Flux-augmented bifurcation analysis in chemical reaction network systems

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The dynamics of biochemical reaction networks are considered to be responsible for biological functions in living systems. Since real networks are immense and complicated, it is difficult to determine which reactions can cause a significant change of dynamical behaviors, namely, bifurcations. Also to what extent numerical results of network systems depend on the chosen kinetic rate parameters is not known. In this paper, an analytical setting that splits the information of the dynamics into the network structure and reaction kinetics is introduced. This setting possesses a factorization structure for some class of network systems which allows one to determine which subnetworks are responsible for the occurrence of a bifurcation. Subsequently, the bifurcation criteria are reformulated in a manner that allows the efficient determination of relevant reactions for bifurcations.

I. INTRODUCTION

In biochemical systems, it is commonly accepted that the dynamical behaviors of biochemical reaction networks are responsible for biological functions, and they can be described by ordinary differential equations (ODEs). A dramatic change in dynamical behaviors of a network system indicates a significant change in the corresponding biological behaviors. For instance, bistability, where a system has two stable states, suggests a switching behavior between states. This switching behavior is considered to underlie some fundamental biological processes, such as cell division, cell differentiation, and cancer onset [1,2]. The bistability can be realized, for example, via a pitchfork bifurcation, where one family of stable steady states transfers its stability properties to two families of steady states after the bifurcation point.

In fact, a bifurcation occurs when a slight variation in a parameter (bifurcation parameter) of a system causes a sudden qualitative or topological change of its dynamical behavior. Note that as a bifurcation parameter crosses a critical value, the local stability properties of steady states or periodic orbits can change. The tuning of a bifurcation parameter can correspond to enzyme modulations or external conditions in biochemical systems. A central question is whether or not a given network system can admit a bifurcation. Chemical reaction networks in biological systems are modeled based on the mass-action law (see, e.g., [3,4]), and the Michaelis-Menten and other kinetics [5,6].

To analytically study the dynamical behaviors in reaction network systems, Clarke [7–9] introduced the stoichiometric network analysis (SNA) to derive the constraints among rate parameters for which bistability or oscillations of network systems can occur. His analysis is based on two assumptions: (i) Each admissible positive steady state can be characterized as a convex linear combination of nonnegative kernel vectors of the stoichiometric matrix of the system (extreme currents); and (ii) the reaction rate kinetics obey the mass-action law (i.e., reaction rate kinetics are given by products of positive rate constants and monomials in the species concentrations). The former assumption was rigorously justified by Gatermann, Eiswirth, and Sensse [10] via the theory of algebraic geometry in the mass-action scheme. The latter assumption allows the Jacobian of the system evaluated at positive steady states to have a form that can be easily manipulated. Later, under the mass-action kinetics assumption, Feinberg and his coauthors [11–14] studied the question on whether a given network system can admit bistability or multiple existences of positive steady states. Finally, using Clarke's framework and mass-action assumptions on rate kinetics, Domijan and Kirkilionis [15] reformulated

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classical bifurcation criteria, such as saddle-node and Hopf bifurcations.

These previous works are based on the assumption that rate kinetics obey mass-action law. Further, the stability of steady states is determined by the Jacobian matrix, $\boldsymbol{J}$, of network systems, which could be tedious to analyze systematically, particularly if the reaction kinetics are not of the mass-action type and the network size is large. On the other hand, employing specific reaction kinetics and parameter values, numerical approaches [16] can efficiently solve the stability of steady states. However, determining the actual kinetics experimentally is still difficult especially *in vivo*. In fact, estimated values of kinetic parameters in the literature can be different by orders of magnitude (as can be seen from Refs. [17] and [18]). Furthermore, novel allosteric regulations continue to be discovered [19], and there may still exist unidentified regulations in the central carbon metabolism network. Thus, the essence of biological systems may not be fully captured only by numerical methods. Thus, the aim of this study is twofold: (i) An analytical setting for the stability analysis of steady states in network systems without mass-action laws imposed on reaction kinetics is introduced. (ii) With this setting, criteria on whether a change of reaction rate parameters in a network system can admit a bifurcation are proposed. The paper is organized as follows. In Sec. II, the augmented matrix $\mathbf{A}$ for the setting of the stability analysis of steady states is introduced. Next, in Sec. III, a correspondence between the spectrum of the Jacobian matrix, $\boldsymbol{J}$, of a network system and that of the augmented matrix, $\mathbf{A}$, is established. Section IV contains our main results, where the criteria of bifurcation (of codimension 1) are reformulated using our setting. One distinct advantage of the proposed formalism is that the conditions on bifurcations are rephrased in terms of reaction rate functions and network topology directly. In Sec. V, the setting is employed to derive the localization principle for bifurcations. In Sec. VI, the proposed theory is illustrated using hypothetical and realistic networks. Finally, the conclusion and discussion are provided in Sec. VII. To make the presentation clear, we focus on the saddle-point bifurcation in the main text which is a typical codimension-1 bifurcation. The reformulation of other codimension-1 bifurcations such as transcritical and pitchfork bifurcations and their demonstrations are presented in Appendixes D and E.

II. THE SETTING AND THE AUGMENTED MATRIX A

In this section, we will lay out our setting and introduce the key augmented matrix $\mathbf{A}$.

We consider a spatially homogeneous chemical reaction system consisting of M chemicals and N reactions:

$$y_1^n X_1 + \cdots + y_M^n X_M \xrightarrow{k_n} \tilde{y}_1^n X_1 + \cdots + \tilde{y}_M^n X_M,$$

for $n = 1, \ldots, N$, and the coefficients y_m^n and $\tilde{y}_m^n$ are the numbers of the chemical X_m participating in the nth reaction E_n at reactant and product stages, respectively. We do not assume specific kinetics such as the mass-action kinetics and the Michaelis-Menten kinetics, but assume that it depends on its substrate concentrations and also on some parameter k_n. For example, in metabolic networks, X are metabolites, and k_n are, for example, catalytic enzyme activities.

Let $r_n(\boldsymbol{x}, k_n)$ denote the reaction rate function of the nth reaction ($n = 1, \ldots, N$), where $\boldsymbol{x} = (x_1, \ldots, x_M)^\top$, x_m is the concentration of chemical X_m, and $\top$ denotes the transpose operator of a matrix. Then the time evolution of the chemical concentration $\boldsymbol{x}$ is governed by the following ODEs:

$$\frac{dx_m}{dt} = f_m(x_1, \ldots, x_M; k_1, \ldots, k_n)$$
$$:= \sum_{n=1}^{N} \boldsymbol{v}_{mn} r_n(\boldsymbol{x}; k_n), \quad m = 1, \ldots, M. \tag{1}$$

The matrix $\boldsymbol{v} = (\boldsymbol{v}_{mn})_{M \times N} = (\bar{y}_m^n - y_m^n)_{M \times N}$, each of which represents the net difference of consumption and production in the reaction, is called the stoichiometric matrix. For ease of notations, set

$$\boldsymbol{f} = (f_1, \ldots, f_M)^\top, \boldsymbol{r} = (r_1, \ldots, r_N)^\top,$$
$$\boldsymbol{k} = (k_1, \ldots, k_N)^\top.$$

Then (1) can be rewritten as follows:

$$\frac{d\boldsymbol{x}}{dt} = \boldsymbol{f}(\boldsymbol{x}; \boldsymbol{k}) := \boldsymbol{v}\boldsymbol{r}. \tag{2}$$

To present the key idea, we assume that the stoichiometric matrix $\boldsymbol{v}$ does not have nonzero cokernel vectors, implying that

$$\operatorname{rank}(\boldsymbol{v}) = M \leqslant N, \tag{3}$$

and the steady-state concentrations $\bar{\boldsymbol{x}}$ and fluxes $\bar{\boldsymbol{r}}$ are continuous functions of parameters $\{k_n\}_{n=1}^{N}$. Nonzero cokernel vectors of $\boldsymbol{v}$ correspond to linear combinations of concentrations that are conserved in time. When a system has nonzero cokernel vectors, (3) can be satisfied after eliminating some of the variables. For a steady state $\bar{\boldsymbol{x}}$, $0 = \boldsymbol{v}\boldsymbol{r}$ means that the flux $\bar{\boldsymbol{r}}$ is expressed, in terms of any basis $\{\boldsymbol{c}_\alpha\}_{\alpha=1}^{N-M}$ of the kernel space of $\boldsymbol{v}$, as

$$\bar{\boldsymbol{r}} = \sum_{\alpha=1}^{K} \zeta^\alpha \boldsymbol{c}_\alpha, \quad K := N - M = \dim \ker \boldsymbol{v}. \tag{4}$$

Note that the basis $\{\boldsymbol{c}_\alpha\}_{\alpha=1}^{N-M}$ depends only on the structure of networks, not on the reaction rate function $\boldsymbol{r}$. The numbers ζ^α are the coordinates in the space of the steady-state flux.

Note that in monomolecular-reaction systems (see [20] for examples of monomolecular-reaction systems), the kernel vectors of ν have an interpretation as "cycles." Such a graphical interpretation is still useful to find kernel vectors visually for general reaction systems.

The following augmented $N \times N$ matrix $\mathbf{A}$ plays a pivotal role throughout the present paper:

$$\mathbf{A} = \left(\left. \frac{\partial \boldsymbol{r}}{\partial \boldsymbol{x}} \right|_{\boldsymbol{x} = \bar{\boldsymbol{x}}} \boldsymbol{K}_\nu \right) := \left(\left. \frac{\partial \boldsymbol{r}}{\partial \boldsymbol{x}} \right|_{\boldsymbol{x} = \bar{\boldsymbol{x}}} - \boldsymbol{c}_1 \ldots - \boldsymbol{c}_K \right). \tag{5}$$

The row length is $M + \dim \ker \boldsymbol{v} = N$, because of the assumption $\dim \operatorname{coker} \boldsymbol{v} = 0$. The augmented matrix $\mathbf{A}$ consists of two submatrices: The submatrix $\frac{\partial \boldsymbol{r}}{\partial \boldsymbol{x}}|_{\boldsymbol{x} = \bar{\boldsymbol{x}}} = (\frac{\partial r_n}{\partial x_m}|_{\boldsymbol{x} = \bar{\boldsymbol{x}}})_{N \times M}$ contains the information of reaction rate functions only, while the submatrix $\boldsymbol{K}_\nu$ is composed of the kernel vectors of $\boldsymbol{v}$ and contains the information of network structure only.

The most important feature of the matrix $\mathbf{A}$ is that it possesses the block structure. To see this, suppose that the whole

chemical reaction system Γ has a subnetwork Γ_s consisting of a subset of species and a subset of reactions. By permutating the column and row indices of the the matrix $\mathbf{A}$, it can be rewritten as

$$\mathbf{A} = \left(\begin{array}{c|c} \mathbf{A}_{\Gamma_s} & * \\ \hline \mathbf{0} & \mathbf{A}_{\bar{\Gamma}_s} \end{array} \right), \qquad (6)$$

where the rows (columns) of $\mathbf{A}_{\Gamma_s}$ are associated with the reactions (chemicals and cycles) in Γ_s, and those of $\mathbf{A}_{\bar{\Gamma}_s}$ are associated with the complement $\bar{\Gamma}_s := \Gamma \backslash \Gamma_s$. This block structure of $\mathbf{A}$ allows one to deduce the information of Γ_s without being obscured by the parameters involved in $\bar{\Gamma}_s$. For more details, see Example 1 in Sec. VI.

The augmented matrix $\mathbf{A}$ allows one to extract qualitative properties of system (2) without resorting to the specific form of reaction rate functions. In fact, under perturbation $k_{\hat{n}} \rightarrow k_{\hat{n}} + \delta k_{\hat{n}}$ ($\hat{n} = 1, \dots, N$), the augmented matrix $\mathbf{A}$ determines how the concentration changes $\delta_{\hat{n}} x_m$ and the flux changes $\delta_{\hat{n}} r_n$ are at the steady state $\bar{x}$. Precisely, Mochizuki and Fiedler [21] showed that if the augmented matrix $\mathbf{A}$ at the steady state $\bar{x}$ is nonsingular, then the sensitivities of the chemical concentrations and the steady-state flux coordinates are given by

$$\frac{\partial \bar{x}_m}{\partial k_n} = -(\mathbf{A}^{-1})_{mn} \left(\frac{\partial r_n(\boldsymbol{x}; k_n)}{\partial k_n} \right)\bigg|_{\boldsymbol{x}=\bar{\boldsymbol{x}}},$$
$$\frac{\partial \zeta^\alpha}{\partial k_n} = -(\mathbf{A}^{-1})_{\alpha n} \left(\frac{\partial r_n(\boldsymbol{x}; k_n)}{\partial k_n} \right)\bigg|_{\boldsymbol{x}=\bar{\boldsymbol{x}}}. \qquad (7)$$

To make the present paper self-contained, we review the derivation in Appendix A. From (4), the sensitivities of reaction rates are then obtained as follows:

$$\frac{\partial \bar{r}_n}{\partial k_{n'}} = \sum_{\alpha=1}^K \frac{\partial \zeta^\alpha}{\partial k_{n'}} \boldsymbol{c}_\alpha.$$

In practical applications, we usually know the signs of the factors $\left(\frac{\partial r_n(\boldsymbol{x}; k_n)}{\partial k_n} \right)\big|_{\boldsymbol{x}=\bar{\boldsymbol{x}}}$. Therefore, the signs of the entries of the inverse matrix of $\mathbf{A}$ completely determine those of the concentration changes $\delta_{\hat{n}} x_m$ and the flux changes $\delta_{\hat{n}} r_n$ as reaction rate parameters are varied. We remark that this method works away from bifurcation points, where $\mathbf{A}$ becomes singular (i.e., $\det \mathbf{A} = 0$).

Recently, some of the authors have made an observation [22] that the $\det \mathbf{A}$ is proportional to the determinant of the Jacobian matrix $\boldsymbol{J}$ of system (2) at the steady state $\bar{x}$. This observation suggests that the augmented matrix $\mathbf{A}$ can determine the critical parameter value at which a bifurcation of system (2) can occur. Further, for reaction networks with specific topology, the factorization property of the augmented matrix $\mathbf{A}$ will allow one to determine which subnetwork may exhibit a bifurcation. However, this observation cannot determine whether the spectrum information of a steady state is encoded in the augmented matrix $\mathbf{A}$. Also, it does not provide any information about which type of bifurcation can occur. In the coming section, we will use the (generalized) spectrum of $\mathbf{A}$ to characterize the spectrum of the Jacobian matrix $\boldsymbol{J}$.

III. EQUIVALENCE BETWEEN SPECTRUM OF THE JACOBIAN $\boldsymbol{J}$ AND THE AUGMENTED MATRIX A

In this section, we establish that spectral properties of steady states of system (2) can be characterized by the augmented matrix $\mathbf{A}$. In the next section, by using the characterization, we will reformulate the criterion for classical bifurcations such as saddle-node bifurcation.

