Relating Attractors and Singular Steady States in the Logical Analysis of Bioregulatory Networks*

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Abstract. In 1973 R. Thomas introduced a logical approach to modeling and analysis of bioregulatory networks. Given a set of Boolean functions describing the regulatory interactions, a state transition graph is constructed that captures the dynamics of the system. In the late eighties, Snoussi and Thomas extended the original framework by including singular values corresponding to interaction thresholds. They showed that these are needed for a refined understanding of the network dynamics. In this paper, we study systematically singular steady states, which are characteristic of feedback circuits in the interaction graph, and relate them to the type, number and cardinality of attractors in the state transition graph. In particular, we derive sufficient conditions for regulatory networks to exhibit multistationarity or oscillatory behavior, thus giving a partial converse to the well-known Thomas conjectures.

1 Introduction

Suggested more than 30 years ago, the logical approach to modeling bioregulatory networks has become increasingly popular in the recent past. In the Boolean setting, components of the networks correspond to variables, which can take the values 0 and 1. Interactions between the components are described by logical equations capturing the evolution of the system. R. Thomas contributed a number of papers on the logical analysis of biological networks, starting with [8]. The distinctive feature of his method is the way he derives a representation of the dynamics from the given Boolean functions. Rather than executing all indicated changes in the components at the same time, an asynchronous updating rule is employed to obtain a non-deterministic state transition graph. It has been shown that this approach captures essential qualitative features of the dynamical behavior of complex biological networks, see [9] and [10] for an overview.

In the following years the framework was extended to allow not only for Boolean but multi-valued variables that describe different activity levels of the regulatory components in the network. Each interaction in the network was

* Supported by the DFG Research Center Matheon "Mathematics for key technologies"

associated with a unique threshold value, which determines when the interaction becomes effective. Snoussi and Thomas realized that a closer inspection of the impact of the threshold values, which they called singular values, would further improve the understanding of the system's dynamics. In [6] they introduced the notion of singular steady states and linked them to feedback circuits in the interaction graph describing the structure of the network. The importance of feedback circuits for the analysis of the dynamical behavior has long been recognized. Thomas conjectured in 1981 that the existence of a positive circuit, resp. a negative circuit, in the interaction graph is a necessary condition for the existence of two distinct attractors, resp. an attractor with cycle structure, in the state transition graph. The conjectures have been proven in different settings (see e.g. [7], [3] and [4]). In the framework of Thomas and Snoussi it is possible to specify the parameters of a given bioregulatory system such that the existence of the appropriate circuit is not only necessary but sufficient to cause the corresponding behavior in the state transition graph. However, those specifications may essentially amount to deleting certain interactions from the network, which is not always desirable.

When trying to incorporate Snoussi's and Thomas' idea of singular states in a Boolean framework, we are faced with several difficulties. On this level of abstraction, every interaction is associated with the same threshold value, a symbolic value between 0 and 1. Thus when crossing the threshold we do not have the advantage of knowing that one and only one interaction becomes effective. As a result we cannot link singular states to circuits in the interaction graph in a non-ambiguous way, while still preserving some essential features known from the multi-valued setting. Despite those complications and the high level of abstraction, this paper shows that the introduction of singular states is a useful tool for refining our understanding of the relation between structure and dynamics of bioregulatory networks.

The organization of the paper is as follows. In Section 2 we give a short overview of the Boolean description of biological networks and introduce the notion of an attractor of a state transition graph. In Section 3 we extend the framework by establishing the concept of singular states. We give different characterizations of singular steady states using the notion of circuit characteristic states and regular adjacent states. In the main section of this paper, we prove several statements that allow us to derive information on the attractors of the state transition graph from the existence of singular steady states. Conversely, we can deduce the existence of a singular steady state if we have specific knowledge about the attractors of the state transition graph. We conclude by outlining ideas for future work.

2 Structure and Dynamics of Regulatory Networks

In the following we introduce the Boolean formalism of R. Thomas for modeling regulatory networks (see for example [9]). We mainly use the notation introduced in [1] and [5]. Throughout the text $\mathcal{B}$ will denote the set $\{0, 1\}$.

Definition 1. *An* interaction graph *(or bioregulatory graph) $\mathcal{I}$ is a labeled directed graph with vertex set $V := \{\alpha_1, \ldots, \alpha_n\}$, $n \in \mathbb{N}$, and edge set E. Each edge $\alpha_j \to \alpha_i$ is labeled with a sign $\varepsilon_{ij} \in \{+, -\}$.*

The only information on a regulatory component we incorporate in the model for now is whether or not it is active. A vertex α_i can be seen as a variable that adopts values in $\mathcal{B}$, where the value 1 indicates that the component is active. By abuse of notation, we identify each vertex α_i with its index i in order to simplify notation.

An edge $\alpha_j \to \alpha_i$ signifies that α_j influences α_i in a positive or negative way depending on the sign ε_{ij}. For each α_i we denote by $Pred(\alpha_i)$ the set of *predecessors* of α_i, i. e., the set of vertices α_j such that $\alpha_j \to \alpha_i$ is an edge in E.

We will be mainly interested in the following structures of the interaction graph. A tuple $(\alpha_{i_1}, \ldots, \alpha_{i_k})$ of distinct vertices of $\mathcal{I}$ is called a *circuit* if $\mathcal{I}$ contains an edge from α_{i_j} to $\alpha_{i_{j+1}}$ for all $j \in \{1, \ldots, k-1\}$ as well as an edge from α_{i_k} to α_{i_1}. The *sign* of a circuit is the product of the sign of its edges.

Definition 1 captures structural aspects of the network. In the following we consider the corresponding dynamical behavior.

Definition 2. *Let $\mathcal{I}$ be an interaction graph comprising n vertices. A* state *of the system described by $\mathcal{I}$ is a tuple $s \in \mathcal{B}^n$. The set of (regular)* resources *$R_i(s) = R_i^{\mathcal{I}}(s)$ of α_i in state s is the set*

$$\{\alpha_j \in Pred(\alpha_i)\,;\, (\varepsilon_{ij} = + \,\wedge\, s_j = 1) \vee (\varepsilon_{ij} = - \,\wedge\, s_j = 0)\}.$$

Given a set

$$K(\mathcal{I}) := \{K_{i,\omega}\,;\, i \in \{1, \ldots, n\}, \omega \subset Pred(\alpha_i)\}$$

of (logical) parameters*, which adopt values in $\mathcal{B}$, we define the Boolean function $f = f^{K(\mathcal{I})} : \mathcal{B}^n \to \mathcal{B}^n$, $s \mapsto (K_{1,R_1(s)}, \ldots, K_{n,R_n(s)})$. The pair $N := (\mathcal{I}, f)$ is called* bioregulatory network*.*

The set of resources $R_i(s)$ provides information about the presence of activators and the absence of inhibitors for some regulatory component α_i in state s. The value of the parameter $K_{i,R_i(s)}$ indicates how the level of activity α_i will evolve. It will increase (decrease) if the parameter value is greater (smaller) than s_i. The activity level stays the same if both values are equal. Thus, the function f maps a state s to the state the system tends to evolve to.

