

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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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 *structural sensitivity analysis* is based on the directed graph structure of the monomolecular reaction network, only. In fact, our function-free approach does not require numerical input. We work with general, not necessarily monotone reaction rate functions. Based on the graph structure alone, we derive which steady state concentrations and reaction fluxes are sensitive to, and thus affected by, a rate change – and which are not. Moreover, we establish a transitivity property for the influence of a rate perturbation, at any reaction, on all 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 sensitivity 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. Because our mathematical analysis will be based on the stoichiometric graph structure of the metabolic or chemical reaction network, only, we call our approach a *structural sensitivity analysis*.

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, \bar{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 $m \in \mathbf{M}$ *metabolites* or *reactants*. 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*



from $m^j \in \mathbf{M} \cup \{0\}$ to a different $\bar{m}^j \in \mathbf{M} \cup \{0\}$. *Feed reactions* j have $m^j = 0$, and *exit reactions* have $\bar{m}^j = 0$. Any ordered pair (m, \bar{m}) is connected by at most one arrow. Self-loops $\bar{m} = m$ are forbidden, but reverse arrows from \bar{m} to $m \neq \bar{m}$ are allowed. A reaction $j: m \rightarrow \bar{m}$ is called *reversible* if the reverse reaction $\bar{j}: \bar{m} \rightarrow m$ also occurs in Γ .

In chemistry the reactant m^j is called an *educt* and \bar{m}^j is a *product* of reaction j . Although monomolecular reactions are usually allowed to yield several products, in chemistry, we are only able to deal with single products in the present paper. Our graph representation of chemical networks therefore coincides with the graphs on reaction complexes of [Fe95]. In fact the monomolecular reactions which we consider are a special case of the deficiency zero networks studied in [Fe95], as we will see in section 2. We do not restrict attention to mass action kinetics, though, and allow for rather general reaction rate functions.

A *path* in a directed graph Γ is any succession of vertices and edges, 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* 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*. A *bi-cycle* is a (non-oriented) cycle which decomposes into two parallel di-path arcs, each with at least one edge. The arcs are required to be disjoint, except for their shared start and termination vertices. Section 4 contains a detailed analysis of the role of cyclicity for flux influence.

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

$$(1.2) \quad \mathbf{m} : \mathbf{E} \rightarrow \mathbf{M} \cup \{0\}$$

such that $\mathbf{m}(j) = m^j$ is the mother of j . The reaction products \bar{m}^j are obtained by a map $\bar{\mathbf{m}}(j) := \bar{m}^j$. The feed reactions j are the elements of $\mathbf{E}_0 := \mathbf{m}^{-1}(0)$. The elements of $\bar{\mathbf{m}}^{-1}(0)$ are the exit reactions.

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

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

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 $r_j \in C^1$,

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

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

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

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).

Let us illustrate the role of the artificial zero-complex 0 in a simple example. Consider the reaction network $0 \xrightarrow{1} A \xrightarrow{2} 0$ with a single metabolite $\mathbf{M} = \{A\}$, $M = 1$. The network consists of only two reactions $\mathbf{E} = \{1, 2\}$: the feed reaction $j = 1$, and the exit reaction $j = 2$. Indeed $m(1) = \bar{m}(2) = 0$. The unit “vector” $e_A = 1 \in \mathbb{R}^M$ is scalar, as is $e_0 = 0$. Therefore the reaction rates are $r_1 = r_1(x_0) = r_1(1)$ and $r_2 = r_2(x_A)$. Note that r_1 is just a constant. Let us put these pieces together and abbreviate $x := x_A$. Then the single ODE (1.3) of the network $0 \xrightarrow{1} A \xrightarrow{2} 0$ becomes $\dot{x} = r_1 - r_2(x)$.

Our final assumption requires the general network to be *regular* at the steady state \mathbf{x}^* of (1.3): 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:

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

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

$$(1.7) \quad \mathbf{r}^\varepsilon := \mathbf{r} + \varepsilon \rho$$

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

$$(1.8) \quad f_r \cdot \rho + f_x \cdot \frac{d}{d\varepsilon} x^* = 0$$

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

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

$$(1.10) \quad \rho_{j^*}(x_{\mathbf{m}(j^*)}^*) = 1.$$

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

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

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*

$$(1.12) \quad \delta x_m^{j^*} \neq 0$$

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^* .*

Here and below, *disjoint* means that the paths do not share any edge, or any vertex besides m^* . The condition on γ^m may appear 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

$$(1.13) \quad \delta x_{m^*}^{j^*} \neq 0;$$

see proposition 2.1 below.

If the perturbed reaction j^* is a feed reaction to the reaction network then the mother vertex m^* of j^* is the zero-complex, $m^* = 0$. In this case we may choose

γ^0 to consist of the vertex $m^* = 0$, only, without any edges. Then (i) – (iv) only require a path γ^m which starts with the perturbed reaction edge j^* , from m^* , and terminates at $m \neq 0$ before reaching the zero-complex.

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.

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

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

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

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:

$$(1.16) \quad j^* \rightsquigarrow j' \iff \Phi_{j'j^*} \neq 0.$$

The next theorem initiates our structural sensitivity analysis of flux influence. Like the concentration response above, we show how an external perturbation at j^* either propagates downward along a directed path γ' starting with m^* and j^* , or else spills over to a side branch γ' from $j^* \in \gamma^0 \setminus \gamma'$.

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*

$$(1.17) \quad \Phi_{j'j^*} \neq 0$$

algebraically, if, and only if, there exist two directed paths γ^0 and γ' 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 exit path γ^0 terminates at vertex 0, and the influence path γ' terminates with reaction edge j' : $m' \rightarrow \bar{m}'$, but omitting the product (head) vertex $\bar{m}' = \bar{\mathbf{m}}(j')$ of j' ;*

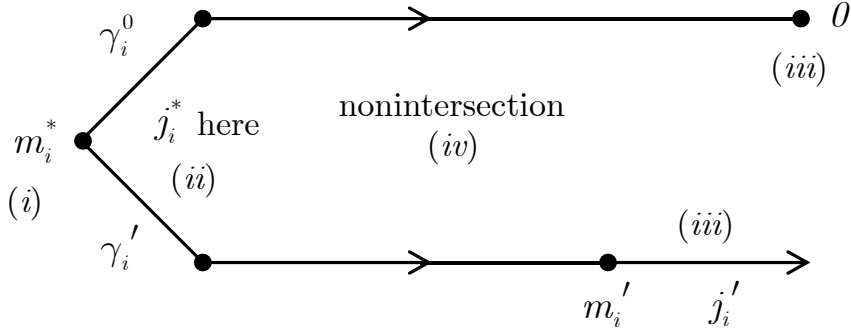


Figure 1.1: Summarizing properties (i) – (iv) of theorem 1.2 for the exit di-paths γ_i^0 , top, and the influence di-paths γ_i' , bottom. Property (i): both paths emanate from m_i^* ; for (ii): one of them contains j_i^* ; for (iii): termination is at vertex 0 and edge j_i' respectively; for (iv): the two paths are disjoint except for m_i^* . The index i will be used in section 5.

(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 the influence path 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

$$(1.18) \quad \Phi_{j^*j^*} = 0,$$

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

$$(1.19) \quad \delta x_{m^*}^{j^*} = -1/r_{j^*m^*} \neq 0.$$

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

$$(1.20) \quad \delta x_m^{j^*} = 0$$

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*

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

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

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

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

$$(1.23) \quad j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_3 \quad \text{implies} \quad j_1 \rightsquigarrow j_3.$$

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:

$$(1.24) \quad j \approx j,$$

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

$$(1.25) \quad j_1 \approx j_2 \iff j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_1.$$

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. In other words, the flux influence graph $F(\Gamma)$ depicts the partial order defined on the flux components F_i by flux influence.

