SENSITIVITY OF CHEMICAL REACTION NETWORKS: A STRUCTURAL APPROACH.

1. EXAMPLES AND THE CARBON METABOLIC NETWORK

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Abstract

In biological cells, chemical reaction pathways lead to complex network systems like metabolic networks. One experimental approach to the dynamics of such systems examines their "sensitivity": each enzyme mediating a reaction in the system is increased/decreased or knocked out separately, and the responses in the concentrations of chemicals or their fluxes are observed. In this study, we present a mathematical method to determine the sensitivity of reaction systems from information on the network alone. We investigate how the sensitivity responses of chemicals in a reaction network depend on the structure of the network, and on the position of the perturbed reaction in the network. We establish and prove some general rules which relate the sensitivity response to the structure of the underlying network. We describe a hierarchical pattern in the flux response which is governed by branchings in the network. We apply our method to several hypothetical and real life chemical reaction networks, including the metabolic network of the E. coli TCA cycle.

Keywords: Sensitivity; Reaction network; TCA cycle; Function-free; Structural approach

1. Introduction

The biological functions of a cell arise from a large set of chemical reactions. In the living cell the reactions define an inter-connected large system, where products of one reaction act as reactants of other reactions. The relationship between chemicals and reactions of such a system are represented by a directed graph, where a node represents one or several species of chemicals and a reaction arrow represents a state-transition of chemicals. Such graphs are called chemical reaction networks.

The carbon metabolic system is one of the most prominent examples of chemical reaction networks in biology. Any living cell obtains energy by the system of carbon metabolites including glycolysis and the tricarboxylic acid (TCA) cycle. Indeed the basic structure of this network is universally shared from bacteria to higher organisms including vertebrates.

Many examples of complex chemical reaction networks are available in databases. They compile the quintessence of chemical reactions, which have been derived by the accumulation of experimental results in biochemistry. On the other hand, the dynamics resulting from, and encoded in, such complex network systems is not understood sufficiently.

One possible experimental method to understand the dynamics of such systems examines their "sensitivity": each enzyme mediating a reaction in the system is increased/decreased or knocked out separately, and the responses in concentrations of chemicals or their fluxes are observed. One recent experimental approach to the behavior of metabolic network, as a whole, is called metabolome analysis: the concentration response of a large number of (species of) chemicals in a system is measured, simultaneously and quantitatively, by mass spectrometry. From such

perturbation sensitivity analysis, reseachers have tried to derive some properties of the systems. However, the responses of metabolic networks in such perturbation experiments seem difficult to understand.

For example, Ishii et al (2007) examined the sensitivity responses of the carbon metabolite network of E. coli towards a knockout, separately, of many enzymes in the system. For each knockout strain of about one third of the known enzymes they measured many metabolites included in the system. Surprisingly, a large percentage of the chemical species did not show any significant response to many knockout experiments. In addition, some responses turned out rather "counter-intuitive". For example, one of the largest response among all chemicals in all experiments was an increase of the concentration of a chemical which was a "reactant" of the perturbed reaction, i.e. an input chemical of the perturbed reaction, upward in the directed graph. The other largest response was the increase of a chemical which was on the side branch of the perturbed pathway. The authors concluded that the carbon metabolite system is robust. The experimental sensitivity results suggested, it was speculated, that the system might possess as yet unidentified bypass reactions.

In the following we develop a method to determine the sensitivity responses of chemical reaction networks towards perturbations of any reaction in the system, in a structural manner. We determine the qualitative change in the concentration of chemicals in the system from information on the reaction network, only, without any assumptions on either the specific functions modelling the reactions, or their reaction parameters.

There are some studies to analyze sensitivity of chemical reaction networks. Kacser and Burns (1973) and Heinrich and Rapoport (1974) independently proposed a

mathematical idea, which has been called "metabolic control analysis" later (Fell, 1992; Stephanopoulos *et al*., 1998). The analysis provides a mathematical framework to determine the sensitivity of a single pathway of chemical reactions and of some cases of branched system. This metabolic control analysis has been applied to some examples of small chemical reaction networks. We will present a different, and simpler, mathematical framework which enables us to calculate the sensitivity of large systems. We study the sensitivity by a structural approach and determine the relation between the structure of the network and the sensitivity responses of the system. We observe that some chemicals are not susceptible to certain perturbations, and we determine such chemicals from the network structure, only.

In spirit, though not in technical detail, our approach is influenced by Feinberg's ground breaking analysis of mass action kinetics in networks of low deficiency. See for example Feinberg (1995) and the many earlier references there, as well as the recent generalizations by Shinar and Feinberg (2013). Our sensitivity analysis, however, is not limited to reaction rates of mass action type and is not concerned with steady state multiplicity. On the other hand, we only study the steady state response to rate perturbations and not the global dynamics of the network. Low Feinberg deficiency greatly simplifies our network analysis, as well, but is violated by many biological relevant networks such as the TCA cycle of section 5 below.

Pallson (2005) proposed flux balance analysis and discussed some conditions on fluxes at steady state. For example, the maximum yield of fluxes was calculated by linear programming. His setting provides conditions on reaction fluxes at steady state. However, it is difficult to measure the fluxes of reactions in real biological systems.

In modern biology we have large data bases on networks. But it remains

difficult to determine complete quantitative details of the dynamics, including the types of reaction functions, reaction parameters, or initial states. This renders any numerical simulation grossly unreliable. As a viable alternative, our structural approach provides a mathematical analysis of real life biological network systems which are expected to include many unknown reaction functions or reaction rate parameters.

2. Mathematical formulation

We study systems of ordinary differential equations (ODE) of the following form:

$$\frac{d}{dt}\mathbf{x} = S\mathbf{w}. \qquad (1.a)$$

Here $\mathbf{x} = (x_m)_{m\in\mathbf{M}}$ is a vector of concentrations x_m of chemicals, $\mathbf{w} = (w_j)_{j\in\mathbf{E}}$ is a vector of fluxes, and $S : \mathbb{R}^E \to \mathbb{R}^M$ is a stoichiometric matrix. The sets $\mathbf{M} = \{1,\ldots,M\}$ and $\mathbf{E} = \{1,\ldots,E\}$ denote the species of chemicals and enumerate their reactions, respectively. Following Feinberg (1995), the stoichiometric matrix S arises as follow. Each reaction $j \in \mathbf{E}$ is conventionally denoted as

$$y_1^j X_1 + \ldots + y_M^j X_M \to \overline{y}_1^j X_1 + \ldots + \overline{y}_M^j X_M \qquad (1.b)$$

with suitable nonnegative real coefficient vectors $y^j, \overline{y}^j \in \mathbb{R}^M$. Here y_m^j and $\overline{y}_m^j$ indicate how many molecules X_m are consumed by reaction j, as reactants, and appear as products, respectively. Usually the vectors $y^j, \overline{y}^j$ are nonnegative integer,

although nonnegative real components are equally acceptable. The concentration of X_m is denoted by x_m. The stoichiometric matrix S is defined by

$$S\mathbf{w} := \sum_{j\in\mathbf{E}} w_j\left(\overline{y}^j - y^j\right) \qquad (1.c)$$

for any vector $\mathbf{w} \in \mathbb{R}_+^E$ of strictly positive real components. The case $y^j = 0$, alias the reaction $0 \to \overline{y}^j$, describes a constant feed reaction which does not consume any X_m. Similarly, $y^j \to 0$ denotes a reaction towards some final products which do not enter as reactants and are therefore omitted in the list $X_1,\ldots,X_M$ of participating chemicals.

In the ODE (1.a), accordingly, the flux w_j is assumed to only depend on those reactants x_m for which the stoichiometric input coefficient y_m^j is strictly positive. More precisely, we assume throughout this paper, that the positive reaction rates w_j satisfy

$$\begin{aligned} &w_j > 0, \\ &r_{jm} := \partial_{x_m} w_j > 0 \Leftrightarrow y_m^j > 0, \end{aligned} \qquad (1.d)$$

for all $j \in \mathbf{E}$ and all $m \in \mathbf{M}$, and for all positive concentration vectors $\mathbf{x}$. Clearly this monotonicity assumption holds for mass action and Michaelis-Menten type kinetics, as well as many variants, independently of their specific reaction parameter. In this sense we call our approach "structural". It is reasonable to assume such monotonicity of chemical reaction functions because non-monotonic reaction functions have been hardly observed in biochemistry.

In the present sensitivity analysis we only study the equilibrium response to arbitrary perturbations of the reactions $w_j = w_j\left(k_j, \mathbf{x}\right)$. We represent this perturbation by (implicit) differentiation with respect to a (formal) reaction parameter k_j. For a precise mathematical formulation and complete technical details we refer to the accompanying mathematical presentation in Fiedler and Mochizuki (2014).

By definition, the left-hand-side of (1) is equal to 0 at the equilibrium. Thus the flux $\mathbf{w}^*$ at equilibrium is in the kernel space of S, i.e. $S\mathbf{w}^* = 0$. Let $\delta\mathbf{w}^*$ denote the total change of the flux at equilibrium caused by a small perturbation of the formal reaction parameter k_{j^*}. Then $\delta\mathbf{w}^*$ satisfies the equilibrium condition (1):

$$S\delta\mathbf{w}^* = 0 \quad . \tag{2}$$

Let $j^* \in \mathbf{E}$ indicate the perturbed reaction rate parameter k_{j^*}. Implicit differentiation of (2) with respect to k_{j^*} then implies

$$S\left[\begin{pmatrix} \\ \dfrac{\partial w_j}{\partial k_{j^*}} \\ \\ \end{pmatrix} + \begin{pmatrix} \\ \dfrac{\partial w_j}{\partial x_m} \\ \\ \end{pmatrix}\begin{pmatrix} \\ \delta x_m^{j^*} \\ \\ \end{pmatrix}\right] = 0 . \tag{3}$$

Here $\delta x_m^{j^*} := \partial x_m / \partial k_{j^*}$ denotes the response sensitivity of the equilibrium concentration x_m, i.e. the total change of the concentration of chemical X_m caused by

a perturbation of the formal reaction parameter k_{j^*}. The decomposed flux change $\delta \mathbf{w}^*$ has two terms: the direct change of fluxes $\partial w_j / \partial k_{j^*}$ by the perturbation of k_{j^*}, and the changes of fluxes caused indirectly through the resulting changes $\delta x_m^{j^*}$ in equilibrium concentrations x_m. The indirect change is the product of a matrix $\left(\partial w_j / \partial x_m\right)$, which we call dependence matrix, and the change $\delta x_m^{j^*}$ in the concentration of chemicals X_m in the system.

The elements of the dependence matrix are given symbolically by parameters r_{jm}. By our assumption (1.d) we have the alternative:

$\frac{\partial w_j}{\partial x_m} = r_{jm} > 0$: the flux of reaction j increases with concentration x_m, because $y_m^j > 0$; or

$\frac{\partial w_j}{\partial x_m} = 0$: the flux of reaction j does not depend on the concentration x_m, because $y_m^j = 0$.

Since the stoichiometric coefficients $y_m^j \geq 0$ are nonnegative, this alternative exhausts all possibilities.

In the present paper the nonzero elements $\partial w_j / \partial x_m \neq 0$ of the dependence matrix indicate the reactant X_m of reaction j, and they are always assumed positive. However, our method generalize to the case that the flux function w_j depends not only on the reactants but also on the products or other regulator chemicals. We can generalize the dependence matrix to arbitrary influences of chemical X_m on the reaction j. In

particular nonzero elements may be negative ($\partial w_j / \partial x_m < 0$) to express a suppressive regulation of reaction j by X_m.

We obtain the response sensitivity $\delta x_m^{j^*}$ of (3) in symbolic form, for any given perturbation $\partial w_j / \partial k_{j^*}$ and the symbolically given dependencies $\left(\partial w_j / \partial x_m\right) = \left(r_{jm}\right)$. In some cases the response sensitivity $\delta x_m^{j^*}$ will turn out to be identically zero: the chemical X_m is insensitive to a rate perturbation of reaction j^*. In other cases the sign of $\delta x_m^{j^*}$ may be determined even in the symbolic solution. In such cases we can determine the qualitative sensitivity response of the concentration x_m of chemical X_m to the given perturbation of k_{j^*} independently of any quantitative detail of the reaction functions or their parameters. Specifically, x_m may be predicted to increase or decrease in response to an increase of the rate parameter k_{j^*}, depending on the determined sign of $\delta x_m^{j^*}$.

Here we introduce a systematic method for the rate sensitivity of chemical reaction networks. Our method is based on a symbolic matrix which is determined from the structure of network directly. In the next argument we assume the following nondegeneracy of the network:

$$\operatorname{range}\left(r_{jm}\right) + \ker S = \mathbb{R}^E. \qquad (4)$$

See Fiedler and Mochizuki (2014) for a detailed discussion.

The equations (2), (3) can then be rewritten as

$$\delta \mathbf{w}^{*} = \left(\frac{\partial w_j}{\partial k_{j^*}} \right) + \left(\quad \frac{\partial w_j}{\partial x_m} \quad \right) \left(\delta x_m^{j^*} \right) = \sum_{k=1}^{K} \mu_k \left(c_k^j \right). \qquad (5)$$

Here the basis vectors $\mathbf{c}_k = \left(c_k^j\right)_{j \in \mathbf{E}}$, $k \in \mathbf{K} = \{1, \ldots, K\}$ span the K-dimensional kernel of the stoichiometric matrix S, and $\mu_k \in \mathbb{R}$ denote their coefficients. If we consider a response $\delta x_m^{j^*}$ of chemical X_m to the normalized perturbation $\partial w_{j^*} / \partial k_{j^*} = 1$ of reaction $j^* \in \mathbf{E}$, equation (5) takes the form

$$\left(\frac{\partial w_j}{\partial x_m} \;\vdots\; -\mathbf{c}_k \right) \begin{pmatrix} \delta x_m^{j^*} \\ \mu_k \end{pmatrix} = -e_{j^*} \qquad (6)$$

in block matrix notation. More explicitly

$$\left(\begin{array}{ccc:ccc} \frac{\partial w_1}{\partial x_1} & \cdots & \frac{\partial w_1}{\partial x_M} & -c_1^1 & \cdots & -c_K^1 \\ & & & & & \\ \frac{\partial w_{|E|}}{\partial x_1} & \cdots & \frac{\partial w_E}{\partial x_M} & -c_1^E & \cdots & -c_K^E \end{array} \right) \begin{pmatrix} \delta x_1 \\ \vdots \\ \delta x_M \\ \mu_1 \\ \\ \mu_K \end{pmatrix} = -e_{j^*}, \qquad (7)$$

where $e_{j^*} = \left(\partial w_j / \partial k_{j^*} \right)$ is the j^*-th unit vector in $\mathbb{R}^E$. In the following the block matrix

$$\mathbf{A} = \left(\frac{\partial w_j}{\partial x_m} \;\vdots\; -\mathbf{c}_k \right) \tag{8}$$

will be called the *augmented matrix* or the $\mathbf{A}$-matrix. The square $E \times E$ sensitivity matrix $\mathbf{S}$ of a system to the normalized perturbation of reaction $j^* \in \mathbf{E}$ is given by the negative inverse matrix of $\mathbf{A}$ as:

$$\mathbf{S} := \left(\begin{array}{ccc} \delta x_1^1 & \cdots & \delta x_1^E \\ \vdots & & \vdots \\ \delta x_M^1 & \cdots & \delta x_M^E \\ \hline \mu_1^1 & \cdots & \mu_1^E \\ \vdots & & \vdots \\ \mu_K^1 & \cdots & \mu_K^E \end{array} \right) = -\mathbf{A}^{-1}. \tag{9}$$

The columns $j^* \in \mathbf{E} = \{1, \ldots, E\}$ of S indicate perturbed reactions. By (6), row $m \in \mathbf{M} = \{1, \ldots, M\}$ collects the sensitivity responses $\delta x_m^{j^*}$, $j^* \in \mathbf{E}$, of all chemicals X_m. Responses of fluxes to a normalized perturbation of the reaction rate k_{j^*} are represented by the coefficient column $\mu_k^{j^*}$, $k = 1, \ldots, K$, in the bottom part of the sensitivity matrix $\mathbf{S}$. Indeed $\mu_k^{j^*}$ are the coefficients of the basis vectors $\mathbf{c}_k$ of the kernel $\ker S$ of the stoichiometric matrix S, in our expression (5) for the total flux change $\delta \mathbf{w}^*$ caused by a normalized perturbation of the reaction rate k_{j^*}.

