The Number of Equilibrium Points of Perturbed Nonlinear Positive Dynamical Systems (Extended Version)*

Cameron McBride and Domitilla Del Vecchio

Keywords: multistability, nonlinear dynamical systems, degree theory, homotopy methods, genetic circuits, systems biology

Abstract The number of equilibrium points of a dynamical system dictates important qualitative properties such as the ability of the system to store different memory states, and may be significantly affected by state-dependent perturbations. In this paper, we develop a methodology based on tools from degree theory to determine whether the number of equilibrium points in a positive dynamical system changes due to structured state-dependent perturbations. Positive dynamical systems are particularly well suited to describe biological systems where the states are always positive. We prove two main theorems that utilize the determinant of the system’s Jacobian to find algebraic conditions on the parameters determining whether the number of equilibrium points is guaranteed either to change or to remain the same when a nominal system is compared to its perturbed counterpart. We demonstrate the application of the theoretical results to genetic circuits where state-dependent perturbations arise due to fluctuations in cellular resources. These fluctuations constitute a major problem for predicting the behavior of genetic circuits. Our results allow us to determine whether such fluctuations change the genetic circuit’s intended number of steady states.

1 Introduction

The number of equilibrium points of a dynamical system is of general theoretical interest [1–3], and is specifically relevant to applications in systems biology [4, 5], population dynamics [6, 7], electrical systems [8], and, more recently, in synthetic biology [9, 10]. In the design or analysis of systems, simplified models are commonly used due to their tractability; however, perturbations to the dynamics may change the qualitative behavior of the system, destroying the predictions made. Thus, it is of interest to determine whether perturbations to a system’s dynamics change the qualitative behavior. In this paper, we address this issue by focusing on the number of equilibrium points, which is a relevant feature of natural or engineered dynamical systems [8, 11, 12] and of genetic circuits in particular [13–15].

We consider the class of positive dynamical systems—systems where all states are positive—which are commonly used to capture the dynamics of biological systems where the states of the system represent concentrations of chemical species. We present a mathematical framework for determining situations where a positive dynamical system maintains its number of equilibrium points as it is affected by a structured state-dependent perturbation to its dynamics. We present a novel methodology to accomplish this using tools from degree theory [16, 17] by choosing sets such that the determinant of the system’s Jacobian does or does not change sign over the set as the system is perturbed. This methodology of choosing sets enables us to find algebraic conditions on the system’s parameters determining whether the number of equilibrium points does or does not change without having to solve for equilibrium points explicitly. Our first result, Theorem 1, provides conditions guaranteeing that the number of equilibrium points of a system does not change as the perturbations is considered. The next result, Theorem 2, provides conditions guaranteeing that the number of equilibrium points of a system changes as the perturbations are considered. These results give easily verifiable algebraic conditions for determining when nominal dynamics captures the qualitative behavior of a perturbed system and for determining the robustness of the qualitative behavior of a system to structured, state-dependent perturbations.

*This work was supported by National Science Foundation under award no. 1521925
We illustrate the application of our results to gene regulatory networks where state-dependent perturbations arise from changes in the availability of resources necessary for the system to function. Fluctuations in the availability of resources has recently appeared as a major bottleneck to the ability of predicting the behavior of genetic circuits [18–21]. In this work, we provide a predictive tool that can be used to practically design genetic circuits that behave as expected from theory, as we illustrate with application examples.

**Related work.** There is a large body of theoretical work aimed at determining structural conditions for chemical reaction networks under which a chemical network exhibits a single positive steady state, most notably deficiency theory [11, 12, 22, 23]. Unfortunately, many systems of practical interest, such as those considered in this paper, do not have a deficiency of zero or one, so these results are often not applicable. The authors of [24] elaborate on tools of deficiency theory and provide results about the number of equilibrium points of a chemical reaction network; however, they require the system to be described by mass-action kinetics [25], which leads to large systems of ODEs that are prohibitive for design. Other structural conditions exist to provide insight into qualitative changes in dynamical system behavior—most notably, they examine the sign pattern of the Jacobian relating to the signs of cycles in the associated graph of the system [14, 15, 26]. However, these methods do not take parameters into account. Related work also exists specifically for monotone systems [5].

This paper is organized as follows. We first present a motivating example in Section 2. Next, we formulate the problem, provide mathematical background, and state our main results in Sections 3, 4, and 5. Finally, in Section 6, we demonstrate the theoretical results through examples of genetic circuits with resource sharing to illustrate the practical relevance of these results.

# 2 Problem Motivation

The general problem of when state-dependent perturbations change the qualitative behavior of a dynamical system (i.e., the number of equilibrium points) is of relevance to many application domains. In this section, we illustrate an instance of this problem in the context of gene regulatory networks, in which the perturbation arises due to fluctuations in the amount of resources available to the network, which are necessary for its functioning. Fluctuation in the availability of resources has recently appeared as a major bottleneck to predicting the behavior of genetic circuits, and therefore limits our ability to design circuits that behave as intended [19–21]. In turn, unpredicted changes to the number of equilibrium points may completely disrupt a circuit’s intended function. As an example, consider the toggle switch, which is currently the most widely used genetic circuit in biotechnology applications [27, 28, 7, 28, 7, 28]. It is a bistable system that can switch an output of interest on or off depending on the input. One of its recent applications is in the design of kill switches, which are safety mechanisms embedded in genetically modified cells that trigger cell death if the functionality of the cell has been compromised—resulting in a biohazard [28, 7]. If, due to fluctuations in gene expression resources, the toggle switch becomes monostable, as we show may occur in the following, cell death may not be triggered when needed and harmful cells may be kept alive in the environment.

A standard non-dimensionalized model of the toggle switch realized by mutual activations (Figure 1), in which perturbations in available resources are not included, can be written as follows:

\[
\begin{align*}
\dot{x}_1 &= F_1(u, x_2) - x_1 \\
\dot{x}_2 &= F_2(x_1) - x_2
\end{align*}
\]  

(1)

where \(x_1\) and \(x_2\) represent the concentration of proteins \(x_1\) and \(x_2\), \(u\) represents the concentration of an input, \(F_1(\cdot)\) and \(F_2(\cdot)\) are smooth functions in the form of Hill functions [29] and are continuous, increasing, bounded, and positive for positive inputs. Note that this system is a positive system—all states are nonnegative for all time if the initial condition is positive. Additionally, it is straightforward to show that the states of this system are bounded since \(F_1(\cdot)\) and \(F_2(\cdot)\) are bounded. We will use these properties in proving our results in Section 5. Biological systems require resources such as enzymes for the production and degradation of proteins, which will be referred to throughout the paper as production or degradation resources, respectively. We now consider the same genetic circuit, except we include the fact that production resources are finite. Then, the dynamical system becomes the perturbed system

\[
\begin{align*}
\dot{x}_1 &= F_1(u, x_2) \\
&= \frac{F_1(u, x_2)}{1 + J_1 F_1(u, x_2) + J_2 F_2(x_1)} - x_1 \\
\dot{x}_2 &= F_2(x_1) \\
&= \frac{F_2(x_1)}{1 + J_1 F_1(u, x_2) + J_2 F_2(x_1)} - x_2,
\end{align*}
\]  

(2a, 2b)
as derived in [30] and experimentally validated in [21]. Here $J_1$ and $J_2$ represent the resource demand by proteins $x_1$ and $x_2$. We consider this type of structured, state-dependent perturbations throughout the paper. We now simulate (1) and (2) by slowly varying the input, $u$, and observing the corresponding steady state concentration of the output, $x_2$, shown in Figure 1.

$$F_1(u, x_2) = u + \frac{1+0.0774(x_2)^3}{1+0.01(x_2)^3} \quad \text{and} \quad F_2(x_1) = \frac{1+0.0774(x_1)^3}{1+0.01(x_1)^3}, \quad \text{and} \quad J_1 = J_2 = 0.03.$$

As it can be seen in Figure 1, the two systems have different steady state responses. The nominal system (1) has a region of bistability for $u$ between 0.48 and 1.18, as desired, while the perturbed system (2) has one equilibrium point for all values of $u$. Thus, the state-dependent perturbation causes this nominally bistable system to undergo a change in its number of equilibrium points resulting in the loss of bistability and a failure in the system’s behavior. This difference in the number of equilibrium points between the nominal and perturbed systems is not easily predicted by inspection of the dynamics.

We will investigate general theoretical conditions under which this difference in the steady state landscapes does or does not occur when we compare systems with and without resource sharing effects. We will use these conditions to enable the analysis and design of systems with perturbed dynamics. Using our results, we will identify systems that are robust to unmodeled dynamics such as resource sharing, as well as ensuring that a simplified dynamical system is similar to the full system. This will be shown in Section 6.

### 3 Problem Formulation

In this section, we present a framework to determine the effects of state-dependent perturbations of a general form that can capture the fluctuations in both production and degradation resources in a genetic network. We do so by comparing two systems: a nominal system and a perturbed one. We then represent these two systems as a single parameterized system, and, using this representation, we present easily checkable analytical conditions to address the question of when the number of equilibrium points differ between the nominal and the perturbed systems.

In this section, we present a framework to determine the effects of state-dependent perturbations of a general form that can capture the fluctuations in both production and degradation resources in a genetic network. We do so by comparing two systems: a nominal system and a perturbed one. We then represent these two systems as a single parameterized system, and, using this representation, we present easily checkable
analytical conditions to address the question of when the number of equilibrium points differ between the nominal and the perturbed systems.

We consider a nominal system in the form
\[ \dot{x} = h(x) - \Lambda x, \]  
where \( x \in \mathbb{R}_+^n \), \( h : \mathbb{R}^n \to \mathbb{R}^n \) is bounded and positive for all positive arguments, and \( \Lambda \) is a diagonal matrix with strictly positive entries. Eq. (3) may represent a model of a biomolecular network in the absence of perturbations on production and degradation resources [9]. We now consider the perturbed system
\[ \dot{x} = h(x) \odot \alpha(x) + g(x) - \Lambda x, \]  
where \( \odot \) represents the element-wise product, \( \alpha : \mathbb{R}^n \to \mathbb{R}^n \) may represent a perturbation on production resources, and \( g : \mathbb{R}^n \to \mathbb{R}^n \) may represent a perturbation on degradation resources [21,31,32]. We are interested in comparing the number of equilibrium points of the nominal system (3) and the number of equilibrium points of the perturbed system (4). To this end, consider the two-parameter system
\[ \dot{x} = h(x) \odot [\mathbb{I} + \mu(\alpha(x) - \mathbb{I})] + \lambda g(x) - \Lambda x, \]  
where \( \mathbb{I} \) represents a vector of 1's, and \( \mu, \lambda \in [0,1] \times [0,1] \) are control parameters and are allowed to vary between 0 and 1. For \( \mu = \lambda = 0 \), (5) becomes the nominal system (3), while for \( \mu = \lambda = 1 \), (5) becomes the perturbed system (4). Our goal is to determine conditions under which the nominal system (3) and the perturbed system (4) are guaranteed to have the same number of equilibrium points. This may be addressed by analyzing the number of equilibrium points of the parameterized system (5) as the parameters change between 0 and 1. Thus, the problem of comparing the number of equilibrium points of the systems (3) and (4) may be restated as

**Problem 1.** Determine conditions under which the number of equilibrium points of (5) is guaranteed to be constant or is guaranteed to change as \( \mu \) and \( \lambda \) are varied between 0 and 1.

### 4 Mathematical Preliminaries

We introduce mathematical objects necessary to state our results. Additional mathematical background and all the proofs of lemmas are given in Appendix A.

