Bistable biological systems: a characterization through local compact input-to-state stability

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***Abstract*—Many biological systems have the capacity to operate in two distinct modes, in a stable manner. Typically, the system can switch from one stable mode to the other in response to a specific external input. Mathematically, these bistable systems are usually described by models that exhibit (at least) two distinct stable steady states. On the other hand, to capture biological variability, it seems more natural to associate to each stable mode of operation an appropriate invariant set in the state space rather than a single fixed point. A general formulation is proposed in this paper, which allows freedom in the form of kinetic interactions, and is suitable for establishing conditions on the existence of one or more disjoint forward-invariant sets for the given system. Stability with respect to each set is studied in terms of a local notion of input-to-state stability with respect to compact sets. Two well known systems that exhibit bistability are analyzed in this framework: the *lac* operon and an apoptosis network. For the first example, the question of designing an input that drives the system to switch between modes is also considered.**

***Index Terms*—Bistability; Compact input-to-state stability; Biological networks.**

I. Introduction

BISTABILITY is a recurrent motif in biology, and there are many examples of systems which can operate, in a stable manner, in two very distinct modes. For instance, the well known *lac* operon in the bacteria *Escherichia coli*, a group of genes which are repressed in the presence of glucose but transcribed in the absence of glucose and presence of lactose [1], [2]. Another striking example is the phage λ virus, which may exist in either of two states. Under "normal" conditions, this virus can exist in a dormant (lysogentic) state, and survive indefinitely within its host, *E. coli*. However, under "adverse" conditions, for example after irradiation with ultra-violet light [3], the phage can switch to a reproducible (lytic) mode, leading to bacterial lysis (that is, the bacteria burst). Yet another example is the complex system of cross-talking pathways that regulates the decision of cells to enter the process of programmed cell death, also known as apoptosis, as opposed to continuing normal development [4]–[6]. From a failure in the pro- and anti-apoptotic signaling pathways various diseases may result, including cancer (where damaged cells that fail to undergo apoptosis, continue to reproduce).

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Bistable behavior has been experimentally detected at the single cell level (for example, the *lac* operon in *E. Coli* [2] and the cell cycle oscillator in *Xenopus laevis* [7]). These beautiful experiments show that each individual cell can indeed only exist in one of two distinct states, and upon stimulation with an appropriate input, a clear jump-like transition is observed, from one state to another. To understand how each bistable system works, many mathematical models have been proposed, but a common feature is the existence of an appropriate positive feedback loop (see, for instance, [6], [8] for analysis of a caspase cascade at the heart of apoptosis). A general method for multistability in a large class of biological systems is provided in [9], using the concept of monotone systems. On the other hand, at the population level, a graded response to increasing stimuli is typically observed [2], [10]. This means that each cell has its own "threshold", its own particular point where it will jump from one steady state to the other. Since this threshold varies from cell to cell, a population experiment should reflect the fraction of cells in a given steady state for each given stimulus concentration.

This introduces a fundamental issue of concern when modeling and studying biological systems: the inherent variability encountered among different "realizations" of the same system. Various modeling techniques have been suggested and used to deal with the problem of variability, and obtain ever more realistic descriptions of the biological systems. Just to cite some examples, among many others: stochastic models [11], [12], discrete/logical models which provide more qualitative descriptions [13]–[16], and more recently hybrid models [17], and in particular piecewise linear models [18]–[22]. The system under study, its complexity, and the knowledge and experimental data available, often determine the most suitable method for modeling a given system. In the case of genetic regulatory networks, although exact forms for the interactions are often not known, the presence (or expression) of a given protein or mRNA is typically due to the appropriate combination of presence or absence of another group of species [23].

An alternative approach is proposed here, which provides an intuitive bridge between continuous models and the class of piecewise linear hybrid models. This approach is specially attractive for the type of systems whose interactions can be described as combinations of "activation" and "inhibition" functions. These functions will be generally formulated in the sense that, instead of a specific mathematical formula, they are bounded within appropriate "tubes". In this context, one may expect a mathematical model for a bistable biological system to exhibit two distinct, disjoint, forward-invariant sets

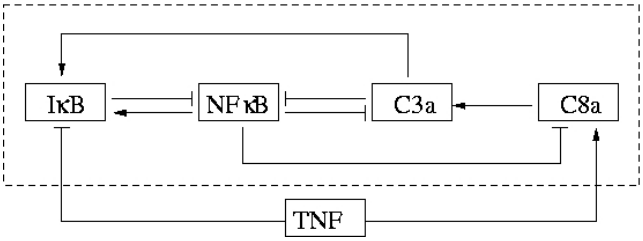

Fig. 1. A simplified schematic view of the interactions between the NFκB pathway and the apoptosis network (see Section IV and also [29]).

in its state space: each invariant set representing one stable mode of operation. An invariant set, as opposed to a single fixed point, captures variability or small disturbances in the system's trajectories while maintaining the same qualitative behavior. The system is able to switch from one invariant set to another only in response to an appropriate external input. An ideal framework to analyze such mathematical systems, and characterize their stability with respect to inputs, is the notion of input-to-state stability (ISS) with respect to compact sets [24]. This can be viewed as a generalization of the original ISS concept [25], [26]. A powerful and extremely useful tool for analysis of control systems, ISS has been adapted to deal with positive systems [27], [28], and in the present case will be adapted to a "local" property, and thus allow for co-existence of two disjoint forward-invariant sets. The original ISS notion is global and, for the zero-input case, ISS implies global asymptotic stability (to the origin or, more generally, the given compact set). The definition suggested here will make use of a local region (one for each forward-invariant set), containing the compact set, over which the input-to-state stability estimates hold. The definitions of "activation" and "inhibition" functions are introduced in Section II. The local notion of ISS with respect to compact sets is then given in Section III, together with a characterization through ISS Lyapunov functions. These ideas are then illustrated with the examples of an apoptosis network and the *lac* operon (Sections IV, V, respectively), and results are compared and discussed in Section VI.

II. A GENERAL FRAMEWORK

We will presently focus on biological networks consisting only of activation or inhibition links, such as the network depitect in Fig. 1 (see Section IV for a description of the system). For these networks, the exact form of interactions is usually not known, and various options can be used in mathematical models. The interactions typically involve threshold concentrations, above (or below) which the activation or inhibition of one species by another is not significant. Such functions are often described mathematically by saturation functions (e.g. Hill type), which involve estimating and choosing fixed parameters that represent an average behaviour (for instance, in a group of cells of the same type). Such functions will not satisfactorily capture the variability, but rather an average behavior. The first step in setting up a general framework, is to associate to each activation (resp. inhibition) link an *activation (resp. inhibition) function* that is defined inside a

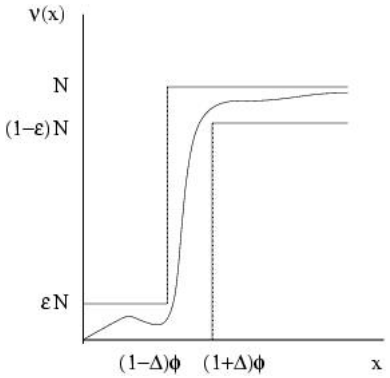

Fig. 2. An activation function $\nu(x)$.

tube (see [29] and Fig. 2). The second step is to consider that each of the variables is produced according to the overall result of the several activation and inhibition links particular to that node and, in addition, is freely degraded. The resulting model will depict the principal interconnetions among the system's variables, but without specifying particular kinetic laws for interactions.

Definition 2.1: Let $N \in \mathbb{R}_+$. A function $\nu : [0,\infty) \to [0,N]$ is an *activation function* if:

(i) ν is continuously differentiable;
(ii) $0 < x < \infty$ implies $\nu(x) > 0$ and $\nu(0) = 0$;
(iii) There exists a threshold value $0 < \phi < \infty$ and constants $\varepsilon, \Delta \in (0,1)$ such that

$$x \in [0, \phi(1-\Delta)) \;\Rightarrow\; \nu(x) \in [0, \varepsilon N),$$
$$x \in (\phi(1+\Delta), \infty) \;\Rightarrow\; \nu(x) \in (N(1-\varepsilon), N].$$

Definition 2.2: Let $M \in \mathbb{R}_+$. A function $\mu : [0,\infty) \to [0,M]$ is an *inhibition function* if:

(i) μ is continuously differentiable;
(ii) $0 < x < \infty$ implies $\mu(x) > 0$ and $\mu(0) = M$;
(iii) There exists a threshold value $0 < \theta < \infty$ and constants $\varepsilon, \Delta \in (0,1)$ such that

$$x \in [0, \theta(1-\Delta)) \;\Rightarrow\; \mu(x) \in (M(1-\varepsilon), M],$$
$$x \in (\theta(1+\Delta), \infty) \;\Rightarrow\; \mu(x) \in [0, \varepsilon M).$$

These definitions are more general than those given in [29], as the restriction for the functions to be strictly monotone has been lifted. Instead, an extra assumption is added, as point (ii) in both Definitions 2.1, 2.2. This property means that an activation function is zero (an inhibition function equals its maximal value) if and only if $x = 0$. This property is not unnatural, and will be useful (together with continuity) in providing a strictly positive minimum value for a function μ in any compact set with $x > 0$. Note that property (ii) allows $\lim_{x\to\infty} \mu(x) = 0$. Another difference regarding the definitions given in [29] is the fact that the value ε (resp., Δ) now represents a *fraction* of the maximal activity (resp., activity threshold).

In the networks depicted in Figs. 1 and 4, nodes inside the dashed rectangle constitute the system's variables, and nodes outside the dashed rectangle form the system's set of inputs. The effect of an activating input on a given variable (link of

the form $\rightarrow$) will be represented as an additive term, and an inhibitory input (link of the form $\dashv$) will be represented as a product with the other terms in the corresponding variable dynamics. The dynamical system for the network in Fig. 1 can then be written, using the notation $x = [\text{NF}\kappa\text{B}]$, $y = [\text{I}\kappa\text{B}]$, $w = [\text{C8a }]$, $z = [\text{C3a}]$, and $u = [\text{TNF}]$:

$$\begin{aligned} \dot{x} &= -k_x x + \mu_1(y)\,\mu_3(z) \\ \dot{y} &= -k_y y + \nu_1(x)\,\nu_2(z)\,\mu_5(u) \\ \dot{w} &= -k_w w + \mu_4(x) + \nu_3(u) \\ \dot{z} &= -k_z z + \mu_2(x)\nu_4(w). \end{aligned} \tag{1}$$

The term $\mu_1(y)\mu_3(z)$ should be interpreted as a total production rate for NFκB, which depends only on how large the concentrations of IκB and C3a are at each instant. Similar interpretation holds for the other production terms. TNF stimulation may be assumed constant, either zero or positive (see Section IV).

