UNIQUENESS OF EQUILIBRIA FOR COMPLEX CHEMICAL REACTION NETWORKS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Haixia Ji, M.S.

Graduate Program in Mathematics

The Ohio State University

2011

Dissertation Committee:

Professor Martin R. Feinberg, Advisor

Professor Boris Pittel

Professor David Terman

ABSTRACT

Each chemical reaction network taken with mass action kinetics gives rise to a system of polynomial differential equations that govern the species concentrations, and in those equations many parameters (rate constants) appear. Even for a moderately sized network with several species and several reactions the resulting equations can be highly intricate. This thesis addresses the problem of determining whether a given chemical reaction network, taken with mass action kinetics, has the capacity to admit multiple positive steady states – that is, whether for the network there are rate constant values such that the resulting polynomial differential equations admit two distinct stoichiometrically-compatible steady states in which all species concentrations are positive. The theory developed extends earlier work by Ellison and Feinberg and is implemented in a Windows-based computer program that has been made internet-available.

To my family and friends for their love and support

ACKNOWLEDGMENTS

The research presented in this thesis could not have been accomplished without the advice, guidance and encouragement of my advisor, Professor Martin Feinberg. I would also like to thank him and Daniel Knight for their patient and constructive critiscism during the writing of this thesis. I also owe a big thanks to Phillipp Ellison, whose Ph.D. research together with Professor Feinberg's earlier work set up the foundation of my research. I would like to thank Yangzhong Tang for introducing me to Dephi programming and Yongjia Fan for her support and accompany during my research.

I am grateful to the Department of Mathematics at OSU with special thanks to Cindy Bernlohr, the National Science Foundation (grants No. BES-0425459 and No. EF-1038394), and US National Institutes of Health (grant No. 1R01GM086881-01) for their financial support of my doctoral studies.

VITA

2003	B.S. in Mathematics, Nanjing University, China, P.R.C.
2007	M.S. in Mathematics and M.A.S. in Applied Statistics, The Ohio State University
2003-2011	Graduate Teaching Associate, Graduate Research Associate, The Ohio State University

PUBLICATIONS

G. Shinar, A. Mayo, H. Ji and M. Feinberg, Constraints on Reciprocal Flux Sensitivities in Biochemical Reaction Networks. *Biophysical Journal* 100(6), 1383-1391, 2011.

FIELDS OF STUDY

Major Field: Mathematics

Specialization: Chemical Reaction Network Theory

CONTENTS

Chapter 1

INTRODUCTION

1.1 Questions of Interest

We will consider a special kind of ordinary differential equations, those derived from chemical reaction networks. In particular, we will consider the question of how reaction network constructure influences the capacity of the corresponding differential equations to admit more than one equilibrium. (More precisely, we will be interested in the possibility of two "stoichiometrically compatible" equilibria in which all species concentrations are positive.)

To see how these ordinary differential equations are derived, let us consider the simple example shown below as (1.1.1).

Suppose A, B, C, and D are chemical species, and suppose that a molecule of A can react with a molecule of B to form a molecule of C, a molecule of C can decompose into two molecules of D, and two molecules of D can form a molecule of C. The chemical reactions occurring among all species are then $A + B \to C$, $C \to 2D$, and $2D \to C$. The set of objects $\{A + B, C, 2D\}$ that lie on either side of the reaction arrow are called the ***complexes*** of the reaction network.

We write down the ***standard reaction diagram*** for this reaction network, in which each complex appears just once:

$$A + B \to C \rightleftharpoons 2D. \tag{1.1.1}$$

Next, imagine that we put a certain amount of species A, B, C and D into a pot. We

will refer the pot as a reactor. We suppose the pot is stirred constantly so its contents remain spatially homogeneous over time. We also suppose the contents in the pot are maintained at a fixed temperature and total volume. We will consider the instantaneous molar concentrations of the species, denoted by $c_A(t)$, $c_B(t)$, $c_C(t)$ and $c_D(t)$. We define a vector, containing these instantaneous concentrations as its components, as a ***composition vector*** $c(t)$, where $c(t) = [c_A(t), c_B(t), c_C(t), c_D(t)]$. We can then write the differential equations describing the evolution of these molar concentrations. Note that the chemical reactions are the causes by which the concentrations change, so the rate of change of the concentrations depends on how fast the reactions are going. In general, we suppose that the instantaneous occurence rate of each reaction depends on the instantaneous composition vector c. For example, we define a continuous nonnegative real-valued function $\mathscr{K}_{A+B\to C}(\cdot)$ such that $\mathscr{K}_{A+B\to C}(c)$ is the instantaneous occurrence rate of reaction $A + B \to C$ (per unit volume of mixture) when the instantaneous composition vector is c. We can similarly define functions $\mathscr{K}_{C\to 2D}(\cdot)$ and $\mathscr{K}_{2D\to C}(\cdot)$ for the reactions $C \to 2D$ and $2D \to C$, respectively. We then define a ***kinetics*** for a reaction network as an assignment of a rate function to each reaction in the network.

Assuming the reaction network (1.1.1) is equipped with a kinetics, we can write down the system of differential equations that govern the reactor. Supposing the reactor has some instantaneous composition c, we can consider the instantaneous rates of change for all entries in c, c_A, c_B, c_C and c_D. For example, let us consider the instantaneous rate of change of c_A. Note that every time the reaction $A + B \to C$ occurs, we lose a molecule of A at the occurrence rate $\mathscr{K}_{A+B\to C}(c)$. For the other two reactions $C \to 2D$ and $2D \to C$, when they occur, there is no gain or loss of molecules of A. Therefore, we can express the instantaneous rate of change of concentration of species A as follows:

$$\dot{c}_A = -\mathscr{K}_{A+B\to C}(c)$$

As for species D, every time the reaction $C \to 2D$ occurs, two molecules of D are

gained at the rate $\mathscr{K}_{C\to 2D}(c)$, every time the reaction $2D \to C$ occurs, two molecules of D are lost at the rate $\mathscr{K}_{2D\to C}(c)$, and the occurence of reaction $A + B \to C$ does not affect the number of molecules of D. Therefore, we can write down the instantaneous rate change of concentration of species A, B, C and D, in a similar fashion.

$$\begin{aligned}
\dot{c}_A &= -\mathscr{K}_{A+B\to C}(c) \\
\dot{c}_B &= -\mathscr{K}_{A+B\to C}(c) \\
\dot{c}_C &= \mathscr{K}_{A+B\to C}(c) - \mathscr{K}_{C\to 2D}(c) + \mathscr{K}_{2D\to C}(c) \\
\dot{c}_D &= 2\mathscr{K}_{C\to 2D}(c) - 2\mathscr{K}_{2D\to C}(c)
\end{aligned} \tag{1.1.2}$$

In this thesis, we assume that the kinetics are mass action, which we will now describe.

We assume that the instantaneous occurrence rate of $A + B \to C$ is proportional to the current value of $c_A c_B$. This is based on the assumption that the probability of a molecule of A encountering a molecule of B, which is required to make the reaction $A+B \to C$ occur, is proportional to $c_A c_B$. Thus we write $\mathscr{K}_{A+B\to C}(c) = k_{A+B\to C} c_A c_B$, where $k_{A+B\to C}$ is a positive ***rate constant*** for the reaction $A + B \to C$.

We assume the instantaneous occurrence rate of $C \to 2D$ is proportional to the current concentration of species C. This is based on the assumption that the higher the concentration of C, the higher will be the number of occurrences per time of the reaction $C \to 2D$. So we can write $\mathscr{K}_{C\to 2D}(c) = k_{C\to 2D} c_D$, where $k_{C\to 2D}$ is a positive rate constant for the reaction $C \to 2D$.

We assume that the instantaneous occurence rate of $2D \to C$ is proportional to the current value of c_D^2. This is based on the assumption that the probability of a molecule of D encountering another molecule of D, which is required to make the reaction $2D \to C$ occur, is proportional to c_D^2. Thus we write $\mathscr{K}_{2D\to C}(c) = k_{2D\to C} c_D^2$, where $k_{2D\to C}$ is a positive rate constant for the reaction $2D \to C$.

Therefore, from our mass action kinetics assumption, we have that

$$\begin{aligned}
\mathscr{K}_{A+B\to C}(c) &= k_{A+B\to C}c_A c_B \\
\mathscr{K}_{C\to 2D}(c) &= k_{C\to 2D}c_C \\
\mathscr{K}_{2D\to C}(c) &= k_{2D\to C}c_D^2
\end{aligned}$$

where $k_{A+B\to C}$, $k_{C\to 2D}$, $k_{2D\to C}$ are the rate constants for the corresponding reactions. We can rewrite (1.1.2) as follows:

$$\begin{aligned}
\dot{c}_A &= -k_{A+B\to C}c_A c_B \\
\dot{c}_B &= -k_{A+B\to C}c_A c_B \\
\dot{c}_C &= k_{A+B\to C}c_A c_B - k_{C\to 2D}c_C + k_{2D\to C}c_D^2 \\
\dot{c}_D &= 2k_{C\to 2D}c_C - 2k_{2D\to C}c_D^2
\end{aligned} \tag{1.1.3}$$

We now are at the point that the differential equations are written for the mass action governed reaction network (1.1.1).

Remark 1.1.1. *Besides reactions derived from true chemistry, there are sometimes so-called pseudo-reactions (see [13]). For example, we can add pseudo-reactions* $A \rightleftharpoons 0$*,* $B \rightleftharpoons 0$*,* $C \rightleftharpoons 0$*,* $D \to 0$*, where* 0 *is called the zero complex, to reaction network (1.1.1). We then have the following reaction network:*

$$\begin{array}{rcl}
A + B \to & C & \rightleftharpoons 2D \\
& \updownarrow & \\
A \rightleftharpoons & 0 & \rightleftharpoons B \\
& \uparrow & \\
& D &
\end{array} \tag{1.1.4}$$

We will explain why the pseudo-reactions might be added to reaction network (1.1.1) to form reaction network (1.1.4). Suppose we change the reactor for reaction network (1.1.1)

so that it has an inflow and outflow stream. We suppose that as the reactions are occurring inside the reactor, a mixture of species A, B, and C is continuously supplied to the reactor at a constant volumetric flow rate r (volume/time), and species A, B, C and D are continuously removed from the reactor in a stream having the same volumetric flow rate r. Let us denote by c_A^f, c_B^f, and c_C^f the instantaneous molar concentrations (moles/volume) of species A, B, and C in the inflow stream. c_A, c_B, c_C, and c_D will still represent the instantaneous molar concentrations of species A, B, C and D, respectively, within the reactor. Therefore c_A, c_B, c_C and c_D are also the instantaneous molar concentrations of species A, B, C and D, respectively, in the outflow stream. Let V be the total volume of the mixture within the reactor.

If we consider the reaction network (1.1.4), the inflow and outflow streams are also causes for changes in concentrations, besides the reactions occurring inside the reactor. So the corresponding mass action differential equations are :

$$\begin{aligned}
\dot{c}_A &= (r/V)c_A^f - (r/V)c_A - k_{A+B\to C}c_Ac_B \qquad (1.1.5)\\
\dot{c}_B &= (r/V)c_B^f - (r/V)c_B - k_{A+B\to C}c_Ac_B\\
\dot{c}_C &= (r/V)c_C^f - (r/V)c_C + k_{A+B\to C}c_Ac_B - k_{C\to 2D}c_C + k_{2D\to C}c_D^2\\
\dot{c}_D &= -(r/V)c_D + 2k_{C\to 2D}c_C - 2k_{2D\to C}c_D^2
\end{aligned}$$

Let $k_{0\to A} = (r/V)c_A^f$, $k_{0\to B} = (r/V)c_B^f$, $k_{0\to C} = (r/V)c_C^f$, $k_{A\to 0} = r/V$, $k_{B\to 0} = r/V$, $k_{C\to 0} = r/V$, and $k_{D\to 0} = r/V$. With these definitions we can rewrite (1.1.5) as follows:

$$\begin{aligned}
\dot{c}_A &= k_{0\to A} - k_{A\to 0}c_A - k_{A+B\to C}c_Ac_B \qquad (1.1.6)\\
\dot{c}_B &= k_{0\to B} - k_{B\to 0}c_B - k_{A+B\to C}c_Ac_B\\
\dot{c}_C &= k_{0\to C} - k_{C\to 0}c_C + k_{A+B\to C}c_Ac_B - k_{C\to 2D}c_C + k_{2D\to C}c_D^2\\
\dot{c}_D &= -k_{D\to 0}c_D + 2k_{C\to 2D}c_C - 2k_{2D\to C}c_D^2
\end{aligned}$$

We can then call $k_{0\to A}$, $k_{0\to B}$, $k_{0\to C}$, $k_{A\to 0}$, $k_{B\to 0}$, $k_{C\to 0}$ and $k_{D\to 0}$ the rate constants for the pseudo reactions $0 \to A$, $0 \to B$, $0 \to C$, $A \to 0$, $B \to 0$, $C \to 0$ and $D \to 0$, respectively. By convention, with mass action kinetics, a reaction of the form $0 \to A$ is taken to have constant rate, the rate being $k_{0\to A}$, i.e., the rate constant for the reaction $0 \to A$. In this way, the mass action differential equations (1.1.6) derive from the network (1.1.4).

For any given reaction network, the construction of the mass action differential equations proceeds in the same way, as in the example. The resulting system of mass action differential equations will usually be far more complicated than (1.1.3). We will give an example of a more complex reaction network below:

$$\begin{aligned} E_1 + S_1 \rightleftharpoons E_1S_1 \to E_1 + S_2 \rightleftharpoons E_1S_2 \to E_1 + S_3 \rightleftharpoons E_1S_3 \to E_1 + S_4 \\ E_2 + S_4 \rightleftharpoons E_2S_4 \to E_2 + S_3 \rightleftharpoons E_2S_3 \to E_2 + S_2 \rightleftharpoons E_2S_2 \to E_2 + S_1 \\ E_3 + S_1 \rightleftharpoons E_3S_1 \to E_3 + S_2 \rightleftharpoons E_3S_2 \to E_3 + S_3 \\ E_4 + S_4 \rightleftharpoons E_4S_4 \to E_4 + S_3 \rightleftharpoons E_4S_3 \to E_4 + S_2 \end{aligned} \tag{1.1.7}$$

with its corresponding mass action differential equations (1.1.8), where $\dot{c}_s$ is the instantaneous rate of change of concentration of species s, c_s is the instantaneous concentration of species s and $k_{y\to y'}$ is the rate constant for the reaction $y \to y'$:

$$
\begin{aligned}
\dot{c}_{E_1} = \quad & -k_{E_1+S_1\to E_1S_1}c_{E_1}c_{S_1} + k_{E_1S_1\to E_1+S_1}c_{E_1S_1} + k_{E_1S_1\to E_1+S_2}c_{E_1S_1} \\
& -k_{E_1+S_2\to E_1S_2}c_{E_1}c_{S_2} + k_{E_1S_2\to E_1+S_2}c_{E_1S_2} + k_{E_1S_2\to E_1+S_3}c_{E_1S_2} \\
& -k_{E_1+S_3\to E_1S_3}c_{E_1}c_{S_3} + k_{E_1S_3\to E_1+S_3}c_{E_1S_3} + k_{E_1S_3\to E_1+S_4}c_{E_1S_3} \\
\dot{c}_{E_2} = \quad & -k_{E_2+S_4\to E_2S_4}c_{E_2}c_{S_4} + k_{E_2S_4\to E_2+S_4}c_{E_2S_4} + k_{E_2S_4\to E_2+S_3}c_{E_2S_4} \\
& -k_{E_2+S_3\to E_2S_3}c_{E_2}c_{S_3} + k_{E_2S_3\to E_2+S_3}c_{E_2S_3} + k_{E_2S_3\to E_2+S_2}c_{E_2S_3} \\
& -k_{E_2+S_2\to E_2S_2}c_{E_2}c_{S_2} + k_{E_2S_2\to E_2+S_2}c_{E_2S_2} + k_{E_2S_2\to E_2+S_1}c_{E_2S_2} \\
\dot{c}_{E_3} = \quad & -k_{E_3+S_1\to E_3S_1}c_{E_3}c_{S_1} + k_{E_3S_1\to E_3+S_1}c_{E_3S_1} + k_{E_3S_1\to E_3+S_2}c_{E_3S_1} \\
& -k_{E_3+S_2\to E_3S_2}c_{E_3}c_{S_2} + k_{E_3S_2\to E_3+S_2}c_{E_3S_2} + k_{E_3S_2\to E_3+S_3}c_{E_3S_2} \\
\dot{c}_{E_4} = \quad & -k_{E_4+S_4\to E_4S_4}c_{E_4}c_{S_4} + k_{E_4S_4\to E_4+S_4}c_{E_4S_4} + k_{E_4S_4\to E_4+S_3}c_{E_4S_4} \\
& -k_{E_4+S_3\to E_4S_3}c_{E_4}c_{S_3} + k_{E_4S_3\to E_4+S_3}c_{E_4S_3} + k_{E_4S_3\to E_4+S_2}c_{E_4S_3} \\
\dot{c}_{S_1} = \quad & -k_{E_1+S_1\to E_1S_1}c_{E_1}c_{S_1} + k_{E_1S_1\to E_1+S_1}c_{E_1S_1} + k_{E_2S_2\to E_2+S_1}c_{E_2S_2} \\
& -k_{E_3+S_1\to E_3S_1}c_{E_3}c_{S_1} + k_{E_3S_1\to E_3+S_1}c_{E_3S_1} \\
\dot{c}_{S_2} = \quad & k_{E_1S_1\to E_1+S_2}c_{E_1S_1} - k_{E_1+S_2\to E_1S_2}c_{E_1}c_{S_2} + k_{E_1S_2\to E_1+S_2}c_{E_1S_2} \\
& +k_{E_2S_3\to E_2+S_2}c_{E_2S_3} - k_{E_2+S_2\to E_2S_2}c_{E_2}c_{S_2} + k_{E_2S_2\to E_2+S_2}c_{E_2S_2} \\
& +k_{E_3S_1\to E_3+S_2}c_{E_3S_1} - k_{E_3+S_2\to E_3S_2}c_{E_3}c_{S_2} + k_{E_3S_2\to E_3+S_2}c_{E_3S_2} \\
& +k_{E_4S_3\to E_4+S_2}c_{E_4S_3} \\
\dot{c}_{S_3} = \quad & k_{E_1S_2\to E_1+S_3}c_{E_1S_2} - k_{E_1+S_3\to E_1S_3}c_{E_1}c_{S_3} + k_{E_1S_3\to E_1+S_3}c_{E_1S_3} \\
& +k_{E_2S_4\to E_2+S_3}c_{E_2S_4} - k_{E_2+S_3\to E_2S_3}c_{E_2}c_{S_3} + k_{E_2S_3\to E_2+S_3}c_{E_2S_3} \\
& +k_{E_3S_2\to E_3+S_3}c_{E_3S_2} + k_{E_4S_4\to E_4+S_3}c_{E_4S_4} - k_{E_4+S_3\to E_4S_3}c_{E_4}c_{S_3} \\
& +k_{E_4S_3\to E_4+S_3}c_{E_4S_3} \\
\dot{c}_{S_4} = \quad & k_{E_1S_3\to E_1+S_4}c_{E_1S_3} - k_{E_2+S_4\to E_2S_4}c_{E_2}c_{S_4} + k_{E_2S_4\to E_2+S_4}c_{E_2S_4} \\
& -k_{E_4+S_4\to E_4S_4}c_{E_4}c_{S_4} + k_{E_4S_4\to E_4+S_4}c_{E_4S_4}
\end{aligned}
\tag{1.1.8}
$$

$$
\begin{aligned}
\dot{c}_{E_1S_1} = \quad & k_{E_1+S_1\to E_1S_1}c_{E_1}c_{S_1} - k_{E_1S_1\to E_1+S_1}c_{E_1S_1} - k_{E_1S_1\to E_1+S_2}c_{E_1S_1} \\
\dot{c}_{E_1S_2} = \quad & k_{E_1+S_2\to E_1S_2}c_{E_1}c_{S_2} - k_{E_1S_2\to E_1+S_2}c_{E_1S_2} - k_{E_1S_2\to E_1+S_3}c_{E_1S_2} \\
\dot{c}_{E_1S_3} = \quad & k_{E_1+S_3\to E_1S_3}c_{E_1}c_{S_3} - k_{E_1S_3\to E_1+S_3}c_{E_1S_3} - k_{E_1S_3\to E_1+S_4}c_{E_1S_3} \\
\dot{c}_{E_2S_4} = \quad & k_{E_2+S_4\to E_2S_4}c_{E_2}c_{S_4} - k_{E_2S_4\to E_2+S_4}c_{E_2S_4} - k_{E_2S_4\to E_2+S_3}c_{E_2S_4} \\
\dot{c}_{E_2S_3} = \quad & k_{E_2+S_3\to E_2S_3}c_{E_2}c_{S_3} - k_{E_2S_3\to E_2+S_3}c_{E_2S_3} - k_{E_2S_3\to E_2+S_2}c_{E_2S_3} \\
\dot{c}_{E_2S_2} = \quad & k_{E_2+S_2\to E_2S_2}c_{E_2}c_{S_2} - k_{E_2S_2\to E_2+S_2}c_{E_2S_2} - k_{E_2S_2\to E_2+S_1}c_{E_2S_2} \\
\dot{c}_{E_3S_1} = \quad & k_{E_3+S_1\to E_3S_1}c_{E_3}c_{S_1} - k_{E_3S_1\to E_3+S_1}c_{E_3S_1} - k_{E_3S_1\to E_3+S_2}c_{E_3S_1} \\
\dot{c}_{E_4S_4} = \quad & k_{E_4+S_4\to E_4S_4}c_{E_4}c_{S_4} - k_{E_4S_4\to E_4+S_4}c_{E_4S_4} - k_{E_4S_4\to E_4+S_3}c_{E_4S_4} \\
\dot{c}_{E_4S_3} = \quad & k_{E_4+S_3\to E_4S_3}c_{E_4}c_{S_3} - k_{E_4S_3\to E_4+S_3}c_{E_4S_3} - k_{E_4S_3\to E_4+S_2}c_{E_4S_3}
\end{aligned}
$$

We propose our questions for this thesis: For a given reaction network, how can we determine if the corresponding mass action differential equations have the capacity to admit multiple positive equilibria (in a sense to be defined more precisely later)? (By a positive equilibrium, we mean one in which all species concentrations are positive.) That is, are there rate constant values for which multiple positive equilibria will be obtained? And how does the answer depend on the reaction network structure? This thesis is about answering these questions.

Remark 1.1.2. *There are certain physical assumptions underlying the work. All reactions are assumed to be governed by mass action kinetics. All mixtures are assumed to have a constant density, independent of composition. All mixtures are assumed to be spatially homogeneous ("well-stirred") so the concentrations in the reactor are uniform. The reactors are assumed to operate isothermally. These assumptions are made to focus solely on changes in the concentrations due to the reactions in the given reaction network.*

1.2 Overview

The main objective is to construct the Higher Deficiency Theory, a theory which is proposed to study the equilibrium states of chemical reaction networks. We will try to provide a systematic way to answer the question of whether a given reaction network has the capacity to admit multiple steady states. The Higher Deficiency Theory translates the question of multiple steady states into questions about solving systems of *linear* inequalities and equalities. An algorithm, called the Higher Deficiency Algorithm, is designed and implemented in a user-friendly Windows-based program called the Chemical Reaction Network Toolbox [16], according to the theory. The Higher Deficiency Theory and Algorithm are reformulations and extensions of earlier work by Feinberg (see [1], [2], [3], [4], [5], and [6]) and Ellison (see [7], [8], [9], [10] and [11]).

The other components of this thesis are the Mass Action Injectivity Test and the Concordance Test. Both serve as supportive tools for the Higher Deficiency Theory and Algorithm in answering the question of multiple steady states. The Mass Action Injectivity Test and the Concordance Test can only conclude that a given reaction network *cannot* support multiple steady states; they can never conclude that a reaction network can support multiple steady states. The Concordance Test also gives information about networks with kinetics that are not mass action.

If a network passes the Mass Action Injectivity Test or the Concordance Test, then (in the mass action case) the given reaction network cannot support multiple steady states, no matter what positive values the rate constants (of the reactions in the network) take. In the case that the computational algorithm for the Higher Deficiency Theory needs to take a somewhat longer time to answer or cannot answer the question, the Mass Action Injectivity Test and the Concordance Test might nevertheless give information, and perhaps quickly.

1.3 Terms and Definitions

In this section, the terms and definitions for understanding chemical reaction network theory are introduced.

Let I be a finite set. We denote by $\#(I)$ or $|I|$ the number of elements in I. We denote $\mathbb{R}^I$ as the vector space of real-valued functions with domain I. Let $x \in \mathbb{R}^I$, and $i \in I$, then $x(i)$ will usually be denoted x_i. In particular, $\mathbb{R}^I_+$ means the vector space of positive-valued functions with domain I and $\bar{\mathbb{R}}^I_+$ means the vector space of nonnegative-valued functions with domain I. We define the standard basis $\{\omega_i\}_{i\in I}$ of $\mathbb{R}^I$ as follows:

$$\omega_i(j) = \begin{cases} 1, & \text{if } j = i \\ 0, & \text{otherwise.} \end{cases}$$

Addition, subtraction, and scalar multiplication in $\mathbb{R}^I$ are defined in the usual way. We then define the vector multiplication, exponential, natural logarithmic functions and standard scalar product in $\mathbb{R}^I$ as follows:

Let $x, z \in \mathbb{R}^I$, then $xz \in \mathbb{R}^I$ is defined via $(xz)_i = x_i z_i$, $e^x \in \mathbb{R}^I$ is defined via $(e^x)_i = e^{x_i}$, $\ln x \in \mathbb{R}^I$ is defined via $(\ln x)_i = \ln x_i$, $x^z \in \mathbb{R}$ is defined via $x^z = \prod_{i\in I} x_i^{z_i}$, and $x \cdot z \in \mathbb{R}$ is defined via $x \cdot z = \sum_{i\in I} x_i z_i$.

We also define the ***support*** of $x \in \mathbb{R}^I$, denoted by supp x, as follows:

supp $x := \{i \in I : x_i \neq 0\}$.

We define the support of a set $A = \{x^1, x^2, ..., x^n\} \subset \mathbb{R}^I$ as follows:

supp $A := \{i \in I :$ there exists $x^j \in A$ such that $x^j_i \neq 0\}$.

In general, a reaction network consists of three sets $\mathscr{S}$, $\mathscr{C}$, and $\mathscr{R}$, where $\mathscr{S}$ is the set of all species in the reaction network, $\mathscr{C}$ is the set of all complexes in the reaction network, and $\mathscr{R}$ is the set of all reactions in the reaction network. In reaction network (1.1.1), $\mathscr{S} = \{A, B, C, D\}$, $\mathscr{C} = \{A + B, C, 2D\}$, and $\mathscr{R} = \{A + B \to C, C \to 2D, 2D \to C\}$.

In a reaction network, each complex is associated with a ***complex vector*** in $\mathbb{R}^{\mathscr{S}}$. The

complex vector y for the complex is defined as $\sum_{s\in\mathscr{S}} y_s\omega_s$, where y_s is the stoichiometric coefficient of the species s for the complex.

In the reaction network theory, we will often find it convenient to identify the name of a complex with its complex vector. For example, the complex $A + B$ is regarded as a symbol for $\omega_A + \omega_B \in \mathbb{R}^{\mathscr{S}}$. Thus, $\mathscr{C}$ is regarded as a subset of $\bar{\mathbb{R}}^{\mathscr{S}}_+$. A reaction, then, will typically be denoted by a symbol such as $y \to y'$, where y and y' are members of $\mathscr{C} \subset \bar{\mathbb{R}}^{\mathscr{S}}_+$ (e.g. $y = A + B$, $y' = C$). In reaction network (1.1.1), the complexes are $A + B$, C, and $2D$, and the corresponding complex vectors are $\omega_A + \omega_B$, ω_C, and $2\omega_D$. Thus $A + B$ is regarded as a symbol for $\omega_A + \omega_B \in \mathbb{R}^{\mathscr{S}}$, C is regarded as a symbol for $\omega_C \in \mathbb{R}^{\mathscr{S}}$, and D is regarded as a symbol for $\omega_D \in \mathbb{R}^{\mathscr{S}}$.

For a reaction in a reaction network with an arrow $\to$, the complex lying in front of the tail of the arrow is called a ***reactant complex*** for the reaction, and the one lying next to the head of the arrow is called a ***product complex***. In other words, if, in a reaction network, there exists a reaction $y \to y\prime$, then y is the reactant complex of this reaction, and y' is the product complex of this reaction.

The ***reaction vector*** for the reaction $y \to y'$ is defined as $y' - y \in \mathbb{R}^{\mathscr{S}}$, and $(y' - y)_s$ represents the net change in the number of molecules for species $s \in \mathscr{S}$ from one occurrence of reaction $y \to y'$. In reaction network (1.1.1), the reaction vectors are $\omega_C - \omega_A - \omega_B$, $2\omega_D - \omega_C$, and $\omega_C - 2\omega_D$, which are associated with the reactions $A + B \to C, C \to 2D$, and $2D \to C$, respectively. We then define the ***stoichiometric subspace*** S as the linear subspace of $\mathbb{R}^{\mathscr{S}}$ spanned by all reaction vectors in the reaction network, $i.e.$, $S = \text{span}\,\{y' - y : y \to y' \in \mathscr{R}\}$. In reaction network (1.1.1), the stoichiometric subspace is the linear space spanned by the set $\{\omega_C - \omega_A - \omega_B, 2\omega_D - \omega_C, \omega_C - 2\omega_D\}$. Therefore, a basis of the stoichiometric subspace is $\{\omega_C - \omega_A - \omega_B, 2\omega_D - \omega_C\}$ and the dimension of the stoichiometric subspace is 2.

Recall that $\{\omega_s : s \in \mathscr{S}\}$ is the standard basis for $\mathbb{R}^{\mathscr{S}}$. In a mixture in which the

molar concentration of species $s \in \mathscr{S}$ is c_s, the ***composition vector*** $c \in \mathbb{R}^{\mathscr{S}}$ is defined by $c := \sum_{s \in \mathscr{S}} c_s \omega_s$. In mass action kinetics, the rate of the reaction $y \to y'$ at a composition c is equal to $k_{y \to y'} c^y$, where $k_{y \to y'}$ is the rate constant for the reaction $y \to y'$. Here c^y is defined as follows:

$$c^y = \prod_{s \in \mathscr{S}} c_s^{y_s}, \tag{1.3.1}$$

where y_s is the stoichiometric coefficient of the species s for the complex y.

The rate of change of composition $\dot{c}$ for a given composition c can then be described by the following equation (see [1]):

$$\dot{c} = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y). \tag{1.3.2}$$

From here we can see that the rate of change of composition $\dot{c}$ for a given composition c lies in the stoichiometric subspace S.

Two composition vectors in $\bar{\mathbb{R}}_+^{\mathscr{S}}$, c^* and c^{**}, are called ***stoichiometrically compatible*** if $c^* - c^{**} \in S$.

As usual, we say that R is an equivalence relation on a set X, if for any $x, x_1, x_2, x_3 \in X$, the following are satisfied:

(i) Reflexivity: xRx.

(ii) Symmetry: If $x_1 R x_2$ then $x_2 R x_1$.

(iii) Transitivity: If $x_1 R x_2$ and $x_2 R x_3$, then $x_1 R x_3$.

We can see that stoichiometric compatibility defines an equivalence relation. Therefore, it can be used to partition compositions in $\bar{\mathbb{R}}_+^{\mathscr{S}}$ into ***stoichiometric compatibility classes***. If we consider instead two composition vectors in $\mathbb{R}_+^{\mathscr{S}}$, we can similarly claim that the stoichiometric compatibility can partition $\mathbb{R}_+^{\mathscr{S}}$ into ***positive stoichiometric compatiblity classes***. In other words, we define a positive stoichiometric compatibility class as the subset of a stoichiometric compatibility class that contains all of the strictly positive

compositions of that stoichiometric compatibility class. In particular, the positive stoichiometric compatibility class containing $c^* \in \mathbb{R}^{\mathscr{S}}_+$ is $(c^* + S) \cap \mathbb{R}^{\mathscr{S}}_+$.

A reaction network is said to ***support multiple positive steady states*** *if there exist positive rate constants such that the corresponding differential equation (1.3.2) admits two distinct steady states in the same positive stoichiometric compatibility class.*

We have defined basic terms that describe a reaction network and the quantitative terms associated with a reaction network. Next we will introduce some terms that describe the general structure of a reaction network.

A reaction $y \to y'$ in a reaction network $\mathscr{R}$ is called ***reversible*** if $y' \to y$ also lies in the reaction network; otherwise that reaction is called ***irreversible***.

A reaction network is called ***reversible*** if every reaction in the network is reversible. A reaction network is called ***weakly reversible*** if for every reaction $y \to y' \in \mathscr{R}$, there exist complexes $y_1, y_2, ...$ such that $y' \to y_1 \to y_2 \to ... \to y$ lie in $\mathscr{R}$. Note that each reversible network is also a weakly reversible network.

Two complexes, y and y' in a reaction network are called ***directly linked*** if the reaction network contains either the reaction $y \to y'$ or the reaction $y' \to y$ or both reactions. If y and y' are directly linked, then we denote it as $y \leftrightarrow y'$. Two complexes, y and y' in a reaction network are called ***linked*** if they are the same complex or if there exist $y_1, y_2, ...$ such that $y \leftrightarrow y_1 \leftrightarrow y_2 \leftrightarrow ... \leftrightarrow y'$.

We can claim that linkage defines an equivalence relation:

(i) Reflexivity: $y \leftrightarrow y$.

(ii) Symmetry: If $y \leftrightarrow y'$, then $y' \leftrightarrow y$.

(iii) Transitivity: If $y_1 \leftrightarrow y_2$ and $y_2 \leftrightarrow y_3$, then $y_1 \leftrightarrow y_3$.

The linkage relation can be used to partition complexes in $\mathscr{C}$ into ***linkage classes***. In reaction network (1.1.1), there is only one linkage class.

A complex y is said to ***react to*** complex y' if $y \to y'$ lies in the reaction network. A

complex y is said to ***ultimately react to*** complex y' if they are the same complex or if there exist $y_1, y_2, ...$ such that $y \to y_1 \to y_2 \to ... \to y'$ lie in the reaction network.

Two complexes, y and y', are called to be ***strongly linked*** if both y ultimately reacts to y' and y' ultimately reacts to y.

We can claim that strong linkage defines an equivalence relation:

(i) Reflexivity: y is strongly linked to y.

(ii) Symmetry: If y is strongly linked to y', then y' is strongly linked to y.

(iii) Transitivity: Suppose that y is strongly linked to $\tilde{y}$ and $\tilde{y}$ is strongly linked to $\hat{y}$. Then there exists paths from y to $\tilde{y}$, from $\tilde{y}$ to y, from $\tilde{y}$ to $\hat{y}$ and from $\hat{y}$ to $\tilde{y}$. Therefore, there exist a path from y to $\hat{y}$ and a path from $\hat{y}$ to y, i.e., y is strongly linked to $\hat{y}$.

The strong linkage relation can be used to partition complexes in $\mathscr{C}$ into ***strong linkage classes***. A strong linkage class is called ***terminal*** if no complex in the strong linkage class reacts to a complex that does not lie in the strong linkage class; otherwise it is called a ***nonterminal strong linkage class***. A strong linkage class is called ***trivial*** if it contains only one complex in the strong linkage class; otherwise it is called ***nontrivial***. In reaction network (1.1.1), the strong linkage classes are $\{A + B\}$ and $\{C, 2D\}$. $\{A + B\}$ is trivial and nonterminal, and $\{C, 2D\}$ is nontrivial and terminal in reaction network (1.1.1).

We will give another example of reaction network:

$$\begin{array}{rccl} A + B + 2C \to & D + E & \rightleftharpoons & 3F \\ C + E \leftarrow & 2F & \rightleftharpoons & 2A + 2B \\ & \downarrow & \nearrow & \\ & 2A + C & & \\ 2B + 2C & \rightleftharpoons & D + F & \end{array} \tag{1.3.3}$$

There are three linkage classes in the reaction network (1.3.3). The strong linkage classes are $\{A + B + 2C\}$, $\{D + E, 3F\}$, $\{C + E\}$, $\{2F, 2A + C, 2A + 2B\}$, and $\{2B + 2C, D +$

$F\}$. $\{A + B + 2C\}$ is nonterminal and trivial, $\{D + E, 3F\}$ is terminal and nontrivial, $\{C + E\}$ is terminal and trivial, $\{2F, 2A + C, 2A + 2B\}$ is nonterminal and nontrivial, and $\{2B + 2C, D + F\}$ is terminal and nontrivial.

Remark 1.3.1. *It is easy to see that in a finite reaction network (with a finite number of complexes), every linkage class contains at least one terminal strong linkage class.*

Let $\#(\mathscr{C})$ be the number of complexes in the reaction network, let dim S be the dimension of the stoichiometric space S spanned by all reaction vectors in the reaction network, and let $\#(\text{linkage classes})$ be the number of linkage classes in the reaction network. The ***deficiency of a reaction network***, denoted by δ, is defined as $\delta = \#(\mathscr{C}) - \#(\text{linkage classes}) - \dim S$. For reaction network (1.1.1), the deficiency of the network is equal to $3 - 1 - 2 = 0$. For reaction network (1.3.3), the deficiency of the network is equal to $9 - 3 - 5 = 1$.

Note that the stoichiometric subspace S is a subspace of $\mathbb{R}^{\mathscr{S}}$. A vector, $v \in \mathbb{R}^{\mathscr{S}}$ is called ***sign-compatible with the stoichiometric subspace*** S, if there exists a vector $\sigma \in S$ and a set of positive numbers $\{p_s : s \in \mathscr{S}\}$, such that $v_s = p_s\sigma_s$, for all $s \in \mathscr{S}$.

In Sections 1.4 to 1.7.2, we will review older work by Feinberg and Ellison.

1.4 Deficiency Zero Theorem

The Deficiency Zero Theorem (see [1] and [2]) provides decisive results on reaction networks with deficiency zero. We will in this section state the Deficiency Zero Theorem and give an example to see the theorem in application. To see more information on the Deficiency Zero Theorem, see [1] and [2].

Before stating the Deficiency Zero Theorem, we restrict "arbitrary kinetics" in the theorem statement to be such that the value of the rate function assigned to a reaction is strictly positive at a given composition if and only if all species in the support of its reactant

complex vector have strictly positive concentrations. In other words, given any reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, for any $y \to y' \in \mathscr{R}$, we assume that the rate function $\mathscr{K}_{y\to y'}(\cdot)$ has the following property: $\mathscr{K}_{y\to y'}(c) > 0$ if and only if for all $s \in$ supp y, $c_s > 0$. We also require the rate functions to be continuous.

Theorem 1.4.1. *(The Deficiency Zero Theorem): For any reaction network of deficiency zero, the following statements hold true:*

(i) If the network is not weakly reversible, then for arbitrary kinetics (not necessarily mass action), the differential equations for the corresponding reaction system cannot admit a positive steady state (i.e. a steady state in $\mathbb{R}^{\mathscr{S}}_+$).

(ii) If the network is not weakly reversible, then for arbitrary kinetics (not necessarily mass action), the differential equations for the corresponding reaction system cannot admit a cyclic composition trajectory along which all species concentrations are positive.

(iii) If the network is weakly reversible, then for mass action kinetics (but regardless of the positive values the rate constants take), the resulting differential equations have the following properties:

There exists within each positive stoichiometric compatibility class precisely one steady state; that steady state is asymptotically stable; there is no nontrivial cyclic composition trajectory along which all species concentrations are positive.

Let us consider the following reaction network.

$$\begin{aligned} A + C &\rightleftharpoons F \\ E + C &\rightleftharpoons H \\ H &\rightleftharpoons B + F \\ B + D &\rightleftharpoons G \\ G &\rightleftharpoons 2A + D \end{aligned} \tag{1.4.1}$$

The reaction network (1.4.1) is a reversible deficiency zero network. Therefore the Deficiency Zero Theorem implies that for any assignment of positive rate constants to the individual reactions, the corresponding mass action differential equations admit precisely one steady state (in each positive stoichiometric compatibility class), that steady state is asymptotically stable, and there are no cyclic composition trajectories.

1.5 Deficiency One Theorem

The Deficiency One Theorem (see [1], [2] and [4]) provides results similar to the Deficiency Zero Theorem. The Deficiency Zero Theorem gives more powerful results and can apply to reaction networks governed by arbitrary kinetics (see the note about "arbitrary kinetics" before the statement of the Deficiency Zero Theorem). The Deficiency One Theorem can only provide results for reaction networks governed by mass action kinetics. However, the Deficiency One Theorem applies to a larger set of reaction networks in terms of the deficiency. On the other hand, the Deficiency One Theorem gives no stability information. To get a full description of the Deficiency One Theorem, see [1], [2] and [4].

To state the Deficiency One Theorem, we need to first introduce a term called the ***deficiency of a linkage class***. Each linkage class, together with the reactions that connect all the complexes in the linkage class, can be treated as a subnetwork of the original reaction network. By the deficiency of a linkage class, we mean the deficiency of the subnetwork associated with that linkage class in the obvious way.

Let us consider the following reaction network.

$$\begin{array}{lll} 2A \rightleftharpoons & B \rightleftharpoons & 2C \\ & \uparrow \quad \swarrow & \\ & A + C & \\ 2B \rightleftharpoons & B + D & \rightleftharpoons 3C \end{array} \tag{1.5.1}$$

For network (1.5.1), $\mathscr{C} = \{2A, B, 2C, A + C, 2B, B + D, 3C\}$, S =span $\{2\omega_A - \omega_B, \omega_A - \omega_C, \omega_B - \omega_D, \omega_B + \omega_D - 3\omega_C\}$, and there are two linkage classes. It is a deficiency one network as $\#(\mathscr{C}) - \#(\text{linkage classes}) - \dim S = 7 - 2 - 4 = 1$. For the first linkage class $\{2A, B, 2C, A + C\}$, the deficiency is $4 - 1 - 2 = 1$, and for the second linkage class $\{2B, B + D, 3C\}$, the deficiency is $3 - 1 - 2 = 0$.

Next we will state the Deficiency One Theorem.

Theorem 1.5.1. *(The Deficiency One Theorem): Consider a mass action system for which the underlying reaction network has l linkage classes. Let δ be the deficiency of the network and let δ_θ be the deficiency of the θ^{th} individual linkage class. Suppose that the following conditions hold:*

(i) $\delta_\theta \leq 1, \theta = 1, 2, ..., l$

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

(iii) *Each linkage class contains only one terminal strong linkage class.*

Then, no matter what (positive) values the rate constants take, the corresponding differential equations can admit no more than one steady state within a positive stoichiometric compatibility class. If the network is weakly reversible, the differential equations admit precisely one steady state in each positive stoichiometric compatibility class.

Consider the following reaction network that satisfies the conditions in the Deficiency One Theorem,

$$2A \leftarrow A + B \rightleftharpoons 2B \tag{1.5.2}$$

The reaction network (1.5.2) is not weakly reversible, has deficiency one and only one linkage class with one terminal strong linkage class in it. From the Deficiency One Theorem, we can claim that no matter what (positive) values the rate constants take, the corresponding differential equations can admit no more than one steady state within a positive stoichiometric compatibility class.

Reaction network (1.5.1) also satisfies the conditions in the Deficiency One Theorem. It is weakly reversible, so the corresponding mass action differential equations admit precisely one steady state in each positive stoichiometric compatibility class.

There are reaction networks with deficiency one that do not satisfy the conditions in the Deficiency One Theorem. Consider the following reaction network:

$$2A \leftarrow A + B \rightarrow 2B \tag{1.5.3}$$

Reaction network (1.5.3) violates the third condition that each linkage class can only contain one terminal strong linkage class.

The following example is another reaction network that does not satisfy the conditions of the Deficiency One Theorem:

$$\begin{aligned} A + 2B &\rightleftharpoons 3A \\ A &\rightleftharpoons 0 \rightleftharpoons B \end{aligned} \tag{1.5.4}$$

Note that in reaction network (1.5.4), the deficiency of the entire network is 1, the deficiency of the first linkage class is 0 and the deficiency of the second linkage class is 0. So it does not satisfy the second condition of the Deficiency One Theorem.

For the reaction networks that do not satisfy the conditions of the Deficiency One Theorem, the theorem stays silent. For a deficiency one reaction network that does not satisfy the conditions of the Deficiency One Theorem, the Deficiency One Algorithm (see [3], [5] and [6] for more information) was created to solve that case.

1.6 Deficiency One Algorithm

The Deficiency One Algorithm analyzes a regular deficiency one reaction network's capacity for multiple steady states by generating and solving systems of *linear* equalities and inequalities (A network is "regular" if it satisfies weak conditions to be discussed shortly).

The systems of linear equalities and inequalities are corresponding to "shelving" partitions of the reactant complexes (The "shelving" partitions are about assigning reactant complexes to three different subsets called "shelves," which are also to be discussed shortly.). All linear systems generated are in terms of an unknown vector $\mu \in \mathbb{R}^{\mathscr{S}}$. The equalities of the linear systems all have the form $y \cdot \mu = y' \cdot \mu$ and the inequalities of the linear systems all have the form $y \cdot \mu > y' \cdot \mu$, where y and y' are complexes. The Deficiency One Algorithm indicates how these inequality systems are to be formed. To see more about the Deficiency One Algorithm, please see [3], [5] and [6].

In the Deficiency One Algorithm, we say that such a linear system of equalities and inequalities has a solution if there exists a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with the stoichiometric subspace S and satisfies all the equalities and inequalities in the linear system. We call the linear system a ***signature*** for the reaction network if it has such a solution. If in the algorithm we find a signature for the reaction network, then the network has the capacity to support multiple steady states. If no signature exists for the network, then the network cannot admit multiple steady states, no matter what positive values the rate constants take.

The Deficiency One Algorithm is implemented in the Chemical Reaction Network Toolbox [6].

Before we state the steps of the Deficiency One Algorithm, we first define a few terms (see [3], [5] and [6] for more information).

A pair of complexes y, y' in a reaction network is a ***cut pair***, if the following conditions hold:

(i) y and y' are directly linked.

(ii) By removing the reaction(s) between y and y', the linkage class containing y and y' will break into two linkage classes (of the new network without the reaction(s) between y and y'), $\mathscr{W}(y)$ and $\mathscr{W}(y')$, where $y \in \mathscr{W}(y)$ and $y' \in \mathscr{W}(y')$.

For reaction network (1.5.4), by the removal of reactions between $A + 2B$ and $3A$, we have that $\mathscr{W}(A + 2B) = \{A + 2B\}$ and $\mathscr{W}(3A) = \{3A\}$; by the removal of reactions between A and 0, we have that $\mathscr{W}(A) = \{A\}$ and $\mathscr{W}(0) = \{0, B\}$; by the removal of the reactions between 0 and B, we have that $\mathscr{W}(0) = \{A, 0\}$ and $\mathscr{W}(B) = \{B\}$. There are three cut pairs in reaction network (1.5.4), $\{A + 2B, 3A\}$, $\{A, 0\}$ and $\{0, B\}$.

For reaction network (1.5.1), there are three cut pairs, $\{2A, B\}$, $\{2B, B + D\}$ and $\{B + D, 3C\}$. Note that $\{B, 2C\}$, $\{B, A + C\}$ and $\{2C, A + C\}$ are not cut pairs as the removal of the reaction(s) between each pair does not disconnect the linkage class.

A network is ***regular*** if it satisfies three conditions:

(i) The reaction vectors of the network are positively dependent, i.e., there exists $\alpha \in \mathbb{R}^{\mathscr{R}}_+$ such that

$$\sum_{y \to y' \in \mathscr{R}} \alpha_{y \to y'}(y' - y) = 0. \tag{1.6.1}$$

(ii) Each linkage class contains only one terminal strong linkage class.

(iii) Each pair of directly linked complexes in a terminal strong linkage class is a cut pair.

Remark 1.6.1. *The reaction networks we consider in the Deficiency One Algorithm are regular deficiency one reaction networks.*

Reaction network (1.5.4) satisfies all three conditions for being a regular network. Let $\alpha \in \mathbb{R}^{\mathscr{R}}_+$ be such that $\alpha_{A+2B\to 3A} = -\alpha_{3A\to A+2B} = \alpha_{A\to 0} = -\alpha_{0\to A} = \alpha_{0\to B} = -\alpha_{B\to 0} = 1$, then $\sum_{y \to y' \in \mathscr{R}} \alpha_{y \to y'}(y' - y) = 0$. Each linkage class is a strong linkage class, so it contains only one terminal strong linkage class. All adjacent complexes in the terminal strong linkage class, $\{A + 2B, 3A\}$, $\{A, 0\}$ and $\{0, B\}$ are cut pairs.

Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, a vector $g \in \mathbb{R}^{\mathscr{C}}$ is a ***confluence vector*** if the following conditions hold:

(I) $\sum_{y \in \mathscr{C}} g_y y = 0$.

(II) For each linkage class $\mathscr{L}$, $\sum_{y\in\mathscr{L}} g_y = 0$.

(III) For each terminal strong linkage class Λ that is not a linkage class, $\sum_{y\in\Lambda} g_y > 0$.

For reaction network (1.5.4), a confluence vector is g such that $g_{A+2B} = 1$, $g_{3A} = -1$, $g_A = 2$, $g_0 = 0$, $g_B = -2$. To see that, note that

(I) $g_{A+2B}(\omega_A + 2\omega_B) + g_{3A}(3\omega_A) + g_A(\omega_A) + g_0(0) + g_B(\omega_B)$= $(1)(\omega_A + 2\omega_B) + (-1)(3\omega_A) + (2)(\omega_A) + (0)(0) + (-2)(\omega_B) = 0$.

(II) $g_{A+2B} + g_{3A} = 1 + (-1) = 0$, $g_A + g_0 + g_B = 2 + 0 + (-2) = 0$.

(III) is satisfied trivially as each terminal strong linkage class is a entire linkage class.

Let $g \in \mathbb{R}^{\mathscr{C}}$ be a confluence vector. If y and y' is a cut pair, we define $[g, y \to y', y] = \sum_{p\in\mathscr{W}(y)} g_p$, where the removal of the reaction(s) between y and y' break the linkage class $\mathscr{L}$ into two linkage classes (of the new network without the reaction(s) between y and y'), $\mathscr{W}(y)$ and $\mathscr{W}(y')$, where $y \in \mathscr{W}(y)$ and $y' \in \mathscr{W}(y')$. We claim that $[g, y \to y', y] = -[g, y' \to y, y']$, as $0 = \sum_{p\in\mathscr{L}} g_p = \sum_{p\in\mathscr{W}(y)} g_p + \sum_{p\in\mathscr{W}(y')} g_p = [g, y \to y', y] + [g, y' \to y, y']$.

For reaction network (1.5.4), from the choice of confluence vector g above, $[g, A + 2B \to 3A, A + 2B] = -[g, 3A \to A + 2B, 3A] = g_{A+2B} = 1$, $[g, A \to 0, A] = -[g, 0 \to A, 0] = g_A = 2$, and $[g, 0 \to B, 0] = -[g, B \to 0, B] = -g_B = 2$.

Recall that for a given reaction network $\mathscr{R}$, a complex y is a reactant complex if there is another complex y' such that $y \to y' \in \mathscr{R}$. An ***upper-middle-lower partition*** is a partition of its reactant complexes into three sets ("shelves"), $\mathscr{U}$, $\mathscr{M}$, and $\mathscr{L}$, called the upper, middle and lower shelves, such that

(i) All non-terminal complexes are put in the middle shelf $\mathscr{M}$.

(ii) All complexes in the same nontrivial terminal strong linkage class are put in the same shelf.

(iii) If there are no trivial terminal strong linkage classes, then neither the upper shelf nor the lower shelf can be empty.

(iv) If there is exactly one trivial terminal strong linkage class, then the upper shelf and the lower shelf cannot both be empty.

We will use reaction network (1.5.4) to illustrate the steps listed in the Deficiency One Algorithm. Recall that the Deficiency One Algorithm applies to regular deficiency one reaction networks. For more information on the algorithm, please see [3], [5] and [6].

Step 1: Find a Confluence Vector

The first step is to find a confluence vector g for the reaction network. For a regular deficiency one reaction network, all confluence vectors are colinear to each other. If in addition the reaction network is not weakly reversible, then all the confluence vectors will point to the same direction. If it is weakly reversible, then there are two possible directions for the confluence vector g. For more information or proof, see [3], [5] and [6].

For the reaction network (1.5.4), it is weakly reversible and therefore there are two directions for the confluence vector. At this step, we choose $g \in \mathbb{R}^{\mathscr{C}}$ such that $g_{A+2B} = 1$, $g_{3A} = -1$, $g_A = 2$, $g_0 = 0$, and $g_B = -2$.

Step 2: Choose a "shelf" Partition for the Reactant Complexes

Choose a partition for all the reactant complexes in the reaction network according to the definition of the upper-middle-lower partition.

For reaction network (1.5.4), there is no trivial terminal strong linkage class, so the only possible partitions are: $\mathscr{U} = \{A + 2B, 3A\}$, $\mathscr{M} = \{\}$, $\mathscr{L} = \{A, 0, B\}$, or $\mathscr{U} = \{A, 0, B\}$, $\mathscr{M} = \{\}$, $\mathscr{L} = \{A + 2B, 3A\}$. Let us choose $\mathscr{U} = \{A + 2B, 3A\}$, $\mathscr{M} = \{\}$, $\mathscr{L} = \{A, 0, B\}$ for this step.

Step 3: Add Shelving Inequalities

All the inequalities comparing $y \cdot \mu$'s between complexes on different shelves are generated for the linear system.

We say y is on a higher shelf than y', if y is put on the upper shelf and y' is put on the middle or lower shelf, or y is put on the middle shelf and y' is put on the lower shelf. For all reactant complexes, if y is on a higher shelf than y', then $y \cdot \mu > y' \cdot \mu$ is added to the linear system.

For reaction network (1.5.4), the inequalities that arise from Step 3 according to the partition in Step 2 are: $\mu_A + 2\mu_B > \mu_A$, $\mu_A + 2\mu_B > 0$, $\mu_A + 2\mu_B > \mu_B$, $3\mu_A > \mu_A$, $3\mu_A > 0$, and $3\mu_A > \mu_B$.

Step 4: Add Shelving Equalities

Equalities comparing $y \cdot \mu$'s between complexes that are put on the middle shelf are added to the linear system. If y and y' are both put on the middle shelf, then $y \cdot \mu = y' \cdot \mu$ is added to the linear system.

For reaction network (1.5.4), since there are no complexes in the middle shelf for the partition in Step 2, there are no equalities added to the linear system in Step 4 for the partition chosen in Step 2.

Step 5: Add Upper and Lower Shelf Equalities and Inequalities

Equalities and Inequalities comparing $y \cdot \mu$'s between complexes of a cut pair on the upper and lower shelves are added to the linear system.

If y and y' is a cut pair on the upper shelf, then an equality or inequality is added to the linear system based on the sign of $[g, y \leftrightarrow y', y]$:

If $[g, y \leftrightarrow y', y] > 0$, then $y \cdot \mu > y' \cdot \mu$ is added to the linear system.

If $[g, y \leftrightarrow y', y] < 0$, then $y \cdot \mu < y' \cdot \mu$ is added to the linear system.

If $[g, y \leftrightarrow y', y] = 0$, then $y \cdot \mu = y' \cdot \mu$ is added to the linear system.

If y and y' is a cut pair on the lower shelf, then an equality or inequality is added to the linear system based on the sign of $[g, y \leftrightarrow y', y]$:

If $[g, y \leftrightarrow y', y] > 0$, then $y \cdot \mu < y' \cdot \mu$ is added to the linear system.

If $[g, y \leftrightarrow y', y] < 0$, then $y \cdot \mu > y' \cdot \mu$ is added to the linear system.

If $[g, y \leftrightarrow y', y] = 0$, then $y \cdot \mu = y' \cdot \mu$ is added to the linear system.

The inequalities and equalities generated from Step 3 through Step 5 form a complete linear system. By complete we mean no other inequality or equality needs to be added to the linear system before we check if it is a signature or not.

We will then check if the complete linear system has a solution of a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S. If yes, we claim that taken with mass action kinetics, the reaction network has the capacity to admit multiple steady states and exit the algorithm. If not, we will move to the next step.

Remark 1.6.2. *Note that in this step only the sign of $[g, y \to y', y]$ matters, and the sign of $[g, y \to y']$ depends only on the direction of the confluence vector g but not on the particular choice of g.*

Note that for a cut pair y and y', if the reactions between y and y' are reversible, then there is no difference using $[g, y \to y', y]$ or $[g, y' \to y, y']$. To see that, note that $[g, y \to y', y] = -[g, y' \to y, y']$. Let y and y' be a cut pair on the upper shelf, and suppose $[g, y \leftrightarrow y', y] > 0$. Therefore $y \cdot \mu > y' \cdot \mu$ is added to the linear system. Note that $[g, y' \to y, y'] < 0$, so $y' \cdot \mu < y \cdot \mu$ is added to the linear system, which is the same as $y \cdot \mu > y' \cdot \mu$. There is no difference using $y \to y'$ or $y' \to y$ in this case. We can show this for all cases of shelves (upper or lower) and signs of $[g, y \to y']$. Therefore, for a cut pair with reversible reactions between them, we can pick any one direction of the two reactions and use it to generate an equality or inequality for the linear system in this step.

For reaction network (1.5.4), note that $g_{A+2B} = 1$, $g_{3A} = -1$, $g_A = 2$, $g_0 = 0$, and $g_B = -2$. Therefore,we have

(i) $[g, A + 2B \to 3A, A + 2B] = -[g, 3A \to A + 2B, 3A] = g_{A+2B} = 1$,

(ii) $[g, A \to 0, A] = -[g, 0 \to A, 0] = g_A = 2$, and

(iii) $[g, 0 \to B, 0] = -[g, B \to 0, B] = -g_B = 2$.

Note that in Step 2, the partition is chosen that $\mathscr{U} = \{A + 2B, 3A\}$, $\mathscr{M} = \{\}$, $\mathscr{L} = \{A, 0, B\}$. Therefore $\mu_A + 2\mu_B > 3\mu_A$, $\mu_A < 0$ and $0 < \mu_B$ are added to the linear system.

Therefore, the inequality system corresponding to the confluence vector g such that $g_{A+2B} = 1$, $g_{3A} = -1$, $g_A = 2$, $g_0 = 0$, and $g_B = -2$, and the "shelf" partition with $\mathscr{U} = \{A + 2B, 3A\}$, $\mathscr{M} = \{\}$, and $\mathscr{L} = \{A, 0, B\}$, is as follows:

$$\begin{aligned}
\mu_A + 2\mu_B &> \mu_A \\
\mu_A + 2\mu_B &> 0 \\
\mu_A + 2\mu_B &> \mu_B \\
3\mu_A &> \mu_A \\
3\mu_A &> 0 \\
3\mu_A &> \mu_B \\
\mu_A + 2\mu_B &> 3\mu_A \\
\mu_A &< 0 \\
0 &< \mu_B
\end{aligned}$$

If we simplify the linear system above, it becomes $\mu_A + 2\mu_B > 3\mu_A > \mu_B > 0 > \mu_A$. Note that $S = \text{span}\ \{\omega_A, \omega_B\} = \mathbb{R}^{\mathscr{S}}$, so any solution of $\mu \in \mathbb{R}^{\mathscr{S}}$ from the linear system shall be sign-compatible with S. We simply need to solve the linear system for a solution of a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$. From the simplified inequality system, we have $\mu_A > 0 > \mu_A$. Then there is no solution to the linear system. We will move to the next step.

Step 6: Repeat Steps 2 to 5

In Step 2 a "shelf" partition is chosen, and from Steps 3 to 5, the linear system are constructed. We will do this for all possible partitions in Step 2.

However, for the cases that the two partitions are only different by exactly exchanging

the upper and lower shelves, the inequality system of the one is just an inversion of the other. We only need to consider one of them.

We will check the linear systems corresponding to all possible "shelf" partitions in Step 2. If there exists a nonzero μ that is sign-compatible with S satisfying a linear system, then we claim that taken with mass action kinetics, the reaction network has the capacity to admit multiple steady states and exit the algorithm. Otherwise, after we test all linear systems generated from Steps 3 to 5 corresponding to all possible "shelf" partitions, we move to the next step.

For reaction network (1.5.4), the two possible partitions are different by exactly exchanging the upper and lower shelf. In this case, we only need to consider the partition we chose in Step 2. We move to the next step.

Step 7: Repeat Steps 1 to 6

Note that as pointed out in Step 5, only the sign of $[g, y \to y', y]$ (depending on the direction of g) matters. As mentioned earlier about a regular deficiency one reaction network, the confluence vectors are all colinear and if the reaction network is not weakly reversible, then all confluence vectors point in the same direction. Therefore, nothing needs to be done for a non-weakly reversible regular deficiency one reaction network. However, if the regular deficiency one reaction network is weakly reversible, then there are two possible directions. In this case, Steps 1 to 6 are repeated for the other orientation of the confluence vector for the weakly reversible regular deficiency one reaction network. Note that the inequalities/equalities from Steps 3 and 4 are based on the choice of partition for reactant complexes, and those from Step 5 are based on the direction of the confluence vector. Therefore, to construct a linear system for this new direction of the confluence vector with some choice of partition for reactant complexes, we only need to change the linear system for the old direction with the same partition choice, in the following way: the inequalities

from Step 5 need to be reversed, and inequalities and equalities from Steps 3 and 4 are kept unchanged.

We will check the linear systems corresponding to all possible directions of confluence vectors (for weakly reversible regular networks there are two directions in total) in Step 1 and all "shelf" partitions in Step 2. If there exists a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S satisfying a linear system, then we claim that the reaction network has the capacity to admit multiple steady states and exit the algorithm. Otherwise, after we test all linear systems generated in Steps 3 to 5 corresponding to all possible directions of confluence vectors and all "shelf" partitions, we claim that taken with mass action kinetics, the reaction network does not have the capacity to admit multiple steady states, no matter what (positive) values the rate constants might take.

For reaction network (1.5.4), we will repeat Steps 1 to 6 by choosing another confluence vector with opposite orientation as g such that $g_{A+2B} = -1$, $g_{3A} = 1$, $g_A = -2$, $g_0 = 0$, and $g_B = 2$. In Step 2, we choose the partition of the reactant complexes as:

$\mathscr{U} = \{A + 2B, 3A\}, \mathscr{M} = \{\}, \mathscr{L} = \{A, 0, B\}$.

In Step 3, with the partition chose in Step 2, we have

$\mu_A + 2\mu_B > \mu_A$, $\mu_A + 2\mu_B > 0$, $\mu_A + 2\mu_B > \mu_B$, $3\mu_A > \mu_A$, $3\mu_A > 0$, and $3\mu_A > \mu_B$.

In Step 4, there are no equalities arose from it.

In Step 5, note that $g_{A+2B} = 1$, $g_{3A} = -1$, $g_A = 2$, $g_0 = 0$, $g_B = -2$. It is known that

(i) $[g, A + 2B \to 3A, A + 2B] = -[g, 3A \to A + 2B, 3A] = g_{A+2B} = -1$,

(ii) $[g, A \to 0, A] = -[g, 0 \to A, 0] = g_A = -2$, and

(iii) $[g, 0 \to B, 0] = -[g, B \to 0, B] = -g_B = -2$.

