Algebraic tools in the study of Multistationarity of Chemical Reaction Networks

PhD thesis by

AmirHosein Sadeghimanesh

Department of Mathematical Sciences
University of Copenhagen
Denmark

PhD School of Science - Faculty of Science - University of Copenhagen

AmirHosein Sadeghimanesh
Department of Mathematical Sciences
University of Copenhagen
Universitetsparken 5
DK-2100 København Ø
Denmark

amir@math.ku.dk
a.h.sadeghimanesh@gmail.com

PhD thesis submitted to the PhD School of Science, Faculty of Science, University of Copenhagen, Denmark, in August 2018.

Academic advisor: Elisenda Feliu
University of Copenhagen, Denmark

Assessment Committee: Carsten Wiuf (chair)
University of Copenhagen, Denmark

Georg Regensburger
Johannes Kepler University Linz, Austria

Jonathan Hauenstein
University of Notre Dame, US

ISBN 978-87-7078-914-1

Acknowledgements

This dissertation is submitted in partial fulfillment of the requirements for the Ph.D. degree at the Faculty of Science, University of Copenhagen, Denmark. The work was carried out at the Department of Mathematical Sciences, University of Copenhagen, from September 2015 to August 2018.

I should give a special thank to my supervisor who opened the door of a new world, chemical reaction network theory, to me and taught me how to use algebraic tools in biology. I also thank other members of the Feliu and Wiuf group, Mathematics of reaction networks (MBIO) of the math department of the University of Copenhagen, both former and current members. And finally not many people can be successful without the warm support of their family and I'm no exception.

AmirHosein Sadeghimanesh
Copenhagen, August 2018

Summary

This thesis consists of three articles:

In the first article, we studied how Gröbner bases and binomiality of the steady state ideal behave with respect to the addition or removal of intermediate species to a reaction network. This work is currently submitted, and available on arXiv: Sadeghimanesh and Feliu (2018a).

After gaining a knowledge about binomiality of networks with intermediates in the first article, the second article studies multistationarity of reaction networks with intermediates and that have a core binomial network. This work is also submitted, and available on arXiv: Sadeghimanesh and Feliu (2018b).

The last work concerns the use of Kac-Rice formulas to study and divide the parameter region of a reaction network according to the number of steady states. A nice implication of this work is the definition of a measure of robustness for multistationarity. A preliminary draft of this work is presented here, Sadeghimanesh and Feliu (2018c).

Dansk resumé

Denne afhandling består af tre artikler:

I den første artikel studerede vi, hvorledes Gröbner-baser og binomialitet af ligevægts-idealet opfører sig under tilføjelse eller fjernelse af mellemliggende specier i et reaktionsnetværk. Dette arbejde er i øjeblikket indsendt og tilgængeligt på arXiv: Sadeghimanesh and Feliu (2018a).

Efter at have fået viden om binomialitet af netværk med mellemprodukter i den første artikel, studeres i den anden artikel multistationaritet af reaktionsnetværk med mellemprodukter indeholdende et indre binomialnetværk. Dette arbejde er også indsendt og tilgængeligt på arXiv: Sadeghimanesh and Feliu (2018b).

Det sidste arbejde vedrører anvendelsen af Kac-Rice-formler til at studere og opdele parameterområdet for et reaktionsnetværk i henhold til antallet af stabile tilstande. En tiltalende implikation af dette arbejde er definitionen af et mål for robustheden af multistationaritet. Et foreløbigt udkast af dette arbejde er præsenteret her, Sadeghimanesh and Feliu (2018c).

Contents

1 Introduction

One can say that the study of chemical reaction networks, CRNs, started with the introduction of the Law of mass-action, in the works of the Norwegian mathematician and chemist, Maximilian Guldberg and the Norwegian chemist, Peter Waage, written in Danish in 1864 for the first time. Mass-action kinetics is based on molecular collisions. In simple words, it assumes that the number of times a reaction happens is proportional to the concentration of the reactants and for a fixed set of conditions there is a reaction rate constant which is the constant of proportionality.

When a reaction takes place, the concentrations of the species in the chemical experiment change, giving rise to a dynamical system. To study the dynamics of a CRN, one key step is the study of its equilibria, steady states. In 1972, works from Horn and Jackson (1972), and Feinberg (1972) introduced the mathematical framework for CRNs.

Mathematical results on CRNs have a vast area of applications in ecology, epidemiology, sociophysics, genetics, cancer, pharmacokinetics, etc. Questions of interest in CRN theory are related to the detection of qualitative behaviors including persistence, number of steady states, stability of steady states and oscillations, and the region in the parameter space where these behaviors take place. This thesis focuses on multistationarity, that is the possibility of having more than one steady state. Multistationarity plays the role of memory in cells and is also needed to produce switch-like behaviors, Kothamachu et al. (2015); Conradi and Flockerzi (2012). Classical results regarding the detection of multistationarity are the deficiency zero and deficiency one theorems, the deficiency one algorithm and the higher deficiency algorithm Feinberg (1987, 1988); Ellison (1998). These results answer whether a network satisfying some conditions is capable of exhibiting multistationary or not and are implemented in a software called CRNToolbox (Ji et al. (2015)). In the case the network is multistationary, the software provides one choice of parameters for which the network has several steady states. Another class of results focus on an injectivity condition which precludes the possibility of multistationarity (see Craciun and Feinberg (2005); Feliu (2015); Müller et al. (2016)). In Pérez Millán and Dickenstein (2018) multistationarity of a class of networks called MESSI networks is studied.

Other than the detection of multistationarity, giving the region in the parameter space or at least a choice of parameters that produce multistationary behavior has also its importance for the experimentalists. In this direction there are works using cylindrical algebraic decomposition such as Bradford et al. (2017) or numerical homotopy methods such as Harrington et al. (2016).

As it can be seen from these works, there are many algorithms arising in the study of CRNs. A major problem when studying realistic examples is the high number of variables and parameters. This makes complexity of computations beyond the power of current computers. Thus model reduction strategies are desirable. One type of model reduction such as in Feliu and Wiuf (2012, 2013a,b), eliminates a class of species called intermediates and gives a smaller network. Then under some conditions the properties of the smaller network such as multistationarity, remain valid for the original network. An advantage of these methods is that the smaller network has a smaller number of variables and parameters and so computations can be done faster. Similarly, Joshi and Shiu (2013) look for minimal networks with specific behavior such as multistationarity, that can transmit this behavior to another network whenever they appear as a motif inside them. Further Banaji and Pantea (2017) present a list of procedures that one can use to extend a network to a larger network preserving multistationarity.

This thesis also, besides answering its target questions, provides new algorithms to optimize the computation time.

1.1 Thesis structure

In Chapter 2 the definition of a CRN is presented. Then it proceeds to introduce the questions that this thesis wants to study. A quick review on the former approaches in the literature is given together with a quick review on some tools used in the new algorithms introduced in the three papers of the thesis. In Chapter 3 a short summary of the results of the papers of this thesis is presented. In this chapter we use the notation Theorem I.3.4 to denote Theorem 3.4 of paper 1. Chapter 4 discusses some possible future work. A bibliography for the references of Chapters 1-4 comes afterwards. Finally, the papers containing our contributions are collected, each one equipped with its own bibliography.

2

Background on Chemical Reaction Network Theory and Computational Algebra

This chapter introduces briefly what a Chemical Reaction Network (CRN) is and what questions this thesis aims at studying, with the help of a biological example. The example that is going to be used is a gene regulatory network. So before giving the example, we briefly explain how a protein is produced in a cell in simple words. First, a promoter and transcription factor(s) attach to DNA at a suitable start transcription site. Then they start to read the DNA and make an mRNA molecule from nucleotides. The mRNA gets some modifications and in case of eukaryotes, mRNA leaves the nucleus. Afterwards ribosomes use these recipes and assemble the corresponding protein from amino acids. Proteins are subsequently used in processes inside the cell or leave the cell. Protein and mRNA molecules degrade at some rate.

Now consider two proteins A and B, such that the expression of A suppresses B and vice versa. Let M_A, P_A, E and D_A stand for mRNA, protein, transcription factors and promoter of protein A respectively and M_B, P_B, F and D_B analogous for protein B. Assume that the promoter of protein A, D_A, has two binding sites that molecules of P_B can bind cooperatively, meaning that binding one protein will make it easier for the second protein to bind (for more about cooperatively binding, see Section 3.3 of Ingalls (2012)). If one of these binding sites is occupied by protein B, then the promoter cannot attach to DNA and initiate the process of transcription of protein A. Let D_A^b and D_A^{bb} denote D_A when one or two of its binding sites are occupied by protein B. The first reaction in our model is

$$D_A + E \xrightarrow{k} D_A + E + M_A. \tag{2.1}$$

It encodes that one molecule of D_A and one molecule (a set of molecules) of E should meet (attach to the DNA) and then under a rate k they become one molecule of D_A, one molecule of E and a molecule of M_A. In other words, one D_A and one E will produce one M_A without being used themselves. The rate k is called the *reaction*

$$
\begin{array}{ll}
D_A + E \xrightarrow{k_1} D_A + E + M_A & D_B + F \xrightarrow{k_2} D_B + F + M_B \\
M_A \xrightarrow{k_3} M_A + P_A & M_B \xrightarrow{k_4} M_B + P_B \\
M_A \xrightarrow{k_5} 0 & M_B \xrightarrow{k_6} 0 \\
P_A \xrightarrow{k_7} 0 & P_B \xrightarrow{k_8} 0 \\
D_A + P_B \underset{k_{11}}{\overset{k_9}{\rightleftharpoons}} D_A^b & D_B + P_A \underset{k_{12}}{\overset{k_{10}}{\rightleftharpoons}} D_B^b \\
D_A^b + P_B \underset{k_{15}}{\overset{k_{13}}{\rightleftharpoons}} D_A^{bb} & D_B^b + P_A \underset{k_{16}}{\overset{k_{14}}{\rightleftharpoons}} D_B^{bb}
\end{array}
$$

Figure 2.1: Gene regulatory network of proteins A and B with inhibitory effect on expression of each other.

rate constant. The next reaction is

$$M_A \longrightarrow M_A + P_A \tag{2.2}$$

which stands for the production of protein A in the ribosome. The number of ribosomes is assumed to be constant and hence not modeled. Then there are degradation reactions for M_A and P_A,

$$M_A \longrightarrow 0 \quad \text{and} \quad P_A \longrightarrow 0. \tag{2.3}$$

Denoting 0 at the right side of a reaction means the molecules in the left side are degraded or exit the environment (such as the cell). Finally, inhibitory bindings of protein B to the promoter of A are modeled as

$$D_A + P_B \rightleftharpoons D_A^b \quad \text{and} \quad D_A^b + P_B \rightleftharpoons D_A^{bb}. \tag{2.4}$$

The symbol $\rightleftharpoons$ indicates that the reaction is reversible, so $D_A + P_B \rightleftharpoons D_A^b$ consists of the two reactions $D_A + P_B \longrightarrow D_A^b$ and $D_A^b \longrightarrow D_A + P_B$. We use k_i with subindex i for the i-th reaction to denote reaction rate constants. Figure 2.1 represents all reactions of our model together, by repeating (2.1), (2.2), (2.3) and (2.4) for protein B.

Figure 2.1 gives an example of a CRN. Formally a CRN consists of three finite sets:

- A set of *species* denoted by $\mathcal{S}$, here

$$\mathcal{S} = \{M_A, M_B, P_A, P_B, E, F, D_A, D_B, D_A^b, D_B^b, D_A^{bb}, D_B^{bb}\}.$$

- A set of *complexes* denoted by $\mathcal{C}$. A complex is a linear combination of species with non-negative integer coefficients appearing in one side of a reaction such as $D_A + E$, M_A or 0.

- A set of *reactions* denoted by $\mathcal{R} \subseteq \mathcal{C} \times \mathcal{C}$. A reversible reaction is considered as two irreversible reactions.

Every reaction has two sides, the complex in the left side of the reaction is called *reactant* (or *educt*) and the complex in the right side is called *product*. Assume $\mathcal{S} = \{X_1, \dots, X_n\}$. Then a complex, c, has the form $c_1X_1 + \cdots + c_nX_n$. The coefficient c_i in this representation is called the *stoichiometric coefficient* of the species X_i in the complex c. The complex c is also represented by the vector $(c_1, \dots, c_n)$ and we denote this vector again by the symbol c.

2.1 ODE system of the network and steady states

ODE system of evolution of concentration of species. Denote the concentration of species with lower-case letters, for example m_A denote the concentration of M_A. The occurrence of a reaction changes the value of these variables. Consider the reaction $D_A + P_B \xrightarrow{k_9} D_A^b$. Each time this reaction happens, one molecule of D_A and one molecule of P_B are consumed and one molecule of D_A^b is produced. Under mass action law, which is one of the many choices for the kinetics (see the subsection on other kinetics), each reaction happens with a rate proportional to the concentrations of the species in the reactant. If the reactant is $a_1X_1 + \cdots + a_nX_n$ and the reaction rate constant is k, then the rate of this reaction is $kx_1^{a_1} \dots x_n^{a_n}$. Let $\dot{x}_i$ denotes the derivative of the concentration of the species X_i with respect to time. Then the variation of the concentration of the species in a CRN under mass action kinetics is modeled by the following ordinary differential equation (ODE) system.

$$\dot{x}_i = F_{k,i}(x) = \sum_{\sum_{j=1}^n a_j X_j \xrightarrow{k} \sum_{j=1}^n b_j X_j \in \mathcal{R}} (b_i - a_i) k x_1^{a_1} \dots x_n^{a_n}, \quad i = 1, \dots, n, \quad x \in \mathbb{R}^n_{\geq 0}. \tag{2.5}$$

The non-negative orthant is forward invariant with respect to the ODE system (2.5), Sontag (2001); (Feinberg, 1987, Appendix I); (Banaji and Pantea, 2016, Lemma B.1). It means that if the initial condition of (2.5) belongs to $\mathbb{R}^n_{\geq 0}$, then so does the trajectory for any positive time. The same holds for $\mathbb{R}^n_{>0}$. There are other properties regarding the behavior of trajectories of the ODE system of the network with a positive initial condition called persistence and permanence. These are not the topic of this thesis and we refer the reader to de Freitas et al. (2016); Gnacadja (2011a,b,c); Craciun et al. (2013). The ODE system for our gene regulatory network of proteins A and B is shown in Figure 2.2.

Conservation laws. By looking at the equations in Figure (2.2), one easily notices that

$$\dot{e} = \dot{f} = 0 \quad \text{and} \quad \dot{d_A} + \dot{d_A^b} + \dot{d_A^{bb}} = \dot{d_B} + \dot{d_B^b} + \dot{d_B^{bb}} = 0.$$

$$\begin{cases} \dot{m_A} &= k_1 d_A e - k_5 m_A \\ \dot{m_B} &= k_2 d_B f - k_6 m_B \\ \dot{p_A} &= k_3 m_A - k_7 p_A - k_{10} d_B p_A + k_{12} d_B^b - k_{14} d_B^b p_A + k_{16} d_B^{bb} \\ \dot{p_B} &= k_4 m_B - k_8 p_B - k_9 d_A p_B + k_{11} d_A^b - k_{13} d_A^b p_B + k_{15} d_A^{bb} \\ \dot{e} &= 0 \\ \dot{f} &= 0 \\ \dot{d_A} &= -k_9 d_A p_B + k_{11} d_A^b \\ \dot{d_B} &= -k_{10} d_B p_A + k_{12} d_B^b \\ \dot{d_A^b} &= k_9 d_A p_B - k_{11} d_A^b - k_{13} d_A^b p_B + k_{15} d_A^{bb} \\ \dot{d_B^b} &= k_{10} d_B p_A - k_{12} d_B^b - k_{14} d_B^b p_A + k_{16} d_B^{bb} \\ \dot{d_A^{bb}} &= k_{13} d_A^b p_B - k_{15} d_A^{bb} \\ \dot{d_B^{bb}} &= k_{14} d_B^b p_A - k_{16} d_B^{bb}. \end{cases}$$

Figure 2.2: The ODE system of the gene regulatory network of proteins A and B in Figure 2.1 under the assumption of mass action kinetics.

This means that for each trajectory, there exist constants $T_1, \ldots, T_4$ such that

$$e = T_1, \quad f = T_2, \quad d_A + d_A^b + d_A^{bb} = T_3, \quad d_B + d_B^b + d_B^{bb} = T_4.$$

These linear invariants are called *conservation laws* and their constants are called constants of conservation laws. Since these linear combinations of concentrations of the species remain constant along the trajectories of the ODE, one can determine constants of conservation laws from the initial conditions of the ODE system. Remember the vector representation of complexes with its entries being the stoichiometric coefficients of the species. Let N be the matrix having $c' - c$ for the i-th column if $c \to c'$ is the i-th reaction. The matrix N is called the *stoichiometric matrix*. Of course the stoichiometric matrix depends on the order of reactions and species. Let $\psi(x)$ be the vector having $x_1^{c_1} \ldots x_n^{c_n}$ as its i-th entry if c is the reactant of the i-th reaction. Then the functions $F_{k,i}(x)$ in (2.5) can be computed by

$$N \operatorname{diag}(k)\psi(x) = (F_{k,1}(x), \ldots, F_{k,n}(x))^t \tag{2.6}$$

where $\operatorname{diag}(k)$ is the matrix with $k = (k_1, \ldots, k_{|\mathcal{R}|})$ on its diagonal and zero elsewhere. It is clear from (2.5) and (2.6) that if v is a vector in the kernel of N^t, then $v \cdot \dot{x} = 0$ or equivalently $v \cdot x$ is a linear invariant of trajectories of our ODE.

Definition 1. Let $\mathcal{N}$ be a reaction network with stoichiometric matrix N and n species. The rank of the network is $\operatorname{rank}(N)$. Let d be the corank of N, that is $n - \operatorname{rank}(N)$. A matrix $Z \in \mathbb{R}^{d \times n}$ whose rows form a basis of $\ker(N^t)$ is called a *matrix of conservation laws*. A *stoichiometric compability class* for a given initial condition x_0 is defined as

$$\{x \in \mathbb{R}^n_{\geq 0} \mid Zx = Zx_0\}.$$

By (Feinberg, 1995a, Remark 3.4) the stoichiometric compability classes are forward invariant with respect to our ODE system.

Steady states. To find the steady states of the ODE system one solves the system of equations obtained from Equation (2.5) by letting $\dot{x_i} = 0$. Since the concentration of the species cannot be complex numbers or even negative reals, only non-negative real solutions are meaningful. Moreover, in many applications we do not want fully consumption or extinction of a species. So by *steady state* in this thesis, we mean an equilibrium where all x_i's are positive. Because the reaction rate constants are (positive) real numbers, the expression $F_{k,i}(x)$ in (2.5) is a polynomial in $\mathbb{R}[x]$. This polynomial is called the *steady state polynomial* of X_i. If the reaction rate constants are treated as parameters, then the steady state polynomials are in $\mathbb{R}(k)[x]$ and we drop k from their subindex, $F_i(x)$. The ideal generated by the steady state polynomials is called the *steady state ideal* and is denoted by I. It is clear that I can be generated by $\text{rank}(\mathcal{N})$ polynomials because $n - \text{rank}(\mathcal{N})$ of the steady state polynomials can be written as linear combinations of the rest.

For example, in the gene regulatory network of proteins A and B, the steady state polynomials of E, F, D_A^b and D_B^b can be ignored when one studies steady states. The steady state ideal of this network is

$$\begin{aligned} I = \langle & k_1 d_A e - k_5 m_A,\ k_2 d_B f - k_6 m_B,\ k_3 m_A - k_7 p_A,\ k_4 m_B - k_8 p_B \\ & k_9 d_A p_B - k_{11} d_A^b,\ k_{10} d_B p_A - k_{12} d_B^b,\ k_{13} d_A^b p_B - k_{15} d_A^{bb},\ k_{14} d_B^b p_A - k_{16} d_B^{bb} \rangle. \end{aligned}$$

The number of steady states in a stochiometric compability class is important. Hence we have the following definition.

Definition 2. A network is *multistationary* if there exist $k \in \mathbb{R}^{\mathcal{R}}_{>0}$ and $T \in \mathbb{R}^d$ such that the system

$$F_{k,1}(x) = \cdots = F_{k,n}(x) = 0, \quad Zx - T = 0 \tag{2.7}$$

has more than one positive solution. In other words, there is more than one positive steady state in a stoichiometric compability class for a choice of reaction rate constants. A *monostationary* network is a network that has one steady state in each stoichiometric compability class.

The system of equations to study multistationarity of the gene regulatory network of proteins A and B is as follows;

$$\begin{array}{ll} k_1 d_A e - k_5 m_A = 0 & k_2 d_B f - k_6 m_B = 0 \\ k_3 m_A - k_7 p_A = 0 & k_4 m_B - k_8 p_B = 0 \\ k_9 d_A p_B - k_{11} d_A^b = 0 & k_{10} d_B p_A - k_{12} d_B^b = 0 \\ k_{13} d_A^b p_B - k_{15} d_A^{bb} = 0 & k_{14} d_B^b p_A - k_{16} d_B^{bb} = 0 \\ e = T_1 & f = T_2 \\ d_A + d_A^b + d_A^{bb} = T_3 & d_B + d_B^b + d_B^{bb} = T_4. \end{array} \tag{2.8}$$

There are some classes of steady states like detailed balancing equilibria and complex balanced equilibria that have been studied for a long time. For example if a network equipped with mass action kinetics has a complex balancing equilibrium, then it is monostationary, see Horn and Jackson (1972); Feinberg (1987) For reading more about these classes of equilibriums we refer the reader to Horn and Jackson (1972); Horn (1972); Feliu et al. (2018); Craciun et al. (2009); Müller and Regensburger (2012).

2.2 Intermediates

A type of species useful in model reduction strategies is called intermediate.

Definition 3. A species Y is an *intermediate* if

1. the stoichiometric coefficient of Y in every complex other than
$$Y = (1)Y + \sum_{X \in \mathcal{S}\setminus\{Y\}} (0)X$$
is zero,
2. there exists a reaction with Y as its reactant,
3. there exists a reaction with Y as its product.

In the gene regulatory network of Figure 2.1, the species D_A^{bb} and D_B^{bb} are intermediates. Intermediate species are abundant in realistic examples. For example, in the ERK activation network (Sadeghimanesh and Feliu, 2018a, Figure 1), 15 of 29 species are intermediates or in the MAPK network (Sadeghimanesh and Feliu, 2018a, Example 2.6), 6 of 11 species are intermediates.

Definition 4. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ and $\widetilde{\mathcal{N}} = (\widetilde{\mathcal{S}}, \widetilde{\mathcal{C}}, \widetilde{\mathcal{R}})$ be two reaction networks. The network $\widetilde{\mathcal{N}}$ is called an *extension* of $\mathcal{N}$ via the addition of intermediates $Y_1, \dots, Y_m$ if

(i) $\mathcal{Y} = \{Y_1, \dots, Y_m\}$ is a set of intermediates of $\widetilde{\mathcal{N}}$.

(ii) $\mathcal{S} \cup \mathcal{Y} \subseteq \widetilde{\mathcal{S}}$ and $\mathcal{C} \cup \mathcal{Y} \subseteq \widetilde{\mathcal{C}}$.

(iii) $c \to c' \in \mathcal{R}$ if and only if there is a sequence of reactions
$$c_0 \longrightarrow c_1, c_1 \longrightarrow c_2, \dots, c_{n-1} \longrightarrow c_n \tag{2.9}$$
in $\widetilde{\mathcal{R}}$ with $c_0 = c$, $c_n = c'$ and $c_1, \dots, c_{n-1} \in \mathcal{Y}$ if $n > 1$.

In this case $\mathcal{N}$ is called the *core network* of $\widetilde{\mathcal{N}}$.

Depending on the property of the network that we are studying, we may change item (ii) of Definition 4 to $\mathcal{S}\cup\mathcal{Y} = \widetilde{\mathcal{S}}$ and $\mathcal{C}\cup\mathcal{Y} = \widetilde{\mathcal{C}}$. The elements of $\widetilde{\mathcal{S}}-\mathcal{Y}$ are called non-intermediates and the elements of $\widetilde{\mathcal{C}}-\mathcal{Y}$ are called non-intermediate complexes. A non-intermediate complex c is an *input* for an intermediate Y if there is a set of reactions like in (2.9) in $\widetilde{\mathcal{R}}$ with $c_0 = c$, $c_n = Y$ and $c_1, \ldots, c_n \in \mathcal{Y}$. An intermediate Y is called an ℓ-input intermediate if there are ℓ inputs for Y.

Consider the following simple network

$$X_1 + X_2 \rightleftharpoons Y_1 \longrightarrow Y_2 \rightleftharpoons X_1 + X_3.$$

The species Y_1 and Y_2 are intermediates. The non-intermediate complex $X_1 + X_2$ is an input for both Y_1 and Y_2 while $X_1 + X_3$ is only an input for Y_2. Therefore Y_1 is a 1-input intermediate and Y_2 is a 2-input intermediate. The core network associated with this network consists of just one reaction $X_1 + X_2 \longrightarrow X_1 + X_3$. We use a tilde above symbols to denote objects related to extended networks, for example $\widetilde{I}$ denotes the steady state ideal of $\widetilde{\mathcal{N}}$. But for the reaction rate constants of the extended network we use κ instead of $\widetilde{k}$.

There are many properties of networks that are preserved by adding or removing intermediates, mainly studied within the group Mathematics of Reaction Networks at University of Copenhagen. To name a few, one can mention lifting of multistationarity and multistability Feliu and Wiuf (2013a), the relation to persistence and monotonicity de Freitas et al. (2016, 2017), and trajectories Cappelletti and Wiuf (2017) and in stochastic modeling Cappelletti and Wiuf (2016). There is also a generalization of intermediates called non-interacting species, see Feliu and Wiuf (2012); Sáez et al. (2017). We contribute to this by studying the effect of intermediates on Gröbner bases Sadeghimanesh and Feliu (2018a).

2.3 Detection of multistationarity

One of the applications of multistationarity is to build switch-like behavior in biological circuits. Consider the gene regulatory network of proteins A and B. One can build a biological switch using this network in the following way. Assume that there exists a suitable choice of parameter values such that there are two (stable) steady states with ratio of the concentration of P_A to the concentration of P_B at one of these two steady states higher than a threshold a and in the other steady state, lower than another threshold b ($b \leq a$). Then one steady state corresponds to the switch being on and the other to the switch being off.

A well-known software for chemical reaction network theory is CRNToolbox Ji et al. (2015). The CRNToolbox software uses results such as deficiency one and higher deficiency algorithms, deficiency zero and deficiency one theorems, to check multistationarity of a network. For example, we consider the deficiency one algorithm, which applies to deficiency one networks that are regular (Feinberg, 1995b, conditions R.1-R.3). If the network satisfies the required assumptions, then the algorithm assigns a set of vectors and a set of partitions on the set of complexes. For each pair of a vector and a partition, the algorithm forms a system of linear inequalities in n variables (n number of species). The network is multistationary if and only if at least one of these systems has a nonzero solution, whose sign agree entry-wise with the sign of a vector in the column space of the stoichiometric matrix (Feinberg, 1995b, Corollary 4.1). Another software to test multistationarity is CoNtRol Donnell et al. (2014).

Giving the gene regulatory network of proteins A and B to CRNToolbox, it reports that the network has deficiency four and is suitable for the application of the higher deficiency algorithm. Then, it reports that there are reaction rate constants that give rise to two or more steady states. It also provides one example of such reaction rate constants

$$\begin{array}{lllll} k_1 = 1.7182818, & k_2 = 2 & k_3 = 1, & k_4 = 1, & k_5 = 1, \\ k_6 = 1, & k_7 = 1, & k_8 = 1, & k_9 = 1.7182818, & k_{10} = 2, \\ k_{11} = 1.7182818, & k_{12} = 2, & k_{13} = 1, & k_{14} = 3.1639534, & k_{15} = 0.63212055, \\ k_{16} = 5.4365636, & & & & \end{array}$$

and gives two steady states corresponding to this reaction rate constants. The chosen constants of the conservation laws can be calculated from the steady states the software provides. In the first steady state

$$\begin{array}{lllll} m_A = 1, & m_B = 1, & p_A = 1, & p_B = 1, & e = 1, \\ f = 1, & d_A = 0.5819767, & d_B = 0.5, & d_A^b = 1, & d_B^b = 1.5819767, \\ d_A^{bb} = 0.31606027, & d_B^{bb} = 0.18393972, & & & \end{array}$$

and in the second steady state

$$\begin{array}{llll} m_A = 2.7182818, & m_B = 0.36787944, & p_A = 2.7182818, & p_B = 0.36787944, \\ e = 1, & f = 1, & d_A = 1.5819767, & d_B = 0.18393972, \\ d_A^b = 1, & d_B^b = 0.5819767, & d_A^{bb} = 0.31606027, & d_B^{bb} = 0.5. \end{array}$$

In some cases, the system (2.7) can be simplified to the study of one univariate polynomial like in Kothamachu et al. (2015). In such cases one can use Descartes' rule of sign or Sturm's theorem to study the number of positive roots of the polynomial. There are also new generalizations of Descartes' rule of signs for multivariate system

of polynomials such as (Müller et al., 2016, Theorem 1.5) or Bihan and Dickenstein (2017).

Binomial networks. Looking at Equation (2.8), the polynomials other than the four conservation laws are binomials, that is have at most two terms. Therefore one can use methods developed to study binomial systems. An ideal is called a *binomial ideal* if it admits a basis consisting of only binomials. A network with binomial steady state ideal is called a *binomial network*. By Corollary 1.2 in Eisenbud and Sturmfels (1996) to check whether an ideal is binomial one needs to compute a reduced Gröbner basis. But computing Gröbner bases can be time consuming. This is one of the concerns that the first paper in this thesis deals with.

Results of Müller et al. (2016) in general can only preclude multistationarity, but when the steady states are solutions to a binomial system, those results can be used to detect multistationarity as well. These results use some conditions which we call (surj), (sign) and (det). As an example of this case, Pérez Millán et al. (2012) uses the (surj) and (sign) conditions to detect multistationarity of binomial networks, when binomiality can be detected by linear algebra. Pérez Millán and Dickenstein (2018) use the same ideas and the (det) condition, but only focus on MESSI networks that are also binomial.

The (surj) condition is equivalent to $\ker(N)\cap\mathbb{R}^n_{>0} \neq \emptyset$ (see Lemma 2.5 in Sadeghimanesh and Feliu (2018b)). To check this condition we use an algorithm in Appendix B of Schilling et al. (2000), which we explain now.

Let N be the stoichiometric matrix of the network and id_m the identity matrix of size m. Define T_0 to be the matrix $\left[\begin{array}{c|c} id_{|\mathcal{R}|} & N^t \end{array}\right]$. Then choose one of the nonzero columns of the matrix in the right side. We form a new matrix T_1 as follows. Keep the rows of T_0 whose entries in the chosen column are zero. Consider all rows with nonzero entries in the chosen column. For each pair of these rows that have different signs in that column, say r_i and r_j, introduce a new vector $r'_{i,j} = |a_j|r_i + |a_i|r_j$ where a_i and a_j are entries of r_i and r_j in the chosen column respectively. Remove all rows of T_0 with nonzero entries in the chosen column and add the new $r'_{i,j}$ vectors as new rows to T_1. Now take T_1 and construct T_2 in the same way. Proceed in this way until the right side matrix becomes a zero matrix. Let A be the set of vectors that are rows of the left matrix. If the support of a vector of A, v_i, is a subset of the support of another vector of A, v_j, then remove v_i from A. The set A is an extremal generating set for $\ker(N) \cap \mathbb{R}^n_{\geq 0}$. The set $\ker(N) \cap \mathbb{R}^n_{\geq 0}$ is nonempty if and only if sum of vectors in its extremal generating set belongs to $\mathbb{R}^n_{>0}$.

Let e_i be the vector having 1 in its i-th entry and 0 elsewhere. We apply the above algorithm on the stoichiometric matrix of the gene regulatory network of the proteins A and B, and the order of dealing with columns of the right hand side

matrices T_i as 1, 2, 7, 8, 9, 11, 3, 4. Then an extremal generating set is

$$\{e_1+e_5, e_2+e_6, e_9+e_{11}, e_{10}+e_{12}, e_{13}+e_{15}, e_{14}+e_{16}, e_3+e_7, e_4+e_8\}.$$

(surj) is equivalent with $\sum_{v\in A} v \in \mathbb{R}^{|\mathcal{R}|}_{>0}$ which holds for this example.

This algorithm is a variant of the double description method for computing extreme rays of a polyhedral cone, see Motzkin et al. (1953); Fukuda and Prodon (1996), and in the context of reaction networks Gagneur and Klamt (2004).

2.4 Parameter region of multistationarity

Knowing that a network is multistationary, one needs the parameter region where the network has more than one positive steady state. In this section we discuss the explored strategies to find the region of multistationarity. Consider the gene regulatory network of proteins A and B. For making it possible to illustrate in figures what follows, we fix all parameters except two of them. Assume we have some control on the reaction rate constants k_7 and k_8, which are the most important ones, since they include the extraction of the proteins from the cell too. For the rest of parameters let

$$\begin{aligned} &k_1=k_2=k_3=k_4=1, k_5=0.0082, k_6=0.0149, k_9=k_{10}=0.01, k_{11}=k_{12}=10000,\\ &k_{13}=2, k_{14}=25, k_{15}=1, k_{16}=9, T_1=T_2=T_3=1, T_4=4. \end{aligned} \tag{2.10}$$

We look for values of k_7 and k_8 that make the network to be multistationary in the box $0 \leq k_7, k_8 \leq 0.1$. The CRNToolbox, and most of the other methods do not have any input for conditions on reaction rate constants such as restricting to some inequalities as a box, or fixing a parameter etc.

A simple idea is to choose a large number of random choices for (k_7, k_8), solve the system of steady states and draw a colored point on the (k_7, k_8)-plane, depending on the number of solution. See Figure 2.3.

Since there are perturbations in experimental environment, one may want to consider an average number of steady states in a neighborhood of each point. So maybe instead of the above idea, one divides the box to some smaller sub-boxes, repeats the computation for the sub-boxes, and then associates the average number of solutions to each sub-box. Figure 2.4 is an implementation of this idea.

There are other methods that provides system of polynomial inequalities for the parameters describing the region of multistationarity. Some of such methods use Descartes' rule of signs, Sturm's theorem Conradi et al. (2017), polyhedral methods Giaroli et al. (2018); Bihan et al. (2018). But looking at a system of polynomial

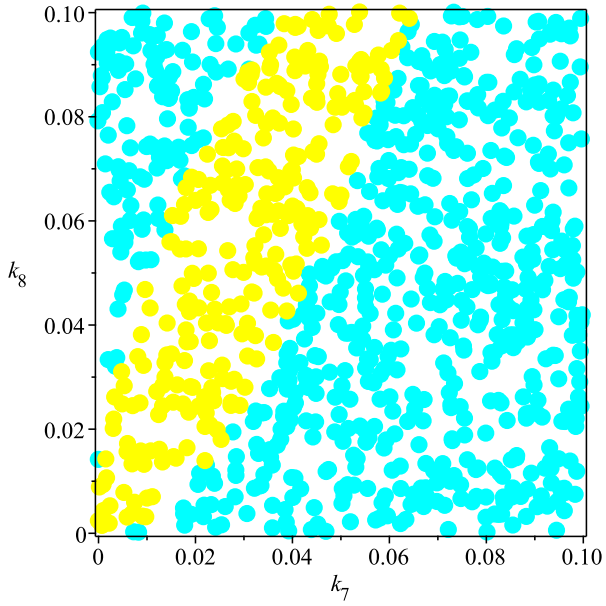


Figure 2.3: 1000 random sample points from the box $[0, 0.1] \times [0, 0.1]$ with uniform distribution for (k_7, k_8) are chosen. Then the system (2.8) is evaluated at (2.10) and these points. The random points are colored by yellow, black and sky blue if the system obtained by evaluation at them has three, two or one positive solutions respectively.

inequalities does not often tell us about the geometry of the region directly such as connectedness.

Cylindrical Algebraic Decomposition. A deterministic method is to use cylindrical algebraic decomposition (CAD). This method gives the exact boundaries between different regions in the parameter space where the system has different number of steady states. Figure 2.5 shows the result of using CAD for our example.

Before explaining CAD, we review the definition of discriminant variety. The discriminant variety of a parametric system is a variety in the parameter space where the system with parameters chosen from this variety has a solution with multiplicity greater than one. Consider the following simple multivariate parametric system:

$$\begin{cases} x^2 - y^3 = 0 \\ xy - T = 0. \end{cases} \tag{2.11}$$

Variables are x and y and the parameter is T. When $T \neq 0$, the system has one real solution with multiplicity one, and when $T = 0$, the system has one real solution with multiplicity 2. Hence it is expected that the discriminant variety is $T = 0$.

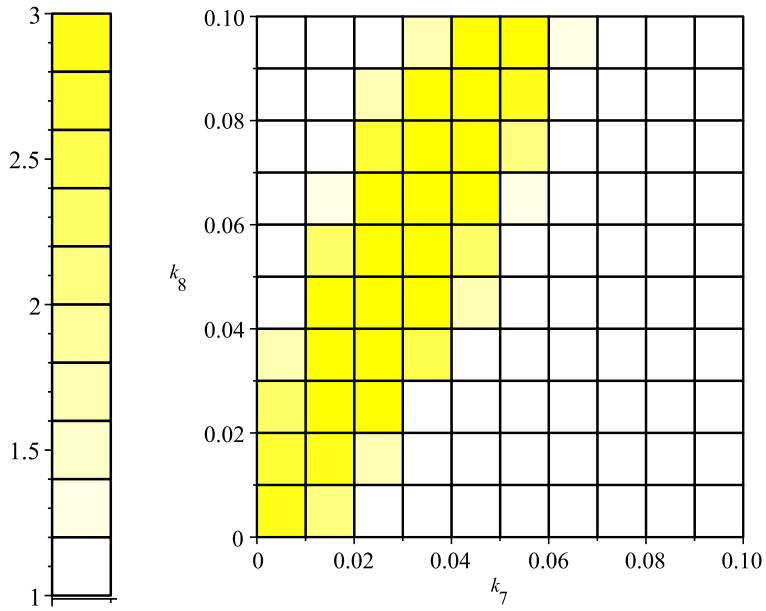


Figure 2.4: The box $[0, 0.1] \times [0, 0.1]$ is divided into 100 equal smaller boxes. For each sub-box, 10 random points with uniform distribution are chosen. Then the system (2.8) is evaluated at (2.10) and the random points in the sub-box. Finally, the average number of positive solutions for the points in the sub-boxes are calculated and assigned to the sub-boxes. Strong yellow means that the average number of positive solutions is three and white means it is one.

When the coefficient field of the polynomial ring of equations of the system is a perfect field, the concepts singularity and nonsmoothness are the same Cutkosky (2004). Since $\mathbb{R}$ is a field of characteristic zero, it is a perfect field. Therefore to compute the singular locus of the solutions set of the system, one can use the Jacobian matrix. The Jacobian matrix of (2.11) is;

$$J_x f = \begin{bmatrix} 2x & -3y^2 \\ y & x \end{bmatrix}.$$

Since the solution set of the system is a zero-dimensional variety and there are 2 variables, the singular locus is the common solutions to the system (2.11) and the ℓ-fitting ideal of the Jacobian matrix with $\ell = 2 - 0 = 2$. The ℓ-fitting ideal of a matrix with polynomial entries, is the ideal generated by ℓ-minors of the matrix. In examples from CRN the system, (2.7) usually has a zero-dimensional solution set as well. The system (2.7) is always possible to be reduced to a square system, in that case we only need to add the determinant of the Jacobian matrix to the system for finding the discriminant variety. Now the last step is to eliminate the variables from

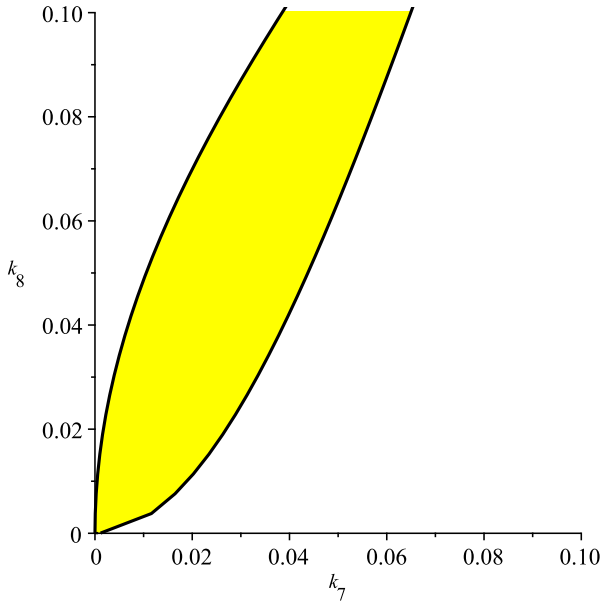


Figure 2.5: Applying CAD on the system (2.8) after being evaluated at (2.10). CAD divides the positive orthant into several cells, five of them are open cells and only one of these open cells have three positive solutions. Intersection of this cell with the box $[0, 0.1] \times [0, 0.1]$ is colored in yellow.

the singular locus variety.

$$\langle x^2 - y^3, xy - T, 2x^2 + 3y^3 \rangle \cap \mathbb{R}[T] = \langle T^2 \rangle.$$

Note that the solution set of $T^2 = 0$ and $T = 0$ agree.

In order to change the number of real solutions of a system, parameters should cross the discriminant variety. A simple example is the system $x^2 + bx + c = 0$. The discriminant variety of this system is defined by $b^2 - 4c = 0$. But crossing the discriminant variety does not always change the number of real solutions as it is the case for (2.11). For system (2.7) of a CRN, other than the change in the number of real solutions, one should take into account where the sign of the solutions change. CAD computes polynomials defining the discriminant variety. Then it eliminates parameters from it one by one. At each step it keeps the leading coefficients of the polynomials, the resultants and the discriminants with respect to the eliminated parameter. It proceeds until we have a union of univariate polynomial systems on the last parameter. It solves them and takes a sample point between every two consecutive roots. Then it evaluates the bivariate polynomial systems of one step back in the projection phase and obtains univariate systems on another parameter. Proceeding this way back until covering all parameters, the result is a set of points and

polynomial inequalities associated with each point in the lifting phase. These polynomial inequalities define open cells that are completely inside one of the connected components of the complement of the discriminant variety. Therefore the number of real solutions to the original system is the same for every two parameter choices in the same cell. To know the number of real points in each cell, it is enough to solve the system at the given sample point.

There is a Maple package called RootFinding[Parametric] Liang et al. (2009) that computes this discriminant variety. The goal of this package is to compute CAD using ideas and algorithms explained in Corvez and Rouillier (2004); Lazard and Rouillier (2007).

The number of open cells can be more than the number of components of the complement of the discriminant variety. The number of cells grows very fast, specially, it is doubly exponential in the sum of the number of variables and parameters (England et al., 2015, Theorem 5). Therefore the problem with CAD is that it only works well for small systems.

