The Number of Equilibrium Points of Perturbed Nonlinear Positive Dynamical Systems ⋆

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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 networks where state-dependent perturbations arise due to disturbances in cellular resources. These disturbances constitute a major problem for predicting the behavior of genetic networks. Our results determine whether such perturbations change a genetic network's number of steady states. The framework and results presented can be applied to a broad class of nonlinear dynamical systems beyond genetic networks.

Key words: multistability, nonlinear dynamical systems, degree theory, homotopy methods, genetic networks, systems biology

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 particular, multistability is a central property of dynamical models of biological regulatory network motifs implicated in cell-fate determination. In these models, each steady state is typically associated with a distinct cellular phenotype and transitions among steady states capture the process of cellular differentiation [11]. A change in the number of equilibria may reflect a change in the phenotypic diversity of a multi-cellular organism and is, therefore, a relevant feature to consider.

Most mathematical models of both natural and synthetic biological network motifs assume the network to be "isolated" from the cellular context. This is rarely true in practice, since a number of interactions exist between the network under study and the rest of the cell. A class of such unwanted interactions, whose effects have been well characterized, consists of interactions due to sharing a limited amount of cellular resources [16]. These interactions manifest themselves as a state-dependent perturbation in the dynamical model of the network and may result in a dramatic change in the qualitative behavior of the system [17]. So far, a theoretical investigation of the potential consequences of these perturbations on the emergent features of a biological network, such as the network's number of equilibria, has been missing.

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 [12, 13, 18, 19]. 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 [20] 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 [21], which leads to large systems of ODEs that are prohibitive for design

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and analysis. Other structural conditions exist to provide insight into qualitative changes in dynamical system behavior—most notably, these conditions examine the sign pattern of the Jacobian relating to the signs of cycles in the associated graph of the system [14, 15, 22]. However, these methods do not take parameters into account, whose ranges are often known for synthetic genetic networks. Related work also exists specifically for monotone systems [5].

We consider the class of positive dynamical systems—systems where all states are positive—which are commonly used to capture the dynamics of biological networks 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 when it is affected by a structured state-dependent perturbation to its dynamics. This framework is useful for analyzing biological systems but may also be applicable to other fields. We present a novel methodology to accomplish this using tools from degree theory [23, 24]. This requires checking whether the determinant of the system's Jacobian does or does not change sign over a subset of the state space that contains the equilibrium points as the system is perturbed. This methodology enables us to find algebraic conditions on the system's parameters under which the number of equilibrium points does or does not change without having to solve for the equilibrium points explicitly. Our first result, Theorem 10, provides conditions guaranteeing that the number of equilibrium points of a system does not change when the perturbation is considered. The next result, Theorem 12, provides conditions guaranteeing that the number of equilibrium points of a system changes as the perturbation is considered. These results give easily verifiable algebraic conditions for determining the robustness of the qualitative behavior of a system to a class of structured, state-dependent perturbations. 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 networks [16, 17, 25, 26]. In this work, we provide a predictive tool that can be used to practically analyze and design genetic networks that behave as expected from theory.

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 networks 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 several 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 the network's operation. Fluctuation in the availability of resources has recently appeared as a major bottleneck to predicting the behavior of genetic network, and therefore limits our ability to design networks that behave as intended [17, 25–27]. In turn, unpredicted changes to the number of equilibrium points may completely disrupt a network's intended function. As an example, consider the toggle switch, which is currently the most widely used genetic network in biotechnology applications [28–31]. 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 [29, 31]. 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:

$$\dot{x}_1 = F_1(u, x_2) - x_1 \qquad \dot{x}_2 = F_2(x_1) - x_2 \tag{1}$$

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 [32] 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 network, except we include the fact that production resources are finite. Then, the dynamical system becomes the perturbed system

$$\dot{x}_1 = \frac{F_1(u, x_2)}{1 + J_1 F_1(u, x_2) + J_2 F_2(x_1)} - x_1 \quad \text{(2a)}$$

$$\dot{x}_2 = \frac{F_2(x_1)}{1 + J_1 F_1(u, x_2) + J_2 F_2(x_1)} - x_2, \quad \text{(2b)}$$

as derived in [33] and experimentally validated in [17]. Here J_1 and J_2 represent the resource demand coefficients by proteins x_1 and x_2, respectively. 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.

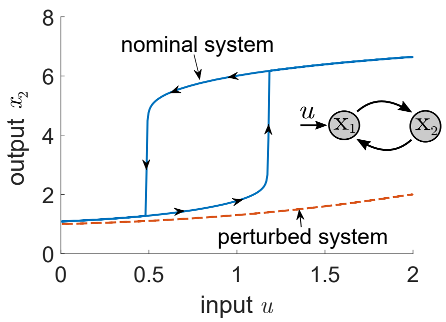

Fig. 1. Simulation of genetic toggle switch in (1) and (2) showing differing number of equilibrium points from state dependent perturbations due to fluctuations in the availability of resources. Parameters used for the simulation are $F_1(u, x_2) = u + \frac{1+0.0774(x_2)^3}{1+0.01(x_2)^3}$ and $F_2(x_1) = \frac{1+0.0774(x_1)^3}{1+0.01(x_1)^3}$, and $J_1 = J_2 = 0.03$.

As it can be seen in Figure 1, the two systems have different steady state responses. The nominal system (1) exhibits bistability for an input range of u between 0.48 and 1.18 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.

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.

We consider a nominal system in the form

$$\dot{x} = h(x) - \Lambda x, \quad (3)$$

where $x \in \mathbb{R}^n_{\geq 0}$, $h : \mathbb{R}^n \to \mathbb{R}^n$ is $\mathcal{C}^1$ and bounded and positive for all positive arguments, and Λ 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

$$\dot{x} = h(x) \odot \alpha(x) + g(x) - \Lambda x, \quad (4)$$

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 [17, 34, 35]. 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

$$\dot{x} = h(x) \odot [\mathbb{1} + \mu(\alpha(x) - \mathbb{1})] + \lambda g(x) - \Lambda x, \quad (5)$$

where $\mathbb{1}$ 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* μ *and* λ *are varied between* 0 *and* 1.