The choice of parameters specifies the model given by the graph $\mathcal{I}$. Depending on their values, edges in the graph may or may not be *functional* in the following sense. Clearly, if there is an edge $\alpha_j \to \alpha_i$ and $K_{i,M} = K_{i,M\setminus\{\alpha_j\}}$ for all $M \subset Pred(\alpha_i)$, then the edge $\alpha_j \to \alpha_i$ has no influence on the dynamics of the system. Eliminating this edge from the interaction graph does not change the function f. Thus we may assume for every $N := (\mathcal{I}, f)$ that whenever there is an edge $\alpha_j \to \alpha_i$ in $\mathcal{I}$, there exists a set $M \subset Pred(\alpha_i)$ such that $K_{i,M} \neq K_{i,M\setminus\{\alpha_j\}}$.

To derive the dynamics of the system from the function f we take the following consideration into account. In a biological system, the time delays corresponding to changes in the activity level of distinct components will most likely

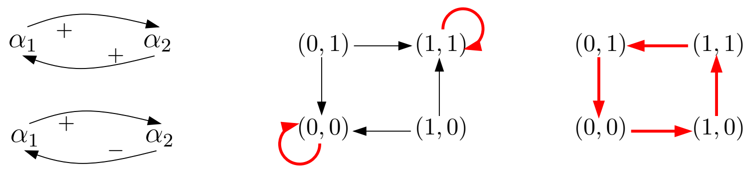

Fig. 1. Two interaction graphs consisting of a positive resp. a negative circuit. In both cases we choose $K_{1,\{2\}} = K_{2,\{1\}} = 1$ and $K_{1,\emptyset} = K_{2,\emptyset} = 0$. The state transition graph corresponding to the positive circuit is in the middle, the one corresponding to the negative circuit is on the right. Attractors are indicated by colored, fat lines.

differ. Thus we may assume that in each state transition at most one component is modified. This procedure is called *asynchronous update* in Thomas' framework. We obtain the following definition.

Definition 3. *The* state transition graph $\mathcal{S}_N$ *describing the dynamics of the network N is a directed graph with vertex set $\mathcal{B}^n$. There is an edge $s \to s'$ if and only if $s' = f(s) = s$ or $s'_i = f_i(s)$ for some $i \in \{1, \dots, n\}$ satisfying $s_i \neq f_i(s)$ and $s'_j = s_j$ for all $j \neq i$.*

In the following we introduce some basic structures in this graph that are of biological interest. In addition we use standard terminology from graph theory, such as paths and cycles.

Definition 4. *Let $\mathcal{S}_N$ be a state transition graph. An infinite path $(s_0, s_1, s_2, \dots)$ in $\mathcal{S}_N$ is called* trajectory*. A nonempty set of states A is called* attractor *if for any $s^1, s^2 \in A$ there is a path from s^1 to s^2 in $\mathcal{S}_N$ and if every trajectory starting in a state in A never leaves A. A state s^0 is called* steady state*, if s^0 is a fixed point of f, that is, if there is an edge from s^0 to itself. A cycle $C := (s^1, \dots, s^r, s^1)$, $r \geq 2$, is called a* trap cycle *if every s^j, $j \in \{1, \dots, r\}$, has only one outgoing edge in $\mathcal{S}_N$, that is, the trajectory starting in s^1 is unique.*

It is easy to see that steady states and trap cycles are attractors. In Figure 1 we show two simple interaction graphs. The positive circuit generates a state transition graph with two steady states. The graph derived from the negative circuit consists of a trap cycle, that is, we find an attractor of cardinality greater than one.

Attractors represent regions of predictability and stability in the behavior of the system. It is not surprising that an attractor can often be associated with a meaningful aspect of the system's role in biological processes. A fixed point in a gene regulatory network associated with cell differentiation, for example, may represent the stable state reached at the end of a developmental process. Attractors of cardinality greater than one imply cyclic behavior, and thus can often be identified with homeostasis of sustained oscillatory activity, as can be found in the cell cycle or circadian rhythm.

The following proposition is an easy observation concerning attractors.

Proposition 1. *In every state transition graph $\mathcal{S}_N$ there is at least one attractor.*

Proof. Since $\mathcal{S}_N$ consists of finitely many states, there exists for every trajectory $T = (s^0, s^1, \dots)$ some $m_T \in \mathbb{N}$ such that the set $\{s^0, s^1, \dots, s^{m_T}\}$ is the set of all states traversed by T. Moreover, for any state s^0 we can find a trajectory $T_0 = (s^0, s^1, \dots)$ such that for every trajectory T that coincides with T_0 in the first $m := m_{T_0}$ states the set of states traversed by T is contained in $M := \{s^0, s^1, \dots, s^m\}$, that is T_0 is maximal in that sense. Let $B := \bigcap_{k \in \mathbb{N}} \{s^i \,;\, i \geq k\}$ be the set of states that T_0 visits infinitely many times. Then B is not empty since $\mathcal{S}_N$ is finite. Let C be the set of states s such that there exists a path from a state in B to s as well as a path from s to a state in B.

Let $A := B \cup C$. It is easy to see that for every $x^1, x^2 \in B$ there exists a path from x^1 to x^2 such that every state on the path is also contained in B. It follows that there exists a path from x^1 to x^2 in $\mathcal{S}_N$ for all $x^1, x^2 \in A$. Since we chose T_0 maximal w. r. t. the set of states visited by T_0, we can deduce that C is a subset of M. If there was a trajectory that starts in a state in A and leaves A at some point we could easily construct a trajectory that violates the maximality of T_0. Thus the set A is an attractor. □

Note that the above proof shows that for every state in the state transition graph there is a trajectory leading to an attractor.

The number of states in the state transition graph grows exponentially with the number of regulatory components in N. Thus our aim is to infer from $N = (\mathcal{I}, f)$ as much information on the structure of $\mathcal{S}_N$ as possible, without having to calculate $\mathcal{S}_N$ explicitly. We pursue this endeavor in the remaining sections.

3 Singular States

In the following, we incorporate threshold values of interactions into the formalism. We mainly use the framework introduced in [5].

Definition 5. *Set $\mathcal{B}_\theta := \{0, \theta, 1\}$, where θ is a symbolic representation of the threshold value and satisfies the order $0 < \theta < 1$. We allow each regulatory component α_i to take values in $\mathcal{B}_\theta$. The values 0 and 1 are called* regular values *and θ is called* singular value*. The elements of $\mathcal{B}_\theta^n$ are called* states*. If a state comprises only regular components it is called* regular state*. Otherwise it is called* singular state*. For every state s we define $J(s) := \{i \in \{1, \dots, n\} \,;\, s_i = \theta\}$.*

To describe the dynamics of the system we have to augment the definition of resources.

Definition 6. *Let $s \in \mathcal{B}_\theta^n$. In addition to the set $R_i(s)$ of regular resources introduced in Definition 2, we define the set $R_i^\theta(s)$ of* singular resources *of α_i in s as the set*

$$R_i^\theta(s) := \{\alpha_j \in Pred(\alpha_i) \,;\, s_j = \theta\}.$$

The definition of a set of logical parameters $K(\mathcal{I})$ remains the same as in Definition 2. In particular, the logical parameters can only adopt regular values.

