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Steady state equivalence among autocatalytic peroxidase-oxidase reactions

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Peroxidase-oxidase is an enzymatic reaction that can exhibit dynamical scenarios such as bistability, sustained oscillations, and Shilnikov chaos. In this work, we apply the chemical reaction network theory approach to find kinetic constants such that the associated mass action kinetics ordinary differential equations induced by three four dimensional structurally different enzymatic reaction systems can support the same steady states for several chemical species despite differences in their chemical nature. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4968554]

I. INTRODUCTION

Peroxidases are enzymes which can be found in all living forms and catalyze the oxidation of several substrates using H_2O_2.[1,2] Nonetheless, peroxidases are capable of using O_2 instead of H_2O_2 as a substrate to promote the oxidation; that is, they might act as oxidases too.[2] Such bivalence is found in biochemistry as the *Peroxidase-Oxidase* (PO) enzymatic reaction, which stands for the oxidation of NADH (reduced nicotinamide adenine dinucleotide) by means of the following global reaction:

$$2\,NADH + O_2 + 2\,H^+ \rightarrow 2\,NAD^. + 2\,H_2O.$$
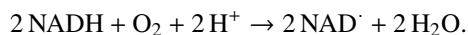
The PO reaction plays an important role in signaling pathways that involve leukocyte dynamics[3,4] and neurodegenerative diseases such as Alzheimer's.[5] From a dynamical point of view, the PO reaction caught the attention of the scientific community due to its wide variety of experimental and theoretical dynamical scenarios such as multiple steady states, complex oscillations, and chaos.[6–13] Among the most detailed models, the Bronnikova, Fed'kina, Schaffer, and Olsen (BFSO) reaction network[14] is capable of displaying all the dynamical features observed in the oxidation of NADH. Due to this fact, the BFSO model was modified by Sensse *et al.*[12] to derive a new model with six chemical species and thirteen reactions, and further decomposed into a core chemical reaction network which contained three chemical species: NAD$^.$ radicals, O_2, and compound III (coIII = Per^{+6}). Three extensions of the core network were developed to include a fourth chemical species: O_2^-, Per^{+2}, or Per^{+3}. The addition of a fourth chemical species to an activation-inhibitor reacting system leads to the emergence of chaotic behaviour provided that certain feedback loop conditions held.[12,15,16] The identification of feedback loops that lead to chaos relied on a linearization using the stoichiometric network analysis approach.[17] Thus, despite differences in the chemical nature of the fourth chemical species, all three extended networks shared the same route to chaos.[12] In view

of this evidence, we ask whether there are parametric conditions such that all three PO extended reaction networks share the same steady states no matter the chemical nature of the fourth species.

In order to address such question, we took advantage of the stoichiometric reaction diagrams reported by Sensse *et al.*[12,16] for the three PO extended networks. These graphical representations were modelled with Mass Action Kinetics (MAK) Ordinary Differential Equations (ODEs). In this contribution, we depart from the MAK ODEs to derive an alternate graphical representation that allows us to apply the so-called Chemical Reaction Network Theory (CRNT) and its *deficiency analysis*.[18,19] The deficiency analysis provides a straightforward framework to find a set of kinetic constants such that the associated MAK ODEs support multiple steady states. Furthermore, once we have a pair of steady states, we can test the possibility of sharing the same pair of steady states among the different PO extended reaction networks, thus considering them as *steady state equivalents* if their associated MAK ODEs can indeed support the specified pair of steady states.

This contribution is organised as follows: Section II presents the set of MAK ODEs for each of the three extended PO networks along with their MAK graphical representation. In Section III, we briefly review the CRNT and its deficiency oriented analysis. The steady state equivalence among PO extended reaction networks is presented in Section IV. Possible scenarios are highlighted in Section V. Some conclusions are drawn in Section VI.

II. PEROXIDASE-OXIDASE REACTION NETWORKS

According to Sensse *et al.*,[12] the interplay among the core PO reaction network chemical species, i.e., NAD$^.$, O_2, and Per^{+6}, with an additional fourth chemical species, O_2^-, Per^{+2}, or Per^{+3}, gives rise to three four dimensional MAK ODEs. We shall denote with capital X_i, $i = 1 \ldots 4$ the chemical species; lower case letters x_i, $i = 1 \ldots 4$ stand for the chemical species concentration.

