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Feedback control of stochastic gene switches using PIDE models ⋆

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Abstract: Achieving control of gene regulatory circuits is one of the goals of synthetic biology, as a way to regulate cellular functions for useful purposes (in biomedical, environmental or industrial applications). The inherent stochastic nature of gene expression makes it challenging to control the behavior of gene regulatory networks, and increasing efforts are being devoted in the field to address different control problems.
In this work, we combine the efficient modeling of stochastic gene regulatory networks by means of Partial Integro-Differential Equations with feedback control, in order to keep protein levels at the target (pre-defined) stationary probability distribution. In particular, we achieve the closed-loop stabilization of bi-modal toggle-switches in the stochastic regime within the region of low probability (around the minimum located between the two modes of the uncontrolled system).

Keywords: Synthetic Biology, Molecular Noise, Bimodality, Gene Regulatory Network, Partial Integro Differential Equations, Stochastic Models, Bistability.

1. INTRODUCTION

One of the challenges of synthetic biology is to control the behaviour of synthetic gene regulatory circuits in cellular contexts of high molecular noise. Gene expression is a stochastic phenomenon, and molecular noise might have a crucial effect on the behaviour of gene regulatory networks when the molecules involved are in low numbers (as is often the case in bacteria). The dynamics of inherently stochastic gene regulatory networks (GRNs) are captured by a Chemical Master Equation (CME) that is mathematically untractable in most cases for realistic scenarios. One widespread approximation of the CME is based on the computation of many realizations of the Stochastic Simulation Algorithm (SSA) Gillespie (1977) (which might be computationally involved).

Pájaro et al. (2017) developed a continuous approximation of the CME for gene regulatory networks of arbitrary dimension (involving self and cross regulation) by a set of Partial Integro Differential Equations (PIDEs), that provides directly the evolution of the probability distributions in time, by extending the result by Friedman et al. (2006) for unidimensional networks (i.e. valid for one self-regulated gene). The approximation is based on the phenomenon of protein bursting (mRNAs are degraded faster than their protein products), a mild assumption that holds for most prokaryotic and eukaryotic organisms (Dar et al., 2012; Shahrezaei and Swain, 2008). A very efficient semilagrangian numerical method for the simulation of the multidimensional PIDE model has been developed by Pájaro et al. (2017) and implemented in the toolbox SELANSI Pájaro et al. (2018). In this work, we make use of the PIDE models and semilagrangian method to develop controllers for stochastic gene regulatory circuits.

Control of biocircuits is an active area of research in synthetic biology. Two of the most prominent results during the last years have been, on the one hand, the antithetic controller Briat et al. (2016); Aoki et al. (2019), a realization of integral control for genetic circuits that has been proved to achieve robust perfect adaptation in presence of noise; and, on the other hand, the balancing of a synthetic gene switch by feedback control, based on single cell ODE modeling by Lugagne et al. (2017). Here, we address the control problem of balancing gene switches by feedback control at the population level, taking into account the inherent molecular noise. To this aim, we design feedback controllers based on the PIDE model, stabilizing the cell population around the region of low probability of the toggle switch. The two modes of the genetic switch are separated by a region with a very low probability in the protein space. In the uncontrolled system, this region acts as a barrier that hinders transitions between the modes (Pájaro et al., 2019).

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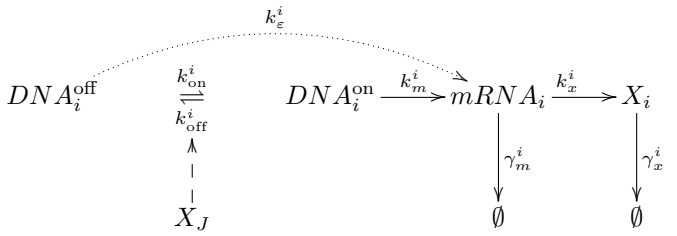

Fig. 1. Scheme representation of a gene regulatory network. The promoter regions of each gene are assumed to switch between active and inactive states (DNA_i^{on}, DNA_i^{off}) at frequencies k_{on}^i, k_{off}^i respectively. The transition is regulated by the binding/unbinding of a number of X_j-protein molecules with $j \in J$. Transcription of messenger RNA ($mRNA_i$) from the active DNA_i form, and translation into protein X_i occur at rates (per unit time) k_m^i and k_x^i. k_ε^i is the transcriptional leakage rate constant. Degradation of $mRNA_i$ and X_i is assumed to occur by first order processes with rate constants γ_m^i, γ_m^i.