However, we need further restrictions for the choice of parameters. In the following every set of logical parameters satisfies

$$\omega \subset \omega' \Rightarrow K_{i,\omega} \leq K_{i,\omega'} \tag{1}$$

for all $i \in \{1, \ldots, n\}$. The condition signifies that an effective activator or a non-effective inhibitor cannot induce the decrease of the activity level of α_i. These constraints are needed to define a function f^θ describing the dynamics of the network.

We call $|a, b|$ a *qualitative value* if $a, b \in \mathcal{B}$ and $a \leq b$. The qualitative value $|0, 0|$ is identified with the regular value 0, $|1, 1|$ with the regular value 1, and $|0, 1|$ with the singular value θ. The relations $<$, $>$, and $=$ are used with respect to this identification.

Definition 7. *Let $K(\mathcal{I})$ be a set of parameters. We define*

$$f^\theta = f^{K(\mathcal{I}),\theta} : \mathcal{B}_\theta^n \to \mathcal{B}_\theta^n \quad \text{by} \quad f_i^\theta(s) = |K_{i,R_i(s)}, K_{i,R_i(s) \cup R_i^\theta(s)}|$$

for all $i \in \{1, \ldots, n\}$.

Note that whenever s is a regular state, then $f^\theta(s)$ is regular, too, since any set of singular resources in a regular state is empty. We have $f^\theta(s) = f(s)$ for all $s \in \mathcal{B}^n$. Thus the state transition graph corresponding to $N = (\mathcal{I}, f)$ is consistent with f^θ. Extending the definition in the previous section, we call s a *steady state* if $f^\theta(s) = s$. The notion of functionality of an edge remains the same as in Section 2. We consider only those edges that effectively influence the dynamical evolution of the system.

We may relate a singular state s to structures in the interaction graph $\mathcal{I}$ by considering the subgraphs of $\mathcal{I}$ induced by the vertices α_j with singular values, that is $j \in J(s)$. The following definition proves useful and was first introduced by E. H. Snoussi in [6], albeit in a different framework. The remainder of this section adapts ideas presented in [6].

Definition 8. *Let $C = (\alpha_{i_1}, \ldots, \alpha_{i_r})$ be a circuit in $\mathcal{I}$. A state $s \in \mathcal{B}_\theta^n$ is called* characteristic state *of C if $s_{i_l} = \theta$ for all $l \in \{1, \ldots, r\}$.*

A characteristic state of a circuit is not unique unless all the regulatory components of the network are contained in the circuit. In this case the state $(\theta, \ldots, \theta)$ is the unique characteristic state. Obviously, the state $(\theta, \ldots, \theta)$ is characteristic of each circuit in $\mathcal{I}$.

Another simple observation is the following. Whenever $R_j^\theta(s) \neq \emptyset$ holds for all singular components $j \in J(s)$, the state s is characteristic of some circuit in $\mathcal{I}$. This is due to the fact that every resource of some regulatory component α_i is a predecessor of α_i and that there are only finitely many components in the network. With that in mind we can easily prove the next statement.

Theorem 1. *Every singular steady state is characteristic of some circuit in $\mathcal{I}$.*

Proof. Let s be a singular state that is not characteristic of any circuit in $\mathcal{I}$. Then there is $i \in \{1, \ldots, n\}$ such that $s_i = \theta$ and $R_i^\theta(s) = \emptyset$. It follows that $f_i^\theta(s) = |K_{i,R_i(s)}, K_{i,R_i(s)}| = K_{i,R_i(s)} \neq \theta = s_i$, since the parameters take only regular values. Thus s is not a steady state. ☐

It is possible to give a characterization of the singular steady states using only regular states and the function f.

Definition 9. *Let $s \in \mathcal{B}_\theta^n$ and $k \in \{1, \ldots, n\}$. Let $s^{k,+}$ and $s^{k,-}$ be states that satisfy $s_i^{k,+} := s_i^{k,-} := s_i$ for all $i \notin J(s)$ and*

$$s_i^{k,+} := \begin{cases} 1 & , \quad \varepsilon_{ki} = + \\ 0 & , \quad \varepsilon_{ki} = - \end{cases} \quad \textit{and} \quad s_i^{k,-} := \begin{cases} 1 & , \quad \varepsilon_{ki} = - \\ 0 & , \quad \varepsilon_{ki} = + \end{cases} \tag{2}$$

for all $i \in J(s)$ satisfying $\alpha_i \in R_k^\theta(s)$. Then $s^{k,+}$ and $s^{k,-}$ are called a maximal *resp.* minimal adjacent state *of s with respect to k.*

There are generally many states $s^{k,+}$, $s^{k,-}$ that satisfy the above conditions. If the sets $R_k^\theta(s)$, $k \in \{1, \ldots, n\}$, are disjoint, then we can define states s^+ and s^- which are maximal resp. minimal adjacent states of s with respect to every $k \in \{1, \ldots, n\}$. If, in addition, the union of all sets $R_k^\theta(s)$ is equal to the set $\{\alpha_j \,;\, j \in J\}$, then s^+ and s^- are unique and are called the maximal resp. minimal adjacent state of s.

Theorem 2. *A state $s \in \mathcal{B}_\theta^n$ is steady if and only if $f_k(s^{k,+}) = s_k^{k,+} = s_k^{k,-} = f_k(s^{k,-})$ for all $k \notin J(s)$, and $f_k(s^{k,-}) < \theta < f_k(s^{k,+})$ for all $k \in J(s)$.*

Proof. We show that $R_k(s^{k,+}) = R_k(s) \cup R_k^\theta(s)$ and $R_k(s^{k,-}) = R_k(s)$ for all $k \in \{1, \ldots, n\}$.

First, let $\alpha_i \in R_k(s^{k,+})$. Then α_i is a predecessor of α_k. If $i \notin J := J(s)$, then $s_i = s_i^{k,+}$, and thus $\alpha_i \in R_k(s)$. If $i \in J$, we have $s_i = \theta$, and thus $\alpha_i \in R_k^\theta(s)$. Now, let $\alpha_i \in R_k(s) \cup R_k^\theta(s)$. Again $\alpha_i \in \mathit{Pred}(\alpha_k)$. If $\alpha_i \in R_k(s)$, then $i \notin J$. It follows that $s_i = s_i^{k,+}$, and thus $\alpha_i \in R_k(s^{k,+})$. If $\alpha_i \in R_k^\theta(s)$, then $\alpha_i \in R_k(s^{k,+})$ according to (2). Analogous reasoning provides the second statement.

Now, suppose that the last condition of the theorem is true. Then $f_k^\theta(s) = |K_{k,R_k(s)}, K_{k,R_k(s) \cup R_k^\theta(s)}| = |K_{k,R_k(s^{k,-})}, K_{k,R_k(s^{k,+})}| = |f_k(s^{k,-}), f_k(s^{k,+})|$ for all $k \in \{1, \ldots, n\}$. According to the assumption we have $|f_k(s^{k,-}), f_k(s^{k,+})| = s_k^{k,+} = s_k$ for $k \notin J$, and $|f_k(s^{k,-}), f_k(s^{k,+})| = |0, 1| = s_k$ for all $k \in J$. Thus s is a steady state. Similar reasoning can be employed to show the inverse statement. $\square$

The theorem and the definition of $s^{k,+}$ and $s^{k,-}$ imply that whenever every regulatory component in the network can be influenced in its behavior by some other regulatory components, the state containing only singular entries is a steady state. In other words, if for every α_k there exists $\omega \subset \mathit{Pred}(\alpha_k)$ such that $K_{\alpha_k,\emptyset} = 0$ and $1 = K_{\alpha_k,\omega} \leq K_{\alpha_k,\mathit{Pred}(\alpha_k)}$, then the state $(\theta, \ldots, \theta)$ is a steady state.

