Local Structure and Behavior of Boolean Bioregulatory Networks*

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Abstract. A well-known discrete approach to modeling biological regulatory networks is the logical framework developed by R. Thomas. The network structure is captured in an interaction graph, which, together with a set of Boolean parameters, gives rise to a state transition graph describing the dynamical behavior. Together with E. H. Snoussi, Thomas later extended the framework by including singular values representing the threshold values of interactions. A systematic approach was taken in [10] to link circuits in the interaction graph with character and number of attractors in the state transition graph by using the information inherent in singular steady states. In this paper, we employ the concept of local interaction graphs to strengthen the results in [10]. Using the local interaction graph of a singular steady state, we are able to construct attractors of the regulatory network from attractors of certain subnetworks. As a comprehensive generalization of the framework introduced in [10], we drop constraints concerning the choice of parameter values to include so-called context sensitive networks.

1 Introduction

In biology, regulatory networks are often visualized as cartoons that illustrate which components of a system interact with each other. In addition, a verbal description of the system's behavior is provided that clarifies the processes captured graphically in the cartoon. Logical approaches are an intuitive way to model such systems in a mathematical framework. In the 70's, R. Thomas introduced a discrete formalism, which, over the years, has been further developed and successfully applied to biological problems (see [13], [14] and references therein). Network components are represented by Boolean variables. The structure of the network is captured in a directed, signed graph called interaction graph. Edges represent interactions between network components. The sign of an edge signifies whether an activating or inhibiting influence is exerted, provided the tail component of the edge is active, i. e., has value 1. Boolean parameter values specify a function that determines the behavior of the system. Thomas used the so-called asynchronous update method to derive a state transition graph from the Boolean

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function. Two successive states differ in at most one component, resulting in a non-deterministic representation of all possible behavior of the system.

This framework has been extended over the years. Network components were allowed to have more than two activity levels, interactions were associated with a threshold value determining when the interaction becomes effective. Thomas, together with E.H. Snoussi, used the threshold values, which they called *singular values*, to obtain a better understanding of the system's dynamics. In [11], they focussed on the relation between singular steady states and feedback circuits in the interaction graph of the network. We adapted these ideas to a Boolean setting in [10]. Despite the high level of abstraction, the introduction of singular states proved a useful tool for uncovering relations between structure and dynamics of bioregulatory networks. In this paper, we generalize and develop the results in [10] further. As a first step, we allow characteristics, i. e., the sign of network interactions to depend on the current state of the system. Whether a component has an activating or inhibiting influence on its target may depend on the activity of certain co-factors. We call systems including such interactions *context sensitive.* Adaptations in the definition of interaction graphs, parameters and singular steady states allow us to include context sensitive systems in our considerations. Furthermore, we exploit the concept of local interactions graphs. It was already successfully used in [6] and [5], and allows for a better understanding of what structures in the interaction graph influence the system's behavior in a given state. This view enables us to focus on the behavior of subnetworks obtained by projection, and from that draw conclusions about the network dynamics.

The organization of the paper is as follows. in Sect.2 we introduce the Boolean framework used to describe regulatory networks. This framework includes systems that display context sensitivity, since we allow parallel edges in the interaction graph. We show in Sect.3 that the set of functions arising from interaction graphs and associated parameter values corresponds to the set of Boolean function $f : \mathcal{B}^n \to \mathcal{B}^n$. We then define the local interaction graph of a given state. Subsequently, we introduce singular steady states. In Sect.5, we employ the concept of local interaction graphs for singular steady states. The resulting view on the network dynamics allows us to derive certain characteristics of the state transition graph from the behavior of suitable subnetworks. We end the paper with concluding remarks and perspectives for future work.

2 Regulatory Networks

As mentioned in the introduction, a directed, signed graph is used in the Thomas formalism to capture the network structure of a regulatory system. However, we are interested in a more general representation that allows for the sign of the interaction, to depend on the current state of the system. To accurately describe the structure of such context sensitive networks we use directed multigraphs that allow for parallel edges. Multigraphs have been used in a similar way in [2]

Throughout the text we set $\mathcal{B} := \{0, 1\}$.

Definition 1. *An* interaction (multi-)graph *(or bioregulatory (multi-)graph)* $\mathcal{I}$ *is a labeled directed multigraph with vertex set* $V := \{\alpha_1, \ldots, \alpha_n\}$*,* $n \in \mathbb{N}$*, and edge set* $E \subseteq V \times V \times \{+, -\}$*.*

The vertices $\alpha_1, \ldots, \alpha_n$ represent the components of the regulatory network such as genes, RNA, or proteins. We view each component α_i as a variable that adopts values in $\mathcal{B}$. The value 1 signifies that the component is active, i. e., it influences its interaction targets according to the interaction signs. For example, if some substance concentration needs to cross a threshold in order to influence some target component, then the corresponding Boolean value is 0 as long as the concentration is below, and 1 if the concentration is above the threshold.

When analyzing the interaction graph of a network we are interested in certain structural motives. In this paper we focus on so-called *(feedback) circuits*. Here, a circuit is a tuple $(e_1, \ldots, e_r)$ of edges $e_i = (k^i, l^i, \varepsilon) \in E$ such that all k^i, $i \in \{1, \ldots, r\}$, are pairwise distinct, and $l^i = k^{i+1}$ for all $i \in \{1, \ldots, r\}$ modulo r. The *sign of a circuit* is the product of the signs of its edges. Note that in a multigraph a circuit is not uniquely determined by its vertices. Figure 1 shows an interaction graph that contains two different circuits consisting of the vertices α_2 and α_3: the positive circuit $((\alpha_2, \alpha_3, +), (\alpha_3, \alpha_2, +))$ and the negative circuit $((\alpha_2, \alpha_3, +), (\alpha_3, \alpha_2, -))$.

To simplify notation, we identify each vertex α_i with its index i. Furthermore, we denote $e_{ij}^{\varepsilon} := (i, j, \varepsilon)$ for all $(i, j, \varepsilon) \in E$. 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 there is an edge $(\alpha_j, \alpha_i, \varepsilon)$ for some $\varepsilon \in \{+, -\}$ in E.

