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Quasi-Steady-State and Singular Perturbation Reduction for Reaction Networks with Noninteracting Species∗

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Abstract. Quasi-steady state (QSS) reduction is a commonly used method to lower the dimension of a differential equation model of a chemical reaction network. From a mathematical perspective, QSS reduction is generally interpreted as a special type of singular perturbation reduction, but in many instances the correspondence is not worked out rigorously, and the QSS reduction may yield incorrect results. The present paper contains a thorough discussion of QSS reduction and its relation to singular perturbation reduction for the special, but important, case when the right-hand side of the differential equation is linear in the variables to be eliminated (but the differential equation model might otherwise be nonlinear). For this class we give necessary and sufficient conditions for a singular perturbation reduction (in the sense of Tikhonov and Fenichel) to exist, and to agree with QSS reduction. We then apply the general results to chemical reaction networks with noninteracting species, generalizing earlier results and methods for steady states to QSS scenarios. We provide easy-to-check graphical conditions to select parameter values for which the singular perturbation reduction applies, and additionally, we identify when the singular perturbation reduction agrees with the QSS reduction. Finally we consider a number of examples.

Key words. reaction networks, dimension reduction, noninteracting sets, linear elimination, invariant sets, critical manifold

AMS subject classifications. 92C45, 34E15, 80A30, 13P10

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1. Introduction. Mathematical modeling of chemical reaction networks naturally yields a large class of (typically) polynomial ordinary differential equations (ODEs). Beyond chemistry and biochemistry, this class of differential equations is also useful in applications to ecology, epidemiology, and genetics. These ODE systems often contain many variables (e.g., concentrations of chemical species) and parameters (e.g., rate constants), and moreover the parameters may not be exactly identifiable by experiments. Therefore a general study of sys-

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tems with undetermined parameters is appropriate, and possible reduction of such systems to smaller dimension is particularly relevant.

Quasi-steady-state (QSS) reduction is commonly used in (bio)chemistry and related fields, but rarely for differential equations in other areas of application. The method was introduced for a model of an enzyme-catalyzed reaction, by Michaelis and Menten in 1913 [15] and by Briggs and Haldane in 1925 [3], based on different assumptions. The reasoning of these authors used intuition about the specific system. In contrast, the insight that singular perturbation theory (based on Tikhonov’s 1952 paper [20]) can explain QSS phenomena is due to Heineken, Tsuchiya, and Aris in 1967 [13], and the analysis of the basic Michaelis–Menten reduction was brought to a conclusion by Segel and Slemrod in 1989 [17]. Nowadays, QSS is widely seen as a special type of a singular perturbation scenario in the sense of Tikhonov [20] and Fenichel [8], although many authors still use QSS reduction without verifying the necessary conditions for singular perturbation reduction. For the reader’s convenience, we give a brief outline of singular perturbation reduction (including a coordinate-free version) in the appendix.

A general mathematical study of QSS reduction, including consistency and validity requirements, and establishing an agreement between QSS and singular perturbation reduction under rather restrictive conditions, is carried out in [12]. In the present paper, we continue this study for a special (but quite relevant) class of differential equations. Building on [10, 12], we study ODE systems that depend linearly on the variables to be eliminated. To compute the singular perturbation reduction, we use the coordinate-free approach introduced in [10] (see also [12, Appendix 4]). We thus determine explicitly the two types of reduction for this general class of systems, and then obtain necessary and sufficient conditions for their agreement (up to higher order terms).

The motivation for studying this class of differential equations comes from reaction networks where the linear structure of the ODE system can be identified by means of a set of noninteracting species (assuming mass-action kinetics), as introduced in [6, 7, 16]. A set of noninteracting species (variables) can be inferred from the reactions of the network alone without scrutinizing the analytical form of the ODE system, and their concentrations will appear linearly in the differential equations. Though many QSS examples in the literature can be phrased in terms of noninteracting species, the original motivation for studying systems with noninteracting species simply came from the desire to reduce the steady state equations of an ODE system to a smaller (potentially more manageable) set of equations [7, 16]. It was, however, noticed that classical QSS examples formed a rich source of systems with noninteracting species.

In the setting of noninteracting species, we give general necessary and sufficient criteria for the existence of a singular perturbation reduction, and furthermore we provide necessary and sufficient criteria for the singular perturbation reduction to agree with the QSS reduction. The linear structure of the ODE system (in the variables to be eliminated) provides easy-to-check sufficient graphical criteria. For smaller reaction networks, the graph and the criteria can easily be constructed and checked by hand, providing criteria that are readily usable by application-oriented scientists.

We end the paper with a number of examples to illustrate the usefulness and limits of the graphical approach. In particular we study so-called posttranslational modification (PTM) systems of which the classical Michaelis–Menten system is a special case, and further examples from the chemical and ecological literature.
2. Linear elimination and reduction.

2.1. Motivation and background: Reaction networks. We consider a system of ODEs arising from a reaction network. A reaction network (or network for short) with species set \( S = \{X_1, \ldots, X_n\} \) consists of a set of reactions \( R = \{r_1, \ldots, r_m\} \), such that the \( i \)th reaction takes the form

\[
\sum_{j=1}^{n} \gamma_{ij} X_j \longrightarrow \sum_{j=1}^{n} \gamma'_{ij} X_j,
\]

where \( \gamma_{ij}, \gamma'_{ij} \in \mathbb{N}_0 \), are nonnegative integers. The left-hand side is called the reactant of the reaction, the right-hand side, the product, and jointly they are complexes. We will assume that any species takes part in at least one reaction and that the reactant and product sides are never identical, that is, it cannot be that \( \gamma_{ij} = \gamma'_{ij} \) for all \( j = 1, \ldots, n \).

A reaction network gives rise to an ODE system of the form

\[
\dot{y} = Nv(y), \quad y \in \mathbb{R}^n_{\geq 0},
\]

where \( \dot{y} \) denotes the derivative with respect to time \( t \), \( N \) is the stoichiometric matrix, that is, the \( i \)th column of \( N \) is the vector with \( j \)th entry \( \gamma'_{ij} - \gamma_{ij} \), and \( v(y) \) is a vector of rate functions defined on an open neighborhood of \( \mathbb{R}^m_{>0} \) and nonnegative on \( \mathbb{R}^m_{\geq 0} \). Under the additional assumption that \( v_i(y) \) vanishes whenever \( y_j = 0 \) and \( \gamma'_{ij} < \gamma_{ij} \), that is, whenever \( X_j \) is consumed by the reaction, the nonnegative orthant \( \mathbb{R}^n_{\geq 0} \) as well as the positive orthant \( \mathbb{R}^n_{>0} \) are forward invariant by the trajectories of the system \([18]\).

Of particular interest is mass-action kinetics with

\[
v = (v_1, \ldots, v_m), \quad v_i(y) = \kappa_i \prod_{j=1}^{n} y_j^{\gamma_{ij}}, \quad \kappa_i > 0,
\]

where \( \kappa_i \) is the (nonnegative) reaction rate constant. Formally, the borderline case \( \kappa_i = 0 \) corresponds to removing a reaction, and we are also interested in such scenarios.

In [6, 7, 16], a reduction procedure was introduced for the computation and discussion of steady states of an ODE system (2.2). It centers around the algebraic elimination at steady state of variables representing the concentrations of so-called noninteracting species.

Definition 2.1. Let \( Z = \{Z_1, \ldots, Z_P\} \subseteq S \) be a subset of the species set and let \( \mathcal{X} = S \setminus Z = \{X_1, \ldots, X_n\} \) be the complementary subset (where the species potentially are relabeled compared to (2.1)). The set \( Z \) is said to be noninteracting and its elements are called noninteracting species if after writing the reactions as

\[
\sum_{j=1}^{n} \beta_{ij} X_j + \sum_{j=1}^{P} \delta_{ij} Z_j \longrightarrow \sum_{j=1}^{n} \beta'_{ij} X_j + \sum_{j=1}^{P} \delta'_{ij} Z_j, \quad i = 1, \ldots, m,
\]

the conditions

\[
\sum_{j=1}^{P} \delta_{ij} \leq 1, \quad \sum_{j=1}^{P} \delta'_{ij} \leq 1
\]

are satisfied.
Two noninteracting species are never in the same reactant nor in the same product of a reaction, but one could be in the reactant and the other in the product. For mass-action kinetics, if \( Z \) is a noninteracting set, then the variables corresponding to noninteracting species appear linearly in the ODE system. The vector of concentrations of the noninteracting species and the remaining species will be denoted by \( z = (z_1, \ldots, z_P) \) and \( x = (x_1, \ldots, x_n) \), respectively. Hence in this terminology \( y = (x, z) \in \mathbb{R}^{n+P} \). It was shown in [7] that one may parameterize \( z_1, \ldots, z_P \) by \( x \) at steady state, given suitable regularity conditions on the Jacobian matrix and assuming the rate functions are linear in \( z \). In the present paper we will extend this reduction procedure to the case of QSS reduction.

**Example 1.** Consider the reaction network with species set \( S = \{X_1, X_2, Z_1, Z_2, Z_3, Z_4\} \) and reactions

\[
\begin{align*}
X_1 + 2X_2 & \overset{\kappa_1}{\rightarrow} Z_1, \\
0 & \overset{\kappa_2}{\rightarrow} X_1, \\
Z_1 & \overset{\kappa_3}{\rightarrow} 0, \\
X_1 + Z_4 & \overset{\kappa_4}{\rightarrow} Z_2 \overset{\kappa_5}{\rightarrow} Z_3 \overset{\kappa_7}{\rightarrow} X_2 + Z_4.
\end{align*}
\]

The labels of the reactions indicate the use of mass-action kinetics with the label being the reaction rate constants. They also indicate the order of the reaction set that we consider. The stoichiometric matrix and vector of rate functions \( v(x) \) under mass-action kinetics are

\[
N = \begin{bmatrix}
-1 & 1 & 0 & -1 & 0 & 0 & 0 \\
-2 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & -1 \\
0 & 0 & 0 & -1 & 0 & 0 & 1
\end{bmatrix},
\]

\[
v(x) = (\kappa_1 x_1^2 z_2, \kappa_2, \kappa_3 z_1, \kappa_4 x_1 z_4, \kappa_5 z_2, \kappa_6 z_3, \kappa_7 z_3).
\]

This yields an ODE system that models the species concentrations over time:

\[
\begin{align*}
\dot{x}_1 &= -\kappa_1 x_1 x_2^2 - \kappa_4 x_1 z_4 + \kappa_2, \\
\dot{x}_2 &= -2\kappa_1 x_1 x_2^2 + \kappa_7 z_3, \\
\dot{z}_1 &= -\kappa_4 x_1 z_4 + \kappa_7 z_3, \\
\dot{z}_2 &= \kappa_4 x_1 z_4 - \kappa_5 z_2 + \kappa_6 z_3, \\
\dot{z}_3 &= \kappa_5 z_2 - \kappa_6 z_3 - \kappa_7 z_3, \\
\dot{z}_4 &= \kappa_1 x_1 x_2^2 - \kappa_3 z_1.
\end{align*}
\]

The subset \( Z = \{Z_1, Z_2, Z_3, Z_4\} \subseteq S \) is noninteracting, as no species in the set appear together on the same side of a reaction, and all appear with coefficient at most one in any complex. The complementary set is \( X = \{X_1, X_2\} \). In this case, \( Z \) is maximal in the sense that there is not a larger noninteracting set containing it, although maximality is not required. For example, any subset of \( Z \) is also noninteracting. Observe that (2.4) is linear in \( z_1, z_2, z_3, z_4 \), as is the case for any noninteracting set and mass-action kinetics.

**2.2. The general setting.** We first discuss the reduction procedures in a context that is not restricted to reaction networks, just keeping the characteristic property of the reaction equations with noninteracting species, namely, the linearity in \( z \). This class of equations, and their reductions, may be of interest beyond reaction networks.

We consider a parameter dependent ODE system that is linear in \( z \), that is,

\[
\begin{align*}
\dot{x} &= a(x, \pi) + A(x, \pi)z, \\
\dot{z} &= b(x, \pi) + B(x, \pi)z
\end{align*}
\]

with \( x \in V_1 \subseteq \mathbb{R}^n, z \in V_2 \subseteq \mathbb{R}^p \), and \( \pi \in \Pi \subseteq \mathbb{R}^q \), satisfying the following conditions:
For every \( \tilde{\pi} \in \Pi \) there is a smooth curve \( \sigma: [0, 1] \to \Pi \) such that \( \sigma(0) = \tilde{\pi} \) and \( \sigma(s) \in \text{int}(\Pi) \) for all \( s > 0 \); in particular \( \text{int}(\Pi) \) is dense in \( \Pi \).

- For any \( \pi \in \Pi \), define the sets
  \[
  \Omega_{\pi} := \{ x \in \mathbb{R}^n | a(x, \pi), A(x, \pi), b(x, \pi), B(x, \pi) \text{ are defined and sufficiently differentiable} \},
  \]
  \[
  \Omega^*_{\pi} := \{ x \in \Omega_{\pi} | B(x, \pi) \text{ is invertible} \},
  \]
  noting that both are open subsets of \( \mathbb{R}^n \) and \( V_1 \subseteq \Omega_{\pi} \).

The general question is whether (and how) it is possible to eliminate \( z \), thus obtaining a reduced system in \( x \) alone. For reaction networks, the “classical” QSS reduction (see Briggs and Haldane [3], Segel and Slemrod [17] for the Michaelis–Menten system, and many others) has been in use for a long time. For this heuristic reduction procedure one assumes invertibility of \( B(x, \pi) \) for all \( x \) and furthermore assumes that the rate of change for \( z \) is equal to zero (or rather, almost zero in a relevant time regime). Then the ensuing algebraic relation for \( x \in \Omega^*_{\pi} \),

\[
0 = \dot{z} = b(x, \pi) + B(x, \pi)z \quad \Leftrightarrow \quad z = -B(x, \pi)^{-1}b(x, \pi),
\]

yields the QSS reduced system

\[
\dot{x} = a(x, \pi) - A(x, \pi)B(x, \pi)^{-1}b(x, \pi), \quad x \in \Omega^*_\pi.
\]
QSS and Singular Perturbation Reduction

Consider a curve $\varepsilon \mapsto \hat{\varepsilon} + \varepsilon \pi^* + \cdots \in \Pi$ and expand the functions for $x \in \Omega_{\hat{\varepsilon}}$

\[
\begin{align*}
  a(x, \hat{\varepsilon} + \varepsilon \pi^* + \cdots) &= a_0(x) + \varepsilon a_1(x) + \cdots, \\
  A(x, \hat{\varepsilon} + \varepsilon \pi^* + \cdots) &= A_0(x) + \varepsilon A_1(x) + \cdots, \\
  b(x, \hat{\varepsilon} + \varepsilon \pi^* + \cdots) &= b_0(x) + \varepsilon b_1(x) + \cdots, \\
  B(x, \hat{\varepsilon} + \varepsilon \pi^* + \cdots) &= B_0(x) + \varepsilon B_1(x) + \cdots,
\end{align*}
\]

where $a_0(x) = a(x, \hat{\varepsilon}) \in \mathbb{R}^n$, $A_0(x) = A(x, \hat{\varepsilon}) \in \mathbb{R}^{n \times n}$, $b_0(x) = b(x, \hat{\varepsilon}) \in \mathbb{R}^{p}$, $B_0(x) = B(x, \hat{\varepsilon}) \in \mathbb{R}^{p \times p}$. We obtain a system with small parameter $\varepsilon$, which we rewrite in the form

\[
\begin{align*}
  \dot{x} &= a_0(x) + A_0(x)z + \varepsilon (a_1(x) + A_1(x)z) + \cdots, \\
  \dot{z} &= b_0(x) + B_0(x)z + \varepsilon (b_1(x) + B_1(x)z) + \cdots
\end{align*}
\]

for $x \in V_1$ and $z \in V_2$. We define the following QSS-critical set:

\[
Y_{\hat{\varepsilon}} := \{(x, z) \in \Omega_{\hat{\varepsilon}} \times \mathbb{R}^p | B_0(x)z + b_0(x) = 0\} = \{(x, z) \in \Omega_{\hat{\varepsilon}} \times \mathbb{R}^p | z = -B_0(x)^{-1}b_0(x)\},
\]

where it is used that $B_0(x)$ is invertible for $x \in \Omega_{\hat{\varepsilon}}^*$; cf. (2.7).

Further properties are required to ensure a singular perturbation reduction to the QSS-critical set. The following two conditions are crucial.

