

Nonlinear Analysis of Toggle Switch

Ayush Pandey

Abstract—A toggle switch is a bistable system that can be engineered out of synthetic biological parts. Since the first demonstration of the toggle switch [1] in 2000, there have been various studies and experiments in the past decade that involve the use of such dynamics. In this paper, we study and analyze the nonlinear characteristics of a toggle switch model. Particularly, we give theoretical results regarding the stability of the equilibrium points of the two protein toggle switch circuit. Using this analysis, we give an estimate of the domain of attraction of a stable equilibrium point using Lyapunov methods. We also show that the toggle switch system is input to state stable and give appropriate bounds and convergence rates for it.

I. INTRODUCTION

Over the past two decades there has been a big push towards applications of control and feedback to design and engineer biomolecular systems. The field of synthetic biology [2] aims to use engineering principles to design biological systems to have a desired behavior. In this paper, we will focus on the modeling and analysis of a canonical biomolecular system – the toggle switch. The toggle switch is one of the most commonly found motifs in biological systems in nature. As shown in [1], it can also be engineered from synthetic biological parts. The bistable nature of the toggle switch makes it an interesting and useful sub-module for many different applications and system designs [3]. However, due to various factors such as the nonlinear dynamics of biological processes, the stochasticity and unknown noise effects, lack of information about the various interactions or context effects from other parts of the system, and other uncertainty effects, the design of biological systems has not been driven by models and systems’ analysis. This is unlike other engineering disciplines where models are used as a primary tool to understand and design a system. In synthetic biology, models have mostly been used to get a vague qualitative understanding of the experiments and to support the inferences and the results observed. To use modeling and control theoretic approaches in a predictive manner for the design of biological circuits and experiments, we need to make scientific progress in the understanding of the systems better and we also need to bridge many research gaps that would enable us to use control theoretic approaches more efficiently for biological systems. One of these challenges is to understand, analyze, and use to our advantage the nonlinear dynamics of different biological systems. In this paper, we will focus on nonlinear analysis of the toggle switch dynamics to make theoretical claims that relate to the circuit design and experimental predictions. Using the nonlinear analysis,

we make claims about the stability of the system and study it under disturbance from leaky parameters. The theoretical results also give guarantees and bounds to the system states that may be used in experiment design to reduce the design space or to infer other experimental characteristics.

The rest of the paper is organized as follows - in Section II, we will briefly discuss some of the preliminary results from the literature regarding modeling of biological systems. We will then present our main results in Section III and follow it up with simulations supporting the claims and finally the discussions in Section IV.

II. PRELIMINARIES

A. Chemical reaction network modeling

Although there are many abstractions to which one can model a biological system, the mass action kinetic modeling using reaction rate equations for biomolecular reactions is one of the most commonly used approaches. We can often create a chemical reaction network (CRN) model for a biological system, especially in the field of systems and synthetic biology, which can be used to give insightful predictions about the system. The CRN models are simple enough to construct and are useful because they capture the essential processes that govern a particular function in a system. But a thorough mathematical analysis and use for control design is often complicated because these models are nonlinear.

1) *Generalized Chemical Reaction Network Model:* In general, given a CRN consisting of a set of species S_i , $i = 1, 2, \dots, n$, and a set of reactions R_j , $j = 1, 2, \dots, l$, we can write the mass action kinetics in the form

$$\dot{x} = Nf(x)$$

where x is the state variable denoting the species, $N \in \mathbb{R}^{n \times l}$ is the stoichiometry matrix for the system and $f(x) \in \mathbb{R}^l$ is the reaction flux vector. Refer to [3] for more details.

2) *A Simple Example:* Consider the reaction $A + 2B \xrightleftharpoons[k_b]{k_f} C$, then let the states of the system be the concentrations of the species, $x_1 = [A]$, $x_2 = [B]$, $x_3 = [C]$, then we have the nonlinear differential equations of states given by,

$$\dot{x} = \begin{bmatrix} -1 & 1 \\ -1 & 1 \\ 1 & -1 \end{bmatrix} \cdot \begin{bmatrix} k_f x_1 x_2^2 \\ k_b x_3 \end{bmatrix}$$

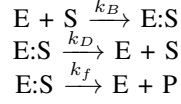
We may also write a model for controlled biochemical system by defining a set of species as the input, and some output species. Then, we can write the state equation in the standard form $\dot{x} = f(x, u)$ and $y = g(x, u)$. Using this modeling framework, we can model important processes such as transcription

The author is with the Electrical Engineering department at California Institute of Technology (Caltech). The paper is a technical report done as part of the coursework of Nonlinear Analysis course for the Winter 2018-19 term at Caltech.

of a DNA in a cell to form mRNA and its translation to form proteins which are essential building blocks of all life. Refer to Chapter 2 in [3] for more details.

B. Hill function modeling

As an example of the CRN modeling, consider the following example,



where $E:S$ denotes the complex formed by the binding of the enzyme (E) and the substrate (S), and P denotes the product at the end of it. The binding rate is given by k_B , the dissociation rate is given by k_D , and the rate for product formation is given by k_f . Let $[E]$, $[S]$, $[E : S]$, $[P]$ denote the concentrations for the enzyme, the substrate, the complex $E:S$, and the product respectively. The nonlinear differential equations describing the CRN above are given below.

$$\frac{d[S]}{dt} = k_D[E : S] - k_B[E][S] \quad (1)$$

$$\frac{d[E]}{dt} = k_f[E : S] + k_D[E : S] - k_B[E][S] \quad (2)$$

$$\frac{d[E : S]}{dt} = k_B[E][S] - (k_f + k_D)[E : S] \quad (3)$$

$$\frac{dP}{dt} = k_f[E : S] \quad (4)$$

Using quasi-steady state assumption for the complex $E : S$, we get the following reduced order model for this system

$$\frac{dP}{dt} = \frac{V_{max}(S_{tot} - P)}{(S_{tot} - P) + K_m} \quad (5)$$

where $V_{max} = kE_{tot}$, and E_{tot}, S_{tot} denote the total amount of E and S respectively. For more details about how to construct such models, refer to [3]. These models are also called Hill function models. This modeling approach will be used to obtain the dynamic model for the toggle switch.