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

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

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 partial order of

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 6, 3, and 5, respectively. Section 4 is devoted to an analysis of di-cycles and bi-cycles in the 2-edge-connected influence set $I(j^*)$. See theorem 4.2 and corollaries 4.1, 4.3.

In section 7 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.

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2 Positivity, connectivity, and cycles

In the present section we collect some further notions about graphs. In lemma 2.3 and corollary 2.4 we express regularity assumption (1.6) in graph terminology: the Jacobian determinant of $f'(x^*)$ 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 2.5.

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 an undirected path joining m and m' . In di-graphs, $m \approx m'$ are strongly connected if there exist two di-paths: one from m to m' and one from m' to m . Without any edge, $m = m'$ implies $m \sim m'$ and $m \approx m'$. 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

$$(2.1) \quad r_{jm} = r_j'(x_m^*),$$

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

$$(2.2) \quad f'(x^*) = \mathbf{S}\mathbf{R}$$

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* $\mathbf{S}: \mathbb{R}^E \rightarrow \mathbb{R}^M$ is defined by

$$(2.3) \quad \mathbf{S}e_j = e_{\bar{\mathbf{m}}(j)} - e_{\mathbf{m}(j)}.$$

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 $e_0 := 0$. Note $\mathbf{S}e_j = e_{\bar{\mathbf{m}}(j)}$ for feed reactions and $\mathbf{S}e_j = -e_{\mathbf{m}(j)}$ for exit reactions j .

Our main tool in the proof of lemma 2.3 below, as well as in the theorems, is the construction of an *augmented matrix* $\mathbf{A}: \mathbb{R}^M \times \mathbb{R}^N \rightarrow \mathbb{R}^E$, where $N := \dim \ker \mathbf{S}$ accounts for the kernel of the stoichiometric matrix $\mathbf{S}: \mathbb{R}^E \rightarrow \mathbb{R}^M$. Let $c^1, \dots, c^N \in \mathbb{R}^E$ be any basis for $\ker \mathbf{S}$, with components c_j^k , and define the $E \times N$ matrix

$$(2.4) \quad \mathbf{C} := (c_j^k)_{j \in \mathbf{E}, k \in \{1, \dots, N\}}.$$

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

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

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

$$(2.6) \quad \mathbf{S} = \mathbf{P}_0 \mathbf{D}$$

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

$$(2.7) \quad \mathbf{D}e_j := e_{\bar{\mathbf{m}}(j)} - e_{\mathbf{m}(j)}.$$

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

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

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

$$(2.9) \quad \chi(\Gamma) := \dim H_0 - \dim H_1 = M + 1 - E,$$

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 forest* T of an undirected graph Γ is any acyclic subgraph of Γ , which contains an (undirected) cycle as soon as 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 an undirected cycle c^j in $T \cup \{j\}$, by maximality of T . The cycle c^j is unique, by acyclicity of T . Note how $c^j \in \ker \mathbf{D} = H_1(\Gamma)$

are linearly independent. By maximality and acyclicity of T , the cycles c^j form a basis for H_1 , and hence

$$(2.10) \quad \dim H_1 = E - E(T)$$

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

In case the graph Γ is (weakly) connected, any maximal spanning forest T of Γ is also connected and hence is a spanning tree. We deviate slightly from standard terminology and call T a *maximal spanning tree*, in the connected case, to recall and emphasize that T consists of a maximal number of edges.

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,*

$$(2.11) \quad \dim H_1(\Gamma) = N := \dim \ker \mathbf{S}.$$

Moreover the following properties are mutually equivalent

- (i) $\mathbf{S}: \mathbb{R}^E \rightarrow \mathbb{R}^M$ is surjective;
- (ii) $N := \dim \ker \mathbf{S} = E - M$;
- (iii) the matrix $\mathbf{A}: \mathbb{R}^{M+N} \rightarrow \mathbb{R}^E$ is square;
- (iv) Γ is (weakly) connected, i.e.

$$(2.12) \quad \dim H_0(\Gamma) = 1.$$

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

$$(2.13) \quad e_0 \notin \text{range } \mathbf{D} = H_0(\Gamma)^\perp.$$

Admittedly $e_0 \perp 1_W$, for the indicator function of any weakly connected component W 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):

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

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

$$(2.15) \quad E - M = \text{def } \mathbf{S} - \text{cork } \mathbf{S} = N - \text{cork } \mathbf{S}.$$

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

$$(2.16) \quad \dim H_0 = \dim H_1 + M + 1 - E = 1 + N - (E - M).$$

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

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 can be 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).

Proposition 2.2. *Consider any directed graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ as in (1.1).*

Then

$$(2.17) \quad \det \mathbf{SR} \neq 0 \iff \det \mathbf{A} \neq 0.$$

Proof. We first show that $\det \mathbf{SR} \neq 0$ implies

$$(2.18) \quad \det \mathbf{A} \neq 0.$$

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

$$(2.19) \quad 0 = \mathbf{R}\xi + \mathbf{C}\mu.$$

Applying \mathbf{S} we obtain $\mathbf{SR}\xi = 0$. Indeed definition (2.4) of the \mathbf{S} -kernel part \mathbf{C} of \mathbf{A} implies $\mathbf{SC} = 0$. Our assumption $\det \mathbf{SR} \neq 0$ then implies $\xi = 0$. Linear independence of the columns c^k of \mathbf{C} , a basis for $\ker \mathbf{S}$, therefore implies $\mu = 0$ in (2.19). This proves claim (2.18).

To show, conversely, that $\det \mathbf{A} \neq 0$ implies $\mathbf{SR} \neq 0$, we show that \mathbf{SR} possesses trivial kernel. Let $\mathbf{SR}\xi = 0$. Then $\mathbf{R}\xi \in \ker \mathbf{S} = \text{span}\{c^1, \dots, c^N\}$ 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 $\xi = 0$, and the lemma is proved. \boxtimes

We now view $\det(\mathbf{SR})$ as a formal polynomial in the nontrivial derivative variables r_{jm} , for $j \in \mathbf{E}$ and $m = \mathbf{m}(j)$. We say that $\det \mathbf{SR} \neq 0$ *algebraically*, if this polynomial does not vanish identically.

Lemma 2.3. Consider any directed graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ as in (1.1).

Then

$$(2.20) \quad \det \mathbf{SR} \neq 0$$

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

Proof. By proposition 2.2, (2.17) we may replace $\det \mathbf{SR} \neq 0$ in (2.20) by $\det \mathbf{A} \neq 0$. By definition $\det \mathbf{A} \neq 0$ holds, algebraically, if and only if the expansion of the determinant contains any nontrivial monomial in the nontrivial entries r_{jm} of \mathbf{R} .

Consider any *child selection map*

$$(2.21) \quad \begin{aligned} J: \quad \mathbf{M} &\rightarrow \mathbf{E} \\ m &\rightarrow J(m). \end{aligned}$$

Then the mother map $\mathbf{m}: \mathbf{E} \rightarrow \mathbf{M} \cup \{0\}$ is a left inverse of the child selection map J :

$$(2.22) \quad \mathbf{m} \circ J = \text{id}_{\mathbf{M}}.$$

Now consider the polynomial expansion

$$(2.23) \quad \det \mathbf{A} = \sum_J a_J r^J.$$

The generating nontrivial monomials of this expansion are

$$(2.24) \quad r^J := \prod_{m \in \mathbf{M}} r_{J(m), m} = \prod_{j \in J(\mathbf{M})} r_j'(x_{\mathbf{m}(j)}).$$

The monomials r^J are in one-to-one correspondence with the child selection maps J . In fact $\det \mathbf{A} \neq 0$ algebraically if, and only if, at least one coefficient a_J of the nontrivial monomials r^J in (2.23) is nonzero. The coefficient a_J is the sub-determinant

$$(2.25) \quad a_J = \pm \det(c_j^k)_{j \in \mathbf{E} \setminus J(\mathbf{M}), k \in \{1, \dots, N\}}$$

of the kernel part \mathbf{C} of the augmented matrix \mathbf{A} .

