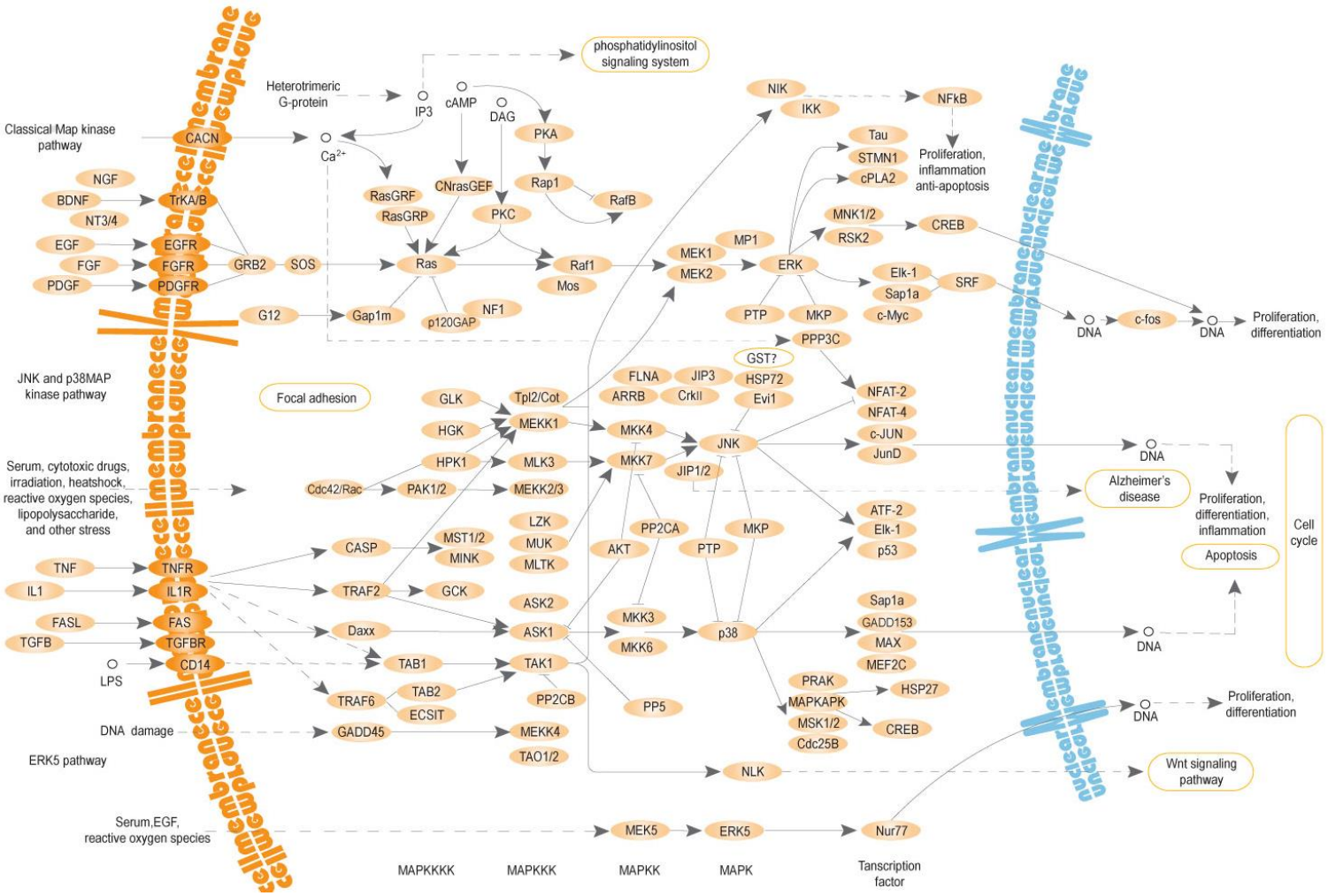

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Influence of Space in the Steady States of Chemical Reaction Networks

Dissertação de Mestrado em Matemática, Área de Especialização em Análise Aplicada e Computação, orientada pela Professora Doutora Maria Paula de Oliveira e apresentada ao Departamento de Matemática da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Abstract

Cells are constantly subject to stimuli that trigger sequences of chemical reactions (signalling pathways) which culminate in an appropriate response. If the chemical reaction networks involved have more than one steady state, then there is more than one possible response, resulting in increased flexibility in cellular decision-making.

The aim of this study is to determine if space can influence the steady states of chemical reaction networks. This influence is assessed in the sense of the capacity of the networks for more than one steady state under specific conditions, often called multistationarity.

The intricacy of signalling pathways requires simplifications prior to the analysis, which usually lead to the exclusion of space. However, signalling pathways are often influenced by space. For instance, some chemical species are required to move to a specific location before they act or even shuttle to a distinct compartment.

The approach considers two spatial factors: compartments and diffusion. Chemical Reaction Network Theory (CRNT) acts as the starting point, describing the networks as systems of Ordinary Differential Equations (ODEs). These can be modified to account for compartments or include diffusion terms resulting in reaction-diffusion systems.

On the one hand, to analyse the original ODEs models and the ones with compartments, assuming mass-action kinetics, the steady states are represented by roots of a polynomial, which can be studied with Injectivity Methods. On the other hand, the initial steady states are homogeneous solutions of the reaction-diffusion systems. Using Linear Stability Analysis, we evaluate their diffusion-driven instability, which could lead to spatial pattern formation.

According to the results, when adding compartments, some networks gain the capacity for multistationarity, while others lose it. However, none has linear diffusion-driven instability. The results prove that space can influence the steady states of chemical reaction networks.

Further research could be conducted on the influence of diffusion, through different representations of the networks, namely with Graph Theory, as well as a combination of compartments and diffusion that resembles the eukaryotic cell organisation.

Keywords: Signalling pathways; chemical reaction networks; multistationarity; compartments; diffusion-driven instability

Resumo

As células estão constantemente sujeitas a estímulos que ativam sequências de reações químicas (redes de sinalização), culminando numa resposta apropriada. Se as redes de reações químicas envolvidas apresentarem mais do que um estado de equilíbrio, há mais do que uma resposta possível, resultando num aumento da flexibilidade de decisão celular.

O objectivo do estudo é determinar se o espaço tem influência nos estados de equilíbrio de redes de reações químicas. Para testar esta influência é considerada a capacidade das redes para apresentarem mais do que um estado de equilíbrio sob condições específicas, denominada de multi-estacionariedade.

A complexidade das redes de sinalização requer simplificações antes da análise, normalmente ignorando o espaço. Contudo, as redes de sinalização são geralmente influenciadas pelo espaço. Por exemplo, algumas espécies químicas necessitam mover-se para um local específico na célula antes de atuar, ou mesmo mudar de compartimento.

A abordagem seguida considera duas aproximações espaciais: compartimentos e difusão. A Teoria das Redes de Reações Químicas (CRNT) serve de ponto de partida, descrevendo as redes como sistemas de equações com derivadas ordinárias (EDOs). Estes podem ser modificados para considerar compartimentos ou termos difusivos resultando em sistemas de reação-difusão.

Por um lado, para analisar os modelos originais com EDOs e os modelos com compartimentos, partinda da lei de ação das massas, os estados de equilíbrio são representados pelas raízes de um polinómio, que podem ser estudadas através de Métodos de Injetividade. Por outro lado, os estados de equilíbrio iniciais são soluções homogéneas dos sistemas de reação-difusão. Recorrendo a Análise de Estabilidade Linear, avalia-se a sua instabilidade na presença de difusão, que pode levar à formação de padrões espaciais.

De acordo com os resultados, com compartimentos, algumas redes ganham a capacidade para múltiplos estados de equilíbrio, enquanto outras a perdem. No entanto, nenhuma tem instabilidade na presença de difusão. Os resultados provam que o espaço tem influência nos estados de equilíbrio de redes de reações químicas.

Poder-se-ia estender a pesquisa à influência da difusão, através de novas representações das redes, por exemplo Teoria de Grafos, assim como à combinação de compartimentos e difusão para aproximar a organização interna das células eucarióticas.

Palavras-chave: Vias de sinalização; redes de reações químicas; multi-estacionariedade; compartimentos; instabilidade de Turing

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Chapter 1

Introduction

Cells, as the basis of life, must adapt to their environment and act appropriately to stimuli. Receptors interpret internal and external signals, triggering a sequence of chemical reactions that ultimately lead to an appropriate response. These highly complex chemical networks are involved in cellular regulatory activities, such as cell division and growth, chemotaxis (chemically directed movement) and apoptosis (programmed death). Therefore, studying signalling pathways is crucial to understand cellular activities and diseases that arise from their errors, such as tumours.

A chemical system is said to be at steady state when the concentrations of the chemical species involved do not change in time. Steady states are directly linked to the flexibility of cells decision-making. On the one hand, if a signalling pathway has a unique steady state, then there is only one response to the stimulus. On the other hand, multistationarity, as the existence of more than one steady state under specific conditions, allows for more complex behaviour of cells. Switch-like behaviour and irreversibility are some of the phenomena associated with multiple steady states. [7, 8]

As a result of the intricacy of signalling pathways, they are modelled through systems of Ordinary Differential Equations (ODEs), focused on the temporal evolution of concentrations of chemical species. Such a simplification disregards the influence of space on the systems. However, many pathways require molecules to diffuse to a specific location before an event occurs, while sometimes they must change compartments. For instance, in Synthetic Biology, the creation of microcompartments is a rising experimental tool. [1, 2, 4, 9]

Despite the advancements in computational tools, it is still difficult to analyse a complete pathway. However, there are specific mechanisms that are ubiquitous to such networks and that give insight on their behaviour. Studying some motifs, simplified patterns of activation and inhibition between a small number of species, acts as a reference point for the complexities of general pathways. The thesis focuses on signalling and phosphorylation motifs. Within the former, feedback and feedforward are two of the most relevant, associated with cellular memory and differentiation, and gene regulatory activity, respectively. The latter refers to the activation of substrates by action of an enzyme called kinase, by adding a phosphate group. [17]

As space has a role on cellular activities, we intend to assess its influence on the steady states of chemical reactions. To that end, space will be considered in two settings: temporal models with two compartments and spatial models in one compartment with diffusion. The two approaches intend to

assess the influence of compartmentalisation and of diffusion, as two ways of accounting for space without an impractical increase on the complexity of the study.

Regardless of the biological inspiration, the analysis is purely mathematical. Firstly, Chemical Reaction Network Theory (CRNT) provides a translation of chemical reactions to an autonomous system of ODEs, describing the temporal evolution of species concentrations. Secondly, analysing the steady states amounts to studying the zeros of the function that determines the system, namely the existence of more than one positive zero, equivalent to the multistationarity of the network. Thirdly, compartmentalisation is achieved by considering reactions happening in two compartments simultaneously, with some species moving between them. Lastly, a one-dimensional system, obtained from the system of ODEs by adding diffusion terms, is studied using Linear Stability Analysis to determine the capacity for diffusion-driven instability.

Chapter 2

Chemical reactions and injectivity

The evolution of chemistry led to the need for an analytical theory to represent and study chemical reactions. The first important step in this direction was made by Aris [3], who layed the foundations for the study, by proposing principles somehow analogous to those of continuum mechanics, as himself recognises:

> *"By 'stoichiometric' we undersand the calculus of changes in composition that take place by reaction; it corresponds to kinematics in the analogy with continuum mechanics. By 'kinetics' we understand the relations that govern the speed of the composition changes and this bears some analogy to the dynamics of continua."* [3, pp. 81–82]

Afterwards, Chemical Reaction Network Theory grew to become the language and the tool to study chemical reactions, specially their properties and equilibria. The work by Feinberg [5] focuses on representing chemical networks with ODEs, determining existence, multiplicity and stability of equilibrium. The ODEs systems only describe the temporal evolution of the chemical species, a simplification that requires assumptions on the homogeneity, temperature and volume of the vessel where the network takes place.

Assuming mass-action kinetics, the systems of ordinary differential equations (ODEs) are autonomous, that is of form $\frac{\mathrm{d}x}{\mathrm{d}t} = f(x)$, where the vector function f has polynomial coordinate functions. The steady states can be studied through appropriate algebraic methods applied to the system $f(x) = 0$, focusing on the injectivity of f, as proposed in [6] with an algorithm sustained by algebraic results detailed in [11]. Such methods exploit a matrix representation for generalised polynomials and do not fix the parameters of the model, therefore reaching general conclusions. Moreover, the methods only test solutions that are positive and that satisfy conservation laws.

2.1 Chemical Reaction Network Theory

A chemical reaction is the transformation of one set of chemical substances (or chemical species) into a different one. The consumed substances are the reactants and the ones created are the products. Intuitively, a chemical reaction network is a set of linked chemical reactions, where the product of one reaction is the reactant of another. CRNT formalises these notions, allowing for a rigorous analysis of the behaviour of the systems.

Chemical reactions usually take place in heterogeneous media, influenced by temperature and volume. However, to simplify the set up of the analysis, CRNT assumes that the medium is continuously stirred (homogeneous), temperature and volume are constant, and if there is a continuous inflow, it is balanced by a continuous outflow with the same rate.

Definition 1. A *chemical reaction network* is a set $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$, with

- $\mathscr{S} = \{X_1,\dots,X_n\}$ the set of chemical species.
- $\mathscr{C}$ the set of complexes, i.e, the left-hand and right-hand side of reactions.
- $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$ the set of reactions, i.e, the relations between complexes.

Each species must be in at least one complex, and each complex in at least one reaction. There are no reactions between identical complexes.
It is possible to consider a *zero complex*, representing the inflow and outflow of a species, if it lies on the left-hand or right-hand side of a reaction, respectively.

Example 1. Let us consider the chemical network

$$\begin{aligned} \mathrm{X_1 + X_2} &\xrightarrow{\mathrm{k_1}} \mathrm{X_3} \\ \mathrm{2\,X_3} &\underset{\mathrm{k_3}}{\overset{\mathrm{k_2}}{\rightleftharpoons}} \mathrm{X_2 + X_4} \\ \mathrm{X_4} &\xrightarrow{\mathrm{k_4}} \mathrm{X_1 + X_2} \end{aligned}$$

where k_i, $i = 1,\dots,4$, stand for the reaction rates. To this network correspond the following sets

$$\begin{aligned} \mathscr{S} &= \{X_1,X_2,X_3,X_4\} \\ \mathscr{C} &= \{X_1+X_2, X_3, 2X_3, X_2+X_4, X_4\} \\ \mathscr{R} &= \{\mathrm{X_1 + X_2} \longrightarrow \mathrm{X_3}, \mathrm{2\,X_3} \longrightarrow \mathrm{X_2 + X_4}, \mathrm{X_2 + X_4} \longrightarrow \mathrm{2\,X_3}, \mathrm{X_4} \longrightarrow \mathrm{X_1 + X_2}\}. \end{aligned}$$

The aim is to compute the state of the system, which is uniquely defined by the concentrations of the species in a given instant. Let $\mathbb{P}$ be the set of non-negative real numbers. Then, the concentration of species in an instant t is represented by $x(t) = (x_1(t),\dots,x_n(t)) \in \mathbb{P}^n$. In addition, a chemical reaction system requires the definition of a function for each reaction, determining the rate of its occurrence.

Definition 2. A *rate function* for a reaction y $\longrightarrow$ y' is a continuous function $k_{\mathrm{y} \longrightarrow \mathrm{y'}} : \mathbb{P}^n \to \mathbb{P}$ which satisfies

$$k_{\mathrm{y} \longrightarrow \mathrm{y'}}(x) > 0 \iff supp(y) \subset supp(x), \tag{2.1}$$

with *supp* the support function in $\mathbb{P}^n$ and x the concentration vector. The *kinetics* of a chemical reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ is defined by the assignment of a rate function to each reaction in $\mathscr{R}$.

Remark 1. Condition (2.1) means that the reaction rate is positive for concentrations x if and only if the reactants have positive concentration in the vector x. Therefore, the reaction takes place if and only if all the reactants are present in the system.

One of the simplest laws of kinetics, introduced by Waage and Gulberg [18], is *mass-action kinetics*, used throughout the text.

Definition 3 (Law of mass-action)**.** The rate of a reaction is proportional to the concentration of its reactants. If $x = (x_1, \ldots, x_l)$ is the concentration vector of the reactants, there exist positive constants k, $\alpha_1, \ldots, \alpha_l$, such that the speed of the reaction is $k \prod_{i=1}^{l} x_i^{\alpha_i}$, with α_i the *stoichiometric coefficient* of x_i in the reaction, and k the *rate constant*.

Therefore, the speed of the reactions is uniquely defined by the positive rate constants associated to each reaction, $\kappa = (k_1, \ldots, k_r)$, with r the number of reactions. After identifying the four components of the chemical system, $\{\mathscr{S}, \mathscr{C}, \mathscr{R}, \kappa\}$, the evolution of concentrations is defined by an autonomous system of ODEs, whose second members are polynomial functions.

Example 2. The network in Example 1 is described by the following ODEs system.

$$\begin{aligned}
\frac{\mathrm{d}x_1}{\mathrm{d}t} &= -k_1 x_1 x_2 + k_4 x_4 \\
\frac{\mathrm{d}x_2}{\mathrm{d}t} &= -(k_1 x_1 + k_3 x_4) x_2 + k_2 x_3^2 + k_4 x_4 \\
\frac{\mathrm{d}x_3}{\mathrm{d}t} &= (k_1 x_1 + 2k_3 x_4) x_2 - 2k_2 x_3^2 \\
\frac{\mathrm{d}x_4}{\mathrm{d}t} &= k_2 x_3^2 - k_3 x_2 x_4 - k_4 x_4
\end{aligned} \tag{2.2}$$

It is possible that a group of equations adds up to zero. This is equivalent to saying that the sum of their time derivatives is equal to zero at all times, thus the sum of their concentrations does not change over time, retaining the initial total amount. The resulting equations are called *conservation laws*.

In Chapters 2, 3 and 4 of this thesis we focus on the *steady states* of chemical reaction networks, i.e, the set of species concentrations for which the system does not change in time. These are the solutions of $\frac{\mathrm{d}x}{\mathrm{d}t} = f_\kappa(x) = 0$. Assuming mass-action kinetics, the steady state system is polynomial and determining equilibria is the same as calculating the roots of a polynomial function.

As $f_\kappa(x)$ is a polynomial vector function, it has a matrix representation easily deducted from the underlying reaction network. Let $V \subset \mathbb{R}^{n \times r}$ be the *matrix of exponents*, with each column, v_j, corresponding to a reaction, and v_{ij} the coefficient of X_i in the reactants of the j-th reaction. To simplify the notation, $x^{v_j} := x_1^{v_{1j}} \cdot \ldots \cdot x_n^{v_{nj}}$ and $x^V := (x^{v_1}, \ldots, x^{v_r})$. In addition, let $A \subset \mathbb{R}^{n \times r}$ be the *stoichiometric matrix* that satisfies

$$\frac{dx}{dt} = f_\kappa(x) = A(\kappa \circ x^V), \tag{2.3}$$

where $\circ$ represents the Hadamard product, i.e, product element-wise.

