Reprogramming cooperative monotone dynamical systems*

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Abstract— **Multistable dynamical systems are ubiquitous in nature, especially in the context of regulatory networks controlling cell fate decisions, wherein stable steady states correspond to different cell phenotypes. In the past decade, it has become experimentally possible to "reprogram" the fate of a cell by suitable externally imposed input stimulations. In several of these reprogramming instances, the underlying regulatory network has a known structure and often it falls in the class of cooperative monotone dynamical systems. In this paper, we therefore leverage this structure to provide concrete guidance on the choice of inputs that reprogram a cooperative dynamical system to a desired target steady state. Our results are parameter-independent and therefore can serve as a practical guidance to cell-fate reprogramming experiments.**

I. INTRODUCTION

Multistability, that is, the co-existence of multiple asymptotically stable steady states, is a common feature of many dynamical systems, especially of those capturing the dynamics of gene regulatory networks (GRNs) implicated in cell fate decisions [1]. In these systems, each stable steady state typically represents one specific cell phenotype, such as skin, blood, or pluripotent cell types, and transitions from less differentiated to more specialized phenotypes are orchestrated in the natural process of cell differentiation [2]. For decades, a popular metaphor due to Waddington [3] was used to explain the concept that the process of cell differentiation is irreversible: a ball (the cell phenotype) rolls down a hill under the effect of gravity starting from the top of the hill (pluripotent stem cell type) and ending in the lowest basins (terminally differentiated cells).

It was only in recent years that ground-breaking experiments demonstrated that the process can actually be reversed [4], although with very low efficiency [5], and that cell types can also be interconverted [6], that is, the fate of a cell can be reprogrammed [7]. In reprogramming practices, external (positive or negative) stimulations are applied to select nodes of a GRN, by increasing the rate of production of the transcription factor (TF) in the node (most common approach) or by enhancing its degradation [8]. Selecting the nodes where the input stimulation needs to be applied and the required stimulation type (positive or negative) for triggering a desired state transition typically relies on biological intuition and on trial-and-error experiments [9].

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Many GRNs involved in important cell fate decisions have been experimentally characterized, such that at least the topology of the network is known [7], [10], [11], [12]. Examples include the so-called fully connected triad [10], describing the core pluripotency network controlling maintenance of pluripotency; the PU.1/GATA1 network [12] controlling transition to the myeloid lineage or to the erythroid lineage from the multipotent common myeloid progenitor cell type; and more extended regulatory networks in which these core motifs are included (see [13], for example). It turns out that these core network motifs belong to the class of monotone dynamical systems (cooperative or competitive) [14] or can be decomposed into interconnection of monotone systems [15], [16]. In particular, the pluripotency network (see [17]) and the PU.1/GATA1 network, as we demonstrate in this paper, belong to the class of generalized cooperative systems [14].

Theoretical studies of multistability in monotone dynamical systems have appeared before, most notably in the works of [18], [19], [20], which provide easily checkable graphical conditions for characterizing global stability behavior and apply these general checks to biological systems. Apart from these theoretical works, most of the available studies of multistability typically take a computational approach through either bifurcation tools [21] or through sampling-based methods to determine parameter conditions for a desired stability landscape [22], [23]. Multi-stability of specific systems such as the pluripotency network and the PU.1/GATA1 network has been subject of a number of studies in the systems biology literature [11], [12]. These works investigate parameter conditions under which the system under study can be bistable or tristable, and some of these also study how some input parameters can be transiently changed in order to trigger a transition between the steady states. The approaches used in these studies commonly rely on graphical methods, such as nullcline analysis for systems in two dimensions, bifurcation analysis of one parameter at the time, and computational simulation to explore parameter spaces with sampling-based methods.

In this paper we focus on the class of generalized cooperative dynamical systems with inputs and address the question of what nodes need to be stimulated with what input (positive or negative) to trigger a transition to a desired target stable steady state. In particular, we leverage the theory of generalized cooperative dynamical systems [14] to provide general criteria based only on system's structure (as opposed to parameter values) and input type (positive or negative) to select appropriate stimulation for a given state reprogramming task. To this end, the paper is organized as

follows. In Section II, we describe the PU.1/GATA1 network as a motivating example. In Section III, we formally define generalized cooperative monotone dynamical systems, and state the problem definition. In Section IV, we present our results, and apply them to the PU.1/GATA1 network in Section V. Finally, in Section VI, we present our conclusions.

II. MOTIVATING EXAMPLE

We consider the interaction network between transcription factors PU.1 and GATA1, known to be the core network controlling lineage specification of hematopoietic stem cells (HSCs), which give rise to all the blood cells [12]. PU.1 and GATA1 mutually repress each other, while also undergoing self-activation. This interaction network motif is shown in Fig. 1A. The motif results in three stable steady states: one characterized by a high concentration of PU.1 and a low concentration of GATA1, which corresponds to the myeloid lineage; one characterized by a low concentration of PU.1 and a high concentration of GATA1, which corresponds to the erythrocyte lineage; and one characterized by an intermediate level of PU.1 and GATA1, which corresponds to the progenitor cell.

Multiple ordinary differential equation (ODE) models that capture these interactions and give rise to tristability have been proposed [24]. For the purpose of this example, we use the following Hill function based description of the system:

$$\dot{x}_1 = \frac{\beta_1 + \alpha_1 (x_1/k_1)^{n_1}}{1 + (x_1/k_1)^{n_1} + (x_2/k_2)^{n_2}} - \gamma_1 x_1,$$
$$\dot{x}_2 = \frac{\beta_2 + \alpha_2 (x_2/k_3)^{n_3}}{1 + (x_2/k_3)^{n_3} + (x_1/k_4)^{n_4}} - \gamma_2 x_2. \quad (1)$$

Here, x_1 and x_2 are the concentrations of the two species, PU.1 and GATA1, β_1, β_2 are the rate constants of leaky expression of the species, α_1, α_2 are the activation rate constants, k_1, k_2, k_3 and k_4 are the apparent dissociation constants, n_1, n_2, n_3 and n_4 are the Hill function coefficients, and γ_1, γ_2 are the decay rate constants of the species.

