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Global stabilization of a genetic positive feedback loop via the design of a synthetic auto-repression [★]

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Abstract: Genetic positive feedback loops are essential for cell differentiation processes. They are accurately modeled with N-dimensional non-linear monotone dynamical systems that display bi-stability: the two stable fixed points represent two distinct cell differentiated states, whereas the unstable fixed point is interpreted as a cell undifferentiated state. This paper shows that the synthetic design of a simple self-inhibition of one gene in the loop is able to globally stabilize the unstable fixed point of the network. This modification may lead to a promising cell dedifferentiation process during which cells regress from a specialized state to an earlier developmental state. Compared to a similar experiment designed for the Toggle Switch, this new synthetic circuit prevents the use of any input and measurement devices, reducing greatly the complexity of the biological set-up. In order to take into account inherent biological uncertainties, the cell undifferentiated state is later considered as a region of the state space around the unstable fixed point and is shown to be globally attractive with the same simple synthetic modification of the loop. Some conditions are given such that all the possible fixed points of the circuit are confined in the undifferentiated region and the global results are proved with the theory of monotone dynamical systems.

Keywords: Gene regulatory networks, Positive feedback loop, Cooperative systems, Monotone systems, Global stability

1. INTRODUCTION

Positive feedback loops are essential and recurrent genetic motifs in living organisms. They are known to be responsible for cell differentiation processes (Lugagne et al., 2017). These mechanisms allow undifferentiated cells, called stem cells, to differentiate into any specialized cells. For few years now, many studies have been focused on the underlying mechanisms of differentiation process and stem cells due to their promising role in therapy improvements. Among these issues, the understanding and the regulation of dedifferentiation process, during which a specialized cell is able to regress to a stem cell, is of really high interest (Cai et al., 2007).

In order to tackle these problems, the control and manipulation of genetic systems have been facilitated by the great improvement of biological techniques and devices. The new synthetic biological tools allow the design and the control of new artificial systems (Gardner et al., 2000). The work recently done by Lugagne et al. (2017) is an example of synthetic biology success story: thanks to the tight

control of two inducer molecules, the authors were able to regulate the differentiation process of a two-dimensional positive feedback loop and force the cells to dedifferentiate. However, these types of control strategies often lead to tedious and expensive experiments. Indeed, control and measurement devices must be of really high quality and precision due to usual biological uncertainties. Moreover, the inherent cells specificities and heterogeneities often induce non-robust and non long-term viable strategies.

Mathematical modeling and the theory of dynamical systems and control provide valuable tools in order to help conceive the pre-design of biological control and synthetic modification strategies as simple, robust, and efficient as possible. Interestingly, a significant number of biological networks are part of monotone dynamical systems that generate order-preserving flows (Smith, 2008). This property greatly restricts their possible behaviors and dynamics and allows the statement of global stability and convergence results, even for highly non-linear systems (Ji-Fa, 1994; Dancer, 1998). More recently, these monotone notions and results have also been extended to dynamical systems with inputs and outputs (Sontag, 2005), that are nicely appropriate for synthetic biology problems.

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This article presents a N -dimensional monotone genetic positive feedback loop that accurately models cell differentiation behavior. In the context of cell dedifferentiation process, the following problem will be addressed: *is it possible to globally stabilize the undifferentiated state of this positive loop by only modifying intrinsically the genetic circuit?* It will be shown that the addition of a simple synthetic self-inhibition of one gene in the loop is indeed able to solve the problem, forcing the cells to dedifferentiate. Compared to the real experiment done in Lugagne et al. (2017) previously introduced, this strategy prevents the use of any input and measurements devices. Only the synthetic redesign of the cells is needed, greatly simplifying the biological set-up for equivalent results.

The N -dimensional positive feedback loop model is introduced in section 2. The synthetic addition of the self-inhibition is presented in section 3 and is shown to globally stabilize the unstable fixed point \bar{x} of the system under tight conditions in section 4. These conditions are relaxed in section 5 when biological uncertainties are taken into account. In this case, the undifferentiated state is defined as a small region around \bar{x} . Thanks to monotone properties of the network, it is shown that the undifferentiated region is globally attractive as soon as all the possible fixed points of the synthetic circuit are confined into it.

2. POSITIVE FEEDBACK LOOP MODEL

With N components, a genetic positive feedback loop is commonly described by the following system (Goodwin et al., 1963):

$$\begin{cases} \dot{x}_1(x_1, x_N) = \kappa_{01} + \kappa_1 h^+(x_N, \theta_N, n_N) - \gamma_1 x_1, \\ \dot{x}_i(x_i, x_{i-1}) = \kappa_{0i} + \kappa_i h^+(x_{i-1}, \theta_{i-1}, n_{i-1}) - \gamma_i x_i, \end{cases} \quad (1)$$

$\forall i \in \{2, \dots, N\}$ where $h^+(x, \theta, n) = x^n / (\theta^n + x^n)$ is a sigmoid function, called Hill function, that models activation between variables with threshold $\theta > 0$ and steepness $n \geq 2$. The parameter n is an integer representing the number of transcription factors that need to bind to the promoter of the target gene in order to enhance its transcription. In addition, biological components are produced with a basal rate $\kappa_{0i} > 0$, degraded with a rate $\gamma_i > 0$, and experience interaction with intensity $\kappa_i > 0$. The graph of this motif is represented in Fig. 1.

