Chapter 4

Model Order Reduction of Transcription and Translation Mass Action Models in the Presence of Resource Consumption

4.1 Introduction

Modeling polymerization reactions like transcription and translation is often used in the study of metabolic pathways [26] in systems biology, and gene regulatory pathways in synthetic biology.

Such modeling can be carried out using either stochastic or deterministic frameworks, each which offers distinct advantages. Stochastic models give us the ability to study the evolution of the probability distributions of species, and work well at low molecular counts, but are computationally expensive. Deterministic models on the other hand, are much less computationally demanding to simulate, but also provide less information than stochastic models.

Models can also exist at various levels of detail. Often, the appropriate level of detail, exemplified by models used in [15], involves the production of RNA and proteins as single steps. In other cases, much more detailed models of transcription incorporating the formation of pre-initiation complexes, release from proximal promoter regions, individual elongation steps, and detailed termination are appropriate [27, 36]. Similarly, models of translation have also been studied at various levels of detail [14, 30].

The choice of which framework to use, stochastic versus deterministic, detailed versus lumped, depends on the specified purpose of the model, and on the computational complexity the user is willing to work with. A detailed stochastic model may be reduced in two different directions: it may be made deterministic under the infinite volume limit [42], or reactions and mechanisms may be lumped into simplified models [51].

The use of models of transcription and translation in cell-free extracts has made it necessary to explicitly account for the consumption and loading of resources that occurs due to gene expression. However, incorporating the consumption of nucleotides and amino acids in the elongation process is done using detailed models, which account for elongation steps individually, as was done in [1] for the case of transcription.

In this chapter, we start with a detailed deterministic ordinary differential equation (ODE) models of transcription similar to the one found in [1], and demonstrate a lumping procedure for reactions that maintains the ability of the model to account for resource consumption. We begin by demonstrating the main idea for the reduction to a single transcription step, and then generalize this to incorporate the possibility of multiple intermediate stages in the transcription process. This general case is required when intermediate nascent transcripts can have some function other than being a precursor to the next elongation step within transcription. An example of this is when non coding RNAs are used as regulatory elements [7, 8]. The model reduction requires the use of the rapid equilibrium assumption [54], which can be rigorously justified using singular perturbation theory [39, 41, 67, 68]. Due to a structural feature of the chemical reaction network describing transcription, when species concentrations are used as state variables in the model, these state variables all possess boundary layer behavior, and converge to a quasi-steady state ([39], Section 1.6). This makes it difficult to bring the differential equations into the standard singular perturbation form, and a change of coordinates, described in Section 4.4.1, is needed before such a form can be achieved.

4.2 Consumption Model

One may divide the stages of transcription and translation into initiation, elongation and termination. Each of these stages involves a complex set of reactions, and may be divided into various smaller stages. For illustrative purposes, we will work with transcription in the rest of this chapter, but a similar reduction procedure may be carried out for translation.

We start with a model similar to the one shown in ([1], Figure 1A), with a few simplifications: we group the different nucleotides into a single species (N), and remove the production of the inorganic pyrophosphate. Defining the notation X:Y to denote the species X and Y bound together into a new species, our resulting model is

$$\begin{aligned}
\mathrm{P} + \mathrm{D} &\underset{k_{Pr}}{\overset{k_{Pf}}{\rightleftharpoons}} \mathrm{P{:}D_1{:}m_0}, && \text{Polymerase binding,}\\
\mathrm{P{:}D_1{:}m_0} + \mathrm{N} &\underset{k_{Nr}}{\overset{k_{Nf}}{\rightleftharpoons}} \mathrm{P{:}D_1{:}m_0{:}N}, && \text{Nucleotide binding,}\\
\mathrm{P{:}D_1{:}m_0{:}N} &\xrightarrow{k_{tx}} \mathrm{P{:}D_2{:}m_1}, && \text{Elongation,}\\
&\vdots &&\\
\mathrm{P{:}D_n{:}m_{n-1}{:}N} &\xrightarrow{k_{tx}} \mathrm{P{:}D_t} + \mathrm{m_n}, && \text{Elongation,}\\
\mathrm{P{:}D_t} &\xrightarrow{k_{\text{term}}} \mathrm{P} + \mathrm{D}, && \text{Termination.}
\end{aligned} \tag{4.1}$$

Here, the entire initiation stage is lumped into a single reaction where an RNA polymerase molecule (P) binds to a DNA molecule (D). Elongation then proceeds iteratively, with each iteration consisting a reversible nucleotide binding reaction and an irreversible elongation step. Finally, termination is modeled as the dissociation of the complex comprising the RNA polymerase bound to the final location on the DNA ($\mathrm{P{:}D_t}$).

We can write this network in terms of ODEs using mass action kinetics

$$
\begin{aligned}
\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} &= -k_{Pf}[\mathrm{P}][\mathrm{D}] + k_{Pr}[\mathrm{P{:}D_1{:}m_0}] + k_{\mathrm{term}}[\mathrm{P{:}D_t}],\\
\frac{\mathrm{d}[\mathrm{D}]}{\mathrm{d}t} &= -k_{Pf}[\mathrm{P}][\mathrm{D}] + k_{Pr}[\mathrm{P{:}D_1{:}m_0}] + k_{\mathrm{term}}[\mathrm{P{:}D_t}],\\
\frac{\mathrm{d}[\mathrm{P{:}D_1{:}m_0}]}{\mathrm{d}t} &= k_{Pf}[\mathrm{P}][\mathrm{D}] - k_{Pr}[\mathrm{P{:}D_1{:}m_0}] - k_{Nf}[\mathrm{P{:}D_1{:}m_0}][\mathrm{N}] + k_{Nr}[\mathrm{P{:}D_1{:}m_0{:}N}],\\
\frac{\mathrm{d}[\mathrm{P{:}D_1{:}m_0{:}N}]}{\mathrm{d}t} &= k_{Nf}[\mathrm{P{:}D_1{:}m_0}][\mathrm{N}] - k_{Nr}[\mathrm{P{:}D_1{:}m_0{:}N}] - k_{tx}[\mathrm{P{:}D_1{:}m_0{:}N}],\\
\frac{\mathrm{d}[\mathrm{P{:}D_2{:}m_1}]}{\mathrm{d}t} &= k_{tx}[\mathrm{P{:}D_1{:}m_0{:}N}] - k_{Nf}[\mathrm{P{:}D_2{:}m_1}][\mathrm{N}] + k_{Nr}[\mathrm{P{:}D_2{:}m_1{:}N}],\\
&\vdots\\
\frac{\mathrm{d}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}]}{\mathrm{d}t} &= -k_{tx}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}] + k_{Nf}[\mathrm{P{:}D_n{:}m_{n-1}}][\mathrm{N}] - k_{Nr}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}],\\
\frac{\mathrm{d}[\mathrm{P{:}D_t}]}{\mathrm{d}t} &= k_{tx}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}] - k_{\mathrm{term}}[\mathrm{P{:}D_t}],\\
\frac{\mathrm{d}[\mathrm{N}]}{\mathrm{d}t} &= \sum_{k=1}^{n}\left(k_{Nr}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}] - k_{Nf}[\mathrm{P{:}D_k{:}m_{k-1}}][\mathrm{N}]\right),\\
\frac{\mathrm{d}[\mathrm{m_n}]}{\mathrm{d}t} &= k_{tx}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}].
\end{aligned}
\tag{4.2}
$$

We may wish to lump all the elongation steps into a single or a few steps, while maintaining the correct average rates of RNA production and nucleotide consumption. A simple model is given by

$$
\begin{aligned}
\mathrm{P} + \mathrm{D} &\underset{k_{Pr}}{\overset{k_{Pf}}{\rightleftharpoons}} \mathrm{P{:}D}, && \text{Polymerase binding},\\
\mathrm{P{:}D} + \mathrm{nN} &\underset{k_{Nr}}{\overset{k_{Nf}}{\rightleftharpoons}} \mathrm{P{:}D{:}nN}, && \text{Nucleotide binding},\\
\mathrm{P{:}D{:}nN} &\xrightarrow{k_{tx}} \mathrm{P{:}D_t} + \mathrm{m}, && \text{RNA production},\\
\mathrm{P{:}D_t} &\xrightarrow{k_{\mathrm{term}}} \mathrm{P} + \mathrm{D}, && \text{Termination},
\end{aligned}
\tag{4.3}
$$

where n is the number of nucleotides needed to create a single RNA transcript. The names of the relevant rate constants are shown on the arrows in the model. While this simple model preserves the stoichiometry of the consumption of substrate nucleotides and the production of RNA, it models the kinetics of the system incorrectly; it is describing the scenario where n nucleotides simultaneously collide with the P:D complex to form a larger

complex. This is both biologically implausible and computationally intractable, due to the appearance of n as an exponent in some of the terms in the mass action ODEs. Figure 4.1B shows the results of attempting to simulate the resulting model for various transcript lengths.

To circumvent this problem, we propose modeling the consumption of nucleotides separately from the production of RNA, and scaling the RNA production rate by n to get the nucleotide consumption rate. The resulting *consumption model* is given by the equations

$$\begin{aligned}
\mathrm{P} + \mathrm{D} &\underset{k_{Pr}}{\overset{k_{Pf}}{\rightleftharpoons}} \mathrm{P{:}D}, && \text{Polymerase binding,} \\
\mathrm{P{:}D} + \mathrm{N} &\underset{k_{\mathrm{Nr}}}{\overset{k_{\mathrm{Nf}}}{\rightleftharpoons}} \mathrm{P{:}D{:}N}, && \text{Nucleotide binding,} \\
\mathrm{P{:}D{:}N} &\xrightarrow{k_{\mathrm{con}}} \mathrm{P{:}D}, && \text{Consumption,} \\
\mathrm{P{:}D{:}N} &\xrightarrow{k_{\mathrm{reduced}}} \mathrm{P{:}D_t} + \mathrm{R}, && \text{RNA production,} \\
\mathrm{P{:}D_t} &\xrightarrow{k_{\mathrm{term}}} \mathrm{P} + \mathrm{D}, && \text{Termination}
\end{aligned} \tag{4.4}$$

with k_{tx} denoting the transcription rate, and $k_{\mathrm{con}} = (n-1) \times k_{tx}$ the rate of a *consumption reaction*. A pictorial representation of this scheme is shown in Figure 4.1A.

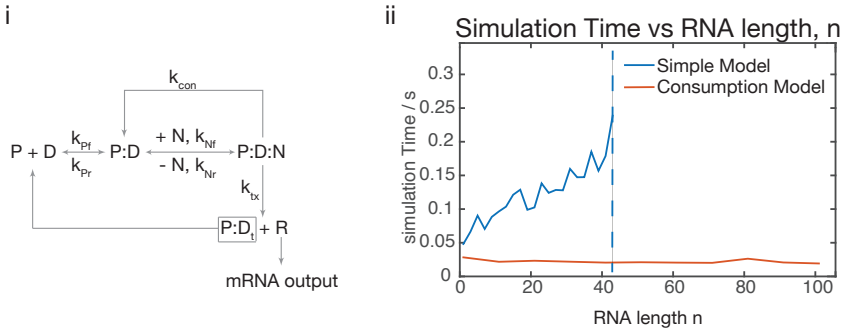

Figure 4.1: (A) Schematic illustrating the consumption reaction. (B) Time required to simulate 10000 seconds of transcription using the simple model (4.3) and the consumption model (4.4). At about n = 42, and MATLAB® is no longer able to complete the simulation.

The simple model (4.3) was able to simulate the production of RNA for up to an n of about 42, after which MATLAB® returned a simulation error. The consumption model (4.4) was able to simulate transcription for all n tested ($n > 2000$). The results for $n < 100$ are shown in Figure 4.1B.

We assume that that at any time, there is only one polymerase molecule bound to the DNA. For this single occupancy model, the features that are preserved between the full and reduced model are that the rate of consumption of nucleotides is independent of the transcript length, while the rate of production of RNA scales inversely with the length. To see this, let k_{tx} be the rate at which the elongation step occurs in the full model (4.1). Then, setting $k_{\text{reduced}} = k_{\text{tx}}/n$ and $k_{\text{con}} = (n-1)k_{\text{reduced}}$ gives us the rate of RNA production as

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{R}]}{\mathrm{d}t} &= k_{\text{reduced}}[\mathrm{P{:}D{:}N}] \\ &= \frac{k_{tx}}{n}[\mathrm{P{:}D{:}N}].\end{aligned}$$

To compute the rate of nucleotide consumption, we define a species concentration $[\mathrm{N}_{\text{uninc}}](t)$, which is the concentration of nucleotides not incorporated into RNA at time t. I.e., $[\mathrm{N}_{\text{uninc}}] = [\mathrm{P{:}D{:}N}] + [\mathrm{N}]$. The rate of consumption of nucleotides, then, does not directly depend on the length n of the RNA, and is n times the rate of RNA production,

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{N}_{\text{uninc}}]}{\mathrm{d}t} &= \frac{\mathrm{d}([\mathrm{P{:}D{:}N}])}{\mathrm{d}t} + \frac{\mathrm{d}([\mathrm{N}])}{\mathrm{d}t} \\ &= -(k_{\text{reduced}} + k_{\text{con}})[\mathrm{P{:}D{:}N}] \\ &= -k_{\text{tx}}[\mathrm{P{:}D{:}N}] \\ &= -n\frac{\mathrm{d}[\mathrm{R}]}{\mathrm{d}t}.\end{aligned}$$

4.3 Mathematical Preliminaries

In this section, we introduce some ideas from chemical reaction network theory (CRNT) and singular perturbation theory, and use them to prove a couple of results which will be useful when we try to carry out our model reduction in Section 4.6. We begin by introducing basic definitions and notions from CRNT.

4.3.1 The Zero Deficiency Theorem and Asymptotic Stability

Let $\{x_1, \ldots, x_s\}$ be a set of s species which participate in r reactions

$$\sum_{j=1}^{s} A_{ij} x_j \xrightarrow{k_i} \sum_{j=1}^{s} B_{ij} x_j, \qquad i \in \{1, \ldots r\}, \tag{4.5}$$

where the $A_{ij} \in \mathbb{R}_{\geq 0}$ are called the stoichiometric coefficients of the system, and the reaction rate of the ith reaction is given by $k_i > 0$. We will call Equation (4.5) a *chemical reaction network* or *reaction network* for short. In the representation above, reversible reactions are modeled as two separate irreversible reactions. The reactants $\sum_{j=1}^{s} A_{ij} x_j$ and products $\sum_{j=1}^{s} B_{ij} x_j$ are called the *complexes* of this reaction network. Let m denote the number of distinct complexes in a reaction network, and label them by $c_1, c_2, \ldots c_m$.

In matrix form we may write this reaction network as

$$Ax \xrightarrow{k} Bx,$$

where the species concentration vector is $x \triangleq [x_1, \ldots, x_s]^T \in \mathbb{R}^s_{\geq 0}$, coefficient matrices are $A \triangleq [A_{ij}] \in \mathbb{R}^{r \times s}_{\geq 0}$, $B \triangleq [B_{ij}] \in \mathbb{R}^{r \times s}_{\geq 0}$, and the reaction rate vector is $k \triangleq (k_1, \ldots, k_r)^T \in \mathbb{R}^r_{>0}$. We define the stoichiometric matrix $S \triangleq (B - A)^T$. Recall that using standard mass action kinetics, we can write the dynamics of the network given by Equation (4.5) as

$$\frac{\mathrm{d}x}{\mathrm{d}t} = S\, v(x, k), \qquad t \geq 0, \quad x(0) = x_0, \tag{4.6}$$

where $v(x, k)$ is a vector function whose ith component gives the velocity of the ith reaction.