Remark 2. Each column of A corresponds to a reaction and the value of each entry is the difference in coefficients of the corresponding species, as a product and as a reactant. For instance, if a species is consumed in the same proportion as it is produced in a reaction, the entry in A is zero. If it is only a product, the value will be positive, and if it is only a reactant, it will be negative. Additionally, the orthogonal space of $Im(A)$ is the subspace of the conservation laws of the system.

Example 3. The matrices for the system (2.2) are

$$A = \begin{pmatrix} -1 & 0 & 0 & 1 \\ -1 & 1 & -1 & 1 \\ 1 & -2 & 2 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix}, \; V = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 2 & 0 & 0 \\ 0 & 0 & 1 & 1 \end{pmatrix},$$

and $x_2 + x_3 + x_4 = x_T$, with x_T a positive constant defined by the initial conditions, is the only conservation law.

2.2 Injectivity criteria

The capacity for multiple steady states is intrinsic to networks, therefore to the function $f_\kappa(x)$ determining the ODEs system. Analysing its injectivity provides insight on the steady states. If the function is injective, there can be at most one steady state. There are injectivity methods that exploit the structure in (2.3), without fixing rate constants κ, to prove the existence of rates that allow for multistationarity. The results in this section are based on [11], thus they are also applicable to generalised polynomials and not only those which arise from chemical networks.

The relevant steady states are positive for all species and correspond to the same initial conditions, $x^* \in \mathbb{P}^n$. Considering the *stoichiometric subspace*, $S := span\{y - x \in \mathbb{R}^n : \mathrm{x} \longrightarrow \mathrm{y} \in \mathscr{R}\} = Im(A)$, it is clear that $f_\kappa(x) \in S$ for all $x \in \mathbb{P}^n$, thus the trajectories of f_κ lie in $(x^* + S)$. In order to disregard the initial conditions, we focus the analysis on the zeros $x, y \in \mathbb{P}^n$ of f_κ such that $x - y \in S$, through the notions of S-injectivity and multiple S-zeros. The chemical system is *S-injective* if for all rate constants κ, for all distinct $x, y \in \mathbb{P}^n$ such that $x - y \in S$, $f_\kappa(x) \neq f_\kappa(y)$. Conversely, the system has *multiple S-zeros* if there exist rate constants κ for which there are distinct $x, y \in \mathbb{P}^n$ such that $x - y \in S$ and $f_\kappa(x) = f_\kappa(y) = 0$.

Remark 3. The restriction to the stoichiometric subspace ensures the signs of the rate constants follow mass-action kinetics and guarantees that they appear in components of x involved in a common reaction complex.
Additionally, $S^\perp$ represents the conservation laws of the system. A basis for the subspace identifies the species whose combined concentrations do not change in time.

The results in this section use the following notations:

$\boldsymbol{\sigma}$ stands for the sign function, and $\sigma(x)$, $x \in \mathbb{R}^n$, is the result of applying the sign function element-wise to x.

$\boldsymbol{\sigma(S)} = \{\sigma(x) : x \in S\}$.

$\boldsymbol{\Sigma(S)} = \sigma^{-1}(\sigma(S)) = \{\lambda \circ x : \lambda \in \mathbb{P}^n, x \in S\}$.

$\boldsymbol{S^*} = S \setminus \{0\}$.

$\boldsymbol{S_V} = \left\{x^V - y^V : x, y \in \mathbb{P}^n, x - y \in S\right\}$.

$\boldsymbol{\Lambda(S)} = \{\ln(x) - \ln(y) : x, y \in \mathbb{P}^n, x - y \in S\}$.

$\boldsymbol{diag(\lambda)}$ is the diagonal matrix with diagonal entries equal to $\lambda \in \mathbb{R}^n$, and $\boldsymbol{A_\lambda} = A \cdot diag(\lambda)$.

$\boldsymbol{[n]} = \{1, \ldots, n\}$.

Remark 4. Note that following this notation $f_\kappa(x) = A\left(\kappa \circ x^V\right) = A_\kappa x^V$.

The *Determinant Criterion* (Theorem 1) assesses the S-injectivity of the polynomial function, considering conservation laws, by analysing the sign of a specific determinant.

Theorem 1 (Determinant Criterion)**.** *Let $A \in \mathbb{R}^{n\times r}$, with $rank(A) = s$, $V \in \mathbb{R}^{n\times r}$, $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$,* $\dim(S) = s$, *$Z \in \mathbb{R}^{(n-s)\times n}$ whose rows form a basis for $S^\perp$, $\tilde{A} \in \mathbb{R}^{s\times r}$ and* $\ker(\tilde{A}) = \ker(A)$. *Let*

$$\Gamma_{\kappa,\lambda} = \begin{pmatrix} Z \\ \tilde{A}_\kappa V_\lambda^t \end{pmatrix}. \tag{2.4}$$

The following statements are equivalent,

1. *The map $f_\kappa(x) = A_\kappa x^V$ is S-injective for all $\kappa \in \mathbb{P}^n$.*
2. *The determinant of $\Gamma_{\kappa,\lambda}$, when regarded as a polynomial in κ and λ, is not identically zero and its coefficients have the same sign.*

Before proving Theorem 1, we require some auxiliary results. The proofs can be found in Appendix A.

Lemma 1. *Let $X, Y \subset \mathbb{R}^n$, then*

$$\Sigma(X) \cap Y = \emptyset \iff \sigma(X) \cap \sigma(Y) = \emptyset \iff X \cap \Sigma(Y) = \emptyset.$$

Lemma 2. *Let $B \in \mathbb{R}^{r\times n}$ and $S \subset \mathbb{R}^n$. The following are equivalent.*

1. $\ker(B_\lambda) \cap S = \emptyset$ *for all* $\lambda \in \mathbb{P}^n$.
2. $\sigma(\ker(B)) \cap \sigma(S) = \emptyset$.

Lemma 3. $\Sigma(S) = \Lambda(S)$

Let $A \in \mathbb{R}^{n\times n}$, $I, J \subset [n]$ sets of l indeces of A, with $1 \leq l < n$, corresponding to rows and columns, respectively. Additionally, $A_{I,J}$ is the submatrix of A obtained by keeping the entries on the intersection of the list of indices I and J, and I^c is the complementary set of I in $[n]$. The *generalised Laplace expansion* (2.5) gives an expression for the determinant of A.

$$\det(A) = (-1)^{\tau(I)} \sum_J (-1)^{\tau(J)} \det(A_{I,J}) \det(A_{I^c,J^c}), \tag{2.5}$$

with $\tau(I)$ the parity of the permutation that sends $[n]$ to $I^c \cup I$, with I^c and I each sorted in ascending order.

Considering matrices $B \in \mathbb{R}^{m\times n}$ and $C \in \mathbb{R}^{n\times m}$, $m < n$, the following is the *Cauchy-Binet Formula*

$$\det(BC) = \sum_I \det(B_{[m],I}) \det(C_{I,[m]}), \tag{2.6}$$

with sum over the different sets of $I \subset [n]$ with m elements.

Lemma 4. *Let $\Gamma_{\kappa,\lambda}$ as defined in Theorem 1, then*

$$\det(\Gamma_{\kappa,\lambda}) = \sum_{I,J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \det\left(\tilde{A}_{[s],I}\right) \det\left(V^t_{I,J}\right) \kappa^I \lambda^J,$$

with sum over all sets $I \subset [r]$, $J \subset [n]$ with s elements.

Lemma 5. *Let $q(c) \in \mathbb{R}[c_1,\ldots,c_l]$ represent a homogeneous polynomial not identically zero, with degree at most 1 in each variable. Then, there exists $c^* \in \mathbb{P}^l$ root of q if and only if not all the coefficients of $q(c)$ have the same sign.*

Since the proof of Theorem 1 is long, the following equivalences are required to reach the final result.

Proof of Theorem 1. The following steps are needed to prove the equivalence of the statements.

1. f_κ S-injective for all $\kappa \in \mathbb{P}^r \iff \sigma(\ker(A)) \cap \sigma\left(V^t\left(\Sigma(S^*)\right)\right) = \emptyset$.

   f_κ being S-injective for all $\kappa \in \mathbb{P}^r$ means that for all $x, y \in \mathbb{P}^n$ such that $x - y \in S^*$, $A_\kappa\left(x^V - y^V\right) \neq 0$, for all $\kappa \in \mathbb{P}^r$. Thus, it is equivalent to $\ker(A_\kappa) \cap S^*_V = \emptyset$, for all $\kappa \in \mathbb{P}^r$. Applying Lemma 2, the equivalence with $\sigma(\ker(A)) \cap \sigma(S^*_V) = \emptyset$ is clear. Therefore,

$$\begin{aligned}
\sigma\left(S^*_V\right) &= \sigma\left(\left\{x^V - y^V : x - y \in S^*\right\}\right) \\
&= \sigma\left(\left\{V^t(\ln x - \ln y) : x - y \in S^*\right\}\right) \\
&= \sigma\left(V^t\left(\Lambda(S^*)\right)\right) \\
&= \sigma\left(V^t\left(\Sigma(S^*)\right)\right),
\end{aligned}$$

   the last equality is a consequence of Lemma 3.

2. $\sigma(\ker(A)) \cap \sigma\left(V^t\left(\Sigma(S^*)\right)\right) = \emptyset \iff \ker\left(A_k V^t_\lambda\right) \cap S^* = \emptyset$ for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$.

   Note that $0 = A_k V^t_\lambda x = A\left(\kappa \circ \left(V^t\left(\lambda \circ x\right)\right)\right)$, then, using Lemma 1 for the first equivalence,

$$\begin{aligned}
\sigma(\ker(A)) \cap \sigma\left(V^t\left(\Sigma(S^*)\right)\right) = \emptyset &\iff \ker(A) \cap \Sigma\left(V^t\left(\Sigma(S^*)\right)\right) = \emptyset \\
&\iff \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n, x \in S^* : A\left(\kappa \circ \left(V^t\left(\lambda \circ x\right)\right)\right) \neq 0 \\
&\iff \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n, x \in S^* : A_k V^t_\lambda x \neq 0 \\
&\iff \ker(A_\kappa V^t_\lambda) \cap S^* = \emptyset, \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n
\end{aligned}$$

3. $\ker\left(A_k V^t_\lambda\right) \cap S^* = \emptyset$ for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n \iff \Gamma_{\kappa,\lambda} x \neq 0$ for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$, $x \in \mathbb{R}^n \setminus \{0\}$.

As a consequence of the definition of $\Gamma_{\kappa,\lambda}$, $\Gamma_{\kappa,\lambda}x = 0 \iff Zx = 0 \wedge A_\kappa V_\lambda^t x = 0$. Therefore

$$\begin{aligned}\ker\left(A_k V_\lambda^t\right) \cap S^* = \emptyset, \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n &\iff \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n, x \in S^* : A_\kappa V_\lambda^t x \neq 0 \\ &\iff \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n, x \in S^* : \Gamma_{\kappa,\lambda} x \neq 0 \\ &\iff \forall \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n, x \in \mathbb{R}^n \setminus \{0\} : \Gamma_{\kappa,\lambda} x \neq 0.\end{aligned}$$

The last equivalence comes from the definition of Z, as S is a solution set of $Zx = 0$.

4. $\Gamma_{\kappa,\lambda}x \neq 0$ for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$, $x \in \mathbb{R}^n \setminus \{0\} \iff \det\left(\Gamma_{\kappa,\lambda}\right) \neq 0$ for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$.

   Since $\Gamma_{\kappa,\lambda}$ is a square matrix, its determinant exists. Thus, $\Gamma_{\kappa,\lambda}x \neq 0$, for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$, $x \in \mathbb{R}^n \setminus \{0\}$, is equivalent to $\Gamma_{\kappa,\lambda}$ being nonsingular for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$, which is equivalent to $\det\left(\Gamma_{\kappa,\lambda}\right) \neq 0$, for all $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$.

5. According to Lemma 4, $\det(\Gamma_{\kappa,\lambda})$ is a homogeneous polynomial in κ and λ, with degree at most 1 in each variable.

6. Since $\det\left(\Gamma_{\kappa,\lambda}\right) \neq 0$ and it is a homogeneous polynomial with degree at most 1 in each variable, according to Lemma 5, all its coefficients have the same sign.

□

If $S \subset \mathbb{R}^n$ is a vector space, S-injectivity of the polynomial map is related to the determinant of its Jacobian matrix. To prove the main result, Lemma 6 is essential.

Lemma 6. *Let $f_\kappa : \mathbb{P}^n \to \mathbb{R}^m$, $f_\kappa(x) = A_\kappa x^V$. Then, the set of Jacobian matrices $J_{f_\kappa}(x)$ and the set of matrices $A_\kappa V_\lambda^t$ coincide:*

$$\{J_{f_\kappa}(x) : \kappa \in \mathbb{P}^r, x \in \mathbb{P}^n\} = \{A_\kappa V_\lambda^t : \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n\}.$$

It is now possible to relate the result of Theorem 1 and the Jacobian matrix of f_κ.

Proposition 1 (Jacobian Injectivity Criterion)**.** *Let $A \in \mathbb{R}^{n\times r}$, $rank(A) = s$, $V \in \mathbb{R}^{n\times r}$, $\kappa \in \mathbb{P}^r$, $\lambda \in \mathbb{P}^n$, $S \subset \mathbb{R}^n$ subspace with dimension s, $x \in \mathbb{P}^n$, $f_\kappa(x) = A_\kappa x^V$.*

If $J_{f_\kappa}(x)$ is the Jacobian matrix of f_κ, $\det(J_{f_\kappa}) \neq 0$ and all its coefficients have the same sign, then f_κ is S-injective.

Proof. To prove Theorem 1 we showed the equivalence between S-injectivity of f_κ, for all κ, and $\ker\left(J_{f_\kappa}(x)\right) \cap S^* = \emptyset$, for all $x \in \mathbb{R}^n \setminus \{0\}, \kappa \in \mathbb{P}^r$, taking Lemma 6 into account. Since $J_{f_\kappa}(x)$ is a square matrix, the former is equivalent to $\det\left(J_{f_\kappa}(x)\right) \neq 0$ for all $x \in \mathbb{R}^n \setminus \{0\}, \kappa \in \mathbb{P}^r$. As $x \in \mathbb{P}^n$ and $\kappa \in \mathbb{P}^r$, if $\det\left(J_{f_\kappa}(x)\right)$ is not identically zero and, when seen as a polynomial in x and κ, its coefficients all have the same sign, its determinant is not identically zero. Consequently, the assumptions imply S-injectivity of f_κ for all $\kappa \in \mathbb{P}^r$. □

2.3 Summary

CRNT provides tools to describe chemical reaction networks as systems of ODEs, assuming the networks take place in controlled conditions of temperature and pressure, in a homogeneous medium.

When assuming mass-action kinetics, the second members of the system are polynomial functions. Thus, determining the steady states of the network is equivalent to calculating the roots of polynomials. Note that the relevant steady states lie in an affine subspace $(x^* + S)$, with $x^* \in \mathbb{P}^n$ the initial conditions and $S = Im(A)$, the stoichiometric subspace.

Despite not having a linear system, it is possible to use a matrix representation for the system of ODEs, which is defined by the stoichiometric matrix A and the matrix of exponents V. Additionally, there is conservation of total amounts of some species, which is conveyed by the conservation laws. Such information is encoded in $S^{\perp}$.

To assess the existence of multiple steady states, proving the S-injectivity of the determining function acts as an exclusion criterion. For instance, the Determinant Criterion exploits the matrix representation of the polynomial, relating its S-injectivity to the determinant of a relevant matrix, $\Gamma_{\kappa,\lambda}$. As the steady states must be consistent with the system and positive, this information is taken into account when the search for injectivity is confined to elements x, y such that $x - y \in S$. The Jacobian Injectivity Criterion is a weaker result, only providing a sufficient condition for S-injectivity.

Chapter 3

Motifs and algorithm

The complexity of signalling pathways, as a consequence of the large number of species and reactions involved, results in intricate models. Thus, simplifications are required to allow for their analysis. By considering recurrent mechanisms in such pathways, as feedback and feedforward, it is possible to gain insight on their behaviour, while avoiding analysing the full system.

In order to conduct an extensive study, the analysis focused on eleven motifs and possible variations, classified as signalling and phosphorylation networks. The former refers to a sequence of chemical reactions activated by an exterior signal, while the latter involves phosphorylation cycles. The motifs were based on [1, 2, 7, 14].

The criteria defined in Section 2.2 give information on the capacity for multistationarity. Furthermore, they can be used indirectly to gather more information. To that end, an algorithm based on [6] was implemented to calculate and test appropriate matrices A and V, deducted from the original matrices. In some cases, the method was inconclusive, which required the usage of the CRNT Toolbox.[1] Subsection 3.2.4 includes examples of the application of the algorithm, namely concluding S-injectivity, a unique zero and multiple S-zeros.

Considering the algorithm in subsection 3.2.3, it is possible to obtain initial results regarding the steady states of the motifs, which act as the control group for the study of the influence of space on the equilibria.

3.1 Motifs

Signalling pathways are present in both cellular regulatory activities and intercellular communication. There are different signal transductions, where the signal is converted into a chemical cascade inside the cell, leading to a response. Such cascades involve activation and deactivation of species. Moreover, the process may require the action of enzymes, as is the case of phosphorylation cycles.

Phosphorylation is the process of adding a phosphate group to a molecule, with the intervention of enzymes called kinases. The opposite process, dephosphorylation, is regulated by phosphatases. Phosphorylation is essential for enzymatic regulation, as it activates and deactivates enzymes.