Definition 1 : Transcriptional Regulation The process of transcription (DNA converting to mRNA), is regulated by various proteins in biological systems. Two of the most common mechanisms for regulation are called activation and repression. We say that a protein activates the production of another protein, if it promotes the transcription of that protein's DNA. Similarly, we say that a protein represses the production of another protein if it prevents transcription. These two transcriptional regulation mechanisms can be used to study a large variety of naturally existing control mechanisms in biological systems and also in synthetic systems. For two species A and B , we have 4 possibilities — A and B activate each other, A represses B , B activates A , A activates B , B represses A , and finally both A and B repress each other.

For the toggle switch, we have both species repressing each other using transcriptional regulation defined above.

Definition 2 : Hill function of a state variable in general can be written as, $h(x) = \frac{x^n}{K^n + x^n}$, where n is called the Hill coefficient and K is the dissociation constant

Definition 3 : Toggle Switch is a two species system where both proteins A and B repress each other. We consider the following toggle switch system with proteins TetR and LacI repressing each others expression. LacI binds with the promoter for protein TetR to prevent the transcription of the TetR gene to form its mRNA and hence repressing the production of protein TetR. Similarly, TetR binds with the promoter of the LacI gene to prevent its transcription to form mRNA, which in turn causes the repression in the expression of LacI protein. This simple two species transcriptional regulation system is shown in Fig. (1).

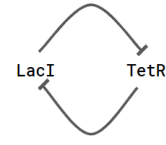


Fig. 1. A two species toggle switch where LacI and TetR repress each others expression. We can control the strength of each repression using inducers, which effectively control the values of the parameters b_T and b_L in the model (Eq. 6)

It shows bistability and has interesting dynamical properties that we will explore in this paper. The derivation for the nonlinear ODEs of this system can be done by writing down the chemical reaction network and then using Hill function modeling described earlier. The detailed derivation is given in Chapter 2 and 5 in [3]. The dynamic model ODEs are,

$$\begin{aligned} \dot{m}_T &= \frac{Kb_T^n}{b_T^n + p_L^n} - d_T m_T \\ \dot{m}_L &= \frac{Kb_L^n}{b_L^n + p_T^n} - d_L m_L \\ \dot{p}_T &= \beta_T m_T - \delta_T p_T \\ \dot{p}_L &= \beta_L m_L - \delta_L p_L \end{aligned} \quad (6)$$

where n is the cooperativity of the hill functions and the state vector is $x = [m_T \ m_L \ p_T \ p_L]^T$ are the states and we define $\mathbb{P} \in \mathbb{R}_{\geq 0}^9$ as the parameter vector consisting of all of the parameters defined above. We can write $\dot{x} = f(x, \mathbb{P})$, $f : E \times \mathbb{R}^9 \rightarrow \mathbb{R}^4$, where E is open and connected subset of \mathbb{R}^4 given by

$$E = \{x \in \mathbb{R}^4 : x_1, x_2 \in (0, m_{max}), x_3, x_4 \in (0, p_{max})\}$$

where p_{max} and m_{max} are positive constants.

III. RESULTS

To study the solutions of the toggle switch dynamic model, we use the following theorem to establish the existence and uniqueness of solutions.

Theorem 1: Lipschitz continuity: The toggle switch system,

Eq. (6), is globally Lipschitz continuous with Lipschitz constant

$$L = \max \left(\delta_L + \frac{K}{b_T}, \beta_L + d_T, \beta_T + d_L, \delta_T + \frac{K}{b_L} \right) \quad (7)$$

and as a consequence of Theorem 2.3 in [6], there exists a unique solution to Eq. (6) in E .

Proof: We have,

$$E = \{x \in \mathbb{R}^4 : x_1, x_2 \in [0, m_{max}], x_3, x_4 \in [0, p_{max}]\}$$

We need to show that, $f : E \times \mathbb{R}^9 \rightarrow \mathbb{R}^4$ is Lipschitz continuous, i.e. (for notational simplicity, we are ignoring the constant parameter vector \mathbb{P} , and just writing $f(x)$ for the dynamics)

$$\|f(x) - f(y)\| \leq L \|x - y\|$$

We have for $x, y \in E$, and without loss of generality, assuming $n = 1$ for simplicity

$$\begin{bmatrix} \frac{Kb_T}{b_T+x_4} - d_T x_1 \\ \frac{Kb_L}{b_L+x_3} - d_L x_2 \\ \beta_T x_1 - \delta_T x_3 \\ \beta_L x_2 - \delta_L x_4 \end{bmatrix} - \begin{bmatrix} \frac{Kb_T}{b_T+y_4} - d_T y_1 \\ \frac{Kb_L}{b_L+y_3} - d_L y_2 \\ \beta_T y_1 - \delta_T y_3 \\ \beta_L y_2 - \delta_L y_4 \end{bmatrix}$$

Since we are in \mathbb{R}^4 , all norms are equivalent, we can show the result for any norm of our choice. Hence, taking the 1-norm of the above, we can write

$$\begin{aligned} &= \left| \frac{Kb_T(y_4 - x_4)}{(b_T + x_4)(b_T + y_4)} - d_T(x_1 - y_1) \right| \\ &+ \left| \frac{Kb_L(y_3 - x_3)}{(b_L + x_3)(b_L + y_3)} - d_L(x_2 - y_2) \right| \\ &+ |\beta_T(x_1 - y_1) - \delta_T(x_3 - y_3)| \\ &+ |\beta_L(x_2 - y_2) - \delta_L(x_4 - y_4)| \end{aligned}$$