Definitions 2.1 and 2.2 imply that there is a "tube" inside which the functions must lie. Examples of such functions include not only Hill and other sigmoidal shaped functions (Fig. 2), but also hyperbolic functions, such as Michaelis-Menten or Monod type kinetics. Numbers ε, Δ can be found to construct a tube around a hyperbolic function (see next paragraph, $n = 1$); however, such a tube might not be sharp enough for some applications. Observe that the limiting case $\varepsilon \equiv \Delta \equiv 0$ reduces essentially to the piecewise linear systems introduced first by Glass and Kauffman [18], and more recently used to study gene regulatory networks in [19]–[22].

The advantage of such an approach is in its general formulation: consider a batch of cells of the same type, to be used in single cell experiments. A model could be generated from experiments with a few cells as "calibration", and then used to extract new information from each of the single cell experiments. If a specific Hill function is chosen say, $Vx^\ell/(k^\ell + x^\ell)$, then the new results will not be as accurate as they could be, if each cell will have slightly different $\tilde{V}$, $\tilde{k}$, and $\tilde{\ell}$. Defining general functions as those in Definitions 2.1 and 2.2, allows the same model to be used for all cells in the batch, as intervals for parameters V, k, and ℓ can be incorporated into μ and ν functions. To write a Hill or Michaelis-Menten type function ($\ell \geq 1$) as an activation function, one may choose: $N = V$, $\phi = k$, and numbers ε, Δ so that $\frac{1-\varepsilon}{\varepsilon} \leq \min\{\frac{1}{(1-\Delta)^\ell}, (1+\Delta)^\ell\}$.

The next property is straightforward from the definitions:

Fact 1: A continuously differentiable function μ is an inhibition function with constants M, θ, ε, Δ, if and only if $\nu = M - \mu$ is an activation function with constants $N = M$, $\phi = \theta$ and ε, Δ.

It is clear that property (iii) is equivalent in both cases since: $x \in [0, (1-\Delta)\theta)$ implies $\mu(x) > M(1-\varepsilon)$, which in turn implies $\nu(x) = M - \mu(x) < M - M(1-\varepsilon) = \varepsilon M$. (The converse implication is similar.) If properties (i) and (ii) of Definition 2.2 hold for μ, then immediately (i) and (ii) of Definition 2.1 hold for $\nu = M - \mu$, and conversely.

Before stating another simple property, recall some standard functions (e.g., [26]), which will be used later. A function $\gamma : \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}_{\geq 0}$ is said to be of class $\mathcal{K}$ if it is continuous, strictly increasing, and zero at the origin. It is of class $\mathcal{K}_\infty$ if, in addition, $\lim_{r\rightarrow\infty} \gamma(r) = \infty$. A function $\beta : \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0} \rightarrow \mathbb{R}_{\geq 0}$ is said to be of class $\mathcal{KL}$, if $\beta(\cdot, t)$ is a $\mathcal{K}_\infty$ function for each fixed $t \geq 0$, and $\beta(r, \cdot)$ is strictly decreasing and satisfies $\lim_{t\rightarrow\infty} \beta(r, t) = 0$ for each fixed r.

Fact 2: Let ν be an activation function. Then there exists a class $\mathcal{K}_\infty$ function γ such that $\nu(x) \leq \gamma(x)$ for all $x \geq 0$.

To see this, let $\tilde{\gamma}(r) = \max\{\nu(x) : \ x \in [0, r]\}$. Then $\tilde{\gamma}(0) = \nu(0) = 0$. $\tilde{\gamma}$ is nondecreasing by construction and continuous because ν is. Then, an appropriate $\mathcal{K}_\infty$ function γ with $\gamma(x) \geq \tilde{\gamma}(x) \geq \nu(x)$ can be found.

For simplicity, throughout this paper it will be assumed that the constants ε and Δ are the same for all activation and inhibition functions in the network (however, the results can be easily extended to the case where ε and Δ are distinct for each activation or inhibition function).

III. Input-to-state stability with respect to compact sets

As in example (1), consider the following model for genetic networks:

$$\dot{x} = -K_{deg} x + F(x, u) \tag{2}$$

where K_{deg} is an $n \times n$ diagonal matrix, containing in its ii-th entry, the degradation rate for species x_i. The function $F(x, u) : \mathbb{R}^n_{\geq 0} \times \mathbb{R}^m_{\geq 0} \rightarrow \mathbb{R}^n_{\geq 0}$ is a sum of terms, each term a product of activation or inhibition functions. Since exact functions are not provided, fixed points cannot be computed. But the objective here is to carry out an equivalent analysis, by identifying forward invariant sets (as opposed to fixed points) in the state space. The existence of forward invariant sets for a system of the form (2), will depend on the relationships among the various threshold and maximal rate constants. Using once more the analogy with the batch of same type cells, suppose that each cell has its own steady state point, which varies from individual cell to cell. But all these steady state points will belong to the same invariant set of system (2). Thus, even if exhibiting slight variations, all cells can be expected to have the same qualitative behavior, characterized by a system of the form (2) and its forward invariant sets.

A very natural concept from control theory to help characterize existence (and stability) of invariant sets, is that of input-to-state stability (ISS) with respect to compact sets [24]. This can be viewed as a generalization of the original ISS notion [25], in which case the compact set is simply the origin $\{0\}$. The concept of ISS has revealed itself an extremely powerful notion in many situations, for characterizing stability of systems, robustness with respect to state, and output disturbances, cascaded systems, and other applications [26], [30], [31]. The definitions to be formulated next, adapt compact ISS to a local property, in the sense that estimates are required to hold only while the trajectories of the system remain within some appropriate set. Similar notions have already been introduced to deal with positive, biochemical networks (for instance [27], [28]).

A. Local notions of compact ISS

In the definitions to follow next, for simplicity consider a system with inputs $\dot{x} = f(x,u)$, evolving in a set $\mathcal{X} \subset \mathbb{R}^n_{\geq 0}$, where $f(\cdot,u)$ is continuously differentiable for each fixed u, and define an *input* to be a locally Lipschitz function $w : \mathbb{R}_{\geq 0} \to \mathbb{R}^m_{\geq 0}$. Let $|u|$ denote the usual Euclidean norm for matrices and define also:

$$\|u\| = \text{ess. sup}.\{|u(t)| : \ t \in [0,+\infty)\}.$$

In the next definition, let $0 < T_{\max} \leq \infty$ and assume that $J_{x_0,w} = [0, T_{\max})$ is the interval where the maximal solution of a system $\dot{x} = f(x,u)$, for an initial condtion x_0 and input w, is defined.

Definition 3.1: A set P is *forward-invariant* for the system $\dot{x} = f(x,u)$ if, for each initial state $x(0) = x_0 \in P$, and each input $w(\cdot)$, the corresponding maximal solution $x(t,x_0,w)$, which is defined on an interval $J_{x_0,w} = [0, T_{\max})$, satisfies $x(t,x_0,w) \in P$ for all $t \in J_{x_0,w}$. The system is P-forward complete if P is a forward invariant set for the system and, in addition, $J_{x_0,w} = [0,\infty)$, for each $x(0) = x_0 \in P$ and each input $w(\cdot)$.

Following [24], let $\mathcal{Q}$ be a nonempty compact set of $\mathbb{R}^n_{\geq 0}$. Then define the usual point-to-set distance:

$$|x|_{\mathcal{Q}} = \inf\{|x-q|, \ q \in \mathcal{Q}\}.$$

In our examples, as in many biological systems, the set $\mathcal{X}$ is a product of intervals $\Pi_{i=1}^n [0, a_i]$, for finite a_i, $i = 1, \ldots, n$. The compact sets to be considered will often touch the boundary of $\mathcal{X}$, for instance $\mathcal{Q} = \{x \in \mathcal{X} : 0 \leq x_1 \leq \varepsilon a_1\}$, with $0 < \varepsilon < 1$. In this context, we will still say that $\mathcal{Q}$ *is contained in the interior of* $\mathcal{X}$. More generally we define:

$$\text{int}_{\mathcal{X}} R := \{x \in R : \ x \in \text{int } R \ \text{ or } \ x \in \partial R \cap \partial \mathcal{X}\}. \qquad (3)$$

Definition 3.2: Assume that the system $\dot{x} = f(x,u)$, is $\mathcal{X}$-forward complete. Then the system is *locally input-to-state stable with respect to a compact set* $\mathcal{Q}$ if there exists a set $R \subset \mathcal{X}$ with $\mathcal{Q} \subset \text{int}_{\mathcal{X}} R$, and functions $\beta = \beta_R$ of class $\mathcal{KL}$ and $\varphi = \varphi_R$ of class $\mathcal{K}_\infty$ such that, for every initial condition $x_0 \in R$ and each input $w(\cdot)$:

$$|x(t,x_0,w)|_{\mathcal{Q}} \leq \beta(|x_0|_{\mathcal{Q}}, t) + \varphi(\|w\|), \qquad (4)$$

for all $t \geq 0$ such that $x(s) \in R$ for all $s \in [0,t]$.

If $R = \mathcal{X}$ then the system is *globally input-to-state stable with respect to the compact set* $\mathcal{Q}$.

Definition 3.3: A continuously differentiable function $V : \mathbb{R}^n_{\geq 0} \to \mathbb{R}_{\geq 0}$ is a *local ISS Lyapunov function with respect to a compact set* $\mathcal{Q}$ for the system $\dot{x} = f(x,u)$, if:

(i) there exist functions $\nu_1, \nu_2 \in \mathcal{K}_\infty$, so that

$$\nu_1(|x|_{\mathcal{Q}}) \leq V(x) \leq \nu_2(|x|_{\mathcal{Q}})$$

for all $x \in \mathbb{R}^n_{\geq 0}$.

(ii) there exists a set $R \subset \mathcal{X}$ with $\mathcal{Q} \subset \text{int}_{\mathcal{X}} R$, and functions $\alpha = \alpha_R, \gamma = \gamma_R \in \mathcal{K}_\infty$ such that

$$\nabla V(x) \ f(x,u) \leq -\alpha(|x|_{\mathcal{Q}}) + \gamma(|u|)$$

for every $x \in R$.

If $R = \mathcal{X}$, then the function V is a *global ISS Lyapunov function with respect to the compact set* $\mathcal{Q}$ for the system.

The local condition means that the ISS estimate will remain valid as long as the trajectory evolves within the given set R. As in the case of the original definition of an ISS system, the existence of an ISS-Lyapunov function with respect to a compact set $\mathcal{Q}$ implies that the system is input-to-state stable with respect to that compact set $\mathcal{Q}$. The proof of this result is very similar to the original one, and follows closely the argument given in [26], hence we do not include it (see also [27]).