In this subsection, we will use the setting of the augmented matrix $\mathbf{A}$ to characterize the stability of steady states. Following the standard method, we examine the stability of a steady state by linearization around it. We write $\boldsymbol{x}(t) = \bar{\boldsymbol{x}} + \delta \boldsymbol{x}_\lambda e^{\lambda t}$, where $\delta \boldsymbol{x}_\lambda$ is the eigenvector of the Jacobian with eigenvalue λ. By substituting this into (2), we obtain

$$\lambda \delta \boldsymbol{x}_\lambda = \boldsymbol{\nu} \mathbf{D} \delta \boldsymbol{x}_\lambda, \qquad (8)$$

where $\mathbf{D}$ is the $N \times N$ matrix defined by $\mathbf{D}_{nm} = \frac{\partial r_n}{\partial x_m}|_{\boldsymbol{x}=\bar{\boldsymbol{x}}}$. To proceed, we introduce the pseudoinverse matrix $\boldsymbol{\rho}$ of $\boldsymbol{\nu}$, which is an $N \times M$ matrix defined by

$$\boldsymbol{\nu} \boldsymbol{\rho} = \mathbf{1}_M. \qquad (9)$$

By multiplying the left-hand side of (8) by the identity matrix $\mathbf{1}_M = \boldsymbol{\nu} \boldsymbol{\rho}$, we have

$$\boldsymbol{\nu} (\lambda \boldsymbol{\rho} - \mathbf{D}) \delta \boldsymbol{x}_\lambda = \mathbf{0}. \qquad (10)$$

This means that, similarly to (4), we can eliminate $\boldsymbol{\nu}$ by expanding the left-hand side in terms of the kernel vectors of $\boldsymbol{\nu}$:

$$(\lambda \boldsymbol{\rho} - \mathbf{D}) \delta \boldsymbol{x}_\lambda = -\sum_{\alpha=1}^K \zeta_\lambda^\alpha \mathbf{c}_\alpha. \qquad (11)$$

Here, ζ_λ^α are the coefficients of the expansion, which depend on λ. This can be expressed more compactly as

$$\mathbf{A}_{\rho,\lambda} \begin{pmatrix} \delta \boldsymbol{x}_\lambda \\ \zeta_\lambda \end{pmatrix} = \mathbf{0},$$

where $\mathbf{A}_{\rho,\lambda}$ is the $N \times N$ matrix defined by

$$\mathbf{A}_{\rho,\lambda} := [\mathbf{D} - \lambda \boldsymbol{\rho} \,|\, \boldsymbol{K}_\nu]. \qquad (12)$$

To clarify the notation, we show these matrices for an example network, shown in the top-left panel of Fig. 1. The stoichiometry matrix $\boldsymbol{\nu}$ is given by

$$\boldsymbol{\nu} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}. \qquad (13)$$

The matrix $\boldsymbol{K}_\nu$, which consists of kernel vectors of $\boldsymbol{\nu}$, is

$$\boldsymbol{K}_\nu = \begin{pmatrix} 1 & 1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{pmatrix}. \qquad (14)$$

These two kernel vectors of $\boldsymbol{\nu}$ can be easily constructed by finding flows such that, for each molecule, its outflow and inflow are exactly balanced. As we commented, they can also be regarded as cycles, if we identify the "empty-set node" $\emptyset$ appearing in 1: $\emptyset \rightarrow$ A, 3: B $\rightarrow \emptyset$, and 5: C $\rightarrow \emptyset$. The matrix

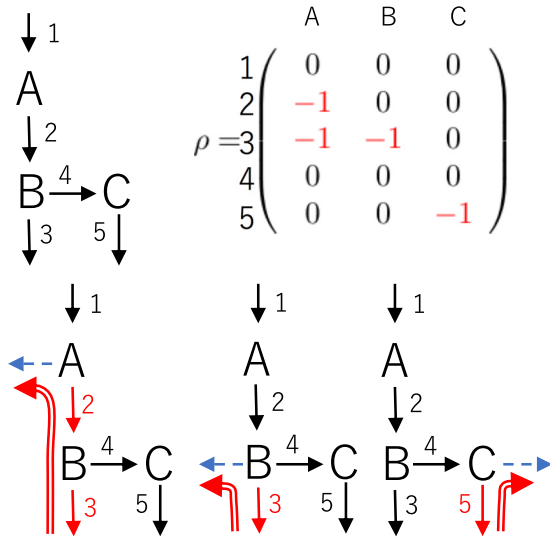FIG. 1. A graphical construction of the matrix ρ. Reaction 1: $\emptyset \to A$ (inflow), 2: $A \to B$, 3: $B \to \emptyset$, 4: $B \to C$, 5: $C \to \emptyset$ (degradation). For each chemical, add an outflow from it (the dashed blue arrow), and compute the steady-state flux (red double line), which gives the associated column of ρ.

ρ is given by

$$\rho = \begin{pmatrix} 0 & 0 & 0 \\ -1 & 0 & 0 \\ -1 & -1 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -1 \end{pmatrix}. \tag{15}$$

We remark that ρ is not unique because null vectors of ν can be added to columns of ρ. We assume that each reaction rate depends on the concentration of the molecule from which the reaction edge emanates. The Jacobian is given by

$$\nu D = \begin{pmatrix} -r_{2,A} & 0 & 0 \\ r_{2,A} & r_{4,B} - r_{3,B} & 0 \\ 0 & r_{4,B} & -r_{5,C} \end{pmatrix}, \tag{16}$$

and the matrix $A_{\rho,\lambda}$ is given by

$$A_{\rho,\lambda} = \begin{pmatrix} 0 & 0 & 0 & 1 & 1 \\ r_{2,A} - \lambda & 0 & 0 & 1 & 1 \\ -\lambda & r_{3,B} - \lambda & 0 & 1 & 0 \\ 0 & r_{4,B} & 0 & 0 & 1 \\ 0 & 0 & r_{5,C} - \lambda & 0 & 1 \end{pmatrix}. \tag{17}$$

It turns out that for a given eigenvector $\mathbf{u}$ of the Jacobian νD associated with the eigenvalue λ, there exists a unique null vector of $A_{\rho,\lambda}$. The converse is also true. That is, $A_{\rho,\lambda}$ completely encodes the information about the spectrum of the Jacobian matrix. To state this correspondence explicitly, we introduce the $N \times N$ matrix $P_0 = \mathbf{1}_N - \rho\nu$, which is a projection operator onto $\ker \nu$ as it satisfies $P_0^2 = P_0$ and $\nu P_0 = \mathbf{0}_{M \times N}$. Then, the following theorem holds (see Appendix B for the proof).

Theorem 1. For each $\lambda \in \mathbb{C}$, the linear mapping

$$\mathcal{F} \colon \ker(\nu D - \lambda \mathbf{1}_M) \to \ker(A_{\rho,\lambda}),$$

defined by

$$\mathcal{F}(\mathbf{u}) = (\mathbf{u}, \mathbf{v})^\top \quad \text{for } \mathbf{u} \in \ker(\nu D - \lambda \mathbf{1}_M),$$

where $\mathbf{v} \in \mathbb{C}^{N-M}$ is uniquely determined by the equation

$$K_\nu(\mathbf{v}) = -P_0(D\mathbf{u}), \tag{18}$$

is a one-to-one correspondence.

We emphasize here that the spectrum of the Jacobian matrix νD is not equivalent to the usual spectrum of A, which is obtained from $\det(A - \lambda \mathbf{1}_N) = 0$, but to a generalized one, which is obtained from $\det A_{\rho,\lambda} = \det(A - \lambda \hat{\rho}) = 0$, where $\hat{\rho} := (\rho \mid \mathbf{0}_{N \times K})$.

In order to establish the criterion for bifurcation in terms of our setting, we also need the correspondence between the transposed spaces $\ker(D^\top \nu^\top - \lambda \mathbf{1}_M)$ and $\ker(A_{\rho,\lambda}^\top)$. Recall the $N \times N$ matrix $A_{\rho,\lambda} = [D - \lambda\rho \mid K_\nu]$. Thus,

$$A_{\rho,\lambda}^\top := \begin{pmatrix} D^\top - \lambda\rho^\top \\ \hline K_\nu^\top \end{pmatrix}.$$

Now we state the correspondence between the spaces $\ker(D^\top \nu^\top - \lambda \mathbf{1}_M)$ and $\ker(A_{\rho,\lambda}^\top)$ in the following theorem (see Appendix C for the proof).

Theorem 2. For each $\lambda \in \mathbb{C}$, the linear mapping

$$\tilde{\mathcal{F}} \colon \ker(D^\top \nu^\top - \lambda \mathbf{1}_M) \to \ker(A_{\rho,\lambda}^\top)$$

defined by

$$\tilde{\mathcal{F}}(\mathbf{w}) = \nu^\top \mathbf{w} \quad \text{for } \mathbf{w} \in \ker(D^\top \nu^\top - \lambda \mathbf{1}_M)$$

is a one-to-one correspondence.

Interpretation of the map ρ. We note that the matrix ρ has an intuitive construction as a null vector of a stoichiometry matrix, namely as a steady-state flux. If we write the ith column as ρ_i $(i = 1, \ldots, M)$, $\nu\rho = \mathbf{1}_M$ is equivalent to

$$(\nu \mid -\mathbf{e}_i)\begin{pmatrix} \rho_i \\ 1 \end{pmatrix} = \mathbf{0}, \tag{19}$$

where the first factor is the augmented matrix obtained by combining ν with the unit column vector $-\mathbf{e}_i = (0, \ldots, \underset{i\text{-th}}{-1}, \ldots, 0)^\top$. The addition of the column $-\mathbf{e}_i$ is equivalent to adding the decay reaction from the ith chemical, $X_i \to \phi$, to the original reaction network. Therefore, $\binom{\rho_i}{1}$, and so ρ_i, can be obtained by considering the underlying network system with the decay reaction of X_i, and then computing the steady-state flux vector whose component for the decay reaction is equal to 1.

As an illustration, consider again the network shown in the top-left panel of Fig. 1. The matrices ν and ρ are given in (13) and (15). For example, to obtain the first column of ρ, which is associated with chemical A, we add an outflow from chemical A (see the dashed arrow in the bottom-left panel of Fig. 1). In this modified network, there is a new steady-state flux that goes through reactions 2, 3 and the added outflow. Therefore, the first column of ρ is given by $(0, -1, -1, 0, 0)^\top$. The sign is negative since the flux goes though the two reactions in the opposite direction. Note that ρ is not unique, and correspondingly, there are infinitely many choices of the flux—for example, the flux going through reaction 1 and the outflow, which gives $(1, 0, 0, 0, 0)^\top$, or one going through reactions 5,

4, 2 and the outflow, which gives $(0, -1, 0, -1, -1)^\top$. The other columns of ρ can be constructed similarly.

IV. CRITERIA ON BIFURCATION WITH SIMPLE ZERO EIGENVALUE

A change of kinetic rate parameter in a biochemical reaction network can correspond to an experimental protocol such as enzyme knockdown of some specific reaction or the transcript inhibition of target proteins on microRNAs. Therefore, in this section, we study codimension-1 bifurcations. To clarify the essence of our approach, the saddle-node bifurcation, which is the typical codimension-1 bifurcation, is presented in the main text, and other codimension-1 bifurcations with specific symmetries such as transcritical and pitchfork bifurcations are presented in Appendixes D–F.

We will use our setting to give conditions under which a bifurcation can occur, by using the correspondence between the spectrum of the Jacobian matrix and that of the augmented matrix **A** defined in the previous subsection. These reformulated bifurcation criteria are not only elegant, but also identify which reactions can cause a bifurcation of the underlying system. Below, we use $D_{\boldsymbol{x}}\boldsymbol{g}$ to denote the matrix of partial derivatives of the components of $\boldsymbol{g}$ with respect to the components of $\boldsymbol{x}$, and $\boldsymbol{g}_\mu$ to denote the vector of partial derivatives of the components of $\boldsymbol{g}$ with respect to the scalar parameter μ, namely, $(D_{\boldsymbol{x}}\boldsymbol{g})_{mm'} = \frac{\partial g_m}{\partial x_{m'}}$ and $(\boldsymbol{g}_\mu)_m = \frac{\partial g_m}{\partial \mu}$. Also, $D_{\boldsymbol{x}}^2\boldsymbol{g}(\boldsymbol{x}_0; k_0)(\mathbf{u}, \mathbf{u}) = \sum_{m,m'} \frac{\partial^2 \boldsymbol{g}(\boldsymbol{x}_0; k_0)}{\partial x_m \partial x_{m'}} u_m u_{m'}$ is an N-dimensional vector obtained by contracting the tensor $D_{\boldsymbol{x}}^2\boldsymbol{g}(\boldsymbol{x}_0; k_0)$ with $\mathbf{u}$.

Saddle-node bifurcation

We begin with the following classical theorem by Sotomayer, which gives a sufficient condition under which the system undergoes a saddle-node bifurcation. See [[23], Theorem 8.12] and [[24], Theorem 3.4.1] for a proof.

Theorem by Sotomayer [25]. Let $\dot{\boldsymbol{x}} = \boldsymbol{g}(\boldsymbol{x}; \mu)$ be a system of differential equations in $\mathbb{R}^M$ depending on the single parameter μ. When $\mu = \mu_0$, assume that there is a steady state $\boldsymbol{x}_0$ for which the following hypotheses are satisfied:

(SN1°) $D_{\boldsymbol{x}}\boldsymbol{g}(\boldsymbol{x}_0; \mu_0)$ has a simple eigenvalue 0 with left eigenvector $\mathbf{w}$ and right eigenvector $\mathbf{u}$.

(SN2°) The left eigenvector $\mathbf{w}$ satisfies $\mathbf{w}^\top \boldsymbol{g}_\mu(\boldsymbol{x}_0; \mu_0) \neq 0$.

(SN3°) The eigenvectors $\mathbf{w}$ and $\mathbf{u}$ satisfy $\mathbf{w}^\top [D_{\boldsymbol{x}}^2\boldsymbol{g}(\boldsymbol{x}_0; k_0)(\mathbf{u}, \mathbf{u})] \neq 0$.