As a first step in our analysis, we present the set of MAK ODEs that model every PO extended reaction system. We then

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used the fact that given a set of MAK ODEs it is always possible to derive their associated (MAK) reaction network,[20,21] enabling us to apply the CRNT along with its deficiency analysis. Before presenting the CRNT in more detail, we shall first introduce the MAK ODEs for the PO extended reaction networks and their graphical representation as needed for a deficiency analysis.[18,22,23]

A. PO extended network No. 1

The set of MAK ODEs for the first extended reaction network as reported by Sensse *et al.*[12] is

$$
\begin{aligned}
\dot{x}_1 &= k_1 x_1 x_3 - k_2 x_1 x_2 - 2k_3 x_1^2 + k_4 \\
\dot{x}_2 &= -k_2 x_1 x_2 - k_5 x_2 + k_6 + k_{13} \hat{x}_4^2 \\
\dot{x}_3 &= -k_1 x_1 x_3 - k_7 x_3 + k_8 \\
\hat{x}_4 &= k_2 x_1 x_2 - 2k_{13} \hat{x}_4^2,
\end{aligned}
\tag{1}
$$

where $x_1 = \mathrm{NAD}^\cdot$, $x_2 = O_2$, $x_3 = \mathrm{coIII} = \mathrm{Per}^{+6}$, and the fourth species $\hat{x}_4 = O_2^-$. We note that in Sensse *et al.* paper,[12] the O_2^- is denoted as x_6 but treated as the fourth variable. Following the algorithm to derive a MAK reaction network from MAK ODEs,[20,21] we have that Eq. (1) induces the reaction network depicted in Fig. 1.

B. PO extended network No. 2

The fourth chemical species added to the core PO network is $\tilde{x}_4 = \mathrm{Per}^{+2}$ (although denoted x_5 in Sensse *et al.* paper,[12] it is treated as the fourth variable), which lead to the following set of MAK ODEs:

$$
\begin{aligned}
\dot{x}_1 &= k_1 x_1 x_3 - k_2 x_1 x_2 - 2k_3 x_1^2 + k_4 - k_{11} x_1, \\
\dot{x}_2 &= -k_2 x_1 x_2 - k_5 x_2 + k_6 - k_{12} x_2 \tilde{x}_4, \\
\dot{x}_3 &= -k_1 x_1 x_3 - k_7 x_3 + k_8 + k_{12} x_2 \tilde{x}_4, \\
\dot{\tilde{x}}_4 &= k_{11} x_1 - k_{12} x_2 \tilde{x}_4
\end{aligned}
\tag{2}
$$

again with $x_1 = \mathrm{NAD}^\cdot$, $x_2 = O_2$, $x_3 = \mathrm{coIII} = \mathrm{Per}^{+6}$. Fig. 2 shows the reaction network induced by MAK ODEs (2).
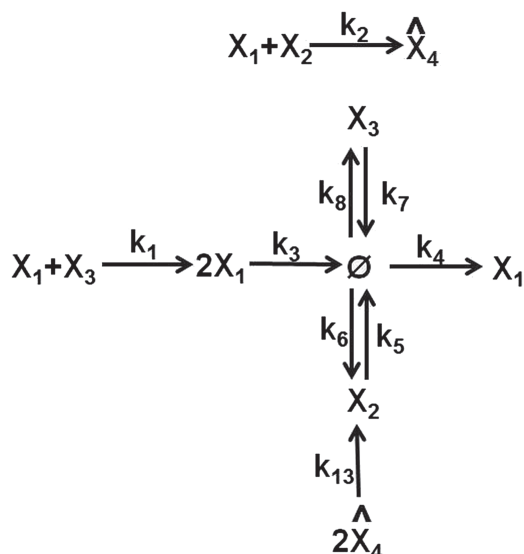

FIG. 1. PO extended network No 1. Chemical species: $X_1 = \mathrm{NAD}^\cdot$, $X_2 = O_2$, $X_3 = \mathrm{coIII} = \mathrm{Per}^{+6}$, and $\hat{X}_4 = O_2^-$. The zero complex $\emptyset$ represents the surroundings.
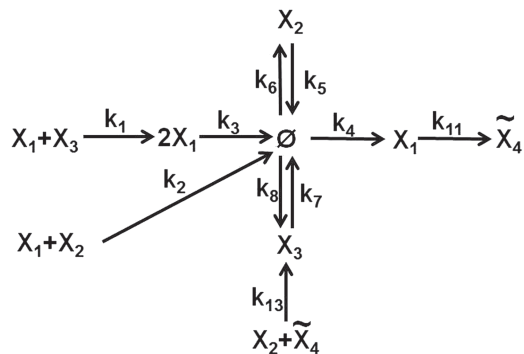

FIG. 2. PO extended network No. 2. Chemical species: $X_1 = \mathrm{NAD}^\cdot$, $X_2 = O_2$, $X_3 = \mathrm{coIII} = \mathrm{Per}^{+6}$, and $\tilde{X}_4 = \mathrm{Per}^{+2}$. The zero complex $\emptyset$ represents the surroundings.

