SADDLE-NODE BIFURCATIONS IN CHEMICAL REACTION NETWORKS

NICOLA VASSENA

Freie Universität Berlin

ABSTRACT. Motivated by investigating multistationarity in biochemical systems, we address saddle-node bifurcations for chemical reaction networks endowed with general kinetics. At positive equilibria, we identify structural network conditions that guarantee the bifurcation behavior and we develop a method to identify the proper bifurcation parameters. As a relevant example, we explicitly provide such bifurcation parameters for Michaelis-Menten and Hill’s kinetics. Examples of application include reversible feedback cycles, the central carbon metabolism of E. Coli, and autocatalytic networks.

CONTENTS

1. Introduction 1
2. Setting 6
3. Child Selections and Partial Child Selections 7
4. Main results 9
5. Eigenvalues zero 10
6. Algebraic multiplicity 11
7. Nonlinear unfolding 11
8. Michaelis-Menten and Hill’s kinetics 12
9. Examples 15
9.1. Example I: Reversible feedback cycles 15
9.2. Example II: Glyoxylate cycle vs TCA cycle in E. Coli 16
9.3. Example III: Mass action 18
9.4. Example IV: Degenerate saddle-node for Michaelis-Menten 20
10. Discussion 21
11. Proofs 24
References 32

1. INTRODUCTION

Multistationarity is the property of a chemical system to exhibit two or more distinct equilibria, under identical conditions, and it has been proposed as an explanation for many epigenetic processes, including cell differentiation: see the groundbreaking

E-mail address: nicola.vassena@fu-berlin.de
work [38] by Thomas and Kaufman, and the many biological references therein. Hence, it is no surprise that investigating multistationarity for chemical systems has become a hot topic. See among others the works by Soulé [36], Craciun and Feinberg [13, 14], Mincheva and Roussel [28], Banaji and Craciun [6], Joshi and Shiu [25], Banaji and Pantea [7], Conradi et al. [11]. Under the restrictive assumption of mass action, see the works by Rendall and coauthors [18,21,32], Dickenstein et al. [15], Shiu and de Wolff [34], Feliu et al. [17].

One attractive mathematical possibility to detect multistationarity is to identify a saddle-node (SN) bifurcation. A bifurcation is a sudden qualitative change in the system behavior according to a small change in the parameter values. A saddle-node bifurcation occurs when two equilibria, e.g. one stable and one unstable, collide in a single saddle equilibrium and disappear. Hence, the occurrence of such a bifurcation at positive equilibria automatically implies an area of parameters with at least two positive equilibria. Saddle-node bifurcations for biochemical systems, under the assumption of mass action, have been attempted by Conradi et al. [12] and Domijan and Kirkilionis [16]. In both these contributions, the abstract conditions leading to the bifurcation have been reformulated in the polynomial language of mass action. Otero–Muras and coauthors used computational methods to detect saddle-node bifurcations in biochemical systems, see for example [31]. Okada et al. [30] translated the bifurcation conditions from the Jacobian to an augmented matrix, which allows them to confine a potential bifurcation behavior in a certain subnetwork. To the best of our knowledge, abstract network conditions that characterize saddle-node bifurcations have not yet been obtained for systems arising from chemical reaction networks. This is the focus of the present paper.

The standard saddle-node bifurcation theorem for ordinary differential equations (ODEs) reads as follows.

**Theorem 1.1** (Saddle-node bifurcation). Let \( \dot{x} = g(x, \lambda) \) be an ODEs system in \( \mathbb{R}^M \) depending on a single parameter \( \lambda \). When \( \lambda = \lambda^* \), assume that there is an equilibrium \( \bar{x} \) for which the following hypotheses are satisfied:

(SN1) The Jacobian \( G(\bar{x}, \lambda^*) := D_x g(\bar{x}, \lambda^*) \) has an algebraically simple eigenvalue 0 with right eigenvector \( v \) and left eigenvector \( w \). \( G(\bar{x}, \lambda^*) \) has \( \kappa \) eigenvalues with negative real part and \( (M - \kappa - 1) \) eigenvalues with positive real parts (counting multiplicity).

(SN2) \( \langle w, \partial_{\lambda} g(\bar{x}, \lambda^*) \rangle \neq 0 \)

(SN3) \( w^T \partial^2 g(\bar{x}, \lambda^*)[v,v] \neq 0 \)

Then there is a smooth curve of equilibria in \( \mathbb{R}^M \times \mathbb{R} \) passing through \( (\bar{x}, \lambda^*) \), tangent to the hyperplane \( \mathbb{R}^M \times \{\lambda^*\} \). Depending on the signs of the expressions in (SN2) and (SN3), there are no equilibria near \( (\bar{x}, \lambda^*) \) when \( \lambda < \lambda^* \) (\( \lambda > \lambda^* \) and two equilibria near \( (\bar{x}, \lambda^*) \) for each parameter value \( \lambda > \lambda^* \) (\( \lambda < \lambda^* \)). The two equilibria for \( \dot{x} = g(x, \lambda) \) near \( (\bar{x}, \lambda^*) \) are hyperbolic and have stable manifolds of dimensions \( \kappa \) and \( \kappa + 1 \), respectively. The set of equations \( \dot{x} = g(x, \lambda) \) which satisfy (SN1)-(SN3) is open and dense in the space of \( C^\infty \) one parameter families of vector fields with an equilibrium at \( (\bar{x}, \lambda^*) \) with a zero eigenvalue.
Condition (SN1) is the necessary spectral condition: an algebraically simple eigenvalue zero of the Jacobian, at an equilibrium. Conditions (SN2) and (SN3) sufficiently guarantee the proper nonlinear unfolding of the bifurcation. We call degenerate saddle-node the situation when conditions (SN1) and (SN2) are satisfied, but not (SN3). Nondegenerate saddle-node indicates then the complete case where conditions (SN1)–(SN3) hold. Moreover, we will refer to properties that hold on an open and dense subset as generic, albeit often in literature a generic set is more generally defined as a set of second Baire category \cite{3}, i.e., a countable intersection of open and dense subsets. Theorem 1.1 is stated this way by Guckenheimer and Holmes \cite{20}, without an explicit proof, for which Vanderbauwhede \cite{39} is a reference. The genericity part has also been addressed and elaborated by Sotomayor \cite{35}.

A chemical reaction turns reactants into products. Several connected reactions constitute a chemical reaction network. We investigate which networks can sustain saddle-node bifurcations, and consequently multistationarity. More precisely, to any network $\Gamma$ we associate the following ODEs dynamical system:

\[ \dot{x} = g(x) = S f(x), \]

where $x(t) > 0 \in \mathbb{R}^M$ is the vector of concentrations of the chemical species; the $M \times E$ matrix $S$ is the stoichiometric matrix, the incidence matrix of the network; $f(x) \in \mathbb{R}^E$ is the vector of the reaction functions. We stress that we consider only strictly positive concentrations $x > 0$: boundary equilibria, where some of the concentrations $x_m$ are zero, fall beyond the scope of the present work. We address and answer the following question:

For which networks $\Gamma$ there exists a choice of $f$ such that the associated dynamical system admits a saddle-node bifurcation?

The precise form of $f$ is typically unknown in applications. Therefore, it is of great interest to obtain conditions only based on the network structure. Following this precise intention, we do not prescribe any specific form to $f$ but rather look into the entire set of functions satisfying only a few meaningful assumptions that make them reasonable as reaction functions, according to the following definition.

**Definition 1 (monotone chemical functions).** Let $j$ be a reaction and $f_j$ the associated reaction function. We call $f_j$ chemical if

1. $f_j$ depends only on the concentrations of the reactants of the reaction $j$;
2. $f_j$ is positive, i.e., $f(x) > 0$, for every $x > 0$.

We call a chemical function $f_j$ monotone if

3. $f'_{jm}(x) := \frac{\partial f_j(x)}{\partial x_m} > 0$, for any species $m$ reactant of $j$ and $x > 0$.

Widely used kinetic schemes as mass action \cite{23}, Michaelis–Menten \cite{27}, and Hill’s kinetics \cite{22} follow Definition 1.

We address the bifurcation conditions symbolically. For a related approach in bifurcation analysis on networks, see the work by Fiedler \cite{19} that concerns global Hopf bifurcation. This symbolic strategy relates to the theory of jets \cite{2}. Aiming at a self-contained
presentation, we proceed from scratch. We call \( r \in \mathbb{R}^M \) the equilibria conditions that \( f(\bar{x}) \) has to satisfy, at an equilibrium \( \bar{x} \). The equilibria constraints simply read
\[(2) \quad S r = 0, \quad \text{with } r_j > 0 \quad \text{for every } j.\]

In particular, \( r \) is any positive right kernel vector of the stoichiometric matrix \( S \). Throughout the paper, we only consider networks whose stoichiometric matrix \( S \) admits a positive right kernel vector, i.e., admitting an equilibrium for a certain choice of chemical functions \( f \). Without this basic assumption, addressing equilibria bifurcations would be meaningless. On the other hand, the bifurcation constraints concern derivatives. We use the notation
\[r' = \{r'_{jm}\}_{j \in E, m \in M}\]
for the values, which the nonvanishing first derivatives \( f'_{jm}(\bar{x}) \) attain at the bifurcating equilibrium \( \bar{x} \). Analogously, we use the notation
\[r'' = \{r''_{jmn}\}_{j \in E, m, n \in M}\]
for the values of the second derivatives \( f''_{jmn}(\bar{x}) := \frac{\partial^2 f_j}{\partial x_m \partial x_n}(\bar{x}) \).

Firstly, we address symbolically conditions (SN1) and (SN2) in terms of the values \( r' \) alone. Secondly, we address the condition (SN3) in terms of the values \( r'' \) alone. Finally, if conditions (2) and (SN1)–(SN3) are satisfied by an independent choice \( (\bar{r}, \bar{r}', \bar{r}'') \), we find proper \( f \) such that
\[(3) \quad f(\bar{x}) = \bar{r}, \quad f'_{jm}(\bar{x}) = \bar{r}'_{jm} \quad \text{and} \quad f''_{jmn}(\bar{x}) = \bar{r}''_{jmn},\]
for a positive equilibrium value \( \bar{x} \) and any \( j, m, \) and \( n \).

In this sense, we say that a chemical network \( \Gamma \) admits a saddle-node bifurcation if there is a choice of \( f(x, \lambda) \), within the class of monotone chemical functions, such that the assumptions of Theorem 1.1 hold. Of course, proving independently the conditions in terms of \( (r, r', r'') \) always implies the existence of a monotone chemical function \( f \) for which all the bifurcation conditions (SN1)–(SN3) are satisfied, at any positive \( \bar{x} \) at will: the class of monotone chemical functions is clearly wide enough to include a nonlinearity \( f \) satisfying (3). However, even in its generosity, nature may not always provide us with such a freedom of choice, and typically given parametric class of functions (kinetics) are used to model the reaction network. The validity of our results, when restricted to a certain kinetics must be further checked. In particular, we need the parametric freedom to assign independently the function value \( r \) and its first derivative value \( r' \), at least. We prove that this is possible in the parametric class of Michaelis-Menten kinetics. The only obstacle for multistationarity might reside in the tangency of the curve of equilibria, condition (SN3) of Theorem 1.1 see example 9.4. The slightly more general Hill’s kinetics already provides the parametric freedom to conclude always a nondegenerate bifurcation result, in our setting. On the contrary, polynomial mass action kinetics does not equally provide such parametric freedom. We show a mass action example undergoing a saddle-node bifurcation in 9.3, to foster discussion.
We base our results on the language of Child Selections. A Child Selection $\mathbf{J}$ is an injective map associating to each species $m$ a reaction $j$, in which $m$ participates as a reactant, see Definition 2. The Jacobian determinant of the system, $\det G$, can be expanded along Child Selections (Proposition 2.1 of [40]) as:

$$\det G = \sum_{\mathbf{J}} \alpha_{\mathbf{J}} \prod_{m \in M} r'_m (m) J_m,$$

where $\alpha_{\mathbf{J}}$ is a coefficient structurally associated to any Child Selection. Note that $\det G$ can then be interpreted as a multilinear homogenous polynomial $P(r') := \det G(r')$, considering $r' > 0$ as independent real variables. Throughout, for simplicity of presentation, we assume the existence of at least one Child Selection $\mathbf{J}$ with $\alpha_{\mathbf{J}} \neq 0$, implying

$$P(r') \neq 0.$$

This excludes a permanent eigenvalue zero of $G$ and allows us to focus directly on solving $P(r') = 0$ without considering any reduced system. This assumption also excludes conserved linear combinations of the concentrations $x_m(t)$ for the whole network and may not be restrictive in itself: for instance, many metabolites in metabolic networks have a decay outflow reaction. Our first main result characterizes the solvability of $P(r') = 0$ in terms of Child Selections.

**Theorem 4.1.** The multilinear homogeneous polynomial

$$P(r') := \det G(r')$$

has a positive root $r' > 0$ if and only if there exist two Child Selections $\mathbf{J}_1, \mathbf{J}_2$ such that

$$\alpha_{\mathbf{J}_1} \alpha_{\mathbf{J}_2} < 0.$$

Theorem 4.1 characterizes the networks admitting a singular Jacobian. As stated in 1.1, genericity of saddle-node bifurcations suggests that singular Jacobians indicate a nondegenerate bifurcation in most applications. However, [41] presents a “pathological” network whose Jacobian $G$ possesses either no or multiple eigenvalue zero, for any choice of monotone chemical functions $f$. Even if rare and unexpected, such a case must be technically excluded. The second main result, Theorem 4.2, provides a sufficient structural condition to have a saddle-node bifurcation. We define a saddle-node pair (SN-pair) of Child Selections, satisfying a further algebraic condition excluding multiple eigenvalues zero. The presence of an SN-pair of Child Selections in the network guarantees the bifurcation behavior. Theorem 4.2 essentially reads: If the network possesses an SN-pair of Child Selections, then the network admits a saddle-node bifurcation. The bifurcation parameter $\lambda$ is introduced parametrizing one single reaction function $f_j$, identified by an SN-pair of Child Selections.

