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Design principles for robust biochemical reaction networks: What works, what cannot work, and what might almost work

Guy Shinar[1]
Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100

Martin Feinberg[2]
The William G. Lowrie Department of Chemical & Biomolecular Engineering and Department of Mathematics, Ohio State University, 140 W. 19th Avenue, Columbus, OH, USA 43210

Abstract

We bring together recent results that connect the structure of a mass-action reaction network to its capacity for concentration robustness — that is, its capacity to keep the concentration of a critical bio-active species within narrow limits, even against large fluctuations in the overall supply of the network's constituents.

Keywords

network; reaction network; robust; systems biology; design principles code (2000)

1. Introduction

Case-by-case experiments and mathematical models are the primary means by which we try to understand structure-function relations in biochemical reaction networks. Experience with even a large number of cases, however, can provide only limited *general* understanding of structure-function relations in the great variety of reaction networks that play their individual roles in the life of a cell. It is natural, then, to seek an overarching theory that could, for a specified function, delineate precisely the class of networks capable of implementing it.

Often, theory at our disposal will not be suffciently well-developed as to identify the full class of networks that might operate in a specified way. Rather, the theory might point to a narrower class containing some, but not all, of the network structures that can implement a particular function. In such instances the theory will furnish us with design principles relative to the particular function that are su cient, but not necessary, for implementation.

In other instances, theory might instead identify classes of networks that are *precluded* from implementing the desired function. Despite being “negative” in their nature, such findings can be no less important than their “positive” counterparts, especially when the function-

guy.shinar@weizmann.ac.il (Guy Shinar), feinberg.14@osu.edu (Martin Feinberg).

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precluded class is large and rich, for then we can begin to understand network properties that are inimical to the realization of various behaviors. Such understanding also contributes, in its own way, to our grasp of design principles. Indeed, in some contexts the *impossibility* of a certain behavior might in itself be a desirable and even crucial aspect of network function.

Our present interest is in design principles that a biochemical reaction network might employ if it is to promote *robust* operation. Loosely speaking, a network is said to behave robustly if it maintains its function despite environmental or structural disruptions [1]–[20]. Robustness properties appear across many scales of biological organization, from simple biochemical circuits (robust exact adaptation in bacterial chemotaxis and input-output robustness [2, 3, 16]) through large metabolic networks (robustness of metabolic function to structural changes caused by mutations [11]), all the way to embryo development (robustness of morphogen gradients to network protein levels [8, 9, 12, 21].)

In this article we are chiefly concerned with what we call *absolute concentration robustness* (ACR) [20]. A model biochemical system has ACR relative to a particular bio-active molecular species if the system admits at least one positive steady state[3] and if the concentration of that species is the same in all of the positive steady states that the system might admit, regardless of the overall supplies of the various network constituents. In this way, the function of an ACR possessing system can remain protected against fluctuations in the total concentrations of its component proteins: While such fluctuations will generally take the system from one steady-state to another, the steady-state concentration of the crucial species will nevertheless remain unchanged, because it is invariant over the entire set of positive steady states.

Of course we should not expect real biochemical systems to exhibit absolute concentration robustness. We shall, however, discuss examples of real systems that exhibit quite strong, but imperfect, concentration robustness [10, 22]. Accurate, highly-detailed models of such systems should then reflect the same strong, but imperfect, robustness observed in the laboratory. But then we might wonder what it is in the architecture of these models that results in the robustness they exhibit. At the core of such a model network might lie a subnetwork which, taken by itself, *does* give rise to ACR and which, to the extent that it approximates the more completely articulated parent network, can impart to the fuller model imperfect but nevertheless strong robustness. Thus, by uncovering network design principles that foster *absolute* concentration robustness, we would have means to understand structural sources of strong concentration robustness observed experimentally [20].

Our principal aim here is to bring together, in a single article, some recent but separate results that bear on the capacity of a mass-action reaction network to exhibit ACR. Taken together, these results help to paint a quite large (but still incomplete) picture of the relationship between network structure and the extent to which ACR might be realized. We shall see that there are indeed network structures that can realize the ACR ideal [20]. We shall also see that there is a large and well-defined class of networks for which ACR cannot be realized [18]. In fact, within this last class, there are precise limits, derived from reaction network structure, on the extent to which ACR can be approached [18]. Moreover, these limits are striking from a biological standpoint: They involve the extent to which the network's constituent molecules can be regarded to be built from elemental units that appear within the various species multiplicitously and that combine with each other gregariously [18].

[3]A positive steady state is one in which all species concentrations are positive.

The narrative will be informal, with reliance on other literature for more formal definitions and proofs. The emphasis will be on mass-action networks. This emphasis derives from two considerations: First, many complex enzyme-catalysis mechanisms, as well as the metabolic networks to which they give rise, can be unraveled to the mass-action level. This has the advantage that no additional approximations need be made, approximations that often require justification on a case-by-case basis. Second, the mass-action formalism, which is tied tightly to the underlying reaction network structure, makes available mathematical results originating in chemical reaction network theory (CRNT), a mathematical theory that connects network attributes with dynamic behavior [23]–[26]. (Of course, mass action models have their limitations, especially when the number of copies of the various species is small.)

The principal results we state are organized around the CRNT concept of a network's deficiency. The *deficiency* of a reaction network is an integer index that assumes non-negative values. Loosely speaking, the deficiency measures the amount of “linear independence” among the reactions of the network. The lowest value that the deficiency can assume, which is zero, is associated with the highest possible extent of linear independence among the individual reactions, consistent with the network's structure as a directed graph. The higher the deficiency, the lower the extent of linear independence.

The remainder of this article is organized as follows: In Section 2 we consider “toy” mass-action networks, with the intent of making the notion of ACR more concrete. In Section 3 we review two ACR-possessing mass-action models of real biochemical circuits [17, 19]. In the experimental systems corresponding to these models — the EnvZ-OmpR osmoregulation system of *Escherischia coli* and the IDHKP-IDH glyoxylate bypass regulation system of *E. coli* — laboratory evidence shows strong (although imperfect) concentration robustness [10, 22]. In Section 4 we provide some vocabulary that is necessary for stating our main results. In Section 5 we describe a large class of networks that cannot realize ACR. More specifically, we show that ACR is *impossible* in any conservative mass-action network of deficiency zero, no matter how large and complicated it might be. This will serve as a backdrop for Section 6, in which we state a design principle for ACR, one that ensures ACR and that exerts itself in all of the examples considered in Section 3. It is noteworthy that these examples all have a deficiency of one, and, indeed, the design principle that underlies them requires a deficiency of one as a precondition. (There is more that is required of these networks beyond a deficiency of one.) In Section 7 we explore the question of whether ACR-resembling behavior can occur, albeit approximately, in deficiency zero networks and in deficiency one networks that fail to satisfy the other conditions of Section 6. In Section 8 we summarize our results and also indicate some of their limitations. Finally, in Section 9, we o er a concluding remark.