We still have a choice for the specific basis c^1, \dots, c^N of $\ker \mathbf{S}$. We choose \mathbf{C} to be defined by the cycles c^k of a maximal spanning forest T_0 , as in (2.10). In fact T_0 is a maximal spanning tree because the graph Γ is connected. This follows from our assumption $\det \mathbf{SR} \neq 0$, surjectivity of the stoichiometric matrix \mathbf{S} , and proposition 2.1 (i), (iv).

In (2.25) the \mathbf{C} -rows of $J(\mathbf{M})$ are omitted. We introduce the abbreviation $\check{C}(J(\mathbf{M}))$ for that square matrix. It is now crucial to observe that

$$(2.26) \quad \det \check{C}(\mathbf{E}') \neq 0$$

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 maximal spanning tree T with vertices $\mathbf{M} \cup \{0\}$ and edges in $\mathbf{E}' = J(\mathbf{M})$ 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 di-path γ^0 cannot return to itself, ever. Therefore γ^0 can only 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. Then Γ is (weakly) connected. To show $\det \mathbf{A} \neq 0$ algebraically, it is then sufficient to construct a child selection $J: \mathbf{M} \rightarrow \mathbf{E}$ 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 exit di-path. Attach any missing vertex $m \in \mathbf{M}$ by following its acyclic exit di-path γ^0 until it bumps into any vertex which has been taken care of before. Inductively, this selects a unique child arrow $J(m)$, for any mother vertex $m \in \mathbf{M}$. The child selection J , in turn, defines a maximal spanning tree $T = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ which satisfies (2.22). In particular J defines a nontrivial monomial (2.24) of $\det \mathbf{A}$ with nonzero coefficient a_J as in (2.23), (2.25). Therefore $\det \mathbf{A} \neq 0$ algebraically. Invoking lemma 2.2 proves the lemma. \boxtimes

The above proof also shows the following variant in terms of child selection maps $J: \mathbf{M} \rightarrow \mathbf{E}$ which are defined to possess the mother map $\mathbf{m}: \mathbf{E} \rightarrow \mathbf{M} \cup \{0\}$ as a left inverse, $\mathbf{m} \circ J = \text{id}_{\mathbf{M}}$. See (2.21), (2.22).

Corollary 2.4. *In the setting of lemma 2.3, $\det \mathbf{SR} \neq 0$ holds algebraically if, and only if, there exists a child selection map $J: \mathbf{M} \rightarrow \mathbf{E}$ such that $T = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ is a maximal spanning tree of the network $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$.*

By construction via the child selection map J 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 reaction j emanating from 0;

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

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

Lemma 2.5. *Let positivity assumptions (1.4), (1.5) hold.*

Then any weakly 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 within any weak connected component of Γ . Consider a minimal component W , in this partial 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. \boxtimes

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 2.3. We crucially rely on maximal spanning trees T and on the augmented matrix \mathbf{A} ; see (2.4), (2.5) and proposition 2.1.

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 $\delta x^* := (\delta x_m^{j^*})_{m \in \mathbf{M}}$ of (1.11) then implies

$$(3.1) \quad \mathbf{S} e^* + \mathbf{SR} \delta x^* = 0.$$

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

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

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

$$(3.3) \quad \Phi^* = e^* + \mathbf{R} \delta x^* = -\mathbf{C} \mu^*$$

by definition (1.14) of the flux response. By regularity assumption (1.6) the Jacobian $f'(x^*) = \mathbf{S}\mathbf{R}$ is invertible. Therefore \mathbf{A} is invertible by proposition 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

$$(3.4) \quad -\mu_k^* \det \mathbf{A} = (-1)^{M+k+j^*} \det \check{A}_{j^*, M+k},$$

for $k = 1, \dots, N$. 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

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

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

$$(3.6) \quad \text{row } j^* : (0 \dots 0, c_{j'}^1 \dots c_{j'}^N);$$

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

for $j' \neq j^*$. The comma “,” separates the first M columns of $\mathbf{R} = (r_{jm})$ from the subsequent N columns of $\mathbf{C} = (c_j^k)$ in $\mathbf{A} = (\mathbf{R}, \mathbf{C})$. To prove (3.5)–(3.7), we have interpreted the sum in the third line of (3.5) as an expansion of $\det \mathbf{A}$ with respect to row j^* , after the replacement (3.6). Subtracting row j^* from row j' , in a second step, provides the replacement (3.7) in row j' to arrive at $\det A_{j'j^*}$. In case $j' = j^*$ we analogously obtain $A_{j^*j^*}$ with only the first replacement (3.6).

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). By (3.5), the assumptions $\Phi_{j'j^*} \neq 0$ and $\det \mathbf{A} \neq 0$ imply $\det A_{j'j^*} \neq 0$, algebraically.

Our algebraic analysis of $\det A_{j'j^*} \neq 0$ for the modified matrix $A_{j'j^*}$ now proceeds analogously to our analysis of $\det \mathbf{A} \neq 0$ in the proof of lemma 2.3; see (2.21) – (2.26). This time, we can assert the existence of a child selection $J: \mathbf{M} \rightarrow \mathbf{E}$, i.e. with the mother map $\mathbf{m}: \mathbf{E} \rightarrow \mathbf{M} \cup \{0\}$ as a left inverse $\mathbf{m} \circ J = \text{id}_{\mathbf{M}}$, 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

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

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

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

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 in

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

Due to these modifications the child selection graph $T := (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ of the modified matrix $A_{j'j^*}$ need not be a maximal spanning tree, this time, as it had been for \mathbf{A} before. Let us therefore replace the child selection graph $T := (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ by

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

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

Then T' is a maximal spanning tree, because $\det A_{j'j^*} \neq 0$ algebraically. However, T' is not defined by child selection. In fact T' possesses a single forward branch point at the mother $m^* = \mathbf{m}(j^*)$ in case $m' := \mathbf{m}(j') \neq m^* \neq 0$.

We use the edge directions in T' to choose the directed paths γ^0 and γ' as follows. If $j' \neq j^*$ have the same mother $m^* = m' \neq 0$, then the influence path γ' is the edge j' from vertex $m^* = m'$ to $\bar{m}' = \bar{\mathbf{m}}(j')$. The exit path γ^0 is the path from m^* along j^* to 0 procured in lemma 2.3.

If the mothers m' and m^* are different, and both different from 0, i.e. $0 \neq m' \neq m^* \neq 0$, 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 di-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 di-paths cannot return to themselves because T' is acyclic. For the same reason, the di-paths remain disjoint after starting at m^* . In particular exactly one of the di-paths extends to 0; this path is the exit di-path γ^0 . The other di-path reaches m' . Extended by j' but omitting the head $\bar{m}' := \bar{\mathbf{m}}(j')$, this is the influence di-path γ' .

If $m^* = 0 \neq m'$ we choose the exit path $\gamma^0 = \{m^*\}$ without any edge. To define the di-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 the influence di-path γ' consists of the edge j^* with its mother m^* . To construct the exit di-path $\gamma^0 \not\supseteq j^*$ we note that $j^* \notin J(\mathbf{M})$ still holds, as in (3.8). 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 j^* and j' with distinct nonzero mothers $m^* = \mathbf{m}(j^*) \neq \mathbf{m}(j')$, leaving the remaining cases as straightforward exercises.