Then system $\dot{\boldsymbol{x}} = \boldsymbol{g}(\boldsymbol{x}; \mu)$ exhibits a saddle-node bifurcation at the steady state $\boldsymbol{x}_0$ as the parameter μ varies through the bifurcation value $\mu = \mu_0$. Precisely, there exist a $\delta > 0$ and a smooth curve $\Upsilon: J = (-\delta, \delta) \to \mathbb{R}^M \times \mathbb{R}$ such that the following holds:

$$\Upsilon(s) = (\bar{\boldsymbol{x}}(s), \mu(s)) \text{ and } \boldsymbol{g}(\bar{\boldsymbol{x}}(s); \mu(s)) = \mathbf{0} \text{ for } s \in J,$$

$$(\bar{\boldsymbol{x}}(0), \mu(0)) = (\boldsymbol{x}_0, \mu_0) \text{ and } \dot{\bar{\boldsymbol{x}}}(0) = \mathbf{u},$$

$$\dot{\mu}(0) = 0 \text{ and } \ddot{\mu}(0) = -\frac{\mathbf{w}^\top[D_{\boldsymbol{x}}^2\boldsymbol{g}(\boldsymbol{x}_0; \mu_0)(\mathbf{u}, \mathbf{u})]}{\mathbf{w}^\top \boldsymbol{g}_\mu(\boldsymbol{x}_0; \mu_0)}.$$

Moreover, each of the steady states $\bar{\boldsymbol{x}}(s)$ with $s > 0$ and with $s < 0$ is hyperbolic.

If we apply this classical theorem to a chemical reaction system, the information on the network structure and that on the kinetics will be mixed up since the theorem is formulated in terms of the product of them, i.e., $D_{\boldsymbol{x}}\boldsymbol{g} = \boldsymbol{\nu}D_{\boldsymbol{x}}\boldsymbol{r}$. On the other hand, our setting deals with these two separately, and the Sotomayer theorem can be reformulated as follows.

Criteria on a saddle-node bifurcation. Suppose that system (2) admits a steady state $\bar{\boldsymbol{x}}^b$ at $k_n = k_n^b$ associated with the nth reaction r_n. Assume that the following conditions hold:

(SN1) The augmented matrix **A** at $(\bar{\boldsymbol{x}}^b, k_n^b)$ has a simple eigenvalue 0 with left eigenvector **W** and right eigenvector **U**.

(SN2) The quantity $W_n \frac{\partial r_n}{\partial k_n}(\bar{\boldsymbol{x}}^b; k_n^b)$ is nonzero.

(SN3) The eigenvectors **W** and **U** satisfy $\mathbf{W}^\top[D_{\boldsymbol{x}}^2\boldsymbol{r}(\bar{\boldsymbol{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})] \neq 0$ where $\mathbf{U} = (\mathbf{u}^\top, \mathbf{v}^\top)^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$ determined by Theorem 1.

Then system (2) exhibits a saddle-node bifurcation at the steady state $\bar{\boldsymbol{x}}^b$ as the parameter k_n varies through the bifurcation value $k_n = k_n^b$. Precisely, there exist a $\delta > 0$ and a smooth curve $\Upsilon: J := (-\delta, \delta) \to \mathbb{R}^M \times \mathbb{R}$ such that the following holds:

$$\Upsilon(s) = (\bar{\boldsymbol{x}}(s), k_n(s)) \text{ and } \boldsymbol{f}(\bar{\boldsymbol{x}}(s); k_n(s)) = \mathbf{0} \text{ for } s \in J,$$

$$(\bar{\boldsymbol{x}}(0), k_n(0)) = (\bar{\boldsymbol{x}}^b, k_n^b) \text{ and } \dot{\bar{\boldsymbol{x}}}(0) = \mathbf{u},$$

$$\dot{k}_n(0) = 0 \text{ and } \ddot{k}_n(0) = -\frac{\mathbf{W}^\top[D_{\boldsymbol{x}}^2\boldsymbol{r}(\bar{\boldsymbol{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})]}{\frac{\partial r_n}{\partial k_n}(\bar{\boldsymbol{x}}^b; k_n^b)},$$

where $\mathbf{u}$ and $\mathbf{U}$ are related through the decomposition $\mathbf{U} = (\mathbf{u}^\top, \mathbf{v}^\top)^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$ determined by Theorem 1. Moreover, each steady state $\bar{\boldsymbol{x}}(s)$ with $s \neq 0$ is hyperbolic.

Proof. From Theorem 1 (with $\lambda = 0$), $D_{\boldsymbol{x}}\boldsymbol{f}(\bar{\boldsymbol{x}}^b; k_n^b)$ has a simple eigenvalue 0. Write **U** as $\mathbf{U} = (\mathbf{u}, \mathbf{v})^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$. Then $\mathbf{u}$ is a right eigenvector of $D_{\boldsymbol{x}}\boldsymbol{f}(\bar{\boldsymbol{x}}^b; k_n^b)$ by Theorem 1. Let $\mathbf{w}$ be its corresponding left eigenvector. Then applying Theorem 2 (with $\lambda = 0$) and using a suitable scaling if necessary, we may assume that $\mathbf{W} = \boldsymbol{\nu}^\top \mathbf{w}$. Thus, we can conclude that (SN1) implies (SN1°).

Now we claim that (SN2) implies (SN2°). To see this, recall that $\boldsymbol{f} = \boldsymbol{\nu}\boldsymbol{r}$, so $\boldsymbol{f}_{k_n}(\bar{\boldsymbol{x}}^b; k_n^b) = \boldsymbol{\nu}\boldsymbol{r}_{k_n}(\bar{\boldsymbol{x}}^b; k_n^b)$. Then it follows that

$$\mathbf{w}^\top \boldsymbol{f}_{k_n}(\bar{\boldsymbol{x}}^b; k_n^b) = \mathbf{W}^\top \boldsymbol{r}_{k_n}(\bar{\boldsymbol{x}}^b; k_n^b) = W_n \frac{\partial r_n}{\partial k_n}(\bar{\boldsymbol{x}}^b; k_n^b),$$

where in the last equality we have used the fact that $\partial r_{n'}/\partial k_n = 0$ for each $n' \neq n$. Thus (SN2) implies (SN2°).

Finally, we claim that (SN3) implies (SN3°). Indeed, from $D_{\boldsymbol{x}}^2\boldsymbol{f}(\bar{\boldsymbol{x}}^b; k_n^b) = \boldsymbol{\nu}D_{\boldsymbol{x}}^2\boldsymbol{r}(\bar{\boldsymbol{x}}^b; k_n^b)$ it thus follows that

$$\mathbf{w}^\top[D_{\boldsymbol{x}}^2\boldsymbol{f}(\bar{\boldsymbol{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})] = \mathbf{W}^\top[D_{\boldsymbol{x}}^2\boldsymbol{r}(\bar{\boldsymbol{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})].$$

Hence (SN3) implies (SN3°).

Therefore, all of the conditions of Sotomayer's theorem are fulfilled. Hence, an application of Sotomayer's theorem establishes the assertions of this theorem.

V. BIFURCATION THEORY FOR BUFFERING STRUCTURE

In Sec. V A, we review a theorem called the *localization principle* [26,27], which states that for a subnetwork Γ satis-

fying a certain topological condition, the concentrations and fluxes outside Γ are independent of the values of parameters inside Γ. An important remark is that this principle is proved under the assumption that the augmented matrix **A** is non-singular. A natural question is what happens at a bifurcation point, where this assumption breaks down. In Sec. V B, we show that the localization principle can be extended including bifurcation points.

A. Review of localization principle

In order to explain the localization principle, we introduce some definitions. Construct a subnetwork $\Gamma = (\mathfrak{m}, \mathfrak{n})$, a pair of chemicals and reactions, as follows:

(1) Choose a subset $\mathfrak{m}$ of chemicals.

(2) Choose a subset $\mathfrak{n}$ of reactions such that $\mathfrak{n}$ includes all reactions whose reaction rates $r_n(\mathbf{x})$ depend on at least one member in $\mathfrak{m}$.

In other words, we construct $\mathfrak{n}$ by collecting all the reactions whose rates depend on at least one of $\mathfrak{m}$ and any other reactions. Note that for a given $\mathfrak{m}$, $\mathfrak{n}$ is not unique since we can add any other reactions to $\mathfrak{n}$. Below, we consider a subnetwork constructed this way and simply call it a *subnetwork*.

We then count the number, # cycles($\mathfrak{n}$), of independent cycles constructed from reaction arrows in $\mathfrak{n}$. More precisely, # cycles($\mathfrak{n}$) is defined as follows: For a reaction subset $\mathfrak{n}$, we introduce an associated vector space $V(\mathfrak{n}) \subset \mathbb{R}^N$:

$$V(\mathfrak{n}) := \text{ span} \left\{ \mathbf{v} \mid \mathbf{v} \in \ker \nu, P^{\mathfrak{n}} \mathbf{v} = \mathbf{v} \right\}.$$

Here, $P^{\mathfrak{n}}$ is an $N \times N$ projection matrix onto the space associated with $\mathfrak{n}$ defined by

$$P^{\mathfrak{n}}_{n,n'} = \delta_{n,n'} \text{ if } n, n' \in \mathfrak{n}, \text{ and } P^{\mathfrak{n}}_{n,n'} = 0 \text{ otherwise.}$$

In other words, $V(\mathfrak{n})$ is a vector space kernel consisting of kernel vectors of ν whose supports are contained in $\mathfrak{n}$. Then, the number of cycles, # cycles($\mathfrak{n}$), is defined as the dimension of the vector space, dim $V(\mathfrak{n})$.

The localization principle. Now, we state the theorem, which was originally proved in [26] (see also Appendix A for the review of the proof). For a subnetwork $\Gamma = (\mathfrak{m}, \mathfrak{n})$, we define a nonpositive *index* by

$$\chi(\Gamma) = |\mathfrak{m}| - |\mathfrak{n}| + (\text{\# cycles}). \tag{20}$$

If $\chi(\Gamma) = 0$, we call the subnetwork a *buffering structure*. The localization principle states that parameters outside a buffering structure Γ do not influence values of steady-state concentrations and fluxes outside Γ. More precisely, it holds that

$$\frac{\partial \bar{x}_m}{\partial k_{n'}} = 0, \quad \frac{\partial \bar{r}_n}{\partial k_{n'}} = 0, \tag{21}$$

for any $n' \in \mathfrak{n}$, $m \in \mathfrak{m}^c$, and $n \in \mathfrak{n}^c$, where $\mathfrak{m}^c = \mathbb{X} \setminus \mathfrak{m}$ and $\mathfrak{n}^c = \mathbb{E} \setminus \mathfrak{n}$ are the complementary set of $\mathfrak{m}$ and $\mathfrak{n}$, respectively.

B. Localization principle for bifurcation arising from buffering structure

Here, we prove that, when a bifurcation is caused by a reaction parameter inside Γ, then concentrations outside Γ will remain constant as the bifurcation parameter is varied

(see below for the precise statement). Note that this fact was observed numerically in a previous work [22], but a formal proof has been missing.

Localization principle for bifurcation arising from buffering structure. Let $\Gamma = (\mathfrak{m}, \mathfrak{n})$ be a buffering structure with $r_n \in \mathfrak{n}$. Suppose that a bifurcation of system (2) occurs at the bifurcation point $(\mathbf{x}, k_n) = (\bar{\mathbf{x}}^b, k_n^b)$ as the the rate parameter k_n associated with the reaction rate function r_n is varied through k_n^b. Also suppose that system (2) possesses a smooth bifurcation curve $(\bar{\mathbf{x}}(s), k_n(s))$ [here $s \in J := (-\delta, \delta)$ for some $\delta > 0$] of steady states in the $(\mathbf{x}, k_n)$ space, such that $(\bar{\mathbf{x}}(0), k_n(0)) = (\bar{\mathbf{x}}^b, k_n^b)$ and the augmented matrix **A** associated with $(\bar{\mathbf{x}}(s), k_n(s))$ is nonsingular for $s \in J \setminus \{0\}$. Then it holds that

$$x_m(s) = \bar{x}_m^b \text{ for each } m \notin \mathfrak{m} \text{ and } s \in J.$$

Proof. Since the **A** matrix is nonsingular for $s \in (-\delta, \delta) \setminus \{0\}$, the Localization principle (21) holds;

$$\frac{d\bar{x}_m(s)}{dk_n} = 0 \quad \forall m \notin \mathfrak{m} \text{ and } s \in J \setminus \{0\}.$$

With the use of the chain rule, this implies

$$\frac{d\bar{x}_m(s)}{ds} = \frac{d\bar{x}_m(s)}{dk_n} \cdot \frac{dk_n(s)}{ds} = 0$$

for $m \notin \mathfrak{m}$ and $s \in J \setminus \{0\}$. Since $(\bar{\mathbf{x}}(s), k_n(s))$ is smooth by assumption, we have $\frac{d\bar{x}_m}{ds}(0) = 0$ for $m \notin \mathfrak{m}$. Taken together, it follows that $\bar{x}_m(s) = \bar{x}_m(0) = \bar{x}_m^b$ for all $m \notin \mathfrak{m}$ and $s \in J$. The proof is thus completed.

VI. EXAMPLES

In this section, we illustrate our theory using hypothetical and real networks.

A. Saddle-node bifurcation: Example 1

Consider the following reaction system whose reaction scheme is depicted in Fig. 2:

$$\frac{d}{dt}\begin{pmatrix} x_A \\ x_B \\ x_C \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & 1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \end{pmatrix}$$

$$= \begin{pmatrix} 1 & -1 & 0 & 1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} k_1 \\ k_2 x_A \\ k_3 x_B \\ f(x_A; k_4) x_c \\ k_5 x_C \end{pmatrix}$$

$$= \begin{pmatrix} k_1 - k_2 x_A + f(x_A; k_4) x_c \\ k_2 x_A - k_3 x_B \\ k_3 x_B - f(x_A; k_4) x_c - k_5 x_C \end{pmatrix}. \tag{22}$$

Here we assume mass-action kinetics for rate functions r_i, except that $r_4 = x_C f(x_A; k_4)$ is regulated by chemical A through the function $f(x_A; k_4)$. We use the parameter k_4 as a bifurcation parameter, and fix a set of positive parameters (k_1, k_2, k_3, k_5). As we described in Sec. IV, our formalism allows us to express the condition of a bifurcation occurrence
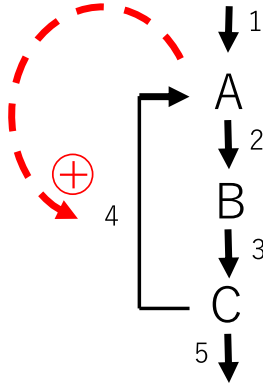
FIG. 2. Reaction scheme consisting of five reactions: 1: $\emptyset \to$ A, 2: A $\to$ B, 3: B $\to$ C, 4: C $\to$ A, 5: C $\to \emptyset$. The fourth reaction is positively regulated by A, i.e., $r_4 = r_4(x_A, x_C)$.

in terms of reactions. As demonstrated below, the bifurcation condition is expressed as a constraint on reaction r_4 (or equivalently on f_4).

