Population-Level Model of the Transient Dynamics of a Toggle Switch with Growth Feedback

Dylan Hirsch[1] and Domitilla Del Vecchio[2]

***Abstract*— Bistable genetic circuits that can be toggled between two states have been engineered in bacterial cells for a variety of applications. These circuits often impose state-dependent resource loads on the cell, creating growth feedback. In the context of a population of cells, each with a copy of the genetic circuit, cells in either circuit state grow at different rates, thereby affecting the emergent population-level dynamics. It is generally difficult to predict how this growth heterogeneity will affect the composition of the population over time. In this work, we consider an ODE population model and evaluate its ability to predict the transient dynamics of the fraction of cells in either state. These dynamics are driven by two processes. The first is due to the difference in growth rate between the cells in the two states, while the second process arises from the probability that the circuit switches state. For the latter, we compute switching rates for the toggle switch using a Markov chain two-dimensional model and exploit the system's structure for efficient computation. We demonstrate via simulations that the ODE model well approximates the dynamics of the system obtained by a published population simulation algorithm for sufficiently large molecular counts and population sizes. The ability to approximate via ODEs the population-level dynamics of cells engineered with multi-stable circuits will be especially relevant to forward engineer such circuits for desired population dynamics.**

I. INTRODUCTION

Understanding how the feedback between a genetic circuit and cellular growth rate affects population-level dynamics is a significant challenge in synthetic biology. For example, genetic toggle switches (Fig. 1a) are used to produce cellular memory, with exposure to some signal causing the cell to preferentially adopt one of two stable states [1]. Due to molecular noise, a cell will eventually switch between these stable states, losing memory of the earlier signal. As a result, a population of cells initially biased to one of the stable states will ultimately return to an equilibrium independent of its initial state, with possible coexistence of both states. When one of the proteins used to implement the toggle switch places a higher burden on the cell growth rate (Fig. 1b), however, cells producing high levels of that protein will grow slower, thereby affecting the dynamics of the population [2], [3].

Monte Carlo methods can be used to simulate the behavior of cell populations in the presence of growth feedback [2]. In particular, the "Next Family" method can be used to run an adaptation of the Stochastic Simulation Algorithm (SSA) at a population level in $\mathcal{O}(NlogN)$ time, where N is the number of cells in the population [2], [4]. Even this method, however, can be computationally intensive when N is large or when the time between reactions is small compared to the overall simulation time. Most importantly, simulation-based approaches offer little insight to forward engineer a genetic circuit that results in desired population-level dynamics.

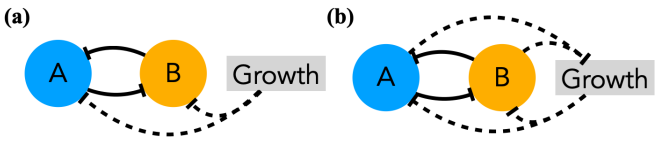

Fig. 1. (a) Circuit diagram for a bistable toggle switch with proteins A and B that each repress the production of the other. A and B concentrations in the cell are also lowered due to dilution. (b) Circuit diagram for a toggle switch in which each protein also suppresses cellular growth rate, creating a feedback loop between growth rate and circuit state.

In [2], an ordinary differential equation (ODE) model was used to approximate the steady-state fraction of cells in either state of the toggle switch. However, setting the rate constants of this ODE model requires repeatedly simulating a single cell via Monte Carlo. Additionally, the approximation accuracy of this model was only investigated at the population's steady-state, without evaluation during the transient. In this work, we introduce a similar ODE model to approximate the subpopulation fraction dynamics of both states. Unlike [2], we set the model parameters without requiring simulation, but instead by computing suitable hitting times in a Markov chain model of the toggle switch circuit. Like in [2], we will assume throughout that the cells are exponentially growing in a fixed-sized population, with a cell removed from the population at random each time a cell divides. We refer to such a setting as an "ideal turbidostat" [5]. We evaluate via simulation how the agreement between the ODE model and the Monte Carlo model changes as molecular counts in each cell are increased and as population size increases. We demonstrate via simulation that the approximation error can be made small for sufficiently large molecular counts and population sizes.

The paper is structured as follows: in Section II, we describe the system; in Section III, we introduce the ODE model and parameterization procedure; in Section IV, we define our approximation metric; in Section V, we compare the ODE model output to simulations from the Next Family method.

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[1] Dylan Hirsch is with the Department of Biological Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA dylanhir@mit.edu

[2] Domitilla Del Vecchio is with the Department of Mechanical Engineering, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA ddv@mit.edu

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II. SYSTEM DESCRIPTION AND ASSUMPTIONS

In this section, we describe the system under investigation, which is composed of a population of N cells, each with an identical copy of a genetic toggle switch. Each cell can stochastically produce and eliminate A and B molecules and divide. Upon division, a cell produces a daughter cell with the same number of A molecules and the same number of B molecules. The daughter cell replaces another cell in the population at random, keeping the population size fixed. Below, we introduce the determinstic model for a toggle switch with growth feedback, and then we precisely describe the stochastic population model we take as ground truth for evaluation of the ODE approximation.

A. Deterministic toggle switch model

We consider the following ODE model for a toggle switch with growth feedback:

$$\frac{d}{dt}[A] = \sigma\beta_A \frac{1}{1+\left(\frac{[B]}{\sigma K_A}\right)^2} - (\gamma'_\sigma([A],[B]) + \delta_A)[A] \tag{1}$$

$$\frac{d}{dt}[B] = \sigma\beta_B \frac{1}{1+\left(\frac{[A]}{\sigma K_B}\right)^2} - (\gamma'_\sigma([A],[B]) + \delta_B)[B] \tag{2}$$

$$\gamma'_\sigma([A],[B]) = \frac{\gamma}{1+\frac{[A]}{\sigma J_A}+\frac{[B]}{\sigma J_B}}, \tag{3}$$

in which $[A]$ and $[B]$ are the concentrations of proteins A and B within the cell, $\sigma\beta_X$, σK_X, σJ_X, and δ_X are, respectively, the maximal production rate, DNA dissociation constant, growth feedback parameter, and degradation constant of protein X ($X \in \{A,B\}$), and γ is the basal growth rate constant [1], [2], [3]. Here, $\sigma > 0$ is a unitless concentration-scaling factor. Indeed, by dividing the left and right hand sides of (1) and (2) by σ and defining $\overline{[A]} = [A]/\sigma$ and $\overline{[B]} = [B]/\sigma$, we obtain a σ-independent toggle switch model described by the scaled quantities $\overline{[A]}$ and $\overline{[B]}$ and parameters β_X, K_X, J_X, δ_X, and γ.