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

For the vertices m of $\gamma^0 \cup \gamma'$, excepting the head $\bar{m}' = \bar{\mathbf{m}}(j')$ 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 di-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 2.3 there exists a di-path γ^m from m to 0. We proceed by iteration on m . At each step we terminate the di-path γ^m as soon as it hits any vertex m_0 where J has already been constructed. The di-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 (undirected) 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 undirected 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. \boxtimes

4 Cycles of influence

Our proof of flux influence theorem 1.2 in section 3 has been based on the construction of a maximal spanning tree T' from a suitable child selection graph T , as an intermediate step; see (3.8) – (3.11). In corollary 4.1 we summarize the resulting variant of theorem 1.2 as a separate characterization of algebraically nonzero fluxes $\Phi_{j'j^*} \neq 0$. In theorem 4.2 and corollary 4.3 we attempt to elucidate how the perturbation influence of reaction j^* spreads to j' via di-cycles, bi-cycles, and side branches.

Corollary 4.1. *Let $j' \neq j^*$ be any two distinct reactions and let the assumptions of theorem 1.2 hold. Then algebraically nonzero flux influence $\Phi_{j'j^*}$, as stated in (1.17) is equivalent to the existence of a child selection $J: \mathbf{M} \rightarrow \mathbf{E} \setminus \{j^*\}$ such that the modified graph T' defined in (3.10), (3.11) is a maximal spanning tree of the reaction network $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$.*

The next theorem describes the cyclicity properties of j' . Let $T' \cup j'$ denote the maximal spanning tree T' with added edge j' , i.e.

$$(4.1) \quad T' \cup j' = (\mathbf{M} \cup \{0\}, J(\mathbf{M}) \cup \{j^*\}) = T \cup j^*.$$

Recall that the child selection graph $T = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ of J need not be a maximal spanning tree. Because T' is a maximal spanning tree, in contrast, $T' \cup j'$ contains a unique cycle c' , not necessarily directed. Also recall the disjoint directed paths γ^0 and γ' from the mother vertex m^* to 0 and j' , respectively, as provided by theorem 1.2.

We can now describe a trichotomy of cyclicity cases for reactions j' in the influence set $I(j^*)$ of the perturbed reaction j^* . A directed cycle is called di-cycle. A bi-cycle is a non-oriented cycle which decomposes into two parallel di-path arcs, each with at least one edge. The arcs are required to be disjoint, except for their shared start and termination vertices. See fig. 4.1 for an illustration of the three cases arising in theorem 4.2.

Theorem 4.2. *Fix the assumptions and notations of theorem 1.2 and corollary 4.1 above. Let j^* influence $j' \neq j^*$. Then exactly one of the following three mutually exclusive cases can arise:*

- (i) *The cycle c' of j' is directed and does not contain j^* . Then the influence di-path γ' need not be contained in c' . The exit di-path γ^0 intersects $\gamma' \cup c'$ at the mother vertex m^* of j^* , only.*
- (ii) *The cycle c' of j' is directed and contains j^* . Then the influence di-path γ' also contains j^* and is contained in c' . The cycle c' intersects the exit di-path γ^0 only at the mother vertex m^* of j^* .*

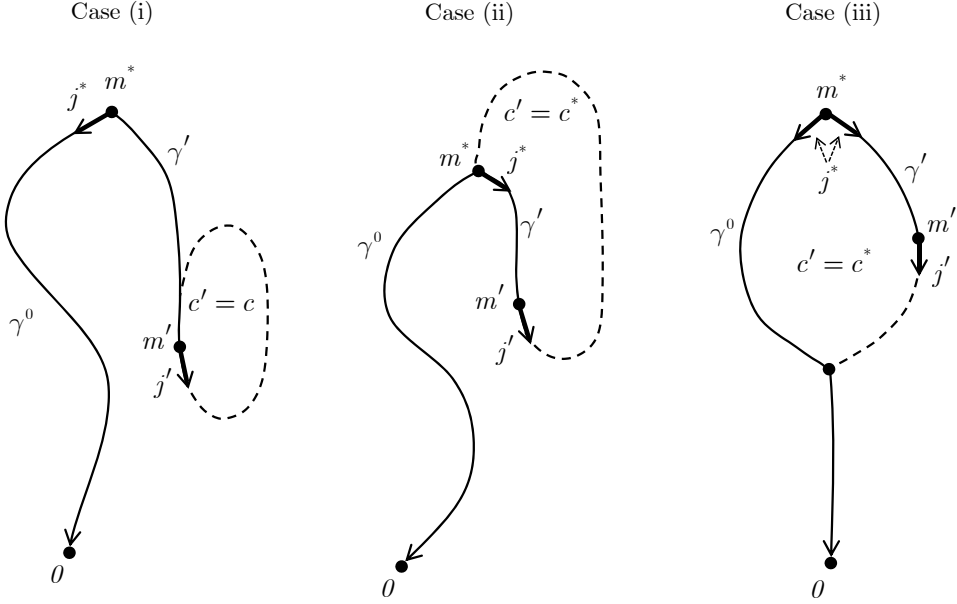


Figure 4.1: The trichotomy of cycle configurations, exit di-paths γ^0 , and influence di-paths γ' arising in theorem 4.2. Case (i): unique di-cycle c in the child selection graph T . Cases (ii) and (iii): di-cycle and bi-cycle $c' = c^*$ in $T' \cup j' = T \cup j^*$ with acyclic child selection graphs T , respectively.

(iii) The cycle c' of j' is not directed. Then c' is a bi-cycle and contains j^* . The two parallel directed arcs of the bi-cycle c' both emanate from the mother vertex m^* of j^* . One arc contains the influence di-path γ' . The other arc is the intersection of the exit di-path γ^0 with c' . The perturbed edge j^* may be contained in either arc.

Proof. It is evident a priori that the three cases (i) – (iii) are mutually exclusive. It is less evident that no other case can occur. Instead of justifying the trichotomy (i) – (iii) directly, we therefore proceed via a complete list of cases for the child selection graph $T = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$. We show below that case (i) occurs if T contains any directed or undirected cycle c . Next suppose T is acyclic. Then T is also a maximal spanning tree, like T' itself, because T and T' have the same number of edges. Therefore the edge $j^* \notin T$ defines a unique undirected cycle c^* in $T \cup j^*$. Below we show

$$(4.2) \quad c^* = c'.$$

Depending on whether c' is directed or not, this will provide the two remaining cases (ii) and (iii) of the theorem. Invoking theorem 1.2 then proves that j^* influences all edges on c' .

Suppose first that the child selection graph T of $J: \mathbf{M} \rightarrow \mathbf{E} \setminus \{j^*\}$ contains any

directed or undirected cycle c . We claim

$$(4.3) \quad c = c',$$

and in particular uniqueness of c . Indeed (4.1) implies that

$$(4.4) \quad c \subseteq T \subseteq T' \cup j'$$

is a cycle in $T' \cup j'$, like c' . By uniqueness of c' in $T' \cup j'$ this proves $c = c'$.

