On the conversion of multivalued gene regulatory networks to Boolean dynamics

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Abstract

We consider the modeling approach introduced by R. Thomas for the qualitative study of gene regulatory networks. Tools and results on regulatory networks are often concerned only with the Boolean case of this formalism. However, multivalued approaches are sometimes more suited to model biological situations. Multivalued networks can be converted to partial Boolean maps, in a way that preserves the asynchronous dynamics. We ask whether this map can provide information on the original multivalued function, in particular via the application of Thomas’ rules. The problem of extending these partial Boolean maps to non-admissible states, i.e. states that do not have a multivalued counterpart, is also investigated. We observe that attractors are preserved if a “unitary” version of the original function is considered for conversion. Different extensions of the Boolean counterpart affect the structure of the regulatory graph in different ways. A particular technique for extending the Boolean unitary version of the network is identified, that ensures that no new circuits are added. This property, combined with the preservation of the asymptotic behaviour, can prove useful for the application of results and analyses defined in the Boolean setting to multivalued networks, and vice versa. By considering the conversion of a known example for the discrete multivalued case, we create a Boolean map showing that, for $n \geq 6$, the absence of fixed points is compatible with the absence of local negative circuits in the regulatory graph.

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1 Introduction

The logical framework introduced by Thomas [15, 16] models the qualitative behaviour of gene regulatory networks. In a system with \( n \) genes or regulatory components, the possible expression levels of each gene are assumed to be in a finite interval of integers, and the evolution of the expression levels is defined by a map \( f : \mathcal{X} \to \mathcal{X} \), where \( \mathcal{X} \) is the product of the \( n \) intervals. A regulatory graph can be associated to \( f \) as a graph on \( \{1, \ldots, n\} \), with an edge from \( j \) to \( i \) representing the influence that the expression level of gene \( j \) has on the expression level of gene \( i \). Such regulatory graph can depend on the state \( x \in \mathcal{X} \); the union of the local regulatory graphs is referred to as the global regulatory graph of the network.

Even though the literature on multivalued networks has been expanding in the last few years (e.g., [12, 9, 10, 1]), many results and tools focus on the Boolean setting. To enable a possible application of these results to the more general multivalued case, a mapping of discrete maps to Boolean has been considered, that defines a Boolean variable for each expression level of each gene [17, 4]. The resulting map associating discrete states to Boolean states is one-to-one, neighbour-preserving and regulatory-preserving, meaning that both the dynamics and the regulatory structure of the discrete map can be reconstructed from the Boolean version [4]. The Boolean map obtained is however defined on a subset of the Boolean configurations, whereas results and tools of gene regulatory network theory usually require maps to be defined on all Boolean states. Among these results are proofs of the renowned conjectures of Thomas [15, 8, 10, 12], which establish connections between the asymptotic behaviour of the network dynamics and the structure of the regulatory graph.

Here we consider possible ways of extending the Boolean version of a multivalued map to the states called “non-admissible” [4], i.e. Boolean states that do not have a discrete counterpart. We investigate some properties of this conversion from multivalued to Boolean networks, focusing on the relation between asymptotic behaviour and presence of circuits in the regulatory structure. We show that, if the “unitary” version of the discrete map is converted, and the non-admissibles states are mapped to admissible states, then the attractors of the asynchronous dynamics are preserved. Then, we ask whether results on the asymptotic behaviour can be derived without considering an explicit extension of the Boolean map. We show that, although, in general, circuits in the discrete regulatory structure do not always have a counterpart in the Boolean version, if a circuit is contained in a more restrictive regulatory graph - the graph associated to the “non-usual” Jacobian introduced in [12] - then the regulatory graph of the Boolean version admits a circuit with the same sign. As a consequence, in presence of multiple fixed points, the regulatory graph of the Boolean version must contain a local positive circuit. We then show that it is possible to exclude the presence of cyclic attractors in the original discrete map using a result for the Boolean counterpart. In addition, we identify a particular extension of the partial Boolean map that has the following property: if the regulatory graph of the Boolean version contains a circuit, then so does the regulatory graph of the original discrete map.

We apply the techniques identified to answer the following question, introduced by Richard [10]:

**Question 1.** Is the absence of a local negative circuit in the regulatory graph a sufficient condition for the map \( f \) to admit at least one fixed point?

Richard showed that the answer is negative in the discrete case [10], and proved that a local negative circuit necessarily exists for a Boolean network with no fixed points if \( f \) is non-expanding [11], for all \( n \). Ruet [14] showed that the answer to Question 1 is negative in the Boolean case as well, exhibiting a counterexample for \( n = 7 \). For \( n \leq 3 \), one can prove by exhaustion that a local negative circuit is required in absence of fixed points [8]. Here we present an example of a Boolean map with no local fixed
points, a unique cyclic attractor and no local negative circuits in the regulatory graph, for \( n = 6 \). We create the counterexample by applying the results of the paper to the conversion of Richard’s discrete counterexample to a Boolean network.

The work is organized as follows. In the first section we introduce some definitions from the theory of gene regulatory networks, and summarize some results connected to Thomas’ conjectures. In Section 3 we consider the map for conversion of a discrete multivalued network to a Boolean network introduced by Van Ham [17], and investigate the effects that extending the map can have on the asymptotic and regulatory properties of the network. Finally, in Section 4 we give a negative answer to Question [1] for \( n = 6 \).

## 2 Background

This section introduces the terminology and definitions used throughout the paper.

### 2.1 Boolean and multivalued gene regulatory networks

A regulatory network is defined by a set of \( n \) regulatory components, also called genes in the following. The definition of regulatory network captures the idea that the expression level of a regulatory component can influence the expression level of another. In the Boolean setting, the expression level of a gene is considered to be either 0, if the gene is not expressed, or 1, if the gene is expressed; the state space of the system is therefore \( \mathcal{X} = \{0, 1\}^n \). In the more general discrete multivalued scenario, a state of the system is an element of the product \( \mathcal{X} = \mathcal{X}_1 \times \cdots \times \mathcal{X}_n \), with \( \mathcal{X}_i = \{0, \ldots, m_i\} \), where \( n \) is the number of genes, and \( m_i \in \mathbb{N} \) is the maximum level of expression for the gene \( i \).

The dynamics of the regulatory network is given by a map

\[
\begin{align*}
  f &: \mathcal{X} \to \mathcal{X} \\
  f &: x \mapsto f(x) = (f_1(x), \ldots, f_n(x)).
\end{align*}
\]

The synchronous dynamics or synchronous state transition graph of the regulatory network is defined as the set \( \{(x, f(x)) \mid x \in \mathcal{X}, x \neq f(x)\} \). A large part of the literature on gene regulatory networks is concerned with the study of the asynchronous dynamics associated to \( f \), whereby only the level of expression of one gene is assumed to change at each iteration of the dynamics. Moreover, it is common to assume that the expression level of a gene can only undergo a unitary change at each step. In other words, the expression level of a gene \( x_i \) can only change to \( x_i + 1 \) if \( f_i(x) > x_i \), or to \( x_i - 1 \) if \( f_i(x) < x_i \).