**Notation.** A domain is an open, connected set in \( \mathbb{R}^n \). A set, \( \Omega \subset \mathbb{R}^n \), is called a bounded domain if it is open, connected, and there exists a ball with finite radius, \( r \), such that \( \Omega \subset B(0,r) \). The closure of a set \( \Omega \) is denoted as \( \overline{\Omega} \), the interior \( \text{int}(\Omega) \) is the largest open set contained in \( \Omega \), and the boundary of a domain \( \Omega \) is denoted as \( \partial \Omega = \overline{\Omega} \setminus \text{int}(\Omega) \). \( x \geq 0, x \in \mathbb{R}^n \) denotes a vector with all components nonnegative. The positive orthant is the set \( \mathbb{R}^n_{\geq 0} = \{x : x \geq 0\} \). Given a family of functions \( f_{\mu,\lambda}(x) \) that are continuous with respect to \( \mu \) and \( \lambda \), we denote the set of zeros as \( S_{\mu,\lambda} = \{x > 0 : f_{\mu,\lambda}(x) = 0\} \) for any fixed \( \mu, \lambda \).

**Definition 1.** Given a \( C^1 \) vector field \( f : \mathbb{R}^n \to \mathbb{R}^n \), a point \( x_0 \in \mathbb{R}^n \), is called degenerate if \( \det \left( \frac{\partial f(x_0)}{\partial x} \right) = 0 \). Additionally, \( x_0 \) is called a degenerate zero if \( f(x_0) = 0 \) and \( \det \left( \frac{\partial f(x_0)}{\partial x} \right) = 0 \).

**Definition 2.** Let \( \Omega \subset \mathbb{R}^n \) be a bounded domain, let \( f : \overline{\Omega} \to \mathbb{R}^n \) be \( C^1 \), and assume \( f \) has no degenerate zeros and has no zeros on the boundary of \( \Omega \). Then the topological degree of \( f \) with respect to zero, or more briefly, the degree of \( f \), is \( \deg(f, \Omega) = \sum_{z \in f^{-1}(0) \cap \Omega} \text{sign} \left( \det \left( \frac{\partial f(z)}{\partial x} \right) \right) \) where \( f^{-1}(0) \) is the set of zeros of \( f \) in \( \Omega \) and \( \text{sign}(\cdot) \) is the sign function.

Lemma 1 provides a condition under which the cardinality of the set of zeros of a family of vector fields is constant.

**Lemma 1.** Consider a bounded domain \( \Omega \subset \mathbb{R}^n \). Let \( f_{\lambda} : \overline{\Omega} \to \mathbb{R}^n \) be a \( C^1 \) family of vector fields and continuous with respect to \( \lambda \). Fix \( \lambda^* \geq 0 \) and assume that, for all \( x \in \partial \Omega \), \( f_{\lambda}(x) \neq 0 \) for every \( \lambda \in [0, \lambda^*] \). If, for all \( \lambda \in [0, \lambda^*] \), \( \det \left( \frac{\partial f_{\lambda}(x)}{\partial x} \right) \neq 0 \) for all \( x \in S_{\lambda} \), then the cardinality of \( S_{\lambda} \) does not depend on \( \lambda \).
Now, consider the system of ordinary differential equations (ODEs)

$$\dot{x} = f(x)$$

(6)

where $x \in \mathbb{R}^n$ and $f : \mathbb{R}^n \to \mathbb{R}^n$ is a $C^1$ vector field. We say a point $x \in \mathbb{R}^n$ is an equilibrium point if $f(x) = 0$.

**Definition 3.** A vector field, $f : \mathbb{R}^n \to \mathbb{R}^n$, is positive invariant if for every $i = 1, \ldots, n$, whenever $x \geq 0$ and $x_1 = 0$, then $f_i(x) \geq 0$. We say that a dynamical system is positive invariant if it has dynamics of the form (6) and $f(x)$ is a positive invariant vector field.

**Definition 4.** Given a domain $\Omega \subset \mathbb{R}^n$, a continuous vector field $h : \Omega \to \mathbb{R}^n$ is bounded over $\Omega$ if there exists an $M \in \mathbb{R}$ such that $\|h(x)\| \leq M$ for all $x \in \Omega$. Given a dynamical system $\dot{x} = f(x)$, where $f : \mathbb{R}^n \to \mathbb{R}^n$ is continuous, a trajectory of the dynamical system is bounded if there exists an $M, T \in \mathbb{R}_{>0}$ such that $\|x(t)\| < M$ for all $t \geq T$. We say a dynamical system is bounded if all trajectories are bounded.

**Definition 5.** A function $g : \mathbb{R}^n \to \mathbb{R}^n$ is mass dissipating if there exists some $m \in \mathbb{R}_{n\geq0}$ such that $m \cdot g(x) \leq 0$ for all $x \in \mathbb{R}_{n\geq0}$.

5 Main Results

We now present the main theoretical results of the paper. Theorem 1 provides a sufficient condition on the determinant of the Jacobian of (5) where if the Jacobian is nonsingular over a set containing all equilibrium points, then the dynamical system is guaranteed not to change its number of equilibrium points as $\mu$ and $\lambda$ are varied. Next, we state a converse theorem, Theorem 2, which provides a condition under which the number of equilibrium points of a dynamical system in the form of (5) changes as $\mu$ and $\lambda$ are varied. This result utilizes a similar check to that in Theorem 1 on the determinant of the Jacobian of (5) over a set containing at least one equilibrium point. The use of these results is illustrated in Section 6. Finally, Theorem 3 finds a set guaranteed to contain at least one equilibrium point for all values of the parameter $\mu$. Before stating our main results, we present a lemma that demonstrates that our general form (5) satisfies all assumptions required by Lemma 1.

**Lemma 2.** Consider the continuous time dynamical system

$$\dot{x} = h(x) \circ [1 + \mu(\alpha(x) - 1)] + \lambda g(x) - \Lambda x \equiv f_{\mu,\lambda}(x),$$

(7)

where $x \in \mathbb{R}_{n\geq0}$, $h : \mathbb{R}^n \to \mathbb{R}^n$ and $g : \mathbb{R}^n \to \mathbb{R}^n$ are positive invariant $C^1$ vector fields, $\alpha : \mathbb{R}^n \to \mathbb{R}^n$ is a $C^1$ vector field, $\Lambda$ is a diagonal matrix with strictly positive diagonal entries. Assume that $0 < \alpha(x) \leq 1$, $g$ is mass dissipating, $h(x)$ has no zeros on the boundary of the positive orthant, and $\dot{x} = f_{0,0}(x)$ is bounded. Fix $\mu^* \in [0,1]$ and $\lambda^* \geq 0$. Then, for all $\mu, \lambda \in [0, \mu^*] \times [0, \lambda^*]$,

(a) (7) is positive invariant;

(b) There exists a positive vector $m$, a positive scalar $M$, and a set $\Omega = \{x \in \mathbb{R}_{n\geq0} : m \cdot (\Lambda x) < M\}$ such that $S_{\mu,\lambda} \subset \text{int}(\Omega);

(c) $\deg(f_{\mu,\lambda}, \Omega) = (-1)^n$ and $S_{\mu,\lambda} \neq \emptyset$.

**Theorem 1.** Consider the dynamical system (7). Choose a fixed $\mu^* \in [0,1]$ and $\lambda^* \geq 0$. If there exists a set $A_{\mu,\lambda} \subset \mathbb{R}_{n\geq0}$ such that $S_{\mu,\lambda} \subset A_{\mu,\lambda}$ and $\det\left(\frac{\partial f_{\mu,\lambda}(x)}{\partial x}\right) \neq 0$ for all $\mu, \lambda \in [0, \mu^*] \times [0, \lambda^*]$, then $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu^*,\lambda^*}(x)$ have the same number of equilibrium points in the positive orthant.

**Proof.** Fix $\mu^* \in [0,1]$ and $\lambda^* \geq 0$. By Lemma 2, system (7) is positive invariant and there exists $\Omega \subset \mathbb{R}_{n\geq0}$ such that $S_{\mu,\lambda} \subset \text{int}(\Omega)$ for all $\mu, \lambda \in [0, \mu^*] \times [0, \lambda^*]$ so Lemma 1 may be applied over this $\Omega$ for $\mu^* \in [0,1]$ and $\lambda^* \geq 0$. Choose a set $A_{\mu,\lambda}$ such that $S_{\mu,\lambda} \subset A_{\mu,\lambda}$. Now, fix $\lambda = 0$ and vary $\mu$ from 0 to $\mu^*$. For each $\mu \in [0, \mu^*]$, if $\det\left(\frac{\partial f_{\mu,0}(x)}{\partial x}\right) \neq 0$ for all $x \in A_{\mu,0}$, then, by Lemma 1, $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu,\lambda^*}(x)$ have the same number of equilibrium points in $\Omega$ and therefore the positive orthant. Next, fix $\mu = \mu^*$ and vary $\lambda$ from
0 to $\lambda^*$. For each $\lambda \in [0, \lambda^*]$, if \( \det \left( \frac{\partial f_{0,0}(x)}{\partial x} \right) \neq 0 \) for all $x \in A_{\mu^*,\lambda}$, then, by Lemma 1, $\dot{x} = f_{\mu^*,0}(x)$ and $\dot{x} = f_{\mu^*,\lambda}(x)$ have the same number of equilibrium points in the positive orthant. Finally, by the transitive property of equality on the numbers of equilibrium points, $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu^*,\lambda}(x)$ have the same number of equilibrium points in the positive orthant. The condition we proved is that $\det \left( \frac{\partial f_{0,0}(x)}{\partial x} \right) \neq 0$ along the path $\mu \in [0, \mu^*], \lambda = 0; \mu = \mu^*, \lambda \in [0, \lambda^*]$. This path is contained in $[0, \mu^*] \times [0, \lambda^*]$, which implies the statement in the theorem.

**Remark 1.** The condition in Theorem 1 must be checked for all $\mu, \lambda \in [0, \mu^*] \times [0, \lambda^*]$. It is not possible to check just the endpoints $(\mu, \lambda) = (0, 0)$ and $(\mu, \lambda) = (\mu^*, \lambda^*)$. For example, (if we suppose $\lambda^* = 0$) System (7) may undergo a pitchfork or saddle-node bifurcation when $\mu = \mu^*/2$. The determinant over $A_{\mu^*,0}$ may be non-zero; however, the number of equilibrium points at $\mu = 0$ and $\mu = \mu^*$ are not guaranteed to be equal.

Special cases of (7) may be considered by letting either $\mu^* = 0$ or $\lambda^* = 0$ and are relevant when considering systems where only production or degradation resources are shared. The construction of the set $A_{\mu^*,\lambda}$ in Theorem 1 allows us to avoid calculating the equilibrium points of the system explicitly. This enables us to provide analytical characterization of conditions to guarantee that the number of equilibrium points remains constant, thus avoiding resorting to numerical methods.

Theorem 1 represents a significant sharpening and generalization of the results presented in [33]. The system considered in [33] is a one-parameter system and is required to be linear and non-degenerate when $\lambda = 0$. Thus, it has one equilibrium point, while in Theorem 1, it is not required for the system with $\mu = \lambda = 0$ to be either linear or to have one equilibrium point. In the case where the system has one equilibrium point when $\mu = \lambda = 0$ and $A_{\mu^*,\lambda} = \mathbb{R}^n_0$. Theorem 1 and the global implicit function theorem are equivalent [34]. However, these two theorems are not equivalent in general: the global implicit function theorem provides conditions under which a system has one equilibrium point, while Theorem 1 guarantees that two systems have the same number of equilibrium points.

