Sensitivity of chemical reaction networks: a structural approach. 2. Regular monomolecular systems

– Dedicated to Messoud Efendiev on the occasion of his 60th birthday –

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version of June 12, 2014

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Abstract

For the deceptively innocent case of monomolecular reactions, only, we embark on a systematic mathematical analysis of the steady state response to perturbations of reaction rates. Our function-free approach does not require numerical input. We work with general, not necessarily monotone reaction rate functions. Based on the directed graph structure of the monomolecular reaction network, only, we derive which steady state concentrations and reaction fluxes are affected by a rate change, and which are not. Moreover, we establish a transitivity property of the mutual influence of reaction fluxes. The results and concepts developed here, from a mathematical view point, are of applied relevance including metabolic networks in biology; see our companion paper [MoFi14].

1 Introduction

We study the response of steady states to perturbations of reaction rates in chemical reaction networks. Strong motivation for our study comes from recent advances of experimental techniques in systems biology of metabolic networks. In our complementary companion paper [MoFi14], for example, we address the steady state response of the tricarboxylic citric acid cycle (TCAC) in the glycolytic carbon metabolism of *E. coli* bacteria. Perturbations are effected, experimentally, by changing the levels of enzymes which selectively catalyze the various reactions in the network; see [Ishetal07]. Based on computer algebra we have calculated symbolic response matrices, in [MoFi14]. We obtained partial conclusions on the increase, decrease, or zero change of steady state concentrations and reaction flux rates. We also observed a transitivity property of the flux response in several specific examples. Notably our results were based on the reaction network structure, only, together with mild positivity and monotonicity conditions on the reaction rates. In that sense our results were *function-free*.

In the present paper, we offer a first step towards a mathematical understanding of the observations in [MoFi14]. Specifically we show how to predict zero versus nonzero flux changes $\Phi_{j'j^*}$ of reaction j', as a result of a rate increase of reaction j^*. Our prediction is based on the reaction network structure only; see theorem 1.1 below. We say that j^* *influences* j', in symbols $j^* \rightsquigarrow j'$, if $\Phi_{j'j^*} \neq 0$. In theorem 1.2 we show that the relation $\rightsquigarrow$ is transitive, indeed, as was first observed in the examples of [MoFi14]. In theorem 1.3 we draw conclusions on the changes δx_m of steady state concentrations x_m, for the metabolite m, from the flux changes $\Phi_{j'j^*}$ of theorem 1.1. This is of practical relevance because experiments are not able to measure the flux response, usually, but determine the concentration response to rate perturbations.

At present, our mathematical results can only be called a first step because they are limited to *monomolecular* reactions: any reaction j just converts one metabolite m^j into another one, $\overline{m}^j$; see our assumption (1.1) below. This is a severe restriction which, for example, excludes the TCAC cycle. Nonetheless we find it worthwhile to diligently settle the monomolecular case, which is less intuitive than it might appear at first sight, before jumping to general conclusions prematurely.

Our mathematical setting and notation is as follows. A *monomolecular reaction network* is a directed graph Γ with vertex set $\mathbf{M} \cup \{0\}$ and directed edges (alias arrows) $j \in \mathbf{E}$. We call $m \in \mathbf{M}$ the *metabolites*. Their total number is M, and they are distinct from the *zero-complex* 0 introduced by Feinberg; see [Fe95] and the references there. The E distinct arrows $j \in \mathbf{E}$ are also called *reactions*

$$j: \quad m^j \to \overline{m}^j \tag{1.1}$$

from $m^j \in \mathbf{M} \cup \{0\}$ to a different $\overline{m}^j \in \mathbf{M} \cup \{0\}$. Any ordered pair $(m, \overline{m})$ is connected by at most one arrow. Self-loops $\overline{m} = m$ are forbidden, but reverse arrows from $\overline{m}$ to $m \neq \overline{m}$ are allowed.

A reaction j: $m \to \overline{m}$ is called *reversible* if the reverse reaction $\overline{j}$: $\overline{m} \to m$ also occurs in Γ. A *path* in Γ is a succession of edges and vertices, without any self-intersection. Unless stated otherwise, all paths are considered to be directed, i.e. they follow edges by their arrow orientation. We speak of a *di-path* occasionally, to emphasize direction. An undirected path where the two end points, only, coincide is called a *cycle*. If the cycle is directed we speak of a *di-cycle*.

Deviating from standard terminology, we call the tail m^j of the arrow j in (1.1) the *mother* metabolite of reaction j. Thus we have a map

$$\mathbf{m}: \quad \mathbf{E} \to \mathbf{M} \cup \{0\} \tag{1.2}$$

such that $\mathbf{m}(j) = m^j$ is the mother of j. In more standard chemical terminology m^j is called the *reactant* or *educt* of reaction j, in contrast to the *reaction product* $\overline{m}^j$ obtained by a map $\overline{\mathbf{m}}(j) := \overline{m}^j$. We call the reactions j in $\mathbf{E}_0 := \mathbf{m}^{-1}(0)$ the *feed reactions* j: $0 \to \overline{m}^j$, and the reactions j: $m^j \to 0$ the *exit reactions* $\overline{\mathbf{m}}^{-1}(0)$.

Let $e_m \in \mathbb{R}^M$ be the m-th unit vector, for any nonzero metabolite $m \in \mathbf{M}$, and define $e_0 = 0 \in \mathbb{R}^M$. Then the dynamics of the vector $x = (x_m)_{m \in \mathbf{M}}$ of *concentrations* x_m of the metabolites $m \in \mathbf{M}$ is given by the ODE

$$\dot{x} = f(\mathbf{r}, x) := \sum_{j \in \mathbf{E}} r_j(x_{\mathbf{m}(j)}) \, (e_{\overline{\mathbf{m}}(j)} - e_{\mathbf{m}(j)}) \, . \tag{1.3}$$

Here we consider the reaction rate functions $\mathbf{r} = (r_j)_{j \in \mathbf{E}}$ as given parameters. We define $x_0 := 1$. We assume *positivity* of the reaction rate functions $r_j \in C^1$,

$$r_j(\xi) > 0 \quad \text{for} \quad \xi > 0 \, , \tag{1.4}$$

as well as the existence of a *positive steady state* $x^* > 0$, i.e.

$$0 = f(\mathbf{r}, x^*) \, , \tag{1.5}$$

for some x^* with all components $x^*_m > 0$. In practice (1.5) just means that we omit zero components of x^*. Likewise we omit vanishing reactions in (1.4).

Our final assumption requires the network to be *regular* at the steady state $\mathbf{x}^*$, i.e. the Jacobian $f_x(\mathbf{r}, x^*)$ of the partial derivatives with respect to $\mathbf{x}$ of the ODE vector field f is required to be nonsingular:

$$\det f_x(\mathbf{r}, x^*) \neq 0 \, . \tag{1.6}$$

This enables us to study the steady state response to any perturbation of the rate function r_{j^*} of any reaction j^*, by the standard implicit function theorem. For any continuously differentiable function $\rho \in C^1$, the C^1-small perturbation

$$\mathbf{r}^\varepsilon := \mathbf{r} + \varepsilon \rho \tag{1.7}$$

of $\mathbf{r} = \mathbf{r}^0$ induces a response curve $x^*(\varepsilon)$, for small real ε, such that

$$f_r \cdot \rho + f_x \cdot \tfrac{d}{d\varepsilon} x^* = 0 \tag{1.8}$$

at $\mathbf{r}^\varepsilon$, $x^*(\varepsilon)$. By regularity (1.6), this determines the resulting perturbation of x^*. Let us consider the special case where $\rho = (\rho_j)_{j\in\mathbf{E}}$, $\rho_j = \rho_j(x_{\mathbf{m}(j)})$ does not change the reaction network and only perturbs reaction j^*, so that

$$\rho_j(\xi) = 0\,, \quad \text{for} \quad j \neq j^*\,, \quad \text{and} \tag{1.9}$$

$$\rho_{j^*}(x^*_{\mathbf{m}(j^*)}) = 1\,. \tag{1.10}$$

Here we have normalized the perturbation of rate j^*, without loss of generality. For this particular choice, we define the resulting (infinitesimal) *concentration response* $\delta x_m^{j^*}$ of metabolite m at steady state as

$$\delta x_m^{j^*} := \left.\frac{d}{d\varepsilon}\right|_{\varepsilon=0} x_m^*(\varepsilon)\,. \tag{1.11}$$

The precise numerical values $\delta x_{m_0}^{j^*}$ of the concentration responses depend on the precise numerical values of the derivatives $r_{jm} := r_j{}'(x_m^*)$, for $m = \mathbf{m}(j)$. Our function-free approach does not rely on such numerical data, which are often unknown. We do not even require positivity of $r_j{}'$, in the present paper. Instead we consider these r_{jm} as variables which enter the responses $\delta x_{m_0}^{j^*}$ via certain rational expressions. We call $\delta x_{m_0}^{j}$ *algebraically nonzero* if $\delta x_{m_0}^{j^*} \neq 0$, as a rational function of the variables r_{jm} with $m = \mathbf{m}(j)$. In particular this implies $\delta x_{m_0}^{j^*} \neq 0$, numerically, except on real algebraic varieties of codimension at least 1 in the space of data r_{jm}.

On the other hand, this view point relies on the derivative variables $r_{jm} = r_j{}'(x_m^*)$ to be independent of the equilibrium flux values $r_j(x_m^*)$ themselves. This independence fails, evidently, in exceptional cases like $r_j = a \cdot \exp(x_m)$. More generally, independence fails whenever the class of the nonlinearities r_j itself solves one and the same ODE $r_j{}' = h_j(r_j)$ for some fixed function h_j. But already two-parameter families of functions r_j are rich enough to justify our function-free approach via algebraic independence of r_j and $r_j{}'$. Rate functions of Michaelis-Menten or Langmuir-Hinshelwood type, as opposed to mere mass action kinetics, are favorable specific examples.

Theorem 1.1. *Let positivity and regularity assumptions* (1.4), (1.5) *hold for the monomolecular reaction network* (1.1) – (1.3). *Moreover assume the Jacobian determinant in* (1.6) *is nonzero, algebraically.*

Then the concentration response $\delta x_m^{j^*}$ *of any metabolite* $m \in \mathbf{M}$ *to a rate perturbation* (1.7)– (1.10) *of any reaction* $j^* \in \mathbf{E}$ *satisfies*

$$\delta x_m^{j^*} \neq 0 \tag{1.12}$$

algebraically if, and only if, there exist two directed paths γ^0 *and* γ^m *for which the following four conditions all hold true:*

(i) both paths emanate from the mother reactant $m^* = \mathbf{m}(j^*)$ *of reaction* j^**;*

(ii) one of the paths contains reaction j^*;

(iii) the paths γ^0 and γ^m terminate at the vertices 0 and m, respectively;

(iv) the two paths are disjoint except for their shared starting vertex m^*.

The condition on γ^m may appear to be straightforward, perhaps, to describe some "domain of influence" of the perturbation j^*. The condition on the exit path γ^0, however, is less intuitive – particularly when the effects of $j^* \in \gamma^0$ spill over to the side branch γ^m.

In the special case of $m = m^*$ the path $\gamma^m = \{m^*\}$ does not contain any edges. If, in addition, reaction j^* is the only child arrow emanating from mother vertex m^*, then a path γ^0 from m^* to 0 always exists and

$$\delta x_{m^*}^{j^*} \neq 0\,; \tag{1.13}$$

see proposition 2.1 below.