To give the definition of asynchronous dynamics, we first define the map \( \tilde{f} : \mathcal{X} \to \mathcal{X} \) by setting

\[
\tilde{f}_i(x) = x_i + \text{sign}(f_i(x) - x_i).
\]

The map \( \tilde{f} \) is a “unitary” version of the map \( f \), that only admits changes by one for each gene expression at each step. We will say that a map \( f \) is unitary if it coincides with its unitary version \( \tilde{f} \). In addition, define for each \( i \in \{1, \ldots, n\} \) the map

\[
\begin{align*}
  F^i &: \mathcal{X} \to \mathcal{X} \\
  F^i &: x \mapsto F^i(x) = (x_1, \ldots, x_{i-1}, \tilde{f}_i(x), x_{i+1}, \ldots, x_n).
\end{align*}
\]

Then the (unitary) asynchronous state transition graph \( AD_f \) for the regulatory network is defined by the set

\[
AD_f = \{(x, F^i(x)) \mid x \in \mathcal{X}, i \in \{1, \ldots, n\}, x_i \neq f_i(x)\}.
\]
In the Boolean case, the synchronous dynamics is uniquely defined by the asynchronous version. In the multivalued case, multiple synchronous dynamics admit the same unitary asynchronous state transition graph. In this work, we will be solely concerned with the study of properties of the asynchronous dynamics of gene regulatory networks.

2.2 Regulatory graphs

We start by giving the classical definition of regulatory graph, that can be found, for example, in [8] (Definition 2.1) for the Boolean case, and in [10] (Definition 8) for the multivalued case. We denote by $e^j$ the state in $X$ with $e^j_i = 1$ and $e^j_i = 0$ for all $i \neq j$, with $j \in \{1, \ldots, n\}$. To simplify the notation, in the examples we will denote an element of the state space $x = (x_1, \ldots, x_n)$ as $x_1 \cdots x_n$.

**Definition 1.** The (local) regulatory graph at $x \in X$ for the network defined by $f$ is the finite labeled directed graph $G_f(x)$ with nodes the set $\{1, \ldots, n\}$, and an edge from $j$ to $i$, labeled with $s = s_1(\text{sign}(f_i(x + s_1 e^j) - f_i(x)))$, for $s_1 \in \{-1, 1\}$ and $x + s_1 e^j \in X$, whenever $s \neq 0$. We will say that the edge is positive if $s = 1$, and negative if $s = -1$, and we will call $s_1$ and $j$ the variation and direction of the edge, respectively.

The global regulatory graph $G_f$ of a Boolean map $f$ is the union of all the local regulatory graphs $G_f = \bigcup_{x \in X} G_f(x)$.

We consider some graphs that are subgraphs of the standard regulatory graph.

**Definition 2.** If $I \subseteq \{1, \ldots, n\}$ is a set of indices, the graph $G_f^I(x)$ is the subgraph of the graph $G_f(x)$ obtained by considering only directions $j$ in $I$.

If $A$ is a subset of the state space $X$, and $x \in A$, we write $G_f(A)(x)$ for the subgraph of the graph $G_f(x)$ obtained by considering only variations $s_1$ and directions $j$ such that $x + s_1 e^j$ is in $A$.

If $x$ and $y$ are two states, we will denote by $I(x, y)$ the set of indices $i \in \{1, \ldots, n\}$ such that $x_i \neq y_i$.

Richard and Comet [12] introduced the following definition of local regulatory graph, referring to it as the graph associated to the “non-usual” Jacobian matrix (Definition 2). It is used to prove a discrete version of Thomas’ first conjecture (see Section 2.3).

**Definition 3.** The non-usual local regulatory graph $\tilde{G}_f(x, y)$ of the map $f : X \to X$ at a state $x \in X$ with variations in direction of $y$ is a graph on $\{1, \ldots, n\}$, with an edge from a node $j$ to a node $i$ of sign $s$, with $i, j \in I(x, y)$, whenever $x + \epsilon_j e^j \in X$ and $s = \epsilon_j \text{sign}(f_i(x + \epsilon_j e^j) - f_i(x))$, with $\epsilon_k = \text{sign}(y_k - x_k)$ for all $k \in I(x, y)$, and, in addition,

$$\min \{f_i(x), f_i(x + \epsilon_j e^j)\} < x_i + \frac{\epsilon_i}{2} < \max \{f_i(x), f_i(x + \epsilon_j e^j)\}.$$

Any non-usual local regulatory graph at a state $x \in X$ is clearly a subgraph of $G_f(x)$. Moreover, non-usual local regulatory graphs are identified by the asynchronous dynamics ([12], Remark 1).

The following definition of regulatory graph was introduced in [10], where it is used to prove a multivalued version of Thomas’ second conjecture (see Section 2.3).

**Definition 4.** ([10], Definition 5) $G_f(x)$ is a graph on $\{1, \ldots, n\}$ that contains an edge from $j$ to $i$ of sign $s \in \{-1, 1\}$ if

(i) $\text{sign}(f_i(x) - x_i) \neq \text{sign}(f_i(F^j_i(x)) - F^j_i(x))$ and

(ii) $s = \text{sign}(f_j(x) - x_j)\text{sign}(f_i(F^j_i(x)) - F^j_i(x)).$
Notice that, in contrast to [10], we consider this definition only for maps $F_i$ that are unitary. The following lemma shows that the graph $G_f(x)$ of the last definition is a subgraph of the graph $G_f(x)$ (it is a variation on Lemma 6 in [10]).

**Lemma 1.** For a map $f : X \to X$ and for all $x \in X$, $G_f(x)$ is a subgraph of $G_f(x)$.

**Proof.** Let $j \to i$ be an edge of $G_f(x)$ of sign $s$. Then from point (ii) of Definition [4] we have that $f_j(x) \neq x_j$ and $f_i(F_j^i(x)) \neq F_j^i(x)$. Therefore we can write $f_j(x) - x_j = s_1 k_1$, $F_j(x) = x + s_1 e^j$ and $f_i(F_j^i(x)) - F_j^i(x) = s_2 k_2$, with $k_1, k_2 > 0$, $s_1, s_2 \in \{-1, +1\}$ and $s = s_1 s_2$.

Moreover, from (i), we find that $f_i(x) - x_i = -s_2 h_2$ for some $h_2 \geq 0$. To conclude that $G_f(x)$ admits an edge from $j$ to $i$ of sign $s$, we show that $\text{sign}(f_i(x + s_1 e^j) - f_i(x)) = s_2$.

If $i \neq j$, we have $F_j^i(x) = x_i$ and we can write

$$f_i(x + s_1 e^j) - f_i(x) = f_i(x + s_1 e^j) - F_j^i(x) + x_i - f_i(x) = s_2 (k_2 + h_2).$$

If instead $i = j$, then necessarily $s_2 = -s_1$ and $h_2 > 0$, and

$$f_i(x + s_1 e^j) - f_i(x) = f_i(x + s_1 e^j) - F_j^i(x) + x_i + s_1 - f_i(x) = s_2 (k_2 + h_2) + s_1 = s_2 (k_2 + h_2 - 1) \neq 0,$$

which concludes the proof.

**Remark 1.** The graph $G_f(x)$ contains edges of $G_f(x)$ that are calculated for directions $j$ such that $f_j(x) \neq x_j$. In other words, $G_f(x)$ is calculated by considering only the states $x \pm e^j$ such that $(x, x \pm e^j)$ is in the asynchronous state transition graph $AD_f$ of $f$.

A path in a directed graph $G$ is a sequence of nodes $(i_1, \ldots, i_k)$ such that $G$ admits an edge from $i_h$ to $i_{h+1}$ for $h = 1, \ldots, k - 1$. If $i_k = i_1$, the path is called a circuit, and if $k = 1$, it is called a loop.