Equations (1)-(2) are a standard model of a toggle switch [1], with the exception of the state-dependent growth rate constant (3), which takes a similar form as in [2], [3]. The growth-rate model assumes exponentially growing cells, which is reasonable for population setups that are continuously infused with new rich media and in which cell counts remain constant, such as in a turbidostat [5].

Assumption 1. *We assume that the parameters γ, β_X, K_X, δ_X, and J_X ($X \in \{A,B\}$) are all positive real numbers such that (1)-(3) admits exactly two asymptotically stable steady states, $([A]_0,[B]_0)$ and $([A]_1,[B]_1)$, and one unstable steady state, $([A]_{us},[B]_{us})$, such that $[A]_0 > [A]_{us} > [A]_1$ and $[B]_0 < [B]_{us} < [B]_1$. We denote the stable steady states of (1)-(2) with $\sigma = 1$ by $(\overline{[A]}_0,\overline{[B]}_0)$ and $(\overline{[A]}_1,\overline{[B]}_1)$, where $\overline{[A]}_0 > \overline{[A]}_1$ and $\overline{[B]}_0 < \overline{[B]}_1$. Then for general $\sigma > 0$, the stable steady states are $([A]_0,[B]_0) = (\sigma\overline{[A]}_0, \sigma\overline{[B]}_0)$ and $([A]_1,[B]_1) = (\sigma\overline{[A]}_1, \sigma\overline{[B]}_1)$. We view state $(\sigma\overline{[A]}_0, \sigma\overline{[B]}_0)$ as being the "A-high, B-low" equilibrium state and $(\sigma\overline{[A]}_1, \sigma\overline{[B]}_1)$ as the "A-low, B-high" equilibrium state.*

B. Stochastic population model

We consider a population with N cells. Accordingly, we define the set of states of the system to be $\mathbb{N}_0^{2N}$, where the vector $x = (a_1,b_1,...,a_N,b_N)$ corresponds to the state in which cell ℓ has a_ℓ copies of molecule A and b_ℓ copies of molecule B. We henceforth refer to x as a "population microstate." Using the production and decay terms in (1)-(2), we define the following four functions, which represent the propensities for an individual cell to produce (+) and eliminate (-) an A or B molecule given that it currently has a copies of A and b copies of B:

$$r^\sigma_{+A}(a,b) = \sigma\beta_A V \frac{1}{1+\left(\frac{b}{\sigma K_A V}\right)^2} \tag{4}$$

$$r^\sigma_{+B}(a,b) = \sigma\beta_B V \frac{1}{1+\left(\frac{b}{\sigma K_B V}\right)^2} \tag{5}$$

$$r^\sigma_{-A}(a,b) = \left(\gamma'_\sigma\left(\frac{a}{V},\frac{b}{V}\right) + \delta_A\right) a \tag{6}$$

$$r^\sigma_{-B}(a,b) = \left(\gamma'_\sigma\left(\frac{a}{V},\frac{b}{V}\right) + \delta_B\right) b. \tag{7}$$

In the above, $V > 0$ represents the cellular volume (treated as constant), and the other parameters are as in (1)-(3). We also define a function representing the rate at which a cell with a molecules of A and b molecules of B divides:

$$r^\sigma_\gamma(a,b) = \gamma'_\sigma\left(\frac{a}{V},\frac{b}{V}\right). \tag{8}$$

We explicitly write the (infinite) infinitesimal generator matrix Q for the minimal, continuous-time Markov chain (CTMC) describing the population microstate as follows. Index the population microstates $\mathbb{N}_0^{2N}$ using a bijective function $\iota_N : \mathbb{N}_0^{2N} \to \mathbb{N}$. For each $\ell,m \in \{1,...,N\}$ and each $i,j \in \mathbb{N}$, define (infinite) matrices $\tilde{Q}^{\ell,\sigma,N}$ and $\tilde{Q}^{\ell,m,\sigma,N}$ element-wise:

$$\tilde{Q}^{\ell,\sigma,N}_{i,j} = \begin{cases} r^\sigma_{+A}(x_{2\ell-1},x_{2\ell}) & x' = x + e_{2\ell-1} \\ r^\sigma_{+B}(x_{2\ell-1},x_{2\ell}) & x' = x + e_{2\ell} \\ r^\sigma_{-A}(x_{2\ell-1},x_{2\ell}) & x' = x - e_{2\ell-1} \\ r^\sigma_{-B}(x_{2\ell-1},x_{2\ell}) & x' = x - e_{2\ell} \\ 0 & \text{else,} \end{cases}$$

$$\tilde{Q}^{\ell,m,\sigma,N}_{i,j} = \begin{cases} \frac{r^\sigma_\gamma(x_{2\ell-1},x_{2\ell})}{N} & x' = x + \Delta a_{m,\ell} e_{2m-1} + \Delta b_{m,\ell} e_{2m} \\ 0 & \text{else,} \end{cases}$$

where e_r is the r-th standard unit vector, $x = \iota_N^{-1}(i)$, $x' = \iota_N^{-1}(j)$, $\Delta a_{m,\ell} = x_{2\ell-1} - x_{2m-1}$, and $\Delta b_{m,\ell} = x_{2\ell} - x_{2m}$. We then define

$$\tilde{Q}^{\sigma,N} = \sum_{\ell=1}^N \tilde{Q}^{\ell,\sigma,N} + \sum_{\ell=1}^N \sum_{m=1}^N \tilde{Q}^{\ell,m,\sigma,N},$$
$$Q^{\sigma,N} = \tilde{Q}^{\sigma,N} - D(\tilde{Q}^{\sigma,N}), \tag{9}$$

where $D(X)$ is the diagonal matrix with $D(X)_{k,k} = \sum_{r=1}^\infty X_{k,r}$.

