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Critical Parameters for Singular Perturbation Reductions of Chemical Reaction Networks

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Abstract

We are concerned with polynomial ordinary differential systems that arise from modelling chemical reaction networks. For such systems, which may be of high dimension and may depend on many parameters, it is frequently of interest to obtain a reduction of dimension in certain parameter ranges. Singular perturbation theory, as initiated by Tikhonov and Fenichel, provides a path towards such reductions. In the present paper, we discuss parameter values that lead to singular perturbation reductions (so-called Tikhonov–Fenichel parameter values, or TFPVs). An algorithmic approach is known, but it is feasible for small dimensions only. Here, we characterize conditions for classes of reaction networks for which TFPVs arise by turning off reactions (by setting rate parameters to zero) or by removing certain species (which relates to the classical quasi-steady state approach to model reduction). In particular, we obtain definitive results for the class of complex-balanced reaction networks (of deficiency zero) and first-order reaction networks.

Keywords Reaction networks · Dimension reduction · Invariant sets · Critical manifold · Quasi-steady state

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1 Introduction

The modelling of chemical reaction networks frequently leads to high-dimensional parameter dependent systems of ordinary differential equations (ODEs). Even in the presence of a well-established structure theory for large classes of reaction networks, reducing the dimension of such systems is desirable for several reasons: From a quantitative perspective in the laboratory, parameter identification is frequently unfeasible for the full system but might be possible for a reduced equation. The Michaelis–Menten system and generalizations can be seen as examples of this; see, e.g. Segel and Slemrod (1989) and Keener and Sneyd (2009). From a qualitative vantage point, one strategy to prove special features such as the existence of periodic solutions, or multistationarity, is to prove such features for a reduced system and show that they persist for the full system in some parameter range. For a recent example of this strategy, see Feliu et al. (2020). Thus it is of general interest to identify parameter domains where a systematic reduction is possible.

Typically (although not exclusively) the reduction procedures are based on singular perturbation theory as developed by Tikhonov (1952) and Fenichel (1979). In the present paper, we will discuss singular perturbation reductions and critical parameters that permit reductions of this kind. The focus will be on characterizing such critical parameters that correspond naturally to structural features of the chemical reaction network.

A frequently used approach to finding appropriate parameters for singular perturbation scenarios goes back to a classical paper by Heineken et al. (1967). The method relies on an adroit scaling of suitable variables (based on an intuitive understanding of the processes in the reaction network) and ideally leads to a system with slow and fast variables to which Tikhonov's and Fenichel's theorems are applicable. From another perspective, a singular perturbation approach for systems with prescribed slow and fast reactions was discussed by Schauer and Heinrich (1983). More recently, a complete characterization of the parameter values (called Tikhonov–Fenichel parameter values, briefly TFPVs) which give rise to singular perturbations, and of their critical manifolds, was obtained in A. Goeke's dissertation (Goeke 2013) and the ensuing papers (Goeke and Walcher 2014; Goeke et al. 2015, 2017) by Goeke et al. Moreover for polynomial or rational systems, an algorithmic path exists towards determining these parameter values. The theory was applied to a number of reaction networks, including standard reaction networks from biochemistry (Keener and Sneyd 2009), and for these all possible singular perturbation reductions could be determined. In addition, it turned out that the algorithmically determined TFPVs for these systems readily admit an interpretation in terms of chemical species concentrations and reaction rates: Frequently these TFPVs correspond to a "switching off" of certain reactions or a removal of certain chemical species. This is the vantage point for the present paper. Since there is a natural limit to any algorithmic approach for systems with large numbers of variables or parameters, generalizing such structural insights is of interest.

From a mathematical as well as from a chemical perspective it seems desirable to understand whether (and how) special properties of reaction networks imply the existence of particular classes of TFPVs. The purpose of the present paper is to contribute towards this understanding. We will focus on reaction networks with mass-action kinetics, hence on polynomial differential equations. Our goal is to employ the structure of chemical reaction networks to obtain heuristics for finding TFPV candidates (respectively, candidates for scaling) in a first step and then, in a second step, proceed to verify the TFPV property for some reasonably large and relevant classes of reaction networks. We make substantial use of the structure theory going back to Horn and Jackson (1972), Feinberg (1972) and others. In terms of chemical reaction networks, we are concerned with slow and fast reactions, on the one hand. On the other hand, we investigate the provenance of quasi-steady state phenomena for chemical species, and their naturally associated "slow–fast" systems. Our main results apply in particular to weakly reversible reaction networks of deficiency zero.

Specifically, in Sect. 3 we first consider TFPVs that arise from turning off reactions, and identify graphical means for their identification (Theorems 3.9 and 3.10). We also provide an explanation of why TFPVs in many cases belong to proper coordinate subspaces (Proposition 3.3). In particular, we obtain a complete characterization for weakly reversible systems of deficiency zero. Continuing, in Sect. 4, we characterize sets of species (so-called LTC species sets) that "shut down" the reaction network when the corresponding variables are zero (hence, the species are present in zero concentration). Such species sets naturally lead to slow–fast systems (in a weak sense), and we further investigate their relation to linear first integrals and give conditions for when an LTC species set is the support of a linear first integral (Proposition 4.6). We proceed to discuss conditions for TFPV for systems on stoichiometric compatibility classes (Proposition 4.10 and its corollaries).

The paper is organized as follows. Section 2 contains preliminaries on reaction networks, TFPVs and (in a weak sense, formally) slow–fast dynamical systems. Section 3, in the context of reaction networks, discusses TFPVs defined by rate parameters. Section 4 builds on Sect. 2 and connects results of Sect. 3 to the classical scaling approach and slow–fast systems. In Appendix, we recall, for the reader's convenience, some results about TFPVs and Laplacian matrices, respectively, and we briefly discuss combining the approaches to turn off certain reactions and to remove certain species. Throughout, the results are illustrated by examples, including standard textbook reaction networks. In particular, we will use the reversible uncompetitive inhibition network as an accompanying example.

2 Preliminaries

We let $\mathbb{R}$, $\mathbb{R}_{\geq 0}$, $\mathbb{R}_{>0}$ denote the sets of real, non-negative real and positive real numbers, respectively. Also, we let $\mathbb{N}_0$ denote the set of non-negative integers. Given $m \in \mathbb{N}_0$, a *coordinate subspace* of $\mathbb{R}^m$ is defined by $x_{i_1} = \cdots = x_{i_k} = 0$ for some $k \in \{0, \ldots, m\}$ and $i_1 < \cdots < i_k$. It is *proper* if $k > 0$. The *support* $\operatorname{supp}(x)$ of $x \in \mathbb{R}^m$ is the set of all indices i with $x_i \neq 0$, $i = 1, \ldots, n$. For $y = (y_1, \ldots, y_n)^\top \in \mathbb{N}_0^n$ and $x = (x_1, \ldots, x_n)^\top \in \mathbb{R}_{\geq 0}^n$ (where $^\top$ denotes the transpose), we define $x^y = \prod_{i=1}^n x_i^{y_i}$.

If $M = (m_1 \dots m_k)$, $m_i \in \mathbb{N}_0^n$, $i = 1, \dots, k$, is an $(n \times k)$-matrix, then we define x^M as the vector $(x^{m_1}, \dots, x^{m_k})^\top \in \mathbb{R}^k_{\geq 0}$.

2.1 Reaction Networks

We consider spatially homogeneous chemical reaction networks with constant thermodynamical parameters and kinetics of mass-action type. The mathematical theory of these reaction networks was initiated and developed in seminal work by Horn and Jackson (1972) and Feinberg (1972). We will refer to Feinberg's recent monograph (Feinberg 2019) as a basic source. First we introduce the notion of a reaction network and fix some terminology.

Definition 2.1 A *mass-action reaction network* over a set of species $\mathcal{X} = \{X_1, \dots, X_n\}$ is a finite labelled directed graph $G = (\mathcal{Y}, \mathcal{R}, \kappa)$ with node set $\mathcal{Y}$ and edge set $\mathcal{R}$ such that

$$\mathcal{Y} \subseteq \left\{ \sum_{i=1}^{n} \alpha_i X_i \mid \alpha_i \in \mathbb{N}_0,\ i = 1, \dots, n \right\}$$

consists of non-negative integer linear combinations in $\mathcal{X}$, and κ labels edges by positive real numbers. Isolated nodes, but not self-edges, are allowed. We refer to the nodes as *complexes*, to the edges as *reactions*, and to the labels as *rate parameters*. Every species is assumed to be in some complex with a positive coefficient. Throughout we let d be the cardinality of $\mathcal{Y}$ and m the cardinality of $\mathcal{R}$.

A reaction network $\widetilde{G}$ is a *subnetwork* of another reaction network G with species set $\mathcal{X}$, if $\widetilde{G}$ is a subdigraph of G.

We enumerate the set of complexes in some way, and thus write $Y_j = \sum_{i=1}^n y_{ij} X_i$ with $y_{ij} \in \mathbb{N}_0$. The y_{ij}'s are referred to as *stochiometric coefficients*. A labelled reaction between the complexes Y_j and Y_ℓ is written as

$$Y_j \xrightarrow{\kappa_{\ell j}} Y_\ell, \qquad \kappa_{\ell j} > 0.$$

Here, Y_j is called a *reactant* complex and Y_ℓ a *product* complex. Note the reversal of the subindex of κ in the labels. A numbering of the elements of $\mathcal{R}$ by $1, \dots, m$, provides an ordering of $\mathcal{R}$ and we identify the collection of $\kappa_{\ell j}$ with a vector $\kappa \in \mathbb{R}^m_{>0}$, ordered in the same way as $\mathcal{R}$, such that $\kappa_i = \kappa_{\ell j}$ if $Y_j \xrightarrow{\kappa_{\ell j}} Y_\ell$ is the i-th reaction. We will use this convention without further reference.

The zero complex 0 is allowed by definition. Reactions with reactant 0 are called *inflow reactions*, and account for production or influx of species.

As a reaction network is given as a directed graph, terminology and properties from graph theory apply. Moreover, special terminology has been developed for CRN, parallel to terminology in graph theory. We will refer to a reaction network where all connected components of the digraph are strongly connected as *weakly reversible*, and otherwise apply standard terminology.

The evolution of the species concentrations in time is modelled by means of a system of ODEs, assuming *mass-action kinetics*. Denote by $x(t) = (x_1(t), \ldots, x_n(t))$ the vector of concentrations of the species $X_1, \ldots, X_n$ at time t. Define the *complex matrix* by

$$Y = \big(y_{ij}\big)_{1\le i\le n,\, 1\le j\le d} \in \mathbb{R}^{n\times d},$$

consisting of the stoichiometric coefficients of the complexes, and let $y_1, \ldots, y_d$ denote its columns. We let B be the *reactant matrix* with i-th column y_j if Y_j is the reactant of the i-th reaction, and $N \in \mathbb{R}^{n\times m}$ the matrix, referred to as the *stoichiometric matrix*, with i-th column given by $y_\ell - y_j$ if $Y_j \longrightarrow Y_\ell$ is the i-th reaction. With this notation, the system of ODEs becomes:

$$\dot{x} = N\ \mathrm{diag}(\kappa)\, x^B, \qquad x \in \mathbb{R}^n_{\ge 0}, \tag{1}$$

where reference to t is omitted and $\kappa \in \mathbb{R}^m_{>0}$. The sets $\mathbb{R}^n_{>0}$ and $\mathbb{R}^n_{\ge 0}$ are positively invariant for (1) (Volpert 1972). Furthermore, there is a useful decomposition of the right-hand side of (1) in terms of the Laplacian of the reaction network. The *Laplacian matrix* $A(\kappa) = (a_{ij})_{1\le i,j\le d} \in \mathbb{R}^{d\times d}$ is given by

$$a_{ij} = \kappa_{ij}, \quad i \ne j, \qquad a_{jj} = -\sum_{j\ne\ell} \kappa_{\ell j}, \qquad \text{for} \quad i, j = 1, \ldots, d,$$

where $\kappa_{ij} = 0$ if there is no reaction $Y_j \to Y_i$. Then, (1) agrees with

$$\dot{x} = Y A(\kappa)\, x^Y, \qquad x \in \mathbb{R}^n_{\ge 0}. \tag{2}$$

(See also Feinberg (2019, Subsection 16.1) for further background.)

System (1) often admits *stoichiometric first integrals*. These are nonzero linear forms

$$\phi(x) = \alpha_1 x_1 + \cdots + \alpha_n x_n$$

with coefficients $\alpha_i \in \mathbb{R}$, $i = 1, \ldots, n$, such that

$$(\alpha_1, \ldots, \alpha_n) \cdot N = 0 \qquad \big(\text{equivalently,} \quad (\alpha_1, \ldots, \alpha_n) \cdot Y A(\kappa) = 0 \quad \text{for all } \kappa \in \mathbb{R}^m_{>0}\big).$$

Note that $\alpha_1, \ldots, \alpha_n$ might be chosen as integers, since N has integer entries.

Definition 2.2 The image of the stoichiometric matrix N is the *stoichiometric subspace*, and the intersection of every coset of this subspace with the non-negative orthant is a *stoichiometric compatibility class (SCC)*.

The *dimension* (respectively, *codimension*) of the mass-action reaction network is by definition the dimension (respectively, codimension) of the stoichiometric subspace.

While it is possible at the outset to reduce the dimension of system (1) via stoichiometric first integrals, for the purpose of the present paper it seems appropriate to keep the representation (1) until at a later stage.

In principle, system (1) might admit further linear first integrals. However, the following result says that it does not happen for *realistic* networks.

Lemma 2.3 (Feinberg and Horn 1977) *If every connected component of a reaction network has exactly one terminal strongly connected component, then every linear first integral of* (1) *is stoichiometric.*

Example 2.4 Consider the mass-action reaction network with species X_1, X_2 and reactions

$$\begin{array}{ccccc} 0 & \xleftarrow{\kappa_2} & X_1 & \xrightarrow{\kappa_1} & 2X_1 \\ & & \underset{\kappa_4}{\uparrow}\downarrow{\scriptstyle \kappa_3} & & \\ & & X_2. & & \end{array} \tag{3}$$

The corresponding ODE system is given by

$$\dot{x}_1 = (\kappa_1 - \kappa_2)x_1 - \kappa_3 x_1 + \kappa_4 x_2, \qquad \dot{x}_2 = \kappa_3 x_1 - \kappa_4 x_2.$$

The reaction network has no linear first integrals for generic κ, but when $\kappa_1 = \kappa_2$, the vector $(1, 1)$ defines one. This reaction network has one connected component, but two terminal strongly connected components, namely $\{0\}$ and $\{2X_1\}$.

For the network $X_2 \overset{\kappa_1}{\longleftarrow} X_1 \overset{\kappa_2}{\longrightarrow} X_3$, the dimension is 1, but for all κ there are two linearly independent linear first integrals: a stoichiometric linear first integral $\phi_1 = x_1 + x_2 + x_3$, and a non-stoichiometric first integral $\phi_2 = \kappa_2 x_2 - \kappa_1 x_3$.

The following example will be used to illustrate notions and results throughout the paper.

