

# AN EXPLORATION OF MULTISTATIONARITY IN CHEMICAL REACTION NETWORKS

MATH40000 PROJECT

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## Abstract

Systems made up of a set of *species*, or *chemicals*, can be described as a network defined by the chemical interactions which they give rise to. In turn, these can be understood as a system of differential equations, from which much can be inferred. Throughout this project, the question “When does the concentration in a Chemical Reaction Network admit multiple *positive* equilibria?” will be at the forefront of our minds. This is an interesting question that can tell us a great deal about the system we are modelling, but in general it is difficult to answer. However, within the framework we define there exist reliable methods to help us answer this question. We begin by introducing the notion of a reaction network, and put in place certain assumptions – some of which we subsequently attempt to relax. The first half typically deals with *deterministic* systems, but later on a *stochastic* approach is assumed, which draws upon the previous work. The introduction gives a general overview of the subject and covers most of the notation, as well as dealing with the *Law of Mass Action*. The next three chapters then explore the structure of a reaction network and describe how this information can be used to help us answer our main question, culminating in the Deficiency Zero Theorem. In the final chapter we explore these systems in a stochastic setting, in an attempt to make our theory more widely applicable.

# Chapter 1

## Introduction

To be able to derive a system of differential equations from a given *Chemical Reaction Network*, we first need to understand what this means. Of course, it is important to be rigorous in how we define such things if we are to use definitions as a platform for further understanding. It is also valuable to see the motivation for such work, so as to get on board with the ideas put forward. Before going straight into heavy notation which may require more from the reader than a casual skim read, an extended example is presented, which should serve to address these two points for the time being, and hopefully whet the readers interest.

Let us say that  $A, B, C, D$  are chemical species, which can react in the following ways,



where the numbers are on the arrows for ease of reference. That is, in reaction 1, one part of species  $A$  can turn into one part of species  $B$  and one part of species  $C$  (and vice versa, in reaction 2). Similarly, in reaction 3 one part of  $A$  and one part of  $B$  can come together to form two parts of  $D$  (and vice versa in reaction 4). This activity begs the question – how does the *amount* of each of the species change over time, due to these reactions? Suppose we mix together a great deal of each species so they are well distributed, and we maintain their temperature (for reasons that will become clear a little later). Then the *amount* of a species becomes synonymous with its *concentration* in this mix. Let us refer to the concentration of a species at some

given point in time, for example  $A$  at time  $t$ , by  $c_A(t)$ , which will make up the entries of a *concentration vector*,  $c(t)$ . Typically we will only write  $c$ . Now, we have an intuitive way of quantifying how a reaction changes the concentration of a species, but what's left to determine is the rate at which each reaction occurs. A *kinetics* is an assignment of a rate function to each reaction in a network<sup>[1]</sup>. Note here that a change in temperature can affect the rate at which a given reaction occurs, which is why earlier we demanded that temperature be held constant. Let us give this system a kinetics so that we are able to derive the equations which govern its dynamics – we do this by simply defining for each reaction a function of the concentration,  $c$ . So for reaction 1,  $A \rightarrow B + C$ , denote the rate function by  $K_{A \rightarrow B+C}(\cdot)$ . This function is non-negative and real valued, and when evaluated at  $c$  gives the instantaneous occurrence rate of the reaction at that concentration. The rate functions for the other reactions are defined in a similar way. Now all there is to do is combine the two! Let's focus first on the rate of change of the concentration of species  $A$ , denoted  $\dot{c}_A$ . After reaction 1 occurs we are left with one less *unit* of  $A$ , so we acquire the term  $-K_{A \rightarrow B+C}(c)$ . Similarly, reaction 2 gives us the term  $K_{B+C \rightarrow A}(c)$ .

**Remark** If we had lost or gained  $n$  units of  $A$ , the multiple of the value the corresponding rate function takes at  $c$  would be  $-n$  or  $n$  respectively, i.e.  $-nK_{nA \rightarrow B+C}$  or  $nK_{B+C \rightarrow nA}$ .

Applying this reasoning to the other three species we obtain the following system of coupled ODE's.

$$\begin{aligned}\dot{c}_A &= -K_{A \rightarrow B+C}(c) + K_{B+C \rightarrow A}(c) - K_{A+B \rightarrow 2D}(c) + K_{2D \rightarrow A+B}(c), \\ \dot{c}_B &= K_{A \rightarrow B+C}(c) - K_{B+C \rightarrow A}(c) - K_{A+B \rightarrow 2D}(c) + K_{2D \rightarrow A+B}(c), \\ \dot{c}_C &= K_{A \rightarrow B+C}(c) - K_{B+C \rightarrow A}(c), \\ \dot{c}_D &= 2K_{A+B \rightarrow 2D}(c) - 2K_{2D \rightarrow A+B}(c).\end{aligned}$$

Deriving the system of coupled ODE's for any given reaction network is as simple as what has just been presented. However, the equations themselves could be very long and difficult to solve. For example, how would one determine if any stationary points for the above system exist? And if they do, how are we to find them? Answering these questions for this particular system is not a great step forward – there are infinitely

many possible systems to deal with. We need to develop a much more general approach to this problem, which will require some formalisation.

**Notation** We denote by  $\mathbb{R}^I$  the vector space of all formal sums  $\sum_{i \in I} \alpha_i i$ ,  $\alpha_i \in \mathbb{R}$  for some arbitrary finite set  $I$ . Naturally,  $I$  forms a basis for  $\mathbb{R}^I$ . If we restrict the  $\alpha_i$  to be positive, we write  $\mathbb{R}_+^I$ , and we write  $\overline{\mathbb{R}}_+^I$  for when the  $\alpha_i$ 's are non-negative.

Typically, the set  $I$  will be a collection of some given chemicals, or ‘species’, for example  $I = \{A, B, C, X, Y\}$ . Given this set, some examples of elements of  $\overline{\mathbb{R}}_+^I$  would be,

$$2A + 1B + 7X, \quad \sqrt{3}C + 17.4Y, \quad \pi B.$$

In general the coefficients, the  $\alpha_i$ 's, will be non-negative *integers*, since we will often want to talk about the number of molecules of a species, but we will stick with the real numbers, as this is needlessly restrictive.

## 1.1 What is a Chemical Reaction Network?

In the theory of chemical reaction networks (CRN's), a reaction network is typically defined as follows:

**Definition 1.1** A chemical reaction network,  $\mathcal{N}$ , is made up of three *finite* sets:

1. a set,  $\mathcal{S}$ , of species,
2. a set,  $\mathcal{C} \subset \overline{\mathbb{R}}_+^{\mathcal{S}}$ , of complexes, and
3. a set,  $\mathcal{R} = \mathcal{C} \times \mathcal{C}$ , of reactions such that,

- (a)  $(y, y) \notin \mathcal{R} \ \forall y \in \mathcal{C}$ , and
- (b)  $\forall y \in \mathcal{C}, \exists y' \in \mathcal{C}$  such that  $(y, y') \in \mathcal{R}$  or  $(y', y) \in \mathcal{R}$ .

**Remark** • The element  $(y, y')$  represents the reaction between the complex  $y$  and the complex  $y'$  and we write  $y \rightarrow y'$ , as is the accepted standard in chemistry. We say that complex  $y$  *reacts to form* complex  $y'$ .

- We reserve  $n$  and  $m$  for the number of species and reactions respectively.

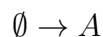
**Example 1.2** Take the example of a Michaelis-Menten system:



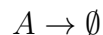
where  $E$  is an enzyme,  $S$  is a substrate,  $C$  is an enzyme–substrate complex<sup>a</sup> and  $P$  is the product.

In this case,  $\mathcal{S} = \{E, S, C, P\}$ ,  $\mathcal{C} = \{E+S, C, E+P\}$ , and  $\mathcal{R} = \{(E+S, C), (C, E+S), (C, E+P)\}$ .

These reaction networks, in general, are not closed systems. In nature, compositions do not change solely from chemical reactions – the external world interferes. We can aim to model this with the consideration of a homogeneous reactor, in which certain chemical species are fed in or taken out. Specifically, we shall refer to the *continuous flow stirred-tank reactor* (CFSTR), where we suppose the mixture is kept at constant temperature and is well mixed throughout (i.e. the same concentration everywhere). In order to facilitate this expansion, we must define so called “pseudo-reactions” as follows:



is written for the in flow of a chemical species  $A$ , and



for the removal of chemical species  $A$ . We refer to networks with these pseudo-reactions included as “augmented”. We can distinguish between these reactions by splitting  $\mathcal{R}$  into three subsets –  $\mathcal{R}_t$ ,  $\mathcal{R}_f$ , and  $\mathcal{R}_o$  representing the reactions occurring within the reactor (so called true reactions), feed reactions, and outflow reactions respectively.

Eventually, we are going to want to talk about the rate of change of the concentration of each species in a CFSTR. For an augmented network, this will require us to have some way of measuring the three following occurrences:

1. How much of a specific species is entering the reactor over a given time period?
2. How frequently do reactions occur among interacting species within the reactor?

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<sup>a</sup>note that this use of the word ‘complex’ is distinct from our previous use, given in Definition 1.1



3. How much of a specific species is leaving the reactor over a given time period?

In order to answer these questions (in particular the second), we must introduce a way of understanding how species interact with one another.

## 1.2 The Law of Mass Action

The law of mass action is the assumption that the rate at which a specific chemical reaction occurs is proportional to the concentration of the chemical species involved (the reactants). Define  $c(t) \in \mathbb{R}^n$  to be the vector whose entries are the concentrations (in moles/volume) of species in some CFSTR at a given time  $t \geq 0$ . Thus the molar concentration of chemical species  $A$  at a time  $t \geq 0$  will be given by  $c_A(t)$ . The rate of a reaction, say  $A \rightarrow B$ , according to the law of mass action will be given by  $k_{A \rightarrow B} c_A(t)$ , where  $k_{A \rightarrow B}$  is some positive real constant called the *rate constant*.

One can generalise this with the introduction of the non-negative, real valued rate function:

**Definition 1.3** Let  $u, v \in \overline{\mathbb{R}}_+^S$ , and define  $u^v = \prod_{s \in S} (u_s)^{v_s}$ . Define the function  $K_{y \rightarrow y'} : \overline{\mathbb{R}}_+^n \rightarrow \overline{\mathbb{R}}_+$  by  $K_{y \rightarrow y'}(c) = k_{y \rightarrow y'} c^y$ , where  $k_{y \rightarrow y'}$  is a non-negative real constant, and  $y, y'$  are complexes.

**Remark** The observant reader will have noticed that the law of mass action simply allows us to define a particular kinetics on a system of equations, namely, *mass action kinetics*.

**Example 1.4** Suppose we have a CRN with species  $\{A, B, C\}$  and two reactions given by  $(A + B, C)$ ,  $(2A, C)$ . Let  $c(t) = (c_A(t), c_B(t), c_C(t))^T$  give the concentration of each species at time  $t \geq 0$ .

Then at some instantaneous moment,

$$K_{A+B \rightarrow C}(c) = k_{A+B \rightarrow C} c^{(A+B)} = k_{A+B \rightarrow C} c_A c_B,$$

and

$$K_{2A \rightarrow C}(c) = k_{2A \rightarrow C} c^{2A} = k_{2A \rightarrow C} c_A^2.$$

How does this mass action law apply to our pseudo-reactions? Well, let's try to think of what they are actually representing, since they aren't really chemical reactions as we typically think of them. The reaction  $\emptyset \rightarrow A$  isn't actually saying that  $A$  spontaneously appears in the mix – it's being physically fed in to the reactor, and the rate at which this is happening is called the *volumetric flow rate*,  $g$  (vol/time). We therefore need to define how much of each chemical species is being fed into the reactor, that is, we need to define the feed concentrations,  $c^f$ . Let  $V$  denote the total volume of the mixture inside the reactor. Supposing the only reaction occurring is  $\emptyset \rightarrow A$ , then  $V\dot{c}_A = gc_A^f$ , so that the rate function for our feed reaction will give us  $(g/V)c_A^f$ . For the reaction  $A \rightarrow \emptyset$ , the rate function gives us  $k_{A \rightarrow \emptyset}c_A$ . If we assume the effluent stream has the same composition as the overall mixture and we remove the mixture at the same rate as we feed it in,  $g$ , then  $k_{A \rightarrow \emptyset} = g/V$  also.

### 1.3 Stoichiometry

Now that we have introduced mass action and understand how rate functions work in a chemical reaction network, we can directly relate the occurrence of reactions to the change in concentration of a particular species in this context. For example, take the reaction  $A + B \rightarrow 2C$ . Using our newly obtained knowledge, it is clear that we arrive at  $k_{A+B}c_Ac_B$  for the rate function. How does this affect the concentration of each species?

Each time this reaction occurs, we lose one molecule of  $A$  and  $B$  respectively, and we gain two molecules of  $C$ . So the contribution to the rate of change of each of these species (from this particular reaction) is simply as follows:

$$\dot{c}_A = -k_{A+B}c_Ac_B$$

$$\dot{c}_B = -k_{A+B}c_Ac_B$$

$$\dot{c}_C = 2k_{A+B}c_Ac_B.$$

This observation of the change in molecules of a species due to a reaction, that is, the number of molecules in the reactant complex subtracted from the number of molecules in the formed complex, is referred to as the *net stoichiometry*. For a general network with  $n$  species and  $m$  reactions, we can form an  $n \times m$  matrix,  $N$ , whose  $ij$ th entry is

the net stoichiometry of the  $i$ th species in the  $j$ th reaction.

We can now consider ourselves to be in a position to talk about the rate of change of concentration of each species in a given CRN. Let us illustrate how, again using the Michaelis-Menten example.

**Example 1.5** Recall,



where  $E$  is an enzyme,  $S$  is a substrate,  $C$  is an enzyme-substrate complex and  $P$  is the product. Let us order the species as  $\{E, S, C, P\}$ , and order the reactions by their rate constants as  $\{k_{E+S \rightarrow C}, k_{C \rightarrow E+S}, k_{C \rightarrow E+P}\}$ . Then we get the  $4 \times 3$  stoichiometric matrix

$$N = \begin{bmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}.$$

Note that  $N_{32}$ , for example, takes the value  $-1$  since one molecule of the third species,  $C$ , is lost every time the second reaction,  $C \rightarrow E + S$ , occurs. Using the law of mass action, we easily find the equations for the rate of change of concentrations of each species to be

$$\begin{aligned} \dot{c}_E &= -k_{E+S \rightarrow C} c_E c_S + k_{C \rightarrow E+S} c_C + k_{C \rightarrow E+P} c_C \\ \dot{c}_S &= -k_{E+S \rightarrow C} c_E c_S + k_{C \rightarrow E+S} c_C \\ \dot{c}_C &= k_{E+S \rightarrow C} c_E c_S - k_{C \rightarrow E+S} c_C - k_{C \rightarrow E+P} c_C \\ \dot{c}_P &= k_{C \rightarrow E+P} c_C. \end{aligned}$$

**Remark** The right hand sides of the above equations can be obtained by computing the simple matrix-vector multiplication  $NK(c)$ , where  $K(c)$  denotes the vector whose elements are a product of the rate constants and corresponding powers of species.

What about if we wanted to model the augmented system? That is, within a CFSTR. We simply include the pseudo reactions  $\emptyset \rightarrow X$  and  $X \rightarrow \emptyset$  at the beginning,

and get

$$N = \begin{bmatrix} 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & -1 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 1 \end{bmatrix} = \left[ I_4 \mid -I_4 \mid N' \right]$$

where  $N'$  is the stoichiometric matrix of the non-augmented system,  $I_4$  is the  $4 \times 4$  identity matrix, and  $g/V = 1$  for ease of calculation. From this, we simply obtain

$$\begin{aligned} \dot{c}_E &= (c_E^f - c_E) - k_{E \rightarrow S \rightarrow C} c_E c_S + k_{C \rightarrow E+S} c_C + k_{C \rightarrow E+P} c_C \\ \dot{c}_S &= (c_S^f - c_S) - k_{E \rightarrow S \rightarrow C} c_E c_S + k_{C \rightarrow E+S} c_C \\ \dot{c}_C &= (c_C^f - c_C) + k_{E \rightarrow S \rightarrow C} c_E c_S - k_{C \rightarrow E+S} c_C - k_{C \rightarrow E+P} c_C \\ \dot{c}_P &= (c_P^f - c_P) + k_{C \rightarrow E+P} c_C. \end{aligned}$$

**Definition 1.6** The *stoichiometric subspace*, denoted  $S$ , is defined as

$$S = \text{span}\{y' - y \mid y \rightarrow y' \in \mathcal{R}\}.$$

For  $c \in \mathbb{R}^n$ , we say  $c+S$  and  $(c+S) \cap \overline{\mathbb{R}}_+^n$  are the *stoichiometric compatibility classes* and *positive stoichiometric compatibility classes* of the network, respectively. We reserve  $s$  to denote  $\dim(S)$ , the dimension of  $S$ .