It is easy to see that there is an equivalence between: $\bar{x} = (\bar{x}_1, \dots, \bar{x}_N)$ is a fixed point of (1) and \tilde{x}_1 is a fixed point of $S_1(x) = H_1 \circ H_N \circ H_{N-1} \circ \dots \circ H_3 \circ H_2(x)$ where:

$$H_i(x) = \frac{\kappa_{0i} + \kappa_i h^+(x, \theta_{i-1}, n_{i-1})}{\gamma_i} \quad (2)$$

$\forall i \in \{1, \dots, N\}$ where $i - 1 = N$ when $i = 1$. From its sigmoidal shape, $S_1(x)$ cannot have more than three fixed points (Ahsen et al., 2015). The previous equivalence proves that system (1) cannot have more than three fixed points neither: two locally stable, called \bar{x}_{inf} and \bar{x}_{sup} , and one locally unstable called \bar{x} . This bistable system, better known as ‘‘Toggle Switch’’ in dimension 2 (Lugagne et al., 2017), properly models cell differentiation. From an undifferentiated state, represented by the unstable fixed point, a cell may differentiate into one type or another type by converging either towards the first stable fixed point or the second stable fixed point.

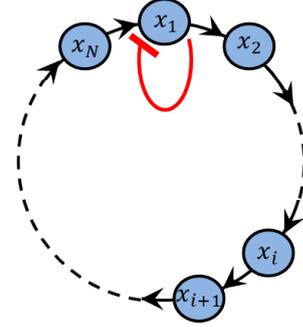


Fig. 1. The black arrows illustrate the classical positive feedback loop (1). The supplementary red repression illustrates the synthetic modified circuit (3).

Remark 1. System (1) is bounded: $x_i \in [\kappa_{0i}/\gamma_i, (\kappa_{0i} + \kappa_i)/\gamma_i[$ for $i \in \{1, \dots, N\}$. Moreover, the unstable fixed point \bar{x} verifies: $\bar{x}_i \in]\kappa_{0i}/\gamma_i, (\kappa_{0i} + \kappa_i)/\gamma_i[$ for $i \in \{1, \dots, N\}$.

Hereafter, in the context of dedifferentiation as presented in the introduction, the parameters of system (1) are assumed to generate three fixed points. The goal of next section is to synthetically modify this positive loop in order to globally stabilize the undifferentiated state \bar{x} .

Remark 2. The variable x_{i-1} is said to inhibit the variable x_i when $h^+(x_{i-1}, \theta_{i-1}, n_{i-1})$ in \dot{x}_i is replaced by $h^-(x_{i-1}, \theta_{i-1}, n_{i-1}) = 1 - h^+(x_{i-1}, \theta_{i-1}, n_{i-1})$. All these loops are fully equivalent after a change of variable as long as the number of inhibitions is even. It follows that the results presented in this article also apply to this more general structure of positive feedback loop. The results will be illustrated in the whole paper with the Toggle Switch presented in Lugagne et al. (2017), composed of two variables *LacI* and *TetR* that inhibit each other. As explained in the introduction, the authors successfully designed a biological control device based on the introduction of two inducer molecules in order to stabilize the cells around their undifferentiated state.

3. AUTO-REPRESSION OF THE FIRST GENE

In dimension 2, a previous study showed that a piecewise constant control law was able to make the unstable fixed point \bar{x} of system (1) globally asymptotically stable (Chambon and Gouzé, 2019). For biological purpose, this control law was only dependent on qualitative measurements of the first gene x_1 and acted on its own expression: when the first gene was weakly expressed ($x_1 < \bar{x}_1$), the input enhanced its production, and at the opposite when the first gene was highly expressed ($x_1 > \bar{x}_1$), the input inhibited its production. Inspired by these results, it seems possible to modify intrinsically the genetic motif by adding a self-inhibition of the first gene x_1 in order to stabilize the unstable fixed point. This hypothesis leads to the following new differential system:

$$\begin{cases} \dot{x}_1 = \kappa_{01} + u(x_1)\kappa_1 h^+(x_N, \theta_N, n_N) - \gamma_1 x_1, \\ \dot{x}_i = \kappa_{0i} + \kappa_i h^+(x_{i-1}, \theta_{i-1}, n_{i-1}) - \gamma_i x_i, \end{cases} \quad (3)$$

$\forall i \in \{2, \dots, N\}$, where $u(x_1) = \alpha h^-(x_1, \theta, n)$. The expression of the first gene is both activated by the production of the last gene N and inhibited by its own production through the decreasing sigmoid function $h^-(x, \theta, n)$

where $n \geq 2$ and $\theta > 0$. The two sigmoid functions $h^+(x_N, \theta_N, n_N)$ and $h^-(x_1, \theta, n)$ are multiplied as it is considered that the transcription of the first gene is restricted as soon as the repressor x_1 is present. Practically, this is often the case in biology when the repressor binds to the DNA and prevents the binding of RNA Polymerase. The parameter $\alpha > 0$ is playing the same role as κ_1 : it allows to tune the sensitivity of the interaction between x_N and x_1 . The graph of this new circuit is presented in Fig. 1. Importantly, this modified system is still a priori bounded:

Lemma 3. System (3) verifies: $x_i \in [\kappa_{0i}/\gamma_i, (\kappa_{0i} + \kappa_i)/\gamma_i]$ $\forall i \in \{2, \dots, N\}$ and $x_1 \in [\kappa_{01}/\gamma_1, x_{1sup}]$.