Example 2.5 The uncompetitive inhibition network with reversible product formation (Keener and Sneyd 2009),

$$X_1 + X_2 \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} X_3 \underset{\kappa_{-2}}{\overset{\kappa_2}{\rightleftharpoons}} X_4 + X_2, \qquad X_3 + X_5 \underset{\kappa_{-3}}{\overset{\kappa_3}{\rightleftharpoons}} X_6,$$

is an extension of the classical reversible Michaelis–Menten network (see Appendix, Example A.2), with an inhibitor X_5 binding to complex X_3. The system has five complexes and two linkage classes. Here, we find

$$Y = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{pmatrix}, \quad N = \begin{pmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ -1 & 1 & 1 & -1 & 0 & 0 \\ 1 & -1 & -1 & 1 & -1 & 1 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix},$$

hence the dimension of the network equals three. One has stoichiometric first integrals

$$\phi_1 = x_2 + x_3 + x_6, \qquad \phi_2 = x_5 + x_6, \qquad \phi_3 = x_1 + x_3 + x_4 + x_6. \tag{4}$$

Moreover

$$A(\kappa) = \begin{pmatrix} -\kappa_1 & \kappa_{-1} & 0 & 0 & 0 \\ \kappa_1 & -(\kappa_{-1}+\kappa_2) & \kappa_{-2} & 0 & 0 \\ 0 & \kappa_2 & -\kappa_{-2} & 0 & 0 \\ 0 & 0 & 0 & -\kappa_3 & \kappa_{-3} \\ 0 & 0 & 0 & \kappa_3 & -\kappa_{-3} \end{pmatrix}, \quad x^Y = \begin{pmatrix} x_1x_2 \\ x_3 \\ x_2x_4 \\ x_3x_5 \\ x_6 \end{pmatrix}.$$

The full ODE system is given by

$$\begin{aligned} \dot{x}_1 &= -\kappa_1 x_1 x_2 + \kappa_{-1} x_3 \\ \dot{x}_2 &= -\kappa_1 x_1 x_2 + (\kappa_{-1} + \kappa_2) x_3 - \kappa_{-2} x_2 x_4 \\ \dot{x}_3 &= \kappa_1 x_1 x_2 - (\kappa_{-1} + \kappa_2) x_3 + \kappa_{-2} x_2 x_4 - \kappa_3 x_3 x_5 + \kappa_{-3} x_6 \\ \dot{x}_4 &= \kappa_2 x_3 - \kappa_{-2} x_2 x_4 \\ \dot{x}_5 &= -\kappa_3 x_3 x_5 + \kappa_{-3} x_6 \\ \dot{x}_6 &= +\kappa_3 x_3 x_5 - \kappa_{-3} x_6. \end{aligned} \tag{5}$$

With typical initial values $x_1(0) = s_0$, $x_2(0) = e_0$, $x_5(0) = f_0$, $x_3(0) = x_4(0) = x_6(0) = 0$, and using the stoichiometric first integrals from (4) to reduce the dimension, one arrives at a three-dimensional system

$$\begin{aligned} \dot{x}_1 &= -\kappa_1(e_0 - x_3 - x_6)x_1 + \kappa_{-1} x_3 \\ \dot{x}_3 &= \kappa_1(e_0 - x_3 - x_6)x_1 - (\kappa_{-1} + \kappa_2)x_3 + \kappa_{-2}(e_0 - x_3 - x_6)(s_0 - x_1 - x_3 - x_6) \\ &\quad - \kappa_3 x_3 (f_0 - x_6) + \kappa_{-3} x_6 \\ \dot{x}_6 &= \kappa_3 x_3 (f_0 - x_6) - \kappa_{-3} x_6. \end{aligned} \tag{6}$$

2.2 Tikhonov–Fenichel Parameter Values (TFPVs)

Throughout, when referring to *singular perturbation reduction*, we mean this in the sense of Tikhonov (1952) and Fenichel (1979). In order to identify parameters that give rise to singular perturbation reductions the following approach was taken in Goeke's dissertation (Goeke 2013) and the subsequent papers (Goeke and Walcher 2014; Goeke et al. 2015).

Consider a parameter-dependent ODE system,

$$\dot{x} = h(x, \pi), \quad x \in \Omega \subseteq \mathbb{R}^n, \quad \pi \in \Pi \subseteq \mathbb{R}^m \tag{7}$$

with $h(x, \pi)$ polynomial in x and π. We let $D_1 h(x, \pi)$ and $D_2 h(x, \pi)$ denote the matrices of partial derivatives with respect to the entries of x and π, respectively.

Given $\pi \in \Pi$, we denote by $\mathcal{V}(h(\cdot, \pi))$ the zero set of $x \mapsto h(x, \pi)$, and let $n - s^*$ be the generic dimension (with respect to π) of the vector subspace generated by the $h(x, \pi)$, with $x \in \Omega$. In addition, we require that the generic rank of $D_1 h(x, \pi)$, with $x \in \mathbb{R}^n$, equals $n - s^*$.

In the setting of mass-action reaction networks, this subspace is equal to the stoichiometric subspace under the hypotheses of Lemma 2.3. In this case, s^* is the codimension of the reaction network, according to Definition 2.2.

The existence of singular perturbation reductions corresponds to the existence of *Tikhonov–Fenichel parameter values* (TFPVs). The following definition extends the one given in Goeke et al. (2015).

Definition 2.6 A TFPV *for dimension* s ($s^* < s < n$) of system (7) is a parameter $\widehat{\pi} \in \Pi$, such that the following hold:

(i) The *critical variety* $\mathcal{V}(h(\cdot, \widehat{\pi})) \cap \Omega$ contains an irreducible component Z of dimension s.

(ii) There is a Zariski open subset $\widetilde{Z} \subseteq Z$ such that for all $x \in \widetilde{Z}$ one has

$$\operatorname{rank} D_1 h(x, \widehat{\pi}) = n - s \quad \text{and} \quad \mathbb{R}^n = \ker D_1 h(x, \widehat{\pi}) \oplus \operatorname{Im} D_1 h(x, \widehat{\pi}).$$

(iii) There exists $x_0 \in \widetilde{Z}$ such that all nonzero eigenvalues of $D_1 h(x_0, \widehat{\pi})$ have negative real part.

We let $\Pi_s \subseteq \Pi$ denote the set of TFPVs for dimension $s > s^*$.

If Π and Ω are semi-algebraic sets (typically, these are positive orthants), then Π_s is a semi-algebraic set as well (Goeke et al. 2015), hence defined by polynomial equations and inequalities. If one is only interested in the defining equations, this amounts to considering the Zariski closure of Π_s, which is denoted by

$$W_s := \overline{\Pi_s}^{Zar}. \tag{8}$$

For a number of standard reaction networks in biochemistry (in particular those described in the first chapter of Keener and Sneyd 2009), all TFPVs were determined algorithmically in Goeke's dissertation (Goeke 2013) and in the subsequent papers (Goeke et al. 2015, 2017). It turned out that every irreducible component of W_s is just a coordinate subspace, and that all of these admit an interpretation as a degenerate scenario in reaction network terms, via "switched off" reactions or missing species (and in some cases a combination of these). Based on these observations, and employing the theory of reaction networks, we will investigate conditions on reaction networks that guarantee the existence of singular perturbation scenarios.

Note that provided (i) holds, then (ii) and (iii) are together equivalent to

(ii') There exists $x_0 \in \widetilde{Z}$ such that $D_1 h(x_0, \widehat{\pi})$ has exactly $n - s$ nonzero eigenvalues (counted with multiplicity), which additionally have negative real part.

For further information about TFPVs see Sect. A.1 in Appendix.

We note the relation to the more restrictive definition from Goeke et al. (2015).

Lemma 2.7 *Given the mass-action reaction network (7), with s^* as defined following (7), let $\widehat{\pi}$ be a TFPV for dimension $s > s^*$ of (7). Then $\widehat{\pi}$ is a TFPV for dimension $s - s^*$ for the restriction to any SCC that has non-trivial intersection with $\widetilde{Z}$ and admits only isolated stationary points.*

For a proof see Sect. A.1 in Appendix.

2.3 Slow–Fast Systems, Scalings and Reductions

In the present paper, we call a smooth system of the form

$$\begin{aligned} \dot{u}_1 &= f_1(u_1, u_2, \varepsilon), \\ \dot{u}_2 &= \varepsilon\, f_2(u_1, u_2, \varepsilon), \end{aligned} \tag{9}$$

on an open subset of $\mathbb{R}^s \times \mathbb{R}^r \times \mathbb{R}$, with a parameter ε in a neighbourhood of 0, a *slow–fast system*. One is interested in the behaviour as $\varepsilon \to 0$ (and mostly $\varepsilon \geq 0$).

The classical statement of Tikhonov's theorem (and Fenichel's local theory) starts from a slow–fast system which satisfies additional conditions amounting to those given in Definition 2.6. In this situation, the equation $f_1(u_1, u_2, 0) = 0$ locally defines $u_1 = \phi(u_2)$ as a function of u_2, and a reduced system in slow time $\tau = \varepsilon\, t$ can be defined as $\frac{du_2}{d\tau} = f_2(\phi(u_2), u_2, 0)$. The trajectories of the reduced system approximate in a specific sense the trajectories of the original system (9).

A well-known approach to a rigorous foundation of quasi-steady state phenomena in chemical reaction networks goes back to Heineken et al. (1967): In order to obtain a slow–fast system from (7) some variables of the system that satisfy a certain compatibility condition are scaled by a positive parameter (frequently called "small parameter") ε, and one considers the system as $\varepsilon \to 0$. We outline a simplified version of this technique: Given a smooth curve $\varepsilon \mapsto \pi^* + \varepsilon\rho + \ldots$ in the parameter space (with π^* not necessarily a TFPV), we obtain a system

$$h(x, \pi^* + \varepsilon\rho + \cdots) = h^{(0)}(x) + \varepsilon h^{(1)}(x) + \varepsilon^2 h^{(2)}(x) + \cdots =: h^*(x, \varepsilon) \tag{10}$$

with small parameter ε. Note that

$$h^{(0)}(x) = h(x, \pi^*).$$

If one starts this procedure from a TFPV π^*, then one obtains a singular perturbation reduction, as noted in Appendix, Proposition A.1.

Remark 2.8 Given the coordinate-independent setting (10), if π^* is a TFPV, then the actual determination of a reduced equation has been discussed in Goeke and Walcher (2014) for the case $s^* = 0$, and we briefly recall it: There exists[1] a decomposition

$$h^{(0)}(x) = P(x) \cdot \mu(x),$$

[1] The decomposition is not unique, but the reduced equation on $\widetilde{Z}$ is.

with P an $n \times s$ matrix, and μ an $s \times 1$ matrix with rational functions as entries, such that $\widetilde{Z}$ is locally the zero set of μ and the rank of $D_1\mu$ equals s, as does the rank of P. With I the identity matrix, define the projection matrix

$$Q(x) := I - P(x)\,(D_1\mu(x)P(x))^{-1}\,D_1\mu(x).$$

Then the reduced equation is given by

$$\dot{x} = Q(x) \cdot h^{(1)}(x) \quad \text{on the invariant manifold } \widetilde{Z}. \tag{11}$$

We illustrate the reduction procedure by an example which has not yet been recorded in the literature. (For a further example see Sect. A.1.)

Example 2.9 The restriction (6) of the uncompetitive inhibition system admits a TFPV with $e_0 = 0$ and all other parameters positive. With $e_0 = \varepsilon e_0^*$, one finds

$$P(x) = \begin{pmatrix} \kappa_1 x_1 + \kappa_{-1} & \kappa_1 x_1 \\ -\kappa_1 x_1 - \kappa_{-1} - \kappa_2 - \kappa_{-2}(s_0 - x_1) - \kappa_3 f_0 & -\kappa_1 x_1 - \kappa_{-2}(s_0 - x_1) + \kappa_{-3} \\ \kappa_3 f_0 & -\kappa_{-3} \end{pmatrix}$$

and the critical manifold $\widetilde{Z}$ is given by $x_3 = x_6 = 0$. Thus one has

$$D_1\mu(x) = \begin{pmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

and $D_1\mu \cdot P$ is the 2×2 matrix consisting of the last two rows of P. The following computations are straightforward (if perhaps tedious). The reduced equation on $\widetilde{Z}$ yields

$$\dot{x}_1 = -\frac{\kappa_{-3} e_0 \cdot (\kappa_2 \kappa_1 x_1 - \kappa_{-1}\kappa_{-2}(s_0 - x_1))}{\kappa_{-2}(\kappa_3 f_0 + \kappa_{-3})(s_0 - x_1) + (\kappa_1 x_1 + \kappa_{-1} + \kappa_2)\kappa_{-3} + \kappa_1 \kappa_3 f_0 x_1}$$

and $\dot{x}_3 = \dot{x}_6 = 0$.

We return to the more general setting of slow–fast systems. The above-mentioned compatibility (LTC) condition ensures that scaling of the chosen variables preserves smoothness. We recall it from Lax and Walcher (2020).

Definition 2.10 An index set

$$\{i_1, \ldots, i_r\}, \quad 1 \le i_1 < \cdots < i_r \le n, \quad 1 \le r < n,$$

is called an *LTC index set* for (10), and the set of corresponding variables $\{x_{i_1}, \ldots, x_{i_r}\}$ an *LTC variable set*, if

$$h^{(0)}(x) = 0, \quad \text{whenever} \quad x_{i_1} = \cdots = x_{i_r} = 0, \tag{12}$$

thus the coordinate subspace defined by $x_{i_1}, \ldots, x_{i_r}$ is contained in $\mathcal{V}(h^{(0)})$. The acronym stands for "locally Tikhonov consistent" (Lax and Walcher 2020).

If the ODE system models a reaction network, then the corresponding species set $\{X_{i_1}, \ldots, X_{i_r}\}$ is called a set of *LTC species*. If the concentrations of all the species in an LCT set are all zero, then no reaction can take place. Note that (12) cannot be fulfilled if there are inflow reactions in the reaction network, as $h^{(0)}(x)$ contains a nonzero constant monomial.

For an LTC index set $\{i_1, \ldots, i_r\}$, define

$$u_1 := \begin{pmatrix} x_{i_1} \\ \vdots \\ x_{i_r} \end{pmatrix},$$

and collect the remaining variables in u_2. Partitioning

$$x = \begin{pmatrix} u_1 \\ u_2 \end{pmatrix},$$

and rewriting $h^*(x, \varepsilon) =: g(u_1, u_2, \varepsilon)$, one obtains a system

$$\begin{aligned} \dot{u}_1 &= g_1(u_1, u_2, \varepsilon), \\ \dot{u}_2 &= g_2(u_1, u_2, \varepsilon), \end{aligned} \tag{13}$$

with $g(0, u_2, \varepsilon) = 0$. Scaling $u_1 = \varepsilon\, u_1^*$, one can write $g_i(\varepsilon\, u_1^*, u_2, \varepsilon) = \varepsilon\, \widehat{g}_i(u_1^*, u_2, \varepsilon)$, with $\widehat{g}_1$, $\widehat{g}_2$ being polynomials, provided that g_1, g_2 are so, arriving at the slow–fast system as in (9),

$$\begin{aligned} \dot{u}_1^* &= \widehat{g}_1(u_1^*, u_2, \varepsilon), \\ \dot{u}_2 &= \varepsilon\, \widehat{g}_2(u_1^*, u_2, \varepsilon). \end{aligned} \tag{14}$$

In the singular perturbation reduction following (Heineken et al. 1967), one applies Tikhonov's theorem to (14), upon verifying the necessary conditions. In the literature, a frequently used shortcut is to directly solve $g_1(u_1, u_2, \varepsilon) = 0$ (with small ε) for u_1 and substitute the result into the second equation of (13). We will refer to this procedure as *classical QSS reduction.* Note that without further analysis, e.g. verifying the hypotheses for Tikhonov's theorem, this is a purely formal procedure.

Obviously, any superset of an LTC species set is also an LTC species set; generally one will first consider minimal ones.

Since solutions of (14) are bounded on compact subsets of their maximal existence interval, one finds $u_1 = O(\varepsilon)$ on these compact subintervals. But it is not guaranteed that system (13) admits a local $(n-r)$-dimensional invariant manifold close to $u_1 = 0$ for small positive ε, hence there remains the question whether some reduction exists. Thus, LTC variable sets provide candidates for Tikhonov–Fenichel reductions, but these need further investigation. Moreover, even in the singular perturbation setting, there may not be a connection to TFPVs. We will get back to this later.

In some cases, direct application of Tikhonov–Fenichel does not work, but singular perturbation reduction with a critical variety of higher dimension is possible. For instance, Schneider and Wilhelm (2000) considered a scenario, where the fast part of (14) admits non-trivial first integrals. In such a setting, the partial derivative $D_1\widehat{g}_1$ cannot have full rank, but if the rank is full on every level set of the first integrals, and the nonzero eigenvalues have negative real parts, then reduction works. Conversely, the local existence of such first integrals is also necessary (Goeke and Walcher 2014, Prop. 2).

Example 2.11 For the uncompetitive inhibition network (see Example 2.5, equation (5)) one finds LTC variable sets $\{x_2,\ x_3,\ x_6\}$ and $\{x_1,\ x_3,\ x_4,\ x_6\}$. For the first set, one obtains (employing first integrals as detailed in Lax and Walcher 2020) the same reduction to dimension one as in Example 2.9.

3 TFPVs for Reaction Networks

3.1 General Considerations

While the notion of TFPV applies to all parameter-dependent polynomial (and more general) vector fields, special properties of reaction networks impose restrictions. We give an elementary illustration of this fact.

