# Local Structure and Behavior of Boolean Bioregulatory Networks

Heike Siebert

DFG Research Center Matheon,
Freie Universität Berlin, Arnimallee 6, D-14195 Berlin, Germany
siebert@mi.fu-berlin.de

**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. A verbal description of the system's behavior clarifies the processes captured 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 has been continuously 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 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 dynamical behavior. Biologically realistic rules are employed to derive a state transition graph from the Boolean function, which amounts to a non-deterministic representation of all possible behaviors 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. R. Thomas and 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. A well-known example is the DNA-binding protein TCF which can repress as well as activate the same target genes. TCF acts as activator in the presence of $\beta$-catenin, induced by WNT signaling, while the co-expression of the protein TLE converts TCF into a repressor. We call systems including such ambiguous 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 (possibly context sensitive) regulatory networks. 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 already mentioned, a directed, signed graph is used in the Thomas formalism to capture the network structure of a regulatory system. We are now interested in a more general representation that allows for the interaction sign 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]. 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 $\alpha_i$ 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. 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 with two circuits consisting of the vertices $\alpha_2$ and $\alpha_3$: 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 $\alpha_i$ with its index $i$, and denote $e_{ij}^{\varepsilon} := (i, j, \varepsilon)$ for all $(i, j, \varepsilon) \in E$. For each $\alpha_i$ we denote by $Pred(\alpha_i)$ the set of *predecessors* of $\alpha_i$, i. e., the set of vertices $\alpha_j$ such that there is an edge $(\alpha_j, \alpha_i, \varepsilon)$ for some $\varepsilon \in \{+, -\}$ in $E$. To identify parallel edges we set $E'' = \{(i, j) \mid \exists e_{i,j}^{\varepsilon}, e_{i,j}^{\varepsilon'} \in E : \varepsilon \neq \varepsilon'\}$ and $E' = E \setminus E''$.

An interaction graph holds no information about dynamical behavior. Next we give a formal definition of the term *bioregulatory network* that includes information on structure as well as dynamics. 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 $\alpha_j$ in state $s = (s_1, \ldots, s_n)$ is the set*

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

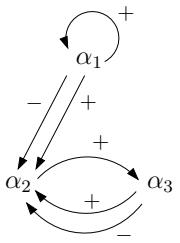
*Given a set*

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

*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 $\alpha_j$ 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 $\alpha_j$ 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 for every $s \in \mathcal{B}^n$ there is $\varepsilon \in \{+, -\}$ such that $e_{ij}^{\varepsilon} \in R_j(s)$, if $(i, j) \in E''$. If $(i, j) \in E'$, then $R_j(s)$ may or may not contain the corresponding edge $e_{i,j}^{\varepsilon}$, depending on $s$.

For $j \in \{1, \ldots, n\}$, set $M_j^{\mathcal{I}} := M_j := \{R_j(s) \mid s \in \mathcal{B}^n\}$. Then, by the above considerations, each $M \in M_j$ can be written as $M = \bigcup_{i \in Pred(j)} L_i$ with




$$
\begin{array}{lcl}
K_{1,\emptyset} & = & 0 \\
K_{1,\{e^{+}_{11}\}} & = & 1 \\
K_{2,\{e^{+}_{12},e^{+}_{32}\}} & = & 0 \\
K_{2,\{e^{+}_{12},e^{-}_{32}\}} & = & 1 \\
K_{2,\{e^{-}_{12},e^{+}_{32}\}} & = & 1 \\
K_{2,\{e^{-}_{12},e^{-}_{32}\}} & = & 0 \\
K_{3,\emptyset} & = & 0 \\
K_{3,\{e^{+}_{23}\}} & = & 1
\end{array}
$$

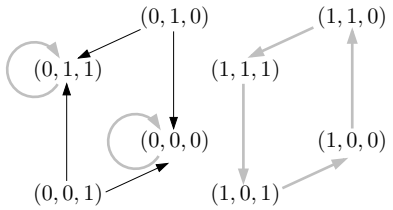



**Fig. 1.** Interaction graph of a system comprising three components, a list of all parameters with an assignment of Boolean values, and the corresponding state transition graph. The heavier gray edges indicate attractors.