C. PO extended network No. 3

For this extended system, $x_4 = \mathrm{Per}^{+3}$ is the same as in Sensse *et al.* paper.[12] The addition of $x_4 = \mathrm{Per}^{+3}$ to the core PO network gives the MAK ODEs,

$$
\begin{aligned}
\dot{x}_1 &= k_1 x_1 x_3 - k_2 x_1 x_2 - 2k_3 x_1^2 + k_4, \\
\dot{x}_2 &= -k_2 x_1 x_2 - k_5 x_2 + k_6, \\
\dot{x}_3 &= -k_1 x_1 x_3 - k_7 x_3 + k_8 + k_9 x_4, \\
\dot{x}_4 &= k_1 x_1 x_3 - k_9 x_4 - k_{10} x_4.
\end{aligned}
\tag{3}
$$

Chemical species $x_1, x_2,$ and x_3 are the same as in both the previous PO extended networks. The MAK graphical representation of ODEs (3) is shown in Fig. 3.

Now, we turn our attention to a brief review of CRNT and the dynamical information that can be quarried from the network structure as depicted in Figs. 1–3.
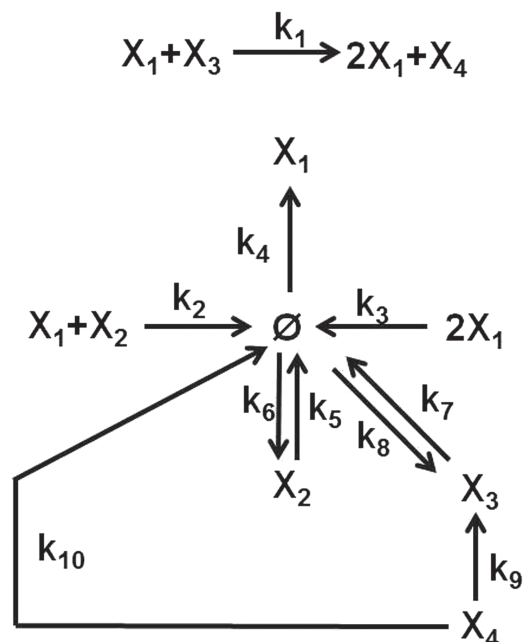

FIG. 3. PO extended network No. 3. Chemical species: $X_1 = \mathrm{NAD}^\cdot$, $X_2 = O_2$, $X_3 = \mathrm{coIII} = \mathrm{Per}^{+6}$, and $X_4 = \mathrm{Per}^{+3}$. The zero complex $\emptyset$ represents the surroundings.

III. CHEMICAL REACTION NETWORK THEORY

The representation of chemical reactions as a network connecting reactants, intermediates, and products has led to general results concerning the expected dynamics as well as robustness without an explicit knowledge of kinetic parameters.[24,25] Based on a non-negative index, $\delta \geq 0$, called *deficiency*, it is possible to establish whether a reaction network can support multiple steady states using the network structure alone.[18,19] The deficiency is computed using the following equation:

$$\delta = C - \mathcal{L} - d, \tag{4}$$

where C is the number of *complexes*, $\mathcal{L}$ is the number of *linkage classes*, and d is the rank of the reaction network, i.e., the rank of the stoichiometric matrix.[18,19] Complexes are the linear combination of chemical species that appear before and after the chemical reaction arrow; linkage classes are the sub-graphs that compound the whole graph. If $\delta = 0$, then no matter the value the kinetic constants might take, the associated MAK ODEs cannot admit multiple steady states or oscillations.[18] On the other hand, for reaction networks with $\delta \geq 1$, the Deficiency One (DO) and the Advance Deficiency (AD) algorithms can determine whether the network admits multiple steady states.[18,19,23] The computational implementation of DO and AD algorithms also provides a set of kinetic constants such that the MAK ODEs can support multiple steady states.[26,27] We stress the fact that δ is not a measure of the reaction network size.[22] Moreover, $\delta \geq 1$ is not a sufficient condition for multistationarity.[18,19,22]

To illustrate the aforementioned concepts, let us consider the following reaction network:

$$2\,X_1 + X_2 \xrightarrow{k_1} 3\,X_1,$$
$$X_1 \xrightarrow{k_2} X_2. \tag{5}$$
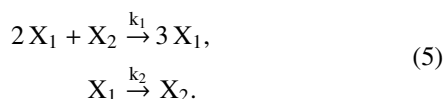
The set of complexes is $\{2\,X_1 + X_2,\ 3\,X_1,\ X_1,\ X_2\}$; thus, $C = 4$. The network is composed of two sub-graphs, that is two linkage classes, $\mathcal{L} = 2$: $\{2\,X_1 + X_2 \rightarrow 3\,X_1\}$ and $\{X_1 \rightarrow X_2\}$. The dynamics of network (5) are governed by the following MAK ODEs:

$$\dot{x}_1 = k_1 x_1^2 x_2 - k_2 x_1,$$
$$\dot{x}_2 = -k_1 x_1^2 x_2 + k_2 x_1. \tag{6}$$