The nodes $i_1, \ldots, i_k$ are distinct, the circuit is called elementary. The sign of a path is the product of the labels of its edges. A circuit in a regulatory graph $G_f$ is said to be local if it contained in a local graph $G_f(x)$ for some state $x \in X$.

We conclude this section by asking how the regulatory graph $G_f$ of a discrete multivalued map $f$ and the regulatory graph $G_f$ of its unitary version $\tilde{f}$ compare. The example in Figure 1 shows that the regulatory graph $G_f$ can contain some autoregulations that are not observed in the regulatory structure of $f$, while edges in the regulatory graph $G_f$ of $f$ are not necessarily contained in $G_f$. We can establish, however, the following relationship between the two regulatory graphs.

**Proposition 1.** Let $\tilde{f}$ be the unitary version of a map $f : X \to X$. If the regulatory graph $G_f(x)$ at some state $x$ contains an edge from $j$ to $i$, with $j \neq i$, then $G_f(x)$ contains an edge from $j$ to $i$ with the same sign.

**Proof.** If $j \to i$ is an edge in $G_f(x)$ with $j \neq i$ and sign $s$, then from $\text{sign}(\tilde{f}_i(x + s_1 e^j) - \tilde{f}_i(x)) = s \cdot s_1$ we get

$$\text{sign}(\text{sign}(f_i(x + s_1 e^j) - x_i) - \text{sign}(f_i(x) - x_i)) = s \cdot s_1 \neq 0,$$

i.e., $f_i(x + s_1 e^j)$ and $f_i(x)$ are on opposite sides of $x_i$. Writing $f_i(x + s_1 e^j) - x_i = s_2 k$, $f_i(x) - x_i = -s_2 h$, with $k, h \geq 0$, $h + k > 0$, we find that $s_2 = s \cdot s_1$ and

$$f_i(x + s_1 e^j) - f(x_i) = f_i(x + s_1 e^j) - x_i - (f_i(x) - x_i) = s \cdot s_1 (h + k),$$

which implies

$$\text{sign}(f_i(x + s_1 e^j) - f(x_i)) = s \cdot s_1,$$

i.e., $G_f(x)$ contains an edge from $j$ to $i$ of sign $s$.
Example 1. Didier et al. [4] analyse a multivalued representation of a p53/Mdm2 network. The synchronous dynamics for this network is in Figure 2a, together with its regulatory graph $G_f$. In Figure 2b, we show the unitary version $\tilde{f}$ of this map, with the resulting regulatory graph. The regulatory graph of the unitary version contains a positive autoregulation for the first component that is not part of the regulatory graph of the original map.

2.3 Rules of Thomas

Thomas suggested general rules connecting the presence of multiple steady states (first conjecture) or oscillations (second conjecture) to the existence respectively of positive or negative circuits in the regulatory graph [15].

Definition 5. A trap domain for $AD_f$ is a non-empty subset $A$ of $\mathcal{X}$ such that $x \in A$ and $(x, y) \in AD_f$ imply $y \in A$. A trap domain that does not admit any proper subset $B \subset A$ that is also a trap domain is called an attractor for $AD_f$. If $A$ is an attractor and $A = \{x^*\}$ for some $x^* \in \mathcal{X}$, then $x^*$ is called a fixed point. If the attractor has cardinality greater than one, than it is called a cyclic attractor.

Remy et al. [8] proved that, in the Boolean case, the presence of at least two fixed points requires the existence of a positive circuit in a local regulatory graph $G_f(x)$ for some state $x \in \mathcal{X}$. The proof of the first conjecture was then generalized by Richard and Comet to the multivalued discrete case [12]:

Theorem 1. ([12], Corollary 1) If $AD_f$ admits two distinct fixed points $x$ and $y$, then there exists a state $z \in \mathcal{X}$ such that $\tilde{G}_f(z, y)$ has a positive circuit.

A version of the second conjecture for the Boolean case was also proved by Remy et al. (8, Theorem 4.4), and later generalized by Richard [10]:

Theorem 2. ([10], Theorem 2) If $AD_f$ has a cyclic attractor, then $G_f = \bigcup_{x \in \mathcal{X}} G_f(x)$ admits a negative circuit.

Remark 2. Theorems 4.4 in [8] and 2 in [10] give additional information on the negative circuit associated to a cyclic attractor: if $A$ is a cyclic attractor, then a negative circuit can be found in the graph $\bigcup_{x \in A} G_f(x)$. If a region of the state space $\mathcal{A}$ is known to be a trap domain, the range of search for negative circuits that could be associated to cyclic attractors is therefore restricted to the union of the
\begin{align*}
  x \in X & \mapsto f(x) \\
  0 0 0 & \quad 0 0 0 \\
  0 0 1 & \quad 0 0 1 \\
  0 1 0 & \quad 0 1 0 \\
  0 1 1 & \quad 0 1 1 \\
  1 0 0 & \quad 1 0 0 \\
  1 0 1 & \quad 1 0 1 \\
  1 1 0 & \quad 1 1 0 \\
  1 1 1 & \quad 1 1 1 \\

  \vdots
\end{align*}

\begin{align*}
  x \in X & \mapsto \tilde{f}(x) \\
  0 0 0 & \quad 0 0 1 \\
  0 0 1 & \quad 0 0 1 \\
  0 1 0 & \quad 0 1 1 \\
  0 1 1 & \quad 0 1 1 \\
  1 0 0 & \quad 1 0 0 \\
  1 0 1 & \quad 1 1 0 \\
  1 1 0 & \quad 1 1 1 \\
  1 1 1 & \quad 1 1 1 \\

  \vdots
\end{align*}

Figure 2: (a): Synchronous discrete dynamics $f : \{0, 1, 2\} \times \{0, 1\} \times \{0, 1\} \to \{0, 1, 2\} \times \{0, 1\} \times \{0, 1\}$ and regulatory graph for the p53/Mdm2 network considered in Section 3.2 of [4]. (b) Synchronous discrete unitary version $\tilde{f}$ of $f$, with the corresponding regulatory graph.

The results on the first conjecture show that the presence of a local positive circuit is required for multistationarity, whereas for attractive cycles only the presence of negative circuits in the global regulatory graph is shown to be necessary. The question of whether the presence of a cyclic attractor in $AD_f$ also requires the presence of a local negative circuit has been often investigated [8, 10, 11, 13, 14]. Richard showed in [10] that the answer is negative in the discrete case, and found a positive answer for the case of non-expanding maps in the Boolean setting [11]. Recently Ruet [14] introduced a technique for delocalizing circuits, and used it to exhibit a counterexample for the Boolean case, for $n \geq 7$. In this work (Section 4) we present a counterexample for $n = 6$, constructed using a different method, which consists in creating a particular Boolean version of Richard’s multivalued counterexample.

3 Mapping multivalued regulatory networks to Boolean dynamics

Research efforts on discrete maps often focus on the Boolean case only, and many tools for the analysis of gene regulatory networks are developed to deal exclusively with the Boolean case. Conversions of multivalued maps to Boolean are therefore of interest. Here we consider the conversion map introduced by Van Ham [17], and shown by Didier et al. [4] to be the only map that can preserve both the regulatory structure and the dynamical properties of the system, when asynchronous updating is considered.