**Remark** • The stoichiometric subspace of a network can also be defined as the column space of its stoichiometric matrix,  $\text{col}(N)$ . In other words, the span of the columns of the matrix  $N$ . Hence  $s$  can be determined by simply finding the rank of  $N$ .

- This definition may seem a bit out of place here, but it will prove quite useful in later sections.

One can generalise the equations given at the end of Example 1.5 to describe the time evolution of any chemical reaction network as follows:

**Definition 1.7** The rate of change in concentration,  $c$ , of a reaction network under the assumption of mass action kinetics, is given by

$$\frac{dc}{dt} = \sum_{y \rightarrow y' \in \mathcal{R}} K_{y \rightarrow y'}(c)(y' - y) = \sum_{y \rightarrow y' \in \mathcal{R}} k_{y \rightarrow y'} c^y (y' - y),$$

where  $y, y' \in \mathcal{C}$  are complexes and  $k_{y \rightarrow y'}$  is given by some rate function.

We have all the tools at our disposal now to ask the following question: *Does a given system of ODE's derived from a chemical reaction network admit multiple positive equilibria for all positive rate constants, and all non-negative feed concentrations?*

We can continue with our current example to press this question, and perhaps help us to understand what else we might need to allow us to answer it.

Since we are interested in equilibria, letting  $\dot{c} = 0$ , and  $g/V = 1$  without loss of generality, we can rearrange the right hand side to get

$$\begin{aligned} c_E^f &= c_E + k_{E+S \rightarrow C} c_E c_S - k_{C \rightarrow E+S} c_C - k_{C \rightarrow E+P} c_C \\ c_S^f &= c_S + k_{E+S \rightarrow C} c_E c_S - k_{C \rightarrow E+S} c_C \\ c_C^f &= c_C - k_{E+S \rightarrow C} c_E c_S + k_{C \rightarrow E+S} c_C + k_{C \rightarrow E+P} c_C \\ c_P^f &= c_P - k_{C \rightarrow E+P} c_C. \end{aligned}$$

Define  $k$  to be the vector of rate constants for a CRN,  $\mathcal{N}$ . We can denote by  $p_{\mathcal{N}}(c, k)$  the vector of right hand sides of the above set of equations. We choose  $p$  since the entries in this vector are *polynomials* in the species concentrations. In general,  $p_{\mathcal{N}}(c, k)$  is referred to as the polynomial function associated to the reaction network.

Suppose there exist two distinct positive concentrations,  $c_1$  and  $c_2$ , such that  $p_{\mathcal{N}}(c_1, k) = p_{\mathcal{N}}(c_2, k) = c^f$ . This shows that our system has (at least) two positive concentrations for which equilibrium is achieved. If this happens to be the case, then the function  $c \rightarrow p_{\mathcal{N}}(c, k)$  is not injective. In other words, injectivity of  $c \rightarrow p_{\mathcal{N}}(c, k)$  is sufficient for the absence of multiple equilibria. This observation leads us into the next section.

## Chapter 2

# Injectivity of a Reaction Network

WHAT use to us is this newly defined polynomial function? It turns out that we can use certain algebraic properties to help us decide if a network,  $\mathcal{N}$ , is injective. First, what exactly does it mean for a network,  $\mathcal{N}$ , to be injective?

## 2.1 Properties of Reaction Networks

**Definition 2.1** We say a chemical reaction network  $\mathcal{N}$  is injective if the polynomial function  $c \rightarrow p_{\mathcal{N}}(c, k)$  is injective for all  $k \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}$ .

**Remark** It is important not to confuse the order of implications with injectivity and capacity for multiple equilibria. We are only able to conclude that, if  $\mathcal{N}$  is injective, then the network *cannot* support multiple equilibria. Thus injectivity is a sufficient condition for the absence of multiple equilibria.

But  $p_{\mathcal{N}}(\cdot, \cdot) : \mathbb{R}_+^{\mathcal{S}} \times \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o} \rightarrow \mathbb{R}_+^{\mathcal{S}}$  is a multidimensional function with many unknown parameters, and so typically injectivity is a difficult property to check. The following theorem allows us to overcome this barrier:

**Theorem 2.2** A reaction network  $\mathcal{N}$  is injective if and only if we have

$$\det \left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right) \neq 0 \tag{2.1}$$

for all  $c \in \mathbb{R}_+^{\mathcal{S}}$  and all  $k \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}$ .

A proof of this theorem is given in a paper by Craciun and Feinberg<sup>[2]</sup>, and is given here for completeness. Note we prove the contrapositive. Before this we define a new scalar product over  $\mathbb{R}^S$  first given in Feinberg's 1995 paper<sup>[3]</sup>.

**Definition 2.3** Define the scalar product  $*$  by

$$t * z = \sum_{s \in S} \frac{t_s z_s}{c_s},$$

where  $c_s$  defines the concentration of each the species  $s$ .

*Proof of Theorem 2.2.* We will prove that the negation of equation (2.1) implies non-injectivity of  $\mathcal{N}$  through a set of equivalences. Let  $\xi \in \mathbb{R}^S$  be an arbitrary vector. Applying  $\frac{\partial p_{\mathcal{N}}}{\partial c}(c, k)$  to  $\xi$  gives

$$\left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right) (\xi) = \sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} k_{y \rightarrow y'} c^y (y * \xi) (y - y').$$

The negation of (2.1) can be phrased as follows:

$$\det \left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right) (\xi) = 0 \text{ for some } c \in \mathbb{R}_+^S, k \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \xi \in \mathbb{R}^S, \xi \neq 0, \quad (2.2)$$

or equivalently

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} k_{y \rightarrow y'} c^y (y * \xi) (y - y') = 0 \text{ for some } c \in \mathbb{R}_+^S, k \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \quad (2.3)$$

$$\xi \in \mathbb{R}^S, \xi \neq 0.$$

Using the change of variables  $\eta_{y \rightarrow y'} = k_{y \rightarrow y'} c^y$  and  $\delta_s = \xi_s / c_s$ , (2.3) becomes

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} \eta_{y \rightarrow y'} (y \cdot \delta) (y - y') = 0 \text{ for some } \eta \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \quad (2.4)$$

$$\text{and some } \delta \in \mathbb{R}^S, \delta \neq 0,$$

where  $\cdot$  is the usual scalar product over  $\mathbb{R}^S$ . Now, if  $y \cdot \delta < 0$ , then  $e^{y \cdot \delta} < 1$ , or  $e^{y \cdot \delta} - 1 < 0$ . And similarly if  $y \cdot \delta > 0$ , then  $e^{y \cdot \delta} - 1 > 0$ . In other words, both  $y \cdot \delta$  and  $e^{y \cdot \delta} - 1$  are the same sign, regardless of the value of  $y \cdot \delta$ , and so (2.4) can equivalently be written as

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} K_{y \rightarrow y'} (e^{y \cdot \delta} - 1) (y - y') = 0 \text{ for some } K \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \quad (2.5)$$

$$\text{and some } \delta \in \mathbb{R}^S, \delta \neq 0.$$

With the change of variables  $\frac{b_s}{a_s} = e^{\delta_s}$ , this becomes

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} K_{y \rightarrow y'} \left( \frac{b^y}{a^y} - 1 \right) (y - y') = 0 \text{ for some } K \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \quad (2.6)$$

and some  $a \neq b \in \mathbb{R}_+^{\mathcal{S}}$ .

Note that  $a \neq b$  if and only if  $\delta \neq 0$ . Finally, with the change  $\kappa_{y \rightarrow y} = \frac{K_{y \rightarrow y}}{a^y}$ , we arrive at

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} \kappa_{y \rightarrow y'} (b^y - a^y) (y - y') = 0 \text{ for some } \kappa \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}, \quad (2.7)$$

and some  $a \neq b \in \mathbb{R}_+^{\mathcal{S}}$ .

If we rearrange this equation, the result then becomes quite clear.

$$\sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} \kappa_{y \rightarrow y'} b^y (y - y') = \sum_{y \rightarrow y' \in \mathcal{R}_t \cup \mathcal{R}_o} \kappa_{y \rightarrow y'} a^y (y - y') \quad (2.8)$$

In other words, for some  $\kappa \in \mathbb{R}_+^{\mathcal{R}_t \cup \mathcal{R}_o}$ , and some  $a \neq b \in \mathbb{R}_+^{\mathcal{S}}$ , we have shown  $p_{\mathcal{N}}(a, \kappa) = p_{\mathcal{N}}(b, \kappa)$ . This violates the injectivity property, and so  $p_{\mathcal{N}}(\cdot, \kappa)$  is not injective on  $\mathbb{R}_+^{\mathcal{S}}$ . Thus the reaction network  $\mathcal{N}$  is injective if and only if (2.1) is true.  $\square$

As we shall see in the next section,  $\det \left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right)$  is a sum of monomials in  $c$  and  $k$ . Now, in theory, equation (2.1) could be satisfied if some of the coefficients were negative. Take for example the fact that the polynomial  $x^2 - x + 1$  takes positive (i.e. nonzero) values for all  $x$ , even though one of its coefficients is negative. However, the following theorem<sup>[2]</sup> tells us that, in the context of CRNT, this can't be the case.

**Theorem** (Theorem 3.3 – Craciun and Feinberg, 2005) *A reaction network  $\mathcal{N}$  is injective if and only if all the coefficients<sup>a</sup> in the expansion of  $\det \left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right)$  are non-negative.*

This result relies on Theorem 2.13, for which a sketch proof is given. First, we will need a technical lemma.

## 2.2 A Technical Lemma for Theorem 2.13

This following section concerns a remark made in the above cited paper, which will be proved here, and then used in the sketch proof of Theorem 2.13.

---

<sup>a</sup>I refer to here, and from now on, the coefficients of the monomials with respect to  $c$  and  $k$ .



When the reaction kinetics are given by the Law of Mass Action, the determinant of the Jacobian (see equation (2.13)) is a polynomial whose constituent monomials are products of concentrations  $c_i$  and mass action rate constants,  $k_j$ . Thus, for example, the Jacobian for the CFSTR version of the Michaelis-Menten system (see Section 2.2.4 for details) is

$$c_2 k_1 k_3 k_5 k_7 + c_1 k_1 k_4 k_6 k_7 + c_2 k_1 k_5 k_6 k_7 + k_2 k_4 k_5 k_7 + k_3 k_4 k_5 k_7 + k_4 k_5 k_6 k_7. \quad (2.9)$$

Craciun and Feinberg assert that, in general, rate constants only appear in  $\det(J)$  with exponent 1, so that, for example, a term such as  $k_1^2 c_1 c_2$  can never appear. Here it is proved as a technical lemma, and used to give a sketch proof of the theorem in an alternative way.

### 2.2.1 A Brief Recap of Notation

A chemical reaction network,  $\mathcal{N}$ , contains  $n$  chemical species, and  $m$  reactions. In earlier examples I have used capital letters, such as  $X, Y, Z$ , to denote separate species. Here I will be more formal, and say the species are represented by  $X_1, \dots, X_n$ . We can then define the  $i$ -th reaction with the expression

$$\sum_{j=1}^n \alpha_{ij} X_j \rightarrow \sum_{j=1}^n \beta_{ij} X_j, \quad (2.10)$$

where the  $n$ -tuples  $\alpha_i, \beta_i \in \mathbb{R}^n$  list the coefficients appearing on, respectively, the reactant and product complexes of the  $i$ -th reaction. Hence from this, we easily obtain the stoichiometric matrix,

$$N = \begin{bmatrix} \beta_{11} - \alpha_{11} & \beta_{12} - \alpha_{12} & \cdots & \beta_{1n} - \alpha_{1n} \\ \beta_{21} - \alpha_{21} & \beta_{22} - \alpha_{22} & \cdots & \beta_{2n} - \alpha_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \beta_{m1} - \alpha_{m1} & \beta_{m2} - \alpha_{m2} & \cdots & \beta_{mn} - \alpha_{mn} \end{bmatrix}.$$

By mass action kinetics, the ODEs for the concentrations then take the form

$$\frac{dc}{dt} = N \cdot K(c) \quad (2.11)$$

where

- $c \in \overline{\mathbb{R}}_+^n$  is the column vector of non-negative chemical concentrations;

- $N$  is the *stoichiometric matrix*, and
- $K(c)$ , which I will typically write as  $K$ , is the column vector whose elements are a product of the rate constants and corresponding powers of species, as remarked directly after Definition 1.7. Note that these elements are sometimes called *fluxes*, and I will refer to them as such from here.

Some of the results in Section 2.2.3 hold in greater generality than others. To help make the necessary distinctions, I'll define some notation for dynamics of a chemical reaction network with arbitrary fluxes.

**Definition 2.4** The rate of change of the concentration of the  $i$ -th species will be abbreviated  $f_i(c, k)$ , so that

$$f_i(c, k) \equiv \frac{dc_i}{dt} = \sum_{r=1}^m N_{ir} \cdot K_r(c). \quad (2.12)$$

This definition also provides a concise notation for the entries in the Jacobian  $J$  of the dynamics.

$$J_{ij} = \frac{\partial f_i}{\partial c_j}(c, k) = \sum_{r=1}^m N_{ir} \frac{\partial K_r}{\partial c_j}, \quad (2.13)$$

which means that we can think of a row of  $J$  as a gradient.

**Definition 2.5** The gradient with respect to the concentrations of a function  $g(c, k)$  is

$$\nabla_c g(c, k) \equiv \left( \frac{\partial g}{\partial c_1}, \dots, \frac{\partial g}{\partial c_n} \right).$$

Using this notation, we can write the  $i$ -th row of the Jacobian,  $J_i$ , as

$$J_i = \nabla_c f_i(c, k) = \left( \frac{\partial f_i}{\partial c_1}, \dots, \frac{\partial f_i}{\partial c_n} \right). \quad (2.14)$$

## 2.2.2 Mass Action Kinetics

For mass action kinetics we have that  $K_i$ , the flux for the  $i$ -th reaction, is given by

$$K_i = k_i c^{\alpha_i} \equiv k_i \prod_{j=1}^n c_j^{\alpha_{ij}}. \quad (2.15)$$

In this case there is exactly one parameter—a positive rate coefficient—per reaction and so  $k \in \overline{\mathbb{R}}_+^m$ . It'll prove convenient in what follows to have a name for that part of  $K_i$  that depends on the concentrations alone, so I'll introduce:

**Definition 2.6** For a system with mass action kinetics, the concentration monomial associated with the flux  $K_r(c)$  of the reaction  $\sum_{j=1}^n \alpha_{rj} X_j \xrightarrow{k_r} \sum_{j=1}^n \beta_{rj} X_j$  is

$$u_r(c) \equiv \frac{K_r(c)}{k_r} = c^{\alpha_r} = \prod_{s=1}^n c_s^{\alpha_{rs}}.$$

For mass action kinetics the functions  $f_i(c, k)$  take the special form

$$\begin{aligned} f_i(c, k) &= \sum_{r=1}^m N_{ir} K_r \\ &= \sum_{r=1}^m N_{ir} k_r u_r(c) \\ &= \sum_{r=1}^m (\beta_{ri} - \alpha_{ri}) k_r c^{\alpha_r}, \end{aligned} \tag{2.16}$$

while the entries in the Jacobian become

$$\begin{aligned} J_{ij} &= \sum_{r=1}^m N_{ir} k_r \frac{\partial u_r}{\partial c_j} \\ &= \sum_{r=1}^m (\beta_{ri} - \alpha_{ri}) k_r \alpha_{rj} \frac{c^{\alpha_r}}{c_j}. \end{aligned} \tag{2.17}$$

### 2.2.3 Main Result

Let's begin with an observation about products of sums,

$$\left( \sum_{i=1}^m a_i \right) \left( \sum_{j=1}^m b_j \right) = \sum_{(i,j) \in \mathbb{N}_m \times \mathbb{N}_m} a_i b_j,$$

where  $\mathbb{N}_m = \{1, \dots, m\}$ . That is, we can interchange the order of summing and product-taking to convert a product of two sums into a sum over pairs of indices. Each term in this new sum will be a product of two factors, one from each of the original sums. More generally, we have the following:

**Proposition 2.7** (Products of sums) *One can write a product of  $n$  sums as a sum over  $n$ -tuples of indices, each term of which is a product of  $n$  factors.*

$$\prod_{i=1}^n \left( \sum_{j=1}^m a_{ij} \right) = \sum_{(j_1, \dots, j_n) \in \mathbb{N}_m^n} \prod_{i=1}^n a_{ij_i} = \sum_{\omega \in \mathbb{N}_m^n} \prod_{i=1}^n a_{i\omega_i}, \tag{2.18}$$

where

$$\mathbb{N}_m^n = \underbrace{\mathbb{N}_m \times \dots \times \mathbb{N}_m}_{n \text{ times}}$$

and  $\omega = (\omega_1, \dots, \omega_n)$ .