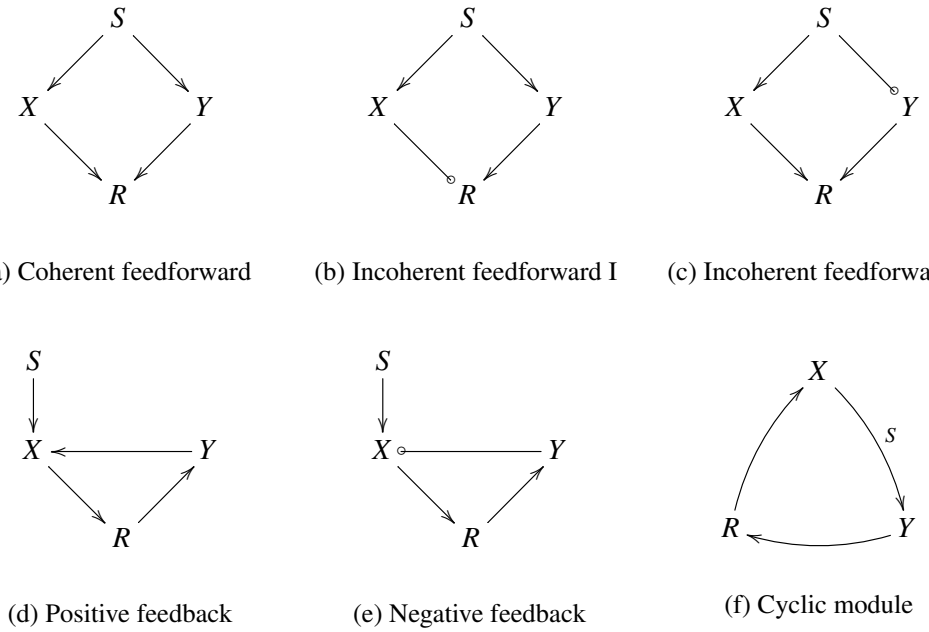
[1] CRNT Toolbox is available at http://crnt.osu.edu/CRNTWin

The motifs have a representation as systems of ODEs, based on CRNT. To avoid presenting duplicate information and a system with an equation for each species, the following sections include a mix of ODEs and conservation laws for each motif.

3.1.1 Signalling networks

Fig. 3.1 represents the signalling networks in study, with S the signal, X, Y and R the chemical species which are activated and deactivated, with the active R as the response. The activated forms act on other species, either activating (arrow with a tip) or deactivating (arrow with a circle), except for the *cyclic module* for which each species spontaneously becomes the next one. The signal is constant in time.

Note that for all the networks apart from the *cyclic module*, there is spontaneous activation and deactivation of species, which is not featured in Fig. 3.1.



(a) Coherent feedforward (b) Incoherent feedforward I (c) Incoherent feedforward II

(d) Positive feedback (e) Negative feedback (f) Cyclic module

Fig. 3.1 Signalling networks

We present in what follows the systems of ODEs that describe the signalling networks in Fig 3.1. Note that an arrow represents activation of the element at its end by the active form of the element at the beginning, while an arrow ending in a circle represents deactivation. Moreover, the active forms are marked with a '*'. For the cyclic module (Fig. 3.1f), each species transforms into the next one spontaneously, apart from X, that does so with help of S.

Coherent feedforward

The signal S activates X and Y, and both activate the response, R (Fig. 3.1a). X_T, Y_T and R_T are total amounts.

$$
\begin{aligned}
S + X &\xrightarrow{k_1} S + X^* \\
S + Y &\xrightarrow{k_2} S + Y^* \\
X^* + R &\xrightarrow{k_3} X^* + R^* \\
Y^* + R &\xrightarrow{k_4} Y^* + R^* \\
X &\underset{k_6}{\overset{k_5}{\rightleftharpoons}} X^* \\
Y &\underset{k_8}{\overset{k_7}{\rightleftharpoons}} Y^* \\
R &\underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} R^*
\end{aligned}
$$

$$
\begin{aligned}
\frac{dX^*}{dt} &= (k_1 S + k_5)X - k_6 X^* \\
\frac{dY^*}{dt} &= (k_2 S + k_7)Y - k_8 Y^* \\
\frac{dR^*}{dt} &= (k_3 X^* + k_4 Y^* + k_9)R - k_{10} R^* \\
X_T &= X + X^* \\
Y_T &= Y + Y^* \\
R_T &= R + R^*
\end{aligned}
$$

Incoherent feedforward

There are two possible representations for this mechanism. Either, S activates X and Y, which have different actions over the response (Fig. 3.1b), or S activates X and inhibits Y, with both X and Y activating the response R (Fig. 3.1c). X_T, Y_T and R_T are total amounts.

$$
\begin{aligned}
S + X &\xrightarrow{k_1} S + X^* \\
S + Y &\xrightarrow{k_2} S + Y^* \\
X^* + R^* &\xrightarrow{k_3} X^* + R \\
Y^* + R &\xrightarrow{k_4} Y^* + R^* \\
X &\underset{k_6}{\overset{k_5}{\rightleftharpoons}} X^* \\
Y &\underset{k_8}{\overset{k_7}{\rightleftharpoons}} Y^* \\
R &\underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} R^*
\end{aligned}
$$

$$
\begin{aligned}
\frac{dX^*}{dt} &= (k_1 S + k_5)X - k_6 X^* \\
\frac{dY^*}{dt} &= (k_2 S + k_7)Y - k_8 Y^* \\
\frac{dR^*}{dt} &= (k_4 Y^* + k_9)R - (k_3 X^* + k_{10})R^* \\
X_T &= X + X^* \\
Y_T &= Y + Y^* \\
R_T &= R + R^*
\end{aligned}
$$

$$
\begin{aligned}
S + X &\xrightarrow{k_1} S + X^* \\
S + Y^* &\xrightarrow{k_2} S + Y \\
X^* + R &\xrightarrow{k_3} X^* + R^* \\
Y^* + R &\xrightarrow{k_4} Y^* + R^* \\
X &\underset{k_6}{\overset{k_5}{\rightleftharpoons}} X^* \\
Y &\underset{k_8}{\overset{k_7}{\rightleftharpoons}} Y^* \\
R &\underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} R^*
\end{aligned}
$$

$$
\begin{aligned}
\frac{dX^*}{dt} &= (k_1 S + k_5)X - k_6 X^* \\
\frac{dY^*}{dt} &= k_7 Y - (k_2 S + k_8)Y^* \\
\frac{dR^*}{dt} &= (k_3 X^* + k_4 Y^* + k_9)R - k_{10} R^* \\
X_T &= X + X^* \\
Y_T &= Y + Y^* \\
R_T &= R + R^*
\end{aligned}
$$

Positive feedback

In this model, the signal S activates X, initiating a cycle of activations, of X, R and Y, sequentially (Fig. 3.1d). X_T, Y_T and R_T are total amounts.

$$
\begin{aligned}
\mathrm{S} + \mathrm{X} &\xrightarrow{k_1} \mathrm{S} + \mathrm{X}^* \\
\mathrm{X}^* + \mathrm{R} &\xrightarrow{k_2} \mathrm{X}^* + \mathrm{R}^* \\
\mathrm{R}^* + \mathrm{Y} &\xrightarrow{k_3} \mathrm{R}^* + \mathrm{Y}^* \\
\mathrm{Y}^* + \mathrm{X} &\xrightarrow{k_4} \mathrm{Y}^* + \mathrm{X}^* \\
\mathrm{X} &\underset{k_6}{\overset{k_5}{\rightleftharpoons}} \mathrm{X}^* \\
\mathrm{Y} &\underset{k_8}{\overset{k_7}{\rightleftharpoons}} \mathrm{Y}^* \\
\mathrm{R} &\underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} \mathrm{R}^*
\end{aligned}
\qquad
\begin{aligned}
\frac{dX^*}{dt} &= (k_1 S + k_4 Y^* + k_5)X - k_6 X^* \\
\frac{dY^*}{dt} &= (k_3 R^* + k_7)Y - k_8 Y^* \\
\frac{dR^*}{dt} &= (k_2 X^* + k_9)R - k_{10} R^* \\
X_T &= X + X^* \\
Y_T &= Y + Y^* \\
R_T &= R + R^*
\end{aligned}
$$

Negative feedback

Similarly to the previous one, the signal S activates X, which activates the response R. The response also activates Y but Y acts as an inhibitor of X (Fig. 3.1e). X_T, Y_T and R_T are total amounts.

$$
\begin{aligned}
\mathrm{S} + \mathrm{X} &\xrightarrow{k_1} \mathrm{S} + \mathrm{X}^* \\
\mathrm{X}^* + \mathrm{R} &\xrightarrow{k_2} \mathrm{X}^* + \mathrm{R}^* \\
\mathrm{R}^* + \mathrm{Y} &\xrightarrow{k_3} \mathrm{R}^* + \mathrm{Y}^* \\
\mathrm{Y}^* + \mathrm{X}^* &\xrightarrow{k_4} \mathrm{Y}^* + \mathrm{X} \\
\mathrm{X} &\underset{k_6}{\overset{k_5}{\rightleftharpoons}} \mathrm{X}^* \\
\mathrm{Y} &\underset{k_8}{\overset{k_7}{\rightleftharpoons}} \mathrm{Y}^* \\
\mathrm{R} &\underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} \mathrm{R}^*
\end{aligned}
\qquad
\begin{aligned}
\frac{dX^*}{dt} &= (k_1 S + k_5)X - (k_4 Y^* + k_6)X^* \\
\frac{dY^*}{dt} &= (k_3 R^* + k_7)Y - k_8 Y^* \\
\frac{dR^*}{dt} &= (k_2 X^* + k_9)R - k_{10} R^* \\
X_T &= X + X^* \\
Y_T &= Y + Y^* \\
R_T &= R + R^*
\end{aligned}
$$

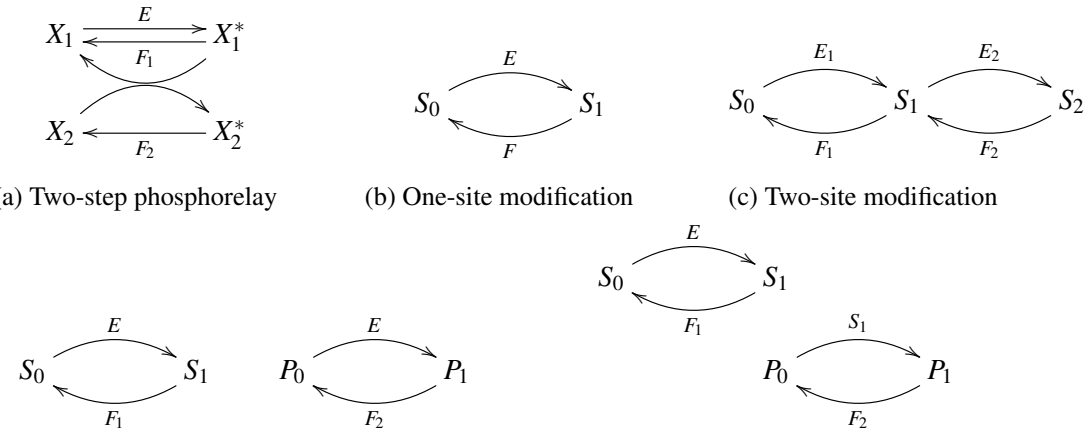
Cyclic module

This model has a cycle of activations that starts with X with the action of S, and follows to Y and Z. The activations are spontaneous (Fig. 3.1f).

$$
\begin{aligned}
\mathrm{S} + \mathrm{X} &\xrightarrow{k_1} \mathrm{S} + \mathrm{Y} \\
\mathrm{Y} &\xrightarrow{k_2} \mathrm{R} \\
\mathrm{R} &\xrightarrow{k_3} \mathrm{X}
\end{aligned}
\qquad
\begin{aligned}
\frac{dX}{dt} &= k_3 R - k_1 X S \\
\frac{dY}{dt} &= k_1 X S - k_2 Y \\
R_T &= X + Y + R
\end{aligned}
$$

3.1.2 Phosphorylation networks

Fig. 3.2 represents the phosphorylation networks in study, with the enzymes identified above the arrows. The intermediate complexes have not been represented Fig. 3.2.



(a) Two-step phosphorelay (b) One-site modification (c) Two-site modification

(d) Modification of two substrates (e) Two-layer cascade

Fig. 3.2 Phosphorylation networks

According to the analysis in [7], sharing enzymes can influence the capacity for multiple steady states, therefore for the motifs in Figs. 3.2c, 3.2d and 3.2e, the study extends to having $E_1 = E_2$ and $F_1 = F_2$, if appropriate.

Two-step phosphorelay

X_1 is activated by kinase E, and there is phosphotransfer from X_1^* to X_2, activating the latter. Both suffer phosphorylation by respective phosphatases F_1 and F_2 (Fig. 3.2a). E_T, F_{1T}, F_{2T}, X_{1T} and X_{2T} are total amounts.

$$
\begin{aligned}
&\mathrm{X_1 + E} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{Y_1} \xrightarrow{\mathrm{k_3}} \mathrm{X_1^* + E}\\
&\mathrm{X_1^* + F_1} \underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftharpoons}} \mathrm{Y_2} \xrightarrow{\mathrm{k_6}} \mathrm{X_1 + F_1}\\
&\mathrm{X_2 + X_1^*} \xrightarrow{\mathrm{k_7}} \mathrm{X_2^* + X_1}\\
&\mathrm{X_2^* + F_2} \underset{\mathrm{k_9}}{\overset{\mathrm{k_8}}{\rightleftharpoons}} \mathrm{Y_3} \xrightarrow{\mathrm{k_{10}}} \mathrm{X_2 + F_2}
\end{aligned}
\qquad
\begin{aligned}
\frac{\mathrm{d}X_1^*}{\mathrm{d}t} &= k_3Y_1 + k_5Y_2 - (k_4F_1 + k_7X_2)X_1^*\\
\frac{\mathrm{d}X_2^*}{\mathrm{d}t} &= k_9Y_3 + k_7X_1^*X_2 - k_8X_2^*F_2\\
\frac{\mathrm{d}Y_1}{\mathrm{d}t} &= -(k_2 + k_3)Y_1 + k_1X_1E\\
\frac{\mathrm{d}Y_2}{\mathrm{d}t} &= -(k_5 + k_6)Y_2 + k_4X_1^*F_1\\
\frac{\mathrm{d}Y_3}{\mathrm{d}t} &= -(k_9 + k_{10})Y_3 + k_8X_2^*F_2
\end{aligned}
$$

with conservation laws $E_T = E + Y_1$, $F_{1T} = F_1 + Y_2$, $F_{2T} = F_2 + Y_3$, $X_{1T} = X_1^* + X_1 + Y_1 + Y_2$ and $X_{2T} = X_2^* + X_2 + Y_3$.

One-site modification

S_0 is a substrate phosphorylated by E to S_1, with phosphatase F. X and Y are intermediate complexes (Fig. 3.2b).

$$\mathrm{S_0 + E} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftarrows}} \mathrm{X} \xrightarrow{\mathrm{k_3}} \mathrm{S_1 + E}$$
$$\mathrm{S_1 + F} \underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftarrows}} \mathrm{Y} \xrightarrow{\mathrm{k_6}} \mathrm{S_0 + F}$$

$$\frac{\mathrm{d}S_0}{\mathrm{d}t} = k_2X + k_6Y - k_1S_0E$$
$$\frac{\mathrm{d}S_1}{\mathrm{d}t} = k_3X + k_5Y - k_4S_1F$$
$$\frac{\mathrm{d}X}{\mathrm{d}t} = k_1S_0E - (k_2 + k_3)X$$
$$\frac{\mathrm{d}Y}{\mathrm{d}t} = k_4S_1F - (k_5 + k_6)Y$$

with conservation laws $E_T = E + X$, $F_T = F + Y$ and $S_T = S_0 + S_1 + X + Y$.

Two-site modification

S_0 and S_1 are substrates, with kinases E_1 and E_2, and phosphatases F_1 and F_2, respectively. There are two one-site phosphorylations, from S_0 to S_1 and from S_1 to S_2. The intermediate complexes are X_1, X_2, Y_1 and Y_2 (Fig. 3.2c).

$$\mathrm{S_0 + E_1} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftarrows}} \mathrm{X_1} \xrightarrow{\mathrm{k_3}} \mathrm{S_1 + E_1}$$
$$\mathrm{S_1 + F_1} \underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftarrows}} \mathrm{Y_1} \xrightarrow{\mathrm{k_6}} \mathrm{S_0 + F_1}$$
$$\mathrm{S_1 + E_2} \underset{\mathrm{k_8}}{\overset{\mathrm{k_7}}{\rightleftarrows}} \mathrm{X_2} \xrightarrow{\mathrm{k_9}} \mathrm{S_2 + E_2}$$
$$\mathrm{S_2 + F_2} \underset{\mathrm{k_{11}}}{\overset{\mathrm{k_{10}}}{\rightleftarrows}} \mathrm{Y_2} \xrightarrow{\mathrm{k_{12}}} \mathrm{S_1 + F_2}$$

$$\frac{\mathrm{d}S_0}{\mathrm{d}t} = k_2X_1 + k_6Y_1 - k_1S_0E_1$$
$$\frac{\mathrm{d}S_1}{\mathrm{d}t} = k_3X_1 + k_5Y_1 + k_8X_2 + k_{12}Y_2 - (k_4F_1 + k_7E_2)S_1$$
$$\frac{\mathrm{d}S_2}{\mathrm{d}t} = k_9X_2 + k_{11}Y_2 - k_{10}S_2F_2$$
$$\frac{\mathrm{d}X_1}{\mathrm{d}t} = k_1S_0E_1 - (k_2 + k_3)X_1$$
$$\frac{\mathrm{d}Y_1}{\mathrm{d}t} = k_4S_1F_1 - (k_5 + k_6)Y_1$$
$$\frac{\mathrm{d}X_2}{\mathrm{d}t} = k_7S_1E_2 - (k_8 + k_9)X_2$$
$$\frac{\mathrm{d}Y_2}{\mathrm{d}t} = k_{10}S_2F_2 - (k_{11} + k_{12})Y_2$$

with conservation laws $E_{1T} = E_1 + X_1$, $F_{1T} = F_1 + Y_1$, $E_{2T} = E_2 + X_2$, $F_{2T} = F_2 + Y_2$ and $S_T = S_0 + S_1 + S_2 + X_1 + Y_1 + X_2 + Y_2$.

