac{\partial J_{\hat{y}\to\hat{y}'}}{\partial k_{y\to y'}}\right)_{k^*}. \quad (8)$$

For the case of mass-action networks, the following result obtains, regardless of network structure, as a corollary of the summation theorem of metabolic control analysis:

At each positive network steady state (c^*, k^*) and for each reaction $\hat{y}\to\hat{y}'$ of the network,

$$\sum_{y\to y'\in\mathcal{R}} C^{\hat{y}\to\hat{y}'}_{y\to y'}(k^*) = 1. \quad (9)$$

(A simple proof based on Euler's homogeneous function theorem is given in Section S9 in the Supporting Material. A proof of the general case of the summation theorem can be found in Heinrich and Schuster (22) and Gunawardena (23).)

We observe that if the flux control coefficients are all known to be nonnegative, as is the case in certain important models such as linear Michaelis-Menten chains (3–5), then Eq. 9 guarantees that each flux control coefficient is between 0 and 1. However, for an arbitrary network there is no assurance that all of the flux control coefficients are indeed nonnegative: if at least one of the flux control coefficients is negative, then Eq. 9 no longer implies that the absolute value of each flux control coefficient is bounded by 1.

As an example, consider the metabolic bifurcation point of Fig. 1 *A*, taken with rate constants k^* as indicated in Fig. 1 *A*, and with the positive composition c^* in which each species concentration is equal to 1. It is not difficult to verify that (c^*, k^*) is a network steady state. Using the computational means provided in Section S8 in the Supporting Material, we find that some of the flux control coefficients take extraordinarily large values. For example,

$$C^{E2S0\to E2+P2}_{E1S0\to E1+P1}(k^*) \approx -171.62. \quad (10)$$

The example of Fig. 1 *A* proves that there is no mathematical guarantee that the flux control coefficients in an arbitrary mass-action network are small in absolute value.

MATERIALS AND METHODS

All numerical calculations of flux control coefficients were performed according to the computational method of Section S8 in the Supporting Material using a standard computer algebra software package (Mathematica 6.0; Wolfram Research, Champaign, IL).

RESULTS

We begin by noting that, for any injective network, the steady-state flux mappings $J_{y\to y'}(\cdot)$ described in the previous section will be well defined at all positive network steady states (see Section S7 in the Supporting Material). Moreover, for each positive-network steady state of an injective network, the flux control coefficients given by Eq. 8 are also well defined. With this in mind, we present the main result of this article, whose proof is given in Section S7 in the Supporting Material.

Reciprocity Theorem. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be an injective mass-action reaction network. At each positive network steady-state (c^*, k^*), the following relations obtain:

1. For each reaction $y \to y'$ in the network,
$$0 \le C^{y\to y'}_{y\to y'}(k^*) \le 1.$$
2. For each pair of distinct reactions $\hat{y}\to\hat{y}'$ and $y \to y'$ in the network,
$$-1 \le C^{\hat{y}\to\hat{y}'}_{y\to y'}(k^*) \le 1$$
or
$$-1 \le C^{y\to y'}_{\hat{y}\to\hat{y}'}(k^*) \le 1.$$

Stated informally, the theorem concludes that for any positive network steady-state of any injective mass-action network, all of the diagonal elements of the flux sensitivity matrix are nonnegative and bounded from above by 1, and for each pair of mutually reciprocal, nondiagonal elements of the flux sensitivity matrix, at most one can exceed 1 in absolute value. Thus, a "large" flux control coefficient in such a "reciprocal" pair will invariably imply that the other flux control coefficient in the pair is "small."

We note that the theorem's injectivity requirement is a condition imposed on network structure alone. Therefore, the conclusion of the theorem will hold for each positive steady state that an injective network might admit.

We observe that if the injectivity condition is not satisfied, then neither of the two conclusions of the theorem need obtain. Consider the noninjective, random-binding mechanism in Entry 6 of Table 1, taken with rate constants k^* as indicated in Fig. 4, and with the positive composition c^* in which each species concentration is equal to 1. It is not difficult to verify that (c^*, k^*) is a network steady state.

