External control of a genetic toggle switch via Reinforcement Learning

Sara Maria Brancato¹, Francesco De Lellis¹, Davide Salzano¹, Giovanni Russo²∗, Mario di Bernardo¹∗

Abstract—We use a learning-based strategy to stabilize a synthetic toggle switch via an external control approach, adopting a sim-to-real paradigm to overcome the data efficiency problem that would render the algorithm infeasible for practical use in synthetic biology. Here, the policy is learnt via training on a simple model capturing the main dynamical features of a toggle switch and is then validated in-silico using stochastic agent-based simulations. Our in-silico experiments confirm the viability of the approach suggesting its potential use for in-vivo control implementation in microfluidics.

I. INTRODUCTION

The problem of conceiving controllers to manage the dynamics of synthetic biological systems is becoming crucial in synthetic biology (see [17, 6]) giving rise to the field of Cybergenetics, as presented in [22]. A possible approach towards the implementation of control algorithms for living cells, termed external control, relies on providing external stimuli (in the form of light or chemical species) according to some policy coded in a PC or microcontroller. Examples of this paradigm include those presented in [28, 20, 24]. (See [17] for a review of other results from the literature.) Model-based controllers, such as Proportional Integral (PI) or Model Predictive Control (MPC) strategies, are the most used external controllers (see [13] for a comparison of different techniques), despite the fact that there is uncertainty in modeling the system under control and the sensed data are noisy [6]. An alternative approach to overcome the need for an accurate, well calibrated mathematical model is to leverage learning-based control methods to learn the policy by directly interacting with the system. However, the learning process can be sample inefficient, requiring long times and a large number of experimental data to learn the policy, which could hinder its use in biology (see [2, 1]).

A possible solution to learn a control policy without the need for a large set of experimental data comes from the sim-to-real approach introduced in [16], where the control policy is learnt on simulated environments and subsequently exported on the real system. To apply this approach, the gap between simulations and real world experiments must be closed.

In this paper, we use, as a case study to validate this approach in synthetic biology, the problem of balancing a synthetic toggle switch about its unstable equilibrium point. Such a problem is a widely adopted benchmark in control applications to synthetic biology, and has been proposed as the equivalent in synthetic biology of the swing-up problem of an inverted pendulum [20]. It is also a problem of practical interest as this bistable system is a fundamental synthetic circuit to endow cells with memory-like features [15] or to differentiate mono-strain cultures in different populations [19, 23].

Recently, this regulation problem was solved via model-based open loop external control, which exhibits poor robustness with respect to parameter variations [20]. Moreover, based on a reduced model developed in [8], a closed-loop proportional-integral pulse width modulation controller (PI-PWM) and a MPC strategy were proposed to stabilize the unstable equilibrium of a toggle switch in [13]. Although exhibiting good performance and robustness properties, the PI-PWM requires the offline calibration of the control gains via an exhaustive search. On the other hand, the MPC requires adequate computing power from the experimental platform in order to cope, in real-time, with the computationally demanding optimization. In addition, both of these strategies heavily rely on model identification of the system dynamics, which is usually subject to uncertainties in structure and parameters [3]. Similarly, in [4], a piecewise linear switched control is designed to either stabilize a simplified toggle switch model or to switch between its stable equilibria.

Despite the many results available, the literature on solving this problem via learning-based control is sparse. To the best of our knowledge, some results in this direction were reported in [26], where fitted Q-learning was used to toggle the switch between its stable equilibria, and only later in [25], where an extension of the approach to track periodic references was reported.

Here, we explore the use of tabular learning methods to regulate a Toggle Switch onto its unstable equilibrium, accounting for realistic actuation and sensing constraints imposed by a microfluidic based experimental set-up. In line with the sim-to-real approach, we learnt a policy training an agent using a simplified model of the toggle switch, validating then the controller via realistic agent-based simulations. Note that, even though partial knowledge of the system’s dynamics is needed, a simple yet accurate model capturing the main dynamical features of a Toggle Switch can be calibrated using a limited number of open-loop experiments. We show via a set of exhaustive in-silico experiments that the sim-to-real gap can be filled and that the control performances learnt using a simple, inaccurate model can be transferred to realistic agent-based simulations. To benchmark our results against other state-
of-the-art algorithms for the toggle switch stabilization, we define and use a set of appropriate control metrics that confirm the effectiveness and viability of the proposed approach.

