Analysis of Discrete Bioregulatory Networks Using Symbolic Steady States

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Abstract. A discrete model of a biological regulatory network can be represented by a discrete function that contains all available information on interactions between network components and the rules governing the evolution of the network in a finite state space. Since the state space size grows exponentially with the number of network components, analysis of large networks is a complex problem. In this paper, we introduce the notion of symbolic steady state that allows us to identify subnetworks that govern the dynamics of the original network in some region of state space. We state rules to explicitly construct attractors of the system from subnetwork attractors. Using the results, we formulate sufficient conditions for the existence of multiple attractors resp. a cyclic attractor based on the existence of positive resp. negative feedback circuits in the graph representing the structure of the system. In addition, we discuss approaches to finding symbolic steady states. We focus both on dynamics derived via synchronous as well as asynchronous update rules. Lastly, we illustrate the results by analyzing a model of T helper cell differentiation.

1 Introduction

Discrete methods of modeling biological regulatory networks are often used if the available data is rather qualitative in nature. Each component of the network is associated with a finite number of activity levels representing e. g. a concentration interval of a substance, activity of a gene or presence or absence of a signal. The state space of the system then consists of vectors of the component activity levels, and the network dynamics is derived from a discrete function f capturing the rules of component interactions in the system. Here, two fundamentally different methods of calculating trajectories of the system are in use. The so-called synchronous update renders a deterministic representation by defining the successor of a given state as its image under f. In contrast, we obtain a non-deterministic version if we require, motivated by the assumption of distinct time delays associated with component value changes, that a state and its successor differ in one component only, but consider all successor possibilities in agreement with f. Both approaches have been used successfully, the synchronous method having advantages regarding the complexity of the analysis, the asynchronous update often allowing for a more realistic representation of the system's behavior (see e. g. [11], [34], [12] and references therein).

When analyzing large and complex networks, one is often interested in identifying subnetworks that are in some sense significant for the system. The idea of network decomposition is well-established in systems biology, and has been approached from many different angles (see e. g. [9, 4, 20, 19, 1]). We are particularly interested in subsystems that play crucial roles in the dynamics of the system. Analysis of such networks in isolation may then yield information on the dynamical behavior of the original network. Clearly, the difficulty is that further components and interactions influence such a network building block once it is again embedded in the network. Conditions to identify suitable subnetworks that retain their behavior once re-embedded are needed to derive useful information on the network dynamics.

In this paper we generalize and extend corresponding ideas developed for Boolean functions and asynchronous dynamics in [31] to multi-valued discrete functions, considering synchronous as well as asynchronous dynamics. We identify regions of state space where a number of network components remain fixed in the dynamics independent of the values of the remaining components by exploiting the properties of *symbolic steady states*. These are fixed points of an adapted function f^θ that coincides with f for the most part, but also allows the consideration of a symbolic value θ for the network components. The value θ can be identified with the whole activity level range of a given component, representing uncertainty of the actual value of that component in a network state. The *regular* components, i. e. those with a specified activity level, of a symbolic steady state act as a boundary between dynamically active subnetworks similar to the notion of *frozen core* introduced in the context of random Boolean networks (see [11]). We obtain a detailed structural representation of the active subnetworks by considering the local interaction graph associated with the symbolic steady state. Assembly of attractors of the isolated subnetworks with respect to the symbolic steady state then yields attractors of the original network. With this fundamental property in mind, we are able to proof more general statements concerning the relation between structure and dynamics of the network. Here, we proof that the existence of a positive resp. a negative circuit under certain conditions implies the existence of multiple attractors resp. a cyclic attractor in the synchronous as well as the asynchronous dynamics.

The results mentioned thus far concern analysis using symbolic steady states. We also address the problem of determining symbolic steady states, exploiting structural as well as dynamical characteristics of f. All results are illustrated using a model of T helper cell differentiation.

The paper is organized as follows. In the next section we introduce the modeling framework used in this paper. In Sect. 3 we establish the notion of symbolic steady state as well as some important properties, followed by the main results on compositional attractors of subnetworks derived from symbolic steady states in Sect. 4. The results are then used to obtain statements linking the existence of feedback circuits in the network to number and size of attractors in Sect.5. In the following section, we examine different methods for deriving symbolic steady states. The results are applied to a model of T helper cell differentiation in Sect. 7. We end the paper with concluding remarks and perspectives for future work.

2 Regulatory Networks

Throughout the text let us consider a network with $n \in \mathbb{N}$ components $\alpha_1, \dots, \alpha_n$. To simplify notation, we identify each component α_i with its index i. Each component is understood as a discrete variable the values of which signify the different activity levels of the component in the network. Activity levels may represent different biological characteristics, e. g. substance concentration, gene activity, absence or presence of a signal and so on. The number of activity levels of different components may differ, depending on function of components and available data. Thus, every component α_i is associated with a *range* $X_i := \{0, 1, \dots, p_i\}$ of activity levels, where $p_i \in \mathbb{N}$ denotes the maximal activity level of α_i. The set $X := X_1 \times \cdots \times X_n$ comprises all possible vectors of activity levels of the network and thus represents the state space of the system. Interaction of network components and rules governing the network's dynamics are then captured by a discrete function $f = (f_1, \dots, f_n) : X \to X$. If $p_i = 1$ for all maximal activity levels p_i, then f is a Boolean function.

2.1 Structure

In a next step, we want to derive the network structure from the function f. As commonly done, we represent the structure as a signed directed (multi-)graph with vertex set $V := \{\alpha_1, \dots, \alpha_n\}$ and edges representing interactions between components. The sign of an edge describes the character of the interaction, negative sign signifying an inhibiting, positive an activating effect. However, in some cases the influence of one component on another depends on the current state of the network. For example, if two substances form a complex that activates some target gene, then in general the presence of only one of those substances is not sufficient to induce gene expression. So, one of the substances can only effectively influence the gene when the other substance is present. Another possibility is that the character of an interaction changes depending on the state of the network. A well-known example is the DNA-binding protein TCF which can be involved in repression as well as activation of the same target genes (see e. g. [30]). Such refined structural information is of great interest when linking structural and dynamical aspects and thus we want to include it in the structural representation of the network. This is done by considering *local interaction graphs*. This notion was introduced for Boolean functions in [23] and is used for multi-value functions in the form considered here in [25].

Definition 1. *Let $x \in X$. By $G(x) := G(f)(x)$ we denote the directed signed (multi-)graph with vertex set $\{\alpha_1, \dots, \alpha_n\}$ and edge set $E(x) \subseteq V \times V \times \{+, -\}$. An edge (i, j, ε) belongs to $E(x)$ iff there exists $c_i \in \{-1, +1\}$ such that $x_i + c_i \in X_i$ and*

$$\operatorname{sgn} \frac{f_j((x_1, \dots, x_{i-1}, x_i + c_i, x_{i+1}, \dots, x_n)) - f_j(x)}{c_i} = \varepsilon\,.$$

We call $G(x)$ the local interaction graph of f in x.

To obtain the local interaction graph we consider changes in the values of the coordinate functions depending on small changes, i. e. changes by absolute value 1, in one component. The local interaction graph in x is thus closely related to the discrete Jacobian matrix, which was introduced in [28] in the Boolean case. Note that in the multi-value other than in the Boolean case it is possible that $G(x)$ contains parallel edges. By definition, there are at most two parallel edges from one vertex to another which then have opposite sign, one resulting from an increase, the other from a decrease of the component value in Def. 1.

If we combine the structural information of the local interaction graphs for a set of states M we obtain a graph that contains all interactions influencing the network's dynamics in M.

Definition 2. *Let $M \subseteq X$. We denote by $G(M) := G(f)(M)$ the union of the graphs $G(x)$, $x \in M$. For $M = X$ we set $G(f) := G(X)$ and call $G(f)$ the* global interaction graph *of f.*

The global interaction graph contains all interactions influencing the network's dynamics in at least some part of state space.

When analyzing interaction graphs we are interested in certain structural motifs. 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.

In Fig. 1 we see on the left interaction graphs of the function $f = (f_1, f_2, f_3) : X \to X$, $X := \{0,1\}^2 \times \{0,1,2\}$, introduced in the caption of the figure. Here, f_2 models that α_2 is influenced by α_1 and α_3 via an OR-gate as long as the activity level of α_3 is below 2. However, if $\alpha_3 = 2$, then α_2 is repressed. The way α_3 influences α_2 thus depends on the current state of the system. If the system is in state $(1,0,0)$, then a small change in the α_3 value is not enough to reach the value 2. Thus, f_2 corresponds to a logical OR-function. Since $\alpha_1 = 1$, we have $f_2((1,0,x_3)) = 1$ for $x_3 \in \{0,1\}$. It follows that in the state $(1,0,0)$ small changes in α_3 do not influence the component value α_2. As a result, we see in Fig. 1 (a) that there is no edge from α_3 to α_2 in $G((1,0,0))$. In comparison, if we look at the state $(0,0,0)$, we have $f_2((0,0,1)) - f_2((0,0,0)) = 1$ and therefore we get a positive edge from α_3 to α_2 in $G((0,0,0))$. Lastly, when looking at state (0,0,1), α_3 influences α_2 via a positive edge, since the argument we just made is still valid. However, if we increase the activity level of α_3 to 2, then by definition of f_2 we have a negative influence of α_3 on α_2. Thus in the local interaction graph $G((0,0,1))$ there is a negative as well as a positive edge from α_3 to α_1. By definition all the local interaction graphs are subgraphs of the global interaction graph of f, which is shown in Fig. 1(d).