The paper is structured as follows: in the Methods section is introduced the PIDE model used to simulate the dynamics of gene regulatory networks, as well as the control strategy proposed to balancing stochastic genetic toggle switches. In the Results section, two different case studies are presented to illustrate the effectiveness of the methodology *in silico*, using feedback control to balance, at the population level: i) a classical gene toggle switch, regulated at the protein-promoter level (this type of switch has been previously stabilized at the single cell level by Lugagne et al. (2017)), and the toggle switch with CRISPRi mediated regulation by Santos-Moreno et al. (2020). Finally, we end up with some conclusions and further work.

2. METHODS

2.1 Stochastic PIDE model

We consider a gene regulatory network involving n different genes ($DNA_1, \ldots, DNA_n$) which are transcribed into n messenger RNA types ($mRNA_1, \ldots, mRNA_n$) and translated into n protein species ($X_1, \ldots, X_n$) following the central dogma. Each protein can interact with the gene responsible for its expression (self-regulation) and/or with any other genes in the network (cross-regulation). In Fig. 1 we show the general scheme of the transcription-translation mechanisms for gene expression.

We assume that proteins are produced in bursts, which means that the life of each messenger RNA ($mRNA_i$) is much shorter than the life of the corresponding protein (X_i). Therefore, $\frac{\gamma_m^i}{\gamma_x^i} \gg 1$ for every $i = 1, \ldots, n$. Under this assumption, the multidimensional PIDE model formulated in Pájaro et al. (2017) describes the dynamics of a gene regulatory network with an arbitrary number n of genes with self and/or cross regulation. The generalized Friedman (or multidimensional PIDE) model reads:

$$\frac{\partial p}{\partial t}(t,\mathbf{x}) = \sum_{i=1}^{n} \frac{\partial}{\partial x_i}\left[\gamma_x^i x_i p(t,\mathbf{x})\right] + \sum_{i=1}^{n}\left(k_m^i \int_0^{x_i} \beta_i(x_i - y_i) c_i(\mathbf{y}_i) p(t,\mathbf{y}_i)\,\mathrm{d}y_i\right), \tag{1}$$

with $p : \mathbb{R}_+ \times \mathbb{R}_+^n \to \mathbb{R}_+ \backslash \{0\}$ being the temporal evolution of the protein probability density function, and β_i such that:

$$\beta_i(x_i - y_i) = \frac{1}{b_i} \exp\left[-\frac{(x_i - y_i)}{b_i}\right] - \delta(x_i - y_i)\,, \tag{2}$$

where $\mathbf{x} \in \mathbb{R}_+^n$ denotes a continuous approximation to the number of proteins and $\mathbf{y}_i$ is obtained from $\mathbf{x}$ by just replacing its ith component by y_i, (that is, $(\mathbf{y}_i)_j = x_j$ if $j \neq i$ and $(\mathbf{y}_i)_j = y_i$ if $j = i$). The parameter $b_i = \frac{k_x^i}{\gamma_m^i}$ represents the burst size for all $i = 1, \ldots, n$. δ is the Dirac delta function and $c_i(x)$ is an input function that describes the regulation exerted over gene DNA_i by the proteins expressed by the genes in the network, which is normally given by a general expression, G, of Hill functions (Alon, 2007; Pájaro et al., 2017):

$$c_i(\mathbf{x}) = G(\rho_{11}, \ldots, \rho_{1n}, \ldots, \rho_{n1}, \ldots, \rho_{nn}), \tag{3}$$

where ρ_{ij} is the probability of gene i to be in the off state by the action of protein X_j:

$$\rho_{ij}(x_j) = \frac{x_j^{H_{ij}}}{x_j^{H_{ij}} + K_{ij}^{H_{ij}}},\ i,j \in \{1, \cdots, n\} \tag{4}$$

with H_{ij} being the Hill coefficient of the regulation of gene i by protein j, and K_{ij} being the corresponding Hill constant.