Example 3.1 Consider the linear differential equation in $\mathbb{R}^2$,

$$\dot{x} = \begin{pmatrix} \alpha_{11} & \alpha_{12} \\ \alpha_{21} & \alpha_{22} \end{pmatrix} x + \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} = A\,x + b,$$

where the second equality defines A and b, and $\alpha_{ij},\ \beta_i \in \mathbb{R}$, $i,\ j = 1, 2$. Thus the parameter space consists of all $(\alpha_{11}, \alpha_{12}, \alpha_{21}, \alpha_{22}, \beta_1, \beta_2)$. By Definition 2.6(i) and Lemma A.3(v), a TFPV for dimension $s = 1$ satisfies

$$0 = \det A = \alpha_{11}\alpha_{22} - \alpha_{12}\alpha_{21}.$$

This relation defines a hypersurface in the parameter space. On the other hand, by (2), every linear 2×2 system describing a first-order reaction network with two species (hence, the reaction network has the complexes X_1, X_2 and possibly 0), takes the form

$$\dot{x} = \begin{pmatrix} -\kappa_{21} - \kappa_{31} & \kappa_{12} \\ \kappa_{21} & -\kappa_{12} - \kappa_{32} \end{pmatrix} x + \begin{pmatrix} \kappa_{13} \\ \kappa_{23} \end{pmatrix},$$

with non-negative κ_{ij} (κ_{ij} is zero if the corresponding reaction does not exist). The determinant condition on the Jacobian of the system simplifies to

$$\kappa_{21}\kappa_{32} + \kappa_{31}\kappa_{12} + \kappa_{31}\kappa_{32} = 0 \quad \Leftrightarrow \quad \kappa_{21}\kappa_{32} = \kappa_{31}\kappa_{12} = \kappa_{31}\kappa_{32} = 0,$$

due to non-negativity. (In addition, the existence of stationary points requires conditions on κ_{13} and κ_{23}.) Evaluating the TFPV conditions, one sees that they all admit an interpretation in the reaction network framework: Certain reactions are being "switched off". Furthermore, the conditions for TFPVs to exist yield very simple irreducible components of the Zariski closure W_s of Π_s (see (8)), namely coordinate subspaces.

Indeed, it is not easy to find (realistic) systems where some component of W_s is not a coordinate subspace. This may be the case when non-stoichiometric first integrals exist for only some κ: Let s^* be the codimension of the reaction network (the number of independent stoichiometric first integrals). Assume the set $\widetilde{\Pi}$ of κ's that give rise to extra linear first integrals is a proper algebraic variety and hence has measure zero (as for the reaction network in (3) in Example 2.4). Any point in $\widetilde{\Pi}$ is a candidate for a TFPV in dimension $s > s^*$, if furthermore the critical manifold intersects the non-negative orthant and is attracting. Going back to network (3), the set $\widetilde{\Pi}$ consists of TFPVs and is characterized by the condition $\kappa_1 = \kappa_2$, as one easily verifies that there exists a linearly attracting critical manifold.

An artificial way to construct further examples where the set of TFPVs is not included in a coordinate subspace, is to consider any parametrized polynomial system for which the dimension of the set of stationary points is larger than s^* for some choice of parameters, and furthermore, all negative monomials of the i-th polynomial are multiples of x_i. The latter is enough to constructively interpret the system as arising from a mass-action reaction network (Érdi and Tóth 1989), though the networks obtained in this way are typically not realistic. The following example is generated in this way.

Example 3.2 Consider the following mass-action reaction network

$$X_2 \xrightarrow{\kappa_1} X_1 + X_2 \xrightarrow{\kappa_2} X_1 \xrightarrow{\kappa_3} 0 \qquad 2X_1 \xrightarrow{\kappa_4} X_2 + 2X_1.$$

The associated ODE system in $\mathbb{R}^2_{\geq 0}$ is

$$\dot{x}_1 = \kappa_1 x_2 - \kappa_3 x_1, \qquad \dot{x}_2 = -\kappa_2 x_1 x_2 + \kappa_4 x_1^2 = x_1(-\kappa_2 x_2 + \kappa_4 x_1).$$

Generically, the variety of stationary points consists of the point $(0, 0)$ and has dimension $s^* = 0$. However, when $\kappa_1\kappa_4 = \kappa_2\kappa_3$, then the variety has dimension one and consists of the line $\kappa_1 x_2 = \kappa_3 x_1$. Additionally, a direct computation shows that the critical manifold is attracting for $(x_1, x_2) \in \mathbb{R}^2_{\geq 0}$. Hence, $\kappa_1\kappa_4 = \kappa_2\kappa_3$ defines a set of TFPVs for dimension one. In this case, there are no linear first integrals.

We now turn to system (1), and first establish conditions to ensure that every TFPV lies in some proper coordinate subspace, thus every irreducible component of W_s is contained in some coordinate subspace. If we require the critical manifold to intersect the positive orthant, the existence of TFPVs $\widehat{\kappa} \in \mathbb{R}^m_{>0}$ is easily precluded for important classes of reaction networks.

In preparation for Proposition 3.3, we introduce some objects and some notation. Let $N' \in \mathbb{R}^{s^* \times m}$ consist of s^* linearly independent rows of the stoichiometric matrix

N. Moreover let $E \in \mathbb{R}^{m\times q}$ be a matrix whose columns are the extreme rays of the polyhedral cone $\ker(N) \cap \mathbb{R}^m_{\geq 0}$, and for $\lambda \in \mathbb{R}^q$ denote by $\operatorname{diag}(E\lambda)$ the matrix with the entries of $E\lambda$ in the diagonal, and zeros off-diagonal. Consider the matrix

$$N' \operatorname{diag}(E\lambda) B^\top \tag{15}$$

(with B the reactant matrix, see Sect. 2.1). Finally, let Λ be the set of $\lambda \in \mathbb{R}^q_{\geq 0}$ such that $E\lambda \in \mathbb{R}^m_{>0}$. (The particular choice of N' will be irrelevant.)

Proposition 3.3 *Let G be a mass-action reaction network of codimension s^*. With the notation introduced above, assume G belongs to one of the following cases:*

(a) The set of positive stationary points $\mathcal{V}_\kappa \subseteq \mathbb{R}^n_{>0}$ admits a smooth parametrization of the form $\mathbb{R}^{s^*}_{>0} \xrightarrow{\varphi_\kappa} \mathcal{V}_\kappa$, with $\operatorname{im}(\varphi_\kappa) = \mathcal{V}_\kappa$ for all $\kappa \in \mathbb{R}^m_{>0}$.
(b) The reaction network is injective (Feliu and Wiuf 2012a)*, hence the coefficient $\sigma_{n-s^*}(x,\kappa)$ of τ^{s^*} of the characteristic polynomial of the Jacobian of system (1) is a polynomial in x and κ with only non-negative coefficients.*
(c) For all $\lambda \in \Lambda$, at least one of the minors of $N' \operatorname{diag}(E\lambda) B^\top$ is nonzero.

Then, there are no TFPVs $\widehat{\kappa} \in \mathbb{R}^m_{>0}$ for which some irreducible component Z of the critical variety intersects the positive orthant.

Proof (a) The parametrization gives that the dimension of $\mathcal{V}_\kappa$ is s^* for all $\kappa \in \mathbb{R}^m_{>0}$. (b) By Lemma A.3 in Appendix, for $\widehat{\kappa}$ to be a TFPV we need that $\sigma_{n-s^*}(x_0, \widehat{\kappa}) = 0$ for some $x_0 \in Z$. Assumption (b) now gives that this can happen only if $\widehat{\kappa}$ or x_0 belong to a coordinate subspace. (c) The condition implies that the Jacobian of (1) has rank $n - s^*$ at any positive stationary point (Pascual-Escudero and Feliu 2021). □

Remark 3.4 We make a few observations regarding the relevance of the criteria in Proposition 3.3.

- Condition (c) holds for surprisingly many networks and is computationally easy to verify. When $\Lambda = \mathbb{R}^q_{>0}$, which occurs often, then condition (c) holds if there is one minor with all nonzero coefficients of the same sign.
- Many realistic reaction networks admit parametrizations in the sense of Proposition 3.3(a): Among these are reaction networks admitting toric steady states (Millán et al. 2012), complex-balancing equilibria (Horn and Jackson 1972; Feinberg 1972; Craciun et al. 2009) (see also Sect. 3.2), and there are many reaction networks for which parametrizations can be found using linear elimination of some variables in terms of the rest (Feliu and Wiuf 2012b; Sáez et al. 2019) (see Conradi et al. 2017 for a short account on how to find parametrizations).
- Injective reaction networks admit at most one equilibrium in each SCC (Craciun and Feinberg 2005; Feliu and Wiuf 2012a), and several criteria, in addition to the one stated in Proposition 3.3(b), have been established. These criteria involve graphical conditions (Banaji and Craciun 2010, 2009) and sign vectors (Müller et al. 2016).

To include TFPVs with critical manifold intersecting the positive orthant, it is appropriate (and necessary in the cases covered in Proposition 3.3) to deviate from

the convention in Definition 2.1 and allow the rate parameters to be zero, thus change the parameter range of κ to $\mathbb{R}^m_{\geq 0}$. Passing from generic $\kappa \in \mathbb{R}^m_{>0}$ to a special $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ may be seen as considering a subnetwork of the original reaction network. To indicate this, we make the following definition.

Definition 3.5 Let $\kappa \in \mathbb{R}^m_{\geq 0}$. We denote by $G(\kappa)$ the subnetwork obtained from G by removing the reactions with indices in $\{1, \ldots, m\} \setminus \operatorname{supp}(\kappa)$, that is, the i-th reaction is removed if $\kappa_i = 0$, for $i = 1, \ldots, m$. Isolated nodes are not removed from $G(\kappa)$, and hence G and $G(\kappa)$ have the same set of complexes and species.

Note that $G(\kappa) = G(\widetilde{\kappa})$ as long as $\operatorname{supp}(\kappa) = \operatorname{supp}(\widetilde{\kappa})$. Recall that the codimension of a network is defined as the codimension of its stoichiometric subspace.

Proposition 3.6 *Let G be a mass-action reaction network of codimension s^*. Let $h(x, \kappa)$ denote the right-hand side of (2).*

(a) Let $\kappa^* \in \mathbb{R}^m_{>0}$. If there exists $x^* \in \mathbb{R}^n_{>0} \cap \mathcal{V}(h(\cdot, \kappa^*))$ such that $D_1 h(x^*, \kappa^*)$ has rank $n - s^*$ (respectively, additionally $n - s^*$ eigenvalues with negative real part), then the same holds for a norm-open neighbourhood of κ^*, and thus for a Zariski dense subset of $\mathbb{R}^m_{>0}$ containing κ^*. In particular, an irreducible component of $\mathcal{V}(h(\cdot, \kappa^*))$ has dimension s^* and intersects the positive orthant.

(b) If $\kappa^* \in \mathbb{R}^m_{\geq 0}$ is a TFPV for dimension $s > s^*$ with s the codimension of $G(\kappa^*)$, then the minimal coordinate subspace containing κ^* is contained in W_s.

Proof (a) We will use that $D_1 h(x^*, \kappa^*)$ has $n - s^*$ eigenvalues with negative real part, if and only if the corresponding $n - s^*$ Hurwitz determinants of its characteristic polynomial, divided by τ^{s^*}, are positive, see Gantmacher (2005, Ch. V, section 6). The rank of $D_1 h(x^*, \kappa^*)$ being $n - s^*$ implies that an irreducible component of $\mathcal{V}(h(\cdot, \kappa^*))$ has dimension s^* and intersects the positive orthant (Cox et al. 2007, §9.6 Thm 9). Let V be the real algebraic variety in the variables x, κ consisting of points where $h(x, \kappa) = 0$ and $D_1 h(x, \kappa)$ has rank strictly smaller than $n - s^*$, respectively, at least one of the Hurwitz determinants vanishes. By hypothesis, there exists $(x^*, \kappa^*) \in \mathbb{R}^{n+m}_{>0} \setminus V$ satisfying $h(x^*, \kappa^*) = 0$. Let $U \subseteq \mathbb{R}^{n+m}_{>0} \setminus V$ be an open Euclidean ball containing (x^*, κ^*). The intersection of U and the zero set of h, which is non-empty, consists of points (x, κ) such that $x \in \mathcal{V}(h(\cdot, \kappa))$ and the Jacobian has maximal rank $n - s^*$, respectively, all Hurwitz determinants are positive. The projection $\widehat{U}$ of U onto $\mathbb{R}^m_{>0}$ in the variable κ contains a non-empty open Euclidian ball of parameters κ_0 for which there exists $x_0 \in \mathbb{R}^n_{>0}$ such that $D_1 h(x_0, \kappa_0)$ has rank $n - s^*$, respectively, additional $n - s^*$ eigenvalues with negative real part. By the Implicit Function Theorem applied to h at (x^*, κ^*), $\widehat{U}$ contains an open ball centred at κ^* such that $\mathbb{R}^n_{>0} \cap \mathcal{V}(h(\cdot, \kappa)) \neq \emptyset$ for all κ in the ball. As any Euclidean ball is Zariski dense, this concludes the proof of (a).

(b) Let C be the minimal coordinate subspace containing κ^*. The parameter κ^* and the reaction network $G(\kappa^*)$ satisfy the hypotheses of (a), after restricting $\mathbb{R}^m_{>0}$ to C. Therefore, there exists a norm-open and Zariski dense set U (relative to C) such that any $\kappa' \in U \subseteq C \cap \mathbb{R}^m_{>0}$ is a TFPV for dimension s and thus $U \subseteq W_s$. Since U is Zariski dense in C, its Zariski closure is C and it follows that $C \subseteq W_s$. □

Proposition 3.6(b) does not imply that all rate parameters in the coordinate subspace are TFPVs given that one is a TFPV, but only that this is the case in an open set relative to the coordinate subspace. The next example illustrates this.

Example 3.7 Consider the following mass-action reaction network,

$$X_1 + X_2 \xrightarrow{\kappa_1} 2X_1, \qquad X_1 + 2X_2 \xrightarrow{\kappa_3} 3X_1, \qquad 0 \underset{\kappa_6}{\overset{\kappa_5}{\rightleftharpoons}} X_1,$$
$$X_1 + X_2 \xrightarrow{\kappa_2} 2X_2, \qquad X_1 + 2X_2 \xrightarrow{\kappa_4} 3X_2.$$

The associated ODE system in $\mathbb{R}^2_{\geq 0}$ is

$$\dot{x}_1 = (\kappa_1 - \kappa_2)x_1x_2 + (2\kappa_3 - \kappa_4)x_1x_2^2 + \kappa_5 - \kappa_6 x_1,$$
$$\dot{x}_2 = (-\kappa_1 + \kappa_2)x_1x_2 + (-2\kappa_3 + \kappa_4)x_1x_2^2.$$

The codimension of the reaction network is $s^* = 0$, and the reaction network has one positive equilibrium $(\frac{\kappa_5}{\kappa_6}, \frac{\kappa_2-\kappa_1}{2\kappa_3-\kappa_4})$, provided the second entry is positive. Consider a parameter value of the form $\widehat{\kappa} = (\kappa_1, \kappa_2, \kappa_3, \kappa_4, 0, 0)$, which corresponds to removing the pair of reactions $0 \rightleftharpoons X_1$. Then, the stoichiometric subspace of $G(\widehat{\kappa})$ has codimension $s = 1$, and the stationary variety consists of the two coordinate axes together with the line $x_2 = \frac{\kappa_2-\kappa_1}{2\kappa_3-\kappa_4}$, provided this expression is positive. In this case, there is a critical manifold of dimension one in $\mathbb{R}^2_{>0}$. The line intersecting the positive orthant is attracting if $\kappa_1 < \kappa_2$ and repelling if $\kappa_2 < \kappa_1$. Hence, $\widehat{\kappa}$ is a TFPV for dimension one if and only if $\kappa_1 < \kappa_2$ and $\kappa_4 < 2\kappa_3$. We also have that the minimal coordinate subspace C containing $\widehat{\kappa}$ belongs to W_1.

If we now consider $\widehat{\kappa} = (0, \kappa_2, \kappa_3, 0, 0, 0)$, the codimension of $G(\widehat{\kappa})$ is also $s = 1$, and the positive part of the stationary variety consists of the attracting line $x_2 = \frac{\kappa_2}{2\kappa_3}$. Hence, $\widehat{\kappa}$ is a TFPV for dimension one. The minimal coordinate subspace containing $\widehat{\kappa}$ is not an irreducible component of W_1, as it is a proper Zariski closed set of the coordinate subspace C.