Now, using the triangle inequality, and clubbing up terms together, we can write

$$\begin{aligned} &\leq |(x_4 - y_4)| \left| \delta_L + \frac{Kb_T}{(b_T + x_4)(b_T + y_4)} \right| \\ &+ |(x_3 - y_3)| \left| \delta_T + \frac{Kb_L}{(b_L + x_3)(b_L + y_3)} \right| \\ &+ |(\beta_T + d_L)(x_1 - y_1)| \\ &+ |(\beta_L + d_T)(x_2 - y_2)| \end{aligned}$$

Observe that all parameters are positive and the domain given above implies that all states are also always positive, hence we can bound the above term by using,

$$\begin{aligned} \delta_L + \frac{Kb_T}{(b_T + x_4)(b_T + y_4)} &\leq \delta_L + \frac{K}{b_T} \\ \delta_T + \frac{Kb_L}{(b_L + x_3)(b_L + y_3)} &\leq \delta_T + \frac{K}{b_L} \end{aligned}$$

We can now write,

$$\|f(x) - f(y)\|_1 \leq L \|x - y\|_1$$

where L is as given in the theorem statement. Since, all parameters are fixed, it is possible to obtain L above and hence, our system is globally Lipschitz in the domain defined above, i.e. the value for L above holds for all $x \in E$. Q.E.D.

We can numerically evaluate the solutions of the nonlinear dynamic model given for the toggle switch.

A. Numerical solutions to the system

The solution for all of the four states solved numerically is shown in Fig. (2). It is clear from this figure that the state $x_3 = p_T$ has higher expression than the other protein. If we introduce an induction signal that changes the parameters of the system, the other protein x_4 will be active, and hence we observe the toggling behavior. This is characteristic behavior of a toggle switch where we observe bistable response in the protein that is expressed the most.

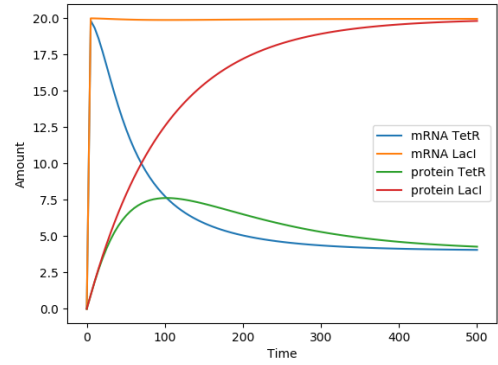


Fig. 2. Numerical solution of the ODEs for a fixed parameter range

We can obtain the numerical solution for this system using Picard's method of approximation. In Picard's iteration, for zero initial condition, we have,

$$\begin{aligned} z_1(t) &= \begin{bmatrix} Kt \\ Kt \\ 0 \\ 0 \end{bmatrix} \\ z_2(t) &= \begin{bmatrix} K - d_T Kt \\ K - d_L Kt \\ \beta_T Kt \\ \beta_L Kt \end{bmatrix} \\ z_3(t) &= \begin{bmatrix} \frac{Kb_T}{b_T + \frac{\beta_L Kt^2}{2}} - d_T \left(Kt - d_T \frac{Kt^2}{2} \right) \\ \frac{Kb_L}{b_L + \frac{\beta_T Kt^2}{2}} - d_L \left(Kt - d_L \frac{Kt^2}{2} \right) \\ \beta_T Kt - \frac{d_T Kt^2}{2} - \delta_T \frac{\beta_T Kt^2}{2} \\ \beta_L Kt - \frac{d_L Kt^2}{2} - \delta_L \frac{\beta_L Kt^2}{2} \end{bmatrix} \end{aligned}$$

and so on...The approximation converges for the fixed point x^* .

$$x^* = P(x^*)$$

Numerically, we can see that the above discrete time system indeed converges to the actual solution. The approximate solution iteration using the Picard's operator compared alongside the numerical solution of the nonlinear ODEs are shown in Fig. (3). In the figure, only one of the outputs of the system is shown for clarity.

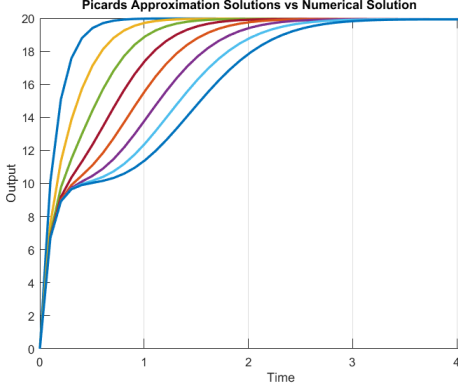


Fig. 3. Picard's iterative solution compared with the numerical solution. The blue trajectory corresponds to the numerical solution to which all the other trajectories converge to as the number of iterations is increased.

Moving on, we can further conclude about the continuity of the solutions of the system with respect to parameters and initial condition perturbations. We can use known bounds from the theory to conclude that the solutions to the toggle switch system are continuous with respect to perturbations in initial conditions and perturbations. We give simulations to support this theoretical analysis.