The steady state $\bar{x} = (\bar{x}_A, \bar{x}_B, \bar{x}_C)$ is given by

$$\frac{k_2 k_5}{k_1} \bar{x}_A - k_5 = f(\bar{x}_A; k_4), \quad \bar{x}_B = \frac{k_2}{k_3} \bar{x}_A, \quad \bar{x}_C = \frac{k_1}{k_5}. \quad (23)$$

By permuting the row indices as {2, 3, 4, 5, 1} and the column indices as {A, B, c_1, C, c_2}, the matrix **A** becomes

$$\mathbf{A} = \begin{pmatrix} k_2 & 0 & 1 & 0 & 1 \\ 0 & k_3 & 1 & 0 & 1 \\ \bar{x}_C \frac{\partial f}{\partial x_A}(\bar{x}_A; k_4) & 0 & 1 & f(\bar{x}_A; k_4) & 0 \\ 0 & 0 & 0 & k_5 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \quad (24)$$

Since the determinant is $\det \mathbf{A} = (k_2 - \bar{x}_C \frac{\partial f}{\partial x_A}) k_3 k_5$, a bifurcation occurs provided that the bifurcation parameter value $k_4 = k_4^b$ is determined by the constraint

$$k_2 - x_C^b \frac{\partial f}{\partial x_A}(x_A^b; k_4^b) = 0. \quad (25)$$

Then, for such a bifurcation parameter value k_4^b, the matrix **A** given by (24) becomes

$$\mathbf{A} = \begin{pmatrix} k_2 & 0 & 1 & 0 & 1 \\ 0 & k_3 & 1 & 0 & 1 \\ k_2 & 0 & 1 & f(x_A^b; k_4^b) & 0 \\ 0 & 0 & 0 & k_5 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}. \quad (26)$$

One can verify that 0 is a simple eigenvalue of **A**, and the corresponding right and left null vectors are, respectively, given by

$$\mathbf{U} = \begin{pmatrix} u_A \\ u_B \\ v_1 \\ u_C \\ v_2 \end{pmatrix} = \begin{pmatrix} -\frac{1}{k_2} \\ -\frac{1}{k_3} \\ 1 \\ 0 \\ 0 \end{pmatrix}, \quad \mathbf{W}^\top = \begin{pmatrix} W_2 \\ W_3 \\ W_4 \\ W_5 \\ W_1 \end{pmatrix} = \begin{pmatrix} -k_5 \\ 0 \\ k_5 \\ -f(x_A^b; k_4^b) \\ k_5 + f(x_A^b; k_4^b) \end{pmatrix}. \quad (27)$$

Next, we will examine (SN2) and (SN3), which can be regarded as constraints on the reaction rate function, $r_4 \propto$
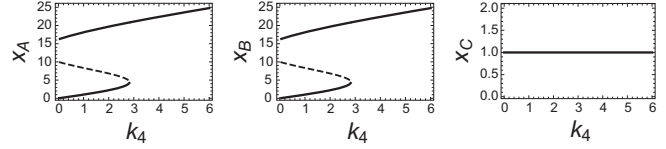
$f(x_A, k_4)$. The condition (SN2), $W_4 \frac{\partial r_4}{\partial k_4} \neq 0$, gives

$$\left. \frac{\partial f(x_A; k_4)}{\partial k_4} \right|_{x_A = x_A^b, k_4 = k_4^b} \neq 0. \quad (28)$$

The condition (SN3) is $\sum_{n=1}^{5} \sum_{m,m'=A,B,C} W_n \frac{\partial^2 r_n}{\partial x_m \partial x_{m'}} u_m u_{m'} \neq 0$. Note that only the reaction $r_4 = f(x_A; k_4) x_C$ is a nonlinear function and contributes to the sum on the left-hand side. Further, since $\frac{\partial r_4}{\partial x_B} = 0$ and $u_C = 0$, only the contribution from $m, m' = A$ is nonzero. Thus, the condition (SN3) is equivalent to

$$\left. \frac{\partial^2 f(x_A; k_4)}{\partial x_A^2} \right|_{x_A = x_A^b, k_4 = k_4^b} \neq 0. \quad (29)$$

Therefore, for $f(x_A; k_4)$ satisfying the conditions (25) and (28)–(29), the system (22) exhibits a saddle-node bifurcation. Figure 3 shows a numerical illustration, assuming a particular choice of $f(x_A, k_4)$.

The conditions (25) and (28)–(29) give local behaviors of the function $f(\bar{x}_A; k_4)$ near (x_A^b, k_4^b). From (23), the component $\bar{x}_A$ of the steady states is given by the intersection point of the line l: $y = \frac{k_2 k_5}{k_1} \bar{x}_A - k_5$ and the curve $\mathcal{C}_{k_4}$: $y = f(\bar{x}_A; k_4)$ in the $\bar{x}_A y$ plane (see Fig. 4). Then, these conditions mean that the line l is the tangent line to the curve $\mathcal{C}_{k_4}$, and, as k_4 is varied, the line l is passed by the curve $\mathcal{C}_{k_4}$ locally, leaving a pair of fixed points.

The numerical result in Fig. 3 shows that for the bifurcation triggered by the parameter k_4 associated with the reaction
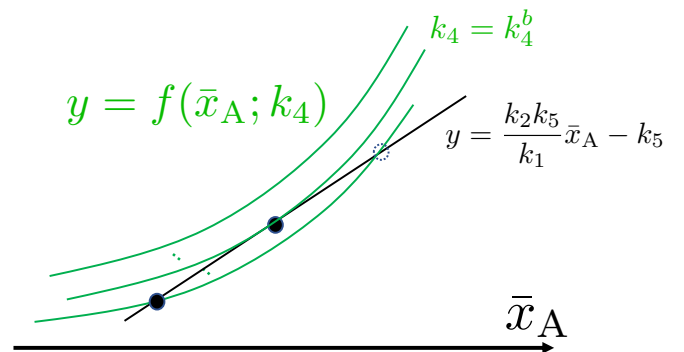

FIG. 3. Bifurcation diagram of steady states $(\bar{x}_A, \bar{x}_B, \bar{x}_C)$ as the bifurcation parameter k_4 is varied. The stable branches and unstable one are represented by solid and dashed lines, respectively. The middle branch and the lower one collide around $k_4 = 2.8303$. Here, we choose $k_1 = 1$, $k_2 = 1$, $k_3 = 1$, $k_5 = 1$, and $f = 20 \frac{(0.1 x_A)^2}{1 + (0.1 x_A)^2} + k_4$.

FIG. 4. The intersection of the curve $\mathcal{C}_{k_4}$: $y = f(\bar{x}_A; k_4)$ and the line l: $y = \frac{k_2 k_5}{k_1} \bar{x}_A - k_5$ gives the component $\bar{x}_A$ of the steady states. The green curves represent the curve $\mathcal{C}_{k_4}$ for different values of k_4. When a saddle-node bifurcation occurs, the number of intersection points changes from zero to two as the parameter k_4 is varied.

r_4, the bifurcating behavior is observed only in chemicals A and B. In fact, this is the case independently of the choice about detailed kinetics, such as the mass-action or Michaelis-Menten types. To see this, we use the localization principle (see Sec. V). In this system, $\Gamma = (\{A, B\}, \{2, 3, 4\})$ is a buffering structure since Γ contains two chemicals, three reactions, and one cycle, composed of the reactions 2, 3, 4, i.e., $\chi = 2 - 3 + 1 = 0$. Therefore, according to the localization principle, any parameter associated with a reaction inside Γ does not trigger a bifurcation of the species outside Γ, namely C (and also fluxes $\bar{r}_1$ and $\bar{r}_5$). Thus, when k_4, which is a parameter inside Γ, is used as a bifurcation parameter, $\bar{x}_C$ cannot exhibit bifurcations (see the third panel of Fig. 3). Hence, the localization of bifurcating behavior in a buffering structure is due to the structural property of networks, that is, independent of the choice of reaction kinetics. We remark that, although we employed k_4 as a bifurcation parameter, a qualitatively similar bifurcation may also be observed by changing the other parameters. In general, different parameters may trigger qualitatively different bifurcations. On the other hand, we can systematically determine from network topology the set of parameters that can trigger qualitatively similar bifurcation behaviors which are shared by the same set of chemicals, by examining the inclusion relationship among buffering structures (see [22] for details).

B. Saddle-node bifurcation: Example 2: Glycolysis network

Consider the glycolysis network with outflow from PYR and outflow from G6P as depicted in Fig. 5, with allosteric regulations. This is a part of the central carbon metabolism pathway, wherein the glucose taken from the environment is decomposed into the metabolite pyruvate. The basic network structure is shared between bacteria and human. The details of the kinetics and parameter values in the glycolysis have not been fully understood. In fact, the reported values of parameters vary by orders of magnitude, depending on literature [17,18]. Here, we will examine the occurrence of bifurcations by using the information of the glycolysis network structure, without resorting to specific forms of reaction rates and parameter values. To do this, we only assume that $\frac{\partial r_n}{\partial x_m} > 0$ when $\boldsymbol{v}_{m,n} < 0$, as in the mass-action kinetics, and also take into account the three allosteric regulations [18]: $\frac{\partial r_5}{\partial x_{F26BP}} > 0$, $\frac{\partial r_6}{\partial x_{PEP}} < 0$, and $\frac{\partial r_{14}}{\partial x_{F16BP}} > 0$.

The stoichiometry matrix $\boldsymbol{v}$ for this reaction network is given by

$$\boldsymbol{v} = \begin{pmatrix}
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 1 & -1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1
\end{pmatrix}, \tag{30}$$

where the row indices are {GLC, G6P, F6P, F26BP, F16BP, DHAP, GAP, G13BPG, 3PG, 2PG, PEP, PYR}. The matrix **A** is

$$\mathbf{A} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
r_{2,1} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & r_{3,2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 \\
0 & 0 & r_{4,3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & r_{5,3} & \check{r}_{5,4} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\
0 & 0 & r_{6,3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \check{r}_{6,11} & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & r_{7,4} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & r_{8,5} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & r_{9,6} & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & r_{10,7} & 0 & 0 & 0 & 0 & 0 & 2 & 2 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{11,8} & 0 & 0 & 0 & 0 & 2 & 2 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{12,9} & 0 & 0 & 0 & 2 & 2 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{13,10} & 0 & 0 & 2 & 2 & 0 & 0 \\
0 & 0 & 0 & 0 & \check{r}_{14,5} & 0 & 0 & 0 & 0 & 0 & r_{14,11} & 0 & 2 & 2 & 0 & 0 \\
0 & r_{15,2} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{16,12} & 2 & 2 & 0 & 0
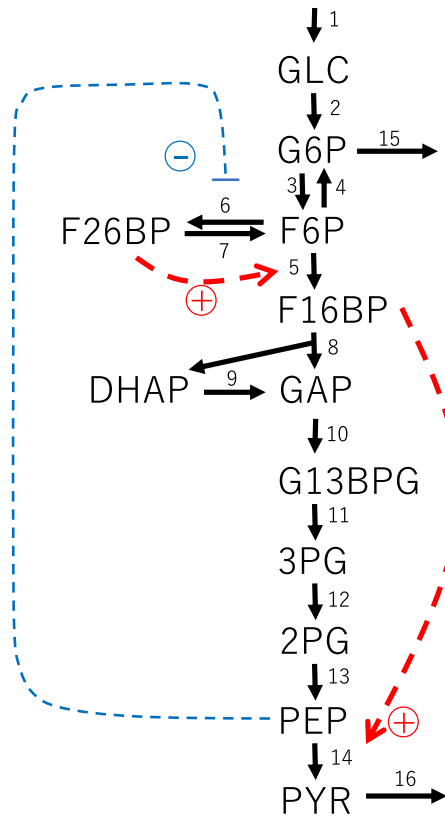\end{pmatrix}, \tag{31}$$

FIG. 5. Reaction network of the glycolysis pathway. The red and the blue dashed lines represent positive and negative allosteric regulations, respectively.

where $r_{n,m} = \frac{\partial r_n}{\partial x_m}|_{x=\bar{x}}$. In order to emphasize the allosteric regulations $r_n (n = 5, 6, 14)$, we use $\check{r}_{n,m}$ instead of $r_{n,m}$. The

determinant of the matrix $\mathbf{A}$ is

$$
\begin{aligned}
\det\mathbf{A} = r_{2,1}r_{9,6}r_{10,7}r_{11,8}r_{12,9}r_{13,10}r_{16,12}\{&r_{7,4}r_{8,5}r_{14,11}[r_{3,2}r_{5,3} \\
&+ (r_{4,3} + r_{5,3})r_{15,2}] + \check{r}_{5,4}(r_{3,2}r_{6,3}r_{8,5}r_{14,11} \\
&+ r_{15,2}[r_{6,3}r_{8,5}r_{14,11} + r_{4,3}\check{r}_{6,11}(-2r_{8,5} + \check{r}_{14,5})])\}.
\end{aligned}
$$
(32)

Since only $\check{r}_{6,11} = \frac{\partial r_6}{\partial x_{\text{PEP}}}$ is negative, this expression has a form like $\det\mathbf{A} = C_1 + C_2\check{r}_{5,4}\check{r}_{6,11}\check{r}_{14,5}$ with $C_i > 0$, where the product $\check{r}_{5,4}\check{r}_{6,11}\check{r}_{14,5}$, coming from the three allosteric regulations, is negative. Therefore, the sign of det $\mathbf{A}$ is generally indefinite, and so there is a possibility that the matrix $\mathbf{A}$ has a null eigenvector at some point of the parameter space. Note that for occurrence of bifurcations, the existence of all three allosteric regulations is necessary because otherwise the determinant would be $\det\mathbf{A} = C_1$, which is always positive.