Homotopy continuation and numerical algebraic geometry. Numerical homotopy methods are methods based on homotopy path tracking and numerical methods of ODE solving to study solutions of systems of equations. We explain the idea on a simple example. Consider the following simple system consisting of one polynomial equation in one variable and no parameter:

$$f(x) = 2x^2 + 2x - 1 = 0.$$

Instead of solving $f(x) = 0$ directly, we consider a simpler system

$$g(x) = x^2 - 1 = 0.$$

The system $g(x) = 0$ has two simple roots $x = \pm 1$. Define a map $H(x,t)$, $t \in [0,1]$ satisfying $H(x,0) = g(x)$ and $H(x,1) = f(x)$, for example $tf(x) + (1-t)g(x)$. Now the idea is to track solutions of $g(x) = 0$ along $H(x,t) = 0$ until the solutions of $f(x) = 0$. To do that, we define an ODE system with trajectories playing the role of paths connecting these solutions. If $x_1(t)$ and $x_2(t)$, $t \in [0,1]$ denote these two paths, then for every $t \in [0,1]$ we have $H\big(x_i(t),t\big) = 0$, $i = 1,2$. Taking derivative with respect to t we have

$$\begin{aligned}\frac{\partial H\big(x(t),t\big)}{\partial t} = 0 &\Longrightarrow \frac{\partial H\big(x(t),t\big)}{\partial x(t)}\frac{dx(t)}{dt} + \frac{\partial H\big(x(t),t\big)}{\partial t} = 0\\ &\Longrightarrow \frac{\partial H}{\partial x}|_{x=x(t)}x'(t) + \frac{\partial H}{\partial t}|_{x=x(t)} = 0.\end{aligned}$$

Therefore in our case we get the following differential equation

$$x'(t) = \frac{-x(t)}{tx(t) + x(t) + t}$$

with the initial conditions $x(0) = \pm 1$. Now use a numerical ODE solving method. For illustration, we consider the Euler method. Let $h = \frac{1}{n}$ and $t_i = \frac{i}{n}$, $i = 0, 1, \ldots, n$, and use

$$x(t_i) = x(t_{i-1}) + hx'(t_{i-1}), \quad i = 1, \ldots, n$$

recursively to approximate the end points of the two paths. Taking $n = 10$ we get $x_1(1) = 0.3204874924$ and $x_2(1) = -1.375719799$. Increasing n to 100, we get $x_1(1) = 0.3616822442$ and $x_2(1) = -1.366972812$. The exact solutions to $f(x) = 0$ are $x_1 = \frac{1-\sqrt{3}}{2} \simeq 0.3660254038$ and $x_2 = \frac{-1-\sqrt{3}}{2} \simeq -1.366025404$.

This was just an example to get familiar with numerical homotopy methods. To read more about numerical homotopy methods see Sommese et al. (2005); Bates et al. (2013). An advantage of numerical homotopy methods is that for big and complicated systems they are faster than solving the system directly. These methods can be used to study the discriminant variety of the system as well. For example one can mention algorithms 1 and 2 in Harrington et al. (2016). (Griffin and Hauenstein, 2005, Proposition 8) uses numerical homotopy methods to find real critical solutions to the system. (Harrington et al., 2016, Algorithm 1) uses such results to find the discriminant variety of a system of polynomials with only one parameter, which is a finite set of points in a line.

(Harrington et al., 2016, Algorithm 2) targets polynomial systems with two parameters, p and q. Denote the vector of variables with x, and the system with $F_{p,q}(x) = 0$. Let $\Delta(x, p, q)$ denote the set of polynomials arising from the ℓ-fitting ideal of the Jacobian matrix of the system as discussed before with $\ell =$ number of variables - dimension of the variety. For a fixed random point $(a, b) \in \mathbb{R}^2$ and a new parameter $d \in \mathbb{R}$, define the following system

$$F_{p,q}(x) = 0, \quad ap + bq - d = 0, \quad \Delta(x, p, q) = 0, \tag{2.12}$$

with one parameter d and x, p and q as variables. The solutions of (2.12) are the intersections of the line $ap+bq = d$ in the (p, q)-plane with the discriminant variety of the original system. The critical points of (2.12), which can be found by (Harrington et al., 2016, Algorithm 1), determine where the number of intersection points of the line $ap + bq = d$ and the discriminant variety change. Therefore, it suffices to take sample values of d between every two consecutive such critical values. Restricting the original system to parameters on the lines $ap+bq = d^\star$, where the $d^\star$'s are either critical or chosen sample values for d, converts the system to a polynomial system with one-dimensional parameter space, since $q = \frac{d^\star - ap}{b}$. Now, by (Harrington et al., 2016, Algorithm 1) one finds the critical values of the restricted one-dimensional system. Gathering the information obtained from these computations one can draw the discriminant variety for the original system in the (p, q)-plane.

The problem with these algorithms is that they are not practical for higher-dimensional parameter spaces. One needs to fix all parameters except a few to use these algorithms or to modify them with some other methods.

Kac-Rice formula. Marc Kac used a formula to compute the expected number of solutions to a univariate polynomial with random coefficients in Kac (1943). Later this formula got extended, see (Adler and Taylor, 2007, Theorem 11.2.1). Here we derive a simple form of this formula, which is used in the third paper of this thesis.

Let δ_x be the Dirac delta function at x,

$$\delta_x(u) = \begin{cases} 1 & \text{if} \quad u = x, \\ 0 & \text{if} \quad u \neq x. \end{cases}$$

Assume f is a well-behaved function for the following computations, such as a polynomial. First note that

$$\int_{\mathbb{R}} \delta_x(y) f(y) dy = f(x).$$

Now let $I \subseteq \mathbb{R}$ be an interval in which $f(t) = 0$ has only one isolated simple solution, Then, by a change of variables

$$1 = \int_{\mathbb{R}} \delta_0(y) dy = \int_I \delta_0\big(f(t)\big) |f'(t)| dt.$$

If all positive solutions of $f(t) = 0$ are simple (have multiplicity one), then

$$|f^{-1}(0) \cap \mathbb{R}_{>0}| = \int_0^\infty \delta_0\big(f(t)\big) |f'(t)| dt.$$

Now let $f(t)$ be a random polynomial, for example, such that its coefficients are polynomials in random parameters. Assume the density distribution functions are well-behaved for the following computations. Since $f(t)$ is a random polynomial, its derivative is also a random polynomial. Let $p_t(x, y)$ and $p_t(x)$ denote the joint density distribution of $\big(f(t) = x, f'(t) = y\big)$ at a given t, and density distribution of $f(t) = x$ at a given t respectively. Then

$$\begin{aligned}
\mathbb{E}\big(|f^{-1}(0) \cap \mathbb{R}_{>0}|\big) &= \int_y \int_x \Big(\int_0^\infty \delta_0(x) |y| dt \Big) p_t(x, y) dx dy \\
&= \int_0^\infty \int_y \big(\int_x \delta_0(x) |y| p_t(x, y) dx \big) dy dt \\
&= \int_0^\infty \int_{x=0,y} |y| p_t(0, y) dy dt \\
&= \int_0^\infty \mathbb{E}(|y| \mid x = 0) p_t(0) dt \\
&= \int_0^\infty \mathbb{E}\big(|f'(t)| \mid f(t) = 0\big) p_t(0) dt.
\end{aligned}$$

This formula is known as the Kac-Rice formula.

Monte-Carlo method for computing integrals. For numerical higher dimensional integration the one-dimensional ideas such as Riemannian sum or Simpson's rule get very slow. One method which is usually faster than one-dimensional ideas for multiple integration is Monte-Carlo integration. The idea is simple. Let I be the following multiple integration on a region $M \subseteq \mathbb{R}^n$;

$$I = \int_M f(x_1, \dots, x_n) dx_1 \dots dx_n.$$

Choose a probability distribution on M and let $p(x_1, \dots, x_n)$ be the density function of this distribution. Then

$$I = \int_M \frac{f(x)}{p(x)} p(x) dx = \mathbb{E}\big(\frac{f(x)}{p(x)}\big).$$

Then by the law of Large Numbers, for a big enough $N \in \mathbb{N}$ and $x^{(1)}, \dots, x^{(N)}$ being N random samples of the random vector x, we have

$$I \simeq \frac{1}{N} \sum_{i=1}^{N} \frac{f(x^{(i)})}{p(x^{(i)})}. \tag{2.13}$$

Let $\hat{I}$ denote the sum in (2.13). Then the standard error of this approximation is

$$\hat{e} = \sqrt{\frac{\sum_{i=1}^{N} \big(\frac{f(x^{(i)})}{p(x^{(i)})} - \hat{I}\big)^2}{N(N-1)}}. \tag{2.14}$$

To read more about Monte-Carlo method we refer the reader to Owen (2013).

2.5 Other kinetics

Mass action kinetics is suitable when some conditions hold for the network such as having a homogeneous and dilute solution. There are other kinetics that can be used when different assumptions on the solution and environment of the network hold. An example of such kinetics is generalized mass action kinetics. The ODE system of the network in this kinetics is written as for mass action kinetics, with the only difference that the monomials of each reaction are not necessarily determined by the stoichiometric vector of the reactant. So when the network is equipped with the generalized mass action kinetics, an extra complex called *kinetic complex* is associated with each reaction. To read more about this kinetics we refer the reader to Müller and Regensburger (2012).

Other than kinetics that are introduced for different solutions, there are kinetics that are introduced when one reduces the network and uses approximations. Two well-known such kinetics are Michaelis-Menten and Hill kinetics. Michaelis–Menten kinetics is used when there is a separation of time scale in some enzymatic reactions like

$$X_1 + E \rightleftharpoons Y \longrightarrow X_2 + E.$$

In this reaction, the species X_1 forms an intermediate complex with the enzyme E and then is modified to the species X_2. But if the reactions $X_1 + E \rightleftharpoons Y$ occur at a faster scale then after writing the ODE system using mass action and using approximations, one can replace the above enzymatic reactions with $X_1 \longrightarrow X_2$ with a fractional rate $\frac{\alpha x_1}{\beta + x_1}$. To read how to do this in detail we refer the reader to (Ingalls, 2012, Subsection 3.1.1).

We explain how Hill-kinetics arise from an approximation of the ODE system written with mass action kinetics with the gene regulatory network of proteins A and B. Remember that in our model the inhibitory bindings on promoters were cooperative. A cooperative binding means occupation of a binding site facilitates occupation of the other binding site and the affinity to bind is higher than unbinding. Mathematically speaking in our model it means

$$0 \ll \frac{k_9}{k_{11}} \ll \frac{k_{13}}{k_{15}} \quad \text{and} \quad 0 \ll \frac{k_{10}}{k_{12}} \ll \frac{k_{14}}{k_{16}}. \tag{2.15}$$

Consider the system (2.8). Using equations $k_9 d_A p_B - k_{11} d_A^b = 0$ and $k_{13} d_A^b p_B - k_{15} d_A^{bb} = 0$ and the conservation law $d_A + d_A^b + d_A^{bb} = T_3$ one can write

$$d_A = \frac{T_3}{1 + \frac{k_9}{k_{11}} p_B + \frac{k_{13}}{k_{15}} p_B^2}. \tag{2.16}$$

Using (2.15) one can approximate Equation (2.16) as follows

$$d_A \approx \frac{T_3}{1 + \frac{k_{13}}{k_{15}} P_B^2}.$$

Using this approximation in the steady state polynomial of M_A one has

$$mi_A = \frac{(k_1 T_1 T_3)}{1 + (\frac{k_{13}}{k_{15}}) p_B^2} - k_5 m_A.$$

The first term is a Hill function. Hill kinetics arises from eliminating some intermediates in cooperative bindings, which is a similar approach to Michaelis–Menten kinetics. To read more about Hill kinetics see Bhaskaran et al. (2015) and (Ingalls, 2012, Subsection 3.3).

2.6 Closing

The results in the papers of this thesis deal with several tasks and questions. To give an overview of the motivation behind these three papers we highlight five tasks:

Given a reaction network;

- compute a reduced Gröbner basis for its steady state ideal.
- detect if it is a binomial network.
- detect if it is multistationary.
- give a choice of parameters in a given box in the parameter space for which the network exhibits multistationary behavior.
- decide what parameter choices are more robust to produce a specific number of steady states.

3 Contribution to the state of the art

3.1 Summary of contributions

As mentioned in Section 2.3, regarding detecting binomiality of the steady state ideal of a network one can use reduced Gröbner basis. Because the computation of Gröbner bases usually takes a long time, it is always preferred to use alternative methods. There are works and attempts to give better alternative methods mostly using linear algebra such as Conradi and Kahle (2015). Not using Gröbner bases, makes these methods fast, but they provide only sufficient conditions for binomiality. Our work in the first paper provides a sufficient and necessary condition to check binomiality by exploiting the properties of intermediate species. It is faster than computing a reduced Gröbner basis of the steady state ideal of the original network. In this work we also provide faster algorithms to compute reduced Gröbner bases of the steady state ideal of networks with intermediate species.

The second paper targets detection of multistationarity. There are several works introducing methods to detect multistationarity of networks with binomial steady state ideal, but usually in papers on injectivity of polynomial systems and papers studying a class of binomial networks. So we gathered the literature on how to detect multistationarity of a binomial network in one place. Further, we introduced a new algorithm to study multistationarity of a network with intermediate species and a binomial core network, which are not necessarily binomial themselves.

Finally we start studying the parameter region of multistationarity and questions related to parameter choice for producing a multistationary behavior. In this direction there are many works. The works can be divided into two categories. One group give exact descriptions of the region in the parameter space where the network exhibits multistationary behavior, but they are limited to low-dimensional parameter space. So one needs to fix all parameters except one or two and then implement these methods to find a suitable description for the remaining parameters. Another group of methods use random sampling and solving the system in many points.

These approaches are very time consuming. We suggested using the Kac-Rice formula and introduce new algorithms to study questions regarding parameter region of multistationarity. A more interesting new idea introduced in this work is a measure of robustness with respect to the number of steady states. We provide a tool to compare two parameter choices that produce the same number of steady states. Our algorithm decides which parameter choice is stronger under pertubations without knowing the description of the region in the parameter space where the network has that number of steady states.

3.2 Overview of Paper 1

Remember that the definition of intermediates, core and extended networks was given in Section 2.2. Before going into any result, we briefly explain the reduction process via eliminating intermediate species. Consider the following simple network.

$$X_0 + X_2 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \xrightarrow{\kappa_3} Y_2 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} X_1 + X_2$$
$$X_1 \xrightarrow{\kappa_6} X_0.$$

The species Y_1 and Y_2 are intermediates. The steady state polynomials of these two species are

$$F_1 = F_{Y_1}(y, x) = \kappa_1 x_0 x_2 - \kappa_2 y_1 - \kappa_3 y_1,$$
$$F_2 = F_{Y_2}(y, x) = \kappa_3 y_1 - \kappa_4 y_2 + \kappa_5 x_1 x_2.$$

The system $F_1 = F_2 = 0$ is linear with respect to y_1 and y_2.

$$(\kappa_2 + \kappa_3) y_1 + (0) y_2 = \kappa_1 x_0 x_2$$
$$(-\kappa_3) y_1 + (\kappa_4) y_2 = \kappa_5 x_1 x_2.$$

Solving this linear system, we have

$$y_1 = \tfrac{\kappa_1}{\kappa_2+\kappa_3} x_0 x_2, \quad y_2 = \tfrac{\kappa_5}{\kappa_4} x_1 x_2 + \tfrac{\kappa_3 \kappa_1}{\kappa_4(\kappa_2+\kappa_3)} x_0 x_2.$$

Let $\mu_{i,c}$ denote the coefficient of the monomial corresponding to the non-intermediate complex c in the solution of y_i. So

$$\mu_{X_0+X_2,1} = \tfrac{\kappa_1}{\kappa_2+\kappa_3}, \quad \mu_{X_1+X_2,1} = 0, \quad \mu_{X_0+X_2,2} = \tfrac{\kappa_3\kappa_1}{\kappa_4(\kappa_2+\kappa_3)}, \quad \mu_{X_1+X_2,2} = \tfrac{\kappa_5}{\kappa_4},$$
$$\mu_{X_1,1} = 0, \quad \mu_{X_0,1} = 0, \quad \mu_{X_1,2} = 0 \quad \mu_{X_0,2} = 0.$$

As it can be seen, $\mu_{i,c}$ is nonzero only when c is an input for Y_i. After eliminating Y_1 and Y_2 from the network, the core network is

$$X_0 + X_2 \xrightarrow{k_1} X_1 + X_2, \quad X_1 \xrightarrow{k_2} X_0.$$

Define the following map

$$\begin{cases} \phi\colon & \mathbb{R}[k][x] & \to & \mathbb{R}(\kappa)[y,x] \\ & k_{c\to c'} & \mapsto & \phi_{c\to c'}(\kappa) = \kappa_{c\to c'} + \sum_{i=1}^{m} \kappa_{Y_i\to c'}\mu_{i,c}. \end{cases}$$

Here m denote the number of intermediates. If $\{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}\}$ ($\mathcal{R}$ is the set of reactions for the core network) is algebraically independent over $\mathbb{R}$, then the map ϕ can be extended to $\mathbb{R}(k)[x]$. Denote it by Φ. Finally define the following polynomials

$$H_i(y,x) = y_i - \sum_{c \text{ input for } Y_i} \mu_{i,c}x^c.$$

While reducing a network via the elimination of its intermediates, to keep information of the original network we need three objects together; the core network, the map Φ, the set of polynomials $H_i(x,y)$. Gröbner bases of the steady state ideal of the extended network and the steady state ideal of the core network are related as the following theorem states.

Theorem 1 (Theorem I.3.4)**.** *Fix a monomial order on $\mathbb{R}(k)[x]$ associated with an $n \times n$ matrix Q, and let G be a Gröbner basis of I with this order. Then, $\widetilde{G} = \Phi(G) \cup \{H_i(y,x)\}$ is a Gröbner basis of $\widetilde{I}$ with the monomial order on $\mathbb{R}(\kappa)[y,x]$ associated with the matrix*

$$\widetilde{Q} = \begin{bmatrix} id_m & 0 \\ 0 & Q \end{bmatrix}, \tag{3.1}$$

where id_m is the identity matrix of size m.

If G is reduced, then $\Phi(G) \cup \left\{ y_i - \operatorname{rem}\left(\sum_{c\in\mathcal{C}} \mu_{i,c}x^c, \Phi(G)\right)\right\}$ is the reduced Gröbner basis of $\widetilde{I}$.

By $\operatorname{rem}(f,B)$ we mean a remainder of the division of a polynomial f by a set of polynomials B. The remainder is unique if B is a Gröbner basis.

Theorem 1 suggests a new algorithm to compute (reduced) Gröbner basis for the steady state ideal of networks with intermediates.

Algorithm 2.

Input: *A network with intermediates and a monomial order such as lex, grlex, grevlex, etc. or if the non-intermediate species are already determined, an arbitrary monomial order on them.*

Output: *A (reduced) Gröbner basis for the steady state ideal in the monomial order associated with the matrix $\widetilde{Q}$.*

Procedure:

1. *Find intermediates.*
2. *Solve the linear system of intermediates to get $\mu_{i,c}$'s and H_i's.*
3. *Simplify the network by eliminating intermediates to get the core network.*
4. *Compute (reduced) Gröbner basis of the steady state ideal of the core network in the given monomial order associated with the matrix Q in the input. Denote this Gröbner basis by G.*
5. *Let $\widetilde{G} = \Phi(G) \cup \{H_i \mid i = 1, \ldots, m\}$ (or $\widetilde{G} = \Phi(G) \cup \{\text{rem}\left(H_i, \Phi(G)\right) \mid i = 1, \ldots, m\}$ for the reduced case). Return $\widetilde{G}$ as the output.*

The ERK activation network is used as an example, Example I.3.5, to demonstrate that using Algorithm 2 is faster than computing a Gröbner basis of the extended network directly. Comparison of methods is given in Remarks I.3.6 and I.3.7.

One application of Gröbner bases in CRN is in model discrimination Gunawardena (2007); Manrai and Gunawardena (2008); Karp et al. (2012); Harrington et al. (2012); Meshkat et al. (2016). When several models are suggested, one way to exclude wrong models is to use invariants. *Invariants* are relations, including only some observable species, that are satisfied at steady states. A correct model should have invariants compatible with the experimental observations. Corollary I.3.8 explains how one can save time using Theorem 1 to compute invariants of a model not involving the concentration of intermediates.

Another application of Gröbner bases is to detect if an ideal can be generated by binomials. Remember from Section 2.3 that there are methods developed to study multistationarity of binomial networks. Theorem I.3.10 suggests a less time consuming method to detect binomiality of the steady state ideal of the extended networks.

Theorem 3 (Theorem I.3.10)**.** *Let $\mathcal{N}$ be a reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of m intermediates $Y_1, \ldots, Y_m$.*

The steady state ideal $\widetilde{I}$ is binomial if and only if

- *I is binomial, and,*
- *for any reduced Gröbner basis G of I and for every $i = 1, \ldots, m$, the remainder of the division of $\sum_{c \in \mathcal{C}} \mu_{i,c} x^c$ by $\Phi(G)$ has at most one term.*

Corollary I.3.11 is a result of Theorem 3 that states that an extended network with 1-input intermediates has a binomial steady state ideal if and only if its core network has a binomial steady state ideal.

Section I.4 is devoted to the study of the algebraic independence condition of the set $\{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}_2\}$ over $\mathbb{R}$. Lemma I.4.4 makes this condition equivalent with the algebraic independence of several smaller subsets. Corollary I.4.6 and Lemma I.4.7 go further and show that the algebraic independence condition of many of those smaller subsets hold. In fact they cover the algebraic independence condition of typical realistic examples. Therefore most of the time just by looking at the graphical representation of the network one can see that this condition holds and there is no need to do any computation.

Finally Section I.5 investigates if there exist similar results discussed so far for model reductions using removal of enzymes instead of removal of intermediates. An enzyme is a species for which the stoichiometric coefficients on both sides of every reactions are equal. For example in the gene regulatory network of proteins A and B in Figure 2.1, the species E and F are enzymes.

We finish this section by applying the above discussion on the gene regulatory network of proteins A and B.

Example 1. Consider the network in Figure 2.1. The species D_A^{bb} and D_B^{bb} are intermediates. The algebraically independence condition holds by Corollary I.4.6. The core network after removal of D_A^{bb} and D_B^{bb} and the complexes $D_A^b + P_B$ and $D_B^b + P_A$ has two new intermediates D_A^b and D_B^b. We removed the isolated complexes because they are no longer involved in any reaction and thus not affecting the steady state ideal. The algebraically independence condition again holds by the same reason. The new core network after removal of D_A^b and D_B^b and the complexes not involved in any reaction has the following ODE system;

$$\begin{aligned} \dot{m_A} &= k_1 d_A e - k_5 m_A & \dot{m_B} &= k_2 d_B f - k_6 m_B \\ \dot{p_A} &= k_3 m_A - k_7 p_A & \dot{p_B} &= k_4 m_B - k_8 p_B \\ \dot{e} &= \dot{f} = \dot{d_A} = \dot{d_B} = 0. \end{aligned}$$

The steady state ideal is trivially binomial since the steady state polynomials are already binomials. Because in both reduction steps, intermediates are 1-input, by Theorem 3 the original network is also binomial.

The theorem in fact provides more than just detecting binomiality. Adding steady state polynomials of D_A^b and D_B^b of the middle extended network and the steady state polynomials of D_A^{bb} and D_B^{bb} of the original extended network, which are binomials, to the binomial generator of the final core network, will give a binomial basis of the steady state ideal.

3.3 Overview of Paper 2

Recall the definition of intermediates, inputs, extended and core networks from Section 2.2. A specific simple but important type of extended networks are called canonical extensions.

Definition 5 (Definition II.3.2)**.** Let $\mathcal{N}$ be a network and $C = \{c_1, \ldots, c_m\} \subseteq \mathcal{C}$. The *canonical extension* of $\mathcal{N}$ associated with C, denoted by $\widetilde{\mathcal{N}}_C = (\widetilde{\mathcal{S}}, \widetilde{\mathcal{C}}, \widetilde{\mathcal{R}})$, is the extension of $\mathcal{N}$ via the addition of 1-input intermediates $Y_1, \ldots, Y_m$ such that

$$\widetilde{\mathcal{R}} = \mathcal{R} \cup \left\{c_i \rightleftharpoons Y_i\right\}_{i=1}^{m}.$$

The canonical network associated with $C = \mathcal{C}$ is called the *largest canonical network*.

From results of Feliu and Wiuf (2013a) under a condition called the generalized realization condition, multistationarity of an extended network is equivalent to multistationarity of a canonical extension with the same core network and the same set of inputs. Other than the generalized realization condition, there is another condition in Feliu and Wiuf (2013a) called the realization condition, these conditions are studied in Section II.5. One method to check these conditions is to use CAD. But as it is mentioned in Section 2.4, CAD is applicable on small size examples. Similar to Section I.4, in Section II.5, these realization conditions of the original network are shown to be equivalent with realization conditions of several smaller subnetworks. Proposition II.5.3 introduces some classes of subnetworks for which these conditions hold.

A binomial network is called *complete* if it fulfills (surj) and its steady state ideal has a basis consisting of $\operatorname{rank}(\mathcal{N})$ binomials. Assume that the steady state ideal of the network is generated by $\operatorname{rank}(\mathcal{N})$ binomials

$$p_i(k)x_1^{c_{i,1}} \ldots x_n^{c_{i,n}} - p_i'(k)x_1^{c'_{i,1}} \ldots x_n^{c'_{i,n}}, \quad i = 1, \ldots, \operatorname{rank}(\mathcal{N}).$$

Define the following matrices

$$M = \left[c_{i,j} - c'_{i,j}\right] \in \mathbb{R}^{\operatorname{rank}(\mathcal{N}) \times n} \text{ and } \Gamma = \begin{bmatrix} M \operatorname{diag}(\lambda) \\ Z \end{bmatrix}, \tag{3.2}$$

where $\lambda = (\lambda_1, \ldots, \lambda_n)$ is a vector of auxiliary indeterminates. Lemma II.3.9 and Proposition II.3.10 show that a canonical extension of a complete binomial network is again a complete binomial network. Thus one has Theorem II.3.11.

Theorem 4 (Theorem II.3.11)**.** *Let $\mathcal{N}$ be a complete binomial network and $\widetilde{\mathcal{N}}$ an extended network satisfying the generalized realization condition with the input set $C \subseteq \mathcal{C}$. Then $\widetilde{\mathcal{N}}$ is multistationary if and only if* $\det(\widetilde{\Gamma}_C)$ *is either identically zero or has both positive and negative coefficients.*

Lemma II.4.1 relates $\det(\Gamma)$ of extended and core binomial networks. Theorem II.4.2 restates Theorem 5.1 of Feliu and Wiuf (2013a), that is an extended network of a multistationary network is multistationary, but with different assumptions.

Theorem 5 (Theorem II.4.2)**.** *Let $\widetilde{\mathcal{N}}$ be a binomial extension of a complete binomial network $\mathcal{N}$ via the addition of intermediates and let Γ be as in (3.2) for $\mathcal{N}$. Assume in addition that (surj) holds for $\widetilde{\mathcal{N}}$. If $\mathcal{N}$ is multistationary and $\det(\Gamma) \neq 0$, then $\widetilde{\mathcal{N}}$ is multistationary.*

Definition 6 (Definition II.4.5)**.** Let $\mathcal{N}$ be a reaction network with the set of complexes $\mathcal{C}$. Let Mult be the set of all subsets of complexes $C \subseteq \mathcal{C}$ for which the canonical extension $\widetilde{\mathcal{N}}_C$ of $\mathcal{N}$ associated with C is multistationary. Denote the set of minimal elements of Mult with respect to inclusion by Circuits.

The set Mult is called the *multistationarity structure* of $\mathcal{N}$ and the elements of Circuits are called *circuits of the multistationarity structure* of $\mathcal{N}$.

Since the multistationarity structure of a multistationary network is just the whole power set of $\mathcal{C}$, we focus on core networks that are not multistationary. Lemmas II.4.6 and II.4.7 build the steps towards Algorithm II.4.8. This algorithm takes a complete binomial core network that is not multistationary as the input and gives the multistationarity structure as the output. Remark II.4.10 compares the use of Algorithm II.4.8 with other search approaches for finding the multistationarity structure. Section II.4.3 applies Algorithm II.4.8 on the core n-site phosphorylation network, Equation (II.18).

Theorem 6 (Theorem II.4.12)**.** *An extension of the core n-site phosphorylation network via the addition of intermediates satisfying the generalized realization condition is multistationarity if and only if there exists an intermediate having at least one of $X_0 + E, \ldots, X_{n-2} + E, X_n + F, \ldots, X_2 + F$ among its inputs.*

Below we compare Algorithm II.4.8 with CRNToolbox:

- To find the multistationarity structure of a reaction network using CRNToolbox one needs to give 2^m networks to the software (or at least as many as needed to use the 1st or the 2nd approaches in Remark II.4.10). But using Algorithm II.4.8, it is possible to get the multistationarity structure in one go.

- The other advantage of Algorithm II.4.8 is that one can do symbolic computation such as finding the multistationarity structure of the n-site phosphorylation for arbitrary n.

- The CRNToolbox only determines whether the network is multistationary while Algorithm II.4.8 determines which complexes can cause multistationarity by being input of an intermediate. Therefore to change the multistationarity of the network you know what intermediate(s) should be added or removed.

We finish this section by applying the above discussion on our simple gene regulatory network of Chapter 2.

Example 2. Consider the network in Figure 2.1. As mentioned in Example 1 this network is the result of extending the second core network in Figure 3.1. Contrary to Section 3.2, here we do not remove the isolated complexes from the graph of the network. By Definition 5, both of these two extensions are canonical, so the generalized

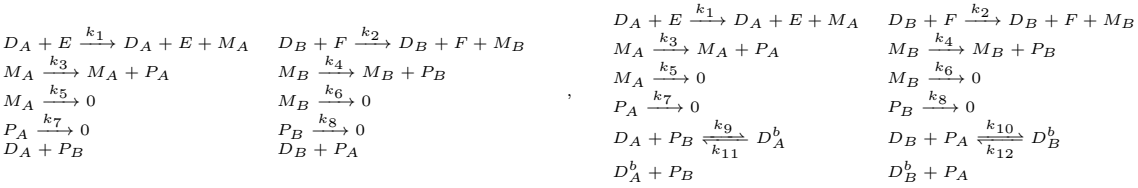


Figure 3.1: From left to right, the second core and the first core networks.

realization condition holds. The second core network is a complete binomial network and $\det(\Gamma) = \lambda_1\lambda_2\lambda_3\lambda_4$. By Theorem II.2.7, it is not multistationary. Running Algorithm II.4.8 on this network, we get that an extension of it is multistationary if and only if it has both complexes D_A+P_B and D_B+P_A as inputs. Therefore the first core network is already multistationary and so are its extensions including the original network. As a conclusion we do not only detect that the network is multistationary, but also figured out that the reason is the inhibitory bindings, without them, the network is not multistationary.

3.4 Overview of Paper 3

Recall the discussion on parameter regions of multistationarity from Section 2.4. CRNToolbox only provides one parameter choice and is not possible to impose conditions on the parameters in this software. Therefore CRNToolbox is not a software to study the region of multistationarity. There are four other methods mentioned in Section 2.4;

1- solving the system in random points from the parameter space,

2- solving the system in random points in sub-boxes and taking average of the number of steady states for each sub-box,

3- CAD,

4- using numerical homotopy methods to approximate the discriminant variety.

In this section we refer to these methods as methods 1-4. Section III.2 reviews method 2. Section III.3 introduces a new method to study the region of multistationarity of CRNs using the Kac-Rice formula. For some networks, studying the positive solutions of the system (2.7) can be reduced to studying the positive roots of a univariate polynomial $f(t)$. An example of this case is the network in Example III.1.2 which is studied in Kothamachu et al. (2015); Conradi et al. (2017). Theorem III.3.1 states the Kac-Rice formula in the setting needed to study positive roots of a univariate polynomial with coefficients being polynomials of parameters, in our case reaction rate constants and constants of conservation laws. Proposition III.3.2 simplifies this formula for the case that $f(t)$ is linear in one parameter, k_1, and the coefficient of k_1 is always positive for any acceptable (positive) choice of other parameters and t.

Proposition 7 (Proposition III.3.2)**.** *Let $f\colon \mathbb{R}_{>0} \to \mathbb{R}$ be a polynomial map with coefficients being polynomials in $k_1, \dots, k_m$. Assume that each parameter k_i follows a continuous random distribution with support in $\mathcal{R}_{>0}$ and density ρ_i, that ρ_i is continuous except maybe in a finite number of points. Assume also that $k_1, \dots, k_m$ are independently distributed. Further assume that $f_k(t) = h_1(k_2, \dots, k_m, t)k_1 + h_2(k_2, \dots, k_m, t)$ is linear in k_1, and that $h_1(k_2, \dots, k_m, t)$ is a polynomial in $t, k_2, \dots, k_m$ with all coefficients positive. Let*

$$g(k_2, \dots, k_m, t, x) = \frac{x - h_2(k_2, \dots, k_m, t)}{h_1(k_2, \dots, k_m, t)}.$$

Then the Kac-Rice integral is

$$\int_0^\infty \mathbb{E}\big(|f'(t)| \mid f(t) = 0\big) p_t(0) dt = \int_0^\infty A(t) \cdot B(t) dt,$$

where, for $\bar{\rho}(k_2, \dots, k_m, t) = \rho_1(g(k_2, \dots, k_m, t)) \cdot \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m)$ we have

$$A = \int_{\mathcal{R}_{>0}^{m-1}} |f_k'(t)_{|_{k_1 = g(k_2, \dots, k_m, t, 0)}}| \; \frac{\bar{\rho}(k_2, \dots, k_m, t)}{\int_{\mathcal{R}_{>0}^{m-1}} \bar{\rho}(s_2, \dots, s_m, t) ds_2 \dots ds_m} dk_2 \dots dk_m,$$

$$B = \int_{\mathcal{R}_{>0}^{m-1}} \frac{\bar{\rho}(k_2, \dots, k_m, t)}{h_1(k_2, \dots, k_m, t)} dk_2 \dots dk_m.$$

The idea of using the Kac-Rice formula to find the region of multistationarity is explained in Example III.3.3. To be able to compare the result with the result obtained by method 3, the example implements the new method on a two-dimensional parameter space. The method divides the box of interest to sub-boxes and then computes the average number of steady states in each sub-box with the Kac-Rice integral and equipping the parameters with uniform distribution.

The first difference of this approach with method 2 is that the Kac-Rice integral gives the exact expected number of steady states, see Example 3 below. One may instead of computing the Kac-Rice integral exactly, which is not always possible, use numerical integration. Both for using 1-dimensional numerical integration methods or higher dimensional numerical integrations such as Monte-Carlo, it is only needed to evaluate the integrand function in sample points, while in method 2 (or method 1) one needs to solve the system in the sample points which needs more computations. The advantage of this new method to methods 3 and 4 is clear due to these methods are not practical for higher dimensional parameter spaces. But using this new method one can store the expected number of steady states of sub-boxes in an n-dimensional array.

Example 3. Consider the following simple network:

$$X \underset{d}{\overset{c}{\rightleftharpoons}} 0, \quad 2X \xrightarrow{a} 3X. \tag{3.3}$$

There is only one steady state polynomial and no conservation law. The system (2.7) is

$$f(t) = at^2 - bt + c.$$

To compute the average number of steady states of this network in the parameter region restricted to the box $[0,1]\times[0,1]\times[0,1]$, we equip the parameters a, b, c with the uniform distribution $U([0,1])$ independently. The system $f(t) = 0$ has infinite positive solutions when $a = b = c = 0$, one positive solution when $a = 0, b, c \neq 0$ or $c = 0, a, b \neq 0$ or $a \neq 0, b^2 = 4ac$ (the double root is treated as one root). All of these regions are of measure zero. There are two remaining regions, which are not of measure zero.

In the region between the planes $a = 0$, $b = 0$ and $c = 1$ and the surface $b^2 = 4ac$, the system $f(t) = 0$ has two positive roots and outside of all mentioned regions it has no real root. Thus

$$\begin{aligned}
\mathbb{E}(|f^{-1}(0)\cap\mathbb{R}_{>0}|) &= 2\int_0^1\int_0^{\frac{1}{4}\wedge 1}\int_{2\sqrt{ac}}^1 db\,dc\,da \\
&= 2\Big(\int_{\frac{1}{4}}^1\int_0^{\frac{1}{4a}}\int_{2\sqrt{ac}}^1 db\,dc\,da + \int_0^{\frac{1}{4}}\int_0^1\int_{2\sqrt{ac}}^1 db\,dc\,da\Big) \\
&= 2\big(\tfrac{\ln(2)}{6} + \tfrac{5}{36}\big).
\end{aligned}$$

Now we use the Kac-Rice formula. Since $f(t)$ is linear in a (and c) with coefficients independent of b, c (and a, b), we can use the Equation III.(8) and write

$$\mathbb{E}(|f^{-1}(0)\cap\mathbb{R}_{>0}|) = \int_0^1 \frac{1}{1}\int_0^1\int_0^1 |f'(t)|_{c=bt-at^2}\chi_{[0,1]}(bt-at^2)da\,db\,dt + \int_1^\infty \frac{1}{t^2}\int_0^1\int_0^1 |f'(t)|_{a=\frac{bt-c}{t^2}}\chi_{[0,1]}(\tfrac{bt-c}{t^2})dc\,db\,dt.$$

Now by solving $0 < bt - at^2 < 1$ assuming $0 < a, b, t < 1$ one has

$$\int_0^1\int_0^1\int_0^1 |f'(t)|_{c=bt-at^2}\chi_{[0,1]}(bt-at^2)da\,db\,dt = \int_0^1\int_0^t\int_0^{\frac{1}{t}b} |f'(t)|_{c=bt-at^2}da\,db\,dt + \int_0^1\int_t^1\int_0^1 |f'(t)|_{c=bt-at^2}da\,db\,dt.$$

Similarly, by solving $0 < \frac{bt-c}{t^2} < 1$ assuming $0 < b, c$ and $1 < t$ one has

$$\int_1^\infty \frac{1}{t^2}\int_0^1\int_0^1 |f'(t)|_{a=\frac{bt-c}{t^2}}\chi_{[0,1]}(\tfrac{bt-c}{t^2})dc\,db\,dt = \int_1^\infty\int_0^{\frac{1}{t}}\int_0^{tb}\frac{1}{t^2}|f'(t)|_{a=\frac{bt-c}{t^2}}dc\,db\,dt + \int_1^\infty\int_{\frac{1}{t}}^1\int_0^1\frac{1}{t^2}|f'(t)|_{a=\frac{bt-c}{t^2}}dc\,db\,dt.$$

These last integrals can be computed exactly and we have

$$\mathbb{E}(|f^{-1}(0)\cap\mathbb{R}_{>0}|) = 2(\tfrac{1}{18}+\tfrac{1}{12}+\tfrac{\ln(2)}{6}) = 2(\tfrac{\ln(2)}{6}+\tfrac{5}{36}).$$

Section III.3.3 introduces a new concept "measure of robustness" which determines how deep in a parameter region with a fixed number of steady states the point is without knowing the description of the region. The idea is to compute the expected number of steady states for a small neighborhood of the point and then increasing the size of the neighborhood until the expected number of steady states drops. This will happen if the neighborhood intersect another region of parameters where the network has less number of steady states.

If one wants to compute such a quantity using methods 3 and 4, if possible, one needs to find the polynomial inequalities defining the region and then computing the distance of the point from the boundaries of this region. Trying to implement the same idea but using the same approach of methods 1 and 2, again one needs to do more computations due to he needs to solve the system in sample points. Using Kac-Rice integral he needs to evaluate the integrand function in sample points. Example III.3.5 computes the measure of robustness for two points from the multistationarity

region depicted in the 2-dimensional parameter region in Examples III.2.1 and III.3.3 to compare the result with visual figures. We exemplify by computing measure of robustness with 8 parameters to show that this approach is not limited to small size systems, as for methods 3 and 4. Section III.4 discusses the numerical methods used for computation of the integrals.

4

Perspective

In this chapter we discuss some perspective suggested by the work done in the papers I-III.

Gröbner basis. As it is mentioned in the introduction of the first paper, the Algorithm 2 can be used for arbitrary parametric ideals. Instead of intermediates one needs to find variables that appear linearly in a set of generators of the ideal. But then one needs to know how to define Φ and what conditions are necessary on Φ to have an equivalent version of Theorem I.3.4. A future work is to find what conditions are needed, also how to check them. For example, we used the graphical representation of the network to check the algebraic independence of $\{\phi_{c\to c'} \mid c \to c' \in \mathcal{R}\}$, while it is not the case for an arbitrary parametric ideal which is not the steady state ideal of a network.

In Section I.5 we saw that enzymes are not as well-behaved as intermediates for studying Gröbner bases. So one cannot formulate a similar result as Theorem I.3.4 for enzymes. But instead one can think about the existence of a condition under which an analogous result becomes true. For example, in the case of the ERK activation network (Figure I.1) eliminating intermediates and enzymes recursively and considering a monomial order having enzymes smaller than non-enzymes and intermediates larger than non-intermediates, one gets a reduced Gröbner basis with 27 polynomials which has an even smaller number of polynomials than the number of steady state polynomials.

Regarding Gröbner bases, not only computation time is an issue. As it can be seen with different monomial orders, the reduced Gröbner basis can have different number and different lengths (number of terms in each polynomial) of polynomials. It is not worthful for a biologist to use a reduced Gröbner basis of the steady state ideal instead of the steady state polynomials if it has too many polynomials or too long polynomials. Using the monomial order given in Theorem I.3.4 we got less number of polynomials with shorter lengths in Example I.3.5 than in the reduced Gröbner basis in the grevlex order. A future work can be to investigate this property formally.

Multistationarity of networks with binomial core network. In Section II.4.3, Algorithm II.4.8 is applied on the n-site distributive sequential phosphoryliation core network. Future work can be to implement this algorithm on other class of networks with binomial core networks, for example when there is a cascade of n-site phosphorylations.

Region of multistationarity and measure of robustness. In the third paper the Kac-Rice formula, Theorem III.3.1, is given for a univariate polynomial. A future work can be giving the Kac-Rice formula in the setting of studying multistationarity of CRNs similar to Theorem III.3.1, but for a multivariate polynomial system. In the Kac-Rice formula of (Adler and Taylor, 2007, Theorem 11.2.1) one needs the number of polynomials in the system be equal to the number of variables. This is always possible for CRNs. Let $n = |\mathcal{S}|$ and $d = n - \text{rank}(\mathcal{N})$. In (2.7), d of the steady state polynomials can be removed since they are linear combinations of the rest. Then the system (2.7) is a square system with n polynomials and n variables.

Another future work is to implement the method with other distributions such as truncated normal and log-normal distributions. It is more natural to use truncated normal distribution when studying measure of robustness or effect of perturbation.

And finally working on the speed of numerical integrations. For different distributions, the Monte-Carlo method has a different speed of convergence. Even with the same type of distribution but different parameters of distribution, the speed can be different. There are many methods in the literature to choose better distributions to increase the speed of Monte-Carlo integration. Increasing the speed of convergence of the Monte-Carlo method means that one needs fewer number of sample points and so less number of evaluation of the integrand function. Therefore it will reduce the time needed to implement the introduced methods of paper 3, in particular of the measure of robustness.