Here, edges in an interaction graph are not labeled with additional information pertaining the activity level values of the tail vertex which allow that edge to have an effect on the dynamics. Thus, an edge (i, j, ε) may represent several

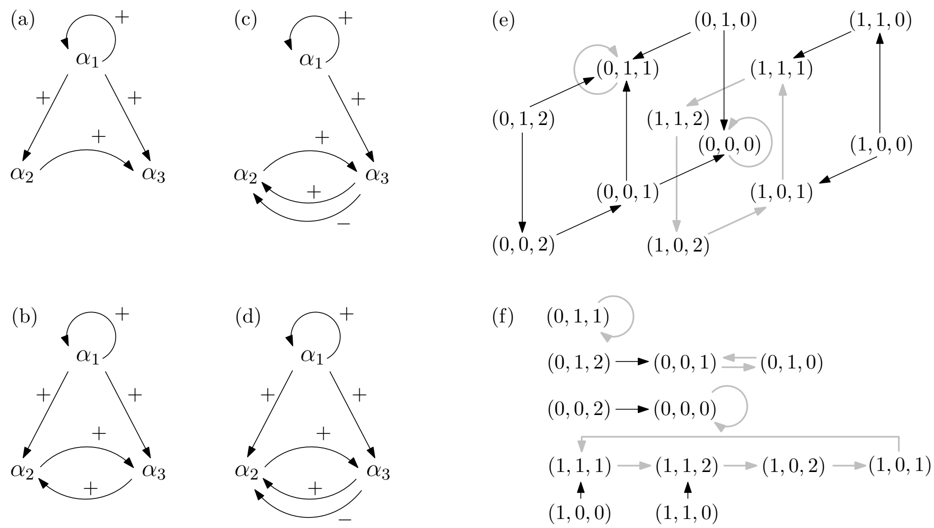


Fig. 1. In (a) to (c) local interaction graphs in states (1,0,0), (0,0,0) and (0,0,1), respectively, of the function $f = (f_1, f_2, f_3) : \{0,1\}^2 \times \{0,1,2\} \to \{0,1\}^2 \times \{0,1,2\}$ with $f_1(x) = x_1$, $f_2(x) = 0$ if $x_3 = 2$ and $f_2(x) = x_1 + x_3 - x_1 \cdot x_3$ otherwise, and $f_3(x) = x_1 + x_2$ for $x = (x_1, x_2, x_3) \in \{0,1\}^2 \times \{0,1,2\}$. In (d) the global interaction graph of f. In (e) and (f) the asynchronous and synchronous state transition graph, respectively. Heavier gray edges indicate attractors.

influences of sign ε from i on j, which may differ in strength and depend on the current value of α_i. Based on this observation we introduce the following notion.

Definition 3. *Let $M \subseteq X$ and let $e := (i, j, \varepsilon)$ be an edge in $G(M)$. We call e* unique in M *if there exists $t^{ij} \in \{0, \dots, p_{i-1}\}$ such that $f_j(x) = f_j(x')$ for all $x, x' \in M$ satisfying $x_i, x'_i \in \{0, \dots, t^{ij}\}$ or $x_i, x'_i \in \{t^{ij}+1, \dots, p_i\}$, and $x_k = x'_k$ for all $k \neq i$.*

Whether or not the edge e has an impact on the dynamical behavior may still be dictated by the values of components other than α_i. However, if all component values x_l, $l \neq i$, are fixed, the value of $f_j(x)$ solely depends on whether x_i is above or below the threshold t^{ij}.

By definition every edge of a circuit influences the behavior of its head vertex at least in some state. When analyzing circuits a stronger property is often useful.

Definition 4. *Let $C = (e_1, \dots, e_r)$ be a circuit such that every edge $e_k = (i_k, i_{k+1}, \varepsilon_k)$ is unique in $M \subseteq X$. We call e_k* functional in C (with respect to M) *if there exists $x \in M$ such that $\overline{x} \in M$ and $f_{i_{k+1}}(x) \leq t^{i_{k+1}, i_{k+2}} < f_{i_{k+1}}(\overline{x})$ or $f_{i_{k+1}}(\overline{x}) \leq t^{i_{k+1}, i_{k+2}} < f_{i_{k+1}}(x)$, where $\overline{x}$ is the sum of x and the i_k-th unit vector.*

For our purposes it is sufficient to introduce the notion of functionality of interactions in circuits for circuits composed of unique edges. For a more general discussion see [18].

2.2 Dynamics

The function f determines the behavior of the network. However, there are different possibilities to derive the dynamics of the system. The entirety of the dynamical behavior, in any case, is captured in a *state transition graph* the paths of which represent all possible behaviors. The most straightforward approach leads to the following definition.

Definition 5. *With $S^s := S^s(f)$ we denote the directed graph with vertex set X and edge set $\{(x, f(x)) \mid x \in X\}$. We call S^s the* synchronous state transition graph of f.

Here, each state has a unique successor. The underlying assumption concerning the evolution of the system is that all activity level changes indicated by f are executed concurrently. This is a highly simplifying assumption. Changes in activity level may represent very different biological processes and it is not realistic to assume that these processes have the exact same duration. If we want to incorporate this observation, we can make the assumption that a state differs from its successor in at most one component. Since data on such time delays is often lacking, we have no way to decide which component value should change in a state where multiple component activity level changes are indicated. Therefore, we consider all possibilities and derive a non-deterministic representation of the dynamical behavior. Furthermore, we take into account that although f may indicate an activity level change of absolute value greater than one, the system nevertheless will behave in some sense continuously. That is, the activity levels of a state and its successor should differ by at most 1.

Definition 6. *Let $S^a := S^a(f)$ be a directed graph with vertex set X. For states $x = (x_1, \dots, x_n), x' = (x'_1, \dots, x'_n) \in X$ there is an edge $x \to x'$ if and only if $x' = f(x) = x$ or $x'_i = x_i + \operatorname{sgn}(f_i(x) - x_i)$ for some $i \in \{1, \dots, n\}$ satisfying $x_i \neq f_i(x)$, and $x'_j = x_j$ for all $j \neq i$. We call S^a the* asynchronous state transition graph of f.

To analyze state transition graphs we use, in addition to standard terminology from graph theory such as paths and cycles, the following concepts.

Definition 7. *Let $S \in \{S^s, S^a\}$. An infinite path $(x^0, x^1, \dots)$ in S 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 $x^1, x^2 \in A$ there is a path from x^1 to x^2 in S. Attractors of cardinality greater than one are called* cyclic *attractors. A cycle $C := (x^1, \dots, x^r, x^1)$, $r \geq 2$, is called a* trap cycle *if every x^j, $j \in \{1, \dots, r\}$, has only one outgoing edge in S, i. e., the trajectory starting in x^1 is unique. A state x is called* steady state*, if there exists an edge $x \to x$, i. e. if $f(x) = x$.*

In other words, the attractors correspond to the terminal strongly connected components of the graph. In a synchronous state transition graph the trajectory starting from some initial state is unique. In consequence, every attractor is either a fixed point of f or a trap cycle, i. e. a periodic point of f. Since the state space is finite, every trajectory leads to an attractor. This is not true for asynchronous state transition graphs. However, it is easy to see that for every state x, there exists a trajectory starting in x leading to an attractor. Steady states and trap cycles are attractors, but there may also be attractors of cardinality greater than one which are not trap cycles. Since steady states are fixed points of f, the steady states, other than attractors of cardinality greater than one, coincide in the synchronous and the asynchronous state transition graph.

In Fig. 1 (e) and (f) we see the asynchronous and synchronous state transition graph of the function f defined in the caption. The system has two steady states, (0,1,1) and (0,0,0). The asynchronous state transition graph contains one cyclic attractor, namely $\{(1,0,1),(1,1,1),(1,1,2),(1,0,2)\}$, which is also an attractor in S^s. In the synchronous state transition graph we find a further cyclic attractor, $\{(0,0,1),(0,1,0)\}$.

We close this section with the following observation. If some coordinate function f_i is constant with value c, then $x_i = c$ for every state x in an attractor Similarly, we know the values x_j of every component j such that f_j depends only on values of components whose dynamics are described by constant coordinate functions. That is, we can easily determine the dynamical behavior of such components, which leads to the same fixed values of those components in every attractor of the system. Throughout the remainder of the paper we assume that *no coordinate function of f is constant*. We still allow the system to have input values in the sense of components maintaining their current activity level independent of the values of the other components. They can be modeled with the coordinate function $f_i(x) = x_i$.

3 Symbolic Steady States

Analysis of complex network dynamics, in particular of asynchronous state transition graphs, is costly. However, complex networks are often composed of smaller building blocks – modules and motifs (see e. g. [9, 5, 1]). When analyzed in isolation such building blocks often reveal specific biological functions. The question of interest is whether or not the behavior observed in isolation can be rediscovered in the complex network. Here, the building blocks themselves interact and influence each others behavior. One goal of this paper is to find conditions that allow to infer behavioral properties of the complex system from the dynamics of suitable subnetworks. The central notions in this endeavor are introduced in the following two definitions. It has already been used for Boolean functions in [33, 31] and uses notation first introduced in [27].

Definition 8. *For all $i \in \{1,\dots,n\}$ we set $X_i^\theta := \{0,\dots,p_i,\theta\}$ and $X^\theta := X_1^\theta \times \dots \times X_n^\theta$, where θ is a symbolic value. We call the elements of X^θ* states. *If no component of a state has value θ, the state is called* regular state*, otherwise* *it is called* symbolic state. *We denote* $J(x) := \{i \in \{1, \ldots, n\} \mid x_i = \theta\}$ *for all* $x \in X^\theta$.

The value θ is used to describe uncertainty of a component value. Following this idea, we define a so-called *qualitative value* $|a, b|$ for $a, b \in \{0, \ldots, \max_i p_i\}$, $a \leq b$ by setting $|a, a| := a$ and $|a, b| := \theta$ if $a < b$. Furthermore, we denote $[x] := \{x' \in X \mid x'_j = x_j \text{ for all } j \notin J(x)\}$ for all $x \in X^\theta$. The set $[x]$ constitutes an affine subspace of the global state space X. However, $[x]$ is not necessarily closed with respect to the dynamics of f, meaning trajectories starting in $[x]$ may leave $[x]$.