In what follows, we consider TFPVs for two classes of reaction networks, namely first-order reaction networks and complex-balanced reaction networks. Due to special properties of the Laplacian matrix, TFPVs for complex-balanced reaction networks can be identified. Our results build on the understanding of the kernel of $A(\kappa)$ in (2). In Appendix, Sect. A.2 we review key results about Laplacian matrices and especially their kernels, using a graphical approach.

3.2 Complex-Balancing and TFPVs

We turn to an important class of reaction networks called complex-balanced reaction networks and the existence of TFPVs for this class. Complex-balanced reaction networks are characterized by their equilibria, called *complex-balanced* equilibria. According to Horn and Jackson (1972) (see also Feinberg 2019, Ch. 15ff.), a *positive*

equilibrium $z \in \mathbb{R}^n_{>0}$ of (2) is complex-balanced for the parameter value κ^* if

$$A(\kappa^*)z^Y = 0.$$

By Lemma A.5(b), the existence of a positive complex-balanced equilibrium implies that the reaction network is weakly reversible (Feinberg 2019, Proposition 16.5.7). However, weak reversibility is not a sufficient condition. Since z is an equilibrium for system (2) if and only if $YA(\kappa)z^Y = 0$, one needs to understand the relation between $\ker\, YA(\kappa)$ and $\ker\, A(\kappa)$. Obviously, the latter is a subset of the former.

The following proposition gathers well-known facts (Feinberg 2019, Thm 15.2.2, Thm 15.2.4, Lemma 16.3.1).

Proposition 3.8 *Let $G = (\mathcal{Y}, \mathcal{R}, \kappa)$ be a mass-action reaction network with d complexes, r connected components and codimension s^*.*

- *Let $e_1, \ldots, e_d$ denote the standard basis of $\mathbb{R}^d$ and let $\Delta := \left\{ e_j - e_i \mid Y_i \to Y_j \in \mathcal{R} \right\} \subseteq \mathbb{R}^d$. Then*

$$\ker\, YA(\kappa) = \ker\, A(\kappa) \oplus (\ker\, Y \cap \operatorname{span} \Delta)\,.$$

  The dimension δ of $\ker\, Y \cap \operatorname{span} \Delta$ *is called the* deficiency, *and satisfies $\delta = d - (n - s^*) - r$.*
- *If system (2) admits a complex-balanced equilibrium in $\mathbb{R}^n_{>0}$, then every SCC contains precisely one positive equilibrium, which also is complex-balanced, and the Jacobian has $n - s^*$ eigenvalues with negative real part and s^* zero eigenvalues (counted with multiplicity). As a consequence, the positive equilibria of the system form a manifold of dimension s^*.*

If G is weakly reversible and $\delta = 0$, then all positive equilibria are complex-balanced, irrespective of the (positive) reaction rate constants. In general, there are δ algebraically independent relations on the rate parameters κ, characterizing when the reaction network admits positive complex-balanced equilibria. These relations are explicit (Craciun et al. 2009; Dickenstein and Millán 2011; Feliu et al. 2018). Complex-balanced equilibria form a manifold of dimension s^*, and the rank of the Jacobian of system (2) evaluated at the equilibrium is $n - s^*$.

In the following theorem we use the notation $G(\widehat{\kappa})$ introduced in Definition 3.5.

Theorem 3.9 *Let G be a mass-action reaction network of codimension s^*. Let $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ such that*

- *$G(\widehat{\kappa})$ is weakly reversible of codimension $s > s^*$.*
- *The nonzero coordinates of $\widehat{\kappa}$ satisfy the relations for the existence of positive complex-balanced equilibria in $G(\widehat{\kappa})$.*

Then, $\widehat{\kappa}$ is a TFPV for dimension s of system (2). Furthermore, the minimal coordinate subspace containing $\widehat{\kappa}$ is contained in W_s.

Proof We show that $\widehat{\kappa}$ is a TFPV by showing that properties (iv)-(vi) in Lemma A.3 hold. By Proposition 3.8, the dimension of the set of positive equilibria of $G(\widehat{\kappa})$ is s, giving (iv). Properties (v)-(vi) follow from the properties of complex-balanced equilibria in Proposition 3.8. The last statement follows from Proposition 3.6(b). □

An immediate consequence of Theorem 3.9 arises when G is weakly reversible and has deficiency zero. A key point is that the deficiency of any subnetwork obtained from G by removing reactions can only decrease (Joshi and Shiu 2015, Prop. 8.2). In particular, if G has deficiency zero, then so does any subnetwork.

Theorem 3.10 *Let a weakly reversible reaction network G of deficiency zero be given, with dynamics governed by system (2). Let $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ be such that the induced subnetwork $G(\widehat{\kappa})$ is weakly reversible and has more connected components than G.*

Then $\widehat{\kappa}$ is a TFPV of system (2) for dimension $n - d + r$, with d and r the number of complexes, respectively, connected components of $G(\widehat{\kappa})$. This dimension equals the codimension of $G(\widehat{\kappa})$.

Proof Let r^* be the number of connected components of G. As the deficiencies of G and $G(\widehat{\kappa})$ are zero, the codimensions of G and $G(\widehat{\kappa})$ are $s^* = n - d + r^*$ and $s = n - d + r$, respectively. As $r > r^*$, we have $s > s^*$. Furthermore, all parameter values $\widehat{\kappa}$ yield complex-balanced equilibria for $G(\widehat{\kappa})$ as the deficiency is zero. The statement now follows from Theorem 3.9. □

In particular, when the hypotheses of Theorem 3.10 hold, then $\widehat{\kappa}$ lies in a coordinate subspace of the parameter space. Moreover, the connected components of $G(\widehat{\kappa})$ identify a coordinate subspace in W_s for the appropriate $s > s^*$. For some classes of reaction networks, including weakly reversible reaction networks of deficiency zero, an explicit formula for the singular perturbation reduction was derived in Feliu et al. (2020).

Example 3.11 The classical reversible Michaelis–Menten system (see Appendix, Example A.2) has deficiency zero and codimension $s^* = 2$. By Theorem 3.10, setting either $\kappa_1 = \kappa_{-1} = 0$ or $\kappa_2 = \kappa_{-2} = 0$, the number of connected components increases, and the resulting rate parameters are TFPVs for dimension 3.

Example 3.12 We continue the discussion of the uncompetitive inhibition network from Example 2.5. This reaction network is weakly reversible with deficiency zero (five complexes, two linkage classes and stoichiometric subspace of dimension three). By Theorem 3.10, setting either $\kappa_1 = \kappa_{-1} = 0$, or $\kappa_2 = \kappa_{-2} = 0$, or $\kappa_3 = \kappa_{-3} = 0$, the number of connected components increases by one, and the resulting rate parameters are TFPVs for dimension 4. In addition, choosing two of the three pairs to be zero, one obtains TFPVs for dimension 5. Restricting to stoichiometric compatibility classes one has TFPVs for dimension one and two, respectively, for system (6), by Lemma 2.7.

We take a closer look at the reduction of (6) induced by the TFPV with $\kappa_1 = \kappa_{-1} = \kappa_3 = \kappa_{-3} = 0$, thus $\kappa_1 = \varepsilon\kappa_1^*$, $\kappa_{-1} = \varepsilon\kappa_{-1}^*$, $\kappa_3 = \varepsilon\kappa_3^*$, $\kappa_{-3} = \varepsilon\kappa_{-3}^*$. Here, we have, using (10) that

$$h^{(0)}(x) = \begin{pmatrix} 0 \\ -\kappa_2 x_3 + \kappa_{-2}(e_0 - x_3 - x_6)(s_0 - x_1 - x_3 - x_6) \\ 0 \end{pmatrix}$$
$$h^{(1)}(x) = \begin{pmatrix} -\kappa_1(e_0 - x_3 - x_6)x_1 + \kappa_{-1}x_3 \\ \kappa_1(e_0 - x_3 - x_6)x_1 - \kappa_{-1}x_3 - \kappa_3 x_3(f_0 - x_6) + \kappa_{-3}x_6 \\ \kappa_3 x_3(f_0 - x_6) - \kappa_{-3}x_6 \end{pmatrix}.$$

By Remark 2.8, we find the decomposition of $h^{(0)}$ in terms of

$$P(x) = \begin{pmatrix} 0 \\ 1 \\ 0 \end{pmatrix}, \qquad \mu(x) = -\kappa_2 x_3 + \kappa_{-2}(e_0 - x_3 - x_6)(s_0 - x_1 - x_3 - x_6),$$

and obtain the projection matrix

$$Q(x) = \frac{1}{\Delta(x)} \begin{pmatrix} \Delta(x) & 0 & 0 \\ -\kappa_{-2}(e_0 - x_3 - x_6) & 0 & \kappa_2 \\ 0 & 0 & \Delta(x) \end{pmatrix};$$
$$\Delta(x) = \kappa_2 + \kappa_{-2}(e_0 - x_3 - x_6 + s_0 - x_1 - x_3 - x_6).$$

By (11), one obtains the reduced system on the invariant variety given by $\mu = 0$. Of the various versions, the one with the simplest appearance is

$$\dot{x}_1 = -\kappa_1(e_0 - x_3 - x_6)x_1 + \kappa_{-1}x_3$$
$$\dot{x}_6 = \kappa_3 x_3(f_0 - x_6) - \kappa_{-3}x_6,$$

with x_3 to be determined from $\mu = 0$, a quadratic equation in x_3.

Example 3.13 Consider the following reaction network, which is the futile cycle with one phosphorylation site (Wang and Sontag 2008):

$$X_1 + X_3 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} X_5 \overset{\kappa_3}{\to} X_1 + X_4 \qquad X_2 + X_4 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} X_6 \overset{\kappa_6}{\to} X_2 + X_3.$$

This reaction network is not weakly reversible and has codimension 3. An easy computation shows that the stationary set admits a parametrization with three free variables x_1, x_2, x_3, and hence has dimension 3. Proposition 3.3(a) applies.

Alternatively, Proposition 3.3(c) is applicable: For a specific choice of N', the matrix $N' \operatorname{diag}(E\lambda) B^\top$ in (15) equals

$$\begin{pmatrix} 0 & -\lambda_2 & 0 & -\lambda_2 & \lambda_2 & \lambda_1 + \lambda_2 \\ \lambda_1 & 0 & \lambda_1 & 0 & -\lambda_2 - \lambda_3 & 0 \\ 0 & \lambda_2 & 0 & \lambda_2 & 0 & -\lambda_1 - 2\lambda_2 - \lambda_3 \end{pmatrix}.$$

We have $\Lambda = \mathbb{R}^3_{>0}$. The minor given by columns 1, 2, 5 is $\lambda_1\lambda_2^2$, which is nonzero.

In conclusion, no TFPVs with positive entries and critical manifold intersecting the positive orthant exist. Upon setting $\kappa_3 = \kappa_6 = 0$, the resulting reaction network is weakly reversible and has deficiency 0 with codimension $s = 4$. Hence, by Theorem 3.9, any rate parameter of the form $(\kappa_1, \kappa_2, 0, \kappa_4, \kappa_5, 0)$ with nonzero entries being positive, is a TFPV for dimension 4.

Example 3.14 Consider the following reaction network modelling an allosteric kinase (Feng et al. 2016):

$$X_1 + X_5 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} X_3 \xrightarrow{\kappa_9} X_1 + X_6, \qquad X_3 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} X_4, \qquad X_6 \xrightarrow{\kappa_{11}} X_5,$$
$$X_2 + X_5 \underset{\kappa_5}{\overset{\kappa_6}{\rightleftharpoons}} X_4 \xrightarrow{\kappa_{10}} X_2 + X_6, \qquad X_1 \underset{\kappa_8}{\overset{\kappa_7}{\rightleftharpoons}} X_2.$$

The positive part of the stationary set admits a parametrization with $s^* = 2$ free variables. Upon setting $\kappa_9 = \kappa_{10} = \kappa_{11} = 0$, the reaction network becomes weakly reversible of deficiency 1 and codimension 3. The condition that characterizes when complex-balanced equilibria exist, referred to in Theorem 3.9, is $\kappa_2\kappa_4\kappa_6\kappa_7 = \kappa_1\kappa_3\kappa_5\kappa_8$ (Craciun et al. 2009). Thus any $\widehat{\kappa} = (\kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5, \kappa_6, \kappa_7, \kappa_8, 0, 0, 0)$, fulfilling this condition, is a TFPV for dimension 3 and the corresponding coordinate subspace is included in W_3.

In this case, the positive part of the stationary variety of $G(\widehat{\kappa})$ always admits a parametrization. Using Hurwitz determinants, one confirms that the variety is linearly attracting. Therefore, the whole positive part of this particular coordinate subspace is formed by TFPVs.

We conclude with an example of a weakly reversible reaction network admitting TFPVs that are not included in a proper coordinate subspace.

Example 3.15 This example is introduced in Boros et al. (2020, Example 4.1), where the purpose is to show the existence of weakly reversible reaction networks with infinitely many equilibria in some SCC. The reaction network consists of four connected components, written in rows for convenience:

$$0 \xrightarrow{\kappa_1} X_1 \xrightarrow{\kappa_2} X_1 + X_2 \xrightarrow{\kappa_3} X_2 \xrightarrow{\kappa_4} 0,$$
$$2X_1 \xrightarrow{\kappa_5} 3X_1 \xrightarrow{\kappa_6} 3X_1 + X_2 \xrightarrow{\kappa_7} 2X_1 + X_2 \xrightarrow{\kappa_8} 2X_1,$$
$$2X_2 \xrightarrow{\kappa_9} X_1 + 2X_2 \xrightarrow{\kappa_{10}} X_1 + 3X_2 \xrightarrow{\kappa_{11}} 3X_2 \xrightarrow{\kappa_{12}} 2X_2,$$
$$2X_1 + 2X_2 \xrightarrow{\kappa_{13}} 3X_1 + 2X_2 \xrightarrow{\kappa_{14}} 3X_1 + 3X_2 \xrightarrow{\kappa_{15}} 2X_1 + 3X_2 \xrightarrow{\kappa_{16}} 2X_1 + 2X_2.$$

This reaction network is weakly reversible of codimension $s^* = 0$. When all parameters are set to 1 except for $\kappa_3 = \kappa_8 = \kappa_{10} = \kappa_{13} = a$, with $a > 5$, then the stationary variety has dimension 1: it consists of one unstable point (1, 1) and one attracting closed curve around (1, 1). Hence, any such rate parameter is a TFPV for dimension 1, which does not belong to a proper coordinate subspace. One might note that the reaction network is of the form discussed in Example 3.2 with all negative terms in the ODE system being multiples of x_2.

3.3 TFPVs for First-Order Reaction Networks

In this section, we consider the special case of a mass-action reaction network $G = (\mathcal{Y}, \mathcal{R}, \kappa)$ containing only first-order reactions; thus $d = n$ or $d = n + 1$ and nonzero complexes may be identified with species. In the formulation

$$\dot{x} = Y A(\kappa)\, x^Y, \qquad x \in \mathbb{R}^n_{\geq 0}, \tag{16}$$

the matrix Y is simply the identity matrix if $0 \notin \mathcal{Y}$ and the identity matrix with an extra zero column otherwise. Hence either $x^Y = x$ or $x^Y = \begin{pmatrix} x \\ 1 \end{pmatrix}$.

Lemma 3.16 *A first-order mass-action reaction network has deficiency zero.*

Proof With the notation introduced in Proposition 3.8, one has $\ker Y \cap \operatorname{span} \Delta = \{0\}$, due to the form of Y. The assertion follows. □

Remark 3.17 By Lemma A.5, the rank of $A(\kappa)$ does not depend on $\kappa \in \mathbb{R}^m_{>0}$. Let T be the number of terminal strongly connected components of G and s^* be the codimension of G. We make the following observations:

- If $0 \notin \mathcal{Y}$, then Y is the identity matrix, and the solution set to (16) in $\mathbb{R}^n_{\geq 0}$ is $\ker A(\kappa)$ and $s^* = T$.
- If $0 \in \mathcal{Y}$, then $s^* = T - 1$.
  - If 0 belongs to a terminal strongly connected component of G, then the solution set to (16) in $\mathbb{R}^n_{\geq 0}$ is the linear affine subspace of $\ker A(\kappa) \cap \mathbb{R}^n_{\geq 0}$ with last coordinate equal to 1. By the description of $\ker A(\kappa)$ in Lemma A.5, this subspace has dimension $T - 1$.
  - If 0 does not belong to a terminal strongly connected component of G, then (16) has no solution. Indeed, the last entry of x^Y is equal to 1, and hence positive, but any vector in $\ker A(\kappa)$ has last entry zero.