**Blanket conditions:**

(i) $\Omega_{\hat{\varepsilon}}^* \cap V_1 \neq \emptyset$, that is $B_0(x)$ is invertible for some $x \in V_1$.

(ii) All points in the QSS-critical set $Y_{\hat{\varepsilon}}$ are stationary points of system (2.9) for $\varepsilon = 0$, thus

\[
a_0(x) - A_0(x)B_0(x)^{-1}b_0(x) = 0 \quad \text{for all } x \in \Omega_{\hat{\varepsilon}}^* \cap V_1.
\]

It will be convenient to introduce

\[
w(x) := B_0(x)^{-1}b_0(x) \in \mathbb{R}^p \text{ for } x \in \Omega_{\hat{\varepsilon}}^*,
\]

and thus have

\[
z = -w(x) \text{ on } Y_{\hat{\varepsilon}}.
\]

By definition and blanket condition (ii), on $Y_{\hat{\varepsilon}}$ we have

\[
a_0(x) + A_0(x)z = A_0(x)(w(x) + z), \quad b_0(x) + B_0(x)z = B_0(x)(w(x) + z),
\]

that is,

\[
\begin{bmatrix}
a_0(x) + A_0(x)z \\
b_0(x) + B_0(x)z
\end{bmatrix} = \begin{bmatrix}
A_0(x) \\
B_0(x)
\end{bmatrix} \cdot (w(x) + z), \quad (x, z) \in Y_{\hat{\varepsilon}}.
\]

We are now in position to state the decomposition and reduction procedure from [10, Theorem 1, Remarks 1 and 2], in three propositions. The first provides existence of a singular perturbation reduction, the second the form of the reduced system, and the third the relationship to the QSS reduction. Define

\[
h^{(0)}(x, z) = \begin{bmatrix}
a_0(x) + A_0(x)z \\
b_0(x) + B_0(x)z
\end{bmatrix}, \quad h^{(1)}(x, z) = \begin{bmatrix}
a_1(x) + A_1(x)z \\
b_1(x) + B_1(x)z
\end{bmatrix},
\]
and

\begin{equation}
\Delta(x) = Dw(x) A_0(x) + B_0(x) \in \mathbb{R}^{p \times p} \text{ on } Y_\pi,
\end{equation}

where $D$ denotes the derivative with respect to $x$. The following is a straightforward application of [10]. A proof is sketched in Appendix A.

**Proposition 2.2.** Assume blanket conditions (i) and (ii) hold for $\pi \in \Pi$.

(a) Condition for reducibility: A singular perturbation reduction with a linearly attractive critical manifold $Y_\pi$ exists locally if and only if the open set

\begin{equation*}
\Omega^*_\pi := \{ x \in \Omega_\pi \mid \text{all eigenvalues of } \Delta(x) \text{ lie in the open left half-plane } \} \subseteq \Omega^*_\pi
\end{equation*}

is nonempty. In this case, for every compact subset of $Y_\pi \cap \{(x, z) \mid x \in \Omega^*_\pi, z \in \mathbb{R}^p\}$ there exist a reduced system in slow time scale $\tau = \varepsilon t$, a fixed time $T > 0$, and a neighborhood in which, as $\varepsilon \to 0$, all solutions converge uniformly to solutions of the reduced system in the interval $[t_0, T]$ with $t_0 > 0$ arbitrary.

(b) Under the conditions stated in (a), the reduced system on $Y_\pi$ in slow time scale $\tau = \varepsilon t$, is

\begin{equation}
\begin{bmatrix}
\frac{dx}{d\tau} \\
\frac{dz}{d\tau}
\end{bmatrix} = Q(x) h^{(1)}(x, w(x)) = Q(x) \begin{bmatrix}
a_1(x) - A_1(x)w(x) \\
b_1(x) - B_1(x)w(x)
\end{bmatrix}
\end{equation}

with

\begin{equation}
Q(x) := I_{n+p} - \begin{bmatrix}
A_0(\Delta(x))^{-1} \\
B_0(\Delta(x))^{-1}
\end{bmatrix} \begin{bmatrix}
Dw(x) & I_p
\end{bmatrix},
\end{equation}

where $I_p$ denotes the $p \times p$ identity matrix.

To keep notation manageable, we suppress the argument $x$ in $a_0, a_1, A_0, A_1, b_0, b_1 B_0, B_1 w, \Delta$, and their derivatives. The essential part of the reduction is given in the following proposition. Note that on $Y_\pi$, it suffices to consider the equation for $x$.

**Proposition 2.3.** Given the blanket conditions (i) and (ii), assume the conditions in Lemma 2.2(a) hold. The reduced equation in slow time $\tau = \varepsilon t$, on $Y_\pi$, yields the system

\begin{equation}
\frac{dx}{d\tau} = (I_n - A_0 (Dw A_0 + B_0)^{-1} Dw) (a_1 - A_1 w) - A_0 (Dw A_0 + B_0)^{-1} (b_1 - B_1 w).
\end{equation}

**Proof.** The reduced system is given in Lemma 2.2(b). The first $n$ rows of the matrix $Q$ in (2.14), given in blocks of size $n \times n$ and $n \times p$, respectively, are

\[ [I_n - A_0 \Delta^{-1} Dw - A_0 \Delta^{-1}] = [I_n - A_0 (Dw A_0 + B_0)^{-1} Dw - A_0 (Dw A_0 + B_0)^{-1}] \]

where we use the definition of $\Delta$ in (2.13). Multiplication of this matrix with $h^{(1)}$ from (2.12) gives $\frac{dx}{d\tau}$ as in the statement of the proposition.

Having obtained the singular perturbation reduction, we compare it to the classical QSS reduction.
Proposition 2.4. Assume blanket conditions (i) and (ii) hold for $\hat{\tau} \in \Pi$.
(a) The classical QSS reduction of system (2.9) yields the system

\[
\frac{dx}{d\tau} = \left(a_1 - A_1 w - A_0 B_0^{-1} (b_1 - B_1 w)\right) + \varepsilon(\cdots)
\]

in slow time.
(b) The classical QSS reduction agrees with the singular perturbation reduction (up to higher order terms in $\varepsilon$) if and only if

\[
A_0 (Dw A_0 + B_0)^{-1} Dw \left(A_0 B_0^{-1} (B_1 w - b_1) - (A_1 w - a_1)\right) = 0.
\]

Given this condition, the conclusion in Proposition 2.2(a) also applies to the QSS reduction.

Proof. The second equation in (2.9) is

\[
z = -(B_0 + \varepsilon B_1 + \cdots)^{-1}(b_0 + \varepsilon b_1 + \cdots).
\]

With the geometric series one has

\[
(B_0 + \varepsilon B_1 + \cdots)^{-1} = B_0^{-1}(I + \varepsilon B_1 B_0^{-1} + \cdots)^{-1} = B_0^{-1} - \varepsilon B_0^{-1} B_1 B_0^{-1} + \cdots
\]

and therefore

\[
z = -B_0^{-1}b_0 + \varepsilon(B_0^{-1}B_1 B_0^{-1}b_0 - B_0^{-1}b_1) + \cdots.
\]

Substitution into the first equation of (2.9), replacing $b_0 = B_0w$, and using blanket condition (ii), and further collecting terms yields the assertion of part (a). As for part (b), comparing (2.15) and (2.16), necessary and sufficient conditions are obtained:

\[
A_0 B_0^{-1} (B_1 w - b_1) = A_0 (Dw A_0 + B_0)^{-1} (B_1 w - b_1)
\]

\[
+ A_0 (Dw A_0 + B_0)^{-1} Dw (A_1 w - a_1)
\]

\[
\Leftrightarrow A_0 (Dw A_0 + B_0)^{-1} ((Dw A_0 + B_0) B_0^{-1} - I_p) (B_1 w - b_1)
\]

\[
= A_0 (Dw A_0 + B_0)^{-1} Dw (A_1 w - a_1)
\]

\[
\Leftrightarrow A_0 (Dw A_0 + B_0)^{-1} Dw A_0 B_0^{-1} (B_1 w - b_1)
\]

\[
= A_0 (Dw A_0 + B_0)^{-1} Dw (A_1 w - a_1).
\]

The last assertion holds since higher order terms in $\varepsilon$ are irrelevant for the conclusion of Proposition 2.2(a) to hold [12].

The general reduction formula of Proposition 2.3 may seem rather unwieldy, given the seemingly simple starting point (2.5). For the purpose of illustration we look at the smallest dimension and consider the case $w = 0$ (and recover a special case of [12, Prop. 5]).

Proposition 2.5. The following simpler scenarios hold.

- For $n = p = 1$ the reduced system in slow time is given by

\[
\frac{dx}{d\tau} = \frac{(B_0 a_1 - A_0 b_1) - (B_0 A_1 - A_0 B_1)w}{B_0 + w'A_0}.
\]
When \( w \) is constant (in particular when \( b_0 = 0 \)), then the reduced equation is given by
\[
\frac{dx}{d\tau} = a_1 - A_1w - A_0B_0^{-1}(b_1 - B_1w).
\]

In particular, if \( w = 0 \) (thus, the critical manifold is given by \( z = 0 \)), then the reduced equation in slow time reads
\[
\frac{dx}{d\tau} = a_1 - A_0B_0^{-1}b_1.
\]

In general the QSS reduction and singular perturbation reduction yield substantially different results, and the reduction by QSS is incorrect. However, we emphasize that for the following notable cases, the two reductions are in agreement (Proposition 2.5).

- \( w \) is constant, thus \( Dw = 0 \). (As will turn out, this case occurs for many reaction networks.)
- \( A_0 = 0 \). Here, system (2.12) is in Tikhonov standard form with slow and fast variables separated.
- \( A_0B_0^{-1}(B_1w - b_1) = A_1w - a_1 \). Here, both reductions have the right-hand side of the ODE system equal to zero.

In dimension 2, this list is complete, as seen by inspection of (2.17).

To summarize: Given the blanket conditions (i) and (ii) and the eigenvalue condition in Lemma 2.2(a), we have determined a closed-form version of the reduced system by singular perturbation and clarified its relation to the classical QSS reduction. The next step will be to apply these results to reaction networks, making use of their special properties.

### 3. Application to reaction networks

We return to the setting of reaction networks from section 2.1, and extend results from [6, 5] on steady states to QSS. Specifically, in [6, 5], a QSS system is obtained upon elimination of noninteracting species (cf. Definition 2.1), but the relation between the dynamics of the original and reduced systems is not studied. We show here that, under certain conditions, parameter vectors \( \hat{\pi} \) can be identified for which Lemma 2.2(a) and Proposition 2.4(b) hold, interpreting the reduced system in [6, 5] as a reduced system in the sense of Tikhonov and Fenichel, as explained in the previous section. In this section we focus on the analytic aspects of this construction, while in the next section we provide a graphical approach to identify suitable parameter vectors \( \hat{\pi} \).

We assume that all the conditions stated after (2.5) hold, with
\[
\mathbb{R}^q_{>0} \subset \Pi \subset \mathbb{R}^q_{\geq 0}, \quad V_1 = \mathbb{R}^n_{>0}, \quad V_2 = \mathbb{R}^p_{>0},
\]
where \( p \leq P \) will be defined below in Lemma 3.2. Furthermore, the following assumptions and definitions are introduced. Note that all requirements are satisfied for systems with mass-action kinetics.

We consider a noninteracting set \( Z = \{Z_1, \ldots, Z_P\} \subseteq S \) and let \( \mathcal{X} = S \setminus Z = \{X_1, \ldots, X_n\} \), as in Definition 2.1. We assume the following:

- The species are ordered as \( X_1, \ldots, X_n, Z_1, \ldots, Z_P \), such that the concentration vector is \( (x, z) \).
• The rate function of a reaction involving the non-interacting species \( Z_i, i = 1, \ldots, P \), in the reactant is linear in \( z_i \) and does not depend on any other \( z_j, j \neq i, j = 1, \ldots, P \).

• The rate function of a reaction that does not involve any noninteracting species in the reactant is constant in \( z_i, i = 1, \ldots, P \).

• We order the set of reactions such that the first \( m_1 \) reactions only have species in \( \mathcal{X} \) in the reactant (without restrictions on the product, and the last \( m_2 \) reactions all have one noninteracting species in the reactant (and at most one in the product). Thus, \( m = m_1 + m_2 \). We let

\[
\Pi = \{1, \ldots, m_1\}
\]

be the set of indices of the reactions that do not involve any species in \( \mathcal{Z} \) (neither in the reactant nor in the product), that is, \( \sum_{j=1}^{P} \delta_{ij} = \sum_{j=1}^{P} \delta_{ij} = 0 \) for \( i \in I \) with notation as in Definition 2.1.

Let \( v_1(x, \kappa), \kappa = (\kappa_1, \ldots, \kappa_n) \in \Pi \), denote the vector of rate functions for the first \( m_1 \) reactions (which by assumption do not depend on \( z \)) and let \( v_2(x, z, \kappa) \) be the vector of rate functions for the last \( m_2 \) reactions (by assumption, each component is linear in the concentration of the noninteracting species in the reactant). By assumption, these functions are defined for all \( (x, \kappa) \) in an open neighborhood of \( \mathcal{V}_1 \times \Pi = \mathbb{R}_{\geq 0}^n \times \mathbb{R}_{\geq 0}^q \), and all \( z \in \mathbb{R}^p \) (by linearity). For \( \kappa \in \Pi \), the set \( \Omega_\kappa \) consists of all \( x \in \mathbb{R}^n \) for which the rate functions are defined; see (2.6). In particular, \( \mathcal{V}_1 = \mathbb{R}_{\geq 0}^n \subseteq \Omega_\kappa \) and \( \Omega_\kappa \cap \mathbb{R}_{\geq 0}^n = \mathbb{R}_{\geq 0}^n \) for any \( \kappa \in \Pi \).

Under the above assumptions, the dynamical system (2.2), which evolves in \( \mathbb{R}^{n+P}_{\geq 0} \) (by invariance), may be written as

\[
\begin{bmatrix}
\dot{x} \\
\dot{z}
\end{bmatrix} = Nv(x, z, \kappa) = [N_{11} N_{12}] [v_1(x, \kappa)] + [N_{21} N_{22}] [v_2(x, z, \kappa)]
\]

\[
= [N_{11}] v_1(x, \kappa) + [N_{21}] v_2(x, z, \kappa),
\]

\[
= [N_{11}] v_1(x, \kappa) + [K_1(x, \kappa)] z
\]

(3.2)

with \( N_{11} \in \mathbb{R}^{n \times m_1}, N_{21} \in \mathbb{R}^{P \times m_1}, N_{12} \in \mathbb{R}^{n \times m_2}, N_{22} \in \mathbb{R}^{P \times m_2}, K_1 \in \mathbb{R}^{n \times P}, \) and \( K_2 \in \mathbb{R}^{P \times P} \). We might take system (3.2) to be of the form (2.5). However, we will refrain from doing so here, as the matrix \( B(x, \kappa) = K_2(x, \kappa) \) in (2.5) might not be invertible, in which case the set \( \Omega^*_\kappa \) (where \( B(x, \kappa) \) is invertible) in (2.7) is empty, and singular perturbation reductions are not possible. Instead, we will modify the system before making the identification with (2.5). The set \( \Omega^*_\kappa \) will remain unchanged before and after the modification.