2. Absolute concentration robustness: “Toy” examples

Consider the “toy” mass-action system

$$\begin{aligned} A+B &\xrightarrow{\alpha} 2B \\ B &\xrightarrow{\beta} A. \end{aligned} \tag{1}$$

A denotes the active form of some protein, and *B* denotes the inactive form. The positive numbers α and β are rate constants. We assume that *A* and *B* are synthesized and degraded over timescales that are much longer than the equilibration timescale of the system. Consequently, the total concentration of protein in the system can be regarded as constant over the equilibration timescale.

The mass-action differential equations describing the evolution of the molar concentrations of A and B, denoted c_A and c_B, are

$$\begin{aligned}\dot{c}_A &= -\alpha c_A c_B + \beta c_B \\ \dot{c}_B &= \alpha c_A c_B - \beta c_B.\end{aligned} \tag{2}$$

The positive steady states of equations (2) are given by

$$\begin{aligned}c_A &= \frac{\beta}{\alpha} \\ c_B &= T - \frac{\beta}{\alpha},\end{aligned} \tag{3}$$

where

$$T = c_A(t) + c_B(t) = c_A(0) + c_B(0) \tag{4}$$

is the conserved total protein concentration.

Equation (3) shows that system (1) has ACR: All of the positive steady states of the system have exactly the same value for c_A, regardless of the total protein concentration T. Thus, if synthesis or degradation processes vary T over timescales much longer than the equilibration timescale of the system, the output of the system, determined by c_A, remains essentially unchanged. The system is robust to (suffciently slow) variations in T.

In contrast, consider the mass-action system

$$A \underset{\alpha}{\overset{\beta}{\rightleftarrows}} B. \tag{5}$$

Suppose, as before, that A is the active form of a protein and that B is the inactive form. Here, the positive steady states are given by

$$\begin{aligned}c_A &= \frac{\beta}{\alpha+\beta} T \\ c_B &= \frac{\alpha}{\alpha+\beta} T,\end{aligned} \tag{6}$$

where T, the total protein concentration, is again described by eq (4). Equation (6) shows that the system fails to have ACR: As T varies, both c_A and c_B vary in step. The system is not robust to variations in T.

3. ACR-possessing models of real biochemical circuits

In this Section we survey two published mass-action models that possess ACR [17, 19, 20]. We also cite empirical evidence supporting the notion that the experimental systems corresponding to these models show strong, although inperfect, concentration robustness [10, 22].

3.1. The EnvZ-OmpR osmoregulation system

The *E. coli* EnvZ-OmpR osmoregulation system consists of the sensor kinase EnvZ, denoted X, and the response-regulator OmpR, denoted Y. Both the sensor and the response-regulator

have phosphorylated forms, denoted X_p and Y_p. X phosphorylates itself by binding and breaking down adenosine triphosphate (ATP) [27, 28], X_p catalyzes the transfer of a phosphoryl group to free Y [27, 28], and X, together with ATP or adenosine diphosphate (ADP) as a cofactor, dephosphorylates Y_p [29]–[31]. The crucial chemical species in the system is Y_p, a transcription factor that regulates the expression of various protein pores, including OmpC and OmpF [27, 28]. Note that the basic architecture of the EnvZ-OmpR system is shared by hundreds of so-called two-component signal transduction systems in bacteria [27, 28].

A mass-action model in which Y_p dephosphorylation requires ATP alone is displayed in (7) [17]. (XT denotes the X-ATP compound.) A similar model, in which Y_p dephosphorylation requires only ADP is displayed in (8) [20]. (XD denotes the X-ADP compound.) In both models we assume that the ATP and ADP levels, denoted [T] and [D], are too high to be significantly affected by the action of the system, and are therefore treated as parameters.

$$\begin{aligned} X &\underset{k_{-1}}{\overset{k_1[T]}{\rightleftarrows}} XT \xrightarrow{k_2} X_p \\ X_p+Y &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} X_pY \xrightarrow{k_4} X+Y_p \\ XT+Y_p &\underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} XTY_p \xrightarrow{k_6} XT+Y \end{aligned} \tag{7}$$

$$\begin{aligned} XD &\underset{k_9[D]}{\overset{k_{-9}}{\rightleftarrows}} X \underset{k_{-1}}{\overset{k_1[T]}{\rightleftarrows}} XT \xrightarrow{k_2} X_p \\ X_p+Y &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} X_pY \xrightarrow{k_4} X+Y_p \\ XD+Y_p &\underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} XDY_p \xrightarrow{k_8} XD+Y \end{aligned} \tag{8}$$

Each of the mass-action models above gives rise to mass-action differential equations whose positive steady states can be obtained analytically [17, 20]. In fact, such ad-hoc calculations show that both models exhibit ACR in Y_p: In both cases the steady state concentration of Y_p is independent of the conserved total concentrations of the X and the Y proteins. (In (7), the conserved total concentration of the X protein is given by $c_X + c_{X_p} + c_{XT} + c_{X_pY} + c_{XTY_p}$, where c_X denotes the molar concentration of X, c_{X_p} denotes the molar concentration of X_p and so on; the conserved total concentration of the Y protein is given by $c_Y + c_{Y_p} + c_{X_pY} + c_{XTY_p}$.)

The *absolute* concentration robustness in models (7) and (8) is suggestive of the strong robustness empirically observed in the EnvZ-OmpR system. In vivo experiments by Batchelor and Goulian [10] examined how the ratio of expression of the protein membrane pores OmpC and OmpF varies as a function of the total concentration of the X and the Y proteins. As both OmpC and OmpF are regulated by Y_p, robustness in the OmpC to OmpF expression ratio is thought to be indicative of robustness in the concentration of Y_p. Indeed, the experiments show that the OmpC to OmpF expression ratio can remain quite flat over order of magnitude changes in the total X and the total Y concentrations, across a wide range of external conditions (different osmolarity levels), indicating strong concentration robustness in Y_p [10].

