Sensitivity of metabolic networks

Dissertation zur Erlangung des Grades eines
Doktors der Naturwissenschaften

eingereicht am
Fachbereich Mathematik und Informatik
der Freien Universität Berlin

vorgelegt von

Nicola Vassena

October 31, 2019

Erstgutachter und Betreuer:
Prof. Dr. Bernold Fiedler, Freie Universität Berlin

Zweitgutachter:
Prof. Dr. Peter Szmolyan, Technische Universität Wien

Abstract

In this thesis we present a comprehensive analysis of sensitivity of metabolic chemical reaction networks, with general kinetics. Sensitivity studies the network response to perturbations. We consider local perturbations of the network components - metabolite concentrations or reaction rates - at a dynamical equilibrium. We investigate the responses in the network, both of the metabolite concentrations and of the reaction fluxes. Firstly, we describe which components of the network respond, at all. Secondly, we analyze whether their responses are positive, negative, or whether the sign depends on the parameters of the system.
Sign changes of the Jacobian determinant play an important overall role both in sensitivity analysis and in the bifurcation of equilibria. The first part of this thesis distinguishes reaction network Jacobians with constant sign from the bifurcation case, where that sign depends on specific values of reaction rates.

Our approach is purely qualitative, rather than quantitative. In fact, our analysis is based, solely, on the stoichiometry of the reaction network. We do not require any quantitative information on the reaction rates. Instead, the description is done only in algebraic terms, and the only data required is the network structure.

Biological applications include detection of multistationarity, enzyme knock-out experiments, and metabolic control.

Acknowledgements

First of all I must thank Bernold Fiedler. The amount of scientific knowledge he transmitted me is incommensurable. He taught me definitely much more than science: things that are expressible and things that are inexpressible. *Fliegt der Schnee mir ins Gesicht, schüttl' ich ihn herunter. Wenn mein Herz im Busen spricht, sing' ich hell und munter.* A very careful listen (or to some extent less careful play) of Schubert's F-minor Fantasia for four hands is needed.

I am very grateful to Peter Szmolyan to have accepted to read and evaluate my thesis.

The entire nonlinear dynamics group at the Free University constituted the fundamental diverse background throughout these years. The characters in this play are so close to me that given names suffice: Yuya, Mark, Isabelle, Adem, Babette, Dennis, Bernhard, Martin, Juliette, Abderrahim, Stefan, Pavel, Nacho. In particular, Nikita, Dr.Jia, Alejandro, and Phillipo proofread this thesis; last-minute walks with Hannes greatly clarified some troublesome points; Anna kindly allowed me to use her magisterial picture; Ulrike, Punta, and Patricia saved so many different situations in so many different ways. I owe them all very much.

I greatly enjoyed proficuous scientific (and non) discussions with Timo de Wolff, Murad Banaji, Polly Yu, and Markus Köbis.

Carlos and Isabela Rocha were very warm hosts while I was writing this thesis in Lisbon. Many pastéis de nata, the wind on the ocean, and two silhouettes on the blue are also to be thanked.

My family is the basis: metaphorically and literally speaking. No doctoral thesis in science is required to understand what happens to whatever lacking a basis.

No nearer am I to exhaust all the people I should thank, ma lo spazio è tiranno. I will plainly say: "I thank all my friends". Plain but deep sentence.

This doctoral thesis has been supported by the Collaborative Research Center 910, the Berlin Mathematical School and the viennese Erwin Schrödinger Institute.

Contents

In the dime stores and bus stations,
People talk of situations,
Read books, repeat quotations,
Draw conclusions on the wall.

Bob Dylan

Chapter 1

Preambulum

The main aim of this thesis is to establish a rational approach to sensitivity analysis of equilibria for metabolic chemical reaction networks. Sensitivity analysis, loosely speaking, investigates how a network responds to perturbations.

Let us wander a bit through etymology, first. The word *sensitivity* forms irregularly from the Latin verb *sentire*, which means *to feel*. Main tools of perception are indeed the *senses*, from the same etymological root. Sentimental, in English, is a person who overfeels. The wonderful jazz standard *"In a sentimental mood"*, by Duke Ellington, perfectly describes this feature. By extension of the signification, the Italian word *sensitivo* has taken on the meaning of medium, clairvoyant, psychic. Namely, a person who feels something. Literally, thus, sensitivity is the capacity of a network to feel.

What does a network feel? The network can feel, for example, external intrusions. Here, we are interested in intrusions, which do not alter the structure of the network. We can perturb some network components, such as a reaction rate or a metabolite concentration and we may naturally ask, then, which other components have been *influenced* by our direct perturbation, and which have remained unaffected. This concept applies to a large variety of phenomena. Parameter fluctuations, for instance, can easily be induced both by environmental as well as genetic agents. The impact of parameter fluctuations on the network is, therefore, a matter of crucial importance.

More specifically, and for simplicity of presentation, we focus on networks at a *dynamical equilibrium*. Here, dynamical equilibrium means, simply, that metabolite concentrations are stationary in time. Also for simplicity, at first, we study the effect of small perturbations. Then, mathematically, the central object of sensitivity analysis is the *sensitivity matrix*. This matrix encodes the partial derivatives of the responsive components with respect to the parameters, at equilibrium. For a chemical network case, natural responsive components to be considered are the concentration of chemicals, and the reaction fluxes.
In applications, precise measurements are often very difficult - if not impossible - and reaction rates remain largely unknown in most specific cases. For this reason,

we aim for a comprehensive *qualitative analysis* rather than quantitative numerical simulations for one or another set of guesswork parameters. Our qualitative analysis is based on the structure of the network, only.

Various types of sensitivity analysis are common in the frame of chemistry. We refer to the survey paper [SRTC05] for more detailed references. An interesting approach, in a deterministic context, has been developed by Shinar, Feinberg, and co-authors [SAF09, SF10, SF11]. In this body of work, the concept of *absolute conentration robustness* (ACR) has been introduced. In the authors' words [SF11], "a model biochemical system has ACR relative to a particular bio-active molecular species if [...] the concentration of that species is the same in all of the positive steady states that the system might admit, regardless of the overall supplies of the various network constituents". ACR thus indicates zero sensitivity of the concentration of a certain species with respect to the other network components. Moreover, in [SMJF11] Shinar and co-authors were able to derive quantitative bounds on the entries of the sensitivity matrix for reaction fluxes, in a mass-action kinetics context and for a regular class of networks.

In parallel, Fiedler and Mochizuki pursued a sensitivity analysis in a more metabolically oriented context [MF15,FM15], with applications to the central glucose metabolism of Escherichia Coli. These works, and the generalization [BF18], were the starting point for the present thesis.

We consider general metabolic chemical reaction networks $\mathbf{\Gamma}$ with M metabolites and N reactions. For notation, we use labels $A, B, C, D, ...$ for metabolites and $1, 2, 3, ...$ for reactions. We call $\mathbf{M}$ the set of metabolites and $\mathbf{E}$ the set of reactions, such that $|\mathbf{M}| = M$ and $|\mathbf{E}| = N$. We use the small letter $m \in \mathbf{M}$ for a generic metabolite and the small letter $j \in \mathbf{E}$ for a generic reaction.

A chemical reaction j is represented as

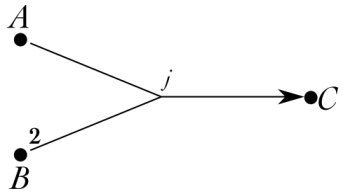
$$j: \quad s_1^j m_1 + ... + s_M^j m_M \underset{j}{\longrightarrow} \bar{s}_1^j m_1 + ... + \bar{s}_M^j m_M, \tag{1.1}$$

with nonnegative stoichiometric coefficients $s^j, \bar{s}^j \in \mathbb{R}$. In a metabolic context, often, these coefficients are only 0 or 1. However, the work of this thesis possibly applies to real stoichiometric coefficients, as well.
A metabolite m is called an *input* or a *reactant* of the reaction j, if $s_m^j \neq 0$. Respectively, m is called an *output* or a *product* of the reaction j, if $\bar{s}_m^j \neq 0$. We say, conversely, that a reaction j is *outgoing* from the metabolite m if m is an input of reaction j. We say that a reaction j is an *ingoing* reaction of the metabolite m if m is an output of the reaction j.
Metabolic systems are intrinsically open systems, that is, they exchange chemicals with the outside environment by feed and exit reactions. Within our settings, the constant *feed reactions*, or *inflows*, are reactions with no inputs ($s^j = 0$) and the *exit reactions*, or *outflows*, are reactions with no outputs ($\bar{s}^j = 0$).

Graphically, we represent a reaction

$$j: \quad A + 2B \underset{j}{\longrightarrow} C, \tag{1.2}$$

where we have omitted stoichiometrically zero terms, as follows.



(1.3)

In (1.3), the arrow orientation is inherited from (1.2). The stoichiometric coefficient 2 of metabolite B is indicated as a weight in the lower tail of the directed arrow j, and stoichiometric coefficients 1 are omitted, as well as non-participating other reactants. In particular, this graphical representation considers the metabolites as vertices and the reactions as arrows of the network, and it is one natural representation widely used in chemistry, biology, and mathematics.

Explicit autocatalytic reactions j are defined as reactions for which a metabolite m is both an input and an output of the reaction. In symbols, $s_m^j, \bar{s}_m^j \neq 0$, for at least one metabolite m. **Throughout this thesis, we exclude explicit autocatalytic reactions**. In particular, self-loops are not allowed in the graphical representation of the network.

To construct the $M \times N$ stoichiometric matrix S, let us consider any reaction j. We associate to any stoichiometric coefficient s_m^j of an *input* metabolite m of the reaction j a *negative* stoichiometric entry of the stoichiometric matrix S, that is:

$$S_{mj} := -s_m^j\,, \qquad \text{for } m \text{ input of } j. \tag{1.4}$$

Conversely, we associate to any stoichiometric coefficient $\bar{s}_m^j$ of an *output* metabolite m of the reaction j a *positive* stoichiometric entry of S, that is:

$$S_{mj} := \bar{s}_m^j\,, \qquad \text{for } m \text{ output of } j. \tag{1.5}$$

For example, a *monomolecular reaction* j is a reaction which possesses as input one single metabolite m_1 and as output one single metabolite m_2,

$$m_1 \underset{j}{\longrightarrow} m_2. \tag{1.6}$$

Such a reaction translates into the j^{th} column of the stoichiometric matrix S as

$$S^j = \begin{array}{c} \\ m_1 \\ m_2 \\ m_3 \\ \dots \\ m_M \end{array} \begin{array}{c} j \\ \begin{pmatrix} -1 \\ 1 \\ 0 \\ \dots \\ 0 \end{pmatrix} \end{array}. \tag{1.7}$$

Here we have indicated the rows by $m_1, ..., m_M$, explicitly. With this construction, in particular, we model a reversible reaction

$$j: \quad A + B \underset{j}{\longleftrightarrow} C \tag{1.8}$$

simply as two irreversible reactions

$$j_1: \quad A + B \underset{j_1}{\longrightarrow} C \quad \text{and} \quad j_2: \quad C \underset{j_2}{\longrightarrow} A + B. \tag{1.9}$$

Columns associated to feed reactions possess only positive entries and the ones associated to exit reactions possess only negative entries. All other columns possess both positive and negative entries.

Let $x_m(t)$ be the time evolution of the concentration of the metabolite m. The isothermal dynamics of the vector $x \in \mathbb{R}^M$ of the concentrations is described by the system of differential equations

$$\dot{x} = f(x) := S\mathbf{r}(x). \tag{1.10}$$

The $M \times N$ matrix S is the stoichiometric matrix constructed above. The N-dimensional vector $\mathbf{r}(x)$ represents the reaction rates as functions of x: the *kinetics* of the system. The feed reactions are represented by constant functions. That is:

$$r_{j_f}(x) \equiv K_{j_f}, \tag{1.11}$$

for a feed reaction j_f. **Throughout this thesis, we pose the following assumptions on the reaction rates $\mathbf{r}(x)$:**

1. We assume the reaction rates $r_j(x)$ to depend only on those concentrations x_m such that the metabolite m is an input metabolite of reaction j. In particular,

$$\frac{\partial r_j(x)}{\partial x_m} \equiv 0, \quad \text{unless } m \text{ is an input of } j. \tag{1.12}$$

   Moreover, we use the notation r_{jm} for the nonzero partial derivatives, i.e.,

$$r_{jm} := \frac{\partial r_j(x)}{\partial x_m} \neq 0 \tag{1.13}$$

   if m is an input of reaction j.

2. We consider strictly positive monotone reaction rate functions $r_j(x) \in C^1$, for every $j = 1, ..., N$:

$$r_j(x) > 0 \quad \text{for} \quad x > 0, \tag{1.14}$$

   and, for the nonzero partial derivatives r_{jm}, strictly positive slopes

$$r_{jm} > 0. \tag{1.15}$$

   This monotonicity restriction is indeed satisfied for most, but not all, chemical reaction schemes. Without any constraint on the sign of r_{jm}, we will not be able to predict the sign of sensitivity, of course.

With these assumptions, *all required information of the network is completely encoded in the stoichiometric matrix S, only.* This fact perfectly suits with our initial intent to develop a theory requiring solely the structure of the network as a data. In particular, we do not specify the mathematical form of the kinetics. Chemical kinetics is a hugely vast field of study; for further reading, and better reference, see the encyclopedia [CCK].

Great effort in the mathematical community has been spent in finding network characterizations of the existence and the uniqueness of equilibrium solutions of (1.10). See the account of Horn and Jackson [HJ72], for example, and the comprehensive book by Martin Feinberg [Fei19], for an extensive reference.
With our approach, we do not address this question at all. In fact, **throughout this thesis, we assume the existence of a dynamical equilibrium** x^* that solves

$$0 = f(x^*) := S\mathbf{r}(x^*). \tag{1.16}$$

The assumption of the existence of a dynamical equilibrium is not smoothly untroubled. In particular, linear constraints have been implicitly imposed on the reaction rates $\mathbf{r}$, because of (1.16). Note that these constraints do not necessarily fix the precise value of an equilibrium x^*, and can be considered posed a priori, so that the existence of the equilibrium is an assumption on the reaction rates $\mathbf{r}$, only. Here, our analysis is based entirely on the derivatives r_{jm} of the reaction rates and we do not want to be concerned by the constraints (1.16). To avoid this, we must assume a certain independence of the derivatives r_{jm} from the reaction rates themselves. In particular, we require the possibility of choosing freely the value of any r_{jm}, independently from each other and from the equilibrium constraints $S\mathbf{r} = 0$. In this sense, the partial derivatives r_{jm} can be considered *positive free parameters.* This requires a certain mathematical complexity of the reaction rates r_j. In fact: too mathematically 'simple' kinetics fail to satisfy this assumption. As an example, for polynomial mass-action kinetics, the value of $r_j(x)$ and $r_{jm}(x)$ are related, a priori, at any value x, and for any j and m. In particular, the theory developed here does not apply to mass-action kinetics. In contrast, Michealis-Menten kinetics satisfy our independence assumption [Fie19].

In general, the value and even the existence of a positive equilibrium x^* depends on the constant feed reactions. However, practically, the constant rates disappear once differentiated. Consequently, the feed reactions do not play a role in our analysis, once we have assumed a priori existence of an equilibrium. For this reason, we will often avoid mentioning feed reactions, at all, especially when formulating examples. As a disclaimer, any of our examples should be intended enlarged with suitable feed reactions, for a feasible case of equilibrium analysis.

As mentioned above, a sensitivity analysis of equilibria, within our settings, has been started in 2015 by Fiedler and Mochizuki in a biological [MF15] and a mathematical paper [FM15]. This analysis, and the subsequent work with Brehm [BF18], was restricted to perturbation of reaction rates, and left untouched the case of metabolite concentrations perturbation. Their main interest was to develop a mathematical the-

ory able to model enzyme knock-out experiments on the central glucose metabolism of Escherichia Coli. The experimental paper for their reference was the fundamental contribution [INB+07] by Ishii et al., which investigated the responses of Escherichia Coli to genetic perturbations of knock-out type. The biological paper [MF15] outlined the modeling approach and symbolically computed the responses for a model of the central metabolism of Escherichia Coli.

The approach of Fiedler and Mochizuki used the Implicit Function Theorem (IFT) to address targeted reaction rate perturbations. They considered a perturbed version of the equilibrium system (1.16) of the form

$$0 = S\mathbf{r}^{\varepsilon}(x^*), \qquad \text{where} \qquad \mathbf{r}^{\varepsilon}(x) = \mathbf{r} + \varepsilon e_{j^*}. \tag{1.17}$$

Above, e_{j^*} indicates the j^*-th unit vector in $\mathbb{R}^N$. In this way, only the reaction rate of j^* is perturbed (*targeted perturbation*). For small ε, and under a mild *nondegeneracy condition*, the IFT guarantees the existence of a family of equilibrium solutions $x^*(\varepsilon)$ for the perturbed equation (1.17). By differentiation of (1.17) with respect to ε, Mochizuki and Fielder defined the *metabolite response* $\delta x^{j^*}_{m'}$ of m' to a perturbation of reaction j^* as

$$\delta x^{j^*}_{m'} := \left.\frac{\partial x^*_{m'}(\varepsilon)}{\partial \varepsilon}\right|_{\varepsilon=0}, \tag{1.18}$$

and the *flux response* $\Phi^{j^*}_{j'}$ of reaction j' to a perturbation of reaction j^* as

$$\Phi^{j^*}_{j'} := \left.\frac{\partial \mathbf{r}_{j'}(x^*(\varepsilon))}{\partial \varepsilon}\right|_{\varepsilon=0}. \tag{1.19}$$

These responses constitute the entries of the sensitivity matrix. The nondegeneracy condition of the standard IFT requires the Jacobian matrix

$$G := f_x, \tag{1.20}$$

to be nonsingular:

$$\det G \neq 0. \tag{1.21}$$

The first part of this thesis studies in detail the sign properties of $\det G$, see Chapter 2. Throughout the second part, Chapters 3-6, we assume the nondegeneracy condition (1.21). See Section 3.2 for a detailed discussion on this assumption, from a network point of view.

In practice, the computation of the responses (1.18) and (1.19) requires intense computational effort. Interestingly, the responses computed in [MF15] showed an unexpected and intriguing pattern feature, with a high number of zero responses (*sparsity of sensitivity*) and interrelated responses.
The mathematical companion paper [FM15] started from this intuition, highlighting the algebraic structures responsible for those patterns. The analysis, there, was restricted to the simpler case of monomolecular networks, which allowed a full

description in terms of directed graphs. The nonzero response of m', j', to a perturbation of j^* has been called *nonzero influence of j^* on m', j'*, and it has been denoted with the graphical representation

$$j^* \rightsquigarrow m' \quad , \quad j^* \rightsquigarrow j' \,. \tag{1.22}$$

In particular, transitivity of flux influence

$$j^* \rightsquigarrow j' \quad \text{and} \quad j' \rightsquigarrow j'' \quad \Rightarrow \quad j^* \rightsquigarrow j'' \tag{1.23}$$

was established, for the monomolecular case. The transitivity statement (1.23) looks deceptively simple but turned out to be a delicate topic. In fact, the perturbation spreads also along other components of the network, and this effect needs to be taken in account. In particular, for example, the present thesis shows, in 6.2, how transitivity does not hold in the case of metabolite influence for general multimolecular networks:

$$m^* \rightsquigarrow m' \quad \text{and} \quad m' \rightsquigarrow m'' \quad \not\Rightarrow \quad m^* \rightsquigarrow m''. \tag{1.24}$$

The pattern formation has been further studied by Okada and co-authors in [OM16, OM17, OTM18]. Connections with the existing sensitivity and robustness theory, as developed by Shinar and co-authors, has been investigated by Sasha Siegmund in his Master's Thesis [Sie16]. In the Master's Thesis [Vas16], and in a following joint paper with Matano [VM17], we have given a more elegant formulation, in the monomolecular case, of the transitivity result of [FM15] and we have described the structure of the *influenced sets* $\mathbf{I}(j^*) := \{j' : \Phi^{j^*}_{j'} \neq 0\}$. In 2018, Brehm and Fiedler [BF18] addressed, with similar settings, the multimolecular case, again only for reaction rates perturbation. They achieved an algebraic description of the responses and established transitivity of flux influence (1.23) also for this general case. The main tool of the analysis in [BF18] are the *Child Selections*. A Child Selection map $\mathbf{J}$ is an injective map from the metabolite set $\mathbf{M}$ to the reaction set $\mathbf{E}$, associating to any input *mother* metabolite m an output *child* reaction j, outgoing from m. A Child Selection identifies reshuffled square minors $S^{\mathbf{J}}$ of the stoichiometric matrix, whose m^{th} column corresponds to the stoichiometric column of the reaction $j = \mathbf{J}(m)$. Child Selections, and in particular the minors $S^{\mathbf{J}}$, play a central role in the analysis of the present work.

This thesis was started with two explicit goals:

1. Completing the analysis for the new case of perturbation of the concentration of metabolites.

2. Addressing the question of the sign of the responses.

Chapter 3 and 6 are concerned with the first goal.
In fact, in Chapter 3 a complete analysis of nonzero sensitivity has been done. It is not restricted to targeted perturbations of single components, but investigates any vector perturbation case. Consequently, the analysis covers both cases of perturbation, (metabolite concentrations and reaction rates) and both cases of response

(metabolite concentrations and reaction fluxes). The response of metabolite concentrations to a perturbation of concentrations has been identified with the inverse of the Jacobian matrix, with opposite sign. This is in accordance with the *ecology community*, which studied similar problems. See for example [Yod88] and [Nak92], where the sensitivity matrix for '*food webs*' and '*flow networks*' has been studied.
Interestingly, Section 3.3.4 argues that a metabolite perturbation can be reduced to a reaction perturbation, from a mathematical perspective. In fact: a perturbation of a metabolite m^* corresponds identically to a perturbation of an artificially added exit reaction $j^0_{m^*}$ from m^*, with reverted sign.
Chapter 6 concentrates on the transitivity problem for the missing case of metabolite perturbation and shows, with a simple counterexample, that the Brehm-Fiedler result [BF18] does not extend to any other case of influence.

The sign analysis of the responses is more involved. First of all, the responses turn out to be rational functions of the r_{jm} variables. Because of the Implicit Function Theorem approach, the rational function response presents the Jacobian determinant of the system as the denominator. It is then clear that a sign analysis has firstly to deal with the sign of the Jacobian determinant in the following sense:

When does the sign of the Jacobian determinant depend on the parameters r_{jm}? (1.25)

This and similar questions abound in the literature, both for mass-action and for more general kinetics. However, most of the results are directed towards finding sufficient conditions for the Jacobian to be of fixed sign, that is, which does not depend on the parameters r_{jm}. Fundamental concepts as *deficiency* [Fei87, Fei95], *injectivity* [GN65, CF06, BDB07, BC10], and *concordance* [SF12, SF13] have been developed towards this purpose. In particular, saddle-node bifurcations are excluded.
In contrast, the first part of this thesis, Chapter 2, fully characterizes, on a graphical level, the answer to the question (1.25). In particular we distinguish *good* Child Selections, whose associated minor $S^{\mathbf{J}}$ does not affect the sign, from *bad* Child Selections, which produce a possible change of sign in the determinant, with consequent instability and bifurcation phenomena. Moreover, in Section 2.6, we find bifurcation parameters responsible for a change of sign of the determinant. This last result hints at the possibility of saddle-node bifurcations, caused by such bifurcation parameters.

Chapter 4 addresses the question of the sign of the responses. A major role is played, in this context, by certain elementary kernel vectors v of the stoichiometric matrix S, see Theorem 4.2.2. Kernel vectors of such type are always associated with a Child Selection $\mathbf{J}$, in the sense that nonzero entries of the vector are always in connection with reactions obtained as image of a fixed Child Selection map. Moreover, they need to be *elementary* in the sense that their support, i.e. nonzero entries, is minimal. That is, their support does not properly contain the support of any other kernel vector. Objects of this kind have firstly been studied mathematically by Rockafellar [Roc69] in the sixties, and recently by Klamt and co-authors [KRG+17] in a metabolic context.
In some cases, this kernel analysis alone is not sufficient, see Theorem 4.2.3. Here, we find that the sign of the responses is related to cokernel vectors of some minors

$S^{\mathbf{J}}$. It is well known that cokernel vectors of the whole stoiochiometric matrix identify *conservation laws*. Cokernel vectors of some minors of S may be interpreted as *conservation laws* for the subsystem identified by that minor. However, note that our analysis excludes *conservation laws* for the entire system, a priori, because of the nondegeneracy assumption (1.21).
Intuitively, if the sign of the Jacobian depends on the parameters r_{jm}, the sign of the responses in the system is expected to depend on those parameters as well. Surprisingly, this is not always the case, as Example II of Section 4.3 shows.

Finally, the 'singleton' Chapter 5 illustrates our sign sensitivity theory for the much simpler class of monomolecular networks. The description is in terms of directed paths in the network. For example, Theorem 5.4.1 addresses the case of a metabolite perturbation of m^*, and states that the response of an element p, either metabolite or reaction, is nonzero if and only if p is reachable from m^* via a directed path, in the usual graph theory sense. Moreover, the responses are always positive.

Last, but not least, our work has been driven with the hope to find some realistic biological explanations. On one hand, indeed, often the explanation of real phenomena has been left to mere simulations, for lack of interpretation and structural understanding. On the other hand, applied mathematics is sometimes too self-centered in finding in applications a selfish reason to unfold itself.
In our opinion, the explanations should rather give us insights on how certain real biological phenomena happens, or at least honestly attempt to. It is our hope that this thesis behaves exactly in this direction.

Part I

The Jacobian of metabolic networks

Chapter 2

Good children and bad children

2.1 Introduction

The Jacobian matrix of a dynamical system plays a central role in the stability analysis of equilibria. The sign of its eigenvalues is an indication of stability and a change of sign of its determinant hints therefore to a change of stability and to bifurcation phenomena.

For the metabolic chemical reaction system

$$\dot{x} = f(x) := S\mathbf{r}(x), \tag{2.1}$$

the Jacobian matrix reads

$$f_x = SR =: G. \tag{2.2}$$

The *reactivity matrix* R of the partial derivatives is an $N \times M$ matrix, whose entries R_{jm} are given by:

$$R_{jm} := \frac{\partial}{\partial x_m} r_j(x) = \begin{cases} r_{jm} & \text{if } \frac{\partial r_j(x)}{\partial x_m} \neq 0 \\ 0 & \text{otherwise} \end{cases} . \tag{2.3}$$

The entry R_{jm} is nonzero, i.e. $R_{jm} = r_{jm}$, if and only if the metabolite m is an input of the reaction j. The algebraic structure of G is thus completely characterized by the network structure, only. In particular, we may consider a matrix of the type of G as a purely linear algebra object. Consider indeed S to be any $M \times N$ real matrix, and let us define the *negative sign-pattern* S^- of S as

$$S^-_{mj} = \begin{cases} 0 & \text{if } S_{mj} \geq 0 \\ r_{mj} & \text{if } S_{mj} < 0 \end{cases} , \tag{2.4}$$

with $r_{mj} > 0$ strictly positive symbolic entry. In this way, the algebraic structure of the matrices $(S^-)^T$ and R coincides. Now, defining G as

$$G := S(S^-)^T, \tag{2.5}$$

the algebraic form of the abstract $G = S(S^-)^T$ and of the Jacobian $G = SR$ is identical.

The leading question of this chapter is the following:

When is $\det G$ *of fixed sign?*

That is:

When - for any *choice of positive parameters* r_{jm} *- does the determinant carry the same sign?*

In the continuation of this chapter we provide answers to this question. In particular, in Section 2.2, we introduce *Child Selections* and we use the Cauchy-Binet formula to expand the Jacobian determinant in a polynomial, in which each monomial summand is associated to a Child Selection. Depending on the sign of the coefficients of these monomials, each Child Selection is abstractly classified in *good* or *bad*. The coexistence of a good and a bad Child Selection characterizes the condition of *indeterminate sign* Jacobian. We provide motivating examples in Section 2.3. In Section 2.4, the main Theorem 2.4.1 abstractly characterizes whether a given Child Selection is good or bad and Section 2.5 translates this abstract condition into a pure network condition. Consequently, Section 2.6 uses the developed concepts to find a bifurcation parameter responsible for a change of sign in the Jacobian determinant, with possible consequent bifurcation phenomena. Section 2.7 contains some arguments regarding the eigenvalues of the system. Section 2.8 analyzes in detail an example of an autocatalytic network. The case in which the Jacobian determinants admits a factorization is briefly studied in Section 2.9. The last Section 2.10 contains an example of an application for the central metabolism of E.Coli.
This thesis wants to be a contribution for applications in metabolic network theory, mainly. For this reason, we have left a more general version of Theorem 2.4 in Appendix 2.A and some computational considerations in Appendix 2.B.

Works in an analogous direction have been pursued by many people, see for a chemical/metabolic perspective [BDB07, BC10, MC13, BR11] and for a purely linear algebra approach [JKVdD77, BS09, LOvdD18], among others.

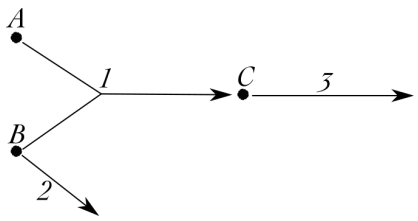
2.2 Cauchy-Binet analysis via Child Selections

The first definition, due to Brehm and Fiedler [BF18], is crucial for the entire thesis.

Definition 2.1 (Child Selections, mothers, children)**.** A *Child Selection* is an injective map $\mathbf{J} : \mathbf{M} \longrightarrow \mathbf{E}$, which associates to every metabolite $m \in \mathbf{M}$ a reaction $j \in \mathbf{E}$ such that m is an input metabolite of reaction j.
We call the reaction $j = \mathbf{J}(m)$ *child* of m, and the metabolite $m = \mathbf{J}^{-1}(j)$ *mother* of the reaction j.

Remark 1. Equivalently, a Child Selection is an injective map $\mathbf{J} : \mathbf{M} \longrightarrow \mathbf{E}$ such that $\mathbf{J}(m) = j$ with strictly negative stoichiometric entry $S_{mj} < 0$, for every m.

Remark 2. It is possible that a metabolite m is an input of j but not a mother of j, due to injectivity of Child Selections. Indeed, consider the following example:



(2.6)

In this minimal case, metabolite B is an input of reaction 1, but never a mother. In fact, 1 is the only outgoing reaction from metabolite A. Therefore, due to injectivity, $\mathbf{J}^{-1}(1) = A$.

For a matrix $\mathcal{A}$, we use the notation $\mathcal{A}^{\mathcal{E}}_{\mathcal{F}}$ to denote the submatrix of $\mathcal{A}$ consisting of columns $\mathcal{E}$ and rows $\mathcal{F}$. For simplicity of notation, we omit the braces $\{m\}$ for single elements, so that, for example, S^j indicates the j^{th} column and S_m indicates the m^{th} row of S. The following Jacobian analysis, based on the Cauchy-Binet formula, is developed from a previous result in [BF18].

Proposition 2.2.1. *Let G be a network Jacobian, in the above settings. Then:*

$$\det G = \sum_{\mathbf{J}} \det S^{\mathbf{J}} \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}, \tag{2.7}$$

where $S^{\mathbf{J}}$ is the matrix whose m^{th} column is the $\mathbf{J}(m)^{th}$ column of S.

Proof. We apply the Cauchy-Binet formula on $G = SR$ to obtain:

$$\det G = \sum_{|\mathcal{E}|=M} \det S^{\mathcal{E}} \cdot \det R_{\mathcal{E}} = \sum_{|\mathcal{E}|=M} \det S^{\mathcal{E}} \, (\sum_{\pi} \operatorname{sgn}(\pi) \cdot \prod_{m \in \mathbf{M}} r_{\pi(m)m}). \tag{2.8}$$

Here π indicates a permutation of M elements and $\operatorname{sgn}(\pi)$ is the signature (or parity) of π. Note that $\prod_{m \in \mathbf{M}} r_{\pi(m)m} \neq 0$ if and only if there is an associated Child Selection $\mathbf{J}$ such that $r_{\mathbf{J}(m)m} = r_{\pi(m)m}$, for every m. In particular, the sum runs non trivially only for the selected minors $S^{\mathcal{E}}$ such that the set $\mathcal{E}$ is the image of $\mathbf{M}$ through a Child Selection $\mathbf{J}$. Now,

$$\begin{aligned} \sum_{|\mathcal{E}|=M} \det S^{\mathcal{E}} \, (\sum_{\pi} \operatorname{sgn}(\pi) \cdot \prod_{m \in \mathbf{M}} r_{\pi(m)m}) &= \sum_{\mathcal{E}=\mathbf{J}(\mathbf{M})} \det S^{\mathcal{E}} \, (\sum_{\mathbf{J}} \operatorname{sgn}(\mathbf{J}) \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}) \\ &= \sum_{\mathbf{J}} \det S^{\mathbf{J}} \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}. \end{aligned} \tag{2.9}$$

Last step is the observation:

$$\det S^{\mathcal{E}=\mathbf{J}(\mathbf{M})} \cdot \operatorname{sgn}(\mathbf{J}) = \det S^{\mathbf{J}}. \tag{2.10}$$

□

Remark 3. Note that, by construction, $S^{\mathbf{J}}_{mm} < 0$, for any Child Selection $\mathbf{J}$ and any metabolite m.

