GOOD AND BAD CHILDREN

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Abstract. Equilibrium bifurcations arise from sign changes of Jacobian determinants, as parameters are varied. Therefore we address the Jacobian determinant for metabolic networks with general reaction kinetics. Our approach is based on the concept of Child Selections: each (mother) metabolite is mapped, injectively, to one of those (child) reactions which it drives as an input. Our analysis distinguishes reaction network Jacobians with constant sign from the bifurcation case, where that sign depends on specific reaction rates. In particular we distinguish “good” Child Selections, which do not affect the sign, from more interesting and mischievous “bad” children, which gang up towards sign changes, instability, and bifurcation.

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1. Introduction

We consider here general Metabolic Chemical Reaction Networks (MCRN) $\Gamma$ with $M$ metabolites and $N$ reactions. For notation, we use capital letters $A,B,C,D,...$ for metabolites and numbers $1,2,3,...$ for reactions. We call $M$ the metabolites set and $E$ the set of reactions, such that $|M|=M$ and $|E|=N$. We use the small letter $m \in M$ for a generic metabolite and the small letter $j \in E$ for a generic reaction.

The dynamics of metabolite concentrations $x$ is described, in general, by the system of differential equations:

$$\dot{x} = f(x) := S r(x) + F.$$ 

The $M \times N$ matrix $S$ is the stoichiometric matrix. The constant $M$-th dimensional vector $F$ represents the inflows of the network (feed reactions). The $N$-th dimensional vector $r(x)$ encodes reaction rate functions.

Here, we assume total irreversibility of the system, that is, we consider strictly positive reaction rates $r_i(x) > 0$, for every $i = 1,...,N$. In particular, we model a possibly reversible reaction

$$(2) \quad s_j^1 m_1 + \ldots + s_{j}^p m_p \leftrightarrow j s_q^1 m_q + \ldots + s_s^1 m_s,$$

simply as two different irreversible reactions

$$(3) \quad s_j^1 m_1 + \ldots + s_{j}^p m_p \rightarrow j s_q^1 m_q + \ldots + s_s^1 m_s \quad \text{and} \quad s_j^2 m_1 + \ldots + s_{j}^p m_p \leftarrow j s_q^2 m_q + \ldots + s_s^2 m_s.$$ 

In this way, the whole information of the network is completely encoded in the stoichiometric matrix $S$, only. We comment about this point in more detail in the framed Remark 1 below.

Moreover, we exclude here explicit autocatalytic reactions of the form:

$$(4) \quad s_j^a m_{ac} + \ldots \rightarrow j s_j^a m_{ac} + \ldots,$$

where a metabolite $m_{ac}$ appears on both sides of the reaction. That is, self-loops are not allowed in the network.

See Example B1 in Subsection 2.2 and Subsection 3.2 below for further comments on autocatalytic reactions.

With these assumptions, we associate to any stoichiometric coefficient $s_j^m$ of an input metabolite $m$ of reaction $j$ a negative stoichiometric entry of the stoichiometric matrix $S$, that is:

$$(5) \quad S_{mj} = -s_j^m, \text{ for } m \text{ input of } j.$$ 

Conversely, we associate to any stoichiometric coefficient $s_j^{m'}$ of an output metabolite $m'$ of reaction $j$ a positive stoichiometric entry of $S$, that is:

$$(6) \quad S_{m'j} = s_j^{m'}, \text{ for } m' \text{ output of } j.$$
In this way, for example, a (monomolecular) reaction $j$ with input $m_1$ and output $m_2$, 
\[
1 \cdot m_1 \rightarrow_j 1 \cdot m_2,
\]

translates into the $j$-th column of the stoichiometric matrix $S$ as

\[
S^j = \begin{pmatrix}
m_1 \\
m_2 \\
m_3 \\
... \\
m_M
\end{pmatrix} \begin{pmatrix}
-1 \\
1 \\
0 \\
...
\end{pmatrix}.
\]

Throughout the work, we assume the existence of a dynamical equilibrium $x^*$, that is:

\[
0 = f(x^*) := Sr(x^*) + F.
\]

Note that, in general, the equilibrium $x^*$ depends on $F$.

However, all the work here is based solely on an analysis of derivatives and Jacobian matrices. Consequently, we will not make further use of the constant vector $F$, at all. In fact, any constant factor disappears once differentiated, and hence it does not play a role in our analysis. For this reason, and uniquely for the purposes of this work, we may consider $F$ to be 0, once we have assumed a priori the existence of an equilibrium, and we shall think no more about feed reactions. We will proceed in this way, by considering $F = 0$.

The Jacobian matrix of the network at an equilibrium $x^*$ reads:

\[
f_x = SR := G,
\]

where the reactivity matrix $R$ of partial derivatives is a $N \times M$ matrix, whose entries $r_{jm}$ are given by:

\[
r_{jm} := \frac{\partial}{\partial x_m} r_j(x^*_m).
\]

As a crucial further assumption, we assume monotonicity of reaction rates (monotone kinetics). That is, we consider the partial derivatives $r_{jm}$ as positive given parameters, in the sense explained here. In fact: we stress the meaning of the word parameters, with the following last assumption. At a fixed dynamical equilibrium $x^*$, we consider the value $r_j(x^*)$ and $r_{jm}(x^*)$ to be possibly chosen independently from each other, for any reaction $j$ and metabolite $m$.

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 given values $x$, and for any $j$ and $m$. In contrast, Michealis-Menten kinetics and Langmuir-Hinshelwood kinetics satisfy our independence assumption.

For further and extensive reading on chemical kinetics, see [6]. For previous work based on this ‘genericity’ assumption see [4,8,20,23–25].
Remark 1. We may consider as well a matrix of the type of $G$ as a general linear algebra object, defined as follows:

Let $S$ be any $M \times N$ ($M$ rows $\times$ $N$ columns) real matrix (entries $S_{mj} \in \mathbb{R}$).

Let us define $S^{-}$ as the negative sign-pattern of $S$. That is:

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

with $r_{mj}$ strictly positive symbolic entry.

Let now $G$ be the $M \times M$ matrix defined by

$$G := S(S^{-})^T.$$

In fact, note how the algebraic form of this abstract $G$ and of Jacobian matrices $SR$ coincide.

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 dimension, and a change of sign of the determinant hints therefore to a change of stability.

In particular, the leading question of this work 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?

A Jacobian determinant of fixed sign excludes certain kind of bifurcation phenomena, such as, for example, saddle-node bifurcations. In the continuation of this work we provide answers to this question.

The work is organized as follows: Section 2 introduces the main tools and states the main results. We develop some further arguments about eigenvalues and bifurcations analysis in Section 3.

This work wants to be a contribution for applications in metabolic network theory, mainly. For this reason, we have left a more general version of Theorem 2.3 in Appendix A and some computational considerations in Appendix B. Appendix C concludes the work with an example of an applied application for the central metabolism of E.Coli.

Works in an analogous direction have been pursued by many people, see for a Chemical/Metabolic prospective [1][8][19] and for a purely linear algebra approach [5][17][18], among others.
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2. Corpus

We introduce in Subsection 2.1 some tools and definitions. We provide motivating examples in Subsection 2.2. The main result is contained in Subsection 2.3. We conclude with some applications in Subsection 2.4.

2.1. Cauchy-Binet analysis via Child Selections. The first definition, due to Brehm and Fiedler [4], is crucial for the entire work.

Definition 1 (Child Selection). A Child Selection is an injective map \( J : M \rightarrow E \), which associates to every metabolite \( m \in M \) a reaction \( j \in E \) such that \( m \) is an input (mother) metabolite of reaction \( j \).

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

The analysis, which uses primarily Cauchy-Binet formula, is developed from a previous result by Brehm and Fiedler contained in [4].

Proposition 2.1. Let \( G \) be the Jacobian matrix of a MCRN, in the above settings. Then:

\[
\det G = \sum_J \det S^J \cdot \prod_{m \in M} r_{mJ(m)},
\]

where \( S^J \) is the matrix whose \( i \)th column is the \( J(i) \)th column of \( S \).

Proof. We apply Cauchy-Binet formula on \( G = SR \) to obtain:

\[
\det G = \sum_{|\xi| = M} \det S^\xi \cdot \det R^\xi
\]

\[
= \sum_{|\xi| = M} \det S^\xi \left( \prod_{m \in M} \text{sgn}(\pi) \cdot \prod_{m \in M} r_{m\pi(m)} \right).
\]

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

Now,

$$\sum_{|E|=M} \det S^E \left( \sum_{\pi} \text{sgn}(\pi) \cdot \prod_{m \in M} r_{m \pi(m)} \right) = \sum_{E=J(M)} \det S^E \left( \sum_{J} \text{sgn}(J) \cdot \prod_{m \in M} r_{m J(m)} \right) = \sum_{J} \det S^J \cdot \prod_{m \in M} r_{m J(m)}. $$

Last step is the observation:

$$\det S^{E=J(M)} \cdot \text{sgn}(J) = \det S^J. $$

Remark 2. Note that, by construction, $S^J_{mm} < 0$, for any $m$.

Remark 3. If there are no Child Selections, at all, then $\det(G) \equiv 0$ for any choice of parameters $r_{jm}$. As already noted in [4], Proposition 2.1 implies that the Jacobian determinant of $G$ is *algebraically nonzero* if and only if there exists at least one Child Selection $J$ such that $\det S^J \neq 0$. Here, *algebraically nonzero* means as a multilinear homogenous polynomial in the variables $r_{jm}$.