3.2. The IDHKP-IDH glyoxylate bypass regulation system

The *E. coli* IDHKP-IDH glyoxylate bypass regulation system controls the partitioning of carbon flux between the tricarboxylic acid (TCA) cycle and the glyoxylate bypass. Precise regulation of flux to the glyoxylate bypass is essential when the bacterium grows on substances such as acetate that contain only two carbon atoms. Without the glyoxylate bypass, both carbon atoms would be converted to CO_2 by the TCA cycle, thereby leaving no carbon available for biosynthesis of cell constituents. Hence, growth on acetate requires directing some of the carbon flux to the glyoxylate bypass, thereby avoiding carbon loss.

Glyoxylate bypass control is achieved by regulating the phosphorylation level of the TCA cycle enzyme isocitrate dehydrogenase (IDH), denoted I. The phosphorylated form of IDH is denoted I_p. Only the unphosphorylated form I is active, whereas the I_p form is virtually inactive. Thus, the level of I essentially determines how much of the carbon flux will flow through the TCA cycle, and how much will flow through the glyoxylate bypass [32]–[35].

The means by which the I protein is phosphorylated and dephosphorylated is the bi-functional enzyme isocitrate dehydrogenase kinase phosphatase (IDHKP) [36, 37], denoted E. At the heart of several putative mass-action models [19] of how the bifunctional enzyme E regulates the level of I lies the following "core" mass-action system:

$$\begin{aligned} EI_p + I &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EI_pI \xrightarrow{k_2} EI_p + I_p \\ E + I_p &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EI_p \xrightarrow{k_4} E + I. \end{aligned} \tag{9}$$

Ad hoc solution of the mass-action differential equations corresponding to (9) shows that the positive steady state concentration of (unphosphorylated) I is completely independent of the total concentrations of the I and the E proteins [19]. Thus, model (9) shows ACR in I.

The ACR indicated by the model is suggestive of the strong concentration robustness empirically observed by LaPorte, Thorsness, and Koshland [22]. It was found that during growth on acetate, the activity of unphosphorylated I (which is proportional to its concentration) is highly robust to changes in the total I concentration: 15-fold variation in the total I concentration resulted in less than 20% change in the active, unphosphorylated I level.

4. Reaction network structure: Ideas from chemical reaction network theory

In our discussion of design principles that foster concentration robustness, it will turn out that distinctions between one network and another can arise from very subtle architectural features. For this reason, we will need suitable ideas and vocabulary with which reaction network structure might be described. It is the purpose of this section to provide these in an informal way. More formal treatments are contained in [38] and [39]. For a more extensive informal discussion with more examples see [24]. Figure 1 will provide the basis for much of our discussion.

4.1. The complexes and linkage classes of a network

By the *complexes* of a reaction network we mean the objects that appear before and after the reaction arrows. Thus, for the network shown in Figure 1a, the complexes are $2A$, B, C, D, $B + C$, E, $2B$ and $A + F$. When we want to speak about a generic complex, we might refer to "the complex y" or "the complex y'". Similarly, we might write $y \to y'$ to indicate a generic

reaction whereby complex y reacts to complex y'; in this case y is the *reactant complex* of the reaction, and y' is its *product complex*.

Each network depicted in Figure 1 is displayed as a *standard reaction diagram*. In such a diagram, each distinct complex appears just once, with arrows indicating how the various complexes are related by reactions.

Note that the standard reaction diagram shown in Figure 1a is composed of two disconnected "pieces," each contained within a dashed blue rectangle. One contains the mutually linked complexes $\{2A, B, C, D\}$, while the other containing the mutually linked complexes $\{B + C, E, 2B, A + F\}$. (Here we are *not* concerned with the directions of the reaction arrows; we are focusing instead on whether certain complexes are linked by arrows, however indirectly.) Such sets of mutually linked complexes are called the *linkage classes* of the network. Later, we shall need to consider the number of linkage classes in a network; this is synonymous with the number of "pieces" of which its standard reaction diagram is composed. The slightly different networks depicted in Figures 1a and 1b both have two linkage classes, indicated by the dashed blue rectangles.

4.2. Strong linkage classes, terminal strong linkage classes, weak reversibility

In the preceding sub-section we were not concerned with the direction of the reaction arrows, but here we will be. Two complexes are *strongly linked* if there is a *directed* arrow path from one to the other, and also a *directed* arrow path from the second back to the first. In Figure 1a complexes $2A$ and D are strongly linked: There is a directed arrow path from $2A$ to D (via complex B) and also a directed arrow path from D to $2A$. Similarly, $B + C$ is strongly linked to E. We adopt the convention that every complex in a reaction network is strongly linked to itself.

A *strong-linkage* class of a reaction network is a maximal subset of its complexes that are strongly linked to each other. In Figure 1c we repeat the network shown in Figure 1a, this time with the strong-linkage classes indicated by dotted rectangles. In Figure 1d, which is slightly different from Figure 1b, the strong linkage classes are identical to the linkage classes. By a *terminal strong-linkage class* we mean a strong-linkage class in which no complex reacts to a complex in another strong-linkage class. (Terminal strong linkage classes are indicated in Figures 1c,d by red dotted rectangles.) In Figure 1c the terminal strong-linkage classes are $\{C\}$ and $\{2B, A + F\}$. In Figure 1d the only two strong-linkage classes are both terminal. A complex is *terminal* if it belongs to a terminal strong-linkage class; otherwise the complex is *non-terminal*. For example, in Figure 1c the non-terminal complexes are $2A$, B, D, $B + C$, and E. The nonterminal complexes in the figure are contained within green dotted rectangles. (See also Figure 2 in Section 6 for more illustrations, with particular reference to networks (7),(8) and (9).)

Chemists say that a reaction network is *reversible* if every reaction is accompanied by its reverse — that is, if the presence of reaction $y \to y'$ in the network implies the presence of $y' \to y$. None of the networks shown in Figure 1 is reversible. On the other hand, the network depicted in Figure 1d possesses a weaker kind of reversibility: We say that a reaction network is *weakly reversible* if, whenever there is a directed arrow path from a complex y to a complex y' there is also a directed arrow path from y' back to y. (Note that every reversible network is also weakly reversible, so any statement we make about weakly reversible networks applies to reversible networks in particular.) In a weakly reversible network, the linkage classes, strong linkage classes, and terminal strong linkage classes coincide.

