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A multi-time-scale analysis of chemical reaction networks: I. Deterministic systems

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Abstract We consider deterministic descriptions of reaction networks in which different reactions occur on at least two distinct time scales. We show that when a certain Jacobian is nonsingular there is a coordinate system in which the evolution equations for slow and fast variables are separated, and we obtain the appropriate initial conditions for the transformed system. We also discuss topological properties which guarantee that the nonsingularity condition is satisfied, and show that in the new coordinate frame the evolution of the slow variables on the slow time scale is independent of the fast variables to lowest order in a small parameter. Several examples that illustrate the numerical accuracy of the reduction are presented, and an extension of the reduction method to three or more time scale networks is discussed.

Keywords Chemical reaction networks · Multi-time-scale analysis · Reduction of graph of reaction networks

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1 Background

In many complex reaction networks the reactions occur on vastly different time scales. Some reactions dominate the initial dynamics and may reach a pseudo-steady state

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quickly, whereas others occur slowly and may dominate the dynamics on a long time scale. To fix terminology, we call the former fast reactions and the latter slow reactions. Regardless of whether a deterministic or stochastic model is used, the dynamics of such systems are described by a large number of variables and differential equations with kinetic parameters of widely-differing orders of magnitude. As a result, accurate computations that resolve the fast and slow time scale dynamics for very large networks of the kind that arise in studies of metabolism, signal transduction, gene control, and developing systems are computationally challenging. Moreover, the slow dynamics are often of primary interest, and to analyze them one has to construct the governing equations for slowly-varying quantities. In this paper we introduce techniques for identifying these equations for deterministic models of reaction networks; a multi-time-scale stochastic analysis will be developed in a sequel. Throughout we deal only with well-mixed systems at constant temperature and volume, which can be described by systems of ordinary differential equations.

Classical singular perturbation techniques are based on a separation of variables into those that vary rapidly and those that change slowly on the chosen time scale. This leads to a system of equations of the form

$$\begin{aligned} \frac{dx}{dt} &= f(x, y, \epsilon) \\ \epsilon \frac{dy}{dt} &= g(x, y, \epsilon) \end{aligned} \tag{1}$$

wherein ϵ is a small parameter and x (resp., y) is a slow (resp., fast) variable on the t time scale. Well-known results such as Tikhonov's theorem (Tikhonov 1952) give conditions on the function g under which y can be eliminated on the slow time-scale, and establish precise estimates of how well the reduced system obtained by eliminating y approximates the dynamics of the full system. In the literature on chemical and biochemical networks, the reduction is known as the partial-equilibrium assumption (PEA) if it is obtained by setting the net reaction rate of certain reactions to be zero, while the quasi-steady-state hypothesis (QSSH) or the quasi-steady-state assumption (QSSA) is obtained by setting the time derivative of the concentration of fast species or radicals to be zero (Lam 1993; Lam and Goussis 1994); the Michaelis-Menten approximation of enzyme kinetics is an example of an application of the QSSA. One of the earliest rigorous analyses of QSSA in the reaction kinetics is by Acrivos et al. (1963), who analyzed four kinetic schemes in the framework of singular perturbation theory; another is due to Heineken et al. (1967) who did a rigorous analysis of the Michaelis-Menten scheme. A review of the technique and other applications to reaction problems is given in Segel and Slemrod (1982).

However, application of the classical singular perturbation techniques requires a classification of the *variables* into slow and fast types, whereas in complex reaction networks it is the processes i.e., *reactions* that are classified as either fast or slow, and species can participate in both fast and slow processes. Typically kinetic equations are written in the form

$$\frac{dc}{dt} = F(c) \tag{2}$$

wherein c is a vector of species concentrations (or some other measure of the amounts) and $F(c)$ is generated by summing all contributions to the rate of change of a species due to the reactions in which it participates. Except in simple cases such as leads to the Michaelis-Menten equation, one cannot separate species into fast and slow types, since a species may participate in both fast and slow reactions, and thus there are both large and small terms on the right-hand side of (2). Thus a preliminary step is needed in which one identifies new variables that can be classified as either fast or slow.

There is a long history of different analytical approaches to the reduction of chemical reaction networks and solution of the underlying equations (King and Altman 1956; Kistiakowsky and Shaw 1953; Park 1974; Snow 1966). For example, King and Altman (1956) were one of the first to apply graph-theoretic methods for the solution of linear algebraic systems to find steady state distributions in networks of first-order reactions. More recently Kijma and Kijima (1982) developed procedures for simplifying first-order reversible reactions in which there are slow and fast steps by applying the QSSA. They classified a species as fast or slow according to whether the species is a reactant of a fast reaction or not, and lumped species into groups characterized by the connectivity within the group. It will be seen later that the graph-theoretic framework we develop subsumes their approach. Schauer and Heinrich considered the outer solution (which is the slowly varying component) in general nonlinear reactions directly by constructing a regular expansion in the small parameter and deriving equations for the evolution of slow variables. Our approach is similar in spirit but more complete, in that we derive explicit evolution equations for both the slow and the fast variables. Their approach has been discussed in the framework of Fenichel's theory of singular perturbation (Stiefenhofer 1998), but the reduction to evolution equations is not carried out. More closely related to the present work is that in Kumar and Daoutidis (1999), where reductions of some typical models in chemical engineering are considered.

In the last twenty-five years there has also been a great deal of work aimed at computational algorithms for the reduction of multi-time-scale chemical kinetics problems and approximation of the slow evolution. A geometric description of the steady-state (SSA) and equilibrium approximations (EA) for low-order systems (Fraser 1988), and more accurate and implicit representations for the slow manifold in enzyme kinetics models amenable to iterative approximation schemes have appeared (Roussel and Fraser 1990, 1991). In a later work (Roussel and Fraser 2001), these authors developed geometric methods for model reduction of enzyme kinetics and obtained reduced equations from the governing differential equations. Mass and Pope developed the intrinsic low-dimensional manifold (ILDM) method which gives an approximation of the slow manifold (Maas and Pope 1992). An explicitly computational approach, called computational singular perturbation (CSP) hinges on decomposing the right-hand side of (2) as a sum of elementary modes and deriving equations for the amplitudes of the modes (Lam 1993; Lam and Goussis 1994). More recently the CSP method has been developed into an efficient method for model reduction by many researchers. A detailed review of the CSP method is given in Gorban and Karlin (2003). Extensions of the CSP method in the form of improved algorithms for approximating fast and slow dynamics of stiff systems (Goussis and Valorani 2006) or for simplication of chemical kinetics (Valorani et al. 2006) have been developed. Furthermore, it is shown in Kaper and Kaper (2004) and Zagaris et al. (2004) that the CSP method gives the simultaneous approximation of the slow manifold and the tangent spaces to the fast fibers at their base points and each iteration of the CSP algorithm improves the accuracy of the approximation by one order of ϵ. It will be of interest to study the computational efficiency of CSP with an implementation of the method developed herein in the future.

Our objectives here are to reduce the underlying graph of complex reaction networks, to develop the methods for identifying fast and slow variables and their corresponding evolution equations in the reduced reaction networks, to identify the correct initial conditions for the these new variables, to clarify the geometric meaning of the QSSA in such systems, and to obtain the equations for the slow dynamics explicitly. Under the nonsingularity condition stated later, our reduction involves setting the rates of fast reactions equal to zero, and thus there is a similarity to the previously-described PEA, but our approach guarantees the existence of a local coordinate system based in part on the level sets of the rates of the fast reactions. We illustrate the numerical accuracy of the reduction method by applying it to several examples.

2 The deterministic description of chemical reaction networks

We begin with some background for a general deterministic description of reacting systems; a more detailed exposition is given in Gadgil et al. (2005), Othmer (1979) and Othmer (1981).

Suppose that the reacting mixture contains the set $\mathcal{M}$ of m chemical species $\mathcal{M}_i$ that participate in a total of r reactions. Let ν_{ij} be the stoichiometric coefficient of the ith species in the jth reaction. The ν_{ij} are non-negative integers that represent the normalized molar proportions or stoichiometric coefficients of the species in a reaction. Each reaction is written in the form

$$\sum_i^{reac.} \nu_{ij}^{reac} \mathcal{M}_i \rightarrow \sum_i^{prod} \nu_{ij}^{prod} \mathcal{M}_i \qquad j = 1, \ldots r, \tag{3}$$

where the sums are over reactants and products, respectively in the jth reaction. In this formulation, the forward and reverse reaction of a reversible pair are considered separately, as two irreversible reactions. Once the reactants and products for each reaction are specified, the significant entities so far as the network topology is concerned are not the species themselves, but rather the linear combinations of species that appear as reactants or products in the various elementary steps. These linear combinations of species are *complexes* (Horn and Jackson 1972), and we suppose that there are p of them. A species may also be a complex as is the case for first-order reactions. Once the complexes are fixed, their composition is specified unambiguously, and we let ν denote the $m \times p$ matrix whose jth column encodes the stoichiometric amounts of the species in the jth complex. Note that we allow proportional columns of ν to allow reactions such as $A \rightarrow B$ and $2A \rightarrow 2B$, which are distinct. Finally, we assume that changes in temperature, pressure and volume V of the mixture during reaction are negligible. Thus the state of the system is specified by the concentration vector

$c = (c_1, \ldots, c_m)^T \in \bar{\mathbf{R}}_m^+$, where c_i is the non-negative concentration of species $\mathcal{M}_i$ measured in moles/liter.

The set of reactions gives rise to a directed graph $\mathcal{G}$ as follows. Each complex is identified with a vertex V_k in $\mathcal{G}$ and a directed edge E_ℓ is introduced into $\mathcal{G}$ for each reaction. The topology of $\mathcal{G}$ is encoded in its vertex-edge *incidence matrix* $\mathcal{E}$, which is defined as follows.

$$\mathcal{E}_{i\ell} = \begin{cases} +1 & \text{if } E_\ell \text{ is incident at } V_i \text{ and is directed toward it} \\ -1 & \text{if } E_\ell \text{ is incident at } V_i \text{ and is directed away from it} \\ 0 & \text{otherwise} \end{cases} \tag{4}$$

Since there p complexes and r reactions, $\mathcal{E}$ has p rows and r columns, and every column has exactly one $+1$ and one -1. Each edge carries a nonnegative weight $R_\ell(c)$ given by the intrinsic rate of the corresponding reaction.

An *undirected graph* $\mathcal{G}^0$ is obtained from $\mathcal{G}$ by ignoring the orientation of the edges, i.e. $\mathcal{G}^0$ consists of the set of vertices and a set of unordered pairs $(V_i, V_j) \in V$ that are undirected edges. There are at most two edges connecting any pair of vertices in $\mathcal{G}^0$ and when it is necessary to distinguish between them they can be written $(i, j)_1$ and $(i, j)_2$. Vertices V_i and V_j are said to be adjacent if (i, j) is in the edge set of $\mathcal{G}$. An *edge sequence* of length $k - 1$ is a finite sequence of the form $(V_{i_1}, V_{i_2})(V_{i_2}, V_{i_3}) \ldots (V_{i_{k-1}}, V_{i_k}), k \geq 2$. When the edges in an edge sequence are all oriented in the same direction, the sequence is a *directed edge sequence* in $\mathcal{G}$. When $i_1 = i_k$, the sequence is closed, and otherwise it is open. A *path* in $\mathcal{G}^0$ is an open edge sequence in which all vertices are distinct. A *cycle* in $\mathcal{G}^0$ is a closed path in which internal vertices are distinct. Directed paths and directed cycles in $\mathcal{G}$ are defined analogously to their counterparts in $\mathcal{G}^0$, and V_j is said to reachable from V_i if there is a directed path from V_i to V_j.

An *oriented cycle* in $\mathcal{G}$ is a cycle in $\mathcal{G}_0$ with an orientation assigned by an ordering of the vertices in the cycle. A *cycle matrix* $\mathcal{B}$ associated with $\mathcal{G}$ is defined as follows:

$$\mathcal{B}_{ij} = \begin{cases} +1 & \text{if } E_j \text{ is in the } i\text{th oriented cycle, and the cycle} \\ & \quad \text{and edge orientation coincide} \\ -1 & \text{if } E_j \text{ is in the } i\text{th oriented cycle, and the cycle} \\ & \quad \text{and edge orientation are opposite} \\ 0 & \text{otherwise} \end{cases}$$

$\mathcal{B}$ is an $r' \times r$ matrix, where r' is the number of independent cycles in $\mathcal{G}^0$. It has a row in which all nonzero entries have the same sign for every directed cycle in $\mathcal{G}$. $\mathcal{G}^0$(resp., $\mathcal{G}$) is said to be acyclic if it contains no cycles (resp., directed cycles). $\mathcal{G}^0$ is *connected* if every pair of vertices is connected by a path. A subgraph of $\mathcal{G}^0$ is a *tree* if it is connected and acyclic, and a *spanning tree* if it is a tree that contains all the vertices of $\mathcal{G}^0$. A directed graph $\mathcal{G}$ is a *directed tree* if the corresponding graph in $\mathcal{G}^0$ is a tree, and a subgraph of $\mathcal{G}$ is a *directed spanning tree* if the tree is directed and contains all the vertices of $\mathcal{G}$.

A *component* is a connected subgraph $\mathcal{G}_1 \subset \mathcal{G}^0$ that is maximal with respect to the inclusion of edges, i.e. if $\mathcal{G}_2$ is a connected subgraph and $\mathcal{G}_1 \subset \mathcal{G}_2 \subset \mathcal{G}^0$, then $\mathcal{G}_1 = \mathcal{G}_2$. An isolated vertex is a component and every vertex is contained in one and only one component. A directed graph $\mathcal{G}$ is *strongly connected* if for every pair of vertices (V_i, V_j), V_i is reachable from V_j and vice-versa. A *strongly connected component* of $\mathcal{G}$ (a strong component for short) is a strongly-connected subgraph of a directed graph $\mathcal{G}$ that is maximal with respect to inclusion of edges. As in the undirected graph, an isolated vertex is a strong component. A directed graph is strongly connected if and only if there exists a closed, directed edge sequence that contains all the edges in the graph. Strong components in the directed graph $\mathcal{G}$ are classified into three different types: sources, internal strong components and absorbing strong components. A source is a strong component in which no edges from other strong components terminate. An internal strong component is a strong component in which edges from other strong components terminate and from which edges to other strong components originate. An absorbing strong component is a strong component from which no edges to other strong components originate. If $\mathcal{G}$ has p vertices and q components then it is easily shown that the rank of $\mathcal{E}$ is $\rho(\mathcal{E}) = p - q$ (Chen 1971).

A *cocycle* of $\mathcal{G}^0$ is a minimal set of edges whose removal increases the number of components by one. A *cutset* is a cocycle or an edge-disjoint union of cocycles, and an *oriented cutset* in $\mathcal{G}$ is a cutset in $\mathcal{G}^0$ with an orientation defined as follows. If V_1 and V_2 are the disjoint subsets into which V is partitioned by a cutset, the orientation of the cutset is specified by ordering the subsets as (V_1, V_2) or as (V_2, V_1). The *cutset matrix* $\mathcal{Q}$ of a directed graph $\mathcal{G}$ is the matrix obtained by setting

$$\mathcal{Q}_{ij} = \begin{cases} +1 & \text{if } E_j \text{ is in cutset } i \text{ and the orientations of the cutset and edge coincide} \\ -1 & \text{if } E_j \text{ is in cutset } i \text{ and the orientations of the cutset and edge} \\ & \quad \text{are opposite} \\ 0 & \text{otherwise} \end{cases}$$

In the current framework the evolution of the composition of a reacting mixture is governed by

$$\frac{dc}{dt} = \nu \mathcal{E} R(c), \quad c(0) = c_0 \tag{5}$$

where the jth column of ν gives the composition of the jth complex and $R_\ell(c)$ is the rate of the ℓth reaction, or equivalently, the flow on the ℓth edge of $\mathcal{G}$. A *flow* on $\mathcal{G}$ is a real-valued function on the edge set of $\mathcal{G}$, and for a given choice of cycles and cutsets, has the unique decomposition

$$f = f^0 + f^1 = \mathcal{B}^T w + \mathcal{Q}^T z \tag{6}$$

where $f^0 \in \mathcal{N}(\mathcal{E})$ and $f^1 \in \mathcal{R}(\mathcal{E}^T)$. The vectors w and z are the cycle and cutset weights associated with the flow f. A flow is *balanced* when $z = 0$ $(f^1 = 0)$, *cobalanced* when $w = 0$ $(f^0 = 0)$, and *positive, nonnegative* or *strictly nonnegative* according as $f > 0$, $f \geqq 0$ or $f \geq 0$, respectively.[1,2] One can prove that there is a positive balanced flow at steady state if and only if every component of $\mathcal{G}$ is strongly connected (Othmer 1979).

The matrix $\hat{\nu} \equiv \nu\mathcal{E}$ is called the stoichiometric matrix when the composition of complexes and the topology of $\mathcal{G}$ are not encoded separately, as we do here (Aris 1965). One can interpret the factored form in (5) as follows: the vector R gives the flows on edges due to reactions of the complexes, the incidence matrix maps this flow to the sum of all flows entering and leaving a given node (a complex), and the matrix ν converts the net change in a complex to the appropriate change in the molecular species.

A special class of rate functions is that in which the rate of the ℓth reaction can be written as

$$R_\ell(c) = k_\ell P_j(c) \tag{7}$$

for every reaction that involves the jth complex as the reactant. This includes ideal and non-ideal mass action rate laws, in which the rate is proportional to the product of the concentrations or activities of the species in the reactant complex, each concentration or activity raised to a power equal to the stoichiometric coefficient of the corresponding species in the complex. Since elementary chemical steps almost always involve at most two reactants, this form is sufficiently general for most purposes. In the case of ideal mass action kinetics (IMAK), which are used later,

$$P_j = \prod_{i=1}^{n} (c_i)^{\nu_{ij}}. \tag{8}$$

For IMAK (7) implies that

$$R(c) = KP(c) \tag{9}$$

where K is an $r \times p$ matrix with $k_{\ell j} > 0$ if and only if the ℓth edge leaves the jth vertex, and $k_{\ell j} = 0$ otherwise.

The topology of the underlying graph $\mathcal{G}$ enters into K as follows. Define the exit matrix $\mathcal{E}_e$ of $\mathcal{G}$ by replacing all 1's in $\mathcal{E}$ by zeroes, and changing the sign of the resulting matrix. Let $\hat{K}$ be the $r \times r$ diagonal matrix with the k_ℓ's, $\ell = 1, \ldots r$, along the diagonal. Then it is easy to see that $K = \hat{K}\mathcal{E}_e^T$ and therefore

$$\frac{dc}{dt} = \nu\mathcal{E}KP(c) = \nu\mathcal{E}\hat{K}\mathcal{E}_e^T P(c). \tag{10}$$

It follows from the definitions that (i) the (p, q)th entry, $p \neq q$, of $\mathcal{E}\hat{K}\mathcal{E}_e^T$ is nonzero (and positive) if and only if there is a directed edge $(q, p) \in \mathcal{G}$, (ii) each diagonal entry

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[1] Here and hereafter, $y > 0$ means all components are positive, $y \geq 0$ means $y_i \geq 0$ and not all are zero, and $y \geqq 0$ means all may vanish.

[2] The balanced flows defined here correspond to the complex-balanced flows in Horn (1972).

of $\mathcal{E}\hat{K}\mathcal{E}_e^T$ is minus the sum of the k's for all edges that leave the jth vertex, and (iii) the columns of $\mathcal{E}\hat{K}\mathcal{E}_e^T$ all sum to zero, and so the rank of $\mathcal{E}\hat{K}\mathcal{E}_e^T$ is $\leq p-1$. When all complexes are species and all reactions are first-order, $\nu = \mathrm{I}$ for a closed system and $\nu = [\mathrm{I}|\mathbf{0}]$ for an open system, where I is the $m \times s$ identity matrix and $\mathbf{0}$ is the zero vector. In this case the right-hand side of (10) reduces to the usual form Kc where K is defined as above.

As it stands, (8) includes all reacting species, but those whose concentration is constant on the time scale of interest can be deleted from each of the complexes in which it appears and its concentration can be absorbed into the rate constant of any reaction in which it participates as reactant. As a result of these deletions, it will appear that reactions which involve constant species do not necessarily conserve mass. Furthermore, some complexes may not comprise any time-dependent species; these will be called zero or null complexes. Each null complex gives rise to a column of zeroes in ν and the rate of any reaction in which the reactant complex is a null complex is usually constant. For instance, any transport reaction of the form $\mathcal{M}^0 \rightarrow \mathcal{M}_i$ introduces a null complex and the corresponding flux of $\mathcal{M}_i$ represents a constant input to the reaction network, provided that the rate of the transport step does not depend on the concentration of a time-dependent species. Of course, a constant species that appears in a complex which also contains a variable species likewise represents an input to the network, and to distinguish these from inputs due to null complexes, the former are called *implicit inputs* and the latter are called *explicit inputs*.

2.1 Reaction invariants

Combinations of species that are invariant under the flow defined by (5) play an important part in the reduction of any system with multiple time scales, and in this section we analyze the existence of such invariants. Given the evolution equation

$$\frac{dc}{dt} = \nu\mathcal{E}R(c), \tag{11}$$

a vector $a \in \mathbf{R}_m$ defines an invariant linear combination of concentrations if

$$\langle a, \nu\mathcal{E}R(c)\rangle = 0, \tag{12}$$

for then

$$\langle a, c(t)\rangle = \langle a, c(0)\rangle$$

where $\langle , \rangle$ denotes the Euclidean inner product in $\mathbf{R}_m$. The set of solutions a of (12) can be represented by the direct sum of three disjoint subspaces I_j defined as follows.

$$\begin{aligned} I_1 &= \mathcal{N}[\nu^T] \\ I_2 &= span\{a \in \mathbf{R}_m |\ a \in \mathcal{R}[\nu],\ \ \nu^T a \in \mathcal{N}[\mathcal{E}^T]\} \\ I_3 &= span\{a \in \mathbf{R}_m |\ \langle \mathcal{E}^T \nu^T a, R(c)\rangle = 0 \text{ for all } c \in \bar{\mathbf{R}}_m^+ \text{ and } \mathcal{E}^T \nu^T a \neq 0\}. \end{aligned}$$

Hereafter $i_j \equiv dim I_j$, $j = 1, 2, 3$.