We proceed with a classification for Child Selections, according to the sign of the determinant $\det S^J$.

**Definition 2.** Let $J$ be a Child Selection. We say that $J$ well-behaves if $\text{sign}(\det S^J) = (-1)^{M}$. We say that $J$ bad-behaves if $\text{sign}(\det S^J) = (-1)^{M-1}$. If $\det S^J = 0$, we say that $J$ zero-behaves.

The choice of the terminology has been done carefully. In fact, in a metabolic network context, large classes of Child Selections well-behave, as we will see later. See preliminary examples of Section 2.2.

With Definition 2, this straightforward Corollary to Proposition 2.1 is derived:

**Corollary 2.2.** $\det G$ is of fixed sign if and only if there are no two Child Selections $J_1$ and $J_2$ such that $J_1$ well-behaves and $J_2$ bad-behaves.

The moral corollary, roughly speaking, is that for given real examples of networks, the Jacobian is of fixed sign if there are no bad Child Selections, i.e., Child Selections that bad-behave. In this sense, the presence of bad Child Selections is a strong indication for a possible change in the sign of the determinant, in a metabolic network context.
2.2. Preliminary examples.

2.2.1. Good Child Selections. Here we list three examples of Child Selections which well-behave.

**Example G1: Monomolecular Child Selections**

A monomolecular reaction is a reaction of the form:

\[
\begin{align*}
  m^i_{j} \rightarrow m'^i_{j},
\end{align*}
\]

where one single metabolite input \(m\) is converted into another single metabolite output \(m'\).

Monomolecular Child Selections never bad-behaves. Indeed, in the monomolecular case, any \(i\)-th column of \(S_j\), corresponding to reaction \(J(m_i)\) has at most two nonzero entries: the diagonal entry \(S_{ii}^j = -1\) and another single nonzero entry +1, corresponding to the metabolite \(m'_i\) product of the reaction. Exception is only the case of an outflow exit reaction from metabolite \(m_i\), which has stoichiometrically associated a negative unit vector \(-e_i\). In this case the corresponding column of \(S^j\) has one single nonzero entry, negative.

We can see \(S^j\) as the incidence matrix of a directed graph and \(\det S^j \neq 0\) holds if and only if the directed graph is acyclic.

If \(\det S^j \neq 0\) we can implement Gaussian elimination to obtain the diagonal matrix \(-Id\), which carries the same determinant. Alternatively and more abstractly, Gershgorin disk Theorem (see [10] and Section 3) guarantees that all eigenvalues are negative, therefore:

\[
\text{sign}(\det S^j) = (-1)^M.
\]

In particular, the Jacobian of a monomolecular network is *always of fixed sign*.

**Example G2: acyclic Child Selection**

\[
\begin{align*}
  S_j = \begin{bmatrix}
    1 & 0 & 0 \\
    0 & -1 & 0 \\
    0 & 1 & -1 \\
  \end{bmatrix}, \quad \det S^j = (-1)^3 = -1.
\end{align*}
\]
Example G3

\[
\begin{align*}
S^J = & \begin{bmatrix}
A & J(A) & J(B) & J(C) \\
B & -1 & 1 & 0 \\
C & 1 & 0 & -1 \\
\end{bmatrix}, \\
\text{det } S^J &= (-1)^3 = -1.
\end{align*}
\]

2.2.2. Bad Child Selections. Here we list three examples of Child Selections which bad-behave.

Example B1: Autocatalysis

Didn’t we exclude autocatalysis from our analysis? Yes, we had excluded explicit autocatalytic reactions such as, for example,

\[
A \xrightarrow{j} 2A.
\]

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:

\[
\begin{align*}
S^J &= \begin{bmatrix}
A & J(A) & J(B) \\
B & -1 & 2 \\
\end{bmatrix}, \\
\text{det } S^J &= (-1)^2 = -1.
\end{align*}
\]
Example B2: Inverse of Example G3
We take above example G3 and invert orientation of all reactions. We obtain the following Child Selection:

\[
J(A) \quad J(B) \quad J(C)
\]

(25)

This Child Selection, as opposite in sign to Example G3, bad-behaves.

\[
S^J = \begin{bmatrix}
A \quad -1 & 0 & 1 \\
B \quad 1 & -1 & 0 \\
C \quad 1 & 1 & -1
\end{bmatrix}, \quad \text{det} S^J = (-1)^{3-1} = +1.
\]

Example B3

(27)

\[
S^J = \begin{bmatrix}
A \quad -1 & 0 & 0 & 0 & 0 \\
B \quad -1 & -1 & 0 & 0 & 0 \\
C \quad 0 & -1 & -1 & 1 & 0 \\
D \quad 1 & 0 & 0 & -1 & 0 \\
E \quad 0 & 1 & 0 & 0 & -1
\end{bmatrix}, \quad \text{det} S^J = (-1)^{5-1} = +1.
\]
2.3. **Main Theorem.** Although our results apply to general matrices $S$ with entries in $\mathbb{R}$, we are here mainly interested in applications for metabolic networks where the entries of the stoichiometric matrix $S$ are mostly $\{-1, 0, 1\}$.

For this reason, now, we proceed in the analysis assuming that $S$ has only entries $S_{ij} \in \{-1, 0, 1\}$. In particular, the diagonal entries are $S_{ii} = -1$, for any $i$.

We will comment later in the dedicated Appendix A about a generalization for matrices with entries in $\mathbb{R}$.

In this section we make a structural analysis of $\det S^J$ in order to characterize whether a given Child Selection $J$ well-behaves or bad-behaves.

Note, however, that most importance of the result is hidden in its interpretation, see Subsection 2.4.

Leibniz expansion formula for the determinant, applied to $S^J$, reads:

\[
\det S^J = \sum_{\pi} \text{sgn}(\pi) \prod_{k=1}^{M} S^J_{\pi(k)k}.
\]

Here, again, $\pi$ indicates a permutation of $M$ elements. Let us define:

\[
E(\pi) := \text{sgn}(\pi) \prod_{k=1}^{M} S^J_{\pi(k)k},
\]

and note that $E(\text{Id}) = (-1)^M$.

Let $\pi \neq \text{Id}$ be a permutation such that $E(\pi) \neq 0$.

As a permutation, combinatorially, $\pi$ can be expressed as product of disjoint cycles $c_i$. That is,

\[
\pi = \prod_{i=1}^{\theta} c_i.
\]

For notation, we consider here only cycles with length $l > 1$. That is, fixed points of the permutation do not belong to any cycle.

**Definition 3** (odd/even-completions, odd/even-cycles). We call $\pi$ an **odd-completion** if

\[
\prod_{k: \pi(k) \neq k} S^J_{\pi(k)k} = (-1)^{\theta}.
\]

We call $\pi$ an **even-completion** if

\[
\prod_{k: \pi(k) \neq k} S^J_{\pi(k)k} = (-1)^{\theta-1}.
\]

Here, $\theta$ is the number of cycles in the permutation expansion.

If $\theta = 1$ we call the odd (resp. even)-completion an **odd** (resp. **even**)-cycle.

Given the above definition, we state the main result:
Theorem 2.3. Let $J$ be a Child Selection and let $o$ and $e$ be the number of odd and even completions, respectively. That is, $o = \#o$-completions; $e = \#e$-completions.

Then:

1. The Child Selection $J$ well-behaves if $o > e - 1$.

2. The Child Selection $J$ bad-behaves if $o < e - 1$.

3. The Child Selection $J$ zero-behaves if $o = e - 1$.

Proof. The proof follows an idea of Banaji and Craciun contained in [1].

Firstly let us note the following:

$$
\det S_J(-1)^M = \det S^J \cdot E(Id) = \sum_{\pi} E(\pi)E(Id)
$$

Therefore, contributions to the determinant are given by the expressions $E(\pi)E(Id)$ for $\pi \neq Id$ and $E(\pi) \neq 0$.

We write the following lines forgetting for a bit that $S^J_{ij} = \{0, 1, -1\}$, because we want to underline the symbolic relation, which holds also for general matrices.

Let $h$ be the number of elements $k$ such that $\pi(k) \neq k$. That is, $h$ is the number of elements of $\pi$ which belongs to a permutation cycle.

$$
E(\pi)E(Id) = \text{sgn}(\pi) \prod_{k=1}^{M} S^J_{\pi(k)k} \cdot \text{sgn}(Id) \prod_{k=1}^{M} S^J_{kk}
$$

$$
= \prod_{k: \pi(k) = k} (S^J_{kk})^2 \cdot \prod_{i=1}^{\theta} \text{sgn}(c_i) \cdot \prod_{k: \pi(k) \neq k} (S^J_{\pi(k)k}S^J_{\pi(k)k})
$$

$$
= (-1)^h \prod_{i=1}^{\theta} \text{sgn}(c_i) \prod_{k: \pi(k) \neq k} S^J_{\pi(k)k}
$$

Steps above are made by noting that $(S^J_{kk})^2 = 1$, for any $k$ and that, for a cycle $c$ of length $l$, $\text{sgn}(c)(-1)^l = -1$.