For the mass-action kinetics or the Michaelis-Menten kinetics, the dependence of reaction rates on parameters is monotonic, and so $\frac{\partial r_n}{\partial k_n}|_{x=\bar{x}} \neq 0$ for $\bar{x} > 0$. Therefore, we can exclude possibilities of transcritical and pitchfork bifurcations as long as the concentrations of steady states are positive. In fact, the system can exhibit saddle-node bifurcations as shown Fig. 6, where, for illustration purposes, we assume the following simple kinetics:

$$
\begin{aligned}
\mathbf{r} = \Big\{&k_1, k_2x_{\text{GLC}}, k_3x_{\text{G6P}}, k_4x_{\text{F6P}}, \\
&k_5x_{\text{F6P}}\big(1 + R_5x_{\text{F26BP}}^2\big), \frac{k_6x_{\text{F6P}}}{1 + R_6x_{\text{PEP}}^2}, \\
&k_7x_{\text{F26BP}}, k_8x_{\text{F16BP}}, k_9x_{\text{DHAP}}, k_{10}x_{\text{GAP}}, \\
&k_{11}x_{\text{G13BPG}}, k_{12}x_{\text{3PG}}, k_{13}x_{\text{2PG}}, \\
&k_{14}x_{\text{PEP}}\big(1 + R_{14}x_{\text{F16BP}}^2\big), k_{15}x_{\text{G6P}}, k_{16}x_{\text{PYR}}\Big\}
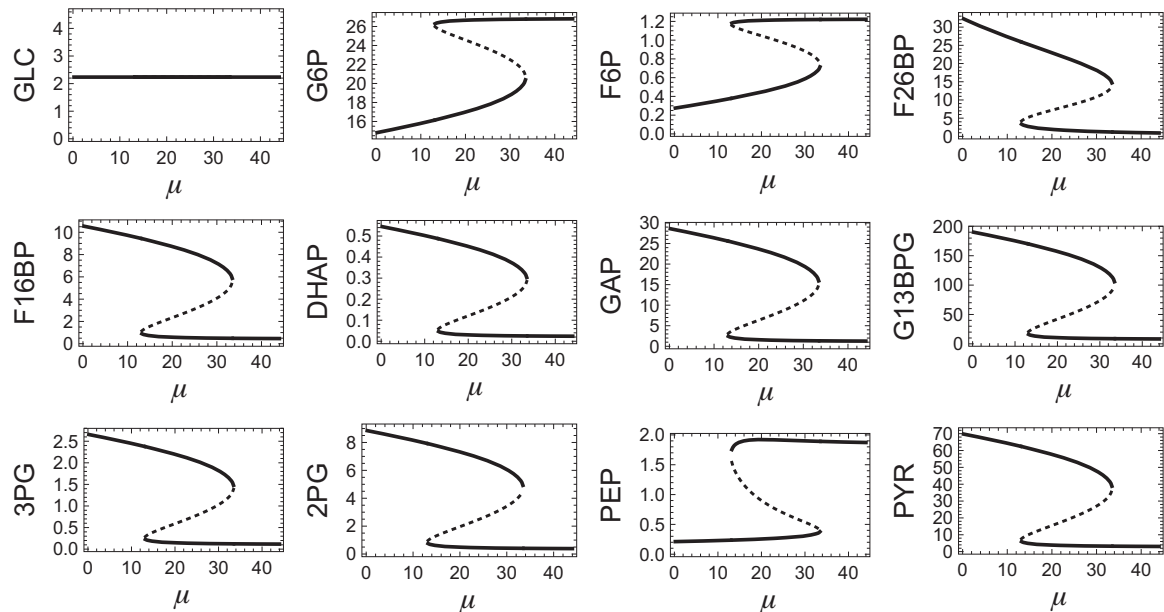\end{aligned}
$$
(33)

FIG. 6. Bifurcation diagram for the glycolysis network. The bifurcation parameter μ is the rate parameter for the allosteric regulation of reaction 6 by PEP, and the system exhibits saddle-node bifurcations at $\mu \approx 12.99$ and $\mu = 33.49$. Note that GLC does not exhibit bifurcations due to the localization principle.

with parameter values

$$k = \{10., 4.6, 0.54, 12., 0.16, 6.5, 0.055, 0.45, 8.7,$$

$$0.33, 0.050, 3.6, 1.1, 0.14, 0.38, 0.14\}, \quad (34)$$

$$\{R_5, R_6, R_{14}\} = \{0.1, \mu, 18\},$$

and use the strength of allosteric regulation of reaction 6 by PEP as a bifurcation parameter. One can confirm the conditions (SN2) and (SN3) for the saddle-node bifurcation numerically.

Note that the component $\bar{x}_{\mathrm{GLC}}$ of steady states remains constant as $\mu = R_6$ is varied (see the upper leftmost panel of Fig. 6). This can be again understood from the localization principle. In this network system, the subnetwork $\Gamma = (\mathfrak{m}, \mathfrak{r})$, where the set $\mathfrak{m}$ consists of all of the chemicals except GLC, and the set $\mathfrak{r}$ consists of reaction arrows emanating from all of the chemicals in $\mathfrak{m}$ (i.e., $\mathfrak{r} = \{3, 4, \ldots, 16\}$), forms a large buffering structure because the subnetwork Γ has 11 chemicals, 14 reactions, and 3 cycles, and so $\chi = 11 - 14 + 3 = 0$. Note that the cycles are given by the last three columns in (31), whose components are nonzero only for reactions within Γ. Therefore, from the localization principle, R_6, which is a parameter inside this buffering structure, does not influence the concentration $\bar{x}_{\mathrm{GLC}}$ (and also the fluxes $\bar{r}_1, \bar{r}_2$), which is outside the buffering structure Γ.

VII. CONCLUSION AND DISCUSSION

In modern biology, many reactions and their vast networks have been identified through the progress of biochemistry and molecular biology. In contrast, since the number of the ordinary differential equations (ODEs) associated with such network systems is extremely large, it is a challenging task to determine which rate parameter change can provide significant change in dynamical behavior, i.e., the occurrence of bifurcations. In this paper, a setting for bifurcation analysis of reaction networks is presented. Specifically, an augmented matrix, $\mathbf{A}$, is introduced, which splits the information of the reaction networks into two parts: the network structure and the reaction kinetics of networks. Then, a correspondence between the spectrum of the Jacobian matrix of a network system and that of the augmented matrix, $\mathbf{A}$, is established. This correspondence allows us to reformulate classical bifurcation criteria in terms of reaction kinetics and network structures (kernel and cokernel of $\mathbf{A}$) directly.

One of the advantages of the proposed setting is that the augmented matrix $\mathbf{A}$ allows us to distinguish between topology-dependent predictions and model-dependent ones. The reaction kinetics of the network systems do not necessarily obey mass-action laws, which are assumed in a number of previous theoretical studies (e.g., Clarke [7–9] and Feinberg and Craciun [11–14,28]). This feature of our approach is crucial in analyzing realistic network systems since it is difficult to determine the actual forms of reaction kinetics and rate parameter values in living cells.

Another advantage of this formalism is that the bifurcation criteria in terms of this framework not only appear elegant, but also indicate which reaction may cause specific bifurcations. For example, suppose that system (2) admits a steady state, $\bar{x}^b$, at $k_n = k_n^b$, associated with the nth reaction, r_n. Then,

the proposed theory indicates that depending on whether the quantity, $\frac{\partial r_n}{\partial k_n}(\bar{x}^b; k_n^b)$, is nonzero, system (2) admits a saddle-node bifurcation or transcritical/pitchfork bifurcation. Hence, this formalism enables efficient determination of relevant reactions for bifurcations.

We can expect that our method is useful to identify important regulations and reactions for biological functions. Our prior knowledge of network topology is possibly incomplete. By formulating bifurcation phenomena in terms of network structures, we can determine which reactions or subnetworks are responsible for considered biological functions. Our structural approach may also suggest a structural origin of the robustness and plasticity of biological systems, because parameters k_i for living cells can be affected by environmental and extrinsic factors.

In this study, Hopf bifurcations are not investigated, which are essential for oscillatory behavior of network systems. For Hopf bifurcations, the corresponding eigenspace of the Jacobian matrix, $\mathbf{J}$, and augmented matrix, $\mathbf{A}$, are not isomorphic. The fundamental theorem (Theorem 1) indicates that to fix this issue, an additional structure matrix, $\boldsymbol{\rho}$, has to be employed to form matrix $\mathbf{A}_{\rho, \lambda}$ defined by (12). Although the modified matrix, $\mathbf{A}_{\rho, \lambda}$, may not share desirable properties of matrix $\mathbf{A}$, such as factorization, its eigenspace is isomorphic to that of the Jacobian matrix, $\mathbf{J}$. The Hopf bifurcations using the proposed setting will be investigated in a future study.

APPENDIX A: REVIEW OF SOME PREVIOUS RESULTS

In order to make the paper self-contained, we review some of the previous results which we use in the main text.

1. Derivation of (7)

We review the derivation of (7) following [21]. Here, as in the main text, we assume that the stoichiometry matrix $\boldsymbol{\nu}$ does not have nonzero cokernel vectors. See [27] for the generalization to the case of dim coker $\nu > 0$, namely, systems with conserved quantities.

Consider the steady-state $\bar{x}$ of the system (2). In (4), the steady-state reaction rate $\bar{r}_n$ depends not only on its own parameter k_n but also on other parameters through $\boldsymbol{x}$, namely $\bar{r}_n = \bar{r}_n(\bar{x}; (k)k_n)$, and ζ^α also depends on $\boldsymbol{k}$. By differentiating the nth component of (4) with respect to the parameter $k_{n'}$ of the n'th reaction, we have

$$\sum_{m=1}^M \frac{\partial r_n}{\partial x_m}\bigg|_{\boldsymbol{x}=\bar{\boldsymbol{x}}} \frac{\partial \bar{x}_m}{\partial k_{n'}} + \delta_{nn'} \frac{\partial r_n}{\partial k_{n'}}\bigg|_{\boldsymbol{x}=\bar{\boldsymbol{x}}} = \sum_\alpha \frac{\partial \zeta^\alpha}{\partial k_{n'}} (\mathbf{c}_\alpha)_n. \quad \text{(A1)}$$

By using the augmented matrix **A** defined in (5), this equation can be rewritten in matrix notation as

$$
\mathbf{A}\begin{pmatrix} \frac{\partial \boldsymbol{x}}{\partial k_{n'}} \\ \frac{\partial \boldsymbol{\zeta}}{\partial k_{n'}} \end{pmatrix} = -\mathrm{diag}\Big(0,\ldots,\underbrace{\frac{\partial r_{n'}}{\partial k_{n'}}\Big|_{\boldsymbol{x}=\bar{\boldsymbol{x}}}}_{n'\text{th}},\ldots,0\Big), \qquad (A2)
$$

where the right-hand side is the diagonal matrix whose n'th element is nonzero and given by $\frac{\partial \bar{r}'_n}{\partial k'_n}\big|_{\boldsymbol{x}=\bar{\boldsymbol{x}}}$. If **A** is nonsingular, by inverting the matrix, we obtain (7).

2. Derivation of (21)

Here, we prove (21) assuming that the stoichiometry matrix $\boldsymbol{v}$ does not have nonzero cokernel vectors. See [27] for the generalization to the case of dim coker $v > 0$.

Suppose that the system (1) has a buffering structure Γ. By permutating the column and row indices, the augmented matrix **A** can be written as follows [26]:

$$
\mathbf{A} = \; |\mathfrak{n}|\updownarrow \overset{\overset{|\mathfrak{m}|+(\#cycle)}{\longleftrightarrow}}{\left(\begin{array}{c|c} \mathbf{A}_\Gamma \\ \text{square} & * \\ \hline 0 & \mathbf{A}_{\bar{\Gamma}} \end{array}\right)}, \qquad (A3)
$$

where the rows (columns) of $\mathbf{A}_\Gamma$ are associated with the reactions (chemicals and cycles) in Γ. Similarly, those of $\mathbf{A}_{\bar{\Gamma}}$ are associated with the complement $\bar{\Gamma}$ of the subnetwork Γ. The fact that the bottom-left block of (A3) is the zero matrix comes from the following two properties: (i) From the construction of the subnetwork Γ, the reactions that are regulated by $\mathfrak{m}$ must be included in $\mathfrak{n}$. In other words, rates of reactions in $\mathfrak{n}^c$ are independent of chemicals in $\mathfrak{m}$, that is, $\frac{\partial r_n}{\partial x_m} = 0$ for $n \in \mathfrak{n}^c$ and $m \in \mathfrak{m}$. (ii) By definition, the cycles in Γ, which appear in columns in the left part of (A3), do not have support in $\mathfrak{n}^c$.

The condition $\chi(\Gamma) = 0$ of a buffering structure implies that the upper-left matrix of (A3) is a square matrix, and so is the bottom-right matrix. A matrix with such a block structure can be easily inverted, and the inverse also preserves the same block structure,

$$
\mathbf{A}^{-1} = \left(\begin{array}{c|c} \mathbf{A}_\Gamma^{-1} & * \\ \hline 0 & \mathbf{A}_{\bar{\Gamma}}^{-1} \end{array}\right). \qquad (A4)
$$

Recall that the steady-state sensitivity is given by inverting the augmented matrix **A** [see (7)]. The zero matrix appearing in the left-bottom corner of (A4), in particular, implies the first equation of (21), $\frac{\partial \bar{x}_m}{\partial k_{n'}} = 0$ for $m \in \mathfrak{m}^c$ and $n' \in \mathfrak{n}$. Then, the second equation immediately follows since, for $n \in \mathfrak{n}'$ and $n \in \mathfrak{n}^c$,

$$
\frac{\partial r_n}{\partial k_{n'}} = \sum_{m=1}^M \frac{\partial r_n}{\partial x_m}\Big|_{\boldsymbol{x}=\bar{\boldsymbol{x}}} \frac{\partial \bar{x}_m}{\partial k_{n'}} = \sum_{m\in\mathfrak{m}^c} \frac{\partial r_n}{\partial x_m}\Big|_{\boldsymbol{x}=\bar{\boldsymbol{x}}} \frac{\partial \bar{x}_m}{\partial k_{n'}} = 0. \quad (A5)
$$

APPENDIX B: PROOF OF THEOREM 1

In order to facilitate notations, we will not distinguish the linear transformation from its matrix representation.