The paper is organized as follows: Section 2 formalizes the mathematical setting, and Section 3 introduces the language of Child Selections. The main results are presented in Section 4. Sections 5, 6, 7, and 8 build up the arguments needed to prove the main results. In particular, Section 5 discusses networks possessing an eigenvalue zero; Section 6 addresses the multiplicity of such eigenvalue; Section 7 presents the unfolding of the bifurcation; Section 8 reads our results with explicit parameter choices for Hill’s and...
Michaelis-Menten kinetics, serving both as a specific example and as a general procedure on how to implement our results in given dynamical models. Section 9 lists four examples: 9.1 a network motif giving rise to saddle-node bifurcation; 9.2 a saddle-node bifurcation identified in the central carbon metabolism of *E. Coli*; 9.3 a mass action example; 9.4 an example of a network that admits only a degenerate saddle-node when endowed with Michaelis-Menten. Section 10 concludes the paper with the discussion. Section 11 lists all proofs.

Acknowledgments We are deeply indebted to Bernold Fiedler for many inspiring discussions. Jia-Yuan Dai helped improve the paper with useful comments. This work has been supported by the Collaborative Research Center 910 of the Deutsche Forschungsgemeinschaft, project A4.

2. Setting

A chemical reaction network $\Gamma$ is a pair of sets $\{M, E\}$: $M$ is the set of chemical species or metabolites, and $E$ is the set of reactions. Both sets are finite with cardinalities $|M| = M$ and $|E| = E$. Letters $m, n \in M$ and $j, h \in E$ refer to species and reactions, respectively.

A reaction $j$ is an ordered association of two positive linear combinations of species:

$$j : \ s^j_1 m_1 + \ldots + s^j_M m_M \rightarrow \bar{s}^j_1 m_1 + \ldots + \bar{s}^j_M m_M.$$

The nonnegative coefficients $s^j, \bar{s}^j$ are called stoichiometric coefficients. Chemical networks deal with integer stoichiometric coefficients, but we can consider real $s^j_m, \bar{s}^j_m \in \mathbb{R}_{\geq 0}$. The reactants (resp., products) of the reaction $j$ are the species appearing at the left (resp., right) of (5) with nonzero stoichiometric coefficient. Chemical systems are often open systems: inflow reactions are then reactions with no reactants ($s^j_m = 0$ for every $m$) and outflow reactions are reactions with no products ($\bar{s}^j_m = 0$ for every $m$). The $M \times E$ stoichiometric matrix $S$ is the matrix of all ordered stoichiometric coefficients:

$$S_{mj} := \bar{s}^j_m - s^j_m.$$

This way we assign a fixed order to each reaction: we model a reversible reaction

$$j : \ A + 2B \leftrightarrow A + 2C$$

simply as two irreversible reactions

$$j_1 : \ A + 2B \rightarrow j_1 A + 2C \quad \text{and} \quad j_2 : \ A + 2C \rightarrow j_2 A + 2B.$$

We use the notation $S^j$ for the column of the stoichiometric matrix $S$ associated to the reaction $j$. For example, in a network of four species $\{A, B, C, D\}$, reaction $j_1$ in (7) is
represented as the $j_1^{th}$ column of the stoichiometric matrix $S$ as

$$S^{j_1} = \begin{pmatrix} A & 0 \\ B & -2 \\ C & 2 \\ D & 0 \end{pmatrix}.$$ 

Let $x \geq 0$ be the $M$-vector of chemical concentrations. Under the assumption that the reactor is well mixed, spatially homogeneous, and isothermal, the dynamics $x(t)$ of the concentrations satisfy the following system of ODEs:

$$\dot{x} = g(x) := S f(x),$$

where $S$ is the $M \times E$ stoichiometric matrix (6) and $f(x)$ is the $E$-vector of the reaction functions. Without any reactant, we consider as constant the reaction function of inflow reactions $j_f$:

$$f_{j_f}(x) \equiv F_{j_f}.$$ 

For any other reaction $j$, we only require that $f$ is monotone chemical, as defined in [1].

3. Child Selections and Partial Child Selections

We introduce the main tools.

**Definition 2** (Child Selections [9]). A **Child Selection** is an injective map $J : M \rightarrow E$, which associates to every species $m \in M$ a reaction $j \in E$ such that $m$ is a reactant of reaction $j$.

The notation $j \in J$ indicates that there exists a species $m \in M$ such that $j = J(m)$. Let now $S^J$ indicate the matrix whose $m^{th}$ column is the $J(m)^{th}$ column of $S$. In particular, the columns of $S^J$ correspond one-to-one and following the order to the reactions $J(m_1), J(m_2), \ldots, J(m_{M-1}), J(m_M)$.

We associate to each Child Selection $J$ the coefficient

$$\alpha_J := \det S^J.$$ 

Let $G := \partial_x g(x)$ indicate the Jacobian matrix of (1). The determinant of $G$ can be expressed in terms of Child Selections [9,10]:

$$\det G = \sum_J \alpha_J \cdot \prod_{m \in M} f'_{J(m)m}(x),$$

The sum runs on all Child Selections. Let us consider the matrix $G$ symbolically, i.e., we consider the nonvanishing partial derivatives $f'_{J(m)m}$ as independent positive variables $r'_{jm} := f'_{J(m)m}$. We then interpret (8) as a multilinear homogenous polynomial of order $M$, in the variables $r'$. The notation $r'[J]$ indicates the monomial of the variables $r'_{jm} = r'_{J(m)m}$. That is,

$$r'[J] = \prod_{m \in M} r'_{J(m)m}.$$
In this sense,

\[ P(r') := \det G(r') = \sum_J \alpha_J r[J] \]

is the symbolic version of polynomial (8).

We call \( \alpha_J \) the behavior coefficient. Depending on the sign of \( \alpha_J \) we classify a Child Selection as follows. We call a Child Selection \( J \) zero if \( \alpha_J = 0 \). On the contrary, we call \( J \) a nonzero Child Selection if \( \alpha_J \neq 0 \). In the latter case, we say that \( J \) is good if \( \text{sign} \alpha_J = (-1)^M \), and bad otherwise. To clarify the naming, let us consider a system that possesses only stable equilibria for any choice of \( f \). This requires the Jacobian of such equilibria to have either only eigenvalues with negative real part or pairs of purely imaginary complex conjugated eigenvalues, and it excludes saddle-node bifurcations, of course. Assuming at least an eigenvalue with negative real part, the sign of a nonsingular Jacobian is

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

Via (9), this “stable” sign is automatically implied if there are no bad Child Selections. In the opposite direction, a possible loss of stability of an equilibrium necessarily implies the existence of at least one bad Child Selection. Furthermore, any Child Selection naturally identifies a subnetwork consisting only of reactions \( j \in J(M) \). In [40], the behavior of any Child Selection has been structurally characterized. That analysis showed that certain classes of Child Selections, commonly found in metabolic networks, are always good. As a consequence, we observe a clear predominance of good Child Selections in metabolic networks, whereas the few bad Child Selections hint at stability change and bifurcations.

A natural distance can be assigned to the set of Child Selections.

**Definition 3** (Distance of Child Selections). Let \( J_1, J_2 \) be two Child Selections. The distance \( d(J_1, J_2) \) is the number of species \( m \in M \) such that \( J_1(m) \neq J_2(m) \).

**Remark 1.** In literature, this type of distance is often called Hamming distance [29].

This definition admits a natural concept of minimality, in the following sense.

**Definition 4** (Minimal distance). Let \( J_1, J_2 \) be two nonzero Child Selections at distance \( d = \delta \). We say that \( J_1 \) and \( J_2 \) are at minimal distance if any Child Selection \( J_3 \) such that

\[ \begin{cases} d(J_1, J_3) < \delta \\ d(J_2, J_3) < \delta \end{cases} \]

is zero, i.e., \( \alpha_{J_3} = 0 \).

**Remark 2.** The above definition always applies if \( J_1 \) and \( J_2 \) are at distance \( d = 1 \), since no Child Selection \( J_3 \) satisfies (10).

We conclude this section with a related concept: the Partial Child Selections.

**Definition 5** (Partial Child Selections). A Partial Child Selection \( J^m \) is an injective map:

\[ J^m : M \setminus \{m\} \to E, \]
associating to each species \( n \neq m \) a reaction \( j \) such that \( n \) is a reactant of \( j \).

Let us pick a metabolite \( m_i \) and consider \( 1, \ldots, i, \ldots M \) without loss of generality. In analogy to the submatrix \( S^j \) for a Child Selection \( J \), the expression \( S_{vi}^{vm} \) indicates the \( M \times (M-1) \) matrix with columns corresponding one-to-one, and following the order, to the reactions

\[
J_{vi}^{vm}(m_1), \ldots, J_{vi}^{vm}(m_{i-1}), J_{vi}^{vm}(m_{i+1}), \ldots, J_{vi}^{vm}(m_M).
\]

The first column is the stoichiometric column \( S^j \) of the reaction \( j_1 = J_{vi}^{vm}(m_1) \) and the \( i \)th column is the stoichiometric column \( S^j \) of the reaction \( j_i = J_{vi}^{vm}(m_{i+1}) \), and so on. We associate to each Partial Child Selection \( J_{vi}^{vm} \) the behavior coefficient

\[
\beta_{J_{vi}^{vm}} := \det S_{vi}^{vm},
\]

where the notation \( S_{vi}^{vm} \) indicates the \( (M-1) \times (M-1) \) matrix obtained from \( S^{vm} \) by removing the \( m \)th row. If the behavior coefficient \( \beta_{J_{vi}^{vm}} \) is zero (resp., nonzero) we call the Partial Child Selection \( J_{vi}^{vm} \) zero (resp., nonzero), accordingly.

4. Main results

The first result is a characterization of networks that admit a singular Jacobian.

**Theorem 4.1.** The multilinear homogeneous polynomial

\[
P(r') := \det G(r')
\]

has a positive root \( r' > 0 \) if and only if there exist two Child Selections \( J_1, J_2 \) such that

\[
\alpha_{J_1} \alpha_{J_2} < 0.
\]

Theorem 4.1 is the structural characterization of a necessary spectral condition for a saddle-node bifurcation. However, the existence of two Child Selections \( J_1, J_2 \) with \( \alpha_{J_1} \alpha_{J_2} < 0 \) does not guarantee that there exists a positive root \( r' \) of \( P(r') \) such that the associated Jacobian \( G(r') \) has an algebraically simple eigenvalue zero. See [41] for a counterexample. For the simplicity, we need a further condition.

**Definition 6** (SN-pair of Child Selections). We call two nonzero Child Selections \( J_1, J_2 \) a saddle-node pair (SN-pair) if the following conditions all hold true:

1. \( J_1 \) and \( J_2 \) are at minimal distance;
2. \( \alpha_{J_1} \alpha_{J_2} < 0 \);
3. there exists a species \( \tilde{m} \) with \( J_1(\tilde{m}) \neq J_2(\tilde{m}) \) and a nonzero Partial Child Selection \( J_{vi}^{vm} \) such that \( J_{vi}^{vm}(n) = J_1(n) \) or \( J_{vi}^{vm}(n) = J_2(n) \) for every \( n \neq \tilde{m} \).

We can now state the main result of this paper.

**Theorem 4.2.** Assume that the network possesses an SN-pair of Child Selections \( J_1, J_2 \). Then there exists a choice of monotone chemical functions \( f \) such that the associated dynamical system

\[
\dot{x} = S f(x, \lambda)
\]

undergoes a saddle-node bifurcation at a positive equilibrium \( \bar{x} \) for a bifurcation value \( \lambda^* \). The bifurcation parameter \( \lambda \) parametrizes the function \( f_\eta \) of a reaction \( \eta \) such that \( J_1(m^*) = \eta \neq J_2(m^*) \), for a species \( m^* \).
Theorem 4.2 states that the existence of an SN-pair of Child Selections is a sufficient condition for the network to admit a saddle-node bifurcation. We derive the following corollary that provides a method to identify bifurcation motifs in reaction networks.

**Corollary 4.3.** Let a network \( \Gamma = (\mathbf{M}, \mathbf{E}) \) possess an SN-pair of Child Selections \((\mathbf{J}_1, \mathbf{J}_2)\) and let \( \tilde{\Gamma} = (\mathbf{\tilde{M}}, \mathbf{\tilde{E}}) \) be a network having \( \Gamma \) as a subnetwork: i.e., \( \mathbf{M} \subseteq \mathbf{\tilde{M}} \), \( \mathbf{E} \subseteq \mathbf{\tilde{E}} \). Assume there exists a pair of nonzero Child Selections of \( \tilde{\Gamma} \), \((\tilde{\mathbf{J}}_1, \tilde{\mathbf{J}}_2)\), at minimal distance in \( \tilde{\Gamma} \), such that

\[
\begin{align*}
\tilde{\mathbf{J}}_1(m) &= \mathbf{J}_1(m) \\
\tilde{\mathbf{J}}_2(m) &= \mathbf{J}_2(m)
\end{align*}
\]

for every \( m \in \mathbf{M} \), and \( \tilde{\mathbf{J}}_1(m) = \tilde{\mathbf{J}}_2(m) \) for every \( m \in \mathbf{\tilde{M}} \setminus \mathbf{M} \). Then \((\tilde{\mathbf{J}}_1, \tilde{\mathbf{J}}_2)\) form an SN-pair of Child Selections of \( \tilde{\Gamma} \) and, in particular, \( \tilde{\Gamma} \) admits a saddle-node bifurcation.

