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

Heike Siebert and Alexander Bockmayr

DFG Research Center Matheon,
Freie Universität Berlin, Arnimallee 3, D-14195 Berlin, Germany
siebert@mi.fu-berlin.de, bockmayr@mi.fu-berlin.de

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 [10]. 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 [11] and [12] 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 associated with a unique threshold value, which determines when the interaction becomes effective. Snoussi and Thomas realized that a closer inspection of the

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impact of the threshold values, which they called singular values, would further improve the understanding of the system's dynamics. In [8] 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 (resp. negative) circuit, in the interaction graph is a necessary condition for the existence of two distinct attractors (resp. a cyclic attractor) in the state transition graph. The conjectures have been proven in different settings (see e.g. [9], [4] and [5]). In [2] it is shown, that isolated elementary regulatory circuits result in fundamentally different dynamics depending on their sign. A positive circuit can be linked to the occurrence of two stable states, while a negative circuit causes an attractor comprising dynamical cycles. However, the situation becomes more difficult to grasp as soon as the circuits are embedded in larger and more complex networks.

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 in the Boolean case 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 [11]). We mainly use the notation introduced in [1] and [6]. 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 α_i is active. To simplify notation, we identify each vertex α_i with its index i.

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 $(\alpha_{i_1}, \dots, \alpha_{i_k})$ 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 $j \in \{1, \dots, k-1\}$ 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. Now 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*

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

Given a set

$$K(\mathcal{I}) := \{K_{i,\omega} \mid i \in \{1, \dots, n\}, \omega \subseteq Pred(\alpha_i)\}$$

of (logical) parameters*, which adopt values in $\mathcal{B}$, we define the Boolean function $f = f^{K(\mathcal{I})} : \mathcal{B}^n \to \mathcal{B}^n$, $s \mapsto (K_{1,R_1(s)}, \dots, K_{n,R_n(s)})$. 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. It contains all genes that contribute to an activation of α_i in state s. Note that the absence of an inhibitor is interpreted as an activating influence on the target gene. The value of the parameter $K_{i,R_i(s)}$ indicates how the level of activity α_i will evolve. It will increase (resp. decrease) if the parameter value is greater (resp. 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. Snoussi and Thomas posed the following condition on the parameter values of the systems they considered:

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

for all $i \in \{1, \dots, n\}$. The condition signifies that an effective activator or a non-effective inhibitor cannot induce the decrease of the activity level of α_i. In the following we always assume that this condition is valid.

The choice of parameters completes the definition of 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 $M \subseteq Pred(\alpha_i)$, then the edge $\alpha_j \to \alpha_i$ has no influence on the dynamics of the system. Eliminating this edge from the interaction graph

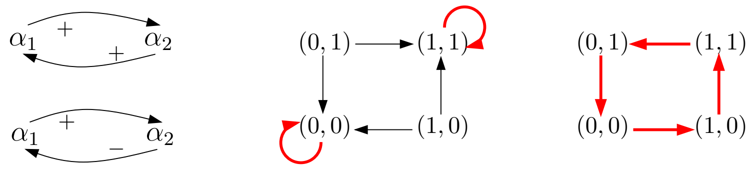


Fig. 1. Two interaction graphs consisting of a positive resp. a negative circuit. In both cases we choose $K_{1,\{2\}} = K_{2,\{1\}} = 1$ and $K_{1,\emptyset} = K_{2,\emptyset} = 0$. The state transition graph corresponding to the positive circuit is in the middle, the one corresponding to the negative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

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 $M \subseteq \ Pred(\alpha_i)$ 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 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* $(s_0, s_1, \dots)$ *in* $\mathcal{S}_N$ *is called* trajectory*. A nonempty set of states* D *is called* trap set *if every trajectory starting in* D *never leaves* D*. A trap set* A *is called* attractor *if for any* $s^1, s^2 \in A$ *there is a path from* s^1 *to* s^2 *in* $\mathcal{S}_N$*. 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* $\mathcal{S}_N$*, i. e., the trajectory starting in* s^1 *is unique.*

Thus, the attractors of $\mathcal{S}_N$ correspond to the terminal strongly connected components of the graph. 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. This corresponds to the typical behavior assigned to positive (resp. negative) circuits mentioned in the introduction.