II. THE CONTROL PROBLEM

The genetic toggle switch is a gene regulatory network first engineered in E.coli in the early 2000s by [10]. It is constructed around two repressors, LacI and TetR, each inhibiting the expression of the other (Fig. 1). The double inhibition chain enables the creation of hysteresis, making this circuit a simple implementation of a binary memory element. From a dynamical system viewpoint, this genetic pathway is a bistable dynamical system, exhibiting at steady-state either of two stable phenotypes where one of the repressors is fully expressed and the other is scarcely present. The steady-state phenotype being exhibited can be modified by laboratory interventions that induce the transition between the two stable states. Specifically, by adding two chemical inducers that diffuse through the cell membrane, i.e. anhydrotetracycline (aTc) and isopropyl-β-D-thiogalactoside (IPTG), it is possible to sequestrate TetR and LacI, respectively, relieving their inhibition on the competing repressor and toggling the system state. In this work, we consider the problem of balancing the genetic toggle switch around its unstable equilibrium by modulating the concentration of the inducers in the growth environment.

We address this problem by learning the policy via a model-free reinforcement learning algorithm. In our design, we explicitly account for biological and technological constraints of a real experimental set-up [24, 7], schematically shown in Fig. 2. Here, the external control loop is implemented via a combination of microfluidics and inverted microscopy, through which it is possible to measure the fluorescence reporters expression level and to dynamically modify the composition of the growth medium. Specifically, the expression levels of the fluorescent reporters transcribed together with the repressors are measured at a single-cell level by leveraging image segmentation algorithms. These signals are then compared with the desired set point and a control input is decided. Finally, a system of actuated syringes is used to deliver the appropriate concentration of the media to the microfluidic chip via hydrostatic pressure. This experimental setup introduces constraints on the time interval we can use to sample the state of the toggle switch and on the structure of the control input we can use to deliver to the cells. Namely, the constraints are:

C1. The state of each cell can be sampled up to once every 5 min, to avoid excessive phototoxicity.
C2. The concentration of the inducer molecules (i.e. the control inputs) can only be changed once every 15 min to limit osmotic stress on the cells.
C3. The inputs need to be a convex combination of their concentration present in the reservoirs. This is due to the specific implementation of the microfluidic device of choice.

III. CONTROL DESIGN

We propose the use of a Q-learning algorithm (QL) in its classical tabular version to train an artificial agent using a standard ε-greedy policy as described in [29]. Differently from [26], the goal here is to stabilize the switch onto its unstable equilibrium rather than toggling it from one stable equilibrium to the other. To overcome the challenge of limited in-vivo experiments to learn the control policy, we carry out the training using synthetic data generated via a simplified model of the switch, which can be parametrized using a limited number of open-loop experiments. We considered for the training the succeeding model from [8]:

![Experimental set-up. A microfluidic device hosts a population of E.coli endowed with a genetic toggle switch. A microscope measures the average fluorescence levels of the reporter proteins RFP and GFP. This information is fed to the trained artificial agent that computes the control input. The control signal is delivered via two motorized syringes.](image-url)
\[
\begin{align*}
\frac{dx_1}{dt} &= k_1^0 + \frac{k_1}{1 + x_2^2} \left(1 + u_1\right) - x_1 \quad (1a) \\
\frac{dx_2}{dt} &= k_2^0 + \frac{k_2}{1 + x_1^2} \left(1 + u_2\right) - x_2 \quad (1b)
\end{align*}
\]

where \(x_1, x_2, u_1, u_2\) are the non-dimensional concentrations of the proteins LacI and TetR and of the inducers IPTG and aTc. In our design, we fulfilled constraints C1 and C2 (see Sec. II) by allowing the learning algorithm to measure the state of the system and modify the control inputs only once every 15 minutes. Finally, we fulfilled constraint C3 by enforcing the following conditions on \(u_1\) and \(u_2\):

\[
u_1 = \phi u_{1,\text{max}}, \quad u_2 = (1 - \phi) u_{2,\text{max}}
\]