Symbolic notation for logical states, often called schema, has been used before (see e. g. [8, 2, 14, 35]). Schemata are mostly used to obtain a compact representation of state space. For example, the different states of a cycle can be represented by a single schema. Schemata representations can also be used as a measure of complexity. In [35] basins of attraction of a system are represented by a set of schemata constructed based on a minimum description principle. The size of the schemata set reflects the complexity of the structure of the basin of attraction in state space.

Although we may encode state sets of relevance for the system's dynamics, the representation using schemata itself is inherently static, i. e., there is no notion of the dynamical behavior of a schema. We now want to analyze the dynamics of the system with respect to the set of states represented by a symbolic state, reminiscent of symbolic dynamics of real or complex dynamical systems (see e. g. [29]). However, instead of encoding trajectories in sequences of identifiers of state space subsets such that the system's dynamics is determined by the shift operation on such a sequence, we define a function that allows us to analyze the dynamics of symbolic states on X^θ and preserves the dynamical behavior derived from f.

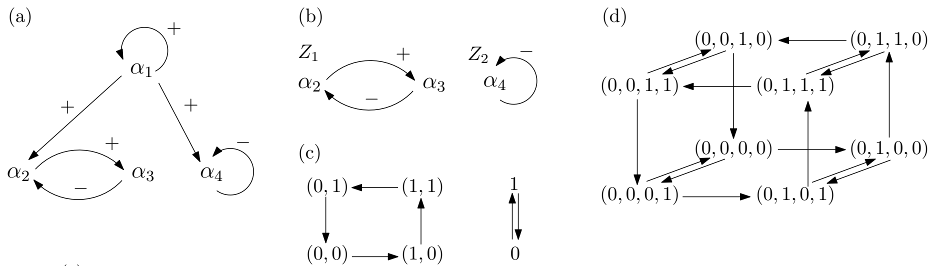
Definition 9. *For all* $i \in \{1, \ldots, n\}$ *we define* $f_i^{\min} : X^\theta \to X_i^\theta$, $f_i^{\min}(x) := \min\{f_i(x') \mid x' \in [x]\}$ *and* $f_i^{\max} : X^\theta \to X_i^\theta$, $f_i^{\max}(x) := \max\{f_i(x') \mid x' \in [x]\}$. *Then we define*

$$f^\theta : X^\theta \to X^\theta \quad \text{by} \quad f_i^\theta(x) = |f_i^{\min}(x), f_i^{\max}(x)|, \; i \in \{1, \ldots, n\}.$$

We call $x \in X^\theta$ *a* steady state *if* $f^\theta(x) = x$.

By definition we have $f^\theta|_X = f$, since regular states are mapped to regular states. If $f_i(x) \in X_i$ for a symbolic state x, we can deduce that $f_i(x) = f_i(y)$ for all $y \in [x]$. That is, the information inherent in the regular components of x is sufficient to determine the evolution of the i-th component. This can be visualized by looking at the local interaction graph $G([x])$, which then does not contain edges from any $\alpha_j \in J(x)$ to α_i.

Since we made the assumption that no coordinate function of f is constant, we know that the state $(\theta, \ldots, \theta)$ is a steady state. In the following we are interested in symbolic steady states x with $J(x) \neq \{1, \ldots, n\}$. The set of components not belonging to $J(x)$ remains dynamically stable regardless of value changes in the symbolic components. This allows us to consider a reduced network for



(e)

$f(x) = x$	attractors (async)	attractors (sync)
$(0, \theta, \theta, \theta)$	$\{x \in X \mid x_1 = 0\}$	$\{(0,0,0,0), (0,1,0,1), (0,1,1,0), (0,0,1,1)\}$
		$\{(0,0,0,1), (0,1,0,0), (0,1,1,1), (0,0,1,0)\}$
$(1, 1, 1, \theta)$	$\{(1,1,1,0), (1,1,1,1)\}$	$\{(1,1,1,0), (1,1,1,1)\}$
$(2, 1, 1, 1)$	$\{(2,1,1,1)\}$	$\{(2,1,1,1)\}$

Fig. 2. In (a) the global interaction graph of $f = (f_1, f_2, f_3, f_4) : X \to X$, $X := \{0,1,2\} \times \{0,1\}^3$ with $f_1(x) = x_1$, $f_2(x) = 1$ if $x_1 \geq 1$ or $x_3 = 0$, and $f_2(x) = 0$ otherwise, $f_3(x) = x_2$, and $f_4(x) = 1$ if $x_1 = 2$ or $x_4 = 0$, and $f_4(x) = 0$ otherwise. The graph $G^\theta([(0, \theta, \theta, \theta)])$ is shown in (b). The corresponding state transition graphs, and the derived compositional attractor in S^a of f in (c) and (d), respectively. In (e) a table of attractors derived from subgraphs induced by steady states given in the left column.

analyzing the system's behavior in the subset $[x]$ of state space without loosing information. The regular components of the symbolic steady state act as a stable or *frozen core*, as was described first by S. Kauffman for random Boolean networks (see [11] for an overview).

To formalize the impact of a symbolic steady state on the dynamics and relate certain structural characteristics, we introduce the following notation for a symbolic steady state x. By $G^\theta([x])$ we denote the (multi-)graph with vertex set $V^\theta[x] := J(x)$ and edge set $E^\theta[x] := \{(i, j, \varepsilon) \in G([x]) \mid i, j \in J(x)\}$. We call a graph $Z = (V_Z, E_Z)$ component of $G^\theta([x])$, if the undirected graph derived from Z is a maximal connected subgraph of the undirected graph derived from $G^\theta([x])$. Figure 2 (b) shows the graph $G^\theta([x])$ for the symbolic steady state $x := (0, \theta, \theta, \theta)$ of the function f defined in the corresponding caption.

Proposition 1. *Let $x \in X^\theta$ be a symbolic steady state and let Z be a component of $G^\theta([x])$. Then every vertex of Z has at least one predecessor in Z. In particular, Z contains a circuit.*

Proof. Let α_i be a vertex in Z. Since $f_i(x) = x_i = \theta$, we have $f_i^{\min}(x) \neq f_i^{\max}(x)$. Thus, according to the definition, f_i depends on some $\alpha_j \in J(x)$ and we find an edge (j, i, ε) in Z for some $\varepsilon \in \{+, -\}$. Since the vertex set of Z is finite, there has to be a circuit in Z. □

In the next lemma we show that the regular components of a symbolic steady state x stay fixed regardless of value changes in $J(x)$, and that the components of $G^\theta([x])$ act dynamically independent from each other in the state set $[x]$. In particular, $[x]$ is not only a subspace of state space but also a trap set. This has already been shown for Boolean functions in [31] and the proof can be adapted easily.

Lemma 1. *Let $x \in X^\theta$ be a symbolic steady state, and let $Z_1, \ldots, Z_m$ be the components of $G^\theta([x])$. Consider a union Z of arbitrary components Z_j. Let $\tilde{x} \in X^\theta$ such that $\tilde{x}_i = x_i$ for all $i \notin Z$. Then $f_i^\theta(\tilde{x}) = f_i^\theta(x) = x_i = \tilde{x}_i$ for all $i \notin Z$.*

Proof. First, let us consider $i \notin J(x)$. Since $x_j = \theta$ for all $j \in Z$, we have $J(\tilde{x}) \subseteq J(x)$. Therefore, $[\tilde{x}] \subseteq [x]$. It follows that $f_i^{\min}(x) \leq f_i^{\min}(\tilde{x}) \leq f_i^{\max}(\tilde{x}) \leq f_i^{\max}(x)$. Since $f_i^\theta(x) = x_i$ is regular, we know $f_i^{\min}(x) = f_i^{\max}(x) = x_i$. Thus, $f_i^{\min}(\tilde{x}) = f_i^{\max}(\tilde{x}) = x_i$ and $f_i^\theta(\tilde{x}) = x_i = \tilde{x}_i$.

Now, let us consider $i \in J(x) \setminus Z$. We need to show that $f_i^\theta(\tilde{x}) = \theta$. Assume $f_i^\theta(\tilde{x}) = c \in X_i$, i.e. $f_i^{\min}(\tilde{x}) = f_i^{\max}(\tilde{x}) = c$. Since $f_i^\theta(x) = \theta$ there exist $y, y' \in [x]$ and $a, b \in X_i$ such that $f_i(y) = a < b = f_i(y')$. We may assume that $a \neq c$, since $a \neq c$ or $b \neq c$. It follows that $y \notin [\tilde{x}] \subseteq [x]$. Since x and $\tilde{x}$ only differ in Z-components, there exists $\tilde{y} \in [\tilde{x}]$ with $y_j = \tilde{y}_j$ for all $j \notin Z$. Then we find regular states $y =: y^1, y^2, \ldots, y^k := \tilde{y}$ in $[x]$ such that for each $l < k$ exists $j^l \in Z$ with $|y^l_{j^l} - y^{l+1}_{j^l}| = 1$ and $y^l_m = y^{l+1}_m$ for $m \neq j^l$, and $y^l \notin [\tilde{x}]$ for all $l < k$. Since $f_i(y^k) = c \neq a$, we find y^l such that $f_i(x^l) = a \neq f_i(y^{l+1})$. According to the definition of the local interaction graph, we then find an edge from j^l to i in $G(y^l)$, and thus also in $G([x])$. This is a contradition to Z being a union of components of $G^\theta([x])$, since $j^l \in J(x) \setminus Z$. □

This lemma allows us to focus on the dynamics of the subnetworks represented by the components of $G^\theta([x])$ and to derive the dynamical behavior of the original network in $[x]$ from that, as we will see in the next section.

4 Compositional Attractors

Attractors of a complex system can be constructed from subsystem attractors if the subsystems are independent of each other. Such subsystems are of interest both in a mathematical and a biological context (see e. g. [3, 15]). In the following we show that symbolic steady states allow us to identify suitable subsystems.