Invariants in I_1 depend only on the stoichiometry of the complexes and represent linear combinations of species that are preserved by the reactions. Since the ν_{ij} are non-negative, there is no non-trivial $a \in I_2$ for which $a \geq 0$, by which we mean $a_i \geq 0$ for all i. Thus these invariants represent differences of species that are preserved.

For the second type $I_2 \subset \mathcal{R}[\nu]$, and more precisely,

$$I_2 = \text{preimage}\left(\mathcal{R}[\nu^T] \cap \mathcal{N}[\mathcal{E}^T]\right).$$

All invariants in $I_1 \oplus I_2$ are independent of the reaction rate functions and thus are called kinematic invariants. Since $\mathcal{N}[(\nu\mathcal{E})^T] = I_1 \oplus I_2$, a vector $a \in \mathbf{R}_m$ is a *kinematic invariant* of the kinetics if

$$a \in \mathcal{N}[(\nu\mathcal{E})^T] = \mathcal{N}[\hat{\nu}^T].$$

We have the orthogonal direct sum decomposition

$$\mathbf{R}_m = \mathcal{N}[(\nu\mathcal{E})^T] \oplus \mathcal{R}[\nu\mathcal{E}].$$

and call $\mathcal{R}[\nu\mathcal{E}]$ the reaction subspace. The intersection of the coset of $\mathcal{R}[\nu\mathcal{E}]$ through a point $c_0 \in \bar{\mathbf{R}}_m^+$ with $\bar{\mathbf{R}}_m^+$ is called the *reaction simplex*, denoted Ω, through c_0, i.e.,

$$\Omega(c_0) = \{c_0 + \mathcal{R}[\nu\mathcal{E}]\} \cap \bar{\mathbf{R}}_m^+.$$

Given an initial condition c_0, the solution of (5) can be written as

$$c(t) - c_0 = \nu\mathcal{E} \int_0^t R(c(\tau))d\tau$$

and so $c(t) - c_0 \in \mathcal{R}[\nu\mathcal{E}]$. Therefore all trajectories of the Eq. (5) with an initial condition c_0 lie on the reaction simplex $\Omega(c_0)$. Clearly the structure of $\Omega(c_0)$ is determined by the kinematic invariants, and for example, the existence of a positive kinematic invariant is equivalent to compactness of the reaction simplex. The following is proven in Othmer (1979).

Theorem 1 *Let* $0 \leq c_0 < \infty$ *be given. Then* $\Omega(c_0)$ *is compact if and only if there is a vector* $y > 0$ *in* $\mathcal{N}[(\nu\mathcal{E})^T]$

It follows from this theorem that $\Omega(c_0)$ is compact for all closed systems, since the total mass of the reacting species is conserved, and this implies that there is a positive y in $\mathcal{N}[(\nu\mathcal{E})^T]$. If the system is open, which implies that there is a null complex, one can show that there is no positive element in $\mathcal{N}[(\nu\mathcal{E})^T]$, and so the reaction simplex is not compact (Othmer 1979). Thus there is no *a priori* guarantee that solutions are bounded, and further analysis is required.

2.2 The deficiency of a kinetic mechanism

For later purposes we relate the number of kinematic invariants to other indices of the network as follows. First note that

$$\mathcal{R}[\nu^T] \cup \mathcal{N}[\mathcal{E}^T] = \mathcal{N}[\nu]^\perp \cup \mathcal{R}[\mathcal{E}]^\perp = (\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}])^\perp,$$

where $[S]^\perp$ denotes an orthogonal complement of a subspace S. Thus, it follows that

$$dim\mathcal{R}[\nu^T] + dim\mathcal{N}[\mathcal{E}^T] - dim(\mathcal{R}[\nu^T] \cap \mathcal{N}[\mathcal{E}^T]) = dim(N[\nu] \cap \mathcal{R}[\mathcal{E}])^\perp$$

and so

$$m - i_1 + q - i_2 = s + q = p - dim(\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]).$$

where $s \equiv m - (i_1 + i_2)$. The integer $\delta \equiv dim(\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}])$ is called the deficiency (Horn and Jackson 1972), and is alternatively given by

$$\delta = p - q - s = \rho(\mathcal{E}) - \rho(\nu\mathcal{E}).$$

Thus δ is the difference between the maximal number of independent reactions based on the structure of the graph and the actual number of independent reactions when the stoichiometry is taken into account. When it vanishes ν does not annihilate any elements in $\mathcal{R}[\mathcal{E}]$, i.e., ν is one-to-one from $\mathcal{R}[\mathcal{E}]$ to $\mathcal{R}[\nu\mathcal{E}]$ and so the reaction subspace is isomorphic to $\mathcal{R}[\mathcal{E}]$. It is clear that if $\delta = 0$, then either a steady-state flow vanishes identically or is balanced, and thus an alternate definition of δ is that it is the maximum number of independent cutsets in any flow that is annihilated by $\nu\mathcal{E}$ (Othmer 1979).

The dimension of the third subspace of invariants, I_3, can be determined as follows: First, notice that one can write $R = R_1 + R_2$, where $R_1 \in \mathcal{N}[\mathcal{E}]$ and $R_2 \in \mathcal{R}[\mathcal{E}^T]$. Any $\Omega \in I_3$ can be written as $\Omega = \Omega_1 + \Omega_2$, where $\Omega_1 \in \mathcal{N}[\mathcal{E}^T\nu^T]$ and $\Omega_2 \in \mathcal{R}[\nu\mathcal{E}]$. Thus,

$$\langle \mathcal{E}^T\nu^T, R(c)\rangle = \langle \Omega_2, \nu\mathcal{E}R(c)\rangle$$

and so it is necessary that either $R_2(c) = 0$, in which case $R(c)$ is identically proportional to an oriented cycle, or the cutset part must satisfy

$$\langle \Omega_2, \nu\mathcal{E}R_2(c)\rangle = 0.$$

The latter requires that either $\Omega_2 = 0$, which means that Ω_2 is not in I_3, or $\nu\mathcal{E}R_2(c)$ must vanish identically. Consequently, i_3 is certainly zero if $\delta = 0$ and $\mathcal{G}$ is acyclic, and if $\delta > 0$, $i_3 > 0$ only if the cutset part is such that $\mathcal{E}R_2(c) \in \mathcal{N}[\nu]$ for all $c \in \bar{\mathbf{R}}_m^+$. Therefore $i_3 \le \delta$ whenever it is positive, and $dc/dt = 0$ in this case. Obviously this is a very degenerate situation, and we do not consider it further.

Next we show that one can choose a basis for $\mathcal{N}(\nu\mathcal{E})^T$ of vectors with integer components. To see this note that since the components of the stoichiometric matrix $\nu\mathcal{E}$ are integers, the system of linear equations

$$(\nu\mathcal{E})^T \cdot a = 0. \tag{13}$$

has integer-valued coefficients. Therefore the solutions of this system are vectors in $\mathbf{Q}_m$, the m-dimensional set of vectors with rational entries, as can be seen by using Gaussian elimination or another method. Define $i_{\mathcal{N}} \equiv i_1 + i_2 = dim[\mathcal{N}(\nu\mathcal{E})^T]$ and define $\{a_i', i = 1, \ldots, i_{\mathcal{N}}\}$ as a set of independent solutions of (13) over Q. Let L be the least common multiplier of denominators of components of all a_i', $i = 1, \ldots, i_{\mathcal{N}}$, and set $a_i = L \cdot a_i'$ for $i = 1, \ldots, i_{\mathcal{N}}$. Then $\{a_i, i = 1, \ldots, i_{\mathcal{N}}\}$ is a basis of $\mathcal{N}[(\nu\mathcal{E})^T]$ with integer components. We define the matrix A over the integers $\mathbf{Z}$ as the $i_{\mathcal{N}} \times m$ matrix whose rows are the vectors a_i, i.e.,

$$A = [a_1 | \cdots | a_{i_{\mathcal{N}}}]^T.$$

For any $c \geq 0 \in R_m$, $\langle a_i, c\rangle$ for $i = 1, \ldots, i_{\mathcal{N}}$ is constant under the flow of (5), i.e.

$$Ac = Ac_0$$

where c_0 is an initial condition. Therefore the scalar equation $\sum_j A_{ij} c_j = b_i$ defines a conserved quantity for each $i = 1, \ldots, i_{\mathcal{N}}$. Since $\mathcal{N}[A] = \mathcal{R}[\nu\mathcal{E}]$, the set of all the points that satisfy the conservation relations is just the reaction simplex $\Omega(c_0)$.

With the further restriction that the reaction simplex is compact, it can be shown that one can choose a basis of $\mathcal{N}[(\nu\mathcal{E})^T]$ in which the components are nonnegative integers. Thus if the reaction simplex is compact, the equation $A \cdot c = b$ implies conservation relations for the positive sums of concentrations of certain species.

Theorem 2 *If the reaction simplex $\Omega(c_0)$ is compact, then there is a basis for $\mathcal{N}[(\nu\mathcal{E})^T]$ for which all basis vectors have nonnegative integer components.*

Proof First note that if $\Omega(c_0)$ is compact, Theorem 1 implies that there is a vector $z > 0$ such that $z \in \mathcal{N}[(\nu\mathcal{E})^T]$. Consider a basis $\{b_1, \ldots, b_{m-r}\}$ of $\mathcal{N}[(\nu\mathcal{E})^T]$, where $r = \rho(\nu\mathcal{E})$. One can choose each $b_i \in \mathbf{Z}_m$ since $(\nu\mathcal{E})^T$ is a matrix over $\mathbf{Z}$ and furthermore, one may assume by Theorem 1 that one of b_i, say, b_1 is positive. Thus for each $i = 2, \ldots, m-r$ there are positive integers λ_i such that

$$7b_i' \equiv b_i + \lambda_i b_1 \geq 0.$$

Set $b_1' = b_1$. Then the set $\{b_1', \ldots, b_{m-r}'\}$ is linearly independent and $b_i' \in \mathcal{N}[(\nu\mathcal{E})^T] \cap \bar{\mathbf{Z}}_m^+$. Therefore $\{b_1', \ldots, b_{m-r}'\}$ is a basis of $\mathcal{N}[(\nu\mathcal{E})^T]$.

2.3 Kinetic equilibria

A point c at which $\nu\mathcal{E}R(c) = 0$ is called a kinetic equilibrium point of the system, and the set of such points

$$\mathcal{K} \equiv \{c : \nu\mathcal{E}R(c) = 0\} \tag{14}$$

is called the kinetic equilibrium manifold. If $\nu\mathcal{E}$ has full rank, then the kinetic equilibrium manifold can be represented as a set

$$\mathcal{K} \equiv \{c : \mathcal{R}(c) = 0\}.$$

If the rank of the Jacobian matrix $D_c[\nu\mathcal{E}R(c)]$ (of $D_c[R(c)]$ if $\nu\mathcal{E}$ has full rank) is s, then Eq. (2.3) has s functionally independent relations, and it follows from the implicit function theorem that the set $\mathcal{K}$ locally defines an $m-s$ dimensional submanifold.

3 Reduction of the reaction graph

For the analysis done later, and for the general analysis of a large system, it is advantageous to convert the system into a dynamically equivalent system by identifying equal complexes, removing the cycles, and removing the deficiency.

Step 1: Identification of equal complexes.

A large reaction system may have inflows from many different sources and outflows into many different sinks. These are all null complexes in the current framework, and such reaction networks can be simplified by identifying all explicit inputs and outputs and redefining the graph as follows.

Suppose that the ith complex $C(i)$ is identical to the jth complex $C(j)$, where $i \neq j$. Let $\nu_{(i)}$ denote the ith column of ν and let $\mathcal{E}^{(j)}$ denote the jth row of $\mathcal{E}$. Then $\nu_{(i)} = \nu_{(j)}$. In this case, we can remove one of $\nu_{(i)}$ or $\nu_{(j)}$, say, $\nu_{(j)}$ by reducing

$$\nu\mathcal{E} = [\nu_{(1)} \dots \nu_{(i)} \dots \nu_{(j)} \dots \nu_{(p)}] \begin{bmatrix} \mathcal{E}^{(1)} \\ \dots \\ \mathcal{E}^{(i)} \\ \dots \\ \mathcal{E}^{(j)} \\ \dots \\ \mathcal{E}^{(p)} \end{bmatrix} \quad \text{to}$$

$$\nu'\mathcal{E}' = [\nu_{(1)} \dots \nu_{(i)} \dots \nu_{(p)}] \begin{bmatrix} \mathcal{E}^{(1)} \\ \dots \\ \mathcal{E}^{(i)} + \mathcal{E}^{(j)} \\ \dots \\ \mathcal{E}^{(p)} \end{bmatrix}.$$

Here $\mathcal{E}'$ is the incidence matrix of a graph $\mathcal{G}'$ obtained from $\mathcal{G}$ by moving all edges incident at the jth node to the ith node and removing the jth node. Notice that $\mathcal{G}'$ may have cycles even if $\mathcal{G}$ has none.

Step 2: Removal of cycles.

We suppose that the directed graph $\mathcal{G}$ of a system includes at least one cycle. We can choose a spanning tree in $\mathcal{G}$ and write

$$\mathcal{E} = [\mathcal{E}_0 | \mathcal{E}_1],$$

where $\mathcal{E}_0$ contains the edges in the chosen tree, and $\mathcal{E}_1$ contains all other edges. Since there are p nodes and q components in $\mathcal{G}$, a set of $p - q$ independent cutsets can be chosen so that every edge of the tree is in one and only one cutset, and so that the orientation of the cutset through a tree edge agrees with the orientation of the tree edge. The resulting cutsets comprise a fundamental set and the cutset matrix for this set is

$$\mathcal{Q} = [I | \mathcal{Q}_1],$$

where $\mathcal{Q}_1$ contains the edges not in the tree.

Since an edge of the tree intersects exactly one cutset, $\mathcal{E}_1 = \mathcal{E}_0 \mathcal{Q}_1$, and therefore $\mathcal{E} = \mathcal{E}_0 [I | \mathcal{Q}_1]$. Thus,

$$\nu \mathcal{E} R(c) = \nu \mathcal{E}_0 [I | \mathcal{Q}_1] R(c) = \nu \mathcal{E}_0 R_0(c),$$

where $R_0(c) = [I | \mathcal{Q}_1] R(c)$. Since $\mathcal{E}_0$ is the incidence matrix for a tree, the new network, whose incidence matrix is $\mathcal{E}_0$, contains no cycles. However, in removing the cycles we have to reassign the rates on edges not in the tree to the tree edges. The definition of $R_0(c)$ shows that the new rate on a tree edge is the sum of the signed rates associated with the edges in the unique cutset containing the tree edge, with the sign of each rate in the sum fixed by the orientation of the corresponding edge.

It may not be easy to find a spanning tree and identify all cycles in the directed graph $\mathcal{G}$ in large networks, but there are established algorithms for doing this. The well-known algorithms for finding a spanning tree are "depth-first search" and "breadth-first search" algorithms, and both types have computational complexity $O(\max(p, r))$ where p is the number of vertices and r is the number of edges in the directed graph (Gibbons 1985). Moreover, the computational complexity for finding all cycles in a connected graph is $O(p^3)$ (Gibbons 1985). Thus in the general case in which the graph $\mathcal{G}$ of the system consists of q components $\mathcal{G}_i$, $i = 1, \ldots, q$, each of which has r_i edges and p_i nodes, the computational complexity for finding all cycles in $\mathcal{G}$ is $O(q\hat{p}^3)$, where $\hat{p} = \max_i \{p_i\}$.

Step 3: Removal of elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]$.

Finally, we remove the elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]$. Suppose that the intersection is spanned by δ column vectors $\hat{\mathcal{E}}_{(j)}$ such that

$$\hat{\mathcal{E}}_{(j)} = \sum_{i=1}^{p-q} d_{ij} \mathcal{E}_{0,(i)} \qquad j = 1, \ldots, \delta,$$

where $\mathcal{E}_{0,(i)}$ denotes ith column of $\mathcal{E}_0$. Let $D = [d_{ij}]$ so that D is $(p-q) \times \delta$ matrix. Since there are no $\mathcal{E}_{0,(i)} \in \mathcal{N}[\nu]$ after equal complexes are identified, we can assume without loss of generality that the first $p-q-\delta$ columns of $\mathcal{E}_0$ span $(\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}])^c \cap \mathcal{R}[\mathcal{E}]$, the complement of $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]$ in $\mathcal{R}[\mathcal{E}]$. Then

$$\begin{aligned}\mathcal{E}R(c) &= \mathcal{E}_0[I|\mathcal{Q}_1]R(c) = \mathcal{E}_0 R_0(c)\\ &= \mathcal{E}_0 H H^{-1} R_0(c) \equiv \tilde{\mathcal{E}}\tilde{R}(c)\end{aligned}$$

where the matrix H is a $(p-q) \times (p-q)$ matrix defined as $H = [C|D]$ and C is a $(p-q) \times (p-q-\delta)$ matrix, which can be determined by which reactions are chosen to be retained, so that

$$\tilde{\mathcal{E}} = \mathcal{E}_0 H = [\tilde{\mathcal{E}}_0 \mid \hat{\mathcal{E}}_{(1)} \cdots \hat{\mathcal{E}}_{(\delta)}],$$

where $\tilde{\mathcal{E}}_0 \equiv \mathcal{E}_0 C$ is a $p \times (p-q-\delta)$ submatrix of $\mathcal{E}_0$.

The matrix $\tilde{\mathcal{E}}$ is not the incidence matrix of a graph in general, but we recover one from it by dropping the last δ columns. The truncated $\tilde{\mathcal{E}}$ defines the graph of the network $\tilde{\mathcal{G}}$ equivalent to $\mathcal{G}$. Of course the last δ rows of H^{-1} must also be dropped, and the resulting vector $\tilde{R}(c)$ gives the rate vector for $\tilde{\mathcal{G}}$. It can happen that in the reduced system there are non-reacting complexes, as indicated by zero rows in $\tilde{\mathcal{E}}$. These can be removed from ν and $\tilde{\mathcal{E}}$ can be collapsed vertically. It should also be noted that we do not remove all $z \in \mathcal{N}[\nu]$, but only those in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]$. Certain dependencies between complexes are dynamically irrelevant, and when $\delta = 0$ they all are. The foregoing shows that every network is dynamically equivalent to one for which $\delta = 0$. The following example shows the reduction steps in detail.

Example The Prigogine–Lefever mechanism We consider the Prigogine–Lefever mechanism (Prigogine and Lefever 1968), which is defined as:

$$A \xrightarrow{k_1} X \xrightarrow{k_2} E \qquad B + X \xrightarrow{k_3} Y + D \qquad 2X + Y \xrightarrow{k_4} 3X.$$

Assume that A, B, D, E are time-invariant, and thus null complexes. $C(1) = X$, $C(2) = Y + D$, $C(3) = 2X + Y$, $C(4) = 3X$, $C(5) = A$, $C(6) = E$, $C(7) = B + X$.

Step 1: Identification of the equal complexes Since A, B, D and E are null complexes, we can identify $C(5) = C(6)$ and $C(1) = C(7) = X$ and $C(2) = Y$. Then the graph is

$$C(5) \overset{3}{\underset{4}{\rightleftharpoons}} C(1) \xrightarrow{1} C(2), \quad C(3) \xrightarrow{2} C(4). \tag{15}$$

We see that

$$\nu = \begin{bmatrix} 1 & 0 & 2 & 3 & 0 \\ 0 & 1 & 1 & 0 & 0 \end{bmatrix},$$

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

and $R_1 = k_3 b c_1$, $R_2 = k_4 c_1^2 c_2$, $R_3 = k_2 c_1$, $R_4 = k_1 a$, where c_1 and c_2 are concentrations of species X and Y and a and b are constant concentrations of A and B, respectively.

Step 2: Removal of cycles By dropping reaction 4 in the graph (15), we define the spanning tree

$$C(5) \leftarrow C(1) \rightarrow C(2), \quad C(3) \rightarrow C(4).$$

We define the cutsets in terms of the reactions

$$C(1) \overset{1}{\rightarrow} C(2), \quad C(3) \overset{2}{\rightarrow} C(4), \quad C(1) \overset{3}{\rightarrow} C(5) \tag{16}$$

and assume that the orientation of each cut coincides with the orientation of the edge. The incidence matrix is

$$\mathcal{E}_0 = \begin{bmatrix} -1 & 0 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix},$$

the cutset matrix is

$$\mathcal{Q} = \left[\begin{array}{ccc|c} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{array}\right] = [I_3 | \mathcal{Q}_1].$$

and the rate function is

$$R_0 = \mathcal{Q}R = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \end{bmatrix} = \begin{bmatrix} R_1 \\ R_2 \\ R_3 - R_4 \end{bmatrix}.$$

Thus, after removal of the cycle we have a reduced graph

$$C(5) \xleftarrow{R_3 - R_4} C(1) \xrightarrow{R_1} C(2), \quad C(3) \xrightarrow{R_2} C(4).$$

Step 3: Removal of elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}]$ Since $\rho(\mathcal{E}) = 3$, $\delta = 1$ and $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}] = \text{span}\,\{(-1, 1, -1, 1, 0)^T\}$. If we choose to retain the reactions 1 and 3 in (16),

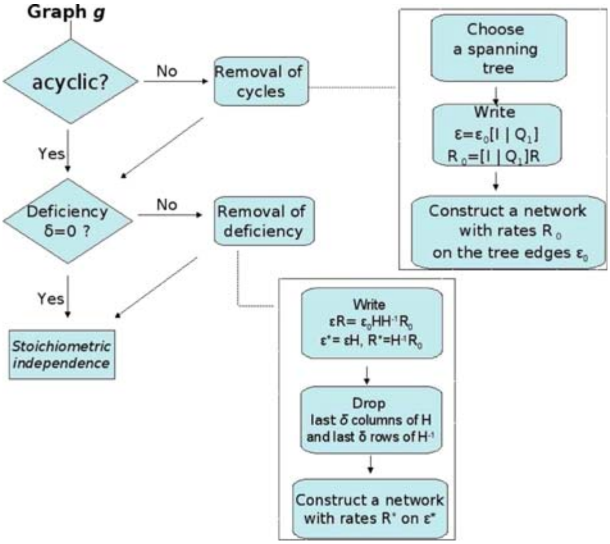

Fig. 1 A flow chart that describes the procedure for the reduction of a graph

then we have

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

After some elementary computations, we obtain a reduced graph, which is dynamically equivalent to the given system,

$$C(5) \xleftarrow{R_3-R_4} C(1) \xrightarrow{R_1-R_2} C(2).$$

One can also see that if we retain reactions 2 and 3 in (16), then a reduced graph is

$$C(1) \xrightarrow{R_3-R_4} C(5), \quad C(3) \xrightarrow{-R_1+R_2} C(4).$$

The scheme for reducing a general network to a dynamically equivalent network is shown in detail in Fig. 1.