Consider the $E \times E$ flux sensitivity matrix Φ with columns $\delta \mathbf{w}^*$,

$j \in \mathbf{E} = \{1,\ldots,E\}$. Then Φ is given explicitly by the basis $\mathbf{c}_k$, coefficients μ_k^j in (9) and, respectively, the dependence matrix $\mathbf{R} = \left(r_{jm}\right) = \left(\partial w_j / \partial x_m\right)$ and the concentration sensitivities δx_m^j in (9) as:

$$\begin{aligned}\Phi &:= \mathrm{id}_{E\times E} + \left(r_{jm}\right)\begin{pmatrix} \delta x_1^1 & \cdots & \delta x_1^E \\ \vdots & & \vdots \\ \delta x_M^1 & \cdots & \delta x_M^E \end{pmatrix} \\ &= \left(\mathbf{c}_k\right)\left(\mu_k\right) \\ &= \begin{pmatrix} c_1^1 & \cdots & c_K^1 \\ \vdots & & \vdots \\ c_1^E & \cdots & c_K^E \end{pmatrix}\begin{pmatrix} \mu_1^1 & \cdots & \mu_1^E \\ \vdots & & \vdots \\ \mu_K^1 & \cdots & \mu_K^E \end{pmatrix}. \end{aligned} \qquad (10)$$

The equation (3) is linear, and the concentration sensitivity $\delta x_m^{j^*}$ is the partial derivative $\partial x_m / \partial k_{j^*}$ of the equilibrium concentration with respect to the reaction rate parameter k_{j^*}. This means that the obtained solution $\delta x_m^{j^*}$ is the response of chemical X_m to a small disturbance of the formal parameter k_{j^*} of reaction j^*. Suppose $\delta x_m^{j^*} = \partial x_m / \partial k_{j^*}$ is zero, or the nonzero sign does not depend on the concentration vector $\mathbf{x}$. Then we can apply the same result to large disturbances as well. In particular our result extend to global perturbations like knock-out experiments for such $\delta x_m^{j^*}$. The same statement holds true whenever we encounter a definite zero or nonzero sign of the response $\delta x_m^{j^*}$, independently of the specific partial derivatives $r_{jm} \geq 0$ in (1.d).

3. Examples

In this section we discuss several examples to illustrate the scope of our function-free approach to sensitivity analysis. The examples 3-1 of single pathways, and 3-2 of a single loop feedback circuit, are networks of monomolecular reactions: any reaction j is driven by only one input reactant. Monomolecular networks are also studied in Fiedler and Mochizuki (2014), from a more general mathematical view point. Example 3-3, in contrast, contains a bi-molecular reaction and is not addressed in Fiedler and Mochizuki (2014).

3-1. Single pathway

As our first example we consider a single reaction pathway which does not include any branches; see Figure 1. The $\mathbf{A}$-matrix is given as

$$\mathbf{A} = \left(\begin{array}{ccc|c} 0 & 0 & 0 & -1 \\ r_{2A} & 0 & 0 & -1 \\ 0 & r_{3B} & 0 & -1 \\ 0 & 0 & r_{4C} & -1 \end{array}\right), \tag{11}$$

where r_{2A}, r_{3B}, r_{4C} are positive constants reflecting the dependence of reactions on chemicals. Here $\mathbf{M} = \{A, B, C\}$, $M = 3$, $\mathbf{E} = \{1, 2, 3, 4\}$, $E = 4$. The sensitivity $\mathbf{S}$ is given in (9) as the negative inverse matrix of $\mathbf{A}$,

$$\mathbf{S} = \begin{pmatrix} 1/r_{2A} & -1/r_{2A} & 0 & 0 \\ 1/r_{3B} & 0 & -1/r_{3B} & 0 \\ 1/r_{4C} & 0 & 0 & -1/r_{4C} \\ \hline 1 & 0 & 0 & 0 \end{pmatrix} . \qquad (12)$$

The columns indicate perturbed reactions $j^* = 1, \ldots, 4$, and the rows indicate the responses δx_A, δx_B, δx_C of chemical concentrations (first three rows) and of the flux (last row). The result shows that the flux changes if, and only if, the top reaction 1 (input to the system) is perturbed. Then all chemical concentrations in the system increase, accordingly. When reactions 2, 3 or 4 are perturbed, however, only the input reactant chemical of the perturbed reaction changes. There is no change in any other chemical concentrations and fluxes. We understand this result easily: a suitable decrease in the upstream chemical reactant concentration compensates for the increase in the reaction rate of its reaction. This buffering effect prevents the perturbation to propagate beyond its original locus, either downward or upward. We calculated the equilibria of the single pathway by numerical simulations for several reaction functions. Of course, these simulations all confirmed our mathematical result.

A complete knock out of one enzyme, e.g. like $r_{2A} \to 0$, produces a singular situation with $\delta x_A \to +\infty$, of course. Indeed chemical A then possesses input, but cannot react further. In this case we consider our results as remaining valid in the limit of arbitrarily small, but positive, rates r_{2A}.

3-2. Feedback circuit

Our second example is the small network shown in Fig. 2. It consists of 6 reactions and 4 chemicals, including one feedback loop via one side branch. The $\mathbf{A}$-matrix is given as:

$$\mathbf{A} = \left(\begin{array}{cccc|cc} 0 & 0 & 0 & 0 & -1 & 0 \\ r_{2A} & 0 & 0 & 0 & -1 & -1 \\ 0 & r_{3B} & 0 & 0 & -1 & -1 \\ 0 & 0 & r_{4C} & 0 & 0 & -1 \\ 0 & 0 & 0 & r_{5D} & 0 & -1 \\ 0 & 0 & r_{6C} & 0 & -1 & 0 \end{array}\right). \qquad (13)$$

The inverse of the $\mathbf{A}$-matrix gives sensitivity, responses of chemical concentrations and of fluxes to the perturbations as:

$$\mathbf{S} = \left(\begin{array}{ccccc} (r_{4C}+r_{6C})/r_{2A}r_{6C} & -1/r_{2A} & 0 & 1/r_{2A} & 0 & -r_{4C}/r_{2A}r_{6C} \\ (r_{4C}+r_{6C})/r_{3B}r_{6C} & 0 & -1/r_{3B} & 1/r_{3B} & 0 & -r_{4C}/r_{3B}r_{6C} \\ 1/r_{6C} & 0 & 0 & 0 & 0 & -1/r_{6C} \\ r_{4C}/r_{5D}r_{6C} & 0 & 0 & 1/r_{5D} & -1/r_{5D} & -r_{4C}/r_{5D}r_{6C} \\ \hline 1 & 0 & 0 & 0 & 0 & 0 \\ r_{4C}/r_{6C} & 0 & 0 & 1 & 0 & -r_{4C}/r_{6C} \end{array}\right). \qquad (14)$$

The columns indicate the perturbed reactions $j^* = 1,\ldots,6$. The rows indicate the sensitivity response of the chemical concentrations A, B, C, D (first four rows) and of fluxes (last two rows). Again, only a change of the top feed reaction w_1 is able to simultaneously affect all chemicals and fluxes. See Fig. 2a. Reactions $j^* = 2, 3, 5$ are buffered by their input reactants A, B, D, respectively, and their perturbations

do not propagate further in the network; see Fig. 2b, c, e.

Let us compare the remaining results of perturbations to reaction $j^* = 4$ (fourth column, Fig. 2d) and $j^* = 6$ (last column, Fig. 2f). A large part of the network is affected by the increase of the reaction constant of reaction 4 as shown in Fig. 2d. We may easily interpret the result: the chemical concentrations D, A, B along the reaction cycle 4, 5, 2, 3 downward of the perturbed reaction w_4 simply increase, along with the fluxes of these reactions. We also observe changes in a large part of the network when reaction $j^* = 6$ is perturbed, as shown in Fig. 2f. This time, however, the changes in concentrations do not appear in the chemical concentrations further downstream, but in the side branch cycle 4, 5, 2, 3 of the perturbed reaction. Also, the signs of concentration and flux changes are reversed from Fig. 2d to 2f. In other words, the signs of the sensitivity response of the system are asymmetric, even though the perturbations are applied to each of the two reaction branches $j^* = 4, 6$ which emanate symmetrically from chemical C.

To understand the sensitivity response of chemical reaction networks, we discuss two different restrictions on the chemical reaction system at equilibrium. The first restriction is the flux balance, i.e. the total influx should be equal to the total outflux at each of the chemicals in the system. This Kirchhoff balance implies that the fluxes along a single pathway without branching should be the same everywhere. We may call this the "restriction along pathways". The second restriction appears at a branching node of a reaction network, where multiple directed reaction edges emanate from a single node. Any change in the concentration of that single chemical will affect all branching pathways simultaneously. We may call this condition "restriction at branching pathways". We can describe and understand some characteristic behavior of

sensitivity in chemical reaction networks by these restrictions.

In Fig. 2f, for example, the concentration of chemical C decreases by the perturbation to reaction $j^* = 6$. This is understood as a compensation to keep the output flow (w_6 of reaction 6) at the same level as the unchanged feed flow w_1 (restriction along the vertical pathways). On the other hand, any change in chemical C spreads to the second branch, reaction 4, which emanates from C. Thus the flux and concentrations in the feedback loop decrease, by the restriction at branching pathways, as a side effect of the decrease of x_C which, in turn, buffers the output flow w_6 to remain unchanged in spite of the externally applied perturbation.

3-3. Hypothetical system

Our method applies to arbitrary chemical reaction networks. Consider the hypothetical network in Figure 3. Our previous examples 3-1 and 3-2 have been monomolecular, i.e. $y_m^j > 0$ held true for at most one unique "mother" reactant X_m of any reaction $j \in \mathbf{E}$. Now, in contrast, reaction 10 is bi-molecular: $G + H \rightarrow I$ in the notation of (1.b). The $\mathbf{A}$-matrix for the network is shown in (15).

$$
\mathbf{A} = \begin{array}{c}
\begin{array}{cccccccccc|ccccc}
A & B & C & D & E & F & G & H & I & J & \mathbf{c}_1 & \mathbf{c}_2 & \mathbf{c}_3 & \mathbf{c}_4 & \mathbf{c}_5
\end{array} \\
\left(\begin{array}{cccccccccc|ccccc}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 1 \\
r_{3A} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & -1 \\
0 & r_{4B} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 1 \\
0 & 0 & r_{5C} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 \\
0 & 0 & r_{6C} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 1 & 0 \\
0 & 0 & 0 & r_{7D} & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & r_{8F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & r_{9E} & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & r_{10F} & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & r_{11G} & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & r_{12G} & r_{12H} & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{14I} & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{15J} & -1 & 0 & 0 & 0 & 0
\end{array}\right)
\begin{array}{c}
1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \\ 10 \\ 11 \\ 12 \\ 13 \\ 14 \\ 15
\end{array}
\end{array}
$$

(15)

We can see that the dependence matrix $\left(r_{jm}\right)$ with $j \in \mathbf{E} = \{1,2,\ldots,15\}$, $E = 15$ and $m \in \mathbf{M} = \{A,\ldots,J\}$, $M = 10$, is sparse: only a few entries r_{jm} are nonzero. The symbolic sensitivity response matrix $\mathbf{S}$ of (9) is the negative inverse of the above $\mathbf{A}$-matrix:

$$
\mathbf{S} = D_1 \left(\begin{array}{ccccccccccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 & 15 \\
2r_{10F}+r_{13F} & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 2r_{10F}+r_{13F} & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
r_{8F}+r_{10F}+r_{13F} & r_{8F}-r_{10F} & 0 & 0 & -1 & 0 & 0 & 1 & 0 & -2r_{8F}-r_{13F} & 0 & 0 & -r_{8F}+r_{10F} & 0 & 0 \\
r_{8F}+r_{10F}+r_{13F} & r_{8F}+r_{10F}+r_{13F} & 0 & 0 & 0 & 0 & -1 & 1 & 0 & -2r_{8F}-r_{13F} & 0 & 0 & -r_{8F}+r_{10F} & 0 & 0 \\
R_1 & R_2+r_{5C}r_{10F} & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{5C}+r_{6C} & -r_{5C} & -R_8+r_{5C}r_{13F} & 0 & 0 & -R_2-r_{5C}r_{10F} & 0 & 0 \\
1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 2 & 0 & 0 & -1 & 0 & 0 \\
R_1-r_{5C}r_{10F} & R_2 & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{5C}+r_{6C} & 0 & -R_8 & -r_{5C} & 0 & -R_2 & 0 & 0 \\
r_{5C}r_{10F}\left(r_{11G}+r_{12G}\right)-r_{12G}R_1 & r_{5C}r_{10F}r_{11G}-r_{12G}R_2 & 0 & 0 & r_{6C}r_{12G} & -r_{12G} & 0 & -r_{12G}\left(r_{5C}+r_{6C}\right) & 0 & r_{12G}R_8+r_{5C}r_{11G}r_{13F} & r_{5C}r_{12G} & -r_{5C}r_{11G} & r_{12G}R_2-r_{5C}r_{10F}r_{11G} & 0 & 0 \\
r_{10F} & r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & -r_{10F} & -1 & 0 \\
r_{13F} & r_{13F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2r_{13F} & 0 & 0 & 2r_{10F} & 0 & -1 \\
\hline
r_{13F} & r_{13F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2r_{13F} & 0 & 0 & 2r_{10F} & 0 & 0 \\
r_{10F} & r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & -r_{10F} & 0 & 0 \\
R_1-r_{5C}r_{10F} & R_2 & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{5C}+r_{6C} & 0 & -R_8 & 0 & 0 & -R_2 & 0 & 0 \\
r_{8F} & r_{8F} & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -2r_{8F} & 0 & 0 & -r_{8F} & 0 & 0 \\
r_{10F}+r_{13F} & -r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -r_{13F} & 0 & 0 & r_{10F} & 0 & 0
\end{array}\right) D_2
$$