We need the following notation. Let $x \in X^\theta$ be a symbolic steady state, and let Z be a component of $G^\theta([x])$. Let $k := \operatorname{card} V_Z$ be the cardinality of V_Z. We may assume that $V_Z = \{\alpha_{l+1}, \ldots, \alpha_{l+k}\}$ for some $l \in \{0, \ldots, n-1\}$. Set $X^Z := X_{l+1} \times \cdots \times X_{l+k}$. We define $f^Z : X^Z \to X^Z$, $f^Z := \pi^Z \circ f^\theta \circ \rho^Z$, where $\rho^Z : X^Z \to X^\theta$ with $\rho_i^Z(z) = x_i$ for $i \notin Z$ and $\rho_i^Z(z) = z_{i-l}$ for $i \in Z$, and $\pi^Z : X^\theta \to X^Z$ is the projection on the components of Z. The function f^Z maps regular states to regular states, since Z is disjoint from all other components $\alpha_j \in J(x)$ in $G^\theta([x])$ and thus $f_i^\theta(\rho(z)) \in X_i$ for $i \in Z$. Proposition 1 ensures that no coordinate function of f^Z is constant. Furthermore, it is easy to see that the global interaction graph $G(f^z)$ is isomorphic to Z. We denote the synchronous and asynchronous state transition graph derived from f^Z by S^s_Z and S^a_Z, respectively.

In the remainder of the section let x be a symbolic steady state of f, and let $Z_1, \ldots, Z_m$ be the components of $G^\theta([x])$. W.l.o.g. we may assume that Z_1 contains the vertices $\alpha_1, \ldots, \alpha_{\operatorname{card} Z_1}$, and $V_{Z_i} = \{\alpha_{1+\sum_{j=1}^{i-1} \operatorname{card} Z_j}, \ldots, \alpha_{\sum_{j=1}^{i} \operatorname{card} Z_j}\}$ for all $i \in \{2, \ldots, m\}$, and thus $\{1, \ldots, n\} \setminus J(x) = \{k, \ldots, n\}$ for $k := 1 + \sum_{j=1}^{m} \operatorname{card} Z_j$. To simplify notation we identify subsets of $X^{Z_1} \times \cdots \times X^{Z_m} \times \{(x_k, \ldots, x_n)\}$ with subsets of X.

Theorem 1. *For all $i \in \{1, \ldots, m\}$ let A_i be an attractor in $S^a_{Z_i}$. Then $A := A_1 \times \cdots \times A_m \times \{(x_k, \ldots, x_n)\}$ is an attractor of the asynchronous state transition graph S^a of f. Moreover, every attractor in S^a with all its vertices in $[x]$ can be represented in this manner as Cartesian product of attractors in $S^a_{Z_i}$, $i \in \{1, \ldots, m\}$, and $\{(x_k, \ldots, x_n)\}$.*

Proof. We observe that $\pi^{Z_j}(f(x')) = \pi^{Z_j}(f^\theta(x')) = \pi^{Z_j}(f^\theta(\rho^{Z_j}(\pi^{Z_j}(x')))) = f^{Z_j}(\pi^{Z_j}(x'))$ for all $x' \in [x]$ and all $j \in \{1, \ldots, m\}$ according to the definition of ρ^{Z_j}, π^{Z_j} and Lemma 1.

By definition we have $A \subseteq [x]$. We show that A is a trap set in S^a which is strongly connected. Let $a \in A$. If $f(a) = a$ then A is an attractor. Otherwise choose $i \in \{1, \ldots, n\}$ such that $f_i(a) \neq a_i$. Consider $a' \in X$ with $a'_i = a_i + \operatorname{sgn}(f_i(a) - a_i)$ and $a'_j = a_j$ for $j \neq i$. Since $a \in [x]$ we have $f_j(a) = a_j$ for all $j \in \{k, \ldots, n\}$. Thus there is $l \in \{1, \ldots, m\}$ with $i \in Z_l$. Then we choose k^l such that $i = j^l + k^l$ with $j^l := \sum_{j=1}^{l-1} \operatorname{card} Z_j$. We have $a_i \neq f_i(a) = f^{Z_l}_{k^l}(\pi^{Z^l}(a))$. It follows that $a_i + \operatorname{sgn}(f_i(a) - a_i) = (\pi^{Z_l}(a))_{k^l} + \operatorname{sgn}(f^{Z_l}_{k^l}(\pi^{Z_l}(a)) - (\pi^{Z_l}(a))_{k^l})$. Since A^l is a trap set in $S^a_{Z_l}$, it follows that $\pi^{Z_l}(a') \in A_l$, and thus $a' \in A$.

To obtain a path from $a, a' \in A$ in S^a we construct the path componentwise in the attractors A_i. More precisely, we exploit the fact that if γ is a path in $S^a_{Z_i}$ from z to z' for $z, z' \in X^{Z_i}$, then we find a path γ' in S^a from y to y' with $y_j = y'_j$ for all $j \notin Z_i$ which is projected on γ by π^{Z_i}. This is possible since the dynamics in $Z_1, \ldots, Z_m$ do not influence each other according to Lemma 1 and since in the asynchronous state transition graph state changes indicated by f are executed componentwise. For a detailed elaboration of this argument in the Boolean case see [31], proof of Theor. 5.6. It follows, that A is strongly connected.

The same reasoning ensures that the projection $\pi^{Z_j}(A)$, $j \in \{1, \ldots, m\}$, of an arbitrary attractor A of S^a is an attractor of $S^a_{Z_j}$. Edges leaving $\pi^{Z_j}(A)$ in $S^a_{Z_j}$ would generate edges leaving A in S^a, and paths in A are projected on sequences of states in X^{Z_j} that constitute a path in $S^a_{Z_j}$, if we eliminate all but one consecutive identical states (due to non-injectivity of π^{Z_l}) in the sequence. $\square$

The proof suggests that the compositional properties inherent in x are not restricted to the construction of attractors. We have shown in [31] in the Boolean case that in fact the subgraph of S^a with vertex set $[x]$ is the composition of the graphs $S^a_{Z_i}$, $i \in \{1, \ldots, m\}$, and $\{x_k, \ldots, x_n\}$. The reasoning can be adopted for multi-valued functions.

In Fig. 2 (e) we see in the second column of the table attractors derived from the corresponding symbolic steady state in the first column. For the state $(0, \theta, \theta, \theta)$ we obtain two components of $G^\theta([x])$ as shown in Fig. 2 (b). In (c) the corresponding graphs $S^a_{Z_1}$ and $S^a_{Z_2}$ are shown, and in (d) we see the compositional attractor in the state transition graph S^a of f.

In [7, 6] the authors consider the case that $G(f)$ is not connected and show how to derive attractors of the synchronous state transition graph from the subnetwork dynamics corresponding to the graph components. Lemma 1 allows us to apply their results to the components $Z_1, \dots, Z_m$ and obtain the following statement concerning the synchronous state transition graph of f.

Theorem 2. *For all $i \in \{1, \dots, m\}$ let A_i be an attractor in $S^s_{Z_i}$. Then the set $\mathcal{A} := A_1 \times \cdots \times A_m \times \{(x_k, \dots, x_n)\}$ is a union of attractors in S^s such that the cardinality of each attractor $A \in \mathcal{A}$ is the least common multiple of the cardinalities of the attractors A_i, $i \in \{1, \dots, m\}$, and, as a consequence, the number of attractors in $\mathcal{A}$ is $\prod_{j=2}^m ((\mathrm{card}\, A_1 \bullet \mathrm{card}\, A_2) \bullet \cdots \bullet \mathrm{card}\, A_{j-1}) \star \mathrm{card}\, A_j$, where $\bullet$ denotes the least common multiple and $\star$ the greatest common divisor operation. Moreover, $\pi^{Z_j}(A)$ is an attractor in $S^s_{Z_j}$ for every attractor $A \subseteq [x]$ in S^s and every $j \in \{1, \dots, m\}$.*

It is easy, but tedious, to describe the states of a compositional attractor in S^s. It is basically a concatenation of the steady states resp. cycles of the subsystems. Since we update all components at once in every step, cycles in $S^s_{Z_i}$ can generate more than one cycle in S^s. This is illustrated by the example in Fig. 2. The last column of the table in (e) shows attractors in S^s derived from different symbolic steady states. The composition of the two attractors shown in (c) yield two cycles in the synchronous dynamics.

When comparing the synchronous and the asynchronous case the differences become apparent when looking at the attractors derived from component attractors of cardinality greater than one. In the synchronous case composition of component attractors may result in a greater number of attractors, while in the asynchronous case only the attractor size increases. A more detailed description of the relation between cyclic attractors in synchronous and asynchronous dynamics necessarily would include observations on the way edges are generated during composition of attractors in the asynchronous case as well as on effects of the gradual activity level change of the asynchronous update.

We close this section with a simple corollary from the two preceding theorems, which for the synchronous case was already formulated in [6].

Corollary 1. *For $i \in \{1, \dots, m\}$ let $S^\delta_{Z_i}$, $\delta \in \{a, s\}$, contain N_i attractors with cardinalities L_{ij}, $j \in \{1, \dots, N_i\}$. Set $I := I_1 \times \cdots \times I_m$ with $I_l := \{1, \dots, N_l\}$.*

- *In the asynchronous state transition graph of f, the number of attractors with vertices in $[x]$ is $\prod_{j=1}^m N_j$ and the maximal attractor cardinality in $[x]$ is $\max_{(k_1,\dots,k_m)\in I} \prod_{j=1}^m L_{jk_j}$.*
- *In the synchronous state transition graph of f, the number of attractors with vertices in $[x]$ is $\sum_{(k_1,\dots,k_m)\in I} \prod_{j=2}^m ((L_{1k_1} \bullet L_{2k_2}) \bullet \cdots \bullet L_{j-1k_{j-1}}) \star L_{jk_j}$, and*

$\max_{(k_1,\ldots,k_m)\in I}((L_{1k_1} \bullet L_{2k_2}) \bullet \cdots \bullet L_{mk_m})$ *is the maximal attractor cardinality in* $[x]$.