Modification of two substrates

There are two distinct substrates S_0 and P_0, phosphorylated by kinase E to S_1 and P_1, respectively. Each has a phosphatase, F_1 and F_2, respectively. X_1, X_2, Y_1 and Y_2 are intermediate complexes. (Fig. 3.2d).

$$
\begin{aligned}
&\mathrm{S_0 + E} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{X_1} \xrightarrow{\mathrm{k_3}} \mathrm{S_1 + E} \\
&\mathrm{S_1 + F_1} \underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftharpoons}} \mathrm{Y_1} \xrightarrow{\mathrm{k_6}} \mathrm{S_0 + F_1} \\
&\mathrm{P_0 + E} \underset{\mathrm{k_8}}{\overset{\mathrm{k_7}}{\rightleftharpoons}} \mathrm{X_2} \xrightarrow{\mathrm{k_9}} \mathrm{P_1 + E} \\
&\mathrm{P_1 + F_2} \underset{\mathrm{k_{11}}}{\overset{\mathrm{k_{10}}}{\rightleftharpoons}} \mathrm{Y_2} \xrightarrow{\mathrm{k_{12}}} \mathrm{P_0 + F_2}
\end{aligned}
\qquad
\begin{aligned}
\frac{\mathrm{d}S_0}{\mathrm{d}t} &= k_2X_1 + k_6Y_1 - k_1S_0E \\
\frac{\mathrm{d}S_1}{\mathrm{d}t} &= k_3X_1 + k_5Y_1 - k_4S_1F_1 \\
\frac{\mathrm{d}P_0}{\mathrm{d}t} &= k_8X_2 + k_{12}Y_2 - k_7P_0E \\
\frac{\mathrm{d}P_1}{\mathrm{d}t} &= k_9X_2 + k_{11}Y_2 - k_{10}P_1F_2 \\
\frac{\mathrm{d}X_1}{\mathrm{d}t} &= k_1S_0E - (k_2 + k_3)X_1 \\
\frac{\mathrm{d}Y_1}{\mathrm{d}t} &= k_4S_1F_1 - (k_5 + k_6)Y_1 \\
\frac{\mathrm{d}X_2}{\mathrm{d}t} &= k_7P_0E - (k_8 + k_9)X_2 \\
\frac{\mathrm{d}Y_2}{\mathrm{d}t} &= k_{10}P_1F_2 - (k_{11} + k_{12})Y_2
\end{aligned}
$$

with conservation laws $E_T = E + X_1 + X_2$, $F_{1T} = F_1 + Y_1$, $F_{2T} = F_2 + Y_2$, $S_T = S_0 + S_1 + X_1 + Y_1$ and $P_T = P_0 + P_1 + X_2 + Y_2$.

Two-layer cascade

There is a one-site modification from S_0 to S_1, with kinase E and phosphatase F_1. Then, S_1 acts as kinase to modify the substrate P_0 into P_1, which has phosphatase F_2. X_1, X_2, X_3, Y_1 and Y_2 are intermediate complexes (Fig. 3.2e).

$$
\begin{aligned}
&\mathrm{S_0 + E} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{X_1} \xrightarrow{\mathrm{k_3}} \mathrm{S_1 + E} \\
&\mathrm{S_1 + F_1} \underset{\mathrm{k_5}}{\overset{\mathrm{k_4}}{\rightleftharpoons}} \mathrm{Y_1} \xrightarrow{\mathrm{k_6}} \mathrm{S_0 + F_1} \\
&\mathrm{P_0 + S_1} \underset{\mathrm{k_8}}{\overset{\mathrm{k_7}}{\rightleftharpoons}} \mathrm{X_2} \xrightarrow{\mathrm{k_9}} \mathrm{P_1 + S_1} \\
&\mathrm{P_1 + F_2} \underset{\mathrm{k_{11}}}{\overset{\mathrm{k_{10}}}{\rightleftharpoons}} \mathrm{Y_2} \xrightarrow{\mathrm{k_{12}}} \mathrm{P_0 + F_2}
\end{aligned}
\qquad
\begin{aligned}
\frac{\mathrm{d}S_0}{\mathrm{d}t} &= k_2X_1 + k_6Y_1 - k_1S_0E \\
\frac{\mathrm{d}S_1}{\mathrm{d}t} &= k_3X_1 + k_5Y_1 + k_8X_2 + k_9Y_2 - (k_4F_1 + k_7P_0)S_1 \\
\frac{\mathrm{d}P_0}{\mathrm{d}t} &= k_8X_1 + k_{12}Y_2 - k_7P_0S_1 \\
\frac{\mathrm{d}P_1}{\mathrm{d}t} &= k_9X_2 + k_{11}Y_2 - k_{10}P_1F_2 \\
\frac{\mathrm{d}X_1}{\mathrm{d}t} &= k_1S_0E - (k_2 + k_3)X_1 \\
\frac{\mathrm{d}Y_1}{\mathrm{d}t} &= k_4S_1F_1 - (k_5 + k_6)Y_1 \\
\frac{\mathrm{d}X_2}{\mathrm{d}t} &= k_7P_0S_1 - (k_8 + k_9)X_2 \\
\frac{\mathrm{d}Y_2}{\mathrm{d}t} &= k_{10}P_1F_2 - (k_{11} + k_{12})Y_2
\end{aligned}
$$

with conservation laws $E_T = E + X_1$, $F_{1T} = F_1 + Y_1$, $F_{2T} = F_2 + Y_2$, $S_T = S_0 + S_1 + X_1 + Y_1 + X_2$ and $P_T = P_0 + P_1 + X_2 + Y_2$.

3.2 Method

Focusing on the injectivity of the determining function, it is possible to assess if the system has more than one zero. Consequently, there is information on the capacity of the underlying motif for multistationarity. If the polynomial map is S-injective, there can be at most one positive steady state. Otherwise, there may exist rate constants that allow for multistationarity.

In order to analyse some motifs, a method based on the algorithm in [6] was implemented using the software *Mathematica*, systematically applying the criteria in Theorem 1 and Proposition 1.[2] If these were inconclusive, we resorted to the CRNT Toolbox.

The proofs of the results can be found in [6].

3.2.1 Injectivity

We aim to determine the injectivity of function f_κ with respect to S, despite of the value of the rate constants κ. Therefore, the family of functions in study is the following

$$\mathscr{F}_{A,V} = \left\{ f_\kappa = A\left(\kappa \circ x^V\right) : \kappa \in \mathbb{P}^r \right\}. \tag{3.1}$$

This set of functions is adequate for the application of the Determinant Criterion. The relevant stoichiometric subspace in the context of chemical reactions is $S = Im(A)$, which will be considered throughout the text. If the family is S-injective, the chemical network has, at most, one positive steady state. Otherwise, it is not possible to conclude multistationarity: there may exist $x, y \in \mathbb{P}^n$, with $x - y \in S$ but $f_\kappa(x) = f_\kappa(y) \neq 0$.

The relevant information in A is in its kernel, therefore let $\tilde{A} \in \mathbb{R}^{s\times r}$ such that $\ker(A) = \ker\left(\tilde{A}\right)$ and $\tilde{A} = [\mathrm{Id}_s | A_1]$. It is possible to swap the columns of $\tilde{A}$ and V, and entries of κ simultaneously to obtain such a matrix, without influencing the dynamics they represent. Using the structure,

$$\begin{aligned} 0 = \tilde{A}(\kappa \circ x^V) &\iff \begin{pmatrix} k_1 x^{v_1} \\ \vdots \\ k_s x^{v_s} \end{pmatrix} = -A_1 \begin{pmatrix} k_{s+1} x^{v_{s+1}} \\ \vdots \\ k_r x^{v_r} \end{pmatrix} \\ &\iff \begin{pmatrix} k_1 \\ \vdots \\ k_s \end{pmatrix} = - \begin{pmatrix} x^{-v_1} \\ \vdots \\ x^{-v_s} \end{pmatrix} \circ A_1 \begin{pmatrix} k_{s+1} x^{v_{s+1}} \\ \vdots \\ k_r x^{v_r} \end{pmatrix} =: g_{\hat{\kappa}}. \end{aligned}$$

A new family of functions arises

$$\mathscr{G}_{A,V} = \left\{ g_{\hat{\kappa}} : \hat{\kappa} \in \mathbb{P}^{r-s} \right\}.$$

If $g_{\hat{\kappa}}$ is S-injective, then f_κ has at most one positive zero. However, $g_{\hat{\kappa}}$ does not have the matrix representation needed for the Determinant Criterion. As $g_{\hat{\kappa}}$ is a generalised polynomial map, it is possible to calculate A' and V' such that $g_{\hat{\kappa}}(x) = f_\eta(x) = A'\left(\eta \circ x^{V'}\right)$.

[2] The code is available at http://www.mat.uc.pt/~mat1224/Tese/.

These matrices are calculated directly from $g_{\hat{\kappa}}$, with V' including the exponents of the monomials in x in its columns, and A' the coefficients. Note that the same $\hat{\kappa}_i$ can be the coefficient for more than one monomial, which forces the introduction of a new parameter $\eta \in \mathbb{P}^{r'}$, with r' the number of distinct monomials of $g_{\hat{\kappa}}$, and

$$\mathscr{G}_{A,V} = \left\{ f_\eta \in \mathscr{F}_{A',V'} : \eta \in \mathbb{P}^{r'},\, \eta_i = \eta_j \text{ if } i,j \in I_k, \text{ for some } k \in [q] \right\}, \tag{3.2}$$

with $I_1 \cup \ldots I_q = [r']$ an adequate partition.

Given that $\mathscr{G}_{A,V} \subset \mathscr{F}_{A',V'}$, it is possible to apply the Determinant Criterion to A' and V'. If the family $\mathscr{F}_{A',V'}$ is S-injective, then f_κ cannot have more than one positive zero, but the reverse conclusion is not immediate as a consequence of the strict inclusion.

To illustrate the previous notions, consider the following example.

Example 4. Let

$$\tilde{A} = \begin{pmatrix} 1 & 0 & -1 & -1 \\ 0 & 1 & -1 & 0 \end{pmatrix},\ V = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 2 \end{pmatrix} \Longrightarrow A_1 = \begin{pmatrix} -1 & -1 \\ -1 & 0 \end{pmatrix}. \tag{3.3}$$

It is possible to determine $g_{\hat{\kappa}}$,

$$g_{\hat{\kappa}} = -\begin{pmatrix} x_1^{-1} \\ x_1^{-1}x_2^{-1} \end{pmatrix} \circ A_1 \begin{pmatrix} k_3 x_2 \\ k_4 x_2^2 \end{pmatrix} = \begin{pmatrix} k_3 x_1^{-1} x_2 + k_4 x_1^{-1} x_2^2 \\ k_3 x_1^{-1}. \end{pmatrix}$$

Consequently, there are three distinct monomials, $\eta = (\eta_1, \eta_2, \eta_3)$,

$$A' = \begin{pmatrix} 1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},\ V' = \begin{pmatrix} -1 & -1 & -1 \\ 1 & 2 & 0 \end{pmatrix},$$

and $I_1 = \{1,3\}$, $I_2 = \{2\}$, thus $g_{\hat{\kappa}} \in \left\{ f_\eta \in \mathscr{F}_{A',V'} : \eta \in \mathbb{P}^3, \eta_1 = \eta_3 \right\}$.

3.2.2 Multiple zeros

Assuming that $\mathscr{F}_{A',V'}$ is not S-injective, it is not possible to conclude that $\mathscr{G}_{A,V}$ is also not S-injective. Therefore, it is necessary to define a new family of functions $\mathscr{F}_{\hat{A},\hat{V}}$ with $\hat{A}$ and $\hat{V}$ defined as follows,

- $\hat{A} \in \mathbb{R}^{s \times s(r-s)}$ the block diagonal matrix whose blocks are the symmetric of the rows of A_1 and remaining entries equal to zero.
- $\hat{V} \in \mathbb{R}^{n \times s(r-s)}$ with l-th column given by

$$\hat{v}^l = v^{j+s} - v^i, \text{ if } l = (i-1)(r-s) + j, \text{ with } i \in [s], j \in [r-s].$$

The definition becomes clear with Example 5.

Example 5. Considering matrices (3.3), from Example 4,

$$\hat{A} = \begin{pmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \end{pmatrix}, \quad \hat{V} = \begin{pmatrix} -1 & -1 & -1 & -1 \\ 1 & 2 & 0 & 1 \end{pmatrix}.$$

Therefore, $\mathscr{G}_{A,V} \subset \mathscr{F}_{A',V'} \subset \mathscr{F}_{\hat{A},\hat{V}}$, and assuming that $\mathscr{F}_{A',V'}$ is not S-injective implies that $\mathscr{F}_{\hat{A},\hat{V}}$ is not S-injective as well. Therefore, there exist $\eta \in \mathbb{P}^{s(r-s)}$, distinct $x, y \in \mathbb{P}^n$ such that $x - y \in S$ and $f_\eta(x) = f_\eta(y)$.

Proposition 2 gives sufficient and necessary conditions on η for $f_\eta \in \mathscr{F}_{\hat{A},\hat{V}}$ to belong in $\mathscr{G}_{A,V}$.

Proposition 2. *Assuming that $f_\eta \in \mathscr{F}_{\hat{A},\hat{V}}$, then $f_\eta \in \mathscr{G}_{A,V}$ if and only if*

$$\eta_{j+(b-1)(r-s)} = \eta_{j+(c-1)(r-s)}, \tag{3.4}$$

for $j \in [r-s]$, $b, c \in [s]$ and $b, c \in \alpha_j$, with α_j the support of the j-th column of A_1.

To conclude that f_κ has multiple S-zeros it is sufficient to prove that there exist $\hat{\kappa} \in \mathbb{P}^{r-s}$, distinct $x, y \in \mathbb{P}^n$, $x - y \in S$, such that

$$g_{\hat{\kappa}}(x) = g_{\hat{\kappa}}(y) \tag{3.5}$$

$$g_{\hat{\kappa}}(x) > 0 \tag{3.6}$$

If 3.5 and 3.6 are verified, then $\kappa := (g_{\hat{\kappa}}(x), \hat{\kappa}) \in \mathbb{P}^r$ is a constant rate vector for which $f_\kappa(x) = f_\kappa(y) = 0$ as a consequence of the definition of $g_{\hat{\kappa}}$ and κ.

As $f_\eta(x) = f_\eta(y)$ and $f_\eta \in \mathscr{F}_{\hat{A},\hat{V}}$, we want to modify η to another parameter $\hat{\eta}$ in order to have $f_{\hat{\eta}} \in \mathscr{G}_{A,V}$ and $f_{\hat{\eta}}(x) = f_{\hat{\eta}}(y)$. Considering $\varepsilon \in \mathbb{P}^s$, $\hat{\varepsilon} := (\varepsilon_1, \ldots, \varepsilon_1, \ldots, \varepsilon_s, \ldots, \varepsilon_s) \in \mathbb{P}^{s(r-s)}$ and $\hat{\eta} := \hat{\varepsilon} \circ \eta$, then

$$f_{\hat{\eta}} = f_{\hat{\varepsilon} \circ \eta} = \varepsilon \circ f_\eta \implies f_{\hat{\eta}}(x) = f_{\hat{\eta}}(y).$$

Consequently, to prove that $\mathscr{G}_{A,V}$ is not S-injective, it is sufficient to define a modifying parameter ε such that $\hat{\eta}$ satisfies the assumptions of Proposition 2. To that end, Proposition 3 gives a sufficient condition on A_1 for the existence of such ε.

Proposition 3. *Let $\eta \in \mathbb{P}^{s(r-s)}$. Assuming that for each $j \in [r-s]$, there is an index $l_j \in [s]$ such that $\alpha_j \cap \alpha_{j'} = \{l_j\} = \{l_{j'}\}$ for all j, j' such that the cardinality of $\alpha_j, \alpha_{j'}$ is at least two and $\alpha_j \cap \alpha_{j'} \neq \emptyset$. Then, there exists $\varepsilon \in \mathbb{P}^s$ with $\hat{\varepsilon} \circ \eta$ satisfying condition (3.4).*

Remark 5. The assumption in Proposition 3 can be described in the following way: for each column j of A_1 with at least two non-zero entries, if its support intersects the support of another such column, then the intersection has one element. If the columns of A_1 have disjoint supports, the hypothesis is trivially verified.

Proposition 4 gives a sufficient condition for 3.6.

Proposition 4. *If all the entries of A_1 are non-positive and each row has at least one negative element, then $g_{\hat{\kappa}}$ is positive for all $\hat{\kappa} \in \mathbb{P}^{r-s}$.*

Let a_{ij} be the entries of A_1, then

$$g_{\hat{\kappa}}(x) = \begin{pmatrix} x^{-v_1}\sum_{j=1}^{r-s}(-a_{1j})x^{v_{s+j}}k_{s+j} \\ \vdots \\ x^{-v_s}\sum_{j=1}^{r-s}(-a_{sj})x^{v_{s+j}}k_{s+j} \end{pmatrix},$$

all the entries are positive when the assumptions in Proposition 4 are verified.

To sum up, assuming that $\mathscr{F}_{A',V'}$ is not S-injective, the following conditions on A_1 are sufficient to conclude that $\mathscr{G}_{A,V}$ is not S-injective, proving that $\mathscr{F}_{A,V}$ has multiple S-zeros.

(a) For each column of A_1 with at least two non-zero elements, if its support intersects the support of another such column, the intersection has a single point.

(b) All entries must be non-positive, with each row having at least one negative element.

3.2.3 Algorithm

A method based on Theorem 1 and Proposition 1, applied to the families of functions defined in Chapter 3, can be used to identify the uniqueness of steady state or assess the capacity for multistationarity. The algorithmic translation of the method consists of the following steps:

1. Apply the Determinant Criterion to A and V, with $S = Im(A)$. If it concludes S-injectivity, stop.
2. Calculate A' and V' such that $g_{\hat{\kappa}} = f_\eta \in \mathscr{F}_{A',V'}$, and apply the Determinant Criterion. If it concludes S-injectivity, stop.
3. Check sufficient conditions (a) and (b) in Subsection 3.2.2. If verified, there are multiple S-zeros for some κ, stop.
4. Permute the columns of A, and corresponding columns of V, to obtain a new set of s linearly independent columns as the first of A. Return to step 1.
5. Apply the Jacobian Criterion to the polynomial function, after reduction using conservation laws. If it concludes S-injectivity, stop.
6. Use CRNT Toolbox.

Since only the first s columns of A and V are relevant for the Determinant Criterion, in case the test is inconclusive, it is required to permute the columns to test all possible combinations. It is sufficient to consider all sets of s linearly independent columns.

3.2.4 Examples

To clarify the application of the algorithm described in Section 3.2.3, consider the following examples. In Example 6, it is possible to conclude S-injectivity by applying the Determinant Criterion. In Example 7, the Determinant Criterion shows that $\mathscr{F}_{A,V}$ is not S-injective, but when calculating $g_{\hat{\kappa}}$, A' and V', we prove the uniqueness of the steady state. For Example 8, $\mathscr{F}_{A',V'}$ is not S-injective and A_1 satisfies conditions (a) and (b), proving that $\mathscr{F}_{A,V}$ has multiple S-zeros.

Example 6. Consider the *cyclic model* (Fig. 3.1f), with S constant and included in constant k_1. Then, the following matrices define the system,

$$A = \begin{pmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{pmatrix}, \; V = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}.$$

Then, $Z = (1,1,1)$ and, since $rank(A) = 2$, choosing the last two rows of A for $\tilde{A}$,

$$\Gamma_{\kappa,\lambda} = \begin{pmatrix} 1 & 1 & 1 \\ k_1\lambda_1 & -k_2\lambda_2 & 0 \\ 0 & k_2\lambda_2 & -k_3\lambda_3 \end{pmatrix},$$

and $\det\left(\Gamma_{\kappa,\lambda}\right) = k_1k_2\lambda_1\lambda_2 + k_1k_3\lambda_1\lambda_3 + k_2k_3\lambda_2\lambda_3$, which is not identically zero and has the same sign for all coefficients. The conclusion is S-injectivity for all κ, and, therefore, the existence of at most one positive steady state.