Each $\tilde{Q}^{\ell,\sigma,N}$ is a matrix, whose non-diagonal entries represent the rates at which the population microstate changes due to cell ℓ producing or eliminating an A or B molecule. Each $\tilde{Q}^{\ell,m,\sigma,N}$ is a matrix, whose non-diagonal entries represent

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the rates at which the population microstate changes due to cell ℓ dividing and its daughter cell replacing cell m. $Q_{i,j}^{\sigma,N}$ then represents the rate at which the population (directly) transitions from the population microstate with index i to the one with index j (with $i \neq j$).

For convenience, if the population microstate is $(a_1, b_1, ..., a_N, b_N)$, we refer to (a_ℓ, b_ℓ) as the microstate of cell ℓ. Fix a rational number $F_{0,0} \in [0,1]$ such that $k = F_{0,0}N$ is an integer. $F_{0,0}$ represents the fraction of cells whose initial A and B concentrations $({}^{a}/_{V}, {}^{b}/_{V})$ are near the state 0 equilibrium point $(\sigma\overline{[A]}_0, \sigma\overline{[B]}_0)$, and $1 - F_{0,0}$ represents the fraction with initial concentrations near $(\sigma\overline{[A]}_1, \sigma\overline{[B]}_1)$. Specifically, we will assume the initial microstates of cells $1, ..., k$ are each $(\lfloor \sigma\overline{[A]}_0 V \rfloor, \lceil \sigma\overline{[B]}_0 V \rceil)$ and that the initial microstates of cells $k+1, ..., N$ are each $(\lceil \sigma\overline{[A]}_1 V \rceil, \lfloor \sigma\overline{[B]}_1 V \rfloor)$. This assumption is made for simplicity and can be relaxed, as shown in the results section. Denote the index of this initial population microstate by $i_0^{\sigma,N}$.

Define $Z^{\sigma,N} = (Z_t^{\sigma,N})_{t \geq 0}$ to be a minimal CTMC on state space $\mathbb{N}$ with infinitesimal generator matrix $Q^{\sigma,N}$ and initial state $i_0^{\sigma,N}$. We define the stochastic process representing the population microstate as

$$\left(a_1^{\sigma,N}(t), b_1^{\sigma,N}(t), ..., a_N^{\sigma,N}(t), b_N^{\sigma,N}(t)\right) = \iota_N^{-1}(Z_t^{\sigma,N}).$$

We define the state 0 subpopulation fraction at time t to be

$$F_0^{\sigma,N}(t) = \frac{1}{N} \sum_{\ell=1}^{N} \theta \left(\frac{a_\ell^{\sigma,N}(t)}{\overline{[A]}_0}, \frac{b_\ell^{\sigma,N}(t)}{\overline{[B]}_1} \right), \tag{10}$$

where $\theta(x,y)$ is 0 if $x < y$, ${}^1/_2$ if $x = y$, and 1 if $x > y$.

C. Simulation Algorithm

The stochastic population model described in Section II.B is simulated via the Next Family method [2], where each cell has the reactions $\varnothing \to A$, $\varnothing \to B$, $A \to \varnothing$, $B \to \varnothing$, with associated propensity functions (4)-(7) and division propensity function (8). At regular time intervals through the simulation, the fraction of cells in which ${}^{a}/_{[A]_0}$ is larger than ${}^{b}/_{[B]_1}$ (with (a,b) the cell microstate) is computed to produce an empirical value of $F_0^{\sigma,N}(t)$. Note that cells in which these numbers are equal provide a count of ${}^1/_2$ (see (10)).

III. ODE MODEL OF SUBPOPULATION FRACTIONS

We here introduce a two-state cell population ODE model, similar to that introduced in [6] and used in [2] for prediction of the steady-state fractions of cells in either toggle switch state. We then define and compute this model's parameters, and we investigate when this model provides a good approximation to $F_0^{\sigma,N}(t)$.

Consider a population of N cells with the following properties. Each of the cells can be in either of two states, denoted as 0 and 1, and switch instantaneously between these states. The cells in state i have growth rate constants γ_i and switch to the opposite state with rate constant α_i. Cells in either state are also removed from the population with equal rate coefficients, such that the total population size remains constant. More precisely, let $\hat{N}_0(t)$ and $\hat{N}_1(t)$ denote the number of cells in the population in each state at time t. The rate at which the number of cells in state i grows due to division minus the rate at which these cells are removed is $\gamma_i \hat{N}_i - \frac{\gamma_i \hat{N}_i + \gamma_j \hat{N}_j}{N} \hat{N}_i = \gamma_i \frac{\hat{N}_j}{N} \hat{N}_i - \gamma_j \frac{\hat{N}_i}{N} \hat{N}_j$, where j is the other state. Assuming $\hat{N}_0(t)$ and $\hat{N}_1(t)$ are sufficiently large [7], we can then write ODEs for the number of cells in either state as follows:

$$\frac{d}{dt}\hat{N}_0 = \gamma_0 \frac{\hat{N}_1}{N} \hat{N}_0 - \gamma_1 \frac{\hat{N}_0}{N} \hat{N}_1 + \alpha_1 \hat{N}_1 - \alpha_0 \hat{N}_0$$
$$\frac{d}{dt}\hat{N}_1 = \gamma_1 \frac{\hat{N}_0}{N} \hat{N}_1 - \gamma_0 \frac{\hat{N}_1}{N} \hat{N}_0 + \alpha_0 \hat{N}_0 - \alpha_1 \hat{N}_1.$$

Let $\hat{F}_0(t) = {}^{\hat{N}_0(t)}/_N$ and $\hat{F}_1(t) = {}^{\hat{N}_1(t)}/_N$ denote the fraction of cells in state 0 and 1, respectively. We can rearrange the first ODE and use the fact that $\hat{F}_1(t) = 1 - \hat{F}_0(t)$ to find

$$\frac{d}{dt}\hat{F}_0 = -\Delta\gamma \hat{F}_0^2 + (\Delta\gamma - \alpha_1 - \alpha_0)\hat{F}_0 + \alpha_1, \tag{11}$$

where $\Delta\gamma = \gamma_0 - \gamma_1$. This equation gives the dynamics of the state 0 subpopulation fraction ($\hat{F}_1$ can be found as $1 - \hat{F}_0$). It should be noted that while the ODEs for the total number of cells in either state are different in the case of a growing population (as in [6]), it can be shown that (11) is the same in either setting.