4 Relating Singular Steady States and Attractors

We have seen that singular steady states can be characterized by regular states and that they are closely related to circuits in the interaction graph. In the following we show what kind of information on the state transition graph we can infer from the existence of a singular steady state. First, we need some additional notations.

Let $s \in \mathcal{B}_\theta^n$ be a singular state. Recall that $J(s)$ is the set of components corresponding to the singular values of s. With $\mathcal{I}^\theta(s)$ we denote the graph with vertex set $V^\theta(s) := J(s)$ and edge set $E^\theta(s)$ consisting of those $\{\alpha_i, \alpha_j\}$ with $i, j \in J(s)$ such that $\alpha_i \to \alpha_j$ or $\alpha_j \to \alpha_i$ is an edge in $\mathcal{I}$. The graph $\mathcal{I}^\theta(s)$ is undirected. It represents the existence of a dependency between singular components, without specifying the type of interaction. A (connected) *component* of $\mathcal{I}^\theta(s)$ is a maximal connected subgraph of $\mathcal{I}^\theta(s)$. Vertices of different components of $\mathcal{I}^\theta(s)$ represent regulatory components in $\mathcal{I}$ that do not influence each other directly. Figure 2 illustrates the concept on a small example.

Let C be a circuit composed of vertices in $J(s)$. Then there is a component of $\mathcal{I}^\theta(s)$ which contains C. We denote that component by $J_C(s)$.

The following lemma shows that value changes in one component of $\mathcal{I}^\theta(s)$ do not influence the image $f^\theta(s)$ outside that component, if s is a singular steady state.

Lemma 1. *Let s be a singular steady state, and let $Z_1, \dots, Z_m$ be the components of the graph $\mathcal{I}^\theta(s)$. Choose $k \in \mathbb{N}$ and $\tilde{s} \in \mathcal{B}_\theta$ satisfying $\tilde{s}_i = s_i$ for all $i \notin Z_k$. Then $f_i^\theta(\tilde{s}) = \tilde{s}_i$ for all $i \notin Z_k$.*

Proof. For $i \in J(s) \setminus Z_k$ we know that $R_i(s) = R_i(\tilde{s})$ and $R_i^\theta(s) = R_i^\theta(\tilde{s})$, since no element of Z_k is a predecessor of α_i. Thus $f_i^\theta(\tilde{s}) = f_i^\theta(s) = s_i$ for all $i \in J(s) \setminus Z_k$. For $i \notin J(s)$ we have $R_i(s) \subset R_i(\tilde{s})$, since a singular resource of α_i may have turned into a regular resource. In addition, $R_i(\tilde{s}) \cup R_i^\theta(\tilde{s}) \subset R_i(s) \cup R_i^\theta(s)$, since a singular resource of α_i might have been eliminated by turning its value to a regular value not contributing to activation. In summary we obtain $R_i(s) \subset R_i(\tilde{s}) \subset R_i(\tilde{s}) \cup R_i^\theta(\tilde{s}) \subset R_i(s) \cup R_i^\theta(s)$ and with condition (1) we derive

$$K_{i,R_i(s)} \leq K_{i,R_i(\tilde{s})} \leq K_{i,R_i(\tilde{s}) \cup R_i^\theta(\tilde{s})} \leq K_{R_i(s) \cup R_i^\theta(s)}.$$

Moreover, we know that $K_{i,R_i(s)} = K_{i,R_i(s) \cup R_i^\theta(s)}$ since $f_i^\theta(s) = s_i$. Thus the above inequality becomes an equality and yields $f_i^\theta(\tilde{s}) = K_{i,R_i(s)} = s_i = \tilde{s}_i$ for all $i \notin J(s)$. $\square$

The above lemma allows us to focus on the possible dynamical behavior in the isolated parts of the biological network corresponding to the components $Z_1, \dots, Z_m$ and leads us to the following theorem.

Theorem 3. *For every singular steady state s there is an attractor A in $\mathcal{S}_N$ such that $u_i = s_i$ holds for all $u \in A$ and $i \notin J(s)$.*

Proof. Let $Z_1, \dots, Z_k$ again be the components of the graph $\mathcal{I}^\theta(s)$. Without loss of generality we may assume that $\alpha_1, \dots, \alpha_n$ are ordered as follows. The set Z_1 contains $\alpha_1, \dots, \alpha_{|Z_1|}$, Z_2 contains $\alpha_{|Z_1|+1}, \dots, \alpha_{|Z_1|+|Z_2|}$ etc., where $|Z_i|$ denotes the cardinality of the set Z_i. The regular components of s are associated with $\alpha_p, \dots, \alpha_n$, with $p := 1 + \sum_{i=1}^k |Z_i|$. According to Proposition 1 we find an attractor A_i in the state transition graph corresponding to the function $f^{(Z_i)}$ for all $i \in \{1, \dots, k\}$. Here $f^{(Z_i)} : \mathcal{B}^{|Z_i|} \to \mathcal{B}^{|Z_i|}$, where $f_j^{(Z_i)}(x) := f_{q+j}(s_1, \dots, s_q, x_1, \dots, x_{|Z_i|}, s_{q+|Z_i|+1}, \dots, s_n)$ with $q = \sum_{l=0}^{i-1} |Z_l|$ and $|Z_0| = 0$.

Next we show that the set $A := A_1 \times \dots \times A_k \times \{(s_p, \dots, s_n)\}$ is an attractor in $\mathcal{S}_N$. We use the fact that each attractor A_i arises from the dynamics induced by the projection of the function f to the components of Z_i. Recall that, as shown above, changes in the values of elements belonging to some Z_i do not influence the values of the other components of s when applying f. Since f determines $\mathcal{S}_N$ via the asynchronous update rule, we obtain the following statement. There is a path from a^i to b^i for states $a^i, b^i \in A_i$ in the state transition graph corresponding to $f^{(Z_i)}$ if and only if there is a path from $(x^1, \dots, x^{i-1}, a^i, x^{i+1}, \dots, x^k, s_p, \dots, s_n)$ to $(x^1, \dots, x^{i-1}, b^i, x^{i+1}, \dots, x^k, s_p, \dots, s_n)$ in $\mathcal{S}_N$ for some states $x^j \in A^j$. It follows easily from this observation that A is an attractor in $\mathcal{S}_N$. $\square$

To illustrate the above theorem and its proof we examine the example given in Figure 2. The components of $\mathcal{I}^\theta(s)$ given there are $Z_1 = \{\alpha_1\}$ and $Z_2 = \{\alpha_2\}$. The function $f^{(Z_1)} : \mathcal{B} \to \mathcal{B}, x \mapsto f_1^\theta(x, \theta, 1)$ generates a state transition graph that consists of a cycle comprising the states 0 and 1. Thus it has a single attractor $A_1 = \{0, 1\}$. The state transition graph corresponding to $f^{(Z_2)}$ consists of the two attractors $A_2^1 = \{0\}$ and $A_2^2 = \{1\}$. Thus we can derive two attractors in $\mathcal{S}_N$, namely $A^1 = \{(0,0,1), (1,0,1)\}$ and $A^2 = \{(0,1,1), (1,1,1)\}$.

