Shaping Pulses to Control Bistable Biological Systems.

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***Abstract*— In this paper, we present a framework for shaping pulses to control biological systems, and specifically systems in synthetic biology. By shaping we mean computing the magnitude and the length of a pulse, application of which results in reaching the desired control objective. Hence the control signals have only two parameters, which makes these signals amenable to wetlab implementations. We focus on the problem of switching between steady states in a bistable system. We show how to estimate the set of the switching pulses, if the trajectories of the controlled system can be bounded from above and below by the trajectories of monotone systems. We then generalise this result to systems with parametric uncertainty under some mild assumptions on the set of admissible parameters, thus providing some robustness guarantees. We illustrate the results on some example genetic circuits.**

***Index Terms*— monotone systems, near-monotone systems, toggle switch, control by pulses, open-loop control**

I. Introduction

The external control of microbes is an important challenge in synthetic biology. Recent seminal works [2]–[4] successfully developed the first implementations of computer-based control of yeast populations. These works used light-based [5], [6] and biochemical interfaces, respectively, to actuate the gene expression machinery. The feedback loop with an external controller, which was either an MPC or a PID controller (cf. [7]), was closed by using sophisticated measurement techniques. Despite these recent successes, we are still far away from a general approach to feedback control of living cells. The main algorithmic difficulty is dealing with stochasticity, which was partially addressed by using reinforcement learning algorithms in [8], [9]. One of the practical difficulties in feedback control of cells is that the optimal control signal may be time-varying (for example, a sine curve), which is very difficult to implement in many wetlab setups. Hence, we shifted our focus to designing control strategies, which are easy to implement with the available wetlab technologies and easy to compute. Specifically, we consider temporal pulses of the following form:

$$u(t) = \mu h(t,\tau) \qquad h(t,\tau) = \begin{cases} 1 & 0 \le t \le \tau, \\ 0 & t > \tau. \end{cases} \tag{1}$$

We focus on the problem of switching from one stable steady state to another in a bistable system. Our goal is to estimate the set of all pairs (μ, τ) that can switch the system between the stable steady states and the set of all pairs (μ, τ) that cannot. We will refer to these sets as *the switching sets*. We also consider a boundary between these switching sets and call it *the switching separatrix*. Theoretically, the switching can be achieved by applying a constant control signal with a large value of μ. In practice, an exposure to a pulse with a large magnitude μ or a large length τ can have unintended consequences for the system. In the case of light-induction as a control mechanism for microbes, for example, this may lead to overexpression of heterologus proteins. This in turn induces cellular burden which can slow the growth rate of the culture. This can also lead to the so-called "photo-bleaching", which means that the cells stop responding to light stimuli after being exposed to high intensities of light for long periods of time. Hence, the pairs (μ, τ) which lie close to the switching separatrix are of particular importance.

We start by showing that for monotone control systems (cf. [10]) the switching separatrix is a monotone curve, and hence the switching sets can be computed efficiently. We then extend this result to a class of non-monotone systems, the vector fields of which can be bounded from below and above by the vector fields of some monotone systems. We show that the switching sets of the bounding monotone systems are inner and outer approximations of the switching sets of a non-monotone one. Empirically, the switching sets of the bounding systems provide useful approximations on the switching sets of a non-monotone system, if the approximated system exhibits a *near-monotone* behaviour. A near-monotone system is defined as a system which becomes monotone by removing particular interactions between the states [11]. Although near-monotonicity is still quite a restrictive assumption, it was recently noticed that biological systems tend to be near-monotone [11]. This justifies the applicability of our results in the biological setting. We then generalise these results to systems with parametric uncertainty under some mild assumptions on the set of admissible parameters. Hence, we provide robustness guarantees towards parameter variations for open-loop switching between steady states.

The development of our results is in the spirit of [12], [13], where the authors considered the problem of computing reachability sets of a monotone system. We also acknowledge

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a connection with [14], [15], where easy feedback controllers for monotone control systems were proposed. Our main contribution, however, focuses on open-loop control by shaped pulses.

The rest of the paper is organised as follows. In Section II we present the general formulation of the problem and the main theoretical results on the properties of the switching separatrix for bistable systems. In Section III we prove the theoretical results, while in Section IV we illustrate the main ideas of the paper on some example genetic circuits. Some of the proofs and the algorithms for computing the separatrix can be found in the full version of the paper [1] (available online). In [1], we also apply the shaping pulses framework to a different control problem. Namely, we consider a problem of inducing an oscillatory behaviour in an eight species generalised repressilator, which is a monotone system.

Notation. Let $\|\cdot\|_2$ stand for the Euclidean norm in $\mathbb{R}^n$, $X\backslash Y$ stands for the relative complement of X in Y, $\mathrm{int}(Y)$ stand for the interior of the set Y, and $\mathrm{cl}(Y)$ for its closure. Let $x \succeq_x y$ stand for a partial order in $\mathbb{R}^n$ induced by the non-negative orthant $\mathbb{R}^n_{\geq 0}$. That is the relation $x \succeq_x y$ is true for vectors x and y if and only if $x_i \geq y_i$, for all i (or $x - y \in \mathbb{R}^n_{\geq 0}$). Let $x \gg_x y$ be true if and only if $x_i > y_i$, for all i (or $x - y \in \mathbb{R}^n_{>0}$). This order is typically referred to as *a standard partial order*. For a general definition of the partial order we refer the reader to [16]. We write $x \not\succeq_x y$, if the relation $x \succeq_x y$ does not hold. The partial order $u \succeq_u v$ on the space of control signals $u(t)$ is defined as an element-wise comparison $u_i(t) \geq v_i(t)$ for all i and t.