In what follows, we set $\Delta\gamma$, α_0, and α_1 using the parameters σ, β_X, K_X, J_X, δ_X, and γ, such that (on a finite time interval $[0,T]$) the solution $\hat{F}_0(t)$ to (11) provides a good approximation to $F_0^{\sigma,N}(t)$.

A. Defining $\Delta\gamma$

To set the value of $\Delta\gamma$, we first compute the growth rate constants γ_i given by (3) at the equilibrium point $(\sigma\overline{[A]}_i, \sigma\overline{[B]}_i)$ of (1)-(2) and define:

$$\gamma_0 = \frac{\gamma}{1 + \frac{\overline{[A]}_0}{J_A} + \frac{\overline{[B]}_0}{J_B}}, \quad \gamma_1 = \frac{\gamma}{1 + \frac{\overline{[A]}_1}{J_A} + \frac{\overline{[B]}_1}{J_B}}.$$

We then define

$$\Delta\gamma = \gamma_0 - \gamma_1. \tag{12}$$

B. Defining α_0 and α_1

We define α_0 and α_1 in terms of the Markov chain that describes the evolution of a single cell's microstate. We make the dependence of these rates on σ explicit with the notation $\alpha_0 = \alpha_0^\sigma$ and $\alpha_1 = \alpha_1^\sigma$.

Specifically, index the set of cell microstates $(a,b) \in \mathbb{N}_0^2$ with a bijective function $\kappa : \mathbb{N}_0^2 \to \mathbb{N}$. For each $\sigma > 0$, define the (infinite) infinitesimal generator matrix R^σ element-wise:

$$R_{i,j}^\sigma = \begin{cases} r_{+A}^\sigma(a,b) & a' = a+1, b' = b \\ r_{+B}^\sigma(a,b) & a' = a, b' = b+1 \\ r_{-A}^\sigma(a,b) & a' = a-1, b' = b \\ r_{-B}^\sigma(a,b) & a' = a, b' = b-1 \\ -\sum_{s \in \{+A,+B,-A,-B\}} r_s^\sigma(a,b) & a' = a, b' = b \\ 0 & \text{else,} \end{cases} \tag{13}$$

where $(a,b) = \kappa^{-1}(i)$ and $(a',b') = \kappa^{-1}(j)$.

Let $X^\sigma = (X_t^\sigma)_{t\geq 0}$ be a minimal CTMC with infinitesimal generator matrix R^σ. Given a set $\mathscr{S} \subset \mathbb{N}$ we define the first passage time (FPT) of X^σ into $\mathscr{S}$, as

$$\tau^{\sigma,\mathscr{S}} = \inf\{t \geq 0 : X_t^\sigma \in \mathscr{S}\}. \tag{14}$$

Given an index $p \in \mathbb{N}$, we define the mean first passage time (MFPT) of X^σ from p to $\mathscr{S}$ as

$$k_p^{\sigma,\mathscr{S}} = \mathbb{E}\left[\tau^{\sigma,\mathscr{S}} \mid X_0^\sigma = p\right]. \tag{15}$$

Recall that $\overline{[A]}_i$ and $\overline{[B]}_i$ are defined such that $(\sigma\overline{[A]}_0, \sigma\overline{[B]}_0)$ and $(\sigma\overline{[A]}_1, \sigma\overline{[B]}_1)$ are the stable equilibria of system (1)-(2), with $\sigma\overline{[A]}_0 > \sigma\overline{[A]}_1$ and $\sigma\overline{[B]}_0 < \sigma\overline{[B]}_1$. Define the sets $\mathscr{A}^\sigma = \{\kappa(a,b) : a \geq \sigma\overline{[A]}_0 V, b \leq \sigma\overline{[B]}_0 V\}$ and $\mathscr{B}^\sigma = \{\kappa(a,b) : a \leq \sigma\overline{[A]}_0 V, b \geq \sigma\overline{[B]}_0 V\}$. Define $u^\sigma = \kappa(\lfloor\sigma\overline{[A]}_0 V\rfloor, \lceil\sigma\overline{[B]}_0 V\rceil)$ and $v^\sigma = \kappa(\lceil\sigma\overline{[A]}_1 V\rceil, \lfloor\sigma\overline{[B]}_1 V\rfloor)$. We then define

$$\alpha_0^\sigma = \left(k_{u^\sigma}^{\sigma,\mathscr{B}^\sigma}\right)^{-1}, \qquad \alpha_1^\sigma = \left(k_{v^\sigma}^{\sigma,\mathscr{A}^\sigma}\right)^{-1}. \tag{16a,b}$$

C. Computing α_0 and α_1

To compute α_0 and α_1, we use a similar approach to [8]. Specifically, given a set $\mathscr{S} \subset \mathbb{N}$, define $k^{\sigma,\mathscr{S}}$ to be the (infinite) vector with i-th element $k_i^{\sigma,\mathscr{S}}$. Note that $R_{i,i}^\sigma > 0$ for all $i \in \mathbb{N}$. Then a standard result for MFPTs (see for example [9], Theorem 3.3.3) of countable state CTMCs indicates that $k^{\sigma,\mathscr{S}}$ is the minimal non-negative solution to the equations

$$\begin{cases} k_i^{\sigma,\mathscr{S}} = 0 & i \in \mathscr{S} \\ -\sum_{j\in\mathbb{N}} R_{i,j}^\sigma k_j^{\sigma,\mathscr{S}} = 1 & i \notin \mathscr{S}. \end{cases} \tag{17}$$