5 Circuits and Attractors

In the preceding section we constructed attractors in the dynamics of f from attractors of functions f^{Z_i} associated with certain subnetworks. The same reasoning allows us to formulate more general relations between structural characteristics of the subnetworks and the dynamics of f. We focus in this section on the impact of feedback circuits in the structure on number and size of attractors of f.

For multi-valued discrete functions with asynchronous update it was shown in [26] that the existence of a positive circuit in the global interaction graph (and even in certain local interaction graphs) is a necessary condition for the existence of two attractors in the asynchronous state transition graph. Complementary, the existence of a negative circuit in the global interaction graph is necessary for the existence of a cyclic attractor (see [24]). Obviously, the result on positive circuits also holds in the synchronous case if we specify the attractors to be steady states. However, simple examples show that the second result is false in the synchronous case.

We now focus on functions whose global interaction graph contains only one circuit.

Lemma 2. *Assume the global interaction graph* $G(f)$ *contains only one circuit* C*, and let* V_C *be the set of vertices visited by* C*. Then* $f_i(x) = f_i(x')$ *for all* $i \in V_C$ *and* $x, x' \in X$ *with* $x_j = x'_j$ *for all* $j \in V_C$*.*

Proof. Assume we find $i \in V_C$ and $x, x' \in X$ such that $x_j = x'_j$ for all $j \in V_C$ and $f_i(x) \neq f_i(x')$. Then there exist $y, y' \in X$ and $k \notin V_C$ such that $y_j = y'_j$ for all $j \neq k$, $|y_k - y'_k| = 1$ and $f_i(y) \neq f_i(y'_k)$ (see proof of Lemma 1). It follows that $G(f)$ contains an edge from k to i. Since we always assume that no coordinate function is constant, every vertex in $G(f)$ has a predecessor. Since the set of vertices is finite, we then find a circuit other than C in $G(f)$ which is a contradiction. □

In [22] it is shown that Boolean functions associated with isolated circuits always display a characteristic behavior depending on their sign, both in the synchronous and the asynchronous case. We use this result to prove the following lemma.

Lemma 3. *Assume the global interaction graph* $G(f)$ *contains only one circuit* C*, whose edges are unique in* X *and functional in* C*. Then there are at least two attractors in* S^s *as well as* S^a*, if* C *is a positive circuit. If* C *is negative, there exists a cyclic attractor in* S^s *and in* S^a*.*

Proof. Let us assume C corresponds to the vertex sequence $(\alpha_1, \ldots, \alpha_r)$. Set $X_C := X_1 \times \cdots \times X_r$ and let $z_i \in X_i$ for $i \in \{r+1, \ldots, n\}$. We set $f^C : X^C \to X^C$

by $f^C := \pi^C \circ f \circ \rho^C$, where $\rho^C : X^C \to X, y \mapsto (y_1, \ldots, y_r, z_{r+1}, \ldots, z_n)$ and π^C is the projection on the components of C. According to Lemma 2, we have $f_i^C(\pi^C(x)) = \pi^C(f(x))$ for all $x \in X$. It follows that $G(f^C) = C$, i. e., values of coordinate function f_i^C depend only on the value of the predecessor in C. Since furthermore all edges of C are unique (see Def. 3), we find for all $i \in \{1, \ldots, r\}$ values $t^{i,i+1} \in \{0, \ldots, p_i - 1\}$ such that $f_{i+1}^C(y) = f_{i+1}^C(y')$ for all $y, y' \in X^C$ with either $y_i, y'_i \leq t^{i,i+1}$ or $y_i, y'_i > t^{i,i+1}$, indices taken modulo r. In addition, there exist $y, y' \in X^C$ with either $y_i \leq t^{i,i+1} < y'_i$ or $y'_i \leq t^{i,i+1} < y_i$ such that $f_{i+1}^C(y) \leq t^{i+1,i+2} < f_{i+1}(y')$, since the edge from α_i to α_{i+1} is functional in C. This allows us to define a Boolean function $f^{\mathcal{B}} : \{0,1\}^r \to \{0,1\}^r$ by $f^{\mathcal{B}} := \pi^t \circ f^C \circ \rho^t$ with $\rho^t : \{0,1\}^r \to X^C$, $\rho_j^t(0) = c_j$, $\rho_j^t(1) = c'_j$ for arbitrary but fixed $c_j \in \{0, \ldots, t^{j,j+1}\}$, $c'_j \in \{t^{j,j+1} + 1, \ldots, p_j\}$, and $\pi^t : X^C \to \{0,1\}^r$, $\pi_j^t(y) = 0$ if $y_j \leq t^{j,j+1}$ and $\pi_j^t(y) = 1$ if $y_j > t^{j,j+1}$. Note that $f^{\mathcal{B}}$ does not depend on the choice of c_j, c'_j. It is easy to see that $G(f^{\mathcal{B}})$ coincides with C.

In [22] was shown that $f^{\mathcal{B}}$ has at least two fixed points, if C is positive. Let b be a fixed point of $f^{\mathcal{B}}$. Choose $y' \in (\pi^t)^{-1}(b)$ and set $y_i := f_i^C(y')$ for all $i \in \{1, \ldots, r\}$. Since b is a fixed point, we can deduce that $y = (y_1, \ldots, y_r) = f^C(y') \in (\pi^t)^{-1}(b)$. It follows from the definition of π^t that for all $i \in \{1, \ldots, r\}$ either $y_i, y'_i \leq t^{i,i+1}$ or $y_i, y'_i > t^{i,i+1}$. The uniqueness condition then yields $f_i^C(y) = f_i^C(y') = y_i$. Thus y is a fixed point of f^C.

We then find a trajectory in S^δ, $\delta \in \{a, s\}$, starting in y and leading to an attractor A. According to Lemma 2 we then have $f_i(a) = y_i$ for all $i \in \{1, \ldots, r\}$ and every $a \in A$. Since we have two different fixed points of f^C, if C is positive, we also find two different attractors in S^δ.

If C is negative, then $f^{\mathcal{B}}$ does not have a fixed point (see again [22]). It follows from the definition of $f^{\mathcal{B}}$, in particular from its independence of the choice of c_j and c'_j, that f^C does not have a fixed point either. Thus, the synchronous as well as the asynchronous state transition graph of f^C contain a cyclic attractor. Lemma 2 then again yields the existence of a cyclic attractor in the asynchronous and synchronous state transition graph of f. □

The uniqueness of edges is exploited in the proof to obtain a suitable projection on the Boolean case. However, it seems likely that the statement remains true when dropping the condition. A finer partition of the range of a component corresponding to multiple thresholds could clarify the situation.

By applying the above lemma and Theorems 1 and 2 (or Cor. 1) we immediately obtain the following theorem.

Theorem 3. *Let x be a symbolic steady state. Assume a component Z of $G^\theta([x])$ contains only one circuit C and that all edges of C are unique in $[x]$ and functional in C with respect to $[x]$. If C is positive, then there exist at least two attractors in S^a as well as in S^s. If C is negative, then there is a cyclic attractor in both S^a and S^s.*

The theorem basically states that circuits embedded in complex networks imprint some of the characteristics they show in isolation on the whole network, if we can in some sense recover isolation in at least a part of state space. The statement does not hold, if the circuit C is not the only circuit in Z (see Boolean examples in [33, 31]) Furthermore, the hypothesis given in the last theorem is a sufficient but not a necessary condition for the existence of multiple resp. cyclic attractors. This is illustrated by an example given in [33], Fig. 4.

6 Determining symbolic steady states

We have seen that symbolic steady states can be very useful in the analysis of the dynamics. In this section, we address the problem of determining symbolic steady states of a given system.

6.1 Seeds

In the following we are interested in states that have component values that stay fixed under iteration.

Definition 10. *We call a state $s \in X^\theta$* seed of f *if $(f^\theta)^k_i(s) = s_i$ for all $i \in \{1, \dots, n\} \setminus J(s)$ and $k \in \mathbb{N}$, where $(f^\theta)^k$ denotes the k-th iterate of f^θ.*

By definition, the state $(\theta, \theta, \dots, \theta)$ is a seed. We have mentioned in Sect. 3 that it is also a steady state. In general, a seed is not a steady state, however, we can easily derive a steady state from a seed.

Lemma 4. *Let $s \in X^\theta$ be a seed of f. Then $((f^\theta)^k(s))_{k\in\mathbb{N}}$ converges with respect to the discrete metric on X^θ to a symbolic steady state, which we call the* symbolic steady state generated by s.

Proof. If $J(s) = \{1, \dots, n\}$ then s is a steady state as mentioned above. So let us assume that $\{1, \dots, n\} \setminus J(s)$ is not empty. We set $s^0 := s$ and $s^l := f^\theta(s^{l-1})$ for all $l \in \mathbb{N}$, and show by induction: for all $l \in \mathbb{N}$ and for all $i \in \{1, \dots, n\}$, if $s^l_i \neq \theta$, then $s^l_i = s^m_i$ for all $m \geq l$. This is true for $l = 0$ since s is a seed.

Let us now assume the hypothesis holds for $l \in \mathbb{N}$. It follows that $[s^m] \subseteq [s^l]$ for all $m \geq l$. Let $i \in \{1, \dots, n\}$ such that $s^{l+1}_i \neq \theta$. Then $s^{l+1}_i = f^{\min}_i(s^l) \leq f^{\min}_i(s^m) \leq f^{\max}_i(s^m) \leq f^{\max}_i(s^l) = s^{l+1}_i$. It follows that $s^{m+1}_i = f^{\min}_i(s^m) = f^{\max}_i(s^m) = s - i^{l+1}$ for all $m \geq l$, which proves our claim. The statement of the lemma follows immediately. □

The proof shows that we reach a symbolic steady state after at most card $J(s)$ iteration steps. Moreover, it shows that a trap set contained in $[s]$ but not in $[x]$, where x is the symbolic steady state generated by s, does not constitute a strongly connected component of either the synchronous or the asynchronous state transition graph. This leads to the following statement.