Remark 4. If there are no Child Selections, at all, then $\det(G) \equiv 0$ for any choice of parameters r_{jm}.

We state a classification of Child Selections, according to the sign of the determinant of the reshuffled minor $S^{\mathbf{J}}$.

Definition 2.2 (Child Selection behavior)**.** Let $\mathbf{J}$ be a Child Selection.
We say that $\mathbf{J}$ is *good*, or $\mathbf{J}$ *well-behaves*, if $\operatorname{sign}(\det S^{\mathbf{J}}) = (-1)^M$.
We say that $\mathbf{J}$ is *bad*, or $\mathbf{J}$ *ill-behaves*, if $\operatorname{sign}(\det S^{\mathbf{J}}) = (-1)^{M-1}$.
If $\det S^{\mathbf{J}} = 0$, we say that $\mathbf{J}$ *zero-behaves*.
Moreover, we define the *behavior coefficient* β as

$$\beta(\mathbf{J}) = \operatorname{sign}(\det S^{\mathbf{J}}). \tag{2.11}$$

That is,

$$\beta(\mathbf{J}) = \begin{cases} (-1)^M & \text{if } \mathbf{J} \text{ well-behaves} \\ (-1)^{M-1} & \text{if } \mathbf{J} \text{ ill-behaves} \\ 0 & \text{if } \mathbf{J} \text{ zero-behaves} \end{cases}.$$

The choice of the terminology has been done carefully. In fact, in a metabolic network context, important classes of Child Selections well-behave, see Section 2.5. For instance, acyclic Child Selections well-behave. Moreover, a 'stable' Child Selection $\mathbf{J}$, in which all the eigenvalues of $S^{\mathbf{J}}$ are negative, well-behaves. Note, however, that the classification is not strictly related with stability, but only with the sign parity of eigenvalues.

At this point, the reader may wonder whether Definition 2.2 is well-posed. That is, whether the behavior of a Child Selection depends on the specific labeling of the network. Section 2.5, Remark 8, clarifies this point, assuring the well-posedness of the definition.

With Definition 2.2, the following straightforward Corollary to Proposition 2.2.1 is derived.

Corollary 2.2.2. *The Jacobian* $\det G$ *is of fixed sign if and only if there are no two Child Selections* $\mathbf{J}_1$ *and* $\mathbf{J}_2$ *such that* $\mathbf{J}_1$ *is good and* $\mathbf{J}_2$ *is bad.*

Stoichiometric matrices of metabolic networks are sparse. Indeed, usually, metabolic reactions are at most bimolecular and many of them are monomolecular. We see in the continuation of this chapter that this sparsity feature constitutes a reason why it is likely to find good Child Selections, easily, in real metabolic networks. The moral interpretation or Corollary 2.2.2, then, is that the Jacobian is of fixed sign if there are no bad Child Selections, for real network examples. In this sense, the presence of bad Child Selections is a strong indication of a possible sign change of the determinant, and it becomes important to be able to recognize them.

2.3 Preliminary examples

In this subsection we present six examples: three of good Child Selections and three of bad.

2.3.1 Good Child Selections

Example G1: monomolecular Child Selections
A monomolecular Child Selection consists only of monomolecular reactions j of the form

$$j: \quad m_1 \xrightarrow[j]{} m_2, \tag{2.12}$$

where one single metabolite input m_1 is converted into a different single metabolite output m_2. The stoichiometry of these networks is particularly simple. In fact, columns S^j of the stoichiometric matrix S have at most one negative entry -1 and one positive entry $+1$. Columns $S^{j^0_m}$ associated to outflow exit reactions j^0_m

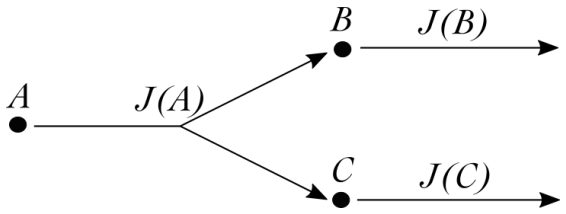
$$j^0_m: \quad m \xrightarrow[j^0_m]{} \tag{2.13}$$

have only a negative entry -1, located in the m^{th} row. For an extensive discussion on monomolecular networks, see Chapter 5.
Monomolecular Child Selections are never bad. In fact, because of the simple stoichiometry structure of S, if $\det S^{\mathbf{J}} \neq 0$ we can implement a simple Gaussian elimination to obtain the M-dimensional identity matrix $-\mathrm{Id}_{\mathrm{M}}$. Clearly then,

$$\det S^{\mathbf{J}} = \det(-\mathrm{Id}_{\mathrm{M}}) = (-1)^M. \tag{2.14}$$

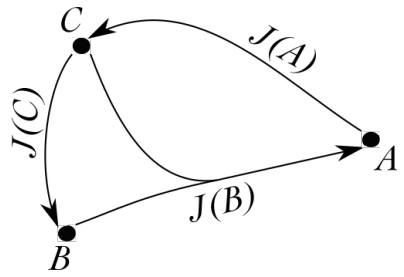
Alternatively, a more abstract argument is provided by the Gershgorin disk Theorem (see [Ger31] and Section 2.7). In particular, for monomolecular networks, the Jacobian is always of fixed sign.

Example G2: Child Selection without cycles
This is a specific example, for which the computation is done directly. However, it points at a general feature: all acyclic Child Selections well-behave, see Corollary 2.5.2.



$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) \end{array} \\ \begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 1 & 0 & -1 \end{bmatrix} \end{array}, \quad \det S^J = (-1)^3 = -1. \tag{2.15}$$

Example G3: Child Selection with cycles



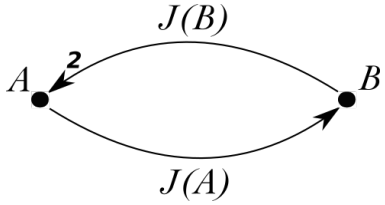
$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) \end{array} \\ \begin{bmatrix} -1 & 1 & 0 \\ 0 & -1 & 1 \\ 1 & -1 & -1 \end{bmatrix} \end{array}, \quad \det S^{\mathbf{J}} = (-1)^3 = -1. \tag{2.16}$$

2.3.2 Bad Child Selections

Example B1: autocatalytic Child Selection
Didn't we exclude autocatalysis from our analysis? Yes, we had excluded *explicit* autocatalytic reactions such as, for example,

$$A \xrightarrow[j]{} 2A. \tag{2.17}$$

However, it is straightforward to insert an intermediate metabolic step B in the above reaction j. In this way, the system does not possess an explicit autocatalytic reaction anymore and it is completely admissible in our approach. Thus it becomes:



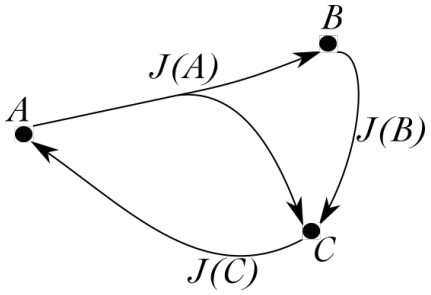
(2.18)

This Child Selection ill-behaves:

$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cc} \mathbf{J}(A) & \mathbf{J}(B) \end{array} \\ \begin{bmatrix} -1 & 2 \\ 1 & -1 \end{bmatrix} \end{array}, \quad \det S^{J} = (-1)^{2-1} = -1. \tag{2.19}$$

Example B2: reverse arrow orientation of Example G3
We take the above example **G3** and revert the orientation of all reactions. We

obtain the following Child Selection:

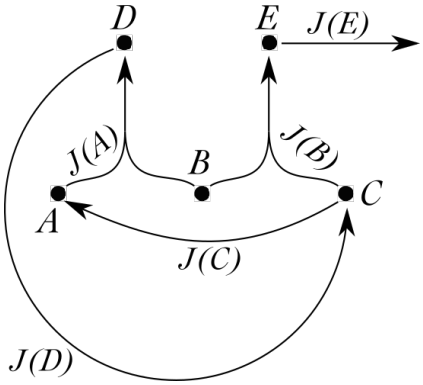


(2.20)

This Child Selection, opposite in sign to Example **G3**, ill-behaves.

$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) \end{array} \\ \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 1 & 1 & -1 \end{bmatrix} \end{array}, \quad \det S^J = (-1)^{3-1} = +1. \tag{2.21}$$

Example B3: double cycle



(2.22)

$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \begin{array}{c} \begin{array}{ccccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) & \mathbf{J}(D) & \mathbf{J}(E) \end{array} \\ \begin{bmatrix} -1 & 0 & 1 & 0 & 0 \\ -1 & -1 & 0 & 0 & 0 \\ 0 & -1 & -1 & 1 & 0 \\ 1 & 0 & 0 & -1 & 0 \\ 0 & 1 & 0 & 0 & -1 \end{bmatrix} \end{array}, \quad \det S^J = (-1)^{5-1} = +1. \tag{2.23}$$

2.4 Main result

In metabolic networks, stoichiometric coefficients are mostly 0 and 1. For this reason, in this section we assume that S has entries $S_{mj} \in \{-1, 0, 1\}$. By 2.2, Remark 3, then, the diagonal entries of the reshuffled minor $S^{\mathbf{J}}$ are $S^{\mathbf{J}}_{mm} \equiv -1$, for any m. We

comment in the dedicated Appendix 2.A about the generalization to stoichiometric matrices with general entries $S_{mj} \in \mathbb{R}$.

Here, via a structural analysis of $\det S^{\mathbf{J}}$, we characterize whether the given Child Selection $\mathbf{J}$ well-behaves or ill-behaves. Note, however, that the importance of the result is mainly revealed in its interpretation, see Section 2.5.

The Leibniz expansion formula for the determinant, applied to $S^{\mathbf{J}}$, reads

$$\det S^{\mathbf{J}} = \sum_{\pi} \operatorname{sgn}(\pi) \prod_{m \in \mathbf{M}} S^{\mathbf{J}}_{\pi(m)m}. \tag{2.24}$$

Again, π indicates permutations of M elements and $\operatorname{sgn}(\pi)$ is the signature of π. Let

$$\mathcal{E}(\pi) := \operatorname{sgn}(\pi) \prod_{m \in \mathbf{M}} S^{\mathbf{J}}_{\pi(m)m} \tag{2.25}$$

denote the summand associated to the permutation π in the Leibniz expansion. For example, denoting as Id the identity permutation,

$$\mathcal{E}(\mathit{Id}) = (-1)^M. \tag{2.26}$$

Let $\pi \neq \mathit{Id}$ be a permutation such that $\mathcal{E}(\pi) \neq 0$. Combinatorially, the permutation π can be expressed as the product of ϑ disjoint permutation cycles c_i of length $l_i > 1$,

$$\pi = \prod_{i=1}^{\vartheta} c_i. \tag{2.27}$$

Definition 2.3 (good/bad-completions, good/bad-cycles)**.** Given a Child Selection $\mathbf{J}$, we call π a *good-completion* if

$$\prod_{m:\, \pi(m) \neq m} S^{\mathbf{J}}_{\pi(m)m} = (-1)^{\vartheta}. \tag{2.28}$$

We call π a *bad-completion* if

$$\prod_{m:\, \pi(m) \neq m} S^{\mathbf{J}}_{\pi(m)m} = (-1)^{\vartheta - 1}. \tag{2.29}$$

Again, ϑ is the number of cycles in the permutation expansion. If π consists of a single cycle c, i.e. for $\vartheta = 1$, we call the good (resp. bad)-completion a *good(resp. bad)-cycle*.

We clarify in the next Section 2.5 what does a completion complete, as it requires some further arguments. Firstly, given the above definition, we state the main result of this section.

Theorem 2.4.1. *Let* $\mathbf{J}$ *be a Child Selection and let* $\mathcal{G}$ *and* $\mathcal{B}$ *be the number of good and bad completions, respectively. Then, in the sense of Definition 2.2,*

1. *The Child Selection* $\mathbf{J}$ *well-behaves if* $\mathcal{G} > \mathcal{B} - 1$.
2. *The Child Selection* $\mathbf{J}$ *ill-behaves if* $\mathcal{G} < \mathcal{B} - 1$.
3. *The Child Selection* $\mathbf{J}$ *zero-behaves if* $\mathcal{G} = \mathcal{B} - 1$.

Proof. The proof follows an idea of Banaji and Craciun [BC10]. Firstly note:

$$\begin{aligned}(\det S^{\mathbf{J}})(-1)^M =&(\det S^{\mathbf{J}})\ \mathcal{E}(\mathit{Id})\\ =&\sum_{\pi}\mathcal{E}(\pi)\mathcal{E}(\mathit{Id})\\ =&1+\sum_{\pi\neq \mathit{Id}}\mathcal{E}(\pi)\mathcal{E}(\mathit{Id}).\end{aligned} \tag{2.30}$$

Let h be the number of elements m such that $\pi(m)\neq m$. That is, h is the number of elements of π which are not fixed points of the permutation, but belong to a permutation cycle.

$$\begin{aligned}\mathcal{E}(\pi)\mathcal{E}(\mathit{Id}) =&\operatorname{sgn}(\pi)\left(\prod_{m\in\mathbf{M}}S^{\mathbf{J}}_{\pi(m)m}\right)\operatorname{sgn}(\mathit{Id})\prod_{m\in\mathbf{M}}S^{\mathbf{J}}_{mm}\\ =&\left(\prod_{m:\,\pi(m)=m}(S^{\mathbf{J}}_{mm})^2\right)\prod_{i=1}^{\vartheta}\operatorname{sgn}(c_i)\left(\prod_{m:\,\pi(m)\neq m}(S^{\mathbf{J}}_{\pi(m)m}S^{\mathbf{J}}_{mm})\right)\\ =&(-1)^h\prod_{i=1}^{\vartheta}\operatorname{sgn}(c_i)\prod_{m:\,\pi(m)\neq m}S^{\mathbf{J}}_{\pi(m)m}\\ =&(-1)^{\vartheta}\prod_{m:\,\pi(m)\neq m}S^{\mathbf{J}}_{\pi(m)m}.\end{aligned} \tag{2.31}$$

The steps above are made noting that $(S^{\mathbf{J}}_{mm})^2\equiv 1$, for any m and that, for a cycle c of length ℓ, $\operatorname{sgn}(c)(-1)^{\ell}=-1$. We conclude the proof by observing that

$$(-1)^{\vartheta}\prod_{m:\,\pi(m)\neq m}S^{\mathbf{J}}_{\pi(m)m}=1\ \text{(-1, respectively)} \tag{2.32}$$

if π is a good-completion (bad-completion, respectively). This yields to the identity

$$\det S^{\mathbf{J}}(-1)^M=1+\mathcal{G}-\mathcal{B}, \tag{2.33}$$

which proves the Theorem. □

2.5 Interpretation of the result

The *Metabolite-Reaction graph* (MR-graph) is an undirected bipartite graph with two sets of vertices, given by the metabolites $m_1,...,m_M$ and the reactions $j_1,...,j_E$, respectively. For a metabolite m participating in a reaction j, edges $e=(m,j)$ are adjacent to a metabolite vertex m and a reaction vertex j. With this construction, then, edges in the MR-graph are in one-to-one relation with the nonzero entries of stoichiometric matrix S. In particular, with $S_{mj}<0$ of

$$j:\quad m+...\xrightarrow[j]{}... \tag{2.34}$$

in mind, we call *negative* the edges $e=(m,j)$ where m is input to j. Conversely, with $S_{mj}>0$ of

$$j:\quad ...\xrightarrow[j]{}m+... \tag{2.35}$$

in mind, we call *positive* the edges $e = (m, j)$ where m is output to j. See Figure 2.1 for a comparison between different kinds of representation graphs for the same network. Under the name Species-Reaction graph (SR-graph), this was considered by [CF06] and others.

We proceed with two definitions and a proposition.

Definition 2.4 (**J**-selected edges)**.** For any Child Selection **J**, we call the negative edges $e = (m, \mathbf{J}(m))$ in the MR-graph to be **J**-*selected*.

Remark 5. In particular, **J**-selected edges are such that the corresponding stoichiometric entry lies on the diagonal of $S^{\mathbf{J}}$.

Remark 6. Injectivity of a Child Selection **J** directly implies that two **J**-selected edges e_1 and e_2 never share a vertex, in the MR-graph.

Definition 2.5 (Completion Cycle)**.** For a Child Selection **J**, a completion cycle in the MR-graph is a cycle of length $2l$, $\ell \leq M$, such that ℓ edges are **J**-selected.

Remark 7. Equivalently, because of Remark 6 above, a completion cycle is a cycle in the MR-graph of length $2l$, such that ℓ **J**-selected edges alternate with ℓ non **J**-selected edges.

Proposition 2.5.1. *For any given Child Selection* **J***, there is a one-to-one correspondence between completion cycles and nonzero permutation cycles, that is, cycles c such that*

$$\prod_{m:\, c(m)\neq m} S^{\mathbf{J}}_{c(m)m} \neq 0. \tag{2.36}$$

Proof. Let us consider the computation (2.31) in the proof of Theorem 2.4.1. Consider, for simplicity, a single cycle permutation $\pi = c$ and concentrate on the expression

$$\prod_{m:\, c(m)\neq m} S^{\mathbf{J}}_{c(m)m} S^{\mathbf{J}}_{mm}. \tag{2.37}$$

Note that the diagonal elements $S^{\mathbf{J}}_{mm}$ and $S^{\mathbf{J}}_{c(m)c(m)}$ represents **J**-selected edges. $S_{c(m)m}$ shares the same column (i.e., reaction vertex) with $S^{\mathbf{J}}_{mm}$ and the same row (i.e., metabolite vertex) with $S^{\mathbf{J}}_{c(m)c(m)}$. Following the order of the cycle c in the Expression (2.37) leads to the desired identification. □

It becomes now clear what the word 'completion' refers to. In fact, any completion cycle is constructed by completing ℓ **J**-selected elements to a cycle of length $2l$ in the MR-graph. In this sense, a good-completion $\pi = \prod_{i=1}^{\vartheta} c_i$ can be seen, in the MR-graph, as a collection of ϑ completion cycles c_i, such that the number of good-cycles has the same parity of ϑ. Respectively, a bad-completion is a collection of ϑ completion cycles, such that the number of good-cycles has opposite parity of ϑ.

Remark 8. Obviously, being a network structure, the definition of a completion cycle does not depend on the specific labeling of the network. Proposition 2.5.1, together with Theorem 2.4.1, guarantees in particular that also Definition 2.2 does not depend on any labeling and, thus, is well-posed.

Finally, we list some consequences of Theorem 2.4.1, useful for applications.

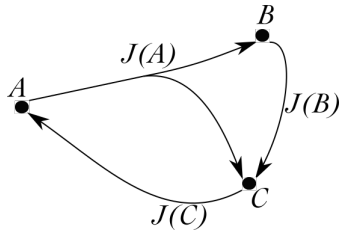
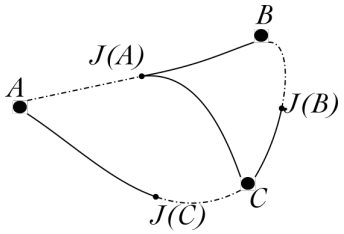
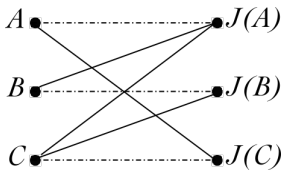
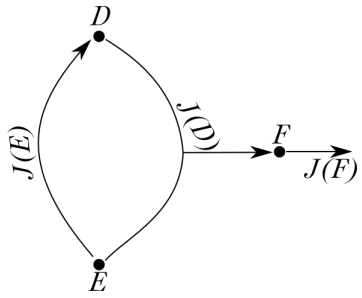
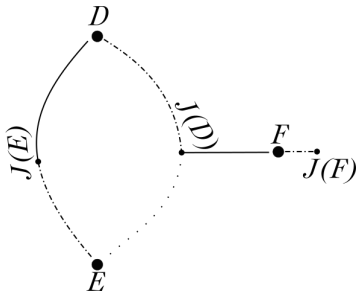
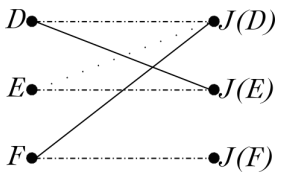
Examples	*BIOLOGICAL*	*MR-GRAPH* *Biological*	*MR-GRAPH* *Combinatorial*	*MATRIX*
1				$\begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) \end{array} \\ \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 1 & 1 & -1 \end{bmatrix} \end{array}$
2				$\begin{array}{c} \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{J}(D) & \mathbf{J}(E) & \mathbf{J}(F) \end{array} \\ \begin{bmatrix} -1 & 1 & 0 \\ -1 & -1 & 0 \\ 1 & 0 & -1 \end{bmatrix} \end{array}$

Figure 2.1: For two examples of Child Selections, four different ways of representation: biological, MR-Graph (in a biological shape), MR-graph (in a combinatorial shape), matrix. Note that, when labeled, the four representations are equivalent. In the MR-graphs, *negative edges* ***J****-selected* are indicated with a dotted-dashed line, the sparse dotted line indicates *negative edges not* ***J****-selected*, the continuous line indicates *positive edges*. In the combinatorial shape, the edges **J**-selected are the horizontal ones. Example 1 possesses two completion cycles: $c_1 = A - \mathbf{J}(A) - C - \mathbf{J}(C) - A$ and $c_2 = A - \mathbf{J}(A) - B - \mathbf{J}(B) - C - \mathbf{J}(C) - A$, both bad. Example 2 possesses only one good completion cycle: $c = D - \mathbf{J}(D) - E - \mathbf{J}(E) - D$. Consequently, Example 1 represents a bad Child Selection, and Example 2 represents a good one.

Corollary 2.5.2 (Examples of application)**.** *The following statements hold true:*

1. *Acyclic Child Selections well-behave;*
2. *A Child Selection containing one good-cycle, and no other cycles, well-behaves;*
3. *A Child Selection containing one bad-cycle, and no other cycles, zero-behaves;*
4. *A Child Selection containing two intersecting bad-cycles, and no other cycles, ill-behaves;*
5. *Any given nonzero Child Selection of a network which possesses only monomolecular reactions and one single bimolecular reaction*

$$j: \quad A + B \underset{j}{\longrightarrow} C \tag{2.38}$$

well-behaves.

Proof. We only sketch the proofs.
1-4) The statements are a simple check via Formula (2.33).
5) We have seen in the preliminary Example **G1** that nonzero monomolecular Child Selections well-behaves. This can be easily seen, by the above MR-graph point of view, as all possible completion cycles in a monomolecular network are disjoint and bad. In particular, in a monomolecular network, either $\mathcal{G} = \mathcal{B} = 0$ in the case of acyclic Child Selections, or $\mathcal{G} = \mathcal{B} - 1$ in the case of Child Selections containing cycles. The underlying combinatorial argument for disjoint cycles is made explicit in the Appendix 2.B. Note that, adding one single bimolecular reaction of the form of (2.38) to a monomolecular network, we only add the possibility to have good-cycles. For analogous combinatorial reasons as sketched above, then, $\mathcal{G} - \mathcal{B}$ does not decrease after adding such a bimolecular reaction. In particular, then, since any nonzero monomolecular Child Selection well-behaves, any nonzero Child Selection of a network which possesses only monomolecular reactions and one single bimolecular reaction as (2.38) well-behaves. □

2.6 Hunting saddle-node bifurcations

Here, we give a simple network condition under which there is the possibility, for certain parameters, of a saddle-node bifurcation of equilibria. We also identify bifurcation parameters responsible for the change of sign of the determinant and consequent change of stability of any equilibrium.

Theorem 2.6.1 (Change of Stability)**.** *Suppose there exist two Child Selections* $\mathbf{J}_1$, $\mathbf{J}_2$, *and a metabolite* m_b, *such that* $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b)$ *and* $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ *for any* $m \neq m_b$. *Assume moreover that* $\mathbf{J}_1$ *well-behaves and* $\mathbf{J}_2$ *ill-behaves. Then the Jacobian determinant of* G *takes the form*

$$\det G = (a r_{\mathbf{J}_1(m_b)m_b} - b r_{\mathbf{J}_2(m_b)m_b}) r_{\mathbf{J}_1(m_{n+1})m_{n+1}} \cdots r_{\mathbf{J}_1(m_m)m_m} \quad + \quad \dots \quad , \tag{2.39}$$

where the omitted terms can be chosen arbitrarily small, and a, b *are coefficients of the same sign. In particular, the parameter*

$$\xi = a r_{\mathbf{J}_1(m_b)m_b} - b r_{\mathbf{J}_2(m_b)m_b} \tag{2.40}$$

may serve as a bifurcation parameter for the bifurcation of nontrivial equilibrium solutions of the system (2.1).

Theorem 2.6.1 does not require to fix an equilibrium and it holds in more general settings. Nevertheless, for simplicity, we are thinking here of an equilibrium situation. The parameter $\xi = a r_{\mathbf{J}_1(m_b)m_b} - b r_{\mathbf{J}_2(m_b)m_b}$ is 'localized' in a single metabolite m_b. In fact, the change of stability is driven by the difference between the derivatives with respect to the same metabolite m_b of the reaction rates of two child reactions of m_b itself. This suggests *a simple biological scheme for modifying the unstable dimension of the equilibrium.*

To prove Theorem 2.6.1, we introduce some concepts, first. The set of Child Selections $\{\mathbf{J}\}$ carries a natural integer-valued distance d.

Definition 2.6. Let $\mathbf{J}_1$, $\mathbf{J}_2$ be two Child Selections. We define the distance $d(\mathbf{J}_1, \mathbf{J}_2)$ as the number of metabolites $m \in \mathbf{M}$ such that $\mathbf{J}_1(m) \neq \mathbf{J}_2(m)$.

It is straightforward to verify that d is a distance on the set of Child Selections. We consider now Child Selections at distance $d = 1$. These are Child Selections $\mathbf{J}_1$, $\mathbf{J}_2$ such that $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b)$ for a single metabolite m_b and $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ for any $m \neq m_b$ different from m_b, as in Theorem 2.6.1. Clearly:

$$\begin{aligned} & \det S^{\mathbf{J}_1} \prod_m r_{\mathbf{J}_1(m)m} + \det S^{\mathbf{J}_2} \prod_m r_{\mathbf{J}_2(m)m} \\ = \quad & r_{\mathbf{J}_1(m_1)m_1} \cdot \ldots (\det S^{\mathbf{J}_1} r_{\mathbf{J}_1(m_b)m_b} + \det S^{\mathbf{J}_2} r_{\mathbf{J}_2(m_b)m_b}) \ldots \cdot r_{\mathbf{J}_1(m_m)m_m} \end{aligned} \tag{2.41}$$

If we further assume that $\mathbf{J}_1$ and $\mathbf{J}_2$ are such that one well-behaves and the other ill-behaves we have:

$$\det S^{\mathbf{J}_1} r_{\mathbf{J}_1(m_b)m_b} + \det S^{\mathbf{J}_2} r_{\mathbf{J}_2(m_b)m_b} = a \cdot r_{\mathbf{J}_1(m_b)m_b} - b \cdot r_{\mathbf{J}_2(m_b)m_b}, \tag{2.42}$$

with a and b constants of the same sign.

By the mere fact that d is an integer-valued distance, any other Child Selection satisfies

$$d(\mathbf{J}_k, \mathbf{J}_1), d(\mathbf{J}_k, \mathbf{J}_2) \geq 1, \quad \text{for any } k \neq 1, 2. \tag{2.43}$$

In particular, we have the following Lemma:

Lemma 2.6.2. *Let $\mathbf{J}_1$ and $\mathbf{J}_2$ be Child Selections at distance $d = 1$, that is, $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b)$ and $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ for any $m \neq m_b$. For any other Child Selection $\mathbf{J}_k$, there is a metabolite m_k such that $\mathbf{J}_k(m_k) \neq \mathbf{J}_1(m_k)$ and $\mathbf{J}_k(m_k) \neq \mathbf{J}_2(m_k)$.*
Moreover if $d(\mathbf{J}_k, \mathbf{J}_1) = d(\mathbf{J}_k, \mathbf{J}_2) = 1$, then $m_k = m_b$.

Proof. Let us consider any m_k such that $\mathbf{J}_1(m_k) \neq \mathbf{J}_k(m_k)$. If $\mathbf{J}_2(m_k) \neq \mathbf{J}_k(m_k)$, we are done. Assume then that $\mathbf{J}_2(m_k) = \mathbf{J}_k(m_k)$. By construction, $m_k = m_b$. Consider now any $\tilde{m}_k$ such that $\mathbf{J}_2(\tilde{m}_k) \neq \mathbf{J}_k(\tilde{m}_k)$ and remember that $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ for any $m \neq m_b$. We conclude that $\mathbf{J}_1(\tilde{m}_k) \neq \mathbf{J}_k(\tilde{m}_k)$. Otherwise, we would have found two metabolites m_k and $\tilde{m}_k$ such that $\mathbf{J}_1(m_k) \neq \mathbf{J}_2(m_k)$ and $\mathbf{J}_1(\tilde{m}_k) \neq \mathbf{J}_2(\tilde{m}_k)$, contradicting $d(\mathbf{J}_1, \mathbf{J}_2) = 1$.

In the above argument, note that if $\mathbf{J}_2(m_k) = \mathbf{J}_k(m_k)$, then $d(\mathbf{J}_1, \mathbf{J}_k) \geq 2$. Hence, if $d(\mathbf{J}_1, \mathbf{J}_k) = d(\mathbf{J}_2, \mathbf{J}_k) = 1$ we conclude that $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b) \neq \mathbf{J}_k(m_b)$ and hence $m_k = m_b$. □

We are now ready to prove Theorem 2.6.1.

Proof of Theorem 2.6.1. Let $\mathbf{J}_1$ and $\mathbf{J}_2$ be Child Selections, as above. In particular, distance $d(\mathbf{J}_1, \mathbf{J}_2) = 1$. By Lemma 2.6.2, for any other Child Selection $\mathbf{J}_k \neq \mathbf{J}_1, \mathbf{J}_2$ we can find m_k such that

$$\mathbf{J}_1(m_k), \mathbf{J}_2(m_k) \neq \mathbf{J}_k(m_k). \tag{2.44}$$

We can consider, then, an ϵ-small choice of reaction rate parameter such that

$$r_{\mathbf{J}_k(m_k)m_k} < \epsilon. \tag{2.45}$$

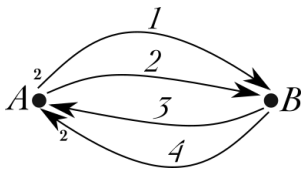
Then, for this ϵ-choice of reaction rates,

$$\det G = (ar_{\mathbf{J}_1(m_b)m_b} - br_{\mathbf{J}_2(m_b)m_b})r_{\mathbf{J}_1(m_{n+1})m_{n+1}} \ldots r_{\mathbf{J}_1(m_m)m_m} + \epsilon. \tag{2.46}$$

The parameter $\xi = ar_{\mathbf{J}_1(m_b)m_b} - br_{\mathbf{J}_2(m_b)m_b}$ becomes then a bifurcation parameter for the sign of the Jacobian determinant. □

Remark 9. The mere existence of two Child Selections with opposite behavior good/bad at a distance $d > 1$ does not always imply the existence of two Child Selections at distance $d = 1$. We illustrate this in the following concluding example:

$$S = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cccc} 1 & 2 & 3 & 4 \end{array} \\ \begin{bmatrix} -2 & -1 & 1 & 2 \\ 1 & 1 & -1 & -1 \end{bmatrix} \end{array}, \tag{2.47}$$



In this abstract example there are *four* Child Selections:

1. $\mathbf{J}_{13} = \{\mathbf{J}_{13}(A) = 1, \mathbf{J}_{13}(B) = 3\}$;
2. $\mathbf{J}_{14} = \{\mathbf{J}_{14}(A) = 1, \mathbf{J}_{14}(B) = 4\}$;
3. $\mathbf{J}_{23} = \{\mathbf{J}_{23}(A) = 2, \mathbf{J}_{23}(B) = 3\}$;
4. $\mathbf{J}_{24} = \{\mathbf{J}_{24}(A) = 2, \mathbf{J}(B)_{24} = 4\}$.

$\mathbf{J}_{13}$ well-behaves, $\mathbf{J}_{14}$, $\mathbf{J}_{23}$ zero-behave, and $\mathbf{J}_{24}$ ill-behaves. Note that $d(\mathbf{J}_{13}, \mathbf{J}_{24}) = 2$ and their behavior is opposite, but all other Child Selections ($\mathbf{J}_{14}$ and $\mathbf{J}_{23}$) zero-behave. Therefore we cannot find two Child Selections with opposite behavior at distance $d = 1$. In this example above, and in analogous situations, however, it is still

possible to find a bifurcation parameter, albeit more involved than the parameter ξ in (2.40). In such a network, in fact, we may consider the parameter

$$\chi = r_{\mathbf{J}_{13}(A)} r_{\mathbf{J}_{13}(B)} - r_{\mathbf{J}_{24}(A)} r_{\mathbf{J}_{24}(B)}. \tag{2.48}$$

Analogously as in Theorem 2.6.1, for a suitable choice of reaction rates, the Jacobian determinant changes sign as the bifurcation parameter χ does. This parameter depends on two metabolites A and B, since the two Child Selections $\mathbf{J}_{13}$ and $\mathbf{J}_{24}$, opposite in behavior, are such that $d(\mathbf{J}_{13}, \mathbf{J}_{24}) = 2$. In particular, the complexity of the parameter is given by the distance of the two considered Child Selections.

