# Modelling the behaviour of the Genetic Toggle Switch

Yasinthan Vickneswaran

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## 1 Introduction

Synthetic biology is the ability to rationally design novel synthetic circuits that implement new or previously known cellular behaviours to function effectively in organisms. The Genetic Toggle Switch is a basic synthetic circuit consisting of two genes that inhibit each other's expression. This toggle switch exhibits bistability which means it can exist in two stable states—similar to an ON and OFF state—and can switch between these states under specific conditions. The functions of the Genetic Toggle Switch are important in key biological processes such as controlling gene expression, creating cellular memory, and designing synthetic biological systems. Ordinary differential equations (ODEs) can simply model for protein production and loss. In order to understand the behaviour of this genetic switch, researchers can use different modelling techniques such as deterministic methods that can describe the average behaviour or stochastic techniques that can account for random fluctuations.

In this report, we will look at the bistability of the toggle switch system, the unsteady stable state, bifurcation analysis and stochastic simulations. By comparing and evaluating different modelling strategies, we can better understand the toggle switch's behaviour and how it can be used in practical applications.

## 2 Exploration of Repressor Kinetics used in the Toggle Switch Model

The law of mass action essentially posits that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants. The central dogma of biology is $gene \rightarrow mRNA \rightarrow protein$. In order to build a model, in which the concentration of the expressed proteins reach a steady state, additional reactions that remove mRNA and protein need to be included. We are assuming other relevant molecular species that are directly involved in transcription, translation and loss remain at constant reactions so that these reactions can be modelled as mass action processes. The model can be written as a system of ODEs.

## 2.1 Model parameters

- $k_{m_0}$ is the basal transcription of the gene
- $k_m$ is the maximal expression rate.
- $K$ is a parameter equal to the concentration of [A] or [R] giving half maximal expression rate.
- $n$ (Hill coefficient) is the cooperativity coefficient where $n = 1$ means no cooperativity and $n > 1$ results in cooperativity.
- $k_{dm}$ is the rate constant for the degradation of mRNA.
- $k_p$ is the rate constant for the production of protein.
- $k_{dp}$ is the rate constant for the degradation of protein.

## 2.2 Model equations

We assume the concentration of the gene and the concentration of the promoter are constant

$$\frac{dM}{dt}\text{production} = k_{m_0} + k_m \times \frac{K^n}{P_R^n + K^n}$$

$$\frac{dM}{dt}\text{loss} = k_{dm} \times M$$

$$\frac{dP}{dt}\text{production} = k_p \times M$$

$$\frac{dP}{dt}\text{loss} = k_{dp} \times P$$

$$\frac{dM}{dt} = \frac{dM}{dt}\text{production} - \frac{dM}{dt}\text{loss}$$

$$\frac{dP}{dt} = \frac{dP}{dt}\text{production} - \frac{dP}{dt}\text{loss}$$

## 2.3 Nullcline analysis

As $\frac{dM}{dt} = 0$,

$$k_{m_0} + k_m \times \frac{K^n}{P_R^n + K^n} - k_{dm} \times M = 0$$

$$M = \frac{k_{m_0}}{k_{dm}} + \frac{k_m}{k_{dm}} \times \frac{K^n}{P_R^n + K^n}$$

When repressor protein is absent (Pr = 0), then $M = 36.147$ (3.dp)
When repressor protein is at high levels (Pr = 3000), then $M = 35.719$ (3.dp)

As $\frac{dP}{dt} = 0$,

$$k_p \times M - k_{dp} \times P = 0$$
$$P = \frac{k_p \times M}{k_{dp}}$$

When repressor protein is absent (Pr = 0), then $P = 2628.873$ (3.dp)
When repressor protein is at high levels (Pr = 3000), then $P = 2597.776$ (3.dp)

## 2.4 Protein Expression with Repression

If genes were unregulated and left "on" all the time, it would not be possible for organisms to develop such rich biological complexity as there is no variation in gene expression. The simplest way to regulate gene expression is through repressors. Repressor proteins can bind to specific binding sites at or near a promoter to inhibit its activity. However, the strength of its inhibition is dependent on external factors.