As a complement to the above theorem on the concentration sensitivity $\delta x_m^{j^*}$ of metabolite m in response to a rate perturbation of reaction j^*, we address *flux sensitivity* next. Let $\Phi_{j'j^*}$ denote the (infinitesimal) change of the flux through reaction arrow j', in response to a rate perturbation of reaction j^*, i.e.

$$\Phi_{j'j^*} := \delta_{j'j^*} + r_{j'\mathbf{m}(j')}\, \delta x_{\mathbf{m}(j')}^{j^*}\,. \tag{1.14}$$

The Kronecker-delta $\delta_{j'j^*}$ indicates the explicit flux change caused by the external perturbation at j^* in reaction $j' = j^*$, only. The second term accounts for the flux change in any reaction j' which is caused, implicitly, by the concentration response $\delta x_{\mathbf{m}(j')}^{j^*}$ of the mother reactant $\mathbf{m}(j')$ to the external perturbation at j^*. We denote partial derivatives of reaction rates at the equilibrium x^* by

$$r_{jm} := \frac{\partial}{\partial x_m}\, r_j(x^*) = \begin{cases} r_j{}'(x_m^*) & \text{for} \quad m = \mathbf{m}(j)\,, \\ 0 & \text{otherwise}\,. \end{cases} \tag{1.15}$$

See also assumption (1.4). We say that reaction j^* *influences* reaction j', in symbols: $j^* \rightsquigarrow j'$, if the flux response $\Phi_{j'j^*}$ is algebraically nonzero:

$$j^* \rightsquigarrow j' \quad \Longleftrightarrow \quad \Phi_{j'j^*} \neq 0\,. \tag{1.16}$$

Theorem 1.2. *Let the assumptions of theorem 1.1 hold, and consider any pair of edges j', $j^* \in \mathbf{E}$, not necessarily distinct.*

Then j^* influences j', i.e. the flux response $\Phi_{j'j^*}$ of reaction j' to a rate perturbation (1.7) – (1.10) *of reaction j^* satisfies*

$$\Phi_{j'j^*} \neq 0 \tag{1.17}$$

algebraically, if, and only if, there exist two directed paths γ^0 and γ' for which the following four conditions all hold true:

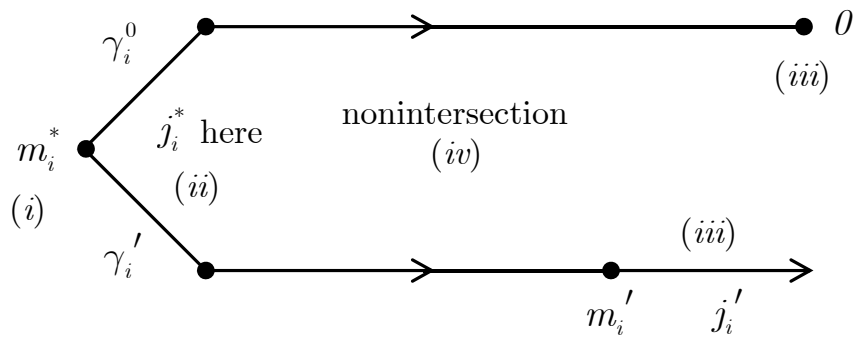

Figure 1.1: *Summarizing properties (i) – (iv) of theorem 1.2 for the paths γ_i^0, top, and γ_i', bottom. Property (i): both paths emanate from m_i^*; for (ii): one of them contains j_i^*; for (iii): termination is at vertex 0 and edge j_i' respectively; for (iv): the two paths are disjoint except for m_i^*. The index i will be used in section 4.*

(i) both paths emanate from the mother reactant $m^* = \mathbf{m}(j^*)$ of reaction j^*;

(ii) one of the paths contains reaction j^*;

(iii) the path γ^0 terminates at vertex 0, and γ' terminates with reaction edge j': $m' \to \overline{m}'$, but omitting the product (head) vertex $\overline{m}' = \overline{\mathbf{m}}(j')$ of j';

(iv) except for their shared starting vertex m^*, the two paths γ^0 and γ' are disjoint.

For an illustration see fig. 1.1. The conditions on the paths γ^0 and γ' in theorem 1.2 are quite similar to those on γ^0 and γ^m in theorem 1.1. However, there are some subtle differences. Let $m{:=}\,\mathbf{m}(j')$ denote the mother reactant of reaction j'. Then γ' just is γ^m, with the edge j' appended. In particular γ' always contains the edge j' and then terminates.

Consider the single child case, for example, where j^* is the only child arrow emanating from the mother vertex m^*. Then

$$\Phi_{j'j^*} = 0\,, \tag{1.18}$$

for all $j' \in \mathbf{E}$ by theorem 1.2, even though $\delta x_{m^*}^{j^*} \neq 0$ algebraically, by (1.13) and theorem 1.1. This follows because the disjointness condition (iv), in case $\Phi_{j^*j^*} \neq 0$, requires two different di-paths γ^0, γ' to emanate, by (i), from the same single-child mother m^* – a contradiction. This effect is owed to the Kronecker-delta in the flux sensitivity (1.14), of course. It is also easy to prove (1.18) directly. Indeed (1.8) – (1.10), (1.15) at the single-child vertex m^* imply

$$\delta x_{m^*}^{j^*} = -1/r_{j^*m^*} \neq 0\,. \tag{1.19}$$

This implicit response compensates the explicit external flux increase by ρ, at j^*, so that (1.14) implies $\Phi_{j^*j^*} = 0$. Moreover (1.8) then implies

$$\delta x_m^{j^*} = 0 \tag{1.20}$$

for all other metabolites $m \neq m^*$. This first example is compatible, of course, with our statements of theorems 1.1 and 1.2.

Interesting reaction networks contain more reaction edges than metabolites. The *flux sensitivity matrix*

$$\Phi := (\Phi_{j'j^*})_{j',\, j*\in\mathbf{E}} \tag{1.21}$$

of theorem 1.2 is therefore larger than the more concise *concentration sensitivity matrix*

$$\delta x := (\delta x_m^{j^*})_{j^*\in\mathbf{E},\, m\in\mathbf{M}}\,. \tag{1.22}$$

On the other hand, the square flux sensitivity matrix Φ allows for the concept of *transitivity of influence*: we call the flux influence relation $j^* \rightsquigarrow j'$, alias $\Phi_{j'j^*} \neq 0$ algebraically, *transitive* if

$$j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_3 \quad \text{implies} \quad j_1 \rightsquigarrow j_3\,. \tag{1.23}$$

Transitivity sounds completely tautological: if a change in the rate of reaction j_1 produces a change in j_2, and a change of the rate of j_2 propagates to j_3, then j_1 also ought to have influence on j_3. Due to the implicit concentration responses δx of the network, however, this is far from obvious. Alas, it is true.

Theorem 1.3. *Let the assumptions of theorem 1.1 hold. Then the flux sensitivity matrix* Φ *of* (1.21) *is transitive. In other words, transitivity* (1.23) *holds true for the flux influence relation* $j^* \rightsquigarrow j'$ *defined by* $\Phi_{j'j^*} \neq 0$ *algebraically in* (1.16).

Based on flux transitivity of the influence relation $\rightsquigarrow$, we can define an *influence equivalence* relation $\approx$ on the set $\mathbf{E}$ of reaction edges j as follows:

$$j \approx j\,, \tag{1.24}$$

and, for all edges $j_1 \neq j_2$,

$$j_1 \approx j_2 \quad \Longleftrightarrow \quad j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_1\,. \tag{1.25}$$

Reflexivity (1.24) has to be assumed separately because self-influence $j \rightsquigarrow j$ fails, for example, when edge j is a single child. The equivalence classes of $\approx$ are called *flux components* F_i. They form the vertices of an acyclic directed *flux influence graph* $F(\Gamma)$. A directed edge from any vertex F_1 to any other vertex F_2 indicates that for some $j_1 \in F_1$ and $j_2 \in F_2$, and hence for all, we have the influence $j_1 \rightsquigarrow j_2$, but not vice versa.

The flux influence graph is a very convenient concept to visualize the hierarchy of reactions and their influence. For example define the *influence sets*

$$I(j^*) := \{j' \in \mathbf{E};\, j^* \rightsquigarrow j'\}\,, \tag{1.26}$$

for any reaction $j^* \in \mathbf{E}$. In case j^* does not influence itself, i.e. for $\Phi_{j^*j^*} = 0$, the influence set $I(j^*)$ is the set of all reactions j' "below" j^* in the flux influence graph. In other words, $I(j^*)$ consists of the union of j' in those other flux components F_i which can be reached from j^*. In case of self-influence $j^* \rightsquigarrow j^*$, i.e. for $\Phi_{j^*j^*} \neq 0$ algebraically, the flux component of j^* itself is added to $I(j^*)$.

The remaining paper is organized as follows. Section 2 introduces some more graph jargon and collects some consequences of our positivity and regularity assumptions (1.4) – (1.6) for the monomolecular network (1.1) – (1.3). In particular we show that the reaction di-graph is strongly connected. Moreover we discuss di-cycles and spanning trees. Theorems 1.1, 1.2, and 1.3 are proved in sections 5, 3, and 4, respectively.

In section 6 we discuss several explicit illustrative examples of artificial, but instructive, monomolecular reaction networks, their concentration and flux sensitivities, and their influence graphs. Realistic networks are rarely monomolecular, of course. For more realistic examples we have already referred to [MoFi14] and the references there. Somewhat to our surprise, these networks also exhibited flux transitivity. For the carbon metabolism of the *E. coli* TCA cycle, the flux influence graph was particularly helpful to identify and understand the control hierarchy of its functional constituents.

Acknowledgement. Generous hospitality during extended very fruitful and enjoyable working visits which made this work possible is mutually acknowledged with particular gratitude. We express our sincere thanks to the following people for their useful comments: Yoh Iwasa, Shuji Ishihara, Hirohisa Kishino, Hiroshi Kokubu, Hiroshi Matano, Yasumasa Nishiura, Hiroe Oka, Kiyotaka Okada, Shingo Iwami, and Hannes Stuke. Delightful typesetting of a messy manuscript was accomplished by Ulrike Geiger. This work was supported in part by the JST CREST program of Japan, and the Deutsche Forschungsgemeinschaft, SFB 910 "Control of Self-Organizing Nonlinear Systems".

2 Positivity, connectivity, and cycles

In the present section we collect some further notions about graphs. In lemma 2.2 and corollary 2.3 we express regularity assumption (1.6) in graph terminology: the Jacobian determinant $f'(x^*)$ is algebraically nonzero if, and only if, there exists a directed path to 0 from every metabolite $m \in \mathbf{M}$. In particular the monomolecular reaction network is (weakly) connected by its (undirected) edges. The complementary positivity assumptions (1.4), (1.5) for the stationary reaction

rates r_j, on the other hand, ensure that each (weakly) connected component is strongly connected; see lemma 2.4.

Our definition of *weak* and *strong connectivity* of vertices m, m' is standard. In undirected graphs we say $m \sim m'$ are weakly connected if there exists a possibly empty, undirected path joining m and m'. In di-graphs, $m \approx m'$ are strongly connected if there exist two possibly empty di-paths: one from m to m' and one from m' to m. The equivalence classes of the vertex equivalence relations $\sim$ and $\approx$ are called weak and strong connected components, respectively. The flux equivalence $\approx$ defined in (1.24), (1.25) above is another example of strong connectivity on the directed *flux graph* with vertices $j \in \mathbf{E}$ and directed edges $j_1 \rightsquigarrow j_2$ defined by flux influence. The flux components are the strong connected components of the flux graph.

Strong connectivity has been called weak reversibility by Feinberg; see [Fe95] for an overview. In the more restrictive setting of mass action kinetics, but for more general reaction networks of Feinberg deficiency $\delta_F = 0$, uniqueness and asymptotic stability of the positive equilibrium x^* has been proved, in each stoichiometric compatibility class. As a simple consequence of proposition 2.1 below we observe that monomolecular networks are of deficiency zero. In a very interesting recent development, uniqueness of the positive equilibrium x^* has also been shown, under much less restrictive kinetic assumptions, for "concordant" networks; see [ShFe13].

Our discussion of regularity assumption (1.6) is purely algebraic, in terms of the abstract independent variables

$$r_{jm} = r_j{}'(x^*_m)\,, \tag{2.1}$$

with $m = \mathbf{m}(j) \in \mathbf{M} \cup \{0\}$ the mother of reaction $j \in E$. We conveniently, but only temporarily, forget that x^* is a stationary solution (1.5) of the ODE (1.3), and we do not require positivity (1.4). We decompose

$$f'(x^*) = \mathbf{SR} \tag{2.2}$$

where $\mathbf{R} = (r_{jm})_{j\in\mathbf{E},\, m\in\mathbf{M}}$ is the $E \times M$ reactivity matrix of the nontrivial derivatives $r_{jm} = r_j{}'(x^*_m)$ with $m = \mathbf{m}(j)$, viewed as independent variables, and filled up by zeros as in (1.15). The $M \times E$ *stoichiometric matrix* $\mathbf{S}$: $\mathbb{R}^E \to \mathbb{R}^M$ is defined by

$$\mathbf{S}e_j = \begin{cases} e_{\overline{\mathbf{m}}(j)} - e_{\mathbf{m}(j)} & \text{if} \quad \mathbf{m}(j) \neq 0\,, \\ e_{\overline{\mathbf{m}}(j)} & \text{if} \quad \mathbf{m}(j) = 0\,. \end{cases} \tag{2.3}$$

Here e_j defines the j-th unit vector in $\mathbb{R}^E$, and e_m the m-th unit vector in $\mathbb{R}^M$ with the convention $e_0 := 0$.