A converse theorem is now presented, which provides conditions to guarantee that the number of equilibrium points change as $\mu$ and $\lambda$ are varied.

**Theorem 2.** Consider the dynamical system (7) where $f_{0,0}(x) = 0$ has one solution, $x_{0,0}$, for $x \in \mathbb{R}^n_0$. Denote a nonempty subset $\hat{S}_{\mu,\lambda} \subset S_{\mu,\lambda}$. If there exists a set $B_{\mu^*,\lambda^*}$ for some fixed $\mu^* \in [0, 1]$ and $\lambda^* \geq 0$ such that $\hat{S}_{\mu^*,\lambda^*} \subset \text{int}(B_{\mu^*,\lambda^*})$ and $\text{sign} \left( \det \left( \frac{\partial f_{0,0}(x_{0,0})}{\partial x} \right) \right) \neq \text{sign} \left( \det \left( \frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x} \right) \right)$ for all $x \in B_{\mu^*,\lambda^*}$, then $\dot{x} = f_{\mu^*,\lambda^*}(x)$ has more than one equilibrium point.

**Proof.** Fix $\mu^* \in [0, 1]$ and $\lambda^* \geq 0$. Suppose that the number of equilibrium points is constant and equal to 1 for all $\mu, \lambda \in [0, \mu^*] \times [0, \lambda^*]$. Without loss of generality, assume that when $\mu = \lambda = 0$, $\det \left( \frac{\partial f_{0,0}(x_{0,0})}{\partial x} \right) > 0$ for $x_{0,0} \in S_{0,0}$. Choose $\Omega$ as in Lemma 2. Then, $\deg \left( f_{0,0}, \Omega \right) = +1$ by Lemma 2 and the definition of degree. Now, suppose that there exists a set $B_{\mu^*,\lambda^*}$ such that a nonempty subset $\hat{S}_{\mu^*,\lambda^*} \subset S_{\mu^*,\lambda^*}$ is $\hat{S}_{\mu^*,\lambda^*} \subset \text{int}(B_{\mu^*,\lambda^*})$, and suppose $\text{det} \left( \frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x} \right) < 0$ for all $x \in \text{int}(B_{\mu^*,\lambda^*})$. Then $\deg \left( f_{\mu^*,\lambda^*}, \Omega \right) < 1$. This is a contradiction since, by Theorem 4, $\deg \left( f_{0,0}, \Omega \right) = \deg \left( f_{\mu^*,\lambda^*}, \Omega \right) = 1$. Therefore, the number of equilibrium points of $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu^*,\lambda^*}(x)$ are different. Furthermore, since $\deg \left( f_{0,0}, \Omega \right)$ is odd, then $\dot{x} = f_{\mu^*,\lambda^*}(x)$ must have an odd number of equilibrium points strictly greater than one by Theorem 4 since the degree over $\Omega$ constant. Note that there exists at least one degenerate point for some $(\mu, \lambda) \in [0, \mu^*] \times [0, \lambda^*]$; however, Theorem 4 still applies, since Theorem 4 applies for more general definitions of degree that allows for the existence of degenerate points.

Theorem 2 allows us to analytically find conditions under which the number of equilibrium points change as $\mu$ or $\lambda$ are varied. The condition that the system $\dot{x} = f_{0,0}(x)$ has one equilibrium point rules out all local bifurcations in which equilibrium points collide and exchange stability properties without changing the number of equilibrium points present overall (e.g. transcritical bifurcations [3]). Using Theorems 1 and 2, it is possible to determine conditions under which the number of equilibrium points change or remain constant as $\mu$ and $\lambda$ are varied. Theorems 1 and 2 are applied to a few examples in Section 6.

We now present a result that characterizes the region in which an equilibrium point of (5) resides. This result is helpful for choosing $A_{\mu,\lambda}$ as required by Theorem 1 when $\lambda^* = 0$ to guarantee that (7) maintains its number of equilibrium points as $\mu$ is varied.
Definition 6. A (possibly nonsymmetric) square matrix $A$ is positive (negative) semidefinite, denoted by $A \succeq 0$ ($A \preceq 0$), if $x^T Ax \geq 0$ ($x^T Ax \leq 0$).

Note that in (7), if all elements of $\alpha(x)$ are the same, then $\alpha(x)$ may be considered to be scalar and the element-wise product becomes scalar multiplication. In the following theorem, this is the case, i.e. $\alpha : \mathbb{R}^n \to \mathbb{R}$.

Theorem 3. Consider a dynamical system in the form

$$\dot{x} = [1 + \mu(\alpha(x) - 1)] h(x) - \Lambda x \triangleq f_\mu$$

for $x \in \mathbb{R}^n_{\geq 0}$, where $h : \mathbb{R}^n \to \mathbb{R}^n$ is $C^1$ and positive invariant and has no zeros on the boundary of the positive orthant, $\alpha : \mathbb{R}^n \to \mathbb{R}$ is $C^1$ and $0 < \alpha(x) \leq 1$ for all $x \in \mathbb{R}^n_{\geq 0}$, and $\Lambda$ is a diagonal matrix with strictly positive entries. Fix $\mu^* \in [0, 1]$. If $\frac{\partial f_\mu}{\partial x} = \frac{\partial h}{\partial x} - \Lambda + \mu ((\alpha(x) - 1) \frac{\partial h}{\partial x} + h(x) \frac{\partial \alpha}{\partial x}) \preceq 0$ for all $\mu \in [0, \mu^*]$ and for all $x \in \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ for some $x_0 \in S_0$, then there exists exactly one equilibrium point, $x_\mu$, such that $x_\mu^T \Lambda x_\mu \leq x_0^T \Lambda x_0$ for all $\mu \in [0, \mu^*]$.

Proof. First, (8) is positive invariant by Lemma 2, and may be written as $\dot{x} = f_\mu(x)$. Setting $\dot{x} = 0$ and differentiating $f_\mu(x) = 0$ with respect to $\mu$ (which can be done since $h$ and $\alpha$ are $C^1$ and $\mu$ appears linearly in $f_\mu$) gives

$$\frac{\partial f_\mu}{\partial x} \frac{\partial x_\mu}{\partial \mu} + \frac{\partial f_\mu}{\partial \mu} = 0$$

where $\frac{\partial f_\mu}{\partial \mu} = (\alpha(x_\mu) - 1) h(x_\mu)$. Then, rearranging (9), substituting, and multiplying both sides by $\frac{\partial x_\mu}{\partial \mu}^T$, we have

$$\frac{\partial x_\mu}{\partial \mu}^T \left( \frac{\partial f_\mu}{\partial x} \right) \frac{\partial x_\mu}{\partial \mu} = (1 - \alpha(x_\mu)) \frac{\partial x_\mu}{\partial \mu}^T h(x_\mu).$$

Additionally, when $\dot{x} = 0$ in (8), we have

$$h(x_\mu) = \left( \frac{1}{1 + \mu(\alpha(x_\mu) - 1)} \right) \Lambda x_\mu$$

at the equilibrium point, $x_\mu$. Then, substituting (11) into (10) gives $\frac{\partial x_\mu}{\partial \mu}^T \left( \frac{\partial f_\mu}{\partial x} \right) \frac{\partial x_\mu}{\partial \mu} = \frac{1 - \alpha(x_\mu)}{1 + \mu(\alpha(x_\mu) - 1)} \frac{\partial x_\mu}{\partial \mu}^T \Lambda x_\mu$.

Fix $\mu^* \in [0, 1]$ and suppose there exists a set $D \subset \mathbb{R}^n_{\geq 0}$ such that $x_\mu \in D$ and $|\frac{\partial f_\mu(x)}{\partial x}|_{x \in D} \preceq 0$ for all $\mu \in [0, \mu^*]$. Then $\frac{\partial x_\mu}{\partial \mu}^T \left( \frac{\partial f_\mu}{\partial x} \right) \frac{\partial x_\mu}{\partial \mu} \preceq 0$ for all $x \in D$. Since $0 < \alpha(x) \leq 1$, then $\frac{1 - \alpha(x)}{1 + \mu(\alpha(x) - 1)} \geq 0$ for all $x \in \mathbb{R}^n_{\geq 0}$ and all $\mu \in [0, \mu^*]$, which gives $\frac{\partial x_\mu}{\partial \mu}^T \Lambda x_\mu \leq 0$. Integrating by parts gives

$$\int_0^\mu \frac{\partial x_\mu}{\partial \mu}^T \Lambda x_\mu d\mu = x_\mu^T \Lambda x_\mu|_0^\mu - \int_0^\mu x_\mu^T \Lambda \frac{\partial x_\mu}{\partial \mu} d\mu \leq 0,$$

and, since $\Lambda$ is symmetric, this implies that

$$\int_0^\mu \frac{\partial x_\mu}{\partial \mu}^T \Lambda x_\mu d\mu = \frac{1}{2} (x_\mu^T \Lambda x_\mu - x_0^T \Lambda x_0) \leq 0.$$

In particular, $x_\mu^T \Lambda x_\mu \preceq x_0^T \Lambda x_0$. Additionally, $D$ exists and $D = \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ since we have just shown that $x_\mu \in \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ for all $\mu \in [0, \mu^*]$.

Theorem 3 guarantees that (8) always has one equilibrium point contained in the set $\{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$. Note that it is not required for (8) to have one equilibrium point globally—there may exist other equilibrium points outside the set $\{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$. For systems with one equilibrium point, Theorem 3 may be used in conjunction with Theorem 1 to show that the equilibrium point in the set $\{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ is unique as $\mu$ is varied from 0 to $\mu^*$ by choosing $A_\mu = \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$. We will illustrate this in Section 6 through an example.
6 Application of Theory

In this section, we present application examples to demonstrate the use of the theorems in Section 3 to genetic circuits where fluctuations in production or degradation resources are captured by state-dependent perturbations in the form of $\alpha$ and $g$ in the form of system (5). In Example 6.1, we revisit the design of a genetic toggle switch circuit. Specifically, we use Theorems 1 and 2 to find conditions under which the system has multiple equilibrium points and show that different designs of the genetic toggle switch behave differently when considering perturbations in production resources. We show that one of the toggle switch designs is more robust than the other when these perturbations are considered. In Example 6.2, we consider a genetic cascade and use Theorems 1 and 3 to find conditions under which the system is guaranteed to maintain its number of equilibrium points. Subsequently, we use Theorem 2 to find conditions under which the system with production resource perturbations is guaranteed to change its number of equilibrium points.

In Example 6.3, we consider a cascade with degradation resource perturbations and apply Theorem 1 to find conditions under which this system is guaranteed to maintain its number of equilibrium points despite resource perturbation effects. Example 6.3 is a system with four states, illustrating how the theorems in Section 3 apply to higher dimensional systems. To simplify analysis, we use nondimensionalized system equations (See Appendix B.1 for more information on nondimensionalization of genetic circuit models). A general guide for applying the theoretical results to genetic circuits is given in Section 6.4.