Bibliography

Adler, R. J. and Taylor, J. E. (2007). *Random Fields and Geometry*. Springer-Verlag, New York.

Banaji, M. and Pantea, C. (2016). Some results on injectivity and multistationarity in chemical reaction networks. *SIAM J. Appl. Dyn. Syst.*, 15(2):807–869.

Banaji, M. and Pantea, C. (2017). The inheritance of nondegenerate multistationarity in chemical reaction networks. *arXiv: 1608.08400v3*.

Bates, D. J., Hauenstein, J. D., Sommese, A. J., and Wampler, C. W. (2013). *Numerically Solving Polynomial Systems with Bertini*. Society for Industrial and Applied Mathematics, Philadelphia, PA, USA.

Bhaskaran, S., Umesh, P., and Nair, A. S. (2015). Hill equation in modeling transcriptional regulation. In Singh, V. and Dhar, P. K., editors, *Systems and Synthetic Biology*, pages 77–92. Springer, Dordrecht.

Bihan, F. and Dickenstein, A. (2017). Descartes' rule of signs for polynomial systems supported on circuits. *Int. Math. Res. Notices*, 2017(22):6867–6893.

Bihan, F., Dickenstein, A., and Giaroli, M. (2018). Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. *arXiv: 1807.05157*.

Bradford, R., Davenport, J. H., England, M., Errami, H., Gerdt, V., Grigoriev, D., Hoyt, C., Košta, M., Radulescu, O., Sturm, T., and Weber, A. (2017). A case study on the parametric occurrence of multiple steady states. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, page 45–52. Association for Computing Machinery.

Cappelletti, D. and Wiuf, C. (2016). Elimination of intermediate species in multiscale stochastic reaction networks. *Ann. Appl. Probab.*, 26(5):2915–2958.

Cappelletti, D. and Wiuf, C. (2017). Uniform approximation of solutions by elimination of intermediate species in deterministic reaction networks. *SIAM J. Appl. Dyn. Syst.*, 16(4):2259–2286.

Conradi, C., Feliu, E., Mincheva, M., and Wiuf, C. (2017). Identifying parameter regions for multistationarity. *PLoS Comput. Biol.*, 13(10):e1005751.

Conradi, C. and Flockerzi, D. (2012). Switching in mass action networks based on linear inequalities. *SIAM J. Appl. Dyn. Syst.*, 11(1):110–134.

Conradi, C. and Kahle, T. (2015). Detecting binomiality. *Adv. Appl. Math.*, 71:52–67.

Corvez, S. and Rouillier, F. (2004). Using computer algebra tools to classify serial manipulators. In Winkler, F., editor, *Automated Deduction in Geometry. ADG 2002. Lecture Notes in Computer Science*, pages 31–43. Springer, Berlin, Heidelberg.

Craciun, G., Dickenstein, A., Shiu, A., and Sturmfels, B. (2009). Toric dynamical systems. *J. Symb. Comput.*, 44(11):1551–1565.

Craciun, G. and Feinberg, M. (2005). Multiple equilibria in complex chemical reaction networks: I. the injectivity property. *SIAM J. Appl. Math.*, 65(5):1526–1546.

Craciun, G., Nazarov, F., and Pantea, C. (2013). Persistence and permanence of mass-action and power-law dynamical systems. *SIAM J. Appl. Math.*, 73(1):305–329.

Cutkosky, S. D. (2004). *Resolution of Singularities*. American Mathematical Society, Providence Rhode Island.

de Freitas, M. M., Feliu, E., and Wiuf, C. (2016). Intermediates, catalysts, persistence, and boundary steady states. *J. Math. Biol.*, 74(4):887–932.

de Freitas, M. M., Wiuf, C., and Feliu, E. (2017). Intermediates and generic convergence to equilibria. *B. Math. Biol.*, 79(7):1662–1686.

Donnell, P., Banaji, M., Marginean, A., and Pantea, C. (2014). Control: an open source framework for the analysis of chemical reaction networks. *Bioinformatics*, 30(11):1633–1634.

Eisenbud, D. and Sturmfels, B. (1996). Binomial ideals. *Duke Math. J.*, 84(1):1–45.

Ellison, P. R. (1998). *The Advanced Deficiency Algorithm and Its Applications to Mechanism Discrimination*. PhD thesis, The University of Rochester, Eastman School of Music. AAI9905373.

England, M., Bradford, R., and Davenport, J. H. (2015). Improving the use of equational constraints in cylindrical algebraic decomposition. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 165–172. Association for Computing Machinery.

Feinberg, M. (1972). Complex balancing in general kinetic systems. *Arch. Ration. Mech. Anal.*, 49:187–194.

Feinberg, M. (1987). Chemical reaction network structure and the stability of complex isothermal reactors—i. the deficiency zero and deficiency one theorems. *Chem. Eng. Sci.*, 42(10):2229–2268.

Feinberg, M. (1988). Chemical reaction network structure and the stability of complex isothermal reactors—ii. multiple steady states for networks of deficiency one. *Chem. Eng. Sci.*, 43(1):1–25.

Feinberg, M. (1995a). The existence and uniqueness of steady states for a class of chemical reaction networks. *Arch. Rational Mech. Anal.*, 132(4):311–370.

Feinberg, M. (1995b). Multiple steady states for chemical reaction networks of deficiency one. *Arch. Rational Mech. Anal.*, 132(4):371–406.

Feliu, E. (2015). Injectivity, multiple zeros and multistationarity in reaction networks. *Proc. R. Soc. A*, 471(2173):20140530.

Feliu, E., Cappelletti, D., and Wiuf, C. (2018). Node balanced steady states: Unifying and generalizing complex and detailed balanced steady states. *Math. Biosci.*, 301:68–82.

Feliu, E. and Wiuf, C. (2012). Variable elimination in chemical reaction networks with mass-action kinetics. *SIAM J. Appl. Math.*, 72(4):959–981.

Feliu, E. and Wiuf, C. (2013a). Simplifying biochemical models with intermediate species. *J. R. Soc. Interface*, 10(87):20130484.

Feliu, E. and Wiuf, C. (2013b). Variable elimination in post-translational modification reaction networks with mass-action kinetics. *J. Math. Biol.*, 66(1-2):281–310.

Fukuda, K. and Prodon, A. (1996). Combinatorics and computer science. In Deza, M. and Euler, R., editors, *SIAM J. Appl. Dyn. Syst.*, pages 91–111. Springer, Berlin, Heidelberg.

Gagneur, J. and Klamt, S. (2004). Computation of elementary modes: a unifying framework and the new binary approach. *BMC Bioinformatics*, 5(1):807–869.

Giaroli, M., Bihan, F., and Dickenstein, A. (2018). Regions of multistationarity in cascades of Goldbeter-Koshland loops. *arXiv: 1807.08400*.

Gnacadja, G. (2011a). Reachability, persistence, and constructive chemical reaction networks (part i): reachability approach to the persistence of chemical reaction networks. *J. Math. Chem.*, 49(10):2117–2136.

Gnacadja, G. (2011b). Reachability, persistence, and constructive chemical reaction networks (part ii): formalism for species composition in chemical reaction network theory and application to persistence. *J. Math. Chem.*, 49(10):2137–2157.

Gnacadja, G. (2011c). Reachability, persistence, and constructive chemical reaction networks (part iii): a mathematical formalism for binary enzymatic networks and application to persistence. *J. Math. Chem.*, 49(10):2158–2176.

Griffin, Z. A. and Hauenstein, J. D. (2005). Real solutions to systems of polynomial equations and parameter continuation. *Adv. Geom.*, 15(2):173–181.

Gunawardena, J. (2007). Distributivity and processivity in multisite phosphorylation can be distinguished through steady-state invariants. *Biophys. J.*, 83(11):3828–3834.

Harrington, H. A., Ho, K. L., Thorne, T., and Stumpf, M. P. (2012). Parameter-free model discrimination criterion based on steady-state coplanarity. *Proc. Natl. Acad. Sci. U. S. A.*, 109(39):15746–15751.

Harrington, H. A., Mehta, D., Byrne, H. M., and Hauenstein, J. D. (2016). Decomposing the parameter space of biological networks via a numerical discriminant approach. *arXiv: 1604.02623v1*.

Horn, F. (1972). Necessary and sufficient conditions for complex balancing in chemical kinetics. *Arch. Ration. Mech. Anal.*, 49(3):172–186.

Horn, F. and Jackson, R. (1972). General mass action kinetics. *Arch. Ration. Mech. Anal.*, 47:81–116.

Ingalls, B. (2012). Mathematical modelling in systems biology: An introduction. http://www.math.uwaterloo.ca/~bingalls/MMSB/Notes.pdf.

Ji, H., Ellison, P., Knight, D., and Feinberg, M. (2015). CRNToolbox Version 2-3 — The Chemical Reaction Toolbox. http://crnt.osu.edu/CRNTWin.

Joshi, B. and Shiu, A. (2013). Atoms of multistationarity in chemical reaction networks. *J. Math. Chem.*, 51(1):153–178.

Kac, M. (1943). On the average number of real roots of a random algebraic equation. *Bull. Amer. Math. Soc.*, 49(4):314–320.

Karp, R. L., Pérez Millán, M., Dasgupta, T., Dickenstein, A., and Gunawardena, J. (2012). Complex-linear invariants of biochemical networks. *J. Theor. Biol.*, 311(21):130–138.

Kothamachu, V. B., Feliu, E., Cardelli, L., and Soyer, O. S. (2015). Unlimited multistability and boolean logic in microbial signalling. *J. R. Soc. Interface*, 12(108):20150234.

Lazard, D. and Rouillier, F. (2007). Solving parametric polynomial systems. *J. Symb. Comput.*, 42(6):636–667.

Liang, S., Gerhard, J., Jeffrey, D. J., and Moroz, G. (2009). A package for solving parametric polynomial systems. *ACM Communications in Computer Algebra*, 43(3):61–72.

Manrai, A. K. and Gunawardena, J. (2008). The geometry of multisite phosphorylation. *Biophys. J.*, 95(12):5533–5543.

Meshkat, N., Rosen, Z., and Sullivant, S. (2016). Algebraic tools for the analysis of state space models. *arXiv: 1609.07985*.

Motzkin, T. S., Raiffa, H., Thompson, G. L., and Thrall, R. M. (1953). The double description method. In Kuhn, H. W. and Tucker, A. W., editors, *Contributions to the Theory of Games II*, pages 31–43. Princeton University Press, Princeton.

Müller, S., Feliu, E., Regensburger, G., Conradi, C., Shiu, A., and Dickenstein, A. (2016). Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found. Comput. Math.*, 16(1):69–97.

Müller, S. and Regensburger, G. (2012). Generalized mass action systems: complex balancing equilibria and sign vectors of the stoichiometric and kinetic-order subspaces. *SIAM J. Appl. Math.*, 72(6):1926–1947.

Owen, A. B. (2013). Monte carlo theory, methods and examples. http://statweb.stanford.edu/~owen/mc/.

Pérez Millán, M. and Dickenstein, A. (2018). The structure of MESSI biological systems. *SIAM J. Appl. Dyn. Syst.*, 17(2):1650–1682.

Pérez Millán, M., Dickenstein, A., Shiu, A., and Conradi, C. (2012). Chemical reaction systems with toric steady states. *Bull. Math. Biol.*, 74(5):1027–1065.

Sadeghimanesh, A. H. and Feliu, E. (2018a). Gröbner bases of reaction networks with intermediate species. *arXiv: 1804.01381*.

Sadeghimanesh, A. H. and Feliu, E. (2018b). The multistationarity structure of networks with intermediates and a binomial core network. *arXiv: 1808.07548*.

Sadeghimanesh, A. H. and Feliu, E. (2018c). The robustness of parameter regions for multistationarity. *In preparation*.

Sáez, M., Wiuf, C., and Feliu, E. (2017). Graphical reduction of reaction networks by linear elimination of species. *SIAM J. Appl. Dyn. Syst.*, 74(1-2):195–237.

Schilling, C. H., Letscher, D., and Palsson, B. Ø.. (2000). Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented prespective. *J. Theor. Biol.*, 203(3):229–248.

Sommese, A. J., Verschelde, J., and Wampler, C. W. (2005). Introduction to numerical algebraic geometry. In Bronstein, M., Cohen, A. M., Cohen, H., Eisenbud, D., Sturmfels, B., Dickenstein, A., and Emiris, I. Z., editors, *Solving Polynomial Equations. Algorithms and Computation in Mathematics*, pages 301–337. Springer, Berlin, Heidelberg.

Sontag, E. D. (2001). Structure and stability of certain chemical networks and applications to the kinetic proofreading model of t-cell receptor signal transduction. *Institute of Electrical and Electronics Engineers. Transactions on Automatic Control*, 46(7):1028–1047.

Papers

I

Gröbner bases of reaction networks with intermediate species

AmirHosein Sadeghimanesh
Department of Mathematical Sciences
University of Copenhagen

Elisenda Feliu
Department of Mathematical Sciences
University of Copenhagen

Publication details

Submited to Advances in Applied Mathematics. Available on arXiv.
Sadeghimanesh, A. H. and Feliu, E. (2018a). Gröbner bases of reaction networks with intermediate species. arXiv: 1804.01381.

GRÖBNER BASES OF REACTION NETWORKS WITH INTERMEDIATE SPECIES

AMIRHOSEIN SADEGHIMANESH[1], ELISENDA FELIU[1,2]

Abstract. In this work we consider the computation of Gröbner bases of the steady state ideal of reaction networks equipped with mass-action kinetics. Specifically, we focus on the role of *intermediate* species and the relation between the extended network (with intermediate species) and the core network (without intermediate species).

We show that a Gröbner basis of the steady state ideal of the core network always lifts to a Gröbner basis of the steady state ideal of the extended network by means of linear algebra, with a suitable choice of monomial order. As illustrated with examples, this contributes to a substantial reduction of the computation time, due mainly to the reduction in the number of variables and polynomials. We further show that if the steady state ideal of the core network is binomial, then so is the case for the extended network, as long as an extra condition is fulfilled. For standard networks, this extra condition can be visually explored from the network structure alone.

Keywords: binomial ideals, mass-action kinetics, steady state ideal, invariant, Gröbner basis

Introduction

Parametric polynomial systems of equations arise in the natural sciences when modeling ecosystems, cell behavior, the spread of an illness, and molecular interactions within the cell, to name a few examples. In these scenarios questions of interest often boil down to describing the solutions to these systems for varying values of the parameters. Although only non-negative solutions are typically meaningful, the standard tool in computational algebraic geometry to study algebraic varieties, namely Gröbner bases, has proven useful. However, due to the parametric coefficients, the computation of a reduced Gröbner basis can be time consuming for realistic examples, which typically involve many variables and parameters. The computation time depends mainly on the degree of the polynomials, the number of variables and coefficients, the choice of the monomial order and the used method [1, 2, 5, 9, 29]. These universal considerations target generic polynomial systems, but, in applications, the structure of the particular system of interest might favor one method or one monomial order over another.

[1]Department of Mathematical Sciences, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark.

[2]Corresponding author: efeliu@math.ku.dk

Date: October 10, 2018.

We focus on a specific type of polynomial systems that arise when modeling chemical reaction networks with mass-action kinetics [10, 12]. Specifically, the evolution of the concentrations of the species of a chemical reaction network in time is described under mass-action by a system of ordinary differential equations in $\mathbb{R}^n$

$$\frac{dx_i}{dt} = f_{\kappa,i}(x), \qquad i = 1, \dots, n$$

with $f_{\kappa,i}(x)$ polynomial. The monomials of each $f_{\kappa,i}(x)$ depend on the reaction network structure alone, and the coefficients depend on the *reaction rate constants* κ, which are often unknown and thus treated as parameters. The steady states, or equilibrium points, of the system are the *non-negative* points of the variety defined by the *steady state ideal* $I_\kappa = \langle f_{\kappa,1}(x), \dots, f_{\kappa,n}(x)\rangle$.

The question of restricting to non-negative steady states remains challenging and no straightforward solutions have been proposed. Despite of this, Gröbner bases have been for example used for model discrimination [13, 14, 18–20]. They are also used to decide whether the steady state ideal is binomial, that is, whether any reduced Gröbner basis consists of polynomials with at most two terms. If this is the case, then methods to detect the existence of multiple steady states can be applied [22, 25].

In this work we exploit the specific structure of the steady state ideal, which reflects the structure of the reaction network, to guide the selection of good monomial orders and to compute reduced Gröbner bases faster. Specifically, we consider a frequent and nicely-behaved class of species introduced in [11] called *intermediate species* (or intermediates, for short). Intermediates give rise to linear terms in the steady state polynomials, and they can be removed from a reaction network resulting in a smaller *core network* with only the non-intermediates. A key property is that steady states of the core network can be lifted to steady states of the *extended network*.

The first main result of this work is Theorem 3.4, where we show how to obtain a Gröbner basis of the extended network from one of the core network using linear algebra. The result implicitly gives good monomial orders, namely, those for which the concentration of the intermediates are larger than for the non-intermediates, and are lexicographic in the variables corresponding to the intermediates. Example 3.5 illustrates the computational advantage of using our approach compared with other methods. Additionally, we conclude that the analysis of the steady state ideal of the core network is sufficient for model discrimination.

The second main result, Theorem 3.10, addresses how to decide whether the steady state ideal is binomial. We show that if the steady state ideal of the core network is binomial, then this is also the case for the steady state ideal of the extended network provided an extra condition is fulfilled. In typical networks, this extra condition can be readily checked from the network structure alone. When the core network has a homogeneous steady state ideal (which happens frequently for realistic reaction networks), then one can employ the linear algebra-based method introduced in [4] to detect whether the steady state ideal of the core network is binomial. Then, combined with our result, we obtain a faster method to address whether the steady state ideal of the original network is binomial, which does not rely on the computation of a Gröbner basis.

The key property behind our results is that intermediates define a square linear subsystem of full rank among the steady state polynomials. Its solution and posterior substitution into the remaining polynomials gives rise to a smaller ideal in the non-intermediates. A Gröbner basis of the small ideal can then be lifted to a Gröbner basis of the original ideal. Our approach can be theoretically applied to arbitrary parametric ideals, after detection of linear subsystems among a set of generators. However, technical conditions that are necessary for our results to hold might not be straightforward to check, since we overcome this difficulty by exploiting the network structure.

The structure of the paper is as follows. We start by introducing reaction networks and basic concepts such as the steady state ideal. Intermediates are introduced in Section 2. In Section 3 we address Gröbner bases of networks with intermediates, discuss binomial steady state ideals and relate our work to [4]. In Section 4 a technical condition of algebraic independence of a set of rational functions, which is assumed in the former sections, is discussed. Finally, in the last section, we discuss another class of special species, namely enzymes, that might lead to similar results concerning the computation of Gröbner bases.

1. The steady state ideal of a reaction network

We follow the formalism of [11]. See also [10, 12] for an introduction to reaction networks. Subscripts $\geq 0, > 0$ on $\mathbb{R}$ (resp. $\mathbb{Z}$) refer to the non-negative and positive real numbers (resp. integer numbers).

A *reaction network* is an ordered triple $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ where $\mathcal{S}$, $\mathcal{C}$ and $\mathcal{R}$ are three sets called the set of *species*, *complexes* and *reactions*, respectively. Here $\mathcal{S}$ is a finite set and $\mathcal{C}$ is a finite set of linear combinations of elements of $\mathcal{S}$ with coefficients in $\mathbb{Z}_{\geq 0}$. A *reaction* is an ordered pair of complexes (c, c') in $\mathcal{C}^2$, usually denoted as $c \to c'$. For the reaction $c \to c'$, the complex c is called the *reactant* and c' is called the *product*.

A digraph is associated with a reaction network as follows. The vertex set is $\mathcal{C}$ and there is a directed edge from the reactant to the product of every reaction. If both reactions $c \to c'$ and $c' \to c$ for two complexes c and c' exist, then the notation $c \rightleftharpoons c'$ is used and the reaction is said to be reversible.

Complexes that are not part of any reaction or species that are not part of any complex do not appear in the digraph. Therefore, the reaction network cannot uniquely be determined from the digraph alone. For simplicity, however, we often introduce a reaction network by its digraph and implicitly assume that the set of complexes equals the set of vertices and the set of species consists of the species that appear in at least one complex.

Write $\mathcal{S} = \{X_1, \ldots, X_n\}$, such that the set of species is implicitly ordered. Then a complex c is of the form $c_1X_1 + \cdots + c_nX_n$, which we also write in vector form as $c = (c_1, c_2, \ldots, c_n) \in \mathbb{Z}^n_{\geq 0}$. With this representation, c_i is called the *stoichiometric coefficient* of X_i in c.

Example 1.1. Let $\mathcal{S} = \{X_1, X_2, X_3, X_4\}$, $\mathcal{C} = \{X_1 + X_3, X_4, X_2 + X_3\}$, $\mathcal{R} = \{X_1 + X_3 \to X_4, X_4 \to X_1 + X_3, X_4 \to X_2 + X_3\}$. The network $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is

represented with the following digraph

$$X_1 + X_3 \rightleftarrows X_4 \longrightarrow X_2 + X_3.$$

The complexes X_1+X_3 and X_4 appear both as reactants and products while X_2+X_3 appears only as a product.

We next construct a system of Ordinary Differential Equations (ODEs) that models the variation of the concentration of each species in time and introduce the relevant polynomials $F_i(x)$ that are the focus of this work. We denote the concentration of each species X_i in lower-case x_i. For each reaction $c \to c'$, we introduce a parameter $k_{c\to c'}$, and a polynomial $F_i(x)$ is associated with every species X_i as follows:

$$F_i(x) = \sum_{c\to c'\in\mathcal{R}} (c'_i - c_i)k_{c\to c'}\, x^c \ \in\ \mathbb{R}(k)[x], \tag{1}$$

where $x^c = x_1^{c_1}\dots x_n^{c_n}$. Here $x = (x_1,\dots,x_n)$ and $\mathbb{R}(k)$ is the field of rational functions with variables $k_{c\to c'}$ and real coefficients. The symbol k stands for the parameter vector

$$k = (k_{c\to c'} \mid c \to c' \in \mathcal{R}).$$

For a chosen positive value $k^\star \in \mathbb{R}^{\mathcal{R}}_{>0}$ of the parameter vector, we let $F_{k^\star,i}(x) \in \mathbb{R}[x]$ denote the image of $F_i(x)$ under the evaluation map

$$\mathbb{R}[k] \to \mathbb{R}, \qquad k_{c\to c'} \mapsto k^\star_{c\to c'}.$$

With this choice of $k^\star$, the ODE system of the reaction network under *mass-action kinetics* is

$$\dot{x}_i = F_{k^\star,i}(x), \quad i = 1,\dots,n, \qquad x \in \mathbb{R}^n_{\geq 0}. \tag{2}$$

The value $k^\star_{c\to c'} > 0$ is called the *reaction rate constant* of $c \to c'$ and is usually depicted as a label of the reaction in the associated digraph. By [27], if the starting condition of (2) belongs to $\mathbb{R}^n_{>0}$ (resp. $\mathbb{R}^n_{\geq 0}$), then so does the trajectory for all positive times in the interval of definition.

The *steady states* of the network are the common zeros of $F_{k^\star,i}(x)$, $i = 1,\dots,n$. In applications, only non-negative real solutions have meaning and mostly, positive steady states are interesting, meaning all concentrations are positive. Since the values of the reaction rate constants are in general unknown, they are treated as parameters of the system. Thus we aim at studying the zeros of the system of polynomials $F_i(x) = 0$, for $i = 1,\dots,n$ in $\mathbb{R}(k)$ and specially the positive zeros after specifying values for k.

Definition 1.2. Let $\mathcal{N} = (\mathcal{S},\mathcal{C},\mathcal{R})$ be a reaction network with $\mathcal{S} = \{X_1,\dots,X_n\}$.

(a) $F_i(x) \in \mathbb{R}(k)[x]$ is called the *steady state polynomial* of X_i.
(b) The ideal generated by the steady state polynomials of all the species in the network in the ring $\mathbb{R}(k)[x]$ is called the *steady state ideal* of the network:

$$I_{\mathcal{N}} = \langle F_i(x) \mid i = 1,\dots,n\rangle \ \subseteq \mathbb{R}(k)[x].$$

The set of steady states for a vector of reaction rate constants $k^\star$ is thus the solution set to any basis (set of generators) of $I_\mathcal{N}$ specialized to $k^\star$.

It follows from (1) and (2) that for all $x \in \mathbb{R}^n$, the vector

$$F_k(x) = (F_{k,1}(x), \ldots, F_{k,n}(x))$$

lies in the vector subspace $S = \operatorname{span}(\{c - c' \mid c \to c' \in \mathcal{R}\}) \subseteq \mathbb{R}^n$. If $s = \dim(S)$, then $n - s$ of the steady state polynomials can be written as linear combinations of the remaining s polynomials. We conclude that it is always possible to find a basis of $I_\mathcal{N}$ with cardinality $\dim(S)$.

Example 1.3. (continued from Example 1.1) The ODE system of the reaction network with digraph

$$X_1 + X_3 \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_4 \xrightarrow{k_3} X_2 + X_3$$

is

$$\begin{aligned} \dot{x}_1 &= -k_1 x_1 x_3 + k_2 x_4 & \dot{x}_2 &= k_3 x_4 \\ \dot{x}_3 &= -k_1 x_1 x_3 + k_2 x_4 + k_3 x_4 & \dot{x}_4 &= k_1 x_1 x_3 - k_2 x_4 - k_3 x_4. \end{aligned}$$

In this case $\dim(S) = 2$, $k = (k_1, k_2, k_3)$ and the steady state ideal is

$$I_\mathcal{N} = \big\langle -k_1 x_1 x_3 + k_2 x_4, k_3 x_4 \big\rangle \subseteq \mathbb{R}(k)[x].$$

2. Intermediates and steady states

In this subsection we introduce a special type of species of interest: intermediates.

Definition 2.1. We say that $\mathcal{Y} \subseteq \mathcal{S}$ is a subset of *intermediates* if each $Y \in \mathcal{Y}$ fulfills:

- $Y \in \mathcal{C}$ and the stoichiometric coefficient of Y in all other complexes is zero, and
- there exists at least one reaction having Y as reactant and at least one reaction having Y as product.

Each $Y \in \mathcal{Y}$ is called an *intermediate*.

Whenever a set of intermediates $\mathcal{Y}$ is given, we partition the set of species into two disjoint subsets $\mathcal{Y} = \{Y_1, \ldots, Y_m\}$ and $\mathcal{X} = \{X_1, \ldots, X_n\}$ of non-intermediates. We assume further that the set of species is ordered such that the species $Y_1, \ldots, Y_m$ are first. With this convention, we let (y, x) denote the concentration vector of all species: x is the concentration vector of the species in $\mathcal{X}$ and y of the species in $\mathcal{Y}$. A complex is either an intermediate in $\mathcal{Y}$ or it contains only non-intermediates. In the latter case we say that c is a *non-intermediate complex*.

Note that given $\mathcal{Y}$, we refer to the intermediates of the network as the species in $\mathcal{Y}$, even though there might be other species in $\mathcal{X}$, regarded as non-intermediates, that fulfill the two items in Definition 2.1.

Example 2.2. The most common mechanism involving intermediates is of the following form:

$$X + E \longrightarrow Y \longrightarrow X' + E$$

or variations of it by letting one or both reactions being reversible. *Isomerism mechanisms* among intermediates are also common:

$$Y \longrightarrow Y' \qquad Y \rightleftharpoons Y'.$$

Combination of these mechanisms yields to more elaborate networks involving intermediates, as in Examples 2.6 and 3.5 below.

Definition 2.3. Let $\mathcal{Y}$ be a set of intermediates and $Y \in \mathcal{Y}$.

- A non-intermediate complex c is called an *input* for Y if there is a directed path from c to Y in the digraph associated with the network, such that all vertices other than c belong to $\mathcal{Y}$.
- Y is called an ℓ-input intermediate if there are ℓ inputs for Y.

Example 2.4. Consider the following network with $\mathcal{Y} = \{Y_1, Y_2, Y_3\}$:

$$X_1 + X_2 \longrightarrow Y_1 \rightleftharpoons Y_2 \rightleftharpoons Y_3 \longrightarrow X_3 + X_4.$$

There are two non-intermediate complexes, $X_1 + X_2$ and $X_3 + X_4$. The species Y_1, Y_2, Y_3 are all 1-input intermediates. Note that Y_2 is however the product of two reactions.

Consider now the following network with $\mathcal{Y} = \{Y\}$:

$$X_1 + X_2 \rightleftharpoons Y \rightleftharpoons X_3 + X_4.$$

The species Y is a 2-input intermediate and $X_1 + X_2$ and $X_3 + X_4$ are both inputs for Y.

2.1. **Intermediates and steady states.** Let $\widetilde{\mathcal{N}}$ be a reaction network with a set of intermediates $\mathcal{Y} = \{Y_1, \ldots, Y_m\}$. Consider the steady state polynomials of the intermediates and denote the parameter vector of reaction rate constants by κ (the reason why will be made clear below). By definition, for every intermediate Y_i, the variable y_i is only part of the monomial y_i in (1). Thus, the system with m equations

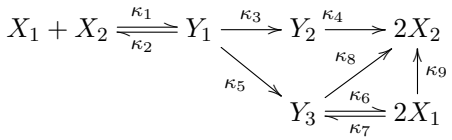
$$F_1(y, x) = \cdots = F_m(y, x) = 0$$

is linear in $y_1, \ldots, y_m$. It is shown in [11] that this system has a unique solution for fixed positive values of κ and x, which is further positive. The solution is of the form

$$y_i = \sum_{c \in \mathcal{C}} \mu_{i,c}\, x^c, \qquad \text{where} \quad \mu_{i,c} \in \mathbb{R}_{\geq 0}(\kappa), \quad i = 1, \ldots, m.$$

The explicit dependence of $\mu_{i,c}$ on κ is omitted from the notation for simplicity. An explicit description of $\mu_{i,c}$ can be found using the Matrix-Tree theorem on a suitable labeled digraph, see [11].

Example 2.5. Consider the following reaction network with $\mathcal{X} = \{X_1, X_2, X_3\}$ and $\mathcal{Y} = \{Y_1, Y_2, Y_3\}$:



The linear system in y_1, y_2, y_3 that the steady state polynomials of Y_1, Y_2, Y_3 define is:

$$\begin{aligned}\kappa_1 x_1 x_2 - (\kappa_2 + \kappa_3 + \kappa_5) y_1 &= 0,\\ \kappa_3 y_1 - \kappa_4 y_2 &= 0,\\ \kappa_5 y_1 - (\kappa_6 + \kappa_8) y_3 + \kappa_7 x_1^2 &= 0,\end{aligned}$$

and its solution is

$$\begin{aligned} y_1 &= \tfrac{\kappa_1}{\kappa_2+\kappa_3+\kappa_5} x_1 x_2, & y_2 &= \tfrac{\kappa_1\kappa_3}{\kappa_4(\kappa_2+\kappa_3+\kappa_5)} x_1 x_2,\\ y_3 &= \tfrac{\kappa_1\kappa_5}{(\kappa_6+\kappa_8)(\kappa_2+\kappa_3+\kappa_5)} x_1 x_2 + \tfrac{\kappa_7}{\kappa_6+\kappa_8} x_1^2. \end{aligned}$$

This gives

$$\begin{aligned} \mu_{1,X_1+X_2} &= \tfrac{\kappa_1}{\kappa_2+\kappa_3+\kappa_5}, & \mu_{1,2X_1} &= 0, & \mu_{1,2X_2} &= 0,\\ \mu_{2,X_1+X_2} &= \tfrac{\kappa_1\kappa_3}{\kappa_4(\kappa_2+\kappa_3+\kappa_5)}, & \mu_{2,2X_1} &= 0, & \mu_{2,2X_2} &= 0,\\ \mu_{3,X_1+X_2} &= \tfrac{\kappa_1\kappa_5}{(\kappa_6+\kappa_8)(\kappa_2+\kappa_3+\kappa_5)}, & \mu_{3,2X_1} &= \tfrac{\kappa_7}{\kappa_6+\kappa_8}, & \mu_{3,2X_2} &= 0. \end{aligned}$$

Example 2.6. The following digraph corresponds to the Mitogen-Activated Protein Kinase cascade (MAPK) given in [3]:

$$\begin{aligned} &X_0 + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} Y_1 \xrightarrow{\kappa_3} X_1 + E \underset{\kappa_5}{\overset{\kappa_4}{\rightleftarrows}} Y_2 \xrightarrow{\kappa_6} X_2 + E\\ &X_2 + F \underset{\kappa_8}{\overset{\kappa_7}{\rightleftarrows}} Y_3 \xrightarrow{\kappa_9} Y_4 \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftarrows}} X_1 + F \underset{\kappa_{13}}{\overset{\kappa_{12}}{\rightleftarrows}} Y_5 \xrightarrow{\kappa_{14}} Y_6 \underset{\kappa_{16}}{\overset{\kappa_{15}}{\rightleftarrows}} X_0 + F. \end{aligned}$$

Species $Y_1, \ldots, Y_6$ are intermediates. The non-zero coefficients $\mu_{i,c}$ are:

$$\begin{aligned} \mu_{1,X_0+E} &= \tfrac{\kappa_1}{\kappa_2+\kappa_3}, & \mu_{2,X_1+E} &= \tfrac{\kappa_4}{\kappa_5+\kappa_6}, & \mu_{3,X_2+F} &= \tfrac{\kappa_7}{\kappa_8+\kappa_9},\\ \mu_{4,X_2+F} &= \tfrac{\kappa_7\kappa_9}{(\kappa_8+\kappa_9)\kappa_{10}}, & \mu_{4,X_1+F} &= \tfrac{\kappa_{11}}{\kappa_{10}}, & \mu_{5,X_1+F} &= \tfrac{\kappa_{12}}{\kappa_{13}+\kappa_{14}},\\ \mu_{6,X_1+F} &= \tfrac{\kappa_{12}\kappa_{14}}{(\kappa_{13}+\kappa_{14})\kappa_{15}}, & \mu_{6,X_0+F} &= \tfrac{\kappa_{16}}{\kappa_{15}}. \end{aligned}$$

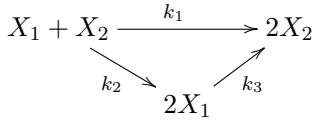
2.2. **Extended and core networks.**

Definition 2.7. Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ and $\widetilde{\mathcal{N}} = (\widetilde{\mathcal{S}}, \widetilde{\mathcal{C}}, \widetilde{\mathcal{R}})$ be two reaction networks. We say that $\widetilde{\mathcal{N}}$ is an *extension* of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$ if

(i) $\mathcal{Y} = \{Y_1, \ldots, Y_m\}$ is a set of intermediates of $\widetilde{\mathcal{N}}$.
(ii) $\mathcal{S} \cup \mathcal{Y} \subseteq \widetilde{\mathcal{S}}$ and $\mathcal{C} \cup \mathcal{Y} \subseteq \widetilde{\mathcal{C}}$.
(iii) $c \to c' \in \mathcal{R}$ if and only if there is a directed path from c to c' in the digraph associated with $\widetilde{\mathcal{N}}$, such that all vertices other than c and c' belong to $\mathcal{Y}$ (there might be none).

In this case $\mathcal{N}$ is called the *core network* of $\widetilde{\mathcal{N}}$.

Example 2.8. The core network associated with the network in Example 2.5 is:



Example 2.9. The core network of the network in Example 2.6 has digraph

$$X_0 + E \xrightarrow{k_1} X_1 + E \xrightarrow{k_2} X_2 + E \qquad X_2 + F \xrightarrow{k_3} X_1 + F \xrightarrow{k_4} X_0 + F.$$

Notations $\kappa, \widetilde{I}, \widetilde{F}$ are used to address reaction rate constants, steady state ideal and steady state polynomials of the extended network respectively. This notation is fixed from now on whenever we study extensions via the addition of intermediates.

Given $\widetilde{\mathcal{N}}$ an extension of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$, we define a map

$$\begin{array}{rccc} \phi\colon & \mathbb{R}[k] & \longrightarrow & \mathbb{R}(\kappa) \\ & k_{c\to c'} & \longmapsto & \phi_{c\to c'}(\kappa), \end{array}$$

such that for every reaction $c \to c' \in \mathcal{R}$, $\phi_{c\to c'}(\kappa)$ is the rational function

$$\phi_{c\to c'}(\kappa) = \kappa_{c\to c'} + \sum_{i=1}^{m} \kappa_{Y_i\to c'}\, \mu_{i,c}, \tag{3}$$

where it is understood that $\kappa_{c\to c'} = 0$, $\kappa_{Y_i\to c'} = 0$ if respectively $c \to c'$, $Y_i \to c'$ do not belong to $\widetilde{\mathcal{R}}$. Note that $\phi_{c\to c'}(\kappa) \neq 0$ for all $c \to c'$ by Definition 2.7(iii) and that $\phi_{c\to c'}(\kappa)$ is a rational function with positive coefficients.

The map ϕ extends to a map

$$\Phi\colon \mathbb{R}[k][x] \to \mathbb{R}(\kappa)[y, x].$$

For example, if F_i is a steady state polynomial of $\mathcal{N}$, $\Phi(F_i)$ is the polynomial obtained by replacing $k_{c\to c'}$ by the rational function $\phi_{c\to c'}(\kappa)$. If the rational functions $\phi_{c\to c'}(\kappa)$ are algebraically independent over $\mathbb{R}$, then ϕ extends to a map of polynomial rings

$$\Phi\colon \mathbb{R}(k)[x] \to \mathbb{R}(\kappa)[y, x].$$

We explore in Section 4 ways to check whether the algebraic independence condition holds, and provide types of intermediates for which it holds and no extra check is required.

We introduce the following polynomials

$$H_i(y, x) = y_i - \sum_{c\in\mathcal{C}} \mu_{i,c}\, x^c \ \in \mathbb{R}(\kappa)[y, x], \qquad i = 1, \ldots, m. \tag{4}$$

Theorem 2.10. ([11, Theorems 3.1 and 3.2]) *Let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$.*

(i) The coefficient $\mu_{i,c}$ is nonzero if and only if the non-intermediate complex c is an input for Y_i in $\widetilde{\mathcal{N}}$.

(ii) The set of steady state polynomials of non-intermediate species and the polynomials $H_1, \ldots, H_m$ in (4) form a basis of $\widetilde{I}$.

(iii) $\widetilde{F}_i\Big(\sum_{c\in\mathcal{C}} \mu_{1,c}\, x^c, \ldots, \sum_{c\in\mathcal{C}} \mu_{m,c}\, x^c, x_1, \ldots, x_n\Big) = \Phi(F_i(x))$ for $i = 1, \ldots, n$.

Statements (ii) and (iii) of the previous theorem constitute the proof of the following corollary.

Corollary 2.11. *Let B be the set of steady state polynomials of $\mathcal{N}$. Then*

$$\widetilde{I} = \Big\langle \Phi(B) \cup \{H_1(y,x), \ldots, H_m(y,x)\} \Big\rangle.$$

We conclude this section with basic properties of Φ.

Lemma 2.12. *With the notation above, assume $\phi_{c\to c'}(\kappa)$ for all $c \to c' \in \mathcal{R}$ are algebraically independent over $\mathbb{R}$. Let $B = \{f_1, \ldots, f_\ell\}$ and $B' = \{f'_1, \ldots, f'_{\ell'}\}$ be two sets in $\mathbb{R}(k)[x]$.*

(i) If $f \in \langle B\rangle$, then $\Phi(f) \in \langle \Phi(B)\rangle$.
(ii) If $\langle B\rangle = \langle B'\rangle$, then $\langle \Phi(B)\rangle = \langle \Phi(B')\rangle$. Thus $\Phi(\langle B\rangle)$ is well defined.

Proof. (i) Write $f = \sum_{j=1}^{\ell} \alpha_j f_j$ with $\alpha_j \in \mathbb{R}(k)[x]$. Then

$$\Phi(f) = \sum_{j=1}^{\ell} \Phi(\alpha_j)\Phi(f_j) \in \langle \Phi(B)\rangle.$$

(ii) It is enough to show inclusion $\subseteq$, since the other inclusion is analogous. If $g \in \langle \Phi(B)\rangle$, we have

$$g = \sum_{i=1}^{\ell} \lambda_i \Phi(f_i), \qquad \lambda_i \in \mathbb{R}(\kappa)[y,x].$$

Since $f_i \in \langle B'\rangle$, we have by (i) that $\Phi(f_i) \in \langle \Phi(B')\rangle$. In particular, g is an algebraic combination of the polynomials $\Phi(f'_1), \ldots, \Phi(f'_{\ell'})$ with coefficients in $\mathbb{R}(\kappa)[y,x]$. Thus $g \in \langle \Phi(B')\rangle$. □

3. Gröbner bases and intermediates

Typically, the values of the reaction rate constants are unknown and reaction networks of interest involve a considerable number of variables. As a consequence, finding a Gröbner basis of the steady state ideal over the field $\mathbb{R}(\kappa)$ can be a demanding task, and sometimes even impossible with standard computers. However, the presence of intermediates, a common feature of reaction networks, can reduce the computation time substantially, by exploiting the structure of the steady state polynomials associated with intermediates given in Theorem 2.10. The main result of this section is Theorem 3.4. Example 3.5 illustrates how the computation time can be reduced by applying our results.

We start with some concepts from computational algebraic geometry.

3.1. Monomial orders and Gröbner bases.

We follow the notation on Gröbner bases from [5]. We give here a brief overview of the results required in this text.

Given a monomial order on $R = K[x_1, \ldots, x_n]$, let $\mathrm{LM}(f)$ and $\mathrm{LT}(f)$ denote respectively the *leading monomial* and *leading term* of f. That is, $\mathrm{LT}(f) = \alpha\,\mathrm{LM}(f)$ if α is the coefficient of the greatest monomial of f. Then, for a subset $A \subseteq R$, one defines $\mathrm{LT}(A) = \big\{\,\mathrm{LT}(f) \mid f \in A\big\}$ and $\mathrm{LM}(A) = \big\{\,\mathrm{LM}(f) \mid f \in A\big\}$. Clearly,

$$\langle \mathrm{LT}(A)\rangle = \langle \mathrm{LM}(A)\rangle. \tag{5}$$

For an ideal I, the *initial ideal* is the ideal generated by the leading terms of the elements of I, $\langle \mathrm{LT}(I) \rangle$. A subset $G \subseteq I$ is called a *Gröbner basis* for I if

$$\big\langle \mathrm{LT}(I) \big\rangle = \big\langle \mathrm{LT}(G) \big\rangle, \qquad (\text{equiv. } \big\langle \mathrm{LM}(I) \big\rangle = \big\langle \mathrm{LM}(G) \big\rangle).$$

A Gröbner basis is a basis of I as well. Further, G is a *reduced Gröbner basis* if additionally for every element $g \in G$ none of its terms can be divided by the leading monomial of an element in $G - \{g\}$, and the coefficient of $\mathrm{LM}(g)$ is 1.

Whether a basis of an ideal is a Gröbner basis depends on the chosen monomial order. Given an ideal and a monomial order, the Gröbner basis is not unique but there is a unique reduced Gröbner basis (see [5]).

