MASTER THESIS

FLUX SENSITIVITY ANALYSIS IN CHEMICAL REACTION NETWORKS

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ABSTRACT

Flux sensitivity analysis in chemical reaction networks is the study of steady state flux responses due to perturbations of reaction rates. In this thesis we give an introduction and a detailed comparison of two recent approaches to flux sensitivity analysis. Fiedler and Mochizuki provide a structural approach based on the directed graph structure of the network in the case of monomolecular reaction networks. Their approach allows to determine zero and nonzero flux responses, without any numerical input. Another intriguing approach, proposed by Feinberg, Shinar, Mayo, and Ji, reveals a connection between the structure of a mass action network and constraints in the order of magnitude on the sensitivity of steady state fluxes against a rate perturbation. We check the consistence of both approaches by means of several examples, and further investigate response patterns determined from the local structure of the network. By this means, we provide two new ideas on motif rules describing the branching in reaction networks, namely the feedback behaviour and the splitting behaviour.

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INTRODUCTION

To understand the biology of living cells, the theory of chemical reaction network attempts to model the relationship between chemicals and reactions by a directed graph. The vertices of the graph represent reactants and products of the reactions, consisting of one or several species of chemicals and the edges are the reaction arrows, representing a state-transition of these chemicals.

Pioneering work in search of the general law of chemical reactions was made by Waage and Guldberg in 1864 with their paper about mass action laws [33]. Independently, van't Hoff rediscovered this law in 1877 and published in 1884 the first book about chemical dynamics "Études de Dynamique Chimique" [17]. Among numerous distinctions, in 1901 van't Hoff was awarded with the first Nobel Prize in Chemistry "in recognition of the extraordinary services he has rendered by the discovery of the laws of chemical dynamics and osmotic pressure in solutions".

A first milestone in analysis of chemical reaction networks was Wegscheider's work [34] on networks which consist of several elementary reactions in 1901. Wegscheider pointed out that an equilibrium of a reaction network may not be an equilibrium of each reaction from the network if their rate constants are independently defined. This situation became known as Wegscheider's paradox. Furthermore, he gave detailed balance conditions on the reaction rate constants which are necessary and sufficient for the equilibrium of the network to coincide with the joint equilibrium of their elementary reactions.

The question how the dynamics of a reaction network is affected by its structure was approached by Semenov [25] in 1935 and by Hinshelwood [16] in 1940 with their theory of chain reactions. "For their researches into the mechanism of chemical reactions", Semenov and Hinshelwood were awarded with the Nobel Prize in Chemistry in 1956. In 1965, Aris' program [1, 2] on the detailed systematization of mathematical ideas and approaches led to fundamental results by Horn and Jackson [18], and Feinberg [6, 8].

Also in the 1960s, Higgins published his ground breaking work [15] on the application of sensitivity analysis to biochemistry. The sensitivity analysis is a framework to determine the relation between the structure of a system and the magnitude of the impact of some small perturbation in a parameter, like enzyme activity on a metabolic system property, such as the flux or the concentration of a metabolite. This method became a fundamental tool in biochemical systems theory, which was developed by Savageau [24], and also in metabolic control analysis pioneered independently by Kacser and Burns [20] and by Heinrich and Rapoport [13, 14]. For a survey of the large area of metabolic control analysis and its development see for example [10, 31].

This thesis deals with two recent approaches of sensitivity analysis in chemical reaction networks. The structural approach by Fiedler and Mochizuki (2015) [11, 22] provides a sensitivity analysis based on the directed graph structure of the network for the case of monomolecular reactions. In fact, to determine which steady state concentrations and reaction fluxes are sensitive to a specific rate change does not require any numerical input, and therefore their results are function-free. Furthermore, Fiedler and Mochizuki observed a concept of transitivity of influence which establishes a hierarchy of the influence of a rate perturbation.

Another interesting approach to this issue has been proposed by Shinar, Mayo, Ji and Feinberg (2011) [29, 30]. The second approach we will study in detail shows a connection between the structure of a mass action network and the sensitivity of their steady state fluxes against a rate perturbation. In particular, for the case of injective reaction networks there are constraints on the flux control coefficients for all positive steady states.

In this thesis we will especially consider the results of both approaches regarding flux sensitivity analysis and its fundamental tool the flux sensitivity matrix. The sensitivity matrix is an array whose elements are the partial derivatives of the concentrations or fluxes with respect to the rate parameters, evaluated at a particular steady state. Examination of the flux sensitivity matrix will indicate which fluxes are affected by a rate change of a specific reaction, and which are not.

Experimentally, one possible method to determine the sensitivity of concentrations and fluxes are knockout experiments. During these experiments, each enzyme which mediates a reaction in the network is knocked out separately and the response of the system is observed in terms of changes in the concentration of chemicals or their fluxes. For large data bases on chemical and metabolic pathways see for example [21, 23].

Concerning flux sensitivity analysis, the approach by Fiedler and Mochizuki provides a theory based only on the structure of the metabolic or chemical reaction network, i. e., it only considers its stoichiometric graph structure and thus is called structural sensitivity analysis. Fundamental assumptions are the existence of a positive steady state of the system and positivity of the reaction rates. In addition, the network is required to be regular at steady states. For reactions j^* and j' we denote $j^* \rightsquigarrow j'$ as j^* influences j', if $\Phi_{j'j^*} \neq 0$. Their main result regarding flux changes $\Phi_{j'j^*}$ of reaction j' due to a rate increase of reaction j^* gives certain conditions to determine if j^* influences j', i. e., if the flux change is algebraically nonzero, or not.

The results apply to almost every choice of reaction rate functions $r_j \in C^1$, provided that their equilibrium flux values $r_j(x^*_m)$ can be considered as algebraically independent to their derivatives $r'_j(x^*_m)$ at the steady state $x^* = (x^*_m)_{m \in \mathbf{M}}$, where x_m is the concentration of the metabolite $m \in \mathbf{M}$. Therefore, the theory does not apply to pure mass action kinetics, since only a single rate constant is available for each reaction. But already rate functions of Michaelis-Menten or Langmuir-Hinshelwood type are rich enough to satisfy the algebraic independence. More generally, two parameter families of functions r_j fulfill these assumptions.

At present, this approach is limited to monomolecular reactions and therefore only offers a first step towards understanding the sensitivity behaviour of chemical reaction networks, mathematically. But in a very recent advance Brehm and Fiedler [3] were able to enhance the theory for regular multimolecular systems. In fact, in some sense, they provide a completely different approach which is more powerful.

We next want to take a closer look at the approach by Shinar, Mayo, Ji and Feinberg. Their theory is designed for mass action networks that possess an injectivity property. Injectivity is a network property which is closely related to the uniqueness of positive equilibria in the system. In fact, we will see that noninjectivity is a necessary condition for the existence of a mass action network possessing multiple equilibria in the same positive stoichiometric compatibility class. Since injectivity is a condition imposed on the network structure alone, the approach is based only on the network structure, too.

Their main result provides bounds on the diagonal elements of the corresponding flux sensitivity matrix in each positive steady state to lie between 0 and 1. Furthermore, it gives constraints on the values that any pair of reciprocal off-diagonal elements of the matrix may take. Specifically, in each pair of reciprocal flux control coefficients at least one coefficient lies between -1 and 1. In the case of noninjective networks these sensitivity bounds can be violated, thus the injectivity requirement is crucial.

Besides the fact that both approaches only rely on the underlying structure of the chemical reaction network, on the first glance, the approaches seem to have complementary assumptions. Indeed, where the approach by Shinar et al. requires injective mass action reaction networks, Fiedler and Mochizuki allow a very general class of rate functions, with exception of pure mass action kinetics. Furthermore, the theory by Fiedler and Mochizuki is limited to monomolecular reaction networks at present, opposed to Shinar et al. not having such limitations.

Let us briefly compare the results. We see that the approach by Shinar et al. gives bounds on the diagonal elements of the flux sensitivity matrix, in addition to constraints on the reciprocal off-diagonal elements. In contrast, the theory by Fiedler and Mochizuki provides a framework, which is able to derive zero and nonzero flux changes, i. e., to determine zero and nonzero entries of the flux sensitivity matrix.

In this thesis we aim for a detailed comparison of the both approaches. We will apply both theories to several examples and examine them at great length. Moreover, we will observe characteristic response patterns in the flux sensitivity matrices of the examples, which are determined from the local structure of the network. By this means, we will provide some ideas to expand the motif rules given by Mochizuki and Fiedler [22]. Another interesting observation is that the regularity assumption on the network from the approach by Fiedler and Mochizuki is very close to the injectivity property of the network by Shinar, Mayo, Ji and Feinberg. In fact, if we forget about the restrictions on the reaction rate functions for a second, the injectivity property implies the regularity assumption.

The thesis is organized as follows. We will introduce basic notation and definitions of the approach by Fiedler and Mochizuki in Chapter 2. We discuss the necessary assumptions, state the main result regarding flux sensitivity analysis, and give a commented proof. Furthermore, we examine the flux sensitivity matrix, which is a fundamental tool in sensitivity analysis, in detail.

Chapter 3 is devoted to the introduction of the approach by Shinar, Mayo, Ji and Feinberg. Here we will adopt the notation from Fiedler and Mochizuki, but give detailed comments about the changes and the standard notation from Feinberg. After introducing the necessary definitions, we state and prove the main result.

Chapter 4 provides a comparison of the both approaches. We will examine the similarities and differences in the fundamental ideas and the model, as well as in the assumptions and the results. This way, we also give a short recall of the basic principles and definitions from the previous two chapters. The reader who is already familiar with the theory by Fiedler and Mochizuki, and by Shinar, Mayo, Ji and Feinberg can therefore start with this chapter and return to Chapter 2 or Chapter 3 as needed.

In Chapter 5 we apply the results of both approaches to several examples. In particular, we derive the corresponding flux sensitivity matrices according to the theory by Fiedler and Mochizuki and check for the constraints and bounds given by Shinar et al. Furthermore, we study characteristic patterns in the flux sensitivity matrices and provide new ideas on motif rules regarding the branching in reaction networks. For the flux sensitivity matrices of even more examples see Appendix A. Finally, we discuss and summarize our ideas and findings in Chapter 6.

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2

A STRUCTURAL APPROACH TO SENSITIVITY ANALYSIS

This chapter presents a detailed introduction to the structural approach to flux sensitivity analysis by Fiedler and Mochizuki [11, 22]. In the following sections we will give the basic notation and definitions for monomolecular reaction networks, their dynamics and sensitivity analysis. We provide some insight with regards to the ideas and necessary assumptions of the main result. Knowing the preliminaries, we are able to state the main result and give a commented proof. Furthermore, we study a fundamental tool in sensitivity analysis, the flux sensitivity matrix. We also present a method to calculate the flux sensitivity matrix, as well as some of its interesting properties.

2.1 MONOMOLECULAR REACTION NETWORKS AND THEIR DYNAMICS

A monomolecular chemical reaction network consists of a set of metabolites $\mathbf{M}$, namely reactants and products, and of a set of reactions $\mathbf{E}$, representing a state-transition of these chemicals. The reaction network theory models the chemical system as a directed graph Γ with vertex set $\mathbf{M} \cup \{0\}$, made of metabolites $m \in \mathbf{M}$ and the zero complex 0, and directed edges $j \in \mathbf{E}$ standing for the reactions. The total number of metabolites is denoted as $M := |\mathbf{M}|$ and the total number of edges is $E := |\mathbf{E}|$. We emphasize here that the metabolites $m \in \mathbf{M}$ are distinct from the zero complex 0. The zero complex 0 is a vertex in which no metabolite is supported. In the graph it acts as a tool to describe feed reactions and exit reactions. The essential role of the zero complex, which was introduced by Feinberg [7, 8], will become clear when we consider feed and exit reactions and in particular in an example at the end of this section.

The approach by Fiedler and Mochizuki is limited to monomolecular reactions. But in a recent advance Brehm and Fiedler were able to enhance the theory for regular multimolecular systems, see [3]. However, this is beyond the scope of this thesis, we regard the monomolecular case, only. Thus, any reaction $j \in \mathbf{E}$ just converts one metabolite m^j, called reactant or educt into another one $\overline{m}^j$, called product. In symbols

$$j: \quad m^j \longrightarrow \overline{m}^j \tag{2.1.1}$$

represents a reaction, where $m^j, \overline{m}^j$ are different elements from the vertex set $\mathbf{M} \cup \{0\}$. Special reactions are feed reactions j with $m^j = 0$ and exit reactions j with $\overline{m}^j = 0$. On the graph structure of the reaction network we impose the conditions that any order pair $(m, \overline{m})$ is connected by at most one directed edge. Further we prohibit the existence of self-loops $\overline{m} = m$. Note that in graph theory this is called simple directed graph. If for a reaction $j: \; m^j \longrightarrow \overline{m}^j$ the reverse reaction $\overline{j}: \; \overline{m}^j \longrightarrow m^j$ also occurs in Γ, we call the reaction j reversible.

A path in a graph is a sequence of edges $j \in \mathbf{E}$ which connect a sequence of vertices $m \in \mathbf{M} \cup \{0\}$ without any self-intersections. We call a directed path, abbreviated a di-path, a path with added restriction that all edges are directed in the same direction. If the two end points of an undirected path coincide we speak of a cycle. A di-cycle is a cycle with an underlying directed path. Another variant of a cycle is called bi-cycle, which consists of two parallel di-path arcs. Each arc is required to have at least one edge and both arcs are disjoint, except for their shared start and end vertices. Of course, in order to respect our conditions on the graph structure, at least one of the arcs needs at least two edges joined by a vertex.

We next define the notions of weak and strong connectivity of vertices $m, m' \in \mathbf{M} \cup \{0\}$. For the case of an undirected graph m, m' are weakly connected, in symbols $m \sim m'$, if there exists an undirected path connecting m and m'. The corresponding equivalence class of the equivalence relation $\sim$ is called weakly connected component. In a di-graph, we call m, m' strongly connected, in symbols $m \approx m'$, if there exists a di-path from m to m' and a different one from m' to m. Analogously, the equivalence class of the relation $\approx$ is called strong connected components. Note that $m = m'$ without any edge implies $m \sim m'$ and $m \approx m'$.

We call a undirected graph Γ acyclic, if it does not contain any cycles. A maximal spanning forest $\mathcal{T}$ of an undirected graph Γ is an acyclic subgraph of Γ, which is maximal. $\mathcal{T}$ has the properties that it contains all vertices $\mathbf{M} \cup \{0\}$ of Γ, i.e., $\mathcal{T} = (\mathbf{M} \cup \{0\}, \mathbf{E}(\mathcal{T}))$, and by maximality of $\mathcal{T}$, that any added edge $j \notin \mathbf{E}(\mathcal{T})$ produces an undirected cycle c^j in $\mathcal{T} \cup \{j\}$. Note that the cycle c^j is unique, since $\mathcal{T}$ is acyclic. We call $\mathcal{T}$ a spanning tree if the graph Γ is weakly connected, since any maximal spanning forest is also connected. Here, deviating from the standard graph terminology, the term maximal spanning tree $\mathcal{T}$ is used in the connected case, to emphasize that $\mathcal{T}$ consists of a maximal number of edges.

Consider a reaction $j: \ m^j \longrightarrow \overline{m}^j$. We call the reactant or tail m^j of the arrow j the mother metabolite of the corresponding reaction and the product or head $\overline{m}^j$ the child metabolite. This terminology gives rise to the mother map, which is a map given by

$$\mathbf{m}: \quad \mathbf{E} \to \mathbf{M} \cup \{0\} \tag{2.1.2}$$

such that $\mathbf{m}(j) = m^j$ is the mother of j, as well as the map $\overline{\mathbf{m}}(j) := \overline{m}^j$ which yields the reaction product $\overline{m}^j$, respectively. Thus, we can characterize the feed reactions j as the elements of $\mathbf{E}_0 := \mathbf{m}^{-1}(0)$ and the elements of $\overline{\mathbf{m}}^{-1}(0)$ as the exit reactions. The right inverse of the mother map $\mathbf{m}$ is called child selection map

$$\begin{aligned} J: \quad & \mathbf{M} \to \mathbf{E}, \\ & m \mapsto J(m). \end{aligned} \tag{2.1.3}$$

Therefore, we have

$$\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}} \tag{2.1.4}$$

as identity relation between the mother map and the child selection map.

To study the dynamics of the chemical reaction network, we first define their ordinary differential equation. The ODE

$$\dot{x} = f(\mathbf{r}, x) := \sum_{j \in \mathbf{E}} r_j(x_{\mathbf{m}(j)})(X_{\overline{\mathbf{m}}(j)} - X_{\mathbf{m}(j)}) \tag{2.1.5}$$

describes the dynamics of the vector $x = (x_m)_{m \in \mathbf{M}}$ of concentrations x_m of the metabolites $m \in \mathbf{M}$. Here $X_m \in \mathbb{R}^M$ defines the m-th unit vector for any nonzero metabolite $m \in \mathbf{M}$. In addition, we define $X_0 := 0 \in \mathbb{R}^M$ and $x_0 := 1$. Furthermore, we view the reaction rate functions $\mathbf{r} = (r_j)_{j \in \mathbf{E}}$, i. e., the rates at which reaction j is active per time unit, as given parameters. For brevity, we define the derivatives of the reaction rates at the equilibrium x^* as

$$r_{jm} := \frac{\partial}{\partial x_m} r_j(x^*) = \begin{cases} r_j'(x_m^*) & \text{for} \quad m = \mathbf{m}(j), \\ 0 & \text{otherwise.} \end{cases} \tag{2.1.6}$$

As promised, we give a simple example to illustrate the essential role of the zero complex 0 and also an application of the ODE (2.1.5). Lets consider the reaction network

$$0 \xrightarrow{1} A \xrightarrow{2} 0 \, .$$

Note that the two zeros in the reaction network are actually the same zero vertex 0, but separated to emphasize feed and exit reactions. In this sense, we can view the graph as a di-cycle and also as strongly connected, even reversible. Since A is the only metabolite, we have $\mathbf{M} = \{A\}$ and hence $M = 1$. We see that $\mathbb{R}^M$ is one-dimensional, so the unit vector $X_A = 1 \in \mathbb{R}^M$ is scalar, as is $X_0 = 0$. The only two reactions that appear are the feed reaction $j = 1$ and the exit reaction $j = 2$, thus we have $\mathbf{E} = \{1, 2\}$ with $\mathbf{m}(1) = \overline{\mathbf{m}}(2) = 0$. Therefore, the reaction rates become $r_1 = r_1(x_0) = r_1(1)$, which is constant and $r_2 = r_2(x_A) = r_2(x)$ if we abbreviate $x := x_A$. So we can conclude that the single ODE (2.1.5) for our example network is given by

$$\dot{x} = r_1 - r_2(x).$$

2.2 MAIN IDEA AND ASSUMPTIONS ON SENSITIVITY ANALYSIS

We next take a closer look at the crucial assumptions and ideas on the flux sensitivity analysis provided by this theory. The first assumptions regard the ODE (2.1.5). On the reaction rate functions $r_j \in C^1$ we assume positivity, i. e.,

$$r_j(\xi) > 0 \quad \text{for} \quad \xi > 0. \tag{2.2.1}$$

This basically means that we omit vanishing reactions. But we do not require the positivity of their derivatives r_j'. Note that the definition (2.1.6) for the derivatives of the reaction rates respects the positivity assumption. Furthermore, we assume the existence of a positive steady state $x^* > 0$ that is

$$f(\mathbf{r}, x^*) = 0 \tag{2.2.2}$$

for some x^* which satisfies in all components $x_m^* > 0$. Again, in practise the positivity in this assumption just omits steady states with zero components of x^*. Finally, we assume the general reaction network to be regular at steady states of (2.1.5). This means we require the Jacobian $f_x(\mathbf{r}, x^*)$ of the partial derivatives with respect to x of the ODE vector field f to be nonsingular, i. e.,

$$\det f_x(\mathbf{r}, x^*) \neq 0. \tag{2.2.3}$$

The last assumption gives rise to a fundamental idea of the approach. We can use the implicit function theorem to study for any reaction j^* the steady state response to any perturbation of the rate function r_{j^*}. Let $\rho \in C^1$ be a continuously differentiable function and $\varepsilon \in \mathbb{R}$ small. Then the C^1-small perturbation

$$\mathbf{r}^{\varepsilon} := \mathbf{r} + \varepsilon\rho \tag{2.2.4}$$

of $\mathbf{r} = \mathbf{r}^0$ induces a response curve $x^*(\varepsilon)$, such that we have at $\mathbf{r}^{\varepsilon}$ and $x^*(\varepsilon)$

$$f_r \cdot \rho + f_x \cdot \frac{d}{d\varepsilon} x^* = 0. \tag{2.2.5}$$

Since we know by (2.2.3) that the Jacobian of f is nonsingular, this yields the resulting perturbation of x^*. Considering a special case of the perturbation, where $\rho = (\rho_j)_{j\in\mathbf{E}}$ with $\rho_j = \rho_j(x_{\mathbf{m}(j)})$ has no effect on the reaction network, but only changes reaction j^*, we are now able to define the concentration response of a metabolite. From the restriction above we have

$$\rho_j(\xi) = 0, \quad \text{for} \quad j \neq j^*, \quad \text{and} \tag{2.2.6}$$

$$\rho_{j^*}(x^*_{\mathbf{m}(j^*)}) = 1 \tag{2.2.7}$$

where we normalized the perturbation of rate j^*, without loss of generality. The resulting (infinitesimal) concentration response $\delta x^{j^*}_m$ of metabolite m at steady state is defined as

$$\delta x^{j^*}_m := \left.\frac{d}{d\varepsilon}\right|_{\varepsilon=0} x^*_m(\varepsilon) \tag{2.2.8}$$

for the particular case (2.2.6), (2.2.7). For this function-free approach no knowledge of numerical data is required to make a prediction on concentration responses $\delta x^{j^*}_m$ to be zero or nonzero, but to determine their precise numerical values, the numerical values of the derivatives r_{jm} are necessary.

One fundamental idea behind the theory is to consider the derivatives r_{jm} as abstract independent variables which enter the response $\delta x^{j^*}_{m_0}$ via certain rational expressions. In this spirit, we define $\delta x^{j^*}_{m_0}$ algebraically nonzero if

$$\delta x^{j^*}_{m_0} \neq 0, \tag{2.2.9}$$

as a rational function of the variables r_{jm} with $m = \mathbf{m}(j)$. As emphasized above, we see here that this implies $\delta x^{j^*}_{m_0} \neq 0$, except on real algebraic varieties of codimension at least 1 in the space of data r_{jm}.

However, this view point puts a further restriction of the approach into force. We require that the derivative variables r_{jm} are independent of the equilibrium flux values $r_j(x_m^*)$. But this independence fails if the class of nonlinearities r_j itself solves the same ODE $r_j' = h_j(r_j)$ for some fixed function h_j, for example in cases like $r_j = a \cdot \exp(x_m)$. Thus we need for the algebraic independence of r_j and r_j' at least a two parameter family of functions r_j. This means we have to exclude pure mass action kinetics, but already slightly richer rate functions of Michaelis-Menten and Langmuir-Hinshelwood type are permissible.

Note that the theory by Fiedler and Mochizuki also provides significant results on concentration sensitivity analysis. In fact, it states precise conditions on the stoichiometric graph structure of the chemical reaction network in order to possess nonzero concentration responses $\delta x_m^{j^*}$ of any metabolite $m \in \mathbf{M}$ in response to a rate perturbation (2.2.4) - (2.2.7) of any reaction $j^* \in \mathbf{E}$. Since we focus especially on flux sensitivity analysis here, this is beyond the scope of this thesis.

2.3 MAIN RESULT ON FLUX CHANGES

Before we state the main result on flux changes due to a perturbation of reaction rates, let us discover the idea behind the construction of the sensitivity of reaction fluxes. The (infinitesimal) flux response $\Phi_{j'j^*}$ of the flux through reaction j' due to a rate perturbation of reaction j^* is defined as follows:

$$\Phi_{j'j^*} := \delta_{j'j^*} + r_{j'\mathbf{m}(j')}\delta x_{\mathbf{m}(j')}^{j^*}. \tag{2.3.1}$$

The flux response $\Phi_{j'j^*}$ consists of two terms, the first one, the Kronecker-delta $\delta_{j'j^*}$, represents the explicit flux change due to the external perturbation at j^* in reaction $j' = j^*$. The second term indicates the implicit flux change in any reaction j', which is caused by the concentration response $\delta x_{\mathbf{m}(j')}^{j^*}$ of the mother reactant $\mathbf{m}(j')$ due to the external perturbation at j^*.

The main result on structural sensitivity analysis of flux influences, which we state now, provides insight on the impact of an external perturbation at reaction j^* to either propagate downward along a directed path γ' starting with vertex $\mathbf{m}(j^*) = m^*$ and edge j^*, or else to spill over to a side branch γ' from $j^* \in \gamma^0 \backslash \gamma'$.

Theorem 2.1 (Fiedler & Mochizuki). *Let positivity and existence assumptions (2.2.1), (2.2.2) hold for the monomolecular reaction network (2.1.1), (2.1.2) and (2.1.5). Moreover assume the Jacobian determinant in (2.2.3) is nonzero, algebraically. Consider any pair of edges $j', j^* \in \mathbf{E}$, not necessarily distinct.*

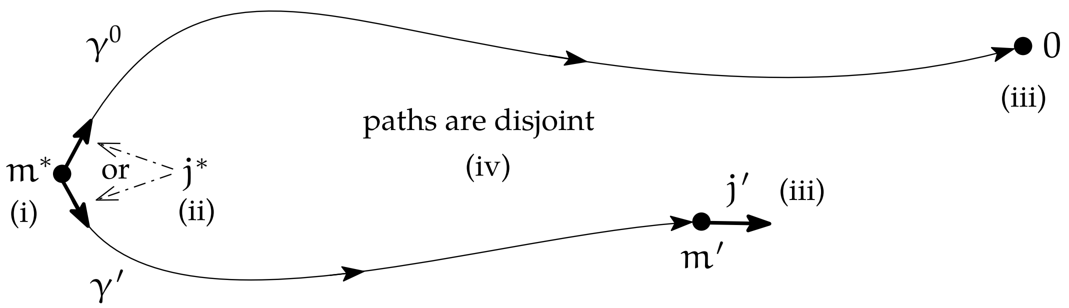


Figure 2.3.1: Illustrating the properties (i) - (iv) of Theorem 2.1: the exit di-path γ^0 is at the top and the influence di-path γ' at the bottom, property (i): both paths emanate from m^*; (ii): one of the paths contains j^*; (iii): γ^0 terminates at vertex 0 and γ' with edge j'; (iv): the paths are disjoint except for m^*.

Then j^* *influences* j'*, i.e., the flux response* $\Phi_{j'j^*}$ *of reaction* j' *to a rate perturbation (2.2.4) - (2.2.7) of reaction* j^* *satisfies*

$$\Phi_{j'j^*} \neq 0 \tag{2.3.2}$$

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* $j' : \ m' \longrightarrow \overline{m}'$ *, but omitting the product 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.*

Where disjoint means that the paths do not share any edge, or any vertex besides m^*. For an illustration of the four conditions see Figure 2.3.1. The idea of the conditions on γ' is to describe some domain of influence of the perturbation j^*. Also note that γ' always contains the edge j' and then terminates. However, the idea of the exit path γ^0 is less intuitive, in particular in the case when the effects of $j^* \in \gamma^0$ spill over to the side branch γ'.

A simple application is the single child case. In this case j^* is the only child edge of the mother vertex m^*. According to the Theorem 2.1 we need two different di-paths γ^0 and γ', which are disjoint, by property *(iv)* and emanate, by *(i)*, from the same mother vertex m^* for a nonzero flux response $\Phi_{j^*j^*} \neq 0$. Since we only have a single child edge of m^*, this is a contradiction. Therefore, we have

$$\Phi_{j'j^*} = 0, \tag{2.3.3}$$

for all $j' \in \mathbf{E}$. To verify this result, we also want to prove (2.3.3) directly. By (2.1.6) and (2.2.5) - (2.2.8) at the single-child vertex m^* we conclude

$$\delta x_{m^*}^{j^*} = \frac{-1}{r_{j^*m^*}} \neq 0. \tag{2.3.4}$$

Looking at (2.3.1), we see that this implicit response compensates for the external flux increase by ρ, at j^*, and therefore $\Phi_{j^*j^*} = 0$. Furthermore, we note that the zero flux response in this example is caused by the Kronecker-delta in the flux sensitivity (2.3.1).

A further intriguing result by Fielder and Mochizuki, which we just want to mention here, is the concept of transitivity of influence. We say that reaction j^* influences reaction j', in terms $j^* \rightsquigarrow j'$, if the flux response $\Phi_{j'j^*}$ is algebraically nonzero:

$$j^* \rightsquigarrow j' \quad \Longleftrightarrow \quad \Phi_{j'j^*} \neq 0. \tag{2.3.5}$$

In addition, we call the flux influence relation (2.3.5) transitive if

$$j_1 \rightsquigarrow j_2 \quad \text{and} \quad j_2 \rightsquigarrow j_3 \quad \text{implies} \quad j_1 \rightsquigarrow j_3, \tag{2.3.6}$$

i. e., if a perturbation in the reaction rate of j_1 causes a change in j_2, and a change of the rate of reaction j_2 propagates to j_3, then j_1 also influences j_3. Under the assumptions of Theorem 2.1 Fiedler and Mochizuki proved that transitivity (2.3.6) holds true for the flux influence relation $j^* \rightsquigarrow j'$. Furthermore, we like to mention that Nicola Vassena [32] has provided a simplified proof of this transitivity result, based on more advanced graph theory. His proof utilizes standard connectivity concepts from graph theory, Menger's Theorem, and what he calls obliged elements.

2.4 CONSTRUCTION AND CALCULATION OF THE FLUX SENSITIVITY MATRIX

The flux sensitivity matrix

$$\Phi := \left(\Phi_{j'j^*}\right)_{j',j^* \in \mathbf{E}} \tag{2.4.1}$$

is an array whose elements are the flux responses $\Phi_{j'j^*} = \delta_{j'j^*} + r_{j'\mathbf{m}(j')}\delta x^{j^*}_{\mathbf{m}(j')}$, see (2.3.1), for the whole reaction network. Similarly, the array of concentration sensitivities $\delta x^{j^*}_m$ is called concentration sensitivity matrix, in terms

$$\delta x := \left(\delta x^{j^*}_m\right)_{j^* \in \mathbf{E}, m \in \mathbf{M}}. \tag{2.4.2}$$

Since involved reaction networks contain more edges than metabolites, the first thing we notice about both matrices is that the flux sensitivity matrix is larger than the concentration sensitivity matrix.

We going to develop a method to calculate the flux sensitivity matrix and in addition gain insight into its precise construction. We also give an example how to derive the matrix for a simple network, afterwards. For this purpose we define the stoichiometric matrix $\mathbf{S}$ with dimensions $M \times E$ by

$$\begin{aligned} \mathbf{S}: \quad & \mathbb{R}^E \to \mathbb{R}^M, \\ \mathbf{S}e_j := & X_{\overline{\mathbf{m}}(j)} - X_{\mathbf{m}(j)}, \end{aligned} \tag{2.4.3}$$

with e_j as the j-th unit vector in $\mathbb{R}^E$, and we already defined X_m as the m-th unit vector in R^M, with $X_0 := 0$. By looking at the feed and exit reactions we can see an application of the convention $X_0 = 0$. For the feed reactions j we have $\mathbf{S}e_j = X_{\overline{\mathbf{m}}(j)} - X_0 = X_{\overline{\mathbf{m}}(j)}$, and for the exit reactions j we get $\mathbf{S}e_j = X_0 - X_{\mathbf{m}(j)} = -X_{\mathbf{m}(j)}$. Moreover, the stoichiometric matrix $\mathbf{S}$ allows us to reformulate the ODE (2.1.5) as

$$\dot{x} = \mathbf{S}\mathbf{r}(x), \tag{2.4.4}$$

where $x = (x_m)_{m \in \mathbf{M}}$ and $\mathbf{r} = (r_j)_{j \in \mathbf{E}}$.