Attractors represent regions of predictability and stability in the behavior of the system. It is not surprising that an attractor often has a biological interpretation. 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. *Every state transition graph $\mathcal{S}_N$ contains at least one attractor.*

Proof. For $s \in \mathcal{B}^n$ we denote by $D(s)$ the set of states reachable from s by a path in $\mathcal{S}_N$. Then $D(s)$ is a trap set for every $s \in \mathcal{B}^n$. Fix $s \in \mathcal{B}^n$ and choose $A \subseteq D(s)$ a minimal trap set, i. e., every proper subset of A is not a trap set. Let $x, y \in A$. Then $D(x) \subseteq A$, since A is a trap set. Since A is minimal, we have $A = D(x)$. Consequently, there is a path from x to y. Thus, 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 restrictions of f to sets of vertices obtained by considering certain subgraphs of $\mathcal{I}$ as much information on the structure of $\mathcal{S}_N$ as possible.

3 Singular States

In the following, we incorporate threshold values of interactions into the formalism to get a more complete understanding of the dynamics of the system. We mainly use the framework introduced in [6].

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 $J(s) := \{i \in \{1, \ldots, n\} \mid s_i = \theta\}$.*

To describe the dynamics of the system we have to extend 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*

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

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.

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

The map f^θ is well defined since condition (1) ensures that $K_{i,R_i(s)} \leq K_{i,R_i(s)\cup R_i^\theta(s)}$ 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 $s \in \mathcal{B}_\theta^n$ 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 [8], albeit in a different framework. The remainder of this section adapts ideas presented in [8].

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. □

If the network consists of a single circuit, then the corresponding characteristic state is always steady under our standard assumption that every edge in the graph is functional. As mentioned before, such a circuit displays a characteristic behavior depending on its sign. In general, the existence of a steady characteristic state of a circuit does not always result in the corresponding dynamical behavior, as will be illustrated in the next section.

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 $k \in \{1,\dots,n\}$. Let $s^{k,+}$ and $s^{k,-}$ be regular states that satisfy $s_i^{k,+} := s_i^{k,-} := s_i$ for all $i \notin J(s)$ and*

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

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 $R_k^\theta(s)$, $k \in \{1,\dots,n\}$, are disjoint, then we can define states s^+ and s^- which are maximal resp. minimal adjacent states of s with respect to every $k \in \{1,\dots,n\}$. 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 iff for all $k \in \{1,\dots,n\}$ there is some choice of $s^{k,+}$, $s^{k,-}$ such that $f_k(s^{k,+}) = s_k^{k,+} = s_k^{k,-} = f_k(s^{k,-})$, if $k \notin J(s)$, and $f_k(s^{k,-}) < \theta < f_k(s^{k,+})$, if $k \in J(s)$.*

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 $k \in \{1,\dots,n\}$. 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 $\alpha_i \in Pred(\alpha_k)$. 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 $k \in \{1,\dots,n\}$. 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 used to show the inverse statement. □

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 we have $K_{\alpha_k,\emptyset} = 0$ and $K_{\alpha_k,Pred(\alpha_k)} = 1$, then the state $(\theta,\dots,\theta)$ 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 can be inferred 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 indices corresponding to the singular values of s and that we identify each vertex α_i with its index i. 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 $\mathcal{I}^\theta(s)$. By abuse of notation we denote the vertex set of a component Z of $\mathcal{I}^\theta(s)$ also with Z. 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 the vertices of C. We denote this component by $J_C(s)$.

The next lemma shows that for a singular steady state s value changes in a component of $\mathcal{I}^\theta(s)$ do not influence the image $f^\theta(s)$ outside that component. It will play an important role in all the following considerations.