Proof. For $i \in \{2, \dots, N\}$ the result is straightforward as the x_i -nullclines are the same as in system (1). For $i = 1$, the determination of the x_1 -nullcline gives the condition $h^+(x_N, \theta_N, n_N) = F(x_1)$ where $F(x_1) = (\gamma_1 x_1 - \kappa_{01})(\theta^n + x_1^n) / (\kappa_1 \alpha \theta^n)$. The conditions on x_1 such that $F(x_1) \in [0, 1]$ are investigated. First, $F(x_1) \geq 0 \Leftrightarrow x_1 \geq \kappa_{01}/\gamma_1$, and $F(\kappa_{01}/\gamma_1) = 0$. Furthermore:

$$\frac{\partial F}{\partial x_1} = \frac{\gamma_1 (\theta^n + x_1^n) + n x_1^{n-1} (\gamma_1 x_1 - \kappa_{01})}{\kappa_1 \alpha \theta^n}.$$

Hence, for $x_1 \geq \kappa_{01}/\gamma_1$, $\partial F/\partial x_1 > 0$. Then, $F(x_1)$ is strictly increasing and positive for $x_1 \geq \kappa_{01}/\gamma_1$. Moreover, $\lim_{x_1 \rightarrow +\infty} F(x_1) = +\infty$. Finally, there exists $x_{1sup} > \kappa_{01}/\gamma_1$ such that $F(x_{1sup}) = 1$. The x_1 -nullcline is then defined as $x_1 = H(x_N) = F^{-1}(h^+(x_N, \theta_N, n_N))$ for $x_N \geq 0$. As F is strictly increasing on $\kappa_{01}/\gamma_1 \leq x_1 < x_{1sup}$, then H is strictly increasing, with $H(0) = \kappa_{01}/\gamma_1$ and $\lim_{x_N \rightarrow +\infty} H(x_N) = x_{1sup}$. Finally, $\dot{x}_1(x_1, x_N) = 0 \Leftrightarrow x_1 = H(x_N)$. It is easy to check that the sign of the x_1 -vector field is separated by the curve $x_1 = H(x_N)$: $\dot{x}_1(x_1, x_N) > 0$ (resp. < 0) $\Leftrightarrow x_1 < H(x_N)$ (resp. $>$). The trajectories are bounded between the lower bound and the upper bound of H , namely $x_1 \in [\kappa_{01}/\gamma_1, x_{1sup}]$.

Besides being bounded, the modified system has the following fundamental property:

Proposition 4. System (3) is cooperative and irreducible on \mathbb{R}_+^N .

Proof. For cooperativity, it is easy to check that $\forall i \in \{1, \dots, N\}$, $\partial \dot{x}_i(x_i, x_{i-1})/\partial x_j \geq 0 \forall j \in \{1, \dots, N\}$, $j \neq i$. Moreover, the Jacobian of system (3) is irreducible on \mathbb{R}_+^N .

Cooperative systems are part of monotone dynamical systems and generate order preserving flows. From this property, many results about asymptotic behavior and stability have been stated (Smith, 2008). Proposition 4 will be essential in next section in order to prove that there exist n , θ , and α such that \bar{x} becomes globally asymptotically stable.

4. GLOBAL ASYMPTOTIC STABILITY OF \bar{X}

Obviously, in order to stabilize the unstable fixed point \bar{x} of system (1), \bar{x} must at least be a fixed point of system (3). This is verified with the following constraint:

Hypothesis 5.

$$\bar{x}_1 = \theta(\alpha - 1)^{1/n}.$$

The goal of this section is to determine conditions on the self-inhibition in order to remove the bi-stability and keep

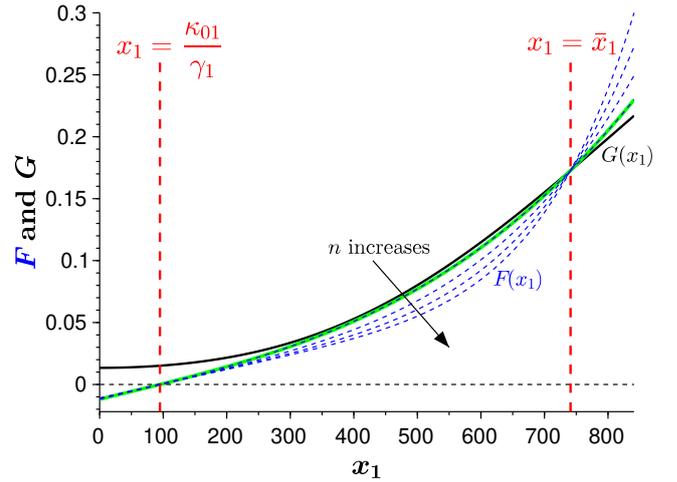


Fig. 2. Illustration of the proof of Lemma 6 with $N = 2$. The parameters are calibrated so that the three fixed points of system (1) are the same as in Lugugue et al. (2017): $\kappa_{01} \approx 1.56$, $\kappa_{02} \approx 1.47$, $\kappa_1 \approx 61.7$, $\kappa_2 \approx 17.6$, $n_1 = n_2 = 2$, $\theta_1 \approx 362.7$, $\theta_2 \approx 134.9$, $\gamma_1 = \gamma_2 = 0.0165$ leading to $\bar{x} = (741, 295.2)$. The self-inhibition parameters are fixed to: $\theta = 700$, $n = \tilde{n} = 2$ and $\alpha \approx 2.12$. The black line is G . The blue dashed lines are different plots of F with $n \in \{2, 3, 4, 5\}$. The plain green line is F with $n = \tilde{n}$.

the unstable fixed point \bar{x} as the unique fixed point, leading to the first important lemma:

Lemma 6. If $\theta < \bar{x}_1$, it is possible to find $\tilde{n} \geq 2$ such that $\forall n \geq \tilde{n}$, system (3) has a unique fixed point \bar{x} .

