University of Nottingham

School of Mathematical Sciences

Graph properties of biological interaction networks

Elisa Tonello

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Abstract

This thesis considers two modelling frameworks for interaction networks in biology. The first models the interacting species qualitatively as discrete variables, with the regulatory graphs expressing their mutual influence. Circuits in the regulatory structure are known to be indicative of some asymptotic behaviours. We investigate the relationship between local negative circuits and sustained oscillations, presenting new examples of Boolean networks without local negative circuits and admitting a cyclic attractor. We then show how regulatory properties of Boolean networks can be investigated via satisfiability problems, and use the technique to examine the role of local negative circuits in networks of small dimension.

To enable the application of Boolean techniques to the study of multivalued networks, a mapping of discrete networks to Boolean can be considered. The Boolean version, however, is defined only on a subset of the Boolean states. We propose a method for extending the Boolean version that preserves both the attractors and the regulatory structure of the network.

Chemical reaction network theory models the dynamics of species concentrations via systems of ordinary differential equations, establishing connections between the network structure and the dynamics. Some results assume mass action kinetics, whereas biochemical models often adopt other rate forms. We propose algorithms for elimination of intermediate species, that can be used to find whether a mass action network simplifies to a given chemical system.

We then consider the problem of identification of generalised mass action networks that give rise to a given mass action dynamics, while displaying useful structural properties, such as weak reversibility. In particular, we investigate systems obtained by preserving the reaction vectors of the mass action network, and outline a new algorithmic approach.

Acknowledgements

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CONTENTS

1

INTRODUCTION

The understanding of biological interaction networks poses a notoriously difficult challenge. Biological systems display an extensive variety of sophisticated regulatory mechanisms arising from the interplay of a vast number of molecular species. The study and prediction of their dynamical behaviour is aided by the continuous advancements in experimental methods and the resulting considerable availability of data. Nevertheless, for many systems a comprehensive quantitative understanding is still out of reach. Mathematical models that integrate the available qualitative information provide therefore valuable languages and frameworks for the investigation of these systems. Complex networks in systems biology are studied via a variety of mathematical and computational approaches [KS08, MCR$^+$11]. Perhaps at two extremes are the modelling frameworks considered in this thesis.

Boolean networks, first introduced by Glass and Kauffman [GK73] and Thomas [Tho73, Td90, Tho91], and their generalisation to discrete multilevel maps offer a genuinely qualitative approach to the investigation of the structure and dynamical behaviour of regulatory networks. At the core of the formalism is the definition of regulatory graph, which records the effects that the changes in some species concentrations have on the state of the system. The species or genes activate or inhibit other species or themselves, i.e. act as positive or negative regulators. The different regulatory configurations result in an explosion of possible dynamical behaviours.

The conjectures of Thomas and Kauffmann [Tho81, Td90, KST07] theorised connections between feedbacks or circuits in the regulatory structure and the dynamical fate of the system. The role of regulatory circuits has since been extensively investigated in different modelling frameworks (see for instance [Sno98, Sou04, KST07, RRT08, Ric08, Ric10, Rue16]). For Boolean networks, positive circuits (i.e., regulatory circuits containing an even number of negative regulations) have been found as necessary for the existence of multiple steady states, whereas negative circuits play a similar role for the permanence of oscillatory behaviour. Part of this thesis is dedicated to investigating whether *local* negative circuits are necessary for oscillatory behaviour. We identify counterexamples for $n \geq 6$, and we study the problem for $n \leq 5$ using Boolean satisfiability problems, showing that the smallest counterexample is indeed found for $n = 6$.

Multilevel discrete networks have the capacity to capture system behaviours in a more accurate fashion, but are less studied. Both the dynamics and the regulatory structure of multilevel maps can be faithfully represented using Boolean variables [DRC11], with the drawback that the Boolean dynamics is not defined on a number of Boolean states, called "non-admissible", that do not have a multilevel counterpart. Another problem examined in this work is that of extending Boolean versions of multilevel maps to the non-admissible states in a way that preserves both the asymptotic dynamical behaviour and the regulatory circuits.

Chemical reaction network theory, the second modelling approach considered in this thesis, models the evolution of the concentrations of chemical species using systems

of ordinary differential equations. The theory explores for instance the questions of existence and number of equilibria, stability or robustness of steady state concentrations, identifying results that rely on characteristics of the network structure and not on the precise knowledge of the parameters (e.g. [Fei79, Fei87, Fei88, Hor72]). In classical chemical reaction network theory, reaction rates are assumed to follow the law of mass action, i.e. are proportional to the product of the reactant concentrations; the theory has since expanded to consider milder assumptions on the kinetics. Recently, some results on complex balancing steady states have been extended to kinetics that allow for rates that are "generalised monomials" that do not necessarily reflect the stoichiometric coefficients of the reactants [MR12, MR14]. Some results require the network to be weakly reversible, i.e., covered by cycles, and can not always be directly applied. Networks with different structures can however give rise to the same dynamics, and one can try to extend the applicability of these results by finding networks with the same associated system of differential equations and the right structural properties. Here we investigate methods for the identification of weakly reversible generalised mass action networks admitting a given dynamics. In particular, we consider an approach that consists in replacing a reaction with another with the same reaction vector, i.e., a "translation" [Joh14, Joh15], and design an algorithm that can identify weakly reversible or deficiency zero translations of reaction networks.

Many biochemical models adopt non-mass action kinetic rates, often derived by applying some simplifications to a mass action system of differential equations. To verify the applicability of results that assume mass action kinetics, one can try to check whether some mass action networks reduce to one with a given dynamics. To make this process more systematic, we introduce some algorithms that allow to identify chemical systems with a required associated dynamics, starting from a mass action network.

Outline of the thesis

The two overview chapters introduce gene regulatory networks and chemical reaction networks informally, and give a survey of the problems and approaches considered in this thesis. After the introduction of the main definitions relating to regulatory networks, Chapter 3 presents a characterisation of Boolean networks with regulatory graphs given by a union of disjoint circuits, restating for a large part known results. The calculation of the number of equivalence classes under symmetry is a new contribution. Propositions 3.2.1 and 3.2.11 are included in [TFC18]. Chapter 4 discusses the conversion of multilevel networks to Boolean maps, and is an extended version of [Ton17]; I thank Claudine Chaouiya, Adrien Fauré and Shizuo Kaji for useful comments on this work. Chapter 5 investigates the relationships between local negative circuits and cyclic attractors. Some results of the chapter are included in [Ton17] and [TFC18].

Chapter 7 establishes the notations and terminology of chemical reaction network theory, including the concept of robustness. Theorems 7.5.6 and 7.5.7 are included in [TJ17]. Chapter 8 describes some approaches to the elimination of species and derivation of kinetic rates. The idea of iterative elimination described as method one and the examples on the elimination order are contributions to [MLNT16, MTLN16, MTLN17]. In Chapter 9, we discuss the definition of translation of a chemical reaction network and some connections to robustness, concluding with the main result of [TJ17]. In the last chapter we survey some methods for the identification of dynamically equivalent weakly reversible generalised mass action networks, detailing in particular the computational method included in [TJ17].

Software tools

To aid the exploration of examples and investigation of properties of networks of interest I have relied significantly on software tools. Some of the scripts used for the analysis of circuits in Boolean networks with SAT are available at github.com/etonello/regulatory-network-sat). In addition, some simple python programs for the investigation of regulatory networks can be found at my github page. The programs allow to create small networks and switch between different formats (synchronous and asynchronous, polynomial form, truth table), as well as explore different regulatory structures, and calculate fixed points and attractors.

To investigate chemical reaction networks I created a python library which is available at https://github.com/etonello/crnpy. The tool provides an easy way of defining examples, by writing reactions for instance as `A+B ->(k1) C` or `2A <-> 2B`. Models can also be read from SBML files. In addition, network models from the BioModels database [LDR$^+$10] are provided in human-readable format. The library provides simple commands for checking basic properties of reaction networks, such as the deficiency, linkage classes, associated matrices and equations, etc. It facilitates the definition of algorithms for manipulation of networks (e.g. elimination of species) or generation of other associated graphs (e.g influence graphs, translated reactions), for instance.

Part I

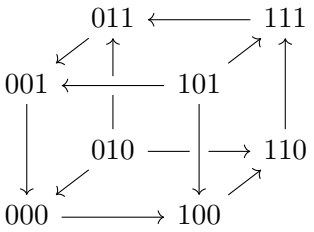
DISCRETE MODELLING OF REGULATORY NETWORKS

2

OVERVIEW OF PART I

In this part of the thesis, we consider the qualitative approach based on Boolean networks, originating from the work of Glass and Kauffman [GK73] and Thomas [Tho73, Td90, Tho91]. To define a Boolean regulatory network, the regulatory components are assumed to be either present (level 1) or absent (level 0) — in the language of genetics, a gene is either expressed or not. If we have n regulatory components, to describe a possible state of the system we use therefore an element of $\{0,1\}^n$. Then, we need a description of the dynamics that will determine which states can be reached from a given state. Usually, the *asynchronous* update scheme is assumed, meaning that only one component can change at each step of the dynamics. If we visualise the dynamics on the hypercube, this means that from one node we can only move to an adjacent node. The graph with nodes the set $\{0,1\}^n$ and edges the possible transitions between states is called the *asynchronous state transition graph* of the regulatory network. The *regulatory graph* instead is defined to capture the idea that a regulatory component or a combination of regulatory components can activate or inhibit the expression of one or multiple components.

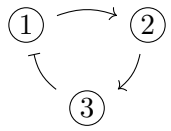
We can illustrate these concepts with an example. Consider a system with 3 regulatory components, and denote x_1, x_2 and x_3 their expression levels. Assume that the system moves from a state (x_1, x_2, x_3) towards the state $(1 - x_3, x_1, x_2)$ (the function $f\colon (x_1, x_2, x_3) \mapsto (1 - x_3, x_1, x_2)$ is called the *synchronous dynamics*). This means, for instance, that the system can transition from the state 000 to the state 100 (we drop the parentheses and commas to simplify the notation), and from the state 101 towards 010 — meaning, to any of the states 001, 110, and 100. The state transition graph for this network can be visualised as follows:



(1)

To describe the regulatory graph associated to this Boolean system, we need to ask, for each pair of regulatory components i and j, does component i change when j changes? For instance, say we start from the state $p = 000$, and we want to ascertain the effect that a variation in the first component has on the second component. We identify the neighbour of p in the direction of the first component, i.e. the state $p_1 = 100$, and compare the target states for p and p_1: since p is sent to 100 and p_1 is sent to 110, the second component shows an increase when the first increases, meaning that the first component activates the second. We denote this with an arrow $1 \to 2$. Using the same states, we see that the first component has no effect on the third. We can repeat the same procedure for the other directions: $p_2 = 010$, neighbour of p in the second direction,

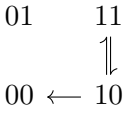
is mapped to 101, meaning that we can draw an edge $2 \to 3$. When we consider the third neighbour $p_3 = 001$ of p we find that the target value for the first component, which is 0 for p, is 1 for p_3. In this case, a positive change in x_3 gives a negative change in x_1. We deem this an inhibitory effect, which we denote $3 \dashv 1$. The regulatory graph that we just described is a circuit:



(2)

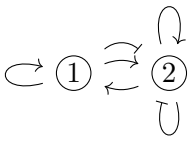
This circuit has *negative sign*, since it combines two positive effects and one negative effect. Perhaps not surprisingly, we could have derived these effects just by taking the derivatives of each component of f with respect to the three variables. In fact, the regulatory graph can be seen as the adjacency matrix of the *discrete Jacobian* of the Boolean network.

Notice that we could have found the same graph by choosing any of the states as our starting point, but this is not the case in general. Consider the following smaller example with 2 variables, defined by the map $(x_1, x_2) \mapsto (x_1x_2, x_1 + x_2 - 2x_1x_2)$. The asynchronous state transition graph is the following:



(3)

We can apply the procedure described above using the state $p = 00$, and detect the regulations $1 \to 2$ and $2 \to 2$. If we calculate the regulations from the state $p_1 = 10$ instead, by changing the first component, we detect the regulation $1 \to 1$, and by changing the second component, two regulations $2 \to 1$ and $2 \dashv 1$. It is necessary therefore to speak of *local regulatory graphs*, indexed on the elements of $\{0,1\}^n$, which are the regulatory graphs with nodes $\{1, \ldots, n\}$ and with edges the regulations that can be detected at a given state with the procedure above. The *global regulatory graph* is the regulatory graph that contains all the regulations that can be detected somewhere in the state space; unlike the local regulatory graph, the global regulatory graph is not necessarily simple, i.e., it can contain parallel edges. For instance, the global regulatory graph corresponding to the state transition graph in (3) is

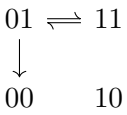


(4)

One of the objectives of the theory of Boolean networks is that of establishing properties of the dynamical behaviour starting from the regulatory graph. In particular, one is interested in characterising the *attractors* of the state transition graphs, i.e. the minimal regions of the state space that the dynamics can eventually enter, and never leave. For the dynamics represented in (3), there are two attractors, consisting of the two *fixed points* 00 and 01. For the state transition graph in (1), we can detect a *cyclic attractor*: $000 \to 100 \to 110 \to 111 \to 011 \to 001 \to 000$. This is a special type of cyclic attractor, consisting of a single cycle.

To have an idea of the size of the problem at hand, we can calculate the number of possible regulatory networks on n regulatory components. The number grows very quickly with n — there are $(2^n)^{2^n}$ maps from $\{0,1\}^n$ to itself. That is, for instance, for

$n = 3$ we can count more than 16.7 million maps. However, many of them represent the same type of behaviour. For instance, compare the state transition graph



to the one in (3): clearly, we can find one graph from the other just by transposing the two components x_1 and x_2. Similarly, we could swap the roles of the values 0 and 1, and obtain a transition graph with exactly the same structure. In Chapter 3, after introducing the terminology of Boolean networks, we investigate the problem of determining how many different state transition graphs exist once the symmetries just described have been taken into account. The number still grows very quickly, but it is smaller by some orders of magnitude. For instance, for $n = 3$ we count just over 352 thousand maps. We also investigate the effect of the symmetries on the regulatory graphs. We find that maps that are equivalent under symmetry have regulatory graphs with the same structure, but the sign of the interactions can change. Interestingly, however, the signs of the circuits are unchanged.

The circuits of the regulatory graph and their relationship with the dynamics of the asynchronous state transition graph have long been under investigation. The dynamics of Boolean networks with regulatory graph consisting exactly of a single, isolated circuit, such as the one in (2), can be completely characterised [RMCT03]: if the circuit is negative, the dynamics admits a unique cyclic attractor, as we noticed for the graph in (1); if the circuit is positive, there are exactly two fixed points. R. Thomas suggested the following two conjectures, which have inspired research in both the discrete and differential frameworks:

- A positive circuit in the regulatory graph is a necessary condition for multistability.
- A negative circuit in the regulatory graph is a necessary condition for the existence of a cyclic attractor.

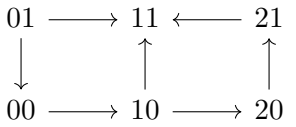
For the Boolean setting, both conjectures have been proved, in different incarnations [RRT08, Ric08, Ric10, Rue16]. For proofs in the differential case, see for example [PMO95, Gou98, Sno98, Sou04, KST07, AHS09]. The end of Chapter 3 presents an overview of the results on Boolean networks related to these two conjectures. A main asymmetry between the two results is the following: the circuit identified by the proofs of the first conjecture is *local*, meaning, it is contained in a local regulatory graph; in presence of an attractive cycle, however, the proof of the second conjectures can only establish the existence of a negative circuit in the *global* regulatory graph. A question that motivated some of the work in this thesis is the following [RRT08, Ric10, Ric11, RR13]:

Question 1. Does the presence of a cyclic attractor imply the existence of a local negative circuit in the regulatory graph?

Before considering Question 1, we devote some attention to discrete multilevel regulatory networks, a generalisation of Boolean networks that allow for more than two expression levels. Multilevel networks provide a step towards a more faithful account of the dynamical intricacies of biological interaction systems. The synchronous dynamics in the discrete multilevel case is a map from a product of n intervals of integers $\{0, \dots, m_1\} \times \cdots \times \{0, \dots, m_n\}$ to itself. We are still interested, however, in asynchronous state transition graphs that only allow a unitary change for each component, meaning that the system can not transition for instance from the state $(0, 0)$ directly to

the state $(2,0)$, but can only move towards the target state. If the synchronous dynamics maps $(0,0)$ to $(2,0)$, the asynchronous state transition graph contains therefore the transition $(0,0) \to (1,0)$.

The local and global regulatory graphs are defined as for the Boolean case, but this time we might have two directions to check for a given component. For example, let us consider a network consisting of two regulatory components, the first one admitting three possible expression levels, and the second only two. We have therefore a map on $\{0,1,2\} \times \{0,1\}$, which we define as $f_1(x_1,x_2) = 2 - x_2$, $f_2(x_1,x_2) = 0$ if $x_1 = 0$, and 1 otherwise. The associated asynchronous state transition graph and global regulatory graph are as follows:

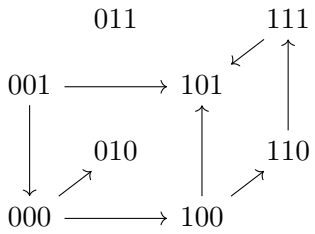


(5)

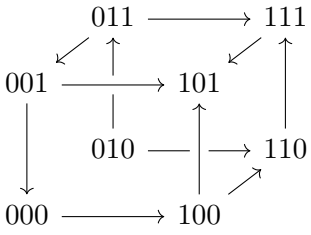
To find the regulations, consider for example the state $(1,1)$. The first component can change either to 0 or to 1. If we change it to 1, we can detect the regulation $1 \to 2$, whereas no change in the target values takes place if we change it to 2.

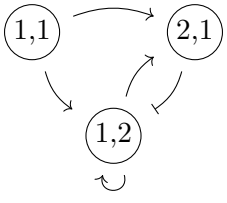
Given the larger availability of tools for the Boolean case, it is natural to consider a mapping of multivalued networks into Boolean ones. Indeed, any multivalued network can be converted to a Boolean network on a larger number of variables, without losing any dynamical or regulatory property [DRC11]. The number of Boolean variables required is equal to $m = \sum_{i=1}^{n} m_i$. For example, consider the map defined above, which is defined on 6 states. To embed these states into a Boolean space, we need to consider 3 dimensions. For instance, we can send the states $(0,a)$ to $(0,0,a)$, $(1,a)$ to $(1,0,a)$, and $(2,a)$ to $(1,1,a)$, for $a \in \{0,1\}$, obtaining the synchronous map and asynchronous graph

$$
\begin{aligned}
000 &\mapsto 110\\
001 &\mapsto 100\\
100 &\mapsto 111\\
101 &\mapsto 101\\
110 &\mapsto 111\\
111 &\mapsto 101
\end{aligned}
$$



This is, in substance, the method described in [DRC11] to define a Boolean version of a multilevel network. This does not fully determine a Boolean network, however, as it is only defined on a subset of the Boolean states, called the *admissible* states. In addition, the asynchronous transitions can leave the admissible states, hence the definition of the dynamics outside the admissible region can affect the asymptotic behaviour. In Chapter 4, we consider the problem of defining a Boolean version on the entire space $\{0,1\}^m$, while still faithfully reproducing the dynamics of the multilevel map. We will propose a procedure to obtain a Boolean version with asynchronous dynamics admitting the same attractors as the multilevel map, and the same local regulatory circuits (except for loops, that might be added or removed). For example, for the map in (5), we will identify the following Boolean version and global regulatory graph:





where we used $(1,1)$ and $(1,2)$ to denote the two Boolean variables corresponding to the first multilevel component, and $(2,1)$ to denote the Boolean variable for the second component. Other conversion methods have been proposed recently, in particular, a related approach appeared in [FK18]; we will give a brief account and comparison.

Chapter 5 is devoted to answering Question 1. For the multilevel case, Richard [Ric10] established that, although a global negative circuit is necessary for the presence of a cyclic attractor, the existence of a local negative circuit is not required. The first counterexamples for the Boolean case were recently identified by Ruet [Rue17]. Using the mapping from multilevel to Boolean dynamics described in Chapter 4, we identify a counterexample for the Boolean case for $n = 6$, i.e., a map with a cyclic attractor and no local negative circuits (an alternative conversion of the same example was introduced by Fauré and Kaji [FK18]). We also present a map with an attractive cycle and no local negative circuits, derived with a variation on Ruet's construction in [Rue17].

We then ask, is 6 the minimum dimension such that a map with a cyclic attractor and no local regulatory circuit exists? For $n = 2$, it is easy to see by hand that any map with a cyclic attractor must have a local negative circuit. The case $n = 3$ is already difficult to cover by hand. Since the argument for $n = 2$ seems difficult to generalise, we investigate a different approach. Given that the functions under consideration take Boolean values, we consider whether it is possible to express some properties as Boolean formulas, using `True` to indicate 1 and `False` to indicate 0. If this is the case, we can use a satisfiability solver (SAT solver) to check if a map with some given properties exists. Since a Boolean map in dimension n is identified by the values of the n coordinates on the 2^n states, we can create Boolean formulas with $2^n n$ variables. For example, suppose we want to check with a satisfiability solver if there are any 2-dimensional Boolean maps with no fixed point. We want to identify a Boolean map on $\{0,1\}^2$, hence we consider the 8 variables

$$f_1(00), f_2(00), f_1(01), f_2(01), f_1(10), f_2(10), f_1(11), f_2(11). \tag{6}$$

To ask that the state 00 is not a fixed point, we need either $f_1(00)$ to be 1, or $f_2(00)$ to be 1, hence, we write the Boolean formula $f_1(00) \vee f_2(00)$. For the state 01 we need to require $f_1(01) \vee \neg f_2(01)$, and so forth. The formula that encodes the absence of fixed points is therefore

$$(f_1(00) \vee f_2(00)) \wedge (f_1(01) \vee \neg f_2(01)) \wedge (\neg f_1(10) \vee f_2(10)) \wedge (\neg f_1(11) \vee \neg f_2(11)). \tag{7}$$

Then, a satisfiability solver can provide us with an assignment for the variables in (6) that is such that the formula in (7) evaluates to `True` (for instance, $f_1(00) = 1$, $f_2(00) = f_i(x) = 0$ for $i = 1, 2$, and $x \neq 00$). The idea of studying properties of Boolean networks using Boolean satisfiability problems is another contribution of this thesis. We show how we can translate dynamical or regulatory properties of Boolean networks into Boolean expressions. In particular, we describe how to encode the absence of local negative circuits, using, for small values of n, a manageable amount of constraints. As seen above, imposing the absence of fixed points is quite easy; therefore we can ask a satisfiability solver to answer the following question: does the absence of fixed points imply the existence of a local circuit in the regulatory graph? The solvers quickly find counterexamples for $n = 6$, and determine the unsatisfiability for $n \leq 5$. We then proceed to answer Question 1 in full, by implementing a necessary condition for the existence of a cyclic attractor. This condition is more complex and requires some work to reduce the search space. For the case $n = 5$, we write a formula in conjunctive normal form (a conjunction of disjunction clauses) with 2.6M `or` clauses, and a SAT solver finds it unsatisfiable. The matter of finding a human-readable proof remains open. The necessary condition implemented in the cnf formula might provide some useful clues in this direction.

3

BOOLEAN NETWORKS

In this chapter we introduce some definitions used in the logical modelling of gene regulatory networks. These can be found with slightly different terminologies for instance in [RMCT03, RRT08, RR08, Rue16]. We then study how the asynchronous dynamics and regulatory graphs of Boolean networks change under permutations of the coordinates or of the variable levels, i.e., under symmetries of the hypercube graph (Section 3.2). We find that the asynchronous dynamics, the regulatory structure, and the signs of the regulatory circuits are preserved. In addition, using a characterisation of the dynamics of Boolean networks with regulatory graph given by a circuit [RMCT03], we discuss the problem of determining how many classes of equivalent Boolean networks exist when the symmetries are taken into account. We conclude the chapter with a summary of known results connecting circuits and asymptotic behaviour of regulatory networks (Section 3.3).

Notations introduced in this chapter

The set $\{0,1\}$ will be denoted $\mathbb{B}$. We write $\mathbf{0}$ and $\mathbf{1}$ for the points of $\mathbb{B}^n$ with all components equal to 0 or 1, respectively. Given an element x of $\mathbb{B}^n$ and a set of indices $J \subseteq \{1,\dots,n\}$, we denote by $\bar{x}^J$ the element of $\mathbb{B}^n$ that satisfies $\bar{x}^J_i = 1 - x_i$ for $i \in J$, and $\bar{x}^J_i = x_i$ for $i \notin J$. We will write $\bar{x}$ for $\bar{x}^J$ with $J = \{1,\dots,n\}$, and for $i \in \{1,\dots,n\}$, we will write $\bar{x}^i$ for $\bar{x}^{\{i\}}$. χ_J will denote the indicator function of the set J, and $\mathcal{P}(J)$ the power set of J.

Given two points $x, y \in \mathbb{B}^n$, $d(x,y)$ will denote the Hamming distance. In addition, define $I(x,y) = \{i \in \{1,\dots,n\} \mid y_i \neq x_i\}$. If $x \neq y$, the set $[x,y] = \{z \in \mathbb{B}^n \mid z_i = x_i \text{ for } i \notin I(x,y)\}$ is called a *subcube* of $\mathbb{B}^n$. Given a subcube $\kappa = [x,y]$ we write $\pi_\kappa \colon \mathbb{B}^n \to \kappa$ for the projection on the subcube, i.e. the map defined by $(\pi_\kappa(z))_i = z_i$ for $i \in I(x,y)$ and $(\pi_\kappa(z))_i = x_i$ for $i \notin I(x,y)$, for $z \in \mathbb{B}^n$ and $i \in \{1,\dots,n\}$.

We call n-dimensional *hypercube graph* the directed graph on $\mathbb{B}^n$ with an edge from $x \in \mathbb{B}^n$ to $y \in \mathbb{B}^n$ whenever $d(x,y) = 1$. An edge with source x and target y in the hypercube graph will be denoted (x,y). Given $I \subseteq \{1,\dots,n\}$, we write ψ_I for the map defined by $\psi_I(x) = \bar{x}^I$ for all $x \in \mathbb{B}^n$. S_n denotes the permutations of a set of n elements. Given $\sigma \in S_n$ and $I \subseteq \{1,\dots,n\}$, we write $\sigma(I)$ for $\{\sigma(i) | i \in I\}$; σ will also denote the corresponding transformation on $\mathbb{B}^n$ (i.e., $\sigma(x)_i = x_{\sigma^{-1}(i)}$, for $x \in \mathbb{B}^n$). $|I|$ denotes the cardinality of the set I.

We write $\mathcal{F}_n$ for the set of all maps from $\mathbb{B}^n$ to itself. For each $f \in \mathcal{F}_n$ and $x \in \mathbb{B}^n$, we define $I_f(x) = I(x, f(x))$. The subscript f will be dropped when there is no ambiguity about the map f in question.

For $f \in \mathcal{F}_n$ and $\kappa \subseteq \mathbb{B}^n$ a subcube of $\mathbb{B}^n$, we define $f|_\kappa = \pi_\kappa \circ f \circ \iota_\kappa \colon \kappa \to \kappa$, where $\iota_\kappa \colon \kappa \to \mathbb{B}^n$ is the inclusion, and π_κ is the projection on κ.

We will give examples of Boolean maps either by defining a truth table, or in polynomial form (see Section 3.1.2). To simplify the notation, in the examples an element $x = (x_1, \ldots, x_n) \in \mathbb{B}^n$ will be denoted $x_1 \cdots x_n$.

3.1 DEFINITIONS

In the Boolean modelling approach, the concentrations of the species of interest are simplified to two possible levels. When Boolean networks are used to model gene regulatory networks, the n species under study are called *genes*, and the two levels represent either expression or absence of each gene. A state of the system is defined by an element of $\mathbb{B}^n$, and a *Boolean network* is simply defined by a map $f\colon \mathbb{B}^n \to \mathbb{B}^n$.

The definition of Boolean network is sometimes given in the following alternative and equivalent form, which puts emphasis on the regulatory roles (see for instance in [RMCT03, CRRT04]). A Boolean (regulatory) network is defined by the following three elements:

- the set of n regulatory components or genes;
- for each component i, the set $\mathcal{I}(i) \subseteq \{1, \ldots, n\}$ of components that regulate i;
- for each component i, a map $K_i\colon \mathcal{P}(\mathcal{I}(i)) \to \mathbb{B}$.

The set $\mathcal{I}(i)$ and map K_i uniquely define the i^{th} component of a Boolean network $f\colon \mathbb{B}^n \to \mathbb{B}^n$: each state $x \in \mathbb{B}^n$ defines a subset X of $\mathcal{I}(i)$ (the indices $j \in \mathcal{I}(i)$ such that $x_j = 1$), and $f_i(x)$ can be set to $K_i(X)$. Conversely, given a Boolean network f, we can define $\mathcal{I}(i) = \{j \in \{1, \ldots, n\} \mid f_i(\bar{x}^j) \neq f_i(x) \text{ for some } x \in \mathbb{B}^n\}$, and $K_i(X) = f_i(x)$, with x any state such that $x_j = 1$ for $j \in X$, and $x_j = 0$ for $j \in \mathcal{I}(i) \setminus X$.

A Boolean network f determines two possible interpretations of the dynamics. We call the dynamics *synchronous* when the values of all variables are updated at the same time. In other words, if the system is in state x at time t, it will be in state $f(x)$ at time $t+1$. Another approach that is often considered is the *asynchronous*, non-deterministic update scheme: if the system is in state x at time t, then at time $t+1$ the system can be in any of the neighbour states that take x one step closer to its target state $f(x)$. The asynchronous update is considered more realistic for biological systems [Td90], since the concentrations of multiple species are unlikely to change level in exactly the same time interval. Mixed synchronous and asynchronous updating schemes are sometimes also investigated [FNCT06].

Definition 3.1.1. The *asynchronous state transition graph* AD_f of a Boolean network $f\colon \mathbb{B}^n \to \mathbb{B}^n$ is a directed graph with nodes the set $\mathbb{B}^n$, and, for each $x \in \mathbb{B}^n$ and $i \in \{1, \ldots, n\}$, an edge with source x and target $\bar{x}^i$ if $f_i(x) \neq x_i$.

The asynchronous state transition graph is therefore a subgraph of the n-hypercube graph. Each subgraph G of the hypercube graph, on the other hand, defines a unique map from $\mathbb{B}^n$ to itself: for each $x \in \mathbb{B}^n$, let $I \subseteq \{1, \ldots, n\}$ be the set of indices i such that $(x, \bar{x}^i)$ is an edge in G, and define $f(x) = \bar{x}^I$. Then, G is the asynchronous state transition graph associated to f. In other words, asynchronous and synchronous dynamics are in one-to-one correspondence.

Many works on regulatory network are concerned with the study of asymptotic properties of the dynamics. A non-empty subset $D \subseteq \mathbb{B}^n$ is *trap domain* for AD_f if, for every edge (x, y), $x \in D$ implies $y \in D$. The minimal trap domains with respect to the inclusion are called *attractors* for the asynchronous dynamics of the network. Attractors that consist of a single point are called *fixed points*; the other attractors are referred to as *cyclic attractors*.

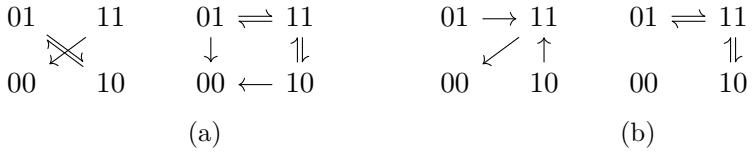


Figure 1: *(a): Synchronous and asynchronous state transition graphs for the map* $f(x_1, x_2) = ((1 - x_1)x_2, x_1(1 - x_2))$. *(b) Synchronous and asynchronous state transition graphs for the map* $f(x_1, x_2) = (x_1 + x_2 - 2x_1x_2, x_1 + x_2 - 2x_1x_2)$.

The steady states of the synchronous and asynchronous dynamics necessarily coincide; the same does not hold for cyclic attractors, as seen in the following example (an analysis of the relationship between synchronous and asynchronous attractors is given in [GDCX$^+$08]).

Example 3.1.2. The synchronous state transition graph associated to the map

$$f(x_1, x_2) = ((1 - x_1)x_2, x_1(1 - x_2))$$

admits two attractors, the fixed point 00 and the type 2 attractive cycle $\{01, 10\}$. The fixed point is the only attractor for the associated asynchronous dynamics instead (see Figure 1a).

The state 00 is the only attractor for the synchronous dynamics defined by

$$f(x_1, x_2) = (x_1 + x_2 - 2x_1x_2, x_1 + x_2 - 2x_1x_2),$$

wheres the associated asynchronous state transition graph admits the additional cyclic attractor $\{01, 11, 10\}$ (see Figure 1b).

The design of algorithms for the identification of fixed points or attractors of Boolean networks and their reachability properties is still an area of active research. An overview of the approaches and of the software tools available for the modelling of regulatory network can be found in the recent survey [AJTM$^+$16].

A significant part of the Boolean network literature motivated by the study of gene regulatory networks is concerned with the identification of properties of the network dynamics from the analysis of a smaller graph associated to the network, that encapsulates its regulatory structure.

3.1.1 *Regulatory graphs and Jacobian matrices*

Boolean networks are used to model the interactions between regulatory components, which are derived from a Boolean map f as follows.

Definition 3.1.3. For each state $x \in \mathbb{B}^n$, the *local regulatory graph* $G_f(x)$ of f at $x \in \mathbb{B}^n$ is a labelled directed graph with nodes the set $\{1, \ldots, n\}$. The graph $G_f(x)$ admits an edge from node j to node i when $f_i(\bar{x}^j) \neq f_i(x)$; the edge is represented as $j \to i$ and is labelled with

$$s = \frac{f_i(\bar{x}^j) - f_i(x)}{\bar{x}^j_j - x_j}. \tag{8}$$

The label s is also called the *sign* of the edge.

The edges of a regulatory graph are therefore labelled with -1 or $+1$; these represent inhibition or activation, respectively. It is easy to see that the sign of an edge $j \to i$ is positive if and only if $x_j = f_i(x)$.

Definition 3.1.4. The *global regulatory graph* G_f of f is the multi-directed labelled graph on $\{1,\dots,n\}$ that admits an edge $j \to i$ of sign s if there exists a local regulatory graph $G_f(x)$ for f that admits an edge $j \to i$ of sign s.

The sign of a path $i_1 \to i_2 \to \cdots \to i_k$ in a regulatory graph is defined as the product of the signs of its edges. A *circuit* in a regulatory graph is a path $i_1 \to i_2 \to \cdots \to i_k$ with $i_1 = i_k$ and such that the elements $i_1, \dots, i_{k-1}$ are all distinct. Hence a circuit is an elementary cycle — a cycle where no nodes are repeated. If $k = 1$, the circuit is called a *loop*. A circuit in a regulatory graph G_f is said to be *local* if it is contained in a local graph $G_f(x)$ for some state $x \in \mathcal{X}$.

From regulatory graphs to Boolean networks

The global regulatory graph of a network can be seen as comprising all the possible regulations between the components; some regulations, however, might be active only when the system is in some specific states. Whereas the regulatory graphs are uniquely defined given a network, we might be interested in studying a biological system starting from a given set of regulations, and ask, for example: given a signed graph G on $\{1,\dots,n\}$, does a regulatory network admitting G as global regulatory graph always exist? And how many such networks exist? Can each regulation be active at every state?

Proposition 3.1.5. *A simple directed graph G on $\{1,\dots,n\}$ with edges labelled with signs $\{-1,1\}$ coincides with all the local regulatory graphs of at least one network f if and only if each node has indegree at most one. Moreover, G identifies 2^r possible dynamics, where r is the number of nodes with indegree zero.*

Proof. Suppose that a node i has indegree 2, i.e., it is regulated by two components j_1 and j_2, with signs of the regulations s_1 and s_2 respectively. It is then sufficient to take a state x such that $x_{j_1} = 0$, and $x_{j_2} = 1$ if $s_1 = s_2$, and 0 otherwise, to show a contradiction.

For the second statement, it is sufficient to observe that if a component i is not regulated, then f_i is constant, and if i is regulated by j, then $f_i(x)$ is uniquely determined by x_j, for all $x \in \mathbb{B}^n$. □

Given a graph that satisfies the hypotheses of Proposition 3.1.5, we can create all the Boolean dynamics that admit G as their regulatory graph as follows: if i is a node with exactly one incoming edge with source j, then we define $f_i(x) = x_j$ if the label of the edge is positive, and $f_i(x) = \bar{x}_j = 1 - x_j$ if the label is negative. All other components of f, corresponding to unregulated nodes, will be constantly equal to either 0 or 1. If a node i in G as indegree greater than 1, we can only ask that, for each edge $j \to i$ there exists at least one state $x \in \mathbb{B}^n$ such that $j \to i$ belongs to the local regulatory graph $G_f(x)$.

More in general, suppose that we are given an assignment of a local regulatory graph $G(x)$ for each state $x \in \mathbb{B}^n$. If, in each graph $G(x)$, every component is regulated by at least one regulatory component, then there exists at most one regulatory network f that satisfies $G_f(x) = G(x)$ for all $x \in \mathbb{B}^n$ (each regulation $j \to i$ in $G(x)$ determines the value taken by f_i at x). If components that are not regulated are allowed, then more than one map can exist admitting $G(x)$ as local regulatory graph at x, for every $x \in \mathbb{B}^n$. Maps with identical local regulatory graphs can have different asymptotic behaviours, see for example the maps in Figure 2.

A question we can ask is: given a signed graph G on $\{1,\dots,n\}$, how many Boolean networks on n variables admit G as their global regulatory graph? Suppose that the

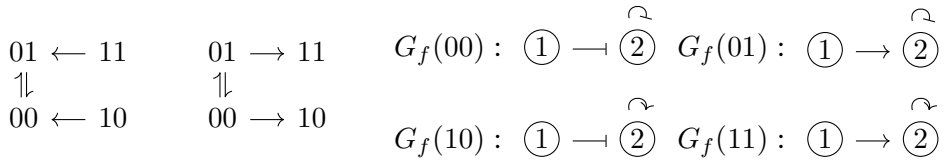


Figure 2: *Asynchronous dynamics of two Boolean networks (defined by* $(x_1, x_2) \mapsto (0, 2x_1x_2 - x_1 - x_2 + 1)$ *and* $(x_1, x_2) \mapsto (1, 2x_1x_2 - x_1 - x_2 + 1)$ *respectively) admitting the same local regulatory graphs (shown on the right). The first map has a single cyclic attractor, the second has two fixed points.*

graph G is *simple*, i.e., it does not admit parallel edges. Then the number of choices for component i is given by the number of non-degenerate monotone Boolean functions of k variables, where k is the number of regulators of i. This number is known up to $k = 8$ [Slo17, A006126].

In Section 3.2 we will give a characterisation of the maps with regulatory graph given by a circuit, whereas in Section 5.2 we will show how one can write requirements on the regulatory structure as Boolean expressions.

Jacobian matrices

For each $x \in \mathbb{B}^n$, define the $n \times n$ matrix $J_f(x)$ as follows: $(J_f(x))_{ij} = 1$ if there exists an edge from j to i, and $(J_f(x))_{ij} = 0$ otherwise. The matrix $J_f(x)$ is called the *discrete Jacobian matrix* or simply *Jacobian* of f at x [RRT08, Rue16]. The Jacobian at x is therefore the transpose of the adjacency matrix [GY04] of the regulatory graph $G_f(x)$.

To reason about the graphs associated to regulatory networks it is sometimes useful to consider a signed version of this matrix, which we call *signed Jacobian*, and denote $\mathfrak{J}_f(x)$. This is a *sign pattern matrix* [Hog06], i.e., a matrix with entries in $\{-1, 0, +1\}$. $\mathfrak{J}_f(x)$ has the same support as the Jacobian $J_f(x)$, and the entry $(\mathfrak{J}_f(x))_{ij}$ is the sign of the edge from j to i (8). Hence the Jacobian $J_f(x)$ is the zero pattern of $\mathfrak{J}_f(x)$, and the local graph $G_f(x)$ is the signed directed graph defined by the transpose of $\mathfrak{J}_f(x)$.

A signed Jacobian matrix can also be associated to the global regulatory graph, by introducing an additional label $\sharp$, that represents the presence of edges with the same source and target and different signs. Operations of addition and multiplication can be defined on the set $\mathcal{S} = \{-1, 0, 1, \sharp\}$ as follows [Hog06, AI04]:

$$
\begin{array}{lll}
0 + s = s = s + 0, & 0 \cdot s = s = s \cdot 0, & \text{for } s \in \mathcal{S}, \\
\sharp + s = \sharp = s + \sharp, & \sharp \cdot s = \sharp = s \cdot \sharp, & \text{for } s \in \mathcal{S}, s \neq 0, \\
1 + 1 = 1,\ -1 + (-1) = -1, & 1 \cdot 1 = 1 = -1 \cdot (-1), & \\
1 + (-1) = \sharp = -1 + (+1), & 1 \cdot (-1) = -1 = -1 \cdot (+1). &
\end{array}
$$

With the two operations defined above, the set $\mathcal{S}$ can be viewed as a commutative semiring with identity, and the matrices $\mathcal{M}_n(\mathcal{S})$ over $\mathcal{S}$, called *generalised sign pattern matrices*, form a monoid with the usual matrix multiplication.

The global signed Jacobian $\mathfrak{J}_f$ can be defined as the sum of all the local signed Jacobians. The signed directed graph $\mathfrak{G}_f$ associated to $\mathfrak{J}_f$ is a version of the global regulatory graph that admits at most one edge from each pair of source and target nodes. The sign of a path in $\mathfrak{G}_f$ can be defined again as the product of the signs of the edges; the existence of a path of sign $\sharp$ in $\mathfrak{G}_f$ from i to j corresponds to the existence of paths of positive and negative signs from i to j in the global regulatory graph.

It is known that, if S is a generalised sign pattern matrix, the entry S^k_{ij} gives the sum of the signs of all paths of length k from i to j in the signed directed graph G associated to S ([AI04], Proposition 3).

If a map f is such that the (signed) Jacobian $J_f(x)$ ($\mathfrak{J}_f(x)$) is independent of x, we say that f has constant (signed) Jacobian. If f has constant signed Jacobian, then clearly it has constant Jacobian, whereas the converse does not hold: consider for instance the map $f = (x_1, x_1 + x_2 - 2x_1x_2)$ with signed Jacobian at x $\mathfrak{J}_f(x) = \begin{vmatrix} 1 & 0 \\ 1-2x_2 & 1-2x_1 \end{vmatrix}$.

Example 3.1.6. For $I \subseteq \{1, \dots, n\}$, the signed Jacobian of ψ_I is constant and diagonal, with $(\mathfrak{J}_{\psi_I})_{ii} = -1$ if $i \in I$ and $(\mathfrak{J}_{\psi_I})_{ii} = +1$ otherwise.

Example 3.1.7. The signed Jacobian of the map σ defined by a permutation of the coordinates $\sigma \in S_n$ is constant and coincides with the Jacobian. It satisfies $(\mathfrak{J}_\sigma)_{ij} = 1$ if $\sigma(j) = i$, and $(\mathfrak{J}_\sigma)_{ij} = 0$ otherwise. The (signed) Jacobian of the inverse of σ is the transpose of the (signed) Jacobian of σ.

Given two maps $f, g \colon \mathbb{B}^n \to \mathbb{B}^n$, it is not in general true that $J_{f\circ g}(x) = J_f(g(x))J_g(x)$ or $\mathfrak{J}_{f\circ g}(x) = \mathfrak{J}_f(g(x))\mathfrak{J}_g(x)$, not even if g has constant (signed) Jacobian, as seen in the following example.

Example 3.1.8. Consider the maps $f(x_1, x_2) = (x_1x_2, x_1x_2)$, $g(x_1, x_2) = (x_2, x_2)$. The Jacobian and signed Jacobian of g are constant and both equal to $\begin{vmatrix} 0 & 1 \\ 0 & 1 \end{vmatrix}$. In addition, $f \circ g = g$, and therefore the Jacobians of $f \circ g$ are also independent of x and given by the same matrix. However, the (signed) Jacobian of f at $(0,0) = g(0,0)$ is $\begin{vmatrix} 0 & 0 \\ 0 & 0 \end{vmatrix}$, and therefore we cannot have $J_{f\circ g}(x) = J_f(g(x))J_g(x)$.

A counterexample is also given in [Rue16], where part (ii) of the following result is also observed.

Proposition 3.1.9. *Consider $f, g \colon \mathbb{B}^n \to \mathbb{B}^n$.*

(i) If the signed Jacobian of f is constant, then $\mathfrak{J}_{f\circ g}(x) = \mathfrak{J}_f(g(x))\mathfrak{J}_g(x)$.

(ii) If the Jacobian of f is constant, then $J_{f\circ g}(x) = J_f(g(x))J_g(x) \mod 2$.

(iii) if g is of the form $\psi_I \circ \sigma$ for some $I \subseteq \{1, \dots, n\}$ and $\sigma \in S_n$, then $\mathfrak{J}_{f\circ g}(x) = \mathfrak{J}_f(g(x))\mathfrak{J}_g(x)$ and $J_{f\circ g}(x) = J_f(g(x))J_g(x) \mod 2$.

Proof. (i) If the signed Jacobian of f is constant, then from Proposition 3.1.5 we have that each component is regulated by at most one variable. In other words, for each $i \in \{1, \dots, n\}$ there exists a unique $i' \in \{1, \dots, n\}$ such that $(\mathfrak{J}_f(x))_{ii'} \neq 0$, and f_i writes as $f_i = (\mathfrak{J}_f(x))_{ii'}x_{i'} + c_i$, with $c_i \in \{-1, 0, 1\}$. As a consequence

$$\begin{aligned}(\mathfrak{J}_{f\circ g}(x))_{ij} &= \frac{f_i(g(\bar{x}^j)) - f_i(g(x))}{\bar{x}^j_j - x_j} = (\mathfrak{J}_f(x))_{ii'}\frac{g_{i'}(\bar{x}^j) - g_{i'}(x)}{\bar{x}^j_j - x_j} \\ &= \sum_{k=1}^{n}(\mathfrak{J}_f)_{ik}(\mathfrak{J}_g(x))_{kj} = (\mathfrak{J}_f\mathfrak{J}_g(x))_{ij}.\end{aligned}$$

(ii) If the Jacobian of f is constant, then each component f_i of f writes, modulo 2, as $f_i = \sum_{k=1}^{n} J_{ik}x_k + c_i$, for some $c_i \in \{0, 1\}$. We find the following equality modulo 2:

$$\begin{aligned}(J_{f\circ g}(x))_{ij} &= \frac{f_i(g(\bar{x}^j)) - f_i(g(x))}{\bar{x}^j_j - x_j} = \sum_{k=1}^{n} J_{ik}\frac{g_k(\bar{x}^j) - g_k(x)}{\bar{x}^j_j - x_j} \\ &= \sum_{k=1}^{n}(J_f)_{ik}(J_g(x))_{kj} = (J_fJ_g(x))_{ij}.\end{aligned}$$

(*iii*) We can give the proof in two steps, considering the cases $g = \psi_I$ and $g = \sigma$ separately. Using the observation in example 3.1.6, in the first case, we have

$$(\mathfrak{J}_{f\circ\psi_I}(x))_{ij} = \frac{f_i(\psi_I(\bar{x}^j)) - f_i(\psi_I(x))}{\bar{x}^j_j - x_j}$$
$$= \frac{f_i(\overline{\psi_I(x)}^j) - f_i(\psi_I(x))}{\overline{\psi_I(x)}^j_j - \psi_I(x)_j} \frac{\overline{\psi_I(x)}^j_j - \psi_I(x)_j}{\bar{x}^j_j - x_j} = (\mathfrak{J}_f(\psi_I(x))\mathfrak{J}_{\psi_I}(x))_{ij}.$$

Using example 3.1.7, we find

$$(\mathfrak{J}_{f\circ\sigma}(x))_{ij} = \frac{f_i(\sigma(\bar{x}^j)) - f_i(\sigma(x))}{\bar{x}^j_j - x_j}$$
$$= \frac{f_i(\overline{\sigma(x)}^{\sigma^{-1}(j)}) - f_i(\sigma(x))}{\overline{\sigma(x)}^{\sigma^{-1}(j)}_{\sigma^{-1}(j)} - \sigma(x)_{\sigma^{-1}(j)}} \frac{\sigma(\bar{x}^j)_{\sigma^{-1}(j)} - \sigma(x)_{\sigma^{-1}(j)}}{\bar{x}^j_j - x_j} = (\mathfrak{J}_f(\sigma(x))\mathfrak{J}_\sigma(x))_{ij}.$$

The proof for the Jacobian equality proceeds similarly. □

3.1.2 *Boolean networks as polynomials*

It is well known that Boolean functions, seen as maps from $\mathbb{F}_2^n$ to $\mathbb{F}_2$, can be written as polynomials. Here we adopt a polynomial representation of Boolean functions which has the additional property of allowing for the distinction between positive and negative regulations. Each component f of a map from $\{0,1\}^n$ to itself can be written as a polynomial in $\mathbb{Z}[x_1,\ldots,x_n]$ by associating to each state $a \in \mathbb{B}^n$ a term $\prod_{i,a_i=1} x_i \prod_{i,a_i=0}(1-x_i)$, summing over all states a such that $f(a) = 1$:

$$f(x) = \sum_{a\in\{0,1\}^n} f(a) \prod_{i=1}^{n} x_i^{a_i}(1-x_i)^{1-a_i}. \tag{9}$$

This polynomial has the property that the sign of an edge from j to i in the regulatory graph at x as defined in the previous section (8) can be found simply by calculating the derivative of the polynomial with respect to x_j. To see this, observe that

$$f(\bar{x}^j) - f(x) = \sum_{a\in\{0,1\}^n} f(a) \prod_{i\neq j} x_i^{a_i}(1-x_i)^{1-a_i}(x_j^{1-a_j}(1-x_j)^{a_j} - x_j^{a_j}(1-x_j)^{1-a_j}).$$

Since, if $a_j = 0$, we have $x_j^{1-a_j}(1-x_j)^{a_j} - x_j^{a_j}(1-x_j)^{1-a_j} = x_j - (1-x_j) = x_j - \bar{x}_j$, and if $a_j = 1$, $x_j^{1-a_j}(1-x_j)^{a_j} - x_j^{a_j}(1-x_j)^{1-a_j} = (1-x_j) - x_j = \bar{x}_j - x_j$, we can write

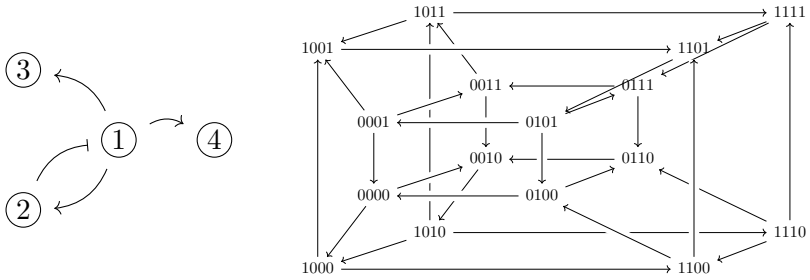
$$\frac{f(\bar{x}^j) - f(x)}{\bar{x}_j - x_j} = \sum_{a\in\{0,1\}^n} f(a) \prod_{i\neq j} x_i^{a_i}(1-x_i)^{1-a_i}(-1)^{a_j+1},$$

which is the same expression we find by taking the derivative of (9) with respect to x_j. Notice also that, if we replace each product in (9) with `and` and each sum with `or`, the polynomial gives the disjunctive normal form of f.

For example, we write a `xor` between x_1 and x_2 as $x_1 + x_2 - 2x_1x_2$, so that we can calculate the derivatives as $1 - 2x_2$ and $1 - 2x_1$.

Example 3.1.10. In [CRRT04], a core model for the cell cycle in Drosophila is studied. The network involves four regulatory components, MPF (Mitosis Promoting Factor) and

the proteins Fizzy, Wee1 and String. The regulatory graph and asynchronous dynamics considered in [CRRT04] are as follows:



The corresponding synchronous dynamics can be written in polynomial form as

$$f(x_1, x_2, x_3, x_4) = (1 - x_2, x_1, 1 - x_1, x_1). \tag{10}$$

With the system written in form (10), it is easy to see that the network does not have any fixed point: any such point must satisfy $1 - x_2 = x_1 = x_2$. The asynchronous state transition graph must therefore admit at least one cyclic attractor. It is more difficult to see that there is only one attractor, which covers the entire state space.

3.2 SYMMETRIES OF THE HYPERCUBE GRAPH

Recall that an *isomorphism* of graphs $G = (V, E)$ and $G' = (V', E')$ is a bijective map $\varphi\colon E \to E'$ such that $(i, j) \in E$ if and only if $(\varphi(i), \varphi(j)) \in E'$. If the graphs are directed, φ must preserve the direction of the edges. If an isomorphism exists between G and G', the two graphs are said to be isomorphic. If $G = G'$, the map φ is called an *automorphism*. For some background terminology of graph theory, see for instance [GY04].

The group of automorphisms of the n-hypercube graph is given by the semidirect product $O_n := (\prod_{i=1}^n S_2) \rtimes S_n$, where S_n is the symmetric group on a finite set of cardinality n (see for instance [Sle53]). The symmetric group S_n acts on $\mathbb{B}^n$ by permuting the coordinates: for each $\sigma \in S_n$ and $x \in \mathbb{B}^n$, $\sigma(x) = (x_{\sigma^{-1}(1)}, \ldots, x_{\sigma^{-1}(n)})$. The product $\prod_{i=1}^n S_2$ acts on $\mathbb{B}^n$ by transposing 0 and 1 on the n coordinates. Given $I \subseteq \{1, \ldots, n\}$, we write ψ_I for the map that swaps the levels 0 and 1 for the coordinates in I. The symmetries of the hypercube graph correspond therefore to the maps that can be written as the composition of a map ψ_I and a permutation of the coordinates σ. It can be easily verified that $\psi_I \circ \sigma = \sigma \circ \psi_{\sigma^{-1}(I)}$. Given maps $U = \psi_I \circ \sigma$ and $f : \mathbb{B}^n \to \mathbb{B}^n$, we write $f^U = U \circ f \circ U^{-1}$.

Proposition 3.2.1. *For each $U = \psi_I \circ \sigma$ and $f : \mathbb{B}^n \to \mathbb{B}^n$, the state transition graphs AD_f and AD_{f^U} are isomorphic.*

Proof. We have that $(x, \bar{x}^i)$ is in AD_f if and only if $(U(x), U(\bar{x}^i) = \overline{U(x)}^{\sigma(i)})$ is in AD_{f^U}, so that the graph isomorphism is given by U. This follows from the observation that

$$f^U_{\sigma(i)}(U(x)) = \overline{\sigma(f(x))}^I_{\sigma(i)} = \overline{f(x)}_i^{\sigma^{-1}(I)}, \tag{11}$$

and $U(x)_{\sigma(i)} = \overline{\sigma(x)}^I_{\sigma(i)} = \overline{x}_i^{\sigma^{-1}(I)}$, and therefore $f^U_{\sigma(i)}(U(x)) \neq U(x)_{\sigma(i)}$ if and only if $f_i(x) \neq x_i$. □

This property can be interpreted as follows: the order we assign to the regulatory components, as well as the stipulation of labels (0 for not expressed, 1 for expressed, or vice versa) are arbitrary. Boolean networks that can be mapped to one another via a

permutations of the coordinates, or by swapping the levels 0 and 1 can be considered as the same Boolean network. For example, the maps $f(x_1, x_2) = ((1 - x_1)x_2, x_1 + x_2 - x_1x_2)$ and $g(x_1, x_2) = (x_1x_2 - x_2 + 1, 1 - x_1x_2)$, with asynchronous state transition graphs

are to all effects the same Boolean network, as g is obtained from f by swapping the first component with the second, and negating the second. To have an idea of the number of objects we are studying, we want to have an estimate of how many different Boolean networks exist after factoring for symmetry. The number of Boolean networks with no auto-regulations is discussed in [EG00], whereas the types of Boolean functions under symmetry is enumerated in [Sle53].

The number of equivalence classes of Boolean networks under symmetry

The group O_n acts on the set $\mathcal{F}_n$ by conjugation: for each $U \in O_n$, $U \cdot f = U \circ f \circ U^{-1}$. To find the number of different Boolean maps under symmetry, we need to count the number of orbits $o(n)$ under the action of O_n on $\mathcal{F}_n$. A lower bound for the number of Boolean networks modulo symmetries is found by observing that the size of each orbit is bounded above by the number of symmetries. Since the cardinality of O_n is $n!2^n$, we have that the number of different Boolean networks $o(n)$ satisfies

$$o(n) \geq \frac{(2^n)^{2^n}}{n!2^n}. \tag{12}$$

To count the number of orbits $o(n)$ we can use a result known as Burnside's Lemma (see for example [Har64]): the number of orbits is the average of the number of elements that are fixed by each symmetry. Denote by $I(U)$ the maps fixed under U: $I(U) = \{f \in \mathcal{F}_n |\ U \circ f \circ U^{-1} = f\}$. Then

$$o(n) = \frac{1}{|O_n|} \sum_{U \in O_n} |I(U)|. \tag{13}$$

Recall that every permutation on m elements admits a unique decomposition into disjoint cycles. Given a permutation τ of a set of m elements, denote by $c_\tau(k)$ the number of cycles of length k in the decomposition of τ, for $k = 1, \ldots, m$. To count the number of fixed maps for each automorphism, we can use the following result ([Dav53, Theorem 6], [Har64, Theorem 5.1]): given a permutation τ of a set X of m elements, the number of maps $f : X \to X$ fixed by τ is given by

$$I(\tau) = \prod_{i=1}^{m} \left(\sum_{k|i} k c_\tau(k) \right)^{c_\tau(i)}. \tag{14}$$

To find the number of orbits using (13) and (14), we need therefore to identify the cyclic structure of the permutation of $\mathbb{B}^n$ defined by the symmetry. For instance, for $n = 1$, we have two symmetries, the identity and the symmetry s that swaps 0 and 1, and four maps:

(a) (b) (c) (d)

n	sym.s	Lower bound	n. equiv. classes	n. Boolean maps
1	2	2	3	4
2	8	32	43	256
3	48	349 525	352 744	16 777 216
				18
4	384	48 038 396 025 285 290	48 038 431 091 088 416	446 744 073 709 551 616
		380 599 384	380 599 384	1 461 501 637 330
		721 589 301 615 542 925	721 589 407 253 467 900	902 918 203 684 832 716
5	3840	186 532 036 368 732 433	488 697 416 122 580 992	283 019 655 932 542 976
		855	855	39 402 006
		078 259 470 366 302 349	078 259 470 366 302 349	196 394 479 212 279 040
		805 557 728 811 063 478	805 557 728 811 063 553	100 143 613 805 079 739
		292 952 918 086 950 259	678 427 681 966 288 752	270 465 446 667 948 293
		294 561 724 082 503 721	681 049 145 413 905 791	404 245 721 771 497 210
		727 660 837 983 208 656	637 049 449 915 971 376	611 414 266 254 884 915
6	46080	356 676 232 782 725 484	284 411 737 392 807 936	640 806 627 990 306 816

Table 1: *Number of symmetries, lower bound on the number of different Boolean networks* $\frac{(2^n)^{2^n}}{n!2^n}$, *number of equivalence classes under* O_n *and number of Boolean networks, for* $n \leq 6$.

The identity has 4 fixed points. The symmetry s consists of one cycle of length 2, hence the number of maps fixed by s is $I(s) = \prod_{i=1}^{2} \left(\sum_{k|i} k c_s(k)\right)^{c_s(i)} = 2$ (the maps (a) and (b) above). Using (13), we find that there are 3 types of maps in dimension 1: these are given by the maps (a), (b) and (c) above.

In the next paragraph we study the cycle decomposition $(c_\tau(i))_{i\in\{1,\dots,m\}}$ defined by a symmetry $\psi_I \circ \sigma$ on $\mathbb{B}^n$, that we can use to find the number of equivalence classes under symmetry for small n. The counts $o(n)$ up to $n \leq 6$, as well as the lower bounds provided in (12), and the total number of Boolean networks are given in Table 1. The lower bounds give a fair approximation of the number of types of Boolean networks – or, in other words, many maps can be identified under symmetry.

Remark 3.2.2. Some networks can have asynchronous state transition graphs that are isomorphic, but not equivalent under symmetry. This is the case for example for the asynchronous state transition graphs associated to $(x_1, x_2) \mapsto (x_1, 0)$ and $(x_1, x_2) \mapsto (x_1, 1 - x_1)$, which are respectively

This example was suggested by A. Fauré. Another related question, which we do not answer here, is therefore: how many non-isomorphic asynchronous state transition graphs exist, for each n?

3.2.1 *Synchronous dynamics*

In this section we look at a map of the form $f = \psi_I \circ \sigma$ for some $I \subseteq \{1, \dots, n\}$ and $\sigma \in S_n$ as a Boolean network, and give a description of its synchronous dynamics. Using the remarks in examples 3.1.6 and 3.1.7, we can observe that the regulatory graphs of such maps consists of a union of disjoint circuits. Special cases of these maps are therefore given by Boolean networks with regulatory structure consisting of an isolated circuit. These maps are studied in detail in [RMCT03]. For these maps, the permutation

σ consists of a single circuit, and $|I|$ determines the sign of the circuit, which equals $(-1)^{|I|}$. We will write s for $|I|$.