Lemma 1. *Let s be a singular steady state, and let $Z_1, \ldots, Z_m$ be the components of $\mathcal{I}^\theta(s)$. Consider a union Z of arbitrary components Z_j. Let $\tilde{s} \in \mathcal{B}_\theta^n$ such that $\tilde{s}_i = s_i$ for all $i \notin Z$. Then $f_i^\theta(\tilde{s}) = f_i^\theta(s) = s_i = \tilde{s}_i$ for all $i \notin Z$.*

Proof. For $i \in J(s) \setminus Z$ 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 is a predecessor of α_i. Thus $f_i^\theta(\tilde{s}) = f_i^\theta(s) = s_i$ for all $i \in J(s) \setminus Z$. For $i \notin J(s)$ we have $R_i(s) \subseteq R_i(\tilde{s})$, since a singular resource of α_i may have turned into a regular resource. In addition, $R_i(\tilde{s}) \cup R_i^\theta(\tilde{s}) \subseteq R_i(s) \cup R_i^\theta(s)$, 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 $R_i(s) \subseteq R_i(\tilde{s}) \subseteq R_i(\tilde{s}) \cup R_i^\theta(\tilde{s}) \subseteq R_i(s) \cup R_i^\theta(s)$ 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, $K_{i,R_i(s)} = K_{i,R_i(s) \cup R_i^\theta(s)}$, since $f_i^\theta(s) = s_i$. Thus the above inequality becomes an equality and $f_i^\theta(\tilde{s}) = K_{i,R_i(s)} = s_i = \tilde{s}_i$ for all $i \notin J(s)$. □

The above lemma allows us to focus on the possible dynamical behavior in the isolated parts of the biological network corresponding to the components $Z_1, \ldots, Z_m$ 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. Set $P := \{x \in \mathcal{B}^n \mid \forall i \notin J(s) : x_i = s_i\}$. Then $f_i(x) = x_i = s_i$ for all $i \notin J(s)$ according to Lemma 1, i. e., $f(x) \in P$. Thus all successors of x in $\mathcal{S}_N$ are also in P. It follows that P is a trap set. Like in the proof of Prop. 1 we deduce that P contains an attractor A, and $u_i = s_i$ for all $u \in A$ and $i \notin J(s)$. □

It is not difficult to see that we can derive such an attractor A from attractors $A_1, \ldots, A_k$ arising in the system's dynamical behavior restricted to the components $Z_1, \ldots, Z_k$ of $\mathcal{I}^\theta(s)$. To illustrate this we examine the example given in

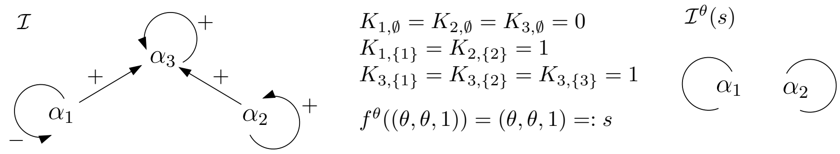


Fig. 2. An interaction graph $\mathcal{I}$ and a specification of the parameters. Missing parameter values follow from condition (1). The graph $\mathcal{I}^{\theta}(s)$ for $s := (\theta, \theta, 1)$ has two components.

Figure 2. The state $(\theta, \theta, 1)$ is steady, the components of $\mathcal{I}^{\theta}(s)$ are $Z_1 = \{\alpha_1\}$ and $Z_2 = \{\alpha_2\}$. We consider the dynamics restricted to Z_1 given by the projection $f^{(Z_1)} : \mathcal{B} \to \mathcal{B}, x \mapsto f_1^{\theta}(x, \theta, 1)$. It 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 the analogously defined function $f^{(Z_2)}$ consists of the two attractors $A_2^1 = \{0\}$ and $A_2^2 = \{1\}$. According to Lemma 1, the value of the third component of s will remain fixed, regardless of the values of the first two components. Thus we can derive two attractors in $\mathcal{S}_N$, namely $A^1 = A_1 \times A_2^1 \times \{s_3\} = \{(0,0,1),(1,0,1)\}$ and $A^2 = A_1 \times A_2^2 \times \{s_3\} = \{(0,1,1),(1,1,1)\}$.