It can be observed that $\dot{x}_1 + \dot{x}_2 = 0$, therefore $x_1(0) + x_2(0) = w = x_1(t) + x_2(t)$[28] for all t, with $w \in \mathbb{R}_+$. As a consequence, the rank of the stoichiometric matrix is one instead of two, that is, $d = 1$. Now, computing the deficiency of network (5), we have $\delta = 4 - 2 - 1 = 1$; thus, the DO algorithm applies. Using the computational implemented DO algorithm[27] for network (5), we find that the network under study can support multiple steady states with kinetic constants: $k_1 = 18.911\,781$, $k_2 = 3.718\,281\,8$. The pair of steady states are $SS_1 = (0.27, 0.73)$, $SS_2 = (0.73, 0.27)$, whereof the conservation relation holds $x_1(0) + x_2(0) = 1 = x_1(t) + x_2(t)$. More detailed examples along with the limits of the deficiency analysis for reaction networks can be found in the review by Feinberg.[18,19]

Next, we turn our attention to a complementary feature of the deficiency analysis[29] that provides a means to identify

TABLE I. Finding kinetic constants from a pair of steady states situation.

SS_1	Chemical species concentration	SS_2
0.27	x_1	0.73
High	x_2	Low

if there is a set of kinetic constants such that the corresponding MAK ODEs induced by a reaction network can support a specific pair of steady states.[22,23,30]

Let us suppose that two steady states have been experimentally observed and we want to test the feasibility of several reaction networks (and their MAK ODEs) to produce the same pair of steady states. Moreover, let us consider the (common) situation where only one chemical species concentration was measured and the second one was only estimated to be higher at one steady state than at the second steady state by means of an indirect measurement (see Table I). Even in the presence of qualitative data, it is possible to test candidate reaction networks using the DO and AD algorithms for a specified pair of steady states, albeit a solution cannot always be found for higher deficiency ($\delta > 1$) due to nonlinear inequality restrictions.[22,23,30]

We can test if network (5) is consistent with the data specified in Table I with the help of the DO algorithm.[23] By doing so, we obtain a slightly modified set of the original kinetic constants presented above $k_1 = 18.790\,987$, $k_2 = 3.703\,703\,7$. The pair of steady states supported by MAK ODEs of network (5) is consistent with those in Table I (actually, the same pair of steady states reported above). As a conclusion, we have evidence that network (5) and its set of MAK ODEs are a valid model for the experiments summarised in Table I.

Although the DO and AD algorithms can be used to discard reaction networks given a pair of steady states, we take advantage of the fact that all three PO extended networks possess bistability as activator-inhibitor reacting systems,[15,16] to test whether the extended PO networks and their associated MAK ODEs support the pair of steady states between them, despite differences in the chemical nature of the fourth species. In particular, given a pair of steady states produced by MAK ODEs induced by PO extended network No. 1, is there a particular choice of kinetic constants such that the MAK ODEs derived from PO extended network No. 2 support the same pair of steady states? In the affirmative case, we say that both the reaction extended networks are *steady state equivalents* even in the presence of chemical species concentrations whose numerical values are not identical but fulfill the higher/lower criteria.

In Sec. IV, we apply the CRNT to the PO extended networks displayed in Section II to obtain the set of steady states and then find a set of kinetic constants from a pair of steady states for every pair combination among PO extended network MAK ODES.

IV. STEADY STATE EQUIVALENCE

We already have all the necessary elements to compute the deficiency of every PO extended network. Table II summarises

TABLE II. Computation of deficiencies for PO extended reaction networks using Eq. (4).

PO extended network	C	$\mathcal{L}$	d	δ	Multiple steady states?
No. 1	9	2	4	3	Yes
No. 2	9	1	4	4	Yes
No. 3	9	2	4	3	Yes

term by term the application of Equation (4) to networks displayed in Sections II A–II C.