The $E \times M$-dimensional reactivity matrix $\mathbf{R}$ is defined by

$$\mathbf{R} := \left(r_{jm}\right)_{j \in \mathbf{E}, m \in \mathbf{M}}, \tag{2.4.5}$$

where $r_{jm} = r'_j(x^*_m)$ are the nontrivial derivatives of the reaction rate functions for $m = \mathbf{m}(j)$, viewed as independent variables. By (2.1.6) we fill the matrix with zeros for missing entries. The idea behind the reactivity matrix $\mathbf{R}$ is the representation of the reaction aspect of the stoichiometric matrix $\mathbf{S}$. Let $N := \dim\ker\mathbf{S}$ be the dimension of the kernel of the stoichiometric matrix and take an arbitrary basis $c^1, \ldots, c^N \in \mathbb{R}^E$ for $\ker\mathbf{S}$, with components c^k_j. We define the $E \times N$-matrix

$$\mathbf{C} := \left(c^k_j\right)_{j\in E, k\in\{1,\ldots,N\}}, \tag{2.4.6}$$

which accounts for the graph aspect of $\mathbf{S}$. Now we are able to define a mandatory tool for the calculation of the flux sensitivity matrix and also for the proof of the main result, the augmented matrix $\mathbf{A} : \mathbb{R}^M \times \mathbb{R}^N \to \mathbb{R}^E$. This matrix, defined in block form as

$$\mathbf{A} := (\mathbf{R}, \mathbf{C}), \tag{2.4.7}$$

with dimensions $E \times (M+N)$, on the one hand combines the reaction aspect $\mathbf{R}$ and the graph aspect $\mathbf{C}$ of the stoichiometric matrix $\mathbf{S}$. On the other hand, both aspects stay separated and can therefore be addressed without any effort.

For the sake of a more elegant appearance of the calculation method, we define for the rest of this section the augmented matrix $\mathbf{A}$ as

$$\mathbf{A} = \left(\begin{array}{ccc:ccc} r_{11} & \ldots & r_{1M} & -c^1_1 & \ldots & -c^N_1 \\ \vdots & & \vdots & \vdots & & \vdots \\ r_{E1} & \ldots & r_{EM} & -c^1_E & \ldots & -c^N_E \end{array}\right), \tag{2.4.8}$$

which is equivalent to (2.4.7) by the freedom of choice of the basis of $\ker\mathbf{S}$, but saves us from a lot of minus signs. The $\mathbf{C}$-part can easily be determined by virtue of Proposition 2.2, which we will state and prove in the next section. For that purpose, we consider a maximal spanning tree $\mathcal{T}$ of the reaction network Γ and generate cycles c^j in $\mathcal{T} \cup \{j\}$ by adding edges $j \notin \mathbf{E}(\mathcal{T})$. These cycles are unique, linearly independent and form a basis of $\ker\mathbf{S}$. Therefore we just need to write the cycles c^j, i. e., the edges they are made of, in the columns of the $\mathbf{C}$-part.

The negative inverse of the augmented matrix $\mathbf{A}$ is called sensitivity matrix $\mathcal{S}$ and give by

$$\mathcal{S} := \left(\begin{array}{ccc} \delta x^1_1 & \ldots & \delta x^E_1 \\ \vdots & & \vdots \\ \delta x^1_M & \ldots & \delta x^E_M \\ \hdashline \mu^1_1 & \ldots & \mu^E_1 \\ \vdots & & \vdots \\ \mu^1_N & \ldots & \mu^E_N \end{array}\right) = -\mathbf{A}^{-1}, \tag{2.4.9}$$

where $\mu_k^j \in \mathbb{R}$ are coefficients of a suitable linear combination of kernel vectors $(c_j^k)_{j \in \mathbf{E}}$ of $\mathbf{S}$ such that we can express the flux response vector $\Phi^* := (\Phi_{j'j^*})_{j' \in \mathbf{E}}$ to a perturbation of reaction j^* as

$$\Phi^* = \mathbf{C}(\mu_k^j)_{k \in \{1,\ldots,N\}}. \tag{2.4.10}$$

The sensitivity matrix $\mathcal{S}$ of a system, with columns $j^* \in \mathbf{E}$ indicating perturbed reactions, is a square $E \times E$ matrix. That the relation $E = M + N$ holds true, we will see in Proposition 2.2, in the next section. Let us emphasize that the sensitivity matrix $\mathcal{S}$ may not be confused with the concentration sensitivity matrix δx, albeit the first M rows of $\mathcal{S}$ collect the sensitivity responses $\delta x_m^{j^*}$ of all metabolites. The bottom part of the $\mathcal{S}$ matrix contains the responses of fluxes to a normalized perturbation of the reaction j^* in the coefficient column $(\mu_k^{j^*})_{k \in \{1,\ldots,N\}}$.

Motivated by (2.3.1), our first attempt to construct the $E \times E$ flux sensitivity matrix

$$\Phi = \mathrm{id}_{E \times E} + \mathbf{R}\delta x \tag{2.4.11}$$

involves the identity matrix $\mathrm{id}_{E \times E}$, the concentration sensitivity matrix δx, as well as the reactivity matrix $\mathbf{R}$. On the other hand, since the flux response vectors Φ^* for $j^* \in \mathbf{E}$ are the columns of the flux sensitivity matrix Φ, we can use (2.4.10) to reformulate (2.4.11) as

$$\Phi = \begin{pmatrix} c_1^1 & \cdots & c_1^N \\ \vdots & & \vdots \\ c_E^1 & \cdots & c_E^N \end{pmatrix} \begin{pmatrix} \mu_1^1 & \cdots & \mu_1^E \\ \vdots & & \vdots \\ \mu_N^1 & \cdots & \mu_N^E \end{pmatrix}. \tag{2.4.12}$$

For an actual calculation of the flux sensitivity matrix Φ, this means, we need to set up the augmented matrix $\mathbf{A}$ for the negative of the $\mathbf{C}$-part, determine the sensitivity matrix $\mathcal{S}$, i.e., compute the negative inverse of $\mathbf{A}$ for its bottom part and multiply both parts with each other, as in (2.4.12). Details on the abstract construction of the augmented matrix $\mathbf{A}$ and the correctness of the equations (2.4.9) and (2.4.10) are given in the proof of the main result in Section 2.6.

To demonstrate the method for calculation of the flux sensitivity matrix we give an example of a simple chemical reaction network, the single path way. This example also illustrates our findings on the single child case in Section 2.3. The single path way Γ is made of three metabolites $\mathbf{M} = \{A, B, C\}$ and four reactions $\mathbf{E} = \{1, 2, 3, 4\}$, which are arranged as a metabolic chain without any side branches, see Figure 2.4.1. The network is monomolecular and fulfills the regularity condition (2.2.3),

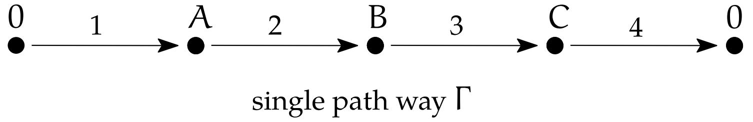


Figure 2.4.1: Single path way Γ containing three metabolites $\mathbf{M} = \{A, B, C\}$ and four reactions $\mathbf{E} = \{1, 2, 3, 4\}$ arranged as metabolic chain without side branches.

which can be easily checked with the help of Lemma 2.4, which we will see in the next section. First we need to determine the augmented matrix $\mathbf{A}$. We know that the network has $E = 4$ reactions, thus we deal with a 4×4 matrix. Since the single path way has three metabolites, i. e., $M = 3$, the first three columns of $\mathbf{A}$ are occupied by the $\mathbf{R}$-part and only the last column is reserved for the $\mathbf{C}$-part of $\mathbf{A}$. Indeed, by $N = E - M$ the kernel of the stoichiometric matrix $\mathbf{S}$ is one dimensional. The augmented matrix $\mathbf{A}$ is given as

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

where the $\mathbf{C}$-part is made of a single cycle, the single path way itself. We next compute the sensitivity matrix $\mathcal{S}$ by taking the negative inverse of $\mathbf{A}$,

$$\mathcal{S} = -\mathbf{A}^{-1} = \left(\begin{array}{cccc} \frac{1}{r_{2A}} & -\frac{1}{r_{2A}} & 0 & 0 \\ \frac{1}{r_{3B}} & 0 & -\frac{1}{r_{3B}} & 0 \\ \frac{1}{r_{4C}} & 0 & 0 & -\frac{1}{r_{4C}} \\ \hline 1 & 0 & 0 & 0 \end{array} \right).$$

The perturbed reactions $j^* = 1, \ldots, 4$ are indicated by the columns. The first three rows represent the concentration responses $\delta x_A, \delta x_B, \delta x_C$ of the metabolites and the last row the response of the flux. Therefore, the last row shows that the flux only changes if reaction 1, the input in the system, is perturbed. Then all concentrations in the network increase, accordingly. However, when perturbing the other reactions 2, 3, or 4, only the input reactant of the perturbed reaction changes.

To obtain the flux sensitivity matrix Φ, finally, we take the negative of the $\mathbf{C}$-part of the augmented matrix $\mathbf{A}$ and multiply it with the bottom part of the sensitivity matrix $\mathcal{S}$, according to (2.4.12),

$$\Phi = \begin{pmatrix} 1 \\ 1 \\ 1 \\ 1 \end{pmatrix} \cdot \begin{pmatrix} 1 & 0 & 0 & 0 \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix}.$$

As mentioned before, this example illustrates our findings on the single child case, since any reaction j^* other than the feed reaction 1 is a single child of a mother vertex $m = \mathbf{m}(j^*)$. Therefore, we have for all $j^* \neq 1$ and all j' a zero flux response $\Phi_{j'j^*} = 0$. For the feed case $j^* = 1$, $m^* = 0$ we have to take the trivial path $\gamma^0 = \{0\}$ as exit path and see that any edge j' is reachable from the leading edge $j^* = 1$ by a di-path γ' from $m^* = 0$, therefore Theorem 2.1 implies $\Phi_{j'1} \neq 0$ for all $j' \in \mathbf{E}$.

The calculation of a prolonged single path way with any finite number of metabolites is straightforward and the flux sensitivity matrix will look the same way, but with adapted dimensions.

2.5 GRAPH THEORETICAL PROPERTIES FOR REACTION NETWORKS

This section mainly concerns a reformulation of the regularity assumption (2.2.3) on the reaction network in terms of graph theory, as well as the implications of the positivity assumptions (2.2.1), (2.2.2) for the stationary reaction rates r_j on the graph structure of the network.

Our view point on the regularity assumption (2.2.3) is a purely algebraic one, in terms of the independent variables $r_{jm} = r_j'(x_m^*)$, where $j \in \mathbf{E}$ is a reaction with mother vertex $m = \mathbf{m}(j) \in \mathbf{M} \cup \{0\}$. Let us temporarily consider x^* as a generic point on a solution curve of the ODE (2.1.5), instead of our usual assumption to be a stationary solution (2.2.2). We also do not require positivity (2.2.1). We decompose

$$f'(x^*) = \mathbf{SR} \tag{2.5.1}$$

into the reactivity matrix $\mathbf{R} = (r_{jm})_{j \in \mathbf{E}, m \in \mathbf{M}}$ and the stoichiometric matrix $\mathbf{S}$, see (2.4.3), (2.4.5). From the last section we know that the augmented matrix $\mathbf{A} =$

$(\mathbf{R}, \mathbf{C})$ separates the reaction aspect $\mathbf{R}$ and the graph aspect $\mathbf{C}$ of the stoichiometric matrix $\mathbf{S}$.

We first study the graph aspect of $\mathbf{S}$ and consider for this purpose the graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ as undirected. This means, we disregard all orientations of the edges j, for the moment. We define the boundary map $\mathbf{D}$, mapping edges $j \in \mathbf{E}$ to vertices $m \in \mathbf{M} \cup \{0\}$ in Γ by

$$\begin{aligned} \mathbf{D}: \quad & \mathbb{R}^{E} \to \mathbb{R}^{M+1}, \\ \mathbf{D}e_j := {} & \mathcal{X}_{\overline{\mathbf{m}}(j)} - \mathcal{X}_{\mathbf{m}(j)}. \end{aligned} \tag{2.5.2}$$

Furthermore, we define the projection $\mathbf{P}_0$, which simply drops the last component associated to the vertex 0 in Γ, by

$$\mathbf{P}_0: \quad \mathbb{R}^{M+1} \to \mathbb{R}^{M}. \tag{2.5.3}$$

We can use the projection $\mathbf{P}_0$ and the boundary map $\mathbf{D}$ to decompose the stoichiometric matrix $\mathbf{S}$ as

$$\mathbf{S} = \mathbf{P}_0 \mathbf{D}. \tag{2.5.4}$$

For the boundary map $\mathbf{D}$ the homologies $H_0(\Gamma)$ and $H_1(\Gamma)$ are defined as

$$H_0(\Gamma) := (\text{range } \mathbf{D})^{\perp} \quad \leqslant \mathbb{R}^{M+1}, \tag{2.5.5}$$

$$H_1(\Gamma) := \ker \mathbf{D} \qquad\quad \leqslant \mathbb{R}^{E}. \tag{2.5.6}$$

By the definition of $H_0(\Gamma)$ we note that $\dim H_0$ counts the weakly connected components W of the graph Γ. Thus, a basis of H_0 is given by the characteristic functions 1_W of the component vertices. The Euler characteristic

$$\chi(\Gamma) := \dim H_0 - \dim H_1 = M + 1 - E \tag{2.5.7}$$

is the alternating sum of the dimensions of the homologies $H_0(\Gamma)$ and $H_1(\Gamma)$. From another view point, the Euler characteristic $\chi(\Gamma)$ is the negative Fredholm index of the boundary map $\mathbf{D}$. An important observation here is that the cycles $c^j \in \ker \mathbf{D} = H_1(\Gamma)$, which are produced by adding edges $j \notin \mathbf{E}(\mathcal{T})$ to a maximal spanning forest $\mathcal{T}$ of Γ, are linearly independent, and by maximality and acyclicity of $\mathcal{T}$ therefore form a basis of $H_1(\Gamma)$. Hence

$$\dim H_1 = E - E(\mathcal{T}), \tag{2.5.8}$$

where $E(\mathcal{T})$ is the number of edges of the maximal spanning forest $\mathcal{T}$ of Γ.

With the next proposition we are going to justify some of the properties, which we already used in the previous section on calculating the flux sensitivity matrix Φ.

Proposition 2.2 (Fiedler & Mochizuki). *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,*

$$\dim H_1(\Gamma) = N := \dim \ker \mathbf{S}. \tag{2.5.9}$$

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.,*

$$\dim H_0(\Gamma) = 1. \tag{2.5.10}$$

Proof. For brevity, we define def $\mathbf{S} := \dim \ker \mathbf{S}$ and cork $\mathbf{S} :=$ codim range $\mathbf{S}$ for the stoichiometric matrix $\mathbf{S} : \mathbb{R}^E \to \mathbb{R}^M$. Our first step is to prove $\ker \mathbf{D} = \ker \mathbf{S}$ and consequently (2.5.9), too. Let $e_0 \in \ker \mathbf{P}_0$ be the spanning element which is omitted by $\mathbf{P}_0$. By definition, we know $e_0 \perp 1_W$ for the characteristic function 1_W of any weakly connected component W of Γ which does not contain vertex 0, and $e_0^T \cdot 1_{W_0} = 1$ for the weakly connected component W_0 of the vertex 0 itself. Therefore, e_0 satisfies

$$e_0 \notin \text{range}\, \mathbf{D} = H_0(\Gamma)^\perp, \tag{2.5.11}$$

if, and only if, $\ker \mathbf{D} \geqslant \ker \mathbf{S}$. Furthermore, $\mathbf{S} = \mathbf{P}_0 \mathbf{D}$ implies $\ker \mathbf{D} \leqslant \ker \mathbf{S}$ and hence the equality $\ker \mathbf{D} = \ker \mathbf{S}$ holds true. This also proves (2.5.9):

$$\dim H_1(\Gamma) := \dim \ker \mathbf{D} = \dim \ker \mathbf{S} =: N. \tag{2.5.12}$$

In the next step we address the equivalence of *(i)* - *(iv)*. The equivalence *(i)* $\Longleftrightarrow$ *(ii)* is true, since

$$E - M = \text{def}\, \mathbf{S} - \text{cork}\, \mathbf{S} = N - \text{cork}\, \mathbf{S} = N, \tag{2.5.13}$$

if, and only if, $\mathbf{S}$ is surjective. Equivalence *(ii)* $\Longleftrightarrow$ *(iii)* is trivial, since $M + N = E$. Lastly, we prove the equivalence *(ii)* $\Longleftrightarrow$ *(iv)*, i. e., (2.5.10). According to the Euler characteristic (2.5.7) we know the first equality of

$$\dim H_0 = \dim H_1 + M + 1 - E = 1 + N - (E - M) = 1, \tag{2.5.14}$$

and (2.5.9) provides the second equality. This completes the proof. □

We next study the symbolic reaction part $\mathbf{R}$ of the stoichiometric matrix $\mathbf{S}$, its influence on the Jacobian matrix $f'(x^*) = \mathbf{SR}$, and its role in the augmented matrix $\mathbf{A} = (\mathbf{R}, \mathbf{C})$, see (2.5.1) and (2.4.7).

Proposition 2.3 (Fiedler & Mochizuki)**.** *Consider any directed graph* $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$*, as in* (2.1.1)*. Then*

$$\det \mathbf{SR} \neq 0 \iff \det \mathbf{A} \neq 0. \tag{2.5.15}$$

Proof. The proof is fairly short. First we show that

$$\det \mathbf{SR} \neq 0 \implies \det \mathbf{A} \neq 0, \tag{2.5.16}$$

and the converse claim afterwards. Suppose that $\det \mathbf{SR} \neq 0$. By Proposition 2.2 *(i)* and *(iii)*, this implies that $\mathbf{S}$ is surjective, and therefore $\mathbf{A} = (\mathbf{R}, \mathbf{C})$ is a square matrix. To show that $\ker \mathbf{A}$ is trivial, we consider $\xi \in \mathbb{R}^M$ and $\mu \in \mathbb{R}^N$ such that

$$0 = \mathbf{R}\xi + \mathbf{C}\mu. \tag{2.5.17}$$

If we multiply (2.5.17) by $\mathbf{S}$, the $\mathbf{C}$ term cancels out, since it describes the kernel of $\mathbf{S}$, i. e., $\mathbf{SC} = 0$. Thus, we obtain $\mathbf{SR}\xi = 0$. We further conclude that $\xi = 0$, by the assumption $\det \mathbf{SR} \neq 0$. For the remaining part $\mathbf{C}\mu$ of (2.5.17) we recollect that the columns c^k of $\mathbf{C}$ are a basis for $\ker \mathbf{S}$ and thus linearly independent. This implies that $\mu = 0$ and hence we proved claim (2.5.16).

To address converse direction that $\det \mathbf{A} \neq 0$ implies $\det \mathbf{SR} \neq 0$, we show that $\mathbf{SR}$ possesses a trivial kernel. Suppose $\mathbf{SR}\xi = 0$. Thus $\mathbf{R}\xi \in \ker \mathbf{S} = \operatorname{span}\{c^1, \dots, c^N\}$ means that there exists a linear combination $\mu \in \mathbb{R}^N$ such that (2.5.17) is fulfilled. But $\det \mathbf{A} \neq 0$ let us draw the conclusion $\xi = 0$. This proves the proposition. $\square$

Now we want to regard $\det \mathbf{SR}$ as a polynomial in the nontrivial derivative variables r_{jm}, for $j \in \mathbf{E}$ and $m = \mathbf{m}(j)$. We call $\det \mathbf{SR} \neq 0$ algebraically, if this polynomial does not vanish identically.

Lemma 2.4 (Fiedler & Mochizuki)**.** *Consider any directed graph* $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ *as in* (2.1.1)*. Then*

$$\det \mathbf{SR} \neq 0 \tag{2.5.18}$$

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. For a clear structure, we divide the proof into five steps, since it is more involved. The first step reminds of the child selection map (2.1.3), (2.1.4) and uses it to replace $\det \mathbf{SR}$ by a polynomial expression. In the second step, we look for a suitable choice for a basis of $\ker \mathbf{S}$ to simplify this polynomial expression. Now, in the third step, we are able to find a directed exit path γ^0 and thus prove the first direction. Step 4 concerns itself with the converse direction by reducing the statement to the construction of a suitable child selection map and Step 5 completes the proof with this construction.

Step 1: We may replace $\det \mathbf{SR} \neq 0$ by $\det \mathbf{A} \neq 0$, according to Proposition 2.3. Our view point on $\det \mathbf{A}$ is purely algebraical, in the sense that we regard it as polynomial in the nontrivial derivative variables r_{jm}. Thus, $\det \mathbf{A} \neq$ holds, algebraically, if, and only if the polynomial expansion of the determinant does not vanish identically in the nontrivial entries r_{jm} of $\mathbf{R}$. By (2.1.4), we know that the mother map $\mathbf{m} : \mathbf{E} \to \mathbf{M} \cup \{0\}$ is a left inverse of the child selection map $J : \mathbf{M} \to \mathbf{E}$, i.e., $\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}$. Consider any child selection map J. The polynomial expansion

$$\det \mathbf{A} = \sum_{J} a_J r^J \tag{2.5.19}$$

has the generating nontrivial monomials r^J with the coefficients a_J. The generating monomials of this expansion, given as

$$r^J := \prod_{m \in \mathbf{M}} r_{J(m),m} = \prod_{j \in J(\mathbf{M})} r'_j(x_{\mathbf{m}(j)}), \tag{2.5.20}$$

are in one-to-one correspondence with the child selection maps J. The coefficients a_J are the subdeterminants of the kernel part $\mathbf{C}$ of the augmented matrix $\mathbf{A}$, in terms

$$a_J = \pm \det(c_j^k)_{j \in \mathbf{E} \setminus J(\mathbf{M}), k \in \{1,\dots,N\}}. \tag{2.5.21}$$

In fact $\det \mathbf{A} \neq 0$ holds, algebraically, if, and only if, at least one coefficient a_J of the nontrivial monomials r^J in (2.5.19) is nonzero.

Step 2: Now we want to choose a suitable basis $c^1, \dots, c^N$ of $\ker \mathbf{S}$ for our situation. Let $\mathcal{T}_0$ be a maximal spanning forest of the graph Γ. Since Γ is connected, by our assumption $\det \mathbf{SR} \neq 0$, which implies surjectivity of the stoichiometric matrix $\mathbf{S}$, combined with Proposition 2.2 *(i)* and *(iv)*, $\mathcal{T}_0$ is even a maximal spanning tree. We define $\mathbf{C}$ by the cycles c^k of the maximal spanning tree $\mathcal{T}_0$. In (2.5.21) the rows of $J(\mathbf{M})$ are omitted, so let us denote this square matrix as $\check{C}(J(\mathbf{M}))$, as well as $\mathbf{E}' := J(\mathbf{M})$. An crucial observation here is that

$$\det \check{C}(\mathbf{E}') \neq 0 \tag{2.5.22}$$

holds, if, and only if, $\mathcal{T} = (\mathbf{M} \cup \{0\}, \mathbf{E}')$ is a maximal spanning tree of Γ. So $\check{C}(\mathbf{E}')$ defines a change of the basis of $\ker \mathbf{S}$ from the cycle basis given by the maximal spanning tree $\mathcal{T}_0$ to the new cycle basis defined by $\mathcal{T}$.

Step 3: To find a directed exit path γ^0, we reactivate the edge orientations. Consider $m_0 \in \mathbf{M}$. Since the maximal spanning tree $\mathcal{T}$ selects one unique child edge $J(m)$ out of any mother vertex $m \in \mathbf{E}$, this defines a unique directed path γ^0 starting at m_0. By acyclicity of the maximal spanning tree $\mathcal{T}$, the di-path γ^0 can never return to itself. Therefore, γ^0 can only terminate at the exit vertex $0 \notin \mathbf{M}$. This shows the first direction.

Step 4: Now we want to deal with the converse direction. Suppose there exists a di-path γ^0 in $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ from any $m \in \mathbf{M}$ to 0. The first thing we observe is that Γ is weakly connected. Therefore, it is sufficient to construct a child selection map $J : \mathbf{M} \to \mathbf{E}$ as is in (2.1.4), to show that $\det \mathbf{A} \neq 0$ algebraically.

Step 5: We are going to inductively construct J from the paths γ^0. To accomplish this, consider any acyclic exit di-path γ^0 and attach any missing vertex $m \in \mathbf{M}$ by following its acyclic exit di-path γ^0 until it reaches a vertex which already has been taken care of. This method selects a unique child arrow $J(m)$, for any mother vertex $m \in \mathbf{M}$, gradually. In return, the child selection J produces a nontrivial monomial (2.5.19) of $\det \mathbf{A}$ with nonzero coefficients a_J, as in (2.5.20), (2.5.21), and hence $\det \mathbf{A} \neq 0$, algebraically. This completes the proof of the lemma. □

The previous proof also yields a variant formulation for the child selection map J, which are in a way defined that they posses the mother map $\mathbf{m}$ as a left inverse, i. e., $\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}$. Also see (2.1.3), (2.1.4). The next corollary concerns itself with this issue.

Corollary 2.5 (Fiedler & Mochizuki)**.** *In the setting of Lemma 2.4,* $\det \mathbf{SR} \neq 0$ *holds algebraically if, and only if, there exists a child selection map* $J : \mathbf{M} \to \mathbf{E}$ *such that* $\mathcal{T} = (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ *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* $\mathcal{T}$ *possesses the following additional properties:*

(i) any di-path in $\mathcal{T}$ *terminates at* 0*;*

(ii) for any edge $j \notin \mathcal{T}$*, the following alternative holds true:*

  (a) either, the unique cycle c^j *in* $\mathcal{T} \cup \{j\}$ *is a di-cycle,*

(b) *or else, the short-cut* $\mathrm{j} \notin \mathcal{T}$ *runs parallel to the di-path* $\mathrm{c}^{\mathrm{j}} \cap \mathcal{T}$ *in* $\mathcal{T}$*;*

(iii) $\mathcal{T}$ *does not contain any feed reaction* j *emanating from* 0*;*

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

The last lemma for this chapter deals with the implications of the positivity assumptions (2.2.1) and (2.2.2) for the stationary reaction rates r_j on the graph structure of the reaction network. In fact, the positivity assumptions ensure that each weakly connected component is also strongly connected.

Lemma 2.6 (Fiedler & Mochizuki)**.** *Let positivity assumptions* (2.2.1), (2.2.2) *hold.*

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

In particular, suppose that regularity assumption (2.2.3) *holds in addition, i. e.,* $\det f'(x^*) \neq 0$*. Then* Γ *is strongly connected.*

Proof. For the first part of the lemma, consider the acyclic induced di-graph on the strongly connected components within any weak connected component of Γ. The remaining directed edge of a minimal component W, if any, then leads to W, and not away from W. Since the reaction fluxes r_j satisfy Kirchhoff's law at any vertex m, we know that the total in-flow to W vanishes. By assumption, all reaction fluxes r_j are strictly positive, thus there cannot exist any directed edge pointing towards W. Therefore strong component W and its weak component coincide.

Part two follows from part one, in addition to Proposition 2.2 *(i)*, *(iv)* applied to the surjective $f'(x^*) = \mathbf{SR}$. Therefore, the lemma is proved. □

2.6 PROOF OF THE MAIN RESULT

This section concerns itself with the proof of the main result on flux sensitivity analysis, Theorem 2.1 by Fiedler and Mochizuki, only. Before we start the proof, let us mention here that Nicola Vassena [32] has provided a reformulation of the theorem, based on more advanced graph theory and his obliged elements.

Throughout the whole section we assume positivity and existence assumptions (2.2.1), (2.2.2), and regularity assumption (2.2.3). Analogously as in the proof of Lemma 2.4, maximal spanning trees $\mathcal{T}$ and the augmented matrix $\mathbf{A}$ will play a crucial role. So you may want to have another look at (2.4.6), (2.4.7), and Proposition 2.2.

To provide a clear structure for an easier understanding, we divide the proof into eight steps and give a brief overview. In the first step we calculate the flux response vector Φ^* in terms of the reactivity matrix $\mathbf{R}$ and the $\mathbf{C}$-matrix. Step 2 makes use of the Cramer rule and some calculation to reformulate the determinant of the augmented matrix $\mathbf{A}$. The third step just sets our goals in the new setting. We need to consider two cases for the construction of a maximal spanning tree. Step 4 concerns itself with the general case and Step 5 uses the results to construct the required exit and influence path. The easy case is shown in Step 6 and finishes the only-if-direction of the theorem. We lay out the plan for the other direction in step 7, in particular, we have to show that the reformulated $\det \mathbf{A}$ is algebraically nonzero. The eight step finally carries out the plan and closes the proof.

Step 1: For fixed $j^* \in \mathbf{E}$, we start with calculating the flux response vector $\Phi^* = (\Phi_{j'j^*})_{j' \in \mathbf{E}}$ to the perturbation vector $\rho = e^* =: e_{j^*}$ of the reaction j^*, see (2.2.4) - (2.2.7). If we look at the concentration response $\delta x^* := (\delta x^{j^*}_m)_{m \in \mathbf{E}}$ of (2.2.8), we can conclude by the implicit function theorem (2.2.5)

$$\mathbf{S}e^* + \mathbf{SR}\delta x^* = 0. \tag{2.6.1}$$

We can rewrite this expression, according to the definition of the kernel matrix $\mathbf{C}$ of the stoichiometric matrix $\mathbf{S}$, as

$$e^* + \mathbf{A}\begin{pmatrix} \delta x^* \\ \mu^* \end{pmatrix} = e^* + \mathbf{R}\delta x^* + \mathbf{C}\mu^* = 0, \tag{2.6.2}$$

for some suitable linear combination μ^* of kernel vectors $(c^k_j)_{j \in \mathbf{E}}$ of $\mathbf{S}$. By definition (2.3.1) of the flux response, we can therefore conclude

$$\Phi^* = e^* + \mathbf{R}\delta x^* = -\mathbf{C}\mu^*. \tag{2.6.3}$$

By regularity assumption (2.2.3), the Jacobian $f'(x^*) = \mathbf{SR}$ is invertible, and hence $\mathbf{A}$ is invertible by Proposition 2.3, (2.5.15), too. Therefore, we can calculate the flux response vector Φ^* by solving (2.6.2) for μ^* and insert the result in (2.6.3).