**Remark 3.** In the case of minimal distance \( d = 1 \), the minimality in the subnetwork \( \Gamma \) is always inherited by \( \tilde{\Gamma} \).

In the following sections, we will present all the arguments which prove our results.

5. **Eigenvalues zero**

We solve \( P(r') = 0 \) by considering a rescaling of the variables \( r' \) with the introduction of a further parameter \( \varepsilon > 0 \). The rescaling identifies two "leading" monomials corresponding to two Child Selections at minimal distance.

**Lemma 5.1.** Let \( \mathbf{J}_1 \) and \( \mathbf{J}_2 \) be two Child Selections at minimal distance. Then there exists an \( \varepsilon \)-rescaling of \( r' \) such that

\[
P(r') = \alpha_{\mathbf{J}_1} r'[\mathbf{J}_1] + \alpha_{\mathbf{J}_2} r'[\mathbf{J}_2] + q(\varepsilon),
\]

where \( q(\varepsilon) \) is a polynomial with \( q(0) = 0 \).

Throughout the paper, we extensively refer to such \( \varepsilon \)-rescaling. If \( \alpha_{\mathbf{J}_1} \alpha_{\mathbf{J}_2} < 0 \),

\[
\alpha_{\mathbf{J}_1} r'[\mathbf{J}_1] = -\alpha_{\mathbf{J}_2} r'[\mathbf{J}_2]
\]

is a positive solution of \( P = 0 \) at the limit \( \varepsilon = 0 \). In the proof of Theorem 4.1 we employ the implicit function theorem to extend this solution to positive \( \varepsilon > 0 \). The bridge between Lemma 5.1 and Theorem 4.1 is then provided by the following lemma.

**Lemma 5.2.** There exist two Child Selections \( \mathbf{J}_1 \) and \( \mathbf{J}_2 \) with \( \alpha_{\mathbf{J}_1} \alpha_{\mathbf{J}_2} < 0 \) if and only if there exist two Child Selections \( \mathbf{J}_3 \) and \( \mathbf{J}_4 \), at minimal distance, with \( \alpha_{\mathbf{J}_3} \alpha_{\mathbf{J}_4} < 0 \).

Lemma 5.2 is inspired by Balinski’s theorem on convex polyhedra [4]. Informally: consider the Newton polytope \( N \) generated by the determinant polynomial \( P(r') \). By convexity, an expansion of the type (11) is possible if and only if there is an edge \( e \) of \( N \) adjacent to the vertices associated to \( \mathbf{J}_1 \) and \( \mathbf{J}_2 \). Moreover, the expansion (11) provides a positive root of \( P(r') \) if and only if the coefficient sign of adjacent vertices of \( e \) is opposite. There is such an edge if and only if there are vertices of different sign: in fact, Balinski’s theorem states the connectedness of the graph associated to \( N \); hence it is enough to consider any path joining two vertices of different sign and find the first sign-switch. Even though this context is inspirational for our results, we proceed in a more elementary setting and we do not require any knowledge of convex polytopes.
6. Algebraic multiplicity

Theorem 4.1 characterizes a network for which the Jacobian $G$ of the associated system admits a zero eigenvalue. To address the multiplicity of such an eigenvalue zero, we study the adjugate matrix (transpose of the cofactor matrix) of $G$, $\text{Adj } G$. We recall two propositions from [41].

Proposition 6.1 ([41]). The Jacobian $G(r')$ has an algebraically simple eigenvalue zero at $r' > 0$ if and only if

\[
\begin{cases}
P(r') := \det G(r') = 0; \\
A(r') := \text{tr Adj } G(r') \neq 0.
\end{cases}
\]

In analogy to the expansion (9) for $P(r')$, the polynomial $A(r')$ can be expanded along Partial Child Selections.

Proposition 6.2 ([41]). Let $G$ be the Jacobian matrix of the system (1) and let $\text{Adj } G^m_m$ indicate the $m$th diagonal entry of its adjugate. Then the following expansion holds:

\[
\text{Adj } G^m_m(r') = \sum_{J^{\text{vm}}} \beta_{J^{\text{vm}}} r'[J^{\text{vm}}],
\]

where $J^{\text{vm}}$ are Partial Child Selections and the notation $r'[J^{\text{vm}}]$ indicates the multilinear monomial of degree $M - 1$:

\[
r'[J^{\text{vm}}] = \prod_{n \neq m} r'_J[J^{\text{vm}}(n) n].
\]

In particular,

\[
A(r') := \text{tr Adj } G(r') = \sum_{m \epsilon M} \sum_{J^{\text{vm}}} \beta_{J^{\text{vm}}} r'[J^{\text{vm}}].
\]

The paper [41] also presents a degenerate network for which $\det G(\bar{r}') = 0 \Rightarrow \text{tr Adj } G(\bar{r}') = 0$, and hence the system (12) is never satisfied. The presence of an SN-pair of Child Selection excludes this degeneracy, as the following lemma states.

Lemma 6.3. Assume that the network possesses an SN-pair of Child Selections. Then the polynomial system (12) has a positive solution $\bar{r}' > 0$. In particular, at $\bar{r}'$ the Jacobian $G(\bar{r}')$ possesses an algebraically simple eigenvalue zero.

7. Nonlinear unfolding

Let us consider a network with an SN-pair of Child Selections $J_1$ and $J_2$. Lemma 6.3 guarantees the existence of a positive choice $\bar{r}' > 0$, such that the Jacobian $G(\bar{r}')$ possesses an algebraically simple eigenvalue zero, i.e., spectral condition (SN1) of 1.1. The nonlinear unfolding comprises conditions (SN2) and (SN3). Let $\eta$ be a reaction such that $J_1(m^*) = \eta \neq j_2 = J_2(m^*)$, for a species $m^*$. We unfold the bifurcation by a $\lambda$-parametrization of the reaction $\eta$. In particular, the bifurcation parameter $\lambda$ appears in the reaction function $f_\eta$, only. To the bifurcation point $(\bar{x}, \lambda^*)$ corresponds the bifurcation value $\bar{r}'$. The nondegeneracy condition (SN2) requires that the derivative
of the vector field with respect to $\lambda$ is not in the range of the Jacobian at the bifurcation point:
\[ \langle w, \partial_\lambda g(\bar{x}, \lambda^*) \rangle \neq 0, \]

for $w$ left eigenvector of the Jacobian $G(\bar{r}')$. The first consequence of our parametrization choice is that the vector $\partial_\lambda g$ is parallel to the stoichiometric vector $S^\eta$ of reaction $\eta$ and
\[ \langle w, \partial_\lambda g(\bar{x}, \lambda^*) \rangle \neq 0 \iff \langle w, S^\eta \rangle \neq 0. \]

We have the following lemma.

**Lemma 7.1 (SN2).** Assume that the network possesses an SN-pair of Child Selections $J_1$ and $J_2$. Let $\eta$ be a reaction such that $J_1(m^*) = \eta \neq j_2 = J_2(m^*)$. Then there exists a positive root $\bar{r}' > 0$ of (12) such that
\[ \langle w, S^\eta \rangle \neq 0, \]

where $w$ is a left kernel vector of the Jacobian $G(\bar{r}')$ and $S^\eta$ is the stoichiometric column of reaction $\eta$.

Lemma 7.1 shows that condition (SN2) is always satisfied by our choice of $f(x, \lambda)$ at the bifurcation point $(\bar{x}, \lambda^*)$. The last step is discussing the tangency of the curve of equilibria at the bifurcation point. Condition (SN3) states that a quadratic tangency is sufficient.

**Lemma 7.2 (SN3).** Let $\bar{r}'$ be any positive root of the system (12), with $w$ and $v$ respectively left and right kernel vectors of the Jacobian $G(\bar{r}')$. Then
\[ w^T \partial^2 g(\bar{x}, \lambda^*)[v, v] \neq 0, \]

as a function of the second derivatives $r'' = f''(\bar{x})$.

Lemma 7.2 concludes that the system admits a saddle-node bifurcation, if we have enough parametric freedom to assign $r''$ freely and independently from $r$ and $r'$. We discuss this in detail in Section 8, where we present the applicability of our results for two kinetics of interest.

### 8. Michaelis-Menten and Hill’s kinetics

In this section, we apply our results to given kinetics. We discuss Hill’s kinetics, as a general mathematical form that comprises also Michaelis-Menten and mass action kinetics as particular cases. Hill’s kinetics is a relevant example of monotone chemical functions. The mathematical form of a reaction $j$ according to Hill’s is:

\begin{equation}
(14) \quad f_j(x) := a_j \prod_{m \in M} \left( \frac{x_m^{c_j^m}}{(1 + b_j^m x_m^{c_j^m})} \right)^{s_{jm}},
\end{equation}

where $s_{jm}$ is the stoichiometric coefficient of species $m$ as reactant of the reaction $j$, and $a_j, b_j^m, c_j^m$ are positive parameters. Typically, $a_j, b_j^m$ are real, while $c_j^m$ is an integer, though irrelevant for the present mathematical description. Michaelis-Menten kinetics fixes $c_j^m = 1$ for all $j, m$. Mass action kinetics is recovered by considering the limit case $b_j^m = 0, c_j^m = 1$ for all $j, m$. We write $a$ to refer to the set of parameters $a_j$ for all reactions $j$. Analogously, we write $b$ (resp., $c$) for the set of parameters $b_j^m$ (resp., $c_j^m$), for all $j$.
and \( m \).

The results of this section can be summarized as follows: at any concentration value \( \bar{x} \), the parametric freedom of Michaelis-Menten allows us to consider the values \( r \) of the function \( f \), and the values \( r' \) of their derivatives \( f' \) as independent parameters, via a careful choice of parameters \( a, b \). In contrast, the value of the second derivatives \( r'' \) cannot be independently chosen. As a consequence, under the assumptions of Theorem 4.2 we can always conclude that the network possesses a positive equilibrium satisfying conditions (SN1) and (SN2) of Theorem 1.1. This is presented in Theorem 8.1. However, we may never be able to find parameters that jointly satisfy also (SN3) of 1.1.

We present in Example 9.4 a network showing such degeneracy under Michaelis-Menten kinetics. Theorem 8.2 provides then a sufficient condition to exclude this degeneracy in a network endowed with Michaelis-Menten kinetics. Positively, the degeneracy can always be avoided in the more general case of Hill’s kinetics, by a proper choice of the further available parameters \( c \), Theorem 8.3. Negatively, on the other side of the parametric freedom, our network assumptions do not conclude a bifurcation result in the case of mass action kinetics. Nevertheless, Example 9.3 presents a mass action system undergoing a saddle-node bifurcation, where the construction is inspired by our results.

We first present the two theorems for Michaelis-Menten kinetics, hence fixing \( c^j_m = 1 \) for all reactions \( j \) and species \( m \) in the nonlinearity (14). We are thus left only with the choice of \( a, b \).

**Theorem 8.1.** Assume that the network possesses an SN-pair of Child Selections \( J_1, J_2 \). Let \( \eta \) be a reaction such that \( J_1(m^*) = \eta \neq J_2(m^*) \), for the species \( m^* \). Choose as bifurcation parameter \( \lambda := b^\eta \). Then there exists a choice of \( a, b \) such that the Michaelis-Menten system admits a positive equilibrium satisfying conditions (SN1) and (SN2) of Theorem 1.1.

Michaelis-Menten kinetics does not guarantee a parameter choice such that the curve of equilibria at the bifurcation point has a quadratic tangency (SN3). For this reason, we present a condition that characterizes the nondegeneracy of a saddle-node bifurcation under Michaelis-Menten kinetics for the case where the SN-pair of Child Selections is at distance \( d = 1 \). The general case requires too much technicality, and we omit it here.

**Theorem 8.2.** Let \((J_1, J_2)\) be an SN-pair of Child Selections at distance \( d = 1 \). Let \( m^* \) be the unique species such that \( J_1(m^*) = \eta \neq J_2(m^*) \). Choose as bifurcation parameter \( \lambda = b^\eta \). Assume the following condition holds:

\[
\frac{\alpha_{J_2}}{\bar{r}_\eta} \left( 1 + \frac{1}{s^\eta_{m^*}} \right) \neq -\frac{\alpha_{J_1}}{\bar{r}_{j_2}} \left( 1 + \frac{1}{s^{j_2}_{m^*}} \right),
\]

where \( \bar{r}_\eta \) and \( \bar{r}_{j_2} \) indicate the equilibrium constraints (2) relative to reaction \( \eta \) and \( j_2 \), respectively. Then, there exists a choice of \( a, b \) such that the Michaelis-Menten system undergoes a saddle-node bifurcation according to the parameter \( \lambda \).
In particular, Theorem 8.2 states that the degeneracy of the saddle-node depends on the ratio
\[ \frac{\bar{r}_\eta}{\bar{r}_j}, \]
which is not uniquely fixed in most applications. The degeneracy can be thus most often avoided by a proper choice of the equilibrium constraints \( \mathbf{r} \). See again Example 9.4.

For the more general Hill’s kinetics, we can choose also parameters \( c \neq 1 \). Our result reads as follows.