4 The QSSA in deterministic systems

By a fast or slow reaction we mean one that occurs more or less frequently compared to other reactions, and this is determined by the magnitude of the reaction rate function R for the reaction in question in the particular region of composition space that is of interest. Of course a reaction that is fast in one region of the state space may be slow in other regions, and the definition of fast and slow reactions may not be uniform through the entire state space. For example, consider a cycle, $A \overset{k_1}{\to} B \overset{k_2}{\to} C \overset{k_3}{\to} A$, where $k_1 \gg k_2, k_3$. For the initial condition $(A, B, C) = (100, 1, 1)$, it is obvious that the rate of the reaction $A \overset{k_1}{\to} B$ is much larger than the others initially. However, as the system evolves the rate of this reaction decreases and eventually, at steady state, it becomes the same as the rates of the other two reactions. Thus, it may seem contradictory to separate these, but in fact the separation stems from the *initial* differences in the rates. If the QSSA is applicable the fast reactions quickly evolve to a region of state space in which their rates are small and comparable to the rates of the slow reactions. In the formal reduction, the leading-order approximation is to solve for the manifold defined by the vanishing of the rates of the fast reactions. However, as we indicated in the Introduction, a difficulty in reducing kinetic networks that involve both fast and slow steps is the identification of appropriate fast and slow variables, since they are usually not chemical species. To illustrate this we consider a system of the form

$$\frac{dc}{d\tau} = (K_0 + \epsilon K_1)c, \tag{17}$$

where $c \in \mathbf{R}_m^+$. As written this is a regular perturbation problem, but as it stands we cannot determine which components vary rapidly and which components vary slowly. However it is clear that, to lowest order in ϵ, the slow reactions encoded in K_1 only affect the stationary modes of the fast dynamics encoded in K_0.

We assume that the underlying graph of the kinetic network consists of a single strongly–connected component, for otherwise we apply the following to each component. It follows that $\rho(K_0 + \epsilon K_1) = m - 1$ and a left eigenvector corresponding to the zero eigenvalue is $u_m \equiv (1, 1, \ldots, 1)^T$ (Othmer 1979). Let $\mathcal{P} : \mathbf{R}_m \to \mathcal{N}(K_0^T)$; then $I - \mathcal{P} : \mathbf{R}_m \to \mathcal{R}(K_0)$ and we have

$$c = \mathcal{P}c + (I - \mathcal{P})c \equiv \xi + \eta. \tag{18}$$

Using this in (17) leads to

$$\frac{d\xi}{d\tau} + \frac{d\eta}{d\tau} = (K_0 + \epsilon K_1)(\xi + \eta), \tag{19}$$

and applying $\mathcal{P}$ and $I - \mathcal{P}$ leads to

$$\frac{d\xi}{d\tau} = \epsilon \mathcal{P} K_1(\xi + \eta) \tag{20}$$

$$\frac{d\eta}{d\tau} = K_0\eta + \epsilon((I - \mathcal{P})K_1)(\xi + \eta). \tag{21}$$

The first of these gives the slow dynamics (on the τ-scale) and the second gives the fast dynamics. On the slow scale $t = \epsilon\tau$, we rewrite (20) and (21) to lowest order as

$$\frac{d\xi}{dt} = \mathcal{P} K_1(\xi + \eta). \tag{22}$$

$$\eta = u_m \tag{23}$$

The second of these defines the slow manifold to lowest order in ϵ, and the first defines the flow on that manifold, again to lowest order. This example illustrates the general procedure concisely: first one must find a suitable representation of the solutions of the 'fast' dynamics (governed by K_0 in this example), and then eliminate these fast variables to obtain an evolution equation for the slowly-varying quantities. Of course the slow manifold has to be 'attracting' in a suitable sense, else the reduction is of no interest. From this example one sees that in general neither the fast nor the slow variables are chemical species. In addition, when some of the reactions are bimolecular the reduction is more difficult.

Hereafter we assume that for initial conditions in a specified region of state space there is a significant separation in the rates of the reactions in the network. When all slow reactions are switched off in the network, i.e. all arrows for slow reactions are removed in the directed graph of the system, the remaining system is called the fast subsystem of the full system. The graph of the fast subsystem, which we denote by $\mathcal{G}^f$, is the disjoint union of the components, denoted by $\mathcal{G}^f_\alpha$, which consist of the edges associated with the fast reactions and nodes connected by those edges. Note that if a complex is affected by only slow reactions, i.e. a node in $\mathcal{G}$ has only edges associated with slow reactions incident at it, then the node is an isolated node in $\mathcal{G}^f$, and so it is a component $\mathcal{G}^f_\alpha$ for some α. Thus, in general the graph $\mathcal{G}^f$ has more components than $\mathcal{G}$. We assume that there are r_f fast reaction and $r_s = r - r_f$ slow reactions in the region of interest in concentration space.

In a reaction network with two distinct time scales one can separate the reactions into fast and slow reactions according to their rates, and the governing evolution equation takes the form

$$\begin{aligned}\frac{dc}{d\tau} &= \nu\mathcal{E}^f R^f(c) + \epsilon\nu\mathcal{E}^s R^s(c)\\ &= \Big[\nu\mathcal{E}^f | \nu\mathcal{E}^s\Big]\begin{bmatrix} R^f(c) \\ \epsilon R^s(c)\end{bmatrix} = \nu\mathcal{E}R(c)\end{aligned} \tag{24}$$

on the fast time scale. Here $\mathcal{E}^f$, which is $p \times r_f$, and $\mathcal{E}^s$, which is $p \times r_s$, denote the incidence matrices of fast and slow reactions, respectively, and R^f and R^s denote the scaled rate functions (now of the same order of magnitude), associated with fast and slow reactions, respectively. Hereafter we denote the ranks of $\nu\mathcal{E}^f$, $\nu\mathcal{E}^s$ and $\nu\mathcal{E}$ by $\rho(\nu\mathcal{E}^f)$ $\rho(\nu\mathcal{E}^s)$ and $\rho(\nu\mathcal{E})$, respectively. Until stated otherwise, we assume that the graph of the fast subsystem has been reduced following the procedure in Sect. 3, and therefore $\nu\mathcal{E}^f$ has full rank, i.e., $\rho(\nu\mathcal{E}^f) = r_f$, but $\nu\mathcal{E}^s$ may not have full rank unless the slow reactions are independent. Clearly $\mathcal{R}(\nu\mathcal{E}^f) \subseteq \mathcal{R}(\nu\mathcal{E})$ with equality only if there are no slow reactions.

When there are fast and slow reactions one can also define the kinematic invariants for the fast subsystem by replacing $\mathcal{N}[(\nu\mathcal{E})^T]$ by $\mathcal{N}[(\nu\mathcal{E}^f)^T]$. Notice that $\mathcal{N}[(\nu\mathcal{E})^T] \subset \mathcal{N}[(\nu\mathcal{E}^f)^T]$ and so the basis elements of $\mathcal{N}[(\nu\mathcal{E})^T]$ are represented by linear combinations of the basis elements of $\mathcal{N}[(\nu\mathcal{E}^f)^T]$. This implies that conservation relations for the whole system also hold for the fast subsystem, but not necessarily vice-versa. As a result, one can define the map $P^f : \mathbf{R}_m \rightarrow \mathbf{R}_{m-r_f}$ for the fast subsystem that represents a vector in $\mathcal{N}[(\nu\mathcal{E}^f)^T]$ in terms of intrinsic coordinates on $\mathcal{N}[(\nu\mathcal{E}^f)^T]$. The associated matrix $\mathcal{P}^f$ has rows given by basis vectors with integer components of $\mathcal{N}[(\nu\mathcal{E}^f)^T]$. It follows that the reaction simplex for the fast subsystem is given by

$$\Omega_f(c_0) \equiv \{c\ : c \in c_0 + \mathcal{R}[\nu\mathcal{E}^f]\} \cap \bar{\mathbf{R}}_m^+ = \{c : \mathcal{P}^f c = \mathcal{P}^f c_0 \equiv \tilde{c} \in \mathbf{R}_{m-r_f}\} \cap \bar{\mathbf{R}}_m^+,$$

Note that $\tilde{c}$ represents a conserved quantity for the fast subsystem, but it may vary as slow reactions occur.

The kinetic equilibria for the fast subsystem in a two-time scale network is defined as in the full system; a point c which satisfies the equation

$$\nu\mathcal{E}^f R^f(c) = 0$$

is called a kinetic equilibrium point for the fast subsystem, and the kinetic equilibrium manifold $\mathcal{K}_f$ for the fast subsystem is defined as

$$\mathcal{K}_f \equiv \{c : \nu\mathcal{E}^f R^f(c) = 0\}. \tag{25}$$

Since it is assumed that $\nu\mathcal{E}^f$ has full rank, this reduces to

$$\mathcal{K}_f \equiv \{c : R^f(c) = 0\}. \tag{26}$$

Clearly $\mathcal{K}_f$ is the set of all the kinetic equilibrium points of the fast subsystem when all the slow reactions are switched off, and hereafter we will assume the existence of $\mathcal{K}_f$ for the fast subsystem. For a more detailed analysis on the existence of kinetic equilibrium state of a reaction network and in particular the relationship between kinetic equilibria and thermodynamic equilibria, see Othmer (1976).

4.1 Preliminary steps in the reduction

If we pattern the reduction of (24) after that of the linear system, we represent c as in (18), and we split (24) by using a projection $\mathcal{P}$ onto $\mathcal{N}[\nu\mathcal{E}^f]$, and its complement $I - \mathcal{P}$. The result is the system

$$\frac{d\xi}{d\tau} = \epsilon\mathcal{P}\nu\mathcal{E}^s R^s(\xi + \eta) \tag{27}$$

$$\frac{d\eta}{d\tau} = \nu\mathcal{E}^f R^f(\xi + \eta) + \epsilon(I - \mathcal{P})\nu\mathcal{E}^s R^s(\xi + \eta). \tag{28}$$

This is the analog of (20) and (21). On the slow time scale t the equations become

$$\frac{d\xi}{dt} = \mathcal{P}\nu\mathcal{E}^s R^s(\xi + \eta) \tag{29}$$

$$\epsilon\frac{d\eta}{dt} = \nu\mathcal{E}^f R^f(\xi + \eta) + \epsilon\nu\mathcal{E}^s R^s(\xi + \eta) \tag{30}$$

and to lowest order the second of these leads to

$$R^f(\xi + \eta) = 0. \tag{31}$$

It appears from this that ξ is a suitable slow variable and η a suitable fast variable. If one can solve (31) to obtain $\eta = \Xi(\xi)$, then using this in (29) gives an explicit relation for the slow dynamics. However (31) suggests that a more natural coordinate system may be based on the level sets of $R^f(c) = 0$, for then the fast dynamics relax to $R^f(c) = 0$ under suitable conditions. The following shows how the remaining coordinates arise naturally.

We begin with a formal reduction of the full system (24), and assume that the solution depends on two time scales as follows:

$$c(t, \epsilon) = \sum_{i=0}^{\infty} \epsilon^i r_i(t) + \sum_{i=1}^{\infty} \epsilon^i s_i\left(\frac{t}{\epsilon}\right). \tag{32}$$

The singular part describes the initial dynamics, and by expansion of this part it follows that

$$\epsilon \sum_{i=0}^{\infty} \epsilon^i \frac{ds_i\left(\frac{t}{\epsilon}\right)}{dt} = \epsilon \nu \mathcal{E}^s R^s\left(\sum_{i=0}^{\infty} \epsilon^i s_i\left(\frac{t}{\epsilon}\right)\right) + \nu \mathcal{E}^f R^f\left(\sum_{i=0}^{\infty} \epsilon^i s_i\left(\frac{t}{\epsilon}\right)\right)$$

Replacing t by $\tau = \frac{t}{\epsilon}$ we obtain

$$\sum_{i=0}^{\infty} \epsilon^i \frac{ds_i(\tau)}{d\tau} = \epsilon \nu \mathcal{E}^s R^s\left(\sum_{i=0}^{\infty} \epsilon^i s_i(\tau)\right) + \nu \mathcal{E}^f R^f\left(\sum_{i=0}^{\infty} \epsilon^i s_i(\tau)\right)$$

By expanding and comparing powers of ϵ^i term we obtain

$$\epsilon^0 : \frac{ds_0}{d\tau} = \nu \mathcal{E}^f R^f(s_0) \tag{33}$$

$$\epsilon^1 : \frac{ds_1}{d\tau} = \nu \mathcal{E}^s R^s(s_0) + \nu \mathcal{E}^f D_{s_0}[R^f(s_0)] \cdot s_1 \tag{34}$$

$$\vdots \quad \vdots$$

where $D_{s_0}[\cdot]$ denotes the Jacobian of a function $\cdot$ with respect to s_0. Since our main interest lies in the dynamics on the slow time scale, we will not consider the singular part further, nor will we discuss the matching between the inner and outer solutions.

A detailed prescription for the latter can be found, for example, in Lin and Segel (1988).

To obtain the slow dynamics we use the expansion of the regular part $\sum_{i=0}^{\infty}\epsilon^i r_i(t)$ in (24) to obtain

$$\epsilon\sum_{i=0}^{\infty}\epsilon^i\frac{dr_i}{dt}=\nu\mathcal{E}^f R^f\left(\sum_{i=0}^{\infty}\epsilon^i r_i\right)+\epsilon\nu\mathcal{E}^s R^s\left(\sum_{i=0}^{\infty}\epsilon^i r_i\right)$$

Comparing the ϵ^0 and ϵ^1 terms, we obtain

$$\epsilon^0 : 0=\nu\mathcal{E}^f R^f(r_0) \tag{35}$$

$$\epsilon^1 : \frac{dr_0}{dt}=\nu\mathcal{E}^s R^s(r_0)+\nu\mathcal{E}^f D_{r_0}[R^f(r_0)]\cdot r_1 \tag{36}$$

$$\vdots \quad \vdots$$

We will approximate the solution $c(t)$ by $r_0(t)$, and the next step is to determine $r_0(t)$ from the Eqs. (35) and (36). First note that (35) defines the kinetic equilibrium of the fast subsystem and the set of solutions of this equation is the kinetic equilibrium manifold. Since $\nu\mathcal{E}^f$ is assumed to have full rank, the manifold is defined as $\mathcal{M}_f=\{c : R^f(c)=0\}$ and $dim(\mathcal{M}_f)\le m-s$, where $s\equiv\rho(D_c[R^f(c)])\le r_f$. Since $s<m$, we have an $(m-s)$-parameter family of solutions, and r_0 is not fully determined by (35).

Thus we have to obtain an evolution equation for r_0 from (36) that does not involve r_1, and this equation should determine the evolution of r_0 on $\mathcal{K}_f$. This can be done by utilizing the kinematic invariants determined by the stoichiometry $\nu\mathcal{E}^f$ of the fast subsystem. Recall that $\tilde{c}=\mathcal{P}^f c$, and multiply (36) by $\mathcal{P}^f$ to obtain

$$\begin{aligned}\frac{d\mathcal{P}^f r_0}{dt}&=\mathcal{P}^f\nu\mathcal{E}^s R^s(r_0)+\mathcal{P}^f\nu\mathcal{E}^f D_{r_0}[R^f(r_0)]\cdot r_1\\&=\mathcal{P}^f\nu\mathcal{E}^s R^s(r_0),\end{aligned}$$

where the last step follows from the fact that $\mathcal{P}^f\nu\mathcal{E}^f=0$. Since $c=r_0+\mathcal{O}(\epsilon)$, to $\mathcal{O}(\epsilon)$ we obtain the reduced equation

$$\frac{d\tilde{c}}{dt}=\mathcal{P}^f\cdot\nu\mathcal{E}^s R^s(c), \tag{37}$$

where c lies on the equilibrium manifold $\mathcal{K}_f$ of the fast subsystem. We earlier saw that $\tilde{c}$ is invariant under the fast dynamics, and now see how it varies under the slow reactions.

The reduced equation (37) is a system of $m-r_f$ differential equations which depend implicitly on the variable c. The equilibrium manifold $\mathcal{K}_f$ of the fast subsystem has dimension $m-s$, and if $s<r_f$ then (37) does not define a vector field on $\mathcal{K}_f$ since some directions are inaccessible. Thus we have to require that $s=r_f$, i.e., that $\rho(D_c[R^f(c)])=\rho(\nu\mathcal{E}^f)$, and we call this "the rank condition". In essence this means that the dimension of the range of the fast stoichiometric matrix $\nu\mathcal{E}^f$ is equal to that of the local tangent space to $\mathcal{K}_f$.

4.2 Reduction to the singularly-perturbed form

We have now motivated using as natural coordinates the level sets of the fast reaction rate R^f and coordinates related to the invariants of the fast dynamics. In this section we reformulate equation (24) into a standard form with a distinct separation of fast and slow variables through a local coordinate change, and clarify the geometric meaning of the reduction. This is done under the condition that the product $D_c(R^f(c))\ \nu\mathcal{E}^f$ is nonsingular on every positive semiorbit of the governing equation

$$\frac{dc}{dt} = \frac{1}{\epsilon}\nu\mathcal{E}^f R^f(c) + \nu\mathcal{E}^s R^s(c), \tag{38}$$

in the domain of attraction $\mathcal{A}(\mathcal{K}_f)$ of the manifold $\mathcal{K}_f$, and we call this the nonsingularity condition.[3] We do not formalize this neighborhood, but trajectories are always understood to remain in this neighborhood. The formal result is stated as follows.

Theorem 3 *Let $T(c)$ be the coordinate change defined by*

$$T(c) = \begin{bmatrix} \mathcal{P}^f \cdot c \\ R^f(c) \end{bmatrix} \equiv \begin{bmatrix} \alpha \\ \beta \end{bmatrix},$$

Then the system (38) can be transformed into the two-time-scale singularly-perturbed system

$$\frac{d\alpha}{dt} = \mathcal{P}^f \cdot \nu\mathcal{E}^s R^s(c) \tag{39}$$

$$\epsilon\frac{d\beta}{dt} = \epsilon D_c(R^f(c))\nu\mathcal{E}^s R^s(c) + D_c(R^f(c))\nu\mathcal{E}^f \beta \tag{40}$$

$$\alpha(0) = \mathcal{P}^f c_0 \tag{41}$$

$$\beta(0) = \mathcal{R}^f(c_0), \tag{42}$$

where $c = T^{-1}(\alpha, \beta)$, if and only if the matrix $D_c(R^f(c))\ \nu\mathcal{E}^f$ is nonsingular in $\mathcal{A}(\mathcal{K}_f)$.

Proof First we show that Jacobian of the transformation T is nonsingular (and so T is a local diffeomorphism) if and only if $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular. Suppose first that the matrix $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular. Since

$$D_c T = \begin{bmatrix} \mathcal{P}^f \\ D_c R^f \end{bmatrix},$$

$\mathcal{N}[D_c T] = \mathcal{N}[\mathcal{P}^f] \cap \mathcal{N}[D_c(R^f)]$, and nonsingularity of the Jacobian of the transformation T is equivalent to

$$\mathcal{N}[\mathcal{P}^f] \cap \mathcal{N}[D_c(R^f)] = \{0\}.$$

---

[3] It is easy to see that the nonsingularity condition implies the rank condition stated earlier.

It is clear that nonsingularity of $D_c(R^f)\ \nu\mathcal{E}^f$ on the solution trajectory implies that no column of $\nu\mathcal{E}^f$ lies in $\mathcal{N}[D_c R^f(c)]$ at any c in $\mathcal{A}(\mathcal{K}_f)$, and so $\mathcal{R}[\nu\mathcal{E}^f] \cap \mathcal{N}[D_c(R^f)] = \{0\}$. Since $\mathcal{N}[\mathcal{P}^f] = \mathcal{R}[\nu\mathcal{E}^f]$,

$$\mathcal{N}[\mathcal{P}^f] \cap \mathcal{N}[D_c(R^f)] = \{0\},$$

which is equivalent to the nonsingularity of the Jacobian of the transformation T.

Next we suppose that T is a diffeomorphism. Thus the Jacobian of T is nonsingular and so

$$\mathcal{R}[\nu\mathcal{E}^f] \cap \mathcal{N}[D_c R^f] = \{0\}. \tag{43}$$

If $D_c R^f\ \nu\mathcal{E}^f$ is singular there is $y \neq 0$ such that

$$D_c R^f \cdot \nu\mathcal{E}^f y = 0,$$

which implies that $\nu\mathcal{E}^f y \in \mathcal{N}[D_c R^f]$. Notice that $\nu\mathcal{E}^f y \neq 0$ by the fact that $y \neq 0$ and $(\nu\mathcal{E}^f)$ has full rank. Since $\nu\mathcal{E}^f y \in \mathcal{R}[\nu\mathcal{E}^f]$, one has

$$0 \neq \nu\mathcal{E}^f y \in \mathcal{R}[\nu\mathcal{E}^f] \cap \mathcal{N}[D_c R^f],$$

which is a contradiction to (43). Thus we proved that T is a diffeomorphism if and only if $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular.