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 $i \notin J(s^1) \cup J(s^2)$.

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 $\mathcal{I}$ with vertices 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 steady characteristic state of C. Assume that C is the only circuit in $\mathcal{I}$ with all its vertices contained in $J_C(s)$. Then $R_{i_j}^{\theta}(s) = \{\alpha_{i_{j-1}}\}$ for all $j \in \{1, \dots, m\}$ with indices taken modulo m.*

Proof. Set $J := J(s)$ and $J_C := J_C(s)$. Clearly, $\alpha_{i_{j-1}} \in R_{i_j}^{\theta}(s)$ 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 $\alpha_l \in R_{i_k}^{\theta}(s)$. 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 $R_j^{\theta}(s) \neq \emptyset$ for all $j \in J$. Furthermore, $R_j^{\theta}(s) \subseteq J_C$ 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. □

Note that there may be vertices in $J_C(s)$ that have more than one singular resource. Lemma 2 allows us to represent $J_C(s)$ by a chain of nested sets.

Lemma 3. *Under the hypotheses of Lemma 2, there exist sets* $M_1, \dots, M_l \subseteq J_C(s)$ *such that* $M_1 = \{i_1, \dots, i_m\}$, $M_l = J_C(s)$, $M_i \subsetneq M_{i+1}$ *and* $R^\theta_j(s) \subseteq M_i$ *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 $R^\theta_j(s) \subseteq M_1$. 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 $M_2 := \{j \in J_C(s)\,;\, R^\theta_j(s) \subseteq M_1\}$ we obtain a set strictly containing M_1. Since $J_C(s)$ is finite, we can repeat the procedure until we get $M_l := \{j \in J_C(s)\,;\, R^\theta_j(s) \subseteq M_{l-1}\} = J_C(s)$. □

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 steady characteristic state of* C. *Assume that* C *is the only circuit in* $\mathcal{I}$ *with all its vertices contained 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 determine states $s^0, s^1 \in \mathcal{B}^n_\theta$ by an iterative process such that s, s^0 and s^1 are fixed points of f^θ. Initially, we set $s^0_i := s^1_i := s_i$ for all $i \notin J_C$ and choose the other components of s^0 and s^1 arbitrary.

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 $i \in \{1, \dots, r-1\}$. 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}. As is easy to see we also have

$$s^l_{i+1} = \begin{cases} s^l_i & , \quad \varepsilon_{i+1,i} = + \\ 1 - s^l_i & , \quad \varepsilon_{i+1,i} = - \end{cases} .$$

It follows for all $i \in \{1, \dots, r-1\}$ that $s^l_{i+1} = s^l_1$ if $\prod_{j=1}^{i} \varepsilon_{j+1,j}$ is positive, and $s^l_{i+1} = s^l_1$ if $\prod_{j=1}^{i} \varepsilon_{j+1,j}$ is negative. 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 $i \in \{1, \dots, r\}$, indices again taken modulo r. Thus $R^\theta_i(s^l) = \emptyset$ for all $i \in \{1, \dots, r\}$. Moreover, we have

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

for all $i \in \{1, \dots, r\}$. Since $f_i^\theta(s) = |K_{i,R_i(s)}, K_{i,R_i(s)\cup R_i^\theta(s)}| = |0, 1|$, it follows from the definition of s_i^l and condition (1) that

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

Thus, we have $f_i^\theta(s^0) = s_i^0$ and $f_i^\theta(s^1) = s_i^1$ for all $i \in \{1, \dots, r\}$, not depending on the values of the components in $J_C \setminus \{1, \dots, r\}$.