We will use the following lemma, which follows from Lemma 2.3.1 and Theorem 2.3.2 of [15].

Lemma 3.1. *Let B be a basis of I. If the leading monomials of every pair $f, g \in B$ are relatively prime, then B is a Gröbner basis.*

All monomial orders are defined via a matrix in the following way (though not all matrices M define a monomial order in this way, [5, 26]). For $M \in \mathbb{R}^{n \times n}$ with full rank, the associated order fulfills $x^{c_1} > x^{c_2}$ if the first non-zero entry of the vector $M(c_1 - c_2)$ is positive

A typical order is the lexicographic monomial order, *lex*. After choosing a variable order $x_{a_1} > \cdots > x_{a_n}$, $\mathrm{lex}(x_{a_1}, \ldots, x_{a_n})$ is the order defined by the matrix with 1 in positions (i, a_i) for all $i = 1, \ldots, n$ and zero otherwise.

Another monomial order of interest is the graded reverse-lexicographic order, abbreviated *grevlex*. With this order, $x^{c_1} > x^{c_2}$ if the total degree of the first monomial is larger than the second. If they are equal, then the monomial with the smallest variable with least exponent is the greatest one. Grevlex with order of variables $x_1 > \cdots > x_n$ is defined by the matrix

$$\begin{pmatrix} 1 & 1 & \ldots & 1 & 1 \\ 0 & 0 & \ldots & 0 & -1 \\ 0 & 0 & \ldots & -1 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & -1 & \ldots & 0 & 0 \end{pmatrix}.$$

The choice of order plays an important role in the computation time for Gröbner bases, performing lex typically worse than grevlex. However, lex, as any other elimination type order, has a crucial property on elimination of variables. Given a partitioning of the set of variables, $\{x_1, \ldots, x_n\} = \{x_{j_1}, \ldots, x_{j_{n-s}}\} \cup \{x_{i_1}, \ldots, x_{i_s}\}$, a monomial order is of elimination type if x_{j_ℓ}, for $\ell = 1, \ldots, n-s$, is larger than any monomial in $K[x_{i_1}, \ldots, x_{i_s}]$ [6, §3.1, Exercise 5]. Clearly, $\mathrm{lex}(x_{j_1}, \ldots, x_{j_{n-s}}, x_{i_1}, \ldots, x_{i_s})$ is of elimination type. If G is a Gröbner basis of I with respect to an elimination type order as above, then $G \cap K[x_{i_1}, \ldots, x_{i_s}]$ is a Gröbner basis of $I \cap K[x_{i_1}, \ldots, x_{i_s}]$ with respect to the induced monomial on $K[x_{i_1}, \ldots, x_{i_s}]$, which for lex is $\mathrm{lex}(x_{i_1}, \ldots, x_{i_s})$.

3.2. **Gröbner bases and intermediates.** In this subsection we fix a reaction network $\mathcal{N}$ and an extension $\widetilde{\mathcal{N}}$ via the addition of intermediates $Y_1, \ldots, Y_m$. We show that any Gröbner basis of the steady state ideal of $\mathcal{N}$ can be extended to one of

$\widetilde{\mathcal{N}}$ by simply adding the polynomials $H_1, \ldots, H_m$ given in Equation (4). By default, we order the variables $y_1 > \cdots > y_m > x_1 > \cdots > x_n$. We start with some general lemmas.

Lemma 3.2. *Let $I = \langle f_0, f_1, \ldots, f_s \rangle \subseteq K[y, x_1, \ldots, x_n]$ be an ideal such that $f_i \in K[x_1, \ldots, x_n]$ for $i = 1, \ldots, s$ and $f_0 = y + f_0'$, with $f_0' \in K[x_1, \ldots, x_n]$. Consider a monomial order defined by a matrix M whose first row is $\begin{pmatrix} 1 & 0 & \ldots & 0 \end{pmatrix}$. Then*

$$\big\langle \operatorname{LT}(I) \big\rangle = \big\langle y \big\rangle + \big\langle \operatorname{LT}(\langle f_1, \ldots, f_s \rangle) \big\rangle.$$

Further given $G \subseteq K[x_1, \ldots, x_n]$, G is a Gröbner basis of $\langle f_1, \ldots, f_s \rangle$ if and only if $\{f_0\} \cup G$ is a Gröbner basis of I.

Proof. By the choice of monomial order, the monomial y is larger than any monomial not involving y. Consider a reduced Gröbner basis G' of $\langle f_1, \ldots, f_s \rangle$. Then the leading terms of the elements in G' are relatively prime with each other and with the leading term of f_0. Since $\{f_0\} \cup G'$ is a basis of I, then by Lemma 3.1 $\{f_0\} \cup G'$ is a Gröbner basis of I. Now, the initial ideal of I is generated by the leading terms of $\{f_0\} \cup G'$. So:

$$\begin{aligned} \langle \operatorname{LT}(I) \rangle &= \langle \operatorname{LT}(\{f_0\} \cup G') \rangle = \langle \{y\} \cup \operatorname{LT}(G') \rangle = \langle y \rangle + \langle \operatorname{LT}(G') \rangle \\ &= \langle y \rangle + \langle \operatorname{LT}(\langle f_1, \ldots, f_s \rangle) \rangle. \end{aligned}$$

This proves the first part of the lemma.

For the second part, note that

$$\langle y \rangle + \langle \operatorname{LT}(G) \rangle = \langle \{\operatorname{LT}(f_0)\} \cup \operatorname{LT}(G) \rangle = \langle \operatorname{LT}(\{f_0\} \cup G) \rangle.$$

Using this equality and the first part of the lemma, we have $\{f_0\} \cup G$ is a Gröbner basis of I if and only if $\langle y \rangle + \langle \operatorname{LT}(G) \rangle = \langle y \rangle + \langle \operatorname{LT}(\langle f_1, \ldots, f_s \rangle) \rangle$. Since y is not part of any polynomial in G, this equality holds if and only if $\langle \operatorname{LT}(G) \rangle = \langle \operatorname{LT}(\langle f_1, \ldots, f_s \rangle) \rangle$, i.e. G is a Gröbner basis of $\langle f_1, \ldots, f_s \rangle$. □

Recall that we write $I \subseteq \mathbb{R}(k)[x_1, \ldots, x_n]$ and $\widetilde{I} \subseteq \mathbb{R}(\kappa)[y_1, \ldots, y_m, x_1, \ldots, x_n]$ for the steady state ideals of $\mathcal{N}$ and $\widetilde{\mathcal{N}}$ respectively. For the rest of the section, we assume that the rational functions $\phi_{c \to c'}(\kappa)$ are **algebraically independent** over $\mathbb{R}$, such that $\Phi(A)$ is defined for all subsets A of $\mathbb{R}(k)[x]$.

For an arbitrary basis B of I, define

$$\widetilde{B} = \Phi(B) \cup \big\{ H_1(y, x), \ldots, H_m(y, x) \big\} \ \subseteq \mathbb{R}(\kappa)[y, x]. \tag{6}$$

Lemma 3.3. *If B is a basis of $I \subseteq \mathbb{R}(k)[x]$, then $\widetilde{B}$ is a basis of $\widetilde{I} \subseteq \mathbb{R}(\kappa)[y, x]$.*

Proof. Let B' be the set of steady state polynomials of $\mathcal{N}$. By Corollary 2.11

$$\widetilde{I} = \Big\langle \Phi(B') \cup \{H_1(y, x), \ldots, H_m(y, x)\} \Big\rangle.$$

Let now B be an arbitrary basis of I. Then $\langle B \rangle = I = \langle B' \rangle$ and thus by Lemma 2.12(ii), $\langle \Phi(B) \rangle = \langle \Phi(B') \rangle$. Therefore

$$\Big\langle \Phi(B) \cup \{H_1(y, x), \ldots, H_m(y, x)\} \Big\rangle = \Big\langle \Phi(B') \cup \{H_1(y, x), \ldots, H_m(y, x)\} \Big\rangle = \widetilde{I}.$$

This completes the proof. □

Let $\operatorname{rem}(p, B)$ be the remainder of the division of the polynomial p by a set of polynomials B.

Theorem 3.4. *Fix a monomial order on $\mathbb{R}(k)[x]$ associated with an $n \times n$ matrix Q, and let G be a Gröbner basis of I with this order. Then, $\widetilde{G}$ is a Gröbner basis of $\widetilde{I}$ with the monomial order on $\mathbb{R}(\kappa)[y, x]$ associated with the matrix*

$$\widetilde{Q} = \begin{pmatrix} \mathrm{Id}_m & 0 \\ 0 & Q \end{pmatrix}, \tag{7}$$

where Id_m is the identity matrix of size m.

If G is reduced, then $\Phi(G) \cup \left\{ y_i - \operatorname{rem}\left(\sum_{c \in \mathcal{C}} \mu_{i,c} x^c, \Phi(G) \right) \right\}$ is the reduced Gröbner basis of $\widetilde{I}$.

Proof. First note that by the monomial order given by $\widetilde{Q}$, we have $y_1 > \cdots > y_m > x_i$ for all $i = 1, \ldots, n$. Also, the polynomial H_i has degree one in y_i and none of the elements of $\Phi(G) \cup \{H_j \mid j \neq i\}$ involves y_i.

Let us assume we have shown that $\Phi(G)$ is a Gröbner basis of $\langle \Phi(G) \rangle$ with the given order, that is

$$\big\langle \operatorname{LT}(\langle \Phi(G) \rangle) \big\rangle = \big\langle \operatorname{LT}(\Phi(G)) \big\rangle. \tag{8}$$

Then by Lemmas 3.2 and 3.3, $\Phi(G) \cup \{H_1(y, x), \ldots, H_m(y, x)\}$ is a Gröbner basis of $\widetilde{I}$. Therefore the first part of the statement holds provided (8) holds.

Let us show (8). We start by noting that for a subset J in $\mathbb{R}(k)[x]$, the set $\operatorname{LM}(J)$ consists only of monomials in $x_1, \ldots, x_n$, and thus is naturally included in $\mathbb{R}(\kappa)[y, x]$ as well. Further

$$\operatorname{LM}(J) = \operatorname{LM}(\Phi(J)). \tag{9}$$

Let G' be a reduced Gröbner basis of I. Since G' is reduced, pairs of monomials in $\operatorname{LM}(G') = \operatorname{LM}(\Phi(G'))$ are relatively prime. Since $\Phi(G')$ is a basis of $\langle \Phi(G') \rangle$, then by Lemma 3.1 and Equation (5), it is actually a Gröbner basis and (8) holds for G'. Now, consider an arbitrary Gröbner basis G of I. In $\mathbb{R}(k)[x]$ it holds

$$\langle \operatorname{LM}(G) \rangle = \langle \operatorname{LM}(G') \rangle. \tag{10}$$

This means that every monomial in $\langle \operatorname{LM}(G') \rangle$ is divisible by a monomial in $\langle \operatorname{LM}(G) \rangle$ and viceversa [5, §2.4, Lemma 2]. Since this fact holds also in $\mathbb{R}(\kappa)[y, x]$, (10) holds also in $\mathbb{R}(\kappa)[y, x]$. Combined with (9) this gives

$$\big\langle \operatorname{LM}(\Phi(G)) \big\rangle = \big\langle \operatorname{LM}(\Phi(G')) \big\rangle.$$

By Lemma 2.12(ii), $\langle G \rangle = \langle G' \rangle$ in $\mathbb{R}(k)[x]$ implies $\langle \Phi(G) \rangle = \langle \Phi(G') \rangle$. Thus in $\mathbb{R}(\kappa)[y, x]$ we have

$$\big\langle \operatorname{LM}(\Phi(G)) \big\rangle = \big\langle \operatorname{LM}(\Phi(G')) \big\rangle = \big\langle \operatorname{LM}(\langle \Phi(G') \rangle) \big\rangle = \big\langle \operatorname{LM}(\langle \Phi(G) \rangle) \big\rangle.$$

This shows that (8) holds.

The second part of the lemma is clear from the definition of a reduced Gröbner basis and using that $\Phi(G) \cup \{ y_i - \operatorname{rem}\left(\sum_{c \in \mathcal{C}} \mu_{i,c} x^c, \Phi(G) \right) \}$ is also a Gröbner basis. □

$$
\begin{aligned}
X_1 + X_2 &\underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} X_3 \xrightarrow{\kappa_3} X_2 + X_4\\
X_4 + X_{18} &\underset{\kappa_{38}}{\overset{\kappa_{37}}{\rightleftarrows}} X_{25} \xrightarrow{\kappa_{39}} X_{18} + X_{26}\\
X_5 + X_{26} &\underset{\kappa_{41}}{\overset{\kappa_{40}}{\rightleftarrows}} X_{27} \xrightarrow{\kappa_{42}} X_4 + X_5 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftarrows}} X_6 \xrightarrow{\kappa_6} X_1 + X_5\\
X_4 + X_7 &\underset{\kappa_8}{\overset{\kappa_7}{\rightleftarrows}} X_8 \xrightarrow{\kappa_9} X_4 + X_9 \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftarrows}} X_{10} \xrightarrow{\kappa_{12}} X_4 + X_{11}\\
X_5 + X_{11} &\underset{\kappa_{14}}{\overset{\kappa_{13}}{\rightleftarrows}} X_{12} \xrightarrow{\kappa_{15}} X_5 + X_9 \underset{\kappa_{17}}{\overset{\kappa_{16}}{\rightleftarrows}} X_{13} \xrightarrow{\kappa_{18}} X_5 + X_7\\
X_{11} + X_{14} &\underset{\kappa_{20}}{\overset{\kappa_{19}}{\rightleftarrows}} X_{15} \xrightarrow{\kappa_{21}} X_{11} + X_{16} \underset{\kappa_{23}}{\overset{\kappa_{22}}{\rightleftarrows}} X_{17} \xrightarrow{\kappa_{24}} X_{11} + X_{18}\\
X_{18} + X_{19} &\underset{\kappa_{26}}{\overset{\kappa_{25}}{\rightleftarrows}} X_{20} \xrightarrow{\kappa_{27}} X_{16} + X_{19} \underset{\kappa_{29}}{\overset{\kappa_{28}}{\rightleftarrows}} X_{21} \xrightarrow{\kappa_{30}} X_{14} + X_{19}\\
X_{18} + X_{22} &\underset{\kappa_{32}}{\overset{\kappa_{31}}{\rightleftarrows}} X_{23} \xrightarrow{\kappa_{33}} X_{16} + X_{22}\\
X_5 + X_{16} &\underset{\kappa_{35}}{\overset{\kappa_{34}}{\rightleftarrows}} X_{24} \xrightarrow{\kappa_{36}} X_5 + X_{14}\\
X_{18} + X_{28} &\underset{\kappa_{44}}{\overset{\kappa_{43}}{\rightleftarrows}} X_{29} \xrightarrow{\kappa_{45}} X_{18} + X_{19}\\
X_{19} &\xrightarrow{\kappa_{46}} X_{28}
\end{aligned}
$$

FIGURE 1. Reaction network of Example 3.5.

From the computational point of view, Theorem 3.4 is very useful. Instead of computing a Gröbner basis of $\widetilde{I}$ directly, one can first compute a Gröbner basis G for the core network, with a smaller number of variables and polynomials, then add the polynomials $y_i - \sum_{c\in\mathcal{C}} \mu_{i,c}x^c$, and, finally, simplify them using polynomial division by $\Phi(G)$. The second step involves only linear algebra. A possible issue here is to verify that the rational functions $\phi_{c\to c'}$ are algebraically independent. We provide in Section 4 a list of network structures involving intermediates for which the condition is fulfilled.

Example 3.5. An interesting example to show the advantage of using Theorem 3.4 is Example 4.4 of [4]. We consider the reaction network $\widetilde{\mathcal{N}}$ with associated digraph given in Figure 1.

This reaction network has 29 species and 46 reactions. Therefore the steady state ideal is generated by 29 polynomials in 29 variables and 46 parameters. Using Singular [7] and monomial order grevlex with $x_1 > \cdots > x_{29}$ (the same monomial order that is used in [4]), it took between 110 and 115 seconds[1] to compute the reduced Gröbner basis. This basis consists of 169 polynomials.

[1]Information about the processor: Intel(R) Core(TM) i5-3570 CPU @3.4GHz 3.4GHz with 8GB RAM. We report the interval of obtained times after several runs of Singular, computed in milliseconds.

Now we consider the monomial order introduced in Theorem 3.4 for the removal of the 15 intermediates:

$$X_3, X_6, X_8, X_{10}, X_{12}, X_{13}, X_{15}, X_{17}, X_{20}, X_{21}, X_{23}, X_{24}, X_{25}, X_{27}, X_{29}.$$

The original network $\widetilde{\mathcal{N}}$ is an extension of the following core network $\mathcal{N}$ with 14 species and 16 reactions:

$$\begin{aligned}
X_1 + X_2 &\xrightarrow{k_1} X_2 + X_4 & X_5 + X_{26} &\xrightarrow{k_3} X_4 + X_5 \xrightarrow{k_4} X_1 + X_5\\
X_4 + X_{18} &\xrightarrow{k_2} X_{18} + X_{26} & X_4 + X_7 &\xrightarrow{k_5} X_4 + X_9 \xrightarrow{k_6} X_4 + X_{11}\\
X_{18} + X_{22} &\xrightarrow{k_{13}} X_{16} + X_{22} & X_5 + X_{11} &\xrightarrow{k_7} X_5 + X_9 \xrightarrow{k_8} X_5 + X_7\\
X_5 + X_{16} &\xrightarrow{k_{14}} X_5 + X_{14} & X_{11} + X_{14} &\xrightarrow{k_9} X_{11} + X_{16} \xrightarrow{k_{10}} X_{11} + X_{18}\\
X_{18} + X_{28} &\xrightarrow{k_{15}} X_{18} + X_{19} & X_{18} + X_{19} &\xrightarrow{k_{11}} X_{16} + X_{19} \xrightarrow{k_{12}} X_{14} + X_{19}\\
X_{19} &\xrightarrow{k_{16}} X_{28}.
\end{aligned}$$

The functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$ by Corollary 4.6 in Section 4. We consider grevlex with $x_1 > \cdots > x_{28}$ for the monomials corresponding to $\mathcal{N}$. The monomial order in Theorem 3.4 is then associated with the following matrix

$$\widetilde{Q} = \left(\begin{array}{ccc:cccccc}
1 & & 0 & & & & & & \\
& \ddots & & & & 0 & & & \\
0 & & 1 & & & & & & \\
\hdashline
 & & & 1 & 1 & \dots & 1 & 1 \\
 & & & 0 & 0 & \dots & 0 & -1 \\
 & 0 & & 0 & 0 & \dots & -1 & 0 \\
 & & & \vdots & \vdots & & \vdots & \vdots \\
 & & & 0 & -1 & \dots & 0 & 0
\end{array}\right),$$

and order of variables

$$\begin{aligned}
&x_3 > x_6 > x_8 > x_{10} > x_{12} > x_{13} > x_{15} > x_{17} > x_{20} > x_{21} > x_{23} > x_{24}\\
&\quad > x_{25} > x_{27} > x_{29} > x_1 > x_2 > x_4 > x_5 > x_7 > x_9 > x_{11} > x_{14} > x_{16}\\
&\quad > x_{18} > x_{19} > x_{22} > x_{26} > x_{28}.
\end{aligned}$$

The reduced Gröbner basis of $\widetilde{I}$ with this monomial order has 33 polynomials and it takes about 96 seconds to compute it directly with Singular. Alternatively the strategy outlined in Theorem 3.4 can be applied. The steady state ideal I of $\mathcal{N}$ is generated by 11 polynomials in 14 variables and 16 parameters. Using Singular, the reduced Gröbner basis of I has 18 polynomials and its computation takes less than a millisecond. The computation time for the polynomials $H_i(y, x)$ is neglectable, since they are found by solving 15 independent linear equations. Therefore the reduced Gröbner basis of the ideal of the original system has 18+15=33 polynomials and can be computed in less than a millisecond.

We conclude that in general, regarding computational time, the monomial order introduced in Theorem 3.4 is a good choice for networks with intermediates, and further, by applying the strategy of Theorem 3.4 we reduce the computation time considerably, compared with direct computation of the reduced Gröbner basis.

Remark 3.6. Theorem 3.4 holds regardless the choice of method to compute a Gröbner basis. Since the computation of the polynomials H_i is simple linear algebra, even for the fastest available methods for the computation of Gröbner bases, decomposing the computation as in Theorem 3.4 should be faster than direct computation of the basis of the steady state ideal of $\widetilde{\mathcal{N}}$.

Remark 3.7. For polynomials with integer coefficients, it is usually faster to compute a Gröbner basis using the so-called *p-modular* approach, see e.g. [23, 29]. These methods first choose a so-called lucky prime and compute a Gröbner basis of the ideal in $\overline{\mathbb{Z}}_p[x]$. Then the coefficients of this Gröbner basis are lifted to a Gröbner basis in $\mathbb{Q}[x]$. For the sake of comparison, we also computed how long it takes to find a Gröbner basis using p-modular approaches on the extended network in Example 3.5 with grevlex and $x_1 > \cdots > x_{29}$. Using the largest prime number in Singular, $p = 32003$, it takes 127 seconds to compute the Gröbner basis over $\overline{\mathbb{Z}}_{32003}$. Since coefficients in the starting basis are 1 or -1, one may think that $p = 2$ is a lucky prime. It took 97 seconds to compute the Gröbner basis over $\overline{\mathbb{Z}}_2$. These times are larger than the times reported in Example 3.5 (and these Gröbner bases still need to be lifted to $\mathbb{Q}(\kappa)[y, x]$).

An important consequence of Theorem 3.4 concerns parameter-free model discrimination. In this setting one seeks elements of the steady state ideal $\widetilde{I}$ involving only the concentrations of species that are experimentally measurable. These elements are called *invariants*. Each invariant implies that there is a set of monomials that lie on a hyperplane, and the hypothesis of coplanary is then tested using experimental data [13, 14, 18–20]. This approach is attractive because it does not require knowing the values of the reaction rate constants.

Experimentally measurable species do not typically involve intermediates. In this case, Theorem 3.4 tells us that invariants on the non-intermediate species can be computed directly from the core network, using elimination ideals.

Corollary 3.8. *Let $\mathcal{N}$ be a reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of m intermediates $Y_1, \ldots, Y_m$. Let $X_{i_1}, \ldots, X_{i_p}$ be non-intermediates. Then*

$$\widetilde{I} \cap \mathbb{R}(\kappa)[x_{i_1}, \ldots, x_{i_p}] = \Phi(I \cap \mathbb{R}(k)[x_{i_1}, \ldots, x_{i_p}]).$$

Proof. For simplicity, assume $\{i_1, \ldots, i_p\} = \{n-p+1, \ldots, n\}$ and let $\overline{x} = (x_{n-p+1}, \ldots, x_n)$. Consider the monomial order $\mathrm{lex}(y_1, \ldots, y_m, x_1, \ldots, x_n)$ on $\mathbb{R}(\kappa)[y, x]$, and $\mathrm{lex}(x_1, \ldots, x_n)$ on $\mathbb{R}(k)[x]$. Let G be a Gröbner basis of I. By Theorem 3.4, $\widetilde{G}$ is a Gröbner basis of $\widetilde{I}$. By the properties of lex and Lemma 2.12(ii) we have

$$\widetilde{I} \cap \mathbb{R}(\kappa)[\overline{x}] = \langle \widetilde{G} \cap \mathbb{R}(\kappa)[\overline{x}] \rangle = \langle \Phi(G \cap \mathbb{R}(k)[\overline{x}]) \rangle = \Phi(I \cap \mathbb{R}(k)[\overline{x}]).$$

This concludes the proof. □

Note that the monomial order on $\mathbb{R}(\kappa)[y, x]$ given in Theorem 3.4 is of elimination type with respect to the partition $\{y_1, \ldots, y_m\} \cup \{x_1, \ldots, x_n\}$.

Example 3.9. Consider the network in Example 2.6 and its core network in Example 2.9. In order to find invariants of the extended network involving the concentration of the non-intermediate species E, X_0, X_1, X_2, we consider the ideal $I \cap$

$\mathbb{R}(k)[e, x_0, x_1, x_2]$, which is generated by the polynomial

$$e\,(k_1k_3x_0x_2 - k_2k_4x_1^2).$$

We have

$$\phi(k_1, k_2, k_3, k_4) = \left(\tfrac{\kappa_1\kappa_3}{\kappa_2+\kappa_3}, \tfrac{\kappa_4\kappa_6}{\kappa_5+\kappa_6}, \tfrac{\kappa_7\kappa_9}{\kappa_8+\kappa_9}, \tfrac{\kappa_{12}\kappa_{14}}{\kappa_{13}+\kappa_{14}}\right).$$

The functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$ by Corollary 4.6. By Corollary 3.8 the ideal $\widetilde{I} \cap \mathbb{R}(\kappa)[e, x_0, x_1, x_2]$ is generated by the polynomial

$$e\left(\tfrac{\kappa_1\kappa_3}{\kappa_2+\kappa_3}\tfrac{\kappa_7\kappa_9}{\kappa_8+\kappa_9}x_0x_2 - \tfrac{\kappa_4\kappa_6}{\kappa_5+\kappa_6}\tfrac{\kappa_{12}\kappa_{14}}{\kappa_{13}+\kappa_{14}}x_1^2\right).$$

3.3. **Detecting binomial steady state ideals.** A *binomial* is a polynomial having at most two terms. An ideal is said to be binomial if it admits a set of generators consisting of binomials only. By [8, Corollary 1.2], an ideal is binomial if and only if any reduced Gröbner basis (with respect to any monomial order) consists of binomials.

It is of biological relevance in the study of reaction networks to determine whether there exists a choice of reaction rate constants k for which there are multiple positive steady states in some coset $x_0 + S$ defined by the vector subspace S that contains the image of F_k (see Section 1). This property is termed *multistationarity*. If the steady state ideal is binomial, then there exist efficient ways to determine whether the network admits multistationarity [22, 24, 25]. This leads to the problem of determining whether an ideal is binomial, and in case it is, of finding a binomial basis of it. As noted, both questions can be addressed by finding a Gröbner basis of the steady state ideal of the network. Thus, for networks with intermediates, our results can be applied also to detect binomial steady state ideals.

Recall that we are assuming that the rational functions $\phi_{c\to c'}(\kappa)$ are **algebraically independent** over $\mathbb{R}$.

Theorem 3.10. *Let $\mathcal{N}$ be a reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of m intermediates $Y_1, \ldots, Y_m$.*

The steady state $\widetilde{I}$ is binomial if and only if

- *I is binomial, and,*
- *for any reduced Gröbner basis G of I and for every $i = 1, \ldots, m$, the remainder of the division of $\sum_{c\in\mathcal{C}} \mu_{i,c}x^c$ by $\Phi(G)$ has at most one term.*

Proof. Fix any monomial order on $\mathbb{R}(k)[x_1, \ldots, x_n]$ associated with an $n \times n$ matrix Q and consider the monomial order with matrix $\widetilde{Q}$ from Theorem 3.4. Let G be the reduced Gröbner basis of I and

$$\widetilde{G}' = \Phi(G) \cup \Big\{ y_i - \operatorname{rem}\big(\sum_{c\in\mathcal{C}} \mu_{i,c}x^c, \Phi(G)\big)\Big\}$$

the reduced Gröbner basis of $\widetilde{I}$ (cf. Theorem 3.4). Using that an ideal is binomial if and only if any reduced Gröbner basis consists of binomials, the theorem is a consequence of the following two facts:

- By definition, $\widetilde{G}'$ consists of binomials if and only if $\Phi(G)$ is a set of binomials and the remainder of the division of $\sum_{c\in\mathcal{C}} \mu_{i,c}x^c$ by $\Phi(G)$ has at most one term.

- By the algebraic independence of $\phi_{c\to c'}$, $\Phi(G)$ consists of binomials if and only if G does.

□

Since the polynomial $\sum_{c\in\mathcal{C}} \mu_{i,c}x^c$ has exactly one term for 1-input intermediates, we readily obtain the following corollary.

Corollary 3.11. *Let $\mathcal{N}$ be a reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of m* 1-input *intermediates $Y_1,\dots,Y_m$. Then $\widetilde{I}$ is binomial if and only if I is binomial.*

Since 1-input intermediates are the most abundant form of intermediates found in realistic networks, this corollary implies that in order to check whether a steady state ideal is binomial, we can often remove intermediates and check whether the steady state ideal of the core network is binomial.

Example 3.12. Consider the network in Example 2.5 and its core network given in Example 2.8. The functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$ by Example 4.1. Since the steady state ideal of $\mathcal{N}$ is

$$\langle -(k_1-k_2)x_1x_2 - 2k_3x_1^2, (k_1-k_2)x_1x_2 + 2k_3x_1^2\rangle,$$

the core network has a binomial steady state ideal. The reduced Gröbner basis for this ideal with monomial order $\mathrm{lex}(x_1,x_2,x_3)$ is

$$G = \left\{ x_1^2 - \tfrac{(k_1-k_2)}{2k_3}x_1x_2 \right\}.$$

We apply Theorem 3.10 to conclude that the steady state ideal of the extended network is also binomial. The intermediates Y_1, Y_2 are 1-input intermediates and hence the remainder condition of the theorem is automatically fulfilled. For the intermediate Y_3, $\mathrm{rem}\left(\mu_{3,X_1+X_2}x_1x_2 + \mu_{3,X_2+X_3}x_2x_3, \Phi(G)\right)$ has a single term with monomial x_1x_2. Therefore we conclude that the extended network also has a binomial steady state ideal.

The following example shows that extended networks with multi-input intermediates might not have binomial steady state ideals, even though their core networks have.

Example 3.13. Consider the network given in Example 2.6 and its core network given in Example 2.9. The steady state ideal of the core network is binomial with basis $B = \{k_1x_0e - k_4x_1f, k_2x_1e - k_3x_2f\}$. The intermediates Y_4 and Y_6 are 2-input intermediates. The remainder of the division of $\mu_{4,X_2+E}x_2f + \mu_{4,X_1+F}x_1f$ by $\Phi(G)$ for G the reduced Gröbner basis of I with the monomial order $\mathrm{lex}(x_2,x_1,x_0,f,e)$ is

$$\tfrac{\kappa_{11}}{\kappa_{10}}x_1f + \tfrac{\kappa_7\kappa_9}{\kappa_8\kappa_{10}+\kappa_9\kappa_{10}}x_2f,$$

which has two terms. Therefore by Theorem 3.10 the steady state ideal of the network in Example 2.6 is not binomial.

Remark 3.14. In [4], a method for determining whether a *homogeneous* ideal is binomial is introduced. The method avoids the computation of Gröbner bases and

is regarded as a fast method. If the steady state ideal of the core network is homogeneous, then Theorem 3.10 or Corollary 3.11 in combination with this method provide a fast procedure to detect binomial steady state ideals.

Interestingly, steady state polynomials of core networks are often homogeneous of degree two, since it is common that non-intermediate species appear in complexes of the form $X_i + X_j$, yielding quadratic terms in the steady state polynomials. This is for example the case for so-called Post-Translational Modification Networks [28].

4. Algebraic independence

In this section we discuss how to check whether the functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$ and provide classes of intermediates for which this property holds. Consider a set of rational functions $A = \{\frac{f_1}{g_1}, \ldots, \frac{f_m}{g_m}\} \subseteq \mathbb{R}(x_1, \ldots, x_n)$. By §III.7, Theorem III, in [16], the set A is algebraically independent over $\mathbb{R}$ if and only if the rank of the associated Jacobian matrix $\left(\frac{\partial(f_i/g_i)}{\partial x_j}\right)_{i,j}$ over $\mathbb{R}(x)$ is m.

Another way to check algebraic independence that requires the computation of a Gröbner basis is as follows. Let φ be the function on $\mathbb{R}^n$ minus the zero locus of the product $g_1 \cdots g_m$ defined by

$$x = (x_1, \ldots, x_n) \mapsto \left(\frac{f_1(x)}{g_1(x)}, \ldots, \frac{f_m(x)}{g_m(x)}\right).$$

By §3.3, Theorem 2, in [6], the closure of $\mathrm{Im}(\varphi)$ is the variety associated with the ideal

$$J := \langle g_1T_1 - f_1, \ldots, g_mT_m - f_m, 1 - yg_1 \cdots g_m\rangle \cap \mathbb{R}[T_1, \ldots, T_m].$$

Since the sets of polynomials vanishing on a set and on its closure agree (see [6] after Definition 2 in §4.4), A is algebraically independent over $\mathbb{R}$ if and only if $J = \{0\}$.

Example 4.1. The functions $\phi_{c\to c'}$ of Examples 2.5 and 2.8 are

$$\begin{aligned}
\phi_{X_1+X_2\to 2X_2}(\kappa) &= \kappa_4\,\mu_{2,X_1+X_2} + \kappa_8\,\mu_{3,X_1+X_2} = \tfrac{\kappa_1\kappa_3}{\kappa_2+\kappa_3+\kappa_5} + \tfrac{\kappa_1\kappa_5\kappa_8}{(\kappa_6+\kappa_8)(\kappa_2+\kappa_3+\kappa_5)},\\
\phi_{X_1+X_2\to 2X_1}(\kappa) &= \kappa_6\,\mu_{3,X_1+X_2} = \tfrac{\kappa_1\kappa_5\kappa_6}{(\kappa_6+\kappa_8)(\kappa_2+\kappa_3+\kappa_5)},\\
\phi_{2X_1\to 2X_2}(\kappa) &= \kappa_9 + \kappa_8\,\mu_{3,2X_1} = \kappa_9 + \tfrac{\kappa_7\kappa_8}{\kappa_6+\kappa_8}.
\end{aligned}$$

We find that $J = \{0\}$. Hence the algebraic independence condition holds for the network in Example 2.8. Alternatively, one easily checks that the associated Jacobian matrix has rank 3.

The computations above can be simplified by taking into account what parameters occur in each of the rational functions.

Definition 4.2. Let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of the intermediates $\{Y_1, \ldots, Y_m\}$. Consider the digraph associated with $\widetilde{\mathcal{N}}$. Let $\mathcal{Y}_1, \ldots, \mathcal{Y}_{t'}$ denote the vertex sets of the connected components of the subgraph induced by the subset of vertices $\{Y_1, \ldots, Y_m\}$.

Let $\mathcal{R}' \subseteq \mathcal{R}$ be the subset of reactions of the core network that are not in $\widetilde{\mathcal{R}}$. These reactions arise necessarily from paths through intermediates. We say that

two reactions $r_1\colon c_1 \to c_1', r_2\colon c_2 \to c_2' \in \mathcal{R}'$ *overlap* if there exist paths through intermediates

$$c_1 \to Y_{i_1} \to \cdots \to Y_{i_p} \to c_1', \qquad c_2 \to Y_{j_1} \to \cdots \to Y_{j_q} \to c_2'$$

with all intermediates belonging to the same set $\mathcal{Y}_i$.

Consider the equivalence relation on $\mathcal{R}'$ generated by the overlap relation: $r \sim r'$ if and only if there exist $r_0 = r, r_1, \ldots, r_p = r'$ such that r_i, r_{i+1} overlap for all $i = 0, \ldots, p-1$. Let $\mathcal{R}_1', \ldots, \mathcal{R}_t'$ be the equivalence classes of this equivalence relation.

Example 4.3. Consider the network in Example 2.8. The set $\mathcal{R}'$ consists of two reactions $X_1 + X_2 \to 2X_2$ and $X_1 + X_2 \to 2X_1$. The subgraph of the digraph associated with $\widetilde{\mathcal{N}}$ induced by the set of intermediates is connected. Thus the two reactions of $\mathcal{R}'$ are equivalent and there is one equivalence class.

Lemma 4.4. *The set $\{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}\}$ is algebraically independent over $\mathbb{R}$ if and only if the set $\{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}_i'\}$ is algebraically independent over $\mathbb{R}$ for all $i = 1, \ldots, t$.*

Proof. Since $\mathcal{R}_i' \subseteq \mathcal{R}$ for all $i = 1, \ldots, t$, the forward implication is clear.

To prove the reverse implication, assume that the sets $T_i = \{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}_i'\}$ are algebraically independent over $\mathbb{R}$ for all $i = 1, \ldots, t$. By construction, the sets of parameters appearing in the rational functions $\phi_{c\to c'}(\kappa)$ are disjoint for two reactions in different equivalence classes. Therefore the union of the sets $T_1, \ldots, T_t$ is algebraically independent over $\mathbb{R}$. Furthermore if $c \to c' \in \mathcal{R} \setminus \mathcal{R}'$, then the parameter $\kappa_{c\to c'}$ appears only in $\phi_{c\to c'}(\kappa)$. As a consequence the set

$$\bigcup_{i=1}^{t} T_i \cup \{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R} \setminus \mathcal{R}'\} = \{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}\}$$

is algebraically independent over $\mathbb{R}$. □

Example 4.5. Consider the network in Example 4.3. The algebraic independence of the functions $\phi_{c\to c'}(\kappa)$ for all reactions $c \to c'$ in $\mathcal{R}$ follows in this case from the algebraic independence of the functions $\phi_{c\to c'}(\kappa)$ for the reactions $X_1 + X_2 \to 2X_2$ and $X_1 + X_2 \to 2X_1$.

Corollary 4.6. *If $\mathcal{R}' = \emptyset$ or each of the equivalence classes $\mathcal{R}_1', \ldots, \mathcal{R}_t'$ consist of one reaction, then the rational functions $\phi_{c\to c'}(\kappa)$ are algebraically independent over $\mathbb{R}$.*

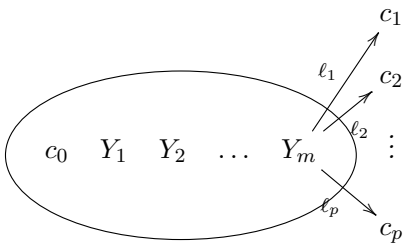
For the networks in Example 2.6 and Example 3.5, each of the equivalence classes consist of one reaction. Therefore, by Corollary 4.6, the algebraic independence condition holds.

We next show that the algebraic independence condition holds for specific classes of intermediates without the need of doing any extra computation.

Lemma 4.7. *For the following extension networks, with intermediates $Y_1, \ldots, Y_m$, the set $\{\phi_{c\to c'}(\kappa) \mid c \to c' \in \mathcal{R}\}$ is algebraically independent over $\mathbb{R}$.*

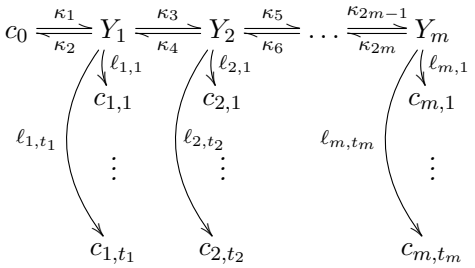
(i) $c \longleftrightarrow Y_1 \longleftrightarrow Y_2 \longleftrightarrow \ldots \longleftrightarrow Y_m \longleftrightarrow c'$, provided $\{Y_1, \ldots, Y_m\}$ is a set of intermediates and where $\longleftrightarrow$ means the reaction can be irreversible or reversible.

(ii)



with an arbitrary digraph structure among the complexes $c_0, Y_1, \dots, Y_m$ such that there exists a directed path from c_0 to Y_m.

(iii)



where some of the reactions with label κ_{2i} might not exist, and for each $1 \leq i \leq m$, either $t_i \geq 0$.

Proof. We start by recalling how to find $\mu_{i,c}$ using a labeled digraph (see proof of Theorem 2 of the electronic supplementary material of [11]). For each non-intermediate complex c, consider the labeled digraph $\widehat{G}_c$ with vertex set $\{Y_1, \dots, Y_m, \star\}$ and labeled edges $Y_i \xrightarrow{\kappa_{Y_i \to Y_j}} Y_j$ if $Y_i \to Y_j \in \widetilde{\mathcal{R}}$, $\star \xrightarrow{\kappa_{c \to Y_i} x^c} Y_i$ if $c \to Y_i \in \widetilde{\mathcal{R}}$ and $Y_i \xrightarrow{\beta_i} \star$ with $\beta_i = \sum_{Y_i \to c'} \kappa_{Y_i \to c'}$ if $\beta_i \neq 0$.

For every vertex v of $\widehat{G}_c$ define $\theta(v)$ as the set of all spanning trees rooted at v.[2] Given such a tree τ, let $\pi(\tau)$ be the product of the labels of the edges of τ. Then

$$\mu_{i,c} = \frac{\sum_{\tau \in \theta(Y_i)} \pi(\tau)}{\sum_{\tau \in \theta(\star)} \pi(\tau)}. \tag{11}$$

(i) If one of the reactions is irreversible, then the core network consists of exactly one reaction, either $c \to c'$ or $c' \to c$, and the set $\{\phi_{c \to c'}(\kappa) \mid c \to c' \in \mathcal{R}\}$ is algebraically independent over $\mathbb{R}$.

If all reactions are reversible, we write

$$c \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} Y_2 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} \dots \underset{\kappa_{2m}}{\overset{\kappa_{2m-1}}{\rightleftharpoons}} Y_m \underset{\kappa_{2m+2}}{\overset{\kappa_{2m+1}}{\rightleftharpoons}} c',$$

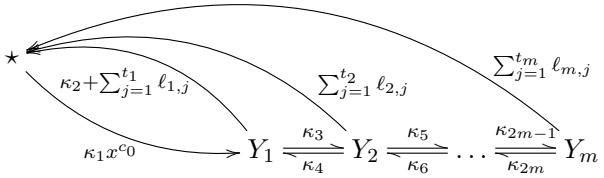
and we have $\phi_{c' \to c}(\kappa) = \kappa_2 \mu_{1,c'}$, $\phi_{c \to c'}(\kappa) = \kappa_{2m+1} \mu_{m,c}$. By the expressions for $\mu_{1,c'}$ and $\mu_{m,c}$ in (11), both rational functions have the same denominator and κ_{2m+1} is not part of their numerator. Therefore, algebraic independence of $\kappa_2 \mu_{1,c'}$ and $\kappa_{2m+1} \mu_{m,c}$

[2] a spanning tree is rooted at v if v is the only vertex with no outgoing edges

follows from the algebraic independence of the numerators of these two rational functions. Since κ_{2m+1} is a factor of $\phi_{c\to c'}(\kappa)$ and is not part of the numerator of $\phi_{c'\to c}(\kappa)$, the two functions $\phi_{c\to c'}(\kappa), \phi_{c'\to c}(\kappa)$ are algebraically independent over $\mathbb{R}$.

(ii) We have $\phi_{c_0\to c_i}(\kappa) = \ell_i\mu_{m,c_0}$ for $i = 1, \ldots, p$. Thus the set $\{\phi_{c_0\to c_i} \mid 1 \leq i \leq p\}$ is algebraically independent over $\mathbb{R}$ if and only if $\{\ell_i \mid 1 \leq i \leq p\}$ is, which clearly holds.

(iii) The reactions of the core network are of the form $c_0 \to c_{i,j}$. We consider the graph $\widehat{G}_{c_0}$ (removing the edges for which there is no reaction):



We have $\phi_{c_0\to c_{i,j}}(\kappa) = \ell_{i,j}\mu_{i,c_0}$. The denominators of the rational functions μ_{i,c_0} as given in (11) agree. Therefore it is enough to check that the polynomials $\rho_{i,j} := \ell_{i,j}\sum_{\tau\in\theta(Y_i)}\pi(\tau)$ for all i, j are algebraically independent over $\mathbb{R}$.