**Example 2.** In Example 1, we find \( n = 2, q = 7, P = 4, m = 7, m_1 = 2, m_2 = 5 \). Furthermore, \( \Omega_\kappa = \mathbb{R}^2 \) for all \( \kappa \in \Pi = \mathbb{R}^q_{\geq 0} \). The set of reactions is already ordered such that the first two reactions are the only ones without species in \( \mathcal{Z} \) in the reactant. Hence \( v_1(x, \kappa) = (\kappa_1 x_1^2, \kappa_2)^\top \) and
Using (3.3) and the form of $N$

\[
(3.3) \quad v_2(x, z, \kappa) = (\kappa_3 z_1, \kappa_4 x_1 z_4, \kappa_5 z_2, \kappa_6 z_3, \kappa_7 z_3) \top = \begin{bmatrix}
\kappa_3 & 0 & 0 & 0 \\
0 & 0 & 0 & \kappa_4 x_1 \\
0 & \kappa_5 & 0 & 0 \\
0 & 0 & \kappa_6 & 0 \\
0 & 0 & \kappa_7 & 0
\end{bmatrix} \begin{bmatrix}
z_1 \\
z_2 \\
z_3 \\
z_4
\end{bmatrix}.
\]

The matrices $N_{11}, N_{12}, N_{21}, N_{22}$ come from the following block decomposition:

\[N = \begin{bmatrix}
-1 & 1 & 0 & -1 & 0 & 0 & 0 \\
-2 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & -1 & 1 \\
0 & 0 & 0 & -1 & 0 & 0 & 1
\end{bmatrix}.
\]

Using (3.3) and the form of $N_{22}$, the matrix $K_2(x, \kappa) \in \mathbb{R}^{4 \times 4}$ is

\[
K_2(x, \kappa) = \begin{bmatrix}
-1 & 0 & 0 & 0 \\
0 & 1 & -1 & 1 \\
0 & 0 & 1 & -1 \\
0 & -1 & 0 & 0
\end{bmatrix} \begin{bmatrix}
\kappa_3 & 0 & 0 & 0 \\
0 & \kappa_4 x_1 & 0 & 0 \\
0 & \kappa_5 & 0 & 0 \\
0 & 0 & \kappa_6 & 0 \\
0 & 0 & \kappa_7 & 0
\end{bmatrix} = \begin{bmatrix}
-\kappa_3 & 0 & 0 & 0 \\
0 & -\kappa_5 & \kappa_6 & \kappa_4 x_1 \\
0 & \kappa_5 & -\kappa_6 - \kappa_7 & 0 \\
0 & 0 & \kappa_7 & -\kappa_4 x_1
\end{bmatrix}.
\]

This matrix is precisely the coefficient matrix of the ODE system for $\dot{z}_1, \ldots, \dot{z}_4$ as a system in $z_1, \ldots, z_4$; see (2.4). Analogously, $K_1(x, \kappa)$ is the coefficient matrix for $\dot{x}_1, \dot{x}_2$, and hence

\[K_1(x, \kappa) = \begin{bmatrix}
0 & 0 & 0 & \kappa_4 x_1 \\
0 & 0 & \kappa_7 & 0
\end{bmatrix}.
\]

Observe that the last three rows of $K_2(x, \kappa)$ sum to zero, and hence $K_2(x, \kappa)$ does not have full rank for any $\kappa \in \Pi$ and $x \in \mathbb{R}^2 (= \Omega_\kappa)$. Specifically, the vector $\lambda = (0, 1, 1, 1)$ satisfies $\lambda^\top K_2(x, \kappa) = 0$. As the bottom three rows of $N_{21}$ are zero, the vector $(0, 0, 0, 1, 1, 1)$ defines a linear first integral of system (2.4). Indeed, $\dot{z}_2 + \dot{z}_3 + \dot{z}_4 = 0$.

Motivated by the previous example, we note some crucial properties of $K_2(x, \kappa)$, in particular, those related to its rank. Most of these results are already shown elsewhere [6, 5].

**Lemma 3.1.** Consider the system (3.2) and let $\kappa \in \Pi$. Then, the following hold.

(a) For all $x \in \mathbb{R}^n_{\geq 0}$, $K_2(x, \kappa)$ is a compartmental matrix, that is, the diagonal entries of $K_2$ are nonpositive, the off-diagonal entries nonnegative, and all column sums are nonpositive.

(b) For all $x \in \mathbb{R}^n_{\geq 0}$, the nonzero eigenvalues of $K_2(x, \kappa)$ have negative real parts, and for the eigenvalue 0 (if it occurs) the geometric and algebraic multiplicity are equal.

(c) Assume the rank of $K_2(x, \kappa)$ is equal to $p = P - k < P$ for all $x$ in an open set $\Omega_\kappa \subseteq \mathbb{R}^n_{\geq 0}$ and assume there are linearly independent linear forms $\lambda_1, \ldots, \lambda_k$ on $\mathbb{R}^P$ such that $\lambda_i(K_2(x, \kappa)) = 0$ for all $x \in \Omega_\kappa$, and $1 \leq i \leq k$. Then $K_2(x, \kappa)$, $x \in \Omega_\kappa$, restricts to a linear map on $\operatorname{Ker} \lambda_1 \cap \cdots \cap \operatorname{Ker} \lambda_k$. This map is invertible, and its eigenvalues are just the nonzero eigenvalues of $K_2(x, \kappa)$, $x \in \Omega_\kappa$. 
Proof. (a) A column of $N_{22}$ contains one entry $-1$ and one entry 1 (with all other entries zero) if it corresponds to a reaction in (2.3) with noninteracting species appearing on both sides of the reaction, and contains just one entry $-1$ (with all other entries equal to zero) if it corresponds to a reaction with a noninteracting species appearing just on the left-hand side. Since the rate functions are nonnegative for $x \in \mathbb{R}_{\geq 0}^n$, assertion (a) follows.

(b) For $x \in \mathbb{R}_{\geq 0}^n$, it follows from (a) that the matrix is compartmental. The first assertion of (b) is well known; see, e.g., Anderson [1, Thm. 12.1], or Chapter 6 of Berman and Plemmons [2] (noting that compartmental matrices are negative M-matrices). We include a proof of the second statement (which also is known) for the sake of completeness: Abbreviate $F := K_2(x, \kappa)$ with $(x, \kappa)$ fixed, and consider the linear differential equation $\dot{z} = F \cdot z$. For this equation the positive orthant is positively invariant, and the equation admits the Lyapunov function $\sum_{i=1}^P z_i$, whence all solutions in the positive orthant are bounded for positive times. The existence of a nontrivial Jordan block for the eigenvalue 0 would imply the existence of unbounded solutions for positive times; a contradiction.

(c) We have shown in (b) that $\mathbb{R}^P$ is the direct sum of the kernel and the image of $F$. Since the image is contained in $\text{Ker} \lambda_1 \cap \cdots \cap \text{Ker} \lambda_k$, and both have dimension $P - k$, they are equal. This shows invertibility and the assertion about the eigenvalues, since $\text{Im} F$ is the sum of generalized eigenspaces for nonzero eigenvalues.

Any vector $\omega$ in the left kernel of the matrix $[N_{21} \quad N_{22}]$ defines a linear first integral of the system (3.2) involving $z_1, \ldots, z_P$, and hence a linear form as in Lemma 3.1(c). In particular, any such form is independent of $x$ and $\kappa$, and we say that it is induced by stoichiometry. In what follows, we will require that the linear forms $\lambda_1, \ldots, \lambda_k$ in Lemma 3.1(c) all arise in this way for $\Omega_k = \mathbb{R}_{\geq 0}^n$. That is, the rank of $K_2(x, \kappa)$ (equivalently, of $[N_{21} \quad N_{22}]$) is $p := P - k$ for all $x \in \mathbb{R}_{\geq 0}^n$ with $k$ the dimension of the left kernel of $[N_{21} \quad N_{22}]$. This situation is quite common for chemical reaction networks [6, 5, 16]; see also Example 2.

By considering a basis of the left kernel of $[N_{21} \quad N_{22}]$ we define the matrix $W \in \mathbb{R}^{k \times P}$ such that $WN_{21} = WN_{22} = 0$. The following is shown in [5, 16].

Lemma 3.2. One may choose a basis of the left kernel of $[N_{21} \quad N_{22}]$ with pairwise disjoint support and coefficients 0 and 1 only. Thus, up to reordering of the $z_j$, $j = 1, \ldots, P$, one might assume the matrix $W$, such that $WN_{21} = WN_{22} = 0$, to be of the form

$$W = [W' \quad I_k] \in \mathbb{R}^{k \times P},$$

and any level set $Wz = \alpha \in \mathbb{R}_{\geq 0}^k$ might be rewritten in the form

$$\begin{bmatrix} z_{p+1} \\ z_p \end{bmatrix} = \begin{bmatrix} \alpha_1 \\ \vdots \\ \alpha_k \end{bmatrix} - W' \begin{bmatrix} z_1 \\ \vdots \\ z_p \end{bmatrix}. \tag{3.4}$$

Moreover, if all linear forms in Lemma 3.1(c) are induced by stoichiometry, that is, the rank of $K_2(x, \kappa)$ is $p = P - k$, then for any $\alpha \in \mathbb{R}_{\geq 0}^k$ and $x$ in $\mathbb{R}_{\geq 0}^n$ the linear system in $z$

$$\alpha = Wz, \quad 0 = N_{21}v_1(x, \kappa) + K_2(x, \kappa)z,$$

has a unique solution, which is nonnegative.
In view of Lemma 3.2, we use (3.4) to reduce the dimension of system (3.2) by eliminating the variables \(z_{p+1}, \ldots, z_p\). In this way we obtain a system of the form (2.5), where the corresponding matrix \(B\) has full rank. The parameter vector of the new system is \((\kappa, \alpha) \in \Pi \times \mathbb{R}^k_{\geq 0}\).

**Proposition 3.3.** Let \(p\) be the rank of \(\begin{bmatrix} N_{21} & N_{22} \end{bmatrix}\), and consider \(\kappa \in \Pi\), such that \(K_2(x, \kappa)\) has rank \(p\) for all \(x \in \mathbb{R}^n_{\geq 0}\). Consider \(W\) given as in Lemma 3.2. Denote by \(\tilde{K}_2\) the \((p \times P)\)-matrix containing the first \(p\) rows of \(K_2\), by \(\tilde{N}_{21}\) the matrix containing the first \(p\) rows of \(N_{21}\), and partition

\[
K_1 = \begin{bmatrix} K_{11} & K_{12} \end{bmatrix}, \quad \tilde{K}_2 = \begin{bmatrix} \tilde{K}_{21} & \tilde{K}_{22} \end{bmatrix}
\]

into matrices with \(p\), respectively, \(k = P - p\) columns. Then, for any \(\alpha \in \mathbb{R}^k_{\geq 0}\), the restriction of (3.2) to the level set \(Wz = \alpha\) induces the following system in \(\mathbb{R}^{n+p}_{\geq 0}\):

\[
\begin{aligned}
\dot{x} &= N_{11}v_1(x, \kappa) + K_{12}(x, \kappa)\alpha + \left(K_{11}(x, \kappa) - K_{12}(x, \kappa)W'\right)z_{1:p}, \\
\dot{z}_{1:p} &= \tilde{N}_{21}v_1(x, \kappa) + \tilde{K}_{22}(x, \kappa)\alpha + \left(\tilde{K}_{21}(x, \kappa) - \tilde{K}_{22}(x, \kappa)W'\right)z_{1:p},
\end{aligned}
\]

(3.5)

where \(z_{1:p} = (z_1, \ldots, z_p)\).

**Proof.** This follows from replacing \((z_{p+1}, \ldots, z_p)\) in (3.2) using (3.4) of Lemma 3.2.

With this in place, we consider a small parameter and determine situations where singular perturbation reductions are possible. We consider a curve in the joint parameter space of \(\kappa\) and \(\alpha\), \((\kappa, \alpha) = (\widehat{\kappa}, \widehat{\alpha}) + \varepsilon(\kappa^*, \alpha^*) + \cdots \in \Pi \times \mathbb{R}^k_{\geq 0}\). Then system (3.5) can be written in the form of (2.9) with

\[
\begin{aligned}
a_0(x) &= N_{11}v_1(x, \widehat{\kappa}) + K_{12}(x, \widehat{\kappa})\widehat{\alpha}, \\
a_0(x) &= K_{11}(x, \widehat{\kappa}) - K_{12}(x, \widehat{\kappa})W', \\
b_0(x) &= \tilde{N}_{21}v_1(x, \widehat{\kappa}) + \tilde{K}_{22}(x, \widehat{\kappa})\widehat{\alpha}, \\
b_0(x) &= \tilde{K}_{21}(x, \widehat{\kappa}) - \tilde{K}_{22}(x, \widehat{\kappa})W'.
\end{aligned}
\]

(3.6) (3.7)

**Proposition 3.4.** Let \(p\) be the rank of \(\begin{bmatrix} N_{21} & N_{22} \end{bmatrix}\). Assume the notation as in Proposition 3.3. Consider system (3.5), and the matrix \(\Delta\) and the function \(w\) from (2.13) and (2.11), respectively.

(a) If \(\widehat{\kappa} \in \Pi\) is such that \(K_2(x, \widehat{\kappa})\) has rank \(p\) in \(\mathbb{R}^n_{\geq 0}\), then \(B_0(x) = \tilde{K}_{21}(x, \widehat{\kappa}) - \tilde{K}_{22}(x, \widehat{\kappa})W'\) is invertible and all its eigenvalues have negative real part for all \(x \in \mathbb{R}^n_{\geq 0}\). In particular blanket condition (i) for system (3.5) holds.

(b) If additionally blanket condition (ii) for system (3.5) holds and \(w\) is constant, then \(\Delta = B_0\) and a singular perturbation reduction with a linearly attractive slow manifold exists. Hence, Propositions 2.4 and 2.5 apply and the singular perturbation reduction of system (3.5) with respect to a curve in the parameter space starting at \((\widehat{\kappa}, \widehat{\alpha})\), agrees with the classical QSS reduction obtained by elimination of the noninteracting species.

(c) If (a) holds, \(v_1(x, \widehat{\kappa}) = 0\), and \(\widehat{\alpha} = 0\), then \(a_0(x) = 0\), \(b_0(x) = 0\), and \(w = 0\), and blanket condition (ii) is satisfied. Hence also (b) holds.

**Proof.** The first statement of part (a) is just a reformulation of Lemma 3.1(c) using the discussion above. The remaining assertions are clear.

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Recall that $\Omega^*_R$ in (2.6) by definition is the set where $B_0(x)$ is invertible. In the setting considered here, $\Omega^*_R = \mathbb{R}^n_{\geq 0}$.

It is shown in [16] that the classical QSS reduction system can be interpreted as the ODE system associated with a reduced network in the species of $X$ with an appropriate choice of kinetics. We will not go further into this here.

Remark 1. When each rate function is a multiple of some entry of $\kappa$, as is the case for mass-action kinetics, it follows from system (3.5) and the assumptions and notation of system (2.9), that

$$
\begin{align*}
a_1(x) &= N_{11}v_1(x, \kappa^*) + K_{12}(x, \kappa^*)\widehat{\alpha} + K_{12}(x, \hat{\kappa})\alpha^*, \\
b_1(x) &= \tilde{N}_{21}v_1(x, \kappa^*) + \tilde{K}_{22}(x, \kappa^*)\widehat{\alpha} + \tilde{K}_{22}(x, \hat{\kappa})\alpha^*, \\
A_1(x) &= K_{11}(x, \kappa^*) - K_{12}(x, \kappa^*)W', \\
b_1(x) &= \tilde{K}_{21}(x, \kappa^*) - \tilde{K}_{22}(x, \kappa^*)W'.
\end{align*}
$$

Hence $A_0, A_1$, respectively, $B_0, B_1$, are the same functions evaluated in different parameter values (cf. (3.6), (3.7)). Recall from Propositions 2.3 and 2.4, that these functions define the reduced system on the critical manifold.