Any generic distribution can be used as initial condition to solve equation (1):

$$p(0,\mathbf{x}) = p_0(\mathbf{x}), \tag{5}$$

with $p_0(\mathbf{x})$ being a probability density function. The generalized Friedman model, Eqn (1), described by a set of PIDEs can be numerically solved very efficiently by a semilagrangian scheme, which has been developed by (Pájaro et al., 2017) and available in the MATLAB toolbox SELANSI (Pájaro et al., 2018).

2.2 Control

The balancing of a genetic toggle switch around the unstable equilibrium was first performed by Lugagne et al. (2017) for a single cell, applying a PI controller to the deterministic model of Ordinary Differential Equations, or to single realizations of the SSA Gillespie (1977). The authors demonstrated the effectiveness of the control approach *in vivo*. Our purpose is to address the control of gene regulatory networks at the population level, applying feedback control to the PIDE model in order to drive the protein distributions to some predetermined state. More specifically, in this work we aim to stabilize the protein distribution of genetic toggle switches around an intermediate state (a state of low probability in the open loop system, which lies between the two more probable states of the bimodal distribution). In practice, considering a deterministic description of the system, this target state is often close to the unstable attractor of the deterministic system, and the two modes of the distribution lie close to the stable steady states of the deterministic model.

The achievements on the control of synthetic biology circuits mentioned in the Introduction (Briat et al., 2016; Aoki et al., 2019; Lugagne et al., 2017) rely on integral (I) and proportional-integral (PI) controllers. The full PID control algorithm (e.g. Åström and Hägglund, 1995) takes the following expression:

$$u(t) = K_P e(t) + K_I \int_0^t e(t)\mathrm{d}t + K_D \frac{\mathrm{d}e(t)}{\mathrm{d}t}, \qquad (6)$$

consists of three terms, respectively proportional to: the error, the integral of the error, and the error derivative. The reference state $y*$ is computed as the minimum between the two modes from the marginal distributions of the proteins. The modes of the marginal distributions are computed, M_i, in order to drive they to the target modes, y_i, being the error the difference between the last two quantities $y_i - M_i$.

The vector $y(t)$ denotes the mode of the distribution, and $y*$ is the reference mode chosen *a priori*, belonging to the separatrix (low probability barrier). This reference state $y*$ is computed as the minimum between the two modes from the marginal distributions of the proteins.

One of the most common experimental set-ups to perform exogenous control of synbio circuits relies on the use of microfluidic devices with flow controllers, in which the input concentrations of inducers of gene expression can be manipulated in real time (Lugagne et al., 2017).

3. RESULTS AND DISCUSSION

Next, we illustrate the methodology proposed to stabilize gene regulatory Toggle Switches at the population level, through two case studies of relevance to the synthetic biology field: i) a classical toggle switch relying on repressor proteins (this type of gene switch has been previously stabilized by Lugagne et al. (2017) at the single cell level), and ii) the CRISPRi Toggle Switch by Santos-Moreno et al. (2020), both in E. coli.

The classical toggle switch configuration consists of two repressible promoters, $P1$ and $P2$, arranged in a mutually inhibitory network. The expression of each of the promoters is tuned by the addition of the inducers I_1 and I_2 respectively, see Fig. 2.

We build the PIDE model (1) describing a toggle switch regulatory network mechanism in presence of molecular noise. See Table 1 where k_m^i, k_x^i, γ_m^i and γ_x^i are the

Table 1. Parameter values for the toggle switch PIDE model.

X_i	k_m^i	k_x^i	γ_m^i	γ_x^i	θ_{X_i}	θ_{I_i}
P1	12.0	50.4	8.4	1	31.94	11.65
P2	7.0	93.6	8.4	1	30.0	$9.06 \cdot 10^{-2}$

transcription, translation, mRNA degradation, and protein degradation rate constants, respectively. The parameters θ_{I_i} and θ_{X_i} are associated to the inducers effects in the proteins regulation, as in Lugagne et al. (2017). We consider that X_1 and X_2 are the $P1$ and $P2$ proteins, respectively.