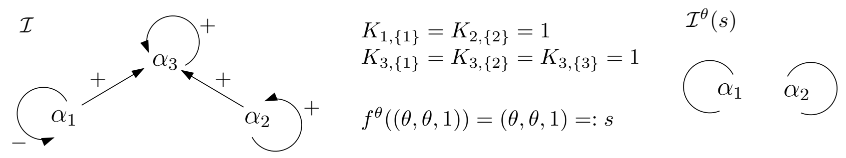

Fig. 2. Given is an interaction graph $\mathcal{I}$ and a specification of the parameters. We listed only the non-zero parameters. The graph $\mathcal{I}^\theta(s)$ for the singular steady state $s := (\theta, \theta, 1)$ has two components.

We have seen above that we can link a singular steady state to a regular attractor. However, different singular steady states s^1 and s^2 may give rise to the same regular attractor. The above proof shows that this possibility is precluded if s^1 and s^2 differ in a component $i \notin J(s^1) \cup J(s^2)$. The same line of thinking shows that the resulting attractors are different, if there exists a component Z_l^1 of $\mathcal{I}^\theta(s^1)$ disjoint of $J(s^2)$ that gives rise to an attractor A_l of the state transition graph corresponding to $f^{(Z_l^1)}$ with $|A| > 1$.

A more precise analysis of the correspondence of attractors and singular steady states is possible if we take into account structural information on the underlying interaction graph $\mathcal{I}$. In the preceding section we have seen that every singular steady state s is characteristic of some circuit C of the interaction graph $\mathcal{I}$. If we know in addition that s is not characteristic of any other circuit in the connected component $J_C(s)$ of $\mathcal{I}^\theta(s)$, we can derive information on the singular valued predecessors of vertices belonging to C. This is shown in the next lemma.

Lemma 2. *Let $C = (\alpha_{i_1}, \dots, \alpha_{i_m})$ be a circuit in $\mathcal{I}$ and let s be a characteristic state of C which is steady. Assume that C is the only circuit in $J_C(s)$. Then $R^\theta_{i_j}(s) = \{\alpha_{i_{j-1}}\}$ for all $j \in \{1, \dots, m\}$ with indices taken modulo m.*

Proof. Set $J := J(s)$ and $J_C := J_C(s)$. Clearly, $\alpha_{i_{j-1}} \in R^\theta_{i_j}(s)$ for all $j \in \{1, \dots, m\}$. Assume that there is $k \in \{1, \dots, m\}$ such that there exists $l \in J$ satisfying $\alpha_l \neq \alpha_{i_{k-1}}$ and $\alpha_l \in R^\theta_{i_k}(s)$. Then $\alpha_l \in Pred(\alpha_{i_k})$ and thus $l \in J_C$. If $l = i_j$ for some $j \neq k-1$, then $(\alpha_{i_j}, \alpha_{i_k}, \dots, \alpha_{i_{j-1}})$ is a circuit other than C in J_C. This contradicts the hypothesis. Thus α_l is not a vertex of C.

Since s is a steady state, we know that $R^\theta_j(s) \neq \emptyset$ for all $j \in J$. Furthermore, $R^\theta_j(s) \subset J_C$ for all $j \in J_C$. Thus for every $j \in J_C$ we find $i \in J_C$, such that $\alpha_i \to \alpha_j$ is an edge in $\mathcal{I}$. Since there are only finitely many vertices in J_C, there is a circuit in $\{\alpha_j \in J_C\,;\, \exists \text{ path from } \alpha_j \text{ to } \alpha_l \text{ in } \mathcal{I}\}$ that differs from C. Again, this leads to a contradiction. ◻

Lemma 2 allows us to represent $J_C(s)$ by a chain of nested sets.

Lemma 3. *Assume the same hypothesis as in Lemma 2. Then there exist sets $M_1, \dots, M_l \subset J_C(s)$ such that $M_1 = C$, $M_l = J_C(s)$, $M_i \subsetneq M_{i+1}$ and $R^\theta_j(s) \subset M_i$ for all $j \in M_{i+1}$ and $i \in \{1, \dots, l-1\}$.*

Proof. Set $M_1 := \{i_1, \dots, i_m\}$. If $J_C(s) \setminus M_1 \neq \emptyset$, then there exists at least one element $j \in J_C(s) \setminus M_1$ such that $R^\theta_j(s) \subset M_1$. Otherwise for every $j \in J_C(s) \setminus M_1$ there is $k_j \in J_C(s) \setminus M_1$ such that α_{k_j} is a predecessor of α_j in $\mathcal{I}$. That would imply the existence of a circuit other than C in $J_C(s)$, since $J_C(s) \setminus M_1$ is finite. Thus by defining $M_2 := \{j \in J_C(s)\,;\, R^\theta_j(s) \subset M_1\}$ we obtain a set strictly contained in M_1. Since $J_C(s)$ is finite, we can repeat the procedure until we get $M_l := \{j \in J_C(s)\,;\, R^\theta_j(s) \subset M_{l-1}\} = J_C(s)$. ◻

In the following we make use of the information on the sign of the circuit C.

Theorem 4. *Let C be a positive circuit in $\mathcal{I}$ and let s be a characteristic state of C which is steady. Assume that C is the only circuit in $J_C(s)$. Then f^θ has at least three fixed points.*

Proof. Set $J := J(s)$ and $J_C := J_C(s)$. Without loss of generality we may assume that $C = (\alpha_1, \dots, \alpha_r)$ for some $r \in \{1, \dots, n\}$. We define states $s^0, s^1 \in \mathcal{B}^n_\theta$ such that s, s^0 and s^1 are fixed points of f^θ. First, we set $s^0_i = s^1_i = s_i$ for all $i \notin J_C$ and, for now, choose the other components of s^0 and s^1 arbitrary. We will determine the values for all components step by step. By abuse of notation, we denote the resulting states again by s^0 and s^1.

From Lemma 1 it follows that $f^\theta_i(s^0) = s^0_i$ and $f^\theta_i(s^1) = s^1_i$ for all $i \notin J_C$. Next, we define the values s^0_i and s^1_i for $i \in \{1, \dots, r\}$. We set $s^0_1 := 0$, $s^1_1 := 1$, and for $l \in \{0, 1\}$

$$s^l_{i+1} := \begin{cases} 0 & , \quad (s^l_i = 0 \wedge \varepsilon_{i+1,i} = +) \vee (s^l_i = 1 \wedge \varepsilon_{i+1,i} = -) \\ 1 & , \quad (s^l_i = 1 \wedge \varepsilon_{i+1,i} = +) \vee (s^l_i = 0 \wedge \varepsilon_{i+1,i} = -) \end{cases}$$

for all $i \in \{1, \ldots, r-1\}$. This definition amounts to setting $s^l_{i+1} = 1$ iff the value of s^l_i characterizes α_i as regular resource of α_{i+1}. Since C is a positive circuit, the value of s^l_1 is consistent with the value we obtain by using the above definition for $i = r$, that is we do not contradict the definition of s^l if we use the above iterative formula modulo r. Note that s^0_1, s^1_1 and s_1 are distinct.