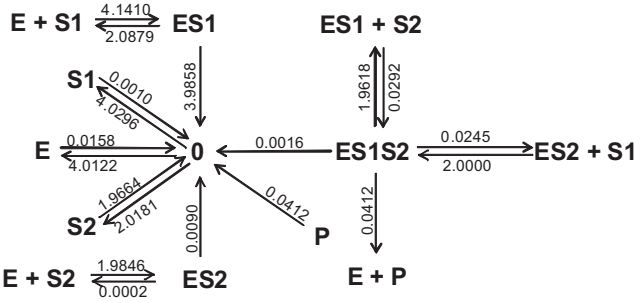

FIGURE 4 A fully articulated mass-action system corresponding to the random binding mechanism in Entry 6 of Table 1. Rate constant values are indicated next to the reaction arrows to which they correspond.

Here, the flux control coefficients are well defined (see Section S5 in the Supporting Material). Using the computational method presented in Section S8 in the Supporting Material, we can calculate a diagonal flux control coefficient that lies outside the range 0 to 1:

$$C^{ES1S2\to E+P}_{ES1S2\to E+P}(k^*)\approx 524.7. \tag{11}$$

We can also calculate a "reciprocal" pair of flux control coefficients for which both coefficients lie outside the range −1 to 1:

$$\begin{aligned} C^{ES1S2\to E+P}_{E+S1\to ES1}(k^*)&\approx -134.9,\\ C^{E+S1\to ES1}_{ES1S2\to E+P}(k^*)&\approx -256.2. \end{aligned} \tag{12}$$

This noninjective counter-example and the simple noninjective counter-example of Eq. 20 below (analyzed fully in Section S8 in the Supporting Material) indicate the importance of the injectivity requirement in the theorem statement.

Finally, we note again that even in the case of injective networks, flux control coefficients can be negative or larger than 1 in absolute value. This is evident from the injective network of Fig. 1 *A* and Eq. 10. Thus, the information provided by the theorem is not a result of some broader theorem that, for injective networks, forces all of the flux control coefficients corresponding to a positive steady state to be positive or small.

A connection between our results and metabolic control analysis

Here, we point out an intriguing connection between the first conclusion of the reciprocity theorem and a classic result of metabolic control analysis.

Consider our example network in Eq. 1, which happens to be injective, and focus on species *A*. For given molar concentrations c_A, c_B, the net formation rate of *A* is given by the coefficient of *A* on the right-hand side of Eq. 5:

$$v_A(c,k) = 2k_{B\to 2A}c_B - 2k_{2A\to B}(c_A)^2. \tag{13}$$

The right-hand side of Eq. 13 shows that the net rate of production of *A* is composed of a positive part and a negative part. Applying metabolic supply-demand analysis (24), we note that the positive part of v_A, defined by

$$v_A^{Supply}(c,k) = 2k_{B\to 2A}c_B, \tag{14}$$

indicates the effective rate in which the part of the network producing *A* supplies it to the rest of the network, whereas the negative part of v_A, defined by

$$v_A^{Demand}(c,k) = 2k_{2A\to B}(c_A)^2, \tag{15}$$

indicates the rate in which the part of the network consuming *A* demands it from the rest of the network.

We note that the total protein concentration $T = c_A + 2c_B$ is conserved over time. This and Eqs. 14 and 15 result in supply-and-demand rates for *A* expressed as functions of c_A, *T*, and *k*:

$$\begin{aligned} \overline{v}_A^{Supply}(c_A,T,k) &= k_{B\to 2A}(T-c_A),\\ \overline{v}_A^{Demand}(c_A,T,k) &= 2k_{2A\to B}(c_A)^2. \end{aligned} \tag{16}$$