We can also introduce this definition, about the determinant of a matrix.

**Definition 2.8** (Leibniz formula for determinants) Let  $A$  be an  $n \times n$  matrix and  $S_n$  denote the symmetric group on  $n$  elements. Then a formula for the determinant of  $A$  is given by

$$\sum_{\sigma \in S_n} \text{sgn}(\sigma) \prod_{i=1}^n a_{\sigma(i)i},$$

where  $\text{sgn}(\sigma)$  gives the parity of a permutation  $\sigma \in S_n$ .

Now recall Eqn. (2.13), which says that the entries in the Jacobian are sums over the reactions,

$$J_{ij} = \sum_{r=1}^m N_{ir} \frac{\partial K_r}{\partial c_j}.$$

This means that  $\det(J)$  involves just the sorts of products governed by Prop. 2.7 and so we can rewrite it as follows:

$$\begin{aligned} \det(J) &= \sum_{\sigma \in S_n} \text{sgn}(\sigma) \prod_{i=1}^n J_{i\sigma(i)} \\ &= \sum_{\sigma \in S_n} \text{sgn}(\sigma) \prod_{i=1}^n \left( \sum_{r=1}^m N_{ir} \frac{\partial K_r}{\partial c_{\sigma(i)}} \right) \\ &= \sum_{\sigma \in S_n} \text{sgn}(\sigma) \left( \sum_{\omega \in \mathbb{N}_m^n} \prod_{i=1}^n N_{i\omega_i} \frac{\partial K_{\omega_i}}{\partial c_{\sigma(i)}} \right) \\ &= \sum_{\omega \in \mathbb{N}_m^n} \left( \sum_{\sigma \in S_n} \text{sgn}(\sigma) \prod_{i=1}^n N_{i\omega_i} \frac{\partial K_{\omega_i}}{\partial c_{\sigma(i)}} \right) \end{aligned} \quad (2.19)$$

where Prop. 2.7 gets us from the second line to the third and then swapping the order of the sums over  $\sigma$  and  $\omega$  takes us from the third to the fourth.

The product in parentheses in Eqn. (2.19) is—separately, for each  $\omega \in \mathbb{N}_m^n$ —also a determinant, but not of the full Jacobian. Instead it's  $\det(J_\omega)$ , where  $J_\omega$  is an  $n \times n$  matrix whose  $i$ -th row is

$$[J_\omega]_{i\cdot} = N_{i\omega_i} \nabla_c K_{\omega_i} \quad (2.20)$$

This observation is the key to the following proposition, which has our main result as an immediate consequence.

**Proposition 2.9** ( $J_\omega$  vanishes for many  $\omega$ ) *If  $J_\omega$  is defined as in Eqn. (2.20) and if  $\omega \in \mathbb{N}_m^n$  contains repeated entries—that is, if  $\omega_i = \omega_j$  for some  $i \neq j$ —then*

$$\det(J_\omega) = 0.$$

*Proof.* The rows of  $J_\omega$  are given by Eqn. (2.20), which implies that if  $\omega_i = \omega_j$  for some  $i \neq j$ , then one of the following two things is true.

- $N_{i\omega_i} = 0$  or  $N_{j\omega_j} = 0$ . In this case  $J_\omega$  contains one or more rows of zeroes.
- $N_{i\omega_i} \neq 0$  and  $N_{j\omega_j} \neq 0$ . In this case we have that

$$\begin{aligned} [J_\omega]_{i\cdot} &= N_{i\omega_i} \nabla_c K_{\omega_i} \\ &= N_{i\omega_i} \nabla_c K_{\omega_j} \\ &= \left( \frac{N_{i\omega_i}}{N_{j\omega_j}} \right) N_{j\omega_j} \nabla_c K_{\omega_j} \\ &= \left( \frac{N_{i\omega_i}}{N_{j\omega_j}} \right) [J_\omega]_{j\cdot} \end{aligned}$$

which means that the rows  $[J_\omega]_{i\cdot}$  and  $[J_\omega]_{j\cdot}$  are parallel.

In either case,  $\det(J_\omega) = 0$

□

**Lemma 2.10** ( $\det(J)$  for mass action systems) *For chemical reaction networks with mass action kinetics in which the number of reactions  $m$  exceeds the number of chemical species  $n$ , the determinant  $\det(J)$  of the Jacobian of the dynamics can be written as a sum of the form*

$$\sum_{\substack{U \subseteq \mathbb{N}_m \\ |U|=n}} \left( \prod_{j \in U} k_j \right) p_U(c)$$

where  $p_U(c)$  is a polynomial in the concentrations.

The following definition simplifies the proof a bit.

**Definition 2.11** The **support** of  $\omega \in \mathbb{N}_m^n$ , written  $\text{supp}(\omega)$ , is the set

$$\text{supp}(\omega) \equiv \{j \in \mathbb{N}_m \mid \omega_i = j \text{ for some } 1 \leq i \leq n\}.$$

Note that  $|\text{supp}(\omega)| = n$  if and only if all the entries in  $\omega$  are distinct.

**Example 2.12** If we have  $\omega = \underbrace{(1, 1, \dots, 1)}_{n \text{ times}}$ , then  $\text{supp}(\omega) = \{1\}$ . Or, we may have  $\omega = (1, 2, \dots, n)$ , in which case  $\text{supp}(\omega) = \{1, 2, \dots, n\}$ . Clearly in the latter instance,  $|\text{supp}(\omega)| = n$ .

*Proof of Lemma 2.10.* As the network has mass action kinetics, we can use Eqn. (2.15) to say

$$\begin{aligned}
 \det(J_\omega) &= \sum_{\sigma \in S_n} \operatorname{sgn}(\sigma) \prod_{i=1}^n N_{i\omega_i} \frac{\partial K_{\omega_i}}{\partial c_{\sigma(i)}} \\
 &= \sum_{\sigma \in S_n} \operatorname{sgn}(\sigma) \prod_{i=1}^n N_{i\omega_i} k_{\omega_i} \frac{\partial u_{\omega_i}}{\partial c_{\sigma(i)}} \\
 &= \sum_{\sigma \in S_n} \operatorname{sgn}(\sigma) \left( \prod_{i=1}^n k_{\omega_i} \right) \left( \prod_{j=1}^n N_{j\omega_j} \frac{\partial u_{\omega_j}}{\partial c_{\sigma(j)}} \right) \\
 &= \left( \prod_{i=1}^n k_{\omega_i} \right) \sum_{\sigma \in S_n} \operatorname{sgn}(\sigma) \prod_{j=1}^n N_{j\omega_j} \frac{\partial u_{\omega_j}}{\partial c_{\sigma(j)}} \\
 &= \left( \prod_{i=1}^n k_{\omega_i} \right) p_\omega(c),
 \end{aligned} \tag{2.21}$$

where

$$p_\omega(c) = \sum_{\sigma \in S_n} \operatorname{sgn}(\sigma) \prod_{j=1}^n N_{j\omega_j} \frac{\partial u_{\omega_j}}{\partial c_{\sigma(j)}} \tag{2.22}$$

is a polynomial that depends on the concentrations alone. This is because  $u_r$ , the concentration monomial for the  $r$ -th reaction, depends only on the concentrations. We've thus established that  $\det(J_\omega)$  is the product of a monomial in the parameters  $k_i$  with a polynomial in the concentrations.

At this stage in the argument we still need to entertain the possibility that the monomial in the  $k_j$  contains factors with exponents bigger than 1, which happens if and only if  $\omega_i = \omega_j$  for some  $i \neq j$ . But then Prop. 2.9 comes into play. It tells us that whenever the  $k$ -monomial in Eqn. (2.21) has some factor with an exponent bigger than 1,  $\det(J_\omega) = 0$ . Equivalently,  $\det(J_\omega) \neq 0$  implies that all the entries in  $\omega$  are distinct.

Further, the ordering of the entries in  $\omega$  does not affect the product  $\prod_{i=1}^n k_{\omega_i}$ . This means that we can rewrite  $\det(J)$  as follows

$$\begin{aligned}
 \det(J) &= \sum_{\omega \in \mathbb{N}_m^n} \det(J_\omega) \\
 &= \sum_{\substack{\omega \in \mathbb{N}_m^n \\ |\operatorname{supp}(\omega)|=n}} \det(J_\omega) \\
 &= \sum_{\substack{U \subset \mathbb{N}_m \\ |U|=n}} \sum_{\substack{\omega \in \mathbb{N}_m^n \\ \operatorname{supp}(\omega)=U}} \det(J_\omega)
 \end{aligned}$$

$$\begin{aligned}
 &= \sum_{\substack{U \subset \mathbb{N}_m \\ |U|=n}} \sum_{\substack{\omega \in \mathbb{N}_m^n \\ \text{supp}(\omega)=U}} \left( \prod_{i=1}^n k_{\omega_i} \right) p_{\omega}(c) \\
 &= \sum_{\substack{U \subset \mathbb{N}_m \\ |U|=n}} \left( \prod_{j \in U} k_j \right) \sum_{\substack{\omega \in \mathbb{N}_m^n \\ \text{supp}(\omega)=U}} p_{\omega}(c) \\
 &= \sum_{\substack{U \subset \mathbb{N}_m \\ |U|=n}} \left( \prod_{j \in U} k_j \right) p_U(c),
 \end{aligned} \tag{2.23}$$

where

$$p_U(c) = \sum_{\substack{\omega \in \mathbb{N}_m^n \\ \text{supp}(\omega)=U}} p_{\omega}(c).$$

The sum in Eqn. (2.23) has the exactly the form described in the conclusion of Lemma 2.10, so we are finished.  $\square$

### 2.2.4 The Michaelis-Menten CFSTR

If we order the species as in  $(S, E, C, P)$  and the reactions as listed in Table 2.1, then the system of ODEs (of the augmented system) is specified by

$$\frac{dc}{dt} = \begin{bmatrix} -1 & 1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ -1 & 1 & 1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} k_1 c_1 c_2 \\ k_2 c_3 \\ k_3 c_3 \\ k_4 c_1 \\ k_5 c_2 \\ k_6 c_3 \\ k_7 c_4 \\ k_8 \\ k_9 \\ k_{10} \\ k_{11} \end{bmatrix} \tag{2.24}$$

or

$$\frac{d}{dt} \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{bmatrix} = \begin{bmatrix} k_8 - k_1 c_1 c_2 + k_2 c_3 - k_4 c_1 \\ k_9 - k_1 c_1 c_2 + k_2 c_3 + k_3 c_3 - k_5 c_2 \\ k_{10} + k_1 c_1 c_2 - k_2 c_3 - k_3 c_3 - k_6 c_3 \\ k_{10} + k_3 c_3 - k_7 c_4 \end{bmatrix}. \tag{2.25}$$

<i>Type</i>	<i>Name</i>	<i>Reaction</i>	<i>Flux</i>
True reactions	complex formation	$S + E \xrightarrow{k_1} C$	$k_1 c_1 c_2$
	complex dissociation	$C \xrightarrow{k_2} S + E$	$k_2 c_3$
	product formation	$C \xrightarrow{k_3} P + E$	$k_3 c_3$
Outflow reactions	substrate outflow	$S \xrightarrow{k_4} \emptyset$	$k_4 c_1 = (g/V) c_1$
	enzyme outflow	$E \xrightarrow{k_5} \emptyset$	$k_5 c_2 = (g/V) c_2$
	complex outflow	$C \xrightarrow{k_6} \emptyset$	$k_6 c_3 = (g/V) c_3$
	product outflow	$P \xrightarrow{k_7} \emptyset$	$k_7 c_4 = (g/V) c_4$
Inflow reactions	substrate inflow	$\emptyset \xrightarrow{k_8} S$	$k_8 = (g/V) c_{1\star}$
	enzyme inflow	$\emptyset \xrightarrow{k_9} E$	$k_9 = (g/V) c_{2\star}$
	complex inflow	$\emptyset \xrightarrow{k_{10}} C$	$k_{10} = (g/V) c_{3\star}$
	product inflow	$\emptyset \xrightarrow{k_{11}} P$	$k_{11} = (g/V) c_{4\star}$

Table 2.1: The reactions for a CFSTR in which the Michaelis-Menten system is operating. Here the species are ordered  $(S, E, C, P)$ , and  $g$  is the rate of inflow (in, say, liters/sec), while  $V$  is the volume of the reactor and  $c_{j\star}$  is the concentration of species  $j$  in the inflow stream.



**Remark** Note here that both the ordering of species *and* reactions is different to when we previously looked at the Michaelis-Menten system in Example 1.5, but it is entirely similar (upto labelling), and causes no problems as long as we are consistent within our example.

### The determinant of $J$ as a sum over subsets

The Jacobian of the system in Eqn. (2.25) is

$$J = \begin{bmatrix} -c_2k_1 - k_4 & -c_1k_1 & k_2 & 0 \\ -c_2k_1 & -c_1k_1 - k_5 & k_2 + k_3 & 0 \\ c_2k_1 & c_1k_1 & -k_2 - k_3 - k_6 & 0 \\ 0 & 0 & k_3 & -k_7 \end{bmatrix}$$

and its determinant is

$$c_2k_1k_3k_5k_7 + c_1k_1k_4k_6k_7 + c_2k_1k_5k_6k_7 + k_2k_4k_5k_7 + k_3k_4k_5k_7 + k_4k_5k_6k_7. \quad (2.26)$$

Here  $n = 4$  and  $m = 11$ , so if we wanted to compute  $\det(J)$  using the sum in Eqn. (2.23) we'd have to consider

$$\binom{11}{4} = \frac{11!}{4!7!} = 330$$

subsets of  $\mathbb{N}_{11}$ . Even if we exclude the outflow reactions—whose rates don't depend on concentration and hence don't contribute to  $\det(J)$ —there would still be

$$\binom{7}{4} = \frac{7!}{4!3!} = 35$$

subsets, each of which has an associated set of  $4! = 24$  index vectors  $\omega \in \mathbb{N}_7^4$ . All the same, it is interesting to work out a few of the terms.

If we take  $U = \{1, 3, 5, 7\}$  we should recover the first term in Eqn. (2.26). Table 2.2 shows a few of the index vectors  $\omega$  with  $\text{supp}(\omega) = U$ , as well as the associated matrices  $J_\omega$  given by Eqn. (2.20) and their determinants.

$\omega$	$J_\omega$	$\det(J_\omega)$
$(1, 3, 5, 7)$	$\begin{bmatrix} -c_2k_1 & -c_1k_1 & 0 & 0 \\ 0 & 0 & k_3 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_7 \end{bmatrix}$	0
$(1, 5, 3, 7)$	$\begin{bmatrix} -c_2k_1 & -c_1k_1 & 0 & 0 \\ 0 & -k_5 & 0 & 0 \\ 0 & 0 & -k_3 & 0 \\ 0 & 0 & 0 & -k_7 \end{bmatrix}$	$c_2k_1k_3k_5k_7$
$(3, 7, 1, 5)$	$\begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ c_2k_1 & c_1k_1 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$	0
$(7, 5, 1, 3)$	$\begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & -k_5 & 0 & 0 \\ c_2k_1 & c_1k_1 & 0 & 0 \\ 0 & 0 & k_3 & 0 \end{bmatrix}$	0

Table 2.2: Some of the  $4! = 24$  index vectors with  $\text{supp}(\omega) = \{1, 3, 5, 7\}$ . The only one for which  $\det(J_w) \neq 0$  is  $\omega = (1, 5, 3, 7)$ .

## 2.3 A Result About Coefficients

Now that we have our required result, we can outline a possible way to prove the following theorem. It is not presented here with as much rigour as is required in order to call it a true proof, but the sketch should hopefully give the reader an idea of how it could be done. For those dissatisfied with only a sketch, a proof can be found in the original paper.