**Theorem 8.3.** Assume that the network possesses an SN-pair of Child Selections \( \mathbf{J}_1, \mathbf{J}_2 \). Let \( \eta \) be a reaction such that \( \mathbf{J}_1(m^*) = \eta = \mathbf{J}_2(m^*) \), for the species \( m^* \). Choose as bifurcation parameter \( \lambda := b_{m^*}^\eta \). Then there exists a choice of \( a, b, c \) such that the Hill’s system undergoes a saddle-node bifurcation according to the parameter \( \lambda \).

Let us be explicit in the parameter choice: assume there exist positive \( \bar{x}, \bar{r}, \bar{r}' \) such that:

\[
\begin{align*}
S\bar{r} &= 0; \\
\det G(\bar{r}') &= 0; \\
\frac{\bar{r}_j}{\bar{r}_{jm}} &\geq \frac{\bar{x}_m}{s_m} \quad \text{for every reaction } j \text{ and species } m.
\end{align*}
\]

(16)

Note that the three constraints (16) can be always satisfied for a network admitting a choice \( \bar{r}, \bar{r}' \) satisfying the first two constraints: the third constraint follows by choosing big enough equilibrium flux \( \bar{r} \). We fix

\[
0 < b_j^m := \left( \frac{\bar{r}_j s_m^j}{\bar{r}_{jm} \bar{x}_m} - 1 \right) \frac{1}{\bar{x}_m},
\]

(17)

and

\[
a_j := \bar{r}_j \prod_{m \in \mathcal{M}} \left( \frac{\bar{x}_m^{c_m}}{1 + b_j^m \bar{x}_m} \right)^{-s_m^j}.
\]

(18)

A straightforward computation shows that the Hill’s function

\[
f_j(x_m) := a_j \prod_{m \in \mathcal{M}} \left( \frac{x_m^{c_m}}{1 + b_j^m x_m} \right)^{s_m^j}
\]

satisfies

\[
\begin{align*}
f_j(\bar{x}_m) &= \bar{r}_j; \\
f_{jm}(\bar{x}_m) &= \bar{r}_{jm}'.
\end{align*}
\]

(19)

Note that (17) and (18) do not require a fixed choice of \( c \) and hence hold true also for Michaelis-Menten, i.e. \( c = 1 \). Furthermore, parameters \( c \) can be used to nudge

\[ w^T \partial_2^2 g(\bar{x}, \lambda^*)[v, v] \]

away from the degenerate value 0, in the Hill’s case. We discuss it in detail in the proof of Theorem 8.3.
9. Examples

9.1. Example I: Reversible feedback cycles. We present a family of networks admitting saddle-node bifurcations. Consider a reversible feedback cycle of length $M$:

\[ m_1 \overset{1}{\leftrightarrow} 4 \quad m_2 \overset{3}{\leftrightarrow} 6 \quad \cdots \quad 2M-3 \overset{2M}{\leftrightarrow} m_M \overset{2M-1}{\leftrightarrow} 2 \quad 2m_1. \]

The feedback cycles generalize autocatalytic processes: walking along the cycle from left to right, one single molecule of $m_1$ produces two molecules of $m_1$, while from right to left, two molecules of $m_1$ reduce to one single molecule of $m_1$. We show that such a structure admits saddle-node bifurcations. More specifically, we can identify $2M$ different parameters triggering a saddle-node bifurcation. The system of $M$ differential equations reads:

\[
\begin{align*}
\dot{x}_1 &= -r_1(x_1) - 2r_2(x_1) + r_4(x_2) + 2r_{2M-1}(x_M); \\
\dot{x}_i &= -r_{2i-1}(x_i) - r_{2i}(x_i) + r_{2i+2}(x_{i+1}) + r_{2i-3}(x_{i-1}), \quad \text{for } i = 2, \ldots, M-1; \\
\dot{x}_M &= -r_{2M-1}(x_m) - r_{2M}(x_M) + 2r_2(x_1) + r_{2M-3}(x_{M-1}).
\end{align*}
\]

where $x_i$ is the concentration of $m_i$. An equilibrium is given by

\[ r_j = \bar{r} \in \mathbb{R}_{>0}, \quad \text{for every } j. \]

There are only two nonzero Child Selections:

\[
\begin{align*}
\mathbf{J}_1(x_i) &= r_{2i-1}, \quad \text{for every } i = 1, \ldots, M; \\
\mathbf{J}_2(x_i) &= r_{2i}, \quad \text{for every } i = 1, \ldots, M.
\end{align*}
\]

Since there are no other nonzero Child Selections, $\mathbf{J}_1$ and $\mathbf{J}_2$ are obviously at minimal distance $d(\mathbf{J}_1, \mathbf{J}_2) = M$. The behavior coefficients are opposite: $\alpha_{\mathbf{J}_1} = (-1)^{M-1}$ and $\alpha_{\mathbf{J}_2} = (-1)^M$, thus

\[ \det G(r') = (-1)^{M-1}r'[\mathbf{J}_1] + (-1)^M r'[\mathbf{J}_2]. \]

The determinant is zero if and only if $r'[\mathbf{J}_1] = r'[\mathbf{J}_2]$. Any Partial Child Selection

\[ \mathbf{J}_i^{\text{vm}}(\mathbf{M} \setminus \{m\}) = \mathbf{J}_i(\mathbf{M} \setminus \{m\}), \]

with $i = 1$ or $i = 2$, is nonzero. Hence $\mathbf{J}_1$ and $\mathbf{J}_2$ form an SN-pair of Child Selections, and the system admits a saddle-node bifurcation, via Theorem 4.2.

To exemplify further, we compute all conditions explicitly under the assumption of Michaelis-Menten kinetics. We operate as described in Section 8 choosing arbitrary values. We fix $\bar{x}_i = 1$ for every $i$, the values $\bar{r}_j = 4$ for every $j$, and $\bar{r}_{jm} = 1$ for every $j$ and $m$. Computing $a, b$ as in (17), (18), the reaction functions $f_j$ read:

\[
f_j(x) = \begin{cases} 
256\left(\frac{\bar{x}_j}{1+\bar{x}_j}\right)^2 & \text{if } j = 2; \\
16\frac{\bar{x}_j}{1+3x_j} & \text{if } j = 2i-1 \text{ or } j = 2i, \text{ } j \neq 2.
\end{cases}
\]
We compute the Jacobian $G(\bar{r}')$ at $\bar{r}' = 1$.

$$G(1) = \begin{bmatrix}
-3 & 1 & 0 & \ldots & 0 & 0 & 2 \\
1 & -2 & 1 & \ldots & 0 & 0 & 0 \\
0 & 1 & -2 & \ldots & 0 & 0 & 0 \\
\vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
0 & 0 & 0 & \ldots & -2 & 1 & 0 \\
0 & 0 & 0 & \ldots & 1 & -2 & 1 \\
1 & 0 & 0 & \ldots & 0 & 1 & -2 \\
\end{bmatrix},$$

with right kernel vector $v = (1, 1, \ldots, 1)^T$ and left kernel vector $w = (M, M+1, \ldots, 2M-1)^T$.

Let us first check the condition (SN3),

$$w^T \partial^2_x g(x)[v, v] = w^T \sum_i \frac{\partial^2 g}{\partial x_i^2} (v_i)^2.$$

A simple computation shows:

$$(M, M + 1, \ldots, 2M - 1) - \frac{\partial^2 g}{\partial x_i^2} = f''(2i-1)_{m,m} - f''(2i)_{m,m},$$

which is nonzero if and only if $i = 1$. In fact, note that $f_{2i} \equiv f_{2i-1}$, unless $i = 1$. In the case of $i = 1$, we have

$$f''_{1_{m_1m_1}} - f''_{2_{m_1m_1}} = -\frac{13}{8} + \frac{3}{2} = -\frac{1}{8} \neq 0,$$

and thus

$$w^T \partial^2_x g(x)[v, v] = w^T \frac{\partial^2 g}{\partial x_1^2} \neq 0.$$

Via Lemma 7.1 or a direct check, we have that

$$\langle w, S' \rangle \neq 0,$$

for any reaction $j$: the condition (SN2) is satisfied. In conclusion, the bifurcation point can be unfolded along $2M$ different parameters $b'_j$, for $i = 1, \ldots, M$, $j = 2i$ or $j = 2i - 1$: such a system admits $2M$ saddle-node bifurcations.

9.2. Example II: Glyoxylate cycle vs TCA cycle in E.Coli. The central carbon metabolism is a fundamental metabolic process in living beings. An important part of this process is the tricarboxylic acid (TCA) cycle, a cyclic sequence of reactions generating energy in form of ATP. Described for the first time in 1957 by Kornberg and Krebs, the glyoxylate cycle is a suggested variation of the TCA cycle. We refer to [26] for more detailed biological explanations. We consider the network structure combining TCA and Glyoxylate cycle, as presented in [26], with further added “cell synthesis” outflow reactions from [24], which play an important mathematical role. We show that this structure admits a saddle-node bifurcation. The structure is the following:
We identify an SN-pair of Child Selections. Consider 2 and 11. Reactions 3, 8, and 12 are outflow reactions considered in [24]. The system of not in the Glyoxylate cycle: reactions 1, 3, and 4. On the contrary, dotted-dashed arrows indicate reactions in the TCA cycle that do not appear in the TCA cycle: reactions 2 and 11. Reactions 3, 8, and 12 are outflow reactions considered in [24]. The system of differential equations is the following:

\[
\begin{align*}
\dot{A} &= -r_1(x_A) - r_2(x_A) + r_{10}(x_G); \\
\dot{B} &= r_1(x_A) - r_3(x_B) - r_4(x_B); \\
\dot{C} &= r_4(x_B) + r_2(x_A) - r_5(x_C); \\
\dot{D} &= r_5(x_C) - r_6(x_D); \\
\dot{E} &= r_6(x_D) - r_7(x_E) + r_{11}(x_H, x_I); \\
\dot{F} &= r_7(x_E) - r_8(x_F) - r_9(x_F, x_I); \\
\dot{G} &= r_9(x_F, x_I) - r_{10}(x_G); \\
\dot{H} &= r_2(x_A) - r_{11}(x_H, x_I); \\
\dot{I} &= -r_9(x_F, x_I) - r_{11}(x_H, x_I) - r_{12}(x_I) + F_I,
\end{align*}
\]

Fix arbitrarily \(\bar{r}_3, \bar{r}_4, \bar{r}_8, \bar{r}_{12} > 0\). The equilibria constraints are:

\[
\begin{align*}
F_I &= 3\bar{r}_3 + \bar{r}_4 + 2\bar{r}_8 + \bar{r}_{12}; \\
\bar{r}_1 &= \bar{r}_3 + \bar{r}_4; \\
\bar{r}_2 &= \bar{r}_3 + \bar{r}_8; \\
\bar{r}_3 &= \bar{r}_3; \\
\bar{r}_4 &= \bar{r}_4; \\
\bar{r}_5 &= \bar{r}_3 + \bar{r}_4 + \bar{r}_8; \\
\bar{r}_6 &= \bar{r}_3 + \bar{r}_4 + \bar{r}_8; \\
\bar{r}_7 &= 2\bar{r}_3 + \bar{r}_4 + 2\bar{r}_8; \\
\bar{r}_8 &= \bar{r}_8; \\
\bar{r}_9 &= 2\bar{r}_3 + \bar{r}_4 + \bar{r}_9; \\
\bar{r}_{10} &= 2\bar{r}_3 + \bar{r}_4 + \bar{r}_8; \\
\bar{r}_{11} &= \bar{r}_3 + \bar{r}_8; \\
\bar{r}_{12} &= \bar{r}_{12}.
\end{align*}
\]

We identify an SN-pair of Child Selections. Consider

\[
\mathbf{J}_1(A, B, C, D, E, F, G, H, I) = (1, 3, 5, 6, 7, 9, 10, 11, 12),
\]
and
\[ J_2(A, B, C, D, E, F, G, H, I) = (2, 3, 5, 6, 7, 9, 10, 11, 12). \]

\( J_1 \) and \( J_2 \) are

1. at minimal distance 1: only the species \( A \) is such that \( J_1(A) \neq J_2(A) \);
2. \( \alpha_{J_1} = -1 \) and \( \alpha_{J_2} = +1 \);
3. the Partial Child Selection
   \[ J^\wedge A(B, C, D, E, F, G, H, I) = (3, 5, 6, 7, 9, 10, 11, 12) \]
   has nonzero coefficient \( \beta_{J^\wedge A} = 1 \neq 0 \).

Hence \( J_1 \) and \( J_2 \) form an SN-pair of Child Selections. The system thus admits a saddle-node bifurcation according to a parametrization of either reaction 1 or 2. Reactions 1 and 2 mark the difference between the TCA cycle and the Glyoxylate cycle. Even when endowed with Michaelis-Menten kinetics, via Theorem 8.2, the system exhibit a saddle-node bifurcation. In this case, it is required to choose \( \bar{r}_1 \neq \bar{r}_2 \), which is allowed by the equilibria constraints (19).