Lemma 3.4: Consider an $\mathbb{R}^n_{\geq 0}$- forward complete system $\dot{x} = f(x,u)$. Suppose that V is a local (resp., global) ISS Lyapunov function with respect to the compact set $\mathcal{Q} \subset \mathbb{R}^n_{\geq 0}$. Then, the system is locally (resp., globally) input-to-state stable with respect to the compact set $\mathcal{Q}$. □

If the system is globally ISS with respect to a compact set $\mathcal{Q}$, then this set is said to be *0-invariant* for the system, that is the solution of

$$\dot{x} = f(x,0), \ x(0) = x_0 \in \mathcal{Q}$$

remains in $\mathcal{Q}$ for all $t \geq 0$, that is, $x(t,x_0,0) \in \mathcal{Q}$ whenever $x_0 \in \mathcal{Q}$. Furthermore, if a system is globally ISS with respect to $\mathcal{Q}$, then in the case $u(t) \equiv 0$, the trajectories globally asymptotically converge to $\mathcal{Q}$. It is not difficult to check that the definition of local compact ISS also implies 0-invariance of the set $\mathcal{Q}$. One needs only to verify that, when $u(t) \equiv 0$ and $x_0 \in \mathcal{Q}$, the trajectories do not leave the set R. To see this, simply note that (4) together with $u(t) \equiv 0$ and $x_0 \in \mathcal{Q}$, in fact imply $|x(t,x_0,w)|_{\mathcal{Q}} \leq 0$ for all times. Using Lemma 3.4 the following result holds.

Lemma 3.5: If there exists a local ISS Lyapunov function with respect to the compact set $\mathcal{Q}$ for the system $\dot{x} = f(x,u)$, then $\mathcal{Q}$ is a 0-invariant set for the system. ∎

The definition in local terms is useful when there exist two (or more) disjoint 0-invariant sets for the system (as is the case with bistable systems). In this case, global asymptotic stability to either set (in the case $u \equiv 0$) clearly does not make sense, but it is still meaningful to characterize the regions of state space (the set R) from where it is possible to eventually converge to one of the sets. In addition, if starting inside one of the invariant sets, local ISS with respect to a compact set quantifies the magnitude of disturbances allowed before the system leaves that set.

B. ISS Lyapunov functions with respect to cubes

For systems of the form (2) and for compact sets which are products of closed intervals, it is possible to use "piece-wise" quadratic functions to systematically construct an ISS Lyapunov function with respect to a given cube. Define the scalar function:

$$\rho(r) = \begin{cases} \frac{1}{2} r^2, & r \geq 0 \\ 0, & r < 0 \ . \end{cases}$$

This function is continuously differentiable and satisfies:

$$r \ \frac{d\rho}{dr} = r \ \sqrt{2\rho(r)} = 2 \ \rho(r). \qquad (5)$$

Now consider a set of the form

$$\mathcal{Q} = [x_1^a, x_1^b] \times \cdots \times [x_n^a, x_n^b].$$

Then our candidate Lyapunov function will be:

$$V(x) = \frac{1}{2}|x|_{\mathcal{Q}}^2 = \sum_{i=1}^{n} \rho(x_i^a - x_i) + \rho(x_i - x_i^b). \tag{6}$$

This is the squared point-to-set distance to a cube-shaped compact set, and hence one may set $\nu_1 = \nu_2 = V(x) = \frac{1}{2}|x|_{\mathcal{Q}}^2$.

Using this V, and noticing that the function $F(x,u)$ in (2) is bounded (as a finite sum of products of activation and inhibition functions), it is easy to prove the following result.

Lemma 3.6: Define $\bar{F}_i = \max_{x,u} F_i(x,u)$ and consider the set

$$P = \left[0, \frac{\bar{F}_1}{k_1}\right] \times \cdots \times \left[0, \frac{\bar{F}_n}{k_n}\right]. \tag{7}$$

Then system (2) is P-forward complete.

Proof: The function $-K_{deg}x + F(x,u)$ is continuously differentiable on $\mathbb{R}^n_{\geq 0}$ for each fixed u, and locally integrable on $\mathbb{R}^m_{\geq 0}$ for each fixed $x \in \mathbb{R}^n_{\geq 0}$. For each continuous input w, and initial condition $x_0 \in P$, let $x(t, x_0, w)$ denote the maximal solution of the initial value problem $\dot{x}(t) = -K_{deg}x(t) + F(x(t), w(t))$, $x(0) = x_0$, and suppose it is defined on an interval $[0, T_{\max})$. Consider now the distance function

$$V(x) = \frac{1}{2}|x|_P^2 = \sum_{i=1}^{n} \rho\left(x_i - \frac{\bar{F}_i}{k_i}\right),$$

since the system is defined only for nonnegative coordinates. Then (writing $x_i = (x_i - \bar{F}/k_i) + \bar{F}/k_i$)

$$\begin{aligned}
\nabla V \ f(x,u) &= \sum_{i=1}^{n} \sqrt{2\rho\left(x_i - \frac{\bar{F}_i}{k_i}\right)} \ \left(-k_i(x_i - \frac{\bar{F}_i}{k_i})\right) \\
&+ \sum_{i=1}^{n} \sqrt{2\rho\left(x_i - \frac{\bar{F}_i}{k_i}\right)} \ (-\bar{F}_i + F_i(x,u)) \\
&\leq \sum_{i=1}^{n} -k_i 2 \ \rho\left(x_i - \frac{\bar{F}_i}{k_i}\right) \\
&\leq -2 \min_i k_i \ |x|_P^2
\end{aligned}$$

because (by definition of $\bar{F}$) $-\bar{F}_i + F_i(x,u) \leq 0$ for all i and all x, u. It is clear that $V(x(t, x_0, w))$ is a nonincreasing function so, for all $t > 0$,

$$|x(t, x_0, w)|_P^2 \leq |x_0|_P^2,$$

implying that the trajectory remains bounded for all times, and hence $T_{\max} = \infty$. By a comparison principle, it also holds that: $V(x(t, x_0, w)) \leq exp(-c|x(t, x_0, w)|_P^2)$ (where $c = 2\min_i k_i$). Therefore, the trajectories of system (2) are asymptotically convergent to the compact set P. Finally, if the initial condition is $x_0 \in P$, then $|x(t, x_0, w)|_P^2 \equiv 0$ for all t, meaning that system (2) is indeed P-forward complete. ∎

From now on, without loss of generality, we will consider only trajectories of (2) evolving in P. For system (1) this set becomes:

$$\begin{aligned}
P_{ap} = &\left[0, \frac{M_1 M_3}{k_x}\right] \times \left[0, \frac{N_1 N_2 M_5}{k_y}\right] \\
\times &\left[0, \frac{M_4 + N_3}{k_w}\right] \times \left[0, \frac{M_2 N_4}{k_z}\right].
\end{aligned}$$

IV. Life and death decision in an apoptosis network

The apoptosis network is responsible for programmed cell death in response to certain stimuli. Apoptosis enables the organisms to eliminate unwanted cells and thus prevent, for instance, replication of damaged cells (see for example [4]). Cancer, as well as other diseases, may develop if the apoptosis network fails to respond in an appropriate manner. At the heart of the apoptosis network is a family of proteins (caspases, each existing in a pro-form and an active form), which are activated in a cascade (for more references see [4] and also [6]). Caspase 3 (C3) is a prominent downstream member of this cascade, and it is responsible for the cleavage (and destruction) of various and critical proteins in the cell: thus high abundance of active C3 (C3a) typically leads to cell death. Other pathways interact with the apoptosis network, in particular the well known Nuclear Factor κB (NFκB) pathway [4]. NFκB is a transcription factor responsible for transcription of various genes, including one for its own inhibitor (IκB), and another for an inhibitor of C3a (IAP). Thus, the presence of NFκB (or, more precisely, its transcription products) typically promotes survival of the cell. While the NFκB pathway can be generally considered an anti-apoptotic pathway, it is often activated in parallel with the pro-apoptotic caspase cascade. A common signal is stimulation of extrinsic death receptors, for example, Tumor Necrosis Factor (TNF) activating its receptor TNFR1. TNFR1 activation leads to deactivation of IκB. On the other hand, TNF activates caspase 8, which in turn activates caspase 3, and NFκB also functions as an inhibitor of this step (through the activity of FLIP, an inhibitor of caspase 8 activation and IAPs, inhibitors of C3a) [4]. The interaction among pro- and anti-apoptotic modules will influence and fine tune the cellular decision to survive or undergo apoptosis [32]. Thus, in model (1) a "living" response corresponds to low concentration of C3a (and high concentration of NFκB), and conversely an "apoptotic" response corresponds to high concentration of C3a (and low concentration of NFκB).

Next we will establish conditions on the degradation and production rates, that guarantee existence of both the "living" (set $\mathcal{L}$, Proposition 4.1) and "apoptotic" (set $\mathcal{A}$, Proposition 4.2) responses, or only one of them (Propositions 4.3 and 4.4). The sets $\mathcal{L}$ and $\mathcal{A}$ are both contained in the larger set P_{ap} (Fig. 3). Note that conditions (L1)-(L2) and (A1)-(A3) can indeed be simultaneously satisfied (see more details below). These sets are disjoint if $M_1 M_3 \varepsilon < m_1 M_3 (1 - \varepsilon)$. This is guaranteed, for instance, for all $\varepsilon < m_1/(m_1 + M_1)$ and $\varepsilon + \sqrt{\varepsilon} < 1$.

As a remark, we would like to point out that the sets $\mathcal{L}$ and $\mathcal{A}$ (or $\mathcal{L}_*$ and $\mathcal{A}_*$) are not necessarily unique, nor the largest invariant sets with the "living" and "apoptotic" qualitative properties. In fact, bistable behavior could be established by

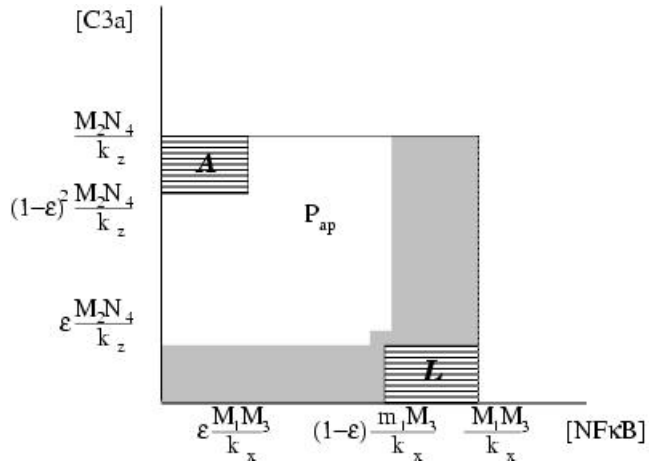

Fig. 3. The "living" ($\mathcal{L}$) and "apoptosis" ($\mathcal{A}$) 0-invariant sets, projected into the plane $x = [\text{NF}\kappa\text{B}]$, $z = [\text{C3a}]$. Also shown (shaded) is the local set R for the "living set".

finding any other suitable pair of disjoint 0-invariant compact sets, say $\tilde{\mathcal{L}}$ and $\tilde{\mathcal{A}}$, with the properties "high x / low w" and "low x / high w", under different assumptions on the parameters of the network. The goal here is to show that the network has the capacity for bistability, by identifying conditions for which at least one pair of sets $\mathcal{L}$, $\mathcal{A}$ co-exist. Or, alternatively, conditions on the parameters for which bistability is lost and only one of the sets is invariant.