First, we can observe that, for each $x \in \mathbb{B}^n$, we have

$$f(\bar{x}^j) = \overline{f(x)}^{\sigma(j)}. \tag{15}$$

Remark 3.2.3. It can be easily verified that, for each $I \subseteq \{1, \ldots, n\}$, $I_{\psi_I}(\psi_I(x)) = I_{\psi_I}(x)$, and for each $\sigma \in S_n$, $I_\sigma(\sigma(x)) = \sigma(I_\sigma(x))$. As a consequence, if $f = \psi_I \circ \sigma$,

$$I_f(f(x)) = \sigma(I_f(x)). \tag{16}$$

Proposition 3.2.4. *Consider a map $f = \psi_I \circ \sigma : \mathbb{B}^n \to \mathbb{B}^n$, with $s = |I|$. Then $|I(x)| = s \bmod 2$ for all $x \in \mathbb{B}^n$.*

Proof. We can write

$$\sum_{i=1}^{n} f_i(x) = \sum_{i=1}^{n} \psi_I(x_{\sigma^{-1}(i)}) = \sum_{\substack{i=1 \\ \sigma^{-1}(i) \notin I}}^{n} x_{\sigma^{-1}(i)} + \sum_{\substack{i=1 \\ \sigma^{-1}(i) \in I}}^{n} (1 - x_{\sigma^{-1}(i)}) = \sum_{i=1}^{n} x_i + s \bmod 2.$$

On the other hand, we have

$$\sum_{i=1}^{n} f_i(x) = \sum_{i \in I(x)} f_i(x) + \sum_{i \notin I(x)} f_i(x) = \sum_{i \in I(x)} (1 - x_i) + \sum_{i \notin I(x)} x_i = \sum_{i=1}^{n} x_i + |I(x)| \bmod 2,$$

which gives $|I(x)| = s \bmod 2$. □

For any integer $k \geq 0$, define the set

$$\mathcal{A}_k = \{x \in \mathbb{B}^n \mid |I(x)| = k\}.$$

For odd s, from Proposition 3.2.4 we find that $\mathcal{A}_k = \emptyset$ for each even k, whereas the same is true for even s and odd k. In particular, if s is odd, f does not have any fixed point. From (16), we have that each state in $\mathcal{A}_k$ is mapped by f to another state in $\mathcal{A}_k$.

To give a characterisation of the synchronous dynamics, we first look at the special cases with σ consisting of a single cycle.

Remark 3.2.5. If σ is a cycle, then, for each $k \geq 0$, $k = s \bmod 2$, and for each $J \subseteq \{1, \ldots, n\}$ with cardinality k, there are exactly two elements $x, y \in \mathbb{B}^n$ such that $I(x) = I(y) = J$. Moreover, $y = \bar{x}$. This is a consequence of the fact that, for each $j \in \{1, \ldots, n\}$, $x_{\sigma^{-1}(j)} = x_j$ if $j \in J \cap I$ or $j \notin J \cup I$, and $x_{\sigma^{-1}(j)} = \bar{x}_j$ otherwise. Each set $\mathcal{A}_k$ has therefore cardinality $2\binom{n}{k}$, and, if s is even, f has exactly two fixed points.

Equation (16) gives that, at each iteration of f, the set $I(x)$ changes according to σ. Write $\mathcal{I}_k$ for the subsets of $\{1, \ldots, n\}$ of cardinality k, and, for $J \in \mathcal{I}_k$, define $\mathcal{A}_k(J) = \{x \in \mathcal{A}_k \mid I(x) = \sigma^i(J) \text{ for some } i \in \{1, \ldots, n\}\}$. To study the synchronous dynamics in $\mathcal{A}_k$ we look at the orbits of the elements of $\mathcal{I}_k$ under σ.

Recall that the Möbius function $\mu : \mathbb{N}_{>0} \to \{-1, 0, 1\}$ is defined as $\mu(n) = (-1)^k$ if n is square-free with k prime factors, and zero otherwise. We extend the definition of the binomial coefficient by setting $\binom{n}{k} = 0$ when k is not integer.

Proposition 3.2.6. *Consider the action of $G = \mathbb{Z}/n\mathbb{Z}$ on $\mathcal{I}_k$. The number of orbits of cardinality m is*

$$\theta_k(m) = \frac{1}{m} \sum_{l \mid m} \binom{l}{\frac{kl}{n}} \mu\left(\frac{m}{l}\right). \tag{17}$$

Proof. Fix a subgroup H of G of cardinality d. First observe that H is contained in the stabiliser of a set $S \in \mathcal{I}_k$ if and only if S is the union of right cosets of H, i.e., $S = H \cdot T$ for some $T \subseteq G$. As a consequence, the number $\tilde{\Theta}(d)$ of elements of $\mathcal{I}_k$ with stabiliser that contains H is given by $\binom{n/d}{k/d}$ (the cosets of H are $\frac{n}{d}$, and we need $\frac{k}{d}$ cosets to obtain k elements).

On the other hand, if we write $\tilde{\theta}(h)$ for the number of elements with stabilizer of cardinality h, we have $\tilde{\Theta}(d) = \sum_{d|h,h|n} \tilde{\theta}(h)$. Writing $\theta(m)$ for the number of elements with orbit of cardinality m, and replacing $\frac{h}{d}$ with l, we get $\sum_{l|m} \theta(l) = \binom{m}{\frac{km}{n}}$. Using the inclusion-exclusion principle [Aig07], we find $\theta(m) = \sum_{l|m} \binom{l}{\frac{kl}{n}} \mu(\frac{m}{l})$, that divided by m gives the number of orbits of cardinality m. □

Proposition 3.2.7. *Consider a $k \geq 1$, $k = s \bmod 2$, and a set $J \in \mathcal{I}_k$ with orbit of cardinality $m = \frac{n}{d}$. Then the set $\mathcal{A}_k(J)$ consists of two cycles of size m if $\frac{k}{d}$ is even, and a cycle of size $2m$ if $\frac{k}{d}$ is odd.*

Proof. Fix an index $j \in J$. Since $J = I(x) = I(f^m(x))$ for some x, from Remark 3.2.5 $f^m(x)$ is either equal to x or to $\bar{x}$. It is therefore sufficient to observe that the j^{th} component of $\{x, f(x), f^2(x), \ldots, f^m(x)\}$ changes exactly $\frac{k}{d}$ times. This is because the number of times the j^{th} component changes is the number of times that j falls in $L = J \cup \sigma(J) \cup \cdots \cup \sigma^{m-1}(J)$. Since all indices in $\{1, \ldots, n\}$ appears the same number of times t in L, and L has $k \cdot m$ elements, we have $t \cdot n = k \cdot m$, and $t = \frac{k}{d}$. □

To each orbit in $\mathcal{I}_k$ with cardinality m correspond therefore either one cycle or two cycles in $\mathcal{A}_k$. We have derived the characterisation of the synchronous dynamics presented in [RMCT03], and provided in addition equation (17), which allows us to count the number of cycles of each order. For each m that divides $2n$, the number of cycles of cardinality m is given by

$$\sum_{\substack{k=1,\ldots,n,\ k=s \bmod 2,\\ \frac{n}{m}|k,\ \frac{km}{n}=0 \bmod 2}} 2\theta_k(m)$$

if m divides n, plus

$$\sum_{\substack{k=1,\ldots,n,\ k=s \bmod 2,\\ \frac{2n}{m}|k,\ \frac{km}{2n}=1 \bmod 2}} \theta_k\left(\frac{m}{2}\right)$$

if m is even.

The synchronous dynamics of some Boolean networks with regulatory graph consisting of a single circuit involving all variables are represented, for $n \leq 4$, in Table 2.

We now move on to describe the topology of the synchronous dynamics for networks with regulatory graph given by the union of disjoint circuits.

Proposition 3.2.8. *Suppose that σ admits a decomposition in circuits $\sigma_1 \cdots \sigma_N$, on the indices $L_1, \ldots, L_N$ respectively. Define $I_i = I \cap L_i$, $i = 1, \ldots, N$, and suppose that the map $\psi_{I_i} \circ \sigma_i$ admits $\gamma_i(m)$ cycles of size m, $i = 1, \ldots, N$, $m = 1, \ldots, n$. Then $\psi_I \circ \sigma$ admits*

$$\frac{1}{l} \sum_{\substack{m_1,\ldots,m_N\\ \mathrm{lcm}(\{m_i\}_{i=1,\ldots,N})=l}} \left(\prod_{i=1}^{N} \gamma_i(m_i)\right)\left(\prod_{i=1}^{N} m_i\right)$$

cycles of size l.

Proof. Suppose that the map $\psi_{I_i} \circ \sigma_i$ admits $\gamma_i(m_i)$ cycles of size m_i, $i = 1, \ldots, N$. To each choice of cycle with support X_i for $\psi_{I_i} \circ \sigma_i$ of size m_i, $i = 1, \ldots, N$ corresponds a (trap) set in $\mathbb{B}^n$ which is given by the product $X = \prod_{i=1}^{N} X_i$. The cardinality of each

n	Positive circuit		Negative circuit	
1		$\boxed{0}$ $\boxed{1}$ $k=0$		$\boxed{0} \rightleftharpoons \boxed{1}$ $k=1$
2		$\boxed{00}$ $\boxed{11}$ $k=0$ $\boxed{01} \rightleftharpoons \boxed{10}$ $k=2$		$\boxed{01} \leftarrow \boxed{11}$ $k=1$ $\downarrow \quad \uparrow$ $\boxed{00} \rightarrow \boxed{10}$
3		$\boxed{000}$ $\boxed{111}$ $k=0$ $\boxed{010}$ $\boxed{101} \leftarrow \boxed{011}$ $k=2$ $\boxed{001} \rightarrow \boxed{100}$ $\boxed{110}$		$\boxed{001} \leftarrow \boxed{011} \leftarrow \boxed{111}$ $k=1$ $\downarrow \quad \uparrow$ $\boxed{000} \rightarrow \boxed{100} \rightarrow \boxed{110}$ $\boxed{010} \rightleftharpoons \boxed{101}$ $k=3$
4		$\boxed{0000}$ $\boxed{1111}$ $k=0$ $\boxed{0010} \leftarrow \boxed{0100}$ $k=2$ $\downarrow \quad \uparrow$ $\boxed{0001} \rightarrow \boxed{1000}$ $m=4$ $\boxed{1101} \leftarrow \boxed{1011}$ $\downarrow \quad \uparrow$ $\boxed{1110} \rightarrow \boxed{0111}$ $\boxed{1001} \leftarrow \boxed{0011}$ $\downarrow \quad \uparrow m=2$ $\boxed{1100} \rightarrow \boxed{0110}$ $\boxed{1010} \rightleftharpoons \boxed{0101}$ $k=4$		$\boxed{0011} \leftarrow \boxed{0111} \leftarrow \boxed{1111}$ $k=1$ $\downarrow \quad \uparrow$ $\boxed{0001} \quad \boxed{1110}$ $\downarrow \quad \uparrow$ $\boxed{0000} \rightarrow \boxed{1000} \rightarrow \boxed{1100}$ $\boxed{1011} \leftarrow \boxed{0110} \leftarrow \boxed{1101}$ $k=3$ $\downarrow \quad \uparrow$ $\boxed{0101} \quad \boxed{1010}$ $\downarrow \quad \uparrow$ $\boxed{0010} \rightarrow \boxed{1001} \rightarrow \boxed{0100}$

Table 2: *Synchronous dynamics of Boolean networks with a single regulatory circuit for $n \leq 4$.*

cycle in X under $\psi_I \circ \sigma$ is $l = \mathrm{lcm}(\{m_i\}_{i=1,\dots,N})$, hence X contains $\frac{1}{l}\prod_{i=1}^{N} m_i$ cycles. The conclusion follows. □

From the Proposition we gather, for instance, that $\psi_I \circ \sigma$ admits fixed points if and only if all the cycles in the decomposition of σ satisfy $|I_i| = 0 \bmod 2$.

Example 3.2.9. Consider for instance the symmetry $f = \psi_I \circ \sigma$ for $n = 5$ with $I = \{3\}$ and $\sigma = (123)(45)$. The regulatory graph is therefore given by a negative circuit of length 3 and a positive circuit of length 2. The synchronous dynamics associated to $\psi_{\{3\}} \circ \sigma_1 = (123)$ and $\sigma_2 = (45)$ are described in Table 2: for the first map we have one cycle of length 6 and one of length 2, i.e. $\gamma_1(6) = \gamma_1(2) = 1$, whereas for the second map we have two fixed points and one cycle of length 2, so that $\gamma_2(1) = 2$ and $\gamma_2(2) = 1$. $\gamma_1(m)$ and $\gamma_2(m)$ are zero for any other value of m. Following Proposition 3.2.8, we have that the synchronous dynamics of f admits cycles of length 2 and length 6. The cycles of length 2 are $\frac{1}{2}(\gamma_1(2)\gamma_2(1)2 + \gamma_1(2)\gamma_2(2)4) = 4$, whereas the cycles of length 6 are $\frac{1}{6}(\gamma_1(6)\gamma_2(1)6 + \gamma_1(6)\gamma_2(2)12) = 4$:

$$
\begin{array}{lll}
\begin{array}{ccccc} 00100 & \leftarrow & 01100 & \leftarrow & 11100 \\ \downarrow & & & & \uparrow \\ 00000 & \rightarrow & 10000 & \rightarrow & 11000 \end{array} &
\begin{array}{ccccc} 00101 & \leftarrow & 01110 & \leftarrow & 11101 \\ \downarrow & & & & \uparrow \\ 00010 & \rightarrow & 10001 & \rightarrow & 11010 \end{array} &
\begin{array}{c} 01001 \rightleftharpoons 10110 \\ 01000 \rightleftharpoons 10100 \end{array} \\
\begin{array}{ccccc} 00111 & \leftarrow & 01111 & \leftarrow & 11111 \\ \downarrow & & & & \uparrow \\ 00011 & \rightarrow & 10011 & \rightarrow & 11011 \end{array} &
\begin{array}{ccccc} 00001 & \leftarrow & 00110 & \leftarrow & 01101 \\ \downarrow & & & & \uparrow \\ 10010 & \rightarrow & 11001 & \rightarrow & 11110 \end{array} &
\begin{array}{c} 01010 \rightleftharpoons 10101 \\ 01011 \rightleftharpoons 10111 \end{array}
\end{array}
$$

3.2.2 *Asynchronous dynamics*

Having analysed the synchronous behaviours of maps of the form $\psi_I \circ \sigma$, in this section we want to give a characterisation of their asynchronous dynamics. The following proposition shows that the cardinality of $I(x)$ can only change by 0 or 2 at each step.

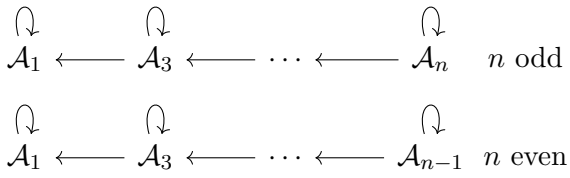


Figure 3: *Asynchronous transitions between the sets $\mathcal{A}_k$ for negative circuits.*

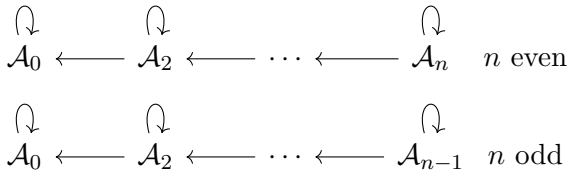


Figure 4: *Asynchronous transitions between the sets $\mathcal{A}_k$ for positive circuits.*

Proposition 3.2.10. *Consider a map $\psi_I \circ \sigma$, a state $x \in \mathbb{B}^n$ and an index $j \in I(x)$ such that $\sigma(j) \neq j$. Then*

(i) if $\sigma(j) \in I(x)$, then $I(\bar{x}^j) = I(x) \setminus \{j, \sigma(j)\}$;

(ii) if $\sigma(j) \notin I(x)$, then $I(\bar{x}^j) = (I(x) \setminus \{j\}) \cup \{\sigma(j)\}$.

Proof. From (15) we find

$$\begin{cases} f_i(\bar{x}^j) & = \overline{f_i(x)} \quad \text{if } i = \sigma(j), \\ f_i(\bar{x}^j) & = f_i(x) \quad \text{if } i \neq \sigma(j). \end{cases}$$

In particular, we have that $j \notin I(\bar{x}^j)$. In addition, since $f_{\sigma(j)}(\bar{x}^j) \neq f_{\sigma(j)}(x)$, and $\bar{x}^j_{\sigma(j)} = x_{\sigma(j)}$, we find

$$\sigma(j) \in I(x) \Leftrightarrow \sigma(j) \notin I(\bar{x}^j).$$

Finally, since $f_i(\bar{x}^j) = f_i(x)$ and $\bar{x}^j_i = x_i$ for $i \neq j$, $i \neq \sigma(j)$, we get

$$i \in I(x) \Leftrightarrow i \in I(\bar{x}^j).$$

□

From Proposition 3.2.10 we can deduce the following: the states $x \in \mathcal{A}_k$ with $I(x) \cap \sigma(I(x)) \neq \emptyset$ have at least a successor in $\mathcal{A}_{k-2}$. If $x \in \mathcal{A}_k$ and $\sigma(I(x)) = I(x)$, with $k > 1$, then all of the k successors of x are in $\mathcal{A}_{k-2}$; otherwise, x admits at least a successor in $\mathcal{A}_k$.

Consider now maps $\psi_I \circ \sigma$ with σ consisting of a single circuit. In this case, all the sets $\mathcal{A}_k$ with $0 < k < n$ are strongly connected: given a state $x \in \mathcal{A}_k$, and a subset of indices $J \in \mathcal{I}_k$, there is a path from x to a state y with $I(y) = J$; in addition, x can reach the other state y with $I(y) = I(x)$. In particular, the attractors of the dynamics are either the two fixed points in $\mathcal{A}_0$ for positive circuits, or the unique cyclic attractor in $\mathcal{A}_1$, for negative circuits. A summary of the asynchronous transitions between the sets $\mathcal{A}_k$ is represented in Figures 3 and 4.

Suppose that the permutation σ decomposes into cycles $\sigma_1 \cdots \sigma_N$ on the indices $L_1, \ldots, L_N$ respectively, with $I_i = I \cap L_i$, $i = 1, \ldots, N$. For each state x, each asynchronous transition connects x to a neighbour state $\bar{x}^j$; if j is in L_i, this means that $(\psi_{I_i} \circ \sigma_i(x))_j \neq x_j$, whereas $(\psi_{I_h} \circ \sigma_h(x))_k = x_k$ for any $k = 1, \ldots, n$, $k \neq j$ and $h = 1, \ldots, N$, $h \neq i$. In other words, the asynchronous state transition graph of $\psi_I \circ \sigma$

is the Cartesian product of the asynchronous state transition graphs of the $\psi_{I_i} \circ \sigma_i$, $i = 1, \dots, N$. In particular, the attractors of the asynchronous dynamics are fixed points if and only if all the circuits are positive. In this case, there are 2^N fixed points. On the other hand, if all the circuits are negative, then the asynchronous dynamics admits exactly one cyclic attractor. If N^+ of the circuits are positive, then there are 2^{N^+} attractors, which are all cyclic attractors if at least one circuit is negative.

3.2.3 *Regulatory graphs*

The next point we want to clarify is how regulatory graphs of Boolean networks change under conjugation with a symmetry. Here we show that the structure of the graphs is preserved, and, albeit the signs of corresponding edges can differ, the signs of the circuits are preserved.

Proposition 3.2.11. *Consider maps $U = \psi_I \circ \sigma$ and $f : \mathbb{B}^n \to \mathbb{B}^n$. Then,*

(i) for each $x \in \mathbb{B}^n$, the graphs $G_f(x)$ and $G_{f^U}(U(x))$, seen as unlabelled directed graphs, are isomorphic. In addition, corresponding cycles have the same sign.

(ii) the graphs G_f and G_{f^U}, seen as unlabelled directed graphs, are isomorphic. In addition, corresponding cycles have the same sign.

Proof. The graph $G_{f^U}(U(x))$ contains the interaction $\sigma(j) \to \sigma(i)$ if and only if $f^U_{\sigma(i)}(\overline{U(x)}^{\sigma(j)}) \neq f^U_{\sigma(i)}(U(x))$. Since $\overline{U(x)}^{\sigma(j)} = U(\bar{x}^j)$, we have from (11) that $f^U_{\sigma(i)}(\overline{U(x)}^{\sigma(j)}) = \overline{f(\bar{x}^j)}_i^{\sigma^{-1}(I)}$, hence the graph $G_{f^U}(U(x))$ contains the interaction $\sigma(j) \to \sigma(i)$ if and only if $f_i(\bar{x}^j) \neq f_i(x)$, i.e. if and only if $j \to i$ is an interaction in $G_f(x)$.

To describe how the signs of the edges change under U, let us call D and P the signed Jacobians of ψ_I and σ, respectively, as described in examples 3.1.6 and 3.1.7. By Proposition 3.1.9, points (i) and (iii), the signed Jacobian of f^U at $U(x)$ is given by $\mathfrak{J}_{f^U}(U(x)) = DP\mathfrak{J}_f(x)P^TD$, i.e., $(\mathfrak{J}_{f^U}(U(x)))_{ij} = (-1)^{\chi_I(i)+\chi_I(j)}(\mathfrak{J}_f(x))_{\sigma^{-1}(i)\sigma^{-1}(j)}$ for all $i, j \in \{1, \dots, n\}$. In other words, a permutation of the coordinates does not change the sign of any edge, whereas under conjugation by the map ψ_I, the sign of an edge $j \to i$ changes if and only if one and only one of the indices i, j is in I. Hence, if

$$i_1 \xrightarrow{s_1} i_2 \xrightarrow{s_2} i_3 \to \cdots \to i_{k-1} \xrightarrow{s_{k-1}} i_k \xrightarrow{s_k} i_{k+1} = i_1$$

is a cycle in G_f (possibly local), then the sign of the corresponding cycle in G_{f^U} is

$$\prod_{j=1}^{k} s_j(-1)^{\chi_I(i_j)+\chi_I(i_{j+1})} = \prod_{j=1}^{k} s_j,$$

which concludes. □

3.3 REGULATORY CIRCUITS AND ASYMPTOTIC BEHAVIOUR

To motivate the work of the next two chapters, we summarise some results concerning the identification of asymptotic properties of the dynamics of Boolean networks from their regulatory structure. The treatment here is not exhaustive; for other references see for instance the survey [PR12].

As seen in [RMCT03] and recapitulated in Sections 3.2.1 and 3.2.2, if the regulatory graph of a Boolean network consists of a single positive circuit, then the associated

asynchronous dynamics admits two fixed states, and no additional attractors. If the regulatory graph is composed of a single negative circuit instead, the asynchronous state transition graph admits a unique cyclic attractor. Other characterisations of the dynamics associated to particular types of regulatory graphs are provided in [DR12], where graphs consisting of multiple circuits that share a common component are considered, and in [RMT16], where the authors provide a characterisation of the synchronous and asynchronous dynamics of Boolean networks with regulatory graphs consisting of a circuit with the addition of a chord.

René Thomas conjectured that positive circuits in the regulatory graph are necessary for the presence of multiple steady states (first conjecture), and that negative circuits are necessary for sustained oscillations (second conjecture) [Tho81]. Since then, a series of results have been proved that connect the asymptotic behaviour of regulatory networks to properties of the circuits of the regulatory graph.

Shih and Dong [SD05] established that, if no local regulatory circuit exists, then the map admits a unique fixed point. The result was extended to the multilevel setting by Richard [Ric08].

Remy et al. [RRT08] proved that, in the Boolean case, the presence of at least two fixed points requires the existence of a local positive circuit in the regulatory graph. The proof of the first conjecture was then generalised by Richard and Comet [RC07]: if a multilevel or Boolean map admits multiple attractors, then its regulatory graph admits a local positive circuit.

A version of the second conjecture showing the existence of a negative circuit in the global regulatory graph was also proved by Remy et al. [RRT08], for the case of an attractive cycle in the Boolean setting. The result was later generalised by Richard [Ric10], who showed that a negative circuit in the regulatory graph is required for the existence of a cyclic attractor, in the Boolean and multilevel case.

Sufficient conditions for multistationarity and oscillations in terms of the regulatory circuits have also been investigated: in [RR08], Remy and Ruet show that if a local circuit is *globally minimal*, i.e. minimal in the global regulatory graph with respect to the inclusion, then the restriction of the Boolean network to the coordinates involved in the circuit admits two fixed points if the circuit is positive, and an attractive cycle if the circuit is negative.

These and other results have inspired some attempts at classifying circuits according to their established connections with the asymptotic properties of the network dynamics. In [CNR+13] several definitions of circuit *functionality* are given, that organise circuits according to the subgraphs of the regulatory graph they appear in; for instance, a type 1 circuit is a local circuit, and type 2 is a stronger form of functionality, requiring the circuit to be contained in all the local graphs of a subcube. The analysis emphasises the difficulty in establishing when a circuit can be regarded as "generating" a certain behaviour, and serves to highlight some asymmetries between the results about positive and negative circuits.

Recently, the necessary conditions for multistationarity and oscillations in [RRT08] and [Ric10], as well as the result of [SD05] have found a common explanation in a result due to Ruet [Rue16]. A Boolean network f is said to admit a *mirror pair* if there exists a state $x \in \mathbb{B}^n$ such that $f|_{x[I]}(\bar{x}^I) = \overline{f|_{x[I]}(x)}^I$. A mirror pair for f exists for instance if f has a cyclic attractor (in particular, if it has no fixed points) or if f admits multiple attractors. Ruet [Rue16] shows that if f admits a mirror pair, then there are two states $y \neq z \in \mathbb{B}^n$ such that $G_f(y)$ and $G_f(z)$ admit a circuit. If the map admits two attractors, then the circuits are positive. If the map admits a cyclic attractor, on the other hand, then it also admits a circuit at two different local regulatory graphs, but no conclusion can be established on the sign of the circuit.

A first result to target the question of whether a *local negative* circuit is necessary for the presence of a cyclic attractor in the Boolean case is Richard's Theorem on non-expansive networks [Ric11]. A Boolean network f is non-expansive if the Hamming distance of any pair of states cannot increase under f. The result of [Ric11] shows that if a non-expansive network does not have any fixed point, then it admits two local regulatory graphs with a common negative circuit. In general, however, cyclic attractors are compatible with the absence of local negative circuits. This was first shown in [Ric10] in the multilevel case. In [Rue17] Ruet gave counterexamples in the Boolean case, in the form of maps with an antipodal attractive cycle (see Definition 5.1.1 in Section 5.1.2) for $n \geq 7$, and in the form of an and-net with no fixed point, for $n = 12$ (in an and-net, each component is a conjunction of the x_i or their negations). In Section 5.1, we present other counterexamples. One counterexample for $n = 6$ is found by converting Richard's multilevel counterexample to Boolean, using the conversion map described in Chapter 4. An alternative counterexample was created by Fauré and Kaji [FK18] with a similar approach (a description of their method is given in Section 4.4). In Section 5.1.2 we give a variation of Ruet's construction [Rue17], that works for $n \geq 6$. We then study the cases with $n \leq 5$ by writing the question as a Boolean satisfiability problem, and, using a satisfiability solver, we find that, in Boolean networks with 5 regulatory components or fewer, a local negative circuit is necessary for the existence of a cyclic attractor.

4

MULTILEVEL TO BOOLEAN REGULATORY NETWORKS

Behaviours of gene regulatory networks are sometimes more appropriately described by considering more than two expression levels for some of the regulatory components [DRC11]. Every multivalued network can, however, be seen as a Boolean network by increasing the number of variables [VH79, DRC11]. In this chapter we consider multilevel discrete systems and the generalisation of some of the definitions given in Chapter 3. The conversion from multivalued to Boolean dynamics studied in [VH79, DRC11] defines a Boolean map on a subset of the Boolean states, called the "admissible states". We show that versions of the conjectures of Thomas can be stated for these partial Boolean maps (Section 4.2). We then present a new approach to the conversion of asynchronous dynamics of multivalued networks to Boolean, that defines a Boolean map on the entire state space, while preserving the attractors and the regulatory circuits. We show that the conversion can be used to give multivalued versions of results on Boolean networks (Section 4.3), whereas in Chapter 5 we use the conversion to create a Boolean map with a cyclic attractor and no local negative circuit, starting from a known multivalued example. A comparison to an alternative conversion approach recently introduced by Fauré and Kaji [FK18] is given in Section 4.4.

Notations introduced in this chapter

$\mathcal{X}$ will denote a set of the form $\{0,\dots,m_1\}\times\cdots\times\{0,\dots,m_n\}$, for some $n\in\mathbb{N}$, $m_1,\dots,m_n\in\mathbb{N}$. Given $x,y\in\mathcal{X}$, we will write $I(x,y)$ for the set of indices $i\in\{1,\dots,n\}$ such that $x_i\neq y_i$. We denote by e^j the element of $\mathcal{X}$ with $\mathrm{e}^j_j=1$ and $\mathrm{e}^j_i=0$ for all $i\neq j$, with $j\in\{1,\dots,n\}$.

Given some directed labelled graphs $(G_i)_{i\in A}$ indexed on some set A and each with set of nodes N, we will denote by $\bigcup_{i\in A}G_i$ the graph with nodes the set N, and an edge from a node a to a node b with label l if there exists an i such that the graph G_i admits an edge from a to b with label l.

4.1 MULTILEVEL GENE REGULATORY NETWORKS

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 $\mathcal{X}_i=\{0,\dots,m_i\}$, where n is the number of genes, and $m_i\in\mathbb{N}$ is the maximum level of expression for the gene i, and the synchronous dynamics of the regulatory network is given by a map $f:\mathcal{X}\to\mathcal{X}$.

To give the definition of asynchronous dynamics in the multivalued case, we first define the map $\tilde{f}:\mathcal{X}\to\mathcal{X}$ by setting

$$\tilde{f}_i(x)=x_i+\mathrm{sign}(f_i(x)-x_i).$$

The map $\tilde{f}$ is the *stepwise* 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 stepwise if it coincides with its stepwise version $\tilde{f}$. In addition, define for each $i \in \{1, \dots, n\}$ the map

$$F^i : \mathcal{X} \to \mathcal{X}$$
$$F^i : x \mapsto F^i(x) = (x_1, \dots, x_{i-1}, \tilde{f}_i(x), x_{i+1}, \dots, x_n).$$

Then the *asynchronous state transition graph* AD_f is a graph with nodes the set $\mathcal{X}$ and edges $(x, F^i(x))$ for all $x \in \mathcal{X}$, $i \in \{1, \dots, n\}$ such that $x_i \neq f_i(x)$. In other words, the expression of one gene only can change at each iteration, and the change is stepwise, i.e. 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 $f_i(x) < x_i$.

Unlike in the Boolean case, in the multivalued case multiple synchronous dynamics admit the same asynchronous state transition graph.

4.1.1 *Regulatory graphs*

The following definition of regulatory graph can be found and in [Ric10] (Definition 8).

Definition 4.1.1. The *(local) regulatory graph* at $x \in \mathcal{X}$ for the network defined by f is the finite labeled directed graph $G_f(x)$ with nodes the set $\{1, \dots, n\}$, 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 $x + s_1 e^j \in \mathcal{X}$, 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 defined as $G_f = \bigcup_{x \in X} G_f(x)$.

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

Definition 4.1.2. If $I \subseteq \{1, \dots, n\}$ 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 $\mathcal{A}$ is a subset of the state space $\mathcal{X}$, and $x \in \mathcal{A}$, we write $G_f^{I_{\mathcal{A}}(x)}(x)$ 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 $\mathcal{A}$.

Richard and Comet [RC07] 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 3.3).

Definition 4.1.3. The *non-usual local regulatory graph* $\tilde{G}_f(x, y)$ of the map $f : \mathcal{X} \to \mathcal{X}$ at a state $x \in \mathcal{X}$ *with variations in direction of* y is a graph on $\{1, \dots, n\}$, with an edge from a node j to a node i of sign s, with $i, j \in I(x, y)$, whenever $x + \epsilon_j e^j \in \mathcal{X}$ and $s = \epsilon_j \text{sign}(f_i(x + \epsilon_j e^j) - f_i(x)) \neq 0$, 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 $x \in \mathcal{X}$ is clearly a subgraph of $G_f(x)$. Moreover, non-usual local regulatory graphs are identified by the asynchronous dynamics ([RC07], Remark 1).

The following definition of regulatory graph was introduced in [Ric10], where it is used to prove a multivalued version of Thomas' second conjecture (see Section 3.3).

Definition 4.1.4. ([Ric10], Definition 5) $\mathcal{G}_f(x)$ is a graph on $\{1,\dots,n\}$ that contains an edge from j to i of sign $s \in \{-1,1\}$ if

(i) $\operatorname{sign}(f_i(x) - x_i) \neq \operatorname{sign}(f_i(F^j(x)) - F_i^j(x))$ and

(ii) $s = \operatorname{sign}(f_j(x) - x_j)\operatorname{sign}(f_i(F^j(x)) - F_i^j(x))$.

Notice that, in contrast to [Ric10], we consider this definition only for maps F^i that are stepwise. The following lemma shows that the graph $\mathcal{G}_f(x)$ of the last definition is a subgraph of the graph $G_f(x)$ (it is a variation on Lemma 6 in [Ric10]).

Lemma 4.1.5. *For a map $f : \mathcal{X} \to \mathcal{X}$ and for all $x \in \mathcal{X}$, $\mathcal{G}_f(x)$ is a subgraph of $G_f(x)$.*

Proof. Let $j \to i$ be an edge of $\mathcal{G}_f(x)$ of sign s. Then from point (ii) of Definition 4.1.4 we have that $f_j(x) \neq x_j$ and $f_i(F^j(x)) \neq F_i^j(x)$. Therefore we can write $f_j(x) - x_j = s_1 k_1$, $F^j(x) = x + s_1 \mathrm{e}^j$ and $f_i(F^j(x)) - F_i^j(x) = s_2 k_2$, 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 $\operatorname{sign}(f_i(x + s_1\mathrm{e}^j) - f_i(x)) = s_2$.

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

$$f_i(x + s_1\mathrm{e}^j) - f_i(x) = f_i(x + s_1\mathrm{e}^j) - F_i^j(x) + x_i - f_i(x) = s_2(k_2 + h_2).$$

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

$$\begin{aligned} f_i(x + s_1\mathrm{e}^j) - f_i(x) &= f_i(x + s_1\mathrm{e}^j) - F_i^j(x) + x_i + s_1 - f_i(x) \\ &= s_2(k_2 + h_2) + s_1 = s_2(k_2 + h_2 - 1) \neq 0, \end{aligned}$$

which concludes the proof. □

Remark 4.1.6. The graph $\mathcal{G}_f(x)$ contains edges of $G_f(x)$ that are calculated for directions j such that $f_j(x) \neq x_j$. In other words, $\mathcal{G}_f(x)$ is calculated by considering only the states $x \pm \mathrm{e}^j$ such that $(x, x \pm \mathrm{e}^j)$ is in the asynchronous state transition graph AD_f of f.

We conclude this section by asking how the regulatory graph G_f of a discrete multivalued map f and the regulatory graph $G_{\tilde{f}}$ of its stepwise version $\tilde{f}$ compare. The example in Figure 5 shows that the regulatory graph $G_{\tilde{f}}$ 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 $G_{\tilde{f}}$. We can establish, however, the following relationship between the two regulatory graphs.

Proposition 4.1.7. *Let $\tilde{f}$ be the stepwise version of a map $f : \mathcal{X} \to \mathcal{X}$. If the regulatory graph $G_{\tilde{f}}(x)$ 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 $G_{\tilde{f}}(x)$ with $j \neq i$ and sign s, then from $\operatorname{sign}(\tilde{f}_i(x + s_1\mathrm{e}^j) - \tilde{f}_i(x)) = s \cdot s_1$ we get

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

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

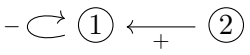
$$f_i(x + s_1\mathrm{e}^j) - f_i(x) = f_i(x + s_1\mathrm{e}^j) - x_i - (f_i(x) - x_i) = s \cdot s_1(h + k),$$

which implies

$$\operatorname{sign}(f_i(x + s_1\mathrm{e}^j) - f_i(x)) = s \cdot s_1,$$

i.e., $G_f(x)$ contains an edge from j to i of sign s. □

$x \in \mathcal{X}$	$f(x)$
0 0	1 2
0 1	2 2
0 2	2 2
1 0	1 2
1 1	1 2
1 2	1 2
2 0	1 2
2 1	1 2
2 2	1 2



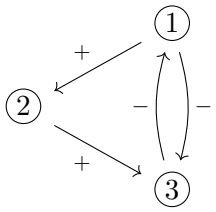
(a)

$x \in \mathcal{X}$	$\tilde{f}(x)$
0 0	1 1
0 1	1 2
0 2	1 2
1 0	1 1
1 1	1 2
1 2	1 2
2 0	1 1
2 1	1 2
2 2	1 2

(b)

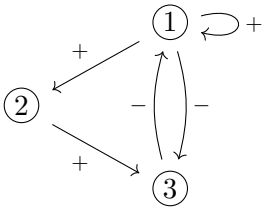
Figure 5: *(a): A map $f : \{0,1,2\}^2 \to \{0,1,2\}^2$ and the corresponding regulatory graph. (b) Synchronous discrete stepwise version $\tilde{f}$ of f, with the associated regulatory graph.*

$x \in \mathcal{X}$	$f(x)$
0 0 0	2 0 1
0 0 1	0 0 1
0 1 0	2 0 1
0 1 1	0 0 1
1 0 0	2 0 0
1 0 1	0 0 0
1 1 0	2 0 1
1 1 1	0 0 1
2 0 0	2 1 0
2 0 1	0 1 0
2 1 0	2 1 1
2 1 1	0 1 1



(a)

$x \in \mathcal{X}$	$\tilde{f}(x)$
0 0 0	1 0 1
0 0 1	0 0 1
0 1 0	1 0 1
0 1 1	0 0 1
1 0 0	2 0 0
1 0 1	0 0 0
1 1 0	2 0 1
1 1 1	0 0 1
2 0 0	2 1 0
2 0 1	1 1 0
2 1 0	2 1 1
2 1 1	1 1 1



(b)

Figure 6: *(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 [DRC11]. (b) Synchronous discrete stepwise version $\tilde{f}$ of f, with the corresponding regulatory graph.*

Example 4.1.8. Didier et al. [DRC11] analyse a multivalued representation of a p53/Mdm2 network. The synchronous dynamics for this network is in Figure 6a, together with its regulatory graph G_f. In Figure 6b, we show the stepwise version $\tilde{f}$ of this map, with the resulting regulatory graph. The regulatory graph of the stepwise version contains a positive autoregulation for the first component that is not part of the regulatory graph of the original map.

4.2 ADMISSIBLE STATES

Research efforts on discrete maps often focus on the Boolean case only, and many tools for the analysis of gene regulatory networks are often designed 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 [VH79], and shown by Didier et al. [DRC11] 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, $i \in \{1, \dots, n\}$, with $\mathcal{X}$ defined as in Section 4.1. Write $m = \sum_{i=1}^{n} m_i$ and consider the set $\mathcal{Y} = \{0,1\}^m$. We define m functions $\varphi_{i,j} : \mathcal{X} \to \{0,1\}$ as $\varphi_{i,j}(x) = \chi_{[j,m_i]}(x_i)$, for $i = 1, \dots, n$, $j = 1, \dots, m_i$, where χ_A is the indicator function of the set A.

We denote by φ the one-to-one map defined by Van Ham [VH79] and studied by Didier et al. [DRC11] that converts a multivalued discrete state of $\mathcal{X}$ to a Boolean state in $\mathcal{Y}$, and is defined by

$$\varphi(x_1, \dots, x_n) = (\varphi_{1,1}(x), \dots, \varphi_{1,m_1}(x), \varphi_{2,1}(x), \dots, \varphi_{2,m_2}(x), \dots, \varphi_{n,m_n}(x)).$$

For convenience, we will index the components of elements of $\mathcal{Y}$ with two indices, as for the components of φ; 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

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

Moreover, we will use the notation $\bar{y}^{i,j}$ 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 $\{(i,j)\}_{1 \le j \le m_i}$, for $i = 1, \dots, n$.

The set $\mathcal{A} = \varphi(\mathcal{X}) \subseteq \mathcal{Y}$ is called the set of *admissible states*. These are the states such that, if $y_{i,j} = 1$ for some $i = 1, \dots, n$, $j = 1, \dots, m_i$, then $y_{i,h} = 1$ for all $h = 1, \dots, j$. We will refer to the states in $\mathcal{Y} \setminus \mathcal{A}$ 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 $F^b : \mathcal{Y} \to \mathcal{Y}$ defined so that the following diagram is commutative:

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

i.e. F^b satisfies $F^b\big|_{\mathcal{A}} \circ \varphi = \varphi \circ f$. We will write $f^b : \mathcal{A} \to \mathcal{A}$ for the map $f^b = \varphi \circ f \circ \varphi^{-1}$. In addition, if x is an admissible state, we will write $G_{f^b}(x)$ for the graph $G_{F^b}^{I_{\mathcal{A}}(x)}(x)$, and G_{f^b} for the graph $\bigcup_{x \in \mathcal{A}} G_{f^b}(x)$.

Lemma 4.2.1. *Consider a Boolean conversion F^b of a map $f : \mathcal{X} \to \mathcal{X}$, and let x be a state in $\mathcal{X}$. If a local graph $G_{f^b}(\varphi(x))$ contains an edge from a node in I_j to a node in I_i with sign s, with $i, j \in \{1, \dots, n\}$, then the graph $G_f(x)$ contains an edge from j to i with sign s.*

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

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

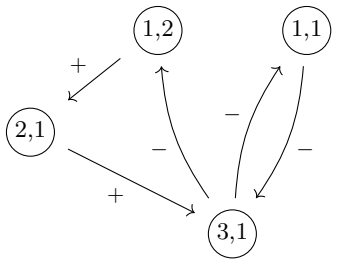
and

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

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

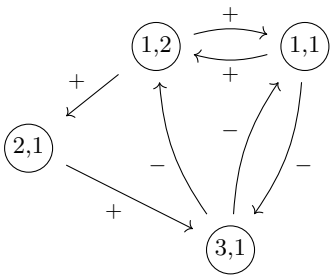
Lemma 4.2.2. *Consider a Boolean conversion F^b of a map $f : \mathcal{X} \to \mathcal{X}$, and let x be a state in $\mathcal{X}$. If the graph $G_f(x)$ contains an edge from j to i with sign s and variation s_1 in direction j, then the local graph $G_{f^b}(\varphi(x))$ contains edges from the node $(j, x_j + \frac{s_1+1}{2}) \in I_j$ to nodes $(i,k') \in I_i$, for all $k' \in \left]\min\{f_i(x), f_i(x + s_1 \mathrm{e}^j)\}, \max\{f_i(x), f_i(x + s_1 \mathrm{e}^j)\}\right]$, with sign s.*

$x \in \mathcal{X}$	$f^b(x)$
0 0 0 0	1 1 0 1
0 0 0 1	0 0 0 1
0 0 1 0	1 1 0 1
0 0 1 1	0 0 0 1
1 0 0 0	1 1 0 0
1 0 0 1	0 0 0 0
1 0 1 0	1 1 0 1
1 0 1 1	0 0 0 1
1 1 0 0	1 1 1 0
1 1 0 1	0 0 1 0
1 1 1 0	1 1 1 1
1 1 1 1	0 0 1 1



(a)

$x \in \mathcal{X}$	$\tilde{f}^b(x)$
0 0 0 0	1 0 0 1
0 0 0 1	0 0 0 1
0 0 1 0	1 0 0 1
0 0 1 1	0 0 0 1
1 0 0 0	1 1 0 0
1 0 0 1	0 0 0 0
1 0 1 0	1 1 0 1
1 0 1 1	0 0 0 1
1 1 0 0	1 1 1 0
1 1 0 1	1 0 1 0
1 1 1 0	1 1 1 1
1 1 1 1	1 0 1 1



(b)

Figure 7: *(a): Conversion to Boolean on the admissible states for the synchronous discrete dynamics f of the example in Figure 6, and the resulting regulatory graph. (b) Conversion to Boolean of the synchronous discrete stepwise version $\tilde{f}$ of f, with the corresponding regulatory graph.*

Proof. Suppose that $s = s_1 \mathrm{sign}(f_i(x + s_1 \mathrm{e}^j) - f_i(x))$ for some $s, s_1 \in \{-1, 1\}$, $i, j \in \{1, \ldots, n\}$. First observe that, if x and $x + s_1 \mathrm{e}^j$ are in $\mathcal{X}$, and $y = \varphi(x)$, then $y + s_1 \mathrm{e}^{j,k} = \varphi(x + s_1 \mathrm{e}^j)$, with $k = x_j + \frac{s_1+1}{2}$.

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

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

as required. □

Example 4.2.3. The conversion to Boolean on the admissible states for the map in Example 4.1.8 is in Figure 7a, together with the corresponding regulatory graph. In 7b is the conversion of the stepwise version of the map, restricted to the admissible states, with the corresponding regulatory graph.

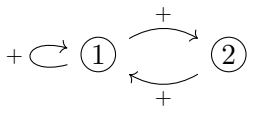
With the following example we observe that local circuits in the regulatory graph of f are not necessarily preserved by the conversion to Boolean.

Example 4.2.4. Consider the maps f and F defined on $\{0, 1, 2\} \times \{0, 1\}$ and $\{0, 1\}^3$ respectively, as in Figure 8. The map f is stepwise, 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 4.1.3 are considered. It is a consequence of the following lemma.

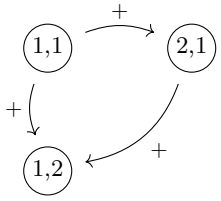
Lemma 4.2.5. *Consider a Boolean conversion F^b of a map $f : \mathcal{X} \to \mathcal{X}$, and let x be a state in $\mathcal{X}$. If the non-usual local regulatory graph $\tilde{G}_f(x, y)$ at x with variations in the direction of y contains an edge from j to i of sign s, and $\epsilon_k = \mathrm{sign}(y_k - x_k)$ for $k \in I(x, y)$, then the local graph $G_{f^b}^{I(\varphi(x),\varphi(y))}(\varphi(x))$ contains an edge from $(j, x_j + \frac{\epsilon_j+1}{2})$ to $(i, x_i + \frac{\epsilon_i+1}{2})$, with sign s.*

$x \in \mathcal{X}$		$f(x)$	
0	0	1	0
0	1	1	0
1	0	1	1
1	1	2	1
2	0	1	1
2	1	2	1



(a)

$y \in \mathcal{Y}$			$F(y)$		
0	0	0	1	0	0
0	0	1	1	0	0
0	1	0	1	0	0
0	1	1	1	0	0
1	0	0	1	0	1
1	0	1	1	1	1
1	1	0	1	0	1
1	1	1	1	1	1



(b)

Figure 8: *(a): Multivalued map $f : \{0,1,2\} \times \{0,1\} \to \{0,1,2\} \times \{0,1\}$ with regulatory graph admitting a local positive circuit (Example 4.2.4). (b): The regulatory graph of a conversion F of f to a Boolean dynamics does not admit any positive circuit.*

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

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

which gives

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

The conclusion follows from Lemma 4.2.2. □

Proposition 4.2.6. *If the non-usual local regulatory graph $\tilde{G}_f(x,y)$ of the map $f : \mathcal{X} \to \mathcal{X}$ at a state $x \in \mathcal{X}$ with variations in direction of y admits a circuit of sign s, then the local graph $G_{f^b}^{I(\varphi(x),\varphi(y))}(\varphi(x))$ admits a circuit of sign s.*

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 $\epsilon_k = \mathrm{sign}(y_k - x_k)$, $k \in I(x,y)$. By Lemma 4.2.5, the graph $G_{f^b}^{I(\varphi(x),\varphi(y))}(\varphi(x))$ contains an edge from $(i_h, x_h + \frac{\epsilon_h+1}{2})$ 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. □

The existence of a circuit in the local regulatory graph of f^b does not imply the existence of a regulatory circuit in the non-usual local regulatory graph of f. If the local circuit in G_{f^b} involves only one Boolean variable per regulatory component, then the non-usual regulatory graph of f contains a corresponding circuit.

Example 4.2.7. Consider the following multilevel stepwise map on $\{0,1,2\}^2$ and the corresponding Boolean version on the admissible states:

$x \in \mathcal{X}$		$f(x)$	
0	0	1	0
0	1	1	0
0	2	0	1
1	0	2	1
1	1	1	1
1	2	0	1
2	0	2	1
2	1	1	2
2	2	1	2

$y \in \mathcal{A}$				$f^b(y)$			
0	0	0	0	1	0	0	0
0	0	1	0	1	0	0	0
0	0	1	1	0	0	1	0
1	0	0	0	1	1	1	0
1	0	1	0	1	0	1	0
1	0	1	1	0	0	1	0
1	1	0	0	1	1	1	0
1	1	1	0	1	0	1	1
1	1	1	1	1	0	1	1

It can be seen that the non-usual regulatory graph does not admit any circuit. In particular, it does not admit any local circuit at 11. In contrast, the local regulatory graph of f^b admits the circuit $1,1 \to 2,1 \to 1,2 \to 2,2 \to 1,1$.

Proposition 4.2.8. *Consider $x \in \mathcal{X}$ and a map $f\colon \mathcal{X} \to \mathcal{X}$. If the local graph $G_{f^b}(\varphi(x))$ admits a circuit of sign s of the form $(i_1, j_1) \to \cdots \to (i_k, j_k) \to (i_{k+1}, j_{k+1}) = (i_1, j_1)$, with $i_h \neq i_l$ for all $h \neq l$, $h, l = 1, \ldots, k$, then there exists a $y \in \mathcal{X}$ such that the non-usual local regulatory graph $\tilde{G}_f(x, y)$ of f at a state $x \in \mathcal{X}$ with variations in direction of y admits a circuit of sign s.*

Proof. Suppose that the variations determined by $(i_1, j_1), \ldots, (i_k, j_k)$ are $s_1^1, \ldots, s_1^k$ respectively, and the signs of the edges are $s^1, \ldots, s^k$. Define $y = x + \sum_{h=1}^k s_1^h \mathrm{e}^{i_h}$. Then $\varphi(y) = \varphi(x) + \sum_{h=1}^k s_1^h \mathrm{e}^{i_h, j_h}$. Since the i_h are all different, $y_{i_h} \neq x_{i_h}$ for all $h = 1, \ldots, k$.

Since $s^h = s_1^h(f^b_{i_{h+1}, j_{h+1}}(\varphi(x) + s_1^h e^{i_h, j_h}) - f^b_{i_{h+1}, j_{h+1}}(\varphi(x)))$, we have that, for $h = 1, \ldots, k$, $s^h = s_1^h(\varphi_{i_{h+1}, j_{h+1}}(f(x + s_1^h \mathrm{e}^{i_h})) - \varphi_{i_{h+1}, j_{h+1}}(f(x))$, so that $i_1 \xrightarrow{s_1} \cdots \to i_k \xrightarrow{s_k} i_{k+1} = i_1$ is a circuit in $G_f(x)$. To conclude that the circuit is in $\tilde{G}_f(x, y)$, we have to show that, for $h = 1 \ldots, k$,

$$\min\{f_{i_{h+1}}(x), f_{i_{h+1}}(x + s_1^h \mathrm{e}^{i_h})\} < x_{i_{h+1}} + \frac{s_1^{h+1}}{2} < \max\{f_{i_{h+1}}(x), f_{i_{h+1}}(x + s_1^h \mathrm{e}^{i_h})\}. \tag{18}$$

Since the variation on component i_{h+1} is in the direction (i_{h+1}, j_{h+1}), we have that $x_{i_{h+1}} = j_{h+1} - 1$ or $x_{i_{h+1}} = j_{h+1}$. In both cases $j_{h+1} - 1 < x_{i_{h+1}} + \frac{s_1^{h+1}}{2} < j_{h+1}$. By hypothesis, $\varphi_{i_{h+1}, j_{h+1}}(f(x)) \neq \varphi_{i_{h+1}, j_{h+1}}(f(x + s_1^h \mathrm{e}^{i_h}))$, hence either $f_{i_{h+1}}(x)$ or $f_{i_{h+1}}(x + s_1^h \mathrm{e}^{i_h})$ must greater or equal to j_{h+1}, and the other must be smaller or equal to $j_{h+1} - 1$, which gives (18). □

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 $\{0, 1\}^m$. GINsim [CNT12], 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 4.2.3 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 stepwise version of f instead, as shown in the following proposition.

Proposition 4.2.9. *Let $f : \mathcal{X} \to \mathcal{X}$ define a multivalued regulatory network, with stepwise map $\tilde{f}$. Let $F^b : \mathcal{Y} \to \mathcal{Y}$ 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 $F^b_{i,j}(a) \neq a_{i,j}$, or, in other words, that $(a, \bar{a}^{i,j})$ is in AD_{F^b}. We want to prove that $\bar{a}^{i,j}$ is admissible. We will show that $\bar{a}^{i,j} = \varphi(x + \epsilon \mathrm{e}^i)$, with $\epsilon = \tilde{f}_i(x) - x_i$.

First observe that, since $F^b_{i,j}(a) \neq a_{i,j}$, we have $\bar{a}^{i,j}_{i,j} = F^b_{i,j}(a) = \tilde{f}^b_{i,j}(a) = \varphi_{i,j}(\tilde{f}(x)) = \varphi_{i,j}(x + \mathrm{sign}(f_i(x) - x_i)\mathrm{e}^i) = \varphi_{i,j}(x + \epsilon \mathrm{e}^i)$.

On the other hand, it follows from the definition of φ that

$$\varphi(x + \mathrm{e}^i) = \varphi(x) + \mathrm{e}^{i,x_i+1} \text{ and } \varphi(x - \mathrm{e}^i) = \varphi(x) - \mathrm{e}^{i,x_i}.$$

As a consequence, we have that $x_i = j - 1$ if $\epsilon = +1$, and $x_i = j$ if $\epsilon = -1$. In both cases we find $\bar{a}^{i,j}_{k,h} = \varphi_{k,h}(x + \epsilon \mathrm{e}^i)$ for all $(k, h) \neq (i, j)$.

□

Since $\mathcal{A}$ is a trap domain for F^b, to obtain a one-to-one correspondence between the attractors of AD_f and the attractors of AD_{F^b}, it is sufficient to ensure that, for each state in $\mathcal{Y} \setminus \mathcal{A}$, there exists a path to the set of admissible states. The following Proposition gives a sufficient condition: it requires that the non-admissible states are mapped to the admissible, and that the map is stepwise also outside of the admissible states.

Proposition 4.2.10. *Let $f : \mathcal{X} \to \mathcal{X}$ define a multivalued regulatory network. Consider a conversion to Boolean $F^b : \mathcal{Y} \to \mathcal{Y}$ that satisfies $F^b(x) \in \mathcal{A}$ and $|\sum_{j=1}^{m_i} F^b_{i,j}(x) - \sum_{j=1}^{m_i} x_{i,j}| \leq 1$ for all $x \in \mathcal{Y}$ and $i = 1, \ldots, n$. Then for each $x \in \mathcal{Y}$ there exists a path from x to $\mathcal{A}$ in AD_{F^b}.*

Proof. We proceed by induction on the distance $d(x, \mathcal{A})$. If $d(x, \mathcal{A}) = 0$, then x is in $\mathcal{A}$ and there is nothing to prove. If $d(x, \mathcal{A}) > 0$, we show that x admits a successor $y \in \mathcal{Y}$ in the asynchronous dynamics that satisfies $d(y, \mathcal{A}) = d(x, \mathcal{A}) - 1$, and the conclusion follows by the induction hypothesis. Since x is not admissible, there exists a pair of indices i, j such that $x_{i,j} < x_{i,j+1}$. Define $j_1 = \min\{1 \leq j \leq m_i | x_{i,j} = 0\}$, $j_2 = \max\{1 \leq j \leq m_i | x_{i,j} = 1\}$. Observe that, if I is a set of indices such that $\bar{x}^I \in \mathcal{A}$, then $(i, j_1) \in I$ or $(i, j_2) \in I$. In addition, since the distance between $\sum_{j=1}^{m_i} F^b_{i,j}(x)$ and $\sum_{j=1}^{m_i} x_{i,j}$ is at most 1 and $F(x)$ is admissible, we have $F^b_{i,j}(x) = 1$ for all $j < j_1$, $F^b_{i,j}(x) = 0$ for all $j > j_2$, and

$$\left| \sum_{k=j_1}^{j_2} (F^b_{i,k}(x) - x_{i,k}) \right| \leq 1. \tag{19}$$

Consider first the case with $F^b_{i,j_2}(x) = 1$, which implies $F^b_{i,k}(x) = 1$ for all $k \leq j_2$. Using 19, we find that $x_{i,k} = 1$ for all $j_1 < k \leq j_2$. Hence, if I is a set of indices such that $\bar{x}^I \in \mathcal{A}$ and $|I| = d(x, \mathcal{A})$, the cardinality of $I \cap \{(i, 1), \ldots, (i, m_i)\}$ is exactly one, and $y = \bar{x}^{i,j_1}$ is a successor for x in AD_{F^b} that satisfies $d(y, \mathcal{A}) = d(x, \mathcal{A}) - 1$.

Suppose now that $F^b_{i,j_2}(x) = 0$. If (i, j_2) belongs to a set of indices I such that $|I| = d(x, \mathcal{A})$ and $\bar{x}^I \in \mathcal{A}$, then $\bar{x}^{i,j_2}$ is a successor for x with $d(y, \mathcal{A}) = d(x, \mathcal{A}) - 1$. Otherwise, for any set of indices I with $|I| = d(x, \mathcal{A})$ and $\bar{x}^I \in \mathcal{A}$, we must have $(i, j_1) \in I$, and, in addition, there must be an index h with $j_1 < h < j_2$ such that $x_h = 1$. Using 19, we find that $F^b_{i,j_1}(x) = 1$, and $\bar{x}^{i,j_1}$ is the required successor.

□

For brevity, in the remainder of the work we will say that a map F^b is a *compatible* conversion of a map $f : \mathcal{X} \to \mathcal{X}$ to a Boolean dynamics if it is a conversion of the stepwise version of f, and, for each $x \in \mathcal{Y} \setminus \mathcal{A}$, there is a path in AD_{F^b} from x to the set of admissible states $\mathcal{A}$.

Exclusion of multiple steady states

Here we ask whether a version of the first conjecture holds for the map f^b defined on $\mathcal{A}$. Recall that the proof of the first conjecture was generalised by Richard and Comet to the multivalued discrete case [RC07]:

Theorem 4.2.11. *([RC07, 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.*

We have seen in Example 4.2.4 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 4.2.6). We can therefore state the following result on the existence of local positive circuits in presence of multistationarity for F^b.

Theorem 4.2.12. *Consider a stepwise map $f : \mathcal{X} \to \mathcal{X}$, 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 $G_{f^b}^{I(z,y)}(z)$ has a positive circuit.*

Proof. It is sufficient to combine Theorem 4.2.11 and Proposition 4.2.6. □

Example 4.2.13. To exclude the presence of multiple fixed points for the map in Example 4.2.4, it is sufficient, by Theorem 4.2.12, to consider the regulatory graph of the map $f^b : \mathcal{A} \to \mathcal{A}$, without specifying a particular extension for f^b to the non-admissible states 010 and 011.

Alternative proof

In this section, we prove a version of Theorem 4.2.11 in the Boolean case that gives an alternative proof for Theorem 4.2.12. We first introduce some new notation. If $I \subseteq \{1, \dots, m\}$ is a set indices, we say that a state x is I-fixed if $f_i(x) = x_i$ for all $i \in I$. Given two states $x, y \in \mathbb{B}^m$, we will write $I(x, y)$ for the set $\{i \in \{1, \dots, m\} \mid x_i \neq y_i\}$. We call a subset $\mathcal{A}$ of $\mathbb{B}^m$ an *admissible set* if it can be obtained as the set of admissible states corresponding to some multilevel space $\mathcal{X}$.

The following result clearly implies Theorem 4.2.12:

Theorem 4.2.14. *Consider an admissible set $\mathcal{A} \subseteq \mathbb{B}^n$ and a map $f \colon \mathcal{A} \to \mathcal{A}$. Suppose that $x, y \in \mathcal{A}$ are two distinct $I(x, y)$-fixed states. Then, there exists a state $z \in \mathcal{A}$ such that $G_f^{I(z,y) \cap I_{\mathcal{A}}(z)}$ has a positive circuit.*

The theorem gives some information on the location of positive circuits associated to multistationarity, when the regulatory network admits a trap set $\mathcal{A}$ in the form of an "admissible" set. Using Propositions 3.2.1 and 3.2.11 the conclusion can be extended to settings where $\mathcal{A}$ is a set obtained from an admissible set by application of a symmetry of the hypercube. To prove the result, we will need the following lemma on the signs of local circuits. Theorem 4.2.14 will be a consequence of Lemma 4.2.17.

Lemma 4.2.15. *[Ric11, Remark 1], [Rue16, Lemma 5.2] Let C be a circuit of $G_f(x)$ with set of vertices I. If the cardinality of $\{i \in I |\ f_i(x) \neq x_i\}$ is even (resp., odd), then C is a positive (resp. negative) circuit. In particular, if x is a fixed point, $G_f(x)$ cannot admit any negative circuit.*

Proof. Write the cycle C as $i_1 \to i_2 \to \cdots \to i_k \to i_1$. Then the sign s of the cycle is

$$\begin{aligned} s &= \frac{f_{i_2}(\bar{x}^{i_1}) - f_{i_2}(x)}{\bar{x}_{i_1}^{i_1} - x_{i_1}} \cdot \frac{f_{i_3}(\bar{x}^{i_2}) - f_{i_3}(x)}{\bar{x}_{i_2}^{i_2} - x_{i_2}} \cdots \frac{f_{i_1}(\bar{x}^{i_k}) - f_{i_1}(x)}{\bar{x}_{i_k}^{i_k} - x_{i_k}} \\ &= \frac{f_{i_2}(\bar{x}^{i_1}) - f_{i_2}(x)}{\bar{x}_{i_2}^{i_2} - x_{i_2}} \cdot \frac{f_{i_3}(\bar{x}^{i_2}) - f_{i_3}(x)}{\bar{x}_{i_3}^{i_3} - x_{i_3}} \cdots \frac{f_{i_1}(\bar{x}^{i_k}) - f_{i_1}(x)}{\bar{x}_{i_1}^{i_1} - x_{i_1}}. \end{aligned}$$

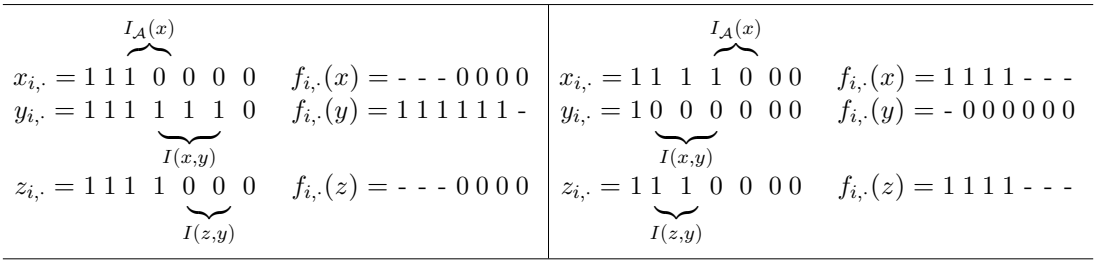


Figure 9: *If $x_{i,k} \neq y_{i,k}$ for some $k \in \{1, \dots, m_i\}$, the sets $I_{\mathcal{A}}(x)$ and $I(x,y)_i$ have one index in common. If $(i,k_i) \in I_{\mathcal{A}}(x) \cap I(x,y)_i$ and $z = \bar{x}^{i,k_i}$, then $I(z,y)_i = I(x,y)_i \setminus \{(i,k_i)\}$. If x is $I(x,y)$-fixed, z is $I(z,y)$-fixed.*

The conclusion follows from the fact that each term $\frac{f_{i_j}(\bar{x}^{i_{j-1}}) - f_{i_j}(x)}{\bar{x}^{i_j}_{i_j} - x_{i_j}}$ of the product is negative if and only if $f_{i_j}(x) \neq x_{i_j}$. □

Lemma 4.2.16. *Consider an admissible set $\mathcal{A} \subseteq \mathbb{B}^m$ and a map $f\colon \mathcal{A} \to \mathcal{A}$. Suppose that $x, y \in \mathcal{A}$ are two distinct states such that x is $I(x,y)$-fixed. If $G_f^{I(x,y)\cap I_{\mathcal{A}}(x)}(x)$ has no positive circuit, then there exists a $I(z,y)$-fixed state $z \in \mathcal{A}$ such that (z,x) is in AD_f.*

Proof. We denote the elements of $\{1, \dots, m\}$ with pairs of indices $I_i = \{(i,j)\}_{1 \leq j \leq m_i}$, $i = 1, \dots, n$, as identified by the definition of the set $\mathcal{A}$. First, observe that $I(x,y)$ is a union of intervals: if i is in $\{1, \dots, n\}$, $I(x,y)_i$ is given by the pairs of indices (i,k), with $1 \leq k \leq m_i$, such that $x_{i,k} + y_{i,k} = 1 \mod 2$. Moreover, for each $i = 1, \dots, n$, the set $(I_{\mathcal{A}}(x) \cap I(x,y))_i$ is either empty, or contains only one index (i,k_i) (see Figure 9).