9.3. Example III: Mass action. The law of mass action for a reaction \( j \) assumes
\[ f_j(x) := k_j \prod_{m \in M} x_m^{s^j_m}, \]
where \( k_j > 0 \) is a positive constant and \( s^j_m \) is the stoichiometric coefficient of the species \( m \) as reactant of the reaction \( j \). Assuming (20) for all reaction functions \( f_j \) translates [I] into a polynomial system. In contrast to Michaelis-Menten and Hill’s, a striking feature is that each reaction function \( f_j \) is parametrized by only one parameter \( k_j \). This impedes our approach, which assumes enough parametric freedom to discuss separately the equilibria constraints from the bifurcation constraints: it is not the case for mass action. In particular, there is not enough choice of parameters to harness the value of the derivative
\[ f'_{jm}(x) = s^j_m x_m^{(s^j_m-1)} k_j \prod_{n \neq m} x_n^{s^j_n} = \frac{s^j_m}{x_m} f_j, \]
once the value \( r_j = f_j(x) \) is fixed. It is not possible using without concern the value \( x_m \) as a parameter, as \( x_m \) appears also in the mathematical expression of any other derivative \( f_{hm} \), for a reaction \( h \neq j \) where \( m \) participates as reactant. For this reason, the mass action case deserves further dedication and work, not addressed in this paper. Nevertheless, we derive two observations and produce an example of a network undergoing a saddle-node bifurcation under the assumption of mass action. We keep this discussion as informal and self-contained as possible.

Essentially, our scheme to detect saddle-node bifurcations is to find two Child Selections \( J_1, J_2 \) with opposite behavior and at minimal distance. Firstly, a trivial necessary condition to have a saddle-node is the nonlinearity of the system: for linear systems, condition (SN3) of Theorem [I,I] is never satisfied. Under the assumption of mass action, this requires reactions with more than one reactant or with a stoichiometric coefficient
bigger than one for the unique reactant. In spirit with our results, the nonlinearity should be precisely in reactions $j$ with $J_1(m^*) = j \neq J_2(m^*)$, for a species $m$. Secondy, in the mass action case, we are not able to discuss independently equilibria constraints and bifurcation constraints, as shown in [21]. Thus, it is helpful having constant inflow reactions to the species $m^*$ with $J_1(m^*) = j \neq J_2(m^*)$. The constant inflow reactions help solve the equilibria equation, but they do not play any role in the bifurcation conditions, disappearing upon differentiation. Following these two observations we present the following example:

\[
\begin{array}{ccc}
F_A & A & 1 \\
F_B & B & 3 \\
A + B & C & 4 \\
\end{array}
\]

where reactions $F_A, F_B$ are inflows and reactions 1, 3 are outflows to species $A,B$, respectively. From the network we derive the following system of differential equations:

\[
\begin{align*}
\dot{x}_A &= -f_1(x_A) - f_2(x_A, x_B) + 2f_4(x_C) + F_A = -k_1x_A - k_2xAx_B + 2k_4x_C + F_A; \\
\dot{x}_B &= -f_3(x_B) - f_2(x_AxB) + 2f_4(x_C) + F_B = -k_3x_B - k_2xAx_B + 2k_4x_C + F_B; \\
\dot{x}_C &= f_2(x_A,B) - f_4(x_C) = k_2xAx_B - k_4x_C.
\end{align*}
\]

Note that reaction 2 has two reactants, and hence the function $f_2 = k_2 x_A x_B$ is nonlinear. Fix arbitrarily $\bar{r}_1, \bar{r}_2, \bar{r}_3 > 0$ such that $\bar{r}_2 < \bar{r}_1, \bar{r}_3$; the equilibrium constraints read:

\[
\begin{pmatrix}
F_A \\
F_B \\
f_1 \\
f_2 \\
f_3 \\
f_4
\end{pmatrix} =
\begin{pmatrix}
\bar{r}_1 - \bar{r}_2 \\
\bar{r}_3 - \bar{r}_2 \\
\bar{r}_1 \\
\bar{r}_2 \\
\bar{r}_3 \\
\bar{r}_2
\end{pmatrix}
\]

The Jacobian of the system is

\[
G = \begin{pmatrix}
-k_1 - k_2x_B & -k_2xA & 2k_4 \\
-k_2x_B & -k_3 - k_2xA & 2k_4 \\
k_2xB & k_2xA & -k_4
\end{pmatrix}, \quad \text{with } \det G = -k_1k_3k_4 + k_1k_2k_4x_A + k_2k_3k_4x_B.
\]

Let us consider the point $\bar{x} = (\bar{x}_A, \bar{x}_B, \bar{x}_C) = (1,1,1)$, and the rates $k_1 = k_3 = 2$, $k_2, k_4 = 1$. This solves $\det G = 0$ with a simple eigenvalue zero and fixes $F_A = F_B = 1$. Let us consider $k_3$ as a bifurcation parameter, and thus $k_3^2 = 1$ as its bifurcation value. The Jacobian $G$ at the bifurcation point reads:

\[
G_{(\bar{x}, k_3^2)} = \begin{pmatrix}
-3 & -1 & 2 \\
-1 & -3 & 2 \\
1 & 1 & -1
\end{pmatrix}
\]

with right kernel vector $v = (1,1,2)^T$ and left kernel vector $w = (1,1,4)$. Condition (SN2) is satisfied:

\[
\langle w, \partial_{k_3} g \rangle = (1,1,4)(-1,-1,1)^T \neq 0,
\]

as well as (SN3):

\[
w^T \partial^2_{k_3} g[v, v] = (1,1,4)(-2,-2,2)^T \neq 0.
\]
The fact that up to a constant the two conditions (SN2) and (SN3) are the same is not a coincidence, but the central idea of this example: the only nonlinear reaction function \( f_2 \) is also the only reaction function where the bifurcation parameter appears, hence \( \partial k_2 g \) must be parallel to \( \partial^2_x g[v, v] \). In conclusion, for \( k_1 = k_3 = 2 \) and \( F_A = F_B = k_4 = 1 \), the equilibrium \((x_A, x_B, x_C) = (1, 1, 1)\) undergoes a saddle-node bifurcation for the parameter \( k_2 = 1 \). In particular, for \( k_2 < 1 \) we have multistationarity.

9.4. Example IV: Degenerate saddle-node for Michaelis-Menten. This example presents a network that, when endowed with Michaelis-Menten kinetics, admits an equilibrium \( \bar{x} \) satisfying conditions (SN1) and (SN2) but for which (SN3) is never satisfied. The network is the following:

\[
\begin{array}{ccc}
\xrightarrow{0} & A & \xrightarrow{1} \ B & \xrightarrow{2} \ 2A \\
\end{array}
\]

where reaction 0 is an outflow from \( A \). The system possesses only two Child Selections \( J_1 \) and \( J_2 \):

\[
J_1(A, B) = (0, 2) \quad \text{and} \quad J_2(A, B) = (1, 2).
\]

\( J_1 \) and \( J_2 \) form an SN-pair of Child Selections. In fact, they are at minimal distance \( d(J_1, J_2) = 1 \), \( \alpha_{J_1} \alpha_{J_2} = 1 \cdot (-1) = -1 \), and the unique Partial Child Selection \( J^{\bigvee}A(B) = 2 \) is nonzero. Theorem 4.2 guarantees that there exist monotone chemical functions such that the associated dynamical system undergoes a saddle-node bifurcation according to a parametrization of the reaction \( \eta = 0 \) or \( \eta = 1 \). However, when restricted to Michaelis-Menten kinetics the saddle-node is always degenerate. Consider the associated system

\[
\begin{align*}
\dot{x}_A &= -r_0(x_A) - r_1(x_A) + 2r_2(x_B); \\
\dot{x}_B &= r_1(x_A) - r_2(x_B).
\end{align*}
\]

The equilibrium constraints fix \( \bar{r}_0 = \bar{r}_1 = \bar{r}_2 \), and hence condition [15] is never satisfied.

However, the degenerate situation is easily fixable: let us consider the same system with an added inflow to species \( A \):

\[
\xrightarrow{F_A} A.
\]

The system of ODEs now reads

\[
\begin{align*}
\dot{x}_A &= -r_0(x_A) - r_1(x_A) + 2r_2(x_B) + F_A; \\
\dot{x}_B &= r_1(x_A) - r_2(x_B),
\end{align*}
\]

with the equilibrium constraints:

\[
\bar{r}_1 = \bar{r}_2 = \bar{r}_0 - F_A.
\]

Thus, for \( 0 < F_A < \bar{r}_0 \), we have now equilibria for which \( \bar{r}_0 \neq \bar{r}_1 \). The case \( F_A = 0 \) recovers the degenerate example. If \( \bar{r}_0 \neq \bar{r}_1 \) we have a nondegenerate saddle-node bifurcation, even in the Michaelis-Menten case.
The network has two Child Selections with opposite behavior.

\[ (1) \Leftrightarrow \]

The network has two Child Selections at minimal distance with opposite behavior.

\[ (2) \quad \text{“} \Leftrightarrow \text{”} \]

The network admits a saddle-node bifurcation.

\[ (3) \Rightarrow \]

The network admits multistationarity.

**Figure 1.** The conceptual map of this paper. Implication (1) is Lemma 5.2. Implication (2) is technically only a necessary condition \( \Leftarrow \), but many “pathological” counterexamples are not biologically relevant, hence in realistic networks, the implication is expected to be also sufficient. Implication (3) concludes the logical chain by assessing multistationarity.

10. **Discussion**

In this paper, we have presented a comprehensive saddle-node bifurcation analysis for chemical reaction networks. Via a symbolic approach, we have analyzed which networks admit the occurrence of a bifurcation behavior. Our work has two direct consequences. Theoretically, we have described the structures, which guarantee that the network can sustain multistationarity. Practically, we have identified the proper parameters to unfold a saddle-node bifurcation.

The key structure we have described are SN-pairs of Child Selections, i.e., two nonzero Child Selections \((J_1, J_2)\) that satisfy three conditions:

1. they are at minimal distance \(\delta\);
2. their behavior coefficient is opposite in sign: \(\alpha_{J_1, \alpha_{J_2}} < 0\);
3. a technical condition excluding multiple eigenvalues zero.

If the network possesses an SN-pair of Child Selections, then a saddle-node bifurcation occurs for a choice of monotone chemical functions \(f(x, \lambda)\) parametrized by a single parameter \(\lambda\). The bifurcation parameter \(\lambda\) parametrizes only the function \(f_\eta\) of an arbitrary reaction \(\eta = J_1(m^*) \neq J_2(m^*)\), for one of the \(\delta\) species \(m^*\) with \(J_1(m^*) \neq J_2(m^*)\). The existence of a pair of Child Selections satisfying conditions (1) and (2) above is only necessary for a bifurcation behavior. However, the identification of a counterexample [41] to the sufficiency of conditions (1) and (2) suggests that this is an issue for mathematicians, with seemingly no biological relevance. That is, for realistic biological networks, conditions (1) and (2) above essentially characterize the bifurcation behavior. On the other hand, the three conditions (1)–(3) are technically only sufficient. See Figure 1 for a conceptual map.
Our symbolic approach considers the derivatives $r'$ of the reaction functions as positive independent variables. We have applied a geometrical perturbation argument with an $\varepsilon$-rescaling of the variables $r'$. At the limit $\varepsilon = 0$, the only nonzero variables are the ones identified by the SN-pair $(J_1, J_2)$ of Child Selections, that is:

$$r'_{jm} \neq 0 \quad \iff \quad (j, m) = (J_i(m), m) \quad \text{for } i = 1, 2.$$ 

In this sense, the bifurcation behavior of the SN-pair of Child Selections is inherited by the full network. Inheritance of dynamical features figures in recent works by Banaji [5, 8]. However, these works have not yet discussed the inheritance of bifurcation behavior, and focused on modified networks, rather than identifying some leading subnetworks that encode the dynamics, as we did. In particular, any network for which a pair of Child Selections is an SN-pair admits a saddle-node bifurcation. Corollaries 4.3 exploits and clarifies this idea, and can be used to find small and simple network motifs for saddle-node bifurcation in larger networks.

Network motifs connected to multistationarity have been discussed in the literature on various levels of abstraction and empiricism. A central role is often claimed by autocatalysis [33]. Autocatalytic reactions are those in which at least one of the products is also a reactant. For example, the reversible reaction

$$m \xleftrightarrow{j_{\text{aut}}^-} 2m$$

is autocatalytic. We refer to reaction $j_{\text{aut}}^+$ as positively autocatalytic and to $j_{\text{aut}}^-$ as negatively autocatalytic. These ideas can be generalized to autocatalytic sequences of reactions (feedback loops) or autocatalytic networks, leading to a more general concept of autocatalysis, which is still under debate [1]. We do not enter here such a formal discussion, but we observe that our results are consistent with the many independent observations on the centrality of autocatalysis. In particular, positive autocatalysis can trigger a saddle-node bifurcation and consequent multistationarity. Example 9.1 shows how reversible feedback loops admit saddle-node bifurcations. Such feedback loops are a simple generalization of autocatalytic reactions and their connection with multistationarity has been pioneered by Thomas [37] in a related context. Example 9.2 identifies a saddle-node bifurcation in the central carbon metabolism of E. Coli. Along the glyoxylate cycle, one molecule of Isocitrate transforms into two molecules, similarly to $j_{\text{aut}}^+$. Example 9.3 contains two reactions

$$A + B \xrightarrow{1} C \xrightarrow{4} 2A + 2B,$$

with a clear analogy to $j_{\text{aut}}^+$. Example 9.4 contains a positive feedback loop of two reactions

$$A \xrightarrow{1} B \xrightarrow{2} 2A.$$

More simply, let us consider a toy network $\Gamma$ with a single species $A$. The behavior coefficient of any Child Selection on $\Gamma$ is then just

$$\alpha_J = \det S^J = S_{AJ(A)} = s^J_A - s^J_A.$$
Clearly, a Child Selection $J$ is bad if and only if $J(A)$ is positively autocatalytic. This observation can be generalized to any dimension and highlights the connection between positive autocatalysis and bad Child Selections. Previous work \[40\] argued on the prevalence of good Child Selections in biochemical networks. In light of such an argument, the mere presence of positive autocatalysis points to the first step of the logical chain of Figure 1 and the consequent possibility of multistationarity. The formalization of these presented arguments will be included in future work.

