MATCH
Communications in Mathematical and in Computer Chemistry

MATCH Commun. Math. Comput. Chem. **91** (2024) 337–365
ISSN: 0340–6253
doi: 10.46793/match.91-2.337V

Derivation of Steady State Parametrizations of Chemical Reaction Networks with n Independent and Identical Subnetworks

Kean Arkhei M. Villareal[a], Bryan S. Hernandez[a,*], Patrick Vincent N. Lubenia[b]

[a] *Institute of Mathematics, University of the Philippines Diliman, Quezon City 1101, Philippines*

[b] *Systems and Computational Biology Research Unit, Center for Natural Sciences and Environmental Research, Manila 0922, Philippines*

kmvillareal@up.edu.ph, bryan.hernandez@upd.edu.ph, pnlubenia@upd.edu.ph

(Received July 15, 2023)

Abstract

The long-term behavior of a chemical reaction network (CRN) is usually described by steady states. Recently, Hernandez et al. provided a method and a computational package for deriving positive steady states of CRNs via the concept of network decomposition. In particular, a given CRN is decomposed into stoichiometrically independent subnetworks; then, positive steady state parametrizations of these subnetworks are derived individually and merged to obtain a positive steady state parametrization of the given network. However, the framework applies to a fixed number of subnetworks. In this work, we establish a systematic approach to solving steady state parametrizations of CRNs that can be decomposed into n stoichiometrically independent and structurally identical subnetworks, where $n \geq 2$ is any positive integer. Specifically, we apply the method to the n-site processive phosphorylation/dephosphorylation model. That is, we compute the positive steady state parametrization for the case when $n = 2$ via the concept of network decomposition using the result of parametrizing positive steady states of

*Corresponding author.

the network when $n = 1$. Then, we generalize the parametrization for any positive integer $n \geq 2$ via the principle of mathematical induction.

1 Introduction

Steady states usually describe the long-term behaviors of chemical (or biochemical) systems. In recent years, network-based approaches to deriving parametrizations of positive steady states have received much attention [11–13]. In 2023, Hernandez et al. [9] proposed a novel framework for deriving positive steady state parametrizations via the concept of network decomposition. That is, the underlying chemical reaction network (CRN) of a chemical system is decomposed into stoichiometrically independent subnetworks (or simply called independent subnetworks).

Martin Feinberg established the result that the set of positive steady states of a CRN coincides with the intersection of the set of positive steady states of its independent subnetworks [5–8]. Thus, after decomposing the CRN into its independent subnetworks, we can consider computing a steady state parametrization of each subnetwork. Finally, the individual parametrizations are merged to obtain a positive steady state parametrization of the given network itself. Importantly, Hernandez et al. [9] provided a computational package, which they called COMPILES (COMPutIng anaLytic stEady States), to facilitate this computation.

However, the mentioned framework applies to a fixed number of subnetworks. In this work, we provide an approach to solving parametrizations of positive steady states of CRNs, under the assumption of mass-action rate laws, that can be decomposed into n stoichiometrically independent and yet structurally identical subnetworks, where $n \geq 2$ is any positive integer. Some CRNs that satisfy such properties are the following:

1. the reversible network considered in [14]: $S_0 \rightleftarrows S_1 \rightleftarrows \ldots \rightleftarrows S_n$,
2. a modified version of the previous network with a catalyst K based on [1]: $S_0 + K \rightleftarrows S_1 + K \rightleftarrows \ldots \rightleftarrows S_n + K$, and

3. the n-site processive phosphorylation/dephosphorylation (PD) network [2]:

$$\begin{array}{c} S_0 + K \rightleftarrows S_0K \to S_1 + K \rightleftarrows S_1K \to S_2 + K \rightleftarrows ... \to S_n + K \\ S_n + F \rightleftarrows ... \to S_2 + F \rightleftarrows S_2F \to S_1 + F \rightleftarrows S_1F \to S_0 + F \end{array}.$$

In this work, we focus on illustrating the method of parametrization of positive steady states for the third network, that is, the n-site PD network. We start describing the 1-site PD network (i.e., the n-site PD network when $n = 1$) as follows:

$$\begin{array}{c} S_0 + K \rightleftarrows S_0K \to S_1 + K \\ S_1 + F \rightleftarrows S_1F \to S_0 + F \end{array}.$$

Here, substrate S_0 binds with an enzyme K (or kinase) forming a substrate enzyme complex S_0K (a reversible reaction), which produces a modified substrate S_1 from which the catalyst K dissociates. Moreover, substrate S_0 can be returned by another enzyme F (or phosphatase). This happens when the modified substrate S_1 binds to F to produce the complex S_1F (a reversible reaction). After which, this complex S_1F can return the substrate S_0 with the enzyme F [2]. The CRN associated with this 1-site PD network is also called a *futile cycle*.

Next, the 2-site network is given as follows:

$$\begin{array}{c} S_0 + K \rightleftarrows S_0K \to S_1 + K \rightleftarrows S_1K \to S_2 + K \\ S_2 + F \rightleftarrows S_2F \to S_1 + F \rightleftarrows S_1F \to S_0 + F \end{array}.$$

Here, continuing from the 1-site network, the modified substrate S_1 binds with an enzyme K to form the complex S_1K (a reversible reaction), which then produces another modified substrate S_2 from which the enzyme K dissociates. Furthermore, this modified substrate S_2 binds to the enzyme F to form a complex S_2F (a reversible reaction). This complex S_2F can return the substrate S_1 with the enzyme F.

In general, the following reaction network describes the n-site PD net-

work:

$$S_0 + K \rightleftarrows S_0K \to S_1 + K \rightleftarrows S_1K \to S_2 + K \rightleftarrows ... \to S_n + K$$
$$S_n + F \rightleftarrows ... \to S_2 + F \rightleftarrows S_2F \to S_1 + F \rightleftarrows S_1F \to S_0 + F \quad .$$

There could be many substrates to which the enzymes K and F attach themselves to form or undo a modification. The n-site network, thus, illustrates the mechanisms of the enzymes and several substrates.

The larger the network, the more challenging it is to parametrize its set of steady state solution. For example, the n-site PD network could have varying sizes since n is arbitrary. In effect, it would be difficult to derive the steady states of the n-site PD for each value of n. To overcome this difficulty, in this work, we compute the positive steady state parametrization for the case when $n = 2$ via network decomposition using the result when $n = 1$. Then, we generalize the parametrization for any $n \geq 2$ using the principle of mathematical induction.

2 Preliminaries

We start this section with the formal definition of chemical reaction networks as follows.

Definition 1. A *chemical reaction network* (CRN) is a triple of nonempty and finite sets $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ where

a. $\mathcal{S} = \{A_1, A_2, \ldots, A_m\}$ is the set of *species*,

b. $\mathcal{C} = \{B_1, B_2, \ldots, B_n\}$ is the set of *complexes*, which are non-negative linear combinations of the species, and

c. $\mathcal{R} = \{R_1, R_2, \ldots, R_k\} \subset \mathcal{C} \times \mathcal{C}$ is the set of *reactions*.

A reaction (y, y') is often denoted as $y \to y'$. Here, the complex y is called a *reactant complex* and the complex y' is called a *product complex*. Furthermore, a *reaction vector* of $y \to y'$ is the difference $y' - y$.

To describe the dynamics of the concentrations of species over time in a chemical system, a CRN is endowed with kinetics. In particular, when

the kinetics is *mass-action*, the rate function of each reaction is proportional to the product of the concentration of the species in its reactant complex. That is, the rate function is a proportionality constant, called *rate constant*, multiplied by the product of each concentration raised to the stoichiometric coefficient of the associated species that occurs in the reactant complex of the associated reaction.

Remark. Throughout this work, we denote the species concentration with the lowercase equivalent of the species notation together with a bar on top. In particular, given the species A_1, F, and A_1F, the species concentrations are indicated by $\overline{a_1}$, $\overline{f}$, and $\overline{a_1 f}$, respectively. The bar was placed on top of the species concentrations to avoid confusion on the multiplication operation, e.g., $\overline{a_1}\,\overline{f}$, which is the product of concentrations of species A_1 F, is evidently different from $\overline{a_1 f}$, which is the concentration of species A_1F as compared to when no bar on top of species concentration is used.