Step 2: Now we reformulate the determinant of the augmented matrix $\mathbf{A}$. For this purpose we use the Cramer rule and obtain

$$-\mu^*_k \det \mathbf{A} = (-1)^{M+k+j^*} \det \check{A}_{j^*,M+k}, \tag{2.6.4}$$

for $k = 1, \ldots, N$. The matrix $\check{A}_{j^*,M+k}$ is the augmented matrix $\mathbf{A} = (\mathbf{R}, \mathbf{C})$ with omitted row j^* and column $M + k$, which in fact is the k-th column of $\mathbf{C}$. Now we insert (2.6.4) into (2.6.3)

$$\begin{aligned} \Phi_{j'j^*} \det \mathbf{A} &= (\Phi^*)_{j'} \det \mathbf{A} \\ &= (\mathbf{C} \cdot (-\mu^*))_{j'} \det \mathbf{A} \\ &= \sum_{k=1}^{N} c^k_{j'} \cdot (-\mu^*_k \det \mathbf{A}) \\ &= \sum_{k=1}^{N} (-1)^{M+k+j^*} c^k_{j'} \cdot \det \check{A}_{j^*,M+k} \\ &= \det A_{j'j^*}, \end{aligned} \tag{2.6.5}$$

where the matrix $A_{j'j^*}$ coincides with the augmented matrix $\mathbf{A}$ but with the replacement of the rows

$$\text{row } j^* : \quad \left(\begin{array}{ccc:ccc} 0 & \ldots & 0 & c^1_{j'} & \ldots & c^N_{j'} \end{array} \right); \tag{2.6.6}$$

$$\text{row } j' : \quad \left(\begin{array}{ccc:ccc} r_{j'1} & \ldots & r_{j'M} & 0 & \ldots & 0 \end{array} \right); \tag{2.6.7}$$

for $j' \neq j^*$. In (2.6.6) and (2.6.7) we separated the first M columns of $\mathbf{R} = (r_{jm})$ from the subsequent N columns of $\mathbf{C} = (c^k_j)$ in $\mathbf{A} = (\mathbf{R}, \mathbf{C})$. Let us provide some explanations for the calculation in (2.6.5). After replacing row j^* by (2.6.6), we interpreted the sum in the third line as an expansion of $\det \mathbf{A}$ with respect to row j^*. The next step is subtracting row j^* from row j' and afterwards replacing row j' with (2.6.7), which finally yields $\det A_{j'j^*}$. In the case, $j' = j^*$ the replacement (2.6.6) is sufficient to obtain $A_{j^*j^*}$.

Step 3: To prove the flux response theorem 2.1, we need to show the equivalence of the nonzero flux influence condition $\Phi_{j'j^*} \neq 0$ with the path conditions *(i) - (iv)*. We start with the only-if-direction, so let us suppose $\Phi_{j'j^*} \neq 0$. First thing we note is that (2.6.5), together with assumptions $\Phi_{j'j^*} \neq 0$ and $\det \mathbf{A} \neq 0$ implies $\det A_{j'j^*} \neq 0$. Therefore, we can proceed with our algebraic analysis of $\det A_{j'j^*} \neq 0$ analogously as in Lemma 2.4 regarding the analysis of $\det \mathbf{A} \neq 0$. On the existence of a child selection map $J : \mathbf{M} \to \mathbf{E}$, such that the mother map $\mathbf{m} : \mathbf{E} \to \mathbf{M} \cup \{0\}$ is a left inverse $\mathbf{m} \circ J = \mathrm{id}_{\mathbf{M}}$, we can insist. But we need 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 $\ker \mathbf{S}$, which is slightly more delicate due to the replacements (2.6.6) and (2.6.7) in $A_{j'j^*}$. As mentioned before, hence we need to consider two cases now, the general case $j' \neq j^*$ and the easy case $j' = j^*$, which we will see in Step 6.

Step 4: We start with general case $j' \neq j^*$. For a nonzero contribution $a_J r^J$ to $\det A_{j'j^*}$, the zero entries in (2.6.6) for the first M columns requires

$$j^* \notin J(\mathbf{M}). \tag{2.6.8}$$

On the other hand, the zero entries in (2.6.7) in the right part, for the subsequent N columns requires

$$j' \in J(\mathbf{M}), \tag{2.6.9}$$

and especially $m' := \mathbf{m}(j') \in \mathbf{M}$, thus $m' \neq 0$. However, the rest stays the same. The remaining rows $\mathbf{E} \setminus J(\mathbf{M})$ of $A_{j'j^*}$ correspond to the rows of $\mathbf{A}$ in

$$\begin{aligned} J^c &:= \mathbf{E} \setminus J', \quad \text{with} \\ J' &:= (J(\mathbf{M}) \setminus \{j'\}) \cup \{j^*\}. \end{aligned} \tag{2.6.10}$$

Since we modified matrix $A_{j'j^*}$, the child selection graph $\mathcal{T} := (\mathbf{M} \cup \{0\}, J(\mathbf{M}))$ is not necessarily a maximal spanning tree, as it had been for $\mathbf{A}$ before. But if we replace the child selection graph by

$$\mathcal{T}' := (\mathbf{M} \cup \{0\}, J'), \tag{2.6.11}$$

where J' excludes j' of $\mathcal{T}$, and instead takes j^* into account, then $\mathcal{T}'$ is a maximal spanning tree, since $\det A_{j'j^*} \neq 0$ algebraically. However, we lose the property that $\mathcal{T}'$ is defined by a child selection. Indeed, in case $m' = \mathbf{m}(j') \neq m^*$ with $m^* \neq 0$ $\mathcal{T}'$ possesses a single forward branch at the mother vertex $m^* = \mathbf{m}(j^*)$.

Step 5: We next construct the exit path γ^0 and the influence path γ'. For this purpose we use the edge directions in $\mathcal{T}'$ and consider the cases that $j' \neq j^*$ have either the same mother vertex $m^* = m' \neq 0$, the mother vertices are different and both not 0, i. e., $0 \neq m' \neq m^* \neq 0$, or they are different but one of them is the zero vertex, $m^* = 0 \neq m'$. If $j' \neq j^*$ have the same mother vertex $m^* = m' \neq 0$, then we choose the directed influence path γ' as the edge j' from vertex $m^* = m'$ to $\overline{m}' = \overline{\mathbf{m}}(j')$. The exit path γ^0 is the path from m^* along j^* to 0 procured in Lemma 2.4.

Then again, if the mother vertices m' and m^* are different, and both different from 0, i. e., $0 \neq m' \neq m^* \neq 0$, the maximal spanning tree $\mathcal{T}'$ is in possession of a single branch point at m^*. Since any other vertex $m \in \mathbf{M}$ of $\mathcal{T}'$ possesses a unique outgoing arrow $j = J(m)$, we can uniquely extend the two arising di-paths $\gamma^* \ni j^*$ and $\gamma^J \ni J(m^*) \neq j^*$, which emanate from m^*. We just extend forward by J in the acyclic tree $\mathcal{T}'$, as in $\mathcal{T}$, until they either hit 0 or m', and by acyclicity of $\mathcal{T}'$, the di-paths cannot return to themselves. After starting at m^*, for the same reason, the di-paths stay disjoint. By this means, only one of the di-paths leads to 0, which we will set as exit di-path γ^0. The other di-path, extended by j' but omitting $\overline{m}' = \overline{\mathbf{m}}(j')$, reaches m'. This is the influence di-path γ'.

For the last case, $m^* = 0 \neq m'$, we choose the exit di-path $\gamma^0 = \{m^*\}$ without any edge. We let the influence di-path γ' start at $m^* = 0$ along j^* in $\mathcal{T}'$, following the orientation of $\mathcal{T}$, J, as before. Hence this path cannot terminate at 0, or else it would create a cycle in $\mathcal{T}'$, which contradicts the acyclicity of $\mathcal{T}'$. Therefore it terminates at m' and we can append j' again.

Step 6: We now turn to the easy case $j' = j^*$, with (2.6.6). We know that the influence di-path γ' consists of the edge j^* with mother vertex m^*. Since $j^* \notin J(\mathbf{M})$ still holds, we have $\mathcal{T}' = \mathcal{T}$ and thus acyclicity of $\mathcal{T}$. To construct the exit di-path $\gamma^0 \not\ni j^*$, we follow the edge $J(m^*) \neq j^*$ emanating from m^* and leading along the orientation of $\mathcal{T}' = \mathcal{T}$. This way, we reach the 0-vertex and obtain the exit di-path γ^0. So we just proved the only-if-direction of Theorem 2.1.

Step 7: For the converse if-direction, i.e., the necessity of $\Phi_{j'j^*} \neq 0$, suppose the existence of two disjoint paths γ^0 from $m^* = \mathbf{m}(j^*)$ to 0, and γ' from m^* to j', such that properties *(i)* - *(iv)* of Theorem 2.1 all hold for both paths. We only concern the main case of j^* and j' with distinct nonzero mother vertices $m^* = \mathbf{m}(j^*) \neq \mathbf{m}(j')$. The other cases are straightforward, but lengthy.

By (2.6.5), if we show that $\det A_{j'j^*} \neq 0$ algebraically holds, this already implies $\Phi_{j'j^*} \neq 0$. So, equivalently, we also can construct a child selection map $J : \mathbf{M} \to \mathbf{E}$, which satisfies (2.6.8) and (2.6.9), such that $\mathcal{T}'$, defined as in (2.6.10), (2.6.11) becomes a maximal spanning tree of the network $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$.

Step 8: We start this construction by defining $J(m)$ to be the unique edge j in $\gamma^0 \cup \gamma'$ which emanated from the vertex m of $\gamma^0 \cup \gamma'$, except for vertex $\overline{m}' = \overline{\mathbf{m}}(j')$ of j' and the mother vertex $m^* = \mathbf{m}(j^*)$ of j^*. At m^* we have two edges, namely j^* and one other such edge. To define $J(m^*)$, we pick the other one. Thus, our construction satisfies $j^* \notin J(\mathbf{M})$ and $j' = J(m') \in J(\mathbf{M})$, as well as uniqueness by properties *(i)*, *(ii)* and *(iv)* of the di-paths γ^0 and γ'. Consider the remaining vertices $m \in \mathbf{M}$. To complete the construction of J, we invoke Lemma 2.4. Thereby we know that there exists a di-path γ^m from m to 0. By iteration on m, the di-path γ^m extends J to all previous vertices on γ^m. For this purpose we terminate the di-path γ^m as soon as it hits any vertex m_0 where J has already been constructed, at each step.

On the one hand, $\gamma^0 \cup (\gamma' \setminus \{j'\})$ is acyclic, by nonintersection property *(iv)* of γ' and assuming acyclicity before each induction step preserves acyclicity. On the other hand, the path γ^m is acyclic, because γ^m starts at m, outside the previous construction, and terminates on the first contact. Hence, it cannot create any new undirected cycle. Therefore, by construction of J is the resulting graph $\mathcal{T}'$ acyclic. Note that $\mathcal{T}'$ omits $E - M = N$ edges. But $\dim H_1 = N$ is the number of independent cycles. According to Proposition 2.2, the subgraph $\mathcal{T}'$ is therefore a maximal spanning tree. Finally, we can conclude that $\det \Phi_{j'j^*} = \det A_{j'j^*} \neq 0$ algebraically. This completes the proof of Theorem 2.1. $\square$

3

CONSTRAINTS ON FLUX SENSITIVITIES

In this chapter, we provide a detailed introduction to the approach to flux sensitivity analysis by Shinar, Mayo, Ji and Feinberg [29, 30]. Specifically, we look at their results on constraints on reciprocal flux sensitivities. In the first section we give basic definitions and concepts for chemical reaction networks. For a clear and precise comparison of the approaches in the next chapter, we want to stay consistent in the notation we use and therefore adopt the notation from Fiedler and Mochizuki, which we discussed in the previous chapter. However, we will make comments on the precise changes and also show the standard notation by Feinberg. We study the flux control coefficient and the concentration control coefficient, as well as the relation between them in the second section. In Section 3 we will state the main result on flux sensitivities, after looking at a fundamental assumption of the theorem, the injectivity property of reaction networks. We give a commented proof to the main result in the fourth section. The last section provides a computational method to determine the control coefficients.

3.1 BASIC DEFINITIONS FOR CHEMICAL REACTION NETWORKS

As already mentioned, we deviate from the standard notation by Feinberg and present this approach to flux sensitivity analysis in the notation from Chapter 2, by Fielder and Mochizuki. For a full introduction to the theory and notation by Feinberg see for example [7, 8].

When considering Feinberg's notation for the theory of chemical reaction networks, we need to change our point of view on how to set up a vector associated with the vertices or edges of a directed graph. Let $\mathscr{I}$ be a finite set, for example, of edges or vertices. For his theory it is useful to associate $\{x_i \in \mathbb{R} \mid i \in \mathscr{I}\}$ as the components of a vector x in the vector space of real-valued functions $\mathbb{R}^{\mathscr{I}}$, having domain $\mathscr{I}$. Thus for $x \in \mathbb{R}^{\mathscr{I}}$ and $i \in \mathscr{I}$, x_i is the value assigned to i by x. We denote the subset of $\mathbb{R}^{\mathscr{I}}$ which contains those functions that take only positive values as $\mathbb{R}_+^{\mathscr{I}}$, and for functions with nonnegative value as $\bar{\mathbb{R}}_+^{\mathscr{I}}$. The symbol $\ln z$, for $z \in \mathbb{R}_+^{\mathscr{I}}$ denotes the element of $\mathbb{R}^{\mathscr{I}}$ defined by

$$(\ln z)_i := \ln z_i, \quad \text{for all } i \in \mathscr{I}, \tag{3.1.1}$$

componentwise. In the same way we define $x \circ x'$ for $x, x' \in \mathbb{R}^{\mathscr{I}}$ as the elements of $\mathbb{R}^{\mathscr{I}}$ fulfilling

$$(x \circ x')_i := x_i x'_i, \quad \text{for all } i \in \mathscr{I}. \tag{3.1.2}$$

And for each $z \in \mathbb{R}_+^{\mathscr{I}}$, we denote with $\frac{1}{z}$ the elements of $\mathbb{R}_+^{\mathscr{I}}$ such that

$$\left(\frac{1}{z}\right)_i := \frac{1}{z_i}, \quad \text{for all } i \in \mathscr{I}. \tag{3.1.3}$$

Let $\mathscr{J}$ be a subset of $\mathscr{I}$. We denote the characteristic function on $\mathscr{J}$ as $\omega_{\mathscr{J}}$ and in particular if $\mathscr{J}$ is the singleton $\{j\} \subset \mathscr{I}$, we write for brevity ω_j instead of the formal $\omega_{\{j\}}$. Hence, the standard basis for $\mathbb{R}^{\mathscr{I}}$ is the set $\{\omega_i \in \mathbb{R}^{\mathscr{I}} \mid i \in \mathscr{I}\}$ and for each $x \in \mathbb{R}^{\mathscr{I}}$ the representation $x = \sum_{i \in \mathscr{I}} x_i \omega_i$ arises naturally. Let x and x' be elements of $\mathbb{R}^{\mathscr{I}}$, we define the standard scalar product in $\mathbb{R}^{\mathscr{I}}$ in this setting as

$$x \cdot x' = \sum_{i \in \mathscr{I}} x_i x'_i. \tag{3.1.4}$$

With respect to this scalar product, the standard basis of $\mathbb{R}^{\mathscr{I}}$ is orthonormal. Furthermore, we consider $\mathbb{R}^{\mathscr{I}}$ carrying the norm and topology derived from the standard scalar product.

In Feinberg's notation, a chemical reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ consists of three finite sets:

(i) a set $\mathscr{S}$ of distinct species of the network;

(ii) a set $\mathscr{C} \subset \bar{\mathbb{R}}_+^{\mathscr{S}}$ of distinct complexes of the network;

(iii) a set $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$ of distinct reactions, with the following properties:

  (a) $(y, y) \notin \mathscr{R}$ for any $y \in \mathscr{C}$;

  (b) for each $y \in \mathscr{C}$ there exists $y' \in \mathscr{C}$ such that $(y, y') \in \mathscr{R}$ or such that $(y', y) \in \mathscr{R}$.

We say that a complex y reacts to a different complex y', in symbols

$$y \longrightarrow y', \tag{3.1.5}$$

if the ordered pair (y, y') is a member of the reaction set $\mathscr{R}$. As before, we call the complex at the tail of a reaction arrow the reactant and the complex at the head of a reaction arrow the product. For more clearness, we will abbreviate ω_s for $s \in \mathscr{S}$ with s itself, and thus view $\mathbb{R}^{\mathscr{S}}$ as the vector space of all formal sums of species $s \in \mathscr{S}$. Consider for example two species A and B, which build the complex $y = \omega_A + 2\omega_B$, henceforward we denote this complex as $y = A + 2B$ and take it as member of $\mathbb{R}^{\mathscr{S}}$.

The stoichiometric coefficient is the nonnegative number $y_s \in \mathbb{R}_+$ of species that participate in a complex $y \in \mathbb{R}_+^{\mathscr{S}}$. In our example the stoichiometric coefficient of the species A is one and the stoichiometric coefficient of B is two.

A reaction diagram of a chemical reaction network is constructed such that each complex appears precisely once and a reaction from one complex to another is indicated by an arrow (3.1.5). The zero complex 0, in which all stoichiometric coefficients are zero, is a special complex that can appear in a reaction network. It acts as a tool to describe the input and the output of a chemical system. A detailed introduction into the use of the zero complex can be found in [7].

To adopt the notation by Fiedler and Mochizuki, there are just a few essential changes. The set $\mathscr{S}$ of distinct species s will be replaced by the set $\mathbf{M}$ of metabolites m. We write $M := |\mathbf{M}|$ for the total number of metabolites. Furthermore, we denote the unit vector $\omega_s \in \mathbb{R}^{\mathscr{S}}$ of any nonzero species s as the m-th unit vector $X_m \in \mathbb{R}^M$, and hence write $\mathbb{R}^M$ for the corresponding vector space instead of $\mathbb{R}^{\mathscr{S}}$. A complex $y \in \mathscr{C}$ is a linear combination of unit vectors X_m with stoichiometric coefficients y_m, so we denote the vector y as

$$y_1 X_1 + \cdots + y_M X_M = y \in \mathbb{R}^M. \tag{3.1.6}$$

The zero complex, which has $y_m = 0$ for all $m \in \mathbf{M}$, will eventually be denoted as $0 \in \mathbb{R}^M$. Note that the zero complex is distinct from the metabolites $m \in \mathbf{M}$. A reaction $y \longrightarrow y' \in \mathscr{R}$ will now be denoted as $j \in \mathbf{E}$, where $\mathbf{E}$ is the set of reactions with total number $E := |\mathbf{E}|$. Since each reaction $j \in \mathbf{E}$ of a network is represented by a arrow in the corresponding diagram, $\mathbf{E}$ may also be called the set of reaction arrows. The elements $j \in \mathbf{E}$ represent the reactions

$$y_1^j X_1 + \cdots + y_M^j X_M \longrightarrow \bar{y}_1^j X_1 + \cdots + \bar{y}_M^j X_M \,, \tag{3.1.7}$$

where $y^j, \bar{y}^j \in \mathbb{R}^M$ are suitable nonnegative real coefficient vectors with components y_m^j and $\bar{y}_m^j$, respectively. To address the tail of a reaction arrow $j \in \mathbf{E}$, i.e., the reactant complex we conventionally use $\mathbf{m}(j) = y^j$, and $\overline{\mathbf{m}}(j) = \bar{y}^j$ for the head of the arrow j, i. e., the product complex. In summary, we replace the notation of the chemical reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ by the sets $\mathbf{M}$ of metabolites m and $\mathbf{E}$ of reactions j.

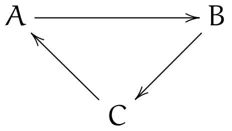
Henceforth, we state all definitions in the new setting. We say that a complex $y \in \mathbb{R}^M$ ultimately reacts to another complex $\bar{y} \in \mathbb{R}^M$ if one of the following conditions hold true:

(i) $y^j \longrightarrow \bar{y}^j$, i. e., $j \in \mathbf{E}$ exists;

(ii) there exists a sequence of complexes $y', y'', \dots, y^{(k)} \in \mathbb{R}^M$ such that

$$y \longrightarrow y' \longrightarrow y'' \longrightarrow \dots \longrightarrow y^{(k)} \longrightarrow \bar{y} \, . \tag{3.1.8}$$

In this sense, we call a reaction network weakly reversible if for each $y, \bar{y} \in \mathbb{R}^M$, y ultimately reacts to $\bar{y}$ implies that $\bar{y}$ ultimately reacts to y, and reversible if $\bar{y}^j \longrightarrow y^j$ whenever $y^j \longrightarrow \bar{y}^j$, $j \in \mathbf{E}$. Therefore, any reversible reaction network is also weakly reversible. For example consider the simple network



which is weakly reversible but not reversible. Note that a weakly reversible network possesses the property that any arrow resides in a cycle of arrows in the corresponding diagram.

The difference of product complex $\bar{y}^j \in \mathbb{R}^M$ and reactant complex $y^j \in \mathbb{R}^M$ of a reaction $j \in \mathbf{E}$ is called reaction vector, and is also an element in $\mathbb{R}^M$. The set of all reaction vectors is denoted as

$$\{\bar{y}^j - y^j \in \mathbb{R}^M \mid j \in \mathbf{E}\}. \tag{3.1.9}$$

The rank of the set of reaction vectors denotes also the rank of the reaction network itself. The linear subspace of $\mathbb{R}^M$ defined by the linear hull of all reaction vectors, i. e.,

$$S := \operatorname{span}\{\bar{y}^j - y^j \in \mathbb{R}^M \mid j \in \mathbf{E}\} \tag{3.1.10}$$

is called the stoichiometric subspace S of a reaction network. Notable is that the dimension of S coincides with the rank of the network.

We call the number of distinct parts of a reaction network, i. e., sets of complexes that are not linked by a reaction, the linkage class l of the network. This definition will be clear if we consider the following example:

$$A + B \longrightarrow C$$

$$\begin{tikzcd} D & E + F \arrow[l] \arrow[r, bend left] & G \arrow[l, bend left] \end{tikzcd}$$

Here the network consists of the two distinct complex sets $\{A + B, C\}$ and $\{D, E + F, G\}$ and thus has linkage class $l = 2$.

The deficiency of a network, introduced by Feinberg to classify reaction networks, is defined by

$$\delta_F := n - l - s, \tag{3.1.11}$$

where n is the number of complexes in the network, l the linkage class, and s the rank of the network. Note that the deficiency is an nonnegative integer. For a more detailed introduction see [7, 8].

A mixture state will conventionally be represented by a composition vector $x = (x_m)_{m \in \mathbf{M}} \in \bar{\mathbb{R}}_+^M$, with nonnegative components x_m understood as the molar concentrations for $m \in \mathbf{M}$. We say that a reaction network is a mass action system if we equip the reaction network with a positive element $k = (k_j)_{j \in \mathbf{E}} \in \mathbb{R}_+^E$. The components k_j are the rate constants for the reactions $j \in \mathbf{E}$. The ordinary differential equation of a mass action system is given as

$$\dot{x} = f(x, k), \tag{3.1.12}$$

where $f : \bar{\mathbb{R}}_+^M \times \mathbb{R}_+^E \to S$ is the mass action rate function, defined by

$$f(x, k) := \sum_{j \in \mathbf{E}} k_j x^{y^j} (\bar{y}^j - y^j) \tag{3.1.13}$$

with

$$x^{y^j} := \prod_{m \in \mathbf{M}} (x_m)^{y^j_m}. \tag{3.1.14}$$

The mass action rate function is determined by the network and in this sense a network attribute. Another point of view on the mass action rate function is to fix the reaction rate vector $k \in \mathbb{R}_+^E$. This induces a map $r : \bar{\mathbb{R}}_+^M \to S$ defined by

$$r(x) := f(x, k), \tag{3.1.15}$$

where r is called the species formation rate function.

By virtue of (3.1.12) - (3.1.14), we observe that in every point, $\dot{x}$ invariably lies in the stoichiometric subspace S of the corresponding reaction network. Therefore, if we consider a solution curve of ODE (3.1.12), the difference of any two compositions $x, x' \in \bar{\mathbb{R}}^M_+$ along these curve will remain in S. We say that two compositions x and x' in $\bar{\mathbb{R}}^M_+$ are stoichiometrically compatible if

$$x' - x \in S, \tag{3.1.16}$$

i. e., if composition x can follow x' along the same solution curve. Stoichiometric compatibility is an equivalence relation, which partitions $\bar{\mathbb{R}}^M_+$ into equivalence classes, called stoichiometric compatibility classes. For example, the stoichiometric compatibility class which contains the composition x is given by

$$(x + S) \cap \bar{\mathbb{R}}^M_+ = \{x' \in \bar{\mathbb{R}}^M_+ \mid x' - x \in S\}. \tag{3.1.17}$$

To check for stoichiometrically compatibility, we can use the following conservation condition. Let us define the orthogonal complement with respect to the standard scalar product in $\bar{\mathbb{R}}^M_+$ of the stoichiometric subspace S by $S^\perp$. Denote the dimension of $S^\perp$ as $\dim S^\perp = p$. If $\{O_1, \dots O_p\}$ is an arbitrary basis of $S^\perp$, we can use the conservation condition

$$O_i \cdot x = O_i \cdot x', \quad \text{for all} \quad i = 1, \dots, p \tag{3.1.18}$$

to verify that two compositions $x, x' \in \bar{\mathbb{R}}^M_+$ are stoichiometrically compatible.

A positive equilibrium of the network is a point $(x, k) \in \mathbb{R}^M_+ \times \mathbb{R}^E_+$, satisfying $f(x, k) = 0$. The set of all positive equilibria is denoted as

$$\mathcal{E} := \{(x, k) \in \mathbb{R}^M_+ \times \mathbb{R}^E_+ \mid f(x, k) = 0\}. \tag{3.1.19}$$

For a fixed composition $x^* \in \mathbb{R}^M_+$ we define a scalar product $*$ in $\mathbb{R}^M$ by

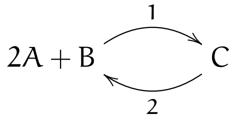
$$x * x' := x \cdot (\frac{1}{x^*} \circ x') \equiv \sum_{m \in \mathbf{M}} \frac{x_m x'_m}{x^*_m}. \tag{3.1.20}$$

This scalar product will be useful in the following definition of the linear transformation $T_{*\eta}$. Let S be the stoichiometric subspace of the reaction network and fix $x^* \in \mathbb{R}^M_+$, $\eta \in \mathbb{R}^E_+$. The linear map $T_{*\eta} : S \to S$ is defined by

$$T_{*\eta}\sigma := \sum_{j \in \mathbf{E}} \eta_j (y^j * \sigma)(\bar{y}^j - y^j), \tag{3.1.21}$$

where $*$ is the scalar product corresponding to x^* as defined in (3.1.20).

Before we close the section, let us provide an example to illustrate most of the definitions. Consider the reaction network



with metabolites $\mathbf{M} = \{A, B, C\}$ and edges $\mathbf{E} = \{1, 2\}$. The complexes of the network are $2A + B$ and C and the set of all reaction vectors is given by $\{2A + B - C, C - 2A - B\}$. The stoichiometric subspace for the network is the one dimensional subspace spanned by $2A + B - C$, and thus the rank of the network is one. Furthermore, the network is reversible and has just one linkage class, which contains all complexes. Hence the network has the deficiency $\delta_F = 2 - 1 - 1 = 0$. The corresponding ODE for the network, according to (3.1.12) - (3.1.14) is given by

$$\begin{aligned}
\dot{x}_A &= -2k_1 x_A^2 x_B + 2k_2 x_C, \\
\dot{x}_B &= -k_1 x_A^2 x_B + k_2 x_C, \\
\dot{x}_C &= k_1 x_A^2 x_B - k_2 x_C.
\end{aligned}$$

3.2 flux - and concentration control coefficients, and their relation

In this section, we establish the fundamental concepts of the flux control coefficients and the concentration control coefficients, as well as a relation between them. Beforehand, in order to define the control coefficients properly, we give conditions for the existence of local functions that map rate constant vectors to unique network equilibria. Furthermore, we provide some technical preliminaries, which are required for the main result in the next chapter.

Consider a mass action network with metabolites $m \in \mathbf{M}$ and reactions $j \in \mathbf{E}$ and let the positive state $(x^*, k^*) \in \mathbb{R}_+^M \times \mathbb{R}_+^E$ be fixed. We define the positive element $\kappa \in \mathbb{R}_+^E$ by

$$\kappa := \sum_{j \in \mathbf{E}} k_j^* (x^*)^{y^j} e_j, \tag{3.2.1}$$

where e_j denotes the j-th unit vector in $\mathbb{R}^E$ and y^j the reactant of the reaction j. Therefore, whenever the mass action system with rate constant vector k^* is observed at composition x^*, the flux through reaction $j \in \mathbf{E}$ is equal to κ_j.

Proposition 3.1 (Shinar, Mayo, Ji & Feinberg)**.** *Let* S *be the stoichiometric subspace of the mass action network with metabolite set* $\mathbf{M}$ *and edge set* $\mathbf{E}$*. Suppose that* $(x^*, k^*) \in \mathcal{E}$ *is a positive network equilibrium. If the map* $T_{*K} : S \to S$ *is nonsingular, then there exists an open set* $K \subset \mathbb{R}_+^E$ *containing* k^**, an open set* $C \subset (x^* + S) \cap \mathbb{R}_+^M$ *containing* x^**, and a unique differentiable function* $\hat{x} : K \to C$ *such that* $x^* = \hat{x}(k^*)$ *and*

$$(x, k) \in \mathcal{E} \cap (C \times K) \textit{ if, and only if, } x = \hat{x}(k). \tag{3.2.2}$$

By an open set $C \subset (x^* + S) \cap \mathbb{R}_+^M$ we mean open in the relative topology that $x^* + S$ inherits from $\mathbb{R}_+^M$.