Consider the linear transformations

$$
\boldsymbol{K}_{\boldsymbol{v}}: \; \mathbb{C}^{N-M} \to \mathbb{C}^N, \quad \text{with } \mathrm{Im}(\boldsymbol{K}_{\boldsymbol{v}}) = \ker(\boldsymbol{v}), \text{ and}
$$

$$
\boldsymbol{\rho}: \; \mathbb{C}^M \to \mathbb{C}^N, \quad \text{with } \boldsymbol{v}\boldsymbol{\rho} = id_{\mathbb{C}^M},
$$

where $\mathrm{Im}(\boldsymbol{K}_{\boldsymbol{v}})$ is the image of $\boldsymbol{K}_{\boldsymbol{v}}$. Note that due to the one-to-one property of $\boldsymbol{v}$ and the relation $\boldsymbol{v}\boldsymbol{\rho} = I_{\mathbb{C}^M}$, $\boldsymbol{\rho}$ is one-to-one and its image $\mathrm{Im}(\boldsymbol{\rho})$ is equal to the row space $\mathrm{row}(\boldsymbol{v})$ of $\boldsymbol{v}$. Thus, $\mathbb{C}^N$ is the direct sum of $\mathrm{Im}(\boldsymbol{\rho})$ and $\ker(\boldsymbol{v})$, i.e.,

$$
\mathbb{C}^N = \mathrm{Im}(\boldsymbol{\rho}) \oplus \ker(\boldsymbol{v}).
$$

Thus, each $u \in \mathbb{C}^N$ can be uniquely expressed as $u = x + y$ with $x \in \ker(\boldsymbol{v})$ and $y \in \mathrm{Im}(\boldsymbol{\rho})$. This allows us to define the following two projection maps:

$$
P_0: \mathbb{C}^N \to \ker(\boldsymbol{v}), \quad P_0(u) = x, \text{ and}
$$

$$
P_1: \mathbb{C}^N \to \mathrm{Im}(\boldsymbol{\rho}), \quad P_1(u) = y.
$$

Therefore, P_0 is the projection of $\mathbb{C}^N$ onto $\ker(\boldsymbol{v})$ along $\mathrm{Im}(\boldsymbol{\rho})$, while P_1 is the projection of $\mathbb{C}^N$ onto $\mathrm{Im}(\boldsymbol{\rho})$ along $\ker(\boldsymbol{v})$. In the proof of the following theorem, we will see that

$$
P_1 \equiv \boldsymbol{\rho} \circ \boldsymbol{v} \text{ on } \mathbb{C}^N.
$$

Also recall the following $N \times N$ matrix:

$$
\mathbf{A}_{\boldsymbol{\rho},\lambda} := [\mathbf{D} - \lambda\boldsymbol{\rho} \,|\, \boldsymbol{K}_{\boldsymbol{v}}].
$$

First, we show that $\mathcal{F}$ is well defined. Indeed, for each $\mathbf{u} \in \mathbb{C}^M$, since P_0 is the projection onto $\ker(\boldsymbol{v})$ and $\boldsymbol{K}_{\boldsymbol{v}}$ is one-to-one, the existence and uniqueness of $\mathbf{v}$ determined by (18) is guaranteed. Also the $\mathbf{v}$ depends linearly on $\mathbf{u}$ since the map $\mathbf{D}$, P_0, and $\boldsymbol{K}_{\boldsymbol{v}}$ are linear. Next, we claim that for each $\mathbf{u} \in \ker(\boldsymbol{v}\mathbf{D} - \lambda\mathbf{1}_M)$, $(\mathbf{u}, \mathbf{v}) \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda})$ with $\mathbf{v} \in \mathbb{C}^{N-M}$ defined by (18). Indeed, $\boldsymbol{v}\mathbf{D}\mathbf{u} - \lambda\mathbf{u} = 0$, and so

$$
\boldsymbol{\rho}(\boldsymbol{v}\mathbf{D}\mathbf{u}) - \lambda\boldsymbol{\rho}(\mathbf{u}) = 0. \qquad (B1)
$$

Let $\{\mathbf{e}_1, \ldots, \mathbf{e}_M\}$ be the canonical basis of $\mathbb{C}^M$, and set $\boldsymbol{\rho}_m = \boldsymbol{\rho}(\mathbf{e}_m)$ for $m = 1, \ldots, M$. Thus, $\{\boldsymbol{\rho}_1, \ldots, \boldsymbol{\rho}_M\}$ is a basis of $\mathrm{Im}(\boldsymbol{\rho})$, and so $P_1(\mathbf{D}\mathbf{u}) = \sum_{m=1}^M b_m \boldsymbol{\rho}_m$ for some $b_m \in \mathbb{C}$ and $m = 1, \ldots, M$. Then we have

$$
\boldsymbol{\rho}(\boldsymbol{v}\mathbf{D}\mathbf{u}) = \boldsymbol{\rho}(\boldsymbol{v}(P_0(\mathbf{D}\mathbf{u}) + P_1(\mathbf{D}\mathbf{u})))
$$
$$
= \boldsymbol{\rho}(\boldsymbol{v}(P_1(\mathbf{D}\mathbf{u})))
$$
$$
= \sum_{m=1}^M b_m \boldsymbol{\rho}(\boldsymbol{v}\boldsymbol{\rho}_m)
$$
$$
= \sum_{m=1}^M b_m \boldsymbol{\rho}(\mathbf{e}_m) \quad (\text{by } \boldsymbol{v}\boldsymbol{\rho} = I_{\mathbb{C}^M})
$$
$$
= P_1(\mathbf{D}\mathbf{u}).
$$

This, together with (18) and (B1), gives

$$
\mathbf{D}\mathbf{u} = P_1(\mathbf{D}\mathbf{u}) + P_0(\mathbf{D}\mathbf{u})
$$
$$
= \boldsymbol{\rho}(\boldsymbol{v}\mathbf{D}\mathbf{u}) - \boldsymbol{K}_{\boldsymbol{v}}(\mathbf{v})
$$
$$
= \lambda\boldsymbol{\rho}(\mathbf{u}) - \boldsymbol{K}_{\boldsymbol{v}}(\mathbf{v}).
$$

Thus, we have $(\mathbf{D} - \lambda\boldsymbol{\rho})(\mathbf{u}) + \boldsymbol{K}_{\boldsymbol{v}}(\mathbf{v}) = 0$, so $(\mathbf{u}, \mathbf{v}) \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda})$.

Now we show that $\mathcal{F}$ is one-to-one. For this, let $(\mathbf{u}, \mathbf{v}) \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda})$ with $\mathbf{v} \in \mathbb{C}^{N-M}$ defined by (18). We claim that $\mathbf{u} \in \ker(\boldsymbol{v}\mathbf{D} - \lambda\mathbf{1}_M)$. Indeed, we have $(\mathbf{D} - \lambda\boldsymbol{\rho})(\mathbf{u}) + \boldsymbol{K}_{\boldsymbol{v}}(\mathbf{v}) = 0$. Now applying $\boldsymbol{v}$ to this equation and using the relations $\mathrm{Im}(\boldsymbol{K}_{\boldsymbol{v}}) = \ker(\boldsymbol{v})$ and $\boldsymbol{v}\boldsymbol{\rho} = I_{\mathbb{C}^M}$, we have $\boldsymbol{v}\mathbf{D}\mathbf{u} - \lambda\mathbf{u} = 0$. This establishes $\mathbf{u} \in \ker(\boldsymbol{v}\mathbf{D} - \lambda\mathbf{1}_M)$. Finally, from the definition

of $\mathcal{F}$ it follows that $\mathcal{F}$ is one-to-one. The proof is thus completed.

APPENDIX C: PROOF OF THEOREM 2

Here, we give a proof of Theorem 2, which states the correspondence between the spaces $\ker(\mathbf{D}^\top \boldsymbol{\nu}^\top - \lambda \mathbf{1}_M)$ and $\ker(\mathbf{A}_{\boldsymbol{\rho},\lambda}^\top)$. To proceed, recall that the linear transformation $\boldsymbol{\rho}\colon \mathbb{C}^M \to \mathbb{C}^N$ satisfies $\boldsymbol{\nu}\boldsymbol{\rho} = I_{\mathbb{C}^M}$. Thus,

$$\boldsymbol{\rho}^\top\colon \mathbb{C}^N \to \mathbb{C}^M \text{ satisfies } \boldsymbol{\rho}^\top \boldsymbol{\nu}^\top = I_{\mathbb{C}^M}.$$

Note that due to the fact that $\ker(\boldsymbol{\nu}^\top) = 0$, we have

$$\mathrm{Im}(\boldsymbol{\rho}^\top) = \mathbb{C}^M.$$

Also recall the $N \times N$ matrix $\mathbf{A}_{\boldsymbol{\rho},\lambda} := [\mathbf{D} - \lambda \boldsymbol{\rho} \,|\, K_{\boldsymbol{\nu}}]$. Thus,

$$\mathbf{A}_{\boldsymbol{\rho},\lambda}^\top := \left(\frac{\mathbf{D}^\top - \lambda \boldsymbol{\rho}^\top}{K_{\boldsymbol{\nu}}^\top}\right).$$

We first show that $\tilde{\mathcal{F}}$ is well defined. For this, we claim that $\boldsymbol{\nu}^\top \mathbf{w} \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda}^\top)$ for $\mathbf{w} \in \ker(\mathbf{D}^\top \boldsymbol{\nu}^\top - \lambda \mathbf{1}_M)$. Indeed, $\mathbf{D}^\top \boldsymbol{\nu}^\top \mathbf{w} - \lambda \mathbf{w} = 0$ by the definition of $\mathbf{w}$. Now applying $\boldsymbol{\rho}^\top \boldsymbol{\nu}^\top$ to this equation and using the relation $\boldsymbol{\rho}^\top \boldsymbol{\nu}^\top = I_{\mathbb{C}^M}$, we have $\mathbf{D}^\top(\boldsymbol{\nu}^\top \mathbf{w}) - \lambda \boldsymbol{\rho}^\top(\boldsymbol{\nu}^\top \mathbf{w}) = 0$. This establishes $\boldsymbol{\nu}^\top \mathbf{w} \in \ker(\mathbf{D}^\top - \lambda \boldsymbol{\rho}^\top)$. Also from $\mathrm{Im}(K_{\boldsymbol{\nu}}) = \ker(\boldsymbol{\nu})$, it follows that $\boldsymbol{\nu}^\top \mathbf{w} \in \ker(K_{\boldsymbol{\nu}}^\top)$. Taken together, these establish $\boldsymbol{\nu}^\top \mathbf{w} \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda}^\top)$.

Now we show that $\tilde{\mathcal{F}}$ is one-to-one. Let $\mathbf{W} \in \ker(\mathbf{A}_{\boldsymbol{\rho},\lambda}^\top)$. Then $K_{\boldsymbol{\nu}}^\top(\mathbf{W}) = \mathbf{0}$, and so $\mathbf{u}^\top \mathbf{W} = 0$ for each $\mathbf{u} \in \mathrm{Im}(K_{\boldsymbol{\nu}}) = \ker(\boldsymbol{\nu})$. Thus $\mathbf{W}$ is in the row space $\mathrm{row}(\boldsymbol{\nu})$, and so $\mathbf{W} = \boldsymbol{\nu}^\top \mathbf{w}$ for some $\mathbf{w} \in \mathbb{C}^M$. This, together with $\mathbf{W} \in \ker(\mathbf{D}^\top - \lambda \boldsymbol{\rho}^\top)$, gives $D^\top(\boldsymbol{\nu}^\top \mathbf{w}) - \lambda \boldsymbol{\rho}^\top \boldsymbol{\nu}^\top \mathbf{w} = 0$. Recall that $\boldsymbol{\rho}^\top \boldsymbol{\nu}^\top = I_{\mathbb{C}^M}$. This in turn implies $\mathbf{w} \in \ker(\mathbf{D}^\top \boldsymbol{\nu}^\top - \lambda \mathbf{1}_M)$, and so $\tilde{\mathcal{F}}$ is one-to-one.

Finally, $\tilde{\mathcal{F}}$ is one-to-one since $\boldsymbol{\nu}^\top$ is one-to-one. The proof is thus completed.

APPENDIX D: REFORMULATION OF BIFURCATION CRITERIA OF TRANSCRITICAL AND PITCHFORK BIFURCATIONS

1. Transcritical bifurcation

As with the saddle-node bifurcation, we can reformulate the classical theorems on the other two types of bifurcations in terms of the augmented matrix $\mathbf{A}$. The following theorem states the conditions for a transcritical bifurcation in terms of our setting. The proof is given in Appendix E.

Criteria on a transcritical bifurcation. Suppose that system (2) admits a steady state $\bar{\mathbf{x}}^b$ at $k_n = k_n^b$ associated with the nth reaction r_n. Assume that the following conditions hold:

(T1) The augmented matrix $\mathbf{A}$ at $(\bar{\mathbf{x}}^b, k_n^b)$ has a simple eigenvalue 0 with left eigenvector $\mathbf{W}$ and right eigenvector $\mathbf{U}$.

(T2) The quantity $\frac{\partial r_n}{\partial k_n}(\bar{\mathbf{x}}^b; k_n^b)$ is zero.

(T3) The eigenvectors $\mathbf{W}$ and $\mathbf{U}$ satisfy

$$\begin{cases} W_n\big[\nabla_{\boldsymbol{x}}\big(\frac{\partial r_n}{\partial k_n}\big)(\bar{\mathbf{x}}^b; k_n^b) \cdot \mathbf{u}\big] \neq 0, \\ \mathbf{W}^\top\big[D_{\boldsymbol{x}}^2 \boldsymbol{r}(\bar{\mathbf{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})\big] \neq 0, \end{cases}$$

where the vectors $\mathbf{u}$ and $\mathbf{U}$ are related through the decomposition $\mathbf{U} = (\mathbf{u}^\top, \mathbf{v}^\top)^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$ determined by Theorem 1.

Then similar conclusions in Theorem I hold for system (2). Precisely, there exist a $\delta \in (0, \delta_0)$ and two smooth functions $\bar{\mathbf{x}}^\pm\colon J := (k_n^b - \delta, k_n^b + \delta) \to \mathbb{R}^M$ such that the following holds:

$$\bar{\mathbf{x}}^\pm(k_n^b) = \bar{\mathbf{x}}^b \text{ and } \boldsymbol{f}(\bar{\mathbf{x}}^\pm(k_n); k_n) = 0 \text{ for } k_n \in J,$$

$$\text{and} \begin{cases} \dot{\bar{\mathbf{x}}}^-(k_n^b) = \mathbf{0}, \\ \dot{\bar{\mathbf{x}}}^+(k_n^b) = \dfrac{-2W_n\big[\nabla_{\boldsymbol{x}}\big(\frac{\partial r_n}{\partial k_n}\big)(\bar{\mathbf{x}}^b; k_n^b) \cdot \mathbf{u}\big]}{\mathbf{W}^\top\big[D_{\boldsymbol{x}}^2 \boldsymbol{r}(\bar{\mathbf{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})\big]}. \end{cases}$$

Moreover, each steady state $\bar{\mathbf{x}}^\pm(k_n)$ with $k_n \neq k_n^b$ is hyperbolic.

2. Pitchfork bifurcation

The following theorem states the conditions for a pitchfork bifurcation in terms of our setting.

Criteria on a pitchfork bifurcation. Suppose that system (2) admits a steady state $\bar{\mathbf{x}}^b$ at $k_n = k_n^b$ associated with the nth reaction r_n. Assume that the following conditions hold:

(P1) The augmented matrix $\mathbf{A}$ at $(\bar{\mathbf{x}}^b, k_n^b)$ has a simple eigenvalue 0 with left eigenvector $\mathbf{W}$ and right eigenvector $\mathbf{U}$.

(P2) The quantity $\frac{\partial r_n}{\partial k_n}(\bar{\mathbf{x}}^b; k_n^b)$ is zero.

(P3) The eigenvectors $\mathbf{W}$ and $\mathbf{U}$ satisfy

$$\begin{cases} W_n\big[\nabla_{\boldsymbol{x}}\big(\frac{\partial r_n}{\partial k_n}\big)(\bar{\mathbf{x}}^b; k_n^b) \cdot \mathbf{u}\big] \neq 0, \\ \mathbf{W}^\top\big[D_{\boldsymbol{x}}^2 \boldsymbol{r}(\bar{\mathbf{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u})\big] = 0, \\ \mathbf{W}^\top\big[D_{\boldsymbol{x}}^3 \boldsymbol{r}(\bar{\mathbf{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u}, \mathbf{u})\big] \neq 0, \end{cases}$$

where the vectors $\mathbf{u}$ and $\mathbf{U}$ are related through the decomposition $\mathbf{U} = (\mathbf{u}^\top, \mathbf{v}^\top)^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$ determined by Theorem 1.