According to Lemma 2 we have $R^\theta_i(s) = \{\alpha_{i-1}\}$ for all $i \in \{1, \ldots, r\}$, indices again taken modulo r. Thus $R^\theta_i(s^l) = \emptyset$ for all $i \in \{1, \ldots, r\}$. Moreover, we have

$$R_i(s^l) = \begin{cases} R_i(s) & , \quad (s^l_{i-1} = 0 \,\wedge\, \varepsilon_{i,i-1} = +) \vee (s^l_{i-1} = 1 \,\wedge\, \varepsilon_{i,i-1} = -) \\ R_i(s) \cup R^\theta_i(s) & , \quad (s^l_{i-1} = 1 \,\wedge\, \varepsilon_{i,i-1} = +) \vee (s^l_{i-1} = 0 \,\wedge\, \varepsilon_{i,i-1} = -) \end{cases}$$

for all $i \in \{1, \ldots, r\}$. Since $f^\theta_i(s) = |K_{i,R_i(s)}, K_{i,R_i(s)\cup R^\theta_i(s)}| = |0, 1|$, it follows from the definition of s^l_i and condition (1) that

$$f^\theta_i(s^l) = K_{i,R_i(s^l)} = \begin{cases} K_{i,R_i(s)} = 0 & , \quad s^l_i = 0 \\ K_{i,R_i(s)\cup R^\theta_i(s)} = 1 & , \quad s^l_i = 1 \end{cases} .$$

Thus, we have $f^\theta_i(s^0) = s^0_i$ and $f^\theta_i(s^1) = s^1_i$ for all $i \in \{1, \ldots, r\}$, not depending on the values of the components in $J_C \setminus \{1, \ldots, r\}$.

Finally, we have to specify s^l_i for all $i \in J_C \setminus \{1, \ldots, r\}$ and $l \in \{0, 1\}$. According to Lemma 3 we find sets $M_1, \ldots, M_k \subset J_C$ satisfying $M_1 = C$, $M_k = J_C$, $M_j \subsetneq M_{j+1}$ and $R^\theta_i(s) \subset M_j$ for all $i \in M_{j+1}$ and $j \in \{1, \ldots, k-1\}$. Thus, for every $i \in M_2$ we have $R^\theta_i(s^l) = \emptyset$ since all components of $M_1 = C$ have regular values. Moreover, it follows that $R_i(s^l) \cap J_C \setminus C = \emptyset$. Therefore we can determine the new values of all components of s^l contained in M_2 by $s^l_i := K_{i,R^l_i(s)}$. Note that this parameter depends only on components previously specified. Since α_i does not have singular resources in state s^l for all $i \in M_2$, we have $f^\theta_i(s^l) = K_{i,R_i(s^l)} = s^l_i$ for all $i \in M_2$. Because the sets M_j are nested, we can repeat the above procedure for consecutive sets without encountering contradictions. Thus we are able to specify all components s^l_i for $i \in J_C \setminus C$, such that $f^\theta_i(s^l) = s^l_i$.

We have shown that the resulting states s^0 and s^1 are fixed points of f^θ. Since s, s^0, and s^1 are distinct, f^θ has at least three fixed points. □

The proof shows that at least two fixed points of f^θ differ in a regular component. Applying Theorem 3 and the subsequent observations we immediately obtain the following statement.

Corollary 1. *If the conditions in Theorem 4 hold, then there are at least two distinct attractors in the corresponding state transition graph.*

The corollary is illustrated in Figure 3 (a) and (c). The singular steady state $(1, \theta, 0)$ is characteristic of the positive circuit comprising α_2 and of no other circuit. The resulting state transition graph shows two distinct attractors. The importance of the condition concerning the circuit C and the component $J_C(s)$ is demonstrated in Figure 3 (b). The state (θ, θ, θ) is steady and characteristic of the positive circuit comprising α_2. Moreover, the state $(\theta, 0, \theta)$ is steady and

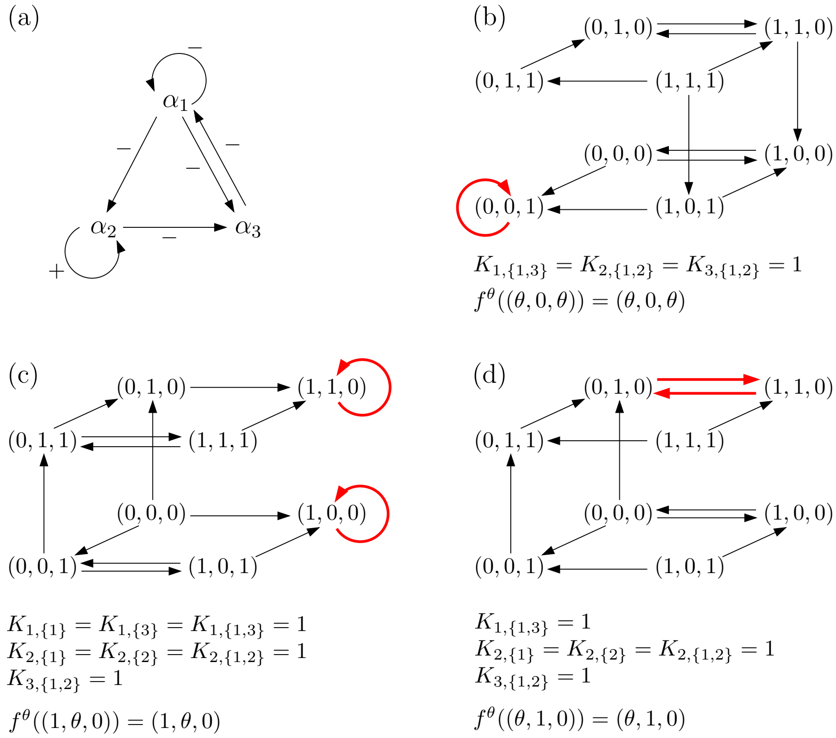

Fig. 3. An interaction graph comprising three components is given in (a). Figures (b)-(d) show the state transition graphs corresponding to the chosen parameter values. We only listed the non-zero parameters. Attractors are indicated by colored, fat lines. For each choice of parameters one singular steady state other than (θ, θ, θ) is given.

characteristic of the positive circuit comprising α_1 and α_3. In both cases the states are characteristic of further circuits in the same component, and the state transition graph has only one attractor. Similar examples can be given to show the importance of this condition for the validity of Theorem 4.

The next theorem clarifies the impact of a negative circuit.

Theorem 5. *Let C be a negative circuit in $\mathcal{I}$ and let s be a characteristic state of C which is steady. Assume that C is the only circuit in $J_C(s)$. Then there exists an attractor with cardinality greater than one.*

Proof. Again we set $J := J(s)$ and $J_C := J_C(s)$ and assume that $C = (\alpha_1, \dots, \alpha_r)$ for some $r \in \{1, \dots, n\}$. First, we examine the dynamical behavior of the system restricted to the first r components.