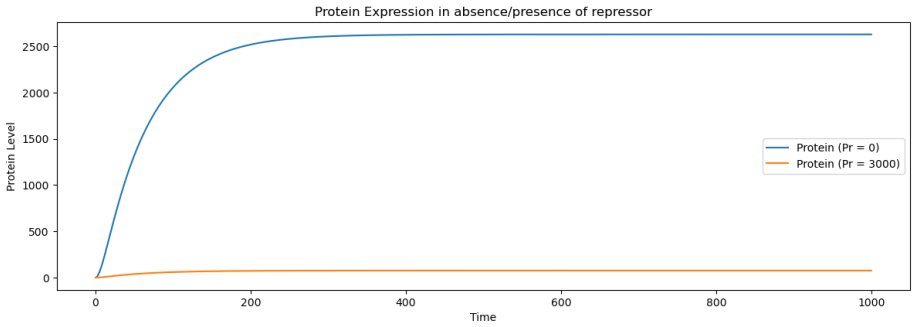


Figure 1: A graph showing how expressed mRNA and protein levels evolve to a steady state when repressor protein (Pr) is absent and when Pr is at high levels (i.e. Pr = 3000)

Figure 1 shows that even with high levels of repression, there is a basal gene expression level. This is modelled by the addition of the constant term $k_{m_0}$.

### 2.4.1 Steady State Analysis for Different Hill Coefficients ($n$)

The Hill coefficient ($n$) is used in the modelling of enzyme-substrate reactions and describes the cooperativity of the enzyme binding process. For this model, the Hill coefficient ($n$) determines the sensitivity to changes in the concentrations of the repressor proteins($P_R$).

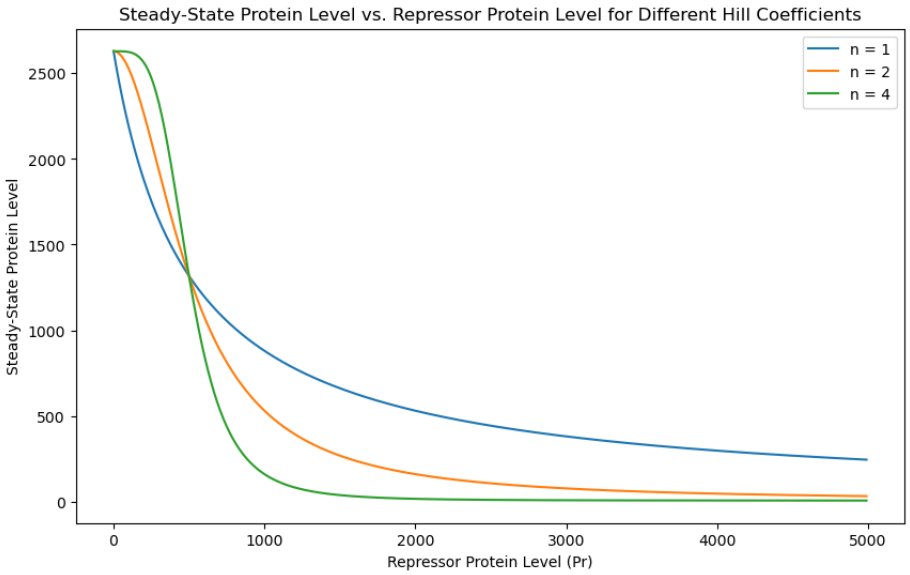


Figure 2: Protein steady state levels under different Hill coefficients ($n$)

When $n = 1$, the curve demonstrates no cooperativity, it follows simple Michaelis-Menten kinetics. The system behaves linearly, and the levels of protein at steady state are relatively resistant to changes in changes of concentration of repressor proteins($P_R$).