Consider $n$ genes with maximum expression levels $m_i$, $i \in \{1, \ldots, n\}$, with $X$ defined as in Section 2.1. Write $m = \sum_{i=1}^{n} m_i$ and consider the set $\mathcal{Y} = \{0, 1\}^m$. We define $m$ functions $\varphi_{i,j} : X \to \mathcal{Y}$ as $\varphi_{i,j}(x) = \chi_{[j,m_i]}(x_i)$, for $i = 1, \ldots, n$, $j = 1, \ldots, m_i$, where $\chi_A$ is the indicator function of the set $A$.

We denote by $\varphi$ the one-to-one map defined by Van Ham [17] and studied by Didier et al. [4] that converts a multivalued discrete state of $X$ to a Boolean state in $\mathcal{Y}$, and is defined by

$$\varphi(x_1, \ldots, x_n) = (\varphi_{1,1}(x_1), \ldots, \varphi_{1,m_1}(x_1), \varphi_{2,1}(x_2), \ldots, \varphi_{2,m_2}(x_2), \ldots, \varphi_{n,m_n}(x_n)).$$
For convenience, we will index the components of elements of $\mathcal{Y}$ with two indices, as for the components of $\varphi$; the first index corresponds to a gene, the second to the level of expression of the gene. I.e. we will denote a state $y \in \mathcal{Y}$ as

$$(y_{1,1}, \ldots, y_{1,m_1}, y_{2,1}, \ldots, y_{n,m_n}).$$

Moreover, we will use the notation $\tilde{y}_{i,j}$ to denote the state obtained from $y$ by changing the value of the component at the position identified by $i$ and $j$, and we will denote by $I_i$ the set of pairs of indices $\{(i,j)\}_{1 \leq j \leq m_i}$, for $i = 1, \ldots, n$.

The set $A = \varphi(X) \subseteq \mathcal{Y}$ is called the set of admissible states. These are the states such that, if $y_{i,j} = 1$ for some $i = 1, \ldots, n$, $j = 1, \ldots, m_i$, then $y_{i,h} = 1$ for all $h = 1, \ldots, j$. We will refer to the states in $\mathcal{Y} \setminus A$ as the non-admissible states.

Given a multivalued discrete dynamics $f$ on $\mathcal{X}$, a conversion of $f$ to a Boolean dynamics is a map $F^b : \mathcal{Y} \rightarrow \mathcal{Y}$ defined so that the following diagram is commutative:

$$
\begin{array}{ccc}
\mathcal{X} & \xrightarrow{f} & \mathcal{X} \\
\downarrow\varphi & & \downarrow\varphi \\
\mathcal{Y} & \xrightarrow{F^b} & \mathcal{Y}
\end{array}
$$

i.e. $F^b$ satisfies $F^b|_A \circ \varphi = \varphi \circ f$. We will write $F^b : A \rightarrow A$ for the map $f^b = \varphi \circ f \circ \varphi^{-1}$. In addition, if $x$ is an admissible state, we will write $G^b_f(x)$ for the graph $G^{I_A(x)}(x)$, and $G^b_f$ for the union of graphs $\bigcup_{x \in A} G^b_f(x)$.

**Lemma 2.** Consider a Boolean conversion $F^b$ of a map $f : \mathcal{X} \rightarrow \mathcal{X}$, and let $x$ be a state in $\mathcal{X}$. If a local graph $G^b_f(\varphi(x))$ contains an edge from a node in $I_j$ to a node in $I_i$, with sign $s$, with $i, j \in \{1, \ldots, n\}$, then the graph $G^b_f(x)$ contains an edge from $j$ to $i$ with sign $s$.

**Proof.** Suppose that $y = \varphi(x)$ for some $x \in \mathcal{X}$, and that $G^b_f(y)$ contains an edge from $j, k$ to $i, k'$. Recall that the graph $G^b_f(y) = G^{I_A(y)}(y)$ contains an edge of $G^b_f(y)$ with source node $(j, k)$ and variation $s_1$ only if the state $y + s_1 e^{j,k}$ is in $A$, i.e. is admissible. In this case, we have $y + s_1 e^{j,k} = \varphi(x + s_1 e^j)$.

We can write

$$f^b_{i,k'}(y + s_1 e^{j,k}) - f^b_{i,k'}(y) = \varphi_{i,k'}(f(x + s_1 e^j)) - \varphi_{i,k'}(f(x)),$$

and

$$s = s_1 \text{sign}(f^b_{i,k'}(y + s_1 e^{j,k}) - f^b_{i,k'}(y)) = s_1 \text{sign}(\varphi_{i,k'}(f(x + s_1 e^j)) - \varphi_{i,k'}(f(x))).$$

This implies $s = s_1 \text{sign}(f_i(x + s_1 e^j) - f_i(x))$ as required. \hfill $\Box$

**Lemma 3.** Consider a Boolean conversion $F^b$ of a map $f : \mathcal{X} \rightarrow \mathcal{X}$, and let $x$ be a state in $\mathcal{X}$. If the graph $G^b_f(x)$ contains an edge from $j$ to $i$ with sign $s$ and variation $s_1$ in direction $j$, then the local graph $G^b_f(\varphi(x))$ contains edges from the node $(j, x_j + \frac{s_1 + 1}{2}) \in I_j$ to nodes $(i, k') \in I_i$, for all $k' \in \{f_i(x), f_i(x + s_1 e^j)\}$, with sign $s$.

**Proof.** Suppose that $s = s_1 \text{sign}(f_i(x + s_1 e^j) - f_i(x))$ for some $s, s_1 \in \{-1, 1\}$, $i, j \in \{1, \ldots, n\}$. First observe that, if $x$ and $x + s_1 e^j$ are in $\mathcal{X}$, and $y = \varphi(x)$, then $y + s_1 e^{j,k} = \varphi(x + s_1 e^j)$, with $k = x_j + s_1 + 1$.

Take $k = x_j + \frac{s_1 + 1}{2}$, and $k' \in \{f_i(x), f_i(x + e^j e^j)\}$, $\{f_i(x), f_i(x + e^j e^j)\}$, $\max \{f_i(x), f_i(x + e^j e^j)\}$. We have

$$s_1 s = \text{sign}(f_i(x + s_1 e^j) - f_i(x)) = \text{sign}(\varphi_{i,k'}(f(x + s_1 e^j)) - \varphi_{i,k'}(f(x))) = \text{sign}(f^b_{i,k'}(y + s_1 e^{j,k}) - f^b_{i,k'}(y)),$$

as required. \hfill $\Box$
Example 2. The conversion to Boolean on the admissible states for the map in Example 1 is in Figure 3a together with the corresponding regulatory graph. In Figure 3b is the conversion of the unitary version of the map, restricted to the admissible states, with the corresponding regulatory graph.

With the following example we observe that local circuits in the regulatory graph of \( f \) are not necessarily preserved by the conversion to Boolean.

Example 3. Consider the maps \( f \) and \( F \) defined on \( \{0,1,2\} \times \{0,1\} \) and \( \{0,1\}^3 \) respectively, as in Figure 4. The map \( f \) is unitary, and \( F \) extends \( f^b \). The regulatory graph of \( f \) admits positive local circuits, whereas the regulatory graph \( G_F \) of the Boolean version admits no circuits.

A positive result on the preservation of circuits in the regulatory structure holds when the non-usual regulatory graphs of Definition 3 are considered. It is a consequence of the following lemma.