$L_i = \{e^{\varepsilon}_{i,j}\}$ for some $\varepsilon \in \{+,-\}$, if $(i,j) \in E''$, and $L_i = \emptyset$ or $L_i = \{e^{\varepsilon}_{i,j}\} \subset E$, if $(i,j) \in E'$. By definition we have $K(\mathcal{I}) = \{K_{j,M} \mid j \in \{1,\ldots,n\},\, M \in M_j\}$. The choice of parameter values should be consistent with the information inherent in the interaction graph. We require that each edge represented in the interaction graph should have a notable effect on the system's dynamics. Moreover, the edge's character given by its sign should be reflected in its dynamical impact. To formalize this requirement we again have to distingish between edges in $E'$ and $E''$. For $e = (i,j,\varepsilon)$, $(i,j) \in E'$, we have $M \cup \{e\} \in M_j$ for all $M \in M_j$, and we demand that $K_{j,M} \leq K_{j,M\cup\{e\}}$ for all $M \in M_j$. Recall that the addition of an edge to the set of resources always signifies increasing activating influence, since the absence of inhibition is interpreted as activating influence. The condition ensures that increasing activating influence does not result in a decrease of component activity level. To ensure that $e$, at least for some state, has a notable impact on the dynamics, we extend the condition and get:

$$\forall\, M \in M_j \,:\, K_{j,M} \leq K_{j,M\cup\{e\}} \quad \text{and} \quad \exists\, M' \in M_j \,:\, K_{j,M'} < K_{j,M'\cup\{e\}}\,. \quad (1)$$

In the case $(i,j) \in E''$, there exists $e' = (i,j,\varepsilon')$ with $\varepsilon \neq \varepsilon'$. Since $\alpha_i$ influences $\alpha_j$ positively as well as negatively depending on the current state, we cannot impose a general monotonicity condition on the parameters as in the first part of (1). However, again we require that there is at least one state where the addition of $e$ to the set of resources induces an increase in the parameter value. Otherwise the edge $e$ would be superfluous. Since in every given state either $e$ or $e'$ is contained in the set of resources, we compare parameter values for sets $M \in M_j$ and $(M \setminus \{e'\}) \cup \{e\}$. We obtain the condition

$$\exists\, M' \in M_j \,:\, K_{j,M'} < K_{j,(M'\setminus\{e'\})\cup\{e\}}\,. \quad (2)$$

We call edges that satisfy condition (1) resp. (2) *functional*. This concept of functionality is an adaptation of the notion of functionality introduced in [10]. In the following, we always assume that all edges in the interaction graph are *functional*.

In Fig. 1 an interaction graph and a choice of parameter values are given. For $\alpha_1$ and $\alpha_3$ the parameters depend on whether or not the single positive edge ending in $\alpha_1$ resp. $\alpha_3$ is effective or ineffective. We have $R_1(s) = \emptyset$ for all states $s$ with $s_1 = 0$, and $R_1(s) = \{e_{11}^+\}$ for all $s$ with $s_1 = 1$. Thus $M_1 = \{\emptyset, \{e_{11}^+\}\}$, and similarly $M_3 = \{\emptyset, \{e_{23}^+\}\}$. 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 $\alpha_2$ is influenced by both $\alpha_1$ and $\alpha_3$ via two parallel edges, respectively. Thus the set of resources is never empty. For example, we have $R_2((0,0,1)) = \{e_{12}^-, e_{32}^+\}$. Overall, we get $M_2 = \{\{e_{12}^+, e_{32}^+\}, \{e_{12}^+, e_{32}^-\}, \{e_{12}^-, e_{32}^+\}, \{e_{12}^-, e_{32}^-\}\}$. Again the choice of parameter values renders all edges functional. A closer look allows the following interpretation. If $\alpha_1$ has activity level 0, then the influence of $\alpha_3$ on $\alpha_2$ corresponds to an activating influence: if $\alpha_3$ is inactive, $\alpha_2$ tends to inactivity represented by the parameter $K_{2,\{e_{12}^-, e_{32}^-\}} = 0$, and if $\alpha_3$ is active $\alpha_2$ tends to activity since $K_{2,\{e_{12}^-, e_{32}^+\}} = 1$. If $\alpha_1$ has value 1, then the situation is reversed and $\alpha_3$ inhibits $\alpha_2$. The system is context sensitive.