Proof. If \tilde{x} is a fixed point then $\dot{x}_i(\tilde{x}_i, \tilde{x}_{i-1}) = 0 \forall i \in \{1, \dots, N\}$ where $i - 1 = N$ when $i = 1$, leading to $\tilde{x}_i = H_i(\tilde{x}_{i-1})$ for $i \in \{2, \dots, N\}$. It is easy to see that by induction, the equation of the fixed points reduces to $G(\tilde{x}_1) = F(\tilde{x}_1)$ where F was already introduced in the proof of Lemma 3 and $G(x_1) = h^+(H_N \circ H_{N-1} \circ \dots \circ H_3 \circ H_2(x_1), \theta_N, n_N)$. G is positive and strictly increasing (for an illustration, see Fig. 2). On the other hand, $F \geq 0 \Leftrightarrow x_1 \geq \kappa_{01}/\gamma_1$ and $F(\kappa_{01}/\gamma_1) = 0$. Hence, the intersection between F and G can only occur for $x_1 \geq \kappa_{01}/\gamma_1$. Moreover, for $x_1 \geq \kappa_{01}/\gamma_1$, F is strictly increasing. From Hypothesis 5, $\alpha = (\theta^n + \bar{x}_1^n)/\theta^n$, leading to $F(\bar{x}_1) = (\gamma_1 \bar{x}_1 - \kappa_{01})/\kappa_1$. This implies that $F(\bar{x}_1)$ and $F(\kappa_{01}/\gamma_1)$ neither depend on θ nor n .

Without any conditions on n and θ , F and G can intersect more than once on $x_1 \geq \kappa_{01}/\gamma_1$. In order to prevent this to happen, the influence of the parameter n on F is investigated: $\partial F/\partial n =$

$$\frac{(\gamma_1 x_1 - \kappa_{01})}{\kappa_1} \left[\frac{\theta^n \bar{x}_1^n \ln(\frac{\theta}{\bar{x}_1}) + x_1^n \theta^n \ln(\frac{x_1}{\theta}) + x_1^n \bar{x}_1^n \ln(\frac{x_1}{\bar{x}_1})}{(\theta^n + \bar{x}_1^n)^2} \right].$$

For $x_1 \geq \kappa_{01}/\gamma_1$: $\partial F/\partial n \geq 0 \Leftrightarrow h(x_1) \geq \theta^n \bar{x}_1^n \ln(\bar{x}_1/\theta)$ where $h(x_1) = x_1^n \theta^n \ln(x_1/\theta) + x_1^n \bar{x}_1^n \ln(x_1/\bar{x}_1)$. This new function h is examined:

$$\frac{\partial h}{\partial x_1} = x_1^{n-1} \left[n\theta^n \ln(\frac{x_1}{\theta}) + \bar{x}_1^n n \ln(\frac{x_1}{\bar{x}_1}) + \theta^n + \bar{x}_1^n \right].$$

From this expression it is possible to deduce that:

$$\frac{\partial h}{\partial x_1} \geq 0 \Leftrightarrow x_1 \geq \exp\left(\frac{\theta^n \ln(\theta) + \bar{x}_1^n \ln(\bar{x}_1)}{\theta^n + \bar{x}_1^n} - \frac{1}{n}\right) = X_1.$$

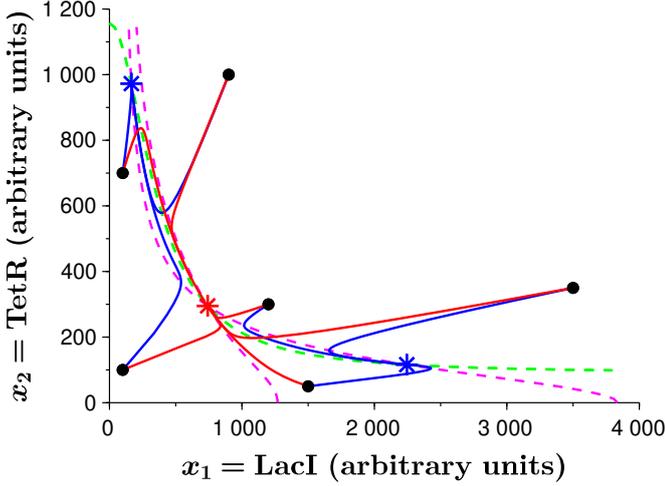


Fig. 3. Simulation of systems (1) and (3) with $N = 2$. The parameters are the same as in Fig. 2. The green (resp. pink) dashed lines are the x_2 (resp x_1) nullclines. The blue stars are \bar{x}_{inf} and \bar{x}_{sup} and the red star is \bar{x} . The six initial conditions are depicted with black dots. The blue lines are the trajectories of system (1). The red lines are the trajectories of system (3) with $\theta = 700$, $n = 3$, and $\alpha \approx 2.19$.