II. Problem Formulation and Main Results

Throughout the paper we consider single input control systems in the following form

$$\dot{x} = f(x, u), \quad x(0) = x_0, \tag{2}$$

where $f : \mathcal{D} \times \mathcal{U} \to \mathbb{R}^n$, $u : \mathbb{R}_{\geq 0} \to \mathcal{U}$, $\mathcal{D} \subset \mathbb{R}^n$, $\mathcal{U} \subset \mathbb{R}$ and $u(\cdot)$ belongs to the space $\mathcal{U}_\infty$ of Lebesgue measurable functions with values from $\mathcal{U}$. We define the flow map $\phi_f : \mathbb{R} \times \mathcal{D} \times \mathcal{U}_\infty \to \mathbb{R}^n$, where $\phi_f(t; x_0, u)$ is a solution to the system (2) with an initial condition x_0 and a control signal u. We consider the control signals in the shape of a pulse, that is signals from the set $\mathcal{S} = \{\mu h(\cdot, \tau) : \mu, \tau \in \mathbb{R}_{>0}\}$, where $h(\cdot, \tau)$ is the step function defined in (1). We confine the class of considered control systems by making the following **assumptions:**

A1. Let $f(x, u)$ be continuous in (x, u) on $\mathcal{D}_f \times \mathcal{U}$. Moreover, for each compact sets $C_1 \subset \mathcal{D}_f$ and $C_2 \subset \mathcal{U}$, let there exist a constant k such that $\|f(\xi, u) - f(\zeta, u)\|_2 \leq k\|\xi - \zeta\|_2$ for all $\xi, \zeta \in C_1$ and $u \in C_2$.

A2. Let the unforced system (2) (that is, with $u = 0$) have two stable steady states in $\mathcal{D}_f$, denoted as s_f^0 and s_f^1,

A3. Let $\mathcal{D}_f = \mathrm{cl}(\mathcal{A}(s_f^0) \cup \mathcal{A}(s_f^1))$, where $\mathcal{A}(s_f^i)$ stands for the domain of attraction of the steady state s_f^i for $i = 0, 1$ of the unforced system (2),

A4. For any $u \in \mathcal{S}$ let $\phi_f(t; s_f^0, u)$ belong to $\mathcal{D}_f$. Moreover, let the sets

$$\mathcal{S}_f^+ = \{\mu, \tau > 0 \Big| \lim_{t\to\infty} \phi_f(t; s_f^0, \mu h(\cdot, \tau)) = s_f^1\}$$
$$\mathcal{S}_f^- = \{\mu, \tau > 0 \Big| \lim_{t\to\infty} \phi_f(t; s_f^0, \mu h(\cdot, \tau)) = s_f^0\}$$

have non-empty interiors.

Assumption A1 guarantees existence, uniqueness and continuity of solutions to (2), while Assumptions A2–A4 define a bistable system on a set $\mathcal{D}_f$ controlled by pulses. Note that the system can be multistable on $\mathbb{R}^n$. We formulate our **control problem** as computing the sets $\mathcal{S}_f^+$, $\mathcal{S}_f^-$, to which we will refer as *the switching sets*. Assumption A4 guarantees that this control problem is well-posed.

We note that in many practical applications, the sets $\mathrm{cl}(\mathcal{S}_f^+)$ and $\mathrm{cl}(\mathbb{R}^2_{>0}\backslash\mathcal{S}_f^-)$ are equal, however, showing this result may require additional assumptions. Therefore in order to simplify the presentation we study only the properties of the set $\mathcal{S}_f^-$ (and consequently, the properties of the set $\mathrm{cl}(\mathbb{R}^2_{>0}\backslash\mathcal{S}_f^-)$).

1) Switching Sets for Monotone Systems: In order to avoid confusion, we will reserve the notation $f(x, u)$ for the vector field of a non-monotone system, while the systems

$$\dot{x} = g(x, u), \qquad x(0) = x_0, \tag{3}$$
$$\dot{x} = r(x, u), \qquad x(0) = x_0, \tag{4}$$

will denote so-called *monotone systems* throughout the paper.

Definition 1: The system (3) is called monotone on $\mathcal{D}_M \times \mathcal{U}_\infty$ with respect to the partial orders $\succeq_x$, $\succeq_u$, if for all $x, y \in \mathcal{D}_M$ and $u, v \in \mathcal{U}_\infty$ such that $x \succeq_x y$ and $u \succeq_u v$, we have $\phi_g(t; x, u) \succeq_x \phi_g(t; y, v)$ for all $t > 0$ when $\phi_g(t; x, u), \phi_g(t; y, v) \in \mathcal{D}_M$.