Definition 11. The *stoichiometric subspace* associated with the mass action Equation (4.6) is given by $\mathcal{S} \triangleq \mathrm{Im}((B - A)^T)$, and is a subspace of $\mathbb{R}^s$. The *rank of the reaction network* (4.6) is given by $q \triangleq \mathrm{rank}(B - A)^T$, a q dimensional manifold called the *stoichiometric compatibility class* is defined by the affine space $(x_0 + \mathcal{S}) \cap \mathbb{R}^s_{\geq 0}$.

Remark 12. The stoichiometric compatibility class is an important concept when defining properties of trajectories, and in particular those of equilibria. These properties include the existence and multiplicity of equilibria, and whether these equilibria are (asymptotically) stable. Feinberg [18] describes the issues involved in Section 5.2 of his paper. We simply note that trajectories beginning at x_0 stay in the stoichiometric compatibility class $(\mathcal{S}+x_0)\cap\mathbb{R}^s_{\geq 0}$ containing x_0. The standard notion of asymptotic stability will be understood to be with respect to the stoichiometric compatibility class containing the trajectory being considered. More precisely, we will consider an equilibrium x^* to be *asymptotically stable* if any trajectory beginning sufficiently close to x^* and within the stoichiometric compatibility class containing x^* stays close to x^* and approaches x^* in the limit $t \to \infty$. ⋄

Definition 12 ([5], Definition 6.3)**.** Let c_i and c_j be complexes in the reaction network (4.5). We say there is a *direct path* from c_i to c_j if $c_i \to c_j$, an *indirect path* from c_i to c_j if there exists a sequence of complexes $(c_i, c_{i_1}, \ldots, c_{i_p}, c_j)$ such that $c_i \to c_{i_1}$, $c_{i_1} \to c_{i_2}$, ... $c_{i_p} \to c_j$. There esists a *path* from c_i to c_j if there exists a direct or indirect path from c_i to c_j. The complexes c_i and c_j are *linked* if $c_i = c_j$, or if there is a direct or indirect path from one to the other. This definition of linkage can be used to separate a chemical reaction network into equivalence classes known as *linkage classes*. Finally, we call a reaction network (4.5) *weakly reversible* if, for each pair (c_i, c_j), the existence of a path from c_i to c_j implies the existence of a path from c_j to c_i.

Definition 13 ([5], Definition 6.2)**.** The *deficiency* of the network (4.5) is given by $\delta \triangleq m-l-q$, where l is the number of distinct linkage classes and $q = \text{rank}(\nu)$.

Theorem 2. *[[18], Theorem 4.1] Assume that the reaction network* (4.6) *has zero deficiency and is weakly reversible. Then, for arbitrary positive rate constants, the system* (4.6) *has the following properties: Each stoichiometric compatibility class contains precisely one equilibrium, this equilibrium is asymptotically stable (see remark below), and there is no nontrivial periodic orbit in* $\mathbb{R}^s_{\geq 0}$.

Remark 13. As in the remark above, asymptotic stability in Theorem 2 is taken with respect to the stoichiometric compatibility class containing that equilibrium, defined by the initial conditions of the system. A pictorial depiction of this situation is given in [17, Fig. 1, 2] ⋄

4.3.2 Relationship Between Nucleotide Consumption Rate and RNA Production Rate

We state a few results used in carrying out the model reduction in Section 4.6. Ideally, we would like to determine the relationship between the rate of production of RNA and the rate of consumption of nucleotides in the full model (4.1). The approach we will take involves first partitioning the corresponding mass action equations (4.2) into subsets of equations as follows:

$$\frac{\mathrm{d}\xi}{\mathrm{d}t} = F(\xi, [\mathrm{N}]), \qquad \xi \in \mathbb{R}^{2n+3}_{\geq 0}, \tag{4.7a}$$

$$\frac{\mathrm{d}[\mathrm{N}]}{\mathrm{d}t} = G(\xi, [\mathrm{N}]), \tag{4.7b}$$

$$\frac{\mathrm{d}[\mathrm{m_n}]}{\mathrm{d}t} = H(\xi), \tag{4.7c}$$

where ξ is a vector comprising the concentrations of all the species except the completed RNA transcript $\mathrm{m_n}$ and the free nucleotides, N. F, G and H are functions defined using mass action kinetics, which, with their respective arguments, give the rates of change of the vector ξ and the scalars $[\mathrm{N}]$ and $[\mathrm{m_n}]$. This decomposition allows us to consider the rate of production of RNA, a species that does not participate anywhere else in the network, separately from the rate of consumption of the nucleotides, which affect dynamics of many reactions in the network. In particular, we note that in equations (4.7a)–(4.7c), the functions F, G and H do not have $[\mathrm{m_n}]$ as an argument, while both F and G depend on $[\mathrm{N}]$.

We will show that when the concentration of nucleotides, $[\mathrm{N}]$ as an argument of F in Equation (4.7a) is held constant, the trajectories of ξ reach an asymptotically stable equilibrium, ξ_e. At this equilibrium, which can be thought of as an operating point for the local dynamics of $[\mathrm{N}]$ in Equation (4.7b) and of $[\mathrm{m_n}]$ in Equation (4.7c), the rate of consumption of nucleotides is proportional to the rate of production of RNA.

The assumption to hold the concentration of some species constant in order to determine the properties of a network requires some justification. To this end, we note that it has been used in the theory of chemical reactions, for instance by Feinberg ([18], Remark 4.3.1), who notes that when a species is in great excess, then over some 'reason-

able' time-scale, one could expect the concentration of the excess species to not change appreciably, while the remaining species can display non-constant dynamics. One domain where nucleotide concentration is in excess for most of the duration of interest is in cell-free extracts, which were the primary motivation for this study. Another domain of relevance for the constancy of nucleotides is in cells, where nucleotide concentrations are regulated, and one might wish to calculate the consumption rate to obtain a measure for the loading of the cell's metabolic machinery.

We now state a proposition which establishes the relationship between the rate of production of RNA and that of the consumption of nucleotides at this steady state, and furthermore provides steady state relationships among species concentrations, which will turn out to be useful for the model reduction procedure in Section 4.6.

Proposition 3. *Consider the full model given by equations* (4.1) *and* (4.2)*, and its decomposition into subsystems F, G and H given by equations (4.7a)–(4.7c). When the nucleotide concentration is held constant, $[\mathrm{N}] = [\mathrm{N}]_{const}$, in the subsystem F, the trajectories of ξ reach an asymptotically stable equilibrium, ξ_e, in the sense of Remarks 12 and 13. Furthermore, substituting ξ_e and $[\mathrm{N}]_{const}$ into the subsystems G and H gives the relationship*

$$\frac{\mathrm{d}[\mathrm{N}]_{uninc}}{\mathrm{d}t} = -n\frac{\mathrm{d}[\mathrm{m_n}]}{\mathrm{d}t}, \tag{4.8}$$

where n is the length of the RNA, $\mathrm{m_n}$, in nucleotides, and $[\mathrm{N}]_{uninc}$ is the total concentration of nucleotides not incorporated into RNA, i.e., $[\mathrm{N}]_{uninc} \triangleq [\mathrm{N}] + \sum_{k=1}^{n}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}]$.

Proof. We first prove the stability of the equilibrium ξ_e of the subsystem

$$\frac{\mathrm{d}\xi}{\mathrm{d}t} = F(\xi, [\mathrm{N}]_{\mathrm{const}}). \tag{4.9}$$

Using the technique from Feinberg ([18], Section 4.3), we can write out the dynamical Equation (4.9) as a chemical reaction network with certain rate constants modified by the

constant scalar $[\mathrm{N}]_{\mathrm{const}}$

$$
\begin{aligned}
\mathrm{P}+\mathrm{D} &\underset{k_{Pr}}{\overset{k_{Pf}}{\rightleftharpoons}} \mathrm{P{:}D_1{:}m_0} \\
\mathrm{P{:}D_1{:}m_0} &\underset{k_{Nr}}{\overset{k_{Nf}[\mathrm{N}]_{\mathrm{const}}}{\rightleftharpoons}} \mathrm{P{:}D_1{:}m_0{:}N} \\
\mathrm{P{:}D_1{:}m_0{:}N} &\xrightarrow{k_{tx}} \mathrm{P{:}D_2{:}m_1} \\
&\vdots \\
\mathrm{P{:}D_n{:}m_{n-1}} &\underset{k_{Nr}}{\overset{k_{Nf}[\mathrm{N}]_{\mathrm{const}}}{\rightleftharpoons}} \mathrm{P{:}D_n{:}m_{n-1}{:}N} \\
\mathrm{P{:}D_n{:}m_{n-1}{:}N} &\xrightarrow{k_{tx}} \mathrm{P{:}D_t} \\
\mathrm{P{:}D_t} &\xrightarrow{k_{\mathrm{term}}} \mathrm{P}+\mathrm{D}.
\end{aligned}
\tag{4.10}
$$

According to Theorem 2, if we can show that the network given by (4.10) has deficiency zero and is weakly reversible, we would have shown that it possesses an asymptotically stable equilibrium. The set of complexes in the network is $\{\mathrm{P{+}D}, \mathrm{P{:}D_1{:}m_0}, \mathrm{P{:}D_1{:}m_0{:}N}, \ldots, \mathrm{P{:}D_n{:}m_{n-1}}, \mathrm{P{:}D_n{:}m}$ Thus, there are $c \triangleq 2n+2$ complexes in the network. Note that there is a cyclic path through the set of complexes, given by $\mathrm{P{+}D} \to \mathrm{P{:}D_1{:}m_0} \to \mathrm{P{:}D_1{:}m_0{:}N} \to \cdots \to \mathrm{P{:}D_n{:}m_{n-1}} \to \mathrm{P{:}D_n{:}m_{n-1}{:}N} \to \mathrm{P{:}D_t} \to \mathrm{P{+}D}$. Thus, the network is weakly reversible, and has only one linkage class ($l = 1$). Finally, we compute the rank of the stoichiometric matrix as follows. The network can be written in matrix form as

$$
\frac{\mathrm{d}\xi}{\mathrm{d}t} = M\, v(\xi, [\mathrm{N}]_{\mathrm{const}}),
$$

where

$$\frac{\mathrm{d}\xi}{\mathrm{d}t} = \frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} [\mathrm{P}] \\ [\mathrm{D}] \\ [\mathrm{P{:}D_1{:}m_0}] \\ [\mathrm{P{:}D_1{:}m_0{:}N}] \\ [\mathrm{P{:}D_2{:}m_1}] \\ [\mathrm{P{:}D_2{:}m_1{:}N}] \\ \vdots \\ [\mathrm{P{:}D_n{:}m_{n-1}}] \\ [\mathrm{P{:}D_n{:}m_{n-1}{:}N}] \\ [\mathrm{P{:}D_t}] \end{pmatrix}, \qquad \nu(\xi, [\mathrm{N}]_{\mathrm{const}}) = \begin{pmatrix} k_{Pf}[\mathrm{P}][\mathrm{D}] \\ k_{Pr}[\mathrm{P{:}D_1{:}m_0}] \\ k_{Nf}[\mathrm{N}]_{\mathrm{const}}[\mathrm{P{:}D_1{:}m_0}] \\ k_{Nr}[\mathrm{P{:}D_1{:}m_0{:}N}] \\ k_{tx}[\mathrm{P{:}D_1{:}m_0{:}N}] \\ k_{Nf}[\mathrm{N}]_{\mathrm{const}}[\mathrm{P{:}D_2{:}m_1}] \\ k_{Nr}[\mathrm{P{:}D_2{:}m_1{:}N}] \\ k_{tx}[\mathrm{P{:}D_2{:}m_1{:}N}] \\ \vdots \\ k_{Nf}[\mathrm{N}]_{\mathrm{const}}[\mathrm{P{:}D_n{:}m_{n-1}}] \\ k_{Nr}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}] \\ k_{tx}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}] \\ k_{\mathrm{term}}[\mathrm{P{:}D_t}] \end{pmatrix}, \tag{4.11}$$

and

$$M = \begin{array}{c} \\ r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_6 \\ \vdots \\ r_{2n+1} \\ r_{2n+2} \\ r_{2n+3} \end{array} \begin{array}{c} \begin{array}{cccccccccccc} c_1 & c_2 & c_3 & c_4 & c_5 & c_6 & c_7 & c_8 & \dots & c_{3n} & c_{3n+1} & c_{3n+2} & c_{3n+3} \end{array} \\ \begin{pmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 & 1 \\ -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 & 1 \\ 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & & 0 & 0 & 0 & 0 \\ & & & & & & & & \ddots & & & & \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 & -1 \end{pmatrix} \end{array},$$

where we denote the rows and columns of the matrix M using r_i and c_i, $i = 1, \dots 2n+2$, respectively. We determine the rank of M as follows. Remove r_1 since it is a duplicate of r_2, and therefore does not affect the rank of the matrix. Also remove columns $\{c_2, c_4, c_7, \dots, c_{3i+1}, \dots, c_{3n+1}\}$, which are all scalar multiples of the columns preceding them. We are then left with the $2n+2 \times 2n+2$ matrix

$$\tilde{M} = \begin{array}{c} \\ \tilde{r}_2 \\ \tilde{r}_3 \\ \tilde{r}_4 \\ \tilde{r}_5 \\ \tilde{r}_6 \\ \vdots \\ \tilde{r}_{2n+1} \\ \tilde{r}_{2n+2} \\ \tilde{r}_{2n+3} \end{array} \begin{array}{c} \begin{array}{ccccccccc} \tilde{c}_1 & \tilde{c}_3 & \tilde{c}_5 & \tilde{c}_6 & \tilde{c}_8 & \dots & \tilde{c}_{3n} & \tilde{c}_{3n+2} & \tilde{c}_{3n+3} \end{array} \\ \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 \\ 1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \\ & & & & & \ddots & & & \\ 0 & 0 & 0 & 0 & 0 & & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \end{pmatrix} \end{array},$$

which has the same rank as M. The sub-matrix $\tilde{M}_1$ obtained by removing $\tilde{c}_{3n+3}$ and $\tilde{r}_{2n+3}$ is lower triangular with nonzero diagonal entries, and thus has a (full) rank of $2n+1$, giving $\mathrm{rank}(M) \geq 2n+1$. We also know that $\mathrm{rank}(M) = \mathrm{rank}(\tilde{M}) \leq 2n+2$. Finally, note that $\tilde{r}_{2n+3}$ can be written as a linear combination of the remaining rows in $\tilde{M}$ as

$$\tilde{r}_{2n+3} = -\sum_{i=2}^{2n+2} \tilde{r}_i.$$

Thus $q \triangleq \mathrm{rank}(M) = 2n+1$. I.e., this network has zero deficiency $\delta = c - l - q = 2n+2-1-(2n+1) = 0$, and is weakly reversible and using Theorem 2, we conclude that there exists a positive equilibrium of the subsystem (4.7a), asymptotically stable relative to its stoichiometric compatibility class.