This ODE model, for certain parameter values, is tristable (with three stable steady states, and two unstable steady states). The nullclines and steady states for such a tristable system are shown in Fig. 1B. Here, steady states S_1 and S_2 represent the differentiated states, the erythrocyte lineage and the myeloid lineage, respectively. The state S_0 represents the undifferentiated progenitor cell. The key question for reprogramming cells (converting one cell type to another using external inputs) is then a question of reachability of these different steady states. In particular, we consider constant external inputs such that the trajectory of the system under this input converges inside the region of attraction of the desired steady state. Once this external input is removed, the system's trajectory then converges to this steady state. The question we ask, then, is when such an input exists, that can trigger a transition to a given steady state, for example S_0, starting from either a particular initial state (such as S_1 or S_2) or from any initial state, and further, what this input is. For a specific 2D system as in eqn. (1), it is
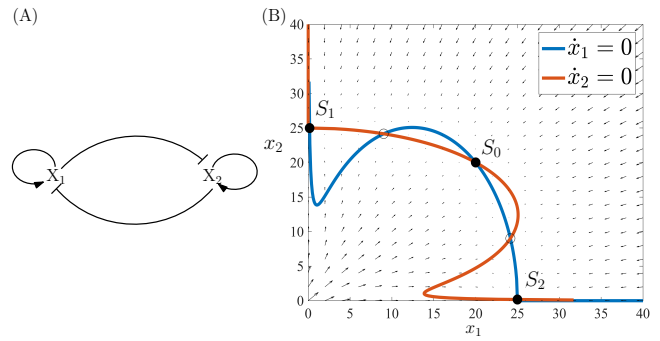

Fig. 1: The PU.1-GATA1 system. (A) The interaction graph between the two species: PU.1 denoted here as X_1, and GATA1 denoted here as X_2. Each species represses the other, while also self-regulating in the form of self-activation. (B) The nullclines of system (1), steady states (stable represented by filled and unstable by empty circles) and the vector-field. Steady state S_1 with high GATA1 and low PU.1 represents the erythrocyte lineage, steady state S_2 with low GATA1 and high PU.1 represents the myeloid lineage, and the intermediate steady state S_0 represents the progenitor state. The parameter values used are: $\alpha_1 = \alpha_2 = 5$ nM/s, $\beta_1 = \beta_2 = 5$ nM/s, $k_1 = k_3 = 1$ nM, $k_2 = k_4 = 2$ nM, $\gamma_1 = \gamma_2 = 5$ s^{-1}, $n_1 = n_2 = n_3 = n_4 = 2$.

possible to gain insight into these questions using geometric intuition from nullcline analysis. However, the way in which these nullclines change with parameters may be non-trivial, and hence it may be difficult to obtain a definite answer. For systems with dimension higher than two, geometric intuition is often not possible. Therefore we seek a strategy for selecting the appropriate inputs for reprogramming based on the structure of the underlying network (and not specific parameter values) and valid for high-dimensional systems. To this end, we consider the reprogramming problem for multi-stable, cooperative monotone dynamical systems, of which the PU.1/GATA1 network of Fig1(A) is an example. The next section formally defines these terms.

III. BACKGROUND: SYSTEM AND PROBLEM DEFINITION

A. Cooperative monotone dynamical systems

This section formally defines cooperative monotone dynamical systems. We first define a partial order " $\leq$ " to compare two vectors in $\mathbb{R}^n$. We then use this definition of a partial order to define a cooperative monotone dynamical system. These systems describe some commonly occurring multi-stable biological network motifs. They have properties that allow geometric reasoning to be used to obtain strong results on reprogrammability, and further, are easily recognized by their graphical structure.

Definition 1: A partial order $\leq$ on a set S is a binary relation that is reflexive, antisymmetric, and transitive. That is, for all $a, b, c \in S$, the following are true:

(i) Reflexivity: $a \leq a$.
(ii) Antisymmetry: $a \leq b$ and $b \leq a$ implies that $a = b$.
(iii) Transitivity: $a \leq b$ and $b \leq c$ implies that $a \leq c$.
Examples. On the set $S = \mathbb{R}^n$, the following are partial orders:

(i) $x \leq y$ if $x_i \leq y_i$ for all $i \in \{1, ..., n\}$.

(ii) $x \leq y$ if $x_i \leq y_i$ for $i \in I_1$ and $x_j \geq y_j$ for $j \in I_2$, where $I_1 \cup I_2 = \{1, ..., n\}$.

To more easily represent the partial orders above, we introduce some notations from [14]. Let $m = (m_1, m_2, ..., m_n)$, where $m_i \in \{0, 1\}$, and

$$K_m = \{x \in \mathbb{R}^n : (-1)^{m_i} x_i \geq 0, \ 1 \leq i \leq n\}.$$

K_m is an orthant in $\mathbb{R}^n$, and generates the partial order $\leq_m$ defined by $x \leq_m y$ if and only if $y - x \in K_m$. We write $x <_m y$ when $x \leq_m y$ and $x \neq y$, and $x \ll_m y$ when $x \leq_m y$ and $x_i \neq y_i, \forall i \in \{1, ..., n\}$. Note that, for the examples above, the corresponding m is: (i) $m_i = 0 \ \forall i \in \{1, ..., n\}$, i.e., $K_m = \mathbb{R}^n_+$; (ii) $m_i = 0, \forall i \in I_1$, and $m_j = 1, \forall j \in I_2$.

We consider a system Σ_u of the form: $\dot{x} = f(x, u)$ with $x \in X \subset \mathbb{R}^n$ and $u \in U \subset \mathbb{R}^p_+$ a constant input vector. Let the flow of system Σ_u starting from $x = x_0$ be denoted by $\phi_u(t, x_0)$. The flow of the system with $u = 0$ is denoted by $\phi_0(t, x_0)$. The domain X is said to be p_m-convex if $tx + (1 - t)y \in X$ whenever $x, y \in X$, $0 < t < 1$, and $x \leq_m y$ [14].