Define the (infinite) vector λ^σ element-wise as $\lambda_i^\sigma = -\frac{1}{R_{i,i}^\sigma}$, and let P^σ be the jump matrix of X^σ, that is:

$$P_{i,j}^\sigma = \begin{cases} 0 & i = j \\ -\frac{R_{i,j}^\sigma}{R_{i,i}^\sigma} & i \neq j. \end{cases} \tag{18}$$

Denote by $\bar{P}^{\sigma,\mathscr{S}}$ the matrix P^σ with row and column indexes in $\mathscr{S}$ removed, and denote by $\bar{k}^{\sigma,\mathscr{S}}$ and $\bar{\lambda}^{\sigma,\mathscr{S}}$, respectively, the vectors $k^{\sigma,\mathscr{S}}$ and λ^σ with row indexes in $\mathscr{S}$ removed. Manipulation of (17) gives the matrix equation (cf. [8])

$$(I - \bar{P}^{\sigma,\mathscr{S}})\bar{k}^{\sigma,\mathscr{S}} = \bar{\lambda}^{\sigma,\mathscr{S}}. \tag{19}$$

The matrices and vectors in (19) when $\mathscr{S} = \mathscr{A}^\sigma$ or $\mathscr{S} = \mathscr{B}^\sigma$ are infinite, so we use a truncation-based approach to compute the MFPTs. For each $r \in \mathbb{N}_0$, define the set $\mathscr{E}_r = \{\kappa(a,b) : a+b \leq r\}$ and $\mathscr{S}_r = \mathscr{S} \cup \mathscr{E}_r^C$.

Proposition 1. *Fix $p \in \mathbb{N}$ and $\mathscr{S} \subset \mathbb{N}$ nonempty. Let $\kappa : \mathbb{N}_0^2 \to \mathbb{N}$ be bijective and for all $i,j \in \mathbb{N}$, and define the element $R_{i,j}^\sigma$ in the i-th row and j-th column of the infinitesimal generator matrix R^σ using (3),(4)-(7),(13), where $(a,b) = \kappa^{-1}(i)$ and $(a',b') = \kappa^{-1}(j)$. Let $X^\sigma = (X_t^\sigma)_{t\geq 0}$ be a minimal, countable-state CTMC with infinitesimal generator matrix R^σ, and define $k_p^{\sigma,\mathscr{S}}$ and $k_p^{\sigma,\mathscr{S}_r}$ using (15). Also let $\mathscr{E}_r$ and $\mathscr{S}_r$ be as above. Then*

$$k_p^{\sigma,\mathscr{S}} = \lim_{r\to\infty} k_p^{\sigma,\mathscr{S}_r}. \tag{20}$$

The above proposition shows that rather than solve (19) directly, we can compute an approximate value for α_0^σ by finding the minimal non-negative solution $\bar{k}^{\sigma,\mathscr{S}_r}$ to

$$(I - \bar{P}^{\sigma,\mathscr{S}_r})\bar{k}^{\sigma,\mathscr{S}_r} = \bar{\lambda}^{\sigma,\mathscr{S}_r}, \tag{21}$$

with r sufficiently large.

In particular, let $\bar{u}^\sigma = u^\sigma - |\{j \in \mathscr{B}^\sigma \cup \mathscr{E}_r^C : j < u^\sigma\}|$ and $\bar{v}^\sigma = v^\sigma - |\{j \in \mathscr{A}^\sigma \cup \mathscr{E}_r^C : j < v^\sigma\}|$, with r sufficiently large. Then $\bar{k}_{\bar{u}^\sigma}^{\sigma,\mathscr{B}^\sigma\cup\mathscr{E}_r^C} = k_{u^\sigma}^{\sigma,\mathscr{B}^\sigma\cup\mathscr{E}_r^C}$, and $\bar{k}_{\bar{v}^\sigma}^{\sigma,\mathscr{A}^\sigma\cup\mathscr{E}_r^C} = k_{v^\sigma}^{\sigma,\mathscr{A}^\sigma\cup\mathscr{E}_r^C}$. We can then approximate the switching rates by

$$\alpha_0^\sigma \approx \left(\bar{k}_{\bar{u}^\sigma}^{\sigma,\mathscr{B}^\sigma\cup\mathscr{E}_r^C}\right)^{-1}, \quad \alpha_1^\sigma \approx \left(\bar{k}_{\bar{v}^\sigma}^{\sigma,\mathscr{A}^\sigma\cup\mathscr{E}_r^C}\right)^{-1}. \tag{22a,b}$$

Remark 1. *The matrices $U = I - \bar{P}^{\sigma,\mathscr{B}^\sigma\cup\mathscr{E}_r^C}$ and $V = I - \bar{P}^{\sigma,\mathscr{A}^\sigma\cup\mathscr{E}_r^C}$ are both invertible because $\bar{P}^{\sigma,\mathscr{B}^\sigma\cup\mathscr{E}_r^C}$ and $\bar{P}^{\sigma,\mathscr{A}^\sigma\cup\mathscr{E}_r^C}$ are irreducible and have row sums greater than or equal to 1, with some rows having sums strictly greater than 1 due to the removed states (see Lemma 1 in [10]). Then (21) indeed has a unique solution. Solving a linear equation involving a r^2-dimensional square matrix generally takes $\mathscr{O}(r^6)$ operations. However, the indexing function κ can be chosen such that U and V are banded matrices with bandwidth of order r (for example $\kappa(0,0) = 1$, $\kappa(0,1) = 2$, $\kappa(1,0) = 3$, $\kappa(0,2) = 4$, $\kappa(1,1) = 5$,...). Using standard banded matrix linear equation algorithms, (21) can then be solved in $\mathscr{O}(r^4)$ computation time [11].*

IV. PROBLEM FORMULATION

Fix parameters β_A, β_B, K_A, K_B, δ_A, δ_B, J_A, J_B, γ, such that Assumption 1 is satisfied. Let $N \in \mathbb{N}$ be the population size, $\sigma > 0$ the concentration-scaling parameter, and $F_{0,0} \in [0,1] \cap \mathbb{Q}$ the initial state 0 subpopulation fraction, where $F_{0,0}N \in \mathbb{N}_0$.