Our first theoretical result reveals that if a bistable system $\dot{x} = g(x, u)$ is monotone, then the sets $\mathcal{S}_g^+$ and $\mathcal{S}_g^-$ can be separated by a non-increasing curve in τ. This is formally stated below.

Theorem 1: Let the system (3) satisfy Assumptions A1–A4 and be monotone on $\mathcal{D}_g \times \mathcal{S}$. The set $\mathcal{S}_g^-$ is simply connected and lies between the points with $\mu = 0$, $\tau = 0$ and a curve $\mu_g(\tau)$, which is a set of maximal elements of $\mathcal{S}_g^-$ in the standard partial order. Moreover, the curve $\mu_g(\tau)$ is such that for any $\mu_1 \in \mu_g(\tau_1)$ and $\mu_2 \in \mu_g(\tau_2)$, $\mu_1 \geq \mu_2$ for $\tau_1 < \tau_2$.

We call the curve $\mu_g(\tau)$ *the switching separatrix*, referring to the separation of the set $\mathcal{S}_g^-$ from the set $\mathcal{S}_g^+$. Theorem 1 shows that the computation of the set $\mathcal{S}_g^-$ is reduced to the computation of a curve $\mu_g(\tau)$, which can be done efficiently as described in [1].

2) Switching Sets for a Class of Non-Monotone Systems: If the system $\dot{x} = f(x, u)$ to be controlled is not monotone, then the set $\mathcal{S}_f^-$ is generally not simply connected making it harder to compute. Instead, we can obtain inner and outer bounds on the switching set provided that the vector field of the system can be bounded from above and below by the vector fields of monotone systems. This is formally stated in the next result.

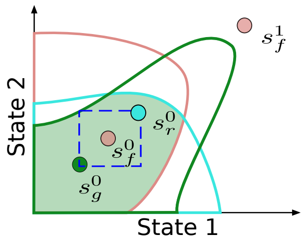

Fig. 1. An illustration of the conditions (6) and (7) for a two-state system. The areas bounded by green, red, and cyan curves are regions of attraction of the stable steady states s_f^0, s_g^0, s_r^0 of the systems (2), (3), (4), respectively. The condition (6) ensures that all the steady states lie in the intersection of the corresponding regions of attractions (light green area). The steady state s_f^1 cannot lie in the dashed blue box due to condition (7).

Theorem 2: Let systems (2), (3), (4) satisfy Assumptions A1–A4. Let $\mathcal{D}_M = \mathcal{D}_g \cup \mathcal{D}_f \cup \mathcal{D}_r$, the systems (3) and (4) be monotone on $\mathcal{D}_M \times \mathcal{S}$ and

$$g(x,u) \preceq_x f(x,u) \preceq_x r(x,u) \text{ on } \mathcal{D}_M \times \mathcal{U}. \tag{5}$$

Additionally assume that the stable steady states s_g^0, s_f^0, s_r^0, s_f^1 satisfy

$$s_g^0, s_f^0, s_r^0 \in \operatorname{int}\left(\mathcal{A}(s_g^0) \cap \mathcal{A}(s_f^0) \cap \mathcal{A}(s_r^0)\right), \tag{6}$$
$$s_f^1 \notin \left\{z | s_g^0 \preceq_x z \preceq_x s_r^0\right\} \tag{7}$$

Then the set $\mathcal{S}_f^-$ of the system (2) can be approximated as follows:

$$\mathcal{S}_g^- \supseteq \mathcal{S}_f^- \supseteq \mathcal{S}_r^-. \tag{8}$$

The technical conditions in (6), (7) are crucial to the proof and are generally easy to satisfy. An illustration of these conditions is provided in Figure 1. Checking the condition (7) reduces to the computation of the stable steady states, as does checking the condition (6). Indeed, to verify that s_f^0 belongs to the intersection of $\mathcal{A}(s_g^0)$, $\mathcal{A}(s_f^0)$, $\mathcal{A}(s_r^0)$, we check if the trajectories of the systems (3), (4) initialised at s_f^0 with $u = 0$ converge to s_g^0 and s_r^0, respectively, which is done by numerical integration. The computation of stable steady states can be done using numerical methods, e.g. [17].

Note that the lower bounding system (3) is used to compute an outer approximation of $\mathcal{S}_f^-$, which corresponds to the set of pulses not switching the system (2). In practice, this means that $\mathcal{S}_g^+$ is an inner approximation of $\mathcal{S}_f^+$. Hence any pulse that switches the system (3) also switches the system (2). In terms of separatrices, we can say that $\mu_g(\tau)$ dominates $\mu_f(\tau)$ for every τ. Therefore, in many practical applications, we will be only interested in finding the system (3). In this case the condition (7) is not required and the condition (6) is transformed to $s_g^0, s_f^0 \in \operatorname{int}\left(\mathcal{A}(s_g^0) \cap \mathcal{A}(s_f^0)\right)$. A straightforward procedure to compute the bounding systems can be found in [1].

3) Robustness Towards Parameter Variations: Theorem 2 provides also a way of estimating the switching set under parametric uncertainty in the system dynamics. This is shown in the next corollary, which is a direct application of Theorem 2.