(16)

where

$$D_1 = \mathrm{diag}\left(r_{3A}, r_{4B}, r_{5C}, r_{7D}, r_{5C}r_{9E}, 1, r_{5C}r_{11G}, r_{5C}r_{11G}r_{12H}, r_{14I}, r_{15J}, 1, 1, r_{5C}, 1, 1\right)^{-1},$$

$$D_2 = \mathrm{diag}\left(2r_{10F}+r_{13F}, 2r_{10F}+r_{13F}, 1, 1, 1, 1, 1, 1, 1, 2r_{10F}+r_{13F}, 1, 1, 2r_{10F}+r_{13F}, 1, 1\right)^{-1},$$

and $R_1 = r_{8F}\left(r_{5C}+r_{6C}\right)+r_{6C}\left(r_{10F}+r_{13F}\right)$, $R_2 = \left(r_{5C}+r_{6C}\right)\left(r_{8F}-r_{10F}\right)$,

$R_8 = \left(r_{5C}+r_{6C}\right)\left(2r_{8F}+r_{13F}\right)$. (17)

As before, the 15 columns j^* indicates the perturbed reactions from reaction for $j^* = 1, \ldots, 15$. The rows indicate the responses of chemicals A, ..., J (first 10 rows) and fluxes (last 5 rows). If we discuss only the signs of the changes $\delta x_m^{j^*}$ in chemical concentrations and μ_k of the fluxes $\mathbf{c}_k$, we can rewrite the sensitivity matrix $\mathbf{S}$ as follows:

$$
\mathbf{S}' = \begin{array}{c}
\begin{array}{ccccccccccccccc}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 & 15
\end{array} \\
\left(\begin{array}{ccccccccccccccc}
+ & 0 & - & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & + & 0 & - & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
+ & \pm & 0 & 0 & - & 0 & 0 & + & 0 & - & 0 & 0 & \pm & 0 & 0 \\
+ & + & 0 & 0 & 0 & 0 & - & + & 0 & - & 0 & 0 & \pm & 0 & 0 \\
+ & \pm & 0 & 0 & - & + & 0 & + & - & - & 0 & 0 & \pm & 0 & 0 \\
+ & + & 0 & 0 & 0 & 0 & 0 & 0 & 0 & - & 0 & 0 & - & 0 & 0 \\
\pm & \pm & 0 & 0 & - & + & 0 & + & 0 & - & - & 0 & \pm & 0 & 0 \\
\pm & \pm & 0 & 0 & + & - & 0 & - & 0 & + & + & - & \pm & 0 & 0 \\
+ & + & 0 & 0 & 0 & 0 & 0 & 0 & 0 & + & 0 & 0 & - & - & 0 \\
+ & + & 0 & 0 & 0 & 0 & 0 & 0 & 0 & - & 0 & 0 & + & 0 & - \\
\hline
+ & + & 0 & 0 & 0 & 0 & 0 & 0 & 0 & - & 0 & 0 & + & 0 & 0 \\
+ & + & 0 & 0 & 0 & 0 & 0 & 0 & 0 & + & 0 & 0 & - & 0 & 0 \\
\pm & \pm & 0 & 0 & - & + & 0 & + & 0 & - & 0 & 0 & \pm & 0 & 0 \\
+ & + & 0 & 0 & 0 & 0 & 0 & + & 0 & - & 0 & 0 & - & 0 & 0 \\
+ & - & 0 & 0 & 0 & 0 & 0 & 0 & 0 & - & 0 & 0 & + & 0 & 0
\end{array}\right)
\end{array}
\begin{array}{c}
A \\ B \\ C \\ D \\ E \\ F \\ G \\ H \\ I \\ J \\ \hline \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \\ \mu_5
\end{array}
$$

(18)

Here the sign (+, −, 0) indicates that the chemical concentration x_m (or the flux coefficient μ_k) increases, decreases or remains unaffected, by the corresponding perturbation $j*$ (increase of the reaction coefficient k_{j*}). Some entries have the "$\pm$" mark, which indicates that the change of the concentration (or of the flux coefficient) is typically nonzero, but the response sign (+ or −) cannot be determined from the structure of the network alone. Rather, that sign depends on the specific values of the partial derivatives $r_{jm} > 0$ at the equilibrium under consideration. The indeterminacy signs $\pm$ are not independent of each other; some of them can be seen to flip in

synchrony, or in a certain order, from the full symbolic form (16) of the sensitivity matrix $\mathbf{S}$.

Figure 3 shows some examples of changes $\delta x_m^{j^*}$ in concentrations of chemicals X_m due to the perturbation of select reactions j^*. Fig. 3a addresses the case $j^* = 5$ where chemicals on the side-branch cycle $j' = 6, 9, 11$ of the perturbed reaction j^* decrease their concentrations. This follows because $\mu_3 < 0$ is the only flux responses to $j^* = 5$ in (18), and because $j' = 6, 9, 11$ is the cycle $\mathbf{c}_3$ of the third last column of the $\mathbf{A}$-matrix in (15). Mediated by this effect on the side-branch cycle $j' = 6, 9, 11$, a perturbation of $j^* = 5$ also affects both reactants G, H of the only bimolecular reaction $j = 12$. However, the reactant concentrations change in opposite directions such that their effect on the bimolecular reaction flux at $j = 12$ cancels. In Fig. 3f the bimolecular reaction $j^* = 12$: $G + H \rightarrow I$ itself is perturbed. Remarkably, and quite asymmetrically, only one of the two reactant chemicals G, H changes its concentration by the perturbation of this bimolecular reaction. In Fig. 3c, the chemicals E, G, C and the fluxes on the reaction cycle $j' = 9, 11, 6$ of $\mathbf{c}_3$ directly downstream of the perturbed reaction $j^* = 8$ increase by the perturbation. The perturbation also propagates downstream reaction through the alternative flux cycle $j' = 9, 11, 5, 7, 8$ of $\mathbf{c}_3 + \mathbf{c}_4$ with chemicals E, G, C, D. However, the input reactant F of the perturbed reaction $j^* = 8$ itself remains unchanged.

We also examined these predictions of equilibrium sensitivity by computer simulations using hypothetically given reaction functions and parameter values. We confirmed that the changes $\delta x_m^{j^*}$ in the concentration of all chemicals X_m at

equilibrium always follow the above predictions, independently of the chosen reaction functions and their parameter values.

4. Analysis of the response pattern

The responses to perturbations of reaction rates k_{j^*} in chemical reaction networks exhibit characteristic patterns related to the structure of the network. We analyze the response patterns in two different ways, based on the local network structures, and on the global flux patterns.

4-1. Patterns determined from local structures of networks (Motif rules)

The rate sensitivities of some local structures of networks are determined a priori from the **A** -matrix. Such responses are summarized as "rules of sensitivity". They derive from local structures of chemical reaction networks as follows.

(1) single pathway without branching; Fig. 4 (1)

Let a perturbation be induced into reaction j^*. Assume that the unique "mother" reactant m^* possesses no other output arrow $j \neq j^*$ on the network. In other words, reaction j^* is a "single child" of m^*. Then the sensitivity response of the system is confined to the concentration change $\delta x_m^{j^*}$ of the reactant m^* of the perturbed reaction j^*. Neither do the fluxes change in any reaction in the network, nor do any other concentrations. The normalized increase in the reaction constant of k_{j^*} is fully compensated by a decrease $\delta x_{m^*}^{j^*} = -1/r_{j^*m^*} < 0$ in the concentration of the reactant chemical X_{m^*}. See also Fiedler and Mochizuki (2014), equation (1.19). Example 3-1 and the results of perturbations to reactions $j^* = 2, 3, 5$ in example 3-2, and to $j^* = 3, 4, 7, 9, 14, 15$ in example 3-3 are explained by this rule. We emphasize

that this rule requires the perturbed reaction j^* to possess a mother reactant m^*. Perturbations to feed reactions j^* of the form $0 \to \overline{y}^{j^*}$, i.e. with $y^{j^*} = 0$, cannot be compensated by any chemicals. Instead multiple nonzero responses of chemical concentrations and fluxes may ensue downstream; see the results of perturbations to reactions 1 in example 3-1, 3-2, and to reactions 1 and 2 in example 3-3.

(2) branching; Fig. 4 (2-1) and (2-2)

From the above we derive another rule (2) which complements rule (1). Besides the case of perturbation to top feed reactions, we will observe multiple nonzero responses of chemical concentrations and fluxes only when j^* is not a single child. In other words, a perturbation is induced into a reaction j^* of an input reactant m^* with multiple output arrows. We now consider the simplest case of two output arrows. The sensitivity responses of the system may then be asymmetric with respect to the perturbed output arrows at m^*, depending on the global structure of the network. We will observe (2-1): responses in the chemical concentrations directly downward of the perturbed reaction j^*, or (2-2): responses in the chemical concentrations of a side branch $j \neq j^*$ of the perturbed reaction. In either case the patterns of concentration and flux responses coincide, albeit with opposite signs.

The results of perturbations to reactions $j^* = 4$ and to $j^* = 6$ in example 3-2 are explained by (2-1) and (2-2), respectively; see Fig. 2d and 2f. See also the examples in Fiedler and Mochizuki (2014), (6.1) -- (6.4). The local structure of the network is not sufficient to determine which type of response the system will observe. The global response patterns of concentrations and fluxes in the network are related to the basis vectors $\mathbf{c}_k$ spanning the kernel of the stoichiometric matrix S, as we will discuss later.

(3) bimolecular reactions; Fig. 4 (3)

Consider a case where two chemicals $m*$, $n*$ are input reactants of a bimolecular reaction 2: $m*+n* \rightarrow p$, such that $n*$ contributes input only to this bimolecular reaction, whereas reactant $m*$ possesses exactly one and only one other child reaction, 1, which is not necessarily monomolecular. Hence $m*$ also figures as input reactant to reaction 1.

(3-1): If the reaction rate of the bimolecular reaction $j* = 2$ is increased, then only the concentration of chemical $n*$ will decrease, to buffer the reaction flux w_2. All fluxes and all other concentrations remain unchanged. The result of perturbations to reaction $j* = 12$ in example 3-3 can be explained by this rule; see Fig. 3f.

(3-2): If the reaction rate of the other output arrow $j* = 1$ of chemical $m*$ is increased, then the concentration of $m*$ will decrease and that of the counterpart $n*$ of the bimolecular reaction 2 will increase. The two reactant concentration changes $\delta x_{m*} < 0 < \delta x_{n*}$ will act in opposite directions. Again, there is no change of any flux or of any other concentration in the network. Indeed, the decrease in the reactant $m*$ compensates and buffers the increase in the perturbed reaction rate $j* = 1$. The increase of the other reactant chemical $n*$ compensates for the decrease of the reactant $m*$ such that the flux of the bimolecular reaction 2 also remains unchanged. The result of perturbations to reaction $j* = 11$ in example 3-3 is explained by this rule; see Fig. 3e.

(4) reversible reactions; Fig. 4 (4)

Consider a case where two chemicals $m*$, $n*$ are involved in a reversible reaction $\pm$: $m* \leftrightarrow n*$. Let the chemical $m*$ be the input reactant of the forward reaction +: $m* \rightarrow n*$. Similarly B is the input reactant of the backward reaction $-$:

$n^* \rightarrow m^*$. We also assume that m^* contributes as input reactant to another reaction 2: $m^* \rightarrow p$, but the other chemical n^* does not contribute as input to any other reactions. (4-1): If the reaction rate $k_\pm$ of any one of the reversible reactions $\pm$ is perturbed, then only δx_{n^*} will be nonzero. The direction of change depends on the direction of the perturbed reactions: $\pm\delta x_{n^*} > 0$ when the reaction rate $k_\pm$ is increased, respectively. However, neither x_{m^*} nor the concentration of any other chemical will change. Likewise, any fluxes remain unchanged.

(4-2): When we increase the reaction rate k_2 of the other, irreversible output arrow of reactant m^*, then the concentration of x_{m^*} will decrease, to compensate. At the same time the other reactant n^* of the reversible reaction will decrease, accordingly. Note that reactants m^*, n^* will both change in the same direction: $\delta x_{m^*} < 0$ and $\delta x_{n^*} < 0$. Again we encounter two compensating concentration changes. The net flux of the reversible reactions $w_\pm$ remains unchanged: $\delta w_+ - \delta w_- = 0$, with $\delta w_+ = \delta w_- < 0$. All remaining fluxes remain unchanged.

The response rules (1), (3) and (4) are proved from the patterns of nonzero entries in the $\mathbf{A}$-matrix. See Appendix A. We note some similarity between rules (3) and (4), i.e. the flux and concentration sensitivity responses to perturbations involving bimolecular reactions and reversible loops are closely related, and neither propagates into the remaining network.

4-2. Analysis of global patterns of flux responses

The flux sensitivity is given by (10) as $\Phi := \mathrm{id} + \left(r_{jm}\right)\delta x = \left(c_k^j\right)\mu$. We analyzed the patterns of flux responses in the system given by the distribution of

nonzero entries in the sensitivity matrix $\mathbf{S}$. For example, the flux sensitivity Φ of the network shown in Figure 3 is:

$$\Phi = \tilde{D}_1 \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
r_{8F}+r_{10F}+r_{13F} & r_{8F}-r_{10F} & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -2r_{8F}-r_{13F} & 0 & 0 & -r_{8F}-r_{10F} & 0 & 0 \\
r_{6C}(r_{8F}+r_{10F}+r_{13F}) & r_{6C}(r_{8F}-r_{10F}) & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{6C} & 0 & -r_{6C}(2r_{8F}+r_{13F}) & 0 & 0 & r_{6C}(-r_{8F}+r_{10F}) & 0 & 0 \\
r_{8F}+r_{10F}+r_{13F} & r_{8F}+r_{10F}+r_{13F} & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -2r_{8F}-r_{13F} & 0 & 0 & -r_{8F}+r_{10F} & 0 & 0 \\
r_{8F} & r_{8F} & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -2r_{8F} & 0 & 0 & -r_{8F} & 0 & 0 \\
R_1 & R_2+r_{5C}r_{10F} & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{5C}+r_{6C} & 0 & -R_8+r_{5C}r_{13F} & 0 & 0 & -R_2-r_{5C}r_{10F} & 0 & 0 \\
r_{10F} & r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & -r_{10F} & 0 & 0 \\
R_1-r_{5C}r_{10F} & R_2 & 0 & 0 & -r_{6C} & r_{5C} & 0 & r_{5C}+r_{6C} & 0 & -R_8 & 0 & 0 & -R_2 & 0 & 0 \\
r_{10F} & r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & -r_{10F} & 0 & 0 \\
r_{13F} & r_{13F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2r_{13F} & 0 & 0 & 2r_{10F} & 0 & 0 \\
r_{10F} & r_{10F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13F} & 0 & 0 & -r_{10F} & 0 & 0 \\
r_{13F} & r_{13F} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2r_{13F} & 0 & 0 & 2r_{10F} & 0 & 0
\end{pmatrix} D_2 \tag{19}$$

where $\tilde{D}_1 = \mathrm{diag}\left(1,1,1,1,1,r_{3C}^{-1},1,1,r_{3C}^{-1},1,r_{3C}^{-1},1,1,1,1\right)$, this time, and D_2, R_1, R_2 and R_8 were defined in (17). The column j^* and row j' indicates the response $\Phi_{j'j^*}$ of flux j' to a perturbation of reaction j^*. If we distinguish zero and nonzero flux responses, only, we obtain the following sparse pattern of nonzero entries:

$$\Phi = \begin{array}{c} \begin{array}{ccccccccccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 & 15 \end{array} \\ \begin{pmatrix} * & & & & & & & & & & & & & & \\ & * & & & & & & & & & & & & & \\ * & & & & & & & & & & & & & & \\ & * & & & & & & & & & & & & & \\ * & * & & & & & & * & & * & & & * & & \\ * & * & & & * & * & & * & & * & & & * & & \\ * & * & & & & & & * & & * & & & * & & \\ * & * & & & & & & * & & * & & & * & & \\ * & * & & & * & * & & * & & * & & & * & & \\ * & * & & & & & & & & * & & & * & & \\ * & * & & & * & * & & * & & * & & & * & & \\ * & * & & & & & & & & * & & & * & & \\ * & * & & & & & & & & * & & & * & & \\ * & * & & & & & & & & * & & & * & & \\ * & * & & & & & & & & * & & & * & & \end{pmatrix} \end{array}$$

(19')

Here "*" denotes any nonzero entry $\Phi_{j'j^*}$ of the flux response matrix Φ. Zero entries are omitted, for legibility.