4.3. The rank of a reaction network

So far, our consideration of reaction network structure has focused exclusively on the graphical properties of a network as manifested in its standard reaction diagram, with the complexes playing the role of vertices and reactions playing the role of directed edges. Here stoichiometry will begin to exert itself, as we begin to consider more algebraic aspects of reaction network structure. In particular, we will be concerned with the extent to which the reactions are "linearly independent."

We can make this precise in the following way: With each reaction in a network we associate a *reaction vector*, formed by subtracting the reactant complex from the product complex. Thus, the reaction $2A \rightarrow B$ gives rise to the reaction vector $B - 2A$. Similarly, $A + F \rightarrow 2B$ gives rise to $2B - A - F$. (We regard the reaction vectors so formed to reside in the vector space of formal real linear combinations of the network's species.)

By the *rank* of a reaction network we mean the number of elements in a maximal linearly independent set that can be formed from its reaction vectors. Thus, the rank of the network shown in Figure 1a is five: The five-member reaction vector set

$$\{B - 2A, D - B, E - B - C, 2B - E, A+F - 2B\}$$

is linearly independent, and any other reaction vector for the network can be written as a linear combination of these. (The rank of a reaction network is identical to the rank of what is sometimes called the "stoichiometric matrix" [40].)

Note that the network shown in Figure 1b differs only slightly from that shown in Figure 1a; the complex $B + G$ has replaced the complex $B + C$. It is easy to see that the rank of network 1b is six, as is the rank of its weakly reversible variant, 1d. (In general, changes of the arrow structure within a linkage class will not affect the rank.)

4.4. The deficiency of a reaction network

The deficiency of a reaction network is a non-negative integer index with which reaction networks can be classified; it is defined as follows:

$$deficiency = \#(complexes) - \#(linkage\ classes) - rank. \tag{10}$$

For the network displayed in Figure 1a, there are eight complexes, two linkage classes, and its rank is five, so the deficiency of the network is *one*. Networks 1b and 1d of the figure both have deficiencies of *zero*; in each case there are eight complexes, two linkage classes, and the rank is six.

For deficiency-zero and deficiency-one mass-action networks there are well-developed theories [24, 25, 38, 39, 41] of the extent to which these can give rise to various categories of dynamical phenomena (e.g., multiple steady states, periodic composition trajectories), but here our focus will be only on robustness issues.

4.5. Conservative networks

We normally expect a reaction network to respect conservation of mass. We can make this precise in the following way: Let $\mathcal{S}$ denote the set of species in a network, and let $\{M_*^S\}_{s\in\mathcal{S}}$ denote their molecular weights. If $y \rightarrow y'$ is any reaction of the network, we expect that

$$\sum_{s\epsilon\mathcal{S}} M^s_* y_s = \sum_{s\epsilon\mathcal{S}} M^s_* y'_s, \tag{11}$$

where y_s is the stoichiometric coefficent of species s in the reactant complex y, and y'_s is its stoichimetric coefficient in the product complex y'. Thus, for a reaction such as $A + B \to C$, eq (11) asserts that $M^*_A + M^*_B = M^*_C$.

Following Horn and Jackson [42] we say that a reaction network is *conservative* if there is *some* set of positive numbers $\{M^s\}_{s\epsilon\mathcal{S}}$ such that, for every reaction $y \to y'$ in the network, we have

$$\sum_{s\epsilon\mathcal{S}} M^s y_s = \sum_{s\epsilon\mathcal{S}} M^s y'_s. \tag{12}$$

In particular, a network that respects mass conservation in the sense described above is conservative in the more general Horn and Jackson sense. All reaction network examples considered in this article are conservative.

Remark 4.1. Software tools for determining various aspects of network structure automatically (e.g., rank, deficiency, terminal strong linkage classes) — and much more in the way of reaction network behavior — are readily available. See [43] and [44].

5. Absolute concentration robustness: Designs that cannot work

5.1. Another motivating example

When we speak of a *mass-action system* we mean a reaction network of the kind shown in Figure 1, taken together with an assignment of a positive rate constant to each reaction. Each mass-action system gives rise, in a natural way, to a system of first order polynomial differential equations for the species concentrations. A simple example was given in the passage from the mass-action system shown in eq (1) to the differential equations shown in eqs (2), but usually the result is far more complicated.[4] The differential equations so-generated are deemed to describe the evolution of a perfectly stirred solution held in a closed vessel and maintained at a fixed temperature.

In this closed-vessel context, it will be helpful to return to an example used once before in [18]. Network (13) is motivated by a regulated recruitment picture [46] of a gene transcription control mechanism. For our purposes, it will suffice to think of *A*, *B* and *C* as three distinct biomolecules. *A* can dimerize to form A_2, and *B* can bind to the dimer to form A_2B, which can then bind to *C* to form the critical bio-active compound A_2BC. We regard all reactions to be reversible, and we assume that the kinetics is mass-action.

$$2A \rightleftarrows A_2, \quad A_2 + B \rightleftarrows A_2B, \quad A_2B + C \rightleftarrows A_2BC \tag{13}$$

Note that the monomer A appears not only explicitly as a network species but also latently in the network species A_2, A_2B, and A_2BC. Note also that the reactions in network (13) conserve the number of units of *A* monomer. Although the molar concentrations of the four *A*-containing species might each vary in time in accordance with the governing mass-action

[4] Formation of the governing differential equations for a mass-action system is discussed, for example, in [24] and [45].

differential equations, we would nevertheless expect them to conform to a conservation condition: At each instant t,

$$c_A(t) + 2c_{A_2}(t) + 2c_{A_2B}(t) + 2c_{A_2BC}(t) = T_A, \tag{14}$$

where T_A is the time-invariant *total* molar concentration of *A*-monomer in the vessel, regardless of whether *A* is free or bound to other units. Similarly, we would expect the species concentrations to respect conservation of the *B* and *C* units: At each instant t,

$$c_{A_2B}(t) + c_B(t) + c_{A_2BC}(t) = T_B, \tag{15}$$

$$c_C(t) + c_{A_2BC}(t) = T_C, \tag{16}$$

where T_B and T_C are the total molar concentrations of *B* and *C* units within the vessel.