A. Bistability of PO extended networks

All PO extended networks have higher deficiency ($\delta > 1$), therefore the AD algorithm applies[23,26] to obtain a pair of steady states for all PO extended networks, which are reported in Table III. Kinetic constants are reported in Table S2 of the supplementary material. We note that all three PO extended networks have the same values for chemical species concentrations x_1 and x_2 at SS_1, whereas for x_3 it has a higher value at SS_1 than at SS_2; the opposite qualitative pattern of x_3 holds for $\hat{x}_4/\tilde{x}_4/x_4$. Thus, if we measure x_1 and x_2 we shall not distinguish between them at the steady state. In addition, we cannot decide which PO extended network is operative at the steady state, either network No. 1 or No. 3, because for these networks both x_1 and x_2 are identical. The remaining chemical species (x_3, $\hat{x}_4/\tilde{x}_4/x_4$) can be distinguished at the steady state. Nevertheless, the qualitative pattern of high/low concentrations at the steady states is shared among all three PO extended networks: x_1 and $\hat{x}_4/\tilde{x}_4/x_4$ have higher values at SS_1 than in SS_2, whereas x_2 and x_3 have lower values at SS_1 than in SS_2.

B. Steady state equivalence cases

We have shown that the steady state equivalence is possible among all three PO extended networks when $x_1 = \text{NAD}^{\cdot}$ or $x_2 = O_2$ is measured. Next, we investigate the existence of kinetic constants such that the chemical species x_3 and $\hat{x}_4/\tilde{x}_4/x_4$ could also be steady state equivalents, despite chemical differences in the fourth chemical species among all three PO extended reaction networks. There are three possible choices considering pairs of MAK ODEs. That is, we ask whether the set of MAK ODEs (1) can exhibit the same pair of steady states of MAK ODEs (2) and (3); and whether the MAK ODEs (2) can support the steady states of MAK ODEs (3).

TABLE III. Pairs of steady states from PO extended reaction networks.[a]

PO extended network No. 1	$x_1 = \text{NAD}^{\cdot}$	$x_2 = O_2$	$x_3 = \text{Per}^{+6}$	$\hat{x}_4 = O_2^{-}$
SS_1	3.16	1	8.60	1.27
SS_2	0.16	7.39	23.37	0.77

PO extended network No. 2	$x_1 = \text{NAD}^{\cdot}$	$x_2 = O_2$	$x_3 = \text{Per}^{+6}$	$\tilde{x}_4 = \text{Per}^{+2}$
SS_1	3.16	1	1.16	6.01
SS_2	0.16	20.09	3.16	0.015

PO extended network No. 3	$x_1 = \text{NAD}^{\cdot}$	$x_2 = O_2$	$x_3 = \text{Per}^{+6}$	$x_4 = \text{Per}^{+3}$
SS_1	3.16	1	1	1.99
SS_2	0.16	7.39	2.72	0.27

[a]Using Windows CRNT Toolbox version.

1. Steady state equivalence between PO extended networks No. 1 and No. 2

Within each pair combination of MAK ODEs, there are fifteen possible cases for the steady state equivalence among the four chemical species (see Table S1 in the supplementary material). The most restrictive case is the identity, that is, the case where all four chemical species are required to have the same concentration at both the steady states (not fulfilled; see
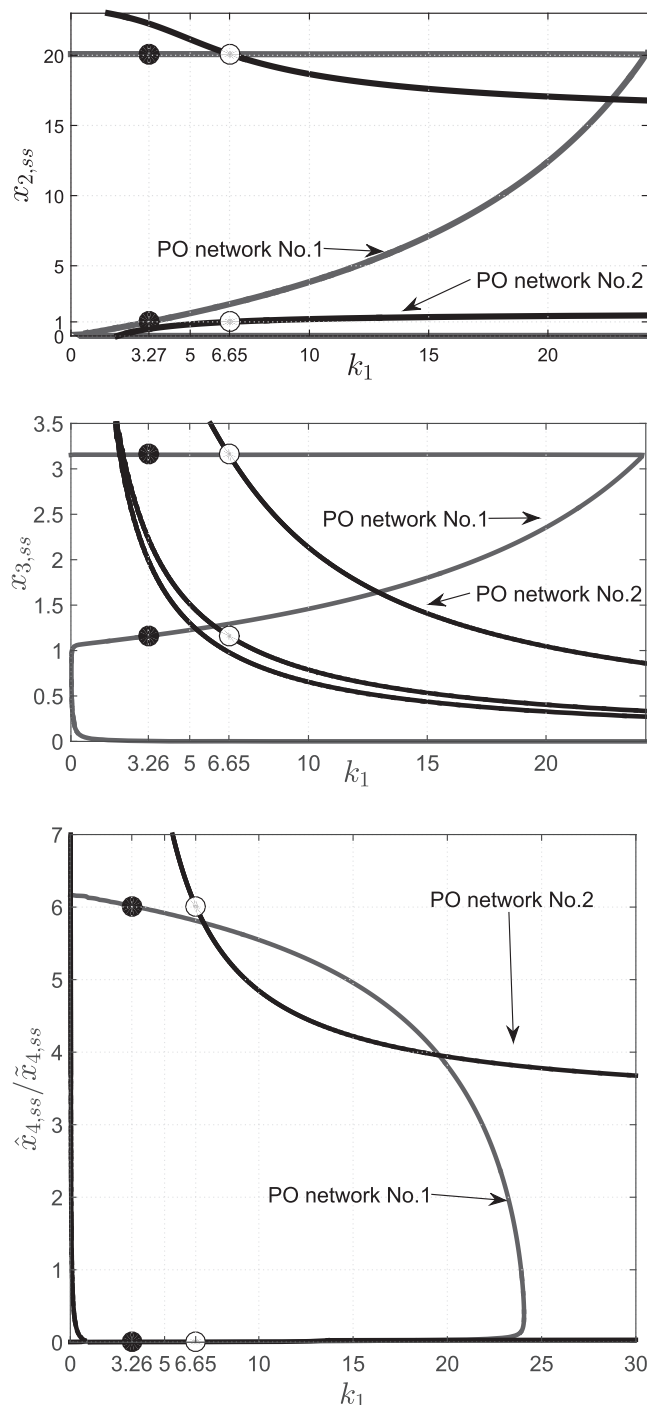