The QL was then used to learn the policy adjusting \(\phi \in [0, 1]\) (rather than for \(u_1\) and \(u_2\)). In equation (2), \(u_{i,\text{max}}, i \in \{1, 2\}\), are the maximum values allowed for the inducers’ concentrations, chosen to be at 35 ng/mL for aTc and at 0.35 mM for IPTG so as to enable bistability when \(\phi = 0.5\) and monostability when \(\phi = 0 \land \phi = 1\). For the sake of comparison, we assumed, as in [13], that the goal is to stabilize the system around the unstable equilibrium point \(x_{\text{ref}} = 23.48, 10.00\)^T, corresponding to \(\text{Lac}_{\text{ref}} = 750\), \(\text{Tet}_{\text{ref}} = 300\).

We discretized the action-state space \(\{x_k, \phi_k\}\) as follows. The action space was sampled in 11 equally spaced values, while the states were discretized non-uniformly in the region \((z_1, z_2) \in [0, 150] \times [0, 150]\). Specifically, we discretized each state in the region \((x_1, x_2) \in [x_{1,\text{ref}} - 3, x_{1,\text{ref}} + 3] \times [x_{2,\text{ref}} - 3, x_{2,\text{ref}} + 3]\) with a step of 0.5, while using a discretization step of 1.5 elsewhere. By doing so, the artificial agent has increased precision around the set point \(x_{\text{ref}}\), reducing the steady-state error.

As reward function, we selected the quadratic function

\[
r(x_k) = - \left( \left( \frac{x_{1,\text{ref}} - x_{1,k}}{x_{1,\text{ref}}} \right)^2 + \left( \frac{x_{2,\text{ref}} - x_{2,k}}{x_{2,\text{ref}}} \right)^2 \right)
\]

describing the relative squared error. We trained the virtual agent by running \(S = 10\) trials of \(E = 35000\) episodes each. Each episode consists of an in-silico control experiment lasting \(T = 72\) hours, starting from random, uniformly sampled, initial conditions. We empirically set the learning rate as \(\alpha = 0.8\), the probability of taking an exploratory move \(\epsilon = 0.1\) and the discount factor \(\gamma = 0.9\).

IV. IN SILICO VALIDATION

With the aim of showing that the sim-to-real gap can be filled, we validated the performance of the control policy learnt on the simpler model (1) by applying it to control an accurate model of the Toggle Switch parameterized on in vivo experimental data which was derived in [20]. First, we considered a deterministic description describing the response of the circuit at a population level. Subsequently, we tested the control algorithm taking into account noise intrinsic to biochemical systems (see Appendix VI for details on the models). Finally, we leveraged an agent-based simulator to include in our numerical experiments cells bio-mechanics, growth and division, as well as a realistic spatiotemporal evolution of chemical inducers provided in the culture media, complementing the simulation of the synthetic network dynamics with accurate modeling of the cells’ growth environment.

A. Metrics

We compared the performance of the control algorithm developed in this work with the PI-PWM and MPC strategies presented in [13]. Specifically, we quantified the effectiveness of each control strategy using the set of metrics introduced in [9, 13]. Namely, we computed the Integral Square Error (ISE), defined as

\[
ISE = \int_{t_0}^{T} \tau(\tau)^2 d\tau,
\]

which measures the average transient and steady-state performances, the Integral Time-weighted Absolute Error (ITAE) defined as

\[
ITAE = \int_{t_0}^{T} \tau |\tau(\tau)| d\tau
\]

which weighs the error more as time progresses, making residual errors at steady-state more relevant to the computation of the control metric.
In Equations (4)-(5) $\dot{e}$ is computed as

$$\dot{e}(t) = \left[ \frac{\text{LacI} - \text{LacI}_{\text{ref}}}{\text{LacI}_{\text{ref}}} - \frac{\text{TetR} - \text{TetR}_{\text{ref}}}{\text{TetR}_{\text{ref}}} \right]_t^w$$

(6)

where LacI(t) and TetR(t) are the evolutions of the concentrations of the proteins, whereas LacI(0) and TetR(0) are the corresponding moving averages over a window of width $w = 240\text{min}$, formally defined as

$$\text{LacI}(t) = \frac{1}{w} \int_{t-w}^{t} \text{LacI}(\tau) d\tau,$$

$$\text{TetR}(t) = \frac{1}{w} \int_{t-w}^{t} \text{TetR}(\tau) d\tau.$$

Both metrics are evaluated from $t_0 = t_w$ up to the control horizon $T$.