We define states $s^1, \dots, s^{2r}$ satisfying $s^l_i = s_i$ for all $i > r$. Furthermore, we set $s^1_1 := 1$ and

$$s^l_{i+1} := \begin{cases} 0 & , \quad (s^l_i = 0 \wedge \varepsilon_{i+1,i} = +) \vee (s^l_i = 1 \wedge \varepsilon_{i+1,i} = -) \\ 1 & , \quad (s^l_i = 1 \wedge \varepsilon_{i+1,i} = +) \vee (s^l_i = 0 \wedge \varepsilon_{i+1,i} = -) \end{cases}$$

for all $i \in \{1, \dots, r-1\}$. So $s_{i+1}^l = 1$ iff the value of s_i^l characterizes α_i as regular resource of α_{i+1}. Since C is a negative circuit, the definition above implies that α_r is not a resource of α_1 in state s^1. According to Lemma 2, the only singular resource of α_1 in s is α_r. Taking into account that $f_1^\theta(s) = \theta$ and thus $K_{1,R_1(s)} = 0$, it follows that $f_1^\theta(s^1) = K_{1,R_1(s^1)} = K_{1,R_1(s)} = 0$. For $1 < i \leq r$ we can repeat the argument employed in the proof of Theorem 4 to obtain

$$f_i^\theta(s^1) = K_{i,R_i(s^l)} = \begin{cases} K_{i,R_i(s)} = 0 & , \quad s_i^1 = 0 \\ K_{i,R_i(s) \cup R_i^\theta(s)} = 1 & , \quad s_i^1 = 1 \end{cases} .$$

Set $s^2 := f^\theta(s^1)$. Then we have $s_1^2 = 1 - s_1^1$ and $s_i^2 = s_i^1$ for $1 < i \leq r$. Furthermore, $s_i^2 = s_i^1$ for all $i \notin J_C$ according to Lemma 1.

We repeat the procedure above and obtain for $1 < j \leq 2r$ states $s^j := f^\theta(s^{j-1})$ satisfying $s_i^j = s_i^1$ for all $i \notin J_C$. Furthermore, we have

$$s_{j-1}^j = 1 - s_{j-1}^{j-1} \text{ and } s_i^j = s_i^{j-1} \text{ for all } i \in \{1, \dots, r\} \setminus \{j-1\},\ 2 \leq j \leq r+1\,,$$

and, since C is a negative circuit,

$$s_{j-1}^{j+r} = 1 - s_{j-1}^{j+r-1} \text{ and } s_i^{j+r} = s_i^{j+r-1} \text{ for all } i \in \{1, \dots, r\} \setminus \{j-1\},\ 2 \leq j \leq r\,.$$

The same reasoning shows that $f_i^\theta(s^{2r}) = s_i^1$ for all $i \notin J_C \setminus C$.

Now we examine the dynamics in the part of the state space that corresponds to J_C, that is, in $\mathcal{B}^{|J_C|}$, where $|J_C|$ again describes the cardinality of J_C. Choose $x \in \mathcal{B}^{|J_C|}$ such that $x_i = s_i^1$ for all $i \in \{1, \dots, r\}$ and $x_i = 0$ for all $i \geq r$. We have seen in the proof of Proposition 1 that there is a trajectory $T = (x, x^1, x^2, \dots)$ leading to an attractor A in the projection to the components of J_C of the state transition graph of f. From the definition of the states s^j it follows that $(x_1^i, \dots, x_r^i) \in \{(s_1^j, \dots, s_r^j)\,;\, 1 \leq j \leq 2r\}$ for all $i \in \mathbb{N}$. In particular, there is $a \in A$ such that the first r components of a correspond to the first r components of one of the vectors s^j. Since all trajectories starting in a remain in A we can deduce that every state $(s_1^j, \dots, s_r^j, a_{r+1}, \dots, a_{|J_C|})$, $j \in \{1, \dots, 2r\}$, belongs to A. Therefore, the cardinality of A is greater than one. We have shown in the proof of Theorem 3 that there is at least one attractor of the state transition graph $\mathcal{S}_N$ that coincides with A in the components corresponding to J_C. Thus, we find an attractor in $\mathcal{S}_N$ with cardinality greater than one. $\square$

Figure 3 illustrates the theorem. In (d) we give a parameter specification that allows for the state $(\theta, 1, 0)$ to be steady. This state is characteristic of the negative circuit comprising α_1. The resulting state transition graph contains the attractor $\{(0,1,0), (1,1,0)\}$. As for Theorem 4, Figure 3 (b) illustrates the importance of C being the only circuit in $J_C(s)$. Although $(\theta, 0, \theta)$ is characteristic of the negative circuit comprising α_1, and (θ, θ, θ) is characteristic of the negative circuit comprising α_1, α_2 and α_3, the only attractor in the state transition graph consists of a single state.

The proofs of Theorems 4 and 5 show that the situation is easy to grasp in case that the only components with singular values are those of the circuit C. In the context of Theorem 4, we then obtain two regular fixed points, that is two steady states in the state transition graph. Those can be explicitly constructed as shown in the proof of Theorem 4. If C is a negative circuit, we find a trap cycle in the state transition graph. It is composed of the states $s^1, \ldots, s^{2r}$ constructed in the proof of Theorem 5.

If we detect the above mentioned structures in the state transition graph, we can conversely derive singular steady states.

Proposition 2. *Let $x, y \in \mathcal{B}^n$ be steady states in the state transition graph $\mathcal{S}_N$. Let I be the set of components i satisfying $x_i \neq y_i$. Then there exists a singular steady state s such that $s_i = \theta$ for all $i \in I$.*

Proof. Let $s^1 \in \mathcal{B}_\theta$ satisfy $s^1_i = \theta$ for all $i \in I$ and $s^1_i = x_i = y_i$ for all $i \notin I$. Let $i \in I$. For $z \in \{x, y\}$ we have $R_i(s^1) \subset R_i(z) \subset R_i(s^1) \cup R^\theta_i(s^1)$. We may assume that $x_i = f_i(x) = K_{i,R_i(x)} = 0$ and $y_i = f_i(y) = K_{i,R_i(y)} = 1$. From condition (1) we can deduce that $K_{i,R_i(s^1)} = 0$ and $K_{i,R_i(s^1)\cup R^\theta_i(s^1)} = 1$. Thus $f^\theta_i(s^1) = \theta = s^1_i$. For $i \notin I = J(s^1)$ and for $z \in \{x, y\}$ we also have $R_i(s^1) \subset R_i(z) \subset R_i(s^1) \cup R^\theta_i(s^1)$, and thus $K_{i,R_i(s^1)} \leq K_{i,R_i(z)} \leq K_{i,R_i(s^1)\cup R^\theta_i(s^1)}$. If $f^\theta_i(s^1)$ is a regular value, it coincides with $x_i = y_i = s^1_i$.

The state s^1 may not be a fixed point, but we can show that we obtain a fixed point by iterating the state s^1. Consider the sequence $s^1, s^2, \ldots$ with $s^j := f^\theta(s^{j-1})$. We show by induction that the sets of singular components satisfy $J(s^j) \subset J(s^j + 1)$ for all $j \in \mathbb{N}$ and $s^{j+1}_i = s^j_i$ for all $i \notin J(s^{j+1})$. This is true for $j = 1$ as shown above, since $J(s^1) = I$.