Example 1. Reconsider the 1-site PD network as follows:

$$\begin{array}{l} S_0 + K \rightleftarrows S_0K \to S_1 + K \\ S_1 + F \rightleftarrows S_1F \to S_0 + F \end{array} .$$

In this CRN, there are six species $(K, S_0, S_0K, F, S_1, S_1F)$, six complexes $(S_0+K, S_0K, S_1+K, S_1+F, S_1F, S_0+F)$, and six reactions $(R_1 : S_0+K \to S_0K,\ R_2 : S_0K \to S_0 + K,\ R_3 : S_0K \to S_1 + K,\ R_4 : S_1 + F \to S_1F,\ R_5 : S_1F \to S_1 + F,\ R_6 : S_1F \to S_0 + F)$.

Let r_o $(o = 1, 2, \ldots, 6)$ be the rate constant for each reaction R_o. Let $\overline{k}, \overline{s_0}, \overline{s_0 k}, \overline{f}, \overline{s_1}, \overline{s_1 f}$ be the concentrations of species $K, S_0, S_0K, F, S_1, S_1F$, respectively. To obtain the rate functions, raise each species concentration to its associated stoichiometric coefficient if the species is present in the reactant, and raise it to 0 otherwise, and then multiply them altogether. Next, multiply the rate constant by the previously obtained product. For instance, in the first reaction $R_1 :\ S_0 + K \to S_0K$, only species S_0 and K are present in the reactant complex of R_1, and so only species concentration $\overline{s_0}$ and $\overline{k}$ are raised to 1 while other concentrations are raised to 0. This gives us the rate function $r_1 \overline{k}^1 \overline{s_0}^1 \overline{s_0 k}^0 \overline{f}^0 \overline{s_1}^0 \overline{s_1 f}^0 = r_1 \overline{k}\,\overline{s_0}$ for the reaction

R_1. The complete rate functions are given by:

$$
\begin{aligned}
R_1 &: S_0 + K \to S_0K & r_1\overline{k}^{1}\overline{s_0}^{1}\overline{s_0k}^{0}\overline{f}^{0}\overline{s_1}^{0}\overline{s_1f}^{0} &= r_1\overline{k}\overline{s_0}\\
R_2 &: S_0K \to S_0 + K & r_2\overline{k}^{0}\overline{s_0}^{0}\overline{s_0k}^{1}\overline{f}^{0}\overline{s_1}^{0}\overline{s_1f}^{0} &= r_2\overline{s_0k}\\
R_3 &: S_0K \to S_1 + K & r_3\overline{k}^{0}\overline{s_0}^{0}\overline{s_0k}^{1}\overline{f}^{0}\overline{s_1}^{0}\overline{s_1f}^{0} &= r_3\overline{s_0k}\\
R_4 &: S_1 + F \to S_1F & r_4\overline{k}^{0}\overline{s_0}^{0}\overline{s_0k}^{0}\overline{f}^{1}\overline{s_1}^{1}\overline{s_1f}^{0} &= r_4\overline{f}\overline{s_1}\\
R_5 &: S_1F \to S_1 + F & r_5\overline{k}^{0}\overline{s_0}^{0}\overline{s_0k}^{0}\overline{f}^{0}\overline{s_1}^{0}\overline{s_1f}^{1} &= r_5\overline{s_1f}\\
R_6 &: S_1F \to S_0 + F & r_6\overline{k}^{0}\overline{s_0}^{0}\overline{s_0k}^{0}\overline{f}^{0}\overline{s_1}^{0}\overline{s_1f}^{1} &= r_6\overline{s_1f}.
\end{aligned}
$$

The reaction vectors, written as column vectors, for the network are given as follows:

$$
\begin{aligned}
R_1 &: (S_0K) - (S_0 + K) = -S_0 - K + S_0K = [-1,-1,1,0,0,0]^\top\\
R_2 &: (S_0 + K) - (S_0K) = S_0 + K - S_0K = [1,1,-1,0,0,0]^\top\\
R_3 &: (S_1 + K) - (S_0K) = K - S_0K + S_1 = [0,1,-1,1,0,0]^\top\\
R_4 &: (S_1F) - (S_1 + F) = -S_1 - F + S_1F = [0,0,0,-1,-1,1]^\top\\
R_5 &: (S_1 + F) - (S_1F) = S_1 + F - S_1F = [0,0,0,1,1,-1]^\top\\
R_6 &: (S_0 + F) - (S_1F) = S_0 + F - S_1F = [1,0,0,0,1,-1]^\top.
\end{aligned}
$$

The set of ordinary differential equations (ODEs) is obtained by multiplying the oth rate function to reaction vector (in columns) of the oth reaction. That is, we obtain the following:

$$
\frac{d}{dt}\begin{pmatrix}\overline{k}\\ \overline{s_0}\\ \overline{s_0k}\\ \overline{f}\\ \overline{s_1}\\ \overline{s_1f}\end{pmatrix} = r_1\overline{k}\overline{s_0}\begin{pmatrix}-1\\-1\\1\\0\\0\\0\end{pmatrix} + r_2\overline{s_0k}\begin{pmatrix}1\\1\\-1\\0\\0\\0\end{pmatrix} + r_3\overline{s_0k}\begin{pmatrix}1\\0\\-1\\0\\1\\0\end{pmatrix} +
$$

$$r_4\overline{f}\,\overline{s_1}\begin{pmatrix}0\\0\\0\\-1\\-1\\1\end{pmatrix}+r_5\overline{s_1f}\begin{pmatrix}0\\0\\0\\1\\1\\-1\end{pmatrix}+r_6\overline{s_1f}\begin{pmatrix}0\\1\\0\\1\\0\\-1\end{pmatrix},$$

where $\frac{d\overline{k}}{dt}, \frac{d\overline{s_0}}{dt}, \frac{d\overline{s_0k}}{dt}, \frac{d\overline{f}}{dt}, \frac{d\overline{s_1}}{dt}, \frac{d\overline{s_1f}}{dt}$ are the time derivatives of the concentration functions of species $K, S_0, S_0K, F, S_1, S_1F$, respectively. Then, the set of ODEs is:

$$\frac{d\overline{k}}{dt} = -r_1\overline{k}\,\overline{s_0} + r_2\overline{s_0k} + r_3\overline{s_0k} \tag{1a}$$

$$\frac{d\overline{s_0}}{dt} = -r_1\overline{k}\,\overline{s_0} + r_2\overline{s_0k} + r_6\overline{s_1f} \tag{1b}$$

$$\frac{d\overline{s_0k}}{dt} = r_1\overline{k}\,\overline{s_0} - r_2\overline{s_0k} - r_3\overline{s_0k} \tag{1c}$$

$$\frac{d\overline{f}}{dt} = -r_4\overline{f}\,\overline{s_1} + r_5\overline{s_1f} + r_6\overline{s_1f} \tag{1d}$$

$$\frac{d\overline{s_1}}{dt} = r_3\overline{s_0k} - r_4\overline{f}\,\overline{s_1} + r_5\overline{s_1f} \tag{1e}$$

$$\frac{d\overline{s_1f}}{dt} = r_4\overline{f}\,\overline{s_1} - r_5\overline{s_1f} - r_6\overline{s_1f}. \tag{1f}$$

Definition 2. A *steady state* is a vector of species concentrations that makes all time derivatives equal to zero. A *positive steady state* is a steady state where each concentration is positive.

A CRN can be decomposed into smaller pieces of networks called *subnetworks* [5, 6, 8]. This can be done by partitioning its reaction set into disjoint subsets. For instance, the 1-site CRN $\mathcal{N}$ in Example 1 can be decomposed into two subnetworks N_1 and N_2 where the first subnetwork consists of the reactions R_1, R_2, R_3 and R_4, while the second subnetwork consists of the reactions R_5 and R_6. Hence, it is obvious that there can be several ways of decomposing a given CRN.