If $G_f^{I(x,y)\cap I_{\mathcal{A}}(x)}(x)$ does not contain a positive circuit, then by Lemma 4.2.15, it cannot contain any circuit. Therefore it admits a node (j,k_j) that is the source of no edges. Define $z = \bar{x}^{j,k_j}$. Since (j,k_j) is in $I_{\mathcal{A}}(x)$, z is admissible. In addition, $I(z,y) = I(x,y) \setminus \{(j,k_j)\}$ (see Figure 9). Since j,k_j is not autoregulated, we have that $f_{j,k_j}(z) = f_{j,k_j}(\bar{x}^{j,k_j}) = f_{j,k_j}(x) = x_{j,k_j} \neq z_{j,k_j}$, and therefore (z,x) is in AD_{f^b}.

For all indices $i,k_i \neq j,k_j$ in $I(x,y) \cap I_{\mathcal{A}}(x)$, since j,k_j does not regulate i,k_i, we find $f_{i,k_i}(z) = f_{i,k_i}(x) = x_{i,k_i} = z_{i,k_i}$. This implies $f_{i,k_i'}(z) = f_{i,k_i'}(x) = x_{i,k_i'} = z_{i,k_i'}$ for any other index k_i' in $I(z,y)_i = I(x,y)_i$. In addition, if $x_{j,k_j} = 0$, we have that $I(x,y)_j = [k_j, k_j']$ for some k_j', and $z_{j,h} = f_{j,h}(z) = f_{j,h}(x) = 0$ for all $h \in [k_j + 1, k_j']$, whereas if $x_{j,k_j} = 1$, we have that $I(x,y)_j = [k_j', k_j]$ for some $k_j' < k_j$, and $z_{j,h} = f_{j,h}(z) = f_{j,h}(x) = 1$ for all $h \in [k_j', k_j - 1]$. We conclude that z is $I(z,y)$-fixed. □

Lemma 4.2.17. *Consider an admissible set $\mathcal{A} \subseteq \mathbb{B}^m$ and a map $f\colon \mathcal{A} \to \mathcal{A}$. Suppose that $x, y \in \mathcal{A}$ are two states such that x is $I(x,y)$-fixed, and that, for all states $z \in \mathcal{A}$, the graph $G_f^{I(z,y)\cap I_{\mathcal{A}}(z)}(z)$ does not admit a positive circuit. Then there exists a path in AD_f from x to y.*

Proof. We proceed by induction on the cardinality of $I(x,y)$. If $I(x,y)$ is empty, there is nothing to prove. Suppose that the cardinality of $I(x,y)$ is $k+1$, and that the statement of the Lemma is valid for cardinality equal to k. Since $G_f^{I(x,y)\cap I_{\mathcal{A}}(x)}$ contains no positive circuits, by Lemma 4.2.16 we can find an admissible state z such that (z,x) is in AD_f and the cardinality of $I(z,y)$ is k. Using the induction hypothesis, we conclude that there exists a path from x in y in the asynchronous dynamics. □

Exclusion of cyclic attractors

A version of the second conjecture in the multilevel case was proved by Richard [Ric10]:

Theorem 4.2.18. *([Ric10, Theorem 2]) If AD_f has a cyclic attractor $\mathcal{A}$, then the graph $\bigcup_{x\in\mathcal{A}} \mathcal{G}_f(x) \subseteq \mathcal{G}_f$ admits a negative circuit.*

The theorem gives some information on the location of the negative circuits: 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 contained in $\mathcal{A}$ is restricted to the graph $\bigcup_{x\in\mathcal{A}} \mathcal{G}_f(x)$. Moreover, following Remark 4.1.6 and Lemma 4.1.5, the search can be limited to the graph $\bigcup_{x\in\mathcal{A}} G_f^{I_\mathcal{A}(x)}(x)$: the relevant edges can be found by considering only directions and variations that are compatible with the set $\mathcal{A}$.

We can now observe that, if f is a discrete map and F^b is a conversion to a Boolean dynamics of its stepwise 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_{f^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.

Theorem 4.2.19. *Consider a stepwise map $f : \mathcal{X} \to \mathcal{X}$, and F^b a compatible conversion of f to a Boolean dynamics, with $f^b = F^b\big|_\mathcal{A}$. Suppose that AD_f admits a cyclic attractor. Then the graph $\bigcup_{x\in\mathcal{A}} G_{F^b}^{I_\mathcal{A}(x)}(x)$ admits a negative circuit.*

Proof. Consequence of Theorem 4.2.18 and Proposition 4.2.10. □

4.3 AN EXTENSION TO NON-ADMISSIBLE STATES

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 $\psi : \mathcal{Y} \to \mathcal{A} \subset \mathcal{Y}$ defined as follows. For each $i = 1, \dots, n$ and $j = 1, \dots, m_i$, we set

$$\psi_{i,j}(y_{1,1}, \dots, y_{1,m_1}, y_{2,1}, \dots, y_{n,m_n}) = \chi_{[j,m_i]}(\sum_{k=1}^{m_i} y_{i,k}). \tag{20}$$

The map ψ therefore sends a state y to the admissible state z such that, for each $i = 1, \dots, n$, $\sum_{j=1}^{m_i} y_{i,j} = \sum_{j=1}^{m_i} z_{i,j}$. 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 φ of the state 211.

Clearly ψ leaves the admissible states fixed. The next lemma states that the map ψ is *neighbour-preserving* in the sense of the definition introduced in [DRC11]: two direct neighbour states in $\mathcal{Y}$ are mapped by ψ to two direct neighbour states in $\mathcal{A}$.

Lemma 4.3.1. *For each $x \in \mathcal{Y}$, $i \in \{1, \dots, n\}$ and $j \in \{1, \dots, m_i\}$, there exists a unique $j' \in \{1, \dots, m_i\}$ such that $\psi(\bar{x}^{i,j}) = \overline{\psi(x)}^{i,j'}$. Moreover, $x_{i,j} = 0$ if and only if $\psi_{i,j'}(x) = 0$.*

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

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

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

Remark 4.3.2. Given $x \in \mathcal{A}$, $i \in \{1, \dots, n\}$ and $k, t \in \{1, \dots, m_i\}$, any state $y \in \mathcal{Y}$ with $\psi(y) = x$ and such that $y_{i,t} = x_{i,k}$ verifies $\psi(\bar{y}^{i,t}) = \bar{x}^{i,k}$.

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

$$F^b = f^b \circ \psi.$$

If f is stepwise, then F^b verifies the hypotheses of Proposition 4.2.10 and is a compatible conversion of f.

Example 4.3.3. Consider the regulatory network $\tilde{f}$ of Examples 4.1.8 and 4.2.3. In Figure 10a is the conversion of $\tilde{f}$ given by $F^b = \tilde{f}^b \circ \psi$. The non-admissible state 0100 is mapped by ψ 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 $\bar{x}^{1,1} = 1000$, $\bar{x}^{1,2} = 0100$, $\bar{x}^{2,1} = 0010$ and $\bar{x}^{3,1} = 0001$. The states $\bar{x}^{1,1}$, $\bar{x}^{2,1}$ and $\bar{x}^{3,1}$ 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:

$$\begin{aligned} x &= 0000, & \tilde{f}^b(x) &= 1001, \\ \bar{x}^{1,1} &= 1000, & \tilde{f}^b(\bar{x}^{1,1}) &= 1100, \\ \bar{x}^{2,1} &= 0010, & \tilde{f}^b(\bar{x}^{2,1}) &= 1001, \\ \bar{x}^{3,1} &= 0001, & \tilde{f}^b(\bar{x}^{3,1}) &= 0001. \end{aligned}$$

We identify the edges $(1,1) \xrightarrow{+} (1,2)$, $(1,1) \xrightarrow{-} (3,1)$ and $(3,1) \xrightarrow{-} (1,1)$. 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 $\bar{x}^{1,2} = 0100$:

$$\begin{aligned} x &= 0000, & \tilde{f}^b(x) &= 1001, \\ \bar{x}^{1,2} &= 0100, \quad F^b(\bar{x}^{1,2}) = & \tilde{f}^b(\bar{x}^{1,1}) &= 1100. \end{aligned}$$

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 $(1,2) \xrightarrow{+} (1,2)$ and $(1,2) \xrightarrow{-} (3,1)$ (see Figure 10b).

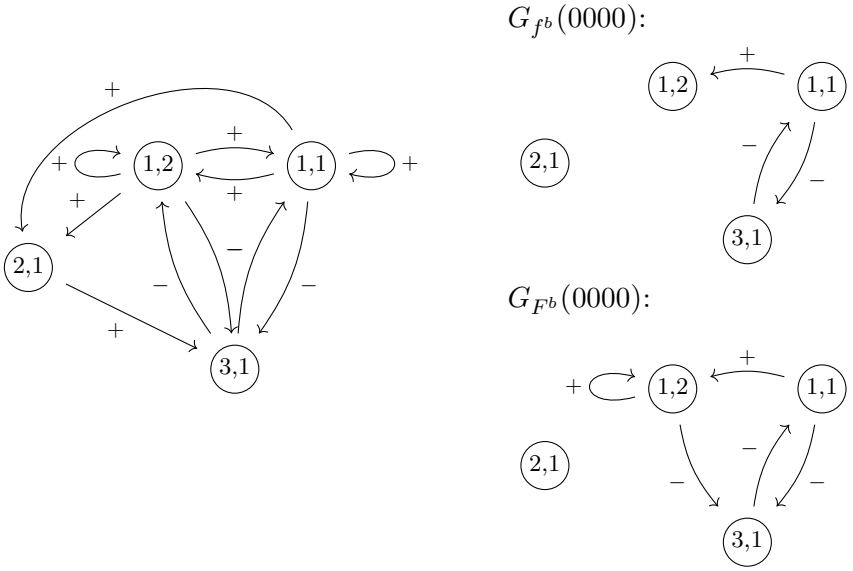
To find, for instance, the graph $G_{F^b}(y)$ at the non-admissible state $y = 0100$, we need to compare $\tilde{f}^b(y) = \tilde{f}^b(1000)$ to the images of the (admissible and non-admissible) neighbours of y:

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

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 $(2,1)$.

$x \in \mathcal{X}$	$F^b(x)$
0 0 0 0	1 0 0 1
0 0 0 1	0 0 0 1
0 0 1 0	1 0 0 1
0 0 1 1	0 0 0 1
0 1 0 0	1 1 0 0
0 1 0 1	0 0 0 0
0 1 1 0	1 1 0 1
0 1 1 1	0 0 0 1
1 0 0 0	1 1 0 0
1 0 0 1	0 0 0 0
1 0 1 0	1 1 0 1
1 0 1 1	0 0 0 1
1 1 0 0	1 1 1 0
1 1 0 1	1 0 1 0
1 1 1 0	1 1 1 1
1 1 1 1	1 0 1 1

(a)



(b)

Figure 10: *(a): The conversion to Boolean $F^b = f^b \circ \psi$ for the map of the example in Figure 6, and the resulting regulatory graph G_{F^b}. (b) Regulatory graphs $G_{f^b}(0000)$ and $G_{F^b}(0000)$.*

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

Lemma 4.3.4. *For each $x \in \mathcal{Y}$, if 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,h) in I_i with sign s, with $i,j \in \{1,\dots,n\}$, then the graph $G_{f^b}(\psi(x))$ contains an edge from some node in I_j to the node (i,h), with sign s.*

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

$$F^b(\bar{x}^{j,k}) - F^b(x) = f^b(\psi(\bar{x}^{j,k})) - f^b(\psi(x)).$$

Using Lemma 4.3.1, we find that

$$\begin{aligned}\frac{F^b_{i,h}(\bar{x}^{j,k}) - F^b_{i,h}(x)}{\bar{x}^{j,k}_{j,k} - x_{j,k}} &= \frac{f^b_{i,h}(\overline{\psi(x)}^{j,k'}) - f^b_{i,h}(\psi(x))}{\overline{\psi(x)}^{j,k'}_{j,k'} - \psi(x)_{j,k'}} \cdot \frac{\overline{\psi(x)}^{j,k'}_{j,k'} - \psi(x)_{j,k'}}{\bar{x}^{j,k}_{j,k} - x_{j,k}} \\ &= \frac{f^b_{i,h}(\overline{\psi(x)}^{j,k'}) - f^b_{i,h}(\psi(x))}{\overline{\psi(x)}^{j,k'}_{j,k'} - \psi(x)_{j,k'}},\end{aligned}$$

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

Lemma 4.3.5. *Suppose that the regulatory graph $G_{f^b}(x)$ at $x \in \mathcal{Y}$ admits an edge $(j,k) \to (i,h)$ of sign s. Take $y \in \mathcal{Y}$ such that $\psi(y) = x$, and $y_{j,t} = x_{j,k}$ for some $t \in \{1,\dots,m_j\}$. Then the graph $G_{F^b}(y)$ admits an edge from (j,t) to (i,h) of sign s.*

Proof. Using Remark 4.3.2, we have that

$$\frac{F^b_{i,h}(\bar{y}^{j,t}) - F^b_{i,h}(y)}{\bar{y}^{j,t}_{j,t} - y_{j,t}} = \frac{f^b_{i,h}(\psi(\bar{y}^{j,t})) - f^b_{i,h}(\psi(y))}{\bar{y}^{j,t}_{j,t} - y_{j,t}} = \frac{f^b_{i,h}(\bar{x}^{j,k}) - f^b_{i,h}(x)}{\bar{x}^{j,k}_{j,k} - x_{j,k}}.$$

□

We are now ready to show that, if the regulatory graph of the original multivalued map f has a circuit, then a corresponding circuit appears in the regulatory graph of the conversion $f^b \circ \psi$.

Theorem 4.3.6. *Consider a map $f : \mathcal{X} \to \mathcal{X}$, and define $F^b = f^b \circ \psi : \mathcal{Y} \to \mathcal{Y}$.*

(i) If, for some $x \in \mathcal{X}$, the graph $G_f(x)$ admits a circuit of sign s, then there exists a $y \in \mathcal{Y}$ with $\psi(y) = \varphi(x)$ such that $G_{F^b}(y)$ admits a circuit of sign s.

(ii) If the regulatory graph of f admits a circuit of sign s, then the regulatory graph of F^b admits a circuit of sign s.

Proof. Consider the case of a local circuit $i_1 \xrightarrow{s_1} i_2 \to \cdots \to i_k \xrightarrow{s_k} i_1$ at $G_f(x)$, $x \in \mathcal{X}$. By Lemma 4.2.2, the graph $G_{f^b}(\varphi(x))$ admits edges

$$(i_1, h_1) \xrightarrow{s_1} (i_2, j_2),\ (i_2, h_2) \xrightarrow{s_2} (i_3, j_3),\ \ldots,\ (i_{k-1}, h_{k-1}) \xrightarrow{s_{k-1}} (i_k, j_k),\ (i_k, h_k) \xrightarrow{s_k} (i_1, j_1).$$

Take $y \in \mathcal{Y}$ such that $\psi(y) = x$ and $y_{i_1,j_1} = x_{i_1,h_1}, \ldots, y_{i_k,j_k} = x_{i_k,h_k}$. By Lemma 4.3.5 the graph $G_{F^b}(y)$ contains the circuit

$$(i_1, j_1) \xrightarrow{s_1} (i_2, j_2) \xrightarrow{s_2} (i_3, j_3) \to \cdots \to (i_{k-1}, j_{k-1}) \xrightarrow{s_{k-1}} (i_k, j_k) \xrightarrow{s_k} (i_1, j_1).$$

The proof for the case of a circuit in the global regulatory graph proceeds similarly. □

Notice that if the circuit in the multivalued version is local, the corresponding circuit does not necessarily appear in the regulatory graph at the corresponding admissible state, but appears in one of the states mapped to the admissible state by ψ. This means that circuits in the multivalued map might not have a corresponding circuit in the graph of f^b, but do have a corresponding circuit in the regulatory graph of F^b (see Example 4.3.8).

Finally, we show that the converse also holds: any circuit in G_{F^b} corresponds to some cycle in G_f.

Theorem 4.3.7. *Consider a map $f : \mathcal{X} \to \mathcal{X}$, and define $F^b = f^b \circ \psi$.*

(i) If, for some $y \in \mathcal{Y}$, the graph $G_{F^b}(y)$ contains a circuit, then the regulatory graph $G_f(x)$ contains a circuit, where $x \in \mathcal{X}$ is such that $\varphi(x) = \psi(y)$. If the circuit in $G_{F^b}(y)$ is negative, then $G_f(x)$ contains a negative circuit.

(ii) If the regulatory graph of F^b contains a circuit, then the regulatory graph G_f of f contains a circuit. If the circuit in G_{F^b} is negative, then G_f contains a negative circuit.

Proof. If $(i_1, h_1) \xrightarrow{s_1} \cdots \xrightarrow{s_{k-1}} (i_k, h_k) \xrightarrow{s_k} (i_1, h_1)$ is a circuit in $G_{F^b}(x)$ for some state $x \in \mathcal{Y}$, then by Lemma 4.3.4 the graph $G_{f^b}(\psi(x))$ contains edges

$$(i_1, h'_1) \xrightarrow{s_1} (i_2, h_2), (i_2, h'_2) \xrightarrow{s_2} (i_3, h_3), \ldots, (i_k, h'_k) \xrightarrow{s_k} (i_1, h_1).$$

The conclusion follows from Lemma 4.2.1. The proof for the second part is similar. □

If the regulatory graph of the network $F^b = f^b \circ \psi$ contains a (local) circuit of negative sign, then the regulatory graph of f contains a (local) circuit of negative sign. On the other hand, a (local) positive circuit in G_{F^b} could correspond to a (local) non-elementary positive cycle in G_f (see Example 4.3.8).

If a discrete map f has no (local) circuits of sign s, the map ψ allows therefore to define an extension of the Boolean version of f that also admits no (local) circuits of

Multilevel $f\colon \mathcal{X} \to \mathcal{X}$		**Multilevel stepwise** $\tilde{f}\colon \mathcal{X} \to \mathcal{X}$	
$j \xrightarrow{s} i \in G_f(x)$, $i \neq j$	$\begin{matrix}\Rightarrow \\ \not\Leftarrow\end{matrix}$	$j \xrightarrow{s} i \in G_{\tilde{f}}(x)$	Figure 5, Proposition 4.1.7
$i \xrightarrow{s} i \in G_f(x)$, $i \neq j$	$\begin{matrix}\not\Rightarrow \\ \Leftarrow\end{matrix}$	$i \xrightarrow{s} i \in G_{\tilde{f}}(x)$	Figure 5
Multilevel $f\colon \mathcal{X} \to \mathcal{X}$		**Boolean on admissible** $f^b\colon \mathcal{A} \to \mathcal{A}$	
$j \xrightarrow{s} i \in G_f(x)$, var. s_1	$\Longleftarrow$	$(j,k) \xrightarrow{s} (i,k') \in G_{f^b}(\varphi(x))$, var. s_1	Lemma 4.2.1
$j \xrightarrow{s} i \in G_f(x)$, var. s_1	$\Longrightarrow$	$(j, x_j + \frac{s_1+1}{2}) \xrightarrow{s} (i,k') \in G_{f^b}(\varphi(x))$, $k' \in \left]\min\{f_i(x), f_i(x+s_1\mathrm{e}^j)\}, \max\{f_i(x), f_i(x+s_1\mathrm{e}^j)\}\right]$	Lemma 4.2.2
$j \xrightarrow{s} i \in \tilde{G}_f(x,y)$	$\Longrightarrow$	$(j, x_j + \frac{\epsilon_j+1}{2}) \xrightarrow{s} (i, x_i + \frac{\epsilon_i+1}{2}) \in G_{f^b}^{I(\varphi(x),\varphi(y))}(\varphi(x))$, $\epsilon_k = \operatorname{sign}(y_k - x_k)$	Lemma 4.2.5
circuit with sign s in $\tilde{G}_f(x,y)$	$\begin{matrix}\Rightarrow \\ \Leftarrow\end{matrix}$	circuit with sign s in $G_{f^b}^{I(\varphi(x),\varphi(y))}(\varphi(x))$	Propositions 4.2.6, 4.2.8
circuit with sign s in $\tilde{G}_f(x,y)$	$\not\Leftarrow$	circuit with sign s in $G_{f^b}(\varphi(x))$	Example 4.2.7
Boolean $f^b\colon \mathcal{A} \to \mathcal{A}$		**Boolean** $F^b = f^b \circ \psi\colon \mathcal{Y} \to \mathcal{Y}$	
$(j,k') \xrightarrow{s} (i,h) \in G_{f^b}(\psi(x))$, $\psi(\bar{x}^{j,k}) = \overline{\psi(x)}^{j,k'}$	$\Longleftarrow$	$(j,k) \xrightarrow{s} (i,h) \in G_{F^b}(x)$	Lemma 4.3.4
$(j,k) \xrightarrow{s} (i,h) \in G_{f^b}(x)$	$\Longrightarrow$	$(j,t) \xrightarrow{s} (i,h) \in G_{F^b}(y)$, $\psi(y) = x$, $y_{j,t} = x_{j,k}$	Lemma 4.3.5
Multilevel $f\colon \mathcal{X} \to \mathcal{X}$		**Boolean** $F^b = f^b \circ \psi\colon \mathcal{Y} \to \mathcal{Y}$	
(local) circuit of sign s	$\Longrightarrow$	(local) circuit of sign s	Theorem 4.3.6
(local) (negative) circuit	$\Longleftarrow$	(local) (negative) circuit	Theorem 4.3.7
(local) positive circuit	$\not\Leftarrow$	(local) positive circuit	Example 4.3.8

Table 3: *Summary of some results relating the regulatory graphs of multilevel maps and their Boolean versions.*

sign s. We will use this result in the next chapter to identify a Boolean map with no local negative circuits.

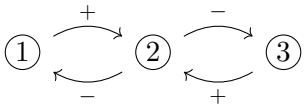
A summary of the results concerning edges and circuits in the regulatory graphs of multilevel maps and their Boolean versions is given in Table 3.

Example 4.3.8. Consider the following multivalued map f with $n = 3$, $m_1 = m_3 = 1$, $m_2 = 2$, and its conversion to Boolean F^b:

$x \in \mathcal{X}$			$f(x)$		
0	0	0	1	0	0
0	0	1	1	0	0
0	1	0	1	0	0
0	1	1	1	0	0
0	2	0	0	1	0
0	2	1	1	1	0
1	0	0	1	1	1
1	0	1	1	1	1
1	1	0	1	1	0
1	1	1	1	2	0
1	2	0	0	1	0
1	2	1	1	2	0

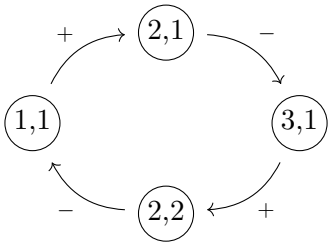
$y \in \mathcal{Y}$				$F^b(y)$			
0	0	0	0	1	0	0	0
0	0	0	1	1	0	0	0
0	0	1	0	1	0	0	0
0	0	1	1	1	0	0	0
0	1	0	0	1	0	0	0
0	1	0	1	1	0	0	0
0	1	1	0	0	1	0	0
0	1	1	1	1	1	0	0
1	0	0	0	1	1	0	1
1	0	0	1	1	1	0	1
1	0	1	0	1	1	0	0
1	0	1	1	1	1	1	0
1	1	0	0	1	1	0	0
1	1	0	1	1	1	1	0
1	1	1	0	0	1	0	0
1	1	1	1	1	1	1	0

It can be verified that the local regulatory graphs of f do not admit positive elementary circuits that involve more than one variable. The local regulatory graph at 110 consists of two negative circuits, with node 2 in common:

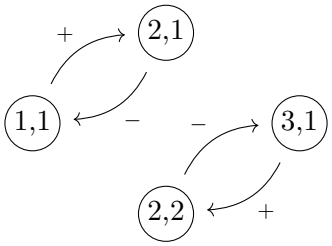


(21)

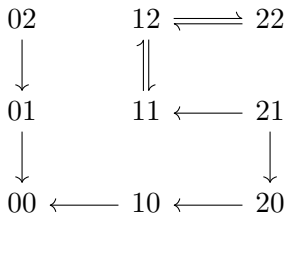
In the conversion, two variables $y_{2,1}$ and $y_{2,2}$ are introduced to represent the second regulatory component. The regulatory graph at 1100 consists of a single elementary positive circuit that involves all four variables:

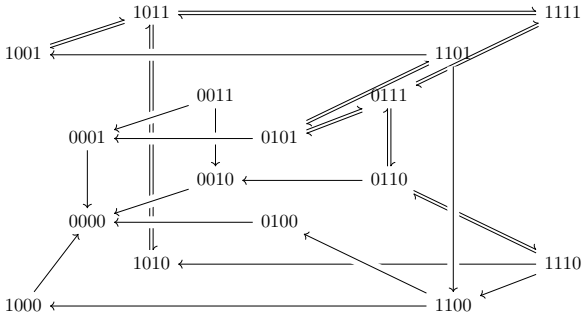


This elementary circuit corresponds therefore to a non-elementary cycle found at $G_f(110)$ as the composition of the two negative circuits (21). Two negative circuits corresponding to the two circuits in $G_f(110)$ (21) appear in the local regulatory graph of F^b at 1010:



Example 4.3.9. The results on the preservation of circuits do not require the map to be stepwise. Consider the non-stepwise map f defined on $\{0,1,2\}^2$ by mapping 11 to 12, 12 to 20, 22 to 02 and all other states to 00. The asynchronous graphs for f and the Boolean version defined by $F^b = f^b \circ \psi$ are as follows:





Notice that the multilevel asynchronous dynamics has two attractors, given by the sets $\{00\}$ and $\{11, 12, 22\}$. In the Boolean version, only the fixed point remains, since the asynchronous dynamics contains a path from 1111 to 0000 through non-admissible states.

As a consequence of Theorem 4.2.11, the regulatory graph of f contains a local circuit of positive sign, and by Theorem 4.3.6 a local positive circuit also appears in the regulatory graph of F^b. Although the asynchronous graph of F^b only has one attractor, the positive circuit can be seen as necessary for the multistationarity of the dynamics obtained by considering only the admissible states.

We can now consider some results about Boolean networks and use the Boolean version of a multilevel map to state analogous results for the multilevel case. The following result is due to Shih and Dong [SD05].

Theorem 4.3.10. *([SD05]) If the regulatory graph of $f : \mathcal{X} \to \mathcal{X}$ contains no circuits, then f has exactly one fixed point.*

We can state a multilevel version of this result.

Theorem 4.3.11. *If the regulatory graph of a multilevel map f contains no local circuits, then f has exactly one fixed point.*

Proof. If the regulatory graph of f contains no local circuits, then by Theorem 4.3.7 the regulatory graph of the map $F^b = f^b \circ \psi$ contains no local circuits. As a consequence of Theorem 4.3.10, F^b has a unique fixed point, and therefore so have f^b and f. □

Notice however that Richard [Ric08] proved the following stronger version of 4.3.11.

Theorem 4.3.12. *([Ric08, Theorem 1]) If the non-usual regulatory graphs of $f\colon \mathcal{X} \to \mathcal{X}$ contain no circuits, then f has exactly one fixed point.*

To state a recent result of Ruet [Rue16, Theorem 5.1] we need the following definition.

Definition 4.3.13. A pair of distinct states $x, y \in \mathbb{B}^n$ is called a *mirror pair* for a map $f\colon \mathbb{B}^n \to \mathbb{B}^n$ if $f|_{[x,y]}(x) = \overline{f|_{[x,y]}(y)}^I$, where $I = I(x,y) = \{i \in \{1,\dots,n\} \mid x_i \neq y_i\}$.

We prove a multilevel version of the following result:

Theorem 4.3.14. *([Rue16, Theorem 5.1]) If $f\colon \mathbb{B}^n \to \mathbb{B}^n$ admits a mirror pair, then there exist two different states $x, y \in \mathbb{B}^n$ such that $G_f(x)$ and $G_f(y)$ contain a circuit, and x, y is a mirror pair for f.*

For multivalued maps, we generalise the definition of mirror pair as follows.

Definition 4.3.15. A pair of distinct states $x, y \in \mathcal{X}$ is called a *mirror pair* for a map $f\colon \mathcal{X} \to \mathcal{X}$ if

$$f_i(x) \leq x_i, y_i \leq f_i(y) \text{ or } f_i(y) \leq x_i, y_i \leq f_i(x) \text{ for all } i \in I,$$

where $I = I(x,y) = \{i \in \{1,\dots,n\} \mid x_i \neq y_i\}$.

Lemma 4.3.16. *$x, y \in \mathcal{X}$ is a mirror pair for $f\colon \mathcal{X} \to \mathcal{X}$ if and only if $\varphi(x)$, $\varphi(y)$ is a mirror pair for $F^b = f^b \circ \psi\colon \mathcal{Y} \to \mathcal{Y}$.*

Proof. For each $i \in I(x,y)$, we can assume $x_i < y_i$. Then, for all $j \in \{1,\dots,m_i\}$, $\varphi_{i,j}(x) \neq \varphi_{i,j}(y)$ if and only if $x_i < j \leq y_i$.

Suppose that x, y is a mirror pair for f. Take $(i,j) \in I(\varphi(x), \varphi(y))$, then either $f_i(x) \leq x_i < j \leq y_i \leq f_i(y)$ or $f_i(y) \leq x_i < j \leq y_i \leq f_i(x)$, and in both cases $f^b_{i,j}(\varphi(x)) \neq f^b_{i,j}(\varphi(y))$.

Conversely, if $\varphi(x)$, $\varphi(y)$ is a mirror pair for f^b, then for any j such that $x_i < j \leq y_i$ we have $f^b_{i,j}(\varphi(x)) \neq f^b_{i,j}(\varphi(y))$, which means that either $f_i(x) < j$ and $f_i(y) \geq j$ or $f_i(y) < j$ and $f_i(x) \geq j$. Taking $j = x_i + 1$ and $j = y_i$ we find $f_i(x) \leq x_i < y_i$ and $f_i(y) \geq y_i > x_i$ or $f_i(y) \leq x_i < y_i$ and $f_i(x) \geq y_i > x_i$. □

Theorem 4.3.17. *If $f\colon \mathcal{X} \to \mathcal{X}$ admits a mirror pair, then there exist two different states $x, y \in \mathcal{X}$ such that $G_f(x)$ and $G_f(y)$ contain a circuit.*

Proof. If f admits a mirror pair, then by Lemma 4.3.16 the map $F^b\colon \mathbb{B}^m \to \mathbb{B}^m$ admits a mirror pair. Theorem 4.3.14 then gives two states $x \neq y \in \mathbb{B}^m$ such that $G_{F^b}(x)$ and $G_{F^b}(y)$ contain a circuit. Taking $x' = \varphi^{-1}(\psi(x))$ and $y' = \varphi^{-1}(\psi(y))$, by Theorem 4.3.7, we have that the graphs $G_f(x')$ and $G_f(y')$ contain a circuit. In addition, since x, y is a mirror pair, we have that $\psi(x) \neq \psi(y)$, since otherwise $F^b(x) = F^b(y)$. Therefore x' and y' are distinct.

□

4.4 COMPARISON TO AN ALTERNATIVE BOOLEAN VERSION

In this chapter, we studied the mapping of multilevel to Boolean networks that relies on the embedding φ of multilevel states to Boolean states, and we suggested a method for defining a Boolean version on the full state space which preserves the regulatory structure. An alternative approach to the mapping of multilevel networks to Boolean networks was recently presented in [FK18]. To describe the approach, we need to introduce another definition. Given a multilevel map $f : \mathcal{X} \to \mathcal{X}$, the *asympotic* version $\hat{f} : \mathcal{X} \to \mathcal{X}$ of f is defined as follows:

$$\hat{f}_i(x) = \begin{cases} 0 & \text{if } f_i(x) < x_i, \\ x_i & \text{if } f_i(x) = x_i, \\ m_i & \text{if } f_i(x) > x_i. \end{cases}$$

A map f is called *asymptotic* if it coincides with its asymptotic version. The regulatory graphs of the asymptotic and stepwise version of a map f can differ in terms of the autoregulations, but they otherwise coincide.

Example 4.4.1. Consider $\mathcal{X} = \{0, 1, 2\}$. The map f on $\mathcal{X}$ that is constantly zero coincides with its asymptotic version $\hat{f}$; its stepwise version is given by $\tilde{f}(0) = \tilde{f}(1) = 0$, $\tilde{f}(2) = 1$. The regulatory graph of $\hat{f}$ does not have any edge, whereas the regulatory graph of $\tilde{f}$ contains an edge $1 \xrightarrow{+} 1$.

The map f on $\mathcal{X}$ that is constantly 1 coincides with its stepwise version $\tilde{f}$; its asymptotic version is given by $\hat{f}(0) = 2$, $\hat{f}(1) = 1$, $\hat{f}(2) = 0$. The regulatory graph of $\tilde{f}$ is empty, whereas the regulatory graph of $\hat{f}$ contains an edge $1 \xrightarrow{-} 1$.

Proposition 4.4.2. *For every $x \in \mathcal{X}$, the graph $G_{\tilde{f}}(x)$ contains an edge from j to i, $i \neq j$, with sign s, if and only if the graph $G_{\hat{f}}(x)$ contains an edge from j to i, with the same sign.*

Proof. If is sufficient to observe that $\operatorname{sign}(\hat{f}_i(x) - x_i) = \operatorname{sign}(\tilde{f}_i(x) - x_i)$ and, since $i \neq j$ and $s \neq 0$,

$$\begin{aligned} \operatorname{sign}(\hat{f}_i(x + s_1 e^j) - \hat{f}_i(x)) &= \operatorname{sign}((\hat{f}_i(x + s_1 e^j) - x_i) - (\hat{f}_i(x) - x_i)) \\ &= \operatorname{sign}(\operatorname{sign}(\hat{f}_i(x + s_1 e^j) - x_i) - \operatorname{sign}(\hat{f}_i(x) - x_i)) \\ &= \operatorname{sign}(\operatorname{sign}(\tilde{f}_i(x + s_1 e^j) - x_i) - \operatorname{sign}(\tilde{f}_i(x) - x_i)). \end{aligned}$$

□

Rather than considering a Boolean version of f that is defined only on the admissible states, Fauré and Kaji [FK18] define a Boolean network on the full state space $\mathcal{Y}$. They call this Boolean network the *binarisation* of f. Consider the map

$$\begin{aligned} &\psi^* : \mathcal{Y} \to \mathcal{X} \\ &\psi^* : (y_{11}, \ldots, y_{1,m_1}, y_{2,1}, \ldots, y_{n,m_n}) \mapsto (\sum_{k=1}^{m_1} y_{1,k}, \ldots, \sum_{k=1}^{m_n} y_{n,k}), \end{aligned}$$

which is called ψ in [FK18]. The *binarisation* $\mathbb{B}(f) : \mathcal{Y} \to \mathcal{Y}$ of f is defined by

$$\mathbb{B}(f)_{i,j}(x) = \begin{cases} 0 & \text{if } f_i(\psi^*(x)) < \psi_i^*(x), \\ x_{i,j} & \text{if } f_i(\psi^*(x)) = \psi_i^*(x), \\ 1 & \text{if } f_i(\psi^*(x)) > \psi_i^*(x). \end{cases}$$

A useful property of this method is that the binarisation defines a bijection between the set of asymptotic networks on $\mathcal{X}$ and the functions on $\mathcal{Y}$ that are invariant under the action of $S_{m_1} \times \cdots \times S_{m_n}$ on $\mathcal{Y}$. This symmetry property does not hold for the conversion introduced in 4.3. To see this, it is sufficient to consider the stepwise multilevel map on $\{0, 1, 2\}$ that is constantly equal to 1, and the permutation that swaps the two Boolean variables of the conversion, since $F^b(0, 0) = (1, 0) \neq (0, 1)$.

The binarisation $\mathbb{B}(f)$ admits a fixed point if and only if f admits a fixed point, and the asynchronous dynamics of $\mathbb{B}(f)$ admits a cyclic attractor if and only if the asynchronous dynamics of f admits a cyclic attractor. In addition, the attractors of the asynchronous dynamics of $\mathbb{B}(f)$ map surjectively onto the attractors of the asynchronous dynamics of f, and to an attractor in AD_f could correspond multiple attractors in $AD_{\mathbb{B}(f)}$.

Example 4.4.3. Reconsider the map of example 4.3.8. Its asymptotic version and binarisation are given by

$x \in \mathcal{X}$			$f(x)$		
0	0	0	1	0	0
0	0	1	1	0	0
0	1	0	1	0	0
0	1	1	1	0	0
0	2	0	0	0	0
0	2	1	1	0	0
1	0	0	1	2	1
1	0	1	1	2	1
1	1	0	1	1	0
1	1	1	1	2	0
1	2	0	0	0	0
1	2	1	1	2	0

$y \in \mathcal{Y}$				$\mathbb{B}(f)(y)$			
0	0	0	0	1	0	0	0
0	0	0	1	1	0	0	0
0	0	1	0	1	0	0	0
0	0	1	1	1	0	0	0
0	1	0	0	1	0	0	0
0	1	0	1	1	0	0	0
0	1	1	0	0	0	0	0
0	1	1	1	1	0	0	0
1	0	0	0	1	1	1	1
1	0	0	1	1	1	1	1
1	0	1	0	1	0	1	0
1	0	1	1	1	1	1	0
1	1	0	0	1	1	0	0
1	1	0	1	1	1	1	0
1	1	1	0	0	0	0	0
1	1	1	1	1	1	1	0

The unique fixed point 110 of f give rise to two fixed points 1010 and 1100 for $\mathbb{B}(f)$. In addition, all the local circuits in the regulatory graph of $\mathbb{B}(f)$ are positive.

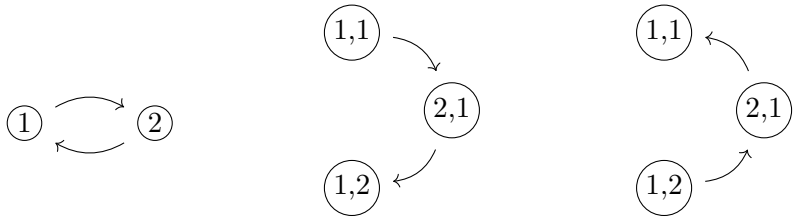
For the conversion $F^b = f^b \circ \psi$ described in Section 4.3, we proved that the existence of a circuit in the regulatory graph of f implies the existence of a circuit in the regulatory graph of F^b, and vice versa (Theorem 4.3.7 and Theorem 4.3.6). A local circuit in $G_{\mathbb{B}(f)}$ requires the existence of a local circuit in G_f. However, to a local circuit in the regulatory graph of the multivalued map does not necessarily correspond a local circuit in the regulatory graph of the binarisation. In this case, one can conclude that the circuit does not play a role in determining the asymptotic behaviour.

Example 4.4.4. Consider the following asymptotic map f and its binarisation:

$x \in \mathcal{X}$		$f(x)$	
0	0	0	0
0	1	0	0
1	0	0	0
1	1	1	0
2	0	0	1
2	1	0	0

$y \in \mathcal{Y}$			$\mathbb{B}(f)(y)$		
0	0	0	0	0	0
0	0	1	0	0	0
0	1	0	0	0	0
0	1	1	0	1	0
1	0	0	0	0	0
1	0	1	1	0	0
1	1	0	0	0	1
1	1	1	0	0	0

The regulatory graphs $G_f(10)$, and the regulatory graphs of $\mathbb{B}(f)$ at the two states mapped to 01 by ψ^*, 010 and 100 are as follows:



Hence the regulatory circuit in $G_f(10)$ is not converted to a circuit in the regulatory graphs $G_{\mathbb{B}(f)}(010)$ and $G_{\mathbb{B}(f)}(100)$. The regulatory graph of the conversion to Boolean f^b for the stepwise version of f on the admissible state 100 does not admit any circuit. The regulatory graph of F^b at 100 also does not admit any circuit; however, the regulatory graph $G_{F^b}(010)$ contains a circuit involving the two regulatory components (nodes $1,1$ and $2,1$).

In the next chapter, we will consider the relationship between local negative circuits and cyclic attractors. In particular, we will present another application of the conversion of multivalued maps to Boolean dynamics, with the construction of a Boolean map with a cyclic attractor and no local negative circuits for $n = 6$, and we will investigate local negative circuits in networks of small dimension.

5

LOCAL NEGATIVE CIRCUITS AND CYCLIC ATTRACTORS

In this chapter, we consider the following question:

Question 1. For a Boolean network, does the presence of a cyclic attractor imply the existence of a *local* negative circuit in the regulatory graph?

See Section 3.3 for some background on the question and an overview of results on circuits and dynamical behaviour of regulatory networks. The first counterexamples to this conjecture can be found in [Rue17], where two constructions are presented: one consists of a 12-dimensional and-net with a cyclic attractor, no local negative circuits and no fixed states; the second is a family of maps, one for each $n \geq 7$, with an attractive cycle and no local negative circuit. A counterexample in the multilevel case was presented by Richard [Ric10]. In this chapter, we identify other counterexamples for the Boolean case. The first we identify is a Boolean version of Richard's multilevel counterexample (Section 5.1.1), created using the mapping described in the previous chapter. We then present a variation on Ruet's construction in [Rue17] for maps with attractive cycles and no local negative circuits, for $n \geq 6$ (Section 5.1.2).

For $n \geq 6$ we find therefore that cyclic attractors, attractive cycles and absence of fixed points are all compatible with the absence of local negative circuits. We do not know, however, if there are counterexamples for smaller n. To find an answer for the remaining cases, we show that the question can be encoded as a Boolean formula, that can be given in input to a satisfiability solver (Section 5.2). We approach the problem as follows: for a fixed number n of regulatory components, we consider $n \cdot 2^n$ Boolean variables, representing the values taken by the n components of the Boolean map on the 2^n states in the state space. Features of the dynamics or the regulatory structures of Boolean networks can be translated into Boolean formulas on these $n \cdot 2^n$ variables. For instance, we can look for maps with a given regulatory graph, or with a desired number of fixed states. In particular, the features investigated by Question 1 — existence of a local negative circuit, and of a cyclic attractor — can be encoded as Boolean expressions on the $n \cdot 2^n$ variables. In more detail, we implement a Boolean formula the encodes the absence of local negative circuits and a necessary condition for the presence of a cyclic attractor. Using the fact that asynchronous dynamics and the signs of the circuits are preserved by symmetries of hypercube (see Section 3.2), we reduce the search space so that, for small n, the problem can be analysed by a satisfiability solver in a few hours. The solver finds the formula unsatisfiable for $n \leq 5$, and identifies other examples for $n = 6$.

5.1 COUNTEREXAMPLES

5.1.1 *Conversion of the multilevel counterexample*

In [Ric10], 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

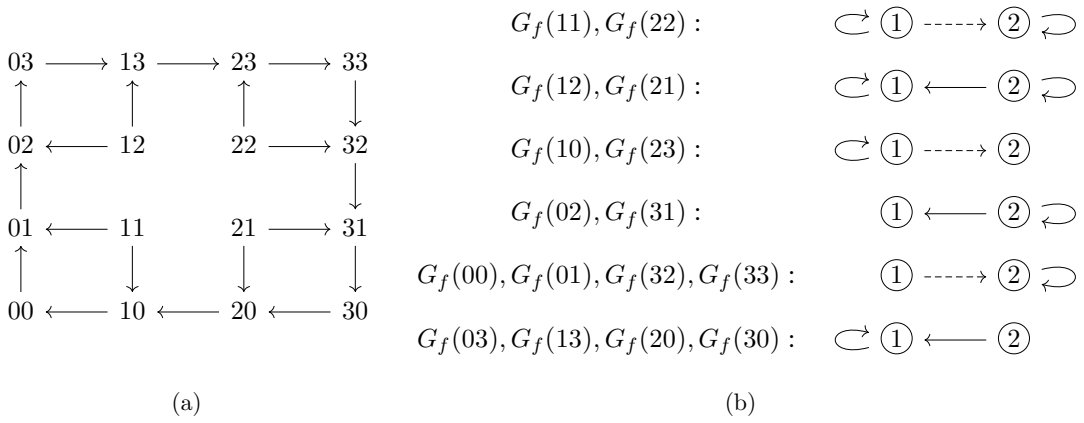


Figure 11: *(a): Asynchronous state transition graph for a map $f : \{0,1,2,3\}^2 \to \{0,1,2,3\}^2$ with one cyclic attractor (Example 6 in [Ric10]). (b): Local regulatory graphs of f (negative edges are dashed).*

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 11 is the stepwise version f of the map introduced by Richard, together with its local regulatory graphs, which do not contain any negative circuit. 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 13a. Since the asynchronous dynamics of f admits a unique cyclic attractor, AD_{f^b} 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 ψ the map defined in (20). By Proposition 4.2.10, the map $F^b = f^b \circ \psi$ is a compatible conversion of f, and hence F^b has a unique cyclic attractor. By application of Theorem 4.3.7, 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 12. The global regulatory graph for F^b takes the form given in Figure 13b. We inspect two local regulatory graphs in detail, to illustrate the 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 $G_{f^b}(x)$, we compare the images under f^b:

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

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

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

$G_{F^b}(x)$ admits therefore additional edges with signs and targets given by the signs and targets of the edges in $G_{f^b}(x)$. The additional edges are $(1,2) \xrightarrow{-} (2,1)$, $(1,3) \xrightarrow{-} (2,1)$,

x	$F^b(x)$	x	$F^b(x)$	x	$F^b(x)$	x	$F^b(x)$
000000	000100	010000	000000	100000	000000	110000	100000
000001	000110	010001	000000	100001	000000	110001	111000
000010	000110	010010	000000	100010	000000	110010	111000
000011	000111	010011	000111	100011	000111	110011	111111
000100	000110	010100	000000	100100	000000	110100	111000
000101	000111	010101	000111	100101	000111	110101	111111
000110	000111	010110	000111	100110	000111	110110	111111
000111	100111	010111	110111	100111	110111	110111	111111
001000	000000	011000	100000	101000	100000	111000	110000
001001	000000	011001	111000	101001	111000	111001	111000
001010	000000	011010	111000	101010	111000	111010	111000
001011	000111	011011	111111	101011	111111	111011	111100
001100	000000	011100	111000	101100	111000	111100	111000
001101	000111	011101	111111	101101	111111	111101	111100
001110	000111	011110	111111	101110	111111	111110	111100
001111	110111	011111	111111	101111	111111	111111	111110

Figure 12: *A map $F^b : \{0,1\}^6 \to \{0,1\}^6$ with no fixed points and with regulatory graph admitting no local negative circuits. The rows corresponding to the admissible states are highlighted in gray.*

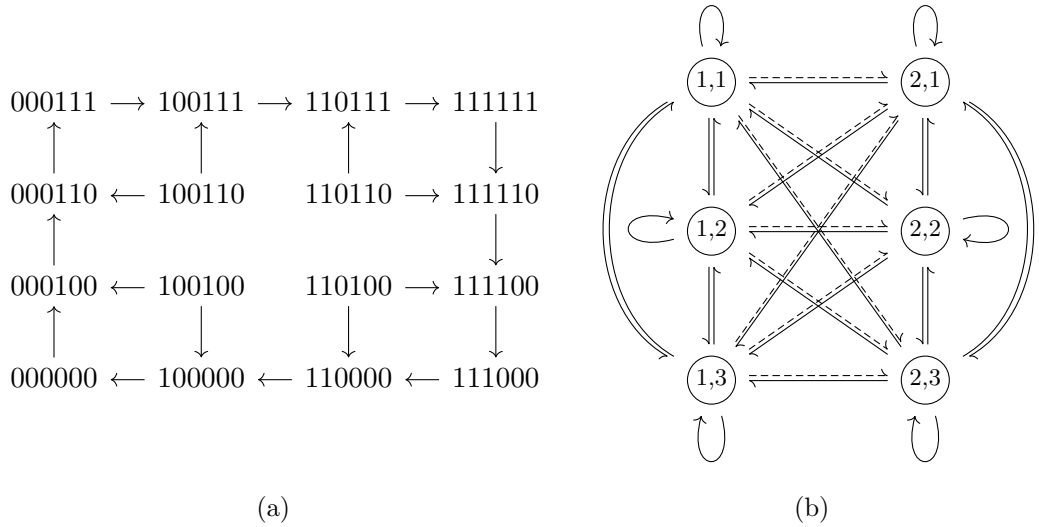


Figure 13: *(a): Asynchronous state transition graph on the admissible states for the map F^b in Figure 12. (b): Global regulatory graph of F^b (negative edges are dashed).*

$(2,2) \xrightarrow{+} (2,2)$ 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:

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

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 $(1,3) \xrightarrow{+} (1,1)$.

The map can be used to show that the absence of local negative circuits in the regulatory graph does not imply the existence of a fixed point for $n \geq 6$ as follows: for each $n \geq 6$, define a map F^n by setting $F^n_i = F^b_i$ for $i = 1, \ldots, 6$, and $F^n_i = x_i$ for $i > 6$. Then, for each $x \in \{0,1\}^n$, 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.

5.1.2 *Maps with antipodal attractive cycles*

Recall that e^i denotes the element of $\mathbb{B}^n$ with $\mathrm{e}^i_i = 1$ and $\mathrm{e}^i_j = 0$ for $j \neq i$. The following definition is given in [Rue16, Rue17].

Definition 5.1.1. A cycle is called *antipodal attractive cycle* if it is obtained from the cycle

$$(\mathbf{0}, \mathrm{e}^1, \mathrm{e}^1 + \mathrm{e}^2, \ldots, \mathrm{e}^1 + \cdots + \mathrm{e}^n, \mathrm{e}^2 + \cdots + \mathrm{e}^n, \ldots, \mathrm{e}^n, \mathbf{0})$$

by application of a map $\psi_I \circ \sigma$, where $\psi_I(x) = \bar{x}^I$ for some $I \subseteq \{1, \ldots, n\}$ and all $x \in \mathbb{B}^n$, and σ is a permutation of the coordinates.

In [Rue17], Ruet presents a method for constructing a Boolean map with an antipodal attractive cycle and no local negative circuits in the regulatory graph, for $n \geq 7$. Here we describe a variation on Ruet's construction, to identify maps with similar properties, for $n \geq 6$.

Definition 5.1.2. Given maps $f, U : \mathbb{B}^n \to \mathbb{B}^n$, f is *U-equivariant* if it satisfies

$$f \circ U = U \circ f.$$

The following Lemma, due to Ruet [Rue17], can be seen as a consequence of 3.2.11, part (i).

Lemma 5.1.3. *[Rue17, Lemma 6] Consider a map $U = \psi_I \circ \sigma$, with $I \subseteq \{1, \ldots, n\}$ and $\sigma \in S_n$. If $f : \mathbb{B}^n \to \mathbb{B}^n$ is U-equivariant, then for any $x \in \mathbb{B}^n$ the graphs $G_f(x)$ and $G_f(U(x))$ are isomorphic. Moreover, corresponding cycles have the same sign.*

The idea of the construction is the following. The regulatory graph of the map consisting of the antipodal attractive cycle $\mathcal{C}$, and all other states fixed, admits many local negative circuits. These circuits belong to graphs $G_f(x)$ with $x \in \mathcal{C}$, since by Lemma 4.2.15 the regulatory graph at fixed points cannot admit any negative circuit. By carefully modifying the map around the antipodal cycle, one can eliminate the local negative circuits, while maintaining the other states fixed. The proof that the regulatory graph admits no local negative circuits is simplified by the fact that the resulting map is T-equivariant, where T is the map defined by

$$T(x_1, \dots, x_n) = (\bar{x}_n, x_1, \dots, x_{n-1}). \tag{22}$$

We start by setting the notation for the states in the antipodal cycle. We set

$$a^i = \sum_{k=1}^{i-1} \mathrm{e}^i,$$

$$a^{n+i} = \overline{a^i},$$

for $i = 1, \dots, n$. Observe that $a^{i+1} = a^i + \mathrm{e}^i$, and that the antipodal cycle is defined by $(a^1 = 0, a^2, \dots, a^n, a^{n+1}, \dots, a^{2n}, a^1)$. We extend the notation for the e^i by setting $\mathrm{e}^{i+kn} = \mathrm{e}^i$ for $i \in \{1, \dots, n\}$, $k \in \mathbb{Z}$. Then, we define

$$\begin{aligned} b^i &= a^i + \mathrm{e}^{i+1}, \\ c^i &= a^i + \mathrm{e}^{i+1} + \mathrm{e}^{i+2} = b^i + \mathrm{e}^{i+2}, \\ d^i &= a^i + \mathrm{e}^{i+1} + \mathrm{e}^{i+3} = b^i + \mathrm{e}^{i+3}, \end{aligned}$$

for $i = 1, \dots, 2n$. Write A, B, C and D for the sets $\bigcup_{i=1}^{2n}\{a^i\}$, $\bigcup_{i=1}^{2n}\{b^i\}$, $\bigcup_{i=1}^{2n}\{c^i\}$ and $\bigcup_{i=1}^{2n}\{d^i\}$ respectively, and set $a^{i+2kn} = a^i$ for $i = \{1, \dots, 2n\}$ and $k \in \mathbb{Z}$, and similarly for the states b^i, c^i and d^i.

We define the map f as follows:

$$\begin{aligned} f(a^i) &= a^{i+1}, \\ f(b^i) &= a^{i+2}, \\ f(c^i) &= a^{i+4}, \\ f(d^i) &= a^{i+4}, \end{aligned}$$

for $i = 1, \dots, 2n$, while all other states are fixed.

First, we need to prove that f is well defined.

Remark 5.1.4. Using $n \geq 6$, we find

1. $a^i = a^j + \mathrm{e}^k$ for some $i \leq j, k$ if and only if $j = i + 1$ modulo $2n$, $k = i$ modulo n, and $a^i = a^{i+1} + \mathrm{e}^i$.
2. $a^i = a^j + \mathrm{e}^{k_1} + \mathrm{e}^{k_2}$ for some $i < j, k_1 < k_2$ if and only if $j = i + 2$ modulo $2n$, $k_1, k_2 = i, i + 1$ modulo n.
3. $a^i = a^j + \mathrm{e}^{k_1} + \mathrm{e}^{k_2} + \mathrm{e}^{k_3}$ for some $i < j, k_1 < k_2 < k_3$ if and only if $j = i + 3$ modulo $2n$, $k_1, k_2, k_3 = i, i + 1, i + 2$ modulo n.
4. $a^i = a^j + \mathrm{e}^{k_1} + \mathrm{e}^{k_2} + \mathrm{e}^{k_3} + \mathrm{e}^{k_4}$ for some $i < j, k_1 < k_2 < k_3 < k_4$ if and only if $j = i + 4$ modulo $2n$, $k_1, k_2, k_3, k_4 = i, i + 1, i + 2, i + 3$ modulo n.

Lemma 5.1.5. *For $n \geq 6$, the points a^i, b^i, c^i, d^i, $i = 1, \dots, 2n$ are all distinct.*

Proof. The points on the antipodal cycle are clearly all distinct. Using Remark 5.1.4, point (2), one can show that the states in B are all distinct, and similarly for C and D using point (4).

Suppose that $a^i = b^j$ for some i, j. Then $a^i = a^j + \mathrm{e}^{j+1}$, and using Remark 5.1.4, point (1), we conclude that the sets A and B do not intersect. Suppose now that $a^i = c^j$ or $a^i = d^j$ for some i, j. Then $a^i = a^j + \mathrm{e}^{j+1} + \mathrm{e}^{j+2}$ or $a^i = a^j + \mathrm{e}^{j+1} + \mathrm{e}^{j+3}$, and we can use Remark 5.1.4, point (2) to see that the set A does not intersect the sets C and D. Similarly, using Remark 5.1.4, points (3) and (4), we find that B and C, B and D and C and D do not intersect. □

For $n \leq 5$, the $4 \cdot 2 \cdot n$ points cannot be all distinct, since there are 2^n states (for $n = 5$, the set D contains only two states).

As in [Rue17], the map we constructed is T-equivariant.

Lemma 5.1.6. *f is T-equivariant.*

Proof. We have to verify that $f(T(x)) = T(f(x))$ for all $x \in \mathcal{X}$. We start by observing that, as in Lemma 5 of [Rue17], for any $i \in 1, \ldots, 2n$

$$T(a^i) = a^{i+1},\ T(b^i) = b^{i+1},\ T(c^i) = c^{i+1},\ T(d^i) = d^{i+1}.$$

As a consequence,

$$T(f(a^i)) = T(a^{i+1}) = a^{i+2} = f(a^{i+1}) = f(T(a^i)),$$

and similarly for b^i, c^i and d^i. If follows that, if x is a fixed point for f, $T(x)$ is also a fixed point for f, and $T(f(x)) = T(x) = f(T(x))$. □

Since f is T-equivariant, by Lemma 5.1.3 we can limit the analysis of the regulatory graph to four states.

Remark 5.1.7. From Remark 5.1.4, point (1), we find that each a^i has two neighbours in A:

$$\begin{aligned} a^i + \mathrm{e}^i &= a^{i+1}, \\ a^i + \mathrm{e}^{i-1} &= a^{i-1}, \end{aligned}$$

and using point (2), a^i has two neighbours in B:

$$\begin{aligned} a^i + \mathrm{e}^{i+1} &= b^i, \\ a^i + \mathrm{e}^{i-2} &= b^{i-2}, \end{aligned}$$

and using point (3), a^i has one neighbour in C:

$$a^i + \mathrm{e}^{i-3} = c^{i-3}.$$

Similarly, we can identify the neighbours of b^i

$$\begin{aligned} b^i + \mathrm{e}^i &= a^{i+2}, & b^i + \mathrm{e}^{i+1} &= a^i, \\ b^i + \mathrm{e}^{i+2} &= c^i, & b^i + \mathrm{e}^{i+3} &= d^i, & b^i + \mathrm{e}^{i-2} &= d^{i-2}, \end{aligned}$$

and the neighbours of c^i and d^i:

$$\begin{aligned} c^i + \mathrm{e}^i &= a^{i+3}, & c^i + \mathrm{e}^{i+2} &= b^i, \\ d^i + \mathrm{e}^i &= b^{i+2}, & d^i + \mathrm{e}^{i+3} &= b^i. \end{aligned}$$

Lemma 5.1.8. *For $n \geq 6$, the graph $G_f(x)$ has no negative local circuit for any $x \in \{a^1, b^1, c^1, d^1\}$.*

Proof. If $x \notin A \cup B \cup C \cup D$, then x is a fixed point, and $G_f(x)$ has no local negative circuits by Lemma 4.2.15. Consider the state $x = a^1$. Recall that a^1 is mapped to $a^2 = a^1 + \mathrm{e}^1$ by f. We enumerate the edges in the graph $G_f(a^1)$. By Remark 5.1.7, we need to consider six possible cases for the direction e^j:

- $j = 1$: $a^1 + \mathrm{e}^1 = a^2$ is mapped to $a^3 = a^1 + \mathrm{e}^1 + \mathrm{e}^2$, hence the regulatory graph contains the edge
$$1 \xrightarrow{+} 2.$$
- $j = 2$: $a^1 + \mathrm{e}^2 = b^i$ is mapped to a^3, and we find the edge
$$2 \xrightarrow{+} 2.$$
- $j = n$: $a^1 + \mathrm{e}^n = a^n$ is mapped to a^1, giving the edge
$$n \xrightarrow{-} 1.$$
- $j = n - 1$: $a^1 + \mathrm{e}^{n-1} = b^{n-1}$ is mapped to a^1, giving the edge
$$n - 1 \xrightarrow{-} 1.$$
- $j = n - 2$: $a^1 + \mathrm{e}^{n-2} = c^{n-2}$, mapped to $a^2 = a^1 + \mathrm{e}^1 = f(a^1)$, and there is no edge with source $n - 2$.
- $j \notin \{1, 2, n, n - 1, n - 2\}$: $a^1 + \mathrm{e}^j$ is mapped to itself, and we find the following edges:
$$j \xrightarrow{-} 1 \text{ and } j \xrightarrow{+} j.$$

We conclude that the graph $G_f(a^1)$ has no negative circuits.

Let us consider a state $x = b^1$. Recall that b^1 is mapped to $a^3 = a^1 + e^1 + e^2$ by f. Again, by Remark 5.1.7, we need to consider six possible cases:

- $j = 1$: $b^1 + \mathrm{e}^1 = a^3$ is mapped to $a^4 = a^1 + \mathrm{e}^1 + \mathrm{e}^2 + \mathrm{e}^3$, hence the edge
$$1 \xrightarrow{+} 3.$$
- $j = 2$: $b^1 + \mathrm{e}^2 = a^1$ is mapped to $a^2 = a^1 + \mathrm{e}^1$, and we find the edge
$$2 \xrightarrow{+} 2.$$
- $j = 3$: $b^1 + \mathrm{e}^3 = c^1$ is mapped to a^5, giving the edges
$$3 \xrightarrow{+} 3,\ 3 \xrightarrow{+} 4.$$
- $j = 4$: $b^1 + \mathrm{e}^4 = d^1$ is mapped to a^5, giving the edges
$$4 \xrightarrow{+} 3,\ 4 \xrightarrow{+} 4.$$
- $j = n - 1$: $b^1 + \mathrm{e}^{n-1} = d^{n-1}$, mapped to a^3, and there is no edge with source $n - 1$.