Proof. We restrict the mass action function f to the domain $(x^* + S) \cap \mathbb{R}_+^M$ in $\mathbb{R}_+^M$, i. e., $\hat{f} : ((x^* + S) \cap \mathbb{R}_+^M) \times \mathbb{R}_+^E \to S$. The assumption $(x^*, k^*) \in \mathcal{E}$ implies

$$\hat{f}(x^*, k^*) = 0. \tag{3.2.3}$$

We can view $(x^* + S) \cap \mathbb{R}_+^M$, $\mathbb{R}_+^E$ and S as smooth manifolds, and note that the tangent subspace to $(x^* + S) \cap \mathbb{R}_+^M$ at x^* can be identified with S. We define the partial derivative $d_c\hat{f}(x^*, k^*) : S \to S$ by

$$d_c\hat{f}(x^*, k^*)\sigma = \left.\frac{d\hat{f}(x^* + \theta\sigma, k^*)}{d\theta}\right|_{\theta=0}, \quad \text{for all } \sigma \in S. \tag{3.2.4}$$

By direct calculation, we see that (3.2.4) is identical to T_{*K}. Since T_{*K} is nonsingular, by assumption, we can apply the implicit function theorem, which provides us with the existence of an open set $K \subset \mathbb{R}_+^E$ containing k^* and an open set $C \subset (x^* + S) \cap \mathbb{R}_+^M$ containing x^*. We also obtain a unique differentiable function $\hat{x} : K \to C$ such that $x^* = \hat{x}(k^*)$, and $(x, k) \in C \times K$, $\hat{f}(x, k) = 0$ if, and only if, $x = \hat{x}(k)$. In addition, we note that $(x, k) \in C \times K$ and $\hat{f}(x, k) = 0$ holds if, and only if, $(x, k) \in \mathcal{E} \cap (C \times K)$. This proves the proposition. □

Let the setting as in Proposition 3.1. We define the function $\bar{x} : \ln K \to C$ by

$$\bar{x}(\ln k) = \hat{x}(k). \tag{3.2.5}$$

Note that $\bar{x}$ is a composition of differentiable functions and thus is differentiable, too. Furthermore, by (3.2.2) and (3.2.5) we have

$$(x, k) \in \mathcal{E} \cap (C \times K) \text{ if, and only if, } x = \bar{x}(\ln k). \tag{3.2.6}$$

Another map that we shall interested in is $\bar{\mathscr{K}} : \ln K \to \mathbb{R}^{E}_{+}$, given by

$$\bar{\mathscr{K}}(\ln k) = \sum_{j \in \mathbf{E}} k_j (\bar{x}(\ln k))^{y^j} e_j. \tag{3.2.7}$$

$\bar{\mathscr{K}}$ is a differentiable function, because it can be expressed as a composition of differentiable functions. We want to emphasize that $\bar{\mathscr{K}}_j(\ln k)$ corresponds to the flux through reaction $j \in \mathbf{E}$ if the mass action system is at the positive equilibrium $\bar{x}(\ln k)$. Hence, by (3.2.1), we have the equivalence

$$\bar{\mathscr{K}}_j(\ln k^*) \equiv \kappa_j, \quad \text{for all } j \in \mathbf{E}. \tag{3.2.8}$$

Finally, we define the differentiable maps

$$\begin{aligned} \ln \bar{x} &: \ln K \to \mathbb{R}^{E} \quad \text{by} \quad \ln \bar{x}(\ln k) = \ln(\bar{x}(\ln k)), && (3.2.9)\\ \ln \bar{\mathscr{K}} &: \ln K \to \mathbb{R}^{E} \quad \text{by} \quad \ln \bar{\mathscr{K}}(\ln k) = \ln(\bar{\mathscr{K}}(\ln k)). && (3.2.10) \end{aligned}$$

Now we are able to give a precise definition of the control coefficients. Let $(x^*, k^*) \in \mathcal{E}$ be a fixed positive network equilibrium. Suppose that the map $T_{*\kappa} : S \to S$ is nonsingular. Then the concentration control coefficient for each $m \in \mathbf{M}$ and $j^* \in \mathbf{E}$ is the partial derivative

$$\frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*). \tag{3.2.11}$$

And for each ordered pair $(j', j^*) \in \mathbf{E} \times \mathbf{E}$ we denote the corresponding flux control coefficient by

$$\frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*). \tag{3.2.12}$$

Thus, the flux control coefficient indicates the fractional change in the flux through reaction $j' \in \mathbf{E}$ due to variation in the rate constant of reaction j^*, evaluated at k^*.

By direct computation (3.2.9) - (3.2.12) imply that for each $j^* \in \mathbf{E}$ the following equations hold true:

$$\begin{aligned} \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) &= y^{j^*} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) + 1 = \sum_{m \in \mathbf{M}} y^{j^*}_m \frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*) + 1,\\ \frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*) &= y^{j'} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) = \sum_{m \in \mathbf{M}} y^{j'}_m \frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*), \text{ for all } j' \in \mathbf{E} \setminus \{j^*\}. \end{aligned} \tag{3.2.13}$$

Relation (3.2.13) will be a crucial tool in the proof of the main result in Section 3.4.

The other main tool will be a intriguing relation between the control coefficients, which we are going to derive now. For a reaction network with positive equilibrium $(x^*, k^*) \in \mathcal{E}$ and nonsingular map $T_{*\kappa} : S \to S$, we combine (3.1.13), (3.1.19), (3.2.6), and (3.2.7) to obtain

$$\sum_{j \in \mathbf{E}} \bar{\mathscr{K}}_j(\ln k)(\bar{y}^j - y^j) = 0, \tag{3.2.14}$$

for every $k \in K$. Furthermore, we take the derivative of (3.2.14) with respect to each component of $\ln k$, and evaluate the result at $\ln k^*$. We note that

$$\frac{\partial \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j'}}(\ln k^*) = \kappa_{j^*} \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j'}}(\ln k^*), \tag{3.2.15}$$

for all $j', j^* \in \mathbf{E}$, and use the relation (3.2.13) to finally obtain

$$\begin{aligned} &\kappa_{j^*} \left(y^{j^*} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) + 1 \right) (\bar{y}^{j^*} - y^{j^*}) \\ &\qquad + \sum_{j' \in \mathbf{E} \setminus \{j^*\}} \kappa_{j'} \left(y^{j'} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) \right) (\bar{y}^{j'} - y^{j'}) = 0 \end{aligned} \tag{3.2.16}$$

for each $j' \in \mathbf{E}$.

3.3 CONSTRAINTS ON FLUX SENSITIVITIES FOR INJECTIVE NETWORKS

This section concerns itself with the main result of the approach, the constraints on reciprocal flux sensitivities in injective mass action networks. The necessary injectivity assumption is a network property, which we investigate beforehand.

Let us start with the bare definition of injectivity for chemical reaction networks.

Definition 3.2 (Injectivity property). *We call a network injective if for each* $k \in \mathbb{R}^E_+$ *and for each choice of distinct stoichiometrically compatible compositions* $x, x' \in \mathbb{R}^M_+$,

$$f(x, k) \neq f(x', k), \tag{3.3.1}$$

holds true.

Thus, injectivity is a network property that, regardless of the rate constants, precludes two different stoichiometrically compatible compositions to give rise to production rates that, metabolite-wise, are completely identical.

In other words, the network is not injective if, and only if, there exist $k \in \mathbb{R}_+^E$ and two different stoichiometrically compatible compositions $x, x' \in \mathbb{R}_+^M$ such that

$$f(x, k) = f(x', k). \tag{3.3.2}$$

So noninjectivity is a necessary condition for the existence of a mass action system which possesses multiple equilibria in the same positive stoichiometric compatibility class. Therefore, the concept of injectivity is closely related to the uniqueness of positive equilibria.

We next want to provide an equivalent definition of injectivity that makes use of the linear transformation $T_{*\eta}$, see (3.1.21). Let S be the stoichiometric subspace of the reaction network. The network is injective if, for every choice of $\eta \in \mathbb{R}_+^E$ and for every choice of $x^* \in \mathbb{R}_+^M$ the map $T_{*\eta} : S \to S$ is nonsingular.

To verify injectivity of a specific network is a more involved matter. One graph theoretic approach to ascertain injectivity in the case of fully open networks, in which for each metabolite m the degradation reaction $m \longrightarrow 0$ belongs to $\mathbf{E}$, utilizes the species reaction (SR) graph. For a detailed introduction see [28].

Furthermore, for an injective reaction network and a positive equilibrium $(x^*, k^*) \in \mathcal{E}$ we note that the element $\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*)$ lies in S for each $j \in \mathbf{E}$. Indeed, the image of $\bar{x} : \ln K \to C$ lies in $x^* + S$, implying for each $k \in K$ that the difference $\bar{x}(\ln k) - \bar{x}(\ln k^*)$ is contained in S. Thus, all partial derivatives of $\bar{x}$ must lie in S, too.

We are now ready to state the main result of the approach by Shinar, Mayo, Ji and Feinberg, the Reciprocity Theorem, which we will compare with the main result of the approach by Fiedler and Mochizuki in the next chapter.

Theorem 3.3 (Reciprocity Theorem; Shinar, Mayo, Ji & Feinberg)**.** *Consider an injective mass action network with metabolite set* **M** *and edge set* **E**. *At each positive network equilibrium* $(x^*, k^*) \in \mathcal{E}$ *the following relations hold:*

(i) For each reaction $j^* \in \mathbf{E}$,

$$0 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1. \tag{3.3.3}$$

(ii) For each pair of distinct reactions $j' \in \mathbf{E}$ *and* $j^* \in \mathbf{E}$,

$$-1 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1 \quad \textit{or} \quad -1 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j'}}(\ln k^*) \leqslant 1. \tag{3.3.4}$$

In other words, if we consider the flux sensitivity matrix for any positive network equilibrium of an injective mass action network, all of the diagonal elements are nonnegative and bounded from above by 1, and for each pair of reciprocal nondiagonal elements at least one entry lies between −1 and 1. Particularly in the nondiagonal case, this provides us with the information that one large entry, i. e., greater 1, implies that his reciprocal partner is small, i. e., less or equal to 1.

We want to emphasize again that the injectivity assumption is a condition imposed on the network structure alone, and thus the conclusions of the theorem will hold for each positive equilibrium. Also we will see an example in Section 3.5, which shows that the injectivity requirement is necessary and can not be weakened.

3.4 PROOF OF THE MAIN RESULT

Now we are going to prove the Reciprocity Theorem 3.3. The proof is divided into two propositions, one for each case *(i)* and *(ii)* of the Reciprocity Theorem. To prove the propositions we take advantage of (3.2.13) and (3.2.16), as well as a lemma, which we state beforehand. Finally, a corollary of the second proposition will complete the proof of Theorem 3.3.

Let **M** be the metabolite set and **E** the reaction set of an injective reaction network, with stoichiometric subspace S. Let the positive network equilibrium $(x^*, k^*) \in \mathcal{E}$ be fixed. Suppose that $\mathbf{E}'$ is a non-empty subset of **E**. For brevity we define the vector $\sigma_{\mathbf{E}'}$ by

$$\sigma_{\mathbf{E}'}(k^*) := \sum_{j \in \mathbf{E}'} \frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*). \tag{3.4.1}$$

Note that $\sigma_{\mathbf{E}'}(k^*)$ lies in S, since it is a sum of $\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*)$.

Lemma 3.4 (Shinar, Mayo, Ji & Feinberg)**.** *Let* $(x^*, k^*) \in \mathcal{E}$ *be a fixed, positive equilibrium of the injective mass action network with metabolite set* $\mathbf{M}$ *and reaction set* $\mathbf{E}$*. Suppose that* $\mathbf{E}'$ *is a non-empty subset of* $\mathbf{E}$*. Then there exists a reaction* $j^* \in \mathbf{E}'$ *such that*

$$-1 \leqslant y^{j^*} * \sigma_{\mathbf{E}'}(k^*) \leqslant 0. \tag{3.4.2}$$

Proof. By the injectivity assumption, we know that the map $T_{*\kappa}$ is nonsingular. Thus, we can use (3.2.16) for each $j^* \in \mathbf{E}'$ and obtain with (3.4.1)

$$\sum_{j \in \mathbf{E}'} \kappa_j (y^j * \sigma_{\mathbf{E}'}(k^*) + 1)(\bar{y}^j - y^j) + \sum_{j \in \mathbf{E} \setminus \mathbf{E}'} \kappa_j (y^j * \sigma_{\mathbf{E}'}(k^*))(\bar{y}^j - y^j) = 0. \tag{3.4.3}$$

Suppose that $(y^j * \sigma_{\mathbf{E}'}(k^*) + 1) \notin [-1, 0]$ for each $j \in \mathbf{E}'$. Then there exists a positive number ρ_j such that

$$y^j * \sigma_{\mathbf{E}'}(k^*) + 1 = \rho_j (y^j * \sigma_{\mathbf{E}'}(k^*)) \neq 0, \tag{3.4.4}$$

for each $j \in \mathbf{E}'$. Plugging (3.4.4) into (3.4.3) yields

$$\sum_{j \in \mathbf{E}'} (\kappa_j \rho_j)(y^j * \sigma_{\mathbf{E}'}(k^*))(\bar{y}^j - y^j) + \sum_{j \in \mathbf{E} \setminus \mathbf{E}'} \kappa_j (y^j * \sigma_{\mathbf{E}'}(k^*))(\bar{y}^j - y^j) = 0. \tag{3.4.5}$$

We define $\bar{\eta} \in \mathbb{R}^E_+$ by

$$\bar{\eta} := \sum_{j \in \mathbf{E}'} (\kappa_j \rho_j) e_j + \sum_{j \in \mathbf{E} \setminus \mathbf{E}'} \kappa_j e_j \tag{3.4.6}$$

where e_j is the j-th unit vector in $\mathbb{R}^E$. Combining (3.1.21), (3.4.5), and (3.4.6), provides us with

$$\sigma_{\mathbf{E}'}(k^*) \in \ker T_{*\bar{\eta}}. \tag{3.4.7}$$

Furthermore, by the inequality in (3.4.4) we know that $\sigma_{\mathbf{E}'}(k^*) \neq 0$, and thus the map $T_{*\bar{\eta}}$ is singular. But this contradicts the injectivity assumption. □

The following proposition shows the first part of the Reciprocity Theorem 3.3.

Proposition 3.5 (Shinar, Mayo, Ji & Feinberg)**.** *Let the setting as in Lemma 3.4. Then for each* $j^* \in \mathbf{E}$

$$0 \leqslant \frac{\partial \ln \mathscr{K}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1. \tag{3.4.8}$$

Proof. Fix the reaction $j^* \in \mathbf{E}$, and let $\mathbf{E}' = \{j^*\}$. By (3.4.1), we have

$$\sigma_{\mathbf{E}'}(k^*) = \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*). \tag{3.4.9}$$

Since Lemma 3.4 holds, we can plug the first equation of (3.2.13) and (3.4.9) into (3.4.2) to obtain (3.4.8), and prove the proposition. □

We next show the second property of Theorem 3.3 with the following proposition.

Proposition 3.6 (Shinar, Mayo, Ji & Feinberg)**.** *Let the setting as in Lemma 3.4. Suppose that* $\mathbf{E}'$ *is a subset of* $\mathbf{E}$ *containing at least two reactions. Then there exists a reaction* $j^* \in \mathbf{E}'$ *such that*

$$-1 \leqslant \sum_{j \in \mathbf{E}' \setminus \{j^*\}} \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_j}(\ln k^*) \leqslant 1. \tag{3.4.10}$$

Proof. According to Lemma 3.4 there exists a reaction $j^* \in \mathbf{E}'$ such that (3.4.2) holds. By plugging (3.4.1) into (3.4.2) we obtain

$$-1 \leqslant y^{j^*} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) + \sum_{j \in \mathbf{E}' \setminus \{j^*\}} y^{j^*} * \frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*) \leqslant 0. \tag{3.4.11}$$

If we further use (3.2.13), the equation (3.4.11) becomes

$$0 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) + \sum_{j \in \mathbf{E}' \setminus \{j^*\}} \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_j}(\ln k^*) \leqslant 1. \tag{3.4.12}$$

By Proposition 3.5 we can use (3.4.8) in addition to (3.4.12) to obtain (3.4.10), and close the proof of the proposition. □

Finally, the following corollary completes the proof of Theorem 3.3.

Corollary 3.7 (Shinar, Mayo, Ji & Feinberg)**.** *Let the setting as in Lemma 3.4. Then for each pair of distinct reactions* $j' \in \mathbf{E}$ *and* $j^* \in \mathbf{E}$*,*

$$-1 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j'}}(\ln k^*) \leqslant 1 \quad \textit{or} \quad -1 \leqslant \frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1. \tag{3.4.13}$$

Proof. Let $j' \in \mathbf{E}$ and $j^* \in \mathbf{E}$ be two distinct reactions, and let $\mathbf{E}' = \{j', j^*\}$. Applying Proposition 3.6 to $\mathbf{E}'$ shows the corollary. □

3.5 A METHOD TO COMPUTE THE CONTROL COEFFICIENTS

We provide a method to compute the control coefficients in this section and use it to give an example which shows that the injectivity requirement is essential. Through the whole section we consider a reaction network with metabolite set **M**, reaction set **E**, and stoichiometric subspace S. We further suppose that $(x^*, k^*) \in \mathcal{E}$ is a positive equilibrium of the network, and that the map $T_{*\kappa} : S \to S$ is nonsingular.

Since for each reaction $j \in \mathbf{E}$ the element $\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*)$ lies in S, we can rewrite (3.2.16) as follows

$$T_{*\kappa}\left(\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*)\right) = -\kappa_j(\bar{y}^j - y^j) \neq 0, \tag{3.5.1}$$

where the inequality holds, because $\kappa \in \mathbb{R}_+^{\mathbf{E}}$ is positive and by definition $\bar{y}^j - y^j \neq 0$ if $j \in \mathbf{E}$. The assumption that $T_{*\kappa}$ is nonsingular guarantees a unique solution for the vector $\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*)$. We can rewrite

$$\frac{\partial \ln \bar{x}_m}{\partial \ln k_j}(\ln k^*) = \frac{1}{x_m^*}\frac{\partial \bar{x}_m}{\partial \ln k_j}(\ln k^*), \tag{3.5.2}$$

for every $m \in \mathbf{M}$ and $j \in \mathbf{E}$. Therefore, the equations (3.5.1) and (3.5.2) uniquely determine the concentration control coefficient (3.2.11). By virtue of (3.2.13), we obtain the flux control coefficient, uniquely, as well.

For a computational method we recast (3.5.1) in a form that suits this purpose. Let $\{\sigma^1, \dots, \sigma^s\}$ be an orthonormal basis for S, with $s := \dim S$ and the standard scalar product in $\mathbb{R}^{\mathbf{M}}$. As abbreviation, we define for each $j \in \mathbf{E}$ and $i \in \{1, \dots, s\}$

$$a_i(j, k^*) := \sigma^i \cdot \frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*), \tag{3.5.3}$$

$$b_i(j) := \sigma^i \cdot (\bar{y}^j - y^j). \tag{3.5.4}$$

Furthermore, we can use the orthonormal basis $\{\sigma^1, \dots, \sigma^s\}$ to get a unique representation of the vectors $\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*) \in S$ and $\bar{y}^j - y^j \in S$ for each $j \in \mathbf{E}$ as

$$\frac{\partial \bar{x}}{\partial \ln k_j}(\ln k^*) = \sum_{l=1}^{s} a_l(j, k^*)\sigma^l, \tag{3.5.5}$$

$$\bar{y}^j - y^j = \sum_{k=1}^{s} b_k(j)\sigma^k. \tag{3.5.6}$$

Combining (3.1.21), (3.5.1) and the representations (3.5.5), (3.5.6) we obtain for each $j \in \mathbf{E}$ the linear system

$$A_{*\kappa} a(j, k^*) = -\kappa_j b(j), \tag{3.5.7}$$

where the real $s \times s$ matrix $A_{*\kappa}$ is defined by

$$(A_{*\kappa})_{kl} := \sum_{j \in \mathbf{E}} \kappa_j (\sigma^l * y^j) b_k(j). \tag{3.5.8}$$

The vectors $a(j, k^*)$ and $b(j)$ are defined for every $j \in \mathbf{E}$ in the obvious way:

$$a(j, k^*) := (a_1(j, k^*), \dots, a_s(j, k^*))^T, \tag{3.5.9}$$

$$b(j) := (b_1(j), \dots, b_s(j))^T. \tag{3.5.10}$$

We just derived a matrix representation $A_{*\kappa}$ of the nonsingular linear map $T_{*\kappa}$ regarding the basis $\{\sigma^1, \dots, \sigma^s\}$ of S. Therefore, $A_{*\kappa}$ is nonsingular too. Hence, the inverse $A_{*\kappa}^{-1}$ exists and we can solve the linear system (3.5.7) by multiplying with it. For each $j \in \mathbf{E}$ we obtain

$$a(j, k^*) = -\kappa_j A_{*\kappa}^{-1} b(j). \tag{3.5.11}$$

Pulling all the parts together, in particular (3.5.11), (3.5.2), (3.5.5), and (3.5.6), we obtain an expression that is able to compute the concentration control coefficient with input of the precise numerical data. For each $j \in \mathbf{E}$ the formula

$$\frac{\partial \ln \bar{x}_m}{\partial \ln k_j}(\ln k^*) = \frac{1}{x_m^*} \sum_{l=1}^{s} a_l(j, k^*)(\sigma^l)_m \tag{3.5.12}$$

holds. The flux control coefficient can be computed using (3.2.13) together with (3.5.12).

We next show an example of a simple mass action network, which can admit two positive equilibria in the same stoichiometric compatibility class for each choice of rate constants. In other words, we look at a reaction network that is not injective.

$$2A + B \xrightarrow{1} 3A$$

$$A \xrightarrow{2} B$$

The set of reaction vectors consists of the vectors $A - B$ and $B - A$, and thus the stoichiometric subspace S is one dimensional. We choose the single vector

$$\sigma^1 = \frac{1}{\sqrt{2}}(B - A)$$

as the basis orthonormal basis of S. For $b(j)$ we get according to (3.5.4) and (3.5.10)

$$\begin{aligned} b(1) &= -\sqrt{2}, \\ b(2) &= \sqrt{2}. \end{aligned}$$

Suppose that $(x^*, k^*) \in \mathcal{E}$ is a positive equilibrium of the network. The equilibrium flux κ_j corresponding to (x^*, k^*) through reaction j is given by $\kappa_j = k_j^*(x^*)^{y^j}$, and since $f(x^*, k^*) = 0$ holds for an equilibrium, we have that the equilibrium fluxes for both reactions are equal, i. e.,

$$\kappa_1 = \kappa_2.$$

We next calculate the $A_{*\kappa}$ matrix, which, by virtue of (3.5.7) and (3.5.8), consists only of one entry. We have

$$A_{*\kappa} = \kappa_2 \left(\frac{1}{x_A^*} - \frac{1}{x_B^*} \right).$$

In order to obtain an invertible $A_{*\kappa}$ matrix, we assume $x_A^* \neq x_B^*$. Inverting our matrix yields

$$A_{*\kappa}^{-1} = \frac{1}{\kappa_2 \left(\frac{1}{x_A^*} - \frac{1}{x_B^*} \right)}.$$

By (3.5.9) and (3.5.11), we have

$$\begin{aligned} a(1, k^*) &= \frac{\sqrt{2}}{\frac{1}{x_A^*} - \frac{1}{x_B^*}}, \\ a(2, k^*) &= \frac{-\sqrt{2}}{\frac{1}{x_A^*} - \frac{1}{x_B^*}}. \end{aligned}$$

Finally, we can use (3.5.12) to get the concentration control coefficients:

$$\begin{aligned}
\frac{\partial \ln \bar{x}_A}{\partial \ln k_1}(\ln k^*) &= \frac{1}{\frac{x_A^*}{x_B^*} - 1},\\
\frac{\partial \ln \bar{x}_B}{\partial \ln k_1}(\ln k^*) &= \frac{1}{\frac{x_B^*}{x_A^*} - 1},\\
\frac{\partial \ln \bar{x}_A}{\partial \ln k_2}(\ln k^*) &= \frac{-1}{\frac{x_A^*}{x_B^*} - 1},\\
\frac{\partial \ln \bar{x}_B}{\partial \ln k_2}(\ln k^*) &= \frac{-1}{\frac{x_B^*}{x_A^*} - 1}.
\end{aligned}$$

By (3.2.13), we can further compute the flux control coefficients as:

$$\begin{aligned}
\frac{\partial \ln \bar{\mathscr{K}}_1}{\partial \ln k_1}(\ln k^*) &= \frac{1}{\frac{x_A^*}{x_B^*} - 1},\\
\frac{\partial \ln \bar{\mathscr{K}}_1}{\partial \ln k_2}(\ln k^*) &= \frac{\frac{x_A^*}{x_B^*} - 2}{\frac{x_A^*}{x_B^*} - 1},\\
\frac{\partial \ln \bar{\mathscr{K}}_2}{\partial \ln k_1}(\ln k^*) &= \frac{1}{\frac{x_A^*}{x_B^*} - 1},\\
\frac{\partial \ln \bar{\mathscr{K}}_2}{\partial \ln k_2}(\ln k^*) &= \frac{\frac{x_A^*}{x_B^*} - 2}{\frac{x_A^*}{x_B^*} - 1}.
\end{aligned}$$

Let ε be positive and much smaller than 1, and suppose that $k_1^* = \frac{1}{1-\varepsilon}$, $k_2^* = 1$, $x_A^* = 1 - \varepsilon$, and $x_B^* = 1$. Hence, (x^*, k^*) is a positive equilibrium of our example. The flux control coefficients for this particular choice are

$$\begin{aligned}
\frac{\partial \ln \bar{\mathscr{K}}_1}{\partial \ln k_1}(\ln k^*) &= \frac{-1}{\varepsilon},\\
\frac{\partial \ln \bar{\mathscr{K}}_1}{\partial \ln k_2}(\ln k^*) &= \frac{1+\varepsilon}{\varepsilon},\\
\frac{\partial \ln \bar{\mathscr{K}}_2}{\partial \ln k_1}(\ln k^*) &= \frac{-1}{\varepsilon},\\
\frac{\partial \ln \bar{\mathscr{K}}_2}{\partial \ln k_2}(\ln k^*) &= \frac{1+\varepsilon}{\varepsilon}.
\end{aligned}$$

Both diagonal flux control coefficient are greater than 1, and thus violate the first part of the Reciprocity Theorem 3.3, to lie in the range $[0, 1]$. Furthermore, the reciprocal off-diagonal flux control coefficients lie outside the interval $[-1, 1]$, violating the second part of the theorem. Therefore, we see that the injectivity requirement of the Reciprocity Theorem is crucial and can not be omitted.

4

COMPARISON OF BOTH APPROACHES TO FLUX SENSITIVITY ANALYSIS

After the detailed introductions into the approaches to flux sensitivity analysis by Fiedler and Mochizuki [11, 22] in Chapter 2, and by Shinar, Mayo, Ji and Feinberg [29, 30] in Chapter 3, we are now ready to compare both theories. For this purpose we start by comparing basic definitions and similar elements of both approaches in the first section. This way, we also provide a short recall of the basic principles and ideas from the previous two chapters. The second section compares the assumptions on reaction networks, and also gives detailed explanations on the restrictions they impose. Thus, we will investigate the limitations and the scope of both approaches. Section 3 contributes insight in the construction of the flux sensitivities. Finally, we will compare the main results of both approaches to flux sensitivity analysis and give an example of a simple reaction network that we examine in detail to illustrate the theorems.

4.1 BASIC DEFINITIONS AND SIMILAR ELEMENTS OF BOTH APPROACHES

In this section we compare the basic principles and similar elements of both approaches. Thus, we also provide a short recall of the basic definitions. For a detailed introduction see Chapter 2 for the approach by Fiedler and Mochizuki, and Chapter 3 for the approach by Shinar, Mayo, Ji and Feinberg.

We start with the most basic definition, the chemical reaction network itself. A reaction network in Feinberg's notation consists of three finite sets: a set of species $\mathscr{S}$, a set of complexes $\mathscr{C} \subset \bar{\mathbb{R}}_+^{\mathscr{S}}$, and a set of reactions $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$. If the ordered pair $(y, \bar{y})$ is a member of the reaction set $\mathscr{R}$, we say that the complex y reacts to a different complex $\bar{y}$, in symbols $y \longrightarrow \bar{y}$. We can construct a reaction diagram of the network such that each complex appears precisely once and a reaction from one complex to another is indicated by an arrow. A special complex that may appear in the network is the zero complex, in which all stoichiometric coefficients are zero.

Fiedler and Mochizuki attempt the construction of the reaction diagram directly. They define the equivalent of the species set $\mathscr{S}$, the set of metabolites $m \in \mathbf{M}$ as the set of all vertices in the reaction network, excluding the zero vertex. The total number of metabolites is denoted as $M := |\mathbf{M}|$. Since the theory by Fiedler and Mochizuki only concerns monomolecular reaction networks, there are no complexes, other than the metabolites themselves. But a complex can easily be written as the linear combination of unit vectors $X_m \in \mathbb{R}^M$ with nonnegative stoichiometric coefficients y_m as $y_1 X_1 + \cdots + y_M X_M = y \in \mathbb{R}^M$. The set of all reactions $\mathscr{R}$ is represented by the edge set $\mathbf{E}$, since each reaction $j : \ m^j \longrightarrow \overline{m}^j$; $m^j, \overline{m}^j \in \mathbf{M} \cup \{0\}$ appears precisely once as a edge in the reaction diagram. The total number of edges is denoted as $E := |\mathbf{E}|$.

We can conclude that the graph representation of a monomolecular reaction network from the approach by Fiedler and Mochizuki coincides with the graphs on reaction complexes given by the theory from Feinberg, since each complex, respectively vertex, appears precisely once in the diagram and any ordered pair of them is connected by at most one arrow.

Furthermore, the view on the zero complex is basically the same. In both theories, the zero complex, respectively the zero vertex, appears in the diagram as a tool to describe feed reactions and exit reactions in the model of the chemical system. In fact, the zero vertex from the theory by Fiedler and Mochizuki is motivated by Feinberg's zero complex. However, there are differences in the implementation of the zero complex into the approaches. Feinberg just views the zero complex as a complex, in which all stoichiometric coefficients are zero, and thus as $0 \in \mathbb{R}^{\mathscr{S}}$, not extending the dimension of $\mathbb{R}^{\mathscr{S}}$. In contrast, Fielder and Mochizuki regard the zero vertex as a additional vertex, extending the metabolite set, i. e., $\mathbf{M} \cup \{0\}$, and therefore it also extends the dimension of $\mathbb{R}^M$ to $|\mathbf{M} \cup \{0\}| = M + 1$.

There are further similarities in the notation of the graph theoretical properties of reaction networks. The notion of a reversible reaction is the same in both approaches. But what Feinberg calls a weakly reversible network, is called strong connectivity by Fiedler and Mochizuki, i. e., a network in which for each reaction $y \longrightarrow \bar{y}$ there exits a sequence of reactions from $\bar{y}$ back to y, see (3.1.8).

The linear span of the reaction vectors, i. e., the vectors $\bar{y}^j - y^j \in \mathbb{R}^M$ for each $j \in \mathbf{E}$, is called stoichiometric subspace $S = \operatorname{span}\{\bar{y}^j - y^j \in \mathbb{R}^M \mid j \in \mathbf{E}\}$, see (3.1.10), by Feinberg. This subspace is equivalent to the image of the stoichiometric matrix $\mathbf{S} : \ \mathbb{R}^E \to \mathbb{R}^M$ from the theory by Fielder and Mochizuki, defined by $\mathbf{S} e_j = X_{\overline{\mathbf{m}}(j)} - X_{\mathbf{m}(j)}$, where e_j is the j-th unit vector in $\mathbb{R}^E$, see also (2.4.3). The equivalence becomes obvious, when we note that the stoichiometric matrix **S** maps every edge to the difference of product and reactant complex. Thus, its image is the subspace, spanned by those reaction vectors. Both definitions play an essential role in their corresponding approaches.

The monomolecular reaction networks, described by Fiedler and Mochizuki are a special case of deficiency zero networks, studied by Feinberg. By (3.1.11), we know that the deficiency of a network is defined as $\delta_F = n - l - s$, where n is the number of complexes, l the linkage class and s the rank of the network. In the language of Fiedler and Mochizuki we can formulate the deficiency as $\delta_F = \dim\ker\mathbf{S} - \dim\ker\mathbf{D}$, where **S** is the stoichiometric matrix and **D** the boundary map, given by (2.4.3) and (2.5.2), respectively. Proposition 2.2 shows that $\dim\ker\mathbf{S} = \dim\ker\mathbf{D}$, and therefore that $\delta_F = 0$.