If in the case that the rank of the stoichiometric matrix of the whole

network (the *stoichiometric matrix* is the matrix where the columns are the reaction vectors of the reactions in the network) is the sum of the ranks of the stoichiometric matrices of its subnetworks (as individual networks), then the decomposition is *independent* and the subnetworks are called *independent subnetworks*. We can see the importance of such independent decompositions through this result of Martin Feinberg [5, 6].

Theorem 1. (Feinberg Decomposition Theorem) *Let $\mathcal{N}$ be a CRN endowed with kinetics $\mathcal{K}$ and let $\mathcal{N}$ be decomposed into independent subnetworks $N_1,\ N_2, \ldots, N_n$ such that the rate functions of the reactions in $\mathcal{N}$ are also the rate functions of the reactions in the smaller independent subnetworks. Then the set of positive steady states of the whole network is equal to the intersection of the sets of positive steady states of the n independent subnetworks, i.e.,*

$$E = E_1 \cap E_2 \cap \ldots \cap E_n.$$

The following proposition shows that the n-site PD network can be decomposed into n independent and structurally identical subnetworks.

Proposition 2. *(Proposition 3.22 [10]) Let $\mathcal{N}$ be the CRN for the n-site processive phosphorylation/dephosphorylation given by:*

$$\begin{aligned} &S_0 + K \rightleftharpoons S_0K \to S_1 + K \rightleftharpoons S_1K \to S_2 + K \rightleftharpoons \ \ldots \ \to S_n + K \\ &S_n + F \rightleftharpoons \ \ldots \ \ \to S_2 + F \rightleftharpoons S_2F \to \ S_1 + F \rightleftharpoons S_1F \to S_0 + F \end{aligned}$$

Then, the decomposed independent subnetworks of $\mathcal{N}$ is of the form:

$$\begin{aligned} &S_i + K \ \rightleftharpoons \ S_iK \ \to \ S_{i+1} + K \\ &S_{i+1} + F \rightleftharpoons S_{i+1}F \to \ S_i + F, \qquad i = 0, 1, \ldots, n-1. \end{aligned}$$

We will utilize Theorem 1 to solve positive steady states of CRNs that can be decomposed into n independent and structurally identical subnetworks such as the n-site PD network.

3 Results and discussion

This section illustrates how we derive parametrizations of positive steady states of chemical reaction networks with n independent and identical subnetworks. To demonstrate this approach, we consider the n-site PD network. We do this step by step by considering the case when $n = 1$, $n = 2$, then for any positive integer $n \geq 2$. At the end of the section, we propose a general approach to parametrizing positive steady states of chemical reaction networks with n independent and identical subnetworks for any positive integer $n \geq 2$.

3.1 1-site PD network

Proposition 3. *The 1-site PD network given by*

$$S_0 + K \underset{r_2}{\overset{r_1}{\rightleftharpoons}} S_0K \overset{r_3}{\to} S_1 + K$$
$$S_1 + F \underset{r_5}{\overset{r_4}{\rightleftharpoons}} S_1F \overset{r_6}{\to} S_0 + F$$

has a positive steady state parametrization of the following form.

$\overline{s_0k} = \dfrac{r_6\overline{s_1f}}{r_3}$
$\overline{f} = \dfrac{(r_5 + r_6)\overline{s_1f}}{r_4\overline{s_1}}$
$\overline{k} = \dfrac{r_6(r_2 + r_3)\overline{s_1f}}{r_1r_3\overline{s_0}}$
Free parameters: $\overline{s_0},\ \overline{s_1},\ \overline{s_1f} > 0$

Remark. The parametrization was derived through the computational package COMPILES (see Appendix A).

Let τ_1, τ_2, τ_3 be free parameters. We can verify that the steady states are indeed solutions by plugging-in the following obtained parametrized

steady state solution to each of the ODEs in Example 1:

$$\begin{cases} \overline{s_0} = \tau_1,\ \overline{s_1} = \tau_2,\ \overline{s_1 f} = \tau_3 \\ \overline{k} = \dfrac{r_6(r_2 + r_3)\tau_3}{r_1 r_3 \tau_1} \\ \overline{f} = \dfrac{(r_5 + r_6)\tau_3}{r_4 \tau_2} \\ \overline{s_0 k} = \dfrac{r_6 \tau_3}{r_3} \end{cases}.$$

By substituting the required solution in equation 1a, we get

$$\begin{aligned} \frac{d\overline{k}}{dt} &= -r_1 \overline{k}\overline{s_0} + r_2 \overline{s_0 k} + r_3 \overline{s_0 k} \\ &= -\cancel{r_1}\left(\frac{r_6(r_2 + r_3)\tau_3}{\cancel{r_1} r_3 \cancel{\tau_1}}\right)(\cancel{\tau_1}) + r_2\left(\frac{r_6\tau_3}{r_3}\right) + \cancel{r_3}\left(\frac{r_6\tau_3}{\cancel{r_3}}\right) \\ &= -\frac{r_2 r_6 \tau_3}{r_3} - r_6\tau_3 + \frac{r_2 r_6 \tau_3}{r_3} + r_6\tau_3 = 0 \end{aligned}$$

One can verify that each ODE in Example 1 equates to zero when the obtained steady state solutions are substituted. Hence, the obtained parametrized steady states are correct.

3.2 A general observation

The process of obtaining the steady state solution does not make use of the species indices in the method. It is only important to take note the modification done, that is, a reaction when a species $S_{\omega'}$ binds to enzyme K produces the complex $S_{\omega'}K$ and this complex $S_{\omega'}K$ may yield either the initial substrate $S_{\omega'}$ and K or the modified substrate $S_{\omega'+1}$ and K by other reactions. Hence, we come up with the following proposition.

Proposition 4. *Let $\omega \in \mathbb{N}$ and $\omega' = \omega - 1$. By assigning ω for the index 1 of species in the CRN for the 1-site PD network, one gets:*

$$S_{\omega'} + K \underset{r_{6\omega'+2}}{\overset{r_{6\omega'+1}}{\rightleftharpoons}} S_{\omega'}K \overset{r_{6\omega'+3}}{\rightarrow} S_\omega + K$$

$$S_\omega + F \underset{r_{6\omega'+5}}{\overset{r_{6\omega'+4}}{\rightleftharpoons}} S_\omega F \overset{r_{6\omega'+6}}{\rightarrow} S_{\omega'} + F.$$

Let the CRN above be denoted by N_ω. Then, N_ω has the following steady state solution:

$\overline{s_{\omega'}k} = \dfrac{r_{6\omega'+6}\overline{s_\omega f}}{r_{6\omega'+3}}$
$\overline{f} = \dfrac{(r_{6\omega'+5}+r_{6\omega'+6})\overline{s_\omega f}}{r_{6\omega'+4}\overline{s_\omega}}$
$\overline{k} = \dfrac{r_{6\omega'+6}(r_{6\omega'+2}+r_{6\omega'+3})\overline{s_\omega f}}{r_{6\omega'+1}r_{6\omega'+3}\overline{s_{\omega'}}}$
Free parameters: $\overline{s_{\omega'}},\ \overline{s_\omega},\ \overline{s_\omega f} > 0$

3.3 2-site PD network

We can extend the 1-site PD network when the modified substrate S_1 binds to K to produce a complex S_1K. This complex S_1K may produce the modified substrate S_1 and enzyme K or another modified substrate S_2 and enzyme K. The undoing of the modification follows with the enzyme F. This extension can be illustrated by the 2-site PD network.