- $j \notin \{1,2,3,4,n-1\}$: $b^1 + \mathrm{e}^j = a^1 + \mathrm{e}^2 + \mathrm{e}^j$ is mapped to itself, and we find the following edges:
$$j \xrightarrow{-} 1 \text{ and } j \xrightarrow{+} j.$$

We conclude that the graph $G_f(b^1)$ has no negative circuits.

The states c^1 and d^1 are mapped to a^5. For the graph $G_f(c^1)$, we consider three cases:

- $j = 1$: $c^1 + \mathrm{e}^1 = a^4$, mapped to a^5, and we have no edges with source 1.
- $j = 3$: $c^1 + \mathrm{e}^3 = b^1$ mapped to a^3, hence the edges
$$3 \xrightarrow{+} 3,\ 3 \xrightarrow{+} 4.$$
- $j \notin \{1,3\}$: $c^1 + \mathrm{e}^j = a^1 + \mathrm{e}^2 + \mathrm{e}^3 + \mathrm{e}^j$ is fixed. For $j = 2$ we find the edges
$$2 \xrightarrow{+} 1,\ 2 \xrightarrow{+} 2,\ 2 \xrightarrow{+} 4.$$
If $j = 4$ we have the single edge
$$4 \xrightarrow{-} 1.$$
Otherwise, we find
$$j \xrightarrow{-} 1,\ j \xrightarrow{-} 4, j \xrightarrow{+} j.$$

For d^1, we again have three cases:

- $j = 1$: $d^1 + \mathrm{e}^1 = b^3$, mapped to a^5, and we have no edges with source 1.
- $j = 4$: $d^1 + \mathrm{e}^4 = b^1$ mapped to a^3, hence the edges
$$4 \xrightarrow{+} 3,\ 4 \xrightarrow{+} 4.$$
- $j \notin \{1,4\}$: $c^1 + \mathrm{e}^j = a^1 + \mathrm{e}^2 + \mathrm{e}^4 + \mathrm{e}^j$ is fixed. If $j = 2$ we find the edges
$$2 \xrightarrow{+} 1,\ 2 \xrightarrow{+} 2,\ 2 \xrightarrow{+} 3.$$
If $j = 3$ we have the single edge
$$3 \xrightarrow{-} 1.$$
Otherwise, we find
$$j \xrightarrow{-} 1,\ j \xrightarrow{-} 3,\ j \xrightarrow{+} j.$$

The graphs $G_f(c^1)$ and $G_f(d^1)$ have no negative circuits, and we concluded the proof. □

We can summarise the section with the following proposition.

Proposition 5.1.9. *For $n \geq 6$, there exists a map with an antipodal attractive cycle and admitting no local negative circuits in the regulatory graph.*

The map for $n = 6$ is pictured in Figure 14.

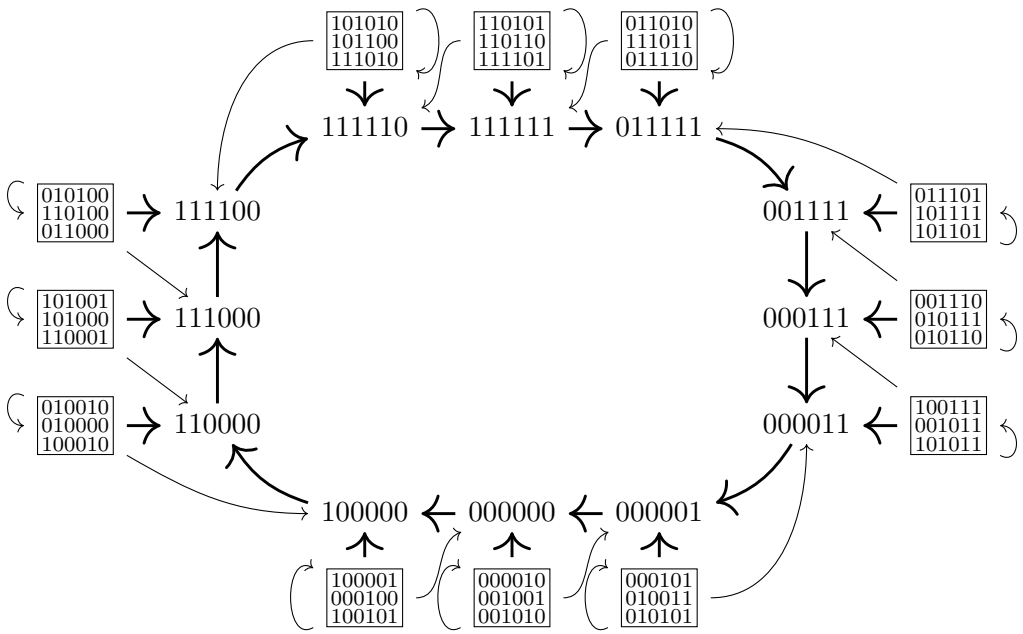


Figure 14: *Dynamics for a regulatory network with an antipodal attractive cycle and admitting no local negative circuits, for $n = 6$. The fixed states are omitted. The synchronous dynamics coincides for the states in the same box, and is represented with bold arrows. The additional transitions are asynchronous.*

5.2 QUESTIONS ON REGULATORY NETWORKS AS BOOLEAN SATISFIABILITY PROBLEMS

In this section we show how some questions about regulatory networks translate naturally into Boolean formulas. SAT solvers then provide powerful tools to determine whether Boolean regulatory networks with some given properties exist. In Section 5.2.1, we will use this approach to answer a question about the existence of negative circuits in presence of cyclic attractors for small networks.

The questions we want to translate into Boolean satisfiability problems are for instance of the form: do regulatory network exist with a given graph as regulatory graph, and admitting a given orbit in the asynchronous state transition graph? What is the smallest dimension n for which such networks exist?

To translate these types of problems into Boolean formulas, for a fixed n, we consider $n \cdot 2^n$ Boolean variables representing the values taken by the hypothetical Boolean map f on the 2^n states in $\mathbb{B}^n$. We denote these variables as

$$f_1(x), \ldots, f_n(x),\ x \in \mathbb{B}^n. \tag{23}$$

We stipulate that a value of *true* for $f_i(x)$ corresponds to 1, and *false* to 0. The conditions on the graphs associated to the regulatory network then translate into Boolean formulas on these variables. For instance, if we want the state $x \in \mathbb{B}^n$ to be mapped to the state $y \in \mathbb{B}^n$ by f, we write the following Boolean formula:

$$\bigwedge_{\substack{1 \leq i \leq n \\ y_i = 0}} \neg f_i(x) \wedge \bigwedge_{\substack{1 \leq i \leq n \\ y_i = 1}} f_i(x). \tag{24}$$

In the following paragraphs, we look at how conditions on the regulatory graphs or on the dynamics of f can be translated into Boolean formulas.

Regulatory graph

For each possible edge (j,i) in the regulatory graph of f, define, for each state $x \in \mathbb{B}^n$,

$$l_x^0(j,i) = f_i(x_1,\dots,x_{j-1},0,x_{j+1},\dots,x_n),$$
$$l_x^1(j,i) = f_i(x_1,\dots,x_{j-1},1,x_{j+1},\dots,x_n).$$

With these definitions, the sign (8) of the edge e is given by the difference between $l_x^1(j,i)$ and $l_x^0(j,i)$. Then, we can require that the sign of edge e is positive, by considering the expression

$$\mathcal{P}^x(j,i) = \neg l_x^0(j,i) \wedge l_x^1(j,i), \tag{25}$$

and to impose a negative edge, the expression

$$\mathcal{N}^x(j,i) = l_x^0(j,i) \wedge \neg l_x^1(j,i). \tag{26}$$

If we want no regulation from j to i at x, we impose

$$\mathcal{O}^x(j,i) = (l_x^0(j,i) \Leftrightarrow l_x^1(j,i)) = (\neg l_x^0(j,i) \vee l_x^1(j,i)) \wedge (l_x^0(j,i) \vee \neg l_x^1(j,i)). \tag{27}$$

Given some positive edges $\mathcal{E}^+$ and some negative edges $\mathcal{E}^-$, we can impose that the regulatory graph of f admits these edges in the global regulatory graph, using the formula

$$\left(\bigwedge_{(j,i)\in\mathcal{E}^+}\bigvee_{x\in\mathbb{B}^n}\mathcal{P}^x(j,i)\right)\wedge\left(\bigwedge_{(j,i)\in\mathcal{E}^-}\bigvee_{x\in\mathbb{B}^n}\mathcal{N}^x(j,i)\right).$$

If we want the regulatory graph of f to contain exclusively the edges in $\mathcal{E}^+$ and $\mathcal{E}^-$, then we add the following clause:

$$\bigwedge_{x\in\mathbb{B}^n}\left(\bigwedge_{\substack{i,j=1,\dots,n\\(j,i)\notin\mathcal{E}^+}}\neg\mathcal{P}^x(j,i)\right)\wedge\left(\bigwedge_{\substack{i,j=1,\dots,n\\(j,i)\notin\mathcal{E}^-}}\neg\mathcal{N}^x(j,i)\right).$$

If we require the edges in $\mathcal{E}^+$ and $\mathcal{E}^-$ to be contained in all of the local graphs at a subset A of $\mathbb{B}^n$, we can use the formula

$$\bigwedge_{x\in A}\left(\bigwedge_{(j,i)\in\mathcal{E}^+}\mathcal{P}^x(j,i)\wedge\bigwedge_{(j,i)\in\mathcal{E}^-}\mathcal{N}^x(j,i)\right). \tag{28}$$

Using the formula in (28), we can impose for instance that the regulatory graph contains a given circuit.

Imposing conditions on the asynchronous state transition graph

We describe a formula that imposes the existence of a given path in the asynchronous state transition graph. Given a pair of states (x,y) such that $d(x,y)=1$, if $x_j \neq y_j$ we can require that the transition (x,y) is in AD_f by imposing

$$f_j(x) \text{ if } y_j = 1, \text{ else } \neg f_j(x). \tag{29}$$

Given a sequence of states $\pi = (x^0, x^1, \dots, x^k)$ such that $d(x^i, x^{i+1}) = 1$, $i = 0,\dots,k-1$, we can require that the sequence defines a path in AD_f by imposing k constraints of the form in (29):

$$\Theta^\pi = \bigwedge_{\substack{0\le i\le k-1\\ j \text{ s.t. } x_j^i\neq x_j^{i+1}\\ x_j^{i+1}=0}}\neg f_j(x^i) \wedge \bigwedge_{\substack{0\le i\le k-1\\ j \text{ s.t. } x_j^i\neq x_j^{i+1}\\ x_j^{i+1}=1}} f_j(x^i). \tag{30}$$

n	number of cycles	number of cycles of length						
		1	2	3	4	5	6	7
2	3	2	1					
3	8	3	3	2				
4	24	4	6	8	6			
5	89	5	10	20	30	24		
6	415	6	15	40	90	144	120	
7	2373	7	21	70	210	504	840	720

Figure 15: *Number of elementary cycles in a complete directed graph with n nodes.*

Given two states x and y with Hamming distance equal to one, we can require that y is the unique state such that (x, y) is in the asynchronous state transition graph by imposing $f(x) = y$, i.e.

$$\bigwedge_{\substack{1\leq i\leq n\\ y_i=1}} f_i(x) \wedge \bigwedge_{\substack{1\leq i\leq n\\ y_i=0}} \neg f_i(x). \tag{31}$$

Using this formula on a cycle $(x^1, \ldots, x^{k-1}, x^k = x^1)$, we can impose that the cycle is attractive for the asynchronous state transition graph of f:

$$\bigwedge_{\substack{1\leq i\leq k-1\\ 1\leq j\leq n\\ x_j^{i+1}=0}} \neg f_j(x^i) \wedge \bigwedge_{\substack{1\leq i\leq k-1\\ 1\leq j\leq n\\ x_j^{i+1}=1}} f_j(x^i). \tag{32}$$

5.2.1 *Attractors and local negative circuits*

In this section, we describe how Question 1 an be translated into a Boolean formula on the $n \cdot 2^n$ Boolean variables in (23). We proceed first to describe how the absence of negative circuits in the local regulatory graph can be translated into a Boolean expression.

Imposing the absence of local negative circuits

To express the sign condition on the circuits, we consider every possible circuit on a graph with n nodes, and impose that the circuit has a non-negative sign. For small values of n, this requirement leads to a satisfiability problem that is computationally manageable. The number of elementary circuits of length k in a complete graph on n nodes is given by $\binom{n}{k}(k-1)!$, hence we have to consider, for instance, 89 circuits for $n = 5$, and 415 circuits for $n = 6$ (see Table 15).

We write $\mathcal{C}_n$ for the set of all possible circuits in the complete directed graph on $\{1, \ldots, n\}$. For each cicuit $c \in \mathcal{C}_n$, and for each edge (j, i) in the circuit, and for each state $x \in \mathbb{B}^n$, we write $\mathcal{N}^x(j, i)$ and $\mathcal{P}^x(j, i)$ for the expressions that impose that the edge e is negative or positive, respectively, as in (26) and (25).

To impose that the label of a circuit c is negative, we require that an odd number of edges are negative, and that the remaining edges are positive:

$$\Phi_c^x = \bigvee_{\substack{1\leq k\leq m,\ k \text{ odd},\\ c=c^-\cup c^+,\ |c^-|=k}} \left(\bigwedge_{(j,i)\in c^-} \mathcal{N}^x(j, i) \wedge \bigwedge_{(j,i)\in c^+} \mathcal{P}^x(j, i) \right). \tag{33}$$

The absence of local negative circuits in the regulatory graph is therefore described by the expression

$$\bigwedge_{\substack{x\in\mathbb{B}^n\\ c\in\mathcal{C}_n}} \neg\Phi_c^x = \bigwedge_{\substack{x\in\mathbb{B}^n\\ c\in\mathcal{C}_n}} \neg\left(\bigvee_{\substack{1\leq k\leq m,\ k \text{ odd},\\ c=c^-\cup c^+,\ |c^-|=k}} \left(\bigwedge_{(j,i)\in c^-} \mathcal{N}^x(j,i) \wedge \bigwedge_{(j,i)\in c^+} \mathcal{P}^x(j,i)\right)\right), \tag{34}$$

which we can write in CNF form as

$$\bigwedge_{\substack{x\in\mathbb{B}^n\\ c\in\mathcal{C}_n}} \neg\Phi_c^x = \bigwedge_{\substack{x\in\mathbb{B}^n\\ c\in\mathcal{C}_n}} \bigwedge_{\substack{1\leq k\leq m,\ k \text{ odd},\\ c=c^-\cup c^+,\ |c^-|=k}} \left(\bigvee_{(j,i)\in c^-} l_x^1(j,i) \vee \neg l_x^0(j,i) \vee \bigvee_{(j,i)\in c^+} \neg l_x^1(j,i) \vee l_x^0(j,i)\right).$$

Absence of fixed points

Before considering Question 1, we describe how a different question can be easily translated into a Boolean satisfiability problem. The question is the following:

Question 2. 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?

Having imposed the absence of local negative circuits as in Section 5.2.1, to ask Question 2, it remains to write formulas that impose the absence of fixed points. To impose that a state $x \in \mathbb{B}^n$ is not a fixed point for f we can write:

$$\mathcal{F}^x = \bigvee_{\substack{1\leq i\leq n\\ x_i=0}} f_i(x) \vee \bigvee_{\substack{1\leq i\leq n\\ x_i=1}} \neg f_i(x), \tag{35}$$

and to impose no fixed points for f:

$$\bigwedge_{x\in\mathbb{B}^n} \mathcal{F}^x = \bigwedge_{x\in\mathbb{B}^n} \left(\bigvee_{\substack{1\leq i\leq n\\ x_i=0}} f_i(x) \vee \bigvee_{\substack{1\leq i\leq n\\ x_i=1}} \neg f_i(x)\right), \tag{36}$$

Since the state $\mathbf{0}$ is not fixed, there exists an index i such that $f_i(\mathbf{0}) = 1$. Consider a permutation $\sigma \in S_n$ that sends i to 1. The map $g = \sigma \circ f \circ \sigma^{-1}$ satisfies $g_1(\mathbf{0}) = 1$; in addition, by Proposition 3.2.11, g and f have local circuits with the same signs. We can therefore reduce the search space by assuming that the first coordinate of $f(\mathbf{0})$ is 1. The formula for Question 2 is therefore:

$$\mathcal{Q}_2 = \left(\bigwedge_{x\in\mathbb{B}^n} \mathcal{F}^x\right) \wedge \left(\bigwedge_{x\in\mathbb{B}^n, c\in\mathcal{C}_n} \neg\Phi_c^x\right) \wedge f_1(\mathbf{0}). \tag{37}$$

The unsatisfiability of this problem is thus determined, for $n = 5$, in minutes, by the satisfiability solvers. By giving the formula in (37) with $n = 6$ in input to a satisfiability solver, we can also identify other examples of maps with no fixed points and no local negative circuits. The existence of a cyclic attractor is less straightforward to impose; we describe our approach in the next section.

Example 5.2.1. Using the formula in (32), we can require a Boolean network to admit an antipodal attractive cycle (see Definition 5.1.1). We can use the expression in (32) in conjunction with the formula in (37), to look for a map with an antipodal attractive cycle, no local negative circuits and no fixed points. Using a SAT solver, we find that the formula is satisfiable.

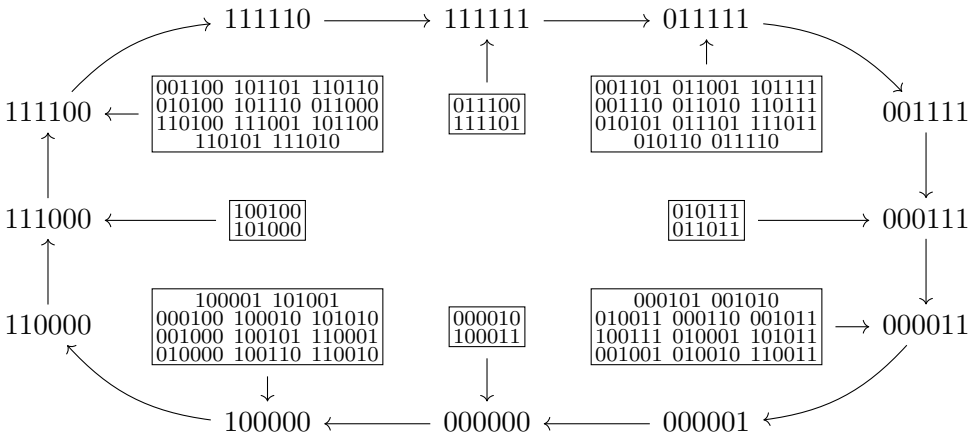


Figure 16: *Synchronous dynamics for a regulatory network in dimension 6 with an antipodal attractive cycle, no fixed points and no local negative circuits.*

We can add for instance the requirement that the map is T-equivariant (see Definition 5.1.2), with T the map in (22). This can achieved with the Boolean formula

$$(f_1(T(x)) \iff \neg f_n(x)) \wedge \bigwedge_{\substack{x\in\mathbb{B}^n \\ 2\leq i\leq n}} (f_i(T(x)) \iff f_{i-1}(x)). \tag{38}$$

In this case we find that the Boolean problem is not satisfiable.

Instead of the formula in (38), we can consider a less strict requirement, asking that the map is T^3-equivariant, using the formula

$$\bigwedge_{\substack{x\in\mathbb{B}^n \\ 1\leq i\leq 3}} (f_i(T^3(x)) \iff \neg f_{n-3+i}(x)) \wedge \bigwedge_{\substack{x\in\mathbb{B}^n \\ 4\leq i\leq n}} (f_i(T^3(x)) \iff f_{i-1}(x)).$$

For additional symmetry, we also impose that the map is S-equivariant, with S the map defined as $S(x) = \bar{x}$ for all $x \in \mathbb{B}^n$, with the formula

$$\bigwedge_{\substack{x\in\mathbb{B}^n \\ 1\leq i\leq n}} (f_i(S(x)) \iff \neg f_i(x)).$$

With these additional requirements, the SAT solver finds for instance the Boolean network represented in Figure 16.

Example 5.2.2. Using the formula in (28) we can impose the existence of a local circuit, or, for instance, of a type 2 functional circuit, i.e., a circuit that is shared by all the local graphs in a subcube of $\mathbb{B}^n$ (see Section 3.3). For $n = 2$, it is easy to see that, if a map admits two fixed points or no fixed point, then its regulatory graph admits a type 2 circuit. Consider the following questions: what is the minimum n such that a map on $\mathbb{B}^n$ admits two fixed points and no type 2 circuit? What is the minimum n such that a map on $\mathbb{B}^n$ has no fixed point and no type 2 circuit? (These questions were suggested by A. Fauré).

We can translate the conditions of the questions to Boolean formulas as follows. Given $x \in \mathbb{B}^n$, to require that the local regulatory graph $G_f(x)$ does not contain a given circuit $c \in \mathcal{C}_n$ we have to impose that at least one of the regulations of the circuit is missing. As a Boolean formula this can be expressed as

$$\bigvee_{(j,i)\in c} \mathcal{O}^x(j,i).$$

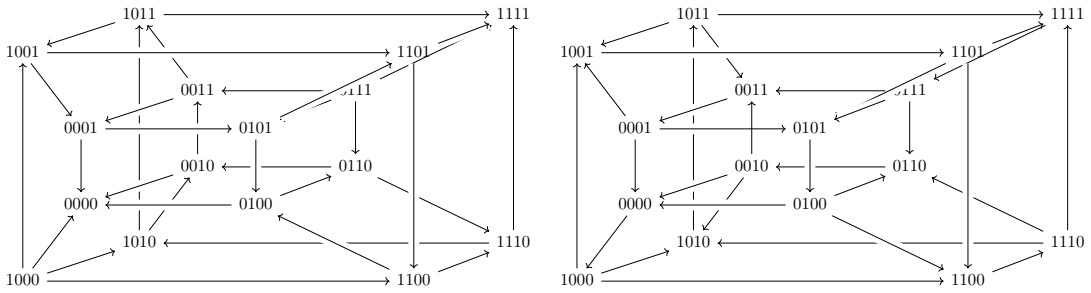


Figure 17: *(a): Asynchronous state transition graph for a map with two fixed points and no type 2 functional circuit. (b) Asynchronous state transition graph for a map with no fixed points and no type 2 functional circuit.*

See equation (27) for the definition of $\mathcal{O}^x$. Write $\mathcal{K}$ for the set of all subcubes of $\mathbb{B}^n$. The circuit c is not a type 2 circuit if, for each subcube $\kappa \in \mathbb{B}^n$, there is a state $x \in \kappa$ such that c is not a circuit in $G_f(x)$:

$$\bigwedge_{\kappa\in\mathcal{K}} \bigvee_{x\in\kappa} \bigvee_{(j,i)\in c} \mathcal{O}^x(j,i).$$

Consider the first question. By propositions 3.2.1 and 3.2.11, we can assume that the fixed points are $\mathbf{0}$ and $\mathbf{1}$. We use (24) to impose $f(\mathbf{0}) = \mathbf{0}$ and $f(\mathbf{1}) = \mathbf{1}$. Hence the Boolean formula for the first question is

$$\left(\bigwedge_{c\in\mathcal{C}_n} \bigwedge_{\kappa\in\mathcal{K}} \bigvee_{x\in\kappa} \bigvee_{(j,i)\in c} \mathcal{O}^x(j,i)\right) \wedge \bigwedge_{1\le i\le n} \neg f_i(\mathbf{0}) \wedge \bigwedge_{1\le i\le n} f_i(\mathbf{1}),$$

and for the second question (see (36)):

$$\left(\bigwedge_{c\in\mathcal{C}_n} \bigwedge_{\kappa\in\mathcal{K}} \bigvee_{x\in\kappa} \bigvee_{(j,i)\in c} \mathcal{O}^x(j,i)\right) \wedge \bigwedge_{x\in\mathbb{B}^n} \mathcal{F}^x.$$

We find that, for $n = 3$, the presence of multiple fixed points and the absence of fixed points imply the existence of a type 2 functional circuit. For $n = 4$, the formulas are satisfiable. We find the maps with no type 2 circuits shown in Figure 17.

A necessary condition for the existence of a cyclic attractor

We now go back to Question 1, and consider therefore the existence of a cyclic attractor in the asynchronous state transition graph of f. The approach is based on the following observation.

Proposition 5.2.3. *The asynchronous state transition graph AD_f of a map $f : \mathbb{B}^n \to \mathbb{B}^n$ admits a cyclic attractor if and only if there exists a state $x \in \mathbb{B}^n$ such that, for any $y \in \mathbb{B}^n$, if there is a path in AD_f from x to y, then y is not a fixed point.*

Proof. If AD_f admits a cyclic attractor, then the conclusion is true for any state x in the cyclic attractor.

Viceversa, suppose that x is a state with the described property, and call R the set of points reachable from x in the asynchronous state transition graph. Then the minimal trap domain contained in R does not contain any fixed point, hence it must contain a cyclic attractor for AD_f. □

Proposition 5.2.3 translates the existence of a cyclic attractor into a condition on the paths in the asynchronous state transition graph. It is, however, computationally problematic to impose that, if AD_f contains a path of any length from x to y, then y is not a fixed point. We therefore consider the following condition instead.

Condition $\Gamma(k)$. There exists a state $x \in \mathbb{B}^n$ such that, for each $y \in \mathbb{B}^n$, if there is an acyclic path in AD_f from x to y of length at most k, then y is not a fixed point.

It is clear from Proposition 5.2.3 that, for each $k \geq 0$, Condition $\Gamma(k)$ is a necessary condition for the existence of a cyclic attractor. Our strategy is therefore to impose the absence of local negative circuits, as well as Condition $\Gamma(k)$ for increasing values of k, until we find that the problem is unsatisfiable.

Given a sequence of states $\pi = (x^0, x^1, \ldots, x^k)$ such that $d(x^i, x^{i+1}) = 1$, $i = 0, \ldots, k-1$, recall that we write Θ^π for the expression that imposes π as a path in the asynchronous dynamics (30). Given a state $x \in \mathbb{B}^n$, denote by $P^k(x)$ the set of acyclic paths in the n-dimensional hypercube graph that start from x and have length less or equal to k. If π is a path in AD_f, we denote by $t(\pi)$ the last node of the path. We impose Condition $\Gamma(k)$ for a state $x \in \mathbb{B}^n$, using (35), as follows:

$$\bigwedge_{\pi \in P^k(x)} \left(\Theta^\pi \Rightarrow \mathcal{F}_p^{t(\pi)}\right) = \bigwedge_{\pi \in P^k(x)} \neg\Theta^\pi \vee \mathcal{F}_p^{t(\pi)}. \tag{39}$$

Condition $\Gamma(k)$ requires the existence of a state $x \in \mathbb{B}^n$ that verifies (39). Suppose that a map f satisfies condition (39) for some $x \in \mathbb{B}^n$, and that its local regulatory graphs do not admit any negative circuit. Consider j such that $f_j(x) \neq x_j$, and call σ the permutation that swaps 1 and j. Define $I = \{i \in \{1, \ldots, n\} | \sigma(x)_i \neq 0\}$. Then, by Proposition 3.2.11, the map f^U with $U = \psi_I \circ \sigma$ admits a cyclic attractor containing $\mathbf{0}$, and its local regulatory graphs do not admit any negative circuit. In addition, $f_1(\mathbf{0}) = 1$. We have therefore that, to exclude the existence of maps with cyclic attractors and no local negative circuits, it is sufficient to consider expression (39) for $x = \mathbf{0}$, and assume $f_1(\mathbf{0}) = 1$. By combining (39) with (34), we find, for fixed k, the Boolean formula

$$\mathcal{Q}_1 = \left(\bigwedge_{\pi \in P^k(\mathbf{0})} \left(\Theta^\pi \Rightarrow \mathcal{F}_p^{t(\pi)}\right)\right) \wedge \left(\bigwedge_{x \in \mathbb{B}^n, c \in \mathcal{C}_n} \neg\Phi_c^x\right) \wedge f_1(\mathbf{0}). \tag{40}$$

Notice that $\mathcal{Q}_1$ is a generalization of (37), where fewer points are required to be non-fixed. Using (39) and (30), (40) is easily written in CNF form. The expressions defined in this section are summarized in Table 4

Results

We created CNF files in DIMACS CNF format, a standard input format accepted by most SAT solvers. The files start with a line that begins with `p cnf` followed by the number of variables and the number of clauses. One line for each clause then follows. Each clause is expressed by listing the indices of the variables involved in the clause separated by spaces, using negative numbers for negated variables. A zero is added at the end of each clause line.

The files were created with a Python script (available at github.com/etonello/regulatory-network-sat). Using the satisfiability solver Lingeling [Bie16], we found that, if k is set to $2, 4, 6, 11$ respectively, for $n = 2, 3, 4, 5$, the problem described by (40) is unsatisfiable. This means that, for $n \leq 5$, all maps that admit a cyclic attractor must have a local negative circuit. The results are summarised in Table 5.

Expression	Description
$\Phi_c^x = \bigvee_{\substack{1\le k\le m,\ k \text{ odd},\\ c=c^-\cup c^+,\ \lvert c^-\rvert=k}} \left(\bigwedge_{(j,i)\in c^-} \mathcal{N}^x(j,i) \wedge \bigwedge_{(j,i)\in c^+} \mathcal{P}^x(j,i) \right)$	c is a local negative circuit at state $x \in \mathbb{B}^n$ (33)
$\bigwedge_{x\in\mathbb{B}^n, c\in\mathcal{C}_n} \neg\Phi_c^x$	absence of local negative circuits (34)
$\mathcal{F}^x = \bigvee_{\substack{1\le i\le n\\ x_i=0}} f_i(x) \vee \bigvee_{\substack{1\le i\le n\\ x_i=1}} \neg f_i(x)$	$x \in \mathbb{B}^n$ is not fixed (35)
$\bigwedge_{x\in\mathbb{B}^n} \mathcal{F}^x$	absence of fixed points (36)
$\Theta^\pi = \bigwedge_{\substack{0\le i\le k-1\\ j \text{ s.t. } x_j^i\neq x_j^{i+1}\\ x_j^{i+1}=0}} \neg f_j(x^i) \wedge \bigwedge_{\substack{0\le i\le k-1\\ j \text{ s.t. } x_j^i\neq x_j^{i+1}\\ x_j^{i+1}=1}} f_j(x^i)$	$\pi = (x^0, x^1, \ldots, x^k)$ is a path in the asynchronous state transition graph (30)
$\bigwedge_{\pi\in P^k(x)} \neg\Theta^\pi \vee \mathcal{F}^{t(\pi)}$	Condition ($\Gamma(k)$)

Table 4: *Expressions considered to answer Question 1.*

n	2	3	4	5
k	2	4	6	11

Table 5: *Minimum path length k such that, in a Boolean model with n variables,* (40) *is unsatisfiable, i.e. if all paths from state* **0** *of length at most k do not reach a fixed point, there must exist a local negative circuit.*

The lengths $k = 2, 4, 6, 11$ are the minimum lengths that lead to the unsatisfiability of the formula in (40). In other words, there exists at least one map in dimension 2 (respectively 3, 4 and 5) such that the paths of length at most 1 (respectively 3, 5 and 10) do not reach a fixed point, and the associated regulatory graph does not admit a local negative circuit. Examples of such maps are given in Figure 18, for $n = 2$ and $n = 3$. Figure 19 illustrates instead the idea of the result obtained for $n = 2$ and $n = 3$, for two special cases of asynchronous state transition graphs admitting a unique path leaving the origin: since this path reaches 3 (respectively 5) different states, the regulatory graph must admit a local negative circuit, somewhere in the state space.

The CNF file for $n = 5$ and $k = 11$ on the 160 variables consists of 2.6 million clauses. The satisfiability solver Lingeling [Bie16] was used to determine the unsatisfiability and to generate a proof, expressed in the standard DRAT notation [WHH14]. For $n = 5$ and $k = 11$, the file for the proof is about 1GB in size. The proof was verified using the SAT checking tool chain GRAT [Lam17].

5.2.2 *Multilevel regulatory networks*

In Chapter 4 we introduced regulatory networks with variables admitting more than two expression levels. Given maximum expression levels $m_1, \ldots, m_n \in \mathbb{N}$, a multilevel regulatory network is a map $f : \mathcal{X} \to \mathcal{X}$, where $\mathcal{X}$ is the product of intervals of integers $\mathcal{X} = \{0, \ldots, m_1\} \times \cdots \times \{0, \ldots, m_n\}$. Taking $\mathcal{Y} = \mathbb{B}^m$ with $m = \sum_{i=1}^n m_i$, we defined a map $\varphi\colon \mathcal{X} \to \mathcal{Y}$ as $\varphi(x) = (\chi_{[1,m_1]}(x_1), \ldots, \chi_{[m_1,m_1]}(x_1), \chi_{[1,m_2]}(x_2), \ldots, \chi_{[m_n,m_n]}(x_n))$

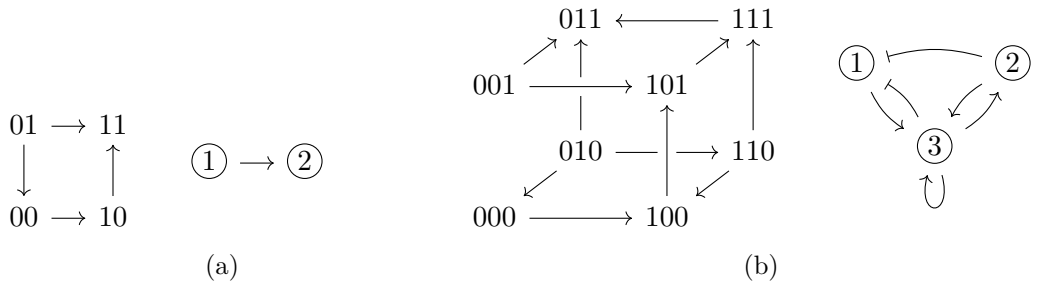


Figure 18: *Example showing that Condition* $\Gamma(k)$ *is compatible with the absence of local negative circuits for* $n = 2$ *with* $k = 1$*, and for* $n = 3$ *with* $k = 3$*. (a): The asynchronous state transition graph and the regulatory graph for the map* $f(x_1, x_2) = (1, x_1)$*. The path of length* 2 *leaving the origin reaches a fixed point, and the regulatory graph does not admit any local circuit. (b) The asynchronous state transition graph and the (global) regulatory graph for the map* $f(x_1, x_2, x_3) = (1 - x_2x_3, x_3, x_1x_2x_3 - x_1x_2 - x_1x_3 - x_2x_3 + x_1 + x_2 + x_3)$*. The path of length* 4 *leaving the origin reaches a fixed point; none of the negative circuits admitted by regulatory graph are local.*

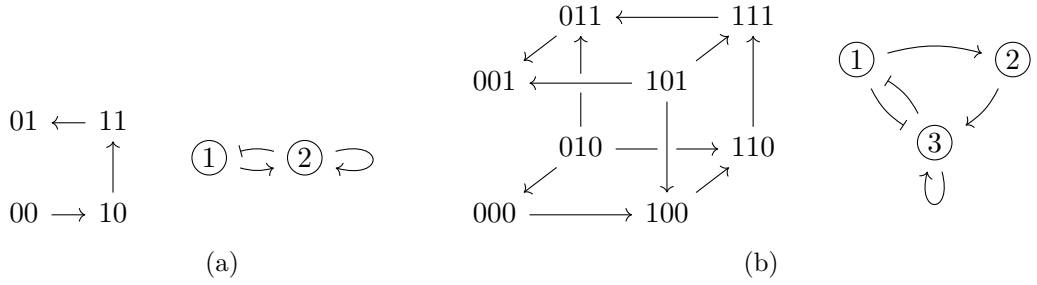


Figure 19: *(a): The asynchronous state transition graph and the regulatory graph for the map* $f(x_1, x_2) = (1 - x_2, x_1 + x_2 - x_1x_2)$*. The paths leaving the origin do not reach a fixed point in* 2 *steps, hence a local negative circuit must exist in the regulatory graph. The unique attractor for the asynchronous state transition graph is a fixed point. (b) The asynchronous state transition graph and the (global) regulatory graph for the map* $f(x_1, x_2, x_3) = (1 - x_3, x_1, x_1x_2x_3 - x_1x_3 - x_2x_3 + x_2 + x_3)$*. No local negative circuit of dimension* 1 *or* 2 *exists; however, since the only path leaving the origin has length* 5*, the regulatory graph must admit a local negative circuit involving all three variables. The unique attractor for the asynchronous state transition graph is a fixed point.*

The map φ is injective, and its image $\mathcal{A}$ is called the set of *admissible states*. The conjugation with φ provides a one-to-one mapping from multilevel networks on $\mathcal{X}$ to maps from $\mathcal{A}$ to itself. We can use the mapping to write constraints on multilevel networks using Boolean variables. Recall that we use pairs of indices (i, j), $i = 1, \ldots, n$, $j = 1, \ldots, m_i$, to identify the components of elements of $\mathcal{Y}$. We need to define $2^m \cdot m$ Boolean variables, and impose that, for each $x \in \mathcal{Y}$, $f(x)$ is in $\mathcal{A}$. Since a state $y \in \mathbb{B}^m$ is in $\mathcal{A}$ if and only if

$$y_{i,j} \geq y_{i,j+1}, \text{ for } i = 1, \ldots, n, \ j = 1, \ldots, m_i - 1,$$

we write the expression

$$\bigwedge_{i=1,\ldots,n} \bigwedge_{j=1,\ldots,m_i-1} f_{i,j+1}(x) \Rightarrow f_{i,j}(x). \tag{41}$$

We can in addition impose that the multilevel map is stepwise by considering the expression

$$\bigwedge_{i=1,\dots,n} \left(\bigwedge_{j=1,\dots,m_i-1} \bigwedge_{\substack{x\in\mathcal{A} \\ x_{i,j}=0}} \neg f_{i,j+1}(x) \wedge \bigwedge_{j=2,\dots,m_i} \bigwedge_{\substack{x\in\mathcal{A} \\ x_{i,j}=1}} f_{i,j-1}(x) \right). \tag{42}$$

Finally, we can require that f satisfies $f \circ \psi = f$, with ψ the map defined in (20). Theorems 4.3.6 and 4.3.7 then guarantee that the regulatory graph of f admits a local negative circuit if and only if the regulatory graph of the multilevel version admits a local negative circuit. The requirement $f \circ \psi = f$ is expressed by the Boolean formula

$$\bigwedge_{i=1,\dots,n} \bigwedge_{j=1,\dots,m_i} f_{i,j}(\psi(x)) \Leftrightarrow f_{i,j}(x). \tag{43}$$

Using the formulas in (41), (42), (43) and (37), and imposing in addition $f_1(1,0) = 0$, we find that the map in Figure 11 is the unique regulatory network on $\{0,1,2,3\}^2$ that satisfies $f_1(1,0) = 0$, is stepwise, does not have any fixed point and does not admit any local negative circuit.

5.3 OPEN QUESTIONS

The following are some open questions related to the topics discussed in this part of the thesis.

(i) Count the number of Boolean networks with non-isomorphic asynchronous state transition graphs (see Remark 3.2.2). Count the number of different asymptotic behaviours of asynchronous dynamics.

(ii) Is the pair of states of Theorem 4.3.17 always a mirror pair?

(iii) Identify a short proof that, for $n \leq 5$, a local negative circuit in the regulatory graph is necessary for the existence of a cyclic attractor or the absence of fixed points.

(iv) Identify classes of cyclic attractors or of maps with cyclic attractors which require the existence of local negative circuits for all n.

Part II

CHEMICAL REACTION NETWORKS

6

OVERVIEW OF PART II

Reaction networks are used in areas such as biology, chemistry and engineering to model processes involving species that interact to form other species. A *chemical reaction* is indicated with an arrow, for example

$$A + B \longrightarrow C \tag{44}$$

represents the combination of a unit of the *chemical species* A and B to form a unit of the species C. The entities appearing on the left and right sides of the arrows are called *complexes*, more precisely, *reactant* complex and *product* complex of the reaction, respectively. For example, a network consisting of three reactions

$$A + B \longrightarrow C, \qquad C \longrightarrow B + D, \qquad B + D \longrightarrow A + B, \tag{45}$$

has four species, A, B, C and D, and three complexes, $A + B$, C and $D + B$. We usually represent the network as a graph with the complexes as nodes:

$$\begin{array}{ccc} A + B & \longrightarrow & C \\ & \nwarrow \quad \swarrow & \\ & B + D & \end{array} \tag{46}$$

This allows to highlight useful properties of the network, such as the number of connected components or the reversibility.

In classical Chemical Reaction Network Theory (see for instance [Fei79, Fei87, Fei88, Hor72]), the reactions are usually assumed to take place in a well-stirred container which is spatially homogeneous. The species concentrations (that we denote for the example above as x_A, x_B, x_C, x_D) are modelled as continuous functions that satisfy a system of ordinary differential equations (ODEs). Other modelling approaches exist, which consider for instance discrete variables for the species concentrations, and stochastic processes to model the evolution in time.

To associate a system of ODEs to (46), a choice needs to be made about the rate at which the reactions take place. Under the assumption of *mass action kinetics* the rates are supposed proportional to the concentrations of the reactant species. The proportionality constants are called *rate constants* or *kinetic parameters*. The reaction in (44) for instance will have a rate of the form $\kappa_1 x_A x_B$. The other two reactions in (45) will have rates $\kappa_2 x_C$ and $\kappa_3 x_B x_D$. To determine a differential equation for species A, one sums the effects of all the reactions that involve A: the species is consumed in the first reaction and produced by the last, so that we can write

$$\frac{dx_A}{dt} = -\kappa_1 x_A x_B + \kappa_3 x_B x_D,$$

and similarly for the other species.

This process leads generally, in practical situations, to large systems that can not be solved analytically. In addition, the equations involve a considerable number of parameters that are often not easy to infer. These factors make the prediction of the system behaviour — for instance, establishing whether the system can display oscillations, or admit more than one stable state — particularly challenging. Results in chemical reaction network theory, however, derive conclusions on the dynamical behaviour of chemical systems from characteristics of the network structure, meaning, from the information encoded in graphs like the one in (46). To demonstrate the idea, we can for instance observe that the graph in (46) contains the following insight on how the system can evolve: if at some point the system contains 5 units in total between species A, C and D (i.e., $x_A + x_C + x_D = 5$), then the system will always verify $x_A + x_C + x_D = 5$. Equations of this form are called *conservation laws*, and their effect is that of restricting the dynamics to the *stoichiometric compatibility classes*, i.e. translations of the *stoichiometric subspace*, which is generated by the vectors expressing the change in the units of species fulfilled by each reaction.

Another structural property that is frequently discussed in chemical reaction network theory is the *deficiency* of the network, which can be calculated by taking the number of complexes and subtracting the number of connected components of the graph and the rank of the stoichiometric subspace. It measures the difference between the rank of the stoichiometric subspace and the rank of the incidence matrix of the graph of complexes, which can be seen as the rank of the stoichiometric subspace that is obtained by replacing each complex with a species. For instance, the network in (46) has 3 complexes and one connected component, and its stoichiometric subspace is generated by the vectors $(-1, -1, 1, 0)$ and $(0, 1, -1, 1)$, and we find that the deficiency is equal to zero. The network is also *weakly reversible*, meaning that if a path exists from a complex c_1 to another complex c_2, then there is also a path back from the complex c_2 to the complex c_1. The network in (46) falls under the hypotheses of one of the first results of chemical reaction network theory, the *Deficiency Zero Theorem* [Fei79, Fei87]. The theorem establishes that mass action systems associated to weakly reversible networks with deficiency zero admit a unique steady state in each stoichiometric compatibility class, which is asymptotically stable. Moreover, the steady states of the system are all *complex balancing*, meaning that at equilibrium the rates of reactions entering a complex balance the rates of reactions exiting the complex. A consequence of this is that for instance the ratio between the product $x_A x_B$ and the concentration x_C does not depend on the initial conditions, but only on the kinetic parameters (we call this a form of *robustness* of the system).

Since the classical deficiency theory results, the literature on chemical reaction network theory has expanded in several directions, exploring for instance convergence and multistationarity, for mass action systems and under more general kinetics (e.g. [CDSS09, DLAS07, AS08, BC09, MDSC12, BM13, MD16]). A recent direction explores a generalisation of mass action systems which allows in particular for reaction rates that are products of concentrations, not necessarily determined by the stoichiometric coefficients of the reactants, and identifies conditions for existence or uniqueness of complex balancing steady states [MR12, MR14]. With generalised mass action kinetics, a reaction rate of the form $\kappa_1 x_A^2$, for instance, is admissible for the reaction in (44). Generalised mass action is a more realistic choice for the kinetics in some biological contexts [MR12, MR14]; in this work we will use it, however, as a tool for the analysis of mass action networks.

Although mass action kinetics is very commonly used, biochemical reaction models often adopt kinetic rates of other forms. Reaction schemes for instance in the study of enzyme kinetics are frequently condensed into a smaller number of reactions, resulting in models involving a smaller number of species or of parameters, and therefore more

manageable. For instance, the first two reactions in (45) might be summarised with a single reaction $A \to D$, with a rate of the form $\frac{\kappa' B_0 x_A}{\kappa'' + x_A}$, that can be derived by eliminating the intermediate species C, using some simplifications referred to in the literature as quasi steady state approximation or rapid equilibrium approximation [Seg75, CB14]. It might be however useful to clarify the underlying mass action structure of a given system, to ascertain whether results connecting features of the structure to properties of the mass action dynamics apply. The detection of the mass action network from a simplified model seeming in the majority of cases a hopeless task, we focus instead on describing methods for the identification of chemical systems that give rise to some desired simplified system of ODEs, starting from a given mass action system. In Chapter 8 we delineate in particular two methods for elimination of intermediate species, that one can use to recover for instance some kinetic laws used in enzyme kinetics. These methods do not provide rationales for the simplifications, but rather, provide some clues about the connections between the structure of the mass action and the simplified systems.

Another fact to take into account is that, if we are interested in studying complex balancing steady states of a system, then the network needs to be in weakly reversible form. A useful observation is that networks with different structures can give rise to the same dynamics (in this case they are called *realisations* of the same dynamics); one can study properties of a given dynamical system by identifying a realisation with desired properties (see for instance [SHT12, JSS13, LSH14, Joh16]). Determining realisations with good structural properties in a parameter-independent way is a possible approach to the study of chemical systems. As a step in this direction, one can observe that, even if a network is not weakly reversible, if the associated mass action system admits some positive steady states, then every reaction must take part in some hidden cycle, that potentially is not directly visible in the graph structure. Take for instance the following reaction network, which is similar to the one in (46):

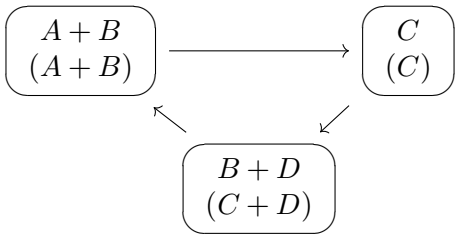
$$A + B \longrightarrow C \longrightarrow B + D, \qquad C + D \longrightarrow A + C. \tag{47}$$

This network has 5 complexes, and is not weakly reversible. The steady states are certainly not complex balancing, since there are complexes with incoming reactions but no outgoing reactions. The cycle in the original network (46) ensures that, if the system contains n_A units of A and n_B units of B, after the three reactions take place, the system still contains the same number n_A and n_B of units. However, the same is still true for the network in (47), even if we cannot see this cycle directly. The idea introduced in [Joh14] allows to change the structure of the graph so that the cycle becomes visible, keeping the same dynamics for the reaction network. This is achieved by *translating* the reaction, i.e. adding the same complex to the reactant and product. In the example above, we take the last reaction and translate it by the "generalised" complex $B - C$:

$$C + D \to A + C \overset{+B-C}{\Longrightarrow} B + D \to A + B.$$

The stoichiometric subspace of the network is unchanged. To ensure that the reaction network obtained gives rise to the right dynamics, the translated reaction can be assigned with the reaction rate of the original reaction (in this case, the rate is of the form $\kappa_3 x_C x_D$). This is permitted in the generalised mass action framework. We find the

graph in (46), with a different assignment of kinetic rates. We write the complexes determining the rates in parenthesis as follows:



(48)

This network satisfies the hypothesis of a generalised mass action version of the Deficiency Zero Theorem. In particular, at steady state the ratio of the monomials determined by the complexes in parenthesis, for instance the ratio between $x_D x_C$ and x_C, is independent of the initial conditions, meaning in this case that positive steady state values of the concentration of species D will not depend on the particular initial state of the system. When this condition is verified for the concentration of a species, the system is said to have *absolute concentration robustness* in the species [SF10]. In Chapter 9 we first discuss the definition of network translations, and introduce an intermediate graphical structure that we call *kinetic graph*, where both the nodes and the edges are assigned a complex. We explore cases where a deficiency zero translation can be determined, and establish some connections to robustness properties.

As we see in Chapter 10, there are other approaches to the identification of weakly reversible dynamically equivalent generalised mass action systems. Since strong conclusions on the steady states can be drawn for networks with deficiency zero or one, we outline a MILP program that allows to search for such realisations in Section 10.2.

7

DEFINITIONS

In this chapter we introduce some background of Chemical Reaction Network Theory (CRNT) (see e.g. [FH77, Fei79, Fei87, Hor72]), which studies properties of systems of differential equations associated to reaction networks and their connections to the network structure. After introducing the graph structure of chemical reaction networks, we define mass action systems and generalised mass action systems, and summarise some classical results around complex balancing steady states, as well as some recent extensions to generalised mass action [MR12, MR14]. We then recall the definition of robustness and the structural conditions for robustness given in [SF10], and state generalised mass action versions of these conditions.

Notations introduced in this chapter

We write $\mathrm{e}^i \in \mathbb{R}^n$ for the vector with $\mathrm{e}^i_i = 1$, $\mathrm{e}^i_j = 0$ for $j \neq i$. The support of a vector $v \in \mathbb{R}^n$ is the set $\mathrm{supp}(v) = \{i \in \{1, \dots, n\} \mid v_i \neq 0\}$.

Given a vector $\mathbf{x} \in \mathbb{R}^n$ and a matrix $\mathbf{M} \in \mathbb{R}^{n \times m}$, we write $\mathbf{x}^{\mathbf{M}} \in \mathbb{R}^m$ for the vector with component j given by $\prod_{i=1}^n x_i^{\mathbf{M}_{ij}}$.

7.1 CHEMICAL REACTION NETWORKS

Given a finite set $\mathcal{S} = \{X_1, \dots, X_n\}$, called the set of *species*, we call a *complex* in the set of species $\mathcal{S}$ an element of $\mathbb{N}^n$, which we denote as an integer linear combination of species. For instance, if $\mathcal{S} = \{A, B\}$, $A + B$ and $2B$ are complexes in $\mathcal{S}$, that stand for the vectors $(1, 1)^t$ and $(0, 2)^t$, respectively. Given $y \in \mathbb{N}^n$, we will call the component y_i the *stoichiometric coefficient* of species X_i in y. The complexes are the entities appearing on the right and left side of arrows representing reactions in the chemical literature. For instance, $A + B \to 2B$ is the reaction that converts the reactant complex $A + B$, consisting of a unit of species A and a unit of species B, to the product complex $2B$ consisting of two units of species B.

Definition 7.1.1. A *chemical reaction network* $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y)$ on the set of species $\mathcal{S}$ is a directed graph with set of nodes $\mathcal{C} = \{1, \dots, c\}$ and set of edges $\mathcal{R}$ that verifies $\mathcal{R} \subseteq \mathcal{C} \times \mathcal{C} \setminus \{(i, i) \mid i \in \mathcal{C}\}$, together with an injective map $y : \mathcal{C} \to \mathbb{N}^n$.

We gave the definition in a slightly non-conventional form, which will be easier to see as a special case of the definition of generalised chemical reaction network (see Section 7.3). A chemical reaction network is therefore a directed graph with nodes labelled by complexes, that does not admit loops and parallel edges.

To simplify the notation, we will write y^i for $y(i)$; a reaction (i, j) will be denoted $y^i \to y^j$; the source complex y^i is called the *reactant* of the reaction, and the target complex the *product* of the reaction. The vector $y^j - y^i \in \mathbb{Z}^n$ is called the *(stoichiometric) reaction*

vector of the reaction. The *stoichiometric subspace* of a chemical reaction network is the subspace of $\mathbb{R}^n$ generated by the reaction vectors of the network:

$$S = \text{span}\{y^j - y^i \mid (i,j) \in \mathcal{R}\}.$$

The dimension $s = \dim(S)$ is called the *rank* of the reaction network.

We write m for the number of reactions in the network, and fix an order for the reactions. We denote by $\mathbf{I}_\mathcal{R}$ the incidence matrix of the graph $(\mathcal{C}, \mathcal{R})$, i.e. the $c \times m$ matrix with entry $(\mathbf{I}_\mathcal{R})_{ij} = -1$ if i is the source of the j^{th} reaction, $(\mathbf{I}_\mathcal{R})_{ij} = 1$ if i is the target of the j^{th} reaction, and $(\mathbf{I}_\mathcal{R})_{ij} = 0$ otherwise. The *stoichiometric matrix* $\mathbf{\Gamma} \in \mathbb{Z}^{n \times m}$ of the reaction network is the matrix with columns the reaction vectors of the reactions in $\mathcal{R}$. The $n \times c$ matrix $\mathbf{Y}$ with columns the vectors $y^1, \ldots, y^c$ is called the *complex matrix* of the reaction network. One can show that the stoichiometric matrix decomposes as the product of the complex matrix and incidence matrix:

$$\mathbf{\Gamma} = \mathbf{Y}\mathbf{I}_\mathcal{R}. \tag{49}$$

CRNT uses some special terminology to indicate the connected components of the graph $(\mathcal{C}, \mathcal{R})$. The strongly connected components are called the *strong linkage classes* of the reaction network. The *linkage classes* $\mathcal{L}_\theta$, $\theta = 1, \ldots, l$, of the reaction network are the connected components of the undirected graph obtained from the graph $(\mathcal{C}, \mathcal{R})$ by ignoring the direction of the reactions. A strong linkage class is said to be terminal if it admits no outgoing edges. A chemical reaction network is said to be *weakly reversible* if its linkage classes coincide with its strong linkage classes.

The *deficiency* of a chemical reaction network is the integer

$$\delta = c - l - s. \tag{50}$$

The difference $c - l$ is coincides with the rank of the incidence matrix $\mathbf{I}_\mathcal{R}$ (see for instance [Fou12]). The deficiency can equivalently be defined as (compare to (49), or see for instance [Joh14, Appendix A])

$$\delta = \dim(\ker(\mathbf{Y}) \cap \text{Im}(\mathbf{I}_\mathcal{R})). \tag{51}$$

In particular, δ is non-negative integer.

Invariants of chemical reaction networks

Borrowing some terminology from metabolic pathway analysis, we define the set P of *steady state fluxes* or simply *fluxes* as

$$P = \ker(\mathbf{\Gamma}) \cap \mathbb{R}^m_{\geq 0}. \tag{52}$$

Contrary to the general metabolic analysis setting, we work only with reactions that have a specific direction, i.e. all reactions are *irreversible*. The set P is a pointed convex polyhedral cone, and therefore admits a minimal set of generators $w^1, \ldots, w^d$, which are unique up to positive scalar multiplication. This means that the fluxes at steady state can be equivalently described as the set $\{\sum_{i=1}^d \alpha_i w^i \mid \alpha_i \in \mathbb{R}_{\geq 0}\}$. The vectors $w^1, \ldots, w^d$ are called *extreme rays* of the cone. An *elementary (flux) mode* w of the network is defined as a non-zero flux with *minimal support* [SH94, KRG+17], meaning that there are no fluxes $v \neq 0$ such that $\text{supp}(v) \subset \text{supp}(w)$. When all reactions are taken as irreversible, the set generators of the flux cone determines the set of elementary modes ([SH94, Theorem 4.1]). For this reason, we call the generators $w^1, \ldots, w^d$ elementary (flux) modes. For the study of flux cones and the related tools in metabolic pathway

analysis, and clarification on the terminology, we refer the reader to the review [KRG$^+$17] and references therein. The relevant background on convex geometry can be found in [Roc70]. We will call *cyclic* the fluxes w that verify $w \in \ker(\mathbf{I}_\mathcal{R})$, and *stoichiometric* the remaining vectors in P.

The left orthogonal complement $\mathbf{\Gamma}^\perp$ of $\mathbf{\Gamma}$ is called the *conservation subspace* of the network.

7.2 MASS ACTION SYSTEMS

We now move on to consider the definition of kinetics for a reaction network. We denote by $x_1, \dots, x_n$ the concentrations of the species in $\mathcal{S}$, that we regard as functions of time. In the examples, we will sometime write x_S for the concentration of species $S \in \mathcal{S}$.

Under the common *mass action kinetic* assumption, the rate of a reaction is proportional to the product of the concentrations of the species appearing in the reactant. The graph defining the chemical reaction network is labelled with an assignment of *rate constants* or *kinetic parameters* $\kappa\colon \mathcal{R} \to \mathbb{R}_{>0}$. For a reaction $r = (i, j)$, we will write κ_{ij} for $\kappa(i, j) = \kappa(r)$. We will denote the reaction also as $i \to j$ or $y^i \xrightarrow{r} y^j$ or $y^i \xrightarrow{\kappa_{ij}} y^j$. In addition, we will sometimes write κ_i for the kinetic parameter of the i^{th} reaction in $\mathcal{R}$, as convenient. For each complex y^i, we call *mass action monomial* the monomial $\mathbf{x}^{y^i} = \prod_{j=1} x_j^{y_j^i}$. Then, under the mass action kinetic assumption the reaction $i \to j$ has rate $\kappa_{ij}\mathbf{x}^{y^i}$. For example, a reaction of the form $A + B \xrightarrow{\kappa_1} 2B$ has rate $\kappa_1 x_A x_B$.

Definition 7.2.1. A *mass action system* is a chemical reaction network $\mathcal{N}$ endowed with a labelling $\kappa\colon \mathcal{R} \to \mathbb{R}_{>0}$ of the edges.

Given a mass action system $(\mathcal{C}, \mathcal{R}, y, \kappa)$, the associated system of differential equations governing the dynamics of the species concentrations is

$$\frac{d\mathbf{x}}{dt} = \sum_{i \to j \in \mathcal{R}} \kappa_{ij}(y^j - y^i)\, \mathbf{x}^{y^i}. \tag{53}$$

Since the left side of (53) is a positive linear combination of reaction vectors, each solution $\mathbf{x}(t)$ of (53) which starts at $\mathbf{x}^0 \in \mathbb{R}^n_{\geq 0}$ is confined to the *stoichiometric compatibility class* $(\mathbf{x}_0 + S) \cap \mathbb{R}^n_{\geq 0}$ associated to $\mathbf{x}_0$.

We will use some reformulations of the system of ODEs in (53). We call *kinetic matrix* the $c \times c$ matrix $\mathbf{A}_\kappa$ with entries $(\mathbf{A}_\kappa)_{ij} = \kappa_{ji}$ for $i \neq j$, and columns that sum to zero. We write $\mathbf{x} = (x_1, \dots, x_n)$ for the vector of species concentrations. Then, the system of differential equations in (53) can be equivalently written as

$$\frac{d\mathbf{x}}{dt} = \mathbf{Y}\mathbf{A}_\kappa \mathbf{x}^{\mathbf{Y}}. \tag{54}$$

Example 7.2.2. Consider the following network, taken from [SF10]:

$$\begin{aligned} A + B &\longrightarrow 2B, \\ B &\longrightarrow A. \end{aligned} \tag{55}$$

The set of species for this network is $\mathcal{S} = \{A, B\}$, and the complexes are $y^1 = A + B$, $y^2 = 2B$, $y^3 = B$ and $y^4 = A$. The linkage classes are $\{A + B, 2B\}$ and $\{B, A\}$, while the strong linkage classes are $\{A + B\}$, $\{2B\}$, $\{B\}$, and $\{A\}$. The network is not weakly reversible and only the strong linkage classes $\{2B\}$ and $\{A\}$ are terminal. The reaction vectors are $y^2 - y^1 = -e^1 + e^2$ and $y^4 - y^3 = -(y^2 - y^1)$ so that the rank of the network

is $s = 1$. The deficiency is $\delta = c - l - s = 4 - 2 - 1 = 1$. The vector $\mathrm{e}^1 + \mathrm{e}^2$ generates the cone $\ker(\mathbf{\Gamma}) \cap \mathbb{R}^m_{\geq 0}$ whereas $\ker(\mathbf{I}_{\mathcal{R}}) = \{0\}$.

Let us consider now an assignment of kinetic parameters $\kappa(1,2) = \kappa_1$ and $\kappa(3,4) = \kappa_2$. Under mass action kinetics, we associate to $1 \to 2$ the rate $\kappa_1 x_A x_B$, and to $3 \to 4$ the rate $\kappa_2 x_B$. The corresponding mass action system (53) is given by

$$\begin{aligned} \frac{dx_A}{dt} &= -\kappa_1 x_A x_B + \kappa_2 x_B, \\ \frac{dx_B}{dt} &= \kappa_1 x_A x_B - \kappa_2 x_B. \end{aligned} \tag{56}$$

Example 7.2.3. Consider the following model of the two-component regulatory system EnvZ-OmpR responsible for osmoregulation in *Escherichia coli*, presented in [SF10]:

$$\begin{aligned} & X \underset{r_2}{\overset{r_1}{\rightleftharpoons}} XT \xrightarrow{r_3} X_p, \\ & X_p + Y \underset{r_5}{\overset{r_4}{\rightleftharpoons}} X_pY \xrightarrow{r_6} X + Y_p, \\ & XT + Y_p \underset{r_8}{\overset{r_7}{\rightleftharpoons}} XTY_p \xrightarrow{r_9} XT + Y. \end{aligned} \tag{57}$$

X represents the histidine kinase EnvZ, Y stands for the response regulator protein OmpR, T denotes ATP, and the subscript p identifies the phosphorylated versions. The species set is $\mathcal{S} = \{X, XT, X_p, Y, X_pY, Y_p, XTY_p, XDY_p\}$, and the complexes are $\mathcal{C} = \{X, XT, X_p, X_p + Y, X_pY, X + Y_p, XT + Y_p, XTY_p, XT + Y\}$. The graph of the network has 3 linkage classes and 3 terminal strong linkage classes, and is not weakly reversible. The dimension of the stoichiometric subspace is 5, so that the deficiency of the network is $\delta = 9 - 3 - 5 = 1$.