Definition 2: System Σ_u is said to be a *cooperative monotone system* with respect to K_m if domain X is p_m-convex and

$$(-1)^{m_i + m_j} \frac{\partial f_i}{\partial x_j}(x, u) \geq 0, \forall i \neq j, \forall x \in X, \forall u \in U. \quad (2)$$

For convenience, we include Proposition 5.1 from [14] here, stated as a Lemma:

Lemma 1: [14] Let X be p_m-convex and f be a continuously differentiable vector field on X such that (2) holds. Let $<_r$ denote any one of the relations $\leq_m, <_m, \ll_m$. If $x <_r y$, $t > 0$ and $\phi_u(t, x)$ and $\phi_u(t, y)$ are defined, then $\phi_u(t, x) <_r \phi_u(t, y)$.

A cooperative monotone dynamical system is easily recognized by its graphical structure. Assume that the system Σ_u is sign-stable (i.e., $\frac{\partial f_i}{\partial x_j}(x, u)$, $i \neq j$ keeps the same sign for all $x \in X$) and sign-symmetric (i.e., $\frac{\partial f_i}{\partial x_j} \frac{\partial f_j}{\partial x_i} \geq 0$ for all $x \in X$). We consider the graph G corresponding to system Σ_u with n nodes where an undirected edge connects two nodes i, j if at least one of $\frac{\partial f_i}{\partial x_j}$ or $\frac{\partial f_j}{\partial x_i}$ has a non-zero value somewhere in X. Assign a "+" or "-" sign depending on the sign of the partial derivative of the edge. Then Σ_u is cooperative in X if and only if *for every closed loop in G, the number of edges with a "-" sign is even* [14].

Consider the extended system Σ'_u:

$$\dot{x} = f(x, u), \quad \dot{u} = 0, \quad (3)$$

with states $x \in X \subset \mathbb{R}^n$ and $u \in U \subset \mathbb{R}^p_+$. Since $\dot{u} = 0$, the trajectories $x(t)$ for this system with $u(0) = u_0$ are the same as that of the original system $\Sigma_{u_0} : \dot{x} = f(x, u_0)$.

Lemma 2: If system $\Sigma_u : \dot{x} = f(x, u)$ is cooperative with respect to K_m for a fixed u, then the extended system Σ'_u is cooperative with respect to $K_m \times K_{m'}$, where $m' = (m'_1, m'_2, ..., m'_p)$ and $m'_k \in \{0, 1\}$, if and only if $\forall i \in \{1, ..., n\}, \forall k \in \{1, ..., p\}, \forall x \in X, \forall u \in U$:

$$(-1)^{m_i + m'_k} \frac{\partial f_i}{\partial u_k}(x, u) \geq 0.$$

Proof: For this extended system with state $(x, u)^T \in \mathbb{R}^{n+p}_+$ to be cooperative, the following must be true for all $x \in X$ and $u \in U$ according to condition (2) for cooperativity:

$$(-1)^{m_i + m_j} \frac{\partial f_i}{\partial x_j}(x, u) \geq 0, \forall i \neq j \in \{1, ..., n\}. \quad (4)$$

$$(-1)^{m_i + m'_k} \frac{\partial f_i}{\partial u_k}(x, u) \geq 0, \forall i \in \{1, .., n\}, \forall k \in \{1, .., p\}. \quad (5)$$

Since the system Σ_u is cooperative with respect to K_m, condition (4) is satisfied. ∎

Corollary 1: Consider the case where the function $f(x, u)$ takes the form $f = (f_1(x, u_1), .., f_i(x, u_i), .., f_n(x, u_n))$, i.e., each state x_i is given a single input u_i, and thus, $p = n$. The extended system is cooperative with respect to $K_m \times K_{m'}$, where $m' = (m'_1, ..., m'_n)$ satisfies the following. If $\frac{\partial f_i}{\partial u_i} \geq 0$ (positive stimulation), then $m'_i = m_i$. If $\frac{\partial f_i}{\partial u_i} \leq 0$ (negative stimulation), then $m'_i = 1 - m_i$.

B. Problem definition: Reprogrammability of multi-stable systems

We consider a dynamical system Σ_u of the form:

$$\dot{x} = f(x, u), \quad (6)$$

where state $x \in X \subset \mathbb{R}^n$ and a constant input vector $u \in U \subset \mathbb{R}^n_+$. Let $\mathbb{S}$ be the set of stable steady states of the system $\Sigma_0 : \dot{x} = f(x, 0)$. Further, we let $\mathcal{R}_u(S)$ denote the region of attraction of a stable steady state S of system Σ_u. The region of attraction $\mathcal{R}_u(S)$ is the set of all states x such that $\lim_{t \to \infty} \phi_u(t, x) = S$ [25].

We define two concepts of reprogrammability. For system Σ_0 to be *strongly reprogrammable* to a steady state $S^0 \in \mathbb{S}$, there must exist an input u such that a trajectory of Σ_u starting from *any* initial condition, must converge inside the region of attraction (defined with respect to Σ_0) of S^0. When the input is removed, then, the system's trajectory converges to the desired steady state S^0. We say that the system Σ_0 is *weakly reprogrammable* to a steady state S from another steady state $\bar{S}$ if there exists an input u such that a trajectory of Σ_u starting from $\bar{S}$ converges to the region of attraction of S, defined with respect to Σ_0. These two concepts are formalized below in Definitions 3 and 4.

Definition 3: We say that system Σ_0 is *strongly reprogrammable* to a steady state $S \in \mathbb{S}$ provided there is an input $u \in U$ such that for system Σ_u, for all $x_0 \in \mathbb{R}^n_+$, the omega-limit set $\omega_u(x_0)$ is such that $\omega_u(x_0) \subset \mathcal{R}_0(S)$.

Definition 4: We say that system Σ_0 is *weakly reprogrammable* to a steady state $S \in \mathbb{S}$ from a steady state $\bar{S} \in \mathbb{S}$, with $S \neq \bar{S}$, provided there exists a $u \in U$ such that the omega-limit set $\omega_u(\bar{S})$ is such that $\omega_u(\bar{S}) \subset \mathcal{R}_0(S)$.