Next, we obtain the relationship between $\mathrm{d}[\mathrm{N_{uninc}}]/\mathrm{d}t$ and $\mathrm{d}[\mathrm{m_n}]/\mathrm{d}t$. Note that

$$\frac{\mathrm{d}[\mathrm{m_n}]}{\mathrm{d}t} = k_{tx}[\mathrm{P{:}D_n{:}m_{n-1}{:}N}], \tag{4.12}$$

$$\begin{aligned}
\frac{\mathrm{d}[\mathrm{N_{uninc}}]}{\mathrm{d}t} &= \frac{\mathrm{d}\left([\mathrm{N}] + \sum_{i=1}^{n}[\mathrm{P{:}D_i{:}m_{i-1}{:}N}]\right)}{\mathrm{d}t} \\
&= \frac{\mathrm{d}[\mathrm{N}]}{\mathrm{d}t} + \sum_{i=1}^{n}\frac{\mathrm{d}[\mathrm{P{:}D_i{:}m_{i-1}{:}N}]}{\mathrm{d}t} \\
&= \sum_{k=1}^{n}\left(k_{Nr}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}] - k_{Nf}[\mathrm{P{:}D_k{:}m_{k-1}}][\mathrm{N}]\right) \\
&\qquad + \sum_{k=1}^{n}\left(k_{Nf}[\mathrm{P{:}D_k{:}m_{k-1}}][\mathrm{N}] - k_{Nr}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}] - k_{tx}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}]\right) \\
&= -k_{tx}\sum_{k=1}^{n}[\mathrm{P{:}D_k{:}m_{k-1}{:}N}].
\end{aligned} \tag{4.13}$$

For the model (4.7a) to be at steady state, the net flux into and out of every species must be zero, and individual fluxes are constant in time. Consider a set of three consecutive reactions from the subsystem (4.10) at an arbitrary $[\mathrm{N}]_{\mathrm{const}}$

$$\mathrm{P{:}D_{i-1}{:}m_{i-2}{:}N} \xrightarrow{k_{tx}} \mathrm{P{:}D_i{:}m_{i-1}}, \tag{4.14}$$

$$\mathrm{P{:}D_i{:}m_{i-1}} \underset{k_{Nr}}{\overset{k_{Nf}[\mathrm{N}]_{\mathrm{const}}}{\rightleftharpoons}} \mathrm{P{:}D_i{:}m_{i-1}{:}N}, \tag{4.15}$$

$$\mathrm{P{:}D_i{:}m_{i-1}{:}N} \xrightarrow{k_{tx}} \mathrm{P{:}D_{i+1}{:}m_i}. \tag{4.16}$$

Since the instantaneous flux into and out of $\mathrm{P{:}D_i{:}m_{i-1}}$ is zero, the flux in due to (4.14) and the flux out due to the reversible reactions (4.15) must balance, we have

$$k_{tx}[\mathrm{P{:}D_{i-1}{:}m_{i-2}{:}N}] = k_{Nf}[\mathrm{P{:}D_i{:}m_{i-1}}][\mathrm{N}]_{\mathrm{const}} - k_{Nr}[\mathrm{P{:}D_i{:}m_{i-1}{:}N}]. \tag{4.17}$$

Similarly, considering the species $\mathrm{P{:}D_i{:}m_{i-1}{:}N}$ in (4.15) and (4.16), we have

$$k_{Nf}[\mathrm{P{:}D_i{:}m_{i-1}}][\mathrm{N}]_{\mathrm{const}} - k_{Nr}[\mathrm{P{:}D_i{:}m_{i-1}{:}N}] = k_{tx}[\mathrm{P{:}D_i{:}m_{i-1}{:}N}]. \tag{4.18}$$

Thus,

$$[\mathrm{P{:}D_{i-1}{:}m_{i-2}{:}N}] = [\mathrm{P{:}D_{i}{:}m_{i-1}{:}N}] \tag{4.19}$$

$$[\mathrm{P{:}D_{i-1}{:}m_{i-2}}] = [\mathrm{P{:}D_{i}{:}m_{i-1}}], \tag{4.20}$$

and by induction, we have that for all i, j in $\{1, 2, \ldots n\}$,

$$[\mathrm{P{:}D_{i}{:}m_{i-1}}] = [\mathrm{P{:}D_{j}{:}m_{j-1}}], \tag{4.21}$$

$$[\mathrm{P{:}D_{i}{:}m_{i-1}{:}N}] = [\mathrm{P{:}D_{j}{:}m_{j-1}{:}N}]. \tag{4.22}$$

Thus, Equation (4.13) can be reduced to

$$\frac{\mathrm{d}[\mathrm{N_{uninc}}]}{\mathrm{d}t} = -k_{tx} \sum_{k=1}^{n} [\mathrm{P{:}D_{k}{:}m_{k-1}{:}N}] \tag{4.23}$$

$$= -n \cdot k_{tx} [\mathrm{P{:}D_{n}{:}m_{n-1}{:}N}] \tag{4.24}$$

$$= -n \frac{\mathrm{d}[\mathrm{m_{n}}]}{\mathrm{d}t}, \tag{4.25}$$

which completes the proof. □

4.4 Overview of Time-Scale Separation in Chemical Kinetics via Singular Perturbation Theory

4.4.1 Singular Perturbation Theory for Chemical Reaction Networks

Singular perturbation theory has been used widely to decompose models of physical systems containing multiple temporal and spatial scales into subsystems at those scales [19]. This decomposition has been carried out for chemical systems too, where some reactions proceed much more quickly than others, or there exist transient short lived species [41, 67, 68].

To begin, we introduce the notion of decomposing a system into *slow* and *fast subsystems*, operating at two different time-scales. Such a decomposition is only possible if we

can write the model for the system in the *standard singular perturbation form*:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(t, x, z, \epsilon), \qquad x(0) = x_0, \qquad x \in \mathbb{R}^n, \tag{4.26}$$

$$\epsilon\frac{\mathrm{d}z}{\mathrm{d}t} = g(t, x, z, \epsilon), \qquad z(0) = z_0, \qquad z \in \mathbb{R}^m, \tag{4.27}$$

where ϵ is a small positive scalar, and f, g are sufficiently many times continuously differentiable in their arguments (t, x, z, ϵ).

The small parameter ϵ in Equation (4.27) is used to capture the effects of large reaction rate constants in chemical kinetics, which lead to fast transient dynamics. These fast dynamics are modeled by the variable z, whose rate of change gets scaled by $1/\epsilon$, and hence becomes very large. Being able to apply tools from singular perturbation theory involves bringing the mass action dynamical equations into the above form as a necessary prerequisite. Singular perturbation theory also requires that there exists at least one asymptotically stable equilibrium (isolated from any others that might exist) to which the trajectories of the variable z, for each allowable x, converge. We defer a discussion of the properties of the equilibria for the moment, and focus on finding a set of state variables that allow us to bring the system into the standard form in the first place. To this end, we will discuss why species concentrations are not appropriate to use as a state variables for the purposes of bringing a system into the standard form, and elaborate on a variable transformation which provides a better system of coordinates.

4.4.1.1 Nonexplicit Time-Scale-Separation

In many applications exhibiting two-time-scale behavior, it is not possible to partition the natural state variables into fast and slow variables to bring the system into the standard singular perturbation form [39]. This is despite the fact that the variables exhibit two-time-scale behavior, where an initial fast transient is followed by slow evolution on an equilibrium manifold [41]. This occurs because these natural state variables are in general a combination of both fast and slow effects, and therefore exhibit *nonexplicit* time-scale-separation. In chemical kinetics, the ODE models are written with species concentrations

as the natural variables, and finding transformations from the natural coordinates to a set of coordinates where the model may be written in the standard form is highly nontrivial. The structural reason for nonexplicit time-scale-separation in chemical kinetics is that each species in a model may participate in both fast and slow reactions.

The problem of finding coordinate changes to allow such models to be written in the standard form has received some attention in the literature. Kokotovic, Khalil and O'Reilly [39] gave a general prescription for constructing such coordinate transformations, while specific ad-hoc transformations for chemical reaction networks were studied in [6,57]. The first systematic procedure for finding a linear coordinate transformation was developed by Van Breusegem and Bastin [67]. These authors first partition the stoichiometric matrix into block matrices corresponding to species participating in fast reactions, both fast and slow reactions, and only slow reactions, and then use these block matrices to construct the desired invertible coordinate transformation. The works of Kumar, Christofidis and Daoutidis [41] and Vora and Daoutidis [68] take an entirely different approach, and develop a very general framework for deriving a family of coordinate transformations that bring nonexplicit two-time-scale models into the standard form. The main idea behind their method involves giving a set of constraints that implicitly define the equilibrium manifold and computing an upper bound on the dimension of this manifold. This allows them to pick an arbitrary subset or transformation of state variables from the original set, and construct an explicit representation of the reduced order model that, after an initial fast transient, evolves on the equilibrium manifold. The generality of this framework arises from the fact that the method is not limited to *isothermal* reaction networks, where the stoichiometric matrix is constant in time, but can instead be used to reduce nonisothermal reaction networks, and even more general systems, like those modeling the dynamics of heat exchange. For isothermal reaction networks, these studies give a method for the explicit construction of the slow variables as a set of linear combinations defined by a basis of the left null space of the subset of the stoichiometric matrix corresponding to the fast reactions.

All of these studies suffer from a set of limitations. We note that the ad-hoc methods

mentioned above require human intuition to find the appropriate coordinate transformation, and such methods do not scale well beyond the simplest models. The remaining methods suffer from the limitation that the transformed state variables do not have a physical interpretation, and are fairly complex. The methods in [41, 68] further suffer from the limitation that even in the case of isothermal reactions, the transformation is nonlinear, and finding the standard form involves inverting this transformation on the equilibrium manifold. Such an inversion is highly nontrivial, and could only be found for the simpler examples in their studies (see, for example, the final step in the esterification example in [68], where no attempt to invert the transformation is made). In this chapter, we provide a general construction for finding a transformation that is simple to construct, allows the transformed variables to have a physical interpretation, and gives a completely explicit representation of the standard form in the transformed coordinates.

4.4.2 Species Concentrations as State Variables

The typical way of reducing an ODE model of a reaction network with two-time-scale behavior into the standard form is to separate the set of mass-action differential equations into those belonging to species participating in slow reactions only, and those participating in fast and (possibly) slow reactions. The enzymatic reaction is a prototypical example of this approach. Consider the reaction

$$\mathrm{Enz} + \mathrm{Su} \underset{\mathrm{d}}{\overset{\mathrm{a}}{\rightleftharpoons}} \mathrm{Cpx} \xrightarrow{k} \mathrm{Pdt} + \mathrm{Enz} \tag{4.28}$$

where Enz is the enzyme, Su the substrate, Cpx the complex, and Pdt the product formed. The binding-unbinding is assumed to be much faster than the catalysis reaction ($a,\ d \gg k$). In differential equations, this is

$$\begin{aligned}
\frac{\mathrm{d}[\mathrm{Cpx}]}{\mathrm{d}t} &= -k[\mathrm{Cpx}] - d[\mathrm{Cpx}] + a[\mathrm{Enz}][\mathrm{Su}] = -\frac{\mathrm{d}[\mathrm{Enz}]}{\mathrm{d}t}, \\
\frac{\mathrm{d}[\mathrm{Su}]}{\mathrm{d}t} &= d[\mathrm{Cpx}] - a[\mathrm{Enz}][\mathrm{Su}], \\
\frac{\mathrm{d}[\mathrm{Pdt}]}{\mathrm{d}t} &= k[\mathrm{Cpx}].
\end{aligned} \tag{4.29}$$

Using $[\mathrm{Enz}] = E_0 - [\mathrm{Cpx}]$, $[\mathrm{Su}] = S_0 - [\mathrm{Cpx}] - [\mathrm{Pdt}]$, $\tau = kt$, $K_d = d/a$, $\epsilon = k/d$ and $K_d x = X$ where $X \in \{[\mathrm{Cpx}],\ [\mathrm{Enz}],\ [\mathrm{Su}],\ [\mathrm{Pdt}],\ S_0,\ E_0\}$ and correspondingly $x \in \{c, e, s, p, s_0, e_0\}$, we arrive at the nondimensionalized model

$$\begin{aligned} \epsilon \frac{\mathrm{d}c}{\mathrm{d}\tau} &= -c - \epsilon c + (e_0 - c)(s_0 - c - p), \\ \frac{\mathrm{d}p}{\mathrm{d}\tau} &= c. \end{aligned} \tag{4.30}$$

Setting $\epsilon = 0$ and using $S_0 - [\mathrm{Cpx}] - [\mathrm{Pdt}] \approx S_0 - [\mathrm{Pdt}]$, allows us to arrive at reduced system

$$\frac{\mathrm{d}[\mathrm{Pdt}]}{\mathrm{d}t} = k[\mathrm{Cpx}] = k\frac{E_0(S_0 - [\mathrm{Pdt}])}{(S_0 - [\mathrm{Pdt}]) + K_d}. \tag{4.31}$$

The reason we are able to write this model in the standard form (4.30) is that the species Pdt only takes part in the slow reaction (rate = k), allowing it to be part of the slow subsystem, while the other species (Enz, Su, Cpx) take part in at least one fast reaction, making them part of the fast subsystem.