Consider $\hat{F}_0^\sigma(t)$, the solution to (11) with initial condition $\hat{F}_0(0) = F_{0,0}$ for t on some interval $t \in [0,T]$, where $\Delta\gamma$ is given by (12), and $\alpha_0 = \alpha_0^\sigma$ and $\alpha_1 = \alpha_1^\sigma$ are given by (16a,b). Let $F_0^{\sigma,N}(t)$ be the "true" state 0 subpopulation fraction defined by (10). We define the approximation error by

$$\varepsilon_{\sigma,N}(t) := \left|\hat{F}_0^\sigma(t) - \mathbb{E}\left[F_0^{\sigma,N}(t)\right]\right|, \tag{23}$$

which we next computationally evaluate for $t \in [0,T]$ as σ and N are increased.

V. MAIN RESULT: COMPUTATIONAL EVALUATION OF APPROXIMATION ERROR

In this section, we compare the trajectory $\hat{F}_0^\sigma(t)$ of the subpopulation fraction model (11) with parameters defined by (12) and (16a,b) to the expected value of $F_0^{\sigma,N}(t)$, simulated as described in Section II.C.

Results are summarised in Fig. 2 and Fig. 3. These results indicate that for a given t, $\varepsilon_{\sigma,N}(t)$ may be made as small as desired by making σ and N sufficiently large. Intuitively, as σ becomes larger and molecular counts grow, the switching rates reduce. We would expect in this case that cells starting near either stable equilibria tend to stay by this equilibria and grow at rates close to the growth rates at the deterministic

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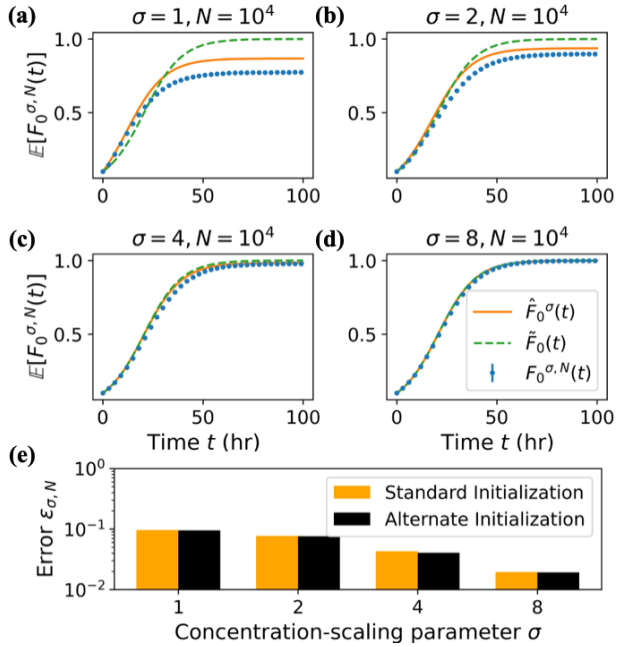

Fig. 2. Comparison for various values of σ of numerical estimates of $\mathbb{E}[F_0^{\sigma,N}(t)]$ (given in (10)) over time with the solution, $\hat{F}_0^{\sigma}(t)$, to (11) with initial value $F_{0,0}$. Parameters were set as $\beta_A = \beta_B = 30$ molecules$\cdot\mu$m^{-3}hr^{-1}, $K_A = K_B = 10$ molecules$\cdot\mu$m^{-3}, $\delta_A = \delta_B = 0.1$hr^{-1}, $J_A = 750$ molecules$\cdot\mu$m^{-3}, $J_B = 150$ molecules$\cdot\mu$m^{-3}, $\gamma = 1$hr^{-1}, $V = 1\mu$m^3, and $F_{0,0} = 0.1$. 900 replicate simulations were performed using $N = 10^4$ cells. Values of $F_0^{\sigma,N}(t)$ were sampled during simulation for each $t \in \{0,...,100\}$ and averaged at each time point. Standard error bars for the numeric estimates are shown but typically too small to see. For each σ, $\Delta\gamma$ was computed using (12) to be approximately 0.1064hr^{-1}, and α_0 and α_1 were computed by using (21)-(22a,b) with $r = 500$. In addition to the primary approximation ($\hat{F}_0^{\sigma}(t)$), the solution to (11) with initial condition $F_{0,0}$, $\Delta\gamma$ given by (12), and $\alpha_0 = \alpha_1 = 0$, which is denoted as $\tilde{F}_0(t)$, is also shown. (a) Results for $\sigma = 1$. $\alpha_0 \approx 1.58 \cdot 10^{-2}$hr^{-1} and $\alpha_1 \approx 1.10 \cdot 10^{-2}$hr^{-1}. (b) Results for $\sigma = 2$. $\alpha_0 \approx 7.01 \cdot 10^{-3}$hr^{-1} and $\alpha_1 \approx 3.46 \cdot 10^{-3}$hr^{-1}. (c) Results for $\sigma = 4$. $\alpha_0 \approx 1.69 \cdot 10^{-3}$hr^{-1} and $\alpha_1 \approx 4.24 \cdot 10^{-4}$hr^{-1}. (d) Results for $\sigma = 8$. $\alpha_0 \approx 9.05 \cdot 10^{-5}$hr^{-1} and $\alpha_1 \approx 5.82 \cdot 10^{-6}$hr^{-1}. (e) Maximal errors across time points in (a)-(d) ("Standard Initialization"). To check for robustness of results with respect to the initial population microstate, trajectories in (a)-(d) were recomputed (not shown) when initial cell microstates were chosen stochastically. Specifically for each cell $\ell \in \{1,...,F_{0,0}N\}$, the microstate, $(a_\ell^{\sigma,N}, b_\ell^{\sigma,N})$, of cell ℓ was initialized as $a_\ell^{\sigma,N}(0) \sim \Lambda\{\sigma[\overline{A}]_0 V\}$ and $b_\ell^{\sigma,N}(0) \sim \Lambda\{\sigma[\overline{B}]_0 V\}$, and for each $\ell \in \{F_{0,0}N+1,...,N\}$, $a_\ell^{\sigma,N}(0) \sim \Lambda\{\sigma[\overline{A}]_1 V\}$ and $b_\ell^{\sigma,N}(0) \sim \Lambda\{\sigma[\overline{B}]_1 V\}$, where $\Lambda\{x\}$ is a Poisson distribution with mean x. The errors for this case are also displayed ("Alternate Initialization").

equilibria, giving plausibility to the deterministic model (11). Computation of the parameters α_0 and α_1 help account for effects due to switching when σ is small or moderate. As N increases, effects associated with the discrete number of cells in the population, such as those due to noise and subpopulation fractions hitting 0, also become attenuated.