2.7 Eigenvalues

In this section we analyze the relation between the structure of the Jacobian matrix G and its eigenvalues. By (2.2), the Jacobian G can be decomposed as $G = SR$. Hence,

$$G_{m_1 m_2} = S_{m_1} R^{m_2}, \tag{2.49}$$

where S_{m_1} is the m_1^{th} row of the stoichiometric matrix S, associated to the metabolite m_1. An entry $S^j_{m_1}$ is nonzero iff the metabolite m_1 participates, as input *or* as output, in the reaction j. The vector R^{m_2} is the m_2^{th} column of the reactivity matrix R, associated to the metabolite m_2. An entry $R^{m_2}_j$ is nonzero iff the metabolite m_2 participates as input in the reaction j. In particular, let $\Omega(m)$ indicate the set of outgoing reactions from m. Our settings exclude explicit autocatalysis, therefore $S^j_m < 0$, always, for any outgoing reaction j of m. Thus the element on the m^{th} diagonal entry of the Jacobian matrix G is

$$G_{mm} = S_m R^m = \sum_{j \in \Omega(m)} -r_{jm}, \tag{2.50}$$

and, in particular, it is strictly negative, if m participates in at least one reaction as input.

We recall the Gershgorin disk theorem [Ger31]. This elementary result provides a useful estimate for the eigenvalues of matrices. For a given square real matrix $\mathcal{A}$, the m^{th} *Gershgorin* disk $\mathcal{D}_m$ is defined as disk in the complex plane centered at $\mathcal{A}_{mm}$ with radius $r_m = \sum_{j \neq m} |\mathcal{A}_{mj}|$. The theorem, then, reads as follows:

Theorem 2.7.1 (Gershgorin, 1931)**.** *For a given real square matrix $\mathcal{A}$, any eigenvalue λ_i lies in at least one Gershgorin disk.*

Consequently, in a metabolic network within our settings, all Gershgorin disks are centered in the negative half-plane, independently from the choice of the reaction rates. For example, if a metabolite m participates only in monomolecular reactions, the entire corresponding Gershgorin disk $\mathcal{D}_m$ is confined in the nonpositive half-plane. Indeed, $\mathcal{D}_m$ is centered at $G_{mm} = -\sum_{j \in \Omega(m)} r_{jm}$, with radius

$$r_m = \sum_{m \neq j} |G_{mj}| \leq |\sum_{j \in \Omega(m)} r_{jm}| = |G_{mm}|. \tag{2.51}$$

Two corollaries follow. We omit the straightforward proofs.

Corollary 2.7.2. *The real part of any eigenvalue of a monomolecular network is nonpositive. In particular, assuming nondegeneracy of the network, i.e.* $\det G \neq 0$*, the real part of any eigenvalue of a monomolecular network is strictly negative, and any possible equilibrium stable.*

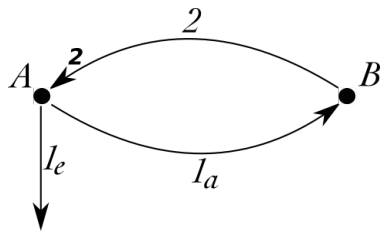
Corollary 2.7.3. *Suppose there is an outflow from a single metabolite* $\tilde{m}$*. That is,* $\tilde{m}$ *participates in an outflow exit reaction* $j^0_{\tilde{m}}$ *such that* $S^{j^0_{\tilde{m}}} = -e_{\tilde{m}}$*. Then, for any fixed choice of rates* r_{jm}*, with* $j \neq j^0_{\tilde{m}}$*, there is always a choice of the outflow parameter* $r_{j^0_{\tilde{m}}\tilde{m}}$ *such that an eigenvalue* λ *has strictly negative real part.*

Fully open networks are defined as networks such that from each metabolite there is an outflow exit reaction. Corollary 2.7.3 directly implies that there always exist a choice of reaction rates, for which any possible equilibrium of a fully open network is stable.

2.8 Example: autocatalytic network

In this section we analyze a simple network, consisting of two metabolites and three reactions. The network graph is

(2.52)



and it has equations

$$\begin{cases} \dot{A} = -r_{1_e}(A) - r_{1_a}(A) + 2r_2(B) \\ \dot{B} = r_{1_a}(A) - r_2(B) \end{cases} . \tag{2.53}$$

The Jacobian determinant reads

$$G = \begin{bmatrix} -r_{1_eA} - r_{1_aA} & 2r_{2B} \\ r_{1_aA} & -r_{2B} \end{bmatrix}, \tag{2.54}$$

with stoichiometric matrix S and reactivity matrix R being

$$S = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{ccc} 1_e & 1_a & 2 \end{array} \\ \begin{bmatrix} -1 & -1 & 2 \\ 0 & 1 & -1 \end{bmatrix} \end{array} \quad \text{and} \quad R = \begin{array}{c} \\ 1_e \\ 1_a \\ 2 \end{array} \begin{array}{c} \begin{array}{cc} A & B \end{array} \\ \begin{bmatrix} r_{1_e} & 0 \\ r_{1_a} & 0 \\ 0 & r_{2s} \end{bmatrix} \end{array}. \tag{2.55}$$

There are two Child Selections, depending on whether the metabolite A chooses the exit reaction 1_e or the autocatalytic reaction 1_a.

1. $\mathbf{J}_{1_e} = \{\mathbf{J}_{1_e}(A) = 1_e; \mathbf{J}_{1_e}(B) = 2\}$. This Child Selection well-behaves. Indeed:

$$S^{\mathbf{J}_{1_e}} = \begin{matrix} \\ A \\ B \end{matrix} \begin{matrix} \begin{matrix} 1_e & 2 \end{matrix} \\ \begin{bmatrix} -1 & 2 \\ 0 & -1 \end{bmatrix} \end{matrix}, \quad \det S^{\mathbf{J}_{1_e}} = +1 \tag{2.56}$$

2. $\mathbf{J}_{1_a} = \{\mathbf{J}_{1_a}(A) = 1_a; \mathbf{J}_{1_a}(B) = 2\}$. This Child Selection ill-behaves. Indeed

$$S^{\mathbf{J}_{1_a}} = \begin{matrix} \\ A \\ B \end{matrix} \begin{matrix} \begin{matrix} 1_a & 2 \end{matrix} \\ \begin{bmatrix} -1 & 2 \\ 1 & -1 \end{bmatrix} \end{matrix}, \quad \det S^{\mathbf{J}_{1_a}} = -1 \tag{2.57}$$

Because $\mathbf{J}_{1_e}$ and $\mathbf{J}_{1_a}$ have opposite behavior, the sign of the Jacobian determinant of G is indeterminate,

$$\det G = (r_{1_eA} - r_{1_aA})r_{2B}. \tag{2.58}$$

The determinant crosses zero when $r_{1_eA} = r_{1_aA}$. In such a simple case, we can explicitly compute the eigenvalues $\lambda_{1,2}$ as roots of the characteristic polynomial P_λ:

$$P_\lambda = \lambda^2 - \operatorname{tr} G\,\lambda + \det G = \lambda^2 + (r_{1_eA} + r_{1_aA} + r_{2B})\lambda + (r_{1_eA} - r_{1_aA})r_{2B}, \tag{2.59}$$

and they have the form:

$$\lambda_{1,2} = \frac{1}{2}[\pm\sqrt{(r_{1_eA} + r_{1_aA} + r_{2B})^2 - 4(r_{1_eA} - r_{1_aA})r_{2B}} - r_{1_eA} - r_{1_aA} - r_{2B}]. \tag{2.60}$$

One of the two eigenvalues is always strictly negative. The other eigenvalue changes sign when the determinant itself does. That is, at value $r_{1_eA} = r_{1_aA}$. A one-parameter bifurcation happens at $r_{1_eA} = r_{1_aA}$, a simple eigenvalue crosses zero and the stability of a possible equilibrium changes.

2.9 Factorizable determinant

Proposition 2.2.1 implies that a network $\mathbf{\Gamma}$ of M metabolites, which possesses only one single Child Selection $\mathbf{J}$, has a Jacobian determinant of the form:

$$\det G = \det S^{\mathbf{J}} \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}. \tag{2.61}$$

Here $\det S^{\mathbf{J}}$ becomes a scalar coefficient of the multilinear monomial $\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}$ of degree M in the variables $r_{\mathbf{J}(m)m}$. In particular, the determinant can be factorized into M independent factors, each of them corresponding to one metabolite $m \in \mathbf{M}$.

Let us now consider any network such that, for *all* Child Selections $\mathbf{J}$,

$$\det S^{\mathbf{J}} \equiv (-1)^M. \tag{2.62}$$

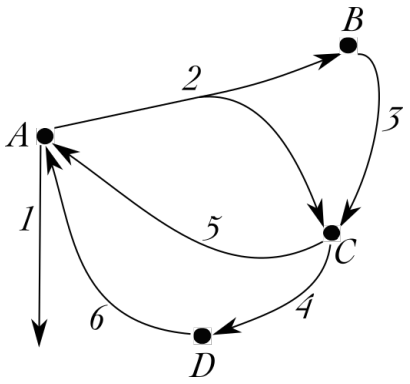
In particular, any Child Selection well-behaves. For instance, this is the case of acyclic monomolecular reaction networks. In this case the Jacobian determinant reads:

$$\det G = \sum_{\mathbf{J}} \det S^{\mathbf{J}} \cdot \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m} = (-1)^M \sum_{\mathbf{J}} \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m} = (-1)^M \prod_{m\in\mathbf{M}} \Big(\sum_{\mathbf{J}(m)} r_{\mathbf{J}(m)m} \Big), \tag{2.63}$$

where the sum $\sum_{\mathbf{J}(m)}$ runs over all the possible children of the metabolite m. In this situation, the determinant is factorizable into M linear subspaces and each of the subspaces depends only on a single metabolite m, as above. In this specific case, each of the linear factors is strictly positive, for any choice of reaction rates. In particular, no eigenvalue can cross zero, and the determinant is always of fixed sign.

The cases described above do not exhaust the casuistry of such factorizations. There are much more diverse examples. Consider, for instance,

$$S = \begin{array}{c} \\ A \\ B \\ C \\ D \end{array} \begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{bmatrix} -1 & -1 & 0 & 0 & 1 & 1 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 \end{bmatrix} \end{array} \tag{2.64}$$



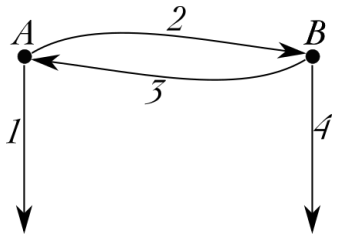
The Jacobian determinant factorizes:

$$\det G = (r_{1A} - r_{2A}) r_{3B} (r_{4C} + r_{5C}) r_{6D}. \tag{2.65}$$

Here, the linear subspace $(r_{1A} - r_{2A})$, corresponding to metabolite A, crosses zero when $r_{1A} = r_{2A}$. This allows the determinant to change sign, hinting at saddle-node bifurcations. In such cases, there is no need to restrict to some ϵ-small choice of parameters of the system, as in Theorem 2.6.1. In fact: for *any* choice of the other parameters, the determinant changes sign when $r_{1A} = r_{2A}$.

On the other hand, extremely simple examples may possess a Jacobian determinant, which does not factorize:

$$S = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cccc} 1 & 2 & 3 & 4 \end{array} \\ \begin{bmatrix} -1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{bmatrix} \end{array} \tag{2.66}$$



In fact, $\det G = r_{1A}r_{3B} + r_{1A}r_{4B} + r_{2A}r_{4B}$ does not factorize.

Therefore, a general interesting question arises:

For which networks, is the Jacobian determinant factorizable?

Equivalently:

For which stoichiometric matrices S does the multilinear polynomial

$$\mathcal{P} := \sum_{\mathbf{J}} \det S^{\mathbf{J}} \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m} \quad \textit{factorize as} \quad \mathcal{P} = \prod_{m} \Big(\sum_{\mathbf{J}(m)} a(\mathbf{J}, m) r_{\mathbf{J}(m)m} \Big)?$$

where $a(\mathbf{J}, m)$ are constants depending on $\mathbf{J}$ and m.

Abstractly considering the space of multilinear polynomials $\mathcal{P}$ of degree M, this is seldom the case. However, stoichiometric matrices of metabolic networks are non-generic, being highly sparse, with few integer entries, only.
These questions, in an algebraic context, have a long history. In fact, they date back to the late 19^{th} century, with the groundbreaking works by Paul Albert Gordan and Alexander von Brill [Gor94] in Germany and Jacques Hadamard [Had99] in France. In these early works, an abstract characterization of algebraic forms factorizing over linear factors was derived. For a more recent reference, see reference book [GKZ08] and the chapter about *Chow Varieties* there. Recent investigations of similar concepts have been done by Yonghui Guan in his doctoral thesis [Gua16] and in [Gua18]. There, connections have been found with the famous conjecture P vs NP, in its algebraic version, firstly posed by Valiant [Val79].

We pose a last question. Let us assume that the Jacobian determinant factorize linearly as above.

Which is the relation between the linear subspace $\sum_{\mathbf{J}(m)} a(\mathbf{J}, m) r_{\mathbf{J}(m)m}$ and the eigenvalue λ_m?

We do not address any of these questions here, leaving them to future work.

2.10 A case study: the central metabolism of E.Coli

The central metabolism of E.Coli consists of different and interconnected parts. In particular, the upper part comprises the so-called *Pentose phosphate pathway* and the *Glycolysis*. The bottom 'cyclic' part includes the *Tricarboxylic acid cycle* and

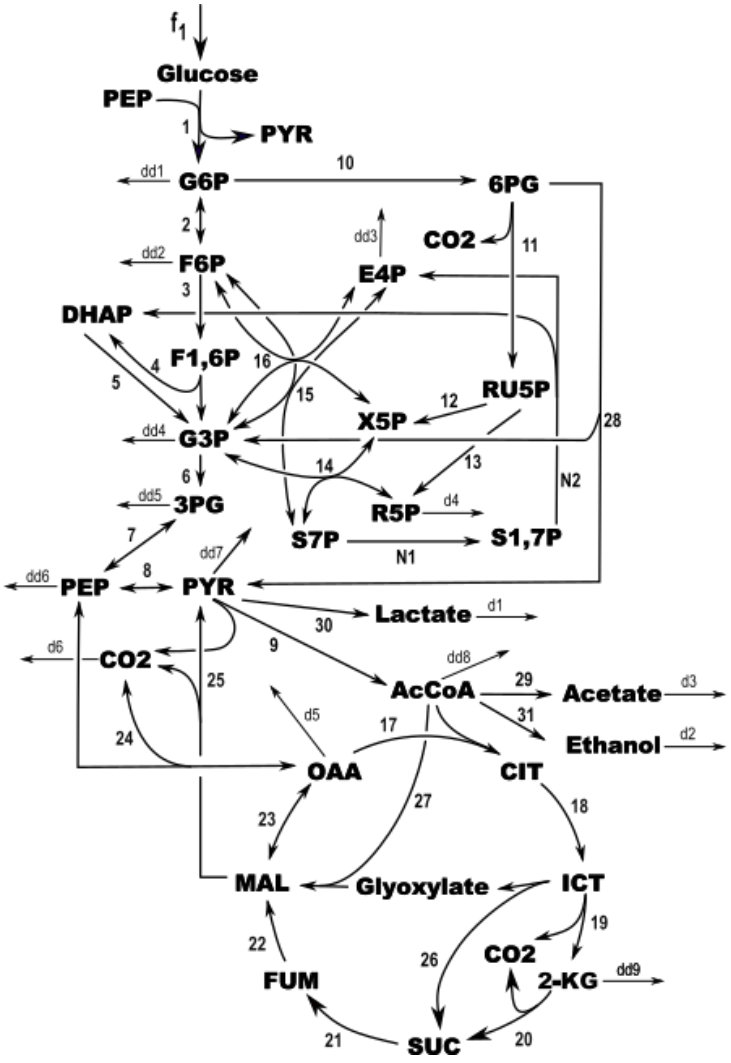


Figure 2.2: This figure has been taken from [BF18] and the graphical representation is courtesy of Anna Karnauhova. Inflow feed reaction is named f_1. Outflow exit reactions are labeled $d1$ – $d6$ and $dd1$ – $dd9$. Here, for image simplicity, a reversible arrow reaction $m \longleftrightarrow \tilde{m}$ encodes two different opposite reactions. Metabolites PEP, PYR and $CO2$ have been graphically repeated, for sake of clarity of the picture.

the *Glyoxylate cycle*. We skip here more detailed biological explanation.

In this section we analyze the network of the central metabolism of Escherichia Coli in Figure 2.2. This network representation is mainly based on the original model proposed by Ishii et al. in [INB+07] with the modifications suggested by Nakahigashi et al. in [NTI+09]. Moreover, in biology papers, 'obvious' outflows exit reactions are frequently omitted. This is the case of reactions $d1-d6$, here. For our mathematical analysis, however, we are bound to include them as well. Note, indeed, that these reactions are the only outgoing reactions of their input metabolites. In particular, their omission would result in an infinite production of their input metabolites and in a mathematical degeneracy of the network.

The network possesses 30 metabolites and 58 reactions. The number of Child Selections is of the order of 10^7. Nevertheless, we can provide interesting biological insights without computing such a huge amount of Child Selections. In the same spirit as Section 2.6, and along its lines, we find here two Child Selections $\mathbf{J}_1$ and $\mathbf{J}_2$ with opposite behavior, at distance $d(\mathbf{J}_1, \mathbf{J}_2) = 1$. That is, $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b)$ for one single metabolite m_b, and $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ for all others metabolite $m \neq m_b$.

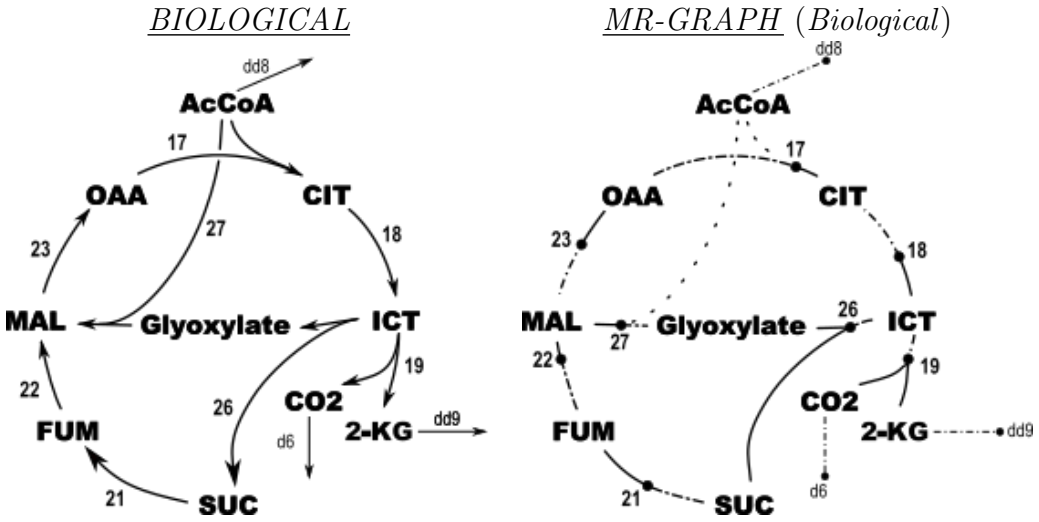


Figure 2.3

This situation, via Theorem 2.6.1, provides a bifurcation parameter responsible for a change of sign in the Jacobian determinant and possible consequent saddle-node bifurcations of equilibria.

To find the two Child Selections $\mathbf{J}_1$ and $\mathbf{J}_2$ as above, we start by attachin certain child reactions j to certain mother metabolites m. We do this arbitrarily, and only for sake of exemplification. Many other choices and analogous constructions are of course possible.
Let us fix the children of the metabolites PEP, PYR, and $CO2$ to be their respective exit reactions, that is:

1. $\mathbf{J}_1(PEP) = \mathbf{J}_2(PEP) = dd6$;
2. $\mathbf{J}_1(PYR) = \mathbf{J}_2(PYR) = dd7$;
3. $\mathbf{J}_1(CO2) = \mathbf{J}_2(CO2) = d6$.

These constraints allow us to consider the upper part (Pent. Phosph. Pathway - Glycolysis) and the bottom bart (Tricarboxylic acid cycle - Glyoxylate cycle) as separate and independent. In fact, any Child Selection $\mathbf{J}$ satisfying the above constraints 1-3, identifies reshuffled minors $S^{\mathbf{J}}$, which are block diagonal. This shows that certain qualitative arguments on the dynamics of the central metabolism may be inferred, separately, from the biological components of the network. For example, for a block diagonal Jacobian matrix in our settings, indeterminate sign determinant of one block trivially implies indeterminate sign determinant for the entire matrix. In particular, we may concentrate on the bottom part of the network, only assuming that $\mathbf{J}_1 = \mathbf{J}_2$ in the upper part.

In Figure 2.3, focusing on the bottom part of the network, we have depicted the chosen subnetwork possessing precisely only the two Child Selections $\mathbf{J}_1$ and $\mathbf{J}_2$. We identify the metabolite m_b, such that $\mathbf{J}_1(m_b) \neq \mathbf{J}_2(m_b)$, as $ICT = m_b$. Indeed, any other metabolite $m \neq ICT$ possesses a single Child $\mathbf{J}_1(m) = \mathbf{J}_2(m)$, and only

ICT possesses two child reactions: reaction 19 and reaction 26. Let us call $\mathbf{J}_1$ the Child Selection such that $\mathbf{J}_1(ICT) = 19$ and $\mathbf{J}_2$ the Child Selection such that $\mathbf{J}_2(ICT) = 26$. In particular, since $\mathbf{J}_1(m) = \mathbf{J}_2(m)$ for any $m \neq ICT$, the two Child Selections are at distance 1, i.e. $d(\mathbf{J}_{19}, \mathbf{J}_{26}) = 1$. With this choice of Child Selections, metabolites *Lactate, Acetate*, and *Ethanol* result disconnected from the rest of the network and have consequently been omitted here.

By looking at the MR-graph representation, we can easily conclude that $\mathbf{J}_1$ well-behaves and $\mathbf{J}_2$ ill-behaves.
Note indeed that $\mathbf{J}_1$ does not contain any completion cycle, and therefore well-behaves. In fact, this Child Selection contains only one network cycle $c = MAL - 23 - OAA - 17 - AcCoa - 27 - MAL$, which is not a completion cycle as the edge $AcCoa - 27$ is not $\mathbf{J}_1$-selected.
On the other hand, the completion cycles structure of $\mathbf{J}_2$ is identical to the one of Example **B2** of Section 2.3, which had provided a simple and recognizable pattern of an ill-behaving Child Selection network. In fact, this Child Selection possesses only two bad completion cycle c_1 and c_2:

1. $c_1 = ICT - 26 - Glyoxylate - 27 - MAL - 23 - OAA - 17 - CIT - 18 - ICT$;
2. $c_2 = ICT - 26 - SUC - 21 - FUM - 22 - MAL - 23 - OAA - 17 - CIT - 18 - ICT$.

Since it possesses only two intersecting bad completion cycles, the Child Selection $\mathbf{J}_{26}$ ill-behaves.
In particular, in accordance to Theorem 2.6.1, the parameter

$$\xi = r_{19m_b} - r_{26m_b}, \text{ (where } m_b = ICT), \tag{2.67}$$

controls a change of sign of the Jacobian determinant of the entire system, for a certain region of parameters.

Remark 10. The choice of reaction 19 and 26 basically highlights the difference between the Tricarboxylic acid cycle (reaction 19) and the Glyoxylate cycle (reaction 26). Our analysis suggests how the control of certain dynamical properties of the metabolism of a cell can be derived from its network structure.

Appendix

2.A Generalizations

Although most stoichiometric entries in metabolic networks are {-1,0,+1}, it is worthwhile to provide a more general version of Theorem 2.4. To this purpose, let $S^{\mathbf{J}}$ be a real $M \times M$ matrix such that $S^{\mathbf{J}}_{mm} < 0$ for any m. Firstly, let us generalize the definition of good/bad-completion as follows:

Definition 2.7 (good/bad-completions, good/bad-cycles - *General form*)**.** In the same notation as Section 2.4, let $\pi = \prod_{i=1}^{\vartheta} c_i \neq \mathit{Id}$ be a nonzero permutation, i.e. $\mathcal{E}(\pi) = \mathrm{sgn}(\pi) \prod_{m \in \mathbf{M}} S^{\mathbf{J}}_{\pi(m)m} \neq 0$.

We call π a *good-completion* if

$$\mathrm{sign}\Big(\prod_{m:\,\pi(m)\neq m} S^{\mathbf{J}}_{\pi(m)m}\Big) = (-1)^{\vartheta}. \tag{2.68}$$

We call π a *bad-completion* if

$$\mathrm{sign}\Big(\prod_{m:\,\pi(m)\neq m} S^{\mathbf{J}}_{\pi(m)m}\Big) = (-1)^{\vartheta-1}. \tag{2.69}$$

Above, ϑ again indicates the number of cycles in the permutation expansion. If $\vartheta = 1$ we call the good(resp. bad)-completion a *good(resp. bad)-cycle*.

For a given completion $\pi \neq \mathit{Id}$ such that $\mathcal{E}(\pi) \neq 0$, let the *value* of π be

$$\mathrm{val}(\pi) = \prod_{m:\,\pi(m)\neq m} \frac{|S^{\mathbf{J}}_{\pi(m)m}|}{|S^{\mathbf{J}}_{mm}|}. \tag{2.70}$$

In particular, note that

$$\mathrm{val}(\pi) = \prod_{i=1}^{\vartheta} \mathrm{val}(c_i). \tag{2.71}$$

The general version of Theorem 2.4.1 reads as follows:

Theorem 2.A.1 (General version)**.** *Let* $\mathbf{J}$ *be a Child Selection.*
Let $\tilde{\mathcal{G}}$ *be*

$$\tilde{\mathcal{G}} = \sum_{\pi\ good} \mathrm{val}(\pi) \tag{2.72}$$

and let $\tilde{B}$ *be*

$$\tilde{\mathcal{B}} = \sum_{\pi\ bad} \mathrm{val}(\pi). \tag{2.73}$$

Then:

1. *The Child Selection* **J** *well-behaves if* $\tilde{\mathcal{G}} > \tilde{B} - 1$.
2. *The Child Selection* **J** *ill-behaves if* $\tilde{\mathcal{G}} < \tilde{B} - 1$.
3. *The Child Selection* **J** *zero-behaves if* $\tilde{\mathcal{G}} = \tilde{B} - 1$.

Proof. The proof is analogous to the proof of Theorem 2.4. The only difference is that we start with a quotient. Indeed:

$$\frac{\det S^{\mathbf{J}}}{\mathcal{E}(Id)} = 1 + \sum_{\pi \neq Id} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} \tag{2.74}$$

Now

$$\begin{aligned} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} =& \frac{\operatorname{sgn}(\pi) \prod_{m=1}^{M} S^{\mathbf{J}}_{\pi(m)m}}{\prod_{m=1}^{M} S^{\mathbf{J}}_{mm}} = \frac{\prod_{i=1}^{\vartheta} \operatorname{sgn}(c_i) \prod_{m:\pi(m)\neq m} S^{\mathbf{J}}_{\pi(m)m}}{(-1)^h |\prod_{m:\pi(m)\neq m} S^{\mathbf{J}}_{mm}|} \\ =& (-1)^{\vartheta} \prod_{m:\pi(m)\neq m} \frac{S^{\mathbf{J}}_{\pi(m)m}}{|S^{\mathbf{J}}_{mm}|} = (-1)^{\mathcal{G}/\mathcal{B}} \operatorname{val}(\pi). \end{aligned} \tag{2.75}$$

Where h is the number of elements of π that belong to a cycle and $(-1)^{\mathcal{G}/\mathcal{B}}$ is 1 if π is good and -1 if π is bad. This leads to the desired equality

$$\frac{\det S^{\mathbf{J}}}{\mathcal{E}(Id)} = 1 + \tilde{\mathcal{G}} - \tilde{\mathcal{B}}, \tag{2.76}$$

which proves the Theorem. Indeed, the sign of the lefthand side is positive, negative, zero if and only if the Child Selection well-behaves, ill-behaves, zero-behaves, respectively. □

Remark 11. We could have started also the proof of Theorem 2.4 with the same ratio argument (2.74). Indeed, for entries $S^{\mathbf{J}}_{mj} = \{-1, 0, +1\}$, a ratio argument is the same as a product argument, of course. In our opinion, however, the product argument illustrated better the concepts leading to MR-graph and completion cycles.

2.B Computational aspects

In relation to the computation (2.74), this appendix contains a simplifying result. Let π be a nonzero permutation, i.e. $\mathcal{E}(\pi) = \operatorname{sgn}(\pi) \prod_{m\in\mathbf{M}} S^{\mathbf{J}}_{\pi(m)m} \neq 0$. Let us assume moreover that $\pi = \prod_{i=1}^{\vartheta} c_i$ with $\vartheta \geq 2$. Let now $\pi' \subseteq \pi$, that is, all c_i cycles of π' are also cycles of π. We are concerned with the following question:

Is it always necessary to compute all $\mathcal{E}(\pi)$*, independently of each other?*

The following proposition provides an answer.

Proposition 2.B.1. *For a permutation* $\pi = \prod_{i=1}^{\vartheta} c_i$*, the following two statements hold true:*

1. *If there exists* at least one *cycle* c_i *satisfying:*

(a) c_i *is a bad-cycle,*

(b) $\mathrm{val}(c_i) = 1$,

then,

$$\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} = -1. \tag{2.77}$$

2. *If* all *cycles* c_i *satisfy:*

(a) c_i *is a good-cycle,*

(b) $\mathrm{val}(c_i) = 1$,

then,

$$\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} = 2^{\vartheta} - 1. \tag{2.78}$$

Proof. With a little abuse of notation, we use now c_i to refer directly to

$$\prod_{m:\, c_i(m) \neq m} \frac{S^{\mathbf{J}}_{c_i(m)m}}{|S^{\mathbf{J}}_{mm}|}. \tag{2.79}$$

1. Let us assume, without loss of generalities, that $c_1 = 1$. That is, c_1 is the bad-cycle with $\mathrm{val}(c_1) = 1$. Consider the sum $\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)}$ written in following form:

$\vartheta' = 1$	-1	$-c_2$	$-c_3$	...	$-c_\vartheta$		
$\vartheta' = 2$	$+1{\cdot}c_2$	$+1{\cdot}c_3$	...	$+1{\cdot}c_\vartheta$	$+c_2c_3$	...	$c_{\vartheta-1}c_\vartheta$
$\vartheta' = 3$	$-1{\cdot}c_2c_3$	...	$-1{\cdot}c_{\vartheta-1}c_\vartheta$	...			
$\vartheta' = ...$	...						
$\vartheta' = \vartheta - 1$	...	$(-1)^{\vartheta-1}c_2c_3\cdots c_\vartheta$					
$\vartheta' = \vartheta$	$(-1)^{\vartheta}1{\cdot}c_2c_3\cdots c_\vartheta$						

(2.80)

Clearly, $\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)}$ is obtained by summing the above rows. Note that each row $\vartheta' > 1$ appears with opposite sign on the right side of the previous row $\vartheta' - 1$. Hence, easy cancellations lead to the result $\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} = -1$

2. The second claim is a well-known property of Pascal triangle. Indeed, we have:

$$\sum_{\pi' \subseteq \pi} \frac{\mathcal{E}(\pi)}{\mathcal{E}(Id)} = \sum_{\vartheta'=1}^{\vartheta} \binom{\vartheta}{\vartheta'} (-1)^{\vartheta'} (-1)^{\vartheta'} = \sum_{\vartheta'=1}^{\vartheta} \binom{\vartheta}{\vartheta'} = 2^{\vartheta} - 1. \tag{2.81}$$

□

Remark 12. For the case in which the stoichiometric matrix has only the values $S^{\mathbf{J}}_{mj} = \{-1, 0, +1\}$, Proposition 2.B.1 always applies.

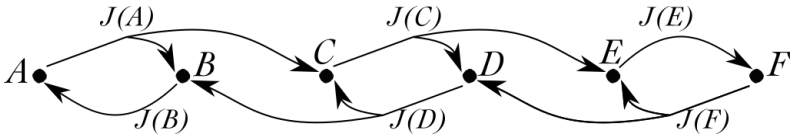
We explain with the following example the use we can make of Proposition 2.B.1. We consider a Child Selection $\mathbf{J}$ with six metabolites.

The matrix $S^{\mathbf{J}}$ is given by:

$$S^{\mathbf{J}} = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{cccccc} \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) & \mathbf{J}(D) & \mathbf{J}(E) & \mathbf{J}(F) \end{array} \\ \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 1 & 0 & 0 \\ 1 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 1 \\ 0 & 0 & 1 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \end{array}, \tag{2.82}$$

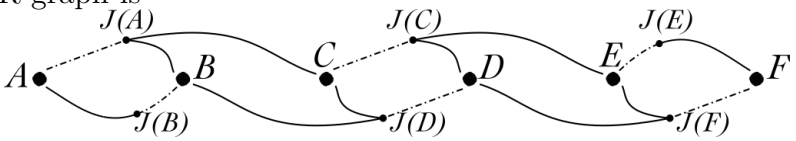
and its determinant, brutally computed, is $\det S^{\mathbf{J}} = (-1)^{6-1} = -1$. In particular, the Child Selection $\mathbf{J}$ ill-behaves. We want here to show how it is possible to use Proposition 2.B.1 to greatly simplify the computation of the determinant.