Corollary 1: Consider a family of systems $\dot{x} = f(x,u,p)$ with a vector of parameters p taking values from a compact set $\mathcal{P}$. Let the systems $\dot{x} = f(x,u,p)$ for every p in $\mathcal{P}$ satisfy Assumptions A1–A4. Assume there exist parameter values $a \in \mathcal{P}$ and $b \in \mathcal{P}$ such that the systems $\dot{x} = f(x,u,a)$ and $\dot{x} = f(x,u,b)$ are monotone on $\mathcal{D}_M \times \mathcal{S}$, where $\mathcal{D}_M = \bigcup_{q \in \mathcal{P}} \mathcal{D}_{f(\cdot,\cdot,q)}$ and

$$f(x,u,a) \preceq_x f(x,u,p) \preceq_x f(x,u,b), \tag{9}$$

for all $(x,u) \in \mathcal{D}_M \times \mathcal{U}$ and for all $p \in \mathcal{P}$. Finally, let the stable steady states $s_{f(\cdot,\cdot,p)}^0$, $s_{f(\cdot,\cdot,p)}^1$ satisfy for all $p \in \mathcal{P}$

$$s_{f(\cdot,\cdot,p)}^0 \in \operatorname{int}\left(\bigcap_{q \in \mathcal{P}} \mathcal{A}(s_{f(\cdot,\cdot,q)}^0)\right) \tag{10}$$
$$s_{f(\cdot,\cdot,p)}^1 \notin \left\{z | s_{f(\cdot,\cdot,a)}^0 \preceq_x z \preceq_x s_{f(\cdot,\cdot,b)}^0\right\}. \tag{11}$$

Then the switching sets $\mathcal{S}_{f(\cdot,\cdot,p)}^-$ can be approximated for all $p \in \mathcal{P}$ as follows:

$$\mathcal{S}_{f(\cdot,\cdot,a)}^- \supseteq \mathcal{S}_{f(\cdot,\cdot,p)}^- \supseteq \mathcal{S}_{f(\cdot,\cdot,b)}^-. \tag{12}$$

The proof follows by setting $g(x,u) = f(x,u,a)$ and $r(x,u) = f(x,u,b)$ and noting that the conditions in (10), (11) imply the conditions in (6), (7) in the premise of Theorem 2. Note that we do not require the system $\dot{x} = f(x,u,p)$ to be monotone for all parameter values p. However, in practice this corollary is hard to apply directly without the monotonicity assumption and the main bottleneck is finding the parameter values a and b. If the system $\dot{x} = f(x,u,p)$ is monotone for all parameter values p, then we can find a and b if there exists a partial order in the parameter space. That is a relation $\preceq_p$ such that for two parameter values p_1 and p_2 satisfying $p_1 \preceq_p p_2$ the following holds

$$f(x,u,p_1) \preceq_x f(x,u,p_2) \; \forall x \in \mathcal{D}, u \in \mathcal{U}.$$

If a partial order is found, the values a and b are computed as minimal and maximal elements of $\mathcal{P}$ in the partial order $\preceq_p$. This idea is equivalent to treating parameters p as inputs and showing that the system $\dot{x} = f(x,u,p)$ is monotone with respect to inputs u and p.

III. Proofs of the Main Results

Proof of Theorem 1: First, we need to prove that if a pair (μ^l, τ^l) belongs to $\mathcal{S}_g^-$, then all pairs (μ, τ) such that $0 < \mu \le \mu^l$, $0 < \tau \le \tau^l$ also belong to $\mathcal{S}_g^-$. By the order in u, for every $0 < \mu \le \mu^l$, $0 < \tau \le \tau^l$ we have $0 \preceq_u \mu h(\cdot,\tau) \preceq_u \mu^l h(\cdot,\tau^l)$. The following relation is then true

$$s_g^0 \preceq_x \phi_g(t; s_g^0, \mu h(\cdot,\tau)) \preceq_x \phi_g(t; s_g^0, \mu^l h(\cdot,\tau^l)).$$

There exists a T such that for all $t > T$ the flow $\phi_g(t; s_g^0, \mu^l h(\cdot,\tau^l))$ belongs to $\mathcal{A}(s_g^0)$ and converges to s_g^0. Therefore $\phi_g(t; s_g^0, \mu h(\cdot,\tau))$ converges to s_g^0 with $t \to +\infty$, and consequently the pair (μ,τ) does not toggle the system and thus belongs to $\mathcal{S}_g^-$.

Above, we have also shown that any point lying in the set $\mathcal{S}_g^-$ is path-wise connected to a point in the neighbourhood of the origin. In order to show that the set is simply connected, it is left to prove that there are no holes in the set $\mathcal{S}_g^-$. Let $\eta(\mu,\tau)$ be a closed curve which lies in $\mathcal{S}_g^-$. Consider the set

$$\mathcal{S}^\eta = \left\{(\mu,\tau) \middle| 0 < \mu \le \mu^\eta, 0 < \tau \le \tau^\eta, (\mu^\eta,\tau^\eta) \in \eta(\mu,\tau)\right\}.$$

Since the set $\mathcal{S}_g^-$ is in $\mathbb{R}^2_{>0}$, the set $\mathcal{S}^\eta$ contains the set enclosed by the curve $\eta(\mu,\tau)$. By the above $\mathcal{S}^\eta$ is a subset of $\mathcal{S}_g^-$, which implies that the set $\mathcal{S}_g^-$ is simply connected.