With this in mind, we obtain the following proposition.

Proposition 3.18 *Let $A(\kappa)$ be the Laplacian matrix of a mass-action reaction network $G = (\mathcal{Y}, \mathcal{R}, \kappa)$ consisting only of first-order reactions with dynamics governed by system (16) in $\mathbb{R}^n_{\geq 0}$. Let T be the number of terminal strongly connected components of G, and s^* the dimension of the solution set to (16).*

(a) If $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ is a TFPV of (16) for dimension $s > s^*$, then $\widehat{\kappa}$ lies in a proper coordinate subspace of $\mathbb{R}^m$.

(b) Let $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ be in a proper coordinate subspace of $\mathbb{R}^m$, and consider the subnetwork $G(\widehat{\kappa})$.
If 0 is not a complex of G, then $\widehat{\kappa}$ is a TFPV if and only if $G(\widehat{\kappa})$ has more than T terminal strongly connected components.
If 0 is a complex of G, then $\widehat{\kappa}$ is a TFPV if and only if $G(\widehat{\kappa})$ has more than T terminal strongly connected components, and additionally the complex 0 belongs to one such component.

(c) Each irreducible component of W_s for $s > s^*$ is a coordinate subspace of $\mathbb{R}^m$.

Proof (a) This is straightforward as the dimension of the solution set to (16) does not depend on $\kappa \in \mathbb{R}^m_{>0}$. (b) We first make a digression. Consider $G(\widehat{\kappa})$ and assume $0 \in \mathcal{Y}$. Then the last column of the matrix Y is zero and the last entry of $v(x) = x^Y$ is 1. Let $\widetilde{A}(\widehat{\kappa})$ be the submatrix of $A(\widehat{\kappa})$ obtained by removing the last row and column. Let $\beta \in \mathbb{R}^{d-1}$ be the vector formed by the first $d-1$ entries of the last column of $A(\kappa)$. Then $YA(\widehat{\kappa})v(x) = \widetilde{A}(\widehat{\kappa})x + \beta$. To prove (b), we apply Lemma A.4 to the compartmental matrices $A(\widehat{\kappa})$ or $\widetilde{A}(\widehat{\kappa})$, depending on whether 0 is a complex of G. (c) is a direct consequence of (a) and (b), as Π_s is a union of coordinate subspaces of $\mathbb{R}^m_{\geq 0}$. □

Rephrasing the statement of Proposition 3.18, all TFPVs are found by setting rate parameters to zero such that the number of terminal strongly connected components increases, and taking into consideration the role of the zero complex. We note that the irreducible components of any W_s can be identified by inspecting the graph G.

If the considered first-order reaction network G in addition is weakly reversible, then for this network Theorem 3.9 and Theorem 3.10 are both consequences of Proposition 3.18. For Theorem 3.9, note that if the subnetwork $G(\widehat{\kappa})$ of G is weakly reversible with codimension $s > s^*$, then it must be that $\widehat{\kappa} \in \mathbb{R}^m_{\geq 0}$ belongs to a proper coordinate subspace of $\mathbb{R}^m$ and the number of terminal strongly connected components of $G(\widehat{\kappa})$ exceeds the number of terminal strongly connected components of G. Hence, the conclusions of Theorem 3.9 follow from Proposition 3.18(b),(c). Note that the second condition of Theorem 3.9 is trivially fulfilled because G has deficiency zero, hence any subnetwork, in particular $G(\widehat{\kappa})$, has also deficiency zero (Joshi and Shiu Joshi and Shiu 2015, Prop. 8.2). For Theorem 3.10, we remark that it is a consequence of Theorem 3.9, hence also of Proposition 3.18. Alternatively, it follows directly from Proposition 3.18 by similar arguments to above.

Example 3.19 Consider a first-order reaction network with three complexes and four reactions,

$$X_1 \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} X_2 \underset{\kappa_{-2}}{\overset{\kappa_2}{\rightleftharpoons}} X_3.$$

This reaction network has one terminal strongly connected component. By Remark 3.17, $s^* = 1$. There are three coordinate subspaces yielding TFPVs for dimension 2. These arise from the three ways to increase the number of terminal strongly connected components: $\kappa_1 = \kappa_{-1} = 0$, or $\kappa_2 = \kappa_{-2} = 0$, or $\kappa_1 = \kappa_{-2} = 0$.

Example 3.20 For the first-order reaction network

$$X_1 \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} X_2, \qquad 0 \xrightarrow{\kappa_2} X_3,$$

we have two connected components and $s^* = 1$ (Remark 3.17), but this reaction network has no stationary points. Upon setting $\kappa_2 = 0$, we have three connected

components and 0 belongs to a terminal strongly connected component. Hence, by Proposition 3.18, $(\kappa_1, \kappa_{-1}, 0)$ is a TFPV for dimension 2. (The critical manifold consists of all $(x_1, x_2, x_3)^\top$ such that $\kappa_1 x_1 = \kappa_{-1} x_2$.)

4 Scalings, Stoichiometry and TFPVs

In this section, we start from LTC variable sets and the scaling approach to singular perturbation reductions of system (2), as initiated by Heineken et al. (1967) (recall Sect. 2.3 on slow–fast systems). A priori, there are no TFPVs that correspond to scalings, but these may appear when the system is restricted to SCCs, as new parameters are introduced. For motivation, we look at a familiar example.

Example 4.1 We consider again the reversible Michaelis–Menten system (see Appendix, Example A.2). The LTC variable set $\{x_2, x_3\}$ corresponds to the stoichiometric first integral $\phi_1 = x_2 + x_3$, and the LTC variable set $\{x_1, x_3, x_4\}$ corresponds to the stoichiometric first integral $\phi_2 = x_1 + x_3 + x_4$. Moreover, on the SCC given by $x_2 + x_3 = e_0$ and $x_1 + x_3 + x_4 = s_0$, one obtains the two-dimensional system

$$\begin{aligned}\dot{x}_1 &= -\kappa_1 x_1 (e_0 - x_3) + \kappa_{-1} x_3\\ \dot{x}_3 &= \kappa_1 x_1 (e_0 - x_3) - (\kappa_{-1} + \kappa_2) x_3 + \kappa_{-2} (e_0 - x_3)(s_0 - x_1 - x_3).\end{aligned}$$

This system admits a TFPV with $e_0 = 0$, and all other parameters > 0, with a degenerate (one dimensional) SCC forming the critical manifold, and a subsequent singular perturbation reduction. (For a TFPV with $s_0 = 0$, the SCC degenerates into a single point.)

Quite generally, LTC variable sets point to bifurcation scenarios, and possibly interesting dynamics may appear for small perturbations. In general, there is no perfect correspondence to stoichiometric first integrals, as shown by examples in Lax and Walcher (2020). But stoichiometric first integrals which correspond to LTC variable sets may, in turn, yield TFPVs of the system on SCCs.

We start by characterizing LTC species sets.

4.1 A Characterization of LTC Species Sets

A useful modification of system (2) is the following, when some complexes are *non-reactant complexes*, that is, they only appear as product complexes. Complex Y_j is non-reactant if and only if column j of $A(\kappa)$ is zero. Thus, one may form Y^* from Y, respectively, $A^*(\kappa)$ from $A(\kappa)$, by removing all columns that correspond to indices of non-reactant complexes, to rewrite (2) as

$$\dot{x} = Y A(\kappa) x^Y = Y A^*(\kappa)\, x^{Y^*}. \tag{17}$$

Let d^* be the number of reactant complexes, hence $Y^* \in \mathbb{N}_0^{n \times d^*}$. Note that $A^*(\kappa)$ is not a square matrix unless all complexes are reactant complexes and thus $A^*(\kappa) = A(\kappa)$.

In parameter-dependent systems, the notion of an LTC species set may depend on particular parameter values. But in the present paper, with the exception of Sect. A.3 in Appendix, we will only discuss sets that are LTC species sets for *all* parameter values $\kappa \in \mathbb{R}^m_{>0}$. The equations $x^Y = 0$, respectively, $x^{Y^*} = 0$ define varieties with coordinate subspaces as irreducible components, and the corresponding variables are obviously LTC variables. We will first show that all LTC variable sets of system (17) (which is the same as system (1)) are of this type.

The following proposition characterizes the LTC species sets. It also shows that the notions of LTC species and LTC variables are of real interest only for nonlinear systems. Recall that reaction networks with inflow reactions do not admit any LTC species sets (remark below Definition 2.10).

Proposition 4.2 *Let system (1) be given. Then $\{i_1, \ldots, i_u\}$ with $u < n$ and $1 \leq i_1 < i_2 \cdots < i_u \leq n$ is an LTC index set for all $\kappa \in \mathbb{R}^m_{>0}$ if and only if*

$$x^{Y^*} = 0, \quad \textit{whenever} \quad x_{i_1} = \cdots = x_{i_u} = 0.$$

Stated differently, LTC species sets are identifiable from the reactant complexes: A set of species $\{X_{i_1}, \ldots, X_{i_u}\}$ is an LTC species set if and only if in every reactant complex, one of the X_{i_k} appears with positive coefficient.

Proof The non-trivial assertion is the "only if" part. The "if" part follows by definition. We need to show that if $h(x, \kappa) = N \operatorname{diag}(\kappa) x^B = 0$ for all $\kappa \in \mathbb{R}^m_{>0}$ whenever $x_{i_1} = \cdots = x_{i_u} = 0$, then also $x^{Y^*} = 0$. We may assume that the LTC index set is $\{1, \ldots, u\}$, and that complexes are ordered such that the first d^* are reactant complexes. We let $y_1, \ldots, y_{d^*}$ denote the columns of Y^*.

We argue by contradiction and assume that some x^{y_i}, $i \in \{1, \ldots, d^*\}$, is nonzero when $x_1 = \cdots = x_u = 0$. For $x_1 = \cdots = x_u = 0$ and $i = 1, \ldots, d^*$, we have

$$x^{y_i} \neq 0 \quad \Longleftrightarrow \quad y_i = \begin{pmatrix} 0 \\ * \end{pmatrix}, \quad \text{with} \quad 0 \in \mathbb{R}^u. \tag{18}$$

We may assume that (18) holds precisely for the indices $d' \leq i \leq d^*$, for some $d' \leq d^*$. Thus, we aim to show $d' = d^*$.

Let K_κ be the $(m \times d^*)$-matrix with non-negative entries such that

$$K_\kappa \, x^{Y^*} = \operatorname{diag}(\kappa) x^B, \qquad \text{hence} \qquad N \operatorname{diag}(\kappa) x^B = N K_\kappa \, x^{Y^*}.$$

Each entry of K_κ is one of the rate parameters: the (i, j)-th entry is $\kappa_{\ell j}$ if the i-th reaction is $Y_j \to Y_\ell$. As y_i, $i = 1, \ldots, d^*$, are pairwise different, the monomials x^{y_i},

$d' \leq i \leq d^*$, are linearly independent over $\mathbb{R}$. Using $NK_\kappa\, x^{Y^*} = 0$, we obtain

$$NK_\kappa \begin{pmatrix} 0 \\ \vdots \\ 0 \\ x^{y_{d'}} \\ \vdots \\ x^{y_{d^*}} \end{pmatrix} = 0 \quad \Rightarrow \quad NK_\kappa = \begin{pmatrix} * & \cdots & * & 0 & \cdots & 0 \end{pmatrix},$$

with the last $d^* - d' + 1 \geq 1$ columns equal to zero. The equality tells us that the last $d^* - d' + 1$ columns of K_κ belong to $\ker(N)$ for all $\kappa \in \mathbb{R}^m_{>0}$. The sum of these columns lies also in $\ker(N)$. The entries of the sum are positive when they correspond to reactions with $Y_{d'}, \ldots, Y_{d^*}$ among the reactant species, and zero otherwise. As κ varies, we thus obtain a relatively open and non-empty subset in some proper coordinate subspace C of $\mathbb{R}^m$. The row space of N is therefore orthogonal to C; hence N has at least one zero column, and we have reached a contradiction, as a reaction network does not have self-edges. □

An enumeration of all LTC species sets may start from those reactant complexes that contain the fewest species (that is, the species appearing with positive stoichiometric coefficients). First, a species that appears alone in some reactant complex is necessarily contained in every LTC species set. Then proceed with complexes containing two species and so on. From this observation, one also finds that LTC species sets for first-order reaction networks (with every complex consisting of one species) are comprised of all species in reactant complexes.

Example 4.3 (a) In the classical reversible Michaelis–Menten reaction network (see Appendix, Example A.2), the reactant complexes are $X_1 + X_2$, X_3 and $X_4 + X_2$. Thus X_3 must lie in every LTC species set, and so must X_2 or X_1. The first alternative yields the LTC species set $\{X_2, X_3\}$, while the second yields the LTC species set $\{X_1, X_3, X_4\}$. These are the only two LTC species sets. In contrast, the irreversible Michaelis–Menten reaction network (omitting the reaction $X_4 + X_2 \xrightarrow{\kappa_{-2}} X_3$) has reactant complexes $X_1 + X_2$ and X_3, with two LTC species sets, $\{X_1, X_2\}$ and $\{X_2, X_3\}$.

(b) For reversible uncompetitive inhibition, all the minimal sets of LTC species for all parameter values are listed in Example 2.11.

Example 4.4 We consider again the futile cycle with one phosphorylation site, see Example 3.13:

$$X_1 + X_3 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} X_5 \xrightarrow{\kappa_3} X_1 + X_4, \qquad X_2 + X_4 \underset{\kappa_5}{\overset{\kappa_4}{\rightleftharpoons}} X_6 \xrightarrow{\kappa_6} X_2 + X_3.$$

Here, X_5 and X_6 are contained in every LTC species set, and altogether one finds the following LCT species sets,

$$\{X_1, X_2, X_5, X_6\}, \quad \{X_1, X_4, X_5, X_6\}, \quad \{X_2, X_3, X_5, X_6\}, \quad \{X_3, X_4, X_5, X_6\}.$$

Only the first and the last of these are also LTC species sets for the fully reversible system with the additional reactions $X_1 + X_4 \xrightarrow{\kappa_7} X_5$ and $X_2 + X_3 \xrightarrow{\kappa_8} X_6$.

4.2 LTC Species and First Integrals

We proceed to study the relation between LTC species sets for all $\kappa \in \mathbb{R}^m$ and linear first integrals. We first note a relation between LTC indices and the complex matrix.

Lemma 4.5 *Let $\{i_1, \ldots, i_u\}$ with $u < n$ and $1 \leq i_1 < i_2 \cdots < i_u \leq n$. Then the following statements are equivalent.*

(a) $\{i_1, \ldots, i_u\}$ is an LTC index set.
(b) The support of every column of Y^* contains some i_k.
(c) There exists a non-negative row $\omega \in \mathbb{N}_0^n$ with support $\{i_1, \ldots, i_u\}$ such that every entry of $\omega \cdot Y^*$ is positive.

Proof The equivalence of (a) and (b) was noted in Proposition 4.2. As for the equivalence of (b) and (c), note that

$$\omega \cdot Y^* = \left(\sum_{i=1}^{n} \omega_i \, y_{i,1}, \ldots, \sum_{i=1}^{n} \omega_i \, y_{i,d^*} \right) = \left(\sum_{k=1}^{u} \omega_{i_k} \, y_{i_k,1}, \ldots, \sum_{k=1}^{u} \omega_{i_k} \, y_{i_k,d^*} \right).$$

Thus, the j-th entry of $\omega \cdot Y^*$ is positive if and only if $y_{i_\ell,j} > 0$ for some i_ℓ. As the (i, j)-entry of Y^* is the stoichiometric coefficient of X_i in the complex Y_j, we have that $(\omega \cdot Y^*)_j > 0$ if and only if the support of the j-th column of Y^* intersects $\{i_1, \ldots, i_u\}$. The assertion follows. □

As a consequence of Lemma 4.5, one finds that the support of certain stoichiometric first integrals consists of LTC indices.

Proposition 4.6 *Let G be a mass-action reaction network with r connected components, such that each connected component has one terminal strongly connected component. Assume that there exists a linear first integral $\phi = \sum_{i=1}^{n} \alpha_i x_i \neq 0$, with non-negative integer coefficients.*

(a) One has $(\alpha_1, \ldots, \alpha_n) \cdot Y \in \ker A(\kappa)$, and therefore, with the notation of Lemma A.5,

$$(\alpha_1, \ldots, \alpha_n) \cdot Y = \sum_{i=1}^{r} \ell_i \left(0, \ldots, 0, e^{(i)}, 0, \ldots, 0\right), \qquad \ell_i \in \mathbb{N}_0.$$

(b) If $\ell_i \neq 0$ for all $i = 1, \ldots, r$, then the indices $i_1, \ldots, i_u$ in the support $\operatorname{supp}(\phi)$ form an LTC index set whenever $u < n$.