There are only six different column patterns of nonzero entries among the 15 columns of the flux response matrix Φ, in our example. For example, an input perturbation in any of the columns $j^* \in \{3, 4, 7, 9, 11, 12, 14, 15\}$ produces an empty set $j' \in \{\ \}$ of nonzero flux responses $\Phi_{j'j^*} = *$. In other words, these columns j^* of the flux response matrix Φ are identically zero. We abbreviate this input $\rightarrow$ output response patterns as $\{3, 4, 7, 9, 11, 12, 14, 15\} \rightarrow \{\}$. Hence the input $\rightarrow$ output patterns

are the following: {3, 4, 7, 9, 11, 12, 14, 15} → {}, {5, 6} → {6, 9, 11}, {8} → {5, 6, …, 9, 11}, {10, 13} → {5, …, 15}, {1} → {1, 3, 5, …, 15}, {2} → {2, 4,…, 15}. Note that any output patterns in our list is strictly contained in all subsequent patterns, except for the last entry.

Let us now define the influence relation

$$j^* \to j' \qquad (20)$$

to indicate $\Phi_{j'j^*} \neq 0$. In other words, j^* influences j', in symbols: $j^* \to j'$, if and only if a perturbation of reaction j^* causes the flux j' to change. Our main observation, in all our examples, is the transitivity of flux influence:

$$j_1 \to j_2 \quad \text{and} \quad j_2 \to j_3 \quad \text{implies} \quad j_1 \to j_3. \qquad (21)$$

For monomolecular reaction networks, only, we prove transitivity of influence in a separate paper; see Fiedler and Mochizuki (2014), theorem 1.3. As a consequence of flux transitivity we are able to define the influence graph $j^* \Rightarrow j'$. See Figure 5 for an example. The vertices of the graph are the reactions $j \in \mathbf{E} = \{1,\ldots,15\}$. The oriented influence graph of Fig. 5 is to be read as follows. The influence $j_1 \to j_2$ holds true if, and only if, there exists a directed path of edges "$\Rightarrow$" from j_1 to j_2 in the influence graph. The response patterns arise as influence sets $I(j^*) := \left\{ j' \in \mathbf{E} \middle| \Phi_{j'j^*} \neq 0 \right\}$ of fixed $j^* \in \mathbf{E}$, i.e. as the subsets of $j' \in \mathbf{E}$ which are influenced by the same j^*. For example there are the seven

$j^* \in \{6,5,8,10,13,1,2\}$ with nonempty influence sets $I(j^*)$. The oriented edge $5 \Rightarrow 6$ and the self-loop $6 \Rightarrow 6$ implies $I(5) = I(6)$. The loop $10 \Rightarrow 13 \Rightarrow 10$ implies $I(10) = I(13)$. This provides the five different nonempty influence sets $I(5) = I(6)$, $I(8)$, $I(10) = I(13)$, $I(1)$, and $I(2)$ listed above. Even though the influence relation itself is unique, the minimal influence graph is not. For example we can omit either the edge $10 \Rightarrow 8$ or $13 \Rightarrow 8$ to represent the same influence relation $\rightarrow$.

In Figure 6 we provide another example to illustrate the hierarchy of flux responses. See also Fiedler and Mochizuki (2014), example 4 in section 6. The network includes a single vertical pathway and two feedbacks. This results in three cycles in the network, which produce the three basis vectors for the kernel $\ker S$ of the stoichiometric matrix S. We call this basis $\mathbf{c}_a$, $\mathbf{c}_b$, $\mathbf{c}_c$, this time. The components of each kernel vector are either 0 or 1. The support of each kernel vector, i.e. the reaction numbers j with nonzero components, is given by the cycles: (a)={1, 2, 3, 4, 5}, (b) ={2, 3, 6, 7} and (c)={3, 4, 8, 9}. The input→output patterns of the resulting flux responses are: {2, 3, 7, 9} → {}, {4, 6} → {2, 3, 6, 7}, {5, 8} → {2, 3, 4, 6, 7, 8, 9}, {1} → {1, 2, 3, 4, 5, 6, 7, 8, 9}. The three response patterns arise from the nontrivial influence sets $I(j^*)$, $j^* \in \{4,6,5,8,1\}$ via $I(4) = I(6)$ and $I(5) = I(8)$. The output parts of these patterns can be understood in terms of the directed cycles (a), (b), (c) as : {2, 3, 7, 9} → {}, {4, 6} → (b), {5, 8} → $(b) \cup (c)$, {1} → $(a) \cup (b) \cup (c)$.

In addition, we recall our motif rules (1) and (2): flux changes of networks only occur, when the perturbations are induced at branching outputs or at a reaction $0 \rightarrow \overline{y}_j$ here the reaction 1. We call such reactions "gates" here. The gates of our basis cycles

(a), (b), (c) are {1}, {4, 6} and {5, 8}, respectively. The basis cycle (c)={3, 4, 8, 9} includes the gate of basis cycle (b). Thus any perturbation $j^* \in \{5,8\}$ induces a flux change in (c), which in turn induces a flux change in (b). Similarly a perturbation in cycle (a) by its gate reaction 1 induces changes in (c) and (b) because the gates of (b) and (c) is included in the cycle (a). In this way, we understand the hierarchy of flux pattern as hierarchy of flux responses: $(a) \to (c) \to (b)$ to be a consequence of the hierarchic relation between cycles at their gates.

5. Metabolic network

As a real life application, we analyze chemical reaction system for the carbon metabolism in E. coli. We use a network presented in Ishii (2007), with minor modifications. In our example, we consider 28 metabolites and 43 reactions. The list of reactions and metabolites is shown in the Appendix B. The reaction network is illustrated in Figure 6. We constructed the $\mathbf{A}$-matrix of (8) and calculated the sensitivity matrix $\mathbf{S}$ of (9) as the negative inverse of $\mathbf{A}$.

The chemical reaction network is large and complex. Some responses are understood by the motif rules as shown in Table 1. We also analyzed the patterns of flux responses as shown in Figure 8. Again, we observe transitivity and clear hierarchy in the response patterns of fluxes; see Figure 9. The analysis of flux response patterns is summarized and shown in colors in Fig. 7. The flux influences of perturbation $j^* \in \mathbf{E}$ to nonzero responses at $j' \in \mathbf{E}$ can be simplified and summarized as follows. The fluxes are categorized into 6 color classes and there is a transitive hierarchical relation among them: "black" → "green", "green" → {"red", "yellow", "blue"}, "red" → "yellow",

"blue" → "light blue". The perturbation to any reactions j^* influences all other members j' in the same class, at least, and all members of classes downward of the perturbed class. It turns out that the sensitivity of the carbon metabolism system possesses reaction sets corresponding to the subsets of the pentose phosphate pathway (red, yellow) and the TCA cycle (blue, light blue). The perturbation to a reaction in these sets influences all members in the sets, but does not influence any others. On the other hand a perturbation to some reactions j^* in glycolysis (green) influence almost all reactions in the network. We emphasize that these insights result from our mathematical analysis of the sensitivity matrix $\mathbf{S}$ and of the flux response matrix Φ in (9), (10), only. No a priori biological knowledge of the metabolism is required, other than the network structure, to arrive at our conclusions.

Let us compare our results with a pioneering experiment for the E. coli system by Ishii et al, (2007). Unfortunately the experiment still lacks measurements of a large part of the network. We focus on some of the largest responses of measured metabolites among the systematic perturbation experiments. One of the largest response is an increase of "F6P" and "G6P" by a knockout of the enzyme *pfkA* mediating reaction 3: "F6P" → "F1,6P". This result is consistent with our analytical calculation: "F6P" and "G6P" decreases by an increase in the rate coefficient of reaction 3; see table 1, row "3" and column "e" and "c". We understand that this is explained by our motif rule (4-2). The other largest response is an increases of "Ru5P" and "R5P" by a knockout of enzyme *rpe* mediating reaction 12: "Ru5P" → "X5P". Again, this result is consistent with our analysis; see table 1, row "12" and columns "m", "o". Indeed "Ru5P" and "R5P" decrease by an increase in the rate coefficient of reaction 12. This result obeys and illustrates our motif rule (2-2).

6. Discussion

We presented a method to analyze the rate sensitivity of chemical reaction networks in a structural and function-free manner: a large part of the sensitivity responses was determined from the structure of the network alone. We introduced the symbolic $\mathbf{A}$-matrix which is determined by the network and its stoichiometry, only. The $\mathbf{A}$-matrix does not depend on the specific choices of reaction functions or their rate parameters, except for some basic monotonicity requirement (1.d). The negative inverse matrix of $\mathbf{A}$ provides the sensitivity matrix $\mathbf{S}$. See (8), (9). A system exhibits characteristic responses depending on the structure of the network and the position j^* of the rate perturbation in the network. We determined some response patterns (motif rules) from the structure of the network and probed the network response, based on the $\mathbf{A}$-matrix. We also analyzed patterns of nonzero responses of fluxes and discovered a clear hierarchy in the flux response. We analyzed some hypothetical networks and the complex real life network of the E. coli metabolism.

Our function-free structural approach makes predictions on the responses of chemical reaction systems, based on their network structure. The method uses information on the reaction network and its stoichiometric coefficients, only, to calculate the sensitivity. Our results do not depend on other quantitative measurements of the systems, like reaction rates, degradation rates, or initial concentrations of the metabolites. On the other hand, our knowledge of chemical reaction networks of many organisms is possibly incomplete, at present. We rather expect to use our theory as a tool to reveal unknown reactions or unknown regulations of chemical networks by combining theory with specific experimental measurements of sensitivity. For example,

we may calculate the sensitivity for hypothetical modifications which introduce, or omit, some reactions or regulations, and may then compare with specific experiments. If such modifications of a "known" network considerably enhance agreement with experimental results, then we may expect the modified network to actually represent the real systems. Disagreement, however, will falsify a network outright -- without any remaining excuses as to more "suitable" reaction functions or fudgy rate constants.

Our assumptions are of a qualitative nature. Besides positivity (1.d) and continuous differentiability of the reaction rates we assume some mild algebraic nondegeneracy of certain Jacobi matrices, to distinguish responses of zero from nonzero sensitivity; see (4) in Section 2. To distinguish concentration or reaction flux increase from decrease, we also require some monotonicity in the form of strictly positive partial derivatives of reaction rates with respect to their reactants; see (1.d) in Section 2. Although strictly negative derivatives, i.e. inhibitory next to excitatory couplings, are equally tractable we do not pursue this generalization here. See also our more mathematical inclined companion paper (Fiedler and Mochizuki, 2014) for a more detailed discussion of these assumptions.

We have developed a function-free approach to sensitivity. Nevertheless, we observed that the responses of chemical reaction systems follow characteristic patterns which only depend on the stoichiometric structure of the network. Some structures are shown to "buffer" the perturbation, and as a result the fluxes do not change at all (motif rules 1, 3, 4). The effect of a perturbation at reaction j^* may also propagate from the lower part to the upper part of a network through feedback reactions. See e.g. Fig. 3 and Fig. 6. The influence region $j' \in I(j^*)$ is mediated by the kernel of the stoichiometric matrix S, which reflects the stoichiometric structure of the network.

The influence $j^* \rightarrow j'$ propagates transitively, in all our examples, and the response patterns follow a corresponding hierarchy. Some of these behaviors are explained analytically, but on an intuitive level, in this paper. We conclude that the equilibrium response of chemical reaction systems obeys strong restrictions as outlined in Section 3-2. Chemical reaction systems are bound to exhibit correspondingly characteristic behavior.

We repeat that our analysis applies to large disturbances of reaction rates. Of course, the mathematical approach is based on the local implicit function theorem via partial derivatives of the reaction functions. However, we can continue to apply our analysis as long as these partial derivatives do not change signs, and as long as equilibria persist. We are not aware of any other results of comparably global scope and impact, except the monumental contributions by Feinberg.

Our method does apply, not only to pure chemical reaction systems but also, to systems including regulatory linkages. For example, "allosteric regulation" is an important topic in metabolic systems, where a product (small molecule) of a reaction may regulate the function of an enzyme to control, not only some other reactions, but even the rate of its own production. Such regulatory effects can be studied by integrating additional nonzero entries in the dependence matrix.

On the other hand, there are limitations to our method. We need to assume the existence of equilibria because our method only determines the response of systems near an equilibrium. We have to examine existence of equilibrium separately by other methods. For some advanced uniqueness and multiplicity results see Feinberg (1995), Shinar and Feinberg (2013). In particular, some networks may possess multiple, coexisting equilibria. An entry $\pm$ of the sensitivity matrix means that the sign of the

change δx_m^{j*} of some focal chemical X_m cannot be determined from the network alone. To determine such a sign requires quantitative information on partial derivatives r_{jm} of the reaction functions. For example, the sensitivity matrix of example 3-3 includes some $\pm$ entries. However, the signs of all such terms depend on the common factor $(r_{8F} - r_{10F})$. This means that we obtain a sign determinate sensitivity response matrix just by measuring the relative magnitude of the partial derivatives of two reaction functions, w_8 and w_{10}, with respect to the single chemical *F*.

Similarly, the sign of $\det \mathbf{A}$ itself may already depend on quantitative information on the partial derivatives r_{jm}. Indeed $\det \mathbf{A}$ is a polynomial of degree M in these variables, jointly, where each nonvanishing term r_{jm} enters at most linearly. If all coefficients of the monomials of $\det \mathbf{A}$ are of the same sign $\sigma = \pm 1$, then $\operatorname{sign} \det \mathbf{A} = \sigma \neq 0$ because all nonvanishing r_{jm} are positive. However, different signs of the coefficients may well occur. Then $\det \mathbf{A}$ depends on quantitative information on the partial derivatives r_{jm}. Equilibrium bifurcations may in fact be heralded by $\det \mathbf{A} = 0$, though only for an algebraic variety of codimension 1 in the space of nonvanishing r_{jm}. The resulting equilibrium bifurcations, in the simplest case of saddle-node type, will feature both signs of $\det \mathbf{A}$. In particular all nonzero sensitivity responses δx_m and μ_k, as well as all nonzero flux changes, will typically be of opposite sign on the two equilibrium branches which join at a saddle-node bifurcation. Still, some sign determinacy will prevail, synchronously in many entries of the sensitivity and flux response matrices. At present however we have not investigated such examples.