Because T is defined by a child selection J , the cycle $c' = c \subseteq T$ cannot possess any vertex with two outgoing edges in c' . Therefore $c' = c \subseteq T$ is directed. Because $c \subseteq T$, the cycle $c' = c$ does not contain edge j^* . The influence di-path γ' in $T' \cup j'$ must enter c' somewhere, to reach j' , and is then trapped in c' by child selection. Here we use that child selection forces unique forward continuation of di-paths. Any intersection vertex of the exit di-path γ^0 with $c' = c$, other than possibly m^* , would likewise trap γ^0 in c' . This would prevent γ^0 from reaching 0. Indeed $0 \notin c' = c \subseteq T$, because child selection $J: \mathbf{M} \rightarrow \mathbf{E}$ prevents any feed reaction to belong to c . Therefore γ^0 and $\gamma' \cup c'$ are disjoint, except possibly for m^* . This proves claim (i).

From now on we suppose that T is acyclic and hence is a maximal spanning tree. Let c^* denote the unique undirected cycle in $T \cup j^* = T' \cup j'$. Again (4.1) implies $c^* = c'$, by uniqueness of the cycle c' in $T' \cup j'$. By construction j^* is in $c^* = c'$, independently of orientability of c' in the resulting cases (ii), (iii).

To address case (ii) assume the cycle $c' = c^*$ is directed. By forward unique continuation of any di-path in the child selection tree T , the di-path γ^* in c' starting from m^* along $j^* \in c'$ must follow c' afterwards, until it reaches $j' \in c'$. Therefore $\gamma' = \gamma^* \subseteq c'$.

We show indirectly that the exit di-path γ^0 does not intersect c' , except at m^* . Indeed $c' = c^* \subseteq T \cup j^*$ implies that child selection holds on c' after m^* . Therefore any other intersection would trap the forward di-path γ^0 on c' until it reaches m^* . This is impossible for the exit di-path γ^0 , from m^* to 0, because paths are defined to be without self-intersection. This shows (ii).

To address case (iii), finally, assume $c' = c^* \subseteq T' \cup j' = T \cup j^*$ is not directed. Because T is defined by child selection, the mother vertex m^* of j^* is the only vertex in $T \cup j^*$ with a forward branching into two outgoing child edges. Therefore the non-oriented cycle c' contains, not only m^* and j^* but, both outgoing child edges of m^* in $T \cup j^*$. Unique forward continuation in T defines two outgoing di-path arcs in c' . Because m^* is the only forward branching vertex in $T \cup j^* \supseteq c'$, the two disjoint arcs emanating from m^* must meet at some first vertex in c' . Hence they decompose c' . Therefore c' is a bi-cycle. Since $j' \in c'$, one of the arcs must contain the influence di-path γ' . The other arc then must be contained in the exit di-path γ^0 . This settles case (iii) and proves the theorem. \boxtimes

Corollary 4.3. *Fix the assumptions and notations of theorem 1.2. Also assume the flux influence set $I(j^*)$, as defined in (1.26), is nonempty.*

Then the influence set $I(j^)$ is 2-edge-connected. More precisely, any reaction $j' \in I(j^*)$ is contained in a di-cycle or bi-cycle $c' \subseteq I(j^*)$.*

Proof. We first consider absent self-influence of $j^* \notin I(j^*) \neq \emptyset$. Then theorem 1.2 implies that any exit di-path γ^0 from m^* to 0 must start along j^* . Moreover $j^* \neq j' \in I(j^*)$. Therefore case (i) of theorem 4.2 applies, and identifies a di-cycle $j' \in c' \subseteq I(j^*)$.

Next suppose $j^* \in I(j^*)$ influences itself. The case $j' \in I(j^*) \setminus \{j^*\}$ has been settled in theorem 4.2. It remains to consider the case $j' = j^* \in I(j^*)$. By theorem 1.2, there exists an exit di-path γ^0 from m^* to 0 which is disjoint from the path γ' . Recall that γ' is given by m^* and the edge $j' = j^*$, but excluding the head $m'' := \overline{\mathbf{m}}(j')$ of j' . Appending any exit di-path γ'' of m'' , until it first meets γ^0 identifies c' as in cases (ii) or (iii) of theorem 4.2. This proves the corollary. \square

5 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

$$(5.1) \quad \Phi_{j_2'j_2^*} \neq 0, \Phi_{j_2^*j_1^*} \neq 0 \implies \Phi_{j_2'j_1^*} \neq 0,$$

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

$$(5.2) \quad j_1^* \neq j_2^* \neq j_2'.$$

Our proof will proceed via the exit and influence di-paths γ_i^0, γ_i' associated to the nonzero flux response $\Phi_{j_i'j_i^*}$ in (5.1), for $i = 1, 2$ and $j_1' := j_2^*$; see theorem 1.2. In subsection 5.1 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 (5.1) 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 5.1. As a warm-up we collect certain special cases in 5.2. The general construction is detailed in 5.3.

5.1 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' : $m_i^* \rightarrow \overline{m}_i'$. See fig. 1.1. In our specific case (5.1) 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 $\bar{m}' := \bar{m}_2'$ such that properties (i) – (iv) hold for γ^0, γ' . In summary,

$$(5.3) \quad m^* = m_1^*, j^* = j_1^*, j_1' = j_2^*, m_2^* = m_1', m' = m_2', j' = j_2', \bar{m}' = \bar{m}_2'.$$

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 $\gamma_i^0 = [m_i^*, 0]_i^0, j_i' = (m_i', \bar{m}_i')_i'$ and $\gamma_i' = [m_i^*, \bar{m}_i']_i'$. 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

$$(5.4) \quad m \in (\gamma_1 \cap \gamma_2) \setminus \{m_1^*\}$$

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 . In other words, the color of m simply tracks the side branch of γ_1 where the intersection m occurs:

$$(5.5) \quad m \in \gamma_1^{c(m)} \cap \gamma_2.$$

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

$$(5.6) \quad m_1^* = m_2^* \quad \text{and} \quad j_1^* \subseteq \gamma_1'.$$

We treat this latter case separately in 5.2, as case 1.

Our general construction of the di-paths $\gamma^0 = [m^*, 0]^0$ and $\gamma' = [m^*, \bar{m}']'$ 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

$$(5.7) \quad \begin{aligned} & \text{(a)} \quad \mu^0 \in \gamma_2^0 \quad \text{and} \quad \mu' \in \gamma_2'; \\ & \text{(b)} \quad \mu^0 \text{ and } \mu' \text{ are of opposite color;} \\ & \text{(c)} \quad \text{any black or white intersection vertex } m \text{ of } \gamma_1 \cap \gamma_2 \\ & \quad \text{which is strictly to the right of } \{\mu^0, \mu'\} \text{ on } \gamma_2 = \gamma_2^0 \cup \gamma_2' \\ & \quad \text{occurs strictly later than } \{\mu^0, \mu'\} \text{ on } \gamma_1 = \gamma_1^0 \cup \gamma_1' \text{ as well.} \end{aligned}$$

With the above notation and terminology our general construction of the paths γ^0, γ' , which will prove transitivity claim (5.1) and theorem 1.3, proceeds as

follows. Let $\{\mu^0, \mu'\}$ be a cut-pair. Define the di-paths

$$(5.8) \quad \begin{aligned} \gamma^0 &:= [m_1^*, \mu^0]_1^{c^0} [\mu^0, 0]_2^0; \\ \gamma' &:= [m_1^*, \mu']_1^{c'} [\mu', \bar{m}'_2]_2' \end{aligned}$$

by concatenation 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 5.3 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 of flux influence.

5.2 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 (5.6). 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 (5.3), and because $m_1^* = m_2^*$, by assumption.