The same trend appears when we look at the transcription model given by Equations (4.7a) – (4.7c). For compactness of notation, let us define $\bar{\eta} \equiv [\mathrm{N}]$, $\bar{\gamma} \equiv [\mathrm{P{:}D_t}]$, $\bar{\rho} \equiv [\mathrm{P}]$, $\bar{d} \equiv [\mathrm{D}]$, $\bar{m}_n \equiv [\mathrm{m_n}]$ and for $i = 1, \ldots, n$, denote $\bar{v}_i \equiv [\mathrm{P{:}D_i{:}m_{i-1}}]$ and $\bar{w}_i \equiv [\mathrm{P{:}D_i{:}m_{i-1}{:}N}]$. We may write the model as

$$
\begin{aligned}
\frac{\mathrm{d}\bar{d}}{\mathrm{d}t} &= k_{\text{term}}\bar{\gamma} - k_{Pf}\bar{\rho}\bar{d} + k_{Pr}\bar{\nu}_1, \\
\frac{\mathrm{d}\bar{\rho}}{\mathrm{d}t} &= k_{\text{term}}\bar{\gamma} - k_{Pf}\bar{\rho}\bar{d} + k_{Pr}\bar{\nu}_1, \\
\frac{\mathrm{d}\bar{\nu}_1}{\mathrm{d}t} &= k_{Pf}\bar{\rho}\bar{d} - k_{Pr}\bar{\nu}_1 - k_{Nf}\bar{\nu}_1\bar{\eta} + k_{Nr}\bar{w}_1, \\
\frac{\mathrm{d}\bar{w}_1}{\mathrm{d}t} &= k_{Nf}\bar{\nu}_1\bar{\eta} - k_{Nr}\bar{w}_1 - k_t\bar{w}_1, \\
\frac{\mathrm{d}\bar{\nu}_2}{\mathrm{d}t} &= k_t\bar{w}_1 - k_{Nf}\bar{\nu}_2\bar{\eta} + k_{Nr}\bar{w}_2, \\
\frac{\mathrm{d}\bar{w}_2}{\mathrm{d}t} &= k_{Nf}\bar{\nu}_2\bar{\eta} - k_{Nr}\bar{w}_2 - k_t\bar{w}_2, \\
&\vdots \\
\frac{\mathrm{d}\bar{\nu}_n}{\mathrm{d}t} &= k_t\bar{w}_2 - k_{Nf}\bar{\nu}_3\bar{\eta}k_{Nr}\bar{w}_3, \\
\frac{\mathrm{d}\bar{w}_n}{\mathrm{d}t} &= k_{Nf}\bar{\nu}_3\bar{\eta} - k_{Nr}\bar{w}_3 - k_t\bar{w}_3, \\
\frac{\mathrm{d}\bar{\gamma}}{\mathrm{d}t} &= k_t\bar{w}_3 - k_{\text{term}}\bar{\gamma}, \\
\frac{\mathrm{d}\bar{\eta}}{\mathrm{d}t} &= \sum_{i=1}^{n} k_{Nr}\bar{w}_i - k_{Nf}\bar{\nu}_i\bar{\eta}, \\
\frac{\mathrm{d}\bar{m}_n}{\mathrm{d}t} &= k_t\bar{w}_n,
\end{aligned}
\tag{4.32}
$$

and use the nondimensionalization scheme $\epsilon = k_t/k_{Nr}$, $\tau = tk_t$, $\alpha_{Pf} = k_{Pf}/k_{Nf}$, $\alpha_{Pr} = k_{Pr}/k_{Nr}$, $\alpha_{term} = k_{\text{term}}/k_t$, $\bar{u} = k_{Nr}/k_{Nf}u$ for all $u \in \{\rho, d, \gamma, \eta, m_n, \nu_1, \ldots, \nu_n, w_1, \ldots, w_n\}$ to

obtain the *nondimensionalized model*

$$
\begin{aligned}
\frac{\mathrm{d}\gamma}{\mathrm{d}\tau} &= w_n - \alpha_{term}\gamma, \\
\frac{\mathrm{d}m_n}{\mathrm{d}\tau} &= w_n, \\
\epsilon\frac{\mathrm{d}d}{\mathrm{d}\tau} &= \epsilon\alpha_{term}\gamma - \alpha_{Pf}\rho d + \alpha_{Pr}v_1, \\
\epsilon\frac{\mathrm{d}\rho}{\mathrm{d}\tau} &= \epsilon\alpha_{term}\gamma - \alpha_{Pf}\rho d + \alpha_{Pr}v_1, \\
\epsilon\frac{\mathrm{d}v_1}{\mathrm{d}\tau} &= \alpha_{Pf}\rho d - \alpha_{Pr}v_1 - v_1\eta + w_1, \\
\epsilon\frac{\mathrm{d}w_1}{\mathrm{d}\tau} &= -\epsilon w_1 + v_1\eta - w_1, \\
\epsilon\frac{\mathrm{d}v_2}{\mathrm{d}\tau} &= \epsilon w_1 - v_2\eta + w_2, \\
\epsilon\frac{\mathrm{d}w_2}{\mathrm{d}\tau} &= -\epsilon w_2 + v_2\eta - w_2, \\
&\vdots \\
\epsilon\frac{\mathrm{d}v_n}{\mathrm{d}\tau} &= \epsilon w_{n-1} - v_n\eta + w_n, \\
\epsilon\frac{\mathrm{d}w_n}{\mathrm{d}\tau} &= -\epsilon w_n + v_n\eta - w_n, \\
\epsilon\frac{\mathrm{d}\eta}{\mathrm{d}\tau} &= \sum_{i=1}^{n}(-v_i\eta + w_i).
\end{aligned}
\tag{4.33}
$$

We see here that only γ and m_n can be separated into the slow subsystem, despite every other species participating in slow reactions as well. If we were to consider a network whereby every species took part in at least one fast reaction, then every equation in the corresponding differential equations would have an epsilon in front of it. Setting $\epsilon = 0$ would lead to a loss of all slow dynamics from our model, and we would only be able to make statements about what happens at the equilibrium for a given set of slow variable values. Due to this nonexplicit time-scale-separation, it would not be possible to have a set of reduced differential equations that can be solved or simulated. In the next section, we develop an invertible transformation from the species concentration coordinates to a different set of coordinates, and show how in these coordinates, the fast and slow dynamics can be separated, and the standard form derived.

4.4.3 Reaction Extents as a Natural and Physically Interpretable Coordinate System

4.4.3.1 Preliminary Reduction by Conservation Laws

The coordinate transformation we are interested in will depend on the stoichiometric matrix being invertible. In this section, we will discuss the procedure of removing linear dependencies in the rows and columns of the stoichiometric matrix. Consider again the ODE description of the chemical reaction system given by Equation (4.6), where $S \in \mathbb{R}^{s\times r}$ is the stoichiometric matrix, and $v \in \mathbb{R}^r$ is the reaction rate velocity vector. We assume that whenever a reversible reaction exists in the system, such that there is a corresponding pair of columns of S which are negatives of each other, the column corresponding to the backward reaction has been removed from the matrix. Furthermore, the element of v corresponding to the backward reaction rate is removed from the vector, and subtracted from the element corresponding to the forward rate, such that we have the net forward rate of the reversible reaction. For example, in the enzymatic reaction in Equation (4.28), we would transform the ODE description

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix}[\mathrm{Enz}]\\ [\mathrm{Su}]\\ [\mathrm{Cpx}]\\ [\mathrm{Pdt}]\end{bmatrix} = \begin{bmatrix}-1 & 1 & 1\\ -1 & 1 & 0\\ 1 & -1 & -1\\ 0 & 0 & 1\end{bmatrix}\begin{bmatrix}a[\mathrm{Enz}][\mathrm{Su}]\\ d[\mathrm{Cpx}]\\ k[\mathrm{Cpx}]\end{bmatrix}, \tag{4.34}$$

where the first two columns of the stoichiometric matrix are negatives of each other, to

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix}[\mathrm{Enz}]\\ [\mathrm{Su}]\\ [\mathrm{Cpx}]\\ [\mathrm{Pdt}]\end{bmatrix} = \begin{bmatrix}-1 & 1\\ -1 & 0\\ 1 & -1\\ 0 & 1\end{bmatrix}\begin{bmatrix}a[\mathrm{Enz}][\mathrm{Su}]-d[\mathrm{Cpx}]\\ k[\mathrm{Cpx}]\end{bmatrix}, \tag{4.35}$$

where the second column has been removed, and the reaction rate vector has been transformed accordingly. We also assume that once all such reversible reaction column pairs

have been converted to single columns, there are no other linear dependencies in the columns of S, and the new number of columns is r'. We expect that a more general case for dependencies between net reactions may be be constructed based on the idea of *reaction pathways*, which are introduced in [58, 59], and refer to reaction cycles arising in the reaction network diagram due to the existence of these linear dependencies.

Once we have removed the linear dependencies in the columns of S, we are ready to do the same for the rows. The argument presented is based on [9] and [56]. Since the columns of S are linearly independent, $\mathrm{rank}(S) = r'$, and there are r' linearly independent rows in S, such that the remaining rows can be written as linear combinations of these r' rows. We may define a full row rank matrix $P \in \mathbb{R}^{r' \times s}$ that picks out the r' linearly independent rows of S. Each row in P is made of all zeros except one element, which is a one. Furthermore, none of the r' rows are equal. By the rank-nullity theorem, we also know that the dimension of the left nullspace of S is $s - r'$, and thus we may find $s - r'$ row vectors which form a basis for this space. Arranging these row vectors into a full row rank matrix $H \in \mathbb{R}^{(s-r') \times n}$, we have $HS = 0$.

We may now use these matrices to define a reduced stoichiometric matrix $S_r \in \mathbb{R}^{r' \times r'}$ such that

$$\begin{bmatrix} S_r \\ 0 \end{bmatrix} \triangleq \begin{bmatrix} P \\ H \end{bmatrix} S, \tag{4.36}$$

where $\begin{bmatrix} P \\ H \end{bmatrix} \in \mathbb{R}^{s \times s}$ is invertible, and 0 is a matrix of zeros of appropriate dimensions.

Similarly, the species concentration vector may be transformed as

$$\begin{bmatrix} x_i \\ x_d \end{bmatrix} \triangleq \begin{bmatrix} P \\ H \end{bmatrix} x, \tag{4.37}$$

where $x_i \in \mathbb{R}^d$ are the dynamic variables in the reduced model, and $x_d \in \mathbb{R}^m$ are combinations of species concentrations which end up being constant, and are determined by the initial concentrations in the system. To see this, apply $\begin{bmatrix} P \\ H \end{bmatrix}$ to Equation (4.6)

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} x_i \\ x_d \end{bmatrix} = \begin{bmatrix} P \\ H \end{bmatrix} \frac{\mathrm{d}x}{\mathrm{d}t} \tag{4.38}$$

$$= \begin{bmatrix} P \\ H \end{bmatrix} S\, v(x) \tag{4.39}$$

$$= \begin{bmatrix} S_r\, v(x) \\ 0 \end{bmatrix} \tag{4.40}$$

$$= \begin{bmatrix} S_r\, v_r(x_i, x_d) \\ 0 \end{bmatrix}, \tag{4.41}$$

$$\begin{bmatrix} x_{i0} \\ x_{d0} \end{bmatrix} \triangleq \begin{bmatrix} x_i \\ x_d \end{bmatrix}(0) \tag{4.42}$$

$$= \begin{bmatrix} P \\ H \end{bmatrix} x_0, \tag{4.43}$$

where

$$v_r(x_i, x_d) \triangleq v(x)\Big|_{x=\left[\begin{smallmatrix} P \\ H \end{smallmatrix}\right]^{-1}\left[\begin{smallmatrix} x_i \\ x_d \end{smallmatrix}\right]}, \tag{4.44}$$

so that we have the reduced system

$$\begin{aligned} \dot{x_i} &= S_r\, v_r(x_i,\, x_d), & x_i(0) &= x_{i0}, \\ \dot{x_d} &= 0, & x_d(t) &= x_{d0} \quad \forall t \geq 0. \end{aligned} \tag{4.45}$$

For example, applying this methodology to the enzymatic reaction (4.28), we may choose $P = \left[\begin{smallmatrix} 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{smallmatrix}\right]$ and $H = \left[\begin{smallmatrix} 1 & 0 & 1 & 0 \\ 0 & 1 & 1 & 1 \end{smallmatrix}\right]$, so that $x_i = \left[\begin{smallmatrix} [\mathrm{Cpx}], & [\mathrm{Pdt}] \end{smallmatrix}\right]^T$ and $x_d = \left[\begin{smallmatrix} [\mathrm{Enz}]+[\mathrm{Cpx}], & [\mathrm{Su}]+[\mathrm{Cpx}]+[\mathrm{Pdt}] \end{smallmatrix}\right]^T$ and the reduced system (4.45) can be written:

$$\begin{aligned} &\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} [\mathrm{Cpx}] \\ [\mathrm{Pdt}] \end{bmatrix} = \begin{bmatrix} 1 & -1 \\ 0 & 1 \end{bmatrix}\begin{bmatrix} a[\mathrm{Enz}][\mathrm{Su}] - d[\mathrm{Cpx}] \\ k[\mathrm{Cpx}] \end{bmatrix}, \quad \begin{bmatrix} [\mathrm{Cpx}] \\ [\mathrm{Pdt}] \end{bmatrix}(0) = \begin{bmatrix} C_0 \\ P_0 \end{bmatrix}, \\ &\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} [\mathrm{Enz}] + [\mathrm{Cpx}] \\ [\mathrm{Su}] + [\mathrm{Cpx}] + [\mathrm{Pdt}] \end{bmatrix} = 0, \qquad \begin{bmatrix} [\mathrm{Enz}] + [\mathrm{Cpx}] \\ [\mathrm{Su}] + [\mathrm{Cpx}] + [\mathrm{Pdt}] \end{bmatrix}(t) = \begin{bmatrix} E_0 \\ S_0 \end{bmatrix}, \ \forall t \geq 0 \end{aligned} \tag{4.46}$$

4.4.3.2 Transforming to Reaction Coordinates

Let us define a coordinate transformation from the $x_i \in \mathbb{R}^{r'}$ variables to a new set of variables $R \in \mathbb{R}^{r'}$,

$$\begin{bmatrix} R \\ x_d \end{bmatrix} \triangleq \left[\begin{array}{c|c} S_r^{-1} & 0 \\ \hline 0 & I_{s-r'} \end{array}\right] \begin{bmatrix} x_i \\ x_d \end{bmatrix}, \tag{4.47}$$

where $I_{s-r'}$ is the identity matrix of dimension $s-r'$. In this new coordinate system, the ODEs are

$$\begin{aligned} \frac{\mathrm{d}R}{\mathrm{d}t} &= S_r^{-1} \frac{\mathrm{d}x_i}{\mathrm{d}t} \\ &= v\left(\begin{bmatrix} P \\ H \end{bmatrix}^{-1} \begin{bmatrix} S_r R \\ x_d \end{bmatrix} \right) \\ &\triangleq v_{rxn}(R, x_d)\,. \end{aligned} \tag{4.48}$$

One of the sources of multiple time-scales in chemical reaction networks is the widely different orders of magnitudes of the reaction rate parameters. Suppose we partition the elements in the reaction velocity vector v into fast and slow rates, as determined by the reaction rate parameters being large or small. In particular, define two matrices $M_s \in \mathbb{R}^{r_s \times r'}$ and $M_f \in \mathbb{R}^{r_f \times r'}$ that pick out the slow and fast reaction velocities respectively. For example, in the enzymatic reaction, recall that the enzyme-substrate binding-unbinding reactions are considered fast, while the product formation reaction is often slow, so that $(a,\ d \gg k)$. Thus, we can partition the elements of v into the set of fast velocities $\{a[\mathrm{Enz}][\mathrm{Su}] - b[\mathrm{Cpx}]\}$ and that of slow velocities $\{k[\mathrm{Cpx}]\}$, with the matrices $M_s = [\,0\ 1\,]$ and $M_f = [\,1\ 0\,]$. Next, partition R using M_s and M_f as

$$\begin{bmatrix} R_s \\ R_f \end{bmatrix} \triangleq \begin{bmatrix} M_s \\ M_f \end{bmatrix} R, \tag{4.49}$$

and note that the above transformation is invertible. With this partitioning scheme in

place, we may write the model as

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} R_s \\ R_f \end{bmatrix} &= \begin{bmatrix} M_s \\ M_f \end{bmatrix}\frac{\mathrm{d}R}{\mathrm{d}t} \\ &= \begin{bmatrix} M_s \\ M_f \end{bmatrix} \nu\left(\begin{bmatrix} P \\ H \end{bmatrix}^{-1}\begin{bmatrix} S_r\begin{bmatrix} M_s \\ M_f \end{bmatrix}^{-1}\begin{bmatrix} R_s \\ R_f \end{bmatrix} \\ x_d \end{bmatrix}\right) \\ &\triangleq \begin{bmatrix} \nu_s \\ \nu_f \end{bmatrix}\left(R_s, R_f\right).\end{aligned} \tag{4.50}$$

Lastly, defining a small parameter ϵ which isolates the effect of the fast reactions, it can be shown that we can bring the above model into the form

$$\begin{aligned}\frac{\mathrm{d}R_s}{\mathrm{d}t} &= \nu_s\left(R_s, R_f\right), \\ \epsilon\frac{\mathrm{d}R_f}{\mathrm{d}t} &= \bar{\nu}_f\left(R_s, R_f\right),\end{aligned} \tag{4.51}$$

where each element of $\bar{\nu}_f = \frac{1}{\epsilon}\nu_f$ has at least one term independent of ϵ. Note that Equations (4.51) can be nondimensionalized if desired. In the context of the enzymatic reaction, Equation (4.50) is

$$\begin{aligned}\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} R_s \\ R_f \end{bmatrix} &= \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix}\nu\left(\begin{bmatrix} E_0 - R_f + R_s \\ S_0 - R_f \\ R_f - R_s \\ R_s \end{bmatrix}\right) \\ &= \begin{bmatrix} k(R_f - R_s) \\ a(E_0 - R_f + R_s)(S_0 - R_f) - d(R_f - R_s) \end{bmatrix},\end{aligned} \tag{4.52}$$

and consequently, Equation (4.51), after nondimensionalization, is

$$\begin{aligned}\frac{\mathrm{d}r_s}{\mathrm{d}\tau} &= r_f - r_s \\ \epsilon\frac{\mathrm{d}r_f}{\mathrm{d}\tau} &= (e_0 - r_f + r_s)(s_0 - r_f) - (r_f - r_s),\end{aligned} \tag{4.53}$$

where $\epsilon = k/d$, $\tau = kt$, and $r = (a/d)R$ for $R \in \{R_f, R_s, E_0, S_0\}$.