Proposition 5. *The CRN for the 2-site PD network given by*

$$S_0 + K \underset{r_2}{\overset{r_1}{\rightleftharpoons}} S_0K \overset{r_3}{\to} S_1 + K \underset{r_8}{\overset{r_7}{\rightleftharpoons}} S_1K \overset{r_9}{\to} S_2 + K$$
$$S_2 + F \underset{r_{11}}{\overset{r_{10}}{\rightleftharpoons}} S_2F \overset{r_{12}}{\to} S_1 + F \underset{r_5}{\overset{r_4}{\rightleftharpoons}} S_1F \overset{r_6}{\to} S_0 + F$$

has a steady state parametrization as follows:

$\overline{s_0k} = \dfrac{r_6\overline{s_1f}}{r_3}$
$\overline{s_1k} = \dfrac{r_{12}\overline{s_2f}}{r_9}$
$\overline{s_0} = \dfrac{r_6r_7r_9r_{10}(r_2+r_3)(r_5+r_6)\overline{s_1f}^2\overline{s_2}}{r_1r_3r_4r_{12}(r_8+r_9)(r_{11}+r_{12})\overline{s_2f}^2}$
$\overline{s_1} = \dfrac{r_{10}(r_5+r_6)\overline{s_1f}\,\overline{s_2}}{r_4(r_{11}+r_{12})\overline{s_2f}}$

$\overline{f} = \dfrac{(r_{11}+r_{12})\overline{s_2 f}}{r_9}$
$\overline{k} = \dfrac{r_2 r_4 (r_8+r_9)(r_{11}+r_{12})\overline{s_2 f}^2}{r_7 r_9 r_{10}(r_5+r_6)\overline{s_1 f}\,\overline{s_2}}$
Free parameters: $\overline{s_2},\ \overline{s_1 f},\ \overline{s_2 f} > 0$

We denote the 2-site PD network by $\mathcal{N}_2$. By Proposition 2, the CRN (Figure 1, upper) can be decomposed into two independent subnetworks N_1 and N_2 (Figure 1, lower).

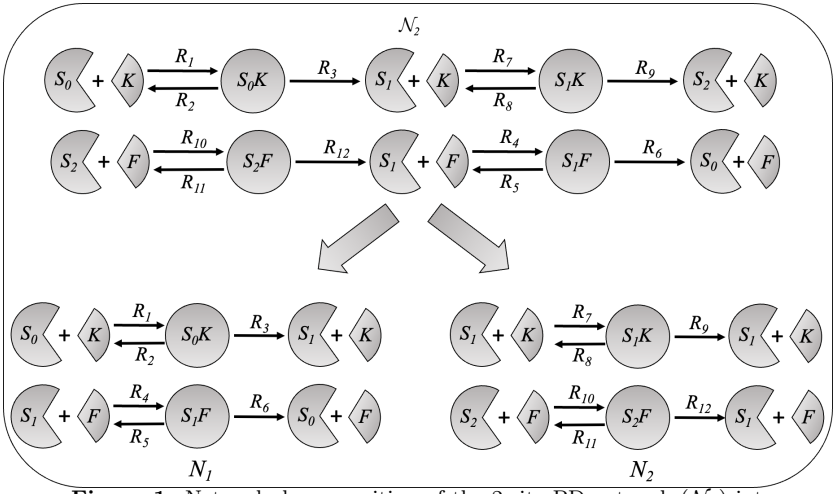

Figure 1. Network decomposition of the 2-site PD network ($\mathcal{N}_2$) into two independent subnetworks (N_1 and N_2)

Note that the independent subnetwork N_1, which is the same network as $\mathcal{N}_1$ in Proposition 3, given by

$$S_0 + K \underset{r_2}{\overset{r_1}{\rightleftharpoons}} S_0K \xrightarrow{r_3} S_1 + K$$

$$S_1 + F \underset{r_5}{\overset{r_4}{\rightleftharpoons}} S_1F \xrightarrow{r_6} S_0 + F$$

has the steady state solution

$$\begin{cases} \overline{s_0 k} = \dfrac{r_6 \overline{s_1 f}}{r_3} \\ \overline{f} = \dfrac{(r_5 + r_6)\overline{s_1 f}}{r_4 \overline{s_1}} \\ \overline{k} = \dfrac{r_6(r_2 + r_3)\overline{s_1 f}}{r_1 r_3 \overline{s_0}} \\ \text{Free parameters: } \overline{s_0},\ \overline{s_1},\ \overline{s_1 f} > 0 \end{cases}, \tag{2}$$

by the same proposition.

If we take $\omega = 2$ in Proposition 4 so that $\omega' = 1$, we would get N_2. That is, we have the CRN

$$S_1 + K \underset{r_{6(1)+2}}{\overset{r_{6(1)+1}}{\rightleftharpoons}} S_1 K \overset{r_{6(1)+3}}{\to} S_2 + K$$
$$S_2 + F \underset{r_{6(1)+5}}{\overset{r_{6(1)+4}}{\rightleftharpoons}} S_2 F \overset{r_{6(1)+6}}{\to} S_1 + F.$$

Hence, the steady state solution of N_2 is:

$$\begin{cases} \overline{s_1 k} = \dfrac{r_{12} \overline{s_2 f}}{r_9} \\ \overline{f} = \dfrac{(r_{11} + r_{12})\overline{s_2 f}}{r_{10} \overline{s_2}} \\ \overline{k} = \dfrac{r_{12}(r_8 + r_9)\overline{s_2 f}}{r_7 r_9 \overline{s_1}} \\ \text{Free parameters: } \overline{s_1},\ \overline{s_2},\ \overline{s_2 f} > 0 \end{cases}. \tag{3}$$

By Theorem 1, the steady state solution of $\mathcal{N}_2$ is the intersection of the steady state solution of N_1 and the steady state solution of N_2. Let $\overline{s_2 f}$ and $\overline{s_2}$ be free parameters. Then, we have the following steady states from N_2:

$$\begin{cases} \overline{s_1 k} = \dfrac{r_{12} \overline{s_2 f}}{r_9} \\ \overline{f} = \dfrac{(r_{11} + r_{12})\overline{s_2 f}}{r_{10} \overline{s_2}} \end{cases}$$

Let $\overline{s_1 f}$ be a free parameter. Then, $\overline{s_0 k} = \dfrac{r_6 \overline{s_1 f}}{r_3}$, from equation 2, is a steady state solution. Now, by equating $\overline{f}$ of equation 2 and equation 3, one obtains

$$\begin{aligned}
\overline{f}_{(N_1)} &= \overline{f}_{(N_2)} \\
\frac{(r_5 + r_6)\overline{s_1 f}}{r_4 \overline{s_1}} &= \frac{(r_{11} + r_{12})\overline{s_2 f}}{r_{10}\overline{s_2}} \\
\overline{s_1} &= \frac{r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}}{r_4(r_{11} + r_{12})\overline{s_2 f}}
\end{aligned}$$

By the same process of equating the steady state solution $\overline{k}$ of equation 2 and equation 3, one gets

$$\overline{s_0} = \frac{r_6 r_7 r_9 r_{10}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^{\,2}\,\overline{s_2}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^{\,2}}.$$

Finally, by substituting the updated solution $\overline{s_1}$ to $\overline{k}$ of equation 3, one obtains

$$\overline{k} = \frac{r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^{\,2}}{r_7 r_9 r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}}$$

Thus, the steady state solution of $\mathcal{N}_2$ is given by

$$\begin{cases}
\overline{s_0 k} = \dfrac{r_6 \overline{s_1 f}}{r_3} \\
\overline{s_1 k} = \dfrac{r_{12}\overline{s_2 f}}{r_9} \\
\overline{s_0} = \dfrac{r_6 r_7 r_9 r_{10}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^{\,2}\,\overline{s_2}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^{\,2}} \\
\overline{s_1} = \dfrac{r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}}{r_4(r_{11} + r_{12})\overline{s_2 f}} \\
\overline{f} = \dfrac{(r_{11} + r_{12})\overline{s_2 f}}{r_{10}\overline{s_2}} \\
\overline{k} = \dfrac{r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^{\,2}}{r_7 r_9 r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}} \\
\text{Free parameters: } \overline{s_2},\ \overline{s_1 f},\ \overline{s_2 f} > 0
\end{cases}.$$

Remark. The parametrization can also be derived through the computational package COMPILES (see Appendix B).

3.4 *n*-site PD network

The 2-site PD network could further be extended up to the nth substrate to obtain the n-site PD.