We conclude the proof by observing that $(-1)^\theta \prod_{k: \pi(k) \neq k} S^J_{\pi(k)k} = 1$ ($-1$, respectively) if $\pi$ is an odd-completion (even-completion, respectively). This yields to the identity:

$$
\det S^J(-1)^M = 1 + o - e,
$$

which proves the Theorem. \qed
2.4. Interpretation of the result.

2.4.1. The Species-Reaction Bipartite Graph. Introduced by Craciun and Feinberg in [7], the Species-Reaction Bipartite Graph (SR-graph) is a bipartite graph which is constructed from a general network graph by associating to each reaction a vertex. See Figure 1 for a comparison between different kinds of representation graphs for the same network.

More in detail, a SR-graph is a bipartite graph, with vertices of two kinds: \( s \) and \( r \). Here, vertices \( s \) correspond to \textit{species} or \textit{metabolites} and vertices \( r \) correspond to reactions.

The undirected edges are always adjacent to a \( s \)-vertex and a \( r \)-vertex.

Given a stoichiometric matrix \( S \), the metabolite rows \( A, B, C, ... \) correspond to the \( s \)-vertices. The reaction columns \( 1, 2, 3, .... \) correspond instead to the \( r \)-vertices. The undirected edges are in 1-to-1 relation with the nonzero entries of the matrix. In this sense it is natural to categorize them as \textit{positive} edges and \textit{negative} edges.

Let us fix a Child Selection \( J \). A negative edge \( e \) is \textit{given by the identity} if \( e \) corresponds to the nonzero entry \( S_{mJ}(m) \) for some \( m \). In other words, for a fixed Child Selection \( J \), a negative edge \( e \) is \textit{given by the identity} if the corresponding entry of \( S^J \) lies on the diagonal.

Considering generic square matrices \( S^J \), indeed, an entry is \textit{given by the identity} if it lies on the diagonal. That is, it belongs to the expansion term of the Leibniz formula associated to the identity permutation.

Naturally, we say that a set of edges \( \{e_1, ..., e_n\} \) is \textit{given by the identity} if there exists one Child Selection \( J \) such that \( e_i \) correspond to the entry \( S_{mJ(m_i)} \) for \( \{m_1, ..., m_n\} \).

2.4.2. Completion Cycles and correspondence to Permutation Cycles.

\textbf{Definition 4.} A completion cycle in the SR-Graph of a fixed Child Selection is a cycle of length \( 2l \), \( l \leq m \), such that \( l \) edges are \textit{given by the identity}.

Equivalently, a completion cycle is a cycle in the SR-Graph of length \( 2l \), such that \( l \) edges given by the identity alternate with \( l \) elements not given by the identity.

\textbf{Proposition 2.4.} There is 1-to-1 correspondence between completion cycles and permutation cycles.

\textit{Proof.} Without losing our generalities, let us assume that \( \pi = c_1 \) (single cycle) and let us consider the expression in the computation \( 35 \) in the proof of Theorem 2.3

\begin{equation}
\prod_{k \in \{k\}} S^{J}_{c_1(k)k} S^{J}_{kk}.
\end{equation}

Note that diagonal elements \( S^{J}_{kk} \) and \( S^{J}_{c_1(k)c_1(k)} \) represents edges given by Identity. \( S^{J}_{c_1(k)k} \) shares same column (i.e., reaction vertex) with \( S^{J}_{kk} \) and same row (i.e., reaction vertex) with \( S^{J}_{c_1(k)c_1(k)} = S^{J}_{kk} \).

Following the order of the cycle \( c_1 \) in Expression \( 37 \) leads to the desired identification. \( \square \)
### Figure 1

For two Examples of Child Selections, four different ways of representation: Biological, SR-Graph (in a biological shape), SR-graph (in a combinatorial shape), Matrix. Note that, for a labeled case, the four representations are equivalent. In the SR-graphs, negative edges given by the identity are indicated with a dotted-dashed line, the sparse dotted line indicates negative edges not given by the identity, the continuous line indicates positive edges. In the combinatorial shape, the edges given by the identity are the horizontal ones. Example 1 possesses two Completion Cycles: \( c_1 = A - J(A) - C - J(C) - A \) and \( c_2 = A - J(A) - B - J(B) - C - J(C) - A \), both even. Example 2 possesses only one odd Completion Cycle: \( c = D - J(D) - E - J(E) - D \).

\[ J(A) \quad J(B) \quad J(C) \\
A \quad -1 \quad 0 \quad 1 \\
B \quad 1 \quad -1 \quad 0 \\
C \quad 1 \quad 1 \quad -1 \\
\]

#### Table

| Examples | BIOLOGICAL | SR-GRAPH | SR-GRAPH | MATRIX |
|----------|------------|-----------|-----------|--------|
|          | Biological | Combinatorial |          |        |
| 1        | ![Diagram](image1.png) | ![Diagram](image2.png) | ![Diagram](image3.png) | ![Matrix](image4.png) |
| 2        | ![Diagram](image5.png) | ![Diagram](image6.png) | ![Diagram](image7.png) | ![Matrix](image8.png) |

**2.4.3. Corollaries for applications.** Here, we summarize some straightforward consequences of Theorem 2.3. This list is intended only to exemplifying how to use the Theorem.

**Corollary 2.5** (Examples of application). The following statements hold true:

1. Acyclic Child Selections well-behave;
2. A Child Selection containing a single o-cycle well-behaves;
3. A Child Selection containing a single e-cycle zero-behaves;
4. Nonzero Child Selections of a network which possesses only monomolecular reactions and a single bimolecular reaction:

\[ A + B \rightarrow C \]

well-behaves.
Proof. Here we only sketch the proofs, as they are completely straightforward.
1-3) Firstly, note that the absence of cycles implies absence of completions. Then check via Formula \[36\].
4) We have seen in preliminary Example \(G1\) that nonzero monomolecular Child Selections well-behaves.
Now let us consider a monomolecular case with the addition of one single bimolecular reaction as \[38\].
Observe that the ‘bimolecular’ structure of reaction \[38\] leads only to \(o\)-cycles. This observation together with the regular structure of monomolecular reactions leads to the conclusion. \(\square\)

For a real given network, our arguments may describe important biological features, such as equilibrium change of stability and bifurcations. See Section \[3\] for further investigation on bifurcations and Appendix \[C\] for an example of an application to the network of central metabolism of E.Coli.

3. Eigenvalues and bifurcations

This section is devoted to eigenvalues and bifurcations.

The general leading question here is:

\[
\text{What can we say about eigenvalues of the Jacobian matrix } G? \\
\]

More specifically:

\[
\text{When is the sign of eigenvalues independent from the choice of reaction rates?} \\
\]

This question may look deceptively analogous to the question about the sign of determinant, but eigenvalues are much more delicate topic.

As a first tool, we recall here Gershgorin disk theorem, appeared firstly in \[10\]. This elementary result provides a useful estimate on the eigenvalues of matrices.

For a given square real matrix \(A\), Gershgorin disks are defined as disks in the complex plane centered in \(A_{ii}\) with radius \(R_i = \sum_{i \neq j} |A_{ij}|\). The theorem, then, reads as follows:

**Theorem 3.1** (Gershgorin, 1931). For a given square real matrix \(A\), any eigenvalue \(\lambda_i\) lies in at least one Gershgorin disk.

Intriguing consequences follow from this theorem, in the case of MCRN, with our settings. In fact, the element on the \(m\)-th entry of the diagonal of the Jacobian matrix \(G\) is

\[
\sum_{J(m)} r_{J(m) m}, \\
\]

where \(J\) are Child Selections. The sum runs on the possible output reactions (children) of metabolite \(m\) and, in particular, the diagonal is negative. This fact implies that all Gershgorin disks are centered in the negative half-plane, independently from the choice of the reaction rates. Moreover, if a metabolite participates only in monomolecular
Two interesting corollaries of Gershgorin Theorem 3.1 can be derived, in our settings. We omit the straightforward proofs.

**Corollary 3.2.** Any eigenvalue of a monomolecular network is non positive. For a nondegenerate network, i.e. for \( \det SR \neq 0 \), any eigenvalue is strictly negative, and any possible equilibrium stable.

**Corollary 3.3.** Assume that there is an outflow from a metabolite \( m_e \), that is, \( m_e \) participates in an outflow exit reaction \( j_e \) such that \( S^{j_e} = -e_{m_e} \). Then, for any fixed choice of parameters \( r_{jm} \), with \( j \neq j_e \), there is always a choice of the outflow parameter \( r_{j_e m} \) such that the associated eigenvalue \( \lambda_m \) is negative.

In particular, consider a fully open network with \( M \) metabolites, that is, such that any metabolite \( m \) participates in an outflow reaction \( j_m^m \). Then, for any fixed choice of parameters \( r_{jm} \) with \( j \neq j_m \), for any \( m \), there is always a choice of the \( M \) outflow parameters \( r_{j_m^m m} \) such that any possible equilibrium is stable.

We can now deepen the analysis and collect some more valuable observations. In Subsection 3.1 we address the case in which the eigenvalues themselves assume Expression 39. Subsection 3.2 analyzes in detail a simple example of a network with autocatalysis. The case in which the Jacobian determinant admits a factorization is briefly studied in Subsection 3.3. In the last Subsection 3.4 we give some arguments to find saddle-node type bifurcations in a region of parameters.