To state our results about reprogrammability for cooperative, monotone dynamical systems, we make the following assumptions on Σ_u.

Assumption 1: The function $f(x, u)$ is C^1 continuous. The trajectories $x(t)$ of Σ_u are bounded for any given u and for all $t \geq 0$.

Assumption 2: The system Σ_u is a monotone cooperative system with respect to some K_m, as defined in Definition 2.

Assumption 3: The function $f(x, u)$ takes the form $f(x, u) = (f_1(x, u_1), .., f_i(x, u_i), .., f_n(x, u_n))$, i.e., each state x_i takes a single input u_i.

Assumption 4: The system Σ_0 takes the form: $f_i(x, 0) = H_i(x) - \gamma_i x$, where $H_i(x) \in C^1$, $0 < H_i(x) \leq H_{iM}, \forall x \in X$, and γ_i is a positive constant. Inputs to the system are given as follows. For positive stimulation, $f_i(x, u_i) = H_i(x) - \gamma_i x + u_i$. For negative stimulation, $f_i(x, u_i) = H_i(x) - (\gamma_i + u_i)x$.
Note that when system Σ_u satisfies Assumption 4, it also satisfies Assumption 1. Since $H_i(x) \in C^1$, the function $f_i(x, u_i) \in C^1$, and therefore $f(x, u)$ is C^1 continuous. Further, for positive stimulation, when $x_i > \frac{H_{iM} + u_i}{\gamma_i}$, $\dot{x}_i < 0$. Thus, $x_i(t) \leq \max(\frac{H_{iM} + u_i}{\gamma_i}, x_i(0))$ for all $t \geq 0$. Similarly, for negative stimulation, $x_i(t) \leq \max(\frac{H_{iM}}{\gamma_i + u_i}, x_i(0))$ for all $t \geq 0$. Since $x \in \mathbb{R}_+^n$, $x_i(t) \geq 0$. Thus, the trajectories of the system Σ_u are bounded for any given u and for all $t \geq 0$.

IV. RESULTS

This section states results about the reprogrammability of steady states in cooperative monotone dynamical systems. The question we wish to address is: for each steady state in $\mathbb{S}$, what inputs, if any, make the system strongly reprogrammable to that steady state, and what inputs, if any, make a given steady state weakly reprogrammable to another given steady state. We first show that the set of steady states of Σ_0, $\mathbb{S}$, has a minimum and a maximum. We then present theorems that provide a strategy for selecting the inputs required to strongly reprogram system Σ_0 to these minimal and maximal steady states of Σ_0. Further, our results rule out certain key input types to strongly reprogram system Σ_0 to other intermediate steady states. Based on this set of results, possible strategies are proposed to reprogram system Σ_0 to intermediate steady states. To present our results, we first define the following exhaustive list of mutually exclusive input types:

(i) Input of **type 1**: An input of type 1 satisfies the following: for all $i \in \{1, ..., n\}$, if $m_i = 0$ then $\partial f_i/\partial u_i \geq 0$ (positive/no stimulation), and if $m_i = 1$ then $\partial f_i/\partial u_i \leq 0$ (negative/no stimulation). Further, for at least one i, $\partial f_i/\partial u_i$ is not identically 0 everywhere.

(ii) Input of **type 2**: An input of type 2 satisfies the following: for all $i \in \{1, ..., n\}$, if $m_i = 1$ then $\partial f_i/\partial u_i \geq 0$ (positive/no stimulation), and if $m_i = 0$ then $\partial f_i/\partial u_i \leq 0$ (negative/no stimulation). Further, for at least one i, $\partial f_i/\partial u_i$ is not identically 0 everywhere.

(iii) Input of **type 3**: An input such that, there exists at least one $i \in \{1, ..., n\}$ such that if $m_i = 0$, $\partial f_i/\partial u_i \geq 0$ and if $m_i = 1$, $\partial f_i/\partial u_i \leq 0$ (and $\partial f_i/\partial u_i$ not identically 0 everywhere); and at least one $j \in \{1, ..., n\}$ such that if $m_j = 0$, $\partial f_j/\partial u_j \geq 0$ and if $m_j = 1$, $\partial f_j/\partial u_j \leq 0$ (and $\partial f_j/\partial u_j$ not identically 0 everywhere).

Lemma 3: Under Assumptions 1 and 2, the set of steady states $\mathbb{S}$ of system Σ_0 has a minimum and a maximum with respect to the partial order $\leq_m$.

Proof: We first prove that the set $\mathbb{S}$ has a maximum with respect to $\leq_m$. Let $\bar{x} \in X$ be such that $\bar{x} \geq_m S$ for all $S \in \mathbb{S}$. Then, by assumption 2, $\omega_0(\bar{x}) \geq_m S$ for all $S \in \mathbb{S}$, under Lemma 1. Since for a cooperative monotone dynamical system that is bounded, by Proposition 2.1 from [14], $\omega_0(\bar{x})$ is a steady state, therefore, $\omega_0(\bar{x}) \in \mathbb{S}$. Thus, $\omega_0(\bar{x}) = \max(\mathbb{S})$, and therefore $\mathbb{S}$ has a maximum. To prove that the set has a minimum, let $\bar{x} \in X$ be such that $\bar{x} \leq_m S$ for all $S \in \mathbb{S}$. Then, similar to the reasoning above, $\omega_0(\bar{x}) \leq_m S$ for all $S \in \mathbb{S}$, and $\omega_0(\bar{x}) \in \mathbb{S}$. Thus, $\mathbb{S}$ has a minimum. ∎

Remark: Recall that $x \leq_m y$ implies that for states where $m_i = 0$, $x_i \leq y_i$, and for states where $m_i = 1$, $x_i \geq y_i$. Thus, a maximum S^* with respect to the partial order $\leq_m$ is such that, for states where $m_i = 0$, $S_i^* = \max_{S \in \mathbb{S}}(S_i)$, and for states where $m_i = 1$, $S_i^* = \min_{S \in \mathbb{S}}(S_i)$. Here, S_i denotes the ith component of the steady state S. Similarly, a minimum S^{**} with respect to the partial order $\leq_m$ is such that, for states where $m_i = 0$, $S_i^{**} = \min_{S \in \mathbb{S}}(S_i)$, and for states where $m_i = 1$, $S_i^{**} = \max_{S \in \mathbb{S}}(S_i)$.