Leading edge property (ii) of γ^ρ claims

$$(5.9) \quad j^* \subseteq \gamma_2^0 \cup \gamma_2'.$$

Assertion (5.3) implies $j^* = j_1^*$, and $j_1^* \subseteq \gamma'_1$ by assumption. Because $m'_1 = m_2^*$ by (5.3), and $m_2^* = m_1^*$ by assumption, we observe that $\gamma'_1 = [m_1^*, \bar{m}'_1]_1' = [m'_1, \bar{m}'_1]_1'$ contains but the single edge j'_1 . In particular $j_1^* = j'_1$. But (5.3) 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 (5.9) 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 (5.3) 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

$$(5.10) \quad \Phi_{j_0 j} = \delta_{j_0 j}$$

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

Now consider the case $m_2' = 0$, with child edge j_2' : $m_2' \rightarrow \bar{m}_2'$. Then (5.10) with $j_0 := j_2'$, $j := j_2^*$ and assumption (5.1) imply $\delta_{j_2' j_2^*} = \Phi_{j_2' j_2^*} \neq 0$, i.e. we are in the trivial case $j_2' = j_2^*$ excluded in 5.2. 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 (5.3) implies $j_2^* = j_1^*$ we can invoke (5.10) with $j_0 := j_2^*$, $j := j_1^*$. Assumption (5.1) 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 (5.2).

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

$$(5.11) \quad \Phi_{j' j^*} \neq 0$$

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 $\Phi_{j_2' j_2^*} \neq 0$ we are also given a di-path γ_2' from $m_2^* = m_1' = \mathbf{m}(j_1')$ to the edge $j' = j_2'$: $m_2' \rightarrow \bar{m}_2'$; see (5.3). Let γ' be defined as the concatenation di-path

$$(5.12) \quad [m^*, m_1']_1' [m_2^*, \bar{m}_2']_2'$$

with shared vertex $m_1' = m_2^*$ and all intermediate loops removed. Then (5.3), (5.11) imply $\Phi_{j_2' j_1^*} = \Phi_{j' j^*} \neq 0$ algebraically, as claimed in (5.1). This settles the case $m_1^* = 0$.

5.3 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 (5.7) (a) – (c). In a second step we have to show that the di-paths γ^0 and γ' defined in (5.8) 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 (5.4) – (5.5). We exclude the cases where zero vertices m_1^* , m_2^* , or m'_2 arise and cyclic paths γ_i^c may occur. We also exclude the only case of (5.6) where m_2^* might be white. These cases have already been treated in 5.2. Therefore m_2^* is colored black and $0 \in \gamma_1^0 \cap \gamma_2^0$ 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 $\mu_2^0 := 0 \in \gamma_1^0 \cap \gamma_2^0$, white, and with μ'_2 as the rightmost colored vertex on γ'_2 . Since $m_2^* = m'_1 \in \gamma'_1 \cap \gamma'_2$ 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 set

$$(5.13) \quad \{\mu^0, \mu'\} := \{\mu_2^\rho, \mu_2^\sigma\}.$$

Actually, we still have to show that the cut-pair properties (5.7) hold with this definition. Property (5.7) (a) of opposite branches holds because $\rho \neq \sigma$. Property (5.7) (b) of opposite colors holds by termination at case 2. Ordering property (5.7) (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 (5.13) 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 (5.8) 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 (5.3), (5.8). Termination property (iii) is immediate, likewise, by construction (5.8) and because (5.3) 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 (5.7) of the cut-pair μ^0, μ' . Indeed (iv) for γ_i^0, γ_i' and $i = 1, 2$ implies

$$(5.14) \quad \{m_1^*\} \subseteq [m_1^*, \mu^0]_1^{c^0} \cap [m_1^*, \mu']_1^{c'} \subseteq \gamma_1^0 \cap \gamma_1' = \{m_1^*\}$$

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

$$(5.15) \quad [\mu^0, 0]_2^0 \cap [\mu', \bar{m}_2']_2 \subseteq (\gamma_2^0 \cap \gamma_2') \setminus \{m_2^*\} = \emptyset.$$

We consider any diagonal intersection vertex

$$(5.16) \quad m \in [m_1^*, \mu^0]_1^{c^0} \cap [\mu', \bar{m}_2']_2$$

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 $m \in [\mu', \bar{m}_2']_2 \cap \gamma_1^{c^0}$ must lie strictly to the right of μ' on γ_2' . Of course $\mu^0 \in \gamma_2^0$ cannot occur on γ_2' except as the leftmost point μ_2^* . Hence m must occur strictly later than μ^0 on $\gamma_1^{c^0}$ by cut-pair property (5.7) (c). This proves that the intersection (5.16) 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 (5.8).

We show next that the di-walks γ^0, γ' defined in (5.8) 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

$$(5.17) \quad [m_1^*, \mu^0]_1^{c^0} \cap [\mu^0, 0]_2^0 = \{\mu^0\}$$

consists of the concatenation point μ^0 , only. Similarly to (5.16), 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 (5.7) (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 (5.17). The analogous argument on γ_2' establishes $[m_1^*, \mu']_1^{c'} \cap [\mu', \bar{m}_2']_2 = \{\mu'\}$. 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 $m_1^* \in \gamma_2^0$. 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 (5.4) – (5.5) 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 (5.8), 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. \square

6 Proof of theorem 1.1: concentration response

Our proof of theorem 1.1 on the concentration response to a perturbation of j^* uses theorem 1.2 on the flux response, which was proved in section 3 above. Fix any metabolite $m \in \mathbf{M}$. We distinguish two cases for the concentration response δx_m^* of the metabolite m to a rate perturbation of reaction $j^* \in \mathbf{E}$. Because we have assumed $\det \mathbf{A} \neq 0$, algebraically, the vertex $m \in \mathbf{M}$ possesses a child reaction $j' := J(m)$ by the child selection map J ; see (2.21) and corollary 2.4. In subsection 6.1 we assume $j' \neq j^*$. Subsection 6.2, in contrast, considers the case where $j' = j^*$ is the only child of m . This covers all cases.

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

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

$$(6.1) \quad \Phi_{j'j^*} = r_{j'm} \delta x_m^*$$

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 5 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 (6.1) and theorem 1.2 imply theorem 1.1.

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

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

$$(6.2) \quad \delta x_m^* = -1/r_{j^*m} \neq 0$$

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 2.3 asserts the 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. \boxtimes

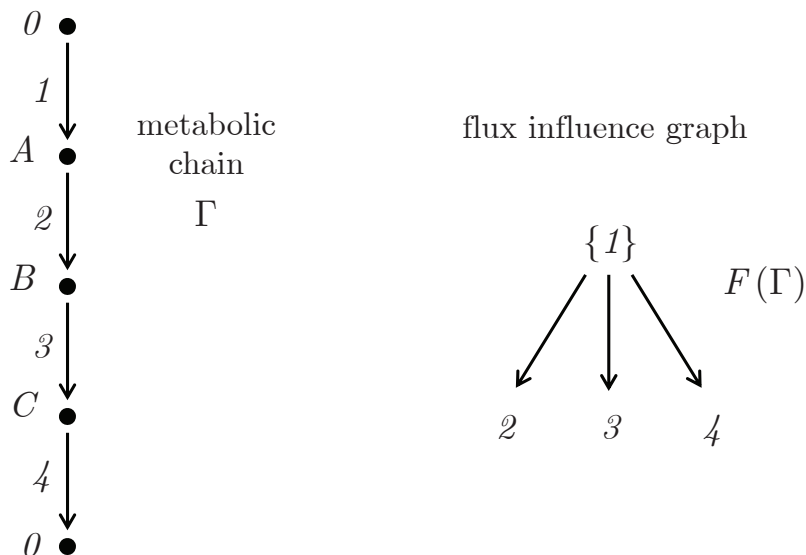


Figure 7.1: *Left: the monomolecular chain Γ of 3 metabolites $\mathbf{M} = \{A, B, C\}$ and 4 reactions $\mathbf{E} = \{1, 2, 3, 4\}$. Right: the flux influence graph $F(\Gamma)$. Only the feed reaction 1 influences all fluxes, including itself. The other reactions are without any influence.*

7 Examples

In this section we review the monomolecular examples of the companion paper [MoFi14] on structural sensitivity analysis, from a flux transitivity point of view. We also discuss several tetrahedral di-graphs on four metabolites with single feed and exit reactions 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 2.3; see (2.20).