When $n = 2$ and $n = 4$, the curve becomes steeper as the Hill coefficient increases thus showing higher levels of cooperativity. Thus, the levels of protein at steady state become more sensitive to the concentration of repressor proteins ($P_R$) as the Hill coefficient increases ($n$)

### 2.4.2 Steady State Analysis for Different Basal Expressions ($k_{m_0}$)

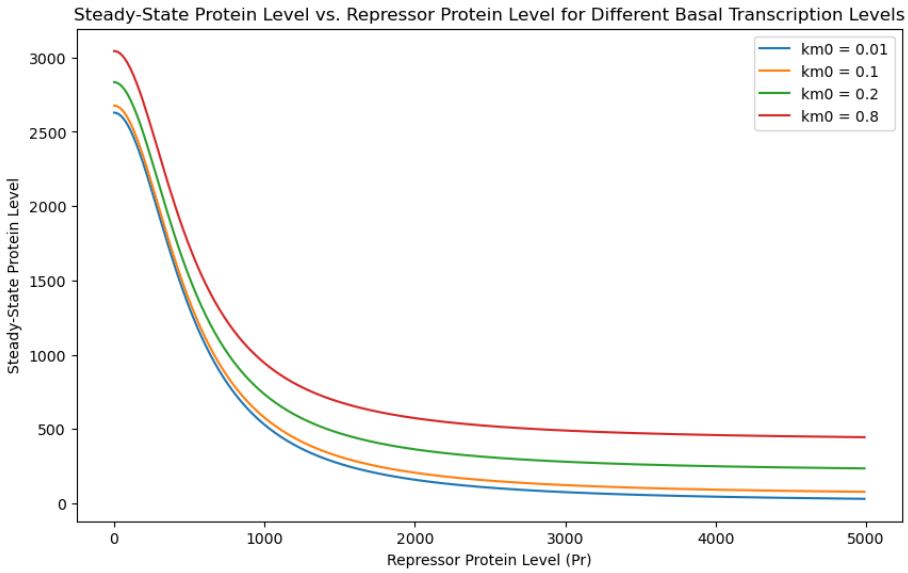


Figure 3: Protein steady state levels under different basal expressions($k_{m_0}$)

Figure 3 shows that the level of basal expression of the gene determines how high it is shifted upwards. The basal expression rate acts as bias shifting the production of steady state protein level upwards as it increases. This is to be expected as the basal expression rate is not dependent on the concentration of the repressor proteins ($P_R$).

# 3 Analysis of the Toggle Switch System

Gardner and Collins established the first genetic toggle switch in *E.coli*. This system consists of two genes (*lacI* and *tetR*) that mutually repress each other to display two stable steady states at equilibrium. (INSERT CITATION).

## 3.1 System variables

- $M_L$: number of LacI mRNA molecules
- $P_L$: number of LacI protein molecules
- $M_T$: number of TetR mRNA molecules
- $P_T$: number of TetR protein molecules

## 3.2 Processes

### 3.2.1 Expression of LacI protein ($P_L$)

$$\frac{dM_L}{dt}\text{production} = k_{m_0 L} + k_{m_L} \times \frac{K_T^n}{P_T^n + K_T^n}$$

$$\frac{dM_L}{dt}\text{loss} = k_{dm_L} \times M_L$$

$$\frac{dP_L}{dt}\text{production} = k_{p_L} \times M_L$$

$$\frac{dP_L}{dt}\text{loss} = k_{dp_L} \times P_L$$

$$\frac{dM_L}{dt} = \frac{dM_L}{dt}\text{production} - \frac{dM_L}{dt}\text{loss}$$

$$\frac{dP_L}{dt} = \frac{dP_L}{dt}\text{production} - \frac{dP_L}{dt}\text{loss}$$

### 3.2.2 Expression of TetR protein ($P_T$)

$$\frac{dM_T}{dt}\text{production} = k_{m_0 T} + k_{m_T} \times \frac{K_L^n}{P_L^n + K_L^n}$$

$$\frac{dM_T}{dt}\text{loss} = k_{dm_T} \times M_T$$

$$\frac{dP_T}{dt}\text{production} = k_{p_T} \times M_T$$

$$\frac{dP_T}{dt}\text{loss} = k_{dp_T} \times P_T$$

$$\frac{dM_T}{dt} = \frac{dM_T}{dt}\text{production} - \frac{dM_T}{dt}\text{loss}$$

$$\frac{dP_T}{dt} = \frac{dP_T}{dt}\text{production} - \frac{dP_T}{dt}\text{loss}$$

## 3.3 Bistability of the Toggle Switch System

As shown by Figure 4 and Figure 5 below, the toggle switch system can exist in either of the two states depending on the initial conditions. Thus, it demonstrates bistable behaviour.