For each $1 \leq i \leq m$, there exists a spanning tree rooted at Y_i involving an edge of the form $Y_j \to \star$ only for $j \gneqq i$. Now consider the smallest index i such that there exists a complex $c_{i,j}$. The parameter $\ell_{i,j}$ appears in a polynomial ρ_{i_1,i_2} only for $i_1 = i$. Hence the polynomials ρ_{i_1,i_2} are algebraically independent if and only if they are for $i_1 > i$. We proceed in the same way now considering the smallest index $k > i$ such that there exists a complex $c_{k,j}$. This process terminates in at most m steps.

□

Corollary 4.6 and Lemma 4.7(i) show that typical rational functions arising from realistic networks, such as those built from the mechanism in Example 2.2, fulfil the algebraic independence condition.

5. Another class of species: enzymes

In this final section we consider another class of species for which reduction mechanisms have also been defined, namely enzymes, and study how Gröbner bases of extended and reduced networks relate.

5.1. **Enzymes.** A species $E \in \mathcal{S}$ is an *enzyme* if for every reaction the stoichiometric coefficient of E in the reactant and the product agree [21]. This automatically gives that the steady state polynomial of E is identically zero, and implies that the concentration of E is constant in time and only depends on the initial amount e_0 of E. For example, E and F are enzymes in the network of Example 2.9.

The core network obtained by removal of E consists of simply removing E from each side of the reaction (this is an example of an embedded network, see [17]). For

example, a reaction

$$X_1 + E \xrightarrow{\kappa_1} X_2 + E \qquad \text{becomes} \qquad X_1 \xrightarrow{k_1} X_2. \tag{12}$$

After fixing the initial amount of enzyme e_0, the steady states of the extended network satisfying that the concentration of E is e_0 agree with the steady states of the core network with $k_1 = e_0\kappa_1$.

This might lead one to think that enzymes are redundant and that similar properties as those that hold for intermediates also hold for enzymes. For example, one might think there is an easy way to obtain a Gröbner basis of the steady state ideal of the extended network from one of the core network, or that a binomial steady state ideal remains binomial upon removal of intermediates. But this is not the case, as the following examples illustrate.

Example 5.1. Let $\mathcal{N}$ be the network

$$2X \xrightarrow{k_1} 3X \xrightarrow{k_2} X, \quad 2X \xrightarrow[k_3]{} X$$

A binomial basis of the steady state ideal is $\{-2k_2x^3 + (k_1 - k_3)x^2\}$. Now consider the following network by adding one enzyme E:

$$2X \xrightarrow{\kappa_1} 3X \xrightarrow{\kappa_2} X \qquad 2X + E \xrightarrow{\kappa_3} X + E.$$

A reduced Gröbner basis of its steady state ideal is $\{x^3 - \frac{\kappa_1}{2\kappa_2}x^2 + \frac{\kappa_3}{2\kappa_2}x^2e\}$, and hence this ideal is not binomial.

The previous example suggests the following: Consider a reaction as in (12). One might obtain a Gröbner basis of the steady state ideal of the extended network by considering a Gröbner basis of the steady state ideal of the core network and substituting the parameter κ_1 by k_1e. The following example gives a negative answer to this question.

Example 5.2. Let $\mathcal{N}$ be the following network

$$\begin{array}{ccc} X_1 & \xrightarrow{k_1} & \\ & & 0 \\ 2X_1 & \xrightarrow{k_2} & \end{array} \qquad 3X_1 \xrightarrow{k_3} X_2.$$

The set of steady state polynomials is

$$\{-k_1x_1 - 2k_2x_1^2 - 3k_3x_1^3,\ k_3x_1^3\}.$$

With every arbitrary monomial order on $\mathbb{R}(k)[x]$, the reduced Gröbner basis of the steady state ideal is $\{x_1\}$.

Let now $\mathcal{N}'$ be the extension of $\mathcal{N}$ via the enzyme E:

$$\begin{array}{ccc} X_1 & \xrightarrow{k_1} & \\ & & 0 \\ 2X_1 & \xrightarrow{k_2} & \end{array} \qquad 3X_1 + E \xrightarrow{k_3} X_2 + E.$$

The set of steady state polynomials of $\mathcal{N}'$ is

$$\{-\kappa_1 x_1 - 2\kappa_2 x_1^2 - 3\kappa_3 x_1 e,\, \kappa_3 x_1^3 e\}.$$

The steady state ideal is different from $\langle x_1 \rangle$. Thus, there is not a monomial order on $\mathbb{R}(\kappa)[x, e]$ for which the reduced Gröbner basis can be obtained from the set $\{x_1\}$ by making the substitution $k_3 = \kappa_3 e$.

Example 5.3. When a binomial basis of the steady state ideal is obtained from linear combinations of the steady state polynomials (see [4]), then the steady state ideal of the core network is binomial if and only if that of the extended network is.

Acknowledgements. This work has been supported by the Danish Research Council for Independent Research. We thank Martin Helmer, Angélica Torres and Carsten Wiuf for comments on previous versions of this manuscript.

References

[1] M. Bardet, J. Faugère, and B. Salvy. On the complexity of the F_5 Gröbner basis algorithm. *J. Symb. Comput.*, 70:49–70, 2015.

[2] D. Bayer and M. Stillman. A theorem on refining division orders by the reverse lexicographc order. *Duke Math. J.*, 55(2):321–328, 1987.

[3] R. Bradford, J. H. Davenport, M. England, H. Errami, V. Gerdt, D. Grigoriev, C. Hoyt, M. Košta, O. Radulescu, T. Sturm, and A. Weber. A case study on the parametric occurrence of multiple steady states. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 45–52. Association for Computing Machinery, 2017.

[4] C. Conradi and T. Kahle. Detecting binomiality. *Adv. Appl. Math.*, 71:52–67, 2015.

[5] D. Cox, J. Little, and D. O'Shea. *Using Algebraic Geometry.* Springer-Verlag New York, 2nd edition, 2005.

[6] D. Cox, J. Little, and D. O'Shea. *Ideals, Varieties, and Algorithms.* Springer International Publishing, 4th edition, 2015.

[7] W. Decker, G. Greuel, G. Pfister, and H. Schönemann. Singular 4-0-2 — A computer algebra system for polynomial computations. `http://www.singular.uni-kl.de`, 2016.

[8] D. Eisenbud and B. Sturmfels. Binomial ideals. *Duke Math. J.*, 84(1):1–45, 1996.

[9] J. Faugère. A new efficient algorithm for computing Gröbner bases (F_4). *J. Pure Appl. Algebr.*, 139:61–88, 1999.

[10] M. Feinberg. Lectures on chemical reaction networks. Available online at `http://www.crnt.osu.edu/LecturesOnReactionNetworks`, 1980.

[11] E. Feliu and C. Wiuf. Simplifying biochemical models with intermediate species. *J. R. Soc. Interface*, 10:20130484, 2013.

[12] J. Gunawardena. Chemical reaction network theory for in-silico biologists. Available online at `http://vcp.med.harvard.edu/papers/crnt.pdf`, 2003.

[13] J. Gunawardena. Distributivity and processivity in multisite phosphorylation can be distinguished through steady-state invariants. *Biophys. J.*, 93(11):3828–3834, 2007.

[14] H. A. Harrington, K. L. Ho, T. Thorne, and M. P. H. Stumpf. Parameter-free model discrimination criterion based on steady-state coplanarity. *Proc. Natl. Acad. Sci. U. S. A.*, 109(39):15746–15751, 2012.

[15] J. Herzog and T. Hibi. *Monomial Ideals*. Springer-Verlag London, 1st edition, 2011.

[16] W. V. D. Hodge and D. Pedoe. *Methods of Algebraic Geometry*, volume 1. Cambridge University Press, 1st edition, 1947.

[17] B. Joshi and Shiu J. Atoms of multistationarity in chemical reaction networks. *J. Math. Chem.*, 51(1):153–178, 2013.

[18] R. L. Karp, M. Pérez Millán, T. Dasgupta, A. Dickenstein, and J. Gunawardena. Complex-linear invariants of biochemical networks. *J. Theor. Biol.*, 311(21):130–138, 2012.

[19] A. L. MacLean, Z. Rosen, H. M. Byrne, and H. A. Harrington. Parameter-free methods distinguish wnt pathway models and guide design of experiments. *Proc. Natl. Acad. Sci. U. S. A.*, 112(9):2652–2657, 2015.

[20] A. K. Manrai and J. Gunawardena. The geometry of multisite phosphorylation. *Biophys. J.*, 95(12):5533–5543, 2008.

[21] M. Marcondes de Freitas, E. Feliu, and C. Wiuf. Intermediates, catalysts, persistence, and boundary steady states. *J. Math. Biol.*, 2016.

[22] S. Müller, E. Feliu, G. Regensburger, C. Conradi, A. Shiu, and A. Dickenstein. Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found. Comput. Math.*, 16(1):69–97, 2016.

[23] M. Noro and K. Yokoyama. Verification of Gröbner basis candidates. In *Mathematical Software – ICMS 2014*, pages 419–424. Springer Berlin Heidelberg, 2014.

[24] M. Pérez Millán and A. Dickenstein. The structure of MESSI biological systems. *arXiv:1612.08763*, 2016.

[25] M. Pérez Millán, A. Dickenstein, A. Shiu, and C. Conradi. Chemical reaction systems with toric steady states. *Bull. Math. Biol.*, 74(5):1027–1065, 2012.

[26] L. Robbiano. Term Orderings on the Polynomial Ring. *Lecture Notes in Computer Science*, 204(93):513–517, 1985.

[27] E. D. Sontag. Structure and stability of certain chemical networks and applications to the kinetic proofreading model of T-cell receptor signal transduction. *Institute of Electrical and Electronics Engineers. Transactions on Automatic Control*, 46(7):1028–1047, 2001.

[28] M. Thomson and J. Gunawardena. The rational parameterization theorem for multisite post-translational modification systems. *J. Theor. Biol.*, 261:626–636, 2009.

[29] F. Winkler. A p-adic approach to the computation of Gröbner bases. *J. Symb. Comput.*, 6:287–304, 1988.

II

The multistationarity structure of networks with intermediates and a binomial core network

AmirHosein Sadeghimanesh
Department of Mathematical Sciences
University of Copenhagen

Elisenda Feliu
Department of Mathematical Sciences
University of Copenhagen

Publication details

Submitted to Bulletin of Mathematical Biology. Available on arXiv.
Sadeghimanesh, A. H. and Feliu, E. (2018b). The multistationarity structure of networks with intermediates and a binomial core network. arXiv: 1808.07548.

The multistationarity structure of networks with intermediates and a binomial core network

AmirHosein Sadeghimanesh[1], Elisenda Feliu[1,2]

August 22, 2018

Abstract

This work addresses whether a reaction network, taken with mass-action kinetics, is multistationary, that is, admits more than one positive steady state in some stoichiometric compatibility class. We build on previous work on the effect that removing or adding intermediates has on multistationarity, and also on methods to detect multistationarity for networks with a binomial steady state ideal. In particular, we provide a new determinant criterion to decide whether a network is multistationary, which applies when the network obtained by removing intermediates has a binomial steady state ideal. We apply this method to easily characterize which subsets of complexes are responsible for multistationarity; this is what we call the *multistationarity structure* of the network. We use our approach to compute the multistationarity structure of the n-site sequential distributive phosphorylation cycle for arbitrary n.

Keywords: binomial ideal, phosphorylation cycle, multistationarity, model reduction, determinant criterion, toric

Introduction

Given a reaction network, an *intermediate* is a species that does not interact with any other species, is produced by at least one reaction, and consumed by at least one reaction. Typical intermediates Y arise in Michaelis-Menten type mechanisms as

$$c \rightleftharpoons Y \longrightarrow c',$$

where c, c' are arbitrary complexes. Removal of the intermediates of a network yields a new network, called the *core network*, as introduced in [8] (and further generalized in [15]). For example, removal of Y from the mechanism above gives the reaction $c \to c'$.

We consider mass-action kinetics, such that the system of ordinary differential equations (ODEs) modeling the evolution of the concentrations of the species in time is polynomial. As shown in [8, Theorem 5.1], multistationarity of the core network implies multistationarity of the original (extended) network, provided a technical realization condition is satisfied. Further, whether an extended network is multistationary depends only on the set of complexes of the core network that react to an intermediate. These complexes are called *inputs*. The subsets of complexes that give rise to multistationarity define the *multistationarity structure* of the core network.

In this work we present an approach to find the multistationarity structure of core networks, and thereby provide a fast way to decide whether a given extension network

[1]Department of Mathematical Sciences, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark

[2]Corresponding author: efeliu@math.ku.dk

is multistationary or not; indeed, it suffices to find the set of inputs of our network and check whether it belongs to the multistationarity structure.

The method applies to core networks that are *binomial* (the ideal generated by the steady state polynomials is binomial). For these networks, a method to decide on multistationarity was introduced in [13], based on the computation of sign vectors. Under some extra assumptions, another method relying on the computation of a symbolic determinant and inspection of the sign of its coefficients is presented in [12] (see Theorem 2.7).

The first main result of this paper is Theorem 3.11, where we combine the results in [8, 12] into a new determinant criterion for multistationarity that applies to extended networks for which the core network is binomial, even though the original network might not be binomial.

The second main result is Theorem 4.2, which removes the technical realization condition in [8] for concluding that an extended network is multistationary provided the core network is. Instead, we require that both the core and the extended networks are binomial (in a compatible way).

The third main contribution is Algorithm 4.8, which returns the multistationarity structure of a binomial core network based on the determinant criterion. The algorithm relies on the study of the signs of a polynomial obtained after the computation of the determinant of a symbolic matrix. Our approach is more direct than testing if the extended network is multistationary for all subsets of input complexes. We apply our method to find the multistationarity structure of the n-site distributive sequential phosphorylation cycle for arbitrary n in §4.3. This illustrates how our results allow us to study a family of networks at once.

We conclude with an investigation of when the technical realization conditions in [8] are satisfied. In particular, we show that the conditions hold for typical types of intermediates like the Michaelis-Menten mechanism above.

The structure of the paper is as follows. In §1 we introduce basic concepts on reaction networks and multistationarity. In §2 we introduce (complete) binomial networks and the determinant criterion for determining multistationarity. In §3 we focus on intermediates and give the determinant criterion for multistationarity applicable to extended networks with a binomial core network. In §4 we link multistationarity of the core and extended networks, and in particular study the multistationary structure. Finally, in §5 we expand on how to check the realization conditions.

Notation. Subscripts ≥ 0, > 0 for $\mathbb{R}$ refer to the non-negative and positive real numbers. The sets $\{1, \ldots, m\}$ and $\{m_1, \ldots, m_2\}$ are respectively denoted by $[m]$ and $[m_1, m_2]$. In particular $[m] = [1, m]$. The cardinality of a set A is denoted by $|A|$.

Consider two vectors $u, v \in \mathbb{R}^n$. The scalar product of u and v is denoted by $u \cdot v$. The vector v^u is defined as $\prod_{j=1}^n v_j^{u_j}$, and for a matrix $M \in \mathbb{R}^{n\times m}$ with column vectors $u^{(i)}$, $i \in [m]$, the vector v^M is the vector whose i-th entry is $v^{u^{(i)}}$. We let $\operatorname{diag}(v)$ be the diagonal matrix with diagonal v and

$$M_v = M \operatorname{diag}(v).$$

The sign vector of v, $\sigma(v) \in \{-1, 0, 1\}^n$, is defined for $i \in [n]$ as

$$\sigma(v)_i = \begin{cases} 1 & \text{if} \quad v_i > 0 \\ 0 & \text{if} \quad v_i = 0 \\ -1 & \text{if} \quad v_i < 0. \end{cases}$$

1 Reaction networks

In this section we briefly introduce the ingredients from chemical reaction network theory needed in the sequel. See for example [5, 10]. A reaction network, also called a *network*, is a triplet of finite sets $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$. The three sets are called respectively the set of *species*, *complexes* and *reactions*. The elements of $\mathcal{C}$ are finite linear combinations of the species with non-negative integer coefficients. The set of reactions consists of ordered pairs (c, c') of complexes, denoted $c \to c'$.

After fixing an order on $\mathcal{S}$, write $\mathcal{S} = \{X_1, \ldots, X_n\}$. We identify a complex $c \in \mathcal{C}$ with the vector in $\mathbb{R}^n$ whose i-th entry is the coefficient of X_i in c. Therefore a complex c is either given as $\sum_{X \in \mathcal{S}} c_X X$ or by the corresponding vector (again denoted c).

There is a natural digraph associated with a network, with vertex set $\mathcal{C}$ and edge set $\mathcal{R}$. We often identify the reaction network with the digraph for simplicity. The stoichiometric matrix N is an $|\mathcal{S}| \times |\mathcal{R}|$ matrix whose column vectors are $c' - c$ for each reaction $c \to c' \in \mathcal{R}$. This matrix depends on a fixed order of the set of reactions. The (real) column space of N is called the *stoichiometric subspace* and is denoted by S. Its dimension, that is, the rank of N, is the *rank* of the network.

In this work we consider so-called *mass-action kinetics*. Under this assumption, the evolution of the concentration of the species in time is modeled by means of a polynomial ODE system as follows. First, a positive real number $k_{c \to c'}$ is assigned to each reaction $c \to c'$. This number is called the *reaction rate constant* and often written as a label of the reaction in the associated digraph. We interchangeably write $k_i = k_{c \to c'}$ if $c \to c'$ is the i-th reaction and write the vector of reaction rate constants $k \in \mathbb{R}^{|\mathcal{R}|}_{>0}$, if the order of $\mathcal{R}$ is relevant, and $k \in \mathbb{R}^{\mathcal{R}}_{>0}$ otherwise.

Next, we let $x = (x_1, \ldots, x_n)$ denote the vector of the concentrations of $X_1, \ldots, X_n$; note that in examples we simply use corresponding lower-case letters to denote concentrations. Given $x \in \mathbb{R}^n$, we define the vector $\psi(x) \in \mathbb{R}^{|\mathcal{R}|}$ as

$$\psi(x)_i = x^c, \qquad \text{if } c \to c' \text{ is the } i\text{-th reaction.}$$

Now the ODE system associated with the network and $k \in \mathbb{R}^{|\mathcal{R}|}_{>0}$ is

$$\frac{dx}{dt} = F_k(x), \qquad \text{where } F_k(x) = N_k \psi(x), \quad x \in \mathbb{R}^n_{\geq 0}. \tag{1}$$

Recall that $N_k = N \operatorname{diag}(k)$.

The solution to (1) with an initial condition $x_0 \in \mathbb{R}^n_{\geq 0}$ is confined to the *stoichiometric compatibility class* of x_0: $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$ [6, Remark 3.4]. Equations for these classes are found as follows. Let d be the corank of the network, that is, $d = n - \operatorname{rank}(N)$. A matrix $Z \in \mathbb{R}^{d \times n}$ whose rows form a basis of the orthogonal complement $S^\perp$ of S is called a *matrix of conservation laws*. Then the set $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$ agrees with the set

$$\{u \in \mathbb{R}^n_{\geq 0} \mid Zu = Zx_0\}.$$

A positive *steady state* is a solution to the system $F_k(x) = 0$ in $\mathbb{R}^n_{>0}$. Since we would like to treat the values of k as unknown, we view the polynomials $F_{k,i}(x)$ as polynomials in the ring $\mathbb{R}(k)[x]$ by regarding k as parameters instead of positive real numbers. When we do this, we write $F_i(x)$. Then $F_1(x), \ldots, F_n(x)$ are called the *steady state polynomials* of the network, and the ideal I they generate is the *steady state ideal*:

$$I = \langle F_1(x), \ldots, F_n(x) \rangle \subseteq \mathbb{R}(k)[x].$$

Throughout this work, given an element or subset B of $\mathbb{R}(k)[x]$, we denote by B_k the specialization of B to a given value of k.

It is always possible to find a basis (a set of generators) of the steady state ideal of a network with cardinality equal to the rank of the network. Indeed, d of the steady state polynomials are redundant since they can be expressed as a linear combination of the $n-d$ remaining polynomials.

Definition 1.1. We say that a reaction network is *multistationary* if there exists a strictly positive vector $k \in \mathbb{R}^{|\mathcal{R}|}_{>0}$ such that the system $F_{k,i}(x) = 0$, $i \in [n]$, has more than one positive solution in a stoichiometric compatibility class. Alternatively, given a matrix of conservation laws Z, the system

$$N_k\psi(x) = 0 \quad \text{and} \quad Zx = T$$

has at least two positive solutions for some positive k and $T \in \mathbb{R}^d$.

2 Binomial networks and multistationarity

In this section we discuss and expand known results on determining whether a network is multistationary when the steady state ideal is binomial. The main references are [12, 13]. An ideal is binomial if it admits a binomial basis, that is, a basis with all polynomials having at most two terms. It is well known that an ideal is binomial if and only if the reduced Gröbner basis in an arbitrary monomial order consists only of binomials [3, Corollary 1.2].

We start with an observation on changing bases in $\mathbb{R}(k)[x]$ for $k = (k_1, \ldots, k_r)$ and $x = (x_1, \ldots, x_n)$. For a set of polynomials A in a polynomial ring $K[x]$, we let $V(A) \subseteq K^n$ denote its solution set, which agrees with $V(\langle A\rangle)$. If B and B' are bases of the same ideal I in $\mathbb{R}(k)[x]$, then $V(B) = V(B') \subseteq \big(\mathbb{R}(k)\big)^n$. However, this does not imply that the specializations to real values $k \in \mathbb{R}^r$ agree, that is, it can happen that $V(B_k) \neq V(B'_k) \subseteq \mathbb{R}^n$. Since we want to study the steady state ideal in $\mathbb{R}(k)[x]$ but obtain results for specific values of k, we introduce the following definition.

Definition 2.1. Let $\mathcal{N}$ be a reaction network and $B \subseteq \mathbb{R}(k)[x]$ the set of steady state polynomials. A basis B' of the steady state ideal of $\mathcal{N}$ is called *admissible* if for every $k \in \mathbb{R}^{\mathcal{R}}_{>0}$ it holds that $V(B_k) = V(B'_k)$. The network $\mathcal{N}$ is a *binomial network* if the steady state ideal has an admissible binomial basis.

We consider a sufficient condition to decide whether the solution sets of two parametric systems agree for any specialization of the parameters, and in particular, for when a basis of the steady state ideal is admissible. Let $B = \{f_1, \ldots, f_\ell\}$ and $B' = \{f'_1, \ldots, f'_{\ell'}\}$. We consider representations of B in terms of B' and *vice versa*, that is, we write

$$f_i = \sum_{j\in[\ell']} \tfrac{h_{ij}}{h_i} f'_j, \quad \text{for } i \in [\ell], \qquad \text{and} \qquad f'_i = \sum_{j\in[\ell]} \tfrac{h'_{ij}}{h'_i} f_j, \quad \text{for } i \in [\ell'], \tag{2}$$

with $h_i, h'_i \in \mathbb{R}[k]$ and $h_{i1}, \ldots, h_{i\ell'}, h'_{i1}, \ldots, h'_{i\ell} \in \mathbb{R}[k][x]$. Note that these representations might not be unique.

Lemma 2.2. *With the notation above, given two bases B and B' of an ideal in $\mathbb{R}(k)[x]$, if $k^\star$ is not in the zero set of $(\prod_{i=1}^{\ell} h_i)(\prod_{i=1}^{\ell'} h'_i)$, then $\langle B_{k^\star}\rangle = \langle B'_{k^\star}\rangle$.*

Proof. For all $i \in [\ell]$ and $j \in [\ell']$ we have that $\frac{h_{k^\star,ij}}{h_{k^\star,i}}, \frac{h'_{k^\star,ji}}{h'_{k^\star,j}} \in \mathbb{R}[x]$ and the equalities in (2) specialize to $k^\star$. Hence $B_{k^\star} \subseteq \langle B'_{k^\star}\rangle$ and $B'_{k^\star} \subseteq \langle B_{k^\star}\rangle$ and so $\langle B_{k^\star}\rangle = \langle B'_{k^\star}\rangle$. □

In particular, if $(\prod_{i=1}^{\ell} h_i)(\prod_{i=1}^{\ell'} h'_i)$ has no positive solution, then $V(B_k) = V(B'_k)$ for all positive k.

Example 2.3. Consider the following reaction network

$$X_1 \xrightarrow{k_1} 2X_1 \xleftarrow{k_3} X_2 \qquad X_1 \xrightarrow{k_2} 2X_2 \xleftarrow{k_4} X_2.$$

The set of steady state polynomials is

$$B = \big\{(k_1 - k_2)x_1 + (2k_3)x_2, (2k_2)x_1 + (-k_3 + k_4)x_2\big\},$$

and the set $B' = \{x_1 - x_2, x_1 - 2x_2\}$ is another basis of the steady state ideal in $\mathbb{R}(k)[x]$. We have that $(a, b) \in \mathbb{R}^2_{>0}$ belongs to $V(B_k)$ with $k = (2\frac{b}{a}+1, 1, 1, 2\frac{a}{b}+1)$. But $V(B'_k) \cap \mathbb{R}^2_{>0} = \emptyset$ for every choice of k. Hence B' is not an admissible basis.

The connection between binomial ideals and multistationarity is as follows. Consider a system of binomial equations in $\mathbb{R}(k)[x]$, say

$$p_1(k)x^{c_1} - p'_1(k)x^{c'_1} = 0, \quad \dots \quad p_s(k)x^{c_s} - p'_s(k)x^{c'_s} = 0. \tag{3}$$

If one of the equations has only one term, or the two terms of a binomial have the same sign, then the system does not admit positive solutions. If $p_i \neq 0$ and $p'_i \neq 0$ in $\mathbb{R}(k)$, the positive solutions to (3) are the positive solutions of the following system

$$x^{c_1 - c'_1} = \tfrac{p'_1(k)}{p_1(k)}, \quad \dots \quad x^{c_s - c'_s} = \tfrac{p'_s(k)}{p_s(k)}.$$

Letting

$$\gamma(k) := \begin{bmatrix} \frac{p'_1(k)}{p_1(k)} \\ \vdots \\ \frac{p'_s(k)}{p_s(k)} \end{bmatrix} \qquad \text{and} \qquad M := \begin{bmatrix} c_1 - c'_1 \\ \vdots \\ c_s - c'_s \end{bmatrix}^t \in \mathbb{R}^{n \times s}, \tag{4}$$

the set of positive solutions of (3) for a positive vector k such that $p_i(k) \neq 0$ for all $i \in [s]$ is

$$\big\{x \in \mathbb{R}^n_{>0} \mid x^M = \gamma(k)\big\}. \tag{5}$$

Let M' be a matrix whose rows form a basis of the orthogonal complement of the row space of M. The solution set of $x^M = \gamma(k)$ is non-empty if and only if $M' \ln\big(\gamma(k)\big) = 0$, where $\ln\big(\gamma(k)\big)$ is defined component-wise. To see why, take the logarithm of both sides, $M^t \ln(x) = \ln\big(\gamma(k)\big)$, and impose that $\ln\big(\gamma(k)\big)$ belongs to the image of M^t.

The parametrization in (5) of positive solutions of a binomial system (3) makes it possible to use results of [12, 13] for detecting multistationarity of binomial networks.

Theorem 2.4 ([12], Proposition 3.9 and Corollary 3.11)**.** *Let $\mathcal{N}$ be a binomial network. Let M and $\gamma(k)$ be as in (4), obtained from an admissible binomial basis B of I, and Z be a matrix of conservation laws. Consider the following conditions:*

(surj) Surjectivity condition*: for all $x \in \mathbb{R}^n_{>0}$ there exists $k \in \mathbb{R}^{\mathcal{R}}_{>0}$ such that $x^M = \gamma(k)$ (equivalently, $x \in V(B_k)$).*

(sign) Sign Condition*: there exist $u, v \in \mathbb{R}^n \backslash \{0\}$ such that $M^t u = Zv = 0$ and $\sigma(u) = \sigma(v)$.*

Then we have:

(i) Assume (surj) is satisfied. Then $\mathcal{N}$ is multistationary if and only if (sign) holds.

(ii) If (sign) does not hold, then $\mathcal{N}$ is not multistationary.

Lemma 2.5 ([13])**.** *A binomial network $\mathcal{N}$ with stoichiometric matrix N satisfies (surj) if and only if* $\ker N \cap \mathbb{R}^{|\mathcal{R}|}_{>0} \neq \emptyset$.

Proof. The solution set of an admissible binomial basis agrees with the solution set of the set of steady state polynomials for every $k \in \mathbb{R}^{\mathcal{R}}_{>0}$. Therefore (surj) is equivalent to the statement

$$\text{for all } x \in \mathbb{R}^n_{>0}, \text{ there exists } k \in \mathbb{R}^{|\mathcal{R}|}_{>0} \text{ such that } N_k\psi(x) = 0.$$

Now $N_k\psi(x) = 0$ is equivalent to $\operatorname{diag}(k)\psi(x) \in \ker N \cap \mathbb{R}^{|\mathcal{R}|}_{>0}$. Thus if $\ker N \cap \mathbb{R}^{|\mathcal{R}|}_{>0} = \emptyset$, then (surj) fails. Conversely given $v \in \ker N \cap \mathbb{R}^{|\mathcal{R}|}_{>0}$ and any $x \in \mathbb{R}^n_{>0}$, by taking $k = \frac{v}{\psi(x)}$ (defined component-wise), we see that (surj) holds. □

Networks fulfilling the condition of the lemma above are often called *consistent*. Using Lemma 2.5 we can check (surj) algorithmically. For a matrix N, $U = \ker(N) \cap \mathbb{R}^{|\mathcal{R}|}_{\geq 0}$ is a convex set. A set of vectors in $\mathbb{R}^{|\mathcal{R}|}_{\geq 0}$ is an *extremal generating set* for U if their non-negative linear combinations generate U and none of them is a non-negative combination of the rest. Then U contains a strictly positive vector if and only if the sum of the vectors in an extremal generating set is positive. To find an extremal generating set for a convex set one can use existing algorithms, e.g. [16, Appendix B].

Let $\mathcal{N}$ be a binomial reaction network with an admissible binomial basis B. Let M be the exponent matrix in the parametrization of its positive steady states as in (5), and Z be a matrix of conservation laws. For $\lambda = (\lambda_1, \ldots, \lambda_n)$ a vector of indeterminates, define

$$\Gamma = \begin{bmatrix} M^t\lambda \\ Z \end{bmatrix} \quad \in \ (\mathbb{R}[\lambda])^{n \times n} \tag{6}$$

and consider the following conditions:

(rank) Rank Condition: The number of elements of B, equivalently the number of columns of M, is equal to the rank of the network.

(det) Determinant Condition: Viewed as a polynomial in λ, $\det(\Gamma)$ is either zero or has at least one positive and at least one negative coefficient.

Note that by [12, Lemma 2.11], $\det(\Gamma)$ is a polynomial in λ that is linear or constant in each λ_i. Theorem 2.13 of [12] states that provided (rank) is fulfilled, then (sign) holds if and only if (det) holds. Combining this with Theorem 2.4, if (surj) and (rank) are satisfied, then $\mathcal{N}$ is multistationary if and only if (det) holds. This yields to the following definition and theorem.

Definition 2.6. A binomial network $\mathcal{N}$ is *complete* if (surj) and (rank) hold for an admissible binomial basis.

Theorem 2.7 (Determinant criterion for complete binomial networks)**.** *Consider a complete binomial network, and let M be as in* (5) *obtained from an admissible binomial basis of the steady state ideal that satisfies both (surj) and (rank). Then the network is multistationary if and only if (det) holds.*

Example 2.8. Consider the following network modeling a simple biological circuit:

$$X_1 \xleftarrow{\kappa_1} 0 \xleftarrow{\kappa_7} X_2 \qquad X_1 + E \underset{\kappa_3}{\overset{\kappa_2}{\rightleftarrows}} Y_1 \xrightarrow{\kappa_4} X_2 + E \qquad 2X_1 + E \underset{\kappa_6}{\overset{\kappa_5}{\rightleftarrows}} Y_2.$$

An admissible binomial basis of the steady state ideal is

$$B = \Big\{\kappa_1 - \tfrac{\kappa_2\kappa_4}{\kappa_3+\kappa_4}x_1e,\ \tfrac{\kappa_2\kappa_4}{\kappa_3+\kappa_4}x_1e - \kappa_7x_2,\ \kappa_2x_1e - (\kappa_3+\kappa_4)y_1,\ \kappa_5x_1^2e - \kappa_6y_2\Big\}.$$

The matrices M, Z and N are

$$M = \begin{bmatrix} 1 & 1 & -1 & -2 \\ 0 & -1 & 0 & 0 \\ 1 & 1 & -1 & -1 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix},\ Z = \begin{bmatrix} 0 & 0 & 1 & 1 & 1 \end{bmatrix},\ N = \begin{bmatrix} 1 & -1 & 1 & 0 & -2 & 2 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & -1 \\ 0 & -1 & 1 & 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix}.$$

The set $P = \{(1,1,0,1,0,0,1), (0,1,1,0,0,0,0), (0,0,0,0,1,1,0)\}$ is an extremal generating set for $\ker(N) \cap \mathbb{R}^7_{\geq 0}$. Since the sum of the vectors in P has all entries positive, (surj) holds by Lemma 2.5. Now taking $u = v = (-1,0,1,0,-1)$, the condition (sign) holds. Therefore by Theorem 2.4 this network is multistationary.

Alternatively, we have $\operatorname{rank}(N) = 4$ and B has 4 elements; hence (rank) holds and this binomial network is complete. We have

$$\det(\Gamma) = \left|\begin{array}{ccccc} \lambda_1 & 0 & \lambda_3 & 0 & 0 \\ \lambda_1 & -\lambda_2 & \lambda_3 & 0 & 0 \\ -\lambda_1 & 0 & -\lambda_3 & \lambda_4 & 0 \\ -2\lambda_1 & 0 & -\lambda_3 & 0 & \lambda_5 \\ \hdashline 0 & 0 & 1 & 1 & 1 \end{array}\right| = \lambda_1\lambda_2\lambda_3\lambda_4 - \lambda_1\lambda_2\lambda_4\lambda_5.$$

The network is multistationary by Theorem 2.7 since (det) holds.

3 Intermediates and multistationarity

In this section we introduce a particular type of species, intermediates, and extended and core networks obtained by adding or removing intermediates. We proceed to present results on multistationarity of extended and core networks from [8] and specifically for binomial networks from [14].

3.1 Intermediates

A species Y is an *intermediate* if it is also a complex, that is belongs to $\mathcal{C}$, only appears in the complex Y, and further both the outdegree and indegree of Y are at least one in the digraph of the network [8]. Given a set of intermediates $\mathcal{Y} = \{Y_1, \ldots, Y_m\}$, let $\mathcal{X} = \mathcal{S} \setminus \mathcal{Y} = \{X_1, \ldots, X_n\}$ be the set of non-intermediates. Then $\mathcal{S}$ is the disjoint union of $\mathcal{X}$ and $\mathcal{Y}$. From now on, the species are ordered such that intermediates are after non-intermediates. Then by (x, y) we mean the vector $(x_1, \ldots, x_n, y_1, \ldots, y_m)$. A complex that is not an intermediate is called a non-intermediate complex.

Given an intermediate Y, an *input* for Y is a non-intermediate complex c such that there exists a directed reaction path from c to Y with all vertices other than c being intermediates. The intermediate Y is an ℓ-input intermediate if it has ℓ inputs [14].

Definition 3.1 ([8])**.** Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ and $\widetilde{\mathcal{N}} = (\widetilde{\mathcal{S}}, \widetilde{\mathcal{C}}, \widetilde{\mathcal{R}})$ be two reaction networks. We say that $\widetilde{\mathcal{N}}$ is an *extension* of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$ if

(i) $\mathcal{Y} = \{Y_1, \ldots, Y_m\}$ is a set of intermediates of $\widetilde{\mathcal{N}}$.

(ii) $\mathcal{S} \cup \mathcal{Y} = \widetilde{\mathcal{S}}$ and $\mathcal{C} \cup \mathcal{Y} = \widetilde{\mathcal{C}}$.

(iii) $c \to c' \in \mathcal{R}$ if and only if there is a directed path from c to c' in the digraph associated with $\widetilde{\mathcal{N}}$, such that all vertices other than c and c' belong to $\mathcal{Y}$ (there might be none).

In this case $\mathcal{N}$ is called the *core network* of $\widetilde{\mathcal{N}}$.

Notations κ, and symbols with tilde are used to refer to reaction rate constants and the corresponding objects of the extended network respectively. Let $\mathcal{N}$ be a reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of m intermediates, $Y_1, \ldots, Y_m$. Choose an input complex c_i for each intermediate Y_i and let $\begin{bmatrix} c_1 & \ldots & c_m \end{bmatrix} \in \mathbb{R}^{n\times m}$ be the matrix whose columns are $c_1, \ldots, c_m$. It follows from Theorem 2.1 of [8] that if Z is a matrix of conservation laws for $\mathcal{N}$, then a matrix of conservation laws for $\widetilde{\mathcal{N}}$ is

$$\widetilde{Z} = \left[\begin{array}{c|c} Z & Z\begin{bmatrix} c_1 & \ldots & c_m \end{bmatrix} \end{array}\right] \in \mathbb{R}^{d\times(n+m)}. \tag{7}$$

In particular the corank of $\mathcal{N}$ and $\widetilde{\mathcal{N}}$ agree and the rank of $\widetilde{\mathcal{N}}$ is the rank of $\mathcal{N}$ plus m.

We next introduce a simple type of extended networks via the addition of intermediates that are useful in the study of multistationarity, see Theorem 3.11.

Definition 3.2. Let $\mathcal{N}$ be a network and $C = \{c_1, \ldots, c_m\} \subseteq \mathcal{C}$. The *canonical extension* of $\mathcal{N}$ associated with C, denoted by $\widetilde{\mathcal{N}}_C = (\widetilde{\mathcal{S}}_C, \widetilde{\mathcal{C}}_C, \widetilde{\mathcal{R}}_C)$, is the extension of $\mathcal{N}$ via the addition of 1-input intermediates $Y_1, \ldots, Y_m$ such that

$$\widetilde{\mathcal{R}}_C = \mathcal{R} \cup \{c_i \rightleftharpoons Y_i \mid i \in [m]\}.$$

The canonical extension associated with $C = \mathcal{C}$ is called the *largest canonical extension.*

We now review the key results in [8] that relate the steady states of extended and core networks. We start by studying the steady state polynomials of the two networks. Let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$ and $C \subseteq \mathcal{C}$ be the set of input complexes. The steady state polynomials associated with the intermediates yield a system $F_{n+1}(x, y) = \cdots = F_{n+m}(x, y) = 0$ that is linear in y and square. As shown in [8], the solution to this linear system is of the form

$$y_i = \sum_{c\in C} \mu_{i,c} x^c, \quad i \in [m] \tag{8}$$

where $\mu_{i,c}$ is a rational function in $\mathbb{R}(\kappa)$ with all non-zero coefficients positive (see also (22)). Consider the following map

$$\begin{array}{rccl} \phi\colon & \mathbb{R}[k] & \longrightarrow & \mathbb{R}(\kappa) \\ & k_{c\to c'} & \longmapsto & \phi_{c\to c'}(\kappa) = \kappa_{c\to c'} + \sum_{i=1}^m \kappa_{Y_i\to c'}\, \mu_{i,c}, \end{array} \tag{9}$$

where it is understood that $\kappa_{c\to c'} = 0$ and $\kappa_{Y_i\to c'} = 0$ if $c \to c'$ and $Y_i \to c'$ do not belong to $\widetilde{\mathcal{R}}$ respectively. Then the steady state polynomials $F, \widetilde{F}$ of $\mathcal{N}$ and $\widetilde{\mathcal{N}}$ for non-intermediate species relate in the following way

$$\widetilde{F}_{\kappa,i}\Big(x, \sum\nolimits_{c\in C} \mu_{1,c} x^c, \ldots, \sum\nolimits_{c\in C} \mu_{m,c} x^c\Big) = F_{\phi(k),i}(x), \qquad i \in [n].$$

Given $f/g \in \mathbb{R}(k)$ such that $\phi(g) \neq 0$, then $\phi(f/g)$ is well defined in $\mathbb{R}(\kappa)$. If $G \in \mathbb{R}(\kappa)[x]$ is a polynomial in x such that all coefficients are rational functions with non-vanishing denominator upon applying ϕ, then we consider the polynomial $\Phi(G)$ in $\mathbb{R}(\kappa)[x, y]$ obtained by applying ϕ on the coefficients of G. In particular, if the rational functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$, then there is no polynomial with coefficients in $\mathbb{R}$ that

identically vanishes when evaluated on the image of ϕ. Then the map ϕ extends to a map of polynomial rings $\Phi\colon \mathbb{R}(k)[x] \to \mathbb{R}(\kappa)[x,y]$. Strategies to check this algebraic independence condition as well as classes of intermediates that satisfy it are described in [14, §4]. In particular the rational functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$ for all canonical extensions by [14, Corollary 4.6].

In order to introduce Theorem 3.3 below, we need to consider the following conditions. Let $\omega_1,\dots,\omega_d$ be a basis of $S^\perp$ and $C'\subseteq C$ consist of the complexes c such that $\omega_j\cdot c\neq 0$ for some $j\in[d]$ (i.e. $c\notin S$). We define two realization conditions on the reaction rate constants of $\mathcal{N}$ and $\widetilde{\mathcal{N}}$:

(i) *Realization condition:*
For all $k\in\mathbb{R}^{\mathcal{R}}_{>0}$, there exists $\kappa\in\mathbb{R}^{\widetilde{\mathcal{R}}}_{>0}$ such that $k=\phi(\kappa)$.

(ii) *Generalized realization condition:*
For all $k\in\mathbb{R}^{\mathcal{R}}_{>0}$ and $r\in\mathbb{R}^{C'}_{>0}$, there exists $\kappa\in\mathbb{R}^{\widetilde{\mathcal{R}}}_{>0}$ such that

$$k=\phi(\kappa)\qquad\text{and}\qquad r_c=\sum_{i\in[m]}\mu_{i,c}\quad\text{for all } c\in C'.$$

Note that $\mu_{i,c}$ depends as well on κ in the last statement. In §5 we focus on how to check whether these realization conditions are satisfied. The proof of the next theorem is found in [8]. The first part is Theorem 5.1, and the second part is discussed in the text.

Theorem 3.3 ([8])**.** *Let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of intermediates $Y_1,\dots,Y_m$.*

(i) If the realization condition holds, then multistationarity of $\mathcal{N}$ implies multistationarity of $\widetilde{\mathcal{N}}$.

(ii) Let $C\subseteq\mathcal{C}$ be the set of inputs of $Y_1,\dots,Y_m$. If the generalized realization condition holds for $\widetilde{\mathcal{N}}$, then $\widetilde{\mathcal{N}}$ is multistationary if and only if the canonical extension $\widetilde{\mathcal{N}}_C$ is multistationary.

Definition 3.4. Let $\mathcal{N}$ be a reaction network and $C\subseteq\mathcal{C}$. The *canonical class* associated with C is the set of all extensions of $\mathcal{N}$ via the addition of intermediates with input set C that satisfy the generalized realization condition.