Differentiating $T(c)$ with respect to t, the first $m - r_f$ components lead to

$$\frac{d\alpha}{dt} = \mathcal{P}^f \frac{dc}{dt} = \mathcal{P}^f \left(\frac{1}{\epsilon}\nu\mathcal{E}^f R^f(c) + \nu\mathcal{E}^s R^s(c)\right) = \mathcal{P}^f \nu\mathcal{E}^s R^s(c),$$

since $\mathcal{P}^f \nu\mathcal{E}^f = 0$. The remaining r_f components lead to

$$\begin{aligned}\frac{d\beta}{dt} &= \frac{dR^f(c)}{dt} = D_c[R^f(c)]\frac{dc}{dt} = D_c[R^f(c)]\left(\frac{1}{\epsilon}\nu\mathcal{E}^f R^f(c) + \nu\mathcal{E}^s R^s(c)\right)\\ &= \frac{1}{\epsilon}D_c[R^f(c)]\nu\mathcal{E}^f R^f(c) + D_c[R^f(c)]\nu\mathcal{E}^s R^s(c)\end{aligned}$$

Thus we obtain the standard form of a singularly-perturbed system written in terms of $m - r_f$ slow variables and r_f fast variables:

$$\begin{aligned}\frac{d\alpha}{dt} &= \mathcal{P}^f \nu\mathcal{E}^s R^s(c)\\ \epsilon\frac{d\beta}{dt} &= \epsilon D_c(R^f(c))\nu\mathcal{E}^s R^s(c) + D_c(R^f(c))\nu\mathcal{E}^f \beta,\end{aligned}$$

where $c = T^{-1}(\alpha, \beta)$. □

According to this theorem, one can identify the fast and slow variables explicitly in any system in which $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular. It should be noted that the nonsingularity of $D_c(R^f)\ \nu\mathcal{E}^f$ depends on the network topology of the fast subsystem but not that of the slow reactions. Examples given later will illustrate this point.

Remark 1 It should be noted that the existence of the coordinate transformation does not require that the kinetics are IMAK; only the nonsingularity condition is needed. However, as stated earlier, there is an implicit assumption that the slow manifold is attracting, and when there is additional structure in the problem this may follow from known results. For instance, if the kinetics are IMAK and the deficiency is zero *in the original network, before reduction* the results of Horn and Jackson (1972) guarantee that the slow manifold is attracting.

Example 1 When the fast system includes only linear reactions, one can explicitly find the inverse of the coordinate transformation T: since all fast reactions are linear, $D_c(R^f(c)) \equiv \hat{K}^f$ is a constant matrix. Thus, one can write

$$\nu\mathcal{E}^f R^f(c) = \nu\mathcal{E}^f \hat{K}^f c \equiv K^f c$$

where K^f is an $m \times m$ rate constant matrix for the fast reactions. In this case, the Eqs. (39) and (42) can be rewritten as

$$\frac{d\alpha}{dt} = \mathcal{P}^f \cdot \nu\mathcal{E}^s R^s(c) \tag{44}$$

$$\epsilon\frac{d\beta}{dt} = \epsilon\hat{K}^f \nu\mathcal{E}^s R^s(c) + \hat{K}^f K^f c, \tag{45}$$

where c can be expressed in terms of α and β by solving the linear system

$$c = \begin{bmatrix} \mathcal{P}^f \\ \hat{K}^f \end{bmatrix}^{-1} \begin{pmatrix} \alpha \\ \beta \end{pmatrix}$$

given that $T = [\mathcal{P}^f|\hat{K}^f]^T$ is nonsingular.

If all slow reactions are also linear (and so the whole system is a linear reaction system), we also have

$$\nu\mathcal{E}^s R^s(c) = \nu\mathcal{E}\hat{K}^s c \equiv K^s c.$$

Thus one can write the governing equation as the system of linear equations

$$\frac{d}{dt}\begin{pmatrix} \alpha \\ \epsilon\beta \end{pmatrix} = \begin{bmatrix} \mathcal{P}^f K^s T^{-1} \\ \epsilon\hat{K}^f K^s T^{-1} + \hat{K}^f K^f T^{-1} \end{bmatrix} \begin{pmatrix} \alpha \\ \beta \end{pmatrix}. \tag{46}$$

4.3 The geometric interpretation of the transformation

One can obtain the fast dynamics of the system to lowest order by defining the time scale by $\tau = t/\epsilon$ and letting $\epsilon \to 0$ in Eqs. (39) and (42) as follows;

$$\frac{d\alpha}{d\tau} = 0 \tag{47}$$

$$\frac{d\beta}{d\tau} = D_c(R^f(c))\nu\mathcal{E}^f\beta. \tag{48}$$

Note that Eq. (47) implies α is constant at the initial value $\alpha_0 = P^f c(0)$. Thus, the system (47) and (48) can be written, under the nonsingularity of $D_c(R^f(c)) \cdot \nu\mathcal{E}^f$, as

$$\frac{d\beta}{d\tau} = D_c(R^f(c))\nu\mathcal{E}^f\beta, \quad \alpha = \alpha_0, \tag{49}$$

where $c = T^{-1}(\alpha_0, \beta)$.

Geometrically the nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$ on the solution trajectory implies that the family of reaction simplexes Ω_f for the fast subsystem is transversal to the level sets $\mathcal{K}_{f,c} \equiv \{\bar{c} : R^f(\bar{c}) = R^f(c)\}$ where c is any point in the solution trajectory. To see this, first notice that since $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular, no column of $\nu\mathcal{E}^f$ lies in $\mathcal{N}[D_c R^f](c)$ at any c in the solution trajectory. Thus $\mathcal{R}[\nu\mathcal{E}^f]$ is non-tangential or transversal to all level sets $\mathcal{K}_{f,c}$ since $\mathcal{N}[D_c R^f](c)$ is a subspace consisting of vectors tangent to the level set $\mathcal{K}_{f,c}$ at any point c. This implies that each $\Omega_f(c) = \{c + \mathcal{R}[\nu\mathcal{E}_f]\} \cap (\bar{\mathbf{R}}_m)^+$ is transversal to all level sets. Thus for any point c on the solution trajectory, one can write

$$\mathbf{R}_m = \Omega_f(c) \oplus T_c\mathcal{K}_{f,c},$$

where $T_c\mathcal{K}_{f,c}$ is the tangent space to $\mathcal{K}_{f,c}$ at c. In the new coordinates the system evolves rapidly along directions transversal to the level sets $\mathcal{K}_{f,c}$ and slowly along the transversal direction to the family of Ω_f. Examples are given in the following section.

By virtue of the assumed nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$, the steady state of the fast dynamics, to lowest order in ϵ, is $\beta = 0$, which then leads to a complete separation of slow and fast variables, since the first equation is independent of β to lowest order. More precisely, if one applies the QSSA by formally setting $\epsilon \to 0$, one has that on the slow time scale

$$\frac{d\alpha}{dt} = \mathcal{P}^f \cdot \nu\mathcal{E}^s R^s(c) \tag{50}$$

$$\alpha_0 = P^f c(0) \tag{51}$$

$$\beta = 0. \tag{52}$$

Under the nonsingularity of $D_c(R^f(c)) \cdot \nu\mathcal{E}^f$, the above equation is equivalent to

$$\frac{d\alpha}{dt} = \mathcal{P}^f \cdot \nu\mathcal{E}^s R^s(T^{-1}(\alpha, 0)) \tag{53}$$

which is independent of β.

Remark 4 If the nonsingularity of $D_c(R_f(c))\nu\mathcal{E}^f$ fails, then on the slow time scale variables are subject to evolve in a manifold $\{c : R^f(c) \in \mathcal{N}[D_c(R^f(c))\nu\mathcal{E}^f]\}$ rather than $\mathcal{K}_f = \{c : R^f(c) = 0\}$, since in Eq. (42), as $\epsilon \to 0$, $D_c(R^f(c))\nu\mathcal{E}^f R^f(c) = 0$, which implies that under the QSS assumption c lies in the manifold $\{c : R^f(c) \in \mathcal{N}[D_c(R^f(c))\nu\mathcal{E}^f]\}$.

5 Sufficient conditions for the nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$

In a number of cases the network topology guarantees the nonsingularity of $D_c(R^f(c))\nu\mathcal{E}^f$. First, one can see that the nonsingularity holds when the fast subsystem consists of only one reversible or irreversible linear reaction: If the reaction is linear, the fast subsystem is either (i) $A \overset{k_f}{\to} B$ or (ii) $A \underset{k_f}{\overset{k_r}{\leftrightharpoons}} B$, and in case (i), the reaction rate function $R^f(c) = k_f c_1$, where c_1 and c_2 are concentrations of A and B, respectively. Thus $D_c R^f \nu\mathcal{E}^f = -k_f < 0$. In the case (ii), $R^f(c) = k_f c_1 - k_r c_2$ and so $D_c R^f \nu\mathcal{E}^f = -(k_f + k_r) < 0$.

Secondly, suppose that the fast subsystem contains one or more bimolecular reactions. One can easily show that the nonsingularity holds when the fast subsystem consists of only one reversible or irreversible bimolecular reaction, for in this case there are only four types of bimolecular reactions:

(i) $A + B \overset{k_f}{\to} C$

(ii) $A + B \underset{k_f}{\overset{k_r}{\leftrightharpoons}} C,$

(iii) $A + B \overset{k_f}{\to} C + D,$

and

(iv) $A + B \underset{k_f}{\overset{k_r}{\leftrightharpoons}} C + D.$

In types (i) and (iii), $R(c) = k_f c_1 c_2$ and $D_c R^f \nu\mathcal{E}^f = -k_f(c_1 + c_2) < 0$ unless $(c_1, c_2) = 0$. In type (ii), $R(c) = k_f c_1 c_2 - k_r c_3$ and $D_c R^f \nu\mathcal{E}^f = -k_f(c_1+c_2) - k_r < 0$. In type (iv), $R(c) = k_f c_1 c_2 - k_r c_3 c_4$ and $D_c R^f \nu\mathcal{E}^f = -(k_f(c_1 + c_2) + k_r(c_3 + c_4)) < 0$ unless $(c_1, c_2, c_3, c_4) = 0$, i.e. the system is degenerate, which is a trivial case. Therefore fast and slow variables can be identified explicitly when there is only one fast reversible or irreversible bimolecular reaction in the system. Moreover, for a system in which any two different fast reactions have no common reactants and products, $D_c(R^f)\nu\mathcal{E}^f$ is also nonsingular, for it is a diagonal matrix with negative diagonal elements.

The following lemma gives more general conditions under which the nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$ holds.

Lemma 5 *Suppose that a reaction network satisfies following three conditions*: (i) *the undirected graph of each fast component* $\mathcal{G}^f_\alpha$ *is originally a tree,* (ii) *any two different fast components have no common species and* (iii) *any two different reactants* *in each fast component contain no common species. Then, the matrix* $D_c(R^f(c))\nu\mathcal{E}^f$ *is nonsingular.*

Proof First we note that $D_c(R^f)$ and $\nu\mathcal{E}^f$ are block diagonal matrices since any two different fast components have no common species. Thus $D_c(R^f)\nu\mathcal{E}^f$ is also a block diagonal matrix if there are at least two different fast components in the graph $\mathcal{G}$, and so nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$ is equivalent to nonsingularity of each block diagonal matrix $[D_c(R^f)\nu\mathcal{E}^f]_\alpha$ which corresponds to each fast component $\mathcal{G}^f_\alpha$. It suffices to consider a fast component $\mathcal{G}^f_\alpha$ and prove that the matrix $[D_c(R^f)\nu\mathcal{E}^f]$ corresponding to $\mathcal{G}^f_\alpha$ is nonsingular. We recall that if the ith species is a reactant (product) of the jth reaction, then $(\nu\mathcal{E}_{ij}) < 0$ (> 0). Thus reaction rate function R^f_j for a reversible reaction is

$$R^f_j(c) = k^f_j \prod_{i=1}^{m} (c_i)^{[(\nu\mathcal{E})_{ij}]^-} - k^r_j \prod_{i=1}^{m} (c_i)^{[(\nu\mathcal{E})_{ij}]^+},$$

where k^f_j and k^r_j are rate constants of the jth forward and backward reactions and $(\nu\mathcal{E})^-_{ij} = -\min\{(\nu\mathcal{E})_{ij}, 0\}$ and $(\nu\mathcal{E})^+_{ij} = \max\{(\nu\mathcal{E})_{ij}, 0\}$. Thus

$$\frac{\partial R^f_j}{\partial c_l} = \begin{cases} k^f_j (\nu\mathcal{E})^-_{lj} c_l^{(\nu\mathcal{E})^-_{lj}-1} \prod_{i\neq l}(c_i)^{(\nu\mathcal{E}_{ij})^-} & \text{if } l\text{th species is a reactant} \\ & \quad \text{of } j\text{th forward reaction} \\ -k^r_j (\nu\mathcal{E})^+_{lj} c_l^{(\nu\mathcal{E})^+_{lj}-1} \prod_{i\neq l}(c_i)^{(\nu\mathcal{E}_{ij})^+} & \text{if } l\text{th species is a product} \\ & \quad \text{of } j\text{th forward reaction} \\ 0 & \text{otherwise.} \end{cases}$$

If the reaction R^f_j is irreversible, then reaction rate function R^f_j is

$$R^f_j(c) = k_j \prod_{i=1}^{m} (c_i)^{(\nu\mathcal{E})^-_{ij}},$$

and so

$$\frac{\partial R^f_j}{\partial c_l} = \begin{cases} k_j (\nu\mathcal{E})^-_{lj} c_l^{(\nu\mathcal{E})^-_{lj}-1} \prod_{i\neq l}(c_i)^{(\nu\mathcal{E})^-_{ij}} & \text{if } l\text{th species is a reactant of } j\text{th reaction} \\ 0 & \text{otherwise} \end{cases}$$

Now we can compute the (j, k)th component of $D_c(R^f)\nu\mathcal{E}^f$ as follows. Note that by labeling each species and reaction properly we can ensure that $[D_c(R^f)\nu\mathcal{E}^f]_{jk} = 0$ if $j < k$, i.e. $[D_c(R^f)\nu\mathcal{E}^f]$ is lower triangular, since any two different reactants in $\mathcal{G}^f_\alpha$ have no common species and $\mathcal{G}^f_\alpha$ has a tree structure.

For $j = k$, first note that if the ith species is a reactant (product) of the jth reaction in the fast component, then $(\nu\mathcal{E}^f)_{ij} < 0 (> 0)$ where $\nu\mathcal{E}^f$ defines the reaction simplex for the fast component $\mathcal{G}^f_\alpha$. If the reaction R^f_j is reversible, then

$$[D_c(R^f)\nu\mathcal{E}^f]_{jj} = -\left[k^f_j\left(\sum_{l=reac}[(\nu\mathcal{E}^f)^-_{lj}]^2 c_l^{(\nu\mathcal{E}^f)^-_{lj}-1}\prod_{i\neq l}(c_i)^{(\nu\mathcal{E}^f)^-_{ij}}\right)\right.$$
$$\left.+\,k^r_j\left(\sum_{m=prod}[(\nu\mathcal{E}^f)^+_{mj}]^2 c_m^{(\nu\mathcal{E}^f)^+_{mj}-1}\prod_{i\neq m}(c_i)^{(\nu\mathcal{E}^f)^+_{ij}}\right)\right] < 0,$$

where l and m denote indices of species in reactants and products of the jth reaction, respectively. If the jth reaction is irreversible, then

$$[D_c(R^f)\nu\mathcal{E}^f]_{jj} = -\left[k^f_j\sum_{l=prod}[(\nu\mathcal{E}^f)^-_{lj}]^2\left(c_l^{(\nu\mathcal{E}^f)^-_{lj}-1}\prod_{i\neq l}(c_i)^{(\nu\mathcal{E}^f)^-_{ij}}\right)\right] < 0$$

Thus $D_c(R^f)\nu\mathcal{E}^f$ is lower triangular with nonzero diagonal elements and so $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular, which completes the proof. □

If a fast subsystem is a linear chain network,

$$A_1 \overset{k_1}{\to} A_2 \overset{k_2}{\to} \cdots \overset{k_{n-1}}{\to} A_n,$$

the matrix $D_c(R^f(c))\nu\mathcal{E}^f$ is nonsingular, since a linear chain satisfies the three conditions in Lemma 5. Furthermore, for a looped linear network

$$A_1 \overset{k_1}{\to} A_2 \overset{k_2}{\to} \cdots \overset{k_{n-1}}{\to} A_n \overset{k_n}{\to} A_1,$$

one can prove that $D_c(R^f(c))\nu\mathcal{E}^f$ is nonsingular as follows: Note that a reduced graph for the looped network is

$$A_1 \overset{R_1-R_n}{\to} A_2 \overset{R_2-R_n}{\to} \cdots \overset{R_{n-1}-R_n}{\to} A_n,$$

where $R_i = k_i c_i$ and c_i is the concentration of A_i for $i = 1, \ldots, n$. From this reduced graph, we can find an $(n-1)\times(n-1)$ matrix

$$D_c(R^f(c))\nu\mathcal{E}^f = \begin{bmatrix} -k_1 & 0 & \cdots & 0 & -k_n \\ k_2 & -k_2 & \cdots & 0 & -k_n \\ 0 & k_3 & -k_3 & \cdots & -k_n \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & k_{n-1} & -(k_n+k_{n-1}) \end{bmatrix}.$$

By the induction argument, one can show that the determinant of $D_c(R^f(c))\nu\mathcal{E}^f$, $|D_c(R^f(c))\nu\mathcal{E}^f| > 0$ if n is odd, and $|D_c(R^f(c))\nu\mathcal{E}^f| < 0$ if n is even. Thus, $D_c(R^f(c))\nu\mathcal{E}^f$ is nonsingular.

For a more generic case that the graph of a fast subsystem consists of components of linear chains or looped linear networks, one can show that $D_c(R^f(c))\nu\mathcal{E}^f$ is invertible as follows: Suppose the directed graph $\mathcal{G}^f$ of a fast subsystem consists of components $\mathcal{G}^f_\alpha, \alpha = 1, \ldots, n$, where each $\mathcal{G}^f_\alpha$ is a linear chain or a looped linear network. Let $[D_c(R^f(c))\nu\mathcal{E}^f]_i, i = 1, \ldots, n$ be the matrices obtained from each component $\mathcal{G}^f_\alpha$. One can easily see that the matrix $D_c(R^f(c))\nu\mathcal{E}^f$ is block-diagonal, since any two different components share no common nodes(=species in a linear reaction). Since each diagonal block is invertible by above lemma, the matrix $D_c(R^f(c))\nu\mathcal{E}^f$ is also invertible.

6 An explicit representation of the slow dynamics for IMAK

For ideal, mass-action kinetics (IMAK) one can find an explicit expression of (53) in terms of the original variable c when the fast subsystem has deficiency zero, i.e. rank of $\nu\mathcal{E}^f$ = rank of $\mathcal{E}^f$. At a steady state of the fast subsystem we have

$$\nu\mathcal{E}^f R^f(c) = \nu\mathcal{E}^f \hat{K}^f (\mathcal{E}^f_e)^T P(c) = 0, \tag{54}$$

where

$$P_j(c) = \prod_{i=1}^{m} c_i^{\nu_{ij}}.$$

and $\hat{K}^f$ is a diagonal matrix with rate constants of fast reactions along the diagonal, and $(\mathcal{E}^f_e)^T$ is the exit matrix obtained by replacing all 1's in $\mathcal{E}^f$ by zeros. Note that since the fast subsystem has deficiency zero, its steady state solutions are completely determined by solving

$$\mathcal{E}^f R^f(c) = \mathcal{E}^f \hat{K}^f (\mathcal{E}^f_e)^T P(c) = 0. \tag{55}$$

To obtain an explicit expression of solution of (55), we first define ν^f as the stoichiometry for complexes which are reactants or products of any fast reactions and ν^s as the stoichiometry for complexes which are reactants or products of only slow reactions. For example, in a system

$$2A_1 \leftrightharpoons A_2 \leftrightharpoons A_3,$$

where the first reversible reaction is fast and the second is slow, $2A_1$ and A_2 are a reactant or a product of a fast reaction and A_3 is a reactant or a product of a slow reaction. Thus, in this case, we have

$$\nu^f = \begin{bmatrix} 2 & 0 \\ 0 & 1 \\ 0 & 0 \end{bmatrix}, \quad \nu^s = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} \quad \text{and} \quad \nu = [\nu^f \mid \nu^s].$$

We suppose there are r_f independent fast reactions and p_f distinct complexes which are reactants or products of the fast reactions. We can write $\nu = [\nu^f \mid \nu^s]$, where ν^f is an $m \times p_f$ submatrix and ν^s is an $m \times (p - p_f)$ submatrix, where p is the number of distinct complexes in the whole system. According to the structure of the ν, we can write

$$\mathcal{E}^f = \left[\frac{\mathcal{E}_1^f}{\mathbf{0}} \right],$$

where $\mathcal{E}_1^f$ is a $p_f \times r_f$ matrix and $\mathbf{0}$ is a $(p - p_f) \times r_f$ null matrix, and

$$P(c) = \left[\frac{P^f(c)}{P^s(c)} \right],$$

where $P_j^f(c) = \prod_{i=1}^m c_i^{\nu_{ij}^f}$ and $P_k^s(c) = \prod_{i=1}^m c_i^{\nu_{ik}^s}$. Note that

$$\nu \mathcal{E}^f = [\nu^f \mid \nu^s] \left[\frac{\mathcal{E}_1^f}{\mathbf{0}} \right] = \nu^f \mathcal{E}_1^f.$$

Furthermore, by the definition of the exit matrix of $\mathcal{E}^f$, we can write the exit matrix

$$\mathcal{E}_e^f = \left[\frac{\mathcal{E}_{1e}^f}{\mathbf{0}} \right],$$

where $\mathcal{E}_{1e}^f$ is the exit matrix of $\mathcal{E}_1^f$ and $\mathbf{0}$ is a $(p - p_f) \times r_f$ null matrix. It follows that

$$\begin{aligned} \mathcal{E}^f \hat{K}^f (\mathcal{E}_e^f)^T P^f(c) &= \left[\frac{\mathcal{E}_1^f}{\mathbf{0}} \right] \hat{K}^f \left[(\mathcal{E}_{1e}^f)^T | \mathbf{0}^T \right] \left[\frac{P^f(c)}{P^s(c)} \right] \\ &= \left(\frac{\mathcal{E}_1^f \hat{K}^f (\mathcal{E}_{1e}^f)^T P^f(c)}{\mathbf{0}_{p-p_f}} \right), \end{aligned} \tag{56}$$

where $\mathbf{0}_{p-p_f}$ is a $(p - p_f) \times 1$ null column vector. Thus, solving equation (55) is equivalent to solving

$$\mathcal{E}_1^f \hat{K}^f (\mathcal{E}_{1e}^f)^T P^f(c) = 0. \tag{57}$$

To solve $\mathcal{E}_1^f \hat{K}^f (\mathcal{E}_{1e}^f)^T P^f(c) = 0$, we first consider a fast subsystem in which each species appears in only one component of the subsystem. Without loss of generality we can assume that the underlying original graph of the system is a single strongly-connected component, for otherwise we can apply the following argument to each component. Note that the single strongly connected component has a positive balanced flow and for positive c_i's, we can write

$$P_j^f(c) = \prod_{i=1}^{m} c_i^{\nu_{ij}^f} = e^{\sum_i \nu_{ij}^f \ln c_i},$$

or in vector form,

$$P^f(c) = e^{(\nu^f)^T \ln c},$$

where $\ln c = (\ln c_1, \ldots, \ln c_m)^T$. Thus, for a positive balanced flow, we have

$$e^{(\nu^f)^T \ln c} = P^f(c) = \lambda Q, \tag{58}$$

where Q is the unique positive eigenvector associated with the zero eigenvalue of $\mathcal{E}_1^f \hat{K}^f (\mathcal{E}_{1e}^f)^T$ and λ is a constant to be determined. Equation (58) is equivalent to

$$[(\nu^f)^T \mid -\mathbf{1}_{p_f}] \left(\frac{\ln c}{\ln \lambda} \right) = \ln Q, \tag{59}$$

where $\mathbf{1}_{p_f}$ is a p_f-dimensional vector $(1, \ldots, 1)^T$. Note that since there is only one component, we have $p_f = r_f + 1$, where $r_f = \rho(\nu \mathcal{E}^f) = \rho(\nu^f \mathcal{E}_1^f)$. Let $B = [(\nu^f)^T \mid -\mathbf{1}_{p_f}]$ and let $\rho(\nu^f) = p'$. Clearly $r_f = \rho(\nu^f \mathcal{E}_1^f) \le \rho(\nu^f) = p'$, and since $r_f = p_f - 1$ and $p' \le p_f$, we have $p_f - 1 \le p' \le p_f$. This implies that $p' = p_f$ or $p_f - 1$.