Since we are not only interested in the structure of a given regulatory network but also in its dynamics, we have to find behavioral rules for the system consistent with the structural description. The notation is based on ideas introduced in [1] and [8].

Definition 2. *Let* $\mathcal{I} = (V, E)$ *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)* resource edges $R_j(s) = R_j^{\mathcal{I}}(s)$ *of* α_j *in state* s *is the set*

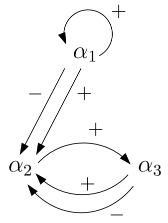
$$\{(\alpha_i, \alpha_j, \varepsilon) \in E \mid (\varepsilon = + \wedge s_i = 1) \vee (\varepsilon = - \wedge s_i = 0)\}.$$

Given a set

$$K(\mathcal{I}) := \{K_{j,R_j(s)} \mid j \in \{1, \ldots, n\},\ s \in \mathcal{B}^n\}$$

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 behavior of a component α_j is determined by the influences its predecessors exert on it. The set of resource edges $R_j(s)$ contains all edges that contribute to an activation of α_j in state s. Note that here the absence of an inhibiting influence (represented by a negative edge) is interpreted as an activating influence on the target component. With this interpretation we have that $R_j(s)$ for all $s \in \mathcal{B}^n$ contains an edge $(\alpha_i, \alpha_j, \varepsilon)$, if there are two edges from α_i to α_j in the interaction



$$
\begin{aligned}
K_{1,\emptyset} &= 0\\
K_{1,\{e_{11}^{+}\}} &= 1\\
K_{2,\{e_{12}^{+},e_{32}^{+}\}} &= 0\\
K_{2,\{e_{12}^{+},e_{32}^{-}\}} &= 1\\
K_{2,\{e_{12}^{-},e_{32}^{+}\}} &= 1\\
K_{2,\{e_{12}^{-},e_{32}^{-}\}} &= 0\\
K_{3,\emptyset} &= 0\\
K_{3,\{e_{23}^{+}\}} &= 1
\end{aligned}
$$

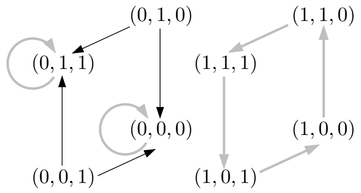


Fig. 1. Interaction graph of a system comprising three components. In the middle, a list of all parameters with an assignment of Boolean values. On the right, the state transition graph corresponding to the interaction graph and parameter values. The heavier gray edges indicate attractors.

graph. If there is only one edge $(\alpha_i, \alpha_j, \varepsilon)$, then $R_j(s)$ may or may not contain that edge depending on ε and the component value s_i. Let $M_j^{\mathcal{I}} = M_j$ be the set of all sets $M \subset E$ such that M contains only edges ending in α_j. More specific, M contains one and only one edge from α_i to α_j, if $\mathcal{I}$ includes two distinct edges from α_i to α_j, and at most one edge from α_i to α_j, if there is only one edge from α_i to α_j in $\mathcal{I}$. Then we have $M_j = \{R_j(s) \mid s \in \mathcal{B}^n\}$ and thus the set of parameters satisfies $K(\mathcal{I}) = \{K_{j,M} \mid j \in \{1, \ldots, n\},\ M \in M_j\}$.

The choice of parameter values should be consistent with the information inherent in the interaction graph. We require that the character of an edge as given by the sign of the edge is reflected in the dynamical impact of that edge. Therefore, we pose the following condition on the parameter values:

$$\forall j \in \{1, \ldots, n\} \quad \forall\ M, M' \in M_j : \quad M \subseteq M' \Rightarrow K_{j,M} \leq K_{j,M'} \ . \qquad (1)$$

The condition signifies that the addition of an activating influence or the removal of an inhibiting influence (both represented by an edge addition to the set of resource edges) cannot induce a decrease in activity level of α_j.

A different aspect of consistency of interaction graph and parameters is the following. Whenever there is an edge in the interaction graph, there should be a notable effect of that edge on the system dynamics. The edge e from α_i to α_j has no dynamical effect, if $K_{j,M} = K_{j,M\setminus\{e\}}$ for all $M \in M_j$ in case there is no other edge from α_i to α_j in the interaction graph. If there is another edge e' from α_i to α_j, then e is dynamically ineffective, if $K_{j,M} = K_{j,M\setminus\{e\}\cup\{e'\}}$ for all $M \in M_j$. We can then delete the edge e from the interaction graph and still obtain the same behavior. In the following, we always assume that all edges in the interaction graph are *functional*, that is, for all $j \in \{1, \ldots, n\}$ and every edge $e = (\alpha_i, \alpha_j, \varepsilon)$ in the interaction graph $\mathcal{I}$ the condition

$$
\begin{aligned}
&\exists\ M \in M_j\ :\ K_{j,M} > K_{j,M\setminus\{e\}}, && \text{if } e \text{ is the only edge from } \alpha_i \text{ to } \alpha_j \text{ in } \mathcal{I},\\
&\exists\ M \in M_j\ :\ K_{j,M} > K_{j,M\setminus\{e\}\cup\{e'\}}, && \text{if } e' \neq e \text{ is an edge from } \alpha_i \text{ to } \alpha_j \text{ in } \mathcal{I},
\end{aligned}
$$

holds. This concept of functionality is an adaptation of the notion of functionality introduced in [10].

In Fig. 1 all the parameters corresponding to the interaction graph given in the figure are listed. For α_1 and α_3 the parameters depend on whether or not the single positive edge ending in α_1 resp. α_3 is effective or ineffective. Thus $M_1 = \{\emptyset, \{e^+_{11}\}\}$ and $M_3 = \{\emptyset, \{e^+_{23}\}\}$. The choice of Boolean values for the parameters satisfies condition (1) and ensures the functionality of the edges e^+_{11} and e^+_{23}. The component α_2 is influenced by both α_1 and α_3 via two parallel edges, respectively. Thus there is no state such that α_2 is without an activating influence, i. e., an ineffective negative edge or an effective positive edge reaching α_2. We have $M_2 = \{\{e^+_{12}, e^+_{32}\}, \{e^+_{12}, e^-_{32}\}, \{e^-_{12}, e^+_{32}\}, \{e^-_{12}, e^-_{32}\}\}$. Again the choice of parameter values renders all edges functional. A closer look allows the following interpretation. If α_1 has activity level 0, then the influence of α_3 on α_2 corresponds to an activating influence. That is, if α_3 is inactive α_2 tends to inactivity represented by the parameter $K_{2,\{e^-_{12},e^-_{32}\}} = 0$, and if α_3 is active α_2 tends to activity since $K_{2,\{e^-_{12},e^+_{32}\}} = 1$. If α_1 has value 1, then the situation is reversed and the influence of α_3 on α_2 is inhibiting. This is an example for a context sensitive system. In [10], the parameters correspond to sets of resource vertices, i. e., the influence of one component on another cannot change depending on the current state of the system. The network shown in Fig.1 cannot be represented with that restriction. However, the notion of resource edges and resource vertices are equivalent, if there are no parallel edges in the interaction graph.