Our main tool in the proof of lemma 2.2 below, as well as in the theorems, is the construction of an *augmented matrix* $\mathbf{A}$: $\mathbb{R}^M \times \mathbb{R}^N \to \mathbb{R}^E$, where $N :=$

$\dim\ker \mathbf{S}$ accounts for the kernel of the stoichiometric matrix $\mathbf{S}$: $\mathbb{R}^E \to \mathbb{R}^M$. Let $c^1, \ldots, c^N \in \mathbb{R}^E$ be any basis for $\ker \mathbf{S}$, with components c^k_j, and define the $E \times N$ matrix

$$\mathbf{C} := (c^k_j)_{j\in\mathbf{E},\, k\in\{1,\ldots,N\}} \,. \tag{2.4}$$

Then the augmented matrix $\mathbf{A}$ is defined as the block matrix

$$\mathbf{A} := (\mathbf{R},\, \mathbf{C}) \,. \tag{2.5}$$

At first it looks redundant to lift issues concerning the $M \times M$ matrix $f'(x^*)$ to the possibly much larger $E \times (M + N)$ matrix $\mathbf{A}$. The reaction aspect $\mathbf{R}$ and the graph aspect $\mathbf{C}$ of $\mathbf{S}$, however, become clearly separated and can now be addressed much more conveniently.

We study the graph aspect first. For the moment we ignore all orientations of the edge arrows j and consider $\Gamma = (\mathbf{M} \cup \{0\},\, \mathbf{E})$ as an undirected graph. We also decompose

$$\mathbf{S} = \mathbf{P}_0\, \mathbf{D} \tag{2.6}$$

where the projection $\mathbf{P}_0$: $\mathbb{R}^{M+1} \to \mathbb{R}^M$ simply drops the last component associated to the vertex 0 in Γ. The matrix $\mathbf{D}$: $\mathbb{R}^E \to \mathbb{R}^{M+1}$ is the *boundary map* from edges $j \in \mathbf{E}$ to vertices $m \in \mathbf{M} \cup \{0\}$ in Γ, defined by

$$\mathbf{D}e_j := e_{\overline{\mathbf{m}}(j)} - e_{\mathbf{m}(j)} \,. \tag{2.7}$$

The *homologies* $H_0(\Gamma)$ and $H_1(\Gamma)$ are defined by

$$\begin{aligned} H_0(\Gamma) &:= (\operatorname{range}\mathbf{D})^\perp \quad \leq \mathbb{R}^{M+1} \,; \\ H_1(\Gamma) &:= \quad \ker \mathbf{D} \qquad\quad \leq \mathbb{R}^E \,. \end{aligned} \tag{2.8}$$

The alternating sum of their dimensions is the *Euler characteristic*

$$X(\Gamma) := \dim H_0 - \dim H_1 = M + 1 - E \,, \tag{2.9}$$

alias the negative Fredholm index of the boundary map $\mathbf{D}$. Note how $\dim H_0$ counts the (weakly) connected components W of Γ; a basis of H_0 is given by the indicator functions 1_W of the component vertices.

A *maximal spanning tree* T of an undirected graph Γ is any acyclic subgraph of Γ which becomes cyclic whenever any edge is added to T. Note that T must contain all vertices $\mathbf{M} \cup \{0\}$ of Γ, i.e. $T = (\mathbf{M} \cup \{0\},\, \mathbf{E}(T))$. Any edge $j \notin \mathbf{E}(T)$ generates a unique undirected cycle c^j in $T \cup \{j\}$, by acyclicity of T. Note how $c^j \in \ker \mathbf{D} = H_1(\Gamma)$ are linearly independent. By maximality of T, the cycles c^j form a basis for H_1, and hence

$$\dim H_1 = E - E(T) \tag{2.10}$$

where $E(T)$ counts the edges of any maximal spanning tree T of Γ.

Proposition 2.1. *In the above setting and notation, the kernel of the stoichiometric matrix* $\mathbf{S}$ *coincides with the first homology:* $H_1(\Gamma) = \ker \mathbf{D} = \ker \mathbf{S}$. *Equivalently,*

$$\dim H_1(\Gamma) = N\,. \tag{2.11}$$

Moreover the following properties are mutually equivalent

(i) $\mathbf{S}$*:* $\mathbb{R}^E \to \mathbb{R}^M$ *is surjective;*

(ii) $N := \dim \ker \mathbf{S} = E - M$*;*

(iii) the matrix $\mathbf{A}$*:* $\mathbb{R}^{M+N} \to \mathbb{R}^E$ *is square;*

(iv) Γ *is (weakly) connected, i.e.*

$$\dim H_0(\Gamma) = 1\,. \tag{2.12}$$

Proof. We prove $\ker \mathbf{D} = \ker \mathbf{S}$ and (2.11) first. Obviously $\mathbf{S} = \mathbf{P}_0\,\mathbf{D}$ implies $\ker \mathbf{D} \leq \ker \mathbf{S}$. Equality holds if, and only if, the spanning element e_0 of $\ker \mathbf{P}_0$, which is omitted by $\mathbf{P}_0$, satisfies

$$e_0 \notin \operatorname{range} \mathbf{D} = H_0(\Gamma)^\perp\,. \tag{2.13}$$

Admittedly $e_0 \perp 1_W$, for the indicator function of any (weakly) connected component of Γ which does not contain vertex 0. However $e_0^T \cdot 1_{W_0} = 1$, by definition, for the (weakly) connected component W_0 of the vertex 0 itself. This proves $\ker \mathbf{D} = \ker \mathbf{S}$, and hence (2.11):

$$\dim H_1(\Gamma) := \ \dim \ker \mathbf{D} = \dim \ker \mathbf{S} := \ N\,. \tag{2.14}$$

To prove the equivalence of (i) – (iv) we invoke elementary linear algebra for the stoichiometric matrix S: $\mathbb{R}^E \to \mathbb{R}^M$. Abbreviate $\operatorname{def} \mathbf{S} := \dim \ker \mathbf{S}$ and $\operatorname{cork} \mathbf{S} := \operatorname{codim} \operatorname{range} \mathbf{S}$. Then

$$E - M = \operatorname{def} \mathbf{S} - \operatorname{cork} \mathbf{S} = N - \operatorname{cork} \mathbf{S}\,. \tag{2.15}$$

This proves the equivalence (i) $\Longleftrightarrow$ (ii). Trivially (ii) $\Longleftrightarrow$ (iii). The equivalence (ii) $\Longleftrightarrow$ (iv), alias (2.12), follows from

$$\dim H_0 = \dim H_1 + M + 1 - E = 1 + N - (E - M)\,. \tag{2.16}$$

Here the first equality is (2.9), and (2.11) provides the second equality. This proves the proposition. $\bowtie$

In the slightly different language of Feinberg, proposition 2.1 shows that monomolecular networks possess deficiency zero; see [Fe95]. Indeed the *Feinberg deficiency* δ_F is defined as $\delta_F := \dim\ker \mathbf{S} - \dim\ker \mathbf{D} = 0$, by (2.14).

We can now return to the role of the symbolic reaction part $\mathbf{R}$ for the determinant of the Jacobian matrix $f'(x^*) = \mathbf{SR}$ and in the augmented matrix $\mathbf{A} = (\mathbf{R}, \mathbf{C})$; see (2.2) and (2.5).

Lemma 2.2. *Consider any directed graph* $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ *as in* (1.1) *and view* $\det(\mathbf{SR})$ *as a formal polynomial in the nontrivial derivative variables* r_{jm}, *for* $j \in \mathbf{E}$ *and* $m = \mathbf{m}(j)$.

Then

$$\det \mathbf{SR} \neq 0 \tag{2.17}$$

algebraically if, and only if, from every vertex $m_0 \in \mathbf{M}$ *there exists a directed path* γ^0 *in* Γ *to vertex* 0.

Moreover (2.17) *is equivalent to* $\det \mathbf{A} \neq 0$ *for the augmented matrix* $\mathbf{A} = (\mathbf{R}, \mathbf{C})$.

Proof of lemma 2.2. We first assume $\det \mathbf{SR} \neq 0$ algebraically, to construct γ^0. We address the converse claim afterwards.

Suppose $\det \mathbf{SR} \neq 0$. Then $\mathbf{S}$ is surjective, and $\mathbf{A} = (\mathbf{R}, \mathbf{C})$ is square by proposition 2.1 (i), (iii). For later use we choose $\mathbf{C}$ to be defined by the cycles c^k of a maximal spanning tree T_0, as in (2.10). We first claim

$$\det \mathbf{A} \neq 0\,. \tag{2.18}$$

We show that $\ker \mathbf{A}$ is trivial. Indeed, consider $\xi = \delta x \in \mathbb{R}^M$ and $\mu \in \mathbb{R}^N$ such that

$$0 = \mathbf{R}\,\xi + \mathbf{C}\,\mu\,. \tag{2.19}$$

Applying $\mathbf{S}$ we obtain $\mathbf{SR}\,\xi = 0$, because $\mathbf{SC} = 0$ by definition (2.4) of the $\mathbf{S}$-kernel part $\mathbf{C}$ of $\mathbf{A}$. Hence $\det \mathbf{SR} \neq 0$ implies $\xi = 0$. Linear independence of the columns c^k of $\mathbf{C}$, a basis for $\ker \mathbf{S}$, implies $\mu = 0$. This proves claim (2.18).

Nonvanishing $\det \mathbf{A} \neq 0$ in turn holds, algebraically, if and only if the expansion of the determinant contains any nontrivial monomial in the nontrivial entries r_{jm} of $\mathbf{R}$. In other words, there exists a *child selection map*

$$\begin{aligned} J: \quad & \mathbf{M} \;\to \mathbf{E} \\ & m \;\to J(m) \end{aligned} \tag{2.20}$$

such that the coefficient a_J of the nontrivial monomial

$$r^J := \prod_{m\in\mathbf{M}} r_{J(m),\,m} = \prod_{j\in J(\mathbf{M})} {r_j}'(x^*) \tag{2.21}$$

is nonzero. The monomial is nontrivial if and only if all factors are of the form $r_{J(m),\,m} = r_j{}'(x^*) = r_{j,\,\mathbf{m}(j)}$, i.e. the mother map $\mathbf{m}\colon\ \mathbf{E} \to \mathbf{M} \cup \{0\}$, is a left inverse of J:

$$\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}\,. \tag{2.22}$$

Summing over all such choices of J we obtain the polynominal expansion

$$\det \mathbf{A} = \sum_J a_J r^J\,, \tag{2.23}$$

where the generating monomials r^J are in one-to-one correspondence with the child selection maps J. The coefficient a_J is given by the sub-determinant

$$a_J = \pm \det(c_j^k)_{j\in\mathbf{E}\setminus J(\mathbf{M}),\ k\in\{1,\dots,N\}} \tag{2.24}$$

of the cycle basis $\mathbf{C}$, with the rows of $J(\mathbf{M})$ omitted. We introduce the abbreviation $\check{C}(J(\mathbf{M}))$ for that square matrix. It is now crucial to observe that

$$\det \check{C}(\mathbf{E}') \neq 0 \tag{2.25}$$

holds if, and only if, $T = (\mathbf{M} \cup \{0\},\, \mathbf{E}')$ is a maximal spanning tree of Γ. Indeed $\check{C}(\mathbf{E}')$ defines the base change in $\ker \mathbf{S}$ from our original cycle basis defined by the maximal spanning tree T_0 to the cycle basis defined by T.