We have introduced two analytical methods to understand sensitivity behavior. We can analyze the local behavior by the motif rules, and the global response by a hierarchy of flux response patterns. We were able to prove some motif rules analytically. However, our explanation of hierarchy patterns is still intuitive. Only in the monomolecular case, so far, we are able to prove the observed transitivity of the influence relation "perturbation of reaction $j*$ implies flux change in reaction j'", $j* \rightarrow j'$, which underlies the hierarchy of flux response patterns and their relation to directed cycles in the reaction network. See Fiedler Mochizuki (2014), because the mathematical details are beyond the scope of our present exposition.

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Appendix A

In the following we discuss the patterns of sensitivity determined from the "local" structure of a network. As we have shown in (9), the sensitivity matrix $\mathbf{S}$ is given by the negative inverse of the matrix $\mathbf{A}$. In other words, the entry s_{mj} of $\mathbf{S}$, for chemical $m \in \mathbf{M}$ and reaction $j \in \mathbf{E}$, is given by:

$$s_{mj} = (-1)^{j+m+1} \det \mathbf{A}^{\vee}_{jm} \Big/ \det \mathbf{A} . \qquad \text{(A3)}$$

Here $\mathbf{A}^{\vee}_{jm}$ denotes the j,m-minor of $\mathbf{A}$, which omits row j and column m from $\mathbf{A}$.

Let us focus on row vectors of the $\mathbf{A}$-matrix $\mathbf{A} = \left(\mathbf{R} \mid \mathbf{C}\right)$, which correspond to reactions. We call the left part $\mathbf{R} = \left(r_{jm}\right)$ and the right part $\mathbf{C} = \left(c_k^j\right)$ of $\mathbf{A}$ the "dependence part" and the "kernel part", respectively. Of course, the kernel part consists of a basis $\mathbf{c}_k$ of the kernel $\ker S$ of the stoichiometric matrix S. The dependence part is determined by the partial derivative r_{jm} of the reaction functions w_j with respect to the metabolite concentrations x_m. In the following we mainly focus on the nonzero entries of the dependence part.

(1) single pathways without branching

In a reaction network, consider a chemical X_{m^*} which has a single outgoing reaction arrow j^* of a monomolecular reaction. Then the m^* column vector of the $\mathbf{A}$-matrix possesses a single nonzero entry $r_{j^*m^*} > 0$. This entry appears in row j^*,

as shown below.

$$\mathbf{A} = \left(\begin{array}{ccccc|c} & & 0 & & & \\ & & \vdots & & & \\ 0 & \cdots & r_{j^*m^*} & 0 & \cdots & \mathbf{C} \\ & & 0 & & & \\ & & \vdots & & & \end{array}\right)$$

The determinant $\det \mathbf{A}^{\vee}_{j^*m}$ of the j^*m-minor $\mathbf{A}^{\vee}_{j^*m}$ will be 0 for all $m \neq m^*$, because the m^*-column of $\mathbf{A}^{\vee}_{j^*m}$ will be the null vector. This implies that the responses $\delta x_m^{j^*}$ of all chemicals $m \neq m^*$ to the perturbation of reaction j^* are zero. On the other hand, our assumption $\det \mathbf{A} \neq 0$ implies $\det \mathbf{A}^{\vee}_{j^*m^*} = (-1)^{j^*+m^*} r_{j^*m^*}^{-1} \det \mathbf{A} \neq 0$. In conclusion the perturbation to reaction j^* whose reactant m^* does not possesses any other output arrows affects only the reactant m^*. In particular all fluxes remain unchanged.

(2) bimolecular reactions

Suppose that reaction j^* is a bimolecular reaction j^*: $X_{m^*} + X_{n^*} \rightarrow X_p$, and that chemical m^* contributes to one other, not necessarily monomolecular reaction $j \neq j^*$. Also assume n^* is a reactant for j^*, only. The corresponding $\mathbf{A}$-matrix is the following:

$$\mathbf{A} = \left(\begin{array}{ccccc|c} & & 0 & 0 & & \\ & & \vdots & \vdots & & \\ 0 & \cdots & r_{j^*m^*} & r_{j^*n^*} & 0 & \mathbf{C} \\ & & 0 & 0 & & \\ & & r_{jm^*} & \vdots & & \\ & & 0 & 0 & & \end{array} \right),$$

where the kernel parts of rows j^* and j may differ, of course.

From the matrix $\mathbf{A}$ we derive some rules for responses to perturbations of the bimolecular reaction j^*. First, $\det \mathbf{A}^{\vee}_{j^*m} = 0$ for all $m \neq n^*$, because the n^*-column of $\mathbf{A}^{\vee}_{j^*m}$ vanishes. This implies that a perturbation of the bimolecular reaction j^* will not affect any chemicals m other than n^*. This implies that δx_{n^*} alone will compensate for the perturbation j^*, and therefore all fluxes will remain unchanged; see Fig. 4 (3-1).

Second consider a perturbation to reaction j, which is the other outgoing reaction branch from reactant chemical m^*. Then $\det \mathbf{A}^{\vee}_{jm} = 0$ for all $m \notin \{m^*, n^*\}$ by linear dependence of the remaining columns m^* and n^* of $\mathbf{A}^{\vee}_{jm}$. This means that the responses of all chemicals are zero except for the reactants m^* and n^* of the bimolecular reaction j^* when the perturbation is applied to the other side branch j of the reactant m^*. Again all flux changes vanish.

(3) reversible reactions

Suppose that reaction $j^+ : X_{m^*} \rightarrow X_{n^*}$ is reversible, and that chemical m^*

contributes as reactant to one other reaction j. Also assume that $n^* \neq m^*$ contributes to the reverse reaction j^-: $X_{n^*} \to X_{m^*}$, only. Then the corresponding $\mathbf{A}$-matrix takes the following shape:

$$\mathbf{A} = \left(\begin{array}{ccccc|c} & & 0 & 0 & 0 & \\ & & \vdots & \vdots & \vdots & \\ 0 & \cdots & r_{j^+m^*} & \vdots & 0 & \\ 0 & \cdots & 0 & r_{j^-n^*} & 0 & \mathbf{C} \\ & & \vdots & 0 & \vdots & \\ & & r_{jm^*} & \vdots & & \\ & & 0 & \vdots & & \end{array}\right),$$

where all r-entries are positive. We derive rules (4-1) and (4-2) on the sensitivity of reversible reactions as follow. First, we study the basis vectors $\left(c_k^j\right)_{j\in\mathbf{E}}$ in the kernel of the stoichiometric matrix S. By (1.c) they satisfy Kirchhoff's law

$$0 = \left(c_k^{j^+} - c_k^{j^-}\right)\left(\overline{y}^{j^+} - y^{j^+}\right) + \sum_{j\notin\left\{j^+,j^-\right\}} c_k^j\left(\overline{y}^j - y^j\right),$$

for all $1 \le k \le K = \dim\ker S$, because $\overline{y}^{j^\pm} = y^{j^\mp}$. We may therefore choose a basis $\mathbf{c}_k$ such that

$c_k^{j^-} = -c_k^{j^+}$, for $1 \le k < K$, and

$$c_K^j = \begin{cases} 1 & \text{for} \quad j \in \left\{j^+, j^-\right\}, \\ 0 & \text{otherwise.} \end{cases}$$

Therefore the $\mathbf{A}$-matrix above takes the more specific form

$$\mathbf{A} = \left(\begin{array}{ccccccc|ccc|c} r_{11} & \cdots & 0 & \cdots & 0 & \cdots & r_{1M} & & & & 0 \\ \vdots & & \vdots & & \vdots & & \vdots & & & & \vdots \\ 0 & \cdots & r_{j^+m^*} & \cdots & 0 & \cdots & 0 & c_1^{j^+} & \cdots & c_{K-1}^{j^+} & 1 \\ 0 & \cdots & 0 & \cdots & r_{j^-n^*} & \cdots & 0 & -c_1^{j^+} & \cdots & -c_{K-1}^{j^+} & 1 \\ \vdots & & \vdots & & \vdots & & \vdots & & & & 0 \\ 0 & \cdots & r_{jm^*} & \cdots & 0 & \cdots & 0 & & & & \vdots \\ \vdots & & \vdots & & \vdots & & \vdots & & & & \\ r_{E1} & \cdots & 0 & \cdots & 0 & \cdots & r_{EM} & & & & 0 \end{array}\right).$$

Now consider a perturbation of reaction j^+ and let $m \neq n^*$. Then

$$\det \mathbf{A}^{\vee}_{j^+m} = 0$$

because the column n^* of $\mathbf{A}$ and the last column K of the kernel-part $\mathbf{C}$ become linearly dependent. Therefore $\delta x_m = 0$ and in particular $\delta x_{m^*} = 0$. This proves that the forward and backwards flux changes of the reactions $j^{\pm}$ cancel, and all (net) fluxes remain unchanged. Moreover $\delta x_{n^*} > 0$ and claim (4-1) is proved; see Fig. 4 (4) left.

The argument for a perturbation of the reverse reaction j^- is even easier, because we obtain a nullvector in column n^*, for $\det \mathbf{A}^{\vee}_{j^-m}$ and any $m \neq n^*$. Since n^* is a single-exit vertex, our above discussion of case (1) also applies, directly.

As our final case, Fig. 4 (4) right, let us consider a perturbation of the side branch reaction j of metabolite $m*$. For any $m \notin \{m*, n*\}$ we then obtain

$$\det \mathbf{A}^{\vee}_{jm} = 0\,,$$

because the remaining columns $m*$ and $n*$ are linearly dependent with the last column $\mathbf{c}_K$ of the kernel-part. Indeed all three columns of $\mathbf{A}^{\vee}_{jm}$ now possess nonzero entries in only the two rows j^+ and j^-. This proves our final claim on reversible reactions.

Appendix B

The list of the 43 reactions of the E. coli metabolism is shown below. The metabolites are denoted by their abbreviated names in quotation marks. The reactions are labeled by numbers. Forward and reverse reactions are distinguished by epithets a and b. For example, the first line indicates that one molecule of the reactants Glucose and PEP together produce one molecule G6P and one molecule PYP. Degradations of the three chemical products, Acetate, Lactate, Ethanol are required for equilibria of the dynamics to exist. We ignored these three degradations because the response of the system to their perturbation is obvious (motif rule 1).

(1) "Glucose" + "PEP" → "G6P" + "PYR"

(2a) "G6P" → "F6P"

(2b) "F6P" → "G6P"

(3) "F6P" → "F1,6P"

(4) "F1,6P" → "G3P" + "DHAP"

(5) "DHAP" → "G3P"

(6) "G3P" → "3PG"

(7a) "3PG" → "PEP"

(7b) "PEP" → "3PG"

(8a) "PEP" → "PYR"

(8b) "PYR" → "PEP"

(9) "PYR" → "AcCoA" + "CO2"

(10) "G6P" → "6PG"

(11) "6PG" → "Ru5P" + "CO2"

(12) "Ru5P" → "X5P"

(13) "Ru5P" → "R5P"

(14a) "X5P" + "R5P" → "G3P" + "S7P"

(14b) "G3P" + "S7P" → "X5P" + "R5P"

(15a) "G3P" + "S7P" → "F6P" + "E4P"

(15b) "F6P" + "E4P" → "G3P" + "S7P"

(16a) "X5P" + "E4P" → "F6P" + "G3P"

(16b) "F6P" + "G3P" → "X5P" + "E4P"

(17) "AcCoA" + "OAA" → "CIT"

(18) "CIT" → "ICT"

(19) "ICT" → "2-KG" + "CO2"

(20) "2-KG" → "SUC" + "CO2"

(21) "SUC" → "FUM"

(22) "FUM" → "MAL"

(23a) "MAL" → "OAA"

(23b) "OAA" → "MAL"

(24a) "PEP" + "CO2" → "OAA"

(24b) "OAA" → "PEP" + "CO2"

(25) "MAL" → "PYR" + "CO2"

(26) "ICT" → "SUC" + "Glyoxylate"

(27) "Glyoxylate" + "AcCoA" → "MAL"

(28) "6PG" → "G3P" + "PYR"

(29) "AcCoA" → "Acetate"

(30) "PYR" → "Lactate"

(31) "AcCoA" → "Ethanol"

(32) "R5P" → (degradation)

(33) "OAA" → (degradation)

(34) "CO2" → (degradation)

(35) (input) → "Glucose"

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Table 1: Sensitivity of metabolite network of E. coli.

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	
1	−																									1
2a	±	±	−	±	+	+	±	+	±	±	±	−	−	±	±	±	±	±	±	±	±	±	±	±	±	
2b	±	±	+	±	−	−	±	−	±	±	±	+	+	±	±	±	±	±	±	±	±	±	±	±	±	
3	±	±	−	±	−	+	±	+	±	±	±	−	−	±	±	±	±	±	±	±	±	±	±	±	±	
4						−																				1
5								−																		1
6							−							±		±	±									4
7a									−																	4
7b									+																	4
8a	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
8b	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
9	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
10	±	±	−	±	−	−	±	−	±	±	±	+	+	±	±	±	±	±	±	±	±	±	±	±	±	
11	±	±	+	±	+	+	±	+	±	±	±	−	+	±	±	±	±	±	±	±	±	±	±	±	±	
12	±	±	+	±	+	+	+	+	±	±	±	+	−	±	−	±	±	±	±	±	±	±	±	±	±	
13	±	±	−	±	−	−	−	−	±	±	±	−	−	±	+	±	±	±	±	±	±	±	±	±	±	
14a														−		+	+									4
14b														+		−	−									4
15a														−		−	+									4
15b														+		+	−									4

16a														–		–	–								4
16b														+		+	+								4
17	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
18																			–							1
19	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
20																					–					1
21																						–				1
22																							–			1
23a	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
23b	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
24a	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
24b	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
25	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
26	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
27																									–	3
28	±	±	–	±	–	–	±	–	±	±	±	–	–	±	±	±	±	±	±	±	±	±	±	±	±	
29	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
30	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
31	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
32														+	–	–	–									4
33	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
34	±	±		±					±	±	±							±	±	±	±	±	±	±	±	
35	±	±	+	±	+	+	+	+	±	±	±	+	+	±	±	±	±	±	±	±	±	±	±	±	±	

The rows $j*$ and columns m indicate perturbed reactions $j*$ and concentration responses δx_m^{j*} of chemicals X_m, respectively. The numbers in the leftmost column indicate the reactions shown in Appendix B and Figure 7. The alphabet in the top row indicates the species of metabolites; a: "Glucose", b: "PEP", c: "G6P", d: "PYR", e: "F6P", f: "F1,6P", g: "G3P", h: "DHAP", i: "3PG", j: "AcCoA", k: "CO2", l: "6PG", m: "Ru5P", n: "X5P", o: "R5P", p: "S7P", q: "E4P", r: "OAA", s: "CIT", t: "ICT", u: "2-KG", v: "SUC", w: "FUM", x: "MAL", y: "Glyoxylate". The system includes 28 metabolites, though we omit the three products Acetate, Lactate and Ethanol, because they only exit the network. The sign (+, −) indicates increase or decrease of chemicals in response to the perturbation of the corresponding reaction. Zero entries indicating no change are omitted, for legibility. The "±" mark indicates nonzero chemical concentration changes δx_m^{j*} to the perturbation of reaction $j*$, generically, but the sign will depend on numerical, rather than symbolic, values. The rightmost column indicates possible explanations by motifs; 1: motif (1), 3: motif (3) , 4: motif (4).