If $p' = p_f$, i.e. $(\nu^f)^T$ has full rank p_f, one can rewrite $B = [(\nu_1^f)^T \mid (\nu_2^f)^T \mid -\mathbf{1}_{p_f}]$, where $(\nu_1^f)^T$ is a $p_f \times (m+1-p_f)$ matrix and $[(\nu_2^f)^T \mid -\mathbf{1}_{p_f}]$ is a $p_f \times p_f$ invertible matrix. Let $B_1 = (\nu_1^f)^T$ and $B_2 = [(\nu_2^f)^T \mid -\mathbf{1}_{p_f}]$, so that $B = [B_1 \mid B_2]$. Let

$$c = \begin{pmatrix} c_b \\ c_a \end{pmatrix},$$

where c_b and c_a are $(m + 1 - p_f)(= m - r_f)$-dimensional and $(p_f - 1)(= r_f)$-dimensional vectors corresponding to $(\nu_1^f)^T$ and $(\nu_2^f)^T$, respectively. Thus, one can rewrite equation (59) as

$$[B_1 \mid B_2] \left(\frac{\ln c_b}{\ln \begin{pmatrix} c_a \\ \lambda \end{pmatrix}} \right) = \ln Q. \tag{60}$$

By multiplying (60) by B_2^{-1}, one obtains

$$[B_2^{-1} B_1 \mid I_{p_f}] \left(\frac{\ln c_b}{\ln \begin{pmatrix} c_a \\ \lambda \end{pmatrix}} \right) = B_2^{-1} \ln Q, \tag{61}$$

and by expanding (61),

$$B_2^{-1} B_1 \ln c_b + \ln \begin{pmatrix} c_a \\ \lambda \end{pmatrix} = B_2^{-1} \ln Q, \tag{62}$$

which gives

$$\begin{pmatrix} c_a \\ \lambda \end{pmatrix} = exp \left(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b \right). \tag{63}$$

Thus, one can write

$$c_a = \left(e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_1}, \ldots, e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_{r_f}} \right) \equiv F(c_b), \tag{64}$$

where $(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_k$ denotes kth entry of the vector $(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)$ for each $k = 1, \ldots, r$.

Next we consider the case $p' = p_f - 1$. As in the above, we define a matrix $B \equiv [\nu_1^T \mid \nu_2^T \mid -\mathbf{1}_{p_f}]$. Note that $\rho(B)$ is either p_f or $p_f - 1$. If $\rho(B) = p_f$, one can write $B = [B_1 \mid B_2]$, where $B_1 = (\nu_1^f)^T$ is a $p_f \times (m + 1 - p_f)$ matrix and $B_2 = [(\nu_2^f)^T \mid -\mathbf{1}_{p_f}]$ is a $p_f \times p_f$ invertible matrix. In this case, by applying the same argument as in the previous case that $p' = p_f$, we can find an explicit expression for the zero-order approximation of the slow manifold: it is the intersection of the manifold defined by

$$c_a = \left(e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_1}, \ldots, e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_{r_f}} \right) \equiv F(c_b). \tag{65}$$

with the reaction simplex.

If $\rho(B) = p_f - 1$ we write $(\nu^f)^T$ as

$$(\nu^f)^T = \left[\begin{array}{c|c|c} (\nu_{11}^f)^T & (\nu_{21}^f)^T & (\nu_{31}^f)^T \\ (\nu_{12}^f)^T & (\nu_{22}^f)^T & (\nu_{32}^f)^T \end{array} \right], \tag{66}$$

where $(\nu_{11}^f)^T$ is a $(p_f - 1) \times (m - p_f + 1)$ submatrix, $(\nu_{21}^f)^T$ is a $(p_f - 1) \times 1$ vector, $(\nu_{31}^f)^T$ is a $(p_f - 1) \times (p_f - 2)$ submatrix, $(\nu_{12}^f)^T$ is a $1 \times (m - p_f + 1)$ vector, $(\nu_{22}^f)^T$ is a 1×1 vector(or a scalar), and $(\nu_{32}^f)^T$ is a $1 \times (p_f - 2)$ vector. We again define a matrix B as

$$B = \left[\begin{array}{c|c|c} B_{11} & B_{21} & B_{31} \\ B_{12} & B_{22} & B_{32} \end{array} \right], \tag{67}$$

where $B_{11} = (\nu_{11}^f)^T$, $B_{21} = (\nu_{21}^f)^T$, $B_{31} = [(\nu_{31}^f)^T | -\mathbf{1}_{p_f-1}]$, $B_{12} = (\nu_{12}^f)^T$, $B_{22} = (\nu_{22}^f)^T$ and $B_{32} = [(\nu_{32}^f)^T | -1]$. Thus, we can rewrite (59) as

$$\left[\begin{array}{c|c|c} B_{11} & B_{21} & B_{31} \\ B_{12} & B_{22} & B_{32} \end{array}\right] \begin{pmatrix} \ln c \\ \ln \lambda \end{pmatrix} = \begin{pmatrix} \ln Q_1 \\ \ln Q_2 \end{pmatrix}. \tag{68}$$

Since B has rank $p_f - 1$, we can reduce the last row of B in Eq. (68) to all zeros by elementary row operations. Thus, after using elementary row operations, we write Eq. (68) as

$$\left[\begin{array}{c|c|c} \hat{B}_{11} & \hat{B}_{21} & \hat{B}_{31} \\ 0 & 0 & 0 \end{array}\right] \begin{pmatrix} \frac{\ln c_b}{\ln c_{a_1}} \\ \hline \ln \begin{pmatrix} c_{a_2} \\ \lambda \end{pmatrix} \end{pmatrix} = \begin{pmatrix} \ln \hat{Q}_1 \\ 0 \end{pmatrix}, \tag{69}$$

where c_b, c_{a_1}, c_{a_2} are $(m - p_f + 1)$, 1, $(p_f - 2)$-dimensional subvectors of c, respectively and $\hat{B}_{11}$, $\hat{B}_{21}$, $\hat{B}_{31}$ and $\ln \hat{Q}_1$ can be obtained after applying elementary row operations. Note that we can choose $\hat{B}_{31}$ as an invertible matrix. Thus, from (69) we obtain

$$\ln \begin{pmatrix} c_{a_2} \\ \lambda \end{pmatrix} = \hat{B}_{31}^{-1} \left(\ln \hat{Q}_1 - \hat{B}_{11} \ln c_b - \hat{B}_{21} \ln c_{a_1} \right), \tag{70}$$

and so

$$\begin{aligned} c_{a_2} &= \left(e^{\left(\hat{B}_{31}^{-1}(\ln R - \hat{B}_{11} \ln c_b - \hat{B}_{21} \ln c_{a1})\right)_1}, \ldots, e^{\left(\hat{B}_{31}^{-1}(\ln R - \hat{B}_{11} \ln c_b - \hat{B}_{21} \ln c_{a_1})\right)_{r_f - 1}} \right) \\ &\equiv F(c_b, c_{a_1}). \end{aligned} \tag{71}$$

Note that if there is a conservation relation that gives a functional relation $c_{a_1} = g(c_b)$ between c_{a_1} and c_b, we can obtain an explicit expression of the slow dynamics from (71) as

$$c_a = (c_{a_1}, c_{a_2}) = (g(c_b), F(c_b, g(c_b)) \equiv H(c_b). \tag{72}$$

Now we consider a system in which some species appear in more than one component. Without loss of generality, we can assume that a species appears in two strongly connected components C_1 and C_2. Let ν_1^f and ν_2^f be stoichiometric matrices for complexes which are reactants or products of fast reactions in the two components C_1 and C_2, respectively. Let p_{f_i} and r_{f_i} be the number of distinct complexes and the number of independent reactions in C_i for each $i = 1, 2$, respectively. Note that $p_f = p_{f_1} + p_{f_2}$, $r_f = r_{f_1} + r_{f_2}$ and $p_{f_i} = r_{f_i} + 1$ for each $i = 1, 2$. For a balanced flow, we have

$$e^{(\nu_1^f)^T \ln c} \equiv P_1^f(c) = \lambda_1 Q_1 \tag{73}$$

$$e^{(\nu_2^f)^T \ln c} \equiv P_2^f(c) = \lambda_2 Q_2, \tag{74}$$

where for each $i = 1, 2$, Q_i is the unique positive eigenvector associated with zero eigenvalue of $\mathcal{E}^f_{1_i} \hat{K}^f_i (\mathcal{E}^f_{1e_i})^T$ corresponding to the component C_i and λ_i is a constant to be determined. From (73) and (74), one obtains

$$\left[\begin{array}{c|c|c} (\nu^f_1)^T & -\mathbf{1}_{p_{f_1}} & \mathbf{0}_{p_{f_1}} \\ (\nu^f_2)^T & \mathbf{0}_{p_{f_2}} & -\mathbf{1}_{p_{f_2}} \end{array}\right] \begin{pmatrix} \ln c \\ \ln \lambda_1 \\ \ln \lambda_2 \end{pmatrix} = \begin{pmatrix} \ln Q_1 \\ \ln Q_2 \end{pmatrix}, \tag{75}$$

where $\mathbf{1}_k$ and $\mathbf{0}_k$ are k-dimensional column vectors $(1, \dots, 1)^T$ and $(0, \dots, 0)^T$, respectively. We let

$$B = \left[\begin{array}{c|c|c} (\nu^f_1)^T & -\mathbf{1}_{p_{f_1}} & \mathbf{0}_{p_{f_1}} \\ (\nu^f_2)^T & \mathbf{0}_{p_{f_2}} & -\mathbf{1}_{p_{f_2}} \end{array}\right].$$

Here note that $r_{f_1} + r_{f_2} = r_f = \rho(\nu^f \mathcal{E}^f_1) \le \rho(\nu^f) = p' \le p_f = p_{f_1} + p_{f_2}$. Since $r_{f_1} = p_{f_1} - 1$ and $r_{f_2} = p_{f_2} - 1$, we have

$$p_{f_1} + p_{f_2} - 2 \le p' \le p_{f_1} + p_{f_2}. \tag{76}$$

Thus, rank of ν^f, p' is $p_{f_1} + p_{f_2}$, $p_{f_1} + p_{f_2} - 1$, or $p_{f_1} + p_{f_2} - 2$.

First we consider the case that $p' = p_{f_1} + p_{f_2}$. Note that in this case, B also has full row rank $p_{f_1} + p_{f_2} = p_f$. We let B_1 be a $p_f \times (m - p_f + 2)$ submatrix of B,

$$\begin{bmatrix} (\nu^f_{11})^T \\ (\nu^f_{21})^T \end{bmatrix}$$

and let B_2 be the $p_f \times p_f$ submatrix of B given by,

$$\left[\begin{array}{c|c|c} (\nu^f_{12})^T & -\mathbf{1}_{p_{f_1}} & \mathbf{0}_{p_{f_1}} \\ (\nu^f_{22})^T & \mathbf{0}_{p_{f_2}} & -\mathbf{1}_{p_{f_2}} \end{array}\right],$$

where ν^f_{11} and ν^f_{12} (ν^f_{21} and ν^f_{22}) are submatrices of ν^f_1 (ν^f_2), so that B_2 can be chosen as an invertible matrix. By applying the same argument as in the case for a single component system to the Eq. (75), one finds

$$c_a = \left(e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_1}, \dots, e^{(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln c_b)_{r_f}} \right) \equiv F(c_b), \tag{77}$$

where c_b and c_a are subvectors of c with dimensions $m - r_f$ and r_f, respectively and $Q = (Q_1^T | Q_2^T)^T$.

Finally, we consider the cases that $\rho(\nu^f)$ is either $p_{f_1} + p_{f_2} - 1$ or $p_{f_1} + p_{f_2} - 2$. In either case, $\rho(B)$ can be $p_{f_1} + p_{f_2}$, $p_{f_1} + p_{f_2} - 1$, or $p_{f_1} + p_{f_2} - 2$. If B has full rank $p_{f_1} + p_{f_2}$, we can obtain the explicit expression (77) by applying a similar argument as in the above. If $\rho(B) = p_{f_1} + p_{f_2} - 1$, by applying the same argument as in the case that rank of $B = p_f - 1$ in the previous single component case, one can again obtain an explicit expression

$$c_{a_2} = F(c_b, c_{a_1}), \tag{78}$$

where $c_a = (c_{a_1}, c_{a_2})$, c_{a_1} is a one-dimensional variable, c_{a_2} is a $(m - p_f + 1)$-dimensional variable and F is a function which can be obtained similarly to (71). Thus, in this case, we can obtain an explicit expression of the slow manifold if c_{a_1} can be written as a function of c_b by a conservation relation.

Similarly, if $\rho(B) = p_{f_1} + p_{f_2} - 2$, one can find an expression

$$(c_{a_2}, c_{a_3}) = F(c_b, c_{a_1}), \tag{79}$$

where $c_a = (c_{a_1}, c_{a_2}, c_{a_3})$ and c_{a_1} and c_{a_2} are one-dimensional variables, c_{a_3} is a $(m - p_f)$-dimensional variable and F is a function which can be obtained similarly to (71). Note that we can obtain an explicit expression of the slow manifold if we can write c_{a_1} as a function of c_b by a conservation relation.

By recalling that $\alpha = \mathcal{P}^f c$, we have

$$\frac{d\alpha}{dt} = \mathcal{P}^f \frac{dc}{dt} = \mathcal{P}^f \frac{d}{dt}\begin{bmatrix} c_b \\ F(c_b) \end{bmatrix} = \mathcal{P}^f \begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} \frac{dc_b}{dt} = S\frac{dc_b}{dt}, \tag{80}$$

where

$$S = \mathcal{P}^f \begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix}.$$

Next we prove that the matrix S is invertible when $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular.

Lemma 6 *The matrix*

$$S = \mathcal{P}^f \begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix}$$

is nonsingular, provided that $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular.

Proof First recall that the nonsingularity of $D_c(R^f)\nu\mathcal{E}^f$ implies that

$$\mathcal{N}[\mathcal{P}^f] \cap \mathcal{N}[D_c(R^f)] = \{0\}. \tag{81}$$

(Recall the proof of Theorem 3.) One can see that

$$D_c(R^f) \begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} = 0, \tag{82}$$

by showing that

$$\begin{aligned} D_c(R^f)\begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} &= \left[\frac{\partial R^f(c)}{\partial c_b} \middle| \frac{\partial R^f(c)}{\partial c_a} \right] \begin{bmatrix} I_{m-r_f} \\ \hline \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} \\ &= \frac{\partial R^f(c)}{\partial c_b} + \frac{\partial R^f(c)}{\partial c_a}\frac{\partial F(c_b)}{\partial c_b} \\ &= \frac{\partial}{\partial c_b} R^f(c_b, F(c_b)) = 0, \quad \text{since } R^f(c_b, F(c_b)) = 0. \end{aligned}$$

Since the nullity of $D_c(R^f) = (m - r_f) =$ rank of $\begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix}$, Eq. (82) implies that

$$\mathcal{N}[D_c(R^f)] = \mathcal{R}\begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix}.$$

By (81), we have

$$\mathcal{N}[\mathcal{P}^f] \cap \mathcal{R}\begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} = \{0\},$$

which implies that the matrix

$$S = \mathcal{P}^f \begin{bmatrix} I_{m-r_f} \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix}$$

is nonsingular. □

From Eq. (80) and the above lemma, if $D_c(R^f)\nu\mathcal{E}^f$ is nonsingular, one can obtain an explicit reduced equation

$$\begin{aligned} \frac{dc_b}{dt} &= S^{-1}\frac{d\alpha}{dt} \\ &= S^{-1}\mathcal{P}^f \nu\mathcal{E}^s R^s(c_b, F(c_b)). \end{aligned} \tag{83}$$

Here one should notice that the initial condition of the explicit reduced equation (83) may not be same as the original initial condition: Note that we obtain Eq. (83) from (37) by assuming the equation for the equilibrium manifold of the fast subsystem, $c_a = F(c_b)$. Thus, the initial condition $c_b(0)$ of Eq. (83) should satisfy $\alpha(0) = \mathcal{P}^f c(0)$ and $c_a(0) = F(c_b(0))$. Details about finding initial condition of the explicit reduced equation will be shown in the next section.

7 Applications

In this section we describe the reduction method in detail for four examples of increasing complexity, and show how the reduced system approximates the full system.

7.1 A system with a fast dimerization and a slow isomerization

We consider a reaction system with a fast dimerization and a slow isomerization:

$$2A_1 \underset{k_1/\epsilon}{\overset{k_{-1}/\epsilon}{\leftrightharpoons}} A_2 \underset{k_2}{\overset{k_{-2}}{\leftrightharpoons}} A_3.$$

We let $c_1(t), c_2(t), c_3(t)$ be concentrations of A_1, A_2, A_3 at time t, respectively. Let $C(1) = 2A_1, C(2) = A_2, C(3) = A_3$, and

$$C(1) \underset{3}{\overset{1}{\leftrightharpoons}} C(2) \underset{4}{\overset{2}{\leftrightharpoons}} C(3).$$

One can find

$$\nu = \begin{bmatrix} 2 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \quad \mathcal{E} = \begin{bmatrix} 1 & 0 & -1 & 0 \\ -1 & 1 & 1 & -1 \\ 0 & -1 & 0 & 1 \end{bmatrix}, \quad \text{and} \quad \mathbf{R}(c) = \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \end{bmatrix} = \begin{bmatrix} k_{-1}c_2/\epsilon \\ k_{-2}c_3 \\ k_1 c_1^2/\epsilon \\ k_2 c_2 \end{bmatrix}.$$

Now we reduce the fast subsystem as follows.

Step 1: Identification of the equal complexes.

Since all complexes consists of distinct species, no identification is needed.

Step 2: Removal of cycle.

We first choose a spanning tree

$$C(1) \longrightarrow C(2),$$

and let $\mathcal{E}^f = [\mathcal{E}_1^f \mid \mathcal{E}_0^f]$, where

$$\mathcal{E}_0^f = \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix} \quad \text{and} \quad \mathcal{E}_1^f = \begin{bmatrix} 1 \\ -1 \\ 0 \end{bmatrix}.$$

One has

$$\mathcal{Q} = [\mathcal{Q}_1|1] = [-1 \mid 1],$$

and so $\mathcal{E}^f = \mathcal{E}_0^f \mathcal{Q}$. Thus one obtains

$$\nu\mathcal{E}^f R^f(c) = \nu\mathcal{E}_0^f [-1 \mid 1] R^f(c),$$

where

$$\nu\mathcal{E}_0^f = \begin{bmatrix} -2 \\ 1 \\ 0 \end{bmatrix},$$

and

$$[-1 \mid 1] R^f(c) = [-1 \mid 1] \begin{pmatrix} R_1 \\ R_3 \end{pmatrix} = -R_1 + R_3 = -k_{-1}c_2/\epsilon + k_1 c_1^2/\epsilon$$

Thus, by the removal of cycle, we have a reduced graph for fast reactions

$$C(1) \overset{-R_1+R_3}{\longrightarrow} C(2).$$

Step 3: Removal of elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f]$.

Since $\rho(\nu\mathcal{E}^f) = 1$ and $\rho(\mathcal{E}^f) = 1, \delta = \rho(\mathcal{E}) - \rho(\nu\mathcal{E}) = 0$. Thus $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f] = \{0\}$, and so there is no element to remove.