### 3.1. Trivial cases of eigenvalues computation

To start with, what about the case in which the computation of eigenvalues is particularly simple? In particular, we give here sufficient conditions for an eigenvalue to be of the form:

\[
\lambda_m = \sum_{j(m)} -r_{j(m)m}.
\]

We call \( \hat{m} \) a single-mother input metabolite if \( \hat{m} \) participates only in reaction of the form:

\[
s^j_{\hat{m}} \hat{m} \rightarrow s^j_1 m_1 + ... + s^j_{i} m_i,
\]

that is, if the flux through reaction \( r_j \) depends only of the concentration of metabolite \( \hat{m} \), i.e., \( r_j = r_j(\hat{m}) \).

We have then the following theorem.

**Theorem 3.4.** Let \( m \) be a single-mother input metabolite. If \( m \) does not belong to any completion cycle for any Child Selection, then the Jacobian
Matrix $G$ possesses a related eigenvalue $\lambda_m$, such that

$$\lambda_m = \sum_{J(m)} -r_{J(m)m}.$$ 

We precede the proof with the following Lemma.

**Lemma 3.5.** A single-mother input metabolite $m$ does not belong to a completion cycle (for any Child Selection) if and only if there exists a labeling $\Lambda$ of the network such that the Jacobian matrix $G$ assumes the block-triangular form:

$$G = \begin{bmatrix} P & 0 & 0 \\ \vdots & \mu & 0 \\ \vdots & \vdots & Q \end{bmatrix}$$

where $P$ and $Q$ are squared matrices (not necessarily of the same dimension) and $\mu = \sum_{J(m)} -r_{J(m)m}$.

**Proof.** Without loss of generalities, we assume that the considered network $\Gamma$ is connected. Indeed, a disconnected metabolic network consisting of $\nu$ connected components may be easily labeled such that its Jacobian is a $\nu$-block-diagonal matrix and the argument is lifted to every single connected component.

Let $a_{kh}$ denote the nonzero entries of the Jacobian of the system [9] that is

$$a_{kh} := \frac{\partial f_k}{\partial x_h} \neq 0, \text{ for any } k, h.$$ 

Above, $f_k$ is such that $\dot{x}_k = f_k(x)$ for a metabolite $m_k$ in system [9] and, in particular, $a_{kh}$ are multilinear polynomials in the variables $r_{jm}$.

Here, we use letters $h, k, p, s, ...$ instead of $m_h, m_k, m_p, m_s ...$ not to overburden the notation.

We consider the following two types of sequences of $a_{kh}$, both starting at $a_{mm}$:

1. $a_{mm}a_{mp1}a_{p1p2}...a_{p\tilde{p}}$  **Horizontal sequence**
2. $a_{mm}a_{s1m}a_{s1s}a_{s2s1}...a_{s\tilde{s}}$  **Vertical sequence**

Note that the two types of sequence are one the inverse of the other. For a finite network, the number of sequences of both kinds is also clearly finite.

The denomination horizontal/vertical comes from the fact that starting at $a_{mm}$, the first element in a horizontal sequence $a_{mp1}$ shares with $a_{mm}$ the same row, but it has different column. Analogously, the first element of a vertical sequence $a_{s1m}$ shares with $a_{mm}$ the same column, but it has different row.

In a certain analogy with previous sections, we might call these sequence completion paths. However, as a caveat, note that here we refer to nonzero entries of the Jacobian matrix $G$, while before we were referring to nonzero entries to certain reshuffled minors of the stoichiometric matrix $S$. 
As a last clarification, we underline that we do not consider the single $a_{mm}$ as a sequence.

We call, now, the set of metabolites $\{\tilde{p}\} \neq m$, for which a horizontal sequence, starting at $a_{\tilde{p}p}$ and terminating at $a_{\tilde{p}m}$, exists, the set of horizontal relatives. Analogously, the set of metabolites $\{\tilde{s} \neq m\}$, for which a vertical sequence, starting at $a_{\tilde{s}s}$ and terminating at $a_{\tilde{s}m}$ exists, is called the set of vertical relatives.

Let us underline the following two considerations:

1. These sets do not coincide, by any means, with sets of ancestors and descendants, nor mothers or children. They should carefully not be misunderstood.
2. Up to now, any of our operation is labeling-free.

The crucial observation is the following:

The metabolite $m$ belongs to a completion cycle $\iff \{\text{horizontal relatives}\} \cap \{\text{vertical relatives}\} \neq 0$.

Indeed, if the metabolite $m$ belongs to a completion cycle, then there exists a sequence of the kind $a_{mm}a_{mp_{1}}a_{p_{1}p_{1}}a_{p_{1}p_{2}}...a_{\tilde{p}_{1}p_{2}}a_{pm}a_{mm}$. By reading the same sequence in the inverse order, we obtain $a_{mm}a_{s_{1}m}a_{s_{1}s_{1}}a_{s_{2}s_{1}}...a_{\tilde{s}_{2}s_{1}}a_{s_{1}m}a_{mm}$. In particular any horizontal relative $p_{i}$ of this sequence is also a vertical relative $s_{k}$ and vice versa, which yields to $\{\text{horizontal relatives}\} \cap \{\text{vertical relatives}\} \neq 0$.

On the other way round, if we assume that there exists a horizontal relative element $p_{s}$, which is also a vertical relative, we can construct a sequence $a_{mm}a_{mp_{1}}a_{p_{1}p_{1}}a_{p_{1}p_{2}}...a_{p_{s}p_{s}}...a_{s_{2}s_{1}}a_{s_{1}s_{1}}a_{s_{1}m}a_{mm}$ and conclude that $m$ lies on a completion cycle.

Let us now construct the block $P$ of horizontal relatives and the block $Q$ of vertical relatives with a proper labeling as above. Let $i$ be the number of metabolites $p$, which are horizontal relatives of $m$. They will be freely labeled metabolite $m_{1}, m_{2}, ..., m_{i}$. Metabolite $m$ will be labeled $m_{i+1}$. Finally, the $k$ elements $s$, which are vertical relatives will be labeled $m_{i+2}, ..., m_{i+k+1}$. Crucially note that all the entries $G_{ik}$ with $i \leq i+1, k \geq i+1$ are $G_{ik} = 0$, with the only exception of $G_{i+1,i+1} = G_{mm} = 0$. If this were not the case we would be able to find a completion cycle on which $m$ is lying, against our hypothesis.

The above equivalence is hence shown, with $a_{mm} = \sum J_{(m)} - r_{j}(\tilde{m}, \bar{m})$. This concludes the proof.  

Observation 1. If $\tilde{m}$ is not a single-mother input metabolite of a reaction $j$ but $\tilde{m}$ participates together with a metabolite $\bar{m}$ to the reaction $j$, then in particular $r_{j} = r_{j}(\tilde{m}, \bar{m})$.

This implies trivially that:

\[
\begin{cases}
\dot{\tilde{m}} = -r_{j}(\tilde{m}, \bar{m}) + ... \\
\dot{\bar{m}} = -r_{j}(\tilde{m}, \bar{m}) + ...
\end{cases}
\]
Consequently, the following entries of the Jacobian matrix $G$ are nonzero: $a_{\tilde{m}\tilde{m}}$, $a_{\bar{m}\tilde{m}}$, $a_{\tilde{m}\bar{m}}$, and $\{\text{horizontal relatives}\} \cap \{\text{vertical relatives}\} \neq 0$ even if $\tilde{m}$ does not belong to any completion cycle. In particular, the entire argument of the proof above breaks down and Lemma 3.5 does not hold.

With Lemma 3.5, we are able to prove Theorem 3.4 as well. It is an easy linear algebra exercise.

**Proof of Theorem 3.4.** Since the metabolite $m$ does not lie on a completion cycle, we may apply Lemma 3.5 and have a Jacobian matrix in the form:

$$G = \begin{pmatrix} P & 0 & 0 \\ \vdots & \mu & 0 \\ \vdots & \vdots & S \end{pmatrix} \text{ with } \mu = \sum_{J(m)} -r_{J(m)m}. \tag{45}$$

The eigenvalues are those complex numbers $\lambda$ such that $\det(G - \lambda \text{Id}) = 0$. The matrix $G - \lambda \text{Id}$ reads, of course:

$$G - \lambda \text{Id} = \begin{pmatrix} P - \lambda \text{Id} & 0 & 0 \\ \vdots & \mu - \lambda & 0 \\ \vdots & \vdots & S - \lambda \text{Id} \end{pmatrix} \tag{46}$$

The matrix is block lower-triagonal and hence its determinant is equal to:

$$\det(G - \lambda \text{Id}) = \det(P - \lambda \text{Id}) \cdot (\mu - \lambda) \cdot \det(S - \lambda \text{Id}). \tag{47}$$

Obviously, $\lambda_i = \mu = \sum_{J(m)} -r_{J(m)m}$ solves the eigenvalue equation and the proposition is therefore proven.

It is now also clear when a Jacobian matrix has lower triangular form, up to relabeling. We state indeed this straightforward corollary.