6.1 Genetic Toggle Switch

We now revisit the example presented in Section 2 and derive analytical conditions for whether the system does or does not change number of equilibrium points with resource perturbations using our results. This example demonstrates that our theorems may be used even for systems that have multiple equilibrium points when $\mu = \lambda = 0$ to guarantee that the system maintains its number of equilibrium points under resource perturbations. Consider a genetic toggle switch shown in Figure 2. We assume the toggle switch may be perturbed by production resource fluctuations. The normalized, nondimensionalized model of the system with resource perturbations in the form of the parameterized system (7) is given as

\[
\begin{align*}
\dot{x}_1 &= 1 + \mu (F_1(x_2) - 1) - x_1 \\
\dot{x}_2 &= 1 + \mu (F_2(x_1) - 1) - x_2,
\end{align*}
\]  

(12a)

(12b)

where $F_1(\cdot), F_2(\cdot)$ have the properties that $0 < F_1(x), F_2(x) \leq 1$ for all $x \geq 0$, $F_1(\cdot)$ and $F_2(\cdot)$ are monotonic functions, $F_1'(\cdot) > 0$ in the activation toggle, $F_2'(\cdot) < 0$ in the repression toggle, and $\mu \in [0, 1]$ is a parameter. Comparing (12) with (7) gives $h(x) = [1, 1]^T$, $\alpha(x) = [F_1(x_2), F_2(x_1)]^T$, $\Lambda = \text{diag}([1, 1])$, and $g(x) = 0$. Since $g(x) = 0$, we simplify notation of the sets $A_{\mu, \lambda}$ and $S_{\mu, \lambda}$ to $A_\mu$ and $S_\mu$, respectively. The system is affine when $\mu = 0$ with the equilibrium point given by $x = [1, 1]^T$, while when $\mu = 1$, the system has the desired dynamics of the toggle switch. The Jacobian of (12) is given as

\[
\frac{\partial f_\mu}{\partial x} = \begin{bmatrix}
-1 & \mu F_1'(x_2) \\
\mu F_2'(x_1) & -1
\end{bmatrix},
\]  

(13)

and the determinant of the Jacobian is $\det \left( \frac{\partial f_\mu}{\partial x} \right) = 1 - \mu^2 F_1'(x_2) F_2'(x_1)$. We simplify the analysis by assuming that $F_1(\cdot) = F_2(\cdot) = F(\cdot)$. From here, it is possible to see that if $|F'(x)| > 1$, then it is possible for (12) to exhibit multiple equilibrium points by Theorem 2. This condition implies that there are two possible designs to construct a toggle switch: either $x_1$ activates $x_2$ and $x_2$ activates $x_1$ ($F_1(\cdot)$ and $F_2(\cdot) > 0$)—which

![Figure 2: Diagrams of possible toggle switch designs. (a) Activation toggle switch design. (b) Repression toggle switch design; ⊥ indicates repression.](image)
we call the activation toggle, or \( x_1 \) represses \( x_2 \) and \( x_2 \) represses \( x_1 \) \((F_1(\cdot)\) and \(F_2(\cdot)<0\)—which we call the repression toggle. Note that, without resource perturbations, both repression and activation designs behave the same according to the condition \(|F'(x)| > 1\). Choose \( \Omega \) from Lemma 2, such that \( S_\mu \subset \Omega \).

Then \( \deg(\mu, \Omega) = 1 \), implying that there is always an odd number of equilibrium points and (12) exhibits symmetry implying that the equilibrium points are always symmetric about the line \( x_1 = x_2 \), with at least one equilibrium point always resides on the line \( x_1 = x_2 \), and the determinant of the Jacobian is symmetric about the line \( x_1 = x_2 \). This implies that (12) always has at least one equilibrium point on the line \( x_1 = x_2 \). Additionally, if (12) exhibits exactly one equilibrium point, the sign of the determinant is positive when evaluated at this equilibrium point on the line \( x_1 = x_2 \), while if there are multiple equilibrium points, then there exists at least one equilibrium point on the line \( x_1 = x_2 \) in which the sign of the determinant is negative. By the determinant of (13) and Theorem 2, if

\[
|F'(x)| > 1, \tag{14}
\]

then there exists a set \( \mathcal{B}_1 \) where \( \det \left( \frac{\partial f_1(x)}{\partial x} \right) < 0 \) for all \( x \in \mathcal{B}_1 \). This condition holds for both activation and repression toggle designs. Choose \( \mathcal{B}_1 = \{ x \in \mathbb{R}^n \_0 : |F'(x)| > 1 \} \). Then the conditions of Theorem 2 are satisfied if (14) is satisfied for \( \mu = 1 \) at the equilibrium point on the line \( x_1 = x_2 \) and (12) has multiple equilibrium points. Additionally, if \( \sup_{x > 0} |F'(x)| \leq 1 \), then (14) is never satisfied and (12) has only one equilibrium point by Theorem 1 and choosing \( \mathcal{A}_n = \mathbb{R}^n \_0 \).

Now, we assume the toggle switch may be perturbed by production resource fluctuations. The normalized, nondimensionalized model of the system with resource perturbations in the form of the parameterized system (7) is given as

\[
\begin{align*}
\dot{x}_1 &= F_1(x_2) \left[ 1 + \nu \left( \frac{1}{1 + J_1 F_1(x_2) + J_2 F_2(x_1)} - 1 \right) \right] - x_1, \tag{15a} \\
\dot{x}_2 &= F_2(x_1) \left[ 1 + \nu \left( \frac{1}{1 + J_1 F_1(x_2) + J_2 F_2(x_1)} - 1 \right) \right] - x_2, \tag{15b}
\end{align*}
\]

where \( \nu \in [0, 1] \) is a parameter. For simplicity, we assume symmetry in the dynamical system parameters: \( J_1 = J_2 = J \) and \( F_1(\cdot) = F_2(\cdot) = F(\cdot) \) and that \( F(\cdot) > 0 \) and bounded for positive inputs. Comparing (15) with (7), \( h(x) = [F_1(x_2), F_2(x_1)]^T, \Lambda = \text{diag}(1, 1), \alpha(x) = \frac{1}{1 + J_1 F_1(x_2) + J_2 F_2(x_1)}, \) and \( g(x) = 0 \). Note that (15) has the dynamics of the toggle switch with resource perturbations (2) when \( \nu = 0 \) and satisfies the assumptions of Lemma 2 required to apply Theorem 1: \( h(x) \) has no zeros on the boundary of the positive orthant, \( 0 < \alpha(x) \leq 1 \), and the states of the system are bounded when \( \mu = 0 \) since \( h(x) \) is a bounded, positive function. The determinant of the Jacobian of (15) is given as

\[
\det \left( \frac{\partial f_\mu(x)}{\partial x} \right) = 1 + \frac{2 \nu JF(x)}{(1+2JF(x))^2} F'(x) - a F'(x)^2, \tag{16}
\]

where \( a = (1 - \nu)^2 + \frac{\nu^2}{(1+2JF(x))^2} + \frac{2(1-\nu)(JF(x)-1)}{(1+2JF(x))^2} \). It may be shown that \( a \in (0, 1) \) for all \( \nu \in (0, 1] \) and \( b \in (0, \nu/4) \).

We now compare the two systems—activation and repression toggle switch designs—in the face of production resource perturbations. We define a metric to compare these two designs by comparing the sets over which (16) is negative for any arbitrary value of \(|F'(x)| \) giving the possibility of multiple equilibrium points by Theorems 1 and 2. Let \( \mathcal{B}_1 = \{ x \in \mathbb{R}^2 \_0 : \det \left( \frac{\partial f_\mu}{\partial x} \right) < 0 \} \) for both designs, i.e. the set over which (16) is negative as in Theorem 2.

**Definition 7.** Suppose that, for some continuous functions \( g_r(x) \) and \( g_a(x), \mathcal{B}_1 \) is nonempty for the repression toggle if and only if \( |F'(x)| \geq g_r(x) \) and for the activation toggle if and only if \( F'(x) \geq g_a(x) \). Then, we say that the repression toggle design is **uniformly better** over the positive orthant than the activation toggle design if

\[
g_r(x) \geq g_a(x) \quad \text{for each } x \in \mathbb{R}^2 \_0, \tag{17}
\]

and the activation toggle design is uniformly better if the inequality is reversed.
By this metric, if the repression toggle design is uniformly better, then, for every value of \(|F'(x)|\) that gives the activation toggle design a nonempty \(B_1\), gives the repression toggle a nonempty \(B_1\), and vice-versa if the activation toggle design is uniformly better. This definition allows us to compare the performance of the two different toggle designs despite the fact that the location of the equilibrium points may not be the same in both systems. Assume that (15) with \(\nu = 0\) has multiple equilibrium points, i.e. the system without resource perturbations behaves as a functioning toggle. From (16), if
\[
a(F'(x))^2 - bF'(x) - 1 > 0
\]
for all \(\nu \in [0,1]\), then there exists a set \(A_\nu\) such that the Jacobian is never singular for any \(x \in A_\nu\) and \(S_\nu \subset A_\nu\) for all \(\nu \in [0,1]\). Then, by Theorem 1, continuity of the determinant of the Jacobian, and connectedness of the set \(\{x \in \mathbb{R}^2_{\geq 0} : |F'(x)| > c\}\) for some constant \(c\), system (15) has 3 equilibrium points. Furthermore, if (18) is satisfied, there exists a region in the positive orthant where the determinant of the Jacobian is negative and at least one equilibrium point exists in this region. To find this region and conditions on \(F'(x)\) such that the system with \(\nu = 1\) has multiple equilibrium points, we solve the quadratic equation in (18) with equality for \(F'(x)\). The two solutions are
\[
\gamma^\pm = \frac{b \pm \sqrt{b^2 + 4a}}{2a},
\]
which are always real because \(a\) and \(b\) are positive. Since \(a > 0\), (18) is satisfied if \(F'(x) > \gamma^+\) or if \(F'(x) < \gamma^-\). Additionally, \(\gamma^+ > 0\), \(\gamma^- < 0\), and \(\gamma^+ + \gamma^- = \frac{b}{a} > 0\) since \(a \in (0,1]\) and \(b > 0\), implying that \(|\gamma^+| > |\gamma^-|\) for all \(x \in \mathbb{R}^2_{\geq 0}\). Then, by (17), the repression toggle design is uniformly better when considering resource perturbations than the activation toggle design. Note that if the equilibrium points were found numerically, it would not be possible to compare the activation and repression toggle designs for robustness to resource perturbations.