B. Deterministic experiments

The designed control strategy successfully stabilizes the unstable equilibrium point over all the training trials (see Figure 3). Table I shows a quantitative comparison between the strategy presented here with the MPC and PI-PWM previously described in [13]. We see that the performances achieved by the QL-based controller are comparable with the model-based ones. In particular, the model-free QL is capable of outperforming the PI-PWM in terms of ISE, reducing the variability of the proteins during the transient.

C. Stochastic experiments

We further validated our control strategy to account for noise coming from the stochastic nature of biochemical processes, as it plays a fundamental role in the transient and asymptotic behavior of synthetic circuits [27]. We selected the Q-table giving the best performance in terms of ITAE and ISE in the deterministic setting, testing its robustness by running a set of in-silico experiments using the stochastic model of the toggle switch using a Euler-Maurayama scheme (for more detail see Appendix VI) with $dt = 0.1$ (see [14] for details on the algorithm). The outcome of the in-silico experiments shown in Fig. 4a confirms the ability of the controller to regulate the Toggle Switch even in the presence of noise. Furthermore, Table I shows that the QL-based controller has slightly better performance when compared with the PI-PWM in the presence of stochasticity, confirming the viability of a sim-to-real paradigm in a biological setting. However, the MPC still outperforms the other control approaches. This is because the algorithm uses a perfectly accurate representation of the genetic circuit for the prediction, which is unrealistic when performing in vivo experiments.

D. Agent-based in-silico experiments

As a final step towards bridging the sim-to-real gap, we used the agent-based platform Bsim [12] to mimic a microfluidic platform, where cells are confined in a small growth chamber and nutrients are constantly provided, allowing the cells to grow in exponential phase. Specifically, we simulated a platform made of a microfluidic device, an inverted microscope, a computer and a set of actuated syringes. The microfluidic chip proposed in [5] is used to trap and grow bacteria. Our agent-based simulations (Figure 4b) confirm the viability of this strategy for in-vivo experiments, ensuring long-term stabilization of the protein levels around the desired equilibrium. Also, the control metrics in Table I highlight how the model-free QL is capable of outperforming the model-based PI-PWM in terms of ISE and ITAE, reducing the variability either in transient and at the steady state. The MPC still performs better but requires good knowledge of the model as shown by the worse performance it exhibits when the simulated circuit differs from the predicted dynamics (e.g. ISE = 178.50 vs ISE = 47.58). Indeed, while the performances of the QL policy do not deteriorate significantly when spatial effects are considered, the increased mismatch between model prediction and system evolution induces a consistent worsening of the MPC performances.

V. CONCLUSION

We investigated the problem of stabilizing the unstable equilibrium of a genetic toggle switch via an external control approach based on machine learning. To overcome the data efficiency problem that would render the algorithm unfeasible for practical use in synthetic biology, we adopted and tested in-silico the use of a sim-to-real paradigm. More precisely, the policy is first learnt via training on a simplified model of the toggle switch and it is then validated numerically. Our in-silico experiments confirm the viability of this approach as a reliable, model-free controller that can regulate the expression levels of the two repressors at the core of the genetic Toggle Switch. This represents a crucial step towards the deployment of learning algorithms to control synthetic biological circuits that can work in vivo. Ongoing research is aimed at testing the proposed strategy in vivo using the microfluidics platform described in [21].