We next turn our attention to the dynamics of the concentrations, i. e., the ODE's which both approaches provide. We will see that they are pretty similar with the exception of a few limitations. The ODE, which Fiedler and Mochizuki introduce, describes the dynamics of the vector $x = (x_m)_{m\in\mathbf{M}}$ of concentrations x_m of the metabolite $m \in \mathbf{M}$ by

$$\dot{x} = f(\mathbf{r}, x) := \sum_{j\in\mathbf{E}} r_j(x_{\mathbf{m}(j)})(X_{\overline{\mathbf{m}}(j)} - X_{\mathbf{m}(j)}), \tag{4.1.1}$$

where $X_0 := 0 \in \mathbb{R}^M$ and $x_0 := 1$, see (2.1.5). The reaction rate functions $\mathbf{r} = (r_j)_{j\in\mathbf{E}}$, which are the rates at which reaction j is active per time unit, are considered as given parameters. Note that $r_j(x_{\mathbf{m}(j)})$ may also be called the flux through reaction j. One fundamental assumption here is that the derivatives of the reaction rates $r_{jm} := r_j'(x_m^*)$ for $m = \mathbf{m}(j)$ at equilibrium x^* are viewed as abstract independent variables. However, this point of view excludes reaction networks taken with pure mass action kinetics. Further assumptions on the ODE are the positivity of reaction rate functions $r_j(\xi) > 0$ for $\xi > 0$, the existence of a positive steady state and the regularity at steady states x^*, $\det f_x(\mathbf{r}, x^*) \neq 0$. See also (2.2.1), (2.2.2), and (2.2.3). We postpone the discussion about the assumptions to the next section.

The approach by Shinar et al. assumes mass action kinetics, i. e., a reaction network equipped with a positive element $k = (k_j)_{j\in\mathbf{E}} \in \mathbb{R}_+^E$. The components k_j are the rate constants for the reactions $j \in \mathbf{E}$. In this setting, the ODE for the vector of concentrations $x = (x_m)_{m\in\mathbf{M}}$ is given by

$$\dot{x} = f(x, k) := \sum_{j\in\mathbf{E}} k_j x^{y^j}(\bar{y}^j - y^j) \tag{4.1.2}$$

with

$$x^{y^j} := \prod_{m \in \mathbf{M}} (x_m)^{y^j_m}. \tag{4.1.3}$$

See (3.1.12) - (3.1.14). We also assume the existence of a positive network equilibrium $(x^*, k^*) \in \mathbb{R}^M_+ \times \mathbb{R}^E_+$. Let such an equilibrium (x^*, k^*) be fixed, we defined the vector of fluxes in (3.2.1) as

$$\kappa = \sum_{j \in \mathbf{E}} k^*_j (x^*)^{y^j} e_j, \tag{4.1.4}$$

with unit vector $e_j \in \mathbb{R}^E$ and y^j as reactant of the reaction j. Thus, the flux through reaction $j \in \mathbf{E}$ is equal to κ_j, when the system with rate constant vector k^* is observed at x^*.

In comparison, the ODE's of both approaches are very similar. Both are constructed as the sum over all reactions of the product of the reaction kinetics and the reaction vector. The differences lie, on the one hand, in the restrictions on the reaction rates. Where Fielder and Mochizuki allow a wide class of reaction rate functions, with exception of pure mass action kinetics, the approach by Shinar et al. is limited to mass action kinetics. On the other hand, the reaction vectors show that the theory by Fielder and Mochizuki is valid for monomolecular reaction networks, only. In contrast, the theory by Shinar et al. has no such limitations. This is just a first glance on the assumptions and their restrictions, we will discuss them extensively in the next section.

4.2 ASSUMPTIONS AND THE RESTRICTIONS THEY IMPOSE

This section concerns the assumptions of both approaches, and the restrictions they impose. We investigate the assumptions on the ODE's, which we already saw in the last section, the assumptions on the network themselves, like the injectivity property or to be monomolecular, as well as the assumptions on the reaction kinetics. Surprisingly, we will also see that the regularity assumption on the reaction network by Fiedler and Mochizuki is kind of a weaker form of the injectivity assumption imposed by Shinar, Mayo, Ji and Feinberg.

See Table 1 for a brief overview of all assumptions and restrictions of the approach by Fiedler and Mochizuki, and the approach by Shinar, Mayo, Ji and Feinberg in contrast to the corresponding assumption from the other theory.

Assumptions and restrictions of the approach by	
Fiedler and Mochizuki	Shinar et al.
• monomolecular reactions	• multimolecular reactions
• general class of reaction kinetics, except for mass action kinetics	• mass action kinetics
• regularity of the network	• injectivity of the network
• positivity of reaction rates	• positivity of reaction rates
• existence of positive steady states	• existence of positive steady states

Table 1: All assumptions and restrictions of both approaches briefly summarized and in contrast to the corresponding assumption from the other theory.

We start our investigation of the assumptions with the fact that the approach by Fielder and Mochizuki is at present limited to monomolecular reaction networks and therefore only offers a first step towards understanding the sensitivity behaviour of chemical reaction networks. In a very recent advance Brehm and Fiedler [3] were able to enhance the theory for regular multimolecular systems. The approach by Shinar et al. does not have such limitations, it is valid for multimolecular reaction networks.

On the other hand, the approach by Shinar et al. also has a fundamental limitation regarding the variety of reaction rate kinetics that can be chosen. The reaction rate functions are restricted to mass action kinetics

$$r_j(x) = k_j x^{y^j} = k_j \prod_{m \in \mathbf{M}} (x_m)^{y^j_m} \tag{4.2.1}$$

as we can see in the ODE (4.1.2), (4.2.3).

In contrast, the approach by Fiedler and Mochizuki allows a very general class of reaction rate functions $r_j \in C^1$, except for pure mass action kinetics. This restriction on the kinetics originates in the fundamental idea that the derivatives of the reaction rate functions r_{jm} are viewed as abstract independent variables. The derivatives participate in the construction of the augmented matrix $\mathbf{A}$, see (2.4.7), via certain rational expressions, as we saw explicitly in the proof of Lemma 2.4. However, we require the derivative variables r_{jm} to be independent of the equilibrium flux values $r_j(x^*_m)$. But if the class of nonlinearities r_j itself solves the same ODE $r'_j = h_j(r_j)$ for some fixed function h_j, this independence evidently fails.

Thus, the algebraic independence of r_j and r_j' requires at least a two parameter family of functions r_j. So let us consider mass action kinetics like in (4.2.1). The partial derivatives of the reaction rate functions $r_j(x)$ are given by

$$r_{jm} = \frac{\partial}{\partial x_m} r_j(x^*) = \frac{y^j_m r_j}{x_m}. \tag{4.2.2}$$

Obviously we are in the case described above, and therefore need to exclude pure mass action kinetics. But already classes like Michaelis-Menten and Langmuir-Hinshelwood kinetics justify the algebraic independence.

We next regard the assumptions on the ODE's of both approaches. We already saw the assumptions (2.2.1), (2.2.2) and (2.2.3) imposed by Fiedler and Mochizuki on the ODE (4.1.1) in the previous section. Let us provide an overview:

- positivity of reaction rates $\quad r_j(\xi) > 0 \quad \text{for} \quad \xi > 0;$ (4.2.3)
- existence of poisitive steady state $\quad x^* > 0 \quad \text{with} \quad f(\mathbf{r}, x^*) = 0;$ (4.2.4)
- regularity at steady states x^* $\quad \det f_x(\mathbf{r}, x^*) \neq 0.$ (4.2.5)

Note that they do not require positivity of the derivatives r_j' of the reaction rates. In practise, the consequences of the positivity assumption (4.2.3) and the positivity of the steady state in (4.2.4) are just that we omit vanishing reactions and steady states with zero components of x^*, respectively. The assumption (4.2.5) on the reaction network to be regular at steady states plays a crucial role in the derivation of the concentration response $\delta x^{j^*}_m$, for a metabolite m. It enables us to use the implicit function theorem for studying the steady state response to any perturbation of the reaction rate r_{j^*} for any reaction j^*. See also (2.2.4) - (2.2.8).

The first two assumptions (4.2.3) and (4.2.4) are present in the approach by Shinar et al., too. They assume the existence of positive network equilibria, see (3.1.19) and implicitly also the positivity of reaction rates, since they consider mass action kinetics, which uses a positive element $k = (k_j)_{j \in \mathbf{E}} \in \mathbb{R}^{\mathbf{E}}_+$, only. Instead of regularity, here we assume the reaction network to be injective, see (3.3.1).

Injectivity is a network property that requires for each $k \in \mathbb{R}^{\mathbf{E}}_+$ and for each choice of distinct stoichiometrically compatible compositions $x, x' \in \mathbb{R}^{\mathbf{M}}_+$,

$$f(x, k) \neq f(x', k), \tag{4.2.6}$$

where f is defined as in (4.1.2). Equivalently, we can assume the linear map $T_{*\eta} : S \to S$, defined by (3.1.21)

$$T_{*\eta}\sigma := \sum_{j \in \mathbf{E}} \eta_j (y^j * \sigma)(\bar{y}^j - y^j) \tag{4.2.7}$$

to be nonsingular for every choice of $\eta \in \mathbb{R}^E_+$ and $x^* \in \mathbb{R}^M_+$. S is the stoichiometric subspace and the scalar product $*$ corresponding to x^* is defined as in (3.1.20). In other words, injectivity, regardless of the rate constants, precludes two different stoichiometrically compatible compositions to give rise to production rates that, metabolite-wise, are completely identical. Thus, injectivity is closely related to the concept of uniqueness of positive equilibria. Indeed, the network is not injective if, and only if, there exists $k \in \mathbb{R}^E_+$ and two different stoichiometrically compatible compositions $x, x' \in \mathbb{R}^M_+$ such that

$$f(x, k) = f(x', k). \tag{4.2.8}$$

Hence, noninjectivity is a necessary condition for the existence of a mass action system, which possesses multiple equilibria in the same positive stoichiometric compatibility class.

The injectivity assumption, i. e., the nonsingularity of the map $T_{*\kappa}$, is essential for the proof of Proposition 3.1, which guarantees the existence of local functions that map rate constant vectors to unique network equilibria. Main step in the proof is to identify the partial derivative of the mass action rate function f with the map $T_{*\kappa}$, see (3.2.4). Since, by assumption, $T_{*\kappa}$ is nonsingular, we can use the implicit function theorem to obtain the existence of the local functions we are looking for. These local functions will play a crucial role in the construction of the concentration control coefficient $\frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*)$, see (3.2.11). Furthermore, the nonsingularity of $T_{*\kappa}$ is utilized in the proof of Lemma 3.4.

Therefore, it seems that the regularity assumption (4.2.5) by Fiedler and Mochizuki, and the injectivity assumption (4.2.6) by Shinar et al. are not as far apart as one would believe on the first glance. In fact, if we forget for a moment about the restrictions on the reaction rate functions in both approaches, we claim that injectivity implies the regularity assumption.

Observation 4.1. *Consider the illegal setting which allows the ODE vector field* f *in* (2.1.5)*, respectively* (3.1.13) *to take any reaction rate functions* r_j*. We claim that the injectivity assumption* (4.2.6) *by Shinar et al. implies the regularity assumption* (4.2.5) *by Fiedler and Mochizuki.*

Indeed, just for a moment, let us allow the ODE vector field f in (2.1.5), respectively (3.1.13) to take any reaction rate functions r_j. We show in this setting that a violation of the regularity assumption implies noninjectivity of the network. Let

$\mathbf{M}$ be the metabolite set and $\mathbf{E}$ be the reaction set of a reaction network with stoichiometric subspace S, and let $\kappa \in \mathbb{R}^{\mathbf{E}}_{+}$. Suppose there exists a positive steady state $x^* \in \mathbb{R}^{\mathbf{M}}_{+}$ such that $\det f_x(\mathbf{r}, x^*) = 0$, i. e., the regularity assumption (4.2.5) is violated. This essentially means that the Jacobian $f_x(\mathbf{r}, x^*)$ of the partial derivatives of f with respect to x evaluated at the steady state x^* is singular. On the other hand, we know that the network is injective, if for every choice of $\kappa \in \mathbb{R}^{\mathbf{E}}_{+}$ and every choice of $x^* \in \mathbb{R}^{\mathbf{M}}_{+}$, the map $T_{*\kappa}$ is nonsingular. By direct calculation we get

$$\left(\frac{\partial f(x,k)}{\partial x}\right)\bigg|_{x=x^*}(\sigma) = \sum_{j\in\mathbf{E}} \kappa_j (y^j * \sigma)(\bar{y}^j - y^j) = T_{*\kappa}\sigma, \tag{4.2.9}$$

also see [9]. Therefore, when ignoring the restrictions on the reaction rate functions, we see that the Jacobian of f with respect to x evaluated at x^* is identical to the linear map $T_{*\kappa}$. Since the Jacobian is singular at x^*, $T_{*\kappa}$ is singular at x^*, too. Hence, by definition of the injectivity property, the reaction network is not injective. Taking the contraposition proves our claim.

Another interesting observation in this illegal setting, in which we forget about the restrictions on the rate functions, is that a singular augmented matrix $\mathbf{A}$, see (2.4.7), for the corresponding network implies noninjectivity. This therefore yields a fairly easy first way to verify noninjectivity, which is not a straightforward matter. Indeed, by Proposition 2.3 and equation (2.5.1) is a singular augmented matrix $\mathbf{A}$ equivalent to a violation of the regularity assumption. We can also check for noninjectivity, in virtue of Lemma 2.4 by just finding a vertex in the reaction diagram $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ that does not possess a directed exit path γ^0 to 0. Thus we have a tool which allows us to check for noninjectivity by examining the graph structure of reaction network, only.

Observation 4.2. *In the illegal setting, in which we forget about the restrictions on the reaction rate functions, we have two simple tools to verify noninjectivity that are equivalent:*

(i) Set up the augmented matrix $\mathbf{A}$ *and show that it is singular.*

(ii) Find a vertex in the reaction network diagram $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$ *without directed exit path to the zero vertex.*

However, keep in mind that this proof is not legal, since the mutually exclusive restrictions on the reaction rate functions forbid the comparison of the ODE vector fields f. We just want to underline the claim that the injectivity assumption and the regularity at a steady state are very similar to each other.

In summary, both approaches are complementary regarding their assumptions about reactions and the variety of permissible reaction rate functions. But they are also similar in their positivity assumption on the reaction rates and the existence of positive steady states. Surprisingly, also the regularity assumption by Fiedler and Mochizuki and the assumption on the reaction network to be injective by Shinar et al. are very close to each other.

In addition, we note that all assumptions are imposed on the structure of the network, only, except for the restrictions on the reaction rate functions. But Fiedler and Mochizuki allow a generic class of rate functions and hence this limitation is negligible. Albeit the restriction to mass action kinetics by Shinar et al. is a significant limitation, this kinetics is widely spread in application. Furthermore, in both of the theories, no numerical data is required in order to utilize their main results. In this sense, both approaches rely only on the underlying network structure.

4.3 COMPARISON OF THE FLUX SENSITIVITIES AND THE MAIN RESULTS

We finally turn our attention to the construction of the flux sensitivities, and also want to compare the main result of both approaches. For illustration and a clear insight into the main results, we apply both theorems to a simple example and provide a detailed discussion.

Regarding the construction of the flux sensitivities, Fiedler and Mochizuki define the flux response $\Phi_{j'j^*}$ of the flux through reaction j' due to a rate perturbation of reaction j^* as follows:

$$\Phi_{j'j^*} := \delta_{j'j^*} + r_{j'\mathbf{m}(j')}\delta x^{j^*}_{\mathbf{m}(j')}, \tag{4.3.1}$$

see also (2.3.1). The Kronecker-delta in the first term accounts for the explicit flux change due to the external perturbation at j^* in reaction $j' = j^*$. The implicit flux change in any reaction j', in the second term, originates due to the concentration response $\delta x^{j^*}_{\mathbf{m}(j')}$ of the mother reactant $\mathbf{m}(j')$, at the steady state x^* caused by the external perturbation at reaction j^*. The flux sensitivity matrix is defined by

$$\Phi := \left(\Phi_{j'j^*}\right)_{j',j^*\in\mathbf{E}}, \tag{4.3.2}$$

see (2.4.1).

Shinar, Mayo, Ji and Feinberg denote the flux control coefficient at the positive network equilibrium $(x^*, k^*) \in \mathcal{E}$ for each ordered pair $(j', j^*) \in \mathbf{E} \times \mathbf{E}$ as the partial derivative $\frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*)$. Thus, the flux control coefficient is the fractional change in the flux through reaction j' caused by variation of the rate constants of reaction j^*, evaluated at k^*. They further provide a relation with the concentration control coefficient $\frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*)$ for two cases, for the diagonal entries of the flux sensitivity matrix and the off-diagonal elements:

$$\begin{aligned} \frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) &= y^{j^*} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) + 1 = \sum_{m \in \mathbf{M}} y_m^{j^*} \frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*) + 1, \\ \frac{\partial \ln \bar{\mathscr{K}}_{j'}}{\partial \ln k_{j^*}}(\ln k^*) &= y^{j'} * \frac{\partial \bar{x}}{\partial \ln k_{j^*}}(\ln k^*) = \sum_{m \in \mathbf{M}} y_m^{j'} \frac{\partial \ln \bar{x}_m}{\partial \ln k_{j^*}}(\ln k^*), \text{ for all } j' \in \mathbf{E} \setminus \{j^*\}, \end{aligned} \tag{4.3.3}$$

for each $j^* \in \mathbf{E}$. Also see (3.2.11) - (3.2.13).

The constructions of the flux sensitivities are very similar, both are essentially made of the concentration response, respectively concentration control coefficient, and account the diagonal elements of the flux sensitivity matrix with a additional plus one.

Since we want to compare the main results of both approaches, the question whether the flux sensitivities are comparable arises. By the construction of the flux response (4.3.1) and the flux control coefficient (4.3.3), which both add a 1 to the diagonal entries of their flux sensitivity matrix, and the examples we saw in Section 2.4 and Section 3.5, respectively, we can conclude that both flux sensitivities lie in the same order of magnitude. To further illustrate that the flux sensitivities also have the same scaling behaviour, we derive the flux sensitivity matrices from both approaches for a simple network in which the flux in the system only changes if the input, i. e., the feed reaction 1 is perturbed. We expect both matrices to show the same behaviour among their entries.

$$0 \xrightarrow{1} A \xrightarrow{2} B \xrightarrow{3} 0 \tag{4.3.4}$$

The example in (4.3.4) is just another variant of the single path way, with two metabolites $\mathbf{M} = \{A, B\}$ and three reactions $\mathbf{E} = \{1, 2, 3\}$. According to the method we explained in Section 3.5, we first calculate the flux sensitivity matrix made of flux control coefficients from the approach by Shinar et al.

The set of reaction vectors of (4.3.4) is $\{A, -B, B-A\}$. Since A and $-B$ are linearly independent, we already found an orthonormal basis

$$\{\sigma^1 = A, \sigma^2 = -B\}$$

of the two dimensional stoichiometric subspace S. We next determine the vectors $b(j) = (b_1(j), b_2(j))^T$ with help of equation $b_i(j) = \sigma^i \cdot (\bar{y}^j - y^j)$, see (3.5.4), for $j \in \mathbf{E}$ and $i = 1, 2$ as

$$b(1) = \begin{pmatrix} 1 \\ 0 \end{pmatrix}, \qquad b(2) = \begin{pmatrix} -1 \\ -1 \end{pmatrix}, \qquad b(3) = \begin{pmatrix} 0 \\ 1 \end{pmatrix}.$$

Let $(x^*, k^*) \in \mathcal{E}$ be a positive equilibrium of the network. Using $f(x^*, k^*) = 0$, we find that the equilibrium fluxes $\kappa_j = k_j^*(x^*)^{y^j}$ through reaction j corresponding to (x^*, k^*) for all three reactions are equal, i. e., we find the relation

$$\kappa_1 = \kappa_2 = \kappa_3.$$

To set up the 2×2 matrix $A_{*\kappa}$, which is the matrix representation of the linear map $T_{*\kappa}$, we use $(A_{*\kappa})_{kl} = \sum_{j \in \mathbf{E}} \kappa_j (\sigma^l * y^j) b_k(j)$, see (3.5.8), and get

$$A_{*\kappa} = \begin{pmatrix} -\frac{\kappa_1}{x_A^*} & 0 \\ -\frac{\kappa_1}{x_A^*} & -\frac{\kappa_1}{x_B^*} \end{pmatrix}.$$

Since the reaction network is injective, we know that $T_{*\kappa}$ is nonsingular, and thus $A_{*\kappa}$ is nonsingular, too. Therefore, the inverse of $A_{*\kappa}$ exists and is given by

$$A_{*\kappa}^{-1} = \begin{pmatrix} -\frac{x_A^*}{\kappa_1} & 0 \\ \frac{x_B^*}{\kappa_1} & -\frac{x_B^*}{\kappa_1} \end{pmatrix}.$$

We can use the inverse $A_{*\kappa}^{-1}$ to solve the linear system $A_{*\kappa} a(j, k^*) = -\kappa_j b(j)$, where $a(j, k^*)$ is defined by (3.5.3) and helps us to determine the concentration control coefficients. According to $a(j, k^*) = -\kappa_j A_{*\kappa}^{-1} b(j)$, we get for $j \in \mathbf{E}$

$$a(1, k^*) = \begin{pmatrix} x_A^* \\ -x_B^* \end{pmatrix}, \qquad a(2, k^*) = \begin{pmatrix} -x_A^* \\ 0 \end{pmatrix}, \qquad a(3, k^*) = \begin{pmatrix} 0 \\ x_B^* \end{pmatrix}.$$

The concentration control coefficients can be determined by the formula

$$\frac{\partial \ln \bar{x}_m}{\partial \ln k_j}(\ln k^*) = \frac{1}{x_m^*} \sum_{l=1}^{2} a_l(j, k^*)(\sigma^l)_m,$$

and further we use (4.3.3) to calculate the flux control coefficients. Arranged as matrix, we get the flux sensitivity matrix to our example by the approach from Shinar et al. as

$$\left(\frac{\partial \ln \bar{\mathscr{K}}_{j^*}}{\partial \ln k_{j'}}(\ln k^*)\right)_{j',j^* \in \mathbf{E}} = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 0 & 0 \end{pmatrix}. \tag{4.3.5}$$

On the other hand, we already know the flux sensitivity matrix of our single path way example (4.3.4) derived by the theory from Fiedler and Mochizuki from Section 2.4. The matrix is given by

$$\Phi = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 0 & 0 \end{pmatrix}. \tag{4.3.6}$$

Comparing both matrices (4.3.5) and (4.3.6) for the single path way (4.3.4) shows that they are identical. This means that their flux sensitivities behave in the same way for this simple network, especially they show the same scaling behaviour. Therefore, we can conclude that the flux sensitivities of both approaches are comparable. Note that in general the theory by Shinar, Mayo, Ji and Feinberg is not able to give such precise matrix entries without numerical input and, in particular, it is not able to predict which matrix entries are zero, and which are not.

Before we compare the main results with the help of an example, let us repeat both theorems as a reminder. The main result by Fiedler and Mochizuki, Theorem 2.1 on structural sensitivity analysis of flux influences basically states that an external perturbation at reaction j^* either propagates downward along a directed path γ' starting with vertex $\mathbf{m}(j^*) = m^*$ and edge j^*, or else spills over to a side branch γ' from $j^* \in \gamma^0 \backslash \gamma'$.

Theorem 2.1. (Fiedler & Mochizuki). *Let positivity and existence assumptions* (4.2.3), (4.2.4) *hold for the monomolecular reaction network (2.1.1), (2.1.2) and (2.1.5). Moreover assume the Jacobian determinant in* (4.2.5) *is nonzero, algebraically. 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 (2.2.4) - (2.2.7) of reaction* j^* *satisfies*

$$\Phi_{j'j^*} \neq 0 \tag{4.3.7}$$

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* $j' : \ m' \longrightarrow \overline{m}'$ *, but omitting the product 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 of the conditions on the directed paths γ^0 and γ' see Figure 2.3.1. Basically, the conditions on γ' describe some domain of influence of the perturbation j^*. The conditions on the exit path γ^0, however, are less intuitive. All conditions are based on the stoichiometric graph structure only, and in that sense the approach is function-free.

The main result of the approach by Shinar, Mayo, Ji and Feinberg is the Reciprocity Theorem 3.3. For all injective mass action networks the theorem provides constraints on the diagonal entries of the flux sensitivity matrix to be nonnegative and bounded from above by 1. Furthermore, for each pair of reciprocal off-diagonal entries of the matrix, at least one element lies between -1 and 1. These conclusions hold for any positive network equilibrium, since the injectivity assumption is a condition imposed on the network structure alone.

Theorem 3.3. (Reciprocity Theorem; Shinar, Mayo, Ji & Feinberg). *Consider an injective mass action network with metabolite set* $\mathbf{M}$ *and edge set* $\mathbf{E}$*. At each positive network equilibrium* $(x^*, k^*) \in \mathcal{E}$ *the following relations hold:*

(i) For each reaction $j^* \in \mathbf{E}$*,*

$$0 \leqslant \frac{\partial \ln \mathscr{K}_{j^*}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1. \tag{4.3.8}$$

(ii) For each pair of distinct reactions $j' \in \mathbf{E}$ *and* $j^* \in \mathbf{E}$*,*

$$-1 \leqslant \frac{\partial \ln \mathscr{K}_{j'}}{\partial \ln k_{j^*}}(\ln k^*) \leqslant 1 \quad \text{or} \quad -1 \leqslant \frac{\partial \ln \mathscr{K}_{j^*}}{\partial \ln k_{j'}}(\ln k^*) \leqslant 1. \tag{4.3.9}$$

When comparing the results, we see that the structural sensitivity analysis by Fiedler and Mochizuki provides us with a framework, which is able to derive

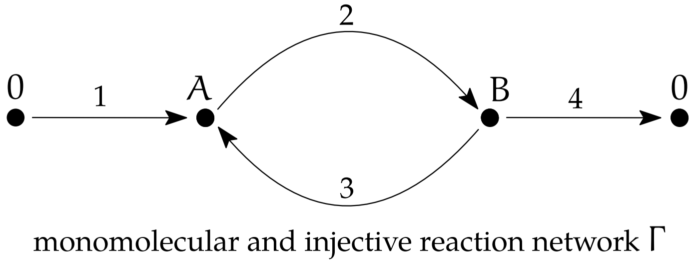


Figure 4.3.1: A reversible chemical reaction network containing two metabolites $\mathbf{M} = \{A, B\}$ and four reactions $\mathbf{E} = \{1, 2, 3, 4\}$, which possesses the injectivity property, fulfills the regularity condition, and is monomolecular.

zero and nonzero flux changes, i. e., to determine zero and nonzero entries of the flux sensitivity matrix. In contrast, the approach by Shinar et al. gives a priori bounds on the diagonal elements of the flux sensitivity matrix to lie between 0 and 1, in addition to constraints on the off-diagonal elements that at least one entry of a reciprocal pair lies in $[-1, 1]$, for any positive network equilibrium. Another interesting fact is that the theory by Fiedler and Mochizuki extracts its conclusions predominantly from the graph structure of the network, where on the contrary the approach by Shinar et al. is a purely analytical one.

To illustrate the theorems and gain a clear insight into the main results, we apply both theorems to the innocent chemical reaction network given in Figure 4.3.1. The monomolecular reaction network consists of two metabolites $\mathbf{M} = \{A, B\}$ and four reactions $\mathbf{E} = \{1, 2, 3, 4\}$. It fulfills the regularity condition (4.2.5), algebraically, as can easily be checked via the existence of exit paths γ^0 from every vertex $m_0 \in \mathbf{M}$ to 0, by virtue of Lemma 2.4. Furthermore, the network possesses the injectivity property (3.1.19) and therefore we will be able to apply the Reciprocity Theorem 3.3.

In order to compare both main results, we first derive the flux sensitivity matrix according to the framework provided by the theory by Fiedler and Mochizuki, in particular see Section 2.4 for a detailed introduction for the calculation of the flux sensitivity matrix.

To calculate the flux sensitivity matrix Φ we start by determining the augmented matrix $\mathbf{A} = (\mathbf{R}, \mathbf{C})$. Since the network has $E = 4$ reactions, we deal with a 4×4 matrix, and by $M = 2$ we know that the $\mathbf{R}$-part of $\mathbf{A}$ occupies two columns. The remaining two columns are reserved by the **C**-part, which we determine via the cycles in the network. Thus the augmented matrix **A** is given by

$$\mathbf{A} = \begin{pmatrix} 0 & 0 & -1 & 0 \\ r_{2A} & 0 & -1 & -1 \\ 0 & r_{3B} & 0 & -1 \\ 0 & r_{4B} & -1 & 0 \end{pmatrix}.$$

Proceeding as in Section 2.4 yields the flux sensitivity matrix Φ for our example:

$$\Phi = \begin{pmatrix} 1 & 0 & 0 & 0 \\ \frac{r_{3B}+r_{4B}}{r_{4B}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} \\ \frac{r_{3B}}{r_{4B}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} \\ 1 & 0 & 0 & 0 \end{pmatrix}. \tag{4.3.10}$$

Therefore, we can use the flux sensitivity matrix, given by the approach by Fielder and Mochizuki, to identify zero and nonzero flux responses, i. e., which reaction fluxes are sensitive to a rate change, and which are not. To obtain precise numerical values for the entries of the matrix, the numerical values of the derivatives r_{jm} are necessary.

The Reciprocity Theorem 3.3 by Shinar et al. provides as first statement constraints on the diagonal elements of the flux sensitivity matrix to lie between 0 and 1. Since our example network is injective, we can apply this conclusion of the theorem. Indeed the diagonal entries of the matrix (4.3.10) are all in the range $[0, 1]$. The second statement of the Reciprocity Theorem tells us that in each pair of reciprocal off-diagonal matrix entries at least one coefficient is constrained to lie in $[-1, 1]$. This conclusion also holds true, since in each pair $\Phi_{j'j_*}$ and $\Phi_{j^*j'}$, with $j^* \neq j'$ one of the flux sensitivities is even zero. Hence, the Reciprocity Theorem acts as intended in a flux sensitivity matrix, which we derived using the framework provided by Fiedler and Mochizuki.

Therefore, for a monomolecular network which satisfies the assumptions of both approaches, to be regular at steady states and injective, and thus for which both main results apply, we see that both theories do not contradict each other. In the next chapter we will examine further examples, but not one of them will yield a contradiction. Even more examples can be found in the appendix.

5

EXAMPLES OF FLUX SENSITIVITY MATRICES AND CHARACTERISTIC PATTERNS

The chapter mainly concerns the presentation and discussion of more examples. We will give six examples of monomolecular reaction networks satisfying the assumptions of both approaches, to be regular at steady states and injective. After checking on the assumptions on the network, we calculate the flux sensitivity matrix and apply the main results of both approaches to verify their conclusions. Furthermore, we investigate response patterns determined from the local structure of the network, i. e., motif rules. We provide the rules for three different kind of patterns and examine their occurrence in the network, respectively in the corresponding flux sensitivity matrix. The first pattern originates from the single path way, which we already saw in Section 2.4. The other two patterns regard the branching in a reaction network. Specifically, we provide new ideas for motif rules with feedback and splitting behaviour as branching in the reaction network.

5.1 IDEAS ON MOTIF RULES AND CHARACTERISTIC RESPONSE PATTERNS

In the next two sections we will present three examples each section with attention to different branching behaviours. In addition, we examine the conclusions of the main results from the approaches by Fiedler and Mochizuki, and by Shinar, Mayo, Ji and Feinberg.

For each example, individually, we will check the necessary assumptions of both theories. According to the theory by Fiedler and Mochizuki, we need to check the regularity condition (4.2.5) for monomolecular reaction networks to use their framework and derive the flux sensitivity matrix Φ. This can easily be done in virtue of Lemma 2.4, which tells us that the regularity of the network holds algebraically if, and only if, for every vertex $m_0 \in \mathbf{M}$ there exists a directed exit path γ^0 from m_0 to the zero vertex 0 in the corresponding graph $\Gamma = (\mathbf{M} \cup \{0\}, \mathbf{E})$. To apply the Reciprocity Theorem 3.3 from the theory by Shinar et al. to the matrix

Φ we need to check on the injectivity property (4.2.6) of the network. Since this is not a straightforward matter, we do not provide the tedious calculations. But the curious reader may use the software 'Chemical reaction network toolbox' [19] to quickly verify the injectivity. For a detailed introduction into the assumptions of both approaches, see Section 4.2.