Figure Legends

Figure 1 Sensitivity analysis of a single reaction pathway of chemical reactions with feed 1 and exit reaction 4. (a-d) Changes in concentrations and fluxes induced by perturbations of reaction rates k_1 to k_4 from top (a) to bottom (d). Perturbed reactions $j^* = 1,\ldots,4$ are indicated by red triangles. Plus, minus, or zero next to circles indicate increase, decrease or no change in the associated concentrations of the chemicals $A,\ B,\ C$, respectively. Red bold circles and arrows indicate increases in concentrations and fluxes. Red dashed circles (and arrows) indicate decrease in concentrations (and fluxes).

Figure 2 Sensitivity analysis of a simple network including one feedback. (a-f) Perturbed reactions are $j^* = 1,\ldots,6$ indicated by red triangles. Changes in concentrations and fluxes induced by perturbations of reaction rates $k_1,\ldots,k_6$ are indicated. See also the legend of Fig. 1.

Figure 3 Sensitivity analysis of a hypothetical network with one bimolecular reaction $j = 12$. Changes in concentrations and fluxes induced by perturbations of reactions $j^* = 5$(a), 6(b), 8(c), 10(d), 11(e) and 12(f). For $j^* = 3$, 4, 7, 9, 14, and 15, only the input reactants x_{m^*} of the perturbed reaction j^* change; see section 4-1. The concentration responses of chemicals *C*, *D*, *E*, *G*, *H*, to a perturbation of reaction $j^* = 13$ are indeterminate. See also the legend of Fig. 1.

Figure 4 Examples of network motifs of chemical reactions and patterns of

sensitivity response. Legend as in Fig. 1.

Figure 5 The transitive influence graph of nonzero response patterns of the hypothetical network shown in Fig. 3. The vertex numbers indicate reactions. Any directed path of arrows from $j*$ to j' indicates the direct influence from $j*$ to j'; i.e. $\Phi_{j'j*} \neq 0$. The set of output responses j' for each input $j*$ is the set of vertices j' which can be reached from $j*$ by a directed path. For example, a perturbation to reaction $j* = 8$ causes changes in reactions $j' \in \{5, 6, 7, 8, 9, 11\}$.

Figure 6 Example of a network with two feedback cycles, (a), and the corresponding hierarchy of response patterns, (b). (a) The network includes one vertical pathway (a) and two feedback cycles (b) and (c). (b) The influence graph of nonzero response patterns of the network shown in (a). Labels and arrows are used as in Fig. 5.

Figure 7 Reaction network of the carbon metabolism TCA cycle of E. coli. We omit some less important degradations which actually exist. The complete set of reactions is shown in Appendix B. Colors summarize the influence patterns of flux responses shown in Fig. 9 with some details omitted. The flux influence patterns are summarized as: i) yellow → yellow, ii) red → {red, yellow}, iii) brown → {red, yellow}, iv) light blue → light blue, v) blue → {blue (including dashed blue), light blue}, vi) green → {green (including dashed), blue, light blue, brown, red, yellow}, vii) black → {black (including dashed), green, blue, light blue, brown, red, yellow}. The differences of responses between perturbations to forward and backward reactions of 7 and 16 are

not shown. See Fig. 9 for exact patterns.

Figure 8 The flux sensitivity matrix $\Phi_{j'j*}$ of the E. coli carbon TCA cycle metabolism. Columns and rows indicate perturbed reactions $j*$ and flux responses j', respectively. The enumerator of reactions follows that shown in Appendix B and Figure 7. The signs (+, −) indicate increase or decrease or no change of flux responses. Zero entries are omitted, for legibility. The "±" mark indicates nonzero flux changes to the perturbation, generically, but the direction depends on numerical values and cannot be determined.

Figure 9 Hierarchy of nonzero response patterns of the carbon metabolism network shown in Fig. 7. Labels and arrows are used in the same way as in Fig. 5 and 6.