**Corollary 3.6.** Let $\Gamma$ be a network consisting only of single-mother input metabolites. The Jacobian matrix $G$ of $\Gamma$ is - up to relabeling - in lower triangular form, if and only if $\Gamma$ does not contain any completion cycle. In particular, if $\Gamma$ does not contain any completion cycle, then for each eigenvalue $\lambda_m$ it holds:

$$\lambda_m = \sum_{J(m)} -r_{J(m)m}. \tag{48}$$

**Observation 2.** We underline that the $SR$-graph does not need to be acyclic in a more generalized sense.

Consider indeed the example system, always with omitted constant feeds:

$$\begin{align*}
\dot{A} &= -r_1(A) \\
\dot{B} &= r_1(A) - r_2(B) \\
\dot{C} &= r_1(A) + r_2(B) - r_3(C)
\end{align*} \tag{49}$$
Which has biological graph:
(50)

and SR-Biograph:
(51)

The graph possesses a cycle $c$, namely
(52) $c = B - 2 - C - 1 - B$.
But it is not a completion cycle since 1 is not a child of $C$, for any Child Selection.
Indeed, the Jacobian of the system is of triangular form:
(53) $G = \begin{bmatrix} -r_{1A} & 0 & 0 \\ r_{1A} & -r_{2B} & 0 \\ r_{1A} & r_{2B} & -r_{3C} \end{bmatrix}$.

3.2. Example: Autocatalysis. In this section we analyze a simple example: the simplest extension to the autocatalytic reaction of Example B1. Indeed, we add to that Child Selection an outflow exit reaction from metabolite $A$.
The goal of this analysis is to show how computing eigenvalues may be extremely difficult, as soon as we leave the safe settings of previous Section 3.1.

GRAPH:
(54)
EQUATIONS:

(55) \[
\begin{align*}
\dot{A} &= f(A, B) = -r_{1e}(A) - r_{1a}(A) + 2r_2(B) \\
\dot{B} &= g(A, B) = r_{1a}(A) - r_2(B)
\end{align*}
\]

JACOBIAN MATRIX:

(56) \[
G = \begin{bmatrix} f_A & f_B \\ g_A & g_B \end{bmatrix} = \begin{bmatrix} -r_{1e}A - r_{1a}A & 2r_2B \\ r_{1a}A & -r_2B \end{bmatrix}
\]

STOICHIOMETRIC AND REACTIVITY MATRICES:

(57) \[
S = \begin{bmatrix} 1_e & 1_a & 2 \\ A & -1 & -1 & 2 \\ B & 0 & 1 & -1 \end{bmatrix}
\]

(58) \[
R = \begin{bmatrix} 1_e & 1_a & 0 \\ A & r_{1e} & 0 \\ 1_a & r_{1a} & 0 \\ 2 & 0 & r_2 \end{bmatrix}
\]

CHILD SELECTIONS, JACOBIAN DETERMINANT AND EIGENVALUES:

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

(1) $J_{1_e} = \{J_{1_e}(A) = 1_e; J_{1_e}(B) = 2 \}$. This Child Selection well-behaves, since it is acyclic.

   Indeed:

(59) \[
S^{J_{1_e}} = \begin{bmatrix} 1_e & 2 \\ A & -1 & 2 \\ B & 0 & -1 \end{bmatrix}, \quad \det S^{J_{1_e}} = +1
\]

(2) $J_{1_a} = \{J_{1_a}(A) = 1_a; J_{1_a}(B) = 2 \}$. This Child Selection bad-behaves, since it contains one single even cycle of value $+2$ (see the generalized Theorem A.1).

   Indeed:

(60) \[
S^{J_{1_a}} = \begin{bmatrix} 1_a & 2 \\ A & -1 & 2 \\ B & 1 & -1 \end{bmatrix}, \quad \det S^{J_{1_a}} = -1
\]
This, in particular, implies that the Jacobian determinant of $G$ has undetermined sign. Indeed:

$$\det G = (r_{1eA} - r_{1aA})r_{2B}. \quad (61)$$

The determinant changes sign when $r_{1eA} = r_{1aA}$. In such a simple case, we can compute explicitly the eigenvalues $\lambda_{1,2}$. They are the roots of the characteristic polynomial $P_\lambda$:

$$P_\lambda = \lambda^2 - \text{tr} G \lambda + \det G = \lambda^2 + (r_{1eA} + r_{1aA} + r_{2B}) \lambda + (r_{1eA} - r_{1aA})r_{2B}, \quad (62)$$

and they have explicit form:

$$\lambda_{1,2} = \frac{1}{2} - (r_{1eA} + r_{1aA} + r_{2B}) \pm \sqrt{((r_{1eA} + r_{1aA} + r_{2B}))^2 - 4(r_{1eA} - r_{1aA})r_{2B}}. \quad (63)$$

One of the two eigenvalues, let’s say $\lambda_1$, is permanently negative. The other, $\lambda_2$, changes sign, as expected, when the determinant itself does. That is, at value $r_{1eA} = r_{1aA}$.

We observe here the following things:

1. A 1-parameter bifurcation happens at $r_{1eA} = r_{1aA}$: in particular, a simple eigenvalue crosses zero and the stability-type of a possible equilibrium changes. In Section 3.4 we see how to generalize this argument.
2. Even for a simple 2-dimensional case, which is not in a triangular form, the eigenvalue expression looks expectedly rather complicated.

In conclusion, we do not exclude better structural means to make claims on the signs of eigenvalues. However, for now, it remains an open question.

### 3.3. Factorizable determinant.

Proposition 2.1 implies that a (sub)network $\Gamma$ of $M$ metabolites which possesses only one single Child Selection $J$ has a Jacobian determinant of the form:

$$\det G = \det S^J \prod_{m \in M} r_{J(m)m}. \quad (64)$$

where $\det S^J$ is a real number, a scalar coefficient of the multilinear monomial $\prod_{m \in M} r_{J(m)m}$ of degree $M$ in the variables $r_{jm}$.

In particular, in this case, the determinant can be factorized in $M$ (linear) independent factors, each of those corresponding to one metabolite $m_i \in \mathbf{M}$. Note that, abstractly, the determinant can be written as product of $M$ eigenvalues, as well. In this section, we make some related considerations, which lead to further questions.
Expression \( \text{Expression 64} \) still holds true, for instance, if the network \( \Gamma \) possesses more than one Child Selection, but all those Child Selections zero-behaves, except only the Child Selection \( J \).

Let us consider now any network such that, for all Child Selection \( J \), it holds

\[
\text{(65)} \quad \det S^J \equiv (-1)^M,
\]

that is, any Child Selection carries the same determinant \( S^J \). In particular, any Child Selection well-behaves. This is, for example, the case of acyclic monomolecular reaction networks. Cfr. Example \( G1 \) of Section \( \text{Section 2.2} \).

In this case, the Jacobian determinant expansion \( \text{2.1} \) reads:

\[
\text{(66)} \quad \det G = \sum_J \det S^J \cdot \prod_{m \in M} r_{J(m)m} = (-1)^M \sum_J \prod_{m \in M} r_{J(m)m} = (-1)^M \prod_{m \in M} \left( \sum_{J(m)} r_{J(m)m} \right),
\]

that is, the determinant is factorizable in \( M \) linear subspaces, as above. Each of the subspaces depends only on a single metabolite \( m_i \), as above. In this case, each of the linear subspaces 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 two classes of networks above do not conclude all the cases in which such factorization can happen.

First of all, easily, we have discussed in Section \( \text{Section 3.4} \) when an eigenvalue \( \lambda_m \) of the system can be written as:

\[
\text{(67)} \quad \lambda_m = \sum_{J(m)} -r_{J(m)m}.
\]

If all eigenvalues can be written as above, we have that the determinant factorizes into the product of eigenvalues:

\[
\text{(68)} \quad \det G = \prod_{m \in M} \lambda_m = \prod_{m \in M} \left( \sum_{J(m)} -r_{J(m)m} \right) = (-1)^M \prod_{m \in M} \left( \sum_{J(m)} r_{J(m)m} \right).
\]

There are, anyway, much more diverse examples. Indeed, consider the following example:

\[
\text{(69)} \quad S = \begin{bmatrix}
1 & 2 & 3 & 4 & 5 & 6 \\
A & -1 & -1 & 0 & 0 & 1 \\
B & 0 & 1 & -1 & 0 & 0 & 0 \\
C & 0 & 1 & 1 & -1 & -1 & 0 \\
D & 0 & 0 & 0 & 1 & 0 & -1
\end{bmatrix}
\]
With simple computation omitted here, we check that the Jacobian determinant factorizes:

\[(70) \quad \det G = (r_{1A} - r_{2A})r_{3B}(r_{4C} + r_{5C})r_{6D}.\]

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 to saddle-node type bifurcations.

On the other hand, some extremely simple examples (even monomolecular) possess a Jacobian determinant, which does not factorize:

\[(71) \quad S = \begin{bmatrix} 1 & 2 & 3 & 4 \\ -1 & -1 & 1 & 1 \\ 0 & 1 & -1 & -1 \end{bmatrix} \]

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

Therefore, a general interesting question arises:

For which networks, the Jacobian determinant is factorizable as above?

Equivalently:

For which stoichiometric matrices \(S\) does the multilinear polynomial

\[(72) \quad P := \sum_{J} \det S^J \prod_{m \in M} r_{J(m)m} \text{ factorize into } P = \prod_{m} (\sum_{J(m)} \alpha(J, m)r_{J(m)m})?\]
where $\alpha(J,m)$ are constants depending on $J$ and $m$.