Proposition 2. *Let $s \in x^\theta$ be a seed of f, and let $x \in X^\theta$ be the symbolic steady state generated by s. Then for every attractor $A \subseteq [s]$ in $S \in \{S^a, S^s\}$ holds $A \subseteq [x]$.*

In consequence, all attractors in $[s]$ in the asynchronous resp. the synchronous state transition graph can be derived from x as described in Theorem 1 resp. 2. In particular, we can find all attractors of the system if we consider a set of seeds $\{s^1, \ldots, s^k\}$ such that $X = \bigcup_{i=1}^{k}[s^i]$.

We illustrate the results using the function f given in Fig. 2. The state $(1, \theta, \theta, \theta)$ is a seed of f. After two iteration steps we obtain the symbolic steady state $(1, 1, 1, \theta) = f^\theta((1, 1, \theta, \theta)) = f^\theta(f^\theta((1, \theta, \theta, \theta)))$. It can easily be checked that the attractors given in Fig. 2 (e) are all attractors of the system. We then see that the attractors derived from $(1, 1, 1, \theta)$ are all the attractors in $[(1, \theta, \theta, \theta)]$.

6.2 Forcing structures

We have seen that we can use seeds to determine symbolic steady states, but we still need methods to find seeds. In the following, we focus on exploiting characteristics of the function describing the system as well as of the interaction graph. We start by introducing the notion of canalyzing functions, which is well-known in the Boolean setting, appearing in several different contexts [11, 10].

Definition 11. *Let $k \in \{1, \ldots, n\}$. A function $g : X \to X_k$ is called* canalyzing *if there exist $i \in \{1, \ldots, n\}$, $c \in X_i$ and $c' \in X_k$ such that $g(x) = c'$ for all $x \in X$ with $x_i = c$. The i-th component is called* canalyzing component*, the value c is called* canalyzing value (of the canalyzing component i) *and c' is called* canalyzed value.

Already in the Boolean case it is possible for a function to have more than one canalyzing value. Consider for example a projection of a k-tuple to the first component. Then, the first component is canalyzing and every possible value of the first component is a canalyzing as well as a canalyzed value.

Recall that we excluded constant coordinate functions. Then it is easy to see that there is an edge from i to j in the global interaction graph, if the j-th coordinate function of f is canalyzing with canalyzing component i. If canalyzing functions interlock along such edges such that the canalyzing and canalyzed values match, then canalyzing values percolate through the network [11]. To make this statement more precise we use the following definitions.

Definition 12. *A pair $(i, j) \in V^2$ is called* forcing connection from i to j *if both f_i and f_j are canalyzing functions, f_j with canalyzing component i, and a canalyzed value of f_i is a canalyzing value of component i of f_j.*

Let $W \subseteq V^2$ and set $V' := \{l \in V \mid \exists k : (l, k) \in W \vee (k, l) \in W\}$. The set W is called forcing structure *if there exist values $c_k \in X_k$, $k \in V'$, such that for every $(i, j) \in W$ the value c_i is a canalyzed value of f_i and a canalyzing value of component i of f_j. The multiset of values c_k, $k \in V'$ is called* assignment of W. *We denote the unsigned directed graph with vertex set V' and edge set W by G_W.*

In general, the assignment of W is not unique. Clearly, every subset of W is also a forcing structure and every $(i, j) \in W$ is a forcing connection.. Every element of W corresponds to an edge in $G(f)$. More precisely, G_W is a subgraph of the graph obtained by dropping the edge signs of the edges of $G(f)$. The properties of canalyzing functions and forcing structures give rise to the following dynamical property. If we consider a state x with $x_j = c_j$ for some $j \in V'$ and some assignment $\{c_i \in X_i \mid i \in V'\}$ of W, then $f_k(x) = c_k$ for all $k \in V'$ with $(j, k) \in W$. That is, in a first iteration step, all successors of j in W adopt the corresponding assignment values, in a second iteration step all their successors in W adopt the corresponding assignment values, and so on. This observation immediately leads to the following statement.

Proposition 3. *Let W be a forcing structure with assignment $\{c_i \in X_i \mid i \in V'\}$. Then, for every trajectory $(x^0, x^1, \dots)$ in S^s, we have $x^k_l = c_l$ for all l with $(i, l) \in W$ and $x^{k-1}_i = c_i$.*

Let $x^0 \in X$ with $x^0_j = c_j$ for some $j \in V'$, and let $(j = j^0, j^1, \dots, j^m)$ be a path in G_W. Then there exists a path $(x^0, x^1, \dots, x^{m'})$, $m' \in \{0, 1, \dots, m\}$, in S^a such that for all $k \in \{0, 1, \dots, m\}$ there is $i_k \in \{0, 1, \dots, m'\}$ with $x^{i_k}_k = c_k$, and $i_{k-1} \leq i_k$ for all $k \in \{1, \dots, m\}$.

The proposition shows that in the synchronous case the influence of canalyzing functions on the dynamics is straightforward. In the asynchronous case we have to keep in mind that the system is non-deterministic and thus the influence of canalyzing values may not be seen along every path in the state transition graph.

In general, the effect of the canalyzing values of a forcing structure on the dynamics is transient, since components outside of the forcing structure may negate it. However, if G_W includes a circuit then outside influences can be neglected as was already observed in [21].

Definition 13. *A forcing structure W such that G_W is a circuit is called* self-freezing circuit*.*

A circuit in G_W corresponds to a circuit in $G(f)$ but we have no information on the sign of the circuit in $G(f)$. In general, it can be positive or negative. A clearer understanding of the relation between self-freezing circuits and the corresponding circuits in $G(f)$ should be possible if one considers the notion of circuit functionality.

The importance of self-freezing circuits for the dynamics is rooted in the following observation.

Lemma 5. *Let W be a self-freezing circuit with assignment $\{c_i \in X_i \mid i \in V'\}$. Then $s \in X^\theta$ with $s_i := c_i$ for all $i \in V'$ and $s_j := \theta$ for $j \in V \setminus V'$ is a seed.*

Proof. Let $i \in V'$. Then there exists $j \in V'$ such that $(j, i) \in W$. Since f_i is canalyzing with canalyzing component j, canalyzing value c_j and canalyzed value c_i, we have $f_i(x) = c_i$ for all $x \in X$ with $x_j = c_j$. It follows that $f^\theta_i(s) = c_i$. So, $f^\theta_l(s) = c_l$ for all $l \in V' = \{1, \dots, n\} \setminus J(s)$. Inductive reasoning then shows $f^\theta(s)^k_l(s) = c_l = s_l$ for all $l \notin J(s)$ and $k \in \mathbb{N}$. □

The seed derived from the self-freezing circuit in turn generates a symbolic steady state as shown in Sect. 6.1. Summarizing the observations up to this point we obtain the following statement.

Theorem 4. *Let W be a self-freezing circuit with assignment $\{c_i \in X_i \mid i \in V'\}$, let $s \in X^\theta$ be a seed derived from W, and let $x \in X^\theta$ be the symbolic steady state it generates. Then all attractors in $[s]$ in the asynchronous resp. the synchronous state transition graph can be derived from x as described in Theorem 1 resp. 2.*

Self-freezing circuits provide a high degree of stability. Perturbations outside of the circuit do not influence the values of the seed derived from the self-freezing circuit. Moreover, the set of attractors reachable from a state obtained by such a perturbation is the set of attractors derived from the symbolic steady state generated by the seed according to Theor. 4. This is true in the synchronous as well as the asynchronous case.

Perturbation of a circuit component may have an effect. However, it is easy to see that the number of circuit components adopting the corresponding assignment value cannot decrease when iterating the perturbed state, since the predecessor of the perturbed component forces its value back to the canalyzing value and only the successor of the perturbed component may adopt a value other than that of the assignment. If outside influences prevent the change of the successor component value at some point, then the component circuit values again remain fixed to their assignment value under iteration. In particular in the Boolean setting this outcome is very likely [21].

The preceding results provide us with a method to find seeds by determining the circuits of $G(f)$ and checking wether the corresponding unsigned circuits are self-freezing. Since circuits play an important role in the dynamics of regulatory networks, determining and analyzing circuits in a given network is often already implemented in software designed for analyzing logical models, as e. g. GINsim [16]. The complexity of the problem to decide whether a circuit is self-freezing depends on the length of the circuit and on the complexity of the coordinate functions of the circuit components. In some cases the task is very easy. Consider for example the system introduced in Fig. 2. We immediately see that the loop (α_1, α_1) is self-freezing since f_1 is the projection on the first component. In this case, it is possible to derive three different seeds from the loop, namely $(0, \theta, \theta, \theta)$, $(1, \theta, \theta, \theta)$ and $(2, \theta, \theta, \theta)$. The symbolic steady states generated by the seeds are $(0, \theta, \theta, \theta)$, $(1, 1, 1, \theta)$ and $(2, 1, 1, 1)$.

In the example we just considered, all attractors of the system can be derived from the symbolic steady states generated by the seeds corresponding to the self-freezing circuit (α_1, α_1). In general, it is not possible to find a self-freezing circuit with that property. In the next section we explore methods to find a set of seeds that allows us to recover all attractors of the system.