Finally, we have to specify s_i^l for all $i \in J_C \setminus \{1, \dots, r\}$ and $l \in \{0, 1\}$. According to Lemma 3 we find sets $M_1, \dots, M_k \subseteq J_C$ satisfying $M_1 = \{1, \dots, r\}$, $M_k = J_C$, $M_j \subsetneq M_{j+1}$ and $R_i^\theta(s) \subseteq M_j$ for all $i \in M_{j+1}$ and $j \in \{1, \dots, k-1\}$. Thus we can deduce that α_i, $i \in M_2$, has no predecessors in $J_C \setminus M_1$, since otherwise they would be in $R_i^\theta(s)$. Furthermore, for every $i \in M_2$ we have $R_i^\theta(s^l) = \emptyset$ since all components corresponding to vertices in C have regular values. Now we set $s_i^l := K_{i,R_i(s^l)}$ for all $i \in M_2$. Note that this parameter depends only on components previously specified, i. e., on the values s_i^l for $i \notin J_C \setminus \{1, \dots, r\}$. Since α_i does not have singular resources in state s^l for all $i \in M_2$, we have $f_i^\theta(s^l) = K_{i,R_i(s^l)} = s_i^l$ 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_i^l for $i \in J_C \setminus \{1, \dots, r\}$, such that $f_i^\theta(s^l) = s_i^l$.

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. *Under the hypotheses of Theorem 4 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 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. Figure 4 shows the importance of C being the only circuit with vertices in $J_C(s)$ for the validity of Theorem 4. The interaction graph given in (a) contains a positive circuit with characteristic state $s := (\theta, \theta, \theta, \theta)$. Together with the parameters given in (b) it gives rise to a system that has no regular fixed point. Moreover, from the logical implications in (d) we can easily deduce that s is the only singular steady state.

The network in Figure 4 (b), together with the parameters given in (c), illustrates that the sufficient condition of Theorem 4 is not necessary. The given

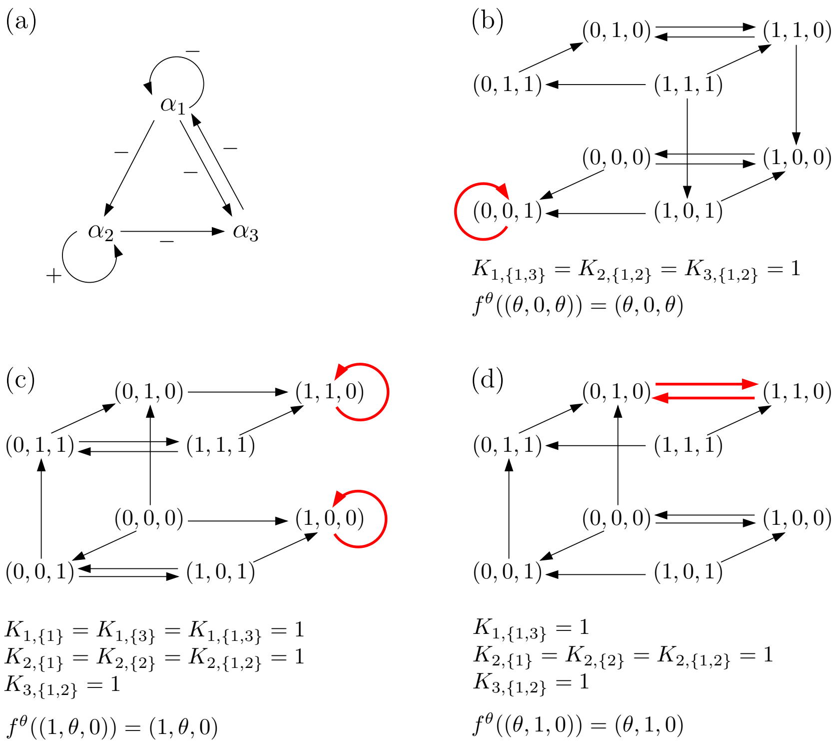


Fig. 3. An interaction graph comprising three components is given in (a). Figures (b)-(d) show the state transition graphs corresponding to the chosen parameter values. We only listed the non-zero parameters. Attractors are indicated by colored, fat lines. For each choice of parameters one singular steady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

system has two regular fixed points, (0,0,0,0) and (1,1,1,1). However, the only steady characteristic state is $s := (\theta, \theta, \theta, \theta)$, as easy to see from the implications in (d). Its components comprise the vertices of all three cycles of the network.