In the next two results, we show that inputs of type 1 and 2 can never make system Σ_0 strongly reprogrammable to an intermediate steady state.

Theorem 1: Under Assumptions 1, 2 and 3, for any input of type 1, system Σ_0 is not strongly reprogrammable to any steady state $S \neq \max(\mathbb{S})$.

Proof: Consider the extended system Σ_u': $\dot{x} = f(x, u), \dot{u} = 0$ with an input of type 1. Then, by Corollary 1, we see that the extended system is a monotone cooperative system with respect to $K_m \times K_{(0,0,...,0)} = K_m \times \mathbb{R}_+^n$. We denote the corresponding partial order by $\leq_{m+}$. For this extended system, we consider the trajectories originating from the following initial conditions: $(\max(\mathbb{S}), 0)$ and $(\max(\mathbb{S}), u_0)$, where $u_0 \in \mathbb{R}_+^n$. Note that these initial conditions are ordered, i.e., $(\max(\mathbb{S}), 0) \leq_{m+} (\max(\mathbb{S}), u_0)$. Since $(\max(\mathbb{S}), 0)$ is a steady state of the extended system, by the cooperativity of the extended system, we have that $\max(\mathbb{S}) \leq_m \phi_{u_0}(t, \max(\mathbb{S}))$, under Lemma 1. Hence, $\omega_{u_0}(\max(\mathbb{S})) \geq_m \max(\mathbb{S})$.

We now consider the system Σ_0: $\dot{x} = f(x, 0)$, starting at an initial condition $z \geq_m \max(\mathbb{S})$. By the cooperativity of Σ_0, we have that $\omega_0(z) \geq_m \max(\mathbb{S})$, under Lemma 1. Since $\omega_0(z) \in \mathbb{S}$, we have that $\omega_0(z) = \max(\mathbb{S})$. Thus, for any $z \geq_m \max(\mathbb{S})$, $z \in \mathcal{R}_0(\max(\mathbb{S}))$. Thus, $\omega_{u_0}(\max(\mathbb{S})) \in \mathcal{R}_0(\max(\mathbb{S}))$. That is, for the system Σ_u with an input of type 1, any trajectory starting at $\max(\mathbb{S})$ will converge to a steady state in the region of attraction (for Σ_0) of $\max(\mathbb{S})$. Thus, Σ_0 is not strongly reprogrammable to any steady state other than $\max(\mathbb{S})$, since there exists an x_0 such that $\omega_u(x_0) \notin \mathcal{R}_u(S), \forall S \neq \max(\mathbb{S})$. ∎

Theorem 2: Under Assumptions 1, 2 and 3, for any input of type 2, system Σ_0 is not strongly reprogrammable to any steady state $S \neq \min(\mathbb{S})$.

Proof: Consider the extended system Σ_u': $\dot{x} =$

$f(x, u), \dot{u} = 0$ with an input of type 2. Then, by Corollary 1, we see that the extended system is a monotone cooperative system with respect to $K_m \times K_{(1,1,...,1)} = K_m \times \mathbb{R}^n_-$. We denote the corresponding partial order by $\leq_{m-}$. For this extended system, we consider the trajectories originating from the following initial conditions: $(\min(\mathbb{S}), 0)$ and $(\min(\mathbb{S}), u_0)$, where $u_0 \in \mathbb{R}^n_+$. Note that these initial conditions are ordered, i.e., $(\min(\mathbb{S}), 0) \geq_{m-} (\min(\mathbb{S}), u_0)$. Since $(\min(\mathbb{S}), 0)$ is a steady state of the extended system, by the cooperativity of the extended system, we have that $\min(\mathbb{S}) \geq_m \phi_{u_0}(t, \min(\mathbb{S}))$, under Lemma 1. Hence, $\omega_{u_0}(\min(\mathbb{S})) \leq_m \min(\mathbb{S})$.

We now consider the system Σ_0: $\dot{x} = f(x, 0)$, starting at an initial condition $z \leq_m \min(\mathbb{S})$. By the cooperativity of Σ_0, we have that $\omega_0(z) \leq_m \min(\mathbb{S})$, under Lemma 1. Since $\omega_0(z) \in \mathbb{S}$, we have that $\omega_0(z) = \min(\mathbb{S})$. Thus, for any $z \leq_m \min(\mathbb{S})$, $z \in \mathcal{R}_0(\min(\mathbb{S}))$. Thus, $\omega_{u_0}(\min(\mathbb{S})) \in \mathcal{R}_0(\min(\mathbb{S}))$. That is, for the system Σ_u with an input of type 2, any trajectory starting at $\min(\mathbb{S})$ will converge to a steady state in the region of attraction (for Σ_0) of $\min(\mathbb{S})$. Thus, Σ_0 is not strongly reprogrammable to any steady state other than $\min(\mathbb{S})$, since there exists an x_0 such that $\omega_u(x_0) \notin \mathcal{R}_u(S)$, $\forall S \neq \min(\mathbb{S})$. ∎

Lemma 4: For system Σ_u satisfying Assumption 4, consider the dynamics of a node with positive stimulation: $\dot{x}_i = H_i(x) + u_i - \gamma_i x$, and $u_i \geq 2H_{iM}$. Then, $\lim_{t \to \infty} x_i(t) \geq \max_{S \in \mathbb{S}}(S_i)$ independent of the initial condition.