From a purely algebraic prospective, considering the space of multilinear polynomials $P$ 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. Hence, it is worth to address this issue.

These questions, in an algebraic context, have long history. In fact, they date back to late 19th century, with the groundbreaking works by Paul Albert Gordan (and Alexander von Brill) [11] in Germany and Jacques Hadamard [15] in France. In these early works, an abstract characterization of algebraic forms factorizing as above was derived. For a more updated reference, see reference book [9], Chapter 4 about Chow Varieties.

Recent investigations of similar concepts has been done by Yonghui Guan in his doctoral thesis [12] and in [13]. Here, connections have been found with the famous conjecture $P$ vs $NP$, in its algebraic version, firstly posed by Valiant [22].

We pose a last question, but very important anyway. Let us assume that the Jacobian determinant factorize as above:

$$\text{Which is the relation between the linear subspace } \sum_{J(m)} \alpha(J,m) r_{J(m)m} \text{ and the eigenvalue } \lambda_m?$$

We do not address here those questions, leaving them to future work. See for some hints the last Section 3.4 below.

3.4. Hunting saddle-node bifurcations. In this section, we develop some theoretical tools useful for a 1-parameter bifurcation analysis.

For a given network $\Gamma$, the set of Child Selections $\{J\}$ carries a natural distance $\delta$. Indeed,

**Definition 5.** Let $J_1$, $J_2$ be two Child Selections of $\Gamma$.

We define the distance $\delta(J_1, J_2)$ as the natural number of metabolites $m \in M$ s.t. $J_1(m) \neq J_2(m)$.

It is straightforward to verify that this is a distance, which we may regard as a multi-valued version of the discrete metric.

With this definition of distance, we can consider Child Selections at distance $\delta = 1$. These are Child Selections $J_1, J_2$ such that $J_1(m_b) \neq J_2(m_b)$ for a single $m_b$ and $J_1(m) = J_2(m)$ for any $m \neq m_b$ different from $m_b$.

Clearly:

$$\det S^{J_1} \prod_m r_{J_1(m)m} + \det S^{J_2} \prod_m r_{J_2(m)m}$$

$$= r_{J_1(m_1)m_1} \cdots (\det S^{J_1} r_{J_1(m_b)m_b} + \det S^{J_2} r_{J_2(m_b)m_b}) \cdots r_{J_1(m_m)m_m}$$

(73)
If we further assume that $J_1$ and $J_2$ are such that one well-behaves and the other bad-behaves we would have:

\begin{equation}
\det S^{J_1} r_{J_1}(m_b) m_b + \det S^{J_2} r_{J_2}(m_b) m_b = \alpha \cdot r_{J_1}(m_b) m_b - \beta \cdot r_{J_2}(m_b) m_b,
\end{equation}

with $\alpha$ and $\beta$ constants of the same sign.

By the mere fact that $\delta$ is a distance, any other Child Selection $J_k \neq J_1, J_2$ is at positive distance to both Child Selections $J_1$ and $J_2$, that is

\begin{equation}
\delta(J_k, J_1), \delta(J_k, J_2) \geq 1, \quad \text{for any } k \neq 1, 2.
\end{equation}

In particular, we have the following Proposition:

**Proposition 3.7.** For $J_1, J_2$ and $J_k$ Child Selections as above, there is an element $m_k$ such that: $J_k(m_k) \neq J_1(m_k)$ and $J_k(m_k) \neq J_2(m_k)$.

Moreover if $\delta(J_k, J_1) = \delta(J_k, J_2) = 1$, then $m_k = m_b$.

**Proof.** Consider any $m_k$ such that $J_1(m_k) \neq J_k(m_k)$, if $J_2(m_k) \neq J_k(m_k)$ we are done. Assume then that $J_2(m_k) = J_k(m_k)$. By construction, $m_k = m_b$ such that $J_1(m_k) \neq J_2(m_k)$.

Consider now $\tilde{m}_k$ such that $J_2(\tilde{m}_k) \neq J_k(\tilde{m}_k)$. Note that $J_1(m) = J_k(m)$ for any $m \neq m_b$. We conclude that $J_1(\tilde{m}_k) \neq J_k(\tilde{m}_k)$. Otherwise indeed we would have found two metabolites $m_k$ and $\tilde{m}_k$ such that $J_1(m_k) = J_2(m_k)$ and $J_1(\tilde{m}_k) = J_2(\tilde{m}_k)$, contradicting $\delta(J_1, J_2) = 1$.

In the above proof, note that if $J_2(m_k) = J_k(m_k)$, then $\delta(J_1, J_k) \geq 2$.

Hence, if $\delta(J_1, J_k) = \delta(J_2, J_k) = 1$ we conclude that $J_1(m_b) \neq J_2(m_b) \neq J_k(m_b)$. \hfill \Box

'Hunting bifurcations' is a very vague expression. In the past decades, research has been done in so many directions that we admittedly do not even try to give a reference list. We avoid completely the discussion about different types of 1-parameter bifurcation (saddle-node, transcritical, pitchfork). For more reference, see \[14\].

Here, we make instead an elementary argument. We give indeed a simple network condition under which there is the possibility, for certain parameters, of a saddle-node bifurcation. More in detail, we provide a bifurcation parameter responsible for the change of sign of the determinant and consequent change of stability.

For a network, let us assume that there exist two Child Selections $J_1$ and $J_2$ as above, with opposite behavior (good/bad) at distance $\delta = 1$.

If the determinant $G$ factorizes, in the sense explained in Section 3.3, we have already marked how the linear space $\sum J(m_b) \alpha(J, m_b) r_{J(m_b)m_b}$ corresponding to the metabolite $m_b$ crosses zero. The linear parameter $\mu = \sum J(m_b) \alpha(J, m_b) r_{J(m_b)m_b}$ becomes a bifurcation parameter, for any choice of other parameters.
If the determinant $G$ does not factorize, we have in particular that there exists at least one other Child Selection $J_k$. By Proposition 3.7, we can find $m_k$ such that

$$J_1(m_k), J_2(m_k) \neq J_k(m_k).$$

We can consider, then, $\varepsilon$-small choice of reaction rate parameter such that

$$r_{J_k(m_k)m_k} = o(\varepsilon).$$

We can extend the same argument to any Child Selection $\tilde{J} \neq J_1, J_2$.

Then, for this $\varepsilon$-choice of reaction rates:

$$\det G = r_{J_1(m_1)m_1} \cdot (\alpha \cdot r_{J_1(m_2)m_2} - \beta \cdot r_{J_2(m_2)m_2}) \cdots r_{J_1(m_n)m_n} + o(\varepsilon).$$

The single bifurcation parameter $\mu = \alpha \cdot r_{J_1(m_2)m_2} - \beta \cdot r_{J_2(m_2)m_2}$ becomes responsible for a sign change in the determinant of the system (up to $\varepsilon$).

We can enlarge the argument and consider any two Child Selections $\tilde{J}_1$ and $\tilde{J}_2$ at any mutual distance $\delta(\tilde{J}_1, \tilde{J}_2) = n$, with $n \leq M$. Without loosing our generalities, we can assume that the $n$ metabolites such that their selected reaction image through $\tilde{J}_1$ and $\tilde{J}_2$ differs are the first $n$. That is, $\tilde{J}_1(m_i) \neq \tilde{J}_2(m_i)$ for metabolite $m_i$, if and only if $i = 1, \ldots, n$.

In this case we can proceed in analogy as before. If we assume that $\tilde{J}_1$ well-behaves and $\tilde{J}_2$ bad-behaves, we find a parameter $\tilde{\mu} = \alpha \cdot \prod_{i=1}^{n} r_{J_1(m_i)m_i} - \beta \cdot \prod_{i=1}^{n} r_{J_2(m_i)m_i}$ responsible for a sign change in the determinant of the system (up to $\varepsilon$).

We have just proven the following conclusive result of this work:

**Theorem 3.8 (Change of Stability).** Let $\Gamma$ be a network. If there exist two Child Selections $J_1$, $J_2$ such that $\delta(J_1, J_2) = n$ and one well-behaves and the other bad-behaves, then there exists a choice of reaction rates such that the sign of the Jacobian determinant of $G$ is driven by the bifurcation parameter (up to $\varepsilon$):

$$\mu = \alpha \cdot \prod_{i=1}^{n} r_{J_1(m_i)m_i} - \beta \cdot \prod_{i=1}^{n} r_{J_2(m_i)m_i}.$$  

**Remark 4.** If $\delta(J_1, J_2) = 1$, the parameter $\mu = \alpha \cdot r_{J_1(m_2)m_2} - \beta \cdot r_{J_2(m_2)m_2}$ reads particularly elegant. Indeed, it is localized in a single metabolite $m_2$. The change of stability is then driven by the difference between the derivatives with respect to $m_2$ of the reaction rates of two children reaction of $m_2$ itself. This suggests a simple biological scheme for controlling stability of the equilibrium.