Now consider $m_0 \in \mathbf{M}$ and let us reactivate edge orientations. The tree T with $\mathbf{E}' = J(\mathbf{M})$ selects one unique child arrow $J(m)$ out of any mother vertex $m \in \mathbf{M}$. This defines a unique directed path γ^0 starting at m_0. Because the tree T is acyclic the path γ^0 cannot return to itself, ever. Therefore γ^0 must terminate at the exit vertex $0 \notin \mathbf{M}$, as claimed in the lemma.

Conversely, let us suppose next that there exists a di-path γ^0 in $\Gamma = (\mathbf{M} \cup \{0\},\, \mathbf{E})$ from any $m \in \mathbf{M}$ to 0. To show $\det \mathbf{SR} \neq 0$ algebraically, it is then sufficient to construct a child selection $J\colon\ \mathbf{M} \to \mathbf{E}$ as in (2.22), such that $T = (\mathbf{M} \cup \{0\},\, J(\mathbf{M}))$ is a maximal spanning tree of Γ. It is easy to construct J from the paths γ^0, inductively. Consider any acyclic di-path. Attach any missing vertex $m \in \mathbf{M}$ by following its acyclic di-path γ^0 until it hits on any vertex which has been taken care of before. By induction, this selects a unique child arrow $J(m)$, for any mother vertex $m \in \mathbf{M}$. The child selection J, in turn, defines an acyclic tree $T = (\mathbf{M} \cup \{0\},\, J(\mathbf{M}))$ which satisfies (2.22). In particular J defines a nontrivial monomial (2.21) of $\det \mathbf{A}$ with nonzero coefficient a_J as in (2.23). Therefore $\det \mathbf{A} \neq 0$ algebraically.

It only remains to show that $\det \mathbf{A} \neq 0$ implies $\det f'(x^*) \neq 0$. In fact we show that $f'(x^*)$ possesses trivial kernel, as follows. Let $0 = f'(x^*)\xi = \mathbf{SR}\,\xi$. Then $\mathbf{R}\,\xi \in \ker \mathbf{S} = \mathrm{span}\,\{c^1,\,\dots,\,c^N\}$ implies that there exists a linear combination $\mu \in \mathbb{R}^N$ such that $0 = \mathbf{R}\,\xi + \mathbf{C}\,\mu$, as in (2.19). But now $\det \mathbf{A} \neq 0$ implies $\xi = 0$. This shows $\det f'(x^*) \neq 0$ algebraically, and the lemma is proved. $\bowtie$

The above proof also shows the following variant in terms of child selection maps J: $\mathbf{M} \to \mathbf{E}$ which are defined to possess the mother map $\mathbf{m}$: $\mathbf{E} \to \mathbf{M} \cup \{0\}$ as a left inverse, $\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}$. See (2.20), (2.22).

Corollary 2.3. *In the setting of lemma 2.2,* $\det \mathbf{SR} \neq 0$ *holds algebraically if, and only if, there exists a child selection map* J: $\mathbf{M} \to \mathbf{E}$ *such that* $T = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ *is a maximal spanning tree of the network* $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$.

The directed tree T possesses the following additional properties:

(i) any di-path in T terminates at 0;

(ii) for any edge $j \notin T$, the following alternative holds true:
(a) either, the unique cycle c^j in $T \cup \{j\}$ is a di-cycle,
(b) or else, the short-cut $j \notin T$ runs parallel to the di-path $c^j \cap T$ in T;

(iii) T does not contain any feed edge j emanating from 0;

(iv) any feed edge j defines a unique di-cycle c^j in $T \cup \{j\}$, which runs from 0 to 0.

We now switch our attention to the positivity assumption (1.4), (1.5) of the stationary reaction rates $r_j = r_j(x^*) > 0$; see also [Fe95].

Lemma 2.4. *Let positivity assumptions* (1.4), (1.5) *hold.*

Then any weak connected component of the reaction network Γ *is strongly connected.*

In particular suppose regularity assumption (1.6) *holds in addition, i.e.* $\det f'(x^*) \neq 0$. *Then* Γ *is strongly connected.*

Proof. Part 2 is a consequence of part 1 and proposition 2.1 (i, iv) applied to surjective $f'(x^*) = \mathbf{SR}$.

Part 1 is well-known. For example, analogously to the end of section 1, consider the acyclic induced di-graph on the strongly connected components W in any weak connected component of Γ. Consider a minimal component W, in this order. Remaining edge arrows, if any, then point towards W, and not away from W. The reaction fluxes r_j satisfy Kirchhoff's law at any vertex m. Therefore the total in-flow to W must vanish. Since all reaction fluxes r_j are strictly positive, there cannot exist any edge arrows pointing towards W. Therefore the strong component W coincides with its weak component. This proves the lemma. $\bowtie$

3 Proof of theorem 1.2: flux response

Throughout the next three proof sections let positivity assumptions (1.4), (1.5) and regularity assumption (1.6) hold. Our proof proceeds somewhat analogously to the proof of lemma 2.2. We crucially rely on maximal spanning trees T and on the augmented matrix $\mathbf{A}$; see (2.4), (2.5) and proposition 2.1.

To show the equivalence of the nonzero flux influence condition $\Phi_{j'j^*} \neq 0$ of (1.17) with the path conditions (i) – (iv) of theorem 1.2 we first show sufficiency of (1.17).

Fix $j^* \in \mathbf{E}$. We first calculate the flux response vector $\Phi^* := (\Phi_{j'j^*})_{j' \in \mathbf{E}}$ to the perturbation vector $\rho = e^* := e_{j^*}$ of the reactions; see (1.7) – (1.10). The implicit function theorem (1.8) for the concentration response $\delta x^* := (\delta x^{j^*}_m)_{m \in \mathbf{M}}$ of (1.11) then implies

$$\mathbf{S}\, e^* + \mathbf{SR}\, \delta x^* = 0\,. \tag{3.1}$$

See (2.2), (2.3) for the stoichiometric matrix $\mathbf{S}$ and the reactivity matrix $\mathbf{R}$. By definition of the kernel matrix $\mathbf{C}$ of $\mathbf{S}$ in (2.4), this is equivalent to

$$e^* + \mathbf{A} \begin{pmatrix} \delta x^* \\ \mu^* \end{pmatrix} = e^* + \mathbf{R}\, \delta x^* + \mathbf{C}\, \mu^* = 0 \tag{3.2}$$

for some suitable linear combination μ^* of kernel vectors $(c^k_j)_{j \in \mathbf{E}}$ of $\mathbf{S}$. On the other hand,

$$\Phi^* = e^* + \mathbf{R}\, \delta x^* = -\mathbf{C}\, \mu^* \tag{3.3}$$

by definition (1.14) of the flux response. By regularity assumption (1.6) the Jacobian $f'(x^*) = \mathbf{SR}$ is invertible. Therefore $\mathbf{A}$ is invertible by lemma 2.2, (2.18). To calculate the flux response Φ^* we can therefore solve (3.2) for μ^* and insert the result in (3.3).

By the Cramer rule we immediately obtain the components

$$-\mu^*_k \det \mathbf{A} \;=\; (-1)^{M+k+j^*} \det \check{A}_{j^*,\, M+k}\,, \tag{3.4}$$

for $k = 1, \ldots, N$. Here $\check{A}_{j^*,\, M+k}$ is the matrix $\mathbf{A} = (\mathbf{R},\, \mathbf{C})$ with omitted row j^* and column $M + k$, alias column k of $\mathbf{C}$. Insertion of (3.4) into (3.3) yields

$$\begin{aligned} \Phi_{j'j^*} \det \mathbf{A} = (\Phi^*)_{j'} \det \mathbf{A} &= (\mathbf{C} \cdot (-\mu^*))_{j'} \det \mathbf{A} = \\ &= \sum_{k=1}^{N} c^k_{j'} \cdot (-\mu^*_k \det \mathbf{A}) = \\ &= \sum_{k=1}^{N} (-1)^{M+k+j^*}\, c^k_{j'} \cdot \det \check{A}_{j^*,\, M+k} = \\ &= \det A_{j'j^*}\,. \end{aligned} \tag{3.5}$$

Here the matrix $A_{j'j^*}$ coincides with the augmented matrix $\mathbf{A}$ except for the following two replacements

$$\text{row } j^*: \quad (0\dots 0,\, c^1_{j'}\dots c^N_{j'})\,; \tag{3.6}$$

$$\text{row } j': \quad (r_{j'1}\dots r_{j'M},\, 0\dots 0); \tag{3.7}$$

for $j' \neq j^*$. This follows by expansion of $\det A_{j'j^*}$ with respect to row j^*. The comma "," separates the first M columns of $\mathbf{R} = (r_{jm})$ from the subsequent N columns of $\mathbf{C} = (c^k_j)$ in $\mathbf{A} = (\mathbf{R}, \mathbf{C})$. In case $j' = j^*$ we analogously obtain $A_{j^*j^*}$ with only the first replacement (3.6). Recall that $\det A_{j'j^*} \neq 0$ by assumption (1.17).

Our algebraic analysis of $\det A_{j'j^*} \neq 0$ now proceeds analogously to our analysis of $\det \mathbf{A} \neq 0$ in the proof of lemma 2.2; see (2.20) – (2.25). This time, we can assert the existence of a child selection $J\colon \mathbf{M} \to \mathbf{E}$, i.e. with the mother map $\mathbf{m}\colon \mathbf{E} \to \mathbf{M} \cup \{0\}$ as a left inverse $\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}$, such that the remaining $N = E - M$ rows $\mathbf{E} \setminus J(\mathbf{M})$ of the $\mathbf{C}$-part $C_{j'j^*}$ of $A_{j'j^*}$ form a cycle basis of $\ker \mathbf{S}$. This is slightly delicate due to the modifications (3.6), (3.7) of $A_{j'j^*}$.

We consider the general case $j' \neq j^*$ of (3.6) *and* (3.7) first. The null vector in the left part of (3.6) requires

$$j^* \notin J(\mathbf{M}) \tag{3.8}$$

for a nonzero contribution $a_J r^J$ to $\det A_{j'j^*}$. The null vector in the right part of (3.7), on the other hand, requires

$$j' \in J(\mathbf{M})\,. \tag{3.9}$$

In particular $m' := \mathbf{m}(j') \in \mathbf{M}$ and hence $m' \neq 0$. The remaining rows $\mathbf{E} \setminus J(\mathbf{M})$ of $A_{j'j^*}$, however, correspond to the $\mathbf{A}$-rows

$$\begin{aligned} J^c :=&\ \mathbf{E} \setminus J'\,, \quad \text{with} \\ J' :=&\ (J(\mathbf{M}) \setminus \{j'\}) \cup \{j^*\}\,. \end{aligned} \tag{3.10}$$

In other words, the acyclic oriented tree $T := (\mathbf{M} \cup \{0\},\, J(\mathbf{M}))$ has been replaced by

$$T' :=\ (\mathbf{M} \cup \{0\},\, J')\,, \tag{3.11}$$

where J' swaps edge j' of T out and edge j^* in.

Because $\det A_{j'j^*} \neq 0$ algebraically, T' is a directed maximal spanning tree – albeit with a branch point at the mother $m^* = \mathbf{m}(j^*)$ in case $m' := \mathbf{m}(j') \neq m^* \neq 0$. We choose the paths γ^0 and γ' in T' as follows.

If $j' \neq j^*$ have the same mother $m^* = m' \neq 0$, then γ' is the edge j' with vertices $m^* = m'$ and $\overline{m}' = \overline{\mathbf{m}}(j')$. The path γ^0 is the path from m^* to 0 procured in lemma 2.2.

If the mothers $m' \neq m^* \neq 0$ are different, and both different from 0, then T' possesses a single branch point at m^*. Any other vertex $m \in \mathbf{M}$ of T' possesses a unique outgoing arrow $j = J(m)$. We can therefore uniquely extend the two paths $\gamma^* \ni j^*$ and $\gamma^J \ni J(m^*) \neq j^*$ emanating from m^* forward, by J in the acyclic tree T', as in T, until they either hit 0 or m'. Indeed the paths cannot return to themselves because T' is acyclic. For the same reason, the paths remain disjoint after starting at m^*. In particular exactly one of the paths extends to 0; this path is γ^0. The other path extends to m'. Extended by j' but omitting its head $\overline{m}' := \overline{\mathbf{m}}(j')$, this is γ'.