FIG. 4. Locus of equilibria of x_2, x_3, $\hat{x}_4/\tilde{x}_4$, and the pair of steady states such that PO reaction extended network No. 1 (black dots and grey solid line) is a steady state equivalent to PO reaction extended network No. 2 (white dots and black solid line). Note: $\hat{x}_4 = O_2^{-}$ in PO extended network No.1 whereas $\tilde{x}_4 = \text{Per}^{+2}$ in PO extended network No. 2.

Table S1 of the supplementary material). The second most restrictive scenario requires that chemical species x_1 fulfill the qualitative high/low condition, meanwhile x_2, x_3, and $\hat{x}_4/\tilde{x}_4$ have identical concentrations at both the steady states (see Figure 4). We note that $\hat{x}_4 = O_2^-$ in PO extended network No. 1, whereas $\tilde{x}_4 = Per^{+2}$ in PO extended network No. 2. There are other cases (not depicted) where PO extended networks No. 1 and No. 2 are the steady state equivalents with respect to the fourth chemical species (see cases 10, 11, and 15 in Table S1 of the supplementary material). However, in case

10 (the only one), the SS_1 produced by MAK ODES (1) is unstable, contrary to the original stability supported by MAK ODES (2); only for SS_2 stability is preserved. The kinetic constants and the stability of the steady states can be consulted in the supplementary material Text S1.

2. Steady state equivalence between PO extended networks No. 1 and No. 3

Figure 5 shows the corresponding steady state equivalence when x_2, x_3, and $\hat{x}_4/x_4$ are required to have the same chemical species concentrations, meanwhile x_1 fulfils the high/low condition. Recall that the fourth chemical species is not the same in both the extended networks. That is, the solution of $\hat{x}_4 = O_2^-$ from MAK ODES (1) will reach the same value that $x_4 = Per^{+3}$ from MAK ODES (3), even though both chemical species are distinct. Nevertheless, solutions from MAK ODES (1) will converge to SS_2 (the low value of x_4) because the stability of SS_1 from PO extended network No. 3 is not preserved (see Text S1 in the supplementary material). The nonpreservation of the stability of at least one steady state is encountered in nine other cases (out of eleven) for the steady state equivalence between PO extended networks No. 1 and 3.