We have obtained, by the reduction of the graph, that

$$2A_1 \overset{-R_1+R_3}{\rightarrow} A_2 \underset{R_4}{\overset{R_2}{\leftrightharpoons}} A_3,$$

where the stoichiometries and reaction rate functions are

$$\nu\mathcal{E} = \begin{bmatrix} -2 & 0 & 0 \\ 1 & 1 & -1 \\ 0 & -1 & 1 \end{bmatrix}, \quad \nu\mathcal{E}^f = \begin{bmatrix} -2 \\ 1 \\ 0 \end{bmatrix}, \quad \nu\mathcal{E}^s = \begin{bmatrix} 0 & 0 \\ 1 & -1 \\ -1 & 1 \end{bmatrix}$$

and

$$R^f = -R_1 + R_3 = (k_1 c_1^2/\epsilon - k_{-1}c_2/\epsilon), \quad R^s = \begin{pmatrix} R_2 \\ R_4 \end{pmatrix} = \begin{pmatrix} k_{-2}c_3 \\ k_2 c_2 \end{pmatrix}.$$

Thus the governing equation is

$$\frac{dc}{dt} = \frac{1}{\epsilon}\nu\mathcal{E}^f R^f + \nu\mathcal{E}^s R^s = \frac{1}{\epsilon}\begin{bmatrix} -2 \\ 1 \\ 0 \end{bmatrix}(k_1 c_1^2 - k_{-1}c_2) + \begin{bmatrix} 0 & 0 \\ 1 & -1 \\ -1 & 1 \end{bmatrix}\begin{pmatrix} k_{-2}c_3 \\ k_2 c_2 \end{pmatrix}. \tag{84}$$

Now we compute invariants. First note that the reaction simplex $\Omega(c_0)$ can be represented by an equation of a plane

$$\{c_0 + \mathcal{R}[\nu\mathcal{E}]\} \cap (\bar{\mathbf{R}}_3)^+ = \{(c_1, c_2, c_3) \geq 0 : c_1 + 2c_2 + 2c_3 = c_{01} + 2c_{02} + 2c_{03}\},$$

where c_0 is the initial condition, and for a point $\bar{c}$ in $\Omega(c_0)$, the fast reaction simplex $\Omega_f(\bar{c})$ in $\Omega(c_0)$ is given by the family of straight lines

$$\{(c_1, c_2, c_3) \geq 0 : c_1 + 2c_2 = \bar{c}_1 + 2\bar{c}_2 \;\; c_3 = \bar{c}_3\}.$$

Moreover, the level sets of R^f are the family of paraboloids

$$\left\{c : R^f(c) = k_1\hat{c}_1^2 - k_{-1}\hat{c}_2\right\} = \left\{(c_1, c_2, c_3) : c_2 = \frac{k_1}{k_{-1}}c_1^2 + \frac{1}{k_{-1}}(k_{-1}\hat{c}_2 - k_1\hat{c}_1^2)\right\},$$

where $\hat{c} = (\hat{c}_1, \hat{c}_2, \hat{c}_3)^T$ is a point in $\Omega(c_0)$, which is unique up to level sets R^f. Thus on the reaction simplex $\Omega(c_0)$ the level sets are described by curves

$$\mathcal{K}_{f,\hat{c}} \equiv \left\{c = (c_1, c_2, c_3) \geq 0 : c_2 = \frac{k_1}{k_{-1}}c_1^2 + \frac{1}{k_{-1}}(k_{-1}\hat{c}_2 - k_1\hat{c}_1^2), \right.$$
$$\left. c_3 = c_{01} + 2c_{02} + 2c_{03} - c_1 - 2c_2\right\}$$

and especially, the equilibrium manifold $\mathcal{K}_f$ on $\Omega(c_0)$ is a curve

$$\left\{c \geq 0 : c_2 = \frac{k_1}{k_{-1}}c_1^2, \;\; c_3 = c_{01} + 2c_{02} + 2c_{03} - c_1 - 2c_2\right\}.$$

Furthermore, the Jacobian of R^f is

$$D_c R^f = [2k_1c_1 \quad -k_{-1} \quad 0]$$

and

$$D_c R^f \nu\mathcal{E}^f = -4k_1c_1 - k_{-1} < 0$$

Since $D_c R^f \cdot \nu\mathcal{E}^f$ is nonsingular, by Theorem 3, the governing equation can be separated into the equations of fast and slow variables as follows.

$$\begin{aligned}\frac{d\alpha}{dt} &= \begin{bmatrix} -2k_2c_2 + 2k_{-2}c_3 \\ k_2c_2 - k_{-2}c_3 \end{bmatrix} \\ \epsilon\frac{d\beta}{dt} &= \epsilon k_{-1}(k_2c_2 - k_{-2}c_3) + (-4k_1c_1 - k_{-1})\beta,\end{aligned}$$

where

$$\begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \beta \end{bmatrix} = T(c) = \begin{bmatrix} \mathcal{P}^f \cdot c \\ R^f(c) \end{bmatrix} = \begin{bmatrix} c_1 + 2c_2 \\ c_3 \\ k_1 c_1^2 - k_{-1} c_2 \end{bmatrix}. \tag{85}$$

From Eq. (85), one can find

$$4k_1 c_2^2 - c_2(4\alpha_1 k_1 + k_{-1}) + k_1 \alpha_1^2 - \beta = 0,$$

and so

$$c_2 = \frac{1}{8k_1}\left[4\alpha_1 k_1 + k_{-1} \pm \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2 + 16 k_1 \beta}\right].$$

Since

$$\begin{aligned} c_2 &= \frac{1}{8k_1}\left[4\alpha_1 k_1 + k_{-1} + \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2 + 16 k_1 \beta}\right] \\ &> \frac{4\alpha_1 k_1}{8k_1} \geq c_2, \end{aligned}$$

we must have

$$c_2 = \frac{1}{8k_1}\left[4\alpha_1 k_1 + k_{-1} - \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2 + 16 k_1 \beta}\right] \equiv f(\alpha_1, \beta).$$

Thus, the explicit equation for α_1, α_2 and β is

$$\frac{d\alpha}{dt} = \begin{bmatrix} -2k_2 f(\alpha_1, \beta) + 2k_{-2}\alpha_2 \\ k_2 f(\alpha_1, \beta) - k_{-2}\alpha_2 \end{bmatrix} \tag{86}$$

$$\epsilon \frac{d\beta}{dt} = \epsilon k_{-1}(k_2 f(\alpha_1, \beta) - k_{-2}\alpha_2) + (-4k_1(\alpha_1 - f(\alpha_1, \beta)) - k_{-1})\beta, \tag{87}$$

Since

$$f(\alpha, 0) = \frac{1}{8k_1}\left[4\alpha_1 k_1 + k_{-1} - \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2}\right], \tag{88}$$

as $\epsilon \to 0$ in (86) and (87), we obtain

$$\frac{d\alpha}{dt} = \begin{bmatrix} -\frac{k_2}{4k_1}\left[4\alpha_1 k_1 + k_{-1} - \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2}\right] + 2k_{-2}\alpha_2 \\ \frac{k_2}{8k_1}\left[4\alpha_1 k_1 + k_{-1} - \sqrt{8\alpha_1 k_1 k_{-1} + k_{-1}^2}\right] - k_{-2}\alpha_2 \end{bmatrix}. \tag{89}$$

Figure 2 illustrates the solutions of the full governing equation and the reduced equation.

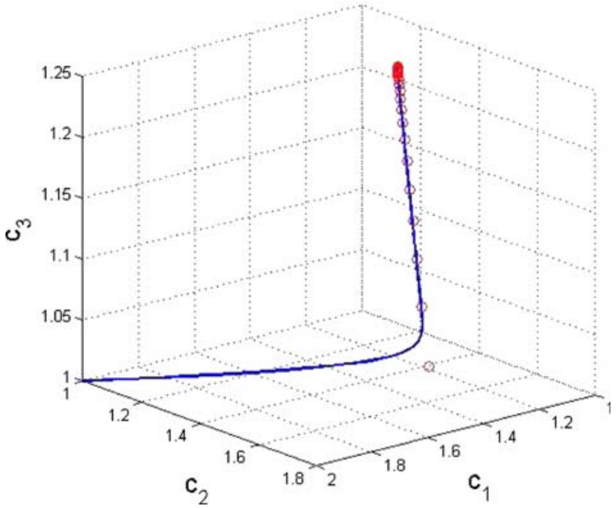

Fig. 2 When $c(0) = (2, 1, 1)$, $\epsilon = 0.1$ and $k_i = 1$ for all i, the evolution of the system in time interval $[0, 100]$. Simulation by MATLAB. Solution trajectory (*blue curve*) versus trajectory by QSS approximation (*red circle*)

Explicit representation of slow dynamics

To obtain an explicit representation of slow dynamics, we let $c = (c_2, c_3, c_1)$, so that we have

$$\nu^f = \begin{bmatrix} 0 & 1 \\ 0 & 0 \\ 2 & 0 \end{bmatrix}, \quad [B_1|B_2]\begin{pmatrix} \ln c \\ \ln \lambda \end{pmatrix} \equiv \left[\begin{array}{cc|cc} 0 & 0 & 2 & -1 \\ 1 & 0 & 0 & -1 \end{array}\right]\begin{pmatrix} \ln c_2 \\ \ln c_3 \\ \ln c_1 \\ \ln \lambda \end{pmatrix} = \ln Q, \tag{90}$$

where $Q = \begin{pmatrix} \frac{k_{-1}}{k_1+k_{-1}} \\ \frac{k_1}{k_1+k_{-1}} \end{pmatrix}$ is the eigenvector corresponding to zero eigenvalue of the matrix $K = \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix}$. Note that since $c = (c_2, c_3, c_1)$, we have

$$\mathcal{P}^f = \begin{bmatrix} 2 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix}, \quad \nu\mathcal{E}^s = \begin{bmatrix} 1 & -1 \\ -1 & 1 \\ 0 & 0 \end{bmatrix}.$$

From Eq. (90), one can obtain

$$\begin{pmatrix} c_1 \\ \lambda \end{pmatrix} = exp\left(B_2^{-1}\ln Q - B_2^{-1}B_1 \ln\begin{pmatrix} c_2 \\ c_3 \end{pmatrix}\right). \tag{91}$$

By solving equation (91), one can obtain

$$c_a \equiv c_1 = \sqrt{\frac{k_{-1}c_2}{k_1}} \equiv F(c_b) = F(c_2, c_3).$$

Since

$$S = \mathcal{P}^f \begin{bmatrix} I_2 \\ \frac{\partial F(c_b)}{\partial c_b} \end{bmatrix} = \begin{bmatrix} 2 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ \frac{1}{2}\sqrt{\frac{k_{-1}}{k_1 c_2}} & 0 \end{bmatrix} = \begin{bmatrix} 2 + \frac{1}{2}\sqrt{\frac{k_{-1}}{k_1 c_2}} & 0 \\ 0 & 1 \end{bmatrix},$$

one obtains

$$S^{-1}\mathcal{P}^f \nu \mathcal{E}^s R^s = \begin{pmatrix} \frac{4\sqrt{c_2}}{4\sqrt{c_2} + \sqrt{k-1/k_1}}\,(k_{-2}c_3 - k_2 c_2) \\ k_2 c_2 - k_{-2}c_3 \end{pmatrix}.$$

Thus, the reduced equation is

$$\frac{d}{dt}\begin{pmatrix} c_2 \\ c_3 \end{pmatrix} = S^{-1}\mathcal{P}^f \nu \mathcal{E}^s R^s = \begin{pmatrix} \frac{4\sqrt{c_2}}{4\sqrt{c_2} + \sqrt{k-1/k_1}}\,(k_{-2}c_3 - k_2 c_2) \\ k_2 c_2 - k_{-2}c_3 \end{pmatrix}. \qquad (92)$$

Note that the initial condition $(c_2(0), c_3(0)) \equiv (A_2, A_3)$ of (92) should satisfy $A_3 = \alpha_2(0)$ and $\sqrt{k_{-1}A_2/k_1} + 2A_2 = \alpha_1(0)$, because the initial condition of (89) is $\alpha_1(0) = c_1(0) + 2c_2(0)$ and $\alpha_2(0) = c_3(0)$. From $\sqrt{k_{-1}A_2/k_1} + 2A_2 = \alpha_1(0)$, we can obtain (See (88))

$$A_2 = \frac{1}{8k_1}\left[4\alpha_1(0)k_1 + k_{-1} - \sqrt{8\alpha_1(0)k_1k_{-1} + k_{-1}^2}\right].$$

Thus, the initial condition of the explicit reduced equation (92) is

$$(c_2(0), c_3(0)) = \left(\frac{1}{8k_1}\left[4\alpha_1(0)k_1 + k_{-1} - \sqrt{8\alpha_1(0)k_1k_{-1} + k_{-1}^2}\right],\ \alpha_2(0)\right).$$

Remark 7 Note that by letting $\epsilon \to 0$ in the governing equation (84), one can obtain $k_1c_1^2 - k_{-1}c_2 = 0$, which is the equation of the equilibrium manifold of the fast subsystem. If we substitute it into Eq. (84), one can simply obtain a reduced equation

$$\frac{d}{dt}\begin{pmatrix} c_2 \\ c_3 \end{pmatrix} = \nu \mathcal{E}^s R^s(c) = \begin{pmatrix} -k_2c_2 + k_{-2}c_3 \\ k_2c_2 - k_{-2}c_3 \end{pmatrix}, \qquad (93)$$

where c_1, c_2 and c_3 are subject to $k_1c_1^2 - k_{-1}c_2 = 0$. One can see that above Eq. (93) is not same as the Eq. (92) obtained by our method. Here we note that Eq. (93) is not an $O(\epsilon)$-approximation, since it includes only the first term $\nu\mathcal{E}^s R^s$ and ignores the second term on the right side of Eq. (36). Thus, if (93) would be used for representing the slow dynamics, errors would be bigger than $O(\epsilon)$. Indeed, when we obtain the reduced equation (37), we do not just ignore the second term in the right side of Eq. (36), but we remove the second term by utilizing the projection operator $\mathcal{P}^f$. Note that the explicit equation (92) is equivalent to Eq. (37), which is, for this example,

$$\frac{d\alpha}{dt} = \mathcal{P}^f \cdot \nu\mathcal{E}^s R^s(c) = \begin{bmatrix} -2k_2c_2 + 2k_{-2}c_3 \\ k_2c_2 - k_{-2}c_3 \end{bmatrix}, \tag{94}$$

where $\alpha = (c_1 + 2c_2, c_3)$, and c_1, c_2, c_3 are subject to $k_1c_1^2 - k_{-1}c_2 = 0$; by applying the operator S on Eq. (92), one can get

$$S\frac{d}{dt}\begin{pmatrix} c_2 \\ c_3 \end{pmatrix} = \mathcal{P}^f \nu\mathcal{E}^s R^s(c) = \begin{bmatrix} -2k_2c_2 + 2k_{-2}c_3 \\ k_2c_2 - k_{-2}c_3 \end{bmatrix}. \tag{95}$$

Since

$$S\frac{d}{dt}\begin{pmatrix} c_2 \\ c_3 \end{pmatrix} = S\frac{dc_b}{dt} = \frac{d\alpha}{dt}$$

on the manifold $\{c \geq 0 : k_1c_1^2 - k_{-1}c_2 = 0\}$ by Eqs. (80), (95) is equivalent to

$$\frac{d\alpha}{dt} = \mathcal{P}^f \nu\mathcal{E}^s R^s(c) = \begin{bmatrix} -2k_2c_2 + 2k_{-2}c_3 \\ k_2c_2 - k_{-2}c_3 \end{bmatrix},$$

where c_1, c_2, c_3 are subject to $k_1c_1^2 - k_{-1}c_2 = 0$. Thus, it is guaranteed by the perturbation analysis done in Sect. 4 that Eq. (92) has errors of at most $\mathcal{O}(\epsilon)$.

7.2 Receptor-Ligand binding

We consider a ligand binding network

$$L + R \underset{k_1}{\overset{k_2}{\leftrightharpoons}} \overline{LR}, \quad \overline{LR} + A_1 \underset{k_3}{\overset{k_4}{\leftrightharpoons}} A_2 \overset{k_5}{\to} \phi.$$

We let $c_1(t), c_2(t), c_3(t), c_4(t), c_5(t)$ be concentrations of $L, R, \overline{LR}, A_1, A_2$ at time t, respectively. We denote each complex by

$$C(1) = L + R, \quad C(2) = \overline{LR}, \quad C(3) = \overline{LR} + A_1, \quad C(4) = A_2, \quad C(5) = \phi$$

and

$$C(1) \underset{1}{\overset{2}{\leftrightharpoons}} C(2), \quad C(3) \underset{3}{\overset{4}{\leftrightharpoons}} C(4) \overset{5}{\to} C(5).$$

Then the stoichiometric matrix for the complexes is

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

Here we assume that the binding and unbinding reactions are fast and others are slow. As a result

$$\mathcal{E} = [\mathcal{E}^f \mid \mathcal{E}^s] = \left[\begin{array}{cc|ccc} -1 & 1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 \\ 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{array}\right].$$

By eliminating the cycle in the first step $L + R \leftrightharpoons \overline{LR}$, one obtains $L + R \rightarrow \overline{LR}$, where the reaction rate function is $k_1c_1c_2 - k_2c_3$ and

$$\mathcal{E} = [\mathcal{E}^f \mid \mathcal{E}^s] = \left[\begin{array}{c|ccc} -1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 1 \end{array}\right], \quad \nu\mathcal{E} = \left[\begin{array}{c|ccc} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{array}\right],$$

$$R^s(c) = \begin{pmatrix} k_3c_3c_4 \\ k_4c_5 \\ k_5c_5 \end{pmatrix},$$

and $\frac{R^f(c)}{\epsilon} = k_1c_1c_2 - k_2c_3$, where $\epsilon > 0$ is a separation parameter. Thus, one can write the governing equation as

$$\frac{d}{dt}\begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \\ c_5 \end{pmatrix} = \begin{pmatrix} -k_1c_1c_2 + k_2c_3 \\ -k_1c_1c_2 + k_2c_3 \\ k_1c_1c_2 - k_2c_3 - k_3c_3c_4 + k_4c_4 \\ -k_3c_3c_4 + k_4c_4 \\ k_3c_3c_4 - k_4c_4 - k_5c_5 \end{pmatrix}, \tag{96}$$

which could of course be obtained directly for this simple example.

Since $D_c(R^f(c))\nu\mathcal{E}^f = \epsilon(k_1c_2, k_1c_1, -k_2, 0, 0)(-1, -1, 1, 0, 0)^T = -\epsilon(k_1(c_1 + c_1) + k_2) < 0$ for all $c_1, c_2 \geq 0$, we can apply Theorem 3 to the system of equations and obtain

$$\begin{aligned}
\frac{d\alpha}{dt} &= \frac{d}{dt}\begin{pmatrix} c_1+c_3 \\ c_2+c_3 \\ c_4 \\ c_5 \end{pmatrix} = \mathcal{P}^f \nu \mathcal{E}^s R^s(c) \\
&= \begin{bmatrix} 1 & 0 & 1 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ -1 & 1 & 0 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \end{bmatrix} \begin{pmatrix} k_3c_3c_4 \\ k_4c_5 \\ k_5c_5 \end{pmatrix} \\
&= \begin{pmatrix} -k_3c_3c_4+k_4c_5 \\ -k_3c_3c_4+k_4c_5 \\ -k_3c_3c_4+k_4c_5 \\ k_3c_3c_4-k_4c_5-k_5c_5 \end{pmatrix},
\end{aligned} \tag{97}$$

where c in the right side satisfies the equation $R^f = 0$, i.e. $k_1c_1c_2 - k_2c_3 = 0$. By using $c_1 = \alpha_1 - c_3$ and $c_2 = \alpha_2 - c_3$, one obtains

$$c_3 = \frac{(k_1(\alpha_1+\alpha_2)+k_2) \pm \sqrt{(k_1(\alpha_1+\alpha_2)+k_2)^2 - 4k_1^2\alpha_1\alpha_2}}{2k_1}. \tag{98}$$

Since

$$\begin{aligned}
&\frac{(k_1(\alpha_1+\alpha_2)+k_2) + \sqrt{(k_1(\alpha_1+\alpha_2)+k_2)^2 - 4k_1^2\alpha_1\alpha_2}}{2k_1} \\
&\geq \frac{k_1(\alpha_1+\alpha_2)+k_2}{2k_1} = \frac{k_1(c_1+c_2+2c_3)+k_2}{2k_1} \\
&> c_3,
\end{aligned}$$

we have

$$c_3 = \frac{(k_1(\alpha_1+\alpha_2)+k_2) - \sqrt{(k_1(\alpha_1+\alpha_2)+k_2)^2 - 4k_1^2\alpha_1\alpha_2}}{2k_1}.$$

Thus, from (97) we obtain the reduced equation

$$\frac{d}{dt}\begin{pmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \\ \alpha_4 \end{pmatrix} = \begin{pmatrix} -k_3c_3\alpha_3+k_4\alpha_4 \\ -k_3c_3\alpha_3+k_4\alpha_4 \\ -k_3c_3\alpha_3+k_4\alpha_4 \\ k_3c_3\alpha_3-k_4\alpha_4-k_5\alpha_4 \end{pmatrix}, \tag{99}$$

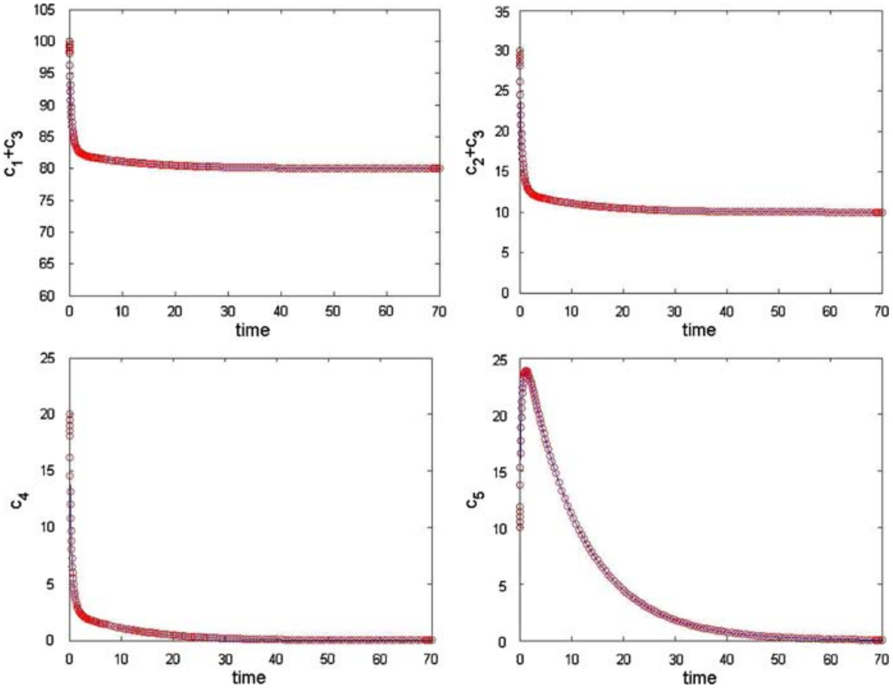

Fig. 3 Evolution of slow variables when $k_1 = k_2 = 1$, $k_3 = k_4 = k_5 = 0.1$ and $(c_1, c_2, c_3, c_4, c_5) = (100, 30, 0, 20, 10)$ initially. Solution of the full equation (*blue solid line*) versus solution of the reduced equation (*red circles*)

where

$$c_3 = \frac{(k_1(\alpha_1 + \alpha_2) + k_2) - \sqrt{(k_1(\alpha_1 + \alpha_2) + k_2)^2 - 4k_1^2\alpha_1\alpha_2}}{2k_1}.$$

Numerical results obtained from the reduced system are compared with the solution of the full system in Fig. 3.