Note that the partition is chosen such that $\mathscr{U} = \{A + 2B, 3A\}, \mathscr{M} = \{\}, \mathscr{L} = \{A, 0, B\}$, so $\mu_A + 2\mu_B < 3\mu_A$, $\mu_A > 0$ and $0 > \mu_B$ are added to the linear system in Step 5. Note that no other partitions of the reactant complexes from Step 2 need to be considered.

We summarized and simplified the linear system corresponding to the confluence vector g such that $g_{A+2B} = -1$, $g_{3A} = 1$, $g_A = -2$, $g_0 = 0$, and $g_B = 2$, and "shelf" partitions $\mathscr{U} = \{A + 2B, 3A\}, \mathscr{M} = \{\}, \mathscr{L} = \{A, 0, B\}$ to get the following inequality system:

$3\mu_A > \mu_A + 2\mu_B > \mu_A > 0 > \mu_B$.

Recall that we argued that we simply need to solve the linear system for a solution of a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$. From the inequality system we have $\mu_B > 0 > \mu_B$, so there is no solution to the linear system. Therefore, we can claim that taken with mass action kinetics, the reaction network (1.5.4) cannot admit multiple positive steady states, no matter what (positive) values the rate constants might take.

Remark 1.6.3. *We have another example which is similar in structure to reaction network (1.5.4) but gives a different answer in terms of the question of multiple steady states.*

$$\begin{aligned} 2A + B &\rightleftharpoons 3A \\ A &\rightleftharpoons 0 \rightleftharpoons B \end{aligned} \tag{1.6.2}$$

For reaction network (1.6.2), the (simplified) linear systems according to different confluence vectors and "shelf" partitions are

(i) $3\mu_A > 2\mu_A + \mu_B > \mu_A > 0 > \mu_B$,

(ii) $2\mu_A + \mu_B > 3\mu_A > \mu_B > 0 > \mu_A$.

Note that $S = \mathbb{R}^{\mathscr{S}}$*, so any solution from (i) or (ii) will be sign-compatible with* S*. Linear system (i) is equivalent to* $\mu_A > -\mu_B > 0 > \mu_B$*, so we can pick* $\mu_A = 2$ *and* $\mu_B = -1$*. Therefore, reaction network (1.6.2), taken with mass action kinetics, does have the capacity for multiple steady states. Of course, according to the algorithm, we could just stop here. However, we still provide here a complete list of the linear systems. Note that linear system (ii) does not have a solution.*

1.7 Advanced Deficiency Theory and Algorithm

1.7.1 Terms and Definitions

The Advanced Deficiency Theory (and Algorithm) is developed on top of the Deficiency One Algorithm. It also invokes the ideas of the inequality systems, the direction of confluence vector and shelving, and generalizes these ideas to reaction networks with deficiencies higher than one. In particular, it introduces the ideas of colinearity classes and coplanar sets, which are to be discussed shortly. The Advanced Deficiency Algorithm is implemented in the Chemical Reaction Network Toolbox [8]. To get more information about Advanced Deficiency Theory and Algorithm, please see [7] and [8].

Let us introduce some terms and definitions that are used in the Advanced Deficiency Theory and Advanced Deficiency Algorithm, developed in the Ph.D. thesis of Phillipp Ellison ([7] and [8]).

An ***orientation*** $\mathscr{O}$ is defined as a subset of the set of all reactions in a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ such that for every reaction $y \to y' \in \mathscr{R}$, either $y \to y' \in \mathscr{O}$ or $y' \to y \in \mathscr{O}$, but not both.

Let the map $L_{\mathscr{O}} : \mathbb{R}^{\mathscr{O}} \to \mathbb{R}^{\mathscr{S}}$ be defined via $L_{\mathscr{O}}\alpha = \sum_{y \to y' \in \mathscr{O}} \alpha_{y \to y'}(y' - y)$.

Let $d := \dim Ker\ L_{\mathscr{O}}$ and let $v^1, ..., v^d$ be a basis for $Ker\ L_{\mathscr{O}}$. Let $y \to y' \in \mathscr{R}$, we define a ***w-vector*** (based on the basis), $w_{y \to y'} \in \mathbb{R}^d$ as follows:

(i) If $y \to y' \in \mathscr{O}$, $w_{y \to y'}(i) = v^i_{y \to y'}$, $\forall 1 \leq i \leq d$.

(ii) If $y \to y' \in \mathscr{R} \backslash \mathscr{O}$, then $y' \to y \in \mathscr{O}$. We define $w_{y \to y'} = w_{y' \to y}$.

The ***colinearity classes*** of a reaction network are defined using w-vectors. We say two reactions, $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are in the same colinearity class if there exists a *nonzero* number α such that $w_{y \to y'} = \alpha w_{\tilde{y} \to \tilde{y}'}$. Therefore, it follows that a reversible pair must be in the same colinearity class.

Remark 1.7.1. *It can be shown that the colinearity classes do not depend on the choice*

of the orientation $\mathcal{O}$, or the basis for $Ker\ L_{\mathcal{O}}$ used to define the w-vectors. For more information and proof, see [7] and [8].

We define the ***zero colinearity class*** as the colinearity class in which the w-vectors of all reactions are zero vectors.

For a reaction network, suppose there exist a zero colinearity class and n nonzero colinearity classes. We denote them as CC_0, CC_1, ..., CC_n, where CC_0 is the zero colinearity class.

We define a colinearity class as a ***reversible colinearity class*** if all the reactions in the colinearity class are reversible, otherwise we call it a ***nonreversible colinearity class***. An empty colinearity class is considered reversible by definition.

Remark 1.7.2. *Note that for a reaction network to have the capacity to admit multiple steady states, the zero colinearity class needs to be reversible. See [7] and [8] for more information.*

Remark 1.7.3. *If a reaction network contains two irreversible reactions, $y \to y'$ and $\tilde{y} \to \tilde{y}'$ in the same colinearity class such that $w_{y\to y'} = \alpha w_{\tilde{y}\to\tilde{y}'}$ for some $\alpha < 0$, then we claim that multiple steady states for the reaction network are not possible. The proof is in [7] and [8].*

Once the colinearity classes are found, we can divide the reaction network into subnetworks where each subnetwork contains exactly all the reactions in one colinearity class. We can then define a ***colinkage set*** as a linkage class in a subnetwork. We can also define ***strong colinkage sets*** and ***terminal strong colinkage sets*** in a similar way.

We define a ***colinearity class vector*** by choosing a representative from all w-vectors within a colinearity class in the following way:

For a nonreversible colinearity class CC_i, we can pick any positive multiple of $w_{y\to y'}$

for some irreversible reaction $y \to y' \in CC_i$. For a reversible colinearity class CC_i, we can pick any nonzero multiple of $w_{y \to y'}$ for some $y \to y' \in CC_i$.

We can see that the colinearity class vector for a zero colinearity class is the zero vector.

A ***coplanar set***, $\mathscr{T}$, is defined as a set of nonzero colinearity classes satisfying the following properties:

(i) The set $\mathscr{T}$ has three or more colinearity classes in it.

(ii) The colinearity class vectors for all colinearity classes in $\mathscr{T}$ lie in the same two-dimensional linear subspace, and all such colinearity classes whose colinearity vectors lie in the two-dimensional linear subspace are in $\mathscr{T}$.

Remark 1.7.4. *The coplanar sets do not partition the colinearity classes. Some colinearity class may not belong to any of the coplanar sets and some may belong to more than one coplanar set. See [7] and [8] for more information.*

After finding all the coplanar sets, we say that two nonzero colinearity classes CC_i and CC_j are ***directly linked***, if there exits a coplanar set $\mathscr{T}$ containing both colinearity classes, denoted by $CC_i \sim CC_j$. We then say that two nonzero colinearity classes CC_i and CC_j are ***connected*** if at least one of the following conditions is satisfied:

(i) They are the same colinearity class.

(ii) $CC_i \sim CC_j$.

(iii) There exist colinearity classes $CC_1, ...CC_m$ such that $CC_i \sim CC_1 \sim ... \sim CC_m \sim CC_j$.

The connected property does partition the nonzero colinearity classes. We call each partitioned set a ***connected class***, i.e., all the colinearity classes in a connected class are linked to each other and all linked colinearity classes are in the same connected class.

After finding all the connected classes, we can construct a ***connecting graph*** where all the vertices are coplanar sets $\mathscr{T}_i$'s and nonzero colinearity classes CC_j's, edges are

only possible between coplanar sets and nonzero colinearity classes, and there is an edge between $\mathscr{T}_i$ and CC_j if $CC_j \in \mathscr{T}_i$.

We can define the ***Independence Linearity Condition*** as follows:

A network satisfies the Independence Linearity Condition if the number of coplanar sets plus the number of connected classes is equal to d, which is the dimension of the $Ker\ L_{\mathscr{O}}$.

The ***Triplet Linearity Condition*** is defined as follows:

A network satisfies the Triplet Linearity Condition if each coplanar set contains exactly three colinearity classes.

1.7.2 The Algorithm

Roughly speaking, in the Advanced Deficiency Algorithm, first the Deficiency One Algorithm is carried out in each colinearity class. Then the coplanar sets are used to connect the colinearity classes and introduce additional inequalities and equalities. The inequality systems produced by applying the Deficiency One Algorithm in each colinearity class, and all the additional inequalities and equalities produced from the coplanar sets, are combined to form the complete inequality systems. The inequality systems generated in the Deficiency One Algorithm are always linear, but this is not always true in the Advance Deficiency Algorithm. The Independence Linearity Condition and Triplet Linearity Condition are used to assess the linearity of the Advanced Deficiency Algorithm; that is, when a linear system will result. For more information on the Advanced Deficiency Theory and Advanced Deficiency Algorithm, please see [7] and [8].

Next we will list the procedures of the Advanced Deficiency Algorithm. We will use the following reaction network to illustrate the steps. To get more information about Advanced

Deficiency Theory and Algorithm, please see [7] and [8].

$$\begin{aligned} A+S &\rightleftharpoons AS \\ B+S &\rightleftharpoons BS \\ A \rightleftharpoons\ 0 &\rightleftharpoons B \\ \uparrow & \\ C & \\ AS+BS &\to C+2S \\ A+BS &\to C+S \end{aligned} \tag{1.7.1}$$

Step 1: Choose an initial Orientation

Choose an initial orientation $\mathscr{O}$ for the reaction network.

For the reaction (1.7.1), we choose an initial orientation $\{A+S \to AS, B+S \to BS, A \to 0, B \to 0, C \to 0, AS+BS \to C+2S, A+BS \to C+S\}$.

Step 2: Find the Colinearity Classes

Find the colinearity classes by definition. We will check if there is a zero colinearity class CC_0. If there exists an irreversible reaction in CC_0, then we claim that the reaction network does not have the capacity to admit multiple steady states (as in Remark 1.7.2). We will exit the algorithm and skip the remaining steps.

For the reaction network, if a colinearity class contains two irreversible reactions, $y \to y'$ and $\tilde{y} \to \tilde{y}'$ such that $w_{y \to y'} = \alpha w_{\tilde{y} \to \tilde{y}'}$ for some $\alpha < 0$, then we claim that the reaction network does not have the capacity to admit multiple steady states (as in Remark 1.7.3). We will exit the algorithm and skip the remaining steps.

For the reaction network (1.7.1), a basis $\{v^1, v^2\}$ for $Ker\ L_{\mathscr{C}}$ is

$$\begin{array}{ll} & \begin{array}{cc} v^1 & v^2 \end{array} \\ \begin{array}{l} A+S \to AS \\ B+S \to BS \\ A \to 0 \\ B \to 0 \\ C \to 0 \\ AS+BS \to C+2S \\ A+BS \to C+S \end{array} & \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ -1 & -1 \\ -1 & -1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix} \end{array}$$

Therefore, from this basis, we find all the w-vectors for the reactions in it. Note that $w_{A+S\to AS} = w_{AS\to A+S} = w_{AS+BS\to C+2S} = [1,0]$, $w_{B+S\to BS} = w_{BS\to B+S} = w_{C\to 0} = [1,1]$, $w_{A\to 0} = w_{0\to A} = w_{B\to 0} = w_{0\to B} = [-1,-1]$, and $w_{A+BS\to C+S} = [0,1]$. We find the following colinearity classes:

$CC_1 = \{A+S \to AS, AS \to A+S, AS+BS \to C+2S\}$,

$CC_2 = \{B+S \to BS, BS \to B+S, A \to 0, 0 \to A, B \to 0, 0 \to B, C \to 0\}$ and

$CC_3 = \{A+BS \to C+S\}$.

There is no zero colinearity class. Each of the nonreversible colinearity classes CC_1, CC_2 and CC_3 only contains one irreversible reaction. We will move to next step.

Step 3: Find the Colinkage Sets

First, we find the subnetwork for each colinearity class. Then, we find the corresponding colinkage sets and strong colinkage sets in each subnetwork.

For the reaction network (1.7.1), the subnetworks for the corresponding colinearity classes are:

$$\begin{array}{rcl} \text{Subnetwork for } CC_1 \text{ is } \mathscr{N}_1 : A+S & \rightleftharpoons & AS \\ AS+BS & \to & C+2S \end{array}$$

Subnetwork for CC_2 is $\mathscr{N}_2 : B + S \rightleftharpoons BS$

$$A \rightleftharpoons 0 \rightleftharpoons B$$
$$\uparrow$$
$$C$$

Subnetwork for CC_3 is $\mathscr{N}_3 : A + BS \to C + S$

The colinkage sets of reaction network (1.7.1) are: $\{A + S, AS\}$ and $\{AS + BS, C + 2S\}$ in $\mathscr{N}_1$, $\{B + S, BS\}$ and $\{A, 0, B, C\}$ in $\mathscr{N}_2$, and $\{A + BS, C + S\}$ in $\mathscr{N}_3$.

The strong colinkage sets of reaction network (1.7.1) are: $\{A + S, AS\}$, $\{AS + BS\}$ and $\{C + 2S\}$ in $\mathscr{N}_1$, $\{B + S, BS\}$, $\{A, 0, B\}$ and $\{C\}$ in $\mathscr{N}_2$, and $\{A + BS\}$ and $\{C + S\}$ in $\mathscr{N}_3$.

Step 4: Choose Colinearity Class Vectors

We choose the colinearity class vector for each colinearity class according to the rules defined. For a nonreversible colinearity class CC_i, we can pick any positive multiple of $w_{y \to y'}$ for some irreversible reaction $y \to y' \in CC_i$. For a reversible colinearity class CC_i, we can pick any nonzero multiple of $w_{y \to y'}$ for some $y \to y' \in CC_i$.

For reaction network (1.7.1), the colinearity class vectors are: $w_1 = [1, 0]$ for CC_1, $w_2 = [1, 1]$ for CC_2, and $w_3 = [0, 1]$ for CC_3.

Step 5: Realign the Orientation

In this step the orientation $\mathscr{O}$ is realigned so that under the realigned orientation, the w-vectors for the reactions in each colinearity class are positive multiples of the colinearity class vector. For a reversible reaction, we will replace $y \to y'$ by $y' \to y$ in $\mathscr{O}$ if $w_{y \to y'}$ is not a positive multiple of the colinearity class vector. Note that we will be able to do this also for the irreversible reactions, as we have made sure that their w-vectors are some

positive multiples of the colinearity class vector, an assumption for moving to Step 3 from Step 2.

For reaction network (1.7.1), we replaced $A \to 0$ with $0 \to A$, $B \to 0$ with $0 \to B$ to make sure that under the realigned orientation, the w-vectors for the reactions in each colinearity class are all positive multiples of the colinearity class vector. The realigned orientation is $\mathscr{O} = \{A + S \to AS, B + S \to BS, 0 \to A, 0 \to B, C \to 0, AS + BS \to C + 2S, A + BS \to C + S\}$.

Step 6: Find Coplanar Sets and Connected Classes

In this step, find all the coplanar sets and connected classes by definition.

For reaction network (1.7.1), the coplanar sets are $\mathscr{T}_1 = \{CC_1, CC_2, CC_3\}$, as $w_1 + w_3 = w_2$. The connecting graph is as follows:

$$CC_1 - \quad \mathscr{T}_1 \quad - CC_2$$

$$\Big|$$

$$CC_3$$

Step 7: Determine Linearity

Note that in Advanced Deficiency Theory and Algorithm, we answer the question of multiple steady states by solving systems of inequalities and equalities. We will determine whether these inequality systems are completely linear in terms of $\mu \in \mathbb{R}^{\mathscr{R}}$, a vector which we have introduced in Deficiency One Algorithm, and some other parameters, which we will introduce in later steps. We will check the linearity of the systems by checking two conditions: the Independence Linearity Condition and the Triplet Linearity Condition.

If both the Independence Linearity Condition and the Triplet Linearity Condition are satisfied, then the systems of inequalities and equalities generated to answer the questions of multiple steady states are completely linear.

Remark 1.7.5. *In Advanced Deficiency Theory and Algorithm, there are the so-called pseudo-colinearity classes. Roughly speaking, pseudo-colinearity classes are colinearity classes which have their colinearity class vectors but have no reactions in them. They could be helpful in the Advanced Deficiency Theory and Algorithm. If the Independence Linearity Condition is not satisfied, then pseudo-colinearity classes can sometimes be added to the reaction network to increase the number of coplanar sets and reduce the number of connected classes, so as to make the adjusted connecting graph satisfy the Independence Linearity Condition. However, the pseudo-colinearity class approach does not always work to make the Independence Linearity Condition satisfied. In the Advanced Deficiency Theory and Algorithm, there is no systematic procedure to check if one can know ahead whether adding pseudo-colinearity classes will help make the Independence Linearity Condition satisfied and how to find these pseudo-colinearity classes. In the Higher Deficiency Theory and Algorithm, we do not need the help of pseudo-colinearity classes to determine linearity. As a result, we will not mention too much details about the pseudo-colinearity class. We say here that if the Independence Linearity Condition fails and adding pseudo-colinearity classes seems not helpful, then nonlinear equalities may need to be added to the inequality system.*

If the Triplet Linearity Condition is not satisfied, then the complete inequality system (for answering the question of multiple steady states) will most likely not be linear as we need to add some nonlinear equalities into the system.

However, sometimes it is still possible to get a definitive answer, for the question of multiple steady states, by looking at the partial (linear) inequality systems. To get more information, see [7] and [8].

For reaction network (1.7.1), there is one coplanar set and one connected classes, which adds up to two, which is equal to the dimension of $Ker\ L_{\mathcal{O}}$. Therefore the Independence Linearity Condition is satisfied. The Triplet Linearity Condition is also satisfied as the only

coplanar set contains exactly three colinearity classes. We then know that the complete inequality systems we will build to answer the question of multiple steady states are linear.

In the next step, we will start to produce the inequality systems.

Step 8: Choose Signs for Colinearity Classes

Each colinearity class is assigned a sign (positive, negative or zero) following the rules below:

(i) The zero colinearity class is assigned a zero sign.

(ii) A nonreversible colinearity class is assigned a positive sign.

(iii) A nonzero reversible colinearity class can be assigned a positive, negative or zero sign as long as the following conditions are satisfied:

(a) If more than one colinearity class in a coplanar set is assigned a zero sign, then every colinearity class in the coplanar set is assigned a zero sign.

(b) Let CC_i, CC_j and CC_k be three colinearity classes from the same coplanar set. If each of these three colinearity classes has a nonzero sign, then there do not exist c_i, c_j and c_k agreeing in sign with their respective colinearity classes such that $c_i w_i + c_j w_j + c_k w_k = 0$. If only one of the three colinearity classes, say CC_i has a zero sign, then there do not exist c_j and c_k agreeing in sign with their respective colinearity classes such that $c_j w_j + c_k w_k$ is a multiple of w_i.

If no choice of signs for colinearity classes can satisfy the conditions listed above, then we claim that the reaction network cannot support multiple steady states; in fact, there are no positive steady states. In this case, we will exit the algorithm and the remaining steps are skipped.

For reaction network (1.7.1), since all the colinearity classes are nonreversible, all three colinearity classes are assigned positive signs.

Step 9: Choose Shelving for Reactions

In this step all reactions are partitioned into sets called ***shelves***. There will be upper, middle and lower shelves for each colinearity class with a nonzero sign.

Given a colinearity class with a nonzero sign, the reactions in this colinearity class are partitioned according to the following conditions:

(i) A reaction whose reactant complex lies in a non-terminal strong colinkage set (relative to the subnetwork of the colinearity class) is placed on the middle shelf of the colinearity class.

(ii) An irreversible reaction is placed on the middle shelf of the colinearity class.

(ii) A reversible reaction network whose reactant complex lies in a terminal strong colinkage set (relative to the subnetwork of the colinearity class) can be placed on upper, middle, or lower shelf of the colinearity class as long as reactions in the same colinkage set (relative to the subnetwork of the colinearity class) are placed in the same shelf of the colinearity class.

In reaction network (1.7.1), we have colinearity classes CC_1, CC_2 and CC_3 all with nonzero signs. For $CC_1 = \{A + S \to AS, AS \to A + S, AS + BS \to C + 2S\}$, $A + S \to AS$ and $AS \to A + S$ can be put on the same upper, middle or lower shelf of CC_1, and $AS+BS \to C+2S$ is put on the middle shelf. For $CC_2 = \{B+S \to BS, BS \to B + S, A \to 0, 0 \to A, B \to 0, 0 \to B, C \to 0\}$, $B + S \to BS$ and $BS \to B + S$ can be put on the same upper, middle, or lower shelf of CC_2, $A \to 0$, $0 \to A$, $B \to 0$ and $0 \to B$ can be put on the same upper, middle, or lower shelf of CC_2, and $C \to 0$ is put on the middle shelf of CC_2. For $CC_3 = \{A + BS \to C + S\}$, $A + BS \to C + S$ is put on the middle shelf of CC_3.

We will pick one shelving choice here, say

$$\mathscr{U}_1 = \{A + S \to AS, AS \to A + S\}, \mathscr{M}_1 = \{AS + BS \to C + 2S\}, \mathscr{L}_1 = \{\}.$$

$$\mathscr{U}_2 = \{B + S \to BS, BS \to B + S, A \to 0, 0 \to A, B \to 0, 0 \to B\},$$

$$\mathscr{M}_2 = \{C \to 0\}, \mathscr{L}_2 = \{\}.$$

$$\mathscr{U}_3 = \{\}, \mathscr{M}_3 = \{A + BS \to C + S\}, \mathscr{L}_3 = \{\}.$$

In Step 10 through Step 13, the inequality systems are produced. We introduce M_i's as a value associated with the middle shelf of the colinearity class CC_i with a nonzero sign.

Step 10: Add Shelving Inequalities

If the reaction $y \to y'$ is placed on the upper shelf of CC_i, then $y \cdot \mu > M_i$ is added to the inequality system. If the reaction $y \to y'$ is placed on the middle shelf of CC_i, then $y \cdot \mu = M_i$ is added to the inequality system. If the reaction $y \to y'$ is placed on the lower shelf of CC_i, then $y \cdot \mu < M_i$ is added to the inequality system.

For reaction network (1.7.1), given the shelving condition chosen in Step 9, the inequalities and equalities added to the inequality system are:

$$\begin{aligned}
\mu_A + \mu_S &> M_1 \\
\mu_{AS} &> M_1 \\
\mu_{AS} + \mu_{BS} &= M_1 \\
\mu_B + \mu_S &> M_2 \\
\mu_{BS} &> M_2 \\
\mu_A &> M_2 \\
0 &> M_2 \\
\mu_B &> M_2 \\
\mu_C &= M_2 \\
\mu_A + \mu_{BS} &= M_3 \\
\mu_C + \mu_S &= M_3
\end{aligned}$$

Step 11: Add Upper and Lower Shelf Inequalities

For each colinearity class with a nonzero sign, inequalities are added to the inequality system for the reactions in the orientation that are on the upper and lower shelves of their colinearity classes.

Suppose that the colinearity class CC_i has a positive sign. If the reaction $y \to y' \in \mathscr{O}$ is on the upper shelf of CC_i, then $y' \cdot \mu > y \cdot \mu$ is added to the inequality system. If it is on the lower shelf of CC_i, then $y \cdot \mu > y' \cdot \mu$ is added to the inequality system.

Suppose that the colinearity class CC_i has a negative sign. If the reaction $y \to y' \in \mathscr{O}$ is on the upper shelf of CC_i, then $y' \cdot \mu < y \cdot \mu$ is added to the inequality system. If it is on the lower shelf of CC_i, then $y \cdot \mu < y' \cdot \mu$ is added to the inequality system.

For reaction network (1.7.1), note that all three colinearity classes have positive signs.

From the shelving conditions chosen in Step 9, we add $\mu_{AS} > \mu_A + \mu_S$, $\mu_{BS} > \mu_B + \mu_S$, $\mu_A > 0$ and $\mu_B > 0$ to the inequality system.

Step 12: Add Equalities for Colinearity Classes with Zero Signs

We add equalities to the inequality system for all colinearity classes with zero signs. Suppose that the colinearity class CC_i has a zero sign. For each $y \to y' \in CC_i$, $y \cdot \mu = y' \cdot \mu$ is added to the inequality system.

For reaction network (1.7.1), all the colinearity classes have nonzero signs, so no equality is added for this step.

Step 13: Add M Inequalities and Equalities

We will add inequalities and/or equalities among M_i's for the colinearity classes CC_i's.

Let CC_i, CC_j and CC_k be three colinearity classes in the same coplanar set.

If all colinearity classes in the coplanar set have zero signs, then no inequalities or equalities are added.

If only one of the colinearity class, say CC_i, has a zero sign, then $M_j = M_k$ is added to the inequality system. In other words, if among all colinearity classes in the same coplanar set, only one colinearity class has a zero sign, then all the M_l's corresponding to the rest of the colinearity classes in this coplanar set are equal.

If all three colinearity classes have nonzero signs, then we will add inequalities/equalities according to the following conditions: given c_i, c_j and c_k agreeing in sign with their respective colinearity classes such that $c_k w_k = c_i w_i + c_j w_j$, either $M_i > M_k > M_j$, $M_i = M_k = M_j$, or $M_i < M_k < M_j$ is added to the inequality system. Note that from the conditions one has to follow to choose signs for colinearity classes in Step 8, we can see that such a choice of c_i, c_j and c_k exists. Note that all colinearity class vectors in the same coplanar set lie in the same two-dimensional linear subspace, so the choice of c_i, c_j and c_k is unique up to a positive multiple.

Remark 1.7.6. *Suppose a coplanar set contains more than three colinearity classes all with nonzero signs. Although it seems that the inequalities or equalities found by considering a triplet at a time, will have many possibilities. However, in the end, there will only be three possibilities: there exists an enumeration of all CC_i's in this coplanar set, say CC_{n_1}, CC_{n_2}, ..., CC_{n_k} such that either $M_{n_1} > M_{n_2} > ... > M_{n_k}$, $M_{n_1} = M_{n_2} = ... = M_{n_k}$, or $M_{n_1} < M_{n_2} < ... < M_{n_k}$ is added to the inequality system. For more information and proof, see [7] and [8].*

For reaction network (1.7.1), there is only one coplanar set with exactly three colinearity classes all with positive signs. Note that $w_1 = [1, 0]$, $w_2 = [1, 1]$ and $w_3 = [0, 1]$, so we can pick $c_1 = c_2 = c_3 = 1 > 0$ such that $c_2 w_2 = c_1 w_1 + c_3 w_3$. Therefore we have three possible choices to add into the inequality system: $M_1 > M_2 > M_3$, $M_1 = M_2 = M_3$, or $M_1 < M_2 < M_3$. For this step, let us choose $M_1 > M_2 > M_3$.

Step 14: Check for Solutions to the Inequality System

In Step 7 we have determined whether the inequality systems are completely linear or not by looking at the Independence Linearity Condition and the Triplet Linearity Condition. We will in this step check whether the inequality system has a solution with a nonzero μ which is sign-compatible with the stoichiometric subspace S. In other words, we will look to see if there exists a set of M_i's and a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S, such that the inequality system is satisfied.

Note that if the inequality systems are determined to be linear, then the inequality system built from Step 10 through Step 13 is a complete system, which means that no other inequalities/equalities need to be included in the system to answer the question of multiple steady states; if some inequality system has such a solution, it will be called a ***signature***. If we find a signature, then we claim that taken with mass action kinetics, the reaction network does have the capacity for multiple steady states and exit the algorithm. Otherwise,

if after we find no solution in any of the inequality systems, we claim that taken with mass action kinetics, the reaction network does not have the capacity for multiple steady states.

If the inequality systems are nonlinear, then we will later need to add some nonlinear equality to the inequality system we have so far, to make it a complete system. In the nonlinear case, if some (linear) inequality system we built from Step 10 through Step 13 has such a solution, it will be called a ***pre-signature***. There is an approach for working on the solution from a pre-signature of a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S, to help decide if the reaction network has the capacity to admit multiple steady states. However, the method is not completely decisive. In other words, it can only possibly lead to the conclusion that the reaction network has the capacity to admit multiple steady states (in this case we can exit the algorithm) but not otherwise (in this case the answer to the question of multiple steady states is inconclusive at the moment). We will not introduce the details here. However, if none of the (linear) inequality systems has such a solution, i.e., there are no pre-signatures, then there is no need to consider the additional nonlinear equality, and we claim that the reaction network does not have the capacity to admit multiple steady states.

For reaction network (1.7.1), we have determined that the complete systems are linear. The complete inequality system based on our choice of signs for colinearity classes,

shelving conditions and M comparisons are:

$$\begin{aligned}
\mu_A + \mu_S &> M_1 \\
\mu_{AS} &> M_1 \\
\mu_{AS} + \mu_{BS} &= M_1 \\
\mu_B + \mu_S &> M_2 \\
\mu_{BS} &> M_2 \\
\mu_A &> M_2 \\
0 &> M_2 \\
\mu_B &> M_2 \\
\mu_C &= M_2 \\
\mu_A + \mu_{BS} &= M_3 \\
\mu_C + \mu_S &= M_3 \\
\mu_{AS} &> \mu_A + \mu_S \\
\mu_{BS} &> \mu_B + \mu_S \\
\mu_A &> 0 \\
\mu_B &> 0 \\
M_1 > M_2 &> M_3
\end{aligned}$$

For this inequality system, there do not exist a set of M_i's and a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S. Therefore this inequality system is not a signature. We will move to next step.

Step 15: Repeat Steps 13 to 14

In this step, Steps 13 and 14 are repeated for every choice of M inequalities and/or equalities.

Supposet that the systems are linear. Once we find a signature in Step 14, we claim that taken with mass action kinetics, the reaction network does have the capacity for multiple steady states and exit the algorithm. Otherwise, after we repeat all the choices of M inequalities and/or equalities and find no signatures, we move to the next step.

Suppose that the systems are nonlinear. Unless we find a pre-signature in Step 14 that will lead to a conclusion that the reaction network does have the capacity for multiple steady states and exit the algorithm, we will repeat all choices of M inequalities and/or equalities and then move to the next step.

For reaction network (1.7.1), there are two more choices of M inequalities and/or equalities, and their corresponding inequality systems are listed in two columns as follows:

$$\begin{aligned}
\mu_A + \mu_S &> M_1 \\
\mu_{AS} &> M_1 \\
\mu_{AS} + \mu_{BS} &= M_1 \\
\mu_B + \mu_S &> M_2 \\
\mu_{BS} &> M_2 \\
\mu_A &> M_2 \\
0 &> M_2 \\
\mu_B &> M_2 \\
\mu_C &= M_2 \\
\mu_A + \mu_{BS} &= M_3 \\
\mu_C + \mu_S &= M_3 \\
\mu_{AS} &> \mu_A + \mu_S \\
\mu_{BS} &> \mu_B + \mu_S \\
\mu_A &> 0 \\
\mu_B &> 0 \\
M_1 &= M_2 = M_3
\end{aligned}
\qquad
\begin{aligned}
\mu_A + \mu_S &> M_1 \\
\mu_{AS} &> M_1 \\
\mu_{AS} + \mu_{BS} &= M_1 \\
\mu_B + \mu_S &> M_2 \\
\mu_{BS} &> M_2 \\
\mu_A &> M_2 \\
0 &> M_2 \\
\mu_B &> M_2 \\
\mu_C &= M_2 \\
\mu_A + \mu_{BS} &= M_3 \\
\mu_C + \mu_S &= M_3 \\
\mu_{AS} &> \mu_A + \mu_S \\
\mu_{BS} &> \mu_B + \mu_S \\
\mu_A &> 0 \\
\mu_B &> 0 \\
M_1 &< M_2 < M_3
\end{aligned}$$

Neither of the two inequality systems listed above has a solution of a set of M_i's and a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S. They are not signatures. We will move to next step.

Step 16: Repeat Steps 9 to 15

In this step all possible shelving condition choices in Step 9 are repeated. Similar to

that of the Deficiency One Algorithm, we can save some time by trying only half of the shelving choices and skipping all the choices which are total inversions of the first half. By an inversion of a shelving assignment we mean a new shelving assignment created by switching all of the upper and lower shelves of the original one. This is true because an inversed system has such a solution if and only if the original system has one.

Suppose that the systems are linear. Once we find a signature in Step 14, we claim that taken with mass action kinetics, the reaction network does have the capacity for multiple steady states and exit the algorithm. Otherwise, after we repeat all the choices of M inequalities and/or equalities and all possible shelving condition choices but find no signatures, we move to the next step.

Suppose that the systems are nonlinear. Unless we find a pre-signature in Step 14 that will lead to a conclusion that the reaction network does have the capacity for multiple steady states and exit the algorithm, we will repeat all choices of M inequalities and/or equalities and all possible shelving condition choices, and then move to the next step.

For reaction network (1.7.1), the flexibility of shelves assignment comes from the reactions $\{A + S \rightleftharpoons AS\}$, $\{B + S \rightleftharpoons BS\}$ and $\{A \rightleftharpoons 0 \rightleftharpoons B\}$. Therefore, we have $3 \times 3 \times 3 = 27$ choices for the shelving assignment. Excluding the inversions, we have 14 choices. We are done with one choice of shelving assignment so far. We will test the remaining 13 choices of shelving assignments.

First, we choose the shelving such that:

$$\mathscr{U}_1 = \{\}, \mathscr{M}_1 = \{AS + BS \to C + 2S\}, \mathscr{L}_1 = \{A + S \to AS, AS \to A + S\}.$$
$$\mathscr{U}_2 = \{B + S \to BS, BS \to B + S, A \to 0, 0 \to A, B \to 0, 0 \to B\},$$
$$\mathscr{M}_2 = \{C \to 0\}, \mathscr{L}_2 = \{\}.$$
$$\mathscr{U}_3 = \{\}, \mathscr{M}_3 = \{A + BS \to C + S\}, \mathscr{L}_3 = \{\}.$$

The three systems produced from repeating Step 10 through Step 15, corresponding to the

shelving conditions above and three different M comparisons, are listed in three columns as follows:

$$\begin{array}{rcc}
\mu_A + \mu_S < M_1 & \mu_A + \mu_S < M_1 & \mu_A + \mu_S < M_1 \\
\mu_{AS} < M_1 & \mu_{AS} < M_1 & \mu_{AS} < M_1 \\
\mu_{AS} + \mu_{BS} = M_1 & \mu_{AS} + \mu_{BS} = M_1 & \mu_{AS} + \mu_{BS} = M_1 \\
\mu_B + \mu_S > M_2 & \mu_B + \mu_S > M_2 & \mu_B + \mu_S > M_2 \\
\mu_{BS} > M_2 & \mu_{BS} > M_2 & \mu_{BS} > M_2 \\
\mu_A > M_2 & \mu_A > M_2 & \mu_A > M_2 \\
0 > M_2 & 0 > M_2 & 0 > M_2 \\
\mu_B > M_2 & \mu_B > M_2 & \mu_B > M_2 \\
\mu_C = M_2 & \mu_C = M_2 & \mu_C = M_2 \\
\mu_A + \mu_{BS} = M_3 & \mu_A + \mu_{BS} = M_3 & \mu_A + \mu_{BS} = M_3 \\
\mu_C + \mu_S = M_3 & \mu_C + \mu_S = M_3 & \mu_C + \mu_S = M_3 \\
\mu_{AS} > \mu_A + \mu_S & \mu_{AS} > \mu_A + \mu_S & \mu_{AS} > \mu_A + \mu_S \\
\mu_{BS} > \mu_B + \mu_S & \mu_{BS} > \mu_B + \mu_S & \mu_{BS} > \mu_B + \mu_S \\
\mu_A > 0 & \mu_A > 0 & \mu_A > 0 \\
\mu_B > 0 & \mu_B > 0 & \mu_B > 0 \\
M_1 > M_2 > M_3 & M_1 = M_2 = M_3 & M_1 < M_2 < M_3
\end{array}$$

The first two (from left to right) of the three inequality systems are not signatures and the third one is a signature. The third inequality system has a solution of a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S: $\mu_A = 1$, $\mu_S = -4$, $\mu_{AS} = -4$, $\mu_B = 4$, $\mu_{BS} = 2$ and $\mu_C = -1$. We will not provide the detailed solution of M_i's here. Since we have found a signature, we claim that taken with mass action kinetics, the reaction network does have the capacity for multiple steady states and exit the algorithm.

Step 17: Repeat Steps 8 to 16

In this step we will repeat all sign choices for the colinearity classes. Note that changing the signs for the colinearity classes may affect the shelving assignments as only colinearity classes with nonzero signs are considered, and the inequality systems built from Step 10 through Step 13 will be affected too. We will check all inequality systems produced by these changes.

Suppose that the systems are linear. Once we find a signature in Step 14, we claim that taken with mass action kinetics, the reaction network does have the capacity for multiple steady states and exit the algorithm. Otherwise, we repeat all the choices of M inequalities and/or equalities, all possible shelving condition choices and all sign choices for the colinearity classes.

Suppose that the systems are nonlinear. Unless we find a pre-signature in Step 14 that will lead to a conclusion that the reaction network does have the capacity for multiple steady states and exit the algorithm, we will repeat all choices of M inequalities and/or equalities, all possible shelving condition choices and all sign choices for the colinearity classes.

If after this step, no signature or pre-signature (for the nonlinear case) has been found, then we can claim that the reaction network cannot support multiple steady states, no matter what positive values the rate constants are. In the nonlinear case, if there are pre-signatures but one cannot conclude that the reaction network can support multiple steady states from any of the pre-signatures, then the answer to the question of multiple steady states is inconclusive. If the answer is inconclusive, then we need to add additional nonlinear equalities and consider the complete (nonlinear) inequality system in order to answer the question of multiple steady states. As for ways of finding the additional nonlinear equalities, we will not present the details here. See [7] for more information.

For reaction network (1.7.1), we already exited the algorithm in Step 16. However, if

we did carry out Step 17, we would find that there is no other choice for the signs of the colinearity classes.

1.8 Higher Deficiency Theory and Algorithm Overview

The Higher Deficiency Theory gives answers very similar to the Advanced Deficiency Theory, both giving a method to study whether the reaction network can support multiple steady states. As a matter of fact, the Higher Deficiency Theory is a reformation and significant extension of the Advanced Deficiency Theory.

For example, let us consider the following reaction network.

$$
\begin{aligned}
&E_1 + S_1 \rightleftharpoons E_1S_1 \to E_1 + S_2 \rightleftharpoons E_1S_2 \to E_1 + S_3 \rightleftharpoons E_1S_3 \to E_1 + S_4 \qquad (1.8.1)\\
&E_2 + S_4 \rightleftharpoons E_2S_4 \to E_2 + S_3 \rightleftharpoons E_2S_3 \to E_2 + S_2 \rightleftharpoons E_2S_2\\
&\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \searrow E_2 + S_1\\
&E_3 + S_1 \rightleftharpoons E_3S_1 \to E_3 + S_2 \rightleftharpoons E_3S_2 \to E_3 + S_3\\
&E_4 + S_4 \rightleftharpoons E_4S_4 \to E_4 + S_3 \rightleftharpoons E_4S_3 \to E_4 + S_2
\end{aligned}
$$

For reaction network (1.8.1), the Advanced Deficiency Theory and Algorithm (see [8]) cannot give a definitive answer, but the Higher Deficiency Theory and Algorithm (see [16]) concludes that taken with mass action kinetics, the reaction network does indeed have the capacity to admit multiple positive steady states. The mass action differential equations for reaction network (1.8.1) are highly complex; they are similar to the system (1.1.8).

We turn next to the Higher Deficiency Theory.

Chapter 2

THE HIGHER DEFICIENCY THEORY

2.1 Main Question

Given a reaction network governed by mass action kinetics, does there exist a set of positive rate constants such that the governing differential equations admit a pair of distinct positive steady states that are stoichiometrically compatible? We will attempt to answer this question algorithmically in this chapter. The algorithm itself has been implemented in a user-friendly Windows-based program available at [16].

Mathematically, the question can be stated as:

Question 1. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and two positive, distinct and stoichiometrically compatible compositions c^* and c^{**}, such that*

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) = 0 \tag{2.1.1}$$

and

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y) = 0 \tag{2.1.2}$$

are satisfied?

We will in this chapter rephrase Question 1 into a sequence of equivalent questions.

2.2 Rephrase the variables

In this section, we will construct new terms $\kappa \in \mathbb{R}^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$ from $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and c^*, $c^{**} \in \bar{\mathbb{R}}_+^{\mathscr{S}}$ so we can convert equations (2.1.1) and (2.1.2) into equations involving κ and μ instead of $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, c^* and c^{**}. With the new variables, Question 1 can be rephrased.

Next we will define $\kappa \in \mathbb{R}^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$, and explore their relationship to $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and c^*, $c^{**} \in \bar{\mathbb{R}}_+^{\mathscr{S}}$.

On one hand, given a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and two positive, distinct, and stoichiometrically compatible compositions c^* and c^{**}, we define $\mu \in \mathbb{R}^{\mathscr{S}}$ and $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ via

$$\mu_s = \ln(\frac{c_s^*}{c_s^{**}}),\ \forall s \in \mathscr{S}, \tag{2.2.1}$$

and

$$\kappa_{y\to y'} = k_{y\to y'} c^{**y},\ \forall y \to y' \in \mathscr{R}. \tag{2.2.2}$$

We claim that μ is a nonzero vector in $\mathbb{R}^{\mathscr{S}}$ that is sign-compatible with the stoichiometric subspace S.

In fact, since $c^* \neq c^{**}$, there exists some $s \in \mathscr{S}$ such that $\mu_s \neq 0$. Therefore, $\mu \neq 0$. From the monotonicity of the $\ln$ function, we know that, for any $s \in \mathscr{S}$, $c_s^* - c_s^{**}$ will have the same sign as $\ln(c_s^*) - \ln(c_s^{**})$. Since $\mu_s = \ln(\frac{c_s^*}{c_s^{**}}) = \ln(c_s^*) - \ln(c_s^{**})$, $c^* - c^{**}$ will be sign-compatible with μ. Note that c^* and c^{**} are presumed to be stoichiometrically compatible, which requires that $c^* - c^{**} \in S$. Therefore, $c^* - c^{**}$ being sign-compatible with μ implies that there exists a vector $c^* - c^{**}$ in S that is sign-compatible with μ, $i.e.$, μ is sign-compatible with S.

On the other hand, assume κ is a member of $\mathbb{R}_+^{\mathscr{R}}$ and that $\mu \in \mathbb{R}^{\mathscr{S}}$ is a nonzero vector

which is sign-compatible with S. Then there exists a nonzero vector, say $\sigma \in S$, that is sign-compatible with μ. We can define c^* and c^{**} in $\bar{\mathbb{R}}^{\mathscr{S}}$ via:

If μ_s and σ_s are not equal to zero, then

$$c_s^* = \frac{\sigma_s e^{\mu_s}}{e^{\mu_s} - 1}, s \in \mathscr{S} \tag{2.2.3}$$

$$c_s^{**} = \frac{\sigma_s}{e^{\mu_s} - 1}, s \in \mathscr{S} \tag{2.2.4}$$

If μ_s and σ_s are equal to 0, then

$$c_s^* = c_s^{**} = p, \text{ for some } p > 0 \tag{2.2.5}$$

Then we can define $k_{y \to y'}$ via:

$$k_{y \to y'} = \kappa_{y \to y'} / ((c^{**})^y) \tag{2.2.6}$$

We claim that $\{k_{y \to y'} : y \to y' \in \mathscr{R}\}$ is a set of positive numbers, and, c^* and c^{**} are two positive, distinct compositions that are stoichiometrically compatible, i.e. $c^* - c^{**} \in S$.

To see this, note that μ being nonzero implies that there exists some $s \in \mathscr{S}$ such that $\mu_s \neq 0$, so $\frac{c_s^*}{c_s^{**}} = e^{\mu_s} \neq 1$. Therefore, $c^* \neq c^{**}$. Note that in the case of $\mu_s \neq 0$, $e^{\mu_s} - 1$ has the same (nonzero) sign as μ_s and σ_s, so c_s^* and c_s^{**} are both positive. Therefore it is true that c_s^* and c_s^{**} are both positive, whether μ_s is equal to 0 or not. Also note that in both cases, $\ln(\frac{c_s^*}{c_s^{**}})$ is equal to μ_s, so equations (2.2.3), (2.2.4) and (2.2.5), are consistent with equation (2.2.1). Finally, note that $c^* - c^{**}$ is equal to σ in both cases, so c^* and c^{**} are stoichiometrically compatible.

Therefore we have the following lemma.

Lemma 2.2.1. *Suppose that we are given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$. Suppose that a set of positive rate constants $\{k_{y \to y'} : y \to y' \in \mathscr{R}\}$ and two distinct, positive, stoichiometrically compatible compositions c^* and $c^{**} \in \bar{\mathbb{R}}_+^{\mathscr{S}}$ are given. Then $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ can be constructed from equation (2.2.2), and $\mu \in \mathbb{R}^{\mathscr{S}}$, constructed from equation (2.2.1), will be nonzero and sign-compatible with the stoichiometric subspace S. Conversely, suppose*

that a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with the stoichiometric subspace S, and $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ are given. Then a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$ and two distinct, positive, stoichiometrically compatible compositions c^* and $c^{**} \in \bar{\mathbb{R}}^{\mathscr{S}}_+$ can be constructed from equations (2.2.3), (2.2.4), (2.2.5) and (2.2.6).

From equations (2.2.1) and (2.2.2), it can be shown that $k_{y\to y'}c^{**y} = \kappa_{y\to y'}$ and $k_{y\to y'}c^{*y} = \kappa_{y\to y'}e^{y\cdot\mu}$. Then equations (2.1.1) and (2.1.2) can be transformed into the following equations:

$$\sum_{y\to y'\in\mathscr{R}} \kappa_{y\to y'}e^{y\cdot\mu}(y'-y) = 0 \tag{2.2.7}$$

$$\sum_{y\to y'\in\mathscr{R}} \kappa_{y\to y'}(y'-y) = 0 \tag{2.2.8}$$

As a result, we can claim the following lemma.

Lemma 2.2.2. *Question 1 can be rewritten in terms of κ and μ as follows:*

Question 2. *Given a reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$, do there exist $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ and a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with the stoichiometric subspace S such that equations (2.2.7) and (2.2.8) are satisfied?*

2.3 Rephrasing the summations

Recall that the summations in Question 1 and 2 are taken over all $\{y \to y' \in \mathscr{R}\}$. Here, some new concepts are introduced to rephrase Question 2 so that the summations are taken over a subset of $\mathscr{R}$.

Define an ***orientation*** $\mathscr{O}$ as a subset of the set of all reactions in the reaction network $\mathscr{R}$ such that for every reaction $y \to y' \in \mathscr{R}$, either $y \to y'$ or $y' \to y$ belongs to $\mathscr{O}$, but not both.

For a given orientation $\mathscr{O}$, define the linear map $L_{\mathscr{O}} : \mathbb{R}^{\mathscr{O}} \to S$ by:

$$L_{\mathscr{O}}\alpha = \sum_{y\to y'\in\mathscr{O}} \alpha_{y\to y'}(y'-y). \tag{2.3.1}$$

For given $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ and nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$, we define $g, h \in \mathbb{R}^{\mathscr{O}}$ as follows:

$$g_{y\to y'} = \begin{cases} \kappa_{y\to y'} - \kappa_{y'\to y}, & \text{if } y \to y' \in \mathscr{O} \text{ is reversible} \\ \kappa_{y\to y'}, & \text{if } y \to y' \in \mathscr{O} \text{ is irreversible.} \end{cases} \tag{2.3.2}$$

and

$$h_{y\to y'} = \begin{cases} \kappa_{y\to y'} e^{y\cdot\mu} - \kappa_{y'\to y} e^{y'\cdot\mu}, & \text{if } y \to y' \in \mathscr{O} \text{ is reversible} \\ \kappa_{y\to y'} e^{y\cdot\mu}, & \text{if } y \to y' \in \mathscr{O} \text{ is irreversible.} \end{cases} \tag{2.3.3}$$

Then equations (2.2.8) and (2.2.7) can be rewritten as

$$L_{\mathscr{O}} g = \sum_{y\to y'\in\mathscr{O}} g_{y\to y'}(y' - y) = 0 \tag{2.3.4}$$

$$L_{\mathscr{O}} h = \sum_{y\to y'\in\mathscr{O}} h_{y\to y'}(y' - y) = 0 \tag{2.3.5}$$

Therefore, if equations (2.2.7) and (2.2.8) are satisfied for some $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ and nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$, then g, h as defined in (2.3.2) and (2.3.3) both lie in $Ker\ L_{\mathscr{O}}$, for any given orientation $\mathscr{O}$ of $\mathscr{R}$.

Remark 2.3.1. *Let us denote the number of reactions in $\mathscr{O}$ by $\#(\mathscr{O})$. For $L_{\mathscr{O}} : \mathbb{R}^{\mathscr{O}} \to S$, we have dim $Ker\ L_{\mathscr{O}}$ = dim $(\mathbb{R}^{\mathscr{O}})$− dim $(Im\ L_{\mathscr{O}})$ = $\#(\mathscr{O})$− dim S, as it is easy to see that $Im\ L_{\mathscr{O}} = S$. Since $\#(\mathscr{O})$ is the same for any orientation $\mathscr{O}$ of $\mathscr{R}$, so is dim $Ker\ L_{\mathscr{O}}$.*

Note that for any orientation $\mathscr{O}$ of $\mathscr{R}$, if dim $Ker\ L_{\mathscr{O}} = 0$, then $g = 0$ and $h = 0$ are the only solutions in (2.3.2) and (2.3.3). In that case, each reaction must be reversible and for each $y \to y' \in \mathscr{O}$, we have $\kappa_{y\to y'} = \kappa_{y'\to y}$ and $\kappa_{y\to y'} e^{y\cdot\mu} - \kappa_{y'\to y} e^{y'\cdot\mu} = 0$. Therefore, for all $y \to y' \in \mathscr{R}$, $e^{y\cdot\mu} = e^{y'\cdot\mu}$, or $(y - y')\cdot\mu = 0$. This implies that $\mu \in S^{\perp}$, for which it follows that μ cannot be sign-compatible with S if $\mu \neq 0$. Therefore the answer to Question 2, and hence Question 1 is no and we are done with our question here.

Therefore, in order to find two distinct positive stoichiometrically compatible steady states c^* and c^{**}, we need to assume that dim $Ker\ L_{\mathscr{O}} \geq 1$. Moreover, we cannot pick g, h to be both zero vectors. Therefore, without loss of generality, let us assume dim $Ker\ L_{\mathscr{O}}$ ≥ 1 (for any orientation $\mathscr{O}$) and $g, h \in Ker\ L_{\mathscr{O}}$ are not both zero vectors from now on.

Let κ and μ satisfy the conditions in Question 2, we want to see if we can derive a question equivalent to Question 2 in terms of μ and $g,\ h \in Ker\ L_{\mathscr{O}}$, where $\mathscr{O}$ is an orientation of $\mathscr{R}$.

First, to simplify our notation, let us introduce a new term. Consider a given orientation $\mathscr{O}$ and a given pair $g, h \in Ker\ L_{\mathscr{O}}$. For $y \to y' \in \mathscr{O}$ such that $g_{y\to y'} \neq 0$, we will define $\rho_{y\to y'}$ as follows:

$$\rho_{y\to y'} = \frac{h_{y\to y'}}{g_{y\to y'}}. \tag{2.3.6}$$

Lemma 2.3.2. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$ are given. Let g, h be defined as in (2.3.2) and (2.3.3), and let $\rho_{y\to y'}$ $(g_{y\to y'} \neq 0)$ be defined as in (2.3.6). Then the following conditions hold:*

(I) If $y \to y' \in \mathscr{O}$ is irreversible, then $g_{y\to y'} > 0$, $h_{y\to y'} > 0$, and $\rho_{y\to y'} = e^{y\cdot\mu}$.

(II) If $y \to y' \in \mathscr{O}$ is reversible, then

(i) If $g_{y\to y'} > 0$, then either $\rho_{y\to y'} > e^{y\cdot\mu} > e^{y'\cdot\mu}$, $\rho_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$, or $\rho_{y\to y'} = e^{y\cdot\mu} = e^{y'\cdot\mu}$.

(ii) If $g_{y\to y'} < 0$, then either $\rho_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$, $\rho_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$, or $\rho_{y\to y'} = e^{y\cdot\mu} = e^{y'\cdot\mu}$.

(iii) If $g_{y\to y'} = 0$ and $h_{y\to y'} > 0$, then $e^{y\cdot\mu} > e^{y'\cdot\mu}$.

(iv) If $g_{y\to y'} = 0$ and $h_{y\to y'} < 0$, then $e^{y\cdot\mu} < e^{y'\cdot\mu}$.

(v) If $g_{y\to y'} = 0$ and $h_{y\to y'} = 0$, then $e^{y\cdot\mu} = e^{y'\cdot\mu}$.

PROOF: We want to show the conditions listed in the lemma hold and we will show it case by case.

If $y \to y' \in \mathscr{O}$ is irreversible, then equations (2.3.2) and (2.3.3) imply that $g_{y\to y'} = \kappa_{y\to y'} > 0$, $h_{y\to y'} = \kappa_{y\to y'} e^{y\cdot\mu} > 0$, and $\rho_{y\to y'} = \dfrac{h_{y\to y'}}{g_{y\to y'}} = e^{y\cdot\mu} > 0$.

If $y \to y' \in \mathscr{O}$ is reversible, then there are the following cases:

(a): Assume $g_{y\to y'} \neq 0$. Then we will discuss two different situations, according to whether $y \cdot \mu$ equals to $y' \cdot \mu$.

If $y \cdot \mu \neq y' \cdot \mu$, then from equations (2.3.2), (2.3.3) and (2.3.6), $\kappa_{y\to y'}$ and $\kappa_{y'\to y}$ can be solved for as follows:

$$\kappa_{y\to y'} = \frac{\rho_{y\to y'} - e^{y'\cdot\mu}}{e^{y\cdot\mu} - e^{y'\cdot\mu}} g_{y\to y'} \tag{2.3.7}$$

$$\kappa_{y'\to y} = \frac{\rho_{y\to y'} - e^{y\cdot\mu}}{e^{y'\cdot\mu} - e^{y\cdot\mu}} g_{y\to y'}. \tag{2.3.8}$$

From $\kappa_{y\to y'} > 0$ and $\kappa_{y'\to y} > 0$, we then have:

If $g_{y\to y'} > 0$, then either $\rho_{y\to y'} > e^{y\cdot\mu} > e^{y'\cdot\mu}$ or $\rho_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$.

If $g_{y\to y'} < 0$, then either $\rho_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$ or $\rho_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$.

If $y \cdot \mu = y' \cdot \mu$, then equations (2.3.2) and (2.3.3) can be simplified to

$$g_{y\to y'} = \kappa_{y\to y'} - \kappa_{y'\to y} \tag{2.3.9}$$

$$h_{y\to y'} = (\kappa_{y\to y'} - \kappa_{y'\to y}) e^{y\cdot\mu}. \tag{2.3.10}$$

Therefore, if $g_{y\to y'} \neq 0$, then $\rho_{y\to y'} = \dfrac{h_{y\to y'}}{g_{y\to y'}} = e^{y\cdot\mu} = e^{y'\cdot\mu}$.

(b): Assume $g_{y\to y'} = 0$. In this case, equations (2.3.2) and (2.3.3) imply that $\kappa_{y\to y'} = \kappa_{y'\to y}$ and $h_{y\to y'} = \kappa_{y\to y'}(e^{y\cdot\mu} - e^{y'\cdot\mu})$. Since $\kappa_{y\to y'} > 0$, we have the following three cases:

If $h_{y\to y'} > 0$, then $e^{y\cdot\mu} > e^{y'\cdot\mu}$.

If $h_{y\to y'} < 0$, then $e^{y\cdot\mu} < e^{y'\cdot\mu}$.

If $h_{y\to y'} = 0$, then $e^{y\cdot\mu} = e^{y'\cdot\mu}$.

We have shown that the conditions listed in the lemma hold. □

Lemma 2.3.3. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, suppose that $\mu \in \mathbb{R}^{\mathscr{S}}$, a pair g, $h \in Ker\ L_{\mathscr{O}}$, and $\rho_{y\to y'}$ $(g_{y\to y'} \neq 0)$ defined as in (2.3.6) satisfy the conditions in Lemma 2.3.2. Then there exists $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ satisfying (2.3.2) and (2.3.3).*

PROOF: Assume that conditions listed in Lemma 2.3.2 hold. Then we want to show that we can solve for $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ from (2.3.2) and (2.3.3).

(a) Assume that $y \to y'$ is irreversible. By the definition of g and h in (2.3.2) and (2.3.3), we have that $g_{y\to y'} = \kappa_{y\to y'}$ and $h_{y\to y'} = \kappa_{y\to y'}e^{y\cdot\mu}$. From the conditions in case (I) in Lemma 2.3.2, we have $g_{y\to y'} > 0$, $h_{y\to y'} > 0$ and $\rho_{y\to y'} = e^{y\cdot\mu}$. Note that $\rho_{y\to y'} = h_{y\to y'}/g_{y\to y'}$, so $h_{y\to y'} = \rho_{y\to y'}g_{y\to y'} = e^{y\cdot\mu}g_{y\to y'}$. Since $h_{y\to y'} = \kappa_{y\to y'}e^{y\cdot\mu}$ in (2.3.3), we have from (2.3.3) that $g_{y\to y'} = \kappa_{y\to y'}$. Therefore two equations (2.3.2) and (2.3.3) become equivalent to each other, and we can easily solve $\kappa_{y\to y'} = g_{y\to y'} > 0$.

(b) Assume that $y \to y'$ is reversible. Then we will discuss three different situations according to the sign of $g_{y\to y'}$.

First, if $g_{y\to y'} > 0$, then we have from condition (i) that either $\rho_{y\to y'} > e^{y\cdot\mu} > e^{y'\cdot\mu}$, $\rho_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$, or $\rho_{y\to y'} = e^{y\cdot\mu} = e^{y'\cdot\mu}$ holds.

If $\rho_{y\to y'} > e^{y\cdot\mu} > e^{y'\cdot\mu}$ or $\rho_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$ holds, then (2.3.2) and (2.3.3) can be rewritten as (2.3.7) and (2.3.8) to solve for $\kappa_{y\to y'}$ and $\kappa_{y'\to y}$, from which we can conclude that $\kappa_{y\to y'} > 0$ and $\kappa_{y'\to y} > 0$.

If $\rho_{y\to y'} = e^{y\cdot\mu} = e^{y'\cdot\mu}$ holds, then since $h_{y\to y'} = \rho_{y\to y'}g_{y\to y'}$, we can see that (2.3.2) and (2.3.3) both convert to (2.3.9). So any $\kappa_{y\to y'}$ and $\kappa_{y'\to y}$ as long as $\kappa_{y\to y'} > \kappa_{y'\to y} > 0$ will satisfy (2.3.2) and (2.3.3).

Secondly, if $g_{y\to y'} < 0$, then we have from condition (ii) that either $\rho_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$, $\rho_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$, or $\rho_{y\to y'} = e^{y'\cdot\mu} = e^{y\cdot\mu}$ holds.

If $\rho_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$ or $\rho_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$ holds, then (2.3.2) and (2.3.3) can be rewritten as (2.3.7) and (2.3.8) to solve for $\kappa_{y\to y'}$ and $\kappa_{y'\to y}$, from which we can conclude that $\kappa_{y\to y'} > 0$ and $\kappa_{y'\to y} > 0$.

If $\rho_{y\to y'} = e^{y'\cdot\mu} = e^{y\cdot\mu}$ holds, then since $h_{y\to y'} = \rho_{y\to y'}g_{y\to y'}$, we can see that (2.3.2) and (2.3.3) both convert to (2.3.9). So any $\kappa_{y\to y'}$ and $\kappa_{y'\to y}$ as long as $\kappa_{y'\to y} > \kappa_{y\to y'} > 0$ will satisfy (2.3.2) and (2.3.3).

Thirdly, if $g_{y\to y'} = 0$, then (2.3.2) and (2.3.3) become

$$\kappa_{y\to y'} = \kappa_{y'\to y}$$

and

$$h_{y\to y'} = \kappa_{y\to y'}(e^{y\cdot\mu} - e^{y'\cdot\mu}).$$

If $h_{y\to y'} > 0$, then from condition (iii) we have $e^{y\cdot\mu} > e^{y'\cdot\mu}$, and therefore $\kappa_{y\to y'} = \kappa_{y'\to y} = h_{y\to y'}/(e^{y\cdot\mu} - e^{y'\cdot\mu}) > 0$.

If $h_{y\to y'} < 0$, then from condition (iv) we have $e^{y'\cdot\mu} > e^{y\cdot\mu}$, and therefore $\kappa_{y\to y'} = \kappa_{y'\to y} = h_{y\to y'}/(e^{y\cdot\mu} - e^{y'\cdot\mu}) > 0$.

If $h_{y\to y'} = 0$, then from condition (v) we have $e^{y\cdot\mu} = e^{y'\cdot\mu}$, and therefore $\kappa_{y\to y'} = \kappa_{y'\to y}$ can be any positive number.

We have shown that if the conditions listed in Lemma 2.3.2 hold, we can always find $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ satisfying (2.3.2) and (2.3.3). □

Recall that Question 2 asked: Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist a set of positive $\{\kappa_{y\to y'} : y \to y' \in \mathscr{R}\}$ and a nonzero μ which is sign-compatible with the stoichiometric subspace S such that equations (2.2.7) and (2.2.8) are satisfied?

From Lemmas 2.3.2 and 2.3.3, we can see that, in order to answer Question 2, we may instead ask the following question:

Question 3. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist an orientation $\mathscr{O}$ of $\mathscr{R}$, a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with the stoichiometric subspace S, a pair g, $h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors, and a set $\{\rho_{y\to y'} = \dfrac{h_{y\to y'}}{g_{y\to y'}} : g_{y\to y'} \neq 0, y \to y' \in \mathscr{O}\}$ such that the conditions in Lemma 2.3.2 are satisfied?*

However, do we have to test against all possible orientations of $\mathscr{R}$? In other words, does the answer to Question 3 depend on the choice of $\mathscr{O}$? The answer is no. We will show next that the choice of orientation $\mathscr{O}$ does not matter here.

To show this, first let us prove the following lemma.