Proof: See [17]. ∎

Lemma 5: For system Σ_u satisfying Assumption 4, consider the dynamics of a node with negative stimulation: $\dot{x}_i = H_i(x) - (\gamma_i + u_i)x_i$, and $u_i \geq \frac{H_{iM}}{\min_{S \in \mathbb{S}}(S_i)} - \gamma_i$. Then, $\lim_{t \to \infty} x_i(t) \leq \min_{S \in \mathbb{S}}(S_i)$ independent of the initial condition.

Proof: Consider the following systems: $\dot{z}_i = -(\gamma_i + u_i)z_i$ and $\dot{\tilde{x}}_i = H_i(\tilde{x}) - (\gamma_i + u_i)\tilde{x}_i$. The second system can be veiwed as the perturbed version of the first system, with $H_i(\tilde{x})$ being the disturbance, which is globally bounded by H_{iM}. Then, we can apply the robustness result from contraction theory [26] to obtain: $\lim_{t \to \infty} |\tilde{x}_i(t) - 0| \leq \frac{H_{iM}}{\gamma_i + u_i}$. Since $\tilde{x}_i(t) \geq 0$ and $u_i \geq \frac{H_{iM}}{\min_{S \in \mathbb{S}}(S_i)} - \gamma_i$, we have that $\lim_{t \to \infty} \tilde{x}_i(t) \leq \min_{S \in \mathbb{S}}(S_i)$. Note that since under Assumption 4, $H_i(x) > 0$, $S_i \neq 0$ for any i and any S, since $f_i(x, 0) = H_i(x) - \gamma_i x_i = H_i(x) \neq 0$ for $x_i = 0$. Thus, $\min_{S \in \mathbb{S}}(S_i) \neq 0$. ∎

In Theorem 3, we show that large enough inputs of type 1 can make Σ_0 strongly reprogrammable to $\max(\mathbb{S})$, and inputs of type 2 can make Σ_0 strongly reprogrammable to $\min(\mathbb{S})$.

Theorem 3: Under Assumptions 2, 3 and 4, a sufficiently large input of type 1 ensures that Σ_0 is strongly reprogrammable to the steady state $\max(\mathbb{S})$, and a sufficiently large input of type 2 ensures that Σ_0 is strongly reprogrammable to the steady state $\min(\mathbb{S})$.

Proof: Consider a $\underline{u}$ such that $\underline{u}_i = 2H_{iM}$ if $m_i = 0$, and $\underline{u}_i = \frac{H_{iM}}{\min_{S \in \mathbb{S}}(S_i)} - \gamma_i$ for $m_i = 1$. Then, using Lemma 4, we have that for $m_i = 0$, $\lim_{t \to \infty} x_i(t) \geq \max_{S \in \mathbb{S}}(S_i)$

for all $x_i(0)$. Using Lemma 5, we have that for $m_i = 1$, $\lim_{t \to \infty} x_i(t) \leq \min_{S \in \mathbb{S}}(S_i)$ for all $x_i(0)$. Note that if x, y are such that for a state where $m_i = 0$, $x_i \leq y_i$, and for a state where $m_i = 1$, $x_i \geq y_i$, then $x \leq_m y$. Thus, $\omega_u(x_0) \geq_m \max(\mathbb{S})$, $\forall x_0$ and $\forall u \geq \underline{u}$ with an input of type 1. By monotonicity, if $z \geq_m \max(\mathbb{S})$, $\omega_0(z) = \max(\mathbb{S})$. Thus, $\omega_u(x_0) \subset \mathcal{R}_0(\max(\mathbb{S}))$ $\forall x_0$. Thus, Σ_0 is strongly reprogrammable to $\max(\mathbb{S})$.

Consider a $\underline{u}$ such that $\underline{u}_i = 2H_{iM}$ if $m_i = 1$, and $\underline{u}_i = \frac{H_{iM}}{\min_{S \in \mathbb{S}}(S_i)} - \gamma_i$ for $m_i = 0$. Then, using Lemma 4, we have that for $m_i = 1$, $\lim_{t \to \infty} x_i(t) \geq \max_{S \in \mathbb{S}}(S_i)$ for all $x_i(0)$. Using Lemma 5, we have that for $m_i = 0$, $\lim_{t \to \infty} x_i(t) \leq \min_{S \in \mathbb{S}}(S_i)$ for all $x_i(0)$. Using the same reasoning as above, we have that $\omega_u(x_0) \leq_m \min(\mathbb{S})$, $\forall x_0$ and $\forall u \geq \underline{u}$ with an input of type 2. Under Lemma 1, if $z \leq_m \min(\mathbb{S})$, $\omega_0(z) = \min(\mathbb{S})$. Thus, $\omega_u(x_0) \subset \mathcal{R}_0(\min(\mathbb{S}))$ $\forall x_0$. Thus, Σ_0 is strongly reprogrammable to $\min(\mathbb{S})$. ∎

Finally, in Theorem 4, we analyze the weak reprogrammability of Σ_0 to intermediate steady states using inputs of type 1 and type 2.

Theorem 4: Consider two steady states $S, \bar{S} \in \mathbb{S}$ such that $S <_m \bar{S}$. Let system Σ_u satisfy assumptions 2, 3 and 4. Then the following is true:

(a) *Σ_0 is not weakly reprogrammable to $\bar{S}$ from S for any input of type 2,*

(b) *There exist a $u_1, u_2 \in \mathbb{R}^n_+$ such that for an input u of type 1 with $u \leq u_1$ or $u \geq u_2$, Σ_0 is not weakly reprogrammable to $\bar{S}$ from S if $\bar{S} \neq max(\mathbb{S})$,*

(c) *Σ_0 is not weakly reprogrammable to S from $\bar{S}$ for any input of type 1, and*

(d) *There exist a $\bar{u}_1, \bar{u}_2 \in \mathbb{R}^n_+$ such that for an input u of type 2 with $u \leq \bar{u}_1$ or $u \geq \bar{u}_2$, Σ_0 is not weakly reprogrammable to S from $\bar{S}$ if $S \neq min(\mathbb{S})$.*

Proof: (a) Consider the extended system Σ'_u: $\dot{x} = f(x, u), \dot{u} = 0$ with an input of type 2. Then, by Corollary 1, we see that the extended system is a monotone cooperative system with respect to $K_m \times K_{(1,1,...,1)} = K_m \times \mathbb{R}^n_-$. We denote the corresponding partial order by $\leq_{m-}$. Then, $(S, 0) \geq_{m-} (S, u)$ for all $u \in \mathbb{R}^n_+$, under Lemma 1. Thus, $\phi_0(t, S) = S \geq_m \phi_u(t, S)$ for all $u \in \mathbb{R}^n_+$. Thus, $\omega_u(S) \leq_m S$, and therefore $\omega_u(S) \notin \mathcal{R}_0(\bar{S})$ since $\bar{S} \geq_m S$, for all u. Thus, for an input of type 2, Σ_0 is not weakly reprogrammable to $\bar{S}$ from S.