We present each example, first, as a network graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$. We label vertices by metabolites $A, B, C, \dots \in \mathbf{M}$ and the zero-complex 0. We label the directed reaction arrows by positive integers $1, 2, 3, \dots \in \mathbf{E}$. 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 denoted 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 $I(j^*)$ of (1.26) from the influence graph $F(\Gamma)$. In each example we sketch the bare-handed derivation of the flux influence graph $F(\Gamma)$ and comment on some peculiarities. All examples illustrate the 2-edge-connectedness of nonempty flux influence sets $I(j^*)$; see corollary 4.3. We also comment on the trichotomy of flux cyclicity; see theorem 4.2. For the closely related concentration response, which is the main concern in experiments, we refer back to section 6 and theorem 1.1.

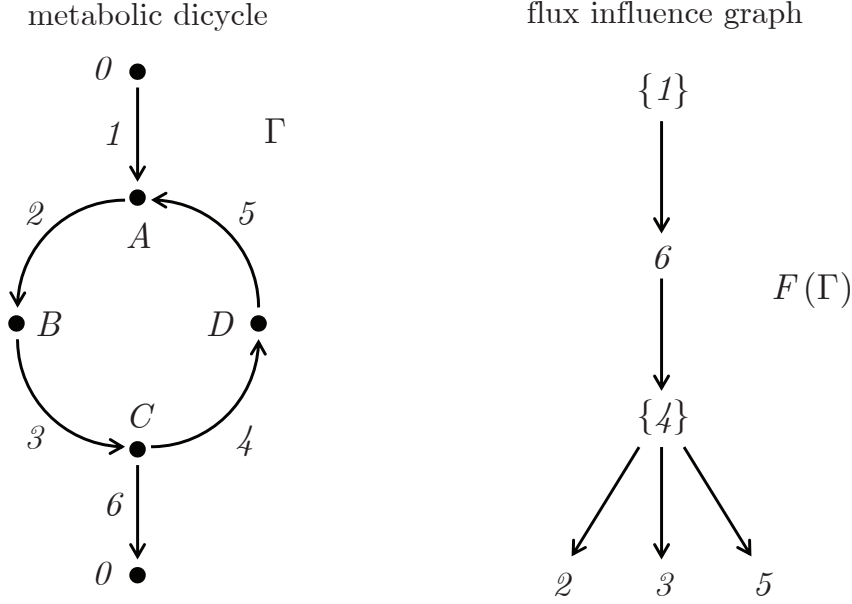


Figure 7.2: *Left: a monomolecular single di-cycle of 4 metabolites $\mathbf{M} = \{A, B, C, D\}$ with 6 reactions $\mathbf{E} = \{1, \dots, 6\}$. Right: the flux influence graph $F(\Gamma)$.*

Our first example is the monomolecular chain of fig. 7.1. 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 $j^* \neq 1$ and all j' ; see (1.18). The feed case $j^* = 1, m^* = 0$ has been discussed in section 5.2, case 2. Specifically (5.11) 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. 7.1. The extension to a monomolecular chain with any finite number of metabolites is straightforward, without further calculation. This example illustrates case (ii) of theorem 4.2, in the special case $m^* = 0$ and $\gamma^0 = \{m^*\}$. See fig. 4.1.

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 from C . See figure 7.2. The single child reactions 2, 3, 5 appear at the bottom level of zero influence in the flux influence graph $F(\Gamma)$.

The entire di-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 $\gamma^0: C \xrightarrow{6} 0$ 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, as before. All three examples illustrate case (ii) of theorem 4.2, again.

Example 3, of figure 7.3, is a variant of figure 7.2 where only the directions of reactions 4, 5 have been reversed. This splits the central di-cycle into a bi-cycle with two parallel arcs $A \xrightarrow{2} B \xrightarrow{3} C$ and $A \xrightarrow{5} D \xrightarrow{4} C$. The single child reactions become 3, 4, 6. Each of the arc entries 2 and 5, separately, now influences both arcs, but not the shared exit $6 \subseteq \gamma^0$. This illustrates case (iii) of theorem 4.2; see also fig. 4.1. As usual the influence of the single feed 1 remains global, by case (ii) with $m^* = 0$ and $\gamma^0 = \{m^*\}$.

Example 4, of figure 7.4, 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 $\gamma^0: C \xrightarrow{4} D \xrightarrow{5} 0$, 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

$$(7.1) \quad I(4) = I(6) = \{2, 3, 7, 6\} = (b)$$

which spans the full di-cycle (b). This illustrates cases (ii) and (i) of theorem 4.2 on di-cycle influence, respectively.

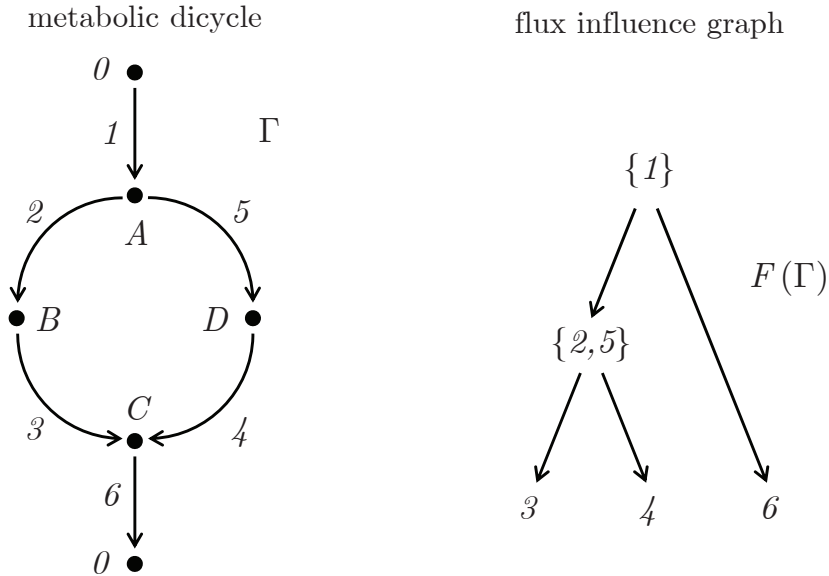


Figure 7.3: Left: monomolecular branching network Γ . The network coincides with fig. 7.2, except for the reversed directions of reactions $4, 5 \in \mathbf{E}$. The central di-cycle becomes a nonoriented cycle with two parallel forward branches 2, 3 and 5, 4 emanating from metabolite A and joining at C . Right: the flux influence graph $F(\Gamma)$ changes drastically, compared to fig. 7.2.