Finally, the input functions $c(\mathbf{x})$ in 1 are given by:

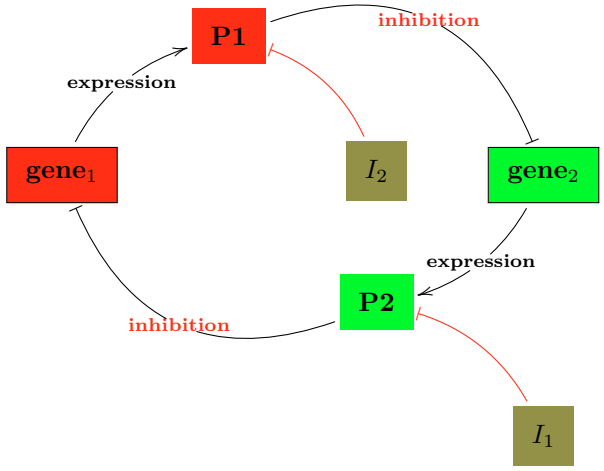

Fig. 2. Scheme of the regulatory network of the classical (relying on protein-based repressors) toggle switch. This configuration will lead to bistability for appropriate cooperativities and kinetic parameters ranges.

$$\begin{aligned} c_1(\mathbf{x}, I_1) =& (1 - \rho_{12}(x_2, I_1)) + \varepsilon_1 \rho_{12}(x_2, I_1), \\ c_2(\mathbf{x}, I_2) =& (1 - \rho_{21}(x_1, I_2)) + \varepsilon_2 \rho_{21}(x_1, I_2), \end{aligned} \qquad (7)$$

where the parameters ε_i represent a basal production from the inactive state, whose values are $\varepsilon_1 = \varepsilon_1 = 0.1$. The ρ_{ij} functions represent the probability of being in the off state the i promoter by the effect of the j protein, as defined in (4). Note that we have incorporated the effect of the inducers in the Hill constant in the ρ functions as follow:

$$\begin{aligned} \rho_{12}(x_2, I_1) =& \frac{x_2^H}{x_2^H + K_{12}(I_1)^H}, \\ \rho_{21}(x_1, I_2) =& \frac{x_1^H}{x_1^H + K_{21}(I_2)^H}, \end{aligned} \qquad (8)$$

with $H = 4$ and the inducer functions defined as:

$$\begin{aligned} K_{12}(I_1) =& \theta_{X_2} \left(1 + \left(\frac{I_1}{\theta_{I_1}}\right)^{\mu_{I_1}}\right), \\ K_{21}(I_2) =& \theta_{X_1} \left(1 + \left(\frac{I_2}{\theta_{I_2}}\right)^{\mu_{I_2}}\right). \end{aligned} \qquad (9)$$

The parameters for this last expression, (9), have been taken from Lugagne Lugagne et al. (2017), where $\mu_{I_1} = \mu_{I_2} = 2$ and the values for parameters θ are in Table 1.

The dynamics of the stochastic toggle switch, with kinetic parameters in Table 1, input functions (7), $H = 4$, $\varepsilon_i = 1$ and $\mu_{I_i} = 2$ for any i, is simulated with SELANSI (Pájaro et al., 2018), obtaining the stationary bimodal distribution in Fig. 3 in absence of inducers ($I_i = 0$).

Our control goal is to steer the probability distribution towards an intermediate state between the two open-loop stationary modes. As a first approximation we apply a simple bang-bang controller, by injecting inducers depending on the current state - target state relationship. Defining the target state by means of the dupla ($P1^*$, $P2^*$), and being I_1, I_2 the concentrations of the inducers, the control is defined as follows:

$$I_1(t) = \begin{cases} I_1^{max}, & \text{if } P2^* > P2(t) \\ I_1^{min}, & \text{if } P2^* \leq P2(t) \end{cases} \qquad (10)$$

$$I_2(t) = \begin{cases} I_2^{max}, & \text{if } P1^* > P1(t) \\ I_2^{min}, & \text{if } P1^* \leq P1(t) \end{cases} \qquad (11)$$