It is a property of the mass-action equations for network (13) that, for specified positive values of the overall monomer concentrations — say T_A^*, T_B^*, and T_C^* — there is precisely one positive steady-state consistent with those values. That is, there is precisely one set of positive species concentrations, c_A^*, c_B^*, c_C^*, $c_{A_2}^*$, $c_{A_2B}^*$, $c_{A_2BC}^*$, that constitutes a steady state for the mass-action differential equations and that also satisfies the equations

$$\begin{aligned} c_A^* + 2c_{A_2}^* + 2c_{A_2B}^* + 2c_{A_2BC}^* &= T_A^*, \\ c_{A_2B}^* + c_B^* + c_{A_2BC}^* &= T_B^*, \\ c_C^* + c_{A_2BC}^* &= T_C^*. \end{aligned} \tag{17}$$

Now suppose that we temporarily open our vessel and add some more of the various species appearing in the network. (How much of each we add does not matter, so long as we add some of at least one of them.) We then close the vessel and stir again. In this case, there will be a different set of overall monomer concentrations, $\{T_A^{**}, T_B^{**}, T_C^{**}\}$, and again there will be precisely one positive steady state, $\{c_A^{**}, c_B^{**}, c_C^{**}, c_{A_2}^{**}, c_{A_2B}^{**}, c_{A_2BC}^{**}\}$, of the mass-action differential equations consistent with the new monomer concentrations.

In fact, the change in overall monomer concentrations will require that at least one of the individual steady-state species concentrations be different from what it was before the addition. This is not to say that there must be a change in the concentration of *every* species, although that might be the case. Indeed, for a *particular* infusion of new material, it is conceivable that the steady-state concentration of a certain species might be greater than, less than, or equal to what it was before the infusion. (Questions about the sign-response were studied in two Ph.D. theses [47, 48] supervised by Michael Reed.) Moreover, for a particular species the *magnitude* of the change might be great or small relative to the magnitudes of the changes in T_A, T_B, and T_C. That is, the steady-state concentration of the individual species might be *highly sensitive* or *highly insensitive* to changes in T_A, T_B, or T_C.

At this point, it will be useful to step away from our closed-vessel picture for a moment to consider its relevance to cell biology. Although the cell is highly dynamic, we can suppose that certain biochemical modules (reaction networks) operate quickly relative to other cellular processes (e.g., relative to the synthesis of proteins or to the transport of smaller

molecules across cell membranes). Thus, the concentrations of species participating in such a “fast” network might be regarded as instantaneously satisfying the corresponding steady-state equations for the network, relative to the *current* supply of the network's constituents (e.g., the supply of monomers in our example). Nevertheless, those supplies might shift on a longer time scale, and we would expect a corresponding shift in the steady state realized by the fast network. In fact, the species concentrations might vary in response to perhaps wild and unpredictable changes in supplies of the network's constituents.

If it is important that the steady-state concentration of a critical network species remains within a narrow range, then one would want a low sensitivity of its concentration to variations in overall supplies of *all* the network's constituents. If, in our example, we are concerned with modulating the steady-state concentration of a particular species, say A_2BC, then we would want that concentration to be highly insensitive to values of T_A, T_B, and T_C, at least over wide ranges of physiological interest. Were this the case, we would say that the mass-action system exhibits *concentration robustness* relative to A_2BC. With this in mind, we return once again to the ideal of absolute concentration robustness.

Recall that a mass-action system exhibits absolute concentration robustness relative to species s if the corresponding differential equations admit at least one positive steady state and, in all positive steady states, the concentrations of s are identical. Recall also that there are indeed mass-action systems that do exhibit ACR. The toy network (1) provided one example, and the experimentally-motivated networks (7), (8) and (9) provided others. (In each case, ACR obtains relative to the critical species regardless of what positive numbers are assigned to the rate constants.) With these examples in mind, we can ask whether, for at least *some assigment* of rate constants, network (13) can exhibit ACR relative to the critical bio-active species A_2BC.

5.2. Network structures that thwart absolute concentration robustness

In fact, network (13) *cannot* exhibit absolute concentration robustness relative to *any* of its species, and this remains true no matter what values the rate constants take. In other words, it is the architecture of the network itself that thwarts absolute concentration robustness.

Indeed, network (13) resides in a very large and easily delineated class of networks which, when taken with mass-action kinetics, *cannot* realize ACR. The following theorem is a special case of a broader result proved in [18].

Theorem 5.1. Consider a mass-action system in which the underlying reaction network is conservative and has a deficiency of zero. Then, no matter what values the rate constants take, there is no species relative to which the system exhibits absolute concentration robustness.

Remark 5.2. Before returning to network (13) and other examples, we should say a little about the status of deficiency zero mass-action systems within the broader class examined in [18]: If the underlying deficiency-zero network is *not* weakly reversible, then the system admits no positive steady states at all [38]. If the underlying network is weakly reversible (and, in particular, reversible) then, regardless of rate constant values, it follows [38] that the system will have what Horn and Jackson [42] called the *quasithermostatic* property, which is to say that the set of positive steady states will have a very special structure. It is the quasithermostatic property that, in [18], precludes ACR for conservative networks. The weakly reversible deficiency-zero mass-action systems are only a subset of the larger set of mass-action systems for which the quasithermostatic property obtains.

Recall that the conservative networks shown in Figures 1b and 1d both have deficiencies of zero. Taken with mass-action kinetics, then, neither can engender ACR. In fact, network 1b is not weakly reversible and so cannot give rise to any positive steady states. (In this case it is easy to see that any steady state must be devoid of species G.) The situation for the weakly reversible network 1d is different. For a given assignment of rate constants, there will be a wealth of positive steady steady states. The resulting differential equations are quite complicated, but the theorem tells us that, regardless of rate constant values, for each choice of species there will be at least two positive steady states in which the concentration of that species differs.

Network (13) is conservative and its deficiency is also zero [#(complexes) = 6, #(linkage classes) = 3, rank = 3]. Thus, a mass-action system derived from (13) could not exhibit ACR relative to the bio-active species A_2BC — or, for that matter, relative to any other network species — no matter what values the rate constants take. In this case, the network is reversible, and therefore weakly reversible. There will be a wealth of positive steady states, corresponding to different combinations of T_A, T_B, and T_C. Theorem 5.1 tells us that, as we scan over the set of positive steady states, there are at least two in which the concentration of A_2BC differs.