Proposition 3.5. *The generalized realization condition holds for canonical extensions. Therefore, a canonical class is not empty.*

Proof. Let $\widetilde{\mathcal{N}}$ be the canonical extension of a network $\mathcal{N}$ associated with $C=\{c_1,\dots,c_m\}\subseteq\mathcal{C}$. For every $i\in[m]$, we have $\mu_{i,c}=\frac{\kappa_{c_i\to Y_i}}{\kappa_{Y_i\to c_i}}$ if $c=c_i$ and zero otherwise. Since no two intermediates have a common input, the generalized realization condition holds if for every $k\in\mathbb{R}^{\mathcal{R}}_{>0}$ and $r\in\mathbb{R}^m_{>0}$, there exists $\kappa\in\mathbb{R}^{\widetilde{\mathcal{R}}}_{>0}$ such that

$$k_{c\to c'}=\kappa_{c\to c'}\quad\text{for all } c\to c'\in\mathcal{R}\quad\text{and}\quad r_i=\tfrac{\kappa_{c_i\to Y_i}}{\kappa_{Y_i\to c_i}}\quad\text{for all } i\in[m].$$

This condition clearly holds. □

Theorem 3.3 implies that multistationarity of an extended network $\widetilde{\mathcal{N}}$ satisfying the generalized realization condition is equivalent to multistationarity of any network in the same canonical class of $\widetilde{\mathcal{N}}$, in particular of the canonical extensions in the class. Canonical extensions have a simple structure and preserve some important properties of $\mathcal{N}$ as we will see below. Hence they are chosen as representatives of the class.

Example 3.6. The following digraph defines a reaction network corresponding to the Mitogen-Activated Protein Kinase cascade [2]:

$$\begin{aligned} &X_0 + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} Y_1 \xrightarrow{\kappa_3} X_1 + E \underset{\kappa_5}{\overset{\kappa_4}{\rightleftarrows}} Y_2 \xrightarrow{\kappa_6} X_2 + E \\ &X_2 + F \underset{\kappa_8}{\overset{\kappa_7}{\rightleftarrows}} Y_3 \xrightarrow{\kappa_9} Y_4 \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftarrows}} X_1 + F \underset{\kappa_{13}}{\overset{\kappa_{12}}{\rightleftarrows}} Y_5 \xrightarrow{\kappa_{14}} Y_6 \underset{\kappa_{16}}{\overset{\kappa_{15}}{\rightleftarrows}} X_0 + F. \end{aligned} \tag{10}$$

If we consider $Y_1, \ldots, Y_6$ as intermediates, then the associated core network is

$$X_0 + E \xrightarrow{k_1} X_1 + E \xrightarrow{k_2} X_2 + E \qquad X_2 + F \xrightarrow{k_3} X_1 + F \xrightarrow{k_4} X_0 + F.$$

The generalized realization condition holds by Example 5.2 in §5. The canonical extension in the canonical class of (10) is the following network:

$$\begin{array}{ccccccccc} Y_1 & & Y_2 & & & & Y_3 & & Y_4 & & Y_5 \\ \uparrow\downarrow & & \uparrow\downarrow & & & & \uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow \\ X_0 + E & \longrightarrow & X_1 + E & \longrightarrow & X_2 + E & \qquad & X_2 + F & \longrightarrow & X_1 + F & \longrightarrow & X_0 + F. \end{array}$$

3.2 Binomial networks and intermediates

In this subsection, building on the results in [14], we relate the condition (det) for the core and extended network. This leads to a determinant criterion for multistationarity of extended networks with a complete binomial core network.

Let $\widetilde{\mathcal{N}}$ be an extension of a binomial reaction network $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$. For a binomial basis B of the steady state ideal $I \subseteq \mathbb{R}(k)[x]$ of $\mathcal{N}$, let $\mathrm{rem}(f, B)$ denote the remainder of the division of a polynomial f by B. Assume $\Phi(B)$ is well defined and consider

$$\widetilde{B} = \Phi(B) \cup \Big\{ y_i - \sum_{c \in \mathcal{C}} \mu_{i,c} x^c, i \in [m] \Big\}, \tag{11}$$

$$\widetilde{B}' = \Phi(B) \cup \Big\{ y_i - \mathrm{rem}\Big(\sum_{c \in \mathcal{C}} \mu_{i,c} x^c, \Phi(B) \Big), i \in [m] \Big\}. \tag{12}$$

If the set on the right-hand side of the union in either $\widetilde{B}$ or $\widetilde{B}'$ consists of binomials, then they have the form

$$\Phi(B) \cup \big\{ y_i - p_i(\kappa) x^{\alpha_i},\ i \in [m] \big\} \tag{13}$$

where $p_i(\kappa) \in \mathbb{R}(\kappa)$ and α_i is a vector of non-negative integers. If all intermediates are 1-input, then a binomial basis of $\widetilde{I}$ is

$$\widetilde{B} = \Phi(B) \cup \{ y_i - \mu_{i,c} x^{c_i},\ i \in [m] \}, \tag{14}$$

where c_i is the only input of Y_i, and this basis is admissible provided B is an admissible binomial basis of I and $\Phi(B)$ is defined. This applies in particular to canonical extensions.

Definition 3.7. Let $\mathcal{N}$ be a binomial reaction network and $\widetilde{\mathcal{N}}$ an extension of it via the addition of intermediates. $\widetilde{\mathcal{N}}$ is a *binomial extension* of $\mathcal{N}$ if there exists an admissible binomial basis B of $\mathcal{N}$ such that $\Phi(B)$ is well defined, no coefficient of B becomes zero under Φ, and further, either $\widetilde{B}$ or $\widetilde{B}'$ is an admissible binomial basis of $\widetilde{\mathcal{N}}$.

Remark 3.8. If the functions $\phi_{c\to c'}$ are algebraically independent over $\mathbb{R}$, then $\Phi(B)$ is well defined and no coefficient of B vanishes. By [14, Lemma 3.3], (11) and (12) are bases of the steady state ideal of the extended network $\widetilde{I} \subseteq \mathbb{R}(\kappa)[x,y]$, and if B is admissible, then so is $\widetilde{B}$. To decide whether $\widetilde{B}'$ is also admissible, it suffices to check that the representations of $y_i - \operatorname{rem}\big(\sum_{c\in\mathcal{C}} \mu_{i,c}x^c, \Phi(G)\big)$ in terms of $\widetilde{B}$ and that of $y_i - \sum_{c\in\mathcal{C}} \mu_{i,c}x^c$ in terms of $\widetilde{B}'$ are well defined for all κ (c.f. Lemma 2.2).

Further, by [14, Theorem 3.10], the steady state ideal $\widetilde{I}$ of $\widetilde{\mathcal{N}}$ is binomial if and only if the steady state ideal I of $\mathcal{N}$ is binomial and for any reduced Gröbner basis G of I, $\operatorname{rem}\big(\sum_{c\in\mathcal{C}} \mu_{i,c}x^c, \Phi(G)\big)$ has at most one term for all $i \in [m]$.

Lemma 3.9. *Let $\widetilde{\mathcal{N}}$ be a binomial extension of a binomial network $\mathcal{N}$. Condition (rank) holds for $\widetilde{\mathcal{N}}$ if and only if it holds for $\mathcal{N}$.*

Proof. Let B and $\overline{B}$ be admissible binomial bases of the steady state ideals of $\mathcal{N}$ and $\widetilde{\mathcal{N}}$ respectively, such that $\overline{B}$ is either $\widetilde{B}$ in (11) or $\widetilde{B}'$ in (12). Then $|\overline{B}| = |B| + m$. If $n - d$ is the rank of $\mathcal{N}$, then by (7) and the text below it, the condition (rank) for $\widetilde{\mathcal{N}}$ is $n + m - d = |\overline{B}| = |B| + m$, which is the rank condition for $\mathcal{N}$, $|B| = n - d$. □

It follows from the lemma above that a binomial extension of a complete binomial network satisfying (surj) is a complete binomial network. In general, for an arbitrary extended network $\widetilde{\mathcal{N}}$, we cannot guarantee that (surj) holds provided it holds for $\mathcal{N}$. However, it does for canonical extensions.

Proposition 3.10. *Let $\mathcal{N}$ be a binomial network. Any canonical extension $\widetilde{\mathcal{N}}_C$ of $\mathcal{N}$ is a binomial extension. Further, (surj) holds for $\mathcal{N}$ with an admissible binomial basis B if and only if (surj) holds for $\widetilde{\mathcal{N}}_C$ with $\widetilde{B}$ as in* (11)*. Therefore, a canonical extension of a complete binomial network is also complete.*

Proof. By [14, Corollary 4.6], the functions $\phi_{c\to c'}$ are algebraically independent for canonical extensions. By (14) and Remark 3.8, $\widetilde{\mathcal{N}}_C$ is a binomial extension.

For the second part of the proposition, write $C = \{c_1, \dots, c_m\}$. Denote the reaction rate constants of $\mathcal{N}$ by $k_1, k_2, \dots, k_r$ following the order of the reaction set. The network $\widetilde{\mathcal{N}}_C$ has $r + 2m$ reactions. We denote the reaction rate constants of the reactions of $\widetilde{\mathcal{N}}_C$ that are also in $\mathcal{N}$ with $\kappa_1, \dots, \kappa_r$ and of the other reactions by $c_i \xrightleftharpoons[\kappa_{r+2i}]{\kappa_{r+2i-1}} Y_i$ for $i \in [m]$. Then $\mu_{i,c_i} = \frac{\kappa_{r+2i-1}}{\kappa_{r+2i}}$. Let M be the matrix constructed in (4) for the basis B. Now (surj) holds for $\widetilde{\mathcal{N}}_C$ if for every $(x,y) \in \mathbb{R}^{n+m}_{>0}$ there exists $(\kappa_1, \dots, \kappa_{r+2m}) \in \mathbb{R}^{r+2m}_{>0}$ such that $x^M = \gamma(\kappa)$ and $\frac{y_i}{x^{c_i}} = \frac{\kappa_{r+2i-1}}{\kappa_{r+2i}}$ for all $i \in [m]$. Since the first part of the system does not depend on $\kappa_{r+1}, \dots, \kappa_{r+2m}$, the second part is always satisfied independently from the first part by letting $\kappa_{r+2i} = x^{c_i}$ and $\kappa_{r+2i-1} = y_i$. The first part of the system is exactly the same as (surj) for $\mathcal{N}$ after replacing k_i by κ_i. Hence (surj) holds for $\widetilde{\mathcal{N}}_C$ if and only if it holds for $\mathcal{N}$. The last statement follows from Lemma 3.9. □

Recall that criterion (det) can be used to determine multistationarity for complete networks, c.f. Theorem 2.7. Consider a binomial extension $\widetilde{\mathcal{N}}$ of a complete binomial network $\mathcal{N}$. The steady state ideal of $\widetilde{\mathcal{N}}$ has an admissible binomial basis $\widetilde{B}$ of the form (13), with B an admissible binomial basis of the steady state ideal of $\mathcal{N}$. Let $M \in \mathbb{R}^{n\times s}$ be the exponent matrix associated with the binomials in B, c.f. (4). Since no coefficient of B becomes zero under ϕ, the exponents of the monomials in B and $\Phi(B)$ agree. Hence

the exponent matrix $\widetilde{M}$ associated with the binomials in $\widetilde{B}$ has the following form:

$$(\widetilde{M})^t = \left[\begin{array}{c:c} M^t & 0 \\ \hdashline \begin{matrix} -\alpha_1 \\ \vdots \\ -\alpha_m \end{matrix} & I_m \end{array}\right] \in \mathbb{R}^{(s+m)\times(n+m)}.$$

Using (7), the corresponding matrices Γ and $\widetilde{\Gamma}$ in (6), for $\mathcal{N}$ and $\widetilde{\mathcal{N}}$ respectively, are:

$$\Gamma = \begin{bmatrix} M_\lambda^t \\ Z \end{bmatrix} \in \mathbb{R}^{n\times n}, \qquad \widetilde{\Gamma} = \left[\begin{array}{c:ccc} M_\lambda^t & \multicolumn{3}{c}{0} \\ \hdashline -\alpha_1 & \lambda_{n+1} & & 0 \\ \vdots & & \ddots & \\ -\alpha_m & 0 & & \lambda_{n+m} \\ \hdashline Z & Zc_1^t & \dots & Zc_m^t \end{array}\right] \in \mathbb{R}^{(n+m)\times(n+m)}, \quad (15)$$

where Z is a matrix of conservation laws for $\mathcal{N}$, $c_1, \dots, c_m$ are chosen inputs for $Y_1, \dots, Y_m$ and $\lambda = (\lambda_1, \dots, \lambda_n)$. If $\widetilde{\mathcal{N}}_C$ is a canonical extension, then $\alpha_i = c_i$ in $\widetilde{\Gamma}$, c.f. (14), in which case we denote the matrix by $\widetilde{\Gamma}_C$.

The results in this subsection combined with Theorem 3.3(ii) imply that we can use (det) to detect multistationarity of networks that are not necessarily binomial, but such that the core network is binomial and complete.

Theorem 3.11 (Determinant criterion for extensions of complete binomial networks)**.** *Let $\mathcal{N}$ be a complete binomial network and $\widetilde{\mathcal{N}}$ an extended network in the canonical class associated with $C \subseteq \mathcal{C}$. Then $\widetilde{\mathcal{N}}$ is multistationary if and only if* $\det(\widetilde{\Gamma}_C)$ *is either identically zero or has both positive and negative coefficients.*

Proof. By Theorem 3.3(ii), $\widetilde{\mathcal{N}}$ is multistationary if and only if $\widetilde{\mathcal{N}}_C$ is. Now $\widetilde{\mathcal{N}}_C$ is a complete binomial network and the matrix $\widetilde{\Gamma}_C$ is defined as in (6) for an admissible binomial basis of the steady state ideal that satisfies both (surj) and (rank). Thus $\widetilde{\mathcal{N}}_C$ is multistationary if and only if (det) holds with the matrix $\widetilde{\Gamma}_C$ by Theorem 2.7. □

Example 3.12. (Continued from Example 3.6) The network in Example 3.6 is not binomial by [14, Example 3.13], but the core network is a complete binomial network. The extended network belongs to the canonical class associated with $C = \{X_0+E, X_1+E, X_2+F, X_1+F, X_0+F\}$. With a suitable choice of basis B, the matrix $\widetilde{\Gamma}_C$ is as follows:

$$\left[\begin{array}{ccccc:ccccc} -\lambda_1 & \lambda_2 & 0 & -\lambda_4 & \lambda_5 & \multicolumn{5}{c}{0} \\ 0 & -\lambda_2 & \lambda_3 & -\lambda_4 & \lambda_5 & & & & & \\ \hdashline -\lambda_1 & 0 & 0 & -\lambda_4 & 0 & \lambda_6 & & & & 0 \\ 0 & -\lambda_2 & 0 & -\lambda_4 & 0 & & \lambda_7 & & & \\ 0 & 0 & -\lambda_3 & 0 & -\lambda_5 & & & \lambda_8 & & \\ 0 & -\lambda_2 & 0 & 0 & -\lambda_5 & & & & \lambda_9 & \\ -\lambda_1 & 0 & 0 & 0 & -\lambda_5 & 0 & & & & \lambda_{10} \\ \hdashline 1 & 1 & 1 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 & 1 \end{array}\right].$$

The polynomial $\det(\widetilde{\Gamma}_C)$ has terms with different signs. Therefore by Theorem 3.11, the network in (10) is multistationary.

4 Lifting multistationarity and the multistationarity structure

In this section we use the following notation: for $J \subseteq [n]$ and λ an n-tuple of indeterminates/numbers, we define $\lambda_J = \prod_{i \in J} \lambda_i$.

4.1 Lifting multistationarity

Theorem 3.3 tells us that multistationarity of the core network implies multistationarity of the extended network if the realization condition is satisfied. In this scenario we informally say that multistationarity is lifted. In this subsection we show that multistationarity is lifted for binomial extensions of complete binomial networks, even if the realization condition is not satisfied. Before that, we start with a lemma on the structure of $\widetilde{\Gamma}$.

Lemma 4.1. *Let $\widetilde{\mathcal{N}}$ be a binomial extension of a complete binomial network $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$, and let $\Gamma, \widetilde{\Gamma}$ be as in* (15)*. Then*

$$\det(\widetilde{\Gamma}) = \lambda_{[n+1,n+m]} \det(\Gamma) + p'(\lambda),$$

where $p'(\lambda)$ is a polynomial in λ such that none of its terms is divisible by $\lambda_{[n+1,n+m]}$.

Proof. Let $s = \operatorname{rank}(N)$ and $\widetilde{\Gamma}_{[s+1,s+m],[n+1,n+m]}$ be the submatrix of $\widetilde{\Gamma}$ obtained by removing the rows with index in $[s+1, s+m]$ and the columns with index in $[n+1, n+m]$. By the generalized Laplacian expansion of $\det(\widetilde{\Gamma})$ along rows $s+1, \ldots, s+m$ we have that

$$\det(\widetilde{\Gamma}) \quad = \lambda_{[n+1,n+m]} \det\left(\widetilde{\Gamma}_{[s+1,s+m],[n+1,n+m]}\right) + p'(\lambda) = \lambda_{[n+1,n+m]} \det(\Gamma) + p'(\lambda),$$

where $p'(\lambda)$ is a polynomial in λ. By construction, $p'(\lambda)$ does not have any monomial multiple of $\lambda_{[n+1,n+m]}$. □

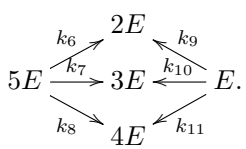
Theorem 4.2 (Lifting multistationarity)**.** *Let $\widetilde{\mathcal{N}}$ be a binomial extension of a complete binomial network $\mathcal{N}$ via the addition of m intermediates $Y_1, \ldots, Y_m$ and let $\Gamma, \widetilde{\Gamma}$ be as in* (15)*. Assume in addition that (surj) holds for $\widetilde{\mathcal{N}}$. If $\mathcal{N}$ is multistationary and $\det(\Gamma) \neq 0$, then $\widetilde{\mathcal{N}}$ is multistationary.*

Proof. Let $\lambda = (\lambda_1, \ldots, \lambda_{n+m})$ and $\bar{\lambda} = (\lambda_1, \ldots, \lambda_n)$. Since $\mathcal{N}$ is multistationary and complete, by Theorem 2.7 we have that $\det(\Gamma)$ is a polynomial in $\lambda_1, \ldots, \lambda_n$ with two terms of different non-zero sign, namely $\alpha\bar{\lambda}^u$ and $-\beta\bar{\lambda}^v$ with $\alpha, \beta > 0$ and $u, v \in \mathbb{Z}^n_{\geq 0}$. By Lemma 4.1, $\det(\widetilde{\Gamma})$ has two terms with different non-zero sign $\alpha\bar{\lambda}^u\lambda_{[n+1,n+m]}$ and $-\beta\bar{\lambda}^v\lambda_{[n+1,n+m]}$. Since $\widetilde{\mathcal{N}}$ is a complete binomial network, $\widetilde{\mathcal{N}}$ is multistationary by Theorem 2.7. □

We finish this subsection with an example where Theorem 4.2 allows us to conclude that an extended network is multistationary, while the realization condition is not satisfied (and hence Theorem 3.3 cannot be applied).

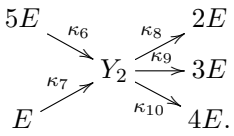
Example 4.3. Consider the following network $\mathcal{N}$:

$$X_0 + E \xrightarrow{k_1} X_1 + E \xrightarrow{k_2} X_2 + E$$
$$X_2 + F \xrightarrow{k_3} Y_1 \xrightarrow{k_4} X_1 + F \xrightarrow{k_5} X_0 + F$$



$\mathcal{N}$ has rank 4 and the steady state ideal has the following admissible binomial basis with 4 elements: $B = \{-k_1x_0e+k_5x_1f, -k_2x_1e+k_3k_4x_2f, k_4y_1-k_3x_2f, (k_9+2k_{10}+3k_{11})e-(3k_6+2k_7+k_8)e^5\}$. Since $(1,1,1,1,1,4,5,6,6,5,4) \in \ker(N)\cap\mathbb{R}^{11}_{>0}$, (surj) holds. Therefore $\mathcal{N}$ is a complete binomial network. Using (det), we see that $\mathcal{N}$ is multistationary and $\det(\Gamma) \neq 0$. Now consider the following extension $\widetilde{\mathcal{N}}$ of $\mathcal{N}$ via the addition of one intermediate Y_2:

$$X_0 + E \xrightarrow{\kappa_1} X_1 + E \xrightarrow{\kappa_2} X_2 + E$$
$$X_2 + F \xrightarrow{\kappa_3} Y_1 \xrightarrow{\kappa_4} X_1 + F \xrightarrow{\kappa_5} X_0 + F$$



This network does not satisfy the realization condition (see §2.2 in the electronic supplementary material of [8]). The well-defined set $\widetilde{B}' = \Phi(B) \cup \{y_2 - \frac{4\kappa_7 e}{3\kappa_8+2\kappa_9+\kappa_{10}}\}$ in (12) makes $\widetilde{\mathcal{N}}$ a binomial extension. Further $(1,1,1,1,1,5,5,4,2,4) \in \ker(\widetilde{N})\cap\mathbb{R}^{10}_{>0}$, and hence (surj) holds for $\widetilde{N}$. By Theorem 4.2, we conclude that $\widetilde{\mathcal{N}}$ is also multistationary.

4.2 Multistationarity structure

In this subsection we introduce the *multistationarity structure* of a core network, consisting of the subsets of complexes that give rise to multistationarity when being the input of some intermediate. Note that if $C_1 \subseteq C_2 \subseteq \mathcal{C}$, then $\widetilde{\mathcal{N}}_{C_2}$ is a binomial extension of $\widetilde{\mathcal{N}}_{C_1}$. Using this and Lemma 4.1, we devise a strategy to determine the multistationarity structure of complete binomial networks by computing the determinant of $\widetilde{\Gamma}_{\mathcal{C}}$ corresponding to the largest canonical extension. We start with an example that illustrates the approach.

Example 4.4. Consider the network in Example 2.8, where Y_1 and Y_2 are intermediates. The associated core network is

$$X_1 \xleftarrow{k_1} 0 \xleftarrow{k_3} X_2 \qquad X_1 + E \xrightarrow{k_2} X_2 + E \qquad 2X_1 + E, \tag{16}$$

which gives $\mathcal{C} = \{0, X_1, X_1+E, X_2+E, 2X_1+E, X_2\}$. An admissible binomial basis of the steady state ideal of (16) is $\{k_1 - k_2x_1e,\ k_2x_1e - k_3x_2\}$. Since the rank of (16) is two and $(1,1,1) \in \ker(N)\cap\mathbb{R}^3_{>0}$, (16) is a complete binomial network. The polynomial $\det(\widetilde{\Gamma}_{\mathcal{C}})$ for the largest canonical extension $\widetilde{\mathcal{N}}_{\mathcal{C}}$ is as follows:

$$\det(\widetilde{\Gamma}_{\mathcal{C}}) = \left|\begin{array}{ccc:cccccc} -\lambda_1 & 0 & -\lambda_3 & & & 0 & & & \\ \lambda_1 & -\lambda_2 & \lambda_3 & & & & & & \\ \hdashline 0 & 0 & 0 & \lambda_4 & & & & & 0 \\ -\lambda_1 & 0 & 0 & & \lambda_5 & & & & \\ -\lambda_1 & 0 & -\lambda_3 & & & \lambda_6 & & & \\ 0 & -\lambda_2 & -\lambda_3 & & & & \lambda_7 & & \\ -2\lambda_1 & 0 & -\lambda_3 & & & & & \lambda_8 & \\ 0 & -\lambda_2 & 0 & 0 & & & & & \lambda_9 \\ \hdashline 0 & 0 & 1 & 0 & 0 & 1 & 1 & 1 & 0 \end{array}\right| = \begin{array}{l} -\lambda_1\lambda_2\lambda_3\lambda_4\lambda_5\lambda_6\lambda_7\lambda_9 \\ +\lambda_1\lambda_2\lambda_3\lambda_4\lambda_5\lambda_6\lambda_8\lambda_9 \\ +\lambda_1\lambda_2\lambda_4\lambda_5\lambda_6\lambda_7\lambda_8\lambda_9. \end{array}$$

Since $\mathcal{N}_{\mathcal{C}}$ is a binomial extension of all canonical extensions, and all canonical extensions are complete, we can use Lemma 4.1 to find $\det(\widetilde{\Gamma}_C)$ for all $C \in \mathcal{C}$. For example, the 3rd and 5th complexes form the set $C = \{X_1 + E, 2X_1 + E\}$ and $\det(\widetilde{\Gamma}_C)$ is the coefficient of $\lambda_4\lambda_5\lambda_7\lambda_9$ in $\det(\widetilde{\Gamma}_{\mathcal{C}})$:

$$\det\big(\widetilde{\Gamma}_{\{X_1+E,2X_1+E\}}\big) = -\lambda_1\lambda_2\lambda_3\lambda_6 + \lambda_1\lambda_2\lambda_6\lambda_8.$$

For any subset $J \subseteq \{4,\dots,9\}$, the coefficient of λ_J in $\det(\widetilde{\Gamma}_{\mathcal{C}})$ is $\det(\widetilde{\Gamma}_C)$ for the set of complexes c_i such that $i+3 \notin J$.

By Theorem 3.11, all networks in the canonical class associated with $\{X_1+E, 2X_1+E\}$ are multistationary. In particular, the network in Example 2.8 belongs to this canonical class (and hence is multistationary), since it satisfies the generalized realization condition:

$$\text{for every } (k,r) \in \mathbb{R}^{3+2}_{>0} \text{ there exists } \kappa \in \mathbb{R}^7_{>0} \text{ such that}$$
$$k_1 = \kappa_1, \quad k_2 = \tfrac{\kappa_2\kappa_4}{\kappa_3+\kappa_4}, \quad k_3 = \kappa_7, \quad r_1 = \tfrac{\kappa_2}{\kappa_3+\kappa_4}, \quad r_2 = \tfrac{\kappa_5}{\kappa_6}.$$

Motivated by this example, we proceed as follows.

Definition 4.5. Let $\mathcal{N}$ be a reaction network, with set of complexes $\mathcal{C}$. Let Mult be the set of all subsets of complexes $C \subseteq \mathcal{C}$ for which the canonical extension $\widetilde{\mathcal{N}}_C$ of $\mathcal{N}$ associated with C is multistationary. Denote the set of minimal elements of Mult with respect to inclusion by Circuits. The set Mult is called the *multistationarity structure* of $\mathcal{N}$ and the elements of Circuits are called the *circuits of multistationarity* of $\mathcal{N}$.

By Theorem 3.3(i), the sets Mult and Circuits associated with a complete binomial network determine each other. Let $D_{\mathcal{N}} = \det(\widetilde{\Gamma}_{\mathcal{C}})$, that is the determinant of $\widetilde{\Gamma}$ associated with the largest canonical extension of $\mathcal{N}$. We assume that the set of complexes is ordered $\mathcal{C} = \{c_1, \ldots, c_m\}$.

Lemma 4.6. *Assume $\mathcal{N}$ is a complete binomial network. Then $C \in$ Mult if and only if the coefficient of $\prod_{c_i \in \mathcal{C}-C} \lambda_{n+i}$ in $D_{\mathcal{N}}$ is zero or has two terms with different non-zero sign.*

Proof. We assume without loss of generality that $C = \{c_1, \ldots, c_t\}$ and $\mathcal{C}-C = \{c_{t+1}, \ldots, c_m\}$. By Lemma 4.1 applied to the complete binomial network $\widetilde{\mathcal{N}}_C$ and the binomial extension $\widetilde{\mathcal{N}}_{\mathcal{C}}$, we have

$$D_{\mathcal{N}} = \det(\widetilde{\Gamma}_C)\lambda_{[n+t+1,n+m]} + p'(\lambda),$$

where $\lambda_{[n+t+1,n+m]}$ does not divide any term of p'. Hence $\det(\widetilde{\Gamma}_C)$ is the coefficient of $\lambda_{[n+t+1,n+m]}$ in $D_{\mathcal{N}}$. The statement now follows by Theorem 2.7. □

Lemma 4.7. *Let $\mathcal{N}$ be a complete binomial network that is not multistationary. Then $C \in$ Mult if and only if a term of $D_{\mathcal{N}}$ is a multiple of $\prod_{c_i \in \mathcal{C}-C} \lambda_{n+i}$ with sign different than the sign of multiples of $\lambda_{[n+1,n+t]}$ in $D_{\mathcal{N}}$.*

Proof. Assume $C = \{c_1, \ldots, c_t\}$ and $\mathcal{C} - C = \{c_{t+1}, \ldots, c_m\}$. We consider $\mathcal{N}$, $\widetilde{\mathcal{N}}_C$ and $\widetilde{\mathcal{N}}_{\mathcal{C}}$, and note that each network is a binomial extension of the previous. We apply Lemma 4.1 twice and obtain

$$D_{\mathcal{N}} = \det(\widetilde{\Gamma}_C)\lambda_{[n+t+1,n+m]} + p' = \big(\det(\Gamma)\lambda_{[n+1,n+t]} + p''\big)\lambda_{[n+t+1,n+m]} + p'.$$

Since $\mathcal{N}$ is not multistationary, then by Theorem 2.7, $\det(\Gamma)$ is non-zero and all terms have the same sign. Hence the coefficient of $\lambda_{[n+1,n+m]}$ is a polynomial where all coefficients have the same sign, σ. Now by Lemma 4.6, $C \in$ Mult if and only if the coefficient of $\lambda_{[n+t+1,n+m]}$ has terms with different sign. Therefore $C \in$ Mult if and only if there is a term in p'' with sign $-\sigma$. This proves the lemma. □

We are now ready to introduce an algorithm to determine the multistationarity structure of a complete binomial network. If the network is multistationary, then all canonical extensions are multistationary and hence Circuits $= \{\emptyset\}$, and Mult is the set of all subsets of $\mathcal{C}$. If $\mathcal{N}$ is not multistationary, then we use the following algorithm.

Algorithm 4.8.

Input: A complete binomial network $\mathcal{N}$ that is not multistationary.

Output: Circuits for $\mathcal{N}$.

Procedure:

Initializing step: Circuits $= \emptyset$.

1. Compute $D_{\mathcal{N}}$ and let σ be the sign of any term divisible by $\lambda_{[n+1,n+m]}$.

2. For every term $T = \alpha\lambda^u$ of $D_{\mathcal{N}}$ with sign $-\sigma$:

2a. Define $C = \{c_i \mid u_{n+i} = 0,\ i = 1, \ldots, m\}$.

2b. Add C to Circuits if no subset of C is already in Circuits, and subsequently remove the supersets of C from Circuits, if any.

Step 2 can be analyzed directly on the exponents u of the terms with sign $-\sigma$: restrict u to the components $n+1, \ldots, n+m$ and choose the corresponding sets C yielding to vectors with maximal support.

Example 4.9. Consider the following complete binomial networks:

$$\mathcal{N}_1\colon\ \begin{array}{l} X_0 + E \longrightarrow X_1 + E \longrightarrow X_2 + E \\ X_2 + F \longrightarrow X_1 + F \longrightarrow X_0 + F, \end{array}$$

$$\mathcal{N}_2\colon\ \begin{array}{ll} S_0 + E \longrightarrow S_1 + E & S_1 + F \longrightarrow S_0 + F \\ P_0 + E \longrightarrow P_1 + E & P_1 + F \longrightarrow P_0 + F, \end{array}$$

$$\mathcal{N}_3\colon\ \begin{array}{ll} S_0 + E \longrightarrow S_1 + E & S_1 + F \longrightarrow S_0 + F \\ P_0 + S_1 \longrightarrow P_1 + S_1 & P_1 + F \longrightarrow P_0 + F. \end{array}$$

We order the species of $\mathcal{N}_1$ as X_0, X_1, X_2, E, F and for $\mathcal{N}_2, \mathcal{N}_3$ we consider P_0, P_1, S_0, S_1, E, F. Complexes are ordered as they appear in the reaction network from left to right and from up to down. For suitable choices of admissible binomial bases, we obtain the following exponent matrices M_1, M_2, M_3 for the three networks respectively, c.f. (4):

$$M_1^t = \begin{bmatrix} -1 & 1 & 0 & -1 & 1 \\ 0 & -1 & 1 & -1 & 1 \end{bmatrix}, \quad M_2^t = \begin{bmatrix} 0 & 0 & -1 & 1 & -1 & 1 \\ -1 & 1 & 0 & 0 & -1 & 1 \end{bmatrix}, \quad M_3^t = \begin{bmatrix} 0 & 0 & -1 & 1 & -1 & 1 \\ -1 & 1 & 0 & -1 & 0 & 1 \end{bmatrix},$$

and we choose the following matrices of conservation laws:

$$Z_1 = \begin{bmatrix} 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}, \qquad Z_2 = Z_3 = \begin{bmatrix} 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}.$$

Using this data, we construct the matrices $\widetilde{\Gamma}_1, \widetilde{\Gamma}_2, \widetilde{\Gamma}_3$ defined as in (15) for the largest canonical extensions associated with $\mathcal{N}_1, \mathcal{N}_2, \mathcal{N}_3$ and find their determinants $D_{\mathcal{N}_1}, D_{\mathcal{N}_2}$ and $D_{\mathcal{N}_3}$. We have $\sigma = 1$ for all three cases. The sets of monomials with negative coefficients in the determinant of $D_{\mathcal{N}_1}, D_{\mathcal{N}_2}$ and $D_{\mathcal{N}_3}$ are respectively

$$\begin{aligned} A_1 =& \big\{\lambda_{\{1,2,3,4,7,8,10,11\}},\ \lambda_{\{1,2,3,5,7,8,10,11\}},\ \lambda_{\{1,2,4,5,7,8,10,11\}},\ \lambda_{\{1,2,4,7,8,9,10,11\}},\ \lambda_{\{1,3,4,5,7,8,10,11\}}, \\ & \lambda_{\{2,3,4,5,7,8,10,11\}},\ \lambda_{\{2,3,5,6,7,8,10,11\}}\big\}, \\ A_2 =& \big\{\lambda_{\{1,4,5,6,7,8,10,12,13,14\}},\ \lambda_{\{2,3,5,6,8,9,10,11,12,14\}}\big\}, \\ A_3 =& \big\{\lambda_{\{1,3,4,5,6,7,9,12,13,14\}},\ \lambda_{\{1,3,4,5,6,7,10,12,13,14\}},\ \lambda_{\{1,3,4,5,6,8,9,12,13,14\}},\ \lambda_{\{1,3,4,5,6,7,9,11,12,14\}}, \\ & \lambda_{\{1,3,4,5,6,7,10,11,12,14\}},\ \lambda_{\{1,3,4,5,6,8,9,11,12,14\}},\ \lambda_{\{1,3,4,5,7,9,10,11,12,14\}},\ \lambda_{\{1,3,4,5,8,9,10,11,12,14\}}, \\ & \lambda_{\{1,3,4,6,7,8,9,12,13,14\}},\ \lambda_{\{1,3,4,6,7,8,10,12,13,14\}},\ \lambda_{\{1,3,4,6,7,8,9,11,12,14\}},\ \lambda_{\{1,3,4,6,7,8,10,11,12,14\}}, \\ & \lambda_{\{1,3,4,6,8,9,10,11,12,14\}},\ \lambda_{\{2,4,6,7,8,9,10,11,12,14\}}\big\}. \end{aligned}$$

According to the algorithm, the monomial $\lambda_{\{1,2,3,4,7,8,10,11\}}$ in A_1 gives rise to the set $C_1 = \{c_6, c_9\}$, while the monomial $\lambda_{\{1,2,4,7,8,9,10,11\}}$ yields $C_2 = \{c_6\}$. Thus C_2 belongs to Circuits_1 while C_1 does not. Proceeding in this way for all monomials, we obtain

$$\begin{aligned}
\text{Circuits}_1 &= \{\{c_6\}, \{c_9\}\} = \{\{X_0 + E\}, \{X_2 + F\}\},\\
\text{Circuits}_2 &= \{\{c_1, c_7\}, \{c_3, c_5\}\} = \{\{S_0 + E, P_1 + F\}, \{S_1 + F, P_0 + E\}\},\\
\text{Circuits}_3 &= \{\{c_7\}, \{c_3, c_5\}, \{c_4, c_5\}\} = \{\{P_1 + F\}, \{S_1 + F, P_0 + S_1\}, \{S_0 + F, P_0 + S_1\}\}.
\end{aligned}$$

We conclude that motifs (g), (i) and (k) in [7] are multistationary, since they are extensions of $\mathcal{N}_1, \mathcal{N}_2, \mathcal{N}_3$ respectively, which satisfy the generalized realization condition and the set of inputs of their intermediates belong to the respective multistationarity structures.

As illustrated by these three examples, the elements of the set of circuits might not have the same cardinality.

Remark 4.10. Algorithm 4.8 provides a direct way to detect the sets of complexes that contribute to multistationarity. The method is appealing because, for small networks, the multistationarity structure can be found by simple visual inspection of one multivariate polynomial. The alternative strategy for finding the multistationarity structure would consist in searching for the circuits by computing $\det(\widetilde{\Gamma}_C)$ for the canonical extensions. One could start from one of the smallest subsets C of $\mathcal{C}$ and compute $\det(\widetilde{\Gamma}_C)$. If this polynomial had terms with different sign or were zero, we would add C to Circuits, and remove C and all its supersets from $\mathcal{C}$ before proceeding in the same way with the next set. The search could alternatively start from the largest subsets C of $\mathcal{C}$ and compute $\det(\widetilde{\Gamma}_C)$. If the determinant did not have terms with different sign or is zero, then we would remove all subsets of C from $\mathcal{C}$. If it had terms with different signs, then we would check the subsets of C with one less element. If none of them were multistationary, then we would add C to Circuits and remove all its subsets from the search.

Going from small to large sets has the advantage of involving the computation of smaller determinants. Our algorithm requires the computation of only one determinant, but it can be large. So, for large networks, it might be advantageous to adopt the search approach starting with small sets described here.

4.3 n-site phosphorylation network

In this section we find the multistationarity structure of the n-site distributive sequential phosphorylation network given as follows (see e.g. [13, 17]):

$$\begin{aligned}
X_0 + E \rightleftharpoons Y_1 \longrightarrow X_1 + E \rightleftharpoons \quad \dots \quad &\longrightarrow X_{n-1} + E \rightleftharpoons Y_n \longrightarrow X_n + E\\
X_n + F \rightleftharpoons Y_{n+1} \longrightarrow X_{n-1} + F \rightleftharpoons \quad \dots \quad &\longrightarrow X_1 + F \rightleftharpoons Y_{2n} \longrightarrow X_0 + F.
\end{aligned} \tag{17}$$

By removing the intermediates $Y_1, \dots, Y_{2n}$, the core network associated with the n-site phosphorylation network is

$$\begin{aligned}
\mathcal{N}\colon\ & X_0 + E \xrightarrow{k_1} X_1 + E \xrightarrow{k_2} \dots \xrightarrow{k_{n-1}} X_{n-1} + E \xrightarrow{k_n} X_n + E\\
& X_n + F \xrightarrow{k_{n+1}} X_{n-1} + F \xrightarrow{k_{n+2}} \dots \xrightarrow{k_{2n-1}} X_1 + F \xrightarrow{k_{2n}} X_0 + F.
\end{aligned} \tag{18}$$

Since $(1, \dots, 1)$ is in the kernel of the stoichiometric matrix of $\mathcal{N}$, (surj) holds. Further, the rank of $\mathcal{N}$ is n and an admissible binomial basis of the steady state ideal is

$$B := \big\{ -k_1 x_0 e + k_{2n} x_1 f, \dots, -k_n x_{n-1} e + k_{n+1} x_n f \big\}.$$

Therefore (rank) also holds and $\mathcal{N}$ is a complete binomial network. By Proposition 5.3 (ii), the generalized realization condition holds for the n-site distributive sequential phosphorylation networks given in (17).

We order the set of species as $X_0, X_1, \ldots, X_n, E, F$, and denote the complexes of the core network as

$$c_1 = X_0 + E, \quad \ldots \quad c_{n+1} = X_n + E, \quad c_{n+2} = X_n + F, \quad \ldots \quad c_{2n+2} = X_0 + F.$$

The largest canonical network consists of the reactions of $\mathcal{N}$ together with the reactions $c_i \rightleftharpoons Y_i$. The matrix $M_\lambda^t \in \mathbb{R}^{n\times(n+3)}$ associated with B and a choice of $Z \in \mathbb{R}^{3\times(n+3)}$ are

$$M_\lambda^t = \begin{bmatrix} -\lambda_1 & \lambda_2 & & & 0 & -\lambda_{n+2} & \lambda_{n+3} \\ & -\lambda_2 & \lambda_3 & & & -\lambda_{n+2} & \lambda_{n+3} \\ & & \ddots & \ddots & & \vdots & \vdots \\ 0 & & & -\lambda_n & \lambda_{n+1} & -\lambda_{n+2} & \lambda_{n+3} \end{bmatrix}, \quad Z = \begin{bmatrix} 1 & \cdots & 1 & 0 & 0 \\ 0 & \ldots & 0 & 1 & 0 \\ 0 & \ldots & 0 & 0 & 1 \end{bmatrix}.$$

Proposition 4.11. *For the n-site phosphorylation network with $n \geq 2$, we have*

$$\text{Circuits} = \{\{c_i\} \mid i \neq n, n{+}1, 2n{+}1, 2n{+}2\} = \big\{X_0{+}E, \ldots, X_{n-2}{+}E,\ X_n{+}F, \ldots, X_2{+}F\big\}.$$

If $n = 1$, then Circuits $= \emptyset$*, since the largest canonical extension is not multistationary.*

Proof. The case $n = 1$ follows by computing the determinant of the largest canonical extension and checking that it is non-zero and that all coefficients have the same sign.

Hence assume that $n \geq 2$. It is enough to first show that $\{c_i\} \in$ Circuits if $i \neq n, n+1, 2n+1, 2n+2$ and then that $\{c_n, c_{n+1}, c_{2n+1}, c_{2n+2}\} \notin$ Mult.