Explicit representation of the slow dynamics

To obtain an explicit representation of the slow dynamics, we first let $c = (c_1, c_2, c_4, c_5, c_3)$, and have

$$\nu^f = \begin{bmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 1 \end{bmatrix}, \text{ and } [B_1|B_2]\begin{pmatrix} \ln c \\ \ln \lambda \end{pmatrix} \equiv \left[\begin{array}{cccc|cc} 1 & 1 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{array}\right] \begin{pmatrix} \ln c_1 \\ \ln c_2 \\ \ln c_4 \\ \ln c_5 \\ \ln c_3 \\ \ln \lambda \end{pmatrix} = \ln Q, \tag{100}$$

where $Q = \begin{pmatrix} \frac{k_2}{k_1+k_2} \\ \frac{k_1}{k_1+k_2} \end{pmatrix}$ is the eigenvector corresponding to zero eigenvalue of the matrix

$$K = \begin{bmatrix} -k_1 & k_2 \\ k_1 & -k_2 \end{bmatrix}.$$

By multiplying equation (100) by $B_2^{-1} = \begin{bmatrix} -1 & 1 \\ -1 & 0 \end{bmatrix}$, we obtain

$$\begin{pmatrix} c_3 \\ \lambda \end{pmatrix} = exp\left(B_2^{-1} \ln Q - B_2^{-1} B_1 \ln (c_1, c_2, c_4, c_5)^T\right), \tag{101}$$

and by solving Eq. (101), one finds that

$$c_a \equiv c_3 = \frac{k_1}{k_2} c_1 c_2 \equiv F(c_b) = F(c_1, c_2, c_4, c_5).$$

Since

$$S = \mathcal{P}^f \begin{bmatrix} I_4 \\ \frac{\partial F}{\partial c_b} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ \frac{k_1}{k_2} c_2 & \frac{k_1}{k_2} c_1 & 0 & 0 \end{bmatrix}$$
$$= \begin{bmatrix} 1 + \frac{k_1}{k_2} c_2 & \frac{k_1}{k_2} c_1 & 0 & 0 \\ \frac{k_1}{k_2} c_2 & 1 + \frac{k_1}{k_2} c_1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix},$$

one obtains

$$S^{-1} = \begin{bmatrix} \frac{k_1 c_1 + k_2}{k_2 + k_1 c_1 + k_1 c_2} & \frac{-k_1 c_1}{k_2 + k_1 c_1 + k_1 c_2} & 0 & 0 \\ \frac{-k_1 c_2}{k_2 + k_1 c_1 + k_1 c_2} & \frac{k_1 c_2 + k_2}{k_2 + k_1 c_1 + k_1 c_2} & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}.$$

Thus the explicit form of the slow dynamics is

$$\frac{d}{dt}\begin{pmatrix} c_1 \\ c_2 \\ c_4 \\ c_5 \end{pmatrix} = S^{-1}\mathcal{P}^f \nu \mathcal{E}^s R^s(c) = \begin{pmatrix} \frac{-k_1 k_3 c_1 c_2 c_4 + k_2 k_4 c_5}{k_2 + k_1 c_1 + k_1 c_2} \\ \frac{-k_1 k_3 c_1 c_2 c_4 + k_2 k_4 c_5}{k_2 + k_1 c_1 + k_1 c_2} \\ \frac{-k_1 k_3}{k_2} c_1 c_2 c_4 + k_4 c_5 \\ \frac{k_1 k_3}{k_2} c_1 c_2 c_4 - k_4 c_5 - k_5 c_5 \end{pmatrix}. \tag{102}$$

Note that the initial condition of Eq. (102) is given by

$$c_1(0) = \alpha_1(0) - c_3(0), \quad c_2(0) = \alpha_2(0) - c_3(0), \quad c_4(0) = \alpha_3(0), \quad c_5(0) = \alpha_4(0),$$

where

$$c_3 = \frac{(k_1(\alpha_1 + \alpha_2) + k_2) - \sqrt{(k_1(\alpha_1 + \alpha_2) + k_2)^2 - 4k_1^2 \alpha_1 \alpha_2}}{2k_1}.$$

7.3 PFK reaction system

We consider a model for the glycolytic reactions given in Othmer and Aldridge (1978). In the model, fructose-6-phosphate (F6P) is phosphorylated to give fructose diphosphate (FDP). Phosphofructokinase (PFK) is activated by AMP and FDP, and inhibited by ATP. Under conditions that lead to oscillations, PFK is fully activated with respect to FDP, and ATP has a negligible effect on activity. The complete set of reactions is as follows.

$$\phi \xrightarrow{k} A_1$$

$$A_1 + E_1 \underset{k_1}{\overset{k_{-1}}{\leftrightharpoons}} E_1 A_1 \xrightarrow{k_2} E_1 + A_2$$

$$A_1 + E_1^* \underset{k_3}{\overset{k_{-3}}{\leftrightharpoons}} E_1^* A_1 \xrightarrow{k_4} E_1^* + A_2$$

$$A_2 + E_2 \underset{k_5}{\overset{k_{-5}}{\leftrightharpoons}} E_2 A_2 \xrightarrow{k_6} E_2 + \text{ Product}$$

$$\hat{E}_1 + A_3 \underset{k_7}{\overset{k_{-7}}{\leftrightharpoons}} \hat{E}_1^*, \qquad 2A_2 \underset{k_8}{\overset{k_{-8}}{\leftrightharpoons}} A_3 + A_4,$$

where we denote F6P, ADP, AMP and ATP by A_1, A_2, A_3 and A_4. E_1 and E_1^* represent the low activity and activated forms of free PFK, respectively. E_2 is the enzyme for the ADP sink reaction. $E_1 A_1$, $E_1^* A_1$ and $E_2 A_2$ represent enzyme-substrate complexes. $\hat{E}_1^*$ and $\hat{E}_1$ are the total activated and low-activity enzymes, both in free and bound form. Here note that we assume that the last two reversible reactions are always at the equilibrium. Thus the equations $k_7[\hat{E}_1][A_3] = k_{-7}[\hat{E}_1^*]$ and $k_8[A_2]^2 = k_{-8}[A_3][A_4]$ hold at any time.

Assuming mass-action kinetics for the various kinetic steps, one obtains the following system of ODEs.

$$\begin{aligned}
\frac{d[A_1]}{dt} &= k - k_1[A_1][E_1] + k_{-1}[E_1A_1] - k_3[A_1][E_1^*] + k_{-3}[E_1^*A_1]\\
\frac{d[A_2]}{dt} &= k_2[E_1A_1] + k_4[E_1^*A_1] - k_5[A_2][E_2] + k_{-5}[E_2A_2] - k_8[A_2]^2\\
&\quad + \frac{1}{2}k_{-8}[A_3][A_4]\\
\frac{d[A_3]}{dt} &= k_7[A_3][\hat{E}_1] - k_{-7}[\hat{E}_1^*] + k_8[A_2]^2 - k_{-8}[A_3][A_4]\\
\frac{d[A_4]}{dt} &= k_8[A_2]^2 - k_8[A_3][A_4]\\
\frac{d[E_1]}{dt} &= -k_1[A_1][E_1] + k_{-1}[E_1A_1] + k_2[E_1A_1]\\
\frac{d[E_1^*]}{dt} &= -k_3[A_1][E_1^*] + k_{-3}[E_1^*A_1] + k_4[E_1^*A_1] + k_7[\hat{E}_1][A_3] - k_{-7}[\hat{E}_1*]\\
\frac{d[E_2]}{dt} &= -k_5[A_2][E_2] + k_{-5}[E_2A_2] + k_6[E_2A_2]\\
\frac{d[\hat{E}_1]}{dt} &= -k_7[\hat{E}_1][A_3] + k_{-1}[\hat{E}_1^*]\\
\frac{d[E_1A_1]}{dt} &= k_1[A_1][E_1] - k_{-1}[E_1A_1] - k_2[E_1A_1]\\
\frac{d[E_1^*A_1]}{dt} &= k_3[A_1][E_1^*] - k_{-3}[E_1^*A_1] - k_4[E_1^*A_1]\\
\frac{d[E_2A_2]}{dt} &= k_5[A_2][E_2] - k_{-5}[E_2A_2] - k_6[E_2A_2]
\end{aligned}$$

To reduce the ODE system by utilizing the QSS assumption, we let $c_1(t)$, $c_2(t)$, $c_3(t)$, $c_4(t)$, $c_5(t)$, $c_6(t)$, $c_7(t)$, $c_8(t)$ and $c_9(t)$ be concentrations of A_1, E_1, E_1A_1, E_1^*, $E_1^*A_1$, A_2, E_2, E_2A_2 and Product at time t respectively. We assume that the three reversible reactions i.e. binding and unbinding of enzymes, are much faster than other three irreversible reactions.

We define

$$C(1)=A_1,\quad C(2)=A_1+E_1,\quad C(3)=E_1A_1,\quad C(4)=E_1+A_2,\quad C(5)=A_1+E_1^*,$$

and

$$\begin{aligned}
C(6) = E_1^*A_1,\quad C(7)=E_1^*+A_2,\quad C(8)=A_2+E_2,\quad C(9)=E_2A_2,\\
C(10) = E_2 + \text{Product},\quad C(11) = \phi.
\end{aligned}$$

Then one obtains the following graph.

$$
\begin{aligned}
&C(11) \xrightarrow{1} C(1)\\
&C(2) \underset{2}{\overset{3}{\leftrightharpoons}} C(3) \xrightarrow{4} C(4)\\
&C(5) \underset{5}{\overset{6}{\leftrightharpoons}} C(6) \xrightarrow{7} C(7)\\
&C(8) \underset{8}{\overset{9}{\leftrightharpoons}} C(9) \xrightarrow{10} C(10)
\end{aligned}
$$

One can write the matrices ν and $\mathcal{E}$ as

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

$$
\mathcal{E} = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1\\
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
$$

and in particular, $\mathcal{E}^f$ and $\nu\mathcal{E}^f$ as

$$
\mathcal{E}^f = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0\\
-1 & 1 & 0 & 0 & 0 & 0\\
1 & -1 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & -1 & 1 & 0 & 0\\
0 & 0 & 1 & -1 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & -1 & 1\\
0 & 0 & 0 & 0 & 1 & -1\\
0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}, \quad
\nu\mathcal{E}^f = \begin{bmatrix}
-1 & 1 & -1 & 1 & 0 & 0\\
-1 & 1 & 0 & 0 & 0 & 0\\
1 & -1 & 0 & 0 & 0 & 0\\
0 & 0 & -1 & 1 & 0 & 0\\
0 & 0 & 1 & -1 & 0 & 0\\
0 & 0 & 0 & 0 & -1 & 1\\
0 & 0 & 0 & 0 & -1 & 1\\
0 & 0 & 0 & 0 & 1 & -1\\
0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
$$

By removing cycles in the fast subsystem, one can obtain

$$\mathcal{E}^f = \begin{bmatrix} 0 & 0 & 0 \\ -1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -1 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}, \quad \nu\mathcal{E}^f = \begin{bmatrix} -1 & -1 & 0 \\ -1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \\ 0 & 0 & -1 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{bmatrix}, \quad \text{and}$$

$$\frac{R^f(c)}{\epsilon} = \begin{pmatrix} k_1c_1c_2 - k_{-1}c_3 \\ k_3c_1c_4 - k_{-3}c_5 \\ k_5c_6c_7 - k_{-5}c_8 \end{pmatrix},$$

where $\epsilon > 0$ is a separation parameter.

One finds that $\rho(\mathcal{E}^f) = 3$ and $\rho(\nu\mathcal{E}^f) = 3$. Thus, the deficiency $\delta = 0$ and so there is no element in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f]$ to be removed.

We compute

$$D_cR^f(c) = \epsilon \begin{bmatrix} k_1c_2 & k_1c_1 & -k_{-1} & 0 & 0 & 0 & 0 & 0 & 0 \\ k_3c_4 & 0 & 0 & k_3c_1 & -k_{-3} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5c_7 & k_5c_6 & -k_{-5} & 0 \end{bmatrix}$$

and

$$D_cR^f \cdot \nu\mathcal{E}^f = \epsilon \begin{bmatrix} -k_1c_2 - k_1c_1 - k_{-1} & -k_1c_2 & 0 \\ -k_3c_4 & -k_3c_4 - k_3c_1 - k_{-3} & 0 \\ 0 & 0 & -k_5c_7 - k_5c_6 - k_{-5} \end{bmatrix}.$$

Thus, the determinant of $D_cR^f \cdot \nu\mathcal{E}_f$ is

$$\begin{aligned} |D_c\mathcal{R}_f \cdot \nu\mathcal{E}_f| &= \epsilon(-k_1c_2 - k_1c_1 - k_{-1})(-k_3c_4 - k_3c_1 - k_{-3})(-k_5c_7 - k_5c_6 - k_{-5}) \\ &\quad + \epsilon k_1c_2k_3c_4(k_5c_7 + k_5c_6 + k_{-5}) \\ &= \epsilon(k_5c_7 + k_5c_6 + k_{-5}) \left[-(k_1c_2 + k_1c_1 + k_{-1})(k_3c_4 + k_3c_1 + k_{-3}) \right. \\ &\quad \left. + k_1k_3c_2c_4\right] \\ &= -\epsilon(k_5c_7 + k_5c_6 + k_{-5}) \left[(k_1c_1 + k_{-1})(k_3c_4 + k_3c_1 + k_{-3}) \right. \\ &\quad \left. + k_1c_2(k_3c_1 + k_{-3})\right] \\ &< 0 \quad \text{for all } c \geq 0. \end{aligned}$$

and so Theorem 3 can be applied to this example.

Since

$$\mathcal{P}^f = \begin{bmatrix} 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad \text{and} \quad \nu\mathcal{E}^s = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix},$$

one can obtain the reduced equation

$$\begin{aligned} \frac{d\alpha}{dt} &= \frac{d}{dt}\begin{pmatrix} c_1 + c_3 + c_5 \\ c_2 + c_3 \\ c_4 + c_5 \\ c_6 + c_8 \\ c_7 + c_8 \\ c_9 \end{pmatrix} \\ &= \mathcal{P}^f \nu\mathcal{E}^s\mathcal{R}^s(c) = \begin{bmatrix} 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & -1 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{pmatrix} k \\ k_2c_3 \\ k_4c_5 \\ k_6c_8 \end{pmatrix} = \begin{pmatrix} k - k_2c_2 - k_4c_5 \\ 0 \\ 0 \\ k_2c_3 + k_4c_5 - k_6c_8 \\ 0 \\ k_6c_8 \end{pmatrix}. \end{aligned}$$

By solving $\mathcal{P}^f c = \alpha$ for c with constraint $R^f(c) = 0$, one can obtain

$$c_2 = \frac{k_{-1}\alpha_2}{k_1c_1 + k_{-1}}, \quad c_3 = \frac{k_1c_1\alpha_2}{k_1c_1 + k_{-1}}, \quad c_5 = \frac{k_3c_1\alpha_3}{k_3c_1 + k_{-3}} \tag{103}$$

and

$$c_8 = \frac{1}{2k_5}\left[k_5(\alpha_4 + \alpha_5) + k_{-5} - \sqrt{k_5^2(\alpha_4 - \alpha_5)^2 + k_{-5}^2 + 2k_5k_{-5}(\alpha_4 + \alpha_5)}\right], \tag{104}$$

where c_1 is a positive solution of the cubic equation

$$\begin{aligned} &k_1k_3c_1^3 + c_1^2(k_1k_{-3} + k_3k_{-1} + k_1k_3\alpha_2 + k_1k_3\alpha_3 - k_1k_3\alpha_1 + c_1(k_{-1}k_{-3} + k_1k_{-3}\alpha_2 \\ &\quad + k_3k_{-1}\alpha_3 - \alpha_1k_{-3}k_1 - \alpha_1k_{-1}k_3) - \alpha_1k_{-3}k_{-1} = 0. \end{aligned} \tag{105}$$

Figure 4 illustrates the numerical accuracy of the reduction method.

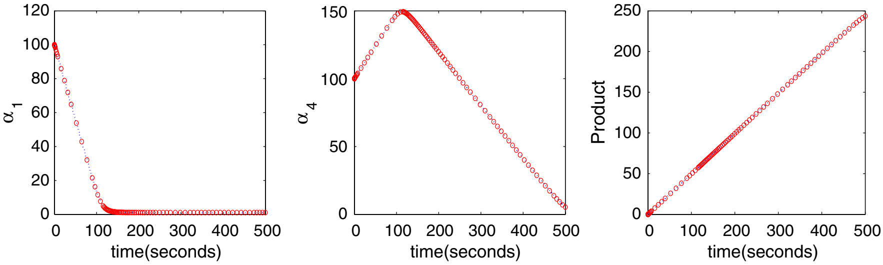

Fig. 4 Comparison of solutions of the reduced ODE system (*circles*) to those of the full system (*dotted*). Time evolution of slow variables α_1, α_4 and α_6(=Product) during 500 seconds when $(A_1, E_1, E_1A_1, E_1^*, E_1^*A_1, A_2, E_2, E_2A_2,$ Product$) = (100, 5, 0, 5, 0, 100, 5, 0, 0)$ initially and reaction rates $(k, k_1, k_{-1}, k_2, k_3, k_{-3}, k_4, k_5, k_{-5}, k_6) = (0.1, 1, 1, 0.1, 1, 1, 0.1, 1, 1, 0.1)$

Explicit representation of slow dynamics

To obtain an explicit representation of slow dynamics, we first let $c = (c_1, c_2, c_4, c_6, c_7, c_9, c_3, c_5, c_8)$, so that we have

$$\nu^f = \begin{bmatrix} 1 & 0 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix},$$

$$[B_1|B_2] \ln c \equiv \left[\begin{array}{cccccc|cccccc} 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 \end{array}\right] \begin{pmatrix} \ln c_1 \\ \ln c_2 \\ \ln c_4 \\ \ln c_6 \\ \ln c_7 \\ \ln c_9 \\ \ln c_3 \\ \ln c_5 \\ \ln c_8 \\ \ln \lambda_1 \\ \ln \lambda_2 \\ \ln \lambda_3 \end{pmatrix}$$

$$= \ln \begin{pmatrix} Q_1 \\ Q_2 \\ Q_3 \end{pmatrix}, \tag{106}$$

where ν^f is the stoichiometry for complexes $C(2)$, $C(3)$, $C(5)$, $C(6)$, $C(8)$ and $C(9)$ which are reactants or products of fast reactions, $Q_1 = \begin{pmatrix} \frac{k_{-1}}{k_1+k_{-1}} \\ \frac{k_1}{k_1+k_{-1}} \end{pmatrix}$, $Q_2 = \begin{pmatrix} \frac{k_{-3}}{k_3+k_{-3}} \\ \frac{k_3}{k_3+k_{-3}} \end{pmatrix}$ and $Q_3 = \begin{pmatrix} \frac{k_{-5}}{k_5+k_{-5}} \\ \frac{k_5}{k_5+k_{-5}} \end{pmatrix}$ are eigenvectors corresponding to zero eigenvalue of the matrices $K_1 = \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix}$, $K_2 = \begin{bmatrix} -k_3 & k_{-3} \\ k_3 & -k_{-3} \end{bmatrix}$ and $K_3 = \begin{bmatrix} -k_5 & k_{-5} \\ k_5 & -k_{-5} \end{bmatrix}$, respectively.