The graph of the Child Selection $\mathbf{J}$ is



(2.83)

and the MR-graph is



(2.84)

This Child Selection possesses six completion cycles:

1. $c_1 := A - \mathbf{J}(A) - B - \mathbf{J}(B) - A$
2. $c_2 := C - \mathbf{J}(C) - D - \mathbf{J}(D) - C$
3. $c_3 := E - \mathbf{J}(E) - F - \mathbf{J}(F) - E$
4. $c_{12} := A - \mathbf{J}(A) - C - \mathbf{J}(C) - D - \mathbf{J}(D) - B - \mathbf{J}(B) - A$
5. $c_{23} := C - \mathbf{J}(C) - E - \mathbf{J}(E) - F - \mathbf{J}(F) - D - \mathbf{J}(D) - C$
6. $c_{123} := A - \mathbf{J}(A) - C - \mathbf{J}(C) - E - \mathbf{J}(E) - F - \mathbf{J}(F) - D - \mathbf{J}(D) - B - \mathbf{J}(B) - A$

From which, combinatorially, we have the list of all nonzero permutations $\pi \neq \mathit{Id}$:

1. $\pi_{123} := c_{123}$
2. $\pi_{12} := c_{12}$
3. $\pi_{12,3} := c_{12} \cdot c_3$
4. $\pi_{23} := c_{23}$
5. $\pi_{1,23} := c_1 \cdot c_{23}$
6. $\pi_{1,2,3} := c_1 \cdot c_2 \cdot c_3$
7. $\pi_{1,2} := c_1 \cdot c_2$
8. $\pi_{1,3} := c_1 \cdot c_3$
9. $\pi_{2,3} := c_2 \cdot c_3$
10. $\pi_1 := c_1$
11. $\pi_2 := c_2$
12. $\pi_3 := c_3$

In this example, all stoichiometric entries $S^{\mathbf{J}}_{mj}$ are {-1,0,1}. In particular, $\mathcal{E}(Id) = 1$ and $\mathrm{val}(c_i) = 1$ for any i. Moreover, all edges not **J**-selected are positive, with stoichiometric entry $S_{mj} = +1$. Therefore we can apply point 1 of Proposition 2.B.1, since all cycles are bad cycles with $\mathrm{val}(c_i) = 1$.
Let us consider permutation $\pi_{1,2,3}$. Note that it contains permutations $\pi_{1,2}, \pi_{2,3}, \pi_{1,3}, \pi_1, \pi_2, \pi_3$. Thus, via Proposition 2.B.1:

$$\mathcal{E}(\pi_{1,2,3}) + \mathcal{E}(\pi_{1,2}) + \mathcal{E}(\pi_{2,3}) + \mathcal{E}(\pi_{1,3}) + \mathcal{E}(\pi_1) + \mathcal{E}(\pi_2) + \mathcal{E}(\pi_3) = -1. \tag{2.85}$$

We still have to compute $\mathcal{E}(\pi_{12,3})$, $\mathcal{E}(\pi_{1,23})$, $\mathcal{E}(\pi_{12,3})$, $\mathcal{E}(\pi_{123})$, $\mathcal{E}(\pi_{12})$, $\mathcal{E}(\pi_{23})$. We use again Proposition 2.B.1 on $\pi_{1,23}$ and $\pi_{12,3}$, that is

$$\mathcal{E}(\pi_{1,23}) + \mathcal{E}(\pi_1) + \mathcal{E}(\pi_{23}) = -1, \tag{2.86}$$

and

$$\mathcal{E}(\pi_{12,3}) + \mathcal{E}(\pi_{12}) + \mathcal{E}(\pi_3) = -1. \tag{2.87}$$

Note that we had already included in the computation $\mathcal{E}(\pi_1)$ and $\mathcal{E}(\pi_3)$.

In conclusion, for the given example, the computation of the determinant is given by the following expression:

$$\begin{aligned}\det S^{\mathbf{J}} &= 1 + \sum_{\pi \neq Id} \mathcal{E}(\pi) \\ &= 1 + \sum_{\pi \subseteq \pi_{1,2,3}} \mathcal{E}(\pi) + \sum_{\pi \subseteq \pi_{12,3}} \mathcal{E}(\pi) + \sum_{\pi \subseteq \pi_{1,23}} \mathcal{E}(\pi) + \mathcal{E}(\pi_{123}) - \mathcal{E}(\pi_1) - \mathcal{E}(\pi_3) \\ &= 1 + -1 - 1 - 1 - 1 + 1 + 1 = -1\end{aligned} \tag{2.88}$$

The last computation has been made observing that c_{123}, c_1, c_3 are all bad cycles, i.e., $\mathcal{E}(\pi_{123}) = \mathcal{E}(\pi_1) = \mathcal{E}(\pi_3) = -1$.

In this example, we have been able to reduce a computation of twelve permutations to a computation of three single-cycle permutations. The argument has been supported only by an observation of the completion cycles of the network.

Part II

Sensitivity

Chapter 3

Nonzero response

3.1 Introduction

For metabolic chemical reaction networks, sensitivity studies the response to external perturbations of the dynamical system

$$\dot{x} = S\mathbf{r}(x). \tag{3.1}$$

Here, the analysis is done at a positive dynamical equilibrium x^*, which solves

$$0 = S\mathbf{r}(x^*). \tag{3.2}$$

We recall that we assume the existence of an equilibrium x^*, throughout, and that we consider strictly positive reaction rates $\mathbf{r}(x)$, such that $r_j(x)$ depends only on those concentrations x_m for which m is an input metabolite to reaction j. For the present nonzero response analysis, only, we do not require monotonicity of the reaction rates $r_j(x)$. The sign of the responses is treated in the dedicated Chapter 4.

In this chapter we address the sensitivity question at its basics:

Which components of the system respond - qualitatively - to external perturbations?

That is:

Which components of the system do respond, at all? (3.3)

Although the question (3.3) is practically a zero/nonzero question, the results in this chapter also constitute the first step for the sign analysis. Hence, this chapter should be considered as a general introduction to sensitivity analysis of equilibria, for the system (3.1).
However, let us not rush with conclusions and interpretations ante tempore, and let us proceed slowly and in order: step by step. Firstly: what do we mean by external perturbations? Simply put, any network consists of M vertices and N arrows. Here, vertices represent metabolite concentrations and arrows represent reactions. Our analysis aims to be qualitative and structural, in the sense that it is solely based on the network structure, which we leave untouched and unaltered throughout. This yields only to two possible targets of an external perturbation, namely the network

components: vertices (metabolites concentrations) or arrows (reactions).

Two apparently distinct questions emerge from the mist: the response of the network to a metabolite perturbation, and the response of the network to a reaction perturbation.

The techniques used for addressing both questions are very similar, and one question can be reduced to the other. For the sake of consistency, we have merged both questions into one. The one perturbed equation, which we consider, reads as follows:

$$0 = S\mathbf{r}^{\varepsilon}(x^*) + \varepsilon\mu, \tag{3.4}$$

where

$$\mathbf{r}^{\varepsilon} = \mathbf{r} + \varepsilon\rho. \tag{3.5}$$

Above, the positive scalar perturbation parameter $\varepsilon > 0$ controls a *reaction perturbation vector* $\rho \in \mathbb{R}^N$ and a *metabolite perturbation vector* $\mu \in \mathbb{R}^M$.
The vector ρ indicates which reaction rates are perturbed, and the mutual linear ratio of the perturbation on different reactions. We refer to this as a *reaction perturbation*. Respectively, the vector μ indicates which metabolite concentrations are perturbed and the mutual linear ratio of the perturbation on different metabolite concentrations. We refer to this as a *metabolite perturbation*.
Naturally, it is possible to consider the question of a reaction perturbation separately from the question of a metabolite perturbation, simply by considering $\mu = 0$ (reaction perturbation) or $\rho = 0$ (metabolite perturbation). The first original account by Fiedler and Mochizuki [FM15] studied the reaction perturbation case $\mu = 0$ for monomolecular networks, tackling the problem with different techniques than the ones used here. We rather follow the approach developed by Brehm and Fiedler in [BF18], where the authors used Child Selections to study the nonzero response of general networks to targeted reaction perturbations, only.

Let us start by defining the *perturbation vector* $\alpha \in \mathbb{R}^{E+M}$ to be $\alpha := (\rho, \mu)$. For an element $p \in \mathbf{M} \cup \mathbf{E}$ of the network, either a metabolite or a reaction, the corresponding entry $\alpha_p > 0$ corresponds to a positive perturbation, $\alpha_p < 0$ corresponds to a negative perturbation, and $\alpha_p = 0$ corresponds to no perturbation at all. As before, α indicates the mutual linear ratio of the perturbation on different components. We refer to this as an *α-perturbation*.

The objects of study, here, is the equilibrium response of the network to ε-small perturbations at a positive dynamical equilibrium x^*. Vaguely, this means that we study the algebraic form of the differentiated components of equation (3.4), with respect to ε, at $\varepsilon = 0$. There are only two 'responsive' components: metabolite concentrations x and reaction fluxes $\mathbf{r}(x)$. Therefore, the objects of study of our sensitivity analysis are:

1. The metabolite concentration response:

$$\delta x^{\alpha} := \left.\frac{\partial x^*}{\partial \varepsilon}\right|_{\varepsilon=0} \tag{3.6}$$

2. The reaction flux response:

$$\Phi^{\alpha} := \left.\frac{\partial \mathbf{r}^{\varepsilon}(x^*)}{\partial \varepsilon}\right|_{\varepsilon=0} = \rho + R\,\delta x^{\alpha} \tag{3.7}$$

Here, R is again the matrix of the partial derivatives r_{jm} introduced in (2.3).

Moreover, we say that a metabolite m' (or a reaction j') is *influenced* by an α-perturbation if $(\delta x)^{\alpha}_{m'} \neq 0$ ($(\Phi)^{\alpha}_{j'} \neq 0$, resp.), *algebraically*, and we denote this by

$$\alpha \rightsquigarrow m' \ (\alpha \rightsquigarrow j', \text{ resp.}). \tag{3.8}$$

Here, *algebraically* means precisely as a rational function of the partial derivatives r_{jm}. It is crucial to clarify this as much as possible, with no fear of being pedantic. In fact, the responses are rational functions of the partial derivatives r_{jm}, as the continuation of this chapter shows. Non-identically zero rational functions of parameters may be zero for some values of the parameters. *Algebraically nonzero* means that the rational function itself of those parameters is non-identically zero. Since our analysis is qualitative, we will not be able to predict a quantitative zero for certain specific reaction rates. Rather, we are only interested in the zeros for all parameters. All statements about any response δx^{α} and Φ^{α} must be intended in this algebraic sense, even if we omit to specify it.

The core tool of analysis here is the Implicit Function Theorem (IFT). Under mild nondegeneracy assumptions, the IFT guarantees the existence of a family of equilibrium solutions $x^*(\varepsilon)$ to equation (3.4), for sufficiently small ε perturbations.

In particular, the following equality holds, by implicit differentiation:

$$0 = \frac{\partial}{\partial \varepsilon}\left(S\mathbf{r}^{\varepsilon}(x^*(\varepsilon)) + \varepsilon\mu\right) = S\left(\rho + R\delta x^{\alpha}\right) + \mu. \tag{3.9}$$

From the equality (3.9), we obtain the fundamental relation

$$S\left(\Phi^{\alpha}\right) = -\mu. \tag{3.10}$$

Remark 13. In the case of a pure reaction perturbation (i.e., $\mu = 0$), equation (3.10) becomes

$$S\left(\Phi^{\rho}\right) = 0. \tag{3.11}$$

This implies a necessary condition of influence: if any reaction j' is influenced by some reaction perturbation, we have that

$$\exists v \in \ker S \text{ such that } v_{j'} \neq 0. \tag{3.12}$$

Above, $v_{j'}$ indicate the j'-th entry of the vector v.

We have not forgotten - of course - that the IFT only holds under a nondegeneracy assumption. Indeed, the determinant of the Jacobian matrix of the unperturbed system has to be nonzero, that is,

$$\det SR \neq 0. \tag{3.13}$$

Throughout the continuation of this thesis we assume the nondegeneracy condition (3.13) and Section 3.2 below is devoted to giving a structural characterization of this condition.
In Chapter 2, we have mainly denoted the Jacobian matrix as G. However, for the sensitivity analysis, it suits better to emphasize the composition of G as $G = SR$. For this reason we proceed using the SR notation.

Keeping in mind that $\alpha = (\rho, \mu)^T$, we define the $(E + M)$-dimensional *sensitivity response vector* to an α-perturbation as

$$z^\alpha := (\Phi^\alpha, \delta x^\alpha). \tag{3.14}$$

Note that z^α satisfies the following identity:

$$\mathcal{B} z^\alpha = \alpha, \tag{3.15}$$

for every α. Here the *Brehm-matrix*, introduced in [BF18], is defined as

$$\mathcal{B} = \left[\begin{array}{c|c} \mathrm{Id}_N & -R \\ \hline -S & 0 \end{array}\right]. \tag{3.16}$$

Indeed, by definition of Φ,

$$\Phi^\alpha - R\delta x^\alpha = \rho, \tag{3.17}$$

and, by equation (3.10),

$$-S(\Phi^\alpha) = \mu. \tag{3.18}$$

In conclusion, to calculate the responses, the only task is to invert the matrix $\mathcal{B}$.

Well, the word "only" may be sometimes misleading. First of all: is $\mathcal{B}$ invertible, at all? Thankfully, simple computation shows:

$$\left[\begin{array}{c|c} \mathrm{Id}_N & 0 \\ \hline S & \mathrm{Id}_M \end{array}\right]\left[\begin{array}{c|c} \mathrm{Id}_N & -R \\ \hline -S & 0 \end{array}\right]\left[\begin{array}{c|c} \mathrm{Id}_N & R \\ \hline 0 & \mathrm{Id}_M \end{array}\right] = \left[\begin{array}{c|c} \mathrm{Id}_N & 0 \\ \hline 0 & -SR \end{array}\right]. \tag{3.19}$$

Hence, via the Binet theorem, invertibility of $\mathcal{B}$ relies on the invertibility of SR, which we have assumed. Furthermore, the determinant is given by

$$\det \mathcal{B} = (-1)^M \det SR, \tag{3.20}$$

where, we repeat, M is the number of metabolites in the network.

By the inversion formula for block-matrices, we obtain the *sensitivity matrix*

$$\Psi := \mathcal{B}^{-1} = \left[\begin{array}{c|c} \mathrm{Id}_N - R(SR)^{-1}S & -R(SR)^{-1} \\ \hline -(SR)^{-1}S & -(SR)^{-1} \end{array}\right]. \tag{3.21}$$

We see that the inverse of the Jacobian, $(SR)^{-1}$, plays a central role. The main problem here, computationally, is that the entries of the Jacobian matrix SR are only symbolic, namely multilinear polynomials in the derivatives r_{jm}. Already for

reasonably small networks, the complexity of the explicit computation of the entries of Ψ is far too high and unfeasible as a routine method of analysis. One of the main goals of this thesis is therefore to simplify the computation, by providing characterizations which circumvent the brutal inversion of the symbolic Jacobian matrix SR.

Let us consider the case

$$(\rho, \mu) = e_p, \tag{3.22}$$

where $p \in \mathbf{M} \cup \mathbf{E}$ is any element of the network and e_p indicates the p^{th} unit vector in $\mathbb{R}^{N+M}$. In this case, when no ambiguity arises, we write $\alpha = p$ instead of $\alpha = e_p$, for simplicity. This corresponds to a targeted perturbation, namely either a single metabolite concentration or a single reaction rate perturbation. Since the family $\{e_p\}_{p=1}^{N+M}$ constitutes a natural basis of $\mathbb{R}^{N+M}$, any vector response $z^\alpha \in \mathbb{R}^{N+M}$ can be expressed as a linear combination of the responses z^p. Here, z^p satisfies

$$\mathcal{B} z^p = e_p. \tag{3.23}$$

This underlines the importance of the sensitivity matrix $\Psi = \mathcal{B}^{-1}$. Indeed:

1. An entry $[-(SR)^{-1}]_{m'}^{m^*}$ in the lower-right $M \times M$ block encodes how a metabolite m' responds to a targeted perturbation of a metabolite m^*;

$$m^* \rightsquigarrow m'.$$

2. An entry $[-R(SR)^{-1}]_{j'}^{m^*}$ in the upper-right $N \times M$ block encodes how the flux of a reaction j' responds to a targeted perturbation of a metabolite m^*;

$$m^* \rightsquigarrow j'.$$

3. An entry $[-(SR)^{-1}S]_{m'}^{j^*}$ in the lower-left $M \times N$ block encodes how a metabolite m' responds to a targeted perturbation of a reaction j^*;

$$j^* \rightsquigarrow m'.$$

4. An entry $[\mathrm{Id}_N - R(SR)^{-1}S]_{j'}^{j^*}$ in the upper-left $N \times N$ block encodes how the flux of a reaction j' responds to a targeted perturbation of a reaction j^*;

$$j^* \rightsquigarrow j'.$$

In this chapter we characterize structurally the entries of the four blocks above in terms of Child Selections. In Section 3.2 we discuss the nondegeneracy condition allowing us to apply the IFT, in the same spirit of Chapter 2. Section 3.3 lists all results: in particular, explicit formulas for the responses are derived. The expression of the responses is characterized to be nonzero according to certain minors of the stoichiometric matrix S. Since the stoichiometric entries are integers, and non symbolic, this leads to computational simplification as well as meaningful insights. For example, Subsection 3.3.2.3 states that a perturbation on a reaction j^*, which is the single outgoing reaction from one of its inputs m^*, influences only m^*. That is, all responses to a perturbation of j^* are zero, except the response of m^*. Subsection 3.3.4 argues that, mathematically, a metabolite perturbation can be considered as a reaction perturbation, and in particular there is no need to develop the metabolite case independently. Section 3.4 concludes the chapter with all the proofs.

3.2 Nondegeneracy condition

As analyzed in Proposition 2.2.1, the Jacobian determinant of SR can be expressed in terms of Child Selections $\mathbf{J}$:

$$\det SR = \sum_{\mathbf{J}} \det S^{\mathbf{J}} \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}. \tag{3.24}$$

As a trivial corollary to (3.24) and Definition 2.2 , we obtain a characterization of a nondegenerate network.

Corollary 3.2.1. *A chemical reaction network is* nondegenerate*, i.e.*

$$\det SR \neq 0, \ \textit{algebraically}, \tag{3.25}$$

if and only if there exists a Child Selection $\mathbf{J}$*, such that* $\mathbf{J}$ *does not zero-behave, i.e.,*

$$\det S^{\mathbf{J}} \neq 0. \tag{3.26}$$

In particular:

1. *any network, which possesses a good Child Selection, is nondegenerate;*
2. *any network, which possesses a bad Child Selection, is nondegenerate.*

We can see, here already, what we mean as computational simplification. In fact, the question whether the Jacobian determinant of SR is nonzero may look of high complexity due to the symbolic entries of SR. Here we are able to verify whether it is nonzero only with the existence of a nonzero minors $S^{\mathbf{J}}$ of the stoichiometric matrix S. Good circumstance: the stoichiometric matrix S has integer entries instead of symbols and computing them is much faster.

3.3 Results

For each of the four blocks of the sensitivity matrix Ψ, (3.21), we give a theorem describing the entries of the block.

3.3.1 Metabolite perturbation

The responses of a targeted metabolite perturbation are encoded in the right blocks of the sensitivity matrix Ψ, that are:

1. $\{(\delta x)^{m^*}_{m'}\} = -(SR)^{-1}$, which describes the metabolites response to metabolite perturbations;
2. $\{(\Phi)^{m^*}_{j'}\} = -R(SR)^{-1}$, which describes the fluxes response to metabolite perturbations.

3.3.1.1 Metabolite response to metabolite perturbation

We analyze the expression

$$(\delta x)^{m^*}_{m'} = -((SR)^{-1})^{m^*}_{m'}, \tag{3.27}$$

which describes the response of the concentration of m' to a perturbation of the concentration of m^*. Here, $((SR)^{-1})^{m^*}_{m'}$ indicates the entry of $(SR)^{-1}$ in the m^*-th column and m'-th row.

The result relies on a modified concept of Child Selections: the *Partial Child Selections* (PCS).

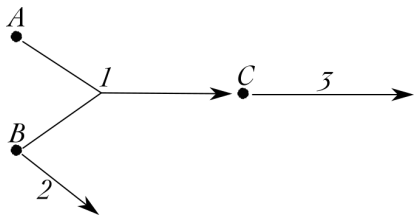
Definition 3.1 (Partial Child Selection)**.** A Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'}$ is an injective map from the metabolite set $\mathbf{M} \setminus \{m'\}$ to the reaction set $\mathbf{E}$, such that to any metabolite $m \neq m'$ is associated an outgoing reaction of m, injectively.

The expression $S^{\mathbf{J}^{\vee\mathbf{m}'}}$ indicates, then, the $M \times (M-1)$ matrix whose i-th column corresponds to the reaction $\mathbf{J}^{\vee\mathbf{m}'}(m_i)$. Of course, a proper relabeling of the index i of m_i has to be taken in account, due to the missing metabolite m'. Intentionally, we are not entirely explicit here, not to linger on heavy and pointless notation. The notation $S^{\mathbf{J}^{\vee\mathbf{m}'}}_{\vee m^*}$ naturally indicates the $(M-1)\times(M-1)$ matrix obtained from $S^{\mathbf{J}^{\vee\mathbf{m}'}}$ by removing the m^*-th row.

Remark 14. We point at an important and deceptive feature of Partial Child Selections. In fact, they may innocently look as a restriction of Child Selections, in the sense that from each Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'}$ it is possible to induce an associated Child Selection $\mathbf{J}$ such that $\mathbf{J}(m) = \mathbf{J}^{\vee\mathbf{m}'}(m)$ for any $m \neq m'$. This is actually not always the case.

Let us clarify Remark 14 with the following minimal example:

$$S = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} 1 & 2 & 3 \end{array} \\ \begin{bmatrix} -1 & 0 & 0 \\ -1 & -1 & 0 \\ 1 & 0 & -1 \end{bmatrix} \end{array}, \tag{3.28}$$



There is only one Child Selection, namely $\mathbf{J} := \{\mathbf{J}(A) = 1; \mathbf{J}(B) = 2; \mathbf{J}(C) = 3\}$. However, considering the Partial Child Selections on the set $\{B, C\}$ only, that is, with vertex A being removed, we have now two Partial Child Selections $\mathbf{J}^{\vee\mathbf{A}}{}_1$ and $\mathbf{J}^{\vee\mathbf{A}}{}_2$. That is,

1. $\mathbf{J}^{\vee\mathbf{A}}{}_1 := \{\mathbf{J}^{\vee\mathbf{A}}{}_1(B) = 1; \mathbf{J}^{\vee\mathbf{A}}{}_1(C) = 3\}$;
2. $\mathbf{J}^{\vee\mathbf{A}}{}_2 := \{\mathbf{J}^{\vee\mathbf{A}}{}_2(B) = 2; \mathbf{J}^{\vee\mathbf{A}}{}_2(C) = 3\}$.

In fact: via a Partial Child Selection $\mathbf{J}^{\vee\mathbf{A}}$, B can freely select both reactions 1 and 2. In contrast, via a Child Selection $\mathbf{J}$, B must select reaction 2, due to injectivity. In fact: reaction 1 must be chosen by A. This observation has important consequences in the analysis below and in Chapter 4.

For the above reason, we make a distinction on Partial Child Selections, depending on whether they can be completed to a Child Selection of the entire network or not.

Definition 3.2 (Deducible/Non-deducible PCS)**.** We call *deducible* Partial Child Selections (dPCS) $\mathbf{J}^{\vee}$ the PCS which can be deduced from a Child Selection by removing a metabolite and its reaction image. In particular, any restriction of a Child Selection $\mathbf{J}$ to the subset $\mathbf{M} \setminus \{m'\}$, coincides with a dPCS $\mathbf{J}^{\vee\mathbf{m}'}$. On the other hand, we say that a Child Selection $\mathbf{J}$ is *induced* from the dPCS $\mathbf{J}^{\vee\mathbf{m}'}$ if $\mathbf{J}(m) = \mathbf{J}^{\vee\mathbf{m}'}(m)$, for any $m \neq m'$.
Respectively, we call *non-deducible* Partial Child Selections (nPCS) $J^{\vee}$ the PCS, which are not deducible.

In Example 3.28 above, $\mathbf{J}^{\vee\mathbf{A}}{}_2$ is *deducible* and $\mathbf{J}^{\vee\mathbf{A}}{}_1$ is *non-deducible*.

The result for metabolite response to a metabolite perturbation reads as follows:

Theorem 3.3.1. *Let m^* and m' be two (not necessarily distinct) metabolites. Then the response $(\delta x)^{m^*}_{m'}$ of metabolite m' to a targeted perturbation of the metabolite m^* is given by the formula:*

$$(\delta x)^{m^*}_{m'} = -\frac{(-1)^{m^*+m'} \sum_{\mathbf{J}^{\vee\mathbf{m}'}} \det S^{\mathbf{J}^{\vee\mathbf{m}'}}_{\vee m^*} \cdot \prod_{m\in\mathbf{M}\setminus m'} r_{\mathbf{J}^{\vee\mathbf{m}'}(m)m}}{\det SR}. \tag{3.29}$$

In particular,

$$(\delta x)^{m^*}_{m'} \neq 0, \text{ algebraically,} \tag{3.30}$$

if, and only if, there exists a Partial Child Selection

$$\mathbf{J}^{\vee\mathbf{m}'} : \mathbf{M} \setminus m' \longrightarrow \mathbf{E} \tag{3.31}$$

such that

$$\det(S^{\mathbf{J}^{\vee\mathbf{m}'}}_{\vee m^*}) \neq 0. \tag{3.32}$$

Note that the expression (3.29) is a rational function, whose numerator is a homogenous multilinear polynomial of degree $M - 1$, and whose denominator is a homogenous multilinear polynomial of degree M (the Jacobian determinant SR). Both polynomials in the numerator and in the denominator are in the variables r_{jm}.

Remark 15. Formula (3.29) is quite general. It simply describes the entries of the inverse of a Jacobian matrix, within our network settings. In particular, let us consider the *adjugate* matrix $\mathrm{Ad}(SR)$ of SR, whose entries are defined as:

$$\mathrm{Ad}(SR)_{mj} := (-1)^{m+j} \det(SR)^{\vee m}_{\vee j}, \tag{3.33}$$

where $(SR)^{\vee m}_{\vee j}$ indicates the $(M-1)\times(M-1)$ minor of SR with removed j^{th} row and m^{th} column. Well-known relation, for the inverse $(SR)^{-1}$, is then:

$$(SR)^{-1} = \frac{\mathrm{Ad}(SR)}{\det(\mathrm{SR})}, \tag{3.34}$$

which implies that the numerator of Formula (3.29) is given by $\mathrm{Ad}(SR)^{m^*}_{m'}$, since $(\delta x)^{m^*}_{m'} = -((SR)^{-1})^{m^*}_{m'}$. Note, in particular that

$$(-1)^{m^*+m'} \det S^{\mathbf{J}^{\vee \mathbf{m}'}}_{\vee m^*} = \mathrm{Ad}(S^{\mathbf{J}})_{m'm^*}. \tag{3.35}$$

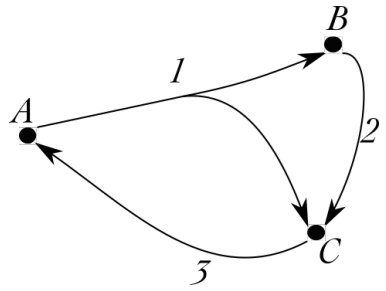
Remark 16. Let the expression

$$S^{\mathbf{J}^{\vee \mathbf{m}'} \cup \mathbf{e}_{\mathbf{m}^*}} \tag{3.36}$$

indicate the $M \times M$ matrix obtained from $S^{\mathbf{J}^{\vee \mathbf{m}'}}$ by inserting, as m'-th column, the unit vector $e_{m^*} \in \mathbb{R}^M$. Note the following equality, due to Laplace determinant expansion:

$$(-1)^{m^*+m'} \det S^{\mathbf{J}^{\vee \mathbf{m}'}}_{\vee m^*} = \det S^{\mathbf{J}^{\vee \mathbf{m}'} \cup \mathbf{e}_{\mathbf{m}^*}}. \tag{3.37}$$

Self-influence does not always happen. Indeed, consider Example **B2** of 2.3:



(3.38)

This dynamical system has equations

$$\begin{cases} \dot{A} = -r_1(A) + r_3(C) \\ \dot{B} = r_1(A) - r_2(B) \\ \dot{C} = r_1(A) + r_2(B) - r_3(C) \end{cases}. \tag{3.39}$$

The Jacobian is easily computed to be:

$$SR = \begin{matrix} \\ A \\ B \\ C \end{matrix} \begin{matrix} \begin{matrix} A & B & C \end{matrix} \\ \begin{bmatrix} -r_{1A} & 0 & r_{3C} \\ r_{1A} & -r_{2B} & 0 \\ r_{1A} & r_{2B} & -r_{3C} \end{bmatrix} \end{matrix}, \quad \det SR = r_{1A} r_{2B} r_{3C}\,. \tag{3.40}$$

The metabolite responses matrix is

$$\{(\delta x)^{m^*}_{m'}\} = -(SR)^{-1} = \frac{1}{\det SR} \begin{matrix} \\ A \\ B \\ C \end{matrix} \begin{matrix} \begin{matrix} A & B & C \end{matrix} \\ \begin{bmatrix} -r_{2B}r_{3C} & -r_{2B}r_{3C} & -r_{2B}r_{3C} \\ -r_{1A}r_{3C} & 0 & -r_{1A}r_{3C} \\ -2r_{1A}r_{2B} & -r_{1A}r_{2B} & -r_{1A}r_{2B} \end{bmatrix} \end{matrix}. \tag{3.41}$$

This example shows that all responses may be nonpositive, and a perturbation of metabolite B does not produce a response on the concentration of B itself. Moreover, we see here a counterexample to metabolite transitivity of influence, although in a rather degenerate case. Indeed B influences C, C influences B, but B does not influence itself. See Chapter 6.

3.3.1.2 Flux response to metabolite perturbation

We analyze the expression

$$(\Phi)^{m^*}_{j'} = R_{j'}(\delta x)^{m^*} = -R_{j'}((SR)^{-1})^{m^*}. \tag{3.42}$$

which describes the response of the flux of j' to a perturbation of the concentration of m^*. Expression (3.42) indicates that we have to compute an inner product between the row vector $R_{j'}$ and the column vector $-((SR)^{-1})^{m^*}$. We know both vectors: $R_{j'}$ by the network structure and $-((SR)^{-1})^{m^*}$ by Theorem 3.3.1. The entries of $R^m_{j'}$ are nonzero if and only if the metabolite m is an input metabolite to reaction j'. The entries of $-((SR)^{-1})^{m^*}_m$ are nonzero if and only if m^* influences m.
However it is not always true that $-R_{j'}((SR)^{-1})^{m^*}$ is nonzero if m^* does influence some input metabolites of reaction j'. In other words, cancellations may happen.

Regarding cancellations, let us be clear with an example, again (3.28). The equations are

$$\begin{cases} \dot{A} = -r_1(A,B) \\ \dot{B} = -r_1(A,B) - r_2(B) \\ \dot{C} = +r_1(A,B) - r_3(C) \end{cases}. \tag{3.43}$$

The Jacobian of the system is:

$$SR = \begin{matrix} \\ A \\ B \\ C \end{matrix} \begin{matrix} \begin{matrix} A & B & C \end{matrix} \\ \begin{bmatrix} -r_{1A} & -r_{1B} & 0 \\ -r_{1A} & -r_{1B} - r_{2B} & 0 \\ r_{1A} & r_{1B} & -r_{3C} \end{bmatrix} \end{matrix}, \tag{3.44}$$

and the metabolite sensitivity matrix corresponds to

$$\{(\delta x)^{m^*}_{m'}\} = -(SR)^{-1} = \frac{1}{\det SR} \begin{matrix} A \\ B \\ C \end{matrix} \begin{bmatrix} \begin{matrix} A \\ -r_{1B}r_{3C} - r_{2B}r_{3C} \\ +r_{1A}r_{3C} \\ -r_{1A}r_{2B} \end{matrix} & \begin{matrix} B \\ +r_{1B}r_{3C} \\ -r_{1A}r_{3C} \\ 0 \end{matrix} & \begin{matrix} C \\ 0 \\ 0 \\ -r_{1A}r_{2B} \end{matrix} \end{bmatrix}. \tag{3.45}$$

Let us concentrate on the B column $(\delta x)^B$, which indicates the responses of the three metabolites A, B, C to a perturbation of B. The response Φ_1^B of the flux through reaction 1 to a perturbation of metabolite B is given by the inner product between and $(\delta x)^B$,

$$\Phi_1^B = \langle R_1^T, (\delta x)^B \rangle \tag{3.46}$$

where $R_1 = (r_{1A}, r_{1B}, 0)$ and $(\delta x)^B = \frac{1}{\det SR} \cdot (r_{1B}r_{3C}, -r_{1A}r_{3C}, 0)^T$. Therefore:

$$\Phi_1^B = \frac{1}{\det SR} \cdot (r_{1A}r_{1B}r_{3C} - r_{1A}r_{1B}r_{3C}) = 0, \tag{3.47}$$

and metabolite B does not influence reaction 1. Note that the expression $r_{1A}r_{1B}r_{3C}$ is not associated with a Child Selection, since reaction 1 appears twice, contradicting injectivity.