4 Mathematical Preliminaries

Here, 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 Ω is denoted as $\overline{\Omega}$, the interior $\mathrm{int}(\Omega)$ is the largest open set contained in Ω, and the boundary of a domain Ω is denoted as

$\partial\Omega = \overline{\Omega}\backslash\mathrm{int}(\Omega)$. $x \geq 0$, $x \in \mathbb{R}^n$ 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 μ and λ, we denote the set of zeros as $\mathcal{S}_{\mu,\lambda} = \{x > 0 : f_{\mu,\lambda}(x) = 0\}$ for any fixed μ, λ.

Definition 2 *Given a $\mathcal{C}^1$ 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 3 *Let $\Omega \subset \mathbb{R}^n$ be a bounded domain, let $f : \overline{\Omega} \to \mathbb{R}^n$ be $\mathcal{C}^1$, and assume f has no degenerate zeros and has no zeros on the boundary of Ω. Then the* topological degree of f with respect to zero, *or more briefly, the* degree *of f, is* $\deg(f, \Omega) = \sum_{z \in f^{-1}(0)\cap\Omega} \mathrm{sign}\left(\det\left(\frac{\partial f(z)}{\partial x}\right)\right)$ *where $f^{-1}(0)$ is the set of zeros of f in Ω and $\mathrm{sign}(\cdot)$ is the sign function.*

Lemma 5 provides a condition under which the cardinality of the set of zeros of a family of vector fields is constant. The following theorem comes from [36] and is one of the main theorems of degree theory. This theorem states that the degree is a topological constant [23, 37] and will be used in the proofs of Lemma 9 and Theorem 12.

Theorem 4 *[36] 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_λ is continuous with respect to λ for $\lambda \in [0, \lambda^*]$, such that f_λ does not have any zeros on the boundary of Ω for all $\lambda \in [0, \lambda^*]$. Then $\deg(f_\lambda, \Omega)$ is constant for all $\lambda \in [0, \lambda^*]$.*

Lemma 5 *Consider a bounded domain $\Omega \subset \mathbb{R}^n$. Let $f_\lambda : \overline{\Omega} \to \mathbb{R}^n$ be a $\mathcal{C}^1$ family of vector fields and continuous with respect to λ. Fix $\lambda^* > 0$ 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 $x \in \mathcal{S}_\lambda$, then the cardinality of $\mathcal{S}_\lambda$ does not depend on λ.*

Now, consider the system of ordinary differential equations (ODEs)

$$\dot{x} = f(x) \tag{6}$$

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

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

Definition 7 *Given a domain $\Omega \subset \mathbb{R}^n$, a continuous vector field $h : \Omega \to \mathbb{R}^n$ is* bounded over Ω *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 8 *A function $g : \mathbb{R}^n \to \mathbb{R}^n$ is* mass dissipating *if there exists some $m \in \mathbb{R}^n_{>0}$ such that $m \cdot g(x) \leq 0$ for all $x \in \mathbb{R}^n_{\geq 0}$.*

5 Main Results

We now present the main theoretical results of the paper. Theorem 10 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 system is guaranteed not to change its number of equilibrium points as μ and λ are varied. Next, we state a converse theorem, Theorem 12, which provides a condition where the number of equilibrium points of a dynamical system in the form of (5) changes as μ and λ are varied, based on the determinant of the system's Jacobian. The use of these results is illustrated in Section 6. Finally, Theorem 14 finds a set guaranteed to contain at least one equilibrium point for all values of the parameter μ. Before stating our main results, we present a lemma that demonstrates that our general form (5) satisfies all assumptions required by Lemma 5.

Lemma 9 *Consider the continuous time dynamical system*

$$\dot{x} = h(x) \odot [\mathbb{1} + \mu(\alpha(x) - \mathbb{1})] + \lambda g(x) - \Lambda x \triangleq f_{\mu,\lambda}(x), \tag{7}$$

where $x \in \mathbb{R}^n_{\geq 0}$, $h : \mathbb{R}^n \to \mathbb{R}^n$ and $g : \mathbb{R}^n \to \mathbb{R}^n$ are positive invariant $\mathcal{C}^1$ vector fields, $\alpha : \mathbb{R}^n \to \mathbb{R}^n$ is a $\mathcal{C}^1$ vector field, Λ 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 on the positive orthant;*
(b) There exists a positive vector m, a positive scalar M, and a set $\Omega = \{x \in \mathbb{R}^n_{>0} : m \cdot (\Lambda x) < M\}$ such that $\mathcal{S}_{\mu,\lambda} \subset \mathrm{int}(\Omega)$;
(c) $\deg(f_{\mu,\lambda}, \Omega) = (-1)^n$ *and $\mathcal{S}_{\mu,\lambda} \neq \emptyset$.*