The parameters determine the behavior of the system as follows. The Boolean value of the parameter $K_{j,R_j(s)}$ indicates how the activity level, i. e., the value of the component α_j will evolve from its value in state s. 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. However, if a state and its image differ in more than one component, we take the following consideration into account. In a biological system two different processes of change in activity level represented by the value change of two distinct components will not take the exact same amount of time. Thus we assume that in the discrete dynamical representation a state differs from its successor in at most one component. This procedure is called *asynchronous update* in Thomas' framework. By applying this idea we derive a non-deterministic representation of the dynamics which we again formalize as a directed graph.

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 $j \neq i$.*

On the right in Fig. 1 we see the state transition graph corresponding to the given interaction graph and parameters. The dynamics are non-deterministic. For example, there are two edges leaving the state (0,1,0), representing two different behaviors of the system.

3 Boolean Functions and Local Interaction Graphs

As we have seen above, the formalism we introduced in the preceding section allows us to model context sensitive systems. The dynamical behavior is determined by a Boolean function that is consistent with the underlying interaction graph. In the following we show that for every Boolean function $g : \mathcal{B}^n \to \mathcal{B}^n$ there exists an interaction graph that is consistent with g.

Proposition 1. *Let $g : \mathcal{B}^n \to \mathcal{B}^n$ be a Boolean function. Then there exists an interaction graph $\mathcal{I} = (V, E)$ and a set of specified parameters $K(\mathcal{I})$ such that $g = f^{K(\mathcal{I})}$.*

Proof. Let $\mathcal{I}^1 = (V, E^1)$ be the interaction graph with $V := \{\alpha_1, \ldots, \alpha_n\}$ and $E^1 := V \times V \times \{+, -\}$, i. e., $\mathcal{I}^1$ includes every possible edge. We set $K_{i,R_i^1(s)} := g_i(s)$ for all $i \in \{1, \ldots, n\}$ and $s \in \mathcal{B}^n$, with $R_i^1(s) := R_i^{\mathcal{I}^1}$. Now, we show that the set of parameters satisfies condition (1).

Let $j \in \{1, \ldots, n\}$. We have shown in Sect. 2 that for every $s \in \mathcal{B}^n$ there exists one and only one $M \in M_j^1$ such that $R_j^1(s) = M$, where $M_j^1 := M_j^{\mathcal{I}^1}$ is the set introduced following Def. 2. By definition, every $M \in M_j^1$ contains one and only one edge from α_i to α_j for all $i \in \{1, \ldots, n\}$. Thus $\operatorname{card} M = n$ for all $M \in M_j^1$, where $\operatorname{card} M$ denotes the cardinality of M. It follows that $M \not\subseteq M'$ for all $M, M' \in M_j^1$. Therefore, every choice of parameter values corresponding to $\mathcal{I}^1$ and in particular the parameter values given above satisfy condition (1).

However, we have yet to consider the functionality of the edges in $\mathcal{I}^1$. This can be done componentwise via the condition characterizing functional edges given in Sect. 2. We then eliminate edges that are not functional and derive a new interaction graph and corresponding parameter values in an iterative procedure.

Let e be an edge ending in α_j. If e is functional, we make no alterations on the interaction graph and parameters. If e is not functional, we define a new interaction graph $\mathcal{I}^2 = (V, E^2)$ with $E^2 := E^1 \setminus \{e\}$. Clearly, we have $M_j^2 := M_j^{\mathcal{I}^2} = \{M \setminus \{e\} \mid M \in M_j\}$. We set $K_{j,M\setminus\{e\}} := K_{j,M}$ for all $M \in M_j^1$, and keep all other parameters as introduced above. It is easy to see that condition (1) holds for $\mathcal{I}^2$ and the corresponding parameters. Let $M, M' \in M_j^1$ with $M \subseteq M'$. In the cases $M, M' \in M_j^1$, $M, M' \notin M_j^1$, and $M \in M_j^1$ and $M' \notin M_j^1$, condition (1) follows from the fact that every set $M'' \in M_j^2 \setminus M_j^1$ satisfies $M'' \cup \{e\} \in M_j^1$, the definition of the parameters corresponding to $\mathcal{I}^2$, and the fact the $\mathcal{I}^1$ with its corresponding parameters satisfies condition (1). If $M \notin M_j^1$ and $M' \in M_j^1$, then $M \cup \{e\} \in M_j^1$. There exists an edge e' parallel to e in $\mathcal{I}^1$ since otherwise $M \in M_j^1$. Then for every $M'' \in M_j^1$ holds either $e \in M''$ or $e' \in M''$. It follows that $e' \in M'$ and $M \cup \{e'\} \in M_j^1$. According to the definition of the parameter values and since e is not functional, we have $K_{j,M} = K_{j,M\cup\{e\}} \leq K_{j,M\cup\{e'\}}$. Since $\mathcal{I}^1$ satisfies (1), we have $K_{j,M\cup\{e'\}} \leq K_{j,M'}$, i. e., (1) holds for $\mathcal{I}^2$ and the corresponding parameters. Clearly, the function $f^{K(\mathcal{I}^{\in})}$ derived from the new parameters still coincides with g.