If $m^* = 0 \neq m'$ we choose the empty path $\gamma^0 = \{m^*\}$. To define the path γ' we start from $m^* = 0$ along j^* in acyclic T', as above, following the orientation of T, J. This path cannot terminate at 0, or else it would define a cycle in T'. Hence it terminates at m' and we can append j' as before.

It only remains to consider the easy case $j' = j^*$, with (3.6). Then γ' consists of the edge j^* with its end point m^*. To construct $\gamma^0 \not\ni j^*$ we note that $j^* \notin J(\mathbf{M})$, as in (3.8), still holds. In consequence we obtain $T' = T$ and acyclicity of T'. Following the edge $J(m^*) \neq j^*$ out of m^* along the orientation of $T' = T$ we reach 0 and obtain γ^0. This proves the only-if-part of theorem 1.2.

To prove the converse if-part of theorem 1.2, i.e. necessity of (1.17), we start from given disjoint paths γ^0 and γ' from $m^* = \mathbf{m}(j^*)$ to 0 and j', such that properties (i) – (iv) of theorem 1.2 all hold. For brevity we only consider the main case of nonzero j^* and j' with nonzero distinct mothers $m^* = \mathbf{m}(j^*) \neq \mathbf{m}(j')$, leaving the remaining cases as straightforward exercises.

We have to show that $\det A_{j'j^*} \neq 0$ algebraically; see (3.5). Equivalently we have to construct a child selection $J\colon \mathbf{M} \to \mathbf{E}$ satisfying (3.8), (3.9), such that T' defined in (3.10), (3.11) becomes a maximal spanning tree of the network $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$.

On the vertices m of $\gamma^0 \cup \gamma'$, excepting the head $\overline{m}' = \overline{\mathbf{m}}(j')$ of j' and the mother m^* of j^*, we define $J(m)$ to be the unique edge j in $\gamma^0 \cup \gamma'$ which emanates from m. At m^* we have j^* and one other such edge; we pick this other one for $J(m^*)$. This construction is feasible and unique by properties (i), (ii), (iv) of the paths γ^0 and γ'. Moreover $j^* \notin J(\mathbf{M})$, so far, and $j' = J(m') \in J(\mathbf{M})$, as required in (3.8) and (3.9). To complete the construction of J consider any remaining vertex $m \in \mathbf{M}$. By lemma 2.2 there exists an oriented path γ^m from m to 0. We proceed by iteration on m. At each step we terminate the path γ^m as soon as it hits any vertex m_0 where J has already been constructed. The path γ^m then extends J to all previous vertices on γ^m. This completes the definition of J.

The resulting graph T' defined in (3.10), (3.11) is acyclic, by the above inductive construction. Indeed $\gamma^0 \cup (\gamma' \setminus j')$ is acyclic, by nonintersection property (iv) of γ'. Assuming acyclicity before each induction step preserves acyclicity. Indeed the path γ^m is acyclic by definition. Because γ^m starts at m, outside the previous construction, and terminates upon first contact, it cannot create any new cycle.

This proves that T' is acyclic. Hence T' is a tree which omits $E - M = N$ edges. Since $\dim H_1 = N$ is the number of independent cycles, by proposition 2.1, the subgraph T' will therefore be a maximal spanning tree, automatically. This shows $\det \Phi_{j'j^*} = \det A_{j'j^*} \neq 0$ algebraically and completes the proof of theorem 1.2. $\bowtie$

Our proof above has used the construction of a maximal spanning tree T' from a maximal spanning tree T, as an intermediate step; see (3.8) – (3.11). We summarize the resulting variants of theorem 1.2 as a separate characterization of algebraically nonzero fluxes $\Phi_{j'j^*} \neq 0$. These corollaries also elucidate how the influence of a perturbation of reaction j^* spreads to j' via di-cycles and side branches.

Corollary 3.1. *Let $j' \neq j^*$ by any two distinct reaction edges and let the assumptions of theorem 1.2 hold. Then algebraically nonzero flux $\Phi_{j'j^*}$ as stated in* (1.17) *implies the following.*

Consider any child selection map $J\colon \mathbf{M} \to \mathbf{E}$ with (2.22) *such that $j^* \notin J(\mathbf{M}) \ni j'$. To J associate the swapped tree $T' := (\mathbf{M} \cup \{0\},\, J')$ which swaps edge j' out of, and edge $j^* \neq j'$ into the oriented tree $T := (\mathbf{M} \cup \{0\},\, J(\mathbf{M}))$ of J; see* (3.10), (3.11). *Then T' is a maximal spanning tree of the reaction network Γ.*

In particular $j' \in T$ lies on the unique cycle through j^* in $J(\mathbf{M}) \cup \{j^*\}$. Suppose this cycle is not oriented. Then the cycle consists of two distinct oriented paths γ^* and γ' emanating from the same mother vertex $m_* = \mathbf{m}(j_*)$. The path γ^* starts with leading edge j^*, and the path γ' contains the edge j'. The paths are disjoint, except for their start at m_* and the second vertex where they meet again to form a cycle with two parallel branched directions.

Corollary 3.2. *Let $j' \neq j^*$ be any two distinct edges and let the assumptions of theorem 1.2 hold. Assume, conversely to corollary 3.1, that there exists a child selection map $J\colon \mathbf{M} \to \mathbf{E}$ such that $j^* \notin J(\mathbf{M}) \ni j'$ and, in addition the associated swapped graph T' does not possess any directed cycle.*

Then the flux $\Phi_{j'j^*}$ is algebraically nonzero.

4 Proof of theorem 1.3: transitivity

To prove transitivity theorem 1.3 we consider any three edges ${j_1}^*, {j_2}^*, {j_2}' \in \mathbf{E}$ such that ${j_1}^* \rightsquigarrow {j_2}^* \rightsquigarrow {j_2}'$. We have to show ${j_1}^* \rightsquigarrow {j_2}'$. In other words we have to show the implication

$$\Phi_{{j_2}'{j_2}^*} \neq 0\,,\ \Phi_{{j_2}^*{j_1}^*} \neq 0 \quad \Longrightarrow \quad \Phi_{{j_2}'{j_1}^*} \neq 0\,, \tag{4.1}$$

for algebraically nonzero flux responses; see (1.23). Omitting trivial cases we may assume

$${j_1}^* \neq {j_2}^* \neq {j_2}'\,. \tag{4.2}$$

Our proof will proceed via the di-paths γ_i^0, γ_i' associated to the nonzero flux response $\Phi_{j_i' j_i^*}$ in (4.1), for $i = 1, 2$ and $j_1' := j_2^*$; see theorem 1.2. In subsection 4.1 we fix some notation on the di-paths γ_i^0, γ_i', their vertices, edges, and concentrations. The transitivity claim $\Phi_{j_2' j_1^*} \neq 0$ of (4.1) is established, again by theorem 1.2, via a general construction of paths γ^0, γ'. Our construction is sketched at the end of subsection 4.1. As a warm-up we collect certain special cases in 4.2. The general construction is detailed in 4.3.

4.1 Notation and terminology

For $i = 1, 2$, let γ_i^0 denote the path from vertex m_i^* to 0 established in theorem 1.2 for $\Phi_{j_i' j_i^*} \neq 0$. Similarly γ_i' denotes the path from m_i^* to the final edge j_i': $m_i' \to \overline{m}_i'$. See fig. 1.1. In our specific case (4.1) we have $j_1' = j_2^*$ and hence $m_2^* = m_1'$. The paths γ_i^0, γ_i' satisfy properties (i) – (iv) of theorem 1.2. Omitting indices, we have to construct paths γ^0 and γ' with $m^* := m_1^*$ and final edge $j' = j_2'$ from $m' := m_2{}'$ to $\overline{m}' := \overline{m}_2'$ such that properties (i) – (iv) hold for γ^0, γ'. In summary,

$$m^* = m_1^*\,,\ j^* = j_1^*\,,\ j_1' = j_2^*\,,\ m_2^* = m_1'\,,\ m' = m_2'\,,\ j' = j_2{}'\,. \tag{4.3}$$

For arbitrary acyclic di-paths γ with designated orientation ordering it is convenient to denote open and closed *intervals* on γ by their bounding vertices as (m_1, m_2), $[m_1, m_2]$ etc., as on the real line. To specify intervals on γ_i' we use the notation $(m_1, m_2)_i'$ etc., and similarly for intervals on γ_i^0. For example $\gamma_i^0 = [m_i^*, 0]_i^0$, $j_i' = (m_i', \overline{m}_i')_i'$ and $\gamma_i' = [m_i^*, \overline{m}_i')_i'$. For vertices m_1, m_2 on the same path γ_1^0 or γ_1' we say that m_2 occurs *later* than m_1 if m_1 precedes m_2 in the order of γ_1^0 or γ_1'. For the same configuration on γ_2^0 or γ_2', in contrast, we say that m_2 occurs *to the right* of m_1.

We use $\gamma_i := \gamma_i^0 \cup \gamma_i'$ to denote the union of the paths γ_i^0 and γ_i', as a set. Consider intersection vertices

$$m \in (\gamma_1 \cap \gamma_2) \setminus \{m_1^*\} \tag{4.4}$$

other than the shared starting vertex $m^* = m_1^*$ of γ_1^0 and γ_1'. Then we call m *white* if $m \in \gamma_1^0$ and *black* for $m \in \gamma_1'$. See nonintersection property (iv) of theorem 1.2. The only shared start vertex $m := m_1^*$, if also present in γ_2, is called *white* if $j^* = j_1{}^* \subseteq \gamma_1'$ and *black* for $j_1^* \subseteq \gamma_1^0$; see property (ii). Correspondingly, we call $c(m) \in \{0, '\}$ the *color* of m. Note

$$m \in \gamma_1^{c(m)} \cap \gamma_2\,. \tag{4.5}$$

Also note that the color of $m_2^* = m_1' \in \gamma_1' \cap \gamma_2^0 \cap \gamma_2'$ is black, unless

$$m_1^* = m_2^* \quad \text{and} \quad j_1^* \subseteq \gamma_1'\,. \tag{4.6}$$

We treat this later case separately in 4.2, as case 1.

Our general construction of the di-paths $\gamma^0 = [m^*, 0]^0$ and $\gamma' = [m^*, \overline{m}')'$ with properties (i) – (iv) of theorem 1.2 hinges on the construction of a *cut-pair* $\{\mu^0, \mu'\}$ of vertices μ^0 and μ' defined by the following three properties

(4.7)
- (a) $\mu^0 \in \gamma_2^0$ and $\mu' \in \gamma_2'$;
- (b) μ^0 and μ' are of opposite color;
- (c) any black or white intersection vertex m of $\gamma_1 \cap \gamma_2$ which is strictly to the right of $\{\mu^0, \mu'\}$ on γ_2 occurs strictly later than $\{\mu^0, \mu'\}$ on γ_1 as well.

With the above notation and terminology our general construction of the paths γ^0, γ' which will prove transitivity claim (4.1) and theorem 1.3 proceeds as follows. Let $\{\mu^0, \mu'\}$ be a cut-pair. Define the di-paths

$$\begin{aligned} \gamma^0 &:= [m_1^*, \mu^0]_1^{c^0} \; [\mu^0, 0]_2^0 \,; \\ \gamma' &:= [m_1^*, \mu']_1^{c'} \; [\mu', \overline{m}_2')_2' \end{aligned} \tag{4.8}$$

by concentration at the cut-vertices μ^0, μ'. Here $c^0 = c(\mu^0)$, $c' = c(\mu') \in \{0, '\}$ denote the colors of the cut vertices μ^0, μ', respectively. In subsection 4.3 below we show that cut-pairs $\{\mu^0, \mu'\}$ exist and that the above di-paths γ^0 and γ' satisfy properties (i) – (iv) of theorem 1.2 – establishing transitivity.

4.2 Special cases

In this subsection we address the construction of di-paths γ^0, γ' with properties (i) – (iv) of theorem 1.2, in several special cases. We begin with the case $m_1^* = m_2^*$, $j_1^* \subseteq \gamma_1'$ of coloring conflict (4.6). Afterwards we address the cases where one of the vertices m_1^*, m_2^*, m_2' is zero.