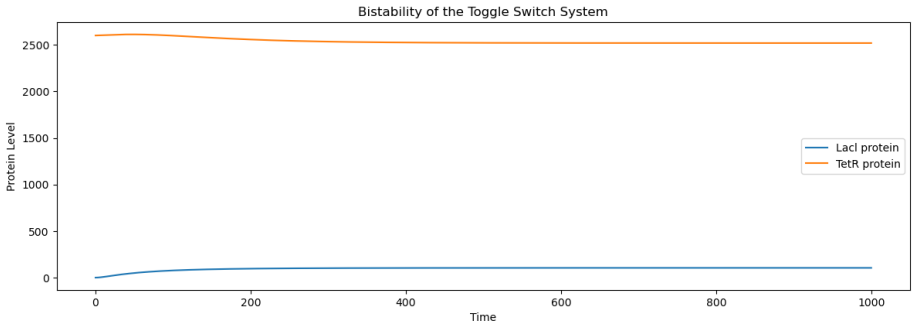


Figure 4: Bistability of the Toggle System when the starting $P_T$ is high

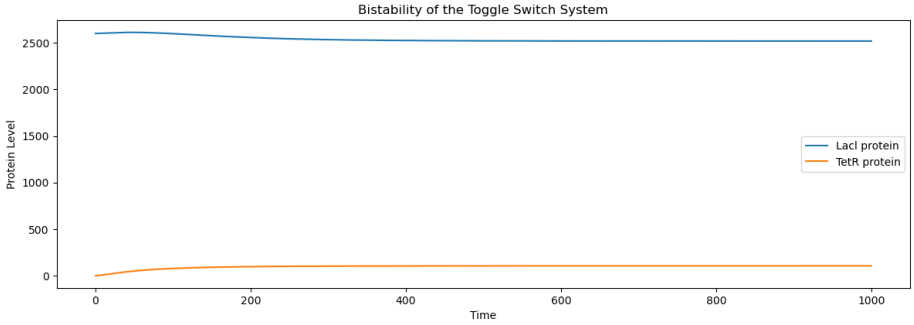


Figure 5: Bistability of the Toggle System when the starting $P_L$ is high

### 3.3.1 Hill coefficient $(n)$

However the stability of the system is dependent on Hill coefficient. When the Hill coefficient $(n)$ is 1 then there is no cooperativity and the levels of both protein tend to the same given enough time to reach a state. Figure 6 shows that when Hill coefficient $(n) = 1$, the system lacks the ability to amplify the small changes in the concentration of repressor proteins $(P_r)$ and is therefore unable to establish two states as it cannot sustain either state. Therefore, the system converges towards a single equilibrium state where both levels of the protein are equal. Figure 7 shows minimum Hill coefficient $(n)$ required to exhibit bistable behaviour.