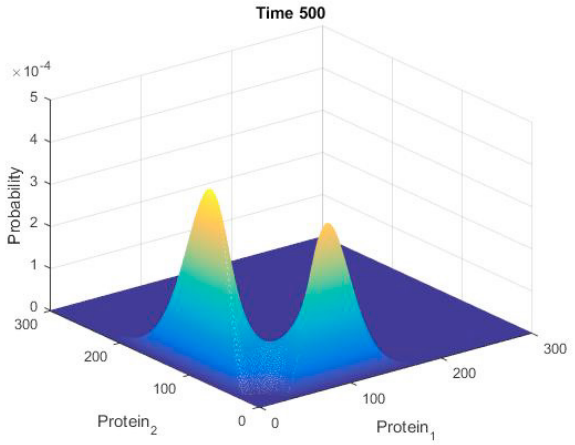

Fig. 3. Open loop stationary distribution ($t = 500$) for the repressor-proteins based stochastic toggle switch showing a bimodal distribution.

being $I_1^{min} = I_2^{min} = 0$, $I_1^{max} = 50$ and $I_2^{max} = 1$. After implementing this controller in SELANSI, the protein distribution has been stabilized in an intermediate state as it is shown in Fig. 4 (left column).

Then, a PI control is applied to drive the protein distribution to the same target, following the strategy described in the Methods section. We consider the expressions and coefficients in Lugagne et al. (2017) for the PI control:

$$I_1(t) = I_1^0 + K_P^L(P2^* - P2(t)) + + K_I^L \int_0^t (P2^* - P2(s))\mathrm{d}s \quad (12)$$

$$I_2(t) = I_2^0 + K_P^T(P1^* - P1(t)) + + K_I^T \int_0^t (P1^* - P1(s))\mathrm{d}s \quad (13)$$

with $K_P^L = 5, K_P^T = 2.5 \cdot 10^{-1}, K_I^L = 2 \cdot 10^{-5}, K_I^T = 6.94 \cdot 10^{-6}$, $I_1^0 = 20$ and $I_2^0 = 0.25$, and the same maximum and minimum values for both inducers as defined above.

After implementation, the results obtained can be seen in Fig. 4 (right column).

3.1 Stabilization of the CRISPRi toggle switch

The second case study is a CRISPRi based toggle switch introduced in Santos-Moreno et al. (2020), more precisely we use the structure that integrates the unspecific binding of dCassg1/2, given in Fig. 5. This structure is proven to lead to bistability (in the deterministic regime) by using the bioswitch toolbox Yordanov et al. (2020) where conditions and algorithms for bistability detection were implemented. We formulate the PIDE model describing the dynamic behaviour of the system in the stochastic regimes in the form of Eqn (1).

In the CRISPRi based toggle switch, genes are regulated by the dCassg1/2 complexes produced through the binding of dCas9 and sgRna1/2, which in turn are transcribed by genes G_1 and G_2. Since the PIDE includes the two steps of protein expression, transcription and translation, we additionally assume that the production rate of dCassg1/2 is proportional to sg1/2 by taking the concentration of Cas9 at steady state. In this way, the CRISPRi toggle switch stochastic dynamics can be adapted to the PIDE modelling framework. Moreover, the bistability of the deterministic toggle switch in Santos-Moreno et al. (2020) relays on the interplay between specific and unspecific binding of dCassg1/2 complexes to DNA. In order to incorporate this mechanism to our model, we derive Hill type input functions, c_i of the form:

$$c_1(\mathbf{x}) = \frac{1}{1 + \left(k_{12}\frac{x_1 x_2}{\alpha^2} + k_{22}\frac{{x_2}^2}{\alpha^2}\right) F(AHL)},$$
$$c_2(\mathbf{x}) = \frac{1}{1 + \left(k_{21}\frac{x_1 x_2}{\alpha^2} + k_{11}\frac{{x_1}^2}{\alpha^2}\right) F(Ara)}, \quad (14)$$

where x_1 and x_2 denote the amount of $dCassg1$ and $dCassg2$, respectively, which are obtained as:

$$dCassg1 = K_1 sg1 \quad \text{and} \quad dCassg_2 = K_2 sg2, \quad (15)$$

with K_1 and K_2 being two constants that we derive from the steady state of $dCas9$ in the deterministic model. The F expressions in (14) incorporating the effects of the AHL and Ara inducers take the following form:

$$F(AHL) = \frac{\left(\frac{AHL}{\theta_{AHL}}\right)^{\mu_{AHL}}}{1 + \left(\frac{AHL}{\theta_{AHL}}\right)^{\mu_{AHL}}}, \quad (16)$$

and

$$F(Ara) = \frac{\left(\frac{Ara}{\theta_{Ara}}\right)^{\mu_{Ara}}}{1 + \left(\frac{Ara}{\theta_{Ara}}\right)^{\mu_{Ara}}}. \quad (17)$$