Lemma 4. Consider a Boolean conversion \( b^b \) of a map \( f : \mathcal{X} \rightarrow \mathcal{X} \), and let \( x \) be a state in \( \mathcal{X} \). If the non-usual local regulatory graph \( G_f(x,y) \) at \( x \) with variations in the direction of \( y \) contains an edge from \( j \) to \( i \) of sign \( s \), and \( \epsilon_k = \text{sign}(y_k - x_k) \) for \( k \in I(x,y) \), then the local graph \( G_f^b(\varphi(x),\varphi(y)) (\varphi(x)) \) contains an edge from \((j, x_j + \epsilon_j + \frac{1}{2})\) to \((i, x_i + \epsilon_i + \frac{1}{2})\), with sign \( s \).

Proof. If the non-usual local regulatory graph \( G_f(x,y) \) at \( x \) contains an edge from \( j \) to \( i \), we have, by definition of non-usual local regulatory graph,

\[
\min \{ f_i(x), f_i(x + \epsilon_j e^j) \} < x_i + \frac{\epsilon_j}{2} < \max \{ f_i(x), f_i(x + \epsilon_j e^j) \},
\]

which gives

\[
\min \{ f_i(x), f_i(x + \epsilon_j e^j) \} < x_i + \epsilon_j + \frac{1}{2} \leq \max \{ f_i(x), f_i(x + \epsilon_j e^j) \}.
\]

The conclusion follows from Lemma 3.

Proposition 2. If the non-usual local regulatory graph \( \tilde{G}_f(x,y) \) of the map \( f : \mathcal{X} \rightarrow \mathcal{X} \) at a state \( x \in \mathcal{X} \) with variations in direction of \( y \) admits a circuit of sign \( s \), then the local graph \( \tilde{G}_f^b(\varphi(x),\varphi(y)) (\varphi(x)) \) admits a circuit of sign \( s \).
sign( the purpose of exploiting the numerous results and tools available for Boolean systems. The description of functions that are defined on all states in Software applications for the analysis of Boolean regulatory networks are developed to work with functions Let Proof. admit any positive circuit. positive circuit (Example 3). (b): The regulatory graph of a conversion $F$ of $f$ to a Boolean dynamics does not admit any positive circuit.

Figure 4: (a): Multivalued map $f: \{0,1,2\} \times \{0,1\} \rightarrow \{0,1,2\} \times \{0,1\}$ with regulatory graph admitting a local positive circuit (Example 3). (b): The regulatory graph of a conversion $F$ of $f$ to a Boolean dynamics does not admit any positive circuit.

Proof. Let $(i_1, \ldots, i_{k-1}, i_k = i_1)$ be a circuit in $\tilde{G}_f(x, y)$, with edge signs $s_1, \ldots, s_{k-1}$, and take $\epsilon_k = \text{sign}(y_k - x_k)$, $k \in I(x, y)$. By Lemma 4, the graph $G_{Ib}^{f}(\varphi(x), \varphi(y))(\varphi(x))$ contains an edge from $(i_h, x_h + \frac{\epsilon_{h+1} + 1}{2})$ to $(i_{h+1}, x_{h+1} + \frac{\epsilon_{h+1} + 1}{2})$ with sign $s_h$, for all $h = 1, \ldots, k - 1$, which concludes the proof.

A conversion of a multivalued regulatory network to a Boolean network could be considered with the purpose of exploiting the numerous results and tools available for Boolean systems. The description above only identifies, however, the behaviour of a Boolean conversion on a subset $\mathcal{A}$ of $\mathcal{Y} = \{0,1\}^m$. Software applications for the analysis of Boolean regulatory networks are developed to work with functions that are defined on all states in $\{0,1\}^m$. GlNsim [2], for example, when provided with a partial truth table, extends the map on the remaining states by sending them to the state $(0, \ldots, 0)$.

It is natural therefore to investigate the properties that different conversion maps $F^b$ can have. Any such extension of the map $f^b$ to the non-admissible states should, ideally, preserve the dynamical properties of the multivalued counterpart $f$. In particular, it is desirable for the set of admissible states to be a trap domain for the dynamics. We can see from Example 2 that simply extending the map $f^b$ to non-admissible states will cause the dynamics to leave the admissible states: for example, the transition $(0000, 0100)$ would be contained in the asynchronous state transition graph. However, this problem is avoided when considering the extension of the conversion of the unitary version of $f$ instead, as shown in the following proposition.

**Proposition 3.** Let $f: \mathcal{X} \rightarrow \mathcal{X}$ define a multivalued regulatory network, with unitary map $\tilde{f}$. Let $F^b: \mathcal{Y} \rightarrow \mathcal{Y}$ be a conversion of $\tilde{f}$ to Boolean. Then the set of admissible states $\mathcal{A}$ is a trap domain for $AD_{F^b}$.

Proof. Let $a$ be an admissible state of $\mathcal{Y}$. Then $a = \varphi(x)$ for some $x \in \mathcal{X}$. Suppose that, for some indices $i, j$, with $i \in \{1, \ldots, n\}$ and $j \in \{1, \ldots, m_i\}$, we have $F_{i,j}^b(a) \neq a_{i,j}$, or, in other words, that $(a, a_{i,j})$ is in $AD_{F^b}$. We want to prove that $a_{i,j}$ is admissible. We will show that $\tilde{a}_{i,j} = \varphi(x + \varepsilon e^j)$, with $\varepsilon = \tilde{f}_i(x) - x_i$.

First observe that, since $F_{i,j}^b(a) \neq a_{i,j}$, we have $\tilde{a}_{i,j} = F_{i,j}^b(a) = \tilde{f}_{i,j}(a) = \varphi_{i,j}(\tilde{f}(x)) = \varphi_{i,j}(x + \text{sign}(f(x) - x)) = \varphi_{i,j}(x + \varepsilon e^j)$.

To conclude, we need to show that $\tilde{a}_{k,h} = \varphi_{k,h}(x + \varepsilon e^j)$ for $(k, h) \neq (i, j)$. This is straightforward for $k \neq i$. For $k = i$ and $h \neq j$, we first consider the case where $a_{i,j} = 0$ and $F_{i,j}^b(a) = 1$. These
conditions imply that \( x_i < j \) and \( x_i + \text{sign}(f_i(x) - x_i) \geq j \), which imply \( x_i = j - 1 \). Therefore we can write
\[
a_{i,h} = \chi_{j-1 \geq h} = \chi_{j \geq h+1} = \chi_{j \geq h} = \varphi_{i,h}(j) = \varphi_{i,h}(x_i + \text{sign}(f_i(x) - x_i)) = \varphi_{i,h}(x + e e^i).
\]

If instead \( a_{i,j} = 1 \) and \( F^b_{i,j}(a) = 0 \), we have that \( x_i \geq j \) and \( x_i + \text{sign}(f_i(x) - x_i) < j \), which imply \( x_i = j \). Therefore
\[
a_{i,h} = \chi_{j \geq h} = \chi_{j \geq h+1} = \chi_{j-1 \geq h} = \varphi_{i,h}(j - 1) = \varphi_{i,h}(x_i + \text{sign}(f_i(x) - x_i)) = \varphi_{i,h}(x + e e^i).
\]

\[
\square
\]

**Proposition 4.** Let \( f : \mathcal{X} \rightarrow \mathcal{X} \) define a multivalued regulatory network, with unitary map \( \tilde{f} \). Let \( F^b : \mathcal{Y} \rightarrow \mathcal{Y} \) be a conversion of \( \tilde{f} \) to Boolean. If \( F^b(x) \in \mathcal{A} \) for all \( x \in \mathcal{X} \setminus \mathcal{A} \), then all the attractors for the asynchronous state transition graph \( AD_{F^b} \) are contained in the set of admissible states \( \mathcal{A} \).