Realistic kinetic models of biochemical networks typically comprise different types of kinetics \[10\]. In this paper, we have explicitly discussed the case of two kinetics of interest: Michaelis-Menten and Hill’s kinetics, and identified the proper bifurcation parameters. In the presence of a SN-pair of Child Selections $(J_1, J_2)$, the bifurcation parameter is $b_{\eta m^*}^*$ in (14) for any reaction $\eta$ and species $m^*$ such that $\eta = J_1(m^*) \neq J_2(m^*)$. We have shown that the parametric richness of Hill’s kinetics always guarantees a saddle-node bifurcation behavior. On the contrary, for Michaelis-Menten we can only guarantee the existence of an equilibrium such that conditions (SN1)-(SN2) of Theorem 1.1 are satisfied, not necessarily (SN3). However, Theorem 8.2 provides a sufficient condition (15) for a nondegenerate saddle-node bifurcation in the case of distance $d(J_1, J_2) = 1$. Condition (15) shows that the degeneracy is quite unlikely, as it requires a uniquely determined equilibrium constraint (2). Example 9.4 shows how an inflow reaction to $m^*$ already guarantees that there exists a choice of equilibrium fluxes $r$ such that (15) is satisfied. Finally, the analysis for mass action kinetics is at present inconclusive, even though Example 9.3 presents a mass action system undergoing a saddle-node bifurcation according to the same structural intuition of the present paper. Further work is needed to clarify it, along the lines of the present contribution.

A natural combinatorial question arises with regard to SN-pairs of Child Selections $(J_1, J_2)$: which structure is required for the case of minimal distance $d(J_1, J_2) > 1$? In Example 9.1 we have presented the case of reversible feedback cycles of length $M$, which possesses an SN-pair of Child Selections at distance $M$. In a work in preparation, we will characterize SN-pairs of Child Selections in terms of a reciprocal permutation structure, generalizing the concept of reversible cycles. This observation stresses how saddle-node bifurcations are triggered either by SN-pairs at distance $d = 1$, or by special (thus recognizable!) structures. Note that even for Michaelis-Menten kinetics we have a sufficient condition for saddle-node for the most relevant case $d = 1$.

In conclusion, we make a non-mathematical consideration. It is a strong impression of the author that bifurcation behavior is essentially characterized in realistic biological networks by simple and recognizable structures, even though mathematics includes much more complex options, and requires a thorough analysis and exclusion of pathological cases. Such pathological cases are of minimal interest for biologists but enhance the technical difficulties of the proofs and diminish the verbal strength of the results. This empirical consideration calls for stronger interaction between mathematicians and theoretical biologists to synthesize the results, with a skimming of biologically irrelevant (but
nevertheless mathematically challenging and intriguing) cases, with the goal of obtaining a clearer picture of the bifurcation behavior in real-world biochemical networks.

11. Proofs

Proofs of Section 5 and Theorem 4.1

Before proving Lemma 5.1, we state and prove a further technical lemma.

Lemma 11.1. Let $J_1, J_2$ be two Child Selections at minimal distance $\delta$. Then for every other nonzero Child Selection $J_3$, there exists a species $m^*$ such that

\[(22) \quad J_3(m^*) \neq J_1(m^*), \quad J_3(m^*) \neq J_2(m^*).\]

Proof. Let $D \subseteq M$ be the set of species $m$ such that $J_1(m) \neq J_2(m)$. If there exists $m^* \in M \setminus D$ such that $J_1(m^*) = J_3(m^*)$ we are done, since together with $J_1(m^*) = J_2(m^*)$ it implies (22). Consequently, let us assume $J_1(m) = J_2(m) = J_3(m)$, for every $m \in M \setminus D$. Take any $m^* \in D$. We have (22). Indeed, assume (22) does not hold. Without loss of generality we have $J_3(m^*) = J_1(m^*)$, which implies

\[d(J_1, J_3) \leq \delta - 1 < \delta,\]

and contradicts the assumption of $\delta$ being the minimal distance.

Proof of Lemma 5.1. For any $r'_{jm} \neq r'_{jm}(m^*)$, for $i = 1, 2$, fix the value

\[r'_{jm} = \varepsilon \bar{r}_{jm},\]

where $\bar{r}_{jm} > 0$ is any positive value. Via Lemma 11.1, $P(r')$ now takes the form:

\[(23) \quad P(r') = \alpha J_1 r'[J_1] + \alpha J_2 r'[J_2] + q(\varepsilon),\]

where $q(\varepsilon)$ indicates all the summands where $\varepsilon$ appears at least linearly. Lemma 11.1 guarantees indeed that there are no other nonzero summands. In particular, $q(\varepsilon)$ is a polynomial in $\varepsilon$ with zero constant term, i.e. $q(0) = 0$.

Proof of Lemma 5.2. The implication $\Leftarrow$ is trivial. We prove the implication $\Rightarrow$. Consider the following two sets of Child Selections:

\[\mathcal{G} = \{ J \mid \alpha J = (-1)^M \} \quad \text{and} \quad \mathcal{B} = \{ J \mid \alpha J = (-1)^{M-1} \}.\]

Since the total number of Child Selections is finite, $\mathcal{G}$ and $\mathcal{B}$ are finite sets and, by assumption, both are nonempty. We can define the distance $d$ of the two sets as:

\[d(\mathcal{G}, \mathcal{B}) := \inf_{J_1 \in \mathcal{G}, J_2 \in \mathcal{B}} d(J_1, J_2) = \min_{J_1 \in \mathcal{G}, J_2 \in \mathcal{B}} d(J_1, J_2) = d(J_3, J_4),\]

for some Child Selections $J_3$ and $J_4$ with $\alpha J_3 \alpha J_4 < 0$. The Child Selections $J_3$ and $J_4$ are at minimal distance, by construction.
Proof of Theorem 4.1. The implication \( \Rightarrow \) is trivial. We prove the implication \( \Leftarrow \). We apply Lemma 5.2 to find two Child Selections \( \mathbf{J}_3, \mathbf{J}_4 \) at minimal distance with \( \alpha_{\mathbf{J}_3}, \alpha_{\mathbf{J}_4} < 0 \). We recall the set \( \mathbf{D} \subseteq \mathbf{M} \) of species \( m \) such that \( \mathbf{J}_3(m) \neq \mathbf{J}_4(m) \). The cardinality of \( \mathbf{D} \) is \( \delta = d(\mathbf{J}_3, \mathbf{J}_4) \). Without loss of generality let us consider \( \mathbf{D} = \{ m_1, \ldots, m_\delta \} \).

We consider the \( \varepsilon \)-rescaling of Lemma 5.1. We define \( m^* := m_1, \eta := \mathbf{J}_3(m^*) \) and \( \rho := r_{jm^*}' \). We want to solve \( P(r') = 0 \) with respect to the variable \( \rho \). To this goal, for any \( r_{jm}' = r_{jm}'(m^*), i = 3, 4, r_{jm}' \neq \rho \), we fix the value

\[
r_{jm}' := r_{jm}',
\]

where \( r_{jm}' > 0 \) is any positive value. Now the polynomial \( [23] \) reads as a bivariate polynomial in the two variables \( \rho \) and \( \varepsilon \):

\[
P(r') = P(\rho, \varepsilon) = \alpha_{\mathbf{J}_3, \rho} r'[\mathbf{J}_3 \setminus \eta] + \alpha_{\mathbf{J}_4, \rho} r'[\mathbf{J}_4] + q(\varepsilon),
\]

where \( r'[\mathbf{J}_3 \setminus \eta] \) indicates the monomial \( \frac{r'[\mathbf{J}_3]}{\rho} \). The value

\[
\rho^* := -\frac{\alpha_{\mathbf{J}_3, \rho} r'[\mathbf{J}_3]}{\alpha_{\mathbf{J}_4, \rho} r'[\mathbf{J}_3 \setminus \eta]}
\]

is positive by assumption, since \( \alpha_{\mathbf{J}_3, \rho} \alpha_{\mathbf{J}_4} < 0 \). Moreover,

\[
P(\rho^*, 0) = 0.
\]

We apply the Implicit Function Theorem to show that a positive solution \( \rho^*(\varepsilon) \) persists also in a positive neighborhood \( \varepsilon > 0 \). We check

\[
P_\rho(\rho, \varepsilon)|_{(\rho^*, 0)} \neq 0.
\]

Indeed,

\[
P_\rho(\rho, \varepsilon)|_{(\rho^*, 0)} = \alpha_{\mathbf{J}_3, \rho} r'[\mathbf{J}_3 \setminus \eta] + q_\rho(\varepsilon)|_{(\rho^*, 0)} = \alpha_{\mathbf{J}_3, \rho} r'[\mathbf{J}_3 \setminus \eta] \neq 0.
\]

Hence, there exists a positive solution \( \rho^*(\varepsilon) \) for \( \varepsilon > 0 \). \( \square \)

Remark 4. In the proof of Theorem 4.1, the choice of \( \rho \) is arbitrary. We could argue analogously, by picking any \( \rho^* := r_{jm}'(m^*), i = 3, 4, \) for any \( m \) such that \( \mathbf{J}_3(m) \neq \mathbf{J}_4(m) \).

Remark 5. Theorem 4.1 can also be proved via intermediate value theorem, in simpler setting. However, the perturbation construction in the presented proof is central to the development of the following results of this paper.

Proofs of Section 6

Proof of Lemma 6.3. Via condition (1) of Definition 6.1 we consider again the \( \varepsilon \)-rescaling of Lemma 5.1 and proceed as in the proof of Theorem 4.1. Without loss of generality, assume again that the \( \delta \) species \( m \) such that \( \mathbf{J}_1(m) \neq \mathbf{J}_2(m) \) are \( m_1, \ldots, m_\delta \), and that \( \tilde{m} = m_1 \) in the Definition 6. Define again \( \eta := \mathbf{J}_1(\tilde{m}) \), and \( \rho := r_{jm}(\tilde{m}) \). With abuse of notation, let \( r' \) indicate all the variables

\[
r' := \{ r_{jm}' \text{ such that } r_{jm}'(m^*), i = 1, 2, r_{jm}' \neq \rho \}.
\]
This way the polynomial \( P(r') \) reads as \( P(\rho, r', \varepsilon) \), in the variables \( \rho, r', \varepsilon \). Let now \( \rho^*(r') \) indicate the function of \( r' \)

\[
\rho^*(r') := -\frac{\alpha_{J_2} r'(J_2)}{\alpha_{J_1} r'(J_1 \setminus \eta)}. 
\]

Condition (2) of Definition 6 guarantees positivity of \( \rho^*(r') > 0 \), and thus

\[
P(\rho^*(r'), r', 0) = 0, \quad \text{for any choice of } r' > 0.
\]

On the other hand, via (13),

\[
\text{Adj} \ G_{\tilde{m}} = \sum_{J_{\tilde{m}}} \beta_{J_{\tilde{m}}} r'[J^\tilde{m}] 
\]

implies that \( \rho \) does not appear in \( \text{Adj} G_{\tilde{m}} \). Moreover, Condition (3) of Definition 6 guarantees the existence of a nonzero Partial Child Selection \( J^\tilde{m} \), i.e. \( \beta_{J_{\tilde{m}}} \neq 0 \). Thus,

\[
\text{Adj} G_{\tilde{m}} \neq 0.
\]

In conclusion, we have that

\[
A(\rho^*(r'), r', 0) = \text{tr} \text{ Adj}(\rho^*, r', 0) \neq 0.
\]

Hence, there exists a choice of \( \bar{r}' \) such that

\[
\begin{cases} 
P(\rho^*(\bar{r}'), \bar{r}', 0) = 0; \\
A(\rho^*(\bar{r}'), \bar{r}', 0) \neq 0. 
\end{cases}
\]

As in the proof of Theorem 4.1 we apply the implicit function theorem obtaining a solution of \( P(\rho^*(\bar{r}', \varepsilon), \bar{r}', \varepsilon) \), for small \( \varepsilon \). By continuity, \( A(\rho^*, \bar{r}', 0) \neq 0 \) persists in a neighborhood of \( \varepsilon \), implying the existence of \( \bar{r}' \) such that:

\[
\begin{cases} 
P(\bar{r}') = 0; \\
A(\bar{r}') \neq 0. 
\end{cases}
\]

\[\square\]

Proofs of Section 7

Proof of Lemma 7.1. Let us consider the \( \varepsilon \)-rescaling of Lemma 5.1 for the SN-pair of Child Selections \( J_1 \) and \( J_2 \). Until the last step of this proof, we consider the limit \( \varepsilon = 0 \). Not to overload notation, we drop the constant reference to \( \varepsilon \). At this limit, we recall that the only nonvanishing variables \( r_{jm}' \) are the ones such that

\[
r_{jm}' = r_{J_1(m)m}' \quad \text{with } i = 1, 2.
\]

Let \( \rho := r_{jj}' \), and consider the \( M \times M \) matrix \( G^\eta \), whose columns \( (G^\eta)^m \) are

\[
(G^\eta)^m = \begin{cases} 
\rho S^\eta & \text{if } m = m^*; \\
G^m & \text{otherwise.}
\end{cases}
\]
We recall that $G^m$ indicates the $m^{th}$ column of $G$ and $S^n$ the stoichiometric column corresponding to reaction $\eta$. The polynomial $P_j(r') := \det G^n$ is nonzero. In fact
\begin{equation}
P_j(r') = \sum_{i \in J} \alpha_{ij} r'[J] = \alpha_{iJ} r'[\mathbf{J}_1] \neq 0, \quad \text{for any choice of } r'[\mathbf{J}_1] > 0.
\end{equation}