Lemma 2.3.4. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, for any orientations $\mathscr{O}_1$ and $\mathscr{O}_2$ of $\mathscr{R}$, $Ker\ L_{\mathscr{O}_1}$ and $Ker\ L_{\mathscr{O}_2}$ are isomorphic, i.e., $Ker\ L_{\mathscr{O}_1} \cong Ker\ L_{\mathscr{O}_2}$.*

PROOF: Recall that an orientation $\mathscr{O} \subseteq \mathscr{R}$ is such that for every $y \to y' \in \mathscr{R}$, either $y \to y'$ or $y' \to y$ belongs to $\mathscr{O}$, but not both. It is obvious that $\#(\mathscr{O}_1) = \#(\mathscr{O}_2)$. Note that $L_{\mathscr{O}}x = \sum_{y \to y' \in \mathscr{O}} x_{y \to y'}(y' - y)$. Let $x^{\mathscr{O}_1} \in Ker\ L_{\mathscr{O}_1}$, we define $x^{\mathscr{O}_2} \in \mathbb{R}^{\mathscr{O}_2}$ as follows:

$$x^{\mathscr{O}_2}_{y \to y'} = \begin{cases} x^{\mathscr{O}_1}_{y \to y'}, & \text{if } y \to y' \in \mathscr{O}_1 \\ -x^{\mathscr{O}_1}_{y' \to y}, & \text{if } y' \to y \in \mathscr{O}_1. \end{cases}$$

We can see that

$$\begin{aligned} L_{\mathscr{O}_2}x^{\mathscr{O}_2} &= \sum_{y \to y' \in \mathscr{O}_2} x^{\mathscr{O}_2}_{y \to y'}(y' - y) \\ &= \sum_{y \to y' \in \mathscr{O}_2 : y \to y' \in \mathscr{O}_1} x^{\mathscr{O}_2}_{y \to y'}(y' - y) + \sum_{y \to y' \in \mathscr{O}_2 : y' \to y \in \mathscr{O}_1} x^{\mathscr{O}_2}_{y \to y'}(y' - y) \\ &= \sum_{y \to y' \in \mathscr{O}_2 : y \to y' \in \mathscr{O}_1} x^{\mathscr{O}_1}_{y \to y'}(y' - y) + \sum_{y \to y' \in \mathscr{O}_2 : y' \to y \in \mathscr{O}_1} (-x^{\mathscr{O}_1}_{y' \to y})(y' - y) \\ &= \sum_{y \to y' \in \mathscr{O}_1 : y \to y' \in \mathscr{O}_2} x^{\mathscr{O}_1}_{y \to y'}(y' - y) + \sum_{y' \to y \in \mathscr{O}_1 : y \to y' \in \mathscr{O}_2} x^{\mathscr{O}_1}_{y' \to y}(y - y') \\ &= \sum_{y \to y' \in \mathscr{O}_1} x^{\mathscr{O}_1}_{y \to y'}(y' - y) \\ &= 0. \end{aligned}$$

So $x^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$. Thus the function F: $Ker\ L_{\mathscr{O}_1} \to Ker\ L_{\mathscr{O}_2}$ via $F(x^{\mathscr{O}_1}) = x^{\mathscr{O}_2}$ is well defined.

On the other hand, let $\tilde{x}^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$, we define $\tilde{x}^{\mathscr{O}_1} \in \mathbb{R}^{\mathscr{O}_1}$ as follows:

$$\tilde{x}^{\mathscr{O}_1}_{y \to y'} = \begin{cases} \tilde{x}^{\mathscr{O}_2}_{y \to y'}, & \text{if } y \to y' \in \mathscr{O}_2 \\ -\tilde{x}^{\mathscr{O}_2}_{y' \to y}, & \text{if } y' \to y \in \mathscr{O}_2. \end{cases}$$

Then similarly we can show that $\tilde{x}^{\mathscr{O}_1} \in Ker\ L_{\mathscr{O}_1}$. Then the function G: $Ker\ L_{\mathscr{O}_1} \to Ker\ L_{\mathscr{O}_2}$ via $G(\tilde{x}^{\mathscr{O}_2}) = \tilde{x}^{\mathscr{O}_1}$ is also well defined. We can verify that, for any $x^{\mathscr{O}_1} \in Ker\ L_{\mathscr{O}_1}$ $G \circ F(x^{\mathscr{O}_1}) = x^{\mathscr{O}_1}$, and for any $x^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$, $F \circ G(x^{\mathscr{O}_2}) = x^{\mathscr{O}_2}$. Therefore, $F = G^{-1}$ and $G = F^{-1}$. We can claim that $Ker\ L_{\mathscr{O}_1} \cong Ker\ L_{\mathscr{O}_2}$. □

Lemma 2.3.5. *Question 3 can be rewritten in terms of g, h, μ, and $\rho_{y_i \to y_i'}$'s as follows:*

Question 4. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, and any orientation $\mathscr{O}$, do there exist a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with the stoichiometric subspace S, a pair g, $h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors, and a set $\{\rho_{y \to y'} = \dfrac{h_{y \to y'}}{g_{y \to y'}} : g_{y \to y'} \neq 0, y \to y' \in \mathscr{O}\}$ such that conditions in Lemma 2.3.2 are satisfied?*

PROOF: It is obvious that if the answer to Question 4 is yes, then the answer to Question 3 is also yes. Thus we want to show the reverse.

Let $\mathscr{O}_1$, $\mathscr{O}_2$ be two orientations of $\mathscr{R}$, and let $\mu \in \mathbb{R}^{\mathscr{S}}$ be nonzero and sign-compatible with S. We will show the following:

Suppose that there exist a pair $g^{\mathscr{O}_1}, h^{\mathscr{O}_1} \in Ker\ L_{\mathscr{O}_1}$ which are not both zero vectors, and a set $\{\rho^{\mathscr{O}_1}_{y \to y'} = \dfrac{h^{\mathscr{O}_1}_{y \to y'}}{g^{\mathscr{O}_1}_{y \to y'}} : g^{\mathscr{O}_1}_{y \to y'} \neq 0\}$, satisfying all the conditions listed in Lemma 2.3.2. Then there exist a pair $g^{\mathscr{O}_2}, h^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$ which are not both zero vectors, and a set $\{\rho^{\mathscr{O}_2}_{y \to y'} = \dfrac{h^{\mathscr{O}_2}_{y \to y'}}{g^{\mathscr{O}_2}_{y \to y'}} : g^{\mathscr{O}_2}_{y \to y'} \neq 0\}$, satisfying all the conditions listed in Lemma 2.3.2.

Note that $g^{\mathscr{O}_1}, h^{\mathscr{O}_1} \in Ker\ L_{\mathscr{O}_1}$. We let $g^{\mathscr{O}_2} = F(g^{\mathscr{O}_1})$ and $h^{\mathscr{O}_2} = F(h^{\mathscr{O}_1})$, where F is defined in Lemma 2.3.4. Then $g^{\mathscr{O}_2}, h^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$ and they are not both zero vectors. Let $y \to y' \in \mathscr{O}_2$, then we have two cases depending on whether $y \to y' \in \mathscr{O}_1$ or $y' \to y \in \mathscr{O}_1$.

Case one: If $y \to y' \in \mathscr{O}_1$, then $g^{\mathscr{O}_2}_{y \to y'} = g^{\mathscr{O}_1}_{y \to y'}$ and $h^{\mathscr{O}_2}_{y \to y'} = h^{\mathscr{O}_1}_{y \to y'}$. If $g^{\mathscr{O}_2}_{y \to y'} \neq 0$, $\rho^{\mathscr{O}_2}_{y \to y'} = \rho^{\mathscr{O}_1}_{y \to y'}$. This case is trivial.

Case two: If $y' \to y \in \mathscr{O}_1$, then $y \to y'$ must be reversible. We have $g^{\mathscr{O}_2}_{y \to y'} = -g^{\mathscr{O}_1}_{y' \to y}$ and $h^{\mathscr{O}_2}_{y \to y'} = -h^{\mathscr{O}_1}_{y' \to y}$. If $g^{\mathscr{O}_2}_{y \to y'} \neq 0$, $\rho^{\mathscr{O}_2}_{y \to y'} = \rho^{\mathscr{O}_1}_{y' \to y}$. We will verify each of the conditions listed in Lemma 2.3.2 for reversible $y \to y' \in \mathscr{O}_2$.

Recall that the conditions in Lemma 2.3.2 are

(I) If $y \to y' \in \mathscr{O}$ is irreversible, then $g_{y \to y'} > 0$, $h_{y \to y'} > 0$, and $\rho_{y \to y'} = e^{y \cdot \mu}$.

(II) If $y \to y' \in \mathscr{O}$ is reversible, then

(i) If $g_{y \to y'} > 0$, then either $\rho_{y \to y'} > e^{y \cdot \mu} > e^{y' \cdot \mu}$, $\rho_{y \to y'} < e^{y \cdot \mu} < e^{y' \cdot \mu}$, or $\rho_{y \to y'} = e^{y \cdot \mu} = e^{y' \cdot \mu}$.

(ii) If $g_{y\to y'} < 0$, then either $\rho_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$, $\rho_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$, or $\rho_{y\to y'} = e^{y\cdot\mu} = e^{y'\cdot\mu}$.

(iii) If $g_{y\to y'} = 0$ and $h_{y\to y'} > 0$, then $e^{y\cdot\mu} > e^{y'\cdot\mu}$.

(iv) If $g_{y\to y'} = 0$ and $h_{y\to y'} < 0$, then $e^{y\cdot\mu} < e^{y'\cdot\mu}$.

(v) If $g_{y\to y'} = 0$ and $h_{y\to y'} = 0$, then $e^{y\cdot\mu} = e^{y'\cdot\mu}$.

Since $y \to y'$ is reversible, (I) is trivially true for $\mathscr{O}_2$.

Next we will show that (II) is true for $\mathscr{O}_2$.

If $g^{\mathscr{O}_2}_{y\to y'} > 0$, then $g^{\mathscr{O}_1}_{y'\to y} < 0$. (II) is true for $y' \to y \in \mathscr{O}_1$, so in particular (ii) is true for $y' \to y \in \mathscr{O}_1$. Therefore we have either $\rho^{\mathscr{O}_1}_{y'\to y} > e^{y\cdot\mu} > e^{y'\cdot\mu}$, $\rho^{\mathscr{O}_1}_{y'\to y} < e^{y\cdot\mu} < e^{y'\cdot\mu}$, or $\rho^{\mathscr{O}_1}_{y'\to y} = e^{y'\cdot\mu} = e^{y\cdot\mu}$. Since $\rho^{\mathscr{O}_1}_{y\to y'} = \rho^{\mathscr{O}_2}_{y'\to y}$, we have either $\rho^{\mathscr{O}_2}_{y\to y'} > e^{y\cdot\mu} > e^{y'\cdot\mu}$, $\rho^{\mathscr{O}_2}_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$, or $\rho^{\mathscr{O}_2}_{y\to y'} = e^{y'\cdot\mu} = e^{y\cdot\mu}$. We have shown that (i) is true for reversible $y \to y' \in \mathscr{O}_2$.

If $g^{\mathscr{O}_2}_{y\to y'} < 0$, then $g^{\mathscr{O}_1}_{y'\to y} > 0$. (II) is true for $y' \to y \in \mathscr{O}_1$, so in particular (i) is true for $y' \to y \in \mathscr{O}_1$. Therefore we have either $\rho^{\mathscr{O}_1}_{y'\to y} > e^{y'\cdot\mu} > e^{y\cdot\mu}$, $\rho^{\mathscr{O}_1}_{y'\to y} < e^{y'\cdot\mu} < e^{y\cdot\mu}$, or $\rho^{\mathscr{O}_1}_{y'\to y} = e^{y'\cdot\mu} = e^{y\cdot\mu}$. Since $\rho^{\mathscr{O}_1}_{y\to y'} = \rho^{\mathscr{O}_2}_{y'\to y}$, we have either $\rho^{\mathscr{O}_2}_{y\to y'} > e^{y'\cdot\mu} > e^{y\cdot\mu}$, $\rho^{\mathscr{O}_2}_{y\to y'} < e^{y'\cdot\mu} < e^{y\cdot\mu}$, or $\rho^{\mathscr{O}_2}_{y\to y'} = e^{y'\cdot\mu} = e^{y\cdot\mu}$. We have shown that (ii) is true for reversible $y \to y' \in \mathscr{O}_2$.

If $g^{\mathscr{O}_2}_{y\to y'} = 0$ and $h^{\mathscr{O}_2}_{y\to y'} > 0$, then $g^{\mathscr{O}_1}_{y'\to y} = 0$ and $h^{\mathscr{O}_1}_{y'\to y} < 0$. (II) is true for $y' \to y \in \mathscr{O}_1$, so in particular (iv) is true for $y' \to y \in \mathscr{O}_1$. Therefore we have $e^{y'\cdot\mu} < e^{y\cdot\mu}$. We have shown that (iii) is true for reversible $y \to y' \in \mathscr{O}_2$.

If $g^{\mathscr{O}_2}_{y\to y'} = 0$ and $h^{\mathscr{O}_2}_{y\to y'} < 0$, then $g^{\mathscr{O}_1}_{y'\to y} = 0$ and $h^{\mathscr{O}_1}_{y'\to y} > 0$. (II) is true for $y' \to y \in \mathscr{O}_1$, so in particular (iii) is true for $y' \to y \in \mathscr{O}_1$. Therefore we have $e^{y'\cdot\mu} > e^{y\cdot\mu}$. We have shown that (iv) is true for reversible $y \to y' \in \mathscr{O}_2$.

If $g^{\mathscr{O}_2}_{y\to y'} = 0$ and $h^{\mathscr{O}_2}_{y\to y'} = 0$, then $g^{\mathscr{O}_1}_{y'\to y} = 0$ and $h^{\mathscr{O}_1}_{y'\to y} = 0$. (II) is true for $y' \to y \in \mathscr{O}_1$,

so in particular (v) is true for $y' \to y \in \mathscr{O}_1$. Therefore we have $e^{y' \cdot \mu} = e^{y \cdot \mu}$. We have shown that (v) is true for reversible $y \to y' \in \mathscr{O}_2$. □

2.4 Partitions on $\mathscr{O}$ and $\mathscr{R}$

Note that in Question 4, we have many parameters: $\{g_{y \to y'} : y \to y' \in \mathscr{O}\}$, $\{h_{y \to y'} : y \to y' \in \mathscr{O}$ such that $g_{y \to y'} = 0\}$, $\{\rho_{y \to y'} : y \to y' \in \mathscr{O}$ such that $g_{y \to y'} \neq 0\}$ and $\{\mu_s : s \in \mathscr{S}\}$. There are $2\#(\mathscr{O}) + \#(\mathscr{S})$ parameters together, where $\#(\mathscr{O})$ is the number of reactions in $\mathscr{O}$ and $\#(\mathscr{S})$ is the number of species in $\mathscr{S}$. We will try to reduce the number of parameters by defining partitions on $\mathscr{O}$ in a way that will reduce the number of $\rho_{y \to y'}$'s needed. Since $g, h \in Ker\ L_{\mathscr{O}}$ and $\rho_{y \to y'} = \dfrac{h_{y \to y'}}{g_{y \to y'}}$ where $g_{y \to y'} \neq 0$, we need to examine $Ker\ L_{\mathscr{O}}$ and $Ker^{\perp}\ L_{\mathscr{O}}$ in order to derive the partition on $\mathscr{O}$.

Recall that $\{\omega_{y \to y'} : y \to y' \in \mathbb{R}^{\mathscr{O}}\}$ is the standard basis for $\mathbb{R}^{\mathscr{O}}$. Let $\alpha \neq 0$. For $y \to y'$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}$, note that the following statements are equivalent:

(i) $x_{y \to y'} = \alpha x_{\tilde{y} \to \tilde{y}'}$, for any $x \in Ker\ L_{\mathscr{O}}$.

(ii) $x \cdot (\omega_{y \to y'} - \alpha \omega_{\tilde{y} \to \tilde{y}'}) = 0$, for any $x \in Ker\ L_{\mathscr{O}}$.

(iii) $\omega_{y \to y'} - \alpha \omega_{\tilde{y} \to \tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

Now we will introduce a relation " $\sim$ " in $\mathscr{O}$ as follows:

For $y \to y'$ and $\tilde{y} \to \tilde{y}' \in \mathscr{O}$, we write $y \to y' \sim \tilde{y} \to \tilde{y}'$ if there exists $\alpha \neq 0$ such that $\omega_{y \to y'} - \alpha \omega_{\tilde{y} \to \tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. The '$\sim$' defined is an equivalence relation. To see that, note that:

(i) Reflexivity: $\omega_{y \to y'} - \omega_{y \to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$, so $y \to y' \sim y \to y'$.

(ii) Symmetry: If $y \to y' \sim \tilde{y} \to \tilde{y}'$, there exists $\alpha \neq 0$ such that $\omega_{y \to y'} - \alpha \omega_{\tilde{y} \to \tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Therefore, $-\dfrac{1}{\alpha}(\omega_{y \to y'} - \alpha \omega_{\tilde{y} \to \tilde{y}'}) \in Ker^{\perp}\ L_{\mathscr{O}}$, i.e., $\omega_{\tilde{y} \to \tilde{y}'} - \dfrac{1}{\alpha}\omega_{y \to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Hence $\tilde{y} \to \tilde{y}' \sim y \to y'$.

(iii) Transitivity: If $y \to y' \sim \tilde{y} \to \tilde{y}'$, and $\tilde{y} \to \tilde{y}' \sim \hat{y} \to \hat{y}'$, then there exist $\alpha_1, \alpha_2 \neq 0$, such that $\omega_{y \to y'} - \alpha_1 \omega_{\tilde{y} \to \tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$ and $\omega_{\tilde{y} \to \tilde{y}'} - \alpha_2 \omega_{\hat{y} \to \hat{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

Therefore, $\omega_{y\to y'} - \alpha_1\omega_{\tilde{y}\to\tilde{y}'} + \alpha_1(\omega_{\tilde{y}\to\tilde{y}'} - \alpha_2\omega_{\hat{y}\to\hat{y}'}) \in Ker^{\perp}\, L_{\mathscr{O}}$, i.e., $\omega_{y\to y'} - \alpha_1\alpha_2\omega_{\hat{y}\to\hat{y}'}$ $\in Ker^{\perp}\, L_{\mathscr{O}}$ where $\alpha_1\alpha_2 \neq 0$. Hence $y \to y' \sim \hat{y} \to \hat{y}'$.

We can define the ***equivalence class*** of $y \to y'$ under '$\sim$' as

$$[y \to y'] = \{\tilde{y} \to \tilde{y}' \in \mathscr{O} : \tilde{y} \to \tilde{y}' \sim y \to y'\}. \tag{2.4.1}$$

Clearly, the following statements are equivalent:

(i) $y \to y' \sim \tilde{y} \to \tilde{y}'$.

(ii) $[y \to y'] = [\tilde{y} \to \tilde{y}']$.

(iii) $y \to y'$ and $\tilde{y} \to \tilde{y}'$ belong to the same equivalence class under '$\sim$'.

If an equivalence class only has one reaction in it, then we call it a ***trivial equivalence class***; otherwise, it is called a ***nontrivial equivalence class***.

Suppose there is a reaction $y \to y' \in \mathscr{O}$ such that $\omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$. Now consider a reaction $\tilde{y} \to \tilde{y}'$ which lies in the same equivalence class with $y \to y'$. Since there exists $\alpha \neq 0$ such that $\omega_{\tilde{y}\to\tilde{y}'} - \alpha\omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$, it must be the case that $\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}$ $L_{\mathscr{O}}$. Conversely, if there exist $y \to y'$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}$, such that $\omega_{y\to y'}$, $\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}}$, then $\omega_{y\to y'} - \omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}}$. Hence $y \to y' \sim \tilde{y} \to \tilde{y}'$, and the two reactions must belong to the same equivalence class. As a result, if there is a reaction $y \to y'$ such that $\omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$, we will name the equivalence class in which $y \to y'$ lies, as P_0. If no such equivalence class exists, we just set $P_0 = \emptyset$.

Remark 2.4.1. *Note that the following statements are equivalent:*

(i) $y \to y' \in P_0$.

(ii) $\omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$.

(iii) For any $x \in Ker\ L_{\mathscr{O}}$, $x \cdot \omega_{y\to y'} = 0$.

(iv) For any $x \in Ker\ L_{\mathscr{O}}$, $x_{y\to y'} = 0$.

Suppose that besides P_0 (if it is not empty), there are additionally w equivalence classes, with w_1 nontrivial equivalence classes and w_2 trivial equivalence classes. Hence $w = w_1 +$

w_2. Let us name these nontrivial equivalence classes $P_1, P_2, ...P_{w_1}$, and trivial equivalence classes $P_{w_1+1}, ..., P_{w_1+w_2}$. The set of all equivalence classes $\{P_i\}_{i=0}^{w}$ of $\mathscr{O}$ defines a partition of $\mathscr{O}$. Let $\tilde{\mathscr{O}} = \mathscr{O} \backslash P_0$. Then $\{P_i\}_{i=1}^{w}$ defines a partition of $\tilde{\mathscr{O}}$.

Remark 2.4.2. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and an orientation $\mathscr{O}$, the following statements are equivalent:*

(i) $y \to y'$ and $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class.

(ii) There exists $\alpha \neq 0$, such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

Let $\alpha \neq 0$. The following statements are equivalent:

(i) $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

(ii) For any $x \in Ker\ L_{\mathscr{O}}$, $x \cdot (\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'}) = 0$.

(iii) For any $x \in Ker\ L_{\mathscr{O}}$, $x_{y\to y'} = \alpha x_{\tilde{y}\to\tilde{y}'}$.

Remark 2.4.3. *Two reactions $y \to y'$ and $\tilde{y}' \to \tilde{y}'$ both lie in P_i $(1 \leq i \leq w)$ if and only if there is a unique $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. The uniqueness of α can be shown by supposing the contrary, i.e. assume there exists $\tilde{\alpha} \neq \alpha$ such that $\omega_{y\to y'} - \tilde{\alpha}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Then we can show that $(\tilde{\alpha} - \alpha)\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Hence $\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Then $\tilde{y} \to \tilde{y}' \in P_0$, which is a contradiction.*

2.5 Digression 1

Before we go on, let us make a few observations about the equivalence classes P_i's $(i \geq 0)$ defined under the orientation $\mathscr{O}$.

For P_i $(i \geq 0)$, let us define $\tilde{\mathscr{N}_i}$ to be the subnetwork generated by reactions in P_i. Note that since $P_i \subset \mathscr{O}$, all the reactions in $\tilde{\mathscr{N}_i}$ are irreversible (with respect to the orientation $\mathscr{O}$).

If we look at the subnetwork as if it is an independent reaction network, then similarly

we can define for this reaction network its linkage classes and deficiency. For example, a linkage class of one of these subnetworks is called a *colinkage set for the reaction network*.

We have defined the reaction vector for a reaction $y \to y'$ as $y' - y$, then the reaction vectors for a reaction network $\tilde{\mathscr{N}_i}$ are $\{y' - y : y \to y' \in \tilde{\mathscr{N}_i}\}$.

Remark 2.5.1. *Note that it is trivial that the single reaction vector in each trivial equivalence class is independent. Therefore, the reaction vector in each* $\tilde{\mathscr{N}_i}$ $(w_1 + 1 \leq i \leq w_1 + w_2)$ *is independent.*

Remark 2.5.2. *We claim that reaction vector(s) for* $\tilde{\mathscr{N}_0}$ *is (are) independent.*

If P_0 *is trivial, then the claim is trivial. If* P_0 *is nontrivial, then to see the independence, let us suppose the contrary, i.e., there exists a set* $\{\gamma_{y\to y'} : y \to y' \in P_0\}$ *whose elements are not all zero, such that,* $\sum_{y\to y'\in P_0} \gamma_{y\to y'}(y' - y) = 0$. *Let* $\beta \in \mathbb{R}^{\mathscr{O}}$ *be defined as follows:*

$$\beta_{y\to y'} = \begin{cases} \gamma_{y\to y'}, & \text{if } y \to y' \in P_0 \\ 0, & \text{otherwise}. \end{cases}$$

Then $\beta \neq 0$ *and* $\beta \in Ker\ L_{\mathscr{O}}$. *Since for all* $y \to y' \in P_0$, $\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$, *we have* $\omega_{y\to y'} \cdot \beta = \beta_{y\to y'} = \gamma_{y\to y'} = 0$, *for all* $y \to y' \in P_0$. *This is a contradiction.*

Therefore, the reaction vector(s) in $\tilde{\mathscr{N}_0}$ *is (are) independent. Consequently, there will be no (undirected) cycle in* $\tilde{\mathscr{N}_0}$, *i.e.,* $\tilde{\mathscr{N}_0}$ *forms a forest.*

Remark 2.5.3. *Consider the subnetwork* $\tilde{\mathscr{N}_i}$ $(1 \leq i \leq w_1)$. *If the reaction vectors for* $\tilde{\mathscr{N}_i}$ *are dependent, then we can claim that for any proper subset* $Q \subset P_i$, *the reaction vectors for the subnetwork generated by reactions in* Q *are independent, i.e., the reaction vectors for* $\tilde{\mathscr{N}_i}$ *are minimally dependent if not independent.*

To show this, let us suppose the contrary, i.e., the reaction vectors for the subnetwork generated by reactions in Q *are dependent. Then there is a set* $\{\gamma_{y\to y'} : y \to y' \in Q\}$

whose elements are not all zero, such that $\sum_{y\to y'\in Q}\gamma_{y\to y'}(y'-y)=0$. *Let* $\beta\in\mathbb{R}^{\mathscr{O}}$ *be defined as follows:*

$$\beta_{y\to y'}=\begin{cases}\gamma_{y\to y'}, & \text{if } y\to y'\in Q\\ 0, & \text{otherwise.}\end{cases}$$

Then $\beta\neq 0$ *and* $\beta\in Ker\ L_{\mathscr{O}}$. *Take* $\tilde{y}\to\tilde{y}'\in P_i\backslash Q$, *and* $\hat{y}\to\hat{y}'\in Q$ *such that* $\gamma_{\hat{y}\to\hat{y}'}\neq 0$. *Because* $\tilde{y}\to\tilde{y}'$ *and* $\hat{y}\to\hat{y}'$ *are both members of* P_i, *there exists* $\alpha_{\tilde{y}\to\tilde{y}'}\neq 0$, *such that* $\omega_{\hat{y}\to\hat{y}'}-\alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'}\in Ker^{\perp}\ L_{\mathscr{O}}$. *Thus we have that* $\beta\cdot(\omega_{\hat{y}\to\hat{y}'}-\alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'})=\beta_{\hat{y}\to\hat{y}'}-\alpha_{\tilde{y}\to\tilde{y}'}\beta_{\tilde{y}\to\tilde{y}'}=\gamma_{\hat{y}\to\hat{y}'}-\alpha_{\tilde{y}\to\tilde{y}'}(0)=\gamma_{\hat{y}\to\hat{y}'}=0$. *This is a contradiction since* $\gamma_{\hat{y}\to\hat{y}'}\neq 0$.

We have shown that the reaction vectors for each $\tilde{\mathscr{N}_i}$ $(1\leq i\leq w_1)$ *are either independent or minimally dependent.*

From Remarks 2.5.1, 2.5.2 and 2.5.3, we know that the following holds:

(i) The reaction vector(s) for $\tilde{\mathscr{N}_0}$ is (are) independent.

(ii) The single reaction vector for each $\tilde{\mathscr{N}_i}$ $(w_1+1\leq i\leq w_1+w_2)$ is independent trivially.

(iii) Reaction vectors for $\tilde{\mathscr{N}_i}$ $(1\leq i\leq w_1)$ are either independent or minimally dependent.

Proposition 2.5.4. *Given a reaction network* $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$, *an orientation* $\mathscr{O}$, *and the* $\tilde{\mathscr{N}_i}$ $(0\leq i\leq w)$ *defined as the subnetwork generated by all reactions in* P_i, *one of the following will hold:*

(i) The reaction vectors for $\tilde{\mathscr{N}_i}$ *are independent, and the subnetwork* $\tilde{\mathscr{N}_i}$ *based on* P_i *forms a forest with deficiency* 0.

(ii) The reaction vectors are minimally dependent, and the subnetwork $\tilde{\mathscr{N}_i}$ *based on* P_i *forms a forest with deficiency* 1.

(iii) The reaction vectors are minimally dependent, and the subnetwork $\tilde{\mathscr{N}_i}$ *based on* P_i *forms a big cycle (a cycle with at least three vertices) with deficiency* 0.

PROOF: To see that, let $\tilde{s}_i$ $(0 \leq i \leq w)$ be the dimension of the linear space spanned by all the reaction vectors in the subnetwork $\tilde{\mathscr{N}_i}$. The deficiency of the subnetwork $\tilde{\mathscr{N}_i}$ generated by reactions in P_i $(0 \leq i \leq w)$ is $\delta_{\tilde{\mathscr{N}_i}} = \#$(complexes in $\tilde{\mathscr{N}_i}) - \#$(linkage classes in $\tilde{\mathscr{N}_i}) - \tilde{s}_i$.

As for i in the range $w_1 + 1 \leq i \leq w_1 + w_2$, note that there is a single reaction in P_i. The reaction vector for each $\tilde{\mathscr{N}_i}$ is independent. $\tilde{\mathscr{N}_i}$ forms a forest, with $\#$(complexes in $\tilde{\mathscr{N}_i}) = 2$, $\#$(linkage classes in $\tilde{\mathscr{N}_i}) = 1$, and $\tilde{s}_i = 1$. Therefore, $\delta_{\tilde{\mathscr{N}_i}} = \#$(complexes in $\tilde{\mathscr{N}_i}) - \#$(linkage classes in $\tilde{\mathscr{N}_i}) - \tilde{s}_i = 2 - 1 - 1 = 0$.

Note that in each P_i $(0 \leq i \leq w_1)$, if $y \to y' \in P_i$, then $y' \to y \notin P_i$. Therefore, the subnetwork generated by reactions in P_i either forms a forest or contains a big (undirected) cycle.

If there is a big (undirected) cycle in $\tilde{\mathscr{N}_i}(1 \leq i \leq w_1)$, then the reaction vectors in the cycle are dependent. Note that if the reactions vectors in $\tilde{\mathscr{N}_i}$ are dependent, then they must be minimally dependent. Therefore, there can be no other reaction other than those in the cycle. In this case, $\tilde{\mathscr{N}_i}$ just forms a big (undirected) cycle. Moreover, $\#$(complexes in $\tilde{\mathscr{N}_i}) = \#(P_i)$, $\#$(linkage classes in $\tilde{\mathscr{N}_i}) = 1$, and $\tilde{s}_i = \#(P_i) - 1$. Thus $\delta_{\tilde{\mathscr{N}_i}} = \#(P_i) - 1 - (\#(P_i) - 1) = 0$.

If there is no big (undirected) cycle in $\tilde{\mathscr{N}_i}$ $(1 \leq i \leq w_1)$, i.e., $\tilde{\mathscr{N}_i}$ forms a forest, then the reaction vectors are either independent or minimally dependent. Note that since it is a forest, $\#$(complexes in $\tilde{\mathscr{N}_i}) = \#(P_i) + \#$(linkage classes in $\tilde{\mathscr{N}_i}$). If the reactions vectors on $\tilde{\mathscr{N}_i}$ are independent, $\tilde{s}_i = \#(P_i)$. Then $\delta_{\tilde{\mathscr{N}_i}} = (\#(P_i) + \#$(linkage classes in $\tilde{\mathscr{N}_i})) - \#$(linkage classes in $\tilde{\mathscr{N}_i}) - \#(P_i) = 0$. If the reaction vectors on $\tilde{\mathscr{N}_i}$ are minimally dependent, $\tilde{s}_i = \#(P_i) - 1$. Then $\delta_{\tilde{\mathscr{N}_i}} = (\#(P_i) + \#$(linkage classes in $\tilde{\mathscr{N}_i})) - \#$(linkage classes in $\tilde{\mathscr{N}_i}) - (\#(P_i) - 1) = 1$. □

We will continue with another proposition for the $\tilde{\mathscr{N}_i}$'s.

Proposition 2.5.5. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, an orientation $\mathscr{O}$ and the $\tilde{\mathscr{N}_i}$*

$(0 \leq i \leq w)$ *defined as the subnetwork generated by all reactions in* P_i*, the following are true:*

i) If dim $Ker\ L_{\mathscr{O}} \geq 1$*, the reaction vectors in* $\cup_{i=1}^{w} \tilde{\mathscr{N}_i}$ *are dependent.*

ii) If we take $\tilde{y} \to \tilde{y}'$ *from* $\tilde{\mathscr{N}_0}$*, then* $\tilde{y}' - \tilde{y}$ *does not lie in the span* $\{y' - y : y \to y' \in \mathscr{O} \backslash \{\tilde{y} \to \tilde{y}'\}\}$.

iii) If we take $\tilde{y} \to \tilde{y}'$ *and* $\hat{y} \to \hat{y}'$ *from the same* $\tilde{\mathscr{N}_i}$ $(1 \leq i \leq w_1)$*, then neither* $\tilde{y}' - \tilde{y}$ *nor* $\hat{y}' - \hat{y}$ *lies in the span* $\{y' - y : y \to y' \in \mathscr{O} \backslash \{\tilde{y} \to \tilde{y}', \hat{y} \to \hat{y}'\}\}$.

PROOF: i) They are dependent because, when dim $Ker\ L_{\mathscr{O}} \geq 1$, there exists a nonzero $x \in Ker\ L_{\mathscr{O}}$:

$$\begin{aligned} 0 &= \sum_{y \to y' \in \mathscr{O}} x_{y \to y'}(y' - y) \\ &= \sum_{y \to y' \in \cup_{i=1}^{w} \tilde{\mathscr{N}_i}} x_{y \to y'}(y' - y) \end{aligned}$$

This last equality holds because from Remark 2.4.1, $x_{y \to y'} = 0$, for any $y \to y' \in P_0$.

ii) Suppose not. Then there exist $\gamma_{y \to y'}$'s not all zero such that

$$\tilde{y}' - \tilde{y} = \sum_{y \to y' \in \mathscr{O} \backslash \{\tilde{y} \to \tilde{y}'\}} \gamma_{y \to y'}(y' - y).$$

We can define $x \in \mathbb{R}^{\mathscr{O}}$ via

$$x_{y \to y'} = \begin{cases} \gamma_{y \to y'}, & \text{if } y \to y' \in \mathscr{O} \backslash \{\tilde{y} \to \tilde{y}'\} \\ -1, & \text{otherwise.} \end{cases}$$

Then $x \in Ker\ L_{\mathscr{O}}$ and $x_{\tilde{y} \to \tilde{y}'} \neq 0$ while $\tilde{y} \to \tilde{y}' \in P_0$. This is a contradiction to the statement in Remark 2.4.1.

iii) To see this, suppose it is not true for $\tilde{y}' - \tilde{y}$. Then there exist $\gamma_{y \to y'}$'s not all zero such that

$$\tilde{y}' - \tilde{y} = \sum_{y \to y' \in \mathscr{O} \backslash \{\tilde{y} \to \tilde{y}', \hat{y} \to \hat{y}'\}} \gamma_{y \to y'}(y' - y).$$

We can define $x \in \mathbb{R}^{\mathscr{O}}$ via

$$x_{y\to y'} = \begin{cases} \gamma_{y\to y'}, & \text{if } y \to y' \in \mathscr{O}\backslash\{\tilde{y}\to\tilde{y}', \hat{y}\to\hat{y}'\} \\ -1, & \tilde{y}\to\tilde{y}' \\ 0, & \hat{y}\to\hat{y}'. \end{cases}$$

Then $x \in Ker\ L_{\mathscr{O}}$. However, since $\tilde{y} \to \tilde{y}', \hat{y} \to \hat{y}' \in P_i$, from Remark 2.4.2, there exists $\alpha_{\hat{y}\to\hat{y}'} \neq 0$ such that $x_{\tilde{y}\to\tilde{y}'} - \alpha_{\hat{y}\to\hat{y}'} x_{\hat{y}\to\hat{y}'} = 0$. From the definition of x, we have $-1 - \alpha_{\hat{y}\to\hat{y}'}(0) = 0$, or $-1 = 0$, which gives a contradiction. The proof for $\hat{y}' - \hat{y}$ is similar. □

2.6 Digression 2

In this section, we will explore another way to define the equivalence classes P_i $(i \geq 0)$ on $\mathscr{O}$, through the properties of the subspaces of S, inspired by statements (ii) and (iii) in Proposition 2.5.5. Recall the definition of equivalence classes defined through $Ker^{\perp}\ L_{\mathscr{O}}$ is that $y \to y'$ and $\tilde{y} \to \tilde{y}'$ belong to the same equivalence class if there exists $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$. To find the definition of equivalence classes through the properties of the subspaces of S, we can use the following approach.

First note that $L_{\mathscr{O}} : \mathbb{R}^{\mathscr{O}} \to S$, where $Im\ L_{\mathscr{O}} = S$. Therefore, the transpose of map $L_{\mathscr{O}}$, $L_{\mathscr{O}}^T : S \to \mathbb{R}^{\mathscr{O}}$, can be defined as follows: for $x \in S$, $L_{\mathscr{O}}^T x = \sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'}$. Note that $Ker^{\perp}\ L_{\mathscr{O}} = Im\ L_{\mathscr{O}}^T$. We have the following two lemmas:

Lemma 2.6.1. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, suppose $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are two distinct reactions in the orientation $\mathscr{O}$. Denote $S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}} =$ span $\{p' - p : p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}\}$. The following statements are equivalent:*

(i) There exists $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

(ii) There exists $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L_{\mathscr{O}}^T$.

(iii) There exists $x \in S\backslash\{0\}$ *and* $\alpha \neq 0$ *such that* $L^T_{\mathscr{O}} x = \sum_{p \to p' \in \mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = \omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L^T_{\mathscr{O}}$.

(iv) There exists $x \in S\backslash\{0\}$ *such that for each* $p \to p' \in \mathscr{O}$, $x\cdot(p'-p) = 0$ *if and only if* $p \to p' \in \mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}$.

(v) $y' - y \notin S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$ *and* $\tilde{y}' - \tilde{y} \notin S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$.

PROOF: Note that since $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are two distinct equations in $\mathscr{O}$, $\omega_{y\to y'}$ and $\omega_{\tilde{y}\to\tilde{y}'}$ are independent.

The equivalence of (i) and (ii) are trivial as $Ker^{\perp}\ L_{\mathscr{O}} = Im\ L^T_{\mathscr{O}}$.

(ii)⇒ (iii): Suppose that there exists $\alpha \neq 0$, such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L^T_{\mathscr{O}}$. Then by the definition of $Im\ L^T_{\mathscr{O}}$, there exists $x \in S$ such that $L^T_{\mathscr{O}} x = \sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = \omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L^T_{\mathscr{O}}$. Note that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \neq 0$ and $L^T_{\mathscr{O}}(0) = 0$, therefore, we claim $x \in S\backslash\{0\}$.

The other direction (iii)⇒ (ii) is trivial.

(iii)⇒ (iv): Suppose (iii) holds, then there exists $x \in S\backslash\{0\}$ and $\alpha \neq 0$, such that $\sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = \omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'}$. Then we can see that $x\cdot(y'-y) = 1 \neq 0$, $x\cdot(\tilde{y}'-\tilde{y}) = -\alpha \neq 0$ and for each $p \to p' \in \mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}$, $x\cdot(p'-p) = 0$.

(iv)⇒ (iii): Suppose that (iv) holds. Let $a = x\cdot(y'-y)) \neq 0$, $b = x\cdot(\tilde{y}'-\tilde{y}) \neq 0$. Note that for each $p \to p' \in \mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}$, $x\cdot(p'-p) = 0$. Therefore, $L^T_{\mathscr{O}}(x) = \sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = a\omega_{y\to y'} - b\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L^T_{\mathscr{O}}$. Since $a, b \neq 0$, there exists $\alpha = b/a \neq 0$, such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Im\ L^T_{\mathscr{O}}$.

The equivalence of (iv) and (v) is slightly complicated. We will show the proof in both directions.

(iv)⇒(v): Suppose (iv) holds, i.e., there exists $x \in S\backslash\{0\}$, such that $x\cdot(y'-y) \neq 0$, $x\cdot(\tilde{y}'-\tilde{y}) \neq 0$ and $x\cdot(p'-p) = 0$ for any $p \to p' \in \mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}$. Therefore $x \perp S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$, $y' - y \notin S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$ and $\tilde{y}' - \tilde{y} \notin S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$.

(v)⇒(iv): Suppose (v) holds. Then $S_{\mathscr{O}\backslash\{y\to y', \tilde{y}\to\tilde{y}'\}}$ is a proper subset of S. Note that

dim $S-2 \leq$ dim $S_{\mathscr{O}\backslash\{y\to y',\tilde{y}\to\tilde{y}'\}} \leq$ dim S. Therefore dim $S-2 \leq$ dim $S_{\mathscr{O}\backslash\{y\to y',\tilde{y}\to\tilde{y}'\}} \leq$ dim $S-1$.

Denote the subspace $S_{\mathscr{O}\backslash\{y\to y',\tilde{y}\to\tilde{y}'\}}$ by X. Denote the subspace $S^{\perp}_{\mathscr{O}\backslash\{y\to y',\tilde{y}\to\tilde{y}'\}} \cap S$ by Y. Then $X \perp Y$, and $S = X \oplus Y$, where $\oplus$ denotes a direct sum. Therefore, dim $Y =$ dim $S-$ dim X. Since dim $S-2 \leq$ dim $X \leq$ dim $S-1$, we have $1 \leq$ dim $Y \leq 2$.

Note that $S = X \oplus Y$. For any $u \in S$, there exists a unique pair $u_X \in X$ and $u_Y \in Y$ such that $u = u_X + u_Y$. Denote $y' - y$ as v and $\tilde{y}' - \tilde{y}$ as $\tilde{v}$. Note that $y' - y, \tilde{y} \to \tilde{y} \notin X$. Therefore, we can write $y' - y = v = v_X + v_Y$, where $v_X \in X$ and $v_Y \in Y\backslash\{0\}$. We can write $\tilde{y}' - \tilde{y} = \tilde{v} = \tilde{v}_X + \tilde{v}_Y$, where $\tilde{v}_X \in X$ and $\tilde{v}_Y \in Y\backslash\{0\}$.

Take $\tilde{x} \in Y\backslash\{0\} \subseteq S\backslash\{0\}$, then $\tilde{x} \perp X$. For any $p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}$, we have $p' - p \in X$, therefore $\tilde{x} \cdot (p' - p) = 0$. Moreover, $\tilde{x} \cdot v_X = 0$ and $\tilde{x} \cdot \tilde{v}_X = 0$, as $v_X, \tilde{v}_X \in X$.

Therefore, $\tilde{x} \cdot (y' - y) = \tilde{x} \cdot v = \tilde{x} \cdot (v_X + v_Y) = \tilde{x} \cdot v_X + \tilde{x} \cdot v_Y = \tilde{x} \cdot v_Y$. Thus $\tilde{x} \cdot (y' - y) = \tilde{x} \cdot v_Y$. Similarly, $\tilde{x} \cdot (\tilde{y}' - \tilde{y}) = \tilde{x} \cdot \tilde{v}_Y$. Note that $\tilde{x}, v_Y, \tilde{v}_Y \neq 0$. We will proceed in two situations based on whether $v_Y \cdot \tilde{v}_Y$ is equal to zero or not.

Suppose that $v_Y \perp \tilde{v}_Y$ (which is possible when dim $Y = 2$), i.e., $v_Y \cdot \tilde{v}_Y = 0$. Then we pick $x = v_Y + \tilde{v}_Y \in Y\backslash\{0\}$ so that $x \cdot (y' - y) = x \cdot v_Y = (v_Y + \tilde{v}_Y) \cdot v_Y = |v_Y|^2 \neq 0$, and $x \cdot (\tilde{y}' - \tilde{y}) = x \cdot \tilde{v}_Y = (v_Y + \tilde{v}_Y) \cdot \tilde{v}_Y = |\tilde{v}_Y|^2 \neq 0$. Note that $x = v_Y + \tilde{v}_Y \in Y\backslash\{0\}$, then $x \cdot (p' - p) = 0$, for any $p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}$.

Suppose that v_Y is not perpendicular to $\tilde{v}_Y$, i.e., $v_Y \cdot \tilde{v}_Y \neq 0$. Then we pick $x = v_Y \in Y\backslash\{0\}$ so that $x \cdot (y' - y) = x \cdot v_Y = |v_Y|^2 \neq 0$, and $x \cdot (\tilde{y}' - \tilde{y}) = x \cdot \tilde{v}_Y = v_Y \cdot \tilde{v}_Y \neq 0$. Note that $x = v_Y \in Y\backslash\{0\}$, then $x \cdot (p' - p) = 0$, for any $p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}$.

Therefore, we can always find $x \in Y\backslash\{0\} \subseteq S\backslash\{0\}$ such that $x \cdot (y' - y) \neq 0$ and $x \cdot (\tilde{y}' - \tilde{y}) \neq 0$, and for each $p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}$, $x \cdot (p' - p) = 0$. Therefore we have shown that there exists $x \in Y\backslash\{0\} \subseteq S\backslash\{0\}$, such that for each $p \to p' \in \mathscr{O}$, $x \cdot (p' - p) = 0$ if and only if $p \to p' \in \mathscr{O}\backslash\{y \to y', \tilde{y} \to \tilde{y}'\}$. $\square$

Lemma 2.6.2. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, and a given orientation $\mathscr{O}$, suppose $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are two distinct reactions in the orientation $\mathscr{O}$. Denote $S_{\mathscr{O}\backslash\{y\to y'\}} =$ span $\{p' - p : p \to p' \in \mathscr{O}\backslash\{y \to y'\}\}$. Then $\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$ if and only if $y' - y \notin S_{\mathscr{O}\backslash\{y\to y'\}}$.*

PROOF: $\Rightarrow$): Suppose that $\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$. Then $\omega_{y\to y'} \in Im\ L_{\mathscr{O}}^T$. There exists $x \in S\backslash\{0\}$, such that $L_{\mathscr{O}}^T x = \sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = \omega_{y\to y'}$. Then for $p \to p' \in \mathscr{O}$, $x \cdot (p' - p) = 0$ if and only if $p \to p' \in \mathscr{O}\backslash\{y \to y'\}$. Then $x \perp S_{\mathscr{O}\backslash\{y\to y'\}}$ and $y' - y \notin S_{\mathscr{O}\backslash\{y\to y'\}}$.

$\Leftarrow$): Suppose that $y' - y \notin S_{\mathscr{O}\backslash\{y\to y'\}}$, then dim $S_{\mathscr{O}\backslash\{y\to y'\}}$ = dim $S - 1$. Let $X = S_{\mathscr{O}\backslash\{y\to y'\}}$, and $Y = S^{\perp}_{\mathscr{O}\backslash\{y\to y'\}} \cap S$. Then $S = X \oplus Y$, and dim $Y = 1$. Let $x \in Y\backslash\{0\}$. For any $p \to p' \in \mathscr{O}\backslash\{y \to y'\}$, $p' - p \in S_{\mathscr{O}\backslash\{y\to y'\}} = X$, then $x \cdot (p' - p) = 0$.

For any $u \in S$, there exists a unique pair $u_X \in X$ and $u_Y \in Y$ such that $u = u_X + u_Y$. Since $y' - y \notin X$, there exists a unique pair $v_X \in X$ and $v_Y \in Y\backslash\{0\}$ such that $y' - y = v_X + v_Y$. Then $x \cdot (y' - y) = x \cdot (v_X + v_Y) = x \cdot v_X + x \cdot v_Y = x \cdot v_Y$. Note that dim $Y = 1$, and $x, v_Y \in Y\backslash\{0\}$, therefore $x \cdot v_Y \neq 0$, i.e., $x \cdot (y' - y) \neq 0$.

We have found that for any $x \in Y\backslash\{0\} \subseteq S\backslash\{0\}$, for $p \to p' \in \mathscr{O}$, $x \cdot (p' - p) = 0$ if and only if $p \to p' \in \mathscr{O}\backslash\{y \to y'\}$. Therefore, $L_{\mathscr{O}}^T x = \sum_{p\to p'\in\mathscr{O}} ((p'-p)\cdot x)\omega_{p\to p'} = \alpha_x \omega_{y\to y'}$ for some $\alpha_x \neq 0$. Then $\omega_{y\to y'} \in Im\ L_{\mathscr{O}}^T = Ker^{\perp} L_{\mathscr{O}}$. □

Recall our definition for an equivalence class: $y \to y'$ and $\tilde{y} \to \tilde{y}'$ to lie in the same equivalence class of $\mathscr{O}$ if and only if there exists $\alpha \neq 0$, such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp} L_{\mathscr{O}}$. In addition, $y \to y'$ lies in the zeroth equivalence class P_0 if and only if $\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$.

Therefore, from Lemmas 2.6.1 and 2.6.2, we can equivalently define $y \to y'$ and $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class of $\mathscr{O}$, if and only if one of the following holds:

(i) They are the same reaction.

(ii) They are distinct reactions and we have $y' - y \notin S_{\mathscr{O}\setminus\{y\to y',\tilde{y}\to\tilde{y}'\}}$ and $\tilde{y}' - \tilde{y} \notin S_{\mathscr{O}\setminus\{y\to y',\tilde{y}\to\tilde{y}'\}}$.

In addition, $y \to y'$ lies in the zeroth equivalence class P_0 if and only if $y' - y \notin S_{\mathscr{O}\setminus\{y\to y'\}}$.

2.7 Fundamental Classes

Next, we will look into the conditions in terms of the partitions on $\mathscr{O}$ in Question 4 so we can rewrite Question 4 in terms of fewer parameters.

Let us define ***fundamental classes*** on $\mathscr{R}$. We say that $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are in the same fundamental class if one of the following holds:

(i) They are the same reaction.

(ii) They are reactions of a reversible pair.

(iii) Either $y \to y'$ or $y' \to y$ and either $\tilde{y} \to \tilde{y}'$ or $\tilde{y}' \to \tilde{y}$ are in the same "$\sim$" equivalence class on $\mathscr{O}$.

$\mathscr{R}$ can then be partitioned into fundamental classes. Obviously each equivalence class P_i $(i \geq 0)$ on $\mathscr{O}$ will be contained in precisely one fundamental class. Each fundamental class will contain an equivalence class on $\mathscr{O}$. We name the fundamental class containing P_i as C_i.

We then define fundamental subnetwork $\mathscr{N}_i$ as the subnetwork formed by reactions from each C_i, $i = 0, ..., w$. This amounts to a partition of the original reaction network into subnetworks; each reaction of $\mathscr{R}$ lies in precisely one such subnetwork.

Let us define a fundamental class as a ***reversible fundamental class*** if the reactions in the fundamental class are all reversible. Otherwise, we say it is a ***nonreversible fundamental class***.

If we look at the subnetwork as if it is an independent reaction network, we can define for this reaction network its linkage classes, strong linkage classes, terminal strong linkage

classes, nonterminal strong linkage classes, and deficiency, etc. A linkage class of one of these subnetworks is a called a ***colinkage set*** for the reaction network. A ***strong colinkage set*** and a ***terminal strong colinkage set*** can be defined in a similar way.

It seems that equivalence classes P_i's depend on the orientation $\mathscr{O}$, and fundamental classes C_i's depend on P_i's and therefore $\mathscr{O}$. However, the fundamental classes does not rely on the orientation, i.e., C_i's are always the same no matter how the orientation is picked.

In other words, we have the following proposition.

Proposition 2.7.1. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, an orientation $\mathscr{O}$ is given and fundamental classes C_i $(0 \leq i \leq w)$ are defined. If $y \to y'$ belongs to the zeroth fundamental class C_0 under an orientation $\mathscr{O}$, then it will belong to C_0 under any orientation. If $y \to y'$ and $\tilde{y} \to \tilde{y}'$ belong to the same fundamental class under an orientation $\mathscr{O}$, then they will belong to the same fundamental class under any orientation.*

PROOF: For the given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, and two orientations $\mathscr{O}_1$ and $\mathscr{O}_2$, we want to consider several cases:

(I) We want to show that if $y \to y' \in P_0^{\mathscr{O}_1}$, then $y \to y'$ (or its reverse if it exists) lies in $P_0^{\mathscr{O}_2}$ and vice versa. So if $y \to y'$ belongs to the zeroth fundamental class C_0 under an orientation $\mathscr{O}$, then it will belong to C_0 under any orientation.

First let us show that if $y \to y' \in P_0^{\mathscr{O}_1}$, then $y \to y'$ (or its reverse if it exists) lies in $P_0^{\mathscr{O}_2}$.

To see that, first note that

$$\begin{aligned} y \to y' \in P_0^{\mathscr{O}_1} \qquad & \text{if and only if } \omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}} \\ & \text{if and only if for any } x^{\mathscr{O}_1} \in Ker\, L_{\mathscr{O}_1}, x^{\mathscr{O}_1}_{y\to y'} = 0. \end{aligned}$$

Let $x^{\mathscr{O}_2} \in Ker\, L_{\mathscr{O}_2}$, we can find $x^{\mathscr{O}_1} = G(x^{\mathscr{O}_2}) \in Ker\, L_{\mathscr{O}_1}$, where G is defined in Lemma 2.3.4. We have the following two possibilities: $y \to y' \in \mathscr{O}_2$ or $y' \to y \in \mathscr{O}_2$.

(i) $y \to y' \in \mathscr{O}_2$. In this case, $x^{\mathscr{O}_2}_{y\to y'} = x^{\mathscr{O}_1}_{y\to y'}$ and we have that

$$x^{\mathscr{O}_2}_{y\to y'} = x^{\mathscr{O}_1}_{y\to y'} = 0. \tag{2.7.1}$$

From the arbitrary choice of $x^{\mathscr{O}_2}$, we have $\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}_2}$.

(ii) $y' \to y \in \mathscr{O}_2$. In this case, $x^{\mathscr{O}_2}_{y'\to y} = -x^{\mathscr{O}_1}_{y\to y'}$ and we have that

$$x^{\mathscr{O}_2}_{y'\to y} = -x^{\mathscr{O}_1}_{y\to y'} = 0. \tag{2.7.2}$$

From the arbitrary choice of $x^{\mathscr{O}_2}$, we have $\omega_{y'\to y} \in Ker^{\perp}\ L_{\mathscr{O}_2}$.

For the other direction, we only need to switch the position of $\mathscr{O}_1$ and $\mathscr{O}_2$, and apply function F (defined also in Lemma 2.3.4) instead of G. The proof is parallel.

Therefore, we can see that if $y \to y'$ lies in the zeroth equivalence class under one orientation, then $y \to y'$ (or its reverse, depending on which one lies in the given orientation) lies in the zeroth equivalence class under any orientation.

(II) We want to show that if $y \to y'$, $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class under orientation $\mathscr{O}_1$, then $y \to y'$ (or its reverse $y' \to y$ if it exists) and $\tilde{y} \to \tilde{y}'$ (or its reverse $\tilde{y}' \to \tilde{y}$ if it exists) lie in the same equivalence class under $\mathscr{O}_2$ and vice versa. Therefore, if $y \to y'$ and $\tilde{y} \to \tilde{y}'$ belong to the same fundamental class under an orientation $\mathscr{O}$, these two reactions will belong to the same fundamental class under any orientation.

First we will show that if $y \to y'$, $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class under orientation $\mathscr{O}_1$, $y \to y'$ (or its reverse $y' \to y$ if it exists) and $\tilde{y} \to \tilde{y}'$ (or its reverse $\tilde{y}' \to \tilde{y}$ if it exists) lie in the same equivalence class under $\mathscr{O}_2$.

Note that $y \to y'$, $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class under $\mathscr{O}_1$ implies that there exists $\alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} \neq 0$ such that $\omega_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}_1}$. To proceed, let $x^{\mathscr{O}_2} \in Ker\ L_{\mathscr{O}_2}$. Let F and G be maps defined as in Lemma 2.3.4. We can find $x^{\mathscr{O}_1} = G(x^{\mathscr{O}_2}) \in Ker\ L_{\mathscr{O}_1}$. We then have the following four possibilities.

(i) Suppose $y \to y'$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}_1 \cap \mathscr{O}_2$.

Claim 1. *There exists* $\alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} \neq 0$ *such that* $\omega_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}_2}$.

Note that in this case $x^{\mathscr{O}_2}_{y\to y'} = x^{\mathscr{O}_1}_{y\to y'}$ and $x^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$. Since $\omega_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_1}$, we can see that $x^{\mathscr{O}_1}_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} = 0$. In terms of $\mathscr{O}_2$, we have $x^{\mathscr{O}_2}_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = 0$.

Since the choice of $x^{\mathscr{O}_2}$ is arbitrary, we have $\omega_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_2}$, where $\alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$.

(ii) $y \to y' \in \mathscr{O}_1 \cap \mathscr{O}_2$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}_1$ and $\tilde{y}' \to \tilde{y} \in \mathscr{O}_2$.

Claim 2. *There exists* $\alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = -\alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} \neq 0$ *such that* $\omega_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}\omega_{\tilde{y}'\to\tilde{y}} \in Ker^{\perp}\, L_{\mathscr{O}_2}$.

Note that in this case $x^{\mathscr{O}_2}_{y\to y'} = x^{\mathscr{O}_1}_{y\to y'}$ and $x^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = -x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$. Since $\omega_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_1}$, we can see that $x^{\mathscr{O}_1}_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} = 0$. In terms of $\mathscr{O}_2$, we have $x^{\mathscr{O}_2}_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}x^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = 0$.

Since the choice of $x^{\mathscr{O}_2}$ is arbitrary, we have $\omega_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}\omega_{\tilde{y}'\to\tilde{y}} \in Ker^{\perp}\, L_{\mathscr{O}_2}$, where $\alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = -\alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$.

(iii) $y \to y' \in \mathscr{O}_1$ and $y' \to y \in \mathscr{O}_2$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}_1 \cap \mathscr{O}_2$.

Claim 3. *There exists* $\alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = -\alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} \neq 0$ *such that* $\omega_{y\to y'} - \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_2}$.

Note that in this case $x^{\mathscr{O}_2}_{y\to y'} = -x^{\mathscr{O}_1}_{y'\to y}$ and $x^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$. Since $\omega_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_1}$, we can see that $x^{\mathscr{O}_1}_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} = 0$. In terms of $\mathscr{O}_2$, we have $-x^{\mathscr{O}_2}_{y'\to y} + \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = 0$.

Since the choice of $x^{\mathscr{O}_2}$ is arbitrary, we have $\omega_{y'\to y} - \alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_2}$, where $\alpha^{\mathscr{O}_2}_{\tilde{y}\to\tilde{y}'} = -\alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$.

(iv) $y \to y' \in \mathscr{O}_1$, $y' \to y \in \mathscr{O}_2$, $\tilde{y} \to \tilde{y}' \in \mathscr{O}_1$, $\tilde{y}' \to \tilde{y} \in \mathscr{O}_2$.

Claim 4. *There exists* $\alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} \neq 0$ *such that* $\omega_{y'\to y} - \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}\omega_{\tilde{y}'\to\tilde{y}} \in Ker^{\perp}\, L_{\mathscr{O}_2}$.

Note that in this case $x^{\mathscr{O}_2}_{y'\to y} = -x^{\mathscr{O}_1}_{y\to y'}$ and $x^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = -x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$. Since $\omega_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\, L_{\mathscr{O}_1}$, we can see that $x^{\mathscr{O}_1}_{y\to y'} - \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}x^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'} = 0$. In terms of $\mathscr{O}_2$, we have $-x^{\mathscr{O}_2}_{y'\to y} + \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}x^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = 0$.

Since the choice of $x^{\mathscr{O}_2}$ is arbitrary, we have $\omega_{y'\to y} - \alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}}\omega_{\tilde{y}'\to\tilde{y}} \in Ker^{\perp} L_{\mathscr{O}_2}$, where $\alpha^{\mathscr{O}_2}_{\tilde{y}'\to\tilde{y}} = \alpha^{\mathscr{O}_1}_{\tilde{y}\to\tilde{y}'}$.

Next, for the other direction, we only need to switch the position of $\mathscr{O}_1$ and $\mathscr{O}_2$, and apply function F instead of G. The proof is parallel.

Now we can see that if $y \to y'$ and $\tilde{y} \to \tilde{y}'$ lie in the same equivalence class under one orientation, then $y \to y'$ (or its reverse, depending on which one lies in the given orientation) and $\tilde{y} \to \tilde{y}'$ (or its reverse, depending on which one lies in the given orientation) lie in the same equivalence class under any orientation. □

Remark 2.7.2. *We claim that given $\mathscr{O}$, all reactions in P_0 must be reversible for the answer to Question 4 to be yes.*

Note for a given orientation $\mathscr{O}$, if there is an irreversible reaction $y \to y' \in P_0$, then $x_{y\to y'} = 0$, for all $x \in Ker\ L_{\mathscr{O}}$ (see Remark 2.4.1). But if $y \to y'$ is irreversible, then for the answer to Question 4 to be yes, we must have $g_{y\to y'} > 0$. However, if $g \in Ker\ L_{\mathscr{O}}$ and $y \to y' \in P_0$ then $g_{y\to y'} = 0$. Then no such $g \in Ker\ L_{\mathscr{O}}$ satisfying (2.3.2) can exist. We know that if there is an irreversible reaction in P_0 under orientation $\mathscr{O}$, there will be an irreversible reaction in P_0 under any orientation. We conclude that the answer to Question 4 and therefore Question 3, 2 and 1 are no and we are done.

From now on let us assume that for any orientation, if P_0 is not empty, then each reaction in P_0 is reversible (by definition if P_0 is empty, it is reversible).

Remark 2.7.3. *Note if there are two distinct irreversible reactions $y \to y'$ and $\tilde{y} \to \tilde{y}'$ in the same equivalence class P_i $(1 \le i \le w_1)$, then there exists a unique $\alpha^{\mathscr{O}}_{\tilde{y}\to\tilde{y}'} \neq 0$, such that $\omega_{y\to y'} - \alpha^{\mathscr{O}}_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp} L_{\mathscr{O}}$. Since $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are irreversible, we have that there exists $\alpha_{\tilde{y}\to\tilde{y}'} \neq 0$, such that $\omega_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp} L_{\mathscr{O}}$, for any orientation $\mathscr{O}$. Then for any orientation $\mathscr{O}$ and any $x \in Ker\ L_{\mathscr{O}}$, $x_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'}x_{\tilde{y}\to\tilde{y}'} = 0$.*

Since $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are irreversible, then for the answer to Question 4 to be yes,

we have $g \in Ker\ L_{\mathscr{O}}$ *with* $g_{y\to y'} > 0$ *and* $g_{\tilde{y}\to\tilde{y}'} > 0$. *Then* $g_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'} g_{\tilde{y}\to\tilde{y}'} = 0$ *implies* $\alpha_{\tilde{y}\to\tilde{y}'} > 0$.

Therefore, if for any given orientation $\mathscr{O}$ *the* $\alpha_{\tilde{y}\to\tilde{y}'}$ *such that* $\omega_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$ *is negative, then the answer to Question 4 and therefore Question 3, 2 and 1 are no and we are done.*

From now on, let us assume for any given orientation $\mathscr{O}$, *if two reactions* $y \to y'$,$\tilde{y} \to \tilde{y}' \in \tilde{\mathscr{O}}$ $(= \mathscr{O}\backslash P_0)$ *are irreversible and belong to the same equivalence class, then there exists a unique* $\alpha_{\tilde{y}\to\tilde{y}'} > 0$, *such that* $\omega_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

Let us define an equivalence class as a ***reversible equivalence class*** if the corresponding fundamental class is reversible; otherwise we say it is a ***nonreversible equivalence class***. We know this reversibility of an equivalence class is independent of the choice of orientation.

We will next try to reduce the number of parameters used in Question 4. Let us pick one ***representative*** from each equivalence class as follows:

If an equivalence class P_i is nonreversible, pick an irreversible (with respect to $\mathscr{R}$) reaction from P_i as the representative for this equivalence class. Otherwise, we pick any one of the reversible (with respect to $\mathscr{R}$) reactions from P_i as the representative. Note for trivial equivalence classes, the representative is the only reaction that lies in the equivalence class.