6.3 Input networks

In Lemma 1 we have shown that we can analyze subnetworks derived from the local interaction graph corresponding to a symbolic steady state independently. This is possible because they do not influence each others behavior in a subspace of state space, which is reflected in the fact that there are no edges between them in the corresponding local interaction graph. The same reasoning applies when considering subgraphs $G' = (V', E')$ of $G(f)$ such that there are no edges from vertices in $V \setminus V'$ to vertices in V' in $G(f)$. We can then define a function $f^{G'} := \pi^{G'} \circ f \circ \rho^{G'} : X^{G'} \to X^{G'}$, $X^{G'} := \prod_{i \in V'} X_i$, analogously to the function f^Z introduced in the beginning of Sect. 4. Here, the coordinate functions $\rho_i^{G'}$ are the projections on the i-th component for all $i \in V'$, while we set $\rho_i^{G'}(z) = c_i$ with $c_i \in X_i$ arbitrary but fixed for all $i \notin V'$, and $\pi^{G'}$ is again the projection on the components of V'.

Definition 14. *Let $V' \subseteq V$. The subgraph $G' = (V', E')$ of $G(f)$ induced by V' is called* input network of f *if*

- *there are no edges from vertices in $V \setminus V'$ to vertices in V' in $G(f)$,*
- *the only attractors of the synchronous and asynchronous state transition graphs of the function $f^{G'}$ are fixed points of $f^{G'}$.*

The definition immediately yields that $f^{G'}$ has at least one fixed point. The notion of input network generalizes the idea of *input vertex*, i.e., a vertex α_i such that $f_i(x) = x_i$, introduced in [31]. An input network does not necessarily have to be connected. If the graph G' consists of several components, then the fixed points of each component can be calculated independently. The fixed points of $f^{G'}$ are then composed of the fixed points of the components. This observation allows for a straightforward approach to networks with input vertices, since the set of fixed point associated with an input vertex coincides with the range of the vertex.

Let us consider the system presented in Fig. 2, in particular the subgraph $G' := (\{\alpha_1\}, \{(\alpha_1, \alpha_1)\})$ of $G(f)$. The only edge leading to α_1 is the one included in G'. The function $f^{G'} : \{0, 1, 2\} \to \{0, 1, 2\}$ is the identity (α_1 is an input vertex). The synchronous as well as the asynchronous state transition graph of $f^{G'}$ consist of three loops originating in the three states of the state space $\{0, 1, 2\}$, i.e., every state is a fixed point of $f^{G'}$, and G' is an input network.

The next statement follows directly from the lack of edges from $V \setminus V'$ to V' in $G(f)$ and the definition of edges in Def. 1.

Proposition 4. *Let $G' = (V', E')$ be an input network of f, and let $y \in X^{G'}$ be a fixed point of $f^{G'}$. Then $s \in X^\theta$ with $s_i := y_i$ for all $i \in V'$ and $s_i := \theta$ for all $i \in V \setminus V'$ is a seed.*

Again, we can use the seeds corresponding to G' to generate symbolic steady states and determine attractors. We obtain all attractors of f if we consider the set of seeds corresponding to the set of fixed points of $f^{G'}$.

Theorem 5. *Let $G' = (V', E')$ be an input network of f. Then all attractors in the asynchronous resp. the synchronous state transition graph can be derived from the symbolic steady states generated by the seeds of G' as described in Theorem 1 resp. 2.*

Proof. Let A be an attractor of f. It suffices to show that there exists a fixed point $y \in X^{G'}$ such that $a_i = y_i$ for all $i \in V'$, $a \in A$. Then $A \subset [s]$, where s is the seed derived from the fixed point y, and the theorem follows with Prop. 2.

It is easy to see in the asynchronous case (compare the second part of the proof of Theorem 1) and even more obvious in the synchronous case that $\pi^{G'}(A)$ is an attractor of $f^{G'}$. Since G' is an input network, there is a fixed point $y \in X^{G'}$ such that $\pi^{G'}(A) = \{y\}$. The assertion follows. □

Lemma 4 and Theorem 5 yield a simple strategy for the analysis of systems with input networks. We first determine the fixed points of the input network. As mentioned above, this is particularly easy for input vertices, which can be used to model input in biological networks, e. g. in signal transduction networks. We then obtain symbolic steady states from the corresponding seeds using the simple iteration procedure described in Lemma 4. Implementation of this procedure is straightforward. The resulting subsystems can then be used to determine the attractors, and to obtain a detailed understanding of the link between structural and dynamical characteristics.

7 T helper cell differentiation

In [13] L. Mendoza proposes a model for a control network regulating differentiation of T helper cells (Th cells), which play an important role in the vertebrate immune system. There exist different types of Th cells involved in different immune responses, namely Th1 and Th2 cells, that originate from a common percursor. Mendoza's model consists of 17 components, represented mostly by Boolean variables, but also by variables with three possible values. The system is described by the logical rules given in Table 1. The global interaction graph is given in Fig. 3. Note that the logical functions associated with the vertices IFN-β, IL-12 and Il-18 listed in Table 1 differ slightly from those given in [13]. Mendoza models all three components with constant functions with value zero, which represents wild type cells in a natural environment. When considering specific artificial environmental conditions, other constant functions are considered. We model the three components as input vertices. In consequence, different environmental conditions correspond to different input values and thus to different subgraphs of state space. In particular, Mendoza's original model corresponds to the situation where all input values are zero.

The vertices $\alpha_1 =$ IFN-β, $\alpha_2 =$ IL-12 and $\alpha_3 =$ Il-18 represent the only input vertices of the network. The graph G' with vertex set $\{\alpha_1, \alpha_2, \alpha_3\}$ and edge set $\{(\alpha_i, \alpha_i, +) \mid i \in \{1, 2, 3\}\}$ is an input network, since every state in $X_1 \times X_2 \times X_3 = \{0, 1\}^3$ is a fixed point of $f^{G'} : \{0, 1\}^3 \to \{0, 1\}^3$, $(x_1, x_2, x_3) \mapsto (x_1, x_2, x_3)$. It follows that every $x \in X = \prod_{i=1}^{17} X_i$ with $x_i \in \{0, 1\}$ for $i \in \{1, 2, 3\}$ and $x_i = \theta$ for $i \in \{4, \ldots, 17\}$ is a seed of the system.

Let us analyze the system for input values $x_i = 0$, $i \in \{1, 2, 3\}$, corresponding to the wild type model in [13]. We start by determining the symbolic steady state generated by the seed $s^0 := (0, 0, 0, \theta, \ldots, \theta)$. Iterating s yields

$$s^1 := f^{\theta}(s^0) = (0, 0, 0, 0, \theta, \theta, \theta, \theta, 0, 0, \theta, \theta, \theta, \theta, \theta, \theta, \theta),$$
$$s^2 := f(s^1) = (0, 0, 0, 0, \theta, \theta, \theta, \theta, 0, 0, \theta, \theta, \theta, 0, 0, \theta, \theta),$$
$$f(s^2) = s^2.$$

IFN-β:	$X_1 = \{0,1\}$,	$f_1(x) = x_1$
IL-12:	$X_2 = \{0,1\}$,	$f_2(x) = x_2$
IL-18:	$X_3 = \{0,1\}$,	$f_3(x) = x_3$
IFN-βR:	$X_4 = \{0,1\}$,	$f_4(x) = x_1$
IFN-γ:	$X_5 = \{0,1,2\}$,	$f_5(x) = 1$ if $(x_{16} = 1 \wedge \neg(x_{14} = 1 \wedge x_{15} = 1)) \vee$
		$(x_{14} = 1 \wedge x_{15} = x_{16} = 0)$,
		$f_5(x) = 2$ if $x_{16} = 2 \vee x_{14} = x_{15} = 1$, and $f_5(x) = 0$ otherwise
IL-4:	$X_6 = \{0,1\}$,	$f_6(x) = 1$ if $x_{12} = 0 \wedge x_{17} = 1$, and $f_6(x) = 0$ otherwise
IFN-γR:	$X_7 = \{0,1,2\}$,	$f_7(x) = 1$ if $x_5 = 1 \vee (x_5 = 2 \wedge x_{11} = 1)$,
		$f_7(x) = 2$ if $x_5 = 2 \wedge x_{11} = 0$, and $f_7(x) = 0$ otherwise
IL-4R:	$X_8 = \{0,1\}$,	$f_8(x) = x_6 \wedge \neg x_{11}$
IL-12R:	$X_9 = \{0,1\}$,	$f_9(x) = x_2 \wedge \neg x_{13}$
IL-18R:	$X_{10} = \{0,1\}$,	$f_{10}(x) = x_3 \wedge \neg x_{13}$
SOCS-1:	$X_{11} = \{0,1\}$,	$f_{11}(x) = 1$ if $x_{12} \geq 1 \vee x_{16} \geq 1$, and $f_{11}(x) = 0$ otherwise
STAT-1:	$X_{12} = \{0,1,2\}$,	$f_{12}(x) = 1$ if $(x_4 = 1 \wedge x_7 = 0) \vee x_7 = 1$,
		$f_{12}(x) = 2$ if $x_7 = 2$, and $f_{12}(x) = 0$ otherwise
STAT-6:	$X_{13} = \{0,1\}$,	$f_{13}(x) = x_8$
STAT-4:	$X_{14} = \{0,1\}$,	$f_4(x) = x_9 \wedge \neg x_{17}$
IRAK:	$X_{15} = \{0,1\}$,	$f_{15}(x) = x_{10}$
T-bet:	$X_{16} = \{0,1,2\}$,	$f_{16}(x) = 1$ if $(x_{17} = 0 \wedge ((x_{12} = 1 \wedge x_{16} \leq 1) \vee$
		$(x_{12} \leq 1 \wedge x_{16} = 1))) \vee (x_{17} = x_{16} = x_{12} = 1)$
		$f_{16}(x) = 2$ if $(x_{17} = 0 \wedge (x_{12} = 2 \vee x_{16} = 2)) \vee$
		$(x_{17} = x_{12} = 1 \wedge x_{16} = 2)$,
		$f_{16}(x) = 0$ otherwise
GATA-3:	$X_{17} = \{0,1\}$,	$f_{17}(x) = 1$ if $x_{13} = 1 \wedge x_{16} = 0$, and $f_{17}(x) = 0$ otherwise

Table 1. Coordinate functions and ranges for the components of the Th cell network.