Case 1: $m_1^* = m_2^*$, $j_1^* \subseteq \gamma_1'$.

We then define $\gamma^\rho := \gamma_2^\rho$ for $\rho \in \{0, '\}$. We have to show properties (i) – (iv) of theorem 1.2 follow for γ^ρ from the corresponding properties for γ_2^ρ; see fig. 1.1.

Emanation property (i) of γ^ρ holds because $m^* = m_1^*$, by assertions (4.3), and because $m_1^* = m_2^*$, by assumption.

Leading edge property (ii) of γ^ρ claims

$$j^* \subseteq \gamma_2^0 \cup \gamma_2' \,. \tag{4.9}$$

Assertion (4.3) implies $j^* = j_1^*$, and $j_1^* \subseteq \gamma_1'$ by assumption. Because $m_1' = m_2^*$ by (4.3), and $m_2^* = m_1^*$ by assumption, we observe that $\gamma_1' =$

$[m_1^*, \overline{m}_1')_1' = [m_1', \overline{m}_1')_1'$ contains but the single edge j_1'. In particular $j_1^* = j_1'$. But (4.3) asserts $j_1' = j_2^*$. Together, we conclude the leading edge property (ii) of $\gamma^\rho = \gamma_2^\rho$ because $j^* = j_1^* = j_1' = j_2^*$ and because (4.9) holds for γ_2^ρ.

Termination property (iii) is identically true for γ^0 and γ_2^0. For γ' it follows from γ_2' because (4.3) asserts $j' = j_2'$. Nonintersection property (iv) holds, identically, for γ^ρ and γ_2^ρ. This settles case 1.

Case 2: At least one of the vertices m_1^*, m_2^*, m_2' is 0.

Consider any feed reaction $j_0 \in \mathbf{E}$, i.e. the mother vertex $m_0 := \mathbf{m}(j_0)$ satisfies $m_0 = 0$. Then r_{j_0} is a constant parameter and hence $r_{j_0 m} = 0$ for all $m \in \mathbf{M}$; see (1.3). Therefore definition (1.14) of the flux response implies

$$\Phi_{j_0 j} = \delta_{j_0 j} \tag{4.10}$$

for all $j \in \mathbf{E}$.

Now consider the case $m_2' = 0$, with child edge j_2': $m_2' \to \overline{m}_2'$. Then (4.10) with $j_0 := j_2'$, $j := j_2^*$ and assumption (4.1) imply $\delta_{j_2' j_2^*} = \Phi_{j_2' j_2^*} \neq 0$, i.e. $j_2' = j_2^*$ and $m_2' = m_2^* = 0$. It therefore remains to consider the cases ${m_1}^* = 0$ and $m_2^* = 0$.

Next suppose $m_2^* = 0$. Since m_2^* is the mother of j_2^* and assertion (4.3) implies $j_2^* = j_1'$ we can invoke (4.10) with $j_0 := j_2^*$, $j := j_1^*$. Assumption (4.1) then yields $\delta_{j_2^* j_1^*} = \Phi_{j_2^* j_1^*} \neq 0$, i.e. $j_1^* = j_2^*$, which has trivially been excluded in (4.2).

In the remaining case $m^* = m_1^* = 0$, we first recall theorem 1.2. Since we have to take the trivial path $\gamma^0 = \{m^*\}$, we see that

$$\Phi_{j' j^*} \neq 0 \tag{4.11}$$

algebraically if, and only if, there exists a di-path γ' from $m^* = 0$ with leading edge j^* and terminating with edge j'. For $\Phi_{j_2^* j_1^*} \neq 0$ we are given such a di-path γ_1' from $m^* = 0$ to $j_1' = j_2^*$. Since $\Phi_{j_2' j_1^*} \neq 0$ we are also given a di-path γ_2' from $m_2^* = m_1' = \mathbf{m}(j_1')$ to the edge $j' = j_2'$: $m_2' \to \overline{m}_2'$; see (4.3). Let γ' be defined as the concatenation di-path

$$[m^*, m_1']_1' \; [m_2^*, \overline{m}_2')_2' \tag{4.12}$$

with shared vertex $m_1' = m_2^*$ and all intermediate loops removed. Then (4.3), (4.11) imply $\Phi_{j_2' j_1^*} = \Phi_{j' j^*} \neq 0$ algebraically, as claimed in (4.1). This settles the case $m_1^* = 0$.

4.3 Cut-pairs and di-paths

To complete the proof of transitivity theorem 1.3, in the general case, two tasks remain. Given general di-paths γ_i^0 and γ_i' with properties (i) – (iv) of theorem 1.2, for $i = 1, 2$, we have to construct a cut-pair $\{\mu^0, \mu'\}$ of vertices with properties (4.7) (a) – (c). In a second step we have to show that the di-paths γ^0 and γ' defined in (4.8) also satisfy properties (i) – (iv) of theorem 1.2.

We show the existence of a cut-pair $\{\mu^0, \mu'\}$ for the paths $\gamma_1 = \gamma_1^0 \cup \gamma_1'$ and $\gamma_2 = \gamma_2^0 \cup \gamma_2'$ as follows. First we color all vertices m of $\gamma_1 \cap \gamma_2$ black or white; see (4.4) – (4.5). We exclude the cases where zero vertices m_1^*, m_2^*, or m_2' arise and cyclic paths γ_i^c may occur; these cases have already been treated in 4.2. Of course we also exclude the only case of (4.6) where m_2^* might be white. Therefore $m_2{}^*$ is colored black and $0 \in \gamma_2^0 \cap \gamma_1^0$ is colored white. We construct the cut-pair $\{\mu^0, \mu'\}$ from a pair of candidates $\mu_2^\rho \in \gamma_2^\rho$, $\rho \in \{0, '\}$, by an iterative process. We start with $\mu_2^0 := 0 \in \gamma_2^0 \cap \gamma_1^0$, white, and with μ_2' as the rightmost colored vertex on γ_2'. Since $m_2^* = m_1' \in \gamma_2' \cap \gamma_1'$ is black, μ_2' indeed exists. Two cases arise.

Case 1: μ_2^0 and μ_2' are both white.

> Let μ_2^ρ denote the earlier of the two vertices μ_2^0 and μ_2' on γ_1^0. We then discard all vertices of γ_1^0 which occur strictly later than μ_2^ρ on γ_1^0, including the other vertex μ_2^σ of μ_2^0, μ_2' defined by $\sigma \neq \rho$. Note $\mu_2^\rho \in \gamma_2^\rho \setminus \{m_2^*\}$ is still white. Define a new vertex μ_2^σ to be the rightmost colored vertex, on the other branch γ_2^σ of γ_2 which has remained on γ_2^σ after the above removal of some white vertices. If μ_2^σ is still white, reiterate the above strict removal process of white vertices, with $\{\mu_2^0, \mu_2'\} := \{\mu_2^\rho, \mu_2^\sigma\}$, until case 2 occurs.

Case 2: μ_2^0 and μ_2' are of opposite color.

> Note that this case has to occur eventually, because the vertex $0 \in \gamma_2^0$ is white and $\{m_2^*\} = \gamma_2^0 \cap \gamma_2'$ is black. When case 2 first occurs we terminate the iteration and define the cut-pair
>
> $$\{\mu^0, \mu'\} := \{\mu_2^\rho, \mu_2^\sigma\}\,. \tag{4.13}$$

Actually, we still have to show that the cut-pair properties (4.7) hold with this definition. Property (4.7) (a) of opposite branches holds because $\rho \neq \sigma$. Property (4.7) (b) of opposite colors holds by termination at case 2. Ordering property (4.7) (c) holds because we only have removed all later white vertices, in case 1, and have always chosen the rightmost remaining vertex μ_2^σ. This proves that (4.13) defines a cut-pair $\{\mu^0, \mu'\}$.

To complete the proof of transitivity theorem 1.3 it now remains to show that the di-walks γ^0 and γ', defined by concatenation (4.8) at the cut-pair $\{\mu^0, \mu'\}$, satisfy properties (i) – (iv) of theorem 1.2; see fig. 1.1. We also have to show that the walks γ^0, γ' are paths, i.e. are without self-intersections.

Emanation property (i) is immediate because γ^0 and γ' both start from $m^* = m_1^*$, see (4.3), (4.8). Termination property (iii) is immediate, likewise, by construction (4.8) and because (4.3) asserts $j' = j_2'$. We address intersections (iv) and self-intersections of γ^0, γ' next, and finish with property (ii) on the leading edge $j^* = j_1^*$ thereafter.

Nonintersection property (iv) follows from the same property for each of the pairs γ_i^0, γ_i' and properties (4.7) of the cut-pair μ^0, μ'. Indeed (iv) for γ_i^0, γ_i' and $i = 1, 2$ implies

$$\{m_1^*\} \subseteq [m_1^*, \mu^0]_1^{c^0} \cap [m_1^*, \mu']_1^{c'} \subseteq \gamma_1^0 \cap \gamma_1' = \{m_1^*\} \tag{4.14}$$

because the colors c^0, c' of μ^0, μ' are opposite. Similarly $\mu^0 \neq \mu'$ and $\mu^\rho \in \gamma_2^\rho$ imply

$$[\mu^0, 0]_2^0 \cap [\mu', \overline{m}_2')_2' \subseteq (\gamma_2^0 \cap \gamma_2') \setminus \{m_2^*\} = \emptyset\,. \tag{4.15}$$

We consider any diagonal intersection vertex

$$m \in [m_1^*, \mu^0]_1^{c^0} \cap [\mu', \overline{m}_2')_2' \tag{4.16}$$

next. Since μ' and μ^0 have opposite color, $c^0 := c(\mu^0)$ implies $\mu' \notin \gamma_1^{c^0}$. Therefore any intersection vertex $m \in [\mu', \overline{m}_2')_2' \cap \gamma_1^{c^0}$ must lie strictly to the right of μ' on γ_2'. Hence m must occur strictly later than μ^0 on $\gamma_1^{c^0}$ by cut-pair property (4.7) (c). This proves that the intersection (4.16) is empty. A precisely analogous argument shows $[m_1^*, \mu']_1^{c'} \cap [\mu^0, 0]_2^0 = \emptyset$. This proves nonintersection property (iv) of the paths γ^0, γ' constructed in (4.8).

We show next that the di-walks γ^0, γ' defined in (4.8) by concatenation of di-paths at the cut-pair $\{\mu^0, \mu'\}$ are actually di-paths. For γ^0 we have to show that the intersection

$$[m_1^*, \mu^0]_1^{c^0} \cap [\mu^0, 0]_2^0 = \{\mu^0\} \tag{4.17}$$

consists of the concatenation point μ^0, only. Similarly to (4.16), suppose there exists any other intersection vertex $m \neq \mu^0$ in the intersection. Then $m \in (\mu^0, 0]_2^0$ lies strictly to the right of cut-vertex μ^0 on γ_2^0. Cut-pair property (4.7) (c) then implies $m \in [m_1^*, \mu^0]_1^{c^0} \subseteq \gamma_1^{c^0} \subseteq \gamma_1$ occurs strictly later than μ^0 on γ_1 – a contradiction. This proves claim (4.17). The analogous argument on γ_2' establishes $[m_1^*, \mu']_1^{c'} \cap [\mu', \overline{m}_2')_2' = \{\mu'\}$. This shows that γ^0, γ' are di-paths.