Let us assume we have picked the representative, say, $y_i \to y_i'$ for each equivalence class P_i $(0 \le i \le w)$. Define $W = \{y_i \to y_i' : i = 1, ..., w\}$, then $W \subseteq \tilde{\mathscr{O}} = \mathscr{O}\backslash P_0$. Recall that from Remark 2.4.3, for $y \to y', \tilde{y} \to \tilde{y}' \in P_i$ $(1 \le i \le w_1)$, since $\omega_{y\to y'}, \omega_{\tilde{y}\to\tilde{y}'} \notin Ker^{\perp}\ L_{\mathscr{O}}$, there exists a unique $\alpha_{\tilde{y}\to\tilde{y}'} \neq 0$ such that $\omega_{y\to y'} - \alpha_{\tilde{y}\to\tilde{y}'}\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

The next step is to readjust the orientation (if applicable) such that for $i = 1, ..., w$, for any $y \to y' \in P_i$, there exists $\alpha_{y\to y'} > 0$, such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Note

for trivial equivalence classes P_i $(w_1 + 1 \leq i \leq w_1 + w_2)$, this is trivial. We just need to make sure it can be done for the nontrivial equivalence classes P_i $(1 \leq i \leq w_1)$.

Note that if $y \to y' \in P_i$ $(1 \leq i \leq w_1)$ is irreversible, then the representative $y_i \to y_i'$ must be irreversible too. Then by Remark 2.7.3, there exists $\alpha_{y\to y'} > 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$.

The only remaining unhandled case is if $y \to y' \in P_i$ $(1 \leq i \leq w_1)$ (under the orientation $\mathscr{O}$) is reversible, and there exists $\alpha_{y\to y'} < 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$.

To deal with this case, we only need to realign the orientation $\mathscr{O}$ so that for all $y \to y' \in \mathscr{O}$ such that there exists $\alpha_{y\to y'} < 0$ and $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$, we replace $y \to y'$ with $y' \to y$ in the orientation. Therefore, in the new orientation $\mathscr{O}$, there exists $\alpha_{y'\to y} = -\alpha_{y\to y'} > 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y'\to y}\omega_{y'\to y} \in Ker^{\perp} L_{\mathscr{O}}$.

From now on we can assume that the orientation, say $\mathscr{O}$, is such that for $i = 1, ..., w$, and for any $y \to y' \in P_i$, there exists $\alpha_{y\to y'} > 0$, such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$. Suppose that $g, h \in Ker\ L_{\mathscr{O}}$, then for all $y \to y' \in P_i$ $(1 \leq i \leq w_1)$ we have $g \cdot (\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'}) = 0$, or $g_{y_i\to y_i'} - \alpha_{y\to y'} g_{y\to y'} = 0$. Similarly, for all $y \to y' \in P_i$ $(1 \leq i \leq w_1)$, we have $h_{y_i\to y_i'} - \alpha_{y\to y'} h_{y\to y'} = 0$. In particular, $g_{y\to y'}$ $(h_{y\to y'})$ will share the same sign (positive/negative/zero) with $g_{y_i\to y_i'}$ $(h_{y_i\to y_i'})$. Also if, in addition, we assume that $g_{y\to y'} \neq 0$, $\rho_{y\to y'}$ is well defined. In this case, for all $y \to y' \in P_i$ $(i \geq 1)$, we have

$$\rho_{y\to y'} = \frac{h_{y\to y'}}{g_{y\to y'}} = \frac{\alpha_{y\to y'} h_{y\to y'}}{\alpha_{y\to y'} g_{y\to y'}} = \frac{h_{y_i\to y_i'}}{g_{y_i\to y_i'}} = \rho_{y_i\to y_i'}.$$

Therefore, in the conditions of Lemma 2.3.2 which were mentioned in Question 4, if $y \to y' \in P_i$, we could replace $\rho_{y\_y'}$ with $\rho_{y_i\to y_i'}$. Thus, in Lemma 2.3.2 and Question 4, instead of considering $\{\rho_{y\to y'} : g_{y\to y'} \neq 0, y \to y' \in \mathscr{O}\}$ for all reactions in the orientation, we only need to consider $\{\rho_{y_i\to y_i'} : g_{y_i\to y_i'} \neq 0, 1 \leq i \leq w\}$ for the nonzeroth equivalence class representatives.

We will rewrite Lemmas 2.3.2 and 2.3.3 in terms of $\{\rho_{y_i \to y_i'} : g_{y_i \to y_i'} \neq 0, 1 \leq i \leq w\}$ shortly.

Recall that an equivalence class P_i is nonreversible if there exists an irreversible reaction (with respect to $\mathscr{R}$) in P_i, and is otherwise reversible. Recall that we have assumed P_0 is reversible. For all $y \to y' \in P_i$, we can now assume that $g_{y \to y'}$ and $h_{y \to y'}$ are sign compatible with $g_{y_i \to y_i'}$ and $h_{y_i \to y_i'}$, respectively; also, we have $\rho_{y \to y'} = \rho_{y_i \to y_i'}$ if $g_{y_i \to y_i'} \neq 0$. If P_i is nonreversible, then $y_i \to y_i'$ is irreversible, $g_{y_i \to y_i'} > 0$, and $h_{y_i \to y_i'} > 0$. Therefore for every $y \to y' \in P_i$ that is irreversible, we have $g_{y \to y'} > 0$ and $h_{y \to y'} > 0$.

As a result, Lemmas 2.3.2 and 2.3.3 can be rewritten as the following two lemmas, respectively.

Lemma 2.7.4. *Suppose that, for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$ are given. Let g, h be defined as in (2.3.2) and (2.3.3), let P_i $(0 \leq i \leq w)$ be the equivalence class defined with representative $y_i \to y_i'$, and define the set $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$. Then the following conditions hold:*

I) If $y \to y' \in P_i$ $(0 \leq i \leq w)$ is irreversible, then $g_{y \to y'} > 0$, $h_{y \to y'} > 0$, and $\rho_{y_i \to y_i'} = e^{y \cdot \mu}$.

II) If $y \to y' \in P_i$ $(0 \leq i \leq w)$ is reversible, then the following holds:

i) If $g_{y_i \to y_i'} > 0$, then either $\rho_{y_i \to y_i'} > e^{y \cdot \mu} > e^{y' \cdot \mu}$, $\rho_{y_i \to y_i'} < e^{y \cdot \mu} < e^{y' \cdot \mu}$, or $\rho_{y_i \to y_i'} = e^{y \cdot \mu} = e^{y' \cdot \mu}$.

ii) If $g_{y_i \to y_i'} < 0$, then either $\rho_{y_i \to y_i'} > e^{y' \cdot \mu} > e^{y \cdot \mu}$, $\rho_{y_i \to y_i'} < e^{y' \cdot \mu} < e^{y \cdot \mu}$, or $\rho_{y_i \to y_i'} = e^{y \cdot \mu} = e^{y' \cdot \mu}$.

iii) If $g_{y_i \to y_i'} = 0$ and $h_{y_i \to y_i'} > 0$, then $e^{y \cdot \mu} > e^{y' \cdot \mu}$.

iv) If $g_{y_i \to y_i'} = 0$ and $h_{y_i \to y_i'} < 0$, then $e^{y \cdot \mu} < e^{y' \cdot \mu}$.

v) If $g_{y_i \to y_i'} = 0$ and $h_{y_i \to y_i'} = 0$, then $e^{y \cdot \mu} = e^{y' \cdot \mu}$.

Lemma 2.7.5. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, suppose that (i) $\mu \in \mathbb{R}^{\mathscr{S}}$, (ii) a pair g, $h \in Ker\ L_{\mathscr{O}}$, (iii) P_i $(0 \leq i \leq w)$ which is an equivalence class with representative $y_i \to y_i'$, and (iv) a set $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$, together satisfy the conditions in Lemma 2.7.4. Then there exists $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ satisfying (2.3.2) and (2.3.3).*

As a result, we will have the following lemma, where given an orientation $\mathscr{O}$, $y_i \to y_i'$ is the representative for each P_i $(i = 0, .., w)$, and $W = \{y_i \to y_i'\}_{i=1}^{w}$.

Lemma 2.7.6. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, the orientation $\mathscr{O}$ and the P_i $(0 \leq i \leq w)$ representatives $\{y_i \to y_i' : i = 0, ..., w\}$ we have chosen, Question 4 can be rewritten in terms of g, h, μ, and $\rho_{y_i \to y_i'}$'s as follows:*

Question 5. *For the given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and the given orientation $\mathscr{O}$, do there exist*

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign compatible with the stoichiometric subspace S,

(ii) a pair g, $h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors, and

(iii) a set $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, 1 \leq i \leq w\}$ where $y_i \to y_i'$ is the representative for P_i,

which together satisfy the conditions in Lemma 2.7.4?

2.8 Sign of Equivalence Class and Shelf Assignments

Note that the conditions in Lemma 2.7.4 are in the format of comparisons among $e^{y \cdot \mu}$, $e^{y' \cdot \mu}$ and $\rho_{y_i \to y_i'}$ $(g_{y_i \to y_i'} \neq 0)$. We will later introduce a helpful analogy stated in terms of w (or fewer) "bookcases", each with upper, middle, and lower shelves, to translate these comparisons among $e^{y \cdot \mu}$, $e^{e^{y' \cdot \mu}}$ and $\rho_{y_i \to y_i'}$ into assignments of the reactions in the same fundamental class into three shelves in a "bookcase."

Suppose that $\mu \in \mathbb{R}^{\mathscr{S}}$, a pair $g, h \in Ker\ L_{\mathscr{O}}$, P_i $(0 \leq i \leq w)$ which is an equivalence class with a representative $y_i \to y_i'$, and a set $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$ satisfy the conditions in Lemma 2.7.4.

Let us define a fundamental class C_i $(0 \leq i \leq w)$ as *degenerate* if $g_{y_i \to y_i'} = 0$. We define a fundamental class C_i $(1 \leq i \leq w)$ as *nondegenerate* if $g_{y_i \to y_i'} \neq 0$.

For each nondegenerate fundamental class C_i $(i \geq 1)$, we assume that we have a 3-shelf bookcase for C_i to store all reactions in C_i. Let us define the ***shelving*** of a reaction in this bookcase. Given $y \to y'$ in a nondegenerate fundamental class C_i $(i \geq 1)$, we assign the reactions to shelves as follows:

i) $y \to y'$ is on the *upper shelf* if $e^{y \cdot \mu} > \rho_{y_i \to y_i'}$.

ii) $y \to y'$ is on the *lower shelf* if $e^{y \cdot \mu} < \rho_{y_i \to y_i'}$.

iii) $y \to y'$ is on the *middle shelf* if $e^{y \cdot \mu} = \rho_{y_i \to y_i'}$.

Proposition 2.8.1. *We are given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with an orientation $\mathscr{O}$. Suppose that $\mu \in \mathbb{R}^{\mathscr{S}}$, a pair g, $h \in Ker\ L_{\mathscr{O}}$, P_i $(0 \leq i \leq w)$ which is an equivalence class with representative $y_i \to y_i'$, and a set $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$ satisfy the conditions in Lemma 2.7.4. Every shelving of reactions in a nondegenerate fundamental class C_i satisfies the following conditions:*

(i) For any irreversible reaction $y \to y' \in C_i$ $(i \geq 1)$, $y \to y'$ must be on the middle shelf.

(ii) If $\rho_{y_i \to y_i'} \leq 0$, then $y \to y' \in C_i$ must be on the upper shelf.

(iii) Both reactions of a reversible pair have to be on the same shelf.

(iv) Any two reactions in the same fundamental class C_i sharing the same reactant complex must lie on the same shelf.

(v) All reactions whose reactant complex lies in a non-terminal strong linkage class must be placed on the middle shelf.

(vi) All reactions whose reactant complex lies in a terminal strong linkage class of the fundamental subnetwork must be put on the same shelf.

(vii) If for a nondegenerate fundamental class C_i $(i \geq 1)$, $\mathscr{N}_i$ forms a big (undirected) cycle (a cycle with at least three vertices), then its reactions are all in a terminal strong linkage class (after the successful realignment of the orientation) and on the middle shelf.

PROOF: (i) If the fundamental class C_i $(i \geq 1)$ is nonreversible, then $\rho_{y_i \to y_i'} > 0$ since $\rho_{y_i \to y_i'} = e^{y_i \cdot \mu}$. For any irreversible reaction $y \to y' \in C_i$ $(i \geq 1)$, $y \to y'$ must be put on the middle shelf since $\rho_{y_i \to y_i'} = e^{y_i \cdot \mu} = e^{y \cdot \mu}$.

(ii) If in a nondegenerate fundamental class, $\rho_{y_i \to y_i'} \leq 0$, then any $y \to y' \in C_i$ can only be put on the upper shelf as $e^{y \cdot \mu} > 0 \geq \rho_{y_i \to y_i'}$, i.e. $e^{y \cdot \mu} > \rho_{y_i \to y_i'}$.

(iii) For two reversible reactions $y \to y'$ and $y' \to y$, note that among the conditions in Lemma 2.7.4, for reversible reactions we have

(a) $e^{y \cdot \mu} > \rho_{y_i \to y_i'}$ if and only if $e^{y' \cdot \mu} > \rho_{y_i \to y_i'}$,

(b) $e^{y \cdot \mu} < \rho_{y_i \to y_i'}$ if and only if $e^{y' \cdot \mu} < \rho_{y_i \to y_i'}$, and

(c) $e^{y \cdot \mu} = \rho_{y_i \to y_i'}$ if and only if $e^{y' \cdot \mu} = \rho_{y_i \to y_i'}$.

Therefore, both reactions of a reversible pair have to be on the same shelf of the fundamental class's bookcase.

(iv) Note that if two reactions in the same fundamental class C_i share the same reactant complex, then the reactions must lie on the same shelf. Because only one of the relationships $e^{y \cdot \mu} > \rho_{y_i \to y_i'}$, $e^{y \cdot \mu} = \rho_{y_i \to y_i'}$, $e^{y \cdot \mu} < \rho_{y_i \to y_i'}$ can be true at a time.

To prove (v) and (vi), let us first prove the following:

All reactions whose reactant complex lies in a strong linkage class of a fundamental subnetwork must be placed on the same shelf.

We will prove the statement above from two cases.

Case one: All the reactions for which the reactant complex lies in the strong linkage class of the fundamental subnetwork are reversible.

In this case, take any two reactions whose reactant complex lies in the strong linkage class, $y \to y'$ and $\tilde{y} \to \tilde{y}'$. If these two reactions are the same reaction or a reversible pair, then they must be put on the same shelf of the fundamental class.

Suppose they are not the same reaction or a reversible pair. Then since y and $\tilde{y}$ both lie in the strong linkage class, there exists a path from y to $\tilde{y}$, say $y \to y_1 \to y_2 \to ...y_{n_y} \to \tilde{y}$. Since $y \to y'$ and $y \to y_1$ (could be the same reaction) share the same reactant complex, they must be put on the same shelf of the fundamental class. Similarly, since $y_1 \to y$ and $y_1 \to y_2$ share the same reactant complex, they must be put on the same shelf of the fundamental class. Therefore, $y \to y'$ and $y_1 \to y_2$ must be put on the same shelf of the fundamental class's bookcase.

Similarly, we can show that $y \to y'$ and $y_{n_y} \to \tilde{y}$ must be put on the same shelf of the fundamental class. However, since $\tilde{y} \to y_{n_y}$ and $\tilde{y} \to \tilde{y}'$ (could be the same reaction) share the same reactant complex, they must be put on the same shelf of the fundamental class. Hence $y \to y'$ and $\tilde{y} \to \tilde{y}'$ must be put on the same shelf of the fundamental class.

Since $y \to y'$ and $\tilde{y} \to \tilde{y}'$ are randomly selected, we claim that all reactions whose reactant complex lies in the strong linkage class of the fundamental class must be put on the same shelf of the fundamental class.

Case two: There exists an irreversible reaction $\tilde{y} \to \tilde{y}'$ whose reactant complex lies in the strong linkage class of the fundamental subnetwork.

In this case, take $\tilde{y} \to \tilde{y}'$ and pick another reaction $y \to y'$ whose reactant complex lies in the strong linkage class. Then there exists a path from y to $\tilde{y}$, say $y \to y_1 \to y_2 \to ...y_{n_y} \to \tilde{y}$. Note that $\tilde{y} \to \tilde{y}'$ must be put on the middle shelf. If $y_{n_y} \to \tilde{y}$ is irreversible, $y_{n_y} \to \tilde{y}$ must be put on the middle shelf. If $y_{n_y} \to \tilde{y}$ is not irreversible, $\tilde{y} \to y_{n_y}$ and $\tilde{y} \to \tilde{y}'$ share the same reactant complex which lies in the strong linkage class. Thus $\tilde{y} \to y_{n_y}$ and $\tilde{y} \to \tilde{y}'$ must be put on the same shelf, i.e., the middle shelf. Either way $y_{n_y} \to \tilde{y}$ must be put on the middle shelf of the fundamental class's bookcase.

Similarly, we can show that if $y_{n_y-1} \to y_{n_y}$ is irreversible, then it must be put on the middle shelf; otherwise, $y_{n_y} \to y_{n_y-1}$ and $y_{n-y} \to \tilde{y}$ must be put on the same middle shelf. Therefore, in either way $y_{n_y-1} \to y_{n_y}$ must be put on the middle shelf. Thus following this approach, we can show that $y \to y_1$ must be put on the middle shelf. Since $y \to y'$ and $y \to y_1$ share the same reactant complex, they must be put on the same shelf of the fundamental class's bookcase. Therefore, $y \to y'$ must be put on the same middle shelf as $\tilde{y} \to \tilde{y}'$.

Since $y \to y'$ is arbitrarily selected, we can claim that if there exists an irreversible reaction whose reactant complex lies in the strong linkage class of the fundamental subnetwork, then all reactions whose reactant complex lies in the strong linkage class of the fundamental subnetwork must be put on the same middle shelf of the fundamental class's bookcase.

(v) To show this, note that in a non-terminal strong linkage class, there exists a complex which reacts to some other complex not in the non-terminal strong linkage class. We know the reaction between these two complex is irreversible. Suppose not, then these two complexes will be in the same strong linkage class, which is a contradiction. Therefore, there exists an irreversible reaction whose reactant complex lies in the strong linkage class but the product complex does not. We can claim from case two that all reactions whose reactant complex lies in a non-terminal strong linkage class of the fundamental subnetwork must be put on the middle shelf of the fundamental class's bookcase.

(vi) As for a terminal strong linkage class, since both case one and case two are possible, we claim that all reactions whose reactant complex lies in terminal strong linkage class of the fundamental subnetwork must be put on the same shelf of the fundamental class's bookcase.

(vii) We suppose that the fundamental class C_i $(i \geq 1)$ is nondegenerate and $\mathscr{N}_i$ forms

a big (undirected) cycle (with at least three vertices). We will show that $\tilde{\mathscr{N}_i}$ forms a direct cycle for the orientation we chose (after successful realignment of the orientation).

Note that $\tilde{\mathscr{N}_i}$ forms a big (undirected) cycle, then from Remark 2.5.3 and Proposition 2.5.4, the reaction vectors for $\tilde{\mathscr{N}_i}$ are minimally dependent. In fact, the vector space formed from the projection of $Ker\ L_{\mathscr{O}}$ on P_i has dimension one, and the components of each vector from this space on P_i all have the same absolute value. After successful realignment of the orientation, we assumed that there exists $\alpha_{y\to y'} > 0$ for all $y \to y' \in P_i$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$. Let $x \in Ker\ L_{\mathscr{O}}$, then for all $y \to y' \in P_i$, we have $x_{y_i\to y_i'} = \alpha_{y\to y'}x_{y\to y'}$. Therefore, for all $y \to y' \in P_i$, all $x_{y\to y'}$'s have the same sign and same absolute value, i.e. all values of $x_{y_\to y'}$'s are equal. We then conclude that given the orientation, $\tilde{\mathscr{N}_i}$ forms a directed cycle.

Therefore, we claim that if $\mathscr{N}_i$ $(i \geq 1)$ forms a big cycle, then under our assumptions about the orientation, the subnetwork is a terminal strong linkage class.

Now we want to show that for such $\mathscr{N}_i$, all reactions in the terminal strong linkage class must be put on the same middle shelf of the fundamental class C_i. To see that, suppose that reactions are put all on the lower shelf or all on the upper shelf.

Note that for every $y \to y' \in \tilde{\mathscr{N}_i}$, there is a cycle $y \to y' \to ... \to y$ in $\tilde{\mathscr{N}_i}$. Recall the conditions listed in Lemma 2.7.4. Suppose that $g_{y_i\to y_i'} > 0$ and all reactions are put on the upper shelf, then we have for all $y \to y' \in P_i$, $\rho_{y\to y'} < e^{y\cdot\mu} < e^{y'\cdot\mu}$, in particular $y \cdot \mu < y' \cdot \mu$. Therefore, working through all reactions in the reaction cycle $y \to y' \to ... \to y$, we will have $y \cdot \mu < y' \cdot \mu < ... < y \cdot \mu$, which is a contradiction. In general, we have two signs of $g_{y_i\to y_i'}$ ($g_{y_i\to y_i'} > 0$ or $g_{y_i\to y_i'} < 0$), and two shelving assignments (the lower or upper shelf). We can show that in either the lower or upper shelf case, we will get $y \cdot \mu < y' \cdot \mu < ... < y \cdot \mu$ or $y \cdot \mu > y' \cdot \mu > ... > y \cdot \mu$ depending on the sign of $g_{y_i\to y_i'}$, which is a contradiction in either case. Therefore, all reactions in a terminal strong linkage class are on the middle shelf of the nondegenerate fundamental class C_i. □

We can rewrite Lemmas 2.7.4 and 2.7.5 in terms of the shelving conditions for the fundamental class C_i.

Lemma 2.8.2. *Suppose that, for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the given orientation $\mathscr{O}$, $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$ are given. Let g, h be defined as in (2.3.2) and (2.3.3), let P_i $(0 \leq i \leq w)$ be the equivalence class defined earlier with the representative $y_i \to y_i'$, and let $\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}}$ be defined for nondegenerate fundamental classes C_i. Then the following conditions hold:*

I) If $y \to y' \in P_i$ $(i \geq 1)$ is irreversible, then $g_{y \to y'} > 0$, $h_{y \to y'} > 0$, and $\rho_{y_i \to y_i'} = e^{y \cdot \mu}$.

II) If $y \to y' \in P_i$ $(i \geq 1)$ is reversible and C_i is nondegenerate, then

i) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the upper shelf, then $\rho_{y_i \to y_i'} < e^{y \cdot \mu} < e^{y' \cdot \mu}$.

ii) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the lower shelf, then $\rho_{y_i \to y_i'} > e^{y \cdot \mu} > e^{y' \cdot \mu}$.

iii) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the middle shelf, then $\rho_{y_i \to y_i'} = e^{y \cdot \mu} = e^{y' \cdot \mu}$.

iv) If $g_{y_i \to y_i'} < 0$, and $y \to y'$ is on the upper shelf, then $\rho_{y_i \to y_i'} < e^{y' \cdot \mu} < e^{y \cdot \mu}$.

v) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the lower shelf, then $\rho_{y_i \to y_i'} > e^{y' \cdot \mu} > e^{y \cdot \mu}$.

vi) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the middle shelf, then $\rho_{y_i \to y_i'} = e^{y \cdot \mu} = e^{y' \cdot \mu}$.

III) If $y \to y' \in P_i$ $(i \geq 1)$ is reversible and C_i is degenerate, then

i) If $h_{y_i \to y_i'} > 0$, then $e^{y \cdot \mu} > e^{y' \cdot \mu}$.

ii) If $h_{y_i \to y_i'} < 0$, then $e^{y \cdot \mu} < e^{y' \cdot \mu}$.

iii) If $h_{y_i \to y_i'} = 0$, then $e^{y \cdot \mu} = e^{y' \cdot \mu}$.

IV) If $y \to y' \in P_0$ (is reversible), then $e^{y \cdot \mu} = e^{y' \cdot \mu}$.

Lemma 2.8.3. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and a given orientation $\mathscr{O}$, suppose that (i) $\mu \in \mathbb{R}^{\mathscr{S}}$, (ii) a pair g, $h \in Ker\ L_{\mathscr{O}}$, (iii) P_i $(0 \leq i \leq w)$ which is an equivalence class*

with representative $y_i \to y_i'$, and (iv) a set $\{\rho_{y_i\to y_i'} = \dfrac{h_{y_i\to y_i'}}{g_{y_i\to y_i'}} : g_{y_i\to y_i'} \neq 0, i = 1, ..., w\}$ defined for nondegenerate fundamental classes, together satisfy the conditions in Lemma 2.8.2. Then there exists $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ satisfying (2.3.2) and (2.3.3).

As a result, we will have the following lemma:

Lemma 2.8.4. *Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the orientation $\mathscr{O}$ and the P_i ($0 \leq i \leq w$) representatives $\{y_i \to y_i' : i = 0, ..., w\}$ we have chosen, Question 5 can be rewritten in terms of g, h, μ, and $\rho_{y_i\to y_i'}$'s as follows:*

Question 6. *For the given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the given orientation $\mathscr{O}$, do there exist*

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign compatible with the stoichiometric subspace S,

(ii) a pair g, $h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors,

(iii) a set $\{\rho_{y_i\to y_i'} = \dfrac{h_{y_i\to y_i'}}{g_{y_i\to y_i'}} : g_{y_i\to y_i'} \neq 0\}$, and

(iv) a choice of shelving assignments for each nondegenerate fundamental class that satisfies the conditions in Proposition 2.8.1,

which together satisfy the conditions in Lemma 2.8.2?

Remark 2.8.5. *Note that from equations (2.2.2), (2.3.2) and (2.3.3), we have that*

$$g_{y\to y'} = \begin{cases} k_{y\to y'}(c^{**})^y - k_{y'\to y}(c^{**})^{y'}, & \text{if } y \to y' \in \mathscr{O} \text{ is reversible} \\ k_{y\to y'}(c^{**})^y, & \text{if } y \to y' \in \mathscr{O} \text{ is irreversible.} \end{cases} \tag{2.8.1}$$

and

$$h_{y\to y'} = \begin{cases} k_{y\to y'}(c^{*})^y - k_{y'\to y}(c^{*})^{y'}, & \text{if } y \to y' \in \mathscr{O} \text{ is reversible} \\ k_{y\to y'}(c^{*})^y, & \text{if } y \to y' \in \mathscr{O} \text{ is irreversible.} \end{cases} \tag{2.8.2}$$

Note also that the definitions of g, h depend on c^* and c^{**}. There is no specific order between c^* and c^{**}, so if we switch these two concentrations, g and h switch. Therefore g and

h are symmetric regarding the choice of orders of two multiple steady states. In particular, if g, h leads us to the two distinct, positive, stoichiometrically compatible steady states c^{**} and c^*, then h, g leads us to the two distinct, positive, stoichiometrically compatible steady states c^* and c^{**}, which are basically identical.

We consider that $g, h \in Ker\ L_{\mathscr{O}}$ which are not both the zero vector. Without loss of generality, we can assume that $g \neq 0$, as if $g = 0$ and $h \neq 0$, we can switch the g and h to make $g \neq 0$.

Remark 2.8.6. *We will find the inequality systems for Question 6 with $g, h \in Ker\ L_{\mathscr{O}}$ for the special case $g \neq 0$ and $h = 0$.*

Suppose that $g \neq 0$ and $h = 0$. Therefore, as long as dim $Ker\ L_{\mathscr{O}} \geq 1$, for any sign pattern that is nonzero and sign compatible with $Ker\ L_{\mathscr{O}}$, we have a solution of nonzero $g \in Ker\ L_{\mathscr{O}}$ with such sign patterns. In this case, all fundamental classes are reversible, as if C_i is nonreversible, then $h_{y_i \to y_i'} > 0$, which is a contradiction to our assumption that $h = 0$. For each $g_{y_i \to y_i'} \neq 0$, $\rho_{y_i \to y_i'} = 0$. Therefore only the upper shelf is possible for reactions in the nondegenerate fundamental classes. Let $y \to y' \in P_i$. We have:

(i) If $g_{y_i \to y_i'} > 0$ and $y \to y'$ is on the upper shelf, $0 < e^{y \cdot \mu} < e^{y' \cdot \mu}$.

(ii) If $g_{y_i \to y_i'} < 0$ and $y \to y'$ is on the upper shelf, $0 < e^{y' \cdot \mu} < e^{y \cdot \mu}$.

(iii) If $g_{y_i \to y_i'} = h_{y_i \to y_i'} = 0$, $e^{y \cdot \mu} = e^{y' \cdot \mu}$.

In other words, if $h = 0$, then sgn $g_{y_i \to y_i'}$ = sgn $e^{y' \cdot \mu} - e^{y \cdot \mu}$.

Note that the conditions in Lemma 2.8.2 are linear in terms of $\rho_{y_i \to y_i'}$, $e^{y \cdot \mu}$ and $e^{y' \cdot \mu}$, and are nonlinear in terms of μ. We will make a transformation for $\rho_{y_i \to y_i'}$ to make the conditions linear in terms of μ.

Suppose that $\mu \in \mathbb{R}^{\mathscr{S}}$, g, $h \in Ker\ L_{\mathscr{O}}$, P_i $(0 \leq i \leq w)$ which is an equivalence class with representative $y_i \to y_i'$, and $\{\rho_{y_i \to y_i'} = \dfrac{h_{y_i \to y_i'}}{g_{y_i \to y_i'}} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$ satisfy the conditions in Lemma 2.8.2.

If $\rho_{y_i \to y_i'} > 0$, we define $M_{y_i \to y_i'} = \ln \rho_{y_i \to y_i'}$. In this case, we can rewrite the shelving condition as follows: for every $y \to y'$ in the nondegenerate fundamental class C_i, $y \to y'$ is on the *upper shelf* if $y \cdot \mu > M_{y_i \to y_i'}$; $y \to y'$ is on the *lower shelf* if $y \cdot \mu < M_{y_i \to y_i'}$; $y \to y'$ is on the *middle shelf* if $y \cdot \mu = M_{y_i \to y_i'}$.

If $\rho_{y_i \to y_i'} \leq 0$, we will take $M_{y_i \to y_i'}$ to be an arbitrary large, negative number (to be found later). Note that for any finite value of $y \cdot \mu$, there always exists $M_{y_i \to y_i'}$ such that $y \cdot \mu > M_{y_i \to y_i'}$. In this case, every $y \to y' \in C_i$ must be on the upper shelf with $y \cdot \mu > M_{y_i \to y_i'}$ for some $M_{y_i \to y_i'}$ to be found later (see Lemma 2.8.2).

We can rewrite Lemmas 2.8.2 and 2.8.3 with the shelving conditions (in terms of $M_{y_i \to y_i'}$'s, not $\rho_{y_i \to y_i'}$'s) for the nondegenerate fundamental class C_i.

Lemma 2.8.7. *Suppose that, for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the given orientation $\mathscr{O}$, $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ and $\mu \in \mathbb{R}^{\mathscr{S}}$ are given. Let g, h be defined as in (2.3.2) and (2.3.3), let P_i ($0 \leq i \leq w$) be the equivalence class defined earlier with the representative $y_i \to y_i'$, and let $M_{y_i \to y_i'}$'s be defined as indicated for nondegenerate fundamental classes. Then the following conditions hold:*

I) If $y \to y' \in P_i$ $(i \geq 1)$ is irreversible, then $g_{y \to y'} > 0$, $h_{y \to y'} > 0$, and $M_{y_i \to y_i'} = y \cdot \mu$.

II) If $y \to y' \in P_i$ $(i \geq 1)$ is reversible and C_i is nondegenerate, then

i) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the upper shelf, then $M_{y_i \to y_i'} < y \cdot \mu < y' \cdot \mu$.

ii) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the lower shelf, then $M_{y_i \to y_i'} > y \cdot \mu > y' \cdot \mu$.

iii) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the middle shelf, then $M_{y_i \to y_i'} = y \cdot \mu = y' \cdot \mu$.

iv) If $g_{y_i \to y_i'} < 0$, and $y \to y'$ is on the upper shelf, then $M_{y_i \to y_i'} < y' \cdot \mu < y \cdot \mu$.

v) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the lower shelf, then $M_{y_i \to y_i'} > y' \cdot \mu > y \cdot \mu$.

vi) If $g_{y_i \to y_i'} > 0$, and $y \to y'$ is on the middle shelf, then $M_{y_i \to y_i'} = y \cdot \mu = y' \cdot \mu$.

III) If $y \to y' \in P_i$ $(i \geq 1)$ is reversible and C_i is degenerate, then

i) If $h_{y_i \to y_i'} > 0$, *then* $y \cdot \mu > y' \cdot \mu$.

ii) If $h_{y_i \to y_i'} < 0$, *then* $y \cdot \mu < y' \cdot \mu$.

iii) If $h_{y_i \to y_i'} = 0$, *then* $y \cdot \mu = y' \cdot \mu$.

IV) If $y \to y' \in P_0$ *(is reversible), then* $y \cdot \mu = y' \cdot \mu$.

Lemma 2.8.8. *For a reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *and a given orientation* $\mathscr{O}$, *suppose that (i)* $\mu \in \mathbb{R}^{\mathscr{S}}$, *(ii) a pair* g, $h \in Ker\ L_{\mathscr{O}}$, *(iii)* P_i $(0 \leq i \leq w)$ *which is an equivalence class with representative* $y_i \to y_i'$, *and (iv)* $\{M_{y_i \to y_i'} : g_{y_i \to y_i'} \neq 0, i = 1, ..., w\}$ *as indicated for nondegenerate fundamental classes, together satisfy the conditions in Lemma 2.8.7. Then there exists* $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ *satisfying (2.3.2) and (2.3.3).*

As a result, we will have the following lemma where the conditions in Lemma 2.8.7 are linear in terms of μ and $M_{y_i \to y_i'}$'s:

Lemma 2.8.9. *Given a reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *with the orientation* $\mathscr{O}$ *and the* P_i $(0 \leq i \leq w)$ *representatives* $\{y_i \to y_i' : i = 0, ..., w\}$ *we have chosen, Question 6 can be rewritten in terms of* g, h, μ, *and* $M_{y_i \to y_i'}$*'s as follows:*

Question 7. *For the given reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *with the given orientation* $\mathscr{O}$, *do there exist*

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ *which is sign compatible with the stoichiometric subspace* S,

(ii) a pair g, $h \in Ker\ L_{\mathscr{O}}$ *which are not both zero vectors,*

(iii) a set $\{M_{y_i \to y_i'} : g_{y_i \to y_i'} \neq 0\}$ *as indicated for nondegenerate fundamental classes, and*

(iv) a choice of shelving assignments for each nondegenerate fundamental class that satisfies the conditions in Proposition 2.8.1,

which together satisfy the conditions in Lemma 2.8.7?

Remark 2.8.10. *We introduced $M_{y_i \to y_i'}$'s (which we will later denote as M_i's in the algorithm) here to provide some sense about the linearity of the resulting inequality system. In next section we will rephrase the requirement in Question 3-7 that g, h be members of $Ker\ L_{\mathscr{O}}$ into explicit conditions in terms of a system of equations. Note that $\rho_{y_i \to y_i'}$ is defined as a ratio of $h_{y_i \to y_i'}$ and $g_{y_i \to y_i'}$, for $g_{y_i \to y_i'} \neq 0$, $1 \leq i \leq w$. It will be more convenient to use $\rho_{y_i \to y_i'}$'s instead of $M_{y_i \to y_i'}$'s. More importantly, in the case that $\rho_{y_i \to y_i'} \leq 0$, $M_{y_i \to y_i'}$ is taken as some large and negative number, which is not related to $\rho_{y_i \to y_i'}$ in a precise way, therefore cannot relate to $g_{y_i \to y_i'}$ and $h_{y_i \to y_i'}$ in a precise way. Therefore, we will continue using $\rho_{y_i \to y_i'}$ to explain our theory. We will develop arguments based on Question 6 instead of Question 7.*

2.9 More about $Ker^{\perp}\ L_{\mathscr{O}}$

In this section, we want to rephrase the requirement that g, h be members of $Ker\ L_{\mathscr{O}}$ into explicit conditions in terms of a system of equations.

Note that $L_{\mathscr{O}} : \mathbb{R}^{\mathscr{O}} \to S$ and $Im\ L_{\mathscr{O}} = S$, so dim $Ker\ L_{\mathscr{O}}$ = dim $\mathbb{R}^{\mathscr{O}}$ − dim S = $\#(\mathscr{O})$ − dim S. Then $d :=$ dim $Ker\ L_{\mathscr{O}} = \#(\mathscr{O})$ − dim S. Therefore dim $Ker^{\perp}\ L_{\mathscr{O}}$ = dim $\mathbb{R}^{\mathscr{O}}$ − dim $Ker\ L_{\mathscr{O}} = \#(\mathscr{O}) - d =$ dim S.

Note that for $x \in \mathbb{R}^{\mathscr{O}}$, $x \in Ker\ L_{\mathscr{O}}$ if and only if x satisfies the set of equations $z^j \cdot x = 0$, where $\{z^j\}_{j=1}^{\dim S}$ is a basis for $Ker^{\perp}\ L_{\mathscr{O}}$. Thus $g, h \in Ker\ L_{\mathscr{O}}$ is equivalent to the fact that g and h satisfy the equations $z^j \cdot g = 0$ and $z^j \cdot h = 0$, $j = 1, ...,$ dim S. However, we need to find a basis for $Ker^{\perp}\ L_{\mathscr{O}}$ first.

Recall that the following two statements are equivalent.

(i) $y \to y' \in P_0$.

(ii) $\omega_{y \to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

The following two statements are equivalent.

(i) $y \to y' \in P_i \backslash \{y_i \to y_i'\}$ $(1 \leq i \leq w_1)$.

(ii) There exists a unique $\alpha_{y\to y'} > 0$, such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp} L_{\mathscr{O}}$.

We denote

$$B_0 := \{\omega_{y\to y'} : y \to y' \in P_0\}, \tag{2.9.1}$$

and

$$B_1 := \{\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} : y \to y' \in P_i\backslash\{y_i \to y_i'\}, i = 1, ..., w_1\}. \tag{2.9.2}$$

It is quite obvious that the vectors in $B_0 \cup B_1$ are independent. Note that

$$\begin{aligned} \#(B_0) + \#(B_1) &= \#(P_0) + \sum_{i=1}^{w_1}(\#(P_i) - 1) = \sum_{i=0}^{w_1}\#(P_i) - w_1 \\ &= \#(\mathscr{O}) - w_2 - w_1 = \#(\mathscr{O}) - w. \end{aligned}$$

So we have found $\#(\mathscr{O}) - w$ independent vectors in $Ker^{\perp} L_{\mathscr{O}}$ and our goal is to find dim$S$ $(= \#(\mathscr{O}) - d)$ independent vectors. If it is true that $w = d$, then we have found a complete set of basis vectors for $Ker^{\perp} L_{\mathscr{O}}$, each with support on one or two reactions from $\mathscr{O}$. In particular, it is not difficult to show that if $d = 1$, then $w = 1$. Otherwise, if we assume that $w > d$, then we will continue to look for more vectors to complete a basis for $Ker^{\perp} L_{\mathscr{O}}$.

Recall that for a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the orientation $\mathscr{O}$, $W = \{y_i \to y_i' : i = 1, ..., w\}$ are the set of representatives for all nonzeroth equivalence classes. Let us define $\Gamma_W = \{x \in \mathbb{R}^{\mathscr{O}} : x \text{ has support in } W\}$. Then we have the following proposition.

Proposition 2.9.1. *dim* $(Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W) = w - d$.

PROOF: First note that

$$\begin{aligned}
\text{dim}\,(Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W) &= \text{dim}\,(\mathbb{R}^{\mathscr{O}}) - \text{dim}\,((Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W)^{\perp}) \\
&= \#(\mathscr{O}) - \text{dim}\,(Ker\; L_{\mathscr{O}} + \Gamma_W^{\perp}) \\
&= \#(\mathscr{O}) - (d + \text{dim}\,\Gamma_W^{\perp} - \text{dim}\,(Ker\; L_{\mathscr{O}} \cap \Gamma_W^{\perp})) \\
&= \#(\mathscr{O}) - d - \text{dim}\,\Gamma_W^{\perp} + \text{dim}\,(Ker\; L_{\mathscr{O}} \cap \Gamma_W^{\perp}) \\
&= \#(\mathscr{O}) - d - (\#(\mathscr{O}) - w) + \text{dim}\,(Ker\; L_{\mathscr{O}} \cap \Gamma_W^{\perp}) \\
&= w - d + \text{dim}(Ker\; L_{\mathscr{O}} \cap \Gamma_W^{\perp})
\end{aligned}$$

To show that dim $(Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W) = w - d$, we only need to show that dim $(Ker\; L_{\mathscr{O}} \cap \Gamma_W^{\perp}) = 0$.

To see this, let $W' = \mathscr{O} \backslash W$, and $\tilde{W} = W' \backslash P_0$. So $\Gamma_W^{\perp} = \Gamma_{W'}$ and $\mathscr{O} = W \cup P_0 \cup \tilde{W}$. Notice that for any vector $x \in Ker\; L_{\mathscr{O}} \cap \Gamma_{W'}$, and any vector $z \in B_0 \cup B_1$, $z \cdot x = 0$. Take $x \in Ker\; L_{\mathscr{O}} \cap \Gamma_{W'}$. For each $z \in B_0$, $z = \omega_{y \to y'}$ for some $y \to y' \in P_0$. Since $\omega_{y \to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$, it implies that $\omega_{y \to y'} \cdot x = 0$, i.e. $x_{y \to y'} = 0$, for all $y \to y' \in P_0$. For each $z \in B_1$, $z = \omega_{y_i \to y_i'} - \alpha_{y \to y'} \omega_{y \to y'}$, for some $y \to y' \in P_i \backslash \{y_i \to y_i'\}$, $1 \leq i \leq w_1$ and some $\alpha_{y \to y'} > 0$. Since $\omega_{y_i \to y_i'} - \alpha_{y \to y'} \omega_{y \to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$, it implies that $(\omega_{y_i \to y_i'} - \alpha_{y \to y'} \omega_{y \to y'}) \cdot x = 0$, or $x_{y_i \to y_i'} = \alpha_{y \to y'} x_{y \to y'}$. Since $x \in Ker\; L_{\mathscr{O}} \cap \Gamma_{W'}$, we know that $x_{y_i \to y_i'} = 0$ for $i = 1, ..., w$. As a result, $x_{y \to y'} = 0$, for all $y \to y' \in P_i$ $(1 \leq i \leq w)$. Now by the definition of $\tilde{W}$, we have $x_{y \to y'} = 0$, for all $y \to y' \in \tilde{W}$. So far we have shown that for all $y \to y' \in \mathscr{O}$, $x_{y \to y'} = 0$, i.e. $x = 0$. So dim $(Ker^{\perp}\; L_{\mathscr{O}} \cap \Gamma_W) = w - d$. □

We can define

$$q := \text{dim}\,(Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W). \tag{2.9.3}$$

Then we have

$$q = w - d, \tag{2.9.4}$$

where w is the number of nonzeroth equivalence classes and $d = \dim Ker\ L_{\mathscr{O}}$.

Remark 2.9.2. *We argue that any vector $z \in Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ must have support on at least three reactions from $W = \{y_i \to y_i'\}_{i=1}^{w}$. If not, then we have two situations. (i) If z has support on two reactions $y_i \to y_i'$ and $y_j \to y_j'$, then these two reactions belong to the same equivalence class, which contradicts with the fact that all reactions in W come from different equivalence classes. (ii) If z has support on one reaction, say $y_i \to y_i'$, it implies that $y_i \to y_i'$ lies in P_0, which contradicts the fact that all reactions in W come from nonzeroth equivalence classes.*

Proposition 2.9.3. *For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the given orientation $\mathscr{O}$, if $B_2 = \{b^j\}_{j=1}^{q}$ is a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, then $B_0 \cup B_1 \cup B_2$ is a basis for $Ker^{\perp}\ L_{\mathscr{O}}$.*

PROOF: Note that each of the basis vectors from B_0 has support on some reaction in P_0. Each of the basis vectors from B_1 has support on two reactions from the same equivalence class P_i, for some $1 \leq i \leq w_1$, with one of them being the representative of P_i. From Remark 2.9.2, each of the basis vectors from B_2 has support on at least three reactions from W. Therefore basis vectors in B_2 for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ are independent of those in B_0 and B_1.

Recall that $\#(B_0) + \#(B_1) = \#(\mathscr{O}) - w$. Note that dim $Ker^{\perp}\ L_{\mathscr{O}} = \#(\mathscr{O}) - d$ as dim $Ker\ L_{\mathscr{O}} = d$. Note that $(\#(\mathscr{O}) - w) + (w - d) = \#(\mathscr{O}) - d$. So the vectors in B_2, which is a basis of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, will be what are needed to complete the basis for $Ker^{\perp}\ L_{\mathscr{O}}$. Therefore $B_0 \cup B_1 \cup B_2$ is a basis for $Ker^{\perp}\ L_{\mathscr{O}}$. □

Now we are ready to write down the explicit conditions necessary and sufficient to ensure that g and h are members of $Ker\ L_{\mathscr{O}}$.

Suppose that a basis $\{b^j\}_{j=1}^{q}$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ is given. Recall that $x \in Ker\ L_{\mathscr{O}}$ if and only if x satisfies the following conditions:

(i) For each $y \to y' \in P_0$, $\omega_{y\to y'} \in B_0$. Therefore, $\omega_{y\to y'} \cdot x = 0$, or $x_{y\to y'} = 0$.

(ii) For each $y \to y' \in P_i \backslash \{y_i \to y_i'\}$ $(1 \leq i \leq w_1)$, there exists a unique $\alpha_{y\to y'} > 0$, such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in B_1$. Therefore, $(\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'}) \cdot x = 0$, or $x_{y_i\to y_i'} - \alpha_{y\to y'}x_{y\to y'} = 0$.

(iii) $b^j \cdot x = 0$, $j = 1, ..., q$, where $b^j \in B_2$.

So $g, h \in Ker\ L_{\mathscr{O}}$ if and only if g and h satisfy the following equations:

$$g_{y\to y'} = 0, \forall y \to y' \in P_0 \tag{2.9.5}$$

$$h_{y\to y'} = 0, \forall y \to y' \in P_0 \tag{2.9.6}$$

$$g_{y_i\to y_i'} - \alpha_{y\to y'}g_{y\to y'} = 0, \forall y \to y' \in P_i \backslash \{y_i \to y_i'\}, 1 \leq i \leq w_1 \tag{2.9.7}$$

$$h_{y_i\to y_i'} - \alpha_{y\to y'}h_{y\to y'} = 0, \forall y \to y' \in P_i \backslash \{y_i \to y_i'\}, 1 \leq i \leq w_1 \tag{2.9.8}$$

$$b^j \cdot g = 0, j = 1, ..., q \tag{2.9.9}$$

$$b^j \cdot h = 0, j = 1, ..., q. \tag{2.9.10}$$

Because $\{b^j : j = 1, ..., q\}$ is a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, equations (2.9.9) and (2.9.10) in terms of g and h can be rewritten in terms of $g_{y_i\to y_i'}$'s and $h_{y_i\to y_i'}$'s as follows:

$$\sum_{y_i\to y_i' \in W} g_{y_i\to y_i'} b^j_{y_i\to y_i'} = 0, j = 1, ..., q \tag{2.9.11}$$

$$\sum_{y_i\to y_i' \in W} h_{y_i\to y_i'} b^j_{y_i\to y_i'} = 0, j = 1, ..., q. \tag{2.9.12}$$

It is obvious that if we have solved for $g, h \in Ker\ L_{\mathscr{O}}$, then $g_{y_i\to y_i'}$ and $h_{y_i\to y_i'}$, for all $i \in \{1, ..., w\}$, satisfy equations (2.9.11) and (2.9.12).

On the other hand, suppose that we have solved for $g_{y_i\to y_i'}$ and $h_{y_i\to y_i'}$ for all $i \in \{1, ..., w\}$, from equations (2.9.11) and (2.9.12). For $1 \leq i \leq w_1$, we can then solve for $g_{y\to y'}$ and $h_{y\to y'}$ from equations (2.9.7) and (2.9.8), for all $y \to y' \in P_i \backslash \{y_i \to y_i'\}$. We also have solved trivially for $g_{y\to y'}$ and $h_{y\to y'}$ for $y \to y' \in P_0$. Therefore, we will have solved for $g, h \in Ker\ L_{\mathscr{O}}$ (but of course not uniquely).

Recall that $W = \{y_i \to y_i' : i = 1, ..., w\}$ is the set of representatives from all nonzeroth equivalence classes. For $g, h \in Ker\ L_{\mathscr{O}}$ and $X \subseteq \mathscr{O}$, we denote $g|_X$ and $h|_X$ as the projections of g and h on Γ_X, respectively, where $\Gamma_X = \{z \in \mathbb{R}^{\mathscr{O}} :\ \text{supp}\ z \subseteq X\}$. Note that if $g, h \in Ker\ L_{\mathscr{O}}$, $g|_W = h|_W = 0$ if and only if $g = h = 0$.

Therefore, the following statements hold:

(i) If there exists a pair $g, h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors, then the vectors $g_W := g|_W$, $h_W := h|_W$ which are not both zero, satisfy equations (2.9.11) and (2.9.12).

(ii) If there exist $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ which are not both zero vectors satisfying equations (2.9.11) and (2.9.12), then we can solve for $g, h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors from $g|_W = g_W$, $h|_W = h_W$, and equations (2.9.5)-(2.9.8).

For a given $g \in Ker\ L_{\mathscr{O}}$, we set $D = \{y_i \to y_i' \in W : g_{y_i \to y_i'} = 0\}$ and $ND = W \backslash D$. Then $g|_D$, $g|_{ND}$, $h|_D$ and $h|_{ND}$ are the projections of g on Γ_D and Γ_{ND}, and h on Γ_D and Γ_{ND}, respectively. Note that $g|_D = 0$.

Recall that in Question 6 (see Remark 2.8.10), we need to test against all choices of $g, h \in Ker\ L_{\mathscr{O}}$ that are not both zero vectors. Note that any choice of shelving for each nondegenerate fundamental class needs to satisfy the conditions of Proposition 2.8.1, and condition (ii) of the proposition is related to the sign of $\rho_{y_i \to y_i'}$, i.e., the sign of the ratio of $h_{y_i \to y_i'}$ and $g_{y_i \to y_i'}$ for $g_{y_i \to y_i'} \neq 0$. Therefore the choice of shelving for each nondegenerate fundamental class depends on the sign pattern of $g|_{ND}$ and $h|_{ND}$, where ND is defined by the sign pattern of $g|_W$. Another point to note in Question 6 is that the inequalities and equalities from Lemma 2.8.2 are based on the choice of shelving assignments for each nondegenerate fundamental class and sign patterns of $g|_{ND}$ and $h|_D$, where D and ND are defined by the sign pattern of $g|_W$.Then given $g, h \in Ker\ L_{\mathscr{O}}$, we only use sign patterns of $g|_W$ and $h|_W$ to set up each of the inequality systems that will lead us to the answer to Question 6. In other words, we only need to find $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ satisfying equations

(2.9.11) and (2.9.12) to set up the inequality systems that will lead us to the answer of Question 6.

From now on then, we will focus on finding $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ satisfying equations (2.9.11) and (2.9.12).

Recall that given the orientation $\mathscr{O}$, P_i $(0 \leq i \leq w)$ is an equivalence class with a representative $y_i \to y_i'$, and $W = \{y_i \to y_i' : i = 1, ..., w\}$.

We have the following lemma:

Lemma 2.9.4. *Given the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with the orientation $\mathscr{O}$, P_i ($0 \leq i \leq w$) which is the equivalence class defined with the representative $y_i \to y_i'$, and $W = \{y_i \to y_i' : i = 1, ..., w\} \subseteq \mathscr{O}$, Question 6 can be rewritten in terms of g_W, h_W, μ, and $\rho_W(y_i \to y_i')$'s as follows:*

Question 8. *For the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and the given orientation $\mathscr{O}$, do there exist*

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign compatible with the stoichiometric subspace S,

(ii) a pair $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ which are not both zero vectors satisfying equations (2.9.11) and (2.9.12),

(iii) a set $\{\rho_W(y_i \to y_i') = \dfrac{h_W(y_i \to y_i')}{g_W(y_i \to y_i')} : g_W(y_i \to y_i') \neq 0, 1 \leq i \leq w\}$, and

(iv) a choice of shelving assignments for each nondegenerate fundamental class that satisfies the conditions in Proposition 2.8.1 (in terms of $\rho_W(y_i \to y_i')$ for condition (ii) in the proposition),

which together satisfy the conditions in Lemma 2.8.2 (in terms of $g_W(y \to y_i')$, $h_W(y_i \to y_i')$ and $\rho_W(y_i \to y_i')$)?

Next we will try to answer Question 8.

The first attempt may be that we make assumptions about the sign patterns of $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, where the pair of sign patterns has to follow some rules. We will state those rules shortly. For now, we will say that if the pair of sign patterns satisfies the rules, we recognize them as a "valid" pair of sign patterns for g_W and h_W.

We then try to solve for all solutions of g_W and h_W with such a pair of sign patterns from equations (2.9.11) and (2.9.12). Suppose that we find g_W and h_W with the pre-selected pair of sign patterns, and we then calculate to find $\{\rho_W(y_i \to y_i') : g_W(y_i \to y_i') \neq 0\}$. Then, for the g_W, h_W and the set $\{\rho_W(y_i \to y_i') : g_W(y_i \to y_i') \neq 0\}$ we find, we will check if the answer to the following Question (a) is "Yes":

Question (a): Do there exist a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S and a choice of shelving for each nondegenerate fundamental class satisfying the conditions in Proposition 2.8.1 (in terms of $\rho_W(y_i \to y_i')$ for condition (ii) in the proposition), such that the conditions in Lemma 2.8.2 are satisfied?

We will repeat the process for all "valid" pairs of sign patterns for g_W and h_W, if necessary. (For reasons to be stated, we may finish before trying all such pairs.)

If for some "valid" pair of sign patterns for g_W and h_W, there exists a solution of g_W and h_W satisfying equations (2.9.11) and (2.9.12), such that the answer to Question (a) is "Yes", then we can claim that the reaction network has the capacity to admit multiple positive steady states and we are done. If there is no "Yes" answer to Question (a) for any "valid" pair of sign patterns for g_W and h_W, then we claim that the reaction network does not have the capacity to admit multiple steady states, no matter what (positive) values the rate constants take.

This procedure would work in general if, given any "valid" pair of sign patterns for g_W and h_W, we can find ALL solutions of g_W and h_W with such sign patterns satisfying equations (2.9.11) and (2.9.12). However, this is impractical. Therefore, we must look for an alternative approach. After assigning sign patterns to g_W and h_W, we will not try to

first solve for g_W, h_W with such sign patterns from equations (2.9.11) and (2.9.12), and then find $\rho_W(y_i \to y_i')$ for $g_W(y_i \to y_i') \neq 0$. Instead, we wish to find the necessary conditions (and in some cases it will also be sufficient) for equations (2.9.11) and (2.9.12) to have a solution of g_W and h_W with such sign patterns. As we will see later, these conditions (let us refer them as conditions $(*)$ for now) will be inequalities and/or equalities in terms of $\rho_W(y_i \to y_i')$'s. For the pre-selected "valid" pair of sign patterns of g_W and h_W, we determine the sign of $\rho_W(y_i \to y_i')$ $(y_i \to y_i' \in ND)$ by the ratio of the signs of $h_W(y_i \to y_i')$ and $g_W(y_i \to y_i')$; we say the set $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$ with such a sign pattern, is ***consistent in sign*** with the pre-selected sign patterns of g_W and h_W. Then for the chosen sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, we check if the answer to the following Question (b) is "Yes":

Question (b): Given the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and the orientation $\mathscr{O}$, do there exist

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign compatible with S,

(ii) a set $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$ which is consistent in sign with the pre-selected sign patterns of g_W and h_W and satisfies these new conditions $(*)$, and

(iii) a choice of shelving assignments for each nondegenerate fundamental class satisfying the conditions in Proposition 2.8.1 (in terms of $\rho_W(y_i \to y_i')$ for condition (ii)),

which together satisfy the conditions in Lemma 2.8.2?

We will repeat the process for all "valid" pairs of sign patterns for g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, if necessary. (For reasons to be stated, we may finish before trying all such pairs.)

We have two situations, depending on whether the necessary conditions (*) are sufficient or not to find from equations (2.9.11) and (2.9.12) a solution of g_W and h_W with the preselected sign patterns.

Suppose the conditions $(*)$ we find are both necessary and sufficient for equations (2.9.11) and (2.9.12) to have a solution of g_W and h_W with the preselected sign patterns. If for a "valid" pair of sign patterns for g_W and h_W, the answer to Question (b) is "Yes", then we can claim that the reaction network has the capacity to admit multiple positive steady states and we are done. If there is no "Yes" answer to Question (b) for any "valid" pair of sign patterns for g_W and h_W, then we claim that the reaction network does not have the capacity to admit multiple steady states, no matter what (positive) values the rate constants take.

If the conditions $(*)$ we find are necessary but not sufficient, then we can only conclude, in the case that there is no "Yes" answer to Question (b) for any "valid" pair of sign patterns for g_W and h_W, the reaction network does not have the capacity to admit multiple steady states. However, if there exists a "valid" pair of sign patterns for g_W and h_W such that the answer to Question (b) is "Yes", and in addition, the solution of $\mu \in \mathbb{R}^{\mathscr{S}}$ from the answer will lead to a solution of $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ from solving equations (2.2.7) and (2.2.8), then we can still conclude the reaction network has the capacity to admit multiple positive steady states and we are done.

We will proceed following this alternative approach.

First of all, let us describe the conditions of being a "valid" pair of sign patterns for g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$.

Consider the equivalence of solving $g, h \in Ker\ L_{\mathscr{O}}$ and solving $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ from equations (2.9.11) and (2.9.12). We know that the "valid" pair of sign patterns of g_W and h_W should include all possible sign patterns for $g|_W$ and $h|_W$, given $g, h \in Ker\ L_{\mathscr{O}}$. We will add two additional constraints that g_W and h_W are not both zero vectors and that for a nonreversible fundamental class C_i, the signs of $g_W(y_i \to y_i')$ and $h_W(y_i \to y_i')$ are both positive.

A pair of sign patterns for g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ is said to be *sign-compatible with* $Ker\ L_{\mathscr{O}}|_W$ if the following two statements hold:

(i) If the fundamental class C_i $(1 \leq i \leq w)$ is nonreversible, then the signs of $g_W(y_i \to y_i')$ and $h_W(y_i \to y_i')$ are both positive.

(ii) There exists $x^1, x^2 \in Ker\ L_{\mathscr{O}}$, such that for every $y_i \to y_i' \in W$, $x^1_{y_i \to y_i'}$ has the same sign as $g_W(y_i \to y_i')$ and $x^2_{y_i \to y_i'}$ has the same sign as $h_W(y_i \to y_i')$.

We say a pair of sign patterns for g_W, $h_W \in \mathbb{R}^{\mathscr{O}}$ is *zero* if we assign that $g_W = h_W = 0$. Note that for $g, h \in Ker\ L_{\mathscr{O}}$, $g|_W = h|_W = 0$ if and only if $g = h = 0$. Note that we want to find $g, h \in Ker\ L_{\mathscr{O}}$ which are not both zero vectors. Therefore, we want to find solutions g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ which are not both zero vectors satisfying equations (2.9.11) and (2.9.12).

With this as background, we say a pair of sign patterns for g_W and h_W is "***valid***" if it is nonzero and sign-compatible with $Ker\ L_{\mathscr{O}}|_W$.

Now suppose we pick a "valid" pair of sign patterns for g_W and h_W. Then $D = \{y_i \to y_i' \in W : g_W(y_i \to y_i') = 0\}$ and $ND = W \backslash D$ are defined. Note that for $y_i \to y_i' \in ND$, $\rho_W(y_i \to y_i') = \dfrac{h_W(y_i \to y_i')}{g_W(y_i \to y_i')}$. Therefore, for each $y_i \to y_i' \in ND$, the sign of $\rho_W(y_i \to y_i')$ is also determined by the ratio of the signs of $h_W(y_i \to y_i')$ and $g_W(y_i \to y_i')$. Suppose we have a set of parameters $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$ with such a sign pattern. Then equations (2.9.11) and (2.9.12) in terms of $g_{y_i \to y_i'}$ and $h_{y_i \to y_i'}$ $(1 \leq i \leq w)$, can be rewritten in terms of $g_W(y_i \to y_i')$, $h_W(y_i \to y_i')$ $(1 \leq i \leq w)$ and $\rho_W(y_i \to y_i')$ $(y_i \to y_i' \in ND)$ as follows:

$$\sum_{y_i \to y_i' \in ND} b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0, \qquad j = 1, ..., q \quad (2.9.13)$$

$$\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') + \sum_{y_i \to y_i' \in ND} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0, \qquad j = 1, ..., q \quad (2.9.14)$$

$$h_W(y_i \to y_i') = \rho_W(y_i \to y_i') g_W(y_i \to y_i'), \qquad y_i \to y_i' \in ND. \quad (2.9.15)$$

To guarantee the existence of a pair of solutions $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15), it is necessary that the set of equations (2.9.13) and (2.9.14) can have nonzero solutions. To find the necessary condition, we will rewrite the set of equations (2.9.13) and (2.9.14) in terms of the format $A_D z = 0$ where A_D and z are defined as follows.

Recall that once a pair of sign patterns of g_W and h_W is chosen, $D = \{y_i \to y_i' \in W : g_W(y_i \to y_i') = 0\}$ and $ND = W \backslash D$ are defined. Let us define a vector $z \in \mathbb{R}^W$ as follows:

$$z_{y_i \to y_i'} = \begin{cases} g_W(y_i \to y_i'), & \text{if } y_i \to y_i' \in ND \\ h_W(y_i \to y_i'), & \text{if } y_i \to y_i' \in D. \end{cases} \tag{2.9.16}$$

Note that equations (2.9.13) and (2.9.14) can be rewritten in terms of $z \in \mathbb{R}^W$ as follows:

$$\sum_{y_i \to y_i' \in ND} b^j_{y_i \to y_i'} z_{y_i \to y_i'} = 0, \qquad j = 1, ..., q \tag{2.9.17}$$

$$\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} z_{y_i \to y_i'} + \sum_{y_i \to y_i' \in ND} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} z_{y_i \to y_i'} = 0, j = 1, ..., q. \tag{2.9.18}$$

Recall that $|W| = w$. We define a $(2q \times w)$ matrix $A_D = (a_{ki})$ as follows:

$$a_{ki} = \begin{cases} b^k_{y_i \to y_i'}, & \text{if } 1 \le k \le q \text{ and } y_i \to y_i' \in ND \\ 0, & \text{if } 1 \le k \le q \text{ and } y_i \to y_i' \in D \\ \rho_W(y_i \to y_i') b^{k-q}_{y_i \to y_i'}, & \text{if } q+1 \le k \le 2q \text{ and } y_i \to y_i' \in ND \\ b^{k-q}_{y_i \to y_i'}, & \text{if } q+1 \le k \le 2q \text{ and } y_i \to y_i' \in D. \end{cases} \tag{2.9.19}$$

Remark 2.9.5. *Note that with the basis $\{b^j\}_{j=1}^q$ of $Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W$ given, the definition of A_D is based on the set D, which is decided by the given sign patterns of g_W and h_W and the given parameter set $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND = W \backslash D\}$.*

From Remark 2.8.6 we can see that the cases of assuming $g_W = 0$ and $h_W \neq 0$ or $g_W \neq 0$ and $h_W = 0$ satisfying equations (2.9.13), (2.9.14) and (2.9.15) (corresponding

to $g = 0$ and $h \neq 0$ or $g \neq 0$ and $h = 0$, where $g, h \in Ker\ L_{\mathscr{O}}$) are special but simple cases, compared to cases where $g_W \neq 0$ and $h_W \neq 0$ satisfying equations (2.9.13), (2.9.14) and (2.9.15) (corresponding to $g \neq 0$ and $h \neq 0$ where $g, h \in Ker\ L_{\mathscr{O}}$). Here we assume that neither g_W nor h_W is assigned to equal zero. Therefore we are looking for a nonzero solution z with a sign pattern determined by pre-selected sign patterns of g_W and h_W, satisfying $A_D z = 0$. Recall that A_D is a $2q \times w$ matrix. We know there exists a nonzero solution z of $A_D z = 0$ if and only if rank $(A_D) < w$. In fact, if rank $(A_D) < w$ then there exists an infinite number of nonzero solutions of $A_D z = 0$.