**Proof.** Using the result of Proposition 3, it is sufficient to observe that, if \( x \in \mathcal{X} \setminus \mathcal{A} \) and \( F^b(x) \in \mathcal{A} \), then there exist \( k \) states \( x = x_1, \ldots, x_k = F^b(x) \in \mathcal{Y} \) such that \( (x_i, x_{i+1}) \in AD_{F^b} \) for \( i = 1, \ldots, k - 1 \).

For brevity, in the remainder of the work we will say that a map \( F^b \) is a compatible conversion of a map \( f : \mathcal{X} \rightarrow \mathcal{X} \) to a Boolean dynamics, if it is a conversion of the unitary version of \( f \), and its image is contained in the set of admissible states \( \mathcal{A} \).

### 3.1 Exclusion of multiple steady states

We have seen in Example 3 that local circuits in the regulatory graph \( G_f \) of \( f \) are not necessarily preserved by the conversion to Boolean. Moreover, in general, the regulatory graph of an extension \( F^b \) of \( f^b \) can contain a local circuit, even if the graph of \( f^b \) does not admit any.

We have, however, that circuits in the non-usual regulatory graph are preserved by the conversion (Proposition 2). We can therefore state the following result on the existence of local positive circuits in presence of multistationarity for \( F^b \).

**Theorem 3.** Consider a unitary map \( f : \mathcal{X} \rightarrow \mathcal{X} \), and \( F^b \) a compatible conversion of \( f \) to a Boolean dynamics, with \( f^b = F^b\big|_{\mathcal{A}} \). Suppose that \( x, y \in \mathcal{A} \) are two distinct fixed points for \( f^b \). Then there exists a state \( z \in \mathcal{A} \) such that \( G^{F^b}_{f^b}(z, y)(z) \) has a positive circuit.

**Proof.** It is sufficient to combine Theorem 1 and Proposition 2.

**Example 4.** To exclude the presence of multiple fixed points for the map in Example 3, it is sufficient, by Theorem 3, to consider the regulatory graph of the map \( f^b : \mathcal{A} \rightarrow \mathcal{A} \), without specifying a particular extension for \( f^b \) to the non-admissible states 010 and 011.

### 3.2 Exclusion of cyclic attractors

The analogue of the result of the previous section for the exclusion of cyclic attractors is the following: if \( f \) is a discrete map and \( F^b \) is a conversion to a Boolean dynamics of its unitary version, then to exclude the presence of a cyclic attractor in the asynchronous dynamics it is sufficient to check for absence of negative circuits in \( G^b \), where \( \mathcal{A} \) is the set of admissible states. The extension of the map \( f^b \) to the non-admissible states might add edges and circuits to the regulatory graph, but the values taken outside the admissible states are not relevant for the exclusion of cyclic attractors for \( f \).
Consider a unitary map \( f : X \rightarrow X \), and \( F^b \) a compatible conversion of \( f \) to a Boolean dynamics, with \( F^b = F^b|_A \). Suppose that \( AD_f \) admits a cyclic attractor. Then the graph \( \bigcup_{x \in A} G^{f^b}_{F^b}(x) \) admits a negative circuit.

**Proof.** Consequence of Theorem 2, Proposition 4 and Remark 2.

In the following example we show how the conversion of a multivalued map to Boolean could sometimes be used to exclude the presence of cyclic attractors, when Theorem 2 fails to be applicable in the discrete case.

**Example 5.** In Figure 5 (a) are the synchronous dynamics and the regulatory graph for a map \( f \) on \( \{0, 1, 2\} \times \{0, 1\} \). In the same figure on the right is the Boolean version \( f^b \) of the same map, with the corresponding regulatory graph. The regulatory graph of \( f \) contains a negative circuit, whereas the regulatory graph of \( f^b \) does not contain any. Moreover, one can obtain a Boolean map on \( \{0, 1\}^3 \) extending \( f^b \) and with regulatory graph containing no negative circuits by sending \( 010 \) and \( 011 \) to \( 100 \). If instead the two non-admissible states are mapped for example to \( 000 \), the resulting regulatory graph admits local negative circuits.

### 3.3 An extension to non-admissible states

As seen in example 5, different extensions for the Boolean map \( f^b \) admit different regulatory graphs. We study here some properties of a particular extension of the map \( f^b \). Consider the function \( \psi : Y \rightarrow A \subset Y \) defined as follows. For each \( i = 1, \ldots, n \) and \( j = 1, \ldots, m_i \), we set

\[
\psi_{i,j}(y_{1,1}, \ldots, y_{1,m_1}, y_{2,1}, \ldots, y_{n,m_n}) = x_{[j,m_i]}(\sum_{k=1}^{m_i} y_{i,k}).
\]

The map \( \psi \) therefore sends a state \( y \) to the admissible state \( z \) such that, for each \( i = 1, \ldots, n \), \( \sum_{j=1}^{m_i} y_{i,j} = \sum_{j=1}^{m_i} z_{i,j} \). For example, take \( n = 3, m_1 = 3, m_2 = m_3 = 2 \), and \( y = 0111001 \in \{0, 1\}^7 \). Then \( \psi(y) = 1101010 \), i.e. \( \psi(y) \) is the image under \( \varphi \) of the state 211.

Clearly \( \psi \) leaves the admissible states fixed. The next lemma states that the map \( \psi \) is neighbour-preserving in the sense of the definition introduced in [4]: two direct neighbour states in \( Y \) are mapped by \( \psi \) to two direct neighbour states in \( A \).
Lemma 5. For each $x \in \mathcal{Y}$, $i \in \{1, \ldots, n\}$ and $j \in \{1, \ldots, m_i\}$, there exists a unique $j' \in \{1, \ldots, m_i\}$ such that $\psi(x^{i,j}) = \tilde{\psi}(x)^{i,j'}$. Moreover, $x_{i,j} = 0$ if and only if $\psi_{i,j}(x) = 0$.

Proof. Consider the case $x_{i,j} = 0$. We have $\sum_{k=1}^{m_i} x_{i,k} = \sum_{k=1}^{m_i} x_{i,k} + 1$, and

$$
\psi_{i,h}(x^{i,j}) = \begin{cases} 1 & \text{if } \sum_{k=1}^{m_i} x_{i,k} \geq h, \\ 1 & \text{if } \sum_{k=1}^{m_i} x_{i,k} + 1 = h, \\ 0 & \text{otherwise}, 
\end{cases}
$$

whereas $\psi_{i',h}(x) = \psi_{i,h}(x^{i,j'})$ for all $i' \neq i$. Therefore $j' = \sum_{k=1}^{m_i} x_{i,k} + 1$ is the only index such that $\psi(x^{i,j}) = \tilde{\psi}(x)^{i,j'}$. In addition, $\psi_{i,j}(x^{i,j}) = 1$. The case $x_{i,j} = 1$ is analogous, with $j' = \sum_{k=1}^{m_i} x_{i,k}$. \hfill \square

Given a unitary map $f : \mathcal{X} \to \mathcal{X}$, consider the map on $\mathcal{Y}$ defined by

$$
F^b = f^b \circ \tilde{\psi}.
$$

Clearly $F^b$ is a compatible conversion of $f$.