Recall that system (1) is P_{ap}-forward complete (Lemma 3.6). Define

$$m_1 = \min\left\{\mu_1(y):\ y \in \left[0, \frac{N_1N_2M_5}{k_y}\right]\right\}, \tag{8}$$

which is a stricly positive constant, because μ_1 is continuous, and by property (ii) of Definition 2.2. To simplify notation, let $\xi = (x, y, w, z)'$, and let $\dot{\xi} = f(\xi, u)$ denote system (1).

Proposition 4.1: Assume that (L1) $\frac{\varepsilon M_2N_4}{k_z} < \theta_3(1-\Delta)$, and (L2) $\frac{m_1M_3(1-\varepsilon)}{k_x} > \max\{\theta_2, \theta_4\}(1+\Delta)$. Then system (1) is locally ISS with respect to the compact set:

$$\begin{aligned}\mathcal{L} \ = \ & \left[\frac{m_1M_3(1-\varepsilon)}{k_x}, \frac{M_1M_3}{k_x}\right] \times \left[0, \frac{N_1N_2M_5}{k_y}\right] \\ \times \ & \left[0, \frac{\varepsilon M_4}{k_w}\right] \times \left[0, \frac{\varepsilon M_2N_4}{k_z}\right].\end{aligned}$$

Proof: By Lemma 3.4, it is enough to show that there exists a local ISS Lyapunov function with respect to $\mathcal{L}$. We will next construct such a function, following (6). Set

$$\begin{aligned}& x^a = \frac{m_1M_3(1-\varepsilon)}{k_x},\ x^b = \frac{M_1M_3}{k_x}, \\ & y^a = 0,\ y^b = \frac{N_1N_2M_5}{k_y}, \\ & w^a = 0,\ w^b = \frac{\varepsilon M_4}{k_w},\ z^a = 0,\ z^b = \frac{\varepsilon M_2N_4}{k_z}.\end{aligned}$$

Since we only consider trajectories evolving in the set P_{ap}, it always holds that $y < y^b$, which implies $\rho(y - y^b) \equiv 0$. In addition, $\rho(y^a - y) = \rho(-y) \equiv 0$. Therefore, the terms on y to be included in the Lyapunov function always vanish. Similar arguments show that also $\rho(x - x^b)$, $\rho(w^a - w)$ and $\rho(z^a - z)$ identically vanish in the state space P_{ap}. Therefore consider the function:

$$V(\xi) = \frac{1}{2}|\xi|^2_{\mathcal{L}} = \rho\left(x^a - x\right) + \rho\left(w - w^b\right) + \rho\left(z - z^b\right).$$

Now choose a number $\delta \in (0, 1)$ such that

$$\begin{aligned}& (1+\delta)\frac{\varepsilon M_2N_4}{k_z} < \theta_3(1-\Delta) \text{ and} \\ & \delta\frac{m_1M_3(1-\varepsilon)}{k_x} > \max\{\theta_2, \theta_4\}(1+\Delta)\end{aligned}$$

(such δ exists, since (L1)-(L2) are strict inequalities), and consider the following set which contains $\mathcal{L}$ in its interior:

$$R = R_1 \cup R_2,$$

$$\begin{aligned}R_1 &= \left\{\xi \in P_{ap} : w \le w^b \text{ and } (x^a \le x \le x^b \text{ or } z \le z^b)\right\} \\ R_2 &= \left\{\xi \in P_{ap} : \delta x^a \le x \le x^b \text{ and } z \le (1+\delta)z^b\right\}\end{aligned}$$

(see also Fig. 3). Then

$$\begin{aligned}\nabla V\ f(\xi,u) \ = \ & -\sqrt{2\rho\left(x^a - x\right)}(-k_x x + \mu_1(y)\mu_3(z)) \\ & +\sqrt{2\rho\left(w - w^b\right)}(-k_w w + \mu_4(x)) \\ & +\sqrt{2\rho\left(z - z^b\right)}(-k_z z + \mu_2(x)\nu_4(w)) \\ & +\nu_3(u)\sqrt{2\rho\left(w - w^b\right)}.\end{aligned}$$

Noting that:

$$-k_x x + \mu_1(y)\mu_3(z) = -k_x(x - x^a) - k_x x^a + \mu_1(y)\mu_3(z)$$

and that

$$-\sqrt{2\rho\left(x^a - x\right)}(-k_x(x - x^a)) = -2k_x\rho\left(x^a - x\right),$$

and similar expressions for the terms in w and z, one can write:

$$\begin{aligned}& \nabla V\ f(\xi,u) \\ \le \ & -2k_x\ \rho\left(x^a - x\right) - 2k_w\ \rho\left(w - w^b\right) - 2k_z\ \rho\left(z - z^b\right) \\ & +g_x(\xi) + g_w(\xi) + g_z(\xi) + \nu_3(u)\sqrt{2\rho\left(w - w^b\right)},\end{aligned}$$

where

$$g_x(\xi) \ = \ -\sqrt{2\rho\left(x^a - x\right)}(-k_x x^a + \mu_1(y)\mu_3(z)) \tag{9}$$

$$g_w(\xi) \ = \ \sqrt{2\rho\left(w - w^b\right)}(-k_w w^b + \mu_4(x)) \tag{10}$$

$$g_z(\xi) \ = \ \sqrt{2\rho\left(z - z^b\right)}(-k_z z^b + \mu_2(x)\nu_4(w)). \tag{11}$$

We will next show that property (ii) of Definition 3.3 holds for the set R. To do this, we only need to show that $g_x(\xi) + g_w(\xi) + g_z(\xi) \le 0$ for all $\xi \in R$. Choose first any point $\xi \in R_1$. The inequality $w \le w^b$ implies $\rho(w - w^b) = 0$ and the term (10) is zero. Suppose first that $x^a \le x \le x^b$. Then $\rho(x^a - x) = 0$ and the term (9) is also zero. By assumption (L2) $x > \theta_2(1+\Delta)$, which implies $\mu_2(x) < \varepsilon M_2$ (by definition of an inhibition function), and so $-k_z z^b + \mu_2(x)\nu_4(w) < -k_z(z^b - \varepsilon M_2N_4/k_z) = 0$. Thus, the term (11) is nonpositive. Suppose now that $0 \le z \le z^b$. Then $\rho(z - z^b) = 0$ and

hence (11) is zero. By assumption (L1), $z < \theta_3(1-\Delta)$, which implies $\mu_3(z) > M_3(1-\varepsilon)$ (by definition of μ_3). Thus

$$k_x x^a - \mu_1(y)\mu_3(z) < k_x\left(x^a - \frac{1}{k_x} m_1 M_3(1-\varepsilon)\right) = 0,$$

and so the term (9) is nonpositive. Therefore, for all points in R_1, the terms (9), (10) and (11), are majorated by zero.

Choose next any point $\xi \in R_2$. By definition of δ we have: $x \geq x^a > \max\{\theta_2, \theta_4\}(1+\Delta)$, which implies (condition (L2)) both $\mu_2(x) < \varepsilon M_2$ and $\mu_4(x) < \varepsilon M_4$. Hence $-k_z z^b + \mu_2(x)\nu_4(w) < -k_z z^b + \varepsilon M_2 N_4 = 0$ and $-k_w w^b + \mu_4(x) < -k_w w^b + \varepsilon M_4 = 0$, and both terms (10) and (11) are nonpositive. Finally, $z \leq (1+\delta) z^b$ implies (by assumption (L1)) $\mu_3(z) > M_3(1-\varepsilon)$. And this again implies that term (9) is nonpositive. Using Fact 2 to get $\nu_3(u) \leq \gamma_3(u)$ and letting $c_3 = \max_{P_{ap}} \sqrt{2\rho\left(w - w^b\right)}$ we have, for all $\xi \in R$,

$$\begin{aligned}\nabla V\ f(\xi,u) &\leq -2k_x\rho\left(x^a - x\right) - 2k_w\rho\left(w - w^b\right) \\ &\quad -2k_z\rho\left(z - z^b\right) + c_3\,\gamma_3(u) \\ &\leq -2\min\{k_x, k_w, k_z\}|\xi|^2_{\mathcal{L}} + \gamma(u),\end{aligned}$$

where $\gamma(r) = c_3\gamma_3(r)$ and $c_3 = (M_4(1-\varepsilon) + N_3)/k_w$. ∎

Proposition 4.2: Assume: (A1) $\frac{M_2 N_4 (1-\varepsilon)^2}{k_z} > \theta_3(1+\Delta)$, (A2) $\frac{M_1 M_3 \varepsilon}{k_x} < \min\{\theta_2, \theta_4\}(1-\Delta)$, and (A3) $\frac{M_4(1-\varepsilon)}{k_w} > \phi_4(1+\Delta)$. Then system (1) is locally ISS with respect to the compact set:

$$\begin{aligned}\mathcal{A} \;=\; & \left[0, \frac{M_1 M_3 \varepsilon}{k_x}\right] \times \left[0, \frac{N_1 N_2 M_5}{k_y}\right] \\ \times\; & \frac{M_4}{k_w}\left[1-\varepsilon, 1\right] \times \frac{M_2 N_4}{k_z}\left[(1-\varepsilon)^2, 1\right].\end{aligned}$$

Proof: By Lemma 3.4, it is enough to show that there exists a local ISS Lyapunov function with respect to $\mathcal{A}$. We will next construct such a function, following (6). Define

$$\begin{aligned}& x^a = 0,\ x^b = \frac{M_1 M_3 \varepsilon}{k_x},\ y^a = 0,\ y^b = \frac{N_1 N_2 M_5}{k_y}, \\ & w^a = \frac{M_4(1-\varepsilon)}{k_w},\ w^b = \frac{M_4}{k_w}, \\ & z^a = \frac{M_2 N_4 (1-\varepsilon)^2}{k_z},\ z^b = \frac{M_2 N_4}{k_z}.\end{aligned}$$

Since we only consider trajectories evolving in the set P_{ap}, it always holds that $y < y^b$, which implies $\rho(y - y^b) \equiv 0$. In addition, $\rho(y^a - y) = \rho(-y) \equiv 0$. Therefore, the terms on y to be included in the Lyapunov function always vanish. Similar arguments show that also $\rho(x^a - x)$ and $\rho(z - z^b)$ vanish in P_{ap}. Therefore consider the function $\frac{1}{2}|\xi|^2_{\mathcal{A}}$:

$$V(\xi) = \rho\left(x - x^b\right) + \rho\left(w^a - w\right) + \rho\left(w - w^b\right) + \rho\left(z^a - z\right).$$

Now choose a number $\delta \in (0,1)$ such that

$$\begin{aligned}& \delta\frac{M_2 N_4 (1-\varepsilon)^2}{k_z} > \theta_3(1+\Delta), \\ & (1+\delta)\frac{M_1 M_3 (1-\varepsilon)}{k_x} < \max\{\theta_2, \theta_4\}(1-\Delta) \\ \text{and}\quad & \delta\frac{M_4(1-\varepsilon)}{k_w} > \phi_4(1+\Delta)\end{aligned}$$