The function h also verifies: $h(0) = 0$, $\lim_{x \rightarrow +\infty} h(x) = +\infty$ and $h(\bar{x}_1) = \theta^n \bar{x}_1^n \ln(\bar{x}_1/\theta)$. This function is decreasing and negative for $x_1 \leq X_1$ and starts increasing for $x_1 \geq X_1$.

The hypothesis $\theta < \bar{x}_1$ on Lemma 6 gives: $h(\bar{x}_1) > 0$, leading to $h(x_1) \geq \theta^n \bar{x}_1^n \ln(\bar{x}_1/\theta) \Leftrightarrow x_1 \geq \bar{x}_1$. Hence, for $x_1 \geq \kappa_{01}/\gamma_1$, the previous equivalence $\partial F/\partial n \geq 0 \Leftrightarrow h(x_1) \geq \theta^n \bar{x}_1^n \ln(\bar{x}_1/\theta)$ becomes $\partial F/\partial n \geq 0 \Leftrightarrow x_1 \geq \bar{x}_1$. Consequently, for $\kappa_{01}/\gamma_1 < x_1 < \bar{x}_1$, $\partial F/\partial n < 0$ and for $x_1 > \bar{x}_1$, $\partial F/\partial n > 0$.

In the end, with $\theta < \bar{x}_1$, an increase of parameter n decreases F for $\kappa_{01}/\gamma_1 < x_1 < \bar{x}_1$ and increases F for $x_1 > \bar{x}_1$. Moreover, it is possible to check that for $\kappa_{01}/\gamma_1 < x_1 < \bar{x}_1$, $\lim_{n \rightarrow +\infty} F(x_1) = 0$ and for $x_1 > \bar{x}_1$, $\lim_{n \rightarrow +\infty} F(x_1) = +\infty$. As a consequence, there exists $\tilde{n} \geq 2$ such that $\forall n \geq \tilde{n}$, $F(x_1) < G(x_1)$ for $\kappa_{01}/\gamma_1 < x_1 < \bar{x}_1$, $F(x_1) > G(x_1)$ for $x_1 > \bar{x}_1$ and $F(\bar{x}_1) = G(\bar{x}_1)$ (for an illustration, see Fig. 2). With these conditions, F and G intersect only once at \bar{x}_1 . Finally, \bar{x} is the unique fixed point of system (3).

This uniqueness property is a fundamental hypothesis for cooperative systems and allows the statement of the main theorem of this section:

Theorem 7. If $\theta < \bar{x}_1$, it is possible to find $\tilde{n} \geq 2$ such that $\forall n \geq \tilde{n}$, \bar{x} is globally asymptotically stable.

Proof. First, from Proposition 4, system (3) is cooperative on \mathbb{R}_+^N . Second, from Lemma 3 every forward semi-orbit has compact closure in \mathbb{R}_+^N . Third, from Lemma 6, if $\theta < \bar{x}_1$ and $n \geq \tilde{n}$, then \bar{x} is the unique fixed point of system (3). Finally, all the conditions of Theorem C stated in Ji-Fa (1994) are met: \bar{x} is globally asymptotically stable.

This theorem finally proves that for any set of parameters of system (1) that leads to the emergence of bi-stability with an unstable fixed point \bar{x} , it is possible to determine

θ and n such that \bar{x} becomes the unique globally asymptotically stable fixed point of system (3). Fig. 3 gives an illustration with $N = 2$: the parameters of system (1) were calibrated to the real Toggle Switch experiment presented in Lugagne et al. (2017). As predicted, all the trajectories converge towards the undifferentiated state \bar{x} . However, from Hypothesis 5, α must be tightly fixed once θ and n are chosen. In next section, biological uncertainties are taken into account in order to relax this constraint.

5. GLOBAL CONVERGENCE TOWARDS AN UNDIFFERENTIATED REGION

For biological reasons, it seems irrelevant to consider the undifferentiated state as the precise fixed point \bar{x} of system (1). Indeed, due to inherent stochasticity in cells, biological quantities are not likely to asymptotically converge towards fixed points in a mathematical sense. Moreover, only partial knowledge of the system is available due to qualitative measurements in biology. Thus, it would be inappropriate to design strict convergence control strategies if measurement devices were not able to detect it. For all these reasons, the undifferentiated state is more likely to be a small region around the unstable fixed point \bar{x} . As a consequence, in this section, the synthetic feedback will be determined in order to obtain global convergence towards a small undifferentiated region instead of \bar{x} . This undifferentiated region is supposed to be an hypercube of length $\delta > 0$ called $\mathcal{B}_\delta = \{x \mid \|x - \bar{x}\|_\infty < \delta\}$. Obviously, the two stable fixed points \bar{x}_{inf} and \bar{x}_{sup} must be outside \mathcal{B}_δ . Thus, δ must verify the following condition:

Hypothesis 8. $\delta < \min_{i \in \{1, \dots, N\}} \{\bar{x}_i - \bar{x}_{inf_i}, \bar{x}_{sup_i} - \bar{x}_i\}$.

In what follows, the three auto-inhibition parameters θ , n , and α will be determined in order to contain all the possible fixed points \tilde{x} of system (3) inside the undifferentiated region \mathcal{B}_δ (there are possibly more than three fixed points). Under these conditions, it will be shown that the cooperative properties of system (3) induce global convergence towards \mathcal{B}_δ .