1) *Continuity of Solutions:* To study the solutions of the system and their dependence on initial condition and parameters, we use the numerical simulations to solve the differential equations under different conditions. From Theorem 3.2 in [6], we have the following result. Note that the 2-norm is chosen for this bound since we are working with errors in states due to perturbations. This choice of the norm is of course without any loss of generality since all norms are equivalent in the space \mathbb{R}^4 in which we are working.

$$\|x(t) - z(t)\|_2 \leq \|x_0 - z_0\|_2 e^{L(t-t_0)} + \mu \times (t - t_0) e^{L(t-t_0)} \quad (8)$$

where L is the Lipschitz constant for the system given by Eq. (7) and μ is defined as follows. Consider the perturbed system dynamics (under disturbance in parameters)

$$\dot{x} = f(x, \mathbb{P})$$

where x is the state variable vector defined above and \mathbb{P} is the parameter vector consisting of all of the 9 parameters. If there is a disturbance in the parameters, we can write

$$\dot{z} = f(z, \mathbb{P} + \Delta)$$

Or equivalently, we can write,

$$\begin{aligned} \dot{z} &= f(z, \mathbb{P}) + f(z, \mathbb{P} + \Delta) - f(z, \mathbb{P}) \\ \dot{z} &= f(z, \mathbb{P}) + g(z, \mathbb{P}) \end{aligned}$$

where $g(z, \mathbb{P})$ is given by the difference and we assume that it is upper bounded in the 2-norm by μ . Next, we will show the calculation of this μ for the toggle switch model with $n = 1$. For our particular case, we only introduce a perturbation in one of the parameters in \mathbb{P} . Using, $K = K + \Delta$, we get the perturbed model,

$$\begin{aligned} \dot{m}_T &= \frac{(K + \Delta)b_T}{b_T + p_L} - d_T m_T \\ \dot{m}_L &= \frac{(K + \Delta)b_L}{b_L + p_T} - d_L m_L \\ \dot{p}_T &= \beta_T m_T - \delta_T p_T \\ \dot{p}_L &= \beta_L m_L - \delta_L p_L \end{aligned}$$

We need to bound $g(z, \mathbb{P})$, since the 2-norm is bounded above by the 1-norm in \mathbb{R}^n , we can write the following by evaluating $g(z, \mathbb{P})$ as $f(z, \mathbb{P} + \Delta) - f(z, \mathbb{P})$

$$\|g(z, \mathbb{P})\|_2 \leq \|g(z, \mathbb{P})\|_1 \leq \left| \frac{\Delta b_T}{b_T + p_L} + \frac{\Delta b_L}{b_L + p_T} \right|$$

Using the triangle inequality

$$\leq \left| \frac{\Delta b_T}{b_T + p_L} \right| + \left| \frac{\Delta b_L}{b_L + p_T} \right|$$

Since, $x \in \mathbb{E} \Rightarrow p_i \geq 0$ for $i = \{L, T\}$, we can write the following bound

$$\leq 2\Delta$$

Hence,

$$\mu = 2 \times \Delta$$

We will now study the continuous dependence of the solutions on the initial condition and on parameters by introducing perturbations to each and visualizing the numerical solution to the system equations.

2) *Dependence on Initial Conditions:* The plot in Fig. (4) shows the dependence of solutions on different initial conditions. We see that for different initial conditions the time traces for all four states vary accordingly. The bound given in Eq. (3.12) in the lecture notes [6] is also satisfied (in this case, there are no perturbations introduced to the parameters). This is demonstrated in Fig. (5).

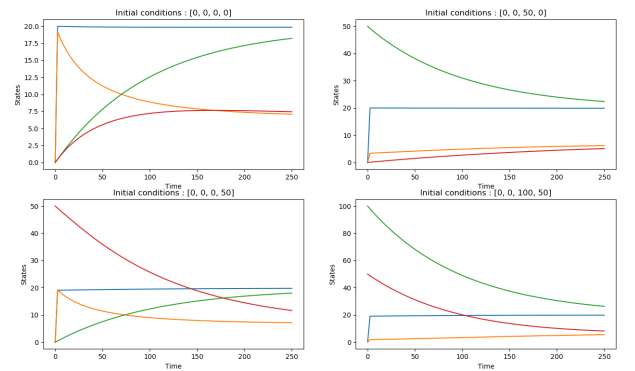


Fig. 4. System solutions for different initial conditions

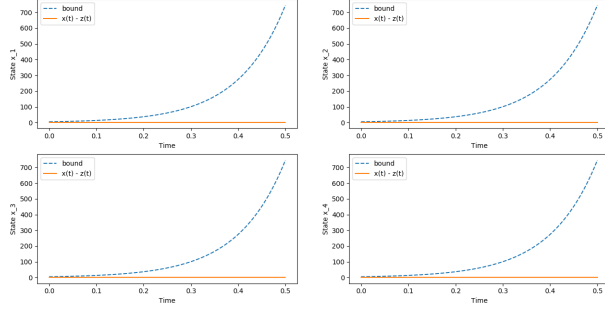


Fig. 5. Continuous dependence of the solution on initial conditions, the bound in Theorem 3.2 is satisfied for all time

3) *Dependence on Parameters:* The plot in Fig. (6) shows the dependence of solutions on different parameter sets. We see that for different parameters, the solutions vary accordingly. Most importantly, we see that by controlling the parameters we can “toggle” the output that we see expressed the most, i.e. the dominant protein concentration toggles according to the changing parameters.

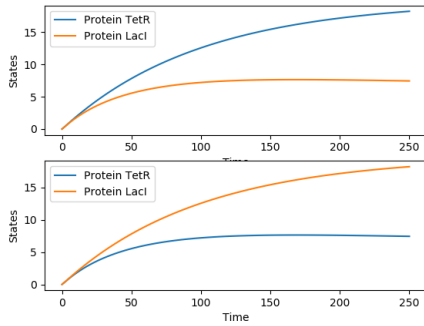


Fig. 6. For two different sets of parameters, we see that the dominant protein expression is toggled depending on the parameters

Finally, we can see in Fig. (7) that the solutions are continuously dependent on the parameters and initial conditions. As we perturb the parameters ($K = K + 10$) and the initial conditions ($z_0 = x_0 + 0.5$), all of the solutions still satisfy the bound described above. It is clear from the plot that the bound is very conservative and hence the Fig. (8) shows the zoomed in behavior establishing the fact that the bound is satisfied for all time.