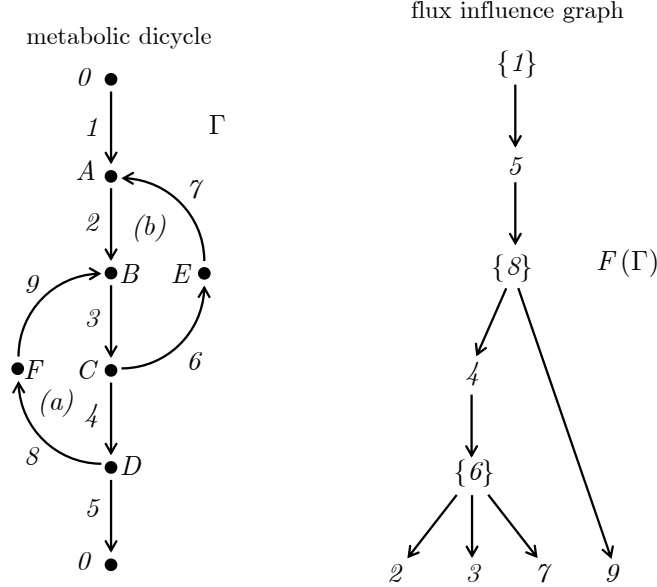


Figure 7.4: *Left: a monomolecular network Γ of two overlapping di-cycles with 6 metabolites $\mathbf{M} = \{A, \dots, F\}$ and 9 reactions $\mathbf{E} = \{1, \dots, 9\}$. Right: the flux influence graph $F(\Gamma)$. Note the global influence of single feed 1, and the absent influence of single children 2, 3, 7, 9. The branching pairs 5, 8 and 4, 6, respectively, share the same influence sets.*

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 $j^* = 5$, without loss of generality. The only possible exit di-path γ^0 from $m^* = D$ is the closed edge $\gamma^0: D \xrightarrow{5} 0$. Again the other path γ' from $m^* = D$ with leading edge 8 can traverse the whole di-cycle (a) to which 8 belongs. See theorem 4.2, (i). 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. Again this illustrates theorem 4.2, (i), and shows that the influence sets

$$(7.2) \quad I(5) = I(8) = \{2, 3, 4, 6, 7, 8, 9\} = (a) \cup (b)$$

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. 7.5, (I) – (IV). They feature the complete graph of 4 vertices $\mathbf{M} = \{A, B, C, D\}$ and 6 reactions 1, ..., 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 2.3 and (2.20).

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 $\gamma^0: m^* = A \xrightarrow{6} C \xrightarrow{8} 0$ is possible and makes edges $j' = 3, 4, 7, 2$, only, accessible to the complementary paths γ' . See case (iii) of theorem 4.2 for the bi-cycle of $j^* = 6$ with $j' = 3, 4$. See case (i) for the di-cycle $j' = 3, 7, 2$. Choosing the other exit di-path $\gamma^0: m^* = A \xrightarrow{3} B \xrightarrow{4} C \xrightarrow{8} 0$ with leading edge 3 makes edge $j' = j^* = 6$ accessible to γ' , only. See theorem 4.2, (iii). This establishes the influence sets

$$(7.3) \quad I(3) = I(6) = \{2, 3, 4, 6, 7\}.$$

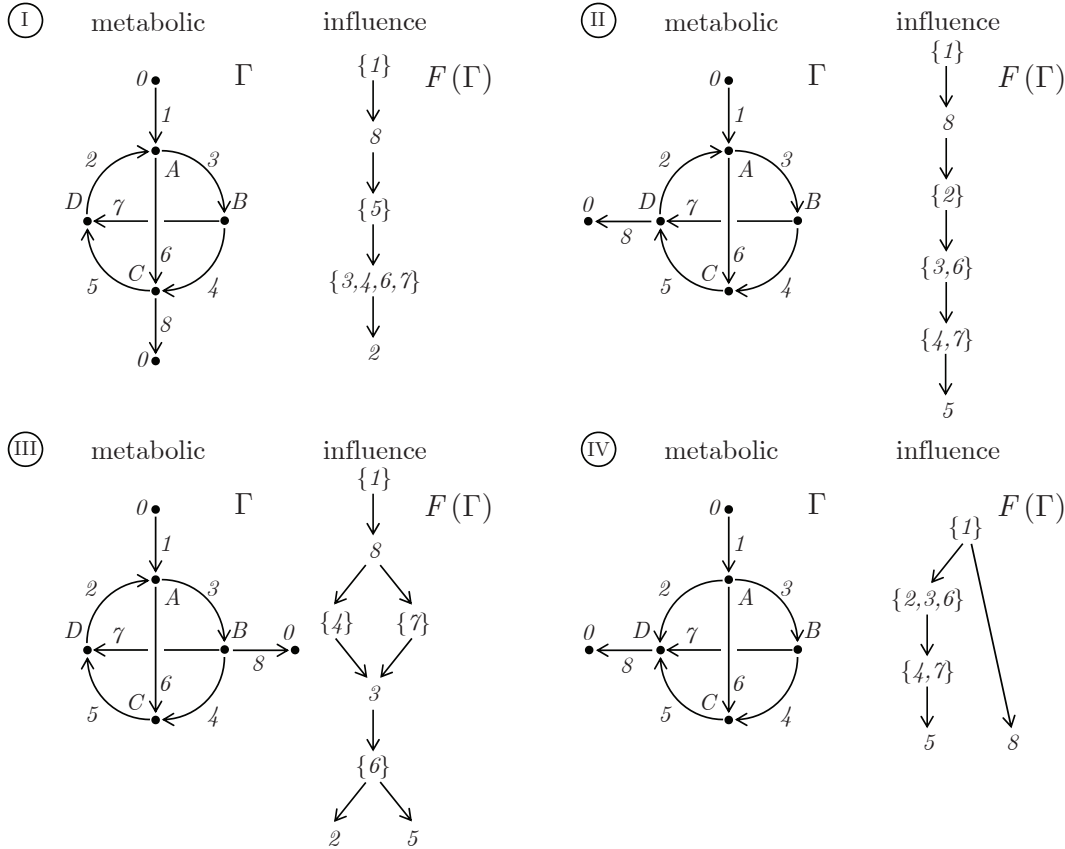


Figure 7.5: Four examples I – IV of monomolecular metabolic networks Γ with a complete irreversible di-graph on 4 metabolites $\mathbf{M} = \{A, B, C, D\}$ with 8 reactions $\mathbf{E} = \{1, \dots, 8\}$ and varying positions of single feed 1 and single exit 8. Examples I – III feature a 4-element di-cycle $A \xrightarrow{3} B \xrightarrow{4} C \xrightarrow{5} D \xrightarrow{2} A$, whereas example IV does not. The flux influence graphs $F(\Gamma)$ are specified to the right of each metabolic network Γ .

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. All cases belong to class (i) of theorem 4.2. This proves

$$(7.4) \quad I(5) = I(8) = \{2, \dots, 7\}$$

and establishes the flux influence graph $F(\Gamma)$ of example I. We omit the rather analogous considerations 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

$$(7.5) \quad C \xrightarrow{5} D \xrightarrow{2} A \xrightarrow{3} B \xrightarrow{8} 0$$

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 $m_* = \mathbf{m}(j^*)$:

$$(7.6) \quad \begin{aligned} j^* = 3, 6; \quad m^* = A; \quad I(3) = I(6) &= \{2, 5, 6\} \\ j^* = 4; \quad m^* = B; \quad I(4) &= \{2, \dots, 7\} \setminus \{7\} \\ j^* = 7; \quad m^* = B; \quad I(7) &= \{2, \dots, 7\} \setminus \{4\} \\ j^* = 8; \quad m^* = B; \quad I(8) &= \{2, \dots, 7\}. \end{aligned}$$

For $m^* = B$ we have used that property (iii) of theorem 1.2 and (7.5) force j^* to be the leading edge of γ' in cases $j^* = 4, 7$, but not 8. The cases $j^* = 4, 7$ illustrate theorem 4.2, (ii). Here $j^* \in I(j^*)$ but the other out-edge of $m^* = B$ is not influenced. For $j^* = 8 \in \gamma^0$ the paths γ' roam all over the tetrahedral edges 2, ..., 7 freely. This illustrates theorem 4.2, (i) and 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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