**Remark 5.** Even in the favorable case of $\delta(J_1, J_2) = 1$, it is not straightforward to infer a saddle-node type bifurcation, in the settings of Theorem 3.8. In fact, we would need to show that one simple eigenvalue crosses zero as the above parameter $\mu$ does. We have seen in the autocatalytic Example 3.2 how this is the case, for this concrete example. In this sense, for certain restricted classes of networks, it might be that Theorem 3.8 reads as a sufficient condition for a saddle node type bifurcation to happen. However, we do not address this question in this work.
Remark 6. The mere existence of two Child Selections with opposite behavior good/bad at a distance $\delta > 1$ does not always imply the existence of two Child Selections at distance $\delta = 1$. We illustrate this in the following concluding example:

$$S = \begin{bmatrix} 1 & 2 & 3 & 4 \\ -2 & -1 & 1 & 2 \\ 1 & 1 & -1 & -1 \end{bmatrix},$$

In this abstract example there are four Child Selections:

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

$J_{13}$ well-behaves, $J_{14}$, $J_{23}$ zero-behave, and $J_{24}$ bad-behaves. Note that $\delta(J_{13}, J_{24}) = 2$ and their behavior is opposite. However, all other Child Selections ($J_{14}$ and $J_{23}$) zero-behave. Therefore we cannot find two Child Selections with opposite behavior at distance $\delta = 1$.

APPENDIX A. GENERAL FORM OF THEOREM 2.3

For any given $M \times M$ matrix $S$, the Leibniz expansion formula for the determinant reads:

$$\det S = \sum_\pi \sgn(\pi) \prod_{i=1}^M S_{\pi(i)i}.$$

This formula should be considered here an ‘upper bound’ to any generalization of Theorem 2.3. This Theorem is indeed only a look upon this formula from an applied point of view of network theory.

Although most stoichiometric entries in metabolic networks are $\{-1,0,+1\}$, it is worth to provide a more general version of Theorem 2.3. We do it here.

Let $S^J$ be a real $M \times M$ matrix such that $S^j_{ii} < 0$ for any $i$.

Firstly, let us generalize naturally the definition of odd/even-completion as follows:

**Definition 6** (odd/even-completions, odd/even-cycles - General form). In the generalities as above, let $\pi = \prod_{k=1}^\theta c_i \neq \text{Id}$ be a permutation such that $E(\pi) = \sgn(\pi) \prod_{k=1}^M S^J_{\pi(k)k} \neq 0$.

We call $\pi$ an **odd-completion** if
We call \( \pi \) an **even-completion** if

\[
\text{sign}\left(\prod_{k=\pi(k) \neq k} S_{\pi(k)k}^J\right) = (-1)^{\theta}.
\]

(82)

With \( \theta \) being the number of cycles in the permutation expansion. If \( \theta = 1 \) we call the odd(resp. even)-completion an **odd**(resp. **even**)-cycle.

For a given permutation \( \pi \neq \text{Id} \) such that \( E(\pi) \neq 0 \), let the **value** of \( \pi \) be

\[
\text{val}(\pi) = \prod_{k=\pi(k) \neq k} \frac{|S_{\pi(k)k}^J|}{|S_{kk}^J|}.
\]

(84)

In particular, note that:

\[
\text{val}(\pi) = \prod_{i=1}^{\theta} \text{val}(c_i).
\]

(85)

The general version of Theorem 2.3 reads as follows:

**Theorem A.1** (General version). Let \( J \) be a Child Selection.

Let \( \tilde{o} \) be

\[
\tilde{o} = \sum_{\pi_o \text{ odd}} \text{val}(\pi_o)
\]

(86)

and let \( \tilde{e} \) be

\[
\tilde{e} = \sum_{\pi_e \text{ even}} \text{val}(\pi_e).
\]

(87)

Then:

1. The Child Selection \( J \) well-behaves if \( \tilde{o} > \tilde{e} - 1 \).
2. The Child Selection \( J \) bad-behaves if \( \tilde{o} < \tilde{e} - 1 \).
3. The Child Selection \( J \) zero-behaves if \( \tilde{o} = \tilde{e} - 1 \).

\textbf{Proof.} The proof is highly analogous as the proof of Theorem 2.3.

The only difference is that we start here with a ratio argument instead of the product argument \( \det S^J \cdot E(\text{Id}) \) of Theorem 2.3. Indeed:

\[
\frac{\det S^J}{E(\text{Id})} = 1 + \sum_{\pi=\text{Id}} \frac{E(\pi)}{E(\text{Id})}
\]

(88)
Now:
\[
\frac{E(\pi)}{E(Id)} = \frac{\text{sgn}(\pi) \prod_{k=1}^{M} S_{\pi(k)k}^{\pi}}{\prod_{k=1}^{M} S_{kk}^{\pi}} = \prod_{k=1}^{\theta} \text{sgn}(c_i) \prod_{k=1}^{\theta} S_{\pi(k)k}^{\pi}
\]
(89)
\[
= (-1)^h \prod_{k=1}^{M} \frac{S_{\pi(k)k}^{\pi}}{S_{kk}^{\pi}} = (-1)^{\theta/2} \text{val}(\pi).
\]

Where \( h \) is the number of elements of \( \pi \) that belongs to a cycle and \((-1)^{\theta/2}\) is 1 if \( \pi \) is odd and \(-1\) if \( \pi \) is even.

These leads to the desired equality:
(90) \[
\text{det} S = 1 + \hat{o} - \hat{e},
\]
which proves the Theorem.

\[\square\]

Remark 7. Of course, we could have started also the proof of Theorem 2.3 with the same ratio argument. Indeed, for \( S_{ij} = \{-1, 0, +1\} \), it is precisely the same. In our opinion, however, the product argument illustrates better the concepts leading to SR-graph and completion cycles. For that reason we have chosen that way in the first place.

Appendix B. Computational aspects

In this section we state a result, which simplifies the computation.

Let \( \pi \) be a permutation with \( \pi = \prod_{i=1}^{\theta} c_i \) with \( \theta \geq 2 \). Let \( \pi' \subseteq \pi \), that is, all \( c_i \) cycles of \( \pi' \) are also cycles of \( \pi \).

We are concerned here with the following question:

**It is always necessary to compute all \( E(\pi) \), independently one to each other?**

The following proposition provides an answer, under absolutely feasible assumptions for the intended case of application.

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

1. **If there exists at least one cycle \( c_i \) satisfying:**
   - (a) \( c_i \) is an even cycle;
   - (b) \( \text{val}(c_i) = 1 \).
   
   then,

   (91)
   \[
   \sum_{\pi' \subseteq \pi} \frac{E(\pi)}{E(Id)} = -1.
   \]

2. **If all cycles \( c_i \) satisfy:**
   - (a) \( c_i \) is an odd cycle;
   - (b) \( \text{val}(c_i) = 1 \).
   
   then,

   (92)
   \[
   \sum_{\pi' \subseteq \pi} \frac{E(\pi)}{E(Id)} = 2^\theta - 1.
   \]
Proof. With a little abuse of notation, we will use now $c_i$ to refer directly to $(93) \prod_{k=1}^{c_i(k)\neq k} \frac{S^J_i(k)k}{|S^J_{kk}|}$

(1) Let us assume, without losing our generalities, that $c_1 = 1$. Consider the sum $\sum_{\pi'\subseteq\pi} \frac{E(\pi)}{E(Id)}$ written in following form:

$$\begin{align*}
\theta' = 1 & \quad -1 \quad -c_2 \quad -c_3 \quad ... \quad -c_{\theta} \\
\theta' = 2 & \quad +1 \cdot c_2 \quad +1 \cdot c_3 \quad ... \quad +1 \cdot c_{\theta} + c_2 c_3 \quad ... \quad c_{\theta-1} c_\theta \\
\theta' = 3 & \quad -1 \cdot c_2 c_3 \quad ... \quad -1 \cdot c_{\theta-1} c_\theta ... \\
\theta' = ... & \quad ... \\
\theta' = \theta - 1 & \quad ... \\
\theta' = \theta & \quad (−1)^{\theta-1} c_2 \cdots c_\theta
\end{align*}$$

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

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

$$\sum_{\pi'\subseteq\pi} \frac{E(\pi)}{E(Id)} = \sum_{\theta'=1}^{\theta} \binom{\theta}{\theta'} (−1)^{\theta'} = \sum_{\theta'=1}^{\theta} \binom{\theta}{\theta'} = 2^{\theta} - 1 \quad \square$$

Remark 8. In the case in which $S_{i\theta} = \{-1, 0, +1\}$ Proposition [B.1] always applies.

We explain with the following example the use we can make of Proposition [B.1]. We consider a Child Selection $J$ with six metabolites.