5.3. Network structures that preclude even an arbitrarily close approach to absolute concentration robustness

Although Theorem 5.1 tells us that network (13) cannot, taken with mass-action kinetics, exhibit *absolute* concentration robustness, no matter what the rate constants are, we might nevertheless wonder whether the network structure permits an *arbitrarily close* approach to perfect robustness relative to A_2BC.

The answer is *no*, and a similar result obtains for all mass-action systems derived from conservative weakly reversible networks of deficiency zero. (We are focusing on the weakly reversible ones, for only those can admit positive equilibria.) The fact is that, within this class, behavior is “bounded away” from absolute concentration robustness, in a sense we will try to make precise in the context of our example.

To begin, we will want to quantify the sensitivity of the steady-state concentration of A_2BC to the supply of the monomers A, B and C. It follows from [18] that there is a smooth function $\bar{c}_{A_2BC}(\cdot, \cdot, \cdot)$ such that, when T_A, T_B, and T_C are the current values of the monomer concentrations, the concentration of A_2BC in the corresponding (unique) positive steady state is given by $\bar{c}_{A_2BC}(\ln T_A, \ln T_B, \ln T_C)$. (It is useful to work with logarithms.) We shall be interested in the fractional change of values of $\bar{c}_{A_2BC}(\cdot, \cdot, \cdot)$ relative to fractional changes in values of the three overall monomer concentrations. In particular, if $T^* := \left[T_A^*, T_B^*, T_C^*\right]$ is a particular specification of the monomer supplies, then we shall be interested in values of the numbers

$$\left\{\left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_A}\right|, \left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_B}\right|, \left|\frac{\partial \ln \bar{c}_{A_2CB}}{\partial \ln T_C}\right|\right\}_{T^*}. \tag{18}$$

.

If it is important that the concentration of A_2BC remain within a narrow range, then we would want a low sensitivity of its concentration to variations in overall supplies of all the network's constituents. In particular we would want the number

$$\Lambda^{A_2BC}(T^*) := \max\left\{\left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_A}\right|, \left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_B}\right|, \left|\frac{\partial \ln \bar{c}_{A_2BC}}{\partial \ln T_C}\right|\right\}_{T^*} \tag{19}$$

to be small for all values of T^* that are of physiological interest. In the context of our example, we say that $\Lambda^{A_2BC}(T^*)$ is the *sensitivity of species* A_2BC at T^*. Thus, concentration robustness in A_2BC requires that the sensitivity of A_2BC be small at physiological values of T^*.

Absolute concentration robustness requires that $\Lambda^{A_2BC}(T^*)$ be *zero* at *all* positive values of T^*. Failing that, we might hope that, for rate constants extant in our mass-action system, $\Lambda^{A_2BC}(T^*)$ is very small, say less than 0.01, over a wide physiological range of T^* values.

In fact, this cannot be: It follows from theory in [18] that, for *all possible* combinations of rate constants and for *all possible* positive specifications of T^*,

$$\Lambda^{A_2BC}(T^*) := \max \geq \frac{1}{3}. \tag{20}$$

In this sense, mass-action systems derived from network (13) are “bounded away” from absolute concentration robustness. The bound in (20) derives from network structure alone, in an interesting way that we shall discuss more fully in Section 7. Not only does the network's architecture thwart ACR, it precludes an arbitrarily close approximation of it.

A similar situation obtains for all conservative weakly reversible networks of deficiency zero [18].

6. Absolute concentration robustness: Designs that work

Having considered network designs that thwart ACR, we turn now to a designs that ensure it. In fact, the seemingly different examples of ACR-possessing networks discussed earlier — the toy network (1), the EnvZ-OmpR osmoregulation networks (7) and (8), and the IDHKP-IDH glyoxylate bypass network (9) — all share subtle architectural attributes that give rise to absolute concentration robustness in their critical species.

To begin, we will need a small amount of vocabulary beyond that provided in Section 4. Recall that for a reaction such as A + B → C we construct the reaction vector $C - A - B$ by subtracting the reactant complex A + B from the product complex C. Here we shall also be interested in the difference between *any* two complexes, not just those connected by reaction arrows. For example, in the toy ACR network (21) considered earlier we can subtract the complex B

$$\begin{aligned} A+B &\rightarrow 2B \\ B &\rightarrow A. \end{aligned} \tag{21}$$

from the complex A + B to obtain the difference $A + B - B = A$. When, as in this example, the difference of two complexes is a non-zero multiple of a single species, s, we say that the two complexes *differ only in species s*. Thus, complexes $A + B$ and B differ only in A.

The following theorem [20] describes a family of mass-action systems for which ACR is ensured.

Theorem 6.1. Consider a mass-action system that admits a positive steady state and for which the underlying reaction network has a deficiency of one. If, in the network, there are distinct non-terminal complexes that differ only in species s, then the system has absolute concentration robustness relative to s.

Network (21) provides a trivial instance of this: For any choice of rate constants the corresponding mass-action system admits the positive steady states given in eqns (3), and the deficiency of the network is one [#(complexes) = 4, #(linkage classes) = 2, rank = 1]. Moreover, the non-terminal complexes $A + B$ and B differ only in species A, so the absolute robustness in A, already in evidence in eqns (3), is ensured.

The network shown in Figure 1c provides a less trivial example. Recall that its deficiency is one. Unlike the very similar deficiency-zero network 1b, there are rate constants for 1c that give rise to positive equilibria. For any such assignment of rate constants there must be absolute concentration robustness in species C: The non-terminal complexes $B + C$ and B differ only in C.

We turn now to the EnvZ-OmpR osmoregulation network (7), displayed again in Figure 2a. It has nine complexes, three linkage classes, and its rank is five, so its deficiency is one. For any assignment of rate constants, the corresponding mass-action system admits positive equilibria. Note the presence of two non-terminal complexes, XT and $XT + Y_p$, that differ only in the critical species Y_p. Theorem 6.1 then ensures absolute concentration robustness in Y_p. The situation for the alternative deficiency-one osmoregulation model network (8) is similar. (See Figure 2b.) In this case ACR relative to Y_p is ensured by the presence of the non-terminal complexes XD and $XD + Y_p$.