6.2 Genetic Cascades

We consider a two-node cascade with a shared pool of production resources, except we consider a more general situation where the arrow between \(x_1\) and \(x_2\) may be either activation or repression. The reduced, nondimensionalized dynamics in the form of (7) are given as
\[
\begin{align*}
x_1 &= F_1(u) \left[1 + \mu \left(\frac{1}{1 + J_1 F_1(u) + J_2 F_2(x_1) - 1}\right)\right] - x_1 \tag{19a} \\
x_2 &= F_2(x_1) \left[1 + \mu \left(\frac{1}{1 + J_1 F_1(u) + J_2 F_2(x_1) - 1}\right)\right] - x_2 \tag{19b}
\end{align*}
\]
where \(F_i(\cdot)\) are \(C^1\), monotonic, positive, bounded functions and \(F_i(0) > 0\) for \(i = 1, 2\). Comparing (19) with (7) gives \(h(x) = [F_1(u), F_2(x_1)]^T\), \(\alpha(x) = \frac{1}{1 + J_1 F_1(u) + J_2 F_2(x_1)}\), \(\lambda^* = 0\), \(\Lambda = \text{diag}([1, 1])\). In this example, \(\lambda^* = 0\) so we will simplify notation of the sets \(A_{\mu}, A_{\mu,\lambda^*}\) and \(S_{\mu,\lambda^*}\) to \(A_{\mu}\) and \(S_{\mu}\), respectively. Additionally, (19) satisfies the assumptions of Lemma 2 required to apply Theorem 1: \(h(x)\) has no zeros on the boundary of the positive orthant, \(0 < \alpha(x) \leq 1\), and the states of the system are bounded when \(\mu = 0\) since \(h(x)\) is a bounded, positive function. The Jacobian of (19) is given as
\[
\frac{\partial f_{\mu}}{\partial x} = \begin{bmatrix}
-1 - \frac{\mu F_1(u) F_2'(x_1)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} & 0 \\
F_2'(x_1) \left[1 - \mu \left(\frac{J_1 F_1(u) + J_2 F_2(x_1)}{(1 + J_1 F_1(u) + J_2 F_2(x_1))^2}\right)\right] & -1
\end{bmatrix},
\]
and the determinant of the Jacobian of (19) is given as
\[
\det \left(\frac{\partial f_{\mu}}{\partial x}\right) = 1 + \mu \frac{F_1(u) F_2'(x_1)}{(1 + J_1 F_1(u) + J_2 F_2(x_1))^2}. \tag{21}
\]
It can be seen from (21) that if \(F_2'(\cdot) \geq 0\) and choosing \(A_{\mu} = \mathbb{R}^2_{\geq 0}\), then \(A_{\mu}\) contains all equilibrium points and \(\det \left(\frac{\partial f_{\mu}}{\partial x}\right) > 0\) for all \(x \in A_{\mu}\) since all terms in (21) are nonnegative. Under these conditions, \(\dot{x} = f_1(x)\)
and \( \dot{x} = f_o(x) \) have the same number of equilibrium points by Theorem 1. These conditions physically correspond to activation of \( x_2 \) by \( x_1 \), so a two-protein activation cascade with ribosome sharing always has one equilibrium point.

We now use Theorem 3 to find another \( A_\mu \) to satisfy the conditions of Theorem 1. When \( \mu = 0 \), the equilibrium point is \( x_1 = F_1(u); x_2 = F_2(F_1(u)) \) and (19) satisfies the conditions of Theorem 3. Choose \( A_\mu = \{ x \in \mathbb{R}^2_0 : x^2_1 + x^2_2 \leq F_1(u)^2 + F_2(F_1(u))^2 \} \). If

\[
-\mu F_1(u) F_2'(x_1) < (1 + J_1 F_1(u) + J_2 F_2(x_1))^2
\]

then (21) is strictly positive for all \( x \) and

\[
4 + 4\mu \frac{F_1(u) F_2'(x_1)}{(1 + J_1 F_1(u) + J_2 F_2(x_1))^2} > F_2^2(x_1) \left[ 1 - \frac{J_1 F_1(u) + 2 J_2 F_2(x_1) + (J_1 F_1(u) + J_2 F_2(x_1))^2}{(1 + J_1 F_1(u) + J_2 F_2(x_1))^2} \right] > 1
\]

for all \( x \in A_\mu \), then, the Jacobian is negative definite over \( A_\mu \) (verified using the principal minors) and, by Theorem 3, \( A_\mu \subseteq \{ x \in \mathbb{R}^2_0 : x^2_1 + x^2_2 \leq F_1(u)^2 + F_2(F_1(u))^2 \} \) for any \( \mu \in [0,1] \). Eq. (22a) and (22b) are satisfied if \( 2 - 2\sqrt{2} < F_2'(x_1) < 2 \) over \( A_\mu \), by solving the quadratic equation in (22b) for \( F_2'(x_1) \) (using the fact that \( 0 < F_1 \leq 1 \)). Furthermore, by Theorem 1, (19) has one equilibrium point.

We now assume that \( F_2'(x) < 0 \) in (19). Observe that if

\[
\sup_{x \geq 0} \{-F_2'(x)\} < \min_{\mu \in [0,1]} \left\{ \inf_{u, x_1 \geq 0} \left\{ \frac{(1 + J_1 F_1(u) + J_2 F_2(x_1))^2}{\mu F_1(u)} \right\} \right\},
\]

then (21) is strictly positive for all \( x \in \mathbb{R}^2_0 \) and all \( \mu \in [0,1] \). The right hand side of (23) is a strictly decreasing function in \( \mu \) with the minimum occurring at \( \mu = 1 \). Then, when \( \mu = 1 \) note that \( x_1 = \frac{F_1(u)}{1 + J_1 F_1(u) + J_2 F_2(x_1)} \) at the equilibrium point, so substituting and simplifying, we have

\[
\sup_{x \geq 0} \{-x F_2'(x)\} < \inf_{u, x_1 \geq 0} \{1 + J_1 F_1(u) + J_2 F_2(x_1)\}.
\]

By substituting parameters for \( F_1(z) = \frac{1+a_1 z^{n_1}}{1+b_1 z^{n_1}} \) and \( F_2(z) = \frac{1+a_2 z^{n_2}}{1+b_2 z^{n_2}} \) in (24) from Appendix B.2, (24) is equivalent to

\[
\frac{n_2}{4} \left( 1 - \frac{a_2}{b_2} \right) < 1 + J_1 \min \left\{ \frac{a_1}{b_1}, \frac{b_1}{a_1} \right\} + J_2 \frac{a_2}{b_2}.
\]

where \( a_1, b_1 \) are parameters belonging to \( F_1(\cdot) \) and \( a_2, b_2, n_2 \) are parameters belonging to \( F_2(\cdot) \). If (24) or (25) is satisfied, then by Theorem 1 with \( \lambda^* = 0 \) and \( A_\mu = \mathbb{R}^2_0 \), (19) has a single equilibrium point. In fact, we can exploit the form of the system to find a tighter set. Observe that since

\[
F_1(u) \left[ 1 + \mu \left( \frac{1}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - 1 \right) \right] \leq F_1(u) \leq 1
\]

\[
F_2(x_1) \left[ 1 + \mu \left( \frac{1}{1 + J_1 F_1(u) + J_2 F_2(x_1)} - 1 \right) \right] \leq F_2(F_1(u)) \leq 1
\]

then any equilibrium point must reside in the set \( \{ x \in \mathbb{R}^2_0 : x_1 \leq F_1(u), x_2 \leq F_2(F_1(u)) \} \). This is a smaller set than \( \{ x \in \mathbb{R}^2_0 : x^T x \leq F_1(u)^2 + F_2(F_1(u))^2 \} \), given by Theorem 3. Thus, if

\[
\max_{0 \leq x \leq F_1(u)} \{-x F_2'(x)\} < \inf_{0 \leq x \leq F_1(u)} \{1 + J_1 F_1(u) + J_2 F_2(x_1)\},
\]

and, if \( b_2 < F_1(u) \), equivalently

\[
\frac{n_2}{4} \left( 1 - \frac{a_2}{b_2} \right) < 1 + J_1 \min \left\{ \frac{a_1}{b_1}, \frac{b_1}{a_1} \right\} + J_2 \frac{1 + a_2}{1 + b_2}.
\]
while if \( b_2 > 1 \), then (27) is equivalent to (25). By Theorem 1, choosing \( \mathcal{A}_\mu = \{ x \in \mathbb{R}^2_{\geq 0} : x_1 \leq F_1(u), x_2 \leq F_2(F_1(u)) \} \), and observing that (27) guarantees \( \det \left( \frac{\partial f}{\partial x} \right) > 0 \) over \( \mathcal{A}_\mu \) for all \( \mu \in [0, 1] \), then \( \dot{x} = f_0(x) \) and \( \dot{x} = f_1(x) \) both have a single unique equilibrium point in the positive orthant.

Now, we use Theorem 2 to find conditions under which (19) has multiple equilibrium points if these exist. Suppose that

\[
\sup_{x \geq 0} \{-x F'_2(x)\} > \sup_{u,x_1 \geq 0} \{1 + J_1 F_1(u) + J_2 F_2(x_1)\},
\]

or, equivalently, substituting \( F_1(z) = \frac{1+a_1 z}{1+b_1 z} \) and \( F_2(z) = \frac{1+a_2 z^2}{1+b_2 z^2} \) from Appendix B.2,

\[
\frac{n_2}{4} \left( 1 - \frac{a_2}{b_2} \right) > 1 + J_1 + J_2,
\]

then it is guaranteed that there exists some \( x \in \mathbb{R}^2_{\geq 0} \) such that \( \det \left( \frac{\partial f_1(x)}{\partial \mu} \right) < 0 \). Choose \( \mathcal{B}_1 = \{ x \in \mathbb{R}^2_{\geq 0} : -x_1 F'_2(x_1) > 1 + J_1 F_1(u) + J_2 F_2(x_1) \} \). From (21) and Theorem 2, if there exists an equilibrium point of (19) with \( \mu = 1 \) in \( \mathcal{B}_1 \), then (19) has multiple equilibrium points. This may be accomplished by choosing \( u \) such that \( x_1 = \frac{J_2}{b_2} \) (which is guaranteed to exist if \( b_2 > 1 + J_1 + J_2 \) since \( \sup_{u \geq 0} \{ F_1(u) \} = 1 \)). Then (19) has multiple equilibrium points for this value of \( u \) by Theorem 2.

We simulated this system with and without resource perturbation effects. Under certain parametric conditions, the system exhibits multiple equilibrium points when resource perturbations are considered. Results of the simulation are shown in Figure 3. Note that the parameters used fail the condition in (28) (meaning we cannot guarantee the system has one equilibrium point) but also the parameters do not satisfy (30).

![Figure 3: Simulation of repression cascade in (1) and (2) showing differing steady state landscapes due to resource perturbations. In the schematic, \( \dagger \) represents repression. Parameters used for the simulation are \( F_1(u) = \frac{1+2 \cdot 10^{-7} u}{1+0.1 u} \) and \( F_2(x_1) = \frac{1+0.02 x_1^2}{1+2 \cdot 10^{-9} x_1^2} \), \( J_1 = 0.25 \), and \( J_2 = 2.5 \).](image)

We have shown that the number of equilibrium points of an activation cascade is more robust to production resource fluctuations than that of a repression cascade. Therefore, if we seek to design a genetic cascade with increasing input/output response, choosing activations is a more robust strategy than choosing repressions. Additionally, the number of equilibrium points of cascades is more robust to production resource fluctuations if the maximum slope of the function \( F_2(\cdot) \) is small. Conversely, if one wishes to create a cascade with multiple equilibrium points due to production resource perturbations, this is guaranteed to be possible in a repression cascade for some parameter conditions if (30) is satisfied for some value of \( u \).
6.3 Genetic Cascade with Degradation Resource Perturbations due to microRNA

This example illustrates how our results can be applied to systems with dimension higher than two. MicroRNA (miRNA) are short RNAs that may bind to sites on mRNA, and with the help of a protein complex known as argonaute, degrade the mRNA transcript [35]. MiRNAs are conserved through this process, and so may be considered a shared resource. MiRNAs have proven to be important in natural genetic systems as well as engineered genetic circuits [36, 37]. We consider a system in which a microRNA degrades the mRNAs of two proteins in a cascade, shown in Figure 4. The set of equations governing this system and derived in Appendix B.3 and nondimensionalized as in Appendix B.1 are given as

\[
\begin{align*}
\dot{m}_1 &= T_1 F_1(u) + \lambda q_1(m_1, m_2) - \gamma m_1 \\
\dot{x}_1 &= m_1 - x_1 \\
\dot{m}_2 &= T_2 F_2(x_1) + \lambda q_2(m_1, m_2) - \gamma m_2 \\
\dot{x}_2 &= m_2 - x_2
\end{align*}
\]

where \(m_1\) and \(m_2\) represent the concentration of mRNAs, \(x_1\) and \(x_2\) represent the concentration of proteins, \(F_1(\cdot)\) and \(F_2(\cdot)\) are normalized nondimensionalized positive monotonic functions, \(u\) is the external input to \(m_1\), and \(\gamma\) represents the ratio of the rate of dilution of mRNA to the rate of dilution of proteins. Additionally, \(q_1\) and \(q_2\) represent degradation of \(m_1\) and \(m_2\) due to miRNA, respectively, and have the form

\[
q_i(m_1, m_2) = \frac{-k_i m_i}{1 + \sum_{j=1}^2 m_j}
\]

for \(i = 1, 2\),

where \(k_i\) is proportional to rate of degradation of the mRNA by microRNA. Then \(q_1\) and \(q_2\) have the following properties:

\[
q_i \leq 0 \quad \text{and} \quad \frac{\partial q_i}{\partial m_j} = \begin{cases} < 0 & \text{if } i = j \\ > 0 & \text{if } i \neq j \end{cases}
\]