Let $j > 1$ and assume the induction hypothesis holds for all $i \leq j - 1$. Then we can derive that $R_i(s^j) \subset R_i(s^{j-1})$ and $R_i(s^{j-1}) \cup R^\theta_i(s^{j-1}) \subset R_i(s^j) \cup R^\theta_i(s^j)$ for all $i \in \{1, \ldots, n\}$. We have $K_{i,R_i(s^{j-1})} = 0$ and $K_{i,R_i(s^{j-1})\cup R^\theta_i(s^{j-1})} = 1$ for all $i \in J(s^j)$, since $s^j_i = f^\theta_i(s^{j-1}) = \theta$ for all $i \in J(s^j)$. It follows with condition (1) that $K_{i,R_i(s^j)} = 0$ and $K_{i,R_i(s^j)\cup R^\theta_i(s^j)} = 1$. Thus $s^{j+1}_i = f^\theta_i(s^j) = \theta$ for all $i \in J(s^j)$, and we obtain $J(s^j) \subset J(s^{j+1})$. If s^{j+1}_i is a regular value, the same is true for $s^j_i = K_{i,R_i(s^{j-1})} = s^{j-1}_i$. In that case, we can deduce from the relation $K_{i,R_i(s^j)} \leq K_{i,R_i(s^{j-1})} \leq K_{i,R_i(s^j)\cup R^\theta_i(s^j)}$ that $s^{j+1}_i = K_{i,R_i(sj-1)} = s^j_i$.

Since a state has only finitely many components, we can derive from the condition $J(s^j) \subset J(s^j + 1)$ for all $j \in \mathbb{N}$ that there exists a minimal $k \in \mathbb{N}$ such that $s^j = s^k$ for all $j \geq k$, that is, s^k is a singular fixed point. $\square$

A similar argument leads the following statement. We omit the proof.

Proposition 3. *Let $C := (x^1, \ldots, x^r, x^1)$ be a trap cycle in the state transition graph $\mathcal{S}_N$. Let I be the set of components i such that there exists j_1, j_2 satisfying $x^{j_1}_i \neq x^{j_2}_i$. Then there is a singular steady state such that $s_i = \theta$ for all $i \in I$.*

In the proof of Proposition 2 we have seen that the singular steady state we derive may be the state $(\theta, \ldots, \theta)$, even when $I \neq \{1, \ldots, n\}$.

5 Perspectives

We have seen in this paper that it is possible to relate systematically singular steady states to attractors in the state transition graph. To do so, we often exploit knowledge about the structure of the associated interaction graph. The results obtained illustrate the possibilities of studying the dynamical behavior of the system without the explicit use of the state transition graph. However, we have focussed on a coarse description, characterizing state transition graphs by the number of their attractors, and distinguishing attractors by their cardinality. In order to tap the full potential of this approach to analyzing the system's dynamics, it should be refined further. A promising starting point for future work is the concept of local interaction graphs introduced in [2]. The authors associate every state of the system with an interaction graph, the union of which is the global interaction graph. This approach allows for a better understanding of what structures in the interaction graph influence the system's behavior in a given state. Combining this local view with our understanding of singular steady states may yield a more detailed description of the resulting dynamical behavior.

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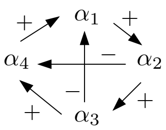
Appendix

A Example

The following example shows that the statement of Theorem 4 is not true, if $J_C(s)$ contains circuits other than C.



$$
\begin{aligned}
K_{\alpha_1,\{\alpha_3\}} &= 1\\
K_{\alpha_1,\{\alpha_4\}} &= 1\\
K_{\alpha_1,\{\alpha_3,\alpha_4\}} &= 1\\
K_{\alpha_2,\{\alpha_1\}} &= 1\\
K_{\alpha_3,\{\alpha_2\}} &= 1\\
K_{\alpha_4,\{\alpha_2,\alpha_3\}} &= 1
\end{aligned}
$$

s	$f^\theta(s)$
0000	1000
1000	1100
1100	1110
1110	0110
1111	1110
1010	0101
1001	1100
1011	1101
1101	1110
0001	1000
0010	0001
0011	1001
0100	1010
0101	1010
0110	0010
0111	1010

$$
\begin{aligned}
s_1 = \theta &\quad\Rightarrow\quad f_2^\theta(s) = \theta\\
s_1 \neq \theta &\quad\Rightarrow\quad f_2^\theta(s) \neq \theta\\
s_2 = \theta &\quad\Rightarrow\quad f_3^\theta(s) = \theta,\ f_4^\theta(s) = 0, \theta\\
s_2 \neq \theta &\quad\Rightarrow\quad f_3^\theta(s) \neq \theta\\
s_3 = \theta &\quad\Rightarrow\quad f_1^\theta(s) = 0, \theta,\ \ f_4^\theta(s) = 0, \theta\\
s_4 = \theta &\quad\Rightarrow\quad f_1^\theta(s) = 0, \theta
\end{aligned}
$$

$$f^\theta(s) = s \quad\Rightarrow\quad s = (\theta, \theta, \theta, \theta)$$

Fig. 4. Interaction graph of a network containing a positive circuit with characteristic state $(\theta, \theta, \theta, \theta)$. Given are the logical parameters not equal to zero. The table shows that f^θ has no regular fixed point. With the help of the implications on the right of the figure it is easy to see that the only singular steady state is $(\theta, \theta, \theta, \theta)$.

B Proposition 3 with Proof

Proposition 3. *Let $C := (x^1, \ldots, x^r, x^1)$ be a trap cycle in the state transition graph $\mathcal{S}_N$. Let I be the set of components i such that there exists j_1, j_2 satisfying $x_i^{j_1} \neq x_i^{j_2}$. Then there is a singular steady state such that $s_i = \theta$ for all $i \in I$.*

Proof. Let $s^1 \in \mathcal{B}_\theta$ satisfy $s_i^1 = \theta$ for all $i \in I$ and $s_i^1 = x_i^1$ for all $i \notin I$. Let $s^2 := f^\theta(s^1)$. For all $j \in \{1, \ldots, r\}$ and $i \in \{1, \ldots, n\}$, we have $R_i(s^1) \subset R_i(x^j) \subset R_i(s^1) \cup R_i^\theta(s^1)$. Let $i \in I$. Since C is a trap cycle, there exist j_1, j_2 such that $f_i(x^{j_1}) = K_{i,R_i(x^{j_1})} = 0$ and $f_i(x^{j_2}) = K_{i,R_i(x^{j_2})} = 1$. It follows from condition (1) that $K_{i,R_i(s^1)} = 0$ and $K_{i,R_i(s^1)\cup R_i\theta(s^1)} = 1$, and thus $f_i^\theta(s^1) = \theta$ for all $i \in\ I = J(s^1)$. If s_i^2 is a regular value, then the relation between the sets of resources and condition (1) imply that $K_{i,R_i(s^1)} = K_{i,R_i(x^j)} = x_i^j = s_i^1$ for all $j \in \{1, \ldots, r\}$. In that case, $s_i^2 = x_i^j = s_i^1$ for all $j \in \{1, \ldots, r\}$.

We can now proceed exactly as shown in the proof of Proposition 2 to obtain a singular steady state s, which satisfies $s_i = \theta$ for all $i \in I$. □