Let a pair (μ^u,τ^u) not belong to $\mathcal{S}_g^-$. If there exists a pair $(\mu,\tau)\in\mathcal{S}_g^-$ such that $\mu\geq\mu^u$, $\tau\geq\tau^u$, then by the arguments above the pair (μ^u,τ^u) must also belong to $\mathcal{S}_g^-$. Hence, all pairs (μ,τ) such that $\mu\geq\mu^u$, $\tau\geq\tau^u$ do not belong to $\mathcal{S}_g^-$. This implies that there exists a set of maximal elements of $\mathcal{S}_g^-$ in the standard partial order, which is a segment of the boundary of $\mathcal{S}_g^-$ excluding the points with μ and τ equal to zero. Let the mapping $\mu_g(\tau)$ denote the set of maximal elements of $\mathcal{S}_g^-$. Since $\mu_g(\tau)$ is the set of maximal elements of $\mathcal{S}_g^-$, for $\tau_1<\tau_2$, we cannot have $\mu_g(\tau_1)<\mu_g(\tau_2)$. Therefore, $\mu_g(\tau_1)\geq\mu_g(\tau_2)$ for $\tau_1<\tau_2$, and μ_g is non-increasing in τ. ■

In order to proceed with the proof of Theorem 2 we need two additional results: one is the so-called comparison principle for control systems and the other is concerned with geometric properties of the regions of attractions of monotone systems. The proofs can be found in [1].

Lemma 1: Consider the dynamical systems $\dot{x}=f(x,u)$ and $\dot{x}=g(x,u)$ satisfying Assumption A1. Let one of the systems be monotone on $\mathcal{D}_M\times\mathcal{U}_\infty$. If $g(x,u)\succeq_x f(x,u)$ for all $(x,u)\in\mathcal{D}_M\times\mathcal{U}$ then for all $t>0$, and for all $x_2\succeq_x x_1$, $u_2\succeq_u u_1$ we have $\phi_g(t;x_2,u_2)\succeq_x\phi_f(t;x_1,u_1)$.

Lemma 2: Let the system $\dot{x}=g(x,0)$ satisfy Assumption A1 and be monotone on $\mathcal{A}(s_g^0)$, where s_g^0 is a stable steady state and $\mathcal{A}(s_g^0)$ is its domain of attraction. Let x^b and x^l belong to $\mathcal{A}(s_g^0)$. Then all points z such that $x^l\preceq_x z\preceq_x x^b$ belong to $\mathcal{A}(s_g^0)$.

Proof of Theorem 2: To prove (8), we proceed by parts.

A. First we note that the assumption in (6) implies that $s_g^0\preceq_x s_f^0\preceq_x s_r^0$. Indeed, take x_0 from the interior of the intersection of the sets $\mathcal{A}(s_g^0)$, $\mathcal{A}(s_f^0)$, $\mathcal{A}(s_r^0)$. By Lemma 1 for all $t>0$, we have

$$\phi_g(t;x_0,0)\preceq_x\phi_f(t;x_0,0)\preceq_x\phi_r(t;x_0,0),$$

and thus taking the limit $t\to\infty$ we get $s_g^0\preceq_x s_f^0\preceq_x s_r^0$.

B. Next we show that $g(x,u)\preceq_x f(x,u)$ for all $(x,u)\in\mathcal{D}_M\times\mathcal{U}$ implies that $\mathcal{S}_g^-\supseteq\mathcal{S}_f^-$. Let $\mathcal{V}\subset\mathcal{S}$ be such that $u=\mu h(\cdot,\tau)\in\mathcal{V}$ if $(\mu,\tau)\in\mathcal{S}_f^-$.

Due to $s_g^0\preceq_x s_f^0$ and $g\preceq_x f$ on $\mathcal{D}_M\times\mathcal{S}$, by Lemma 1, we have that

$$s_g^0\preceq_x\phi_g(t;s_g^0,u)\preceq_x\phi_f(t;s_f^0,u),$$

for all $u\in\mathcal{V}$. Note that the first inequality is due to monotonicity of the system $\dot{x}=g(x,u)$. The flow $\phi_f(t;s_f^0,u)$ converges to s_f^0 with $t\to+\infty$. Therefore, there exists a time T such that for all $t>T$ we have

$$s_g^0\preceq_x\phi_g(t;s_g^0,u)\ll_x s_f^0+\varepsilon\mathbf{1}.$$

for some positive ε. Moreover, we can pick an ε such that $s_f^0+\varepsilon\mathbf{1}$ lies in $\mathcal{A}(s_g^0)$ (due to (6)). Since the system $\dot{x}=g(x,u)$ is monotone, according to Lemma 2, the flow $\phi_g(t;s_g^0,u)$ lies in $\mathcal{A}(s_g^0)$. Hence, no u in $\mathcal{V}$ toggles the system $\dot{x}=g(x,u)$ either and we conclude that $\mathcal{S}_g^-\supseteq\mathcal{S}_f^-$.