Theorem 6. *For every $n \in \mathbb{N}$, $n \geq 2$, the network $\mathcal{N}_n$ for the n-site PD network given by:*

$$S_0 + K \rightleftharpoons S_0K \to S_1 + K \rightleftharpoons S_1K \to S_2 + K \rightleftharpoons \ \ldots \ \to S_n + K$$
$$S_n + F \rightleftharpoons \ \ldots \ \to S_2 + F \rightleftharpoons S_2F \to \ S_1 + F \rightleftharpoons S_1F \to S_0 + F$$

has the steady state solution given by:

$\overline{s_\mu k} = \dfrac{r_{6(\mu+1)}\overline{s_{\mu+1} f}}{r_{6\mu+3}}, \quad \mu = 0, 1, 2, \ldots, n-1$
$\overline{s_0} = \dfrac{r_6 r_7 r_9 r_{6n-2}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^2\,\overline{s_n}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{6n-1} + r_{6n})\overline{s_2 f}\ \overline{s_n f}}$
$\overline{s_\epsilon} = \dfrac{r_{6n-2}(r_{6\epsilon-1} + r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_n}}{r_{6\epsilon-2}(r_{6n-1} + r_{6n})\overline{s_n f}}, \quad \epsilon = 1, 2, \ldots, n-1$
$\overline{f} = \dfrac{(r_{6n-1} + r_{6n})\overline{s_n f}}{r_{6n-2}\overline{s_n}}$
$\overline{k} = \dfrac{r_{6n-8} r_{6n}(r_{6n-4} + r_{6n-3})(r_{6n-1} + r_{6n})\overline{s_n f}^2}{r_{6n-5} r_{6n-3} r_{6n-2}(r_{6n-7} + r_{6n-6})\overline{s_{n-1} f}\,\overline{s_n}}$
Free parameters: $\overline{s_1 f}, \overline{s_2 f}, \ldots, \overline{s_n f}, \overline{s_n} > 0$

Proof. We prove by induction. Let $n \in \mathbb{N}$, $n \geq 2$. Let the network $\mathcal{N}_n$ be the CRN for the n-site PD given by:

$$S_0 + K \rightleftharpoons S_0K \to S_1 + K \rightleftharpoons S_1K \to S_2 + K \rightleftharpoons \ \ldots \ \to S_n + K$$
$$S_n + F \rightleftharpoons \ \ldots \ \to S_2 + F \rightleftharpoons S_2F \to \ S_1 + F \rightleftharpoons S_1F \to S_0 + F$$

Base Step. ($n = 2$) We have obtained in Proposition 5 that the steady

state solution of the 2-site PD is given by:

$$\begin{cases} \overline{s_0 k} = \dfrac{r_6 \overline{s_1 f}}{r_3} \\ \overline{s_1 k} = \dfrac{r_{12} s_2 f}{r_9} \\ \overline{s_0} = \dfrac{r_6 r_7 r_9 r_{10}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^2 \overline{s_2}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^2} \\ \overline{s_1} = \dfrac{r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}}{r_4(r_{11} + r_{12})\overline{s_2 f}} \\ \overline{f} = \dfrac{(r_{11} + r_{12})\overline{s_2 f}}{r_{10}\overline{s_2}} \\ \overline{k} = \dfrac{r_4 r_{12}(r_8 + r_9)(r_{11} + r_{12})\overline{s_2 f}^2}{r_7 r_9 r_{10}(r_5 + r_6)\overline{s_1 f}\,\overline{s_2}} \\ \text{Free parameters: } \overline{s_2},\ \overline{s_1 f},\ \overline{s_2 f} > 0. \end{cases} \tag{4}$$

Observe that equation 4 can be written as:

$$\begin{cases} \overline{s_\mu k} = \dfrac{r_{6(\mu+1)}\overline{s_{\mu+1} f}}{r_{6\mu+3}}, \quad \mu = \mathbf{0}, \mathbf{1} \\ \overline{s_0} = \dfrac{r_6 r_7 r_9 r_{6(\mathbf{2})-2}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^2 \overline{s_2}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{6(\mathbf{2})-1} + r_{6(\mathbf{2})})\overline{s_2 f}\ \overline{s_{\mathbf{2}} f}} \\ \overline{s_\epsilon} = \dfrac{r_{6(\mathbf{2})-2}(r_{6\epsilon-1} + r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_{\mathbf{2}}}}{r_{6\epsilon-2}(r_{6(\mathbf{2})-1} + r_{6(\mathbf{2})})\overline{s_{\mathbf{2}} f}}, \quad \epsilon = \mathbf{1} \\ \overline{f} = \dfrac{(r_{6(\mathbf{2})-1} + r_{6(\mathbf{2})})\overline{s_{\mathbf{2}} f}}{r_{6(\mathbf{2})-2}\overline{s_{\mathbf{2}}}} \\ \overline{k} = \dfrac{r_{6(\mathbf{2})-8} r_{6(\mathbf{2})}(r_{6(\mathbf{2})-4} + r_{6(\mathbf{2})-3})(r_{6(\mathbf{2})-1} + r_{6(\mathbf{2})})\overline{s_{\mathbf{2}} f}^2}{r_{6(\mathbf{2})-5} r_{6(\mathbf{2})-3} r_{6(\mathbf{2})-2}(r_{6(\mathbf{2})-7} + r_{6(\mathbf{2})-6})\overline{s_{\mathbf{2}-1} f}\,\overline{s_{\mathbf{2}}}} \\ \text{Free parameters: } \overline{s_1 f}, \overline{s_2 f}, \overline{s_2} > 0. \end{cases}$$

The theorem holds for $n = 2$.

Inductive Step. Assume that for $n = m$ such that $n > 2$, the theorem holds, i.e., the steady state solution for the m-site PD is given by

$$E'_m = \begin{cases} \overline{s_\mu k} = \dfrac{r_{6(\mu+1)}\overline{s_{\mu+1} f}}{r_{6\mu+3}}, \quad \mu = 0, 1, 2, \ldots, m-1 \\ \overline{s_0} = \dfrac{r_6 r_7 r_9 r_{6m-2}(r_2 + r_3)(r_5 + r_6)\overline{s_1 f}^2 \overline{s_m}}{r_1 r_3 r_4 r_{12}(r_8 + r_9)(r_{6m-1} + r_{6m})\overline{s_2 f}\ \overline{s_m f}} \\ \overline{s_\epsilon} = \dfrac{r_{6m-2}(r_{6\epsilon-1} + r_{6\epsilon})s_\epsilon \overline{f s_m}}{r_{6\epsilon-2}(r_{6m-1} + r_{6m})\overline{s_m f}}, \quad \epsilon = 1, 2, \ldots, m-1 \\ \overline{f} = \dfrac{(r_{6m-1} + r_{6m})s_m f}{r_{6m-2}\overline{s_m}} \\ \overline{k} = \dfrac{r_{6m-8} r_{6m}(r_{6m-4} + r_{6m-3})(r_{6m-1} + r_{6m})\overline{s_m f}^2}{r_{6m-5} r_{6m-3} r_{6m-2}(r_{6m-7} + r_{6m-6})\overline{s_{m-1} f s_m}} \\ \text{Free parameters: } \overline{s_1 f}, \overline{s_2 f}, \ldots, \overline{s_m f}, \overline{s_m} > 0. \end{cases} \tag{5}$$

Note that the decomposed independent subnetworks N_{i+1} of the m-site PD, by Proposition 2, is given by:

$$\begin{aligned} S_i + K &\rightleftharpoons S_i K \rightarrow S_{i+1} + K \\ S_{i+1} + F &\rightleftharpoons S_{i+1} F \rightarrow S_i + F, \qquad i = 0, 1, \ldots, m-1. \end{aligned}$$

Now, by Theorem 1, the steady state solution E'_m of the CRN for the m-site PD network, is obtained by taking the intersection of the steady state solution of each decomposed independent subnetwork, i.e.,

$$E'_m = \bigcap_{i=1}^{m-1} E_{i+1}, \quad i = 0, 1, \ldots, m-1,$$

where E_{i+1} is the steady state solution of the subnetwork N_{i+1}.