A different choice of parameter values illustrates the concept of functionality. If we set $K_{2,\{e_{12}^+, e_{32}^+\}} = K_{2,\{e_{12}^-, e_{32}^+\}} = 1$ and $K_{2,\{e_{12}^+, e_{32}^-\}} = K_{2,\{e_{12}^-, e_{32}^-\}} = 0$, then verification of conditions (1) and (2) shows that $e_{12}^+$, $e_{12}^-$ and $e_{32}^-$ are not functional. Only the edge $e_{32}^+$ is functional and influences the system's dynamics.

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 $\alpha_j$ 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 $\mathcal{B}^n$. For states $s = (s_1, \ldots, s_n)$ and $s' = (s'_1, \ldots, s'_n)$, there is an edge $s \to s'$ if and only if $s' = f(s) = s$ or $s'_i = f_i(s)$ for some $i \in \{1, \ldots, n\}$ 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

In the formalism introduced above 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 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 $K^1_{i,R^1_i(s)} := g_i(s)$ for all $i \in \{1, \ldots, n\}$ and $s \in \mathcal{B}^n$, with $R^1_i(s) := R^{\mathcal{I}^1}_i$.

Now, we have to consider the functionality of the edges in $\mathcal{I}^1$. This can be done componentwise via conditions (1) and (2) 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, starting with $\mathcal{I}^1$ and the parameter values given above. In the $k$-th step, if edge $e = (i, j, \varepsilon)$ is functional, we make no alterations on the interaction graph and parameters. If $e$ is not functional, we define a new interaction graph $\mathcal{I}^{k+1} = (V, E^{k+1})$ with $E^{k+1} := E^k \setminus \{e\}$. Clearly, we have $M^{k+1}_j = \{M \setminus \{e\} \mid M \in M^k_j\}$, since $e$ is eliminated in all sets of resources. We then set $K^{k+1}_{j,M\setminus\{e\}} := K^k_{j,M}$ for all $M \in M^k_j$, and keep all other parameters the same as before. By definition the function $f^{K(\mathcal{I}^{k+1})}$ derived from the new parameters still coincides with $g$.

However, we have to make sure that the order of edges we use to check functionality does not influence the result. That is, we want to show that an edge $e$ is functional in $\mathcal{I}^k$ if and only if it is functional in $\mathcal{I}^{k+1}$, w. r. t. the parameters as chosen above. This follows directly from the relation between parameter values in the $k$-th and $(k+1)$-th step. For example, let $e$ be a functional edge in $\mathcal{I}^k$, and assume that its parallel edge $e'$ is not functional and deleted in the step resulting in $\mathcal{I}^{k+1}$. To check whether $e$ is still functional we now have to consider condition (1) instead of (2). Let us assume the first part of (1) does not hold. Then there exists $M \in M^{k+1}_j$ such that $K^{k+1}_{j,M} > K^{k+1}_{j,M\cup\{e\}}$. It follows that $e \notin M$. Since we deleted the parallel edge $e'$, we know $e' \notin M$, $M \cup \{e\}, M \cup \{e'\} \in M^k_j$ and $K^k_{j,M\cup\{e'\}} = K^{k+1}_{j,M}$. We set $M' := M \cup \{e\}$. Then we get $K^k_{j,(M'\setminus\{e\})\cup\{e'\}} = K^{k+1}_{j,M} > K^{k+1}_{j,M\cup\{e\}} = K^k_{j,M\cup\{e\}} = K^k_{j,M'}$. This contradicts the assumption that (2) does not hold for $e'$ in $\mathcal{I}^k$. Thus $e$ satisfies the first part of (1). All other functionality statements can be shown with similar methods.