4.4.4 Comparison to the Method of Kumar et al. [41]

In this section, we compare the method developed in [41, 68] to the reaction extents method developed in the previous section. Consider the model of the esterificaton of carboxylic acid described in [68]. The state space model with nonexplicit time-scale separation is given by

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \\ \dot{x}_4 \\ \dot{x}_5 \\ \dot{x}_6 \\ \dot{x}_7 \\ \dot{x}_8 \\ \dot{x}_9 \\ \dot{x}_{10} \\ \dot{x}_{11} \end{bmatrix} = \begin{bmatrix} 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & -1 & 0 & -1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ -1 & 1 & 0 & -1 & 1 & 1 & 1 & -1 & 0 & 1 & -1 & -1 \\ 1 & -1 & 1 & 1 & -1 & 0 & -1 & 1 & -1 & -1 & 1 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 \\ 1 & 0 & 0 & 0 & 0 & -1 & -1 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_6 \\ r_7 \\ r_8 \\ r_9 \\ r_{10} \\ r_{11} \\ r_{12} \end{bmatrix}, \tag{4.54}$$

where the reaction rates are given by the expressions $r_1 = k_1 x_6 x_4$, $r_2 = k_2 x_5 x_2$, $r_3 = k_3 x_1 x_7$, $r_4 = k_4 x_2 x_4$,$r_5 = k_5 x_1 x_5$, $r_6 = k_6 x_{11} x_9$, $r_7 = k_7 x_5 x_{11}$, $r_8 = k_8 x_7 x_4$, $r_9 = k_9 x_5 x_3$, $r_{10} = k_{10} x_8 x_5$, $r_{11} = k_{11} x_4 x_9$ and $r_{12} = k_{12} x_{10} x_4$. We note that the reactions indexed 3, 6, 9, and 12 are slow, and the remaining are fast.

The last six columns of this stoichiometric matrix are a scalar multiple of the first six, and so we pick the first six columns as the linearly independent columns. The reaction velocity vector is transformed accordingly, with the new rates being $r_1 - r_7, \ldots, r_6 - r_{12}$. Furthermore, we can use Gauss-Jordan elimination to design the matrix H, and pick linearly

independent rows by inspection to get the matrix P. Then, we can write

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

$$H = \begin{bmatrix} -1/2 & 1/2 & 0 & 0 & 1/2 & 1/2 & 1 & 0 & 0 & 0 & 0 \\ 1/2 & 1/2 & -1 & 0 & -1/2 & -1/2 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & -1 & -1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix},$$

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

$$v(x) = \begin{bmatrix} r_1(x) - r_7(x) \\ r_2(x) - r_8(x) \\ r_3(x) - r_9(x) \\ r_4(x) - r_10(x) \\ r_5(x) - r_11(x) \\ r_6(x) - r_12(x) \end{bmatrix} = \begin{bmatrix} k_1 x_6 x_4 - k_7 x_5 x_{11} \\ k_2 x_5 x_2 - k_8 x_7 x_4 \\ k_3 x_1 x_7 - k_9 x_5 x_3 \\ k_4 x_2 x_4 - k_{10} x_8 x_5 \\ k_5 x_1 x_5 - k_{11} x_4 x_9 \\ k_6 x_{11} x_9 - k_{12} x_{10} x_4 \end{bmatrix},$$

and the matrices M_s and M_f, which pick out the slow and fast reaction rates from $v(x)$

respectively, are given by

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

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

With these definitions, we can bring the system into the standard singular perturbation form by following the method in Section 4.4.3.2. Recall that the coordinate transformation to a new set of variables is $R \triangleq S_r^{-1} P x$, and the transformed system is $\dot{R} = \nu(x)$. We can further partition the entries of R as $\begin{bmatrix} R_s \\ R_f \end{bmatrix} \triangleq \begin{bmatrix} M_s \\ M_f \end{bmatrix} R$. Then, x can be written in terms of R_s and R_f as

$$x(R_s, R_f) = \begin{bmatrix} P \\ H \end{bmatrix}^{-1} \begin{bmatrix} S_r \begin{bmatrix} M_s^T & M_f^T \end{bmatrix} \begin{bmatrix} R_s \\ R_f \end{bmatrix} \\ H x_0 \end{bmatrix}$$

$$= \begin{bmatrix} x_{10} - R_{s1} - R_{f4} - x_{2,0} - x_{7,0} - x_{8,0} + x_{9,0} + x_{10,0} \\ -R_{f2} - R_{f3} \\ R_{s1} + x_{2,0} + x_{3,0} + x_{7,0} + x_{8,0} \\ R_{f2} - R_{f1} - R_{f3} + R_{f4} + R_{s2} + x_{2,0} + x_{4,0} - x_{6,0} + 2x_{8,0} - x_{9,0} - 2x_{10,0} \\ R_{f1} - R_{f2} + R_{f3} - R_{f4} + R_{s1} + x_{5,0} + x_{6,0} + x_{7,0} - x_{8,0} + x_{9,0} + x_{10,0} \\ -R_{f1} \\ R_{f2} - R_{s1} \\ R_{f3} \\ R_{f4} - R_{s2} \\ R_{s2} \\ R_{f1} - R_{s2} + x_{6,0} + x_{10,0} + x_{11,0} \end{bmatrix}.$$

Defining a small parameter $\epsilon = 1/k^*$ such that $k_i/k^* \ll \mathcal{O}(1)$, for $i = 3, 6, 9, 12$, and letting

$k' = k_j/k^*$ for $j = 1,2,4,5,7,8,10,11$, we can write

$$\begin{aligned}
\dot{R}_s &= M_s\, v\left(x\left(R_s, R_f\right)\right) \\
&= \nu_s\left(R_s, R_f\right) \\
&= \begin{bmatrix}
-k_3(R_{f2}-R_{s1})(R_{f4}+R_{s1}-x_{1,0}+x_{2,0}+x_{7,0}+x_{8,0}-x_{9,0}-x_{10,0})-k_9(R_{s1} \\
\qquad +x_{2,0}+x_{3,0}+x_{7,0}+x_{8,0})(R_{f1}-R_{f2}+R_{f3}-R_{f4}+R_{s1}+x_{5,0}+x_{6,0} \\
\qquad +x_{7,0}-x_{8,0}+x_{9,0}+x_{10,0}) \\
k_6(R_{f4}-R_{s2})(R_{f1}-R_{s2}+x_{6,0}+x_{10,0}+x_{11,0})-R_{s2}k_{12}(R_{f2}-R_{f1} \\
\qquad -R_{f3}+R_{f4}+R_{s2}+x_{2,0}+x_{4,0}-x_{6,0}+2x_{8,0}-x_{9,0}-2x_{10,0})
\end{bmatrix},
\end{aligned}$$

$$\begin{aligned}
\dot{R}_f &= M_f\, v\left(x\left(R_s, R_f\right)\right) \\
&= \frac{1}{\epsilon}\,\bar{\nu}_f\left(R_s, R_f\right) \\
&= \frac{1}{\epsilon}\begin{bmatrix}
-R_{f1}k_1'(R_{f2}-R_{f1}-R_{f3}+R_{f4}+R_{s2}+x_{2,0}+x_{4,0}-x_{6,0}+2x_{8,0}-x_{9,0} \\
\qquad -2x_{10,0})-k_7'(R_{f1}-R_{s2}+x_{6,0}+x_{10,0}+x_{11,0})(R_{f1}-R_{f2}+R_{f3} \\
\qquad -R_{f4}+R_{s1}+x_{5,0}+x_{6,0}+x_{7,0}-x_{8,0}+x_{9,0}+x_{10,0}) \\
-k_8'(R_{f2}-R_{s1})(R_{f2}-R_{f1}-R_{f3}+R_{f4}+R_{s2}+x_{2,0}+x_{4,0}-x_{6,0}+2x_{8,0} \\
\qquad -x_{9,0}-2x_{10,0})-k_2'(R_{f2}+R_{f3})(R_{f1}-R_{f2}+R_{f3}-R_{f4} \\
\qquad +R_{s1}+x_{5,0}+x_{6,0}+x_{7,0}-x_{8,0}+x_{9,0}+x_{10,0}) \\
-R_{f3}k_{10}'(R_{f1}-R_{f2}+R_{f3}-R_{f4}+R_{s1}+x_{5,0}+x_{6,0}+x_{7,0}-x_{8,0}+x_{9,0} \\
\qquad +x_{10,0})-k_4'(R_{f2}+R_{f3})(R_{f2}-R_{f1}-R_{f3} \\
\qquad +R_{f4}+R_{s2}+x_{2,0}+x_{4,0}-x_{6,0}+2x_{8,0}-x_{9,0}-2x_{10,0}) \\
-k_{11}'(R_{f4}-R_{s2})(R_{f2}-R_{f1}-R_{f3}+R_{f4}+R_{s2}+x_{2,0}+x_{4,0}-x_{6,0}+2x_{8,0} \\
\qquad -x_{9,0}-2x_{10,0})-k_5'(R_{f4}+R_{s1}-x_{1,0}+x_{2,0}+x_{7,0}+x_{8,0}-x_{9,0}-x_{10,0}) \\
\qquad (R_{f1}-R_{f2}+R_{f3}-R_{f4}+R_{s1}+x_{5,0}+x_{6,0}+x_{7,0}-x_{8,0}+x_{9,0}+x_{10,0})
\end{bmatrix},
\end{aligned}$$

which gives us an entirely explicit form of the model in standard form.

Next, we shall briefly summarize the application of the method of Kumar et al. to this example, as was done in [68]. The original system in Equation (4.54) can be written in the

form with nonexplicit time-scale separation as

$$\dot{x} = f(x) + \frac{1}{\epsilon} V_f \bar{r}_f(x). \tag{4.55}$$

This can be reduced to a set of differential algebraic equations of the form

$$\dot{x} = f(x) + V_f z, \tag{4.56}$$

$$\bar{r}_f = 0, \tag{4.57}$$

where $z = \lim_{\epsilon \to 0} \frac{\bar{r}_f}{\epsilon}$. Equation (4.57) gives a set of algebraic constraints that the state trajectories must respect, effectively specifying a lower dimensional manifold near which the system in Equation (4.55) evolves. In some situations, these constraints may be differentiated in time to yield an explicit expression for the variables z. In particular, we have

$$\frac{\mathrm{d}\,\bar{r}_f}{\mathrm{d}\,t} = \mathcal{L}_f \bar{r}_f(x) + \mathcal{L}_{V_f} \bar{r}_f(x) z = 0, \tag{4.58}$$

where the column vector $\mathcal{L}_b a(x)$ is such that $[\mathcal{L}_b a(x)]_i \triangleq \frac{\partial a_i}{\partial x} b(x)$ is the Lie derivative of the scalar field $a_i(x)$ with respect to the vector field $b(x)$, and a Lie derivative of $\bar{r}_f$ with respect to a matrix V_f is interpreted as the matrix formed by concatenating the Lie derivatives with respect to the individual columns of V_f. When $\mathcal{L}_{V_f} \bar{r}_f(x)$ is nonsingular, we have

$$z = -\left[\mathcal{L}_{V_f} \bar{r}_f(x)\right]^{-1} \mathcal{L}_f \bar{r}_f(x). \tag{4.59}$$

The general form of the coordinate transformation given in [68] is

$$\begin{bmatrix} \zeta \\ \eta \end{bmatrix} \triangleq T(x) \triangleq \begin{bmatrix} \phi(x) \\ \bar{r}_f(x) \end{bmatrix},$$

where ζ and η are the slow and fast variables respectively, and $\zeta = \phi(x)$ is a vector of the same dimension as the equilibrium manifold. The function ϕ can be chosen with considerable flexibility, and in general will lead to slow variables that exhibit fast initial

transients. We can derive an explicit expression for these slow variables by differentiating ζ,

$$\dot{\zeta} = \left\{ \mathcal{L}_f \phi(x) - \mathcal{L}_{V_f} \phi(x) \left[\mathcal{L}_{V_f} \bar{r}_f(x) \right]^{-1} \left[\mathcal{L}_f \bar{r}_f(x) \right] \right\} \Big|_{x = T^{-1}(\zeta, 0)}, \tag{4.60}$$

which reduces to

$$\dot{\zeta} = \mathcal{L}_f \phi(x) |_{x = T^{-1}(\zeta, 0)} \tag{4.61}$$

if $\mathcal{L}_{V_f} \phi(x)$ is identically zero. We note that the second term in Equation (4.60) defines the initial fast transients, and we can get 'true' slow variables if the condition $\mathcal{L}_{V_f} \phi(x) \equiv 0$ holds. Kumar et al. show that this is only possible when the distribution spanned by V_f is involutive, and [68] notes that for the constant stoichiometric matrix, this is always the case, and indeed leads to a matrix Φ, with $\phi(x) = \Phi x$, whose rows are in the left null space of the fast reaction stoichiometric matrix V_f. This condition for finding slow variables that are independent of the fast dynamics was also mentioned in [39].