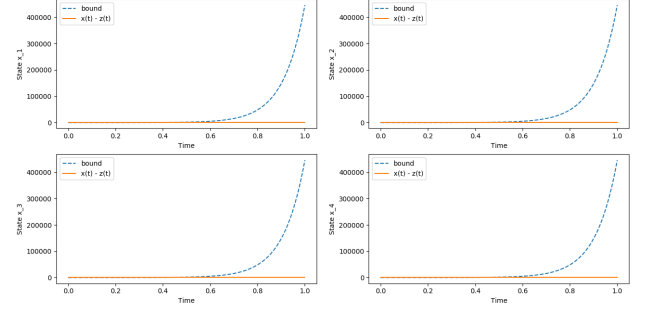


Fig. 7. The bound given in Theorem 3.2 holds true, however it is a very conservative bound

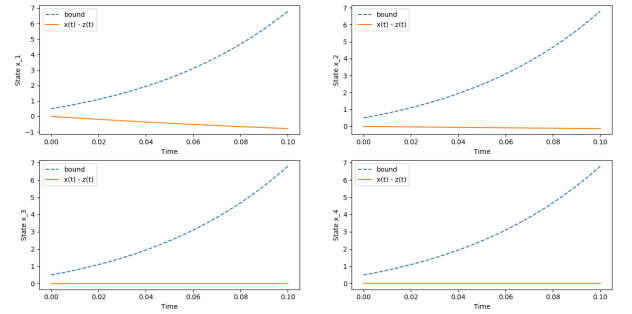


Fig. 8. A zoomed up view of the bound to show that it holds for all time

B. Stability

The following theorem makes important conclusions about the equilibrium points and their stability for the toggle switch nonlinear model.

Theorem 2 : The toggle switch system, Eq. (6) with $n = 2$, has two stable equilibria and one unstable equilibrium point. The stable equilibria are exponentially stable and correspond to expression of one of the proteins. An estimate of the domain of attraction for the stable equilibrium point shifted to the origin is $\mathcal{B}_{0.086}(0) \subset D_{oa}$ for

$$\mathbb{P} = [100 \ 10 \ 100 \ 5 \ 5 \ 0.01 \ 0.01 \ 0.01 \ 0.01]^T$$

Proof: We have,

$$\begin{aligned} \dot{m}_T &= \frac{Kb_T^2}{b_T^2 + p_L^2} - d_T m_T \\ \dot{m}_L &= \frac{Kb_L^2}{b_L^2 + p_T^2} - d_L m_L \\ \dot{p}_T &= \beta_T m_T - \delta_T p_T \\ \dot{p}_L &= \beta_L m_L - \delta_L p_L \end{aligned} \tag{9}$$

$$\tag{10}$$

This system has three equilibrium points, two stable equilibrium points corresponding to the each protein and one unstable

equilibrium point. This fact is clear from the nullcline analysis for the system. Depending on the parameters, the system may be made to toggle between the two stable states. The analysis of the equilibrium points can be found in Chapter 5 of [3]. Here, we focus only on one of the stable equilibrium point obtained for a fixed set of parameters. From symmetry of the model, the following proof easily extends to make similar conclusions for the other stable equilibrium point. We use Lyapunov methods to conclude about the stability of this equilibrium point and then estimate its domain of attraction. Solving for x^* , we get,

$$x^* = \begin{bmatrix} x_{1e} \\ x_{2e} \\ x_{3e} \\ x_{4e} \end{bmatrix} = \begin{bmatrix} x_{1e} \\ x_{2e} \\ x_{3e} \\ x_{4e} \end{bmatrix} = \begin{bmatrix} 4.010 \\ 19.967 \\ 4.010 \\ 19.967 \end{bmatrix} \quad (11)$$

For simplicity in analysis, we can shift this equilibrium point to the origin and change the system dynamics accordingly. Define, $\bar{x} = x - x^*$. The model with the origin as equilibrium point can be written as

$$\begin{aligned} \dot{x}_1 &= \dot{m}_T = \frac{Kb_T^2}{b_T^2 + (x_4 + x_{4e})^2} - d_T x_1 - d_T x_{1e} \\ \dot{x}_2 &= \dot{m}_L = \frac{Kb_L^2}{b_L^2 + (x_3 + x_{3e})^2} - d_L x_2 - d_L x_{2e} \\ \dot{x}_3 &= \dot{p}_T = \beta_T x_1 + \beta_T x_{1e} - \delta_T x_3 - \delta_T x_{3e} \\ \dot{x}_4 &= \dot{p}_L = \beta_L x_2 + \beta_L x_{2e} - \delta_L x_4 - \delta_L x_{4e} \end{aligned} \quad (12)$$

To construct a Lyapunov function for this system, we will use the constructive approach given in [6] that uses the linearization of the system and then derive the Lyapunov function from the linearized system that also works for the nonlinear system locally. The linearized dynamics can be obtained by evaluating the Jacobian at the equilibrium point

$$J = Df(0) \quad (13)$$

$$= \begin{bmatrix} -d_T & 0 & 0 & \frac{-2Kb_T^2 x_{4e}}{(b_T^2 + x_{4e}^2)^2} \\ 0 & -d_L & \frac{-2Kb_L^2 x_{3e}}{(b_L^2 + x_{3e}^2)^2} & 0 \\ \beta_T & 0 & -\delta_T & 0 \\ 0 & -\beta_L & 0 & -\delta_L \end{bmatrix} \quad (14)$$

The eigenvalues of the linearized system are

$$\lambda_J = \begin{bmatrix} -5.002 \\ -4.997 \\ -0.012 \\ -0.007 \end{bmatrix}$$