So let $i \in [n+1]$ and define

$$\Omega(i) = \left[\begin{array}{cccccccc:c} -1 & 1 & & & & & 0 & -1 & 1 & 0 \\ & \ddots & \ddots & & & & & \vdots & \vdots & \vdots \\ & & -1 & 1 & & & & -1 & 1 & 0 \\ & & & -1 & 1 & & & -1 & 1 & 0 \\ & & & & \ddots & \ddots & & \vdots & \vdots & \vdots \\ 0 & & & & & -1 & 1 & -1 & 1 & 0 \\ \hdashline 0 & \cdots & 0 & -1 & 0 & \cdots & 0 & -1 & 0 & 1 \end{array}\right] \in \mathbb{R}^{(n+1)\times(n+4)},$$

where the -1 in the last row is in the i-th column. Then we have that

$$\widetilde{\Gamma}_{\{c_i\}} = \left[\begin{array}{c:c} \Omega(i)\,\mathrm{diag}(\lambda_1, \ldots, \lambda_{n+4}) & \\ \hdashline & \begin{matrix} 1 \\ 0 \\ 0 \end{matrix} \\ Z & \end{array}\right] \in \mathbb{R}^{(n+4)\times(n+4)}.$$

For $J \subseteq [n+4]$ of cardinality 3, we denote by $\Omega(i)_J$ the $(n+1)\times(n+1)$ submatrix of $\Omega(i)$ obtained by deleting the columns with index in J. We expand the determinant of

$\widetilde{\Gamma}_{\{c_i\}}$ along the last three rows and obtain

$$\begin{aligned}
\det\big(\widetilde{\Gamma}_{\{c_i\}}\big) =& \sum_{j=1}^{n+1}(-1)^{5n+14+j}\begin{vmatrix}1&0&0\\0&1&0\\0&0&1\end{vmatrix}\det\big(\Omega(i)_{\{j,n+2,n+3\}}\big)\lambda_{[n+4]\setminus\{j,n+2,n+3\}}+\\
&\sum_{j=1}^{n+1}(-1)^{5n+16+j}\begin{vmatrix}1&0&1\\0&0&1\\0&1&0\end{vmatrix}\det\big(\Omega(i)_{\{j,n+3,n+4\}}\big)\lambda_{[n+4]\setminus\{j,n+3,n+4\}}+\\
&(-1)^{6n+18}\begin{vmatrix}0&0&1\\1&0&1\\0&1&0\end{vmatrix}\det\big(\Omega(i)_{\{n+2,n+3,n+4\}}\big)\lambda_{[n+4]\setminus\{n+2,n+3,n+4\}}\\
=&\sum_{j=1}^{n+1}(-1)^{n+j}\det\big(\Omega(i)_{\{j,n+2,n+3\}}\big)\lambda_{[n+4]\setminus\{j,n+2,n+3\}}+\\
&\sum_{j=1}^{n+1}(-1)^{n+j+1}\det\big(\Omega(i)_{\{j,n+3,n+4\}}\big)\lambda_{[n+4]\setminus\{j,n+3,n+4\}}+\\
&\det\big(\Omega(i)_{\{n+2,n+3,n+4\}}\big)\lambda_{[n+4]\setminus\{n+2,n+3,n+4\}}.
\end{aligned}$$

We see from this expansion that the coefficient of $\lambda_{[n+4]\setminus\{1,n+2,n+3\}}$ is $(-1)^{n+1}\det\big(\Omega(i)_{\{1,n+2,n+3\}}\big)$. We have that

$$\Omega(i)_{\{1,n+2,n+3\}} = \left[\begin{array}{ccccccc:c}
1 & & & & & & 0 & 0\\
-1 & \ddots & & & & & & \\
 & \ddots & \ddots & & & & & \vdots\\
 & & \ddots & \ddots & & & & \\
 & & & \ddots & \ddots & & & \\
0 & & & & & -1 & 1 & 0\\
\hdashline
0 & \cdots & & -1 & \cdots & & 0 & 1
\end{array}\right] \in \mathbb{R}^{(n+1)\times(n+1)}, \tag{19}$$

where the -1 in the last row is in position $i-1$ if $i>1$ and is not there if $i=1$. Clearly, $(-1)^{n+1}\det\big(\Omega(i)_{\{1,n+2,n+3\}}\big) = (-1)^{n+1}$.

Consider now the coefficient of $\lambda_{[n+4]\setminus\{n+1,n+3,n+4\}}$, which is $(-1)^{n+n+1+1}\det\big(\Omega(i)_{\{n+1,n+3,n+4\}}\big)$. We have that

$$\Omega(i)_{\{n+1,n+3,n+4\}} = \left[\begin{array}{ccccccc:c}
-1 & 1 & & & & & 0 & -1\\
 & -1 & \ddots & & & & & \\
 & & \ddots & \ddots & & & & \vdots\\
 & & & \ddots & \ddots & & & \\
 & & & & \ddots & 1 & & \\
0 & & & & & -1 & -1 & -1\\
\hdashline
0 & \cdots & & -1 & \cdots & & 0 & 1
\end{array}\right] \in \mathbb{R}^{(n+1)\times(n+1)},$$

where the -1 in the last row is in position i if $i\le n$, and there is no -1 if $i=n+1$. Replacing the last row of $\Omega(i)_{\{n+1,n+3,n+4\}}$ with minus the sum of the rows from i to n, we obtain the matrix

$$\left[\begin{array}{ccccc:c}
-1 & 1 & & & 0 & -1\\
 & -1 & \ddots & & & \vdots\\
 & & \ddots & & 1 & -1\\
0 & & & & -1 & -1\\
\hdashline
 & & & 0 & & n-i
\end{array}\right] \in \mathbb{R}^{(n+1)\times(n+1)}.$$

It follows that the coefficient of $\lambda_{[n+4]\setminus\{n+1,n+3,n+4\}}$ is $(-1)^n(n-i)$.

This shows that if $i < n$, then the coefficients of $\lambda_{[n+4]\setminus\{n+1,n+3,n+4\}}$ and $\lambda_{[n+4]\setminus\{1,n+2,n+3\}}$ have opposite non-zero signs, and hence $\{c_i\}$ is a circuit. For $n+1 < i \leq 2n$ the claim follows by the symmetry of the network after interchanging E and F and sending $X_0, \ldots, X_n$ to $X_n, \ldots, X_0$.

All that remains is to show that $C = \{c_n, c_{n+1}, c_{2n+1}, c_{2n+2}\} \notin \text{Mult}$. The matrix $\widetilde{\Gamma}_C \in \mathbb{R}^{(n+7)\times(n+7)}$ is:

$$\left[\begin{array}{cccccccc:cccc}
-\lambda_1 & \lambda_2 & & & & 0 & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
 & -\lambda_2 & \lambda_3 & & & & & & & & & \\
 & & \ddots & \ddots & & \vdots & \vdots & \vdots & & 0 & & \\
 & & & \ddots & \lambda_n & 0 & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
0 & & & & -\lambda_n & \lambda_{n+1} & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
\hdashline
0 & 0 & \cdots & \cdots & -\lambda_n & 0 & -\lambda_{n+2} & 0 & \lambda_a & & & 0 \\
0 & 0 & \cdots & \cdots & 0 & -\lambda_{n+1} & -\lambda_{n+2} & 0 & & \lambda_b & & \\
0 & -\lambda_2 & \cdots & \cdots & 0 & 0 & 0 & -\lambda_{n+3} & & & \lambda_c & \\
-\lambda_1 & 0 & \cdots & \cdots & 0 & 0 & 0 & -\lambda_{n+3} & 0 & & & \lambda_d \\
\hdashline
1 & 1 & \cdots & \cdots & 1 & 1 & 0 & 0 & 1 & 1 & 1 & 1 \\
0 & 0 & \cdots & \cdots & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 \\
0 & 0 & \cdots & \cdots & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1
\end{array}\right].$$

By performing row operations, we transform $\widetilde{\Gamma}_C$ into a block triangular matrix with diagonal blocks of size n and 7, respectively, as follows:

- Subtract the sum of the rows $1, \ldots, n$ from the $(n+4)$-th row.
- Subtract the sum of the rows $2, \ldots, n$ from the $(n+3)$-th row.
- Subtract the n-th row from the $(n+1)$-th row.
- Add to the $(n+5)$-th row the following linear combination of the first n rows:

$$\sum_{i=1}^{n} \left(\tfrac{1}{\lambda_1} + \cdots + \tfrac{1}{\lambda_i}\right) f_i,$$

  where f_i is the i-th row.

After these operations, which preserve the determinant, we obtain the following matrix:

$$\left[\begin{array}{cccc:ccc:cccc}
-\lambda_1 & \lambda_2 & & 0 & 0 & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
 & \ddots & \ddots & & \vdots & \vdots & \vdots & & & 0 & \\
 & & \ddots & \lambda_n & 0 & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
0 & & & -\lambda_n & \lambda_{n+1} & -\lambda_{n+2} & \lambda_{n+3} & & & & \\
\hdashline
0 & \cdots & \cdots & 0 & -\lambda_{n+1} & 0 & -\lambda_{n+3} & \lambda_{n+4} & & & 0 \\
0 & \cdots & \cdots & 0 & -\lambda_{n+1} & -\lambda_{n+2} & 0 & & \lambda_{n+5} & & \\
0 & \cdots & \cdots & 0 & -\lambda_{n+1} & (n-1)\lambda_{n+2} & -n\lambda_{n+3} & & & \lambda_{n+6} & \\
0 & \cdots & \cdots & 0 & -\lambda_{n+1} & n\lambda_{n+2} & -(n+1)\lambda_{n+3} & 0 & & & \lambda_{n+7} \\
\hdashline
0 & \cdots & \cdots & 0 & 1+z_1\lambda_{n+1} & -z_2\lambda_{n+2} & z_2\lambda_{n+3} & 1 & 1 & 1 & 1 \\
0 & \cdots & \cdots & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 \\
0 & \cdots & \cdots & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1
\end{array}\right],$$

where

$$z_1 = \sum_{i=1}^{n} \tfrac{1}{\lambda_i}, \qquad z_2 = \sum_{i=1}^{n} \tfrac{n-i+1}{\lambda_i}.$$

The determinant of $\widetilde{\Gamma}_C$ is therefore equal to $(-1)^n\lambda_{[n]}$ times the determinant of the inferior diagonal block of size 7×7 of the matrix above. We compute this determinant and obtain the following expression:

$$\begin{aligned}
&\lambda_{n+2}\lambda_{n+4}\lambda_{n+6}\lambda_{n+7}(1+(z_1+z_2)\lambda_{n+1})+\lambda_{n+1}\lambda_{n+4}(\lambda_{n+5}\lambda_{n+7}+\lambda_{n+6}\lambda_{n+7}+\lambda_{n+5}\lambda_{n+6})\\
&+\lambda_{n+1}\lambda_{n+5}\lambda_{n+6}\lambda_{n+7}(1+z_1\lambda_{n+4}+z_2\lambda_{n+2})+\big((n\,z_1-z_2)\lambda_{n+1}+n\big)\lambda_{n+3}\lambda_{n+4}\lambda_{n+5}\lambda_{n+7}\\
&+\big((n\,z_1+z_1-z_2)\lambda_{n+1}+n+1\big)\lambda_{n+3}\lambda_{n+4}\lambda_{n+5}\lambda_{n+6}+\lambda_{n+4}\lambda_{n+5}\lambda_{n+6}\lambda_{n+7}\\
&+\big(z_1\lambda_{n+1}\lambda_{n+2}\lambda_{n+3}+\lambda_{n+1}\lambda_{n+2}+\lambda_{n+1}\lambda_{n+3}+\lambda_{n+2}\lambda_{n+3}\big)\big((n+1)\lambda_{n+4}\lambda_{n+6}+n\,\lambda_{n+4}\lambda_{n+7}\\
&\qquad+n\,\lambda_{n+5}\lambda_{n+6}+(n-1)\lambda_{n+5}\lambda_{n+7}\big).
\end{aligned}$$

Since $nz_1\geq z_2$ and $n\geq 2$, this determinant is strictly positive. Hence, the determinant of $\widetilde{\Gamma}_C$ has sign $(-1)^n$. By Theorem 2.7, we conclude that $\{c_n, c_{n+1}, c_{2n+1}, c_{2n+2}\}\notin$ Mult. $\square$

In view of Proposition 4.11 and Theorem 3.3(ii) we obtain the following theorem.

Theorem 4.12. *Let $\widetilde{\mathcal{N}}$ be an extension of the core n-site phosphorylation network in* (18) *via the addition of intermediates that satisfies the generalized realization condition. Then $\widetilde{\mathcal{N}}$ is multistationary if and only if at least one of $X_0+E,\ldots,X_{n-2}+E, X_n+F,\ldots,X_2+F$ is an input of an intermediate.*

Note that the network in Example 3.6 is an extension of the 2-site phosphorylation network, with set of inputs $C=\{X_0+E, X_1+E, X_2+F, X_1+F, X_0+F\}$. This network satisfies the generalized realization condition by Example 5.2. By Theorem 4.12, we conclude that the network is multistationary.

For the n-site phosphorylation network for a fixed n, Algorithm 4.8 requires the computation of one large determinant. The search approach described in Remark 4.10, stops after computing $2n+14$ determinants, if we start with the small subsets, while it stops after computing

$$\sum_{i=1}^{3}\binom{2n-2}{i}+\sum_{i=4}^{2n+2}\binom{2n+2}{i}$$

determinants if we start with large subsets. For example, if $n=2,3$, the first approach requires the computation of 18 and 20 determinants, and the second approach requires the computation of 25 and 177 determinants respectively. In these cases, the computation of the determinants takes negligible time, and therefore our algorithm is the fastest strategy.

5 Realization conditions

In this section we discuss methods to decide whether the realization conditions are satisfied. The two realization conditions concern the surjectivity of a rational map on the positive orthant. Specifically, let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of the intermediates $Y_1,\ldots,Y_m$ and C' be the set of input complexes that do not belong to the stoichiometric subspace. Consider the following maps from $\mathbb{R}^{\widetilde{\mathcal{R}}}_{>0}$:

$$\phi(\kappa)=\big(\phi_{c\to c'}(\kappa)\mid c\to c'\in\mathcal{R}\big)\in\mathbb{R}^{\mathcal{R}}_{>0}, \tag{20}$$

$$\phi'(\kappa)=\Big(\phi(\kappa),\big(\textstyle\sum_{i\in[m]}\mu_{i,c}(\kappa)\mid c\in C'\big)\Big)\in\mathbb{R}^{\mathcal{R}}_{>0}\times\mathbb{R}^{C'}_{>0}. \tag{21}$$

The generalized realization condition is equivalent to the surjectivity of ϕ' and the realization condition to the surjectivity of ϕ. So let $f=(\frac{f_1}{g_1},\ldots,\frac{f_m}{g_m})$ be an arbitrary map from $\mathbb{R}^n_{>0}$ to $\mathbb{R}^m_{>0}$, defined by rational functions $\frac{f_i}{g_i}\in\mathbb{R}(x_1,\ldots,x_n)$. Consider the ideal

$I = \langle g_1 y_1 - f_1, \ldots, g_m y_m - f_m, 1 - z\prod_{i=1}^m g_i \rangle \subseteq \mathbb{R}[y_1, \ldots, y_m, x_1, \ldots, x_n, z]$. As discussed in §2.3 of the electronic supplementary material of [8], if $I \cap \mathbb{R}[y_1, \ldots, y_m] \neq \{0\}$, then f is not surjective, but the reverse does not necessarily hold.

Another approach is to use Cylindrical Algebraic Decomposition (CAD) [1, 9, 11]. Consider the parametric multivariate system of equations $(x_1, \ldots, x_n, z) \in V(I)$ with $y_1, \ldots, y_m$ treated as parameters and all variables and parameters constrained to be real and positive. The map f is surjective if and only if this system has at least one positive real solution when evaluated at the sample parameter point of all cells obtained after performing CAD. This approach fully characterizes whether f is surjective, but CAD is computationally expensive. In particular, the number of cells is doubly exponential in the number of variables and parameters, and depends also on the degree and number of polynomials in the system [4, Theorem 5]. Therefore the use of CAD is impractical already in relatively small examples.

Example 5.1. We consider the following core network and its extension via the addition of one intermediate Y:

Using CAD on the system of equations describing the realization condition, we obtain three cells. The sample point of each cell yields a system with infinitely many positive solutions. Therefore the realization condition holds.

In view of the difficulties of checking the realization conditions in practice, we start by understanding how the coefficients $\mu_{i,c}$ are found. Let $\widetilde{\mathcal{N}}$ be an extension of $\mathcal{N}$ via the addition of intermediates $Y_1, \ldots, Y_m$. Consider the digraph associated with $\widetilde{\mathcal{N}}$ and let $\mathcal{Y}_1, \ldots, \mathcal{Y}_{t'}$ denote the vertex sets of the connected components of the subgraph induced by the subset of vertices $\{Y_1, \ldots, Y_m\}$. For each non-intermediate complex c and intermediate Y_i, consider the labeled digraph $G_{i,c}$ with vertex set $\mathcal{Y}_\ell \cup \{\star\}$ if $Y_i \in \mathcal{Y}_\ell$. Labeled edges are $Y_i \xrightarrow{\kappa_{Y_i \to Y_j}} Y_j$ if $Y_i \to Y_j \in \widetilde{\mathcal{R}}$, $\star \xrightarrow{\kappa_{c \to Y_i}} Y_i$ if $c \to Y_i \in \widetilde{\mathcal{R}}$ and $Y_i \xrightarrow{\beta_i} \star$ with $\beta_i = \sum_{Y_i \to c'} \kappa_{Y_i \to c'}$ if $\beta_i \neq 0$. For each vertex v of $G_{i,c}$, define $\Theta_{i,c}(v)$ to be the set of all spanning trees rooted at v, that is, v is the only vertex with zero outdegree. Given a tree τ, let $\pi(\tau)$ be the product of all labels of the edges of τ. Then

$$\mu_{i,c} = \frac{\sum_{\tau \in \Theta_{i,c}(Y_i)} \pi(\tau)}{\sum_{\tau \in \Theta_{i,c}(\star)} \pi(\tau)}. \tag{22}$$

The numerator of $\mu_{i,c}$ is linear in the reaction rate constants of the form $\kappa_{c \to Y_j}$, and these reaction rate constants do not appear in the denominator. To read more about properties of the $\mu_{i,c}$'s and how to compute them using the Matrix-Tree theorem, see [8].

The components of ϕ and ϕ' might not depend on the reaction rate constants of all reactions in the network. Specifically, from (22) and (9) it follows that $\phi_{c \to c'}$ depends on $c \to c'$, if this reaction belongs to $\widetilde{\mathcal{R}}$, and possibly on the reactions involving intermediates in the sets $\mathcal{Y}_j$ such that there exists a path from c to c' with all intermediates in $\mathcal{Y}_j$. So for each reaction, we consider the union of these relevant sets of intermediates $\mathcal{Y}_j$. Then $\phi_{c_1 \to c_1'}$ and $\phi_{c_2 \to c_2'}$ do not depend on a common reaction rate constant if the sets of intermediates corresponding to $c_1 \to c_1'$ and $c_2 \to c_2'$ are disjoint. In this way we obtain a partition of $\widetilde{\mathcal{R}}$ into subsets of reactions, that is, subnetworks, for which surjectivity of the map ϕ can be checked independently on each smaller network.

We proceed similarly for ϕ', but in this case the relevant sets of intermediates $\mathcal{Y}_j$ are those for which there exists a path from c to at least one $Y_i \in \mathcal{Y}_j$ (or equivalently, $\mu_{i,c} \neq 0$).

Example 5.2. We consider the generalized realization condition for Example 3.6. By the discussion above, this condition needs to be checked independently on the following three subnetworks:

$$\mathcal{N}_1\colon X_0 + E \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \xrightarrow{\kappa_3} X_1 + E \qquad \mathcal{N}_2\colon X_1 + E \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} Y_2 \xrightarrow{\kappa_6} X_2 + E$$

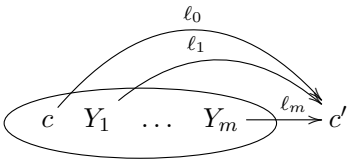
$$\mathcal{N}_3\colon X_2 + F \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} Y_3 \xrightarrow{\kappa_9} Y_4 \underset{\kappa_{11}}{\overset{\kappa_{10}}{\rightleftharpoons}} X_1 + F \underset{\kappa_{13}}{\overset{\kappa_{12}}{\rightleftharpoons}} Y_5 \xrightarrow{\kappa_{14}} Y_6 \underset{\kappa_{16}}{\overset{\kappa_{15}}{\rightleftharpoons}} X_0 + F.$$

For the three subnetworks the generalized realization condition holds due to Proposition 5.3(ii) below.

We next show that the realization condition holds for specific classes of intermediates without the need to do any extra computations.

Proposition 5.3. *The realization condition holds for the following types of extension networks via the addition of intermediates $Y_1, \ldots, Y_m$.*

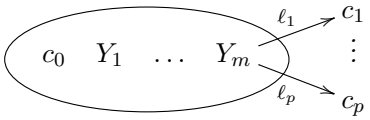
(i)



with an arbitrary digraph structure among the complexes $c, Y_1, \ldots, Y_m$ such that there is a path from c to all Y_i, and where some reactions with label ℓ_i might not exist.

(ii) $c \longleftrightarrow Y_1 \longleftrightarrow Y_2 \longleftrightarrow \ldots \longleftrightarrow Y_m \longleftrightarrow c'$*, provided $\{Y_1, \ldots, Y_m\}$ is a set of intermediates, and where $\longleftrightarrow$ means the reaction can be either irreversible or reversible. These networks satisfy also the generalized realization condition. Further, a union of subnetworks of this form such that the sets of intermediates of each subnetwork do not intersect, satisfies also the generalized realization condition.*

(iii)



with an arbitrary digraph structure among the complexes $c_0, Y_1, \ldots, Y_m$ such that there exists a directed path from c_0 to Y_m.

Proof. (i) The realization condition is equivalent to the scalar-valued map $\ell_0 + \sum_{i=1}^m \ell_i \mu_{i,c}$ being surjective. This map is linear in $\ell_0, \kappa_{c\to Y_1}, \ldots, \kappa_{c\to Y_m}$ (some might be zero, but at least one is non-zero). Hence the statement is clear.

(ii) We start with the case with only one such block. We write

$$\widetilde{\mathcal{N}}\colon \quad c \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} Y_1 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} Y_2 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} \ldots \underset{\kappa_{2m}}{\overset{\kappa_{2m-1}}{\rightleftharpoons}} Y_m \underset{\kappa_{2m+2}}{\overset{\kappa_{2m+1}}{\rightleftharpoons}} c'. \tag{23}$$

If not all reactions are reversible, then we assume that the reaction of the core network is $c \to c'$. This means that all reactions with label with odd subindex are present, and the reverse reactions might or might not be present.

We can assume without loss of generality that neither $c \to c'$ nor $c' \to c$ belong to $\widetilde{\mathcal{N}}$ (if a map is surjective between two positive orthants, adding an extra variable that sums to one component preserves surjectivity).

We have that $\phi_{c\to c'}(\kappa) = \kappa_{2m+1}\mu_{m,c}(\kappa)$ and $\phi_{c'\to c}(\kappa) = \kappa_2\mu_{1,c'}(\kappa)$ (the latter being zero in the irreversible case). Throughout we assume that the set C' used to define ϕ' equals $\{c, c'\}$. This is the worst case scenario.

We show by induction on m that this network satisfies the generalized realization condition. For $m = 1$, if all reverse reactions are present we have that

$$\phi'(\kappa_1,\kappa_2,\kappa_3,\kappa_4) = \big(\phi_{c\to c'}, \phi_{c'\to c}, \mu_{1,c}, \mu_{1,c'}\big) = \big(\tfrac{\kappa_1\kappa_3}{\kappa_2+\kappa_3}, \tfrac{\kappa_2\kappa_4}{\kappa_2+\kappa_3}, \tfrac{\kappa_1}{\kappa_2+\kappa_3}, \tfrac{\kappa_4}{\kappa_2+\kappa_3}\big).$$

A missing reverse reaction corresponds to setting the reaction rate constant equal to zero, and projecting ϕ' away from the components that become zero. We confirm using CAD that this map is surjective when restricted to the positive orthants, in the four scenarios obtained by considering none, one or both reverse reactions.

Assume now that (23) satisfies the generalized realization condition for $m-1$. We view $\widetilde{\mathcal{N}}$ as an extended network of

$$\overline{\mathcal{N}}\colon \quad c \underset{\overline{\kappa}_2}{\overset{\overline{\kappa}_1}{\rightleftharpoons}} Y_2 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} \dots \underset{\kappa_{2m}}{\overset{\kappa_{2m-1}}{\rightleftharpoons}} Y_m \underset{\kappa_{2m+2}}{\overset{\kappa_{2m+1}}{\rightleftharpoons}} c',$$

via the addition of one intermediate Y_1. If we let $\widetilde{\kappa} = (\kappa_1,\kappa_2,\kappa_3,\kappa_4)$, this gives rise to the following relevant functions

$$\widetilde{\mu}_{1,c}(\widetilde{\kappa}) = \tfrac{\kappa_1}{\kappa_2+\kappa_3}, \qquad \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}) = \tfrac{\kappa_4}{\kappa_2+\kappa_3}, \qquad \overline{\kappa}_1 = \kappa_3\widetilde{\mu}_{1,c}(\widetilde{\kappa}), \qquad \overline{\kappa}_2 = \kappa_2\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}). \tag{24}$$

By the case $m = 1$, the right-hand sides of these equalities define a surjective map when restricted to the positive orthant (by omitting the zero components if some reaction rate constants are set to zero). In turn, $\overline{\mathcal{N}}$ is an extended network of $c \rightleftharpoons c'$ via the addition of the intermediates $Y_2,\dots,Y_m$. By the induction hypothesis, $\overline{\mathcal{N}}$ satisfies the generalized realization condition. Let $\overline{\mu}_{i,c}, \overline{\mu}_{i,c'}$ for $i = 2,\dots,m$ correspond to this extension.

Recall that $\mu_{i,c}$ and $\mu_{i,c'}$ are the coefficients of x^c and $x^{c'}$ respectively after writing $y_1,\dots,y_m$ in terms of x by solving the steady state equations corresponding to the intermediates. This system can be solved iteratively, by first finding y_1 and then $y_2,\dots,y_m$. If we let $\varphi(\kappa) = \big(\kappa_3\widetilde{\mu}_{1,c}(\widetilde{\kappa}), \kappa_2\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}), \kappa_5, \kappa_6, \dots, \kappa_{2m+2}\big)$, it follows that

$$\mu_{i,c} = \overline{\mu}_{i,c}(\varphi(\kappa)), \qquad \mu_{i,c'} = \overline{\mu}_{i,c'}(\varphi(\kappa)), \qquad \text{for } i = 2,\dots,m.$$

For $i = 1$, iterative elimination of y_1 and $y_2 = \overline{\mu}_{2,c}(\varphi(\kappa))x^c + \overline{\mu}_{2,c'}(\varphi(\kappa))x^{c'}$ gives that

$$y_1 = \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})y_2 + \widetilde{\mu}_{1,c}(\widetilde{\kappa})x^c = \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c'}(\varphi(\kappa))x^{c'} + \big(\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c}(\varphi(\kappa)) + \widetilde{\mu}_{1,c}(\widetilde{\kappa})\big)x^c.$$

Hence

$$\mu_{1,c}(\kappa) = \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c}(\varphi(\kappa)) + \widetilde{\mu}_{1,c}(\widetilde{\kappa}), \qquad \mu_{1,c'}(\kappa) = \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c'}(\varphi(\kappa)).$$

Therefore $\phi'(\kappa) = \big(\kappa_{2m+1}\mu_{m,c}(\kappa), \kappa_2\mu_{1,c'}(\kappa), \sum_{i=1}^m \mu_{i,c}(\kappa), \sum_{i=1}^m \mu_{i,c'}(\kappa)\big)$ can be written as

$$\begin{aligned}\phi'(\kappa) = {} & \big(\kappa_{2m+1}\overline{\mu}_{m,c}(\varphi(\kappa)), \kappa_2\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c'}(\varphi(\kappa)), \textstyle\sum_{i=2}^m \overline{\mu}_{i,c}(\varphi(\kappa)), \sum_{i=2}^m \overline{\mu}_{i,c'}(\varphi(\kappa))\big) \\ & + \big(0, 0, \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c}(\varphi(\kappa)) + \widetilde{\mu}_{1,c}(\widetilde{\kappa}),\ \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c'}(\varphi(\kappa))\big).\end{aligned}$$

Let $(k_1, k_2, r_c, r_{c'}) \in \mathbb{R}^4_{>0}$, with $k_2 = 0$ in the irreversible case and $r_{c'} = 0$ if c' is not an input of any intermediate. Write $r_c = r_{c,1} + r_{c,2}$, $r_{c'} = r_{c',1} + r_{c',2}$ such that

$r_{c',2} < r_{c,2}$ and $r_{c,1}, r_{c,2}, r_{c',1}, r_{c',2} > 0$ (= 0 as appropriate). We want to show that $(k_1, k_2, r_c, r_{c'}) = \phi'(\kappa)$ for some κ. First note that by the induction hypothesis, we can find $\overline{\kappa} = (\overline{\kappa}_1, \overline{\kappa}_2, \kappa_5, \ldots, \kappa_{2m+2})$ such that

$$(k_1, k_2, r_{c,1}, r_{c',1}) = \Big(\kappa_{2m+1}\overline{\mu}_{m,c}(\overline{\kappa}), \overline{\kappa}_2\overline{\mu}_{2,c'}(\overline{\kappa}), \sum\nolimits_{i=2}^{m} \overline{\mu}_{i,c}(\overline{\kappa}), \sum\nolimits_{i=2}^{m} \overline{\mu}_{i,c'}(\overline{\kappa})\Big).$$

By the last two equalities in (24), the decomposition of $\phi'(\kappa)$ above and the definition of φ, all we need is to show that there exists $\widetilde{\kappa} = (\kappa_1, \kappa_2, \kappa_3, \kappa_4)$ such that

$$\begin{aligned} \overline{\kappa}_1 &= \kappa_3\widetilde{\mu}_{1,c}(\widetilde{\kappa}), & \overline{\kappa}_2 &= \kappa_2\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}), \\ r_{c,2} &= \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c}(\overline{\kappa}) + \widetilde{\mu}_{1,c}(\widetilde{\kappa}), & r_{c',2} &= \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa})\overline{\mu}_{2,c'}(\overline{\kappa}). \end{aligned}$$

This gives in particular that $\overline{\kappa} = \varphi(\kappa)$. Since $\overline{\kappa}$ has now been fixed, we want

$$\overline{\kappa}_1 = \kappa_3\widetilde{\mu}_{1,c}(\widetilde{\kappa}), \quad \overline{\kappa}_2 = \kappa_2\widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}), \quad \widetilde{\mu}_{1,Y_2}(\widetilde{\kappa}) = \frac{r_{c',2}}{\overline{\mu}_{2,c'}(\overline{\kappa})} > 0, \quad \widetilde{\mu}_{1,c}(\widetilde{\kappa}) = r_{c,2} - r_{c',2} > 0.$$

Since the generalized realization condition holds for $m = 1$, there exist $\kappa_1, \ldots, \kappa_4$ such that this system holds (or the equivalent system if some reactions are irreversible). This finishes the proof for the case where there is only one block.

If there are several blocks with the same structure as (23), then we simply need to notice that ϕ' can be written as the Cartesian product of the corresponding map for each block, and $\sum_{i=1}^{m} \mu_{i,c}$ can be split as a sum of the $\mu_{i,c}$'s of each block. Since the generalized realization condition holds for each block, it also holds for the whole network by splitting r_c accordingly for each complex c.

(iii) The core network has p reactions $c_0 \to c_1, \ldots, c_0 \to c_p$. We have $\phi_{c_0 \to c_i}(\kappa) = \ell_i\mu_{m,c_0}$. The denominator of μ_{m,c_0} is a multiple of $\sum_{i=1}^{p} \ell_i$ and $\mu_0 = (\sum_{i=1}^{p} \ell_i)\mu_{m,c_0}$ does not depend on any ℓ_i. Note that the scalar-valued function μ_0 is positive and linear in $(\kappa_{c_0 \to Y_1}, \ldots, \kappa_{c_0 \to Y_m})$. Hence by varying the reaction rate constants different from ℓ_i, μ_0 covers $\mathbb{R}_{>0}$. With this we have that given $k_1, \ldots, k_p > 0$, we define $\ell_i = k_i$ and choose the rest of reaction rate constants such that $\mu_0 = \sum_{i=1}^{p} k_i$. Then $\phi_{c_0 \to c_i}(\kappa) = k_i$, showing that ϕ is surjective. □

Acknowledgements. This work has been supported by the Independent Research Fund of Denmark. We thank Martin Helmer and Angélica Torres for comments on a preliminary version of this manuscript.

References

[1] S. Basu, R. Pollack, and M. F. Coste-Roy. *Algorithms in real algebraic geometry*, volume 10. Springer Science & Business Media, 2007.

[2] R. Bradford, J. H. Davenport, M. England, H. Errami, V. Gerdt, D. Grigoriev, C. Hoyt, M. Košta, O. Radulescu, T. Sturm, and A. Weber. A case study on the parametric occurrence of multiple steady states. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 45–52. Association for Computing Machinery, 2017.

[3] D. Eisenbud and B. Sturmfels. Binomial ideals. *Duke Math. J.*, 84(1):1–45, 1996.

[4] M. England, R. Bradford, and J. H. Davenport. Improving the use of equational constraints in cylindrical algebraic decomposition. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 165–172. Association for Computing Machinery, 2015.

[5] M. Feinberg. Lectures on chemical reaction networks. Available online at `http://www.crnt.osu.edu/LecturesOnReactionNetworks`, 1980.

[6] M. Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Arch. Ration. Mech. Anal.*, 132(4):311–370, 1995.

[7] E. Feliu and C. Wiuf. Enzyme-sharing as a cause of multi-stationarity in signalling systems. *J. R. Soc. Interface*, 9(71):1224–1232, 2012.

[8] E. Feliu and C. Wiuf. Simplifying biochemical models with intermediate species. *J. R. Soc. Interface*, 10(87):20130484, 2013.

[9] J. Gerhard, D. Jeffrey, and G. Moroz. A package for solving parametric polynomial systems. *ACM Commun. Comput. Algebra*, 43(3/4):61–72, 2010.

[10] J. Gunawardena. Chemical reaction network theory for in-silico biologists. Available online at `http://vcp.med.harvard.edu/papers/crnt.pdf`, 2003.

[11] D. Lazard and F. Rouillier. Solving parametric polynomial systems. *J. Symb. Comput.*, 42(6):636–667, 2007.

[12] S. Müller, E. Feliu, G. Regensburger, C. Conradi, A. Shiu, and A. Dickenstein. Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found. Comput. Math.*, 16(1):69–97, 2016.

[13] M. Pérez Millán, A. Dickenstein, A. Shiu, and C. Conradi. Chemical reaction systems with toric steady states. *Bull. Math. Biol.*, 74(5):1027–1065, 2012.

[14] A. H. Sadeghimanesh and E. Feliu. Gröbner bases of reaction networks with intermediate species. *arXiv: 1804.01381*, 2018.

[15] M. Sáez, C. Wiuf, and E. Feliu. Graphical reduction of reaction networks by linear elimination of species. *J. Math. Biol.*, 74(1):195–237, 2017.

[16] C. H. Schilling, D. Letscher, and Palsson B. Ø. Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented prespective. *J. Theor. Biol.*, 203(3):229–248, 2000.

[17] L. Wang and E. D. Sontag. On the number of steady states in a multiple futile cycle. *J. Math. Biol.*, 57(1):29–52, 2008.

III

Region of multistationarity and measure of robustness

AmirHosein Sadeghimanesh
Department of Mathematical Sciences
University of Copenhagen

Elisenda Feliu
Department of Mathematical Sciences
University of Copenhagen

Publication details

Preliminary draft (2018).

The robustness of parameter regions for multistationarity

AmirHosein Sadeghimanesh, Elisenda Feliu

October 10, 2018

Abstract

In this work we explore parameter regions for multistationarity in chemical reaction networks under mass-action kinetics. We further introduce a measure to address how robust a parameter point is with respect to multistationarity, or any given number of steady states. Our approach uses the Kac-Rice formula for the expected number of positive roots a polynomial has, when the coefficients of the polynomial are functions of the parameters, and these parameters follow a given random distribution.

Introduction

Mathematical models of biological systems typically involve a large number of parameters. One might be able to determine an approximate value for some of the parameters or to give some sensible bounds, while for the rest the value is completely unknown. Additionally, parameter values might change depending on the environment and from individual to individual. Combined with the fact that mathematical models can display completely opposite behaviors only after changing the values of the parameters, it makes little sense to study mathematical models for a specific parameter choice, without an understanding of how sensible the conclusions are to the choice. This leads to the use of sensitivity analysis, often performed by varying one parameter at a time.

The ideal situation, however, is to have a full understanding of how the model behaves with respect to the parameter values. That is, given a property of interest, such as *does the system converge to a steady state?*, we would like an explicit partition of the parameter space into the regions where the answer is *yes*, and the regions where the answer is *no*. Unfortunately, one can rarely find such a partition.

The scope of this work is to address the question *how many positive steady states does the system have?*. We focus on mathematical models of the evolution of the concentration of species subject to the occurrence of some reactions. With this in mind, we consider Ordinary Differential Equations (ODEs) obtained under the assumption of mass-action kinetics, although our approach can be applied to any choice of algebraic kinetics (polynomials or rational functions).

With this setting, we have a system of polynomial equations

$$F_k(x) = 0, \qquad x \in \mathbb{R}^n, \tag{1}$$

depending on a vector of parameters $k \in \Omega \subseteq \mathbb{R}^m$, and the question reduces to partition Ω according to the number of positive solutions the system has. Seed work in this direction has focused on the question *does the system has at least two positive steady states for some choice of k?*, a property termed *multistationarity*. There exist numerous methods to determine whether the answer is yes or no, for example [8, 11, 12, 15–18, 21, 24, 26]. As a consequence, for moderately sized systems, this problem can be regarded as solved.

Finding parameter regions where the system displays multistationarity is a much harder question. Most of the existing methods to determine whether multistationarity exists, return an example parameter value for which the system has at least two positive solutions, but offer no possibility to restrict the point to a region of interest, for example. Only very recently, some approaches to partially understand the region of multistationarity are starting to be developed, e.g. [5, 7, 9].

Theoretically, it is possible using Cylindrical Algebraic Decomposition (CAD) to partition the parameter space into regions where the number of positive solutions is constant [4]. However, this approach has a high computational cost and becomes unfeasible already for small systems with three or four parameters and three or four variables. Nevertheless, whenever computational issues can be resolved, this method gives an explicit answer to our question, as for example successfully applied in [6] and also in the present work. Other approaches involve numerical algebraic geometry [20]. But even if the parameter region of interest can be described, the high number of parameters makes it impossible to *understand* the region, and determine, for example, whether the region is connected, or explore the neighborhood of a point.

In this work we explore the use of Kac-Rice formulas to study the parameter space. Kac introduced in [22] a formula to compute the expected number of roots a univariate polynomial with random coefficients has. Later on, this formula was extended to compute the expected number of solutions a random field has on a manifold under some conditions [1]. This yielded to a formula now known as the Kac-Rice formula, and which expresses the expected number of solutions to the system by means of some integrals. The Kac-Rice formula has found applications in many areas such as in regression [27], the theory of random matrices [2], number theory [14] or enumerative geometry [3] to name a few.

Here, we start by showing that if the polynomial system (1) is "nice" enough, meaning that its positive solutions are in one-to-once correspondence with the positive roots of one polynomial, then, combined with Monte-Carlo integration, we can find a good approximation to the number of solutions the system has, even when the number of parameters is not small.

Secondly, we use this formula to introduce a measure of robustness of multistationarity, or more specifically, of a given number of steady states. This is motivated by the following issue. Given a parameter point for which the system has N steady states, how much can one perturb the point such that the system still has N solutions? From the applications point of view, one would like parameter values that can be substantially perturbed and still preserve the same properties. For illustration, consider Figure 3 and the two points marked in the yellow region. For both points, the system has three positive solutions, but clearly, the point to the right is more robust, as it remains in the yellow region under bigger perturbations. In order to answer this question without finding and plotting the region, and also for high number of parameters, we use again the Kac-Rice formula, with a suitable distribution of the parameters around the given point. In this way we explore the neighborhood of the point at once, by numerically computing one integral. We exemplify our method with a running example toy model extracted from [23], and afterwards test the approach with relevant biological examples.

Notation. Subscripts $\geq 0, > 0$ for $\mathbb{R}$ refer to the non-negative and positive real numbers.

1 A parametric polynomial system

In this section we give a brief overview of reaction networks and introduce the polynomial systems of interest.

1.1 Reaction networks and multistationarity

A *reaction network* is a triplet of finite sets $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$. The set $\mathcal{S} = \{X_1, \ldots, X_n\}$ is called the set of *species*. The set $\mathcal{C}$ consists of vectors c in $\mathbb{R}^n_{\geq 0}$, interpreted as linear combinations of species with non-negative integer coefficients, $c = \sum_{i=1}^n c_i X_i$. Each such c is called a *complex*. The set $\mathcal{R}$ consists of ordered pairs of complexes (c, c') represented by an arrow $c \longrightarrow c'$ and is called the set of *reactions*.

Under the assumption of mass-action kinetics, the evolution of the concentrations of the species in time is modeled by means of a polynomial system of ODEs as follows. Each reaction $c \to c'$ is associated a positive real number $k_{c\to c'}$, called the *reaction rate constant*. These values are gathered in a vector $k = (k_{c\to c'} \mid c \to c' \in \mathcal{R}) \in \mathbb{R}^{\mathcal{R}}_{>0}$. The concentration of each species is denoted by corresponding lower-case letters x_i and we let $x = (x_1, \ldots, x_n)$ denote the vector of concentrations. The rate of the reaction $c \to c'$ is assumed to be described by the monomial $k_{c\to c'} x^c$, where $x^c = \prod_{i=1}^n x_i^{c_i}$ and $0^0 = 1$. By considering the net production of each species in each reaction, the concentration of the species in time vary according to

$$\dot{x_i} = F_{k,i}(x),\ i = 1, \ldots, n \qquad \text{where} \qquad F_{k,i}(x) = \sum_{c\to c' \in \mathcal{R}} (c'_i - c_i) k_{c\to c'} x^c. \tag{2}$$

Both the non-negative orthant $\mathbb{R}^n_{\geq 0}$ and the positive orthant $\mathbb{R}^n_{>0}$ are forward-invariant by the trajectories of this system; that is, if the initial condition is in the non-negative (resp. positive) orthant, then so is the solution. Furthermore, this system might have linear invariant sets given as follows. Consider the vector subspace S of $\mathbb{R}^n$ generated by the vectors $c' - c$ for each reaction $c \to c'$ (this is called the *stoichiometric subspace*). Since $F_k(x) = (F_{k,1}(x), \ldots, F_{k,n}(x))$ belongs to S, trajectories are confined to the sets $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$, called stoichiometric compatibility classes, where x_0 is the initial condition of the system. Equations for these classes are found by considering a basis $\{\omega_1, \ldots, \omega_d\}$ of the orthogonal complement $S^\perp$ of S. Specifically, the class $(x_0 + S) \cap \mathbb{R}^n_{\geq 0}$ is described by the equations

$$\omega_i \cdot x = \omega_i \cdot x_0, \qquad i = 1, \ldots, d.$$

Each such equation is called a *conservation law* and the constant $T_i := \omega_i \cdot x_0$ is called a *total amount*.

The *steady states* of the ODE system (2) are the non-negative solutions to the system $F_{k,i}(x) = 0$, $i = 1, \ldots, n$. Thus each $F_{k,i}$ is called a *steady state polynomial*. We say the steady state is positive if all coordinates are different from zero. Then, the steady states within the stoichiometric compatiblity class described by total amounts $T_1, \ldots, T_d$ for a choice of basis of $S^\perp$ are the solutions to the system of equations

$$F_{k,i}(x) = 0, \quad i = 1, \ldots, n, \qquad \omega_1 \cdot x = T_1 \quad \ldots \quad \omega_d \cdot x = T_d. \tag{3}$$

Due to the conservation laws, d steady state polynomials are redundant in (3) and they can be removed from the system. This implies that the system of equations of interest is always transformed into a square system of size n.