Definition 9. $\forall i \in \{2, \dots, N\}$, S_i is the composition of $(i - 1)$ nullcline functions such that $S_i(x) = H_i \circ H_{i-1} \circ \dots \circ H_3 \circ H_2(x)$. Their inverse are called $S_i^{-1}(x)$.

These functions are positive and strictly increasing. Their bounds are called $S_i(0) = S_{imin} > 0$ and $\lim_{x \rightarrow +\infty} S_i(x) = S_{imax}$. Moreover, if \tilde{x} is a fixed point of (3), then $\tilde{x}_i = S_i(\tilde{x}_1)$. Similarly, as the x_i -nullclines are the same for system (1) and (3) $\forall i \in \{2, \dots, N\}$, then $S_i(\bar{x}_{inf_1}) = \bar{x}_{inf_i}$, $S_i(\bar{x}_{sup_1}) = \bar{x}_{sup_i}$ and $S_i(\bar{x}_1) = \bar{x}_i$ (see Fig. 4). These functions allow the construction of bounds of the state space, included in \mathcal{B}_δ :

Definition 10. Under Hypothesis 8, $\forall i \in \{2, \dots, N\}$, $m_i = \bar{x}_1 - S_i^{-1}(\bar{x}_i - \delta)$ and $M_i = S_i^{-1}(\bar{x}_i + \delta) - \bar{x}_1$. From this, it is possible to define $m = \min_{i \in \{2, \dots, N\}} \{m_i\}$, $M = \min_{i \in \{2, \dots, N\}} \{M_i\}$, and $\epsilon = \min \{m, M, \delta\}$.

It is possible to check that these parameters verify two propositions:

Proposition 11. Under Hypothesis 8, $\forall i \in \{2, \dots, N\}$, $m_i > 0$, $M_i > 0$, $\bar{x}_1 - m_i > \bar{x}_{inf_1}$, and $\bar{x}_1 + M_i < \bar{x}_{sup_1}$. Moreover, $\bar{x}_1 - \epsilon > \bar{x}_{inf_1}$ and $\bar{x}_1 + \epsilon < \bar{x}_{sup_1}$.

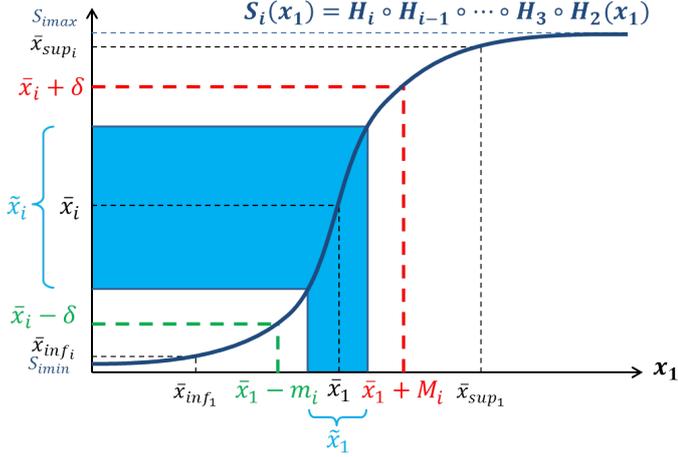


Fig. 4. Illustration of Definitions 9 and 10, and Propositions 11 and 12.

Proposition 12. Under Hypothesis 8, if a fixed point \tilde{x} of system (3) is such that $\tilde{x}_{inf_1} < \tilde{x}_1 - \epsilon < \tilde{x}_1 < \tilde{x}_1 + \epsilon < \tilde{x}_{sup_1}$, then $\tilde{x}_i - \delta < \tilde{x}_i < \tilde{x}_i + \delta \forall i \in \{1, \dots, N\}$.

These propositions are illustrated in Fig. 4 and allow the statement of the following lemma:

Lemma 13. Under Hypothesis 8, if $\tilde{x}_1 - \epsilon < \theta < \tilde{x}_1 + \epsilon$, it is possible to find $\tilde{n} \geq 2$ and $\tilde{\alpha} > 1$ such that $\forall n \geq \tilde{n}$ and $\forall \alpha \geq \tilde{\alpha}$, all the fixed points \tilde{x} of system (3) verify $\tilde{x}_i - \delta < \tilde{x}_i < \tilde{x}_i + \delta \forall i \in \{1, \dots, N\}$.

Proof. As in the proof of Lemma 6, the fixed points \tilde{x} of system (3) verify $G(\tilde{x}_1) = F(\tilde{x}_1)$. As $F(\theta) = 2(\gamma_1\theta - \kappa_{01})/(\alpha\kappa_1)$, then $F(\theta)$ does not depend on n . Moreover, $\partial F/\partial n = ((\gamma_1 x_1 - \kappa_{01})x_1^n \ln(x_1/\theta))/(\alpha\kappa_1\theta^n)$. For $x_1 \geq \kappa_{01}/\gamma_1$, where the intersection between F and G can occur, $(\gamma_1 x_1 - \kappa_{01})/\kappa_1 \geq 0$. Then, $\partial F/\partial n \geq 0 \Leftrightarrow x_1 \geq \theta$. Hence, for $x_1 \geq \kappa_{01}/\gamma_1$ and $x_1 < \theta$, increasing n decreases F and for $x_1 > \theta$, increasing n increases F .