Example 7. Consider the model *positive feedback* (Fig. 3.1d). Note that the numbering of the reactions does not coincide with the numbering in the ODEs system. The following matrices describe the motif

$$A = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & -1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & -1 \\ 1 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix}, V = \begin{pmatrix} 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{pmatrix}.$$

According to the Determinant Criterion, the function is not S-injective. Afterwards, matrices A' and V' are determined. Applying Gauss elimination to A, the submatrix 3×7 next to the identity matrix is

$$A_1 = \begin{pmatrix} 1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & -1 \end{pmatrix}.$$

Consequently,

$$g_{\hat{\kappa}}(x) = -\begin{pmatrix} x_2^{-1}x_6^{-1} \\ x_6^{-1} \\ x_7^{-1} \end{pmatrix} \circ A_1 \begin{pmatrix} k_4x_1x_2 \\ k_5x_2 \\ k_6x_5 \\ k_7x_3x_7 \\ k_8x_3 \\ k_9x_4x_5 \\ k_{10}x_4 \end{pmatrix} = \begin{pmatrix} -k_4x_1x_6^{-1} - k_5x_6^{-1} + k_6x_2^{-1}x_5x_6^{-1} \\ k_7x_3x_6^{-1}x_7 + k_8x_3x_6^{-1} \\ k_9x_4x_5x_7^{-1} + k_{10}x_4x_7^{-1} \end{pmatrix}.$$

Then, $g_{\hat{\kappa}} \in \mathscr{F}_{A',V'}$, with matrices

$$A' = \begin{pmatrix} -1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix}, \; V' = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ -1 & -1 & -1 & -1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 & -1 \end{pmatrix}.$$

When applying the Determinant Criterion to A' and V', S-injectivity is concluded for all κ, therefore *positive feedback* has at most one positive steady state.

Example 8. Consider the chemical network defined by the following matrices,

$$A = \begin{pmatrix} -1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}, \; V = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 & 0 & 1 & 3 & 1 \\ 0 & 1 & 1 & 3 & 0 & 0 & 0 & 0 & 2 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}.$$

The application of the Determinant Criterion leads to the conclusion that the function is not S-injective. The same conclusion is reached for the function defined by A' and V'. Therefore, it is relevant to check if the conditions in Section 3.2.2 are satisfied. Focusing on A_1,

$$A_1 = \begin{pmatrix} -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & -1 \end{pmatrix}$$

Since the supports of the columns of A_1 are disjoint, there are no positive entries and each row has at least one negative entry, there exists a rate constants vector κ such that f_κ has more than one positive zero.

3.3 Initial results

The application of the method in Section 3.2 to the motifs in Section 3.1 leads to the conclusion that most motifs do not have the capacity for more than one positive steady state.

There are some exceptions which have the capacity for multistationarity for some rate constants, namely *two-site modification* when $\{E_1 = E_2\}$ and $\{E_1 = E_2, F_1 = F_2\}$, *modification of two substrates* when $\{F_1 = F_2\}$, *two-layer cascade* when both E and S_1 act as kinases for the second phosphorylation cycle and *two-layer cascade* when $\{F_1 = F_2\}$.

Chapter 4

Compartmentalisation

Despite the trend to disregard space, many authors proposed techniques to assess its influence, as in [1, 2, 9].

To avoid considering a spatial variable and to use CRNT to describe the system, the initial approach would be to consider compartments. Eukaryotic cells have compartments, namely nucleus and cytoplasm, and it is possible that the same network is happening simultaneously in both or that the membranes separate its distinct stages. Feinberg [5] mentioned this potentiality of CRNT, with the idea of having chemical networks occurring in different cells connected by a chemical reaction representing intercellular movement. Similar experiments were conducted by Harrington et al. [9] and Alam-Nazki and Krishnan [2], the latter with different tools.

We focused on two distinct ways to introduce compartments, namely by considering the motifs in parallel or in stages. The former refers to having the same set of reactions taking place in two independent compartments, while the latter focuses on separating the reactions in two compartments. In both cases, a selection of species moves between compartments. With this approach, it is possible to describe the networks using CRNT and to apply the algorithm defined in Chapter 3.

Fig. 4.1 illustrates the stages of the study for the *two-layer cascade*. Firstly, we assess the capacity of the motifs for multistationarity (see Section 3.3). Secondly, we assume the same set of reactions is taking place in two compartments, simultaneously, not necessarily with the same rate constants. The compartments are connected through species that can move between them. Lastly, if the motif can be separated in two stages, each one is placed in one compartment, with the connecting species moving between them. The initial results in Section 3.3 act as the control group, compared with the results in compartments.

It was clear that for some motifs compartmentalisation resulted in changes in the number of steady states. Therefore, space can influence the equilibria of chemical networks.

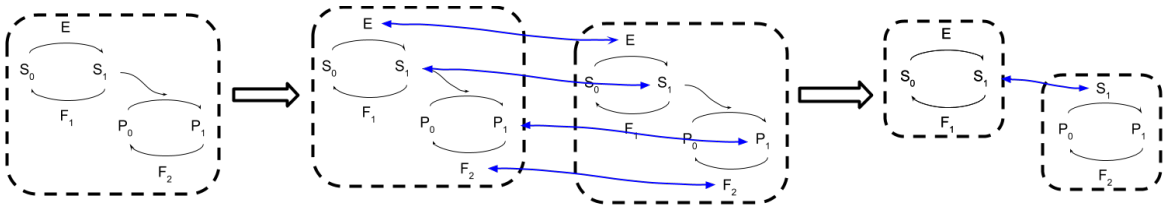


Fig. 4.1 Illustration of the stages of the analysis.

4.1 Models in parallel

Within a cell, the same species and reactions can occur simultaneously in different compartments, and there can be movement through membranes, connecting them. Therefore, the capacity for multistationarity may change when considering the reactions happening in parallel in two compartments.

The compartmentalisation is achieved by considering copies of the reactions in another compartment, indexed appropriately to distinguish their location. To emulate the movement through the barriers, it is possible to add reactions between the same species from different compartments. The system in (4.1) illustrates the reaction between X and Y happening in two compartments, with X moving between them.

$$\begin{aligned} \mathrm{X} &\longrightarrow \mathrm{Y} \\ \mathrm{X}^{\mathrm{c}} &\longrightarrow \mathrm{Y}^{\mathrm{c}} \\ \mathrm{X} &\rightleftharpoons \mathrm{X}^{\mathrm{c}} \end{aligned} \tag{4.1}$$

The change on the steady states will depend on the motifs and the species that are allowed to move between compartments. For instance, if both networks are isolated, there cannot be a change in the behaviour. As a consequence of the high number of possible shuttling species, not all sets were tested. Moreover, the intermediate species were only allowed to shuttle for the *one-site modification*, as it is one of the smallest networks.

The *negative feedback* module was only tested for one or two species, which retained the uniqueness of the steady state, while the *two-site modification* was not possible to analyse with the given method. The *two-step phosphorelay* could only be tested for one or two species, leading to the conclusion that with $\{X_1, X_2^*\}$, $\{X_1^*, F_2\}$, $\{X_2, E\}$, $\{E, F_1\}$, $\{E, F_2\}$ or $\{F_1, F_2\}$ shuttling there is the capacity for multistationarity.

The motifs that had the capacity for multistationarity, namely *modification of two substrates*, with $F_1 = F_2$, *two-layer cascade*, with E acting as a kinase or with $F_1 = F_2$, kept their multistationarity, regardless of the shuttling species, with at most 3 species shuttling.

Some networks were not affected by this approach, and continued to display at most one positive steady state, namely *coherent feedforward*, *incoherent feedforward* and *cyclic module*, for all sets with at most three species.

Positive feedback, *one-site modification*, *modification of two substrates* and *two-layer cascade* have the most interesting results, with a clear change on the number of steady states.

From the results it is clear that one species shuttling is not enough to change the capacity for multistationarity. In addition, adding elements to a set that leads to multistationarity may result in the loss of that property.

Positive feedback

Table 4.1 summarises the results. The original network has at most one positive steady state, which does not change when considering one shuttling species in parallel. The sets marked with $(*)$ have the capacity for multistationarity for some weakly monotonic kinetics (an increase in the concentration

of a reactant implies an increase on the speed of the reaction), not necessarily mass-action kinetics. However, the networks are not injective with mass-action.

Sets that include both the active and inactive forms of a species result in multistationarity. This "continuity" is only visible in this motif.

Table 4.1 Capacity for multistationarity of *positive feedback* in parallel.

Species	**One steady state**	**Multistationarity**
1	All	None
2	$\{X, Y\}$, $\{X, Y^*\}$, $\{X, R\}$, $\{X, R^*\}$, $\{X^*, Y\}$, $\{X^*, Y^*\}$, $\{X^*, R\}$, $\{X^*, R^*\}$, $\{Y, R\}$, $\{Y, R^*\}$, $\{Y^*, R\}$, $\{Y^*, R^*\}$	$\{X, X^*\}^*$, $\{Y, Y^*\}$, $\{R, R^*\}$
3	$\{X, Y, R\}$, $\{X, Y, R^*\}$, $\{X, Y^*, R\}$, $\{X, Y^*, R^*\}$, $\{X^*, Y, R\}$, $\{X^*, Y, R^*\}$, $\{X^*, Y^*, R\}$, $\{X^*, Y^*, R^*\}$	$\{X, X^*, Y\}^*$, $\{X, X^*, Y^*\}^*$, $\{X, X^*, R\}^*$, $\{X, X^*, R^*\}^*$, $\{X, Y, Y^*\}$, $\{X, R, R^*\}$, $\{X^*, Y, Y^*\}$, $\{X^*, R, R^*\}$, $\{Y, Y^*, R\}$, $\{Y, Y^*, R^*\}$, $\{Y, R, R^*\}$, $\{Y^*, R, R^*\}$

One-site modification

Table 4.2 summarises the results. The network displays a unique steady state originally, and the same holds when only one species shuttles. However, there are specific pairs that result in multistationarity.

With three species shuttling, if the set includes one of the following, then it has multistationarity: $\{S_0,S_1\}$, $\{S_0,Y\}$, $\{S_1,X\}$, $\{X,Y\}$.

With the increase in the number of species shuttling, there are more sets that result in multistationarity. However, adding a species to such a set may result in the loss of such a capacity. For example, $\{E,F\}$ results in multistationarity but adding other species does not.

Table 4.2 Capacity for multistationarity of *one-site modification* in parallel.

Species	**One steady state**	**Multistationarity**
1	All	None
2	$\{S_0,S_1\}$, $\{S_0,E\}$, $\{S_0,F\}$, $\{S_0,X\}$, $\{S_1,E\}$, $\{S_1,F\}$, $\{S_1,Y\}$, $\{E,X\}$, $\{E,Y\}$, $\{F,X\}$, $\{F,Y\}$	$\{S_0,Y\}$, $\{S_1,X\}$, $\{E,F\}$, $\{X,Y\}$
3	$\{S_0,E,F\}$, $\{S_0,F,X\}$, $\{S_1,E,F\}$, $\{S_1,E,Y\}$, $\{E,F,X\}$, $\{E,F,Y\}$	$\{S_0,S_1,E\}$, $\{S_0,S_1,F\}$, $\{S_0,S_1,X\}$, $\{S_0,S_1,Y\}$ $\{S_0,E,X\}$, $\{S_0,E,Y\}$, $\{S_0,F,Y\}$, $\{S_0,X,Y\}$, $\{S_1,E,X\}$, $\{S_1,F,X\}$, $\{S_1,F,Y\}$, $\{S_1,X,Y\}$, $\{E,X,Y\}$, $\{F,X,Y\}$
4	$\{S_0,S_1,X,Y\}$, $\{S_0,F,X,Y\}$, $\{S_1,E,X,Y\}$, $\{E,F,X,Y\}$	$\{S_0,S_1,E,F\}$, $\{S_0,S_1,E,X\}$, $\{S_0,S_1,E,Y\}$, $\{S_0,S_1,F,X\}$, $\{S_0,S_1,F,Y\}$, $\{S_0,E,F,X\}$, $\{S_0,E,F,Y\}$, $\{S_0,E,X,Y\}$, $\{S_1,E,F,X\}$, $\{S_1,E,F,Y\}$, $\{S_1,F,X,Y\}$
5	None	All

Modification of two substrates

Table 4.3 summarises the results. The original network has at most one steady state, as well as the network in parallel with one species moving.

Note that sets with three elements that include $\{S_0, S_1\}$ or $\{P_0, P_1\}$ all have multistationarity.

If we consider the sharing of enzymes, namely with $F_1 = F_2$, the original network has multiple steady states, which does not change in parallel with at most three species shuttling.

Table 4.3 Capacity for multistationarity of *modification of two substrates* in parallel.

Species	One steady state	Multistationarity
1	All	None
2	$\{S_0, S_1\}$, $\{S_0, P_0\}$, $\{S_0, P_1\}$,$\{S_0, E\}$, $\{S_0, F_1\}$,$\{S_1, P_0\}$,$\{S_1, E\}$,$\{S_1, F_1\}$, $\{S_1, F_2\}$,$\{P_0, P_1\}$,$\{P_0, E\}$,$\{P_0, F_2\}$, $\{P_1, E\}$,$\{P_1, F_1\}$,$\{P_1, F_2\}$	$\{S_0, F_2\}$, $\{S_1, P_1\}$, $\{P_0, F_1\}$, $\{E, F_1\}$, $\{E, F_2\}$,$\{F_1, F_2\}$
3	$\{S_0, P_0, E\}$, $\{S_0, P_0, F_1\}$, $\{S_0, P_0, F_2\}$, $\{S_0, P_1, E\}$, $\{S_0, P_1, F_1\}$, $\{S_0, P_1, F_2\}$, $\{S_0, E, F_1\}$, $\{S_1, P_0, E\}$, $\{S_1, P_0, F_1\}$, $\{S_1, P_0, F_2\}$, $\{S_1, P_1, E\}$, $\{S_1, E, F_1\}$, $\{S_1, F_1, F_2\}$, $\{P_0, E, F_2\}$, $\{P_1, E, F_2\}$, $\{P_1, F_1, F_2\}$	$\{S_0, S_1, P_0\}$, $\{S_0, S_1, P_1\}$, $\{S_0, S_1, E\}$, $\{S_0, S_1, F_1\}$, $\{S_0, S_1, F_2\}$, $\{S_0, P_0, P_1\}$, $\{S_0, E, F_2\}$, $\{S_0, F_1, F_2\}$, $\{S_1, P_0, P_1\}$, $\{S_1, P_1, F_1\}$, $\{S_1, P_1, F_2\}$, $\{S_1, E, F_2\}$, $\{P_0, P_1, E\}$, $\{P_0, P_1, F_1\}$, $\{P_0, P_1, F_2\}$, $\{P_0, E, F_1\}$, $\{P_0, F_1, F_2\}$, $\{P_1, E, F_1\}$, $\{E, F_1, F_2\}$

Two-layer cascade

Table 4.4 summarises the results. The original network has a unique steady state, and in parallel with one species shuttling it also holds.

It is interesting that sets with three species including $\{P_0, P_1\}$ or $\{S_0, P_0\}$ have multistationarity.

If enzymes are shared, namely $F_1 = F_2$ or E acting as a kinase for P_0, the networks still have the capacity for multistationarity, with at most three species shuttling.

Table 4.4 Capacity for multistationarity of *two-layer cascade* in parallel.

Species	One steady state	Multistationarity
1	All	None
2	$\{S_0, S_1\}$, $\{S_0, P_1\}$, $\{S_0, E\}$, $\{S_0, F_1\}$, $\{S_1, P_0\}$, $\{S_1, P_1\}$, $\{S_1, E\}$, $\{S_1, F_1\}$, $\{P_0, P_1\}$, $\{P_0, E\}$, $\{P_0, F_1\}$, $\{P_0, F_2\}$, $\{P_1, F_1\}$, $\{P_1, F_2\}$, $\{E, F_2\}$	$\{S_0, P_0\}$, $\{S_0, F_2\}$, $\{S_1, F_2\}$, $\{P_1, E\}$, $\{E, F_1\}$, $\{F_1, F_2\}$
3	$\{S_0, S_1, P_1\}$, $\{S_0, P_1, E\}$, $\{S_0, P_1, F_1\}$, $\{S_0, P_1, F_2\}$, $\{S_0, E, F_1\}$, $\{S_1, P_0, E\}$, $\{S_1, P_0, F_1\}$, $\{S_1, P_0, F_2\}$, $\{S_1, P_1, E\}$, $\{S_1, P_1, F_1\}$, $\{S_1, P_1, F_2\}$, $\{S_1, E, F_1\}$, $\{P_0, E, F_2\}$, $\{P_0, F_1, F_2\}$, $\{P_1, F_1, F_2\}$	$\{S_0, S_1, P_0\}$, $\{S_0, S_1, E\}$, $\{S_0, S_1, F_1\}$, $\{S_0, S_1, F_2\}$, $\{S_0, P_0, P_1\}$, $\{S_0, P_0, E\}$, $\{S_0, P_0, F_1\}$, $\{S_0, P_0, F_2\}$, $\{S_0, E, F_2\}$, $\{S_0, F_1, F_2\}$, $\{S_1, P_0, P_1\}$, $\{S_1, E, F_2\}$, $\{S_1, F_1, F_2\}$, $\{P_0, P_1, E\}$, $\{P_0, P_1, F_1\}$, $\{P_0, P_1, F_2\}$, $\{P_0, E, F_1\}$, $\{P_1, E, F_1\}$, $\{P_1, E, F_2\}$, $\{E, F_1, F_2\}$

4.2 Models with stages

There exists a different paradigm of compartmentalisation, in which distinct stages of a network take place in independent compartments, with the relevant species shuttling between them. This analysis was only considered for the motifs where stages are clearly identified, namely *two-step phosphorelay*, *two-site modification* and *two-layer cascade*. Table 4.5 includes a summary of the results.