We repeat the iterative procedure for every edge in $\mathcal{I}^1$. After $2n^2$ steps we obtain an interaction graph and parameters consistent with $g$. □

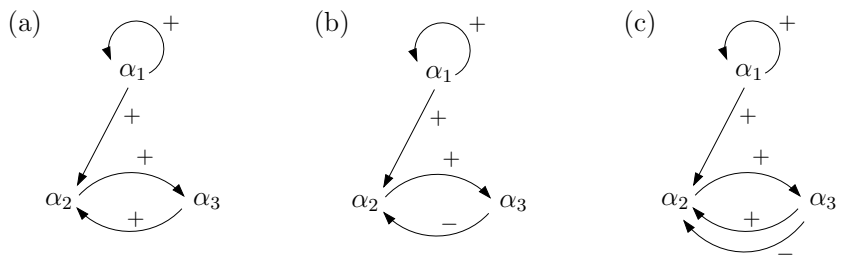



**Fig. 2.** Local interaction graphs corresponding to the graph and parameters given in Fig. 1. $\mathcal{I}((0,0,0))$ in (a), $\mathcal{I}((1,0,0))$ in (b), $\mathcal{I}((\theta,0,0))$ in (c).

We persistently emphasize the point that we only deal with functional edges, i. e. edges that 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 $s = (s_1, \ldots, s_n) \in \mathcal{B}^n$. Then we denote by $\mathcal{I}(s)$ the graph with vertex set $V$ and edge set $E(s) \subseteq E$. An edge $(i, j, \varepsilon)$ 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. 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 $\theta$ is a symbolic representation of the threshold value and satisfies the order $0 < \theta < 1$. We allow each regulatory component $\alpha_i$ to take values in $\mathcal{B}_\theta$. The values 0 and 1 are called* regular values *and $\theta$ is called* singular value. *The elements of $\mathcal{B}_\theta^n$ are called* states. *If all components of a state are regular, it is called* regular state*, else it is called* singular state. *For every state $s = (s_1, \ldots, s_n)$ we define $J(s) := \{i \in \{1, \ldots, n\} \mid s_i = \theta\}$.*

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 $\theta$. The relations $<$, $>$, and $=$ are used with respect to this identification.

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

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

*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 $f^\theta$ 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 $\alpha_i$ have no activating influence on $\alpha_i$ or they all contribute to an activation of $\alpha_i$.

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 $C = (\alpha_{i_1}, \ldots, \alpha_{i_r})$ be a circuit in $\mathcal{I}$. A state $s = (s_1, \ldots, s_n) \in \mathcal{B}_\theta^n$ is called* characteristic state *of $C$ if $s_{i_l} = \theta$ for all $l \in \{1, \ldots, r\}$.*

In general, a characteristic state of a circuit is not unique. The state $(\theta, \ldots, \theta)$ 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 $i \in \{1, \ldots, n\}$. 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 $\alpha_i$ in $\mathcal{I}$ does not have a predecessor, then clearly $a_i = K_{i,\emptyset}$ for every state $a = (a_1, \dots, a_n)$ in an attractor. Similarly, we know the values $a_j$ for vertices the only predecessor of which is $\alpha_i$, 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