When this method is applied to the model of the esterification of carboxylic acid, as was done in [68], we find that the involutivity condition holds, and the transformation $T(x)$ has the components

$$\phi(x) = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & -1 & 0 & 2 & 0 & 0 & 0 \\ -1 & 1 & 0 & -1 & 0 & 1 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} x, \qquad \bar{r}_f(x) = \begin{bmatrix} k_1' x_6 x_4 - k_7' x_5 x_{11} \\ k_2' x_5 x_2 - k_8' x_7 x_4 \\ k_4' x_2 x_4 - k_{10}' x_8 x_5 \\ k_5' x_1 x_5 - k_{11}' x_4 x_9 \end{bmatrix}. \tag{4.62}$$

The resulting final expression for the slow subsystem is given by

$$\dot{\zeta} = \mathcal{L}_f \phi(x)|_{x=T^{-1}(\zeta,0)} \tag{4.63}$$

$$= \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 1 & 0 & 1 & 0 & -1 & 0 & 2 & 0 & 0 & 0 \\ -1 & 1 & 0 & -1 & 0 & 1 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & -1 & 0 \\ 0 & 1 & 0 & -1 \\ 1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 \\ -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 1 \\ 0 & 1 & 0 & -1 \\ 0 & -1 & 0 & 1 \end{bmatrix} \begin{bmatrix} k_3 x_1 x_7 \\ k_6 x_{11} x_9 \\ k_9 x_5 x_3 \\ k_{12} x_{10} x_4 \end{bmatrix} \Bigg|_{x=T^{-1}(\zeta,0)} . \tag{4.64}$$

We note that the right hand side of Equation (4.64) requires an evaluation of T^{-1}, which is nontrivial. A merit of this method is that the slow variables can be chosen with great flexibility, even when the stoichiometric matrix is not constant, and when other nonlinearities exist in the system. In this case, Equation (4.60) gives the dynamics of the slow subsystem in terms of these variables, and reduces to a simpler case when the involutivity condition holds, as it does for isothermal reactions. In this sense, this method gives a unified method for bringing systems with nonexplicit time-scale-separation into the standard form. We also note that the fast variables in [41, 68] are the net reaction velocity expressions for the fast reactions, whereas in our method, these quantities are the rates of change of the fast variables. This is a fundamental difference between the two methods, and allows us to give a physical interpretation to our fast variables as the reaction extents of the fast reactions.

4.5 Application of Reaction Extents to the Reduction of Transcription and Translation Reactions

We now apply the transformations provided in Sections 4.4.3.1 and 4.4.3.2 to the transcription model (4.32) to derive the corresponding standard singular perturbation form. We can write the model (4.32) in matrix notation as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{bmatrix} d \\ \rho \\ v_1 \\ w_1 \\ v_2 \\ w_2 \\ \vdots \\ v_n \\ w_n \\ m_n \\ \eta \\ \gamma \end{bmatrix} = \begin{array}{c} \\ \tilde{r}_{2n+2} \\ \tilde{r}_{2n+1} \\ \tilde{r}_{2n} \\ \tilde{r}_{2n-1} \\ \tilde{r}_{2n-2} \\ \tilde{r}_{2n-3} \\ \vdots \\ \tilde{r}_2 \\ \tilde{r}_1 \\ \tilde{q}_1 \\ \tilde{q}_2 \\ \tilde{q}_3 \end{array} \begin{array}{c} \begin{array}{ccccccccc} \tilde{c}_1 & \tilde{c}_2 & \tilde{c}_3 & \tilde{c}_4 & \tilde{c}_5 & \tilde{c}_6 & \cdots & \tilde{c}_{2n+1} & \tilde{c}_{2n+2} \end{array} \\ \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & & 0 & 0 \\ & & & & & & \ddots & & \\ 0 & 0 & 0 & 0 & 0 & 1 & & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 \\ 0 & 0 & -1 & 0 & -1 & 0 & & -1 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 \end{pmatrix} \end{array} \begin{bmatrix} k_{\mathrm{term}}\gamma \\ k_{Pf}\rho d - k_{Pr}v_1 \\ k_{Nf}v_1\eta - k_{Nr}w_1 \\ k_t w_1 \\ k_{Nf}v_2\eta - k_{Nr}w_2 \\ k_t w_2 \\ \vdots \\ k_{Nf}v_n\eta - k_{Nr}w_n \\ k_t w_n \end{bmatrix}, \tag{4.65}$$

where the $\tilde{q}_i$, $\tilde{r}_i$'s and $\tilde{c}_j$'s are row and column labels we will use for manipulating the matrix $\tilde{S} \in \mathbb{R}^{(2n+5)\times(2n+2)}$. Compactly, we write this as

$$\frac{\mathrm{d}x}{\mathrm{d}t} = S\,v, \qquad x(0) = x_0, \tag{4.66}$$

where we have dropped the bars for notational clarity. Here the species concentration vector is $x \in \mathbb{R}_+^{2n+5}$ and the reaction velocity vector is $v \in \mathbb{R}^{2n+2}$. The stoichiometric matrix

S has linearly dependent rows due to the existence of conservation laws. We can define the matrices P and H as defined in the discussion surrounding Equation (4.36) as follows. The rows $\tilde{r}_{2n+1}$, $\tilde{q}_3$ and $\tilde{q}_1$ are linearly dependent on the remaining rows of the matrix because of the relations $\tilde{r}_{2n+1} = \tilde{r}_{2n+2}$ and $\tilde{q}_3 = -\sum_{i=1}^{2n+1} \tilde{r}_i$, and the argument provided in Lemma 4 in the appendix. The fact that no other linear dependencies exist follows from the fact that the matrix resulting from the removal of these rows is invertible (Lemma 5 in the appendix). Thus, we can define

$$P = \begin{bmatrix} 1 & 0 & 0 & 0 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & & 0 & 0 & 0 & 0 \\ & & & & \ddots & & & & \\ 0 & 0 & 0 & 0 & & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & & 0 & 0 & 1 & 0 \end{bmatrix}, \quad H = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 & 0 & 0 & \dots & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 1 & \dots & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 & 2 & 2 & \dots & n & n & 1 & 0 \end{bmatrix}.$$

With these matrices, we can define S_r, x_i, x_d and R via Equations (4.36), (4.37), (4.44) and (4.47). Let us enumerate the elements of $R \in \mathbb{R}^{2n+2}$ as $R = [\, R_{term}\ R_P\ R_{N_1}\ R_{t_1}\ \dots\ R_{N_n}\ R_{t_n}\,]$. Here, the subscripts are chosen to represent the reactions present in the system, due to

the relationship $\mathrm{d}R/\mathrm{d}t = \nu_{rxn}$ in Equation (4.48) and note that

$$x = \begin{bmatrix} P \\ H \end{bmatrix}^{-1} \begin{bmatrix} S_r R \\ x_d \end{bmatrix} = \begin{bmatrix} R_{term} - R_P \\ R_{term} - R_P - x_{d_{10}} \\ R_P - R_{N_1} \\ R_{N_1} - R_{t_1} \\ R_{t_1} - R_{N_2} \\ \vdots \\ R_{t_{n-1}} - R_{N_n} \\ R_{N_n} - R_{t_n} \\ R_{t_n} - x_{d_{30}}/5 \\ -\sum_{i=1}^{n} R_{N_i} \\ R_{t_n} - R_{term} + x_{d_{10}} + x_{d_{20}} \end{bmatrix}, \tag{4.67}$$

where $x_{d_{m0}}$ for $m \in \{1, 2, 3\}$ are the dependent variables fixed to their intitial conditions (due to Equation (4.45)). Thus, the ODEs in R coordinates for this system (Equation (4.48)) are

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \begin{bmatrix} k_{\mathrm{term}}(R_{t_n} - R_{term} + x_{d_{10}} + x_{d_{20}}) \\ k_{Pf}(R_{term} - R_P - x_{d_{10}})(R_{term} - R_P) - k_{Pr}(R_P - R_{N_1}) \\ k_{Nf}(R_P - R_{N_1})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_1} - R_{t_1}) \\ k_t(R_{N_1} - R_{t_1}) \\ \vdots \\ k_{Nf}(R_{t_{j-1}} - R_{N_j})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_j} - R_{t_j}) \\ k_t(R_{N_j} - R_{t_j}) \\ \vdots \\ k_{Nf}(R_{t_{n-1}} - R_{N_n})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_n} - R_{t_n}) \\ k_t(R_{N_n} - R_{t_n}) \end{bmatrix}, \tag{4.68}$$

and defining M_s, M_f, R_s, R_f to separate out the slow and fast terms, we have,

$$\frac{\mathrm{d}R_s}{\mathrm{d}t} = \begin{bmatrix} k_{\text{term}}(R_{t_n} - R_{term} + x_{d_{10}} + x_{d_{20}}) \\ k_t(R_{N_1} - R_{t_1}) \\ \vdots \\ k_t(R_{N_j} - R_{t_j}) \\ \vdots \\ k_t(R_{N_n} - R_{t_n}) \end{bmatrix}, \tag{4.69}$$

$$\frac{\mathrm{d}R_f}{\mathrm{d}t} = \begin{bmatrix} k_{Pf}(R_{term} - R_P - x_{d_{10}})(R_{term} - R_P) - k_{Pr}(R_P - R_{N_1}) \\ k_{Nf}(R_P - R_{N_1})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_1} - R_{t_1}) \\ \vdots \\ k_{Nf}(R_{t_{j-1}} - R_{N_j})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_j} - R_{t_j}) \\ \vdots \\ k_{Nf}(R_{t_{n-1}} - R_{N_n})(-\sum_{i=1}^{n} R_{N_i}) - k_{Nr}(R_{N_n} - R_{t_n}) \end{bmatrix}. \tag{4.70}$$

We can further transform this into a nondimensional model, where a small parameter ϵ can be defined to capture the effects of the scale separation in the parameter values.

After nondimensionalization, we have

$$\frac{\mathrm{d}r_s}{\mathrm{d}\tau} = \begin{bmatrix} \alpha_{\text{term}}(r_{t_n} - r_{term} + \bar{x}_{d_{10}} + \bar{x}_{d_{20}}) \\ (r_{N_1} - r_{t_1}) \\ \vdots \\ (r_{N_j} - r_{t_j}) \\ \vdots \\ (r_{N_n} - r_{t_n}) \end{bmatrix}, \tag{4.71}$$

$$\epsilon\frac{\mathrm{d}r_f}{\mathrm{d}\tau} = \begin{bmatrix} \alpha_{Pf}(r_{term} - r_P - \bar{x}_{d_{10}})(r_{term} - r_P) - \alpha_{Pr}(r_P - r_{N_1}) \\ (r_P - r_{N_1})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_1} - r_{t_1}) \\ \vdots \\ (r_{t_{j-1}} - r_{N_j})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_j} - r_{t_j}) \\ \vdots \\ (r_{t_{n-1}} - r_{N_n})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_n} - r_{t_n}) \end{bmatrix}, \tag{4.72}$$

with the scheme

$$\begin{bmatrix} r_{t_i} \\ r_{term} \\ r_{N_i} \\ r_P \\ \bar{x}_{d_{j0}} \\ \epsilon \\ \alpha_{Pf} \\ \alpha_{Pr} \\ \tau \end{bmatrix} = \begin{bmatrix} \begin{bmatrix} R_{t_i} \\ R_{term} \\ R_{N_i} \\ R_P \\ x_{d_{j0}} \end{bmatrix} \\ k_t/k_{Nr} \\ k_{Pf}/k_{Nf} \\ k_{Pr}/k_{Nr} \\ k_t t \end{bmatrix}, \qquad \forall i \in \{1, \ldots, n\},\ j \in \{1, 2, 3\}, \tag{4.73}$$

where the α parameters are of order $O(1)$. Setting $\epsilon = 0$ and transforming back to species

concentration coordinates, we have

$$0 = \begin{bmatrix} \alpha_{Pf}(r_{term} - r_P - \bar{x}_{d_{10}})(r_{term} - r_P) - \alpha_{Pr}(r_P - r_{N_1}) \\ (r_P - r_{N_1})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_1} - r_{t_1}) \\ \vdots \\ (r_{t_{j-1}} - r_{N_j})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_j} - r_{t_j}) \\ \vdots \\ (r_{t_{n-1}} - r_{N_n})(-\sum_{i=1}^{n} r_{N_i}) - (r_{N_n} - r_{t_n}) \end{bmatrix} \tag{4.74}$$

$$= \begin{bmatrix} k_{Pf}\rho d - k_{Pr}v_1 \\ k_{Nf}v_1\eta - k_{Nr}w_1 \\ k_{Nf}v_2\eta - k_{Nr}w_2 \\ \vdots \\ k_{Nf}v_n\eta - k_{Nr}w_n \end{bmatrix}. \tag{4.75}$$

4.6 Generalized Consumption Model

In this section, we reduce the full model (4.1) to a generalized version of the consumption model by matching their steady state behaviors. In Section 4.2, we modeled the creation of RNA as a single step transcription reaction, with no intermediate RNA transcripts, and discussed a method for incorporating nucleotide consumption in this model. While this suffices for models where the only regulation of gene expression comes from transcription factor proteins, it becomes inadequate when we wish to model the regulation of transcription via interactions with nascent RNA transcripts, for instance by non-coding RNA like the pT181 transcriptional attenuator [8, 43, 50]. Thus, we wish to have a transcription scheme where the creation of the final RNA transcript occurs in steps, and each intermediate RNA piece is free to interact with other elements of the environment. For an RNA transcript of length n, the detailed model shown in Equation (4.1) has approximately $2n$ chemical reactions and about the same number of differential equations. For typical RNAs of length $500-2000$ bases, these models quickly become unwieldy, especially when modeling large systems composed of multiple genes and regulatory pathways. Thus, we derive a way to

reduce such transcription models while quantifying the error introduced into the dynamics by such reductions.