Therefore, to guarantee the existence of a pair of solutions $g_W, h_W \in (\mathbb{R}^{\mathscr{O}} \cap \Gamma_W) \backslash \{0\}$ with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15), it is necessary that rank $(A_D) < w$ where A_D is defined as in (2.9.19). Note that if $2q < w$, then rank $(A_D) < w$ holds trivially.

Remark 2.9.6. *Note that if $2q < w$, then it is guaranteed there will be a nonzero solution for z of $A_D z = 0$. This is independent of the values that the $\rho_W(y_i \to y_i')$'s take.*

If $2q \geq w$, then $d \leq w$. To have rank $(A_D) < w$, we need to require that any $w \times w$ submatrix of A_D has a determinant of 0. There are C_{2q}^{w} (the number of w-combinations from a given set of $2q$ elements) such submatrices. Note that $q < w$ holds as we have assumed that $d = Ker\ L_{\mathscr{O}} = w - q \geq 1$. In fact, we may assume that $d \geq 2$ as if $d = 1$ then $w = 1$ and $q = 0$. Then any $w \times w$ submatrix of A_D will include at least two rows from rows $q+1$ to $2q$ in A_D. Let $K = \#(ND)$, then K is the number of $\rho_W(y_i \to y_i')$'s shown in A_D. We have assumed that $g_W \neq 0$, therefore $K \geq 1$. Note that $K = \#(ND) \geq 1$, since the sign patterns of $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ are "valid", the rank of the submatrix formed by the first q rows is less than K, therefore the rank of A_D is less than $K + q$. If $K + q \leq w$, then rank $A_D < w$ holds.

Note that we assume that $q + 2 \leq w \leq 2q$, so $q \geq 2$. In general, each $w \times w$ submatrix of A_D is formed by choosing k rows from rows 1 to q and $w - k$ rows from rows $q + 1$ to

$2q$. *Note that* $k \leq q$ *and* $w - k \leq q$, *thus* $d = w - q \leq k \leq q < w$. *If* $k \geq K$, *then the submatrix has a rank less than* $K + (w - k) \leq K + (w - K) = w$. *If* $k < K$, *then the determinant of such submatrix is a multivariate polynomial in terms of* $\rho_W(y_i \to y_i')$*'s of degree max* $\{K - k, 0\}$.

Note that there exists a "valid" sign pattern such that $K = w$. *Then for any submatrix formed with* $K = \#(ND) = w$ *and some* k *such that* $d = w - q \leq k \leq q < w$, *its determinant is a multivariate polynomial in terms of* $\rho_W(y_i \to y_i')$*'s of degree* $w - k$. *In particular, if* $w - k = q$ *or* $k = w - q$, *we will have that in this case the determinant of the corresponding submatrix is a multivariate polynomial in terms of* $\rho_W(y_i \to y_i')$*'s of degree* $q \geq 2$. *By requiring the determinants equal to zero, we will have nonlinear equalities in terms of* $\rho_W(y_i \to y_i')$*'s (or* $M_{y_i \to y_i'}$*'s) adding to the necessary conditions for the existence of a pair of solutions* $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15). We will not consider these nonlinear equalities here.*

In the next section, we will look at other necessary conditions on the existence of a pair of solutions $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15).

Remark 2.9.7. *We will assume the following on the reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *for the following sessions.*

For a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *and the given orientation* $\mathscr{O}$, *suppose that (i)* P_i *(*$0 \leq i \leq w$*) which is the equivalence class defined with the representative* $y_i \to y_i'$, *(ii)* $W = \{y_i \to y_i' : i = 1, ..., w\} \subseteq \mathscr{O}$, *(iii) a basis* $\{b^j\}_{j=1}^q$ *of* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, *and (iv) a "valid" pair of sign patterns for* g_W, h_W, *and a set of parameters* $\{\rho_W(y_i \to y_i') : g_W(y_i \to y_i') \neq 0, 1 \leq i \leq w\}$ *whose sign pattern is such that the sign of* $\rho_W(y_i \to y_i')$ *is the same as the ratio of the signs of* $h_W(y_i \to y_i')$ *and* $g_W(y_i \to y_i')$, *are given.*

2.10 Conditions on ρ's

In this section, we will continue looking for necessary conditions on the existence of a pair of solutions $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15).

Recall that a basis $\{b^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ is given. Note that after the pair of sign patterns for g_W and h_W are chosen, D and ND are also given. We will define a few sets upon this.

For $1 \leq j \leq q$, let $R_+^j = \{y_i \to y_i' \in ND : b^j_{y_i \to y_i'} g_W(y_i \to y_i') > 0\}$, and $R_-^j = \{y_i \to y_i' \in ND : b^j_{y_i \to y_i'} g_W(y_i \to y_i') < 0\}$.

For $1 \leq j \leq q$, let $Q_+^j = \{y_i \to y_i' \in D : b^j_{y_i \to y_i'} h_W(y_i \to y_i') > 0\}$, and $Q_-^j = \{y_i \to y_i' \in D : b^j_{y_i \to y_i'} h_W(y_i \to y_i') < 0\}$.

Let $I_1 = \{y_i \to y_i' \in W : b^j_{y_i \to y_i'} = 0, \forall 1 \leq j \leq q\}$, $I_2 = \{y_i \to y_i' \in W \backslash I_1 : g_W(y_i \to y_i') = h_W(y_i \to y_i') = 0\}$. Then we can see that $W = I_1 \cup I_2 \cup (\cup_{j=1}^q (R_+^j \cup R_-^j \cup Q_+^j \cup Q_-^j))$. Note that for any $1 \leq j \leq q$, R_+^j, R_-^j, Q_+^j, Q_-^j, I_1 and I_2 are disjointed.

Suppose that $y_i \to y_i' \in I_1$. Note that $b^j_{y_i \to y'} = 0$, for all $j = 1, ..., q$. We have two situations. Suppose $y_i \to y_i' \in I_1 \cap ND$. $g_W(y_i \to y_i')$ and $\rho_W(y_i \to y_i')$ do not really appear in equations (2.9.13) and (2.9.14). Then $g_W(y_i \to y_i')$ can be any number that is sign-compatible with the pre-selected sign of $g_W(y_i \to y_i')$, and $h_W(y_i \to y_i')$ can be any number that is sign-compatible with the pre-selected sign of $h_W(y_i \to y_i')$. Suppose $y_i \to y_i' \in I_1 \cap D$. Then $g_W(y_i \to y_i') = 0$. Note that $h_W(y_i \to y_i')$ does not really appear in equations (2.9.14) and (2.9.15). Then $h_W(y_i \to y_i')$ can be any number that is sign-compatible with the pre-selected sign of $h_W(y_i \to y_i')$.

Suppose $y_i \to y_i' \in I_2$. Then $g_W(y_i \to y_i') = h_W(y_i \to y_i') = 0$.

Remark 2.10.1. *Consider* $I_1^j = \{y_i \to y_i' \in W : b^j_{y_i \to y_i'} = 0\}$ *and* $I_2^j = \{y_i \to y_i' \in W \backslash I_1 : g_W(y_i \to y_i') = h_W(y_i \to y_i') = 0\}$. *Then* $W = I_1^j \cup I_2^j \cup R_+^j \cup R_-^j \cup Q_+^j \cup Q_-^j$,

for $1 \leq j \leq q$. Suppose we want to solve for g_W^j and h_W^j sign compatible with the pre-selected sign patterns of g_W and h_W, satisfying the j^{th} case of equations (2.9.13) and (2.9.14), and equations (2.9.15). Then for $y_i \to y_i' \in I_1^j \cup I_2^j$, we can let $g_W^j(y_i \to y_i')$ and $h_W^j(y_i \to y_i')$ be any numbers that are sign compatible with their pre-selected sign patterns, respectively. We then only focus on solving for $g_W^j(y_i \to y_i')$ and $h_W^j(y_i \to y_i')$ for $y_i \to y_i' \in R_+^j \cup R_-^j \cup Q_+^j \cup Q_-^j$ satisfying j^{th} case of equations (2.9.13) and (2.9.14), and equations (2.9.15).

Therefore, we need to solve for $g_W(y_i \to y_i')$ and $h_W(y_i \to y_i')$ for $y_i \to y_i' \in \cup_{j=1}^q (R_+^j \cup R_-^j \cup Q_+^j \cup Q_-^j)$ satisfying equations (2.9.13), (2.9.14) and (2.9.15).

Recall that we are given $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$, a set of parameters in which the sign of $\rho_W(y_i \to y_i')$ is the same as the ratio of the pre-selected signs of $h_W(y_i \to y_i')$ and $g_W(y_i \to y_i')$. Let $\rho_{min}^{u^j} = \min\{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$, $\rho_{max}^{u^j} = \max\{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$, $\rho_{min}^{v^j} = \min\{\rho_W(y_i \to y_i') : y_i \to y_i' \in Q_-^j\}$, and $\rho_{max}^{v^j} = \max\{\rho_W(y_i \to y_i') : y_i \to y_i' \in Q_-^j\}$.

For $y_i \to y_i' \in R_+^j \cup R_-^j$, define

$$u_{y_i \to y_i'}^j = b_{y_i \to y_i'}^j g_W(y_i \to y_i') > 0, \text{for } y_i \to y_i' \in R_+^j \tag{2.10.1}$$

$$v_{y_i \to y_i'}^j = -b_{y_i \to y_i'}^j g_W(y_i \to y_i') > 0, \text{ for } y_i \to y_i' \in R_-^j. \tag{2.10.2}$$

For $y_i \to y_i' \in Q_+^j \cup Q_-^j$, define

$$s_{y_i \to y_i'}^j = b_{y_i \to y_i'}^j h_W(y_i \to y_i') > 0, \text{for } y_i \to y_i' \in Q_+^j \tag{2.10.3}$$

$$t_{y_i \to y_i'}^j = -b_{y_i \to y_i'}^j h_W(y_i \to y_i') > 0, \text{ for } y_i \to y_i' \in Q_-^j. \tag{2.10.4}$$

From equation (2.9.13), we have

$$\sum_{y_i \to y_i' \in ND} b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in R^j_+} b^j_{y_i \to y_i'} g_W(y_i \to y_i') + \sum_{y_i \to y_i' \in R^j_-} b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in R^j_+} b^j_{y_i \to y_i'} g_W(y_i \to y_i') = \sum_{y_i \to y_i' \in R^j_-} -b^j_{y_i \to y_i'} g_W(y_i \to y_i') \tag{2.10.5}$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in R^j_+} u^j_{y_i \to y_i'} = \sum_{y_i \to y_i' \in R^j_-} v^j_{y_i \to y_i'}. \tag{2.10.6}$$

From equation (2.9.14), we have

$$\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') + \sum_{y_i \to y_i' \in ND} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in Q^j_+} b^j_{y_i \to y_i'} h_W(y_i \to y_i') + \sum_{y_i \to y_i' \in Q^j_-} b^j_{y_i \to y_i'} h_W(y_i \to y_i') +$$

$$\sum_{y_i \to y_i' \in R^j_+} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i') + \sum_{y_i \to y_i' \in R^j_-} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i') = 0$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in Q^j_+} b^j_{y_i \to y_i'} h_W(y_i \to y_i') + \sum_{y_i \to y_i' \in R^j_+} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i')$$

$$= \sum_{y_i \to y_i' \in Q^j_-} -b^j_{y_i \to y_i'} g^{new}_{y_i \to y_i'} + \sum_{y_i \to y_i' \in R^j_-} -\rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g_W(y_i \to y_i') \tag{2.10.7}$$

$$\text{iff} \quad \sum_{y_i \to y_i' \in Q^j_+} s^j_{y_i \to y_i'} + \sum_{y_i \to y_i' \in R^j_+} \rho_{y_i \to y_i'} u^j_{y_i \to y_i'}$$

$$= \sum_{y_i \to y_i' \in Q^j_-} t^j_{y_i \to y_i'} + \sum_{y_i \to y_i' \in R^j_-} \rho_{y_i \to y_i'} v^j_{y_i \to y_i'}. \tag{2.10.8}$$

Note that here we are following the convention that if a set $A = \emptyset$, then the sum based on the set A is always 0.

Therefore, we can claim the following lemma.

Lemma 2.10.2. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Suppose there exists $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the given sign pattern satisfying equations (2.9.13), (2.9.14) and (2.9.15). Then for each $1 \leq j \leq q$, there exist positive $u^j_{y_i \to y_i'}$, $v^j_{y_i \to y_i'}$, $s^j_{y_i \to y_i'}$ and $t^j_{y_i \to y_i'}$ defined on R^j_+, R^j_-, Q^j_+ and Q^j_- respectively from (2.10.1), (2.10.2), (2.10.3) and (2.10.4), satisfying (2.10.6) and (2.10.8).*

We will now introduce a few terms. They will help us find the necessary conditions on $\rho_W(y_i \to y_i')$, for the existence of g_W and h_W with pre-selected sign patterns that satisfy the equations (2.9.13), (2.9.14) and (2.9.15).

Let Q_1 and Q_2 be two finite multisets of real numbers. For $i = 1, 2$, let min Q_i be the minimum of elements in Q_i, and max Q_i be the maximum of elements in Q_i.

Let us consider the following conditions:

(a) min $Q_1 \leq$ max Q_2, min $Q_2 \leq$ max Q_1, and min $Q_1 =$ max Q_2 if and only if min Q_2 $=$ max Q_1.

(b) min $Q_1 <$ max Q_2 and min $Q_2 <$ max Q_1, or min $Q_1 =$ max $Q_2 =$ min $Q_2 =$ max Q_2.

(c) max $Q_1 \leq$ min Q_2 and min $Q_1 <$ max Q_2, or max $Q_2 \leq$ min Q_1 and min $Q_2 <$ max Q_1.

(d) min $Q_1 \leq$ max $Q_1 \leq$ min $Q_2 \leq$ max Q_2 and the three equalities do not hold simultaneously, or min $Q_2 \leq$ max $Q_2 \leq$ min $Q_1 \leq$ max Q_1 and the three equalities do not hold simultaneously.

We can easily verify that, (a) and (b) are equivalent, (c) and (d) are equivalent, and, (a) (or (b)) and (c) (or (d)) are complementary of each other.

We define Q_1 and Q_2 as *nonsegregated* if (a) (or (b)) holds. We define Q_1 and Q_2 as *segregated* if (c) (or (d)) holds.

Remark 2.10.3. *Note there is another equivalent way to define nonsegregated multisets. As an alternative definition, we define Q_1 and Q_2 as nonsegregated if one of the following holds:*

(i) One of the elements in one multiset lies between two non-identical elements of the other multiset, i.e. there exists a from one multiset and $b < c$ from the other multiset such that $b < a < c$.

(ii) The underlying sets of elements of the two multisets sets are identical with set cardinality both equal to one or two.

We will show next how these two definitions are equivalent.

On one hand, note that (a) min $Q_1 \leq$ max Q_2, min $Q_2 \leq$ max Q_1, and min Q_1 = max Q_2 iff min Q_2= max Q_1, is equivalent to one of the following holds:

(I) min $Q_1 <$ max Q_2, and min $Q_2 <$ max Q_1.

(II) min $Q_1 =$ max Q_2, and min $Q_2 =$ max Q_1.

(I) implies that min Q_1< min Q_2< max Q_1, min $Q_2 <$ min Q_1< max Q_2, min Q_2 < max Q_1 < max Q_2, min Q_1 < max Q_2 < max Q_1, or min Q_1 = min Q_2 < max Q_1 = max Q_2.

Note that the first four inequalities imply that one element in one multiset lies between two distinct elements in the other multiset. As for the last inequality, for it to hold, both Q_1 and Q_2 must have at least two distinct elements. Therefore, if both Q_1 and Q_2 have exactly two distinct elements, then min Q_1 = min Q_2 < max Q_1 = max Q_2 implies that the underlying sets of elements of the two multisets are identical with set cardinality equal to two. Otherwise, if one of the multisets has at least 3 distinct elements, then the one which is neither minimum nor maximum of this multiset must lie between the minimum and maximum of the other multiset.

(II) implies that all the elements in Q_1 and Q_2 are equal, so the underlying sets of elements of the two multisets are identical with set cardinality equal to one.

On the other hand, note that in (i), without loss of generality, let us assume there exist $a \in Q_1$ and $b < c \in Q_2$ such that $b < a < c$. Then (i) implies that min $Q_1 \leq a <$ c $\leq$ max Q_2 and min $Q_2 \leq b <$ a $\leq$ max Q_1. Thus min Q_1 < max Q_2 and min Q_2 < max Q_1.

In (ii), if the underlying sets of elements of the two multisets sets are identical with set cardinality both equal to one, then all the elements in both multisets are equal. Otherwise, if the underlying sets of elements of the two multisets sets are identical with set cardinality both equal to two, then we have min Q_1 = min Q_2 < max Q_1 = max Q_2.

Remark 2.10.4. *The reason why we introduced the alternative definition of nonsegregated multisets is because it is utilized in the program [16]. In addition, in the algorithm, instead of checking condition (ii) in Remark 2.10.3 for the case of cardinality equal to two, we check instead "if there exists $a < b \in Q_1$ and $c < d \in Q_2$ with $a = c$ and $b = d$". In other words, for the alternative definition of nonsegregated multisets introduced in Remark 2.10.3, we check instead of (ii) that:*

(ii') If all elements in Q_1 and Q_2 are equal or if there exist $a, b \in Q_1$, $c, d \in Q_2$ such that $a < b$, $a = c$ and $b = d$.

To see why replacement of (ii) with (ii') in conditions (i) and (ii) of Remark 2.10.3 is valid, first suppose (ii) is true. Then it is easy to see that (ii') is also true. Next suppose (ii') is true. Note that if (ii) is true, then we are done. Otherwise, suppose (ii) is not true, i.e., not all others are either equal to one or the other. Then, without loss of generality, we assume in Q_1 (Q_2 is just similar) there exists x such that either $x < a < b$ or $a < x < b$ or $a < b < x$. Then in each case we can see that either $x < c < b$, or $c < x < d$, or $a < d < x$, respectively. All of these cases imply that (i) is true. So (i) + (ii) is equivalent to (i) + (ii').

Remark 2.10.5. *We claim that if Q_1 and Q_2 are segregated, then one element from one multiset is strictly greater than one element from the other multiset.*

To see this, note that from previous definition, Q_1 and Q_2 are segregated if

(a) max $Q_1 \leq$ min Q_2 and min Q_1< max Q_2, or max $Q_2 \leq$ min Q_1 and min Q_2< max Q_1, or equivalently,

(b) min $Q_1 \leq$ max $Q_1 \leq$ min $Q_2 \leq$ max Q_2 and the three equalities do not hold simultaneously, or min $Q_2 \leq$ max $Q_2 \leq$ min $Q_1 \leq$ max Q_1 and the three equalities do not hold simultaneously.

Therefore, in any case, one element from one multiset is strictly greater than one element from the other multiset.

Note that the reverse of the claim is not true. In a nonsegregated set, we can easily have a case that one element from one multiset is strictly greater than one element from the other multiset.

We mentioned this since we are going to use this property of segregation later in this section and in the algorithm.

Now we will try to find necessary conditions on $\rho_W(y_i \to y_i')$'s for the existence of positive $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$ defined on R^j_+, R^j_-, Q^j_+ and Q^j_-, respectively, satisfying (2.10.6) and (2.10.8), for a given $1 \le j \le q$. We have the following cases depending on the sign of $\sum_{y_i\to y_i'\in Q^j_+} s^j_{y_i\to y_i'} - \sum_{y_i\to y_i'\in Q^j_-} t^j_{y_i\to y_i'}$:

Case (I): Let us assume that

$$\sum_{y_i\to y_i'\in Q^j_+} s^j_{y_i\to y_i'} = \sum_{y_i\to y_i'\in Q^j_-} t^j_{y_i\to y_i'}. \tag{2.10.9}$$

Then equation (2.10.6) and (2.10.8) become

$$\begin{aligned} \sum_{y_i\to y_i'\in R^j_+} u^j_{y_i\to y_i'} &= \sum_{y_i\to y_i'\in R^j_-} v^j_{y_i\to y_i'} \\ \sum_{y_i\to y_i'\in R^j_+} \rho_W(y_i\to y_i') u^j_{y_i\to y_i'} &= \sum_{y_i\to y_i'\in R^j_-} \rho_W(y_i\to y_i') v^j_{y_i\to y_i'}. \end{aligned} \tag{2.10.10}$$

Lemma 2.10.6. *Suppose the reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u^j_{y_i\to y_i'}$ and $v^j_{y_i\to y_i'}$, defined on R^j_+ and R^j_- respectively, satisfying (2.10.6) and (2.10.10).

(ii) The multisets Q^j_1=$\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ and Q^j_2= $\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ are nonsegregated.

PROOF: (i) $\Rightarrow$ (ii): Suppose not, then we have two situations: either $\rho^{u^j}_{min} \le \rho^{u^j}_{max} \le \rho^{v^j}_{min} \le \rho^{v^j}_{max}$ in which the three equalities do not hold simultaneously, or $\rho^{v^j}_{min} \le \rho^{v^j}_{max} \le \rho^{u^j}_{min} \le \rho^{u^j}_{max}$ in which the three equalities do not hold simultaneously.

Without loss of generality, we may assume $\rho_{min}^{u^j} \leq \rho_{max}^{u^j} \leq \rho_{min}^{v^j} \leq \rho_{max}^{v^j}$, and these three equalities do not hold simultaneously.

Note that for any positive $u_{y_i \to y_i'}^j$ and $v_{y_i \to y_i'}^j$ defined on R_+^j and R_-^j respectively, satisfying (2.10.6), we have

$$\begin{aligned} &\sum_{y_i \to y_i' \in R_+^j} \rho_W(y_i \to y_i') u_{y_i \to y_i'}^j \leq \sum_{y_i \to y_i' \in R_+^j} \rho_{max}^{u^j} u_{y_i \to y_i'}^j \\ \leq &\sum_{y_i \to y_i' \in R_-^j} \rho_{min}^{v^j} v_{y_i \to y_i'}^j \leq \sum_{y_i \to y_i' \in R_-^j} \rho_W(y_i \to y_i') v_{y_i \to y_i'}^j. \end{aligned} \tag{2.10.11}$$

Note that in (2.10.11) from left to right, the first equality holds iff $\rho_{min}^{u^j} = \rho_{max}^{u^j}$, the second equality holds iff $\rho_{max}^{u^j} = \rho_{min}^{v^j}$, and the third equality holds iff $\rho_{min}^{v^j} = \rho_{max}^{v^j}$. Since these three equalities do not hold simultaneously, so do the three equalities in (2.10.11). Therefore, we will have

$$\sum_{y_i \to y_i' \in R_+^j} \rho_W(y_i \to y_i') u_{y_i \to y_i'}^j < \sum_{y_i \to y_i' \in R_-^j} \rho_W(y_i \to y_i') v_{y_i \to y_i'}^j.$$

which is a contradiction to equation (2.10.10).

(ii) $\Rightarrow$ (i): We assume that, given $1 \leq j \leq q$, there exists a multiset $\{\rho_W(y_i \to y_i')\}_{i=1}^w$ such that, for the two disjointed proper (nonempty) subsets of W, R_+^j and R_-^j, the multisets $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$ and $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ are nonsegregated. We want to show that there exist $u_{y_i \to y_i'}^j > 0$ for $y_i \to y_i' \in R_+^j$ and $v_{y_i \to y_i'}^j > 0$ for $y_i \to y_i' \in R_-^j$ satisfying both (2.10.6) and (2.10.10).

To show this, suppose the claim is not true, i.e., given $1 \leq j \leq q$, for any $u_{y_i \to y_i'}^j > 0$ and $v_{y_i \to y_i'}^j > 0$ satisfying (2.10.6), (2.10.10) is not satisfied. Equivalently, we assume that for all $u_{y_i \to y_i'}^j, v_{y_i \to y_i'}^j > 0$ satisfying (2.10.6) (it is obvious that such u, v exist), we have

$$\sum_{y_i \to y_i' \in R_+^j} u_{y_i \to y_i'}^j = \sum_{y_i \to y_i' \in R_-^j} v_{y_i \to y_i'}^j$$

and

$$\sum_{y_i \to y_i' \in R_+^j} \rho_W(y_i \to y_i') u_{y_i \to y_i'}^j \neq \sum_{y_i \to y_i' \in R_-^j} \rho_W(y_i \to y_i') v_{y_i \to y_i'}^j.$$

Without loss of generality, we may assume for any $u^j_{y_i\to y_i'}, v^j_{y_i\to y_i'} > 0$ satisfying (2.10.6), we have

$$\sum_{y_i\to y_i'\in R^j_+} u^j_{y_i\to y_i'} = \sum_{y_i\to y_i'\in R^j_-} v^j_{y_i\to y_i'}$$

and

$$\sum_{y_i\to y_i'\in R^j_+} \rho_W(y_i\to y_i')u^j_{y_i\to y_i'} < \sum_{y_i\to y_i'\in R^j_-} \rho_W(y_i\to y_i')v^j_{y_i\to y_i'}. \tag{2.10.12}$$

Since (2.10.12) is true for any $u^j_{y_i\to y_i'}, v^j_{y_i\to y_i'} > 0$ satisfying (2.10.6), we can take the limit on both sides of (2.10.12) over $u^j_{y_i\to y_i'}$ where $y_i\to y_i'\in R^j_+$, and $v^j_{y_i\to y_i'}$ where $y_i\to y_i'\in R^j_-$, respectively. Let $N = \sum_{y_i\to y_i'\in R^j_+} u^j_{y_i\to y_i'} = \sum_{y_i\to y_i'\in R^j_-} v^j_{y_i\to y_i'}$. Denote $k^j_u = \min\{k : y_k\to y_k'\in R^j_+ \text{ and } \rho_W(y_k\to y_k') = \rho^{u^j}_{max}\}$. Denote $k^j_v = \min\{k : y_k\to y_k'\in R^j_+ \text{ and } \rho_W(y_k\to y_k') = \rho^{v^j}_{min}\}$. Note that $0 < u^j_{y_i\to y_i'}, v^j_{y_i\to y_i'} < N$. We can let $u^j_{y_{k^j_u}\to y'_{k^j_u}}$ and $v^j_{y_{k^j_v}\to y'_{k^j_v}}$ go to N, and let $u^j_{y_i\to y_i'}$ (if $i\neq k^j_u$) and $v^j_{y_i\to y_i'}$ (if $i\neq k^j_v$) go to 0. Then after taking the limit on both sides, we have $N\rho^{u^j}_{max}\le N\rho^{v^j}_{min}$, i.e., $\rho^{u^j}_{max}\le\rho^{v^j}_{min}$. Therefore,

$$\sum_{y_i\to y_i'\in R^j_+} \rho^{u^j}_{max}u^j_{y_i\to y_i'} \le \sum_{y_i\to y_i'\in R^j_-} \rho^{v^j}_{min}v^j_{y_i\to y_i'}. \tag{2.10.13}$$

Combined with (2.10.12), we can see that (2.10.11) holds except the three equalities do not hold simultaneously. Then recall that in (2.10.11), from left to right, the first equality holds iff $\rho^{u^j}_{min} = \rho^{u^j}_{max}$, the second equality holds iff $\rho^{u^j}_{max} = \rho^{v^j}_{min}$, and the third equality holds iff $\rho^{v^j}_{min} = \rho^{v^j}_{max}$. Therefore, $\rho^{u^j}_{min}\le\rho^{u^j}_{max}\le\rho^{v^j}_{min}\le\rho^{v^j}_{max}$ and these three equalities do not hold simultaneously. This means that $\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ and $\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ are segregated. This is a contradiction to our nonsegregation assumption. □

Lemma 2.10.7. *Suppose the reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$, defined on R^j_+, R^j_-, Q^j_+ and Q^j_- respectively, satisfying (2.10.6), (2.10.8) and (2.10.9).

(ii) The multisets Q^j_1=$\{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_+\}$ and Q^j_2= $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_-\}$ are nonsegregated.

PROOF: (i)$\Rightarrow$ (ii): Note that (2.10.8) and (2.10.9) imply that (2.10.10). Hence this direction is done from Lemma 2.10.6.

(ii) $\Rightarrow$ (i): From Lemma 2.10.6, there exist positive $u^j_{y_i\to y_i'}$ and $v^j_{y_i\to y_i'}$, defined on R^j_+ and R^j_- respectively, satisfying (2.10.6) and (2.10.10). Since $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$ are defined on Q^j_+ and Q^j_- respectively, we can choose positive $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$ for $y_i \to y_i' \in Q^j_+$ and $y_i \to y_i' \in Q^j_-$ respectively, satisfying (2.10.9). Note that (2.10.9) and (2.10.10) imply (2.10.8), so there exist positive $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$, defined on R^j_+, R^j_-, Q^j_+ and Q^j_- respectively, satisfying (2.10.6), (2.10.8) and (2.10.9). □

Case (II): Let us assume that

$$\sum_{y_i\to y_i'\in Q^j_+} s^j_{y_i\to y_i'} > \sum_{y_i\to y_i'\in Q^j_-} t^j_{y_i\to y_i'}. \tag{2.10.14}$$

Then from equation (2.10.6) and (2.10.8) we have

$$\begin{aligned} \sum_{y_i\to y_i'\in R^j_+} u^j_{y_i\to y_i'} &= \sum_{y_i\to y_i'\in R^j_-} v^j_{y_i\to y_i'} \\ \sum_{y_i\to y_i'\in R^j_+} \rho_W(y_i \to y_i') u^j_{y_i\to y_i'} &< \sum_{y_i\to y_i'\in R^j_-} \rho_W(y_i \to y_i') v^j_{y_i\to y_i'}. \end{aligned} \tag{2.10.15}$$

Lemma 2.10.8. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u^j_{y_i\to y_i'}$ and $v^j_{y_i\to y_i'}$, defined on R^j_+ and R^j_- respectively, satisfying (2.10.6) and (2.10.15).

(ii) One element in $Q^j_2 = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_-\}$ is strictly greater than one element in $Q^j_1 = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_+\}$.

PROOF: (i) $\Rightarrow$ (ii): Suppose not, i.e. we assume that for any $y_l \to y_l' \in R_+^j$ and any $y_r \to y_r' \in R_+^j$, we always have $\rho_W(y_l \to y_l') \geq \rho_W(y_r \to y_r')$. Then it implies that $\rho_{min}^{u^j} \geq \rho_{max}^{v^j}$. Therefore, for any $u_{y_i \to y_i'}^j > 0$ and $v_{y_i \to y_i'}^j > 0$ satisfying (2.10.6), we have

$$\begin{aligned}
\sum_{y_i \to y_i' \in R_+^j} \rho_W(y_i \to y_i') u_{y_i \to y_i'}^j &\geq \rho_{min}^{u^j} \sum_{y_i \to y_i' \in R_+^j} u_{y_i \to y_i'}^j \\
&\geq \rho_{max}^{v^j} \sum_{y_i \to y_i' \in R_-^j} v_{y_i \to y_i'}^j \\
&\geq \sum_{y_i \to y_i' \in R_-^j} \rho_W(y_i \to y_i') v_{y_i \to y_i'}^j
\end{aligned}$$

which is a contradiction to (2.10.15).

(ii) $\Rightarrow$ (i): Let us assume one element in $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ is strictly greater than one element in $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$. In other words, we assume there exists $y_l \to y_l' \in R_+^j$ and $y_r \to y_r' \in R_-^j$ such that $\rho_W(y_r \to y_r') > \rho_W(y_l \to y_l')$. We can pick $u_{y_l \to y_l'}^j = v_{y_r \to y_r'}^j$ quite large and positive, and the other $u_{y \to y'}^j$'s and $v_{y \to y'}^j$'s quite small and positive, such that (2.10.6) and (2.10.15) are both satisfied. $\square$

Lemma 2.10.9. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u_{y_i \to y_i'}^j$, $v_{y_i \to y_i'}^j$, $s_{y_i \to y_i'}^j$ and $t_{y_i \to y_i'}^j$, defined on R_+^j, R_-^j, Q_+^j and Q_-^j, respectively, satisfying (2.10.6), (2.10.8) and (2.10.14).

(ii) One element in $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ is strictly greater than one element in $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$.

PROOF: (i) $\Rightarrow$ (ii): Note that (2.10.8) and (2.10.14) imply (2.10.15). Hence this direction is done from Lemma 2.10.8.

(ii) $\Rightarrow$ (i): From Lemma 2.10.6, there exist positive $u_{y_i \to y_i'}^j$ and $v_{y_i \to y_i'}^j$, defined on R_+^j and R_-^j, respectively, satisfying (2.10.6) and (2.10.15). Note that $s_{y_i \to y_i'}^j$ and $t_{y_i \to y_i'}^j$ are

defined on Q_+^j and Q_-^j, respectively. Therefore, we can choose positive $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$ for $y_i \to y_i' \in Q_+^j$ and $y_i \to y_i' \in Q_-^j$ respectively, satisfying

$$\begin{aligned} & \sum_{y_i\to y_i'\in Q_+^j} s^j_{y_i\to y_i'} - \sum_{y_i\to y_i'\in Q_-^j} t^j_{y_i\to y_i'} \\ = \quad & - \sum_{y_i\to y_i'\in R_+^j} \rho_W(y_i\to y_i')u^j_{y_i\to y_i'} + \sum_{y_i\to y_i'\in R_-^j} \rho_W(y_i\to y_i')v^j_{y_i\to y_i'}. \end{aligned} \tag{2.10.16}$$

Note that (2.10.16) and (2.10.15) imply (2.10.8) and (2.10.14). There exist positive $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ and $t^j_{y_i\to y_i'}$, defined on R_+^j, R_-^j, Q_+^j and Q_-^j, respectively, satisfying (2.10.6), (2.10.8) and (2.10.14). □

Case (III): Let us assume that

$$\sum_{y_i\to y_i'\in Q_+^j} s^j_{y_i\to y_i'} < \sum_{y_i\to y_i'\in Q_-^j} t^j_{y_i\to y_i'}. \tag{2.10.17}$$

Then from equation (2.10.6) and (2.10.8) we have

$$\begin{aligned} \sum_{y_i\to y_i'\in R_+^j} u^j_{y_i\to y_i'} &= \sum_{y_i\to y_i'\in R_-^j} v^j_{y_i\to y_i'} \\ \sum_{y_i\to y_i'\in R_+^j} \rho_W(y_i\to y_i')u^j_{y_i\to y_i'} &> \sum_{y_i\to y_i'\in R_-^j} \rho_W(y_i\to y_i')v^j_{y_i\to y_i'}. \end{aligned} \tag{2.10.18}$$

Lemma 2.10.10. *Suppose the reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u^j_{y_i\to y_i'}$ and $v^j_{y_i\to y_i'}$ defined on R_+^j and R_-^j respectively satisfying (2.10.6) and (2.10.18).

(ii) One element in $\{\rho_W(y_i\to y_i') : y_i\to y_i' \in R_+^j\}$ is strictly greater than one element in $\{\rho_W(y_i\to y_i') : y_i\to y_i' \in R_-^j\}$.

PROOF: It is quite similar to the proof for Lemma 2.10.8 and we will skip it here. □

Lemma 2.10.11. *Suppose the reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then the following statements are equivalent:*

(i) There exist positive $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ *and* $t^j_{y_i\to y_i'}$, *defined on* R^j_+, R^j_-, Q^j_+ *and* Q^j_- *respectively ,satisfying (2.10.6), (2.10.8) and (2.10.17).*

(ii) One element in $\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ *is strictly greater than one element in* $\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$.

PROOF: It is quite similar to the proof for Lemma 2.10.9 and we will skip it here. □

Note that given any two multisets, they are either nonsegregated or segregated. Recall that for two segregated sets, one element from one multiset is strictly greater than on element from the other multiset. Therefore, for $1\le j\le q$, $Q^j_1=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ and $Q^j_2=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ will satisfy at least one of the following three conditions:

i) The multisets $Q^j_1=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ and $Q^j_2=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ are nonsegregated.

ii) One element in $Q^j_2=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ is strictly greater than one element in $Q^j_1=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$.

iii) One element in $Q^j_1=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ is strictly greater than one element in $Q^j_2=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$.

Proposition 2.10.12. *Suppose the reaction network* $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ *satisfies the conditions in Remark 2.9.7. Suppose that there exist* g_W, $h_W\in\mathbb{R}^{\mathscr{O}}\cap\Gamma_W$ *with the given sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15). Then for each* $1\le j\le q$, *there exist positive* $u^j_{y_i\to y_i'}$, $v^j_{y_i\to y_i'}$, $s^j_{y_i\to y_i'}$ *and* $t^j_{y_i\to y_i'}$ *defined on* R^j_+, R^j_-, Q^j_+ *and* Q^j_- *respectively from (2.10.1), (2.10.2), (2.10.3) and (2.10.4), satisfying (2.10.6) and (2.10.8), and the following holds:*

i) If $\displaystyle\sum_{y_i\to y_i'\in Q^j_+} s^j_{y_i\to y_i'}=\sum_{y_i\to y_i'\in Q^j_-} t^j_{y_i\to y_i'}$, *then the multisets* $Q^j_1=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_+\}$ *and* $Q^j_2=\{\rho_W(y_i\to y_i') : y_i\to y_i'\in R^j_-\}$ *are nonsegregated.*

ii) If $\sum_{y_i \to y_i' \in Q_+^j} s^j_{y_i \to y_i'} > \sum_{y_i \to y_i' \in Q_-^j} t^j_{y_i \to y_i'}$, *then one element in* $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ *is strictly greater than one element in* $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$.

iii) If $\sum_{y_i \to y_i' \in Q_+^j} s^j_{y_i \to y_i'} < \sum_{y_i \to y_i' \in Q_-^j} t^j_{y_i \to y_i'}$, *then one element in* $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$ *is strictly greater than one element in* $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$.

Proposition 2.10.13. *Suppose the reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *satisfies the conditions in Remark 2.9.7. Then for each* $1 \leq j \leq q$, R_+^j, R_-^j, Q_+^j *and* Q_-^j *are defined. If the conditions in Proposition 2.10.12 are satisfied, then for each* $1 \leq j \leq q$, *there exist positive* $u^j_{y_i \to y_i'}$, $v^j_{y_i \to y_i'}$, $s^j_{y_i \to y_i'}$ *and* $t^j_{y_i \to y_i'}$ *defined on* R_+^j, R_-^j, Q_+^j *and* Q_-^j *respectively satisfying (2.10.6) and (2.10.8).*

Note that for any $1 \leq j \leq q$, positive $u^j_{y_i \to y_i'}$, $v^j_{y_i \to y_i'}$, $s^j_{y_i \to y_i'}$ and $t^j_{y_i \to y_i'}$ are defined on R_+^j, R_-^j, Q_+^j and Q_-^j, respectively from (2.10.1), (2.10.2), (2.10.3) and (2.10.4). Therefore, for any $1 \leq j \leq q$, the sign of $\sum_{y_i \to y_i' \in Q_+^j} s^j_{y_i \to y_i'} - \sum_{y_i \to y_i' \in Q_-^j} t^j_{y_i \to y_i'}$ is same as the sign of $\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i')$.

Proposition 2.10.14. *Suppose the reaction network* $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *satisfies the conditions in Remark 2.9.7. If there exist* g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *with the given sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15), then the following has to be held for* $1 \leq j \leq q$:

i) If $\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') = 0$, *then the multisets* $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$ *and* $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ *are nonsegregated.*

ii) If $\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') > 0$, *then one element in* $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$ *is strictly greater than one element in* $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$.

iii) If $\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') < 0$, *then one element in* $Q_1^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_+^j\}$ *is strictly greater than one element in* $Q_2^j = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R_-^j\}$.

Next we will form a proposition in terms of g_W and h_W similar to Proposition 2.10.13.

Suppose that the $\rho_W(y_i \to y_i')$'s satisfy the conditions in Proposition 2.10.14, then equivalently, $\rho_W(y_i \to y_i')$'s satisfy the conditions in Proposition 2.10.12. Then for each $1 \leq j \leq q$, there exist positive $u^j_{y_i \to y_i'}, v^j_{y_i \to y_i'}, s^j_{y_i \to y_i'}, t^j_{y_i \to y_i'}$ on R^j_+, R^j_-, Q^j_+ and Q^j_-, respectively satisfying (2.10.6) and (2.10.8).

We define $g^j_W, h^j_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ as follows:

$$\begin{aligned}
g^j_W(y_i \to y_i') &= \frac{u^j_{y_i \to y_i'}}{b^j_{y_i \to y_i'}}, \text{ for } y_i \to y_i' \in R^j_+ \\
g^j_W(y_i \to y_i') &= -\frac{v^j_{y_i \to y_i'}}{b^j_{y_i \to y_i'}}, \text{ for } y_i \to y_i' \in R^j_- \\
g^j_W(y_i \to y_i') &= \frac{s^j_{y_i \to y_i'}}{b^j_{y_i \to y_i'}}, \text{ for } y_i \to y_i' \in Q^j_+ \\
g^j_W(y_i \to y_i') &= -\frac{t^j_{y_i \to y_i'}}{b^j_{y_i \to y_i'}}, \text{ for } y_i \to y_i' \in Q^j_-.
\end{aligned}$$

and for $y_i \to y_i' \in I^j_1 \cup I^j_2$, we can let $g^j_W(y_i \to y_i')$ and $h^j_W(y_i \to y_i')$ be any numbers that are sign compatible with their pre-selected sign patterns, respectively.

Then $g^j_W, h^j_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ are sign-compatible with the given sign patterns of $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, respectively, and satisfy:

$$\sum_{y_i \to y_i' \in ND} b^j_{y_i \to y_i'} g^j_W(y_i \to y_i') = 0 \tag{2.10.19}$$

$$\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h^j_W(y_i \to y_i') + \sum_{y_i \to y_i' \in ND} \rho_W(y_i \to y_i') b^j_{y_i \to y_i'} g^j_W(y_i \to y_i') = 0 \tag{2.10.20}$$

$$h^j_W(y_i \to y_i') = \rho_W(y_i \to y_i') g^j_W(y_i \to y_i'), y_i \to y_i' \in ND. \tag{2.10.21}$$

Proposition 2.10.15. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Then for each $1 \leq j \leq q$, R^j_+, R^j_-, Q^j_+ and Q^j_- are defined. If the conditions in Proposition 2.10.14 are satisfied, then there exist g^j_W, $h^j_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected sign patterns satisfying equations (2.10.19), (2.10.20) and (2.10.21).*

If we can find a common $g_W = g^j_W$ and $h_W = h^j_W$ for all $1 \leq j \leq q$ in Proposition 2.10.15, then g_W and h_W satisfy equations (2.9.13), (2.9.14) and (2.9.15), i.e. we find

a solution of g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected ("valid") pair of sign patterns. But in general, if the assumption is that $\rho_W(y_i \to y_i')$'s satisfy conditions in Proposition 2.10.14, it is not always the case that we can find a common $g_W = g_W^j$ and $h_W = h_W^j$ for all $1 \leq j \leq q$. In the next section, we will consider the case that the basis vectors $\{b^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ satisfy some special property (to be described later). Under this assumption on the basis vectors and the assumption that $\rho_W(y_i \to y_i')$'s satisfy conditions in Proposition 2.10.14, we can find a common $g_W = g_W^j$ and $h_W = h_W^j$ $(1 \leq j \leq q)$ so as to find a solution of g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected ("valid") pair of sign patterns satisfy equations (2.9.13), (2.9.14) and (2.9.15).

2.11 What is nice about a forest basis?

As mentioned earlier, our goal in this section is to discuss the existence of bases (called "forest bases") for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ having especially attractive properties. If such bases exist, then the three conditions in Proposition 2.10.14 on $\rho_W(y_i \to y_i')$'s are also sufficient for the existence of g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected ("valid") pair of sign patterns satisfy equations (2.9.13), (2.9.14) and (2.9.15).

To continue, first let us introduce some basic terms in graph theory.

In [12], an acyclic graph, one that does not contain any cycles, is called a ***forest***. A connected forest is called a ***tree***. Thus, a forest is a graph whose components are trees. The vertices of degree 1 in a tree are its ***leaves***.

Lemma 2.11.1. *[12] The vertices of a tree G can always be enumerated, say as $v_1, .., v_n$, so that every v_i with $i \geq 2$ has a unique neighbour in $\{v_1, .., v_{i-1}\}$.*

PROOF: If G has only one leaf, then the statement is true trivially.

Assume C is a nontrivial tree, i.e., it has at least two leaves.

Pick v_1 as any vertex in C. Let $G_1 = \{v_1\}$. Assume inductively that $v_1, .., v_i$ have been chosen for some $i < |G|$ such that v_{k+1} has a unique neighbour in G_k ($k < i$).

We can claim that G_i is connected by induction i. To see that, note that G_1 is trivially connected. For $i = 1$, v_2 is chosen so v_2 has a unique neighbour in $G_1 = \{v_1\}$, then G_2 is connected. For all $1 \leq k < i$, if we assume G_k is connected, then since v_{k+1} is connected to G_k via v_k, G_{k+1} is also connected.

Now pick a vertex $v \in G - G_i$, where $G_i = G[v_1..v_i]$ is a subgraph of G by removing all vertices not in $\{v_1, ..., v_i\}$. As G is a tree, there exists a unique $v - v_1$ path P. Choose as v_{i+1} the last vertex of P in $G - G_i$, then we conclude that v_{i+1} has a unique neighbour in G_i. Suppose not, i.e., we assume that v_{i+1} has another neighbour in G_i. Since G_i is connected, there is a path between the two neighbours of v_{i+1} in G_i. Since $v_{i+1} \notin G_i$, then we would have a cycle containing the two neighbours and v_{i+1}, which is a contradiction to the tree assumption for G. Therefore it has been proved that vertices of G can be enumerated as $\{v_1, .., v_{|V(G)|}\}$ such that v_{k+1} has a unique neighbour in G_k ($1 \leq k < |V(G)|$). □

We will define a graph based on a set of basis vectors. Given a set of basis vectors $\{b^j\}_{j=1}^q$ of $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$, we define a bipartite graph G with vertices $V = A \cup B$ and edges E, where $A = \{y_i \to y_i'\}_{i=1}^w$, $B = \{b^j\}_{j=1}^q$ and $E = \cup_{j=1}^q \{\{y_i \to y_i', b^j\} : b^j_{y_i \to y_i'} \neq 0\}$. Given a basis $\{b^j : j = 1..q\}$ for $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$, G is the ***basis graph*** for that basis. For $1 \leq j \leq q$, we define a bipartite graph H_j with edges $V_j = A_j \cup B_j$ and edges E_j, where $A_j = \{y_i \to y_i' \in W : b^j_{y_i \to y_i'} \neq 0\}$, $B_j = \{b^j\}$ and $E_j = \{\{y_i \to y_i', b^j\} : y_i \to y_i' \in A_j\}$. H_j is a star-shaped subgraph of G. For $1 \leq j \leq q$, denote $N_G(b^j)$ the neighours of b^j in G. Then $N_G(b^j) = \{y_i \to y_i' : b^j_{y_i \to y_i'} \neq 0\} = H_j \cap W$. Define a new graph G_b based on G, with vertices $V(G_b)$ and edges $E(G_b)$), where $V(G_b) = \{b^j\}_{j=1}^q$ and $(b^j, b^k) \in E(G_b)$ iff $N_G(b^j) \cap N_G(b^k) \neq \emptyset$ or equivalently, $H_j \cap H_k \neq \emptyset$.

Note we have shown in Remark 2.9.2 that any vector in $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$ shall have support on at least three $y_i \to y_i'$'s, otherwise it will contradict the fact that $y_i \to y_i'$'s

are from different and all non-zeroth equivalence classes. Therefore, for any basis vectors $\{b^j : j = 1, ..., q\}$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, $|\{i \in \{1, ..., w\} : b^j_{y_i \to y'_i} \neq 0\}| \geq 3$, or degree of b^j in G $\deg(b^j) \geq 3$. In particular, if a component of the basis graph is trivial with one leaf, then it must be from set $A = \{y_i \to y'_i\}_{i=1}^{w}$.

We say that $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has ***a forest basis*** if there exists a basis of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ such that the graph based on the basis vectors is a forest.

Remark 2.11.2. *Note that if for the given orientation $\mathscr{O}$ and $W \subseteq \mathscr{O}$, $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis, then we claim that for any orientation $\tilde{\mathscr{O}}$ and $\tilde{W} \subseteq \tilde{\mathscr{O}}$ chosen, $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{\tilde{W}}$ has a forest basis.*

To see this, first we make a few observations.

Recall that $Ker\ L_{\mathscr{O}}$ and $Ker\ L_{\tilde{\mathscr{O}}}$ are isomorphic through linear maps $F : Ker\ L_{\mathscr{O}} \to Ker\ L_{\tilde{\mathscr{O}}}$ and $G : Ker\ L_{\tilde{\mathscr{O}}} \to Ker\ L_{\mathscr{O}}$, defined as in Lemma 2.3.4. We define linear maps $F_1 : Ker^{\perp}\ L_{\mathscr{O}} \to Ker^{\perp}\ L_{\tilde{\mathscr{O}}}$ and $G_1 : Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \to Ker^{\perp}\ L_{\mathscr{O}}$ through the following approach.

Let $z^{\mathscr{O}} \in Ker^{\perp}\ L_{\mathscr{O}}$, we define $z^{\tilde{\mathscr{O}}} \in \mathbb{R}^{\tilde{\mathscr{O}}}$ as follows:

$$z^{\tilde{\mathscr{O}}}_{y \to y'} = \begin{cases} z^{\mathscr{O}}_{y \to y'}, & \text{if } y \to y' \in \mathscr{O} \\ -z^{\mathscr{O}}_{y' \to y}, & \text{if } y' \to y \in \mathscr{O}. \end{cases}$$

Let $x^{\tilde{\mathscr{O}}} \in Ker\ L_{\tilde{\mathscr{O}}}$, then $x^{\mathscr{O}} = G(x^{\tilde{\mathscr{O}}}) \in Ker\ L_{\mathscr{O}}$. We have $z^{\mathscr{O}} \cdot x^{\mathscr{O}} = 0$. We also have that

$$\begin{aligned} z^{\tilde{\mathscr{O}}} \cdot x^{\tilde{\mathscr{O}}} &= \sum_{y \to y' \in \tilde{\mathscr{O}}} z^{\tilde{\mathscr{O}}}_{y \to y'} x^{\tilde{\mathscr{O}}}_{y \to y'} \\ &= \sum_{y \to y' \in \tilde{\mathscr{O}} : y \to y' \in \mathscr{O}} z^{\tilde{\mathscr{O}}}_{y \to y'} x^{\tilde{\mathscr{O}}}_{y \to y'} + \sum_{y \to y' \in \tilde{\mathscr{O}} : y' \to y \in \mathscr{O}} z^{\tilde{\mathscr{O}}}_{y \to y'} x^{\tilde{\mathscr{O}}}_{y \to y'} \\ &= \sum_{y \to y' \in \tilde{\mathscr{O}} : y \to y' \in \mathscr{O}} z^{\mathscr{O}}_{y \to y'} x^{\mathscr{O}}_{y \to y'} + \sum_{y \to y' \in \tilde{\mathscr{O}} : y' \to y \in \mathscr{O}} (-z^{\mathscr{O}}_{y' \to y})(-x^{\mathscr{O}}_{y' \to y}) \\ &= \sum_{y \to y' \in \mathscr{O} : y \to y' \in \tilde{\mathscr{O}}} z^{\mathscr{O}}_{y \to y'} x^{\mathscr{O}}_{y \to y'} + \sum_{y' \to y \in \mathscr{O} : y \to y' \in \tilde{\mathscr{O}}} z^{\mathscr{O}}_{y' \to y} x^{\mathscr{O}}_{y' \to y} \\ &= \sum_{y \to y' \in \mathscr{O}} z^{\mathscr{O}}_{y \to y'} x^{\mathscr{O}}_{y \to y'} = 0. \end{aligned}$$

Note the choice of $x^{\tilde{\mathscr{O}}}$ is aribrary. So we have $z^{\tilde{\mathscr{O}}} \in Ker^{\perp} L_{\tilde{\mathscr{O}}}$. Thus the function F_1: $Ker^{\perp} L_{\mathscr{O}} \to Ker^{\perp} L_{\tilde{\mathscr{O}}}$ via $F_1(z^{\mathscr{O}}) = z^{\tilde{\mathscr{O}}}$ is well defined.

Similarly, let $\tilde{z}^{\tilde{\mathscr{O}}} \in Ker\ L_{\tilde{\mathscr{O}}}$, then we define $\tilde{z}^{\mathscr{O}} \in \mathbb{R}^{\mathscr{O}}$ as follows:

$$\tilde{z}^{\mathscr{O}}_{y \to y'} = \begin{cases} \tilde{z}^{\tilde{\mathscr{O}}}_{y \to y'}, & \text{if } y \to y' \in \tilde{\mathscr{O}} \\ -\tilde{z}^{\tilde{\mathscr{O}}}_{y' \to y}, & \text{if } y' \to y \in \tilde{\mathscr{O}}. \end{cases}$$

Then we can similarly show that the function G_1: $Ker^{\perp} L_{\tilde{\mathscr{O}}} \to Ker^{\perp} L_{\mathscr{O}}$ via $G_1(z^{\tilde{\mathscr{O}}}) = z^{\mathscr{O}}$ is well defined. We can verify that, for any $z^{\mathscr{O}} \in Ker^{\perp} L_{\mathscr{O}}$, $G_1 \circ F_1(z^{\mathscr{O}}) = z^{\mathscr{O}}$, and for any $z^{\tilde{\mathscr{O}}} \in Ker^{\perp} L_{\tilde{\mathscr{O}}}$, $F_1 \circ G_1(z^{\tilde{\mathscr{O}}}) = z^{\tilde{\mathscr{O}}}$. Therefore, $F_1 = G_1^{-1}$ and $G_1 = F_1^{-1}$. We can claim that $Ker^{\perp} L_{\mathscr{O}} \cong Ker^{\perp} L_{\tilde{\mathscr{O}}}$.

For a given orientaion $\mathscr{O}$, the set W is based on the choice of representatives for the nonzeroth equivalence classes. Suppose that for two different orientations $\mathscr{O}$ and $\tilde{\mathscr{O}}$, if we have picked $W^{\mathscr{O}} = \{y_i \to y_i' : 1 \le i \le w\}$ from $\mathscr{O}$, we will pick $W^{\tilde{\mathscr{O}}}$ from $\tilde{\mathscr{O}}$ as follows:

If $y_i \to y_i' \in \tilde{\mathscr{O}}$, then pick $y_i \to y_i'$ as the representative for equivalence class $P_i^{\tilde{\mathscr{O}}}$ in $\tilde{\mathscr{O}}$; otherwise, pick $y_i' \to y_i$.

Let $z^{\tilde{\mathscr{O}}} \in Ker^{\perp} L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^{\tilde{\mathscr{O}}}}$, then we can verify that $z^{\mathscr{O}} = G_1(z^{\tilde{\mathscr{O}}}) \in Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_{W^{\mathscr{O}}}$. Similarly, if $z^{\mathscr{O}} \in Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_{W^{\mathscr{O}}}$, then $z^{\tilde{\mathscr{O}}} = F_1(z^{\mathscr{O}}) \in Ker^{\perp} L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^{\tilde{\mathscr{O}}}}$.

Suppose for a given oriention $\tilde{\mathscr{O}}$, we have two different choices of representatives to form two different sets, say $W^1 = \{p_i \to p_i'\}_{i=1}^w$ and $W^2 = \{y_i \to y_i'\}_{i=1}^w$, where $p_i \to p_i'$ and $y_i \to y_i'$ are two choices (they maybe the same equation) of representatives for equivalence class $P_i^{\tilde{\mathscr{O}}}$ in $\tilde{\mathscr{O}}$. Note that there exists $\alpha_{p_i \to p_i'} \neq 0$ such that $\omega_{y_i \to y_i'} - \alpha_{p_i \to p_i'} \omega_{p_i \to p_i'} \in Ker^{\perp} L_{\tilde{\mathscr{O}}}$.

Suppose $z^1 \in Ker^{\perp} L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^1}$. Let $z^2 \in \mathbb{R}^{\tilde{\mathscr{O}}} \cap \Gamma_{W^2}$ be defined as follows, for $y_i \to y_i' \in W$:

$$z^2_{y_i \to y_i'} = \frac{1}{\alpha_{p_i \to p_i'}} z^1_{p_i \to p_i'}, 1 \le i \le w.$$

Then we can show that $z^2 \in Ker^{\perp} L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^2}$. To see that, let $x^{\tilde{\mathscr{O}}} \in Ker\ L_{\tilde{\mathscr{O}}}$, then

$z^1 \cdot x^{\mathscr{O}} = 0$. *Note also that for any* $1 \leq i \leq w$, $x^{\tilde{\mathscr{O}}} \cdot (\omega_{y_i \to y_i'} - \alpha_{p_i \to p_i'} \omega_{p_i \to p_i'}) = 0$, *or* $x^{\tilde{\mathscr{O}}}_{y_i \to y_i'} = \alpha_{p_i \to p_i'} x^{\tilde{\mathscr{O}}}_{p_i \to p_i'}$. *We have that*

$$\begin{aligned} z^2 \cdot x^{\tilde{\mathscr{O}}} &= \sum_{y_i \to y_i' \in W^2} z^2_{y_i \to y_i'} x^{\tilde{\mathscr{O}}}_{y_i \to y_i'} \\ &= \sum_{p_i \to p_i' \in W^1} \frac{1}{\alpha_{p_i \to p_i'}} z^1_{p_i \to p_i'} \alpha_{p_i \to p_i'} x^{\tilde{\mathscr{O}}}_{p_i \to p_i'} \\ &= \sum_{p_i \to p_i' \in W^1} z^1_{p_i \to p_i'} x^{\tilde{\mathscr{O}}}_{p_i \to p_i'} = 0 \end{aligned}$$

Then the linear map $F_2 : Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^1} \to Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^2}$ *via* $F_2(z^1) = z^2$ *is well defined. Similarly we can construct* $G_2 : Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^2} \to Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^1}$ *such that* $G_2 = F_2^{-1}$ *and* $F_2 = G_2^{-1}$. *Details will be skipped here as it is parallel to the case of* F_2.

Now we are ready to show the claim made at the beginning of the remark.

Let $W^{\mathscr{O}} = \{y_i \to y_i' : 1 \leq i \leq w\}$ *from* $\mathscr{O}$, *we define* W^1 *from* $\tilde{\mathscr{O}}$ *as follows:*

If $y_i \to y_i' \in \tilde{\mathscr{O}}$, *then pick* $y_i \to y_i'$ *as the representative for equivalence class* $P_i^{\tilde{\mathscr{O}}}$ *in* $\tilde{\mathscr{O}}$; *otherwise, pick* $y_i' \to y_i$.

Therefore, we could first map the forest basis vectors for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *into* $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^1}$, *through the linear map* F_1 *(in terms of* $\mathscr{O}$ *with* W *and* $\tilde{\mathscr{O}}$ *with* W^1*). Then we map the corresponding image basis vectors for* $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{W^1}$ *into* $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{\tilde{W}}$, *through the linear map* F_2 *(in terms of* W^1 *and* $\tilde{W}$*).*

Note that the fundamental classes C_i*'s stay the same under all orientation. Note that* W *is the set of representatives for all nonzeroth equivalence classes, so it can be regarded as the set of representatives for all zeroth fundamental classes, except that the representative always comes from its corresponding equivalence class. We can then see that the support of each of the resulting basis vectors in* $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{\tilde{W}}$ *and that of the corresponding original basis vector in* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *are representatives from the same fundamental classes. Therefore the original forest basis in* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *and the resulting basis in* $Ker^{\perp}\ L_{\tilde{\mathscr{O}}} \cap \Gamma_{\tilde{W}}$ *share the same basis graph structure if we replace each representative by*

its corresponding fundamental class in the graph. Therefore $Ker^{\perp} L_{\tilde{\mathscr{O}}} \cap \Gamma_{\tilde{W}}$ *also has a forest basis.*

From Remark 2.11.2, we say that the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has the ***forestal*** property, if for the given $\mathscr{O}$ and $W \subseteq \mathscr{O}$, $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis.

Remark 2.11.3. *Note that we have assumed* $q = dim\ Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W > 1$. *However, if* $q = 0$*, then by definition the reaction network has the forestal property.*

Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. We want to claim that if $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis, then the $\rho_W(y_i \to y_i')$'s satisfying the conditions in Proposition 2.10.14 is also sufficient for the existence of g_W, h_W with the pre-selected ("valid") pair of sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15).

Lemma 2.11.4. *If one of the basis graphs for* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ *is a forest, then* $w > 2q$.

PROOF: Note that for any basis $\{\tilde{a}^j\}_{j=1}^q$ in $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$, each $\tilde{a}^j$ has at least 3-reaction support. Therefore, in the basis graph of $\{\tilde{a}^j\}_{j=1}^q$ each $\tilde{a}^j$ has a degree of 3 or more, i.e., $\deg(\tilde{a}^j) \geq 3$. Suppose that $q > 0$. If the basis graph of $\{\tilde{a}^j\}_{j=1}^q$ is a forest, then since the edge is always between some $\tilde{a}^j$ with some $y_i \to y_i'$, we have

$$\begin{aligned} q + w &= \#(\text{vertices}) = \#(\text{components}) + \#(\text{edges}) \\ &= \#(\text{components}) + \sum_{j=1}^{q} (\deg(\tilde{a}^j)) \geq \#(\text{components}) + 3q. \end{aligned}$$

Therefore, $\#(\text{components}) \leq w - 2q$. Since $\#(\text{components}) \geq 1$, we have $w > 2q$. In particular, in the case that $w = 2q + 1$, if G is a forest, there is only one component, i.e. G is a tree. □

Remark 2.11.5. *We see that in the case that there exists a forest basis for* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$*, we have* $2q < w$. *Therefore, from Remark 2.9.6, the necessary condition we found (in*

section 2.10) for the existence of a pair $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15) is trivially satisfied.

Proposition 2.11.6. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ satisfies the conditions in Remark 2.9.7. Suppose that there exists a basis $\{b^j\}_{j=1}^q$ in $Ker\ L_{\mathscr{O}} \cap \Gamma_W$ such that its basis graph G is a forest. Then there exist g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected ("valid") pair of sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15), if and only if $\rho_W(y_i \to y_i')$'s satisfy the conditions in Proposition 2.10.14.*

PROOF: $\Rightarrow$) We have shown this in Proposition 2.10.14 (from section 2.10) without any constraint on the basis graphs of $Ker\ L_{\mathscr{O}} \cap \Gamma_W$.

$\Leftarrow$) We want to show if $\rho_W(y_i \to y_i')$'s satisfy the conditions in Proposition 2.10.14, then there exists a pair $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the pre-selected ("valid") pair of sign patterns satisfying equations (2.9.13), (2.9.14) and (2.9.15). Note that D and ND are defined by the given sign patterns of g_W and h_W.