The network has 3 cyclic elementary modes with support $\mathrm{e}^1 + \mathrm{e}^2$, $\mathrm{e}^4 + \mathrm{e}^5$ and $\mathrm{e}^7 + \mathrm{e}^8$, respectively, and one stoichiometric elementary mode $\mathrm{e}^1 + \mathrm{e}^3 + \mathrm{e}^4 + \mathrm{e}^6 + \mathrm{e}^7 + \mathrm{e}^9$.

Example 7.2.4. The following network is an alternative model for EnvZ/OmpR system (see S.60 in [SF10]), where ADP is also a cofactor in the dephosphorylation of Y_p:

$$\begin{aligned} & XD \underset{r_2}{\overset{r_1}{\rightleftharpoons}} X \underset{r_4}{\overset{r_3}{\rightleftharpoons}} XT \xrightarrow{r_5} X_p, \\ & X_p + Y \underset{r_7}{\overset{r_6}{\rightleftharpoons}} X_pY \xrightarrow{r_8} X + Y_p, \\ & XT + Y_p \underset{r_{10}}{\overset{r_9}{\rightleftharpoons}} XTY_p \xrightarrow{r_{11}} XT + Y, \\ & XD + Y_p \underset{r_{13}}{\overset{r_{12}}{\rightleftharpoons}} XDY_p \xrightarrow{r_{14}} XD + Y, \end{aligned} \tag{58}$$

The symbols are defined as in Example 7.2.3, with the addition of D representing ADP. The species are $\mathcal{S} = \{XD, X, XT, X_p, Y, X_pY, Y_p, XTY_p, XDY_p\}$, and the set of complexes is $\mathcal{C} = \{X, XT, X_p, X_p + Y, X_pY, X + Y_p, XT + Y_p, XTY_p, XT + Y, XD + Y_p, XDY_p, XD + Y\}$. This network has 4 linkage classes, 8 strong linkage classes, and 4 terminal strong linkage classes, and is not weakly reversible. The rank is $s = 7$ so that the deficiency is $\delta = c - l - s = 13 - 4 - 7 = 2$.

In addition to the 5 cyclic elementary modes $\mathrm{e}^1 + \mathrm{e}^2$, $\mathrm{e}^3 + \mathrm{e}^4$, $\mathrm{e}^6 + \mathrm{e}^7$, $\mathrm{e}^9 + \mathrm{e}^{10}$ and $\mathrm{e}^{12} + \mathrm{e}^{13}$, the network has 2 stoichiometric elementary modes $\mathrm{e}^3 + \mathrm{e}^5 + \mathrm{e}^6 + \mathrm{e}^8 + \mathrm{e}^9 + \mathrm{e}^{11}$ and $\mathrm{e}^3 + \mathrm{e}^5 + \mathrm{e}^6 + \mathrm{e}^8 + \mathrm{e}^{12} + \mathrm{e}^{14}$.

7.3 GENERALISED CHEMICAL REACTION NETWORKS AND SYSTEMS

Generalised chemical reaction networks [MR12, MR14] extend the definition of chemical reaction systems by decoupling the stoichiometric structure and the assignment of the

kinetics. The introduction of separate complexes governing the structure and the kinetics of reaction networks was motivated by the observed limited validity of the mass action kinetic assumption [MR12]; in this work, however, we mostly regard generalised chemical reaction networks as a tool for the analysis of standard chemical reaction systems.

Definition 7.3.1. A *generalised chemical reaction network* $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y, y_\kappa)$ on the set of species $\mathcal{S}$ is a directed graph with set of nodes $\mathcal{C} = \{1, \dots, c\}$, set of edges $\mathcal{R}$ that verifies $\mathcal{R} \subseteq \mathcal{C} \times \mathcal{C} \setminus \{(i,i) \mid i \in \mathcal{C}\}$, together with two maps $y : \mathcal{C} \to \mathbb{R}^n_{\geq 0}$ and $y_\kappa : \mathcal{C}_k \to \mathbb{R}^n_{\geq 0}$, where $\mathcal{C}_k \subseteq \{1, \dots, c\}$ are the nodes with strictly positive outdegree.

The maps y and y_κ define the assignment of the *stoichiometric* and the *kinetic* complexes respectively. The map y_κ is only defined on the nodes that act as reactant for at least one reaction; we will see that these remain, as in the standard case, the only complexes that play a role in the definition of the dynamics. The definition is clearly an extension of 7.1.1; notice the significant additional flexibility in the assignment of the complexes, which can have real coefficients and can be repeated among nodes.

The linkage classes, strong linkage classes, reversibility and weak reversibility are defined as for standard chemical reaction networks. If i is a terminal (nonterminal) node, we will call y^i a terminal (nonterminal) complex and y^i_κ a terminal (nonterminal) kinetic complex.

We will denote the reaction $i \to j$ as $y^i(y^i_\kappa) \to y^j$, with the kinetic complex between parentheses. The stoichiometric reaction vector of a reaction $i \to j$ is defined as $y^j - y^i$ in the standard case, as is the stoichiometric subspace $S = \operatorname{span}\{y^j - y^i \mid (i,j) \in \mathcal{R}\}$. The kinetic reaction vector $y^j_\kappa - y^i_\kappa$ for $i \to j$ is defined only when $\mathcal{C}_\kappa$ coincides with $\mathcal{C}$. In this case the subspace

$$S_\kappa = \operatorname{span}\{y^j_\kappa - y^i_\kappa \mid (i,j) \in \mathcal{R}\}$$

is called the *kinetic-order subspace* of the generalised chemical reaction network. We denote s_κ the dimension of this subspace.

The definitions given in Section 7.1 of stoichiometric matrix, complex matrix and incidence matrix, as well as linkage classes and weak reversibility apply, without change, to generalised chemical reaction networks. We define, in addition, the kinetic complex matrix $\mathbf{Y}_\kappa$ as the $n \times c$ matrix with columns the vectors $y^1_\kappa, \dots, y^c_\kappa$. In addition to the (stoichiometric) deficiency defined as in (50), one can define the *kinetic deficiency* as

$$\delta_\kappa = c - l - s_\kappa.$$

We now consider the definition of kinetic for a generalised mass action network. The reaction rate for a reaction in the generalised setting is still given by a kinetic parameter multiplied by monomial, with the monomial being identified by the map y_κ.

Definition 7.3.2. A *generalised mass action system* is a generalised chemical reaction network $\mathcal{N}$ endowed with a labelling $\kappa : \mathcal{R} \to \mathbb{R}_{>0}$ of the edges.

A reaction $i \to j$ with kinetic parameter κ_{ij} will be denoted $y^i(y^i_\kappa) \xrightarrow{\kappa_{ij}} y^j$. The system of differential equations associated to a generalised mass action system $(\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$ is

$$\frac{d\mathbf{x}}{dt} = \sum_{i \to j \in \mathcal{R}} \kappa_{ij} (y^j - y^i)\, \mathbf{x}^{y^i_\kappa}. \tag{59}$$

The map y determines therefore the *stoichiometric compatibility classes* which are defined as in the mass action case. The map y_κ governs the reaction rates. The kinetic

matrix $\mathbf{A}_\kappa$ can be defined exactly as in the mass action case, and the system of ODEs in 59 reformulated as

$$\frac{d\mathbf{x}}{dt} = \mathbf{Y}\mathbf{A}_\kappa \mathbf{x}^{\mathbf{Y}_\kappa}. \tag{60}$$

Two (generalised) mass action systems $\mathcal{M}$ and $\mathcal{M}'$ will be called *dynamically equivalent* if they admit the same associated system of ODEs. Using the terminology of [Sze10, SHP11], we will also say that $\mathcal{M}$ and $\mathcal{M}'$ are *realisations* of the system of ODEs, or that $\mathcal{M}$ is a realisation of $\mathcal{M}'$.

Example 7.3.3. Consider the following generalised chemical reaction system:

$$\boxed{\begin{matrix} A+B \\ (A+B) \end{matrix}} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \boxed{\begin{matrix} 2B \\ (B) \end{matrix}}. \tag{61}$$

The set of species is $\mathcal{S} = \{A, B\}$, and the graph has two nodes, with $y(1) = y_\kappa(1) = A + B$, $y(2) = 2B$, $y_\kappa(2) = B$. The stoichiometric and kinetic subspaces are given respectively by $S = \text{span}\{(1, -1)\}$, $S_\kappa = \text{span}\{(1, 0)\}$, and the stoichiometric and kinetic deficiencies are both zero. In addition the network is weakly reversible.

The corresponding generalised mass action system is governed by the following dynamical equations:

$$\begin{aligned} \frac{dx_A}{dt} &= -k_{12}x_A x_B + k_{21}x_B, \\ \frac{dx_B}{dt} &= k_{12}x_A x_B - k_{21}x_B. \end{aligned} \tag{62}$$

Notably, we have that (56) coincides with (62). That is, the systems are dynamically equivalent.

7.4 STEADY STATES

A vector of concentrations $\mathbf{x} \in \mathbb{R}^n_{\geq 0}$ is a *steady state* or *equilibrium* for a generalised mass action system $(\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$ if it verifies

$$\mathbf{Y}\mathbf{A}_\kappa \mathbf{x}^{\mathbf{Y}_\kappa} = 0.$$

A *positive steady state* or *positive equilibrium* is an equilibrium in $\mathbb{R}^n_{>0}$. A positive equilibrium is said to be *complex balancing* if it satisfies

$$\mathbf{A}_\kappa \mathbf{x}^{\mathbf{Y}_\kappa} = 0.$$

An equilibrium is therefore a state of the system such that, for each species, the sum of the rates of the reactions producing the species equals the sum of the rates of the reactions consuming the species. If the equilibrium is complex balancing, then a similar property holds for the complexes: for each complex y, the sum of the rates of the reactions having y as a product equals the sum of the rates of the reactions having y as a reactant.

A fundamental result in chemical reaction network theory is the following characterisation of the kernel of $\mathbf{A}_\kappa$.

Theorem 7.4.1. *([FH77, Fei79]) Let $T_1, \ldots, T_t$ denote the terminal strong linkage classes of $(\mathcal{C}, \mathcal{R})$. Then $\ker(\mathbf{A}_\kappa)$ has a basis $b^1, \ldots, b^t \in \mathbb{R}^c_{\geq 0}$ such that $\text{supp}(b^\theta) = T_\theta$, for $\theta = 1, \ldots, t$.*

The elements of the vectors of the basis $b^1, \dots, b^t$ can be calculated from the structure of the graph $(\mathcal{C}, \mathcal{R})$ as follows. For each node i of the graph, a *spanning i-tree* $\mathcal{T}$ is a subgraph of the graph of complexes that contains all complexes in the terminal strong linkage class of i, admits no cycles, and has the complex i as its unique sink. We denote by $\mathcal{T}_i$ the set of all spanning i-trees, and call *tree constant* associated to the complex i the following polynomial in the kinetic parameters:

$$K_i = \sum_{t \in \mathcal{T}_i} \prod_{r \in t} \kappa_r. \tag{63}$$

Then $b_i^\theta = K_i$, where T^θ is the strongly connected component containing i. Equivalently, K_i can be found, modulo a sign, by considering the restriction of the kinetic matrix $\mathbf{A}_\kappa$ to the linkage class containing the complex i, and calculating the minor obtained by deleting the column corresponding to i and any of the rows [CDSS09, Joh14].

From (51) one can observe that, if the deficiency of the network is zero, then all steady states are complex balancing. The asymptotic behaviour of mass action systems on networks of deficiency zero can be characterised further independently of the values of the kinetic parameters.

Theorem 7.4.2. *(Deficiency Zero Theorem [Fei79, Fei87]) Consider a mass action system on a deficiency zero chemical reaction network.*

(i) If the network is not weakly reversible, then the mass action system does not admit any positive steady state.

(ii) If the network is weakly reversible, then the system admits exactly one steady state in each stoichiometric compatibility class. Each steady state admits a strict Lyapunov function in the stoichiometric compatibility class, and is asymptotically stable.

A consequence of Theorem 7.4.1 is that, if the system admits a complex balancing equilibrium, then its underlying graph $(\mathcal{C}, \mathcal{R})$ is weakly reversible (each node belongs to a terminal strongly connected linkage class). Dynamical systems of the form in (60) that admit a complex balancing equilibrium are called *toric dynamical systems* [CDSS09]. In [CDSS09], these systems are studied with techniques of toric geometry and computational algebra. A weakly reversible mass action system admits a complex balancing equilibrium if and only if the kinetic parameters lie in the variety associated to a certain toric ideal in $\mathbb{Q}[K_1, \dots, K_c]$ [CDSS09]. Recall that an ideal is called *binomial* if it is generated by binomials, and *toric* if it is binomial and prime [Stu96]. The following theorem, found in [MR14], gives a characterisation in terms of tree constants and an algorithm to construct a parametrisation of the complex balancing equilibria for generalised mass action systems. We will use the following notation: for a subset $\mathcal{E}$ of the edges of $(\mathcal{C}, \mathcal{R})$ we denote by $\mathbf{I}_\mathcal{E}$ the corresponding incidence matrix. Recall that given a matrix $\mathbf{M} \in \mathbb{R}^{n_1 \times n_2}$, a matrix $\mathbf{H} \in \mathbb{R}^{n_2 \times n_1}$ is a generalised inverse of $\mathbf{M}$ if it verifies $\mathbf{MHM} = \mathbf{M}$.

Theorem 7.4.3. *([MR14], Theorem 1) Let $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y, y_k)$ be a weakly reversible generalised reaction network. Let $\mathcal{E} \subset \mathcal{R}$ be a set of $c - l$ edges such that the incidence matrix $\mathbf{I}_\mathcal{E}$ has rank $c - l$, so that $\mathrm{Im}(\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E}) = S_\kappa$. Given an assignment κ of kinetic parameters for $\mathcal{N}$, define the vector $v \in \mathbb{R}^{c-l}_{>0}$ with $v_k = \frac{K_j}{K_i}$, if $i \to j$ is the k^{th} reaction in $\mathcal{E}$, for $k = 1, \dots, c - l$. Then $\mathbf{x} \in \mathbb{R}^n_{>0}$ is a complex balancing equilibrium if and only if it satisfies*

$$\mathbf{x}^{\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E}} = v.$$

In addition:

(i) The set of complex balancing equilibria is non-empty for all assignments of kinetic parameters κ if and only if $\delta_\kappa = 0$.

(ii) If $\delta_\kappa \neq 0$, then, given an assignment of kinetic parameters κ, the set of complex balancing equilibria is non-empty if an only if $v^{\mathbf{C}} = \mathbf{1}$, where $\mathbf{C}$ is a $(c-l) \times \delta_k$ real matrix that satisfies $\mathrm{Im}(\mathbf{C}) = \ker(\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E})$ and $\ker(\mathbf{C}) = \{\mathbf{0}\}$.

(iii) If, for a given an assignment of kinetic parameters κ, the set of complex balancing equilibria is non-empty, then a complex balancing equilibrium $\mathbf{x}^*$ can be calculated as $\mathbf{x}^* = v^{\mathbf{H}^T}$, where $\mathbf{H} \in \mathbb{R}^{n\times(c-l)}$ is a generalised inverse of $(\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E})^T$.

(iv) For a given an assignment of kinetic parameters κ, if $\mathbf{x}^*$ is a complex balancing equilibrium, then the set of complex balancing equilibria is given by $\{\mathbf{x} \in \mathbb{R}^n_{>0} \mid \ln(\mathbf{x}) - \ln(\mathbf{x}^*) \in S_\kappa^\perp\}$.

If $\mathbf{B} \in \mathbb{R}^{n\times(n-s_\kappa)}$ is such that $\mathrm{Im}(\mathbf{B}) = S_\kappa^\perp$ and $\ker(\mathbf{B}) = \{\mathbf{0}\}$, then the complex balancing equilibria coincide with the set $\{(\mathbf{x}_1^*(\xi^{\mathbf{B}^T})_1, \ldots, \mathbf{x}_n^*(\xi^{\mathbf{B}^T})_n) \mid \xi \in \mathbb{R}^{n-s_\kappa}_{>0}\}$.

The theorem defines an algorithm for the identification of the conditions that the kinetic parameters need to satisfy for the existence of complex balancing equilibria, and for the identification of a binomial parametrisation of the complex balancing equilibria.

Feinberg [Fei87] also proved the following generalisation of Theorem 7.4.2 which applies to a class of networks with positive deficiency.

Theorem 7.4.4. *(Deficiency One Theorem [Fei87]) Consider a chemical reaction network $\mathcal{N}$ with deficiency δ and with l linkage classes with deficiencies $\delta_1, \ldots, \delta_l$. Suppose that each linkage class contains only one terminal strong linkage class, and that*

(i) $\delta_\theta \leq 1$, $\theta = 1, \ldots, l$ and

(ii) $\sum_{\theta=1}^{l} \delta_\theta = \delta$.

Then, any mass action system on $\mathcal{N}$ admits at most one steady state in each stoichiometric compatibility class. In addition, if the network is weakly reversible, then any mass action system on $\mathcal{N}$ admits exactly one steady state in each stoichiometric compatibility class.

Definition 7.4.5. A *direct decomposition* of a network $\mathcal{N}$ or a decomposition of $\mathcal{N}$ in independent subnetworks [Fei87, SF10] is a partition of the set of reactions $\mathcal{R}$ into subsets $\mathcal{R}_1, \ldots, \mathcal{R}_k$ such that, if $\mathcal{N}_i$ is the network consisting of the reactions in $\mathcal{R}_i$, and s_i is the rank of $\mathcal{N}_i$, then

$$\sum_{i=1}^{k} s_i = s.$$

If a network $\mathcal{N}$ admits a direct decomposition in subnetworks $\mathcal{N}_1, \ldots, \mathcal{N}_k$, then an equilibrium of a mass action system $\mathcal{M}$ associated to $\mathcal{N}$ is an equilibrium for each submass action system identified by the $\mathcal{N}_i$. As a consequence, properties of the equilibria of $\mathcal{M}$ can sometimes be derived from the analysis of the subnetworks.

A more general version of the Deficiency One Theorem is stated in [Fei87, Remark 6.2.D]: if the network admits a direct decomposition in subnetworks all with deficiency smaller or equal to one, and if each subnetwork contains only one linkage class and one terminal strong linkage class, then the conclusions of Theorem 7.4.4 still hold.

Reaction networks that fall outside the scope of classical CRNT theorems are common in biology, and several approaches exist, in the literature, to the study of equilibria for such chemical systems. Binomial parametrisations of steady states of mass action

systems are considered for instance in [CDSS09, MDSC12, Joh14, MD16]. Existence of equilibria, multistationarity and convergence have also been extensively investigated under mild assumptions on the network kinetics (e.g. [DLAS07, AS08, BC09, BM13]).

In this work we focus our attention on mass action systems, and the approach we consider consists in interpreting the polynomial system of ODEs of the network as the system of ODEs associated to a generalised mass action system (a simple example of this setting is given in examples 7.2.2 and 7.3.3), as done in [Joh14, Joh15]. Computational techniques for the identification of weakly reversible mass action realisations, that rely for the most part on the knowledge of the kinetic parameters, have been previously described in [JSS12, SHT12, LSH14]. Here we are interested in parameter-independent approaches. We will consider in particular the identification of weakly reversible deficiency zero realisations, which allow the application of Theorem 7.4.3, or of deficiency one realisations, which enable the application of a structural criterion for the identification of robustness (see Section 7.5). We will see in Chapter 10 that there are many kinetic-independent ways of writing a mass action system as a weakly reversible generalised mass action system. We will first consider, in Chapter 9, *translations* of networks [Joh14], i.e. generalised mass action networks admitting the same system of ODEs and the same reaction vectors.

Other binomial parametrisations of positive steady states

In some cases, a mass action system can admit steady states that are parametrised by binomials, but is not toric in the sense of the previous paragraph. Another approach to the identification of binomial parametrisations of steady states is introduced in [MDSC12]. To state this definition, we first need some notation. Consider a generalised chemical reaction network $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y, y_\kappa)$. Having fixed an assignment of kinetic parameters κ, we write $f(\mathbf{x}) = \mathbf{Y}\mathbf{A}_\kappa \mathbf{x}^{\mathbf{Y}_\kappa}$ and call the ideal

$$J_\kappa = \langle f_1, \dots, f_n \rangle \subset \mathbb{R}[x_1, \dots, x_n]$$

the *steady state ideal* of the generalised mass action system $(\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$. Recall that an ideal is called *binomial* if it is generated by binomials (an introduction to the relevant algebraic tools can be found in [CLO92]).

Definition 7.4.6. ([MDSC12], Definition 2.2) We say that the dynamical system in (60) has *toric steady states* if J_κ is a binomial ideal and it admits real zeros.

We introduce the following definition.

Definition 7.4.7. We say that the dynamical system in (60) has *positive binomial steady states* if the positive steady states are positive real zeros of a binomial ideal.

We compare the definition of toric dynamical system and the two definitions above in the following examples.

Example 7.4.8. A chemical reaction network in one species with positive binomial steady states is given by the following reactions:

$$2A \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} A \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} \emptyset,$$

The associated dynamical system

$$\frac{dx_A}{dt} = -\kappa_1 x_A^2 + (\kappa_2 - \kappa_3) x_A + \kappa_4$$

has toric steady states if and only if $\kappa_2 = \kappa_3$. Using the procedure described in Theorem 7.4.3, we can define $\mathbf{I}_{\mathcal{E}} = \begin{vmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{vmatrix}$, $C = \begin{vmatrix} 1 \\ -1 \end{vmatrix}$, $v = (\frac{K_2}{K_1}, \frac{K_3}{K_2})$, $K_1 = \kappa_2\kappa_4$, $K_2 = \kappa_1\kappa_4$, $K_3 = \kappa_1\kappa_3$, and find that the system is toric if and only if $\kappa_1\kappa_4 = \kappa_2\kappa_3$.

Example 7.4.9. (*Triangle network*) Consider the following network, studied in [CDSS09] (Example 1) and [MDSC12] (Example 2.3):

$$\begin{array}{ccc} & 2A & \\ {\scriptstyle\kappa_{13}}\swarrow\ \ \searrow{\scriptstyle\kappa_{12}} & & \\ {\scriptstyle\kappa_{31}}\nearrow\ \ \searrow{\scriptstyle\kappa_{21}} & & \\ 2B & \underset{\kappa_{23}}{\overset{\kappa_{32}}{\rightleftharpoons}} & A+B. \end{array} \tag{64}$$

It is established in [CDSS09] that the dynamical system associated to this network

$$\frac{dx_A}{dt} = -(\kappa_{12}+2\kappa_{13})x_A^2 + (\kappa_{21}-\kappa_{23})x_Ax_B + (2\kappa_{31}+\kappa_{32})x_B^2 = -\frac{dx_B}{dt}, \tag{65}$$

is toric if and only if the kinetic parameters satisfy $(\kappa_{21}\kappa_{31}+\kappa_{21}\kappa_{32}+\kappa_{23}\kappa_{31})(\kappa_{12}\kappa_{23}+\kappa_{13}\kappa_{21}+\kappa_{13}\kappa_{23}) = (\kappa_{12}\kappa_{31}+\kappa_{12}\kappa_{32}+\kappa_{13}\kappa_{32})^2$. In addition, it is shown in [MDSC12] that the system has toric steady states if and only if $\kappa_{21} = \kappa_{23}$ (in this case, the steady state ideal is generated by the binomial $-(\kappa_{12}+2\kappa_{13})x_A^2 + (2\kappa_{31}+\kappa_{32})x_B^2$).

On the other hand, the dynamical system in (65) has positive binomial steady states for any assignment of the rate constants. In fact, the polynomial in (65) can be factorised as

$$\left(\sqrt{2\kappa_{31}+\kappa_{32}}x_B - \frac{\kappa_{23}-\kappa_{21}-\sqrt{(\kappa_{21}-\kappa_{23})^2+4(2\kappa_{31}+\kappa_{32})(\kappa_{12}+2\kappa_{13})}}{2\sqrt{2\kappa_{31}+\kappa_{32}}}x_A\right)\cdot$$
$$\left(\sqrt{2\kappa_{31}+\kappa_{32}}x_B - \frac{\kappa_{23}-\kappa_{21}+\sqrt{(\kappa_{21}-\kappa_{23})^2+4(2\kappa_{31}+\kappa_{32})(\kappa_{12}+2\kappa_{13})}}{2\sqrt{2\kappa_{31}+\kappa_{32}}}x_A\right),$$

and all positive steady states satisfy

$$\sqrt{2\kappa_{31}+\kappa_{32}}x_B - \frac{\kappa_{23}-\kappa_{21}+\sqrt{(\kappa_{21}-\kappa_{23})^2+4(2\kappa_{31}+\kappa_{32})(\kappa_{12}+2\kappa_{13})}}{2\sqrt{2\kappa_{31}+\kappa_{32}}}x_A = 0.$$

Uniqueness of complex balancing equilibria

For a fixed initial condition $\mathbf{x}_0 \in \mathbb{R}^n_{\geq 0}$, the dynamics of a generalised mass action system never leaves the stoichiometric compatibility class $\mathbf{x}_0 + S$. As a consequence, the existence and uniqueness of equilibria are investigated within each stoichiometric compatibility class.

For toric mass action systems, a result known as *Birch Theorem* establishes the existence and uniqueness of a complex balancing equilibrium in each stoichiometric compatibility class [CDSS09]. Müller and Regensburger [MR12] proved a version for generalised mass action systems. To state the result, we introduce some additional notation. Let $\sigma\colon \mathbb{R}^n \to \{-,0,+\}^n$ denote the function defined by $\sigma_i(x) = \operatorname{sign}(x_i)$ for $i = 1,\dots,n$. and, for any $A \subseteq \mathbb{R}^n$, write $\sigma(A) = \{\sigma(x) \mid x \in A\}$. Given a matrix $\mathbf{A} \in \mathbb{R}^{n\times d}$ and sets $I \subseteq \{1,\dots,n\}$ and $J \subseteq \{1,\dots,d\}$, $\mathbf{A}_{I,J}$ denotes the submatrix of $\mathbf{A}$ consisting of the rows indexed by I and the columns indexed by J.

A generalised mass action network $(\mathcal{C},\mathcal{R},y,y_\kappa)$ is said to have the *capacity for multiple complex balancing equilibria* if there exists an assignment of rate constants κ such that the dynamical system associated to $(\mathcal{C},\mathcal{R},y,y_\kappa,\kappa)$ has more than one complex balancing equilibrium in some stoichiometric compatibility class.

Proposition 7.4.10. *([MR12, Proposition 3.1]) Consider a generalised mass action network $(\mathcal{C}, \mathcal{R}, y, y_\kappa)$. If $\sigma(S) \cap \sigma(S_\kappa^\perp) = \{0\}$, then, for each generalised mass action system $(\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$, each stoichiometric compatibility class contains at most one complex balancing equilibrium.*

Proposition 7.4.11. *([MR12, Proposition 3.2]) If a weakly reversible generalised mass action network $(\mathcal{C}, \mathcal{R}, y, y_\kappa)$ is such that $\sigma(S) \cap \sigma(S_\kappa^\perp) \neq \{0\}$, then it has the capacity for multiple complex balancing equilibria.*

To check whether the condition for uniqueness of complex balancing equilibria of Proposition 7.4.10 is verified, one can use the following result:

Proposition 7.4.12. *([MFR+16, Corollary 2.15]) Let $\mathbf{A} \in \mathbb{R}^{n\times d}$ and $\mathbf{B} \in \mathbb{R}^{d\times n}$ be matrices of rank d, and write $I = \{1, \dots, d\}$. Then, $\sigma(\mathrm{Im}(\mathbf{A})) \cap \sigma(\ker(\mathbf{B})) = \{0\}$ if and only if for all subsets $J \subseteq \{1, \dots, n\}$ of cardinality d the product $\det(\mathbf{A}_{J,I}) \det(\mathbf{B}_{I,J})$ either is zero or has the same sign as all other nonzero products, and at least one such product is not zero.*

Existence of a complex balancing equilibrium in each stoichiometric compatibility class can be guaranteed under stronger conditions on the sign vectors.

Theorem 7.4.13. *([MR12, Theorem 3.10]) Let $(\mathcal{C}, \mathcal{R}, y, y_\kappa)$ be a generalised mass action network such that $\sigma(S) = \sigma(S_\kappa)$ and $(+, \dots, +) \in \sigma(S^\perp)$. Then, for each assignment of rate constants κ such that the system $(\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$ admits complex balancing equilibria, there exists a unique complex balancing equilibrium in each stoichiometric compatibility class.*

7.5 STRUCTURAL CONDITIONS FOR ROBUSTNESS

The definition of *absolute concentration robustness* (ACR), introduced in [SF10], describes the ability of a system to maintain some species concentrations within strict bounds in response to fluctuations in the input species, a property that has been observed experimentally [ASBL99, SMMA07, SRA09]. Our interest in robustness will be additionally motivated in Section 9.2, where we will use the knowledge on robustness of certain ratios to derive conclusions on steady state properties of some networks.

In this paragraph, we recall some structural conditions for detection of ACR, and we state a version of the ACR result of Shinar and Feinberg [SF10] for generalised mass action networks. In Chapter 9, we describe how robustness properties can be derived and robustness values calculated from the structure of some generalised mass action networks that are identified by translating reactions.

The following definitions are meaningful only for systems that admit some positive steady states. When discussing robustness properties, we will assume that the system we are working with admits some positive steady states, without explicitly adding this requirement in each statement or example.

Definition 7.5.1. A generalised mass action system $\mathcal{M} = (\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$ is said to have *absolute concentration robustness* in species A if x_A takes the same value at every positive steady state $\mathbf{x} \in \mathbb{R}^n_{>0}$ of (53).

We will be interested in the following generalisation of the definition of absolute concentration robustness.

Definition 7.5.2. A generalised mass action system $\mathcal{M} = (\mathcal{C}, \mathcal{R}, y, y_\kappa, \kappa)$ is said to have a *robust ratio* between complexes u, $u' \in \mathbb{N}^n$ if the ratio $\mathbf{x}^{u'}/\mathbf{x}^u$ takes the same value at every positive steady state $\mathbf{x} \in \mathbb{R}^n_{>0}$ of (53).

If two complexes u and u' have a robust ratio and differ only in a single species, then clearly the system has ACR in this species. The techniques for determining ACR established in [SF10] give conditions for the identification of complexes with robust ratios, which can then be used to check for ACR. We will call *robustness space* $R \subseteq \mathbb{R}^n$ of a mass action system $\mathcal{M}$ a subspace defined by

$$R = \text{span}\{u' - u \mid u \text{ and } u' \text{ have a robust ratio}\}. \tag{66}$$

We now restate an observation made in [SF10, Proposition $S4.1$] and [NGN13, Lemma 11] in slightly different forms.

Lemma 7.5.3. *Consider a chemical reaction network $\mathcal{N}$ with corresponding mass action system $\mathcal{M}$ and robustness space R. If $u, u' \in \mathbb{N}^n$ and $u' - u \in R$, then u and u' have a robust ratio. In particular, if $\mathrm{e}^i \in R$ then $\mathcal{M}$ has ACR in X_i.*

Proof. Suppose that R is generated by $v^1, \dots, v^k$, and that, at each positive steady state $\mathbf{x} \in \mathbb{R}^n_{>0}$, $\mathbf{x}^{v^i} = \alpha_i \in \mathbb{R}_{>0}$. Then $u' - u$ writes as $u' - u = \sum_{i=1}^k \beta_i v^i$, and, given a positive steady state $\mathbf{x} \in \mathbb{R}^n_{>0}$, we have $\mathbf{x}^{u'}/\mathbf{x}^u = \prod_{i=1}^k \alpha_i^{\beta_i}$. The second point follows immediately taking $u' = \mathrm{e}^i$ and $u = 0$. □

The following are techniques for identification of complexes with robust ratios given in [SF10].

Theorem 7.5.4. *If $\mathcal{N}$ is a weakly reversible deficiency zero chemical reaction network, then any mass action system $\mathcal{M}$ on $\mathcal{N}$ has a robust ratio in each pair of complexes u and u' belonging to a common linkage class in $\mathcal{N}$.*

Theorem 7.5.5. *Consider a mass action system $\mathcal{M}$ on a chemical reaction network $\mathcal{N}$. If $\mathcal{N}$ has a deficiency of one, then $\mathcal{M}$ has a robust ratio in every pair of nonterminal complexes u and u' in $\mathcal{N}$.*

We can state generalised mass action versions of the two theorems.

Theorem 7.5.6. *If $\mathcal{N}$ is a weakly reversible deficiency zero generalised chemical reaction network, then any generalised mass action system $\mathcal{M}$ on $\mathcal{N}$ has a robust ratio in each pair of kinetic complexes u and u' belonging to a common linkage class in $\mathcal{N}$.*

Proof. Consider the system of ODEs (60) corresponding to the generalised mass action system $\mathcal{M}$. Since the network has a stoichiometric deficiency of zero, it follows that $\ker(\mathbf{A}_\kappa) = \ker(\mathbf{Y}\mathbf{A}_\kappa)$. Recall that, by Theorem 7.4.1, the kernel of $\mathbf{A}_k$ admits a basis $b^1, \dots, b^t \in \mathbb{R}^c_{\geq 0}$, with b^θ having support on the linkage class T^θ. For any positive equilibrium $\mathbf{x} \in \mathbb{R}^n_{>0}$, we can therefore write $\mathbf{x}^{\mathbf{Y}_\kappa} = \sum_{\theta=1}^t \lambda_\theta b^\theta$ for some $\lambda_0, \dots, \lambda_t \in \mathbb{R}_{>0}$. Given i, j in the same linkage class T^θ, we find $\frac{\mathbf{x}^{y^i_\kappa}}{\mathbf{x}^{y^j_\kappa}} = \frac{b^\theta_i}{b^\theta_j}$. □

Theorem 7.5.7. *Consider a generalised mass action system $\mathcal{M}$ on a generalised chemical reaction network $\mathcal{N}$. If $\mathcal{N}$ has a stoichiometric deficiency of one, then $\mathcal{M}$ has a robust ratio in every pair of nonterminal kinetic complexes u and u' in $\mathcal{N}$.*

Proof. Consider the system of ODEs (60) corresponding to the generalised mass action system $\mathcal{M}$ and suppose that the network has a stoichiometric deficiency of one. From Theorem 7.4.1, $\ker(\mathbf{A}_\kappa)$ admits a basis $\{b^1, \dots, b^t\} \subset \mathbb{R}^c_{\geq 0}$ with support on the t terminal strong linkage classes of $(\mathcal{S}, \mathcal{C}, \mathcal{R})$. Lemma S3.20 in [SF10] gives $\dim(\ker(\mathbf{Y}\mathbf{A}_\kappa)) \leq 1 + t$.

Now consider an arbitrary positive equilibrium $\mathbf{x} \in \mathbb{R}^n_{>0}$. It follows that $\mathbf{x}^{\mathbf{Y}_\kappa} \in \ker(\mathbf{Y}\mathbf{A}_\kappa) \cap \mathbb{R}^c_{>0}$. Since no vectors in $\{b^1, \dots b^t\}$ have support on the nonterminal complexes, we have that $\dim(\ker(\mathbf{Y}\mathbf{A}_\kappa)) = 1 + t$ so that there is a basis of $\ker(\mathbf{Y}\mathbf{A}_\kappa)$ given

by $\{b^0, b^1, \dots, b^t\}$ where only $b^0 \in \mathbb{R}^c_{\geq 0}$ has support on the nonterminal complexes. It follows that $\mathbf{x}^{\mathbf{Y}_\kappa} = \lambda_0 b^0 + \sum_{\theta=1}^t \lambda_\theta b^\theta$ for some $\lambda_0, \dots, \lambda_t \in \mathbb{R}_{>0}$. For any nonterminal nodes $i, j \in \mathcal{C}$, we have $\mathbf{x}^{y^i_\kappa} = \lambda_0 b^0_i$ and $\mathbf{x}^{y^j_\kappa} = \lambda_0 b^0_j$ so that, after solving for λ_0 and rewriting, we have $\frac{\mathbf{x}^{y^i_\kappa}}{\mathbf{x}^{y^j_\kappa}} = \frac{b^0_i}{b^0_j}$. Since the positive equilibrium $\mathbf{x}$ and nonterminal nodes i and j were chosen arbitrarily, we are done. □

Theorems 7.5.4, 7.5.5, 7.5.6 and 7.5.7 apply to networks with deficiency zero or one. In addition, if a reaction network admits a direct decomposition in subnetworks $\mathcal{N}_1, \dots, \mathcal{N}_k$, then robust ratios can be identified by applying the theorems to any of the subnetworks.

More general invariants, specifically, polynomials with monomials the mass action monomials $\mathbf{x}^{y^1}, \dots, \mathbf{x}^{y^c}$ and coefficients given by rational expressions in the kinetic parameters, are considered in [KMD+12], where a linear method for identification of invariants is presented, and Theorem 7.5.5 is derived as a special case.

In [NGN13], the authors observe how invariant flux ratios can be used to identify robust complexes. Two reactions r_i and r_j are called *fully coupled* if there exists $\alpha > 0$ such that, for all flux vectors $v \in \mathbb{R}^m_{\geq 0}$, $v_j = \alpha v_i$. If two reactions are fully coupled then, if the network has mass action network kinetics, the reactant complexes of the two reactions have a robust ratio. The observation extends directly to generalised mass action systems: if the ratio between the rates of two reactions r_i and r_j is the same at each positive steady state, then the kinetic reactant complexes of reactions r_i and r_j have a robust ratio. Fully coupled reactions can be identified by calculating the kernel of $\mathbf{\Gamma}$ or a generating set of elementary modes [NGN13]. Suppose that $w^1, \dots, w^d$ are a basis for the kernel of $\mathbf{\Gamma}$ or a generating set of elementary modes for a generalised chemical reaction network $\mathcal{N}$, and assume that for two indices $i, j \in \{1, \dots, m\}$ there is an $\alpha \in \mathbb{R}_{>0}$ such that $w^k_i = \alpha w^k_j$ for all $k = 1, \dots, d$. Then the ratio between the rates of two reactions r_i and r_j is the same at each positive steady state.

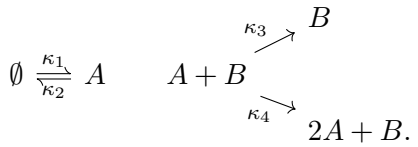
One can state a similar result by considering the kernel of $\mathbf{YA}_\kappa$:

Proposition 7.5.8. *Consider a generalised mass action network $\mathcal{N}$, and let $b^1, \dots, b^d \in \mathbb{R}^c$ be a basis of $\ker(\mathbf{YA}_\kappa)$. Suppose that there are two indices $i, j \in \{1, \dots, m\}$ and an $\alpha \in \mathbb{R}_{>0}$ such that $b^k_i = \alpha b^k_j$ for all $k = 1, \dots, d$. Then, for any generalised mass action system on $\mathcal{N}$, the complexes y^i_κ and y^j_κ have a robust ratio.*

Proof. For any positive steady state $\mathbf{x} \in \mathbb{R}^n_{>0}$, the vector $\mathbf{x}^{\mathbf{Y}_\kappa}$ is in $\ker(\mathbf{YA}_\kappa)$, and therefore writes as $\mathbf{x}^{\mathbf{Y}_\kappa} = \sum_{k=1}^d \beta_k b^k$. Hence $\mathbf{x}^{y^i_\kappa} = \sum_{k=1}^d \beta_k b^k_i = \alpha \sum_{k=1}^d \beta_k b^k_j = \alpha \mathbf{x}^{y^j_\kappa}$. □

Example 7.5.9. Reconsider the network from Example 7.2.2. The network has deficiency one and the nonterminal complexes are $A + B$ and B. Consequently, these complexes have a robust ratio by Theorem 7.5.5. Similarly, the kernel of the stoichiometric matrix is generated by the vector $(1, 1)$, and therefore the reactant complexes $A + B$ and B have a robust ratio. Since $A + B$ and B differ in the species A, ACR in species A follows.

Proposition 7.5.8 could be used to identify sufficient conditions for robustness in terms of the network structure, or of the network kinetics. For a simple example of the latter case, consider the mass action network



Defining $y^1 = \emptyset$, $y^2 = A$, $y^3 = A + B$, $y^4 = B$ and $y^5 = 2A + B$, we have that the kernel of $\mathbf{YA}_\kappa$ is generated by $\frac{\kappa_2}{\kappa_1}\mathrm{e}^1 + \mathrm{e}^2$, $\frac{\kappa_3 - \kappa_4}{\kappa_1}\mathrm{e}^1 + \mathrm{e}^3, \mathrm{e}^4, \mathrm{e}^5$. The condition of the Proposition is verified for $i = 1$, $j = 2$, with $\alpha = \frac{\kappa_2}{\kappa_1}$, if $\kappa_3 = \kappa_4$. In this case, the system has ACR in species A.

Example 7.5.10. Reconsider the deficiency 2 network considered in Example 7.2.4, with an assignment of rate constants κ.

The network admits a subnetwork of deficiency zero given by the two reactions $XD \underset{r_2}{\overset{r_1}{\rightleftharpoons}} X$. By Theorem 7.5.4 applied to this subnetwork, we conclude that the complexes XD and X have a robust ratio.

Reactions r_5 and r_8 take part in the same elementary modes with the same multiplicity, therefore the species XT and X_pY also have a robust ratio.

The kernel of $\mathbf{YA}_\kappa$ is generated by the following vectors (this is observed in [MDSC12]):

$$\mathrm{e}^4, \mathrm{e}^7, \mathrm{e}^{10}, \mathrm{e}^{13},$$
$$\frac{\kappa_{11}(\kappa_4 + \kappa_5)}{\kappa_3\kappa_5}\left(\frac{\kappa_2}{\kappa_1}\mathrm{e}^1 + \mathrm{e}^2\right) + \frac{\kappa_{11}}{\kappa_5}\mathrm{e}^3 + \frac{\kappa_{11}(\kappa_7 + \kappa_8)}{\kappa_6\kappa_8}\mathrm{e}^5 + \frac{\kappa_{11}}{\kappa_8}\mathrm{e}^6 + \frac{\kappa_{10} + \kappa_{11}}{\kappa_9}\mathrm{e}^8 + \mathrm{e}^9,$$
$$\frac{\kappa_{14}(\kappa_4 + \kappa_5)}{\kappa_3\kappa_5}\left(\frac{\kappa_2}{\kappa_1}\mathrm{e}^1 + \mathrm{e}^2\right) + \frac{\kappa_{14}}{\kappa_5}\mathrm{e}^3 + \frac{\kappa_{14}(\kappa_7 + \kappa_8)}{\kappa_6\kappa_8}\mathrm{e}^5 + \frac{\kappa_{14}}{\kappa_8}\mathrm{e}^6 + \frac{\kappa_{13} + \kappa_{14}}{\kappa_{12}}\mathrm{e}^{11} + \mathrm{e}^{12}.$$

The hypothesis of Proposition 7.5.8 is verified for all pair of indices in $\{1, 2, 3, 5, 6\}$ and for $8, 9$ and $11, 12$. Overall, we find that the complexes in $\{XD, X, XT, X_p + Y, X_pY\}$ have a robust ratio, as well as the pairs of complexes $XTY_p, XT + Y_p$ and $XD + Y_p, XDY_p$.

It was observed in [SF10] that the system has ACR in species Y_p. This was established by calculating the concentration of Y_p at steady states directly from the steady state equations:

$$x_{Y_p} = \frac{\kappa_1\kappa_3\kappa_5(\kappa_{10} + \kappa_{11})(\kappa_{13} + \kappa_{14})}{\kappa_1\kappa_3\kappa_9\kappa_{11}(\kappa_{13} + \kappa_{14}) + \kappa_2(\kappa_4 + \kappa_5)(\kappa_{10} + \kappa_{11})\kappa_{12}\kappa_{14}}. \tag{67}$$

An alternative derivation for (67) based on the calculation of linear complex invariants is presented in [KMD+12]. In Chapter 9, we will develop another method which allows to establish ACR in species Y_p and compute the ACR value (53) directly from the graph of a generalised chemical reaction network.

Finally, the following proposition gives a necessary condition for robustness of ratios for (generalised) mass action systems that admit complex balancing states. The condition is also sufficient if all the steady states of the system are complex balanced.

Proposition 7.5.11. *Suppose that the system in* (60) *admits some complex balancing steady states, and take $y \in \mathbb{R}^n$. Then value of $\mathbf{x}^y$ is the same at each complex balancing steady state if and only if the vector y is in the column space of the matrix $\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E}$ defined in Theorem 7.4.3.*

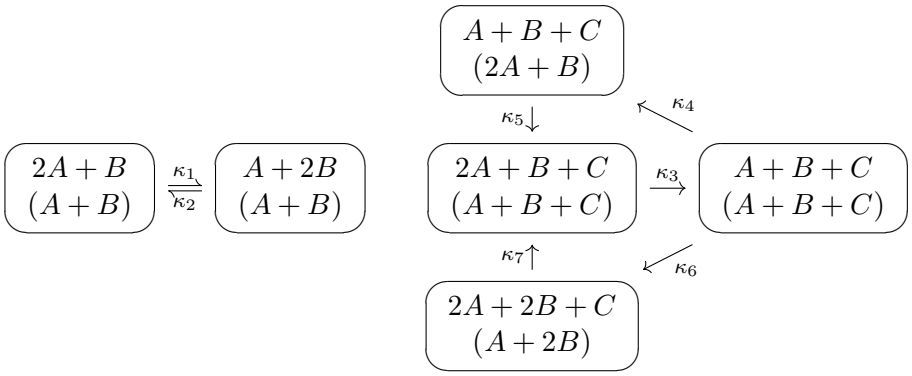
Proof. Suppose that there exists a $u \in \mathbb{R}^{c-l}$ such that $\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E}u = y$. Then each positive steady state $\mathbf{x}$ satisfies

$$\mathbf{x}^y = \mathbf{x}^{\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E}u} = v^u,$$

where v is defined in Theorem 7.4.3. Conversely, suppose that each complex balancing steady state $\mathbf{x}$ satisfies $\mathbf{x}^y = \alpha > 0$. We need to show that y is in the column space of $\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E}$, or, equivalently, that y is in $(\ker((\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^\top))^\perp$. In other words, we have to prove that, if w is in $\ker((\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^\top)$, then $y^tw = 0$. Take $\mathbf{x}$ complex balancing steady state for the system and $w \in \ker((\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^\top)$. Then, we have that $(\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^\top(w + \ln(\mathbf{x}^*)) = (\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^\top \ln(\mathbf{x}) = \ln(v)$. Consider the vector $w' \in \mathbb{R}^n$ defined by $\mathbf{x}'_j = \mathbf{x}_j\mathrm{e}^{w_j}$, $j = 1, \ldots, n$. Then we can write $(\mathbf{Y}_\kappa\mathbf{I}_\mathcal{E})^T \ln(w') = \ln(v)$, i.e., $\mathbf{x}'$ is also a complex balancing steady state of the system. In particular, $\alpha = \mathbf{x}^y = (\mathbf{x}')^y = \alpha\mathrm{e}^{y^tw}$, and $y^tw = 0$. □

If a system admits complex balancing steady states, the proposition gives therefore a necessary condition for ACR ($\mathrm{e}^i \in \mathbb{R}^n$ is in the column space of $\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E}$). If e^i is not in the columns space of $\mathbf{Y}_\kappa \mathbf{I}_\mathcal{E}$, then we can exclude that ACR holds *for some assignments of kinetic parameters*. ACR could still hold for other values of kinetic parameters, as in the following example.

Example 7.5.12. Consider the following generalised mass action system:



(68)

The kinetic deficiency is 2, and the system admits complex balancing steady states if $\kappa_1 = \kappa_2$ and $\kappa_6 = \kappa_3 - \kappa_4$. The kinetic subspace S_κ is generated by $(1, 0, -1)$ and $(0, 1, -1)$, and ACR is excluded in any species by Proposition 7.5.11. However, for $\kappa_6 = \kappa_3$, the equation for x_A associated to (68) is $\frac{dx_A}{dt} = x_A x_B(\kappa_5 x_A - \kappa_1 + \kappa_2)$, and the system has ACR in species A.

In this chapter, we introduced the terminology of chemical reaction networks, and recapitulated some results on complex balancing steady states. We have also seen that "robust ratios" can be identified in deficiency zero weakly reversible and deficiency one networks with generalised mass action kinetics. In chapters 9 and 10 we will discuss methods for the identification of generalised mass action realisations with low deficiency, which enable the application of these results.

8

NETWORKS WITH OTHER KINETICS AND ELIMINATION OF SPECIES

Biochemical reaction models often adopt rate laws that are not mass action. The study of enzyme kinetics in particular is simplified by aggregating elementary steps, resulting in a reduction of the number of variables and the number of parameters (see for instance [Seg75, CB14, HS12]). Since some results in chemical reaction network theory apply to systems with mass action kinetics, it might be useful to write a model in its full extended mass action form, even when the knowledge of the kinetic parameters is limited. Given a network with non-mass action kinetics, it might be difficult to determine the underlying mass action system. Therefore we focus on the opposite task, and seek to provide algorithms that, given a mass action system, can be used to derive simplified networks and kinetics. We will focus on the elimination of intermediate linear species, and our intent is to provide a method for deriving the structure of the reduced network as well as the rate laws such as the enzyme kinetic rate laws, with a general procedure that does not require some ad-hoc calculations. For instance, for the following two-substrate compulsory order scheme

$$\begin{aligned} A + E &\underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA, \\ B + EA &\underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} EAB \xrightarrow{\kappa_5} P + E, \end{aligned} \tag{69}$$

we want to derive the simplified network $A + B \to P$ and the rate law

$$\frac{\kappa_+^{cat} E_0 x_A x_B}{\kappa_A^i \kappa_B^M + \kappa_B^M x_A + \kappa_A^M x_B + x_A x_B},$$

with $\kappa_+^{cat} = \kappa_5$, $\kappa_A^i = \frac{\kappa_2}{\kappa_1}$, $\kappa_A^M = \frac{\kappa_5}{\kappa_1}$ and $\kappa_B^M = \frac{\kappa_4 + \kappa_5}{\kappa_3}$. This rate can be derived using the King-Altman method [CB14] (a detailed application of the method to this network can be found in the documentation for SBMLSqueezer [DHS+08]). Our approach will be instead to consider the stoichiometric structure of a network found by elimination of a single species, and then to define kinetic rates on the given structure that give rise to the required simplified dynamics.

The problem we consider is a special case of the question of the identification, given a system of differential equations, of a reaction network that admits the given system as its associates system of ODEs. In the case we consider, the system of ODEs is obtained by simplification of another system. We emphasise that several approaches exist to this problem. Other systematic reduction methods have been proposed, for example, for general kinetics in Radulescu et al. [RGZN12], and for intermediate linear species in Saez et al. [SWF17]. In [TG09], the authors show how the King-Altman method can be seen as a consequence of the Matrix-Tree theorem. A general method for identification of reaction networks from a system of rational ODEs is given in [GHBS15].

In many practical situations, dealing with a reduced, non-mass action system is the only feasible approach. Since the simplification procedures impact significantly the transient and steady state behaviour of the systems, their application is justified only under

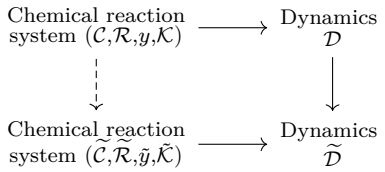


Figure 20: *We describe methods to derive the structure and kinetics of reduced networks (dashed arrow), in a way that makes the diagram commutative.*

some specific conditions, which must be carefully evaluated. An extensive literature is dedicated to the identification of these conditions and the investigation of the effects of simplifications on the network dynamics. For an overview of these techniques, see, for instance, [HS12, GW13]. The work of this chapter stems from the interest in developing a procedure to verify whether a given mass action system can be reduced to some given network, to motivate the study of the mass action system. We do not provide an analysis and comparison of the dynamics and steady states of the mass action and reduced system. For the elimination of isolated intermediate species, we refer the reader to [FW13] for a detailed characterisation of the relationship between steady states of the full and simplified models. It is also to be noted that we do not investigate the efficiency of the algorithms. The kinetic rates that can be generated with the procedures we present quickly become difficult to handle. In the examples we will consider the elimination of only a small number of intermediate species, as is usually done for instance for enzymatic reaction schemes.

8.1 DEFINITIONS

We need first to extend the definition of kinetics for a chemical reaction network.

Definition 8.1.1. A *kinetics* for a reaction network $(\mathcal{C}, \mathcal{R}, y)$ is a function that assigns to each reaction $r \in \mathcal{R}$ a *rate function*

$$\mathcal{K}\colon r \mapsto (\mathcal{K}_r\colon \Omega \to \mathbb{R}_{\geq 0}),$$

with $\mathbb{R}^n_{>0} \subseteq \Omega \subseteq \mathbb{R}^n_{\geq 0}$.

We will write $\mathcal{K}_{ij}$ for $\mathcal{K}_r$ with $r : i \to j$. as convenient. We call a tuple $(\mathcal{C}, \mathcal{R}, y, \mathcal{K})$ a *chemical reaction system*. The system of differential equations associated to $(\mathcal{C}, \mathcal{R}, y, \mathcal{K})$ is defined as

$$\frac{d\mathbf{x}}{dt} = \sum_{i \to j \in \mathcal{R}} \mathcal{K}_{ij}(\mathbf{x})(y^j - y^i). \tag{70}$$

If $\alpha \in \mathbf{\Gamma}^{\perp}$, we have from (70) that $\sum_{i=1}^n \alpha_i \frac{dx_i}{dt} = 0$, and therefore there is a constant $T \in \mathbb{R}$ such that $\sum_{i=1}^n \alpha_i x_i = T$. This expression is called a *conservation law* for the system, and $\sum_{i=1}^n \alpha_i x_i$ is said to be *conserved*.

The goal of this chapter is to identify a chemical reaction system that admits a given system of differential equations $\widetilde{\mathcal{D}}$ as its associated dynamics. The system of differential equations $\widetilde{\mathcal{D}}$ is obtained by eliminating a variable from an another system of differential equations $\mathcal{D}$, that is the dynamics associated to some chemical reaction system $\mathcal{M} = (\mathcal{C}, \mathcal{R}, y, \mathcal{K})$. We are looking therefore to define a chemical reaction system $\widetilde{\mathcal{M}} = (\widetilde{\mathcal{C}}, \widetilde{\mathcal{R}}, \tilde{y}, \tilde{\mathcal{K}})$ whose underlying graph and kinetics are derived from the graph and kinetics of $\mathcal{M}$. This process is illustrated in Figure 20.

To describe the reduction methods, we are guided by the reduction approaches used in enzyme kinetics, and described for instance in [Seg75, CB14, HS12]. We do not discuss

the assumptions under which the simplifications are justified – for these analyses we refer the reader to the references above. We want the methods we describe to be able to derive the kinetic rates used for the classical reaction schemes, such as Michaelis-Menten kinetics, multiple substrate enzyme kinetics, inhibition schemes, etc. For example, the reaction network

$$S + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} ES \xrightarrow{\kappa_3} P + E, \tag{71}$$

is often reduced to a single reaction $S \to P$. We want to recover for this reaction the Henri-Michaelis-Menten rate form

$$\frac{\kappa_3 E_0 x_S}{\kappa_S + x_S}, \tag{72}$$

for some constants E_0 and κ_S.

We are going to describe methods for the identification of chemical reaction system that admit a given system of differential equations as their dynamics. The system of differential equations are obtained from the dynamics of some chemical reaction system by eliminating the concentration of some intermediate species. For convenience, assume that the species whose concentration is being eliminated is the last species X_n. X_n is called an *intermediate species* if it is consumed in at least one reaction, and produced in at least one reaction: there exists a reaction $i \to j$ such that $y_n^j - y_n^i < 0$, and a reaction $i' \to j'$ such that $y_n^{j'} - y_n^{i'} > 0$.

In the case of elimination of a single intermediate, isolated species (i.e., a species that does not interact with other species) from a mass action system, we want the reduced network to coincide in structure with the network identified by the method described in [FW13]. Let us study the special case of a single intermediate isolated species first. Fix a set of species $\mathcal{S} = \{X_1, \ldots, X_n\}$. Consider a chemical reaction network $\mathcal{N}$ on $\mathcal{S}$ and a mass action system $\mathcal{M}$ on $\mathcal{N}$. If X_n is an intermediate isolated species, define the sets of reactions

$$\mathcal{R}' = \{i \to j \in \mathcal{R} \mid y_n^i = 1, y_n^j = 0\},$$
$$\mathcal{R}'' = \{i \to j \in \mathcal{R} \mid y_n^i = 0, y_n^j = 1\}.$$

A reduced (core) model $\widetilde{\mathcal{N}}$ in [FW13] is obtained from $\mathcal{N}$ as follows:

(1) the reactions $\mathcal{R} \setminus (\mathcal{R}' \cup \mathcal{R}'')$, that do not involve X_n in the reactant or product, remain unchanged;

(2) the pairs of reactions $y^i \xrightarrow{r''} X_n \in \mathcal{R}''$, $X_n \xrightarrow{r'} y^j \in \mathcal{R}'$ involving X_n as an intermediate are collapsed to a single reaction $y^i \to y^j$.

The equation $\frac{dx_n}{dt} = 0$ is used to write the steady state concentration of the intermediate as a sum

$$x_n = \sum_i \mu(\kappa)(\mathbf{x}')^{y^i}, \tag{73}$$

where μ a rational function of the rate constants and the $(\mathbf{x}')^{y^i}$ are monomials in the concentrations of the remaining species $\mathbf{x}' = (x_1, \ldots, x_{n-1})$. By substituting the expression for x_n in the system of ODEs associated to $\mathcal{M}$, a system of ODEs associated to a mass action system $\widetilde{\mathcal{M}}$ on the core model $\widetilde{\mathcal{N}}$ is obtained.

For this special case of single, isolated intermediate species eliminated from a mass action network, we can find kinetic parameters for the core model with a simplified approach. Given the assumptions on X_n, we can write the matrices $\mathbf{Y}$ and $\mathbf{A}_k$ in block form as follows:

$$\mathbf{Y} = \begin{bmatrix} \widetilde{\mathbf{Y}} & \mathbf{0} \\ \mathbf{0}^t & 1 \end{bmatrix}, \quad \mathbf{A}_\kappa = \begin{bmatrix} \mathbf{A}'_\kappa & -\mathbf{v} \\ -\mathbf{u}^t & a \end{bmatrix},$$

where $\mathbf{u}, \mathbf{v} \in \mathbb{R}^{c-1}_{\geq 0}$ and $a = \sum_{i=1}^{c-1} v_i$ is the sum of all the kinetic parameters of reactions in $\mathcal{R}''$. Then $\mathbf{x}^{\mathbf{Y}} = ((\mathbf{x}')^{\widetilde{\mathbf{Y}}}, x_n)$ and the system of ODEs (54) for $\mathbf{x}'$ can be written as

$$\frac{d\mathbf{x}'}{dt} = \widetilde{\mathbf{Y}}(\mathbf{A}'_\kappa \mathbf{x}^{\widetilde{\mathbf{Y}}} - x_n \mathbf{v}). \tag{74}$$

The equation for x_n instead writes as

$$\frac{dx_n}{dt} = -\mathbf{u}^t (\mathbf{x}')^{\widetilde{\mathbf{Y}}} + a x_n.$$

From the equation $\frac{dx_n}{dt} = 0$ we derive the expression $x_n = \frac{\mathbf{u}^t (\mathbf{x}')^{\widetilde{\mathbf{Y}}}}{\sum_{i=1}^{c-1} v_i}$. Replacing x_n with this expression in (74), we find

$$\frac{d\mathbf{x}'}{dt} = \widetilde{\mathbf{Y}} \left(\mathbf{A}'_\kappa - \frac{\mathbf{u}^t \mathbf{v}}{\sum_{i=1}^{c-1} v_i} \right) \mathbf{x}^{\widetilde{\mathbf{Y}}} = \widetilde{\mathbf{Y}} \widetilde{\mathbf{A}}_\kappa \mathbf{x}^{\widetilde{\mathbf{Y}}}, \tag{75}$$

with $\widetilde{\mathbf{A}}_\kappa = \mathbf{A}'_\kappa - \frac{\mathbf{u}^t \mathbf{v}}{\sum_{i=1}^{c-1} v_i}$. It is now easy to see that (75) is the system of ODEs associated to a mass action system on $\widetilde{\mathcal{N}}$. The kinetic parameter of a reaction $y^i \to y^j$ in $\widetilde{\mathcal{N}}$ is given by the kinetic parameter of the reaction $y^i \to y^j$ in $\mathcal{N}$, if such reaction exists, plus the sum of all the products of kinetic parameters of pairs of reactions in $\mathcal{R}' \times \mathcal{R}''$, divided by the sum a of all the kinetic parameters of reactions in $\mathcal{R}''$.

For example, consider the reaction network in (71). The associated system of ODEs is the following:

$$\begin{aligned}
\frac{dx_S}{dt} &= -\kappa_1 x_E x_S + \kappa_2 x_{ES}, \\
\frac{dx_E}{dt} &= -\kappa_1 x_E x_S + (\kappa_2 + \kappa_3) x_{ES}, \\
\frac{dx_P}{dt} &= +\kappa_3 x_{ES}, \\
\frac{dx_{ES}}{dt} &= +\kappa_1 x_E x_S - (\kappa_2 + \kappa_3) x_{ES}.
\end{aligned} \tag{76}$$

The intermediate species ES does not interact with any other species. From $\frac{dx_{ES}}{dt} = 0$ we find $x_{ES} = \frac{\kappa_1}{\kappa_2 + \kappa_3} x_E x_S$. The reduced system

$$\begin{aligned}
\frac{dx_S}{dt} &= -\frac{\kappa_1 \kappa_3}{\kappa_2 + \kappa_3} x_E x_S, \\
\frac{dx_E}{dt} &= 0, \\
\frac{dx_P}{dt} &= +\frac{\kappa_1 \kappa_3}{\kappa_2 + \kappa_3} x_E x_S,
\end{aligned}$$

is the system of ODEs associated to the reaction network

$$S + E \xrightarrow{\frac{\kappa_1 \kappa_3}{\kappa_2 + \kappa_3}} P + E. \tag{77}$$

The following is another example of elimination of an intermediate isolated species.