Remark 2. *In Fig. 2 and Fig. 3, approximations using a second model $\tilde{F}_0(t)$ are shown. $\tilde{F}_0(t)$ is defined to be the solution to (11) with initial condition $\tilde{F}_0(t) = F_{0,0}$, when $\Delta\gamma$ is defined by (12) and $\alpha_0 = \alpha_1 = 0$. The results in Fig. 2 and Fig. 3 indicate that this simplified ODE model may also provide a good approximation for $\mathbb{E}[F_0^{\sigma,N}(t)]$ when σ and N are sufficiently large. However, for σ of small or moderate size, this model may produce poor results because it does not account for cells switching between states (Fig. 4). Indeed, as shown in Fig. 4, the simplified model is only accurate when the switching rates are small enough that they need not be accounted for.*

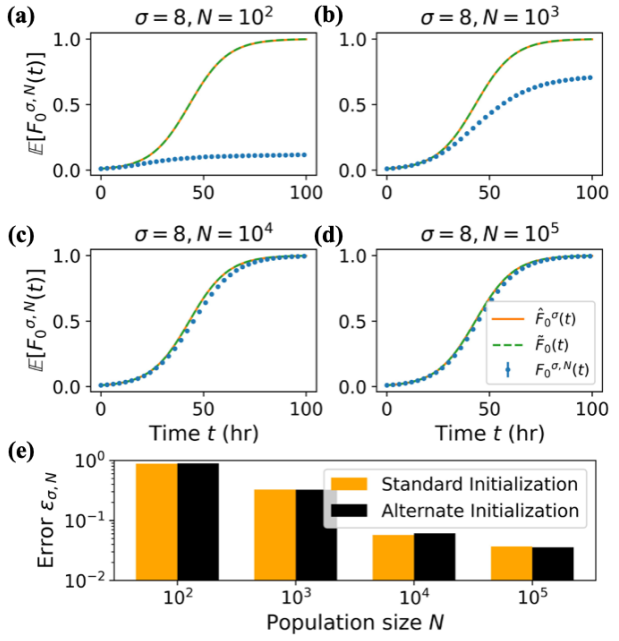

Fig. 3. Results from simulations and ODE approximations with parameters identical to those in Fig. 2 except that $F_{0,0} = 0.01$, $\sigma = 8$ throughout and N is varied rather than σ. (a)-(d) Results for various values of N. In each case, $\alpha_0 \approx 9.05 \cdot 10^{-5}$hr^{-1} and $\alpha_1 \approx 5.82 \cdot 10^{-6}$hr^{-1} were computed using using (21)-(22a,b) with $r = 500$. (e) Maximal errors for each N.

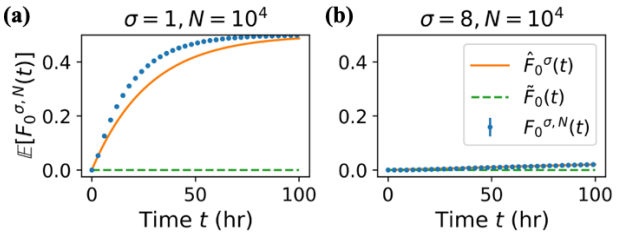

Fig. 4. Results from simulations and ODE approximations with parameters identical to those in Fig. 2 except that $F_{0,0} = 0$, and $J_A = J_B = 750$ molecules$\cdot\mu$m^{-3} so that the toggle switches are symmetric and $\Delta\gamma = 0$. (a) Results for $\sigma = 1$. $\alpha_0 = \alpha_1 \approx 1.82 \cdot 10^{-2}$hr^{-1}. (b) Results for $\sigma = 8$. $\alpha_0 = \alpha_1 \approx 2.09 \cdot 10^{-4}$hr^{-1}. Switching rates were computed using (21)-(22a,b) with $r = 500$.

VI. CONCLUSION

In this paper, we introduced and evaluated an ODE-based approximation for the subpopulation fraction dynamics of exponentially growing cells engineered with toggle switch genetic circuits with growth feedback in a turbidostat. For sufficiently large parameter scaling factors σ and population sizes N, computational analyses show good agreement of the ODE model and numerical population simulations on fixed, finite time intervals. Importantly, the parameters for this approximate model can be computed without simulation. The ability to efficiently quantify and design population-

level behavior of toggle switches via this approximate model may be useful for applications requiring the engineering of populations with subpopulations that partition in a desired way.

APPENDIX

To prove Proposition 1, we need two results. The first is from [12], and the second follows directly from [13] (in particular see their Theorem 1.1, conclusions (iii) and (v)).

Theorem 1. (see [12], Theorem 2.3) *Let R be the infinitesimal generator matrix (assumed to have finite entries and rows that sum to 0) of an irreducible, time-homogeneous, Markov process $X = (X_t)_{t\geq 0}$ on state space $\mathbb{N}$. If for some vector $y = (y_i)_{i\in\mathbb{N}}$ with finite, non-negative elements and for some finite set $F \subset \mathbb{N}$, R satisfies*

$$\forall i \in F^C \quad \sum_j R_{i,j} y_j \leq -1 \tag{i}$$

$$\forall i \in F \quad \sum_j R_{i,j} y_j < \infty, \tag{ii}$$

with $\lim_{i\to\infty} y_i = \infty$, then all states in X are ergodic (with ergodicity of state j defined by the condition $\lim_{t\to\infty} \mathbb{P}(X_t = j | X_0 = j) > 0$).