The re-shuffled stoichiometric matrix $S^J$ is given by:

$$S^J = \begin{bmatrix}
A & J(A) & B & J(B) & C & J(C) & D & J(D) & E & J(E) & F & J(F) \\
-1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 1 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 0 & 0 & 0 & 1 \\
\end{bmatrix}$$

with determinant $\det S^J = (-1)^{6-1} = -1$. In particular, the Child Selection $J$ bad-behaves.
The biological graph is represented by:

\[
\begin{align*}
A & \rightarrow J(A) \\
& \rightarrow B \\
& \rightarrow C \\
& \rightarrow J(C) \\
& \rightarrow D \\
& \rightarrow E \\
& \rightarrow F \\
& \rightarrow J(F) \\
& \rightarrow J(D) \\
& \rightarrow J(E) \\
& \rightarrow J(B) \\
& \rightarrow A
\end{align*}
\]

and the SR-Biograph is:

\[
\begin{align*}
A & \rightarrow J(A) \\
& \rightarrow B \\
& \rightarrow C \\
& \rightarrow J(C) \\
& \rightarrow D \\
& \rightarrow E \\
& \rightarrow J(E) \\
& \rightarrow J(F) \\
& \rightarrow J(D) \\
& \rightarrow J(B) \\
& \rightarrow J(F) \\
& \rightarrow J(D) \\
& \rightarrow J(E) \\
& \rightarrow J(C) \\
& \rightarrow J(B) \\
& \rightarrow J(A) \\
& \rightarrow A
\end{align*}
\]

Now, we list all the six completion cycles in this Child Selection network:

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

And the entire list of permutations \( \pi_i \):

\[
\begin{align*}
(1) & \pi_{123} := c_{123} & (7) & \pi_{1,2} := c_1 \cdot c_2 \\
(2) & \pi_{12} := c_{12} & (8) & \pi_{1,3} := c_1 \cdot c_3 \\
(3) & \pi_{12,3} := c_{12} \cdot c_3 & (9) & \pi_{2,3} := c_2 \cdot c_3 \\
(4) & \pi_{23} := c_{23} & (10) & \pi_1 := c_1 \\
(5) & \pi_{1,23} := c_1 \cdot c_{23} & (11) & \pi_2 := c_2 \\
(6) & \pi_{1,2,3} := c_1 \cdot c_2 \cdot c_3 & (12) & \pi_3 := c_3
\end{align*}
\]

In this example, all stoichiometric entries \( S_{ij} \) are \( \{-1,0,1\} \). In particular, \( \text{val}(c_i) = 1 \) for any \( i \). Moreover, note that all elements not given by identity are equal to +1. Therefore we can apply point 1 of Proposition B.1, since all cycles are even cycles with \( \text{val}(c_i) = 1 \). Finally, here, \( E(\text{Id}) \equiv 1 \)

Let us consider permutation \( \pi_{1,2,3} \). Note that it contains permutations \( \pi_{1,2}, \pi_{2,3}, \pi_{1,3}, \pi_1, \pi_2, \pi_3 \).

Proposition B.1 guarantees that the following equality hold:

\[
E(\pi_{1,2,3}) + E(\pi_{1,2}) + E(\pi_{2,3}) + E(\pi_{1,3}) + E(\pi_1) + E(\pi_2) + E(\pi_3) = -1.
\]

But we still have to compute \( E(\pi_{12,3}), E(\pi_{1,23}), E(\pi_{12,3}), E(\pi_{123}), E(\pi_{12}) \), \( E(\pi_{23}) \). Of course we can argue that

\[
E(\pi_{1,23}) + E(\pi_1) + E(\pi_{23}) = -1,
\]
and proceed in this way with

\[(101) \quad E(\pi_{12,3}) + E(\pi_{12}) + E(\pi_3) = -1,\]

but we should be careful to notice that we had already computed taken in account

\[E(\pi_1) \text{ and } E(\pi_3) \text{ while using Proposition B.1 on } E(\pi_{1,2,3}).\]

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

\[(102) \quad \det S^J = 1 + \sum_{\pi \neq \text{Id}} E(\pi)
= 1 + \sum_{\pi \subseteq \pi_{1,2,3}} E(\pi) + \sum_{\pi \subseteq \pi_{1,2,3}} E(\pi) + \sum_{\pi \subseteq \pi_{1,2,3}} E(\pi) + E(\pi_{123}) - E(\pi_1) - E(\pi_3)
= 1 - 1 - 1 + 1 + 1 = -1\]

The last computation has been made using Proposition B.1 and observing that

\[c_{123}, c_1, c_3 \text{ are all even-cycle, i.e., } E(\pi_{123}) = E(\pi_1) = E(\pi_3) = -1.\]

In the above framed example, we were able to reduce a computation of 12 permutations to a computation of 3 single-cycle permutations. The argument has been supported only by an observation of the completion cycles of the network.

This is far from being an optimal account on how to handle computationally such a problem, it has been only a small and humble account on what may help.

Appendix C. A case of study: the central metabolism of E.Coli

In this section we analyze briefly the network representation of the central metabolism of E.Coli in Figure C.

This network model is mainly based on the model proposed by Ishii et al. in [16]. Moreover, Nakahigashi et al. in [21] incorporates metabolite S1,7P and adds reactions N1 and N2 to the model of [16]. Finally, 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 single children of their mother metabolite. In particular, their omission would result in an infinite production of their mother metabolites and in a mathematical degeneracy of the network since no Child Selection map would exist.

The network possesses 30 metabolites and 58 reactions. The number of Child Selections is of the order of \((10)^7\). This computationally prohibitive number may be deceptive on how our theory may apply. In particular, the theory may provide interesting biological insights even without necessarily compute such a huge amount of Child Selections. Goal
Figure 2. This figure has been taken from [4] and the graphical representation is courtesy of Anna Karnauhova. Inflow feed reaction is named $f_1$. Outflow exit reactions are labeled $d_1 − d_6$ and $dd_1 − dd_9$. Here, for image simplicity, it has been used the convention that a reversible reaction $m \leftrightarrow m'$ encodes two different opposite reactions, as explained in Section 1. Metabolites PEP, PYR and CO2 have been graphically repeated, only for sake of clarity of the picture.
of the following lines is to provide an example of its use.

The central metabolism of E.Coli consists of different and interconnected parts. In particular, the above part comprises the so-called \textit{Pentose phosphate pathway} and \textit{Glycolysis}. The bottom 'cyclic' part includes basically the \textit{Tricarboxylic acid cycle} and the \textit{Glyoxylate cycle}. We skip more detailed biological explanation.

Here, we want to study some dynamical properties of the system, by pointing out some interesting Child Selections and relying on an analogous argument to the one presented in Section 3.4. In other words, we impose certain reaction images $j$ to certain metabolites $m$, and consider the reaction rates not belonging to these 'constraints' to be $\varepsilon$-small.

For example, let us fix the image of metabolites $G6P$, $3PG$, $PEP$ and $PYR$ to be the respective exit reactions, that is:

1) $J(G6P) = dd1$;
2) $J(3PG) = dd5$;
3) $J(PEP) = dd6$;
4) $J(PYR) = dd7$.

Moreover, let us impose to metabolite $F6P$ not to choose reaction 2, that is:

5) $J(F6P) \neq 2$.

Now, note that any Child Selection $J$ satisfying the above constraints 1-5 is, in particular, a disconnected Child Selection. Indeed we have separated the upper part with the bottom part.

This shows that some qualitative arguments on the dynamics of the central metabolism may be inferred separately between (Pent. Phosph. Pathway - Glycolysis) / (TCA cycle - Glyoxylate cycle).

In particular, we see in this Appendix how it is easy to infer the following statement:

\begin{quote}
\textit{The network representation in Figure C has unfixed sign determinant.}
\end{quote}

We do this by considering only the bottom part: the TCA cycle and the Glyoxylate cycle. Indeed, with constraint 1-5 above, we have been able to select a disconnected subnetwork, whose Jacobian matrix $G$ is in particular a block-diagonal matrix, and consequently unfixed sign determinant for one block implies unfixed sign determinant for the entire matrix.

We push this argument forward, by selecting only two Child Selections in the bottom part of the network, such that one well-behaves and the other bad-behaves.

Here below, the chosen subnetwork has been depicted both in Biological form and $SR$-graph.
With this choice of Child Selection, metabolites Lactate, Acetate, and Ethanol result disconnected from the rest of the network and have hence been omitted here. Note that this subnetwork possesses only two Child Selections, depending on whether metabolite ICT chooses reaction 19 or reaction 26. Let us call $J_{19}$ the Child Selection such that $J_{19}(ICT) = 19$ and $J_{26}$ the Child Selection such that $J_{26}(ICT) = 26$. In particular, $J_{19}(m) = J_{26}(m)$ for any $m \neq ICT$ and the two Child Selections are at distance 1, i.e., $\delta(J_{19}, J_{26}) = 1$.

By considering any extension $\tilde{J}$ to a Child Selection of the entire network, such that $\tilde{J}_{19}(m) = \tilde{J}_{26}(m)$ for any $m \neq ICT$, we may lift the following argument to the entire network.

By looking at the SR-graph representation, we see that $J_{19}$ does not contain any completion cycle, and therefore in particular well-behaves. Indeed, this Child Selection contains only one network cycle $c = MAL - 23 - OAA - 17 - AcCoa - 27 - MAL$. However, $c$ is not a completion cycle as the edge $AcCoa - 27$ is not given by the identity.

On the other hand, the completion cycles structure of $J_{26}$ is identical to the one of Example B2 of Section 2.2. This Child Selection possesses only two even 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 even completion cycles, Child Selection $J_{26}$ bad-behaves.

In particular, in accordance to Theorem 3.8 the parameter

(103) \[ \mu = r_{19m} - r_{26m}, \] (where $m = ICT$),

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

We underline here a particularly interesting point, of biological relevance. The choice of reaction 19 and 26 marks, in the basics, the difference between the TCA cycle (reaction
and the Glyoxylate cycle (reaction 26).

Our analysis suggests therefore how the network structure of a cell can control certain dynamical properties of its cell metabolism. In particular it points out certain ways of control those dynamical properties.

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