Example (3.28) constitutes also a counterexample to transitivity of influence of the case *metabolite* $\rightsquigarrow$ *metabolite* $\rightsquigarrow$ *reaction*. Indeed, metabolite A influences reaction 1:

$$\Phi_1^A = \langle R_1^T, (\delta x)^A \rangle = -\frac{1}{\det SR} r_{1A}r_{2B}r_{3C}. \tag{3.48}$$

On the other hand, metabolite B influences metabolite A, since $(\delta x)^B_A = r_{1B}r_{3C}$. Nevertheless, B does not influence reaction 1, i.e. $\Phi_1^B = 0$.

It is possible to prove a result about the flux response analyzing when the above kind of cancellations occur. However, we take here an independent route, so that any of our results can be considered singularly. Let now the expression

$$S^{\mathbf{J} \setminus j' \cup e_{m^*}} \tag{3.49}$$

indicate the $M \times M$ matrix obtained from $S^{\mathbf{J}}$ by removing the column $S^{j'}$, for $j' \in \mathbf{J}$, and replacing it with the unit vector $e_{m^*} \in \mathbb{R}^M$, in the same position of $S^{j'}$.

Our result again characterizes the flux response to metabolite perturbations in terms of Child Selections.

Theorem 3.3.2. *Let m^* be a metabolite and j' be a reaction. Then the flux response $(\Phi)^{m^*}_{j'}$ of reaction j' to a targeted perturbation of the concentration of metabolite m^**

is given by

$$(\Phi)_{j'}^{m^*} = -\frac{\sum_{\mathbf{J}\ni j'} \det S^{\mathbf{J}\setminus j'\cup e_{m^*}} \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m),m}}{\det SR}. \tag{3.50}$$

In particular,

$$(\Phi)_{j'}^{m^*} \neq 0, \ \textit{algebraically,} \tag{3.51}$$

if, and only if, there exists a Child Selection $\mathbf{J}$ *containing* j' *and such that*

$$\det(S^{\mathbf{J}\setminus j'\cup e_{m^*}}) \neq 0. \tag{3.52}$$

The expression (3.50) is a rational function, whose both numerator and denominator are homogenous multilinear polynomials of degree M.

Remark 17. Any metabolite response $(\delta x)_m^{m^*}$, which is expressed by a multilinear polynomial associated to a nPCS must undergo cancellations when computing the inner product $\langle R_{j'}^T, (\delta x)^{m^*}\rangle$. Theorem 3.3.2, indeed, asserts that the flux response expressions are always associated to a Child Selection.

3.3.2 Reaction perturbation

The two results of this section are due to Brehm and Fiedler [BF18]. We present them here for sake of completeness, following our personal point of view. They are concerned with the left blocks of the sensitivity matrix Ψ, that is:

1. $\{(\delta x)_{m'}^{j^*}\} = -(SR)^{-1}S$, which describes the metabolites response to reaction perturbations;
2. $\{(\Phi)_{j'}^{j^*}\} = \mathrm{Id}_N - R(SR)^{-1}S$, which describes the fluxes response to reaction perturbations.

3.3.2.1 Metabolite response to reaction perturbation

We analyze the expression

$$(\delta x)_{m'}^{j^*} = -((SR)^{-1}S^{j^*})_{m'} = -((SR)^{-1})_{m'}S^{j^*}. \tag{3.53}$$

which describes the response of the concentration of m' to a perturbation of the rate of j^*. Equality (3.53) shows the following connected points:

1. The response of metabolite m' to a targeted perturbation of reaction j^* is given by the inner product between the row vector $-((SR)^{-1})_{m'}$ of the m'-th metabolite response to any metabolite perturbation and the stoichiometric column vector S^{j^*} corresponding to reaction j^*.
2. Considering the inner product $\langle -((SR)^{-1})_{m'}^T, S^{j^*}\rangle$, the only possible nonzero summands are given by those entries m such that both $-((SR)^{-1})_{m'}^m = (\delta x)_{m'}^m$ and $S_m^{j^*}$ are nonzero. That is, corresponding to metabolites m, *influencers* of m', that are participating to reaction j^*.

3. Lastly, the inner product is between a vector $-((SR)^{-1})_{m'}$ with entries as rational functions described by Theorem 3.3.1 and a vector S^{j^*} with integer entries. Hence the form of the metabolite response to reaction perturbation must have the same algebraic form as the entries of $-((SR)^{-1})_{m'} = (\delta x)_{m'}$. That is, a rational function whose both numerator and denominator are homogeneous multilinear polynomials. The numerator has degree $M-1$ and the denominator has degree M.

In analogy to (3.36), let

$$S^{\mathbf{J}^{\vee\mathbf{m}'}\cup j^*} \tag{3.54}$$

indicate the $M \times M$ matrix obtained from $S^{\mathbf{J}^{\vee\mathbf{m}'}}$ by inserting, as m'-th column, the column S^{j^*}. The result, which confirms independently the above observations, reads:

Theorem 3.3.3. *Let j^* be a reaction and m' be a metabolite. Then the response $(\delta x)^{j^*}_{m'}$ of metabolite m' to a reaction rate perturbation of j^* is given by*

$$(\delta x)^{j^*}_{m'} = -\frac{\sum_{\mathbf{J}^{\vee\mathbf{m}'}\not\ni j^*} \det S^{\mathbf{J}^{\vee\mathbf{m}'}\cup j^*} \cdot \prod_{m\in\mathbf{M}\setminus m'} r_{\mathbf{J}^{\vee\mathbf{m}'}(m)m}}{\det SR}. \tag{3.55}$$

In particular,

$$(\delta x)^{j^*}_{m'} \neq 0, \ \textit{algebraically,} \tag{3.56}$$

if, and only if, there exists a Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'} : \mathbf{M}\setminus\{m'\} \to \mathbf{E}\setminus\{j^*\}$ such that

$$\det(S^{\mathbf{J}^{\vee\mathbf{m}'}\cup j^*}) \neq 0. \tag{3.57}$$

The responses (3.55) are indeed of the same algebraic form as the metabolite response to metabolite perturbation (3.29). In particular, the numerator of the rational function is given by a homogenous multilinear polynomial of degree $M-1$, expressed by PCS.

Again, considering Example (3.28). We recall the metabolite sensitivity matrix

$$\{(\delta x)^{m^*}_{m'}\} = -(SR)^{-1} = \frac{1}{\det SR} \begin{matrix} \\ A \\ B \\ C \end{matrix} \begin{matrix} \begin{matrix} A & B & C \end{matrix} \\ \begin{bmatrix} -r_{1B}r_{3C} - r_{2B}r_{3C} & r_{1B}r_{3C} & 0 \\ r_{1A}r_{3C} & -r_{1A}r_{3C} & 0 \\ -r_{1A}r_{2B} & 0 & -r_{1A}r_{2B} \end{bmatrix} \end{matrix}. \tag{3.58}$$

and the stoichiometric matrix

$$S = \begin{matrix} \\ A \\ B \\ C \end{matrix} \begin{matrix} \begin{matrix} 1 & 2 & 3 \end{matrix} \\ \begin{bmatrix} -1 & 0 & 0 \\ -1 & -1 & 0 \\ 1 & 0 & -1 \end{bmatrix} \end{matrix}. \tag{3.59}$$

The matrix $\{(\delta x)^{j^*}_{m'}\}$ of the metabolite response to reaction perturbation is then

$$\{(\delta x)^{j^*}_{m'}\} = -(SR)^{-1}S = \frac{1}{\det SR} \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccc} 1 & 2 & 3 \end{array} \\ \begin{bmatrix} r_{2B}r_{3C} & -r_{1B}r_{3C} & 0 \\ 0 & r_{1A}r_{3C} & 0 \\ 0 & 0 & r_{1A}r_{2B} \end{bmatrix} \end{array}. \tag{3.60}$$

Note the zero influence of reaction 1 on metabolite B and C. Both are due to PCS cancellations, e.g. for B, $-(SR)^{-1}_B = (r_{1A}r_{3C}, -r_{1A}r_{3C}, 0)$ and $S^1 = (-1,-1,1)^T$:

$$\langle -((SR)^{-1}_B)^T, S^1\rangle = \frac{1}{\det SR}(-r_{1A}r_{3C} + r_{1A}r_{3C}) = 0. \tag{3.61}$$

3.3.2.2 Flux response to reaction perturbation

We analyze the expression

$$\Phi^{j^*}_{j'} = \delta_{j^*j'} - R_{j'}(SR)^{-1}S^{j^*}, \tag{3.62}$$

which describes the response of the flux of j' to a perturbation of the rate of j^*. Above, $\delta_{j^*j'}$ is the Kronecker delta. We have two slightly different theorems: one corresponding to the self-influence case $j^* = j'$, the other to the case of distinct reactions $j^* \neq j'$.
For the self-influence case $j^* = j'$ the theorem reads as follows:

Theorem 3.3.4. *Let j^* be a reaction. Then the flux response $(\Phi)^{j^*}_{j^*}$ of reaction j^* to an external perturbation of the reaction rate of j^* itself is given by:*

$$(\Phi)^{j^*}_{j^*} = \frac{\sum_{\mathbf{J}\not\ni j^*} \det S^{\mathbf{J}} \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR}. \tag{3.63}$$

In particular,

$$(\Phi)^{j^*}_{j^*} \neq 0, \ \textit{algebraically,} \tag{3.64}$$

if and only if there exists a Child Selection $\mathbf{J}$ such that $j^* \notin \mathbf{J}$ and

$$\det S^{\mathbf{J}} \neq 0. \tag{3.65}$$

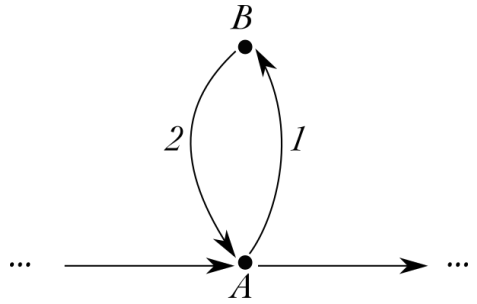
Promptly, we derive the following corollary

Corollary 3.3.5. *Let j^* be a reaction such that, for any Child Selection $\mathbf{J}$ containing j^*, $\det S^{\mathbf{J}} = 0$. Then*

$$(\Phi)^{j^*}_{j^*} \equiv 1. \tag{3.66}$$

Corollary 3.3.5 is completely independent from any choice of reaction rates, as well as from the sign of the Jacobian determinant $\det SR$.

As an example, let us consider a network of the following kind:



(3.67)

The basic structure considered is a metabolite A with more than one outgoing reactions. Among those outgoing reactions, a monomolecular reaction 1 leads to a metabolite B, which possesses a single outgoing child reaction 2 leading back to A such that reactions 1 and 2 construct a directed cycle. The network, outside this basic structure can be freely chosen. In such a case, we have that

$$\Phi_1^1 \equiv 1, \tag{3.68}$$

independently on the rest on the network. Note, indeed, that any Child Selection $\mathbf{J}$ containing 1 has $\det S^{\mathbf{J}} = 0$, since reaction 2 has to be in the image of any Child Selection, being single outgoing child reaction of B. Moreover reaction 1 and reaction 2 have the same opposite stoichiometry. This leads to a linear dependency in the column of $S^{\mathbf{J}}$ and consequently $\det S^{\mathbf{J}} = 0$, if $1 \in \mathbf{J}$.

The case when $j^* \neq j'$ is slightly more involved. In analogy to (3.49), let

$$S^{\mathbf{J}\setminus j' \cup j^*} \tag{3.69}$$

indicate the $M \times M$ matrix obtained from $S^{\mathbf{J}}$ by removing the column $S^{j'}$, for $j' \in \mathbf{J}$, and replacing it with the column S^{j^*}, in the same position of $S^{j'}$.

Theorem 3.3.6. *Let j^*, j' be two distinct reactions. Then a rate perturbation of j^* produces a response $(\Phi)_{j'}^{j^*}$ in the flux of j' given by*

$$(\Phi)_{j'}^{j^*} = -\frac{\sum_{j^* \notin \mathbf{J} \ni j'} \det S^{\mathbf{J}\setminus j' \cup j^*} \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR}, \tag{3.70}$$

In particular,

$$(\Phi)_{j'}^{j^*} \neq 0, \text{ algebraically,} \tag{3.71}$$

if, and only if, there exists a Child Selection $\mathbf{J}$ such that $j^* \notin \mathbf{J} \ni j'$ and

$$\det(S^{\mathbf{J}\setminus j' \cup j^*}) \neq 0. \tag{3.72}$$

3.3.2.3 Single children

This subsection collects two corollaries to reaction perturbation results, Theorems 3.3.3, 3.3.4, and 3.3.6. With little assumptions, and no computations, both corollaries yield strong conclusions.

Corollary 3.3.7. *Let m^* participate as input only in one single reaction j^*. Then the following three conclusions hold true:*

1. $\Phi^{j^*}_{j'} = 0$ *for any* $j' \in \mathbf{E}$;
2. $\Phi^{j^*}_{m'} = 0$ *for any* $m' \neq m^*$;
3. $\Phi^{j^*}_{m^*} < 0$, *assuming* $r_{j^*m^*} > 0$.

Note that, in Corollary 3.3.7, the reaction j^* is required to be a single outgoing reaction from m^*. This condition is stronger than the condition of j^* being a single child, in the sense of $\mathbf{J}(m^*) \equiv j^*$, for any Child Selection $\mathbf{J}$.
Indeed, relaxing the single-outgoing condition to the single-child condition leads to the second corollary of this subsection, which is a milder version of 3.3.7.

Corollary 3.3.8. *Let j^* be the single child of the mother metabolite m^*, that is, $\mathbf{J}(m^*) \equiv j^*$, for any Child Selection $\mathbf{J}$. Then $\Phi^{j^*}_{j'} = 0$ for any $j' \in \mathbf{E}$.*

The corollaries can be summarized in two catchy sentences. The first Corollary 3.3.7 in the statement:

Single outgoing reactions always influence only their input, negatively!

The second Corollary 3.3.8 in the statement:

Single children have no flux-influence!

3.3.3 General α-perturbations

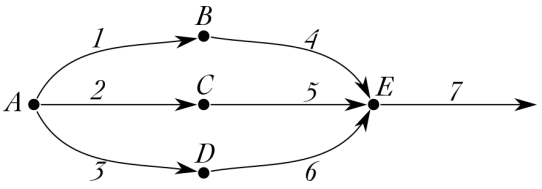
What about general α-perturbations?

Mathematically, when we consider a general perturbation vector $\alpha = (\rho, \mu) \in \mathbb{R}^{E+M}$ and any network single component p, we must be aware about possible cancellations between the summands in the response inner product:

$$z^{\alpha}_{p} = -\langle \Psi^{T}_{p}, \alpha \rangle. \tag{3.73}$$

The following example shows how cancellations are possible.

$$S = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \begin{array}{c} \begin{array}{ccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 \end{array} \\ \begin{bmatrix} -1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 1 & 1 & -1 \end{bmatrix} \end{array}. \tag{3.74}$$



Let us focus on a perturbation of reaction 2 and of reaction 3, and let us check the response of the flux of reaction 4. By Theorem 3.3.6 or by brute calculation, we observe that:

1. $(\Phi)^2_4 = -r_{1A}r_{4B}r_{5C}r_{6D}r_{7E}$;
2. $(\Phi)^3_4 = -r_{1A}r_{4B}r_{5C}r_{6D}r_{7E}$.

That is, $(\Phi)^2_4 = (\Phi)^3_4$, because a symmetry of structure holds. Now, consider α such that $\alpha_2 = +1$, $\alpha_3 = -1$ and $\alpha_i = 0$ for all $i \neq 2, 3$. In this case we would have zero response $(\Phi)^\alpha_4 = 0$, although $(\Phi)^2_4, (\Phi)^3_4 \neq 0$.

It is beyond the interest of this thesis to investigate conditions for not having cancellations. However, we should not bother too much about this problem. In fact, this is an abstract mathematical situation in which the perturbation vector α can be chosen with such a precision to cause cancellations. In a real application, even only for intrinsic operational errors, we cannot expect to choose α in such a way that $|\alpha_k| = |\alpha_i|$, for any k and i. This is indeed a non-generic effect. For this reason, in any feasible biological application, cancellations should be excluded, when investigating a nonzero response.

3.3.4 Reducing metabolite to reaction perturbations

Let us consider Formula (3.50) for the flux response of reaction j' to a metabolite perturbation of m^*

$$(\Phi)^{m^*}_{j'} = -\frac{\sum\limits_{\mathbf{J}\ni j} \det S^{\mathbf{J}\smallsetminus j\cup e^*_m} \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m),m}}{\det SR},$$

and Formula (3.70) for the response of j' to a reaction perturbation of j^*

$$(\Phi)^{j^*}_{j'} = -\frac{\sum_{j^*\notin\mathbf{J}\ni j'} \det S^{\mathbf{J}\smallsetminus j'\cup j^*} \prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR}.$$

For the response of j' to a perturbation of m^*, the matrix $S^{\mathbf{J}\smallsetminus j\cup e^*_m}$ plays a central role. Respectively, for the response of j' to a perturbation of j^*, the matrix $S^{\mathbf{J}\smallsetminus j'\cup j^*}$ plays a central role. We observe that the formulas (3.50) and (3.70) are identical in the mathematical structure. In fact, $S^{\mathbf{J}\smallsetminus j\cup e^*_m}$ is constructed via a swap between the stoichiometric column $S^{j'}$ and the unit vector e_{m^*}, and $S^{\mathbf{J}\smallsetminus j'\cup j^*}$ is constructed via a swap between the stoichiometric columns $S^{j'}$ and S^{j^*}. The crucial observation is that the stoichiometry of e_{m^*} is the same as an exit reaction $j^0_{m^*}$ from m^*, with inverted sign. That is, $S^{j^0_{m^*}} = -e_{m^*}$. This implies that a metabolite perturbation of m^* corresponds identically to a reaction perturbation of an added exit reaction $j^0_{m^*}$, with reverted sign. In this way there is no need to develop an independent theory for metabolite perturbation, as it is already implicitly included in the reaction perturbation.

All other cases follow in complete analogy. Therefore, in the following chapters we concentrate primarily on the reaction perturbation.

3.4 Proofs

This section is devoted to the proofs of the results in this chapter. Again, we repeat that, for a matrix $\mathcal{A}$, the notation $\mathcal{A}^{\mathcal{E}}_{\mathcal{F}}$ denotes the submatrix of $\mathcal{A}$ consisting of columns $\mathcal{E}$ and rows $\mathcal{F}$. For simplicity of notation, we omit the braces $\{m\}$ for single elements, so that, for example, R_j indicates the j^{th} row and R^m indicates the m^{th} column of R.
We prove Theorems 3.3.1, 3.3.2, 3.3.3, 3.3.4, and 3.3.6 independently and not pursuing the reduction argument of Section 3.3.4. We paid a bit to redundancy but we obtained more, we hope, in clarity. With this approach, in particular, Section 3.3.4 reads as a corollary of the independently proven Theorem 3.3.2 and 3.3.6.
These proofs are all based on a careful use of Cramer's rule and Cauchy-Binet formula. They have a basic analogous structure: we invert the Jacobian determinant SR using Cramer's rule, obtaining:

$$((SR)^{-1})^{m^*}_{m'} = \frac{(-1)^{m^*+m'}\det((SR)^{\vee m'}_{\vee m^*})}{\det SR}, \tag{3.75}$$

where $(SR)^{\vee m'}_{\vee m^*}$ indicates the minor of SR taken removing the m^*-th row and the m'-th column. The numerator is analyzed via Cauchy-Binet formula and interpreted - depending on each specific theorem - in terms of Child Selections, in a rather similar flavor to the proof of Proposition 2.2.1. We list the proofs one after the other.

Proof of Theorem 3.3.1. The response $(\delta x)^{m^*}_{m'}$ of a metabolite concentration m' to a perturbation of the metabolite concentration m^* is given by

$$(\delta x)^{m^*}_{m'} = -((SR)^{-1})^{m^*}_{m'}, \tag{3.76}$$

where $((SR)^{-1})^{m^*}_{m'}$ indicates the entry corresponding to the m^*-column and m'-row. We invert SR using Cramer's rule:

$$(\delta x)^{m^*}_{m'} = -((SR)^{-1})^{m^*}_{m'} = -\frac{(-1)^{m^*+m'}\det((SR)^{\vee m'}_{\vee m^*})}{\det SR}. \tag{3.77}$$

Here, again, $(SR)^{\vee m'}_{\vee m^*}$ indicates the minor of SR taken removing the m^*-th row and the column m'-th column. We analyze the numerator, using Cauchy-Binet formula:

$$\begin{aligned} -\det SR\,(\delta x)^{m^*}_{m'} &= (-1)^{m^*+m'}\det((SR)^{\vee m'}_{\vee m^*}) \\ &= (-1)^{m^*+m'}\det(S_{\vee m^*}R^{\vee m'}) \\ &= (-1)^{m^*+m'}\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\det S^{\mathcal{E}'}_{\vee m^*}\cdot\det R^{\vee m'}_{\mathcal{E}'}. \end{aligned} \tag{3.78}$$

We observe that $\det R^{\vee m'}_{\mathcal{E}'} \neq 0$ if and only if there exists a Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'}\colon \mathbf{M}\setminus\{m'\} \longrightarrow \mathbf{E}$, such that $\mathbf{J}^{\vee\mathbf{m}'}(\mathbf{M}\setminus\{m'\}) = \mathcal{E}'$. This observation and the signature argument

$$\operatorname{sgn}(\mathbf{J}^{\vee\mathbf{m}'})\det S^{\mathcal{E}'=\mathbf{J}^{\vee\mathbf{m}'}(\mathbf{M}\setminus m')} = \det S^{\mathbf{J}^{\vee\mathbf{m}'}}_{\vee m^*}, \tag{3.79}$$

leads to the following equality:

$$(\delta x)^{m^*}_{m'} = -\frac{(-1)^{m^*+m'} \sum\limits_{\mathbf{J}\vee\mathbf{m}'} \det S^{\mathbf{J}\vee\mathbf{m}'}_{\vee m^*} \cdot \prod_{m\in\mathbf{M}\smallsetminus m'} r_{(\mathbf{J}\vee\mathbf{m}'(m))m}}{\det SR}, \tag{3.80}$$

which concludes the proof. □

Proof of Theorem 3.3.2. Analogously as above, we analyze the expression $(\Phi)^{m^*}_{j'} = -R_{j'}((SR)^{-1})^{m^*}$ for the flux response of j' to a metabolite perturbation of m^*.

$$\begin{aligned}(\Phi)^{m^*}_{j'} &= -R_{j'}((SR)^{-1})^{m^*}\\ &= -\sum_{m\in\mathbf{M}} R^m_{j'}((SR)^{-1})^{m^*}_m\\ &= -\sum_{m\in\mathbf{M}} R^m_{j'}\frac{(-1)^{m^*+m}\det(SR)^{\vee m}_{\vee m^*}}{\det SR}.\end{aligned} \tag{3.81}$$

That is,

$$\begin{aligned}-\det SR\,(\Phi)^{m^*}_{j'} &= \sum_{m\in\mathbf{M}} R^m_{j'}(-1)^{m^*+m}\det(SR)^{\vee m}_{\vee m^*}\\ &= \sum_{m\in\mathbf{M}} R^m_{j'}(-1)^{m^*+m}\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\det S^{\mathcal{E}'}_{\vee m^*}\cdot\det R^{\vee m}_{\mathcal{E}'}\\ &= \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\sum_{m\in\mathbf{M}}((-1)^{m^*+j'}\det S^{\mathcal{E}'}_{\vee m^*})((-1)^{m+j'}R^m_{j'}\det R^{\vee m}_{\mathcal{E}'})\\ &= \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\det S^{\mathcal{E}'\cup e_{m^*}}\det R_{\mathcal{E}'\cup j'}\\ &= \sum_{\mathbf{J}\ni j'}\det S^{\mathbf{J}(\mathbf{M})\smallsetminus j'\cup e_{m^*}}\operatorname{sgn}(\mathbf{J})\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}\\ &= \sum_{\mathbf{J}\ni j'}\det S^{\mathbf{J}\smallsetminus j'\cup e_{m^*}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}.\end{aligned} \tag{3.82}$$

□

Proof of Theorem 3.3.3. Here we analyze the metabolite response $(\delta x)^{j^*}_{m'} = -((SR)^{-1}S^{j^*})_{m'}$, of m' to a perturbation of reaction j^*.

$$\begin{aligned}(\delta x)^{j^*}_{m'} = -((SR)^{-1}S^{j^*})_{m'} &= -((SR)^{-1})_{m'}S^{j^*}\\ &= -\sum_{m\in\mathbf{M}}((SR)^{-1})^m_{m'}S^{j^*}_m\\ &= -\sum_{m\in\mathbf{M}}\frac{(-1)^{m+m'}\det(SR)^{\vee m'}_{\vee m}}{\det(SR)}S^{j^*}_m.\end{aligned} \tag{3.83}$$

That is,

$$\begin{aligned}-\det SR\,(\delta x)^{j^*}_{m'} &= \sum_{m\in\mathbf{M}}(-1)^{m+m'}\det(SR)^{\vee m'}_{\vee m}S^{j^*}_m\\ &= \sum_{m\in\mathbf{M}}(-1)^{m+m'}\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\det S^{\mathcal{E}'}_{\vee m}\det R^{\vee m'}_{\mathcal{E}'}S^{j^*}_m\\ &= \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\sum_{m\in\mathbf{M}}((-1)^{m+m'}S^{j^*}_m\det S^{\mathcal{E}'}_{\vee m})\det R^{\vee m'}_{\mathcal{E}'}.\end{aligned} \tag{3.84}$$

Again, $\det R^{\vee m'}_{\mathcal{E}'} \neq 0$ if and only if there exists a Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'}\colon \mathbf{M}\setminus m' \longrightarrow \mathbf{E}$, such that $\mathbf{J}^{\vee\mathbf{m}'}(\mathbf{M}\setminus m') = \mathcal{E}'$. This observation and the signature argument

$$\operatorname{sgn}(\mathbf{J}^{\vee\mathbf{m}'}) \det S^{\mathcal{E}'=\mathbf{J}^{\vee\mathbf{m}'}(\mathbf{M}\setminus m')} = \det S^{\mathbf{J}^{\vee\mathbf{m}'}}_{\vee m^*}, \tag{3.85}$$

leads to:

$$\begin{aligned}(\delta x)^{j^*}_{m'} =& -\frac{(-1)^{m+m'} S^{j^*}_m \det S^{\mathcal{E}'=\mathbf{J}^{\vee\mathbf{m}'}(\mathbf{M}\setminus m')}_{\vee m} \operatorname{sgn}(\mathbf{J}^{\vee\mathbf{m}'}) \cdot \prod_{m\in\vee m'} r_{\mathbf{J}^{\vee\mathbf{m}'}(m)m}}{\det SR} \\ =& -\frac{\sum_{\mathbf{J}^{\vee\mathbf{m}'}\not\ni j^*} \det(S^{\mathbf{J}^{\vee\mathbf{m}'}\cup\mathbf{j}^*}) \cdot \prod_{m\in\vee m'} r_{\mathbf{J}^{\vee\mathbf{m}'}(m)m}}{\det SR},\end{aligned} \tag{3.86}$$

concluding the proof. □

Proof of Theorem 3.3.4. The flux response $(\Phi)^{j^*}_{j^*}$ of a reaction j^* to a perturbation of j^* itself reads

$$\begin{aligned}(\Phi)^{j^*}_{j^*} =& 1 - R_{j^*}(SR)^{-1}S^{j^*} \\ =& 1 - \sum_{m_1,m_2\in\mathbf{M}} R^{m_1}_{j^*}((SR)^{-1})^{m_2}_{m_1} S^{j^*}_{m_2} \\ =& 1 - \sum_{m_1,m_2\in\mathbf{M}} R^{m_1}_{j^*}\frac{(-1)^{m_1+m_2}\det(SR)^{\vee m_1}_{\vee m_2}}{\det SR} S^{j^*}_{m_2}.\end{aligned} \tag{3.87}$$

That is,

$$\begin{aligned}\det SR\,(\Phi)^{j^*}_{j^*} =& \det SR - \sum_{m_1,m_2} R^{m_1}_{j^*}(-1)^{m_1+m_2}\Big(\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}} \det S^{\mathcal{E}'}_{\vee m_2}\cdot\det R^{\vee m_1}_{\mathcal{E}'}\Big)S^{j^*}_{m_2} \\ =& \det SR - \\ & \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\Big(\sum_{m_2\in\mathbf{M}}(-1)^{m_2+j^*}S^{j^*}_{m_2}\det S^{\mathcal{E}'}_{\vee m_2}\Big)\Big(\sum_{m_1\in\mathbf{M}}(-1)^{m_1+j^*}R^{m_1}_{j^*}\det R^{\vee m_1}_{\mathcal{E}'}\Big) \\ =& \det SR - \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}} \det S^{\mathcal{E}'\cup j^*}\det R_{\mathcal{E}'\cup j^*}.\end{aligned} \tag{3.88}$$

Moreover, $\det R_{\mathcal{E}'\cup j^*} \neq 0$ if and only if $\mathcal{E}'\cup j^* = \mathbf{J}(\mathbf{M})$, for a Child Selection $\mathbf{J}$. In particular, $\mathbf{J}$ containins j^*. Since, again,

$$\operatorname{sgn}(\mathbf{J})\det S^{\mathcal{E}'=\mathbf{J}(\mathbf{M})} = \det S^{\mathbf{J}}, \tag{3.89}$$

we have that

$$\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}} \det S^{\mathcal{E}'\cup j^*}\det R_{\mathcal{E}'\cup j^*} = \sum_{\mathbf{J}\ni j^*}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}. \tag{3.90}$$

Now, $\det SR$ can be analogously expanded along Child Selections, according to Proposition 2.2.1, leading to

$$\begin{aligned}(\Phi)^{j^*}_{j^*} =& \frac{\sum_{\mathbf{J}}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m} - \sum_{\mathbf{J}\ni j^*}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR} \\ =& \frac{\sum_{\mathbf{J}\not\ni j^*}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR}.\end{aligned} \tag{3.91}$$

□

Proof of Theorem 3.3.6. The flux response $(\Phi)_{j'}^{j^*}$ of a reaction j' to a perturbation of reaction j^*, with $j^* \neq j'$, reads

$$\begin{aligned}(\Phi)_{j'}^{j^*} &= -(R(SR)^{-1}S)_{j'}^{j^*} \\ &= -R_{j'}(SR)^{-1}S^{j^*} \\ &= -\sum_{m_1,m_2\in\mathbf{M}} R_{j'}^{m_1}(SR)^{-1}_{m_1,m_2}S_{m_2}^{j^*} \\ &= -\sum_{m_1,m_2\in\mathbf{M}} R_{j'}^{m_1}\frac{(-1)^{m_1+m_2}\det(SR)^{\vee m_1}_{\vee m_2}}{\det(SR)}S_{m_2}^{j^*},\end{aligned} \tag{3.92}$$

which yields

$$\begin{aligned}-\det SR\,(\Phi)_{j'}^{j^*} &= \sum_{m_1,m_2} R_{j'}^{m_1}(-1)^{m_1+m_2}\det(SR)^{\vee m_1}_{\vee m_2}S_{m_2}^{j^*} \\ &= \sum_{m_1,m_2} R_{j'}^{m_1}(-1)^{m_1+m_2}\Big(\sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\det S^{\mathcal{E}'}_{\vee m_2}\cdot\det R^{\vee m_1}_{\mathcal{E}'}\Big)S_{m_2}^{j^*} \\ &= \sum_{\mathcal{E}'\in\mathcal{E}^{M-1}}\Big(\sum_{m_2\in\mathbf{M}}(-1)^{m_2+j'}S_{m_2}^{j^*}\det S^{\mathcal{E}'}_{\vee m_2}\Big)\Big(\sum_{m_1\in\mathbf{M}}(-1)^{m_1+j'}R_{j'}^{m_1}\det R^{\vee m_1}_{\mathcal{E}'}\Big) \\ &= \sum_{j'\in\mathbf{J}\not\ni j^*}\det S^{\mathbf{J}\setminus j'\cup j^*}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}.\end{aligned} \tag{3.93}$$

The last equality is an analogous determinant contraction as done and commented in the previous proofs. The above computation leads to the desired equality

$$(\Phi)_{j'}^{j^*} = -\frac{\sum_{j'\in\mathbf{J}\not\ni j^*}\det S^{\mathbf{J}\setminus j'\cup j^*}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m}}{\det SR}. \tag{3.94}$$

□

We proceed now with the brief proof of Corollary 3.3.5.