Then (31) has the form of (7) with \(h(x) = [F_1, 1, F_2, 1]^T\), \(\mu^* = 0\), \(g(x) = [q_1, 0, q_2, 0]^T\), and \(\Lambda = \text{diag}([\gamma, 1, \gamma, 1])\). Additionally, when \(\lambda = 0\), (31) is bounded by Proposition 1, and (31) satisfies the conditions in Lemma 2. The Jacobian of (31) is given as

\[
\frac{\partial f_\lambda}{\partial x} = \begin{bmatrix}
\lambda \frac{\partial q_1}{\partial m_1} - \gamma & 0 & \lambda \frac{\partial q_1}{\partial m_2} & 0 \\
1 & -1 & 0 & 0 \\
\lambda \frac{\partial q_2}{\partial m_1} & T_2 F_2'(x_1) & \lambda \frac{\partial q_2}{\partial m_2} - \gamma & 0 \\
0 & 0 & 1 & -1
\end{bmatrix},
\]

and The determinant of the Jacobian is given as

\[
det \left( \frac{\partial f_\lambda}{\partial x} \right) = -\lambda \frac{\partial q_1}{\partial m_2} T_2 F_2'(x_1) + \left( \gamma - \lambda \frac{\partial q_1}{\partial m_1} \right) \left( \gamma - \lambda \frac{\partial q_2}{\partial m_2} \right) - \lambda^2 \frac{\partial q_1}{\partial m_2} \frac{\partial q_2}{\partial m_1} > 0
\]

Figure 4: Diagram of a genetic cascade with mRNA degradation by microRNA.
and the second and third terms are positive for all \( \lambda \in [0,1] \) since 
\[
\lambda^2 \left( \frac{\partial q_1}{\partial m_1} \frac{\partial q_2}{\partial m_2} - \frac{\partial q_1}{\partial m_2} \frac{\partial q_2}{\partial m_1} \right) = \frac{\lambda^2 k_1 k_2}{(1+m_1+m_2)^2} \geq 0.
\]
Note that whenever \( \lambda = 0 \), \( \det \left( \frac{\partial f}{\partial x} \right) > 0 \). Then, if \( F'_2(x_1) \leq 0 \), this system exhibits one equilibrium point by Theorem 1 choosing \( A_\lambda = \mathbb{R}^4_{\geq 0} \), since all terms in \( \det \left( \frac{\partial f}{\partial x} \right) \) are positive. Additionally, if
\[
F'_2(x) < \frac{\gamma}{T_2} \left( \gamma + \lambda \left| \frac{\partial q_1}{\partial m_1} \right| + \lambda \left| \frac{\partial q_2}{\partial m_2} \right| \right) \left( \lambda \frac{\partial q_1}{\partial m_2} \right)^{-1}
\]
for all \( x \in \mathbb{R}^4_{\geq 0} \), then there does not exist a region in the positive orthant where the determinant of the Jacobian is zero. Thus, by Theorem 1, it is guaranteed that perturbations due to microRNA cannot cause a change in the number of equilibrium points in a two-node repression cascade, and it is not possible for the number of equilibrium points of a two-node activation cascade to change due to microRNA perturbations unless the slope of \( F'_2(x) \) is large enough, corresponding to very strong activation.

6.4 General Considerations for the Application of Results

We now summarize a recipe for use of the results in Section 3 in the design of engineered circuits and analysis of natural systems.

1. If the user can verify that the determinant of the Jacobian is nonzero over the entire positive orthant, then by choosing \( A_{\mu,\lambda} = \mathbb{R}^n_{\geq 0} \), Theorem 1 may be applied, proving that (7) has the same number of equilibrium points in the positive orthant for any \( \mu, \lambda \in [0, \mu^*] \times [0, \lambda^*] \).

2. If there does exist at least one point in the positive orthant in which the determinant of the Jacobian is zero, then one must find a set \( A_{\mu,\lambda} \) containing all the equilibrium points over which the determinant of the Jacobian is nonzero. This may be done by bounding the region in which a particular equilibrium point resides. If such an \( A_{\mu,\lambda} \) exists, then Theorem 1 may be applied. Theorem 3 may be used for guidance in the choice of \( A_{\mu} \). By bounding the location of the equilibrium points and choosing this set as \( A_{\mu,\lambda} \) allows one to perform this check in an analytically tractable manner. Additionally, systems may be designed such that \( A_{\mu,\lambda} \) is as large as possible to provide increased robustness.

3. If the set \( A_{\mu,\lambda} \) is not easily found, and the nominal system (\( \mu = \lambda = 0 \)) has one equilibrium point, then one may be able to find the set \( B_{\mu^*,\lambda^*} \), which contains at least one equilibrium point when \( \mu = \mu^*, \lambda = \lambda^* \) over which sign of the determinant of the Jacobian is opposite that of the sign of the determinant of the Jacobian evaluated at equilibrium point in the nominal system. Then, Theorem 2 applies guaranteeing that the number of equilibrium points is different when \( \mu = \mu^*, \lambda = \lambda^* \) versus \( \mu = \lambda = 0 \). It is usually most straightforward to choose \( B_{\mu^*,\lambda^*} \) as the set where the sign of the Jacobian is different than that of the sign of the determinant of the Jacobian evaluated at the equilibrium point when \( \mu = \lambda = 0 \). Then one may verify that an equilibrium point is in the set \( B_{\mu^*,\lambda^*} \) when \( \mu = \mu^*, \lambda = \lambda^* \).

4. If none of the above approaches are possible, one may use brute force numerical solution techniques along with bifurcation software to solve the system.

7 Discussion

The number of equilibrium points is an important qualitative property of dynamical systems. In this paper, we developed a theoretical framework to analyze the number of equilibrium points of a positive dynamical system using tools from degree theory and homotopy theory. We formulated the problem using a general form to compare a nominal system to a perturbed system, and our results then allow for the analysis of the number of equilibrium points without explicitly finding the equilibrium points, which may greatly simplify analysis.

The main results presented are Theorems 1 and 2. Theorem 1 provides a check on the determinant of the Jacobian guaranteeing that two nonlinear systems in the form of (7) have the same number of
equilibrium points and may be used to assess the effects of perturbations on the number of equilibrium points. Theorem 2 provides a check on the determinant of the Jacobian for systems where the nominal system has one equilibrium point to find conditions under which the system has multiple equilibrium points. These theorems may be used without explicitly solving for the equilibrium points of the system. Finally, Theorem 3 may be used for systems with one equilibrium point to find a set in which one equilibrium point is guaranteed to reside—this may aid in selecting a set over which to apply Theorem 1 for systems with one equilibrium point. We demonstrated the use of our mathematical tools with two examples of genetic circuits with resource perturbation effects.

Our results may be used as a high-level design tool for comparing different genetic circuit topologies to determine preferable circuit designs. We demonstrated this procedure in Example 6.1 where we showed that the repression toggle design is more robust to production resource perturbations than the activation toggle. These results provide a framework to enable the analysis and design of complex dynamical systems with unmodeled dynamic effects such as resource perturbations in genetic circuits. They may further be used to analyze simplified systems and guarantee that the simplified system has the same qualitative behavior as a perturbed system.

We applied our tools to genetic circuits as a specific application example. State-dependent perturbations arising from fluctuations in available resources have recently appeared as a major problem to our ability of predicting a circuit’s behavior. We have illustrated our results on a genetic toggle switch and on a genetic cascade to show how to determine parameter conditions under which the number of equilibrium points of the nominal and perturbed systems are guaranteed to be the same or to differ. These conditions allow us to both design circuits in a way such that they are robust to perturbations in resources and to select the most robust circuit topologies.

Acknowledgments: The authors would like to thank Muhammad Ali Al-Radhawi for his helpful comments and for proofreading the paper.

References


A. Additional Proofs and Mathematical Background

The following theorem comes from [33] and is one of the main theorems of degree theory [38]. This theorem states that the degree is a topological constant [16, 38].

**Theorem 4.** Consider a bounded domain \( \Omega \subset \mathbb{R}^n \) and a family of \( \mathcal{C}^1 \) vector fields \( f_\lambda : \Omega \to \mathbb{R}^n \). Let \( \lambda^* > 0 \) and suppose that \( f_\lambda \) is continuous with respect to \( \lambda \) for \( \lambda \in [0, \lambda^*] \), such that \( f_\lambda \) does not have any zeros on the boundary of \( \Omega \) for all \( \lambda \in [0, \lambda^*] \). Then \( \deg(f_\lambda, \Omega) \) is constant for all \( \lambda \in [0, \lambda^*] \) [33].

Intuitively, Theorem 4 may be described as follows: zeros may appear or disappear in pairs from degenerate zeros as \( \lambda \) is varied; however, the zeros must appear or disappear with opposite signs of their determinants. This is required for the degree to remain constant. Local bifurcations show this behavior such as the pitchfork or saddle-node bifurcation [3]. In the case where \( f_\lambda \) has degenerate zeros, Theorem 4 still holds, however the definition of degree needs to be chosen differently [16]. We do not present this more complex definition of degree here.

**Definition 8.** A point \( x_0 \in \mathbb{R}^n \) is an isolated zero of a vector field \( f : \mathbb{R}^n \to \mathbb{R}^n \) if \( f(x_0) = 0 \) and there exists an \( \varepsilon > 0 \) such that \( x_0 \) is the only point in the ball \( B(x_0, \varepsilon) \) satisfying \( f(x) = 0 \).

**Definition 9.** Let \( \Omega \) and \( f \) be as in Definition 2 and suppose \( f \) has only isolated zeros. Let \( x_i \) be a zero of \( f \) and \( \Omega_i \) be a sufficiently small open and bounded neighborhood of \( x_i \in \Omega_i \) such that \( x_i \) is the only solution of \( f(x) = 0 \) in \( \Omega_i \), then the index of an isolated zero of \( f \) is \( \Phi(f, x_i) = \deg(f, \Omega_i) \). 

---


Note that the index essentially is the determinant of the Jacobian of $f$ evaluated at each zero of $f$; however, since the degree is defined over a set $\Omega$, the index is as well. We now state two results related to degree theory that will be used in later proofs. Lemma 3 connects the definition of degree evaluated over a bounded domain with the number of zeros of the vector field in that domain.

**Lemma 3.** Let $\Omega \subseteq \mathbb{R}^n$, $f : \Omega \to \mathbb{R}^n$ be a $C^1$ vector field, and suppose that $\det \left( \frac{\partial f}{\partial x}(x) \right) \neq 0$ for all $x \in f^{-1}(0)$. Then the number of zeros of $f$ in $\Omega$ is equal to the sum of the absolute values of the indexes of $f$ in $\Omega$, i.e. $n = \sum_i |\Phi(f, x_i)|$.