Note that (24) holds only at the limit $\varepsilon = 0$. Let again $r'$ indicate all the variables $r' := \{ r'_{jm} \text{ such that } r'_{j(m,m)}, i = 1, 2, \; r'_{jm} \neq \rho \}$. Analogously as in the proof of Lemma 6.3, we can choose $\bar{r}'$, $\rho^*(\bar{r}')$ such that $G[(\rho^*(\bar{r}'), \bar{r}')$ has an algebraically simple eigenvalue zero. Let $w$ be left kernel vector of $G[(\rho^*(\bar{r}'), \bar{r}')]$. Note that the $m^{th}$ column of $G$ is the $m^{th}$ column of $G^n$, except for $m = m^*$. From the nonsingularity of $G^n$ we conclude:
\begin{equation}
0 \neq w^T G^n = (0, \; \cdots \; m^* \; \cdots \; M)
\end{equation}

implying $(w, S^n) \neq 0$, which persists for small $\varepsilon > 0$, by continuity. \hfill \square

**Proof of Lemma 7.2** By linearity
\begin{equation}
w^T \frac{\partial^2 g(\bar{x}, \lambda^*)}{\partial x_h \partial x_k} (v_h v_k) = w^T \sum_{h,k} \frac{\partial^2 g}{\partial x_h \partial x_k} (v_h v_k)
= \sum_{h,k} w^T \frac{\partial^2 g}{\partial x_h \partial x_k} (v_h v_k).
\end{equation}

For any $m$ and $n$, a second derivative $f''_{jmn}$ appears only in the summand $w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n)$. Hence,
\begin{equation}
w^T \frac{\partial^2 g(\bar{x}, \lambda^*)}{\partial x_h \partial x_k} (v_h v_k) = 0 \quad \iff \quad \sum_{h,k} w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n) = 0 \text{ for any } m \text{ and } n.
\end{equation}

We show that there exists $m \in \mathbf{M}$ such that:
\begin{equation}
w^T \frac{\partial^2 g(\bar{x}, \lambda^*)}{\partial x_m \partial x_n} (v_m v_n) \neq 0.
\end{equation}

Indeed, since $\bar{r}'$ is a positive solution of $P(r') = 0$, there exist $m^*$ and $\eta$ such that
\begin{equation}
\rho^* := \bar{r}'_{jm*} = -\frac{1}{\sum_{i \in J} \alpha_{ij}} \frac{\sum_{j \in J} \alpha_{ij} \bar{r}'[\mathbf{J}]_{ij}}{\sum_{j \in J} \alpha_{ij} \bar{r}'[\mathbf{J} \setminus j]},
\end{equation}

with numerator and denominator of the same sign. In the proof of Theorem 4.1, we have shown how to construct such $\rho^*$ from two Child Selections of opposite behavior. Note that the $m^{th}$ column $G^m = \partial g / \partial x_m$ of the Jacobian $G$ has an identical symbolic structure as the $m$ column vector $g_{mm} := \partial^2 g / (\partial x_m)^2$. Indeed, every first derivative $r'_{jm}$ in $G^m$ is simply substituted with the second derivative $r''_{jm}$ in $g_{mm}$. This implies
\begin{equation}
w^T \frac{\partial^2 g(\bar{x}, \lambda^*)}{(\partial x_m)^2} = 0
\end{equation}
for any \( m \), if \( r'_{jm} = r'_{jmn} \), for every \( m \) and \( j \). Let us focus on \( m^* \). For \( (j, m) \neq (\eta, m^*) \), fix \( \hat{r}'_{jmn} = \hat{r}'_{jm} \), and let \( \hat{r}'_{jnm^*} \neq \rho^* \). Clearly, from (26) this choice of \( \hat{r}' \) implies

\[
 w^T \frac{\partial g^2(\bar{x}, \lambda^*)}{\partial x_m} (\hat{r}'') \neq 0.
\]

To conclude (25), we show that \( v_{m^*} \neq 0 \). Fix \( \hat{r}'_{jm} \) for \( (j, m) \neq (\eta, m^*) \) as in (26) and let \( \rho \) be the only variable. Then the univariate polynomial

\[
 P(\hat{r}') = P(\rho)
\]

is evaluated zero if and only if \( \rho = \rho^* \). But \( \rho \) appears only in the \( m^* \)th column of \( G \). Via \( G(\hat{r}') v = 0 \), we have \( v_{m^*} \neq 0 \).

**Proofs of Section 8**

**Proof of Theorem 8.1.** Via Lemma 6.3, if the network possesses an SN-pair of Child Selections, then there is a choice of \( \bar{x}, \bar{r}', \bar{r}' \) such that (16) is satisfied, with the Jacobian \( G(\bar{r}') \) possessing an algebraically simple eigenvalue zero. We can then choose \( (a, b) = (a, \hat{b}) \), with \( a, \hat{b} \) defined as in (18), (17), respectively. The choice of the bifurcation parameter

\[
 \lambda := b^0_{m^*}, \quad \text{with bifurcation value} \quad \lambda^* := b^0_{m^*},
\]

implies that

\[
 \partial_{\lambda} g = \frac{\partial f_{\eta}}{\partial \lambda} S^\eta,
\]

where \( S^\eta \) is the stoichiometric vector of reaction \( \eta \). Since \( \frac{\partial f_{\eta}}{\partial \lambda} \neq 0 \), Lemma 7.1 implies that the Michaelis-Menten system possesses a simple eigenvalue zero at \( \bar{x} \) for the choice \( (a, b) = (a, \hat{b}) \), which satisfies (SN1) and (SN2) of Theorem 1.1. \( \square \)

**Proof of Theorem 8.2.** Let us consider the \( \epsilon \)-rescaling of Lemma 5.1 for the SN-pair of Child Selections \( J_1, J_2 \). Until the last step of this proof, we consider the limit \( \epsilon = 0 \). Not to overload notation, we drop the constant reference to \( \epsilon \). At this limit, we recall that the only nonvanishing variables \( r'_{jm} \) are the ones such that

\[
 r'_{jm} = r'_{J_i(m) m}, \quad \text{with } i = 1, 2.
\]

Note that distance \( d(J_1, J_2) = 1 \) and injectivity of Child Selections imply

(27)

\[
 r'_{jm} r'_{jn} = 0
\]

for any reaction \( j \) and \( m \neq n \).

At \( \epsilon = 0 \), we can proceed as in the proof of Lemma 6.3. For a proper choice of \( \hat{r}' \) we obtain an algebraically simple eigenvalue zero. Note that

(28)

\[
 P(\hat{r}') = \det G(\hat{r}') = (a_{J_1} r'_{\eta m^*} + a_{J_2} r'_{j_2 m^*}) r'[J_1 \setminus \eta] = 0,
\]

if and only if

\[
 a_{J_1} r'_{\eta m^*} = -a_{J_2} r'_{j_2 m^*}.
\]
Let \( v, w \) be right and left kernel vectors of \( G(\mathbf{r}') \), respectively. Let \( G^m \) indicate the \( m^{th} \) column of the Jacobian \( G \). At the limit \( \varepsilon = 0 \) we have

\[
\begin{align*}
G^m &= S^{J_1(m)} r_{J_1(m)}' \quad \text{for } m \neq m^*; \\
G^{m^*} &= S^{J_2} r_{J_2}'+S^{J_2} r_{J_2^m}.'
\end{align*}
\]

Consequently, \( w^T G(\mathbf{r}') = 0 \) yields

\[
\begin{align*}
w^T G^m &= w^T S^{J_1(m)} r_{J_1(m)}' = 0 \quad \text{for } m \neq m^*; \\
w^T G^{m^*} &= w^T (S^{J_2} r_{J_2}'+S^{J_2} r_{J_2^m}'). = 0.
\end{align*}
\]

In particular, this implies

\[ \tag{29} \begin{cases} w^T S^{J_1(m)} = 0 & \text{for } m \neq m^*; \\
w^T (S^{J_2} r_{J_2}'+S^{J_2} r_{J_2^m}'). = 0 & \text{if and only if } \alpha_{J_1} r_{J_2}'+\alpha_{J_2} r_{J_2^m}.' \neq 0. \end{cases} \]

Moreover, \( (28) \) and \( G(\mathbf{r}) v = 0 \) imply

\[ \forall m^* \neq 0. \]

Let us now fix \( \bar{x} \) and \( (a, b) = (a, \bar{b}) \) as in \( (16), (17), (18). \) For this parameter choice, a straightforward computation yields:

\[
\begin{align*}
f''_{J_1(m)mm} &= \frac{-2r_{J_1(m)m}'}{x_m} + \frac{(r_{J_1(m)m}')^2}{r_{J_1(m)m}} \left( 1 + \frac{1}{s_{J_1(m)}} \right); \\
f''_{J_2(m)nm} &= \frac{f''_{J_2(m)nm}}{r_j} \left( \frac{r_{J_2(m)n}'}{x_m} \right) \left( \frac{r_{J_2(m)n}'}{s_{J_2(m)}} \right) \left( \frac{J_2^m}{s_{J_2^m}} \right)
\end{align*}
\]

For every \( J_i, m, n \), \( (27) \) implies that \( f''_{J_i(m)mm} = 0 \) and hence all mixed second derivatives \( g''_{mn} \) are zero, at \( \varepsilon = 0. \) This yields

\[
w^T \partial^2_x g[v, v] = w^T \sum_{m} \sum_{n} g''_{mn} v_m v_n
\]

\[
= w^T \left( S^{J_2} r_{J_2}'+S^{J_2} r_{J_2^m}.' \right) (v_m) (v_m) + w^T S^{J_1(m)} f_{J_1mm} (v_m)^2
\]

\[
= w^T \left( S^{J_2} r_{J_2}'+S^{J_2} r_{J_2^m}.' \right) (v_m) (v_m) + w^T S^{J_1(m)} f_{J_1mm} (v_m)^2.
\]

Via \( (29), \) thus,

\[
w^T \partial^2_x g[v, v] = 0 \quad \text{if and only if } \alpha_{J_1} f''_{J_2^m} + \alpha_{J_2} f''_{J_2^m} \neq 0. \]

We compute:

\[
\begin{align*}
\alpha_{J_1} f''_{J_2^m} + \alpha_{J_2} f''_{J_2^m} &= \alpha_{J_1} \left( \frac{-2r_{J_2^m}}{x_m} + \frac{(r_{J_2^m}')^2}{r_{J_2^m}} \left( 1 + \frac{1}{s_{J_2^m}} \right) \right) + \alpha_{J_2} \left( \frac{-2r_{J_2^m}}{x_m} + \frac{(r_{J_2^m}')^2}{r_{J_2^m}} \left( 1 + \frac{1}{s_{J_2^m}} \right) \right)
\]

\[
= \alpha_{J_2} \frac{r_{J_2^m}r_{J_2^m}'}{r_{J_2}'} \left( 1 + \frac{1}{s_{J_2^m}} \right) + \alpha_{J_1} \frac{r_{J_2^m}r_{J_2^m}'}{r_{J_2}'} \left( 1 + \frac{1}{s_{J_2^m}} \right)
\]

\[
= \frac{r_{J_2^m}r_{J_2^m}'}{r_{J_2}'} \left( \alpha_{J_2} \left( 1 + \frac{1}{s_{J_2^m}} \right) + \alpha_{J_1} \left( 1 + \frac{1}{s_{J_2^m}} \right) \right).
\]
which is nonzero if and only if

$$\frac{\alpha J_2}{r_\eta} \left( 1 + \frac{1}{s_{m^*}} \right) \neq -\frac{\alpha J_1}{r J_2} \left( 1 + \frac{1}{s_{m^*}} \right).$$

By continuity, $w^T \partial^2 g[v, v] \neq 0$ also for small positive $\varepsilon > 0$. Hence, we find Michaelis-Menten functions $f(x, \lambda)$ satisfying all conditions (SN1)--(SN3) of Theorem 1.1. □