Two-step phosphorelay

The network includes the following reactions

$$\begin{aligned}
X_1 + E &\rightleftharpoons Y_1 \longrightarrow X_1^* + E \\
X_1^* + F_1 &\rightleftharpoons Y_2 \longrightarrow X_1 + F_1 \\
X_2^c + X_1^{*c} &\longrightarrow X_2^{*c} + X_1^c \\
X_2^{*c} + F_2^c &\rightleftharpoons Y_3^c \longrightarrow X_2^c + F_2^c
\end{aligned}$$

$$\begin{aligned}
X_1^* &\rightleftharpoons X_1^{*c} \\
\{X_1 &\rightleftharpoons X_1^c\}
\end{aligned}$$

The original motif continues to have a unique steady state in stages when X_1^* and X_1 shuttle, but has no positive steady states when only X_1^* shuttles.

Two-site modification

The network in stages is described by the following reactions

$$\begin{aligned}
S_0 + E_1 &\rightleftharpoons X_1 \longrightarrow S_1 + E_1 \\
S_1 + F_1 &\rightleftharpoons Y_1 \longrightarrow S_0 + F_1 \\
S_1^c + E_2^c &\rightleftharpoons X_2^c \longrightarrow S_2^c + E_2^c \\
S_2^c + F_2^c &\rightleftharpoons Y_2^c \longrightarrow S_1^c + F_2^c
\end{aligned}$$

$$\begin{aligned}
S_1 &\rightleftharpoons S_1^c \\
\{E &\rightleftharpoons E^c\} \\
\{F &\rightleftharpoons F^c\}
\end{aligned}$$

S_1 is the species that connects both stages, and when it shuttles the network continues to have at most one positive steady state.

If $E_1 = E_2 =: E$, the original motif has the capacity for multistationarity, but it is lost when in stages with S_1 shuttling. However, if $\{S_1, E\}$ shuttle there is multistationarity.

In addition, if $E_1 = E_2 =: E$ and $F_1 = F_2 =: F$, the motif retains its multistationarity if $\{S_1, E\}$, $\{S_1, F\}$ or $\{S_1, E, F\}$ shuttle, while losing this capacity when only S_1 shuttles.

Two-layer cascade

The network in two stages comprises the following reactions

$$\begin{aligned}
S_0 + E &\rightleftharpoons X_1 \longrightarrow S_1 + E \\
S_1 + F_1 &\rightleftharpoons Y_1 \longrightarrow S_0 + F_1 \\
S_1^c + P_0^c &\rightleftharpoons X_2^c \longrightarrow S_1^c + P_1^c \\
P_1^c + F_2^c &\rightleftharpoons Y_2^c \longrightarrow P_0^c + F_2^c
\end{aligned}$$

$$\begin{aligned}
S_1 &\rightleftharpoons S_1^c \\
\{E &\rightleftharpoons E^c\} \\
\{F &\rightleftharpoons F^c\}
\end{aligned}$$

If S_1 shuttles, the uniqueness of the steady state remains.

If both E and S_1 act as kinases for the second phosphorylation cycle, the network has the capacity for multistationarity, but in stages when S_1 and E shuttle there is at most one steady state.

If $F_1 = F_2 =: F$ the network has the capacity for multiple steady states, while there is uniqueness of equilibrium when S_1 shuttles and multiple steady states when $\{S_1, F\}$ shuttle.

Table 4.5 Results for the motifs in stages.

Motif	Sharing	Original	Shuttling	Compartments
Two-step phosphorelay		Injective	X_1^*	No positive zeros
			X_1^*, X_1	Injective
Two-site modification		Injective	S_1	(*)
	E	Multiple zeros, for some rates	S_1	Injective
			S_1, E	Multiple zeros for some rates
	E,F	Multiple zeros, for some rates	S_1	Injective
			S_1, E	Multiple zeros, for some rates
			S_1, F	Multiple zeros, for some rates
			S_1, E, F	Multiple zeros, for some rates
Two-layer cascade		Injective	S_1	Injective
	E	Multiple zeros, for some rates	S_1, E	Injective
	F	Multiples zeros, for some rates	S_1	Injective
			S_1, F	Multiple zeros, for some rates

Remark 6. In Table 4.5, (*) means that the function is not S-injective but there are no multiple zeros.

4.3 Summary

The results suggest that considering compartments can have an influence on the number of steady states, may it be by considering reactions in parallel or in stages, in two separate compartments. Table

4.6 includes a summary of the results, with a check for the motifs that exhibit multistationarity under specific conditions. The cells marked with '–' were not tested with that approach.

For the motifs in study, when in parallel there is no loss of multistationarity and some motifs, *two-step phosphorelay*, *positive feedback*, *one-site modification*, *modification of two substrates* and *two-layer cascade*, with certain sets of shuttling species, gain multistationarity.

Considering the motifs with stages, there is loss of multistationarity for the *two-site modification* when sharing enzymes, and for the *two-layer cascade* when S_1 and E both act as kinases, with distinct and equal phosphatases.

Table 4.6 Capacity for multistationarity of the motifs with compartments.

Module	Sharing	Original	Parallel	Stages
Coherent feedforward				–
Incoherent feedforward I				–
Incoherent feedforward II				–
Positive feedback			✓	–
Negative feedback				–
Cyclic module				–
Two-step phosphorelay			✓	
One-site modification			✓	–
Two-site modification			–	
	E	✓	–	✓
	E,F	✓	–	✓
Modification of two substrates			✓	–
	F	✓	✓	–
Two-layer cascade			✓	
	E	✓	✓	
	F	✓	✓	✓

Chapter 5

Diffusion-driven instability

The results in Chapters 3 and 4 focus on a temporal model for chemical reaction networks, the latter includes compartments as a subterfuge to introduce space without considering a spatial domain.

To continue the analysis of the motifs and of the influence of space on their steady states, the models should include space. To that end, we propose new models based on the ODEs system in Chapter 3, by adding diffusion terms. Thus, the focus is on systems of Partial Differential Equations (PDEs) in a one-dimensional domain, with no-flux boundary conditions.

We intend to assess if diffusion can influence the steady states, using *Linear Stability Analysis* to look for solutions that are not spatially homogeneous. Under specific conditions for diffusion-driven instability, chemical networks can show spatial patterns, proving the influence of space in their equilibria. The starting point are the steady states of the original ODEs systems, which are homogeneous solutions of the reaction-diffusion systems. We want to prove that these are stable solutions without diffusion, and unstable with diffusion, which can result in interesting spatial behaviour.

5.1 Linear Stability Analysis

Diffusion was seen as a stabilising mechanism, until Turing [16] suggested that, under specific conditions, reaction-diffusion systems could reach a spatially heterogeneous steady state. In a coupled reaction-diffusion system with two chemical species, with one acting as an inhibitor and the other as an activator, with drastically different diffusion coefficients, the system could generate a pattern. The context of such study was the formation of biological patterns, such as the prints on animal skin. Nowadays, reaction-diffusion theory is a field of study on its own.

The interest in diffusion-driven instability requires that there is a homogeneous steady state in the absence of diffusion, stable for small spatial perturbations, but which becomes unstable in the presence of diffusion. Therefore, we focus on *linear stability* of homogeneous steady states. The analysis presented follows [12].

Consider a system of chemical reactions, with concentrations $x(t)$ at time t, given by the differential equations and boundary conditions

$$\begin{aligned} \frac{\partial x}{\partial t} &= f(x) + D\nabla^2 x, \text{ in } \Omega \\ \frac{\partial x}{\partial \eta} &= 0, \text{ on } \partial\Omega \end{aligned} \tag{5.1}$$

with f the nonlinear reaction kinetics and D the diagonal matrix with the diffusion coefficients, η the unit exterior normal vector and $\Omega = [0,a]$ the domain, a the length of the one-dimensional domain.

Suppose that x_0 is a positive homogeneous steady state of $f(x) = 0$. We want conditions for the local stability of this steady state. To that end, we linearise around the steady state, considering

$$w = x - x_0. \tag{5.2}$$

For $|w|$ small, the value of $f(x)$ can be linearly approximated,

$$f(x) = f(x_0 + w) = f(x_0) + J_f(x_0)w, \tag{5.3}$$

where J_f represents the Jacobian matrix of f.

Since $f(x_0) = 0$, using (5.3),

$$\frac{\partial w}{\partial t} = \frac{\partial x}{\partial t} = f(x) = J_f(x_0)w, \tag{5.4}$$

with $B := J_f(x_0)$ the *stability matrix*.

We are interested in solutions of the eigenvalue problem $\frac{\partial w}{\partial t} = Bw$, with no-flux boundary conditions. Since the domain is one-dimensional, $w = \alpha e^{\lambda t}$, with λ an eigenvalue. As a consequence, if all the eigenvalues of B have negative real part, $Re(\lambda) < 0$, x_0 is a stable solution.

To prove diffusion-driven instability, we need to prove that the solution for the following system, with no-flux boundary conditions, is unstable.

$$\frac{\partial w}{\partial t} = Bw + D\nabla^2 w. \tag{5.5}$$

Let $w(\theta)$ be time independent solutions of the eigenvalue problem, with k the eigenvalues.

$$\nabla^2 w + k^2 w = 0 \tag{5.6}$$

As the domain is one-dimensional, the eigenfunctions are $w_k(\theta) = \cos\left(\frac{n\pi\theta}{a}\right)$, $n \in \mathbb{Z}$, and $k = \frac{n\pi}{a}$. Let w, the solution of (5.5), have the form

$$w(\theta,t) = \sum_k c_k w_k(\theta) e^{\lambda t}, \tag{5.7}$$

with λ the temporal eigenvalues and c_k determined by the initial conditions.

Then, it satisfies

$$\begin{aligned}\frac{\partial w}{\partial t} &= \sum_k \lambda c_k w_k(\theta) e^{\lambda t} \\ &= Bw + D\nabla^2 w \\ &= B\sum_k c_k w_k(\theta) e^{\lambda t} + D\sum_k c_k \nabla^2 w_k(\theta) e^{\lambda t} \\ &= \sum_k c_k (B - k^2) w_k(\theta) e^{\lambda t},\end{aligned} \tag{5.8}$$

which implies that, for each k,

$$\lambda w_k = B w_k - k^2 D w_k, \tag{5.9}$$

the eigenvalue problem that determines the temporal eigenvalues. Therefore, assuming w_k is not a trivial eigenfunction, λ are the solutions of $\det\left(B - k^2 D - \lambda I\right) = 0$, with I the identity matrix.

The eigenvalues λ are a function of k, $\lambda = \lambda(k)$, and to have diffusion-driven instability, there must exist k such that at least one eigenvalue $\lambda(k)$ of $B(k) := \left(B - k^2 D\right)$ has a positive real part. If this is the case, when $t \to \infty$, the value of w is dominated by the terms corresponding to an eigenvalue with positive real part, that does not vanish.

Remark 7. It is assumed that these unstable eigenfunctions will eventually reach an upper bound, resulting in a non-homogeneous steady state. The proof of such a result requires additional analysis.

5.1.1 Example

To illustrate the relevance of the study, consider the following chemical reaction network, the Oregonator, studied in [13]. The reactions involving X, Y and Z are the following,

$$\begin{gathered}\mathrm{A} + \mathrm{Y} \xrightarrow{\mathrm{k}_1} \mathrm{X} + \mathrm{P}, \quad \mathrm{X} + \mathrm{Y} \xrightarrow{\mathrm{k}_2} 2\,\mathrm{P}, \quad \mathrm{A} + \mathrm{X} \xrightarrow{\mathrm{k}_3} 2\,\mathrm{X} + 2\,\mathrm{Z}, \\ 2\,\mathrm{X} \xrightarrow{\mathrm{k}_4} \mathrm{A} + \mathrm{P}, \quad \mathrm{Z} \xrightarrow{\mathrm{k}_5} \mathrm{fY},\end{gathered}$$

assuming the concentrations of A and P are constant in time, with $\kappa = (k_1, \ldots, k_5)$ the positive rate constants vector, and f a positive constant.

The system of ODEs is described by matrices A and V,

$$A = \begin{pmatrix} 1 & -1 & 1 & -2 & 0 \\ -1 & -1 & 0 & 0 & f \\ 0 & 0 & 2 & 0 & -1 \end{pmatrix}, \quad V = \begin{pmatrix} 0 & 1 & 1 & 2 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix},$$

and has a unique steady state, according to the method in Chapter 3. However, it is possible to prove that for a portion of the parameter space, there is diffusion-driven instability. Such conditions can be determined using the results in Section 5.2. Despite not having more than one homogeneous steady state, the diffusion-driven instability may lead to a spatial pattern, proving that diffusion affects the steady states of chemical networks.

5.2 Necessary and sufficient conditions

The stability matrix B is the object of study of linear stability analysis, especially its eigenvalues. However, calculating the eigenvalues of a matrix and assessing the sign of their real part leads to highly complex inequalities over the parameters. Therefore, it is relevant to consider alternative criteria and to simplify the systems of equations involved, when possible.

Determining if B is negative definite works as an exclusion criterion, as if B is negative definite, then there is no diffusion-driven instability. The Routh-Hurwitz conditions, despite their intricacy, give sufficient and necessary conditions for diffusion-driven instability. Moreover, when B is 3×3, these give rise to easily tested conditions on the diagonal elements and the diagonal cofactors.

5.2.1 Negative definiteness and stability

In [13] the authors noticed the connection between negative definiteness of the stability matrix, B, and the stability of B and $B(k)$. This condition is applicable to any system with a finite number of species.

Definition 4. Matrix $B \in \mathbb{R}^{n\times n}$ is *negative definite* if for all $x \in \mathbb{R}^n$, $x \neq 0$, $x^t Bx < 0$.

Proposition 5 states the relationship between negative definiteness and stability. Note that the converse implication is not valid.

Proposition 5. *If a matrix $B \in \mathbb{R}^{n\times n}$ is negative definite, then it is stable.*

According to Proposition 6, if B is negative definite then $B(k)$ is also negative definite, which proves that if the stability matrix is negative definite there cannot be diffusion-driven instability.

Proposition 6. *If the stability matrix B is negative definite, then so is $B(k)$.*

In summary, a necessary condition for diffusion-driven instability is that the stability matrix is not negative definite. Moreover, being negative definite acts as an exclusion criterion.

5.2.2 Special case $n = 3$

In addition to noticing the relation between negative definiteness and stability, in [13] the authors define a necessary and sufficient condition that depends on the diagonal elements of B and on its diagonal cofactors.

Consider M_{ij} the cofactor of b_{ij}, i.e, $(-1)^{i+j}\det(C_{ij})$, with C_{ij} the submatrix obtained from B after removing row i and column j.

The characteristic polynomial of B is

$$\lambda^3 + p_2\lambda^2 + p_1\lambda + p_0 = 0,$$

with $p_2 = -tr(B)$, $p_1 = M_{11} + M_{22} + M_{33}$ and $p_0 = -\det(B)$.

Considering the Routh-Hurwitz conditions for B, it is stable if and only if

$$\det(B) = -p_0 < 0 \tag{5.10}$$

$$tr(B) = -p_2 < 0 \tag{5.11}$$

$$tr(B)\cdot(M_{11} + M_{22} + M_{33}) - \det(B) = -p_1p_2 + p_0 < 0 \tag{5.12}$$

Note that the coefficients of the characteristic polynomial of $B(k)$ are given by

$$\begin{aligned} p_2(k^2) =& (d_1+d_2+d_3)k^2 - tr(B) \\ p_1(k^2) =& (d_1d_2+d_2d_3+d_1d_3)k^4 - [d_1(b_{22}+b_{33})+d_2(b_{11}+b_{33})+d_3(b_{11}+b_{22})]k^2 \\ &+ (M_{11}+M_{22}+M_{33}) \\ p_0(k^2) =& d_1d_2d_3k^6 - (b_{11}d_2d_3+b_{22}d_1d_3+b_{33}d_1d_2)k^4 \\ &+ (d_1M_{11}+d_2M_{22}+d_3M_{33})k^2 - \det(B). \end{aligned} \tag{5.13}$$

Proposition 7 gives a necessary condition for diffusion-driven instability.

Proposition 7. *If B is stable, with all its diagonal elements negative, and all its diagonal cofactors positive, then $B(k)$ is also stable.*

This condition is not only necessary, it is also sufficient, as stated in Proposition 8.

Proposition 8. *If B is stable and*

1. *the largest diagonal element of B is positive, or*
2. *the smallest digonal cofactor of B is negative,*

then, $B(k)$ is unstable.

To sum up, according to Proposition 7, if all diagonal elements of B are negative and all its diagonal cofactors are positive, then there is no need for further analysis, since if B is stable then $B(k)$ will also be stable and there will be no diffusion-driven instability. However, if the assumptions of Proposition 8 hold, to have diffusion-driven instability the conditions to guarantee that B is stable are sufficient.

5.3 Results

To determine the influence of diffusion on the steady states of the motifs in Chapter 3, we study systems of PDEs which are modified versions of the original ODEs systems, by adding a diffusion term to each equation. Note that to maintain the analysis as generic as possible, the diffusion coefficients are not fixed.

As with CRNT, the one-dimensional domain is isolated, by considering no-flux boundary conditions.

Some of the motifs involve many parameters and reactions. To simplify the study some of the species are substituted by conservation laws or assumed homogeneous in space and constant in time. The conservation laws of the ODEs systems are still valid for the PDEs systems, under specific conditions.

Note that, as a consequence of $\frac{\mathrm{d}\,x_1}{\mathrm{d}\,t}+\ldots+\frac{\mathrm{d}\,x_l}{\mathrm{d}\,t}=0$ for the ODEs system,

$$\frac{\partial x_1}{\partial t}+\ldots+\frac{\partial x_l}{\partial t} = d_1\frac{\partial^2 x_1}{\partial \theta^2}+\ldots+d_l\frac{\partial^2 x_l}{\partial \theta^2}. \tag{5.14}$$

Assuming $d_1 = \ldots = d_l =: d$ and $x = x_1 + \ldots + x_l$, we get the following differential problem,

$$\begin{aligned} \frac{\partial x}{\partial t} &= \frac{\partial^2 x}{\partial \theta^2} && \text{, in } \Omega \\ x(0,\theta) &= X_T && \text{, in } \Omega \\ \frac{\partial x}{\partial \eta} &= 0 && \text{, in } \partial\Omega, \end{aligned} \tag{5.15}$$

with $X_T := x_1(0,\theta) + \ldots + x_l(0,\theta)$ the initial total amount. This differential problem has a unique solution $x = X_T$, which proves that the conservation laws are still valid.

As some conservation laws involve more than two species, to minimise the conditions imposed on the diffusion coefficients, we only account for the conservation laws involving two species. Then, it is possible to substitute one of the species involved by the relation deducted from the law as well as to remove one equation from the system.

After simplification using conservation laws, if possible, we can analyse the motifs. Firstly, focusing on the stability matrix B we calculate eigenvalues and check negative definiteness. For instance, if B is negative definite, there is no diffusion-driven instability, and there is no need for further analysis. However, as the resulting inequalities involve many parameters and do not lead to an easy identification of a portion of the parameter space, they were omitted. Otherwise, we focus on the 3×3 principal submatrices of B, which are the stability matrices for the system if only three species are not spatially homogeneous and constant in time. Then, we check the diagonal elements and diagonal cofactors, according to Subsection 5.2.2.