Our goal is to understand how the number of positive solutions to this system depends on $k = (k_{c\to c'} \mid c \to c' \in \mathcal{R}) \in \mathbb{R}^{\mathcal{R}}_{>0}$ and $T_1, \ldots, T_d$. Thus, we view system (3) as a parametric polynomial system in the two types of parameters: reaction rate constants and total amounts. This gives rise to the following map

$$\begin{array}{rcl} \mathbb{R}^{\mathcal{R}}_{>0} \times \mathbb{R}^d & \to & \mathbb{N} \cup \{+\infty\} \\ (k, T) & \mapsto & \#\{x \in \mathbb{R}^n_{>0} \mid x \text{ is a solution to (3)}\}. \end{array} \tag{4}$$

The image of this map partitions the parameter space $\mathbb{R}^{\mathcal{R}}_{>0} \times \mathbb{R}^d$. In particular, motivated by the following definition, we are interested in the set of parameters that give rise to more than one positive solutions.

Definition 1.1. A reaction network is called *multistationary* if there exists $(k, T) \in \mathbb{R}^{\mathcal{R}}_{>0} \times \mathbb{R}^d$ such that system (3) has at least two positive solutions. The set of all points $(k, T) \in \mathbb{R}^{\mathcal{R}}_{>0} \times \mathbb{R}^d$ for which the system of equations (3) has more than one positive solution is called the *region of multistationarity*.

Example 1.2. The following reactions define a reaction network representing a simplified model of a hybrid Histidine-Kinase [23]:

$$\begin{aligned} X_1 \xrightarrow{k_1} X_2 \xrightarrow{k_2} X_3 \xrightarrow{k_3} X_4 \\ X_3 + X_5 \xrightarrow{k_4} X_1 + X_6 \\ X_4 + X_5 \xrightarrow{k_5} X_2 + X_6 \\ X_6 \xrightarrow{k_6} X_5. \end{aligned}$$

The steady state polynomials are

$$\begin{aligned} F_{k,1}(x) &= k_4x_3x_5 - k_1x_1, & F_{k,2}(x) &= k_5x_4x_5 + k_1x_1 - k_2x_2, \\ F_{k,3}(x) &= -k_4x_3x_5 + k_2x_2 - k_3x_3, & F_{k,4}(x) &= k_3x_4 - k_5x_4x_5, \\ F_{k,5}(x) &= -k_4x_3x_5 - k_5x_4x_5 + k_6x_6, & F_{k,6}(x) &= k_4x_3x_5 + k_5x_4x_5 - k_6x_6. \end{aligned}$$

The stoichiometric subspace $S \subseteq \mathbb{R}^6$ is generated by the vectors $(-1, 1, 0, 0, 0, 0)$, $(0, -1, 1, 0, 0, 0)$, $(0, 0, -1, 1, 0, 0)$,$(0, 0, 0, 0, 1, -1)$, from where it follows that a basis of $S^\perp$ is

$$\{(1, 1, 1, 1, 0, 0), (0, 0, 0, 0, 1, 1)\}.$$

Since $F_{k,4}(x)$ and $F_{k,6}(x)$ can be written as linear combinations of the rest of the steady state polynomials, we replace them with the two conservation laws. Therefore the system of parametric polynomial equations we want to investigate is

$$\begin{aligned} k_4x_3x_5 - k_1x_1 &= 0, \\ k_5x_4x_5 + k_1x_1 - k_2x_2 &= 0, \\ -k_4x_3x_5 + k_2x_2 - k_3x_3 &= 0, \\ -k_4x_3x_5 - k_5x_4x_5 + k_6x_6 &= 0, \\ x_1 + x_2 + x_3 + x_4 - T_1 &= 0, \\ x_5 + x_6 - T_2 &= 0. \end{aligned}$$

It is shown in [23] that the positive solutions to this system are in one-to-one correspondence with the positive solutions to the following univariate polynomial of degree three in $t = x_5$:

$$\begin{aligned} f(t) = (k_1k_4k_5k_6 + k_2k_4k_5k_6)t^3 + (T_1k_1k_2k_4k_5 - T_2k_1k_4k_5k_6 \\ - T_2k_2k_4k_5k_6 + k_1k_2k_5k_6 + k_1k_3k_5k_6)t^2 + (T_1k_1k_2k_3k_5 - T_2k_1k_2k_5k_6 \\ - T_2k_1k_3k_5k_6 + k_1k_2k_3k_6)t - T_2k_1k_2k_3k_6. \end{aligned} \tag{5}$$

Thus, in this example, the goal is to study the number of positive solutions to a degree 3 polynomial as function of the eight parameters $k_1, \ldots, k_6 > 0$ and T_1, T_2. Observe that we necessarily have $T_1, T_2 > 0$ for positive solutions to exist.

1.2 Region of multistationarity

As stated in the introduction, there are numerous methods that answer the yes-no question of "is a network multistationary?", however, other questions regarding specific issues of the parameter space are more complex to deal with. For example, we would like to understand whether the region of multistationarity is connected, convex, etc., but this is out of reach with current techniques even for small networks. In this work we are specifically interested in the following questions:

(i) Find a parameter point for which the network exhibits multistationarity in a given region of interest.

(ii) Find a parameter point such that system (3) has N positive solutions

(iii) Find the maximum number of solutions to system (3).

(iv) Determine the robustness of a parameter point with respect to multistationarity, in the sense of understanding whether small perturbations to the parameters would imply loosing multistationarity.

The region of interest in question (i) arises often from real applications, by taking into consideration the realistic values of k and T for the systems we model. The coarse approach, seen often in practice, is to sample in the parameter space, solve the system of interest for each choice of parameters, and record the number of positive solutions. This is often done in the literature for about 10^4 parameter choices, which, considering the number of parameters even small networks contain, is far from accurate. Also, because the regions with the maximum number of steady states might have weird shapes and be hard to hit by sampling.

A more accurate approach would be to first divide the parameter region into many small sub-boxes. Then, one takes one or many sample points in each sub-box, solves the system of interest and takes the average number of solutions of each system. This method requires solving the system for considerably many parameter choices in order to cover realistically the parameter space. It is very slow and inefficient.

Another strategy is to use numerical homotopy as in [20, Algorithms 1 and 2]. The idea is to use numerical homotopy ideas to get a description of the discriminant locus, that is, the boundary between different regions of the parameter space where the number of steady states change. But the ideas are not easily applicable to higher dimensional parameter spaces and are best suited when all parameters except one or two parameters are fixed.

In what follows we investigate two other approaches: CAD, which gives the exact answer but is impractical as the number of parameters grow; and the use of the so-called Kac-Rice formula to approximate the number of solutions of the system in each sub-box as above. The latter can also be used to assess how close a parameter point is to loose multistationarity after small perturbations are applied to it.

2 Cylindrical Algebraic Decomposition

In this section we explore the use of CAD to partition the parameter space according to the number of solutions to system (3), see [4, 10]. The starting point to apply CAD is system (3), together with the inequalities $x_1, \dots, x_n > 0$ and $k_{c \to c'} > 0$ for all $c \to c' \in \mathcal{R}$. We might also have $T_i > 0$ for some i, if all coefficients of ω_i are non-negative. In this system, $x_1, \dots, x_n$ are our variables, and k, T the parameters.

CAD involves the computation of the discriminants and resultants of systems of polynomials to divide the parameter space into cells, such that the number of solutions of the system of equalities and inequalities is constant within each cell. Then by picking a sample point from each cell and solving the system, we get the number of solutions for all the parameters in the cell. The union of the cells with more than one solution is the region of multistationarity. Each cell has an exact description using polynomial equalities and inequalities. Here we focus only on open cells, in which case there are only inequalities.

Although theoretically this method fully partitions the parameter space as desired, it is impractical because the number of cells grows very fast: it is double exponential in the total number of variables and parameters, and depends also on the number of polynomials and the degree of the system [13]. Thus a standard computer has only memory to handle small systems.

CAD consists of two phases: the projecting phase and the lifting phase. If the number of indeterminates (variables and parameters) is u, the projecting phase proceeds through $u-1$ steps, such that at each step one indeterminate is eliminated and a system involving only the remaining indeterminates is constructed. So at the last step, we are left with a collection of polynomials in one indeterminate.

The lifting phase proceeds as follows. We find the roots to each of the polynomials in the last step that fulfill the inequalities we started with and order them. We pick a point in each interval these roots define. We evaluate at each of these points the collection of polynomials obtained in the step $u-2$ of the projecting phase, which involve this indeterminate and one more. These become polynomials in one indeterminate, and hence we can solve them as in the first step. We repeat these steps until we are back to the original system and are left with a number of sample points for all parameter indeterminates. We find the number of solutions to the original system after evaluating it at each sample point.

We illustrate the CAD approach using Example 1.2.

Example 2.1. Consider the polynomial given in Equation (5), whose positive solutions are in one-to-one correspondence with the positive solutions to (3). Even though we only have one polynomial of degree three in one variable, t, having eight parameters, $k_1, \ldots, k_6, T_1, T_2$ makes the system too large for CAD in a standard computer.

Instead, we fix the reaction rate constants k and understand the region in the parameters (T_1, T_2) according to the number of positive roots of the polynomial. In [7] it is shown that there exists a choice of $(T_1, T_2) \in \mathbb{R}^2_{>0}$ for which the network is multistationary if and only if $k_1 < k_3$. So we fix the following reaction rate constants (from [23, Fig. 2C]):

$$(k_1, \ldots, k_6) = (0.7329, 100, 73.29, 50, 100, 5). \tag{6}$$

Evaluating the univariate polynomial (5) at (6) gives a polynomial $f_{T_1,T_2}(t)$ of degree 3 in t, whose coefficients depend on the two parameters T_1 and T_2:

$$\begin{aligned} f_{T_1,T_2}(t) =& (2518322.5)t^3 + \big((366450)T_1 - (2518322.5)T_2 + 63502.1205\big)t^2 \\ &+ \big((537142.41)T_1 - (63502.1205)T_2 + 26857.1205\big)t - (26857.1205)T_2. \end{aligned} \tag{7}$$

The study of this polynomial is addressable using CAD. The output is 6 open cells in the (T_1, T_2)-space, such that one of them has 3 positive solutions and the rest one positive solution. The steps of CAD are as follows.

Projecting phase:

- The indeterminate t is eliminated and we obtain a degree four polynomial in T_1 and T_2. Name this polynomial $h_2(T_1, T_2)$.

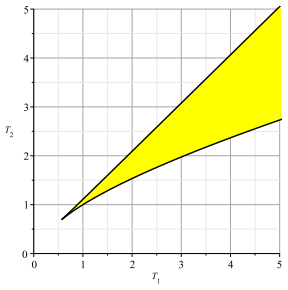


Figure 1: The yellow region is the region where system (3) for the network in Example 2.1 and the choice of k in (6) has three positive solutions. The number of solutions is one in the white region (and there are two solutions on the boundary of the yellow region).

- The indeterminate T_2 is eliminated and we obtain a degree eleven polynomial in T_1 (which is the discriminant of h_2 with respect to T_2). Name this polynomial $h_1(T_1)$.

Lifting phase:

- $h_1(T_1)$ factors as the product of a degree three polynomial to the cube and two linear polynomials. The two linear factors do not have positive roots. The degree three polynomial has only one positive real root β. So we pick two sample points α_1, α_2 for T_1, one smaller and one larger than β.

- In this step the sample values for T_1 are substituted into the polynomial h_2. The result is a univariate polynomial in T_2. For α_1, the resulting polynomial has two linear factors and a factor of degree two, which does not have a real root. Only one of the two linear factors has a positive real root. This gives rise to two cells. For α_2, the resulting polynomial factors as the product of four linear polynomials, three of which admit a positive root. This gives rise to four cells.

Now, there are 6 open cells and a sample point (T_1, T_2) for each cell. We substitute the sample point into $f_{T_1,T_2}(t)$ in (7) and find the positive roots. For one sample point, $f_{T_1,T_2}(t)$ has exactly three positive roots, while for the other five, $f_{T_1,T_2}(t)$ has one positive root. Therefore, the region in T_1, T_2 for this choice of k where the system 3 has three positive solutions is given by one of the cells. In particular, the region is described by the inequalities $T_1 > \beta$ and imposing T_2 to be between the second and third roots of a degree three bivariate polynomial in T_1, T_2 after letting $T_1 > \beta$, see Figure 1.

This example illustrates how to use CAD when the number of parameters is small. As opposed to other coarse methods involving sampling or numerical approaches, CAD gives an exact description of the region of interest. In combination with other methods that provide partial answers on the regions of multistationarity, one can obtain pretty accurate descriptions of them.

For example, in [7] a strategy to obtain parameter regions only on the reaction rate constants is presented, and illustrated with numerous examples (like Example 1.2 here). This allows a user to select realistic values of the reaction rate constants for the network under study. Once this values are selected, CAD can be applied on the total amounts, and, if d is small, a simple figure can be used to lead the selection of realistic values of T. Note also that, in the practical setting of an experiment *in vitro*, total amounts might be easier to control than reaction rate constants.

3 Parameter regions using Kac-Rice formulas

In this section we introduce a new approach to address the parameter space and in particular questions (i)-(iv) posed in Subsection 1.2. The strategy is based on the Kac-Rice formula.

3.1 The Kac-Rice formula

We consider a polynomial $f_k(t)$ in one variable t whose coefficients are polynomials in some parameters $k_1, \ldots, k_m$. We assume the parameters are independently distributed, following distributions with densities $\rho_i(k_i)$ for $i = 1, \ldots, m$ with support in $\mathbb{R}_{>0}$.

The notation $p_t(u)$ is used for the density of $f_k(t) = 0$ for a fixed t, given as $\big(P(f_k(t) \leq x)'_x\big)_{|x=u}$. We let $\mathbb{E}\big(\#(f_k^{-1}(0) \cap \mathbb{R}_{>0})\big)$ be the expected number of zeroes of f_k in the positive orthant, and $\mathbb{E}\big(|f'_k(t)| \mid f_k(t) = 0\big)$ the expected value of $|f'_k(t)|$ (absolute value of $f'_k(t)$) over the set $f_k(t) = 0$ for a fixed t.

The following theorem is a consequence of [1, Theorem 11.2.1] applied to the given setting.

Theorem 3.1. *Let $f\colon \mathbb{R}_{>0} \to \mathbb{R}$ be a polynomial map with coefficients being polynomials in $k_1, \ldots, k_m$. Assume that each parameter k_i follows a continuous random distribution with support in $\mathbb{R}_{>0}$ and density ρ_i, that ρ_i is continuous except maybe in a finite number of points. Assume also that $k_1, \ldots, k_m$ are independently distributed. Then*

$$\mathbb{E}\big(\#(f_k^{-1}(0) \cap \mathbb{R}_{>0})\big) = \int_0^\infty \mathbb{E}\big(|f'(t)| \mid f(t) = 0\big) p_t(0) dt.$$

The formula in the theorem is called the *Kac-Rice* formula, and the expression on the right-hand side is called the *Kac-Rice integral*. The left-hand side of the equality gives the estimated number of positive roots the polynomial has depending on the distribution the parameters follow. By choosing a uniform distribution in some box, then we are finding the mean number of positive solutions when the parameters belong to the box.

In the following special situation, where $f_k(t)$ is linear in the parameter k_1 and the coefficient of k_1 is strictly positive, then the computations of the Kac-Rice integral take the following nice form.

Proposition 3.2. *With the hypothesis in Theorem 3.1, assume that $f_k(t) = h_1(k_2, \ldots, k_m, t)k_1 + h_2(k_2, \ldots, k_m, t)$ is linear in k_1, and that $h_1(k_2, \ldots, k_m, t)$ is a polynomial in $t, k_2, \ldots, k_m$ with all coefficients positive. Let*

$$g(k_2, \ldots, k_m, t, x) = \frac{x - h_2(k_2, \ldots, k_m, t)}{h_1(k_2, \ldots, k_m, t)}.$$

Then the Kac-Rice integral is

$$\int_0^\infty \mathbb{E}\big(|f'(t)| \mid f(t) = 0\big) p_t(0) dt = \int_0^\infty A(t) \cdot B(t) dt,$$

where, for $\bar{\rho}(k_2, \ldots, k_m, t) = \rho_1(g(k_2, \ldots, k_m, t)) \cdot \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m)$ we have

$$A = \int_{\mathbb{R}_{>0}^{m-1}} |f'_k(t)_{|k_1 = g(k_2, \ldots, k_m, t, 0)}| \; \frac{\bar{\rho}(k_2, \ldots, k_m, t)}{\int_{\mathbb{R}_{>0}^{m-1}} \bar{\rho}(s_2, \ldots, s_m, t) ds_2 \ldots ds_m} dk_2 \ldots dk_m,$$

$$B = \int_{\mathbb{R}_{>0}^{m-1}} \frac{\bar{\rho}(k_2, \ldots, k_m, t)}{h_1(k_2, \ldots, k_m, t)} dk_2 \ldots dk_m.$$

Proof. Note that we have $f_k(t) \le x$ if and only if $k_1 \le g(k_2, \dots, k_m, t, x)$ and that $f_k(t) = 0$ if and only if $k_1 = g(k_2, \dots, k_m, t, 0)$. Recall that $p_t(0) = \big(P(f_k(t) \le x)'_x\big)_{|x=0}$. We have

$$\begin{aligned} P(f_k(t) \le x) &= P(k_1 \le g(k_2, \dots, k_m, t, x)) \\ &= \int_{\mathbb{R}_{>0}^{m-1}} \left(\int_{-\infty}^{g(k_2,\dots,k_m,t,x)} \rho_1(k_1) dk_1 \right) \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m) dk_2 \dots dk_m. \end{aligned}$$

By Leibniz's integration rule we have

$$\begin{aligned} \Big(\tfrac{d}{dx} \int_0^{g(k_2,\dots,k_m,t,x)} \rho_1(k_1) dk_1\Big)_{|x=0} &= \rho_1(g(k_2, \dots, k_m, t, 0)) \tfrac{d}{dx}(g(k_2, \dots, k_m, t, x)) \mid_{x=0}, \\ &= \frac{\rho_1(g(k_2, \dots, k_m, t, 0))}{h_1(k_2, \dots, k_m, t)}, \end{aligned}$$

where the first equality holds whenever ρ_1 is continuous at $g(k_2, \dots, k_m, t, 0)$. Since the equation $g(k_2, \dots, k_m, t, 0) = a$ has a finite number of solutions in t and our densities are continuous outside a finite set of points, we can ignore these points when taking the integral over t. So we consider

$$p_t(0) = \int_{\mathbb{R}_{>0}^{m-1}} \frac{\bar{\rho}(k_2, \dots, k_m, t)}{h_1(k_2, \dots, k_m, t)} dk_2 \dots dk_m.$$

Fix now t and let $J_k(t) = f'_k(t)_{|_{k_1 = g(k_2,\dots,k_m,t,0)}}$. Then

$$\mathbb{E}(|f'_k(t)| \mid f_k(t) = 0) = \int_{\mathbb{R}_{>0}^{m-1}} |J_k(t)| \ \rho_{f_k(t)=0}(k_2, \dots, k_m) dk_2 \dots dk_m,$$

where $\rho_{f_k(t)=0}(k_2, \dots, k_m)$ is the density of the joint distribution of $k_2, \dots, k_m$ in $f_k(t) = 0$. We consider the conditional density on the measure zero solution set of $f_k(t) = 0$ and obtain that

$$\rho_{f_k(t)=0}(k_2, \dots, k_m) = \tfrac{1}{\int_{\mathbb{R}_{>0}^{m-1}} \bar{\rho}(s_2,\dots,s_m,t) ds_2 \dots ds_m} \bar{\rho}(k_2, \dots, k_m, t).$$

And finally the Kac-Rice integral is as stated

$$\begin{aligned} \int_0^\infty \mathbb{E}\big(|f'(t)| \mid f(t) = 0\big) p_t(0) dt = \int_0^\infty &\left(\int_{\mathbb{R}_{>0}^{m-1}} |J_k(t)| \tfrac{\bar{\rho}(k_2,\dots,k_m,t)}{\int_{\mathbb{R}_{>0}^{m-1}} \bar{\rho}(s_2,\dots,s_m,t) ds_2 \dots ds_m} dk_2 \dots dk_m \right) \\ &\cdot \left(\int_{\mathbb{R}_{>0}^{m-1}} \frac{\bar{\rho}(k_2, \dots, k_m, t)}{h_1(k_2, \dots, k_m, t)} dk_2 \dots dk_m \right) dt. \end{aligned}$$

□

Note that if $h_1(t) = h_1(k_2, \dots, k_m, t)$ does not depend on $k_2, \dots, k_m$, then the Kac-Rice integral simplifies to

$$\int_0^\infty \frac{1}{h_1(t)} \int_{\mathbb{R}_{>0}^{m-1}} |f'_k(t)_{|_{k_1 = g(k_2,\dots,k_m,t)}}| \ \bar{\rho}(k_2, \dots, k_m, t, 0) dk_2 \dots dk_m dt. \tag{8}$$

3.2 Finding regions of multistationarity

As already discussed, the Kac-Rice formula can be used to approximate the region of multistationarity by considering a grid in the parameter region and assuming uniform distributions in each box of the grid. This can be performed as long as the Kac-Rice integral can be computed.

To illustrate this, consider the reaction network in Example 1.2 with the choice of reaction rate constants in (6). Again, we consider T_1, T_2 as parameters, and equip them with uniform distributions in a box: $T_1 \sim U([a_7, b_7])$ and $T_2 \sim U([a_8, b_8])$. We write the polynomial $f_{T_1,T_2}(t)$ in (7) as $f_{T_1,T_2}(t) = \alpha(t)T_1 - \beta(t)(T_2 - t)$ with

$$\begin{aligned}\alpha(t) &= 366450t^2 + 537142.41t,\\ \beta(t) &= 2518322.5t^2 + 63502.1205t + 26857.1205.\end{aligned}$$

In the notation of Proposition 3.2, this gives $h_1(t) = \alpha(t)$ and $h_2(T_2, t) = -\beta(t)(T_2 - t)$. We are in the scenario where we can use (8). Therefore, the Kac-Rice integral is

$$\int_0^\infty \frac{1}{\alpha(t)} \int_{a_8}^{b_8} \frac{|J_{T_2}(t)|}{b_7 - a_7} \chi_{[a_7,b_7]}\big(\tfrac{\beta(t)(T_2-t)}{\alpha(t)}\big) dT_2\, dt,$$

where $J_{T_2}(t) = f'_{T_1,T_2}(t)_{|T_1=\frac{\beta(t)(T_2-t)}{\alpha(t)}}$ and $\chi_{[a,b]}\big(y\big)$ is the indicator function being 1 if $y \in [a, b]$ and 0 otherwise. In order to compute this integral for fixed values of a_7, b_7, a_8, b_8, we use numerical integration, see Section 4.

Let us assume we are interested in the region of multistationarity in the box $[0, 5]\times[0, 5]$ in (T_1, T_2)-space. We make a grid of this box by dividing each edge into 10 equal parts, such that we obtain 100 sub-boxes. We then compute the Kac-Rice integral above with $T_1 \sim U([a_i, a_{i+1}])$, $T_2 \sim U([b_j, b_{j+1}])$ for each of the sub-boxes. We get, for each sub-box, the mean number of positive steady states the network has when T_1, T_2 belong to the sub-box. In order to visualise this, we color the sub-box with a graduation of yellow: strong yellow means we get the number three, and white means we get one. The strongest the yellow is, the closer the number is to three and vice versa. The result is shown in Figure 2.

Clearly, Figure 2 approximates Figure 1, which shows the exact region where there are three positive steady states. In Figure 2 the sub-boxes that cross the thick line separating the yellow and white regions in Figure 1 have a lighter color, because the sub-box contains parameters with both one and three positive steady states. By making the size of the sub-boxes smaller, we would get more accurate approximations of Figure 1.

This example illustrates how the Kac-Rice formula can be used to approximate the parameter region. The advantage is that the numerical integrals we need to compute require, in principle, less computer power than performing CAD. That is, we could study the parameter region for multistationarity for our example, by varying more parameters. Computing one integral for each sub-box is also faster and more accurate than sampling over all the parameter space and finding the number of positive solutions.

This approach can also be used to numerically assess whether multistationarity occurs in a given box. One might consider a coarse division of the this box and compute the integrals in each sub-box. If some sub-box gives a number larger than one, then we could proceed to subdivide this box and repeat the process, until we can assert with confidence that there is indeed a small region with more than two positive steady states. Further, this approach can be also used to numerically determine the maximal number of positive steady states the network admits.

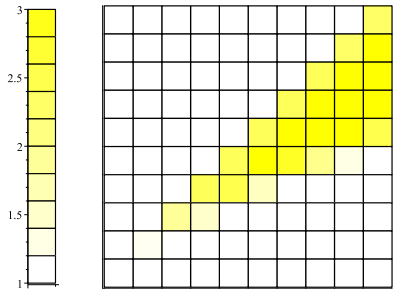


Figure 2: Approximate mean number of positive steady states in the parameters T_1, T_2 for the network in Example 1.2 with reaction rate constants in (6). The mean number is found using the Kac-Rice formula. The strong yellow color means three positive steady states and the white color means one positive steady state; the color bar of the diagram is shown on the left.

3.3 Measure of robustness

In practice, when designing small circuits that are multistationary, we would like to choose parameter values that yield multistationarity, and such that this property is not lost by small perturbations. In plain words, we would like to know how much "inside" the region of multistationarity our parameter point is. Here we focus on comparing the robustness of different parameter choices with respect to the number of positive steady states.

For a small number of parameters, and with an explicit description of the parameter region, one might proceed as follows. Consider Example 2.1. The choices $(2, 1.75)$ and $(4, 3)$ for (T_1, T_2) both provide multistationarity with three positive steady states. However, $(4, 3)$ can be perturbed more than $(2, 1.75)$ and still remain in the yellow region in Figure 3. Specifically, the supremum of the radius of the balls centred at each of the points that are in the region with three steady states is largest for $(4, 3)$. Or equivalently, the distance of the point to the closer thick black line is largest for $(4, 3)$. Thus, we would say that $(4, 3)$ is more robust than $(2, 1.75)$ with respect to multistationarity, and further, by finding the distance of each of the points to the black line in separating the regions in Figure 3, we can give a measure of robustness.

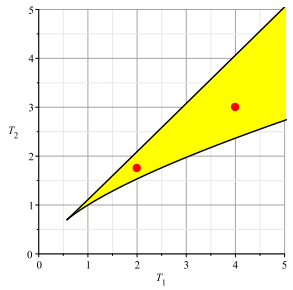


Figure 3: Two points $(2, 1.75)$ and $(4, 3)$ in the region of multistationarity of Example 2.1. A larger neighborhood of the second point is inside the yellow region than for the first point.

An approximation of this computation can be performed using the Kac-Rice formula to measure robustness of a parameter point with respect to multistationarity, when nothing is known about the region of multistationarity. The idea is the following. We consider

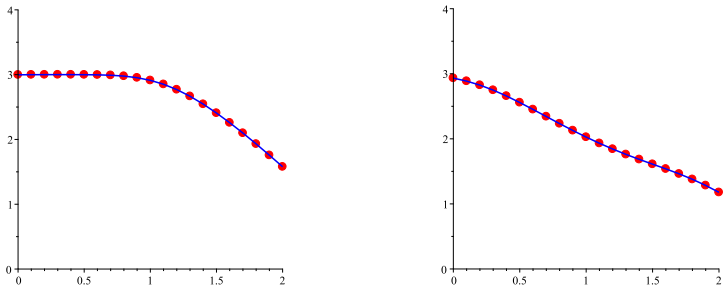


Figure 4: In both graphs the vertical axis represents $\mathbb{E}(\#(f^{-1}(0) \cap \mathbb{R}_{>0}))$ for the truncated normal distribution, and the horizontal axis represents $2 + \log_{10} \sigma^2$. The left graph corresponds to the point $(T_1, T_2) = (4, 3)$ and the right graph corresponds to the point $(T_1, T_2) = (2, 1.75)$. For both points, the normal distribution has support in the box $[T_1 - 1.75, T_1 + 1.75] \times [T_2 - 1.75, T_2 + 1.75]$.

random distributions giving high probability to a neighborhood of the point, and find the expected number of positive steady states according to the distribution. We then increase the size of the neighborhood and plot the expected number of positive steady states with respect to the size, and observe when the expected number decreases.

Specifically, we propose to use the following three distributions:

- Uniform distributions on a box around the parameter point, and we increase the size of the box.
- Normal distributions centred at the parameter point, truncated symmetrically such that the support is in $\mathbb{R}_{>0}$, and we increase the variance.
- Log-normal distributions centred at the parameter point and we increase the variance.

With the uniform distribution we will simply find the expected number of positive steady states in boxes around the point. Since we want that the parameters to be independently distributed, we cannot use a uniform distribution on a ball around the point. For the two other distributions, we give more importance to small perturbations than larger perturbations. This makes sense if our starting parameter point is realistic but determined with some uncertainty due to experimental or numerical errors, or inaccuracies of the model. The log-normal distribution would additionally take into account the order of magnitude of the parameter value.

We consider Example 1.2 again with the points $(4, 3)$ and $(2, 1.75)$, and the truncated normal distribution. By increasing the variance σ^2 from 0.01 to 100, the expected number of positive steady states is as shown in Figure 4. We observe that $(4, 3)$ is more robust than $(2, 1.75)$ because the expected number of steady states drops for a larger variance. We can also deduce from the figure how large the variance can be before we might loose multistationarity.

We knew this already by looking at Figure 3. The advantage of using this approach is that we can make the same study with several parameters. To illustrate this, we have considered the following two points

$$(k_1, \ldots, k_6, T_1, T_2) = (10, 110, 50, 40, 90, 12, 7, 9.25),$$
$$(k_1, \ldots, k_6, T_1, T_2) = (5, 100, 75, 50, 100, 10, 6.5, 7.5),$$

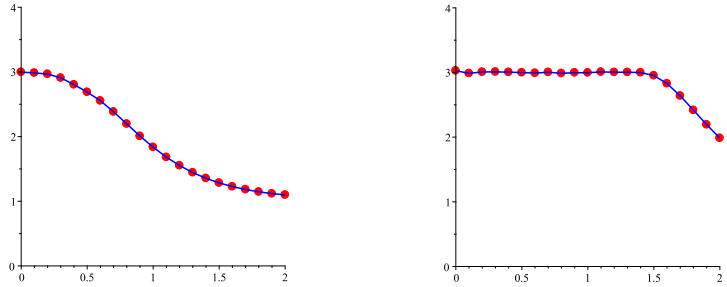


Figure 5: In both graphs the vertical axis represents $\mathbb{E}(\#(f^{-1}(0)\cap\mathbb{R}_{>0}))$ for the uniform distribution $U(\prod_{i=1}^{8}[p_i-d,p_i+d])$, and the horizontal axis represents $2+\log_{10}d$. The left graph corresponds to the point $p=(10,110,50,40,90,12,7,9.25)$ and the right graph corresponds to the point $p=(5,100,75,50,100,10,6.5,7.5)$.

and studied their robustness with respect to the number of positive steady states using the uniform distribution. By varying the size of the support of the distribution as

$$[p_1-d,p_1+d]\times\cdots\times[p_8-d,p_8+d],$$

where p is the chosen parameter point and increasing d from 0.01 to 1, we have obtained that the mean number of positive steady states is as shown in Figure 5. By inspection of the figure, we conclude that the second point is more robust.

The same analysis could be carried out using the log-normal distribution or a truncated normal distribution. Which one to consider depends on whether the order of magnitude needs to be taken into account, or whether we want to give more relevance to the parameter points around our start point.

4 Methods for numerical integration

Although in some cases one might be able to find the exact value of the Kac-Rice integral, in most cases one needs to rely on the numerical computation of the integral. In this case, depending on the dimension of the Kac-Rice integral, we have applied one of the following methods:

(i) If the integral involves a single or double integration, we use the approximation of the integral as a Riemannian sum, combined with the following decompositions of unbounded integrals to a sum of bounded integrals:

$$\begin{aligned}\int_0^\infty g(x)dx &= \int_0^1 g(x)dx+\int_0^1 \tfrac{1}{x^2}g(\tfrac{1}{x})dx,\\ \int_{-\infty}^\infty g(x)dx &= \int_0^1 \tfrac{1}{x^2}g(-\tfrac{1}{x})dx+\int_0^1 g(-x)dx\int_0^1 g(x)dx+\int_0^1 \tfrac{1}{x^2}g(\tfrac{1}{x})dx.\end{aligned}\tag{9}$$

(ii) If the dimension of the integral is larger, then we use Monte-Carlo integration. [25, Chapter 1].

The idea of Monte-Carlo integration is as follows (for more details, see [25]). Let I be an integral on a region $M\subseteq\mathbb{R}^n$ of the form

$$I=\int_M f(x_1,\ldots,x_n)dx_1\ldots dx_n.$$

We start by choosing a probability distribution on M with density function $p(x) = p(x_1, \ldots, x_n)$. Then, we have that

$$I = \int_M \frac{f(x)}{p(x)} p(x) dx = \mathbb{E}\left(\frac{f(x)}{p(x)}\right). \tag{10}$$

For $N \in \mathbb{N}$, consider random samples $x^{(1)}, \ldots, x^{(N)}$ of the random vector x following the probability distribution. By the Law of Large Numbers, if N is large enough, then I is approximated as

$$I \simeq \frac{1}{N} \sum_{i=1}^{N} \frac{f(x^{(i)})}{p(x^{(i)})}. \tag{11}$$

Further, the standard error of this approximation can also be found. Let $\hat{I}$ denote the sum in the right hand side of (11). Then the standard error of the approximation is

$$\hat{e} = \sqrt{\frac{\sum_{i=1}^{N} \left(\frac{f(x^{(i)})}{p(x^{(i)})} - \hat{I}\right)^2}{N(N-1)}}. \tag{12}$$

We apply the approximation given in (11) to the Kac-Rice integral I in Proposition 3.2 as follows. By considering the expressions of A and B in Proposition 3.2, we need to consider one integral on $\mathbb{R}_{>0}$ in t and three integrals with domain $\mathbb{R}_{>0}^{m-1}$ in the parameters, namely

$$\begin{aligned}
I_1 &= \int_{\mathbb{R}_{>0}^{m-1}} \rho_1(g(k_2, \ldots, k_m, t, 0)) \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m) dk_2 \ldots dk_m, \\
I_2 &= \int_{\mathbb{R}_{>0}^{m-1}} |f_k'(t)_{|_{k_1 = g(k_2, \ldots, k_m, t, 0)}}| \ \rho_1(g(k_2, \ldots, k_m, t, 0)) \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m) dk_2 \ldots dk_m, \\
I_3 &= \int_{\mathbb{R}_{>0}^{m-1}} \frac{\rho_1(g(k_2, \ldots, k_m, t, 0)) \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m)}{h_1(k_2, \ldots, k_m, t)} dk_2 \ldots dk_m.
\end{aligned}$$

We need now to choose a probability distribution on the domain of our integrals. First we note that the integration over t in the Kac-Rice integral can sometimes be considered as a bounded integral. In our example, t is the concentration of X_5, x_5, which must be smaller than T_2 for positive solutions to $f_k(t) = 0$ to exist. Thus, when considering robustness around one point, we might restrict t to a fixed number. If the integration over t is bounded in $[0, b]$, we sample using the uniform distribution $U([0, b])$. Alternatively one can use an exponential distribution with parameter λ, $E(\lambda)$, where λ is chosen such that the quantile of $1 - \epsilon$ for a small $\epsilon \in [0, 1]$ is b. If the integral is unbounded, then we use the decomposition given in (9) for $\int_0^\infty g(t) dt$, and use the uniform distribution on $[0, 1]$.

For the integrals over $\mathbb{R}_{>0}^{m-1}$ in the parameters $k_2, \ldots, k_m$, we use the same distribution assigned to them, $\rho_i(k_i)$ in the notation of Proposition 3.2. This gives that for the three integrals I_1, I_2, I_3, the quotients Q_1, Q_2, Q_3 corresponding to $f(x)/p(x)$ in (10) become

$$\begin{aligned}
Q_1 &= \frac{\rho_1(g(k_2, \ldots, k_m, t, 0)) \rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m)}{\rho_2(k_2) \cdot \ldots \cdot \rho_m(k_m)} = \rho_1(g(k_2, \ldots, k_m, t, 0)), \\
Q_2 &= |f_k'(t)_{|_{k_1 = g(k_2, \ldots, k_m, t, 0)}}| \ \rho_1(g(k_2, \ldots, k_m, t, 0)), \\
Q_3 &= \frac{\rho_1(g(k_2, \ldots, k_m, t, 0))}{h_1(k_2, \ldots, k_m, t)}.
\end{aligned}$$

In order to sample from given distributions, we can use build-in functions of the software we use. We have done it in the following way. For each distribution, we sample one point ϵ uniformily in the interval $[0, 1]$, and consider the quantile with respect to the distribution, that is, consider the value in $\mathbb{R}_{>0}$ with cumulative probability ϵ. We do this for the $m-1$ parameters and t, and obtain sample points $t_i, k_{2_i}, \ldots, k_{m_i}$ for $i = 1, \ldots, N$. We sample for large N until $\hat{e}$ is small enough. In the computations performed here, the stop criterion is $N > 1000$ and $\hat{e} < 10^{-2}$.

This method easily allows to be parallelized, by distributing the N sample points into the different processors the server has, and letting each processor evaluate the functions in the point.

Methods. The computations in this work are performed with **Maple 2018**. The package **RootFinding** is used for CAD and plotting the regions of multistationarity [19]. We use the **Statistics** package for sampling and the package **Grid** for doing parallel computations.

Acknowledgements. This work was initiated while A. S. visited MPI for the Mathematical Sciences in Leipzig in the summer of 2017. In particular A. S. learned about the Kac-Rice formula in the *Reading Group on Real Algebraic Geometry* that took place at MPI in June 2017. We thank Paul Breiding for clarifications on the Kac-Rice formula and Bernd Sturmfels for discussions on algebraic approaches to determine parameter regions of multistationarity. We thank Carsten Wiuf and Jimmy Olsson for key and fruitful discussions on conditional probabilities and Monte-Carlo methods for numerical integration.

References

[1] R. J. Adler and J. E. Taylor. *Random Fields and Geometry.* Springer-Verlag New York, 1st edition, 2007.

[2] A. Auffinger, G. Ben Arous, and Černý J. Random matrices and complexity of spin glasses. *Comm. Pure Appl. Math.*, 66(2):165–201, 2012.

[3] S. Basu, A. Lerario, E. Lundberg, and C. Peterson. Random fields and the enumerative geometry of lines on real and complex hypersurfaces. *Arxiv*, (1610.01205), 2016.

[4] S. Basu, R. Pollack, and M.-F. Coste-Roy. *Algorithms in real algebraic geometry*, volume 10. Springer Science & Business Media, 2007.

[5] F. Bihan, A. Dickenstein, and Giaroli M. Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. *ArXiv*, (1807.05157), 2018.

[6] R. Bradford, J. H. Davenport, M. England, H. Errami, V. Gerdt, D. Grigoriev, C. Hoyt, M. Košta, O. Radulescu, T. Sturm, and A. Weber. A case study on the parametric occurrence of multiple steady states. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 45–52. Association for Computing Machinery, 2017.

[7] C. Conradi, E. Feliu, M. Mincheva, and C. Wiuf. Identifying parameter regions for multistationarity. *PLoS Comput. Biol.*, 13(10):e1005751, 2017.

[8] C. Conradi and D. Flockerzi. Switching in mass action networks based on linear inequalities. *SIAM J. Appl. Dyn. Syst.*, 11(1):110–134, 2012.

[9] C. Conradi and M. Mincheva. Catalytic constants enable the emergence of bistability in dual phosphorylation. *J. R. S. Interface*, 11(95), 2014.

[10] S. Corvez and F. Rouillier. Using computer algebra tools to classify serial manipulators. In *4th International Workshop, ADG 2002, Hagenberg Castle, Austria, September 4-6, 2002, Revised Papers*, pages 31–43. Springer-Verlag Berlin Heidelberg, 2004.

[11] P. Donnell, M. Banaji, A. Marginean, and C. Pantea. Control: an open source framework for the analysis of chemical reaction networks. *Bioinformatics*, 30(11), 2014.

[12] P. Ellison, M. Feinberg, H. Ji, and D. Knight. Chemical reaction network toolbox, version 2.2. Available online at `http://www.crnt.osu.edu/CRNTWin`, 2012.

[13] M. England, R. Bradford, and J. H. Davenport. Improving the use of equational constraints in cylindrical algebraic decomposition. In *Proceedings of the International Symposium on Symbolic and Algebraic Computation, ISSAC*, pages 165–172. Association for Computing Machinery, 2015.

[14] E. Evans. The expected number of zeros of a random system of p-adic polynomials. *Electron. Commun. Probab.*, 11(29):278–290, 2006.

[15] M. Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors–II. Multiple steady states for networks of deficiency one. *Chem. Eng. Sci.*, 43(1):1–25, 1988.

[16] M. Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Arch. Ration. Mech. Anal.*, 132(4):311–370, 1995.

[17] E. Feliu and C. Wiuf. A computational method to preclude multistationarity in networks of interacting species. *Bioinformatics*, 29:2327–2334, 2013.

[18] E. Feliu and C. Wiuf. Simplifying biochemical models with intermediate species. *J. R. S. Interface*, 10:20130484, 2013.

[19] J. Gerhard, D. Jeffrey, and G. Moroz. A package for solving parametric polynomial systems. *ACM Commun. Comput. Algebra*, 43(3/4):61–72, 2010.

[20] H. A. Harrington, D. Mehta, H. M. Byrne, and J. D. Hauenstein. Decomposing the parameter space of biological networks via a numerical discriminant approach. *ArXiv*, (1604.02623), 2016.

[21] B. Joshi and A. Shiu. Atoms of multistationarity in chemical reaction networks. *J. Math. Chem.*, 51(1):153–178, 2013.

[22] M. Kac. On the average number of real roots of a random algebraic equation. *Bull. Amer. Math. Soc.*, 49(4):314–320, 1943.

[23] V. B. Kothamachu, E. Feliu, L. Cardelli, and O. S. Soyer. Unlimited multistability and boolean logic in microbial signalling. *J. R. Soc. Interface*, 12(108):20150234, 2015.

[24] S. Müller, E. Feliu, G. Regensburger, C. Conradi, A. Shiu, and A. Dickenstein. Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Found. Comput. Math.*, 16(1):69–97, 2016.

[25] A. B. Owen. Monte carlo theory, methods and examples. http://statweb.stanford.edu/~owen/mc/, 2013.

[26] M. Pérez Millán, A. Dickenstein, A. Shiu, and C. Conradi. Chemical reaction systems with toric steady states. *Bull. Math. Biol.*, 74(5):1027–1065, 2012.

[27] J. E. Taylor, J. R. Loftus, and R. J. Tibshirani. Inference in adaptive regression via the kac-rice formula. *Ann. Statist.*, 44(2):743–770, 2016.