Note that for the chain X in the theorem, ergodicity of a non-absorbing state in this sense is equivalent to positive recurrence of the state (see §1.1.7,§1.1.8 of [14]). Because X is assumed irreducible, positive recurrence of all states in turn implies that the MFPT of X from any state to any set of states is finite (see footnote in [15]).

Theorem 2. (see [13], Theorem 1.1, conclusions (iii),(v)) *Let R be the infinitesimal generator matrix (with finite entries and rows that sum to 0) of a minimal, time-homogeneous, Markov process, $X = (X_t)_{t\geq 0}$, on state space $\mathbb{N}$ for which the initial distribution is 0 for all states outside of some finite set. Consider a sequence $\mathscr{E}_1 \subset \mathscr{E}_2 \subset \ldots$ whose union is $\mathbb{N}$, a set $\mathscr{S} \in \mathbb{N}$, and a sequence of positive real numbers $t_f^1 \leq t_f^2 \leq \ldots$, with $t_f^r \to \infty$ as $r \to \infty$. If $\mathbb{E}[\min(\tau^{\mathscr{S}}, T^\infty)] < \infty$ then*

$$\mathbb{E}[\min(\tau^{\mathscr{S}}, T^\infty)] - \mathbb{E}[\min(\tau^{\mathscr{S}}, t_f^r) 1_{\tau^{\mathscr{S}} \leq \tau^{\mathscr{E}_r^C}}] \to 0 \tag{24}$$

as $r \to \infty$, where $1_\bullet$ is the indicator function that is 1 when its argument is true and 0 otherwise, and $\tau^{\mathscr{S}}$ is the FPT of X into $\mathscr{S}$.

Proof of Proposition 1. To begin, notice that R^σ is irreducible. Indeed for each $(a,b) \in \mathbb{N}_0^2$, $R_{\kappa(a,b),\kappa(a+1,b)}$, $R_{\kappa(a,b),\kappa(a,b+1)}$, $R_{\kappa(a+1,b),\kappa(a,b)}$, and $R_{\kappa(a,b+1),\kappa(a,b)}$ are all positive, and $R_{\kappa(a,b),\kappa(a,b)}$ is finite.

We show that the conditions of Theorem 1 apply to R^σ with $y_{\kappa^{-1}(a,b)} = e^{(a+b)h}$, where h is chosen large enough that $\delta_A > (\delta_A + \gamma)e^{-h}$ and $\delta_B > (\delta_B + \gamma)e^{-h}$ (recall it is assumed that $\delta_A > 0$ and $\delta_B > 0$), and F is chosen such that its complement, F^C, is the set of $\kappa(a,b)$ for which

$$(\sigma\beta_A V + \sigma\beta_B V)e^h + e^{-(a+b)h} \leq (\delta_A - (\delta_A + \gamma)e^{-h})a + (\delta_B - (\delta_B + \gamma)e^{-h})b.$$

Note that this choice of F^C implies F finite. The only nontrivial condition to check is (i). Let (a,b) be such that $\kappa(a,b) \in F^C$. Then, simple manipulation of the above equation gives

$$(\sigma\beta_A V + \sigma\beta_B V)e^{(a+b+1)h} + ((\delta_A + \gamma)a + (\delta_B + \gamma)b)e^{(a+b-1)h} + 1 \leq (\delta_A a + \delta_B b)e^{(a+b)h}.$$

From the definition of y and noticing that $0 \leq r_{+A}^\sigma(a,b) \leq \sigma\beta_A V$, $0 \leq r_{+B}^\sigma(a,b) \leq \sigma\beta_B V$, $\delta_A a \leq r_{-A}^\sigma(a,b) \leq (\delta_A + \gamma)a$, and $\delta_B b \leq r_{-B}^\sigma(a,b) \leq (\delta_B + \gamma)b$, the above equation implies that

$$r_{+A}^\sigma(a,b) y_{\kappa(a+1,b)} + r_{+B}^\sigma(a,b) y_{\kappa(a,b+1)} + r_{-A}^\sigma(a,b) y_{\kappa(a-1,b)} + r_{-B}^\sigma(a,b) y_{\kappa(a,b-1)} + 1 \leq \sum_{s\in\{+A,+B,-A,-B\}} r_s^\sigma(a,b) y_{\kappa(a,b)},$$

which is equivalent to $\sum_j R^\sigma_{\kappa(a,b),j} y_j \leq -1$, i.e. condition (i). Without loss of generality, we may assume that X^σ has initial state p. Because X^σ is positive recurrent, its explosion time $T^\infty = \infty$ almost surely (see [9], Theorem 3.6.3).

Notice that $\mathbb{E}\left[\min(\tau^{\mathscr{S}}, t_f^r) 1_{\tau^{\mathscr{S}} \leq \tau^{\mathscr{E}_r^C}}\right] \leq \mathbb{E}\left[\tau^{\mathscr{S}} 1_{\tau^{\mathscr{S}} \leq \tau^{\mathscr{E}_r^C}}\right] \leq \mathbb{E}\left[\min(\tau^{\mathscr{S}}, \tau^{\mathscr{E}_r^C})\right] = \mathbb{E}\left[\tau^{\mathscr{S}\cup\mathscr{E}_r^C}\right] = \mathbb{E}\left[\tau^{\mathscr{S}_r}\right]$. Thus $\mathbb{E}\left[\min(\tau^{\mathscr{S}}, t_f^r) 1_{\tau^{\mathscr{S}} \leq \tau^{\mathscr{E}_r^C}}\right] \leq \mathbb{E}\left[\tau^{\mathscr{S}_r}\right] \leq \mathbb{E}\left[\tau^{\mathscr{S}}\right]$. Combining this fact with Theorem 2, we see that as $r \to \infty$, $\mathbb{E}\left[\tau^{\mathscr{S}_r}\right] - \mathbb{E}\left[\tau^{\mathscr{S}}\right] \to 0$. Because the initial state of X^σ is assumed to be p, $\mathbb{E}\left[\tau^{\mathscr{S}_r}\right] = k_p^{\sigma,\mathscr{S}_r}$ and $\mathbb{E}\left[\tau^{\mathscr{S}}\right] = k_p^{\sigma,\mathscr{S}}$. Our result follows. □

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