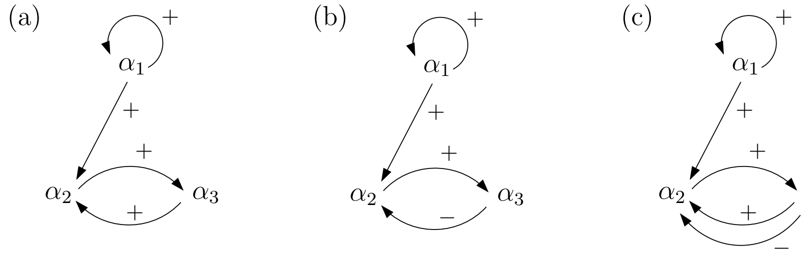


Fig. 2. Local interaction graphs corresponding to the graph and parameters given in Fig. 1. $\mathcal{I}((0,0,0))$ in (a), $\mathcal{I}((1,0,0))$ in (b), $\mathcal{I}((\theta,0,0))$ in (c).

We repeat the procedure, starting always with the last defined interaction graph and corresponding parameters, for every edge in $\mathcal{I}^1$. After $2n^2$ steps we obtain an interaction graph and parameters consistent with g. $\square$

In Sect. 2 we emphasized the point that we only deal with interaction graphs all the edges of which are functional. We demand that each edge has to have an impact on the dynamics. However, this influence does not have to be effective in the whole state space $\mathcal{B}^n$. If we want to understand the way the structure and dynamics of a system relate to each other, then it is useful to have a closer look on the effective interactions depending on the current state of the system. To capture those local structural aspects we introduce the concept of *local interaction graphs*. It has already been used in [6] and [5] (see also references therein). In the following, we denote with $\overline{s}^i$ the state that coincides with s in all components $j \neq i$ and takes the value $1 - s_i$ in the i-th component.

Definition 4. *Let $\mathcal{I} = (V, E)$ be an interaction graph with parameter set $K(\mathcal{I})$. Let $s \in \mathcal{B}^n$. Then we denote by $\mathcal{I}(s)$ the graph with vertex set V and edge set $E(s) \subseteq E$. An edge (i, j, ε) is in $E(s)$ if and only if*

$$K_{j,R_j(s)} \neq K_{j,R_j(\overline{s}^i)} \quad \wedge \quad \varepsilon = + \Leftrightarrow s_i = K_{j,R_j(s)} \,.$$

We call $\mathcal{I}(s)$ the (local) interaction graph in state *s.*

Clearly, every edge in a local interaction graph $\mathcal{I}(s)$ is also contained in $\mathcal{I}$, since we use the parameters of the interaction graph $\mathcal{I}$ to characterize the edges in a local interaction graph. More precisely, $\mathcal{I}$ is the union of all graphs $\mathcal{I}(s)$, $s \in \mathcal{B}^n$. We call $\mathcal{I}$ also the *global interaction graph*. Note that there are no parallel edges in a local interaction graph in state $s \in \mathcal{B}^n$. Figure 2(a) and (b) show the graphs $\mathcal{I}((0,0,0))$ and $\mathcal{I}((1,0,0))$ corresponding to the example given in Fig. 1. The local interaction graphs give us a finer understanding of the way the network components interact. They can be seen as a visualization of the discrete Jacobian matrix of the Boolean function $f^{\mathcal{I}}$ as introduced in [9].

4 Singular States

In our formalism we only consider whether a component is active or not. We now incorporate a threshold value that allows us to express uncertainty in the sense that we do not know if a certain interaction is effective. We already used this concept in [10] for networks without context sensitivity. Again, we mainly use notation introduced in [8]. Throughout this section let $N := (\mathcal{I}, f = f^{K(\mathcal{I})})$ be a bioregulatory network comprising n components.

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

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 6. *We define for all $i \in \{1, \dots, n\}$*

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

where $K_{i,\min(s)} := \min\{\, K_{i,R_i(s')} \mid s' \in \mathcal{B}^n \,,\ s'_j = s_j \text{ for all } j \notin J(s)\}$ and $K_{i,\max(s)} := \max\{\, K_{i,R_i(s')} \mid s' \in \mathcal{B}^n \,,\ s'_j = s_j \text{ for all } j \notin J(s)\}$. We call $s \in \mathcal{B}_\theta^n$ a steady state if $f^\theta(s) = s$.

The definition of $K_{i,\min(s)}$ and $K_{i,\max(s)}$ ensures that the image of a regular state under f^θ is again a regular state. More specific, we have $f^\theta|_{\mathcal{B}^n} = f$. If a state has singular components, then $K_{i,\min(s)}$ and $K_{i,\max(s)}$ reflect the dynamical behavior of the component i in the two extreme cases that either all singular predecessors of α_i have no activating influence on α_i or they all contribute to an activation of α_i.

Thomas and Snoussi already link singular states to circuits in the interaction graph, albeit in a different framework (see [11]). We have adapted their ideas to a Boolean framework without context sensitivity in [10].

Definition 7. *Let $C = (\alpha_{i_1}, \dots, \alpha_{i_r})$ 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 $l \in \{1, \dots, r\}$.*

In general, a characteristic state of a circuit is not unique. The state $(\theta, \dots, \theta)$ is characteristic for every circuit in $\mathcal{I}$. A simple modification of the reasoning in [10] leads to the following statement.

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

A singular steady state s can be characterized using only regular states and the function f. The idea is to check componentwise the behavior for regular states s^+ and s^- that satisfy $K_{i,R_i(s^+)} = K_{i,\max(s)}$ and $K_{i,R_i(s^-)} = K_{i,\min(s)}$ for some $i \in \{1, \dots, n\}$. The proofs for networks that are not context sensitive are given in [10] and can be easily adapted.

5 Attractors and Local Interaction Graphs of Singular Steady States

In this section we link structural properties of (local) interaction graphs with the dynamical behavior of the system by considering singular steady states. Every possible behavior of the system is captured in the corresponding state transition graph introduced in Sect.2. In the following let $N := (\mathcal{I} = (V, E), f = f^{K(\mathcal{I})})$ be a bioregulatory network comprising n components and $\mathcal{S}_N$ the corresponding state transition graph. In addition to standard terminology from graph theory such as paths and cycles we us the following concepts.