(b) Consider Σ_u with u small. Since S is a stable equilibrium for Σ_0, it follows that $\frac{\partial f(x,u)}{\partial x}\Big|_{S,0}$ is Hurwitz and hence non-singular. Since it is a continuous function of u and x, it follows from the implicit function theorem that there is an open ball $B \subset U$ about $u = 0$ such that $\bar{x}(u)$ is a locally unique solution to $f(x, u) = 0$ for $u \in B$; furthermore $\bar{x}(u)$ is a continuous function of u. Therefore, for small u, we will have that $\bar{x}(u)$ is close to $\bar{0} = S$. We can thus pick u small enough such that $\bar{x}(u)$ is in the region of attraction of S. Therefore, there is an input $u_1 > 0$ sufficiently small such that if $u \leq u_1$ the system is not reprogrammed from S to $\bar{S}$. The fact that there exists a u_2 sufficiently large that if $u \geq u_2$, the system is not reprogrammed to S but in fact to $\max S$

follows from Theorem 3.

(c), (d): The proof is similar to that for (a), (b). ∎

Theorem 4 shows that inputs of type 1 and 2 are not suited to achieve even weak reprogrammability to intermediate steady states. We note that, if $u_1 > u_2$ or $\bar{u}_1 > \bar{u}_2$, there is no input of type 1 or 2 that allows weak reprogrammability to an intermediate steady state. Further, even if this were not the case, u_1, u_2, $\bar{u}_1$ and $\bar{u}_2$ depend on the parameters of the system. The success of reprogramming using such an input would therefore be highly susceptible to uncertainty in both parameters and initial states. Therefore, reprogramming to an intermediate steady state may be more promising using inputs of type 3. We note however that, as the system under consideration increases in dimension, the number of type 3 inputs would increase combinatorially with the number of dimensions. We leave this question for future work.

To summarize, we provide an intuitive explanation for why certain input types are better suited to reprogram the system to specific steady states. Inputs of type 1 are the "maximizing" inputs. For nodes where the partial order $\leq_m$ implies $x_i \leq y_i$, we apply a positive stimulation (thus attempting to increase the concentration of that node). For nodes where the partial order $\leq_m$ implies $x_i \geq y_i$, we apply a negative stimulation. Both these make it so that the new state that the system ends up in, is larger (in the sense of the partial order $\leq_m$) than the initial state. Thus, this "maximizing" input reprograms the system to the maximum steady state, when large enough. Similarly, inputs of type 2 are "minimizing" inputs, and reprogram the system to the minimum steady state. Inputs of type 3, on the other hand, are "balancing" inputs. They result in a state that is "disordered" (with respect to $\leq_m$) compared to the initial state of the system, and thus, might work to reprogram the system to intermediate steady states. In the next section, we test these results on the PU.1-GATA1 network.

V. APPLICATION OF RESULTS TO THE MOTIVATING EXAMPLE

In this section, we return to the motivating example of Section II. We apply the results of Section IV and discuss strategies for reprogramming the system to the three different steady states S_0, S_1 and S_2.

We first note that the PU-GATA network satisfies the graphical test for being a cooperative monotone dynamical network: it is sign-stable, sign-symmetric, and for every closed-loop (in this case none) in the interaction graph, the number of edges with a "-" sign is even. We further note that, since $\frac{\partial f_1}{\partial x_2}, \frac{\partial f_2}{\partial x_1} \leq 0$, the system is cooperative with respect to the cone K_m where $m = (0, 1)$. According to this cone, we see that Lemma 3 holds for the set $\mathbb{S} = \{S_0, S_1, S_2\}$ of the stable steady states of the system. We have that $\min(\mathbb{S}) = S_1$ and $\max(\mathbb{S}) = S_2$, where $\min$ and $\max$ are defined with respect to the partial order $\leq_m$.

Under Theorem 1, an input of type 1 for this system, which consists of either a positive stimulation on node 1 (X_1) or a negative stimulation on node 2 (X_2) or both, cannot strongly reprogram system (1) to any steady state besides
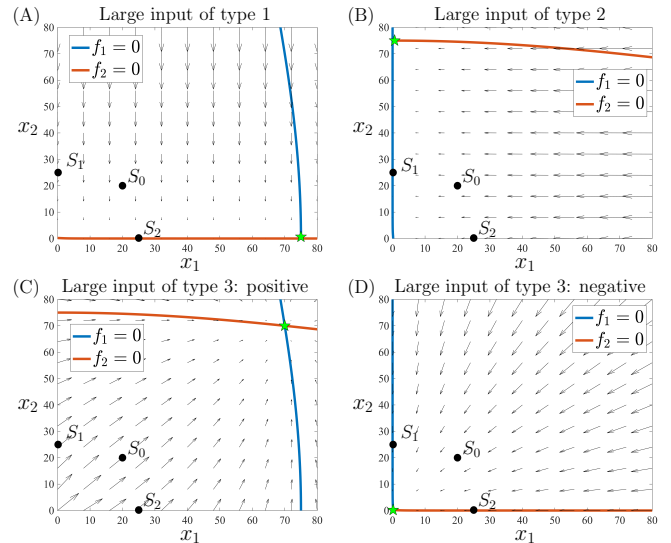