**Theorem 2.13** *Let  $\mathcal{N}$  be a chemical reaction network with  $n$  species. For each coefficient in the expansion of  $\det\left(\frac{\partial p_{\mathcal{N}}}{\partial c}(c, k)\right)$  there is a set of  $n$  reactions  $\{y_1 \rightarrow y'_1, \dots, y_n \rightarrow y'_n\}$  (excluding inflow reactions) such that the coefficient is equal to*

$$\det([y_1, \dots, y_n]) \det([y_1 - y'_1, \dots, y_n - y'_n]). \quad (2.27)$$

*Moreover, for each choice of  $n$  reactions such that (2.27) is not zero, there is a corresponding coefficient in the expansion of  $\det\left(\frac{\partial p_{\mathcal{N}}}{\partial c}(c, k)\right)$ .*

*Sketch proof of Theorem 2.13.* It is worth noting that in the previous section, we have been talking about the Jacobian of the system. Now the Jacobian, although similar to  $p_{\mathcal{N}}(c, k)$ , is not identical. The Jacobian includes all reactions, whereas  $p_{\mathcal{N}}(c, k)$  excludes the ‘pseudo’ feed reactions. In terms of the determinant however, this difference is inconsequential – as we have seen previously the feed reactions simply append an identity matrix to  $N$ , the stoichiometric matrix. We can extend equation (2.17) to help us construct a general form for the Jacobian of the network.

$$J = N \cdot \text{diag}(k) \cdot [y_1, \dots, y_m] \cdot \text{diag}\left(\frac{c^{y_i}}{c_i}\right), \quad (2.28)$$

where

$$\text{diag}(k) = \begin{bmatrix} k_1 & & & \\ & k_2 & & \\ & & \ddots & \\ & & & k_m \end{bmatrix}$$

and

$$\text{diag}\left(\frac{c^{y_i}}{c_i}\right) = \begin{bmatrix} \frac{c^{y_1}}{c_1} & & & \\ & \frac{c^{y_2}}{c_2} & & \\ & & \ddots & \\ & & & \frac{c^{y_m}}{c_m} \end{bmatrix}.$$

We can also write  $N$  as

$$N = [y_1 - y'_1, \dots, y_m - y'_m],$$

so the determinant of  $J$  looks like

$$\begin{aligned} \det(J) &= \det \left( [y_1 - y'_1, \dots, y_m - y'_m] \cdot \text{diag}(k) \cdot [y_1, \dots, y_m] \cdot \text{diag} \left( \frac{c^{y_i}}{c_i} \right) \right) \\ &= \det ([y_1 - y'_1, \dots, y_m - y'_m] \cdot \text{diag}(k) \cdot [y_1, \dots, y_m]) \cdot \det \left( \text{diag} \left( \frac{c^{y_i}}{c_i} \right) \right). \end{aligned}$$

In the second equality I have separated the final term since it does not affect the value of the coefficient. We know that by Lemma 2.10 the determinant of the Jacobian can be written as a sum of the form

$$\sum_{\substack{U \subseteq \mathbb{N}_m \\ |U|=n}} \left( \prod_{j \in U} k_j \right) p_U(c),$$

or in other words, that the rate constants in each term in the expansion of the determinant have multiplicity not exceeding one. We can use this to our advantage here – the set  $U$  in the above expression can correspond to the  $y$ 's in the statement of the theorem. In other words, if we choose some  $n$  complexes  $\{y_1, \dots, y_n\}$ , then the coefficient of the determinant can be found by simply removing any rate constant  $k_i$  from  $\text{diag}(k)$  that does not relate to a  $y_i$  in  $U$ , resulting in

$$\begin{aligned} &\det ([y_1 - y'_1, \dots, y_m - y'_m] \cdot \text{diag}(k)' \cdot [y_1, \dots, y_m]) \\ &= \det ([y_1 - y'_1, \dots, y_n - y'_n] \cdot \text{diag}(k)'' \cdot [y_1, \dots, y_n]) \\ &= \det ([y_1 - y'_1, \dots, y_n - y'_n]) \det ([y_1, \dots, y_n]) \det (\text{diag}(k)'') \end{aligned}$$

where  $\text{diag}(k)'$  is the  $m \times m$  matrix where  $k_i = 0$  if  $y_i \notin U$ , and  $\text{diag}(k)''$  is the  $n \times n$  matrix whose  $i$ th column *and* row are removed if  $y_i \notin U$ . Thus the coefficient is

$$\det ([y_1 - y'_1, \dots, y_n - y'_n]) \det ([y_1, \dots, y_n])$$

giving us the required result.  $\square$

As discussed at the end of section 2.1, the paper<sup>[2]</sup> then goes on to prove (in what they call Theorem 3.3) that  $\mathcal{N}$  is injective if and only if all the coefficients in the expansion of  $\det \left( \frac{\partial p_{\mathcal{N}}}{\partial c}(c, k) \right)$  are non-negative. Using these two results together, Theorem 2.13 and Theorem 3.3, we only need to look at the subnetworks consisting

of exactly  $n$  reactions to determine whether or not a system *cannot* admit multiple positive equilibria. That is, if we can identify one negative coefficient, then  $\mathcal{N}$  is not injective. Unfortunately, we cannot use these results to confirm that a system has the capacity to support multiple equilibria – in other words, injectivity is only a sufficient condition for the absence of multiple equilibria. However, if given a chemical reaction network that *does* admit multiple positive equilibria, these two results let us find exactly which subnetworks create such a capacity – they are simply the sets of reactions  $\{y_1, \dots, y_n\}$  for which  $\det([y_1, \dots, y_n]) \det([y_1 - y'_1, \dots, y_n - y'_n])$  is negative.

# Chapter 3

## The Kernel of A

Now we will attempt to make use of the general structure of a chemical reaction network. This will allow us to take a more algebraic approach to answering our questions.

### 3.1 Some New Functions

We begin by defining some functions that will tell us a great deal about a network in terms of its species and complexes.

**Definition 3.1** We denote  $\mathbb{R}^{\mathcal{C}}$  as ‘complex’ space, i.e. the vector space of the complexes in a chemical reaction network. Let  $U \subseteq \mathcal{C}$ . Then  $(\omega_U)_y$  is the element of  $\mathbb{R}^{\mathcal{C}}$  such that

$$(\omega_U)_y = \begin{cases} 1 & \text{if } y \in U \\ 0 & \text{otherwise,} \end{cases}$$

the so called characteristic function. For a singleton subset  $\{y\}$ , we write  $\omega_y$  in place of  $\omega_{\{y\}}$ , so that  $(\omega_{\{y\}})_{y'} = 1$  only if  $y = y'$ . Then, just as  $\{e_i \mid 1 \leq i \leq n\}$  is a standard basis for  $n$  dimensional Euclidean space, so  $\{\omega_y \mid y \in \mathcal{C}\}$  is a standard basis for complex space. In other words, for each  $x \in \mathbb{R}^{\mathcal{C}}$ , one can write  $x = \sum_{y \in \mathcal{C}} x_y \omega_y$ , where  $x_y$  is the  $y^{\text{th}}$  component of  $x$  with respect to the standard basis.

**Definition 3.2** We also define the three following functions between complex and species space as follows. First,  $Y : \mathbb{R}^{\mathcal{C}} \rightarrow \mathbb{R}^{\mathcal{S}}$  by  $Y(\omega_y) = y$ . Second,  $\Psi : \mathbb{R}^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{C}}$  by

$\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y$ . Finally,  $A_k : \mathbb{R}^{\mathcal{C}} \rightarrow \mathbb{R}^{\mathcal{C}}$  by  $A_k(x) = \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y)$ .

But why should we define these functions? What do they mean? First, let's look at how they behave for a specific example.

**Example 3.3** Once again, we shall consider the Michaelis-Menten system as defined above by



and note that  $\mathcal{C} = \{E + S, C, E + P\}$ . Set up the system in the order  $\{E, S, C, P\}$ , so that  $c = [c_E, c_S, c_C, c_P]^T$ . From what we have just defined, our standard basis for this network is  $\{\omega_{E+S}, \omega_C, \omega_{E+P}\}$ , and for convenience we will write  $k = [k_{E+S \rightarrow C}, k_{C \rightarrow E+S}, k_{C \rightarrow E+P}]^T \equiv [k_1, k_{-1}, k_2]^T$ .

Let us compute, explicitly, each of the functions we have previously defined. Some of the intermediate steps are omitted, and left for the reader to fill in as required.

$$Y(\omega_{E+S}) = E + S$$

$$Y(\omega_C) = C$$

$$Y(\omega_{E+P}) = E + P,$$

and

$$\begin{aligned} \Psi(c) &= \sum_{y \in \mathcal{R}} c^y \omega_y \\ &= c_E c_S \omega_{E+S} + c_C \omega_C + c_E c_P \omega_{E+P} \\ &= \begin{bmatrix} c_E c_S \\ c_C \\ c_E c_P \end{bmatrix}. \end{aligned}$$

For illustrative purposes, let us take  $x \in \mathbb{R}^{\mathcal{C}}$  to be  $x = [1, 1, 1]^T$ . Then,

$$\begin{aligned} A_k(x) &= \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y) \\ &= \begin{bmatrix} -k_1 + k_{-1} \\ k_1 - k_{-1} - k_2 \\ k_2 \end{bmatrix}. \end{aligned}$$

Looking at the composition of these functions,  $Y A_k \Psi(c)$ , we find something rather surprising:

$$\begin{aligned}
 Y(A_k(\Psi(c))) &= Y \left( A_k \left( \sum_{y \in \mathcal{C}} c^y \omega_y \right) \right) \\
 &= Y \left( \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} \left( \sum_{y \in \mathcal{C}} c^y \omega_y \right)_y (\omega_{y'} - \omega_y) \right) \\
 &= Y \left( \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y (\omega_{y'} - \omega_y) \right) \\
 &= \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y (y' - y),
 \end{aligned}$$

since  $\left( \sum_{y \in \mathcal{C}} c^y \omega_y \right)_y = c^y$  by definition. But now, comparing this formula with Definition 1.7, we see they are exactly the same! Thus  $f(c) = Y A_k \Psi(c) = \frac{dc}{dt}$ . We can show this for our example above:

$$\begin{aligned}
 Y A_k \Psi \left( \begin{bmatrix} c_E \\ c_S \\ c_C \\ c_P \end{bmatrix} \right) &= Y A_k \left( \begin{bmatrix} c_E c_S \\ c_C \\ c_E c_P \end{bmatrix} \right) \\
 &= Y \left( \begin{bmatrix} -k_1 c_E c_S + k_{-1} c_C \\ k_1 c_E c_S - k_{-1} c_C - k_2 c_E c_P \\ k_2 c_E c_P \end{bmatrix} \right) \\
 &= (-k_1 c_E c_S + k_{-1} c_C) \cdot \begin{bmatrix} 1 \\ 1 \\ 0 \\ 0 \end{bmatrix} \\
 &\quad + (k_1 c_E c_S - k_{-1} c_C + k_2 c_E c_P) \cdot \begin{bmatrix} 0 \\ 0 \\ 1 \\ 0 \end{bmatrix} + (k_2 c_E c_P) \cdot \begin{bmatrix} 1 \\ 0 \\ 0 \\ 1 \end{bmatrix}
 \end{aligned}$$



$$= \begin{bmatrix} -k_1 c_E c_S + k_{-1} c_C + k_2 c_E c_P \\ -k_1 c_E c_S - k_{-1} c_C \\ k_1 c_E c_S - k_{-1} c_C - k_2 c_E c_P \\ k_2 c_E c_P \end{bmatrix} = \frac{dc}{dt}.$$

The following interesting result requires an additional definition, given about vectors in general.

**Definition 3.4** The logarithm of a vector  $v \in \mathbb{R}^n$  is defined by applying the logarithm function componentwise,

$$\ln(v) = (\ln(v_1), \ln(v_2), \dots, \ln(v_n))^T.$$

**Proposition 3.5**  $\ln(\Psi(c)) = Y^T \ln(c)$ .

*Proof.* First, recall that  $Y(\omega_z) = z$ , and  $\Psi(c) = \sum_{y \in \mathcal{C}} c^y \omega_y$ , and note that  $\ln(c^y) = y \cdot \ln(c) = \langle y, \ln(c) \rangle$ , where  $\langle \cdot, \cdot \rangle$  denotes the inner product of two vectors. Then, for any  $z \in \mathcal{C}$ ,

$$\begin{aligned} \langle \omega_z, \ln(\Psi(c)) \rangle &= \langle \omega_z, \sum_{y \in \mathcal{C}} \langle y, \ln(c) \rangle \omega_y \rangle && \text{(by Definition 3.4)} \\ &= \langle \omega_z, \langle z, \ln(c) \rangle \omega_z \rangle \\ &= \langle z, \ln(c) \rangle \\ &= \langle Y(\omega_z), \ln(c) \rangle \\ &= \langle \omega_z, Y^T \ln(c) \rangle \end{aligned}$$

where the last equality is true by definition, since if  $g: V \rightarrow W$  defines a linear map, then  $g^T: W \rightarrow V$  satisfies  $\langle g(v), w \rangle = \langle v, g^T(w) \rangle$  for all  $v \in V, w \in W$ . Thus,  $\ln(\Psi(c)) = Y^T \ln(c)$  as required.  $\square$

We want to use these functions, which give us important information about the structure of the network, to tell us more about the long term dynamics of the system. Here I will give the first of two definitions of a quantity called the *deficiency* of a chemical reaction network. This definition makes use of the newly defined functions, and can be found in Section 3 of the expanded notes from Gunawardena<sup>[4]</sup>.

**Definition 3.6** The *deficiency* of a chemical reaction network,  $\{\mathcal{S}, \mathcal{R}, \mathcal{C}\}$ , is given by  $\delta = \dim(\ker Y \cap \text{Im } A_k)$ .

This definition has an advantage over the other in the sense that proving the deficiency is a non-negative integer becomes trivial. It also demands greater significance here than in its other (computational) form, given later, since it is placed in direct relation to the structure of the network.

## 3.2 A Graph Theoretical Approach

In light of some of the questions we are going to want to ask involving the new functions we have defined, it will make sense for us to look at reaction networks from a different perspective. It turns out that we can gain a great deal of understanding about a specific network from its underlying structure, and that with the use of some new language we will be able to give the second definition of the *deficiency* of a network. Much of the language used in this section is somewhat specific to CRNT, however the ideas and definitions coincide with many well known concepts in graph theory, which the reader may already be comfortable with. For this reason, I will include here a dictionary of terms, which the reader can use to help familiarise themselves with what is presented here.

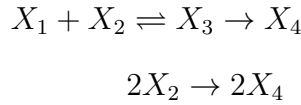
CRNT Term	Graph Theory Term
Complex $y$ ultimately reacts to form complex $y'$	Node $y'$ is reachable from node $y$
Strongly linked	Strongly connected
Linkage class	Weakly connected components
Strong linkage class	Strongly connected components
Linked	Weakly linked

Table 3.1: A dictionary showing the equivalent definitions between CRNT and graph theory.

### 3.2.1 Linkage Classes

When looking at the reaction diagram of a network, it is typically very plain to see which complexes are ‘*linked*’, or in non-technical terms, which sets of complexes have an arrow connecting them (not necessarily directly).

**Example 3.7** Consider the chemical reaction network with  $\mathcal{S} = \{X_1, X_2, X_3, X_4\}$ ,  $\mathcal{C} = \{X_1 + X_2, X_3, X_4, 2X_2, 2X_4\}$ , and  $\mathcal{R} = \{(X_1 + X_2, X_3), (X_3, X_1 + X_2), (X_3, X_4), (2X_2, 2X_4)\}$ . This naturally gives rise to the following reaction diagram:



In some sense we can say that  $X_1 + X_2$  and  $X_4$  are linked, though not in exactly the same way that  $X_3$  and  $X_4$  are.

We can extend this observation to a more formal construction, but first let us set the scene. Consider the *graph of a reaction network*, a directed graph whose nodes refer to the complexes, and whose directed edges refer to reactions between complexes. That is, if a network contains the reaction  $A \rightarrow B$  between the two complexes  $A, B$ , there would exist an edge emanating from the node corresponding to  $A$ , and ending up at the node corresponding to  $B$ . The following properties can now be defined.

**Definition 3.8** A complex  $y \in \mathcal{C}$  *ultimately reacts to form* a complex  $y' \in \mathcal{C}$  if any of the following three conditions hold:

- (i)  $y = y'$ ,
- (ii)  $y \rightarrow y'$ ,
- (iii)  $\mathcal{C}$  contains a sequence  $y_1, \dots, y_k$  (not necessarily all distinct) such that

$$y \rightarrow y_1, y_1 \rightarrow y_2, y_2 \rightarrow y_3, \dots, y_{k-1} \rightarrow y_k, y_k \rightarrow y'.$$

We call this sequence a *directed path*.

We write  $y \Rightarrow y'$  if  $y$  ultimately reacts to form  $y'$ . Two complexes are *strongly linked* if they each ultimately react to the other, and we write  $y \approx y'$  to show this. This equivalence relation  $\approx$  partitions  $\mathcal{C}$  into a family of equivalence classes called the *strong linkage classes* of the network, denoted  $\{\Lambda_j\}$ .