Let us consider the component(s) of G. Let $g_W = 0$ and $h_W = 0$ to start. We will update g_W and h_W step by step until g_W and h_W have the pre-selected sign patterns and satisfy equations (2.9.13), (2.9.14) and (2.9.15).

Let C be a component of G, then we have two cases.

I) Assume C is a nontrivial tree, i.e., it has at least two leaves. Note the edges in G are between vertices in A and B. Therefore, there exists b^j from B and since $\deg(b^j) \geq 3$, $|C| \geq 4$. Let $A_C = \{y_i \to y_i' : y_i \to y_i' \in C\}$ and $B_C := \{b^j : b^j \in C\}$.

Pick any j with $b^j \in B_C$. Note that the $\rho_W(y_i \to y_i')$'s satisfy the conditions in Proposition 2.10.14. There exists a nonzero pair $g_W^j, h_W^j \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ sign compatible with the given sign patterns, respectively, satisfying equations (2.10.19), (2.10.20) and (2.10.21).

We know that vertices of C can be enumerated as $\{v_1, .., v_{|V(C)|}\}$ such that v_{k+1} has a unique neighbour in $\{v_1, .., v_k\}$ $(1 \leq k < |V(C)|)$. Choice of v_1 is arbitrary, so we can pick v_1 from B, say b^{l_1}.

We define a new graph C_b based on C, with vertices $V(C_b)$ and edges $E(C_b)$), where $V(C_b) = B_C = \{b^j : b^j \in C\}$ and $(b^j, b^k) \in E(C_b)$ iff $N_C(b^j) \cap N_C(b^k) \neq \emptyset$, or equivalently, $H_j \cap H_k \neq \emptyset$.

Let $|V(C_b)| = N_b$. We enumerate the vertices in C_b (or elements in B_C) in the same (relative) order as they are in the C enumeration: $\{v_1, ..., v_{|V(C)|}\}$, as $\{b^{l_1}, ..., b^{l_{N_b}}\}$.

We will use induction on $1 \leq n \leq N_b$ in C_b.

For $n = 1$, for $y_i \rightarrow y_i' \in H_{l_1}$, let $g_W(y_i \rightarrow y_i') = g_W^{l_1}(y_i \rightarrow y_i')$, and $h_W(y_i \rightarrow y_i') = h_W^{l_1}(y_i \rightarrow y_i')$. Then g_W and h_W satisfies (2.10.19), (2.10.20) and (2.10.21) for $j = l_1$, and, g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap H_{l_1}}$, respectively.

Assuming that for $n = k$ ($1 \leq k < N_b$), we have found g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_k$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap (\cup_{j \in \{l_1, ..., l_k\}} H_j)}$, respectively. We want to show next that we will update g_W and h_W such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_{k+1}$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap (\cup_{j \in \{l_1, ..., l_{k+1}\}} H_j)}$, respectively.

Note that $b^{l_{k+1}}$ is also in the enumeration $\{v_1, ..., v_{|V(C)|}\}$ in C and the enumeration in C is such that v_{i+1} has a unique neighbor in $\{v_1, ..., v_i\}$. Therefore, if we assume $v_{n_k} = b^{l_{k+1}}$, then $b^{l_{k+1}}$ has a unique neighbor in $\{v_1, ..., v_{n_k - 1}\}$, say v_{m_k}. We know that v_{m_k} is from A_C and $m_k > 1$, since we pick $v_1 = b^{l_1} \in B_C$. Let us assume that $v_{m_k} = y_{i_{m_k}} \rightarrow y'_{i_{m_k}}$. The vertex v_{m_k} has a unique neighbor in $\{v_1, ..., v_{m_k - 1}\}$, say v_{p_k}. Note that v_{p_k} is from B_C, so it is also in the enumeration $\{b^{l_1}, ..., b^{l_{N_b}}\}$. Note that the order of the elements in B_C in the enumeration $\{b^{l_1}, ..., b^{l_{N_b}}\}$ is consistent with their relative orders in $\{v_1, ..., v_{|V(C)|}\}$. If we say $v_{p_k} = b^{l_r}$, then $r < k + 1$. Note that we have found g_W and h_W satisfying (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_k$. We also have there exists $1 \leq r \leq k$, such that

$H_{l_{k+1}}$ and H_{l_r} have a common vertex $v_{m_k} = y_{i_{m_k}} \rightarrow y'_{i_{m_k}}$. For $y_i \rightarrow y'_i \in H_{l_{k+1}}$, we can let

$$g_W(y_i \rightarrow y'_i) = \frac{g_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})}{g_W^{l_{k+1}}(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})} g_W^{l_{k+1}}(y_i \rightarrow y'_i), y_i \rightarrow y'_i \in ND$$

$$h_W(y_i \rightarrow y'_i) = \frac{g_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})}{g_W^{l_{k+1}}(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})} g_W^{l_{k+1}}(y_i \rightarrow y'_i), y_i \rightarrow y'_i \in D$$

$$h_W(y_i \rightarrow y'_i) = \rho_W(y_i \rightarrow y'_i) g_W(y_i \rightarrow y'_i), y_i \rightarrow y'_i \in D.$$

Note that $g_W^{l_{k+1}}$ and $h_W^{l_{k+1}}$ satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_{k+1}$, with the pre-selected sign patterns of g_W and h_W. Therefore, the sign of $g_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ and $g_W^{l_{k+1}}(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ are the same as they both are the same as the pre-selected sign for $g_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$. Similarly, the sign of $h_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ and $h_W^{l_{k+1}}(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ are the same. The updated g_W and h_W then satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_{k+1}$, and $g_W|_{W \cap H_{l_{k+1}}}$ and $h_W|_{W \cap H_{l_{k+1}}}$ are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap H_{l_{k+1}}}$, respectively.

Note that the updated $g_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ and $h_W(y_{i_{m_k}} \rightarrow y'_{i_{m_k}})$ at the $j + k + 1$ step are the same as their values before this step. We need to argue that g_W and h_W we just updated still satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_k$, and, $g_W|_{W \cap (\cup_{j \in \{l_1, ..., l_k\}} H_j)}$ and $h_W|_{W \cap (\cup_{j \in \{l_1, ..., l_k\}} H_j)}$ are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap (\cup_{j \in \{l_1, ..., l_k\}} H_j)}$. We will show that nothing changes for the element values that have been assigned to g_W and h_W before this ($j = k + 1$) step, i.e., we want to show $(\cup_{j \in \{l_1, ..., l_k\}} H_{l_j}) \cap H_{l_{k+1}} = \{v_{m_k}\}$. To see that, suppose not. We assume there exists $y_i \rightarrow y'_i \in ((\cup_{j \in \{l_1, ..., l_k\}} H_{l_j}) \cap H_{l_{k+1}}) \backslash \{v_{m_k}\}$, i.e., there exists $1 \leq t \leq k$ such that $y_i \rightarrow y'_i \in H_{l_t} \cap H_{l_{k+1}}$. Since $b^{l_{k+1}} = v_{n_k}$ has a unique neighbor $v_{m_k} = y_{i_{m_k}} \rightarrow y'_{i_{m_k}}$ in $\{v_1, ..., v_{n_k - 1}\}$, we have $H_{l_{k+1}} \cap \{v_i : 1 \leq i \leq n_k - 1\} = \{v_{m_k}\}$. Therefore, if $y_i \rightarrow y'_i = v_p$, then $p > n_k$. Recall again that the order of the elements in B_C in the enumeration $\{b^{l_1}, ..., b^{l_{N_b}}\}$ is consistent with their relative orders in $\{v_1, ..., v_{|V(C)|}\}$. Therefore $b^{l_t}, b^{l_{k+1}} \in \{v_1, ..., v_{n_k}\}$. Then v_p has at least two neighbors b^{l_t} and $b^{l_{k+1}}$ in $\{v_1, ..., v_{p-1}\}$, which is a contradiction to the assumption of the enumeration in C.

We have shown that we have found g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_{k+1}$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap (\cup_{j \in \{l_1,...,l_{k+1}\}} H_j)}$.

Therefore, by induction, we find g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_{N_b}$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap (\cup_{j \in \{l_1,...,l_{N_b}\}} H_j)}$. In other words, we find g_W and h_W such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = l_1, ..., l_{N_b}$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W projected on $\Gamma_{W \cap C}$.

II) Assume that the component C is a trivial tree, i.e., it only has one leaf, say $y_i \to y_i'$. In other words, C is an isolated vertex $\{y_i \to y_i'\}$ with degree 0. Then for all $1 \leq j \leq q$, $b^j_{y_i \to y_i'} = 0$. Thus $y_i \to y_i' \in I_1$. We can let $g_W(y_i \to y_i')$ and $h_W(y_i \to y_i')$ be any numbers that are sign compatible with their pre-selected sign patterns.

Since each component of G is disjointed, then after working on all nontrivial components and then all trivial components, we find g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ such that (i) g_W and h_W satisfy (2.10.19), (2.10.20) and (2.10.21) for $j = 1, ..., q$, and (ii) g_W and h_W are sign compatible with the pre-selected sign patterns of g_W and h_W. □

Remark 2.11.7. *Recall the comments made in Remark 2.11.3; we can assume that dim* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W = q \geq 0$ *instead of* $q > 0$.

Remark 2.11.8. *Recall that we have introduced the following definitions and assumptions.*

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$*, and a given orientation* $\mathscr{O}$*, we define* P_i $(0 \leq i \leq w)$ *as the* i^{th} *equivalence class of* $\mathscr{O}$*. Each equivalence class* P_i $(0 \leq i \leq w)$ *has a representative* $y_i \to y_i'$*.* C_i $(0 \leq i \leq w)$ *is the fundamental class containing* P_i*.* $W = \{y_i \to y_i'\}_{i=1}^w$ *is the set of all representatives for the nonzeroth equivalence classes.*

In Theorem 2.11.9 and Proposition 2.11.11 below we suppose that for the given orientation $\mathscr{O}$ *of* $\mathscr{R}$*, the following conditions hold:*

(i) dim $Ker\ L_{\mathscr{O}} \geq 1$ and P_0 (and C_0) is reversible (although these have nothing to do with a particular choice of the orientation).

(ii) For any $y \to y' \in P_i$ $(1 \leq i \leq w)$, there exists $\alpha_{y\to y'} > 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

Also recall that a "valid" pair of sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ is nonzero and sign-compatible with $Ker\ L_{\mathscr{O}}|_W$, as in Section 2.9.

Theorem 2.11.9. *Suppose the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has a forestal property. Then reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has the capacity to admit multiple steady states if and only if the following is true: There exist*

(i) a nonzero vector $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with the stoichiometric subspace S,

(ii) a "valid" pair of sign patterns for g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$,

(iii) a set of parameters $\{\rho_W(y_i \to y_i') : g_W(y_i \to y_i') \neq 0\}$ whose sign pattern is such that the sign of $\rho_W(y_i \to y_i')$ is the same as the ratio of the signs of $h_W(y_i \to y_i')$ and $g_W(y_i \to y_i')$, satisfying the conditions in Proposition 2.10.14, and

(iv) a choice of shelving assignments for each nondegenerate (defined upon the sign patterns of g_W) fundamental class satisfying the conditions in Proposition 2.8.1 (in terms of $\rho_W(y_i \to y_i')$ for condition (ii) in the proposition),

which together satisfy the conditions in Lemma 2.8.2 (in terms of $g_W(y \to y_i')$, $h_W(y_i \to y_i')$ and $\rho_W(y_i \to y_i')$).

Remark 2.11.10. *Note that we have introduced $M_i = M_{y_i\to y_i'} = \ln(\rho_W(y_i \to y_i'))$ when $\rho_W(y_i \to y_i') > 0$, and we let M_i be some large and negative number when $\rho_W(y_i \to y_i') \leq 0$. So we can rewrite conditions in Proposition 2.10.14 and Lemma 2.8.2 in terms of M_i's instead of $\rho_W(y_i \to y_i')$'s. We can see that all the constraints are linear in terms of μ_s*

($s \in \mathscr{S}$) and M_i's ($i = 1, .., q$). Therefore, under the assumption of Theorem 2.11.9, the equality system we will construct later in the algorithm will be completely linear in terms of μ and M_i's.

Therefore, we can be certain about the linearity of the resulting systems to answer the question of multiple steady states if the reaction network has such a forestal property.

Proposition 2.11.11. *Suppose that the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has a forestal property. A few definitions and assumptions are given as in Remark 2.11.8 for the reaction network. Then, the question of whether the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ can admit multiple steady states can be converted into finding whether there exists a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with the stoichiometric subspace S, satisfying any of the linear systems of inequalities and/or equalities (generated from the conditions in Lemma 2.8.2 and Proposition 2.10.14 under different "valid" sign patterns of $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ and different shelf assignments satisfying Proposition 2.8.1) in terms of μ and M_i's.*

2.12 Find a Forest Basis

In this section, we will find a systematic way to check whether the reaction network has the forestal property. Note that the forestal property does not rely on the choice of the orientation $\mathscr{O}$ or the set W of all representatives for the nonzeroth equivalence classes. In other words, given the orientation $\mathscr{O}$ and W, we want to see that if there exists a basis $\{b^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$ which has a forest graph. If we find such a basis with a forest basis graph, then we will call the basis a forest basis and conclude that $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis. To do this, let us start with a given basis $\{a^j\}_{j=1}^q$. If $\{a^j\}_{j=1}^q$ has a forest graph, then we are done. From now on, we will follow the steps until either we find a forest basis or we conclude that there is no forest basis for $KerL^\perp\ L_{\mathscr{O}} \cap \Gamma_W$.

Let us find some necessary (and sufficient) conditions for the existence of a forest basis for $KerL^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$.

Note that for a basis $\{a^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, each a^j has at least 3-reaction support from W. Therefore, the component of the graph under $\{a^j\}_{j=1}^q$ containing a^j will not be trivial. The possible trivial component of the graph G under $\{a^j\}_{j=1}^q$ is of the form $y_i \to y_i'$ where $y_i \to y_i' \in W$.

Lemma 2.12.1. *Given the orientation $\mathscr{O}$ and $W = \{y_i \to y_i'\}_{i=1}^w$ where $y_i \to y_i'$ is the representative for the nonzeroth equivalence class P_i, the following statements are equivalent:*

(i) $y \to y'$ is a trivial component of the forest basis graph under $\{b^j\}_{j=1}^q$, which is a basis of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$.

(ii) $y \to y'$ is a trivial component of any basis graph of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$.

(iii) For any given basis $\{a^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, $a^j_{y\to y'} = 0$ for all $1 \le j \le q$.

PROOF: If $y \to y'$ is a trivial component of the basis graph under $\{a^j\}_{j=1}^q$, then $a^j_{y\to y'} = 0$ for all $1 \le j \le q$. For any basis $\{\tilde{a}^j\}_{j=1}^q$, $\tilde{a}^j_{y\to y'} = 0$, for all $1 \le j \le q$. Therefore, if there is a forest basis $\{b^j\}_{j=1}^q$, then $b^j_{y\to y'} = 0$ for all $1 \le j \le q$. Hence, in the forest graph, $y \to y'$ is a trivial component of the basis graph.

On the other hand, if $y \to y'$ is a trivial component of a forest basis graph of $\{b^j\}_{j=1}^q$, then $b^j_{y\to y'} = 0$ for all $1 \le j \le q$. For any basis graph under $\{\tilde{a}^j\}_{j=1}^q$, $\tilde{a}^j_{y\to y'} = 0$ for all $1 \le j \le q$. Therefore $y \to y'$ is a trivial component of any basis graph, in particular, of the basis graph under $\{a^j\}_{j=1}^q$. □

Note that we are given a basis $\{a^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$. Let us find $U_0 \subset W$ of maximum size, such that $a^j|_{U_0} = 0$, for all $1 \le j \le q$. Each $y_i \to y_i' \in U_0$ is a trivial component in the basis graph under $\{a^j\}_{j=1}^q$. In particular, if there is a forest basis graph under $\{b^j\}_{j=1}^q$, then each $y_i \to y_i' \in U_0$ is a trivial component in the forest basis graph. Note that U_0 can be empty. We define $W_1 = W \backslash U_0$.

Next, let us consider the nontrivial components of a forest basis for $q \geq 1$. In particular, we consider $q \geq 2$, as for $q = 1$, the basis graph is trivially a forest.

Note that from graph theory, we know if a component of a forest is a trivial tree, then there is a single leaf. In other words, each trivial component of a forest has exactly one leaf. Note that both ends of the longest path in a nontrivial tree are leaves. If not, then we can show that there is a longer path, which is a contradiction. Therefore, there exists at least two leaves in each nontrivial component of a forest.

Recall that when we define the basis graph G of $\{b^j\}_{j=1}^q$, we also define H_j $(1 \leq j \leq q)$ based on b^j, a bipartite graph with edges $V_j = A_j \cup B_j$ and edges E_j, where $A_j = \{y_i \to y_i' \in W : b^j_{y_i \to y_i'} \neq 0\}$, $B_j = \{b^j\}$ and $E_j = \{\{y_i \to y_i', b^j\} : y_i \to y_i' \in A_j\}$. If we assume that the basis graph G of $\{b^j\}_{j=1}^q$ is a forest, then each of its components is a tree. Let C be a nontrivial component of G; we then find the longest path in C. We know that the ends of the longest path of a nontrivial tree must be leaves of the nontrivial tree. Therefore, we have the two possibilities for the location of the two leaves in the component C.

(i) Suppose that the ends of the longest path in C come from the same H_j in C, say H_l. Then this component C is equal to H_l and we call this component C a nontrivial star.

(ii) Suppose that the ends of the longest path in C come from different H_j's in C, say H_{l_1} and H_{l_2}. Then the component C is not a star, and H_{l_1}, H_{l_2} must connect to $C \backslash H_{l_1}$, $C \backslash H_{l_2}$, via only $y_{n_1} \to y'_{n_1}$, $y_{n_2} \to y'_{n_2}$, respectively. If not, then there exists a longer path in C, which is a contradiction.

Therefore, if there exists a forest basis $\{b^j\}_{j=1}^q$ with a forest graph G, then for a nontrivial component C of G, we have two situations: either (i) C is a nontrivial star and $C = H_l$ for some $1 \leq l \leq q$, or (ii) C is not a star and there exist two distinct H_{l_1} and H_{l_2} in C such that H_{l_i} connects to $C \backslash H_{l_i}$ via only $y_{n_i} \to y'_{n_i}$, for $i = 1, 2$.

Now if we assume that $Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis, say $\{b^j\}_{j=1}^q$ with a forest graph

G, then there is a nontrivial component C in G. We will proceed through the following two cases depending on whether the nontrivial component C is a star or not.

Case I: C is a nontrivial star.

Suppose that the forest basis graph G under $\{b^j\}_{j=1}^q$ has a nontrivial star component, then it is in the form of H_l for some $1 \leq l \leq q$. Note that for all $y \to y' \in H_l$, we have $b^j_{y\to y'} = 0$ if and only if $j \neq l$. Note also that for all $y \to y' \notin H_l$, we have $b^l_{y\to y'} = 0$. Let $U = \{y \to y' \in W_1 : y \to y' \in H_l\} = \{y \to y' : y \to y' \in H_l\}$. Then we have

i) $|U| \geq 3$.

ii) $b^l|_{W_1\setminus U} = 0$.

iii) $b^j|_U = 0$ if and only if $j \neq l$.

iv) Every $y \to y' \in U$ is a leaf of the forest graph in H_l. Reactions in U together with b^l make up H_l.

Therefore, supp $b^l = U$, and the supp $\{b^j : j = 1, ..., q \text{ and } j \neq l\} = W_1\setminus U$. The vectors $\{b^j|_{W_1\setminus U}\}_{j=1}^q$ are dependent as $b^l|_{W_1\setminus U} = 0$. We can also see that $\{b^j|_U\}_{j=1}^q$ are colinear, as $b^j|_U = 0$ if and only if $j \neq l$. However, $\{b^j|_{U'}\}_{j=1}^q$ are not colinear for any subset U' of W_1 that properly contains U. To show that, suppose not, i.e., assume there exists $U \subset U' \subseteq W_1$ such that $\{b^j|_{U'}\}_{j=1}^q$ are colinear. Then there exists $y \to y' \in U'\setminus U$ such that $\{b^j|_{U\cup\{y\to y'\}}\}_{j=1}^q$ are colinear. Note that $b^l|_U \neq 0$ and $b^j|_U = 0$ for $j \neq l$, so from colinearity we have $b^j_{y\to y'} = 0$ for $j \neq l$, no matter what value $b^l_{y\to y'}$ takes. Note that $b^l|_{W_1\setminus U} = 0$; in particular, $b^l_{y\to y'} = 0$. Thus, $b^j_{y\to y'} = 0$ for all $1 \leq j \leq q$. Therefore, we have $y \to y' \in U_0$, which is a contradiction as we have assumed that $y \to y' \in W_1$. We say $\{b^j|_U\}_{j=1}^q$ are *maximally colinear over* W_1 in the case that $\{b^j|_U\}_{j=1}^q$ $(U \subseteq W_1)$ are colinear, and $\{b^j|_{U'}\}_{j=1}^q$ are not colinear for any subset U' of W_1 that properly contains U.

Let $\{a^j\}_{j=1}^q (q \geq 1)$ be the given basis of $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$. Note that $\{a^j\}_{j=1}^q$ and $\{b^j\}_{j=1}^q$ are both bases of $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$. It is easy to see that for any set $\tilde{U} \subseteq W$,

(i) $\{b^j|_{\tilde{U}}\}_{j=1}^q$ are dependent if and only if $\{a^j|_{\tilde{U}}\}_{j=1}^q$ are dependent, and

(ii) $\{b^j|_{\tilde{U}}\}_{j=1}^q$ are colinear if and only if $\{a^j|_{\tilde{U}}\}_{j=1}^q$ are colinear.

Therefore, we have

(i) $\{b^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent if and only if $\{a^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent, and

(ii) $\{b^j|_U\}_{j=1}^q$ are maximally colinear over W_1 if and only if $\{a^j|_U\}_{j=1}^q$ are maximally colinear over W_1.

Lemma 2.12.2. *We are given the orientation $\mathscr{O}$ and $W = \{y_i \to y_i'\}_{i=1}^w$ where $y_i \to y_i'$ is the representative for the nonzeroth equivalence class P_i. Suppose that there is a forest basis $\{b^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$ and the forest graph G under the basis $\{b^j\}_{j=1}^q$ has a nontrivial star component C. Then the following statements hold:*

There exists $U \subseteq W_1$ with $|U| \geq 3$, such that

(i) There exists $1 \leq l \leq q$, such that the support of $b^l \in C$ is U, and the support of $\{b^j : j = 1, ..., q$ and $j \neq l\}$ is $W_1\backslash U$.

(ii) For any given basis $\{a^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$, we have $\{a^j|_U\}_{j=1}^q$ are maximally colinear over W_1 and $\{a^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent.

Lemma 2.12.3. *We are given the orientation $\mathscr{O}$ and $W = \{y_i \to y_i'\}_{i=1}^w$ where $y_i \to y_i'$ is the representative for the nonzeroth equivalence class P_i. Suppose that there is a forest basis $\{b^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$, and for the given basis $\{a^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$, there exists $U \subset W_1$ with $|U| \geq 3$, such that $\{a^j|_U\}_{j=1}^q$ are maximally colinear over W_1 and $\{a^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent. Then there exist a forest basis $\{\tilde{b}^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$ and $1 \leq l \leq q$, such that the support of $\{\tilde{b}^j : 1 \leq j \leq q,$ and $j \neq l\}$ is $W_1\backslash U$ and the support of $\tilde{b}^l$ is U.*

PROOF: If $q = 1$, it is trivial. Let us assume that $q \geq 2$.

Suppose there is a forest basis $\{b^j\}_{j=1}^q$ and for a given basis $\{a^j\}_{j=1}^q$, there exists $U \subseteq W_1$ and $|U| \geq 3$ such that $\{a^j|_U\}_{j=1}^q$ are maximally colinear over W_1, and $\{a^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent. Thus, $\{b^j|_U\}_{j=1}^q$ are maximally colinear over W_1 and $\{b^j|_{W_1\backslash U}\}_{j=1}^q$ are dependent.

For any basis vectors $\{\tilde{a}^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, $\{\tilde{a}^j|_U\}_{j=1}^q$ are maximally colinear over W_1. We assume that there are k $(1 \leq k \leq q)$ nonzero vectors, say $\tilde{a}^{n_1}|_U, ..., \tilde{a}^{n_k}|_U$, and $q-k$ zero vectors $\tilde{a}^j|_U$ for $j \neq n_1, ..., n_k$. Here $k \geq 1$ is because $U \subseteq W_1 = W \backslash U_0$. Note that the support of each $\tilde{a}^{n_1}|_U, ..., \tilde{a}^{n_k}|_U$ is U, as $U \subseteq W_1 = W \backslash U_0$. In the basis graph G of $\{\tilde{a}^j\}_{j=1}^q$, for all $y \to y' \in U$, $\{y \to y', \tilde{a}^j\} \in E(G)$ if and only if $j \in \{n_1, ..., n_k\}$.

Note that k can be any integer between 1 and q, depending on the basis. Note that $|U| \geq 3$. If $k \geq 2$, then there exists a cycle in the basis graph. Thus, if there is a forest basis of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, k must be 1 for this forest basis. Therefore, for the forest basis $\{b^j\}_{j=1}^q$, there exists $1 \leq l \leq q$ such that for all $j \neq l$, $b^j|_U = 0$ and the support of $b^l|_U$ is U. Therefore, the support of b^j (for any $j \neq l$) is contained in $W_1 \backslash U$. In fact, the support of $\{b^j : j = 1, ..., q \text{ and } j \neq l\}$ is $W_1 \backslash U$. Suppose not; then there exists $y \to y' \in W_1 \backslash U$ such that $b^j_{y \to y'} = 0$ for all $j \neq l$. Since $W_1 = W \backslash U_0$, $b^l_{y \to y'} \neq 0$. Therefore, $\{b^j|_{U \cup \{y \to y'\}}\}_{j=1}^q$ are colinear, which contradicts with the fact that $\{b^j|_U\}_{j=1}^q$ are maximally colinear over W_1.

We have that the support of $\{b^j : j = 1, ..., q \text{ and } j \neq l\}$ is $W_1 \backslash U$. Is the support of b^l equal to U? We will not try to answer this directly. Instead, we will show that we can find a forest basis $\{\tilde{b}^j\}_{j=1}^q$ in which the support of $\{\tilde{b}^j\}_{j=1}^q$ is $W_1 \backslash U$ and the support of $\tilde{b}^l$ is U. To show this, consider the forest basis $\{b^j\}_{j=1}^q$. If the support of b^l is U, then we are done. Otherwise, since $\{b^j|_{W_1 \backslash U}\}_{j=1}^q$ are dependent, $b^l|_{W_1 \backslash U}$ can be expressed as a linear combination of $\{b^j|_{W_1 \backslash U} : 1 \leq j \leq q, \text{ and } j \neq l\}$. We could use this fact to zeroize the entries on $b^l|_{W_1 \backslash U}$ (while keeping other entries the same) to get a new set of basis $\{\tilde{b}^j\}_{j=1}^q$ such that the support of $\{\tilde{b}^j : j = 1, ..., q \text{ and } j \neq l\}$ is $W_1 \backslash U$ and the support of $\tilde{b}^l$ is U. Note that $\{b^j\}_{j=1}^q$ is a forest basis, and the basis graph of $\{\tilde{b}^j\}_{j=1}^q$ is a subgraph of the forest basis graph of $\{b^j\}_{j=1}^q$, so $\{\tilde{b}^j\}_{j=1}^q$ is also a forest basis. □

Case II: C is a nontrivial component and not a star.

Suppose that the forest basis graph G under $\{b^j\}_{j=1}^q$ has a nontrivial component which

is not a star. It contains H_{l_1} and H_{l_2} satisfying the following conditions: for $i = 1, 2$, H_{l_i} contains b^{l_i} and at least 3 reactions, and H_{l_i} connects to the rest of the component via $y_{n_i} \to y'_{n_i}$. If there is a forest basis $\{b^j\}_{j=1}^q$ and the forest graph has a nontrivial component which is not a star, then there exists for $i = 1, 2$, $1 \le l_i \le q$, such that

i) For all $y \to y' \in H_{l_i} \backslash \{y_{n_i} \to y'_{n_i}\}$ and $1 \le j \le q$, $b^j_{y \to y'} \neq 0$ if and only if $j \neq l_i$.

ii) $b^{l_i}_{y_{n_i} \to y'_{n_i}} \neq 0$ and there exists $1 \le j \le q$ with $j \neq l_i$ such that $b^j_{y_{n_i} \to y'_{n_i}} \neq 0$.

iii) For all $y \to y' \notin H_{l_i}$, $b^{l_i}_{y \to y'} = 0$.

For $i = 1, 2$, let $U^i = \{y \to y' \in W_1 : y \to y' \in H_{l_i} \backslash \{y_{n_i} \to y'_{n_i}\}\}$, then $|U^i| \ge 2$. Note that a leaf in a nontrivial component has only degree 1. For $i = 1, 2$, H_{l_i} connects to the rest of the component via $y_{n_i} \to y'_{n_i}$. Therefore, U^1 and U^2 are disjoint. For $i = 1, 2$, let $V^i = \{y \to y' \in W_1 : y \to y' \in H_{l_i}\} = \{y \to y' : y \to y' \in H_{l_i}\}$. Then for $i = 1, 2$, $V^i = U^i \cup \{y_{n_i} \to y'_{n_i}\}$. Note that for $i = 1, 2$, we have

i) $|V^i| \ge 3$.

ii) $b^{l_i}|_{W_1 \backslash V^i} = 0$.

iii) For $1 \le j \le q$, $b^j|_{U^i} = 0$ if and only if $j \neq l_i$.

iv) $b^{l_i}_{y_{n_i} \to y'_{n_i}} \neq 0$ and there exists $1 \le j \le q$ with $j \neq l_i$ such that $b^j_{y_{n_i} \to y'_{n_i}} \neq 0$.

v) For every $y \to y' \in U^i$, $y \to y'$ is a leaf of the forest graph in H_{l_i}. Reactions of V^i together with b^{l_i} make up H_{l_i}, and H_{l_i} connects to the rest of the nontrivial and non-star like component through $y_{n_i} \to y'_{n_i}$.

Therefore, the support of b^{l_i} is V^i and the support of $\{b^j : j = 1, ..., q \text{ and } j \neq l_i\}$ is contained in $W_1 \backslash U^i$. Moreover, note that $\{b^j : j = 1, ..., q \text{ and } j \neq l_i\}$ has support on $y_{n_i} \to y'_{n_i}$. Then similarly as in the case I, we can show that the support of $\{b^j : j = 1, ..., q \text{ and } j \neq l_i\}$ is $W_1 \backslash U^i$. The support of $\{b^j : j = 1, ..., q \text{ and } j \neq l_1, l_2\}$ is contained in $(W_1 \backslash U^1) \cup (W_1 \backslash U^2) = W_1 \backslash (U^1 \cup U^2)$. Note that each $a^k|_{U^i}$ $(1 \le k \le q)$ can be represented as a linear combination of $b^j|_{U^i}$, $j = 1, ..., q$ and each $b^j|_{U^i}$ $(1 \le j \le q)$ can

be represented as a linear combination of $a^k|_{U^i}$, $k = 1, ..., q$. Note also that $\{b^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1, so $\{a^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1.

Note that $\{b^j|_{W_1\setminus V^i}\}_{j=1}^q$ are dependent, and we can claim that in case II, for $i = 1, 2$, $\{b^j|_{W_1\setminus U^i}\}_{j=1}^q$ are not dependent. To see that, just suppose the opposite, i.e. $\{b^j|_{W_1\setminus U^i}\}_{j=1}^q$ are dependent. Note that $b^{l_i}|_{W_1\setminus U^i}$ can be expressed as a linear combination of $\{b^j|_{W_1\setminus U^i} : 1 \leq j \leq q, \text{ and } j \neq l_i\}$. We could use this fact to zeroize the entries on $b^{l_i}|_{W_1\setminus U^i}$ (while keeping other entries the same), in particular, $b^{l_i}_{y_{n_i}\to y'_{n_i}}$, to get a new set of basis $\{\tilde{b}^j\}_{j=1}^q$ where the support of $\tilde{b}^{l_i}$ is U^i and the support of $\{\tilde{b}^j : 1 \leq j \leq q, \text{ and } j \neq l_i\}$ is $W_1\setminus U^i$. Note that we have $\{\tilde{b}^j|_{U^i}\}_{j=1}^q$ being maximally colinear over W_1 and $\{\tilde{b}^j|_{W_1\setminus U^i}\}$ are dependent. Note that since each $\tilde{b}^j$ has to have at least 3-reaction support, $|U^i| \geq 3$. Thus, there is a forest basis $\{\tilde{b}^j\}_{j=1}^q$ and there exists $U = U^i$ with $|U| = |U^i| \geq 3$ such that $\{\tilde{b}^j|_U\}_{j=1}^q$ are maximally colinear over W_1 and $\{\tilde{b}^j|_{W_1\setminus U}\}$ are dependent; it then goes back to the case I, which we have discussed. Therefore in case II, we assume that $\{b^j|_{W_1\setminus V^i}\}_{j=1}^q$ are dependent, but $\{b^j|_{W_1\setminus U^i}\}_{j=1}^q$ are not dependent.

Note that each $b^j|_{W_1\setminus V^i}$ $(1 \leq j \leq q)$ can be represented as a linear combination of $a^k|_{W_1\setminus V^i}$, $k = 1, ..., q$. Thus $\{a^j|_{W_1\setminus V^i}\}_{j=1}^q$ are dependent. Note that each $a^k|_{W_1\setminus U^i}$ $(1 \leq k \leq q)$ can be represented as a linear combination of $b^j|_{W_1\setminus U^i}$, $j = 1, ..., q$. Therefore, $\{a^j|_{W_1\setminus U^i}\}_{j=1}^q$ are not dependent.

Lemma 2.12.4. *We are given the orientation $\mathscr{O}$ and $W = \{y_i \to y'_i\}_{i=1}^w$ where $y_i \to y'_i$ is the representative for the nonzeroth equivalence class P_i. Suppose there is a forest basis $\{b^j\}_{j=1}^q$ of $Ker^\perp L_{\mathscr{O}} \cap \Gamma_W$ and the forest graph G has a nontrivial component C which is not a star. Then the following statements hold:*

There exist two disjoint subsets of W_1, U^1 and U^2, where for $i = 1, 2$, $|U^i| \geq 2$, and $V^i = U^i \cup \{y_{n_i} \to y'_{n_i}\}$ for some $y_{n_i} \to y'_{n_i} \in W_1\setminus(U^1 \cup U^2)$, such that

(i) There exists $1 \leq l_i \leq q$, such that the support of b^{l_i} is V^i, and the support of b^j

$(j \neq l_i)$ is contained in $W_1 \backslash U^i$. Furthermore, the support of b^j $(j \neq l_1, l_2)$ is contained in $W_1 \backslash (U^1 \cup U^2)$.

(ii) For any given basis $\{a^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, we have $\{a^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1, $\{a^j|_{W_1 \backslash V^i}\}_{j=1}^q$ are dependent but $\{a^j|_{W_1 \backslash U^i}\}_{j=1}^q$ are not.

Lemma 2.12.5. *We are given the orientation $\mathscr{O}$ and $W = \{y_i \rightarrow y_i'\}_{i=1}^w$ where $y_i \rightarrow y_i'$ is the representative for the nonzeroth equivalence class P_i. Suppose that*

(i) There is a forest basis $\{b^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$.

(ii) For a given basis $\{a^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, there exist two disjoint subsets of W_1, U^1 and U^2, where $|U^1|, |U^2| \geq 2$, and two sets V_1 and V_2, where for $i = 1, 2$, $V^i = U^i \cup \{y_{n_i} \rightarrow y_{n_i}'\}$ for some $y_{n_i} \rightarrow y_{n_i}' \in W_1 \backslash (U^1 \cup U^2)$, such that $\{a^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1, $\{a^j|_{W_1 \backslash V^i}\}_{j=1}^q$ are dependent but $\{a^j|_{W_1 \backslash U^i}\}_{j=1}^q$ are not.

Then for $i = 1, 2$, there exists a forest basis $\{\tilde{b}^{j,i}\}_{j=1}^q$, and $1 \leq l_i \leq q$ such that the support of $\{\tilde{b}^{j,i} : 1 \leq j \leq q,$ and $j \neq l_i\}$ is $W_1 \backslash U^i$ and the support of $\tilde{b}^{l_i,i}$ is V^i.

PROOF: Suppose that the conditions (i) and (ii) hold. Therefore, for $i = 1, 2$, $\{b^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1, $\{b^j|_{W_1 \backslash V^i}\}_{j=1}^q$ are dependent but $\{b^j|_{W_1 \backslash U^i}\}_{j=1}^q$ are not. For $i = 1, 2$ and for any basis vectors $\{\tilde{a}^j\}_{j=1}^q$ of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, $\{\tilde{a}^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1. We assume that there are k_i $(1 \leq k_i \leq q)$ nonzero vectors, say $\tilde{a}^{n_1^i}|_{U^i}, ..., \tilde{a}^{n_{k_i}^i}|_{U^i}$ and $q - k_i$ zero vectors. The support of each $\tilde{a}^{n_1^i}|_{U^i}, ..., \tilde{a}^{n_{k_i}^i}|_{U^i}$ is U^i, as $U^i \subseteq W_1 = W \backslash U_0$. In the basis graph G of $\{\tilde{a}^j\}_{j=1}^q$, for each $y \rightarrow y' \in U^i$, $\{y \rightarrow y', a^j\} \in E(G)$ if and only if $j \in \{n_1^i, ..., n_{k_i}^i\}$. Note that k_i can be any integer between 1 and q, depending on the basis. Note that if $k_i \geq 2$, there exists a cycle in the basis graph. Thus if there is a forest basis, then $k_i = 1$ for this forest basis. Therefore for the forest basis $\{b^j\}_{j=1}^q$, there exists $1 \leq l_i \leq q$ such that the support of $\{b^j : 1 \leq j \leq q,$ and $j \neq l_i\}$ is contained in $W_1 \backslash U^i$. Furthermore, the support of $\{b^j : 1 \leq j \leq q,$ and $j \neq l_i\}$ is $W_1 \backslash U^i$. Suppose not, then there exists $y \rightarrow y' \in W_1 \backslash U^i$ such that $b^j_{y \rightarrow y'} = 0$, for all $j \neq l_i$. By the definition of W_1, $b^{l_i}_{y \rightarrow y'} \neq 0$. Therefore,

$\{b^j|_{U^i\cup\{y\to y'\}}\}_{j=1}^q$ are colinear, which contradicts with the fact that $\{b^j|_U\}_{j=1}^q$ are maximally colinear over W_1.

We have that the support of $\{b^j : 1 \leq j \leq q, \text{ and } j \neq l_i\}$ is $W_1\backslash U^i$. Is the support of b^{l_i} equal to V^i? We will not try to answer this directly. Instead, we will show that, for each $i = 1, 2$, we can find a forest basis $\{\tilde{b}^{j,i}\}_{j=1}^q$ in which the support of $\{\tilde{b}^{j,i} : j = 1, ..., q \text{ and } j \neq l_i\}$ is $W_1\backslash U^i$ and the support of $\tilde{b}^{l_i,i}$ is V^i.

To show this, consider the forest basis $\{b^j\}_{j=1}^q$. We will do it for each $i = 1$ and 2. If the support of b^{l_i} is V^i, then we are done for the case i. Otherwise, since $\{b^j|_{W_1\backslash V^i}\}_{j=1}^q$ are dependent, $b^{l_i}|_{W_1\backslash V^i}$ can be expressed as a linear combination of $\{b^j|_{W_1\backslash V^i} : 1 \leq j \leq q, \text{ and } j \neq l_i\}$. We could use this fact to zeroize the entries on $b^{l_i}|_{W_1\backslash V^i}$ (while keeping other entries the same) to get a new set of basis $\{\tilde{b}^{j,i}\}_{j=1}^q$, such that the support of $\{\tilde{b}^{j,i} : j = 1, ..., q \text{ and } j \neq l_i\}$ is $W_1\backslash U^i$ and the support of $\tilde{b}^{l_i,i}$ is either U^i or V^i. Note that since we assume $\{b^j|_{W_1\backslash V^i}\}$ are dependent and $\{b^j|_{W_1\backslash U^i}\}$ are not dependent, then $\{\tilde{b}^{j,i}|_{W_1\backslash V^i}\}$ are dependent and $\{\tilde{b}^{j,i}|_{W_1\backslash U^i}\}$ are not dependent. Thus $\tilde{b}^{l_i,i}_{y_{n_i}\to y'_{n_i}} \neq 0$, from the formation of $\{\tilde{b}^{j,i}\}_{j=1}^q$ from $\{b^j\}_{j=1}^q$. Therefore, the support of $\tilde{b}^{l_i,i}$ is V^i. Next we want to show that $\{\tilde{b}^{j,i}\}_{j=1}^q$ is a forest basis. We have two cases depending on whether $b^{l_i}_{y_{n_i}\to y'_{n_i}}$ is zero or not.

(i) Note that if $b^{l_i}_{y_{n_i}\to y'_{n_i}} \neq 0$, then the basis graph of $\{\tilde{b}^{j,i}\}_{j=1}^q$ is a subgraph of the forest basis graph of $\{b^j\}_{j=1}^q$. In this case, since $\{b^j\}_{j=1}^q$ is a forest basis $\{\tilde{b}^{j,i}\}_{j=1}^q$ is also a forest basis.

(ii) Note that if $b^{l_i}_{y_{n_i}\to y'_{n_i}} = 0$, then we can still show that $\{\tilde{b}^{j,i}\}_{j=1}^q$ is also a forest basis. To show it, note that the basis graph of $\{\tilde{b}^{j,i}\}_{j=1}^q$ is not a subgraph of the forest basis graph of $\{b^j\}_{j=1}^q$. However, in the basis graph of $\{\tilde{b}^{j,i}\}_{j=1}^q$, if we remove the edge between $y_{n_i} \to y'_{n_i}$ and $\tilde{b}^{l_i,i}$, then the new graph is a subgraph of the forest basis graph of $\{b^j\}_{j=1}^q$. Thus, the new graph is a forest. However, note that in the new graph the component containing $\tilde{b}^{l_i,i}$ is a star and disconnected with the components containing $\tilde{b}^{j,i}$ for $j \neq l_i$. In the new graph,

if we add back the edge between $y_{n_i} \to y'_{n_i}$ and $\tilde{b}^{1,i}$ to get back the basis graph of $\{\tilde{b}^{j,i}\}_{j=1}^q$, we reduce the component by 1, increase the number of edges by 1 and keep the number of vertices. Then #(components)+#(edges)= #(vertices) holds for the new graph as well as for the basis graph of $\{\tilde{b}^{j,i}\}_{j=1}^q$. Therefore, $\{\tilde{b}^{j,i}\}_{j=1}^q$ is also a forest basis. □

Remark 2.12.6. *We have proved the following statement.*

We are given the orientation $\mathscr{O}$ and $W = \{y_i \to y'_i\}_{i=1}^w$ where $y_i \to y'_i$ is the representative for the nonzeroth equivalence class P_i. Given a basis $\{a^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$, if there exists a forest basis $\{b^j\}_{j=1}^q$, then the forest basis graph has a nontrivial component, which is either a star or not a star. Therefore, one of the following must be true:

(a) There exist $U \subset W_1$ with $|U| \geq 3$, and $1 \leq l \leq q$, such that the support of b^l is U and the support of b^j $(j \neq l)$ is contained in $W_1 \backslash U$. Moreover, the support of $\{b^j : j =, ..., q \text{ and } j \neq l\}$ is $W_1 \backslash U$. We have $\{a^j|_U\}_{j=1}^q$ are maximally colinear over $W_1$and $\{a^j|_{W_1 \backslash U}\}$ are dependent.

(b) There exists two disjoint subsets of W_1, U^1 and U^2, where for $|U^1|, |U^2| \geq 2$, and two sets V_1 and V_2, where for $i = 1, 2$, $V^i = U^i \cup \{y_{n_i} \to y'_{n_i}\}$ for some $y_{n_i} \to y'_{n_i} \in W_1 \backslash (U^1 \cup U^2)$, such that

(i) There exists $1 \leq l_i \leq q$ such that the support of b^{l_i} is V^i and the support of $\{b^j : j = 1, ..., q \text{ and } j \neq l_i\}$ is $W_1 \backslash U^i$.

(ii) The support of $\{b^j : j = 1, ..., q \text{ and } j \neq l_1, l_2\}$ is contained in $W_1 \backslash (U^1 \cup U^2)$.

(iii) $\{a^j|_{U^i}\}_{j=1}^q$ are maximally colinear over W_1, $\{a^j|_{W_1 \backslash V^i}\}_{j=1}^q$ are dependent but $\{a^j|_{W_1 \backslash U^i}\}_{j=1}^q$ are not.

Remark 2.12.7. *We have the following statement.*

Suppose that there is a forest basis $\{b^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}} \cap \Gamma_W$. The following statements are equivalent.

(i) Given a basis $\{a^j\}_{j=1}^q$ of $Ker^\perp\ L_{\mathscr{O}}$, and a set $U \subset W_1$ with $|U| \geq 3$, $\{a^j|_U\}_{j=1}^q$ are maximally colinear over W_1 and $\{a^j|_{W_1 \backslash U}\}_{j=1}^q$ are dependent.

(ii) There exists a forest basis $\{\tilde{b}^j\}_{j=1}^q$ *and* $1 \leq l \leq q$*, such that the support of* $\{\tilde{b}^j : 1 \leq j \leq q, \text{ and } j \neq l\}$ *is* $W_1 \backslash U$ *and the support of* $\tilde{b}^l$ *is* U*.*

Remark 2.12.8. *We have the following statement.*

Suppose that there is a forest basis $\{b^j\}_{j=1}^q$ *of* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$*. The following statements are equivalent.*

(i) Given a basis $\{a^j\}_{j=1}^q$ *of* $Ker^{\perp} L_{\mathscr{O}}$*, two disjoint subsets of* W_1*,* U^1 *and* U^2*, where* $|U^1|, |U^2| \geq 2$*, and two sets* V_1 *and* V_2*, where for* $i = 1, 2$*,* $V^i = U^i \cup \{y_{n_i} \rightarrow y'_{n_i}\}$ *for some* $y_{n_i \rightarrow y'_{n_i}} \in W_1 \backslash (U^1 \cup U^2)$*, we have* $\{a^j|_{U^i}\}_{j=1}^q$ *are maximally colinear over* W_1*,* $\{a^j|_{W_1 \backslash V^i}\}_{j=1}^q$ *are dependent but* $\{a^j|_{W_1 \backslash U^i}\}_{j=1}^q$ *are not dependent.*

(ii) For $i = 1, 2$*, there exists a forest basis* $\{\tilde{b}^{j,i}\}_{j=1}^q$ *and* $1 \leq l_i \leq q$*, such that the support of* $\{\tilde{b}^{j,i} : 1 \leq j \leq q, \text{ and } j \neq l_i\}$ *is* $W_1 \backslash U^i$ *and the support of* $\tilde{b}^{l_i,i}$ *is* V^i*.*

From Remarks 2.12.6, 2.12.7 and 2.12.8, we will design an algorithm to check whether $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis or not. If yes, the algorithm will find such a basis.

In the algorithm, we carry out the following steps to check whether there is a forest basis in $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$, given a basis $\{a^j\}_{j=1}^q$. We start with $p = q$.

Step 1:

We first check if one of conditions (a) or (b) in Remark 2.12.6 is true. If not, then we claim $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ does not have a forest basis and we are done. Otherwise, go to Step 2.

Step 2:

First we discuss it in two situations in Remark 2.12.6.

Case (i): If (a) is true, then we let $U_1 = V_1 = U$. Let $W_2 = W_1 \backslash U_1$, and $W_2' = W_1 \backslash V_1$.

Therefore, there exists $U_1 \subseteq W_1$ with $|U_1| \geq 3$, such that $\{a^j|_{U_1}\}_{j=1}^p$ are maximally colinear over W_1 and $\{a^j|_{W_2'}\}$ are dependent. There exist a forest basis $\{b^j\}_{j=1}^p$ and $1 \leq l \leq p$ such that supp $b^l = V_1$, and supp $\{b^j : j = 1, ..., p \text{ and } j \neq l\} = W_2$.

Case (ii): If (b) is true, then we let $U_1 = U^1$, $V^1 = U_1 \cup \{y_{n_1} \to y'_{n_1}\}$, and $l = l_1$. Let $W_2 = W_1 \backslash U_1$, and $W'_2 = W_1 \backslash V_1$.

Therefore, there exists $U_1 \subseteq W_1$ with $|U_1| \geq 2$, such that $\{a^j|_{U_1}\}_{j=1}^p$ are maximally colinear over W_1, $\{a^j|_{W'_2}\}$ are dependent but $\{a^j|_{W_2}\}$ are not. There exist a forest basis $\{b^j\}_{j=1}^p$ and $1 \leq l \leq p$ such that supp $b^l = V_1$, and supp $\{b^j : j = 1, ..., p \text{ and } j \neq l\} = W_2$.

Then, we deal with both cases in one situation.

Without loss of generality, let us assume that $l = p$; let us assume the basis vectors $\{a^j\}_{j=1}^p$ are reordered so that if $a^k|_{U_1} = 0$, then $a^j|_{U_1} = 0$ for all $1 \leq j \leq k$. Note that $\{a^j|_{U_1}\}_{j=1}^p$ are maximally colinear over W_1. There exists β_j for each $1 \leq j \leq p-1$, such that $a^j|_{U_1} = \beta_j a^p|_{U_1}$. We then may replace a^j $1 \leq j \leq p-1$ with $a^j - \beta_j a^p$ in the basis. In the new basis, $a^j|_{U_1} = 0$ for all $1 \leq j \leq p-1$. Because of the maximally colinearity over W_1, we can show that the support of $\{a^j : 1 \leq j \leq p-1\}$ is W_2. Note that $a^p_{U_1}$ will be unique up to a multiple. Since $\{a^j_{W'_2}\}_{j=1}^p$ are dependent, $a^p|_{W'_2}$ lies in the span of $\{a^j|_{W'_2} : 1 \leq j \leq p-1\}$. Therefore, there exists α_j for each $1 \leq j \leq p-1$, such that $a^p_{W'_2} = \sum_{1 \leq j \leq p-1} \alpha_j a^j|_{W'_2}$. In the new basis we may replace a^p with $a^p - \sum_{1 \leq j \leq p-1} \alpha_j a^j$. We will rename the most updated basis vectors as $\{a^{j,1}\}_{j=1}^p$. Now we have that for all $y \to y' \in W_1 \backslash W'_2$, $a^{p,1}_{y \to y'} \neq 0$ from the definition of W_1, and $a^{p,1}|_{W'_2} = 0$. Therefore, the support of $a^{p,1}$ is V_1 and the support of $\{a^{j,1} : 1 \leq j \leq p-1\}$ is W_2. Note that if (a) is true, then $a^{p,1}$ is unique up to a multiple. If (b) is true, then it is still true that $a^{p,1}$ is unique up to a multiple. Suppose not. Note that $a^{p,1}|_{U_1}$ is unique up to a multiple, and we assume $a^{p,1}|_{V_1}$ is not unique up to a multiple, then we can generate a new vector whose support is $V_1 \backslash U_1$. This contradicts with the fact that the support of every vector in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ must contain at least 3 reactions. Note that the support of b^p (we assume $l = p$) is V_1, and the support of $\{b^j : j = 1, ..., p \text{ and } j \neq p\}$ is W_2. In both cases of (a) and (b) we take $b^p = a^{p,1}$.

Let us consider the basis $\{a^{j,1} : 1 \leq j \leq p-1\}$. We can claim that the subspace span $\{a^{j,1} : 1 \leq j \leq p-1\}$ has a forest basis if and only if the subspace span $\{a^{j,1} : 1 \leq j \leq p\}$ has a forest basis. In particular, the subspace span $\{a^{j,1} : 1 \leq j \leq q-1\}$ has a forest basis if and only if $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis. We will only need to prove for the case that $p = q$ and the proof will be given shortly in Lemma 2.12.10. We rename the basis $\{a^{j,1} : 1 \leq j \leq p-1\}$ as $\{a^j\}_{j=1}^{p^{new}}$, where $p^{new} = p-1$. If p^{new} equals 1, we are done and claim that $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis. Otherwise, we update p to be p^{new} and go to Step 1.

In the end, we will have two situations: one is that we conclude that $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ does not have a forest basis; the other is that when $p^{new} = 1$, we find $\{b^j\}_{j=1}^{q}$, which is a forest basis of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ (we know $\{b^j\}_{j=1}^{q}$ is a forest basis by its formation process).

Remark 2.12.9. *Note that if case (b) in Remark 2.12.6 holds, we have two sets U^1 and U^2 and we only use $U_1 = U^1$ in Step 2. To save some time, we may do step two for $U_1 = U^1$ and then do it for $U_1 = U^2$ (update V^1, W_2, and W_2' accordingly) before going back to Step 1.*

Lemma 2.12.10. *We are given the orientation $\mathscr{O}$ and $W = \{y_i \to y_i'\}_{i=1}^{w}$ where $y_i \to y_i'$ is the representative for the nonzeroth equivalence class P_i. The subspace span $\{a^{j,1} : 1 \leq j \leq q-1\}$ (where $p = q$ in Step 2 of the algorithm) has a forest basis if and only if $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis.*

PROOF: If $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis $\{b^j\}_{j=1}^{q}$ as we assumed, then the subspace span $\{a^{j,1} : 1 \leq j \leq q-1\}$ also has a forest basis. To see this, note that the graph of $\{b^j\}_{j=1}^{q-1}$ can be obtained from removing b^q from the graph of $\{b^j\}_{j=1}^{q}$. In case (a), $W_2 = W_2'$, removing b^q reduces the number of the vertices by 1, reduces the number of edges by $|U_1|$, and increases the number of components by $|U_1| - 1$. Therefore, the change of #(vertices)$-$(#(components)+#(edges)) is $-1 - (-|U_1| + |U_1| - 1) = 0$. In case (b),

$W_2 \neq W_2'$. Removing b^q reduces the number of the vertices by 1, reduces the number of edges by $|U_1|+1$, and increases the number of components by $|U_1|$. Therefore, the change of #(vertices)$-$(#(components)+#(edges)) is $-1-(-(|U_1|+1)+|U_1|)=0$.

On the other hand, we assume the subspace span $\{a^{j,1} : 1 \leq j \leq q-1\}$ has a forest basis. We will show $Ker^{\perp}\, L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis. To see this, note that supp $b^q = V_1$ and supp $\{b^j : 1 \leq j \leq q-1\} = W_2$. In case (a), $V_1 \cap W_2 = \emptyset$. If $\{b^j\}_{j=1}^{q-1}$ has a forest graph, then to get the graph of $\{b^j\}_{j=1}^{q}$, we add an independent component to the graph. Therefore, the new graph (of $\{b^j\}_{j=1}^{q}$) is still a forest graph. In case (b), $V_1 \cap W_2 = \{y_{n_1} \rightarrow y'_{n_1}\}$. If $\{b^j\}_{j=1}^{q-1}$ has a forest graph, then to get the graph of $\{b^j\}_{j=1}^{q}$, we just attach an independent component to a component in the old graph of $\{b^j\}_{j=1}^{q-1}$ through an edge. Therefore, the new graph (of $\{b^j\}_{j=1}^{q}$) is still a forest graph. $\square$

Chapter 3

THE HIGHER DEFICIENCY ALGORITHM

3.1 Overview

The Higher Deficiency Algorithm, implemented in [16], gives results which are similar to those of the Advanced Deficiency Algorithm (see [7] and [8]). It is a reformation and extension of the Advanced Deficiency Algorithm. The Higher Deficiency Algorithm always provides a result when the Advanced Deficiency Algorithm gives one and sometimes provides results when the Advanced Deficiency Algorithm stays silent. In terms of the approach to answer the question of whether a given reaction network has the capacity to admit multiple steady states, the Higher Deficiency Algorithm also produces systems of equalities and inequalities in terms of $\mu \in \mathbb{R}^{\mathscr{S}}$ and M_i's, in the same way that the Advanced Deficiency Algorithm does.

In this chapter, we will present the steps for finding the inequality/equality systems, based on the theory in Chapter 2.

3.2 Constructing Inequality/Equality Systems

We will list the steps for finding the inequality/equality systems, and apply the algorithm on reaction network (3.2.1) given below to help illustrate the steps in the algorithm.

$$E_1 + S_1 \rightleftharpoons E_1S_1 \rightleftharpoons E_1 + S_2 \rightleftharpoons E_1S_2 \to E_1 + S_3$$

$$E_2 + S_3 \rightleftharpoons E_2S_3 \to E_2 + S_2 \rightleftharpoons E_2S_2 \to E_2 + S_1$$

$$E_3 + S_1 \rightleftharpoons E_3S_1 \to E_3 + S_3 \rightleftharpoons E_3S_3 \to E_3 + S_2$$

Step 1: Choose an initial Orientation

We choose an orientation $\mathscr{O}$ for the reaction network.

For reaction network (3.2.1), we can choose $\mathscr{O} = \{E_1 + S_1 \to E_1S_1, E_1S_1 \to E_1 + S_2, E_1 + S_2 \to E_1S_2, E_1S_2 \to E_1 + S_3, E_2 + S_3 \to E_2S_3, E_2S_3 \to E_2 + S_2, E_2 + S_2 \to E_2S_2, E_2S_2 \to E_2 + S_1, E_3 + S_1 \to E_3S_1, E_3S_1 \to E_3 + S_3, E_3 + S_3 \to E_3S_3, E_3S_3 \to E_3 + S_2\}$.

Remark 3.2.1. *Let $\{v^l\}_{l=1}^d$ be a basis for $Ker\ L_{\mathscr{O}}$. Let $y \to y' \in \mathscr{O}$. Consider the following two statements.*

(i) $\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

(ii) For all $1 \le l \le d$, $v^l_{y\to y'} = 0$.

It is easy to see that (i) and (ii) are equivalent. Therefore, if for all $1 \le l \le d$, $v^l_{y\to y'} = 0$, then $y \to y'$ lies in the zeroth equivalence class P_0.

Let $y \to y', \tilde{y} \to \tilde{y}' \in \mathscr{O}\backslash P_0$. We consider the following two statements.

(iii) There exists $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

(iv) There exists $\alpha \neq 0$ such that $v^l_{y\to y'} = \alpha v^l_{\tilde{y}\to\tilde{y}'}$, for all $1 \le l \le d$.

We will show that (iii) and (iv) are equivalent. We can then use (iv) to find nonzeroth equivalence classes and therefore fundamental classes. To see that, note that if there exists $\alpha \neq 0$ such that $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$, then $v^l_{y\to y'} = \alpha v^l_{\tilde{y}\to\tilde{y}'}$ for all $1 \le l \le d$. On

the other hand, suppose that for all $1 \leq l \leq d$, $v^l_{y\to y'} = \alpha v^l_{\tilde{y}\to\tilde{y}'}$. *Since any* $v \in Ker\ L_{\mathscr{O}}$ *is a linear combination of* $v^1, ..., v^d$, *we can easily show that* $v_{y\to y'} = \alpha v_{\tilde{y}\to\tilde{y}'}$. *Therefore* $\omega_{y\to y'} - \alpha\omega_{\tilde{y}\to\tilde{y}'} \in Ker^{\perp}\ L_{\mathscr{O}}$.

In the algorithm, we will use the alternative statements (ii) and (iv) to find the equivalence classes and therefore fundamental classes.

Step 2: Find the Equivalence Classes and Fundamental Classes

First, we find a basis for $Ker\ L_{\mathscr{O}}$, say $v^1, ..., v^d$, where $d =$ dim $Ker\ L_{\mathscr{O}}$.

Then based on Remark 3.2.1, we find the equivalence classes by the following rules:

(i) For $y \to y' \in \mathscr{O}$, if for all $1 \leq l \leq d$, $v^l_{y\to y'} = 0$, then $y \to y' \in P_0$, which is the zeroth equivalence class.

(ii) For $y \to y', \tilde{y} \to \tilde{y}' \in \mathscr{O}\backslash P_0$, if there exists $\alpha \neq 0$ such that $v^l_{y\to y'} = \alpha v^l_{\tilde{y}\to\tilde{y}'}$ for all $1 \leq l \leq d$, then $y \to y'$ and $\tilde{y} \to \tilde{y}'$ belong to the same equivalence class.

We then name each equivalence class from (ii) as P_i, $i = 1, .., w$. Recall that each fundamental class C_i consists of all reactions in the same equivalence class and their reverses if there are any. Also recall the comments in Remarks 2.7.2 and 2.7.3. Next, we will check if the following statements hold:

(a) All reactions in the zeroth equivalence class P_0 are reversible (with respect to $\mathscr{R}$). In other words, P_0 and C_0 are reversible.

(b) For two irreversible (with respect to $\mathscr{R}$) reactions $y \to y'$ and $\tilde{y} \to \tilde{y}'$ in the same equivalence class P_i $(1 \leq i \leq w)$, there exists $\alpha > 0$ such that $v^l_{y\to y'} = \alpha v^l_{\tilde{y}\to\tilde{y}'}$ for all $1 \leq l \leq d$.

If one of the statements above does not hold, then we claim the reaction network cannot have the capacity to admit multiple steady states and exit the algorithm.

For reaction network (3.2.1), $d = \dim\, Ker\ L_{\mathscr{O}} = 4$. A basis $\{v^1, v^2, v^3, v^4\}$ for the linear space $Ker\ L_{\mathscr{O}}$, is given as follows:

$$\begin{array}{c} \\ E_1 + S_1 \to E_1S_1 \\ E_1S_1 \to E_1 + S_2 \\ E_1 + S_2 \to E_1S_2 \\ E_1S_2 \to E_1 + S_3 \\ E_2 + S_3 \to E_2S_3 \\ E_2S_3 \to E_2 + S_2 \\ E_2 + S_2 \to E_2S_2 \\ E_2S_2 \to E_2 + S_1 \\ E_3 + S_1 \to E_3S_1 \\ E_3S_1 \to E_3 + S_3 \\ E_3 + S_3 \to E_3S_3 \\ E_3S_3 \to E_3 + S_2 \end{array} \begin{array}{c} \begin{array}{cccc} v^1 & v^2 & v^3 & v^4 \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \end{pmatrix} \end{array}$$

Therefore reaction network (3.2.1) has the following $w = 6$ equivalence classes:

$$P_0 = \{\}$$

$$P_1 = \{E_1 + S_1 \to E_1S_1, E_1S_1 \to E_1 + S_2\}$$

$$P_2 = \{E_1 + S_2 \to E_1S_2, E_1S_2 \to E_1 + S_3\}$$

$$P_3 = \{E_2 + S_3 \to E_2S_3, E_2S_3 \to E_2 + S_2\}$$

$$P_4 = \{E_2 + S_2 \to E_2S_2, E_2S_2 \to E_2 + S_1\}$$

$$P_5 = \{E_3 + S_1 \to E_3S_1, E_3S_1 \to E_3 + S_3\}$$

$$P_6 = \{E_3 + S_3 \to E_3S_3, E_3S_3 \to E_3 + S_2\}$$

Reaction network (3.2.1) has the following fundamental classes:

$$C_0 = \{\}$$

$$C_1 = \{E_1 + S_1 \to E_1S_1, E_1S_1 \to E_1 + S_1, E_1S_1 \to E_1 + S_2, E_1 + S_2 \to E_1S_1\}$$

$$C_2 = \{E_1 + S_2 \to E_1S_2, E_1S_2 \to E_1 + S_2, E_1S_2 \to E_1 + S_3\}$$

$$C_3 = \{E_2 + S_3 \to E_2S_3, E_2S_3 \to E_2 + S_3, E_2S_3 \to E_2 + S_2\}$$

$$C_4 = \{E_2 + S_2 \to E_2S_2, E_2S_2 \to E_2 + S_2, E_2S_2 \to E_2 + S_1\}$$

$$C_5 = \{E_3 + S_1 \to E_3S_1, E_3S_1 \to E_3 + S_1, E_3S_1 \to E_3 + S_3\}$$

$$C_6 = \{E_3 + S_3 \to E_3S_3, E_3S_3 \to E_3 + S_3, E_3S_3 \to E_3 + S_2\}$$

We can verify that statements (a) and (b) (in Step 2) both hold for reaction network (3.2.1), as P_0 is empty and each P_i $(1 \leq i \leq 6)$ has at most one irreversible reaction. We can move on to the next step.