Clearly, all eigenvalues are negative, hence, the linearized system is exponentially stable to the origin. Now, we will construct the Lyapunov function to conclude about the local stability of the origin for the nonlinear system using the Lyapunov function for the nonlinear system. For linear systems, the general form of Lyapunov system is $V(x) = x^T P x$ where $P = P^T > 0$ satisfies the continuous time Lyapunov equation

$$A^T P + P A = -Q$$

where $Q = Q^T > 0$ can be arbitrarily chosen (different choices of Q will give different convergence to the equilibrium point). Choosing $Q = I$, and solving for P numerically, we get,

$$P = \begin{bmatrix} 2.672 & -0.436 & 2.362 & -11.549 \\ -0.436 & 1.886 & -9.662 & 1.960 \\ 2.362 & -9.662 & 52.362 & -10.605 \\ -11.549 & 1.960 & -10.605 & 51.960 \end{bmatrix} \quad (15)$$

The eigenvalues of P are

$$\lambda_P = \begin{bmatrix} 65.392 \\ 43.289 \\ 0.099 \\ 0.100 \end{bmatrix}$$

Since, all eigenvalues are positive, P is a positive definite symmetric matrix, the Lyapunov function $V(x) = x^T P x$ is a valid Lyapunov function. We also have the derivative of the Lyapunov function along the solution trajectories of the system,

$$\dot{V}(x) = x^T (A^T P + P A) x = -x^T Q x$$

Hence, the equilibrium point is exponentially stable for the linearized system. Using Theorem 8.3 from [6], this Lyapunov function establishes exponential stability for the nonlinear system locally. The next part of the proof gives an estimate of how “local” this result is, i.e., we get an estimate of the domain of attraction of the equilibrium point.

$$\left\| \int_0^1 Df(\tau x) d\tau - Df(0) \right\| < \frac{\lambda_{\min}(Q)}{2\lambda_{\max}(P)}, \quad x \in \mathcal{B}_r(0)$$

Solving for this bound, we can get the radius of the ball that will give us the estimate for the domain of attraction

$$\begin{aligned} & \int_0^1 Df(\tau x) d\tau \\ &= \int_0^1 \begin{bmatrix} -d_T & 0 & 0 & \frac{-2Kb_T^2(\tau x_4 + x_{4e})}{(b_T^2 + (\tau x_4 + x_{4e})^2)^2} \\ 0 & -d_L & \frac{-2Kb_L^2(\tau x_3 + x_{3e})}{(b_L^2 + (\tau x_3 + x_{3e})^2)^2} & 0 \\ \beta_T & 0 & -\delta_T & 0 \\ 0 & -\beta_L & 0 & -\delta_L \end{bmatrix} d\tau \end{aligned}$$

To evaluate the integral, substitute

$$\begin{aligned} \tau x_4 + x_{4e} &= \tau_4 \\ \tau x_3 + x_{3e} &= \tau_3 \end{aligned}$$

Subsequently, using $\tau_4^2 = u$ and $\tau_3^2 = v$, we can evaluate the integral

$$\begin{aligned} & \frac{-Kb_T^2}{x_4} \int_{x_{4e}^2}^{(x_{4e} + x_4)^2} \frac{du}{(b_T^2 + u)^2} = \frac{-Kb_T^2}{x_4} \left[\frac{-1}{b_T^2 + u} \right]_{x_{4e}^2}^{(x_{4e} + x_4)^2} \\ &= \frac{-Kb_T^2}{x_4} \left(\frac{1}{b_T^2 + x_{4e}^2} - \frac{1}{b_T^2 + (x_4 + x_{4e})^2} \right) \end{aligned}$$

Similarly, solving for the other integral, we finally get

$$\int_0^1 Df(\tau x) d\tau \quad (16)$$

$$= \begin{bmatrix} -d_T & 0 & 0 & a_1 \\ 0 & -d_L & b_1 & 0 \\ \beta_T & 0 & -\delta_T & 0 \\ 0 & -\beta_L & 0 & -\delta_L \end{bmatrix} \quad (17)$$

where

$$\begin{aligned} a_1 &= \frac{-Kb_T^2}{x_4} \left(\frac{1}{b_T^2 + x_{4e}^2} - \frac{1}{b_T^2 + (x_4 + x_{4e})^2} \right) \\ b_1 &= \frac{-Kb_L^2}{x_3} \left(\frac{1}{b_L^2 + x_{3e}^2} - \frac{1}{b_L^2 + (x_3 + x_{3e})^2} \right) \end{aligned} \quad (18)$$

Now, we can evaluate,

$$\left\| \int_0^1 Df(\tau x) d\tau - Df(0) \right\| < \frac{\lambda_{\min}(Q)}{2\lambda_{\max}(P)}$$

Using the integrals calculated in Eq. (14) and (17), and numerically obtained values for the eigenvalues, we can solve numerically for the radius of the ball that would estimate the domain of attraction. Here, the 2-norm for the matrix norm was chosen and a numerical search for the domain of attraction was done. Solving, we get $r = 0.086$. Therefore, for a ball $B_r(0)$ of radius r the Lyapunov function obtained from the linear system $V(x) = x^T P x$ with the P given in Eq. (15), the nonlinear system is locally exponentially stable on $B_{0.086}(0) \subset D_{oa}$ for the parameter set as given in the Theorem statement. Q.E.D.

For the numerical computation, Python based code available at [7]. It is possible to get estimates of the domain of attraction for different parameter sets. By tuning the choice of Q while obtaining the Lyapunov function, it is also possible to obtain other convergence rates that get traded off with the size of the domain of attraction.

C. Input to state stability

In this section, we will analyze the input to state stability behavior of the toggle switch system. In biological systems, parameter uncertainty and unwanted leaky dynamics of protein expression are very common. Hence, it is important to study the input to state stability of our system. We already established local exponential stability around the stable equilibrium point of our system in the previous section. Here, we will study the case when there is leaky expression in addition to the regulated expression of each protein.