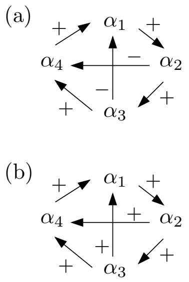
The next theorem clarifies the impact of a negative circuit.

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

Proof. Again set $J := J(s)$ and $J_C := J_C(s)$ and assume that $C = (\alpha_1, \ldots, \alpha_r)$ for some $r \in \{1, \ldots, n\}$. By P_j, $j \in \{1, \ldots, r\}$, we denote the set of all regular states x satisfying $x_k = s_k$ for all $k \notin J$ and

$$x_{i+1} = \begin{cases} x_i & , \quad \varepsilon_{i+1,i} = + \\ 1 - x_i & , \quad \varepsilon_{i+1,i} = - \end{cases} \quad \text{for all } i \in \{1, \ldots, r\} \setminus \{j\},$$

with indices i taken modulo r. Choose $j \in \{1, \ldots, r\}$ and $x \in P_j$. Lemma 1 implies that $f_i(x) = s_i$ for all $i \notin J$. Now set $\tilde{x} = f(x)$ and let $i \in \{1, \ldots, r\}$. Again,



(c)

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

(d)

$$
\begin{aligned}
s_1 = \theta &\Rightarrow f_2^\theta(s) = \theta\\
s_1 \neq \theta &\Rightarrow f_2^\theta(s) \neq \theta\\
s_2 = \theta &\Rightarrow f_3^\theta(s) = \theta,\ f_4^\theta(s) = 0, \theta\\
s_2 \neq \theta &\Rightarrow f_3^\theta(s) \neq \theta\\
s_3 = \theta &\Rightarrow f_1^\theta(s) = \theta, 1,\ f_4^\theta(s) = 0, \theta\\
s_4 = \theta &\Rightarrow f_1^\theta(s) = \theta, 1
\end{aligned}
$$

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

Fig. 4. Interaction graphs and parameter values of networks with only one singular steady state. Given are the non-zero logical parameters. For details see the text.

consider indices modulo r. According to Lemma 2 the only singular resource of α_{i+1} in s is α_i. Furthermore, we know $f_{i+1}^\theta(s) = s_{i+1} = \theta = |0,1|$. Thus, with reasoning similar to that in the proof of Theorem 4, we can deduce that

$$\tilde{x}_{i+1} = K_{i+1,R_{i+1}(x)} = \begin{cases} 0 & , \quad (x_i = 0 \wedge \varepsilon_{i+1,i} = +) \vee (x_i = 1 \wedge \varepsilon_{i+1,i} = -) \\ 1 & , \quad (x_i = 1 \wedge \varepsilon_{i+1,i} = +) \vee (x_i = 0 \wedge \varepsilon_{i+1,i} = -) \end{cases} ,$$

that is

$$\tilde{x}_{i+1} = \begin{cases} x_i & , \quad \varepsilon_{i+1,i} = + \\ 1 - x_i & , \quad \varepsilon_{i+1,i} = - \end{cases} .$$