3. Steady state equivalence between PO extended networks No. 2 and No. 3

Steady state equivalence between PO extended networks No. 2 and 3 covers five cases. In each case, the stability of at least one steady state is unpreserved by the MAK ODES (2) with respect to those supported by MAK ODES (3). Figure 6 shows the only case where x_4 from PO extended network No. 2 has the same pair of steady states as in PO extended network No. 3. Compared to the last two combinations of PO extended networks, in this case $\tilde{x}_4$ is the same chemical species but in a different oxidative state. The stability of steady state SS_2 (low concentration of x_4) is not preserved.
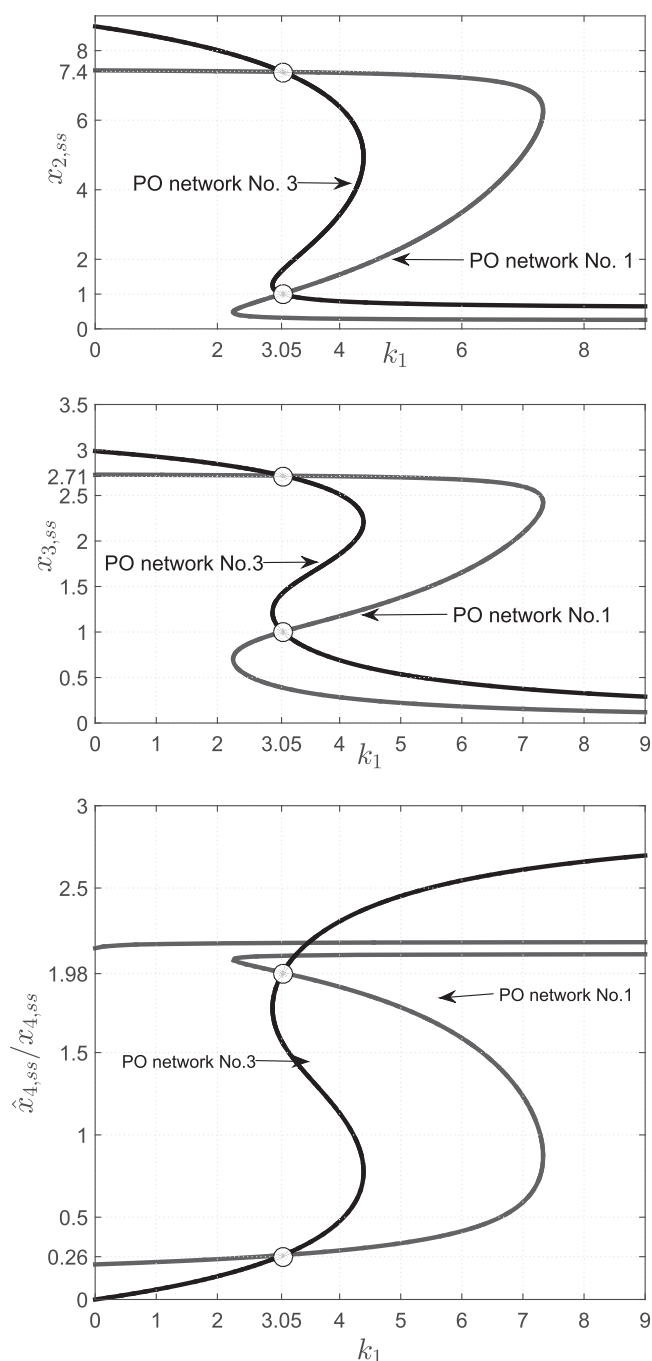

FIG. 5. Locus of equilibria of x_2, x_3, $\hat{x}_4/x_4$, and the pair of steady states such that PO reaction extended network No. 1 (grey solid line) is steady state equivalent to PO reaction extended network No. 3 (black solid line). Note: $\hat{x}_4 = O_2^-$ in PO extended network No. 1 whereas $x_4 = Per^{+3}$ in PO extended network No. 3.
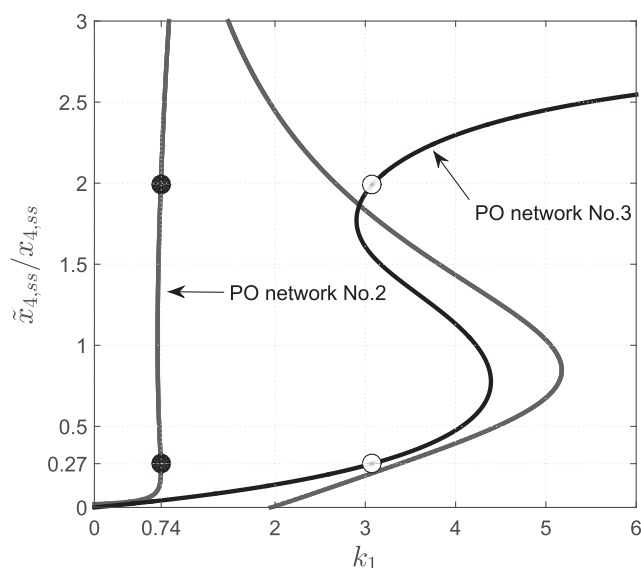

FIG. 6. Locus of equilibria of $\tilde{x}_4/x_4$, and the pair of steady states such that PO reaction extended network No. 2 (black dots, grey solid line) is steady state equivalent to PO reaction extended network No. 3 (white dots, black solid line). Note: $\tilde{x}_4 = Per^{+2}$ in PO extended network No. 2 whereas $x_4 = Per^{+3}$ in PO extended network No. 3.

TABLE IV. PO extended network No. 3 supporting the pair of steady states from PO extended network No. 1: eigenvalues comparison of case 5.[a] The eigenvalues were obtained using the linear stability analysis.