We begin by re-indexing the detailed model (4.1) as follows. Divide the length RNA (of length n) into K segments of lengths n_i, for $i \in \{1, 2, \ldots, K\}$. The species $\mathrm{P{:}D_{k,j}{:}m_{k,j-1}}$, with $k \in \{1, 2, \ldots, K\}$, refers to the species from the kth segment, with the polymerase attached to the jth DNA base pair site of this segment, where j is in $\{1, \ldots n_k\}$, and an RNA of length $\sum_{i=1}^{k-1} n_i + (j-1)$ attached to the polymerase molecule. Finally, identify the last element of a block with the first element of the next block, i.e., $\mathrm{P{:}D_{k,n_k+1}{:}m_{k,n_k}} = \mathrm{P{:}D_{k+1,1}{:}m_{k+1,0}}$. This new indexing scheme is shown on the left in Figure 4.2. We would like to reduce this model to the one shown in Equation (4.76), where the RNA and DNA have been divided into K blocks. The concentration of each species in the reduced model (4.76) is the sum of the corresponding species in the full model (4.1). For example, $[\mathrm{P{:}D{:}R_k}] = \sum_{h=1}^{n_k} [\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$.

$$
\begin{aligned}
\mathrm{P + D} &\underset{k'_{Pr}}{\overset{k'_{Pf}}{\rightleftharpoons}} \mathrm{P{:}D} && \text{RNA polymerase binding,}\\
\mathrm{P{:}D + N} &\underset{k'_{Nr}}{\overset{k'_{Nf}}{\rightleftharpoons}} \mathrm{P{:}D{:}N} && \text{nucleotide binding,}\\
\mathrm{P{:}D{:}N} &\xrightarrow{k_{\mathrm{con}(1)}} \mathrm{P{:}D} && \text{consumption reaction,}\\
\mathrm{P{:}D{:}N} &\xrightarrow{k_{tx(1)}} \mathrm{P{:}D{:}R_1} && \text{lumped RNA production,}\\
&\vdots &&\\
\mathrm{P{:}D{:}R_{K-1} + N} &\underset{k'_{Nr}}{\overset{k'_{Nf}}{\rightleftharpoons}} \mathrm{P{:}D{:}R_{K-1}{:}N} && \text{nucleotide binding,}\\
\mathrm{P{:}D{:}R_{K-1}{:}N} &\xrightarrow{k_{\mathrm{con}(K)}} \mathrm{P{:}D{:}R_{K-1}} && \text{consumption reaction,}\\
\mathrm{P{:}D{:}R_{K-1}{:}N} &\xrightarrow{k_{tx(K)}} \mathrm{P{:}D_t + R_K} && \text{lumped RNA production,}\\
\mathrm{P{:}D_t} &\xrightarrow{k_{\mathrm{term}}} \mathrm{P + D} && \text{termination.}
\end{aligned}
\tag{4.76}
$$

Let D_T be the total DNA concentration in in either model. Mass conservation gives

$$
D_T = \sum_{i=1}^{K} \sum_{h=1}^{n_i} \Big([\mathrm{P{:}D_{(i,h)}{:}m_{(i,h-1)}}] + [\mathrm{P{:}D_{(i,h)}{:}m_{(i,h-1)}{:}N}] \Big) + [\mathrm{D}] + [\mathrm{P{:}D_t}], \tag{4.77}
$$

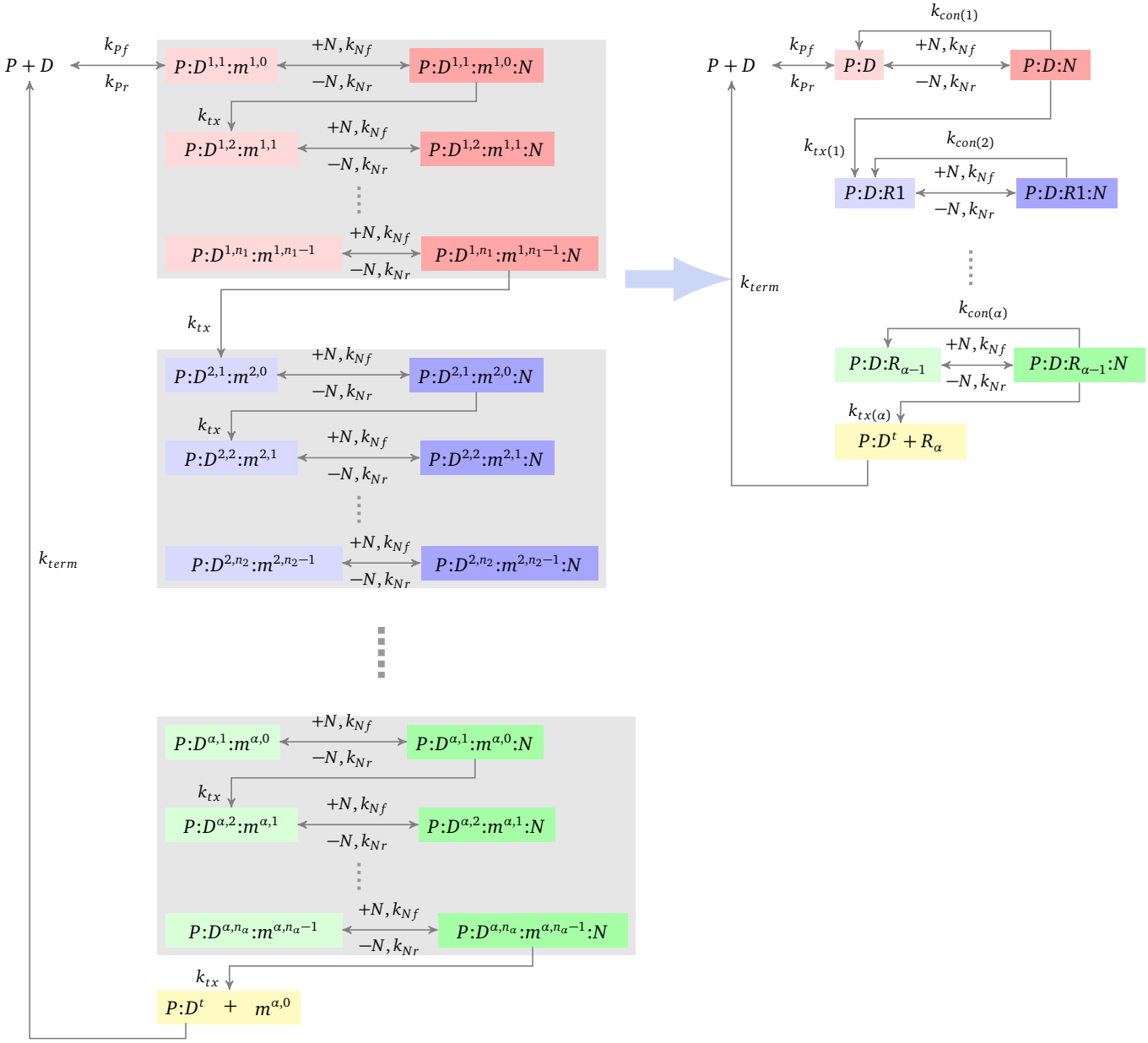

Figure 4.2: The model reduction procedure. Corresponding species are color coded. Each grey 'block' represents the part of the model that transcribes the corresponding segment of the RNA, with the output of the grey block as the intermediate RNA species which will be modeled by the reduced model.

for the full model and

$$D_T = \sum_{i=1}^{K} \left([\mathrm{P{:}D{:}R_{i-1}}] + [\mathrm{P{:}D{:}R_{i-1}{:}N}] \right) + [\mathrm{D}] + [\mathrm{P{:}D_t}], \tag{4.78}$$

with $\mathrm{P{:}D{:}R_0} \triangleq \mathrm{P{:}D}$ and $\mathrm{P{:}D{:}R_0{:}N} \triangleq \mathrm{P{:}D{:}N}$, for the reduced model.

Using the mass balance equations (4.21), (4.22) and rapid equilibrium assumption (4.75), we can express $[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$ in the full model as:

$$[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}] = \frac{1}{n_k} \left(\frac{D_T - [\mathrm{D}] - [\mathrm{P{:}D_t}]}{\left(1 + \frac{k_{Nf}}{k_{Nr}}[\mathrm{N}]\right)} - \sum_{i=1, i\neq k}^{K} n_i [\mathrm{P{:}D_{(i,1)}{:}m_{(i,0)}}] \right). \tag{4.79}$$

The rapid equilibrium assumption for the reduced model can be derived in exactly the same way as that for the full model, and using it gives

$$[\mathrm{P{:}D{:}R_k}] = \left(\frac{D_T - [\mathrm{D}] - [\mathrm{P{:}D_t}]}{\left(1 + \frac{k'_{Nf}}{k'_{Nr}}[\mathrm{N}]\right)} - \sum_{i=0, i\neq k}^{K-1} [\mathrm{P{:}D{:}R_i}] \right). \tag{4.80}$$

We would like the reduced model to be an approximation of the full model at steady state (noting that the steady state is defined in the sense of the discussion preceding Proposition 3, when the nucleotide concentration is sufficiently large to be assumed to be essentially constant in the time-scale of interest). A set of sufficient conditions for this to be true is presented for the remainder of this section.

We first note that the discussion around equations (4.14) to (4.22) in Section 4.3.2 can be applied to the species within a single block to obtain the result that at steady state, corresponding species within a block are in mass balance (i.e., (4.21) and (4.22) hold within each block). This allows us to define and simplify the correspondence $[\mathrm{P{:}D{:}R_k}] = \sum_{h=1}^{n_k} [\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$ between the full and reduced models to

$$[\mathrm{P{:}D{:}R_k}] = n_k [\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}], \qquad \text{Condition 1.} \tag{4.81}$$

The relation (4.81) is a condition we have imposed upon the model to create a corre-

spondence between the species between corresponding blocks of the full and reduced models. This condition, applied to equations (4.79) and (4.80), gives us further correspondences between the full and reduced models. In Equation (4.79), the term $D_T - [\mathrm{D}] - [\mathrm{P{:}D_t}]$ is the total concentration of species in the grey boxes in Figure 4.2, the $\frac{1}{(1+(k_{Nf}/k_{Nr})[\mathrm{N}])}$ term, which arises out of the analysis of Section 4.4.1, picks out the proportion of $[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$ from the total $[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]+[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}{:}N}]$, and so applying it to $D_T - [\mathrm{D}] - [\mathrm{P{:}D_t}]$ gives the total concentration of terms of the form $[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$. Equation (4.80) has a similar interpretation, with the difference that $[\mathrm{P{:}D{:}R_k}]$ is now the concentration of a block in the reduced model, and thus corresponds to the total concentration of terms of the form $[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$ in a single block of the full model, which by Equation (4.81) is $n_k[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}]$.

The next step in using the reduced model to approximate the full model is to equate the fluxes out of corresponding blocks in the two models. We can then obtain a set of (not necessarily unique) relations between the parameters of the two models so that the steady state behaviors match. Consider the rate of production of the species $\mathrm{P{:}D_{(k+1,1)}{:}m_{(k+1,0)}}$, which corresponds to the output of the kth block, or kth intermediate RNA transcript, in the full model,

$$\begin{aligned}[\mathrm{P{:}D_{(k+1,1)}{:}m_{(k+1,0)}}]_{\text{production}} &= k_{tx}[\mathrm{P{:}D_{(k,n_k)}{:}m_{(k,n_k-1)}{:}N}] \\ &= k_{tx}\frac{k_{Nf}}{k_{Nr}}[\mathrm{P{:}D_{(k,h)}{:}m_{(k,h-1)}}][\mathrm{N}],\end{aligned} \tag{4.82}$$

where we recall that $\mathrm{P{:}D_{(k,n_k+1)}{:}m_{(k,n_k)}}$ is identified with $\mathrm{P{:}D_{(k+1,1)}{:}m_{(k+1,0)}}$.

The corresponding expression for the reduced model is

$$\begin{aligned}[\mathrm{P{:}D{:}R_{k+1}}]_{\text{production}} &= k'_{tx(k)}[\mathrm{P{:}D{:}R_k{:}N}] \\ &= k'_{tx(k)}\frac{k'_{Nf}}{k'_{Nr}}[\mathrm{P{:}D{:}R_k}][\mathrm{N}].\end{aligned} \tag{4.83}$$

Equating the equations (4.82) and (4.83), and comparing terms, we can draw a corre-

spondence between the respective coefficients,

$$k'_{tx(k)} = \frac{k_{tx}}{n_k}, \tag{4.84}$$

$$k'_{Nf} = k_{Nf}, \tag{4.85}$$

$$k'_{Nr} = k_{Nr}, \qquad \text{Conditions 2 - 4.} \tag{4.86}$$

We now state a result that generalizes the result of Proposition 3 to the model lumped into blocks, and states that the rate of consumption of nucleotides within the kth block is n_k times the rate of production of the kth nascent transcript. This, in turn, will allow us to derive a generalization for the consumption reaction for the reduced model (4.76).

Proposition 4. *Consider the full model* (4.1) *under the same assumptions as Proposition 3, now considered with the indexing scheme that divides it into separate transcription blocks, as described at the beginning of Section 4.6. Then, in the kth block, for $k \in \{1, 2, \ldots, K\}$, the rate of nucleotide consumption within the block is proportional to the rate of production of the kth intermediate transcript, $\mathrm{P{:}D_{k,n_k+1}{:}m_{k,n_k}}$ (with the Kth transcript being $\mathrm{m_{K,0}}$).*

Proof. From the proof of Proposition 3, we know that the total rate of nucleotide consumption is

$$\frac{\mathrm{d[N_{uninc}]}}{\mathrm{d}t} = -k_{tx} \sum_{i=1}^{n} \mathrm{[P{:}D_i{:}m_{i-1}{:}N]}. \tag{4.87}$$

This sum can be partitioned into terms using the new indexing scheme into contributions

to the consumption rate by each block as follows:

$$\begin{aligned}
\frac{\mathrm{d}[\mathrm{N}_{\mathrm{uninc}}]}{\mathrm{d}t} &= -k_{tx}\sum_{k=1}^{K}\sum_{h=1}^{n_k}[\mathrm{P{:}D}_{(\mathrm{k,h})}{:}\mathrm{m}_{(\mathrm{k,h-1})}{:}\mathrm{N}] \\
&= -k_{tx}\sum_{h=1}^{n_1}[P{:}D_{(1,h)}{:}m_{(1,h-1)}{:}N] \\
&\quad - k_{tx}\sum_{h=1}^{n_2}[P{:}D_{(2,h)}{:}m_{(2,h-1)}{:}N] \\
&\qquad\qquad \vdots \\
&\quad - k_{tx}\sum_{h=1}^{n_K}[\mathrm{P{:}D}_{(\mathrm{k,h})}{:}\mathrm{m}_{(\mathrm{k,h-1})}{:}\mathrm{N}] \\
&= -n_1k_{tx}[P{:}D_{(1,n_1)}{:}m_{(1,n_1-1)}{:}N] \\
&\quad - n_2k_{tx}[P{:}D_{(2,n_2)}{:}m_{(2,n_2-1)}{:}N] \\
&\qquad\qquad \vdots \\
&\quad - n_Kk_{tx}[\mathrm{P{:}D}_{(\mathrm{K,n_K})}{:}\mathrm{m}_{(\mathrm{K,n_K-1})}{:}\mathrm{N}],
\end{aligned} \tag{4.88}$$

where the last line follows from the fact that the species within a block are in flux balance as given by equations (4.21) and (4.22). In each of the terms of the form $-n_k k_{tx}[\mathrm{P{:}D}_{(\mathrm{k,n_k})}{:}\mathrm{m}_{(\mathrm{k,n_k-1})}{:}\mathrm{N}]$ in the above partition, the term $k_{tx}[\mathrm{P{:}D}_{(\mathrm{k,n_k})}{:}\mathrm{m}_{(\mathrm{k,n_k-1})}{:}\mathrm{N}]$ is the production rate of the kth transcript (bound to the RNA polymerase and DNA) for all $k \in \{1, 2, \ldots, K-1\}$ while it is the production rate of $\mathrm{m}_{\mathrm{K,0}}$ for $k = K$. On the other hand, the full term is the contribution of the kth block to the total consumption rate of the nucleotides not yet incorporated into the any RNA. □

The rate of consumption of nucleotides is the same in the full model (4.1) and the reduced model (4.76) if we set

$$k'_{\mathrm{con}(k)} = k'_{tx(k)}(n_k - 1), \qquad \text{Condition 5.} \tag{4.89}$$

Indeed, from Proposition 4, and equations (4.81) and (4.83), the rate of consumption of

N in the kth block of the full model is

$$k_{tx} n_k [\mathrm{P{:}D_{(k,n_k)}{:}m_{(k,n_k-1)}{:}N}] = k_{tx} \frac{n_k}{n_k} [\mathrm{P{:}D{:}R_k{:}N}] \tag{4.90}$$

$$= \frac{k_{tx}}{n_k} [\mathrm{P{:}D{:}R_k{:}N}] + \frac{k_{tx}}{n_k} (n_k - 1) [\mathrm{P{:}D{:}R_k{:}N}] \tag{4.91}$$

$$= [\mathrm{P{:}D{:}R_{k+1}}]_{\text{production}} + k'_{\text{con}(k)} [\mathrm{P{:}D{:}R_k{:}N}]. \tag{4.92}$$

4.7 Discussion

We have introduced a scalable method for incorporating nucleotide resource consumption in a reduced order model of transcription. This method uses a consumption reaction to emulate the usage of nucleotides, instead of modeling each nucleotide binding event separately or binding all the nucleotides simultaneously (Figure 4.1).