Now, if $i \neq j+1$, we can express x_i in terms of x_{i-1}, since x is in P_j. Furthermore, we can then express x_{i-1} in terms of $\tilde{x}_i$ according to the observation above, which is valid for all $i \in \{1, \dots, r\}$. Some easy substitutions yield firstly

$$\tilde{x}_{i+1} = \begin{cases} x_{i-1} & , \quad (\varepsilon_{i+1,i} = + \wedge \varepsilon_{i,i-1} = +) \vee (\varepsilon_{i+1,i} = - \wedge \varepsilon_{i,i-1} = -) \\ 1 - x_{i-1} & , \quad (\varepsilon_{i+1,i} = + \wedge \varepsilon_{i,i-1} = -) \vee (\varepsilon_{i+1,i} = - \wedge \varepsilon_{i,i-1} = +) \end{cases} ,$$

and secondly that $\tilde{x}_{i+1} = \tilde{x}_i$, if $\varepsilon_{i+1,i} = +$, and $\tilde{x}_{i+1} = 1 - \tilde{x}_i$, if $\varepsilon_{i+1,i} = -$. It follows that $\tilde{x} = f(x)$ is an element of P_{j+1}. Furthermore, in case $\varepsilon_{i,i-1} = +$ and $i \neq j+1$, we have $\tilde{x}_i = x_{i-1}$ as seen above and $x_{i-1} = x_i$, since $x \in P_j$. This shows $f_i(x) = \tilde{x}_i = x_i$. The same reasoning leads to $f_i(x) = \tilde{x}_i = x_i$ for $\varepsilon_{i,i-1} = -$ and $i \neq j+1$. It follows that every successor x' of x in the state transition graph is either in P_j, in case $x'_{j+1} = x_{j+1}$, or in P_{j+1}, in case $x'_{j+1} \neq x_{j+1}$. Since our reasoning is true for indices modulo r, we can deduce that the union P of the sets P_j, $j \in \{1, \dots, r\}$, is a trap set and thus contains an attractor A (see the proof of Proposition 1).

Finally, we show that each state in P, and thus in A, has a successor other than itself. For $x \in P_j$ we have

$$x_j = \begin{cases} x_{j+1} & , \quad \varepsilon_{j+2,j+1} \cdot \ldots \cdot \varepsilon_{j,j-1} = + \\ 1 - x_{j+1} & , \quad \varepsilon_{j+2,j+1} \cdot \ldots \cdot \varepsilon_{j,j-1} = - \end{cases} .$$

Furthermore, we know that $\tilde{x}_j = x_j$ with $\tilde{x} := f(x)$ and $\tilde{x} \in P_{j+1}$. It follows that $\tilde{x}_{j+1} = x_j$, if $\varepsilon_{j+1,j} = +$, and $\tilde{x}_{j+1} = 1 - x_j$, if $\varepsilon_{j+1,j} = -$. Thus we obtain

$$\tilde{x}_{j+1} = \begin{cases} x_{j+1} & , \quad \prod_{k=1}^r \varepsilon_{k+1,k} = + \\ 1 - x_{j+1} & , \quad \prod_{k=1}^r \varepsilon_{k+1,k} = - \end{cases} ,$$

with indices k taken modulo r. Since C is negative, we know $\prod_{k=1}^{r} \varepsilon_{k+1,k} = -$, and thus $f_{j+1}(x) \neq x_{j+1}$. Thus x has a successor other than itself in the state transition graph. It follows that the cardinality of A is greater than one. ☐

Figure 3 illustrates the theorem. In (d) we give a parameter specification that allows 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. Figure 4 (a) and (c) specify a system that illustrates that the sufficient condition in Theorem 5 is not necessary. By calculating the corresponding state table we can see that there is no regular steady state of the system. Thus there has to be an attractor with cardinality greater than one. However, from the logical implications given in (d), it follows easily that the only singular steady state is $(\theta,\theta,\theta,\theta)$, which is characteristic for all circuits in the interaction graph given in (a).

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 in the set P introduced 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. The proofs of the next two propositions are omitted for lack of space. They can be found in [7].

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 $i \in I$.*

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 $i \in I$.*

The proofs in [7] show how to derive singular steady states satisfying the statements of Prop. 2 and 3. However, those singular steady states may coincide with $(\theta, \ldots, \theta)$, 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 [3]. 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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