Following the aforementioned approach, we concluded the motifs in study do not have diffusion-driven linear instability.

Coherent feedforward

Considering conservation laws $X + X^* = X_T$, $Y + Y^* = Y_T$ and $R + R^* = R_T$ we arrive at a system with three equations, one positive steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_1 S - k_5 - k_6 & 0 & 0 \\ 0 & -k_7 - k_8 - k_2 S & 0 \\ k_3(R_T - R^*) & k_4(R_T - R^*) & -k_9 - k_{10} - k_3 X^* - k_4 Y^*, \end{pmatrix}$$

As B is triangular, it is easily seen that its eigenvalues are real and positive, and therefore stable. If λ is an eigenvalue of B, then $\lambda - k^2 d$, with d a positive constant, is an eigenvalue of $B(k)$. Consequently, all eigenvalues of $B(k)$ are real and negative, which makes $B(k)$ stable, leading to no diffusion-driven instability.

Incoherent feedforward

As both motifs that represent *coherent feedforward* have very similar systems, consider the one where the signal acts as an activator for both X and Y (Fig. 3.1b). Considering conservation laws $X + X^* = X_T$, $Y + Y^* = Y_T$ and $R + R^* = R_T$ we arrive at a system with three equations, one positive steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_1S - k_5 - k_6 & 0 & 0 \\ 0 & -k_7 - k_8 - k_2S & 0 \\ k_3R^* & k_4(R_T - R^*) & -k_9 - k_{10} - k_3X^* - k_4Y^*, \end{pmatrix}$$

As before, B is a triangular matrix which simplifies the determination of its eigenvalues. As its eigenvalues and those of $B(k)$ are real and negative, both matrices are stable, proving that there is no diffusion-driven instability.

Positive feedback

Considering conservation laws $X + X^* = X_T$, $Y + Y^* = Y_T$ and $R + R^* = R_T$ we arrive at a system with three equations, one positive steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_5 - k_6 - k_1S - k_4Y^* & k_4(X_T - X^*) & 0 \\ 0 & -k_7 - k_8 - k_3R^* & k_3(Y_T - Y^*) \\ k_2(R_T - R^*) & 0 & -k_9 - k_{10} - k_2X^* \end{pmatrix}$$

As the system has only three equations, we focus on the diagonal elements and cofactors of B. The diagonal entries are negative and the diagonal minors are positive,

$$\begin{aligned} M_{11} &= (k_7 + k_8 + k_3R^*)(k_9 + k_{10} + k_2X^*) > 0 \\ M_{22} &= (k_5 + k_6 + k_1S + k_4Y^*)(k_9 + k_{10} + k_2X^*) > 0 \\ M_{33} &= (k_5 + k_6 + k_1S + k_4Y^*)(k_7 + k_8 + k_3R^*) > 0, \end{aligned}$$

according to Proposition 7, if B is stable, then $B(k)$ will be stable. Therefore, the system does not show diffusion-driven instability.

Negative feedback

Considering conservation laws $X + X^* = X_T$, $Y + Y^* = Y_T$ and $R + R^* = R_T$ we arrive at a system with three equations, one positive steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_5 - k_6 - k_1S - k_4Y^* & -k_4X^* & 0 \\ 0 & -k_7 - k_8 - k_3R^* & k_3(Y_T - Y^*) \\ k_2(R_T - R^*) & 0 & -k_9 - k_{10} - k_2X^* \end{pmatrix}$$

As the system has only three equations, we focus on the diagonal elements and cofactors of B. The diagonal entries are negative and the diagonal minors are positive,

$$\begin{aligned} M_{11} &= (k_7 + k_8 + k_3R^*)(k_9 + k_{10} + k_2X^*) > 0 \\ M_{22} &= (k_5 + k_6 + k_1S + k_4Y^*)(k_9 + k_{10} + k_2X^*) > 0 \\ M_{33} &= (k_5 + k_6 + k_1S + k_4Y^*)(k_7 + k_8 + k_3R^*) > 0, \end{aligned}$$

according to Proposition 7, if B is stable, $B(k)$ will be stable. Therefore, the system does not show diffusion-driven instability.

Cyclic module

The original model has conservation law $X_T = X + Y + R$. However, if we consider this conservation law, for it to be valid for the diffusion system, the diffusion coefficients of the species need to be the same. Therefore, we shall consider that at the starting point, one of the species is already at equilibrium and homogeneous over the domain.

As a consequence of the symmetry of the system, the choice for the fixed species is irrelevant. Assume R is already at steady state. As concluded before, the system has one homogeneous positive steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_1 S & 0 \\ k_1 S & -k_2 \end{pmatrix},$$

whose eigenvalues are negative, $-k_1 S$ and $-k_2$, proving B is stable. Since, the eigenvalues of $B(k)$ are $-k_1 S - k^2 d_1 < 0$ and $-k_2 - k^2 d_2 < 0$, $B(k)$ is also stable and there is no diffusion-driven instability.

Two-step phosphorelay

The original model has conservation laws $E_T = Y_1 + E$, $F_{1T} = Y_2 + F_1$, $F_{2T} = Y_3 + F_2$, $X_{1T} = X_1^* + X_1 + Y_1 + Y_2$ and $X_{2T} = X_2^* + X_2 + Y_3$. For the analysis we shall consider only the first three conservation laws, since the last ones require all diffusion coefficients to be the same. The system has one positive homogeneous steady state and the following stability matrix.

$$B = \begin{pmatrix}
-k_7X_2 - k_4(F_{1T} - Y_2) & 0 & 0 & -k_7X_1^* & k_3 & k_5 + k_4X_1^* & 0 \\
k_7X_2 & -k_1(E_T - Y_1) & 0 & k_7X_1^* & k_2 + k_1X_1 & k_6 & 0 \\
k_7X_2 & 0 & -k_8(F_{2T} - Y_3) & k_7X_1^* & 0 & 0 & k_9 + k_8X_2^* \\
-k_7X_2 & 0 & 0 & -k_7X_1^* & 0 & 0 & k_{10} \\
0 & k_1(E_T - Y_1) & 0 & 0 & -k_2 - k_3 - k_1X_1 & 0 & 0 \\
k_4(F_{1T} - Y_2) & 0 & 0 & 0 & 0 & -k_5 - k_6 - k_4X_1^* & 0 \\
0 & 0 & k_8(F_{2T} - Y_3) & 0 & 0 & 0 & -k_{10} - k_9 - k_8X_2^*
\end{pmatrix}$$

The conditions for B being negative definite are complex. Thus, we will focus on systems with three species, with the remaining species considered constant.

```
k1 (et - y1) (f1t k4 + k7 x2 - k4 y2)
k8 (f1t k4 + k7 x2 - k4 y2) (f2t - y3)
k4 k7 x1e (f1t - y2)
(k2 + k3 + k1 x1) (f1t k4 + k7 x2 - k4 y2)
f1t k4 k6 + k5 k7 x2 + k6 k7 x2 + k4 k7 x1e x2 - k4 k6 y2
(k10 + k9 + k8 x2e) (f1t k4 + k7 x2 - k4 y2)
k1 k8 (et - y1) (f2t - y3)
k1 k7 x1e (et - y1)
k1 k3 (et - y1)
k1 (k5 + k6 + k4 x1e) (et - y1)
k1 (k10 + k9 + k8 x2e) (et - y1)
k7 k8 x1e (f2t - y3)
k8 (k2 + k3 + k1 x1) (f2t - y3)
k8 (k5 + k6 + k4 x1e) (f2t - y3)
k10 k8 (f2t - y3)
k7 (k2 + k3 + k1 x1) x1e
k7 x1e (k5 + k6 + k4 x1e)
k7 x1e (k10 + k9 + k8 x2e)
(k2 + k3 + k1 x1) (k5 + k6 + k4 x1e)
(k2 + k3 + k1 x1) (k10 + k9 + k8 x2e)
(k5 + k6 + k4 x1e) (k10 + k9 + k8 x2e)
```

Fig. 5.1 Diagonal cofactors from all 3×3 principal submatrices of B, for the *two-site phosphorelay*.

To that end, we used Mathematica to determine all the possible determinants from 2×2 principal submatrices, which are all the diagonal cofactors of the matrices in study. Given that the diagonal elements are negative, there can only be diffusion-driven instability if one of these cofactors is positive (Fig. 5.1). This method was repeated for all motifs with more than three species after simplification with conservation laws.

As all cofactors are positive, there is no system with three species deducted from this one that has diffusion-driven instability, because if B is stable, since all diagonal elements are negative and all diagonal cofactors are positive, $B(k)$ will be stable.

One-site modification

The original model has conservation laws $E_T = E + X$, $F_T = F + Y$ and $S_T = S_0 + S_1 + X + Y$. For the analysis we shall consider only the first two conservation laws, since the last one requires all diffusion coefficients to be the same. To simplify the analysis, we will focus on systems with three species, with the remaining species considered constant. The system has one positive homogeneous steady state and the following stability matrix.

$$B = \begin{pmatrix} -k_1 (E_T - X) & 0 & k_2 + k_1 S_0 & k_6 \\ 0 & -k_4 (F_T - Y) & k_3 & k_5 + k_4 S_1 \\ k_1 (E_T - X) & 0 & -k_2 - k_3 - k_1 S_0 & 0 \\ 0 & k_4 (F_T - Y) & 0 & -k_5 - k_6 - k_4 S_1 \end{pmatrix}$$

Given that the diagonal elements are negative, there can only be diffusion-driven instability if one of these cofactors is positive (Fig. 5.2).

```
k1 k4 (et - x) (ft - y)
k1 k3 (et - x)
k1 (k5 + k6 + k4 s1) (et - x)
k4 (k2 + k3 + k1 s0) (ft - y)
k4 k6 (ft - y)
(k2 + k3 + k1 s0) (k5 + k6 + k4 s1)
```

Fig. 5.2 Diagonal cofactors from all 3×3 principal submatrices of B, for the *one-site modification*.

As all cofactors are positive, there is no system with three species deducted from the original one that has diffusion-driven instability, because if B is stable, since all diagonal elements are negative and all diagonal cofactors are positive, $B(k)$ will be stable.

Two-site modification

The original model has conservation laws $E_{1T} = E_1 + X_1$, $E_{2T} = E_2 + X_2$, $F_{1T} = F_1 + Y_1$, $F_{2T} = F_2 + Y_2$ and $S_T = S_0 + S_1 + S_2 + X_1 + X_2 + Y_1 + Y_2$. For the analysis, we consider only the ones involving two species. To simplify the analysis, we will focus on systems with three species, with the remaining species considered constant. The system has one positive homogeneous steady state and the following stability matrix.

The stability matrix is

$$B = \begin{pmatrix}
-k_1(E_{1T}-X_1) & 0 & 0 & k_2+k_1S_0 & 0 & k_6 & 0 \\
0 & -k_7(E_{2T}-X_2)-k_4(F_{1T}-Y_1) & 0 & k_3 & k_8+k_7S_1 & k_5+k_4S_1 & k_{12} \\
0 & 0 & -k_{10}(F_{2T}-y_2) & 0 & k_9 & 0 & k_{11}+k_{10}S_2 \\
k_1(E_{1T}-X_1) & 0 & 0 & -k_2-k_3-k_1S_0 & 0 & 0 & 0 \\
0 & k_7(E_{2T}-X_2) & 0 & 0 & -k_8-k_9-k_7S_1 & 0 & 0 \\
0 & k_4(F_{1T}-Y_1) & 0 & 0 & 0 & -k_5-k_6-k_4S_1 & 0 \\
0 & 0 & k_{10}(F_{2T}-Y_2) & 0 & 0 & 0 & -k_{11}-k_{12}-k_{10}S_2
\end{pmatrix}$$

Assuming that B is stable, we focus on its diagonal entries and cofactors. It is clear that its diagonal entries are negative. Additionally, Fig. 5.3 includes all the diagonal cofactors from the relevant 3×3 submatrices of B.

```
k1 (e1t - x1) (f1t k4 + e2t k7 - k7 x2 - k4 y1)
k1 k10 (e1t - x1) (f2t - y2)
k1 k3 (e1t - x1)
k1 (k8 + k9 + k7 s1) (e1t - x1)
k1 (k5 + k6 + k4 s1) (e1t - x1)
k1 (k11 + k12 + k10 s2) (e1t - x1)
k10 (f1t k4 + e2t k7 - k7 x2 - k4 y1) (f2t - y2)
(k2 + k3 + k1 s0) (f1t k4 + e2t k7 - k7 x2 - k4 y1)
e2t k7 k9 + f1t k4 (k8 + k9 + k7 s1) - k7 k9 x2 - k4 k8 y1 - k4 k9 y1 - k4 k7 s1 y1
f1t k4 k6 + e2t k7 (k5 + k6 + k4 s1) - k5 k7 x2 - k6 k7 x2 - k4 k7 s1 x2 - k4 k6 y1
(k11 + k12 + k10 s2) (f1t k4 + e2t k7 - k7 x2 - k4 y1)
k10 (k2 + k3 + k1 s0) (f2t - y2)
k10 (k8 + k9 + k7 s1) (f2t - y2)
k10 (k5 + k6 + k4 s1) (f2t - y2)
k10 k12 (f2t - y2)
(k2 + k3 + k1 s0) (k8 + k9 + k7 s1)
(k2 + k3 + k1 s0) (k5 + k6 + k4 s1)
(k2 + k3 + k1 s0) (k11 + k12 + k10 s2)
(k5 + k6 + k4 s1) (k8 + k9 + k7 s1)
(k8 + k9 + k7 s1) (k11 + k12 + k10 s2)
(k5 + k6 + k4 s1) (k11 + k12 + k10 s2)
```

Fig. 5.3 Diagonal cofactors from all 3×3 principal submatrices of B, for the *two-site modification*.

It is easily seen that all the values are positive. Therefore, assuming that B is stable, according to Proposition 7, $B(k)$ is stable, and there cannot be diffusion-driven instability. For *two-site modification* with enzyme-sharing there is no diffusion-driven instability either, for subsystems with three species.

Modification of two substrates

The original model has conservation laws $E_T = E + X_1 + X_2$, $F_{1T} = F_1 + Y_1$, $F_{2T} = F_2 + Y_2$, $S_T = S_0 + S_1 + X_1 + Y_1$ and $P_T = P_0 + P_1 + X_2 + Y_2$. The only conservation laws considered are the ones that involve only two species. To simplify the analysis, we will focus on systems with three species, with the remaining species considered constant. The system has one positive homogeneous steady state and the following stability matrix.

$$B = \begin{pmatrix}
-k_1E & 0 & 0 & 0 & -k_1S_0 & k_2 & 0 & k_6 & 0 \\
0 & -k_4(F_{1T}-Y_1) & 0 & 0 & 0 & k_3 & 0 & k_5+k_4S_1 & 0 \\
0 & 0 & -k_7E & 0 & -k_7P_0 & 0 & k_8 & 0 & k_{12} \\
0 & 0 & 0 & -k_{10}(F_{2T}-Y_2) & 0 & 0 & k_9 & 0 & k_{11}+k_{10}P_1 \\
-k_1E & 0 & -k_7E & 0 & -k_7P_0-k_1S_0 & k_2+k_3 & k_8+k_9 & 0 & 0 \\
k_1E & 0 & 0 & 0 & k_1S_0 & -k_2-k_3 & 0 & 0 & 0 \\
0 & 0 & k_7E & 0 & k_7P_0 & 0 & -k_8-k_9 & 0 & 0 \\
0 & k_4(F_{1T}-Y_1) & 0 & 0 & 0 & 0 & 0 & -k_5-k_6-k_4S_1 & 0 \\
0 & 0 & 0 & k_{10}(F_{2T}-Y_2) & 0 & 0 & 0 & 0 & -k_{11}-k_{12}-k_{10}P_1
\end{pmatrix}$$

Assuming that B is stable, we focus on its diagonal entries and cofactors. It is clear that its diagonal entries are negative. Additionally, Fig. 5.4 includes all the diagonal cofactors from the relevant 3×3 submatrices of B.

```
e k1 k4 (f1t - y1)
e^2 k1 k7
e k1 k10 (f2t - y2)
e k1 k7 p0
e k1 k3
e k1 (k8 + k9)
e k4 k7 (f1t - y1)
k10 k4 (f1t - y1) (f2t - y2)
k4 (k7 p0 + k1 s0) (f1t - y1)
(k2 + k3) k4 (f1t - y1)
k4 (k8 + k9) (f1t - y1)
e k10 k7 (f2t - y2)
e k1 k7 s0
e (k2 + k3) k7
e k7 k9
k10 (k7 p0 + k1 s0) (f2t - y2)
k10 (k2 + k3) (f2t - y2)
k10 (k8 + k9) (f2t - y2)
(k2 + k3) k7 p0
k1 (k8 + k9) s0
(k2 + k3) (k8 + k9)
```

Fig. 5.4 Diagonal cofactors from all 3×3 principal submatrices of B, for the *modification of two substrates*.

It is clear that all diagonal elements of B are negative and that the cofactors shown in Fig. 5.4 are all positive. Therefore, there cannot be diffusion-driven instability. Moreover, for the *modification of two substrates* with enzyme-sharing there is no diffusion-driven instability either.

Two-layer cascade

The original model has conservation laws $F_{1T} = F_1 + Y_1$, $F_{2T} = F_2 + Y_2$, $E_T = E + X_1$, $S_T = S_0 + S_1 + X_1 + Y_1$ and $P_T = P_0 + P_1 + X_2 + Y_2$. The only conservation laws considered are the ones that involve only two species. To simplify the analysis, we will focus on systems with three species, with the remaining species considered constant. The system has one positive homogeneous steady state and the following stability matrix.

$$B = \begin{pmatrix}
-k_1E & 0 & 0 & 0 & -k_1S_0 & k_2 & 0 & 0 & k_6 & 0 \\
0 & -k_{13}P_0 - k_4(F_{1T} - Y_1) & -k_{13}S_1 & 0 & 0 & k_3 & 0 & k_{14} + k_{15} & k_5 & 0 \\
0 & -k_{13}P_0 & -k_7E - k_{13}S_1 & 0 & -k_7P_0 & 0 & k_8 & k_{14} & 0 & k_{12} \\
0 & 0 & 0 & -k_{10}(F_{2T} - Y_2) & 0 & 0 & k_9 & k_{15} & 0 & k_{11} \\
-k_1E & 0 & -k_7E & 0 & -k_7P_0 - k_1S_0 & k_2 + k_3 & k_8 + k_9 & 0 & 0 & 0 \\
k_1E & 0 & 0 & 0 & k_1S_0 & -k_2 - k_3 & 0 & 0 & 0 & 0 \\
0 & 0 & k_7E & 0 & k_7P_0 & 0 & -k_8 - k_9 & 0 & 0 & 0 \\
0 & k_{13}P_0 & k_{13}S_1 & 0 & 0 & 0 & 0 & -k_{14} - k_{15} & 0 & 0 \\
0 & k_4(F_{1T} - Y_1) & 0 & 0 & 0 & 0 & 0 & 0 & -k_5 - k_6 & 0 \\
0 & 0 & 0 & k_{10}(F_{2T} - Y_2) & 0 & 0 & 0 & 0 & 0 & -k_{11} - k_{12}
\end{pmatrix}$$

Assuming that B is stable, we focus on its diagonal entries and cofactors. It is clear that its diagonal entries are negative. Additionally, Fig. 5.5 includes all the diagonal cofactors from the relevant 3×3 submatrices of B.