We determine the flux sensitivity matrix Φ of the corresponding network by the method explained in Section 2.4. Since we already gave an example how to calculate the flux sensitivity matrix, we will just give the bare matrix in this chapter. But basically, the calculation is straightforward: first set up the augmented matrix $\mathbf{A} = (\mathbf{R}, \mathbf{C})$, invert it to get the sensitivity matrix $\mathcal{S} = -\mathbf{A}^{-1}$ and multiply the $\mathbf{C}$-part of $\mathbf{A}$ with the last N-rows of $\mathcal{S}$ to get Φ. Knowing the matrix Φ, we can verify the constraints given by the Reciprocity Theorem from the approach by Shinar et al. We will see that the flux sensitivity matrices of all examples satisfy the statements that all diagonal elements lie in the range $[0, 1]$, and one entry of each reciprocal off-diagonal pair is bounded by -1 from below and 1 from above. Therefore, we see that both approaches do not contradict each other.

Furthermore, we investigate three motif rules, i. e., response patterns in the flux sensitivity matrix Φ determined from the local structure of the network. The first rule is the single path way, which originates from the single child case that we already discussed in Chapter 2. The other two motif rules, namely the feedback behaviour and the splitting behaviour, which we are going to introduce, expand the branching rule for the sensitivity matrix $\mathcal{S}$ given by Fielder and Mochizuki, in terms of the flux sensitivity matrix Φ. Their motif rule states that the responses in the chemical concentrations propagate directly downward or spills over to a side branch [22].

Let us first take a closer look at the single path way rule. This motif rule describes the behaviour in situations, where the mother vertex $\mathbf{m}(j^*) = m^*$ has only one child edge j^*, i. e., metabolic chain pieces of the network. According to the main theorem 2.1 from Fielder and Mochizuki, we need for a nonzero flux response $\Phi_{j^*j^*} \neq 0$ two different di-paths γ^0 and γ', which are disjoint and emanate from the same mother vertex m^*. But in the single child case, we only have a single child edge of m^*, and hence a contradiction to these conditions. Therefore we have a zero flux response

$$\Phi_{j'j^*} = 0, \tag{5.1.1}$$

for all $j' \in \mathbf{E}$. We already saw the example

$$\Gamma^1 \; : \quad 0 \xrightarrow{1} A \xrightarrow{2} B \xrightarrow{3} C \xrightarrow{4} 0$$

of the single path way rule at the end of Section 2.4. We calculated the flux sensitivity matrix as

$$\Phi^1 = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \end{pmatrix}.$$

In this network, any reaction j^* other than the feed reaction 1 is a single child of a mother vertex $m = \mathbf{m}(j^*)$. By the single path way rule, we therefore have for all reactions $j^* \neq 1$ and all $j' \in \mathbf{E}$ a zero flux response $\Phi_{j'j^*} = 0$. Also see (2.3.3).

The two new motif rules are motivated by the following theorem from the theory by Fiedler and Mochizuki. The theorem describes how the influence of a perturbed reaction j^* spreads to a reaction j' via di-cycles, bi-cycles and side branches.

Theorem 5.1 (Fiedler & Mochizuki). *Let positivity and existence assumptions (2.2.1), (2.2.2) hold for the monomolecular reaction network (2.1.1), (2.1.2), and (2.1.5). Moreover assume the Jacobian determinant in (2.2.3) is nonzero, algebraically. Consider any pair of distinct edges* $j', j^* \in \mathbf{E}$. *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^*.

(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.*

For the proof of Theorem 5.1 see Section 4 of [11]. See Figure 5.1.1 for an illustration of the three cases that can arise.

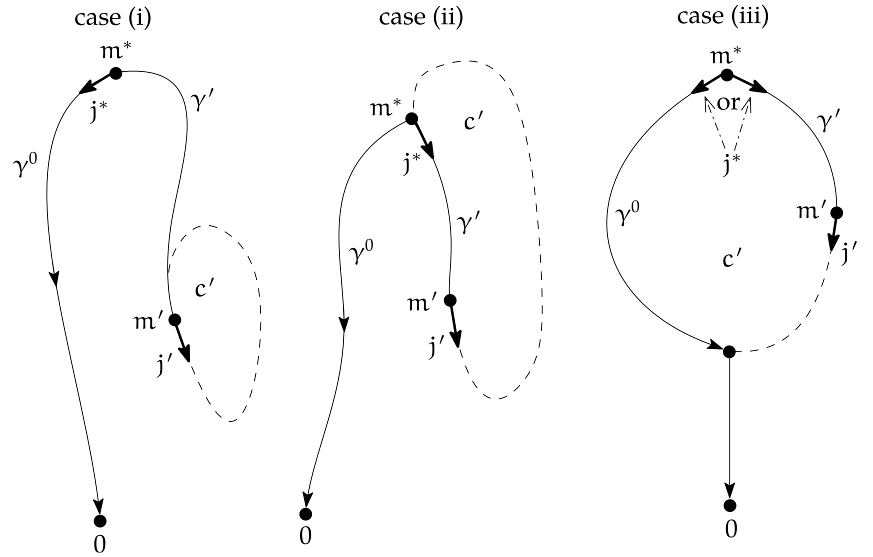


Figure 5.1.1: Illustrating the three cases of Theorem 5.1 with exit path γ^0 and influence dipath γ'. Case (i): γ' not contained in di-cycle c'. Case (ii): di-cycle c' contains γ'. Case (iii): one arc of bi-cycle c' contains γ'. The figure is redrawn from the reference paper [11].

Inspired by case (ii) of Theorem 5.1, we introduce the first new motif rule, the feedback behaviour. The rule attempts situations in which the network looks like:

$$\begin{tikzcd}
 & & \dots \arrow[dl, bend right] & \dots \arrow[l] & & \\
\dots \arrow[r] & m_1 \arrow[r] & \dots \arrow[r] & \dots \arrow[r] & m_2 \arrow[ul, bend right, "j_1"'] \arrow[r, "j_2"] & \dots
\end{tikzcd} \tag{5.1.2}$$

I. e., the network branches at one vertex, here m_2, and one of the branches needs to return to the branching point in order to possess a directed exit path to the zero vertex 0. In other words, if all possible directed exit paths starting with child edge j_1 of the branching point m_2 need necessarily to pass through the branching point m_2 on their way to the zero vertex 0, we call the branch starting with j_1 a feedback branch. Note that the feedback branch does not necessarily need to possess any vertices, in fact the simplest example is a reversible reaction like in Figure 4.3.1.

This behaviour corresponds to the following pattern on the diagonal entries of the flux sensitivity matrix:

$$\Phi = \begin{array}{cc} & \begin{matrix} & j_1 & & j_2 & \end{matrix} \\ \begin{matrix} \\ j_1 \\ \\ j_2 \\ \end{matrix} & \begin{pmatrix} \ddots & & & & \\ & 1 & & & \\ & & \ddots & & \\ & & & 0 & \\ & & & & \ddots \end{pmatrix} \end{array}, \tag{5.1.3}$$

where we omitted all but the interesting matrix entries. Therefore, the feedback behaviour is characterized by a $\Phi_{j_1 j_1} = 1$ on the diagonal of the flux sensitivity matrix for the feedback branch j_1, and by a $\Phi_{j_2 j_2} = 0$ for the other branch j_2, without feedback behaviour. For a clear illustration, we give the matrix pattern only for the particular case given in (5.1.2). Every additional feedback branch j_i just adds another 1 in the corresponding diagonal entry $\Phi_{j_i j_i} = 1$. When examining the flux sensitivity matrix, the 1 on the diagonal for the feedback branch may not be confused with the 1 on the diagonal imposed by the feed reaction of the network. Also see the discussion of the example in Section 2.4.

In summary we have the first conjectured motif rule:

Conjectured Motif Rule 1 (Feedback behaviour). *Consider a reaction network which branches at vertex* m. *If all possible directed exit paths starting with one of the child edges, say* j_1, *of the branching vertex* $m = \mathbf{m}(j_1)$ *need to pass through the branching point* m *on their way to* 0, *we call the branch starting with* j_1 *feedback branch. On the diagonal of the corresponding flux sensitivity matrix, we can find an entry* $\Phi_{j_1 j_1} = 1$ *for the edge* j_1 *with feedback behaviour, and further an entry* $\Phi_{j_2 j_2} = 0$ *for the child edge* j_2 *of* m, *without feedback behaviour.*

The second new motif rule, which we call splitting behaviour, describes the response pattern in the flux sensitivity matrix that occurs in situations like:

$$\begin{tikzcd} & & m_2 \arrow[r] & \dots \\ \dots \arrow[r] & m_1 \arrow[ur, "j_1"] \arrow[r, "j_2"] \arrow[dr, "j_3"] & m_3 \arrow[r] & \dots \\ & & m_4 \arrow[r] & \dots \end{tikzcd} \tag{5.1.4}$$

The network splits at one vertex, here m_1, into two or several branches, and each branch possesses a direct exit path to the zero vertex 0 that does not return to the branching point m_1. Note that the branches later may reunite. This rule is motivated by the third case of Theorem 5.1. We have to take two cases into account for the splitting behaviour. The simple case like in (5.1.4), where the splitting vertex is not influenced by another splitting vertex, and a more involved case. The matrix pattern of the splitting behaviour for the simple case (5.1.4) is given by

$$\Phi = \begin{array}{cc} & \begin{array}{ccccccc} & j_1 & & j_2 & & j_3 & \end{array} \\ \begin{array}{c} \\ j_1 \\ \\ j_2 \\ \\ j_3 \\ \\ \end{array} & \left(\begin{array}{ccccccc} \ddots & & & & & & \\ & \frac{r_{j_2 m_1} + r_{j_3 m_1}}{r_{j_1 m_1} + r_{j_2 m_1} + r_{j_3 m_1}} & & & & & \\ & & \ddots & & & & \\ & & & \frac{r_{j_1 m_1} + r_{j_3 m_1}}{r_{j_1 m_1} + r_{j_2 m_1} + r_{j_3 m_1}} & & & \\ & & & & \ddots & & \\ & & & & & \frac{r_{j_1 m_1} + r_{j_2 m_1}}{r_{j_1 m_1} + r_{j_2 m_1} + r_{j_3 m_1}} & \\ & & & & & & \ddots \end{array} \right) \end{array} , \tag{5.1.5}$$

where r_{jm} are the derivatives of the reaction rate functions for $m = \mathbf{m}(j)$, and we omitted again all uninteresting matrix entries. We can characterize the splitting behaviour in such simple cases on the diagonal of the flux sensitivity matrix by an entry $\Phi_{j_i j_i}$ for each splitting branch j_i given by

$$\Phi_{j_i j_i} = \frac{\sum_{j \in \mathbf{E}_S \setminus \{j_i\}} r_{j m_S}}{\sum_{j \in \mathbf{E}_S} r_{j m_S}}, \tag{5.1.6}$$

where $\mathbf{E}_S$ is the set of all edges that participate in the splitting, here $\mathbf{E}_S = \{j_1, j_2, j_3\}$, and $m_S = \mathbf{m}(j)$ the mother vertex of $j \in \mathbf{E}_S$, here m_1. The more involved case occurs in situations, where the splitting vertex is strongly connected to another splitting vertex, like:

$$\begin{tikzcd} & & m_2 \arrow[r] \arrow[dl, bend right] & \ldots \\ \ldots \arrow[r] & m_1 \arrow[ur] \arrow[r] & m_3 \arrow[r] & \ldots \end{tikzcd} \tag{5.1.7}$$

I. e., if there is a di-path from splitting vertex m_1 to splitting vertex m_2, then there also exists a di-path from m_2 back to m_1. We do not have a formula to predict the diagonal entries of the flux sensitivity matrix for the splitting branches of m_1 or

m_2, since they influence each other. However, summing over all diagonal entries $\Phi_{j_i j_i}$ for the $N := |E_S|$ branches of one splitting vertex yields

$$\sum_{i=1}^{N} \Phi_{j_i j_i} = N - 1, \tag{5.1.8}$$

in both cases. Therefore, the motif rule which describes splitting situations is still valuable for predicting the diagonal entries of the flux sensitivity matrix, or the other way around, for setting up the network diagram with given flux sensitivity matrix.

Let us summarize the second conjectured motif rule:

Conjectured Motif Rule 2 (Splitting behaviour)**.** *Consider a reaction network which branches at vertex m. If each branch possesses a direct exit path which starts with the corresponding child edge j_i of the branching vertex $m = \mathbf{m}(j_i)$ and does not return to the branching point m on its way to 0, we say that m shows splitting behaviour. We need to consider the following two cases:*

(i) In the simple case of the splitting behaviour, the splitting vertex is not influenced by another splitting vertex. In this case, we can predict the corresponding diagonal entry $\Phi_{j_i j_i}$ of the flux sensitivity matrix for the splitting branch starting with the child edge j_i of the branching vertex $m = \mathbf{m}(j_i)$ with the formula

$$\Phi_{j_i j_i} = \frac{\sum_{j \in \mathbf{E}_S \setminus \{j_i\}} r_{j m_S}}{\sum_{j \in \mathbf{E}_S} r_{j m_S}}, \tag{5.1.9}$$

where $\mathbf{E}_S$ is the set of all edges that participate in the splitting.

(ii) In the more involved case, the splitting vertex is strongly connected to a different splitting vertex, and we do not have such a formula.

However, if we take the sum over all diagonal entries $\Phi_{j_i j_i}$ for the $N := |E_S|$ branches of one splitting vertex, we obtain in both cases

$$\sum_{i=1}^{N} \Phi_{j_i j_i} = N - 1. \tag{5.1.10}$$

However, keep in mind that we just provide ideas on new motif rules. We check for consistence of the new motif rules over several examples, see the following sections and in the appendix, but do not prove them properly.

5.2 EXAMPLES WITH FEEDBACK BEHAVIOUR

This section provides three examples, which we examine with regards to the assumptions and the main results of the both approaches by Fiedler and Mochizuki, and by Shinar et al., as explained in Section 5.1. Furthermore, the examples in this section all show feedback behaviour, which we are going to investigate in detail.

The motif rule for the feedback behaviour claims that we can find an entry $\Phi_{j_1j_1} = 1$ on the diagonal of the flux sensitivity matrix Φ for the feedback branch j_1, and an entry $\Phi_{j_2j_2} = 0$ for the other child edge j_2 of the branching point. A feedback branch can be identified by looking for directed exit paths starting with a child edge of the branching point. If all possible exit paths need to return to the branching point on their way to the zero vertex 0, then the corresponding child edge possesses feedback behaviour.

The reaction network of the first example is given in Figure 5.2.1. On the first glance, we can see that all reactions in the network are monomolecular, and since every vertex possesses a direct exit path to the zero vertex, we know by Lemma 2.4 that the network also fulfills the regularity condition (4.2.5). Furthermore, the network is injective (4.2.1) and thus we can apply the main results of both approaches. We use the framework and Theorem 2.1 provided by Fiedler and Mochizuki to determine the flux sensitivity matrix as

$$\Phi^2 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 1+\frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 & 0 \\ 1+\frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 & 0 \\ \frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 & 0 \end{pmatrix}.$$

The Reciprocity Theorem 3.3 by Shinar et al. imposes constraints on the diagonal elements of the flux sensitivity matrix Φ^2, to be nonnegative and bounded from above by 1, which in fact holds true. The second part of the reciprocity theorem states that in each reciprocal off-diagonal pair one of the entries lies in the range $[-1, 1]$. In Φ^2 one element of each reciprocal off-diagonal pair is even zero.

Regarding the new motif rules, the reaction network Γ^2 shows feedback behaviour in the branching point C. Indeed, there is only one possible directed exit path

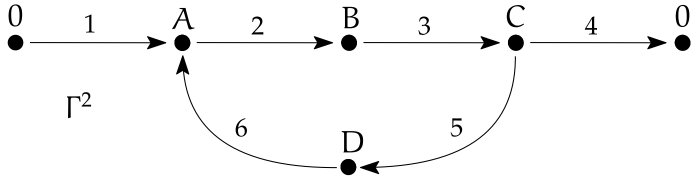


Figure 5.2.1: A chemical reaction network Γ^2, which contains four metabolites $\mathbf{M} = \{A, B, C, D\}$, six reactions $\mathbf{E} = \{1, \ldots, 6\}$, and shows feedback behaviour at branching vertex C.

starting with the child edge 5 of the branching point C and this di-path needs to return to C on its way to 0. Therefore, we expect a diagonal entry $\Phi_{55} = 1$ for the feedback branch, and $\Phi_{44} = 0$ for the other branch that possesses a direct exit path without returning to the branching point. Examining the flux sensitivity matrix confirms our expectations.

The second example Γ^3 in Figure 5.2.2 is taken from the reference papers [11, 22]. It fulfills the assumptions to be monomolecular, injective and regular at steady states, as easily can be checked by virtue of Lemma 2.4. Indeed, every vertex $m_0 \in \mathbf{M}$ possesses a directed exit path in Γ^3 from m_0 to the zero vertex 0. The flux sensitivity matrix can be calculated as described in Section 2.4, which yields

$$\Phi^3 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 + \frac{(r_{5D}+r_{8D})r_{6C}}{r_{4C}r_{5D}} & 0 & 0 & -\frac{r_{6C}}{r_{4C}} & \frac{r_{6C}r_{8D}}{r_{4C}r_{5D}} & 1 & 0 & \frac{r_{6C}}{r_{4C}} & 0 \\ \frac{(r_{5D}+r_{8D})(r_{6C}+r_{4C})}{r_{4C}r_{5D}} & 0 & 0 & -\frac{r_{6C}}{r_{4C}} & -\frac{r_{8D}(r_{6C}+r_{4C})}{r_{4C}r_{5D}} & 1 & 0 & 1 + \frac{r_{6C}}{r_{4C}} & 0 \\ \frac{r_{5D}+r_{8D}}{r_{5D}} & 0 & 0 & 0 & \frac{r_{8G}}{r_{5D}} & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{(r_{5D}+r_{8D})r_{6C}}{r_{4C}r_{5D}} & 0 & 0 & -\frac{r_{6C}}{r_{4C}} & -\frac{r_{6C}r_{8D}}{r_{4C}r_{5D}} & 1 & 0 & \frac{r_{6C}}{r_{4C}} & 0 \\ \frac{(r_{5D}+r_{8D})r_{6C}}{r_{4C}r_{5D}} & 0 & 0 & -\frac{r_{6C}}{r_{4C}} & -\frac{r_{6C}r_{8D}}{r_{4C}r_{5D}} & 1 & 0 & \frac{r_{6C}}{r_{4C}} & 0 \\ \frac{r_{8D}}{r_{5D}} & 0 & 0 & 0 & -\frac{r_{8D}}{r_{5D}} & 0 & 0 & 1 & 0 \\ \frac{r_{8D}}{r_{5D}} & 0 & 0 & 0 & -\frac{r_{8D}}{r_{5D}} & 0 & 0 & 1 & 0 \end{pmatrix}.$$

Since the matrix has only 0 and 1 entries on the diagonal and one element of each reciprocal off-diagonal pair is 0, we see that the flux sensitivity matrix given by the framework from Fiedler and Mochizuki is consistent with the Reciprocity Theorem from the theory by Shinar, Mayo, Ji and Feinberg.

The example Γ^3 has two branching points, the first one in C and the second in D. We start with investigating the first point C. Looking for exit paths starting with

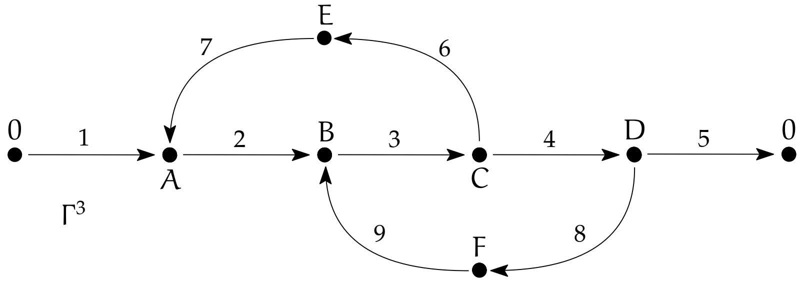


Figure 5.2.2: A chemical reaction network Γ^3, which contains six metabolites $\mathbf{M} = \{A, \ldots, F\}$, nine reactions $\mathbf{E} = \{1, \ldots, 9\}$, and shows feedback behaviour at branching vertex C and also in vertex D.

the child edge 6 of the branching vertex C, we note that all of them pass through the branching point C again, leading to the zero vertex 0. Hence, 6 shows feedback behaviour and the flux sensitivity matrix Φ^3 has an entry $\Phi_{66} = 1$ on the diagonal. The other branch 4 at C possess a exit path directly to 0, without returning to C, and thus is not a feedback branch. It has a matrix entry $\Phi_{44} = 0$ on the diagonal. The same procedure shows for the second branching point D that the child edge 8 is a feedback branch. Therefore, we have the diagonal entry $\Phi_{88} = 1$ for the feedback branch 8 and $\Phi_{55} = 0$ for the other branch 5.

The last example for this section, shown in Figure 5.2.3 is also monomolecular, injective (4.2.5), and fulfills the regularity condition (4.2.1), what can easily be verified as we did before. The flux sensitivity matrix is given by

$$\Phi^4 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{7A}+r_{2A}} & \frac{r_{7A}}{r_{7A}+r_{2A}} & 0 & 0 & 0 & 0 & -\frac{r_{2A}}{r_{7A}+r_{2A}} \\ 1+\frac{r_{6D}}{r_{4D}} & 0 & 0 & -\frac{r_{6D}}{r_{4D}} & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{7A}}{r_{7A}+r_{2A}}+\frac{r_{6D}}{r_{4D}} & -\frac{r_{7A}}{r_{7A}+r_{2A}} & 0 & -\frac{r_{6D}}{r_{4D}} & 0 & 1 & \frac{r_{2A}}{r_{7A}+r_{2A}} \\ \frac{r_{6D}}{r_{4D}} & 0 & 0 & -\frac{r_{6D}}{r_{4D}} & 0 & 1 & 0 \\ \frac{r_{7A}}{r_{7A}+r_{2A}} & -\frac{r_{7A}}{r_{7A}+r_{2A}} & 0 & 0 & 0 & 0 & \frac{r_{2A}}{r_{7A}+r_{2A}} \end{pmatrix}.$$

Examining the flux sensitivity matrix Φ^4 for the example verifies, one more time, the consistence of the conclusions of the Reciprocity Theorem by Shinar et al. in the framework provided by Fiedler and Mochizuki. Indeed, the diagonal entries are all nonnegative and bounded by 1 from above, and one element of each reciprocal off-diagonal pair lies in the range $[-1, 1]$.

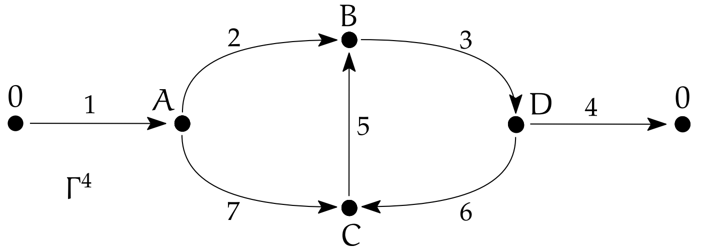


Figure 5.2.3: A chemical reaction network Γ^4, which contains four metabolites $\mathbf{M} = \{A, B, C, D\}$ and seven reactions $\mathbf{E} = \{1, \dots, 7\}$. We can observe feedback behaviour at branching vertex D, as well as splitting behaviour at branching point A.

The example of the reaction network Γ^4 in Figure 5.2.3 has the two branching points A and D. We can observe feedback behaviour in vertex D, since every possible exit path starting with child edge 6 needs to pass through the vertex D before it can reach the zero vertex 0. According to the corresponding motif rule, we get on the diagonal of the flux sensitivity matrix Φ^4 an entry $\Phi_{66} = 1$ for the feedback branch 6 and another entry $\Phi_{44} = 0$ for the other branch 4 originating at the vertex D. In contrast to the previous examples, we also encounter splitting behaviour in the branching vertex A. This motif rule accounts for the diagonal entries $\Phi_{22} = \frac{r_{7A}}{r_{7A}+r_{2A}}$ and $\Phi_{77} = \frac{r_{2A}}{r_{7A}+r_{2A}}$ of the flux sensitivity matrix. We will investigate this behaviour in more detail in the next section.

5.3 EXAMPLES WITH SPLITTING BEHAVIOUR

We discuss another three examples in this section. Again, we examine the examples with regards to the assumptions and main results of the theories by Fiedler and Mochizuki, and by Shinar et al. For a detailed comparison of the approaches see Chapter 4. The example in this section all possess splitting behaviour, which we want to investigate further.

The splitting behaviour is a motif rule, which applies in situations where the network splits at one vertex into two or several branches, and each branch possesses a direct exit path that does not return to the branching point on its way to the zero vertex. In the simple case (5.1.4), the rule now claims that we can find entries of the form

$$\Phi_{j_i j_i} = \frac{\sum_{j \in \mathbf{E}_S \setminus \{j_i\}} r_{j m_S}}{\sum_{j \in \mathbf{E}_S} r_{j m_S}},$$

on the diagonal of the flux sensitivity matrix, for each child edge j_i originating at the branching vertex m_S. We denote the set of all edges that participate in the splitting $\mathbf{E}_S$. In the more involved case (5.1.7), we do not have such a prediction on the diagonal elements of the flux sensitivity matrix. However, in both cases we know that summing over all diagonal entries $\Phi_{j_i j_i}$ for the $N := |E_S|$ branches of one splitting vertex yields

$$\sum_{i=1}^{N} \Phi_{j_i j_i} = N - 1.$$

The first two examples in this section show the simple case of the splitting behaviour and the last example shows the more involved case.

The first example for this section is the reaction network Γ^5, illustrated in Figure 5.3.1. The network is monomolecular and possesses the injectivity property (4.2.1). By Lemma 2.4 is the network regular at steady states, since each vertex in Γ^5 possesses a directed exit path to the zero vertex. According to the method from the approach by Fiedler and Mochizuki, explained in Section 2.4, computing the flux sensitivity matrix yields

$$\Phi^5 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{5A}+r_{2A}} & \frac{r_{5A}}{r_{5A}+r_{2A}} & 0 & 0 & -\frac{r_{2A}}{r_{5A}+r_{2A}} \\ \frac{r_{2A}}{r_{5A}+r_{2A}} & \frac{r_{5A}}{r_{5A}+r_{2A}} & 0 & 0 & -\frac{r_{2A}}{r_{5A}+r_{2A}} \\ 1 & 0 & 0 & 0 & 0 \\ \frac{r_{5A}}{r_{5A}+r_{2A}} & -\frac{r_{5A}}{r_{5A}+r_{2A}} & 0 & 0 & \frac{r_{2A}}{r_{5A}+r_{2A}} \end{pmatrix}.$$

We note that all diagonal entries of the matrix Φ^5 are nonnegative and bounded by 1 from above. Therefore, the matrix verifies the first statement of the Reciprocity Theorem 3.3 from the approach by Shinar et al. The second statement of the theorem claims that one element of each reciprocal off-diagonal pair in the matrix lies between -1 and 1, which is indeed the case. Hence, both approaches do not contradict each other.

The example reaction network Γ^5 has one branching point A, showing splitting behaviour in the simple case. The splitting vertex A has two child edges, 2 and

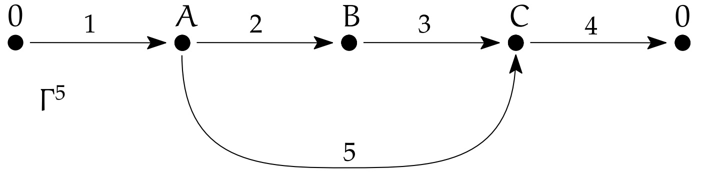


Figure 5.3.1: A chemical reaction network Γ^5, which contains three metabolites $\mathbf{M} = \{A, B, C\}$ and five reactions $\mathbf{E} = \{1, \dots, 5\}$. We can observe splitting behaviour at branching vertex A.

5, which both possess a direct exit path to the zero vertex that does not return to A. Therefore, we can find by the splitting rule two diagonal entries, $\Phi_{22} = \frac{r_{5A}}{r_{5A}+r_{2A}}$ for edge 2 and $\Phi_{55} = \frac{r_{2A}}{r_{5A}+r_{2A}}$ for edge 5 in the flux sensitivity matrix Φ^5. Furthermore, if we sum up both diagonal entries Φ_{22} and Φ_{55} we get $N - 1 = 1$, i. e., the number of splitting branches minus 1.

An illustration of the second example Γ^6 is given in Figure 5.3.2. The network fulfills the assumptions to be monomolecular, injective and regular at steady states. The flux sensitivity matrix is given by

$$\Phi^6 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{3A}+r_{2A}} & \frac{r_{3A}}{r_{3A}+r_{2A}} & -\frac{r_{2A}}{r_{3A}+r_{2A}} & 0 & 0 & 0 & 0 \\ -\frac{r_{3A}}{r_{3A}+r_{2A}} & -\frac{r_{3A}}{r_{3A}+r_{2A}} & \frac{r_{2A}}{r_{3A}+r_{2A}} & 0 & 0 & 0 & 0 \\ \frac{r_{2A}r_{4B}}{\alpha} & \frac{r_{3A}r_{4B}}{\alpha} & -\frac{r_{2A}r_{4B}}{\alpha} & \frac{r_{5B}}{r_{4B}+r_{5B}} & -\frac{r_{4B}}{r_{4B}+r_{5B}} & 0 & 0 \\ \frac{r_{2A}r_{5B}}{\alpha} & \frac{r_{3A}r_{5B}}{\alpha} & -\frac{r_{2A}r_{5B}}{\alpha} & -\frac{r_{5B}}{r_{4B}+r_{5B}} & \frac{r_{4B}}{r_{4B}+r_{5B}} & 0 & 0 \\ \frac{r_{2A}r_{4B}+r_{3A}(r_{4B}+r_{5B})}{\alpha} & -\frac{r_{3A}r_{5B}}{\alpha} & \frac{r_{2A}r_{5B}}{\alpha} & \frac{r_{5B}}{r_{4B}+r_{5B}} & -\frac{r_{4B}}{r_{4B}+r_{5B}} & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix},$$

where $\alpha := (r_{3A} + r_{2A})(r_{4B} + r_{5B})$. According to the Reciprocity Theorem 3.3 we expect the diagonal entries of the flux sensitivity matrix to lie in the range $[0, 1]$ and the reciprocal off-diagonal pairs in $[-1, 1]$, which indeed holds true.

Example Γ^6 shows two branching points with splitting behaviour in the simple case, A and B. We can easily verify the splitting behaviour by looking for the existence of directed exit paths, which do not return to the branching point, for each splitting branch. The splitting in the vertex A accounts for the diagonal entries $\Phi_{22} = \frac{r_{3A}}{r_{3A}+r_{2A}}$ and $\Phi_{33} = \frac{r_{2A}}{r_{3A}+r_{2A}}$ in the flux sensitivity matrix Φ^6, and the child edges 4 and 5 of branching point B for $\Phi_{44} = \frac{r_{5B}}{r_{5B}+r_{4B}}$ and $\Phi_{55} = \frac{r_{4B}}{r_{5B}+r_{4B}}$. Furthermore, the diagonal entries for both splitting situations sum up to 1, separately.