1) *Perturbed dynamics:* We introduce a perturbation in the dynamics that corresponds to the leak of transcription so that there is mRNA expression and in turn downstream protein expression in addition to the regulated expression. In the system dynamic equations given in Eq. (6) with $n = 2$, the first term in each equation for mRNAs corresponds to the production rate due to the transcriptional regulation by

the other protein. In addition to this production, mRNAs for each protein might also be transcribed without this regulation (referred to as leaky expression). In this case, the system dynamic equations are given as,

$$\begin{aligned} \dot{m}_T &= \frac{Kb_T^2}{b_T^2 + p_L^2} - d_T m_T + K_{0T} \\ \dot{m}_L &= \frac{Kb_L^2}{b_L^2 + p_T^2} - d_L m_L + K_{0L} \\ \dot{p}_T &= \beta_T m_T - \delta_T p_T \\ \dot{p}_L &= \beta_L m_L - \delta_L p_L \end{aligned} \quad (19)$$

where K_{0T} and K_{0L} are two new parameters corresponding to the leaky dynamics. Let $\dot{x} = f(x)$ be the original unperturbed dynamics, then define

$$d = \begin{bmatrix} K_{0T} \\ K_{0L} \\ 0 \\ 0 \end{bmatrix}, \quad (20)$$

so that the perturbed dynamics are given by $\dot{x} = f(x) + d$. It is easy to see that the disturbance $\|d\|$ will be bounded by $\|K_{0T}\| + \|K_{0L}\|$. We assume that this disturbance is bounded, hence, we assume that K_{0T} and K_{0L} are both finite so that our disturbance is bounded. We will study the effect of this disturbance on the system stability and performance in the subsequent sections.

Theorem 3 The toggle switch system, Eq. (6) is input to state stable such that there exist $M \geq 0$ and bounded $\|d\|$ such that

$$\|x(t)\| \leq \max \left(\|x(0)\| e^{-\frac{\lambda_{\min}(Q)}{4\lambda_{\max}(P)} t} M^{\frac{1}{2}}, \frac{4\lambda_{\max}(P)}{\lambda_{\min}(P)} \|d\| \right) \quad (21)$$

where P and Q are the matrices obtained in the previous section.

Proof Consider the Lyapunov candidate $V(x) = x^T P x$. We have already shown in the previous section that this is a valid Lyapunov function for the unperturbed system and numerically we have shown that there is a P such that this Lyapunov function gives local exponential stability for the unperturbed system. We have,

$$\lambda_{\min}(P) \|x\|^2 \leq V(x) \leq \lambda_{\max}(P) \|x\|^2$$

Now take the derivative of the Lyapunov function along the trajectories of the solutions of the perturbed system, we have,

$$\begin{aligned} \dot{V}(x) &\leq -\lambda_{\min}(Q) \|x\|^2 + d^T P x + x^T P d \\ &\leq -\lambda_{\min}(Q) \|x\|^2 + d^T P x + x^T P d \end{aligned}$$

Using Cauchy-Schwarz inequality and the properties of the norm, we have,

$$\leq -\lambda_{\min}(Q) \|x\|^2 + 2\lambda_{\max}(P) \|d\| \|x\|$$

For $t \in [0, 1]$

$$\begin{aligned} &= -t\lambda_{\min}(Q) \|x\|^2 - (1-t)\lambda_{\min}(Q) \|x\|^2 \\ &\quad + 2\lambda_{\max}(P) \|d\| \|x\| \end{aligned} \quad (22)$$

Choose $t = \frac{1}{2}$, the choice of t will affect our convergence rate and the set around the equilibrium point that is convergent.

$$= -\frac{1}{2}\lambda_{\min}(Q) \|x\|^2 - \frac{1}{2}\lambda_{\min}(Q) \|x\|^2 + 2\lambda_{\max}(P) \|d\| \|x\|$$

$$\|d\| \leq \frac{1}{4} \frac{\lambda_{\min}}{\lambda_{\max}(P)} \|x\| \iff \|x\| \geq \frac{4\lambda_{\max}(P)}{\lambda_{\min}(Q)} \|d\|$$

$$\Rightarrow \dot{V} \leq -\frac{1}{2}\lambda_{\min}(Q) \|x\|^2$$

Using Proposition 11.1 from [6], where,

$$\rho(r) = \frac{4\lambda_{\max}(P)}{\lambda_{\min}(Q)}, \alpha_1(r) = \lambda_{\min}(P)r^2$$

$$\alpha_2(r) = \lambda_{\max}(P)r^2, \alpha_3(r) = \frac{1}{2}\lambda_{\min}(Q)r^2$$

are class \mathcal{K} functions. Now, we need to solve for $\|x(t)\|$ to find out the set to which it converges. We have from the Lyapunov theorem,

$$\|x(t)\| \leq \alpha_1^{-1}(\beta(\alpha_2(\|x(0)\|), t - t_0))$$

where, β is a class \mathcal{KL} function that is the solution to the following initial value problem

$$\dot{y} = -\alpha_3(y), y(0) = V(x(0)) = x_0^T P x_0$$

$$\dot{y} = -\frac{\lambda_{\min}(Q)}{2\lambda_{\max}(P)} y$$

Solving the one dimensional ODE, we get,

$$y(t) = M e^{-\frac{\lambda_{\min}(Q)}{2\lambda_{\max}(P)} t}$$

where $M \triangleq x_0^T P x_0$. Using $\alpha_1^{-1} = \frac{1}{\lambda_{\min}(P)^{\frac{1}{2}}} r^{\frac{1}{2}}$, we have using Proposition 11.1 in [6] that,

$$\|x(t)\| \leq \max \left(\|x(0)\| e^{-\frac{\lambda_{\min}(Q)}{4\lambda_{\max}(P)} t} M^{\frac{1}{2}}, \frac{4\lambda_{\max}(P)}{\lambda_{\min}(P)} \|d\| \right)$$

and hence we have the result. Q.E.D.