In this model, the following parameter values have been considered: $k_{12} = 93.5831, k_{22} = 30.9418, k_{21} = 33.1719, k_{11} = 5.0981, K_1 = 0.1276, K_2 = 0.1171$ (these six values have been deduced from Santos-Moreno et al. (2020)), $\alpha = 10$ and for F we have worked with $\mu_{AHL} = \mu_{Ara} = 2$, $\theta_{AHL} = 5 \cdot 10^3$ and $\theta_{Ara} = 3 \cdot 10^3$.

The stochastic version of the CRISPRi toggle switch model is simulated into the SELANSI toolbox. We take into account the following assumptions for the parameters associated with transcription, translation, mRNA degradation and protein degradation $(k_m^i, k_x^i, \gamma_m^i, \gamma_x^i)$. First, the quotient between the translation factor and the product of both degradation rates must be equal to one ($\frac{k_x^i}{\gamma_m^i \gamma_x^i} = 1$ for $i = 1, 2$). With the protein at steady state, it follows that the translation factor must be equal to the rate of protein degradation ($k_x^i = \gamma_x^i$ for $i = 1, 2$). Finally, the burst condition ensures that the rate of mRNA degradation is greater than the rate of protein degradation ($\gamma_m^i < \gamma_x^i$) and that the α factor in Eqn 14 allows us to multiply the translation parameter by α. Therefore, we obtain the following values for the parameters of this network, $k_x^i = 10$, $\gamma_m^i = \gamma_x^i = 1$ for $i = 1, 2$, $k_m^1 = 50.72434$ and $k_m^2 = 50.33746$. After these considerations, the PIDE model is simulated in SELANSI, obtaining (in open loop) the bimodal stationary distribution in Fig. 6, with $AHL = 10^5$ and $Ara = 10^5$.

In order to stabilize the system around the unstable equilibrium, we apply PI control as described in the Methods section.