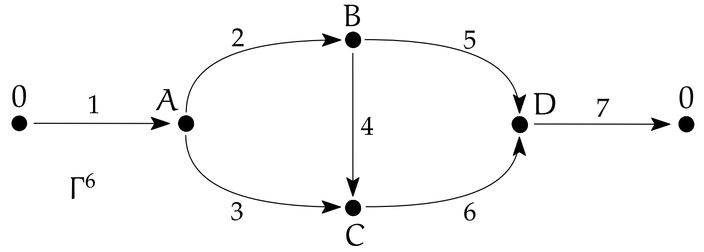


Figure 5.3.2: A chemical reaction network Γ^6, which contains four metabolites $\mathbf{M} = \{A, B, C, D\}$ and seven reactions $\mathbf{E} = \{1, \ldots, 7\}$. We can observe splitting behaviour at branching vertex A and also at B.

The last example Γ^7, shown in Figure 5.3.3, has an interesting behaviour regarding the new motif rules. But first we check the assumptions of both approaches in order to use the framework by Fiedler and Mochizuki and apply the Reciprocity Theorem from the approach by Shinar et al. As explained in Section 5.1, we can easily see that the network is monomolecular, regular at steady states and possess the injectivity property. The flux sensitivity matrix is given by

$$\Phi^7 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}(r_{3B}+r_{4B})}{\alpha} & \frac{r_{5A}(r_{3B}+r_{4B})}{\alpha} & \frac{r_{2A}r_{4B}}{\alpha} & -\frac{r_{2A}r_{3B}}{\alpha} & -\frac{r_{2A}(r_{3B}+r_{4B})}{\alpha} & 0 & 0 \\ \frac{r_{2A}r_{3B}}{\alpha} & \frac{r_{5A}r_{3B}}{\alpha} & \frac{r_{4B}(r_{2A}+r_{5A})}{\alpha} & -\frac{r_{3B}(r_{2A}+r_{5A})}{\alpha} & -\frac{r_{2A}r_{3B}}{\alpha} & 0 & 0 \\ \frac{r_{2A}r_{4B}}{\alpha} & \frac{r_{5A}r_{4B}}{\alpha} & -\frac{r_{4B}r_{5A}}{\alpha} & \frac{r_{3B}r_{5A}}{\alpha} & -\frac{r_{2A}r_{4B}}{\alpha} & 0 & 0 \\ \frac{r_{3B}+r_{4B}}{\alpha} & -\frac{r_{5A}r_{4B}}{\alpha} & \frac{r_{4B}r_{5A}}{\alpha} & -\frac{r_{3B}r_{5A}}{\alpha} & \frac{r_{2A}r_{4B}}{\alpha} & 0 & 0 \\ \frac{r_{3B}+r_{4B}}{\alpha} & -\frac{r_{5A}r_{4B}}{\alpha} & \frac{r_{4B}r_{5A}}{\alpha} & -\frac{r_{3B}r_{5A}}{\alpha} & \frac{r_{2A}r_{4B}}{\alpha} & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix},$$

where $\alpha := r_{2A}r_{4B} + r_{5A}r_{3B} + r_{5A}r_{4B}$. Once again, the conclusions of the Reciprocity Theorem 3.3 by Shinar er al. hold true.

The vertex A of the example Γ^7 obviously shows splitting behaviour, since each branch of the splitting has a directed exit path which do not pass through the vertex A itself. On the first glance, the branching at vertex B seems to possess feedback behaviour. However, since the pretended feedback branch 3 possesses a directed exit path through vertex C and D, which thus do not need to return to the branching point B, we in fact have splitting behaviour in B. Another observation is that the two splitting vertices A and B are strongly connected, even reversible, and therefore can we not use (5.1.6) to predict the diagonal entries of the flux sensitivity matrix. However, the second part of the splitting rule, which says that summing

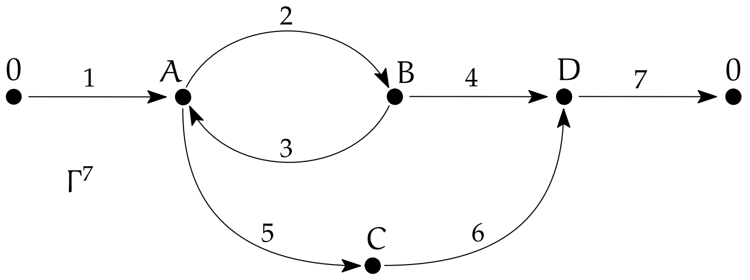


Figure 5.3.3: A chemical reaction network Γ^7, which contains four metabolites $\mathbf{M} = \{A, B, C, D\}$ and seven reactions $\mathbf{E} = \{1, \dots, 7\}$. We can observe splitting behaviour at branching vertex A and also at B.

over the diagonal entries of the N child edges that participate in a splitting yields $N - 1$, still holds true.

In this chapter, we saw six examples of reaction networks which satisfied the assumptions of both approaches and also showed that the conclusions of their main results hold true. Hence, the both theories do not contradict each other. Even more examples can be found in the appendix, but not one of them will yield a contradiction.

6

SUMMARY AND DISCUSSION

In this thesis we gave an introduction and a detailed comparison of two recent approaches to flux sensitivity analysis in chemical reaction networks. The structural approach by Fiedler and Mochizuki (2015) [11, 22] provided a sensitivity analysis based on the directed graph structure of the network for the case of monomolecular reactions. Their approach enabled us to determine zero and nonzero flux responses, without any numerical input. Another intriguing approach to this issue, proposed by Shinar, Mayo, Ji and Feinberg (2011) [29, 30], revealed a connection between the structure of a mass action network and constraints in the order of magnitude on the sensitivity of their steady state fluxes against a rate perturbation.

In order to give a clear and precise comparison of both approaches, we wanted to stay consistent in the notation. Therefore we adopted the notation from the approach by Fiedler and Mochizuki into the setting provided by Feinberg, Shinar, Mayo, and Ji. We saw that the setting of both theories is very similar in their construction of the reaction network diagrams, in fact their graph representations coincide, as well as their view on the dynamics of the concentrations of metabolites. Also we identified corresponding notions in both theories of all the basic principles.

The differences that make the approaches complementary came in with the assumptions that are necessary to apply the main results, and the restrictions they impose. We saw that the approach by Fiedler and Mochizuki can only deal with monomolecular reaction networks, where the approach by Shinar et al. does not have such limitations. But in a very recent advance Brehm and Fiedler [3] were able to enhance the theory for regular multimolecular reaction networks. In fact, in some sense, they provide a completely different approach which is more powerful.

On the other hand, the approach by Shinar et al. also has a fundamental limitation regarding the variety of reaction rate kinetics that can be chosen. The reaction rate functions are restricted to mass action kinetics. In contrast, the approach by

Fiedler and Mochizuki allows a very general class of reaction rate functions, with exception for pure mass action kinetics.

In both theories, positivity assumptions on the reaction rates and the existence of a positive steady state are present. In addition, Fiedler and Mochizuki assume that the reaction network is regular at steady states, i. e., that the Jacobian of the ODE vector field is nonsingular at steady states. Shinar et al. impose, in contrast, an injectivity property on the network. Injectivity is network property that, regardless of the rate constants, precludes two different stoichiometrically compatible compositions to give rise to production rates that are completely identical. Albeit these two assumptions seems to be different on the first glance, if we forget about the restrictions on the reaction rates for a moment, we can show that injectivity implies the regularity assumption. Another interesting observation in this illegal settingis that the framework by Fiedler and Mochizuki, especially Lemma 2.4, provides a simple way to check for noninjectivity, which otherwise is not a straightforward matter. Also notable is that all assumptions of both approaches rely on the underlying network structure, only.

The main result by Fiedler and Mochizuki on structural sensitivity analysis of flux influences basically states that an external perturbation either propagates directly downward in the reaction network, or else spills over to a side branch. I. e., it provides certain conditions to determine which reaction fluxes are sensitive to a rate change, in other words, whether the flux response is nonzero, or not. Since no numerical data is required, their results are function-free.

In contrast, the Reciprocity Theorem from the approach by Shinar et al. provides constraints on the diagonal entries of the flux sensitivity matrix to be nonnegative and bounded from above by 1, for any positive network equilibrium. Furthermore, for each pair of reciprocal off-diagonal entries of the matrix, at least one element lies between -1 and 1.

When comparing the results, we can see that the theory by Fiedler and Mochizuki extracts its conclusions predominantly from the graph structure of the network, whereas on the contrary the approach by Shinar et al. is a purely analytical one. To gain further insight into both approaches, we applied the theorems to several examples. All of the reaction networks in the examples fulfilled the assumptions to be monomolecular, regular at steady states and injective. So we were able to calculate the flux sensitivity matrix according to the framework provided by Fiedler and Mochizuki, and afterwards verify the statements of the Reciprocity Theorem on the matrix entries. Since all statements hold true, we concluded that both approaches do not contradict each other.

Furthermore, we examined the flux sensitivity matrices with regards to characteristic behaviour. We were able to identify three response patterns determined from the local structure of the network, i. e., motif rules. The first one is the single path way rule, which states that the flux influence of a single child edge is zero, for all reactions of the network. Regarding the branching in a reaction network, we gave ideas for two new motif rules, namely the feedback behaviour and the splitting behaviour.

The feedback behaviour occurs in situations, where the network branches at one point and for one of the branches every possible exit path starting with a child edge of the branching vertex needs to pass through the branching vertex on its way to the zero vertex. We call the branch, starting with this child edge, feedback branch. In such situations we can find a matrix entry 1 on the diagonal of the flux sensitivity matrix for each feedback child edge, and a zero entry on the diagonal for the other branch, which possesses a direct exit path.

The splitting behaviour describes the branching of a reaction network, where all branches possess a direct exit path that does not return to the branching vertex. For this motif rule we need to consider two cases. In the simple case, the splitting vertex is not influenced by another splitting vertex, and we can provide a formula, giving detailed predictions on the diagonal entries of the flux sensitivity matrix for the child edges of the branching vertex. The more involved case occurs if the splitting vertex is strongly connected to another splitting vertex. At present, we do not have a formula that gives predictions on the diagonal entries for the more involved case. However, summing over all diagonal entries for the N branches of one splitting vertex yields $N - 1$, in both cases. Therefore, the motif rule, which describes splitting situations is still valuable for predicting the diagonal entries of the flux sensitivity matrix, or the other way around, for setting up the network diagram with given flux sensitivity matrix.

We checked for consistence of these new motif rules over several examples, but do not have proper proofs at present. A proof of the feedback behaviour and the splitting behaviour, as well as finding a formula that combines both cases of the splitting behaviour is still open.

A

APPENDIX: FURTHER EXAMPLES

We provide here further examples of monomolecular reaction networks, which are injective (4.2.1) and satisfy the regularity condition (4.2.5). All of them show the consistence of both approaches, i. e., that the flux sensitivity matrix, which is derived in the framework and Theorem 2.1 by Fiedler and Mochizuki shows also the conclusions of the Reciprocity Theorem 3.3 by Shinar, Mayo, Ji and Feinberg. We provide the reaction network Γ^i with its corresponding flux sensitivity matrix Φ^i, calculated as explained in Section 2.4. For each example, if existent, we give comments on its branching behaviour according to Conjectured Motif Rule 1 for the feedback behaviour and Conjectured Motif Rule 2 for the splitting behaviour, see Section 5.1. For this purpose, we denote the diagonal entry of the flux sensitivity matrix Φ^i for the affected child edge j of the branching vertex $m = \mathbf{m}(j)$ by Φ^i_{jj}. For examples investigated in detail, see Chapter 5.

A.1 FEEDBACK BEHAVIOUR

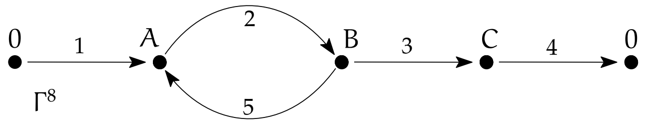


Figure A.1.1: Reaction network Γ^8 with feedback behaviour at branching vertex B. In particular, see the diagonal entries $\Phi^8_{55} = 1$ for the feedback branch 5 and $\Phi^8_{33} = 0$ for the other branch 3. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^8 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 1 + \frac{r_{5B}}{r_{3B}} & 0 & -\frac{r_{5B}}{r_{3B}} & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ \frac{r_{5B}}{r_{3B}} & 0 & -\frac{r_{5B}}{r_{3B}} & 0 & 1 \end{pmatrix}$$

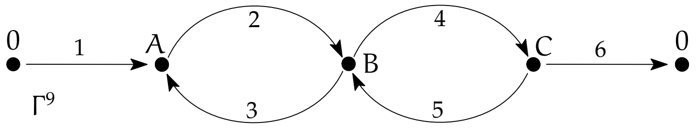


Figure A.1.2: Reaction network Γ^9 with feedback behaviour at branching vertices B and C. In particular, see the diagonal entries $\Phi^9_{33} = 1$ for the feedback branch 3 and $\Phi^9_{44} = 0$ for the other branch 4 of vertex B, and the diagonal entries $\Phi^9_{55} = 1$ for the feedback branch 5 and $\Phi^9_{66} = 0$ for the other branch 6 of vertex C. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^9 = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 1 + \frac{r_{3B}(r_{5C}+r_{6C})}{r_{4B}r_{6C}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} & \frac{r_{3B}}{r_{4B}} & -\frac{r_{3B}r_{5C}}{r_{4B}r_{6C}} \\ \frac{r_{3B}(r_{5C}+r_{6C})}{r_{4B}r_{6C}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} & \frac{r_{3B}}{r_{4B}} & -\frac{r_{3B}r_{5C}}{r_{4B}r_{6C}} \\ \frac{r_{5C}+r_{6C}}{r_{6C}} & 0 & 0 & 0 & 1 & -\frac{r_{5C}}{r_{6C}} \\ \frac{r_{5C}}{r_{6C}} & 0 & 0 & 0 & 1 & -\frac{r_{5C}}{r_{6C}} \\ 1 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

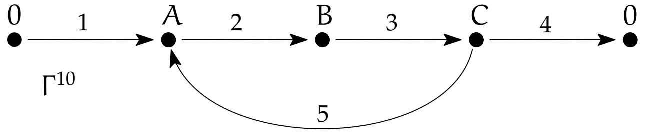


Figure A.1.3: Reaction network Γ^{10} with feedback behaviour at branching vertex C. In particular, see the diagonal entries $\Phi^{10}_{55} = 1$ for the feedback branch 5 and $\Phi^{10}_{44} = 0$ for the other branch 4. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{10} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 1+\frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 \\ 1+\frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 \\ 1 & 0 & 0 & 0 & 0 \\ \frac{r_{5C}}{r_{4C}} & 0 & 0 & -\frac{r_{5C}}{r_{4C}} & 1 \end{pmatrix}$$

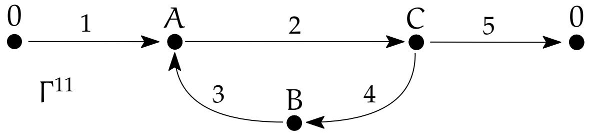


Figure A.1.4: Reaction network Γ^{11} with feedback behaviour at branching vertex C. In particular, see the diagonal entries $\Phi^{11}_{44} = 1$ for the feedback branch 4 and $\Phi^{11}_{55} = 0$ for the other branch 5. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{11} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 1+\frac{r_{4C}}{r_{5C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}} \\ \frac{r_{4C}}{r_{5C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}} \\ \frac{r_{4C}}{r_{5C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}} \\ 1 & 0 & 0 & 0 & 0 \end{pmatrix}$$

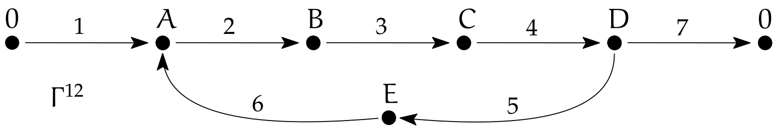


Figure A.1.5: Reaction network Γ^{12} with feedback behaviour at branching vertex D. In particular, see the diagonal entries $\Phi^{12}_{55} = 1$ for the feedback branch 5 and $\Phi^{12}_{77} = 0$ for the other branch 7. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{12} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1+\frac{r_{5D}}{r_{7D}} & 0 & 0 & 0 & 1 & 0 & -\frac{r_{5D}}{r_{7D}} \\ 1+\frac{r_{5D}}{r_{7D}} & 0 & 0 & 0 & 1 & 0 & -\frac{r_{5D}}{r_{7D}} \\ 1+\frac{r_{5D}}{r_{7D}} & 0 & 0 & 0 & 1 & 0 & -\frac{r_{5D}}{r_{7D}} \\ \frac{r_{5D}}{r_{7D}} & 0 & 0 & 0 & 1 & 0 & -\frac{r_{5D}}{r_{7D}} \\ \frac{r_{5D}}{r_{7D}} & 0 & 0 & 0 & 1 & 0 & -\frac{r_{5D}}{r_{7D}} \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

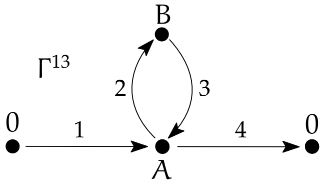


Figure A.1.6: Reaction network Γ^{13} with feedback behaviour at branching vertex A. In particular, see the diagonal entries $\Phi^{13}_{22} = 1$ for the feedback branch 2 and $\Phi^{13}_{44} = 0$ for the other branch 4. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{13} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{4A}} & 1 & 0 & -\frac{r_{2A}}{r_{4A}} \\ \frac{r_{2A}}{r_{4A}} & 1 & 0 & -\frac{r_{2A}}{r_{4A}} \\ 1 & 0 & 0 & 0 \end{pmatrix}$$

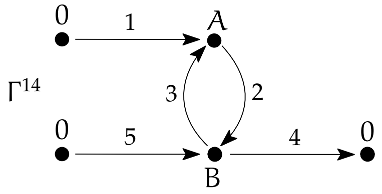


Figure A.1.7: Reaction network Γ^{14} with feedback behaviour at branching vertex B. In particular, see the diagonal entries $\Phi^{14}_{33} = 1$ for the feedback branch 3 and $\Phi^{14}_{44} = 0$ for the other branch 4. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{14} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 1 + \frac{r_{3B}}{r_{4B}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} & \frac{r_{3B}}{r_{4B}} \\ \frac{r_{3B}}{r_{4B}} & 0 & 1 & -\frac{r_{3B}}{r_{4B}} & \frac{r_{3B}}{r_{4B}} \\ 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}$$

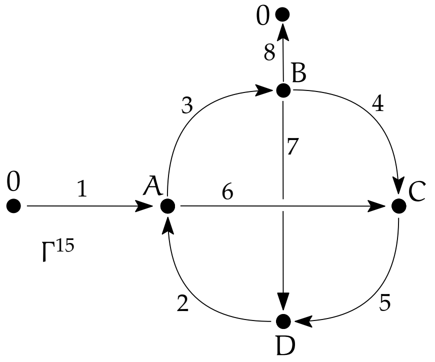


Figure A.1.8: Reaction network Γ^{15} with feedback behaviour at branching vertices A and B. In particular, see the diagonal entries $\Phi^{15}_{66} = 1$ for the feedback branch 6 and $\Phi^{15}_{33} = 0$ for the other branch 3 of vertex A, and the diagonal entries $\Phi^{15}_{44} = 1$ and $\Phi^{15}_{77} = 1$ for the feedback branches 4 and 7, and $\Phi^{15}_{88} = 0$ for the other branch 8 of vertex B. Note that one matrix entry of each reciprocal off-diagonal pair is zero. Define $\alpha := r_{4B} + r_{7B}$, $\beta := r_{3A} + r_{6A}$, and $\omega := r_{8B} + \alpha$.

$$\Phi^{15} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{3A}\alpha + r_{6A}\omega}{r_{3A}r_{8B}} & 0 & -\frac{r_{6A}}{r_{3A}} & \frac{\beta}{r_{3A}} & 0 & 1 & \frac{\beta}{r_{3A}} & -\frac{\alpha\beta}{r_{3A}r_{8B}} \\ \frac{\omega}{r_{8B}} & 0 & 0 & 1 & 0 & 0 & 1 & -\frac{\alpha}{r_{8B}} \\ \frac{r_{4B}}{r_{8B}} & 0 & 0 & 1 & 0 & 0 & 0 & -\frac{r_{4B}}{r_{8B}} \\ \frac{r_{3A}r_{4B} + r_{6A}\omega}{r_{3A}r_{8B}} & 0 & -\frac{r_{6A}}{r_{3A}} & \frac{\beta}{r_{3A}} & 0 & 1 & \frac{r_{6A}}{r_{3A}} & -\frac{(r_{3A}r_{4B} + r_{6A}\alpha)}{r_{3A}r_{8B}} \\ \frac{r_{6A}\omega}{r_{3A}r_{8B}} & 0 & -\frac{r_{6A}}{r_{3A}} & \frac{r_{6A}}{r_{3A}} & 0 & 1 & \frac{r_{6A}}{r_{3A}} & -\frac{r_{6A}\alpha}{r_{3A}r_{8B}} \\ \frac{r_{7B}}{r_{8B}} & 0 & 0 & 0 & 0 & 0 & 1 & -\frac{r_{7B}}{r_{8B}} \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

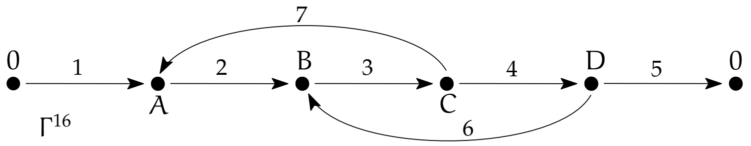


Figure A.1.9: Reaction network Γ^{16} with feedback behaviour at vertices C and D. In particular, see the diagonal entries $\Phi^{16}_{77} = 1$ for the feedback branch 7 and $\Phi^{16}_{44} = 0$ for the other branch 4 of vertex C, and the diagonal entries $\Phi^{16}_{66} = 1$ for the feedback branch 6 and $\Phi^{16}_{55} = 0$ for the other branch 5 of vertex D. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{16} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{5D} r_{4C} + r_{7C}(r_{5D} + r_{6D})}{r_{5D} r_{4C}} & 0 & 0 & -\frac{r_{7C}}{r_{4C}} & -\frac{r_{7C} r_{6D}}{r_{4C} r_{5D}} & \frac{r_{7C}}{r_{4C}} & 1 \\ -\frac{r_{6D}}{r_{5D}} & 0 & 0 & -\frac{r_{7C}}{r_{4C}} & -\frac{r_{6D}(r_{7C} + r_{4C})}{r_{4C} r_{5D}} & \frac{r_{7C} + r_{4C}}{r_{4C}} & 1 \\ \frac{r_{5D} + r_{6D}}{r_{5D}} & 0 & 0 & 0 & -\frac{r_{6D}}{r_{5D}} & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{6D}}{r_{5D}} & 0 & 0 & 0 & -\frac{r_{6D}}{r_{5D}} & 1 & 0 \\ \frac{r_{7C}(r_{5D} + r_{6D})}{r_{5D} r_{4C}} & 0 & 0 & -\frac{r_{7C}}{r_{4C}} & -\frac{r_{7C} r_{6D}}{r_{4C} r_{5D}} & \frac{r_{7C}}{r_{4C}} & 1 \end{pmatrix}$$

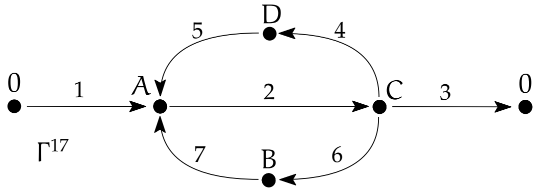


Figure A.1.10: Reaction network Γ^{17} with feedback behaviour at branching vertex C. In particular, see the diagonal entries $\Phi^{17}_{44} = 1$ and $\Phi^{17}_{66} = 1$ for the feedback branches 4 and 6, and $\Phi^{17}_{33} = 0$ for the other branch 3. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{17} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{3C}+r_{4C}+r_{6C}}{r_{2A}r_{3C}} & 0 & -\frac{r_{4C}+r_{6C}}{r_{3C}} & 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{4C}}{r_{3C}} & 0 & -\frac{r_{4C}}{r_{3C}} & 1 & 0 & 0 & 0 \\ \frac{r_{4C}}{r_{3C}} & 0 & -\frac{r_{4C}}{r_{3C}} & 1 & 0 & 0 & 0 \\ \frac{r_{6C}}{r_{3C}} & 0 & -\frac{r_{6C}}{r_{3C}} & 0 & 0 & 1 & 0 \\ \frac{r_{6C}}{r_{3C}} & 0 & -\frac{r_{6C}}{r_{3C}} & 0 & 0 & 1 & 0 \end{pmatrix}$$

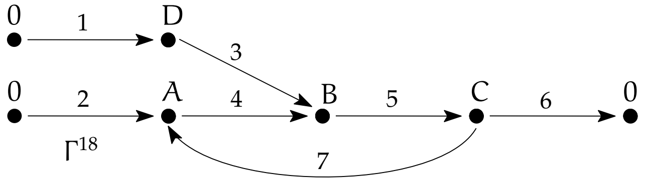


Figure A.1.11: Reaction network Γ^{18} with feedback behaviour at branching vertex C. In particular, see the diagonal entries $\Phi^{18}_{77} = 1$ for the feedback branch 7 and $\Phi^{18}_{66} = 0$ for the other branch 6. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{18} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{7C}}{r_{6C}} & 1 + \frac{r_{7C}}{r_{6C}} & 0 & 0 & 0 & -\frac{r_{7C}}{r_{6C}} & 1 \\ 1 + \frac{r_{7C}}{r_{6C}} & 1 + \frac{r_{7C}}{r_{6C}} & 0 & 0 & 0 & -\frac{r_{7C}}{r_{6C}} & 1 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{7C}}{r_{6C}} & \frac{r_{7C}}{r_{6C}} & 0 & 0 & 0 & -\frac{r_{7C}}{r_{6C}} & 1 \end{pmatrix}$$

A.2 SPLITTING BEHAVIOUR IN THE SIMPLE CASE

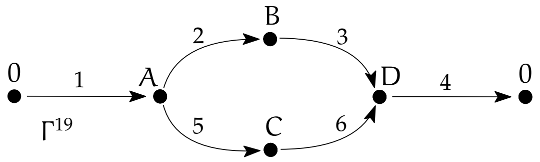


Figure A.2.1: Reaction network Γ^{19} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{19}_{22} and Φ^{19}_{55} characterizing the splitting branches 2 and 5 in the matrix. Note that only the entries Φ^{19}_{25} and Φ^{19}_{52} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{19} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{5A}} & \frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{2A}}{r_{2A}+r_{5A}} & \frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{5A}}{r_{2A}+r_{5A}} & -\frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & \frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{5A}}{r_{2A}+r_{5A}} & -\frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & \frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \end{pmatrix}$$

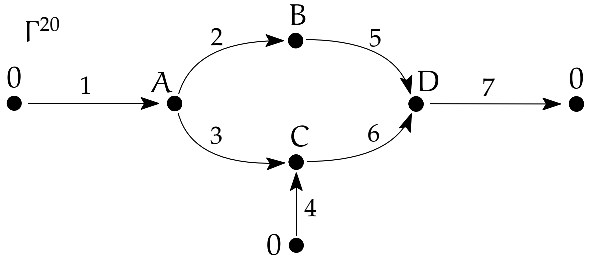


Figure A.2.2: Reaction network Γ^{20} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{20}_{22} and Φ^{20}_{33} characterizing the splitting branches 2 and 3 in the matrix. Note that only the entries Φ^{20}_{23} and Φ^{20}_{32} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{20} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{3A}} & \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\ \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{3A}}{r_{2A}+r_{3A}} & \frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{3A}} & \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\ \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{3A}}{r_{2A}+r_{3A}} & \frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 \end{pmatrix}$$

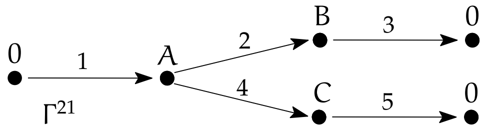


Figure A.2.3: Reaction network Γ^{21} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{21}_{22} and Φ^{21}_{44} characterizing the splitting branches 2 and 4 in the matrix. Note that only the entries Φ^{21}_{24} and Φ^{21}_{42} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{21} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{4A}} & \frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & -\frac{r_{2A}}{r_{2A}+r_{4A}} & 0 \\ \frac{r_{2A}}{r_{2A}+r_{4A}} & \frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & -\frac{r_{2A}}{r_{2A}+r_{4A}} & 0 \\ \frac{r_{4A}}{r_{2A}+r_{4A}} & -\frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & \frac{r_{2A}}{r_{2A}+r_{4A}} & 0 \\ \frac{r_{4A}}{r_{2A}+r_{4A}} & -\frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & \frac{r_{2A}}{r_{2A}+r_{4A}} & 0 \end{pmatrix}$$

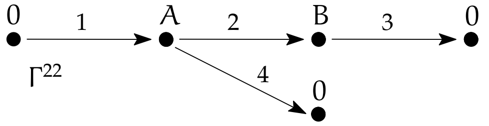


Figure A.2.4: Reaction network Γ^{22} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{22}_{22} and Φ^{22}_{44} characterizing the splitting branches 2 and 4 in the matrix. Note that only the entries Φ^{22}_{24} and Φ^{22}_{42} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{22} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{4A}} & \frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & -\frac{r_{2A}}{r_{2A}+r_{4A}} \\ \frac{r_{2A}}{r_{2A}+r_{4A}} & \frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & -\frac{r_{2A}}{r_{2A}+r_{4A}} \\ \frac{r_{4A}}{r_{2A}+r_{4A}} & -\frac{r_{4A}}{r_{2A}+r_{4A}} & 0 & \frac{r_{2A}}{r_{2A}+r_{4A}} \end{pmatrix}$$

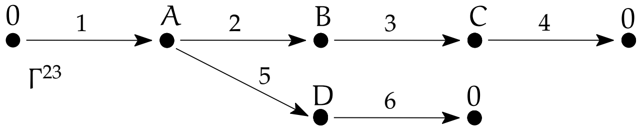


Figure A.2.5: Reaction network Γ^{23} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{23}_{22} and Φ^{23}_{55} characterizing the splitting branches 2 and 5 in the matrix. Note that only the entries Φ^{23}_{25} and Φ^{23}_{52} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{23} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{5A}} & \frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{2A}}{r_{2A}+r_{5A}} & \frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{2A}}{r_{2A}+r_{5A}} & \frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{5A}}{r_{2A}+r_{5A}} & -\frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & \frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \\ \frac{r_{5A}}{r_{2A}+r_{5A}} & -\frac{r_{5A}}{r_{2A}+r_{5A}} & 0 & 0 & \frac{r_{2A}}{r_{2A}+r_{5A}} & 0 \end{pmatrix}$$