Example 3. We continue with Example 2. Lemma 3.2 applies with $k = 1, P = 4, p = 3$, and

$$
W = \begin{bmatrix} 0 & 1 & 1 & 1 \end{bmatrix}, \quad \text{such that } z_4 = \alpha_1 - z_2 - z_3.
$$

System (3.5) is simply obtained by insertion of $z_4 = \alpha_1 - z_2 - z_3$ into (2.4) and removing the ODE for $z_4$. In the matrix notation of (3.5), we find

$$
\begin{align*}
\begin{bmatrix}
\dot{x}_1 \\
\dot{x}_2 \\
\dot{x}_3 \\
\dot{x}_4
\end{bmatrix} &= \begin{bmatrix} -1 & 1 \\
-2 & 0 \\
0 & 0 \\
0 & 0
\end{bmatrix} \begin{bmatrix} x_1 \\
x_2 \\
x_3 \\
x_4
\end{bmatrix} + \begin{bmatrix} \kappa_1x_1x_2^2 \\
\kappa_2x_1 \end{bmatrix} \alpha_1 + \begin{bmatrix} 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \kappa_4x_1 \\
0 \end{bmatrix} \begin{bmatrix} 0 & 1 & 1 \end{bmatrix} \begin{bmatrix} z_1 \\
z_2 \\
z_3 \\
z_4
\end{bmatrix},

\dot{z}_1 &= \begin{bmatrix} 1 & 0 \\
0 & 0 \\
0 & 0 \\
0 & 0
\end{bmatrix} \begin{bmatrix} x_1 \\
x_2 \\
x_3 \\
x_4
\end{bmatrix} \alpha_1 + \begin{bmatrix} -\kappa_3 & 0 & 0 & 0 \\
0 & -\kappa_5 & \kappa_6 & \kappa_7 \\
0 & \kappa_5 & -\kappa_6 & -\kappa_7 \\
0 & 0 & 0 & 0
\end{bmatrix} \begin{bmatrix} 0 \\
1 \\
1 \\
1
\end{bmatrix} \begin{bmatrix} z_1 \\
z_2 \\
z_3 \\
z_4
\end{bmatrix}.
\end{align*}
$$

These expressions allow the matrices and vectors $a_0, b_0, A_0, B_0$ to be easily identified. For $\hat{\kappa} \in \mathbb{R}^7_{\geq 0}$, the matrix $K_2(x, \hat{\kappa})$ in Example 2, repeated here for convenience,

$$
K_2(x, \hat{\kappa}) = \begin{bmatrix} -\hat{\kappa}_3 & 0 & 0 & 0 \\
0 & -\hat{\kappa}_5 & \hat{\kappa}_6 & \hat{\kappa}_4x_1 \\
0 & \hat{\kappa}_5 & -\hat{\kappa}_6 - \hat{\kappa}_7 & 0 \\
0 & 0 & \hat{\kappa}_7 & -\hat{\kappa}_4x_1
\end{bmatrix},
$$

has rank $p = 3$ for $x_1 > 0$, when $\hat{\kappa}_3 \neq 0$ and additionally $\hat{\kappa}_5\hat{\kappa}_7 \neq 0$, or $\hat{\kappa}_4(\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \neq 0$ hold. In this case, the matrix $B_0(x)$ is invertible by Proposition 3.4(a) for $x \in \mathbb{R}^2_{\geq 0}$. For
Proposition 3.4(c) to hold, we require \( \hat{\alpha} = 0 \) and \( \hat{\kappa}_1 x_1^2 = \hat{\kappa}_2 = 0 \), which occurs if \( \hat{\kappa}_1 = \hat{\kappa}_2 = 0 \). For any such \( \hat{\kappa} \), Proposition 3.4(b) holds.

As an example, consider \( \hat{\kappa} = (0, 0, \hat{\kappa}_3, \hat{\kappa}_4, \hat{\kappa}_5, \hat{\kappa}_6, \hat{\kappa}_7) \) with \( \hat{\kappa}_i > 0, i \neq 1, 2, \hat{\alpha} = 0 \), and a curve in the parameter space of the form

\[(\kappa(\varepsilon), \alpha(\varepsilon)) = (\varepsilon \kappa^*_1, \varepsilon \kappa^*_2, \kappa_3, \kappa_4, \kappa_5, \kappa_6, \kappa_7, \varepsilon \alpha^*),\]

such that \( \kappa^*_i \neq 0 \) for \( i \neq 1, 2 \) and \( \alpha^* \neq 0 \). We apply the reduction procedure as given in Proposition 2.2 ff. and Appendix A. As \( w(x) = 0 \), Proposition 2.5 implies that the critical manifold \( Y_{\hat{\kappa}, \hat{\alpha}} \) in (2.10) for system (3.8) is defined by the equations \( z_1 = z_2 = z_3 = 0 \). By the same proposition, the reduced system on the critical manifold in slow time agrees with the QSS reduction and is given by

\[\frac{dx}{d\tau} = a_1(x) - A_0(x)B_0(x)^{-1}b_1(x).\]

Using Remark 1, \( \hat{\alpha} = 0 \), and the form of \((\kappa(\varepsilon), \alpha(\varepsilon))\), the vectors \( a_1(x) \) and \( b_1(x) \) are simply \( a_0(x) \) and \( b_0(x) \), respectively, evaluated at the parameter point \((\kappa(1), \alpha(1))\). This leads to the reduced system in slow time with equations

\[
\begin{align*}
\frac{dx_1}{d\tau} &= -\kappa^*_1 x_1^2 + \hat{\kappa}_4 \alpha x_1^2 + \kappa^*_2 - \frac{\hat{\kappa}_3 (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \alpha \kappa^*_1 x_1^2}{\hat{\kappa}_5 \hat{\kappa}_7 + (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \hat{\kappa}_4 x_1}, \\
\frac{dx_2}{d\tau} &= -2 \kappa^*_1 x_1^2 + \frac{\hat{\kappa}_4 \hat{\kappa}_5 \hat{\kappa}_7 \alpha \kappa^*_1 x_1}{\hat{\kappa}_5 \hat{\kappa}_7 + (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \hat{\kappa}_4 x_1}.
\end{align*}
\]

(3.9)

In particular, system (3.9) agrees with the system obtained by solving the equations \( \dot{z}_1 = \dot{z}_2 = \dot{z}_3 = 0 \) in (3.8) for \( z_1, z_2, z_3 \), and subsequently, plugging the result into the first two equations of (3.8) (as there are no terms of higher order in \( \varepsilon \)). As a consequence, for every compact subset of the critical manifold \( Y_{\hat{\kappa}, \hat{\alpha}} \) there exists \( T > 0 \) and a neighborhood in which, as \( \varepsilon \to 0 \), the solutions of (3.8) with parameter \((\kappa(\varepsilon), \alpha(\varepsilon))\) converge uniformly to solutions of the system (3.9) in the interval \([t_0, T]\) with \( t_0 > 0 \) arbitrary.

We found conditions on the parameters in the example above, that ensure the criterion in Proposition 3.4(a) is fulfilled and blanket condition (i) holds. These conditions are equivalent to the denominators of (3.9) being nonzero.

Finally, we note that the fast fibers of the singularly perturbed system (3.8) are level sets of the first integrals of the linear system

\[
\begin{pmatrix}
\dot{z}_1 \\
\dot{z}_2 \\
\dot{z}_3
\end{pmatrix}
= \begin{pmatrix}
-\hat{\kappa}_3 & 0 & 0 \\
0 & \hat{\kappa}_4 x_1 - \hat{\kappa}_5 & -\hat{\kappa}_4 x_1 + \hat{\kappa}_6 \\
0 & \hat{\kappa}_5 & -\hat{\kappa}_6 - \hat{\kappa}_7
\end{pmatrix}
\begin{pmatrix}
z_1 \\
z_2 \\
z_3
\end{pmatrix},
\]

(3.10)

where \( x_1 \) is “frozen.” Thus, while the critical manifold is a coordinate subspace given by \( z = 0 \), the fast fibers are not coordinate subspaces.

To conclude this section, we show by example that the linear first integrals in Lemma 3.1(c) might not all be induced by stoichiometry.
Example 4. Consider the network $Z_2 \xleftarrow{\kappa_1} Z_1 \xrightarrow{\kappa_2} Z_3$ with only noninteracting species and mass-action kinetics. The matrix $K_2(x, \kappa)$ is found from the decomposition

$$Nv_2(x, z, \kappa) = \begin{bmatrix} -1 & -1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \kappa_1 z_1 \\ \kappa_2 z_1 \end{bmatrix} = \begin{bmatrix} -(\kappa_1 + \kappa_2) & 0 & 0 \\ \kappa_1 & 0 & 0 \\ \kappa_2 & 0 & 0 \end{bmatrix} z.$$ 

This matrix vanishes when evaluated at the two linear forms $z_1 + z_2 + z_3$ and $\kappa_1 z_3 - \kappa_2 z_2$. Both of these forms are independent of $x$, but only the first is independent of $\kappa$. Similarly, consider the network $Z_1 \xleftarrow{\kappa_1} X_1 \xrightarrow{\kappa_2} Z_2$ with two noninteracting species $Z_1, Z_2$ and mass-action kinetics. Now $m_2 = 0$ and the matrix $K_2(x, \kappa)z$ is obtained from

$$N_{22}v_2(x, z, \kappa) = \begin{bmatrix} 0 & 0 \\ 0 & 0 \end{bmatrix} z.$$ 

(Note that $N_{22}$ is a $2 \times 0$ matrix and $v_2(x, z, \kappa)$ is a $0 \times 1$ matrix.) Hence, this matrix vanishes when evaluated at any linear form. However, the ODE system admits only one independent linear first integral in $z_1, z_2$, namely, $\kappa_1 z_3 - \kappa_2 z_2$, which depends on the choice of reaction rate constants.

4. The noninteracting graph and the blanket conditions. Proposition 3.4 characterizes cases where the QSS reduction obtained by elimination of noninteracting species agrees with the reduced system obtained by singular perturbation theory. It establishes properties of the parameter vector $(\hat{\kappa}, \hat{\alpha})$ that give rise to such reductions. We have shown in Example 3 how to verify the conditions by inspection of the relevant matrices. In what follows we show that finding $\hat{\kappa}$ such that Proposition 3.4 is satisfied, can be done by inspection of a labeled multidigraph.

Specifically, we relate the results of the previous section to a particular labeled multidigraph built from the reaction network and the set of noninteracting species. It extends the formalism introduced in [16] from steady states to QSS. The two blanket conditions may be interpreted in terms of conditions on this graph, which (at least for relatively small networks) allows for easy identification of parameters $\hat{\kappa}, \hat{\alpha}$, where Proposition 3.4 applies.

4.1. The noninteracting graph. Throughout the section, we continue with the setup of section 3. Recall that the vector of rate functions has the form $v(x, z, \kappa) = (v_1(x, \kappa), v_2(x, z, \kappa))$ and that $v_2(x, z, \kappa)$ is linear in $z$, with each component depending on only one $z_j, j = 1, \ldots, P$. Therefore, we write

$$v_2(x, z, \kappa) = \begin{bmatrix} \nu_2(x, \kappa, z_j) \\ \nu_2(x, \kappa, i) \end{bmatrix} z_j$$

if $Z_j$ is the noninteracting species in the reactant of the reaction $r_{m_{1+i}}, i = 1, \ldots, m_2$. Importantly, the rate functions are evaluated in the nonnegative orthant only.

Given $\kappa$, we follow [16] and introduce a labeled multidigraph $G_\kappa = (\mathcal{N}, \mathcal{E}_\kappa)$ describing the part of the network relating to the noninteracting species only. Essentially, we identify all complexes involving only species in $\mathcal{X}$ with one node, say $*$, and any other complex is identified with the noninteracting species that appears in it. Edges correspond to reactions, and are labeled to encode the reaction rate functions.
Definition 4.1. For $\kappa \in \Pi$, the labeled multidigraph $G_\kappa = (\mathcal{N}, \mathcal{E}_\kappa)$ has node set
\[ \mathcal{N} = \mathbb{Z} \cup \{\ast\} = \{Z_1, \ldots, Z_P, \ast\} \]
and edge set $\mathcal{E}_\kappa$ consisting of the following edges with corresponding labels:

- $Z_j \xrightarrow{\nu_2(\kappa,j)} Z_k$ if $r_{m_1+i}$ involves $Z_j$ in the reactant and $Z_k$ in the product, $i = 1, \ldots, m_2$,
- $Z_j \xrightarrow{\nu_2(\kappa,j)} \ast$ if $r_{m_1+i}$ involves $Z_j$ in the reactant and no noninteracting species in the product, $i = 1, \ldots, m_2$,
- $\ast \xrightarrow{\nu_1(\kappa,i)} Z_k$ if $r_i$ involves no noninteracting species in the reactant and $Z_k$ in the product, $i = 1, \ldots, m_1$.

We let $\ell_\kappa(e)$ denote the label of the edge $e$, which is a function of $x \in \mathbb{R}^n_{\geq 0}$. For a submultidigraph $G' = (\mathcal{N}', \mathcal{E}')$ of $G_\kappa$, we define the label of $G'$ by
\[ \ell_\kappa(G') = \prod_{e \in \mathcal{E}'} \ell_\kappa(e). \]

Let $G_\kappa = G_\kappa^s \cup G_\kappa^1 \cup \cdots \cup G_\kappa^d$ be the partitioning of $G$ into its connected components $G_\kappa^i = (\mathcal{N}^i, \mathcal{E}_\kappa^i)$, $i = *, 1, \ldots, d$, such that $G_\kappa^*$ is the component containing the node $\ast$. If all edges of $G_\kappa$ are between two noninteracting species, then $G_\kappa^*$ consists of only the node $\ast$. Since all species of the network are in at least one reaction (by assumption), there cannot be any other connected component consisting of one node only. For each $G_\kappa^i$, $i = 1, \ldots, d$, there is a corresponding linear first integral $\lambda_i$ (as in Lemma 3.2) with coefficient one for the entries corresponding to the nodes $V \in G_\kappa^i$ and zero otherwise [5]. Hence $d \leq k$ in Lemma 3.1(e), and the assumption that all linear forms are induced by stoichiometry imposes $d = k$. Let
\[ \alpha_i = \sum_{j=1}^P \lambda_{ij} z_j \]
be the conserved amount of $G_\kappa^i$, and note that $\lambda_{ij} \geq 0$.

Let $\Theta_{\kappa,i}(V)$, $i = \ast, 1, \ldots, d$, be the set of spanning trees of $G_\kappa^i$ rooted at the node $V \in \mathcal{N}^i$. A spanning tree of $G_\kappa^i$ is a subdigraph of $G_\kappa^i$ containing all nodes such that there is precisely one outgoing edge for each $V' \in \mathcal{N}^i$, except for the root $V$. It follows that the edges of the spanning tree are directed towards $V$ (see Figure 1 for examples). Furthermore, the set of spanning trees with positive labels for all $x \in \mathbb{R}^n_{\geq 0}$ is defined as
\[ \Theta_{\kappa,i}^+(V) = \{\tau \mid \tau \in \Theta_{\kappa,i}(V), \ell_\kappa(\tau) > 0 \text{ in } \mathbb{R}^n_{\geq 0}\}, \]
i = \ast, 1, \ldots, d.

Figure 1 shows the graph $G_\kappa$ for Example 1, which has two connected components, so $d = 1$. The component $G_\kappa^*$, containing the node $\ast$, is shown at the top. The second component $G_\kappa^1$ gives the linear first integral $z_2 + z_3 + z_4$, which we already found in Example 2. Figure 1 also shows the set $\Theta_{\kappa,i}(V)$ of spanning trees for each node of the graph and the label of each
spanning tree. If \( \kappa \) is such that the label is nonzero for any \( x \in \mathbb{R}^n_{>0} \), then the tree belongs to \( \Theta_{\kappa,i}^+(V) \) as well.

In order to study blanket condition (ii), we need to introduce some extra objects related to the multidigraph \( \mathcal{G}_\kappa \) for \( \kappa \in \Pi \). Let \( \sigma \) be a (directed) cycle of the graph \( \mathcal{G}_\kappa \), say in the connected component \( \mathcal{G}_\kappa^i \), \( e \) an edge of \( \sigma \), and define

\[
\Gamma_\kappa(\sigma) = \left\{ \tau \cup e \mid \tau \in \Theta_{\kappa,i}(\text{source of } e) \text{ and } \sigma \subseteq \tau \cup e \right\},
\]

where \( \cup \) is applied to both the node set and the edge set. In other words, \( \Gamma_\kappa(\sigma) \) consists of subgraphs build from spanning trees rooted at the source of an edge \( e \) that, after the addition of the edge \( e \), contain the cycle \( \sigma \). It is shown in [16] that \( \Gamma_\kappa(\sigma) \) does not depend on the choice of edge \( e \) of \( \sigma \). For an example of this construction, see Figure 2 and Example 5 below.

For \( i = 1, \ldots, m \), let \( \xi_i \in \mathbb{R}^n \) be the vector with entries

\[
\xi_{ij} = \beta'_{ij} - \beta_{ij}, \quad j = 1, \ldots, n,
\]

with \( \beta_{ij}, \beta'_{ij} \) as in Definition 2.1. That is, \( \xi_i \) consists of the first \( n \) components of the \( i \)th column of the stoichiometric matrix, encoding the net production of the species in \( \mathcal{X} \) in reaction \( r_i \). For a cycle \( \sigma \), define \( \zeta_\sigma \in \mathbb{R}^n \) to be the sum of all vectors \( \xi_i \) corresponding to the edges of \( \sigma \):

\[
\zeta_\sigma = \sum_{r_i \text{ defines an edge of } \sigma} \xi_i.
\]

We consider the set

\[
\Sigma_\kappa = \{ \sigma \in \mathcal{G}_\kappa \mid \zeta_\sigma \neq 0 \text{ and } \Gamma_\kappa(\sigma) \neq \emptyset \}.
\]

The first condition in (4.6) means that the net production of the species in \( \mathcal{X} \) is nonzero in the reaction path composed of the reactions in the cycle. A cycle consisting of two reactions
forming one reversible reaction never satisfies this condition as the net production is zero. We decompose the set $\Sigma_\kappa$ into components corresponding to the connected components of $\mathcal{G}_n$, $\Sigma_\kappa = \Sigma_{\kappa,*} \cup \Sigma_{\kappa,1} \cup \cdots \cup \Sigma_{\kappa,d}$.