Proof of Corollary 3.3.5. Simply note that, in the case $\det S^{\mathbf{J}} = 0$ for any Child Selection $\mathbf{J}$ containing j^*, the numerator of (3.63) reads:

$$\sum_{\mathbf{J}\not\ni j^*}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m} = \sum_{\mathbf{J}}\det S^{\mathbf{J}}\prod_{m\in\mathbf{M}} r_{\mathbf{J}(m)m} = \det SR. \tag{3.95}$$

□

And we conclude with Corollaries 3.3.7 and 3.3.8.

Proof of Corollary 3.3.7. Note that the assumption implies that $\mathbf{J}(m^*) \equiv j^*$, for any Child Selection $\mathbf{J}$. In particular $j^* \in \mathbf{J}$, for any $\mathbf{J}$, and $j^* \in \mathbf{J}^{\vee\mathbf{m}'}$, for any Partial Child Selection $\mathbf{J}^{\vee\mathbf{m}'}$ such that $m' \neq m^*$. These observations leads to the following conclusions.

1. By Formula (3.63) and (3.70), any nonzero flux-influence $\Phi_{j'}^{j^*} \neq 0$ requires the existence of a Child Selection $\mathbf{J}$, such that $j^* \notin \mathbf{J}$. This is excluded by assumption, and therefore $\Phi_{j'}^{j^*} = 0$ for every $j' \in \mathbf{E}$.

2. By Formula (3.55), any nonzero influence $\Phi^{j^*}_{m'} \neq 0$ requires the existence of a Partial Child Selection $\mathbf{J}^{\vee \mathbf{m}'}$ such that $j^* \notin \mathbf{J}^{\vee \mathbf{m}'}$. Again, this condition is always violated by assumption, unless $m' = m^*$. This concludes that $\Phi^{j^*}_{m'} \neq 0$ for every $m' \neq m^*$.

3. The running nondegeneracy assumption $\det SR \neq 0$ implies, via Corollary 3.2.1, the existence of a Child Selection $\mathbf{J}$ such that $\det S^{\mathbf{J}} \neq 0$. In particular, from a nonzero Child Selection $\mathbf{J}$, let us consider a deducible Partial Child Selection $\mathbf{J}^{\vee \mathbf{m}^*}$. Again via Formula (3.55),

$$\Phi^{j^*}_{m^*} \neq 0 \Leftrightarrow \det(S^{\mathbf{J}^{\vee \mathbf{m}^*} \cup j^*}) \neq 0. \tag{3.96}$$

Note, in the considered case, that the following equality holds:

$$(S^{\mathbf{J}^{\vee \mathbf{m}^*} \cup j^*}) = S^{\mathbf{J}}, \tag{3.97}$$

and therefore, $\det S^{\mathbf{J}} \neq 0$ implies $\Phi^{j^*}_{m^*} \neq 0$.
Moreover, the nonzero response $(\Phi)^{j^*}_{m^*}$ can be explicitly computed to be

$$\Phi^{j^*}_{m^*} = -\frac{\partial_{r_{j^*m^*}}(\det SR)}{\det SR} = -\frac{1}{r_{j^*m^*}}, \tag{3.98}$$

and, assuming further $r_{j^*m^*} > 0$, we conclude that $\Phi^{j^*}_{m^*} < 0$.

□

Corollary 3.3.8 has exactly the same proof of point 1 in Corollary 3.3.7, which we do not repeat.

Chapter 4

Signed response

4.1 Introduction

This chapter deepens the analysis of Chapter 3 and addresses the question on the *sign* of the flux and metabolite responses. We consider targeted positive perturbations of $\alpha = e_p$. As before, $p \in \mathbf{M} \cup \mathbf{E}$ indicates a single reaction or a single metabolite. Analogous considerations as in Section 3.3.3 hold for general $\alpha \in \mathbb{R}^{E+M}$. For the targeted case $\alpha = e_p$, we ask:

What is the sign of the responses?

Our previous assumptions for nonzero responses remain in effect. Specifically, we recall that we consider strictly positive reaction rates $\mathbf{r}(x)$, such that $r_j(x)$ depends only on those concentrations x_m for which m is an input metabolite to reaction j. Moreover, the sign question of this chapter requires the further assumption of monotonicity for the reaction rates $\mathbf{r}(x)$, as in Chapter 2. That is, we consider the nonzero partial derivatives r_{jm} to be positive:

$$r_{jm} > 0. \tag{4.1}$$

In particular, the responses are given as rational functions whose denominator and numerator are multilinear homogenous polynomials in the variables r_{jm}. With the monotonicity assumption $r_{jm} > 0$, we restrict the (algebraic) sign analysis to a sign analysis of the coefficients of the response rational functions.

We concentrate mainly on the case $\{(\Phi)^{j^*}_{j'}\}$ of flux response j' to reaction perturbation j^*. In fact, the metabolite perturbation case can be reduced to the reaction one, as already introduced in Section 3.3.4; see also Corollary 4.2.4 below. The metabolite response is treated in Subsection 4.2.2.

Formula (3.70) for the flux response $(\Phi)^{j^*}_{j'}$ of reaction j' to a reaction perturbation of $j^* \neq j'$ tells that

$$\det SR \cdot (\Phi)^{j^*}_{j'} = \sum_{j^* \notin \mathbf{J} \ni j'} (\varphi^{\mathbf{J}})^{j^*}_{j'}, \tag{4.2}$$

where

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} := -\det S^{\mathbf{J} \setminus j' \cup j^*} \prod_{m \in M} r_{\mathbf{J}(m)m}. \tag{4.3}$$

The sign of the flux response $(\Phi)^{j^*}_{j'}$, in particular, depends on the sign of the Jacobian determinant $\det SR$ and on the sign of each *response summand* $(\varphi^{\mathbf{J}})^{j^*}_{j'}$. We have structurally analyzed $\operatorname{sign}(\det SR)$ in Chapter 2. Here, thus, we are only concerned with the sign of the response summands $(\varphi^{\mathbf{J}})^{j^*}_{j'}$.

The main tool of our analysis are the *Enlarged Child Selections* (ECS) $\mathbf{J} \cup j^*$, for $j^* \notin \mathbf{J}$. An ECS naturally identifies an $M \times (M+1)$ matrix $S^{\mathbf{J}\cup j^*}$, where j^* is the column $M+1$ and the first M columns are identical to $S^{\mathbf{J}}$. The two main results of this chapter, contained in Section 4.2, fix a Child Selection $\mathbf{J}$ and describe the sign of $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ for any $j' \in \mathbf{J}$. Specifically, Proposition 4.2.1 shows that the only relevant case is when the dimension of the kernel of $S^{\mathbf{J}\cup j^*}$ is exactly one. Indeed, trivial kernels are excluded by the dimension $M \times (M+1)$ of $S^{\mathbf{J}\cup j^*}$ and kernels of dimension bigger than one indicate zero response summands $(\varphi^{\mathbf{J}})^{j^*}_{j'}$, for all j'. The analysis highlights in particular the important role played by nonzero kernel vectors $0 \neq v \in \mathbb{R}^{M+1}$,

$$S^{\mathbf{J}\cup j^*} v = 0, \tag{4.4}$$

in the one-dimensional kernel situation, $\ker(S^{\mathbf{J}\cup j^*}) = \operatorname{span}\langle v\rangle$. In this case,

The sign pattern of the entries v_j holds the key to the sign pattern of the responses.

In fact, Theorem 4.2.2 states that nonzero response summands $(\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0$ are characterized by nonzero entries $v_{j'} \neq 0$, and the mutual sign of the entries translates to the mutual sign of the response summands. That is, for j'_1 and j'_2,

$$\operatorname{sign}\big((\varphi^{\mathbf{J}})^{j^*}_{j'_1}(\varphi^{\mathbf{J}})^{j^*}_{j'_2}\big) = \operatorname{sign}(v_{j'_1} v_{j'_2}). \tag{4.5}$$

The determination of the specific sign of each summands is addressed in Theorem 4.2.3. Two different cases appear depending on whether the Child Selection $\mathbf{J}$ of the response summand $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ zero-behaves, or not. If the Child Selection $\mathbf{J}$ does not zero-behave, in particular, then the entry v_{j^*} associated to the perturbed reaction j^* is nonzero. The absolute sign of the response summand $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ is then, simply, given by

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} = \beta(\mathbf{J}) \operatorname{sign}(v_{j^*} v_{j'}), \tag{4.6}$$

where $\beta(\mathbf{J}) = \operatorname{sign}(\det S^{\mathbf{J}})$ is again the behavior coefficient introduced in Definition 2.2. The case in which $\mathbf{J}$ zero-behaves is more elaborate and it involves cokernel vectors κ,

$$\kappa S^{\mathbf{J}} = 0. \tag{4.7}$$

We recall that nonzero cokernel vectors κ represent *conservation laws* of the subnetwork identified by the stoichiometric matrix $S^{\mathbf{J}}$. For zero-behaving Child Selections $\mathbf{J}$ and for a uniquely determined choice of the cokernel vector κ, we have that

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} = -\operatorname{sign}\big(\, v_{j'} \langle \kappa, S^{j^*} \rangle \,\big), \tag{4.8}$$

Here S^{j^*} again indicates the stoichiometric column associated to the perturbed reaction j^*.

The above arguments are made precise in Section 4.2, which contains the main results. Section 4.3 presents three examples and Section 4.4 concludes with the proofs.

4.2 Main results

The first proposition provides a necessary condition for any nonzero response summand $(\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0$.

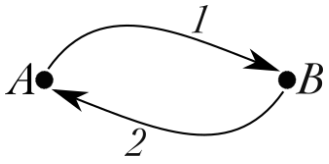
Proposition 4.2.1. *For a response summand* $(\varphi^{\mathbf{J}})^{j^*}_{j'}$*, it holds:*

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} = 0 \text{ for all } j' \quad \Leftrightarrow \quad \dim(\ker(S^{\mathbf{J}\cup j^*})) > 1. \tag{4.9}$$

Remark 18. Consider any (not necessarily square) matrix, with one-dimensional kernel. Note how the support, i.e. nonzero entries, of any nontrivial kernel vector is unique. In particular, kernel vectors of such type are *elementary* in the sense that they do not properly contain the support of any other kernel vectors. In a metabolic context, the importance of *elementary kernel vectors* has already been noted by Klammt and coauthors [KRG+17]. Mathematically, they have previously been studied by Rockafellar [Roc69].

An interesting question arises from the above Remark 18. Let us assume that only a small part $\mathbf{\Gamma}_{sub}$ of a network $\mathbf{\Gamma}$ is known, such as a reversible reaction from metabolite A to metabolite B.

$$S_{sub} = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cc} 1 & 2 \end{array} \\ \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix} \end{array}, \tag{4.10}$$



Above, S_{sub} is the stoichiometric matrix of the subnetwork $\mathbf{\Gamma}_{sub}$. Note, in particular, that $\dim(\ker S_{sub}) = 1$. Let S be the stoichiometric matrix of the whole network $\mathbf{\Gamma}$. Under our standing nondegeneracy assumption $\det SR \neq 0$, for the whole network $\mathbf{\Gamma}$, *can we infer the existence of a Child Selection* $\mathbf{J}$ *for* $\mathbf{\Gamma}$*, such that* $\mathbf{J}(A) = 1$*,* $\mathbf{J}(B) = 2$ *and* $\dim(\ker S^{\mathbf{J}}) = 1$*?*
This question may possibly possible an affirmative answer for the majority of biological networks. However, on a purely mathematical basis the answer is negative: the above claim is wrong. See Example III in Section 4.3

We now state the first main theorem of this chapter, on the relative sign of the responses.

Theorem 4.2.2 (Relative sign of responses)**.** *Suppose* $\dim(\ker(S^{\mathbf{J}\cup j^*}))=1$*, and let* $\ker S^{\mathbf{J}\cup j^*} = \operatorname{span}\langle v\rangle$*. Then,*

1. *The response summand of reaction j' is nonzero if and only if the j'-th entry of v is nonzero, that is*

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0 \quad \Leftrightarrow \quad v_{j'} \neq 0. \tag{4.11}$$

2. *The mutual sign of the response summands of reactions j'_1 and j'_2 is given by the mutual sign of the j'_1-th and j'_2-th entries of v, that is*

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'_1} \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'_2} = \operatorname{sign}(v_{j'_1} v_{j'_2}). \tag{4.12}$$

To proceed towards the second result of the chapter, on the specific sign of each response, we recall some linear algebra concepts, first, [HJ13].

Let $\mathcal{A}$ be any $M \times M$ matrix with a one-dimensional kernel. Straightforwardly, then, also the cokernel is one-dimensional. The *cofactor matrix* $\mathcal{C}(\mathcal{A})$ of $\mathcal{A}$ is the matrix whose entries $\mathcal{C}(\mathcal{A})_{mj}$ are given by

$$\mathcal{C}(\mathcal{A})_{mj} = (-1)^{m+j} \det \mathcal{A}^{\vee j}_{\vee m}, \tag{4.13}$$

where $\mathcal{A}^{\vee j}_{\vee m}$ indicates the $(M-1)\times(M-1)$ minor of $\mathcal{A}$, obtained by removing row m and column j. The *adjugate matrix* of $\mathcal{A}$, $\operatorname{Ad}(\mathcal{A})$, is then defined as the transpose of the cofactor matrix $\mathcal{C}(\mathcal{A})$ of A. That is,

$$\operatorname{Ad}(\mathcal{A}) = \mathcal{C}(\mathcal{A})^T. \tag{4.14}$$

Moreover, we have the relation

$$\mathcal{A} \operatorname{Ad}(\mathcal{A}) = \operatorname{Ad}(\mathcal{A})\mathcal{A} = \det \mathcal{A} = 0. \tag{4.15}$$

Let us fix a kernel vector v, which spans $\ker \mathcal{A}$. Equalities (4.15) imply that there exists a cokernel vector $\kappa = \kappa(v)$, $\operatorname{coker} \mathcal{A} = \operatorname{span}\langle\kappa\rangle$, such that

$$\operatorname{Ad}(\mathcal{A}) = \ v \cdot \kappa^T . \tag{4.16}$$

In particular, any entry $\operatorname{Ad}(\mathcal{A})_{mj}$ of the adjugate matrix can be expressed as:

$$\operatorname{Ad}(\mathcal{A})_{mj} = (-1)^{m+j} \det \mathcal{A}^{\vee m}_{\vee j} = v_m \, \kappa_j \, . \tag{4.17}$$

We are now ready to state the second main Theorem 4.2.3.

Theorem 4.2.3 (Absolute sign of responses)**.** *As in Theorem 4.2.2, let us suppose* $\dim(\ker(S^{\mathbf{J}\cup j^*}))$*=1, and let* $\ker S^{\mathbf{J}\cup j^*} = \operatorname{span}\langle v\rangle$*. There are two cases:*

1. *If the Child Selection* $\mathbf{J}$ *does not zero-behave, then the j^*-th entry of v is nonzero, i.e. $v_{j^*} \neq 0$, and*

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} = \beta(\mathbf{J}) \operatorname{sign}(v_{j^*} v_{j'}). \tag{4.18}$$

2. *If the Child Selection* $\mathbf{J}$ *zero-behaves, then* $v_{j^*} = 0$. *In particular, consider* $\tilde{v} \in \mathbb{R}^M$ *such that* $\ker S^{\mathbf{J}} = \operatorname{span}\langle \tilde{v} \rangle$ *and* $\tilde{v}_j = v_j$, *for any* $j = 1, ..., M$. *For the unique cokernel vector* κ *of* $S^{\mathbf{J}}$ *such that* $\operatorname{Ad}(S^{\mathbf{J}}) = \tilde{v} \cdot \kappa^T$, *we have*

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} = -\operatorname{sign}(v_{j'} \langle \kappa, S^{j^*} \rangle). \tag{4.19}$$

Remark 19. Theorem 4.2.3 confirms that, for self-influence $j^* \rightsquigarrow j^*$,

$$\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j^*} = \beta(\mathbf{J}). \tag{4.20}$$

This can also be computed directly from Formula (3.63) for $(\Phi)^{j^*}_{j^*}$.

As a straightforward consequence, we state a corollary for metabolite perturbations.

Corollary 4.2.4 (Metabolite Perturbation)**.** *For a perturbation of the metabolite concentration of* m^*, *Proposition 4.2.1, Theorems 4.2.2, and 4.2.3 read identically by setting*

$$j^* = e_{m^*}. \tag{4.21}$$

In applications, the system is often required to have a stable equilibrium, for any choice of reaction rates. As commented in Chapter 2, this requirement implies that the system does not possess any Child Selection $\mathbf{J}$, which ill-behaves, that is, $\beta(\mathbf{J}) \neq (-1)^{M-1}$. In particular, the Jacobian has to be of the fixed sign

$$\operatorname{sign}(\det SR) = (-1)^M. \tag{4.22}$$

With this case of application in mind, we state the following Corollary to Theorem 4.2.3.

Corollary 4.2.5 (Fixed Sign Jacobian)**.** *If* $\det SR$ *is of fixed sign, then* $\operatorname{sign}(\det SR) = \beta(\mathbf{J}) \equiv \beta$, *for* any *nonzero Child Selection* $\mathbf{J}$. *In particular, for nonzero Child Selections* $\mathbf{J}$, *we have:*

$$\operatorname{sign} \frac{(\varphi^{\mathbf{J}})^{j^*}_{j'}}{\det SR} = \operatorname{sign}(v_{j^*} v_{j'}). \tag{4.23}$$

In a fixed sign Jacobian situation, Corollary 4.2.5 determines the sign of the response summands for nonzero Child Selections without any determinant computation. However, the zero-behaving Child Selection case, point 2 of Theorem 4.2.3, still involves a determinant computation. This is an unavoidable point in our analysis. Indeed, in our approach of Child Selections behavior, we have only classified the sign of certain maximal reshuffled minors of the stoichiometric matrix. In particular we have identified the nonsingular $M \times M$ Child Selection minors to be either good or bad, depending on the sign of $\det S^{\mathbf{J}}$. Point 2 of Theorem 4.2.3, however, deals with Child Selections, which *are* singular, that is, Child Selections $\mathbf{J}$ such that $\det S^{\mathbf{J}} = 0$. In this case, we have made no statement nor assumption on the sign of non maximal minors.

Stronger network assumptions might possibly simplify the computation also for point 2 of Theorem 4.2.3 and consequently allow a fast and efficient algorithm. For instance, a square matrix $\mathcal{A}$ is called *sign-nonsingular* if all matrices with the same

sign pattern as $\mathcal{A}$ are nonsingular. Then, a square matrix $\mathcal{A}$ is called *Strongly Sign Determined* if all its square minors are either singular or sign-nonsingular. The assumption of the stoichiometric matrix S being Strongly Sign Determined guarantees a regular sign structure of all square minors of the stoichiometric matrix. For more references on these assumptions and structures see [BDB07, BR11, BS09].
However, we underline that these assumptions may be far too strong, depending on the goal, as they exclude the existence of bad Child Selections, and consequently of saddle-node bifurcations.

How to deal with an indeterminate sign Jacobian remains an important open question. We are not addressing this problem in this thesis, and leave it for future work. Suffice it to say that an indeterminate sign Jacobian does not always result in indeterminate sign responses, in contrast to what intuition might suggest. Indeed, cancellations between the denominator and the numerator of the rational expression of the responses may occur, see Example II in the next Section 4.3.

4.2.1 Twin sisters have opposite influence

Note that any ECS $\mathbf{J} \cup j^*$ contains in particular at least two outgoing reactions from a metabolite m^*, one of which is j^*. We call here the reaction $j_s^* = \mathbf{J}(m^*)$ the *sister* of j^*. Let now $\mathbf{J}$ and $\mathbf{J}_s$ be two Child Selections at distance $d = 1$ such that $\mathbf{J}(m^*) = j_s^*$ and $\mathbf{J}_s(m^*) = j^*$. In particular, $\mathbf{J}(m) = \mathbf{J}_s(m)$ for any $m \neq m^*$. The matrix $S^{\mathbf{J}_s \setminus j' \cup j_s^*}$ has opposite determinant to the matrix $S^{\mathbf{J} \setminus j' \cup j^*}$. The two matrices are indeed obtainable one from the other, via a single interchange of the columns S^{j^*} and $S^{j_s^*}$. The change of sign in the determinant is a well-known property of a multilinear alternating form. This implies that

$$(\varphi^{\mathbf{J}})_{j'}^{j^*} = -(\varphi^{\mathbf{J}_s})_{j'}^{j_s^*}. \tag{4.24}$$

Let us now assume that the metabolite m^* participates, as a mother input, *only* to two reactions j^* or j_s^*. In particular, any Child Selection $\mathbf{J}$ contains either j^* or j_s^*, as child reaction of m^*. In this case, the statement (4.24) can be strengthened to the following proposition.

Proposition 4.2.6. *Suppose that any Child Selection* $\mathbf{J}$ *maps the metabolite* m^* *either to* j^* *or to* j_s^**, only. Then*

$$r_{j^* m^*}(\Phi)_{j'}^{j^*} = -r_{j_s^* m^*}(\Phi)_{j'}^{j_s^*}, \quad \textit{for any } j'. \tag{4.25}$$

In particular,

$$\operatorname{sign}(\Phi)_{j'}^{j^*} = -\operatorname{sign}(\Phi)_{j'}^{j_s^*}. \tag{4.26}$$

For further related arguments in the monomolecular case, see [VM17].

4.2.2 Metabolite response

Formula (3.55) for the metabolite response $(\delta x)^{j^*}_{m'}$ of metabolite m' to a reaction perturbation of j^* tells that

$$\det SR \cdot (\delta x)^{j^*}_{m'} = \sum_{\mathbf{J}^{\vee \mathbf{m}'} \not\ni j^*} (\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'}, \tag{4.27}$$

where

$$(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'} = -\det S^{\mathbf{J}^{\vee \mathbf{m}'} \cup j^*} \prod_{m \in \mathbf{M} \setminus m'} r_{\mathbf{J}^{\vee \mathbf{m}'}(m)m}. \tag{4.28}$$

Consider now a reaction j', which is outgoing child of the metabolite m'. Let us start by considering a dPCS $\mathbf{J}^{\vee \mathbf{m}'}$ and one of its induced Child Selections $\mathbf{J}$. A comparison between Formula (3.55) and Formula (3.70) implies that

$$(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'} = \frac{(\varphi^{\mathbf{J}})^{j^*}_{j'}}{r_{j'm'}}, \tag{4.29}$$

and, consequently,

$$\operatorname{sign}(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'} = \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} = \operatorname{sign}(-\det S^{\mathbf{J} \setminus j' \cup j^*}). \tag{4.30}$$

Indeed, the algebraic structure of $S^{\mathbf{J} \setminus j' \cup j^*}$ is identical to, and indistinguishable from, the algebraic structure of $S^{\mathbf{J}^{\vee \mathbf{m}'} \cup j^*}$, for $\mathbf{J}$ Child Selection induced from the dPCS $\mathbf{J}^{\vee \mathbf{m}'}$. In fact: the columns of both matrices possesses no child assigned to m' and both matrices possesses at least two outgoing reactions of m^*, with j^* being in the m'-th column. Therefore, for m' mother input metabolite of n reactions $j'_1, ..., j'_n$, it holds:

$$\operatorname{sign}(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'} = \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'_1} = ... = \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'_n}. \tag{4.31}$$

In particular, fixing $\mathbf{J}^{\vee \mathbf{m}'}$ and $\mathbf{J}$ as above, a proven nonzero influence on one element $m', j'_1, ..., j'_n$ implies nonzero influence for all elements $m', j'_1, ..., j'_n$. Consequently, we have the following Corollary:

Corollary 4.2.7 (Metabolite Response for dPCS)**.** *Let $\mathbf{J}^{\vee \mathbf{m}'}$ be a deducible Partial Child Selection. For the summand response $(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{m'}$ of metabolite concentration m', we have:*

$$\operatorname{sign}(\sigma^{\mathbf{J}^{\vee \mathbf{m}'}})^{j^*}_{j'} = \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'}, \tag{4.32}$$

where $\mathbf{J}$ is an induced Child Selections from $\mathbf{J}^{\vee \mathbf{m}'}$.

The sign-analysis of $(\sigma^{J^{\vee m'}})^{j^*}_{m'}$ when $J^{\vee m'}$ is a nPCS is more involved. For doing this we need to introduce some notation, first. Consider the set $\mathcal{E}'$ of $M-1$ reactions such that $\mathcal{E}' = J^{\vee m'}(\mathbf{M} \setminus m')$. Note that there always exists a Child Selection $\tilde{\mathbf{J}} : \mathbf{M} \longrightarrow \mathbf{E}$ such that

$$\tilde{\mathbf{J}}(\mathbf{M}) = \mathcal{E}' \cup \tilde{j}, \tag{4.33}$$

for some reaction $\tilde{j}$. On the other hand, the map $J^{\vee m' \cup j^*} : \mathbf{M} \longrightarrow \mathbf{E}$, associating to any $m \neq m'$ the corresponding reaction $j = J^{\vee m'}(m)$ and to m' the reaction j^*, can

as well be seen as a permutation of M elements, in analogy to any Child Selection map. Let now τ be

$$\tau = \mathrm{sgn}(J^{\vee m' \cup j^*})\,\mathrm{sgn}(\tilde{\mathbf{J}}), \tag{4.34}$$

where $\mathrm{sgn}(\cdot)$ indicates the signature (or parity). We have the following corollary:

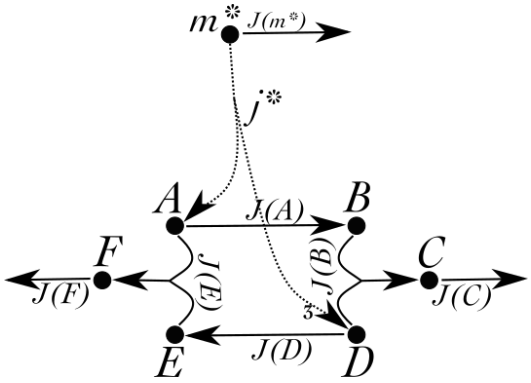
Corollary 4.2.8 (Metabolite Response for nPCS)**.** *Let $J^{\vee m'}$ be a non deducible Partial Child Selection. In the above notation, for the summand response $(\sigma^{J^{\vee m'}})^{j^*}_{m'}$ of metabolite concentration m', we have:*

$$\mathrm{sign}(\sigma^{J^{\vee m'}})^{j^*}_{m'} = \tau\ \mathrm{sign}(\varphi^{\tilde{\mathbf{J}}})^{j^*}_{\tilde{j}}\,. \tag{4.35}$$

Remark 20. Note that there can be more than one Child Selection as $\tilde{\mathbf{J}}$ above, as well as more than one reaction as $\tilde{j}$. The goal of this Section 4.2.2 has only been to reduce, mathematically, the metabolite response to the flux response. Since the flux response has been previously analyzed, Corollaries 4.2.7 and 4.2.8 show that there is no need of an independent analysis for the metabolite response case.

4.3 Examples

Example I. We present a zero-behaving Child Selection $\mathbf{J}$ on seven metabolites: m^*, A, B, C, D, E, F. With our theorems, we want to study the responses $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ of reactions $j' = \mathbf{J}(A)$, $\mathbf{J}(B)$, $\mathbf{J}(C)$, $\mathbf{J}(D)$, $\mathbf{J}(E)$, $\mathbf{J}(F)$, to a perturbation of the dashed reaction j^*.



(4.36)

$$S^{\mathbf{J}} = \begin{array}{c} \\ m^* \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{array}{c} \begin{array}{ccccccc} \mathbf{J}(m^*) & \mathbf{J}(A) & \mathbf{J}(B) & \mathbf{J}(C) & \mathbf{J}(D) & \mathbf{J}(E) & \mathbf{J}(F) \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \end{array}, \quad S^{j^*} = \begin{array}{c} \\ m^* \\ A \\ B \\ C \\ D \\ E \\ F \end{array} \begin{bmatrix} -1 \\ 1 \\ 0 \\ 0 \\ 3 \\ 0 \\ 0 \end{bmatrix};$$

In particular, the vector $v = (0, w, w, w, -w, -w, -w)^T$, $w \in \mathbb{R}$ satisfies

$$S^{\mathbf{J}} v = 0, \tag{4.37}$$

and it is therefore a kernel vector of $S^{\mathbf{J}}$. Note, moreover, that the

$$\dim(\ker S^{\mathbf{J}}) = 1 \qquad \text{and thus} \qquad \ker(S^{\mathbf{J}}) = \operatorname{span}\langle v \rangle. \tag{4.38}$$

Now, the adjugate matrix $\mathrm{Ad}(S^{\mathbf{J}})$ is

$$\mathrm{Ad}(S^{\mathbf{J}}) = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & -1 & -1 & 0 \\ 0 & 1 & 1 & 0 & -1 & -1 & 0 \\ 0 & 1 & 1 & 0 & -1 & -1 & 0 \\ 0 & -1 & -1 & 0 & 1 & 1 & 0 \\ 0 & -1 & -1 & 0 & 1 & 1 & 0 \\ 0 & -1 & -1 & 0 & 1 & 1 & 0 \end{bmatrix}, \tag{4.39}$$

and the choice of the cokernel vector $\kappa = (0, \frac{1}{w}, \frac{1}{w}, 0, -\frac{1}{w} - \frac{1}{w}, 0)^T$ satisfies

$$v \cdot \kappa^T = \mathrm{Ad}(S^{\mathbf{J}}). \tag{4.40}$$

For simplicity of the computation, we can consider in particular the choice $w = y = 1$, so that $v = (0, 1, 1, 1, -1, -1, -1)^T$ and $\kappa = (0, 1, 1, 0, -1 - 1, 0)^T$. Firstly we compute

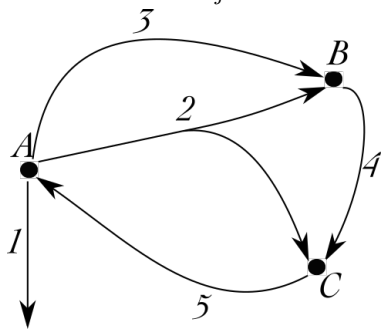
$$-\langle \kappa, S^{j^*} \rangle = -1 \cdot -2 = +2 \tag{4.41}$$

The signs of the single response summands $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ follows, according to Thm 4.2.3:

$$\begin{cases} \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(A)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(A)}) = \operatorname{sign}(+2 \cdot 1) > 0. \\ \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(B)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(B)}) = \operatorname{sign}(+2 \cdot 1) > 0. \\ \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(C)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(C)}) = \operatorname{sign}(+2 \cdot 1) > 0. \\ \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(D)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(D)}) = \operatorname{sign}(+2 \cdot -1) < 0. \\ \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(E)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(E)}) = \operatorname{sign}(+2 \cdot -1) < 0. \\ \operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{\mathbf{J}(F)} = \operatorname{sign}(-\langle \kappa, S^{j^*} \rangle v_{\mathbf{J}(F)}) = \operatorname{sign}(+2 \cdot -1) < 0. \end{cases} \tag{4.42}$$

Example II: Indeterminate sign determinant does not imply indeterminate sign response.

This example has intentionally been designed to illustrate a case of a determined sign response in the case of indeterminate sign Jacobian, due to a cancellation between the numerator and the denominator of $(\Phi)^{j^*}_{j'}$.



(4.43)

$$S = \begin{array}{c} \\ A \\ B \\ C \end{array} \begin{array}{c} \begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array} \\ \begin{bmatrix} -1 & -1 & -1 & 0 & 1 \\ 0 & 1 & 1 & -1 & 0 \\ 0 & 1 & 0 & 1 & -1 \end{bmatrix} \end{array}, \tag{4.44}$$

Metabolites B and C can only choose their single child, reactions 4 and 5, respectively. On the other hand, metabolite A can choose three different children, namely reactions 1, 2, and 3. Consequently, there are three Child Selections.

$$\begin{cases} \mathbf{J}_1 := \{\mathbf{J}_1(A) = 1; \mathbf{J}_1(B) = 4; \mathbf{J}_1(C) = 5\} \\ \mathbf{J}_2 := \{\mathbf{J}_2(A) = 2; \mathbf{J}_2(B) = 4; \mathbf{J}_2(C) = 5\} \\ \mathbf{J}_3 := \{\mathbf{J}_3(A) = 3; \mathbf{J}_3(B) = 4; \mathbf{J}_3(C) = 5\} \end{cases} \tag{4.45}$$

The sign of the Jacobian determinant is indeterminate. Indeed,

$$\begin{aligned} \det SR &= \sum_{\mathbf{J}} \det S^{\mathbf{J}} \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m} \\ &= \det S^{\mathbf{J}_1} \prod_{m \in \mathbf{M}} r_{\mathbf{J}_1(m)m} + \det S^{\mathbf{J}_2} \prod_{m \in \mathbf{M}} r_{\mathbf{J}_2(m)m} + \det S^{\mathbf{J}_3} \prod_{m \in \mathbf{M}} r_{\mathbf{J}_3(m)m} \\ &= -1 \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}_1(m)m} + 1 \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}_2(m)m} + 0 \cdot \prod_{m \in \mathbf{M}} r_{\mathbf{J}_3(m)m} \\ &= (r_{2A} - r_{1A}) r_{4B} r_{5C}. \end{aligned} \tag{4.46}$$

Considering the flux response $(\Phi)_4^3$ of reaction 4 to a perturbation of reaction 3, via Formula (3.70), we have:

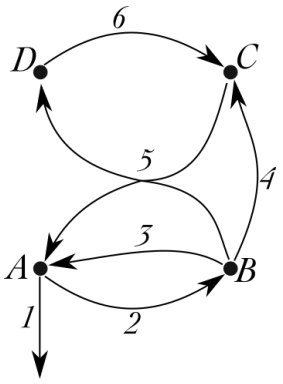
$$\begin{aligned} \det SR\,(\Phi)_4^3 = (r_{2A} - r_{1A}) r_{4B} r_{5C}\,(\Phi)_4^3 &= \sum_{4 \in \mathbf{J} \not\ni 3} (\varphi^{\mathbf{J}})_4^3 = (\varphi^{\mathbf{J}_1})_4^3 + (\varphi^{\mathbf{J}_2})_4^3 \\ &= -\det S^{\mathbf{J}_1 \setminus 4 \cup 3} \prod_{m \in M} r_{\mathbf{J}_1(m)m} - \det S^{\mathbf{J}_2 \setminus 4 \cup 3} \prod_{m \in M} r_{\mathbf{J}_2(m)m} \\ &= -1 \cdot \prod_{m \in M} r_{\mathbf{J}_1(m)m} - (-1) \cdot \prod_{m \in M} r_{\mathbf{J}_2(m)m} \\ &= -(r_{2A} - r_{1A}) r_{4B} r_{5C}. \end{aligned} \tag{4.47}$$

This concludes that $(\Phi)_4^3 \equiv -1$, with no indeterminacy at all, even in presence of indeterminate sign determinant.