Theorem 10 *Consider the dynamical system* (7) *with the same assumptions as Lemma 9. Choose a fixed $\mu^* \in [0, 1]$ and $\lambda^* \geq 0$. If there exists a set $\mathcal{A}_{\mu,\lambda} \subset \mathbb{R}^n_{\geq 0}$ such that $\mathcal{S}_{\mu,\lambda} \subset \mathcal{A}_{\mu,\lambda}$ and* $\det\left(\frac{\partial f_{\mu,\lambda}(x)}{\partial x}\right)\Big|_{\forall x \in \mathcal{A}_{\mu,\lambda}} \neq 0$ *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 9, system (7) is positive invariant and there exists $\Omega \subset \mathbb{R}^n_{\geq 0}$ such that $\mathcal{S}_{\mu,\lambda} \subset \mathrm{int}(\Omega)$ for all $\mu, \lambda \in [0,\mu^*] \times [0,\lambda^*]$ so Lemma 5 may be applied over this Ω for $\mu^* \in [0,1]$ and $\lambda^* \geq 0$. Choose a set $\mathcal{A}_{\mu,\lambda}$ such that $\mathcal{S}_{\mu,\lambda} \subset \mathcal{A}_{\mu,\lambda}$. Now, fix $\lambda = 0$ and vary μ from 0 to μ^*. For each $\mu \in [0,\mu^*]$, if $\det\left(\frac{\partial f_{\mu,0}(x)}{\partial x}\right) \neq 0$ for all $x \in \mathcal{A}_{\mu,0}$, then, by Lemma 5, $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu^*,0}(x)$ have the same number of equilibrium points in Ω and therefore the positive orthant. Next, fix $\mu = \mu^*$ and vary λ from 0 to λ^*. For each $\lambda \in [0,\lambda^*]$, if $\det\left(\frac{\partial f_{\mu^*,\lambda}(x)}{\partial x}\right) \neq 0$ for all $x \in \mathcal{A}_{\mu^*,\lambda}$, then, by Lemma 5, $\dot{x} = f_{\mu^*,0}(x)$ 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 $\det\left(\frac{\partial f_{\mu,\lambda}(x)}{\partial x}\right) \neq 0$ 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. $\square$

Remark 11 *The condition in Theorem 10 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,* (7) *(let $\lambda^* = 0$) may undergo a pitchfork or saddle-node bifurcation when $\mu = \mu^*/2$, resulting in a change in the number of equilibrium points while the determinant of the Jacobian over $\mathcal{A}_{\mu,0}$ with $\mu = \mu^*$ may be non-zero.*

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 $\mathcal{A}_{\mu,\lambda}$ in Theorem 10 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 10 represents a significant sharpening and generalization of the results presented in [36]. The system considered in [36] 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 10, 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 $\mathcal{A}_{\mu,\lambda} = \mathbb{R}^n_{\geq 0}$, Theorem 10 and the global implicit function theorem have similarities [38]. 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 10 guarantees that two systems have the same number of equilibrium points.

A theorem is now presented which provides conditions to guarantee that the number of equilibrium points changes in system (7) as μ and λ are varied.

Theorem 12 *Consider the dynamical system* (7) *with the same assumptions as Lemma 9 and assume that $f_{0,0}(x) = 0$ has one solution, $x_{0,0}$, for $x \in \mathbb{R}^n_{\geq 0}$. Denote a nonempty subset $\hat{\mathcal{S}}_{\mu,\lambda} \subset \mathcal{S}_{\mu,\lambda}$. For some fixed $\mu^* \in [0,1]$ and $\lambda^* \geq 0$, assume that $\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x}\right) \neq 0$ for all $x \in \mathcal{S}_{\mu^*,\lambda^*}$. Then $\dot{x} = f_{\mu^*,\lambda^*}(x)$ has more than one equilibrium point if and only if there exists a set $\mathcal{B}_{\mu^*,\lambda^*}$ such that $\hat{\mathcal{S}}_{\mu^*,\lambda^*} \subset \mathrm{int}(\mathcal{B}_{\mu^*,\lambda^*})$ and $\mathrm{sign}\left(\det\left(\frac{\partial f_{0,0}(x_{0,0})}{\partial x}\right)\right) \neq \mathrm{sign}\left(\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x}\right)\right)$ for all $x \in \mathcal{B}_{\mu^*,\lambda^*}$.*

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 $x_{0,0} \in \mathcal{S}_{0,0}$. Choose Ω as in Lemma 9. Then, $\deg(f_{0,0},\Omega) = +1$ by Lemma 9 and the definition of degree. Now, suppose that there exists a set $\mathcal{B}_{\mu^*,\lambda^*}$ such that a nonempty subset $\hat{\mathcal{S}}_{\mu^*,\lambda^*} \subset \mathcal{S}_{\mu^*,\lambda^*}$ is $\hat{\mathcal{S}}_{\mu^*,\lambda^*} \subset \mathrm{int}(\mathcal{B}_{\mu^*,\lambda^*})$, and suppose $\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x}\right) < 0$ for all $x \in \mathrm{int}(\mathcal{B}_{\mu^*,\lambda^*})$. Then $\deg(f_{\mu^*,\lambda^*},\Omega) < 1$. This is a contradiction since, by Theorem 4, $\deg(f_{0,0},\Omega) = \deg(f_{\mu^*,\lambda^*},\Omega) = 1$. Therefore, the number of equilibrium points of $\dot{x} = f_{0,0}(x)$ and $\dot{x} = f_{\mu^*,\lambda^*}(x)$ are different. Furthermore, since $\deg(f_{0,0},\Omega)$ 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 Ω 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. To prove the converse, suppose (7) has multiple equilibrium points when $\mu = \mu^*$ and $\lambda = \lambda^*$ and, without loss of generality, suppose that $\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x_{\mu^*,\lambda^*})}{\partial x}\right) > 0$ for all $x_{\mu^*,\lambda^*} \in \mathcal{S}_{\mu^*,\lambda^*}$. Then $\deg(f_{\mu^*,\lambda^*},\Omega) > 1$. This contradicts Lemma 9. Then, there exists some $x^* \in \mathcal{S}_{\mu^*,\lambda^*}$ such that $\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x^*_{\mu^*,\lambda^*})}{\partial x}\right) < 0$. Choose $\mathcal{B}_{\mu^*,\lambda^*}$ as a sufficiently small open ball around x^*. Therefore, there exists a set $\mathcal{B}_{\mu^*,\lambda^*}$ such that $\hat{\mathcal{S}}_{\mu^*,\lambda^*} \subset \mathrm{int}(\mathcal{B}_{\mu^*,\lambda^*})$ and $\mathrm{sign}\left(\det\left(\frac{\partial f_{0,0}(x_{0,0})}{\partial x}\right)\right) \neq \mathrm{sign}\left(\det\left(\frac{\partial f_{\mu^*,\lambda^*}(x)}{\partial x}\right)\right)$ for all $x \in \mathcal{B}_{\mu^*,\lambda^*}$. $\square$

Theorem 12 allows us to find conditions where the number of equilibrium points change as μ or λ are varied. The condition that the system $\dot{x} = f_{0,0}(x)$ has one equilibrium point rules out all local bifurcations where equilibrium points collide and exchange stability properties without changing the number of equilibrium points present overall (e.g. transcritical bifurcations [3]). Using Theorems 10 and 12, it is possible to determine conditions where the number of equilibrium points change or remain constant as μ and λ are varied. Theorems 10 and 12 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 $\mathcal{A}_{\mu,\lambda}$ as required by Theorem 10 when $\lambda^* = 0$ to guarantee that (7) maintains its number of equilibrium points as μ is varied.