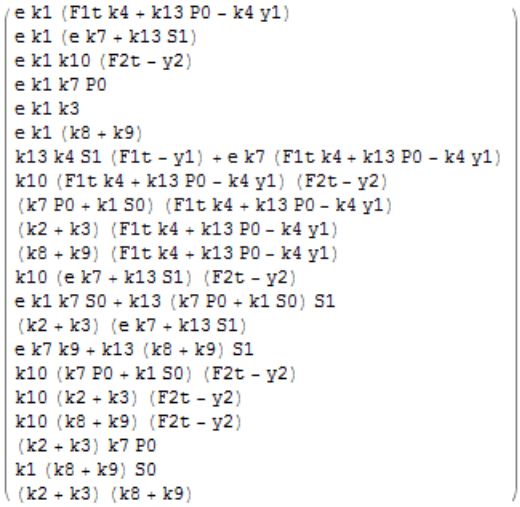


Fig. 5.5 Diagonal cofactors from all 3×3 principal submatrices of B, *for two-layer cascade*.

It is clear that all diagonal elements of B are negative and that the cofactors shown in Fig. 5.5 are all positive. Therefore, there cannot be diffusion-driven instability. Moreover, for the *two-layer cascade* with enzyme-sharing there is no diffusion-driven instability either.

5.4 Summary

Using Linear Stability Analysis it is possible to determine if a reaction-diffusion system may showcase spatial patterns. Our initial analysis of the motifs focused on their steady states which are spatially homogeneous solutions of the reaction-diffusion systems of this Chapter. If the steady states are stable without diffusion and become unstable with diffusion, it may be possible to observe the formation of spatial patterns. If this was the case, there would be an influence of space on the motifs.

The study of diffusion-driven instability focuses on the stability matrix of the system, B, more specifically on its eigenvalues. However, the conditions that arise are not easy to understand, forcing us to simplify the system. As a consequence, our analysis regards the subsystems of three species obtained by assuming the remaining species start at a homogeneous steady state.

None of the three species subsystems in study showed diffusion-driven linear instability.

Chapter 6

Conclusion

Steady states of a cellular signalling pathway are the possible responses for a stimuli, thus multistationarity results in more flexibility in decision-making. The thesis aimed to determine if space can have an influence on steady states of chemical reaction networks, by considering different models to describe them.

CRNT is the classical theory for such study, representing the temporal evolution of species concentrations through systems of ODEs (Chapter 2), therefore disregarding space.

As steady states are intrinsic to the networks, we wanted to consider a general set of chemical reaction networks that represented mechanisms ubiquitous in signalling pathways. To that end, we chose eleven motifs, described in Chapter 3. Moreover, by assuming mass-action kinetics, the steady states are represented by the roots of a polynomial, which can be studied with injectivity methods. As injectivity methods exploit a matrix representation of the polynomials, with matrices independent of the parameters of the model, the results are general, which means we can conclude the uniqueness of steady state for all rate constants or the existence of a set of rate constants that lead to multistationarity.

Space was initially studied through the idea of compartments, leading to models described by similar ODEs systems. In Chapter 4, two types of compartmentalisation are accounted for: in parallel and in stages. For some motifs, there were changes in the capacity for multistationarity, from uniqueness of steady state to multiple steady states and vice-versa.

In Chapter 5 we wanted to define a model in a one-dimensional domain. Accounting for diffusion inside cells, we defined reaction-diffusion models based on the ODEs ones. Determining the influence of space was equivalent to determining the influence of diffusion on the homogeneous solutions, which meant assessing the stability of the original steady states when diffusion is added to the system. The classical theory of diffusion-driven instability relies on Linear Stability Analysis, for systems with two species, and there are few results for three or more. Therefore, the systems were simplified to focus on three species, and none of the motifs displayed linear diffusion-driven instability.

We concluded that space can have an influence in the capacity for multistationarity of chemical reaction networks. Despite not concluding diffusion-driven instability for any motif, with compartments there were changes. For instance, some motifs gained the capacity for multistationarity when in parallel, while its loss was visible for motifs in stages.

There were some limitations to be overcome by future research on the topic. Firstly, assuming mass-action kinetics allowed for the application of injectivity methods (Chapter 3), but considering

other kinetics may lead to different results. For instance, Michaelis-Menten kinetics are often used for phosphorylation networks. Thirdly, we considered two compartments in Chapter 4, which could be extended to more compartments to emulate the communication of a set of cells in a body. Thirdly, in Chapter 5, the lack of linear diffusion-driven instability may be a consequence of the focus on subsystems with three species. There are more general results, focusing on the stability matrix that may be relevant [15] as well as alternative tools that describe the chemical network as a bipartite graph [10]. Moreover, it is possible the motifs exhibit diffusion-driven instability that is not linear. Lastly, it would be insteresting to combine the ideas of compartments and diffusion, as both are present in eukaryotic cells. On the one hand, we could consider boundary conditions emulating membrane binding, with the domain representing the cytoplasm and the boundaries distinct membranes. On the other hand, we could consider a spatial domain with internal membranes, which separate different cellular compartments and allow for a more realistic representation of movement through membranes, not necessarily rate restricted as in Chapter 4.

This work offers a justification for the growth in the spatial study of signalling pathways, while including results that can be useful for other researchers, namely synthetic biologists. By thoroughly analysing a number of motifs and including space through different perspectives, we proved the influence of space in the capacity of chemical reaction networks for multistationarity.

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Appendix A

Auxiliary results used in Chapter 2

Lemma 1. *Let $X, Y \subset \mathbb{R}^n$, then*

$$\Sigma(X) \cap Y = \emptyset \iff \sigma(X) \cap \sigma(Y) = \emptyset \iff X \cap \Sigma(Y) = \emptyset.$$

Proof. It is sufficient to prove the first equivalence, as the second follows by changing the roles of X and Y. If $\Sigma(X) \cap Y = \emptyset$ and $\sigma(X) \cap \sigma(Y) \neq \emptyset$, there are $x \in X$ and $y \in Y$ such that $\sigma(x) = \sigma(y)$. Consequently, there are $\lambda \in \mathbb{P}^n$ with $y = \lambda \circ x$. Thus, $y \in \Sigma(X) \cap Y \neq \emptyset$.

On the other hand, assuming $\Sigma(X) \cap Y \neq \emptyset$ and $\sigma(X) \cap \sigma(Y) = \emptyset$, there are $y \in Y$, $x \in X$, and $\lambda \in \mathbb{P}^n$ such that $y = \lambda \circ x$. Consequently, $\sigma(y) = \sigma(\lambda \circ x) = \sigma(x)$ and $\sigma(Y) \cap \sigma(X) \neq \emptyset$. □

Lemma 2. *Let $B \in \mathbb{R}^{r \times n}$ and $S \subset \mathbb{R}^n$. The following are equivalent.*

1. $\ker(B_\lambda) \cap S = \emptyset$ *for all* $\lambda \in \mathbb{P}^n$.
2. $\sigma(\ker(B)) \cap \sigma(S) = \emptyset$.

Proof. For the proof, take into account the second equivalence from Lemma 1. Assuming $\ker(B_\lambda) \cap S = \emptyset$, for all λ, and $\ker(B) \cap \Sigma(S) \neq \emptyset$, there are $w \in S$ and $\lambda \in \mathbb{P}^n$ such that $B(\lambda \circ w) = 0$. Note that $0 = B(\lambda \circ w) = B\, diag(\lambda) w = B_\lambda w$, thus $w \in \ker(B_\lambda) \cap S \neq \emptyset$.
On the other hand, assuming there is $\lambda \in \mathbb{P}^n$ such that $\ker(B_\lambda) \cap S \neq \emptyset$, and $\ker(B) \cap \Sigma(S) = \emptyset$, then there is $w \in S$ satisfying $B_\lambda w = 0$. Moreover, $0 = B_\lambda w = B(\lambda \circ w)$ and $\lambda \circ w \in \Sigma(S)$. □

Lemma 3. $\Sigma(S) = \Lambda(S)$

Proof. Let $\lambda \in \mathbb{P}^n$ and $w \in S$. Fixing x and y as

$$y_i = \begin{cases} \frac{w_i}{e^{\lambda_i w_i} - 1} & , \text{if } w_i \neq 0 \\ 1 & , \text{if } w_i = 0 \end{cases}$$

and $x_i = e^{\lambda_i w_i} y_i$. Then, $x - y = w$ and $\ln(x) - \ln(y) = \lambda \circ w$.
If x, y, $x - y \in S$, then $\sigma(\ln(x) - \ln(y)) = \sigma(x - y) \in \sigma(S)$, since the logarithm is an increasing function. Thus, $\ln(x) - \ln(y) \in \sigma^{-1}(\sigma(S)) = \Sigma(S)$. □

Lemma 4. *Let $\Gamma_{\kappa,\lambda}$ as defined in Theorem 1, then*

$$\det(\Gamma_{\kappa,\lambda}) = \sum_{I,J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \det\left(\tilde{A}_{[s],I}\right) \det\left(V^t_{I,J}\right) \kappa^I \lambda^J,$$

with sum over all sets $I \subset [r]$, $J \subset [n]$ with s elements.

Proof. Using Laplace expansion (2.5), with $I = [s]$,

$$\det(\Gamma_{\kappa,\lambda}) = \sum_{J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \det\left(\left(\tilde{A}_\kappa V^t_\lambda\right)_{[s],J}\right),$$

with sum over $J \subset [n]$ with s elements. Note that $\tau(I) = 0$.
Considering Cauchy-Binet formula (2.6), with $I \subset [r]$ with s elements,

$$\begin{aligned}
\det(\Gamma_{\kappa,\lambda}) &= \sum_{J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \sum_{I} \det\left(\left(\tilde{A}_\kappa\right)_{[s],I}\right) \det\left(\left(V^t_\lambda\right)_{I,J}\right) \\
&= \sum_{I,J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \det\left(\left(\tilde{A}_\kappa\right)_{[s],I}\right) \det\left(\left(V^t_\lambda\right)_{I,J}\right) \\
&= \sum_{I,J} (-1)^{\tau(J)} \det\left(Z_{[n-s],J^c}\right) \det\left(\left(\tilde{A}\right)_{[s],I}\right) \det\left(\left(V^t\right)_{I,J}\right) \kappa^I \lambda^J.
\end{aligned}$$

□

Lemma 5. *Let $q(c) \in \mathbb{R}[c_1, \ldots, c_l]$ represent a homogeneous polynomial not identically zero, with degree at most 1 in each variable. Then, there exists $c^* \in \mathbb{P}^l$ root of q if and only if not all the coefficients of $q(c)$ have the same sign.*

Proof. If every coefficient has the same sign, there is no positive root. Assuming there is one pair of coefficients with opposite signs. Let αc^v be a monomial of q, then $v \in \{0,1\}^l$. Considering $\varepsilon > 0$, let

$$c_i(\varepsilon) = \begin{cases} \varepsilon & \text{, if } v_i = 1 \\ 1 & \text{, if } v_i = 0 \end{cases}.$$

Then, $q(c(\varepsilon))$ is a polynomial in one variable, ε, with the same degree as q and α as its leading coefficient. Thus, if ε is sufficiently big, the sign of the polynomial is determined by the sign of α. As it is possible to choose two monomials of q whose coefficients have distinct signs, then $q(c)$ takes positive and negative values in $\mathbb{P}^l$. Being a polynomial, it is continuous, therefore it has a positive root. □

Lemma 6. *Let $f_\kappa : \mathbb{P}^n \to \mathbb{R}^m$, $f_\kappa(x) = A_\kappa x^V$. Then, the set of Jacobian matrices $J_{f_\kappa}(x)$ and the set of matrices $A_\kappa V^t_\lambda$ coincide:*

$$\{J_{f_\kappa}(x) : \kappa \in \mathbb{P}^r, x \in \mathbb{P}^n\} = \{A_\kappa V^t_\lambda : \kappa \in \mathbb{P}^r, \lambda \in \mathbb{P}^n\}.$$

Proof. Given that $f_{\kappa,i}(x) = \sum_{j=1}^r a_{ij} \kappa_j x^{v_j}$, the (i,l)-th entry of the Jacobian matrix of f_κ is the following,

$$J_{f_\kappa}(x)_{i,l} = \frac{\partial f_{\kappa,i}(x)}{\partial x_l} = \sum_{j=1}^{r} a_{ij}\kappa_j x^{v_j} v_{lj} x_l^{-1}.$$

Thus, the Jacobian can be written as

$$J_{f_\kappa}(x) = A \operatorname{diag}(\kappa \circ x^V) V^t \operatorname{diag}(x^{-1}) = A_{\kappa'} V_{\lambda'}^t,$$

with $\kappa' = \kappa \circ x^V$ and $\lambda' = x^{-1}$. Clearly, going over κ and x is equivalent to going over κ' and λ'.

□

Appendix B

Auxiliary results used in Chapter 5

Proposition 5. *If a matrix $B \in \mathbb{R}^{n\times n}$ is negative definite, then it is stable.*

Proof. For B to be stable, all its eigenvalues must have negative real part. Let λ be and eigenvalue with eigenvector $v = \alpha + i\beta$.

$$\begin{aligned} 2Re(\lambda)\sum_j |v_j|^2 &= (\lambda+\bar{\lambda})\sum_j v_j\bar{v_j} \\ &= \sum_j (\lambda v_j\bar{v_j} + \bar{\lambda v_j} v_j) \\ &= \sum_{j,l} (b_{j,l} v_l \bar{v_j} + \bar{b_{j,l}} v_l v_j) \\ &= 2\sum_{j,l} \left(\alpha_j b_{j,l}\alpha_l + \beta_j b_{j,l}\beta_l\right) \\ &= 2(\alpha^t B\alpha + \beta^t B\beta) < 0. \end{aligned} \tag{B.1}$$

□

Proposition 6. *If the stability matrix B is negative definite, then so is $B(k)$.*

Proof. Let $y \in \mathbb{R}^n$ such that $y \neq 0$. Then,

$$y^t B(k)\, y = y^t \left(B - k^2 D\right) y = y^t B\, y - k^2 y^t D\, y < -k^2 \sum_j d_j y_j^2 < 0.$$

□

Proposition 7. *If B is stable, with all its diagonal elements negative, and all its diagonal cofactors positive, then $B(k)$ is also stable.*

Proof. It is sufficient to show that under these assumptions, $B(k)$ satisfies conditions 5.10. Calculating the determinant of $B(k)$,

$$\begin{aligned} \det(B(k)) = \det(B) - k^2 (d_1 M_{11} + d_2 M_{22} + d_3 M_{33}) + \\ k^4 (d_2 d_3 b_{11} + d_1 d_3 b_{22} + d_1 d_2 b_{33}) - k^6 d_1 d_2 d_3 < 0, \end{aligned}$$

since $\det(B) < 0$, $M_{ii} > 0$, $d_i > 0$, $b_{ii} < 0$, $i = 1,2,3$.
It is clear that its trace is negative, $tr(B(k)) = tr(B) - k^2(d_1 + d_2 + d_3) < 0$.
For the third condition, using (5.13), and calculating the derivative of $q(k^2) = -p_1(k^2)p_2(k^2) + p_0(k^2)$ with respect to k^2,

$$\begin{aligned}\frac{\partial q}{\partial k^2} =& tr(B)\left(M_{11} + M_{22} + M_{33} - f_1(d,b)\right) \\ &+ k^2\left(2f_2(d,b) + 2tr(B)g_1(d) + g_2(d)f_1(d,b) - tr(B)f_1(d,b)\right) \\ &+ k^4\left(tr(B)g_1(d) - 2g_2(d)g_1(d) - 3g_3(d)\right),\end{aligned} \tag{B.2}$$

with

$$\begin{aligned} f_1(d,b) &= b_{11}d_2 + b_{22}d_1 + b_{11}d_3 + b_{33}d_1 + a_{22}d_3 + b_{33}d_2 \\ f_2(d,b) &= b_{11}d_2d_3 + b_{22}d_1d_3 + b_{33}d_1d_2 \\ g_1(d) &= d_1d_2 + d_1d_3 + d_2d_3 \\ g_2(d) &= d_1 + d_2 + d_3 \\ g_3(d) &= d_1d_2d_3, \end{aligned}$$

which satisfy $f_1(d,b) < 0$, $f_2(d,b) < 0$, $g_1(d) > 0$, $g_2(d) > 0$ and $g_3(d) > 0$. Therefore, as conditions 5.10 are valid for B, the derivative of q is negative, which implies that $q(k^2) < q(0) = -p_1p_2 + p_0 < 0$. □

Proposition 8. *If B is stable and*

1. *the largest diagonal element of B is positive, or*
2. *the smallest digonal cofactor of B is negative,*

then, $B(k)$ is unstable.

Proof. $B(k)$ is unstable if one of the conditions (5.10) is not satisfied. To that end, it is required to find suitable diffusion coefficients d_i, $i = 1,2,3$, and k.
Assuming $M_{33} < 0$, consider $d_1 = d_2 = 0$, then

$$p_0(k^2) = d_3M_{33}k^2 - \det(B).$$

If $k^2 > \frac{\det(B)}{d_3M_{33}}$, then $p_0(k^2) < 0$, and $B(k)$ is unstable. In practical terms, d_3 shouls be considered significantly larger than d_1 and d_2.
If, for example, $b_{11} > 0$, assume $d_2 = d_3 = 1$ and $d_1 = 0$. Then,

$$p_0(k^2) = -b_{11}k^4 + (d_2M_{22} + d_3M_{33})k^2 - \det(B).$$

If k is sufficiently large, the sign of $p_0(k^2)$ will be determined by the sign of the term $-b_{11}k^4$. Therefore, there exists k such that $p_0(k^2) < 0$, i.e., $B(k)$ is unstable. □