Fig. 1

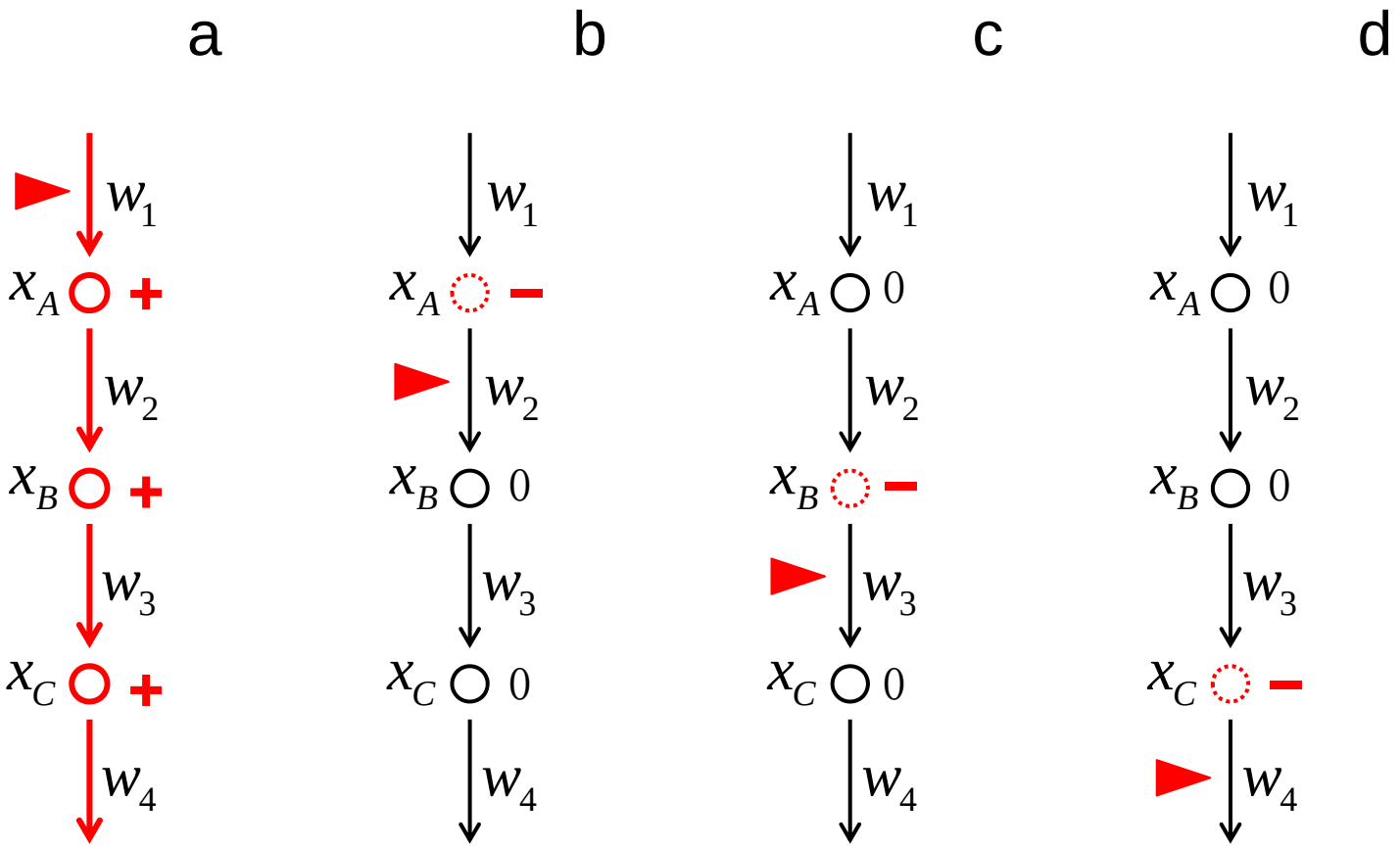


Fig. 2a-c

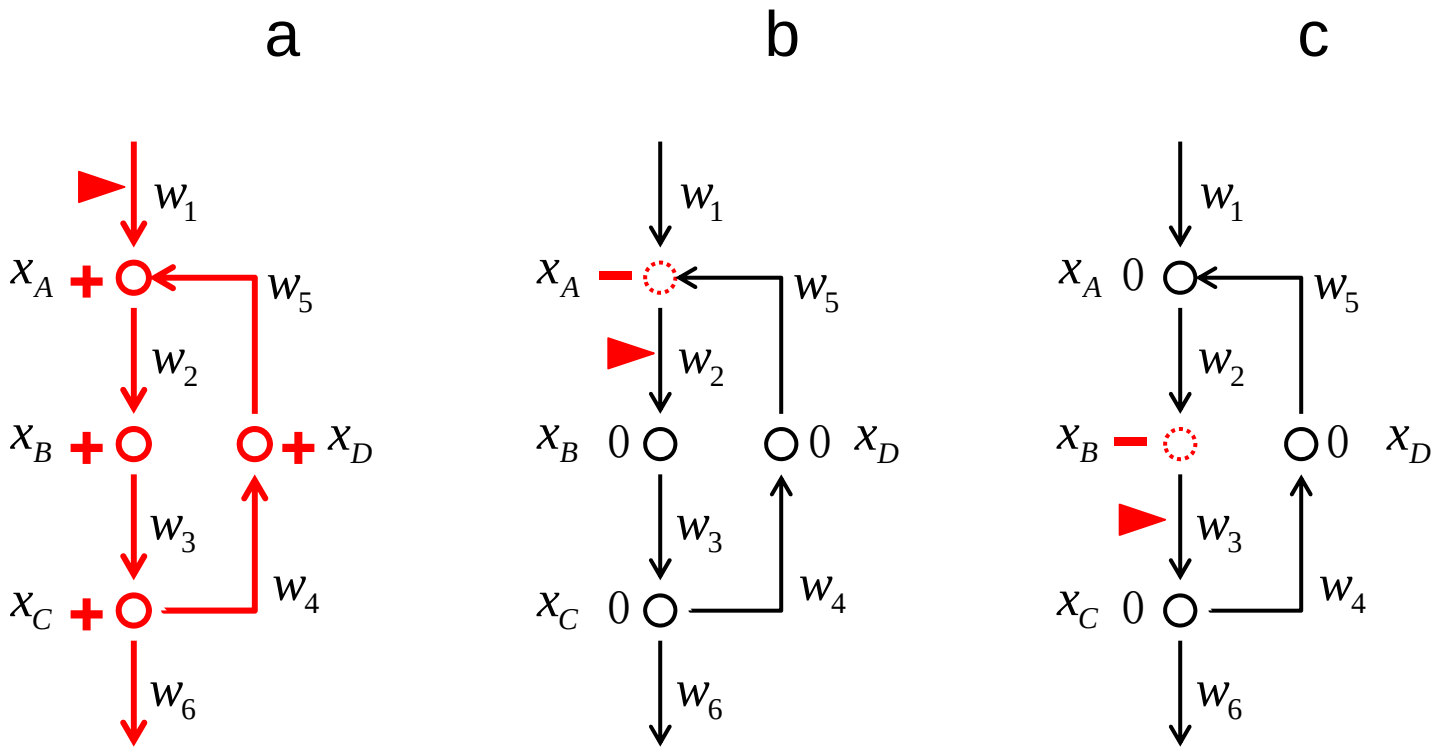


Fig. 2d-f

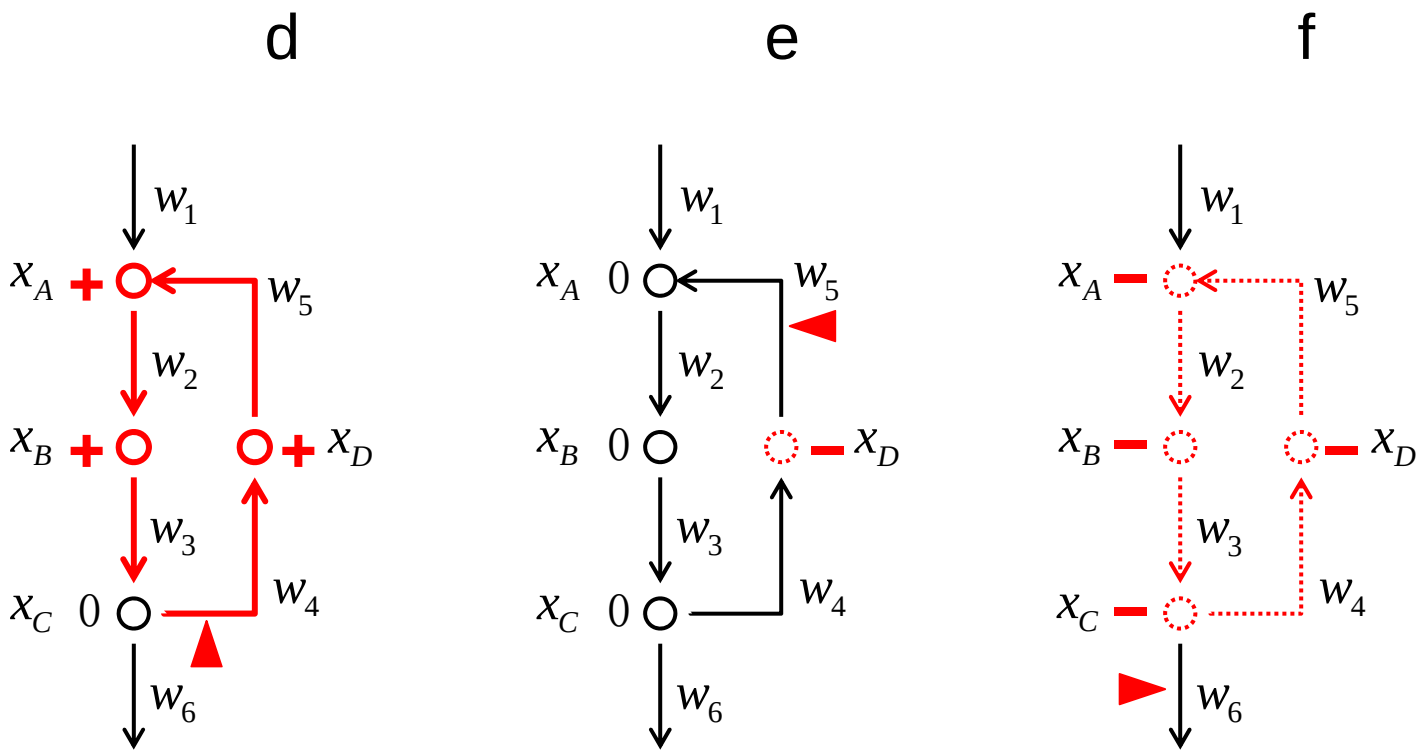


Fig. 3a-c

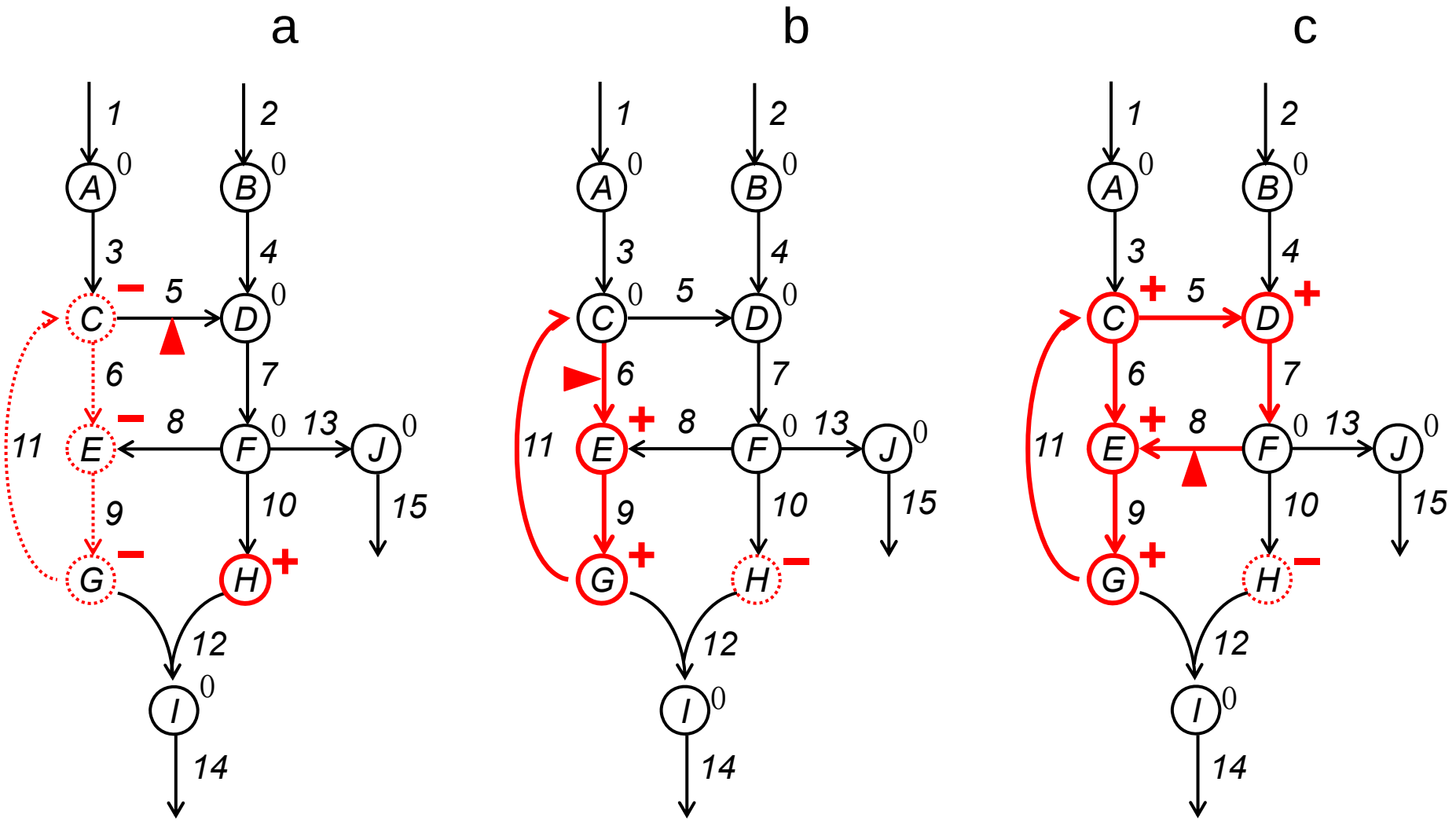


Fig. 3d-f

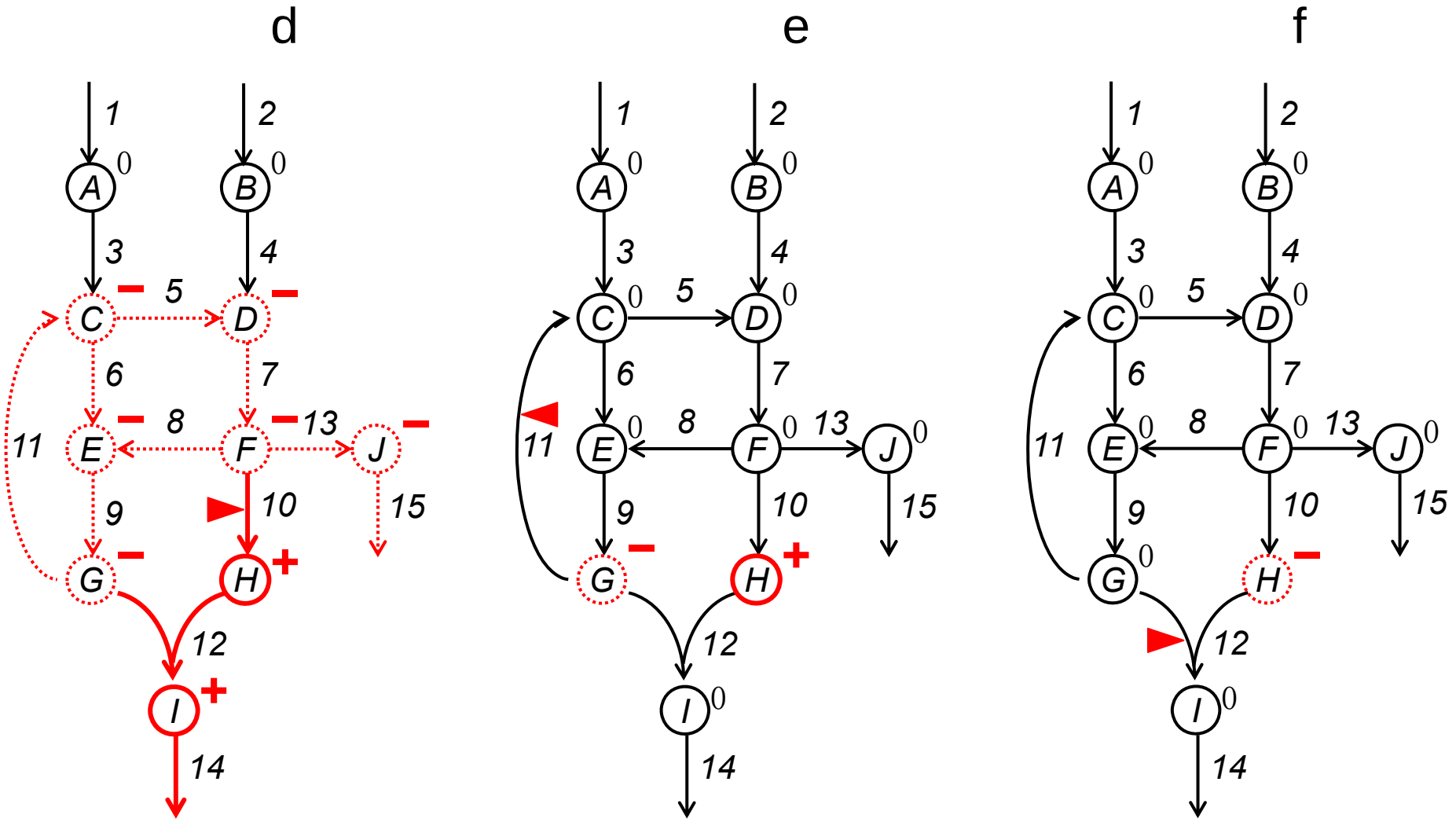


Fig. 4

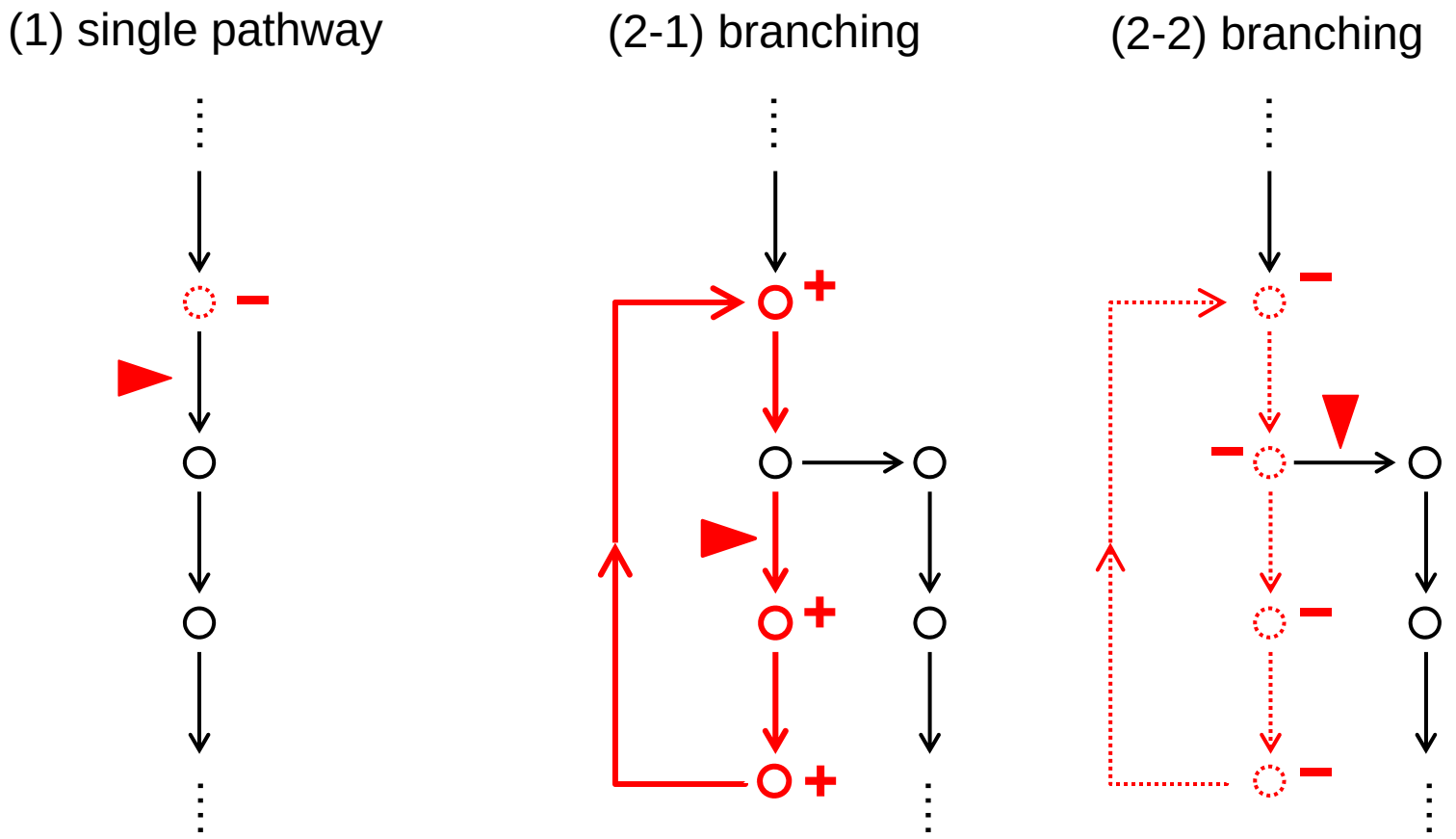
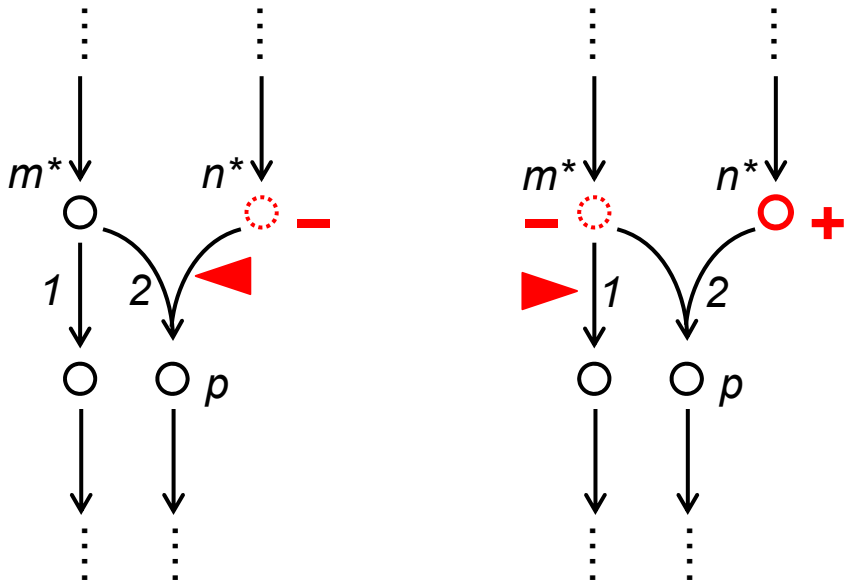


Fig. 4

(3) bimolecular reaction



(4) reversible reaction

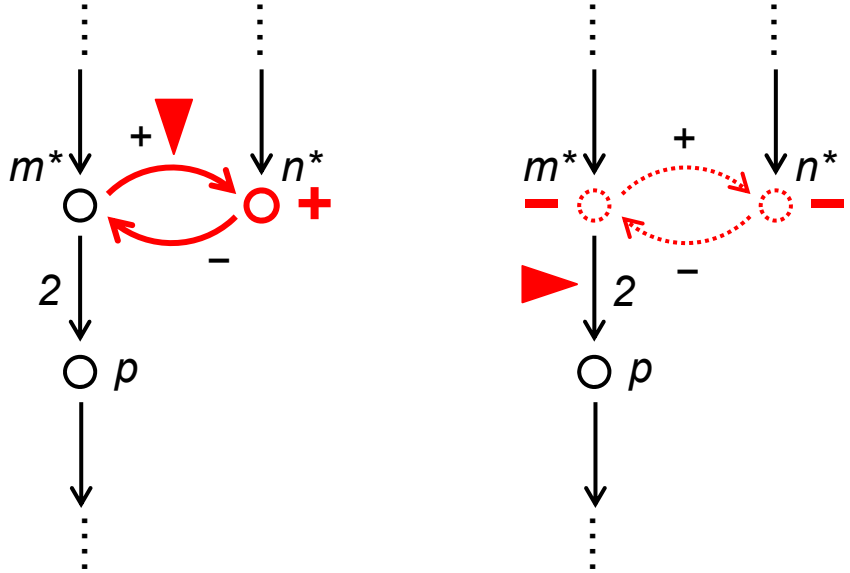


Fig. 5

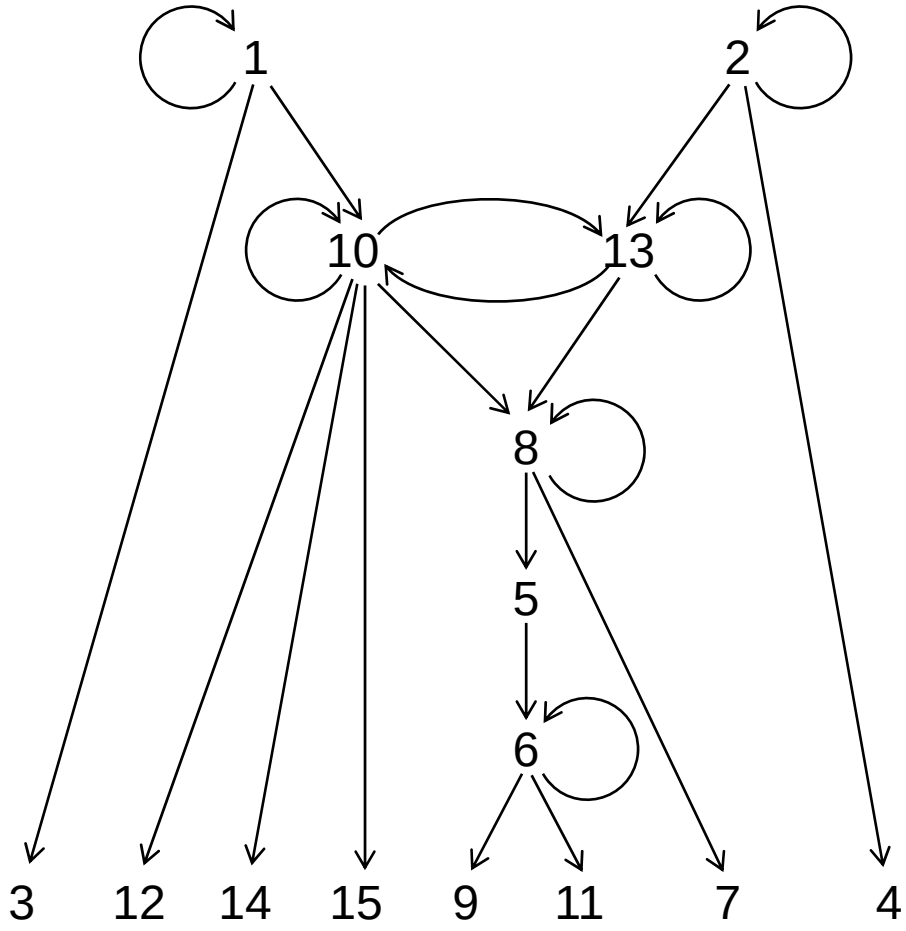
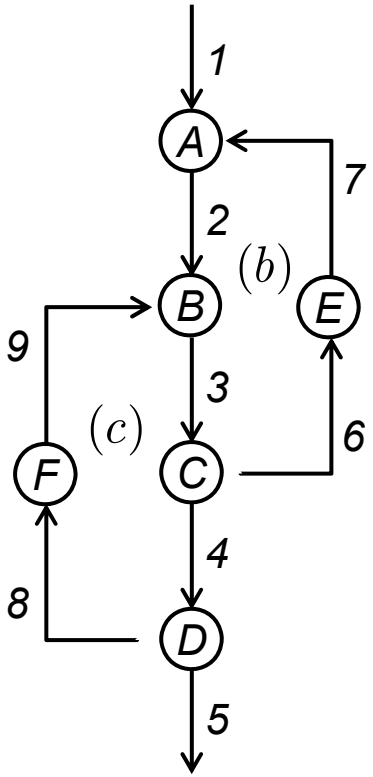