(such δ exists, since (A1)-(A3) are strict inequalities), and the following large set that strictly contains $\mathcal{A}$:

$$R = R_1 \cup R_2,$$

with

$$\begin{aligned}R_1 = \{\xi \in P_{ap} :\quad & w \geq w^a \text{ and} \\ & \left(0 \leq x \leq x^b \text{ or } z^a \leq z \leq z^b\right)\}\end{aligned}$$

and

$$\begin{aligned}R_2 = \{\xi \in P_{ap} :\quad & 0 \leq x \leq (1+\delta)x^b \text{ and} \\ & \delta w^a \leq w \text{ and } \delta z^a \leq z \leq z^b\}.\end{aligned}$$

Then

$$\begin{aligned}\nabla V\ f(\xi,u) = & \sqrt{2\rho\left(x - x^b\right)}(-k_x x + \mu_1(y)\mu_3(z)) \\ & -\sqrt{2\rho\left(w^a - w\right)}(-k_w w + \mu_4(x)) \\ & +\sqrt{2\rho\left(w - w^b\right)}(-k_w w + \mu_4(x)) \\ & -\sqrt{2\rho\left(z^a - z\right)}(-k_z z + \mu_2(x)\nu_4(w)) \\ & +\nu_3(u)\left(-\sqrt{2\rho(w^a - w)} + \sqrt{2\rho(w - w^b)}\right).\end{aligned}$$

Simplifying as in the proof of Proposition 4.1:

$$\begin{aligned}\nabla V\ f(\xi,u) \;\leq\; & -2\min\{k_x, k_w, k_z\}|\xi|^2_{\mathcal{A}} \\ & +g_{x,b}(\xi) + g_{w,a}(\xi) + g_{w,b}(\xi) + g_{z,b}(\xi) \\ & +c_3\,\nu_3(u),\end{aligned}$$

where $c_3 = \max_{P_{ap}}\left\{\sqrt{2\rho(w^a - w)} + \sqrt{2\rho(w - w^b)}\right\}$ and

$$g_{x,b}(\xi) = \sqrt{2\rho\left(x - x^b\right)}(-k_x x^b + \mu_1(y)\mu_3(z)) \quad (12)$$

$$g_{w,a}(\xi) = -\sqrt{2\rho\left(w^a - w\right)}(-k_w w^a + \mu_4(x)) \quad (13)$$

$$g_{w,b}(\xi) = \sqrt{2\rho\left(w - w^b\right)}(-k_w w^b + \mu_4(x)) \quad (14)$$

$$g_{z,a}(\xi) = -\sqrt{2\rho\left(z^a - z\right)}(-k_z z^a + \mu_2(x)\nu_4(w)) \quad (15)$$

We will next show that V satisfies property (ii) of Definition 3.3 for all $\xi \in R$. We verify this only for $\xi \in R_2$, since the verification for $\xi \in R_1$ is analogous. Assume that $\xi \in R_2$ and recall the definition of δ. Then $z > \theta_3(1+\Delta)$ implies $\mu_3(z) < \varepsilon M_3$. Hence $-k_x x^b + \mu_1(y)\mu_3(z)) < -k_x x^b + M_1 M_3 \varepsilon = 0$ and $g_{x,b} \leq 0$. Next, note that $w > \phi_4(1+\Delta)$ implies $\nu_4(w) > N_4(1-\varepsilon)$. And $x \leq \min\{\theta_2, \theta_4\}(1-\Delta)$ implies $\mu_2(x) > M_2(1-\varepsilon)$ and $\mu_4(x) > M_4(1-\varepsilon)$. Then $-k_z z^a + \mu_2(x)\nu_4(w) > -k_z z^a + (1-\varepsilon)^2 M_2 N_4 = 0$, implying that $g_{z,a} \leq 0$. Also $-k_w w^a + \mu_4(x) > -k_w w^a + M_4(1-\varepsilon) > 0$ implying that $g_{w,a} \leq 0$. Finally, note that $-k_w w^b + \mu_4(x) \leq -k_w w^b + M_4 = 0$, so $g_{w,b} \leq 0$.

We conclude that, for all $\xi \in R$, the terms (12), (13), (14), and (15) can all be majorated by zero in the expression $\nabla V\, f(\xi,u)$. Using Fact 2 obtain $\nu_3(u) \leq \gamma_3(u)$, one can say that, for all $\xi \in R$,

$$\nabla V\ f(\xi,u)) \leq -2\min\{k_x, k_w, k_z\}|\xi|^2_{\mathcal{A}} + \gamma(u),$$

where $\gamma(r) = c_3\gamma_3(r)$, with $c_3 = 2((1-\varepsilon)M_4 + N_3/k_w)$. ∎

The next two Propositions provide stricter conditions, which guarantee that only one of the two possible responses may ultimately happen.

Proposition 4.3: Assume: (L1') $\frac{M_2N_4}{k_z} \leq \theta_3(1-\Delta)$. Then system (1) is globally ISS with respect to the compact set $\mathcal{L}_*$:

$$\mathcal{L}_* = \left[\frac{m_1M_3(1-\varepsilon)}{k_x}, \frac{M_1M_3}{k_x}\right] \times \left[0, \frac{N_1N_2M_5}{k_y}\right] \times \left[0, \frac{M_4}{k_w}\right] \times \left[0, \frac{M_2N_4}{k_z}\right].$$

Proof: Define

$$x^a = \frac{m_1M_3(1-\varepsilon)}{k_x},\ x^b = \frac{M_1M_3}{k_x},$$
$$y^a = 0,\ y^b = \frac{N_1N_2M_5}{k_y},$$
$$w^a = 0,\ w^b = \frac{M_4}{k_w},\ z^a = 0,\ z^b = \frac{M_2N_4}{k_z}.$$

As in the proof of Proposition 4.1, consider the function

$$V(\xi) = \frac{1}{2}|\xi|^2_{\mathcal{L}_*} = \rho\left(x^a - x\right) + \rho\left(w - w^b\right) + \rho\left(z - z^b\right).$$

We will show that, under condition (L1'), this function satisfies Definition 3.3, with $R = P_{ap}$, and so is indeed a global Lyapunov function with respect to the compact set $\mathcal{L}_*$. (Recall that only trajectories evolving on P_{ap} are considered.) By definition $\mu_2(x) \leq M_2$ for all x and $\nu_4(w) \leq N_4$ for all w, so the term (11) is always nonpositive. Similarly, $\mu_4(x) \leq M_4$ for all x implies that the term (10) is always nonpositive. By assumption (L1'), $z \leq M_2N_4/k_z \leq \theta_3(1-\Delta)$, so that (using (8) and property (iii) of Definition 2.2) $-k_xx^a + \mu_1(y)\mu_3(z) \geq -k_xx^a + m_1M_3(1-\varepsilon) = 0$. It follows that term (9) is always nonpositive. Therefore, for all $\xi \in P_{ap}$,

$$\begin{aligned}\nabla V\ f(\xi,u) &\leq -2k_x\rho\left(x^a - x\right) - 2k_w\rho\left(w - w^b\right) \\ &\quad -2k_z\rho\left(z - z^b\right) + c_3\,\gamma_3(u) \\ &\leq -2\min\{k_x,k_w,k_z\}|\xi|^2_{\mathcal{L}_*} + \gamma(u),\end{aligned}$$

where $c_3 = \max_{P_{ap}}\sqrt{2\rho(w-w^2)} = N_3/k_w$ and $\gamma(r) = c_3\gamma_3(r)$. We conclude that, under assumption (L1'), V is a global ISS Lyapunov function with respect to the compact set $\mathcal{L}_*$. By Lemma 3.4, system (1) is globally ISS with respect to the same compact set, as we wanted to show. ∎

A very similar proof shows that under some other conditions, the apoptosis set will be an attractor for the system.

Proposition 4.4: Assume: (A2') $\frac{M_1M_3}{k_x} \leq \min\{\theta_2,\theta_4\}(1-\Delta)$. (A3') $\frac{M_4}{k_w} > \phi_4(1+\Delta)$. Then system (1) is globally ISS with respect to the compact set $\mathcal{A}_*$.

$$\mathcal{A}_* = \left[0, \frac{M_1M_3}{k_x}\right] \times \left[0, \frac{N_1N_2M_5}{k_y}\right] \times \frac{M_4}{k_w}\left[1-\varepsilon, 1\right] \times \frac{M_2N_4}{k_z}\left[(1-\varepsilon)^2, 1\right].$$

□

The network depicted in Fig. 1 is capable of bistable behavior, when the conditions (L1), (L2) and (A1)-(A3) are simultaneously satisfied. These can be rewritten as:

$$\frac{1+\Delta}{1-\varepsilon} < \frac{m_1M_3}{k_x\min\{\theta_2,\theta_4\}} < \frac{1-\Delta}{\varepsilon},$$
$$\frac{1+\Delta}{(1-\varepsilon)^2} < \frac{M_2N_4}{k_z\theta_3} < \frac{1-\Delta}{\varepsilon},\quad \frac{1+\Delta}{1-\varepsilon} < \frac{M_4}{\phi_4k_w}.$$

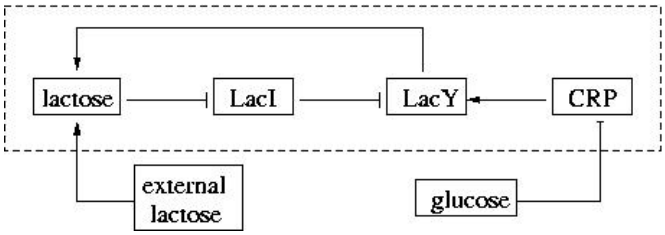

Fig. 4. A simplified *lac* operon regulatory network (similar to the model used in [2]), with two inputs: external lactose and glucose.

Provided that

$$\varepsilon + \sqrt{\varepsilon} < 1 \text{ and } \Delta < \frac{(1-\varepsilon)^2 - \varepsilon}{(1-\varepsilon)^2 + \varepsilon}, \tag{16}$$

many choices of parameters will satisfy these four conditions. Bistability will obtain from a balance between the maximal expression levels of NFκB and C3a, and their mutual inhibition thresholds (see [29]). In the bistable region of parameters, either $\mathcal{L}$ or $\mathcal{A}$ can be reached depending on the initial conditions and input. Our results also show that, under alternative conditions, the network of Fig. 1 can exhibit only monostable behavior. Indeed, if condition (L1') is satisfied, then any trajectory of system (1) (corresponding to a zero input, or after TNF stimulus is turned off) will asymptotically converge to the compact set $\mathcal{L}_*$ (Proposition 4.3). This means that the cell will not go to apoptosis. In a similar manner, conditions (A2')-(A3') guarantee that any trajectory will asymptotically converge to $\mathcal{A}_*$ (Proposition 4.4), that is, C3a will remain at high levels, and the cell will eventually die.