Step 3: Find the Colinkage Sets

Divide the reaction network into subnetworks within which the reactions are from the same fundamental class and all reactions from the same fundamental class are in the same subnetwork.

For reaction network (3.2.1), the subnetworks are:

Fundamental Class C_1 subnetwork: $E_1 + S_1 \rightleftharpoons E_1S_1 \rightleftharpoons E_1 + S_2$

Fundamental Class C_2 subnetwork: $E_1 + S_2 \rightleftharpoons E_1S_2 \to E_1 + S_3$

Fundamental Class C_3 subnetwork: $E_2 + S_3 \rightleftharpoons E_2S_3 \to E_2 + S_2$

Fundamental Class C_4 subnetwork: $E_2 + S_2 \rightleftharpoons E_2S_2 \to E_2 + S_1$

Fundamental Class C_5 subnetwork: $E_3 + S_1 \rightleftharpoons E_3S_1 \to E_3 + S_3$

Fundamental Class C_6 subnetwork: $E_3 + S_3 \rightleftharpoons E_3S_3 \to E_3 + S_2$

Then the colinkage sets of reaction network (3.2.1) are $\{E_1 + S_1, E_1S_1, E_1 + S_2\}$,

$\{E_1 + S_2, E_1S_2, E_1 + S_3\}$, $\{E_2 + S_3, E_2S_3, E_2 + S_2\}$, $\{E_2 + S_2, E_2S_2, E_2 + S_1\}$, $\{E_3 + S_1, E_3S_1, E_3+S_3\}$, and $\{E_3+S_3, E_3S_3, E_3+S_2\}$. The strong colinkage sets are: within C_1, $\{E_1+S_1, E_1S_1, E_1+S_2\}$ (terminal); within C_2, $\{E_1+S_2, E_1S_2\}$ (non-terminal), $\{E_1+S_3\}$ (terminal); within C_3, $\{E_2 + S_3, E_2S_3\}$ (non-terminal), $\{E_2 + S_2\}$ (terminal); within C_4, $\{E_2 + S_2, E_2S_2\}$ (non-terminal), $\{E_2 + S_1\}$ (terminal); within C_5, $\{E_3 + S_1, E_3S_1\}$ (non-terminal), $\{E_3 + S_3\}$ (terminal); within C_6, $\{E_3 + S_3, E_3S_3\}$ (non-terminal), $\{E_3 + S_2\}$ (terminal).

Step 4: Pick $W \subseteq \mathscr{O}$

Recall that if all reactions in an equivalence class are reversible (with respect to $\mathscr{R}$, not $\mathscr{O}$), then it is called a reversible equivalence class; otherwise, it is called a nonreversible equivalence class.

Pick a representative $y_i \to y_i' \in P_i$ for each equivalence class P_i $(0 \leq i \leq w)$ by the following rules:

If the equivalence class is nonreversible, then pick an irreversible reaction as its representative; otherwise, pick any reversible reaction.

Let $W = \{y_i \to y_i' : i = 1, .., w\}$.

For network (3.2.1), we choose $W = \{E_1S_1 \to E_1 + S_2, E_1S_2 \to E_1 + S_3, E_2S_3 \to E_2 + S_2, E_2S_2 \to E_2 + S_1, E_3S_1 \to E_3 + S_3, E_3S_3 \to E_3 + S_2\}$. Note that $|W| = w = 6$. We also name the i^{th} reaction listed in the set W as $y_i \to y_i'$, for $1 \leq i \leq 6$. For example, $y_1 \to y_1' = E_1S_1 \to E_1 + S_2$, and $y_5 \to y_5' = E_3S_1 \to E_3 + S_3$.

Step 5: Realign the Orientation (if necessary)

We will realign the orientation (if necessary) so that the following is true:

In each nonzeroth equivalence class P_i $(1 \leq i \leq w)$, for any $y \to y' \in P_i$, there exists $\alpha_{y\to y'} > 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp}\, L_{\mathscr{O}}$; or equivalently, for a basis $\{v^l\}_{l=1}^{d}$ of $Ker\ L_{\mathscr{O}}$, there exists $\alpha_{y\to y'} > 0$ such that $v^l_{y_i\to y_i'} = \alpha_{y\to y'}v^l_{y\to y'}$ for all $1 \leq l \leq d$.

If the statement above holds for the current orientation $\mathscr{O}$, then nothing needs to be done and we move to the next step. Otherwise, suppose that for $y \to y' \in P_i \backslash \{y_i \to y_i'\}$ there exists $\alpha_{y\to y'} < 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y\to y'}\omega_{y\to y'} \in Ker^{\perp}\ L_{\mathscr{O}}$; or equivalently, for a basis $\{v^l\}_{l=1}^d$ of $Ker\ L_{\mathscr{O}}$, there exists $\alpha_{y\to y'} < 0$ such that $v^l_{y_i\to y_i'} = \alpha_{y\to y'}v^l_{y\to y'}$, for all $1 \leq l \leq d$. Since the statement (b) in Step 2 is satisfied, P_i must be reversible and so is $y \to y'$. We can realign the orientation $\mathscr{O}$ to $\tilde{\mathscr{O}}$ by replacing $y \to y'$ with $y' \to y$. Therefore, in the new orientation $\tilde{\mathscr{O}}$, for $1 \leq i \leq w$, there exists $\alpha_{y'\to y} > 0$ such that $\omega_{y_i\to y_i'} - \alpha_{y'\to y}\omega_{y'\to y} \in Ker^{\perp}\ L_{\tilde{\mathscr{O}}}$; or equivalently, for a basis $\{\tilde{v}^l\}_{l=1}^d$ of $Ker\ L_{\tilde{\mathscr{O}}}$, there exists $\alpha_{y'\to y} > 0$ such that $\tilde{v}^l_{y_i\to y_i'} = \alpha_{y'\to y}\tilde{v}^l_{y'\to y}$ for all $1 \leq l \leq d$. We will repeat this replacement process to update the orientation until the condition mentioned at the beginning of Step 5 is satisfied. Without loss of generality, we will still call the new orientation $\mathscr{O}$.

Note that since we do not replace the representative in the realignment, $W = \{y_i \to y_i' : i = 1, .., w\}$ is unchanged.

For reaction network (3.2.1), there is no change made to the orientation.

Step 6: Find a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$

In this step , we find a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, say $a^1, ..., a^q$, where $q = \dim\ Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W = w - d$.

For reaction network (3.2.1), $q = w - d = 6 - 4 = 2$. A basis $\{a^1, a^2\}$ for $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$, is given as follows:

$$\begin{array}{r} \\ E_1S_1 \to E_1 + S_2 \\ E_1S_2 \to E_1 + S_3 \\ E_2S_3 \to E_2 + S_2 \\ E_2S_2 \to E_2 + S_1 \\ E_3S_1 \to E_3 + S_3 \\ E_3S_3 \to E_3 + S_2 \end{array} \begin{array}{c} \begin{array}{cc} a^1 & a^2 \end{array} \\ \begin{pmatrix} 0 & 1 \\ 1 & 0 \\ -1 & 0 \\ 0 & -1 \\ 1 & 1 \\ -1 & 0 \end{pmatrix} \end{array}$$

Step 7: Determine linearity by whether there exists a forest basis in $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$

We will follow the procedure for finding out whether there exists a forest basis in $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$ as listed in Section 2.12.

Suppose there exists a forest basis in $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$. By trying to solve for a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign compatible with S from the linear inequality/equality systems (in terms of μ and M_i's), we can answer the question of whether the given reaction network has the capacity to admit multiple steady states. In this case, we say the resulting inequality systems are linear.

Suppose there does not exist any forest basis in $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$; then besides the linear inequalities and equalities in the system, we may need additional nonlinear constraints (equalities) on the M_i's (which we will not mention in detail in this thesis) to determine the answer to the question of multiple steady states. In this case, we say the resulting inequality systems are nonlinear.

For reaction network (3.2.1), we find out there exists a forest basis $\{b^1, b^2\}$ of $Ker^{\perp}$ $L_{\mathscr{O}} \cap \Gamma_W$, where $b^1 = a^1 = [0, 1, -1, 0, 1, -1]$ and $b^2 = a^2 = [1, 0, 0, -1, 1, 0]$. Therefore,

the inequality systems we will construct in later steps to answer the question of multiple steady states are linear.

Step 8 : Pick Sign Patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$

We pick two sign patterns for $\mathbb{R}^{\mathscr{O}}$ satisfying the following conditions:

(i) The two sign patterns are not both zero (meaning the zero vector in $\mathbb{R}^{\mathscr{O}}$).

(ii) The two sign patterns are both sign-compatible with $Ker\ L_{\mathscr{O}}$.

(iii) For each sign pattern and each nonreversible equivalence class P_i $(1 \leq i \leq w)$, the corresponding sign projected on the representative of P_i (and all reactions in P_i) is positive.

We then choose the sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ as projections of these two sign patterns on W.

If no such sign patterns exist, we will claim that the reaction network does not have the capacity to admit multiple steady states and exit the algorithm.

In reaction network (3.2.1), all P_i's $(2 \leq i \leq 6)$ are nonreversible. Thus for each $y_i \to y_i' \in W$ $(2 \leq i \leq 6)$, the signs of $g_W(y_i \to y_i')$ and $h_W(y_i \to y_i')$ both have to be positive. Note that P_1 is reversible. We can verify that any pair of sign assignment ((positive, positive), (positive, negative), or (negative, zero), etc.) to $g_W(y_1 \to y_1')$ and $h_W(y_1 \to y_1')$ will make the sign patterns of g_W and h_W satisfy the three conditions (i), (ii) and (iii). For this step, let us pick the signs of $g_W(y_1 \to y_1')$ and $h_W(y_1 \to y_1')$ both to be positive. Therefore, the sign of each $g_W(y_i \to y_i')$ (or $h_W(y_i \to y_i')$) for $1 \leq i \leq 6$ is positive.

Step 9: Choose Shelvings for reactions in nondegenerate fundamental classes

Note that the shelvings assignments are applicable for reactions in nondegenerate fundamental classes C_i's. We assign a shelf to each reaction in a nondegenerate fundamental class following the conditions in Proposition 2.8.1.

For reaction network (3.2.1), note that from Step 8 all signs for $g_W(y_i \to y_i')$'s $(1 \leq$

$i \leq 6$) are positive. Note also that from Step 3, the nontrivial strong colinkage set in fundamental class C_1 is terminal and every nontrivial strong colinkage set in fundamental class C_i $(2 \leq i \leq 6)$ is non-terminal. Therefore, reactions in C_1 can be all on the upper, middle or lower shelf and reactions in C_i $(2 \leq i \leq 6)$ must be all on the middle shelf. One of the shelving assignments is given as follows, where we put reactions in C_1 on the upper shelf:

$$\mathscr{U}_1 = \{E_1 + S_1 \to E_1S_1, E_1S_1 \to E_1 + S_1, E_1S_1 \to E_1 + S_2\}, \mathscr{M}_1 = \{\}, \mathscr{L}_1 = \{\}$$

$$\mathscr{U}_2 = \{\}, \mathscr{M}_2 = \{E_1 + S_2 \to E_1S_2, E_1S_2 \to E_1 + S_2, E_1S_2 \to E_1 + S_3\}, \mathscr{L}_2 = \{\}$$

$$\mathscr{U}_3 = \{\}, \mathscr{M}_3 = \{E_2 + S_3 \to E_2S_3, E_2S_3 \to E_2 + S_3, E_2S_3 \to E_2 + S_2\}, \mathscr{L}_3 = \{\}$$

$$\mathscr{U}_4 = \{\}, \mathscr{M}_4 = \{E_2 + S_2 \to E_2S_2, E_2S_2 \to E_2 + S_2, E_2S_2 \to E_2 + S_1\}, \mathscr{L}_4 = \{\}$$

$$\mathscr{U}_5 = \{\}, \mathscr{M}_5 = \{E_3 + S_1 \to E_3S_1, E_3S_1 \to E_3 + S_1, E_3S_1 \to E_3 + S_3\}, \mathscr{L}_5 = \{\}$$

$$\mathscr{U}_6 = \{\}, \mathscr{M}_6 = \{E_3 + S_3 \to E_3S_3, E_3S_3 \to E_3 + S_3, E_3S_3 \to E_3 + S_2\}, \mathscr{L}_6 = \{\}$$

where $\mathscr{U}_i$, $\mathscr{M}_i$ and $\mathscr{L}_i$ are the upper, middle and lower shelves, respectively, for the nondegenerate fundamental class C_i $(1 \leq i \leq 6)$.

In Steps 10 to 13, we will construct the inequality system according to the sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ chosen in Step 8 and the shelving assignments for nondegenerate fundamental classes chosen in Step 9.

Step 10: Add Shelving Equalities and Inequalities for nondegenerate fundamental classes

Recall that for each nondegenerate fundamental class C_i $(1 \leq i \leq w)$, we let $M_i = \ln(\rho_W(y_i \to y_i'))$ if $\rho_W(y_i \to y_i') > 0$; otherwise, we let M_i be an arbitrary (large and negative) number which is to be solved later.

Suppose that C_i $(1 \leq i \leq w)$ is a nondegenerate fundamental class. Let $y \to y' \in C_i$. If the reaction $y \to y'$ is on the middle shelf of the bookcase corresponding to the fundamental class C_i, then $y \cdot \mu = M_i$ is added to the inequality system. If $y \to y'$ is on the upper shelf,

then $y \cdot \mu > M_i$ is added to the inequality system. If $y \to y'$ is on the lower shelf, then $y \cdot \mu < M_i$ is added to the inequality system.

For reaction network (3.2.1), recall the shelving assignment given in Step 9. Recall also that $W = \{E_1S_1 \to E_1+S_2, E_1S_2 \to E_1+S_3, E_2S_3 \to E_2+S_2, E_2S_2 \to E_2+S_1, E_3S_1 \to E_3+S_3, E_3S_3 \to E_3+S_2\}$ and we named the i^{th} reaction listed in the set W as $y_i \to y_i'$. The inequalities and (simplified) equalities generated in Step 10 according to the shelving assignment given in Step 9 are as follows:

$$\begin{aligned}
\mu_{E_1} + \mu_{S_1} &> M_1 \\
\mu_{E_1S_1} &> M_1 \\
\mu_{E_1} + \mu_{S_2} &> M_1 \\
\mu_{E_1} + \mu_{S_2} &= M_2 = \mu_{E_1S_2} \\
\mu_{E_2} + \mu_{S_3} &= M_3 = \mu_{E_2S_3} \\
\mu_{E_2} + \mu_{S_2} &= M_4 = \mu_{E_2S_2} \\
\mu_{E_3} + \mu_{S_1} &= M_5 = \mu_{E_3S_1} \\
\mu_{E_3} + \mu_{S_3} &= M_6 = \mu_{E_3S_3}
\end{aligned}$$

Step 11: Add Upper and Lower Shelves Inequalities for P_i's with nondegenerate C_i's

Suppose C_i $(1 \leq i \leq w)$ is a nondegenerate fundamental class. Let $y \to y' \in P_i$. If $g_W(y_i \to y_i') > 0$ and $y \to y'$ is on the upper shelf or if $g_W(y_i \to y_i') < 0$ and $y \to y'$ is on the lower shelf, then $y \cdot \mu < y' \cdot \mu$ is added to the inequality system. If $g_W(y_i \to y_i') > 0$ and $y \to y'$ is on the lower shelf or if $g_W(y_i \to y_i') < 0$ and $y \to y'$ is on the upper shelf, then $y \cdot \mu > y' \cdot \mu$ is added to the inequality system.

For reaction network (3.2.1), note that the sign of $g_W(y_1 \to y_1')$ is chosen to be positive in Step 8. Note also that from the shelving assignment given in Step 9, all reactions

in nondegenerate fundamental class C_1 are on the upper shelf. Therefore the following inequalities are added to the system:

$$\mu_{E_1} + \mu_{S_1} < \mu_{E_1 S_1} \tag{3.2.1}$$
$$\mu_{E_1 S_1} < \mu_{E_1} + \mu_{S_2}$$

Step 12: Add Equalities and Inequalities for P_i's with degenerate C_i's

Suppose C_i $(1 \leq i \leq w)$ is a degenerate fundamental class. Let $y \to y' \in P_i$; if $h_W(y_i \to y_i') > 0$, then $y \cdot \mu > y' \cdot \mu$ is added to the inequality system; if $h_W(y_i \to y_i') < 0$, then $y \cdot \mu < y' \cdot \mu$ is added to the system; if $h_W(y_i \to y_i') = 0$, then $y \cdot \mu = y' \cdot \mu$ is added to the inequality system.

For reaction network (3.2.1), this step is not applicable as there is no degenerate fundamental class.

Step 13: Add M Equalities and Inequalities

Note the conditions in Proposition 2.10.14 are comparisons among $\rho_W(y_i \to y_i')$; we can directly convert them to comparisons among M_i's.

Recall that two multisets Q_1 and Q_2 are nonsegregated if one of the following two cases holds:

(I) min $Q_1 <$ max Q_2, and min $Q_2 <$ max Q_1.

(II) min $Q_1 =$ max Q_2, and min $Q_2 =$ max Q_1.

Recall that in Remark 2.10.3, we define two multisets Q_1 and Q_2 as nonsegregated if one of the following two cases holds:

(i) There exist a from one of two multisets and $b < c$ from the other multiset such that $b < a < c$.

(ii) All elements in both multisets are equal or there exists $a \neq b$ such that in each multiset, a portion of elements are equal to a and the rest (both not empty) are equal to b.

Recall that in Remark 2.10.4, we define that two multisets Q_1 and Q_2 are nonsegregated if at least one of the following two cases holds:

(i) There exist a from one of two multisets and $b < c$ from the other multiset such that $b < a < c$.

(ii') All elements in Q_1 and Q_2 are equal, or there exist $a, b \in Q_1$ and $c, d \in Q_2$ such that $c = a < b = d$.

We will use the definition in Remark 2.10.4 here in the algorithm.

Recall that given a sign pattern of $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, we define $D = \{y_i \to y_i' \in W : g_W(y_i \to y_i') \neq 0\}$. For $1 \leq j \leq q$, we have $H_j = \{y_i \to y_i' : b^j_{y_i \to y_i'} \neq 0\} \cup \{b^j\}$. Also recall that for $1 \leq j \leq q$, we define $R^j_+ = \{y_i \to y_i' : b^j_{y_i \to y'} g_W(y_i \to y_i') > 0\}$, $R^j_- = \{y_i \to y_i' : b^j_{y_i \to y'} g_W(y_i \to y_i') < 0\}$, $Q^j_1 = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_+\}$, and $Q^j_2 = \{\rho_W(y_i \to y_i') : y_i \to y_i' \in R^j_-\}$.

Note that $\sum_{y_i \to y_i' \in D} b^j_{y_i \to y_i'} h_W(y_i \to y_i') = \sum_{y_i \to y_i' \in D \cap H_j} b^j_{y_i \to y_i'} h_W(y_i \to y_i')$. Depending on the sign patterns of b^j $(1 \leq j \leq q)$ and $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, the conditions in Proposition 2.10.14 can be rewritten as follows:

(a) If $\sum_{y_i \to y_i' \in D \cap H_j} b^j_{y_i \to y_i'} h_W(y_i \to y_i') > 0$, then one element in Q^j_2 are strictly greater than one element in Q^j_1.

(b) If $\sum_{y_i \to y_i' \in D \cap H_j} b^j_{y_i \to y_i'} h_W(y_i \to y_i') < 0$, then one element in Q^j_1 are strictly greater than one element in Q^j_2.

(c) If $\sum_{y_i \to y_i' \in D \cap H_j} b^j_{y_i \to y_i'} h_W(y_i \to y_i') = 0$, then Q^j_1 and Q^j_2 are nonsegregated. In other words, at least one of the following two cases holds:

(i) There exist a from one of two multisets (Q^j_1 and Q^j_2) and $b < c$ from the other multiset such that $b < a < c$.

(ii') All elements in Q^j_1 and Q^j_2 are equal or there exist $a, b \in Q^j_1$ and $c, d \in Q^j_2$ such that $c = a < b = d$.

Note that for each case (a), (b), and (c), we can derive more subcases with detailed inequalities and/or equalities in terms of the elements $\rho_W(y_i \to y_i')$'s in Q_1^j and Q_2^j. For example, suppose that for a given $j \in \{1, ..., q\}$ such that C_j is nondegenerate, $Q_1^j = \{\rho_W(y_i \to y_i')\}_{i=1}^3$ and $Q_2^j = \{\rho_W(y_i \to y_i')\}_{i=4}^5$. Suppose the sign patterns of b^j and $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ make it possible for $\sum_{y_i \to y_i' \in D \cap H_j} b^j_{y_i \to y_i'} h_W(y_i \to y_i')$ to be positive, then the assumption in case (a) is satisfied. To satisfy case (a), we could have $\rho_W(y_1 \to y_1') < \rho_W(y_4 \to y_4')$ or $\rho_W(y_1 \to y_1') < \rho_W(y_5 \to y_5')$ or $\rho_W(y_2 \to y_2') < \rho_W(y_4 \to y_4')$ or $\rho_W(y_2 \to y_2') < \rho_W(y_5 \to y_5')$ or $\rho_W(y_3 \to y_3') < \rho_W(y_4 \to y_4')$ or $\rho_W(y_3 \to y_3') < \rho_W(y_5 \to y_5')$. Note that since we have the signs of $\rho_W(y_i \to y_i')$'s assigned, we will remove (if applicable) the subcases which cannot hold for the reasons such as a nonnegative $\rho_W(y_i \to y_i')$ cannot be larger than or equal to a positive $\rho_W(y_k \to y_k')$. Note that no subcases will be removed if all $\rho_W(y_i \to y_i')$'s are positive. After we find subcases for (a), we can similarly derive more subcases for (b) and (c) if their respective assumptions are satisfied.

In general, for this step, we pick one subcase (in terms of inequalities and/or equalities in terms of $\rho_W(y_i \to y_i')$'s in Q_1^j and Q_2^j) for each nondegenerate fundamental class C_i $(1 \leq i \leq w)$. We then rephrase all such subcases in terms of M_i's (instead of $\rho_W(y_i \to y_i')$'s) and add these inequalities and equalities of M_i's into the inequality system.

Remark 3.2.2. *Suppose we have a subset of basis vectors of $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ with dimension q, $\{b^j\}_{j=1}^n$ $(n \leq q)$ such that each b^j $(1 \leq j \leq n)$ has support on three reactions, and supp $\{b^j\}_{j=1}^n = \{y_i \to y_i'\}_{i=1}^{n+2}$. For any three reactions from $\{y_i \to y_i'\}_{i=1}^{n+2}$, either there exists b^k $(1 \leq k \leq n)$ with support on these reactions or we can find a vector as a linear combination of the b^j's $(1 \leq j \leq n)$ such that its support is these three reactions. Suppose there exists b^j $(1 \leq j \leq n)$ with support on three reactions $y_{i_1} \to y_{i_1}'$, $y_{i_2} \to y_{i_2}'$ and $y_{i_3} \to y_{i_3}'$. Note that if C_{i_1} and C_{i_2} are both degenerate fundamental classes, then C_{i_3} must*

also be degenerate. Therefore, for fundamental classes C_i's $(1 \leq i \leq n+2)$ we have three possible cases:

(i) All fundamental classes C_i's $(1 \leq i \leq n+2)$ are nondegenerate.

(ii) All but one C_k are nondegenerate.

(iii) All fundamental classes C_i's $(1 \leq i \leq n+2)$ are degenerate.

Therefore, for deriving the $\rho_W(y_i \to y_i')$'s (therefore M_i's) comparisons, we only need to consider the cases (i) and (ii).

Suppose the sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ are given. Based on Remark 1.7.6 of the Advanced Deficiency Theory (see [7] for more information), we claim that the conditions in Proposition 2.10.14 can be rewritten by considering $j = 1, ..., n$ at once:

(I) For C_i's $(1 \leq i \leq n+2)$, if all but one fundamental class, say C_1, are nondegenerate, then we have two subcases:

(i) If $h_W(y_1 \to y_1') = 0$, then all $\rho_W(y_i \to y_i')$'s $(i \neq 1)$ are equal.

(ii) Suppose that $h_W(y_1 \to y_1') \neq 0$. There exists an enumeration of $\{\rho_W(y_i \to y_i')\}_{i=2}^{n+2}$, say $\{\rho_W(y_{k_1} \to y_{k_1}'), ..., \rho_W(y_{k_{n+1}} \to y_{k_{n+1}}')\}$, such that either $\rho_W(y_{k_1} \to y_{k_1}') < ... < \rho_W(y_{k_{n+1}} \to y_{k_{n+1}}')$, $\rho_W(y_{k_1} \to y_{k_1}') = ... = \rho_W(y_{k_{n+1}} \to y_{k_{n+1}}')$, or $\rho_W(y_{k_1} \to y_{k_1}') > ... > \rho_W(y_{k_{n+1}} \to y_{k_{n+1}}')$ is true.

(II) If all C_i's $(1 \leq i \leq n+2)$ are nondegenerate, then there are only three possible cases of $\rho_W(y_i \to y_i')$'s comparison. There exists an enumeration of $\{\rho_W(y_i \to y_i')\}_{i=1}^{n+2}$, say $\{\rho_W(y_{k_1} \to y_{k_1}'), ..., \rho_W(y_{k_{n+2}} \to y_{k_{n+2}}')\}$, such that either $\rho_W(y_{k_1} \to y_{k_1}') < ... < \rho_W(y_{k_{n+2}} \to y_{k_{n+2}}')$, $\rho_W(y_{k_1} \to y_{k_1}') = ... = \rho_W(y_{k_{n+2}} \to y_{k_{n+2}}')$, or $\rho_W(y_{k_1} \to y_{k_1}') > ... > \rho_W(y_{k_{n+2}} \to y_{k_{n+2}}')$ is true.

In the Higher Deficiency Algorithm, we will adjust Step 13 accordingly to make use of these properties. For the case (ii) of (I) and case (II), we may remove some subcases following the given signs of $\rho_W(y_i \to y_i')$'s (especially when they are not having the same signs). Then we can rephrase the subcases into comparisons of M_i's.

Next we will show that both cases (I) and (II) hold.

We will show that case (I) holds. Suppose that $\{b^j\}_{j=1}^n$ *is a subset of basis for* $Ker^\perp$ $L_{\mathscr{O}} \cap \Gamma_W$ *where each* b^j *has support on three reactions, and supp* $\{b^j\}_{j=1}^n = \{y_i \to y_i'\}_{i=1}^{n+2}$. *Also, suppose that a sign pattern of* $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *with* $D \cap$ *supp* $\{b^j\}_{j=1}^n = \{y_1 \to y_1'\}$ *is given. Without loss of generality, let us assume that* b^j $(1 \leq j \leq n)$ *has support on* $y_1 \to y_1'$, $y_2 \to y_2'$ *and* $y_{j+2} \to y_{j+2}'$. *From equations (2.9.13) and (2.9.14) we have*

$$b^j_{y_2 \to y_2'} g_W(y_2 \to y_2') + b^j_{y_{j+2} \to y_{j+2}'} g_W(y_{j+2} \to y_{j+2}') = 0, \qquad j = 1, ..., n \quad (3.2.2)$$

$$b^j_{y_1 \to y_1'} h_W(y_1 \to y_1') + \rho_W(y_2 \to y_2') b^j_{y_2 \to y_2'} g_W(y_2 \to y_2') + \rho_W(y_{j+2} \to y_{j+2}') b^j_{y_{j+2} \to y_{j+2}'} g_W(y_{j+2} \to y_{j+2}') = 0, \quad j = 1, ..., n. \quad (3.2.3)$$

From the j^{th} $(1 \leq j \leq n)$ *case of equations (3.2.2), we have that* $b^j_{y_2 \to y_2'} g_W(y_2 \to y_2') = -b^j_{y_{j+2} \to y_{j+2}'} g_W(y_{j+2} \to y_{j+2}') = 0$. *We then plug it into the* j^{th} *case of equations (3.2.3) to get the following:*

$$b^j_{y_1 \to y_1'} h_W(y_1 \to y_1') + (\rho_W(y_2 \to y_2') - \rho_W(y_{j+2} \to y_{j+2}')) b^j_{y_2 \to y_2'} g_W(y_2 \to y_2') = 0, \qquad j = 1, ..., q. \quad (3.2.4)$$

If $h_W(y_1 \to y_1') = 0$ *as in case (i) of (I), then we have* $\rho_W(y_2 \to y_2') = \rho_W(y_{j+2} \to y_{j+2}')$ *for* $1 \leq j \leq n$. *Then all* $\rho_W(y_i \to y_i')$*'s* $(2 \leq i \leq n+2)$ *are equal.*

Next let us assume that $h_W(y_1 \to y_1') \neq 0$. *We could rescale* b^j*'s by letting* $\tilde{b}^j = b^j / b^j_{y_1 \to y_1'}$ *for all* $1 \leq j \leq n$, *making* $\tilde{b}^j_{y_1 \to y_1'} = 1$ *for all* $1 \leq j \leq n$. *From equations (3.2.4) in terms of* $\tilde{b}^j$*'s* $(1 \leq j \leq n)$ *and the assumption that* $g_W(y_2 \to y_2') \neq 0$, *we have that for any pair* $1 \leq j \neq k \leq n$ *(trivial if* $j = k$*):*

$$\begin{aligned} & (\rho_W(y_2 \to y_2') - \rho_W(y_{j+2} \to y_{j+2}')) \tilde{b}^j_{y_2 \to y_2'} \\ = \quad & (\rho_W(y_2 \to y_2') - \rho_W(y_{k+2} \to y_{k+2}')) \tilde{b}^k_{y_2 \to y_2'} \end{aligned} \quad (3.2.5)$$

Since we have $\tilde{b}^j_{y_1 \to y_1'} = 1$ *for all* $1 \leq j \leq n$, *the* $\tilde{b}^j_{y_2 \to y_2'}$*'s are different (nonzero of course) for all* $1 \leq j \leq n$. *Suppose not, then we will have a vector in* $Ker^\perp$ $L_{\mathscr{O}} \cap \Gamma_W$ *with support*

on two reactions $y_{j+2} \to y'_{j+2}$ *and* $y_{k+2} \to y'_{k+2}$ *for some* $1 \leq j \neq k \leq n$*, which is a contradiction to the fact that all vectors in* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ *have support on at least three reactions.*

Therefore, from equation (3.2.5) we have the following cases:

If $\tilde{b}^j_{y_2 \to y'_2} > \tilde{b}^k_{y_2 \to y'_2} > 0$ *or* $\tilde{b}^j_{y_2 \to y'_2} < \tilde{b}^k_{y_2 \to y'_2} < 0$*, then we have either* $\rho_W(y_2 \to y'_2) < \rho_W(y_{j+2} \to y'_{j+2}) < \rho_W(y_{k+2} \to y'_{k+2})$, $\rho_W(y_2 \to y'_2) > \rho_W(y_{j+2} \to y'_{j+2}) > \rho_W(y_{k+2} \to y'_{k+2})$, *or* $\rho_W(y_2 \to y'_2) = \rho_W(y_{j+2} \to y'_{j+2}) = \rho_W(y_{k+2} \to y'_{k+2})$.

If $\tilde{b}^j_{y_2 \to y'_2} > 0 > \tilde{b}^k_{y_2 \to y'_2}$ *or* $\tilde{b}^k_{y_2 \to y'_2} > 0 > \tilde{b}^j_{y_2 \to y'_2}$*, then we have either* $\rho_W(y_{j+2} \to y'_{j+2}) < \rho_W(y_2 \to y'_2) < \rho_W(y_{k+2} \to y'_{k+2})$, $\rho_W(y_{j+2} \to y'_{j+2}) > \rho_W(y_2 \to y'_2) > \rho_W(y_{k+2} \to y'_{k+2})$, *or* $\rho_W(y_{j+2} \to y'_{j+2}) = \rho_W(y_2 \to y'_2) = \rho_W(y_{k+2} \to y'_{k+2})$.

Let $\hat{b}^j_{y_2 \to y'_2} = 1/\tilde{b}^j_{y_2 \to y'_2}$*, for* $1 \leq j \leq n$*. Then we have that*

If $\hat{b}^j_{y_2 \to y'_2} > \hat{b}^k_{y_2 \to y'_2} > 0 > \hat{b}^l_{y_2 \to y'_2} > \hat{b}^m_{y_2 \to y'_2}$*, then we have either* $\rho_W(y_{j+2} \to y'_{j+2}) < \rho_W(y_{k+2} \to y'_{k+2}) < \rho_W(y_2 \to y'_2) < \rho_W(y_{l+2} \to y'_{l+2}) < \rho_W(y_{m+2} \to y'_{m+2})$, $\rho_W(y_{j+2} \to y'_{j+2}) > \rho_W(y_{k+2} \to y'_{k+2}) > \rho_W(y_2 \to y'_2) > \rho_W(y_{l+2} \to y'_{l+2}) > \rho_W(y_{m+2} \to y'_{m+2})$, *or* $\rho_W(y_{j+2} \to y'_{j+2}) = \rho_W(y_{k+2} \to y'_{k+2}) = \rho_W(y_2 \to y'_2) = \rho_W(y_{l+2} \to y'_{l+2}) = \rho_W(y_{m+2} \to y'_{m+2})$.

Note that if all fundamental classes are nondegenerate, then in the Advanced Deficiency Theory all colinearity classes have nonzero signs (but not vice versa). Therefore, for the proof of case (II), please refer to the proof in [7]. We will, in the algorithm, provide an approach to find the enumeration of the M_i*'s in case (II). We will give a simple example in the next paragraph to illustrate this.*

Suppose that $\{b^j\}_{j=1}^n$ *is a subset of a basis for* $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ *where each* b^j *has support on three reactions and supp* $\{b^j\}_{j=1}^n = \{y_i \to y'_i\}_{i=1}^{n+2}$*. Let us assume that* $n = 5$*. We also suppose that a sign pattern of* $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *with* $D \cap$ *supp* $\{b^j\}_{j=1}^5 = \emptyset$ *is given. We will use* M_i*'s instead of* $\rho_W(y_i \to y'_i)$*'s here and we will assume the most general case that the signs of* $\rho_W(y_i \to y'_i)$*'s allow all three subcases in the case (II) to be possible. First,*

we start with a b^j vector in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ with support on three reactions, say b^1 with support on $y_1 \to y_1'$, $y_2 \to y_2'$ and $y_3 \to y_3'$. Since $D \cap$ supp $\{b^j\}_{j=1}^5 = \emptyset$, we only need to consider case (c), the nonsegregation condition. According to the sign patterns of b^1 and $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, we will order M_1, M_2 and M_3 to satisfy the nonsegregation condition. Without loss of generality, we suppose either $M_1 < M_2 < M_3$, $M_1 > M_2 > M_3$, or $M_1 = M_2 = M_3$. We then have a partial enumeration $\{M_1, M_2, M_3\}$ (or equivalently its complete reverse $\{M_3, M_2, M_1\}$). We then will see if there is a b^j vector in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ with support on $y_1 \to y_1'$, $y_2 \to y_2'$, and $y_4 \to y_4'$. If such a b^j vector is not given, then we should be able to produce it by finding an appropriate linear combination of the given b^j's. Without loss of generality, let us suppose b^2 has support on $y_1 \to y_1'$, $y_2 \to y_2'$, and $y_4 \to y_4'$. According to the sign patterns of b^2 and $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, we will order M_1, M_2 and M_4 to satisfy the nonsegregation condition. We then have several situations in terms of the ordering.

(i) If it is either $M_1 < M_4 < M_2$, $M_1 > M_4 > M_2$, or $M_1 = M_4 = M_2$, then combined with the subcases from b^1, we have either $M_1 < M_4 < M_2 < M_3$, $M_1 > M_4 > M_2 > M_3$, or $M_1 = M_4 = M_2 = M_3$.

(ii) If it is either $M_4 < M_1 < M_2$, $M_4 > M_1 > M_2$ or $M_4 = M_1 = M_2$, then combined with the subcases from b^1, we have either $M_4 < M_1 < M_2 < M_3$, $M_4 > M_1 > M_2 > M_3$ or $M_4 = M_1 = M_2 = M_3$.

(iii) If it is either $M_1 < M_2 < M_4$, $M_1 > M_2 > M_4$ or $M_1 = M_2 = M_4$, then combined with the subcases from b^1, we cannot get a complete ordering for M_1, M_2, M_3 and M_4. In this case, we will find a b^j vector in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ (if it is not given, then produce it from the given ones) with support on $y_2 \to y_2'$, $y_3 \to y_3'$, and $y_4 \to y_4'$, say b^3. According to the sign patterns of b^3 and $g_W \in \mathbb{R}^{\mathscr{O}}$, we will order M_2, M_3 and M_4. We then have several cases to consider for b^3.

sub (i): If it is either $M_2 < M_4 < M_3$, $M_2 > M_4 > M_3$, or $M_2 = M_4 = M_3$, then

combined with the subcases from b^1*, we have either* $M_1 < M_2 < M_4 < M_3$*,* $M_1 > M_2 > M_4 > M_3$*, or* $M_1 = M_2 = M_4 = M_3$*.*

sub (ii): If it is either $M_2 < M_3 < M_4$*,* $M_2 > M_3 > M_4$*, or* $M_2 = M_3 = M_4$*, then combined with the subcases from* b^1*, we have either* $M_1 < M_2 < M_3 < M_4$*,* $M_1 > M_2 > M_3 > M_4$*, or* $M_1 = M_2 = M_3 = M_4$*.*

Note that it cannot be the case that we have either $M_4 < M_2 < M_3$*,* $M_4 > M_2 > M_3$*, or* $M_4 = M_2 = M_3$ *(which will conflict with the assumptions that* M_2 *is between* M_1 *and* M_3 *and* M_2 *is between* M_1 *and* M_4*). We will show that by assuming the contrary, i.e., suppose the case that* M_2 *is between* M_4 *and* M_3 *exists. Then from* b^3 *we see that* $b^3_{y_4\to y_4'} g_W(y_4 \to y_4')$ *and* $b^3_{y_3\to y_3'} g_W(y_3 \to y_3')$ *have the same (nonzero) sign which is the opposite sign of* $b^3_{y_2\to y_2'} g_W(y_2 \to y_2')$*. Note that from* b^1 *we see that* $b^1_{y_1\to y_1'} g_W(y_1 \to y_1')$ *and* $b^1_{y_3\to y_3'} g_W(y_3 \to y_3')$ *have the same (nonzero) sign which is the opposite sign of* $b^1_{y_2\to y_2'} g_W(y_2 \to y_2')$*. From* b^2 *we see that* $b^2_{y_1\to y_1'} g_W(y_1 \to y_1')$ *and* $b^2_{y_4\to y_4'} g_W(y_4 \to y_4')$ *have the same (nonzero) sign which is the opposite sign of* $b^2_{y_2\to y_2'} g_W(y_2 \to y_2')$*. Therefore,* $b^1_{y_2\to y_2'}$ *and* $b^2_{y_2\to y_2'}$ *have the same (nonzero) sign if and only if* $b^1_{y_1\to y_1'}$ *and* $b^2_{y_1\to y_1'}$ *have the same (nonzero) sign. Without loss of generality, let us suppose that* $b^1_{y_2\to y_2'}$ *and* $b^2_{y_2\to y_2'}$ *have the same (nonzero) sign, therefore* $b^1_{y_1\to y_1'}$ *and* $b^2_{y_1\to y_1'}$ *have the same (nonzero) sign. Then* $b^1_{y_2\to y_2'} g_W(y_2 \to y_2')$ *and* $b^2_{y_2\to y_2'} g_W(y_2 \to y_2')$ *have the same (nonzero) sign. Therefore,* $b^1_{y_3\to y_3'} g_W(y_3 \to y_3')$ *and* $b^2_{y_4\to y_4'} g_W(y_4 \to y_4')$ *have the same (nonzero) sign.*

On the other hand, note that we can derive b^3 *(up to a scalar multiple) from* b^1 *and* b^2*. Let* $\tilde{b}^3 = (b^2_{y_1\to y_1'})b^1 - (b^1_{y_1\to y_1'})b^2$*, then* $\tilde{b}^3$ *has support on* $y_2 \to y_2'$*,* $y_3 \to y_3'$*, and* $y_4 \to y_4'$ *with* $\tilde{b}^3_{y_2\to y_2'} = b^2_{y_1\to y_1'} b^1_{y_2\to y_2'} - b^1_{y_1\to y_1'} b^2_{y_2\to y_2'}$*,* $\tilde{b}^3_{y_3\to y_3'} = b^2_{y_1\to y_1'} b^1_{y_3\to y_3'}$*, and* $\tilde{b}^3_{y_4\to y_4'} = -b^1_{y_1\to y_1'} b^2_{y_4\to y_4'}$*.* $\tilde{b}^3$ *is colinear with* b^3*, and we see that from the ordering of* M_2*,* M_3 *and* M_4 *that* $\tilde{b}^3_{y_4\to y_4'} g_W(y_4 \to y_4')$ *and* $\tilde{b}^3_{y_3\to y_3'} g_W(y_3 \to y_3')$ *have the same (nonzero) sign which is the opposite sign of* $\tilde{b}^3_{y_2\to y_2'} g_W(y_2 \to y_2')$*. Written in terms of* b^j*'s, we can see that* $-b^1_{y_1\to y_1'} b^2_{y_4\to y_4'} g_W(y_4 \to y_4')$ *and* $b^2_{y_1\to y_1'} b^1_{y_3\to y_3'} g_W(y_3 \to y_3')$ *have the same (nonzero)*

sign. Note that we have assumed $b^1_{y_1 \to y'_1}$ *and* $b^2_{y_1 \to y'_1}$ *have the same (nonzero) sign, so* $-b^2_{y_4 \to y'_4} g_W(y_4 \to y'_4)$ *and* $b^1_{y_3 \to y'_3} g_W(y_3 \to y'_3)$ *have the same (nonzero) sign. This is a contradiction, as in the previous paragraph we have shown that* $b^1_{y_3 \to y'_3} g_W(y_3 \to y'_3)$ *and* $b^2_{y_4 \to y'_4} g_W(y_4 \to y'_4)$ *have the same (nonzero) sign.*

For now we have a complete ordering of M_1, M_2, M_3 *and* M_4. *Without loss of generality, let us assume it is either* $M_1 < M_2 < M_3 < M_4$, $M_1 > M_2 > M_3 > M_4$, *or* $M_1 = M_2 = M_3 = M_4$. *In other words, we assume the partial enumeration is* $\{M_1, M_2, M_3, M_4\}$. *We then will find a place for* M_5 *in the enumeration. We will first consider a* b^j *vector in* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *with support on* $y_1 \to y'_1$, $y_2 \to y'_2$ *and* $y_5 \to y'_5$. *Without loss of generality, let us suppose it is* b^4. *According to the sign patterns of* b^4 *and* $g_W \in \mathbb{R}^{\mathscr{O}}$, *we will order* M_1, M_2 *and* M_5 *to satisfy the nonsegregation condition. We have the following cases.*

(1) If the ordering is such that M_1 *is between* M_2 *and* M_5 *or* M_5 *is between* M_1 *and* M_2, *then we will have a complete enumeration* $\{M_5, M_1, M_2, M_3, M_4\}$ *or* $\{M_1, M_5, M_2, M_3, M_4\}$.

(2) If in the ordering M_2 *is in the middle, i.e.* $M_1 < M_2 < M_5$ *or* $M_1 > M_2 > M_5$ *or* $M_1 = M_2 = M_5$, *then we need to consider a* b *vector in* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *with support on* $y_2 \to y'_2$, $y_3 \to y'_3$, *and* $y_5 \to y'_5$. *If the ordering is such that* M_5 *is between* M_2 *and* M_3, *then we have a complete enumeration* $\{M_1, M_2, M_5, M_3, M_4\}$. *The other possibility is that* M_3 *is between* M_2 *and* M_5 *(note that* M_2 *cannot be between* M_3 *and* M_5, *for a similar reason that we have shown above), i.e.,* $M_2 < M_3 < M_5$, $M_2 > M_3 > M_5$, *or* $M_2 = M_3 = M_5$. *We need to consider a* b^j *vector in* $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ *with support on* $y_3 \to y'_1$, $y_4 \to y'_3$, *and* $y_5 \to y'_5$. *If the ordering this time is such that* M_5 *is between* M_3 *and* M_4, *then we have a complete enumeration* $\{M_1, M_2, M_3, M_5, M_4\}$. *Otherwise* M_4 *is between* M_3 *and* M_5 *(again* M_3 *cannot be in the middle), then we have a complete enumeration* $\{M_1, M_2, M_3, M_4, M_5\}$. *So we can always find an enumeration for* M_1, M_2, M_3, M_4, *and* M_5, *after considering several* b^j*'s.*

For reaction network (3.2.1), note that $b^1 = [0, 1, -1, 0, 1, -1]$ and $b^2 = [1, 0, 0, -1, 1, 0]$. We have the following:

$$R_+^1 = \{E_1S_2 \to E_1 + S_3, E_3S_1 \to E_3 + S_3\} = \{y_2 \to y_2', y_5 \to y_5'\},$$

$$R_-^1 = \{E_2S_3 \to E_2 + S_2, E_3S_3 \to E_3 + S_2\} = \{y_3 \to y_3', y_6 \to y_6'\},$$

$$Q_+^1 = \{\},$$

$$Q_-^1 = \{\},$$

$$R_+^2 = \{E_1S_1 \to E_1 + S_2, E_3S_1 \to E_3 + S_3\} = \{y_1 \to y_1', y_5 \to y_5'\},$$

$$R_-^2 = \{E_2S_2 \to E_2 + S_1\} = \{y_4 \to y_4'\},$$

$$Q_+^2 = \{\},$$

$$Q_-^2 = \{\}.$$

Therefore, we have

$$Q_1^1 = \{\rho_W(y_2 \to y_2'), \rho_W(y_5 \to y_5')\},$$

$$Q_2^1 = \{\rho_W(y_3 \to y_3'), \rho_W(y_6 \to y_6')\},$$

$$Q_1^2 = \{\rho_W(y_1 \to y_1'), \rho_W(y_5 \to y_5')\},$$

$$Q_2^2 = \{\rho_W(y_4 \to y_4')\}.$$

Since $D = \emptyset$, we only need to consider case (c) where Q_1^j and Q_2^j are nonsegregated. Here all $\rho_W(y_i \to y_i')$'s are positive, so we can directly convert the comparisons among $\rho_W(y_i \to y_i')$'s to comparisons among M_i's, without first removing any subcases from (i), (ii) and (iii) of case (c). We will set up inequalities and/or equalities directly in terms of M_i's.

From b^1, we have that from (i) of case (c), either $M_2 < M_3 < M_5$, $M_2 < M_6 < M_5$, $M_5 < M_2 < M_3$, or $M_5 < M_6 < M_2$, $M_3 < M_2 < M_6$, $M_3 < M_5 < M_6$, $M_6 < M_2 < M_3$, $M_6 < M_5 < M_3$, from (ii'), $M_2 = M_3 = M_5 = M_6$, $M_2 = M_3 < M_5 = M_6$, $M_2 = M_6 < M_5 = M_3$, $M_5 = M_6 < M_2 = M_3$, or $M_5 = M_3 < M_2 = M_6$.

From b^2, we have that from (i) of (c), either $M_1 < M_4 < M_5$, $M_1 > M_4 > M_5$, or from (ii'), $M_1 = M_4 = M_5$.

For this step, let us pick $M_3 < M_2 < M_6$ from b^1 and $M_1 > M_4 > M_5$ from b^2.

Step 14: Check for Solutions to the Inequality System

In Step 7 we have determined whether the inequality systems are completely linear or may include nonlinear equalities by considering if $Ker^{\perp} L_{\mathscr{O}} \cap \Gamma_W$ has a forest basis. We will in this step check whether the inequality system has a solution with a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign compatible with the stoichiometric subspace S. In other words, we will look to see if there exist a set of M_i's and a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ which is sign compatible with S such that the inequality system is satisfied.

If the inequality systems are determined to be linear, then the inequality system built from Step 10 through Step 13 is a complete system. If an inequality system has such a solution, it will be called a ***signature***.

If the inequality systems are nonlinear, then we will need to add some nonlinear constraints (equalities) to the inequality system to make it a complete system. If a partial (linear) inequality system we built from Step 10 through Step 13 has such a solution, it will be called a ***pre-signature***. If none of such inequality systems has such a solution, then there is no need to consider the additional nonlinear constraints (equalities), and we claim that the reaction network does not have the capacity to admit multiple steady states.

Suppose the inequality systems are linear. If the inequality system generated from Steps 10 to 13 is a signature, then we claim that the reaction network has the capacity to admit multiple steady states and exit the algorithm. Otherwise, move to next step.

Suppose that the inequality systems are nonlinear. If the partial (linear) system generated from Steps 10 to 13 is not a pre-signature, move to next step. If the partial (linear) system generated from Steps 10 to 13 is a pre-signature, then we will plug the solution of

$\mu \in \mathbb{R}^{\mathscr{S}}$ from the pre-signature into equations (2.2.7) and (2.2.8). After that, we will see if we can solve for $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ from equations (2.2.7) and (2.2.8). If we find a $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ satisfying equations (2.2.7) and (2.2.8), we claim that the reaction network has the capacity to admit multiple steady states and exit the algorithm. Otherwise, move to next step.

For reaction network (3.2.1), from the inequalities and equalities added in Steps 10 to 13, we have the following inequality system:

$$
\begin{aligned}
&\mu_{E_1} + \mu_{S_1} > M_1 \\
&\mu_{E_1S_1} > M_1 \\
&\mu_{E_1} + \mu_{S_2} > M_1 \\
&\mu_{E_1} + \mu_{S_2} = M_2 = \mu_{E_1S_2} \\
&\mu_{E_2} + \mu_{S_3} = M_3 = \mu_{E_2S_3} \\
&\mu_{E_2} + \mu_{S_2} = M_4 = \mu_{E_2S_2} \\
&\mu_{E_3} + \mu_{S_1} = M_5 = \mu_{E_3S_1} \\
&\mu_{E_3} + \mu_{S_3} = M_6 = \mu_{E_3S_3} \\
&\mu_{E_1} + \mu_{S_1} < \mu_{E_1S_1} \\
&\mu_{E_1S_1} < \mu_{E_1} + \mu_{S_2} \\
&M_3 < M_2 < M_6 \\
&M_1 > M_4 > M_5
\end{aligned}
\tag{3.2.6}
$$

The inequality system (3.2.6) has a nonzero solution $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign compatible with S: $\mu_{E_1} = 3$, $\mu_{E_2} = -5$, $\mu_{E_3} = 0$, $\mu_{S_1} = -5$, $\mu_{S_2} = 1$, $\mu_{S_3} = 8$, $\mu_{E_1S_1} = -1$, $\mu_{E_1S_2} = 4$, $\mu_{E_2S_2} = -4$, $\mu_{E_2S_3} = 3$, $\mu_{E_3S_1} = -5$, $\mu_{E_3S_3} = 8$. Therefore this inequality system is a signature. We can claim that reaction network (3.2.1) has the capacity to admit multiple steady states and exit the algorithm.

Remark 3.2.3. *To see that the solution of μ above is sign compatible with S. Note that in*

network (3.2.1), $\mathscr{S} = \{E_1, E_2, E_3, S_1, S_2, S_3, E_1S_1, E_1S_2, E_2S_2, E_2S_3, E_3S_1, E_3S_3\}$*, and dim* $S = 8$*.*

A basis for S *is*

$$\begin{array}{c} \\ E_1 \\ E_2 \\ E_3 \\ S_1 \\ S_2 \\ S_3 \\ E_1S_1 \\ E_1S_2 \\ E_2S_2 \\ E_2S_3 \\ E_3S_1 \\ E_3S_3 \end{array} \begin{array}{c} \begin{array}{cccccccc} x^1 & x^2 & x^3 & x^4 & x^5 & x^6 & x^7 & x^8 \end{array} \\ \left(\begin{array}{cccccccc} -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 \\ -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{array} \right) \end{array}$$

We let

$$\begin{aligned} \sigma &= 2x^1 + 5x^2 + 5x^3 + 4x^4 + 2x^5 - x^6 - x^7 + x^8 \\ &= [2, -1, 0, -1, 1, 1, -3, 1, -1, 2, -1, 1] \end{aligned}$$

Then $\mu = [3, -5, 0, -5, 1, 8, -1, 4, -4, 3, -5, 8]$ *is sign compatible with* $\sigma \in S$*.*

Step 15: Repeat Steps 13 to Step 14

In this step, Steps 13 and 14 are repeated for every choice of M inequalities and/or equalities. Recall a choice of M inequalities and/or equalities are picking one subcase for each $1 \leq j \leq q$ in Step 13.

For reaction network (3.2.1), we have exited the algorithm in Step 14.

Step 16: Repeat Steps 9 through Step 15

In this step all possible shelving assignments in Step 9 are repeated. Similar to that of the Deficiency One Algorithm (and the Advanced Deficiency Algorithm), we can save some time by trying only half of the choices and leaving out the rest which are just total inversions of the first half (by switching all of the upper and lower shelves). This is true because the inverted system has such a solution if and only if the original system has one.

For reaction network (3.2.1), we have exited the algorithm in Step 14.

Step 17: Repeat Steps 8 through Step 16

In this step we will repeat all sign pattern choices for g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ in Step 8.

Note that changing the sign patterns for g_W, h_W may affect the shelving assignments as only nondegenerate fundamental classes are considered, and the inequality systems built from Step 10 through Step 13 will be affected too. We will check all the inequality systems produced by these changes.

If after this step no signature or pre-signature has been found, then we can claim that the reaction network cannot support multiple steady states, no matter what (positive) values the rate constants take.

If all pre-signatures have been tested and no $\kappa \in \mathbb{R}^{\mathscr{R}}_+$ can be solved for from equations (2.2.7) and (2.2.8) with $\mu \in \mathbb{R}^{\mathscr{S}}$ from the pre-signature, then we claim that the reaction network may still have the capacity to admit multiple steady states; the result is inconclusive. In this case, in order to answer the question of multiple steady states, we need to add additional nonlinear equalities and consider the complete (nonlinear) inequality system. As for finding the additional nonlinear equalities, we will not present the details here.

For reaction network (3.2.1), we have exited the algorithm in Step 14.

Remark 3.2.4. *Recall that in Step* 17*, we will repeat the construction of inequality systems for each "valid" pair of sign patterns for* g_W*,* $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *chosen in Step* 8*. What we*

can do to improve the efficiency of the algorithm is to revise Step 8 *to combine some of the sign patterns for* g_W, $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *together in one step so there will fewer repetitions in Step* 17.

Recall that the conditions (*) (first mentioned in Section 2.9) on $\rho_W(y_i \to y_i')$*'s are the conditions (we later find) in Proposition 2.10.14.*

Recall in Section 2.9, for a "valid" pair of sign patterns for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$, *we check if the answer to the following Question (b) is "Yes":*

Question (b): Given the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ *and the orientation* $\mathscr{O}$, *do there exist*

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ *that is sign compatible with* S,

(ii) a set $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$ *which is consistent in sign with the pre-selected sign patterns of* g_W *and* h_W *and satisfies these new conditions* $(*)$ *(i.e. conditions in Proposition 2.10.14), and*

(iii) a choice of shelving assignments for each nondegenerate fundamental class satisfying the conditions in Proposition 2.8.1 (in terms of $\rho_W(y_i \to y_i')$ *for condition (ii)),*

which together satisfy the conditions in Lemma 2.8.2?

In the algorithm that implemented in [16], we actually use the following approach.

As we can see that although we define a "valid" pair of sign patterns for g_W *and* h_W, *the definition works for a single* g_W *or* h_W *as long as they are not both picked zero at the same time. Thus here we will apply a "valid" sign pattern on* g_W *and* h_W *separately. For the chosen "valid" sign pattern for* $g_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *, we have* $D = \{y_i \to y_i' \in W : g_W(y_i \to y_i') \neq 0\}$. *We then choose a partial sign pattern for* $h_W|_D \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ *such that there exists a "valid" sign pattern for* $h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$. *Therefore, we have a pair of sign patterns for*

g_W and $h_W|_D$ that is "valid". In this case, the signs of $\rho_W(y_i \to y_i')$'s $(y_i \to y'^i \in W \backslash D)$ are not specified. We then check if the answer to the following Question (c) is "Yes":

Question (c): Given the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ and the orientation $\mathscr{O}$, do there exist

(i) a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ that is sign compatible with S,

(ii) a set $\{\rho_W(y_i \to y_i') : y_i \to y_i' \in ND\}$ which satisfies these new conditions $(*)$ (i.e. conditions in Proposition 2.10.14), and

(iii) a choice of shelving assignments for each nondegenerate fundamental class satisfying the conditions in Proposition 2.8.1,

which together satisfy the conditions in Lemma 2.8.2?

Note in this case we cannot specify whether $M_i = \ln(\rho_W(y_i \to y_i'))$ or M_i is some large and negative number. Thus we do not consider if we need to remove subcases from conditions (a), (b) and (c) in Step 13, thus there might be more subcases where not all $\rho_W(y_i \to y_i')$'s are assigned the same signs. However, there will be fewer repetitions in Step 17 in this case, therefore the approach is still more efficient than what has been stated in the algorithm.

Remark 3.2.5. *The following is a comment made on finding a set of rate constants and two positive, distinct and stoichiometrically compatible compositions corresponding to two steady states, if they exist.*

In the linear case, after finding a solution of $\mu \in \mathbb{R}^{\mathscr{S}}$ from a signature, we can find $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ from equations (2.2.7) and (2.2.8). We pick a $\sigma \in S$ that is sign compatible with μ, then find c^*, $c^{**} \in \mathbb{R}_+^{\mathscr{S}}$ from equations (2.2.3) and (2.2.4) or equations (2.2.5), depending on whether μ_s is zero or not. For each $y \to y' \in \mathscr{R}$, we find $k_{y \to y'}$ by equation (2.2.6). Therefore, we find a set of rate constants for the reaction network to admit two steady states with the corresponding compositions c^* and c^{**}.

In the nonlinear case, suppose from a solution of $\mu \in \mathbb{R}^{\mathscr{S}}$ from a pre-signature, we have solved for $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ from equations (2.2.7) and (2.2.8). Then we are ready to find a set of rate constants and the compositions corresponding to two steady states. The process is same as in the linear case.

In the nonlinear case, if all pre-signatures have been tested and no $\kappa \in \mathbb{R}_+^{\mathscr{R}}$ can be found, then we claim that the reaction network may still have the capacity to admit multiple steady states. We need to rely on finding nonlinear equalities to complete the resulting (nonlinear) system to answer the question of multiple steady states. After that, if we conclude that the reaction network has the capacity to admit multiple steady states, then we can find a set of rate constants and the compositions corresponding to two steady states following the same approach as above.

Chapter 4

MASS ACTION INJECTIVITY TEST

4.1 Introduction

The Mass Action Injectivity Test is a test of whether a given reaction network is mass action injective (to be defined shortly). In this chapter, the main focus is on finding an algorithm for the Mass Action Injectivity Test. The algorithm has been implemented in the Chemical Reaction Network Toolbox [16]. To see more information about mass action injectivity, see [13].

We recall a few terms defined in Chapter 1 and introduce a few new terms from [13].

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, the reaction vector corresponding to the reaction $y \to y'$ is $y' - y \in \mathbb{R}^{\mathscr{S}}$. The stoichiometric subspace for the network, denoted by S, is defined via

$$S := \text{span}\{y' - y \in \mathbb{R}^{\mathscr{S}} : y \to y' \in \mathscr{R}\}. \tag{4.1.1}$$

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, a mass action kinetics is an assignment of a reaction rate function to each reaction: for each reaction $y \to y' \in \mathscr{R}$, there is a positive rate constant $k_{y \to y'}$ such that the molar occurrence rate per unit volume of $y \to y'$ is given by $k_{y \to y'} c^y$. Here

$$c^y = \prod_{s \in \mathscr{S}} c_s^{y_s}, \tag{4.1.2}$$

where y_s is the stoichiometric coefficient of species s for complex y.

Recall that the reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ we consider in this thesis will be governed by mass action kinetics (except the next chapter). The ***species-formation-rate function*** r: $\bar{\mathbb{R}}_+^{\mathscr{S}} \to \mathbb{R}^{\mathscr{S}}$ is given by

$$r(c) = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y). \tag{4.1.3}$$

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, the mass action differential equations are $\dot{c} = r(c)$, where the dot over c indicates the time differentiation and $r(\cdot)$ is the corresponding species-formation-rate function.

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, a composition $a \in \bar{R}_+^{\mathscr{S}}$ is an ***equilibrium*** of the mass action differential equations if $r(a) = 0$. An equilibrium a is a positive equilibrium of the mass action differential equations if $a \in \mathbb{R}_+^{\mathscr{S}}$.

Recall that our reactor may have an inflow (feed) stream and an outflow stream. Besides the species that are put into the reactor initially, we can provide several species to the reactor through a continuous inflow stream containing a liquid mixture of these species, and we can remove several species from the reactor through a continuous outflow stream containing these species. For generality, we might allow only certain species to be in the inflow or outflow.

We call the species in the inflow (feed) stream as inflow species and denote the set of inflow species by $\mathscr{S}_f$. We call the species in the outflow stream as outflow species and denote the set of outflow species by $\mathscr{S}_o$. Note that the reaction corresponding to species s in the inflow stream is $0 \to s$, and the reaction corresponding to species s in the outflow stream is $s \to 0$. We can then define $\mathscr{R}_f = \{s \in \mathscr{S} : 0 \to s \in \mathscr{R}\}$ as the set of *inflow reactions*, $\mathscr{R}_o = \{s \in \mathscr{S} : s \to 0\}$ as the set of *outflow reactions*, and $\mathscr{R}_t = \mathscr{R} \backslash (\mathscr{R}_f \cup \mathscr{R}_o)$ as the set of *true reactions*.

Recall that two compositions c and c' are stoichiometrically compatible if $c' - c \in S$.

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, the network has the capacity to ***admit multiple positive equilibria*** if there exists a set of positive rate constants $\{k_{y \to y'} : y \to y' \in \mathscr{R}\}$

such that the mass action differential equations admit (at least) two distinct positive equilibria that are stoichiometrically compatible. In other words, a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ has the capacity for multiple positive equilibria if there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and two stoichiometrically compatible compositions $a \in \mathbb{R}^{\mathscr{S}}_+$ and $b \in \mathbb{R}^{\mathscr{S}}_+$ with $a \neq b$ such that $r(a) = r(b) = 0$.