Following the same argument, the steady state solution E'_{m+1} of the network $\mathcal{N}_{m+1}$ is derived by taking the intersection

$$E'_m \cap E_{m+1},$$

where E_{m+1} is the steady state solution of the subnetwork N_{m+1}. Observe

that the network N_{m+1} is given by

$$S_m + K \underset{r_{6m+2}}{\overset{r_{6m+1}}{\rightleftharpoons}} S_m K \overset{r_{6m+3}}{\rightarrow} S_{m+1} + K$$
$$S_{m+1} + F \underset{r_{6m+5}}{\overset{r_{6m+4}}{\rightleftharpoons}} S_{m+1} F \overset{r_{6m+6}}{\rightarrow} S_m + F.$$

which, by Proposition 4, has the steady state solution

$$\begin{cases} \overline{s_m k} = \dfrac{r_{6m+6}\overline{s_{m+1} f}}{r_{6m+3}} \\ \overline{f} = \dfrac{(r_{6m+5} + r_{6m+6})\overline{s_{m+1} f}}{r_{6m+4}\overline{s_{m+1}}} \\ \overline{k} = \dfrac{r_{6m+6}(r_{6m+2} + r_{6m+3})\overline{s_{m+1} f}}{r_{6m+1} r_{6m+3}\overline{s_m}} \\ \text{Free parameters: } \overline{s_m},\ \overline{s_{m+1}},\ \overline{s_{m+1} f} > 0. \end{cases} \tag{6}$$

Let $\overline{s_1 f}, \overline{s_2 f}, \ldots, \overline{s_{m+1} f}, \overline{s_{m+1}}$ be free parameters. Then, the steady states in equation 5 in terms of the free parameters can be obtained:

$$\overline{s_\mu k} = \frac{r_{6(\mu+1)}\overline{s_{\mu+1} f}}{r_{6\mu+3}}, \quad \mu = 0, 1, 2, \ldots, m-1.$$

Similarly, from the steady states in equation 6, we can get:

$$\begin{cases} \overline{s_m k} = \dfrac{r_{6m+6}\overline{s_{m+1} f}}{r_{6m+3}} = \dfrac{r_{6(m+1)}\overline{s_{m+1} f}}{r_{6m+3}} \\ \overline{f} = \dfrac{(r_{6m+5} + r_{6m+6})\overline{s_{m+1} f}}{r_{6m+4}\overline{s_{m+1}}} \end{cases}$$

By equating $\overline{f}$ of equation 5 to the $\overline{f}$ of equation 6, we can derive $\overline{s_m}$, that is,

$$\begin{aligned} \frac{(r_{6m-1} + r_{6m})\overline{s_m f}}{r_{6m-2}\overline{s_m}} &= \frac{(r_{6m+5} + r_{6m+6})\overline{s_{m+1} f}}{r_{6m+4}\overline{s_{m+1}}} \\ \frac{r_{6m-2}\overline{s_m}}{(r_{6m-1} + r_{6m})\overline{s_m f}} &= \frac{r_{6m+4}\overline{s_{m+1}}}{(r_{6m+5} + r_{6m+6})\overline{s_{m+1} f}} \end{aligned}$$

$$\begin{aligned}
\overline{s_m} &= \frac{\left[r_{6m+4}\overline{s_{m+1}}\right]\left[(r_{6m-1}+r_{6m})\overline{s_m f}\right]}{\left[(r_{6m+5}+r_{6m+6})\overline{s_{m+1}f}\right]\left[r_{6m-2}\right]} \\
\overline{s_m} &= \frac{r_{6m+4}(r_{6m-1}+r_{6m})\overline{s_m f}\,\overline{s_{m+1}}}{r_{6m-2}(r_{6m+5}+r_{6m+6})\overline{s_{m+1}f}} \\
\overline{s_m} &= \frac{r_{6(m+1)-2}(r_{6m-1}+r_{6m})\overline{s_m f}\,\overline{s_{m+1}}}{r_{6m-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}}.
\end{aligned}$$

By substituting this newly obtained parametrization for $\overline{s_m}$ to $\overline{s_\epsilon}$, $\epsilon = 1, 2, \ldots, m-1$, of equation 5, we get:

$$\begin{aligned}
\overline{s_\epsilon} &= \frac{r_{6m-2}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_m}}{r_{6\epsilon-2}(r_{6m-1}+r_{6m})\overline{s_m f}} \\
&= \frac{r_{6m-2}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}}{r_{6\epsilon-2}(r_{6m-1}+r_{6m})\overline{s_m f}} \cdot \frac{r_{6(m+1)-2}(r_{6m-1}+r_{6m})\overline{s_m f}\,\overline{s_{m+1}}}{r_{6m-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}} \\
&= \frac{\cancel{r_{6m-2}}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}}{r_{6\epsilon-2}\cancel{(r_{6m-1}+r_{6m})}\overline{\cancel{s_m f}}} \cdot \frac{r_{6(m+1)-2}\cancel{(r_{6m-1}+r_{6m})}\overline{\cancel{s_m f}}\,\overline{s_{m+1}}}{\cancel{r_{6m-2}}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}} \\
&= \frac{r_{6(m+1)-2}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_{m+1}}}{r_{6\epsilon-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}}.
\end{aligned}$$

Plugging-in the same $\overline{s_m}$ to $\overline{s_0}$ of equation 5, we get:

$$\begin{aligned}
\overline{s_0} &= \frac{r_6 r_7 r_9 r_{6m-2}(r_2+r_3)(r_5+r_6)\overline{s_1 f}^2\,\overline{s_m}}{r_1 r_3 r_4 r_{12}(r_8+r_9)(r_{6m-1}+r_{6m})\overline{s_2 f}\;\overline{s_m f}} \\
&= \frac{r_6 r_7 r_9 \cancel{r_{6m-2}}(r_2+r_3)(r_5+r_6)\overline{s_1 f}^2}{r_1 r_3 r_4 r_{12}(r_8+r_9)\cancel{(r_{6m-1}+r_{6m})}\overline{s_2 f}\;\overline{\cancel{s_m f}}} \\
&\cdot \frac{r_{6(m+1)-2}\cancel{(r_{6m-1}+r_{6m})}\overline{\cancel{s_m f}}\,\overline{s_{m+1}}}{\cancel{r_{6m-2}}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}} \\
&= \frac{r_6 r_7 r_9 r_{6(m+1)-2}(r_2+r_3)(r_5+r_6)\overline{s_1 f}^2\,\overline{s_{m+1}}}{r_1 r_3 r_4 r_{12}(r_8+r_9)(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_2 f}\;\overline{s_{m+1}f}}.
\end{aligned}$$

Finally, by substituting the same $\overline{s_m}$ to $\overline{k}$ of the steady state solution in equation 6, we obtain:

$$\begin{aligned}
\overline{k} &= \frac{r_{6m+6}(r_{6m+2}+r_{6m+3})\overline{s_{m+1}f}}{r_{6m+1}r_{6m+3}\overline{s_m}} \\
&= \frac{r_{6m+6}(r_{6m+2}+r_{6m+3})\overline{s_{m+1}f}}{r_{6m+1}r_{6m+3}} \cdot \frac{r_{6m-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}}{r_{6(m+1)-2}(r_{6m-1}+r_{6m})\overline{s_m f}\,\overline{s_{m+1}}} \\
&= \frac{r_{6(m+1)-8}r_{6(m+1)}(r_{6(m+1)-4}+r_{6(m+1)-3})(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}^2}{r_{6(m+1)-5}r_{6(m+1)-3}r_{6(m+1)-2}(r_{6(m+1)-7}+r_{6(m+1)-6})\overline{s_{(m+1)-1}f}\,\overline{s_{m+1}}}.
\end{aligned}$$