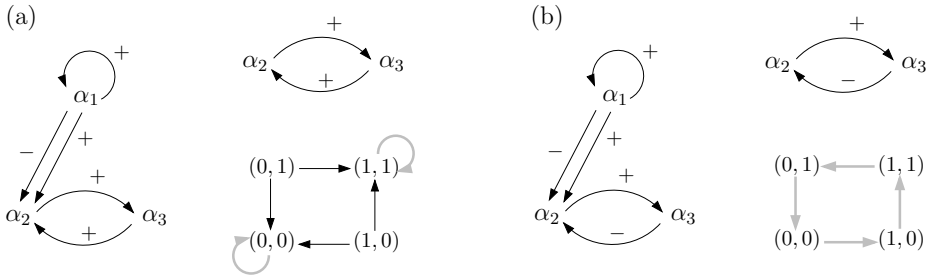



**Fig. 3.** Local interaction graph $\mathcal{I}((0,\theta,\theta))$ on the left, $\mathcal{I}^\theta((0,\theta,\theta))$ and corresponding state transition graph on the right of (a). Local interaction graph $\mathcal{I}((1,\theta,\theta))$ on the left, $\mathcal{I}^\theta((1,\theta,\theta))$ and corresponding 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 $s = (s_1, \ldots, s_n) \in \mathcal{B}_\theta^n$. 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 $s' = (s'_1, \ldots, s'_n)$ 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 $s = (s_1, \ldots, s_n)$ 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 $\tilde{s} = (\tilde{s}_1, \ldots, \tilde{s}_n) \in \mathcal{B}_\theta^n$ 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 $\alpha_i$ in $Z$. Therefore, the sets of resource edges of $\alpha_i$ are not influenced by value changes in $Z$, i. e., $\{R_i(s') \mid s' \in \mathcal{B}^n,\ s'_j = s_j \text{ for all } j \notin J(s)\} = \{R_i(s') \mid s' \in \mathcal{B}^n,\ s'_j = \tilde{s}_j \text{ for all } j \notin J(\tilde{s})\}$. Then $K_{i,\min(s)} = K_{i,\min(\tilde{s})}$ and $K_{i,\max(s)} = K_{i,\max(\tilde{s})}$, and $f_i^\theta(\tilde{s}) = f_i^\theta(s) = s_i = \tilde{s}_i$.

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 $V_Z = \{\alpha_{l+1}, \ldots, \alpha_{l+k}\}$ for some $l \in \{0, \ldots, n-1\}$. 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 $f^{K(Z)} = f^Z : \mathcal{B}^k \to \mathcal{B}^k$, $z \mapsto (K^Z_{1,R_1^Z(z)}, \ldots, K^Z_{k,R_k^Z(z)})$. 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 $Z$. 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 definitions of parameters and $\mathcal{I}^\theta(s)$ ensure that all edges in $Z$ are functional. According to Prop. 2 the graph $\mathcal{S}_{N^Z}$ contains an attractor. This fact leads to the next theorem.

**Theorem 2.** *Let $s = (s_1, \ldots, s_n)$ be a singular steady state, and $Z_1, \ldots, Z_m$ be the components of $\mathcal{I}^\theta(s)$. For all $j \in \{1, \ldots, m\}$ 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 $a = (a_1, \ldots, a_n) \in A$, $i \notin J(s)$, and $\pi^{Z_j}(A) = A_j$ for all $j \in \{1, \ldots, m\}$.*

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

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 $\gamma_1$ 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 $\gamma_2$ 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 $\gamma_i$ 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 $\alpha_2$ and $\alpha_3$. 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 $\alpha_3$ 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 that contains only one circuit $C$. 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 $(\theta, \ldots, \theta)$ 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 $f^\theta$ 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 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 $f^\theta$ 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 $\alpha_1, \ldots, \alpha_r$ for some $r \in \{1, \ldots, N\}$. 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 $i \in \{1, \ldots, r\}$. From Lemma 1 follows that the states $s^1$ and $s^2$ are steady states. Thus $f^\theta$ 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 $\operatorname{card} A' > 1$. Theorem 2 yields an attractor $A$ in $\mathcal{S}_N$ with $\pi^{J_C(s)}(A) = A'$. Thus cardinality of $A$ is also greater than one. □

Theorem 3 is a stronger result than the one obtained in [10], even for networks without context sensitivity. The use of local interaction graphs allows for 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