Definition 13 *A 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 14 *Consider a dynamical system in the form*

$$\dot{x} = [1 + \mu(\alpha(x) - 1)]\, h(x) - \Lambda x \triangleq f_\mu \tag{8}$$

for $x \in \mathbb{R}^n_{\geq 0}$, where $h : \mathbb{R}^n \to \mathbb{R}^n$ is $\mathcal{C}^1$ and positive invariant and has no zeros on the boundary of the positive orthant, $\alpha : \mathbb{R}^n \to \mathbb{R}$ is $\mathcal{C}^1$ and $0 < \alpha(x) \leq 1$ for all $x \in \mathbb{R}^n_{\geq 0}$, and Λ is a diagonal matrix with strictly positive entries. Fix $\mu^* \in [0, 1]$. If $\frac{\partial f_\mu}{\partial x} = \frac{\partial h}{\partial x} - \Lambda + \mu\left((\alpha(x) - 1)\frac{\partial h}{\partial x} + h(x)\frac{\partial \alpha}{\partial x}\right) \preceq 0$ 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 $x_0 \in \mathcal{S}_0$, then there exists exactly one equilibrium point, x_μ, 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 9, and may be written as $\dot{x} = f_\mu(x)$. Setting $\dot{x} = 0$ and differentiating $f_\mu(x) = 0$ with respect to μ (which can be done since h and α are $\mathcal{C}^1$ and μ appears linearly in f_μ) gives

$$\frac{\partial f_\mu}{\partial x}\frac{\partial x_\mu}{\partial \mu} + \frac{\partial f_\mu}{\partial \mu} = 0 \tag{9}$$

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

$$\frac{\partial x_\mu}{\partial \mu}^T \left(\frac{\partial f_\mu}{\partial x}\right)\frac{\partial x_\mu}{\partial \mu} = (1 - \alpha(x_\mu))\frac{\partial x_\mu}{\partial \mu}^T h(x_\mu). \tag{10}$$

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

$$h(x_\mu) = \left(\frac{1}{1 + \mu(\alpha(x_\mu) - 1)}\right)\Lambda x_\mu \tag{11}$$

at the equilibrium point, x_μ. Then, substituting (11) into (10) gives $\frac{\partial x_\mu}{\partial \mu}^T \left(\frac{\partial f_\mu}{\partial x}\right)\frac{\partial x_\mu}{\partial \mu} = \frac{1-\alpha(x_\mu)}{1+\mu(\alpha(x_\mu)-1)}\left(\frac{\partial x_\mu}{\partial \mu}\right)^T \Lambda x_\mu$. Fix $\mu^* \in [0, 1]$ and suppose there exists a set $\mathcal{D} \subset \mathbb{R}^n_{\geq 0}$ such that $x_\mu \in \mathcal{D}$ and $\left.\frac{\partial f_\mu(x)}{\partial x}\right|_{x \in \mathcal{D}} \preceq 0$ for all $\mu \in [0, \mu^*]$. Then $\frac{\partial x_\mu}{\partial \mu}^T \left(\frac{\partial f_\mu}{\partial x}\right)\frac{\partial x_\mu}{\partial \mu} \leq 0$ for all $x \in \mathcal{D}$. 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 $\int_0^\mu \frac{\partial x_{\hat{\mu}}}{\partial \hat{\mu}}^T \Lambda x_{\hat{\mu}} d\hat{\mu} = x_{\hat{\mu}}^T \Lambda x_{\hat{\mu}}\Big|_0^\mu - \int_0^\mu x_{\hat{\mu}}^T \Lambda \frac{\partial x_{\hat{\mu}}}{\partial \hat{\mu}} d\hat{\mu} \leq 0$, and, since Λ is symmetric, this implies that

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

In particular, $x_{\mu^*}^T \Lambda x_{\mu^*} \leq x_0^T \Lambda x_0$. Additionally, $\mathcal{D}$ exists and $\mathcal{D} = \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ 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 14 guarantees that (8) always has one equilibrium point contained in the set $\{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$ when $\frac{\partial f_\mu}{\partial x} \preceq 0$ in that set for all $\mu \in [0, \mu^*]$. 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 14 may be used in conjunction with Theorem 10 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 μ is varied from 0 to μ^* by choosing $\mathcal{A}_\mu = \{x \in \mathbb{R}^n_{\geq 0} : x^T \Lambda x \leq x_0^T \Lambda x_0\}$. We will illustrate this in Section 6 through an example.

6 Application of Theory

In this section, we present examples to demonstrate the use of the theorems in Section 3 to genetic networks where fluctuations in production or degradation resources are captured by state-dependent perturbations α or g, respectively, for systems in the form of (5). In Example 6.1, we consider a genetic cascade and use Theorems 10 and 14 to find conditions where the system is guaranteed to maintain its number of equilibrium points. In Example 6.2, we revisit the design of a genetic toggle switch network. Specifically, we use Theorem 10 to find conditions where 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. Due to space limitations, we demonstrate the application of our results to systems with perturbations of degradation resources and for systems with more than 2 states in the extended version of the paper [39].