APPENDIX

VI. MATHEMATICAL MODEL

We introduce the mathematical model for the toggle switch that we used for the in-silico validations. Letting mRNA$_{\text{LacI}} \in \mathbb{R}_{\geq 0}$ and mRNA$_{\text{TetR}} \in \mathbb{R}_{\geq 0}$ be the concentration of the mRNA associated with the lacI and tetR genes,

| PI-PWM | QL | MPC |
|--------|----|-----|
| **Deterministic in-silico experiments** | | |
| ISE | 876.71 | 707.04 | 47.58 |
| ITAE | 2.07E$+6$ | 3.58E$+6$ | 0.81E$+6$ |
| **Stochastic in-silico experiments** | | |
| ISE | 830.52 | 788.12 | 178.50 |
| ITAE | 2.07E$+6$ | 3.72E$+6$ | 1.98E$+6$ |
| Bsim in-silico experiments | | |
| ISE | 961.29 | 861.71 | 277.18 |
| ITAE | 4.46E$+6$ | 4.13E$+6$ | 1.95E$+6$ |

TABLE I

Control performance comparison via the metrics introduced in the IV-A, for deterministic and stochastic experiments.
LacI ∈ ℝ>0 and TetR ∈ ℝ≥0 the concentration of LacI and TetR, using the pseudo reactions from [20], the dynamics of the system can be described via the following set of ODEs:

\[
\begin{align*}
\frac{d\text{mRNA}_{\text{LacI}}}{dt} &= k_L^{m0} + \frac{k_L^m}{1 + \left(\frac{\text{TetR}}{\theta_{\text{TetR}}}/a_{\text{aTc}}\right)} - g^{\text{LacI}} \text{mRNA}_{\text{LacI}}, \\
\frac{d\text{mRNA}_{\text{TetR}}}{dt} &= k_T^{m0} + \frac{k_T^m}{1 + \left(\frac{\text{LacI}}{\theta_{\text{LacI}}}/\text{IPTG}/\theta_{\text{IPTG}}\right)} - g^{\text{mRNA}_{\text{TetR}}}, \\
\frac{d\text{LacI}}{dt} &= k_L^p \text{mRNA}_{\text{LacI}} - g^{\text{LacI}} \text{LacI}, \\
\frac{d\text{TetR}}{dt} &= k_T^p \text{mRNA}_{\text{TetR}} - g^{\text{TetR}} \text{TetR};
\end{align*}
\]

where \(k_L^{m0}, k_L^m, k_T^{m0}, k_T^m, g_L^{m0}, g_L^m, g_T^{m0}, g_T^m\) are basal transcription, maximal transcription, translation, mRNA degradation and protein degradation rates, respectively. Parameters \(\theta_i\) and \(\eta_i\), \(i \in \{\text{LacI, TetR, aTc, IPTG}\}\) are the thresholds and the cooperativity coefficients of the hill functions. aTc ∈ ℝ>0 and IPTG ∈ ℝ>0 are two inputs: the intra-cellular concentrations of aTc and IPTG, respectively. Note that aTc and IPTG cannot be directly manipulated as we can only modify the concentration of the inducers in the culture media, say \(u_{aTc} \in \mathbb{R}_{≥0} \) and \(u_{\text{IPTG}} \in \mathbb{R}_{≥0}\). Extra-cellular and intra-cellular inducer concentrations are linked together through the diffusion dynamics across the membrane. Specifically, as in [20], we complemented the model in (7) with equations describing diffusion:

\[
\begin{align*}
\frac{da_{\text{aTc}}}{dt} &= \begin{cases} 
  k_{i\text{aTc}}^i (u_{a_{\text{aTc}}}) - a_{\text{aTc}} & \text{if } u_{a_{\text{aTc}}} > a_{\text{aTc}} \\
  k_{o\text{aTc}}^o (u_{a_{\text{aTc}}}) & \text{if } u_{a_{\text{aTc}}} \leq a_{\text{aTc}}
\end{cases} \\
\frac{d\text{IPTG}}{dt} &= \begin{cases} 
  k_{i\text{IPTG}}^i (u_{\text{IPTG}}) - \text{IPTG} & \text{if } u_{\text{IPTG}} > \text{IPTG} \\
  k_{o\text{IPTG}}^o (u_{\text{IPTG}}) & \text{if } u_{\text{IPTG}} \leq \text{IPTG}
\end{cases}
\end{align*}
\]