**Proof.** The proof follows by Definitions 2 and 9. Since $\det \left( \frac{\partial f}{\partial x}(x) \right) \neq 0$ for all $x \in f^{-1}(0)$, all zeros of $f$ are isolated by the Inverse Function Theorem [39] and Definition 9 may be applied. Note that $n = \sum_{x \in f^{-1}(0)} \left| \text{sign} \left( \det \left( \frac{\partial f}{\partial x}(x) \right) \right) \right| = \sum_i |\Phi(f, x_i)|$ since $|\text{sign}(z)| = 1$ for any $z \neq 0$ which is assumed in Definition 2. \[\square\]

**Proof of Lemma 1.** Choose a fixed $\lambda^* > 0$ and let $n$ denote the cardinality of $S_0$. Suppose that $\det \left( \frac{\partial f_\lambda(x)}{\partial x} \right) \neq 0$ for each $x^k \in S_\lambda$ for every $\lambda \in [0, \lambda^*]$, then $n$ is finite since all zeros are isolated. Partition the interval $[0, \lambda^*]$ into $N$ subintervals according to $P = \{0 = \lambda_0, \lambda_1, \ldots, \lambda_{N-1}, \lambda_N = \lambda^*\}$. Consider the $k$th subinterval $[\lambda_k, \lambda_{k+1}]$, where $\lambda_k$ is fixed and $\lambda_{k+1}$ will be chosen later. For $\lambda_k$ and for each $x^k \in S_{\lambda_k}$, there exists an open ball $\Omega^{\lambda_k}_{x_k} = B(x^k, \epsilon^k)$ containing the zero $x^k_{\lambda_k}$ such that $x^k_{\lambda_k}$ is the unique solution of $f_{\lambda_k}(z) = 0$ for $z \in \Omega^{\lambda_k}_{x_k}$ by the Inverse Function Theorem [39]. Note that $x^k_{\lambda_k}$ is continuous, since $f_\lambda$ is continuous with respect to $\lambda$. Choose $\lambda_{k+1}$ such that for all $\lambda \in [\lambda_k, \lambda_{k+1}]$, $x^k_{\lambda_k} \in \Omega^{\lambda_k}_{x_k}$ for all $i = 1, \ldots, n$ since $x^k_{\lambda_k}$ is continuous. Apply Lemma 3 to each $\Omega^{\lambda_k}_{x_k}$ since each zero is isolated, contained in $\Omega^{\lambda_k}_{x_k}$, and the index for each $x^k_{\lambda_k}$ is nonzero for all $\lambda \in [\lambda_k, \lambda_{k+1}]$. Then, for all $\lambda \in [\lambda_k, \lambda_{k+1}]$, the cardinality of the set $S_\lambda$ is constant and equal to $n$. Note that, by Theorem 4, if any zeros appear, they must appear from a degenerate zero, since the degree over any domain with no zeros on the boundary is constant. Repeat over each subinterval until $\lambda_N = \lambda^*$. Then the cardinality of $S_\lambda$ is constant and equal to $n$ for all $\lambda \in [0, \lambda^*]$. \[\square\]

We first present a Lemma that will be used in the proof of Lemma 2.

**Lemma 4.** For any positive invariant vector fields $f : \mathbb{R}^n \to \mathbb{R}^n$ and $g : \mathbb{R}^n \to \mathbb{R}^n$ and for nonnegative scalars, $a, b \in \mathbb{R}_{\geq 0}$, $af(x) + bg(x)$ is positive invariant. Furthermore, for nonnegative vectors $c, d \in \mathbb{R}^n_{\geq 0}$, $c \circ f(x) + d \circ g(x)$ is positive invariant.

**Proof.** We show that $h_1(x) = af(x) + bg(x)$ is a positive invariant vector field for positive constants $a, b \in \mathbb{R}_{\geq 0}$. Consider the boundary of the positive orthant, $\partial \mathbb{R}^n_{\geq 0} = \{x : x_i = 0 \text{ and } x \geq 0 \text{ for each } i = 1, \ldots, n\}$. Since $f(x) \geq 0$ and $g(x) \geq 0$ for all $x \in \partial \mathbb{R}^n_{\geq 0}$ and $a, b > 0$, then $af(x) + bg(x) \geq 0$ for all $x \in \partial \mathbb{R}^n_{\geq 0}$. Thus, $h_1(x)$ is positive invariant. Similarly, for $c, d \in \mathbb{R}^n_{\geq 0}$, $h_2(x) = c \circ f(x) + d \circ g(x)$ is positive invariant, since on $f(x) \geq 0$ and $g(x) \geq 0$ for all $x \in \partial \mathbb{R}^n_{\geq 0}$ and $c, d \geq 0$, then $c \circ f(x) + d \circ g(x) \geq 0$ for all $x \in \partial \mathbb{R}^n_{\geq 0}$. Thus, $h_2(x)$ is positive invariant. \[\square\]

**Proof of Lemma 2.** We first show that (7) is positive invariant. Since $h(x), g(x)$, and $-\Delta x$ are $C^1$ positive invariant functions, $\alpha(x)$ is $C^1$, and $0 < \alpha(x) \leq 1$ for $x \in \mathbb{R}^n_{\geq 0}$, it follows that $\mu(x - \mathbf{1}) + \mathbf{1} > 0$ for any $\mu \in [0, 1]$. Then $f_{\mu, \lambda}(x)$ is $C^1$ positive invariant for all $\mu, \lambda \in [0, 1] \times [0, \infty)$ by Lemma 4. This proves (a).

Next, to prove (b), we construct a bounded domain, $\Omega$, over which we will consider the set of equilibrium points of (7) in the positive orthant. To construct $\Omega$, choose an $m > 0$ such that $m \cdot g(x) \leq 0$, which can be done since $g$ is mass dissipating. Now, by assumption, $x(t)$ is bounded for the system $\dot{x} = h(x) - \Delta x$, so $\Delta x(t)$ is also bounded for all $t \geq 0$. Furthermore, $m \cdot (\Delta x(t))$ is finite. Choose $M > \sup_{t \geq 0} \{m \cdot (\Delta x(t))\}$. We now define $\Omega = \{x \in \mathbb{R}^n_{\geq 0} : m \cdot (\Delta x) < M\}$. We prove that $f_{\mu, \lambda}$ has no zeros on the boundary of $\Omega$. We first observe that $f_{\mu, \lambda}(x)$ has no zeros on the sides of $\Omega : \{x : x_i = 0 \text{ and } x \geq 0 \text{ for each } i = 1, \ldots, n\}$ for all $\mu, \lambda \in [0, 1] \times [0, \infty)$ since $h(x)$ has no zeros in the set $\partial \mathbb{R}^n_{\geq 0} = \{x : x_i = 0 \text{ and } x \geq 0 \text{ for each } i = 1, \ldots, n\}$ and both $g(x)$ and $-\Delta x$ are positive invariant and $\alpha(x) > 0$. Now, we show that $f_{\mu, \lambda}$ has no zeros on the
boundary defined by \( \{ x : m \cdot (Ax) = M \} \) and no zeros in the positive orthant outside of \( \Omega \). To this end, consider

\[
m \cdot f_{\mu,\lambda} = m \cdot (h \circ [1 + \mu(\alpha(x) - 1)]) + m \cdot \lambda g - m \cdot (Ax)
\]

where \( h \) is decomposed into the form of (37), then it is bounded, even if \( m \cdot (\mu(\alpha - 1) \circ h) = m \cdot h + m \cdot \lambda g - m \cdot (Ax) \).

Since \( \alpha \leq 1 \), then \( m \cdot (\mu(\alpha - 1) \circ h) \leq 0 \). It then follows that \( m \cdot f_{\mu,\lambda} \leq m \cdot h + m \cdot \lambda g - m \cdot (Ax) \). Furthermore, since \( g \) is mass dissipating with respect to \( m \), we have \( m \cdot g \leq 0 \) and \( m \cdot f_{\mu,\lambda} \leq m \cdot h - m \cdot (Ax) \). Since \( M > \sup_{t \geq 0} \{ m \cdot (Ax(t)) \} \), this implies that \( \sup_{t \geq 0} \{ m \cdot h(x(t)) \} < M \). Then, for \( \{ x : m \cdot (Ax) \geq M \} \), we have \( m \cdot f_{\mu,\lambda} \leq m \cdot h(x) - m \cdot (Ax) \leq m \cdot h(x) - M < 0 \). So \( m \cdot f_{\mu,\lambda} < 0 \) for all points on the outer boundary of \( \Omega : \{ x : m \cdot (Ax) = M \} \) for all \( \mu, \lambda \in [0, 1] \times [0, \infty) \) since \( m \) is a positive vector. This implies that \( f_{\mu,\lambda} \) has no zeros on the boundary of \( \Omega \) for any \( \mu, \lambda \in [0, 1] \times [0, \infty) \). Similarly, since \( m \cdot f_{\mu,\lambda}(x) < 0 \) for all \( x \in \{ x : m \cdot (Ax) > M \} \), then there exist no zeros in the positive orthant outside of \( \Omega \) for any \( \mu, \lambda \in [0, 1] \times [0, \infty) \). Therefore the interior \( \Omega \) contains all zeros in the positive orthant for all \( \mu, \lambda \in [0, 1] \times [0, \infty) \). This proves (b).

To prove (c), we will find \( \deg(f_{\mu,\lambda}, \Omega) \). Note that by Theorem 4, \( \deg(f_{\mu,\lambda}, \Omega) = \deg(f_{0,0}, \Omega) \) where \( f_{0,0}(x) = 0 \). Since \( x(t) \) is bounded, \( \Omega \) is compact, and, since \( h(x) \) is continuous over \( \Omega \), then \( h(x(t)) \) is bounded over \( \Omega \). We can rewrite as \( h(x) = c \circ \beta(x) \) where \( \beta : \mathbb{R}^n \to \mathbb{R}^n \) is \( C^1 \), \( 0 < \beta(x) \leq 1 \) for all \( x \in \mathbb{R}^n_{>0} \), and \( c_i = \sup \{ h_i(x) \} \) for each \( i = 1, \ldots, n \). We now define the auxiliary function \( f_{\nu}(x) = c \circ [1 + \nu(\beta(x) - 1)] - Ax \) with parameter \( \nu \in [0, 1] \). Then \( f_1(x) = f_{0,0}(x) \) and \( f_0(x) = c - Ax \), which is linear. Since \( 0 < \beta(x) \leq 1 \) and \( c > 0 \), then \( f_{\nu} \) is positive invariant and has no zeros on the boundary of \( \Omega \), as shown previously. Additionally, \( f_0(x) \) has one zero in \( \Omega \), namely \( x = \Lambda^{-1}c \), and the Jacobian is \( \frac{\partial f_0}{\partial x} = -\Lambda \).

Then \( \det \left( \frac{\partial f_0}{\partial x} \right) = \prod_{i=1}^n (-\Lambda_i) \) and \( \sign(\det \left( \frac{\partial f_0}{\partial x} \right)) = (-1)^n \) so \( \deg(f_0(x), \Omega) = (-1)^n \) by Definition 2. Then, by Theorem 4, \( \deg(f_{\mu,\lambda}, \Omega) = \deg(f_{0,0}, \Omega) = \deg(f_1, \Omega) = \deg(f_0, \Omega) = (-1)^n \). Furthermore, \( S_{\mu,\lambda} \neq \emptyset \) by Definition 2. This proves (c).