Example III: A subnetwork with one-dimensional kernel which cannot be completed to a Child Selection with one-dimensional kernel.

We consider the following network $\mathbf{\Gamma}$:

(4.48)



$$S = \begin{array}{c} \\ A \\ B \\ C \\ D \end{array} \begin{array}{c} \begin{array}{cccccc} 1 & 2 & 3 & 4 & 5 & 6 \end{array} \\ \begin{bmatrix} -1 & -1 & 1 & 0 & 1 & 0 \\ 0 & 1 & -1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \end{array}. \tag{4.49}$$

The network is nondegenerate. In fact, there is a well-behaving Child Selection $\mathbf{J}$,

$$\mathbf{J} := \{\mathbf{J}(A) = 1; \mathbf{J}(B) = 4; \mathbf{J}(C) = 5; \mathbf{J}(D) = 6\}, \tag{4.50}$$

with associated nonsingular stoichiometric matrix

$$S^{\tilde{\mathbf{J}}} = \begin{array}{c} \\ A \\ B \\ C \\ D \end{array} \begin{array}{c} \begin{array}{cccc} 1 & 4 & 5 & 6 \end{array} \\ \begin{bmatrix} -1 & 0 & 1 & 0 \\ 0 & -1 & -1 & 0 \\ 0 & 1 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix} \end{array}, \quad \det S^{\tilde{\mathbf{J}}} = 1. \tag{4.51}$$

Reactions 2 and 3 and their input metabolites A and B constitute a degenerate subnetwork $\mathbf{\Gamma}_{sub} \subset \mathbf{\Gamma}$ whose stoichiometric matrix S_{sub} reads:

$$S_{sub} = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cc} 2 & 3 \end{array} \\ \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix} \end{array}. \tag{4.52}$$

Note that $\dim(\ker S_{sub}) = 1$. We show here that it is not possible to extend the Child Selection $\{\tilde{\mathbf{J}}_{sub}(A) = 2,\ \tilde{\mathbf{J}}_{sub}(B) = 3\}$ on $\mathbf{\Gamma}_{sub}$ to a Child Selection $\tilde{\mathbf{J}}$ on $\mathbf{\Gamma}$ such that $\dim(\ker S^{\tilde{\mathbf{J}}}) = 1$. Indeed, reactions 5 and 6 are single children from their mother metabolites C and D. In particular, any Child Selection contains reactions 5 and 6. That is, the only possible extension of the Child Selection $\tilde{\mathbf{J}}_{sub}$ on $\mathbf{\Gamma}_{sub}$ to a Child Selection $\tilde{\mathbf{J}}$ on $\mathbf{\Gamma}$ is

$$\tilde{\mathbf{J}} := \{\tilde{\mathbf{J}}(A) = 2; \tilde{\mathbf{J}}(B) = 3; \tilde{\mathbf{J}}(C) = 5; \tilde{\mathbf{J}}(D) = 6\}, \tag{4.53}$$

with associated stoichiometric matrix

$$S^{\tilde{\mathbf{J}}} = \begin{array}{c} \\ A \\ B \\ C \\ D \end{array} \begin{array}{c} \begin{array}{cccc} 2 & 3 & 5 & 6 \end{array} \\ \begin{bmatrix} -1 & 1 & 1 & 0 \\ 1 & -1 & -1 & 0 \\ 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix} \end{array}, \tag{4.54}$$

which possesses a 2-dimensional kernel, $\ker(S^{\mathbf{J}}) = \operatorname{span}\{v^1, v^2\}$ where $v^1 = (1,1,0,0)^T$ and $v^2 = (1,0,1,1)^T$.

4.4 Proofs

We start this section with the proof of Proposition 4.2.1.

Proof of Proposition 4.2.1. Preliminarily, note that $\ker(S^{\mathbf{J}\cup j^*}) \neq \varnothing$, since $S^{\mathbf{J}\cup j^*}$ is a $M \times (M+1)$ matrix. Hence, the dimension of the kernel is either 1 or greater than 1. Moreover, by Formula (4.3),

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0 \quad \Leftrightarrow \quad \det(S^{\mathbf{J}\smallsetminus j'\cup j^*}) \neq 0. \tag{4.55}$$

Firstly, assume that $\dim(\ker(S^{\mathbf{J}\cup j^*})) > 1$.

$$\begin{aligned} \dim(\ker(S^{\mathbf{J}\cup j^*})) > 1 \quad &\Rightarrow \quad \ker(S^{\mathbf{J}\smallsetminus j'\cup j^*}) \neq \varnothing, \text{ for all } j' \\ &\Rightarrow \quad (\varphi^{\mathbf{J}})^{j^*}_{j'} = 0, \text{ for all } j'. \end{aligned} \tag{4.56}$$

Conversely, assume that $\dim(\ker(S^{\mathbf{J}\cup j^*})) = 1$. We have

$$\begin{aligned} \dim(\ker(S^{\mathbf{J}\cup j^*})) = 1 \quad &\Rightarrow \quad \operatorname{rank} S^{\mathbf{J}\cup j^*} = M \\ &\Rightarrow \quad \exists\, \det(S^{\mathbf{J}\smallsetminus j'\cup j^*}) \neq 0 \quad \Rightarrow \quad \exists\, (\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0. \end{aligned} \tag{4.57}$$

□

Proof of Theorem 4.2.2. The proof is based on a careful use of Cramer's rule.

1) We prove that

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} \neq 0 \quad \Leftrightarrow \quad v_{j'} \neq 0. \tag{4.58}$$

The first step is to make the matrix $S^{\mathbf{J}\cup j^*}$ an invertible $(M+1)\times(M+1)$ matrix N_b by adding in the $(M+1)$-th row a proper row vector b^T, that is

$$N_b := \begin{bmatrix} S^{\mathbf{J}\cup j^*} \\ b^T \end{bmatrix}. \tag{4.59}$$

Secondly, we compute:

$$\begin{bmatrix} S^{\mathbf{J}\cup j^*} \\ b^T \end{bmatrix} \cdot v = \begin{bmatrix} \underline{0} \\ \langle b, v\rangle \end{bmatrix}. \tag{4.60}$$

Above, $\underline{0}$ refers to the M-dimensional zero vector. Note that $\langle b, v\rangle \neq 0$, since N_b is invertible. We now apply Cramer's rule to the j'-th entry of v and find that

$$\begin{aligned}\det(N_b)\, v_{j'} &= \det \begin{bmatrix} 1 & \dots & j' & \dots & M+1 \\ S^{\mathbf{J}(m_1)} & \dots & \underline{0} & \dots & S^{j^*} \\ b_1^T & \dots & \langle b, v\rangle & \dots & b_{M+1}^T \end{bmatrix} \\ &= -\det \begin{bmatrix} 1 & \dots & j' & \dots & M+1 \\ S^{\mathbf{J}(m_1)} & \dots & S^{j^*} & \dots & \underline{0} \\ b_1^T & \dots & b_{M+1}^T & \dots & \langle b, v\rangle \end{bmatrix} \\ &= -\langle b, v\rangle \det S^{\mathbf{J}\setminus j'\cup j^*}.\end{aligned} \tag{4.61}$$

The conclusion follows by noting that

$$v_{j'} \neq 0 \Leftrightarrow \det S^{\mathbf{J}\setminus j'\cup j^*} \neq 0 \Leftrightarrow (\varphi^{\mathbf{J}})_{j'}^{j^*} \neq 0. \tag{4.62}$$

2) Above, (4.61) showed that for any $v'_j \neq v_{j^*}$

$$\det(N_b)\, v_{j'} = -\langle b, v\rangle \det S^{\mathbf{J}\setminus j'\cup j^*}. \tag{4.63}$$

In particular, this holds for any two $v_{j'_1}, v_{j'_2} \neq 0$. We can divide one equality by the other obtaining

$$\frac{v_{j'_1}}{v_{j'_2}} = \frac{\det S^{\mathbf{J}\setminus j'_1\cup j^*}}{\det S^{\mathbf{J}\setminus j'_2\cup j^*}} = \frac{(\varphi^{\mathbf{J}})_{j'_1}^{j^*}}{(\varphi^{\mathbf{J}})_{j'_2}^{j^*}}. \tag{4.64}$$

Passing to the sign operator gives the desired equality. □

Proof of Theorem 4.2.3. Firstly, let us observe that, under the one-dimensional condition $\ker S^{\mathbf{J}\cup j^*} = \operatorname{span}\langle v\rangle$, we have

$$\begin{cases} \det S^{\mathbf{J}} = 0 & \Leftrightarrow \quad v_{j^*} = 0. \\ \det S^{\mathbf{J}} \neq 0 & \Leftrightarrow \quad v_{j^*} \neq 0. \end{cases} \tag{4.65}$$

1) Now, let us assume $\det S^{\mathbf{J}} \neq 0$, i.e. $v_{j^*} \neq 0$. By Cramer's rule,

$$\begin{aligned}\det(N_b)\, v_{j*} &= \det \begin{bmatrix} 1 & \dots & M+1 \\ S^{\mathbf{J}(m_1)} & \dots & \underline{0} \\ b_1^T & \dots & \langle b, v\rangle \end{bmatrix} \\ &= \langle b, v\rangle \det S^{\mathbf{J}}.\end{aligned} \tag{4.66}$$

Comparison of the equalities between (4.61) regarding v'_j and (4.66) regarding v^*_j implies:

$$\frac{v_{j'}}{v_{j^*}} = \frac{-\det S^{\mathbf{J}\setminus j'\cup j^*}}{\det S^{\mathbf{J}}}. \tag{4.67}$$

Passing to the sign operator yields

$$\operatorname{sign}(v_{j'} v_{j^*}) = \beta(\mathbf{J}) \operatorname{sign}(\varphi^{\mathbf{J}})_{j'}^{j^*}. \tag{4.68}$$

2) For this case, we are allowed to choose $b = e_{j'}$, where $e_{j'}$ indicates the j'-th unit vector in $\mathbb{R}^{M+1}$. We consider, then, the nonsingular matrix

$$N_{j'} := \begin{bmatrix} S^{\mathbf{J}\cup j^*} \\ e_{j'}^T \end{bmatrix}. \tag{4.69}$$

By Cramer's rule, we obtain that

$$\det(N_{j'})v_{j'} = -v_{j'}\det(S^{\mathbf{J}\setminus j'\cup j^*}). \tag{4.70}$$

Hence,

$$\operatorname{sign}(\varphi^{\mathbf{J}})_{j'}^{j^*} = -\operatorname{sign}\det(S^{\mathbf{J}\setminus j'\cup j^*}) = \operatorname{sign}\det(N_{j'}). \tag{4.71}$$

To compute $\det(N_{j'})$, we consider

$$\det(N_{j'}) = \det(N_{j'}^T) = \det\begin{bmatrix} (S^{\mathbf{J}})^T & e_{j'} \\ (S^{j^*})^T & 0 \end{bmatrix}. \tag{4.72}$$

Let us consider $\tilde{v} \in \mathbb{R}^M$ such that $\ker S^{\mathbf{J}} = \operatorname{span}\langle\tilde{v}\rangle$ and $\tilde{v}_j = v_j$, for any $j = 1, ..., M$. Now, for square matrices, $\dim\operatorname{coker}(S^{\mathbf{J}}) = \dim\ker(S^{\mathbf{J}})$. Let us choose the vector $\kappa \in \mathbb{R}^M$ such that,

$$\operatorname{coker}(S^{\mathbf{J}}) = \operatorname{span}\langle\kappa\rangle, \tag{4.73}$$

and

$$\operatorname{Ad}(S^{\mathbf{J}}) = \tilde{v}\cdot\kappa^T \tag{4.74}$$

Let us set $\tilde{\kappa} = (\kappa, 0)^T$. Again:

$$N_{j'}^T\cdot\tilde{\kappa} = \begin{bmatrix} \underline{0} \\ \langle S^{j^*}, \kappa\rangle \end{bmatrix}. \tag{4.75}$$

Let us pick an entry $\kappa_i \neq 0$ and, one more time by Cramer's, we obtain:

$$\begin{aligned} \det(N_{j'})^T\,\kappa_i &= \det\begin{bmatrix} & i & & M+1 & \\ \cdots & 0 & \cdots & 0 & 1 \\ \cdots & \cdots & \cdots & \cdots & \\ \cdots & 0 & \cdots & 1_{j'} & j' \\ \cdots & \cdots & \cdots & \cdots & \\ \cdots & \langle S^{j^*}, \kappa\rangle & \cdots & 0 & M+1 \end{bmatrix} \\ &= (-1)^{i+j'+1}\langle S^{j^*}, \kappa\rangle\ \det(S^{\mathbf{J}})_{\vee i}^{\vee j'}. \end{aligned} \tag{4.76}$$

Above, again, $(S^{\mathbf{J}})_{\vee i}^{\vee j'}$ indicates the matrix with removed column j' and row i. Now, noting that

$$(-1)^{i+j'}\det(S^{\mathbf{J}})_{\vee i}^{\vee j'} = (\operatorname{Ad} S^{\mathbf{J}})_i^{j'} = v_{j'}\kappa_i \tag{4.77}$$

leads to the complete chain of equalities:

$$\begin{aligned} \operatorname{sign}(\varphi^{\mathbf{J}})_{j'}^{j^*} &= -\operatorname{sign}\det(S^{\mathbf{J}\setminus j'\cup j^*}) = \operatorname{sign}\det(N_{j'}) = \operatorname{sign}\det(N_{j'})^T \\ &= -\operatorname{sign}(v_j\langle S^{j^*}, \kappa\rangle), \end{aligned} \tag{4.78}$$

which concludes our proof. □

Corollary 4.2.4 is a consequence of reduction arguments of Section 3.3.4 and Corollary 4.2.5 is a simple check via equality (4.18), in the case of fixed sign Jacobian SR.

Proof of Proposition 4.2.6. We have assumed that the metabolite m^* participates, as a mother input, only to two reactions j^* or j_s^*. In particular, any Child Selection $\mathbf{J}$ contains either j^* or j_s^*, as child reaction of m^*.

Let us pick the influence of j^* on any $j' \neq j^*, j_s^*$ and let us brutally compute, via Formula (3.70):

$$\begin{aligned} \det SR \cdot r_{j^*m^*} \cdot (\Phi)_{j'}^{j^*} &= - \sum_{j^* \notin \mathbf{J} \ni j'} \det S^{\mathbf{J} \smallsetminus j' \cup j^*} \cdot r_{j^*m^*} \cdot \prod_{m \in M} r_{\mathbf{J}(m)m} \\ &= + \sum_{j_s^* \notin \tilde{\mathbf{J}} \ni j'} \det S^{\tilde{\mathbf{J}} \smallsetminus j' \cup j_s^*} \cdot r_{j_s^*m^*} \prod_{m \in M} r_{\tilde{\mathbf{J}}(m)m} \\ &= - \det SR \cdot r_{j_s^*m^*} \cdot (\Phi)_{j'}^{j_s^*}. \end{aligned} \tag{4.79}$$

To check the central step above, note that any Child Selection, which does not contain the sister j^*, must contain her twin sister j_s^*. Hence, with only one column swap $j^* \leftrightarrow j_s^*$, the matrix $S^{\mathbf{J} \smallsetminus j' \cup j^*}$, for a Child Selection $\mathbf{J} \not\ni j^*$ becomes the matrix $S^{\tilde{\mathbf{J}} \smallsetminus j' \cup j_s^*}$ for a Child Selection $\tilde{\mathbf{J}} \not\ni j_s^*$. The step follows since the determinant is an alternating form.
Cases $j^* = j'$ and $j_s^* = j'$ follow analogously by considering Formula (3.63) instead. We omit the computation here. □

Corollary 4.2.7 follows from the arguments exposed in Section 4.2.2. We conclude with the proof of Corollary 4.2.8.

Proof of Corollary 4.2.8. Note that, by construction, $S^{J^{\vee m'} \cup j^*}$ possesses same columns set as $S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*}$. Let now $\mathcal{E}'$ be the subset of $\mathbf{E}$ so that $J^{\vee m'} = (\mathcal{E}')$. In particular, $\tilde{\mathbf{J}}(\mathbf{M}) = \mathcal{E}' \cup \tilde{j}$ and

$$S^{\tilde{\mathbf{J}}(\mathbf{M})} \operatorname{sgn}(\tilde{\mathbf{J}}) = S^{\tilde{\mathbf{J}}}. \tag{4.80}$$

Of course, the equality still holds if the stoichiometric column $S^{\tilde{j}}$ is removed and replaced with the stoichiometric column S^{j^*}, on both sides. That is:

$$S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*(\mathbf{M})} \operatorname{sgn}(\tilde{\mathbf{J}}) = S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*}. \tag{4.81}$$

On the other hand,

$$S^{J^{\vee m'} \cup j^*(\mathbf{M})} \operatorname{sgn}\big(J^{\vee m' \cup j^*}\big) = S^{J^{\vee m'} \cup j^*}. \tag{4.82}$$

In conclusion,

$$\begin{aligned} S^{J^{\vee m'} \cup j^*} &= \operatorname{sgn}\big(J^{\vee m' \cup j^*}\big)\, S^{J^{\vee m'} \cup j^*(\mathbf{M})} \\ &= \operatorname{sgn}\big(J^{\vee m' \cup j^*}\big)\, S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*(\mathbf{M})} \\ &= \operatorname{sgn}\big(J^{\vee m' \cup j^*}\big) \operatorname{sgn}(\tilde{\mathbf{J}})\, S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*} \\ &= \tau\, S^{\tilde{\mathbf{J}} \smallsetminus \tilde{j} \cup j^*}. \end{aligned} \tag{4.83}$$

Therefore,

$$\begin{aligned}\operatorname{sign}(\sigma^{J\vee m'})^{j^*}_{m'} &= \operatorname{sign}(\det S^{J^{\vee m'}\cup j^*})\\ &= \tau \operatorname{sign}(\det S^{\tilde{\mathbf{J}}\setminus\tilde{j}\cup j^*}) = \tau \operatorname{sign}(\varphi^{\tilde{\mathbf{J}}})^{j^*}_{\tilde{j}}.\end{aligned} \tag{4.84}$$

□

Chapter 5

Monomolecular networks

5.1 Introduction

A monomolecular reaction network consists of metabolites m, which interact singularly by certain reactions j. That is, a monomolecular reaction network possesses only monomolecular reactions j is of the form

$$j: \quad m_1 \underset{j}{\longrightarrow} m_2, \tag{5.1}$$

where one single metabolite input m_1 is converted into another single metabolite output m_2. The stoichiometry of these networks is particularly simple: the columns S^j of the stoichiometric matrix S have at most one negative entry -1 and one positive entry $+1$. In particular, columns $S^{j^0_m}$ associated to outflow exit reactions j^0_m

$$j^0_m: \quad m \underset{j^0}{\longrightarrow} \mathbf{0} \tag{5.2}$$

have only a negative entry -1 in the m^{th} row.

It is natural to model a monomolecular reaction network as a directed graph with a vertex metabolite set $\mathbf{M} \cup \{\mathbf{0}\}$ and an arrow reaction set $\mathbf{E}$. We require here that there are no self-loops. As in previous chapters, this excludes explicit autocatalytic reactions. A dipath (or directed path) is any acyclic ordered sequence of alternatingly adjacent vertices and arrows. The *zero-complex* $\mathbf{0}$ in the words of Feinberg [Fei87] is *'a complex in which the stoichiometric coefficient of every species is zero'*. Ingoing reactions of the zero-complex $\mathbf{0}$ are called *outflow exit reactions* or simply *exit reactions*. In the previous chapters we had avoided the zero-complex $\mathbf{0}$ as a superfluous tool, in that multimolecular context. Here, we make use of it, since it allows us to represent monomolecular networks as directed graphs.

At the basis of the sensitivity analysis presented in this thesis, there is a nondegeneracy assumption on the network. That is, we assume the Jacobian matrix of the network to be nonsingular, algebraically:

$$\det SR \neq 0. \tag{5.3}$$

In Section 3.2, we have already discussed all implications and characterizations of this nondegeneracy condition, in the general case. In the monomolecular case, it is a known fact (see for example [FM15] and [BB08]) that this condition is equivalent to the following graph condition:

There exists a dipath from any metabolite m to $\mathbf{0}$. (5.4)

Again, at first, we concentrate on the case $\{(\Phi)^{j^*}_{j'}\}$ of flux response j' to reaction perturbation j^*. Fiedler and Mochizuki were able to characterize the nonzero flux response, using only graph means, see [FM15]. The main theorem on the flux response for monomolecular reaction networks reads as follows.

Theorem 5.1.1 (Fiedler&Mochizuki)**.** *Consider any pair of reactions (j^*, j'), not necessarily distinct. Then the flux response $(\Phi)^{j^*}_{j'}$ of j' to a perturbation of j^* is nonzero, algebraically, if and only if there exist two dipaths γ^0 and γ' such that:*

1. *both dipaths emanate from m^*, input metabolite of j^*;*
2. *one of the dipaths contains j^*;*
3. *the* exit dipath *γ^0 terminates at vertex $\mathbf{0}$, and the* influence dipath *γ' terminates with metabolite m', the input vertex of j';*
4. *except for their shared starting vertex m^*, the two dipaths γ^0 and γ' are disjoint.*

We call (γ^0, γ') an exit-influence pair *of (j^*, j').*

In a joint paper with Matano [VM17] we have further clarified the flux response in the monomolecular case, from a network connectivity point of view.

In the present thesis, we significantly improve on the Fiedler-Mochizuki result for monomolecular reaction networks, by addressing the problem of signed (+/-) response. In other words, we investigate and answer the following question:

Is the nonzero flux response positive, negative, or of indeterminate sign?

Moreover, we clarify the precise relation between the choice of an exit-influence pair of dipaths of Theorem 5.1.1 and the explicit rational expression of the flux response $(\Phi)^{j^*}_{j'}$, (3.70). In this sense, our present result provides a deeper interpretation of the above theorem.

The chapter is organized as follows. Section 5.2 contains the main result and its proof. Extensions to the other cases are discussed in Sections 5.3 and 5.4. Section 5.5 concludes the chapter with a commented example. An abridged version of the same results presented here has been previously announced, by the author. See [Vas17].

5.2 Main result: flux response to reaction perturbation

For this result, we assume again monotone kinetics. That is, we assume the nonzero derivatives $r_{jm} = r'_j(x_m)$ to be positive functions.

The main theorem is preceded by a lemma which analyzes the sign of $\det SR$, in the monomolecular case.

Lemma 5.2.1. *For any monomolecular reaction network:*

$$\operatorname{sign}(\det SR) = (-1)^M. \tag{5.5}$$

Proof. In example **G1** of Section 2.3, we have noticed that any nonzero monomolecular Child Selection $\mathbf{J}$ well-behaves, that is:

$$\det S^{\mathbf{J}} \equiv (-1)^M. \tag{5.6}$$

The conclusion of the lemma follows by the determinant expansion of Proposition 2.2.1. □

We recall explicitly formula (3.70) for the flux response $(\Phi)^{j^*}_{j'}$ of j' to a perturbation of j^*:

$$\det SR \cdot (\Phi)^{j^*}_{j'} = \sum_{j^* \notin \mathbf{J} \ni j'} (\varphi^{\mathbf{J}})^{j^*}_{j'}, \tag{5.7}$$

where

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} = -\det(S^{\mathbf{J} \setminus j' \cup j^*}) \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}. \tag{5.8}$$

The main theorem of this chapter reads as follows.

Theorem 5.2.2. *Consider any pair of reactions (j^*, j') in a monomolecular reaction network, not necessarily distinct. Assume that $(\Phi)^{j^*}_{j'}$ is algebraically nonzero, that is, there exist one or several exit-influence pairs of two dipaths (γ^0, γ') satisfying conditions (i)-(iv) of Theorem 5.1.1.*
Then the exit-influence pairs (γ^0, γ') are in one-to-one correspondence with the response summands $(\varphi^{\mathbf{J}})^{j^*}_{j'}$. Moreover,

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} > 0 \ \text{ for } (\gamma^0, \gamma') \quad \Leftrightarrow \quad j^* \in \gamma'; \tag{5.9}$$

$$(\varphi^{\mathbf{J}})^{j^*}_{j'} < 0 \ \text{ for } (\gamma^0, \gamma') \quad \Leftrightarrow \quad j^* \in \gamma^0. \tag{5.10}$$

The concrete interpretation of the theorem, in terms of positive, negative, or indeterminate sign influence, is given by the following straightforward corollary.

Corollary 5.2.3 (Sign of the responses)**.** *There are three possible cases.*

1. *The flux response $(\Phi)^{j^*}_{j'} > 0$ is positive if, and only if, for* any *choice of an exit-influence pair (γ_0, γ'), j^* is in the influence dipath γ'.*

2. *The flux response* $(\Phi)^{j^*}_{j'} < 0$ *is negative if, and only if, for* any *choice of an exit-influence pair* (γ_0, γ'), j^* *is in the exit dipath* γ^0.
3. *The flux response sign is indeterminate if, and only if, there are at least two different choices of exit-influence pairs* (γ_1^0, γ_1') *and* (γ_2^0, γ_2') *such that* $j^* \in \gamma_1^0$ *is in the exit dipath of one pair, but* $j^* \in \gamma_2'$ *is in the influence dipath of the other pair.*

Now, we proceed with the proof of the main Theorem.

Proof of Theorem 5.2.2. We divide the proof in two cases: $j^* \neq j'$ and $j^* = j'$.

Case $j^* \neq j'$. In the monomolecular case, positivity of all nonzero derivatives $r_{\mathbf{J}(m)m}$ and Lemma 5.2.1 imply:

$$\operatorname{sign}((\Phi)^{j^*}_{j'}) = (-1)^{M-1} \operatorname{sign}\Big(\sum_{j^* \notin \mathbf{J} \ni j'} \det(S^{\mathbf{J} \setminus j' \cup j^*}) \prod_{m \in \mathbf{M}} r_{\mathbf{J}(m)m}\Big), \tag{5.11}$$

and the sign of a summand $(\varphi^{\mathbf{J}})^{j^*}_{j'}$ (5.8) is

$$\operatorname{sign}((\varphi^{\mathbf{J}})^{j^*}_{j'}) = \operatorname{sign}(\det(S^{\mathbf{J} \setminus j' \cup j^*})). \tag{5.12}$$

Now, for economy, we refer to the set $\mathbf{J}(\mathbf{M}) \setminus j' \cup j^*$ simply with $\mathbf{J} \setminus j' \cup j^*$. This swapped determinant is nonzero if and only if the set $\mathbf{J} \setminus j' \cup j^* \subseteq \mathbf{E}$ selects, jointly with all the adjacent vertices of the reaction arrows in $\mathbf{J} \setminus j' \cup j^*$, a spanning tree $\mathbf{T}$ of the network. In other words, $\mathbf{T}$ contains all metabolites in $\mathbf{M}$ plus the zero-complex $\mathbf{0}$ and it does not contain cycles. Moreover, the set $\mathbf{J} \setminus j' \cup j^* \subseteq \mathbf{E}$ is a Child Selection except for the swapped elements. Hence all metabolites $m \in \mathbf{M}$ have one single outgoing reaction in the spanning tree $\mathbf{T}$, with the only two exceptions of m' (input of j') and m^* (input of j^*). Indeed, m' has no outgoing reactions and m^* has two outgoing reactions, due to the swapping $\mathbf{J} \setminus j' \cup j^*$.
To compute the determinant, we implement Gaussian elimination and proceed as follows. We start choosing a subset of $\mathbf{M}_0 \subset \mathbf{M}$ such that m^*, $m' \notin \mathbf{M}_0$ and any $m \in \mathbf{M}_0$ has no ingoing reaction in $\mathbf{J} \setminus j' \cup j^*$. Sometimes these vertices are called in graph theory literature *roots* of the tree $\mathbf{T}$. Of course this set might be empty, and in this case we just skip this step. For any m^{th} row of the $S^{\mathbf{J} \setminus j' \cup j^*}$ matrix with $m \in \mathbf{M}_0$, we sum the m^{th} row to the $\mathcal{O}(\mathbf{J}(m))^{th}$ row. With the notation $\mathcal{O}(j)$, we simply indicate the output metabolite of reaction j. As a rough explanation, we are reducing tree branches. After this first step, indeed, all the columns corresponding to $\mathbf{J}(m)$ with $m \in \mathbf{M}_0$ possesses only a nonzero entry, which is -1 on the diagonal.
We iterate this procedure by defining a set $\mathbf{M}_1$ such that any $\tilde{m} \in \mathbf{M}_1$ is an output $\tilde{m} = \mathcal{O}(\mathbf{J}(m))$ of a reaction $j = \mathbf{J}(m)$, with $m \in \mathbf{M}_0$, and no $\tilde{m} \in \mathbf{M}_1$ is in $\gamma^0 \cup \gamma'$. Again, we sum the $\tilde{m}^{th}$ row to the $\mathcal{O}(\mathbf{J}(\tilde{m}))^{th}$ row. We keep on iterating by defining a set $\mathbf{M}_2$, analogously. etc. At the end of this procedure, we have left untouched the rows corresponding exactly to the metabolites contained in $\gamma^0 \cup \gamma'$.
At this stage, the matrix $S^{\mathbf{J} \setminus j' \cup j^*}$ has been modified into another matrix with the same determinant (we have only added rows), such that every column corresponding to reactions $j \notin \gamma^0 \cup \gamma'$ is the opposite of the j^{th} unit vector, i.e., $-e_j$.
We assume now that $j^* \in \gamma'$. We start, with the same procedure, summing the

m^*-th row to the $\mathcal{O}(\mathbf{J}(m^*))^{th}$ row. Note that $\mathbf{J}(m) \neq j^*$. The m^*-th row has -1 both in the $\mathbf{J}(m^*)^{th}$ column and in the j^*-th column. We iterate now this procedure on the $\mathcal{O}(\mathbf{J}(m^*))^{th}$ row and we keep on iterating the procedure as long as we can, namely until we have touched all the elements on γ^0 and reached the zero. This must happen since $j^* \in \gamma'$. Also, the j^*-th column has been filled with -1 in all the rows corresponding to metabolites in γ^0. We call this process a *cascade* of -1 along γ_0. Up to now, the only rows we did not touch correspond exactly to the metabolites contained in $\gamma' \setminus m^*$. At this second stage, the original matrix $S^{\mathbf{J}\setminus j'\cup j^*}$ has changed into another matrix with the same determinant (we have just added rows), such that every column corresponding to reactions $j \notin \gamma'$ is the opposite of the j^{th} unitary vector e_j. In other words, it has only a -1 on the diagonal. Note that j^* is indeed in γ'. Now, lastly, we start adding the $\mathcal{O}(j^*)^{th}$ row with the same procedure of the preceding step. The $\mathcal{O}(j^*)^{th}$ row has a -1 in the column of reaction $\mathbf{J}(\mathcal{O}(j^*))$ and a +1 in the column of reaction j^*, which is in the original position of reaction j'. Therefore, iterating the procedure along γ' we have a cascade of +1 until we reach the m'-th row, where m' is the input of j'. In this way the j^*-th column has been filled with $+1$ in all the rows corresponding to metabolites in γ', including m'-th row. Note that this row has been filled with $+1$ crucially in the diagonal entry, of course. At the end of this third stage, the matrix is almost diagonal, with the only exception of j^*-th column. This transformed matrix has the same determinant of $S^{\mathbf{J}\setminus j'\cup j^*}$ and it has only -1 on the diagonal except for the j^*-th (originally j'-th) column, in which, due to the cascade of $+1$ along γ', there is now +1. Therefore:

$$\operatorname{sign}(\det(S^{\mathbf{J}\setminus j'\cup j^*})) = (-1)^{M-1}.$$

and

$$\begin{aligned}(-1)^{M-1}\operatorname{sign}(\varphi^{\mathbf{J}})^{j^*}_{j'} &= (-1)^{M-1}\operatorname{sign}(\det(S^{\mathbf{J}\setminus j'\cup j^*}))\\ &= (-1)^{M-1}(-1)^{M-1} = +1.\end{aligned}$$

The case in which $j^* \in \gamma^0$ is solved by analogous arguments, and we omit here the redundant details.