V. THE *lac* OPERON

An operon is a group of genes which are adjacent to one another in the chromosome, and are transcribed into a unique mRNA molecule. In *E. coli*, the *lac* operon genes code for three proteins (β-galactosidase or LacZ, lactose permease or LacY, and β-galactoside transacetylase or LacA) that are required for the transport of lactose into the cell and its subsequent breakdown. The *lac* operon has been a widely studied system, since Jacob and Monod [1] first proposed a model and analyzed this regulatory mechanism. *E. coli* will preferably use glucose as a source of carbon but will also use lactose, if glucose is not available. Binding of the lac repressor protein (LacI) to the operator site of the *lac* operon, prevents transcription of the *lac* genes. The presence of lactose (or, more precisely, some of its derivatives) in the interior of the cell, contributes to the inhibition of the protein LacI, thus de-repressing the operon and allowing transcription to be initiated. In the absence of glucose, the cyclic AMP receptor protein (CRP) is activated, and strongly promotes transcription of the three *lac* operon genes, *lac*Z, *lac*Y, and *lac*A. The protein LacY facilitates the uptake of lactose from the exterior to the interior of the cell, while the enzyme β-galactosidase is responsible for lactose breakdown. Thus, the absence of glucose triggers a positive feedback cycle, which drives the cell to increase its lactose uptake and the corresponding metabolism. Here again there is a system exhibiting bistability: the *lac* operon is repressed in the presence of glucose, but

transcribed in the absence of glucose and presence of lactose. In [2], this regulatory system and its response to glucose and a lactose analog was explored: there are two inputs to the system. A schematic view of the system is shown in Fig. 4, where "lactose" stands for intracellular lactose. Letting x = [lactose], y = [LacY], w = [LacI], z = [CRP], u_1 = [extracellular lactose] and u_2 = [glucose], a model for the system depicted in Fig. 4 is:

$$\begin{aligned}\dot{x} &= -k_x x + \nu_1(y) + \nu_4(u_1)\\ \dot{y} &= -k_y y + \mu_1(w)\nu_2(z)\\ \dot{w} &= -k_w w + \mu_2(x)\\ \dot{z} &= -k_z z + \mu_3(u_2)\end{aligned} \tag{17}$$

To simplify notation, let $\xi = (x, y, w, z)'$ and let $\dot{\xi} = f(\xi, u)$ denote system (17). By Lemma 3.6, system (17) is P_{lac}-forward complete. where:

$$P_{lac} = \left[0, \frac{N_1 + N_4}{k_x}\right] \times \left[0, \frac{M_1 N_2}{k_y}\right] \times \left[0, \frac{M_2}{k_w}\right] \times \left[0, \frac{M_3}{k_z}\right].$$

As in the apoptosis example, conditions can be given that guarantee the capacity for bistable behavior. It is convenient to rewrite the equation for z, using Fact 1:

$$\begin{aligned}\dot{z} &= -k_z z + M_3 + (\mu_3(u) - M_3)\\ &= -k_z z + M_3 - \nu_3(u),\end{aligned} \tag{18}$$

where $N_3 = M_3$. In Proposition 5.1 below, the set $\mathcal{L}_{lac}$ represents the response of the *lac* operon in the presence of glucose: LacI (w) represses transcription of the *lac* genes, and only a residual concentration of lactose (x) is present inside the cell.

Proposition 5.1: Assume that (L1) $\frac{\varepsilon M_1 N_2}{k_y} < \phi_1(1-\Delta)$, (L2) $\frac{M_2(1-\varepsilon)}{k_w} > \theta_1(1+\Delta)$, and (L3) $\frac{\varepsilon N_1}{k_x} < \theta_2(1-\Delta)$. Then system (17) is locally ISS with respect to the compact set:

$$\mathcal{L}_{lac} = \left[0, \frac{\varepsilon N_1}{k_x}\right] \times \left[0, \frac{\varepsilon M_1 N_2}{k_y}\right] \times \frac{M_2}{k_w}[1-\varepsilon, 1] \times \left[0, \frac{M_3}{k_z}\right].$$

Proof: By Lemma 3.4, it is enough to show that there exists a local ISS Lyapunov function with respect to $\mathcal{L}_{lac}$. Define

$$\begin{aligned}&x^a = 0,\ x^b = \frac{\varepsilon N_1}{k_x},\ y^a = 0,\ y^b = \frac{\varepsilon M_1 N_2}{k_y},\\ &w^a = \frac{M_2(1-\varepsilon)}{k_w},\ w^b = \frac{M_2}{k_w}.\end{aligned}$$

Following (6), consider the function:

$$V(\xi) = \frac{1}{2}|\xi|^2_{\mathcal{L}_{lac}} = \rho\left(x - x^b\right) + \rho\left(y - y^b\right) + \rho\left(w^a - w\right).$$

This function satisfies property (i) of Definition 3.3, and we will show that it also satisfies property (ii). Find $\delta \in (0,1)$ so that:

$$\begin{aligned}&(1+\delta)\frac{\varepsilon M_1 N_2}{k_y} < \phi_1(1-\Delta),\\ &\delta\frac{M_2(1-\varepsilon)}{k_w} > \theta_1(1+\Delta),\ (1+\delta)\frac{\varepsilon N_1}{k_x} < \theta_2(1-\Delta)\end{aligned}$$

(such a δ exists, since (L1)-(L3) are strict inequalities), and define the following set :

$$R = \{\xi \in P_{lac} : x \le (1+\delta)x^b,\ y \le (1+\delta)y^b,\ \delta w^a \le w\}.$$

This set R clearly contains $\mathcal{L}_{lac}$ in its interior (in the sense defined by (3)). Then

$$\begin{aligned}\nabla V\ f(\xi,u) &= \sqrt{2\rho\left(x - x^b\right)}(-k_x x + \nu_1(y))\\ &\quad + \sqrt{2\rho\left(y - y^b\right)}(-k_y y + \mu_1(w)\nu_2(z))\\ &\quad - \sqrt{2\rho\left(w^a - w\right)}(-k_w w + \mu_2(x))\\ &\quad + \nu_4(u_1)\sqrt{2\rho\left(x - x^b\right)}.\end{aligned}$$

Noticing that $-k_x x + \nu_1(y) = -k_x(x - x^b) - k_x x^b + \nu_1(y)$, and that $\sqrt{2\rho(x-x^b)}(x-x^b) = 2k_x\rho(x-x^b)$, the expression $\nabla V f(\xi,u)$ can be rewritten as

$$\begin{aligned}\nabla V\ f(\xi,u) &= -2k_x\ \rho\left(x - x^b\right) - 2k_y\ \rho\left(y - y^b\right)\\ &\quad -2k_w\ \rho\left(w^a - w\right) + g_{x,b} + g_{y,b} + g_{w,a}\\ &\quad + \nu_4(u_1)\sqrt{2\rho\left(x - x^b\right)},\end{aligned}$$

where

$$g_{x,b} = \sqrt{2\rho\left(x - x^b\right)}(-k_x x^b + \nu_1(y)) \tag{19}$$

$$g_{y,b} = \sqrt{2\rho\left(y - y^b\right)}(-k_y y^b + \mu_1(w)\nu_2(z)) \tag{20}$$

$$g_{w,a} = -\sqrt{2\rho\left(w^a - w\right)}(-k_w w^a + \mu_2(x)). \tag{21}$$

Now, let $\xi \in R$. Recall the definition of δ. Then $y < \phi_1(1-\Delta)$ implies (definition of activation function) $\nu_1(y) < \varepsilon N_1$, and hence $g_{x,b} \le 0$. The fact that $w > \theta_1(1+\Delta)$ implies $\mu_1(w) < \varepsilon M_1$ (by definition of an inhibition function), and so $-k_y y^b + \mu_1(w)\nu_2(z) < -k_y(y^b - \varepsilon M_1 N_2/k_y) = 0$, and $g_{y,b} \le 0$. Since $x < \theta_2(1-\Delta)$ it follows that $\mu_2(x) > M_2(1-\varepsilon)$, and $-k_w w^a + \mu_2(x) > -k_w w^a + M_2(1-\varepsilon) = 0$, so also $g_{w,a} \le 0$. Thus, the terms (19)-(21) are nonpositive. For the input term, use Fact 2 to obtain a $\mathcal{K}_\infty$ function $\gamma_4(r) \ge \nu_4(r)$. In conclusion, for all points of R one can write:

$$\nabla V\ f(\xi,u) \le -2\min\{k_x, k_y, k_w\}|\xi|^2_{\mathcal{L}_{lac}} + \gamma(|u|),$$

where $\gamma(r) = (N_1(1-\varepsilon) + N_4)\gamma_4(r)/k_x$ is a $\mathcal{K}_\infty$ function. ∎

In the next Proposition, the set $\mathcal{A}_{lac}$ represents the state of the operon in the absence of glucose and presence of external lactose. In this mode, both internal lactose and the Lac proteins are present, while the repressor LacI is at a low level.

Proposition 5.2: Assume: (A1) $\frac{M_1 N_2(1-\varepsilon)^2}{k_y} > \phi_1(1+\Delta)$, (A2) $\frac{\varepsilon M_2}{k_w} < \theta_1(1-\Delta)$, (A3) $\frac{N_1(1-\varepsilon)}{k_x} > \theta_2(1+\Delta)$, and (A4) $\frac{M_3(1-\varepsilon)}{k_z} > \phi_2(1+\Delta)$. Then system (17) is locally ISS with respect to the compact set:

$$\begin{aligned}\mathcal{A}_{lac} = &\quad \frac{N_1}{k_x}[1-\varepsilon, 1] \times \frac{M_1 N_2}{k_y}\left[(1-\varepsilon)^2, 1\right]\\ &\times \quad \left[0, \frac{\varepsilon M_2}{k_w}\right] \times \frac{M_3}{k_z}[1-\varepsilon, 1].\end{aligned}$$

Proof: The argument is very similar to that used in Proposition 5.1. Following (6), consider the function $\frac{1}{2}|\xi|^2_{\mathcal{A}_{lac}}$:

$$\begin{aligned}V(\xi) = \rho\left(x^a - x\right) &+ \rho\left(x - x^b\right) + \rho\left(y^a - y\right)\\ &+ \rho\left(w - w^b\right) + \rho\left(z^a - z\right).\end{aligned}$$

This function satisfies property (i) of Definition 3.3, and we will show that it also satisfies property (ii). To simplify notation, let $\xi = (x, y, w, z)'$ and define

$$x^a = \frac{N_1(1-\varepsilon)}{k_x},\ x^b = \frac{N_1}{k_x},$$
$$y^a = \frac{M_1N_2(1-\varepsilon)^2}{k_y},\ y^b = \frac{M_1N_2}{k_y},$$
$$w^a = 0,\ w^b = \frac{M_2\varepsilon}{k_w},\ z^a = \frac{M_3(1-\varepsilon)}{k_z},\ z^b = \frac{M_3}{k_z}.$$

Find $\delta \in (0,1)$ so that:

$$\delta\frac{(1-\varepsilon)^2M_1N_2}{k_y} > \phi_1(1-\Delta),$$
$$(1+\delta)\frac{M_2\varepsilon}{k_w} < \theta_1(1+\Delta),\ \delta\frac{(1-\varepsilon)N_1}{k_x} > \theta_2(1-\Delta),$$
$$\delta\frac{M_3(1-\varepsilon)}{k_z} > \phi_2(1+\Delta),$$

and define the following set:

$$R = \{\xi \in P_{lac}: \quad \delta x^a \le x,\ \delta y^a \le y,\ w \le (1+\delta)w^b,\ \delta z^a \le z\}. \quad (22)$$