The system dynamics converges to the ball around the equilibrium point with radius given by the bound above. The convergence rate to this set is $\frac{\lambda_{\min}(Q)}{4\lambda_{\max}(P)}$. We can get better convergence rates by choosing t in Eq. (22), however, this choice will be a traded off with the size of the set around the equilibrium point that is convergent. We can have higher convergence rates but at the expense that our result will be more “local”, i.e. the set around the equilibrium point in which solutions converge will be smaller. This is evident from the bound given above.

Numerical simulations demonstrate input to state stability of the system under the perturbation to the dynamics introduced. Simulations in Fig. (9) and (10) for different bounded disturbances in the dynamics all show that the system is input to state stable.

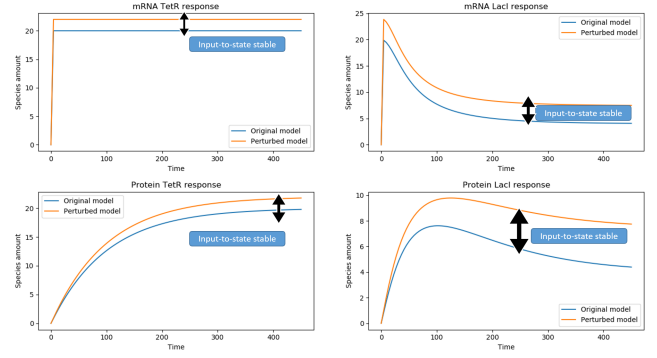


Fig. 9. Under different bounded disturbances for the leaky dynamics given in the model in Eq. (19), the numerical simulations show that the system is input to state stable and the bound derived above is satisfied. Here $\|d\| = 30$.

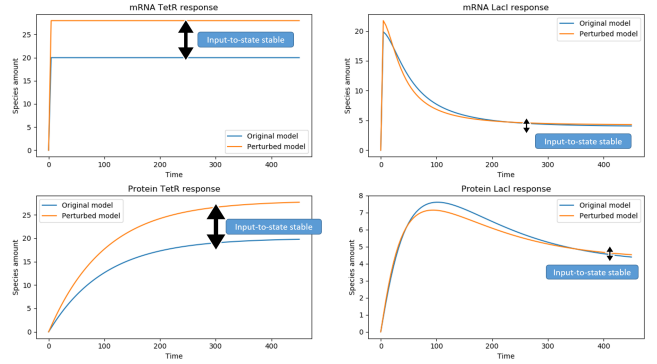


Fig. 10. Under different bounded disturbances for the leaky dynamics given in the model in Eq. (19), the numerical simulations show that the system is input to state stable and the bound derived above is satisfied. Here $\|d\| = 50$.

IV. DISCUSSION

A. Stability analysis

Using Lyapunov methods, we analyzed the stability of an equilibrium point of the toggle switch. It is a trivial extension of Theorem 2 to prove stability using similar approach for other equilibrium points due to the symmetry in the model. It is important to note here that unlike the traditional approach to study the stability of the toggle switch by linearization about the equilibrium points, we have given a constructive approach and derived a Lyapunov function to conclude about the stability of the nonlinear dynamics. Since the CRN level abstraction of modeling biological systems is very useful in terms of how well it can relate to the observations in an experiment, we often use dynamic models as the ones considered in this paper for modeling and analysis of biological circuits. But, it is not easy to come up with Lyapunov functions for such models of biological systems as they differ from mechanical or electrical systems in that there is no obvious concept of energy conservation at the CRN level modeling. Hence, the results regarding stability analysis discussed in the paper are significant as we can use these methods in general to conclude about stability and convergence guarantees for a much wider class of models for biological systems. We could

use these results to guide and predict the experimental designs and hence this is a potentially interesting future research work that could help in bridging the gaps of using systems' theory and modeling to design and engineer biological circuits.

B. Input to state stability

Uncertain parameters, unknown dynamics, and other perturbations are very common in biological systems. It is difficult to model biological systems accurately because it is often the case that these uncertainties can not be modeled due to lack of information about all of the processes that are going on. Further, due to lack of high throughput measurements, it is a big challenge and an active research area to get estimates for different parameter values in the model. With these challenges in mind, we can see that the analysis for input to state stability of nonlinear dynamic models of biological circuits could be a very useful tool. It can be used to imply important and useful results regarding the effects of different perturbations. With the toggle switch, we concluded how the leaky parameters in the protein expression affect the equilibrium reached at steady state. The bounds presented also give us guarantees of convergence to a set and their convergence rate which could be a very important tool that could be used when designing experiments.

REFERENCES

- [1] Gardner, Timothy S., Charles R. Cantor, and James J. Collins. *Construction of a genetic toggle switch in Escherichia coli*. Nature 403.6767 (2000): 339.
- [2] Powell, K. *How biologists are creating life-like cells from scratch*. Nature (2018): 172-175.
- [3] Domitilla Del Vecchio and Richard M. Murray *Biomolecular Feedback Systems*. Princeton University Press, 2014
- [4] Buse O, Prez R, Kuznetsov A. *Dynamical properties of the repressilator model* Physical Review E. 2010 Jun 11;81(6):066206.
- [5] Alon, Uri. *An introduction to systems biology: design principles of biological circuits*. Chapman and Hall/CRC, 2006.
- [6] Ames, A., Tabuada, P *Nonlinear Dynamics : CDS232 Lecture Notes*, Caltech, 2019
- [7] Pandey, Ayush. *Python based tools for nonlinear analysis of the toggle switch biological circuit*, Github, 2019, [Available online](#).