Definition 8. *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 all* $s^1, s^2 \in A$ *there is a path from* s^1 *to* s^2 *in* $\mathcal{S}_N$*. 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.*

In other words, the attractors correspond to the terminal strongly connected components of the graph. Regular steady states as well as trap cycles are attractors. The attractors in the state transition graph given in Fig. 1 are the sets containing the steady states, i. e., $\{(0, 0, 0)\}$ and $\{(0, 1, 1)\}$, and the set containing the states of the trap cycle in the graph, i. e., $\{(1, 0, 0), (1, 1, 0), (1, 1, 1), (1, 0, 0)\}$.

The behavior of a system becomes, at least to some degree, predictable and stable inside an attractor. Often, a sensible biological interpretation can be found for an attractor. In cell differentiation, the different stable states reached at the end of development may be represented by distinct steady states in the state transition graph. 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.

State transition graphs always contain at least one attractor. The proof of the following more precise statement can be found in [10].

Proposition 2. *For every state* $s \in \mathcal{B}^n$ *exists a trajectory in* $\mathcal{S}_N$ *which starts in* s *and leads to an attractor.*

If some vertex α_i in $\mathcal{I}$ does not have a predecessor, then clearly $a_i = K_{i,\emptyset}$ for every state a in an attractor. Similarly, we know the values a_j for vertices the only predecessor of which is α_i, and so on. Throughout this section we assume that every vertex in $\mathcal{I}$ has a predecessor. Note that an input value in the sense of a component that maintains its current activity level independent of the values of the other components is represented as a vertex with its only incoming edge being a positive selfloop.

In the following we have a closer look at the information concerning the network dynamics in general and the attractors in particular that is inherent in the existence and properties of singular steady states. We also want to exploit structural information. As a first step we adapt the concept of local interaction

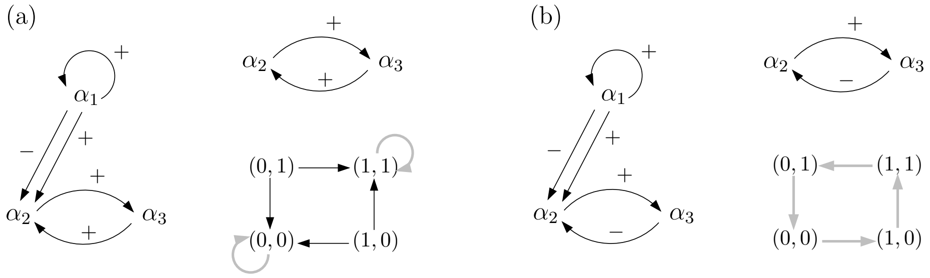


Fig. 3. Local interaction graph $\mathcal{I}((0, \theta, \theta))$ on the left, $\mathcal{I}^{\theta}((0, \theta, \theta))$ and corresponding state transition graph on the right of (a). Local interaction graph $\mathcal{I}((1, \theta, \theta))$ on the left, $\mathcal{I}^{\theta}((1, \theta, \theta))$ and corresponding state transition graph on the right of (b). Attractors are indicated by heavier gray edges.

graphs to singular states. Recall that $J(s)$ is the set of all singular components of a state $s \in \mathcal{B}_{\theta}^{n}$

Definition 9. *Let $s \in \mathcal{B}_{\theta}^{n}$. We denote by $\mathcal{I}(s)$ the (multi-)graph with vertex set V and edge set $E(s)$. An edge e is in $E(s)$ if and only if there exists a regular state s' such that $s'_i = s_i$ for all $i \notin J(s)$ and $e \in E(s')$, where $E(s')$ denotes the edge set of the interaction graph $\mathcal{I}(s')$ in s'. Again, we call $\mathcal{I}(s)$ the (local) interaction graph in s.*

Note that the interaction graph in a singular state may have parallel edges. In Fig. 2 (c) we see the local interaction graph in state $(\theta, 0, 0)$, which is the union of the graphs $\mathcal{I}((0, 0, 0))$ and $\mathcal{I}((1, 0, 0))$ given in (a) and (b).

A singular steady state s yields stability in the dynamical behavior for the components that do not belong to $J(s)$. To make a more precise statement we introduce notation for a specific subgraph of $\mathcal{I}(s)$. By $\mathcal{I}^{\theta}(s)$ we denote the (multi-)graph with vertex set $V^{\theta}(s) := J(s)$ and edge set $E^{\theta}(s) := \{(i, j, \varepsilon) \in E(s) \mid i, j \in J(s)\}$. That is, we only keep the singular components and interactions between them. We call a graph Z component of $\mathcal{I}^{\theta}(s)$, if $Z = (V_Z, E_Z)$ is a maximal subgraph of $\mathcal{I}^{\theta}(s)$ such that for every $k, k' \in V_Z$ exist vertices $k_1, \ldots, k_r \in V_Z$ with $k_1 = k$, $k_r = k'$, and $(k_i, k_{i+1}, \varepsilon) \in E^{\theta}(s)$ or $(k_{i+1}, k_i, \varepsilon) \in E^{\theta}(s)$ for some $\varepsilon \in \{+, -\}$ and all $i \in \{1, \ldots, r-1\}$. In Fig. 3 we see for our running example introduced in Fig. 1 the graphs $\mathcal{I}((0, \theta, \theta))$ and $\mathcal{I}^{\theta}((0, \theta, \theta))$ in (a), as well as the graphs $\mathcal{I}((1, \theta, \theta))$ and $\mathcal{I}^{\theta}((1, \theta, \theta))$ in (b). Lastly, let C be a circuit in $\mathcal{I}(s)$ such that all edges of C are in $\mathcal{I}^{\theta}(s)$. Then there exists a component of $\mathcal{I}^{\theta}(s)$ that contains C. We denote this component by $J_C(s)$.

The next lemma shows that the stability of the regular components of a singular steady state is not influenced by value changes in a component Z of $\mathcal{I}^{\theta}(s)$. Moreover, if $\mathcal{I}^{\theta}(s)$ has more than one component, the component dynamics are independent of each other. This property is crucial for the remaining results in this section. The proof of the lemma is an adaptation of a similar, less general statement in [10]. Note that in [10] a different definition of $\mathcal{I}^{\theta}(s)$ is used that does not take the effectiveness of interactions in state s into account.