This set R clearly contains $\mathcal{A}_{lac}$ in its interior (in the sense defined by (3)). Then computing and simplifying $\nabla V\, f(\xi,u)$:

$$\begin{aligned}\nabla V\ f(\xi,u) \quad = \quad & -2k_x\ \rho\left(x^a - x\right) - 2k_x\ \rho\left(x - x^b\right)\\ & -2k_y\ \rho\left(y^a - y\right) - 2k_w\ \rho\left(w - w^b\right)\\ & -2k_z\ \rho\left(z^a - z\right)\\ & +g_{x,a} + g_{x,b} + g_{y,a} + g_{w,b} + g_{z,a}\\ & +\nu_4(u_1)\left(\sqrt{2\rho\left(x^a - x\right)} + \sqrt{2\rho\left(x - x^b\right)}\right)\\ & +\nu_3(u_2)\sqrt{2\rho\left(z^a - z\right)}\end{aligned}$$

where

$$g_{x,a} = -\sqrt{2\rho\left(x^a - x\right)}(-k_x x^a + \nu_1(y)) \quad (23)$$
$$g_{x,b} = \sqrt{2\rho\left(x - x^b\right)}(-k_x x^b + \nu_1(y)) \quad (24)$$
$$g_{y,a} = -\sqrt{2\rho\left(y^a - y\right)}(-k_y y^a + \mu_1(w)\nu_2(z)) \quad (25)$$
$$g_{w,b} = \sqrt{2\rho\left(w - w^b\right)}(-k_w w^b + \mu_2(x)) \quad (26)$$
$$g_{z,a} = -\sqrt{2\rho\left(z^a - z\right)}(-k_z z^a + M_3). \quad (27)$$

Now, let $\xi \in R$. Recall the definition of δ. Note first that $-k_z z^a + M_3 > 0$, and so $g_{z,a} < 0$. Then $y > \phi_1(1+\Delta)$ implies $\nu_1(y) > (1-\varepsilon)N_1$, and hence $-k_x x^a + \nu_1(y) > -k_x x^a + N_1(1-\varepsilon) = 0$, so that $g_{x,a} \le 0$. Note also that $-k_x x^b + \nu_1(y) \le -k_x x^b + N_1 = 0$, so that $g_{x,b} \le 0$. The fact that $x > \theta_2(1+\Delta)$ implies that $g_{w,b} \le 0$. Now note that $w < \theta_1(1-\Delta)$ implies $\mu_1(w) > (1-\varepsilon)M_1$ and $z > \phi_2(1+\Delta)$ implies $\nu_2(z) > (1-\varepsilon)N_2$. Thus $-k_y y^a + \mu_1(w)\nu_2(z) > -k_y y^a + M_1N_2(1-\varepsilon)^2 = 0$ and $g_{y,a} \le 0$. Thus, the terms (23)-(27) are nonpositive. For the input term, use Fact 2 to obtain a $\mathcal{K}_\infty$ function $\gamma_i(r) \ge \nu_i(r)$, $i = 3, 4$. In conclusion, for all points of R one can write:

$$\nabla V\ f(\xi,u) \le -2\min\{k_x, k_y, k_w, k_z\}|\xi|^2_{\mathcal{A}_{lac}} + \gamma(|u|),$$

where we used $u_i \le |u| = \sqrt{u_1^2 + u_2^2}$ for $i = 1, 2$, and $\gamma(r) = M_3(1-\varepsilon)\gamma_3(r)/k_z + (N_1(1-\varepsilon) + N_4)\gamma_4(r)/k_x$ is a $\mathcal{K}_\infty$ function. ∎

More restrictive conditions can be given, for a monostable system. The next Proposition describes conditions under which the system is prevented from expressing high levels of the Lac proteins (and consequently cannot increase its lactose levels), whether or not glucose is available.

Proposition 5.3: Assume: (L1') $\frac{M_1N_2}{k_y} \le \phi_1(1-\Delta)$ and (L2') $\frac{M_2}{k_w} \ge \theta_1(1+\Delta)$. Then system (17) is globally ISS with respect to the compact set:

$$\mathcal{L}_{lac,*} = \left[0, \frac{\varepsilon N_1}{k_x}\right] \times \left[0, \frac{\varepsilon M_1N_2}{k_y}\right] \times \left[0, \frac{M_2}{k_w}\right] \times \left[0, \frac{M_3}{k_z}\right].$$

Proof: Set

$$x^a = 0,\ x^b = \frac{\varepsilon N_1}{k_x},\ y^a = 0,\ y^b = \frac{\varepsilon M_1N_2}{k_y}.$$

Consider the function:

$$V(\xi) = \frac{1}{2}|\xi|^2_{\mathcal{L}_{lac,*}} = \rho\left(x - x^b\right) + \rho\left(y - y^b\right).$$

It is easy to see that Lemma 3.4 can be applied with $R = P_{lac}$. Indeed, note that

$$\begin{aligned}& \nabla V\ f(\xi,u)\\ \le\ & -2k_x\ \rho\left(x - x^b\right) + \sqrt{2\rho\left(x - x^b\right)}(-k_x x^b + \nu_1(y))\\ & -2k_y\ \rho\left(y - y^b\right) + \sqrt{2\rho\left(y - y^b\right)}(-k_y y^b + N_2\mu_1(w))\\ & +\nu_4(u_1)\sqrt{2\rho\left(x - x^b\right)}.\end{aligned}$$

Assumption (L1') (and recalling the definition of an activation function ν) implies that $-k_x x^b + \nu_1(y) \le -k_x x^b + \varepsilon N_1 = 0$. Assumption (L2') implies that $-k_y y^b + N_2\mu_1(w) \le -k_y y^b + \varepsilon N_2 M_1 = 0$. Therefore, using Fact 2, one can find a $\mathcal{K}_\infty$ function γ such that

$$\nabla V\ f(\xi,u) \le -2\min\{k_x, k_y\}\ |\xi|^2_{\mathcal{L}_{lac,*}} + \gamma(|u|)$$

and Property (ii) of Lemma 3.4 is satisfied. ∎

A similar argument shows that, under alternative conditions, the Lac proteins will always be expressed and lactose metabolism "switched on", independently of glucose concentration. Not surprisingly, the conditions are opposite to those given in Proposition 5.3.

Proposition 5.4: Assume: (A1') $\frac{M_1N_2}{k_y} \ge \phi_1(1+\Delta)$, (A2') $\frac{M_2}{k_w} \le \theta_1(1-\Delta)$, and Then system (17) is globally ISS with respect to the compact set:

$$\begin{aligned}\mathcal{A}_{lac,*} = \quad & \frac{N_1}{k_x}[1-\varepsilon, 1] \times \frac{M_1N_2}{k_y}[1-\varepsilon, 1]\\ \times \quad & \left[0, \frac{M_2}{k_w}\right] \times \left[0, \frac{M_3}{k_z}\right].\end{aligned}$$

□

Just as in the example of the apoptosis network, the *lac* operon system clearly has the capacity for bistable response. This happens when the conditions from Propositions 5.1

and 5.2 are simultaneously satisfied. Putting conditions (L1)-(L3) and (A1)-(A4) together, one has:

$$\begin{aligned}\frac{N_1}{k_x\theta_2},\ \frac{M_2}{k_w\theta_1} &\in \left(\frac{1+\Delta}{1-\varepsilon},\frac{1-\Delta}{\varepsilon}\right)\\ \frac{M_1N_2}{k_y\phi_1} &\in \left(\frac{1+\Delta}{(1-\varepsilon)^2},\frac{1-\Delta}{\varepsilon}\right)\\ \frac{M_3}{k_z\phi_2} &\in \left(\frac{1+\Delta}{1-\varepsilon},\infty\right).\end{aligned} \tag{28}$$

Note that assumption (A4) (condition on k_z) simply reflects the fact that the input function μ_3 should have a sufficiently high maximal production rate: for low levels of glucose, the protein CRP should become activated. It is necessary that $\varepsilon < 1/2$ for $\mathcal{L}_{lac}$ and $\mathcal{A}_{lac}$ to be disjoint sets. In addition, both ε and Δ should satisfy the condition (16) (as for the apoptosis network).

If glucose is available and $\mu_3(u) \approx 0$, then the *lac* operon activator (CRP) is not activated. The system will be evolving in the set $\mathcal{L}_{lac}$. Suppose now that glucose is all used up: then the activator CPR enables and amplifies transcription of the operon genes. A nonzero input of extracellular lactose, together with the positive feedback loop, will repress LacI and induce sucessful transcription of the *lac* operon. The system will eventually be driven to the set $\mathcal{A}_{lac}$. (see Section V-B below). The conditions listed in Propositions 5.3 or 5.4 represent two situations where bistability is not possible. In the absence of inputs, the trajectories *always* converge to the set $\mathcal{L}_{lac,*}$ (resp., $\mathcal{A}_{lac,*}$), which correspond to the mode of repressed (resp., induced) *lac* operon.

A. Comparison to experimental results

The result of Proposition 5.4 can be compared to an experiment reported in [2]. In this paper, the authors detect and measure the bistable response of the *lac* operon. In one of the experiments, a new strain of *E. coli* was constructed, which has extra LacI binding sites introduced. Adding new LacI binding sites is equivalent to increasing the activity threshold θ_1, because a larger number of LacI molecules will be needed to produce the same level of repression of the operon. This new strain of *E. coli* was then exposed to increasing levels of extracellular TMG (a non-metabolizable lactose analogue). Increasing the levels of extracellular lactose corresponds to decreasing the activity threshold ϕ_1, since it becomes easier for permease LacY to recruit lactose. Thus it holds that

- increasing the levels of extracellular lactose ($\sim 1/\phi_1$) leads to validation of condition (A1');
- a large increase in LacI binding sites ($\sim \theta_1$) validates condition (A2').

According to Proposition 5.4, the mode "repressed *lac* operon" is not stable for this new strain. And indeed, the experiment (see [2], Fig. 4c) shows that only one qualitative type of response can be obtained from this strain, corresponding to the induced *lac* operon – as characterized by $\mathcal{A}_{lac,*}$.

B. Controlling the system towards lactose metabolism

A fundamental problem in the analysis of bistable biological systems is that of controlling or switching the system from one stable mode to another. In many cases, while possible inputs or stimuli are known (for instance, TNF in the apoptosis network; or extracellular lactose or glucose in the *lac* operon), it is not always clear how to "design" the control that will drive the system to the desired state. Following our idea that each desired state is represented by a set (as opposed to a single stationary point), our results suggest one method to control the system towards a desired set $\mathcal{Q}$: first, "turn on" the stimulus until the system is in a sufficiently small neighborhood of $\mathcal{Q}$, and then "turn off" stimulus. This is a reasonable protocol from the experimental point of view, as cell stimulation is often achieved through piecewise constant inputs: for instance, the cells are maintained in a medium with fixed external lactose and glucose concentrations (say E and G), for a certain time interval (say $t \in [t_0, t_0 + T]$).