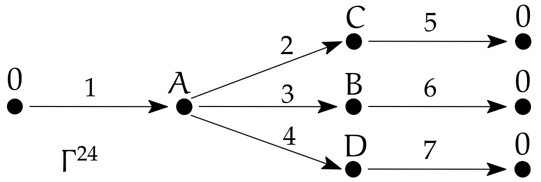


Figure A.2.6: Reaction network Γ^{24} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{24}_{22}, Φ^{24}_{33}, and Φ^{24}_{44} characterizing the splitting branches 2, 3, and 4 in the matrix. Note that only the entries Φ^{24}_{23}, Φ^{24}_{32}, Φ^{24}_{24}, Φ^{24}_{42}, Φ^{24}_{34}, and Φ^{24}_{43} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\omega := r_{2A} + r_{3A} + r_{4A}$.

$$\Phi^{24} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{\omega} & \frac{r_{3A}+r_{4A}}{\omega} & -\frac{r_{2A}}{\omega} & -\frac{r_{2A}}{\omega} & 0 & 0 & 0 \\ \frac{r_{3A}}{\omega} & -\frac{r_{3A}}{\omega} & \frac{r_{2A}+r_{4A}}{\omega} & -\frac{r_{3A}}{\omega} & 0 & 0 & 0 \\ \frac{r_{4A}}{\omega} & -\frac{r_{4A}}{\omega} & -\frac{r_{4A}}{\omega} & \frac{r_{2A}+r_{3A}}{\omega} & 0 & 0 & 0 \\ \frac{r_{2A}}{\omega} & \frac{r_{3A}+r_{4A}}{\omega} & -\frac{r_{2A}}{\omega} & -\frac{r_{2A}}{\omega} & 0 & 0 & 0 \\ \frac{r_{3A}}{\omega} & -\frac{r_{3A}}{\omega} & \frac{r_{2A}+r_{4A}}{\omega} & -\frac{r_{3A}}{\omega} & 0 & 0 & 0 \\ \frac{r_{4A}}{\omega} & -\frac{r_{4A}}{\omega} & -\frac{r_{4A}}{\omega} & \frac{r_{2A}+r_{3A}}{\omega} & 0 & 0 & 0 \end{pmatrix}$$

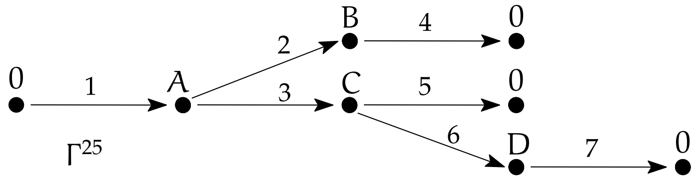


Figure A.2.7: Reaction network Γ^{25} with splitting behaviour in the simple case at branching vertices A and C. In particular, see the diagonal entries Φ^{25}_{22} and Φ^{25}_{33} for the splitting branches 2 and 3 of vertex A, and the diagonal entries Φ^{25}_{55} and Φ^{25}_{66} for the splitting branches 5 and 6 of vertex C. Note that only the entries Φ^{25}_{23}, Φ^{25}_{32} and Φ^{25}_{56}, Φ^{25}_{65} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\alpha := (r_{2A} + r_{3A})(r_{5C} + r_{6C})$.

$$\Phi^{25} = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{r_{2A}}{r_{2A}+r_{3A}} & \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\
\frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{3A}}{r_{2A}+r_{3A}} & \frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\
\frac{r_{2A}}{r_{2A}+r_{3A}} & \frac{r_{3A}}{r_{2A}+r_{3A}} & -\frac{r_{2A}}{r_{2A}+r_{3A}} & 0 & 0 & 0 & 0 \\
\frac{r_{3A}r_{5C}}{\alpha} & -\frac{r_{3A}r_{5C}}{\alpha} & \frac{r_{2A}r_{5C}}{\alpha} & 0 & \frac{r_{6C}}{r_{5C}+r_{6C}} & -\frac{r_{5C}}{r_{5C}+r_{6C}} & 0 \\
\frac{r_{3A}r_{6C}}{\alpha} & -\frac{r_{3A}r_{6C}}{\alpha} & \frac{r_{2A}r_{6C}}{\alpha} & 0 & -\frac{r_{6C}}{r_{5C}+r_{6C}} & \frac{r_{5C}}{r_{5C}+r_{6C}} & 0 \\
\frac{r_{3A}r_{6C}}{\alpha} & -\frac{r_{3A}r_{6C}}{\alpha} & \frac{r_{2A}r_{6C}}{\alpha} & 0 & -\frac{r_{6C}}{r_{5C}+r_{6C}} & \frac{r_{5C}}{r_{5C}+r_{6C}} & 0
\end{pmatrix}$$

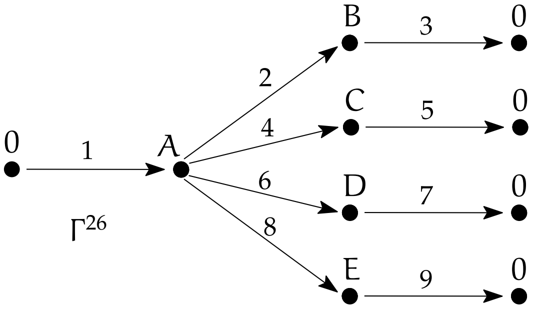


Figure A.2.8: Reaction network Γ^{26} with splitting behaviour in the simple case at branching vertex A. In particular, see the diagonal entries Φ^{26}_{22}, Φ^{26}_{44}, Φ^{26}_{66}, and Φ^{26}_{88} characterizing the splitting branches 2, 4, 6, and 8 in the matrix. Note that the entries Φ^{26}_{24}, Φ^{26}_{42}, Φ^{26}_{26}, Φ^{26}_{62}, Φ^{26}_{28}, Φ^{26}_{82}, Φ^{26}_{46}, Φ^{26}_{64}, Φ^{26}_{48}, Φ^{26}_{84}, Φ^{26}_{68}, and Φ^{26}_{86} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\alpha := r_{2A} + r_{4A} + r_{6A} + r_{8A}$.

$$\Phi^{26} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{\alpha} & \frac{r_{4A}+r_{6A}+r_{8A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 \\ \frac{r_{2A}}{\alpha} & \frac{r_{4A}+r_{6A}+r_{8A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 & -\frac{r_{2A}}{\alpha} & 0 \\ \frac{r_{4A}}{\alpha} & -\frac{r_{4A}}{\alpha} & 0 & \frac{r_{2A}+r_{6A}+r_{8A}}{\alpha} & 0 & -\frac{r_{4A}}{\alpha} & 0 & -\frac{r_{4A}}{\alpha} & 0 \\ \frac{r_{4A}}{\alpha} & -\frac{r_{4A}}{\alpha} & 0 & \frac{r_{2A}+r_{6A}+r_{8A}}{\alpha} & 0 & -\frac{r_{4A}}{\alpha} & 0 & -\frac{r_{4A}}{\alpha} & 0 \\ \frac{r_{6A}}{\alpha} & -\frac{r_{6A}}{\alpha} & 0 & -\frac{r_{6A}}{\alpha} & 0 & \frac{r_{2A}+r_{4A}+r_{8A}}{\alpha} & 0 & -\frac{r_{6A}}{\alpha} & 0 \\ \frac{r_{6A}}{\alpha} & -\frac{r_{6A}}{\alpha} & 0 & -\frac{r_{6A}}{\alpha} & 0 & \frac{r_{2A}+r_{4A}+r_{8A}}{\alpha} & 0 & -\frac{r_{6A}}{\alpha} & 0 \\ \frac{r_{8A}}{\alpha} & -\frac{r_{8A}}{\alpha} & 0 & -\frac{r_{8A}}{\alpha} & 0 & -\frac{r_{8A}}{\alpha} & 0 & \frac{r_{2A}+r_{4A}+r_{6A}}{\alpha} & 0 \\ \frac{r_{8A}}{\alpha} & -\frac{r_{8A}}{\alpha} & 0 & -\frac{r_{8A}}{\alpha} & 0 & -\frac{r_{8A}}{\alpha} & 0 & \frac{r_{2A}+r_{4A}+r_{6A}}{\alpha} & 0 \end{pmatrix}$$

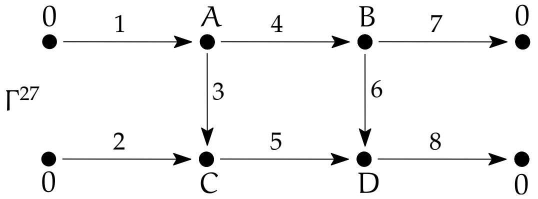


Figure A.2.9: Reaction network Γ^{27} with splitting behaviour in the simple case at branching vertices A and B. In particular, see the diagonal entries Φ^{27}_{33} and Φ^{27}_{44} for the splitting branches 3 and 4 of vertex A, and the diagonal entries Φ^{27}_{66} and Φ^{27}_{77} for the splitting branches 6 and 7 of vertex B. Note that only the entries Φ^{27}_{34}, Φ^{27}_{43} and Φ^{27}_{67}, Φ^{27}_{76} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\alpha := (r_{3A} + r_{4A})(r_{6B} + r_{7B})$

$$\Phi^{27} = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & \frac{r_{4A}}{r_{3A}+r_{4A}} & -\frac{r_{3A}}{r_{3A}+r_{4A}} & 0 & 0 & 0 & 0 \\
\frac{r_{4A}}{r_{3A}+r_{4A}} & 0 & -\frac{r_{4A}}{r_{3A}+r_{4A}} & \frac{r_{3A}}{r_{3A}+r_{4A}} & 0 & 0 & 0 & 0 \\
\frac{r_{3A}}{r_{3A}+r_{4A}} & 1 & \frac{r_{4A}}{r_{3A}+r_{4A}} & -\frac{r_{3A}}{r_{3A}+r_{4A}} & 0 & 0 & 0 & 0 \\
\frac{r_{4A}r_{6B}}{\alpha} & 0 & -\frac{r_{4A}r_{6B}}{\alpha} & \frac{r_{3A}r_{6B}}{\alpha} & 0 & \frac{r_{7B}}{r_{6B}+r_{7B}} & -\frac{r_{6B}}{r_{6B}+r_{7B}} & 0 \\
\frac{r_{4A}r_{7B}}{\alpha} & 0 & -\frac{r_{4A}r_{7B}}{\alpha} & \frac{r_{3A}r_{7B}}{\alpha} & 0 & -\frac{r_{7B}}{r_{6B}+r_{7B}} & \frac{r_{6B}}{r_{6B}+r_{7B}} & 0 \\
\frac{r_{4A}r_{6B}+r_{3A}(r_{6B}+r_{7B})}{\alpha} & 1 & \frac{r_{4A}r_{7B}}{\alpha} & -\frac{r_{3A}r_{7B}}{\alpha} & 0 & \frac{r_{7B}}{r_{6B}+r_{7B}} & -\frac{r_{6B}}{r_{6B}+r_{7B}} & 0
\end{pmatrix}$$

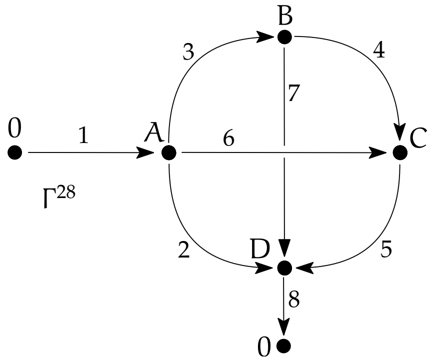


Figure A.2.10: Reaction network Γ^{28} with splitting behaviour in the simple case at branching vertices A and B. In particular, see the diagonal entries Φ^{28}_{22}, Φ^{28}_{33}, and Φ^{28}_{66} for the splitting branches 2, 3, and 6 of vertex A, and the diagonal entries Φ^{28}_{44} and Φ^{28}_{77} for the splitting branches 4 and 7 of vertex B. Note that the entries Φ^{28}_{23}, Φ^{28}_{32}, Φ^{28}_{26}, Φ^{28}_{62}, Φ^{28}_{36}, Φ^{28}_{63}, and Φ^{28}_{47}, Φ^{28}_{74} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\alpha := r_{2A} + r_{3A} + r_{6A}$ and $\beta := r_{4B} + r_{7B}$.

$$\Phi^{28} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{\alpha} & \frac{r_{3A}+r_{6A}}{\alpha} & -\frac{r_{2A}}{\alpha} & 0 & 0 & -\frac{r_{2A}}{\alpha} & 0 & 0 \\ \frac{r_{3A}}{\alpha} & -\frac{r_{3A}}{\alpha} & \frac{r_{2A}+r_{6A}}{\alpha} & 0 & 0 & -\frac{r_{3A}}{\alpha} & 0 & 0 \\ \frac{r_{3A}r_{4B}}{\alpha\beta} & -\frac{r_{3A}r_{4B}}{\alpha\beta} & \frac{r_{4B}(r_{2A}+r_{6A})}{\alpha\beta} & \frac{r_{7B}}{\beta} & 0 & -\frac{r_{3A}r_{4B}}{\alpha\beta} & -\frac{r_{4B}}{\beta} & 0 \\ \frac{r_{6A}\beta+r_{3A}r_{4B}}{\alpha\beta} & -\frac{r_{6A}\beta+r_{3A}r_{4B}}{\alpha\beta} & \frac{r_{4B}r_{2A}-r_{6A}r_{7B}}{\alpha\beta} & \frac{r_{7B}}{\beta} & 0 & \frac{r_{2A}\beta+r_{4B}r_{7B}}{\alpha\beta} & -\frac{r_{4B}}{\beta} & 0 \\ \frac{r_{6A}}{\alpha} & -\frac{r_{6A}}{\alpha} & -\frac{r_{6A}}{\alpha} & 0 & 0 & \frac{r_{2A}+r_{3A}}{\alpha} & 0 & 0 \\ \frac{r_{3A}r_{7B}}{\alpha\beta} & -\frac{r_{3A}r_{7B}}{\alpha\beta} & \frac{r_{7B}(r_{2A}+r_{6A})}{\alpha\beta} & -\frac{r_{7B}}{\beta} & 0 & -\frac{r_{3A}r_{7B}}{\alpha\beta} & \frac{r_{4B}}{\beta} & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

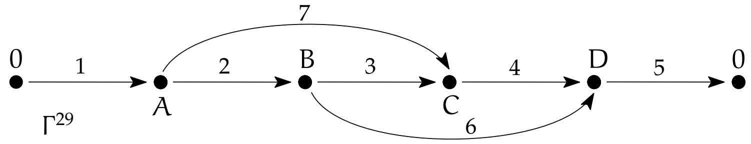


Figure A.2.11: Reaction network Γ^{29} with splitting behaviour in the simple case at branching vertices A and B. In particular, see the diagonal entries Φ^{29}_{22} and Φ^{29}_{77} for the splitting branches 2 and 7 of vertex A, and the diagonal entries Φ^{29}_{33} and Φ^{29}_{66} for the splitting branches 3 and 6 of vertex B. Note that only the entries Φ^{29}_{27}, Φ^{29}_{72} and Φ^{29}_{36}, Φ^{29}_{63} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3. Define $\omega := (r_{2A} + r_{7A})(r_{3B} + r_{6B})$.

$$\Phi^{29} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}}{r_{2A}+r_{7A}} & \frac{r_{7A}}{r_{2A}+r_{7A}} & 0 & 0 & 0 & 0 & -\frac{r_{2A}}{r_{2A}+r_{7A}} \\ \frac{r_{2A}r_{3B}}{\omega} & \frac{r_{7A}r_{3B}}{\omega} & \frac{r_{6B}}{r_{3B}+r_{6B}} & 0 & 0 & -\frac{r_{3B}}{r_{3B}+r_{6B}} & -\frac{r_{2A}r_{3B}}{\omega} \\ \frac{r_{2A}r_{3B}+r_{7A}(r_{3B}+r_{6B})}{\omega} & -\frac{r_{7A}r_{6B}}{\omega} & \frac{r_{6B}}{r_{3B}+r_{6B}} & 0 & 0 & -\frac{r_{3B}}{r_{3B}+r_{6B}} & \frac{r_{2A}r_{6B}}{\omega} \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{2A}r_{6B}}{\omega} & \frac{r_{7A}r_{6B}}{\omega} & -\frac{r_{6B}}{r_{3B}+r_{6B}} & 0 & 0 & \frac{r_{3B}}{r_{3B}+r_{6B}} & -\frac{r_{2A}r_{6B}}{\omega} \\ \frac{r_{7A}}{r_{2A}+r_{7A}} & -\frac{r_{7A}}{r_{2A}+r_{7A}} & 0 & 0 & 0 & 0 & \frac{r_{2A}}{r_{2A}+r_{7A}} \end{pmatrix}$$

A.3 SPLITTING BEHAVIOUR IN THE MORE INVOLVED CASE

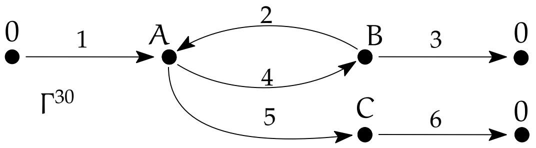


Figure A.3.1: Reaction network Γ^{30} with splitting behaviour in the more involved case at branching vertices A and B, which are strongly connected. In particular, see the diagonal entries Φ^{30}_{44} and Φ^{30}_{55} for the splitting branches 4 and 5 of vertex A, and the diagonal entries Φ^{30}_{22} and Φ^{30}_{33} for the splitting branches 2 and 3 of vertex B. Define $\alpha := r_{2B}r_{5A} + r_{3B}r_{4A} + r_{3B}r_{5A}$.

$$\Phi^{30} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{r_{4A}r_{2B}}{\alpha} & \frac{r_{3B}(r_{5A}+r_{4A})}{\alpha} & -\frac{r_{2B}(r_{5A}+r_{4A})}{\alpha} & \frac{r_{2B}r_{5A}}{\alpha} & -\frac{r_{2B}r_{4A}}{\alpha} & 0 \\ \frac{r_{4A}r_{3B}}{\alpha} & -\frac{r_{3B}r_{5A}}{\alpha} & \frac{r_{2B}r_{5A}}{\alpha} & \frac{r_{3B}r_{5A}}{\alpha} & -\frac{r_{3B}r_{4A}}{\alpha} & 0 \\ \frac{r_{4A}(r_{3B}+r_{2B})}{\alpha} & \frac{r_{3B}r_{4A}}{\alpha} & -\frac{r_{2B}r_{4A}}{\alpha} & \frac{r_{5A}(r_{2B}+r_{3B})}{\alpha} & -\frac{r_{4A}(r_{2B}+r_{3B})}{\alpha} & 0 \\ \frac{r_{5A}(r_{3B}+r_{2B})}{\alpha} & \frac{r_{3B}r_{5A}}{\alpha} & -\frac{r_{2B}r_{5A}}{\alpha} & -\frac{r_{5A}r_{3B}}{\alpha} & \frac{r_{4A}r_{3B}}{\alpha} & 0 \\ \frac{r_{5A}(r_{3B}+r_{2B})}{\alpha} & \frac{r_{3B}r_{5A}}{\alpha} & -\frac{r_{2B}r_{5A}}{\alpha} & -\frac{r_{5A}r_{3B}}{\alpha} & \frac{r_{4A}r_{3B}}{\alpha} & 0 \end{pmatrix}$$

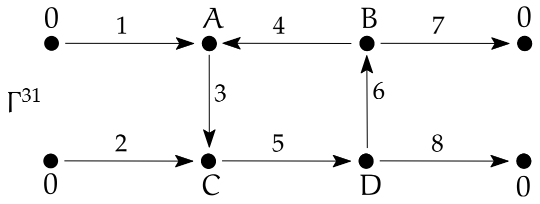


Figure A.3.2: Reaction network Γ^{31} with splitting behaviour in the more involved case at branching vertices B and D, which are strongly connected. In particular, see the diagonal entries Φ^{31}_{44} and Φ^{31}_{77} for the splitting branches 4 and 7 of vertex B, and the diagonal entries Φ^{31}_{66} and Φ^{31}_{88} for the splitting branches 6 and 8 of vertex D. Define $\alpha := r_{4B} + r_{7B}$, $\beta := r_{6D} + r_{8D}$, and $\omega := r_{6D}r_{7B} + \alpha r_{8D}$.

$$\Phi^{31} = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{\alpha\beta}{\omega} & \frac{r_{6D}r_{4B}}{\omega} & 0 & \frac{r_{7B}\beta}{\omega} & 0 & \frac{r_{8D}r_{4B}}{\omega} & -\frac{r_{4B}\beta}{\omega} & -\frac{r_{6D}r_{4B}}{\omega} \\
\frac{r_{6D}r_{4B}}{\omega} & \frac{r_{6D}r_{4B}}{\omega} & 0 & \frac{r_{7B}\beta}{\omega} & 0 & \frac{r_{8D}r_{4B}}{\omega} & -\frac{r_{4B}\beta}{\omega} & -\frac{r_{6D}r_{4B}}{\omega} \\
1 + \frac{r_{6D}r_{4B}}{\omega} & \frac{\alpha\beta}{\omega} & 0 & \frac{r_{7B}\beta}{\omega} & 0 & \frac{r_{8D}r_{4B}}{\omega} & -\frac{r_{4B}\beta}{\omega} & \frac{r_{6D}r_{4B}}{\omega} \\
\frac{r_{6D}r_{7B}}{\omega} & \frac{r_{6D}\alpha}{\beta} & 0 & \frac{r_{7B}r_{6D}}{\omega} & 0 & \frac{r_{8D}\alpha}{\omega} & -\frac{r_{4B}r_{6D}}{\omega} & -\frac{r_{6D}\alpha}{\omega} \\
\frac{r_{6D}r_{7B}}{\omega} & \frac{r_{6D}r_{7B}}{\omega} & 0 & -\frac{r_{7B}r_{8D}}{\omega} & 0 & \frac{r_{8D}r_{7B}}{\omega} & \frac{r_{4B}r_{8D}}{\omega} & -\frac{r_{6D}r_{7B}}{\omega} \\
\frac{r_{8D}\alpha}{\omega} & \frac{r_{8D}\alpha}{\omega} & 0 & \frac{r_{7B}r_{8D}}{\omega} & 0 & -\frac{r_{7B}r_{8D}}{\omega} & -\frac{r_{8D}r_{4B}}{\omega} & \frac{r_{6D}r_{7B}}{\omega}
\end{pmatrix}$$

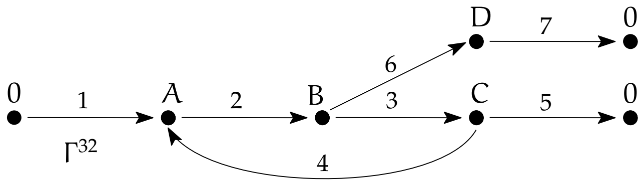


Figure A.3.3: Reaction network Γ^{32} with splitting behaviour in the more involved case at branching vertices B and C, which are strongly connected. In particular, see the diagonal entries Φ^{32}_{33} and Φ^{32}_{66} for the splitting branches 3 and 6 of vertex B, and the diagonal entries Φ^{32}_{44} and Φ^{32}_{55} for the splitting branches 4 and 5 of vertex C. Define $\omega := r_{3B}r_{5C} + r_{6B}(r_{4C} + r_{5C})$.

$$\Phi^{32} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 + \frac{r_{3B}r_{4C}}{\omega} & 0 & \frac{r_{6B}r_{4C}}{\omega} & \frac{r_{5C}(r_{3B}+r_{6B})}{\omega} & -\frac{r_{4C}(r_{3B}+r_{6B})}{\omega} & \frac{r_{3B}r_{4C}}{\omega} & 0 \\ \frac{r_{3B}(r_{4C}+r_{5C})}{\omega} & 0 & \frac{r_{6B}(r_{4C}+r_{5C})}{\omega} & \frac{r_{3B}r_{5C}}{\omega} & -\frac{r_{4C}r_{3B}}{\omega} & \frac{r_{3B}(r_{4C}-r_{5C})}{\omega} & 0 \\ \frac{r_{3B}r_{4C}}{\omega} & 0 & \frac{r_{6B}r_{4C}}{\omega} & \frac{r_{4C}(r_{3B}+r_{6B})}{\omega} & -\frac{r_{4C}(r_{3B}+r_{6B})}{\omega} & \frac{r_{3B}r_{4C}}{\omega} & 0 \\ \frac{r_{6B}r_{5C}}{\omega} & 0 & \frac{r_{6B}r_{5C}}{\omega} & -\frac{r_{6B}r_{5C}}{\omega} & \frac{r_{6B}r_{4C}}{\omega} & -\frac{r_{3B}r_{5C}}{\omega} & 0 \\ \frac{r_{6B}(r_{4C}+r_{5C})}{\omega} & 0 & -\frac{r_{6B}r_{5C}}{\omega} & \frac{r_{6B}r_{5C}}{\omega} & -\frac{r_{6B}r_{4C}}{\omega} & \frac{r_{3B}r_{5C}}{\omega} & 0 \\ \frac{r_{6B}(r_{4C}+r_{5C})}{\omega} & 0 & -\frac{r_{6B}r_{5C}}{\omega} & \frac{r_{6B}r_{5C}}{\omega} & -\frac{r_{6B}r_{4C}}{\omega} & \frac{r_{3B}r_{5C}}{\omega} & 0 \end{pmatrix}$$

A.4 FEEDBACK BEHAVIOUR AND SPLITTING BEHAVIOUR

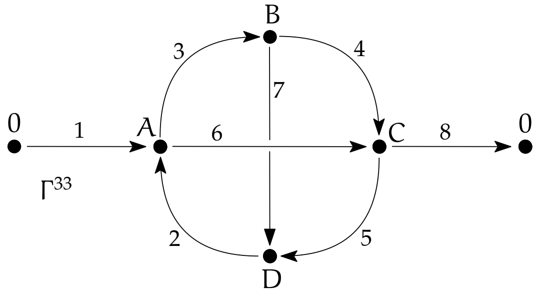


Figure A.4.1: Reaction network Γ^{33} with feedback behaviour at branching vertex C and splitting behaviour in the more involved case at branching vertices A and B, which are strongly connected. In particular, see the diagonal entries $\Phi^{33}_{55} = 1$ for the feedback branch 5 and $\Phi^{33}_{88} = 0$ for the other branch 8 of vertex C. Further, see the diagonal entries Φ^{33}_{33} and Φ^{33}_{66} for the splitting branches 3 and 6 of vertex A, and the diagonal entries Φ^{33}_{44} and Φ^{33}_{77} for the splitting branches 4 and 7 of vertex B. Define $\alpha := r_{4B} + r_{7B}$, $\beta := r_{5C} + r_{8C}$, $\gamma := r_{3A} + r_{6A}$, and $\omega := r_{4B} r_{3A} + r_{6A}\alpha$.

$$\Phi^{33} = \begin{pmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{r_{5C}\omega + r_{7B} r_{3A} \beta}{r_{8C}\omega} & 0 & \frac{r_{6A} r_{7B}}{\omega} & -\frac{r_{7B}\gamma}{\omega} & 1 + \frac{r_{3A} r_{7B}}{\omega} & -\frac{r_{3A} r_{7B}}{\omega} & \frac{r_{4B}\gamma}{\omega} & -\frac{r_{5C}\omega + r_{7B} r_{3A} r_{5C}}{r_{8C}\omega} \\
\frac{r_{3A}\alpha\beta}{r_{8C}\omega} & 0 & \frac{r_{6A}\alpha}{\omega} & -\frac{r_{7B} r_{3A}}{\omega} & \frac{r_{3A}\alpha}{\omega} & -\frac{r_{3A}\alpha}{\omega} & \frac{r_{4B} r_{3A}}{\omega} & \frac{r_{3A} r_{5C}\alpha}{r_{8C}\omega} \\
\frac{r_{3A} r_{4B}\beta}{r_{8C}\omega} & 0 & \frac{r_{6A} r_{4B}}{\omega} & \frac{r_{7B} r_{6A}}{\omega} & \frac{r_{3A} r_{4B}}{\omega} & -\frac{r_{3A} r_{4B}}{\omega} & -\frac{r_{4B} r_{6A}}{\omega} & -\frac{r_{3A} r_{5C} r_{4B}}{r_{8C}\omega} \\
\frac{r_{5C}}{r_{8C}} & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
\frac{r_{6A}\alpha\beta}{r_{8C}\omega} & 0 & -\frac{r_{6A} r_{4B}}{\omega} & -\frac{r_{7B} r_{6A}}{\omega} & \frac{r_{6A}\alpha}{\omega} & \frac{r_{3A} r_{4B}}{\omega} & \frac{r_{4B} r_{6A}}{\omega} & -\frac{r_{6A} r_{5C}\alpha}{r_{8C}\omega} \\
\frac{r_{7B} r_{3A}\beta}{r_{8C}\omega} & 0 & \frac{r_{6A} r_{7B}}{\omega} & -\frac{r_{7B}\gamma}{\omega} & \frac{r_{3A} r_{7B}}{\omega} & -\frac{r_{3A} r_{7B}}{\omega} & \frac{r_{4B}\gamma}{\omega} & -\frac{r_{7B} r_{3A} r_{5C}}{r_{8C}\omega} \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}$$

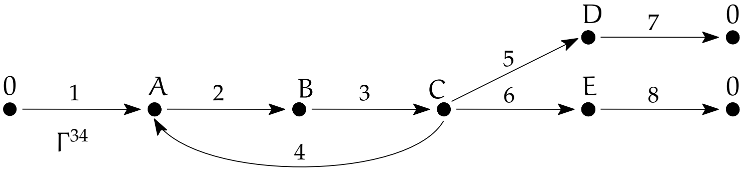


Figure A.4.2: Reaction network Γ^{34} with feedback and splitting behaviour in the simple case at branching vertex C. In particular, see the diagonal entries $\Phi^{34}_{44} = 1$ for the feedback branch 4, and Φ^{34}_{55} and Φ^{34}_{66} characterizing the splitting branches 5 and 6 in the matrix. Note that only the entries Φ^{34}_{56} and Φ^{34}_{65} do not have one matrix entry of each reciprocal off-diagonal pair be zero, but still fulfill the conclusions of the Reciprocity Theorem 3.3.

$$\Phi^{34} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 + \frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}+r_{6C}} & -\frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 \\ 1 + \frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}+r_{6C}} & -\frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 \\ \frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 & 1 & -\frac{r_{4C}}{r_{5C}+r_{6C}} & -\frac{r_{4C}}{r_{5C}+r_{6C}} & 0 & 0 \\ \frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 & 0 & \frac{r_{6C}}{r_{5C}+r_{6C}} & -\frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 \\ \frac{r_{6C}}{r_{5C}+r_{6C}} & 0 & 0 & 0 & -\frac{r_{6C}}{r_{5C}+r_{6C}} & \frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 \\ \frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 & 0 & \frac{r_{6C}}{r_{5C}+r_{6C}} & -\frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 \\ \frac{r_{6C}}{r_{5C}+r_{6C}} & 0 & 0 & 0 & -\frac{r_{6C}}{r_{5C}+r_{6C}} & \frac{r_{5C}}{r_{5C}+r_{6C}} & 0 & 0 \end{pmatrix}$$

MISCELLANEOUS

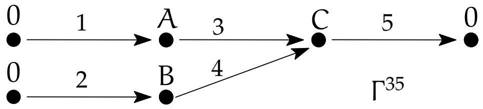


Figure A.4.3: Reaction network Γ^{35}. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{35} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \end{pmatrix}$$

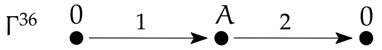


Figure A.4.4: Reaction network Γ^{36} from section 2.1. Note that one matrix entry of each reciprocal off-diagonal pair is zero.

$$\Phi^{36} = \begin{pmatrix} 1 & 0 \\ 1 & 0 \end{pmatrix}$$

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