**Definition 3.9** We say that two complexes,  $y, y' \in \mathcal{C}$  are *directly linked* if either  $y$  reacts to form  $y'$ , or vice versa. We write  $y \leftrightarrow y'$  to convey that  $y$  and  $y'$  are directly linked. Two complexes  $y, y' \in \mathcal{C}$  are *linked* if any of the following three conditions hold:

- (i)  $y = y'$ ,
- (ii)  $y \leftrightarrow y'$ ,
- (iii)  $\mathcal{C}$  contains a sequence  $y_1, \dots, y_k$  (not necessarily all distinct) such that

$$y \leftrightarrow y_1, y_1 \leftrightarrow y_2, y_2 \leftrightarrow y_3, \dots, y_{k-1} \leftrightarrow y_k, y_k \leftrightarrow y'.$$

If  $y, y'$  are linked, we write  $y \sim y'$ . This equivalence relation  $\sim$  partitions  $\mathcal{C}$  into a family of equivalence classes called the *linkage classes* of the network, denoted  $\{L_i\}$ . We reserve  $l$  for the number of linkage classes in a given network,  $\mathcal{N}$ .

**Remark** • By definition,  $\Lambda_i, L_i \neq \emptyset, \forall i$ .

- Instead of writing

$$y \rightarrow y_1, y_1 \rightarrow y_2, y_2 \rightarrow y_3, \dots, y_{k-1} \rightarrow y_k, y_k \rightarrow y',$$

we will write

$$y \rightarrow y_1 \rightarrow y_2 \rightarrow \dots \rightarrow y_k \rightarrow y'.$$

Similarly, we will do this for  $\leftrightarrow$ .

**Lemma 3.10** *If two complexes,  $y, y' \in \mathcal{C}$  are strongly linked, then they are linked. That is,  $y \approx y' \implies y \sim y'$ .*

*Proof.* Suppose that  $y \approx y'$ . Then one of the three conditions given in Definition 3.8 holds, and so we will consider each of these three cases. If  $y = y'$  then, trivially, we are done. So consider the second case, that  $y \rightarrow y'$ . Then by definition,  $y$  and  $y'$  are directly linked, so  $y \leftrightarrow y'$ .

Finally, if  $y \rightarrow y_1 \rightarrow y_2 \rightarrow \dots \rightarrow y_k \rightarrow y'$  for some sequence  $y_1, \dots, y_k$ , then we can apply our result for the second condition repeatedly. As  $y \rightarrow y_1$ , we have  $y \leftrightarrow y_1$ . Similarly,  $y_1 \leftrightarrow y_2, y_2 \leftrightarrow y_3, \dots, y_k \leftrightarrow y'$ , and so once again we conclude that  $y$  and  $y'$  are linked.  $\square$

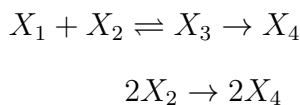
**Corollary 3.11** *Every linkage class is the disjoint union of strong linkage classes.*

*Proof.* Since the equivalence relation,  $\approx$ , partitions  $\mathcal{C}$ , we know that  $\Lambda_i \cap \Lambda_j = \emptyset$  for any pair  $(i, j)$ ,  $i \neq j$ . For the same reason,  $L_i \cap L_j = \emptyset$  for any pair  $(i, j)$ ,  $i \neq j$ . So it is enough for us to prove that each  $\Lambda_i$  is a subset of a unique  $L_j$ . By lemma 3.10, if two complexes are strongly linked ( $y, y' \in \Lambda_i$ ), then they are linked, and so lie in the corresponding linkage class,  $y, y' \in L_j$ , so  $\Lambda_i \subseteq L_j$ .

For uniqueness, suppose  $\Lambda_i \subseteq L_a$  and  $\Lambda_i \subseteq L_b$  for two linkage classes  $L_a, L_b$ . Then  $\Lambda_i \subseteq L_a \cap L_b = \emptyset$ , wherein lies our contradiction (see above remark).  $\square$

**Definition 3.12** A *terminal strong linkage class* is a strong linkage class  $\Lambda$  with the property that no complex in  $\Lambda$  reacts to form a complex not in  $\Lambda$ . In other words,  $y \in \Lambda$  and  $y \rightarrow y' \implies y' \in \Lambda$ . We reserve  $t$  for the number of terminal strong linkage classes in a network  $\mathcal{N}$ .

**Example 3.13** Recall the reaction network of Example 3.7, which is illustrated by the reaction diagram



By using the definitions we have just outlined, we can determine the linkage, strong linkage, and terminal strong linkage classes of this network. Firstly, the linkage classes are visually quite obvious. They are  $L_1 = \{X_1 + X_2, X_3, X_4\}$  and  $L_2 = \{2X_2, 2X_4\}$ . The strong linkage classes are slightly less obvious. They are  $\Lambda_1 = \{X_1 + X_2, X_3\}$ ,  $\Lambda_2 = \{X_4\}$ ,  $\Lambda_3 = \{2X_2\}$ , and  $\Lambda_4 = \{2X_4\}$ . Of these four, the two terminal strong linkage classes are  $\Lambda_2$  and  $\Lambda_4$ .

Now we are in a position to state the second definition of the deficiency of a network. It makes use of the new graph theoretical language with which we are now familiar, and has an obvious advantage to the previous definition.

**Definition 3.14** The *deficiency* of a chemical reaction network,  $\mathcal{N} = \{\mathcal{S}, \mathcal{R}, \mathcal{C}\}$ , is  $\delta = |\mathcal{C}| - l - s$ , where  $|\mathcal{C}|$  is the number of complexes,  $l$  is the number of linkage classes, and  $s$  is the dimension of the stoichiometric subspace of the network<sup>[5] [6]</sup>.

This definition of the deficiency of a network allows us to much more easily compute its value for a given network. All we need to do is count the number of complexes, find the number of linkage classes (usually by inspection of the network diagram), and then compute the dimension of the stoichiometric subspace, or equivalently the rank of the stoichiometric matrix.

**Remark** Although I implied that these definitions are equivalent, that is not strictly true in general. The second is the original definition introduced by Feinberg and Horn. In fact, for equality in these definitions the following proposition, again from Gunawardena<sup>[4]</sup> needs to hold.

**Proposition 3.15** *If each linkage class has precisely one terminal strong linkage class then the deficiency,  $\delta$ , of the network is given by  $\delta = |\mathcal{C}| - l - s$ .*

In other words, the two definitions agree whenever  $t = l$ . One way of overcoming this issue, as illustrated by Feinberg<sup>[1]</sup> is by changing our first definition. Instead of taking the image of  $A_k$ , we will define a different set and take its span.

**Definition 3.16** Define a new set  $\Delta$  by

$$\Delta := \{\omega_{y'} - \omega_y \in \mathbb{R}^c \mid y \sim y'\}.$$

This set proves to be quite useful in the next few results. An important thing to notice about this set is its similarity to something we have already encountered. Rather, its span is where the similarity is much more striking. If we take our function  $Y$  and apply it to the span of  $\Delta$ , a nice relationship emerges.

$$\begin{aligned} Y(\text{span}(\Delta)) &= Y(\text{span}(\{\omega_{y'} - \omega_y \in \mathbb{R}^c \mid y \sim y'\})) \\ &= \text{span}(Y(\{\omega_{y'} - \omega_y \in \mathbb{R}^c \mid y \sim y'\})) \\ &= \text{span}(\{y' - y \in \mathbb{R}^c \mid y \sim y'\}) \\ &= S, \end{aligned} \tag{3.1}$$

the stoichiometric subspace! The second equality requires some justification. I have swapped the order of  $Y$  and  $\text{span}$  since  $Y$  is a linear function. I will try to illustrate why this works with a small example.

**Example 3.17** Let  $Y$  be the function as defined above, and let  $X$  be the set  $X = \{a, b\}$  where  $a, b$  are arbitrary elements. By definition,  $\text{span}(X) = \{\lambda a + \mu b \mid \lambda, \mu \in \mathbb{R}\}$ . We will consider both  $Y(\text{span}(X))$  and  $\text{span}(Y(X))$  separately, and determine conditions for equality. First,

$$\begin{aligned} Y(\text{span}(X)) &= Y(\{\lambda a + \mu b \mid \lambda, \mu \in \mathbb{R}\}) \\ &= \{Y(\lambda a + \mu b) \mid \lambda, \mu \in \mathbb{R}\}, \end{aligned}$$

and

$$\begin{aligned} \text{span}(Y(X)) &= \text{span}(\{Y(a), Y(b)\}) \\ &= \{\lambda Y(a) + \mu Y(b) \mid \lambda, \mu \in \mathbb{R}\}. \end{aligned}$$

We will only find equality in these sets when  $Y(\lambda a + \mu b) = \lambda Y(a) + \mu Y(b)$ . In other words,  $Y$  must be a linear map.

This example extends in a straightforward way for arbitrarily large, yet still finite  $X$ . Since  $\Delta$  is a finite set (potentially arbitrarily large), and  $Y$  is a linear map, this example will serve here as a sufficient justification for the above claim that  $Y(\text{span}(\Delta)) = S$ , the stoichiometric subspace.

**Proposition 3.18** *Let  $\delta$  denote the deficiency of a network  $\mathcal{N}$ , in the sense of Definition 3.14. Then*

$$\delta = \dim(\ker Y \cap \text{span}(\Delta)).$$

Before we are able to prove this proposition, we need to state and prove a result about our new set  $\Delta$ .

**Lemma 3.19** *Let  $\Delta$  be as defined in Definition 3.16. Then*

$$\dim \text{span}(\Delta) = |\mathcal{C}| - l.$$

*Proof.* Let  $\{L_1, \dots, L_l\}$  be the linkage classes of the network, and for each  $i = 1, \dots, l$ , define

$$\Delta_i = \{\omega_{y'} - \omega_y \in \mathbb{R}^{\mathcal{C}} \mid y, y' \in L_i\}.$$

Immediately we can see  $\Delta$  is the union of all the  $\Delta_i$ 's. But what of the span of  $\Delta$ ? For each  $\Delta_i$ , it is quite clear from the definitions that  $\text{span}(\Delta_i) \subseteq \text{span}(\Delta)$ .

Thus  $\bigoplus_{i=1}^l \text{span}(\Delta_i) \subseteq \text{span}(\Delta)$  follows naturally, since we are simply taking a linear combination of the elements already stated to be in  $\text{span}(\Delta)$ . We seek the reverse inclusion to conclude equality. Consider an element  $\nu \in \text{span}(\Delta)$ . It can be written in the following way.

$$\begin{aligned}
 \nu &= \sum_{y \sim y'} \lambda_{y,y'} (\omega_{y'} - \omega_y) \\
 &= \sum_{i=1}^l \sum_{y^i, y'^i \in L_i} \lambda_{y^i, y'^i} (\omega_{y'^i} - \omega_{y^i}) \\
 &= \underbrace{\sum_{y'^1, y^1 \in L_1} \lambda_{y'^1, y^1} (\omega_{y'^1} - \omega_{y^1})}_{\text{an element of } \Delta_1} + \underbrace{\sum_{y'^2, y^2 \in L_2} \lambda_{y'^2, y^2} (\omega_{y'^2} - \omega_{y^2})}_{\text{an element of } \Delta_2} + \cdots \\
 &\quad \cdots + \underbrace{\sum_{y'^l, y^l \in L_l} \lambda_{y'^l, y^l} (\omega_{y'^l} - \omega_{y^l})}_{\text{an element of } \Delta_l},
 \end{aligned}$$

where  $\lambda_{y,y'}$  represents a scalar. So, since each element of  $\text{span}(\Delta)$  can be written as a linear combination of the elements of the span of each  $\Delta_i$ , we find that  $\bigoplus_{i=1}^l \text{span}(\Delta_i) \supseteq \text{span}(\Delta)$ . Hence

$$\text{span}(\Delta) = \text{span}(\Delta_1) \oplus \text{span}(\Delta_2) \oplus \cdots \oplus \text{span}(\Delta_l).$$

Define  $n_i$  to be the number of complexes found in each linkage class,  $L_i$ , so that the set of complexes in  $L_i$  is given by  $\{y_1^i, \dots, y_{n_i}^i\}$ . Then a basis for each  $\Delta_i$  is given by  $\{\omega_{y_2^i} - \omega_{y_1^i}, \omega_{y_3^i} - \omega_{y_1^i}, \dots, \omega_{y_{n_i}^i} - \omega_{y_1^i}\}$ , from which we see  $\dim \text{span}(\Delta_i) = n_i - 1$ . Then, as the linkage classes form a partition,  $\dim(\bigoplus_i \text{span}(\Delta_i)) = \sum_i \dim \text{span}(\Delta_i)$ , so

$$\dim \text{span}(\Delta) = \sum_{i=1}^l (n_i - 1) = |\mathcal{C}| - l.$$

□

**Lemma 3.20** *Let  $f: A \rightarrow B$  be some function mapping an arbitrary set  $A$  to a set  $B$ , and let its restriction be defined by  $\bar{f}: C \rightarrow B$ , where  $C \subseteq A$ . Then*

$$\ker \bar{f} = \ker f \cap C.$$

*Proof.* The kernel of each function can be defined as follows.

$$\ker f = \{a \in A \mid f(a) = 0 \in B\},$$

$$\ker \bar{f} = \{a \in C \mid f(a) = 0 \in B\}.$$



But we can rewrite the second line as

$$\begin{aligned}\ker \bar{f} &= \{a \in C \mid f(a) = 0 \in B\} \\ &= \{a \in A \mid f(a) = 0 \in B\} \cap C \\ &= \ker f \cap C.\end{aligned}$$

□

*Proof of Proposition 3.18.* Let  $\bar{Y}: \text{span}(\Delta) \rightarrow \mathbb{R}^S$  be the restriction of  $Y$  to  $\text{span}(\Delta)$ . Then, by standard properties of a linear transformation,

$$\dim \text{span}(\Delta) = \dim \ker \bar{Y} + \dim \text{Im } \bar{Y}.$$

By Lemma 3.19,  $\dim \text{span}(\Delta) = |\mathcal{C}| - l$ , and from (3.1),

$$\text{Im } \bar{Y} = Y(\text{span}(\Delta)) = S,$$

but  $\dim S = s$  where  $s$  can be thought of simply as the rank of  $N$ , the stoichiometric matrix. So this gives us that

$$\begin{aligned}\dim \ker \bar{Y} &= \dim \text{span}(\Delta) - \dim \text{Im } \bar{Y} \\ &= |\mathcal{C}| - l - s.\end{aligned}$$

Lastly, by Lemma 3.20,

$$\ker \bar{Y} = \ker Y \cap \text{span}(\Delta),$$

from which our result follows. □

**Example 3.21** Let us calculate the deficiency of the network given in example 1.5. The set of complexes is given by  $\mathcal{C} = \{E + S, C, E + P\}$ . Since  $E + S$  reacts to form  $C$ , and  $C$  reacts to form  $E + P$ , we see that there is only one linkage class. Also note that there are two strong linkage classes –  $\{E + S, C\}$  and  $\{E + P\}$ . Lastly, our stoichiometric matrix can be row reduced to echelon form to find its rank as follows.

$$N = \begin{bmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix} \longrightarrow \begin{bmatrix} 1 & -1 & -1 \\ 0 & 0 & -1 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix} \longrightarrow \begin{bmatrix} 1 & -1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

which clearly has rank 2. In other words, the dimension of the stoichiometric subspace of this network is  $s = 2$ . Putting all this together we find that

$$\delta = |\mathcal{C}| - l - s = 3 - 1 - 2 = 0,$$

hence this network has deficiency zero.

**Remark** Networks whose deficiency is zero form an important subclass of chemical reaction networks, which will be discussed soon.

Finally, there is a desirable property for a reaction network to have, that of *reversibility*. That is, when each reaction is accompanied by a corresponding anti-reaction. For example, in the very simple network represented by  $X_1 \rightleftharpoons X_2$ . In later sections we will see how nice reversibility can be, however it only covers a very restricted class of networks. A larger class of networks are those we will call *weakly reversible*.

**Definition 3.22** Let  $y, y' \in \mathcal{C}$  be complexes of a chemical reaction network. We say this network is weakly reversible if, whenever there is a directed path from  $y$  to  $y'$ , there is also a directed path from  $y'$  to  $y$ . Further, if any of the following four (equivalent) statements hold, we say the network is weakly reversible.