With $\overline{v}_A^{Supply}$ and $\overline{v}_A^{Demand}$ at our disposal, *A*'s *supply elasticity* $\varepsilon_A^{Supply}(\cdot,\cdot,\cdot)$ and *demand elasticity* $\varepsilon_A^{Demand}(\cdot,\cdot,\cdot)$ are defined as the following fractional partial derivatives (24):

$$\begin{aligned} \varepsilon_A^{Supply}(c_A,T,k) &= \frac{c_A}{\overline{v}_A^{Supply}(c_A,T,k)}\frac{\partial}{\partial c_A}\overline{v}_A^{Supply}(c_A,T,k),\\ \varepsilon_A^{Demand}(c_A,T,k) &= \frac{c_A}{\overline{v}_A^{Demand}(c_A,T,k)}\frac{\partial}{\partial c_A}\overline{v}_A^{Demand}(c_A,T,k). \end{aligned} \tag{17}$$

From Eqs. 16 and 17, we obtain

$$\begin{aligned} \varepsilon_A^{Supply}(c_A,T,k) &= -\frac{c_A}{T-c_A},\\ \varepsilon_A^{Demand}(c_A,T,k) &= 2. \end{aligned} \tag{18}$$

Suppose that (c^*, k^*) is a positive steady state of the network. Then from the summation and connectivity theorems of metabolic control analysis (24,25) we have that

$$C^{B\to 2A}_{B\to 2A}(k^*) = \left(\frac{\varepsilon_A^{Demand}}{\varepsilon_A^{Demand}-\varepsilon_A^{Supply}}\right)_{(c_A^*,T,k^*)}. \tag{19}$$

In this case, we have from Eq. 18 that the supply elasticity of *A* is always negative, whereas the demand elasticity of *A* is always positive. Together with Eq. 19, this shows that $C^{B\to 2A}_{B\to 2A}(k^*)$ will always reside between 0 and 1.

Equation 19 is a special example of a more general result of metabolic supply-demand analysis (24). This result indicates that the sensitivity of the rate of supply of a given species with respect to an overall upshift in the species' supply curve will always equal the right-hand side of Eq. 19. It will typically be the case that for each positive composition of a network, even one that is large and complicated, the demand elasticity of a given species will always be positive and the supply elasticity will always be negative. Therefore, even in large and complicated networks, the right-hand side of Eq. 19 will typically give a value between 0 and 1.

Although this provides, in typical cases, an intuitive basis for the first conclusion of the reciprocity theorem, we have seen from Eq. 11 that in noninjective networks the first conclusion need not follow.

It is interesting to note that in some noninjective networks the first conclusion of the reciprocity theorem gets violated precisely when the supply elasticity ceases to be negative. Consider, for example, the toy mass-action network

$$\begin{aligned} 2A + B &\to 3A,\\ A &\to B. \end{aligned} \tag{20}$$

(Although unrealistic, it nevertheless illustrates well our mathematical point.) From Section S8 in the Supporting Material,

it follows that in the positive network steady-state $c^*_A = 1/2$, $c^*_B = 1$ (which gives rise to the conserved total concentration $T = c^*_A + c^*_B = 3/2$), $k^*_{2A+B\to 3A} = 2$, and $k^*_{B\to A} = 1$, the diagonal flux control coefficients are outside the range 0 to 1. More generally, it is not difficult to see from Eq. S78 in Section 8 in the Supporting Material that both of the diagonal flux control coefficients violate the first conclusion of the reciprocity theorem precisely for network steady states in which

$$0 \le c_A < 2T/3. \tag{21}$$

By essentially repeating the supply-demand analysis provided in the case of the network in Eq. 1 for the case of the network in Eq. 20, we obtain that

$$\begin{aligned} \varepsilon_A^{Supply}(c_A, T, k) &= 2 - \frac{c_A}{T - c_A}, \\ \varepsilon_A^{Demand}(c_A, T, k) &= 1, \end{aligned} \tag{22}$$

and from the top expression in Eq. 22, we have that whenever Eq. 21 obtains, the supply elasticity is positive, and not negative as intuitively expected. Thus, both of the diagonal flux control coefficients violate the first conclusion of the reciprocity theorem precisely when the supply elasticity of A is positive.

DISCUSSION

We emphasized both in the Introduction and in the Results that the bounds given by the theorem will obtain for any positive steady state of any mass-action network that possesses injectivity. This means that within the class of injective mass-action networks, some degree of robustness will exist in the positive-steady-state fluxes, and such robustness will obtain regardless of the (injective) network's size and complexity, and regardless of the particular positive steady state under consideration. Thus, within the scope of the injective class, some robustness in the positive-steady-state fluxes will result as a nonadaptive side effect even when natural selection is at work on another system property.