6.1 Genetic Cascades

Cascades are one of the most common genetic networks in both natural [40] and engineered systems [9]. We consider a two-node cascade shown in Figure 2 in which protein x_1 either activates (Figure 2a) or represses (Figure 2b) the production of protein x_2. The experimentally verified model [17] with perturbations in production resources in the form of (7) is given as

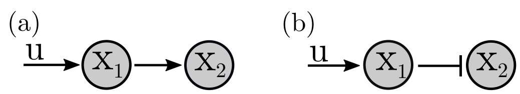

Fig. 2. Diagram of two-node cascade network: (a) Activation cascade (b) Repression cascade

$$\dot{x_1} = F_1(u)\left[1 + \mu\left(\frac{1}{1+J_1F_1(u)+J_2F_2(x_1)} - 1\right)\right] - x_1, \tag{12a}$$

$$\dot{x_2} = F_2(x_1)\left[1 + \mu\left(\frac{1}{1+J_1F_1(u)+J_2F_2(x_1)} - 1\right)\right] - x_2, \tag{12b}$$

where $F_i(z) = \frac{1+a_iz^{n_i}}{1+b_iz^{n_i}}$ with positive constants a_i, b_i, and n_i for $i = 1, 2$. Comparing (12) with (7) gives $h(x) = [F_1(u), F_2(x_1)]^T$, $\alpha(x) = \frac{[1,1]^T}{1+J_1F_1(u)+J_2F_2(x_1)}$, $\lambda^* = 0$, $\Lambda = \mathrm{diag}([1, 1])$. In this example, $\lambda^* = 0$ so we will simplify notation of the sets $\mathcal{A}_{\mu,\lambda}$ and $\mathcal{S}_{\mu,\lambda}$ to $\mathcal{A}_\mu$ and $\mathcal{S}_\mu$, respectively. The determinant of the Jacobian of (12) is given as

$$\det\left(\frac{\partial f_\mu}{\partial x}\right) = 1 + \mu\frac{F_1(u)F_2'(x_1)}{(1 + J_1F_1(u) + J_2F_2(x_1))^2}. \tag{13}$$

It can be seen from (13) that if $F_2'(\cdot) \geq 0$, then choosing $\mathcal{A}_\mu = \mathbb{R}^2_{\geq 0}$, and $\mathcal{A}_\mu$ contains all equilibrium points and $\det\left(\frac{\partial f_\mu}{\partial x}\right) > 0$ for all $x \in \mathcal{A}_\mu$ since all terms in (13) are nonnegative. Under these conditions, $\dot{x} = f_1(x)$ and $\dot{x} = f_0(x)$ have the same number of equilibrium points by Theorem 10. These conditions physically correspond to activation of x_2 by x_1, so *a two-protein activation cascade with perturbations in production resources always has one equilibrium point.*

We now investigate whether a repression cascade where $F_2'(\cdot) < 0$ is guaranteed to have one equilibrium point for any parameters using Theorems 10 and 14. Note that when $\mu = 0$, the system (12) becomes $\dot{x_1} = F_1(u) - x_1; \dot{x_2} = F_2(x_1) - x_2$. The equilibrium point is easily shown to be unique and is given as $x_1 = F_1(u); x_2 = F_2(F_1(u))$ due to the cascade structure of the system. Additionally, (12) with $\mu = 0$ satisfies the conditions of Theorem 14. Choose $\mathcal{A}_\mu = \{x \in \mathbb{R}^2_{\geq 0} : x_1^2 + x_2^2 \leq F_1(u)^2 + F_2(F_1(u))^2\}$. Now, the Jacobian of (12) is negative definite over $\mathcal{A}_\mu$ if

$$-\mu F_1(u)F_2'(x_1) < (1 + J_1F_1(u) + J_2F_2(x_1))^2, \tag{14a}$$

$$4 + 4\mu\beta F_2'(x_1) > \alpha(F_2'(x_1))^2 \tag{14b}$$

for all $x \in \mathcal{A}_\mu$ (derived using the principal minors), where $\beta = \frac{F_1(u)}{(1+J_1F_1(u)+J_2F_2(x_1))^2}$ and $\alpha = \left(1 - \mu\frac{J_1F_1(u)+2J_2F_2(x_1)+(J_1F_1(u)+J_2F_2(x_1))^2}{(1+J_1F_1(u)+J_2F_2(x_1))^2}\right)^2$. Note that (14a) is always satisfied whenever $F_2'(x_1) > -1$ (using the fact that $0 < F_1(\cdot) \leq 1$). Additionally, solving the quadratic equation in (14b) for $F_2'(\cdot)$ by using the fact that since $0 < F_1, F_2 \leq 1$, then $0 < \beta \leq 1$, and $0 \leq \alpha < 1$, we can guarantee that (14b) is satisfied whenever $2 - 2\sqrt{2} < F_2'(x_1) < 2$ over $\mathcal{A}_\mu$. The lower bound is found by maximizing the negative root of (14b) over $\alpha, \beta, \mu \in [0, 1]$, while the upper bound is found by minimizing the positive root of (14b) over $\alpha, \beta, \mu \in [0, 1]$ (i.e. worst case parameters). Then, if $F_2'(x_1)$ satisfies this condition, the equilibrium point is unique and contained in the set $\{x \in \mathbb{R}^2_{\geq 0} : x_1^2 + x_2^2 \leq F_1(u)^2 + F_2(F_1(u))^2\}$ for all $\mu \in [0, 1]$ by Theorem 14. Combining with the previous result that the equilibrium point is unique when $F_2'(x_1) > 0$, (12) is guaranteed to have one equilibrium point for any set of parameters when $F_2'(x) > 2 - 2\sqrt{2}$ for all $x \in \mathbb{R}_{\geq 0}$.

It was shown in [33] that a two-node repression cascade may have multiple equilibrium points. Due to space limitations, we use Theorem 12 to find conditions where the system with resource perturbations is guaranteed to change its number of equilibrium points in the extended version of this paper [39]. 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 of the function $|F_2'(\cdot)|$ is small.