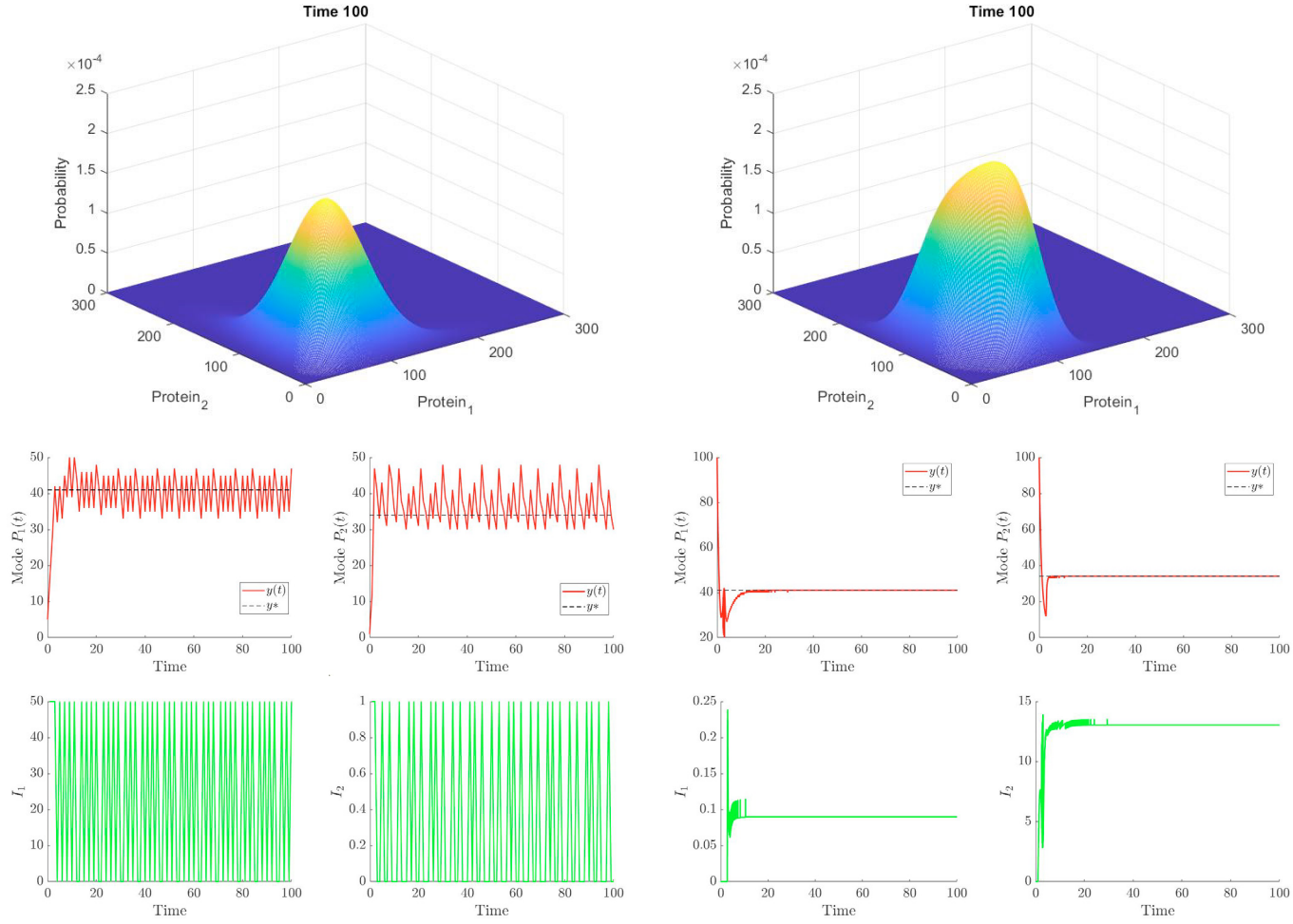

Fig. 4. Closed-loop balancing of the repressor-protein based Toggle Switch by bang-bang control (left column) and PI control (right column). The probability distributions at the stationary state are depicted (top), together with the evolution of "mode X_1" and "mode X_2" (mid) dependent on the evolution of I_1 and I_2 inducers over time (bottom).

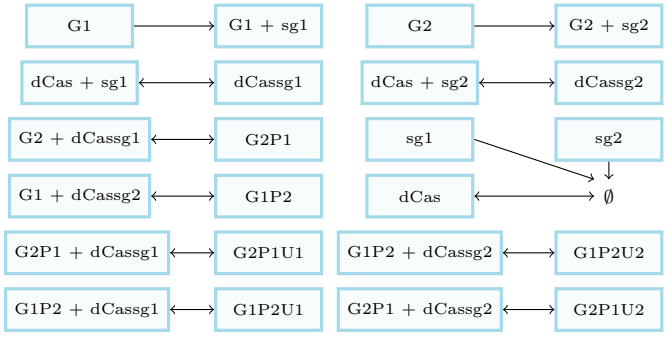

Fig. 5. Biochemical reaction network of the CRISPRi toggle switch by Santos-Moreno et al. (2020), where the dCassg1/2 complexes can bind to a specific target site in G1 and G2 respectively, or unspecifically via PAM sequences to G1/2.

The PI control expressions read:

$$AHL(t) = K_P^1 e_1(t) + K_I^1 \int_0^t e_1(s)ds \tag{18}$$

$$Ara(t) = K_P^2 e_2(t) + K_I^2 \int_0^t e_2(s)ds \tag{19}$$

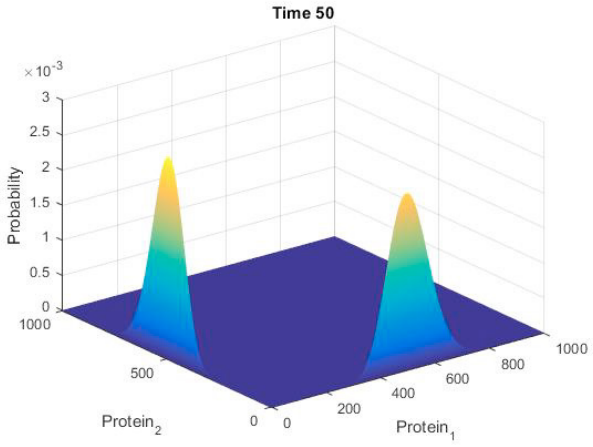

Fig. 6. Steady-state bistability of the CRISPRi model, for a final time of t=50.