Fig. 6

a



b

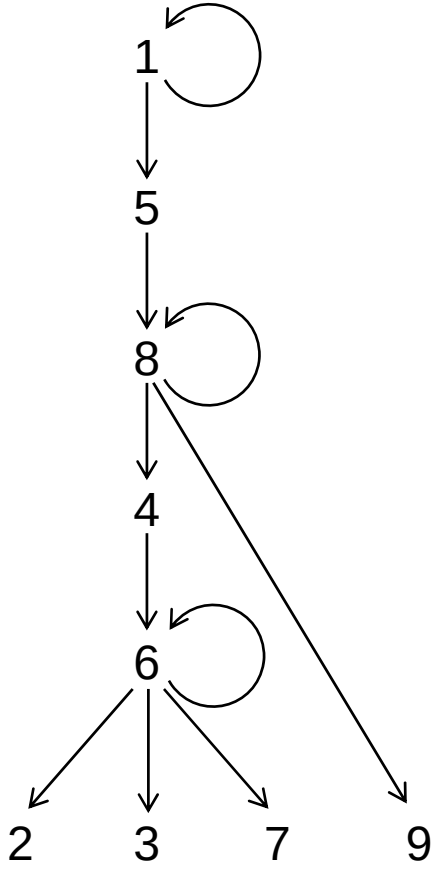
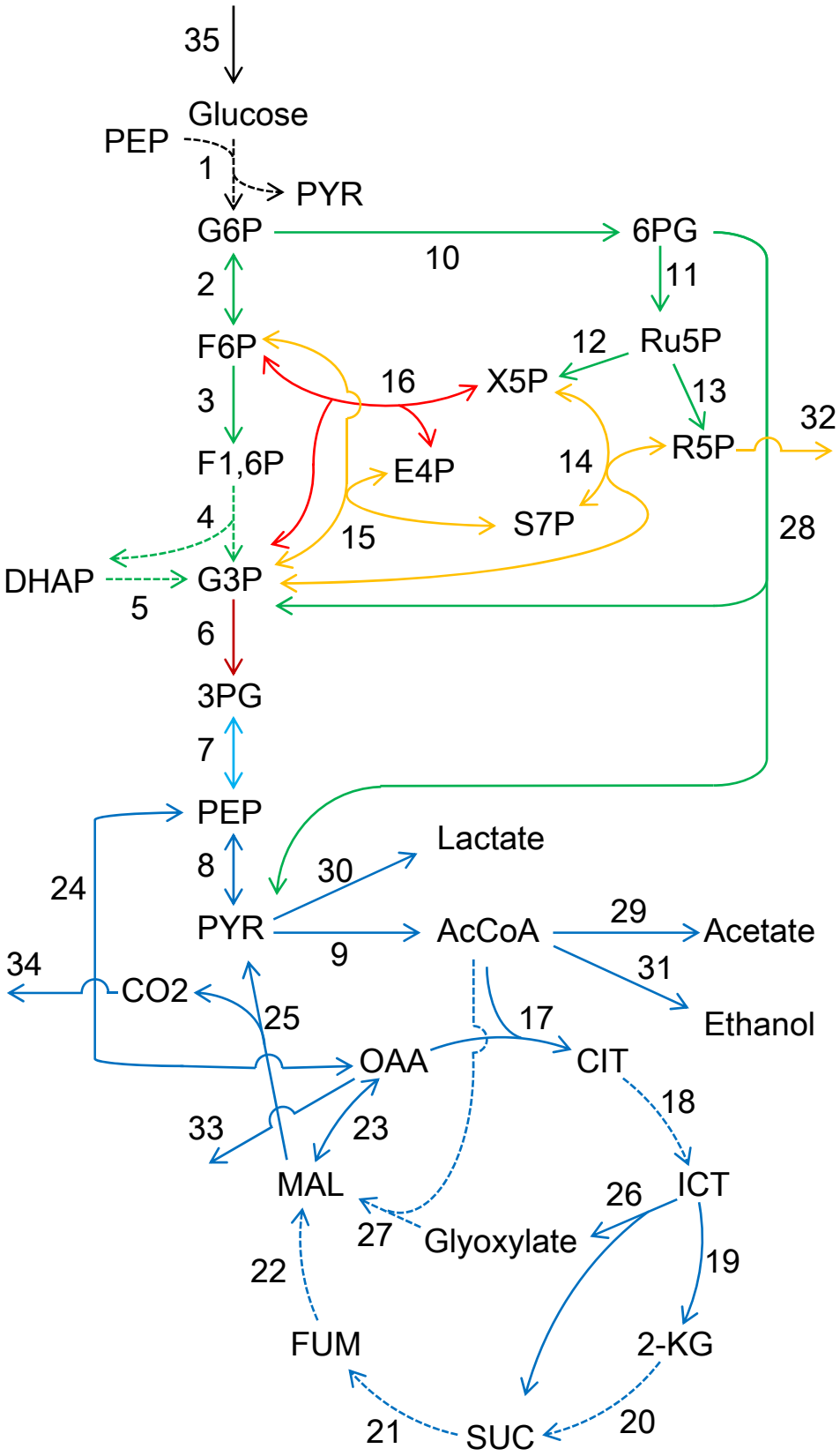


Fig. 7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PYR
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6PG
2
11
F6P
12
Ru5P
16
X5P
3
13
32
F1,6P
E4P
14
R5P
4
S7P
28
15
DHAP
G3P
5
6
3PG
7
PEP
Lactate
8
30
24
PYR
AcCoA
29
Acetate
9
34
31
CO2
25
17
Ethanol
OAA
CIT
18
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MAL
26
ICT
27
Glyoxylate
22
19
FUM
2-KG
21
SUC
20

Fig. 8

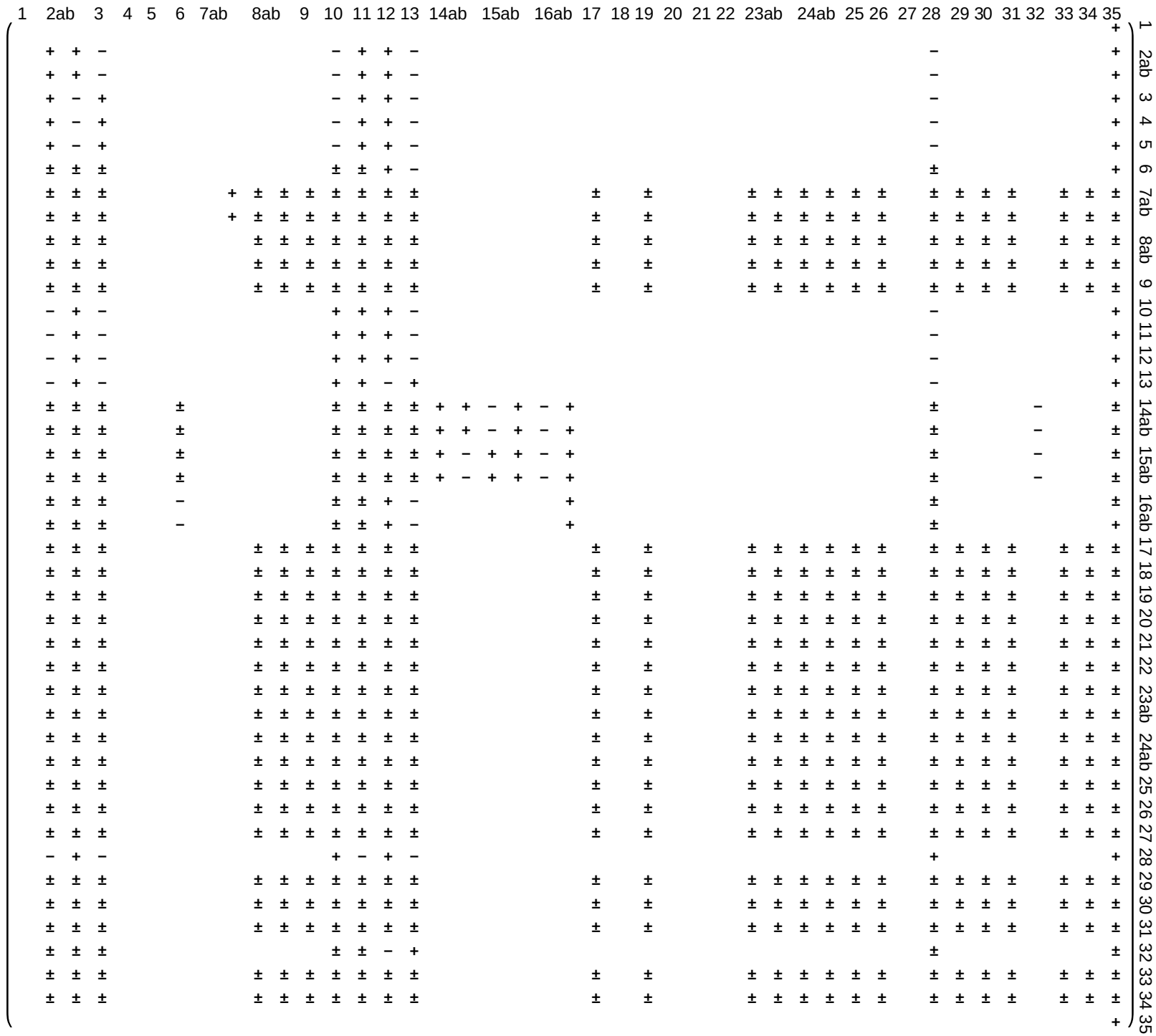
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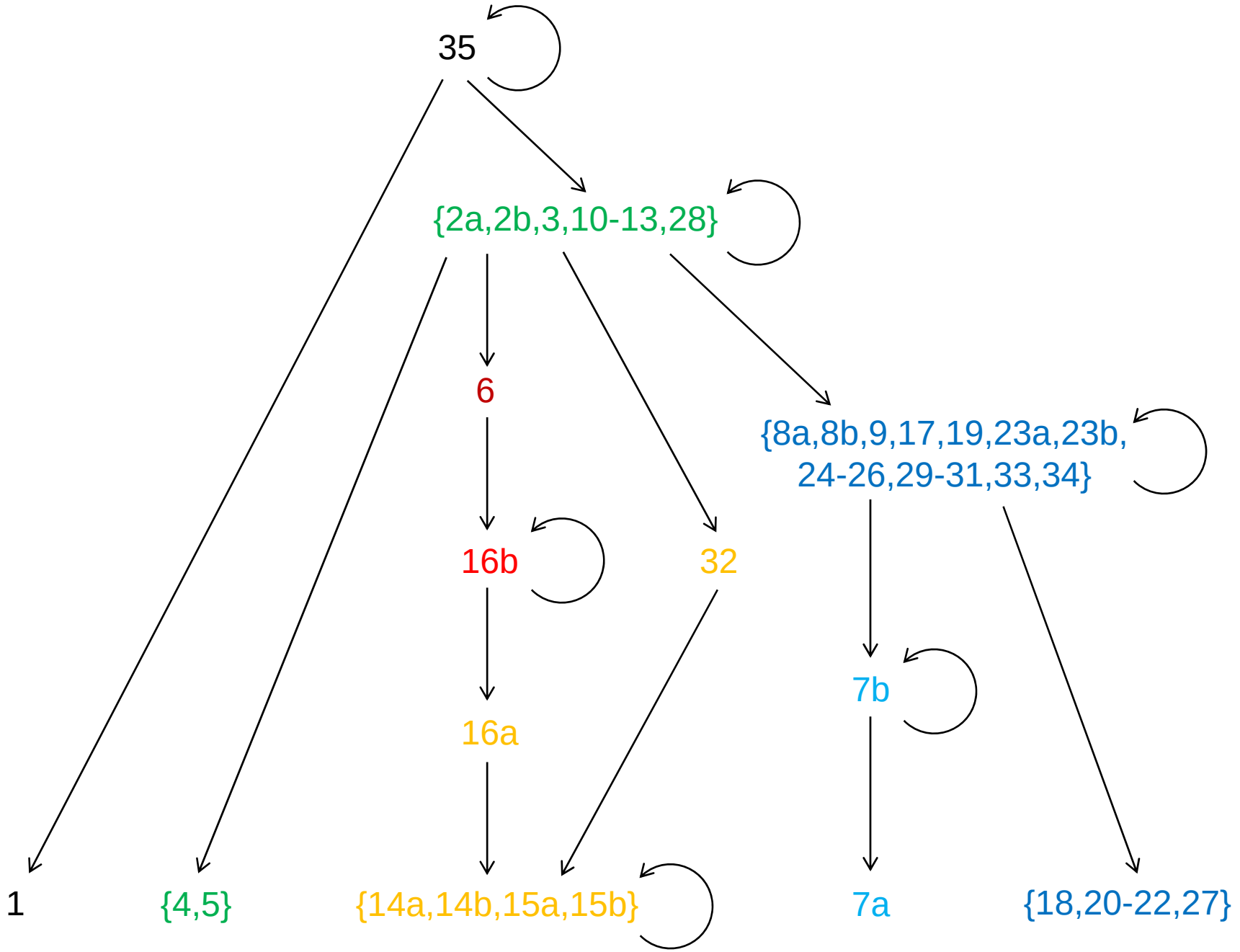
Fig. 9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7a
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