Lemma 1. *Let s be a singular steady state, and let $Z_1, \dots, Z_m$ 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. First, let us consider $i \in J(s) \setminus Z$. Then $s_j = \tilde{s}_j$ for every $j \in Pred(\alpha_i)$, since there are no predecessors of α_i in Z. Therefore, the sets of resource edges of α_i are not influenced by value changes in Z, i. e., $\{R_i(s') \mid s' \in \mathcal{B}^n,\ s'_j = s_j \text{ for all } j \notin J(s)\} = \{R_i(s') \mid s' \in \mathcal{B}^n,\ s'_j = \tilde{s}_j \text{ for all } j \notin J(\tilde{s})\}$. It follows that $K_{i,\min(s)} = K_{i,\min(\tilde{s})}$ and $K_{i,\max(s)} = K_{i,\max \tilde{s}}$, and thus $f_i^\theta(\tilde{s}) = f_i^\theta(s) = s_i = \tilde{s}_i$.

Now, let $i \notin J(s)$. Since $s_j = \theta$ for all $j \in Z$, we have $J(\tilde{s}) \subseteq J(s)$. Therefore, $\{s' \in \mathcal{B}^n \mid s'_j = \tilde{s}_j \text{ for all } j \notin J(\tilde{s})\} \subseteq \{s' \in \mathcal{B}^n \mid s'_j = s_j \text{ for all } j \notin J(s)\}$. It follows that $K_{i,\min(s)} \leq K_{i,\min \tilde{s}} \leq K_{i,\max(\tilde{s})} \leq K_{i,\max(s)}$. Since $f_i^\theta(s) = s_i$ is regular, we know $K_{i,\min(s)} = K_{i,\max(s)} = s_i$. Thus, $K_{i,\min(\tilde{s})} = K_{i,\max(\tilde{s})} = s_i$ and $f_i^\theta(\tilde{s}) = s_i = \tilde{s}_i$. □

The above lemma shows that we can construct attractors of the state transition graph $\mathcal{S}_N$ from attractors of the dynamics restricted to the components of $\mathcal{I}^\theta(s)$. To give a clear understanding of this construction we need the following notation.

Let s be a singular steady state and Z a component of $\mathcal{I}^\theta(s)$ with $k := \operatorname{card} V_Z$. We may assume that $V_Z = \{\alpha_{l+1}, \dots, \alpha_{l+k}\}$ for some $l \in \{0, \dots, n-1\}$. Then Z is an interaction graph comprising k vertices. Now, we want to define the dynamics of Z as the projection of the dynamics of $\mathcal{I}$ with respect to s. We define a parameter set $K(Z)$ according to Def. 2 as the set of all parameters $K^Z_{i,R_i^Z(z)} := K_{i,R_i(\tilde{s})}$ for $z \in \mathcal{B}^k$ and $\tilde{s} \in \mathcal{B}^n$ with $\tilde{s}_i = s_i$ for all $i \notin J(s)$ and $\tilde{s}_i = z_{i-l}$ for all $i \in Z$. The parameters are well defined since there are no predecessors of vertices in Z in $J(s) \setminus Z$. We set $f^{K(Z)} = f^Z : \mathcal{B}^k \to \mathcal{B}^k$, $z \mapsto (K^Z_{1,R_1^Z(z)}, \dots, K^Z_{k,R_k^Z(z)})$. We then have $f^Z = \pi^Z \circ f^\theta \circ \rho^Z$, where $\rho^Z : \mathcal{B}^k \to \mathcal{B}^n$ with $\rho_i^Z(z) = s_i$ for $i \notin Z$ and $\rho_i^Z(z) = z_{i-l}$ for $i \in Z$, and $\pi^Z : \mathcal{B}^n \to \mathcal{B}^k$ is the projection on the components of Z. Again, note that f^Z yields always regular values, since the singular values in $J(s) \setminus Z$ do not influence the components in Z. The resulting network $N^Z := (Z, f^Z)$ inherits condition (1) from $N = (\mathcal{I}, f)$. The definitions of parameters and $\mathcal{I}^\theta(s)$ also ensure that all edges in Z are functional. According to Prop. 2 the state transition graph $\mathcal{S}_{N^Z}$ contains an attractor. This fact leads to the next theorem.

Theorem 2. *Let s be a singular steady state, and $Z_1, \dots, Z_m$ be the components of $\mathcal{I}^\theta(s)$. For all $j \in \{1, \dots, m\}$ let A_j be an attractor of the state transition graph corresponding to the network N^{Z_j} as defined above. Then there exists an attractor A in the state transition graph $\mathcal{S}_N$ such that $a_i = s_i$ for all $a \in A$, $i \notin J(s)$, and $\pi^{Z_j}(A) = A_j$ for all $j \in \{1, \dots, m\}$.*

Proof. Without loss of generality we may assume that Z_1 contains the vertices $\alpha_1, \dots, \alpha_{\operatorname{card} Z_1}$, Z_2 contains the vertices $\alpha_{\operatorname{card} Z_1 + 1}, \dots, \alpha_{\operatorname{card} Z_1 + \operatorname{card} Z_2}$, etc. We set $k := 1 + \sum_{i+1}^m \operatorname{card} Z_i$ and $A := A_1 \times \dots \times A_m \times \{(s_k, \dots, s_n)\}$.

First, we show that A is a trap set, i. e., every successor of a state in A is again in A. Let $x \in A$ and x' be a successor of x in $\mathcal{S}_N$. Assume $x \neq x'$. Then there exists $i \in \{1,\dots,n\}$ such that $x'_i = f^i(x) \neq x_i$ and $x'_j = x_j$ for all $j \neq i$. Lemma 1 yields that $f_j(x) = x_j = s_j$ for all $j \in \{k,\dots,n\}$. Thus, we find $l \in \{1,\dots,m\}$ such that $i \in Z_l$. Now, we only have to show that $\pi^{Z_l}(x') \in A^l$. Per definition we have $f^{Z_l}(\pi^{Z_l}(x)) = \pi^{Z_l} \circ f^\theta \circ \rho^{Z_l}(\pi^{Z_l}(x))$. Lemma 1 allows us to ignore the values of components in $J(s) \setminus Z_l$ and we obtain $\pi^{Z_l} \circ f^\theta \circ \rho^{Z_l}(\pi^{Z_l}(x)) = \pi^{Z_l}(f^\theta(x)) = \pi^{Z_l}(f(x))$. Since $i \in Z_l$, we then have $f^{Z_l}_{i-\operatorname{card} Z_{l-1}}(\pi^{Z_l}(x)) = f_i(x) = x'_i \neq x_i = \pi^{Z_l}_{i-\operatorname{card} Z_{l-1}}(x)$, where we set $\operatorname{card} Z_0 = 0$. Per definition there is an edge between $\pi^{Z_l}(x)$ and $\pi^{Z_l}(x')$ in $\mathcal{S}_{N^{Z_l}}$ and, since A_l is an attractor, we have $\pi^{Z_l}(x') \in A_l$.