where $K_P^1 = -3.60, K_I^1 = 1.6836 \cdot 10^{-11}, K_P^2 = -3.60$ and $K_I^2 = 7.7942 \cdot 10^{-11}$, obtaining the results for the closed loop system illustrated in Fig. 7.

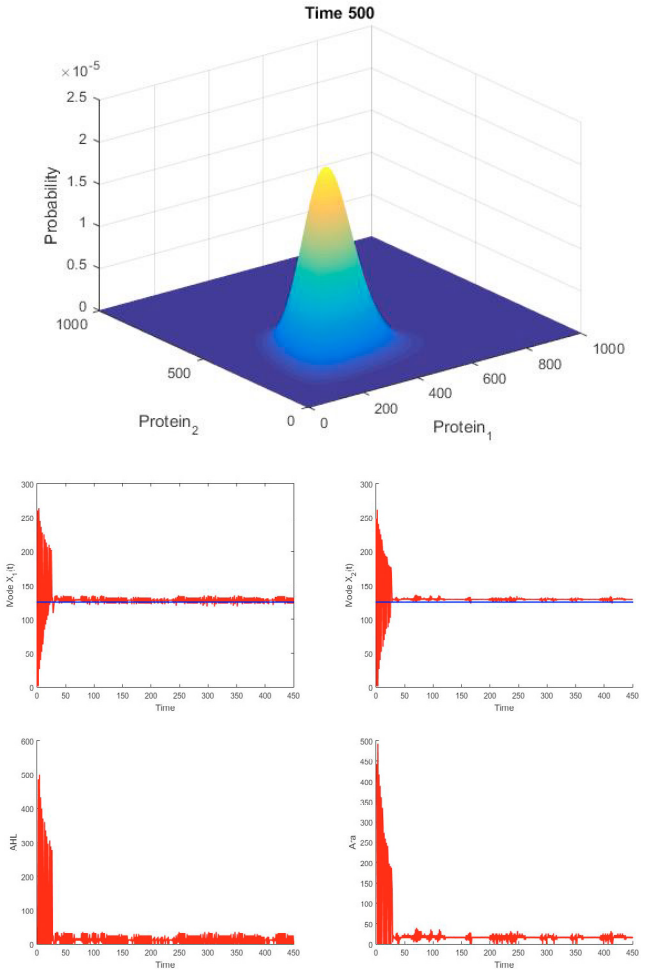

Fig. 7. Closed loop balancing of the CRISPRi Toggle Switch by PI control. Stationary probability distribution (top), evolution of the mode of the probability distribution, "mode X_1" and "mode X_2" (mid) and evolution of the AHL and Ara inducers over time (bottom).

4. CONCLUSIONS

This paper addresses the feedback control of stochastic gene regulatory networks using PIDE models. We have solved a control problem of interest in synthetic biology, the stabilization of bimodal (stochastic) toggle switches within the region of low probability of the uncontrolled system. The control of bistable and bimodal biomolecular systems is also of interest in the context of reaction networks Alonso and Szederkényi (2016). In this approach, we use PI control, obtaining a good result *in silico* by using the marginal distributions to compute the error. We have achieved the desired closed-loop response with a control policy that can be implemented in a microfluidic platform. Importantly, this illustrates the potential of PIDE models for the control of stochastic gene regulatory networks. In a future work, we plan to design more advanced (nonlinear) controllers, to the toggle-switch stabilization problem addressed here, as well as to other control problems of interest in the context of synthetic biocircuits. Moreover, we plan to test *in vivo* the control strategies combining a microfluidic platform with time-lapse microscopy.

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