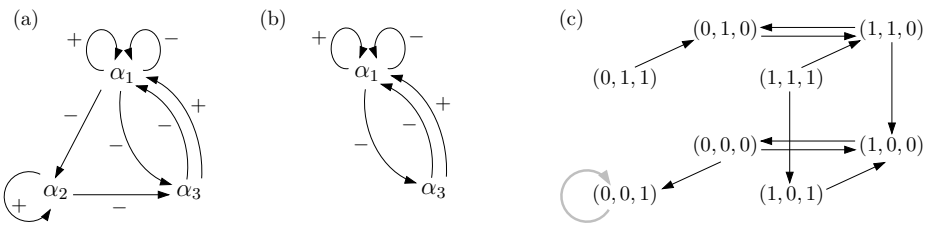



**Fig. 4.** We choose the parameters for the interaction graph in (a) as $K_{1,\{e^+_{11},e^+_{31}\}} = K_{1,\{e^-_{11},e^-_{31}\}} = K_{2,\{e^-_{12},e^+_{22}\}} = K_{3,\{e^-_{13},e^-_{23}\}} = 1$ and set all other parameters 0. In (b) the graph $\mathcal{I}^\theta(s)$ for the singular steady state $s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

derived from the interaction graph in (a) and the parameters specified in the caption. There are four 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). 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 not necessary, as illustrated by an example given in [10], Fig. 4.

## 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.

## References


1. G. Bernot, J.-P. Comet, A. Richard, and J. Guespin. Application of formal methods to biological regulatory networks: extending Thomas' asynchronous logical approach with temporal logic. *J. Theor. Biol.*, 229:339–347, 2004.
2. C. Chaouiya, É. Remy, B. Mossé, and D. Thieffry. Qualitative analysis of regulatory graphs: a computational tool based on a discrete formal framework. In *First Multidisciplinary International Symposium on Positive Systems: Theory and Applications, POSTA 2003*, volume 294 of *LNCIS*, pages 119–126. Springer, 2003.
3. A. Naldi, D. Thieffry, and C. Chaouiya. Decision diagrams for the representation and analysis of logical models of genetic networks. In *Computational Methods in Systems Biology, CMSB 2007, Edinburgh, Scotland*, volume 4695 of *LNBI*, pages 233–247. Springer, 2007.
4. É. Remy, B. Mossé, C. Chaouiya, and D. Thieffry. A description of dynamical graphs associated to elementary regulatory circuits. *Bioinform.*, 19:172–178, 2003.
5. É. Remy and P. Ruet. On differentiation and homeostatic behaviours of Boolean dynamical systems. In *Transactions on Computational Systems Biology VIII*, volume 4780 of *LNCS*, pages 92–101. Springer, 2007.
6. É. Remy, P. Ruet, and D. Thieffry. Graphic requirements for multistability and attractive cycles in a boolean dynamical framework. Prépublication, 2005.
7. A. Richard and J.-P. Comet. Necessary conditions for multistationarity in discrete dynamical systems. Rapport de Recherche, 2005.
8. A. Richard, J.-P. Comet, and G.Bernot. R. Thomas' modeling of biological regulatory networks: introduction of singular states in the qualitative dynamics. *Fundamenta Informaticae*, 65:373–392, 2005.
9. F. Robert. *Discrete Iterations: A Metric Study*, volume 6 of *Springer Series in Computational Mathematics*. Springer, 1986.
10. H. Siebert and A. Bockmayr. Relating attractors and singular steady states in the logical analysis of bioregulatory networks. In *Algebraic Biology, AB 2007, Castle of Hagenberg, Austria*, volume 4545 of *LNCS*, pages 36–50. Springer, 2007.
11. E. H. Snoussi and R. Thomas. Logical identification of all steady states: the concept of feedback loop characteristic states. *Bull. Math. Biol.*, 55:973–991, 1993.
12. C. Soulé. Graphical requirements for multistationarity. *ComPlexUs*, 1:123–133, 2003.
13. R. Thomas and R. d'Ari. *Biological Feedback*. CRC Press, 1990.
14. R. Thomas and M. Kaufman. Multistationarity, the basis of cell differentiation and memory. II. Logical analysis of regulatory networks in terms of feedback circuits. *Chaos*, 11:180–195, 2001.