**Example 5.** Returning to Example 1, it follows from Figures 1 and 2 that the multidigraph $\mathcal{G}_n$ has three cycles, one in $\mathcal{G}_n^*$ and two in $\mathcal{G}_n^1$. The cycle $\sigma_2$ in $\mathcal{G}_n^1$, consisting of the edges $Z_2 \xrightarrow{\kappa_5} Z_3, Z_3 \xrightarrow{\kappa_6} Z_2$, corresponds to a pair of reversible reactions, and hence $\zeta_2 = 0$. Consequently, $\sigma_2 \notin \Sigma_\kappa$.

For the cycle $\sigma_1$ in $\mathcal{G}_n^*$, we have $\zeta_1 = \xi_1 + \xi_3 = (-1, -2) + (0, 0) = (-1, -2) \neq 0$. Furthermore, $\Gamma_\kappa(\sigma_1) = \{ \sigma_1 \} \neq \emptyset$. Hence, $\sigma_1 \in \Sigma_{\kappa,*}$.

Finally, let $\sigma_3$ be the cycle in $\mathcal{G}_n^1$ through all three nodes. Then, $\zeta_3 = \xi_4 + \xi_5 + \xi_7 = (-1, 0) + (0, 0) + (0, 1) = (-1, 1) \neq 0$. Again, $\Gamma_\kappa(\sigma_3) = \{ \sigma_3 \} \neq \emptyset$ and $\sigma_3 \in \Sigma_{\kappa,1}$. We conclude $\Sigma_\kappa = \Sigma_{\kappa,*} \cup \Sigma_{\kappa,1} = \{ \sigma_1, \sigma_3 \}$.

**4.2. Blanket conditions.** In this section, we relate the blanket conditions to conditions on the multidigraph $\mathcal{G}_n$ and the objects introduced in section 4.1.

As in section 3, we consider the joint parameter space of $\kappa$ and $\alpha$, and let $(\kappa, \alpha) = (\hat{\kappa}, \hat{\alpha}) + \varepsilon (\kappa^*, \alpha^*) + \cdots$ with $\hat{\kappa} \in \Pi$, $\hat{\alpha} \in \mathbb{R}^d_{\geq 0}$ be a curve in the joint parameter space for $\varepsilon \geq 0$. Consider the ODE system in (3.5), which using (3.6) and (3.7), might be written as

$$
\dot{x} = a_0(x) + A_0(x)z_{1:p}, \quad \dot{z}_{1:p} = b_0(x) + B_0(x)z_{1:p}.
$$

The next proposition tells us that blanket condition (i) corresponds to the existence of at least one rooted spanning tree with positive label in each connected component, and that the root must be $*$ for the component $\mathcal{G}_n^\kappa$. 

---

**Figure 2.** Shown is the graph $\mathcal{G}_n$ from Figure 1. It has three (directed) cycles, $\sigma_1, \sigma_2, \sigma_3$: one in component $\mathcal{G}_n^*$ and two in component $\mathcal{G}_n^1$. To determine $\Gamma_\kappa(\sigma_1)$ in (4.3), we select an edge $e$ (dashed) and search for spanning trees $\tau$ such that $\sigma_1 \subseteq \tau \cup e$. The edge $e$ might be chosen in two ways. In both cases, there is only one spanning tree (solid line), rooted at the source of $e$, such that $\sigma_1 \subseteq \tau \cup e$ holds. Since $\sigma_1 = \tau \cup e$, then $\Gamma_\kappa(\sigma_1) = \{ \sigma_1 \}$. This illustrates the independence of $e$. Similarly, for $\sigma_2$. For $\sigma_3$, we choose the edge $e$ to be the one with label $\kappa_2$ (dashed). The two possible spanning trees rooted at the source of $e$ (that is, $Z_2$) are shown, together with $e$ (see also Figure 1). Only the second graph contains $\sigma_3$, and in fact agrees with $\sigma_3$. Hence the first graph is disregarded and $\Gamma_\kappa(\sigma_3) = \{ \sigma_3 \}$. In Subsection 5.2, we show an example where $\Gamma_\kappa(\sigma) \neq \{ \sigma \}$. 

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Proposition 4.2. Consider \( \hat{\kappa} \in \Pi \), \( B_0(x) \) in (3.7), and recall the sets \( \Theta_{\hat{\kappa},i}^\pm(V) \), \( i = *, 1, \ldots, d \), in (4.2). Then \( B_0(x) \) is invertible for \( x \in \mathbb{R}_{>0}^n \) if and only if

\[
\Theta_{\hat{\kappa},i}^+(*) \neq \emptyset \quad \text{and} \quad \bigcup_{V \in \mathcal{N}} \Theta_{\hat{\kappa},i}^+(V) \neq \emptyset \quad \text{for all } i = 1, \ldots, d.
\]

Proof. We have \( B_0(x) = \tilde{K}_{21}(x, \hat{\kappa}) - \tilde{K}_{22}(x, \hat{\kappa})W' \). It is shown in [5, 16] (with a proof based on the matrix-tree theorem) that invertibility is equivalent to the condition of the proposition.

If at least one of the two sets of spanning trees in Proposition 4.2 is empty, then there are additional conservation relations among the noninteracting species, as illustrated in Example 4, or the spanning trees have zero labels, implying some reactions have vanishing rate (hence they are not present in practice).

Example 6. Consider Example 1 with the graphical objects in Figure 1. We determine the values of \( \hat{\kappa} \) for which (4.7), hence blanket condition (i), holds for all \( x \in \mathbb{R}_{>0}^n \). By Figure 1, \( \Theta_{\hat{\kappa},i}^+(*) \neq \emptyset \) if and only if \( \hat{\kappa}_3 \neq 0 \). For \( \bigcup_{V \in \mathcal{N}} \Theta_{\hat{\kappa},i}^+(V) \neq \emptyset \), we need one of the following inequalities to hold: \( \hat{\kappa}_5\hat{\kappa}_7 \neq 0 \), \( \hat{\kappa}_4\hat{\kappa}_6 \neq 0 \), \( \hat{\kappa}_4\hat{\kappa}_7 \neq 0 \), \( \hat{\kappa}_4\hat{\kappa}_5 \neq 0 \). That is, either \( \hat{\kappa}_5\hat{\kappa}_7 \neq 0 \), or \( \hat{\kappa}_4\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7 \neq 0 \). We recover the conditions given in Example 3, there found by inspection of the matrix \( K_2(x, \hat{\kappa}) \).

For blanket condition (ii), we proceed as follows. Recall the set \( I \subseteq \{1, \ldots, m_1\} \) defined in (3.1) consisting of the indices of the reactions that do not involve any species in \( Z \) and \( \zeta_\sigma \) from (4.5). Assuming blanket condition (i), then it is shown in [16] that the following equality holds for \( x \in \mathbb{R}_{>0}^n \):

\[
a_0(x) - A_0(x)B_0(x)^{-1}b_0(x) = \sum_{i \in I} v_1(x, \hat{\kappa})_i \xi_i + \sum_{i = *, 1, \ldots, d} \frac{\alpha_i}{q_i(x, \hat{\kappa})} \sum_{\sigma \in \Sigma_{\hat{\kappa},i}} \left( \sum_{\gamma \in \Gamma_{\hat{\kappa}}(\sigma)} \ell_{\hat{\kappa}}(\gamma) \right) \zeta_\sigma,
\]

where

- \( \alpha_* = 1 \) for convenience, and \( \xi_i \in \mathbb{R}^n \) is as in (4.4);
- \( \ell_{\hat{\kappa}}(\gamma) \) is the label of the subgraph \( \gamma \) of \( \Gamma_{\hat{\kappa}}(\sigma) \). Furthermore, \( \ell_{\hat{\kappa}}(\sigma) \) is a factor of \( \ell_{\hat{\kappa}}(\gamma) \);
- the function \( q_i(x, \hat{\kappa}) \), \( i = *, 1, \ldots, d \), is positive if blanket condition (i) holds. In particular, it is the sum of the labels of the spanning trees in \( \Theta_{\hat{\kappa},i}^+(*) \) for \( i = * \) and of the labels of the spanning trees in \( \bigcup_{V \in \mathcal{N}} \Theta_{\hat{\kappa},i}^+(V) \) for \( i = 1, \ldots, d \) (cf. Proposition 4.2).

We remark that in [16], it is assumed the parameter \( \hat{\kappa} \) is positive, but this is only used to guarantee that blanket condition (i) holds.

Blanket condition (ii) states that \( a_0(x) - A_0(x)B_0(x)^{-1}b_0(x) \) vanishes for all \( x \in \mathbb{R}_{>0}^n \). Hence, blanket condition (ii) holds in addition if and only if the right-hand side of (4.8) vanishes. In the next proposition, we obtain a sufficient condition for this to occur, by requiring that all summands in (4.8) vanish.

Proposition 4.3. Consider system (3.5) and \( I \) as in (3.1). Assume blanket condition (i) holds for a fixed \( \hat{\kappa} \in \Pi \) and \( \alpha \in \mathbb{R}_{>0}^d \). Blanket condition (ii) holds if the following three statements are satisfied:

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These conditions are also necessary if the vectors \( \xi_i, i \in I \), and \( \zeta_\sigma, \sigma \in \Sigma_{\hat{R}} \), are linearly independent.

**Sufficient conditions for (b) and (c) to hold are**

- \( \ell_{\hat{R}}(\sigma) = 0 \) if \( \sigma \in \Sigma_{\hat{R}, \kappa} \) is a cycle of \( G_{\hat{R}}^\kappa \);
- \( \hat{\alpha}_i (\sum_{\gamma \in \Gamma_i(\sigma)} \ell_{\hat{R}}(\gamma)) = 0 \) for all cycles \( \sigma \in \Sigma_{\hat{R}, i} \) of \( G_{\hat{R}}^i \), \( i = 1, \ldots, d \).

**Proof.** The statement is a consequence of the form of the right-hand side of (4.8) as all terms vanish under the conditions of the lemma. For the second part, we note that \( \ell_{\hat{R}}(\gamma) \) is a multiple of \( \ell_{\hat{R}}(\gamma) \) as all subgraphs \( \gamma \) contain \( \sigma \).

**Example 7.** We conclude the running Example 1 by analyzing it in the context of Proposition 4.3. Assume the conditions in Example 6 for blanket condition (i) hold, that is, assume \( \hat{\kappa}_2 \neq 0 \), and either \( \hat{\kappa}_2 \hat{\kappa}_7 \neq 0 \) or \( \hat{\kappa}_2 (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \neq 0 \).

The reaction network in Example 1 has one reaction that does not involve any non-interacting species, namely, the second, so \( I = \{ 2 \} \): cf. (3.1). Proposition 4.3(a) requires \( 0 = v_1(x, \hat{n})_2 = \hat{\kappa}_2 \). The set \( \Sigma_{\hat{n}, 0} \) contains one element \( \sigma_1 \): see Example 5. As \( \ell_{\hat{R}}(\sigma_1) = \hat{\kappa}_1 \hat{\kappa}_3 x_1 x_2^2 \) and \( \hat{\kappa}_3 \neq 0 \), Proposition 4.3(b) holds if and only if \( \hat{\kappa}_1 = 0 \). Finally, for Proposition 4.3(c) to hold we require \( \hat{\alpha}_1 \ell_{\hat{R}}(\sigma_3) = \hat{\alpha}_1 \hat{\kappa}_4 \hat{\kappa}_5 \hat{\kappa}_7 x_1 = 0 \) (with \( \sigma_3 \) as in Example 5). Combining this with blanket condition (i), the parameter values satisfying the hypotheses of Proposition 4.3 fulfill one of the following conditions:

- \( \hat{\kappa}_1 = \hat{\kappa}_2 = 0, \hat{\kappa}_3 \neq 0, \hat{\kappa}_5 \hat{\kappa}_7 \neq 0, \) or \( \hat{\kappa}_4 (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \neq 0, \) and \( \hat{\alpha}_1 = 0 \).
- \( \hat{\kappa}_1 = \hat{\kappa}_2 = 0, \hat{\kappa}_3 \neq 0, \hat{\kappa}_5 \hat{\kappa}_7 = 0, \) or \( \hat{\kappa}_4 (\hat{\kappa}_5 + \hat{\kappa}_6 + \hat{\kappa}_7) \neq 0, \) and \( \hat{\alpha}_1 \neq 0 \).

The case studied analytically in Example 3 fulfills the first condition, where \( \hat{\kappa}_i > 0 \) for all \( i > 2 \).

The previous example shows that if \( \hat{\alpha} = 0 \), all rate constants of reactions without non-interacting species in the reactant are zero, and the remaining reaction rate constants are positive, then Proposition 4.3 applies. This statement generalizes to certain types of kinetics: In the special case of mass-action kinetics, or a kinetics for which the rate functions scale in one parameter, the first two conditions in Proposition 4.3 hold, if the parameters are chosen similarly to the above. Specifically, we obtain the following corollary, which is a consequence of Propositions 3.4, 4.2, and 4.3.

**Corollary 4.4.** Consider system (3.5). Assume that \( \Pi = \Pi_1 \times \Pi_2 \subseteq \mathbb{R}^m_{\geq 0} \times \mathbb{R}^{n-m} \), and that the functions \( v_1 \) and \( v_2 \) in (4.1) take the following form,

\[
v_1(x, \kappa', \kappa'')_i = \kappa'_i u_1(x, \kappa'_i), \quad \text{for } i = 1, \ldots, m_1, \\
v_2(x, \kappa', \kappa'')_i = \kappa'_i u_2(x, \kappa'_i), \quad \text{for } i = m_1 + 1, \ldots, m,
\]

where \( \kappa = (\kappa', \kappa'') \), \( \kappa' \in \Pi_1, \kappa'' \in \Pi_2, \) and \( u_1, u_2 \) are functions of \( x \) and \( \kappa'' \), that take positive values for \( x \in \mathbb{R}^n_{\geq 0} \). Assume further that \( \Theta_{\kappa, \kappa}(*) \neq \emptyset \) and \( \bigcup_{V \in \mathcal{N}} \Theta_{\kappa, \kappa}(V) \neq \emptyset \) for all \( i = 1, \ldots, d \) (whether these hold does not depend on \( \kappa \)).
Proposition 4.3 holds trivially as \( \kappa \), then the label of the edge with source \( \kappa \), in \( \Theta \) and hence so does blanket condition (ii).

In particular, there is a choice of parameters for which the QSS reduction obtained after eliminating the noninteracting species, can be seen as a singular perturbation reduction of the original system.

**Proof.** Let \( \hat{\kappa} = (\hat{\kappa}', \hat{\kappa}'') \) with \( \hat{\kappa}' \in \Pi_1 \) and \( \hat{\kappa}'' \in \Pi_2 \). For any edge of a spanning tree in \( \Theta_{\kappa,*(s)} \cup \bigcup_{V \in \mathcal{A}'} \Theta_{\kappa,i}(V) \), the source is an element of \( \mathcal{Z} \), and the label is of the form \( \hat{\kappa}' u_2(x, \hat{\kappa}'') \). By assumption, both \( \hat{\kappa}' \) and \( u_2(x, \hat{\kappa}'') \) are strictly positive, as \( m_1 < j \leq m \). Hence, any such spanning tree has positive label (as nonpositive edges are zero). By Proposition 4.2, blanket condition (i) holds.

Note that the assumptions imply that \( v_1(x, \hat{\kappa}) = 0 \). Hence conditions (a) and (c) in Proposition 4.3 hold trivially as \( \hat{\alpha} = 0 \). Consider a cycle \( \sigma \in \Sigma_{\hat{\kappa},*} \). If \( \sigma \) contains the node \( * \), then the label of the edge with source \( * \) is zero (cf. Definition 4.1), and hence \( \ell_{\hat{\kappa}}(\sigma) = 0 \). If \( * \) is not in \( \sigma \), then consider any subgraph \( \gamma \in \Gamma_{\hat{\kappa}}(\sigma) \neq \emptyset \). The subgraph \( \gamma \) contains a spanning tree with root a node of \( \sigma \). Hence, it must contain an edge with source \( * \), which has zero label, implying that \( \ell_{\hat{\kappa}}(\gamma) = 0 \) for any \( \gamma \in \Gamma_{\hat{\kappa}}(\sigma) \). It follows that Proposition 4.3(b) holds, and hence so does blanket condition (ii).