C. Finally, we show that $\mathcal{S}_f^-\supseteq\mathcal{S}_r^-$. Let $\mathcal{W}$ be such that $u=\mu h(\cdot,\tau)\in\mathcal{W}$ if $(\mu,\tau)\in\mathcal{S}_r^-$.

Due to $s_g^0\preceq_x s_f^0\preceq_x s_r^0$ and $g\preceq_x f\preceq_x r$ on $\mathcal{D}_M\times\mathcal{S}$, by Lemma 1, we have that

$$\phi_g(t;s_g^0,u)\preceq_x\phi_f(t;s_f^0,u)\preceq_x\phi_r(t;s_f^0,u),$$

for all $u\in\mathcal{W}$. Furthermore, monotonicity of $\dot{x}=g(x,u)$ implies that

$$s_g^0\preceq_x\phi_g(t;s_g^0,u). \tag{13}$$

Moreover, due the fact that $\phi_r(t;s_f^0,u)\to s_r^0$ with $t\to+\infty$, there exists a T such that for all $t>T$, $u\in\mathcal{W}$ and some small positive ε the following holds

$$s_g^0\preceq_x\phi_f(t;s_f^0,u)\preceq_x s_r^0+\varepsilon\mathbf{1}.$$

Due to (6), we can choose an ε such that $s_r^0+\varepsilon\mathbf{1}$ lies in $\mathcal{D}_M$. Hence, the flow of $\dot{x}=f(x,u)$ for all $u\in\mathcal{W}$ belongs to the set $\{z|s_g^0\preceq_x z\preceq_x s_r^0+\varepsilon\mathbf{1}\}$ for all $t>T$.

Now, assume there exists $u^c\in\mathcal{W}$ that toggles the system $\dot{x}=f(x,u)$. This implies that the flow $\phi_f(t;s_f^0,u^c)$ converges to s_f^1 with $t\to\infty$. Therefore we have that s_f^1 belongs to the set $\{z|s_g^0\preceq_x z\preceq_x s_r^0+\varepsilon\mathbf{1}\}$ for an arbitrarily small ε, and consequently $s_f^1\preceq_x s_r^0$. This contradicts the condition (7) in the premise of Theorem 1. Hence, no u in $\mathcal{W}$ toggles the system $\dot{x}=f(x,u)$ and $\mathcal{S}_f^-\supseteq\mathcal{S}_r^-$. ■

IV. Illustrative Examples

A. A Monotone Toggle Switch

As a first example we consider a model of a toggle switch, which was a pioneering circuit in synthetic biology [18]. The genetic toggle switch is composed of two mutually repressive genes *LacI* and *TetR*. We consider a control-affine model, which is consistent with a toggle switch actuated by light induction [6]. The dynamical equations can be written as follows.

$$\begin{aligned}\dot{x}_1&=\frac{p_1}{1+(x_2/p_2)^{p_3}}+p_4-p_5x_1+u,\\ \dot{x}_2&=\frac{p_6}{1+(x_1/p_7)^{p_8}}+p_9-p_{10}x_2,\end{aligned} \tag{14}$$

with the following parameter values

$$\begin{aligned}&p_1=40,\quad p_2=1,\quad p_3=4,\quad p_4=0.05,\quad p_5=1,\\ &p_6=30,\quad p_7=1,\quad p_8=4,\quad p_9=0.1,\quad\ \ p_{10}=1.\end{aligned} \tag{15}$$

The repression interactions are modelled here by rational functions $\frac{p_1}{1+(x_2/p_2)^{p_3}}$, $\frac{p_6}{1+(x_1/p_7)^{p_8}}$, which are called Michaelis-Menten functions, if $p_3=p_8=1$, and Hill functions otherwise. The coefficients p_3, p_8 are called Hill coefficients (or cooperativity coefficients), p_2, p_7 are called repression strengths (or thresholds), the coefficients p_4, p_9 model leaky transcriptions, while p_5, p_{10} are degradation rates. The coefficients p_1, p_6 influence the positions of steady states. For all non-negative values of parameters this model is monotone and satisfies Assumptions A1–A4.

The simulation results concern the changes in the switching separatrices with respect to parameter variations for the toggle switch system. In Table I, we specify the systems by

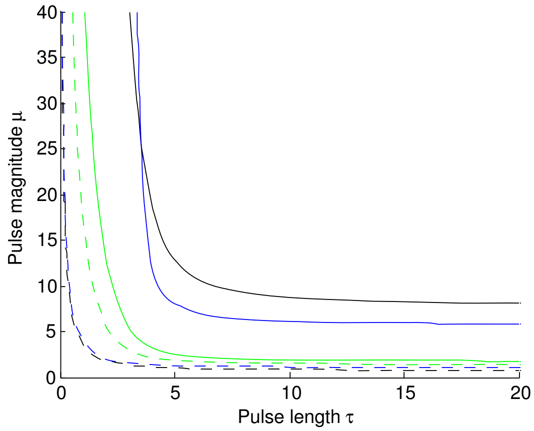

Fig. 2. Switching separatrices for the toggle switch (14) with parameter values specified in Table I. The switching separatrices corresponding to the systems with the subscript lower (respectively, upper) in Table I are depicted with solid (respectively, dashed) curves. The switching separatrices corresponding to systems with superscripts 1, 2 and 3 in Table I are depicted with green, blue and black curves, respectively.