Then similar conclusions in Theorem II hold for system (2). Precisely, there exist a $\delta > 0$, a smooth curve $\Upsilon\colon J_1 := (-\delta, \delta) \to \mathbb{R}^M \times \mathbb{R}$, and a smooth function $\mathbf{z}\colon J_2 := (k_n^b - \delta, k_n^b + \delta) \to \mathbb{R}^M$ such that the following hold:

(i)

$$\Upsilon(s) = (\bar{\mathbf{x}}(s), k_n(s)) \text{ and } \boldsymbol{f}(\bar{\mathbf{x}}(s); k_n(s)) = \mathbf{0} \text{ for } s \in J_1,$$

$$(\bar{\mathbf{x}}(0), k_n(0)) = (\bar{\mathbf{x}}^b, k_n^b) \text{ and } \dot{\bar{\mathbf{x}}}(0) = \mathbf{u},$$

$$\dot{k}_n(0) = 0 \text{ and } \ddot{k}_n(0) = -\frac{\mathbf{W}^\top\big[D_{\boldsymbol{x}}^3 \boldsymbol{r}(\bar{\mathbf{x}}^b; k_n^b)(\mathbf{u}, \mathbf{u}, \mathbf{u})\big]}{3W_n\big[\nabla_{\boldsymbol{x}}\big(\frac{\partial r_n}{\partial k_n}\big)(\bar{\mathbf{x}}^b; k_n^b) \cdot \mathbf{u}\big]}.$$

(ii)

$$\mathbf{z}(k_n^b) = \bar{\mathbf{x}}^b, \ \dot{\mathbf{z}}(k_n^b) = \mathbf{0}, \text{ and } \boldsymbol{f}(\mathbf{z}(k_n); k_n) = 0 \text{ for } k_n \in J_2.$$

Moreover, the steady states $\bar{\mathbf{x}}(s)$ with $s \neq 0$ and $\mathbf{z}(k_n)$ with $k_n \neq k_n^b$ are hyperbolic.

APPENDIX E: PROOF OF THE REFORMULATED CRITERIA OF TRANSCRITICAL AND PITCHFORK BIFURCATIONS

Here, we write the classical theorems which give sufficient conditions for the occurrence of transcritical or pitchfork bifurcation. For the transcritical bifurcation, we also provide the proof of their reformulation in terms of $\mathbf{A}$ written in the main

text. The reformulation for the pitchfork bifurcation can be proved in a similar way.

1. Transcritical bifurcation

The following classical theorem [[29], p. 338] (see also [[23], Chap. 8]) gives a sufficient condition under which the system undergoes a transcritical bifurcation.

Theorem I. Let $\dot{x} = g(x; \mu)$ be a system of differential equations in $\mathbb{R}^M$ depending on the single parameter $\mu \in E := (-\delta_0, \delta_0)$ for some $\delta_0 > 0$. When $\mu = \mu_0 \in E$, assume that there is a steady state x_0 for which the following hypotheses are satisfied:

(T1°) $D_x g(x_0; \mu_0)$ has a simple eigenvalue 0 with left eigenvector $\mathbf{w}$ and right eigenvector $\mathbf{u}$.

(T2°) The left eigenvector $\mathbf{w}$ satisfies $\mathbf{w}^\top g_\mu(x_0; \mu_0) = 0$.

(T3°) The eigenvectors $\mathbf{w}$ and $\mathbf{u}$ satisfy

$$\begin{cases} \mathbf{w}^\top \big[D_x g_\mu(x_0; \mu_0) \mathbf{u} \big] \neq 0, \\ \mathbf{w}^\top \big[D_x^2 g(x_0; \mu_0)(\mathbf{u}, \mathbf{u}) \big] \neq 0. \end{cases}$$

Then system $\dot{x} = g(x; \mu)$ exhibits a transcritical bifurcation at the steady state x_0 as the parameter μ varies through the bifurcation value $\mu = \mu_0$. Precisely, there exist a $\delta \in (0, \delta_0)$ and two smooth functions $\bar{x}^\pm: J := (\mu_0 - \delta, \mu_0 + \delta) \to \mathbb{R}^M$ such that the following holds:

$$\bar{x}^\pm(\mu_0) = x_0 \text{ and } g(\bar{x}^\pm(\mu); \mu) = 0 \text{ for } \mu \in J,$$

$$\text{and} \begin{cases} \dot{\bar{x}}^-(\mu_0) = \mathbf{0}, \\ \dot{\bar{x}}^+(\mu_0) = \dfrac{-2\mathbf{w}^\top \big[D_x g_\mu(x_0; \mu_0) \mathbf{u} \big]}{\mathbf{w}^\top \big[D_x^2 g(x_0; \mu_0)(\mathbf{u}, \mathbf{u}) \big]} \mathbf{u}. \end{cases}$$

Moreover, each steady state $\bar{x}^\pm(\mu)$ with $\mu \neq \mu_0$ is hyperbolic.

Proof of the criteria in Appendix D 1. In this proof, we use the same notations as in Sec. IV A. Then the same arguments in the proof in Sec. IV A show that (T1) and (T2) imply (T1°) and (T2°).

Next, we claim that (T3) implies (T3°). Indeed, we have

$$\mathbf{w}^\top \big[D_x f_{k_n}(\bar{x}^b; k_n^b) \mathbf{u} \big] = \mathbf{W}^\top \big[D_x r_{k_n}(\bar{x}^b; k_n^b) \mathbf{u} \big]$$
$$= W_n \Big[\nabla_x \big(\frac{\partial r_n}{\partial k_n} \big)(\bar{x}^b; k_n^b) \cdot \mathbf{u} \Big],$$

where in the last equality we have used the fact that $\partial r_{n'} / \partial k_n = 0$ for each $n' \neq n$. Also one has

$$\mathbf{w}^\top \big[D_x^2 f(\bar{x}^b; k_n^b)(\mathbf{u}, \mathbf{u}) \big] = \mathbf{W}^\top \big[D_x^2 r(\bar{x}^b; k_n^b)(\mathbf{u}, \mathbf{u}) \big].$$

Thus (T3) implies (T3°).

Finally, an application of Theorem I proves the assertions of this theorem.

2. Pitchfork bifurcation

The following classical theorem [[29], p. 339] (see also [[23], Chap. 8]) gives a sufficient condition under which the system undergoes a pitchfork bifurcation.

Theorem II. Let $\dot{x} = g(x; \mu)$ be a system of differential equations in $\mathbb{R}^M$ depending on the single parameter k. When $\mu = \mu_0$, assume that there is a steady state x_0 for which the following hypotheses are satisfied:

(P1°) $D_x g(x_0; \mu_0)$ has a simple eigenvalue 0 with left eigenvector $\mathbf{w}$ and right eigenvector $\mathbf{u}$.

(P2°) The left eigenvector $\mathbf{w}$ satisfies $\mathbf{w}^\top g_\mu(x_0; \mu_0) = 0$.

(P3°) The eigenvectors $\mathbf{w}$ and $\mathbf{u}$ satisfy

$$\begin{cases} \mathbf{w}^\top \big[D_x g_\mu(x_0; \mu_0) \mathbf{u} \big] \neq 0, \\ \mathbf{w}^\top \big[D_x^2 g(x_0; \mu_0)(\mathbf{u}, \mathbf{u}) \big] = 0, \\ \mathbf{w}^\top \big[D_x^3 g(x_0; \mu_0)(\mathbf{u}, \mathbf{u}, \mathbf{u}) \big] \neq 0. \end{cases}$$

Then system $\dot{x} = g(x; \mu)$ exhibits a pitchfork bifurcation at the steady state x_0 as the parameter μ varies through the bifurcation value $\mu = \mu_0$. Precisely, there exist a $\delta > 0$, a smooth curve $\Upsilon: J_1 := (-\delta, \delta) \to \mathbb{R}^M \times \mathbb{R}$, and a smooth function $\mathbf{z}: J_2 := (\mu_0 - \delta, \mu_0 + \delta) \to \mathbb{R}^M$ such that the following hold:

(i)

$$\Upsilon(s) = (\bar{x}(s), \mu(s)) \text{ and } g(\bar{x}(s); \mu(s)) = \mathbf{0} \text{ for } s \in J_1,$$

$$(\bar{x}(0), \mu(0)) = (x_0, \mu_0) \text{ and } \dot{\bar{x}}(0) = \mathbf{u},$$

$$\dot{\mu}(0) = 0 \text{ and } \ddot{\mu}(0) = -\frac{\mathbf{w}^\top \big[D_x^3 g(x_0; \mu_0)(\mathbf{u}, \mathbf{u}, \mathbf{u}) \big]}{3\mathbf{w}^\top \big[D_x g_\mu(x_0; \mu_0) \mathbf{u} \big]}.$$

(ii)

$$\mathbf{z}(\mu_0) = x_0, \ \dot{\mathbf{z}}(\mu_0) = \mathbf{0}, \text{ and } g(\mathbf{z}(\mu); \mu) = 0 \text{ for } \mu \in J_2.$$
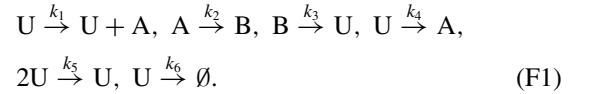
Moreover, the steady states $\bar{x}(s)$ with $s \neq 0$ and $\mathbf{z}(\mu)$ with $\mu \neq \mu_0$ are hyperbolic.

These conditions can be reformulated in term of $\mathbf{A}$ similarly to the other two types of bifurcations.

APPENDIX F: EXAMPLES OF TRANSCRITICAL AND PITCHFORK BIFURCATIONS

1. Transcritical bifurcation

We consider the following system, which is designed by modifying the first Schlögl model [30]:

$$\text{U} \xrightarrow{k_1} \text{U} + \text{A}, \ \text{A} \xrightarrow{k_2} \text{B}, \ \text{B} \xrightarrow{k_3} \text{U}, \ \text{U} \xrightarrow{k_4} \text{A},$$
$$2\text{U} \xrightarrow{k_5} \text{U}, \ \text{U} \xrightarrow{k_6} \emptyset. \tag{F1}$$

The dynamics are described by

$$\begin{pmatrix} \dot{x}_\text{A} \\ \dot{x}_\text{B} \\ \dot{x}_\text{U} \end{pmatrix} = \begin{pmatrix} 1 & -1 & 0 & 1 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & -1 \end{pmatrix} \begin{pmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_6 \end{pmatrix}. \tag{F2}$$

The stoichiometric matrix ν has three independent kernel vectors $c_1 = (1, 1, 1, 0, 0, 1)^\top$, $c_2 = (1, 1, 1, 0, 1, 0)^\top$, $c_3 = (0, 1, 1, 1, 0, 0)^\top$. It contains a buffering structure $\Gamma = (\{A, B\}, \{2, 3\})$, since $\chi = 2 - 2 + 0 = 0$.

Under the mass-action kinetics, reaction rate functions read as follows:

$$(r_1, \ldots, r_6) = \big(k_1 x_\text{U}, k_2 x_\text{A}, k_3 x_\text{B}, k_4 x_\text{U}, k_5 x_\text{U}^2, k_6 x_\text{U} \big).$$

The steady states $\bar{x} = (\bar{x}_A, \bar{x}_B, \bar{x}_U)$ are (Fig. 7)

$$\bar{x} = \left(\frac{(k_1 + k_4)(k_1 - k_6)}{k_2 k_5}, \frac{(k_1 + k_4)(k_1 - k_6)}{k_3 k_5}, \frac{k_1 - k_6}{k_5} \right),$$
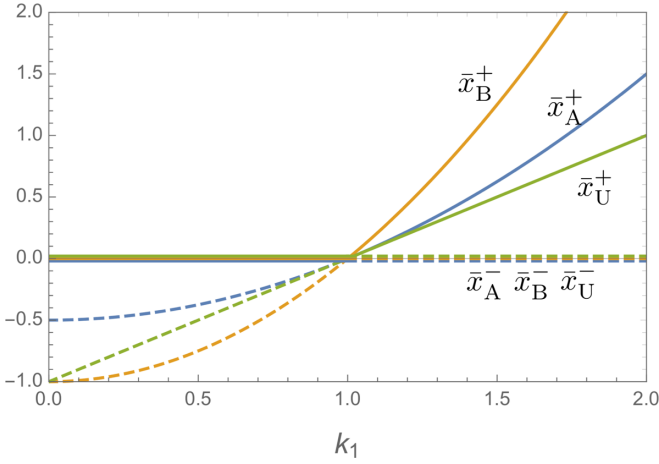$$(0, 0, 0). \tag{F3}$$

FIG. 7. Bifurcation diagram for system (F1). $\boldsymbol{x}^+$ and $\boldsymbol{x}^-$ represent the two solutions in (F4). The solid and dashed branches are stable and unstable, respectively. The parameter values are $k_2 = 2$, $k_4 = 1$, $k_3 = 1$, $k_5 = 1$, and $k_6 = 1$.

From these two solutions, this system exhibits a transcritical bifurcation as the parameter k_1 is varied through the bifurcation value $k_1^b = k_6$.