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, let $a \in \mathbb{R}^{\mathscr{S}}_+$ be a positive equilibrium, then the ***derivative*** of the species-formation-rate function evaluated at a, $\mathrm{D}r(a)$ can be viewed as a linear transformation from S to S.

Recall that $r(c) = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'} c^y (y' - y)$. Then we have, for each $\sigma \in S$,

$$\begin{aligned} \mathrm{D}r(a)\sigma &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(a^y)\frac{y\cdot\sigma}{a}(y'-y) \\ &= \sum_{y\to y'\in\mathscr{R}} \kappa_{y\to y'}\frac{y\cdot\sigma}{a}(y'-y), \end{aligned} \tag{4.1.4}$$

where $\kappa_{y\to y'} = k_{y\to y'}a^y$. Define "$*_a$-scalar product" in $\mathbb{R}^{\mathscr{S}}$ via $u *_a v = \sum_{s\in\mathscr{S}} \frac{u_s v_s}{a_s}$. Then

$$\mathrm{D}r(a)\sigma = \sum_{y\to y'\in\mathscr{R}} \kappa_{y\to y'}(y *_a \sigma)(y'-y) \tag{4.1.5}$$

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, a positive equilibrium $a \in \mathbb{R}^{\mathscr{S}}_+$ is ***degenerate*** if $\mathrm{D}r(a)$ is singular, i.e., there exists a nonzero $\sigma \in S$ such that $\mathrm{D}r(a)\sigma = 0$.

For a given reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with stoichiometric subspace S, we say that the reaction network is ***mass action injective*** if for any $\eta \in \mathbb{R}^{\mathscr{R}}_+$ and any $*_a$-scalar product for $a \in \mathbb{R}^{\mathscr{S}}_+$, the map $T_{*_a,\eta}$: $S \to S$ defined by

$$T_{*_a,\eta}(\sigma) = \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y *_a \sigma)(y'-y). \tag{4.1.6}$$

is nonsingular.

In the next lemma we show that the seemingly *linear* notion of mass action injective actually has implications for injectivity of the *nonlinear* function $r(\cdot)$.

Lemma 4.1.1. *[Feinberg] Let $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ be a reaction network with stoichiometric subspace S. The reaction network $\{\mathscr{S},\mathscr{C},\mathscr{R}\}$ is not mass action injective if and only if there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$ and distinct compositions $c^*, c^{**} \in \mathbb{R}^{\mathscr{S}}_+$, with $c^* - c^{**} \in S$ such that*

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y). \tag{4.1.7}$$

PROOF: $\Leftarrow$): Suppose there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$ and distinct $c^*, c^{**} \in \mathbb{R}^{\mathscr{S}}_+$, with $c^* - c^{**} \in S$ such that

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y).$$

Then we have

$$\begin{aligned} 0 &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y) - \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) \\ &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}((c^{**})^y - (c^*)^y)(y'-y) \\ &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y((c^{**})^y/(c^*)^y - 1)(y'-y) \end{aligned} \tag{4.1.8}$$

Let $\sigma = c^{**} - c^*$ and $\gamma = \ln c^{**} - \ln c^*$, then $\sigma \in S$ and $(c^{**})^y/(c^*)^y = e^{y\cdot\gamma}$. Note that for all $s \in \mathscr{S}$, γ_s and σ_s have the same sign, as $\ln c^*_s - \ln c^{**}_s$ and $c^*_s - c^{**}_s$ have the same sign. We claim that γ is sign-compatible with S, therefore there exists $a \in \mathbb{R}^{\mathscr{S}}_+$ such that $\gamma = \dfrac{1}{a}\sigma$. Note that by the definition of $*_a$-scalar product, $y\cdot\gamma = y *_a \sigma$. We have that

$$\begin{aligned} 0 &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(e^{y\cdot\gamma} - 1)(y'-y) \\ &= \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(e^{y*_a\sigma} - 1)(y'-y) \end{aligned} \tag{4.1.9}$$

Note that $e^{y*_a\sigma}-1$ and $y*_a\sigma$ have the same sign, so there exists $p_y > 0$ such that $e^{y*_a\sigma}-1 = p_y(y *_a \sigma)$. Given $y \to y' \in \mathscr{R}$, we let $\eta_{y\to y'} = k_{y\to y'}(c^*)^y p_y$, then we claim that there exists a $*_a$-scalar product with $a \in \mathbb{R}^{\mathscr{S}}_+$, an $\eta \in \mathbb{R}^{\mathscr{R}}_+$, and a nonzero $\sigma \in S$ such that

$$0 = \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y *_a \sigma)(y'-y),$$

which leads to the conclusion that the reaction network is not mass action injective.

$\Rightarrow$): Suppose that there exists a $*_a$-scalar product where $a \in \mathbb{R}^{\mathscr{S}}_+$, an $\eta \in \mathbb{R}^{\mathscr{R}}_+$, and a nonzero $\sigma \in S$ such that

$$0 \;=\; \sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y *_a \sigma)(y'-y).$$

We can find γ defined by $\gamma = \dfrac{1}{a}\sigma$ such that $y *_a \sigma = y\cdot\gamma$. If we let $\tilde{\eta}_{y\to y'} = \eta_{y\to y'}(y *_a \sigma)/(e^{y*_a\sigma}-1) = \eta_{y\to y'}(y *_a \sigma)/(e^{y\cdot\gamma}-1)$, we will have

$$0 \;=\; \sum_{y\to y'\in\mathscr{R}} \tilde{\eta}_{y\to y'}(e^{y\cdot\gamma}-1)(y'-y).$$

Note that $a \in \mathbb{R}^{\mathscr{S}}_+$, so γ and σ have the same sign. In particular, $\sigma \neq 0$ implies $\gamma \neq 0$. Since γ and $e^\gamma - 1$ have the same sign, there exists $c^* \in \mathbb{R}^{\mathscr{S}}_+$ such that $\sigma = c^*(e^\gamma - 1)$. Let $c^{**} = c^* e^\gamma$, then $c^{**} \neq c^*$, $\gamma = \ln c^{**} - \ln c^*$, and $c^{**} - c^* = c^*(e^\gamma - 1) = \sigma \in S$. Given that $y \to y' \in \mathscr{R}$, we let $k_{y\to y'} = \tilde{\eta}_{y\to y'}/(c^*)^y$, then we claim there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$ and distinct $c^*, c^{**} \in \mathbb{R}^{\mathscr{S}}_+$ with $c^* - c^{**} \in S$ such that

$$0 = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) - \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y)$$

or equivalently,

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^*)^y(y'-y) = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'}(c^{**})^y(y'-y). \quad \square$$

Later in the chapter, we will develop an algorithm to test if the reaction network is mass action injective, which we will call the ***Mass Action Injectivity Test***. But first of all, we want to explain why this is relevant to our question of multiple steady states.

4.2 Why the Mass Action Injectivity Test?

Recall our main question is stated as: Given a reaction network governed by mass action kinetics, does there exist a set of positive rate constants such that the governing differential equations admit a pair of distinct positive steady states which are stoichiometrically compatible?

Mathematically, the question can be stated as:

Question 1: Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist a set of positive rate constants $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$, and two positive, distinct and stoichiometrically compatible compositions c^* and c^{**}, such that

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'} c^{*y}(y'-y) = 0 \tag{4.2.1}$$

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'} c^{**y}(y'-y) = 0 \tag{4.2.2}$$

are satisfied?

It easily follows that, for the answer to Question 1 to be "Yes", we will have the necessary condition that

$$\sum_{y\to y'\in\mathscr{R}} k_{y\to y'} c^{*y}(y'-y) = \sum_{y\to y'\in\mathscr{R}} k_{y\to y'} c^{**y}(y'-y) \tag{4.2.3}$$

where $\{k_{y\to y'} : y \to y' \in \mathscr{R}\}$ is some set of positive constants, and c^* and c^{**} are some pair of positive, distinct and stoichiometrically compatible compositions.

Therefore, from Lemma 4.1.1, we can convert the necessary condition above into the following question which will be tested in the Mass Action Injectivity Test:

Question a: Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist an $\eta \in \mathbb{R}^{\mathscr{R}}_+$, a $*_a$-scalar product where $a \in \mathbb{R}^{\mathscr{S}}_+$, and a nonzero $\sigma \in S$ which is sign-compatible with the stoichiometric space S such that

$$\sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y *_a \sigma)(y'-y) = 0 \tag{4.2.4}$$

is satisfied?

Letting $\gamma = \dfrac{1}{a}\sigma$ makes γ nonzero and sign-compatible with the stoichiometric subspace S. And we can equivalently ask

Question b: Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist an $\eta \in \mathbb{R}^{\mathscr{R}}_+$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with the stoichiometric space S such that

$$\sum_{y\to y'\in\mathscr{R}} \eta_{y\to y'}(y \cdot \gamma)(y'-y) = 0 \tag{4.2.5}$$

is satisfied?

We designed the Higher Deficiency Algorithm to solve the question of multiple steady states in terms of Question 1. An alternative approach to solve the question of the multiple steady states will be the Mass Action Injectivity Test. If the Mass Action Injectivity Test passes, i.e., the reaction network is mass action injective, then we immediately know the answer to Question 1 is no. If the Mass Action Injectivity Test fails, i.e., the reaction network is not mass action injective, we can then try the Higher Deficiency Algorithm. In practice, when the Higher Deficiency Algorithm is not quite efficient (in terms of running time) or accurate (in terms of giving definite answers), the Mass Action Injectivity Test may give a definitive answer and may be faster. Next, an algorithm is designed to carry out the Mass Action Injectivity Test.

4.3 Algorithm

Recall that we want to test the following question:

Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, do there exist a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S and an $\eta \in \mathbb{R}^{\mathscr{R}}_{+}$ satisfying (4.2.5)?

If there exists such a $\gamma \in \mathbb{R}^{\mathscr{S}}$, then we claim that the reaction network is not mass action injective. If there does not exist such a γ, then we claim that the reaction network is mass action injective and therefore the reaction network does not have the capacity to admit multiple steady states, no matter what (positive) values the rate constants take.

To conclude that there does not exist such a γ, we need to run through all valid (nonzero and sign-compatible with S) sign patterns of γ to verify that. In this case, the implementation could be impractical for a large number of species. To improve the efficiency of the algorithm, a so-called "tree" idea is introduced. It first appeared in Feinberg's codes for the Deficiency One Algorithm (see [6]), then in Ellision's codes for the Advanced Deficiency Algorithm (see [8]), and now in the codes for the Higher Deficiency Algorithm (see [16]).

The "tree" idea is used to expedite the process of finding if there exists a nonzero $\mu \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with S, such that a system of linear inequalities and/or equalities in terms of μ is satisfied. We will apply the idea in the Mass Action Injectivity Test.

We will define a few terms related to the "tree" idea.

Given a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$, assume $N = \#(\mathscr{S})$ is the number of species and n is the number of reactant complexes. We will order the species from s_1 to s_N and the reactant complexes from y_1 to y_n.

We can generate a tree representing the signs of γ_s's ($s \in \mathscr{S}$), called a γ ***tree***. There is a root node for the tree representing no signs have been assigned to any σ_s ($s \in \mathscr{S}$). Starting from the root as the zeroth layer of the tree, the root has three children which form the first layer of the tree, which represent the positive, negative, and zero sign assignment, respectively, to species s_1. Each of the nodes in the first layer has three children, which represent the positive, negative, and zero sign assignment, respectively, to species s_2. These nine children form the second layer. We continue this and stop at the N^{th} layer, containing 3^N nodes. All edges are between a mother node and its children nodes. Conversely, each mother node and each of its three children nodes are connected by edge. Note that except for the root node and the nodes in the final layer, all other nodes are both mother nodes and children nodes at the same time. Note that a direct (shortest) path from the root node to some node in the i^{th} layer contains one node from each layer between the zeroth and the i^{th} layer. Also note that nodes in j^{th} layer represent some sign assignment for γ_{s_j}. Therefore, the direct (shortest) path from the root node to some node in the i^{th} layer represents sign assignments for γ_s's where s is the among the first i species.

Similary we can generate a tree representing the signs of $y \cdot \gamma$'s where y is a reactant complex in $\mathscr{R}$, called the $y \cdot \gamma$ ***tree***. Note that the $y \cdot \gamma$ tree has 3^n leaf nodes. We then claim that the (shortest) path from the root to some node in the i^{th} layer represents sign assignments for $y \cdot \gamma$'s where y is the among the first i reactant complexes.

In order to develop the algorithm for the Mass Action Injectivity Test, we make some observations below.

Remember our goal is to find if there exists a positive set of $\{\eta_{y\to y'} : y \to y' \in \mathscr{R}\}$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with S satisfying equation (4.2.5).

Remark 4.3.1. *Note that we only have to check half the sign patterns of γ which are sign-compatible with S, as if there exist an $\eta \in \mathbb{R}^{\mathscr{R}}_+$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with S satisfying equation (4.2.5), then $\eta \in \mathbb{R}^{\mathscr{R}}_+$ and $-\gamma \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S also satisfy equation (4.2.5). We will take this into account in the algorithm.*

We define a linear map $T : \mathbb{R}^{\mathscr{R}} \to S$ as follows:

$$T\alpha = \sum_{y\to y'\in\mathscr{R}} \alpha_{y\to y'}(y' - y), \text{ for } \alpha \in \mathbb{R}^{\mathscr{R}}. \tag{4.3.1}$$

Given $\gamma \in \mathbb{R}^{\mathscr{S}}$, let us define $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ by

$$\tilde{L}_{y\to y'}(\gamma) = y \cdot \gamma, \text{ for all } y \to y' \in \mathscr{R}. \tag{4.3.2}$$

Then we can claim that the following statements are equivalent:

(A) There exist a positive set $\{\eta_{y\to y'} : y \to y' \in \mathscr{R}\}$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with S satisfying equation (4.2.5).

(B) There exists a nonzero γ sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is sign-compatible with $Ker\ T$.

We define S_Y =span$\{y : y \to y' \in \mathscr{R}\}$.

Note that if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S such that $\tilde{L}(\gamma) = 0$, i.e., there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S and also lies in $S_Y^{\perp}$, then statement (B) (therefore (A)) above is satisfied trivially.

Therefore, we have the following lemma.

Lemma 4.3.2. *The following statements are equivalent:*

(A) There exist an $\eta \in \mathbb{R}^{\mathscr{R}}_{+}$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S, satisfying equation (4.2.5).

(B) There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is sign-compatible with $Ker\ T$.

(C) One of the two conditions holds:

(a): There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S and lies in $S_Y^{\perp}$.

(b): There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is nonzero and sign-compatible with $Ker\ T$.

In the algorithm for the Mass Action Injectivity Test ([16]), we check if (B) holds. Note that (C) is merely a reformation of (B) by separating the case $\tilde{L}(\gamma) = 0$ from the case $\tilde{L}(\gamma) \neq 0$. To better explain the "tree" idea, we will first check if condition (a) in (C) holds (a simpler case in terms of the "tree" idea) and then if condition (b) in (C) holds (a more complex one).

First we check if condition (a) holds, i.e., if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S and lies in $S_Y^{\perp}$. If the answer is yes, then we claim that the reaction network is not mass action injective. Note that if $S_Y^{\perp} \subseteq S^{\perp}$, or equivalently $S \subseteq S_Y$, then no such nonzero γ will exist, and the answer to the check of whether condition (a) holds is no. Without loss of generality, let us assume that $S \subseteq S_Y$ does not hold. If the answer to the check of whether condition (a) holds is no, then we will move to check if condition (b) holds, i.e., if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ which is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is nonzero and sign-compatible with $Ker\ T$. We explain first the check of whether condition (a) holds to provide some background on describing the check of whether condition (b) holds.

We will explain the algorithm in terms of the tree idea for checking if condition (a) holds first. We need to introduce a few tree related terms.

Starting from one node and moving through the edges in the tree to reach another node is called a ***walk*** in the γ tree.

Starting from the root node and walking in the γ tree to reach another node a, the direct (shortest) path from the root node to the node a, is called a ***walk trace*** at the node a (with respect to the root node).

We say a ***sign pattern*** is assigned to $\gamma \in \mathbb{R}^{\mathscr{S}}$ if all components of γ have been assigned signs. We say a ***partial sign pattern*** is assigned to $\gamma \in \mathbb{R}^{\mathscr{S}}$, if some (not necessarily all) of its components have been assigned signs.

Recall that each node in the γ tree corresponds to a sign assignment to γ_s for some species s. A walk trace at some node at the i^{th} layer of the γ tree is a direct (shortest) path from the root to this current node, which includes one node from each of the first i layers. Therefore, a walk trace at a node in the i^{th} layer provides a partial sign pattern of γ, in particular, a sign assignment to each γ_{s_j} for $1 \leq j \leq i$.

Before we walk in the γ tree, we want to check if we can fix the sign of some γ_s $(s \in \mathscr{S})$ so we can set up some rules for the walk.

Note that we want to find a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with $S = \text{span}\ \{y' - y : y \to y' \in \mathscr{R}\}$. Therefore, if a species s does not lie in the support of any reaction vector $y' - y$, we assign γ_s to be zero. We say such a species is a species with a fixed sign, or a ***sign-fixed*** species. We require that for the walk in the γ tree, if we move past the nodes corresponding to these species with fixed signs, we will only walk through the zero sign node.

Suppose that we start from the root node, walk in the γ tree, and we are currently at node a in the i^{th} layer. We say the sign of γ_s is ***known*** at node a, if (at least) one of the following holds:

(i) Species s is sign-fixed as indicated earlier (where γ_s is set to be 0).

(ii) Species s is among the first i $(1 \leq i \leq N)$ species, i.e. $s \in \{s_1, ..., s_i\}$.

(iii) The sign of γ_s can be determined (to be explained in the next remark) by the signs of $\gamma_{s'}$'s which are known from (i) and (ii).

Remark 4.3.3. *By saying that γ_s can be determined based on the known signs of $\gamma_{s'}$'s from (i) and (ii), we mean that γ_s is a linear combination of the $\gamma_{s'}$'s from (i) and (ii) in such a way that for the $\gamma_{s'}$'s with a nonzero coefficient, the signs of coefficients of positive $\gamma_{s'}$'s are all the same, and are the opposite to the signs of coefficients of negative $\gamma_{s'}$'s.*

Since we only know that to be sign-compatible with S, $\gamma \notin S^{\perp}$, it is not very clear what the dependences of γ_s's are. Therefore, (iii) is set false by default in the definition of the known sign of γ_s, in the process of checking whether condition (a) is satisfied. We include (iii) in the definition because it will be generalized in the process of checking whether condition (b) is satisfied.

Note that from finding at each node of the γ tree, all the signs of γ_s's that are known, we can get a partial sign pattern for γ at this node.

Next, let us describe two ways of walking in the γ tree: ***move forward*** and ***move backward***.

The rule for moving forward is simple. We start from the current node assumed not to be a leaf node (in the N^{th} layer). Walk toward higher layers (for example, from the i^{th} layer to $(i+1)^{th}$ layer) in the tree until we either reach a positive leaf node or arrive at the positive node of the next earliest species whose sign is not fixed. Note that whenever we walk past the nodes of a sign-fixed species, we will walk through the fixed (zero in this case) sign node.

The rule for moving backward is slightly complicated. Here we will take into account the fact that only half (leaving out the inversions) of the sign patterns of γ which are sign-compatible with S need to be considered. We start from the current node which is not the root node, then follow these rules:

(I) If the current node is a fixed sign node (zero sign in this case), move back along its

walk trace until a node, whose corresponding species is not a sign-fixed species, is reached. If we reach the root node, we exit the moving backward process. Otherwise, go to (II).

(II) If the current node is a zero node, skip the rest and go to (III). If the current node is a positive or negative node, then move to another child of its mother node in the following way:

(i) Suppose current node is a positive node. If the fixed signs all have a sign of zero (true in this case), then check if the partial sign pattern from the walk trace at the mother node of this node contains all zero signs. If the answer is yes, move to the zero sign node (first walk toward lower layer to current node's mother node then walk toward higher layer to the zero sign node that is another child of the mother node). Otherwise, move to the negative sign node.

(ii) If the current node is a negative node, move to the zero node.

(III) If the current node is a zero node, then move back in the tree (along its walk trace) to its mother node. If we reach the root node, we exit the moving backward process. Otherwise, go back to (I).

Now we are ready to describe the algorithm of checking if condition (a) holds. We will proceed according to the following steps.

Step 1:

We have two situations.

(i) If it is the first time walking in the γ tree, we start at the root of the γ tree. We move forward in the γ tree. Skip (ii).

(ii) If it is not the first time walking in the γ tree, then we are currently at some node in the γ tree. Note that if we previously moved backward and arrive at the root of the γ tree, then we terminate the process and claim condition (a) does not hold; therefore the test regarding whether the reaction network is mass action injective is inconclusive by checking condition (a).

Step 2:

If dim $S = \#(\mathscr{S})$, go to Step 3 and skip the rest of Step 2. Otherwise, check if there exists γ with the partial sign pattern, generated from all γ_s's that are known at the current node, that is sign-compatible with S. If yes, go to Step 3. Otherwise we move backward in the γ tree and go back to Step 1.

Step 3:

Check if there exists a nonzero γ in $S_Y^\perp$ with the partial sign pattern generated from the signs of all γ_s's that are known at the current node. If the answer is no, move backward in the γ tree and go back to Step 1. Otherwise, we record the value and sign of each γ_s, for $s \in \mathscr{S}$.

Step 4:

Now that all signs of γ_s's ($s \in \mathscr{S}$) are known, we will check if γ is sign-compatible with S if dim $S < \#(\mathscr{S})$, since if dim $S = \#(\mathscr{S})$ then it is trivially true. If the answer to the check is no, then we move forward in the γ tree (unless we are already at the node of the last species with a non-fixed sign, in which case we move backward in the γ tree) and go back to Step 1. If the answer is yes, then we can let $\eta_{y\to y'} = 1$ for all $y \to y' \in \mathscr{R}$. Note that $\gamma \in S_Y^\perp \backslash \{0\}$. Then $\gamma \in \mathbb{R}^{\mathscr{S}}$ which is nonzero and sign-compatible with S, and $\eta \in \mathbb{R}_+^{\mathscr{R}}$ satisfy equation (4.2.5). We will terminate the process and claim that the network is not mass action injective.

Therefore, from the result of checking whether condition (a) holds, we can either claim that the network is not mass action injective or we must also check if condition (b) holds in order to answer our mass action injectivity question.

Let us move to check if condition (b) holds. Recall that for each $y \to y' \in \mathscr{R}$, we define $\tilde{L}_{y\to y'}(\gamma) = y \cdot \gamma$. Recall that for condition (a), we check if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ sign-compatible with S which also lies in $S_Y^\perp$ (i.e. $\tilde{L}(\gamma) = 0$). For condition (b), we check

if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is nonzero and sign-compatible with $Ker\ T$.

The algorithm that checks if condition (b) holds contains two kinds of trees: the γ tree and $y \cdot \gamma$ tree. Its procedure is similar to the one for checking whether condition (a) holds, but with more complicated rules.

Similarly, we can define terms like *walk* in the γ tree, *walk* in the $y \cdot \gamma$ tree, the *walk trace* at some node (respect to the root node of the γ tree) in the γ tree or $y \cdot \gamma$ tree, a *sign pattern* for γ, a *sign pattern* for $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$, a *partial sign pattern* for γ, a *partial sign pattern* for $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$, etc.

Our algorithm contains a γ tree and at each node (except the root node) of γ tree, a $y \cdot \gamma$ tree is formed using the node as its root node.

Recall that we enumerate the species as s_1,..., s_N where $N = \#(\mathscr{S})$. We also enumerate the reactant complexes as $y_1, ..., y_n$ where n is the number of reactant complexes in $\mathscr{R}$.

Let a be a node (not a root node) in the i^{th} $(i \geq 1)$ layer of the γ tree, then a represents a sign node for γ_{s_i}. The walk trace at node a is the direct (shortest) path from the root of the γ tree to node a, which provides a partial sign pattern for γ (sign assignments to all γ_{s_k}'s for $1 \leq k \leq i$). Let b be a node in the j^{th} ($j \geq 0$ and if $j = 0$ then $b = a$) layer of the $y \cdot \gamma$ tree formed using node a as its root, then b represents a sign node for $y_j \cdot \gamma$ (if $j = 0$ then it means no sign is assigned to any $y \cdot \gamma$'s). The walk trace at node b is defined as the direct (shortest) path from the root node of the γ tree to node a in the γ tree, plus the direct (shortest) path from the node a to node b in the $y \cdot \gamma$ tree. The walk trace at node b in the $y \cdot \gamma$ tree not only provides a partial sign pattern for $y \cdot \gamma$ (sign assignments to all $y_l \cdot \gamma$'s for $1 \leq l \leq j$), but also a partial sign pattern for γ (sign assignments to all γ_{s_k}'s for $1 \leq k \leq i$). If $a = b$, i.e., node b is a root of some $y \cdot \gamma$ tree, then we say we have two partial sign patterns for γ and $y \cdot \gamma$, respectively, except the latter one is empty (i.e. from

the walk trace no sign is assigned to any $y \cdot \gamma$ where y is a reactant complex). Thus we can always consider the general case that node b is in some $y \cdot \gamma$ tree (which includes the case that node a is in the γ tree).

Before we walk in the γ tree or $y \cdot \gamma$ tree, we want to check if we can fix the sign of some γ_s ($s \in \mathscr{S}$) or $y \cdot \gamma$ (y is a reactant complex) to set up some rules for walking on γ tree or $y \cdot \gamma$ tree.

(i) Note that we want to find a nonzero γ that is sign-compatible with $S = \text{span}\,\{y' - y : y \to y' \in \mathscr{R}\}$. Therefore, we can find each of the species s which does not lie in the support of any reaction vector $y' - y$, and assign the corresponding γ_s a sign zero. We call such a species a species with a fixed sign, or a ***sign-fixed*** species. We require that for the walk in the γ tree, if we move past the nodes corresponding to these species, we only walk through the zero sign node.

(ii) If there exists any feed (inflow) reaction, i.e., $\mathscr{R}_f \neq \emptyset$, then the zero complex will appear in the list of reactant complexes. In this case, note that for $y = 0$, $y \cdot \gamma = 0$ for any $\gamma \in \mathbb{R}^{\mathscr{S}}$. We then fix the sign for $y \cdot \gamma = 0$ to be zero, where y is the zero reactant complex. If we walk past the nodes corresponding to the zero reactant complex in the $y \cdot \gamma$ tree, then we only walk through the zero sign node.

Suppose that we start from the root node of the γ tree, walk in the γ tree, and enter the $y \cdot \gamma$ tree formed by node a at the i^{th} layer of the γ tree. We are currently at some node b at the j^{th} layer of the $y \cdot \gamma$ tree generated by node a.

We say the sign of γ_s is ***known*** at this node b if (at least) one of the following holds:

(i) Species s is sign-fixed as indicated earlier (where γ_s is set to be 0).

(ii) The species s is among the first i ($1 \leq i \leq N$) species $\{s_1, ..., s_i\}$.

(iii) The sign of γ_s can be determined (to be defined shortly) by the signs of $\gamma_{s'}$'s that are known from (i) and (ii), and the signs of $y_l \cdot \gamma$'s for $1 \leq l \leq j$.

Remark 4.3.4. *By saying that the sign of γ_s can be determined by the signs of $\gamma_{s'}$'s that*

are known from (i) and (ii) and the signs of $y_l \cdot \gamma$'s for $1 \leq l \leq j$, we mean that γ_s can be written as a linear combination of these $\gamma_{s'}$'s and $y_l \cdot \gamma$'s such that for those $\gamma_{s'}$'s or $y_l \cdot \gamma$'s with nonzero coefficients, the signs of coefficients of positive $\gamma_{s'}$'s and $y_l \cdot \gamma$'s are all the same, and are the opposite to the signs of coefficients of negative $\gamma_{s'}$'s and $y_l \cdot \gamma$'s.

We say the sign of $y \cdot \gamma$ is ***known*** at this node b if (at least) one of the following holds:

(i) The reactant complex y is the zero complex, where the sign of $y \cdot \gamma$ is fixed to be zero.

(ii) The reactant complex y is among the first j $(1 \leq j \leq n)$ reactant complexes $\{y_1, ..., y_j\}$.

(iii) The sign of $y \cdot \gamma$ can be determined (to be defined shortly) based on the signs of γ_s's that are known from (i) and (ii) in the definition of the known signs of γ_s at this node b, and $y_l \cdot \gamma$'s for $1 \leq l \leq j$. In particular, if the sign of $y \cdot \gamma$ can be determined (to be defined shortly) based on the signs of γ_s's that are known from (i) and (ii) in the definition of the known signs of γ_s at this node b, we say the sign of such $y \cdot \gamma$ can be *temporarily fixed* (for the current $y \cdot \gamma$ tree generated by node a).

Remark 4.3.5. *By saying that $y \cdot \gamma$ can be determined based on the known signs of γ_s's or $y_l \cdot \gamma$'s ($1 \leq l \leq j$), we mean that $y \cdot \gamma$ can be written as a linear combination of these γ_s's and $y_l \cdot \gamma$'s such that for those γ_s's or $y_l \cdot \gamma$'s with nonzero coefficients, the signs of coefficients of positive γ_s's and $y_l \cdot \gamma$'s are all the same, and are the opposite to the signs of coefficients of negative γ_s's and $y_l \cdot \gamma$'s.*

From finding at each node of a $y \cdot \gamma$ tree, all the signs of γ_s's and $y \cdot \gamma$'s that are known, we can get a partial sign pattern for γ and a partial sign pattern for $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$ at this node.

Now when we stop at a node in the γ tree, we will use all the known signs of γ_s's to generate a system of linear equalities/inequalities in terms of γ_s's. When we stop at a

node at the $y \cdot \gamma$ tree, we will use all the known signs of γ_s's and $y \cdot \gamma$'s to update the partial sign patterns for γ and $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$, respectively, and update the system of linear equalities/inequalities in terms of γ_s's.

Next, let us describe two ways of walking on the γ and $y \cdot \gamma$ tree: ***move forward*** and ***move backward***.

The rule for moving forward in the γ tree is simple. We start from the current node which is not a leaf node (N^{th} layer) in the γ tree. Walk toward higher layers (for example, from the i^{th} layer to $(i+1)^{th}$ layer) in the γ tree until we either reach a leaf node of positive sign or arrive at the positive node of the next earliest species whose sign is not fixed. Note that whenever we walk past a sign-fixed species (in the γ tree), we will walk through the fixed (zero in this case) sign node.

The rule for moving forward in the $y \cdot \gamma$ tree is similar but slightly complicated. We start from the current node which is not a leaf node (n^{th} layer) in the $y \cdot \gamma$ tree. Walk toward higher layers (for example, from the i^{th} layer to $(i+1)^{th}$ layer) in the $y \cdot \gamma$ tree until we either reach a leaf node of positive sign or arrive at the earliest reactant complex whose corresponding $y \cdot \gamma$ sign is not fixed or temporarily fixed. Note that whenever we walk past a reactant complex whose $y \cdot \gamma$ sign is fixed (i.e. the zero complex in the $y \cdot \gamma$ tree) or temporarily fixed on the way, we will walk through the fixed or temporarily fixed sign node.

The rule for moving backward in the γ tree is slightly complicated. Here we will take into account the fact that only about half (without the inversions) of the sign patterns of γ which are sign-compatible with S need to be considered. We start from the current node which is not the root node of γ tree. Then we follow these rules:

(I) If the current node is a fixed sign node, we walk toward lower layers along the node's walk trace until a node, whose species is not a sign-fixed species, is reached. If we reach the root node, we exit the moving backward process. Otherwise, go to (II).

(II) If the current node is a zero node, skip the rest and go to (III). If the current node is a positive or negative node, then move to another child of its mother node in the following way:

(i) Suppose that the currently stayed node is a positive node. If the fixed signs all have a sign of zero (true in this case), then check if the partial sign pattern from the walk trace at the mother node of this node contains all zero signs. If the answer is yes, move to the zero sign node. Otherwise, move to the negative sign node.

(ii) If the current node is a negative node, move to the zero node.

(III) If the current node is a zero node, then walk toward lower layers in the tree along the node's walk trace to its mother node. Go back to (I).

The rule for moving backward in the $y \cdot \gamma$ tree is similar. We start from the current node which is not the root node in the $y \cdot \gamma$ tree. Then we follow these rules:

(I) If the currently stayed node is a fixed or temporarily fixed sign node, we walk toward lower layers along the node's walk trace until a node, whose reactant complex with $y \cdot \gamma$ sign is not fixed or not temporarily fixed, is reached. Otherwise, go to (II).

(II) If the currently stayed node is a zero node, skip the rest and go to (III). If the current node is a positive or negative node, then move to another child of its mother node in the following way:

(i) If the current node is a positive node, move to the negative sign node.

(ii) If the current node is a negative node, move to the zero sign node.

(III) If the current node is a zero node, then walk toward lower layers in the tree along the node's walk trace to its mother node. Go back to (I).

Now we are ready to talk about the algorithm for checking whether (b) holds. We introduce a boolean variable called *EverFoundSolution*. We will proceed according to the following steps:

Step 0:

EverFoundSolution is set to be False.

Step 1:

We are currently at some node in the γ tree.

(i) If it is the first time walking in the γ tree, i.e., we start at the root of the γ tree, then we will move forward in the γ tree. Skip (ii).

(ii) If it is not the first time walking in the γ tree, then we have already arrived at some node in the γ tree. We will move forward on the γ tree (unless it is already at the last non-fixed species node, then move backward in γ tree) if *EverFoundSolution* is True, and we will move backward on the γ tree if *EverFoundSolution* is False. Thus we arrive at some other node in the γ tree. Note that if after moving backward, we arrive at the root of the γ tree, then we will terminate the process and conclude that condition (b) does not hold. Otherwise we will go to Step 2 and move into the $y \cdot \gamma$ tree generated by the current node as its root.

Step 2:

Set *EverFoundSolution* to be False. We are currently at some node in the $y \cdot \gamma$ tree. We find the known signs of γ_s's and $y \cdot \gamma$'s at the current node and then we have a partial sign pattern for γ and for $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$.

(i) If it is the first time walking in the $y \cdot \gamma$ tree from the γ tree, then we start at the root of the $y \cdot \gamma$ tree. Recall that the known signs of $y \cdot \gamma$'s based on the known signs of γ_s's (s is sign-fixed species or among the first i species where i is the number of the layer the root of the $y \cdot \gamma$ tree lies in the γ tree) are said to be temporarily fixed for the current $y \cdot \gamma$ tree. We will find all the reactant complexes with their corresponding $y \cdot \gamma$ signs temporarily fixed at the root of the $y \cdot \gamma$ tree. We then move forward in the $y \cdot \gamma$ tree. Skip (ii).

(ii) If it is not the first time walking in the $y \cdot \gamma$ tree, then we have already arrived at

some node in the $y \cdot \gamma$ tree. Note that if we previously moved backward and arrive at the root of the $y \cdot \gamma$ tree, then we will go to Step 1 and return to the γ tree.

Step 3:

At the current node of the $y \cdot \gamma$ tree, we update the knowns signs of γ_s's and $y \cdot \gamma$'s and update the corresponding sign patterns of γ and $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$, respectively.

Step 4:

Check if there exist outflow reactions, i.e., $\mathscr{R}_o \neq \emptyset$. If the answer is no, then we skip the following and go to Step 5. Otherwise, we have $\mathscr{R}_o \neq \emptyset$. Note that $\mathscr{R} = \mathscr{R}_t \cup \mathscr{R}_o \cup \mathscr{R}_f$, then equation (4.2.5) can be rewritten as follows:

$$\sum_{y \to y' \in \mathscr{R}_t} \eta_{y \to y'}(y \cdot \gamma)(y' - y) + \sum_{s \to 0 \in \mathscr{R}_o} \eta_{s \to 0}(\gamma_s)(-s) + \sum_{0 \to s \in \mathscr{R}_f} \eta_{s \to 0}(0)(s) = 0$$

which is equivalent to:

$$\sum_{s \to 0 \in \mathscr{R}_o} \eta_{s \to 0}(\gamma_s)(s) = \sum_{y \to y' \in \mathscr{R}_t} \eta_{y \to y'}(y \cdot \gamma)(y' - y) \tag{4.3.3}$$

Given $\gamma \in \mathbb{R}^{\mathscr{S}}$, we define $\gamma^{\text{new}} \in \mathbb{R}^{\mathscr{S}}$ as follows:

$$\gamma_s^{\text{new}} = \begin{cases} \gamma_s, & \text{if } s \to 0 \in \mathscr{R}_o \\ 0, & \text{otherwise.} \end{cases}$$

From the partial sign pattern for γ at the current node and the definition of γ^{new}, γ^{new} has a partial sign pattern at the current node in the $y \cdot \gamma$ tree. Let $S_{\text{true}} = \{y' - y : y \to y' \in \mathscr{R}_t\}$. From equation (4.3.3), we can see that γ^{new} must be sign-compatible with S_{true}. Therefore we will check if there exists $\tilde{\gamma} \in S_{\text{true}}$ with the same partial sign pattern as γ^{new}. If the answer is yes, we move to Step 5; otherwise, we move backward in the $y \cdot \gamma$ tree and go back to Step 2.

Step 5:

If dim $S = \#(\mathscr{S})$, go to Step 6 and skip the rest of Step 5. Otherwise, we check if there exists a nonzero $\beta \in S$ with the same partial sign pattern as γ. If the answer is yes, we go to Step 6; otherwise, we move backward in the $y \cdot \gamma$ tree and go back to Step 2.

Step 6:

Recall that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is defined via $\tilde{L}_{y\to y'}(\gamma) = y \cdot \gamma$. From the partial sign pattern for $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$, $\tilde{L}(\gamma)$ has a partial sign pattern at the current node in the $y \cdot \gamma$ tree. We check if there exists a nonzero $\alpha \in Ker\ T$ with the same partial sign pattern as $\tilde{L}(\gamma)$. If the answer is yes, then we move to Step 7; otherwise, we move backward in the $y \cdot \gamma$ tree and go back to Step 2.

Step 7:

Check if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ satisfying the inequality system generated by the known signs of γ_s's and $y \cdot \gamma$'s. If the answer is no, then we move backward in $y \cdot \gamma$ tree and go back to Step 2. If the answer is yes, then record the value and sign of each γ_s and calculate the value and sign of each $y \cdot \gamma$. We will set *EverFoundSolution* to be true.

Step 8:

Now that all signs of γ_s's and $y \cdot \gamma$'s are known, we have (full) sign patterns for γ and $[y_1 \cdot \gamma, ..., y_n \cdot \gamma]$. We then check if all of the sign-compatible conditions in Step 4 on S_{true} (if applicable), Step 5 on S (if applicable) and Step 6 on $Ker\ T$ are satisfied.

If the answer is no, then we move forward in the $y \cdot \gamma$ tree (unless it is already at the node corresponding to the last reactant complex whose $y \cdot \gamma$ sign is not fixed or temporarily fixed, then we move backward in $y \cdot \gamma$ tree) and go back to Step 2. If the answer is yes, then we can find a solution of $\eta \in \mathbb{R}^{\mathscr{R}}_+$ from equation (4.2.5). We will terminate the process and claim that the network is not mass action injective.

Therefore, after we run the two checks for whether (a) or (b) holds, we can have the following situations:

(i) Neither condition (a) nor (b) holds. We then claim that the reaction network is mass action injective and therefore does not have the capacity to admit more than one steady state.

(ii) Condition (a) or (b) holds. We then claim that the reaction network is not mass

action injective and therefore the answer to whether the network has the capacity to admit multiple steady states is inconclusive. In this case, we need to refer to the Higher Deficiency Algorithm for more information.

Remark 4.3.6. *Recall that in Lemma 4.3.2, we state that the following statements are equivalent:*

(A) There exist an $\eta \in \mathbb{R}^{\mathscr{R}}_{+}$ and a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S, satisfying equation (4.2.5).

(B) There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is sign-compatible with $Ker\ T$.

(C) One of the two conditions holds:

(a): There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S and lies in $S_Y^{\perp}$.

(b): There exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that $\tilde{L}(\gamma) \in \mathbb{R}^{\mathscr{R}}$ is nonzero and sign-compatible with $Ker\ T$.

Note that in Lemma 4.3.2, (B) is a trivial generalization (by allowing $\tilde{L}(\gamma) = 0$) of condition (b) of (C), therefore the algorithms of checking whether condition (b) holds or (B) holds are very similar. The algorithm for the Mass Action Injectivity Test implemented in [16], which tests if (B) holds, is different from that of checking whether condition (b) holds in the following places:

In (ii) of Step 1, we replace the original sentence "Note that if after moving backward, we arrive at the root of the γ tree, then we will terminate the process and conclude that condition (b) does not hold." by "Note that if after moving backward, we arrive at the root of the γ tree, then we will terminate the process and conclude that the reaction network is mass action injective."

In Step 6, we replace the original sentence "We check if there exists a nonzero $\alpha \in Ker$

T with the partial sign pattern of $\tilde{L}(\gamma)$." with "We check if there exists a $\alpha \in Ker\ T$ with the partial sign pattern of $\tilde{L}(\gamma)$."

4.4 Applications

Having explained how the algorithm worked, we will give an example of reaction network (4.4.1). Note that we will write in terms of reactions, not a standard network diagram.

$$
\begin{aligned}
S_1 + E_1 &\rightleftharpoons E_1S_1 \\
S_2 + E_1S_1 &\rightleftharpoons E_1S_1S_2 \to P_1 + E_1 \\
S_2 + E_2 &\rightleftharpoons E_2S_2 \\
S_3 + E_2S_2 &\rightleftharpoons E_2S_2S_3 \to P_2 + E_2 \\
S_3 + E_3 &\rightleftharpoons E_3S_3 \\
S_4 + E_3S_3 &\rightleftharpoons E_3S_3S_4 \to P_3 + E_3 \\
S_4 + E_4 &\rightleftharpoons E_4S_4 \to 2S_1 + E_4 \\
S1 &\rightleftharpoons 0 \rightleftharpoons S2 \\
S3 &\rightleftharpoons 0 \rightleftharpoons S4 \\
P1 &\to 0 \leftarrow P_2 \\
&\uparrow \\
&P_3
\end{aligned}
\tag{4.4.1}
$$

The Mass Action Injectivity Test, implemented in [16], concludes that the reaction network (4.4.1) is mass action injective. Therefore, the network (4.4.1) does not have the capacity to admit more than one steady state.

Chapter 5

CONCORDANCE TEST

5.1 Background

The Concordance Test is a test of whether a given reaction network is concordant (to be defined shortly). The idea of a concordant network comes from a private communication ([17]). As we shall see, one can say much about systems in which the underlying network is concordant, *even when the kinetics is not mass action* (provided that certain weak conditions on the kinetics are satisfied). In this chapter we mainly focus on finding an algorithm to determine whether a network is concordant. The algorithm has been implemented in the Chemical Reaction Network Toolbox [16].

We will present a few terms and a proposition from [17].

The map $L : \mathbb{R}^{\mathscr{R}} \to \mathbb{R}^{\mathscr{S}}$ is defined as follows: for given $\alpha \in \mathbb{R}^{\mathscr{R}}$,

$$L\alpha = \sum_{y \to y' \in \mathscr{R}} \alpha_{y \to y'}(y' - y).$$

A reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is ***concordant*** if there does not exist an $\alpha \in Ker\ L$ and a nonzero $\sigma \in S$ satisfying the following conditions:

(i) For each $y \to y' \in \mathscr{R}$ such that $\alpha_{y \to y'} \neq 0$, supp y contains a species s for which $\text{sgn}(\sigma_s) = \text{sgn}(\alpha_{y \to y'})$.

(ii) For each $y \to y' \in \mathscr{R}$ such that $\alpha_{y \to y'} = 0$, $\sigma_s = 0$ for all $s \in$ supp y or else supp y contains species s and s' for which $\text{sgn}(\sigma_s) = -\text{sgn}(\sigma_{s'})$, both not zero.

Recall that a kinetics $\mathscr{K}$ for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is an assignment of a (continuous nonnegative real-valued) rate function $\mathscr{K}_{y\to y'}(\cdot)$ to each reaction $y \to y'$ in the network.

A reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ governed by some kinetics $\mathscr{K}$ is ***injective*** if, for each of distinct stoichiometrically compatible compositions $c^*, c^{**} \in \bar{\mathbb{R}}^{\mathscr{S}}_+$, at least one of which is strictly positive,

$$\sum_{y\to y'\in\mathscr{R}} \mathscr{K}_{y\to y'}(c^{**})(y'-y) \neq \sum_{y\to y'\in\mathscr{R}} \mathscr{K}_{y\to y'}(c^{*})(y'-y) \tag{5.1.1}$$

A kinetics $\mathscr{K}$ for reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is ***weakly monotonic*** if, for each pair of compositions $c^*, c^{**} \in \bar{\mathbb{R}}^{\mathscr{S}}_+$, the following implications hold for each reaction $y \to y' \in \mathscr{R}$ such that supp $y \subseteq$ supp c^* and supp $y \subseteq$ supp c^{**}:

(i) If $\mathscr{K}(c^{**}) > \mathscr{K}(c^*)$, then there exists a species $s \in$ supp y with $c^{**}_s > c^*_s$.

(ii) If $\mathscr{K}(c^{**}) = \mathscr{K}(c^*)$, then either $c^{**} = c^*$ or there exist species $s, s' \in$ supp y with $c^{**}_s > c^*_s$ and $c^{**}_{s'} < c^*_{s'}$.

Proposition 5.1.1. *[17] A reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ governed by weakly monotonic kinetics is injective whenever its underlying reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is concordant. In particular, if the underlying reaction network is concordant, then the reaction network governed by weakly monotonic kinetics cannot admit two distinct stoichiometrically compatible equilibria, at least one of which is positive.*

Concordant networks have many pleasant properties when taken with any weakly monotonic kinetics. For more information on those other properties, see [17].

5.2 Algorithm

We will describe an algorithm to test if a given reaction network is concordant. From a given nonzero sign pattern for $\sigma \in S$, the definition of concordance restricts the signs that

components of $\alpha \in Ker\ L$ might take. Suppose that $\sigma \in S$ is nonzero and let y be a reactant complex. Then we have the following cases:

(i) If for all $s \in$ supp y, sgn$(\sigma_s) = 0$, then $\alpha_{y\to y'} = 0$; or else if for all $s \in$ supp y, sgn$(\sigma_s) \geq 0$, then $\alpha_{y\to y'} > 0$; or else if for all $s \in$ supp y, sgn$(\sigma_s) \leq 0$, then $\alpha_{y\to y'} < 0$.

(ii) If there exist $s, s' \in$ supp y such that sgn$(\sigma_s) = -$sgn$(\sigma_{s'})$, both nonzero, then the sign of $\alpha_{y\to y'}$ can be free (by free we mean that there is no constraint on the sign, and it could be positive, negative or zero).

Note that $\alpha_{y\to y'}$ having a free sign actually means we do not assign a sign to $\alpha_{y\to y'}$. Thus we have a partial sign pattern for $\alpha \in \mathbb{R}^{\mathscr{R}}$. We then check if such a partial sign pattern is sign compatible with $Ker\ L$. If so, then there exists $\alpha \in Ker\ L$ with such a partial sign pattern; thus we claim that the reaction network is not concordant. If for any nonzero $\sigma \in S$, there does not exist $\alpha \in Ker\ L$ with the partial sign pattern generated from cases (i) and (ii) above, then we claim the reaction network is concordant and the reaction network does not have the capacity to admit multiple steady states (with weakly monotonic kinetics).

Note that since we only care about the sign pattern of $\sigma \in S$, not the value of its component, we may relax the condition of a nonzero $\sigma \in S$ to study any nonzero $\sigma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S. In other words, we check if the following condition (**) holds:

(**): There exists a nonzero $\sigma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S such that there exists $\alpha \in Ker\ L$ with a partial sign pattern generated from cases (i) and (ii) above.

Note that if $\sigma \in \mathbb{R}^{\mathscr{S}}$ and $\alpha \in Ker\ L$ satisfy the condition (**), then $-\sigma$ and $-\alpha$ also satisfy the condition (**). Recall that in Section 4.3 of Chapter 4, for the check of whether condition (a) holds, we check if there exists a nonzero $\gamma \in \mathbb{R}^{\mathscr{S}}$ that is sign-compatible with S and lies in $S_Y^{\perp}$. When checking whether condition (**) holds or whether condition (a) holds (in Section 4.3 of Chapter 4), we only need to consider almost half (leaving out the

inversions) of the sign patterns of γ or σ. Therefore, we can set up a σ tree as we did for a γ tree in the check of whether condition (a) holds for the Mass Action Injectivity Test. We can similarly define the walk in the tree, the known signs of σ_s's at some node, a partial sign pattern for σ, a sign pattern for σ, move forward and move backward in the σ tree, etc. We will also first check if there exists any species s that does not lie in the support of any reaction vectors $y' - y$. Because $\sigma \in \mathbb{R}^{\mathscr{S}}$ is required to be sign-compatible with S, we set the sign of σ_s to be zero for any such species s, and call it a sign-fixed species. As for the sign-fixed species, when we walk past the nodes corresponding such species, we only walk through the one corresponding to the given fixed (zero) sign node.

Recall that in Section 4.3 of Chapter 4, when we checked whether condition (a) holds, we defined two ways of walking in the γ tree: ***move forward*** and ***move backward***. Here we apply the same rules on the σ tree.

The rule for moving forward is simple. We start from the current node which is not a leaf node (in the N^{th} layer, where $N = \#(\mathscr{S})$). We walk toward higher layers (for example, from i^{th} layer to $(i+1)^{th}$ layer) in the σ tree until we either reach a positive leaf node or arrive at the positive node of the next earliest species whose sign is not fixed. Note that whenever we walk past a sign-fixed species, we will walk through the fixed (zero in this case) sign node.

The rule for moving backward is slightly complicated. Here we will take into account the fact that only almost half (leaving out the inversions) of the sign patterns of σ which are sign-compatible with S need to be considered. We start from the current node which is not the root node. Then follow these rules:

(I) If the current node is a fixed sign node (zero sign in this case), walk toward lower layers along its walk trace until a node, whose species is not a sign-fixed species, is reached. If we reach the root node, we exit the moving backward process. Otherwise, go to (II).

(II) If the current node is a zero node, skip the rest and go to (III). If the current node is

a positive or negative node, then move to another child of its mother node in the following way:

(i) Suppose that the current node is a positive node. If the fixed signs all have a sign of zero (true in this case), then check if the partial sign pattern, generated from the known signs of σ_s's at the mother node of this node, contains all zero signs. If the answer is yes, move to the zero sign node. Otherwise, move to the negative sign node.

(ii) If the current node is a negative node, move to the zero node.

(III) If the current node is a zero node, then move back in the tree (along its walk trace) to its mother node. If we reach the root node, we exit the moving backward process. Otherwise, go back to (I).

We will proceed with the Concordance Test according to the following steps.

Step 0:

Start at the root node of the σ tree and move forward.

Step 1:

We are currently at some node. We have a partial sign pattern for σ from the σ_s's that are known at the current node. We then check if there exists a nonzero $\beta \in S$ with such a partial sign pattern.

Step 2:

If the answer is no in Step 1, move backward on the σ tree and go to Step 1. If the answer is yes, then check if we are currently at a leaf node of the σ tree. If the answer is no, move forward and go to Step 1. If the answer is yes, then go to Step 3.

Step 3:

If the sign pattern of σ is such that $\sigma_s = 0$ for all $s \in \mathscr{S}$, then we claim that the reaction network is concordant and exit the algorithm. Otherwise, for the given nonzero sign pattern

of σ, we may restrict the signs of $\alpha_{y\to y'}$'s where y is a reactant complex, according to the following rules:

(i) If for all $s \in$ supp y, sgn$(\sigma_s) = 0$, then $\alpha_{y\to y'} = 0$; or else if for all $s \in$ supp y, sgn$(\sigma_s) \geq 0$, then $\alpha_{y\to y'} > 0$; or else if for all $s \in$ supp y, sgn$(\sigma_s) \leq 0$, then $\alpha_{y\to y'} < 0$.

(ii) If there exist $s, s' \in$ supp y, such that sgn$(\sigma_s) = -$sgn$(\sigma_{s'})$, both nonzero, then the sign of $\alpha_{y\to y'}$ is set to be free (by free we mean that any sign is possible and we do not put any constraint on the sign).

We then obtain a partial sign pattern for $\alpha \in \mathbb{R}^{\mathscr{R}}$. We check if there exists an $\alpha \in Ker\ L$ with such a partial sign pattern. If the answer is yes, then we claim that the reaction network is not concordant and exit the algorithm. If the answer is no, we move backward in the σ tree and go to Step 1.

Therefore, after we run the Concordance Test, we can either claim that the reaction network is concordant and therefore does not have the capacity to admit more than one positive steady state, or the reaction network is not concordant. In the latter case, the test is inconclusive in answering the question of multiple steady states and we may refer to the Higher Deficiency Algorithm or the Mass Action Injectivity Test for more information.

5.3 Applications

We will use the same example of reaction network (4.4.1) as in Chapter 4.

The Concordance Test, implemented in [16], concludes that the reaction network is concordant. Therefore, the network (4.4.1) does not have the capacity to admit more than one positive steady state, so long as the kinetics is weakly monotonic: It does not have to be mass action. From [17], we have that:

Concordant networks have many pleasant properties when taken with any weakly monotonic kinetics, not necessarily mass action. For example, a positive equilibrium is invariably

unique in the following sense: There can be no other equilibrium that is stoichiometrically compatible with it, not even one in which certain species are absent.

For still other properties of concordant networks, see [17] for more information.

Chapter 6

FUTURE RESEARCH

6.1 Overview

In this chapter we will raise a few questions about improving the efficiency (in terms of getting a definite answer) of the Higher Deficiency Algorithm.

Recall that for a reaction network $\{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ with orientation $\mathscr{O}$, we define "$\sim$" equivalence classes P_i $(0 \leq i \leq w)$ and the corresponding fundamental classes C_i $(0 \leq i \leq w)$, where P_0 is the zeroth equivalence class. We pick $y_i \rightarrow y_i'$ as the representative for P_i $(0 \leq i \leq w)$. We then define $W = \{y_i \rightarrow y_i'\}_{i=1}^{w}$. Recall that q is the dimension of $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$. Suppose we pick a "valid" sign pattern for $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ and a parameter set $\{\rho_W(y_i \rightarrow y_i') : g_W(y_i \rightarrow y_i') \neq 0\}$ is also given, consistent in sign with the sign patterns of g_W and h_W. Let $\{b^j\}_{j=1}^{q}$ be a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$. Given the sign patterns for g_W and h_W, for each $1 \leq j \leq q$, we define $Q_+^j = \{\rho_W(y_i \rightarrow y_i') : y_i \rightarrow y_i' \in W$ and $b^j_{y_i \rightarrow y_i'} g_W(y_i \rightarrow y_i') > 0\}$ and $Q_-^j = \{\rho_W(y_i \rightarrow y_i') : y_i \rightarrow y_i' \in W$ and $b^j_{y_i \rightarrow y_i'} g_W(y_i \rightarrow y_i') < 0\}$.

6.2 Nonlinear Conditions on ρ's

From Remark 2.9.6, we know that if $2q \geq w$, then in order for there to exist g_W, h_W satisfying equations (2.9.13), (2.9.14) and (2.9.15), we need to add additional (nonlinear) equalities in terms of $\rho_W(y_i \rightarrow y_i')$'s (from satisfying rank $A_D < w$, where A_D is defined

as in equation (2.9.19)). However, we did not implement this in the algorithm as these are nonlinear equalities in terms of $\rho_W(y_i \rightarrow y_i')$'s. Note that these nonlinear equalities in terms of $\rho_W(y_i \rightarrow y_i')$'s will be nonlinear equalities in terms of e^{M_i}'s. Therefore, the resulting systems are nonlinear in terms of M_i's. (Or we could rephrase a pre-signature in terms of M_i's to be in terms of $\rho_W(y_i \rightarrow y_i')$'s and get a nonlinear system in terms of $\rho_W(y_i \rightarrow y_i')$'s.) In the future, we could implement this as an expanded version of [16]. We will need a package to calculate the determinants (of all $(w \times w)$ submatrices of A_D) and a reliable and freely available nonlinear solver that can be called from [16].

If $2q < w$ and $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ does not have a forest basis, then the conditions (*) (first mentioned in Section 2.9) on the $\rho_W(y_i \rightarrow y_i')$'s, which we later denote as conditions in Proposition 2.10.14, are necessary but not necessarily sufficient for the existence of $g_W, h_W \in \mathbb{R}^{\mathscr{O}} \cap \Gamma_W$ with the given "valid" pair of sign patterns satisfying equations (2.9.13), (2.9.14), and (2.9.15). Therefore, we may require additional equalities in terms of $\rho_W(y_i \rightarrow y_i')$'s besides those arose from zero determinants (of all $(w \times w)$ submatrices of A_D) in the last paragraph to guarantee the existence of such g_W and h_W. We have not built a systematic way to set up these additional nonlinear equalities. For our future study, we could work on finding these equalities.

6.3 More Efficient b-Bases

Note that in the conditions of Proposition 2.10.14 we did not put any assumptions on the size of the support of each b^j $(1 \leq j \leq q)$. However, we know if we could find a basis $\{b^j\}_{j=1}^q$ where each b^j has a relatively small support (meaning the basis vector matrix is quite sparse), then the multisets Q_1^j and Q_2^j in Proposition 2.10.14 are smaller. Therefore, the number of subcases generated in each case of the conditions in Proposition 2.10.14 will be smaller, and the efficiency of the algorithm will be improved. In the linear case, we can

try to find a forest basis $\{b^j\}_{j=1}^q$ where each b^j $(1 \leq j \leq q)$ has a relatively small support, following the algorithm in Section 2.12.

In the case that there does not exist a forest basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$, we would work to find a systematic way to find a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ such that each basis vector has a relatively smaller support. We could exhaustively search basis vectors with 3-support, 4-support, 5-support, etc., to construct a basis. For a basis vector with n-support, we try to find a vector in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ with support on some n reactions. We will test all 3 combinations of the w reactions to find an independent set of vectors in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ with 3-support. If the number of independent vectors we found is less than q, then we need to continue looking for vectors with bigger size support. For the 4-support, we will test all 4 combinations of the w reactions to find an independent set of vectors in $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ with 4-support, which if adding the vectors we find in the 3-support step, will still be an independent set. Of course, we can skip the combinations of four reactions where a basis vector with three of the four reactions has been found in the 3-support step. The process of finding such a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ could be time consuming, especially when the size of the support becomes high and we are still looking for more vectors to complete a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$. Thus currently in [16], when $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$ does not have a forest basis, we only find the basis vectors with 3-support to build a subset of a basis for $Ker^{\perp}\ L_{\mathscr{O}} \cap \Gamma_W$. If we can find a smart way of finding a complete basis where each basis vector has a relatively small support, the efficiency of the algorithm could be improved.

6.4 Shelving conditions

If we can find more necessary conditions as listed in Proposition 2.8.1, we could possibly improve the efficiency of the algorithm.

Recall that in the Deficiency One Algorithm the "shelves" are assigned following the conditions below:

(i) All non-terminal complexes are put in the middle shelf $\mathscr{M}$.

(ii) All complexes in the same nontrivial terminal strong linkage class are put in the same shelf.

(iii) If there are no trivial terminal strong linkage classes, then neither the upper shelf nor the lower shelf can be empty.

(iv) If there is exactly one trivial terminal strong linkage class, then the upper shelf and the lower shelf cannot both be empty.

Recall that we did not generalize conditions (iii) and (iv) above in the Higher Deficiency Algorithm. These generalizations may still exist.

6.5 Merger of Theories

There are three approaches to answer the question of multiple steady states in this thesis as well as in its computer implementation [16]: the Deficiency Theories and Algorithm, the Mass Action Injectivity Test, and the Concordance Test. The Deficiency Zero Theorem and the Deficiency One Theorem are very simple and powerful, without involving too many calculations. However, the Deficiency One Algorithm and the Higher Deficiency Theory may need to produce many inequality systems to solve the question of multiple steady states. The Mass Action Injectivity Test and the Concordance Test are relatively simple in terms of the calculations involved. We have known that there are cases when the Mass Action Injectivity Test and/or the Concordance Test conclude that the reaction network does not have the capacity to admit multiple steady states and yet the High Deficiency Theory stays silent. We in the future could work on merging all of these approaches into some "big" theory that incorporates all of these developed theories.

BIBLIOGRAPHY

[1] M. Feinberg, Lectures on Chemical Reaction Networks, 1979. Written version of lectures given at the Mathematical Research Center, University of Wisconsin, Madison, WI.

http://www.chbmeng.ohio-state.edu/∼feinberg/LecturesOnReactionNetworks.

[2] M. Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors: I. The deficiency zero and deficiency one theorems, Chem. Eng. Science, 42, 2229-2268, 1987.

[3] M. Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors: II. Multiple steady states for networks of deficiency one, Chem. Eng. Science, 43, 1-25, 1988.

[4] M. Feinberg, The existence and uniqueness of steady states for a class of chemical reaction networks, Archive for Rational Mechanics and Analysis, 132, 311-370, 1995.

[5] M. Feinberg, Multiple steady states for chemical reaction networks of deficiency one, Archive for Rational Mechanics and Analysis, 132, 371-406, 1995.

[6] M. Feinberg, The Chemical Reaction Network Toolbox, Ver. 1.02a, 1995.

http://www.chbmeng.ohio-state.edu/∼feinberg/crnt/.

[7] P. Ellison, Ph.D. thesis, The Advanced Deficiency Algorithm and its applications to mechanism discrimination, Department of Chemical Engineering, University of Rochester, 1998.

[8] P. Ellison and M. Feinberg, The Chemical Reaction Network Toolbox, Ver. 1.1a, 1995. http://www.chbmeng.ohio-state.edu/∼feinberg/crnt/.

[9] P. Ellison and M. Feinberg, How catalytic mechanisms reveal themselves in multiple steady-state data: I. Basic principles, Journal of Molecular Catalysis. A, Chemical, 154, 155-167, 2000.

[10] P. Ellison, M. Feinberg, M. H. Yue and H. Saltsburg, How Catalytic Mechanisms Reveal Themselves in Multiple Steady State Data: II. Basic Principles, Journal of Molecular Catalysis. A, Chemical, 154, 169-184, 2000.

[11] P. Ellison, M. Feinberg, M. H. Yue and H. Saltsburg, Corrigendum to "How Catalytic Mechanisms Reveal Themselves in Multiple Steady State Data: II. An Ethylene Hydrogenation Example, Mol. Catal. A: Chem. 154, 169-184, 2000", 260, 306-306, 2006.

[12] S. Axler, F. W. Gehring, K. A. Ribet, Graph Theory, 2nd edition, Springer.

[13] G. Craciun and M. Feinberg, Multiple Equilibria in Comoplex Chemical reaction Networks: I. The Injectivity Property, SIAM Journal on Applied Mathematics, 65, 1526-1546, 2005.

[14] G. Craciun and M. Feinberg, Multiple equilibria in complex chemical reaction networks: II. The species-reaction graph, SIAM Journal on Applied Mathematics, 66, 1321-1338, 2006.

[15] G. Craciun and M. Feinberg, Multiple equilibria in complex chemical reaction networks: extensions to entrapped species models, IEE Proc. Syst. Biol, 153, 179-186, 2006.

[16] H. Ji, P. Ellison and M. Feinberg, The Chemical Reaction Network Toolbox, Ver. 2.X, 2011. http://www.chbmeng.ohio-state.edu/∼feinberg/crntwin/.

[17] G. Shinar and M. Feinberg, On Concordant Reaction Networks, manuscript in preparation, 2011.