Of course, not all biologically relevant properties can be implemented using injective networks—a case in point being switching of the kind that requires two distinct stoichiometrically compatible steady states (9). But other functions, such as the catalysis process

$$S1 + S2 \xrightarrow{E} P$$

of Table 1, can be implemented by either injective (Entry 5) or noninjective (Entry 6) networks. In such cases, the injective solution might enable evolution to adapt, say, the production rate of P, while obtaining some robustness solely as a salutary side effect of the injective network structure.

It is interesting to consider the potential implications of this work in light of both the *adaptive* and the *intrinsic* views on the evolution of robustness (26). The adaptive view, which goes back to Fisher (27,28) (in the context of the evolution of dominance), and Schmalhausen (29), Waddington (30), and Rendel (31) (in the context of canalization), holds that robustness evolves because it increases fitness. (The adaptive view of robustness has gained support from recent work that takes into account the molecular details of certain robust biochemical networks. See, for example, von Dassow et al. (32), Barkai and Leibler (33), Eldar et al. (34), and Alon (35).) The intrinsic view, which goes back to Wright (36,37) and Kacser and Burns (38) (also in the context of the evolution of dominance), holds that robustness is a passive consequence of enzyme biochemistry and the structure of metabolic networks.

Here we showed that within the injective class of mass-action networks, some robustness in the positive-steady-state fluxes will obtain solely as a passive consequence of injectivity, echoing the intrinsic view. Yet even within the injective class, some positive network steady states (as in the network of Fig. 1 *A*) will correspond to extraordinarily large flux control coefficients. Thus, in line with the adaptive view, robustness (in the sense that all flux control coefficients be small) might require that network structure and rate constant values evolve, rather than emerge as a passive consequence of the underlying chemistry.

The "middle-ground" that our analysis strikes between the intrinsic and adaptive views is somewhat related to the conclusions drawn by Cornish-Bowden (39) from the analysis of a simple chain of metabolic reactions. Cornish-Bowden has demonstrated that, in the context of chains of enzyme-catalyzed reactions, network structure alone will not make all of the (Michaelis-Menten) flux control coefficients much less than one. This, in fact, will only occur for certain choices of parameter values. The study presented here shows that in the context of injective networks, of which the mass-action representations of enzyme chains are (simple) members, network structure does somewhat constrain the majority of flux control coefficients. Yet these constraints, by themselves, will not guarantee that all flux control coefficients be small.

We note that, in the context of injective mass-action networks, the theorem highlights the existence of a surprising correlation between "reciprocal" pairs of flux control coefficients at whatever positive steady states might exist. This correlation will persist for all pairs of distinct reactions, regardless of the size and the complexity of the network. That such a connection might exist, even between reactions that have no common species, and that might in some sense be very "distant" from each other, is far from intuitive. Further research might circumscribe other, not necessarily injective, classes of networks in which similar, and perhaps stronger, correlations obtain.

We observe that this work might be related to classic results in metabolic supply-demand analysis (24). Although we provided a simple example of a noninjective network in which the first conclusion of the theorem breaks down precisely when the supply elasticity ceases to be negative, it might be the case that a similar breakdown mechanism might also operate in more general cases. In fact, it is not impossible that injectivity acts to constrain the range of values that the

supply or demand elasticities might assume. We hope that similar connections between the second, less intuitive conclusion of the reciprocity theorem and concepts originating in metabolic control analysis could be found in the future.

Finally, we note that the constraints on flux control coefficients discovered in this article apply only at steady state. Whether similar constraints apply also outside steady state is an interesting topic for future research. We also note that the current investigation on the sensitivity of fluxes with respect to rate constant values is, at present, separate from an earlier investigation of the sensitivity of steady-state concentrations with respect to total building-block concentrations (7). Future work might help bring together these two lines of research.

SUPPORTING MATERIAL

Additional text is available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)00186-X.

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