TABLE I

PARAMETER VALUES FOR SYSTEMS IN SUBSECTION IV-A. THE UNSPECIFIED PARAMETER VALUES ARE THE SAME AS IN (15).

	$\mathcal{F}^i_{\text{lower}}$	$\mathcal{F}^i_{\text{upper}}$
$i=1$	$p_1=40$, $p_4=0.05$ $p_6=30$, $p_9=0.1$	$p_1=20$,$p_4=0.01$, $p_6=45$, $p_9=0.3$,
$i=2$	$p_1=40$, $p_4=0.05$ $p_6=30$, $p_9=0.1$ $p_2=4$, $p_7=1$	$p_1=20$,$p_4=0.01$, $p_6=45$, $p_9=0.3$, $p_2=1$, $p_7=4$,
$i=3$	$p_1=40$, $p_4=0.05$ $p_6=30$, $p_9=0.1$ $p_5=1$, $p_{10}=2$	$p_1=20$,$p_4=0.01$, $p_6=45$, $p_9=0.3$, $p_5=3$, $p_{10}=1$,

varying some of the parameter values (15) of the system (14). After that we compute the switching separatrices and plot them in Figure 2. Note that the subscript lower stands for a lower bounding vector field in the order $\succeq_x$, not the lower bounding separatrix. In fact, the comparison is reversed for separatrices, that is a separatrix for a lower bounding vector field, dominates a separatrix for an upper bounding vector field. The separatrices corresponding to the systems with the subscript lower (respectively, upper) in Table I are depicted with solid (respectively, dashed) curves. The switching separatrices corresponding to systems with superscripts 1, 2 and 3 in Table I are depicted with green, blue and black curves, respectively. Note that the blue and black solid curves in Figure 2 intersect, which happens since the vector fields f_2 and f_3 of systems $\mathcal{F}^2_{\text{lower}}$ and $\mathcal{F}^3_{\text{lower}}$, correspondingly, are not comparable. This means that there exists a set $\mathcal{X} = \{(x,u) \in \mathcal{D} \times \mathcal{U}\}$ on which $f_2(x,u) \not\preceq_x f_3(x,u)$ and $f_3(x,u) \not\preceq_x f_2(x,u)$.

The green curves lie very close to each other despite the number of parameters varied and the level of variations. This is not true for the black or the blue curves for example, which indicates that some parameters are much more sensitive to variations than others. In our case, this happens because the variations in parameters p_5, p_{10}, p_2, and p_7 affect significantly the positions of the stable steady states. Therefore, pulses with significantly smaller magnitudes are required to

TABLE II

PARAMETER VALUES FOR SYSTEMS WITH DIFFERENT PARAMETER VARIATIONS, WHERE $z_0^i(1)$ IS THE FIRST COMPONENT OF THE INITIAL POINT z_0^i OF THE SYSTEM $\mathcal{F}^i$ FOR i EQUAL TO 1 AND 2.

$\mathcal{F}^1$	$\mathcal{G}^1_{\text{upper}}$	$\mathcal{G}^1_{\text{lower}}$
$p_2=0.1$	$p_1=0$	$p_1=0.1z_0^1(1)$
$p_1=p_3=0$	$p_2=p_3=0$	$p_2=p_3=0$
$\mathcal{F}^2$	$\mathcal{G}^2_{\text{upper}}$	$\mathcal{G}^2_{\text{lower}}$
$p_3=0.1$	$p_1=0$	$p_1=0.1\frac{z_0^2(1)}{z_0^2(1)+1}$
$p_1=p_2=0$	$p_2=p_3=0$	$p_2=p_3=0$

switch the systems $\mathcal{F}^2_{\text{upper}}$ and $\mathcal{F}^3_{\text{upper}}$ in comparison with $\mathcal{F}^2_{\text{lower}}$ and $\mathcal{F}^3_{\text{lower}}$, respectively.

B. A Non-Monotone System

Consider the following three state system

$$\mathcal{F} = \begin{cases} \dot{x}_1 &= \dfrac{1000}{1+x_3^2} - 0.4x_1, \\ \dot{x}_2 &= \dfrac{1000}{1+x_1^4} - 4x_2 + u, \\ \dot{x}_3 &= p_1 + p_2 x_1 + p_3\frac{x_1}{x_1+1} + 5x_2 - 0.3x_3, \end{cases} \tag{16}$$

and two nominal systems $\mathcal{F}^1$ and $\mathcal{F}^2$ specified in Table II by changing parameter values for p_1, p_2, p_3. In Table II, the notations $\mathcal{G}^i_{\text{lower}}$ and $\mathcal{G}^i_{\text{upper}}$ stand for the lower and upper bounding system of the system $\mathcal{F}^i$ (for $i=1,2$).