For instance, to switch *E. coli* to the lactose metabolism mode ($\mathcal{A}_{lac}$), glucose and external lactose should, respectively, be removed from and added to the system, and maintained at, respectively, low and high levels, for *a suitable period of time*. To switch off lactose metabolism and go back to glucose metabolism ($\mathcal{L}_{lac}$), it suffices to add an appropriate amount of glucose to the medium and again wait for a sufficiently long interval. Thus, the question of choosing an *appropriate stimulation interval* arises or, more generally, choosing appropriate combinations of E, G and T. The next Proposition provides an answer to this question, by fixing a minimum time interval needed to start lactose metabolism.

Assume that the bistability conditions (28) are satisfied. Assume further that

$$N_4 > N_1. \tag{29}$$

Let $E_0 < \phi_4(1+\Delta)$ and $G_0 < \theta_3(1-\Delta)$, and consider constant inputs of the form:

$$u_1(t) = E_0, \quad u_2(t) = G_0, \quad t \in [0, T], \tag{30}$$

and $u_1(t) = u_2(t) = 0$ for $t > T$. Let $\delta \in (0,1)$ and R be the set constructed in the proof of Proposition 5.2, and define:

$$\begin{aligned}
T_1 &= -\frac{1}{k_x}\ln\left(1 - \frac{k_x\theta_2}{N_4}\frac{1+\Delta}{1-\varepsilon}\right)\\
T_2 &= -\frac{1}{k_x}\ln\left(1 - \frac{N_1}{N_4}\right)\\
T_3 &= T_1 - \frac{1}{k_w}\ln\frac{\varepsilon}{1-\varepsilon}\left(\frac{k_w\theta_1}{M_2}\frac{1-\Delta}{\varepsilon} - 1\right)\\
T_4 &= T_1 - \frac{1}{k_w}\ln\frac{\varepsilon}{1-\varepsilon}\left(\frac{1+\delta}{\varepsilon} - 1\right)\\
T_5 &= -\frac{1}{k_z}\ln\left(1 - \frac{k_z\phi_2}{M_3}\frac{1+\Delta}{1-\varepsilon}\right)\\
T_6 &= -\frac{1}{k_z}\ln(1-\delta)\\
T_7 &= \max\{T_3, T_5\} - \frac{1}{k_y}\ln(1-\delta).
\end{aligned}$$

By assumptions (28), (29), and $\varepsilon < 1/2$, it follows that all arguments inside the logarithms are positive and less than 2. Put

$$T_* = \max\{T_2, T_4, T_6, T_7\}.$$

The next result shows that stimulus should be on for at least $T = T_*$, in order to drive the *lac* operon to switch from lactose to glucose metabolism modes.

Proposition 5.5: Let $\xi(t, \xi_0, u)$ be the solution of system (17) with initial condition $\xi_0 \in \mathcal{L}_{lac}$, and input (30). Then $\xi(t, \xi_0, u)$ evolves in the set R (containing $\mathcal{A}_{lac}$), for $T_* < t \leq T$.

Proof: We will show that, for $T_* \leq t \leq T$, the trajectory evolves inside the set R. For $t \in [0, T]$, for an input of the form (30), it is clear that $\dot{x} \geq -k_x x + (1-\varepsilon)N_4$ and $\dot{z} \geq -k_z z + (1-\varepsilon)M_3$, so that (one may assume, in the worst case, that $x_0 = z_0 = 0$):

$$\begin{aligned} x(t) &\geq \frac{(1-\varepsilon)N_4}{k_x}(1 - e^{-k_x t}) \\ z(t) &\geq \frac{(1-\varepsilon)M_3}{k_z}(1 - e^{-k_z t}). \end{aligned}$$

It is straigthforward to check that:

$$T_1 < t \leq T \quad \Rightarrow \quad x(t) > \theta_2(1+\Delta) \tag{31}$$
$$T_2 < t \leq T \quad \Rightarrow \quad x(t) > (1-\varepsilon)N_1/k_x \tag{32}$$
$$T_5 < t \leq T \quad \Rightarrow \quad z(t) > \phi_2(1+\Delta) \tag{33}$$
$$T_6 < t \leq T \quad \Rightarrow \quad z(t) > \delta(1-\varepsilon)M_3/k_z. \tag{34}$$

Coordinate w starts decreasing as x increases above $\theta_2(1+\Delta)$:

$$w(t) \leq \frac{M_2}{k_w} e^{-k_w(t-T_1)} + \frac{\varepsilon M_2}{k_w}(1 - e^{-k_w(t-T_1)}),$$

and hence:

$$T_3 < t \leq T \quad \Rightarrow \quad w(t) \leq \ \theta_1(1-\Delta) \tag{35}$$
$$T_4 < t \leq T \quad \Rightarrow \quad w(t) \leq \ (1+\delta)\frac{\varepsilon M_2}{k_w}. \tag{36}$$

Expression (35) and (33) imply that $\dot{y} \geq -k_y y + M_1 N_2 (1-\varepsilon)^2$, for $\max\{T_3, T_5\} < t \leq T$ and so, in this time interval,

$$y(t) \geq \frac{(1-\varepsilon)^2 M_1 N_2}{k_y}(1 - e^{-k_y(t-T_{3,5})}).$$

It is clear now that $T_7 < t \leq T$ implies $y(t) \geq \delta \frac{(1-\varepsilon)^2 M_1 N_2}{k_y}$. This together with (32), (34), and (36) finishes the proof. ∎

As indicated by this Proposition, external lactose is needed to "switch" the system from glucose to lactose metabolism ($\mathcal{L}_{lac}$ to $\mathcal{A}_{lac}$). Indeed, glucose should be absent and external lactose available, during a minimum length of time, T_*. The inverse switch ($\mathcal{A}_{lac}$ to $\mathcal{L}_{lac}$) would be obtained by inverting the input conditions (i.e., high glucose, low external lactose).

VI. Discussion

The examples discussed in Sections IV and V illustrate a general formalism for modeling genetic networks, using a class of inhibition and activation functions. These functions are defined by appropriate physiological bounds, and allow the mathematical model to capture the variability often encountered in biological systems. Using this formalism, the possible responses of the network to various stimuli can be characterized by identifying invariant sets of the model. The goal is to identify invariant sets that represent distinct qualitative modes of operation of the system. For instance, the capacity of the network to exhibit bistable behavior is characterized by the co-existence of two disjoint (compact and nonempty) invariant subsets of the state space (named $\mathcal{L}$ and $\mathcal{A}$ in the examples), with low versus high concentrations of some species. Each of these invariant subsets is described by conditions on the parameters (relating maximal activities, activity thresholds and degradation constants), and represents a distinct response of the network: life or cell death in network (1), and *lac* operon repression or transcription in network (17). In all examples, it is shown that the system is locally ISS with respect to both $\mathcal{L}$ and $\mathcal{A}$. This ISS property leads to 0-invariance, that is in the absence of an input, if the system starts in one of the sets, then it will remain in that set. Since there are at least two such invariant sets, the system is indeed capable of operating in two distinct modes, in a stable manner. Furthermore, inputs or perturbations of small magnitude (as given by the corresponding sets R) do not drive the system far out from the 0-invariant set. Therefore, the system exhibits robustness with respect to small fluctuations in the environment, as its qualitative response is basically unchanged.

In contrast, conditions on the parameters that guarantee monostability are also given. Monostability is characterized by the existence of a 0-invariant set (denoted by either $\mathcal{L}_*$ or $\mathcal{A}_*$ in the examples), with respect to which the system is *globally* ISS. Global ISS with respect to a given compact set $\mathcal{L}_*$ guarantees that, in the absence of an input, the trajectories of the system asymptotically converge to $\mathcal{L}_*$, independently of the initial condition, which rules out the capacity for a bistable response of the network.

In both biological systems discussed, the wild type healthy cell has the capacity for bistability, that is, it can respond in two distinct ways, in a stable manner. However, damaged or malfunctioning cells often loose the capacity for bistability. This happens in the apoptosis network [33], where damaged cells seem to loose the capacity to undergo apoptosis, causing various diseases. It has also been verified for the *lac* operon on specially constructed strains of *E. coli*, as in [2] (Section V-A). The conditions developed in Propositions 4.1, 4.3, and 4.2, 4.4, provide a means to classify cells, according to whether they are healthy (both (L1)-(L3) and (A1)-(A4) satisfied), or not (either (L1')-(L2') or (A1')-(A2')). For example, Proposition 4.4 describes a malfunctioning cell, such as a cancerous cell (condition (L1'), low levels of C3a). And we have seen in Section V that Proposition 5.4 correctly describes an *E. coli* strain with extra LacI binding sites.

Our analysis can thus be applied to the detection of malfunctioning or damaged cells. (Note that, if none of the conditions is satisfied, then our analysis is not conclusive). By measuring the maximal production rates, as well as degradation rates and activation/inhibition thresholds for a given network, one can then check which of the conditions (L1)-(L3), (L1')-(L2') and (A1)-(A4), (A1')-(A2') are satisfied. Once the system is thus classified, an appropriate input can be constructed, to control the system to a desired compact set. Observe that if the system (1) is in the living state $\mathcal{L}$, then by sufficiently increasing TNF (and appropriate conditions on μ_5, ν_3) it is possible to drive the system towards apoptosis. Once the

trajectory reaches the set $\mathcal{A}$ (or sufficiently close), the stimulus can be "turned off" and the trajectory will remain in the set $\mathcal{A}$ (or expected to converge towards $\mathcal{A}$, if in its basin of attraction). On the other hand, if the system starts in the apoptosis set $\mathcal{A}$, then no input will drive the system back towards the "living" state – which of course makes sense from the biogical point of view. In the *lac* operon network (Proposition 5.5), it is interesting to note that *two* independent inputs are needed to allow the system to switch between the two stable modes, in both directions.

VII. Conclusion

A general framework has been discussed for modeling genetic regulatory networks, where interactions among genes and proteins are described in terms of a class of free-form activation and inhibition functions. The formalism presented in this paper intuitively relates the class of piecewise linear hybrid models to a class of continuous models: one possible extension of the formalism is to explore this connection to further study and analyze piecewise linear models. Other possible extensions of the current work include introducing more general degradation functions.

The capacity for mono- or bi-stable behavior in a genetic regulatory network can be fully characterized by identifying appropriate 0-invariant compact sets for the system (with respect to which the system is, respectively, globally or locally input-to-state stable). Conditions relating the degradation rates, maximal activities and threshold constants are provided, which guarantee that the system will be capable of bistable or only monostable behavior. Our analysis allows a classification of systems (or cells) according to their capacity for monostable or bistable responses. This classification helps to distinguish among "healthy" and "damaged" or "malfunctioning" cells. An application of this knowledge is the construction of suitable inputs (stimuli) that will drive the system to a desired compact set – and drive the biological network to a desired qualitative response.

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