As for the proof of Lemma 3, it is possible to show that for $x_1 > \theta$, $\lim_{n \rightarrow +\infty} F(x_1) = +\infty$. However, for $\kappa_{01}/\gamma_1 < x_1 < \theta$, $\lim_{n \rightarrow +\infty} F(x_1) = (\gamma_1 x_1 - \kappa_{01})/(\alpha\kappa_1)$. Hence, if $\alpha = 1$, the conditions for the fixed points of system (1) are recovered: this means that for $\kappa_{01}/\gamma_1 < x_1 < \theta$ and $n \rightarrow +\infty$, there is an intersection between F and G at the point \tilde{x}_{inf_1} . This must be prevented. Hence, $\alpha > 1$ is fixed so that the limit function $(\gamma_1 x_1 - \kappa_{01})/(\alpha\kappa_1)$ becomes smaller than $G(x_1)$ for $\kappa_{01}/\gamma_1 < x_1 \leq \tilde{x}_1 - \epsilon$. Besides, if $\tilde{x}_1 - \epsilon < \theta < \tilde{x}_1 + \epsilon$, then from Remark 1 and Proposition 11, $\kappa_{01}/\gamma_1 \leq \tilde{x}_{inf_1} < \tilde{x}_1 - \epsilon < \theta$. Once α is fixed as explained previously, it is possible to find \tilde{n} such that $F(x_1) < G(x_1)$ for $\kappa_{01}/\gamma_1 < x_1 \leq \tilde{x}_1 - \epsilon$ and $F(x_1) > G(x_1)$ for $x_1 \geq \tilde{x}_1 + \epsilon$ (for an illustration, see Fig. 5). It follows that the intersection between F and G can only occur for $x_1 \in [\tilde{x}_1 - \epsilon, \tilde{x}_1 + \epsilon]$. Hence, if $\tilde{x}_1 - \epsilon < \theta < \tilde{x}_1 + \epsilon$, then any fixed point \tilde{x} of system (3) verifies $\tilde{x}_1 - \epsilon < \tilde{x}_1 < \tilde{x}_1 + \epsilon$. Finally, from Proposition 12, $\tilde{x}_i - \delta < \tilde{x}_i < \tilde{x}_i + \delta \forall i \in \{1, \dots, N\}$.

This Lemma gives conditions on θ , n , and α such that all the fixed points \tilde{x} of system (3) are confined inside the undifferentiated region. From this result, the main theorem of this section is introduced:

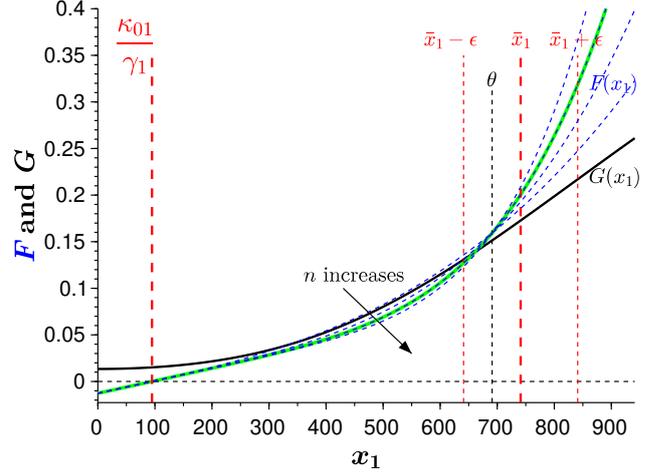


Fig. 5. Illustration of the proof of Lemma 13 with $N = 2$. The parameters are the same as in Fig. 2 with $\delta = 100$ leading to $\epsilon = \delta = 100$. The controlled parameters are fixed to: $\theta \approx 691$ and $\alpha = 2 > \tilde{\alpha}$. It is possible to determine $\tilde{n} = 4$. The black line is G . The blue dashed lines are different plots of F with different values of $n \in \{2, 3, 4, 5\}$. The plain green line is F with $n = \tilde{n}$.

Theorem 14. Under Hypothesis 8, if $\tilde{x}_1 - \epsilon < \theta < \tilde{x}_1 + \epsilon$, it is possible to find $\tilde{n} \geq 2$ and $\tilde{\alpha} > 1$ such that $\forall n \geq \tilde{n}$ and $\forall \alpha \geq \tilde{\alpha}$ the undifferentiated region \mathcal{B}_δ is globally attractive.

Proof. First, from Remark 1, \mathbb{R}_+^N is positively invariant. Furthermore, from Proposition 4, system (3) is cooperative and irreducible in \mathbb{R}_+^N . It follows from the results in Smith (2008) or Dancer (1998) that the flow is strongly monotone and there exist maximal and minimal fixed points called respectively x_M and x_m such that any trajectory $x(t)$ of system (3) will be trapped between these two extreme fixed points: $\lim_{t \rightarrow +\infty} x_i(t) \in [x_{m_i}, x_{M_i}] \forall i \in \{1, \dots, N\}$. On the other hand, from Lemma 13, under Hypothesis 8, if $\tilde{x}_1 - \epsilon < \theta < \tilde{x}_1 + \epsilon$, it is possible to find \tilde{n} and $\tilde{\alpha}$ such that $\forall n \geq \tilde{n}$ and $\forall \alpha \geq \tilde{\alpha}$, all the fixed points \tilde{x} of system (3) verify $\tilde{x}_i - \delta < \tilde{x}_i < \tilde{x}_i + \delta \forall i \in \{1, \dots, N\}$. Hence, the extreme fixed points x_M and x_m verify $\tilde{x}_i - \delta < x_{m_i} < \tilde{x}_i + \delta$ and $\tilde{x}_i - \delta < x_{M_i} < \tilde{x}_i + \delta \forall i \in \{1, \dots, N\}$. Finally, any trajectory $x(t)$ of system (3) will be trapped inside the undifferentiated region: $\lim_{t \rightarrow +\infty} x_i(t) \in [\tilde{x}_i - \delta, \tilde{x}_i + \delta] \forall i \in \{1, \dots, N\}$.