Collecting these derived steady state solution for $\mathcal{N}_{m+1}$, we have:

$$\begin{cases}
\overline{s_\mu k} = \dfrac{r_{6(\mu+1)}\overline{s_{\mu+1}f}}{r_{6\mu+3}}, \quad \mu = 0,1,2,\ldots,m-1 \\
\overline{s_m k} = \dfrac{r_{6(m+1)}\overline{s_{m+1}f}}{r_{6m+3}} \\
\overline{s_0} = \dfrac{r_6 r_7 r_9 r_{6(m+1)-2}(r_2+r_3)(r_5+r_6)\overline{s_1 f}^2\,\overline{s_{m+1}}}{r_1 r_3 r_4 r_{12}(r_8+r_9)(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_2 f}\;\overline{s_{m+1}f}} \\
\overline{s_\epsilon} = \dfrac{r_{6(m+1)-2}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_{m+1}}}{r_{6\epsilon-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}}, \quad \epsilon = 1,2,\ldots,m-1 \\
\overline{s_m} = \dfrac{r_{6(m+1)-2}(r_{6m-1}+r_{6m})\overline{s_m f}\,\overline{s_{m+1}}}{r_{6m-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}} \\
\overline{f} = \dfrac{(r_{6m+5}+r_{6m+6})\overline{s_{m+1}f}}{r_{6m+4}\overline{s_{m+1}}} \\
\overline{k} = \dfrac{r_{6(m+1)-8}r_{6(m+1)}(r_{6(m+1)-4}+r_{6(m+1)-3})(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}^2}{r_{6(m+1)-5}r_{6(m+1)-3}r_{6(m+1)-2}(r_{6(m+1)-7}+r_{6(m+1)-6})\overline{s_{(m+1)-1}f}\,\overline{s_{m+1}}} \\
\text{Free parameters: } \overline{s_1 f}, \overline{s_2 f}, \ldots, \overline{s_{m+1}f}, \overline{s_{m+1}} > 0,
\end{cases}$$

or by merging some of the solution such as the first two ($\overline{s_\mu k}$ for $\mu = 0,1,2,\ldots,m-1$ and $\overline{s_m k}$), and the fourth and the fifth ($\overline{s_\epsilon}$ for $\epsilon = 1,2,\ldots,m-1$ and $\overline{s_m}$), we can group them as follows:

$$\begin{cases}
\overline{s_\mu k} = \dfrac{r_{6(\mu+1)}\overline{s_{\mu+1}f}}{r_{6\mu+3}}, \quad \mu = 0,1,2,\ldots,m \\
\overline{s_0} = \dfrac{r_6 r_7 r_9 r_{6(m+1)-2}(r_2+r_3)(r_5+r_6)\overline{s_1 f}^2\,\overline{s_{m+1}}}{r_1 r_3 r_4 r_{12}(r_8+r_9)(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_2 f}\;\overline{s_{m+1}f}} \\
\overline{s_\epsilon} = \dfrac{r_{6(m+1)-2}(r_{6\epsilon-1}+r_{6\epsilon})\overline{s_\epsilon f}\,\overline{s_{m+1}}}{r_{6\epsilon-2}(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}}, \quad \epsilon = 1,2,\ldots,m \\
\overline{f} = \dfrac{(r_{6m+5}+r_{6m+6})\overline{s_{m+1}f}}{r_{6m+4}\overline{s_{m+1}}} \\
\overline{k} = \dfrac{r_{6(m+1)-8}r_{6(m+1)}(r_{6(m+1)-4}+r_{6(m+1)-3})(r_{6(m+1)-1}+r_{6(m+1)})\overline{s_{m+1}f}^2}{r_{6(m+1)-5}r_{6(m+1)-3}r_{6(m+1)-2}(r_{6(m+1)-7}+r_{6(m+1)-6})\overline{s_{(m+1)-1}f}\,\overline{s_{m+1}}} \\
\text{Free parameters: } \overline{s_1 f}, \overline{s_2 f}, \ldots, \overline{s_{m+1}f}, \overline{s_{m+1}} > 0,
\end{cases}$$

as required. This means that the theorem is true for $(m+1)$-site PD.

Therefore, the theorem holds for all $n \in \mathbb{N}$, $n \geq 2$. ■

3.5 Derivation of positive steady state parametrization of CRNs with n independent and identical subnetworks

We are now in the position to introduce the following steps (S1-S5) of deriving positive steady state parametrization of CRNs, under the assumption of mass-action kinetics, with n independent and identical subnetworks:

S1. Decompose the CRN into n independent and identical subnetworks.

S2. Derive the positive steady state parametrization of the network for the case when $n = 1$ (Section 3.1).

S3. Generalize a formula for a steady state parametrization for any subnetwork using the network in the previous step (S2) (Section 3.2).

S4. Derive a positive steady state parametrization of the network for the case when $n = 2$ by invoking Theorem 1, i.e., getting the intersection of the two parametrizations (coming from the two subnetworks) obtained using the previous step (S3) (Section 3.3).

S5. Apply the principle of mathematical induction with $n = 2$ as the basis step (Section 3.4).

4 Summary and recommendation

In this work, we introduce a framework to derive parametrizations of CRNs, endowed with mass-action kinetics, that can be decomposed into n independent and structurally identical subnetworks where $n \geq 2$ is any positive integer. The approach was illustrated to derive a generalization for the steady state solution of the n-site PD. First, a steady state parametrization of the CRN for the 1-site PD was obtained. Then, we compute the positive steady state parametrization for the case when $n = 2$ via network decomposition (i.e., Theorem 1) using the result when $n = 1$.

Then, we generalize the parametrization for any $n \geq 2$ using the principle of mathematical induction.

Studies on CRN theory (CRNT) often include critical biological properties such as absolute concentration robustness and multistationarity [3, 4, 13, 15]. Parametrizations of positive steady states can be used to facilitate checking these properties. Future studies may include the use of conservation laws in the system after computing the parametrization. One can also look into different types of networks with varying sizes.

Appendix A A parametrization of the positive steady states of the 1-site PD network

In this section, we provide a parametrization of the 1-site PD via the computational package COMPILES (COMPutIng anaLytic stEady States) developed in [9], which is built in MATLAB. It derives a steady state parametrization of a network by decomposing the CRN into independent subnetworks and combines parametrizations of each subnetwork. Here, we present the COMPILES package with the corresponding MATLAB code that we have used to obtain the parametrization in Section 3.1.

Input

1. Gather the m-files **steadyState.m**, **addReaction.m**, **edge.m**, **graph.m**, and **vertex.m** in the same working directory. In the same directory, create another m-file on which the script would be put.

2. In the created m-file, write the model name as the **model.id**. In our code, we used

   ```
   model.id = 'for n=1';
   ```

   for the 1-site PD.

3. List all the reactions. The string in the first line is the visual representation of the reaction. It may be reversible or not, and we use the signs $<$ and $>$ to denote the direction of the reaction. The second line consists of the reactant species (in strings), their respective stoichiometric coefficients (in the cell list), and their respective kinetic order (in the array list). The third line consists of product species (in strings), their respective stoichiometry (in cell list), and their kinetic order if reversible (in array list), which is otherwise empty. The fourth line contains the word *true* (false) if the reaction is reversible (not reversible). In our code, we wrote the first reaction as

```
model = addReaction(model, 'S0+K<->S0K', ...
                           {'S0', 'K'}, {1, 1}, [1, 1], ...
                           {'S0K'}, {1}, [1], ...
                           true);
```

   Do this for all reactions in the CRN.

4. Add the line

```
[equation, species, free_parameter, conservation_law,
    model] = steadyState(model);
```

   for the computation of the parametrized steady state.