We have also generalized this method to allow for multiple intermediate transcripts. The approach here is to find a set of sufficient conditions, for a given nucleotide concentration, for the steady state dynamics of the full and reduced models to match. In a subsequent work, we plan to also study the deviation in the transient behavior of the two models and quantify the tradeoff between model reduction and fidelity.

In the process of attempting the above generalization, we had to use the deficiency zero theorem from chemical reaction network theory to show that part of the system we were considering has a steady state. We also had to define a coordinate transformation to justify the rapid equilibrium assumption that was made, since in the species concentration coordinate, such an assumption was not supported by singular perturbation theory. A technical point to include in a future work is to bring the two-time-scale model into the true *standard singular perturbation form*. This involves showing that the fast subsystem, with $\epsilon = 0$ in equations (4.27) and (4.72), has an isolated root, and that this root is (at least locally) asymptotically stable.

Other directions this work may be extended in is to allow for multiple occupancy of the DNA transcript by RNA polymerase, adding stochastic effects to the model, and to create an analogous scheme for translation, which should be similar in many respects.

Appendices

4.A Detailed Proofs

Lemma 4. *The row labeled $q_{1,n}$ in matrix P_n, defined as*

$$P_n = \begin{array}{c} r_{2n+1,n} \\ r_{2n,n} \\ r_{2n-1,n} \\ r_{2n-2,n} \\ r_{2n-3,n} \\ \vdots \\ r_{2,n} \\ r_{1,n} \\ q_{1,n} \\ q_{2,n} \end{array} \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \\ & & & & & & \ddots & & & \\ 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 \\ 0 & 0 & -1 & 0 & -1 & 0 & & 0 & -1 & 0 \end{pmatrix}, \tag{4.93}$$

is a linear combination of the remaining rows.

Proof. We use induction on the size of the matrix. We will prove that for

$$P_k = \begin{array}{c} \\ r_{2k+1,k} \\ r_{2k,k} \\ r_{2k-1,k} \\ r_{2k-2,k} \\ r_{2k-3,k} \\ \vdots \\ r_{2,k} \\ r_{1,k} \\ q_{1,k} \\ q_{2,k} \end{array} \begin{array}{c} \begin{array}{cccccccccc} 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 1 & -1 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} & & & & & & \ddots & & & \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & -1 & 0 & -1 & 0 & & 0 & -1 & 0 \end{array} \end{array}, \tag{4.94}$$

the row $q_{2,k}$ can be written as the linear combination $q_{2,k} = -(r_{2k-1,k} + r_{2k-2,k}) - 2(r_{2k-3,k} + r_{2k-4,k}) - 3(r_{2k-5,k} + r_{2k-6,k}) - \cdots - k(r_{1,k} + q_{1,k})$, and inverting this relationship gives $q_{1,k}$ as the linear combination

$$q_{1,k} = \frac{-(r_{2k-1,k} + r_{2k-2,k}) - 2(r_{2k-3,k} + r_{2k-4,k}) - \cdots - (k-1)(r_{3,k} + r_{2,k}) - kr_{1,k} - q_{2,k}}{k}. \tag{4.95}$$

Note that the claim holds for P_1 and P_2

$$P_1 = \begin{matrix} r_{3,1} \\ r_{2,1} \\ r_{1,1} \\ q_{1,1} \\ q_{2,1} \end{matrix} \begin{pmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & 0 \end{pmatrix}, \qquad P_2 = \begin{matrix} r_{5,2} \\ r_{4,2} \\ r_{3,2} \\ r_{2,2} \\ r_{1,2} \\ q_{1,2} \\ q_{2,2} \end{matrix} \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & 0 & -1 & 0 \end{pmatrix},$$

$$q_{1,1} = -r_{1,1} - q_{2,1}, \qquad q_{1,2} = \frac{-(r_{3,2} + r_{2,2}) - 2r_{1,2} - q_{2,2}}{2}.$$

Assume the claim holds for $k \geq 2$, as in Equation (4.94) and (4.95). For clarity, we remove rows $r_{2k+1,k}$ and $r_{2k,k}$, and the first two columns from P_k (which do not matter) to get

$$\bar{P}_k = \begin{matrix} \bar{r}_{2k-1,k} \\ \bar{r}_{2k-2,k} \\ \bar{r}_{2k-3,k} \\ \vdots \\ \bar{r}_{2,k} \\ \bar{r}_{1,k} \\ \bar{q}_{1,k} \\ \bar{q}_{2,k} \end{matrix} \begin{pmatrix} 1 & -1 & 0 & 0 & & 0 & 0 \\ 0 & 1 & -1 & 0 & & 0 & 0 \\ 0 & 0 & 1 & -1 & & 0 & 0 \\ & & & & \ddots & & \\ 0 & 0 & 0 & 0 & & -1 & 0 \\ 0 & 0 & 0 & 0 & & 1 & -1 \\ 0 & 0 & 0 & 0 & & 0 & 1 \\ -1 & 0 & -1 & 0 & & -1 & 0 \end{pmatrix}, \tag{4.96}$$

with

$$\bar{q}_{1,k} = \frac{-(\bar{r}_{2k-1,k} + \bar{r}_{2k-2,k}) - 2(\bar{r}_{2k-3,k} + \bar{r}_{2k-4,k}) - \cdots - (k-1)(\bar{r}_{3,k} + \bar{r}_{2,k}) - k\bar{r}_{1,k} - \bar{q}_{2,k}}{k}. \tag{4.97}$$

Now consider the $\bar{P}_{k+1}$, obtained by augmenting $\bar{P}_k$ with two rows and two columns:

$$\bar{P}_{k+1} = \left(\begin{array}{cc|ccccccc} 1 & -1 & 0 & 0 & \dots & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & \dots & 0 & 0 & 0 & 0 \\ \hline 0 & 0 & & & & & & & \\ 0 & 0 & & & & & & & \\ \vdots & & & & & & & & \\ 0 & 0 & & & & \bar{P}_k & & & \\ 0 & 0 & & & & & & & \\ 0 & 0 & & & & & & & \\ -1 & 0 & & & & & & & \end{array}\right) \begin{array}{c} \bar{r}_{2(k+1)-1,k+1} \\ \bar{r}_{2(k+1)-2,k+1} \\ \bar{r}_{2(k+1)-3,k+1} \\ \bar{r}_{2(k+1)-4,k+1} \\ \vdots \\ \bar{r}_{2,k+1} \\ \bar{r}_{1,k+1} \\ \bar{q}_{1,k+1} \\ \bar{q}_{2,k+1} \end{array} \tag{4.98}$$

$$= \left(\begin{array}{cc|ccccccc} 1 & -1 & 0 & 0 & \dots & & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & \dots & & 0 & 0 & 0 \\ \hline 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \\ \vdots & & & & & \ddots & & & \\ 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 \\ -1 & 0 & -1 & 0 & -1 & & 0 & -1 & 0 \end{array}\right) \begin{array}{c} \bar{r}_{2(k+1)-1,k+1} \\ \bar{r}_{2(k+1)-2,k+1} \\ \bar{r}_{2(k+1)-3,k+1} \\ \bar{r}_{2(k+1)-4,k+1} \\ \vdots \\ \bar{r}_{2,k+1} \\ \bar{r}_{1,k+1} \\ \bar{q}_{1,k+1} \\ \bar{q}_{2,k+1} \end{array}, \tag{4.99}$$

where we identify (augmented) rows of the matrix P_k in Equation (4.94) with the corre-

sponding rows of the matrix $\bar{P}_{k+1}$ in Equation(4.98) as follows:

$$\begin{aligned}
\bar{r}_{2(k+1)-2-i,k+1} &= \begin{pmatrix} 0 & 0 & \bar{r}_{2k-i,k} \end{pmatrix} \qquad \forall i \in \{1,\dots,2k-1\},\\
\bar{q}_{1,k+1} &= \begin{pmatrix} 0 & 0 & \bar{q}_{1,k} \end{pmatrix},\\
\bar{q}_{2,k+1} &= \begin{pmatrix} 0 & 0 & \bar{q}_{2,k} \end{pmatrix}.
\end{aligned}$$

We know from Equation (4.97) that

$$\begin{aligned}
\begin{pmatrix} 0 & 0 & \bar{q}_{2,k} \end{pmatrix} = &-(\bar{r}_{2(k+1)-3,k+1} + \bar{r}_{2(k+1)-4,k}) - 2(\bar{r}_{2(k+1)-5,k} + \bar{r}_{2(k+1)-6,k})\\
&-\cdots- (k-1)(\bar{r}_{3,k+1} + \bar{r}_{2,k+1}) - k(\bar{r}_{1,k+1} - \bar{q}_{1,k+1}).
\end{aligned} \tag{4.100}$$

It is also clear that the rows of $\bar{P}_{k+1}$ in Equation (4.98) satisfy

$$-\sum_{i=1}^{2k+1} \bar{r}_{2(k+1)-i,k+1} - \bar{q}_{1,k+1} = \begin{pmatrix} -1 & 0 & 0 & \dots & 0 \end{pmatrix}, \tag{4.101}$$

and together equations (4.100) and (4.101) give us the expression

$$\begin{aligned}
\bar{q}_{2,k+1} &= \begin{pmatrix} 0 & 0 & \bar{q}_{2,k} \end{pmatrix} + \begin{pmatrix} -1 & 0 & 0 & \dots & 0 \end{pmatrix}\\
&= -\sum_{i=1}^{2k+1} \bar{r}_{2(k+1)-i,k+1} - \bar{q}_{1,k+1} - (\bar{r}_{2(k+1)-3,k+1} + \bar{r}_{2(k+1)-4,k}) - 2(\bar{r}_{2(k+1)-5,k} + \bar{r}_{2(k+1)-6,k})\\
&\quad -\cdots- (k-1)(\bar{r}_{3,k+1} + \bar{r}_{2,k+1}) - k(\bar{r}_{1,k+1} - \bar{q}_{1,k+1}).\\
&= -(\bar{r}_{2(k+1)-1,k+1} + \bar{r}_{2(k+1)-2,k+1}) - 2(\bar{r}_{2(k+1)-3,k+1} + \bar{r}_{2(k+1)-4,k+1})\\
&\quad -\cdots- k(\bar{r}_{3,k+1} + \bar{r}_{2,k+1}) - (k+1)(\bar{r}_{1,k+1} + \bar{q}_{1,k+1}),
\end{aligned} \tag{4.102}$$

which can be rewritten in the desired form of Equation (4.95) as:

$$\bar{q}_{1,k+1} = \frac{\begin{array}{r} -(\bar{r}_{2(k+1)-1,k+1} + \bar{r}_{2(k+1)-2,k+1}) - 2(\bar{r}_{2(k+1)-3,k+1} + \bar{r}_{2(k+1)-4,k+1})\\ -\cdots- k(\bar{r}_{3,k+1} + \bar{r}_{2,k+1}) - (k+1)\bar{r}_{1,k+1} - \bar{q}_{2,k+1} \end{array}}{k+1}. \tag{4.103}$$

Adding to $\bar{P}_{k+1}$ analogues of the rows and columns we removed from P_k to get $\bar{P}_k$, we

can construct P_{k+1},

$$P_{k+1} = \left(\begin{array}{cccc|cccccc} 1 & -1 & 0 & 0 & 0 & 0 & \dots & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & \dots & 0 & 0 & 0 \\ \hline 0 & 0 & 1 & -1 & 0 & 0 & \dots & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & \dots & 0 & 0 & 0 \\ \hline 0 & 0 & 0 & 0 & & & & & & \\ 0 & 0 & 0 & 0 & & & & & & \\ \vdots & & \vdots & & & & & & & \\ 0 & 0 & 0 & 0 & & & \bar{P}_k & & & \\ 0 & 0 & 0 & 0 & & & & & & \\ 0 & 0 & 0 & 0 & & & & & & \\ 0 & 0 & -1 & 0 & & & & & & \end{array}\right) \begin{array}{c} r_{2(k+1)+1,k+1} \\ r_{2(k+1),k+1} \\ r_{2(k+1)-1,k+1} \\ r_{2(k+1)-2,k+1} \\ r_{2(k+1)-3,k+1} \\ r_{2(k+1)-4,k+1} \\ \vdots \\ r_{2,k+1} \\ r_{1,k+1} \\ q_{1,k+1} \\ q_{2,k+1} \end{array}, \tag{4.104}$$

and for this matrix, the linear combination relationship (4.103) now becomes

$$q_{1,k+1} = \frac{\begin{array}{r} -(r_{2(k+1)-1,k+1} + r_{2(k+1)-2,k+1}) - 2(r_{2(k+1)-3,k+1} + r_{2(k+1)-4,k+1}) \\ -\dots - k(r_{3,k+1} + r_{2,k+1}) - (k+1)r_{1,k+1} - q_{2,k+1} \end{array}}{k+1}, \tag{4.105}$$

which completes the proof. □

Lemma 5. *The $2n+2$ by $2n+2$ matrix*

$$S = \begin{array}{c} \\ r_{2n+1} \\ r_{2n} \\ r_{2n-1} \\ r_{2n-2} \\ r_{2n-3} \\ \vdots \\ r_2 \\ r_1 \\ q_2 \end{array} \begin{array}{c} \begin{array}{cccccccccc} 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 1 & -1 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} & & & & & & \ddots & & & \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & -1 & 0 & -1 & 0 & & 0 & -1 & 0 \end{array} \end{array} \tag{4.106}$$

is invertible.

Proof. Consider first the upper triangular matrix

$$S_1 = \begin{array}{c} \\ r_{2n+1} \\ r_{2n} \\ r_{2n-1} \\ r_{2n-2} \\ r_{2n-3} \\ \vdots \\ r_2 \\ r_1 \\ q_1 \end{array} \begin{array}{c} \begin{array}{cccccccccc} 1 & -1 & 0 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 1 & -1 & 0 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 1 & -1 & 0 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 1 & -1 & 0 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 1 & -1 & & 0 & 0 & 0 \end{array} \\ \begin{array}{cccccccccc} & & & & & & \ddots & & & \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 1 & -1 & 0 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 1 & -1 \end{array} \\ \begin{array}{cccccccccc} 0 & 0 & 0 & 0 & 0 & 0 & & 0 & 0 & 1 \end{array} \end{array} . \tag{4.107}$$

This is clearly invertible, and the set of its rows $U_1 = \{q_1,\ r_1,\ \ldots,\ r_{2n+1}\}$ forms a basis

for its rowspace $\text{span}(U_1) = \mathbb{R}^{2n+2}$. From Lemma 4, we have $q_1 \in \text{span}(\{q_2, r_1, \ldots, r_{2n+1}\})$, giving us that $U_2 = \{q_2, r_1, \ldots, r_{2n+1}\}$ is also a basis for $\mathbb{R}^{2n+2}$. Thus the rows of the square matrix S form a basis for its rowspace, implying that S is invertible. □