**Proof of Theorem 8.3.** For Hill’s kinetics (14), we can use parameters $a, b, c$. We proceed analogously as in the proof of Theorem 8.2, considering the $\varepsilon$-rescaling of the variables $r'_{jm}$ of Lemma 5.1 at the limit $\varepsilon = 0$. Again not to overload notation, we omit the explicit dependency on $\varepsilon$. Differently from Theorem 8.2, however, we do not have any assumption on the distance $d$. We again fix $(a(c), b(c)) = (a(c), b(c))$ as in (16), (17), (18), now explicitly including the dependence on parameters $c$. We compute again the second derivatives of (14) and obtain

$$f''_{jmm}(c) = \frac{-2c^2_{c'_{j'}}r_{j'}}{x_m} + \left( \frac{r'_{jm}}{r_j} \right)^2 \left( 1 + \frac{\varepsilon c_{c'_{j'}}}{s_m} \right);$$

$$f''_{jmn}(c) = f''_{jnm} = \frac{r'_{jm} r'_{jn}}{r_j}.$$

At the limit $\varepsilon = 0$, $r'_{jm} = 0$ implies $f''_{jmm} = 0$, $f''_{jmn} = 0$, for any choice $c$ and any $n$. Moreover, a parameter $c_{j'}^c$ appears only in the second derivative $f''_{jmm}$. In particular, the mixed derivatives $f''_{jmn}$ do not depend on the parameters $c$:

$$\frac{\partial f''_{jmn}(c)}{\partial c_k} \equiv 0, \quad \text{for any } h \text{ and } k,$$

while on the contrary,

$$\frac{\partial f''_{jmn}(c)}{\partial c_n^h} \neq 0, \quad \text{if and only if } h = j, n = m.$$
We focus on the parameter $c_{m^*}^{n}$. At $\varepsilon = 0$ we have
\[
\begin{align*}
& w^T \partial^2_{x} g(\bar{x}, \lambda^*) [v, v](c_{m^*}^{n}), \\
& = w^T \sum_{m,n} \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n)(c_{m^*}^{n}), \\
& = \sum_{m,n} w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n)(c_{m^*}^{n}) \\
& = \sum_{m} w^T \frac{\partial^2 g}{(\partial x_m)^2} (v_m)^2 (c_{m^*}^{n}) + \sum_{m,n} w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n) \\
& = w^T \frac{\partial^2 g}{(\partial x_m)^2} (v_m)^2 (c_{m^*}^{n}) \\
& + \sum_{m,m^*} w^T \frac{\partial^2 g}{(\partial x_m)^2} (v_m)^2 + \sum_{m,m^*} w^T \frac{g}{\partial x_m} (v_m v_n) \\
& = w^T S^a f''_{jm^*m^*} (c_{m^*}^{n}) \\
& + w^T S^a f''_{m^*m^*} + \sum_{m,m^*} w^T \frac{\partial^2 g}{(\partial x_m)^2} (v_m)^2 + \sum_{m,n} w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n).
\end{align*}
\]

We define
\[
K := w^T S^a f''_{jm^*m^*} + \sum_{m,m^*} w^T \frac{\partial^2 g}{(\partial x_m)^2} (v_m)^2 + \sum_{m,n} w^T \frac{\partial^2 g}{\partial x_m \partial x_n} (v_m v_n).
\]

The constant $K$ does not depend on $c_{m^*}^{n}$, and hence $c_{m^*}^{n}$ can be used to nudge $w^T \partial^2_{x} g[v, v]$ away from the degeneracy in the following way. Let us arbitrarily pick a choice $\tilde{c}$. For $(a(\tilde{c}), b(\tilde{c}), \tilde{c})$, if the equilibrium $\bar{x}$ possesses a singular Jacobian $G$ with left kernel vector $w$ and right kernel vector $v$ such that
\[
w^T \partial_{x} g[v, v](\tilde{c}) \neq 0,
\]
we are done. Otherwise, let us assume
\[
w^T \partial_{x} g[v, v](\tilde{c}) = 0.
\]

Let us choose $\tilde{c}$ such that
\[
\begin{align*}
\tilde{c}_m^j = \tilde{c}_m^n & \quad \text{for } (j, m) \neq (\eta, m^*); \\
\tilde{c}_m^j \neq \tilde{c}_m^n & \quad \text{for } (j, m) = (\eta, m^*).
\end{align*}
\]

As previously noted, the parameter $c_{m^*}^{n}$ appears only in $f''_{jm^*m^*}$ and hence
\[
\begin{align*}
& w^T \partial^2_{x} g(\bar{x}, \lambda^*) [v, v](\tilde{c}) - w^T \partial^2_{x} g(\bar{x}, \lambda^*) [v, v](\tilde{c}) \\
& = w^T S^a f''_{jm^*m^*} (\tilde{c}_{m^*}^{n}) - w^T S^a f''_{jm^*m^*} (\tilde{c}_{m^*}^{n}) \neq 0,
\end{align*}
\]

implying
\[
w^T \partial^2_{x} g(\bar{x}, \lambda^*) [v, v](\tilde{c}) \neq 0.
\]

By continuity, this extends to small $\varepsilon > 0$. □
**Proof of Theorem 4.2 and Corollary 4.3**

**Proof of Theorem 4.2.** The Theorem is just a corollary of Theorem 8.3. Hill’s kinetics is indeed a specific example of monotone chemical functions, which proves the theorem. □

**Proof of Corollary 4.3.** We have only to check that the pair \((\tilde{J}_1, \tilde{J}_2)\) satisfies Definition 6 of SN-pair of Child Selections of \(\Gamma\). Condition (1) is satisfied by assumption. Condition (2) is inherited from the SN-pair of Child Selections \(J_1, J_2\) of \(\Gamma\). In fact, note that

\[
S_{J_3} = \begin{pmatrix} S_{J_1} & B \\ 0 & D \end{pmatrix}
\quad \text{and} \quad
S_{J_4} = \begin{pmatrix} S_{J_2} & B \\ 0 & D \end{pmatrix},
\]

where

\[
\begin{pmatrix} B \\ D \end{pmatrix}
\]

indicates the stoichiometric matrix of the Child Selections \(S_{J_3}\) and \(S_{J_4}\) relative to the reactions \(J_3(m) = J_4(m)\) for \(m \in \tilde{M} \setminus M\). Hence,

\[
\begin{cases}
0 \neq \alpha_{J_1} = \det S_{J_3} = \alpha_{J_1} \det D \\
0 \neq \alpha_{J_2} = \det S_{J_4} = \alpha_{J_2} \det D
\end{cases}
\]

implying \(\alpha_{J_1} \alpha_{J_2} < 0\). Condition 3 is satisfied by considering the same species \(m^*\) and the same nonzero Partial Child Selection \(J_{vm^*}\) for the SN-pair \(J_1, J_2\) of \(\Gamma\) and extending it to a nonzero Partial Child Selection \(\tilde{J}_{vm^*}\) of \(\tilde{\Gamma}\) defined as

\[
\begin{cases}
\tilde{J}_{vm^*}(m) = J_{vm^*}(m) & \text{for } m \in M \setminus \{m^*\} \\
\tilde{J}_{vm^*}(m) = \tilde{J}_1(m) = \tilde{J}_2(m) & \text{for } m \in \tilde{M} \setminus M
\end{cases}
\]

For \(\tilde{J}_{vm^*}\) it holds:

\[
\beta_{\tilde{J}_{vm^*}} = \det S_{\tilde{J}_{vm^*}} = \det S_{J_{vm^*}} \neq 0,
\]

concluding the proof. □

**References**

[1] J. L. Andersen, C. Flamm, D. Merkle, and P. F. Stadler, *Defining autocatalysis in chemical reaction networks*, Journal of Systems Chemistry, 8 (2020), pp. 121–133.

[2] V. I. Arnold, *Geometrical methods in the theory of ordinary differential equations*, vol. 250, Springer Science & Business Media, 2012.

[3] R. Baire, *Sur les fonctions de variables réelles*, Annali di Matematica Pura ed Applicata (1898-1922), 3 (1899), pp. 1–123.

[4] M. L. Balinski, *On the graph structure of convex polyhedra in n-space*, Pacific Journal of Mathematics, 11 (1961), pp. 431–434.

[5] M. Banaji, *Inheritance of oscillation in chemical reaction networks*, Applied Mathematics and Computation, 325 (2018), pp. 191–209.

[6] M. Banaji and G. Craciun, *Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems*, Advances in Applied Mathematics, 44 (2010), pp. 168–184.

[7] M. Banaji and C. Pantea, *Some results on injectivity and multistationarity in chemical reaction networks*, SIAM Journal on Applied Dynamical Systems, 15 (2016), pp. 807–869.

[8] ———, *The inheritance of nondegenerate multistationarity in chemical reaction networks*, SIAM Journal on Applied Mathematics, 78 (2018), pp. 1105–1130.
[9] B. Brehm and B. Fiedler, *Sensitivity of chemical reaction networks: a structural approach. 3. Regular multimolecular systems*, Mathematical Methods in the Applied Sciences, 41 (2018), pp. 1344–1376.

[10] C. Chassagnole, N. Noisommit-Rizzi, J. W. Schmid, K. Mauch, and M. Reuss, *Dynamic modeling of the central carbon metabolism of Escherichia coli*, Biotechnology and bioengineering, 79 (2002), pp. 53–73.

[11] C. Conradi, E. Feliu, M. Mincheva, and C. Wiuf, *Identifying parameter regions for multistationarity*, PLoS computational biology, 13 (2017), p. e1005751.

[12] C. Conradi, D. Flockerzi, and J. Raisch, *Saddle-node bifurcations in biochemical reaction networks with mass action kinetics and application to a double-phosphorylation mechanism*, in 2007 American control conference, IEEE, 2007, pp. 6103–6109.

[13] G. Craciun and M. Feinberg, *Multiple equilibria in complex chemical reaction networks: I. The injectivity property*, SIAM Journal on Applied Mathematics, 65 (2005), pp. 1526–1546.

[14] ------, *Multiple equilibria in complex chemical reaction networks: II. The species-reaction graph*, SIAM Journal on Applied Mathematics, 66 (2006), pp. 1321–1338.

[15] A. Dieckstein, M. P. Millan, A. Shiu, and X. Tang, *Multistationarity in structured reaction networks*, Bulletin of Mathematical Biology, 81 (2019), pp. 1527–1581.

[16] M. Domijan and M. Kirkilionis, *Bistability and oscillations in chemical reaction networks*, Journal of Mathematical Biology, 59 (2009), pp. 467–501.

[17] E. Feliu, N. Kairnsa, T. de Wolff, and O. Yürük, *The kinetic space of multistationarity in dual phosphorylation*, Journal of Dynamics and Differential Equations, (2020), pp. 1–28.

[18] E. Feliu, A. D. Rendall, and C. Wiuf, *A proof of unlimited multistability for phosphorylation cycles*, Nonlinearity, 33 (2020), p. 5629.

[19] B. Fiedler, *Global Hopf bifurcation in networks with fast feedback cycles*, Discrete and Continuous Dynamical Systems - S, 0 (2020).

[20] J. Guckenheimer and P. Holmes, *Nonlinear oscillations, dynamical systems and bifurcations of vector fields*, Springer, 1984.

[21] J. Hell and A. D. Rendall, *A proof of bistability for the dual futile cycle*, Nonlinear Analysis: Real World Applications, 24 (2015), pp. 175–189.

[22] A. V. Hill, *The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves*, J. Physiol., 40 (1910), pp. 4–7.

[23] F. Horn and R. Jackson, *General mass action kinetics*, Archive for Rational Mechanics and Analysis, 47 (1972), pp. 81–116.

[24] N. Ishii, K. Nakahigashi, T. Baba, M. Robert, T. Soga, A. Kanai, T. Hirasesawa, M. Naba, K. Hirai, A. Hoque, et al., *Multiple high-throughput analyses monitor the response of E. coli to perturbations*, Science, 316 (2007), pp. 593–597.

[25] B. Joshi and A. Shiu, *Atoms of multistationarity in chemical reaction networks*, Journal of Mathematical Chemistry, 51 (2013), pp. 153–178.

[26] H. Kornberg and H. A. Krebs, *Synthesis of cell constituents from c2-units by a modified tricarboxylic acid cycle*, Nature, 179 (1957), pp. 988–991.

[27] L. Michaelis and M. L. Menten, *Die kinetik der invertnwirkung*, Biochem. Z., 49 (1913), pp. 333–369.

[28] M. Mincheva and M. R. Roussel, *Graph-theoretic methods for the analysis of chemical and biochemical networks. 1. Multistationarity and oscillations in ordinary differential equation models*, Journal of mathematical biology, 55 (2007), pp. 61–86.

[29] M. A. Nowak, *Evolutionary dynamics: exploring the equations of life*, Harvard university press, 2006.

[30] T. Okada, A. Mochizuki, M. Furuta, and J.-C. Tsai, *Flux-augmented bifurcation analysis in chemical reaction network systems*, Physical Review E, 103 (2021), p. 062212.

[31] I. Otero-Muras and J. R. Banga, *Optimization-based prediction of fold bifurcations in nonlinear ODE models*, IFAC-PapersOnLine, 51 (2018), pp. 485–490.
[32] A. D. Rendall and E. D. Sontag, *Multiple steady states and the form of response functions to antigen in a model for the initiation of T-cell activation*, Royal Society Open Science, 4 (2017), p. 170821.

[33] P. Schuster, *What is special about autocatalysis?*, Monatshefte für Chemie-Chemical Monthly, 150 (2019), pp. 763–775.

[34] A. Shiu and T. de Wolff, *Nondegenerate multistationarity in small reaction networks*, Discrete & Continuous Dynamical Systems-B, 24 (2019), p. 2683.

[35] J. Sotomayor, *Generic bifurcations of dynamical systems*, in Dynamical systems, Elsevier, 1973, pp. 561–582.

[36] C. Soulé, *Graphic requirements for multistationarity*, ComPlexUs, 1 (2003), pp. 123–133.

[37] R. Thomas, *On the relation between the logical structure of systems and their ability to generate multiple steady states or sustained oscillations*, Numerical methods in the study of critical phenomena, (1981), pp. 180–193.

[38] R. Thomas and M. Kaufman, *Multistationarity, the basis of cell differentiation and memory. I. Structural conditions of multistationarity and other nontrivial behavior*, Chaos: An Interdisciplinary Journal of Nonlinear Science, 11 (2001), pp. 170–179.

[39] A. Vanderbauwhede, *Centre manifolds, normal forms and elementary bifurcations*, in Dynamics reported, Springer, 1989, pp. 89–169.

[40] N. Vassena, *Good and bad children in metabolic networks*, Mathematical Biosciences and Engineering, 17 (2020), pp. 7621–7644.

[41] N. Vassena, *Structural obstruction to the simplicity of the eigenvalue zero in chemical reaction networks*, arXiv preprint arXiv:2205.12655, (2022).