Proof (a) Since the complexes are pairwise different, the monomial entries of x^Y are linearly independent over $\mathbb{R}$. Therefore

$$\phi(Y A(\kappa) x^Y) = 0 \quad \text{for all} \quad x \in \mathbb{R}^n_{\geq 0} \qquad \Leftrightarrow \qquad \phi(Y A(\kappa)) = 0.$$

Now, the statement follows from Lemma A.5(c). (b) It follows directly from Lemma 4.5. □

Example 4.7 For the futile cycle from Examples 3.13 and 4.4, the linear first integral $\phi_1 = x_1 + x_2 + x_5 + x_6$ satisfies

$$(1, 1, 0, 0, 1, 1) \cdot Y = (1, 1, 1, 1, 1, 1) = (1, 1, 1, 0, 0, 0) + (0, 0, 0, 1, 1, 1),$$

where in the second equality the vector is written as in Proposition 4.6 with $\ell_1 = \ell_2 = 1$. Hence, by Proposition 4.6, $\{X_1, X_2, X_5, X_6\}$ is an LTC species set. The linear first integral $\phi_2 = x_1 + x_5$ satisfies

$$(1, 0, 0, 0, 1, 0) \cdot Y = (1, 1, 1, 0, 0, 0) = (1, 1, 1, 0, 0, 0) + 0 \cdot (0, 0, 0, 1, 1, 1),$$

and Proposition 4.6 does not apply. In fact, $\{X_1, X_5\}$ is not an LTC species set. The LTC species set $\{X_1, X_4, X_5, X_6\}$ does not correspond to the support of any linear first integral, but it contains the support of one.

The next example shows that non-negativity of the coefficients of the stoichiometric first integral in Proposition 4.6 cannot be discarded in general.

Example 4.8 Consider the reversible Michaelis–Menten mass-action reaction network (Appendix, Example A.2), with degradation of the intermediate complex (the reaction $X_3 \xrightarrow{\kappa_3} 0$), governed by the ODE system,

$$\begin{aligned}
\dot{x}_1 &= -\kappa_1 x_1 x_2 + \kappa_{-1} x_3 \\
\dot{x}_2 &= -\kappa_1 x_1 x_2 + (\kappa_{-1} + \kappa_2) x_3 - \kappa_{-2} x_2 x_4 \\
\dot{x}_3 &= \kappa_1 x_1 x_2 - (\kappa_{-1} + \kappa_2) x_3 + \kappa_{-2} x_2 x_4 - \kappa_3 x_3 \\
\dot{x}_4 &= \kappa_2 x_3 - \kappa_{-2} x_2 x_4,
\end{aligned}$$

with $\kappa_3 > 0$. As in the system without degradation (Example 4.3), $\{X_2, X_3\}$ is an LTC species set, but the only stoichiometric first integral (up to multiples) is $\phi = x_1 - x_2 + x_4$, due to $(1, -1, 0, 1)\, Y = 0$. The set $\{1, 2, 4\}$ is not an LTC index set. Similar to Example 4 in Lax and Walcher (2020), this example also illustrates that the scaling approach may yield singular perturbation scenarios which are not directly related to TFPVs (even after restriction to SCCs). See Lax and Walcher (2020) for details.

Remark 4.9 There remains the question under which conditions the existence of LTC species sets in turn implies the existence of stoichiometric first integrals with corresponding support. We give a characterization for reaction networks with one connected component and one terminal strongly connected component. Thus, let system (2) represent such a reaction network. Assume without loss of generality that $\{X_1, \ldots, X_u\}$ is an LTC species set, and denote by $\bar{y}_1, \ldots, \bar{y}_u$ the first rows of the complex matrix Y. By Lemma A.5 in Appendix and Proposition 4.6 the system admits a stoichiometric first integral if, and only if, $e = (1, \ldots, 1)$ lies in the closed convex hull of $\bar{y}_1, \ldots, \bar{y}_u$.

(Note that due to Lemma 4.5(c), there exist integers $\omega_1 > 0, \ldots, \omega_u > 0$ such that $\sum_{i=1}^{u} \omega_i \bar{y}_i > 0$ (coordinate-wise).)

4.3 Stoichiometry and TFPVs

We now address TFPVs of system (2) versus TFPVs of its restriction to stoichiometric compatibility classes. As seen in Example 4.1, the restricted system may admit additional TFPVs. We first fix some notation.

We introduce the abbreviation

$$h(x, \kappa) = YA(\kappa)x^Y. \tag{19}$$

In the following, we will assume that system (19) admits a maximal set of independent stoichiometric first integrals $\phi_1, \ldots, \phi_{s^*}$. Then every SCC is the intersection of $\mathbb{R}^n_{\geq 0}$ with the common level set

$$\phi_i(x) = \theta_i, \quad 1 \leq i \leq s^*,$$

which we abbreviate as $S_\theta, \theta = (\theta_1, \ldots, \theta_{s^*})$. One may choose $\widehat{x} \in \mathbb{R}^{n-s^*}$ with entries from $x_1, \ldots, x_n$, such that the Jacobian of $(\widehat{x}, \phi_1(x), \ldots, \phi_{s^*}(x))$ has full rank n. This yields an equivalent version

$$\dot{\widehat{x}} = \widehat{h}(\widehat{x}, \kappa, \theta) \quad \text{in} \quad \mathbb{R}^{n-s^*}, \tag{20}$$

which for given θ represents system (19) on S_θ. We are interested in TFPVs of the $(n - s^*)$-dimensional system (20) for dimension $s > 0$. Candidates for TFPVs are as follows.

- TFPVs via "inheritance" from (19): If $\widehat{\kappa}$ is a TFPV of (19) for dimension $s > s^*$, then $(\widehat{\kappa}, \theta)$ is a TFPV of (20) for dimension $s - s^*$ and some θ, according to Lemma 2.7.
- TFPV candidates from stoichiometric first integrals: Let the setting of Proposition 4.6 be given and assume that the stoichiometric first integral ϕ_ℓ with non-negative coefficients corresponds to an LTC variable set (for all $\kappa \in \mathbb{R}^m_{\geq 0}$). If there exists a TFPV $(\widehat{\kappa}, \widehat{\theta})$ with $\widehat{\theta}_\ell = 0$, then the critical variety will be a coordinate subspace, and consequently by Goeke et al. (2017) the singular perturbation reduction will agree with the "classical" QSS reduction (in the sense of Sect. 2.3) for the LTC variables. (We restrict attention to a single first integral here, since we are interested in minimal LTC sets; cf. Sect. 2.3.)

There remains to establish manageable criteria for TFPVs from stoichiometric first integrals. The next result yields conditions for parameter values that are "almost TFPV"; the notion will be specified in Remark 4.12.

Proposition 4.10 *Let system* (1) *be given, and assume that every SCC of this system is compact (equivalently, the left-kernel of N in* (1) *has a vector with all entries positive* (Ben-Israel 1964)*. Moreover assume that there exists a parameter* $\widehat{\theta} \in \mathbb{R}^{s^*}$ *such that:*

(a) No stationary points in $S_{\widehat{\theta}}$ are isolated relative to $S_{\widehat{\theta}}$.
(b) For every $\rho > 0$, there exists some θ such that $\|\theta - \widehat{\theta}\| < \rho$ and $\dot{\widehat{x}} = \widehat{h}(\widehat{x}, \kappa, \theta)$ admits an isolated linearly attracting stationary point. (Here, $\|\cdot\|$ denotes some norm.)

Then, $\dot{\widehat{x}} = \widehat{h}(\widehat{x}, \kappa, \widehat{\theta})$ admits a non-isolated stationary point whose Jacobian has only eigenvalues with non-positive real part, and admits zero as an eigenvalue.

Proof Let κ be fixed in the following. Given a compact subset K^* of $\mathbb{R}^{s^*}$, the union of the SCCs S_θ with $\theta \in K^*$ is compact. In the following, let K be a compact neighbourhood of $S_{\widehat{\theta}}$.

For every positive integer L let $\theta_L \in K^*$ be such that $\|\theta_L - \widehat{\theta}\| < 1/L$ and $\dot{\widehat{x}} = \widehat{h}(\widehat{x}, \kappa, \theta_L)$ admits an isolated linearly attracting stationary point $\widehat{z}_L$. By compactness, the sequence $(\widehat{z}_L)_L$ in $\mathbb{R}^{n-s^*}$ has an accumulation point $\widehat{z}$, in $S_{\widehat{\theta}}$. Since $\widehat{z}$ is not isolated, the Jacobian of $D_1\widehat{h}(\widehat{z}, \kappa, \widehat{\theta})$ has the eigenvalue zero. Moreover, the map which sends $(\widehat{x}, \kappa, \theta)$ to the coefficients of the characteristic polynomial

$$\widehat{\chi}_{(\widehat{x},\kappa,\theta)}(\tau) = \tau^{n-s^*} + \widehat{\sigma}_1(\widehat{x}, \kappa, \theta)\tau^{n-s^*-1} + \cdots + \widehat{\sigma}_{n-s^*}(\widehat{x}, \kappa, \theta) \tag{21}$$

of $D_1\widehat{h}(\widehat{x}, \kappa, \theta)$ is continuous. Thus, if some eigenvalue of the Jacobian had positive real part, the same would hold for some eigenvalue of the Jacobian of $D_1\widehat{h}(\widehat{z}_L, \kappa, \theta_L)$ with L sufficiently large (see, e.g. the reasoning in Gantmacher (2005, Ch. V, section 3). □

Corollary 4.11 *Assume that system (20) describes the dynamics of a weakly reversible deficiency zero reaction network. Let $\widehat{\theta}$ be such that no stationary points are isolated in $S_{\widehat{\theta}}$. Then, $\dot{\widehat{x}} = \widehat{h}(\widehat{x}, \kappa, \widehat{\theta})$ admits a stationary point whose Jacobian has only eigenvalues with non-positive real part, and admits zero as an eigenvalue.*

Remark 4.12 We now clarify what is meant by "almost TFPV" prior to the statement of Proposition 4.10. For this, we discuss the conditions for TFPV in Definition 2.6 in Sect. 2.2 for system (20) and parameter value $\widehat{\theta}$.

- Condition (i) is always satisfied for some dimension > 0, due to condition (a) in Proposition 4.10.
- Condition (ii) requires equality of geometric and algebraic multiplicity for the eigenvalue 0. This holds automatically when the algebraic multiplicity is equal to one. Generally, this property can be checked by algebraic methods: For $\widehat{x}$ in the critical manifold, τ divides the characteristic polynomial in (21). Obtain a new polynomial η from $\widehat{\chi}_{(\widehat{x},\kappa,\theta)}$ by dividing out a power of τ such that a single factor τ remains. Then the multiplicity equals one if and only if η annihilates $D_1\widehat{h}(\widehat{x}, \kappa, \widehat{\theta})$. But [as mentioned, e.g. in Goeke and Walcher 2014, Example 4)] there exist realistic reaction networks for which the direct sum condition on the kernel and the image does not hold.
- Finally, to guarantee condition (iii), one needs to verify that there exist no purely imaginary eigenvalues except 0. However, if (iii) is not satisfied, then the system may admit some interesting dynamics, like zero-Hopf bifurcations.

We note a sharper result for the case of a one-dimensional critical variety.

Corollary 4.13 *In the setting of Proposition 4.10, consider system (20), with characteristic polynomial of the Jacobian given by (21). Let $\widehat{\theta}$ be such that $\widehat{\sigma}_{n-s^*} = 0$ and $\widehat{\sigma}_{n-s^*-1} \neq 0$ for $\theta = \widehat{\theta}$, some $\kappa \in \mathbb{R}^m_{>0}$ and some stationary point $\widehat{z} \in \mathbb{R}^{n-s^*}{}_{\geq 0}$. Then it holds:*

(a) The eigenvalue 0 of $D_1\widehat{h}(\widehat{z}, \kappa, \widehat{\theta})$ has multiplicity one.
(b) There exists a polynomial Φ in $n - s^* - 1$ variables with the following property: The Jacobian $D_1\widehat{h}(\widehat{z}, \kappa, \widehat{\theta})$ admits nonzero purely imaginary eigenvalues if and only if $\Phi(\widehat{\sigma}_1, \ldots, \widehat{\sigma}_{n-s^*-1}) = 0$ at $(\widehat{z}, \kappa, \widehat{\theta})$.

Proof (a) is obvious. (b) There exists a polynomial Φ in the coefficients of the characteristic polynomial that vanishes if and only if a pair of (nonzero) eigenvalues adds up to zero; see, e.g. Kruff and Walcher (2020, Lemma 4.1, Appendix B). Since all eigenvalues have real part ≤ 0, such a pair of eigenvalues must have zero real parts. □

The non-trivial restrictions on the $\widehat{\sigma}_i$ in Corollary 4.13(b) suggest that there will be nonzero purely imaginary eigenvalues only in exceptional cases. There is an obvious (but less readily applicable) extension of Corollary 4.13 to TFPVs for dimension strictly larger than one, with an additional requirement that the geometric and the algebraic multiplicities of the zero eigenvalue are equal in Corollary 4.13(a), and that Corollary 4.13(b) is left unchanged except for the number of variables of Φ.

The polynomial Φ can be determined explicitly. We recall some cases for SCCs of small generic dimension from Kruff and Walcher (2020, Example 1).

Remark 4.14 (a) If the SCCs of system (2) generically have dimension two, and the hypotheses of Proposition 4.10 are satisfied, then $\widehat{\theta}$ is a TFPV for dimension one whenever $\widehat{\sigma}_1 \neq 0$ at $(\widehat{z}, \kappa, \widehat{\theta})$,

(b) If the SCCs of system (2) generically have dimension three, and the hypotheses of Proposition 4.10 are satisfied, then $\widehat{\theta}$ is a TFPV for dimension one whenever $\widehat{\sigma}_1 \neq 0$ and $\widehat{\sigma}_2 \neq 0$ at $(\widehat{z}, \kappa, \widehat{\theta})$.

(c) If the SCCs of system (2) generically have dimension four, and the hypotheses of Proposition 4.10 are satisfied, then $\widehat{\theta}$ is a TFPV for dimension one whenever $\widehat{\sigma}_3 \neq 0$ and $\widehat{\sigma}_1\widehat{\sigma}_2 \neq \widehat{\sigma}_3$ at $(\widehat{z}, \kappa, \widehat{\theta})$.

Example 4.15 Corollary 4.11 is applicable to the reversible competitive inhibition reaction network introduced in Example 2.5, with the critical parameter value $\widehat{\theta}$ having $e_0 = 0$, and all other parameters being positive. The dynamics on an SCC is described by the ODE system (6), and the Jacobian on the SCC with $e_0 = 0$ (thus, on the critical manifold with $x_3 = x_6 = 0$) is equal to:

$$\begin{pmatrix} 0 & \kappa_1 x_1 + \kappa_{-1} & \kappa_1 x_1 \\ 0 & -(\kappa_1 x_1 + \kappa_{-1} + \kappa_2 + \kappa_{-2}(s_0 - x_1)) - \kappa_3 f_0 & -(\kappa_1 x_1 + \kappa_{-2}(s_0 - x_1)) + \kappa_{-3} \\ 0 & \kappa_3 f_0 & -\kappa_{-3} \end{pmatrix}.$$

The coefficients of its characteristic polynomial are

$$\begin{aligned} \widehat{\sigma}_1 &= f_0\kappa_3 + \kappa_{-3} + \kappa_2 + \kappa_{-1} + \kappa_1 x_1 + \kappa_{-2}(s_0 - x_1), \\ \widehat{\sigma}_2 &= \kappa_3 f_0(\kappa_1 x_1 + \kappa_{-2}(s_0 - x_1)) + \kappa_{-3}(\kappa_1 x_1 + \kappa_{-1} + \kappa_2 + \kappa_{-2}(s_0 - x_1)), \\ \widehat{\sigma}_3 &= 0. \end{aligned}$$

Since both $\widehat{\sigma}_1$ and $\widehat{\sigma}_2$ are positive when $0 \leq x_1 \leq s_0$, the conditions in Remark 4.14 and Corollary 4.11 are satisfied, and $\widehat{\theta}$ is a TFPV for dimension one. One may compare this to the discussion of the related competitive inhibition network in Goeke et al. (2012), by direct computation with no reference to reaction network theory. Thus one sees that the approach developed here saves substantial computational effort. Moreover, one verifies that Proposition 4.10 is also applicable to the system with irreversible product formation (that is, $\kappa_{-2} = 0$).