PO extended network No. 3 eigenvalues				
Steady states	λ_1	λ_2	λ_3	λ_4
SS_1	$-12.31 - 2.39\,i$	$-12.31 + 2.39\,i$	-0.47	-1
SS_2	-15.82	-5.68	-0.74	-1
PO extended network No. 3 eigenvalues when supporting the pair of steady states of PO extended network No. 1				
Steady states	λ_1	λ_2	λ_3	λ_4
SS_1	-168.02	9.67	-58.93	-105.65
SS_2	-402.64	-51.21	-11.69	-6.70

[a]See the full cases in the supplementary material.

As a unique feature for the pair combination of MAK ODEs (2) and (3), the AD algorithm[26] remains silent with respect to steady state equivalent cases 2 and 6 because the AD algorithm was unable to find solutions for the nonlinear inequalities induced by PO extended network No. 2, although such MAK examples might exist.[23]

V. DISCUSSION

It has been shown that all three PO extended networks can be steady state equivalents, that is, there are kinetic constants such that the corresponding MAK ODEs can produce the same pair of steady states. In this contribution, the kinetic constants are not scaled for comparison purposes as in the work of Sensse *et al.*[12] No effort has been made to fit the MAK ODEs using the CRNT approach from the experimental data nor has parametric research been carried out to identify routes to chaos. This task will be reported elsewhere.

What we contribute regards comparisons among pairs of MAK ODEs ((1) with (2), (1) with (3), and (2) with (3)) which show that there are pH and temperature conditions, encoded within kinetic constants[11] such that the time evolution of O_2^- in PO extended network No. 1 can reach the same steady states as Per^{+2} and Per^{+3} in PO extended networks 2 and 3, respectively. Such possible scenarios are more likely to occur for steady states from network No. 2 than those from No. 3 because only in one case the stability of the given pair of steady states is unpreserved.

On the other hand, when PO extended networks No. 1 and 3 are compared, neither of the pair of steady states stability is conserved after comparison. The comparison between MAK ODEs induced by PO extended networks No. 2 and 3 lead us to just one case where the fourth chemical species (Per^{+2} and Per^{+3}, respectively) is a steady state equivalent, and with the stability preservation of one steady state, in spite of both MAK ODEs being more alike for the fourth chemical species than the MAK ODEs from PO extended network No. 1. Overall, we found conditions whereby measuring at least one chemical species, there is a possibility of having a steady state equivalence among all three PO extended networks, thus leading to spurious assignments of the actual operative mechanism under some pH or temperature conditions.

The versatility of the extended PO network No. 1 ($\hat{x}_4 = O_2^-$) to support several steady state equivalent scenarios agrees with its suggested dynamical domination of the complete PO dynamics for a large range of kinetic constant values.[12] The biological implications of PO network No. 1 to produce, under some circumstances, the same concentration levels of Per^{+2} and Per^{+3} remains an open question.

Complementary, we stress the fact that finding two equivalent steady states using the CRNT approach does not give information about the local stability of these steady states. In order to evaluate the local stability of the steady states, we exploited the linear stability analysis with the kinetic rate constants obtained from the CRNT for the equivalent steady state cases. We also note that for some equivalent steady state cases the stability may be different between the reaction networks under study. As usual, the eigenvalues indicate if the steady state is stable or not. Table IV summarises the eigenvalues for the steady state equivalence between PO networks No. 1 and No. 3. Although there are kinetic rate constants such that the MAK ODEs of PO network No. 1 give rise to the same steady states of PO network No. 3 (see Table S1 in the supplementary material for details) the stability of the given pair of steady states is not preserved.

A more stringent concept than the steady state equivalence is the topological equivalence concept.[31] In order to ensure a one-to-one correspondence that preserves the stability between pairs of steady states for both networks is that the number of eigenvalues with negative (or positive) real part be the same for both MAK ODEs in the vicinity of the steady states.[31] Other approaches rely on the construction of linear and nonlinear mappings using Lie derivatives of a measured chemical species along the vector fields defined by the MAK ODEs to map their solutions into an equivalent state defined by a second MAK ODE.[32] Commonly, these nonlinear mappings lead to intricate mathematical expressions. In this respect, the approach reported here remains simple to use and more robust when fragmentary or uncertain data come into play, a frequent situation in the study of biochemical reaction systems.

VI. CONCLUSIONS

There are kinetic constants such that the MAK ODEs induced by PO extended network Nos. 1–3 show (exactly) the same pairs of steady states for more than one chemical species, even in the presence of a different chemical species: O_2^-, Per^{+2}, and Per^{+3}. No matter which chemical species is

measured, there are kinetic constants such that at least one MAK ODE from a PO extended network can produce the same pair of steady states for that particular measured chemical species.

SUPPLEMENTARY MATERIAL

See supplementary material for the complete set of steady state equivalence cases and stability among Peroxidase-Oxidase chemical reaction networks along with their corresponding kinetic rate constants.

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[29] Only available in the MS-DOS version of CRNT Toolbox.

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