Now, we have to show that there is a path from x to x' in $\mathcal{S}_N$ for all distinct $x, x' \in A$. First, we prove that if there is an edge from state z to state z', $z \neq z'$, in $\mathcal{S}_{N^{Z_l}}$, $l \in \{1,\dots,m\}$, then there is an edge from x to x' in $\mathcal{S}_N$ for all states $x, x' \in A$ satisfying $\pi^{Z_l}(x) = z$, $\pi^{Z_l}(x') = z'$, and $x_j = x'_j$ for all $j \notin Z_l$. According to the definition there is $i \in Z$ such that $z_p \neq z'_p = f^{Z_l}_p(z)$ with $p = i - \operatorname{card} Z_{l-1}$. With Lemma 1 follows that $z_p \neq f^{Z_l}_p(z) = \pi^{Z_l}_p \circ f^\theta \circ \rho^{Z_l}(z) = \pi^{Z_l}_p(f(x)) = f_i(x)$ for all $x \in A$ with $x_j = z_j$ for all $j \in Z_l$. For every such x the state x' satisfying $x'_j = x_j$ for all $j \neq i$ and $x'_i = f_i(x) \neq z_i = x_i$ is also in A, and there is an edge from x to x' in $\mathcal{S}_N$.

Let $x, x' \in A$. We set $x^1_i := x_i$ for all $i \notin Z_1$ and $x^1_i := x'_i$ for all $i \in Z_1$. For $l \in \{2,\dots,m\}$ we set $x^l_i := x^{l-1}_i$ for all $i \notin Z_l$ and $x^l_i := x'_i$ for all $i \in Z_l$. Then there exists a path in $\mathcal{S}_{N^{Z_1}}$ from $\pi^{Z_1}(x)$ to $\pi^{Z^1}(x^1)$, since A_1 is an attractor. As seen above, we then can find a path γ_1 from x to x^1 in $\mathcal{S}_N$ such that $\tilde{x}_j = x_j$ for every state $\tilde{x} \in \gamma_1$ and every $j \notin Z_1$. In the same fashion we find a path γ_2 from x^1 to x^2 in $\mathcal{S}_n$ such that $\tilde{x}_j = x^1_j$ for all $\tilde{x} \in \gamma_2$ and $j \notin Z_2$. We continue the procedure for $Z_3,\dots,Z_m$. Since $x^m = x'$ per definition, combining the paths γ_i in the order of their indices yields a path from x to x' in $\mathcal{S}_N$. □

We illustrate the theorem by considering our running example in Fig. 1. As shown in Fig. 3 (a), the graph $\mathcal{I}^\theta((0,\theta,\theta))$ has only one component Z consisting of a positive circuit containing α_2 and α_3. We derive the parameters $K(Z)$ from those given in Fig. 1 for the global interaction graph. Since $s_1 = 0$, we obtain, according to the above definition, the parameters $K^Z_{2,\emptyset} := K_{2,\{e^-_{12},e^-_{32}\}} = 0$ and $K^Z_{2,\{e^+_{32}\}} := K_{2,\{e^-_{12},e^+_{32}\}} = 1$. The parameters for α_3 stay the same, i. e., $K^Z_3, \omega = K_{3,\omega}$ for $\omega \in \{\emptyset, \{e^+_{23}\}\}$. The resulting state transition graph $\mathcal{S}^Z_N$ is also given in Fig. 3 (a) and contains the attractors $\{(0,0)\}$ and $\{(1,1)\}$. It follows from Theorem 2 that the sets $\{(0,,0,0)\}$ and $\{(0,1,1)\}$ are attractors in $\mathcal{S}_N$. Similarly, we derive a state transition graph from $\mathcal{I}^\theta((1,\theta,\theta))$ which consists of a negative circuit. The state transition graph is shown in Fig. 3 (b) and contains only one attractor, the set $\{(0,0),(1,0),(1,1),(0,1)\}$, which has cardinality greater than one. Thus, we find an attractor $\{(1,0,0),(1,1,0),(1,1,1),(1,0,1)\}$ in $\mathcal{S}_N$. The state transition graph $\mathcal{S}_N$ is given in Fig. 1 with the attractors emphasized.

In [4] it is shown that isolated circuits always display a characteristic behavior depending on their sign. A positive circuit gives rise to two attractors, more precisely two steady states, a negative circuit results in a cyclic attractor, i. e., an attractor with cardinality greater than one. The situation is much more difficult to analyze if there are many circuits in $\mathcal{I}$, possibly even intertwined.

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 attractors resp. a cyclic attractor in the state transition graph. The conjectures haven been proven in different settings (see e. g. [12], [5] and [7]). For regulatory networks without context sensitivity, we formulated in [10] a sufficient condition for circuits to display their characteristic behavior using singular steady states. The proof in [10] can be easily adapted to show the next statement.

Lemma 2. *Let I be an interaction graph such that there is only one circuit C in $\mathcal{I}$. If C is a positive circuit, then f has two fixed points. If C is negative, then there exists an attractor with cardinality greater than one in the state transition graph.*

We make some short remarks on the proof. Recall our assumption that every vertex in $\mathcal{I}$ has a predecessor. Since every edge is functional, the state $(\theta, \dots, \theta)$ is steady. In [10], it is shown that $\mathcal{I}$ then has a particular structure. It consists of the circuit C with possibly directed trees coming out of vertices of C. Those trees may also be interconnected. This structure allows us to explicitly specify values for the vertices of C that remain fix under f^θ in the case of C being positive, or behave like a trap cycle, if C is negative. From this core behavior we can then infer the behavior of the whole graph. Here, we also have to consider that there may be parallel edges outside the circuit C. However, the proof method is still valid. The necessary technical adaptations to the proofs in [10] correspond to those made in the proof of Lemma 1.