A nice consequence of Corollary 4.4 is the following: If all reactions involve some species of \( \mathcal{Z} \) in the reactant, then regardless of the (positive) values of the reaction rate constants, \( \hat{\alpha} = 0 \) alone guarantees that the singular perturbation reduction exists and agrees with the QSS reduction.

Note that if \( \mathcal{G}_x^* \) is just the node \( * \) itself, then \( b_0(x) = 0 \) (cf. (3.7)), and hence \( w(x) = 0 \).

**Remark 2.** A special scenario occurs for so-called intermediate species [6]: these are species that do not interact with any other species, and are the reactant and the product of at least one reaction. The set of these species is obviously a set of noninteracting species. With mass-action kinetics, \( K_2(x, \hat{\kappa}) \) has full rank and hence there are no linear first integrals in their concentrations. In particular,

\[
b_0(x) = N_{21} v_1(x, \hat{\kappa}), \quad B_0(x) = K_2(x, \hat{\kappa}),
\]

and \( B_0(x) \) is constant in \( x \). Hence, if blanket conditions (i) and (ii) are satisfied, we need only to choose \( \hat{\kappa} \) such that the rate of any reaction producing an intermediate is constant in order to obtain a valid singular perturbation reduction which further agrees with the QSS reduction (up to irrelevant terms of higher order in \( \varepsilon \)).

Let us look at this scenario in more detail. By the condition on the production and degradation of all intermediates, the graph \( \mathcal{G}_{\hat{\kappa}} \) has one connected component, namely, that of \( * \), which necessarily has a spanning tree rooted at \( * \). The label of the spanning tree may be zero depending on \( \hat{\kappa} \). Hence blanket condition (i) is satisfied if and only if there is a directed path from any intermediate species to \( * \) with positive label.

The cycles of \( \mathcal{G}_{\hat{\kappa}} \) are of two kinds. A cycle is not in \( \Sigma_{\hat{\kappa}} \) if it does not go through \( * \), because the reactions corresponding to the cycle only involve noninteracting species and \( \zeta_* = 0 \). If a
cycle goes through $\ast$, then it contains an edge of the form $\ast \rightarrow Z$. By setting the reaction rate constant of all reactions of this form to zero, we are guaranteed that (4.8) is zero, that is, blanket condition (ii) holds. This straightforwardly implies that $b_0(x) = 0$, hence also $w(x) = 0$. Hence by Corollary 4.4 there exists a singular perturbation reduction and it agrees with the QSS reduction. By Proposition 2.5 the reduced system in slow time is

$$\frac{dx}{d\tau} = a_1(x) - A_0(x)B_0^{-1}(x)b_1(x).$$

By the nature of the reactions, the matrices $A_0(x)$ and $B_0(x)$ are constant in the concentrations $x$.

Before moving to the discussion of realistic examples in the next section, we provide an illustrative example to show that the conditions in Proposition 4.3 are sufficient but not necessary.

**Example 8.** Consider the (artificial) reaction network

$$X_1 + Z_1 \xrightarrow{\kappa_1} 2X_1, \quad X_1 \xrightarrow{\kappa_2} 2X_1 + Z_1, \quad X_1 + Z_1 \xrightarrow{\kappa_3} 0, \quad X_1 \xrightarrow{\kappa_4} Z_1$$

with $Z = \{Z_1\}, \mathcal{X} = \{X_1\}$, and assuming mass-action kinetics. The graph $\mathcal{G}_\kappa$ for $\kappa$ is

![Graph](image)

It has exactly two spanning trees rooted at $\ast$, namely, $Z_1 \overset{\kappa_1}{\longrightarrow} X_1 \overset{\kappa_2}{\longrightarrow} Z_1$ and $Z_1 \overset{\kappa_3}{\longrightarrow} X_1 \overset{\kappa_4}{\longrightarrow} Z_1$, so either of these two coefficients must be nonzero for blanket condition (i) to be fulfilled; see Proposition 4.2. The graph has four cycles, but only two are in $\Sigma_\kappa$, namely,

$$\sigma_1: \quad Z_1 \overset{\kappa_1}{\longrightarrow} X_1 \overset{\kappa_2}{\longrightarrow} Z_1, \quad \sigma_2: \quad Z_1 \overset{\kappa_3}{\longrightarrow} X_1 \overset{\kappa_4}{\longrightarrow} Z_1.$$

For each cycle $\sigma$, $\Gamma(\sigma) = \{\sigma_i\}$. Furthermore, $\zeta_{\sigma_1} = 2$ and $\zeta_{\sigma_2} = -2$; cf. (4.5). For the other two cycles of $\mathcal{G}_\kappa$, $\zeta_{\sigma} = 0$ as the net production of $X_1$ equals zero. We have $I = \emptyset$ as all reactions involve species in $Z$ (cf. (3.1)), and the function on the right hand side of (4.8) is

$$\frac{1}{q(x_1, \kappa)} (2\kappa_1\kappa_2 x_1^2 - 2\kappa_3\kappa_4 x_1^2) = \frac{2x_1^2}{q(x_1, \kappa)} (\kappa_1\kappa_2 - \kappa_3\kappa_4),$$

where $q(x_1, \kappa)$ is positive if blanket condition (i) holds, that is, if at least one of $\kappa_1, \kappa_3$ are positive.

Blanket condition (ii) is fulfilled by choosing, for example, $\kappa_2 = \kappa_4 = 0$ according to Proposition 4.3. However, it is clear that the function also vanishes if $\kappa_1\kappa_2 - \kappa_3\kappa_4 = 0$. This shows that the conditions in Proposition 4.3 are only sufficient and not necessary.

To complete the example, we note that

$$a_0(x_1) = (\kappa_2 - \kappa_4)x_1, \quad A_0(x_1) = (\kappa_1 - \kappa_3)x_1,$$

$$b_0(x_1) = (\kappa_2 + \kappa_4)x_1, \quad B_0(x_1) = -(\kappa_1 + \kappa_3)x_1.$$
hence
\[ w(x_1) = B_0(x_1)^{-1} b_0(x_1) = -\frac{\hat{\kappa}_2 + \hat{\kappa}_4}{\hat{\kappa}_1 + \hat{\kappa}_3}, \quad \Delta(x_1) = B_0(x_1) = -(\hat{\kappa}_1 + \hat{\kappa}_3)x_1, \]
and a singular perturbation reduction exists according to Lemma 2.2(a) if and only if \( \hat{\kappa}_1 \hat{\kappa}_2 - \hat{\kappa}_3 \hat{\kappa}_4 = 0 \) and at least one of \( \hat{\kappa}_1, \hat{\kappa}_3 \) is positive. Finally, according to Proposition 2.5, the singular perturbation reduction and the QSS reduction agree since \( w \) is constant.

5. Examples and applications. In this section, we consider realistic and relevant reaction networks. In the examples, we determine possible singular perturbation reductions and compare them to the QSS reduction. The starting linear system is as given in (3.5), and we use the notation from (3.6) and (3.7). Recall the matrix \( \Delta \) and function \( w \) introduced in (2.13) and (2.11), respectively. In each example, for different noninteracting sets \( Z \), we construct the multidigraph \( G_Z \) defined in section 4.1, and find the spanning trees rooted at the different nodes to verify blanket condition (i) via Proposition 4.2. We proceed to verify blanket condition (ii) via Proposition 4.3, by finding the sets \( \Sigma_{\hat{\kappa}_1} \) given in (4.6). We make use of the set \( I \) defined in (3.1).

5.1. The Michaelis–Menten mechanism. For the purpose of illustration, we will discuss the standard enzyme-substrate mechanism for some choices of noninteracting sets with mass-action kinetics. (Note that all possible QSS and singular perturbation reductions of this system are discussed in [11, 12].) The mechanism is
\[ E + S \xrightleftharpoons[\kappa_2]{\kappa_1} C \xrightarrow{\kappa_3} E + P. \]
There are two linear first integrals, which are given by the stoichiometry, namely, \( x_E + x_C \) and \( x_C + x_S + x_P \). The associated ODE system in \( \Omega_\kappa = \mathbb{R}^n_{\geq 0} \) is
\[
\begin{align*}
\dot{x}_E &= -\kappa_1 x_E x_S + (\kappa_2 + \kappa_3) x_C, \\
\dot{x}_C &= \kappa_1 x_E x_S - (\kappa_2 + \kappa_3) x_C, \\
\dot{x}_S &= -\kappa_1 x_E x_S + \kappa_2 x_C, \\
\dot{x}_P &= \kappa_3 x_C.
\end{align*}
\]

We now consider three nonequivalent choices of noninteracting sets, namely, \( Z = \{ S \} \), \( Z = \{ E, C \} \), \( Z = \{ P \} \). Table 1 summarizes the data related to the analysis of each of the sets.

**Case \( Z = \{ S \} \).** By Proposition 4.2, blanket condition (i) holds if and only if \( \hat{\kappa}_1 > 0 \). Using the data in Table 1 and Proposition 4.3, blanket condition (ii) holds if and only if \( \hat{\kappa}_3 = 0 \).

Now let \( \hat{\kappa}_1 > 0 \) and \( \hat{\kappa}_3 = 0 \). We verify the extra condition in Proposition 3.4 and we find
\[ B_0(x) = -\hat{\kappa}_1 x_E, \quad w(x) = B_0(x)^{-1} b_0(x) = -\frac{\hat{\kappa}_2 x_C}{\hat{\kappa}_1 x_E}. \]
If \( \hat{\kappa}_2 = 0 \), then \( w = 0 \) and by Proposition 3.4 a singular perturbation reduction exists and agrees with the QSS reduction.

If \( \hat{\kappa}_2 > 0 \), then Proposition 3.4 is not informative. We find
\[
\Delta(x) = -\left( \hat{\kappa}_1 x_E + \frac{\hat{\kappa}_2 x_C}{x_E} + \hat{\kappa}_2 \right),
\]
Table 1
Noninteracting sets and related objects for the Michaelis–Menten mechanism in section 5.1. Cycles σ of \( \mathcal{G}_\emptyset \) that are not in \( \Sigma_\emptyset \) fail the condition \( \zeta_\sigma \neq 0 \); cf. (4.5).

<table>
<thead>
<tr>
<th>( Z )</th>
<th>( \mathcal{G}_\emptyset )</th>
<th>( \Theta_{+0}(+) )</th>
<th>( \Theta_{+,1}(V) )</th>
<th>( \Sigma_\emptyset )</th>
<th>( I )</th>
</tr>
</thead>
<tbody>
<tr>
<td>{S}</td>
<td>( S \xrightarrow{\eta_1 x_S} \eta_2 x_C ), *</td>
<td>( S \xrightarrow{\eta_1 x_S} \eta_2 x_C ), *</td>
<td>–</td>
<td>\emptyset</td>
<td>{3}</td>
</tr>
<tr>
<td>{E,C}</td>
<td>( E \xrightarrow{\widetilde{\eta}_2} \widetilde{\eta}_1 x_S ), ( \widetilde{\eta}_3 ), ( C )</td>
<td>( E \xrightarrow{\widetilde{\eta}_1 x_S} \underline{C} ), ( C \xrightarrow{\widetilde{\eta}_2} \underline{E} )</td>
<td>( E \xrightarrow{\widetilde{\eta}_1 x_S} \underline{C} ), ( \underline{C} \xrightarrow{\widetilde{\eta}_3} \underline{E} ), ( \emptyset )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>{P}</td>
<td>( \kappa_3 x_P \rightarrow P )</td>
<td>( \emptyset )</td>
<td>–</td>
<td>\emptyset</td>
<td>( \emptyset )</td>
</tr>
</tbody>
</table>

and a singular perturbation reduction exists according to Lemma 2.2(b). However, the reduction agrees with the QSS reduction only in a degenerate setting: By Proposition 2.5, we find that for the two reductions to agree, identity (2.17) must be satisfied. With

\[
A_0 B_0^{-1} (B_1 w - b_1) = \begin{bmatrix} -1 & 1 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \widetilde{\eta}_2 \\ \kappa_1 - \kappa_2 \end{bmatrix} x_C, \quad A_1 w - a_1 = \begin{bmatrix} -1 & 1 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} \widetilde{\eta}_2 \\ \kappa_1 - \kappa_2 \end{bmatrix} x_C + \begin{bmatrix} 1 \\ -1 \end{bmatrix} \kappa_3^* x_C,
\]

this holds if and only if \( \kappa_1^* = 0 \). However, this implies \( \kappa_3 = \widetilde{\kappa}_3 + \varepsilon \cdots \) for the curve in parameter space, hence the reduced system is trivial, providing no information. (Moreover, if one makes the obvious choice \( \kappa_3 = \widetilde{\kappa}_3 \), then the last reaction of the mechanism does not occur at all.)

**Case** \( Z = \{E,C\} \). With the data in Table 1, the only cycle \( \sigma \in \Sigma_\emptyset \) belongs to the connected component with the linear first integral \( x_E + x_C = \alpha_1 \). The set \( \Gamma(\sigma) \) contains only \( \sigma \). By Proposition 4.3, in conjunction with blanket condition (i), blanket condition (ii) holds if and only if \( \alpha_1 \widetilde{\kappa}_1 \widetilde{\kappa}_3 x_S = 0 \). The case that \( x_S \) vanishes identically may be dismissed, since it would amount to \( Z = \{E,C,S\} \). We are left with the following scenarios:

- \( \widetilde{\kappa}_1 > 0 \), and \( \widetilde{\kappa}_3 = 0 \) or \( \widetilde{\alpha}_1 = 0 \);
- \( \widetilde{\kappa}_2 > 0 \), and \( \widetilde{\kappa}_1 = 0 \) or \( \widetilde{\kappa}_3 = 0 \) or \( \widetilde{\alpha}_1 = 0 \);
- \( \widetilde{\kappa}_3 > 0 \), and \( \widetilde{\kappa}_1 = 0 \) or \( \widetilde{\alpha}_1 = 0 \).

By substituting \( x_C = \alpha_1 - x_E \), we are in the setting of (2.9) and (3.7) with

\[
x = \begin{bmatrix} x_S \\ x_P \end{bmatrix} \quad \text{and} \quad z = x_E,
\]

and

\[
b_0 = (\widetilde{\kappa}_2 + \widetilde{\kappa}_3) \widetilde{\alpha}_1, \quad B_0 = -\widetilde{\kappa}_1 x_S - (\widetilde{\kappa}_2 + \widetilde{\kappa}_3), \quad a_0 = \widetilde{\alpha}_1 \begin{bmatrix} \widetilde{\kappa}_2 \\ \widetilde{\kappa}_3 \end{bmatrix}, \quad A_0 = \begin{bmatrix} -\widetilde{\kappa}_1 x_S - \widetilde{\kappa}_2 \\ -\widetilde{\kappa}_3 \end{bmatrix}.
\]

Moreover

\[
w(x) = \frac{- (\widetilde{\kappa}_2 + \widetilde{\kappa}_3) \widetilde{\alpha}_1}{\widetilde{\kappa}_1 x_S + (\widetilde{\kappa}_2 + \widetilde{\kappa}_3)}.
\]

In the cases where \( \widetilde{\alpha}_1 = \widetilde{\kappa}_2 = \widetilde{\kappa}_3 = 0 \), or \( \widetilde{\alpha}_1 \neq 0 \) but \( \widetilde{\kappa}_1 = 0 \), \( w(x) \) is constant and by Proposition 3.4, a singular perturbation reduction exists and agrees with the QSS reduction.
The only scenario left to analyze is when \( \hat{\kappa}_3 = 0 \) and the remaining parameters are positive, thus we have a curve \( \varepsilon \hat{\kappa}_3^* \) in parameter space. One easily checks that \( \Delta(x) < 0 \) for all \( x \). But identity (2.17) would imply (after some computation) that
\[
\kappa_3^* \left( \frac{\hat{\kappa}_2}{\kappa_1 S + \hat{\kappa}_2} - 1 \right) = 0.
\]
This yields a contradiction unless \( \kappa_3^* = 0 \), but the latter characterizes the degenerate case that the last reaction does not occur at all.

**Case \( Z = \{ P \} \)**. This is a case where no singular perturbation reduction (and no QSS reduction) exists, as blanket condition (i) cannot be satisfied by Proposition 4.2; cf. Table 1.