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A Appendix

Here, we first collect some further properties of TFPVs, as well as their use and characterization, and then recall some properties of Laplacian matrices. These two subsections are included (mostly) for the reader's convenience. In a final subsection, we outline a heuristic that combines switching off reactions and removing species, to obtain further singular perturbation reductions. No systematic discussion is intended, but we give relevant examples where this heuristic works.

A.1 Tikhonov–Fenichel Parameter Values: Details

We collect here some facts about TFPV and the reduction procedure, and introduce further notation.

A.1.1 A Proof of Lemma 2.7

Proof One needs to verify that the intersection of $\widetilde{Z}$ with the SCC has dimension $s - s^*$; the remaining properties are clear. Given the fixed TFPV, after a linear coordinate change the system has the form $\dot{w} = f(w)$, in detail

$$\begin{aligned}\dot{w}_1 &= 0\\ &\vdots\\ \dot{w}_{s^*} &= 0\\ \dot{w}_{s^*+1} &= f_{s^*+1}(w)\\ &\vdots\\ \dot{w}_n &= f_n(w).\end{aligned}$$

At a point w_0 in the SCC one has $f(w_0) = 0$ and

$$Df(w_0) = \begin{pmatrix} 0 & 0 \\ A & B \end{pmatrix}$$

with an $(n - s^*) \times (n - s^*)$-matrix B. Now $Df(w_0)$ has rank $n - s$, and so has B by condition (ii)'. With the implicit function theorem one gets (up to renumbering) a local parameterization of the zero set of f in the form

$$\begin{pmatrix} w_{s+1} \\ \vdots \\ w_n \end{pmatrix} = q(w_1, \ldots, w_s).$$

On the SCC the entries $w_1, \ldots, w_{s^*}$ are fixed, hence there remains an $s - s^*$-dimensional manifold. □

A.1.2 TFPV and Reductions

We have by Goeke (2013) and Goeke et al. (2015):

Proposition A.1 *Given a parameter $\pi \in \Pi$ and any smooth curve $\varepsilon \mapsto \varphi(\varepsilon)$ in the parameter space Π with $\varphi(0) = \widehat{\pi}$, in case $s^* = 0$ the system*

$$\dot{x} = h(x, \varphi(\epsilon)) = h(x, \widehat{\pi}) + \varepsilon D_2 h(x, \widehat{\pi})\varphi'(0) + \cdots = h^{(0)}(x) + \varepsilon h^{(1)}(x) + \cdots \quad (22)$$

with $\varepsilon \geq 0$, admits a singular perturbation reduction in the sense of Tikhonov and Fenichel if and only if $\widehat{\pi}$ is a TFPV.

Thus one may think of a TFPV as a ("degenerate") parameter from which singularly perturbed systems emanate.

A.1.3 A Detailed Example

We further illustrate the procedure outlined in Remark 2.8 with a detailed example that seems to be unavailable in the literature. The TFPV property can be verified by computation but is also an immediate consequence of Theorem 3.10 .

Example A.2 Consider the reversible Michaelis–Menten reaction network (Keener and Sneyd 2009),

$$X_1 + X_2 \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} X_3 \underset{\kappa_{-2}}{\overset{\kappa_2}{\rightleftharpoons}} X_4 + X_2, \tag{23}$$

where X_1 chemically is a substrate, X_2 an enzyme, X_3 an intermediate complex, and X_4 a product, formed by conversion of the substrate X_1. Using (1), we obtain the ODE system

$$\begin{aligned}
\dot{x}_1 &= -\kappa_1 x_1 x_2 + \kappa_{-1} x_3,\\
\dot{x}_2 &= -\kappa_1 x_1 x_2 + (\kappa_{-1} + \kappa_2) x_3 - \kappa_{-2} x_2 x_4,\\
\dot{x}_3 &= \kappa_1 x_1 x_2 - (\kappa_{-1} + \kappa_2) x_3 + \kappa_{-2} x_2 x_4,\\
\dot{x}_4 &= \kappa_2 x_3 - \kappa_{-2} x_2 x_4.
\end{aligned}$$

With stoichiometric first integrals and the typical initial values $x_1(0) = s_0,\ x_2(0) = e_0$ and $x_3(0) = x_4(0) = 0$ one has the two-dimensional system

$$\begin{aligned}
\dot{x}_1 &= -\kappa_1 e_0 x_1 + (\kappa_1 x_1 + \kappa_{-1}) x_3,\\
\dot{x}_3 &= \kappa_1 e_0 x_1 - (\kappa_1 x_1 + \kappa_{-1} + \kappa_2) x_3 + \kappa_{-2}(e_0 - x_3)(s_0 - x_1 - x_3).
\end{aligned}$$

This system admits a TFPV with $\kappa_2 = \kappa_{-2} = 0$ and all other parameters positive. With $\kappa_2 = \varepsilon\kappa_2^*,\ \kappa_{-2} = \varepsilon\kappa_{-2}^*$ one gets

$$h^{(0)}(x) = \begin{pmatrix} -\kappa_1 e_0 x_1 + (\kappa_1 x_1 + \kappa_{-1}) x_3 \\ \kappa_1 e_0 x_1 - (\kappa_1 x_1 + \kappa_{-1}) x_3 \end{pmatrix} = \begin{pmatrix} -1 \\ 1 \end{pmatrix} \cdot (\kappa_1 e_0 x_1 - (\kappa_1 x_1 + \kappa_{-1}) x_3),$$

the decomposition being as in Remark 2.8. So, the critical manifold $\widetilde{Z}$ is given by $\mu = 0$, explicitly $x_3 = \frac{\kappa_1 e_0 x_1}{\kappa_1 x_1 + \kappa_{-1}}$, and with $D_1\mu(x) = (\kappa_1(e_0 - x_3),\ -(\kappa_1 x_1 + \kappa_{-1}))$ one finds via straightforward computations:

$$Q(x) = \frac{1}{\kappa_1(e_0 - x_3) + \kappa_1 x_1 + \kappa_{-1}} \begin{pmatrix} \kappa_1 x_1 + \kappa_{-1} & \kappa_1 x_1 + \kappa_{-1} \\ \kappa_1(e_0 - x_3) & \kappa_1(e_0 - x_3) \end{pmatrix}.$$

With

$$h^{(1)}(x) = \begin{pmatrix} 0 \\ -\kappa_2 x_3 + \kappa_{-2}(e_0 - x_3)(s_0 - x_1 - x_3) \end{pmatrix}$$

one obtains a version of the reduced equation on $\widetilde{Z}$. Substituting x_3 via the defining equation yields a version for x_1 alone, viz.

$$\dot{x}_1 = \frac{(-\kappa_2\kappa_1 e_0 x_1 + \kappa_{-2}\kappa_{-1} e_0) \cdot (\kappa_1 x_1 + \kappa_{-1}) - \kappa_{-2}\kappa_{-1} e_0 \cdot \kappa_1 e_0 x_1}{\kappa_1\kappa_{-1} e_0 + (\kappa_1 x_1 + \kappa_{-1})^2}.$$

A.1.4 A Computational Approach to TFPVs

The following is taken from Goeke (2013) and Goeke et al. (2015).

Lemma A.3 *Consider the characteristic polynomial*

$$\chi(\tau, x, \pi) = \tau^n + \sigma_1(x, \pi)\tau^{n-1} + \cdots + \sigma_{n-1}(x, \pi)\tau + \sigma_n(x, \pi)$$

of $D_1 h(x, \pi)$. Then, given $s^* < s < n$, a parameter value $\widehat{\pi}$ is a TFPV with locally exponentially attracting critical manifold $\widetilde{Z}$ (depending on $\widehat{\pi}$) of dimension s, if and only if the following conditions hold for some $x_0 \in \widetilde{Z}$:

(iv) $h(x_0, \widehat{\pi}) = 0$.
(v) The characteristic polynomial $\chi(\tau, x, \pi)$ satisfies
  (1) $\sigma_n(x_0, \widehat{\pi}) = \cdots = \sigma_{n-s+1}(x_0, \widehat{\pi}) = 0$;
  (2) all roots of $\chi(\tau, x_0, \widehat{\pi})/\tau^s$ have negative real part.
(vi) The system $\dot{x} = h(x, \widehat{\pi})$ admits s independent local analytic first integrals at x_0.

Therefore, a starting point for computing TFPVs is as follows: With $h(x_0, \widehat{\pi}) = 0$ and $\sigma_n(x_0, \widehat{\pi}) = \cdots = \sigma_{n-s+1}(x_0, \widehat{\pi}) = 0$, one sees that $(x_0, \widehat{\pi})$ is a solution to $n + s > n$ equations for $x \in \mathbb{R}^n$, given $\widehat{\pi}$. In turn, this allows us to obtain conditions on $\widehat{\pi}$ for general polynomial systems via elimination theory.

The validity of the hypotheses for Tikhonov's and Fenichel's theorems depend on the ambient space, and thus may change when passing to an invariant subspace. As a consequence, the notion of TFPV may also depend on the ambient space. For reaction networks this observation is relevant when passing to SCCs.

For further information on the reduction procedure and its properties, and further examples, we refer to Goeke and Walcher (2014) and Goeke et al. (2017). The latter reference contains an explanation of the simple form of the reduced equation in Example 2.9. Reference Feliu et al. (2020) provides a version of the singular perturbation reduction when a parameterization of the critical manifold is known.

A.2 Some Properties of Laplacians

In this subsection we recall and review some properties of Laplacian and compartmental matrices. For the following known facts refer, e.g. to Jacquez and Simon (1993, Subsection 4.1), Anderson (2013, Thm. 12.1), as well as Berman and Plemmons (1994, Ch. 6) (noting that compartmental matrices are negative M-matrices).

The Laplacian matrix of a directed graph (and thus the Laplacian $A(\kappa)$ of a reaction network) is a compartmental matrix. We recall some notions.

- A square matrix with real entries is called a compartmental matrix if all its off-diagonal entries are ≥ 0 and all its column sums are ≤ 0.

- Given non-negative real numbers σ_{ij}, $1 \le i,\ j \le n$, and τ_k, $1 \le k \le n$, the matrix

$$L(\sigma,\tau) := \begin{pmatrix} -\sum_\ell \sigma_{\ell 1} - \tau_1 & \sigma_{12} & \cdots & \sigma_{1d} \\ \sigma_{21} & & \ddots & \vdots \\ \vdots & \ddots & \ddots & \sigma_{d-1,d} \\ \sigma_{d1} & \cdots & \sigma_{d,d-1} & -\sum_\ell \sigma_{\ell d} - \tau_d \end{pmatrix} \in \mathbb{R}^{d\times d}, \quad (24)$$

  with $\sigma = (\sigma_{ij})$ and $\tau = (\tau_k)$, is compartmental. In turn, every compartmental $d \times d$ matrix has a representation of the form (24), with uniquely determined σ_{ij} and τ_k.
- The Laplacian $A(\kappa)$ of a reaction network satisfies $\sigma = \kappa$ and $\tau = 0$. Hence, $A(\kappa) = L(\kappa, 0)$ and column sums are zero.

Lemma A.4 *Let $L(\sigma,\tau)$ be a compartmental matrix as in (24). Then, all eigenvalues of $L(\sigma,\tau)$ have non-positive real part, and any eigenvalue with real part zero is equal to zero. Moreover $\mathbb{R}^n$ is the direct sum of the kernel and the image of $L(\sigma,\tau)$.*

Proof We give a direct argument for the second assertion, which is not readily found in the cited references: Due to the structure of the matrix, the simplex defined by $x_1 \ge 0, \ldots, x_n \ge 0$ and $x_1 + \cdots + x_n \le 1$ is positively invariant for $\dot{x} = L(\sigma,\tau)\,x$. In particular all solutions starting in the simplex are bounded for positive times. Therefore the eigenvalue 0 cannot admit a non-trivial Jordan block, which would yield unbounded solutions. □

Consider a mass-action reaction network G. Let $G_1, \ldots, G_r$ be the connected components of G and further order the set of complexes according to the connected component they belong to. If $A_i(\kappa)$ stands for the Laplacian matrix of G_i, then $A(\kappa)$ becomes a block diagonal matrix with r blocks,

$$A(\kappa) = \begin{pmatrix} A_1(\kappa) & 0 & \ldots & 0 \\ 0 & A_2(\kappa) & \ldots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \ldots & A_r(\kappa) \end{pmatrix} \in \mathbb{R}^{d\times d}.$$

The form of the kernel of the Laplacian matrix $A(\kappa)$ of a digraph with $\kappa \in \mathbb{R}^m_{>0}$ is well known, in particular, in the context of reaction networks (Feinberg 2019, Thm 16.4.2). It derives from the Matrix-Tree theorem (Tutte 1948; Mirzaev and Gunawardena 2013; Chaiken and Kleitman 1978).

- The dimension of the kernel of $A(\kappa)$ agrees with the number of terminal strongly connected components and is independent of $\kappa \in \mathbb{R}^m_{>0}$.
- If the digraph is strongly connected, then $\dim\ker A(\kappa) = 1$ and a generator of $\ker A(\kappa)$ is given by the sequence of signed principal minors (which are positive).
- If the digraph is not strongly connected, then any complex in the support of a vector in $\ker A(\kappa)$ belongs to a terminal strongly connected component. Furthermore, a basis of $\ker A(\kappa)$ can be chosen such that the support of each vector is exactly

one terminal strongly connected component and the nonzero entries are positive. These entries arise as the signed principal minors of the restriction of the matrix to the nodes in the component.

- The vector $e = (1, \ldots, 1)$ belongs to the left-kernel of $A(\kappa)$, and generates it when the digraph has one terminal strongly connected class.

These facts lead to the following lemma.

Lemma A.5 *Let G be a mass-action reaction network with labelling $\kappa \in \mathbb{R}^m_{>0}$. Let $G_1, \ldots, G_r$ be the connected components of G and assume that the set of complexes is ordered in accordance with the components. Let T be the number of terminal strongly connected components of G.*

Then, the rank of $A(\kappa)$ does not depend on the choice of $\kappa \in \mathbb{R}^m_{>0}$, and in particular

(a) $\dim \ker A(\kappa) = T$.

(b) $\ker A(\kappa)$ *has non-trivial intersection with the positive orthant $\mathbb{R}^d_{>0}$, if and only if G is weakly reversible.*

(c) The left-kernel of $A(\kappa)$ contains the following row vectors, one for each connected component:

$$\left(e^{(1)}, 0, \ldots, 0\right), \ldots, \left(0, \ldots, 0, e^{(r)}\right), \quad \text{with} \quad e^{(i)} = (1, \ldots, 1)$$

of size the number of nodes of G_i. If each connected component of G has exactly one terminal strongly connected component, then these vectors span the left-kernel of $A(\kappa)$.

Proof (a-b) are direct consequences of the properties of the kernel of $A(\kappa)$ discussed above. (c) The column sums in each block $A_i(\kappa)$ are zero, as each submatrix is a Laplacian. The second part follows from (a), as $T = r$. □

With the notation in Lemma A.5, if a connected component of G has more than one terminal strongly connected component, then the vectors given in Lemma A.5(c) do not form a basis of the left-kernel of $A(\kappa)$. To obtain a basis, one has to augment them by vectors that might depend on the particular entries of $A(\kappa)$, that is, on κ; see Example 2.4 for an illustration.

A.3 Partial Scalings: An Outlook

In the main part of this paper, we considered on the one hand TFPVs that, in reaction network interpretation, arise from "switching off" certain reactions (Theorems 3.9 and 3.10). On the other hand, we introduced LTC species sets for all parameters, with the characterizing property that if their concentrations are zero, then all reactions of the reaction network are precluded from taking place, and discussed their relation to TFPVs (Proposition 4.10 and Corollary 4.13). But beyond the proven results, one may also use the underlying strategies as heuristics to obtain particular singular perturbation reductions. It is suggestive to go further and combine these approaches by switching off certain reactions and determining LTC species for the reactant complexes. This combination will be sketched here. In the setting of Sect. 2.3 and in particular expansion

(10), we consider LTC variable sets for a specific choice of the parameter $\pi^* = \widetilde{\kappa}$ in (10), for reaction networks. This yields a slow–fast system which may further admit a Tikhonov–Fenichel reduction. We will not attempt to establish necessary or sufficient conditions for a singular perturbation setting.