6.2 Genetic Toggle Switch

We now revisit the motivating example presented in Section 2 and derive analytical conditions using our results under which the number of equilibrium points of the system changes when perturbed by resource sharing. Consider a genetic toggle switch shown in Figure 3. The toggle switch may be created either where x_1 and x_2 mutually activate each other (activation toggle, Figure 3a) or mutually repress each other (repression toggle, Figure 3b). We assume the toggle switch is perturbed by production resource fluctuations, and we wish to find conditions, when it is possible, for the system to exhibit multiple equilibrium points. The normalized, nondimensionalized model of the system with resource perturbations in the form of the parameterized system (7) is given as

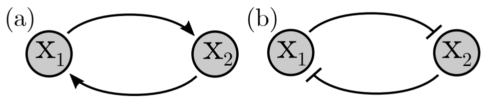

Fig. 3. Diagrams of possible toggle switch designs: (a) Activation toggle switch design. (b) Repression toggle switch design; ⊣ indicates repression.

$$\dot{x}_1 = \beta_1 \left[1 + \mu \left(\frac{F_1(x_2)/\beta_1}{1 + J_1 F_1(x_2) + J_2 F_2(x_1)} - 1\right)\right] - x_1 \tag{15a}$$

$$\dot{x}_2 = \beta_2 \left[1 + \mu \left(\frac{F_2(x_1)/\beta_2}{1 + J_1 F_1(x_2) + J_2 F_2(x_1)} - 1\right)\right] - x_2. \tag{15b}$$

Comparing this with (7), $h(x) = [\beta_1, \beta_2]^T$, $\alpha(x) = \frac{[F_1(x_2)/\beta_1, F_2(x_1)/\beta_2]^T}{1+J_1F_1(x_2)+J_2F_2(x_1)}$, $\Lambda = \mathrm{diag}([1,1])$, and $g(x) = 0$. Since $g(x) = 0$, we drop λ from our notation for clarity. The functions F_1 and F_2 have the form $F_i(x_j) = \frac{1+a_i x_j^{n_i}}{1+b_i x_j^{n_i}}$ for nonnegative constants a_i, b_i and integer $n_i \geq 1$. Additionally, β_1 and β_2 are positive constants such that $F_1(x_2) \leq \beta_1$ and $F_2(x_1) \leq \beta_2$ and all $x \in \mathbb{R}^2_{\geq 0}$. Then (15) satisfies the conditions on h, g, α, and Λ in Lemma 9 and Theorem 12. When $\mu = 0$, (15) is linear with one unique equilibrium point at $x_1 = \beta_1; x_2 = \beta_2$, while when $\mu = 1$, (15) has the dynamics of the toggle switch with resource sharing.

Using Theorem 12, we will find a necessary condition such that (15) has multiple equilibrium points. Note that $\det\left(\frac{\partial f_\mu}{\partial x}\right) > 0$ for all $x \in \mathbb{R}^2_{\geq 0}$ when $\mu = 0$. Let $\mu^* = 1$, and $\mathcal{B}_1 = \left\{x \in \mathbb{R}^2_{\geq 0} : \det\left(\frac{\partial f_1(x)}{\partial x}\right) < 0\right\}$. By Theorem 12, if (15) has multiple equilibrium points, then at least one equilibrium point exists in $\mathcal{B}_1$. We now simplify our reasoning by taking advantage of the symmetry of (15). We assume that $J_1 = J_2 = J$ and $F_1(\cdot) = F_2(\cdot) = F(\cdot)$. Since the dynamics of x_1 and x_2 are symmetric, this implies that the trajectories of (15) are symmetric about the line $x_1 = x_2$ and all equilibrium points must appear symmetrically about the line $x_1 = x_2$. We will now find a condition on $\frac{\partial F(x)}{\partial x}$ such that $\mathcal{B}_1$ is nonempty, which is necessary for (15) to exhibit multiple equilibrium points by Theorem 12.

Since $\deg(f_\mu) = 1$ by Lemma 9, then the number of equilibrium points is odd. Since the determinant of the Jacobian of (15) is symmetric about the line $x_1 = x_2$ and the number of equilibrium points is odd, there always exists an odd number of equilibrium points on the line $x_1 = x_2$. The dynamics of (15) restricted to $x_1 = x_2 = x$ are given as

$$\dot{x} = \beta \left[1 + \mu \left(\frac{F(x)/\beta}{1 + 2JF(x)} - 1\right)\right] - x. \tag{16}$$

By Lemma 9, the degree of (16) is -1, which implies that there exists at least one equilibrium point on the line $x_1 = x_2 = x$ such that $\det\left(\frac{\partial f_1(x)}{\partial x}\right) < 0$ by the definition of degree. Thus, we can restrict our attention to the line $x_1 = x_2$ to find a necessary condition for the existence of $\mathcal{B}_1$ in Theorem 12. We denote $\frac{\partial F(x)}{\partial x}$ as $F'(x)$. Then, the determinant of the Jacobian of (16) is given as

$$\det\left(\frac{\partial f_\mu}{\partial x}\right) = 1 + \frac{2\mu J F(x) F'(x)}{(1 + 2JF(x))^2} - \frac{\mu^2 (F'(x))^2}{(1 + 2JF(x))^3}. \tag{17}$$

We now evaluate (17) when $\mu = 1$ and find an equality to eliminate the dependence of (17) on $F(x)$. With $\mu = 1$ and setting the derivative in (16) to 0, $\frac{F(x^*)}{1+2JF(x^*)} = x^*$ is satisfied at any equilibrium point x^* that lies on the line $x_1 = x_2$. Then, solving for $F(x^*)$, we have

$$F(x^*) = \frac{x^*}{1 - 2Jx^*}. \tag{18}$$

Since $F(x) > 0$ for all $x \geq 0$, then all equilibrium points x^* satisfy $x^* \in [0, \frac{1}{2J})$. Substituting (18) into (17) with $\mu = 1$, we find

$$\det\left(\frac{\partial f_1}{\partial x}\right) = 1 + 2J(1 - 2Jx^*)F'(x^*) - (1 - 2Jx^*)^3 (F'(x^*))^2. \tag{19}$$