Consider first the system $\mathcal{F}^1$. Using the Kamke conditions, it is easy to check that with a positive p_2 this system is not monotone with respect to any orthant. Hence, we need to bound the term $p_2 x_1$ by constants in order to obtain monotone bounding systems in the order $\succeq_x$ endowed by the orthant $\mathrm{diag}([-1,\ 1,\ 1]\mathbb{R}^3_{\geq 0})$. By simulating the system we observe that x_1 lies in a bounded interval between 0 and $z_0^1(1)$, where $z_0^1(1)$ is the first component of the initial point z_0^1. Hence, we can build a lower $\mathcal{G}^1_{\text{lower}}$ and an upper $\mathcal{G}^1_{\text{upper}}$ bounding system for the nominal one $\mathcal{F}^1$ in the order $\succeq_x$. We take the system $\mathcal{G}^1_{\text{lower}}$ with the same parameter values as the nominal one except for p_2, which is equal to zero, and p_1 equal to $0.1z_0^1(1)$. Similarly, we choose the system $\mathcal{G}^1_{\text{upper}}$ with $p_2=0$, and $p_1=0$. The results can be seen in the upper panel of Figure 3. Note that the switching separatix for $\mathcal{F}^1$ appears to be a monotone curve, even though this property cannot be guaranteed. However, this can be guaranteed for the separatrices of the bounding systems, which are monotone on a specific domain.

Now let us compute the bounds on the switching separatrix of the nominal system $\mathcal{F}^2$, where the Michaelis-Menten term $(x_1/(x_1+1))$ prevents the system from being monotone. In a similar fashion as for the case of $\mathcal{F}^1$, we can build a lower $\mathcal{G}^2_{\text{lower}}$ and an upper $\mathcal{G}^2_{\text{upper}}$ bounding system for $\mathcal{F}^2$. This results in the switching separatrices depicted in the lower panel of Figure 3. The bounds on the switching separatrix for $\mathcal{F}^2$ are tighter in comparison with the bounds of the switching separatrix for $\mathcal{F}^1$. Note that in the case of the system $\mathcal{F}^1$, we use the following bound $0 \leq x_1 \leq z_0^1(1)$, while in the case of the system $\mathcal{F}^2$, we use the bound $0 \leq \frac{x_1}{x_1+1} \leq \frac{z_0^2(1)}{z_0^2(1)+1}$. At the same time the numbers $z_0^1(1)$,

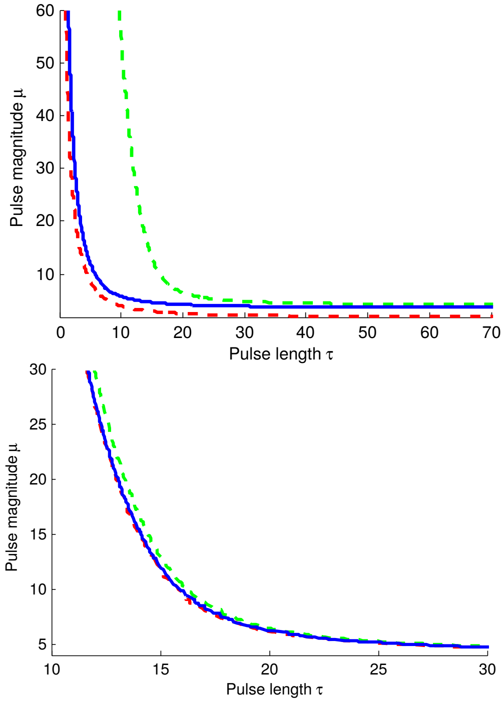

Fig. 3. The switching separatrices (solid blue curves) for systems $\mathcal{F}^1$ (the upper panel) and $\mathcal{F}^2$ (the lower panel) with separatrices for upper (dashed red curves) and lower (dashed green curves) bounding systems.

$z_0^2(1)$ are of order $O(10^3)$, hence clearly $\frac{z_0^2(1)}{z_0^2(1)+1} \ll z_0^1(1)$. This means that the bounds on the vector field of $\mathcal{F}^2$ are tighter than the bounds on the vector field of $\mathcal{F}^1$. This in turn indicates that tighter bounds on the vector field entail tighter bounds on the separatrix.

V. Conclusion and Discussion

In this paper, a framework for shaping pulses to control biological systems is presented. We considered a problem of switching from one stable steady state to another in a bistable system. We have shown that it is possible to estimate pulses, which fulfil the objective, if the flow of the controlled system can be bounded from above and below by the flows of monotone systems. This result can be generalised to systems parametric uncertainty under some mild assumptions on the set of admissible parameters. Hence, even with infinite sampling time (that is *open-loop control*) some robustness guarantees can be provided.

We further specify a number of future research directions:

Experimental validation of the theoretical results. The main application in mind for the experimental validation is synthetic biology. For example, we will consider the problem of toggling a genetic toggle switch using shaped pulses as control inputs. However, the approach can potentially be applicable to other biochemical and biomedical systems.

Relaxing the conditions of the main results. The main result uses only sufficient conditions for finding the bounding systems. Hence, an interesting direction of research is to find the closest monotone system in a given order. Secondly, the conditions for monotonicity of the switching separatrix are also only sufficient and are restrictive. Describing a set of non-monotone systems for which the switching separatrix is monotone is another direction of research.

Extension to the stochastic case. The stochastic case seems to be more relevant for the application of the framework to synthetic biology. Hence, we need to consider stochastically monotone Markov decision processes. Work in this direction has begun in [19].

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