Example 8.1.2. Consider the two-substrate compulsory order scheme in equation 69. The system of ODEs associated to the network is as follows:

$$\begin{aligned}
\frac{dx_A}{dt} &= -\kappa_1 x_A x_E + \kappa_2 x_{EA},\\
\frac{dx_B}{dt} &= -\kappa_3 x_B x_{EA} + \kappa_4 x_{EAB},\\
\frac{dx_E}{dt} &= -\kappa_1 x_A x_E + \kappa_2 x_{EA} + \kappa_5 x_{EAB},\\
\frac{dx_{EA}}{dt} &= +\kappa_1 x_A x_E - \kappa_2 x_{EA} - \kappa_3 x_B x_{EA} + \kappa_4 x_{EAB},\\
\frac{dx_P}{dt} &= \kappa_5 x_{EAB}\\
\frac{dx_{EAB}}{dt} &= \kappa_3 x_B x_{EA} - (\kappa_4 + \kappa_5) x_{EAB}.
\end{aligned} \tag{78}$$

The species EAB is an intermediate isolated species. By collapsing the reaction paths $B + EA \to EAB \to B + EA$ and $B + EA \to EAB \to P + E$, we obtain the chemical reaction network

$$A + E \rightleftharpoons EA, \qquad B + EA \longrightarrow P + E. \tag{79}$$

From $\frac{dx_{EAB}}{dt} = \kappa_3 x_B x_{EA} - (\kappa_4 + \kappa_5) x_{EAB} = 0$ we can write $x_{EAB} = \frac{\kappa_3}{\kappa_4+\kappa_5} x_B x_{EA}$. Substituting x_{EAB} for $\frac{\kappa_3}{\kappa_4+\kappa_5} x_B x_{EA}$ in (78) one obtains the system of ODEs

$$\begin{aligned}
\frac{dx_A}{dt} &= -\kappa_1 x_A x_E + \kappa_2 x_{EA},\\
\frac{dx_B}{dt} &= -\frac{\kappa_3\kappa_5}{\kappa_4 + \kappa_5} x_B x_{EA},\\
\frac{dx_E}{dt} &= -\kappa_1 x_A x_E + \kappa_2 x_{EA} + \frac{\kappa_3\kappa_5}{\kappa_4 + \kappa_5} x_B x_{EA},\\
\frac{dx_{EA}}{dt} &= +\kappa_1 x_A x_E - \kappa_2 x_{EA} - \frac{\kappa_3\kappa_5}{\kappa_4 + \kappa_5} x_B x_{EA},\\
\frac{dx_P}{dt} &= \frac{\kappa_3\kappa_5}{\kappa_4 + \kappa_5} x_B x_{EA}.
\end{aligned} \tag{80}$$

This is the system of ODEs associated to the mass action system on (79) with the following assigement of kinetic parameters:

$$A + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA, \qquad B + EA \xrightarrow{\frac{\kappa_3\kappa_5}{\kappa_4+\kappa_5}} P + E. \tag{81}$$

In this section we want to deal with intermediate species that can interact with other species, still under the assumption that an expression of the form (73) can be derived for x_n from the steady state equation $\frac{dx_n}{dt} = 0$. For instance, we want to consider the elimination of the intermediate species EA in (81).

The species X_n will be called *linear* if the following two conditions are satisfied:

(i) for all $i \in \mathcal{C}$, $y_n^i \leq 1$;

(ii) for each reaction $i \to j$, there exists a function $\mathcal{K}'_{ij} \colon \Omega' \to \mathbb{R}_{\geq 0}$, with $\mathbb{R}^{n-1}_{>0} \subseteq \Omega' \subseteq \mathbb{R}^{n-1}_{\geq 0}$, such that if $y_n^i = 1$, $\mathcal{K}_{ij}(\mathbf{x}) = x_n \mathcal{K}'_{ij}(\mathbf{x}')$ and, if $y_n^i = 0$, $\mathcal{K}_{ij}(\mathbf{x}) = \mathcal{K}'_{ij}(\mathbf{x}')$.

If X_n is an intermediate linear species, then from the steady state equation

$$\frac{dx_n}{dt} = \sum_{i \to j \in \mathcal{R}} \mathcal{K}_{ij}(\mathbf{x})(y_n^j - y_n^i) = -\sum_{i \to j \in \mathcal{R}'} x_n \mathcal{K}'_{ij}(\mathbf{x}') + \sum_{i \to j \in \mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}') = 0$$

we can write the expression for x_n

$$x_n = \frac{\sum_{i\to j\in\mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}')}{\sum_{i\to j\in\mathcal{R}'} \mathcal{K}'_{ij}(\mathbf{x}')}. \tag{82}$$

We write $\varphi(\mathbf{x}')$ for the expression on the right in (82). Denote by $\pi_{n-h}\colon \mathbb{R}^n \to \mathbb{R}^{n-h}$ the projection on the first $n-h$ coordinates, and write π for π_{n-1}. Then the system of ordinary differential equations obtained by replacing x_n with $\varphi(\mathbf{x}')$ in (70) is

$$\frac{d\mathbf{x}'}{dt} = \sum_{i\to j\in\mathcal{R}} \mathcal{K}_{ij}(\mathbf{x}', \varphi(\mathbf{x}'))(\pi(y^j) - \pi(y^i)). \tag{83}$$

8.2 FIRST METHOD

For the method summarised in the previous section, the stoichiometric matrix of the reduced network contains all the reaction vectors of the reactions that do not involve the species X_n, plus all the sums of reaction vectors of pairs of reactions (r', r'') in $\mathcal{R}' \times \mathcal{R}''$, which correspond to the reaction paths $y^i \xrightarrow{\kappa_{r''}} X_n \xrightarrow{\kappa_{r'}} y^j$ collapsed to a reaction $y^i \to y^j$, and assigned with the kinetic parameter $\frac{\kappa_{r'}\kappa_{r''}}{\sum_{r\in\mathcal{R}'}\kappa_r}$. A straightforward generalisation, which we describe as Algorithm 1, consists in considering all pairs of reactions that produce and consume X_n, and combining each pair into a single reaction.

From the point of view of the network structure, the elimination of the intermediate species is realised as a special case of a well-known elimination algorithm. The algorithm appears in the literature in different variations and under different names, for instance, as the Fourier-Motzkin elimination algorithm [DLHK12] or double description method [FP96]. The procedure allows to eliminate a variable from a system of inequalities of the form $\mathbf{Ax} \geq \mathbf{0}$, obtaining a new set of inequalities that describe the projection of the cone $\{\mathbf{x} : \mathbf{Ax} \geq \mathbf{0}\}$ onto $x_n = 0$. In the double description form, the algorithm allows to simultaneously construct a set of generators for the polyhedral cone. In [SS93], the procedure is adapted for the calculation of a generating set of elementary modes of the network, with additional constraints on the fluxes.

Proposition 8.2.1. *The system of ODEs in* (83) *is the dynamics associated to the chemical reaction system identified by Algorithm 1.*

Proof. We can rewrite the system in (83) as follows:

$$\begin{aligned}
\frac{d\mathbf{x}'}{dt} &= \sum_{i\to j\in\mathcal{R}\setminus(\mathcal{R}'\cup\mathcal{R}'')} \mathcal{K}_{ij}(\mathbf{x}', \varphi(\mathbf{x}'))(\pi(y^j) - \pi(y^i)) + \\
&\quad \varphi(\mathbf{x}') \sum_{i\to j\in\mathcal{R}'} \mathcal{K}'_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)) + \sum_{i\to j\in\mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)), \\
&= \sum_{i\to j\in\mathcal{R}\setminus(\mathcal{R}'\cup\mathcal{R}'')} \mathcal{K}_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)) + \\
&\quad \frac{\sum_{i\to j\in\mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}')}{\sum_{i\to j\in\mathcal{R}'} \mathcal{K}'_{ij}(\mathbf{x}')} \sum_{i\to j\in\mathcal{R}'} \mathcal{K}'_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)) + \sum_{i\to j\in\mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)), \\
&= \sum_{i\to j\in\mathcal{R}\setminus(\mathcal{R}'\cup\mathcal{R}'')} \mathcal{K}_{ij}(\mathbf{x}')(\pi(y^j) - \pi(y^i)) + \\
&\quad \sum_{i\to j\in\mathcal{R}''} \sum_{i'\to j'\in\mathcal{R}'} \frac{\mathcal{K}'_{ij}(\mathbf{x}')\mathcal{K}'_{i'j'}(\mathbf{x}')}{\sum_{h\to k\in\mathcal{R}'} \mathcal{K}'_{hk}(\mathbf{x}')} (\pi(y^{j'}) + c - \pi(y^{i'}) - (c + \pi(y^i) - \pi(y^j)).
\end{aligned}$$

□

Algorithm 1: Elimination of the intermediate linear species X_n.

Data: A chemical reaction system $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y, \mathcal{K})$,
X_n intermediate linear species.

$\widetilde{\mathcal{R}} = \emptyset$
$\mathcal{R}' = \{i \to j \in \mathcal{R} \mid y_n^i = 1, y_n^j = 0\}$
$\mathcal{R}'' = \{i \to j \in \mathcal{R} \mid y_n^i = 0, y_n^j = 1\}$
$\varphi(\mathbf{x}') = \frac{\sum_{i\to j\in\mathcal{R}''} \mathcal{K}'_{ij}(\mathbf{x}')}{\sum_{i\to j\in\mathcal{R}'} \mathcal{K}'_{ij}(\mathbf{x}')}$
foreach $i \to j \in \mathcal{R} \setminus (\mathcal{R}' \cup \mathcal{R}'')$ **do**
  add reaction with reactant $\pi(y^i)$ and product $\pi(y^j)$ to $\widetilde{\mathcal{R}}$, with rate function $\mathbf{x}' \mapsto \mathcal{K}_{ij}(\mathbf{x}', \varphi(\mathbf{x}'))$
end
foreach $i \to j \in \mathcal{R}''$ **do**
  foreach $i' \to j' \in \mathcal{R}'$ **do**
    if $y^i + y^{i'} \neq y^j + y^{j'}$ **then**
      $c = \sup(\pi(y^j), \pi(y^{i'}))$
      add reaction with reactant $\pi(y^i) + c - \pi(y^j)$ and product $\pi(y^{j'}) + c - \pi(y^{i'})$ to $\widetilde{\mathcal{R}}$, with rate function $\frac{\mathcal{K}'_{ij}\mathcal{K}'_{i'j'}}{\sum_{h\to k\in\mathcal{R}'} \mathcal{K}'_{hk}}$
    end
  end
end

Remark 8.2.2. As is apparent from the proof of Proposition 8.2.1, we are free to choose other complexes in place of the complex c. The choice for c in Algorithm 1 can be motivated as follows. Given reactions $i \to j \in \mathcal{R}''$ and $i' \to j' \in \mathcal{R}'$, for any $z \geq y^j, y^{i'}$, the original network generates a "reaction path" from $y^i + z - y^j \to y^{j'} + z - y^{i'}$ (i.e., there is a sequence of reactions converting $y^i + z - y^j$ to $y^{j'} + z - y^{i'}$). Choosing $c = \sup(\pi(y^j), \pi(y^{i'}))$ guarantees that these reaction paths are contained in the reduced network.

Example 8.2.3. Reconsider the scheme in equation 69, which, after elimination of the intermediate species EAB, reduced to (81). We can consider the elimination of the species EA. Following Algorithm 1, we define $\mathcal{R}' = \{EA \to A + E, B + EA \to P + E\}$, $\mathcal{R}'' = \{A + E \to EA\}$ and $\varphi(\mathbf{x}') = \frac{\kappa_1(\kappa_4+\kappa_5)x_A x_E}{\kappa_2(\kappa_4+\kappa_5)+\kappa_3\kappa_5 x_B}$. For the reactions $EA \to A + E$ and $A + E \to EA$ we have $y^i + y^{i'} = y^j + y^{j'}$, so that $\widetilde{\mathcal{R}}$ consists of only one reaction, obtained by combining $A + E \to EA$ and $B + EA \to P + E$:

$$A + B + E \longrightarrow P + E, \tag{84}$$

with rate $\frac{\kappa_1\kappa_3\kappa_5 x_A x_B x_E}{\kappa_2(\kappa_4+\kappa_5)+\kappa_3\kappa_5 x_B}$.

Example 8.2.4. In this example, we compare the reduced network obtained with Algorithm 1 to the network obtained using Algorithm 1 in [GHBS15], which offers a general method for identification of chemical systems that give rise to a given rational system of ODEs. Consider the network with three species defined by the reactions

$$2X_1 \xrightarrow{\kappa_1} 2X_1 + X_3, \qquad X_1 + X_3 \xrightarrow{\kappa_2} X_2, \qquad X_3 \xrightarrow{\kappa_3} \emptyset,$$

and with associated system of ODEs

$$\frac{dx_1}{dt} = -\kappa_2 x_1 x_3, \ \frac{dx_2}{dt} = \kappa_2 x_1 x_3, \ \frac{dx_3}{dt} = \kappa_1 x_1^2 - \kappa_2 x_1 x_3 - \kappa_3 x_3.$$

The species X_3 is an intermediate linear species. We have $\varphi(x_1, x_2) = \frac{\kappa_1}{\kappa_3+\kappa_2 x_1} x_1^2$, and the reduced system of ODEs is

$$\frac{dx_1}{dt} = -\frac{\kappa_1\kappa_2}{\kappa_3 + \kappa_2 x_1} x_1^3, \ \frac{dx_2}{dt} = \frac{\kappa_1\kappa_2}{\kappa_3 + \kappa_2 x_1} x_1^3. \tag{85}$$

Applying Algorithm 1, we have $\mathcal{R}' = \{X_3 \to \emptyset, X_1 + X_3 \to X_2\}$, $\mathcal{R}'' = \{2X_1 \to 2X_1 + X_3\}$, hence the reduced network contains only one reaction

$$2X_1 \longrightarrow X_1 + X_2$$

with rate $\frac{\kappa_1\kappa_2}{\kappa_3+\kappa_2 x_1} x_1^3$. The algorithm described in [GHBS15] is a generalisation of the construction of the "canonic mechanism" associated to a system of differential equations presented in [HT81]. It proceeds by considering each differential equation separately. Each differential equation is written as a sum of terms of the form $\pm\kappa\frac{\mathbf{x}^c}{p(\mathbf{x})}$, where c is a complex and $p(\mathbf{x})$ is a polynomial such that $p(0) = 1$, and κ is a kinetic parameter. For each term in the equation for species X_i, the monomial at the numerator determines the reactant complex c. Then, the product complex is defined as $c - X_i$ or $c + X_i$ depending on the sign of the term. The reaction is then assigned the reaction rate $\kappa\frac{\mathbf{x}^c}{p(\mathbf{x})}$.

The equations in (85) can be written in the required form by dividing the numerator and denominator by κ_3. The algorithm gives the reactions

$$3X_1 \longrightarrow 2X_1, \qquad 3X_1 \longrightarrow 3X_1 + X_2,$$

both with rate function $\frac{\kappa_1\kappa_2}{\kappa_3+\kappa_2 x_1} x_1^3$.

Elimination of an intermediate set of species

In this section, we consider the elimination of a set of species. We need to introduce some new notations and definitions first. Given a chemical reaction network $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y)$, and a set of species $\mathcal{I} = \{X_{n-k+1}, \dots, X_n\}$, write $\pi_{\mathcal{I}}$ for the projection on the last k coordinates. Denote by $\mathbf{\Gamma}_{\mathcal{I}}$ the submatrix of the stoichiometric matrix $\mathbf{\Gamma}$ with rows corresponding to the species in $\mathcal{I}$, and write $\mathbf{x}' = (x_1, \dots, x_{n-k})$.

Definition 8.2.5. Consider a set of species $\mathcal{I} = \{X_{n-k+1}, \dots, X_n\}$. $\mathcal{I}$ is called a *linear set of non-interacting species* if

(i) for all $i \in \mathcal{C}$, $\sum_{j=n-k+1}^{n} y_j^i \leq 1$, and

(ii) for each reaction $i \to j$, there exists a function $\mathcal{K}'_{ij} \colon \Omega' \to \mathbb{R}_{\geq 0}$, with $\mathbb{R}_{>0}^{n-k} \subseteq \Omega' \subseteq \mathbb{R}_{\geq 0}^{n-k}$, such that if $\sum_{j=n-k+1}^{n} y_j^i = 0$, $\mathcal{K}_{ij}(\mathbf{x}) = \mathcal{K}'_{ij}(\mathbf{x}')$ and, if $y_h^i = 1$, $\mathcal{K}_{ij}(\mathbf{x}) = x_h \mathcal{K}'_{ij}(\mathbf{x}')$.

The definition is similar to the one considered in [SWF17]. To ensure that the elimination can be applied iteratively, we need an additional condition. Define $\mathcal{R}^{\mathcal{I}}$ as the multidirected graph with nodes the set $\mathcal{I} \cup \{\emptyset\}$, and edges $\{\pi_{\mathcal{I}}(y^i) \to \pi_{\mathcal{I}}(y^j) \mid i \to j \in \mathcal{R}, \pi_{\mathcal{I}}(y^i) \neq \pi_{\mathcal{I}}(y^j)\}$. We will say that $\mathcal{I}$ is an *intermediate set of species* if all the components of the graph $\mathcal{R}^I$ are strongly connected.

Notice that, if $X_{n-k+1}, \dots, X_n$ are intermediate species, then $\{X_{n-k+1}, \dots, X_n\}$ is not necessarily an intermediate set of species. Consider for instance the intermediate species X_3 and X_4 in $X_4 \rightleftharpoons X_3 \to X_1 \rightleftharpoons X_2$. If the species X_4 is removed, then X_3 is not an intermediate species and the elimination algorithm cannot be applied.

Lemma 8.2.6. *Consider a chemical reaction network $(\mathcal{C}, \mathcal{R}, y)$ and an intermediate linear set of non-interacting species $\mathcal{I} = \{X_{n-k+1}, \dots, X_n\}$. Then the set $\mathcal{J} = \{X_{n-k+1}, \dots, X_{n-1}\}$ is an intermediate linear set of non-interacting species for the network obtained by eliminating X_n with Algorithm 1.*

In addition, if the vector $v = \sum_{i=n-k}^{n} \mathrm{e}^i \in \mathbb{R}^n$ is in $\mathbf{\Gamma}^\perp$, then $\pi(v) \in (\mathbf{\Gamma}')^\perp$, where $\mathbf{\Gamma}'$ is the stoichiometric matrix of the reduced network.

Proof. If a reaction in $\widetilde{\mathcal{R}}$ is of the form $\pi(y^i) \to \pi(y^j)$ for some reaction $i \to j \in \mathcal{R}$, then condition (i) of Definition 8.2.5 is verified for both the reactant and product. If $y^i_n = 1$, then the rate is of the form $\varphi(\mathbf{x}')\mathcal{K}(\mathbf{x}')$; otherwise, the rate coincides with the rate of $i \to j$, and satisfies condition (ii) of Definition 8.2.5

Consider now a reaction $i'' \to j'' \in \widetilde{\mathcal{R}}$ obtained as a combination of a reaction $i \to j \in \mathcal{R}''$ and a reaction $i' \to j' \in \mathcal{R}'$. By hypothesis, $\sum_{l=n-k+1}^{n} z \le 1$ for $z \in y^i, y^j, y^{i'}, y^{j'}$, and $y^i_n = y^{j'}_n = 0$, $y^j_n = y^{i'}_n = c_n = 1$, $c = \sup(y^j, y^{i'})$. Hence for the reactant of $i'' \to j''$ we have $\sum_{l=n-k+1}^{n-1}(y^i + c - y^j)_l = \sum_{l=n-k+1}^{n} y^i_l \le 1$, and similarly for the product. In addition, if $y^{i''}_l = 1$ for some $l = n-k+1, \dots, n-1$, then $y^i_l = 1$ and the rate of $i'' \to j''$ is of the form $x_l\mathcal{K}'(\mathbf{x}')$. Otherwise, it is of the form $\mathcal{K}'(\mathbf{x}')$.

Finally, to see that the connected components of the graph $\widetilde{\mathcal{R}}^{\mathcal{J}}$ are strongly connected, it is sufficient to observe that for any pair of nodes $a, b \in \mathcal{J} \cup \{\emptyset\}$ there is a path from a to b in $\widetilde{\mathcal{R}}^{\mathcal{J}}$ if and only if there is a path from a to b in $\mathcal{R}^{\mathcal{I}}$.

For the last statement, observe that the reaction vectors are either of the form $\pi(y^j - y^i)$ with $y^j_n = y^i_n$, or of the form $\pi(y^{j'} - y^{i'} + y^j - y^i)$, with $y^{j'}_n - y^{i'}_n = y^i_n - y^j_n$. □

The next example shows that, when multiple species are eliminated using the Algorithm in 1, the result depends on the order of elimination.

Example 8.2.7. Consider the mass action system

$$X_1 \xrightarrow{\kappa_1} X_2 + X_5 \xrightarrow{\kappa_2} X_4 \xrightarrow{\kappa_3} X_5 \xrightarrow{\kappa_4} X_3, \tag{86}$$

and the elimination of species X_4 and X_5 with the method in Algorithm 1. To eliminate X_4, we simply combine r_2 and r_3, obtaining

$$X_1 \xrightarrow{\kappa_1} X_2 + X_5 \xrightarrow{\kappa_2} X_5 \xrightarrow{\kappa_4} X_3.$$

Then, to eliminate X_5, we need to combine $X_1 \to X_2 + X_5$ and $X_5 \to X_3$. We find the network

$$X_1 \xrightarrow{\tilde{r}_1} X_2 + X_3, \; X_2 \xrightarrow{\tilde{r}_2} \emptyset, \tag{87}$$

with rates $\mathcal{K}_{\tilde{r}_1} = \kappa_1 x_1$ and $\mathcal{K}_{\tilde{r}_2} = \frac{\kappa_1\kappa_2}{\kappa_4} x_1 x_2$.

Now, consider the application of Algorithm 1 to the network in (86) with intermediate X_5 first, and then X_4. For the first step we have $\mathcal{R}' = \{X_2 + X_5 \to X_4, X_5 \to X_3\}$ and $\mathcal{R}'' = \{X_1 \to X_2 + X_5, X_4 \to X_5\}$. Combining these reactions two by two we find the four reactions

$$\begin{array}{ccccc} X_1 & \xrightarrow{r'_1} & X_4 & \xrightarrow{r'_2} & X_3 \\ \downarrow r'_3 & & \uparrow r'_4 & & \\ X_2 + X_3 & & X_2 + X_4 & & \end{array}$$

with rates $\mathcal{K}_{r'_1} = \frac{\kappa_1\kappa_2 x_1 x_2}{\kappa_4 + \kappa_2 x_2}$, $\mathcal{K}_{r'_2} = \frac{\kappa_3\kappa_4 x_4}{\kappa_4 + \kappa_2 x_2}$, $\mathcal{K}_{r'_3} = \frac{\kappa_1\kappa_4 x_1}{\kappa_4 + \kappa_2 x_2}$ and $\mathcal{K}_{r'_4} = \frac{\kappa_2\kappa_3 x_2 x_4}{\kappa_4 + \kappa_2 x_2}$ respectively. To eliminate X_4, we have $\mathcal{R}' = \{X_4 \to X_3\}$ and $\mathcal{R}'' = \{X_1 \to X_4\}$, and we find the network

$$\begin{array}{ccccc} X_1 & \xrightarrow{r''_1} & X_3, & \qquad & X_2 \xrightarrow{r''_2} \emptyset \\ \downarrow r''_3 & & & & \\ X_2 + X_3 & & & & \end{array}$$

with reaction rates $\mathcal{K}_{r_1''} = \frac{\kappa_1\kappa_2 x_1 x_2}{\kappa_4+\kappa_2 x_2}$, $\mathcal{K}_{r_2''} = \frac{\kappa_1\kappa_2^2 x_1 x_2^2}{\kappa_4(\kappa_4+\kappa_2 x_2)}$ and $\mathcal{K}_{r_3''} = \frac{\kappa_1\kappa_4 x_1}{\kappa_4+\kappa_2 x_2}$. Therefore the reduced network depends on the order of elimination of the species.

Eliminating redundant reactions

As seen in Example (8.2.7), the iterative elimination of intermediate linear species based on Algorithm 1 can yield different reduced networks, when different orders for the species are considered. In this section, we introduce a version of Algorithm 1 for elimination of a set of species, that allows for the identification of a reduced network with minimal number of reactions.

As mentioned in the previous section, when a set of species $\mathcal{I}$ is eliminated using Algorithm 1, the structure of the reduced network is found using a special case of a well-studied elimination algorithm [SS93, FP96], that allows for the identification of the generators of the cone $\{\mathbf{\Gamma}_{\mathcal{I}}\mathbf{x} = 0,\ \mathbf{x} \in \mathbb{R}^m_{\geq 0}\} = \{\mathbf{A}\mathbf{x} \geq 0 \mid \mathbf{x} \in \mathbb{R}^m\}$. Except under some elimination orders, many of the generators identified by the algorithm given in the previous section are redundant. To avoid the explosion in the number of generators, and ensure that the minimum number of generators for the cone is obtained (i.e., a set of extreme rays), algorithms such as those in [SS93, FP96] go through an additional step that ensures that redundant rays are not created.

To introduce a similar elimination step that would take into account the network kinetics, we compare the algorithm given in the previous section to the algorithm in [SS93] for the calculation of the extreme rays (in the case where no flux is fixed). The input of the algorithm in [SS93] is the matrix $\mathbf{T}^{(0)} = |\mathbf{\Gamma}_{\mathcal{I}}\ \mathbf{I}_m|^t$, where $\mathbf{I}_m$ is the $m \times m$ identity matrix. At step $j+1$, for $j = 1, \ldots, k-1$, the matrix $\mathbf{T}^{(j)}$ is replaced by a matrix containing:

(i) The columns of $\mathbf{T}^{(j)}$ with index h such that $\mathbf{T}^{(j)}_{(j+1)h} = 0$.

(ii) All the possible combinations of pairs of columns $|\mathbf{T}^{(j)}_{(j+1)h'}|\mathbf{T}^{(j)}_{.h} + |\mathbf{T}^{(j)}_{(j+1)h}|\mathbf{T}^{(j)}_{.h'}$ for indices (h, h') of $\mathbf{T}^{(j)}$ such that $\mathbf{T}^{(j)}_{jh} \cdot \mathbf{T}^{(j)}_{jh'} < 0$, if the following condition is verified: $Z(\mathbf{v}^{j,h}) \cap Z(\mathbf{v}^{j,h'}) \not\subseteq Z(\mathbf{v}^{j,l})$, for $l \neq h, h'$, where $Z(\mathbf{v}) = \{i \mid v_i = 0\}$, and $\mathbf{v}^{j,h}$ is the projection of h^{th} column of $\mathbf{T}^{(j)}$ on the last m coordinates.

At the end of the process, the matrix $\mathbf{R}$ given by the last m rows of the matrix $\mathbf{T}^{(k)}$ contains the generating vectors. The Algorithm in 1, goes through the same steps, in the special case of $|\mathbf{T}^{(j)}_{(j+1)h}| = |\mathbf{T}^{(j)}_{(j+1)h'}| = 1$, but does not discard any combination of columns that satisfy $\mathbf{T}^{(j)}_{jh} \cdot \mathbf{T}^{(j)}_{jh'} < 0$. Observe, in addition, that the reaction vectors of the reduced network are obtained by multiplying the original stoichiometric matrix $\mathbf{\Gamma}$ by $\mathbf{R}$.

Consider a ray $\mathbf{v}$ that has been discarded at step (ii), and write r for the corresponding reaction, and $\mathcal{K}_r$ for the rate obtained as in Algorithm 1. If the reaction corresponding to $\mathbf{v}$ is simply discarded, as in point (ii), then the resulting reaction system does not have the required associated system of ODEs, since the contribution $\mathcal{K}_r(\mathbf{\Gamma}v)$ of the reaction to (70) is lost. To obtain a dynamically equivalent system when the redundant reactions are removed, we need to adjust the kinetic rates of the remaining reactions. Writing $\{\mathbf{v}^{(j+1)l}\}_l$ for the generators identified at step $j+1$, we have that $\mathbf{v}$ verifies $\mathbf{v} = \sum_l \lambda_l \mathbf{v}^{(j+1)l}$ for some $\lambda_l \geq 0$. Since $\mathcal{K}_r(\mathbf{\Gamma}\mathbf{v}) = \sum_l \lambda_l \mathcal{K}_r(\mathbf{\Gamma}\mathbf{v}^{(j+1)l})$, if we add $\lambda_l\mathcal{K}$ to the rate associated to $\mathbf{v}^{(j+1)l}$, the reduced network has the required system of ODEs. Therefore we add the following definitions of the reaction rates for the reactions identified by the steps (i) and (ii) above:

(iii) For a column with indices h at step (i), set the reaction rate to $\mathcal{K}_h^{(j)}(\mathbf{x}', \varphi(\mathbf{x}'))$.

(iv) For each pair of columns with $\mathbf{T}^{(j)}_{(j+1)h} + \mathbf{T}^{(j)}_{(j+1)h'} = 0$, define a reaction rate as in 1.

(v) If $\mathbf{v}$ is a ray corresponding to a reaction with rate $\mathcal{K}_r$, and $\mathbf{v} = \sum_l \lambda_l \mathbf{v}^{(j+1)l}$, $\lambda_l \geq 0$, add $\lambda_l \mathcal{K}_r$ to the rate corresponding to $\mathbf{v}^{(j+1)l}$.

This method ensures that the structure of the reduced network is independent of the order of elimination. There is no guarantee of uniqueness however for the kinetics of the reduced network (see Example 8.2.10).

We summarise the algorithm given above in 2. We denote by $\mathbf{v}^{ij}$ the ray associated to a reaction $i \to j$. Notice that a reduced chemical reaction network is properly defined only at the end of the elimination process: in the intermediate steps, reactions with the same complex as reactant and product are considered, so that the minimal structure can be identified (see also Example 8.2.10). In addition, not all species in the elimination process are intermediate species. In this case, we use a constant instead of the expression φ_h to replace the species concentration.

Algorithm 2: Elimination of an intermediate linear set of non-interacting species.

Data: A chemical reaction system $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y, \mathcal{K})$,
an intermediate linear set of non-interacting species $\{X_{n-k+1}, \dots, X_n\}$,
$\alpha \in \mathbb{R}$.

$\mathcal{R}_{(0)} = \mathcal{R}$
$\mathrm{e}^1, \dots, \mathrm{e}^m$ rays of the reactions in $\mathcal{R}_{(0)}$
foreach $h = 0, \dots, k-1$ **do**
  $\mathcal{R}_{(h+1)} = \emptyset$
  $\mathcal{R}'_{(h)} = \{i \to j \in \mathcal{R}_{(h)} \mid y^i_{n-h} = 1, y^j_{n-h} = 0\}$
  $\mathcal{R}''_{(h)} = \{i \to j \in \mathcal{R}_{(h)} \mid y^i_{n-h} = 0, y^j_{n-h} = 1\}$
  if $|\mathcal{R}'_{(h)}| > 0$ **then** $\varphi_h(\mathbf{x}') = \dfrac{\sum_{i \to j \in \mathcal{R}''_{(h)}} \mathcal{K}_{ij}(\mathbf{x}')}{\sum_{i \to j \in \mathcal{R}'_{(h)}} \mathcal{K}'_{ij}(\mathbf{x}')}$
  else $\varphi_h(\mathbf{x}') = \alpha$
  foreach $i \to j \in \mathcal{R}_{(h)} \setminus (\mathcal{R}'_{(h)} \cup \mathcal{R}''_{(h)})$ **do**
    add a reaction to $\mathcal{R}_{(h+1)}$ with reactant y^i and product y^j, with rate function $\mathbf{x}' \mapsto \mathcal{K}_{ij}(\mathbf{x}', \varphi_h(\mathbf{x}'))$ and ray $\mathbf{v}^{ij}$
  end
  foreach $i \to j \in \mathcal{R}''_{(h)}$ **do**
    foreach $i' \to j' \in \mathcal{R}'_{(h)}$ **do**
      $c = \sup(y^j, y^{i'})$
      add a reaction to $\mathcal{R}_{(h+1)}$ with reactant $y^i + c - y^j$ and product $y^{j'} + c - y^{i'}$, with rate function $\dfrac{\mathcal{K}_{ij}\mathcal{K}'_{i'j'}}{\sum_{h \to k \in \mathcal{R}'_{(h)}} \mathcal{K}'_{hk}}$ and ray $\mathbf{v}^{ij} + \mathbf{v}^{i'j'}$
    end
  end
  foreach $i \to j \in \mathcal{R}_{(h+1)}$ **do**
    if $\mathbf{v}^{ij} = \sum_{i'j'} \lambda_{i'j'} \mathbf{v}^{i'j'}$, $\lambda_{i'j'} \geq 0$, $(i,j) \neq (i',j')$, remove reaction $i \to j$ and add $\lambda_{i'j'}\mathcal{K}_{ij}$ to the rate of $i' \to j'$
  end
end

From the discussion above we have the following.

Proposition 8.2.8. *Consider a chemical reaction network $(\mathcal{C}, \mathcal{R}, y)$ and an intermediate linear set of non-interacting species $\{X_{n-k+1}, \dots, X_n\}$. Then the networks obtained by eliminating $\{X_{n-k+2}, \dots, X_n\}$ with Algorithm 1 or Algorithm 2 are dynamically equivalent.*

Example 8.2.9. Reconsider the network of Example 8.2.7, and the elimination of X_5 and X_4. The elimination of X_5 yields the generating rays

$$\mathbf{v}^1 = \mathrm{e}^1 + \mathrm{e}^2,\ \mathbf{v}^2 = \mathrm{e}^3 + \mathrm{e}^4,\ \mathbf{v}^3 = \mathrm{e}^1 + \mathrm{e}^4,\ \mathbf{v}^4 = \mathrm{e}^2 + \mathrm{e}^3,$$

corresponding to the reactions r_1', r_2', r_3', r_4'. The elimination of X_4 gives the vectors $\mathbf{v} = \mathbf{v}^1 + \mathbf{v}^2$, $\mathbf{v}^3$ and $\mathbf{v}^4$ corresponding to reactions r_1'', r_2'' and r_3''. The vector $\mathbf{v}$ writes as the sum of the remaining rays $\mathbf{v}^3 + \mathbf{v}^4$. Therefore, we remove reaction r_1'' and redefine the rates of reactions r_2'' and r_3'' as $\frac{\kappa_1\kappa_2^2 x_1 x_2^2}{\kappa_4(\kappa_4+\kappa_2 x_2)} + \frac{\kappa_1\kappa_2 x_1 x_2}{\kappa_4+\kappa_2 x_2} = \frac{\kappa_1\kappa_2}{\kappa_4} x_1 x_2$ and $\frac{\kappa_1\kappa_4 x_1}{\kappa_4+\kappa_2 x_2} + \frac{\kappa_1\kappa_2 x_1 x_2}{\kappa_4+\kappa_2 x_2} = \kappa_1 x_1$. This network coincides with the one in (87) identified by eliminating X_4 and then X_5.

Example 8.2.10. Consider the mass action system

$$X_1 \xrightarrow{\kappa_1} X_2 + X_4 \xrightarrow{\kappa_2} X_5 \xrightarrow{\kappa_3} X_6 \underset{\kappa_6}{\overset{\kappa_4}{\rightleftarrows}} \overset{\kappa_5}{} X_4 \qquad X_3.$$

We first eliminate species X_6, obtaining the graph

$$X_1 \xrightarrow{r_1} X_2 + X_4 \xrightarrow{r_2} X_5 \xrightarrow{r_3} X_4 \circlearrowleft r_6 \qquad r_4 \searrow \quad \downarrow r_5 \qquad X_3, \tag{88}$$

with mass action kinetics, kinetic parameters $(\kappa_1, \kappa_2, \frac{\kappa_3\kappa_4}{\kappa_4+\kappa_6}, \frac{\kappa_5\kappa_6}{\kappa_4+\kappa_6}, \frac{\kappa_3\kappa_6}{\kappa_4+\kappa_6}, \frac{\kappa_4\kappa_5}{\kappa_4+\kappa_6})$ and and corresponding generating rays $\mathbf{v}^1 = \mathrm{e}^1$, $\mathbf{v}^2 = \mathrm{e}^2$, $\mathbf{v}^3 = \mathrm{e}^3 + \mathrm{e}^4$, $\mathbf{v}^4 = \mathrm{e}^3 + \mathrm{e}^6$, $\mathbf{v}^5 = \mathrm{e}^5 + \mathrm{e}^6$, $\mathbf{v}^6 = \mathrm{e}^4 + \mathrm{e}^5$.

Now consider the elimination of X_5 and X_4 from (88) in two different orders. If we eliminate X_5 first, we obtain the graph with kinetic parameters

$$X_1 \xrightarrow{\kappa_1} X_2 + X_4 \xrightarrow{\frac{\kappa_2\kappa_4}{\kappa_4+\kappa_6}} X_4 \circlearrowleft \frac{\kappa_4\kappa_5}{\kappa_4+\kappa_6} \qquad \frac{\kappa_2\kappa_6}{\kappa_4+\kappa_6} \searrow \quad \downarrow \frac{\kappa_5\kappa_6}{\kappa_4+\kappa_6} \qquad X_3,$$

and eliminating X_4, the final reduced network

$$X_1 \xrightarrow{r_1'} X_3, \qquad X_1 \xrightarrow{r_2'} X_2 + X_3, \qquad X_2 \xrightarrow{r_3'} \emptyset. \tag{89}$$

with rates $\mathcal{K}_{r_1'}(x_1, x_2) = \frac{\kappa_1\kappa_2 x_1 x_2}{\kappa_5+\kappa_2 x_2}$, $\mathcal{K}_{r_2'}(x_1, x_2) = \frac{\kappa_1\kappa_5 x_1}{\kappa_5+\kappa_2 x_2}$ and $\mathcal{K}_{r_3''}(x_1, x_2) = \frac{\kappa_1\kappa_2\kappa_4 x_1 x_2}{\kappa_6(\kappa_5+\kappa_2 x_2)}$, and corresponding generating vectors $\mathrm{e}^1 + \mathrm{e}^2 + \mathrm{e}^3 + \mathrm{e}^6$, $\mathrm{e}^1 + \mathrm{e}^5 + \mathrm{e}^6$ and $\mathrm{e}^2 + \mathrm{e}^3 + \mathrm{e}^4$.

Now reconsider the network in (88). The elimination of X_4 gives

$$X_1 \xrightarrow{r_1''} X_5 \underset{r_3''}{\overset{r_2''}{\rightrightarrows}} X_3 \qquad \emptyset \circlearrowleft r_6'' \qquad \downarrow r_4'' \qquad r_5'' \uparrow \qquad X_2 + X_3 \qquad X_2 + X_5,$$

with reaction rates $\mathcal{K}_{r_1''}(x_1,x_2,x_5) = \frac{\kappa_1\kappa_2(\kappa_4+\kappa_6)}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}x_1x_2$, $\mathcal{K}_{r_2''}(x_1,x_2,x_5) = \frac{\kappa_3\kappa_6}{\kappa_4+\kappa_6}x_5$, $\mathcal{K}_{r_3''}(x_1,x_2,x_5) = \frac{\kappa_3\kappa_4\kappa_5\kappa_6}{(\kappa_4+\kappa_6)(\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2)}x_5$, $\mathcal{K}_{r_4''}(x_1,x_2,x_5) = \frac{\kappa_1\kappa_5\kappa_6}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}x_1$ and $\mathcal{K}_{r_5''}(x_1,x_2,x_5) = \frac{\kappa_2\kappa_3\kappa_4}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}x_2x_5$ (we do not need to calculate $\mathcal{K}_{r_6''}$ as it will not factor in the next steps). The corresponding generators are $\mathrm{e}^1+\mathrm{e}^2$, $\mathrm{e}^3+\mathrm{e}^6$, $\mathrm{e}^3+\mathrm{e}^4+\mathrm{e}^5+\mathrm{e}^6$, $\mathrm{e}^1+\mathrm{e}^5+\mathrm{e}^6$, $\mathrm{e}^2+\mathrm{e}^3+\mathrm{e}^4$ and $\mathrm{e}^4+\mathrm{e}^5$. The third ray does not have minimal support, therefore r_3'' can be eliminated, and its kinetic rate added to $\mathcal{K}_{r_2''}$ and $\mathcal{K}_{r_6''}$, giving $\mathcal{K}_{r_2''}(x_1,x_2,x_5) = \frac{\kappa_3\kappa_6(\kappa_5+\kappa_2x_2)}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}x_5$.

After eliminating X_5, we find the final reduced network

$$X_1 \xrightarrow{\tilde{r}_1} X_3, \qquad X_1 \xrightarrow{\tilde{r}_2} X_2+X_3, \qquad X_2 \xrightarrow{\tilde{r}_3} \emptyset.$$

The rates we obtain are $\mathcal{K}_{\tilde{r}_1}(x_1,x_2) = \frac{\kappa_1\kappa_2(\kappa_4+\kappa_6)x_1x_2}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}$, $\mathcal{K}_{\tilde{r}_2}(x_1,x_2) = \frac{\kappa_1\kappa_5\kappa_6x_1}{\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2}$ and $\mathcal{K}_{\tilde{r}_3}(x_1,x_2) = \frac{\kappa_1\kappa_2^2\kappa_4(\kappa_4+\kappa_6)x_1x_2^2}{\kappa_6(\kappa_5+\kappa_2x_2)(\kappa_5\kappa_6+\kappa_2(\kappa_4+\kappa_6)x_2)}$. Hence the network coincides in structure with the network in (89), but has different kinetic rates.

8.3 SECOND METHOD

The second method is inspired by another form of approximation adopted in enzyme kinetics, the rapid equilibrium approximation. For the reaction network in (71), for instance, one assumes that the enzyme, substrate and enzyme-substrate complex are at equilibrium, so that an expression of the form $x_{ES} = \frac{\kappa_1}{\kappa_2}x_Ex_S$ can be written for the concentration of x_{ES}. The equation is then used in combination with the conservation law $x_E + x_{ES} = E_0$ and the concentration of ES is eliminated. For a complete description of the assumptions we refer the reader to [Seg75, CB14, HS12].

For this method, we assume that we are given a complex $z \in \mathbb{N}^{n-1}$, and for the concentration of the species X_n an expression of the form

$$x_n = \psi(\mathbf{x}'),\ \psi : \Omega' \to \mathbb{R}_{\geq 0},\ \mathbb{R}^{n-1}_{>0} \subseteq \Omega' \subseteq \mathbb{R}^{n-1}_{\geq 0}. \tag{90}$$

We consider the reduced system of ODEs on $\mathbf{x}'$ obtained from (70) by substituting x_n with $\psi(\mathbf{x}')$, and adjusting the stoichiometric coefficients with z as follows:

$$\frac{d\mathbf{x}'}{dt} = \sum_{i\to j\in\mathcal{R}} \mathcal{K}_{ij}(\mathbf{x}',\psi(\mathbf{x}'))(\pi(y^j)+y^j_nz-\pi(y^i)-y^i_nz). \tag{91}$$

Algorithm 3: Elimination of the intermediate species X_n that replaces X_n with the complex z.

Data: A chemical reaction system $\mathcal{N} = (\mathcal{C},\mathcal{R},y,\mathcal{K})$,
X_n intermediate species,
a complex $z \in \mathbb{N}^n$, and a function $\psi : \Omega' \to \mathbb{R}_{\geq 0}$.

$\widetilde{\mathcal{R}} = \emptyset$
foreach $i \to j \in \mathcal{R}$ **do**
  $c = \pi(y^i) + y^i_nz$
  $c' = \pi(y^j) + y^j_nz$
  if $c \neq c'$ **then**
    add reaction with reactant c and product c' to $\widetilde{\mathcal{R}}$, with rate function $\mathcal{K}_{ij}(\mathbf{x}',\psi(\mathbf{x}'))$
  end
end

The following proposition is straightforward from equation (91) and Algorithm 3.

Proposition 8.3.1. *The system of ODEs in* (91) *is the dynamics associated to the chemical reaction system identified by Algorithm 3.*

Consider the network in (71), and the application of Algorithm 3 on the intermediate species ES, with $z = S + E$ and $\psi(\mathbf{x}') = \frac{\kappa_1}{\kappa_2} x_E x_S$. We obtain the reaction $S + E \to P + E$ with rate $\frac{\kappa_2\kappa_3}{\kappa_1} x_S x_E$, and the associated system of ODEs

$$\begin{aligned} \frac{dx_S}{dt} &= -\frac{\kappa_1\kappa_3}{\kappa_2} x_E x_S, \\ \frac{dx_E}{dt} &= 0, \\ \frac{dx_P}{dt} &= +\frac{\kappa_1\kappa_3}{\kappa_2} x_E x_S. \end{aligned} \tag{92}$$

To find a rate of the form in (72) we need to consider the use of conservation laws (see the next section).

Lemma 8.3.2. *Consider a chemical reaction network $(\mathcal{C}, \mathcal{R}, y)$ and an intermediate linear set of non-interacting species $\mathcal{I} = \{X_{n-k+1}, \dots, X_n\}$. Suppose that the intermediate species X_n is eliminated using Algorithm 3, with z that satisfies $\sum_{i=n-k+1}^{n-1} z_j = 1$, and ψ of the form $\psi(\mathbf{x}') = \varphi(x_1, \dots, x_{n-k})\mathbf{x}^z$ for some $\varphi\colon \Omega' \to \mathbb{R}_{\geq 0}$, with $\mathbb{R}_{>0}^{n-k-1} \subseteq \Omega' \subseteq \mathbb{R}_{\geq 0}^{n-k-1}$. Then the set $\mathcal{J} = \{X_{n-k+1}, \dots, X_{n-1}\}$ is an intermediate linear set of non-interacting species for the reduced network.*

In addition, if the vector $v = \sum_{i=n-k}^{n} \mathrm{e}^i \in \mathbb{R}^n$ is in $\mathbf{\Gamma}^\perp$, then $\pi_{n-h}(v) \in (\mathbf{\Gamma}')^\perp$, where $\mathbf{\Gamma}'$ is the stoichiometric matrix of the reduced network.

Proof. Each complex in the reduced network is of the form $w = \pi(y^i) + y_n^i z$ for some complex $i \in \mathcal{C}$. Therefore if $y_n^i = 0$, $\sum_{j=n-k+1}^{n-1} w_j = \sum_{j=n-k+1}^{n-1} y_j^i \leq 1$, and if $y_n^i = 1$, we have $\sum_{j=n-k+1}^{n-1} w_j = \sum_{i=n-k+1}^{n-1} z_j \leq 1$.

Call h the index such that $z_h = 1$. Consider a reaction r in the reduced network, obtained from the reaction $i \to j \in \mathcal{R}$. If $y_n^i = 0$, then the reaction rate of r coincides with the rate of $i \to j$. Otherwise, the rate of r is of the form $x_h \psi(\mathbf{x}')\mathcal{K}_{ij}(\mathbf{x}')$. In both cases, the rate satisfies the conditions of Definition 8.2.5

The graph $\widetilde{\mathcal{R}}^{\mathcal{J}}$ is obtained from the graph $\mathcal{R}^{\mathcal{I}}$ by identifying the nodes X_n and X_h; therefore all connected components of $\widetilde{\mathcal{R}}^{\mathcal{J}}$ are strongly connected.

For the last statement, observe that the reaction vectors of the reduced network are of the form $w = \pi(y^j - y^i) + (y_n^j - y_n^i)z$, hence $\pi(v)^t w = \pi(v)^t \pi(y^j - y^i) + (y_n^j - y_n^i)\pi(v)^t z = -(y_n^j - y_n^i) + (y_n^j - y_n^i) = 0$. □

Example 8.3.3. Consider the following network, called a non-competitive inhibition scheme in [Seg75]:

$$\begin{aligned} &S + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} ES \xrightarrow{\kappa_3} P + E, \qquad I + E \underset{\kappa_5}{\overset{\kappa_4}{\rightleftarrows}} E_i, \\ &S + E_i \underset{\kappa_7}{\overset{\kappa_6}{\rightleftarrows}} ES_i \underset{\kappa_9}{\overset{\kappa_8}{\rightleftarrows}} ES + I. \end{aligned}$$

Consider the expression $\psi(\mathbf{x}') = \frac{\kappa_4}{\kappa_5} x_E x_I$ for the species E_i obtained by assuming equilibrium for the association-dissociation reactions for E and I, and take $z = E + I$. Using Algorithm 3 we find the reduced network

$$S + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} ES \xrightarrow{\kappa_3} P + E, \qquad S + E + I \underset{\kappa_7}{\overset{\frac{\kappa_4\kappa_6}{\kappa_5}}{\rightleftarrows}} ES_i \underset{\kappa_9}{\overset{\kappa_8}{\rightleftarrows}} ES + I.$$

Similarly, consider the same procedure for species ES_i, replaced by $I + ES$ with $\psi(\mathbf{x}') = \frac{\kappa_9}{\kappa_8} x_{ES} x_I$, we find the network

$$S + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} ES \xrightarrow{\kappa_3} P + E, \qquad S + E + I \underset{\frac{\kappa_7\kappa_9}{\kappa_8}}{\overset{\frac{\kappa_4\kappa_6}{\kappa_5}}{\rightleftarrows}} ES + I.$$

Finally, removing ES using $z = E + S$ and $\psi(\mathbf{x}') = \frac{\kappa_1}{\kappa_2} x_E x_S$ (found by assuming the equilibrium of the first two reactions) we obtain one reaction:

$$S + E \xrightarrow{\frac{\kappa_1\kappa_3}{\kappa_2}} P + E. \tag{93}$$

8.4 CONSERVATION LAWS

The elimination of the enzyme using the conservation law can be seen as a special case of Algorithm 3. To see how conservation laws can be used to derive enzyme kinetic rates, consider the scheme in equation (71) again. The equation

$$x_E + x_{ES} = E_0 \tag{94}$$

is a conservation law for the network. The two elimination methods of the previous sections use expressions for x_{ES} ($x_{ES} = \frac{\kappa_1}{\kappa_2+\kappa_3} x_E x_S$ or $x_{ES} = \frac{\kappa_1}{\kappa_2} x_S x_E$). Inserting these expression in (94), we find $x_E + \frac{\kappa_1}{\kappa_2+\kappa_3} x_E x_S = E_0$ or $x_E + \frac{\kappa_1}{\kappa_2} x_S x_E = E_0$. Both equations can be used to find an expression for x_E in terms of the remaining species. We can then apply Algorithm 3 with $\psi(\mathbf{x}') = \frac{(\kappa_2+\kappa_3)E_0}{\kappa_2+\kappa_3+\kappa_1 x_S}$ or $\psi(\mathbf{x}') = \frac{\kappa_2 E_0}{\kappa_2+\kappa_1 x_S}$, and $z = \emptyset$. The reduced network we find consists of the single reaction $S \to P$, with rate function $\frac{\kappa_3 E_0 x_S}{\frac{\kappa_2+\kappa_3}{\kappa_1}+x_S}$ or $\frac{\kappa_3 E_0 x_S}{\frac{\kappa_2}{\kappa_1}+x_S}$. Both rates are of the form in (72).

Let us describe a setting that allows for the application of the procedure just illustrated. Suppose that $X_{n-k}, \dots, X_n$ are an intermediate linear set of non-interacting species, and that $\sum_{i=n-k}^{n} x_i = T$ is a conservation law for the network, i.e. the vector $v = \sum_{i=n-k}^{n} \mathrm{e}^i \in \mathbb{R}^n$ is in $\mathbf{\Gamma}^\perp$. Suppose that the species $X_{n-k+1}, \dots, X_n$ are eliminated using Algorithm 1 under the hypotheses of Lemma 8.2.6, or Algorithm 3 under the hypotheses of Lemma 8.3.2. Using the expressions obtained for $x_{n-k+1}, \dots, x_n$ to replace these concentrations in the conservation law $\sum_{i=n-k}^{n} x_i = T$, we can derive an expression for x_{n-k} in terms of the remaining variables. Then, we can apply Algorithm 3 using this expression and $z = \emptyset$. We conclude the chapter with some examples that illustrate the procedure just described.

Example 8.4.1. Reconsider the network in examples 8.1.2 and 8.2.3. The equation $x_E + x_{EA} + x_{EAB} = E_0$ is a conservation law for the original network. Replacing x_{EAB} and x_{EA} using $x_{EAB} = \frac{\kappa_3}{\kappa_4+\kappa_5} x_B x_{EA}$ and $x_{EA} = \frac{\kappa_1(\kappa_4+\kappa_5)x_A x_E}{\kappa_2(\kappa_4+\kappa_5)+\kappa_3\kappa_5 x_B}$, we can write $x_E = \frac{(\kappa_2(\kappa_4+\kappa_5)+\kappa_3\kappa_5 x_B)E_0}{\kappa_2(\kappa_4+\kappa_5)+\kappa_3\kappa_5 x_B+\kappa_1(\kappa_4+\kappa_5)x_A+\kappa_1\kappa_3 x_A x_B}$. Proceeding as in Algorithm 3 with $z = \emptyset$ on the reaction network in (84), we find the reaction $A + B \to P$ with rate

$$\frac{\kappa_5 E_0 x_A x_B}{\kappa_2 \frac{\kappa_4+\kappa_5}{\kappa_1\kappa_3} + \frac{\kappa_5}{\kappa_1} x_B + \frac{\kappa_4+\kappa_5}{\kappa_3} x_A + x_A x_B}.$$

Example 8.4.2. The non-competitive inhibition scheme in Example 8.3.3 admits the conservation law $x_E + x_{ES} + x_{ES_i} + x_{E_i} = E_0$. Replacing x_{E_i}, x_{ES_i} and x_{ES} with the expressions considered in Example 8.3.3 we obtain $x_E + (1 + \frac{\kappa_9}{\kappa_8} x_I)\frac{\kappa_1}{\kappa_2} x_E x_S + \frac{\kappa_4}{\kappa_5} x_E x_I = E_0$, which we can use to write an expression for x_E in terms of x_I, x_S and the kinetic parameters. Eliminating E in (93), we find the reaction $S \to P$ with rate

$$\frac{\frac{\kappa_1\kappa_3}{\kappa_2} E_0 x_S}{1 + \frac{\kappa_4}{\kappa_5} x_I + \frac{\kappa_1}{\kappa_2} x_S + \frac{\kappa_1\kappa_9}{\kappa_2\kappa_8} x_I x_S}.$$

Example 8.4.3. Consider a classical reversible two-substrate, two-product compulsory order mechanism [CB14] (a similar mechanism is discussed in [SWF17], Example 12):

$$A+E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA, \qquad B+EA \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} EAB \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} P+EQ, \qquad EQ \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} Q+E.$$

Eliminating EAB using Algorithm 1 one obtains the mass action network

$$A+E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA, \qquad B+EA \underset{\frac{\kappa_4\kappa_5}{\kappa_4+\kappa_5}}{\overset{\frac{\kappa_3\kappa_5}{\kappa_4+\kappa_5}}{\rightleftharpoons}} P+EQ, \qquad EQ \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} Q+E.$$

After eliminating the intermediate species EA and EQ, the remaining reactions are

$$A+B+E \rightleftharpoons P+Q+E, \tag{95}$$

with rates $\frac{\kappa_1\kappa_3\kappa_5\kappa_7 x_A x_B x_E}{\kappa_2(\kappa_4+\kappa_5)\kappa_7+(x_B\kappa_3\kappa_7+x_P\kappa_2\kappa_4)\kappa_5}$ and $\frac{\kappa_2\kappa_4\kappa_5\kappa_8 x_E x_P x_Q}{\kappa_2(\kappa_4+\kappa_5)\kappa_7+(x_B\kappa_3\kappa_7+x_P\kappa_2\kappa_4)\kappa_5}$.

Using the conservation law $x_E + x_{EA} + x_{EQ} + x_{EAB} = E_0$ the network is reduced to $A+B \rightleftharpoons P+Q$, with rates $\kappa_1\kappa_3\kappa_5\kappa_7 E_0 x_A x_B/d$ and $\kappa_2\kappa_4\kappa_5\kappa_8 E_0 x_P x_Q/d$, with

$$\begin{aligned} d =& \kappa_1\kappa_3\kappa_5 x_A x_B x_P + \kappa_1\kappa_3(\kappa_5+\kappa_7)x_A x_B + \kappa_1\kappa_4(\kappa_5+\kappa_7)x_A x_P + \\ & \kappa_1\kappa_5\kappa_7 x_A + \kappa_3\kappa_5\kappa_7 x_B + \kappa_2\kappa_4\kappa_5 x_P + \kappa_2(\kappa_4+\kappa_5)\kappa_8 x_Q + \\ & \kappa_3\kappa_5\kappa_8 x_B x_P x_Q + \kappa_3\kappa_5\kappa_8 x_B x_Q + (\kappa_2+\kappa_4)\kappa_5\kappa_8 x_P x_Q + \kappa_2(\kappa_4+\kappa_5)\kappa_7. \end{aligned}$$

Example 8.4.4. Consider the following reversible two-substrate, two-product random order mechanism [CB14]

$$\begin{array}{llll} A+E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} EA, & B+EA \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} EAB \underset{\kappa_{12}}{\overset{\kappa_{11}}{\rightleftharpoons}} B+EP, & Q+EP \underset{\kappa_{14}}{\overset{\kappa_{13}}{\rightleftharpoons}} EAB \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} EP, & EP \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} P+E, \\ B+E \underset{\kappa_{10}}{\overset{\kappa_9}{\rightleftharpoons}} EB, & A+EB \rightleftharpoons P+EQ, & & EQ \underset{\kappa_{16}}{\overset{\kappa_{15}}{\rightleftharpoons}} Q+E. \end{array}$$

Assumption of equilibrium for the association-dissociation reactions $A+E \rightleftharpoons EA$, $B+E \rightleftharpoons EB$, $P+E \rightleftharpoons EP$ and $Q+E \rightleftharpoons EQ$ gives the expressions $x_{EA} = \frac{\kappa_1}{\kappa_2}x_A x_E$, $x_{EB} = \frac{\kappa_9}{\kappa_{10}}x_B x_E$, $x_{EP} = \frac{\kappa_8}{\kappa_7}x_P x_E$, $x_{EQ} = \frac{\kappa_{16}}{\kappa_{15}}x_Q x_E$. After replacing EA, EB, EP and EQ with $E+A$, $E+B$, $E+P$, $E+Q$ respectively, the network reduces to

$$A+B+E \underset{\kappa_4+\kappa_{12}}{\overset{\frac{k_1k_3k_{10}+k_2k_9k_{11}}{k_2k_{10}}}{\rightleftharpoons}} EAB \underset{\frac{k_7k_{14}k_{16}+k_5k_8k_{15}}{k_7k_{15}}}{\overset{\kappa_5+\kappa_{13}}{\rightleftharpoons}} E+P+Q.$$

Using Algorithm 1 on the species EAB and the conservation law $x_E + x_{EA} + x_{EP} + x_{EP} + x_{EQ} + x_{EAB} = E_0$ we find the network $A+B \rightleftharpoons P+Q$ with rates $\frac{V_f}{\kappa_A^i\kappa_B^m d}$ and $\frac{V_r}{\kappa_P^m\kappa_Q^i d}$, where $V_f = (\kappa_5+\kappa_{13})E_0$, $V_r = (\kappa_4+\kappa_{12})E_0$, $d = 1 + \frac{x_A}{\kappa_A^i} + \frac{x_B}{\kappa_B^i} + \frac{x_P}{\kappa_P^i} + \frac{x_Q}{\kappa_Q^i} + \frac{x_A x_B}{\kappa_A^i\kappa_B^m} + \frac{x_P x_Q}{\kappa_P^m\kappa_Q^i}$, $\kappa_A^i = \frac{\kappa_2}{\kappa_1}$, $\kappa_B^i = \frac{\kappa_{10}}{\kappa_9}$, $\kappa_P^i = \frac{\kappa_7}{\kappa_8}$, $\kappa_Q^i = \frac{\kappa_{15}}{\kappa_{16}}$, $\kappa_B^m =$, $\kappa_B^m = \frac{\kappa_1\kappa_{10}(\kappa_4+\kappa_5+\kappa_{12}+\kappa_{13})}{k_1k_3k_{10}+k_2k_9k_{11}}$. $\kappa_P^m = \frac{\kappa_7\kappa_{16}(\kappa_4+\kappa_5+\kappa_{12}+\kappa_{13})}{k_7k_{14}k_{16}+k_5k_8k_{15}}$.

9

NETWORK TRANSLATION AND STEADY STATE PROPERTIES

In this chapter we discuss weakly reversible generalised mass action realisations of chemical reaction networks, identified using translations of reactions. Given a mass action system on a chemical reaction network, we might want to determine whether the system admits complex balancing steady states or if the steady states admit a binomial parametrisation. Whether a mass action system admits complex balancing steady states depends on the decomposition $\mathbf{Y}\mathbf{A}_\kappa\mathbf{x}^{\mathbf{Y}}$ of the steady state equations determined by the network structure, and complex balancing steady states cannot exist if the network is not in weakly reversible form. Recall that a graph of complexes is weakly reversible if every reaction belongs to at least one cycle. A cycle in the graph of complexes identifies a vector in the kernel of the incidence matrix $\mathbf{I}_\mathcal{R}$. If the system admits positive steady states, then the kernel of the stoichiometric matrix $\mathbf{\Gamma}$ contains at least a positive vector v; if the network is not weakly reversible, then v is not in the kernel of $\mathbf{I}_\mathcal{R}$.

Consider for instance a network with three reactions

$$\begin{aligned} &A + B \xrightarrow{r_1} 2C \xrightarrow{r_2} D + B, \\ &D + E \xrightarrow{r_3} A + E. \end{aligned} \tag{96}$$

The kernel of the stoichiometric matrix for this network contains the vector $v = (1, 1, 1)$; this vector identifies a sequence of reactions r_1, r_2 and r_3, which, when all executed, take the system back to its original state. The reactions cannot be directly composed in the graph of complexes (96), since the product of reaction r_2 differs from the reactant of reaction r_3. To allow for the composition of r_2 and r_3, we can consider an additional operation, which consists in adding some species to the reactant and product of reactions. If we add the species E to reactions r_1 and r_2 and the species B to reaction r_3, then we obtain the following graph:

$$A + B + E \xrightarrow{r_1+E} 2C + E \xrightarrow{r_2+E} D + B + E. \qquad (r_3+B) \tag{97}$$

The vector $(1, 1, 1)$ now identifies a cycle in the graph of complexes. We call the operation of adding (or removing) species from the reactant and product of a reaction a *translation* of the reaction.

Observe that if the graph structure of the network is changed using translation of reactions, the stoichiometric structure of the network is unvaried. In addition, if we assign each translated reaction with the reaction rate of the original reaction — using the generalised mass action framework — we automatically obtain a dynamically equivalent system.

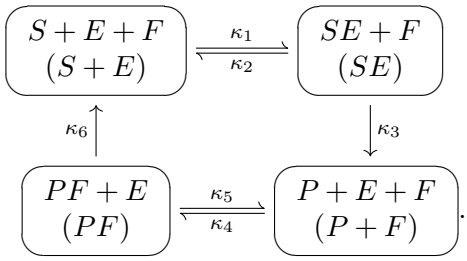
Converting "hidden" cycles to cycles that are visible in the graph of complexes can not only result in the identification of a weakly reversible realisation, but also of a realisation with a lower deficiency. For example, the network in (96) has deficiency one, whereas

the network in (97) has deficiency zero. The following is a biological example to which the procedure just described applies.