Property (ii), that one of the paths γ^0, γ' contains the starting edge $j^* = j_1^*$, is immediate from the same property for the di-paths γ_1^0, γ_1', unless the cut-vertex μ^ρ coincides with the colored vertex $m_1^* \in \gamma_2^\rho$. Let $c^\rho := c(\mu^\rho)$ denote the color

of $\mu^\rho = m_1^*$. Let $\sigma \neq \rho$. Then $\mu^\sigma \neq \mu^\rho = m_1^*$ is the other cut-vertex, and is of the opposite color $c^\sigma := c(\mu^\sigma) \neq c^\rho$. By definition (4.4) – (4.5) of the color c^ρ of $\mu^\rho = m_1^*$, the edge j_1^* emanating from m_1^* is the first edge of $\gamma_1^{c^\rho}$. But by definition (4.8), the path γ^σ contains the piece $[m_1^*, \mu^\sigma]_1^{c^\sigma}$ and hence, by $\mu^\sigma \neq {m_1}^*$, the first edge $j_1^* = j^*$. This proves the leading edge property (ii) for γ^σ. It also completes the proof of theorem 1.3 via theorem 1.2. ⋈

5 Proof of theorem 1.1: concentration response

Our proof of theorem 1.1 on the concentration response uses theorem 1.2 on the flux response, which was proved in section 3 above. We distinguish two cases for the concentration response δx_m^* of the metabolite $m \in \mathbf{M}$ to a rate perturbation of reaction $j^* \in \mathbf{E}$. Because we have assumed $\det \mathbf{A} \neq 0$, algebraically, any vertex $m \in \mathbf{M}$ possesses a child reaction $j' = J(m)$ by the child selection map J; see (2.20) and corollary 2.3. In subsection 5.1 we assume $j' \neq j^*$. Subsection 5.2, in contrast, considers the case where $j' = j^*$ is the only child of m. This covers all cases.

5.1 The case $m = \mathbf{m}(j')$, $j' \neq j^*$.

In this case the flux-concentration relation (1.14) reads

$$\Phi_{j'j^*} = r_{j'm} \delta x_m^* \tag{5.1}$$

with $r_{j'm} \neq 0$, algebraically. Therefore, algebraically, a nonzero concentration response $\delta x_m^* \neq 0$ is equivalent to a nonzero flux response $\Phi_{j'j^*} \neq 0$. Theorem 1.2, equivalently, provides us with paths γ^0 and γ' which satisfy properties (i) – (iv), there. We have already observed how the paths γ^m of theorem 1.1 and γ' of theorem 1.2 only differ by appending/removing the final edge j' of γ'. In the notation of section 4 we therefore define $\gamma^m := [m^*, m]'$ by omission of j' from γ'. We keep $\gamma^0 := [m^*, 0]^0$. Then γ^0, γ^m satisfy the property list (i) – (iv) of theorem 1.1, equivalently to $\delta x_m^* \neq 0$. Therefore (5.1) and theorem 1.2 imply theorem 1.1.

5.2 The case when $j' = j^*$ is the single child of $m = \mathbf{m}(j')$.

In this case we have already observed in (1.19) that

$$\delta x_m^* = -1/r_{j^*m} \neq 0 \tag{5.2}$$

for $m = \mathbf{m}(j') = \mathbf{m}(j^*) = m^*$. With the trivial path $\gamma^m := \{m^*\}$, the statements (i) – (iv) of theorem 1.1 hold true, directly. Indeed lemma 2.2 asserts the

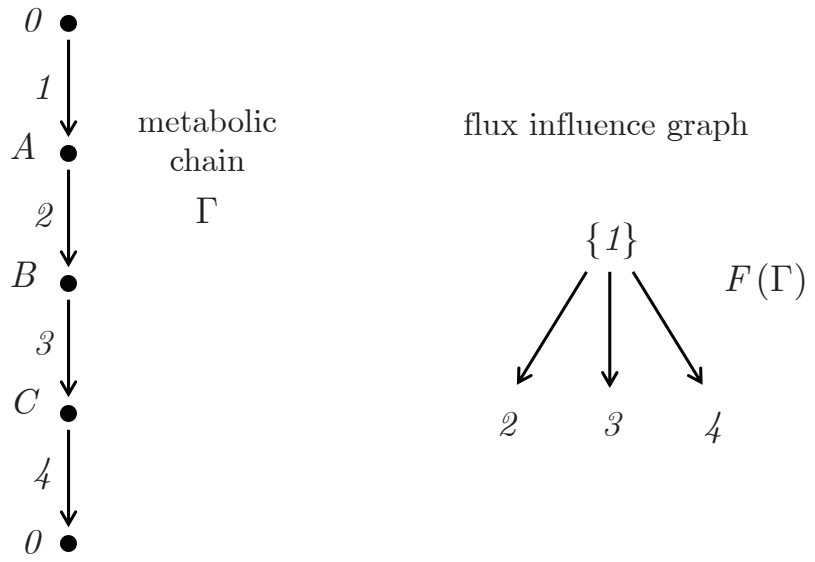

Figure 6.1: *Left: the monomolecular chain* Γ *of 3 metabolites* $\mathbf{M} = \{A, B, C\}$ *and 4 reactions* $\mathbf{E} = \{1, 2, 3, 4\}$. *Right: the flux influence graph* $F(\Gamma)$. *Only the feed reaction 1 influences all fluxes, including itself. The other reactions are without any influence.*

existence of a di-path γ^0 from m^* to 0, under our standing regularity assumption (1.6) that $\det f_x = \det \mathbf{SR} \neq 0$. Evidently the di-path γ^0 contains the single child edge j^* emanating from m^*. By construction, therefore, the paths γ^0, γ^m satisfy all properties (i) – (iv) required in theorem 1.1. This completes the proof of theorem 1.1. $\bowtie$

6 Examples

In this section we review the monomolecular examples of the companion paper [MoFi14] from a flux transitivity point of view. We also discuss several tetrahedral di-graphs on four metabolites with single feed and exit edges from/to vertex 0. All examples satisfy the regularity condition (1.6), algebraically, as is easily checked via the exit paths γ^0 to 0 recommended in lemma 2.2; see (2.17).

We present each example, first, as a network graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$. We label vertices by 0 and metabolites A, B, C, $\cdots \in \mathbf{M}$. We label the directed reaction arrows by positive integers 1, 2, 3, $\cdots \in \mathbf{E}$. Next to the network graph Γ we indicate the flux influence graph $F(\Gamma)$, with the flux components as vertices and flux influences as arrows. Flux components are indicated in braces $\{j_1, j_2, \dots\}$ with the exception of singletons j^*. We use the notation $\{j^*\}$ to indicate true self-loops $\Phi_{j^*j^*} \neq 0$, i.e. nonzero self-influence $j^* \rightsquigarrow j^*$. In case $\Phi_{j^*j^*} = 0$ we omit the braces. This notation facilitates the determination of the influence sets $I(j^*)$ from the influence graph $F(\Gamma)$; see (1.26). In each example we indicate the

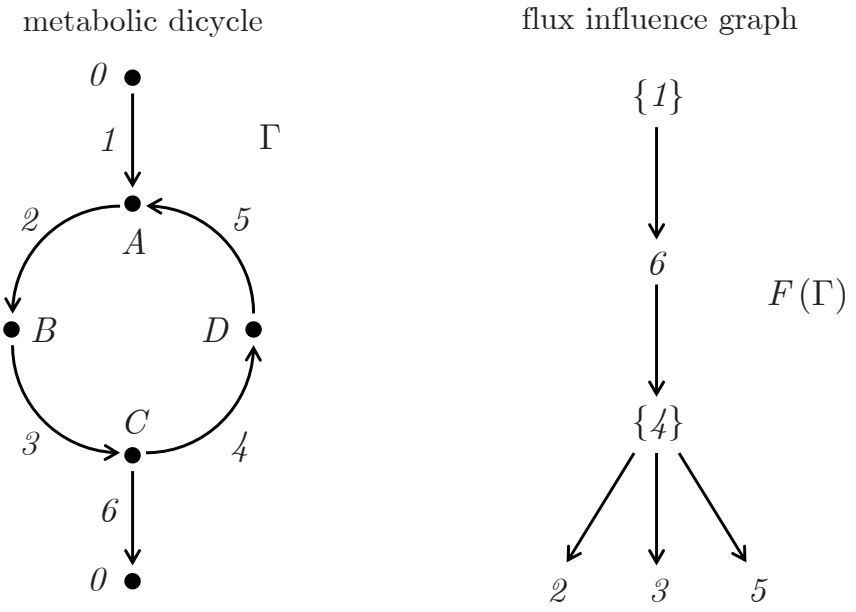

Figure 6.2: *Left: a monomolecular single di-cycle of 4 metabolites* $\mathbf{M} = \{A, B, C, D\}$ *with 6 reactions* $\mathbf{E} = \{1, \ldots, 6\}$. *Right: the flux influence graph* $F(\Gamma)$.

bare-handed derivation of the flux influence graph $F(\Gamma)$ and comment on some peculiarities. For the closely related concentration response, which is the main concern in experiments, we refer back to section 5 and theorem 1.1.

Our first example is the monomolecular chain of fig. 6.1. Any reaction edge j^* other than the feed 1 is a single child of a mother vertex $m = \mathbf{m}(j^*) \in \mathbf{M}$. Therefore $\Phi_{j'j^*} = 0$ for all j'; see (1.18). The feed case $j^* = 1$, $m^* = 0$ has been discussed in section 4.2, case 2. Specifically (4.11) implies $\Phi_{j'1} \neq 0$ for all $j' \in \mathbf{E}$, because any edge j' is reachable from the leading edge $j^* = 1$ by a di-path γ'. This proves the flux influence graph $F(\Gamma)$ shown in fig. 6.1. The extension to a monomolecular chain with any finite number of metabolites is straightforward, without further calculation.

Our second example Γ is the monomolecular single di-cycle $A \xrightarrow{2} B \xrightarrow{3} C \xrightarrow{4} D \xrightarrow{5} A$ with feed 1 to A and exit 6 of C. The single child reactions 2, 3, 5 appear at the bottom level of zero influence in the flux influence graph $F(\Gamma)$. The entire cycle is driven by $j^* = 4$ and path $\gamma^0 = \{C \xrightarrow{6} 0\}$. In fact an admissible path γ', in the sense of properties (i) – (iv) of theorem 1.2, can reach any edge j' in the cycle, from $m^* = C$, and no other edge. Indeed γ^0: $C \xrightarrow{6} 0$ is the closed edge 6 including the two end points $m^* = C$ and 0. The path γ' from $m^* = C$ to $j' = 3$ terminates at the open edge j' and hence does not intersect γ^0 at C. This complies with disjointness property (iv) of theorem 1.2. Edge $j^* = 6$ from $m^* = C$ requires the same exit path γ^0 and hence influences the same other edges of the cycle, but not itself. The single feed 1 influences all edges including itself,

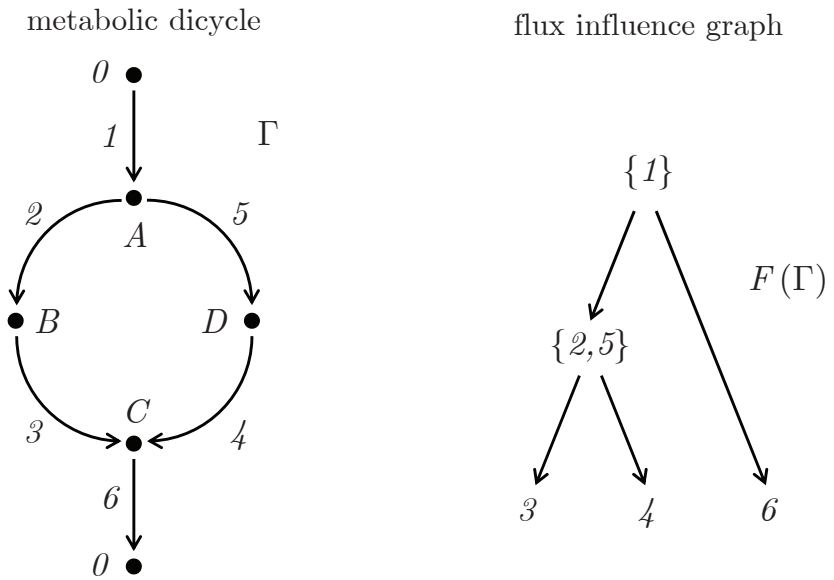

Figure 6.3: *Left: monomolecular branching network* Γ. *The network coincides with fig. 6.2, except for the reversed directions of reactions* 4, 5 $\in \mathbf{E}$. *The central di-cycle becomes a nonoriented cycle with two parallel forward branches* 2, 3 *and* 5, 4 *emanating from metabolite* A *and joining at* C. *Right: the flux influence graph* $F(\Gamma)$ *changes drastically, compared to fig. 6.2.*

as before.