Proposition 1 illustrates that given a dynamical system in the form of (3) or (4), if the dynamics may be decomposed into the form of (37), then it is bounded, even if \( h(x) \) in (3) is not necessarily bounded.

**Proposition 1.** Consider a system of the form

\[
\begin{align*}
\dot{x}_1 &= h_1(x_1, x_2) - \Lambda_1 x_1 \\
\dot{x}_2 &= h_2(x_1) - \Lambda_2 x_2
\end{align*}
\]

(37)

where \( x_1 \in \mathbb{R}^m, x_2 \in \mathbb{R}^p, h_1 : \mathbb{R}^m \times \mathbb{R}^p \to \mathbb{R}^m \) and \( h_2 : \mathbb{R}^m \to \mathbb{R}^p \) are continuous, positive vector fields and \( h_1 \) is bounded over \( \mathbb{R}_{>0}^m \), while \( h_2 \) is not necessarily bounded. Additionally, \( \Lambda_1, \Lambda_2 \) are diagonal matrices with strictly positive diagonal entries. Then, the dynamical system (37) is bounded.

**Proof.** Note that (37) is positive invariant since \( h_1, h_2 > 0 \) for all \( x_1, x_2 > 0 \). Then \( x_1 \) is bounded since \( h_1 \) is bounded so there exists a \( T \in \mathbb{R}_{>0} \) such that for all \( t > T \), \( x_1(t) \leq \sup \{ \Lambda_1^{-1} h_1(x) \} \). Since \( h_2 \) is continuous and depends only on \( x_1 \), then \( h_2(x_1(t)) \) is bounded for bounded \( x_1 \). Therefore, \( x_2(t) \leq \sup \{ \Lambda_2^{-1} h_2(x_1(t)) \} < \infty \) and all trajectories in the positive orthant are bounded. Thus the dynamical system is bounded. 

\[\blacksquare\]
B Background on Biomolecular ODE Models

B.1 Nondimensionalization of Biomolecular ODEs

We consider systems that have the form \([30, 31]\)

\[
\dot{x}_i = T_i F_i(x) \left( \frac{1}{h_i(x)} \right) \left( \frac{1}{1 + \sum_{j=1}^{m} J_{ij} F_j(x)} \right) x_i - \frac{k P_{\text{tot}} x_i}{1 + \sum_{k=1}^{m} x_k K_k} \frac{\delta x_i}{g_i(x)},
\]

where \(x \in \mathbb{R}^m\) represents the concentration of all proteins in the system, \(F_i(x)\) is a normalized Hill function in the form \(\frac{1+a_i x_i^n}{1+b_i x_i^n}\) for positive constants \(a, b,\) and \(n, T_i\) represents a scaling of \(F_i\), and \(J_{ij}\) scales the resource usage by each protein, which appears in the denominator of \(h_i(x)\alpha_i(x)\) for each \(i = 1, \ldots, m\).

It will be helpful to first nondimensionalize (38) to simplify our analysis, eliminating any free parameters. Throughout the nondimensionalization process, we denote nondimensional quantities with \(^\ast\). By choosing the nondimensional concentration \(x_i^\ast = \frac{x_i}{T_i}\) and nondimensional time \(t^\ast = \delta \cdot t\), then (38) becomes

\[
\frac{dx_i^\ast}{dt^\ast} = \frac{1}{T_i} \frac{dz_i}{dt} = \frac{F_i(T \odot x^\ast/\delta)}{1 + \sum_j J_{ij} F_j(T \odot x^\ast/\delta)} \left( \frac{1+a_i(T_i/\delta)^{n_i} x_i^\ast}{1+b_i(T_i/\delta)^{n_i} x_i^\ast} \right) - \frac{k P_{\text{tot}} x_i^\ast}{1 + \sum_k x_k^\ast K_k/\gamma} - x_i^\ast.
\]

For simplicity, we consider single input Hill functions, \(F(\cdot)\); however, this may easily be extended to Hill functions with multiple inputs \([30]\). The nondimensional Hill function is defined as

\[
F_i^\ast(x^\ast) = \frac{1+a_i(T_i/\delta)^{n_i} x_i^\ast}{1+b_i(T_i/\delta)^{n_i} x_i^\ast} \text{ if } a_i \leq b_i
\]

\[
\left( \frac{b_i}{a_i} \right) \frac{1+a_i(T_i/\delta)^{n_i} x_i^\ast}{1+b_i(T_i/\delta)^{n_i} x_i^\ast} \text{ if } a_i > b_i.
\]

Define the nondimensional constants

\[
a_i^\ast = a_i(T_i/\delta)^{n_i},
\]

\[
b_i^\ast = b_i(T_i/\delta)^{n_i},
\]

where \(T_i\) corresponds to the input state \(x_i\). Then, the nondimensionalized Hill function is given as

\[
F_i^\ast(x) = \left\{ \begin{array}{ll}
\frac{1+a_i x_i^\ast}{1+b_i x_i^\ast} & \text{ if } a_i^\ast \leq b_i^\ast \\
\left( \frac{b_i}{a_i} \right) \frac{1+a_i x_i^\ast}{1+b_i x_i^\ast} & \text{ if } a_i^\ast > b_i^\ast.
\end{array} \right.
\]

Additionally, define the nondimensional constants \(k_i^\ast = \frac{k P_{\text{tot}}}{\delta K_i}\) and \(K_i^\ast = \frac{T_i}{K_i \delta}\) for \(i = 1, \ldots, m\). This gives the nondimensionalized version of (38) as

\[
\frac{dx_i^\ast}{dt^\ast} = \frac{F_i^\ast(x^\ast)}{1 + \sum_{j=1}^{m} J_{ij} F_j^\ast(x^\ast)} x_i^\ast - \frac{k_i^\ast x_i^\ast}{1 + \sum_{k=1}^{m} x_k^\ast K_k^\ast} - x_i^\ast.
\]

An alternative method of nondimensionalization may be performed by choosing the nondimensional concentration \(x_i^\ast = x_i/K_i\) where \(K_i\) is a Michaelis-Menten constant and nondimensional time is \(t^\ast = t \cdot \delta\). Then, the nondimensional model is given as

\[
\frac{dx_i^\ast}{dt^\ast} = \frac{T_i^\ast F_i^\ast(x^\ast)}{1 + \sum_{j=1}^{m} J_{ij} F_j^\ast(x^\ast)} - \frac{k_i^\ast x_i^\ast}{1 + \sum_{k=1}^{m} x_k^\ast K_k^\ast} - x_i^\ast.
\]

In the examples, we drop the \(^\ast\) for clarity of presentation.
B.2 Background on Hill Functions

Here we state some useful properties of the standard Hill function commonly used in modeling mRNA or protein production in biomolecular models [9, 10]. These properties are utilized in Section 6. We consider single input Hill function models; however, similar results may be derived in a straightforward manner for multiple inputs. We assume all Hill functions have the form

\[
F(x) = \begin{cases} 
1 + ax^n & \text{if } a \leq b \\
\frac{b}{a} \left(1 + \frac{b}{a}x^n\right)^{-1} & \text{if } a > b 
\end{cases}
\]  

(44)

for positive constants \(a, b, n\). Then, the derivative of the Hill function with respect to its argument is

\[
F'(x) = \begin{cases} 
\frac{(a-b)nx^{n-1}}{(1+bx^n)^2} & \text{if } a \leq b \\
\frac{b}{a} \frac{(a-b)nx^{n-1}}{(1+bx^n)^2} & \text{if } a > b 
\end{cases}
\]  

(45)

Observe that \(F(\cdot)\) has the following properties:

1. \(0 < F(\cdot) \leq 1\) for all positive arguments
2. \(F(\cdot)\) is monotonic. Strictly increasing if \(a > b\) and strictly decreasing if \(a < b\).
3. \(\sup_{x \geq 0} F(x) = 1\)
4. \(\inf_{x \geq 0} F(x) = \min\left\{\frac{a}{b}, \frac{b}{a}\right\} \leq 1\).

Additionally, \(\arg \max_{x \geq 0} |xF'(x)| = b^{-1/n}\) and

\[
\begin{align*}
\min_{x \geq 0} \{xF'(x)\} &= \frac{n}{4} \left(\frac{a}{b} - 1\right) \leq 0 & \text{if } a \leq b \\
\max_{x \geq 0} \{xF'(x)\} &= \frac{n}{4} \left(1 - \frac{b}{a}\right) > 0 & \text{if } a > b
\end{align*}
\]  

(46)

If we consider only the domain \(x \in [0, 1]\) and \(b < 1\), then \(\arg \max_{x \in [0,1]} |xF'(x)| = 1\) and

\[
\begin{align*}
\min_{x \in [0,1]} \{xF'(x)\} &= \frac{n(a-b)}{(1+b)^2} \leq 0 & \text{if } a \leq b \\
\max_{x \in [0,1]} \{xF'(x)\} &= \left(b \frac{n(a-b)}{(1+b)^2} > 0 & \text{if } a > b
\end{align*}
\]  

(47)

B.3 Modeling microRNA Dynamics

We begin with the chemical reactions. We consider a system in which \(n\) different mRNAs are produced and degraded by a single microRNA modeled by the following chemical reactions [35, 40]

\[
\mu + A \xrightleftharpoons[d\mu]{d} R \\
\emptyset \xrightleftharpoons[\delta]{H_i(x)} m_i \\
m_i + R \xrightleftharpoons[k]{d_i}{\alpha_{m_i}} C_i \xrightarrow[k]{k} R \\
m_i \xrightarrow[\alpha]{\alpha_{m_i}} m_i + x_i.
\]  

(48a-d)

Here \(\mu\) is a microRNA, \(A\) is the argonaute protein, \(R\) is the RNA induced silencing complex (RISC) formed from the microRNA and argonaute, \(m_i\) is the mRNA, and \(C_i\) is the complex formed from RISC and mRNA,
\( \dot{H}_i(x) \) is the production rate of mRNA which depends on the concentration of proteins, \( x \) for each \( i = 1, \ldots, n \). Then, using the law of mass action, this becomes the set of ordinary differential equations

\[
\begin{align*}
\dot{x}_i &= \alpha m_i - \delta x_i & (49a) \\
\dot{m}_i &= H_i(x) - a_i m_i R + d_i C_i - \delta m_i & (49b) \\
\dot{C}_i &= a_i m_i R - (d_i + k_i + \delta) C_i & (49c)
\end{align*}
\]

We assume that the argonaute protein is non-limiting and so its dynamics and the dynamics of the miRNA loading into RISC are neglected. Assuming that the dynamics for the complexes are much faster than for mRNA and protein dynamics (quasi-steady state assumption), and noting that \( R \) is conserved

\[
\begin{align*}
\overline{C}_i &= \frac{Rm_i}{K_i} & (50a) \\
R_{tot} &= R + \sum C_i & (50b) \\
R &= \frac{R_{tot}}{1 + \sum \frac{m_j}{K_j}} & (50c)
\end{align*}
\]

where \( K_i = \frac{d_i + k_i}{a_i} \) is the Michaelis-Menten binding constant. Then, substituting (50) into (49) and simplifying we arrive at the desired, reduced dynamics

\[
\begin{align*}
\dot{m}_i &= H_i(x) - \frac{k R_{tot} m_i}{1 + \sum \frac{m_j}{K_j}} - \delta m_i & (51) \\
\dot{x}_i &= \alpha m_i - \delta x_i. & (52)
\end{align*}
\]