### 5.2. A predator-prey system

The following three dimensional predator-prey system was introduced and discussed in [14] in the course of a first-principle derivation of the two dimensional Rosenzweig–MacArthur system:

\[
\begin{align*}
\dot{x}_B &= \kappa_1 x_B (1 - x_B) - \kappa_2 x_B x_H, \\
\dot{x}_S &= -\kappa_3 x_S + \kappa_4 x_B x_H, \\
\dot{x}_H &= \kappa_3 x_S - \kappa_4 x_B x_H + \kappa_5 x_S - \kappa_6 x_H
\end{align*}
\]

with nonnegative parameters \( \kappa_1, \ldots, \kappa_6 \). Here \( x_B \) stands for the abundance of species \( B \), the prey, while \( x_S \), respectively \( x_H \), are abundances of the species \( S \) and \( H \) (resting and hunting predators). The three dimensional system is obtained from an individual based stochastic model (see [14, section 2]), upon scaling the abundance of prey. All parameter values \( \hat{\kappa} \) leading to dimension two singular perturbation reductions and the corresponding reductions, were determined in [14] and its supplementary material. We investigate here what types of reductions arise by means of sets of noninteracting sets and Propositions 4.2 and 4.3.

The ODE may be considered to arise from reaction networks with mass-action kinetics in different ways. We make a choice different from [14] and consider, for example,

\[
\begin{align*}
B &\xrightarrow{\kappa_7} 2B, & B + H &\xrightarrow{\kappa_2} H, & S &\xrightarrow{\kappa_3} H, \\
B + H &\xrightarrow{\kappa_4} B + S, & S &\xrightarrow{\kappa_5} S + H, & H &\xrightarrow{\kappa_6} 0
\end{align*}
\]

with \( \kappa_1 = \kappa_7 \). The network has only two sets of noninteracting species, namely, \( Z = \{ H \} \) and \( Z = \{ S \} \). (The union of these two sets is not a noninteracting set, and neither is \( \{ B \} \). According to [14] there exist QSS reductions with respect to \( B \), hence our approach will not retrieve all possible QSS reductions.)

We will provide a brief analysis of the two noninteracting sets using Propositions 4.2 and 4.3, and compare the results to the detailed analysis carried out in [14]. In that paper it is shown that if \( \hat{\kappa}_3 = 0 \), then \( \hat{\kappa}_2 \hat{\kappa}_3 \hat{\kappa}_6 = 0 \) is a necessary condition for the existence of a singular perturbation reduction. The condition is further divided into 11 cases specifying precisely the parameters that are zero and those that are not in order to obtain validity. We will discuss these cases from the perspective of Propositions 4.2 and 4.3.
4.3 of [14] shows that a singular perturbation reduction exists but is not in agreement with the QSS reduction. Whenever \( \kappa > 0 \), subsection 4.3 of [14] shows that a singular perturbation reduction exists but is not in agreement with the QSS reduction. Here we have retrieved cases 5 and 9 of [14, section 3.4.2].

We see that the second summand of (4.8) vanishes when \( \kappa = 0 \), which gives rise to a new set of parameters satisfying blanket conditions (i) and (ii):

- \( \kappa > 0 \), \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_4 = \kappa_6 = 0 \).

In this case, \( w(x) = 0 \) and hence a singular perturbation reduction exists and agrees with the QSS reduction by Proposition 3.4; the supplementary material to [14] shows that the reduced system is of Volterra–Lotka type. This is case 8 from [14, section 3.4.2].

Case \( \mathcal{Z} = \{ S \} \). By Table 2 and Proposition 4.2, blanket condition (i) holds if and only if \( \Omega^+_{\kappa,0}(\sigma) \neq \emptyset \), hence if and only if \( \kappa > 0 \). By Proposition 4.3, using \( I \) and \( \Sigma_{\tilde{\kappa}} \) from Table 2, blanket conditions (i) and (ii) hold for

- \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_5 = \kappa_6 = 0 \).

(as we assume \( \kappa_1 = \kappa_7 \)). If in addition \( \kappa_4 = 0 \), then Corollary 4.4 applies and a singular perturbation reduction exists and agrees with the QSS reduction. Whenever \( \kappa > 0 \), subsection 4.3 of [14] shows that a singular perturbation reduction exists but is not in agreement with the QSS reduction. Here we have retrieved cases 5 and 9 of [14, section 3.4.2].

If we consider the vanishing of (4.8) directly (characterizing blanket condition (ii)), the set \( \Gamma_{\tilde{\kappa}}(\sigma) \) consists of the graph

\[
\{ S \} \quad \frac{S \overset{\kappa_4 > 0}{\rightarrow} S_{\kappa_4 > 0}}{= \emptyset}
\]

We see that the second summand of (4.8) vanishes when \( \kappa_5 \kappa_4 \kappa_6 = 0 \). This holds when \( \kappa_4 = 0 \), which gives rise to a new set of parameters satisfying blanket conditions (i) and (ii):

- \( \kappa > 0 \), \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_4 = \kappa_6 = 0 \).

In this case, \( w(x) = 0 \) and hence a singular perturbation reduction exists and agrees with the QSS reduction by Proposition 3.4; the supplementary material to [14] shows that the reduced system is of Volterra–Lotka type. This is case 8 from [14, section 3.4.2].

Case \( \mathcal{Z} = \{ H \} \). By Table 2 and Proposition 4.2, blanket condition (i) holds if and only if \( \Omega^+_{\kappa,0}(\sigma) \neq \emptyset \), hence if and only if either \( \kappa > 0 \) or \( \kappa > 0 \). Considering \( I \) and \( \Sigma_{\tilde{\kappa}} \) in Table 2, and Proposition 4.3, the following possibilities guarantee that blanket conditions (i) and (ii) are satisfied:

- \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_5 = \kappa_6 = 0 \),
- \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_3 = \kappa_4 = 0 \),
- \( \kappa > 0 \), \( \kappa > 0 \), \( \kappa_1 = \kappa_2 = \kappa_3 = \kappa_5 = 0 \).

If both \( \kappa = 0 \) (as in the third case), then Corollary 4.4 shows the existence of a singular perturbation reduction and agreement with the QSS reduction. If \( \kappa > 0 \) in the first case or

---

Table 2
Noninteracting sets and relevant objects for the network (5.1).

<table>
<thead>
<tr>
<th>( \mathcal{Z} )</th>
<th>( \mathcal{G}_\kappa )</th>
<th>( \Theta^+_{\kappa,0}(\sigma) )</th>
<th>( \Sigma_{\tilde{\kappa}} )</th>
<th>( I )</th>
</tr>
</thead>
<tbody>
<tr>
<td>{ ( S ) }</td>
<td>( { S } )</td>
<td>( S \quad \kappa \rightarrow S )</td>
<td>( \sigma: S \quad \kappa \rightarrow S )</td>
<td>( { 1, 2, 6, 7 } )</td>
</tr>
<tr>
<td>{ ( H ) }</td>
<td>( { H } )</td>
<td>( H \quad \kappa \rightarrow H )</td>
<td>( \sigma_1: H \quad \kappa \rightarrow H )</td>
<td>( { 1, 7 } )</td>
</tr>
</tbody>
</table>

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\( \hat{\kappa}_S > 0 \) in the second case, then a singular perturbation reduction is still obtained but it does not agree with the QSS reduction; see [14, section 4.2]. We have retrieved cases 5, 6, 7, 10, and 11 of [14, section 3.4.2].

If we consider the explicit form of (4.8) as above, we find that \( \Gamma_r(\sigma_1) \) consists of two graphs:

Furthermore, \( \zeta_{\sigma_1} = (-1, 0)^T \). Hence, the term in (4.8) corresponding to this cycle has numerator \(-\hat{\kappa}_5 x_B x_S (\hat{\kappa}_3 + \hat{\kappa}_5) \) and is zero in the \( x_S \) component. For the other two cycles, the sets \( \Gamma_r(\sigma_i) \) contain only the cycle itself and when added together give the term with numerator \((0, -\hat{\kappa}_3 \hat{\kappa}_6 x_B + \hat{\kappa}_4 \hat{\kappa}_5 x_B x_B)^T \). The detailed analysis of the term arising from the cycle \( \sigma_1 \) gives the following cases for which blanket conditions (i) and (ii) hold:

- \( \hat{\kappa}_4 > 0, \hat{\kappa}_3 = \hat{\kappa}_5 = \hat{\kappa}_6 = 0 \),
- \( \hat{\kappa}_6 > 0, \hat{\kappa}_1 = \hat{\kappa}_3 = \hat{\kappa}_5 = \hat{\kappa}_4 = 0 \),
- \( \hat{\kappa}_4 > 0, \hat{\kappa}_6 > 0, \hat{\kappa}_2 > 0, \hat{\kappa}_1 = \hat{\kappa}_3 = \hat{\kappa}_5 = 0 \).

This gives cases 1, 2, and 3 of [14, section 3.4.2]. In all cases \( b_0 = 0 \), hence \( w = 0 \), and we obtain a Tikhonov–Fenichel reduction which agrees with the QSS reduction.

By our approach we could not identify case 4 of the 11 cases listed in [14, section 3.4.2] corresponding to setting all reaction rate constants to zero except \( \hat{\kappa}_2 > 0 \). For an explanation, note that this case amounts to a QSS reduction with QSS species \( B \).

5.3. A two substrate mechanism. We consider a mechanism that consists of two substrates \( A, B \) that are converted into two products \( P, Q \) through a series of reactions catalyzed by an enzyme \( E \); see Cornish-Bowden [4, Chapter 5]. It is an example of a bi-bi mechanism in the notation of Cleland [9]:

\[
E + A \xrightarrow{\kappa_1 \kappa_2} EA \quad EA + B \xrightarrow{\kappa_3 \kappa_4} EAB \xrightarrow{\kappa_5 \kappa_6} EPQ \xrightarrow{\kappa_7 \kappa_8} EQ + P, \quad EQ \xrightarrow{\kappa_9 \kappa_{10}} E + Q.
\]

Here, the complexes \( EA, EAB, EQ, EQ \) are seen as intermediate or transient complexes in the transformation of \( A, B \) into \( P, Q \).

We discuss here just one set of noninteracting species, namely, \( Z = \{ Z_1, Z_2, Z_3, Z_4, Z_5 \} \), where \( Z_1 = E, Z_2 = EA, Z_3 = EAB, Z_4 = EPQ, \) and \( Z_5 = EQ \) are all species involving the enzyme \( E \). There is a single linear first integral relating only species in \( Z \), which is \( \lambda(z) = z_1 + z_2 + z_3 + z_4 + z_5 (= \alpha) \). We further assume mass-action kinetics.

Let \( (\kappa, \alpha) = (\hat{\kappa}, \hat{\alpha}) + \varepsilon(\kappa^*, \alpha^*) + \cdots \) be a curve in the joint parameter space with \( \hat{\kappa} \in \mathbb{R}_{\geq 0}^{10} \) and \( \hat{\alpha} \in \mathbb{R}_{\geq 0} \). The noninteracting graph \( G_R \) is

\[
\begin{align*}
* & \xrightarrow{\hat{\kappa}_1 \kappa} Z_1 \xrightarrow{\hat{\kappa}_2 \kappa} Z_2 \xrightarrow{\hat{\kappa}_3 \kappa} Z_3 \xrightarrow{\hat{\kappa}_6 \kappa} Z_4 \xrightarrow{\hat{\kappa}_7 \kappa} Z_5. \\
\end{align*}
\]
The set $\Sigma_{\vec{\kappa}}$ has two cycles: The cycle with the edges with labels $\vec{\kappa}_1, \vec{\kappa}_3, \vec{\kappa}_5, \vec{\kappa}_7$, and $\vec{\kappa}_9$ that meets all nodes clockwise, and the cycle with the edges with the rest of the labels that meets all nodes counterclockwise, $\vec{\kappa}_2, \vec{\kappa}_4, \vec{\kappa}_6, \vec{\kappa}_8$ and $\vec{\kappa}_{10}$. In both cases $\Gamma_{\vec{\kappa}}(\sigma)$ is the cycle itself and the labels are, respectively,

$$\vec{\kappa}_1 \vec{\kappa}_3 \vec{\kappa}_5 \vec{\kappa}_7 \vec{\kappa}_9 x_A x_B \quad \text{and} \quad \vec{\kappa}_2 \vec{\kappa}_4 \vec{\kappa}_6 \vec{\kappa}_8 \vec{\kappa}_{10} x_P x_Q,$$

and furthermore one has $I = \emptyset$ as all reactions involve species in $Z$. Blanket condition (i) is satisfied if there is a spanning tree with positive labels of the connected component with nodes $Z$; see Proposition 4.2. The latter can be achieved in various ways:

- $\vec{\kappa}_2 > 0, \vec{\kappa}_4 > 0, \vec{\kappa}_6 > 0, \vec{\kappa}_8 > 0,$
- $\vec{\kappa}_2 > 0, \vec{\kappa}_4 > 0, \vec{\kappa}_6 > 0, \vec{\kappa}_9 > 0,$
- $\vec{\kappa}_2 > 0, \vec{\kappa}_4 > 0, \vec{\kappa}_7 > 0, \vec{\kappa}_9 > 0,$
- $\vec{\kappa}_2 > 0, \vec{\kappa}_5 > 0, \vec{\kappa}_7 > 0, \vec{\kappa}_9 > 0,$
- $\vec{\kappa}_3 > 0, \vec{\kappa}_5 > 0, \vec{\kappa}_7 > 0, \vec{\kappa}_9 > 0,$

assuming $Z_1$ to be the root, and similarly if any other node is the root. This gives 25 different cases. Blanket condition (ii) holds for all positive $x_A, x_B, x_P, x_Q$ if and only if $\vec{\kappa}_1 \vec{\kappa}_3 \vec{\kappa}_5 \vec{\kappa}_7 \vec{\kappa}_9 = 0$ and $\vec{\kappa}_2 \vec{\kappa}_4 \vec{\kappa}_6 \vec{\kappa}_8 \vec{\kappa}_{10} = 0, \text{ or } \hat{\alpha} = 0$.

We have (with $z_3$ eliminated using the linear first integral)

$$a_0(x) = \begin{bmatrix} 0 \\ \hat{\kappa}_4 \hat{\alpha} \\ 0 \\ 0 \\ 0 \end{bmatrix}, \quad b_0(x) = \begin{bmatrix} 0 \\ \hat{\kappa}_3 \hat{\alpha} \\ \hat{\kappa}_5 \hat{\alpha} \\ 0 \\ 0 \end{bmatrix}.$$  

In particular, if $\hat{\alpha} = 0$ or $\hat{\kappa}_4 = \hat{\kappa}_5 = 0$, then $a_0(x) = b_0(x) = 0$ and a singular perturbation reduction exists and agrees with the QSS reduction. By symmetry of the reactions, the same holds if $\hat{\kappa}_6 = \hat{\kappa}_7 = 0$.

### 5.4. PTM systems.

**Generalities.** We will consider here a generalization of the Michaelis–Menten system in section 5.1 known as PTM systems [7]. Mass-action kinetics is assumed throughout. A PTM system consists of reactions of the form

$$S_i + S_j \xrightleftharpoons{\alpha_{i,j}^\ell}{b_{i,j}^\ell} C_\ell, \quad C_\ell \xrightleftharpoons{c_{\ell,k}}{c_{k,\ell}} C_k, \quad S_i \xrightleftharpoons{d_{i,j}}{d_{j,i}} S_j$$

for $i, j, \ell, m$ varying in some index sets and $a_{i,j}^\ell, b_{i,j}^\ell, c_{\ell,k}, d_{i,j} \geq 0$. (Recall that a reaction rate constant is allowed to be zero in which case the corresponding reaction does not take place.) The species $S_i$ are known as substrates and the species $C_\ell$ as intermediates. The Michaelis–Menten system discussed earlier is one example of a PTM system (in which the enzyme also plays the role of a substrate). PTM systems are found in abundance in biological organisms and PTM is considered a general mechanism for signal transmission [7]. The class of PTM systems also includes the MAPK cascade, a layered network of reactions in which a signal is filtered. These systems play pivotal roles in the modeling of cancers and have been studied extensively in the literature, experimentally as well as mathematically.
We will assume that all intermediate species are degraded in the sense that for any $C_\ell$ there exists a sequence of reactions (with positive rate constants) such that

$$C_\ell \xrightarrow{c_\ell,t_\ell} \cdots \xrightarrow{c_{k-1},t_{k-1}} C_k \xrightarrow{b_{k,j}} S_i + S_j.$$  

Next, we will study some generic cases of noninteracting species sets in the light of Propositions 4.2 and 4.3. Let $\kappa$ denote the vector of parameters and assume $\kappa = \widehat{\kappa} + \varepsilon \kappa^* + \cdots$ is a curve in parameter space with $\widehat{\kappa} \in \mathbb{R}^n_{>0}$. The case where $Z$ only consists of intermediate species was discussed in Remark 2. We consider a generalization of the standard Michaelis–Menten reduction by enzyme and substrate. For this, assume furthermore that

- all intermediate species are produced and degraded, that is, for $C \in C$ (in the set of intermediate species) there is a sequence of reactions such that
  
  $$S_i + S_j \longrightarrow \cdots \longrightarrow C \longrightarrow \cdots \longrightarrow S_k + S_\ell;$$

- $Z = C \cup S$, where $S = \{S_{K+1}, \ldots, S_M\}$ (potentially after relabeling) is a subset of the substrate species. Furthermore, assume the graph $G_\kappa$ has two components, $G_\kappa^0$ with $\mathcal{N}^0 = \{\ast\}$ and $G_\kappa^\ast$. Hence there is a linear first integral

$$\sum_{i : C_i \in C} z_i + \sum_{i : S_i \in S} z_i$$

relating the noninteracting species, and if there is a noninteracting species in the reactant (product) of a reaction, then there is one in the product (reactant) of the same reaction.