Example 9.0.1. Consider the classical *futile cycle* reaction scheme with set of species $\mathcal{S} = \{S, P, E, F, SE, PF\}$, defined by the reactions

$$\begin{aligned} S + E &\underset{r_2}{\overset{r_1}{\rightleftharpoons}} SE \xrightarrow{r_3} P + E, \\ P + F &\underset{r_5}{\overset{r_4}{\rightleftharpoons}} PF \xrightarrow{r_6} S + F. \end{aligned} \tag{98}$$

This scheme and its multisite versions (see example 9.1.17) are of significant interest in biology, and have been extensively studied under mass action and more general kinetics assumptions (see for instance [AS08, WS08, MDSC12, Joh14] and references therein). The network is not weakly reversible; however, the reactions r_1, r_3, r_4 and r_6 define a cycle that is "visible" if r_1 and r_3 are translated by F, and r_4 and r_6 are translated by E. Considering a mass action system on (98) with kinetic parameters $\kappa_1, \ldots, \kappa_6$, we can define the generalised mass action network



This is the generalised mass action version presented in [Joh14]. Since the deficiency and kinetic deficiency for this network are both equal to zero, by Theorem 7.4.3 the steady states are parametrised by binomials, for any assignment of kinetic parameters.

The procedure sketched above suggests considering vectors in the cone $\ker(\mathbf{\Gamma}) \cap \mathbb{R}^m_{\geq 0}$ and changing the decomposition of $\mathbf{\Gamma} = \mathbf{Y}\mathbf{I}_{\mathcal{R}}$ so that the vectors belongs to $\ker(\mathbf{I}_{\mathcal{R}})$. For a fixed order of the reactions, imposing that the translation converts a flux to a cycle amounts to writing a linear system for the stoichiometric coefficients of the translation complexes (see Lemma 9.1.10). As previously mentioned, the definition of complex balancing steady states will depend on the decomposition identified. For instance, we could use a single flux involving all reactions (see Chapter 10), and study complex balancing steady states for which all the reactant complexes of the original network have robust ratios.

The identification of a structure with deficiency zero is particularly useful, since under this condition all the steady states are complex balancing. In Chapter 10, we write a mixed-integer linear program, with the objective of maximising the number of generators that are converted to cycles. In several biological examples, this approach allows to identify a realisation with lower deficiency.

Other realisations can be identified by considering multiple translations per reaction, or by rescaling reaction vectors. Consider for instance the network

$$2A \xrightarrow{r_1} 2B, \qquad B \xrightarrow{r_2} A.$$

We can find a weakly reversible structure using r_2 twice, once translated by B and once by A:

$$2A \xrightarrow{r_1} 2B \xrightarrow{r_2+B} A + B, \qquad A + B \xrightarrow{r_2+A} 2A$$

or by dividing the stoichiometric coefficients of the reactant and product of reaction r_1 by 2:

$$A \underset{r_2}{\overset{r_1}{\rightleftharpoons}} B.$$

In both cases, the kinetic parameters need to be defined appropriately to obtain a dynamically equivalent system.

In this chapter, we will limit the translations we consider to a single translation per reaction. Since the reaction rates of translated reactions are determined by the original reactant rather then the new stoichiometric reactant, it will be useful to define an intermediate structure, which we call kinetic graph, that allows for the assignment of kinetic complexes to reactions. We identify a recurring scheme that allows for a deficiency zero kinetic graph to be defined, and we will see how deficiency zero kinetic graphs can sometimes be used to determine robust ratios.

The definition of translation considered in the following will be slightly different from the formalisation used in [Joh14, Joh15] and [TJ17], due to the different definition of generalised mass action network adopted here, which allows for more flexibility in the assignment of stoichiometric and kinetic complexes (see Remark 9.1.7 for a comparison between the two definitions).

9.1 TRANSLATED CHEMICAL REACTION NETWORKS

Given a chemical reaction network $(\mathcal{C}, \mathcal{R}, y)$, we call a *translation scheme* a map $T\colon \mathcal{R} \to \mathbb{Z}^n$ that satisfies $y(i) + T(i,j) \geq 0$ and $y(j) + T(i,j) \geq 0$ for all $(i,j) \in \mathcal{R}$. We say that T assigns *translation complexes* to reactions. Here we use the term complex in a generalised sense, since we allow for translations by negative vectors, as long as the resulting reactant and product vectors are positive. A translation scheme associates reactions to reactions in a generalised reaction network in the following way:

$$y^i \xrightarrow{r} y^j \quad (+T(r)) \quad \Longrightarrow \quad \boxed{\begin{matrix} y^i + T(r) \\ (y^i) \end{matrix}} \xrightarrow{r+T(r)} \boxed{\begin{matrix} y^j + T(r) \\ (-) \end{matrix}}, \tag{99}$$

that is, if we translate a reaction by $T(r)$, the source complex y becomes the kinetic complex associated to the stoichiometric complex $y^i + T(r)$, which reacts to the product complex $y^j + T(r)$.

Since we want to define the rate of the reaction $r + T(r)$ using the reactant of r, it is convenient to introduce another structure, that allows for the kinetic complexes to be assigned to reactions, rather than nodes.

Definition 9.1.1. A *kinetic graph* $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$ on the set of species $\mathcal{S}$ is a (multi)-directed graph with set of nodes $\mathcal{C} = \{1, \dots, c\}$ and set of edges $\mathcal{R}$, without loops, together with an injective map $y : \mathcal{C} \to \mathbb{N}^n$ and a map $z : \mathcal{R} \to \mathbb{N}^n$.

The definition of kinetic graph is a generalisation of the definition of chemical reaction network, where parallel edges are allowed, and reactions are labelled with complexes. Given an assignment of kinetic parameters κ on a kinetic graph $\mathcal{G}$, we associate a system of differential equations to $(\mathcal{G}, \kappa)$ as follows:

$$\frac{d\mathbf{x}}{dt} = \sum_{i \to j \in \mathcal{R}} \kappa_{ij} (y^j - y^i)\, \mathbf{x}^{z(i,j)}.$$

Definition 9.1.2. Given a reaction network $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y)$ and a translation scheme $T\colon \mathcal{R} \to \mathbb{Z}^n$, we call *kinetic translation graph* obtained from $\mathcal{N}$ under the translation T

the kinetic graph obtained by replacing each reaction $r \in \mathcal{R}$ with its translation $r + T(r)$, setting, for $r = (i, j) \in \mathcal{R}$, $z(r + T(r)) = y(i)$, and identifying nodes with the same stoichiometric complex.

An assignment of kinetic parameters κ on $\mathcal{N}$ directly defines an assignment of kinetic parameters on a kinetic translation graph $T(\mathcal{N})$. We will draw a kinetic graph as a chemical reaction network with edges labelled by monomials (which include the kinetic parameters if an assignment of kinetic parameters is given).

Example 9.1.3. Consider the chemical reaction network

$$A \underset{r_2}{\overset{r_1}{\rightleftharpoons}} B \qquad\qquad A + B \xrightarrow{r_3} 2B, \tag{100}$$

and the translation scheme

$$T(r_1) = T(r_2) = \emptyset,\ T(r_3) = -B.$$

The scheme identifies the kinetic graph

$$A \underset{x_A x_B}{\overset{x_A}{\underset{x_B}{\rightleftharpoons}}} B. \tag{101}$$

Definition 9.1.4. We say that a kinetic graph $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$ has *a conflict* at a node $i \in \mathcal{C}$ or at a complex $y(i)$ if there are two reactions $i \to j$ and $i \to j'$ in $\mathcal{R}$ such that $z(i, j) \neq z(i, j')$.

We can say therefore that the kinetic graph in (101) has a conflict at A.

From kinetic graphs to generalised chemical reaction networks

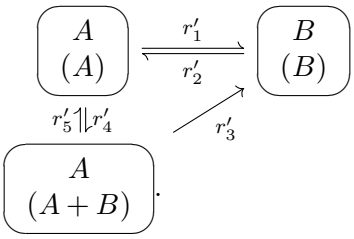
The ultimate goal of the translation process is to identify a generalised chemical reaction network that is dynamically equivalent to the original network. To this end, we consider the following structures associated to a kinetic graph $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$.

- The network $\mathcal{N}'(\mathcal{G})$ is the chemical reaction network obtained by ignoring the assignment of kinetic complexes to reactions z and by merging the parallel edges. We define the stoichiometric matrix and stoichiometric subspace, incidence matrix and deficiency of the kinetic graph using the respective definitions for the underlying chemical reaction network $\mathcal{N}'(\mathcal{G})$.

- We define generalised reaction networks $\widetilde{\mathcal{N}}(\mathcal{G}) = (\widetilde{\mathcal{C}}, \widetilde{\mathcal{R}}, \tilde{y}, \tilde{y}_\kappa)$ as follows: for each node i, create one node i' for each complex $c \in \{z(i, j) \mid i \to j \in \mathcal{R}\}$, setting $\tilde{y}(i') = y(i)$, $\tilde{y}_k(i') = c$. Then, for each reaction $r = (i, j) \in \mathcal{R}$, consider an edge $r' = (i', j')$ with $\tilde{y}(i') = y(i)$, $\tilde{y}_k(i') = z(i)$ and $\tilde{y}(j') = y(j)$. In general, there might be more than one possible choice for j'. In addition, if the kinetic graph is weakly reversible, we can define a weakly reversible generalised reaction network by adding reactions between each pair of nodes with the same stoichiometric complex.

- Given an assignment of kinetic parameters κ to $\mathcal{G}$, it will not be possible, in general, to define a dynamically equivalent mass action system on $\mathcal{N}(\mathcal{G})$. However, we can define a dynamically equivalent generalised mass action system on $\widetilde{\mathcal{N}}(\mathcal{G})$ by setting $\kappa(r') = \kappa(r)$. If additional reactions have been added between nodes with the same stoichiometric coefficients, then we can assign them with an arbitrary kinetic parameter.

Example 9.1.5. Reconsider the network in example 9.1.3. A weakly reversible generalised reaction network associated to the kinetic graph (101) is



(102)

If $\kappa = (\kappa_1, \kappa_2, \kappa_3)$ is an assignment of kinetic parameters for the network in (100), we can define a dynamically equivalent generalised mass action system on (102) by assigning the parameter κ_i to r'_i, $i = 1, 2, 3$, and arbitrary kinetic parameters to r'_4 and r'_5.

The following proposition is a direct consequence of the definition of kinetic translation graph and Theorem 7.4.3.

Proposition 9.1.6. *Consider a mass action system $\mathcal{M}$ on a chemical reaction network $\mathcal{N}$. Suppose that $\mathcal{N}$ admits a weakly reversible translation kinetic graph with no conflicts, and deficiency and kinetic deficiency equal to zero. Then all the steady states associated to $\mathcal{M}$ are complex balancing.*

If a deficiency zero kinetic graph has conflicts, then the associated generalised mass action networks will have positive deficiency. If a deficiency zero kinetic graph $\mathcal{G}$ has only one conflict at i given by exactly two reactions $i \to j$ and $i \to j'$, then we can associate to $\mathcal{G}$ a generalised mass action network with deficiency one: the number of linkage classes of the generalised network and the rank are unvaried, whereas the number of complexes increases by one. In this case we can apply Theorem 7.5.7 to identify robust ratios.

Remark 9.1.7. The approach to the definition of translated reaction network we describe here differs slightly from the approach in [Joh14, Joh15, TJ17]. Both definitions only consider one translation for each reaction in the chemical reaction network. The following are differences between the approaches:

- Kinetic graphs are not considered in [Joh14, Joh15, TJ17], where translations are defined directly as generalised reaction networks.
- In [Joh14, Joh15, TJ17], reactions with the same reactant are translated to reactions with the same reactant. This allows to avoid the assignment of the same kinetic complex to different stoichiometric complexes, requirement that we drop in this work.

Identification of kinetic translation graphs

In this section, we give some sufficient conditions for the existence of deficiency zero kinetic translation graphs, and some examples. We first need some preliminary lemmas.

Lemma 9.1.8. *Consider a chemical reaction network with m reactions, deficiency δ, and a set of generating elementary modes $w^1, \dots, w^d$ such that $\cup_{i=1}^{d} \mathrm{supp}(w^i) = \{1, \dots, m\}$. Suppose that the network has d' stoichiometric elementary modes. Then $\delta \leq d'$.*

Proof. Suppose that $w^1, \dots, w^{d'}$ are stoichiometric elementary modes, and define $v^i = \mathbf{I}_{\mathcal{R}} w^i$, $i = 1, \dots, d'$. Consider a vector $v \in \ker(\mathbf{Y}) \cap \mathrm{Im}(\mathbf{I}_{\mathcal{R}})$ and write $v = \mathbf{I}_{\mathcal{R}} u$. We show

that v is a linear combination of the v^i. By hypothesis, there exists a positive vector $w = \sum_{i=1}^{d} \alpha_i w^i \in \mathbb{R}^m_{>0}$ such that $\mathbf{\Gamma} w = 0$, $\alpha_i \geq 0$, $i = 1, \ldots, d$. Then, for any $\alpha \in \mathbb{R}_{>0}$ the vector $u + \alpha w$ is in $\ker(\mathbf{\Gamma})$, and, for $\alpha > 0$ sufficiently large, $z = u + \alpha w > 0$. Then z writes as $z = \sum_{i=1}^{d} \beta_i w^i$ for some $\beta_i \geq 0$, and $v = \mathbf{I}_{\mathcal{R}} u = \mathbf{I}_{\mathcal{R}}(z - \alpha w) = \mathbf{I}_{\mathcal{R}}(\sum_{i=1}^{d}(\beta_i - \alpha\alpha_i)w^i) = \sum_{i=1}^{d'}(\beta_i - \alpha\alpha_i)(\mathbf{I}_{\mathcal{R}} w^i)$, which concludes the proof. □

To see that the δ can be strictly smaller than d', consider for instance the network

$$2A \underset{r_2}{\overset{r_1}{\rightleftharpoons}} A + B \underset{r_4}{\overset{r_3}{\rightleftharpoons}} 2B.$$

The deficiency of the network is equal to one, and the network admits two stoichiometric elementary modes with support $\{1, 4\}$ and $\{2, 3\}$.

Corollary 9.1.9. *Suppose that a chemical reaction network is weakly reversible and does not admit any stoichiometric elementary mode. Then the network has deficiency zero.*

Lemma 9.1.10. *Let $\mathcal{N}$ be a chemical reaction network. Suppose that $w \in \mathbb{N}^m$ is a flux for $\mathcal{N}$ with support on I, and that $w_i = 1$ for all $i \in I$. Then there exists a translation scheme T for the network $\mathcal{N}$ such that the resulting kinetic graph admits a cycle with support on I.*

Proof. Write $\{r_1, \ldots, r_k\}$ for the reactions identified by the support of w. We have that

$$\sum_{\substack{\theta=1,\ldots,k \\ r_\theta=(i,j)}} y^i = \sum_{\substack{\theta=1,\ldots,k \\ r_\theta=(i,j)}} y^j. \tag{103}$$

Consider, for $r_1, \ldots, r_k$, the translation complexes defined as

$$T(r_h) = \sum_{\substack{\theta=1,\ldots,h-1 \\ r_\theta=(i,j)}} y^j + \sum_{\substack{\theta=h+1,\ldots,k \\ r_\theta=(i,j)}} y^i,\ h = 1, \ldots, k.$$

With this translation scheme, the product of reaction $r_h + T(r_h)$ is

$$\sum_{\substack{\theta=1,\ldots,h \\ r_\theta=(i,j)}} y^j + \sum_{\substack{\theta=h+1,\ldots,k \\ r_\theta=(i,j)}} y^i$$

which coincides with the reactant of $r_{h+1} + T(r_{h+1})$, for $h < k$, and with the reactant of $r_1 + T(r_1)$ for $h = k$, as a consequence of (103). Hence the reactions $r_1 + T(r_1), \ldots, r_k + T(r_k)$ define a cycle. □

Remark 9.1.11. The translation for reaction r_h consists of all the products of the reactions preceding r_h, plus all the reactants of the reactions following r_h. Consider the complex υ with entries

$$\upsilon_l = \min_{1 \leq \theta \leq k} \left(\sum_{\substack{\eta=1,\ldots,\theta-1 \\ r_\eta=(i,j)}} y^j_l + \sum_{\substack{\eta=\theta+1,\ldots,k \\ r_\eta=(i,j)}} y^i_l \right).$$

If the reactions $r_h + T(r_h)$, $h = 1, \ldots, k$, are further translated by the complex $-\upsilon$, the result is still a cycle with complexes with nonnegative stoichiometric coefficients. □

Remark 9.1.12. The translation schemes identified in Lemma 9.1.10 and Remark 9.1.11 depend on the order chosen for the reactions in the support of the elementary mode w.

Proposition 9.1.13. *Consider a chemical reaction network $\mathcal{N} = (\mathcal{C}, \mathcal{R}, y)$ with m reactions and generating elementary modes $w^1, \dots, w^d$ that satisfy $\cup_{h=1}^{d} \mathrm{supp}(w^h) = \{1, \dots, m\}$ and $w_i^h = 1$ for all i such that $w_i^h \neq 0$, $h = 1, \dots, h$. Suppose that one elementary mode is stoichiometric and the others are 2-cycles. Then $\mathcal{N}$ admits a deficiency zero weakly reversible kinetic translation graph.*

Proof. Write I for the support of the stoichiometric elementary mode w. By Lemma 9.1.10, there exists a translation scheme that converts w to a cycle. This defines a translation complex $T(r_i)$ for all $i \in I$. For any 2-cycle with support on reactions r_i and r_j, define a translation as follows: if $i, j \notin I$, set $T(r_i) = T(r_j) = \emptyset$; if $i \in I$ and $j \notin I$, define $T(r_j) = T(r_i)$ (since the elementary modes are vectors with minimal support, we cannot have $i \in I$ and $j \in I$).

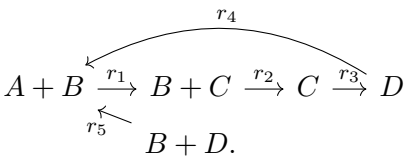
Call $\mathbf{\Gamma}$ the stoichiometric matrix of $\mathcal{N}$, and $\mathbf{I}$ the incidence matrix of $T(\mathcal{N})$. Then $\mathbf{\Gamma}$ decomposes as $\mathbf{YI}$ for some matrix $\mathbf{Y}$, and w^i is in the kernel of $\mathbf{I}$, for $i = 1, \dots, d$. Since the stoichiometric matrix and incidence matrix of the network $\mathcal{N}'(T(\mathcal{N}))$ are obtained from $\mathbf{\Gamma}$ and $\mathbf{I}$ by eliminating some duplicate columns, the reaction network $\mathcal{N}'(T(\mathcal{N}))$ admits only cyclic elementary modes. Therefore, by Corollary 9.1.9, the kinetic translation graph $T(\mathcal{N})$ has deficiency zero. In addition, since the cycles cover all reactions, $T(\mathcal{N})$ is weakly reversible. □

Notice that if $w^1, \dots, w^d$ are generating elementary modes of a network, then the condition $\cup_{i=1}^{d} \mathrm{supp}(w^i) = \{1, \dots, m\}$ is necessary for the existence of positive steady states for any associated mass action system.

Proposition 9.1.13 applies for instance to the futile cycle network in Example 9.0.1.

If a network $\mathcal{N}$ admits a direct decomposition in subnetworks $\mathcal{N}_1, \dots, \mathcal{N}_k$, then each equilibrium for $\mathcal{N}$ is an equilibrium for $\mathcal{N}_i$, $i = 1, \dots, k$. As a consequence, some properties that hold for the steady states of a subnetwork, such as robustness, necessarily hold for the steady states of the full network [SF10]. In particular, one can apply Proposition 9.1.13 to the subnetworks in a direct decomposition and derive properties of the steady states of the full network.

Example 9.1.14. In the statement of Proposition 9.1.13, the requirement that the cycles are all of length 2 is necessary. Consider the network



The cycle with support $\{1, 2, 3, 4\}$ and the stoichiometric elementary mode with support $\{1, 3, 5\}$ are generators of the cone 52. The translation scheme of the proposition identified by the stoichiometric elementary mode with the order $\{1, 3, 5\}$ is such that $B + C + T(r_1) = C + T(r_3)$; but any such translation scheme would convert the cycle $\{1, 2, 3, 4\}$ into a stoichiometric mode. The same happens if we consider the alternative order $\{1, 5, 3\}$.

The translation of Proposition 9.1.13 identifies a deficiency zero kinetic graph, but not necessarily a deficiency zero generalised reaction network. For example, the network in (100) has one stoichiometric elementary mode with support $\{2, 3\}$ and one elementary mode which is a 2-cycle with support $\{1, 2\}$. The kinetic graph (101) identified by the translation in (9.1.3) has deficiency zero, whereas the generalised reaction network in (102) has deficiency $3 - 1 - 1 = 1$.

The stoichiometric subspace of the generalised reaction network identified by Proposition 9.1.13 coincides with the stoichiometric subspace of the original reaction network

$\mathcal{N}$, whereas the kinetic-order subspace is generated by the differences between reactant complexes of $\mathcal{N}$ that are translated to the same linkage class. In the following examples we apply Proposition 9.1.13.

Example 9.1.15. Consider the chemical reaction network

$$\begin{array}{ccccc} & & & \xrightarrow{r_2} & B+C \\ A+B & \xrightarrow{r_1} & 2C & & \\ & & & \xrightarrow{r_3} & A+C. \end{array} \tag{104}$$

This network has only one elementary mode $\mathrm{e}^1+\mathrm{e}^2+\mathrm{e}^3$. We proceed as described in Remark 9.1.11 for the reactions r_1, r_2, r_3 in this order, and identify the translation scheme $T(r_1) = C$, $T(r_2) = C$, $T(r_3) = B$. This defines the generalised chemical reaction network

$$\boxed{\begin{array}{c} A+B+C \\ (A+B) \end{array}} \xrightarrow{r_1+C} \boxed{\begin{array}{c} 3C \\ (2C) \end{array}} \xrightarrow{r_2+C} \boxed{\begin{array}{c} B+2C \\ (2C) \end{array}} \xrightarrow{r_3+B} \boxed{\begin{array}{c} A+B+C \\ (A+B) \end{array}}. \tag{105}$$

The kinetic-order subspace is generated by $(-1,-1,2)^t$ and has dimension 1, hence the kinetic deficiency is $3-1-1=1$.

Consider an assignment of kinetic parameters $\kappa_1, \kappa_2, \kappa_3$ on (104) and (105). Since the network in (105) has deficiency zero, all positive steady states are complex balancing. Theorem 7.4.3 provides us with conditions that the kinetic parameters must satisfy for the existence of positive steady states. We have

$$\mathbf{Y}_\kappa = \begin{vmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 2 & 2 \end{vmatrix}, \ \mathbf{I}_\mathcal{E} = \begin{vmatrix} -1 & 0 \\ 1 & -1 \\ 0 & 1 \end{vmatrix}.$$

We find $\mathbf{C} = |\,0\ 1\,|^t$ and the condition for the existence of complex balancing equilibria $\kappa_2 = \kappa_3$.

Example 9.1.16. We can illustrate the idea of Proposition 9.1.13 using the Envz-Ompr model in Example 7.2.3. The network has only one stoichiometric elementary mode, involving the reactions

$$\begin{aligned} &X \xrightarrow{r_1} XT \xrightarrow{r_3} X_p, \\ &X_p + Y \xrightarrow{r_4} X_pY \xrightarrow{r_6} X + Y_p, \\ &XT + Y_p \xrightarrow{r_7} XTY_p \xrightarrow{r_9} XT + Y. \end{aligned}$$

It is easy to see that by translating the reactions r_1 and r_3 by $Y+XT$, the reactions r_4 and r_6 by XT and the reactions r_7 and r_9 by X we find the cycle

$$\begin{array}{ccccc} X+Y+XT & \xrightarrow{r_1+Y+XT} & 2XT+Y & \xrightarrow{r_3+Y+XT} & X_p+Y+XT \\ {\scriptstyle r_9+X}\uparrow & & & & \downarrow{\scriptstyle r_4+XT} \\ XTY_p+X & \xleftarrow{r_7+X} & X+XT+Y_p & \xleftarrow{r_6+XT} & X_pY+XT \end{array}$$

We can also keep the existing cycles by translating the remaining reactions r_2, r_5 and r_8 by $Y + XT$, XT and X respectively, finding the graph

$$\begin{array}{ccccc} X+Y+XT & \underset{r_2+Y+XT}{\overset{r_1+Y+XT}{\rightleftharpoons}} & 2XT+Y & \xrightarrow{r_3+Y+XT} & X_p+Y+XT \\ {\scriptstyle r_9+X}\uparrow & & & & {\scriptstyle r_5+XT}\uparrow\downarrow{\scriptstyle r_4+XT} \\ XTY_p+X & \underset{r_8+X}{\overset{r_7+X}{\rightleftharpoons}} & X+XT+Y_p & \xleftarrow{r_6+XT} & X_pY+XT \end{array}$$

The translation defines a weakly reversible kinetic graph without conflicts. Given an assignment of kinetic parameters $\kappa_1, \ldots, \kappa_9$ defining a mass action system $\mathcal{M}$, we can define the generalised mass action system

$$\begin{array}{ccccc} \begin{array}{c} X+Y+XT \\ (X) \end{array} & \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} & \begin{array}{c} 2XT+Y \\ (XT) \end{array} & \xrightarrow{\kappa_3} & \begin{array}{c} X_p+Y+XT \\ (X_p+Y) \end{array} \\ {\scriptstyle \kappa_9}\uparrow & & & & {\scriptstyle \kappa_5}\uparrow\downarrow{\scriptstyle \kappa_4} \\ \begin{array}{c} XTY_p+X \\ (XTY_p) \end{array} & \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} & \begin{array}{c} X+XT+Y_p \\ (XT+Y_p) \end{array} & \xleftarrow{\kappa_6} & \begin{array}{c} X_pY+XT \\ (X_pY) \end{array} \end{array}$$

which is dynamically equivalent to $\mathcal{M}$. The deficiency and kinetic deficiency are both zero, hence Proposition 9.1.6 applies. Theorem 7.5.6 also applies, so that $\mathcal{M}$ has a robust ratio in all the reactant complexes of the original network. In particular, $\mathcal{M}$ has ACR in species Y_p, since the latter writes as the difference between the kinetic complexes $XT + Y_p$ and XT. In addition, one can find the value of Y_p at steady state by dividing the tree constants associated to the nodes $X + XT + Y_p(XT + Y_p)$ and $2XT + Y(XT)$, finding $x_{Y_p} = \frac{\kappa_3(\kappa_8+\kappa_9)}{\kappa_7\kappa_9}$.

Example 9.1.17. The *multiple futile cycle* or *n-site phosphorylation network*

$$\begin{array}{ll} S_0 + E \underset{\kappa_{off_0}}{\overset{\kappa_{on_0}}{\rightleftharpoons}} ES_0 \xrightarrow{\kappa_{cat_0}} S_1 + E, & S_1 + F \underset{l_{off_0}}{\overset{l_{on_0}}{\rightleftharpoons}} FS_1 \xrightarrow{l_{cat_0}} S_0 + F, \\ \vdots & \vdots \\ S_{n-1} + E \underset{\kappa_{off_{n-1}}}{\overset{\kappa_{on_{n-1}}}{\rightleftharpoons}} ES_{n-1} \xrightarrow{\kappa_{cat_{n-1}}} S_n + E, & S_n + F \underset{l_{off_{n-1}}}{\overset{l_{on_{n-1}}}{\rightleftharpoons}} FS_n \xrightarrow{l_{cat_{n-1}}} S_{n-1} + F. \end{array} \tag{106}$$

admits, in the mass action case, a binomial parametrisation of the steady states [MDSC12, Joh14]. Multistationarity is possible for $n \geq 2$, and a technique for computing rate constants that give rise to multistationarity is given in [MDSC12]. For results on the number of steady states, see [WS08].

The reaction sets $\mathcal{R}_i = \{S_i + E \rightleftharpoons ES_i \to S_{i+1} + E, S_{i+1} + F \rightleftharpoons FS_{i+1} \to S_i + F\}$, for $i = 0, \ldots, n-1$, define a direct decomposition of the network. Proposition 9.1.13 applies therefore to each subnetwork. The resulting translation graph defines a generalised mass action network with deficiency and kinetic deficiency of zero:

$$\begin{array}{ccccccc} \begin{array}{c} S_0+E+F \\ (S_0+E) \end{array} & \underset{\kappa_{off_0}}{\overset{\kappa_{on_0}}{\rightleftharpoons}} & \begin{array}{c} ES_0+F \\ (ES_0) \end{array} & & \begin{array}{c} S_{n-1}+E+F \\ (S_{n-1}+E) \end{array} & \underset{\kappa_{off_{n-1}}}{\overset{\kappa_{on_{n-1}}}{\rightleftharpoons}} & \begin{array}{c} ES_{n-1}+F \\ (ES_{n-1}) \end{array} \\ {\scriptstyle l_{cat_0}}\uparrow & & \downarrow{\scriptstyle \kappa_{cat_0}} & \cdots & {\scriptstyle l_{cat_{n-1}}}\uparrow & & \downarrow{\scriptstyle \kappa_{cat_{n-1}}} \\ \begin{array}{c} FS_1+E \\ (FS_1) \end{array} & \underset{l_{on_0}}{\overset{l_{off_0}}{\rightleftharpoons}} & \begin{array}{c} S_1+E+F \\ (S_1+F) \end{array} & & \begin{array}{c} FS_n+E \\ (FS_n) \end{array} & \underset{l_{on_{n-1}}}{\overset{l_{off_{n-1}}}{\rightleftharpoons}} & \begin{array}{c} S_n+E+F \\ (S_n+F) \end{array} \end{array}$$

As a consequence, by Theorem 7.4.3 all the steady states of the system are complex balancing. To determine whether the system has the capacity for multiple complex balancing equilibria, we can determine whether $\sigma(S) \cap \sigma(S_\kappa^\perp) = \{0\}$ using Proposition 7.4.12. Since the number of species is $3(n+1)$ and the rank of S and S_κ equals $3n$, we need to calculate $2\binom{3(n+1)}{3n}$ determinants. For $n = 1$ we find that Proposition 7.4.10 applies and the system does not have the capacity for multiple complex balancing equilibria; for $n = 2$ instead Proposition 7.4.11 applies and there exists a mass action system on (106) that admits more than one complex balancing equilibrium in a stoichiometric compatibility class.

In this section, we have shown how it is possible to identify deficiency zero kinetic translation graphs for some reaction networks. In Section 10.2, we will describe a computational method for the identification of kinetic translation graphs. In the next section, we describe some results around the identification of binomial steady states and robustness that rely on the identification of deficiency zero kinetic graphs.

9.2 REMOVING CONFLICTS

If a network admits a weakly reversible kinetic translation graph with deficiency zero and no conflicts, then we can conclude that all the steady states are complex balancing, and use Theorem 7.4.3 to write conditions on the kinetic parameters for the existence of complex balancing steady states. If the kinetic graph has some conflicts, we can still find a dynamically equivalent generalised mass action network with positive deficiency and apply Theorem 7.4.3, with no guarantee, however, that all steady states are complex balancing.

In this section, we discuss some conditions that allow us to "remove some conflicts"; more specifically, the robustness of the kinetic complexes at the nodes with conflicts allows us to find a generalised mass action system on a network with deficiency zero, having the same positive steady states as the original mass action system.

We first need some new terminology. Consider a chemical reaction network $\mathcal{N}$, and an assignment of kinetic parameters κ defining a mass action system $\mathcal{M}$ on $\mathcal{N}$. Let $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$ be a kinetic translation graph on $\mathcal{N}$. For each $i \in \mathcal{C}$, define $J_i = \{j \in \mathcal{C} \mid i \to j \in \mathcal{R}\}$, and choose a map $\gamma : \mathcal{C} \to \mathcal{C}$ such that $\gamma(i) \in J_i$. Define a generalised chemical reaction network $\mathcal{G}_\gamma$ as follows: set the complexes, reactions and stoichiometric complexes to $\mathcal{C}$, $\mathcal{R}$ and y respectively, and set $y^i_k = z(i, \gamma(i))$ for all $i \in \mathcal{C}$. We call $\mathcal{G}_\gamma$ a *resolved* version of the kinetic graph $\mathcal{G}$ under γ, and we call the set of reactions

$$\mathcal{R}_I = \{i \to j \in \mathcal{R} \mid y^i_\kappa \neq z(i, \gamma(i))\}$$

improperly translated reactions.

Proposition 9.2.1. *Suppose that, for all $i \in \mathcal{C}$ and $j \in J_i$, the complexes $z(i, \gamma(i))$ and $z(i, j)$ have a robust ratio, and write α_{ij} for the value of $\mathbf{x}^{z(i,j)}/\mathbf{x}^{z(i,\gamma(i))}$ at positive steady states. Consider the assignment κ' of kinetic parameters on $\mathcal{G}_\gamma$ defined as $\kappa'_{ij} = \alpha_{ij}\kappa_{ij}$ for $j \in J_i$ defining a generalised mass action system $\widetilde{\mathcal{M}}$ on $\mathcal{G}_\gamma$. Then all the positive steady states associated to $\mathcal{M}$ are positive steady states for $\widetilde{\mathcal{M}}$.*

Proof. The system of ODEs associated to $(\mathcal{G}_\gamma, \kappa')$ writes as

$$\frac{d\mathbf{x}}{dt} = \sum_{i \to j \in \mathcal{R}} \alpha_{ij}\kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,\gamma(i))}.$$

On the other hand, every positive steady state $\mathbf{x}$ of $\mathcal{M}$ satisfies

$$0 = \sum_{i\to j\in\mathcal{R}} \kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,j)} = \sum_{i\to j\in\mathcal{R}} \alpha_{ij}\kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,\gamma(i))},$$

where we used $\mathbf{x}^{z(i,j)}/\mathbf{x}^{z(i,\gamma(i))} = \alpha_{ij}$. □

Example 9.2.2. Consider the network $\mathcal{N}$ defined by

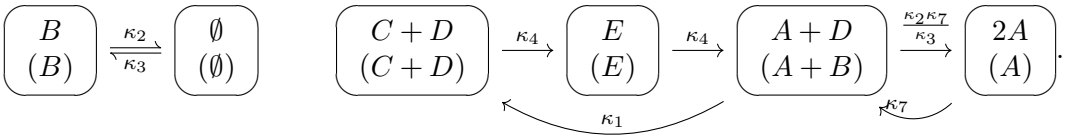
$$A + B \xrightarrow{r_1} B + C \qquad C + D \xrightarrow{r_4} E \xrightarrow{r_5} A + D \xrightarrow{r_6} 2A$$
$$B \underset{r_3}{\overset{r_2}{\rightleftharpoons}} \emptyset \qquad\qquad A \xrightarrow{r_7} D.$$

The network has one cyclic elementary mode with support $\{2,3\}$ and two stoichiometric elementary modes with support $\{1,4,5\}$ and $\{6,7\}$, and the sets $\mathcal{R}_1 = \{r_1, r_4, r_5\}$, $\mathcal{R}_2 = \{r_2, r_3\}$ and $\mathcal{R}_3 = \{r_6, r_7\}$ define a direct decomposition for the network. By applying Theorem 7.5.4 to the deficiency zero network $\mathcal{N}_2$ and Theorem 7.5.5 to the deficiency one network $\mathcal{N}_3$ we find that the network has ACR in B and D. Then, if κ is an assignment of kinetic parameters defining a mass action system $\mathcal{M}$ on $\mathcal{N}$, each positive steady state $\mathbf{x}$ of $\mathcal{M}$ verifies $\frac{x_B}{x_D} = \alpha$ for some $\alpha > 0$ (it can be easily verified that $x_B = \frac{\kappa_3\kappa_6}{\kappa_2\kappa_7}x_D$).

Consider the translation scheme $T(r_1) = D - B$, $T(r_2) = T(r_3) = T(r_4) = T(r_5) = T(r_6) = \emptyset$, $T(r_7) = A$, giving the kinetic translation graph

$$B \underset{\kappa_3}{\overset{\kappa_2 x_B}{\rightleftharpoons}} \emptyset \qquad C + D \underset{\kappa_1 x_A x_B}{\overset{\kappa_4 x_C x_D}{\rightleftharpoons}} E \xrightarrow{\kappa_5 x_E} A + D \underset{\kappa_7 x_A}{\overset{\kappa_6 x_A x_D}{\rightleftharpoons}} 2A,$$

The graph has only one conflict at $A + D$. In addition, the complexes $A + B$ and $A + D$ have a robust ratio. Consider resolving the conflict at $A + D$ by setting the kinetic complex to $A + B$. Applying Proposition 9.2.1 we find that all the positive steady states of $\mathcal{M}$ are positive steady states for the generalised mass action network



(107)

Since this network has deficiency zero, all its positive steady states are complex balancing. The kinetic deficiency is instead equal to 1. Using the procedure described in Theorem 7.4.3, we can find a condition on the kinetic parameters for the existence of complex balancing steady states, that is verified for any values of the kinetic parameters. It follows that $\mathcal{M}$ has positive binomial steady states. Notice, however, that not all the positive steady states of the system associated to $(\mathcal{G}_\gamma, \kappa')$ are steady states for $\mathcal{M}$. For example, $(x_A, x_B, x_C, x_D, x_E) = (\frac{\kappa_2\kappa_4}{\kappa_1\kappa_3}, \frac{\kappa_3}{\kappa_2}, \frac{\kappa_6+1}{\kappa_7}, \frac{\kappa_7}{\kappa_6+1}, \frac{\kappa_4}{\kappa_5})$ is a positive steady state for $(\mathcal{G}_\gamma, \kappa')$, but is not a steady state for $\mathcal{M}$.

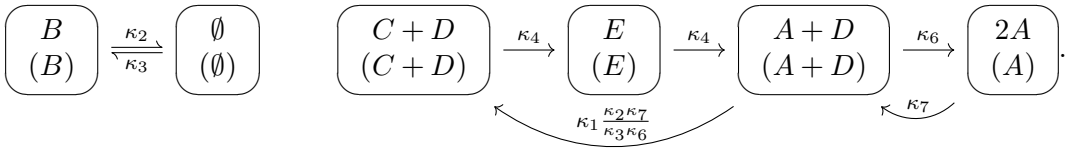
Proposition 9.2.3. *Under the hypotheses of Proposition 9.2.1, suppose that all the positive steady states of the system of ODEs associated to $(\mathcal{G}_\gamma, \kappa')$ satisfy $\mathbf{x}^{z(i,j)}/\mathbf{x}^{z(i,\gamma(i))} = \alpha_{ij}$, for $j \in J_i$. Then the systems of ODEs associated to $\mathcal{M}$ and $(\mathcal{G}_\gamma, \kappa')$ have the same positive steady states.*

Proof. We need to show that a positive steady state $\mathbf{x}$ for $(\mathcal{G}_\gamma, \kappa')$ is a steady state for $\mathcal{M}$. By hypothesis, $\mathbf{x}$ satisfies

$$0 = \sum_{i\to j\in\mathcal{R}} \alpha_{ij}\kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,\gamma(i))} = \sum_{i\to j\in\mathcal{R}} \kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,j)},$$

as required. □

Example 9.2.4. Reconsider the network in example 9.2.2, and this time resolve the conflict at $A+D$ by setting the kinetic complex to $A+D$. The resulting generalised chemical reaction system is



(108)

This weakly reversible network has deficiency and kinetic deficiency equal to zero. Moreover, it is easily verified that, at each positive steady state, $x_B = \frac{\kappa_3}{\kappa_2}$ and $x_D = \frac{\kappa_6}{\kappa_7}$. As a consequence, the equality $\frac{x_B}{x_D} = \frac{\kappa_3\kappa_6}{\kappa_2\kappa_7}$ is verified for all positive steady states associated to (108). If follows from Proposition 9.2.3 that the positive steady states associated to $\mathcal{M}$ coincide with the positive steady states associated to (108).

We now describe a property that is sufficient to guarantee the hypothesis of Proposition 9.2.3, and which is a reformulation of the resolvability condition described in [Joh14] and [TJ17]. The approach gives in particular a method for determining the robustness of a ratio.

Proposition 9.2.5. *Consider a chemical reaction network $\mathcal{N}$, with a weakly reversible deficiency zero kinetic translation graph $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$, and resolved version $\mathcal{G}_\gamma = (\mathcal{C}, \mathcal{R}, y, y_\kappa)$ of $\mathcal{G}$ with set of improperly translated reactions $\mathcal{R}_I$. Write $\mathcal{L}_\theta$, $\theta = 1, \dots, l$ for the linkage classes of $\mathcal{G}_\gamma$, and K_i for the tree constants. Suppose that $c, c' \in \mathbb{N}^n$ verify $c - c' = \sum_{\theta=1}^{l} \sum_{i,j\in\mathcal{L}_\theta} \beta_{ij}(y^i_\kappa - y^j_\kappa)$, and that the expression $\alpha = \prod_{\theta=1}^{l} \prod_{i,j\in\mathcal{L}_\theta} \left(\frac{K_i}{K_j}\right)^{\beta_{ij}}$ does not depend on the kinetic rates of the reactions in $\mathcal{R}_I$. Then, for any mass action system on $\mathcal{N}$, each positive steady state $\mathbf{x}$ satisfies $\frac{\mathbf{x}^c}{\mathbf{x}^{c'}} = \alpha$.*

Proof. Fix an assignment of kinetic parameters κ on $\mathcal{N}$ and a positive steady state $\bar{\mathbf{x}}$ for the associated system of ODEs. The system of ODEs associated to $\mathcal{N}$ and to $\mathcal{G}$ writes as $\frac{d\mathbf{x}}{dt} = \sum_{i\to j\in\mathcal{R}} \kappa_{ij}(y^j - y^i)\mathbf{x}^{z(i,j)}$, whereas the system of ODEs associated to $\mathcal{G}_\gamma$ is $\frac{d\mathbf{x}}{dt} = \sum_{i\to j\in\mathcal{R}} \kappa'_{ij}(y^j - y^i)\mathbf{x}^{z(i,\gamma(i))}$.

Consider a labelling of the reactions of $\mathcal{G}_\gamma$ defined as follows: $\kappa'_{ij} = \kappa_{ij}$ if $i \to j \in \mathcal{R}\setminus\mathcal{R}_I$, and $\kappa'_{ij} = \kappa_{ij}\frac{\bar{\mathbf{x}}^{z(i,j)}}{\bar{\mathbf{x}}^{z(i,\gamma(i))}}$ if $i \to j \in \mathcal{R}_I$. Then, the state $\bar{\mathbf{x}}$ is a positive steady state for $(\mathcal{G}_\gamma, \kappa')$. Since the network is weakly reversible and has a deficiency of zero, each positive steady state satisfies $\frac{\bar{\mathbf{x}}^{y^i_k}}{\bar{\mathbf{x}}^{y^j_\kappa}} = \frac{K_i}{K_j}$, for i and j in the same linkage class. By hypothesis, we have $\frac{\bar{\mathbf{x}}^c}{\bar{\mathbf{x}}^{c'}} = \prod_{\theta=1}^{l} \prod_{i,j\in\mathcal{L}_\theta} \left(\frac{\bar{\mathbf{x}}^{y^i_k}}{\bar{\mathbf{x}}^{y^j_\kappa}}\right)^{\beta_{ij}} = \alpha$, where $\alpha = \prod_{\theta=1}^{l} \prod_{i,j\in\mathcal{L}_\theta} \left(\frac{K_i}{K_j}\right)^{\beta_{ij}}$ does not depend on $\bar{\mathbf{x}}$. This concludes the proof. □

Proposition 9.2.6. *Under the hypothesis of Proposition 9.2.5, for any generalised mass action system on $\mathcal{G}_\gamma$, each positive steady state $\mathbf{x}$ satisfies $\frac{\mathbf{x}^c}{\mathbf{x}^{c'}} = \alpha$.*

Proof. Since the network has deficiency zero, for each positive steady state $\mathbf{x}$ the vector $\mathbf{x}^{\mathbf{Y}_k}$ is in the kernel of $\mathbf{A}_\kappa$. In particular, for i, j in the same linkage class we have $\frac{\mathbf{x}^{y^i_\kappa}}{\mathbf{x}^{y^j_\kappa}} = \frac{K_i}{K_j}$. The conclusion follows. □

The consequence of propositions 9.2.5 and 9.2.6 in terms of resolvability of conflicts is the following: if a conflict exists at node i, and we can prove the robustness of the complexes $z(i,j)$, $j \in J_i$, using the condition described in Proposition 9.2.5, then we can conclude that the original mass action system and the generalised mass action system on the resolved network have the same positive steady states, using propositions 9.2.1 and 9.2.3. We summarise the result in the following theorem.

Theorem 9.2.7. *Consider a mass action system $\mathcal{M}$ on a chemical reaction network $\mathcal{N}$, with a weakly reversible deficiency zero kinetic translation graph $\mathcal{G} = (\mathcal{C}, \mathcal{R}, y, z)$, and resolved version $\mathcal{G}_\gamma = (\mathcal{C}, \mathcal{R}, y, y_\kappa)$ with set of improperly translated reactions $\mathcal{R}_I$. Suppose that, for all $i \in \mathcal{C}$ and $j \in J_i$, the complexes $z(i,j), z(i,\gamma(i))$ verify $z(i,j) - z(i,\gamma(i)) = \sum_{\theta=1}^{l} \sum_{h,k \in \mathcal{L}_\theta} \beta_{hk}(y_\kappa^h - y_\kappa^k)$, and that the expression $\alpha_{ij} = \prod_{\theta=1}^{l} \prod_{h,k \in \mathcal{L}_\theta} \left(\frac{K_h}{K_k}\right)^{\beta_{hk}}$ does not depend on the kinetic rates of the reactions in $\mathcal{R}_I$. Consider the assignment κ' of kinetic parameters on $\mathcal{G}_\gamma$ defined as $\kappa'(i,j) = \alpha_{ij}\kappa_{ij}$ for $j \in J_i$ defining a generalised mass action system $\widetilde{\mathcal{M}}$ on $\mathcal{G}_\gamma$. Then $\mathcal{M}$ and $\widetilde{\mathcal{M}}$ have the same positive steady states.*

We conclude the section with some examples.

Example 9.2.8. Reconsider the network in Examples 9.2.2 and 9.2.4. Consider first the generalised reaction network in (108). In this case, the difference $B - D$ can be written as sum of differences of kinetic complexes in the same linkage class $(B - \emptyset) + ((A + D) - A)$. This guarantees the robustness of the ratio $\frac{x_B}{x_D}$ in the original mass action system and the system on the resolved network.

In case of the generalised network in (107) instead, it is not possible to write $B - D$ as sum of differences of kinetic complexes in the same linkage class, so propositions 9.2.5 and 9.2.6 do not apply.

Example 9.2.9. Reconsider the deficiency 2 network considered in Example 7.2.4, with an assignment of rate constants κ defining a mass action system $\mathcal{M}$. Using the translation scheme proposed in [Joh14]

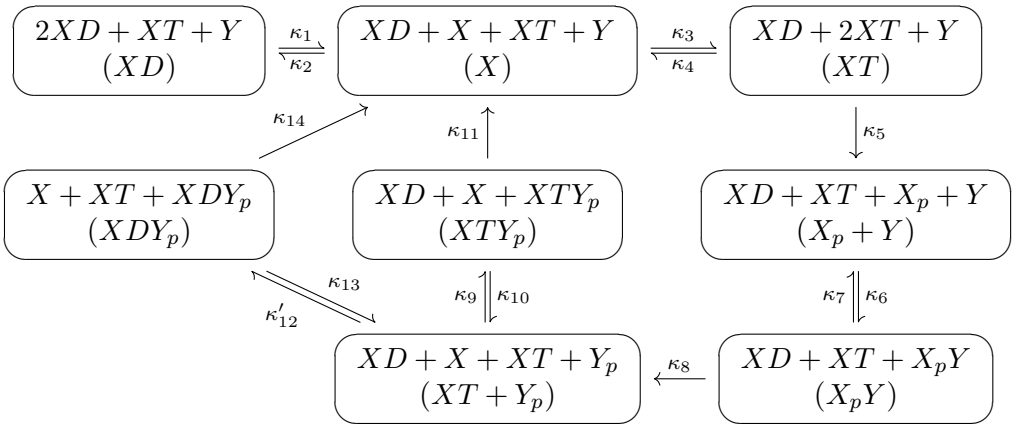
$$\begin{aligned}
&XD \underset{r_2}{\overset{r_1}{\rightleftharpoons}} X \underset{r_4}{\overset{r_3}{\rightleftharpoons}} XT \xrightarrow{r_5} X_p, && (+XD + XT + Y),\\
&X_p + Y \underset{r_7}{\overset{r_6}{\rightleftharpoons}} X_pY \xrightarrow{r_8} X + Y_p, && (+XD + XT),\\
&XT + Y_p \underset{r_{10}}{\overset{r_9}{\rightleftharpoons}} XTY_p \xrightarrow{r_{11}} XT + Y, && (+XD + X),\\
&XD + Y_p \underset{r_{13}}{\overset{r_{12}}{\rightleftharpoons}} XDY_p \xrightarrow{r_{14}} XD + Y, && (+X + XT),
\end{aligned} \tag{109}$$

one obtains the kinetic graph

$$\begin{array}{ccccc}
2XD + XT + Y & \underset{\kappa_2 x_X}{\overset{\kappa_1 x_{XD}}{\rightleftharpoons}} & XD + X + XT + Y & \underset{\kappa_4 x_{XT}}{\overset{\kappa_3 x_X}{\rightleftharpoons}} & XD + 2XT + Y \\
 & \overset{\kappa_{14}x_{XDY_p}}{\nearrow} \quad \underset{\kappa_{11}x_{XTY_p}}{\uparrow} & & & \downarrow \kappa_5 x_{XT} \\
X + XT + XDY_p & & XD + X + XTY_p & & XD + XT + X_p + Y \\
 & \underset{\kappa_{12}x_{XD}x_{Y_p}}{\swarrow} \quad \overset{\kappa_{13}x_{XDY_p}}{\searrow} & & & \kappa_7 x_{X_pY} \uparrow \quad \downarrow \kappa_6 x_{X_p}x_Y \\
 & & XD + X + XT + Y_p & \xleftarrow{\kappa_8 x_{X_pY}} & XD + XT + X_pY
\end{array} \tag{110}$$

The graph has deficiency zero and has a conflict at $XD + X + XT + Y_p$. To find a weakly reversible generalised chemical reaction network with positive steady states that coincide with the positive steady states of $\mathcal{M}$ using Theorem 9.2.7, we choose an assignment of

kinetic complex for the node with stoichiometric complex $XD + X + XT + Y_p$, say $XT + Y_p$, and define the generalised mass action network



(111)

Reaction r_{12} is the only improperly translated reaction. To apply Theorem 9.2.7, we observe that the difference between the kinetic complexes at the node with a conflict $XD + Y_p$ and $XT + Y_p$ coincides with the difference between two kinetic complexes XD and XT belonging to the same linkage class. Hence we just need to calculate the tree constants associated to $2XD + XT + Y(XD)$ and $XD + 2XT + Y(XT)$ and check that their ratio does not depend on the kinetic parameter of the improperly translated reaction κ'_{12}. This is indeed the case: even though the tree constants depend on κ'_{12}, their ratio is given by

$$\alpha = \frac{\kappa_2(\kappa_4+\kappa_5)\kappa_6\kappa_8\big(\kappa_9\kappa_{11}(\kappa_{13}+\kappa_{14}) + (\kappa_{10}+\kappa_{11})\kappa'_{12}\kappa_{14}\big)}{\kappa_1\kappa_3\kappa_6\kappa_8\big(\kappa_9\kappa_{11}(\kappa_{13}+\kappa_{14}) + (\kappa_{10}+\kappa_{11})\kappa'_{12}\kappa_{14}\big)} = \frac{\kappa_2(\kappa_4+\kappa_5)}{\kappa_1\kappa_3}. \tag{112}$$

We can therefore define $\kappa'_{12} = \alpha\kappa_{12}$ and obtain a generalised mass action system with the same positive steady states as $\mathcal{M}$.

Since the network in (110) has deficiency and kinetic deficiency equal to zero, we conclude by Theorem 7.4.3 that all the positive steady states of $\widetilde{\mathcal{M}}$ are complex balancing. As a consequence, $\mathcal{M}$ has positive binomial steady states. In particular, the kinetic complexes XT and $XT + X$ have a robust ratio, thus confirming that the mass action system $\mathcal{M}$ has ACR in species X. We can also determine the value of X at positive steady states by dividing the tree constants associated to $XD + X + XT + Y_p(XT + Y_p)$ and $XD + 2XT + Y(XT)$, finding

$$\begin{aligned} x_{Y_p} &= \frac{\kappa_5(\kappa_{10}+\kappa_{11})(\kappa_{13}+\kappa_{14})}{\kappa_9\kappa_{11}(\kappa_{13}+\kappa_{14}) + (\kappa_{10}+\kappa_{11})\kappa'_{12}\kappa_{14}} \\ &= \frac{\kappa_1\kappa_3\kappa_5(\kappa_{10}+\kappa_{11})(\kappa_{13}+\kappa_{14})}{\kappa_1\kappa_3\kappa_9\kappa_{11}(\kappa_{13}+\kappa_{14}) + \kappa_2(\kappa_4+\kappa_5)(\kappa_{10}+\kappa_{11})\kappa_{12}\kappa_{14}}. \end{aligned}$$

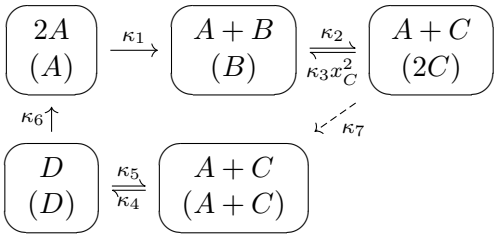
Example 9.2.10. Consider the following network

$$\begin{aligned} A &\xrightarrow{r_1} B \xrightarrow{r_2} C, \\ 2C &\xrightarrow{r_3} B + C, \\ A + C &\underset{r_5}{\overset{r_4}{\rightleftharpoons}} D \xrightarrow{r_6} 2A. \end{aligned} \tag{113}$$

with an assignment of kinetic parameters κ. The network is not weakly reversible and has deficiency two. One can identify (for instance using the algorithm described in Section 10.2) the following dynamically equivalent kinetic graph:

$$\begin{array}{ccc} 2A & \xrightarrow{\kappa_1 x_A} & A+B \\ {\scriptstyle \kappa_6 x_D}\uparrow & & \uparrow{\scriptstyle \kappa_3 x_C^2} \\ D & \underset{\kappa_4 x_A x_C}{\overset{\kappa_5 x_D}{\rightleftharpoons}} & A+C \end{array} \quad {\scriptstyle \kappa_2 x_B} \tag{114}$$

The kinetic graph has deficiency zero, but has a conflict at $A+C$. We can define therefore a dynamically equivalent deficiency one generalised mass action network network as follows:



(115)

Consider the network without the dashed arrow. Since the network has deficiency one, we can apply Theorem 7.5.7 and conclude that every mass action system on (113) has a robust ratio in each pair of non-terminal kinetic complexes $\{A+C, D, A\}$. In particular, the network has ACR in species C. To calculate the value taken by x_C at each positive steady state, we can consider a resolved version of the kinetic graph in (114), or we can use the network in (115) directly. In both cases, we find the tree constants associated to $A+C(A+C)$ and $2A(A)$ and divide them, finding $x_C = \frac{\kappa_1(\kappa_5+\kappa_6)}{\kappa_4\kappa_6}$. In addition, the network in (115) with the dashed arrow is weakly reversible, and has kinetic deficiency zero. We conclude using Theorem 7.4.3 that every mass action system on (115) admits some complex balancing steady states.

In this chapter, we discussed an approach to the identification of deficiency zero realisations for some classes of networks, which relies on an intermediate structure which we called *kinetic graph*. We have seen that in some cases where the kinetic graph does not directly identify a deficiency zero structure, some conclusions can still be drawn by identification of robust ratios. In Chapter 10 we will present further methods for constructing generalised mass action realisations.

10

FROM MASS ACTION TO WEAKLY REVERSIBLE GENERALISED MASS ACTION

A general goal in chemical reaction network theory is the identification of properties of the dynamics of reaction networks from characteristics of the network structures. Dynamically equivalent networks, i.e., networks that admit the same associated system of ODEs (also called *realisations* of the system of ODEs) can exhibit different structural properties, such as different reversibility properties or different deficiency. The problem of the identification of realisations of a given system of ODEs is also known as the *inverse problem* of chemical kinetics, and has been first considered in [HT81] for systems of polynomial equations. Given a system of polynomial ODEs, a simple condition on the signs of the monomials establishes whether the system is "kinetic", i.e. whether it is the system of ODEs associated to some chemical reaction system with mass action. Specifically, the dynamics can be realised by a mass action kinetic system if each monomial with a negative coefficient in the derivative of x_i contains a power of x_i. In [HT81], the authors describe a "canonic mechanism" associated to a system of kinetic polynomial differential equations that can be defined independently of the kinetic parameters. No particular structural properties are guaranteed for the network.

Methods for the identification of mass action realisations with given structural properties have been later proposed. In [Sze10, SHP11], realisations with the minimal or maximal number of reactions or complexes are computed using optimisation problems. Constraints expressing the reversibility of the realisations are also considered. Constraints that ensure weak reversibility, linear conjugacy, minimal deficiency or the conditions for the application of the Deficiency One Theorem are studied in [SHT12, JSS12, JSS13, LSH14, Joh16]. Most of the optimisation problems considered in these works rely on a choice for the rate constants. In [GHBS15], the authors consider more general kinetics, and present a generalisation of the construction in [HT81] for the identification of a reaction network from a system of rational ODEs.

In Chapter 7, we summarised some chemical reaction network theory results that hold for weakly reversible generalised mass action systems, and we showed how some notable mass action networks admit a weakly reversible generalised mass action realisation that allow for kinetic-independent results to be applied. These results motivate the search for more systematic methods for the identification of weakly reversible generalised mass action realisations with low deficiency or kinetic deficiency.

The flexibility of the definition of generalised mass action allows for the existence of many weakly reversible realisations for a given mass action system. In this chapter, we start by giving some examples of constructions of weakly reversible generalised mass action realisations that do not require the knowledge of the kinetic parameters. These methods, however, do not provide us with the ability to control the deficiency of the network. We then present an optimisation problem that aims at the identification of deficiency zero translations of chemical reaction networks. The method is based on the knowledge of the network elementary modes, and realises the objective first sketched in [Joh14, Section 5.1]. We conclude the chapter by considering the application of

the algorithm and of the results of Chapter 9 to two network models of the MAPK signalling cascade. The analysis allows to compare the robustness properties of the complex balancing steady states of the two models.

10.1 GENERALISED MASS ACTION REALISATIONS

Reversible generalised mass action realisations

Any mass action system $\mathcal{M} = (\mathcal{C}, \mathcal{R}, y, \kappa)$ admits a *reversible* generalised mass action realization with the original complexes as stoichiometric and kinetic complexes. Write κ' for an assignment of kinetic parameters on $\mathcal{R}$. For each reaction (i, j) in $\mathcal{R}$ with $(j, i) \notin \mathcal{R}$ consider the pair of reactions

$$\boxed{\begin{matrix} y^i \\ (y^i) \end{matrix}} \underset{\kappa'_{ij}}{\overset{\kappa_{ij}+\kappa'_{ij}}{\rightleftarrows}} \boxed{\begin{matrix} y^j \\ (y^i) \end{matrix}}.$$

These two reactions give the same contribution to the dynamics (59) as the reaction $y^i \xrightarrow{\kappa_{ij}} y^j$.

Example 10.1.1. We can write the network in (55) as reversible generalised mass action network as

$$\boxed{\begin{matrix} A+B \\ (A+B) \end{matrix}} \underset{\kappa'_1}{\overset{\kappa_1+\kappa'_1}{\rightleftarrows}} \boxed{\begin{matrix} 2B \\ (A+B) \end{matrix}}$$
$$\boxed{\begin{matrix} B \\ (B) \end{matrix}} \underset{\kappa'_2}{\overset{\kappa_2+\kappa'_2}{\rightleftarrows}} \boxed{\begin{matrix} A \\ (B) \end{matrix}}.$$

This network has deficiency 1 and kinetic deficiency 2.

Reversible realisations with species and empty complex

Given a system of polynomial equations on $x_1, \ldots, x_n$, it is always possible to create a reversible generalised mass action realization with stoichiometric complexes the set $\{\emptyset, X_1, \ldots, X_n\}$. Suppose that the derivative of the concentration x_h of species X_h is given by the polynomial

$$\sum_{i=1}^{k} \alpha_i \mathbf{x}^{y^i} - \sum_{j=1}^{l} \beta_j \mathbf{x}^{z^j}.$$

Then, for each monomial with positive coefficient we can consider a reaction of the form

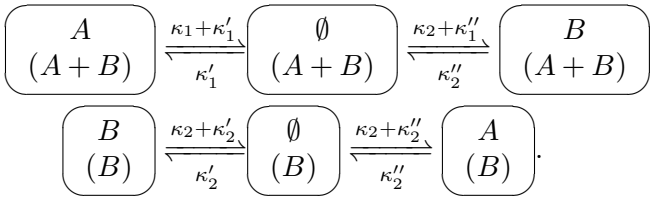
$$\boxed{\begin{matrix} \emptyset \\ (y^i) \end{matrix}} \underset{\alpha'_i}{\overset{\alpha_i+\alpha'_i}{\rightleftarrows}} \boxed{\begin{matrix} X_h \\ (y^i) \end{matrix}},$$

for some $\alpha'_i > 0$, and for each monomial with negative coefficient a reaction of the form

$$\boxed{\begin{matrix} X_h \\ (z^j) \end{matrix}} \underset{\beta'_j}{\overset{\beta_j+\beta'_j}{\rightleftarrows}} \boxed{\begin{matrix} \emptyset \\ (z^j) \end{matrix}},$$

for some $\beta'_j > 0$.

Example 10.1.2. For the network in (55), one can construct the reversible network



This network has deficiency 2 and kinetic deficiency 4.

Weakly reversible translations

In this section we show how a weakly reversible generalised mass action realisation of a chemical reaction network can be defined using translations of reactions and rescaling of reaction vectors.

Consider a mass action system $\mathcal{M} = (\mathcal{C}, \mathcal{R}, y, \kappa)$, and suppose that $\{w^1, \dots, w^d\} \subset \mathbb{R}^m_{\geq 0}$ is a set of fluxes for the network that satisfies $\cup_{h=1}^d \text{supp}(w^h) = \{1, \dots, m\}$. We can use for instance a generating set of elementary modes, or a single flux vector with support on all the reactions. Denote by $\mathbf{Y}$ the complex matrix of $\mathcal{M}$.