Example 3, of figure 6.3, is a variant of figure 6.2 where only the directions of reactions 4, 5 have been reversed. This splits the central di-cycle into the two parallel branches $A \xrightarrow{2} B \xrightarrow{3} C$ and $A \xrightarrow{5} D \xrightarrow{4} C$. The single child reactions become 3, 4, 6. Each of the branch entries 2 and 5, separately, now influences both branches, but not the shared exit $6 \subseteq \gamma^0$. The influence of the single feed 1 remains global.

Example 4, of figure 6.4, features two overlapping di-cycles (a): $B \xrightarrow{3} C \xrightarrow{4} D \xrightarrow{8} F \xrightarrow{9} B$, and (b): $A \xrightarrow{2} B \xrightarrow{3} C \xrightarrow{6} E \xrightarrow{7} A$. As before, single children 2, 3, 7, 9 exert no influence, whereas the influence of the single feed 1 is global.

Let us consider the influence of $j^* = 6$, $m^* = C$, next. By the unique exit di-path γ^0: $C \xrightarrow{4} D \xrightarrow{5} 0$, the edge $j^* = 6$ activates the whole di-cycle (b) via a di-path γ'. Because γ^0 is unique, however, the leading edge $j^* = 6$ cannot influence any other edges. Swapping the perturbed edge j^* from γ' to become the leading edge $j^* = 4$ of the exit path γ^0, instead, produces the same influence set

$$I(4) = I(6) = \{2,\, 3,\, 7,\, 6\} = (b) \tag{6.1}$$

which spans the full di-cycle (b). This illustrates corollary 3.1 on cycle influence.

The influence sets $I(5) = I(8)$ of the two outgoing reactions 5 and 8 of $m^* = D$ are also identical a priori, by swapping of j^*. Let us therefore consider $j^* = 5$,

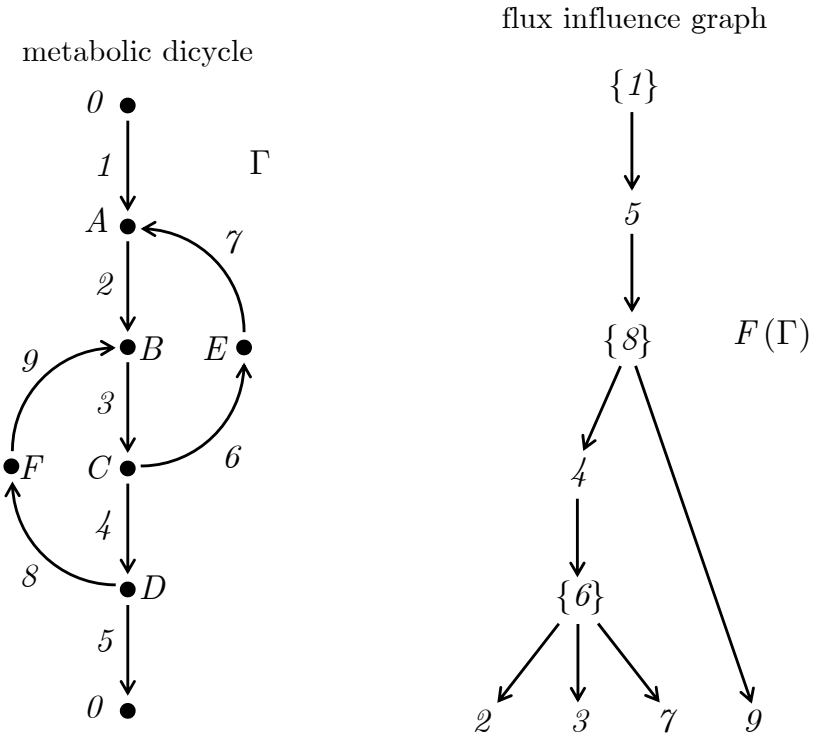

Figure 6.4: *Left: a monomolecular network* Γ *of two overlapping di-cycles with* 6 *metabolites* $\mathbf{M} = \{A, \ldots, F\}$ *and* 9 *reactions* $\mathbf{E} = \{1, \ldots, 9\}$. *Right: the flux influence graph* $F(\Gamma)$. *Note the global influence of single feed* 1*, and the absent influence of single children* 2, 3, 7, 9. *The branching pairs* 5, 8 *and* 4, 6*, respectively, share the same influence sets.*

without loss of generality. The only possible exit path γ^0 from $m^* = D$ is the closed edge γ^0: $D \xrightarrow{5} 0$. Again the other path γ' from $m^* = D$ with leading edge 8 can traverse the whole di-cycle (a) to which 8 belongs. The forward branching 4, 6 at metabolite vertex C in di-cycle (a), however, also opens access to the whole di-cycle (b) via reaction arrow 6. This shows that the influence sets

$$I(5) = I(8) = \{2, 3, 4, 6, 7, 8, 9\} = (a) \cup (b) \tag{6.2}$$

span both di-cycles (a) and (b).

It is an amusing and highly recommended exercise to revert one or both orientations of the reaction branch 6, 7 and of the pair 8, 9, independently. The arising three cases of mixed di-cycles and branching are left to the reader.

Instead we consider the four metabolic networks Γ of fig. 6.5, (I) – (IV). They feature the complete graph of 4 vertices $\mathbf{M} = \{A, B, C, D\}$ and 6 reactions $1, \ldots, 6$. Up to isomorphism, there is only one irreversible orientation with a 4-element di-cycle, and one without; see cases I – III, and IV, respectively. We then choose various single feeds 1 and single exits 8 such that regularity condition (1.6) holds in the guise of lemma 2.2 and (2.17).

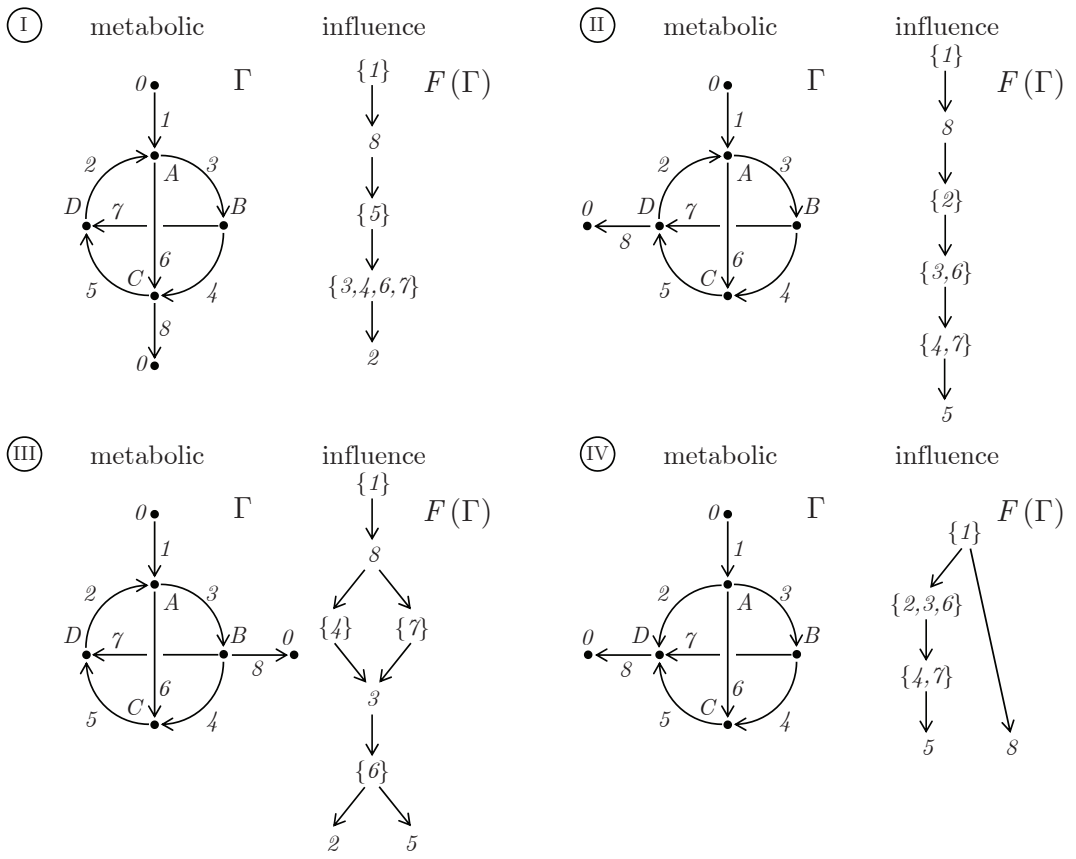

Figure 6.5: *Four examples I – IV of monomolecular metabolic networks* Γ *with a complete irreversible di-graph on* 4 *metabolites* $\mathbf{M} = \{A, B, C, D\}$ *with* 8 *reactions* $\mathbf{E} = \{1, \ldots, 8\}$ *and varying positions of single feed* 1 *and single exit* 8. *Examples I – III feature a* 4*-element di-cycle* $A \xrightarrow{3} B \xrightarrow{4} C \xrightarrow{5} D \xrightarrow{2} A$, *whereas example IV does not. The flux influence graphs* $F(\Gamma)$ *are specified to the right of each metabolic network* Γ.

As always, the single feed 1 exerts global influence, and the single children exert none. In examples I and II, all other mothers have out-degree 2, so that swapping of j^* between the two outgoing edges produces identical results. In example I this applies to the outgoing edge pairs $j^* \in \{3, 6\}$, $\{4, 7\}$, and $\{5, 8\}$; example II has the pairs $\{2, 8\}$, $\{3, 6\}$, $\{4, 7\}$ instead.

Consider example I, $j^* = 6$ with $m^* = A$. The exit di-path γ^0: $m^* = A \xrightarrow{6} C \xrightarrow{8} 0$ is possible and makes edges $j' = 3, 4, 7, 2$, only, accessible to the complementary paths γ'. Choosing the other exit di-path γ^0: $m^* = A \xrightarrow{3} B \xrightarrow{4} C \xrightarrow{8} 0$ with leading edge 3 makes edge $j' = 6$ accessible to γ', only. This establishes the influence sets

$$I(3) = I(6) = \{2, 3, 4, 6, 7\}\,. \tag{6.3}$$

The arguments for $I(4) = I(7) = I(3)$ are similar and are omitted.

Consider example I, $j^* = 8$, $m^* = C$ next. This forces γ^0: $m^* = C \xrightarrow{8} 0$ as the only exit path. The complementary path γ' can then roam all over the tetrahedral edges 2, ..., 7, only. This proves

$$I(5) = I(8) = \{2, \dots, 7\} \tag{6.4}$$

and establishes the flux influence graph $F(\Gamma)$ of example I We omit the rather analogous details which derive $F(\Gamma)$ in example II.

In example III we encounter out-degree 2 at vertex A and out-degree 3 at B. This allows swapping of $j^* \in \{3, 6\}$ at A, and proves $I(3) = I(6)$. We also note that any exit di-path γ^0 from any metabolite m^* must follow the sequence

$$C \xrightarrow{5} D \xrightarrow{2} A \xrightarrow{3} B \xrightarrow{8} 0 \tag{6.5}$$

from vertex m^* on. This seriously restricts the choices of the complementary paths γ' from m^*, and allows us to determine the influence sets $I(j^*)$ easily, via the mother vertex $m_* = \mathbf{m}(j^*)$:

$$\begin{array}{lll} j^* = 3, 6\,; & m^* = A\,; & I(3) = I(6) = \{2, 5\,, 6\} \\ j^* = 4\,; & m^* = B\,; & I(4) = \{2, \dots\,, 7\} \setminus \{7\} \\ j^* = 7\,; & m^* = B\,; & I(7) = \{2, \dots\,, 7\} \setminus \{4\} \\ j^* = 8\,; & m^* = B; & I(8) = \{2, \dots\,, 7\}\,. \end{array} \tag{6.6}$$

For $m^* = B$ we have used that property (iii) of theorem 1.2 and (6.5) force j^* to be the leading edge of γ' in cases $j^* = 4$, 7, but not 8. For $j^* = 8 \subseteq \gamma^0$ the paths γ' roam all over the tetrahedral edges 2, ..., 7 freely. This establishes the flux influence graph $F(\Gamma)$ of example III. The analogous details of example IV are left to the reader as a final exercise.

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