Example 6. Consider the regulatory network $\tilde{f}$ of Examples 1 and 2. In Figure 6a is the conversion of $\tilde{f}$ given by $F^b = \tilde{f}^b \circ \tilde{\psi}$. The non-admissible state 0100 is mapped by $\tilde{\psi}$ to the admissible state 1000, and by $F^b$ to the image of 1000 under $\tilde{f}^b$. The regulatory graph of $F^b$ contains some edges that do not appear in $G_{\tilde{f}^b}$. Consider, for example, the state $x = 0000$, which is mapped to 1001. The neighbours of $x$ are $\tilde{x}^{1,1} = 1000$, $\tilde{x}^{1,2} = 0100$, $\tilde{x}^{2,1} = 0010$ and $\tilde{x}^{3,1} = 0001$. The states $\tilde{x}^{1,1}$, $\tilde{x}^{2,1}$ and $\tilde{x}^{3,1}$ are admissible. To find the regulatory graph $G_{\tilde{f}^b}(x)$, we compare the image of $x$ under $\tilde{f}^b$ to the image of the neighbouring admissible states:

- $x = 0000$, $\tilde{f}^b(x) = 1001$,
- $\tilde{x}^{1,1} = 1000$, $\tilde{f}^b(\tilde{x}^{1,1}) = 1100$,
- $\tilde{x}^{2,1} = 0010$, $\tilde{f}^b(\tilde{x}^{2,1}) = 1001$,
- $\tilde{x}^{3,1} = 0001$, $\tilde{f}^b(\tilde{x}^{3,1}) = 0001$.

We identify the edges $(1, 1) \rightarrow (1, 2)$, $(1, 1) \rightarrow (3, 1)$ and $(3, 1) \rightarrow (1, 1)$. The graph $G_{F^b}(x)$ contains all these edges, plus the edges found by comparing $\tilde{f}^b(x)$ to the image under $F^b$ of the non-admissible state $\tilde{x}^{1,2} = 0100$:

- $x = 0000$, $\tilde{f}^b(x) = 1001$,
- $\tilde{x}^{1,2} = 0100$, $F^b(\tilde{x}^{1,2}) = \tilde{f}^b(\tilde{x}^{1,1}) = 1100$.

We find therefore two edges with source the index $(1, 2)$, and with targets the targets of the edges in $G_{\tilde{f}^b}$ with source $(1, 1)$, i.e. the edges $(1, 2) \rightarrow (1, 2)$ and $(1, 2) \rightarrow (3, 1)$ (see Figure 6b).

To find, for instance, the graph $G_{F^b}(y)$ at the non-admissible state $y = 0100$, we need to compare $\tilde{f}^b(y)$ to the images of the (admissible and non-admissible) neighbours of $y$:

- $y = 0100$, $F^b(y) = \tilde{f}^b(1000) = 1100$,
- $\bar{y}^{1,1} = 1100$, $F^b(\bar{y}^{1,1}) = \tilde{f}^b(\bar{y}^{1,1}) = 1110$,
- $\bar{y}^{1,2} = 0000$, $F^b(\bar{y}^{1,2}) = \tilde{f}^b(\bar{y}^{1,2}) = 1001$,
- $\bar{y}^{2,1} = 0110$, $F^b(\bar{y}^{2,1}) = \tilde{f}^b(1010) = 1101$,
- $\bar{y}^{3,1} = 0101$, $F^b(\bar{y}^{3,1}) = \tilde{f}^b(1001) = 0000$.

The graph $G_{F^b}(y)$ therefore contains, for example, a positive edge from $(1, 1)$ to $(2, 1)$, that derives from the existence of an edge at $\psi(y) = 1000$ with source $(1, 2)$ and target $(2, 1)$.
Proof. Let $F^b$ be a unitary map. Suppose that the regulatory graph $G_{F^b}$ contains a (local) circuit $c$ of sign $s$. Then the regulatory graph $G_f$ of $f$ contains a (local) circuit of sign $s$.

Proof. Consider a state $x \in \mathcal{Y}$, and indices $(i,k_i) \in I_i$ and $(j,k_j) \in I_j$. Then we have

$$F^b(x_i) = f^b(\psi(x_i)),$$

for some indices $(j,k_j) \in I_j$, which concludes the proof.

The next lemma provides information about the relationship between the regulatory graph of $G_{F^b}$ and the regulatory graph of $f^b$.

**Lemma 6.** For each state $x$, the local regulatory graph $G_{F^b}(x)$ of $F^b$ at $x$ contains an edge from a node in $I_j$ to a node $(i,k)$ in $I_i$ with sign $s$, if and only if the graph $G_f(x)$ contains an edge from some node in $I_j$ to the node $(i,k)$, with sign $s$.

Proof. Consider a state $x \in \mathcal{Y}$, and indices $(i,k_i) \in I_i$ and $(j,k_j) \in I_j$. Then we have

$$F^b(x_i) = f^b(\psi(x_i)),$$

for some indices $(j,k_j) \in I_j$, which concludes the proof.

**Proposition 5.** Let $f : \mathcal{X} \to \mathcal{X}$ be a unitary map. Suppose that the regulatory graph of the Boolean map $F^b = f^b \circ \psi$ contains a (local) circuit $c$ of sign $s$. Then the regulatory graph $G_f$ of $f$ contains a (local) circuit of sign $s$.

Proof. If $c = ((i_1, h_{i_1}), \ldots, (i_k, h_{i_k}))$ is a circuit with edge signs $s_1, \ldots, s_k$ in $G_{F^b}$ (resp., in $G_{F^b}(x)$ for some state $x \in \mathcal{Y}$), then by Lemma 6 the graph $G_f$ (resp., the graph $G_f(\psi(x))$) contains edges $(i_1, h_{i_1}) \to (i_2, h_{i_2}), (i_2, h_{i_2}) \to (i_3, h_{i_3}), \ldots, (i_k, h_{i_k}) \to (i_1, h_{i_1})$ with signs $s_1, \ldots, s_k$. The conclusion follows from Lemma 2.
If a discrete unitary map $f$ has no (local) circuits of sign $s$, the map $\psi$ allows therefore to define an extension of the Boolean version of $f$ that also admits no (local) circuits of sign $s$. We use this result in the next section.

**Remark 3.** If the regulatory graph of the network $F^b = f^b \circ \psi$ contains a (local) elementary circuit of negative sign, then the regulatory graph of $f$ contains a (local) elementary circuit of negative sign. The same does not hold for positive circuits: a (local) positive elementary circuit in $G_{F^b}$ could correspond to a non-elementary (local) positive circuit in $G_f$.

### 4 Counterexample for $n = 6$

In [10], Example 6, Richard presented an example of discrete multivalued map with a unique cyclic attractor and no local negative circuits in the regulatory graph. In this section we present a Boolean version of this map, and show that the absence of local negative circuits does not imply the existence of a unique fixed point, for Boolean networks with $n \geq 6$.

In Figure 7 is the unitary version $f$ of the map introduced by Richard, together with its local regulatory graphs. The asynchronous state transition graph for the conversion $f^b$ of this map to a Boolean dynamics on the admissible states is as in Figure 9a. Since the asynchronous dynamics of $f$ admits a unique cyclic attractor, $AD_f$ also admits a unique cyclic attractor.

We define a Boolean map $F^b$ that extends $f^b$ to the non-admissible states as $F^b = f^b \circ \psi$, with $\psi$ the map defined in (1).