This theorem is illustrated in Fig. 6 for the Toggle Switch experimented in Lugagne et al. (2017). As analytically proved, it is possible to find conditions on n , θ , and α such that the undifferentiated region \mathcal{B}_δ becomes globally attractive. With these constraints, it is possible to observe that the new fixed point \tilde{x} of system (3) is contained in \mathcal{B}_δ and all the trajectories converge to it. With different parameters in system (1), it might be possible to have multiple steady states all contained in the region \mathcal{B}_δ . It would mean that the trajectories might not all converge towards the same fixed point inside \mathcal{B}_δ . However, they would all converge inside the undifferentiated region, as expected. Compared to the result presented in section 4, the constraints on the parameters are relaxed: θ can be chosen anywhere in a range, and n and α must be big enough. These constraints seem more realistic for bio-

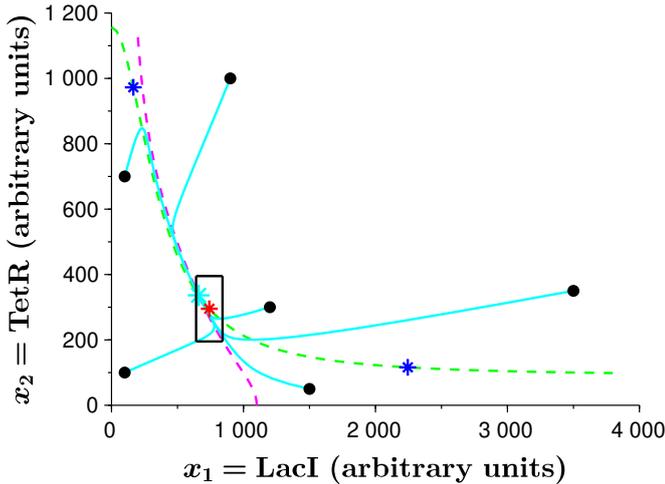


Fig. 6. Simulation of system (3) with $N = 2$. The parameters are the same as in Fig. 5. With $n = 4$, system (3) has a unique fixed point $\tilde{x} \approx (660.2, 336.4)$ represented by the light blue star. The undifferentiated region \mathcal{B}_δ is highlighted by the black square and contains \tilde{x} . The green (resp. pink) dashed line is the x_2 (resp. x_1) nullcline of system (3). The dark blue stars are \bar{x}_{inf} and \bar{x}_{sup} and the red star is \bar{x} . The six initial conditions are depicted with black dots. The light blue lines are the trajectories of system (3).

logical applications. Moreover, as long as the parameters meet the right conditions, the result is valid even if they fluctuate in time. Importantly, this result is also valid if the undifferentiated region is not an hypercube: if the region has any shape around \bar{x} , it is always possible to define an hypercube included in the undifferentiated region that is globally attractive, with the same techniques as explained before.

6. CONCLUSION

In this article, it was shown that a simple synthetic modification of a bistable positive feedback loop in any dimension N was able to globally stabilize the undifferentiated state of the system. First, for mathematical convenience, the cell undifferentiated state was assumed to be represented by the unstable fixed point \bar{x} of the dynamical system. In this context, the parameters of the new synthetic self-inhibition were tightly fixed on order to ensure that \bar{x} was the unique fixed point of the modified circuit. Then, thanks to cooperative properties of the network, \bar{x} was proved to be globally asymptotically stable. In a more relevant biological context, the undifferentiated state was considered as a full region of the state space. The previous conditions on the self-inhibition parameters were relaxed in order to confine all the possible fixed points of the system in the undifferentiated region. Global convergence towards this region was finally proved thanks to cooperative properties of the new synthetic network. These results can also be applied to bistable positive feedback loops with arbitrary monotonically increasing interaction functions instead of Hill functions.

This strategy may be a good alternative to classical biological control techniques as presented in Lugagne et al. (2017). Indeed, the intrinsic modification of the genetic

motif avoids the use of measurements and control devices, that often lead to tedious experiments and generate uncertainties. Moreover, this synthetic modification is probably more adapted to inherent specificity and heterogeneity of each cell, and is hopefully more viable in the long-term thanks to replication machinery and cell division. For this purpose, our current work focuses on the extension of these results in the case of noisy and uncertain parameters.

On the other side, this persistence property may be a drawback if the bi-stability needs to be recovered for any reason. In this case, it may be interesting to study the synthetic modified circuit coupled with a classical biological control mean, such as optogenetics for example, which is able to switch on or off the modified self-inhibition. This idea will probably lead to the study of hybrid systems and seems an interesting extension of this work.

Finally, the same methods can be extended in order to force a cell to differentiate into one particular type. This may be interesting in the context of tissue regeneration for example, where a great amount of specialized cells is needed. In this case, the synthetic modification must lead to the global convergence towards one of the two stable fixed points of the original system.

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