We start again from (17), but now we consider some $\widetilde{\kappa}$ such that $A(\widetilde{\kappa})$ has zero columns, thus there are additional non-reactant complexes in the reaction network $G(\widetilde{\kappa})$. We may assume that the remaining reactant complexes correspond to columns $y_1, \ldots, y_{\widetilde{d}}$ of Y^*, thus

$$A^*(\widetilde{\kappa}) = \begin{pmatrix} * & \cdots & * & 0 & \cdots & 0 \\ \vdots & & \vdots & \vdots & & \vdots \\ * & \cdots & * & 0 & \cdots & 0 \end{pmatrix}.$$

Matrices of this type define some coordinate subspace of parameter space. We denote by Y_1 the matrix with columns $y_1, \ldots, y_{\widetilde{d}}$, and by Y_2, the matrix with the remaining columns of Y^*. LTC variable sets for $G(\widetilde{\kappa})$ can be identified via Proposition 4.2 with the complex matrix Y_1.

Upon relabelling, we may assume that $x_1, \ldots, x_u$ form an LTC variable set for $G(\widetilde{\kappa})$. Considering a curve $\varepsilon \mapsto \widetilde{\kappa} + \varepsilon\rho + \ldots$ in parameter space, we obtain

$$A^*(\widetilde{\kappa} + \varepsilon\rho) = \begin{pmatrix} A_{11} + \varepsilon \cdots \varepsilon A_{12}^* + \varepsilon^2 \cdots \\ A_{21} + \varepsilon \cdots \varepsilon A_{22}^* + \varepsilon^2 \cdots \end{pmatrix}$$

with $A_{11} \in \mathbb{R}^{u \times \widetilde{d}}$. Moreover, set $x_i = \varepsilon x_i^*$ for $1 \leq i \leq u$, then we have

$$x^{y_j} = \varepsilon^{y_{1j} + \cdots + y_{uj}} {x_1^*}^{y_{1j}} \cdots {x_u^*}^{y_{uj}} \cdot x_{u+1}^{y_{u+1,j}} \cdots x_n^{y_{nj}},$$

noting that the exponent of ε is positive for all $j \leq \widetilde{d}$. Abbreviating $w_1(x) = x^{Y_1}$, and $w_2(x) = x^{Y_2}$, one obtains an expansion

$$\begin{aligned} w_1(\varepsilon x_1^*, \ldots, \varepsilon x_u^*, x_{u+1}, \ldots, x_n) &= \varepsilon w_1^*(x_1^*, \ldots, x_u^*, x_{u+1}, \ldots, x_n) + \varepsilon^2 \cdots, \\ w_2(\varepsilon x_1^*, \ldots, \varepsilon x_u^*, x_{u+1}, \ldots, x_n) &= w_2^*(x_1^*, \ldots, x_u^*, x_{u+1}, \ldots, x_n) + \varepsilon \cdots, \end{aligned}$$

and altogether we arrive at the slow–fast system,

$$\frac{d}{dt} \begin{pmatrix} x_1^* \\ \vdots \\ x_u^* \\ x_{u+1} \\ \vdots \\ x_n \end{pmatrix} = \begin{pmatrix} A_{11} & A_{12}^* \\ \varepsilon A_{21} & \varepsilon A_{22}^* \end{pmatrix} \cdot \begin{pmatrix} w_1^*(x_1^*, \ldots, x_u^*, x_{u+1}, \ldots, x_n) \\ w_2^*(x_1^*, \ldots, x_u^*, x_{u+1}, \ldots, x_n) \end{pmatrix} + \begin{pmatrix} \varepsilon \cdots \\ \varepsilon^2 \cdots \end{pmatrix}.$$

One would arrive at the same slow–fast system by starting from a different vantage point: First designate LTC variables and then switch off all reactions whose source complexes do not contain these variables.

Since the fast part of the scaled system involves slow reactions corresponding to A_{12}^*, the results from the previous subsections do not carry over to partial scalings. We will not discuss these matters any further here.

For given reaction networks, the above considerations on partial scalings, while not providing comprehensive results, may be used to identify candidates for singular perturbation reductions, as shown by the following examples.

Example A.6 We continue the Michaelis–Menten reaction network from Example A.2, rewriting (23) in the form (2) with

$$Y = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}, \quad A(\kappa) = \begin{pmatrix} -\kappa_1 & \kappa_{-1} & 0 \\ \kappa_1 & -(\kappa_{-1}+\kappa_2) & \kappa_{-2} \\ 0 & \kappa_2 & -\kappa_{-2} \end{pmatrix}, \quad x^Y = \begin{pmatrix} x_1x_2 \\ x_3 \\ x_2x_4 \end{pmatrix}.$$

If $\widetilde{\kappa}$ is such that $\{x_3\}$ is an LTC variable set for $G(\widetilde{\kappa})$, then we need $\kappa_1 = \kappa_{-2} = 0$ at $\varepsilon = 0$ (slow formation of the intermediate complex from both sides). Then for the curve in parameter space $\widetilde{\kappa} + \epsilon\kappa^* = (\epsilon\kappa_1^*, \kappa_{-1}, \kappa_2, \epsilon\kappa_{-1}^*)$,

$$A(\kappa) = \begin{pmatrix} 0 & \kappa_{-1} & 0 \\ 0 & -(\kappa_{-1}+\kappa_2) & 0 \\ 0 & \kappa_2 & 0 \end{pmatrix} + \varepsilon \begin{pmatrix} -\kappa_1^* & 0 & 0 \\ \kappa_1^* & 0 & \kappa_{-2}^* \\ 0 & 0 & -\kappa_{-2}^* \end{pmatrix},$$

and scaling $x_3 = \varepsilon x_3^*$ yields

$$\begin{aligned} \dot{x}_1 &= \varepsilon(-\kappa_1^* x_1 x_2 + \kappa_{-1} x_3^*), \\ \dot{x}_2 &= \varepsilon(-\kappa_1^* x_1 x_2 + \kappa_{-1} c x_3^* + \kappa_2 x_3^* - \kappa_{-2^*} x_2 x_4), \\ \dot{x}_3^* &= \kappa_1^* x_1 x_2 - \kappa_{-1} x_3^* - \kappa_2 x_3^* + \kappa_{-2}^* x_2 x_4, \\ \dot{x}_4 &= \varepsilon(\kappa_2 x_3^* - \kappa_{-2}^* x_2 x_4). \end{aligned}$$

Here, Tikhonov's theorem is directly applicable, with the reduced system admitting the first integrals $\phi_1 = x_2$ and $\phi_2 = x_1 + x_4$. Thus, we end up with a one-dimensional equation (see Goeke et al. 2015).

Designating the LTC variable set $\{x_2\}$ forces $\kappa_{-1} = \kappa_2 = 0$ at $\varepsilon = 0$ (slow degradation of the intermediate complex in both directions). Proceeding as before, one has

$$A(\kappa) = \begin{pmatrix} -\kappa_1 & 0 & 0 \\ \kappa_1 & 0 & \kappa_{-2} \\ 0 & 0 & -\kappa_{-2} \end{pmatrix} + \varepsilon \begin{pmatrix} 0 & \kappa_{-1}^* & 0 \\ 0 & -(\kappa_{-1}^* + \kappa_2^*) & 0 \\ 0 & \kappa_2^* & 0 \end{pmatrix}.$$

Scaling $x_2 = \varepsilon x_2^*$, one obtains

$$\begin{aligned}
\dot{x}_1 &= \varepsilon(-\kappa_1 x_2^* x_1 + \kappa_{-1}^* x_3),\\
\dot{x}_2^* &= -\kappa_1 x_2^* x_1 + \kappa_{-1}^* c x_3 + \kappa_2^* x_3 - \kappa_{-2} x_2^* x_4,\\
\dot{x}_3 &= \varepsilon(\kappa_1 x_2^* x_1 - \kappa_{-1}^* x_3 - \kappa_2^* x_3 + \kappa_{-2} x_2^* x_4),\\
\dot{x}_4 &= \varepsilon(\kappa_2^* x_3 - \kappa_{-2} x_2^* x_4),
\end{aligned}$$

for which, again, Tikhonov's theorem is directly applicable. The reduced system admits the first integrals $\phi_1 = x_3$ and $\phi_2 = x_1 + x_4$. Thus, again one arrives at a reduction to dimension one; see Goeke et al. (2015) for details.

Hence, as noted earlier, for reversible Michaelis–Menten, by the approaches in the present paper we have obtained all TFPVs that were determined algorithmically in Goeke et al. (2015) for this system.

References

Anderson, D.H.: Compartmental Modeling and Tracer Kinetics. Lecture Notes in Biomathematics, Springer, New York (2013)

Banaji, M., Craciun, G.: Graph-theoretic approaches to injectivity and multiple equilibria in systems of interacting elements. Commun. Math. Sci. **7**(4), 867–900 (2009)

Banaji, M., Craciun, G.: Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems. Adv. Appl. Math. **44**, 168–184 (2010)

Ben-Israel, A.: Notes on linear inequalities, 1: the intersection of the nonnegative orthant with complementary orthogonal subspaces. J. Math. Anal. Appl. **9**, 303–314 (1964)

Berman, A., Plemmons, R.J.: Nonnegative matrices in the mathematical sciences, volume 9 of Classics in Applied Mathematics. Society for Industrial and Applied Mathematics (SIAM), Philadelphia. [Revised reprint of the 1979 original (1994)]

Boros, B., Craciun, G., Yu, P.Y.: Weakly reversible mass-action systems with infinitely many positive steady states. SIAM J. Appl. Math. **80**(4), 1936–1946 (2020)

Chaiken, S., Kleitman, D.: Matrix tree theorems. J. Combin. Theory Ser. A **24**, 377–381 (1978)

Conradi, C., Feliu, E., Mincheva, M., Wiuf, C.: Identifying parameter regions for multistationarity. PLoS Comput. Biol. **13**(10), e1005751 (2017)

Cox, D., Little, J., O'Shea, D.: Ideals, varieties, and algorithms. An Introduction to Computational Algebraic Geometry and Commutative Algebra. Undergraduate Texts in Mathematics, 3rf edn. Springer, New York (2007)

Craciun, G., Feinberg, M.: Multiple equilibria in complex chemical reaction networks I. The injectivity property. SIAM J. Appl. Math. **65**(5), 1526–1546 (2005)

Craciun, G., Dickenstein, A., Shiu, A., Sturmfels, B.: Toric dynamical systems. J. Symbolic Comput. **44**(11), 1551–1565 (2009)

Dickenstein, A., PérezMillán, M.: How far is complex balancing from detailed balancing? Bull. Math. Biol. **73**(4), 811–828 (2011)

Érdi, P., Tóth, J.: Mathematical Models of Chemical Reactions. Theory and Applications of Deterministic and Stochastic Models. Princeton University Press, Princeton (1989)

Feinberg, M.: Complex balancing in general kinetic systems. Arch. Rational Mech. Anal. **49**, 187–194 (1972)

Feinberg, M.: Foundations of Chemical Reaction Network Theory. Applied Mathematical Sciences, vol. 202. Springer (2019)

Feinberg, M., Horn, F.J.M.: Chemical mechanism structure and the coincidence of the stoichiometric and kinetic subspaces. Arch. Rational. Mech. Anal. **66**(1), 83–97 (1977)

Feliu, E., Wiuf, C.: Preclusion of switch behavior in reaction networks with mass-action kinetics. Appl. Math. Comput. **219**, 1449–1467 (2012a)

Feliu, E., Wiuf, C.: Variable elimination in chemical reaction networks with mass-action kinetics. SIAM J. Appl. Math. **72**, 959–981 (2012b)
Feliu, E., Cappelletti, D., Wiuf, C.: Node balanced steady states: unifying and generalizing complex and detailed balanced steady states. Math. Biosci. **301**, 68–82 (2018)
Feliu, E., Kruff, N., Walcher, S.: Tikhonov–Fenichel reduction for parameterized critical manifolds with applications to chemical reaction networks. J. Nonlinear Sci. **30**(4), 1355–1380 (2020)
Feliu, E., Rendall, A.D., Wiuf, C.: A proof of unlimited multistability for phosphorylation cycles. Nonlinearity **33**(11), 5629–5658 (2020)
Feng, S., Sáez, M., Wiuf, C., Feliu, E., Soyer, O.S.: Core signalling motif displaying multistability through multi-state enzymes. J. R. S. Interface **13**(123), 20160524 (2016)
Fenichel, N.: Geometric singular perturbation theory for ordinary differential equations. J. Differ. Equ. **31**(1), 53–98 (1979)
Gantmacher, F.R.: Applications of the Theory of Matrices. Dover, Mineola (2005)
Goeke, A.: Reduktion und asymptotische Reduktion von Reaktionsgleichungen. Doctoral dissertation, RWTH Aachen (2013)
Goeke, A., Walcher, S.: A constructive approach to quasi-steady state reduction. J. Math. Chem. **52**, 2596–2626 (2014)
Goeke, A., Schilli, C., Walcher, S., Zerz, E.: Computing quasi-steady state reductions. J. Math. Chem. **50**, 1495–1513 (2012)
Goeke, A., Walcher, S., Zerz, E.: Determining "small parameters" for quasi-steady state. J. Differ. Equ. **259**, 1149–1180 (2015)
Goeke, A., Walcher, S., Zerz, E.: Classical quasi-steady state reduction—a mathematical characterization. Physica D **345**, 11–26 (2017)
Heineken, F.G., Tsuchiya, H.M., Aris, R.: On the mathematical status of the pseudo-steady state hypothesis of biochemical kinetics. Math. Biosci. **1**, 95–113 (1967)
Horn, F.J.M., Jackson, R.: General mass action kinetics. Arch. Rational Mech. Anal. **47**, 81–116 (1972)
Jacquez, J.A., Simon, C.P.: Qualitative theory of compartmental systems. SIAM Rev. **35**(1), 43–79 (1993)
Joshi, B., Shiu, A.: A survey of methods for deciding whether a reaction network is multistationary. Math. Model. Nat. Phenom. **10**(5), 47–67 (2015)
Keener, J., Sneyd, J.: Mathematical Physiology I: Cellular Physiology, 2nd edn. Springer, New York (2009)
Kruff, N., Walcher, S.: Coordinate-independent criteria for Hopf bifurcations. Discrete Contin. Dyn. Syst. Ser. S **13**(4), 1319–1340 (2020)
Lax, C., Walcher, S.: Singular perturbations and scaling. Discrete Contin. Dyn. Syst. Ser. B **25**, 1–29 (2020)
Millán, M.P., Dickenstein, A., Shiu, A., Conradi, C.: Chemical reaction systems with toric steady states. Bull. Math. Biol. **74**, 1027–1065 (2012)
Mirzaev, I., Gunawardena, J.: Laplacian dynamics on general graphs. Bull. Math. Biol. **75**, 2118–49 (2013)
Müller, S., Feliu, E., Regensburger, G., Conradi, C., Shiu, A., Dickenstein, A.: Sign conditions for the injectivity of polynomial maps in chemical kinetics and real algebraic geometry. Found. Comut. Math. **16**(1), 69–97 (2016)
Pascual-Escudero, B., Feliu, E.: Local and global robustness in systems of polynomial equations. Math. Methods Appl. Sci. **45**(1), 359–382 (2021)
Sáez, M., Wiuf, C., Feliu, E.: Nonnegative linear elimination for chemical reaction networks. SIAM J. Appl. Math. **79**(6), 2434–2455 (2019)
Schauer, M., Heinrich, R.: Quasi-steady-state approximation in the mathematical modeling of biochemical reaction networks. Math. Biosci. **65**(2), 155–170 (1983)
Schneider, K.R., Wilhelm, T.: Model reduction by extended quasi-steady-state approximation. J. Math. Biol. **40**(5), 443–450 (2000)
Segel, L.A., Slemrod, M.: The quasi-steady-state assumption: a case study in perturbation. SIAM Rev. **31**, 446–477 (1989)
Tikhonov, A.N.: Systems of differential equations containing a small parameter multiplying the derivative (in Russian). Math. Sb. **31**, 575–586 (1952)
Tutte, W.T.: The dissection of equilateral triangles into equilateral triangles. Proc. Camb. Philos. Soc. **44**, 463–482 (1948)
Volpert, A.I.: Differential equations on graphs. Math. USSR-Sb **17**, 571–582 (1972)
Wang, L., Sontag, E.D.: On the number of steady states in a multiple futile cycle. J. Math. Biol. **57**(1), 29–52 (2008)