In particular, we have shown that any different choice of couple (γ^0, γ') gives a nonzero summand to the rational expression of the flux-response indicator $(\Phi)^{j^*}_{j'}$.

Case $j^* = j'$. According to the Fiedler-Mochizuki Theorem 5.1.1, $j' = j^*$ always lies in the influence dipath γ'. Therefore, to prove the Theorem 5.2.2 for this case, it is enough to show that any nonzero self-influence is positive, i.e. ,

$$(\Phi)^{j^*}_{j^*} \geq 0. \tag{5.13}$$

Formula (3.63), for the response of the flux of reaction j^* to a perturbation of j^* itself reads:

$$(\Phi)^{j^*}_{j^*} = \frac{\sum_{\mathbf{J}\not\ni j^*} \det S^{\mathbf{J}} \prod_{m\in M} r_{\mathbf{J}(m)m}}{\det SR}. \tag{5.14}$$

Note that the above expression $(\Phi)^{j^*}_{j^*} \neq 0$ can only be nonnegative. Indeed, in the monomolecular case, $\operatorname{sign}(\det SR) = (-1)^M$, and no Child Selection ill-behaves.

Therefore,

$$(-1)^M \det S^{\mathbf{J}} \geq 0, \text{ for any } \mathbf{J}. \tag{5.15}$$

and any nonzero self-influence is positive.

This completes the proof.

□

5.3 Metabolite response to reaction perturbation

For the metabolite response m' to a reaction perturbation j^*, we have the following two relations:

1. If $m' = m^*$ is the input metabolite only of reaction j^*, then

$$\delta x_{m'}^{j^*} = -\frac{1}{r_{j^* m'}} < 0, \tag{5.16}$$

   and therefore the metabolite response $\delta x_{m'}^{j^*}$ is always strictly negative. See Section 3.3.2.3. This case is therefore trivial.

2. If m' is the input metabolite of a reaction $j' \neq j^*$, then

$$(\Phi)_{j'}^{j^*} = (e_{j^*} + R(\delta x)^{j^*})_{j'} = r_{j' m'}\, \delta x_{m'}^{j^*}. \tag{5.17}$$

   Note that the equality $(R(\delta x)^{j^*})_{j'} = r_{j' m'}\, \delta x_{m'}^{j^*}$ holds only for a monomolecular network. This case includes also the case $m' = m^*$, input of j^*.

In the case 2 above, we can conclude that

$$\delta x_{m'}^{j^*} \begin{cases} = 0 & \Leftrightarrow \quad (\Phi)_{j'}^{j^*} = 0 \\ > 0 & \Leftrightarrow \quad (\Phi)_{j'}^{j^*} > 0 \\ < 0 & \Leftrightarrow \quad (\Phi)_{j'}^{j^*} < 0 \\ \text{indet.} & \Leftrightarrow \quad (\Phi)_{j'}^{j^*} \text{ indet.} \end{cases} . \tag{5.18}$$

In particular, Theorem 5.2.2 structurally characterizes the sign of the metabolite response $\delta x_{m'}^{j^*}$ of m' to a perturbation of j^*, in an identical way.

5.4 Metabolite perturbation

We state a simple characterization of the responses to a perturbation of a metabolite concentration m^*, in the monomolecular case. We have the following result:

Theorem 5.4.1. *Let m^* be a metabolite and p' an element, either a metabolite $p' = m'$ or a reaction $p' = j'$, in a monomolecular reaction network. Then a perturbation of m^* produces a response on an element p' if and only if p' is reachable from m^* via a directed path.*
Moreover, any nonzero response is strictly positive.

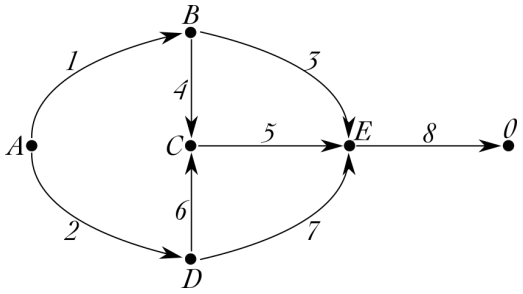
Above, the reachability is intended in the usual graph theory sense. That is, an element p is reachable from a vertex m if there exists a directed path $\gamma[m,p]$, which starts at the element m and ends at the element p.
Before proceeding on to the proof, let us observe that reachability in a graph is a transitive property. This leads to a general metabolite transitivity result for monomolecular network. See for further details Chapter 6.

Proof of Theorem 5.4.1. According to Section 3.3.4, a metabolite perturbation of m^* is identical to a reaction perturbation of an *added* exit reaction $j^0_{m^*}$ from m^*, with reverted sign.
Consider now the previous results in this chapter, based on the existence of two directed paths (γ^0, γ'), both departing from m^*, γ^0 leading to 0, γ' leading to m'. In our case, we should consider $\gamma^0 \equiv j^*_0$. This always provides the existence of γ^0 satisfying the needed conditions. For any reachable p' from m^*, any directed path $\gamma[m^*, p']$ serves as γ', providing the desired exit-influence pair (γ^0, γ').
The perturbed reaction $j^0_{m^*}$ is trivially always in γ^0, so that previous Theorem 5.2.2 guarantees that the response is always negative. By construction, we must revert the sign and we always obtain a positive response. □

5.5 Example

In this section we comment on one example.

$$S = \begin{array}{c} \\ A \\ B \\ C \\ D \\ E \end{array} \begin{array}{c} \begin{array}{cccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \end{array} \\ \begin{bmatrix} -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & -1 & -1 & 0 \\ 0 & 0 & 1 & 0 & 1 & 0 & 1 & -1 \end{bmatrix} \end{array}, \tag{5.19}$$



Here below the table of the responses, obtained by applying the theorems of this chapter. The upper row indicates the perturbed element, the left column indicates the responsive element, as suggested by the arrow ↯.
The signs of response are given by $0, +, -, +/-$ for zero, positive, negative, indeterminate - respectively.

↓	1	2	3	4	5	6	7	8	A	B	C	D	E
1	+	-	0	0	0	0	0	0	+	0	0	0	0
2	-	+	0	0	0	0	0	0	+	0	0	0	0
3	+	-	+	-	0	0	0	0	+	+	0	0	0
4	+	-	-	+	0	0	0	0	+	+	0	0	0
5	+/-	-/+	-	+	0	+	-	0	+	+	+	+	0
6	-	+	0	0	0	+	-	0	+	0	0	+	0
7	-	+	0	0	0	-	+	0	+	0	0	+	0
8	0	0	0	0	0	0	0	0	+	+	+	+	+
A	-	-	0	0	0	0	0	0	+	0	0	0	0
B	+	-	-	-	0	0	0	0	+	+	0	0	0
C	+/-	-/+	-	+	-	+	-	0	+	+	+	0	0
D	-	+	0	0	0	-	-	0	+	0	0	+	0
E	0	0	0	0	0	0	0	-	+	+	+	+	+

The symbol $-/+$ has been used to stress that the response $(\Phi)^1$ is the opposite of $(\Phi)^2$, i.e., $(\Phi)^1 = -(\Phi)^2$ in all flux response components. The same feature can be seen for all the cases of metabolite with only two outgoing reactions, see also Proposition 4.2.6. That is, $(\Phi)^3 = -(\Phi)^4$ and $(\Phi)^6 = -(\Phi)^7$. The metabolite responses present a similar feature, with the important exception of the response of the input of the perturbed reactions, which is always negative. In fact: $(\delta x)^1_A = (\delta x)^2_A < 0$, $(\delta x)^3_B = (\delta x)^4_B < 0$, and $(\delta x)^6_D = (\delta x)^7_D < 0$.
Note that *Single children influence only the mothers, negatively!*, see Section 3.3.2.3. That is, reaction 5 and reaction 8 only influence metabolite C and metabolite E, respectively.
The indetermination of the response $(\Phi)^1_5$ of reaction 5 to a perturbation of reaction 1 can be seen in the network by considering two couples of exit-influence pairs (γ^0_1, γ'_1) and (γ^0_2, γ'_2), where

$$\begin{cases} \gamma^0_1 = [A1B3D80] \\ \gamma'_1 = [A2D6C] \end{cases} \qquad \begin{cases} \gamma^0_2 = [A2D7E80] \\ \gamma'_2 = [A1B4C] \end{cases} .$$

Reaction 1 is in γ^0_1 (providing a negative sign summand), and in γ'_2 (providing a positive sign summand). Identical considerations for the indeterminate responses $(\Phi)^2_5$, $(\Phi)^1_C$ and $(\Phi)^2_C$.
En passant, let us observe here the following things:

1. $1 \rightsquigarrow B$ and $B \rightsquigarrow E, 8$. But $1 \not\rightsquigarrow E, 8$. This constitutes a counterexample to the transitivity of reaction influence and metabolite influence. See Chapter 6.
2. $(\Phi)^1_4 > 0$, $(\Phi)^4_5 > 0$, but $(\Phi)^1_5$ is indeterminate. This constitutes a mild but already significative indication that signed transitivity fails. See Chapter 6.

Chapter 6

Limitations to influence transitivity

6.1 State of the art

In previous works, great effort has been invested into the topic of transitivity of influence. Let us introduce transitivity by considering any p_1, p_2, and p_3 elements in the network, either metabolites or reactions. The *transitivity question* is:

$$\textit{If } p_1 \rightsquigarrow p_2 \textit{ and } p_2 \rightsquigarrow p_3\textit{, can we conclude that } p_1 \rightsquigarrow p_3\textit{?}$$

The relevance of a positive answer to this question is both conceptual and practical. Conceptually, indeed, it explains the patterns observed experimentally in the responses. Practically, it greatly simplifies the computation of the nonzero responses, at least for the nonzero question.

The answer has originally been addressed for the reaction perturbation case, only. In the case of reaction perturbations, nonzero transitivity has been established in the monomolecular case [FM15], [VM17], at first. The general multimolecular case was resolved in [BF18].
The result by Brehm and Fielder in [BF18] actually claims more than the pure reaction perturbation case, and it is worth to recall it here:

Theorem 6.1.1 (Brehm-Fiedler)**.** *Let p_1 and p_2 be elements in a metabolic network. Let j' be any reaction and m' one of its input metabolites.*

1. *If $p_1 \rightsquigarrow m'$ and $j' \rightsquigarrow p_2$, then $p_1 \rightsquigarrow p_2$.*
2. *If $p_1 \rightsquigarrow j'$ and $j' \rightsquigarrow p_2$, then $p_1 \rightsquigarrow p_2$.*

However the general case

$$p_1 \rightsquigarrow p_2 \text{ and } p_2 \rightsquigarrow p_3 \overset{?}{\Longrightarrow} p_1 \rightsquigarrow p_3$$

has remained open, for $p_2 = m$ metabolite.

The following Section 6.2 shows, with an extremely simple example, that theorem 6.1.1 cannot be improved, for the general multimolecular case. That is, any further

transitivity claim of this type fails, in the multimolecular case. In the much simpler case of monomolecular networks, however, it is possible to extend the transitivity to the pure metabolite case, Section 6.3.

Even in the monomolecular case, as seen in Section 5.5, nonetheless,

$$j \rightsquigarrow m \text{ and } m \rightsquigarrow p \not\Rightarrow j \rightsquigarrow p.$$

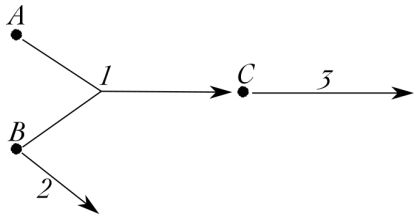
Finally, unfortunately, there is no hope for a signed transitivity result of the kind

$$p_1 \overset{+}{\rightsquigarrow} p_2 \overset{+}{\rightsquigarrow} p_3 \overset{?}{\Longrightarrow} p_1 \overset{+}{\rightsquigarrow} p_3.$$

We show this with a counterexample in Section 6.4. These counterexample further emphasize the subtleties of the transitivity question.

6.2 A general counterexample

The simple example here is again Example (3.28) of Chapter 3:



(6.1)

The sensitivity matrix below can be derived easily:

$\Psi =$

↯	1	2	3	A	B	C
1	0	0	0	+	0	0
2	0	0	0	-	+	0
3	0	0	0	+	0	+
A	-	+	0	+	-	0
B	0	-	0	-	+	0
C	0	0	-	+	0	+

.

Note that the flux-responses to reaction perturbations $(\Phi)_{j'}^{j^*}$ are constantly zero for any reaction j^*, j'. This is due to the fact that there is only one Child Selection, and therefore any reaction is a single child, see 3.3.2.3.

The four counterexamples, which we chose to focus our attention on, are:

1. $B \rightsquigarrow A$ and $A \rightsquigarrow C$, but $B \not\rightsquigarrow C$;
2. $B \rightsquigarrow A$ and $A \rightsquigarrow 3$, but $B \not\rightsquigarrow 3$;
3. $2 \rightsquigarrow A$ and $A \rightsquigarrow C$, but $2 \not\rightsquigarrow C$;
4. $2 \rightsquigarrow A$ and $A \rightsquigarrow 1$, but $2 \not\rightsquigarrow 1$.

Hence, Theorem 6.1.1 covers all the transitivity properties.

6.3 Metabolite transitivity in monomolecular networks

For the special case of monomolecular reaction networks, the following Theorem holds:

Theorem 6.3.1 (Monomolecular Transitivity)**.** *Let m^*, p_1, p_2, be three elements of a monomolecular reaction network, where m^* is a metabolite, and p_1 and p_2 are metabolites or reactions.*
Assume moreover that

$$m^* \rightsquigarrow p_1 \text{ and } p_1 \rightsquigarrow p_2.$$

Then,

$$m^* \rightsquigarrow p_2.$$

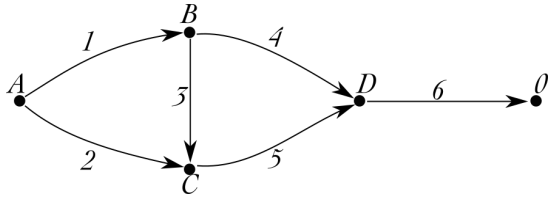
Proof. The case in which p_1 is a reaction reduces to Theorem 6.1.1.
Next, we only need to consider $p_1 = m$ metabolite. This case is solved by the monomolecular Theorem 5.4.1 for metabolite influence, which states that metabolite influence is equivalent to reachability. Reachability in networks is obviously a transitive property. □

6.4 Failure of sign-transitivity

Transitivity of flux-influence (reaction $\rightsquigarrow$ reaction) has been proven to hold; see 6.1.1. In contrast, Section 6.2 above illustrates how transitivityfails for the pure metabolite case (metabolite $\rightsquigarrow$ metabolite), in general. For sign-transitivity, we therefore restrict to the case of flux-influence, only. Let j^*, j', j'' be three distinct reactions. The question of sign-transitivity asks whether it is possible to infer the sign of the influence $j^* \rightsquigarrow j''$, knowing the signs of the influences $j^* \rightsquigarrow j'$, and $j' \rightsquigarrow j''$. In symbols,

$$j^* \overset{+}{\rightsquigarrow} j' \overset{+}{\rightsquigarrow} j'' \overset{?}{\Longrightarrow} j^* \overset{+}{\rightsquigarrow} j'',$$

or any other combinations of signs. We show that sign-transitivity fails, with the following simple monomolecular example.



(6.2)

Consider a perturbation of reaction 1. By Theorem 5.2.2, the flux of reaction 3 responds positively. Consider indeed the dipaths $(\gamma_3^1)^0 = [A2C5D60]$ and $(\gamma_3^1)' = [A1B]$. Since $(\gamma_3^1)^0, (\gamma_3^1)'$ is the only exit-influence pair and 1 belongs to the influence dipath $(\gamma_3^1)'$, we conclude that the response is positive, $(\Phi)_3^1 > 0$. Analogously, there is a single choice of exit-influence pair for a response of reaction 5 to a perturbation of reaction 3, namely $(\gamma_5^3)^0 = [B4D60]$ and $(\gamma_5^3)' = [B3C]$. Here, the perturbed reaction 3 is in the influence dipath $(\gamma_5^3)'$ and therefore the influence is again positive.

However, checking the response of reaction 5 to a perturbation of reaction 1 we see that, again, we have a single choice of exit-influence pair, namely $(\gamma_5^1)^0 = [A1B4D60]$ and $(\gamma_5^1)' = [A2C]$. Here, however, reaction 1 lies in the exit dipath $(\gamma_5^1)^0$ and the influence is therefore negative, contradicting any signed transitivity hope. In conclusion we have shown:

$$1 \overset{+}{\rightsquigarrow} 3 \overset{+}{\rightsquigarrow} 5 \text{ but } 1 \overset{-}{\rightsquigarrow} 5.$$

Other cases follow in analogy, and we omit the computation. From the same example:

$$2 \overset{-}{\rightsquigarrow} 1 \overset{-}{\rightsquigarrow} 5 \text{ but } 2 \overset{+}{\rightsquigarrow} 5.$$

$$1 \overset{+}{\rightsquigarrow} 4 \overset{-}{\rightsquigarrow} 5 \text{ and } 1 \overset{-}{\rightsquigarrow} 5,$$

$$1 \overset{+}{\rightsquigarrow} 4 \overset{-}{\rightsquigarrow} 3 \text{ but } 1 \overset{+}{\rightsquigarrow} 3.$$

$$2 \overset{-}{\rightsquigarrow} 1 \overset{+}{\rightsquigarrow} 3 \text{ and } 2 \overset{-}{\rightsquigarrow} 3.,$$

$$2 \overset{-}{\rightsquigarrow} 3 \overset{+}{\rightsquigarrow} 5 \text{ but } 2 \overset{+}{\rightsquigarrow} 5.$$

The remaining cases are easily constructible with new examples, and we omit them here. See for instance Example 5.19 in Section 5.5, which presents some cases where also indeterminate response is involved.

To close this topic, let us remind that Theorem 5.4.1 states that pure metabolite influence is characterized by reachability, in the monomolecular case. Moreover, the influence is always positive. This implies that metabolite influence is trivially sign-transitive, for the monomolecular case, as it is always positive. With this minimal success, we still consider sign-transitivity to fail, overall.

Chapter 7

Discussion and open questions

In this thesis we have presented a systematic approach to sensitivity analysis for metabolic chemical reaction networks.
In particular, in the new language of Child Selections, we have highlighted the main algebraic players in the signed description of the entries of the sensitivity matrix. These structures, related to kernel and cokernel vectors of the stoichiometric matrix, possess well-known biological significance [KRG+17].

Some delicate issues have not been addressed in detail in this dissertation.
First, our approach is based on the Implicit Function Theorem. In this sense, our analysis is a *local* analysis. However, in real applications, the fluctuation of parameters modeled by a perturbation may not be small, for example enzyme knock-out experiments, where an enzyme catalyzing a reaction is knocked out by a genetic modification of the cell. After this modification, the reaction rate of the perturbed reaction becomes very small or zero. Obviously, this kind of perturbation cannot be considered local. This requires global extension of our arguments to the case of large perturbations. Fortunately, our results also apply to such large perturbations, due to an interpolation argument by Brehm and Fiedler [BF18], which we omit here. Suffice it to say that the core reason, why such an interpolation argument holds, is that our analysis is only based on the stoichiometry of the system, and does not really depend - mathematically - on the specific equilibrium. In fact, in any equilibrium we consider, the responses are the same, qualitatively.
Secondly, the nondegeneracy assumption $\det(SR) \neq 0$ relies on the intended application. Due to the abundant presence of outflows, this assumption practically always holds, in a metabolic context. Mathematically possible, an extension of the present theory allowing stoichiometric subspaces may dangerously result in an overload of mathematical abstraction and confusing notation, mostly, at least without a desired application requiring such a context.

We conclude with three main open questions to drive our future work in this field.

1. The computation of the full sensitivity matrix for a metabolic network of medium size, like the central carbon metabolism of Section 2.10, still requires substantial effort, at present. We have not addressed the design of an efficient algorithm for this problem. In a mathematical context more related to ecology,

Giordano and co-authors [GSFB16] have designed an algorithm of exponential complexity to compute the sign of some sensitivity responses.
Some tools developed in our thesis may help in the construction of an efficient algorithm. For example, the concept of the distance of Child Selections from Section 2.6 implicitly points at a useful lattice structure on the set of Child Selections. In view of the analysis of Chapter 4, a major role is played by Enlarged Child Selections which identify stoichiometric minors with one-dimensional kernel. The elementary kernel vectors v, spanning such one-dimensional kernel spaces, are the central objects in the description of the sensitivity responses. Child Selections at low distance, then, may identify stoichiometric minors possessing the same one-dimensional kernel space, spanned by the same elementary kernel vector. For this reason, different sensitivity terms associated to many low-distance Child Selections may be computed in parallel allowing a strong reduction of computation time. In other words, there may be much fewer elementary kernel vectors v than Child Selections. We hope to develop an efficient algorithm along these lines.

2. As pointed out in Example II of Section 4.3, a Jacobian of indeterminate sign does not always imply indeterminate sign responses. In particular, cancellations may occur in the rational expression of the responses between the numerator of and the Jacobian denominator. We have only cursorily touched upon such cancellations in Corollary 3.3.5. Indeterminate sign responses are of particular interest. In fact: they are controllable, in the sense that there exist choices of parameters for which the responses are positive, negative, or zero. A better comprehension of this topic would be of great help for the control of the response sign, with important consequences for applications.

3. In Section 2.9, we have introduced and discussed the property of a system possessing a factorizable Jacobian determinant. These networks are nongeneric but - interestingly - they present a striking feature for the bifurcation analysis: the presence of two Child Selections with opposite behavior directly implies the existence of a simple bifurcation parameter which controls the sign of the Jacobian determinant of the entire system, *for any choice of parameters*. A complete structural characterization of this class of networks is of great interest.

With the last parting glances upon the unfinished aspects of our present work, which constitute - as always - the real driving force for new future work, we conclude this dissertation.

Bibliography

[BB08] Murad Banaji and Stephen Baigent. Electron transfer networks. *Journal of Mathematical Chemistry*, 43(4):1355–1370, 2008.

[BC10] Murad Banaji and Gheorghe Craciun. Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems. *Advances in Applied Mathematics*, 44(2):168–184, 2010.

[BDB07] Murad Banaji, Pete Donnell, and Stephen Baigent. P matrix properties, injectivity, and stability in chemical reaction systems. *SIAM Journal on Applied Mathematics*, 67(6):1523–1547, 2007.

[BF18] Bernhard Brehm and Bernold Fiedler. Sensitivity of chemical reaction networks: a structural approach. 3. Regular multimolecular systems. *Mathematical Methods in the Applied Sciences*, 41(4):1344–1376, 2018.

[BR11] Murad Banaji and Carrie Rutherford. P-matrices and signed digraphs. *Discrete Mathematics*, 311(4):295–301, 2011.

[BS09] Richard A. Brualdi and Bryan L. Shader. *Matrices of sign-solvable linear systems*. Cambridge University Press, 2009.

[CCK] *Comprehensive chemical kinetics*. Elsevier.

[CF06] Gheorghe Craciun and Martin Feinberg. Multiple equilibria in complex chemical reaction networks: II. The species-reaction graph. *SIAM Journal on Applied Mathematics*, 66(4):1321–1338, 2006.

[Fei87] Martin Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors—I. The deficiency zero and deficiency one theorems. *Chemical Engineering Science*, 42(10):2229–2268, 1987.

[Fei95] Martin Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Archive for Rational Mechanics and Analysis*, 132(4):311–370, 1995.

[Fei19] Martin Feinberg. *Foundations of Chemical Reaction Network Theory*. Springer, 2019.

[Fie19] Bernold Fiedler. Global hopf bifurcation in networks with fast feedback cycles. *arXiv preprint arXiv:1903.09996*, 2019.

[FM15] Bernold Fiedler and Atsushi Mochizuki. Sensitivity of chemical reaction networks: a structural approach. 2. Regular monomolecular systems. *Mathematical Methods in the Applied Sciences*, 38(16):3519–3537, 2015.

[Ger31] Semyon A. Gershgorin. Über die Abgrenzung der Eigenwerte einer Matrix. *Bulletin de l'Académie des Sciences de l'URSS. Classe des sciences mathématiques et naturelles*, (6):749–754, 1931.

[GKZ08] Israel M. Gelfand, Mikhail Kapranov, and Andrei Zelevinsky. *Discriminants, resultants, and multidimensional determinants*. Springer Science and Business Media, 2008.

[GN65] David Gale and Hukukane Nikaido. The Jacobian matrix and global univalence of mappings. *Mathematische Annalen*, 159(2):81–93, 1965.

[Gor94] Paul Gordan. Das Zerfallen der Curven in gerade Linien. *Mathematische Annalen*, 45(3):410–427, 1894.

[GSFB16] Giulia Giordano, Christian C. Samaniego, Elisa Franco, and Franco Blanchini. Computing the structural influence matrix for biological systems. *Journal of Mathematical Biology*, 72(7):1927–1958, 2016.

[Gua16] Yonghui Guan. *Equations for Chow Varieties, Their Secant Varieties and Other Varieties Arising in Complexity Theory*. PhD thesis, 2016.

[Gua18] Yonghui Guan. Brill's equations as a GL(V)-module. *Linear Algebra and its Applications*, 548:273–292, 2018.

[Had99] Jacques Hadamard. Sur les conditions de décomposition des formes. *Bulletin de la Société Mathématique de France*, 27:34–47, 1899.

[HJ72] Fritz Horn and Roy Jackson. General mass action kinetics. *Archive for Rational Mechanics and Analysis*, 47(2):81–116, 1972.

[HJ13] Roger A. Horn and Charles R. Johnson. *Matrix Analysis*. Cambridge University Press, 2013.

[INB+07] Nobuyoshi Ishii, Kenji Nakahigashi, Tomoya Baba, Martin Robert, Tomoyoshi Soga, Akio Kanai, Takashi Hirasawa, Miki Naba, Kenta Hirai, Aminul Hoque, et al. Multiple high-throughput analyses monitor the response of e. coli to perturbations. *Science*, 316(5824):593–597, 2007.

[JKVdD77] Clark Jeffries, Victor Klee, and Pauline Van den Driessche. When is a matrix sign stable? *Canadian Journal of Mathematics*, 29(2):315–326, 1977.

[KRG+17] Steffen Klamt, Georg Regensburger, Matthias P Gerstl, Christian Jungreuthmayer, Stefan Schuster, Radhakrishnan Mahadevan, Jürgen Zanghellini, and Stefan Müller. From elementary flux modes to elementary flux vectors: Metabolic pathway analysis with arbitrary linear flux constraints. *PLoS Computational Biology*, 13(4):e1005409, 2017.

[LOvdD18] Jephian C.-H. Lin, Dale D. Olesky, and Pauline van den Driessche. Sign patterns requiring a unique inertia. *Linear Algebra and its Applications*, 546:67–85, 2018.

[MC13] Maya Mincheva and Gheorghe Craciun. Graph-theoretic conditions for zero-eigenvalue turing instability in general chemical reaction networks. *Mathematical Biosciences and Engineering*, 10(4):1207–1226, 2013.

[MF15] Atsushi Mochizuki and Bernold Fiedler. Sensitivity of chemical reaction networks: a structural approach. 1. Examples and the carbon metabolic network. *Journal of Theoretical Biology*, 367:189–202, 2015.

[Nak92] Hisao Nakajima. Sensitivity and stability of flow networks. *Ecological Modelling*, 62(1-3):123–133, 1992.

[NTI+09] Kenji Nakahigashi, Yoshihiro Toya, Nobuyoshi Ishii, Tomoyoshi Soga, Miki Hasegawa, Hisami Watanabe, Yuki Takai, Masayuki Honma, Hirotada Mori, and Masaru Tomita. Systematic phenome analysis of escherichia coli multiple-knockout mutants reveals hidden reactions in central carbon metabolism. *Molecular Systems Biology*, 5(1):306, 2009.

[OM16] Takashi Okada and Atsushi Mochizuki. Law of localization in chemical reaction networks. *Physical review letters*, 117(4):048101, 2016.

[OM17] Takashi Okada and Atsushi Mochizuki. Sensitivity and network topology in chemical reaction systems. *Physical Review E*, 96(2):022322, 2017.

[OTM18] Takashi Okada, Je-Chiang Tsai, and Atsushi Mochizuki. Structural bifurcation analysis in chemical reaction networks. *Physical Review E*, 98(1):012417, 2018.

[Roc69] Ralph T. Rockafellar. The elementary vectors of a subspace of $\mathbb{R}^n$. In *Proceedings of the Chapel Hill Conf.* University of North Carolina Press, 1969.

[SAF09] Guy Shinar, Uri Alon, and Martin Feinberg. Sensitivity and robustness in chemical reaction networks. *SIAM Journal on Applied Mathematics*, 69(4):977–998, 2009.

[SF10] Guy Shinar and Martin Feinberg. Structural sources of robustness in biochemical reaction networks. *Science*, 327(5971):1389–1391, 2010.

[SF11] Guy Shinar and Martin Feinberg. Design principles for robust biochemical reaction networks: what works, what cannot work, and what might almost work. *Mathematical Biosciences*, 231(1):39–48, 2011.

[SF12] Guy Shinar and Martin Feinberg. Concordant chemical reaction networks. *Mathematical Biosciences*, 240(2):92–113, 2012.

[SF13] Guy Shinar and Martin Feinberg. Concordant chemical reaction networks and the species-reaction graph. *Mathematical Biosciences*, 241(1):1–23, 2013.

[Sie16] Sascha Siegmund. Flux sensitivity analysis in chemical reaction networks. Master's thesis, Freie Universität Berlin, 2016.

[SMJF11] Guy Shinar, Avi Mayo, Haixia Ji, and Martin Feinberg. Constraints on reciprocal flux sensitivities in biochemical reaction networks. *Biophysical Journal*, 100(6):1383–1391, 2011.

[SRTC05] Andrea Saltelli, Marco Ratto, Stefano Tarantola, and Francesca Campolongo. Sensitivity analysis for chemical models. *Chemical Reviews*, 105(7):2811–2828, 2005.

[Val79] Leslie G. Valiant. Completeness classes in algebra. In *Proceedings of the eleventh annual ACM symposium on Theory of computing*. ACM, 1979.

[Vas16] Nicola Vassena. Monomolecular reaction networks: a new proof of flux transitivity. Master's thesis, Free University of Berlin, 2016.

[Vas17] Nicola Vassena. Sensitivity of monomolecular reaction networks: signed flux-response to reaction rate perturbations. In *2017 European Conference on Circuit Theory and Design (ECCTD)*. IEEE, 2017.

[VM17] Nicola Vassena and Hiroshi Matano. Monomolecular reaction networks: Flux-influenced sets and balloons. *Mathematical Methods in the Applied Sciences*, 40(18):7722–7736, 2017.

[Yod88] Peter Yodzis. The indeterminacy of ecological interactions as perceived through perturbation experiments. *Ecology*, 69(2):508–515, 1988.

Zusammenfassung

In dieser Arbeit präsentieren wir eine umfassende Sensitivitätsanalyse metabolischer Reaktionsnetzwerke mit allgemeiner Kinetik. Sensitivitätsanalyse meint hier das systematische Studium der Veränderung des Netzwerkkomponenten durch Störungen. Wir betrachten lokale Störungen eines dynamischen Gleichgewichts, das heißt, Störungen der Metabolitenkonzentrationen bzw. der Reaktionsgeschwindigkeiten. Wir untersuchen die resultierenden Veränderung der Metabolitenkonzentrationen als auch die der Reaktionsflüsse. Zunächst beschreiben wir, auf welche Komponenten des Netzwerks überhaupt eingewirkt wird. Zweitens analysieren wir, ob das Vorzeichen der Veränderung von den Parametern des Systems abhängt.

Dabei spielen Vorzeichenänderungen der Jacobi-Determinante des Netzwerks eine wichtige Rolle, sowohl bei der Sensitivitätsanalyse als auch bei der Bifurkation von Gleichgewichten. Der erste Teil dieser Arbeit unterscheidet den Fall des konstanten Vorzeichens vom Bifurkationsfall, bei dem das Vorzeichen von den Werten der Reaktionsgeschwindigkeiten abhängt.

Unser Ansatz ist eher qualitativ als quantitativ. Tatsächlich basiert unsere Analyse ausschließlich auf der Stöchiometrie des Reaktionsnetzwerks. Quantitative Angaben zu den Reaktionsgeschwindigkeiten sind nicht erforderlich. Stattdessen erfolgt die Beschreibung in rein algebraischen Begriffen, welche nur die Kenntnis der Netzwerkstruktur erfordern.

Biologische Anwendungen umfassen den Nachweis von Multistationarität, Enzym-Knock-Out-Experimente und die Stoffwechselkontrolle.

Selbständigkeitserklärung

Hiermit bestätige ich, VASSENA NICOLA, dass ich die vorliegende Dissertation mit dem Thema

Sensitivity of metabolic networks

selbständig angefertigt und nur die genannten Quellen und Hilfen verwendet habe. Die Arbeit ist erstmalig und nur an der Freien Universität Berlin eingereicht worden.

Berlin, den