The above lemma together with Theorem 2 immediately leads to the following theorem.

Theorem 3. *Let C be a circuit in $\mathcal{I}$ and s a singular steady state characteristic of C. Assume that C is the only circuit in the component $J_C(s)$ of $\mathcal{I}^\theta(s)$. If C is a positive circuit, then f^θ has at least three fixed points and $\mathcal{S}_N$ contains at least two attractors. If C is negative, there is an attractor in $\mathcal{S}_N$ with cardinality greater than one.*

Proof. We may assume that $J_C(s)$ comprises the vertices $\alpha_1, \dots, \alpha_r$ for some $r \in \{1, \dots, N\}$. Let at first C be positive. Then $f^{J_C(s)}$ has two fixed points $x, x' \in \mathcal{B}^r$ according to Lemma 2. We define states s^1 and s^2 in $\mathcal{B}_\theta^n$ by $s_i^1 := s_i^2 := s_i$ for all $i \notin J_C(s)$, $s_i^1 := x_i$ and $s_i^2 := x_i'$ for all $i \in \{1, \dots, r\}$. From Lemma 1 follows that the states s^1 and s^2 are steady states. Thus f^θ has three fixed points, since s is distinct from s^1 and s^2. According to Theorem 2 we find attractors A_1 and A_2 in $\mathcal{S}_N$ such that $\pi^{J_C(s)}(A_1) = \{s^1\}$ and $\pi^{J_C(s)}(A_2) = \{s^2\}$.

If C is negative, we find an attractor A' in the state transition graph of the component graph $J_C(s)$ with cardinality greater than one. Then Theorem 2 yields an attractor A in $\mathcal{S}_N$ with $\pi^{J_C(s)}(A) = A'$. Thus A also has cardinality greater than one. □

The above theorem is a stronger result than the one obtained in [10], even for regulatory networks without context sensitivity. This is due to the fact that we

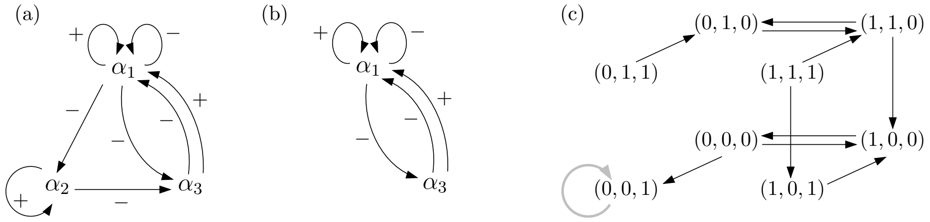


Fig. 4. We choose the parameters corresponding to the interaction graph in (a) as $K_{1,\{e^+_{11},e^+_{31}\}} = K_{1,\{e^-_{11},e^-_{31}\}} = K_{2,\{e^-_{12},e^+_{22}\}} = K_{3,\{e^-_{13},e^-_{23}\}} = 1$ and set all other parameters 0. In (b) the graph $\mathcal{I}^\theta(s)$ for the singular steady state $s = (\theta, 0, \theta)$. In (c) the corresponding state transition graph.

use the notion of local interaction graphs. They render a more refined picture of the dynamics possible in restricted parts of the state space.

Our running example from Fig. 1 together with Fig. 3 illustrates the theorem. Figure 4 shows that the statement does not hold, if the circuit C is not the only circuit in $J_C(s)$. The state $(\theta, 0, \theta)$ is steady for the bioregulatory network derived from the interaction graph in (a) and the parameters specified in the caption. There are four distinct circuits in $\mathcal{I}^\theta((\theta, 0, \theta))$, two negative and two positive circuits. However, the state transition graph contains only one attractor, namely the set $\{(0, 0, 1)\}$, as is shown in (c). Thus, neither the behavior characteristic for positive circuits nor that characteristic for negative circuits is displayed. Further examples can be found in [10]. However, a system may display the behavior characteristic for a circuit of a given sign, although there is no singular steady s such that the circuit is the only one in the corresponding component of $\mathcal{I}^\theta(s)$. The condition is therefore not necessary. Examples illustrating this point are given in Fig. 4 of [10].

6 Conclusion

In [10] we started a systematic investigation of the relation between singular steady states and attractors in the state transition graph of regulatory networks, which are described by an interaction graph and Boolean parameters. Among other results, we found sufficient conditions concerning singular steady states and circuits in the interaction graph ensuring the existence of two distinct attractors resp. a cyclic attractor. In this paper, we considerably refine and generalize the results in [10]. We are now able to deal with systems that display context sensitivity resulting in interaction graphs with parallel edges. In Sect.3 we have shown that in this framework the set of functions arising from interaction graphs and associated parameter values corresponds to the set of Boolean functions $f : \mathcal{B}^n \to \mathcal{B}^n$. To obtain a better understanding of the relation between the structure and the behavior of the system, we employ local interaction graphs, which consist of the interactions influencing the behavior of the system in a given state. Using the local interaction graph of a singular steady state, we are able to construct attractors of the given regulatory network from attractors of subsystems of the network. We also obtain a result linking the existence of circuits in the interaction graph to the existence of multiple attractors resp. an attractor with cardinality greater than one, which generalizes the corresponding statement in [10]. Both results demonstrate possibilities to study the network's dynamics without the explicit use of the state transition graph.

There are several starting points for future work. Although we have a good grasp on the behavior arising from circuits in the interaction graph, which are in some sense isolated, we have no clear understanding of the impact of intertwined circuits. In [3] the authors propose the concept of functionality context of a circuit, describing a set of states that ensure the effectiveness of the circuit interactions. Combining this idea with the notion of singular steady states may yield an approach to analyzing the behavior of networks containing intertwined circuits. Besides extending the results for regulatory networks described by Boolean functions, a further goal is to generalize the approach to multi-valued, discrete functions, since they allow a refined modeling of bioregulatory networks.

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