- (i) The  $(\Rightarrow)$  relation is symmetric, whenever  $y \Rightarrow y', y' \Rightarrow y$ .
- (ii) Each reaction is contained in a directed cycle. That is, whenever  $y \rightarrow y'$ , we have  $y' \Rightarrow y$ .
- (iii) Each linkage class is a strong linkage class.
- (iv) Each linkage class is a terminal strong linkage class.

**Remark** If a network is reversible, then it is weakly reversible. The converse is not true in general.

### 3.2.2 Graphical Structure

We can now start to think about the relationship between those strange functions defined in section 3.1 and the graphical structure of a network. The following proposition is what gives this chapter its name.

**Proposition 3.23** Let  $\mathcal{N} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$  be a reaction network with terminal strong linkage classes  $\{\Lambda_1, \dots, \Lambda_t\}$ , and let  $k$  be any vector in  $\mathbb{R}_+^{\mathcal{R}}$  with  $A_k$  defined as above. Then the kernel of  $A_k$  has a basis  $\{x_1, \dots, x_t\} \subset \overline{\mathbb{R}}_+^{\mathcal{C}}$  such that  $\text{supp}(x_i) = \Lambda_i$ ,  $i = 1, \dots, t$ .

This is proved by a series of lemmata adapted from a paper by Feinberg and Horn, as well as a couple of extra definitions<sup>[7]</sup>.

**Definition 3.24** For each  $y \in \mathcal{C}$ , let

$$\mathcal{R}_{\rightarrow y} = \{y' \rightarrow y \in \mathcal{R} \mid y' \in \mathcal{C}\},$$

and

$$\mathcal{R}_{y \rightarrow} = \{y \rightarrow y' \in \mathcal{R} \mid y' \in \mathcal{C}\}.$$

**Remark** One can see clearly that  $\mathcal{R} = \bigcup_{y \in \mathcal{R}} \mathcal{R}_{\rightarrow y} = \bigcup_{y \in \mathcal{R}} \mathcal{R}_{y \rightarrow}$ .

**Definition 3.25** For every  $x \in \overline{\mathbb{R}}_+^{\mathcal{C}}$ , define  $|x| \in \overline{\mathbb{R}}_+^{\mathcal{C}}$  by

$$|x|_y = |x_y|, \quad \forall y \in \mathcal{C}.$$

Now we will go on to use these definitions to help us form a solid foundation upon which we can prove Proposition 3.23.

**Lemma 3.26** If  $x \in \ker A_k$ , then  $|x| \in \ker A_k$ .

*Proof.* Rearranging  $A_k(x) = \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y)$ , for every  $x \in \mathbb{R}^{\mathcal{C}}$ ,

$$\begin{aligned} A_k(x) &= \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} x_y \omega_{y'} - \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} x_y \omega_y \\ &= \sum_{y \in \mathcal{C}} \left[ \sum_{\mathcal{R}_{\rightarrow y}} k_{y' \rightarrow y} x_{y'} \omega_y - \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} x_y \omega_y \right] \\ &= \sum_{y \in \mathcal{C}} \left[ \sum_{\mathcal{R}_{\rightarrow y}} k_{y' \rightarrow y} x_{y'} - \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) x_y \right] \omega_y. \end{aligned}$$

If either  $\mathcal{R}_{y \rightarrow}$  or  $\mathcal{R}_{\rightarrow y}$  is empty then the sum will be taken to be zero. By definition, if  $x \in \ker A_k$  then  $A_k(x) = 0$ . Since  $\{\omega_y\}_{y \in \mathcal{C}}$  is a linearly independent set,  $x \in \ker A_k$  if and only if the quantity in the square braces above is zero for each  $y \in \mathcal{C}$ .

That is,

$$x \in \ker A_k \implies \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} x_{y'} = \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) x_y. \quad (3.2)$$

Using the simple fact that  $\sum_i |x_i| \geq |\sum_i x_i|$ , where equality is obtained when  $x_i \geq 0$  for all  $i$ , and also that  $k_{y \rightarrow y'} > 0$  for all  $y \rightarrow y'$ , we can obtain the following series of implications.

$$\begin{aligned} \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} x_{y'} &= \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) x_y \\ \implies \left| \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} x_{y'} \right| &= \left| \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) x_y \right|. \end{aligned} \quad (3.3)$$

By the previous remark we know that

$$\sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} |x_{y'}| \geq \left| \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} x_{y'} \right|$$

and that

$$\left| \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) x_y \right| = \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) |x_y|.$$

In turn, continuing our implications from (3.3), we get

$$\begin{aligned} \implies \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} |x_{y'}| &\geq \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) |x_y|, \quad \forall y \in \mathcal{C} \\ \iff \sum_{y \in \mathcal{C}} \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} |x_{y'}| &\geq \sum_{y \in \mathcal{C}} \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} |x_y|. \end{aligned}$$

But in the final line equality certainly holds, hence for each  $x \in \ker A_k$  and for all  $y \in \mathcal{C}$ ,

$$\sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} |x_{y'}| = \left( \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} \right) |x_y|, \quad (3.4)$$

and thus  $|x| \in \ker A_k$ . □

**Lemma 3.27** *If  $x \in \ker A_k$ ,  $x_y = 0$ , and  $y' \Rightarrow y$ , then  $x_{y'} = 0$ .*

*Proof.* If  $y = y'$  the result is trivial. From equation (3.4), if  $x_y = 0$  and  $y' \rightarrow y$  then

$$\begin{aligned} (x_y = 0 \implies) |x_y| = 0 &\implies \sum_{\mathcal{R} \rightarrow y} k_{y' \rightarrow y} |x_{y'}| = 0 \\ &\implies |x_{y'}| = 0 \end{aligned}$$

since rate constants are strictly positive. Finally, suppose that  $y' \rightarrow y_1 \rightarrow y_2 \rightarrow \cdots \rightarrow y_k \rightarrow y$ . Then  $x_y = 0$  implies that  $x_{y_k} = 0$  by our previous argument, which in turn implies  $x_{y_{k-1}} = 0$ , until we eventually arrive at  $x_{y'} = 0$ , proving the lemma.  $\square$

**Lemma 3.28** *Let  $\Lambda$  be any strong linkage class (not necessarily terminal). If  $x \in \ker A_k$ , and  $x_y = 0$  for some  $y \in \Lambda$ , then  $x_{y'} = 0 \ \forall \ y' \in \Lambda$ .*

*Proof.* Since  $\Lambda$  is a strong linkage class,  $y \in \Lambda$  and  $y' \in \Lambda$  implies  $y \approx y'$ . Thus  $y' \Rightarrow y$  and so by lemma 3.27 the result follows.  $\square$

**Lemma 3.29** *Let  $\mathcal{T} \subset \mathcal{C}$  be the set of all complexes which lie in a terminal strong linkage class, and let  $x \in \ker A_k$ . Then  $\text{supp}(x) \subset \mathcal{T}$ . That is, if  $y \notin \mathcal{T}$  then  $x_y = 0$ .*

*Proof.* Recall that we define the support of  $x$  to be the set that satisfies  $\text{supp}(x) = \{y \in \mathcal{C} \mid x_y = 0\}$ . If  $\mathcal{T} = \mathcal{C}$ , the result is trivially true, since  $y \notin \mathcal{T} \implies y \notin \mathcal{C}$ , so by definition  $x_y = 0$ , as  $y$  is not a complex we consider in our reaction network! Suppose then that  $\mathcal{T} \neq \mathcal{C}$ . For each  $y \in \mathcal{T}$ , define

$$\mathcal{R}_{\rightarrow y}^{\text{int.}} = \{y' \rightarrow y \in \mathcal{R}_{\rightarrow y} \mid y' \in \mathcal{T}\},$$

and

$$\mathcal{R}_{\rightarrow y}^{\text{ext.}} = \{y' \rightarrow y \in \mathcal{R}_{\rightarrow y} \mid y' \notin \mathcal{T}\}.$$

Since these two sets partition  $\mathcal{R}_{\rightarrow y}$ , after summing over  $\mathcal{T}$  equation (3.4) becomes

$$\sum_{y \in \mathcal{T}} \sum_{\mathcal{R}_{\rightarrow y}^{\text{int.}}} k_{y' \rightarrow y} |x_{y'}| + \sum_{y \in \mathcal{T}} \sum_{\mathcal{R}_{\rightarrow y}^{\text{ext.}}} k_{y' \rightarrow y} |x_{y'}| = \sum_{y \in \mathcal{T}} \sum_{\mathcal{R}_{y \rightarrow}} k_{y \rightarrow y'} |x_y|.$$

Since both the right hand side of the above equation and the first term on the left hand side are a sum over all the reactions  $y \rightarrow y'$  such that  $y \in \mathcal{T}$  and  $y' \in \mathcal{T}$ , they must be equivalent, which implies

$$\sum_{y \in \mathcal{T}} \sum_{\mathcal{R}_{\rightarrow y}^{\text{ext.}}} k_{y' \rightarrow y} |x_{y'}| = 0. \quad (3.5)$$

Define

$$G = \{y' \in \mathcal{C} \setminus \mathcal{T} \mid y' \rightarrow y \text{ for some } y \in \mathcal{T}\}.$$

Equation (3.5) implies that if  $y' \in G$  then  $x_{y'} = 0$ . Using the fact that  $\mathcal{C}$  is finite, we may conclude that for every  $y'' \in \mathcal{C} \setminus \mathcal{T}$ , there exists  $y' \in G$  such that  $y'' \Rightarrow y'$ . Thus for each such  $y''$ ,  $x_{y''} = 0$  by lemma 3.27, and hence  $\text{supp}(x) \subset \mathcal{T}$ .  $\square$

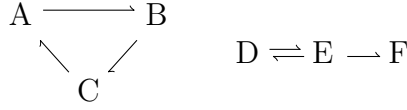


Figure 3.1: A simple reaction network.

**Example 3.30** That last proof got a little conceptually challenging towards the end, so I'll show the result of this lemma in action for one example. Figure 3.1 illustrates a simple reaction network that we can work with. This network admits two terminal strong linkage classes:  $\Lambda_1 = \{A, B, C\}$  and  $\Lambda_2 = \{F\}$ , so  $\mathcal{T} = \{A, B, C, F\}$ , and  $G = \{E\}$ . So by the result of the previous lemma, if  $x \in \ker A_k$ , then since  $E \rightarrow F$ ,  $x_E = 0$  by equation (3.5), and then by Lemma 3.27 we know  $x_D = 0$  since  $D \rightleftharpoons E$ . The point is that  $D$  is our  $y'' \in \mathcal{C} \setminus \mathcal{T}$ , and  $E$  is our  $y' \in G$  from above, and that for any network we encounter these will always exist (unless  $\mathcal{C} = \mathcal{T}$  of course).

If  $\{\Lambda_i\}_{i=1,\dots,t}$  are the terminal strong linkage classes, let  $\Gamma_i$  denote the linear subspace of  $\mathbb{R}^{\mathcal{C}}$  comprised of all elements with support in  $\Lambda_i$ . That is,  $\text{supp}(x) \subset \Lambda_i \implies x \in \Gamma_i$ . Then lemma 3.29 requires that

$$\ker A_k \subset \Gamma_1 \oplus \Gamma_2 \oplus \cdots \oplus \Gamma_t, \quad (3.6)$$

where  $\oplus$  denotes the direct sum.

**Lemma 3.31** *For  $i = 1, 2, \dots, t$ ,  $\Gamma_i$  is invariant under  $A_k$ . That is, if  $x \in \Gamma_i$  then  $A_k(x) \in \Gamma_i$ .*

*Proof.* Let  $\mathcal{R}_i = \{y \rightarrow y' \in \mathcal{R} \mid y \in \Lambda_i\}$ . If  $x \in \Gamma_i$ , then  $y \notin \Lambda_i \implies x_y = 0$ . Consequently, it follows that the sum in the definition of  $A_k(x)$  may be restricted to  $\mathcal{R}_i$  when  $x \in \Gamma_i$ , so for  $x \in \Gamma_i$ ,

$$A_k(x) = \sum_{y \rightarrow y' \in \mathcal{R}_i} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y). \quad (3.7)$$

If  $\mathcal{R}_i$  is empty (as when  $|\Lambda_i| = 1$ ), then  $A_k(x)$  is zero and we are done. Suppose  $\mathcal{R}_i$  is not empty. Since  $\Lambda_i$  is terminal,  $y \rightarrow y' \in \mathcal{R}_i$  implies both  $y, y' \in \Lambda_i$ , in which case  $\omega_{y'} - \omega_y$  has support in  $\Lambda_i$ . Thus every term of equation (3.7) lies in  $\Gamma_i$ , so  $A_k(x) \in \Gamma_i$ .  $\square$

**Lemma 3.32** *For each  $i = 1, 2, \dots, t$ , there exists  $x_i \in \Gamma_i$  with the following properties:*

1.  $A_k(x_i) = 0$ ,
2.  $(x_i)_y > 0$  for all  $y \in \Lambda_i$ ,
3. If  $x'_i \in \Gamma_i$  and  $A_k(x'_i) = 0$ , then  $x'_i = \alpha x_i$  for some number  $\alpha$ .

*Proof.* Let  $A_i: \Gamma_i \rightarrow \Gamma_i$  be the restriction of  $A_k$  to  $\Gamma_i$ . Then, from equation (3.7),

$$A_i(x) \equiv \sum_{y \rightarrow y' \in \mathcal{R}_i} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y).$$

The dimension of  $\text{Im}(A_i)$  is smaller than the dimension of  $\Gamma_i$ ; in fact, we can find an element of  $\Gamma_i$  which is orthogonal to  $\text{Im}(A_i)$ . For each coefficient  $k_{y \rightarrow y'} x_y$  of  $\omega_{y'}$ , the corresponding negation,  $-k_{y \rightarrow y'} x_y$ , is present as the coefficient of  $\omega_y$ . Since this is true for all of these coefficients, if we imagine the vector of ‘ones’ and compute its inner product with  $A_i(x)$ , we get

$$\begin{aligned} [1, 1, \dots, 1] A_i(x) &= [1, 1, \dots, 1] \sum_{y \rightarrow y' \in \mathcal{R}_i} k_{y \rightarrow y'} x_y (\omega_{y'} - \omega_y) \\ &= [1, 1, \dots, 1] \underbrace{\begin{bmatrix} \dots & \vdots & \dots \\ \dots & k_{y \rightarrow y'} x_y & \dots \\ \vdots & \vdots & \vdots \\ \dots & -k_{y \rightarrow y'} x_y & \dots \\ \dots & \vdots & \dots \end{bmatrix}}_{y\text{th complex}} \begin{matrix} \xleftarrow{\omega_{y'} \text{ coefficient}} \\ \xleftarrow{\omega_y \text{ coefficient}} \end{matrix} \\ &= 0. \end{aligned}$$

The equivalent of the vector of ones in terms of  $\omega$ ’s is given by  $\sum_{y \in \Lambda_i} \omega_y \in \Gamma_i$ , and as we have just seen this is orthogonal to  $\text{Im}(A_i)$ . Since we have found an element in  $\Gamma_i$  orthogonal to  $\text{Im}(A_i)$ , it must have a smaller dimension. Thus  $A_i$  has a non-trivial kernel. Let  $x_i$  be a non-zero element of  $\ker A_i$ . Then  $\emptyset \neq \text{supp}(x_i) \subseteq \Lambda_i$ . By lemma 3.28,  $\Lambda_i \subseteq \text{supp}(x_i)$ , and so  $\Lambda_i = \text{supp}(x_i)$ . Hence

$$\begin{aligned} y \in \Lambda_i &\implies (x_i)_y \neq 0, \\ y \notin \Lambda_i &\implies (x_i)_y = 0. \end{aligned}$$

In fact, by lemma 3.26 we can take  $(x_i)_y > 0$  if  $y \in \Lambda_i$ . This proves the first two assertions. Now suppose there exists  $x'_i \in \Gamma_i$  such that  $A_k(x'_i) = 0$ . Then an  $\alpha$  may be

chosen such that  $x'_i - \alpha x_i$  vanishes for some  $y \in \Lambda_i$ . Clearly  $x'_i - \alpha x_i \in \ker A_k$  so that lemma 3.28 requires  $x'_i - \alpha x_i$  vanishes for all  $y \in \Lambda_i$ . Since  $x'_i - \alpha x_i$  has support in  $\Lambda_i$  it follows that  $x'_i - \alpha x_i = 0$ .  $\square$

*Proof of Proposition 3.23.* Let  $\{x_i \in \Gamma_i\}_{i=1,\dots,t}$  be a set of vectors which satisfy the first two results of the previous lemma. We show this is a basis for  $\ker A_k$ . It is certainly linearly independent, so we need only show that  $x \in \ker A_k$  can be expressed as a linear combination of the elements of this set. By equation (3.6),  $x$  has decomposition

$$x = x'_1 + x'_2 + \cdots + x'_t, \quad x'_i \in \Gamma_i, \quad i = 1, \dots, t.$$

Then  $A_k(x) = A_k(x'_1) + A_k(x'_2) + \cdots + A_k(x'_t) = 0$ . From lemma 3.31,  $A_k(x'_i) \in \Gamma_i$ ,  $i = 1, \dots, t$ . Because the  $\Gamma_i$ 's are linearly independent, we have  $A_k(x'_i) = 0$  for every  $i$ . From lemma 3.32(3.), there exist  $\{\alpha_i\}_{i=1,\dots,t}$  such that  $x'_i - \alpha_i x_i = 0$ . In other words,  $x'_i = \alpha_i x_i$ , so

$$x = \alpha_1 x_1 + \alpha_2 x_2 + \cdots + \alpha_t x_t.$$

$\square$

**Remark** Indeed, a consequence of this theorem is that  $\dim \ker(A_k) = t$ . Then, by the Rank-Nullity theorem<sup>a</sup>,  $\dim \operatorname{Im}(A_k) = |\mathcal{C}| - t$ .