In the case of the IDHKP-IDH glyoxylate bypass network (9), shown again in Figure 2c, there are six complexes, two linkage classes, and the rank is three, so the deficiency of the network is one. For any choice of rate constants there are positive steady states. Because the two non-terminal complexes $EI_p + I$ and EI_p differ only in species I, Theorem 6.1 asserts that there is absolute concentration robustness in I.

Thus, the theorem provides a design strategy for ACR — a common architecture shared by all of our diverse ACR examples, including the experimentally motivated ones, that can foster absolute concentration robustness in crucial bio-active species.

We note in passing that the experimentally motivated models (7) and (9) were not constructed using the theorem as a guide. On the contrary, the theorem was discovered *after* these models had appeared. In this sense, Theorem 6.1 serves to unite prior and seemingly different examples within a common framework.

Remark 6.2. The deficiency-one requirement of the theorem cannot be omitted. In fact, there are examples of deficiency-two mass-action systems that otherwise satisfy all the requirements of the theorem but for which ACR does not obtain. On the other hand, there are also such deficiency-two examples for which ACR does obtain. An example of a deficiency-two system possessing ACR is provided in the on-line Supplementary Material associated with [20].

7. Absolute concentration robustness: Designs that might almost work

We do not expect *absolute* concentration robustness in real systems. As we indicated earlier, however, experimental systems do indicate the presence of quite strong, but imperfect, robustness. What, then, are we to make of the information given by Theorem 6.1, which provides conditions under which a reaction network will provide concentration robustness of the most ideal kind?

Reaction network models are, of course, simplifications aimed at capturing the essence of a particular aspect of cell function. For such purposes, they often ignore considerable detail, including the presence of other reactions in which the model's species are also participants. Typically, then, the model under study is viewed as a subnetwork of a larger, more fully articulated network containing additional reactions that are, in some sense, weakly coupled to the subnetwork. In this case, a reaction network model that satisfies the requirements of Theorem 6.1 might act as a subnetwork “core” that imparts to the parent network strong but imperfect robustness in the concentration of a critical bio-active species. (Examples are provided in the Supplementary Material that accompanies [20].)

This is the spirit in which Theorem 6.1 should be viewed. We do believe, however, that there is need for general study of the extent to which ACR-possessing subnetworks might serve to induce “almost-perfect” concentration robustness in the parent network when coupling to the remaining reactions is weak.

In this regard, there is one special consideration that we want to take up here. Some readers will have noticed that a weakly reversible network (in particular, a reversible one) cannot satisfy the requirements of Theorem 6.1, for such networks have no non-terminal complexes at all. For reversible networks, then, Theorem 6.1 stands silent. To make matters worse, the more orthodox chemists generally assert that *every* true chemical reaction is reversible, if only to a very small extent. For them, *all* reaction networks are reversible. With this in mind, we should ask about the extent to which Theorem 6.1 can nevertheless provide information:

7.1. When can ACR be approached arbitrarily closely in a reversible network?

Irreversible reactions are invoked ubiquitously in mass-action models, and we have done so here. In such instances, it is generally supposed that rate constants for the omitted reverse reactions are so small that the reactions them-selves can be omitted without compromising the quality of the information those models provide. Indeed, it is reasonable to expect that mass-actions systems that satisfy the requirements of Theorem 6.1 will exhibit very strong but imperfect concentration robustness (over wide ranges of network-constituent supplies) when reverse reactions are added with very small rate constants. Moreover, it is reasonable to expect that, in some sense, there should be an approach to *absolute* concentration robustness as the reverse-reaction rate constants approach zero. (Again, some examples are studied in the Supplementary Material for [20].) In this sense, Theorem 6.1 provides deficiency-one reversible designs that can almost ensure ACR.

Recall that the situation for reversible *deficiency-zero* mass-action systems is very different: Theorem 5.1 asserted that no deficiency-zero mass-action system, reversible or otherwise, can exhibit ACR. Moreover, in Section 5 we provided a reversible deficiency-zero example — network (13) — for which ACR cannot be approached arbitrarily closely *no matter how the rate constants are chosen*. Recall that the sensitivity of the critical species A_2BC is bounded away from zero by network structure alone: For all values of the monomer supplies $T^* := \left[T_A^*, T_B^*, T_C^*\right]$ we had

$$\Lambda^{A_2BC}(T^*) \geq \frac{1}{3}. \tag{22}$$

Such concentration-robustness-thwarting bounds, derived solely from reaction network structure, exist for *any* conservative weakly reversible deficiency-zero mass-action system [18].

Although this means that a deficiency-zero weakly reversible network cannot provide the foundation for an *arbitrarily* close approach to ACR, it might still happen that the ACR-thwarting sensitivity bound particular to the network at hand is very weak. (Imagine, for example, that the number on the right side of (22) were 10^{-6} rather than $\frac{1}{3}$.)

Indeed, within the class of deficiency-zero weakly reversible networks, there are structural factors that can, in a particular instance, mitigate the sensitivity bounds' severity. These factors are striking in their connection to biology. We discuss them next.

7.2. When molecules are built from gregarious and multiplicitous elements

It will be important to understand something of the way in which sensitivity bounds, of the kind shown in (22), relate to reaction network structure. Although a far wider ranging theory is offered in [18], it will be useful to restrict our discussion here to conservative weakly-reversible deficiency-zero networks such as (13), in which certain elements — for example, the monomers *A*, *B* and *C* in (13) — appear explicitly as species and in which all reactions serve merely to build higher order compounds from those elements. (These were called *constructive networks* in [18].)

We will need some additional vocabulary in order to demonstrate how the sensitivity bounds are deduced.

We denote by $\mathcal{E}$ the set of elements from which all other species in a constructive network are composed. Thus, in our example network (13), $\mathcal{E}=\{A, B, C\}$. For each element $e \in \mathcal{E}$ and for each species s of the network, we denote by M_e^s the content of element e in species s. In our example $M_A^{A_2BC}=2$, $M_B^{A_2BC}=1$, $M_C^{A_2BC}=1$, $M_A^A=1$, $M_B^A=0$, and so on. We denote by M_e^{max} the maximal content of element e in any species. Thus, in (13), $M_A^{max}=2$, $M_B^{max}=1$, and $M_C^{max}=1$.