Fig. 2: Nullclines of the systems with different inputs. The new steady states are denoted by the green star. (A) Large input of type 1: the resulting globally asymptotically stable steady state has very low levels of x_2 (GATA1) and very high levels of x_1 (PU.1). The inputs are $u_1 = 10$nM/s of positive stimulation and $u_2 = 28\text{s}^{-1}$ of negative stimulation. (B) Large input of type 2: the resulting globally asymptotically has very high levels of x_2 and very low levels of x_1. The inputs are $u_1 = 28\text{s}^{-1}$ of negative stimulation and $u_2 = 10$nM/s of positive stimulation. (C) Large input of type 3: the resulting globally asymptotically stable steady state has very high (and comparable) levels of x_2 and x_1. The inputs are $u_1 = 10$nM/s and $u_2 = 10$nM/s of positive stimulation. (D) Large input of type 3: the resulting globally asymptotically stable steady state has very low levels of x_2 and x_1. The inputs are $u_1 = 28\text{s}^{-1}$ of negative stimulation and $u_2 = 28\text{s}^{-1}$ of negative stimulation.

S_2. This is because an input of type 1 causes x_1 to increase and/or x_2 to decrease, and always results in a stable steady state that lies in the region of attraction of S_2. Thus, for any input of type 1, there always exists some initial condition such that the system is reprogrammed to S_2. Thus, an input of type 1 cannot strongly reprogram the system to any steady state besides S_2. Similarly, under Theorem 2, an input of type 2, with either a negative stimulation on X_1, a positive stimulation on X_2, or both, cannot strongly reprogram system (1) to any steady state besides S_1.

Under Theorem 3, a sufficiently large input of type 1 makes system (1) strongly reprogrammable to S_2, and a sufficiently large input of type 2 makes it strongly reprogrammable to S_1. We use nullcline analysis to validate that this is indeed the case. We would like to note here that nullcline analysis is parameter dependent and any result obtained with the nullclines is specific to the parameter set used. The results that we have obtained in Section IV are *parameter independent* and therefore general as they rely only on the graph structure of the system. In Fig. 2A, we show the nullclines for the system with a large input of type 1, and show that there is one globally asymptotically stable steady state. Trajectories starting from any initial condition converge to this steady state. Since this steady state has a very high x_1 and a very low x_2, it is in the region of attraction

of S_2 (compare to Fig. 1B). The removal of this input would the cause the system's trajectory to converge to S_2. Similarly, a large input of type 2 results in the nullclines shown in Fig. 2B. The resulting system has a globally asymptotically stable steady state in the region of attraction of S_1, making the system strongly reprogrammable to S_1.

Finally, we look into reprogramming the system to the intermediate steady state S_0. By Theorems 1 and 2, inputs of type 1 and 2 cannot strongly reprogram the system to S_0. Further, while under Theorem 4, it could be possible to weakly reprogram the system from S_1 to S_0 for a specific range of inputs of type 1, and from S_2 to S_0 for a specific range of inputs of type 2, such ranges, if they do exist, would be dependent on the parameters of the system.

Therefore, instead, we investigate strong reprogrammability to S_0 using inputs of type 3. There are two possibilities for an input of type 3: either a positive stimulation on both nodes, or a negative stimulation on both nodes. We apply large inputs of type 3 to the system, and the resulting nullclines are shown in Fig. 2C (with both nodes receiving positive stimulation) and in Fig. 2D (with both nodes receiving negative stimulation). Large positive stimulation results in a globally asymptotically stable steady state with x_1 and x_2 both large (and comparable). This steady state is in the region of attraction of S_0 (compare to Fig. 1B), making the system strongly reprogrammable to S_0. However, if the difference between u_1 and u_2 were large (or the system were not symmetric), this input might still result in a steady state in the region of attraction of S_1 or S_2. On the other hand, a large negative input on both, even if the two inputs were different, results in a steady state near $(0,0)$ as long as both inputs are large enough. This steady state is in the region of attraction of S_0 (compare to Fig. 1B), making the system strongly reprogrammable to S_0. Thus, we see that both inputs of type 3 can make the system strongly reprogrammable to S_0.

VI. CONCLUSIONS AND DISCUSSION

In this work, we have shown that inputs of type 1 can strongly reprogram a cooperative monotone dynamical system to a steady state if and only if that steady state is the maximal steady state. Similarly, we showed that inputs of type 2 can achieve strong reprogrammability if and only if the desired steady state is the minimal steady state. Since strong reprogrammability implies that the system's state converges to the desired steady state independent of initial conditions, in practice, this can be interpreted to mean that a population of cells (each at a different state in the state-space) could be given the same input, and reprogrammed to the desired state (cell type). Further, we showed that there may or may not exist a range of inputs of type 1 (or type 2) that can weakly reprogram the system from S to $\bar{S}$ (or $\bar{S}$ to S) if $S <_m \bar{S}$ and $\bar{S} \neq \max(\mathbb{S})$ ($S \neq \min(\mathbb{S})$). For the PU.1/GATA1 system, we therefore considered inputs of type 3, which were successful in strongly reprogramming the system to its intermediate steady state S_0.

We would like to point to the core network responsible for pluripotency and the self-renewal of embryonic stem cells [10]. This network is cooperative and can be tristable, with the intermediate steady state corresponding to the pluripotent state [17]. The most common strategy used for reprogramming this system to pluripotency is the over-expression of key factors [5]- an input of type 1. As discussed here, such a strategy is fragile. If a range of type 1 inputs does exist that can (weakly) reprogram the system to pluripotency, it would be parameter-dependent, and possibly hard to achieve experimentally. This could be contributing to the low efficiency of current reprogramming strategies. Based on our work here, we recommend looking at inputs of type 3 to reprogram this system to pluripotency, which we leave for future work.

Like the pluripotency network and the PU.1/GATA1 network, many GRNs controlling cell-fate decisions are monotone (or decomposable into an interconnection of monotone systems) [15], [16]. This work therefore provides a parameter-independent strategy to select inputs that could achieve more efficient cellular reprogramming in cooperative monotone systems.

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