We now confirm the transcritical bifurcation by using our formulation. First, by permuting the row index as $\{2, 3, 1, 4, 5, 6\}$ and the column index as $\{A, B, U, \boldsymbol{c}_1, \boldsymbol{c}_2, \boldsymbol{c}_3\}$, the matrix $\mathbf{A}$ becomes

$$\mathbf{A} = \begin{pmatrix} r_{2,A} & r_{2,B} & 0 & 1 & 1 & 1 \\ r_{3,A} & r_{3,B} & 0 & 1 & 1 & 1 \\ 0 & 0 & r_{1,U} & 1 & 1 & 0 \\ 0 & 0 & r_{4,U} & 0 & 0 & 1 \\ 0 & 0 & r_{5,U} & 0 & 1 & 0 \\ 0 & 0 & r_{6,U} & 1 & 0 & 0 \end{pmatrix}$$

$$= \begin{pmatrix} k_2 & 0 & 0 & 1 & 1 & 1 \\ 0 & k_3 & 0 & 1 & 1 & 1 \\ 0 & 0 & k_1 & 1 & 1 & 0 \\ 0 & 0 & k_4 & 0 & 0 & 1 \\ 0 & 0 & 2k_5 x_U & 0 & 1 & 0 \\ 0 & 0 & k_6 & 1 & 0 & 0 \end{pmatrix}, \quad \text{(F4)}$$

where the upper-left and lower-right blocks, enclosed by lines, correspond to the buffering structure Γ and its complement $\bar{\Gamma}$, respectively. The determinant of the matrix $\mathbf{A}$ evaluated at the steady states is then factorized and given by

$$\det \mathbf{A} = \underbrace{k_2 k_3}_{\det \mathbf{A}_\Gamma} \underbrace{(2k_5 \bar{x}_U - k_1 + k_6)}_{\det \mathbf{A}_{\bar{\Gamma}}} \quad \text{(F5)}$$

$$= \pm k_2 k_3 (k_1 - k_6),$$

where $+$ and $-$ correspond to the first and the second solution in (F3), respectively. At the bifurcation point $k_1 = k_6$, the determinant $\det \mathbf{A}$ vanishes, and the left eigenvector $\mathbf{W}$ and
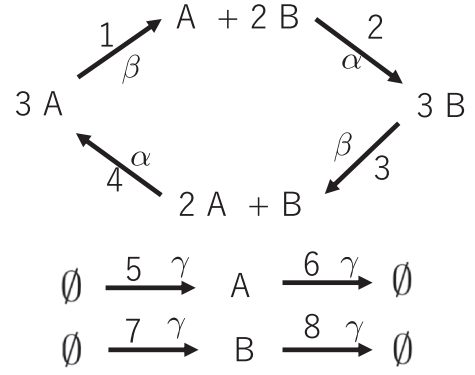

FIG. 8. r_1: 3A $\to$ A + 2B, r_2: A + 2B $\to$ 3B, r_3: 3B $\to$ 2A + B, r_4: 2A + B $\to$ 3A, r_5: $\emptyset \to$ A, r_6: A $\to \emptyset$, r_7: $\emptyset \to$ B, r_8: B $\to \emptyset$.

the right eigenvector $\mathbf{U}$ are given by

$$\mathbf{W} = (0, 0, -1, 0, 1, 1)^\top,$$

$$\mathbf{U} = (U_1, U_2, U_3, U_4, U_5, U_6)^\top$$

$$= (u_1, u_2, u_3, v_1, v_2, v_3)^\top \quad \text{(F6)}$$

$$= \left(\underbrace{\frac{k_4 + k_6}{k_2 k_4}, \frac{k_4 + k_6}{k_3 k_4}, \frac{1}{k_4}}_{\mathbf{u}}, \underbrace{-\frac{k_6}{k_4}, 0, -1}_{\mathbf{v}} \right)^\top \in \mathbb{R}^6.$$

Now we verify the three conditions (T1)–(T3) in Appendix D 1. First, recall that $r_1 = k_1 x_U$ and the bifurcation point $(\bar{\boldsymbol{x}}^b, k_1^b) = (\mathbf{0}, k_6)$. Thus

$$\frac{\partial r_1}{\partial k_1}(\bar{\boldsymbol{x}}^b; k_1^b) = \bar{x}_U^b(k_1^b) = 0, \quad \text{(F7)}$$

where we used $\bar{x}_U^b(k_1^b) = 0$ at the bifurcation point. Hence, the condition (T1) holds. Second, using $r_1 = k_1 x_U$, we have

$$W_3 \left[\nabla_{\boldsymbol{x}} \left(\frac{\partial r_1}{\partial k_1} \right)(\bar{\boldsymbol{x}}^b; k_1^b) \cdot \mathbf{u} \right]$$

$$= W_3 \left[D_{x_U} \left(\frac{\partial r_1}{\partial k_1} \right)(\bar{\boldsymbol{x}}^b; k_1^b) U_3 \right] = (-1) \times \frac{1}{k_4} \neq 0. \quad \text{(F8)}$$

Thus, the condition (T2) holds. Finally, by noting that only $r_5 = k_5 x_U^2$ is a quadratic function,

$$\mathbf{W}^\top \left[D_{\bar{\boldsymbol{x}}}^2 \boldsymbol{r}(\bar{\boldsymbol{x}}^b; k_1^b)(\mathbf{u}, \mathbf{u}) \right] = \mathbf{W}^\top (0, 0, 0, 0, 2k_5, 0)^\top (u_3)^2$$

$$= W_5 \times 2k_5 \times (u_3)^2 = \frac{2k_5}{k_4^2} \neq 0.$$

Therefore, the condition (T3) holds.

2. Pitchfork bifurcation

Consider the reaction system shown in Fig. 8. Under the mass-action kinetics, the dynamics are described
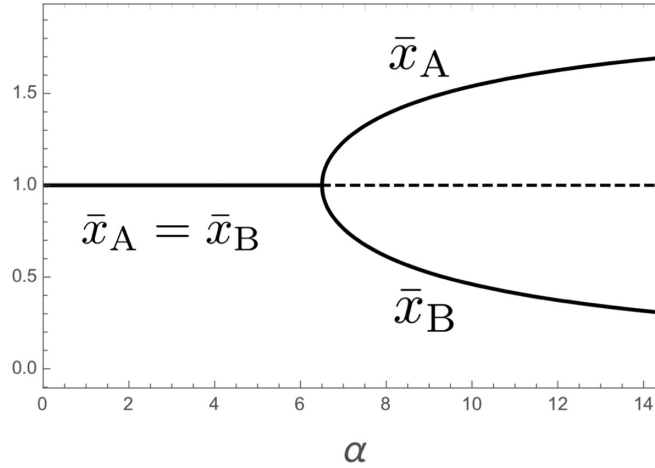
FIG. 9. Bifurcation diagram for the system (F9). The solid lines represent stable branches, while the dashed line represents an unstable one. $\beta = 1$ and $\gamma = 1$.

by

$$\begin{pmatrix} \dot{x}_A \\ \dot{x}_B \end{pmatrix} = \begin{pmatrix} -2 & -1 & 2 & 1 & 1 & -1 & 0 & 0 \\ 2 & 1 & -2 & -1 & 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} r_1(x_A) \\ r_2(x_A, x_B) \\ r_3(x_B) \\ r_4(x_A, x_B) \\ r_5 \\ r_6(x_A) \\ r_7 \\ r_8(x_B) \end{pmatrix}, \tag{F9}$$

where $r_1 = \beta x_A^3$, $r_2 = \alpha x_A x_B^2$, $r_3 = \beta x_B^3$, $r_4 = \alpha x_A^2 x_B$, $r_5 = \gamma$, $r_6 = \gamma x_A$, $r_7 = \gamma$, $r_8 = \gamma x_B$. A similar model was first presented by Feinberg [28], which corresponds to the case without the inflows, r_5, r_7 and the outflows, r_6, r_8, namely $\gamma = 0$. In the original model, the dynamics are essentially one-dimensional since the sum $x_A + x_B$ is conserved and determined from an initial condition. Here, due to the presence of the inflows and outflows, the sum is no longer conserved and the dynamics are indeed two-dimensional. The matrix **A** is

$$\mathbf{A} = \begin{pmatrix} 3\beta x_A^2 & 0 & -1 & 1 & 0 & -1 & -1 & 1 \\ \alpha x_B^2 & 2\alpha x_A x_B & 0 & 0 & 0 & 0 & 0 & -2 \\ 0 & 3\beta x_B^2 & 0 & 0 & 0 & 0 & -1 & 0 \\ 2\alpha x_A x_B & \alpha x_A^2 & 0 & 0 & 0 & -2 & 0 & 0 \\ 0 & 0 & -2 & 2 & -1 & 0 & 0 & 0 \\ \gamma & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -2 & 0 & 0 & 0 & 0 \\ 0 & \gamma & -2 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}. \tag{F10}$$

The steady states are given by

$$(\bar{x}_A, \bar{x}_B) = (1, 1), \text{ or } \left(1 \pm \frac{\sqrt{2\alpha - 12\beta - \gamma}}{\sqrt{2}\sqrt{\alpha + 2\beta}}, 1 \mp \frac{\sqrt{2\alpha - 12\beta - \gamma}}{\sqrt{2}\sqrt{\alpha + 2\beta}}\right). \tag{F11}$$

When $\alpha < \frac{1}{2}(12\beta + \gamma)$, only the first solution $\bar{x} = (1, 1)$ exists and it is stable. When $\alpha > \frac{1}{2}(12\beta + \gamma)$, all of the three solution exist and the first solution is unstable and the other two are stable. See Fig. 9 for the plot of the steady-state concentrations.

We will use α, which enters both of rate functions r_2 and r_4, as a bifurcation parameter μ. Then the parameter value μ associated with the occurrence of bifurcation is given by $\mu^b = \alpha^b = \frac{1}{2}(12\beta + \gamma)$. For this parameter value μ, all of the three

steady states in (F11) are equal to $\bar{x}^b = (1, 1)$, and the matrix **A** given by (F10) becomes

$$
\mathbf{A} = \begin{pmatrix}
3\beta & 0 & -1 & 1 & 0 & -1 & -1 & 1 \\
6\beta + \frac{\gamma}{2} & 12\beta + \gamma & 0 & 0 & 0 & 0 & 0 & -2 \\
0 & 3\beta & 0 & 0 & 0 & 0 & -1 & 0 \\
12\beta + \gamma & 6\beta + \frac{\gamma}{2} & 0 & 0 & 0 & -2 & 0 & 0 \\
0 & 0 & -2 & 2 & -1 & 0 & 0 & 0 \\
\gamma & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & -2 & 0 & 0 & 0 & 0 \\
0 & \gamma & -2 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}.
$$

The right eigenvector **U** and the left eigenvector **W** are then given by

$$
\mathbf{U}^\top = \left(-1, 1, \frac{\gamma}{2}, 0, -\gamma, -\frac{12\beta + \gamma}{4}, 3\beta, \frac{12\beta + \gamma}{4} \right)^\top,
$$

$$
\mathbf{W}^\top = (-4, -2, 4, 2, 1, -1, -1, 1). \tag{F12}
$$

In particular, $\mathbf{u} = (u_A, u_B) = (-1, 1)$.

In the following, we will show that system (F9) exhibits a pitchfork bifurcation at the bifurcation point $(\bar{x}, \mu) = (\bar{x}^b, \alpha^b)$. Note that in this example, the bifurcation parameter μ appears in the expressions of both of reaction rate functions r_2 and r_4, and therefore, one cannot apply the criteria (P1), (P2), and (P3) directly. However, the argument in Appendix D 2 suggests that the conditions of (P2) and (P3) should be replaced by (P2′) and (P3′), as stated here:

(P2′) The quantity $\mathbf{W} \cdot \frac{\partial \mathbf{r}}{\partial \mu}(\bar{x}^b; \mu^b)$ is zero.

(P3′) The eigenvectors **W** and **U** satisfy

$$
\begin{cases}
\mathbf{W}^\top \left[D_{\mathbf{x}} \left(\frac{\partial \mathbf{r}}{\partial \mu} \right)(\bar{x}^b; k_n^b) \cdot \mathbf{u} \right] \neq 0, \\
\mathbf{W}^\top \left[D_{\mathbf{x}}^2 \mathbf{r}(\bar{x}^b; k_n^b)(\mathbf{u}, \mathbf{u}) \right] = 0, \\
\mathbf{W}^\top \left[D_{\mathbf{x}}^3 \mathbf{r}(\bar{x}^b; k_n^b)(\mathbf{u}, \mathbf{u}, \mathbf{u}) \right] \neq 0,
\end{cases}
$$

where the vectors **u** and **U** are related through the decomposition $\mathbf{U} = (\mathbf{u}^\top, \mathbf{v}^\top)^\top$ with $\mathbf{u} \in \mathbb{C}^M$ and $\mathbf{v} \in \mathbb{C}^{N-M}$ determined by Theorem 1.

To verify these two conditions, we first note that

$$
\frac{\partial \mathbf{r}}{\partial \mu} = \frac{\partial \mathbf{r}}{\partial \alpha} = (0, x_A x_B^2, 0, x_A^2 x_B, 0, 0, 0, 0)^\top |_{\mathbf{x} = \bar{x}^b = (1,1)}
$$

$$
= (0, 1, 0, 1, 0, 0, 0, 0)^\top,
$$

and so $\mathbf{W} \cdot \frac{\partial \mathbf{r}}{\partial \mu} = -2 + 2 = 0$. Therefore, (P2′) is satisfied. As for (P3′), since $\frac{\partial}{\partial x_A} \frac{\partial \mathbf{r}}{\partial \mu} = (0, x_B^2, 0, 2x_A x_B, 0, 0, 0, 0)^\top$,

$\frac{\partial}{\partial x_B} \frac{\partial \mathbf{r}}{\partial \mu} = (0, 2x_A x_B, 0, x_A^2, 0, 0, 0, 0)^\top$, and $\mathbf{u} = (u_A, u_B)^\top = (-1, 1)^\top$, it follows that

$$
\mathbf{W}^\top \left[D_{\mathbf{x}} (\frac{\partial \mathbf{r}}{\partial \mu}) \mathbf{u} \right]
$$

$$
= \left(4x_A x_B - 2x_B^2 \right) u_A + \left(-4x_A x_B + 2x_A^2 \right) u_B \Big|_{\mathbf{x} = \bar{x}^b}
$$

$$
= 2u_A - 2u_B = -4 \neq 0.
$$

Next, since $\frac{\partial \mathbf{r}}{\partial x_A^2} = (6\beta x_A, 0, 0, 2\alpha x_B, 0, 0, 0, 0)$, $\frac{\partial \mathbf{r}}{\partial x_B^2} = (0, 2\alpha x_A, 6\beta x_B, 0, 0, 0, 0, 0)$, and $\frac{\partial \mathbf{r}}{\partial x_A \partial x_B} = (0, 2\alpha x_B, 0, 2\alpha x_A, 0, 0, 0, 0)$, it follows that

$$
\mathbf{W}^\top \left[D_{\mathbf{x}}^2 \mathbf{r}(\mathbf{u}, \mathbf{u}) \right]
$$

$$
= (4\alpha x_B - 24\beta x_A) u_A^2 + (24\beta x_B - 4\alpha x_A) u_B^2
$$

$$
+ (4\alpha x_A - 4\alpha x_B) u_A u_B |_{\mathbf{x} = (1,1)} = 0.
$$

Finally, since $\frac{\partial \mathbf{r}}{\partial x_A^3} = (6\beta, 0, 0, 0, 0, 0, 0, 0)$, $\frac{\partial \mathbf{r}}{\partial x_B^3} = (0, 0, 6\beta, 0, 0, 0, 0, 0)$, $\frac{\partial \mathbf{r}}{\partial x_A^2 \partial x_B} = (0, 0, 0, 2\alpha, 0, 0, 0, 0)$, and $\frac{\partial \mathbf{r}}{\partial x_A \partial x_B^2} = (0, 2\alpha, 0, 0, 0, 0, 0, 0)$, it follows that

$$
\mathbf{W}^\top \left[D_{\mathbf{x}}^3 \mathbf{r}(\mathbf{u}, \mathbf{u}, \mathbf{u}) \right]
$$

$$
= (-24\beta) u_A^3 + 24\beta u_B^3 + 4\alpha^b u_A^2 u_B + (-4\alpha^b) u_A u_B^2
$$

$$
= 8\alpha^b + 48\beta \neq 0.
$$

Thus, all of the conditions in (P3′) have been confirmed.

Finally, we remark that the symmetric kinetics between A and B are necessary for the pitchfork bifurcation. Asymmetry would generally lead to a saddle-node bifurcation.

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