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<sup>a</sup>A proof of which can be found in 'Algebra' by Artin [8].



## Chapter 4

# The Deficiency Zero Theorem

WE have already seen an example of a reaction network with deficiency zero. Indeed, there are an infinite number of possible networks with this property. They may be on the whole quite simple, such as the one we have observed, or they may be extremely large and intricate - the following theorem, taken from lectures<sup>[1]</sup> given by Feinberg himself, is one of the main results of chemical reaction network theory. It is the culmination of all our hard work so far – it ties together all of the networks with zero deficiency, and greatly helps us towards answering our initial question.

**Theorem 4.1** (The Deficiency Zero Theorem – Feinberg, 1979) *Let  $\mathcal{N} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$  be a reaction network of deficiency zero.*

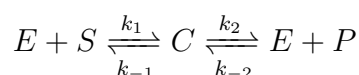
1. *If the network is not weakly reversible, then for arbitrary kinetics  $\mathcal{K}$ , the system of differential equations for  $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$  cannot admit a positive equilibrium.*
2. *If the network is not weakly reversible, then for arbitrary kinetics  $\mathcal{K}$ , the system of differential equations for  $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$  cannot admit a cyclic composition trajectory containing a positive composition.*
3. *If the network is weakly reversible, then for any mass action kinetics  $k$ , the system of differential equations for  $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$  has the following properties:*
  - (a) *There exists within each positive stoichiometric compatibility class precisely one equilibrium,*
  - (b) *that equilibrium is asymptotically stable,*

(c) *there cannot exist a nontrivial cyclic composition trajectory in  $\mathbb{R}_+^S$ .*

**Remark** One of the most surprising things about this theorem is that the assumptions are related to the structure of the network, but its conclusions are related to its dynamical properties.

We now have a powerful tool to help us determine that certain specific networks have no more than one equilibrium per stoichiometric compatibility class. Whilst having zero deficiency is indeed a sufficient condition for this attribute, we don't yet know whether or not it is a necessary condition. This is perhaps jumping the gun a bit, so it seems natural to first consider some examples.

**Example 4.2** The reversible version of our Michaelis-Menten system from Example 1.5 is as follows.

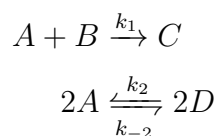


From previous analysis it is quite simple to show that this system has zero deficiency. Ordering the species  $\{E, S, C, P\}$  and the rate constants  $\{k_1, k_{-1}, k_2, k_{-2}\}$  we obtain

$$N = \begin{bmatrix} -1 & 1 & 1 & -1 \\ -1 & 1 & 0 & 0 \\ 1 & -1 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix},$$

which is of rank 2. It is also easy to observe that there is just one linkage class. Thus  $\delta = |\mathcal{C}| - l - s = 3 - 1 - 2 = 0$ . So far this is the same as before. What is it that makes this system different? Well, due to the addition of an extra reaction  $E + P \xrightarrow{k_{-2}} C$  this system is now weakly reversible! Thus the assumptions required for the third condition in Theorem 4.1 hold, and hence this system admits multiple equilibria.

**Example 4.3** One can also consider the system given by



which consists of 4 complexes, two linkage classes, and has stoichiometric matrix

$$N = \begin{bmatrix} -1 & -2 & 2 \\ -1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 2 & -2 \end{bmatrix}$$

which is of rank 2. In other words,  $\delta = 0$ . However, the system is not weakly reversible – there is no way for  $C$  to return to  $A+B$ . Thus for arbitrary kinetics the above system cannot admit a positive equilibrium, or a cyclic composition trajectory with positive composition, by Theorem 4.1.

## Chapter 5

# Moving On – Stochastic Systems

So far we have only dealt with deterministic systems. The conclusions we were able to make relied entirely upon the assumption that we were dealing with a very large amount of molecules. What about if we want to work within very small systems, containing only a few molecules of each species? We are not able to rely on differential equations then, and so must employ strategies that can deal with this new situation, so called stochastic models based on continuous-time Markov chains.

Of course we are still interested in finding equilibrium points. The stochastic counterpart to stationary points is something called the *stationary distribution* of the system. This stationary distribution can be found analytically using the *Kolmogorov forward equation*, or *chemical master equation* in the chemistry literature, however this is in general very difficult. Techniques have been developed that approximate the stationary distribution using Monte Carlo methods<sup>[9] [10] [11] [12]</sup>.

One of the main problems we are now going to encounter is the fact that reactions occur at random time intervals, and this is now a much more important factor. Previously, so many reactions would be occurring that the randomness would not be such a problem and differential equations would offer a good approximation. I will try to illustrate and explain some of what I have already mentioned, and then using an important paper by Anderson, Craciun and Kurtz<sup>[13]</sup>, show how we may end up with an understanding of the stationary distribution of a stochastic system with the help of Theorem 4.1.

## 5.1 Complex and Detailed Balanced Systems

Here I will introduce a few basic concepts from an important paper by Horn and Jackson<sup>[14]</sup>, which will help us to link deterministic and stochastic systems. From now on I will reserve  $\gamma$  to denote the number of complexes, where previously I have used  $|\mathcal{C}|$ .

**Definition 5.1** Denote by  $R(c)$  the *rate matrix* at concentration  $c$ , whose elements  $r_{ij}(c)$  are defined by<sup>a</sup>,

$$r_{ij}(c) = k_{y_j \rightarrow y_i} c^{y_j}.$$

That is, the  $ij$ th element of  $R(c)$  is the rate of formation of the  $i$ th complex,  $y_i$ , due to the  $j$ th complex,  $y_j$ .

**Remark** The rate of creation of complex  $i$ , denoted  $y_i$ , at concentration  $c$  is given by

$$\sum_{j=1}^{\gamma} r_{ij}(c).$$

Similary, its rate of destruction is

$$\sum_{j=1}^{\gamma} r_{ji}(c).$$

This is reflected in the following definition.

**Definition 5.2** The complex formation vector, denoted  $g(c)$ , is given by

$$g(c) = (R(c) - R^T(c)) e_w, \quad (5.1)$$

where  $e_w = [1, \dots, 1]^T \in \mathbb{R}^{\mathcal{C}}$ .

We can now define certain sets to help us pin down what it means to say a system is *complex balanced*, or to say it is *detailed balanced*.

**Definition 5.3** The subset of  $\mathbb{R}^{\mathcal{S}}$  containing all equilibrium concentrations will be called the equilibrium set, denoted by  $E$ :

$$E := \{c \in \mathbb{R}_+^{\mathcal{S}} \mid f(c) = 0\}, \quad (5.2)$$

where  $f$  is defined as in Chapter 3.

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<sup>a</sup>They are defined in this way by our assumption of mass action kinetics.

It follows from previous analysis that  $c \in E$  if and only if  $g(c) \in \ker Y$ .

**Definition 5.4** A reaction network is said to be complex balanced at a concentration  $c$  if the complex formation vector  $g(c)$  is zero at this concentration. The set of all such concentrations is given by

$$C := \{c \in \mathbb{R}_+^S \mid g(c) = 0\}. \quad (5.3)$$

We call a reaction network *complex balanced* if

$$C = E \neq \emptyset. \quad (5.4)$$

**Remark** • In other words, a system is complex balanced if it is complex balanced at all equilibrium concentrations.

- If some concentration  $c$  admits a complex balanced equilibria, i.e.  $c \in C$ , we can conclude that the formation rate of all complexes is zero. Hence, the system must be in equilibrium, since no species are being formed/destroyed. Hence  $c \in E$ . In other words,

$$C \subseteq E.$$

It may interest the reader to know that this concept of complex balancing is very strongly linked to Theorem 4.1. So strongly in fact, that it offers a reformulation of the entire theorem. The following was presented by Fritz Horn in 1972, as Theorem 4A<sup>[6]</sup>.

**Theorem** (Theorem 4A – Horn, 1972) *A mechanism<sup>b</sup> is complex balanced for all sets of rate constants if and only if the following two requirements are met:*

- (a) *The mechanism is weakly reversible.*
- (b) *The deficiency of the mechanism is zero.*

**Remark** Notice that the two requirements of this theorem are precisely those conditions in the third statement of Theorem 4.1.

I will also include here a more general result about weakly reversible networks which follows quite naturally. A proof can be found here<sup>[15]</sup>.

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<sup>b</sup>Horn defines a mechanism to be a collection of mass action systems with certain things in common, such as their species and complex sets, and species and complex space.

**Theorem** *If a chemical reaction network is weakly reversible, then its deficiency,  $\delta$ , corresponds to the number of conditions on the rate constants which need to be satisfied in order for the network to be complex balanced.*

In other words, we now have something to say about the dynamics of a much broader collection of systems. If a system is weakly reversible, then there exist some rate constants, subject to a number of conditions, which guarantee the system is complex balanced. Better still, the proof of the above theorem presents us with a way of determining such conditions, so it is possible for us to find out which values the rate constants can take in order to ensure the existence of precisely one equilibrium per positive stoichiometric compatibility class. Indeed, the Deficiency Zero Theorem is an application of the above theorem, for the case of  $\delta = 0$ .

Another way we can approach the concept of complex balancing is in the context of those functions given in Definition 3.2. Since

$$f(c) = \frac{dc}{dx} = Y A_k \Psi(c)$$

and  $Y$  is a linear operator, an equilibrium concentration for the systems gives

$$A_k \Psi(c) = 0.$$

This observation brings us to the following Proposition, remarked upon by Horn and Jackson<sup>[14]</sup>.

**Proposition 5.5** *Let  $f(c) = Y A_k \Psi(c)$  and  $g(c)$  be as in Definition 5.2. Then*

$$f(c) = Y g(c).$$

*Proof.* Since, by construction,  $f(c) = Y A_k \Psi(c)$ , proving this proposition is equivalent to proving that

$$g(c) = A_k \Psi(c).$$

As we saw in Example 3.3, an expression for  $A_k \Psi(c)$  is given by

$$A_k \Psi(c) = \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y (\omega_{y'} - \omega_y).$$

I will compare the  $i$ th element of both  $A_k\Psi(c)$  and  $g(c)$  to prove equivalence. Firstly,

$$\begin{aligned} g_i(c) &= \sum_{j=1}^{\gamma} r_{ij}(c) - r_{ji}(c) \\ &= \sum_{j=1}^{\gamma} k_{y_j \rightarrow y_i} c^{y_j} - k_{y_i \rightarrow y_j} c^{y_i} \\ &= \sum_{y \in \mathcal{C}} k_{y \rightarrow y_i} c^y - k_{y_i \rightarrow y} c^{y_i}. \end{aligned}$$

To obtain the  $i$ th element of  $A_k\Psi(c)$  we take the inner product with  $\omega_{y_i}$ .

$$\begin{aligned} (A_k\Psi(c))_i &= \omega_{y_i} \cdot A_k\Psi(c) \\ &= \omega_{y_i} \cdot \left( \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y (\omega_{y'} - \omega_y) \right) \\ &= \omega_{y_i} \cdot \left( \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y \omega_{y'} \right) - \omega_{y_i} \cdot \left( \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y \omega_y \right) \\ &= \sum_{y \in \mathcal{C}} k_{y \rightarrow y_i} c^y - \sum_{y' \in \mathcal{C}} k_{y_i \rightarrow y'} c^{y_i} \\ &= \sum_{y \in \mathcal{C}} k_{y \rightarrow y_i} c^y - \sum_{y \in \mathcal{C}} k_{y_i \rightarrow y} c^{y_i} \quad (\text{upto labelling}) \\ &= \sum_{y \in \mathcal{C}} k_{y \rightarrow y_i} c^y - k_{y_i \rightarrow y} c^{y_i}. \end{aligned}$$

These are both the same, hence equivalence holds.  $\square$

**Remark** This shows that the species formation vector,  $f$ , is the image of the complex formation vector  $g$  under  $Y$ .

The result of this proposition is that finding an equilibrium concentration for a system amounts to solving  $A_k\Psi(c) = 0$ , and to say the system is complex balanced at  $c$  is to say that  $A_k\Psi(c) = 0$ . In other words, a system is complex balanced at  $c$  if

$$\sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y (\omega_{y'} - \omega_y) = 0,$$

or equivalently

$$\sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y \omega_{y'} = \sum_{y \rightarrow y' \in \mathcal{C}} k_{y \rightarrow y'} c^y \omega_y. \quad (5.5)$$

We conclude this section with one more definition.



**Definition 5.6** A system is called detailed balanced at concentration  $c$  if its rate matrix is symmetric at  $c$ . That is,

$$R(c) = R^T(c). \quad (5.6)$$

We denote the subset of all such concentrations by

$$D := \{c \in \mathbb{R}_+^S \mid R(c) = R^T(c)\}, \quad (5.7)$$

and conclude from Definition 5.2 that

$$D \subseteq C \subseteq E.$$

Finally, we say that a system is *detailed balanced* if

$$D = C = E \neq \emptyset. \quad (5.8)$$

**Remark** Having  $R(c) = R^T(c)$  is equivalent to having  $r_{ij}(c) = r_{ji}(c)$  for all  $i, j$ . In other words, the rate at which  $i$  is formed due to  $j$  is equal to the rate at which  $j$  is formed due to  $i$ . In this sense, *balanced* is the most appropriate description.

## 5.2 Markov Chains and States

Markov chains are well studied objects surrounded by a lot of rich theory. Regrettably, we will ignore most of this, and use only what is required to achieve the goal of this chapter. This section closely follows Section 3 of Anderson et al. <sup>[13]</sup>, which provides a good introduction to this topic in the context of CRNT.

We denote by  $X(t) \in \overline{\mathbb{Z}}_+^n$  the *state* of the system at time  $t \geq 0$ . It represents the number of molecules of each species at time  $t$ , and each reaction represents a possible transition from this state to another. A continuous-time Markov Chain  $\{X(t), t \geq 0\}$  is a stochastic process, i.e. random evolution of a phenomenon through time, which obeys the memoryless property. We define the memoryless property, also known as the *Markov property*, by

$$\begin{aligned} \mathbb{P}[X(t+s) = j \mid X(s) = i, X(u) = x(u) \text{ for } 0 \leq u \leq s] \\ = \mathbb{P}[X(t+s) = j \mid X(s) = i], \end{aligned} \quad (5.9)$$

where  $j, i$ , and  $x(u)$  represent the  $j$ th,  $i$ th and  $x(u)$ th state of the system respectively. What this means is that the future state of the system, given its current state, is completely independent of any past states. We assume that the number of reactions,  $m$ , is finite. If some reaction  $y \rightarrow y'$  occurs at time  $t$ , then the new state is given by

$$X(t) = X(t-) + y' - y,$$

where  $X(t-)$  represents the state of the system just before the reaction. This is used instead of, say,  $X(t-1)$ , since reactions will occur at random, as opposed to predetermined, time intervals. We can also represent the state of the system at time  $t$  by

$$X(t) = X(0) + \sum_k T_k(t)(y'_k - y_k), \quad (5.10)$$

where  $T_k(t)$  gives the number of times the  $k$ th reaction has occurred by time  $t$ , and  $X(0)$  is the initial state of the system.