By the *connectivity* of an element *e*, denoted conn(*e*), we mean the number of elements, including *e* itself, that participate with *e* in at least one species. (In more colloquial terms, this is just the number of elements that are “collaborators” of *e*.) In (13), A participates with itself in, say, species *A* (and also in A_2 and A_2BC), *B* participates with *A* in A_2BC, and the same is true of *C*. Thus, conn(*A*) = 3. Similar considerations show that conn(*B*) = 3 and conn(*C*) = 3. In this example, the connectivity for each element is maximal because all participate in A_2BC.

We note that for a mass-action system based on a weakly reversible, deficiency-zero, constructive network, there will invariably exist a smooth function $\bar{c}(\cdot)$ that gives, for each specification of the (logarithms of) the total element concentrations $T := [T_e : e \in \mathcal{E}]$, the corresponding (unique) positive steady state $\bar{c}(\ln T)$ [18]. We can therefore define the sensitivity of species *s*, evaluated at *T**, in the way we did in the specific case of eqn (19), using the same rationale:

$$\Lambda^s(T^*) := \max_{e \in \mathcal{E}} \left\{ \left| \frac{\partial \ln \bar{c}_s}{\partial \ln T_e} \right| \right\}_{T^*}. \tag{23}$$

We now have at our disposal all the terminology needed to specify how a sensitivity bound, such as the one appearing in (22), can be calculated, in the context of a mass-action system whose underlying network is conservative, weakly reversible, deficiency-zero, and constructive: For each species s and each specification T^* of total element concentrations,

$$\Lambda^s(T^*) \geq \max_{e \in \mathcal{E}} \left\{ \frac{M_e^s}{\mathrm{conn}(e)\, M_e^{max}} \right\}. \tag{24}$$

Note that the right side of (24) is an attribute of the network itself; it is independent of rate constants or of any particular specification of T^*.

Thus, for our example network (13), the bound given in (22) is derived from (24) in the following way:

$$\max\left\{ \frac{M_A^{A_2BC}}{\mathrm{conn}(A) M_A^{max}}, \frac{M_B^{A_2BC}}{\mathrm{conn}(B) M_B^{max}}, \frac{M_C^{A_2BC}}{\mathrm{conn}(C) M_C^{max}} \right\} = \max\left\{ \frac{2}{3 \cdot 2}, \frac{1}{3 \cdot 1}, \frac{1}{3 \cdot 1} \right\} = \frac{1}{3}. \tag{25}$$

We shall now briefly consider the biological significance of the bound given by (24). For the class of networks to which it applies, (24) serves to limit the extent to which a mass-action system might even *approach* absolute concentration robustness, no matter what the rate constants might be. The *value* of the bound, however, is specific to the particular network at hand, and in this sense some networks might be less intrinsically ACR-thwarting than others: A high value on the right side of (24) is more ACR-thwarting than a low one.

Note that a very low value of the bound on the sensitivity of species s will generally require one or the other of the following (or a mixture of both): (i) high connectivity ($\mathrm{conn}(e) >> 1$) of elements that appear within s, which means that those elements are gregarious with respect to many other elements — they have many collaborators, or (ii) elements that appear within s are present with very high multiplicity, relative to their content in s, in some compound species different from s ($M_e^{max}/M_e^s \gg 1$).

Thus, in the context of mass-action systems based on conservative, weakly reversible, deficiency-zero, constructive networks, intrinsic ACR-thwarting is mitigated by a certain level of network complexity: It helps considerably if the various reactions serve to build up large molecules composed of many gregarious and multiplicitous building blocks. It is striking that this is a situation often encountered in biology. Note that the *sufficient* conditions for ACR given in Theorem 6.1 are completely divorced from any of the considerations we have discussed here.

We conclude this subsection by reminding the reader that our focus on constructive networks was for the sole purpose of a simpler exposition. Far more subtle sensitivity bounds, also derived from network structure alone, are given in [18] for a far wider class of networks, even ones for which no natural choice of “elements” is apparent.

8. Discussion

In this article we brought under the same roof various results connecting the structure of mass-action reaction networks with their capacity to exhibit absolute concentration robustness. The central concept that we used to classify network architecture was the deficiency. We showed that mass-action systems derived from deficiency-zero networks are incapable of ACR, no matter what values the rate constants take, and that — for at least a certain class of networks — their sensitivity is in fact "bounded away" from zero as a result of network structure alone. However, we also argued that these sensitivity bounds are mitigated for networks that are suitably complex, in the sense that their elements, from which larger compound species are composed, either interact gregariously with each other or appear in great multiplicity within the compound species. Finally, we identified a subclass of deficiency-one networks in which ACR does obtain, a subclass that includes models for real biochemical circuits in which strong robustness resembling ACR has been observed in the laboratory.

We note that the ACR-possessing subclass that we identified does not exhaust all networks capable of ACR. Further study of how network architecture is connected to robustness is therefore warranted in higher deficiency (≥ 2) systems.

Again, we do not expect ACR-possessing networks to act in isolation within real biochemical systems. Rather, we expect that they will exert their influence as core subnetworks that induce strong yet imperfect concentration robustness in the parent networks that contain them. Precise conditions under which core ACR networks do indeed induce robustness more broadly remain to be discovered, especially within the context of very large networks with many interacting parts, for which the overall deficiency is likely to exceed one.

9. Conclusions

The results presented in this article provide design principles for absolute concentration robustness in mass-action networks. They also provide information about which network architectural features will thwart ACR, and to what extent. Together, these "positive" and "negative" results provide an initial map of the ACR landscape. We hope that this map will be useful for researchers wishing to venture deeper or broader into that territory.

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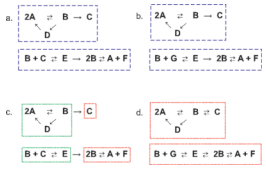

Figure 1.

Aspects of reaction network structure. Linkage classes are surrounded by a dashed blue outline. Terminal [non-terminal] strong linkage classes are surrounded by a dotted red [green] outline.

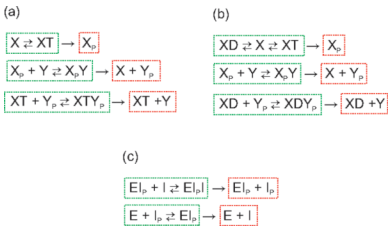

Figure 2.
Some examples revisited. Non-terminal strong linkage classes are surrounded by a green outline. Terminal strong linkage classes are surrounded by a red outline.