5. Run the script file.

Code (used on the script file)

```
model.id = 'for n=1';
model = addReaction(model, 'S0+K<->S0K', ...
                           {'S0', 'K'}, {1, 1}, [1, 1], ...
                           {'S0K'}, {1}, [1], ...
                           true);
model = addReaction(model, 'S0K->S1+K', ...
                           {'S0K'}, {1}, [1], ...
                           {'S1', 'K'}, {1, 1}, [ ], ...
                           false);
model = addReaction(model, 'S1+F<->S1F', ...
                           {'S1', 'F'}, {1, 1}, [1, 1], ...
                           {'S1F'}, {1}, [1], ...
                           true);
```

```
model = addReaction(model, 'S1F->S0+F', ...
                           {'S1F'}, {1}, [1], ...
                           {'S0', 'F'}, {1, 1}, [ ], ...
                           false);

[equation, species, free_parameter, conservation_law, model]
= steadyState(model);
```

Output

1. As an output, we would be given the number of independent decomposition of the CRN.
2. For each of these subnetworks, the reactions associated with it are displayed.
3. Each species in the subnetwork are listed with their corresponding steady states parametrization.
4. After the steady state parametrization for all subnetworks are shown, the final solution by combining these solutions of the subnetworks is presented.
5. The conservation laws for the CRN are also shown.

```
The network has no nontrival independent decomposition.

- Network -

R1: S0+K->S0K
R2: S0K->S0+K
R3: S0K->S1+K
R4: S1+F->S1F
R5: S1F->S1+F
R6: S1F->S0+F

Solving the network...

F = (tau3*(k5 + k6))/(k4*tau2)
K = (k6*tau3*(k2 + k3))/(k1*k3*tau1)
S0 = tau1
S0K = (k6*tau3)/k3
S1 = tau2
S1F = tau3
```

```
Solving positive steady state parametrization of the
network...

The solution is:

F = (S1F*(k5 + k6))/(S1*k4)
K = (S1F*k6*(k2 + k3))/(S0*k1*k3)
S0K = (S1F*k6)/k3
Free parameters: S0, S1, S1F

Conservation laws:
dK/dt + dS0K/dt = 0
-dF/dt - dK/dt + dS0/dt + dS1/dt = 0
dF/dt + dS1F/dt = 0
```

Appendix B A parametrization of the positive steady states of the 2-site PD network

This section is an extension of the 1-site PD network. The script file follows the same steps presented in Appendix A.

Input

Code (used on the script file)

```
model.id = 'for n=2';
model = addReaction(model, 'S0+K<->S0K', ...
                           {'S0', 'K'}, {1, 1}, [1, 1], ...
                           {'S0K'}, {1}, [1], ...
                           true);
model = addReaction(model, 'S0K->S1+K', ...
                           {'S0K'}, {1}, [1], ...
                           {'S1', 'K'}, {1, 1}, [ ], ...
                           false);
model = addReaction(model, 'S1+F<->S1F', ...
                           {'S1', 'F'}, {1, 1}, [1, 1], ...
                           {'S1F'}, {1}, [1], ...
                           true);
```

```
model = addReaction(model, 'S1F->S0+F', ...
                           {'S1F'}, {1}, [1], ...
                           {'S0', 'F'}, {1, 1}, [ ], ...
                           false);
model = addReaction(model, 'S1+K<->S1K', ...
                           {'S1', 'K'}, {1, 1}, [1, 1], ...
                           {'S1K'}, {1}, [1], ...
                           true);
model = addReaction(model, 'S1K->S2+K', ...
                           {'S1K'}, {1}, [1], ...
                           {'S2', 'K'}, {1, 1}, [ ], ...
                           false);
model = addReaction(model, 'S2+F<->S2F', ...
                           {'S2', 'F'}, {1, 1}, [1, 1], ...
                           {'S2F'}, {1}, [1], ...
                           true);
model = addReaction(model, 'S2F->S1+F', ...
                           {'S2F'}, {1}, [1], ...
                           {'S1', 'F'}, {1, 1}, [ ], ...
                           false);
[equation, species, free_parameter, conservation_law, model]
= steadyState(model);
```

Output

```
The network has 2 subnetworks.

- Subnetwork 1 -

R1: S0+K->S0K
R2: S0K->S0+K
R3: S0K->S1+K
R4: S1+F->S1F
R5: S1F->S1+F
R6: S1F->S0+F

Solving Subnetwork 1...

F = (tau3*(k5 + k6))/(k4*tau2)
K = (k6*tau3*(k2 + k3))/(k1*k3*tau1)
S0 = tau1
S0K = (k6*tau3)/k3
S1 = tau2
```

```
S1F = tau3

- Subnetwork 2 -

R7: S1+K->S1K
R8: S1K->S1+K
R9: S1K->S2+K
R10: S2+F->S2F
R11: S2F->S2+F
R12: S2F->S1+F

Solving Subnetwork 2...

F = (tau6*(k11 + k12))/(k10*tau5)
K = (k12*tau6*(k8 + k9))/(k7*k9*tau4)
S1 = tau4
S1K = (k12*tau6)/k9
S2 = tau5
S2F = tau6

Solving positive steady state parametrization of the
entire network...

The solution is:

F = (S2F*k11 + S2F*k12)/(S2*k10)
K = (S2F^2*k4*k12*(k8 + k9)*(k11 + k12))/(S2*S1F*k7*k9*k10
*(k5+ k6))
S0 = (S2*S1F^2*k6*k7*k9*k10*(k2 + k3)*(k5 + k6))/(S2F^2*k1
*k3*k4*k12*(k8 + k9)*(k11 + k12))
S1 = (S2*S1F*k5*k10 + S2*S1F*k6*k10)/(S2F*k4*k11 + S2F*k4*
k12)
S0K = (S1F*k6)/k3
S1K = (S2F*k12)/k9
Free parameters: S2, S1F, S2F

Conservation laws:
dK/dt + dS0K/dt + dS1K/dt = 0
-dF/dt - dK/dt + dS0/dt + dS1/dt + dS2/dt = 0
dF/dt + dS1F/dt + dS2F/dt = 0
```

***Acknowledgment*:** KAMV acknowledges the undergraduate scholarship under the *DOST SEI Merit Program* in the Philippines. BSH was supported by the UP System Enhanced Creative Work and Research Grant (ECWRG-2020-2-11R).

References

[1] R. Anguelov, M. Borisov, A. Iliev, N. Kyurkchiev, S. Markov, On the chemical meaning of some growth models possessing Gompertzian–type property, *Math. Methods Appl. Sci.* **41** (2018) 8365–8376.

[2] C. Conradi, A. Shiu, Dynamics of post–translational modification systems: recent results and future directions, *Biophys. J.* **114** (2018) 507–515.

[3] G. Craciun, M. Feinberg, Multiple equilibria in complex chemical reaction networks: II. The species-reaction graph, *SIAM J. Appl. Math.* **66** (2006) 1321–1338.

[4] A. Dickenstein, M. Millán, A. Shiu, X. Tang, Multistationarity in structured reaction networks, *Bull. Math. Biol.* **81** (2019) 1527–1581.

[5] M. Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors I: The deficiency zero and deficiency one theorems, *Chem. Eng. Sci.* **42** (1987) 2229–2268.

[6] M. Feinberg, *Foundations of Chemical Reaction Network Theory*, Springer, 2019.

[7] B. Hernandez, D. Amistas, R. De la Cruz, L. Fontanil, A. de los Reyes V, E. Mendoza, Independent, incidence independent and weakly reversible decompositions of chemical reaction networks, *MATCH Commun. Math. Comput. Chem.* **87** (2022) 367–396.

[8] B. Hernandez, R. De la Cruz, Independent decompositions of chemical reaction networks, *Bull. Math. Biol.* **83** (2021) #76.

[9] B. Hernandez, P. Lubenia, M. Johnston, J. Kim, A framework for deriving analytic steady states of biochemical reaction networks, *PLoS Comput. Biol.* **19** (2023) e1011039.

[10] B. Hernandez, E. Mendoza, A. de los Reyes V, Fundamental decompositions and multistationarity of power–law kinetic systems, *MATCH Commun. Math. Comput. Chem.* **83** (2020) 403–434.

[11] M. Johnston, A computational approach to steady state correspondence of regular and generalized mass action systems, *Bull. Math. Biol.* **77** (2015) 1065–1100.

[12] M. Johnston, E. Burton, Computing weakly reversible deficiency zero network translations using elementary flux modes, *Bull. Math. Biol.* **81** (2019) 1613–1644.

[13] M. Johnston, S. Müller, C. Pantea, A deficiency-based approach to parametrizing positive equilibria of biochemical reaction systems, *Bull. Math. Biol.* **81** (2019) 1143–1172.

[14] S. Rao, A. van der Schaft, K. van Eunen, B. Bakker, B. Jayawardhana, A model reduction method for biochemical reaction networks, *BMC Syst. Biol.* **8** (2014) #52.

[15] G. Shinar, M. Feinberg, Structural sources of robustness in biochemical reaction networks, *Science* **327** (2010) 1389–1391.