How do we determine how often these reactions occur? In the deterministic setting, we defined a rate function (see Definition 1.3). The stochastic analogue is the *intensity function*, denoted  $\lambda_k(X(t))$ , where  $k$  refers to the reaction under investigation. The form which we will use, though there are many others, is that of stochastic mass action, and is given by

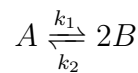
$$\lambda_k(x) = \kappa_k \left( \prod_{l=1}^n (y_k)_l! \right) \binom{x}{y_k} = \kappa_k \prod_{l=1}^n \frac{x_l!}{(x_l - (y_k)_l)!} \cdot 1_{\{x_l \geq (y_k)_l\}}, \quad (5.11)$$

for some constant  $\kappa_k$ . Note that  $(y_k)_l$  represents the  $l$ th component of the complex  $y_k$ . The function  $1_{\{x_l \geq (y_k)_l\}}$  is given by

$$1_{\{x_l \geq (y_k)_l\}} = \begin{cases} 1 & \text{if } x_l \geq (y_k)_l, \\ 0 & \text{otherwise,} \end{cases}$$

and tells us that the probability of reaction  $k$  occurring is zero if there isn't enough reactant(!).

**Example 5.7** Consider the dimerization-dissociation system<sup>[16]</sup> given by



We can look at the intensity functions of  $k_1$  and  $k_2$ , which should give us a more intuitive feel for why the intensities are defined as they are.

$$\begin{aligned}
 \lambda_{k_1}(x) &= \kappa_{k_1} \prod_{l=1}^2 \frac{x_l!}{(x_l - (y_{k_1})_l)!} \cdot 1_{\{x_l \geq (y_{k_1})_l\}} \\
 &= \kappa_{k_1} \left( \frac{x_1!}{(x_1 - 1)!} \cdot 1_{\{x_1 \geq 1\}} \right) \times \left( \frac{x_2!}{(x_2)!} \cdot 1_{\{x_2 \geq 0\}} \right) \\
 &= \kappa_{k_1} \frac{x_1!}{(x_1 - 1)!} \cdot 1_{\{x_1 \geq 1\}} \\
 &= \kappa_{k_1} x_1 \cdot 1_{\{x_1 \geq 1\}}
 \end{aligned}$$

and

$$\begin{aligned}
 \lambda_{k_2}(x) &= \kappa_{k_2} \prod_{l=1}^2 \frac{x_l!}{(x_l - (y_{k_2})_l)!} \cdot 1_{\{x_l \geq (y_{k_2})_l\}} \\
 &= \kappa_{k_2} \left( \frac{x_1!}{(x_1)!} \cdot 1_{\{x_1 \geq 0\}} \right) \times \left( \frac{x_2!}{(x_2 - 2)!} \cdot 1_{\{x_2 \geq 2\}} \right) \\
 &= \kappa_{k_2} \frac{x_2!}{(x_2 - 2)!} \cdot 1_{\{x_2 \geq 2\}} \\
 &= \kappa_{k_2} x_2(x_2 - 1) \cdot 1_{\{x_2 \geq 2\}},
 \end{aligned}$$

where we make the bijection  $(1, 2) \rightarrow (A, B)$ . What this means is that, as long as there is at least one molecule of  $A$ , the stochastic mass action intensity is proportional to the number of molecules of  $A$ , as expected. The intensity function for  $B$  is a little more subtle. One can consider an analogous result from elementary probability theory. Imagine a bag containing  $\alpha$  red balls, and  $\beta$  blue balls. The probability of selecting two red balls is given by  $\frac{\alpha(\alpha-1)}{(\alpha+\beta)(\alpha+\beta-1)} \propto \alpha(\alpha-1)$ . This is exactly what is happening here. We can think of it as first one molecule of  $B$  being chosen for the reaction, and then another molecule of  $B$  chosen from the remaining mixture. In this sense,  $\lambda_k(x)$  is much more intuitive than its formula first portrays.

Now we meet the concept of the *stationary distribution* of a Markov chain. It has one very important property – it describes the long term behaviour of the system.

**Definition 5.8** We say that the probability distribution  $\pi(x)$  is a stationary distribution for the Markov chain  $\{X(t), t \geq 0\}$  if

$$\sum_k \pi(x - y'_k + y_k) \lambda_k(x - y'_k + y_k) = \pi(x) \sum_k \lambda_k(x) \quad (5.12)$$

for all  $x$  in the state space.

If the network is weakly reversible, the state space is a union of closed, irreducible *communicating classes*. This is clear if we think about it in terms of reactions – if a series of reactions take us from one state to another, weak reversibility implies we can, in theory, get back to the original state. If a stationary distribution exists over an irreducible space, then it is unique and

$$\lim_{t \rightarrow \infty} \mathbb{P}(X(t) = x \mid X(0) = y) = \pi(x), \quad (5.13)$$

for all  $x, y$  in the same communicating class.

### 5.3 Stationary Distributions of Complex Balanced Systems

Usually, stationary distributions are hard to determine, with approximate simulation being the typical practical approach. However, in the case of complex balanced systems, we are able to determine the exact form of the associated stationary distribution. In the following proof we define the factorial of a vector by  $x! = \prod_i x_i!$ .

**Theorem 5.9** *Let  $\mathcal{N} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$  be a chemical reaction network with rate constants  $\kappa_k$ , where  $k$  refers to the  $k$ th reaction. Suppose that the system, when modelled deterministically, is complex balanced with complex balanced equilibrium  $c \in \mathbb{R}_+^n$ . Then the stochastically modelled system with intensities given by (5.11) has a stationary distribution,  $\pi(x)$ , consisting of the product of Poisson distributions,*

$$\pi(x) = \prod_{i=1}^n \frac{c_i^{x_i}}{x_i!} e^{-c_i}, \quad x \in \overline{\mathbb{Z}}_+^n. \quad (5.14)$$

*Proof.* To prove this theorem, we prove that taking the stationary distribution to be of the form given above, along with the intensities given by (5.11), conforms to equation (5.12), and that this in turn is equivalent to the system being complex balanced.

Let  $\pi(x)$  be given as in the statement of the theorem, and  $\lambda_k(x)$  be as in (5.11). Also take  $c \in \mathbb{R}_+^n$  to be a concentration for which the system is in complex balanced equilibrium. We compute separately the right and left hand sides of equation (5.12)

as follows.

$$\begin{aligned}
 \pi(x) \sum_k \lambda_k(x) &= \prod_{i=1}^n \frac{c_i^{x_i}}{x_i!} e^{-c_i} \sum_k \kappa_k \prod_{i=1}^n \frac{x_i!}{(x_i - (y_i)_k)!} \cdot 1_{\{x_i \geq (y_i)_k\}} \\
 &= \sum_k \kappa_k C^x \prod_{i=1}^n \frac{e^{-c_i}}{(x_i - (y_i)_k)!} \cdot 1_{\{x_i \geq (y_i)_k\}} \\
 &= \sum_k \kappa_k C^x \frac{1}{(x - y_k)!} \prod_{i=1}^n e^{-c_i} \cdot 1_{\{x_i \geq (y_i)_k\}}.
 \end{aligned} \tag{5.15}$$

The left hand side is given by

$$\begin{aligned}
 \sum_k \pi(x - y'_k + y_k) \lambda_k(x - y'_k + y_k) &= \sum_k \left( \prod_{i=1}^n \frac{c_i^{x_i - (y'_i)_k + (y_i)_k}}{(x_i - (y'_i)_k + (y_i)_k)!} e^{-c_i} \right. \\
 &\quad \left. \times \kappa_k \prod_{i=1}^n \frac{(x_i - (y'_i)_k + (y_i)_k)!}{(x_i - (y'_i)_k)!} \cdot 1_{\{x_i \geq (y'_i)_k\}} \right) \\
 &= \sum_k \kappa_k C^{x - y'_k + y_k} \prod_{i=1}^n \frac{e^{-c_i}}{(x_i - (y'_i)_k)!} \cdot 1_{\{x_i \geq (y'_i)_k\}} \\
 &= \sum_k \kappa_k C^{x - y'_k + y_k} \frac{1}{(x - y'_k)!} \prod_{i=1}^n e^{-c_i} \cdot 1_{\{x_i \geq (y'_i)_k\}}.
 \end{aligned} \tag{5.16}$$

What happens if we set these two expressions to be equal to each other?

$$\sum_k \kappa_k C^{x - y'_k + y_k} \frac{1}{(x - y'_k)!} \prod_{i=1}^n e^{-c_i} \cdot 1_{\{x_i \geq (y'_i)_k\}} = \sum_k \kappa_k C^x \frac{1}{(x - y_k)!} \prod_{i=1}^n e^{-c_i} \cdot 1_{\{x_i \geq (y_i)_k\}}$$

simplifies down to

$$\sum_k \kappa_k C^{y_k - y'_k} \frac{1}{(x - y'_k)!} \prod_{i=1}^n 1_{\{x_i \geq (y'_i)_k\}} = \sum_k \kappa_k \frac{1}{(x - y_k)!} \prod_{i=1}^n 1_{\{x_i \geq (y_i)_k\}},$$

which will be satisfied precisely when, for each complex  $z \in \mathcal{C}$ ,

$$\sum_{\{k | y'_k = z\}} \kappa_k C^{y_k - z} \frac{1}{(x - z)!} \prod_{i=1}^n 1_{\{x_i \geq z_i\}} = \sum_{\{k | y_k = z\}} \kappa_k \frac{1}{(x - z)!} \prod_{i=1}^n 1_{\{x_i \geq z_i\}}. \tag{5.17}$$

Here  $z$  is fixed, the left hand sum is over all reactions where  $z$  is the resultant complex, and the right hand sum is over all reactions where  $z$  is the source complex. This is essentially the same form as in Equation (5.5), but this is precisely what it means for a reaction network to be complex balanced! Hence we have proved the desired result.  $\square$

We may now state the theorem that ties together all of our work so far.

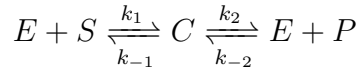
**Theorem 5.10** *Let  $\mathcal{N} = \{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$  be a chemical reaction network with rate constants  $\kappa_k$ , where  $k$  refers to the  $k$ th reaction. Suppose that the system is weakly reversible and has deficiency zero. Then the stochastically modelled system with intensities given by (5.11) has a stationary distribution,  $\pi(x)$ , consisting of the product of Poisson distributions,*

$$\pi(x) = \prod_{i=1}^n \frac{c_i^{x_i}}{x_i!} e^{-c_i}, \quad x \in \mathbb{Z}_+^n,$$

where  $c$  is an equilibrium concentration which is guaranteed to exist and is complex balanced.

*Proof.* The existence of  $c$ , and the proof of the theorem, follows from Theorem 5.9, Horn's Theorem 4A, Theorem 4.1 and the arguments made directly after Proposition 5.5.  $\square$

**Example 5.11** To visualise the final result of this project, let's look at the stationary distribution of our reversible Michaelis-Menten system. Recall the system has reaction diagram

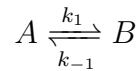


We know from previous analysis that it satisfies the requirements of the theorem, thus there exists an equilibrium concentration  $c$  such that

$$\begin{aligned} \pi(x) &= \prod_{i=1}^4 \frac{c_i^{x_i}}{x_i!} e^{-c_i} \\ &= \frac{c_1 c_2 c_3 c_4}{x_1! x_2! x_3! x_4!} e^{-(c_1 + c_2 + c_3 + c_4)}, \end{aligned}$$

where we make the bijection  $(1, 2, 3, 4) \rightarrow (E, S, C, P)$ .

**Remark** Anderson et al. give an example of the system



and use the fact that  $x_A + x_B$  is a conserved quantity to show that the stationary distribution takes the form of a Binomial distribution. In particular, they use this to make the point that independence in the amount of species does not follow from the existence of a product-form distribution in systems with a conservation relation among species.

# Chapter 6

## Conclusion

THROUGHOUT this paper we have been concerned with one question in particular:

*When does the concentration in a Chemical Reaction Network admit multiple positive equilibria?*

In order to help us answer this question we have developed a broad range of techniques that draw from different areas of mathematics. We first developed our vocabulary, so that we would be able to talk about any chemical reaction network in a formal, mathematical setting.

Our initial approach, in the introduction and chapter 2, was to characterise a bunch of reactions as a coherent system of coupled ordinary differential equations. These systems do not typically lend themselves well to being solved directly, and can be so large as to be practically impossible to solve computationally. Even ignoring these two set backs, this approach would be hopeless – imagine we get lucky and manage to solve the system under scrutiny. What about the next system we should wish to solve, and the one after that? So, we needed to construct some sort of framework, or set of criteria, that would allow us to say something about a chemical reaction network, *in general*. This came in the form of a so called *injectivity criterion*, where we saw that if a reaction network  $\mathcal{N}$  is injective, then it could admit at most one stationary point. We sought a way to determine when a system would admit many equilibria, with Theorems 2.2 and 2.13 acting as a starting point by telling us when a reaction network is or isn't injective. However, this was not enough – the injectivity

property only gives a sufficient condition for the absence of multiple equilibria, so it isn't guaranteed that a reaction network that is *not* injective will definitely have the capacity for multiple equilibria.

From here, we took a related but different approach. In chapter 3 we started to think more about how a network was structured, and how this structure could shed light on stationary points of the resulting system. The three functions  $Y$ ,  $\Psi$  and  $A_k$  that we defined between complex and species space hinted to us that the way complex and species interactions were structured could help us in this pursuit – especially when coupled with the realisation that the composition  $YA_k\Psi(c)$  gave us the rate of change of concentration vector,  $\frac{dc}{dt}$ . This was enough to justify laying down a graph theoretical groundwork that would help us better explore these properties. It also allowed us to give a second (more practical) definition of *deficiency*, the first being given earlier in terms of our three functions. We ended with the proof of a proposition, which told us specifically how the kernel of  $A_k$  is structured. It allowed us to see strong ties between the function and a network's terminal strong linkage classes.

We then arrived at one of the most famous results of chemical reaction network theory – the Deficiency Zero Theorem. It brings together the work of chapter 3 and tells us something about the dynamical properties of certain networks. When first encountered it is not particularly striking, but it quickly becomes apparent that with a small amount of relatively easily obtainable information about the *structure* of  $\mathcal{N}$ , we can now say something about the dynamics of an enormous class of systems. The most obvious downside to this theorem is that it only applies to weakly reversible deficiency zero networks. This was discussed in the next chapter, where an attempt to loosen these restrictions was made.

Loosening restrictions is, in some sense, the main goal of the final chapter. Up to this point in the project we have made one critical assumption – that the amount of species in consideration is great enough for us to get away with using ODE's to talk about the dynamics. There is now a worry that none of our work so far will be of use in the case of a very small number of species, for example at a cellular level, where the number of molecules involved in say, gene expression, are typically of the order  $1 - 1000$ <sup>[17]</sup>. So how do we account for this oversight? Luckily, there exists a rich theory perfectly suited for dealing with the inherent randomness of a



small scale chemical mixture – stochastic processes (in particular, continuous-time Markov chains). Further, efforts to incorporate this into chemical reaction network theory have already been made, and this chapter tries to present some of that work. It is encouraging to discover that much of our work in chapter 3 is of consequence here, and the final result incorporates the Deficiency Zero theorem directly to talk about the dynamics of corresponding stochastic systems. One of my favourite points of this chapter is the discussion after the statement of Theorem 4A. It is here where we discover the Deficiency Zero Theorem is a special case of a much more general result, and that the proof of this result gives us a way to talk about the dynamics of all weakly reversible networks.

## Future Work

In chapter 2, despite all the hard work, a sufficient condition for the existence of multiple positive equilibria is never obtained. Two are outlined by Craciun and Feinberg<sup>[2]</sup>, which structure a general algorithm that could be implemented to determine if  $\mathcal{N}$  has the capacity for multiple positive equilibria, but are not included here.

The greatest opportunity for further work lies in developing the theory in chapter 5. We have only really been able to say something about the stationary distribution of one type of system, whereas in chapter 2 we weren't as restricted. It would be encouraging to be able to construct stochastic chemical reaction network theory in such a way as to consider at least a broader class of models, if not all. A paper by Cappelletti and Wiuf<sup>[18]</sup> develops theory for stochastically complex balanced systems, and expands somewhat on Anderson et al. Another paper by Polletini et al.<sup>[19]</sup> uses the deficiency of a network in application to stochastic thermodynamics, thus showing that something as seemingly abstract as deficiency can have important real world application.

Lastly, Feinberg and Shinar use the structural properties of a reaction network to characterise something they call the *robustness*, “that is, the capacity for sustained and precise function even in the presence of structural or environmental disruption”<sup>[20]</sup>. They say a system shows *absolute concentration robustness* for a species if, for every possible steady state the system might take, the concentration of that species is

the same each time. Although the work is done deterministically, Enciso develops a stochastic counterpart to this theory<sup>[21]</sup> and even uses the deficiency of a network in the main result. This could be an interesting pursuit, especially since a lot of work has been put into understanding the underlying structure of these systems and the biological implications are vast<sup>[22]</sup>.

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