For each reaction r_k, we will consider one reaction for each steady state flux it appears in. For convenience, we denote these reactions $r_k^1, \dots, r_k^d$, for $k = 1, \dots, m$. However, the reactions r_k^h corresponding to $w_k^h = 0$ will be discarded.

- First, for each reaction $r_k : y^i \to y^j$, we define reactions $r_k^1, \dots, r_k^d$ by setting the stoichiometric reactant of r_k^h to $w_k^h y^i$, the product to $w_k^h y^j$ and the kinetic reactant to y^i.

- For each $h = 1, \dots, d$, since $0 = \sum_{k=1}^m (y^j - y^i) w_k^h = \sum_{k=1}^m (w_k^h y^j - w_k^h y^i)$, we can apply Lemma 9.1.10 to find a translation scheme T^h for the reactions $r_1^h, \dots, r_m^h$, so that they form a cycle.

- Finally, for each $k = 1, \dots, m$ we choose an index θ_k such that $w_k^{\theta_k} \neq 0$. We assign to each reaction r_k^h with $h \neq i$ a new kinetic parameter, that we denote κ_k^h, and to r_k^i the kinetic parameter of $(\kappa_k - \sum_{h \neq \theta_k} w_k^h \kappa_k^h)/w_k^{\theta_k}$.

All reactions in the resulting generalised mass action system $\widetilde{\mathcal{M}}$ belong to a cycle, i.e. the network is weakly reversible. In addition, since

$$\left(\sum_{\substack{h=1,\dots,d \\ h \neq \theta_k}} \kappa_k^h w_k^h + \frac{1}{w_k^{\theta_k}} (\kappa_k - \sum_{\substack{h=1,\dots,d \\ h \neq \theta_k}} w_k^h \kappa_k^h) w_k^{\theta_k} \right) (y^j - y^i) \mathbf{x}^{y^i} = \kappa_k (y^j - y^i) \mathbf{x}^{y^i},$$

the generalised mass action system $\widetilde{\mathcal{M}}$ is dynamically equivalent to $\mathcal{M}$. Observe that we can take $\mathbf{Y}$ to be the kinetic complex matrix of $\widetilde{\mathcal{M}}$, and that two kinetic complexes are connected in the underlying graph if and only if they are reactant complexes for two reactions that are in the support of a common flux.

Each tree constant is the product of all kinetic parameters of reactions in the cycle, except one. If $d = 1$, then the deficiency of the network equals the number of reactions minus the rank of the network minus one, whereas the kinetic deficiency equals the number of reactions minus one, minus the rank of the subspace generated by the differences between all reactant complexes, and the δ_κ conditions for existence of complex balancing steady states are binomial in the $\kappa' s$.

Example 10.1.3. Consider the mass action system

$$A + B \xrightarrow{\kappa_1} 2B, \qquad 2A \xrightarrow{\kappa_2} 2B, \qquad B \xrightarrow{\kappa_3} A. \tag{116}$$

which admits the generating set of elementary modes $\{(1,0,1)^t, (0,1,2)^t\}$. Following the construction above, we find the following weakly reversible generalised mass action system:

$$\boxed{\begin{matrix} A+B \\ (A+B) \end{matrix}} \underset{\kappa_3^1}{\overset{\kappa_1}{\rightleftharpoons}} \boxed{\begin{matrix} 2B \\ (B) \end{matrix}} \underset{\kappa_2}{\overset{\frac{\kappa_3 - \kappa_3^1}{2}}{\rightleftharpoons}} \boxed{\begin{matrix} 2A \\ (2A) \end{matrix}}.$$

Example 10.1.4. Reconsider the Envz-Ompr network in examples 7.2.3 and 9.1.16. The generalised mass action system identified using the procedure above on the elementary modes is

$$\begin{array}{ccccc} \boxed{\begin{matrix} X+Y+XT \\ (X) \end{matrix}} & \xrightarrow{\kappa_1^1} & \boxed{\begin{matrix} 2XT+Y \\ (XT) \end{matrix}} & \xrightarrow{\kappa_3} & \boxed{\begin{matrix} X_p+Y+XT \\ (X_p+Y) \end{matrix}} \\ {\scriptstyle \kappa_9}\uparrow & & & & \downarrow{\scriptstyle \kappa_4^1} \\ \boxed{\begin{matrix} XTY_p+X \\ (XTY_p) \end{matrix}} & \xleftarrow{\kappa_7^1} & \boxed{\begin{matrix} X+XT+Y_p \\ (XT+Y_p) \end{matrix}} & \xleftarrow{\kappa_6} & \boxed{\begin{matrix} X_pY+XT \\ (X_pY) \end{matrix}} \end{array} \qquad \begin{array}{l} X \underset{\kappa_2}{\overset{\kappa_1 - \kappa_1^1}{\rightleftharpoons}} XT \\ X_p + Y \underset{\kappa_5}{\overset{\kappa_4 - \kappa_4^1}{\rightleftharpoons}} X_pY \\ XT + Y_p \underset{\kappa_8}{\overset{\kappa_7 - \kappa_7^1}{\rightleftharpoons}} XTY_p \end{array}$$

We can use Theorem 7.4.3 to find conditions for the existence of complex balancing steady states for this system. We find that the parameters $\kappa_1^1, \kappa_4^1, \kappa_7^1$, can always be chosen so that the system admits complex balancing steady states ($\kappa_1^1, \kappa_4^1, \kappa_7^1$ should be set to $\frac{\kappa_1\kappa_3}{\kappa_2+\kappa_3}$, $\frac{\kappa_4\kappa_6}{\kappa_5+\kappa_6}$, and $\frac{\kappa_7\kappa_9}{\kappa_8+\kappa_9}$ respectively).

10.2 MILP FRAMEWORK FOR NETWORK TRANSLATION

In this section we describe an algorithm for the identification of translations of reaction networks. Given a chemical reaction network $\mathcal{N}$ with m reactions, the goal of the algorithm is to identify translation complexes $\upsilon^1, \ldots, \upsilon^m \in \mathbb{Z}^m$, which define a translated kinetic graph which has as low of a stoichiometric deficiency as possible. The observation behind the method, made in [Joh14, Remark 11], can be restated as follows. If the deficiency of a network is zero, then every elementary mode is a cycle. If instead the network has strictly positive deficiency, then it may possess a stoichiometric elementary mode. To find a network with lower deficiency, we therefore attempt to convert stoichiometric elementary modes in the original network into cyclic elementary modes in the translation.

The method described in this section applies to network that satisfy the following hypotheses: the network admit a generating set of elementary modes $\{w^1, \ldots, w^d\}$ such that $w_i^h = 1$ for all $h = 1, \ldots, d$, $i = 1, \ldots, m$ such that $w_i^h \neq 0$. Under this hypothesis, Lemma 9.1.10 suggests a technique for identifying the translation complexes υ^1, …, υ^m as solutions of a system of linear equations. Specifically, suppose that the indices $\{j_1, \ldots, j_k\} \subseteq \{1, \ldots, m\}$ identify a stoichiometric elementary mode, and that $\hat{\upsilon}^{j_1}, \ldots, \hat{\upsilon}^{j_k}$ are translation complexes specific to this stoichiometric elementary mode such that the reactions $r_{j_1} + \hat{\upsilon}^{j_1}, \ldots, r_{j_k} + \hat{\upsilon}^{j_k}$ form a cycle by Lemma 9.1.10. Then, to guarantee that $r_{j_1}, \ldots, r_{j_k}$ are translated to this cycle, we impose that

$$\upsilon_i^{j_1} - \hat{\upsilon}_i^{j_1} = \upsilon_i^{j_2} - \hat{\upsilon}_i^{j_2} = \cdots = \upsilon_i^{j_k} - \hat{\upsilon}_i^{j_k}, \text{ for } i = 1, \ldots, n. \tag{117}$$

The constraint set (117) only imposes that reactions in the stoichiometric elementary mode identified by $r_{j_1}, \ldots, r_{j_k}$, are translated to a cycle. In general, we will have multiple

stoichiometric elementary modes, each with their set of translated complexes $\hat{v}_{j_1}, \dots, \hat{v}_{j_k}$, and it might not be possible to satisfy the constraint (117) simultaneously for all such elementary stoichiometric modes.

The optimisation problem described in the following can be summarised as follows:

- Reactions in a cycle in $\mathcal{N}$ are translated by the same translation complex (Cycle).
- The number of stoichiometric elementary modes that are translated to cycles is maximized (Obj).
- Optionally, permutations of the reactions in the support of a stoichiometric mode can be considered (Perm1), (Perm2), (Perm3), (Perm4).
- We can optionally require that reactions with a common source complex in $\mathcal{N}$ are translated by the same translation complex (React) (this allows to define a translation in the sense of [Joh14, Joh15, TJ17], see Remark 9.1.7).
- Optionally, we can require that each stoichiometric complex is assigned with only one kinetic complex; in the terminology introduced in [Joh14], the translation is proper (Proper1), (Proper2).

In addition to the elements above, we make the following additional considerations:

- For networks that admit a direct decomposition in subnetworks, we can apply the program separately to each subnetwork. If we implement the constraints (React), we need first to merge subnetworks with common complexes.
- Each subnetwork can be translated by additional arbitrary translation complexes, without changing the deficiency or the associated system of ODEs. This can be used to avoid conflicts between nodes.

In the remainder of the chapter, we describe each of the points above in detail. We first need to fix some notations. We define $\mathbb{B} = \{0, 1\}$, and denote by $\mathbf{I}^+$ and $\mathbf{I}^-$ the positive and negative part of the incidence matrix, respectively, and set $\mathbf{\Gamma}^+ = \mathbf{Y}\mathbf{I}^+$, $\mathbf{\Gamma}^- = \mathbf{Y}\mathbf{I}^- \in \mathbb{N}^{n\times m}$. In other words, the columns of $\mathbf{\Gamma}^+$ and $\mathbf{\Gamma}^-$ contain the stoichiometric coefficients of the product and reactant complexes of the network, respectively. Given α cycles and β stoichiometric elementary modes, we denote by $I^h \subseteq \{1, \dots, m\}$, $h = 1, \dots, \alpha$, the indices of reactions in the cyclic elementary modes, and by $J^h \subseteq \{1, \dots, m\}$, $h = 1, \dots, \beta$, the indices of reactions in the stoichiometric elementary modes.

Translation complexes are well defined: We define a matrix of decision variables $\mathbf{\Upsilon} \in \mathbb{R}^{n\times m}$ where the j^{th} column of $\mathbf{\Upsilon}$ corresponds to the translation complex v^j of the reaction r_j, i.e. $\mathbf{\Upsilon}_{\cdot j} = v^j$. We need to make sure that the reactions in the translated graph are well-defined, i.e., that all complexes have positive stoichiometric coefficients. We can optionally require that all the translation complexes are positive, by imposing

$$\mathbf{\Upsilon}_{ij} \geq 0, i = 1, \dots, n, j = 1, \dots, m. \tag{PosT}$$

If we do not impose that the translation complexes have positive coefficients, we need to guarantee that all the coefficients of the complexes resulting from the translations are positive. To this end, we require

$$\mathbf{\Upsilon}_{ij} + \mathbf{\Gamma}^-_{ij} \geq 0, \mathbf{\Upsilon}_{ij} + \mathbf{\Gamma}^+_{ij} \geq 0, i = 1, \dots, n, j = 1, \dots, m. \tag{PosC}$$

To define a translation according to the definition in [Joh14, Joh15, TJ17], since each complex in the original network can be translated to only one stoichiometric complex in

the translated network, any two reactions r_{j_1} and r_{j_2} that have the same reactant need to be translated by the same translation complex:

$$\mathbf{\Gamma}^-_{ij_1} = \mathbf{\Gamma}^-_{ij_2} \text{ for all } i = 1, \dots, n \Rightarrow \mathbf{\Upsilon}_{ij_1} = \mathbf{\Upsilon}_{ij_2} \text{ for all } i = 1, \dots, n. \tag{React}$$

Stoichiometric elementary modes translated to cycles: Suppose now that the reactions $r_{j_1}, \dots, r_{j_k}$ define a cycle. To ensure that these reactions form a cycle in the translated network as well, we impose

$$\mathbf{\Upsilon}_{ij_1} = \mathbf{\Upsilon}_{ij_2} = \cdots = \mathbf{\Upsilon}_{ij_k}, \ i = 1, \dots, n. \tag{Cycle}$$

Consider now the stoichiometric elementary modes. Suppose that the reactions $r_{j_1}, \dots, r_{j_k}$ define the h^{th} stoichiometric elementary mode, and $\hat{v}^{j_1}, \dots, \hat{v}^{j_k}$ are complexes such that the reactions $r_{j_1} + \hat{v}^{j_1}, \dots, r_{j_k} + \hat{v}^{j_k}$ define a cycle by Lemma 9.1.10, for a fixed order of the reactions. We denote by $\widehat{\mathbf{\Upsilon}}^h \in \mathbb{Z}^{n \times |J^h|}$ the matrix with columns the complexes $\hat{v}^{j_1}, \dots, \hat{v}^{j_k}$. Write j' for the position of the index j in J^h, i.e., $j = J^h_{j'}$. Then to guarantee that $r_{j_1}, \dots, r_{j_k}$ are translated to the identified cycle we impose

$$\mathbf{\Upsilon}_{ij_1} - \widehat{\mathbf{\Upsilon}}^h_{ij'_1} = \mathbf{\Upsilon}_{ij_2} - \widehat{\mathbf{\Upsilon}}^h_{ij'_2} = \cdots = \mathbf{\Upsilon}_{ij_k} - \widehat{\mathbf{\Upsilon}}^h_{ij'_k}, \text{ for } h = 1, \dots, \beta, i = 1, \dots, n. \tag{Stoich}$$

For each stoichiometric elementary mode we have therefore a set of constraints of the form (Stoich). These constraints might not be satisfiable at the same time for all stoichiometric elementary modes; hence we want to maximize the number of stoichiometric elementary modes for which these constraints are verified. We do so by introducing additional variables.

Minimize stoichiometric deficiency of translated network: For the h^{th} stoichiometric elementary mode, we introduce a binary variable σ_h that will be equal to 1 if the elementary mode is not translated to a cycle. The restrictions on σ_h are obtained by imposing, for each pair of indices $j_1, j_2 \in J^h$ and for each species X_i the constraint

$$\sigma_h \geq \varepsilon(\mathbf{\Upsilon}_{ij_1} - \widehat{\mathbf{\Upsilon}}^h_{ij'_1} - \mathbf{\Upsilon}_{ij_2} + \widehat{\mathbf{\Upsilon}}^h_{ij'_2}). \tag{Count}$$

Notice that (Count) reduces to (Stoich) if $\sigma_h = 0$. In order to maximize the number of stoichiometric modes which are translated to cycles, we introduce the following objective function:

$$\text{minimize} \ \sum_{h=1}^{\beta} \sigma_h. \tag{Obj}$$

Permutations of stoichiometric modes (optional): As observed in Remark 9.1.12, we can identify a possible cycle for each possible order of the reactions involved in the elementary mode. In general, the existence of a solution can depend on the choice of the order. The following example illustrates this point.

Example 10.2.1. Consider the chemical reaction network

$$\begin{array}{c} A \xrightarrow{r_1} B \xrightarrow{r_2} C, \\ \quad \nwarrow^{r_4} \quad \swarrow_{r_3} \\ \quad\quad D \end{array} \qquad 2B \xrightarrow{r_5} E \xrightarrow{r_6} B + C.$$

The reactions $\{r_1, r_2, r_3, r_4\}$ form a cycle, while the reactions $\{r_1, r_3, r_4, r_5, r_6\}$ identify a stoichiometric elementary mode. The constraints (Cycle) impose that the reactions in the cycle have the same translation:

$$\mathbf{\Upsilon}_{i1} = \mathbf{\Upsilon}_{i2} = \mathbf{\Upsilon}_{i3} = \mathbf{\Upsilon}_{i4}, \text{ for all } i = 1, \dots, 5. \tag{118}$$

The translations that convert the stoichiometric elementary mode to a cycle, when the order above is considered, are $\upsilon^1 = B + C$, $\upsilon^3 = 2B$, $\upsilon^4 = 2B$, $\upsilon^5 = A$ and $\upsilon^6 = A$. Therefore the constraints in (Stoich) include

$$\boldsymbol{\Upsilon}_{21} = \boldsymbol{\Upsilon}_{23} - 1. \tag{119}$$

The system of equations given by (118) and (119) does not admit any solution; however, the translation scheme defined by $T(r_1) = T(r_2) = T(r_3) = T(r_4) = B$, $T(r_5) = T(r_6) = \emptyset$, that can be identified using the order $r_1, r_5, r_6, r_2, r_3, r_4$, defines a deficiency zero weakly reversible kinetic translation graph.

We take the order of the reactions into account by introducing variables and constraints to keep track of the possible permutations of the reactions in the elementary mode. In addition, since the order of reactions in the elementary mode is not fixed, instead of calculating the translations that convert the elementary mode to a cycle, we consider the matrices $\widehat{\boldsymbol{\Upsilon}}^h \in \mathbb{Z}^{n\times|J^h|}$ as matrices of decision variables, and impose the constraint (Count) as done previously in the case of elementary modes with a fixed order.

To keep all the possible orders into account, for the h^{th} stoichiometric elementary mode, with set of reaction indices J^h, we define $|J^h| \times |J^h|$ binary variables $\mathbf{P}^h \in \mathbb{B}^{|J^h|\times|J^h|}$, that will identify the position of each reaction in the possible orders: $\mathbf{P}^h_{tj}$ will be equal to 1 if and only if the j^{th} reaction is in position t.

We have therefore the following constraints. To ensure that each reaction is assigned one and only one position, we impose that each row and column of $\mathbf{P}^h$ sums to 1:

$$\sum_{j=1}^{k} \mathbf{P}^h_{tj} = 1 \text{ for all } t = 1, \ldots, |J^h|, \tag{Perm1}$$

$$\sum_{t=1}^{k} \mathbf{P}^h_{tj} = 1 \text{ for all } j = 1, \ldots, |J^h|. \tag{Perm2}$$

Now, suppose that j'_1, j'_2, t and t' are indices in $\{1, \ldots, |J^h|\}$ such that $t' = t + 1 \pmod{|J^h|}$. Write j_1, j_2 for $J^h_{j'_1}$ and $J^h_{j'_2}$, respectively. If $\mathbf{P}^h_{tj'_1}$ and $\mathbf{P}^h_{tj'_2}$ are both equal to 1, then reaction r_{j_1} is followed by reaction r_{j_2} in the cycle. If this is the case, we want to impose that the product of reaction r_{j_1} is equal to the reactant of reaction r_{j_2}. For each pair of indices $J^h_{j'_1}$, $J^h_{j'_2}$ in the elementary mode we therefore add the following constraints, for each species $i = 1, \ldots, n$:

$$-\frac{1}{\varepsilon}(2 - \mathbf{P}^h_{tj'_1} - \mathbf{P}^h_{t'j'_2}) \leq \boldsymbol{\Gamma}^+_{ij_1} - \boldsymbol{\Gamma}^-_{ij_2} + \widehat{\boldsymbol{\Upsilon}}^h_{ij'_1} - \widehat{\boldsymbol{\Upsilon}}^h_{ij'_2} \leq \frac{1}{\varepsilon}(2 - \mathbf{P}^h_{tj'_1} - \mathbf{P}^h_{t'j'_2}),\ j' = j+1 \pmod{|J^h|}. \tag{Perm3}$$

We can also fix the position of one reaction arbitrarily, and set

$$\mathbf{P}^h_{00} = 1 \text{ for all } h = 1, \ldots, \beta. \tag{Perm4}$$

Proper translations (optional): We might sometimes be interested in identifying a proper translation with minimum deficiency. To impose that distinct complexes in the original network are translated to distinct complexes in the translated network, we introduce $n \times m \times m$ binary variables $\mathbf{U}_{ij_1j_2}$. The variable $\mathbf{U}_{ij_1j_2}$ encodes whether the reactant complexes of reactions r_{j_1} and r_{j_2} have been translated to the same complex with respect to species X_i, i.e.

$$|\boldsymbol{\Gamma}^-_{ij_1} + \boldsymbol{\Upsilon}_{ij_1} - \boldsymbol{\Gamma}^-_{ij_2} - \boldsymbol{\Upsilon}_{ij_2}| = 0 \ \Rightarrow\ \mathbf{U}_{ij_1j_2} = 1.$$

This is achieved by introducing additional $n \times m \times m$ auxiliary binary variables $\mathbf{V}_{ij_1j_2}$, and considering the constraints

$$\begin{cases} \mathbf{\Gamma}^-_{ij_1} + \mathbf{\Upsilon}_{ij_1} - \mathbf{\Gamma}^-_{ij_2} - \mathbf{\Upsilon}_{ij_2} \geq \epsilon(1 - \mathbf{U}_{ij_1j_2}) - M\mathbf{V}_{ij_1j_2}, \\ \mathbf{\Gamma}^-_{ij_1} + \mathbf{\Upsilon}_{ij_1} - \mathbf{\Gamma}^-_{ij_2} - \mathbf{\Upsilon}_{ij_2} \leq -\epsilon(1 - \mathbf{U}_{ij_1j_2}) + M(1 - \mathbf{V}_{ij_1j_2}), \end{cases} \tag{Proper1}$$

as can be easily checked by considering the four possible cases. Finally, we have to impose that, if the reactants of reactions r_{j_1} and r_{j_2} differ, then their corresponding translated complexes differ in at least one species. Since the variables $\mathbf{U}_{ij_1j_2}$ count the number of matching species, we impose, for $j_1, j_2 = 1, \ldots, m$

$$\mathbf{\Gamma}^-_{ij_1} \neq \mathbf{\Gamma}^-_{ij_2} \text{ for any } i = 1, \ldots, n \;\Rightarrow \sum_{i=1}^{n} \mathbf{U}_{ij_1j_2} \leq n - 1. \tag{Proper2}$$

The variables $\mathbf{U}_{ij_1j_2}$ and $\mathbf{V}_{ij_1j_2}$ corresponding to reactions r_{j_1} and r_{j_2} with the same reactant can be omitted. The parameters, variables and constraints of the problem are summarized in the following tables.

Parameters

$n \in \mathbb{N}$	number of species
$c \in \mathbb{N}$	number of complexes
$m \in \mathbb{N}$	number of reactions
$\mathbf{\Gamma} \in \mathbb{N}^{n \times m}$	stoichiometric matrix
$\mathbf{\Gamma}^- \in \mathbb{N}^{n \times m}$	stoichiometric coefficients of the reactants
$\mathbf{\Gamma}^+ \in \mathbb{N}^{n \times m}$	stoichiometric coefficients of the products
$\alpha \in \mathbb{N}$	number of cyclic elementary modes
$\beta \in \mathbb{N}$	number of stoichiometric elementary modes
$I^h \subseteq \{1, \ldots, m\}$, $h = 1, \ldots, \alpha$	indices of reactions in cycles
$J^h \subseteq \{1, \ldots, m\}$, $h = 1, \ldots, \beta$	indices of reactions in stoichiometric elementary modes
$M \in \mathbb{R}_{>0}$	$M \gg 1$
$\epsilon \in \mathbb{R}_{>0}$	$0 < \epsilon \ll 1$

Decision Variables

$\mathbf{\Upsilon} \in \mathbb{R}^{n \times m}$	matrix of translation complexes: $\mathbf{\Upsilon}_{ij} = v_i^j$ is the stoichiometric coefficient of species X_i in the translation of the reaction r_j
$\widehat{\mathbf{\Upsilon}}^h \in \mathbb{Z}^{n \times \lvert J^h \rvert}$, $h = 1, \ldots, \beta$	matrix of translation complexes which convert elementary mode h to cycle: $\widehat{\mathbf{\Upsilon}}^h_{ij} = \hat{v}_i^j$ is the stoichiometric coefficient of species X_i in the translation of the j^{th} reaction in the h^{th} elementary mode to a cycle. $\widehat{\mathbf{\Upsilon}}^h$ is a matrix of variables if permutations of reactions are considered, and is otherwise calculated using Lemma 9.1.10.
$\mathbf{P}^h \in \mathbb{B}^{\lvert J^h \rvert \times \lvert J^h \rvert}$, $h = 1, \ldots, \beta$	orders of reactions in elementary mode h: $\mathbf{P}^h_{tj} = 1$ iff the j^{th} reaction in the h^{th} stoichiometric elementary mode is in position t
$\sigma \in \mathbb{B}^{\beta}$	count the elementary modes that are translated to cycles: $\sigma_h = 1$ iff the h^{th} stoichiometric elementary mode is translated to a cycle
$\mathbf{U} \in \mathbb{B}^{n \times m \times m}$	(optional for proper) count the number of matching species in the translation of the reactants: $\mathbf{U}_{ij_1j_2} = 1$ iff the translations of the reactants of reactions r_{j_1} and r_{j_2} are equal in species i
$\mathbf{V} \in \mathbb{B}^{n \times m \times m}$	(optional for proper) auxiliary variables

Objective

minimize $\sum_{h=1}^{\beta} \sigma_h$ Minimize deficiency by minimizing stoichiometric modes (Obj)

Constraints

$$\begin{cases} \boldsymbol{\Upsilon}_{ij} \geq 0,\ i = 1, \ldots, n,\ j = 1, \ldots, m & \text{translations are positive (optional) (PosT)} \\ \boldsymbol{\Upsilon}_{ij} + \boldsymbol{\Gamma}^{-}_{ij} \geq 0,\ \boldsymbol{\Upsilon}_{ij} + \boldsymbol{\Gamma}^{+}_{ij} \geq 0,\ i = 1, \ldots, n,\ j = 1, \ldots, m & \text{all resulting stoichiometric coefficients are positive (PosC)} \end{cases}$$

$$\begin{cases} \boldsymbol{\Gamma}^{-}_{ij_1} = \boldsymbol{\Gamma}^{-}_{ij_2}, \forall i = 1, \ldots, n \Rightarrow \boldsymbol{\Upsilon}_{ij_1} = \boldsymbol{\Upsilon}_{ij_2}, \forall i = 1, \ldots, n, \\ j_1, j_2 = 1, \ldots, m, j_1 \neq j_2 \end{cases} \quad \text{reactions with the same reactant are translated by the same complex (optional) (React)}$$

$$\begin{cases} \boldsymbol{\Upsilon}_{ij_1} = \boldsymbol{\Upsilon}_{ij_2},\ i = 1, \ldots, n, \\ j_1 = I^h_j,\ j_2 = I^h_{j+1},\ j = 1, \ldots, |I^h| - 1, \\ h = 1, \ldots, \alpha \end{cases} \quad \text{cycles are preserved (Cycle)}$$

$$\begin{cases} \begin{cases} \sigma_h \geq \varepsilon(\boldsymbol{\Upsilon}_{ij_1} - \widehat{\boldsymbol{\Upsilon}}^h_{ij'_1} - \boldsymbol{\Upsilon}_{ij_2} + \widehat{\boldsymbol{\Upsilon}}^h_{ij'_2}), \\ i = 1, \ldots, n, j_1 = J^h_{j'_1}, j_2 = J^h_{j'_2}, \\ j'_1 = 1, \ldots, |J^h|, j'_2 = 1, \ldots, |J^h|, j'_1 \neq j'_2 \end{cases} & \sigma_h = 1 \text{ if the } h^{th} \text{ stoichiometric elementary mode is not translated to a cycle (Count)} \\ \sum_{j=1}^{|J^h|} \mathbf{P}^h_{tj} = 1,\ t = 1, \ldots, |J^h| & \text{each index in the order corresponds to only one reaction (Perm1)} \\ \sum_{t=1}^{|J^h|} \mathbf{P}^h_{tj} = 1,\ j = 1, \ldots, |J^h| & \text{each reaction is assigned only one index in the order (Perm2)} \\ \begin{cases} \boldsymbol{\Gamma}^{+}_{ij_1} - \boldsymbol{\Gamma}^{-}_{ij_2} + \widehat{\boldsymbol{\Upsilon}}^h_{ij'_1} - \widehat{\boldsymbol{\Upsilon}}^h_{ij'_2} \leq \frac{1}{\varepsilon}(2 - \mathbf{P}^h_{tj'_1} - \mathbf{P}^h_{t'j'_2}), \\ \boldsymbol{\Gamma}^{+}_{ij_1} - \boldsymbol{\Gamma}^{-}_{ij_2} + \widehat{\boldsymbol{\Upsilon}}^h_{ij'_1} - \widehat{\boldsymbol{\Upsilon}}^h_{ij'_2} \geq -\frac{1}{\varepsilon}(2 - \mathbf{P}^h_{tj'_1} - \mathbf{P}^h_{t'j'_2}), \\ t = 1, \ldots, |J^h|, t' = t + 1 \pmod{|J^h|}, \\ i = 1, \ldots, n, j_1 = J^h_{j'_1}, j_2 = J^h_{j'_2}, \\ j'_1 = 1, \ldots, |J^h|, j'_2 = 1, \ldots, |J^h|, j'_1 \neq j'_2 \end{cases} & \text{the product of the reaction with index } t \text{ is aligned with the reactant of reaction with index } t + 1 \pmod{|J^h|} \text{ (Perm3)} \\ \mathbf{P}^h_{00} = 1 & \text{first reaction is in first position (Perm4)} \\ h = 1, \ldots, \beta \end{cases}$$

$$\begin{cases} \begin{cases} \boldsymbol{\Gamma}^{-}_{ij_1} + \boldsymbol{\Upsilon}_{ij_1} - \boldsymbol{\Gamma}^{-}_{ij_2} - \boldsymbol{\Upsilon}_{ij_2} \geq \epsilon(1 - \mathbf{U}_{ij_1j_2}) - M\mathbf{V}_{ij_1j_2}, \\ \boldsymbol{\Gamma}^{-}_{ij_1} + \boldsymbol{\Upsilon}_{ij_1} - \boldsymbol{\Gamma}^{-}_{ij_2} - \boldsymbol{\Upsilon}_{ij_2} \leq -\epsilon(1 - \mathbf{U}_{ij_1j_2}) + M(1 - \mathbf{V}_{ij_1j_2}), \\ i = 1, \ldots, n \end{cases} & \text{proper translation (optional) (Proper1)} \\ \boldsymbol{\Gamma}^{-}_{ij_1} \neq \boldsymbol{\Gamma}^{-}_{ij_2} \text{ for any } i = 1, \ldots, n \Rightarrow \sum_{i=1}^{n} \mathbf{U}_{ij_1j_2} \leq n - 1 & \text{(Proper2)} \\ j_1, j_2 = 1, \ldots, m. \end{cases}$$

10.3 EXAMPLE: MAPK SIGNALLING CASCADE

We conclude this chapter with the analysis of a network model of the mitogen-activated protein kinase (MAPK) signalling cascade. A schematic of the model, adapted from [SG12], is given in Figure 21a. The species *Sig* represents the external signal that activates the cascade. The kinase *MKKK* is dephosphorylated by the phosphatase $Phos_2$, *MK* is dephosphorylated by $Phos_1$, and *MKK* is dephosphorilated by both. The model we consider is introduced in [SG12] as model M4, where it is studied in two different forms, an "unsequestrated" and a "sequestrated" version. In the latter, the species resulting from the binding of a phosphorylated kinase with its phosphatase, before the release of the unphosphorylated kinase, converts to an intermediate compound consisting of the unphosphorylated kinase and its phosphatases. In [SG12], the authors observe that the sequestration of a phosphatase by its kinase significantly increases the robustness of the output, defined as the variation in concentration of *MK-PP* in response to changes in kinases, phosphatases and signal concentrations. In this section, using the techniques described in this thesis, we rewrite the networks as weakly reversible generalised mass action networks. At the end of the section, we compare the robustness properties of the complex balancing steady states in the two scenarios. We make the analyses under the assumption that the phosphatase concentrations are controlled by input and output reactions.

The reactions in the unsequestrated version of the network are as follows:

$$\begin{aligned}
&X_1 + X_2 \underset{r_{-1}}{\overset{r_1}{\rightleftarrows}} X_3 \xrightarrow{r_2} X_2 + X_4 \underset{r_{-3}}{\overset{r_3}{\rightleftarrows}} X_5 \xrightarrow{r_4} X_2 + X_6,\\
&X_6 + X_{13} \underset{r_{-5}}{\overset{r_5}{\rightleftarrows}} X_{21} \xrightarrow{r_6} X_4 + X_{13} \underset{r_{-7}}{\overset{r_7}{\rightleftarrows}} X_{22} \xrightarrow{r_8} X_1 + X_{13} \underset{r_9}{\overset{r_{-9}}{\rightleftarrows}} X_{23},\\
&X_7 + X_8 \underset{r_{-10}}{\overset{r_{10}}{\rightleftarrows}} X_9 \xrightarrow{r_{11}} X_8 + X_{10} \underset{r_{-12}}{\overset{r_{12}}{\rightleftarrows}} X_{11} \xrightarrow{r_{13}} X_8 + X_2,\\
&X_2 + X_{13} \underset{r_{-14}}{\overset{r_{14}}{\rightleftarrows}} X_{12} \xrightarrow{r_{15}} X_{10} + X_{13} \underset{r_{-16}}{\overset{r_{16}}{\rightleftarrows}} X_{14} \xrightarrow{r_{17}} X_7 + X_{13} \underset{r_{18}}{\overset{r_{-18}}{\rightleftarrows}} X_{15},\\
&X_{16} + X_{18} \underset{r_{-19}}{\overset{r_{19}}{\rightleftarrows}} X_{17} \xrightarrow{r_{20}} X_8 + X_{18},\\
&X_8 + X_{20} \underset{r_{-21}}{\overset{r_{21}}{\rightleftarrows}} X_{19} \xrightarrow{r_{22}} X_{16} + X_{20} \underset{r_{24}}{\overset{r_{-24}}{\rightleftarrows}} X_{27},\\
&X_2 + X_{20} \underset{r_{-23}}{\overset{r_{23}}{\rightleftarrows}} X_{24} \xrightarrow{r_{25}} X_{10} + X_{20} \underset{r_{-26}}{\overset{r_{26}}{\rightleftarrows}} X_{25} \xrightarrow{r_{27}} X_7 + X_{20} \underset{r_{28}}{\overset{r_{-28}}{\rightleftarrows}} X_{26}.
\end{aligned} \tag{120}$$

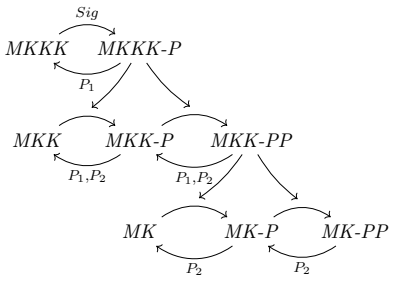
The species have been renamed as shown in Figure 21b. We have used the same labelling of species and reactions provided for the network in the BioModels database [LDR+10] (model 430). To this network, we add two input and output reactions regulating $Phos_1$ and $Phos_2$:

$$X_{20} \underset{r_{-29}}{\overset{r_{29}}{\rightleftarrows}} \emptyset, \quad X_{13} \underset{r_{-30}}{\overset{r_{30}}{\rightleftarrows}} \emptyset. \tag{121}$$

We consider a mass action system on this network, writing κ_i, κ_{-i} for the kinetic rates assigned to reactions r_i, r_{-i}.

We start by partitioning the network in subnetworks, that we can study separately. The reactions in (121) define two subnetworks with deficiency zero; as a consequence, any mass action system on (120) displays ACR in $Phos_1$ and $Phos_2$ (the concentrations at steady state are $\frac{\kappa_{-29}}{\kappa_{29}}$ and $\frac{\kappa_{-30}}{\kappa_{30}}$ respectively). We will use this observation shortly to resolve some conflicts in the translation of the network. The network admits 11 subnetworks, 6 with deficiency zero

$$\{r_9, r_{-9}\}, \quad \{r_{18}, r_{-18}\}, \quad \{r_{24}, r_{-24}\},$$



(a)

$MK = X_1$	$MKK\text{-}P = X_{10}$,	$MKKK\text{-}P\text{-}P_1 = X_{19}$,
$MKK\text{-}PP = X_2$	$MKK\text{-}P\text{-}MKKK\text{-}P = X_{11}$,	$Phos_1 = X_{20}$,
$MK\text{-}MKK\text{-}PP = X_3$	$MKK\text{-}PP\text{-}P_2 = X_{12}$,	$MK\text{-}PP\text{-}P_2 = X_{21}$,
$MK\text{-}P = X_4$	$Phos_2 = X_{13}$,	$MK\text{-}PP_2 = X_{22}$,
$MK\text{-}P\text{-}MKK\text{-}PP = X_5$	$MKK\text{-}P\text{-}P_2 = X_{14}$,	$MK\text{-}P_2 = X_{23}$,
$MK\text{-}PP = X_6$	$MKK\text{-}P_2 = X_{15}$,	$MKK\text{-}PP\text{-}P_1 = X_{24}$,
$MKK = X_7$	$MKKK = X_{16}$,	$MKK\text{-}P\text{-}P_1 = X_{25}$,
$MKKK\text{-}P = X_8$	$MKKK\text{-}Sig = X_{17}$,	$MKK\text{-}P_1 = X_{26}$,
$MKK\text{-}MKKK\text{-}P = X_9$	$Sig = X_{18}$,	$MKKK\text{-}P_1 = X_{27}$.

(b)

Figure 21: (*a*)*: Schematics of the MAPK cascade in network* (120). (*b*)*: Renaming of the species in network* (120).

$$\{r_{28}, r_{-28}\}, \quad \{r_{29}, r_{-29}\}, \{r_{30}, r_{-30}\},$$

3 with deficiency one

$$\{r_1, r_{-1}, r_2, r_7, r_{-7}, r_8\},$$

$$\{r_3, r_{-3}, r_4, r_5, r_{-5}, r_6\},$$

$$\{r_{19}, r_{-19}, r_{20}, r_{21}, r_{-21}, r_{22}\},$$

and 2 the deficiency two subnetworks

$$\{r_{10}, r_{-10}, r_{11}, r_{16}, r_{-16}, r_{17}, r_{26}, r_{-26}, r_{27}\},$$

$$\{r_{12}, r_{-12}, r_{13}, r_{14}, r_{-14}, r_{15}, r_{23}, r_{-23}, r_{25}\}.$$

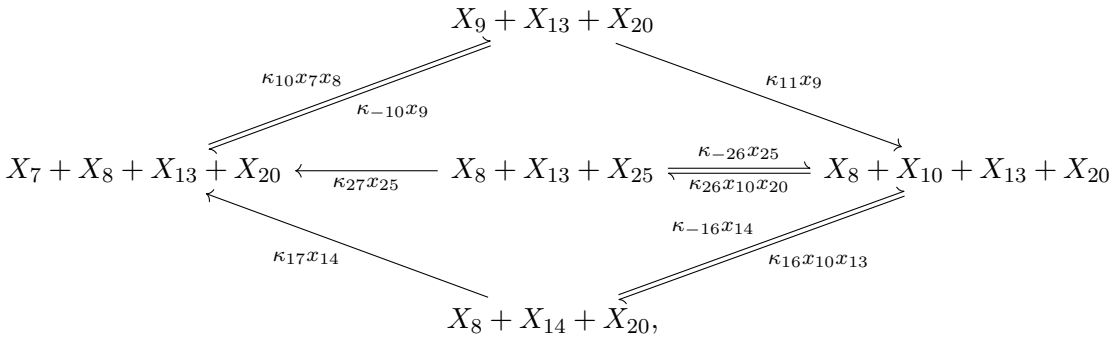
The deficiency one subnetworks fall under the hypothesis of Proposition 9.1.13: they admit only one stoichiometric elementary mode, and some 2-cycles. Using the translation scheme described in Proposition 9.1.10 and Remark 9.1.11, we identify the three weakly reversible generalised mass action networks

$$\begin{array}{ccccccc}
 & & \xleftarrow{\kappa_8} & & & & \\
X_1+X_2+X_{13} & \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftarrows}} & X_3+X_{13} & \xrightarrow{\kappa_2} & X_2+X_4+X_{13} & \underset{\kappa_{-7}}{\overset{\kappa_7}{\rightleftarrows}} & X_2+X_{22} \\
(X_1+X_2) & & (X_3) & & (X_4+X_{13}) & & (X_{22})
\end{array},$$

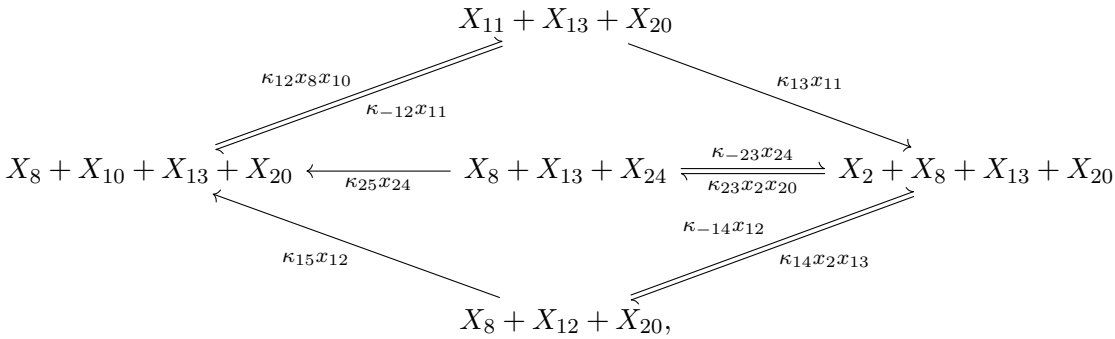
$$\begin{array}{ccccccc}
 & & \xleftarrow{\kappa_6} & & & & \\
X_2+X_4+X_{13} & \underset{\kappa_{-3}}{\overset{\kappa_3}{\rightleftarrows}} & X_5+X_{13} & \xrightarrow{\kappa_4} & X_2+X_6+X_{13} & \underset{\kappa_{-5}}{\overset{\kappa_5}{\rightleftarrows}} & X_2+X_{21} \\
(X_2+X_4) & & (X_5) & & (X_6+X_{13}) & & (X_{21})
\end{array},$$

$$\begin{array}{ccccccc}
 & & \xleftarrow{\kappa_{22}} & & & & \\
X_{16}+X_{18}+X_{20} & \underset{\kappa_{-19}}{\overset{\kappa_{19}}{\rightleftarrows}} & X_{17}+X_{20} & \xrightarrow{\kappa_{20}} & X_8+X_{18}+X_{20} & \underset{\kappa_{-21}}{\overset{\kappa_{21}}{\rightleftarrows}} & X_{18}+X_{19} \\
(X_{16}+X_{18}) & & (X_{17}) & & (X_8+X_{20}) & & (X_{19})
\end{array}. \tag{122}$$

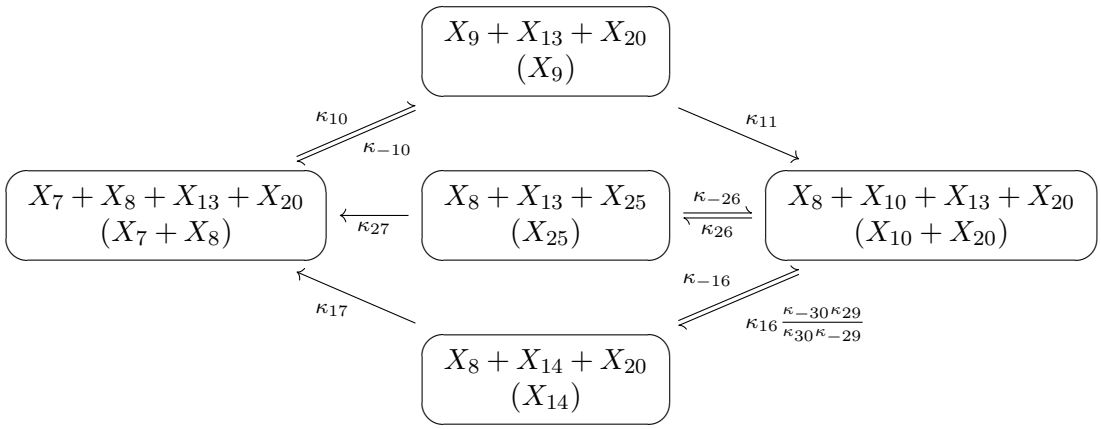
These three networks have deficiency and kinetic deficiency equal to zero. For the two deficiency 2 subnetworks, we use the algorithm of the previous section and find two deficiency zero kinetic translation graphs



and

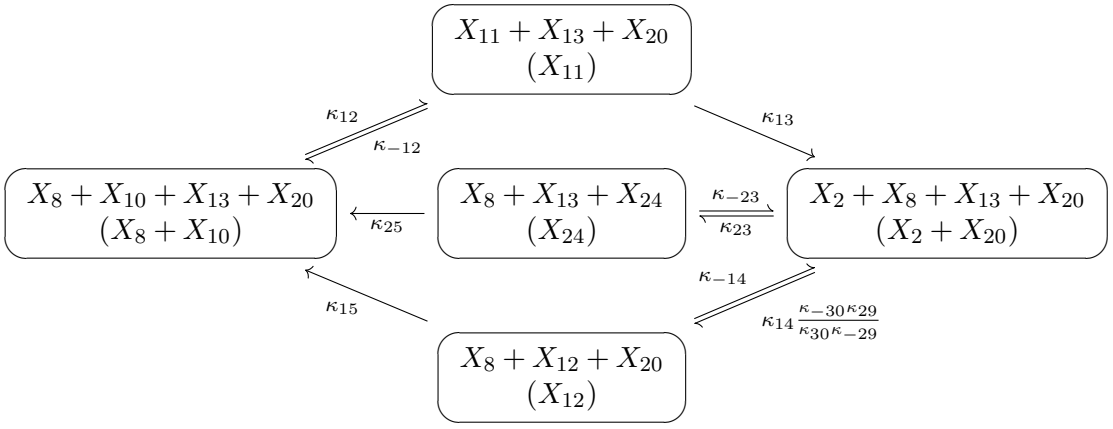


The two kinetic graphs have a conflict at $X_8 + X_{10} + X_{13} + X_{20}$ and one at $X_2 + X_8 + X_{13} + X_{20}$, respectively. Since the network has ACR in X_{13} and X_{20}, the complexes $X_{10} + X_{20}$ and $X_{10} + X_{13}$ have a robust ratio, as do the complexes $X_2 + X_{20}$ and $X_2 + X_{13}$. As a consequence, the conflicts are resolvable, and, choosing the kinetic complexes $X_{10} + X_{20}$ and $X_2 + X_{20}$, we can define the two weakly reversible generalised mass action networks



(123)

and



(124)

Putting together the deficiency zero networks and the networks in (122), (123) and (124), we find a weakly reversible generalised network with deficiency and kinetic deficiency equal to zero. Since all the positive steady states of the associated system $\widetilde{\mathcal{M}}$ also satisfy $x_{20} = \frac{\kappa_{-29}}{\kappa_{29}}$ and $x_{13} = \frac{\kappa_{-30}}{\kappa_{30}}$, by Proposition 9.2.3 the systems $\widetilde{\mathcal{M}}$ and $\mathcal{M}$ have the same positive steady states. By Theorem 7.4.3, the system $\widetilde{\mathcal{M}}$ has complex balancing steady states for any choice of the kinetic parameters. For any pair of kinetic complexes in the same linkage class, the ratio $\frac{\mathbf{x}^{y_\kappa^i}}{\mathbf{x}^{y_\kappa^j}}$ takes the same value at each positive steady state. We can use this to study how the concentration of *MK-PP* at steady state varies as a function of the input species, defined as the input signal X_{18} and the kinases X_1, X_7 and X_{16}. To do so, we can intersect the kinetic-order subspace with the subspace of $\mathbb{R}^n$ generated by e_1, e_6, e_7, e_{16}, e_{18}. We find that the intersection is generated by the vector

$$-e_6 + e_1 + 2e_7 + 4e_{16} + 4e_{18}$$

meaning that the complexes X_6 and $X_1 + 2X_7 + 4X_{16} + 4X_{18}$ have a robust ratio. In other words, there exists a function $f(\kappa)$ of the kinetic parameters such that $x_6 = f(\kappa)x_1x_7^2x_{16}^4x_{18}^4$ holds at each positive steady state. Hence the value at steady state of *MKPP* varies with the fourth power of the input signal *Sig*, for instance.

Let us consider the sequestrated version of the network in (120), defined by the reactions

$$\begin{aligned}
&X_1 + X_2 \underset{r_{-1}}{\overset{r_1}{\rightleftarrows}} X_3 \xrightarrow{r_2} X_2 + X_4 \underset{r_{-3}}{\overset{r_3}{\rightleftarrows}} X_5 \xrightarrow{r_4} X_2 + X_6,\\
&X_6 + X_{13} \underset{r_{-5}}{\overset{r_5}{\rightleftarrows}} X_{21} \xrightarrow{r_6} X_4 + X_{13} \underset{r_{-7}}{\overset{r_7}{\rightleftarrows}} X_{22} \xrightarrow{r_8} X_{23} \underset{r_{-9}}{\overset{r_9}{\rightleftarrows}} X_1 + X_{13},\\
&X_7 + X_8 \underset{r_{-10}}{\overset{r_{10}}{\rightleftarrows}} X_9 \xrightarrow{r_{11}} X_8 + X_{10} \underset{r_{-12}}{\overset{r_{12}}{\rightleftarrows}} X_{11} \xrightarrow{r_{13}} X_8 + X_2,\\
&X_2 + X_{13} \underset{r_{-14}}{\overset{r_{14}}{\rightleftarrows}} X_{12} \xrightarrow{r_{15}} X_{10} + X_{13} \underset{r_{-16}}{\overset{r_{16}}{\rightleftarrows}} X_{14} \xrightarrow{r_{17}} X_{15} \underset{r_{-18}}{\overset{r_{18}}{\rightleftarrows}} X_7 + X_{13},\\
&X_{16} + X_{18} \underset{r_{-19}}{\overset{r_{19}}{\rightleftarrows}} X_{17} \xrightarrow{r_{20}} X_8 + X_{18},\\
&X_8 + X_{20} \underset{r_{-21}}{\overset{r_{21}}{\rightleftarrows}} X_{19} \xrightarrow{r_{22}} X_{27} \underset{r_{-24}}{\overset{r_{24}}{\rightleftarrows}} X_{16} + X_{20},\\
&X_2 + X_{20} \underset{r_{-23}}{\overset{r_{23}}{\rightleftarrows}} X_{24} \xrightarrow{r_{25}} X_{10} + X_{20} \underset{r_{-26}}{\overset{r_{26}}{\rightleftarrows}} X_{25} \xrightarrow{r_{27}} X_{26} \underset{r_{-28}}{\overset{r_{28}}{\rightleftarrows}} X_7 + X_{20},
\end{aligned} \tag{125}$$

to which we add again the reactions in (121). The networks in (120) and (125) differ in reactions r_8, r_{17}, r_{22} and r_{27}: in the sequestrated version, the intermediate species X_{22} converts to the intermediate species X_{23}, instead of dissociating directly to $X_1 + X_{13}$, and similarly for X_{15}, X_{27} and X_{25}. Let us see how these changes affect the analysis

above. First, we calculate a decomposition of the network in subnetworks, finding only two deficiency zero subnetworks

$$\{r_{29}, r_{-29}\}, \{r_{30}, r_{-30}\},$$

3 with deficiency one subnetworks

$$\{r_1, r_{-1}, r_2, r_7, r_{-7}, r_8, r_9, r_{-9}\},$$
$$\{r_3, r_{-3}, r_4, r_5, r_{-5}, r_6\},$$
$$\{r_{19}, r_{-19}, r_{20}, r_{21}, r_{-21}, r_{22}, r_{24}, r_{-24}\},$$

and 2 the deficiency two subnetworks

$$\{r_{10}, r_{-10}, r_{11}, r_{16}, r_{-16}, r_{17}, r_{18}, r_{-18}, r_{26}, r_{-26}, r_{27}, r_{28}, r_{-28}\},$$
$$\{r_{12}, r_{-12}, r_{13}, r_{14}, r_{-14}, r_{15}, r_{23}, r_{-23}, r_{25}\}.$$

The networks $\{r_3, r_{-3}, r_4, r_5, r_{-5}, r_6\}$ and $\{r_{12}, r_{-12}, r_{13}, r_{14}, r_{-14}, r_{15}, r_{23}, r_{-23}, r_{25}\}$ are unchanged, and we can write for them the same deficiency zero generalised mass action networks we found for the unsequestrated case.

For the remaining three networks with positive deficiency, the algorithm identifies the following kinetic graphs:

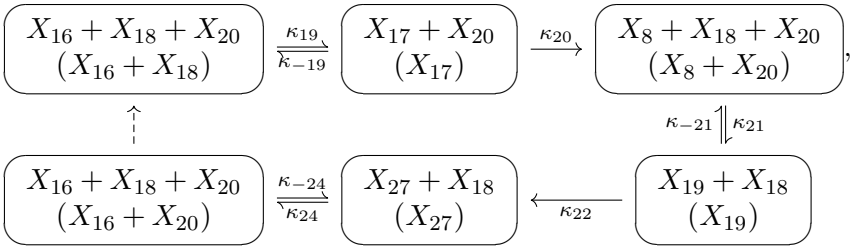
$$\begin{array}{ccccc} X_1+X_2+X_{13} & \underset{\kappa_{-1}x_3}{\overset{\kappa_1 x_1 x_2}{\rightleftharpoons}} & X_3+X_{13} & \xrightarrow{\kappa_2 x_3} & X_2+X_4+X_{13} \\ & {\scriptstyle\kappa_9 x_{23}}\searrow \quad \nearrow{\scriptstyle\kappa_{-9}x_1x_{13}} & & & {\scriptstyle\kappa_{-7}x_{22}}\uparrow \quad \downarrow{\scriptstyle\kappa_7 x_4 x_{13}} \\ & X_2+X_{23} & \xleftarrow[\kappa_8 x_{22}]{} & & X_2+X_{22} \end{array} \tag{126}$$

$$\begin{array}{ccccc} X_{16}+X_{18}+X_{20} & \underset{\kappa_{-19}x_{17}}{\overset{\kappa_{19}x_{16}x_{18}}{\rightleftharpoons}} & X_{17}+X_{20} & \xrightarrow{\kappa_{20}x_{17}} & X_8+X_{18}+X_{20}, \\ & {\scriptstyle\kappa_{24}x_{27}}\searrow \quad \nearrow{\scriptstyle\kappa_{-24}x_{16}x_{20}} & & & {\scriptstyle\kappa_{-21}x_{19}}\uparrow \quad \downarrow{\scriptstyle\kappa_{21}x_8x_{20}} \\ & X_{27}+X_{18} & \xleftarrow[\kappa_{22}x_{19}]{} & & X_{19}+X_{18} \end{array} \tag{127}$$

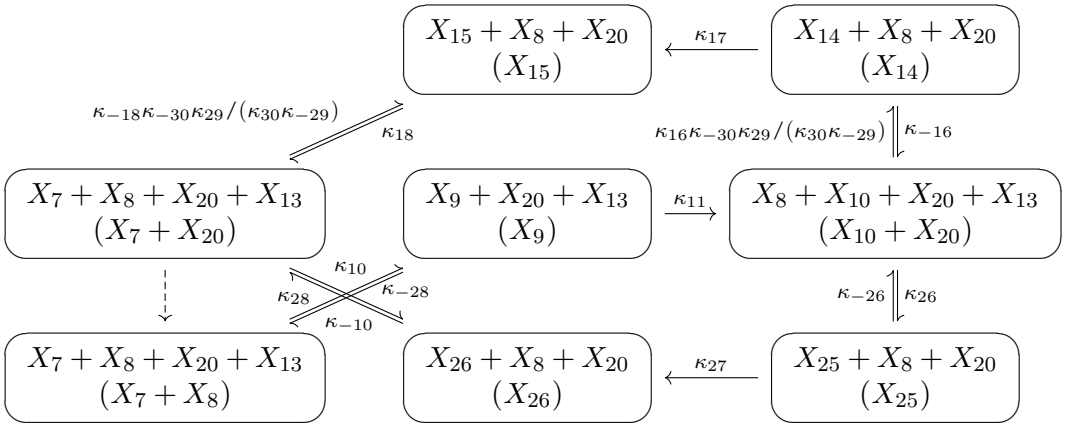
$$\begin{array}{ccccc} & & X_{15}+X_8+X_{20} & \xleftarrow{\kappa_{17}x_{14}} & X_{14}+X_8+X_{20} \\ & & {\scriptstyle\kappa_{-18}x_7x_{13}}\searrow \quad \nearrow{\scriptstyle\kappa_{18}x_{15}} & & {\scriptstyle\kappa_{16}x_{10}x_{13}}\uparrow \quad \downarrow{\scriptstyle\kappa_{-16}x_{14}} \\ X_7+X_8+X_{20}+X_{13} & \underset{\kappa_{-10}x_9}{\overset{\kappa_{10}x_7x_8}{\rightleftharpoons}} & X_9+X_{20}+X_{13} & \xrightarrow{\kappa_{11}x_9} & X_8+X_{10}+X_{20}+X_{13} \\ & {\scriptstyle\kappa_{28}x_{26}}\searrow \quad \nearrow{\scriptstyle\kappa_{-28}x_7x_{20}} & & & {\scriptstyle\kappa_{-26}x_{25}}\uparrow \quad \downarrow{\scriptstyle\kappa_{26}x_{10}x_{20}} \\ & & X_{26}+X_8+X_{20} & \xleftarrow{\kappa_{27}x_{25}} & X_{25}+X_8+X_{20} \end{array} \tag{128}$$

The first kinetic graph has a conflict at $X_1+X_2+X_{13}$, the second has a conflict at $X_{16}+X_{18}+X_{20}$ and the third one at $X_7+X_8+X_{20}+X_{13}$ and one at $X_8+X_{10}+X_{20}+X_{13}$. The difference between the kinetic complexes at $X_8+X_{10}+X_{20}+X_{13}$ is $X_{20}-X_{13}$, therefore this conflict can be resolved as done for the conflicts of the unsequestrated network. The same is true for one of the conflicts at $X_7+X_8+X_{20}+X_{13}$. The remaining conflicts cannot be resolved with the same technique. To associate generalised mass action networks to each of the kinetic graphs, we create separate nodes for each of the kinetic complexes at the nodes with conflicts. We determine the following generalised mass action networks:

$$\begin{array}{ccccc} \boxed{\begin{array}{c}X_1+X_2+X_{13}\\(X_1+X_2)\end{array}} & \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} & \boxed{\begin{array}{c}X_3+X_{13}\\(X_3)\end{array}} & \xrightarrow{\kappa_2} & \boxed{\begin{array}{c}X_2+X_4+X_{13}\\(X_4+X_{13})\end{array}} \\ \uparrow & & & & {\scriptstyle\kappa_{-7}}\uparrow \quad \downarrow{\scriptstyle\kappa_7} \\ \boxed{\begin{array}{c}X_1+X_2+X_{13}\\(X_1+X_{13})\end{array}} & \underset{\kappa_9}{\overset{\kappa_{-9}}{\rightleftharpoons}} & \boxed{\begin{array}{c}X_2+X_{23}\\(X_{23})\end{array}} & \xleftarrow[\kappa_8]{} & \boxed{\begin{array}{c}X_2+X_{22}\\(X_{22})\end{array}} \end{array} \tag{129}$$



(130)



(131)

Each of the three networks has deficiency equal to one and kinetic deficiency equal to zero. Overall, we have obtained a generalised mass action network $\widetilde{\mathcal{N}}$ with deficiency three and kinetic deficiency zero. By Theorem 7.4.3, any mass action system on (125) admits complex balancing steady states. The dashed reactions can be added to obtain the weak reversibility. If the dashed reactions are not added, Theorem 7.5.7 can be applied to (129), (130) and (131) (in each network, all pairs of non-terminal kinetic complexes have a robust ratio). Using this observation one can find that the value of x_6 at each steady state is again proportional to $x_1x_7^2x_{16}^4x_{18}^4$.

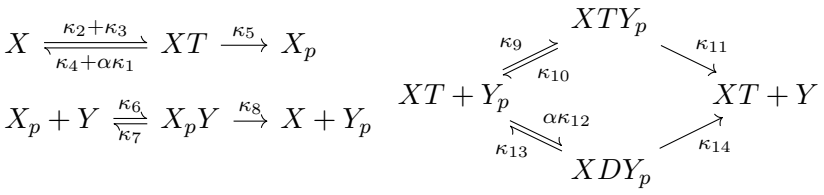
Let us focus now on the complex balancing steady states. Take a pair of kinetic complexes y_κ^i, y_κ^j in $\widetilde{\mathcal{N}}$ the same linkage class. The ratio $\mathbf{x}^{y_\kappa^i}/\mathbf{x}^{y_\kappa^j}$ takes the same value at each complex balancing steady state $\mathbf{x}$. For instance, X_8 and X_{20} have a robust ratio on the complex balancing steady states, but not on all the positive steady states. If we check how the concentration of X_6 writes in terms of the input species when limiting the analysis to the complex balancing steady states (by intersecting the kinetic-order subspace and the subspace generated by e_1, e_6, e_7, e_{16} and e_{18}), we find that x_6 is proportional to x_1, and does not vary when the input concentrations of X_7, X_{16} and X_{18} vary. Could this give a possible explanation for the stronger robustness properties displayed by the sequestrated network? Notice that the proportionality constant between x_1 and x_6 at complex balancing steady states depends on the kinetic parameters chosen for the dashed reactions in (129), (130) and (131), which do not have a clear biological interpretation.

10.4 OPEN QUESTIONS

In this section we describe some open questions and directions of future work.

(i) Can conclusions can be drawn around preservation and number of steady states for the elimination method of Section 8.3? In addition, can robustness properties be used in combination with elimination methods to simplify the analysis of steady states?

Consider for instance the deficiency two Envz-Ompr model model in (58). We know from (112) that, at each positive steady state, $x_{XD} = \frac{\kappa_2(\kappa_4+\kappa_5)}{\kappa_1\kappa_3} x_{XT}$ holds. The mass action system obtained by eliminating the species XD using the reduction method described in 8.3 is the following:



where $\alpha = \frac{\kappa_2(\kappa_4+\kappa_5)}{\kappa_1\kappa_3}$. This is a deficiency one network, that can be analysed for instance by considering a dynamically equivalent generalised mass action network with deficiency and kinetic deficiency zero.

(ii) Identify classes of networks that admit a weakly reversible generalised mass action realisation with deficiency zero or kinetic deficiency zero.

(iii) Describe methods for the identification of low deficiency or weakly reversible generalised mass action realisations that do not rely on the calculation of steady state fluxes. Consider the minimization of the kinetic deficiency.

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