Next, setting $\det\left(\frac{\partial f_1}{\partial x}\right) < 0$ and solving the resulting quadratic inequality in (19) for $F'(x^*)$, we find that if (15) has multiple equilibrium points by Theorem 12, there exists an equilibrium point x^* such that

$$F'(x^*) > \frac{J + \sqrt{J^2 + 1 - 2Jx^*}}{(1 - 2Jx^*)^2}, \text{ or} \tag{20a}$$

$$F'(x^*) < \frac{J - \sqrt{J^2 + 1 - 2Jx^*}}{(1 - 2Jx^*)^2}. \tag{20b}$$

Note that an equilibrium point $x^* \in \mathcal{B}_1$ if and only if x^* satisfies (20). Furthermore, if (20) is never satisfied for any $x^* \in [0, \frac{1}{2J})$, then (20) is never satisfied for any equilibrium point and, by Theorem 12, (15) exhibits one equilibrium point.

We restrict our attention to the activation toggle switch, as presented in Section 2 where $F'(x) > 0$ for all $x \geq 0$. Note that when $J = 0$ (no resource sharing), the right-hand side of (20a) is 1. It can be shown that the right-hand side of (20a) is increasing with increasing J for all $x \in [0, \frac{1}{2J})$ by taking the derivative with respect to J. Increasing J corresponds to increased resource demand by the proteins x_1 and x_2. Thus, an activation toggle switch that meets the condition in (20a) when $J = 0$, may not satisfy it when $J > 0$. Specifically, there exists an $F'(x)$ that satisfies (20) when $J = 0$, but not when $J > 0$, and any $F'(x)$ that satisfies (20) for some $J > 0$ also satisfies (20) when $J = 0$. Therefore, in the activation toggle switch, a system that nominally has multiple equilibrium points may have one equilibrium point when perturbed with resource sharing. Due to space limitations, we use Theorem 10 to find a condition on $F'(x)$ such that (15) is guaranteed to always have one equilibrium point in the extended version [39].

7 Discussion

The number of equilibrium points is an important qualitative property of dynamical systems. In this paper, we developed a theoretical framework to determine algebraic conditions under which the number of equilibrium points of a positive dynamical system changes when state-dependent perturbations are considered. Our results allow for the analysis of this problem without having to explicitly find the equilibrium points, thus allowing us to determine parametric conditions under which the number of equilibrium points of a nominal system and a perturbed system differ.

We applied our tools to genetic networks as a specific application example. State-dependent perturbations such as arising from fluctuations in available resources have recently appeared as a major problem to our ability of predicting a genetic network'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 networks in a way such that they are robust to perturbations in resources, and to select the most robust network topologies.

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A Additional Proofs and Mathematical Background

Definition 15 *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 16 *Let Ω and f be as in Definition 3 and suppose f has only isolated zeros. Let x_i be a zero of f and Ω_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 Ω_i, then the* index of an isolated zero of f *is* $\Phi(f, x_i) = \deg(f, \Omega_i)$.

Lemma 17 *Let $\Omega \subset \mathbb{R}^n$, $f : \Omega \to \mathbb{R}^n$ be a $\mathcal{C}^1$ 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 Ω is equal to the sum of the absolute values of the indexes of f in Ω, i.e. $n = \sum_i |\Phi(f, x_i)|$.*

PROOF. The proof follows by Definitions 3 and 16. 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 [42] and Definition 16 may be applied. Note that $n = \sum\limits_{x \in f^{-1}(0)} \left|\operatorname{sign}\left(\det\left(\frac{\partial f}{\partial x}(x)\right)\right)\right| = \sum_i |\Phi(f, x_i)|$ since $|\operatorname{sign}(z)| = 1$ for any $z \neq 0$ which is assumed in Definition 3. □

PROOF. *Lemma 5.* Choose a fixed $\lambda^* > 0$ and let n denote the cardinality of $\mathcal{S}_0$. Suppose that $\det\left(\frac{\partial f_\lambda(x)}{\partial x}\right) \neq 0$ for each $x_i^\lambda \in \mathcal{S}_\lambda$ 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 $\mathcal{P} = \{0 =$

$\lambda_0, \lambda_1, \ldots, \lambda_{N-1}, \lambda_N = \lambda^*\}$. Consider the kth subinterval $[\lambda_k, \lambda_{k+1}]$, where λ_k is fixed and λ_{k+1} will be chosen later. For λ_k and for each $x_i^{\lambda_k} \in \mathcal{S}_{\lambda_k}$, there exists an open ball $\Omega_i^{\lambda_k} = B(x_i^{\lambda_k}, \epsilon_i^k)$ containing the zero $x_i^{\lambda_k}$ such that $x_i^{\lambda_k}$ is the unique solution of $f_{\lambda_k}(z) = 0$ for $z \in \overline{\Omega}_i^{\lambda_k}$ by the Inverse Function Theorem [42]. Note that x_i^{λ} is continuous, since f_λ is continuous with respect to λ. Choose λ_{k+1} such that for all $\lambda \in [\lambda_k, \lambda_{k+1}]$, $x_i^\lambda \in \Omega_i^{\lambda_k}$ for all $i = 1, \ldots, n$ since x_i^λ is continuous. Apply Lemma 17 to each $\Omega_i^{\lambda_k}$ since each zero is isolated, contained in $\Omega_i^{\lambda_k}$, and the index for each x_i^λ 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 $\mathcal{S}_\lambda$ 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 $\mathcal{S}_\lambda$ is constant and equal to n for all $\lambda \in [0, \lambda^*]$. □

Lemma 18 *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 $c, d \in \mathbb{R}^n_{\geq 0}$, $c \odot f(x) + d \odot g(x)$ 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 $c, d \in \mathbb{R}^n_{\geq 0}$, $h_2(x) = c \odot f(x) + d \odot g(x)$ 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 $c \odot f(x) + d \odot g(x) \geq 0$ for all $x \in \partial\mathbb{R}^n_{\geq 0}$. Thus, $h_2(x)$ is positive invariant. □