The symbolic steady state s^2 has 8 regular components. The graph $G^\theta[s^2]$ is shown in Fig. 4 (a). The state space of the original system consists of 663552 states. Fixing the values of the components α_1, α_2 and α_3 reduces the size to 82944 states. The state space of $f^{G^\theta[s^2]}$ contains only 2592 states. Analyzing the reduced model still renders all attractors of the system for input values $x_i = 0$, $i \in \{1,2,3\}$ according to Theorem 1 resp. 2. The system has four attractors, all of them steady states, namely $(0,0,0,0,0,0,0,0,0)$, $(1,0,1,0,1,1,0,1,0)$, $(2,0,1,0,1,1,0,2,0)$, $(0,1,0,1,0,0,1,0,1) \in X_5 \times X_6 \times X_7 \times X_8 \times X_{11} \times X_{12} \times X_{13} \times X_{16} \times X_{17}$. Expanding the states with the regular components of s^2 then yields the four steady states of the original system. They are in complete agreement with the results in [13], where there is also mentioned that each of the discovered steady states has a clear biological interpretation.

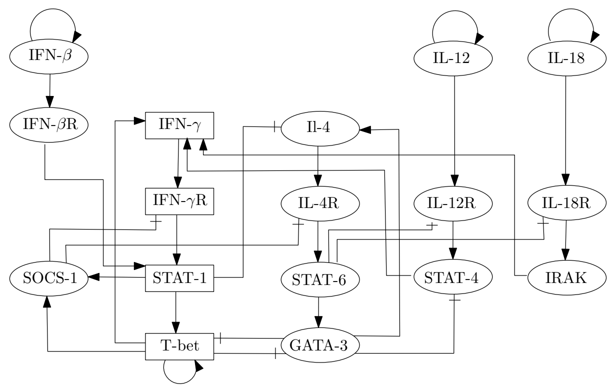


Fig. 3. Global interaction graph of the Th cell differentiation network introduced in [13]. Arrows represent activation, crossed lines represent inhibition.

Instead of calculating the attractors for $f^{G^\theta[s^2]}$, we could also use the idea of self-freezing circuits to simplify the system even further. The graph shown in Fig. 4 (a) contains 10 different circuits, not all of them self-freezing. Let us look at two examples of self-freezing circuits of $f^{G^\theta[s^2]}$. The components $\alpha_6 =$IL-4, $\alpha_8 =$ IL-4R, $\alpha_{13} =$STAT-6 and $\alpha_{17} =$GATA-3 constitute a circuit, and it is easy to see that the corresponding functions f_i are canalyzing. An assignment of the circuit is given by the values $c_i := 0$,$i \in \{6, 8, 13, 17\}$, i. e., $x := (\theta, 0, \theta, 0, \theta, \theta, 0, \theta, 0)$ is a seed of $f^{G^\theta[s^2]}$. One iteration step shows that x is also a symbolic steady state. The global interaction graph of the reduced system is shown in Fig. 4 (b). We derive three attractors of $f^{G^\theta[s^2]}$ from x, namely $(0, 0, 0, 0, 0, 0, 0, 0, 0)$, $(1, 0, 1, 0, 1, 1, 0, 1, 0)$ and $(2, 0, 1, 0, 1, 1, 0, 2, 0)$.

The circuit $(\alpha_5, \alpha_7, \alpha_{12}, \alpha_{16}) = (\text{IFN-}\gamma, \text{IFN-}\gamma\text{R}, \text{STAT-1}, \text{T-bet})$ is also self-freezing. The assignment is given by the values $c_i := 0$, $i \in \{5, 7, 12, 16\}$, i. e., $(0, \theta, 0, \theta, \theta, 0, \theta, 0, \theta)$ is also a seed of $f^{G^\theta[s^2]}$. The corresponding symbolic steady state is $y := (0, \theta, 0, \theta, 0, 0, \theta, 0, \theta)$. The global interaction graph of the reduced system is shown in Fig. 4 (c). Here, we obtain two attractors of $f^{G^\theta[s^2]}$, namely $(0, 0, 0, 0, 0, 0, 0, 0, 0)$ and $(0, 1, 0, 1, 0, 0, 1, 0, 1)$.

Together, the two self-freezing circuits we considered here yield all attractors of the original system. However, the two seeds derived from them do not cover the state space of $f^{G^\theta[s^2]}$ in its entirety, which is the only criterion we have for ensuring that we obtain all attractors of the original system from the reduced systems (as mentioned in Sect. 6.1). It is clear that this is a point of interest for future work.

Thus far we have only focussed on reduction of complexity when analyzing the network using symbolic steady states. But identification of the subnetworks associated with the symbolic steady state is also of interest, since they represent the characteristics of the system responsible for asymptotic dynamical behavior.

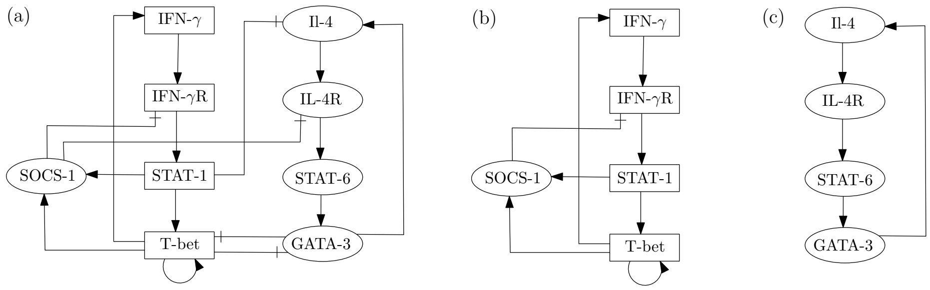


Fig. 4. Subnetworks of the Th cell differentiation network associated with the symbolic fixed points derived from the input values $x_1 = x_2 = x_3 = 0$ in (a) and $x_1 = 1$, $x_2 = x_3 = 0$ in (b).

For example, we have seen that input values $x_i = 0$, $i \in \{1, 2, 3\}$, completely determine the component values of IFN-βR, IL-2R, STAT4, IL-18R and IRAK in the attractors of the system. Their asymptotic behavior is independent of the behavior of the components belonging to the subnetwork represented by the graph in Fig. 4 (a). In contrast, the symbolic steady state $(1, 1, 1, 1, \theta, \ldots, \theta)$ derived from the input values $x_i = 1$, $i \in \{1, 2, 3\}$, has only four regular components. Therefore, the corresponding subnetwork consists of all components of the original system except for the three input vertices and the vertex IFN-βR. This is not very useful in terms of network reduction, however, we can derive information nonetheless. In this case, the system's asymptotic behavior heavily depends on components not belonging to the input network. In particular for signal transduction networks, where signal reception can easily be modeled using input networks, information this coarse is already biologically relevant. A refined understanding of the relation between asymptotic behavior and subnetworks might then be possible using self-freezing circuits.

8 Conclusion

Analyzing complex networks is a difficult task. Even if the number of components of a discrete regulatory network is in some sense manageable, we have to deal with the problem of analyzing the dynamics in an exponentially large state space. A well-known idea to approach this difficulty is to identify smaller building blocks of the system the study of which in isolation still renders information on the dynamics of the whole network. In this paper, we introduce the notion of symbolic steady state which allows us to identify such building blocks, systematically extending ideas developed for Boolean functions and asynchronous dynamics in [33] and [31]. We state explicit rules how to derive attractors of the network from subnetwork attractors valid for synchronous as well as asynchronous dynamics. Illustrating those rules, we derive general conditions for circuits embedded in the network to transfer their behavioral characteristics pertaining number and size of attractors observed in isolation to the complex network. We also propose methods for determining symbolic steady states based on structural and dynamical characteristics of the system.

Stronger results are possible if we refine the representation of component values via the symbolic value. Instead of merging the whole range of a component to one symbolic value we could partition it into several symbolic values, which would allow for a more precise localization in phase space. Such a refinement can be useful for analysis and poses no difficulty from a mathematical point of view, although careful consideration has to be given to the differences generated by the choice of update strategy. We presented some results for the asynchronous case in a workshop contribution [32], where we also considered the Th cell differentiation network. We have not yet considered refinements in the synchronous case.

An even more accurate understanding of the interactions governing asymptotic behavior in the region of state space associated with a symbolic steady state x would be possible when considering the local interaction graph $G(M)$ for a set $M \subseteq [x]$ derived from $[x]$ by eliminating in some sense dynamically irrelevant states. For example, a comparison of $[x]$ with its forward orbit may be helpful.

To make the theory more accessible for testing and using it in the study of logical models, we have to provide procedures for determining symbolic steady states that are suitable for implementation. We presented some ideas addressing this problem in Sect. 6. The results show that circuits in the interaction graph may be key to a more comprehensive understanding. In particular, the relation between self-freezing circuits and functional circuits should be clarified. The notion of functionality context as introduced in [18] could prove very useful in this endeavor. A thorough understanding of the links between the different concepts may also allow for easy integration of procedures calculating symbolic steady states into software capable of analyzing logical models with respect to circuits, as e. g. GINsim [16]. Circuits may also play an important role regarding the extent of network reduction possible using symbolic steady states. In general, networks that are not too densely connected may be better candidates, especially when determining symbolic steady states using seeds derived from input values. As mentioned in Sect. 6.1 we need at most as many iteration steps as there are symbolic components in the seed. However, the iteration procedure may terminate after only a few steps if we deal with densely connected networks. In this case the information we gain is limited.

Future work will also focus on comparing our approach to other network modularization and reduction techniques. A good starting point would be a comparison with reduction techniques available for logical models (see e. g. [17]), but we should also consider methods proposed for other modeling frameworks to gain a clearer understanding of underlying concepts.

Lastly, a very important step is to apply the methods to established biological network models. This would allow not only for testing the suitability of the approach to the dynamical analysis, but also for a comparison of the subnetworks derived from symbolic steady states with network modules of known biological importance.

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