By using time scale separation between the dynamics of mRNAs and proteins is possible to derive the simplified model used in section III, as done in [8]. In addition, in model (1) we introduce normalized time protein concentrations, say \(x_1 = \frac{\text{LacI}}{\theta_{\text{LacI}}}, x_2 = \frac{\text{TetR}}{\theta_{\text{TetR}}}\), \(t' = g^p t\), and cluster the parameters defining \(k_1^0 = (k_L^{m0} k_L^m)/(g_L^p \theta_{\text{LacI}} g^p), k_2^0 = (k_T^{m0} k_T^m)/(g_T^p \theta_{\text{TetR}} g^p), k_2 = (k_L^p)/(g_L^p \theta_{\text{LacI}} g^p)\) and \(k_2 = (k_T^p)/(g_T^p \theta_{\text{TetR}} g^p)\). The values of all the parameters used in the in-silico experiments are reported in Table II.

A. Stochastic model

In our in-silico validations, we also consider the stochastic version of (7) obtained from the Chemical Master Equation [11, 23, 20]. The stochastic dynamics of the toggle switch can be described as

\[
d\mathbf{X}(t) = S \cdot \mathbf{a}(\mathbf{X}(t)) \cdot dt + S \cdot \text{diag}(\sqrt{s}(\mathbf{X}(t))) \cdot d\mathbf{W}
\]

where \(\mathbf{X}(t) = [\text{mRNA}_{\text{LacI}}, \text{mRNA}_{\text{TetR}}, \text{LacI}, \text{TetR}], \)
\(d\mathbf{W} \in \mathbb{R}^8\) are independent standard Wiener process increments, \(S\) is the stoichiometric matrix and \(\mathbf{a}(\cdot)\) is the vector og the propensities associated to each reaction [18]. Given the pseudoreactions introduced in [20], we define \(S = \begin{bmatrix} 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix}\) and
\[
\alpha (\cdot) = [k_{L}^{m0} + k_{L}^{m0} f_1 (\text{TetR}, \text{aTc}), k_{T}^{m0} + k_{T}^{m0} f_2 (\text{LacI}, \text{IPTG}),
\]
\[k_{L}^{P} \text{mRNA}_{\text{LacI}}, k_{T}^{P} \text{mRNA}_{\text{TetR}}, g_{L}^{m0} \text{mRNA}_{\text{LacI}}, g_{T}^{m0} \text{mRNA}_{\text{TetR}},
\]
\[g_{L}^{P} \text{LacI}, g_{T}^{P} \text{TetR}]^{T}, \text{ where } f_1 = \frac{k_{L}^{P}}{1 + \left( \frac{\text{aTc}}{\text{K}_{\text{aTc}}} \right)^{\theta_{\text{aTc}}}} \text{ and } f_2 = \frac{k_{T}^{P}}{1 + \left( \frac{\text{IPTG}}{\text{K}_{\text{IPTG}}} \right)^{\theta_{\text{IPTG}}}}.
\]

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| Table II
| Parameter | Value |
|-----------|-------|
| $k_{m0}^{L}$ | $3.20E^{-2}$ | mRNA $\text{min}^{-1}$ |
| $g_{m0}^{L}$ | $1.19E^{-1}$ | mRNA $\text{min}^{-1}$ |
| $g_{m0}^{T}$ | $8.30$ | mRNA $\text{min}^{-1}$ |
| $k_{m0}^{P}$ | $2.06$ | mRNA $\text{min}^{-1}$ |
| $k_{T}^{m0}$ | $9.72E^{-1}$ | a.u. |
| $g_{T}^{m0}$ | $31.94$ a.u. |
| $g_{T}^{P}$ | $2.00$ |
| $k_{T}^{P}$ | $9.72E^{-1}$ | a.u. |
| $g_{L}^{P}$ | $30.00$ a.u. |
| $k_{L}^{P}$ | $2.75E^{-1}$ | a.u. |
| $g_{L}^{m0}$ | $1.62$ | min $^{-1}$ |
| $g_{L}^{P}$ | $2.00E^{-2}$ | min $^{-1}$ |

TABLE II

VALUE OF THE PARAMETERS FOR THE TOGGLE SWITCH. ALL PARAMETERS ARE TAKEN FROM [20].