PROOF. *Lemma 9.* We first show that (7) is positive invariant. Since $h(x)$, $g(x)$, and $-\Lambda x$ are $\mathcal{C}^1$ positive invariant functions, $\alpha(x)$ is $\mathcal{C}^1$, and $0 < \alpha(x) \leq 1$ for $x \in \mathbb{R}^n_{\geq 0}$, it follows that $\mu(\alpha(x) - \mathbb{1}) + \mathbb{1} > 0$ for any $\mu \in [0, 1]$. Then $f_{\mu,\lambda}(x)$ is $\mathcal{C}^1$ positive invariant for all $\mu, \lambda \in [0, 1] \times [0, \infty)$ by Lemma 18. This proves (a).

Next, to prove (b), we construct a bounded domain, Ω, over which we will consider the set of equilibrium points of (7) in the positive orthant. To construct Ω, 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 $\dot{x} = h(x) - \Lambda x$, so $\Lambda x(t)$ is also bounded for all $t \geq 0$. Furthermore, $m \cdot (\Lambda x(t))$ is finite. Choose $M > \sup_{t \geq 0} \{m \cdot (\Lambda x(t))\}$. We now define $\Omega = \{x \in \mathbb{R}^n_{>0} : m \cdot (\Lambda x) < M\}$. We prove that $f_{\mu,\lambda}$ has no zeros on the boundary of Ω. 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 $-\Lambda x$ are positive invariant and $\alpha(x) > 0$. Now, we show that $f_{\mu,\lambda}$ has no zeros on the boundary defined by $\{x : m \cdot (\Lambda x) = M\}$ and no zeros in the positive orthant outside of Ω. To this end, consider

$$
\begin{aligned}
m \cdot f_{\mu,\lambda} =& m \cdot (h \odot [\mathbb{1} + \mu(\alpha(x) - \mathbb{1})]) + \\
& m \cdot \lambda g - m \cdot (\Lambda x) \\
m \cdot f_{\mu,\lambda} =& m \cdot (\mu(\alpha - \mathbb{1}) \odot h) + m \cdot h + m \cdot \lambda g - \\
& m \cdot \Lambda x.
\end{aligned}
$$

Since $\alpha \leq 1$, then $m \cdot (\mu(\alpha - \mathbb{1}) \odot h) \leq 0$. It then follows that $m \cdot f_{\mu,\lambda} \leq m \cdot h + m \cdot \lambda g - m \cdot (\Lambda x)$. Furthermore, since g is mass dissipating with respect to m, we have $m \cdot g \leq 0$ and $m \cdot f_{\mu,\lambda} \leq m \cdot h - m \cdot (\Lambda x)$. Since $M > \sup_{t \geq 0} \{m \cdot (\Lambda x(t))\}$, this implies that $\sup_t \{m \cdot h(x(t))\} < M$. Then, for $\{x : m \cdot (\Lambda x) \geq M\}$, we have $m \cdot f_{\mu,\lambda} \leq m \cdot h(x) - m \cdot (\Lambda x) \leq m \cdot h(x) - M < 0$. So $m \cdot f_{\mu,\lambda} < 0$ for all points on the outer boundary of $\Omega : \{x : m \cdot (\Lambda x) = M\}$ 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 Ω for any $\mu, \lambda \in [0, 1] \times [0, \infty)$. Similarly, since $m \cdot f_{\mu,\lambda}(x) < 0$ for all $x \in \{x : m \cdot (\Lambda x) > M\}$, then there exist no zeros in the positive orthant outside of Ω for any $\mu, \lambda \in [0, 1] \times [0, \infty)$. Therefore the interior Ω 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 $f_{0,0}(x) = h(x) - \Lambda x$. Since $x(t)$ is bounded, $\overline{\Omega}$ is compact, and, since $h(x)$ is continuous over $\overline{\Omega}$, then $h(x(t))$ is bounded over $\overline{\Omega}$. We can rewrite as $h(x) = c \odot \beta(x)$ where $\beta : \mathbb{R}^n \to \mathbb{R}^n$ is $\mathcal{C}^1$, $0 < \beta(x) \leq 1$ for all $x \in \mathbb{R}^n_{\geq 0}$, and $c_i = \sup_{x \geq 0} \{h_i(x)\}$ for each $i = 1, \ldots, n$. We now define the auxiliary function $\hat{f}_\nu(x) = c \odot [\mathbb{1} + \nu(\beta(x) - \mathbb{1})] - \Lambda x$ with parameter $\nu \in [0, 1]$. Then $\hat{f}_1(x) = f_{0,0}(x)$ and $\hat{f}_0(x) = c - \Lambda x$, which is linear. Since $0 < \beta(x) \leq 1$ and $c > 0$, then $\hat{f}_\nu$ is positive invariant and has no zeros on the boundary of Ω, as shown previously. Additionally, $\hat{f}_0(x)$ has one zero in Ω, namely $x = \Lambda^{-1}c$, and the Jacobian is $\frac{\partial \hat{f}_0}{\partial x} = -\Lambda$. Then $\det\left(\frac{\partial \hat{f}_0}{\partial x}\right) = \prod_{i=1}^n (-\Lambda_{ii})$ and $\text{sign}\left(\det\left(\frac{\partial \hat{f}_0}{\partial x}\right)\right) = (-1)^n$ so $\deg(\hat{f}_0(x), \Omega) = (-1)^n$ by Definition 3. Then, by Theorem 4, $\deg(f_{\mu,\lambda}, \Omega) = \deg(f_{0,0}, \Omega) = \deg(\hat{f}_1, \Omega) = \deg(\hat{f}_0, \Omega) = (-1)^n$. Furthermore, $S_{\mu,\lambda} \neq \emptyset$ by Definition 3. This proves (c). □