From Eq. (106), one can obtain

$$\begin{aligned} c_a &\equiv (c_3,\ c_5,\ c_8,\ \lambda_1,\ \lambda_2,\ \lambda_3)^T \\ &= exp\left(B_2^{-1} \ln Q - B_2^{-1} B_1 \left(\ln c_1,\ \ln c_2,\ \ln c_4,\ \ln c_6,\ \ln c_7,\ \ln c_9,\right)\right), \end{aligned} \tag{107}$$

where

$$B_2^{-1} = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 \end{bmatrix}.$$

By solving equation (107), one can obtain

$$\begin{pmatrix} c_3 \\ c_5 \\ c_8 \end{pmatrix} = \begin{pmatrix} \frac{k_1}{k_{-1}} c_1 c_2 \\ \frac{k_3}{k_{-3}} c_1 c_4 \\ \frac{k_5}{k_{-5}} c_6 c_7 \end{pmatrix} \equiv F(c_b) = F(c_1, c_2, c_4, c_6, c_7, c_9).$$

Thus, one can compute

$$S = \mathcal{P}^f \begin{bmatrix} I_6 \\ \frac{\partial F}{\partial c_b} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}$$

$$\times \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ \frac{k_1}{k_{-1}}c_2 & \frac{k_1}{k_{-1}}c_1 & 0 & 0 & 0 & 0 \\ \frac{k_3}{k_{-3}}c_4 & 0 & \frac{k_3}{k_{-3}}c_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{k_5}{k_{-5}}c_7 & \frac{k_5}{k_{-5}}c_6 & 0 \end{bmatrix}$$

$$= \begin{bmatrix} 1+\frac{k_1}{k_{-1}}c_2+\frac{k_3}{k_{-3}}c_4 & \frac{k_1}{k_{-1}}c_1 & \frac{k_3}{k_{-3}}c_1 & 0 & 0 & 0 \\ \frac{k_1}{k_{-1}}c_2 & 1+\frac{k_1}{k_{-1}}c_1 & 0 & 0 & 0 & 0 \\ \frac{k_3}{k_{-3}}c_4 & 0 & 1+\frac{k_3}{k_{-3}}c_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1+\frac{k_5}{k_{-5}}c_7 & \frac{k_5}{k_{-5}}c_6 & 0 \\ 0 & 0 & 0 & \frac{k_5}{k_{-5}}c_7 & 1+\frac{k_5}{k_{-5}}c_6 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}.$$

After some computations to obtain $S^{-1}\mathcal{P}^f \nu^s R^s(c)$, one obtains the explicit reduced equations

$$\frac{dc_1}{dt} = \frac{(k_{-1}+k_1c_1)(k_{-3}+k_3c_1)\left(k-\frac{k_1k_2}{k_{-1}}c_1c_2-\frac{k_3k_4}{k_{-3}}c_1c_4\right)}{(k_{-1}k_{-3}+k_{-1}k_3c_1+k_{-3}k_1c_1+k_1k_3c_1^2+k_1k_{-3}c_2+k_1k_3c_2c_1+k_3k_{-1}c_4+k_1k_3c_4c_1)}$$

$$\frac{dc_2}{dt} = \frac{(k_{-3}+k_3c_1)\left(-kk_1c_2+\frac{k_1^2k_2}{k_{-1}}c_1c_2^2+\frac{k_1k_3k_4}{k_{-3}}c_1c_2c_4\right)}{(k_{-1}k_{-3}+k_{-1}k_3c_1+k_{-3}k_1c_1+k_1k_3c_1^2+k_1k_{-3}c_2+k_1k_3c_2c_1+k_3k_{-1}c_4+k_1k_3c_4c_1)}$$

$$\frac{dc_4}{dt} = \frac{(k_{-1}+k_1c_1)\left(-k_3c_4k+\frac{k_1k_2k_3}{k_{-1}}c_1c_2c_4+\frac{k_3^2k_4}{k_{-3}}c_1c_4^2\right)}{(k_{-1}k_{-3}+k_{-1}k_3c_1+k_{-3}k_1c_1+k_1k_3c_1^2+k_1k_{-3}c_2+k_1k_3c_2c_1+k_3k_{-1}c_4+k_1k_3c_4c_1)}$$

$$\frac{dc_6}{dt} = \frac{(k_{-5}+k_5c_6)}{(k_{-5}+k_5c_6+k_5c_7)}\left(\frac{k_1k_2}{k_{-1}}c_1c_2+\frac{k_3k_4}{k_{-3}}c_1c_4-\frac{k_5k_6}{k_{-5}}c_6c_7\right)$$

$$\frac{dc_7}{dt} = \frac{k_5c_7}{(k_{-5}+k_5c_6+k_5c_7)}\left(-\frac{k_1k_2}{k_{-1}}c_1c_2-\frac{k_3k_4}{k_{-3}}c_1c_4+\frac{k_5k_6}{k_{-5}}c_6c_7\right)$$

$$\frac{dc_9}{dt} = \frac{k_5k_6}{k_{-5}}c_6c_7.$$

Note that the initial condition $(c_1(0), c_2(0), c_4(0), c_6(0), c_7(0))$ of above ODE system is obtained from Eqs. (103)–(105).

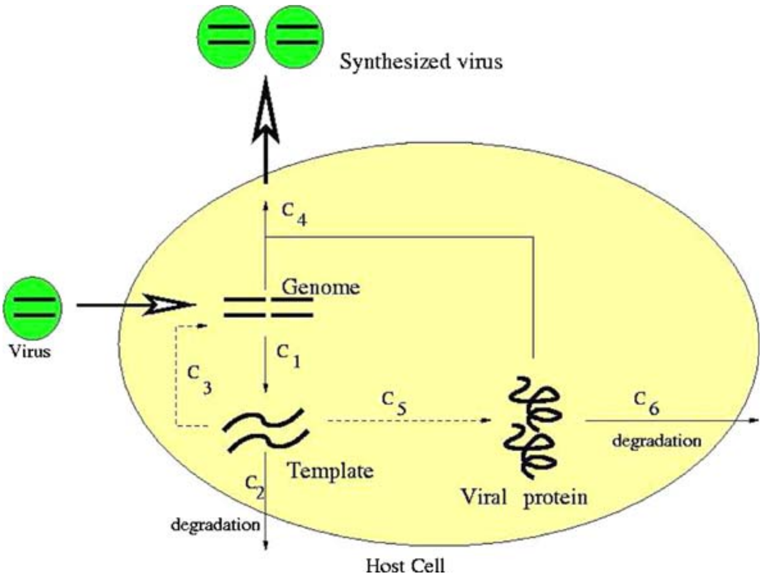

Fig. 5 A model of intracellular viral infection. Dotted lines represent catalytic reactions. Both RNA and proteins are subject to degradation

7.4 Intracellular viral infection model

We consider an intracellular viral infection model proposed by Srivastava et al. (2002) (Fig. 5).

We denote DNA, RNA, viral protein and viral cell by D, R, P and V respectively. Reactions and parameters are obtained from (Srivastava et al. 2002) as follows.

$$R_1 : D \overset{k_1}{\rightarrow} R + D \tag{108}$$

$$R_2 : R \overset{k_2}{\rightarrow} \phi \tag{109}$$

$$R_3 : R \overset{k_3}{\rightarrow} R + D \tag{110}$$

$$R_4 : D + P \overset{k_4}{\rightarrow} V \tag{111}$$

$$R_5 : R \overset{k_5}{\rightarrow} P + R \tag{112}$$

$$R_6 : P \overset{k_6}{\rightarrow} \phi \tag{113}$$

Parameter	Value
k_1	$0.025\,\text{day}^{-1}$
k_2	$0.25\,\text{day}^{-1}$
k_3	$1.0\,\text{day}^{-1}$
k_4	$7.5 \times 10^{-6}\,\text{molecules}^{-1}\text{day}^{-1}$
k_5	$1000\,\text{day}^{-1}$
k_6	$1.99\,\text{day}^{-1}$

We denote the numbers of molecules of D, R, P and V by c_1, c_2, c_3 and c_4.

We assume that two reactions R_5 and R_6 are much faster than other reactions as in Haseltine and Rawlings (2002). Thus, the fast subsystem is given by

$$R_5 : R \overset{k_5}{\rightarrow} P + R \tag{114}$$

$$R_6 : P \overset{k_6}{\rightarrow} \phi \tag{115}$$

We define the complexes as follows:

$$C(1)=D, \quad C(2)=R, \quad C(3)=P, \quad C(4)=V, \quad C(5)=R+D, \quad C(6)=P+R,$$
$$C(7) = D + P, \quad C(8) = \phi.$$

Thus, the stoichiometric matrix ν for complexes is

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

After identification of equal complexes, one can obtain the graph of the reaction system with the complexes:

$$\begin{aligned} C(1) &\overset{1}{\rightarrow} C(5) \\ C(2) &\overset{2}{\rightarrow} C(8) \\ C(2) &\overset{3}{\rightarrow} C(5) \\ C(7) &\overset{4}{\rightarrow} C(4) \\ C(2) &\overset{5}{\rightarrow} C(6) \\ C(3) &\overset{6}{\rightarrow} C(8). \end{aligned}$$

One finds the incidence matrices $\mathcal{E}$ and $\mathcal{E}^f$

$$\mathcal{E} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & -1 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 \end{bmatrix}, \quad \mathcal{E}^f = \begin{bmatrix} 0 & 0 \\ -1 & 0 \\ 0 & -1 \\ 0 & 0 \\ 0 & 0 \\ 1 & 0 \\ 0 & 0 \\ 0 & 1 \end{bmatrix},$$

and the reaction rate functions

$$R_1(c) = k_1c_1, \quad R_2(c) = k_2c_2, \quad R_3(c) = k_3c_2, \quad R_4(c) = k_4c_1c_3, \quad R_5(c) = k_5c_2,$$
$$R_6(c) = k_6c_3.$$

Now we reduce the fast subsystem. One can see that $\rho(\mathcal{E}^f) = 2$ and so $\mathcal{E}$ has full column rank. This implies the graph contains no cycles, which is also obvious from the graph of the fast subsystem.

Next we remove the elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f]$. Since

$$\nu\mathcal{E}^f = \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 1 & -1 \\ 0 & 0 \end{bmatrix},$$

the deficiency is $\delta = \rho(\mathcal{E}^f) - \rho(\nu\mathcal{E}^f) = 1$. Thus, there is a basis vector that spans $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f]$. One can find the basis vector as $(0, -1, -1, 0, 0, 1, 0, 1)^T$, and so $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f] = \text{span}\{(0, -1, -1, 0, 0, 1, 0, 1)^T\}$.

If we choose to retain the edge 5, then we obtain

$$\mathcal{E}^f \begin{pmatrix} R_5 \\ R_6 \end{pmatrix} = \mathcal{E}_0^f H H^{-1} \begin{pmatrix} R_5 \\ R_6 \end{pmatrix} = \tilde{\mathcal{E}}_0^f \tilde{R}_0,$$

where

$$\mathcal{E}_0^f = \mathcal{E}^f, \quad H = \begin{bmatrix} 1 & 1 \\ 0 & 1 \end{bmatrix}, \quad H^{-1} = \begin{bmatrix} 1 & -1 \\ 0 & 1 \end{bmatrix}, \quad \tilde{\mathcal{E}}_0^f = \mathcal{E}_0^f H, \quad \tilde{R}_0 = H^{-1} \begin{pmatrix} R_5 \\ R_6 \end{pmatrix}.$$

Dropping the last $\delta(=1)$ row of H^{-1}, one obtains a reduced graph $C(2) \overset{R_5 - R_6}{\to} C(6)$ for the fast subsystem. Thus, after removal of the elements in $\mathcal{N}[\nu] \cap \mathcal{R}[\mathcal{E}^f]$, one can obtain the graph for the whole system

$$\begin{aligned} C(1) &\overset{R_1}{\to} C(5) \\ C(2) &\overset{R_2}{\to} C(8) \\ C(2) &\overset{R_3}{\to} C(5) \\ C(7) &\overset{R_4}{\to} C(4) \\ C(2) &\overset{R_5 - R_6}{\to} C(6). \end{aligned}$$

In the reduced graph, one obtains the reaction rate functions

$$R(c) = \begin{bmatrix} k_1 c_1 \\ k_2 c_2 \\ k_3 c_2 \\ k_4 c_1 c_3 \\ k_5 c_2 - k_6 c_3 \end{bmatrix}$$

and the stoichiometry

$$\nu\mathcal{E} = \begin{bmatrix} 0 & 0 & 1 & -1 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix}.$$

Since the last reaction is fast and others are slow, one has

$$\frac{R^f}{\epsilon} = k_5 c_2 - k_6 c_3, \quad R^s = \begin{bmatrix} k_1 c_1 \\ k_2 c_2 \\ k_3 c_2 \\ k_4 c_1 c_3 \end{bmatrix},$$

where $\epsilon > 0$ is a separation parameter, and

$$\nu\mathcal{E}^s = \begin{bmatrix} 0 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad \nu\mathcal{E}^f = \begin{bmatrix} 0 \\ 0 \\ 1 \\ 0 \end{bmatrix}.$$

One can see that

$$D_c(R^f)\nu\mathcal{E}^f = -\epsilon k_6 \neq 0.$$

Thus, by Theorem 3, one obtains the reduced equation

$$\begin{aligned} \frac{d\alpha}{dt} &= \mathcal{P}^f \nu\mathcal{E}^s R_s(c) \\ &= \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 0 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} k_1 c_1 \\ k_2 c_2 \\ k_3 c_2 \\ k_4 c_1 c_3 \end{bmatrix} \\ &= \begin{bmatrix} k_3 c_2 - k_4 c_1 c_3 \\ k_1 c_1 - k_2 c_2 \\ k_4 c_1 c_3 \end{bmatrix}, \end{aligned}$$

where

$$\alpha = \mathcal{P}^f c = \begin{bmatrix} c_1 \\ c_2 \\ c_4 \end{bmatrix}.$$

From the equation $0 = R^f = k_5 c_2 - k_6 c_3$, one can obtain an explicit evolution equation for α,

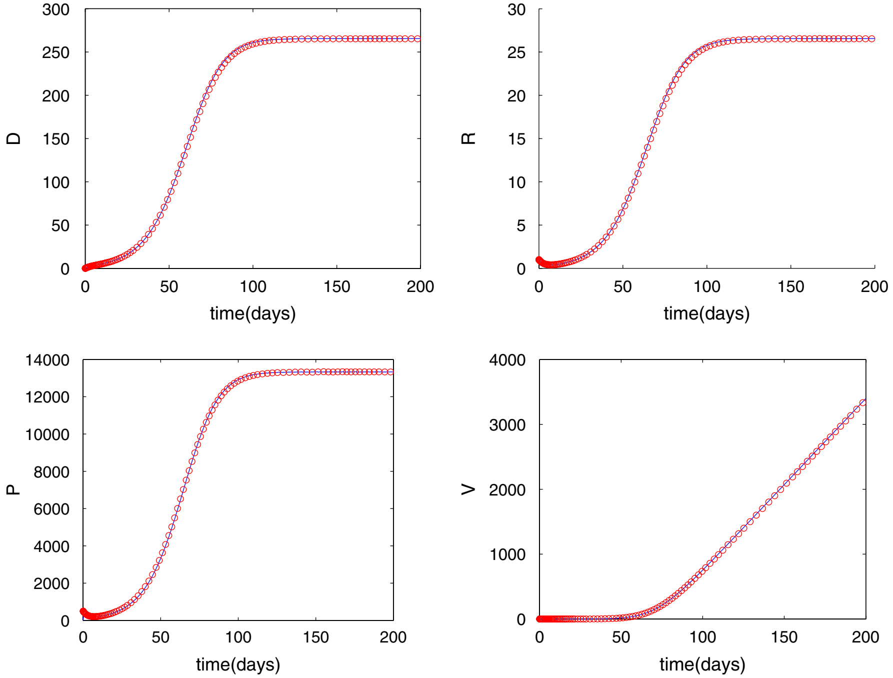

Fig. 6 Evolution of numbers of molecules of DNA, RNA, protein and viral cells when $(D, R, P, V) = (0, 1, 0, 0)$ initially. Solution of the full equation (*blue solid line*) versus solution of the reduced equation (*red circles*)

$$\frac{d\alpha}{dt} = \frac{d}{dt}\begin{bmatrix}\alpha_1\\ \alpha_2\\ \alpha_3\end{bmatrix} = \begin{bmatrix} k_3\alpha_2 - \frac{k_4k_5}{k_6}\alpha_1\alpha_2 \\ k_1\alpha_1 - k_2\alpha_2 \\ \frac{k_4k_5}{k_6}\alpha_1\alpha_2 \end{bmatrix} \tag{116}$$

or alternatively, an explicit expression of the slow dynamics in terms of original variables c_1, c_2 and c_4

$$\frac{d}{dt}\begin{bmatrix}c_1\\ c_2\\ c_4\end{bmatrix} = \begin{bmatrix} k_3c_2 - \frac{k_4k_5}{k_6}c_1c_2 \\ k_1c_1 - k_2c_2 \\ \frac{k_4k_5}{k_6}c_1c_2 \end{bmatrix} \tag{117}$$

A comparison of the numerical results the full and reduced systems is given in Fig. 6.

8 Conclusion

In this paper we presented a reduction method for chemical reaction networks with coupled fast and slow reactions. In the general reduction process, which can be applied to all networks, we first identify null complexes, then remove cycles, and finally reduce the deficiency to zero. The result is a dynamically equivalent systems, and using this we eliminated the fast kinetic steps using singular perturbation. When $D_c(R^f(c))\nu\mathcal{E}^f$ is nonsingular there is a change of coordinates that leads to a standard form in which slow and fast variables are identified explicitly. This reduction also clarifies the geometric meaning of the reduction, proving that under the QSS assumption, the solution of the reduced system is a projection of a solution trajectory onto the steady state manifold along a family of reaction simplexes for the fast subsystem.

We also identified network topologies that guarantee the separation of fast and slow variables, and showed that if the fast subsystem is a linear loop, the nonsingularity condition holds. It also holds if the fast subsystem has a tree structure, with some additional conditions. Several examples of increasing complexity, including a receptor-ligand binding model, an intracellular viral infection model, and a reaction model for the glycolytic reactions were presented to illustrate the reduction method. Using a similar approach, the result for two-time scale reaction networks can be extended to three or more time scale reaction networks (see the appendix.)

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Appendix A: Extension to three or more time scale networks

The analysis presented in previous sections can be extended to three or more time scale reaction networks under suitable conditions. Here we describe the procedure for the reduction of the three-time scale reaction network.

Suppose that reactions are separated into subsets of three different time scale reactions: slow, medium and fast reactions, with $O(1)$, $O(1/\epsilon_1)$ and $O(1/\epsilon_2)$, respectively, where $0 < \epsilon_2 \ll \epsilon_1 \ll 1$. We denote slow, medium and fast reactions by superscripts s, m and f respectively. We let R^k be the reaction rate function, $\nu\mathcal{E}^k$ be the stoichiometric matrix, and r_k be $\rho(\nu\mathcal{E}^k)$ for each $k = s, m, f$. As in the two time scale networks, we can make the reduction of the graphs of fast and medium subsystems, so that we assume a reduced graph of the given system and two stoichiometric matrices $\nu\mathcal{E}^f$ and $\nu\mathcal{E}^m$ have full rank. Thus, one can write the governing equation,

$$\frac{dc}{dt} = \frac{1}{\epsilon_2}\nu\mathcal{E}^f R^f(c) + \frac{1}{\epsilon_1}\nu\mathcal{E}^m R^m(c) + \nu\mathcal{E}^s R^s(c). \tag{118}$$

The QSS approximations on two different time scales is done as follows.

I. Dynamics after the QSS approximation of fast dynamics

We first assume the rank condition, $\rho(\nu\mathcal{E}^f) = \rho(D_c(R^f(c))$. By Theorem 3, if the matrix $D_c(R^f(c))\nu\mathcal{E}^f$ is nonsingular, then by a coordinate change

$$T_1(c) = \begin{bmatrix} \mathcal{P}^f \cdot c \\ R^f(c) \end{bmatrix} \equiv \begin{bmatrix} \alpha \\ \beta \end{bmatrix},$$

we obtain

$$\frac{d\alpha}{dt} = \frac{1}{\epsilon_1}\mathcal{P}^f \cdot \nu\mathcal{E}^m R^m(c) + \mathcal{P}^f \nu\mathcal{E}^s R^s(c) \tag{119}$$

$$\epsilon_2\frac{d\beta}{dt} = \epsilon_2 D_c(R^f(c))\left[\frac{1}{\epsilon_1}\nu\mathcal{E}^m R^m(c) + \nu\mathcal{E}^s R^s(c)\right] + D_c(R^f(c))\nu\mathcal{E}^f R^f(c), \tag{120}$$

where $c = T^{-1}(\alpha, \beta)$.

Under the QSS assumption, i.e. letting $\epsilon_2 \to 0$, we obtain a reduced equation

$$\frac{d\alpha}{dt} = \frac{1}{\epsilon_1}\mathcal{P}^f \cdot \nu\mathcal{E}^m R^m(S(\alpha)) + \mathcal{P}^f \nu\mathcal{E}^s R^s(S(\alpha)), \tag{121}$$

where $c = T^{-1}(\alpha, 0) \equiv S(\alpha)$. Note that the variable c evolves on the manifold $\mathcal{K}_f = \{c : R^f(c) = 0\}$.

II. Slow dynamics after the QSS approximations of fast and medium dynamics

We can obtain a reduced equation on the slow time scale by applying a similar method as in the above to the two-time scale equation (121). To do so, we assume $\mathcal{P}^f \nu\mathcal{E}^m$ has full column rank, and the rank condition, $\rho(\mathcal{P}^f \nu\mathcal{E}^m) = \rho(D_\alpha(R^m S(\alpha)))$.

We define a matrix B^m whose rows are basis vectors of $\mathcal{P}^f \nu\mathcal{E}^m$ and a transformation

$$T_2(\alpha) = \begin{bmatrix} B^m \cdot \alpha \\ R^m S(\alpha) \end{bmatrix} \equiv \begin{bmatrix} \eta \\ \zeta \end{bmatrix}.$$

Under the nonsingularity of $D_\alpha[R^m(S(\alpha))]\mathcal{P}^f \nu\mathcal{E}^m$, we can obtain

$$\frac{d\eta}{dt} = B^m\mathcal{P}^f \nu\mathcal{E}^s R^s S(\alpha) \tag{122}$$

$$\epsilon_1\frac{d\zeta}{dt} = \epsilon_1 D_\alpha(R^m(\alpha))\mathcal{P}^f \nu\mathcal{E}^s R^s S(\alpha) + D_\alpha(R^m S(\alpha))\mathcal{P}^f \nu\mathcal{E}^m \zeta, \tag{123}$$

where $\alpha = T_2^{-1}(\eta, \zeta)$.

Under the QSS assumption, i.e. by letting $\epsilon_1 \to 0$, we obtain

$$\frac{d\eta}{dt} = B^m\mathcal{P}^f \cdot \nu\mathcal{E}^s R^s S T_2^{-1}(\eta, 0). \tag{124}$$

Notice that the original variable c evolves on the set $\mathcal{K}_f \cap \mathcal{K}_m$, where $\mathcal{K}_m = \{c : R^m(c) = 0\}$.

A similar procedure can be used for reaction networks with more than three time scales.

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