**Proposition 6.** For $n = 6$, the absence of local negative circuits in the regulatory graph does not imply the existence of a fixed point.

**Proof.** The image of $F^b = f^b \circ \psi$ is contained in the set of admissible states $A$; moreover, $F^b$ coincides with $f^b$ on the admissible states. In particular, $F^b$ has no fixed points. By Proposition 4, we conclude that $F^b$ has a unique cyclic attractor. Finally, by application of Proposition 5, we find that the regulatory graph of $F^b$ has no local negative circuits.

The synchronous dynamics for the map $F^b$ is given in Figure 8. The global regulatory graph for $F^b$ takes the form given in Figure 9b. We inspect two local regulatory graphs in detail, to illustrate the
Figure 8: A map $F^b : \{0, 1\}^6 \rightarrow \{0, 1\}^6$ with no fixed points and with regulatory graph admitting no local negative circuits. The rows corresponding to the admissible states are highlighted in gray.

Figure 9: (a): Asynchronous state transition graph on the admissible states for the map $F^b$ in Figure 8. (b): Global regulatory graph of $F^b$ (negative edges are dashed).
consequences of the construction. Consider the admissible state $x = 000000$. This state has only two admissible neighbours, $\bar{x}^{1,1} = 100000$ and $\bar{x}^{2,1} = 000100$. To describe the graph $G_f(x)$, we compare the images under $f^b$:

$$
\begin{align*}
x &= 000000, & f^b(x) &= 000100, \\
\bar{x}^{1,1} &= 100000, & f^b(\bar{x}^{1,1}) &= 000000, \\
\bar{x}^{2,1} &= 000100, & f^b(\bar{x}^{2,1}) &= 000110.
\end{align*}
$$

We identify two edges in $G_f(x)$, $(1, 1) \xrightarrow{+} (2, 1)$ and $(2, 1) \xrightarrow{+} (2, 2)$. Now consider the extension $F^b$ of $f^b$, and compare the image of $x$ to the images of its non-admissible neighbours:

$$
\begin{align*}
x &= 000000, & f^b(x) &= 000100, \\
\bar{x}^{1,2} &= 010000, & f^b(\bar{x}^{1,2}) &= f^b(\bar{x}^{1,1}) = 000000, \\
\bar{x}^{1,3} &= 001000, & f^b(\bar{x}^{1,3}) &= f^b(\bar{x}^{1,1}) = 000000, \\
\bar{x}^{2,2} &= 000010, & f^b(\bar{x}^{2,2}) &= f^b(\bar{x}^{2,1}) = 000110, \\
\bar{x}^{2,3} &= 000001, & f^b(\bar{x}^{2,3}) &= f^b(\bar{x}^{2,1}) = 000110.
\end{align*}
$$

$G_{F^b}(x)$ admits therefore additional edges with signs and targets given by the signs and targets of the edges in $G_f(x)$. The additional edges are $(1, 2) \xrightarrow{+} (2, 1)$, $(1, 3) \xrightarrow{+} (2, 1)$, $(2, 2) \xrightarrow{+} (2, 2)$ and $(2, 3) \xrightarrow{+} (2, 2)$. In particular, this local regulatory graph contains a positive loop at $(2, 2)$ (the graph $G_f(00)$ also contains a positive loop at node 2).

Now consider, for illustration purposes, the non-admissible state $y = 010000$. To derive the graph $G_{F^b}(y)$, we compare the images under $F^b$ of $y$ and its neighbouring states:

$$
\begin{align*}
y &= 010000, & F^b(y) &= f^b(100000) = 000000, \\
\bar{y}^{1,1} &= 110000, & F^b(\bar{y}^{1,1}) &= f^b(110000) = 100000, \\
\bar{y}^{1,2} &= 000000, & F^b(\bar{y}^{1,2}) &= f^b(000000) = 000100, \\
\bar{y}^{1,3} &= 011000, & F^b(\bar{y}^{1,3}) &= f^b(110000) = 100000, \\
\bar{y}^{2,1} &= 010100, & F^b(\bar{y}^{2,1}) &= f^b(100100) = 000000, \\
\bar{y}^{2,2} &= 010010, & F^b(\bar{y}^{2,2}) &= f^b(100100) = 000000, \\
\bar{y}^{2,3} &= 010001, & F^b(\bar{y}^{2,3}) &= f^b(100100) = 000000.
\end{align*}
$$

We find that we need to compare the images under $F^b$ of the admissible state 100000 to the images under $F^b$ of the three admissible states 110000, 000000 and 100100. We identify three regulatory edges: $(1, 1) \xrightarrow{+} (1, 1), (1, 2) \xrightarrow{+} (2, 1)$ and $(1, 3) \xrightarrow{+} (1, 1)$.

**Corollary 1.** For $n \geq 6$, the absence of local negative circuits in the regulatory graph does not imply the existence of a fixed point.

**Proof.** For each $n \geq 6$, we define a map $F^n$ with no fixed points and no local negative circuits. Set $F^n_i = F^b_i$ for $i = 1, \ldots, 6$, and $F^n_i = x_i$ for $i > 6$. Then, for each $x \in \{0, 1\}^7$, the regulatory graph $G_{F^n}(x)$ is given by the regulatory graph of $F^b$ at $(x_1, \ldots, x_6)$, with the addition of a positive loop for each node $i$ with $i > 6$, and does not admit any negative circuit. \qed
5 Conclusion and discussion

Discrete asynchronous maps are studied to model gene regulatory networks, with many efforts focusing on establishing connections between the asymptotic behaviour of the dynamics and the presence of circuits in the regulatory structure. To this end, different notions of circuit functionality have been considered in recent years [7, 5, 6, 3]. Informally, a circuit is considered to be functional if it is responsible for the presence of multiple steady states (for a positive circuit) or cyclic attractors (in the case of a negative circuit). For instance, type-1 functionality, introduced in [3] requires all edges in the circuit to be included in at least one local graph.

In this work, we considered a map, previously studied in [17, 4], that associates to a discrete multivalued network a partial Boolean map. We asked whether this conversion can provide information on the asymptotic behaviour of the asynchronous dynamics of the network, in particular by application of some versions of Thomas' first and second conjectures. To answer this question, we considered the problem of extending the map to the Boolean “non-admissible” states, i.e. states that do not have a discrete counterpart. We clarified that the asymptotic behaviour of the asynchronous dynamics is preserved when the unitary version of the original network is converted, and extended so that the non-admissible states are mapped to the admissible. Since the steady states and cyclic attractors are preserved in the conversion, circuits found in the regulatory structure of a multivalued map but not in the regulatory structure of its Boolean representation can be considered as non-functional.

Using previous results for the Boolean and multivalued case concerning special subgraphs of the regulatory graph, we clarified how the absence of positive local circuits or negative global circuits can be used to exclude, respectively, the existence of multiple steady states or of cyclic attractors, independently of the particular extension. We then contributed a technique for extending the Boolean version, that guarantees that no new circuits are added to the regulatory structure. We used this technique to show the existence of a Boolean map, for $n = 6$, with a unique cyclic attractor, no fixed points, and with corresponding regulatory graph admitting no local negative circuits. A negative answer to Question 1 indicates that the definition of type-1 functionality suggested in [3] is not suitable for circuits of negative sign, and more general approaches should be investigated. The conversion map described in this work could be considered further to study characterizations of circuit functionality in the multivalued case via analysis of the Boolean counterpart.

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