Assume that blanket conditions (i) and (ii) are satisfied, for example, by choosing rate constants or conserved amounts such that Propositions 4.2 and 4.3 are applicable. Note that any cycle in $\Sigma_\kappa$ contains both substrates and intermediates. Indeed, a cycle of $G_\kappa$ involving only substrates (resp., intermediates) corresponds to a reaction path with reactions of the form $S_i \longrightarrow S_j$ (resp., $C_i \longrightarrow C_j$), hence the net production of species that are not noninteracting is zero and the cycle is not in $\Sigma_\kappa$.

Since $G_\kappa^0$ contains only the node $\ast$, one has $b_0(x) = 0$ and a singular perturbation reduction exists and agrees with the QSS reduction. Furthermore, it takes the form in Proposition 2.5, where $a_1(x), b_1(x), A_0(x), B_0(x)$ are all linear in $x$, hence the right-hand side of the ODE system is a rational function $p(x)/q(x)$ in $x$ with $p(x), q(x)$ irreducible polynomials in $x$. It follows from [7] that the monomials of $p(x)$ and $q(x)$ only depend on the reactions $S_i \longrightarrow S_j$ and whether $S_i + S_j$ is connected by a reaction path to $S_k + S_\ell$ or not, and not on the chain of intermediate species connecting them nor the structure of the intermediate network as such.

**A class of PTM systems.** As an example, we consider a modified Michaelis–Menten system with enzyme $E$, substrate $S$, product $P$, and an arbitrary number $C_1, \ldots, C_m$ of intermediate complexes (that is, $E, S, P$ are “substrates” in the terminology of the first part). The reactions are

$$E + S \xrightarrow{\kappa_1} C_1, \quad C_i \xrightarrow{\gamma_{ij}} C_j, \quad C_m \xrightarrow{\kappa_2} E + P,$$

and we assume that there is a reaction path from $C_1$ to $C_m$, and mass-action kinetics. We consider the noninteracting set $Z = \{E, C_1, \ldots, C_m\}$ with the linear first integral $\alpha = x_E +$
where the dashed circle represents the edges among the intermediate species and with labels $\gamma_i$. Note that all edges of $G_\kappa$ have a label that is constant in $x_S$, except the edge $E \rightarrow C_1$, which has label $\kappa_1 x_S$.

Blanket condition (i) is satisfied for $\hat{\kappa}$ if the graph $G_{\hat{\kappa}}$ contains at least one spanning tree. All cycles in $\Sigma_{\hat{\kappa}}$ must involve both $E$ and intermediates, and since the net production of either $S$ or $P$ needs to be nonzero, we are left with cycles containing the edges with labels $\hat{\kappa}_1 x_S$ and $\hat{\kappa}_2$.

Hence, in view of Proposition 4.3, assuming the existence of the spanning tree with positive label, $\hat{\alpha} = 0$ or $\hat{\kappa}_1 = 0$ or $\hat{\kappa}_3 = 0$ guarantee that blanket condition (ii) holds. By Corollary 4.4, $\hat{\alpha} = 0$ implies $w = 0$. When $\hat{\kappa}_1 = 0$, $w$ is constant as $v_1(x, \hat{\kappa}) = 0$ and $K_2(x, \kappa)$ becomes constant in $x$. It follows that either $\hat{\alpha} = 0$ or $\hat{\kappa}_1 = 0$ give choices of parameters for which there is a singular perturbation reduction, which further agrees with QSS.

We now find the reduced system in $x_S$, by using the expression in (2.8) and then evaluating at $\hat{\kappa}$. We have

$$\dot{x}_S = -\kappa_1 x_E x_S + \kappa_2 x_{C_1}.$$  

We assume the graph $G_{\hat{\kappa}}$ is strongly connected. Then, by [5], see also [16, 6], the solution to $\dot{x}_C_i = 0$, $i = 1, \ldots, m$, together with the linear first integral is of the form

$$x_E = \frac{\alpha (\kappa_2 \theta_1 + \kappa_3 \theta_m)}{\kappa_1 \delta x_S + \kappa_2 \theta_1 + \kappa_3 \theta_m}, \quad x_{C_i} = \frac{\alpha \kappa_1 \theta_i x_S}{\kappa_1 \delta x_S + \kappa_2 \theta_1 + \kappa_3 \theta_m},$$

where $\theta_i$ is the sum of the labels of all spanning trees of the subgraph of $G_{\hat{\kappa}}$ delimited by the dashed circle in the figure above rooted at $C_i$, and depends only on $\gamma_i$; and $\delta$ is the sum $\theta_1 + \cdots + \theta_m$. Then $\kappa_2 \theta_1 + \kappa_3 \theta_m$ is the sum of the labels of all spanning trees of $G_{\hat{\kappa}}$ rooted at $E$ and $\kappa_1 \theta_i x_S$ is the sum of the labels of all spanning trees of $G_{\hat{\kappa}}$ rooted at $C_i$. The denominator is the sum of all possible spanning trees of $G_{\hat{\kappa}}$. Plugging these expressions into $\dot{x}_S$, we obtain

$$\dot{x}_S = -\kappa_1 x_S \frac{\alpha (\kappa_2 \theta_1 + \kappa_3 \theta_m)}{\kappa_1 \delta x_S + \kappa_2 \theta_1 + \kappa_3 \theta_m} + \frac{\alpha \kappa_1 \theta_1 x_S}{\kappa_1 \delta x_S + \kappa_2 \theta_1 + \kappa_3 \theta_m},$$

This is the QSS reduction of the system, which agrees with the singular perturbation reduction if either $\hat{\alpha} = 0$ or $\hat{\kappa}_1 = 0$, provided $G_{\hat{\kappa}}$ has a rooted spanning tree with positive label. Remarkably, the basic form of the reduced equation (the right-hand side being a quotient of two degree one polynomials) does not depend on the number of intermediates nor on specifics of their interactions, and is identical with the form of the standard Michaelis–Menten equation.
We now look at the specific cases. For $\alpha = 0$, we consider the curve $(\varepsilon \alpha^*, \hat{\kappa}_1, \ldots)$ in parameter space, which in slow time is

$$\frac{dx_S}{d\tau} = \frac{-\hat{\kappa}_1 \hat{\kappa}_3 \alpha^* \hat{\theta}_m x_S}{\hat{\kappa}_1 \delta x_S + \hat{\kappa}_2 \hat{\theta}_1 + \hat{\kappa}_3 \hat{\theta}_m}.$$ 

For $\hat{\kappa}_1 = 0$, we consider the curve $(\hat{\alpha}, \varepsilon \kappa_1^*, \hat{\kappa}_2, \ldots)$, which gives in slow time

$$\frac{dx_S}{d\tau} = \frac{-\kappa_1^* \kappa_3 \hat{\alpha} \delta x_S + \hat{\kappa}_2 \hat{\theta}_1 + \hat{\kappa}_3 \hat{\theta}_m \varepsilon \theta_m}{\hat{\kappa}_2 \hat{\theta}_1 + \hat{\kappa}_3 \hat{\theta}_m}.$$

### Appendix A. Singular perturbations: A brief outline.

Here we give an informal outline on singular perturbation reduction according to Tikhonov [20] and Fenichel [8], and on some related computational issues. In particular, we discuss the coordinate-free setting. In principle, this is contained in Fenichel [8, section 5, (eq. (5.18) and explicitly in Lemma 5.4 in a special case)]; see also Stiefenhofer [19, eq. (2.13) and the geometric discussion therein]. A detailed and complete account is given in the recent monograph by Wechselberger [22, section 5]. As for actual computations, we follow [10].

All functions and vector fields are assumed to be sufficiently differentiable.

1. Consider first a system with small parameter $\varepsilon$ in **standard form**, \[
\begin{align*}
\dot{u}_1 &= \varepsilon f_1(u_1, u_2) + \varepsilon (\ldots), & u_1 \in H \subseteq \mathbb{R}^n, \\
\dot{u}_2 &= f_2(u_1, u_2) + \varepsilon^2 (\ldots), & u_2 \in G \subseteq \mathbb{R}^p.
\end{align*}
\]

Rewritten in **slow time** $\tau = \varepsilon t$ one obtains

$$u_1' = f_1(u_1, u_2) + \cdots, \quad \varepsilon u_2' = f_2(u_1, u_2) + \cdots,$$

where $u_1' = \frac{du_1}{d\tau}$. Given that

- there is a nonempty **critical manifold**
  \[
  \bar{Z} := \left\{ (v_1, v_2)^\top \in H \times G \mid f_2(v_1, v_2) = 0 \right\},
  \]

- there exists $\nu > 0$ such that all eigenvalues of $D_2 f_2(v_1, v_2)$, $(v_1, v_2) \in \bar{Z}$ have real part $\leq -\nu$, where $D_2$ denotes derivative with respect to $v_2$,

then by **Tikhonov’s theorem** there exist $T > 0$ and a neighborhood of $\bar{Z}$ in which, as $\varepsilon \to 0$, all solutions converge uniformly to solutions of

$$u_1' = f_1(u_1, u_2), \quad f_2(u_1, u_2) = 0 \quad \text{on } [t_0, T]$$

with $t_0 > 0$ arbitrary.

For details see the monograph by Verhulst [21, Chapter 8], (in particular Theorem 8.1).
2. More generally, a system may be transformable into standard form, and then admit a singular perturbation reduction, by a coordinate change. We refer to [10] for more about the coordinate-independent version. Thus, we start with a parameter dependent

\[ \dot{x} = h^{(0)}(x) + \varepsilon h^{(1)}(x) + \varepsilon^2 \cdots, \]

with \( x \) in some open subset \( U \subseteq \mathbb{R}^{n+p} \) and \( \varepsilon \) in a neighborhood of 0 in \( \mathbb{R} \). Assume that \( Z := \{ x \in U | h^{(0)}(x) = 0 \} \) has dimension \( n \). This system admits a coordinate transformation into standard form and subsequent singular perturbation reduction near every point of \( Z \) if and only if

(A) \( \text{rank } Dh^{(0)}(x) = p \) for all \( x \in Z \);
(B) for each \( x \in Z \) there exists a direct sum decomposition \( \mathbb{R}^{n+p} = \text{Ker } Dh^{(0)}(x) \oplus \text{Im } Dh^{(0)}(x) \);
(C) for each \( x \in Z \) the nonzero eigenvalues of \( Dh^{(0)}(x) \) have real parts less than \( -\nu < 0 \).

The remaining problem is that an explicit computation of the coordinate transformation is generally impossible. This can be circumvented by the following coordinate-free reduction procedure, which we state for the system

\[ x' = \varepsilon^{-1}h^{(0)}(x) + h^{(1)}(x) + \cdots \]

in slow time. We assume that \( Z \subseteq \mathcal{V}(h^{(0)}) \), the vanishing set of \( h^{(0)} \), satisfies conditions (A), (B), and (C), and let \( a \in Z \). The following hold:

Decomposition: There is an open neighborhood \( U_a \) of \( a \) such that

\[ h^{(0)}(x) = P(x)\mu(x), \quad x \in U_a, \]

with \( \mu(x) \) having values in \( \mathbb{R}^p \), \( P(x) \) having values in \( \mathbb{R}^{(n+p)\times p} \), \( \text{rank } P(a) = p \), \( \text{rank } D\mu(a) = p \), and (without loss of generality) \( \mathcal{V}(h^{(0)}) \cap U_a = \mathcal{V}(\mu) \cap U_a = Z \).

(This is a consequence of the implicit function theorem for the differentiable case.) When \( h^{(0)} \) is rational, then \( P \) and \( \mu \) can be chosen rational, and \( U_a \) is Zariski-open. Note that \( Dh^{(0)}(x) = P(x)D\mu(x) \) on \( Z \). By linear algebra, the nonzero eigenvalues of \( Dh^{(0)}(x) \) and of \( \Delta(x) := D\mu(x)P(x) \) coincide on \( Z \), thus conditions (B) and (C) above are satisfied whenever all eigenvalues of \( \Delta \) have real part \( \leq -\nu \).

Reduction: The system

\[ (A.1) \quad x' = [I_{n+p} - P(x)\Delta(x)^{-1}D\mu(x)] h^{(1)}(x) \]

is defined on \( U_a \) and admits \( Z \) as an invariant set. The restriction to \( Z \) corresponds to the reduction from Tikhonov’s theorem.

3. We turn to the scenario studied in section 2.2, and sketch the proof of Proposition 2.2. For the sake of notational consistency in the appendix (although at the expense of consistency with the main part of the paper), we replace \( x \) in the statement of the proposition by \( x_1 \), and \( z \) by \( x_2 \), and write \( x = (x_1, x_2) \) here. Blanket conditions
(i) and (ii) imply that the critical manifold equals $Z = Y \hat{\pi}$, and is nonempty. The decomposition is obtained as

$$h(0)(x) = \begin{bmatrix} a_0(x_1) + A_0(x_1)x_2 \\ b_0(x_1) + B_0(x_1)x_2 \end{bmatrix} = \begin{bmatrix} A_0(x_1) \\ B_0(x_1) \end{bmatrix} \cdot (w(x_1) + x_2),$$

so $P(x) = \begin{bmatrix} A_0(x_1) \\ B_0(x_1) \end{bmatrix}$ and $\mu(x) = w(x_1) + x_2$. Then

$$D\mu(x) = \begin{bmatrix} Dw(x_1) & I_p \end{bmatrix} \in \mathbb{R}^{p \times (n+p)}, \quad \Delta(x) = D\mu(x)P(x) = Dw(x_1)A_0(x_1) + B_0(x_1)$$

in accordance with the definition in (2.13). Moreover

$$P(x)D\mu(x) = \begin{bmatrix} A_0(x_1) Dw(x_1) & A_0(x_1) \\ B_0(x_1) Dw(x_1) & B_0(x_1) \end{bmatrix},$$

thus condition (A) in item 2 above is satisfied by the invertibility of $B_0$, and the condition in part (a) of the proposition corresponds to (B) and (C).

We note that, in the given situation, reduction formulas provided earlier by Fenichel [8] and Stiefenhofer [19] are also applicable.

4. Finally, we briefly address weaker notions of QSS. As shown in [12, Proposition 2], the minimal requirement for QSS to be consistent for all small perturbations of $\hat{\pi}$ is invariance of the QSS-critical set $Y \hat{\pi}$ for system (2.5) at $\pi = \hat{\pi}$. This requirement guarantees for small perturbations of $\hat{\pi}$ that the $x$-components of solutions to (2.5) with initial value in $Y \hat{\pi}$ remain close to the corresponding solutions of (2.8), and it is also necessary for this property. The invariance condition may be expressed as

$$(Db_0(x) - DB_0(x)(B_0(x)^{-1}b_0(x))) (a_0(x) - A_0(x)B_0(x)^{-1}b_0(x)) = 0, \quad x \in \Omega \hat{\pi}.$$  

To verify this directly, evaluate $\frac{d}{dt}(B_0(x)z + b_0(x)) = 0$ on $Y \hat{\pi}$. See [12] for a general result.

It should be noted, however, that invariance alone is too weak to ensure QSS properties on par with expectations concerning fast-slow timescales.

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**REFERENCES**