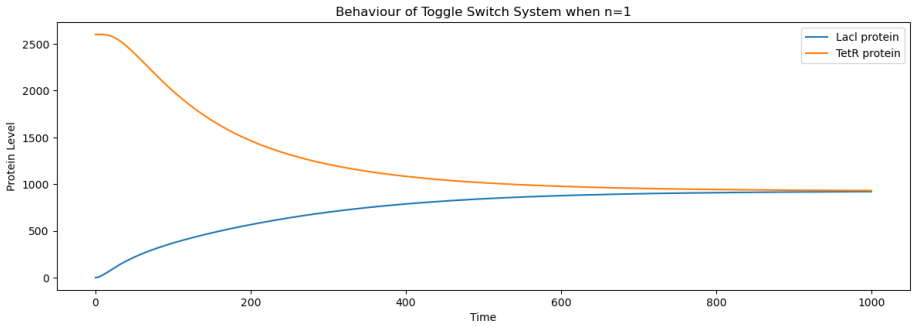


Figure 6: Behaviour of Toggle Switch System when n=1

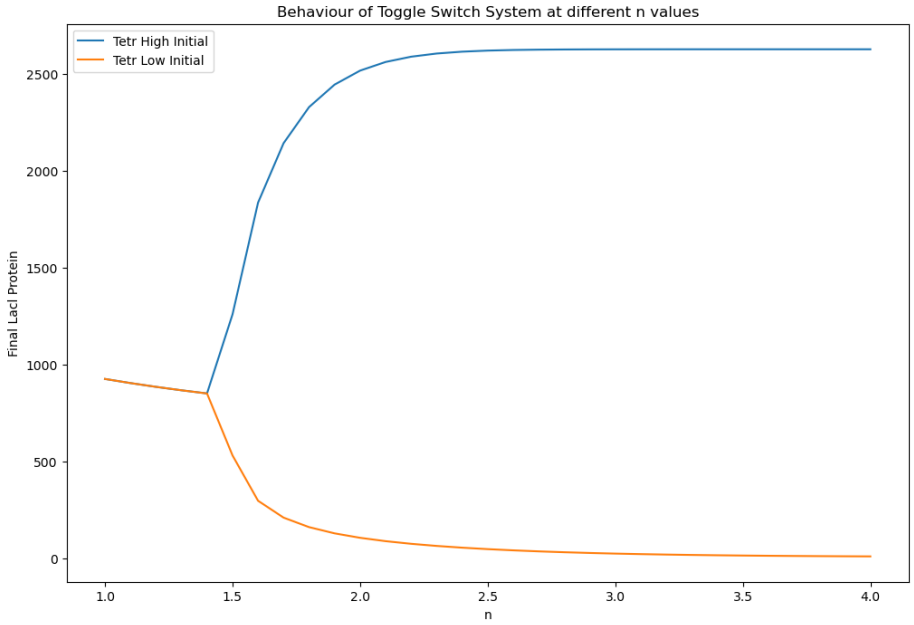


Figure 7: Behaviour of Toggle Switch System at different $n$ values

### 3.3.2 Basal Expression Rate ($k_{m0}$)

Figure 8 shows that as the basal expression rate ($k_{m0}$) increases, the system converges towards a single equilibrium state. Thus, showing sensitivity to the concentration of the repressor protein is essential to establishing bistable behaviour.

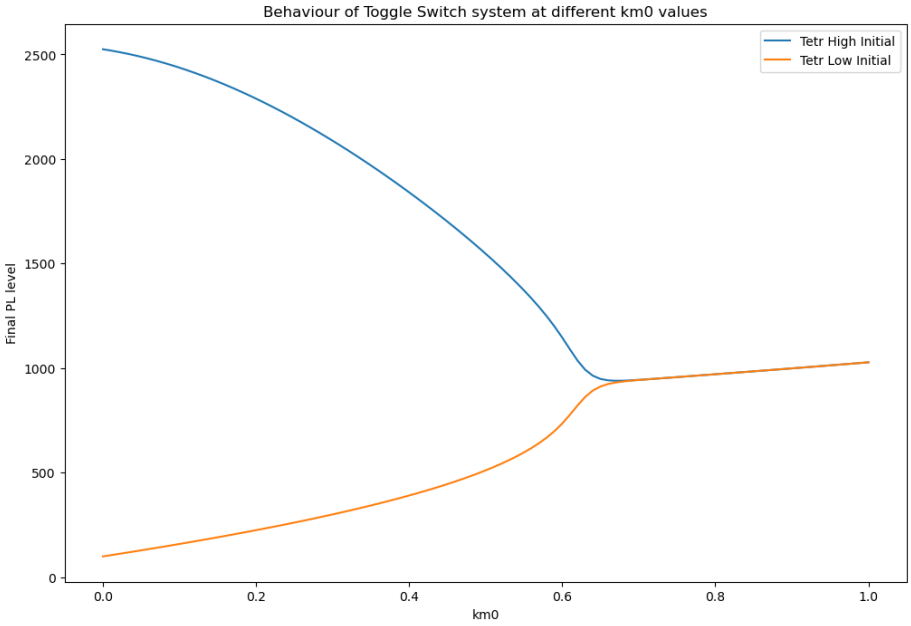


Figure 8: Behaviour of Toggle Switch System at different $k_{m0}$ values

## 3.4 Reduced ODE model

To simplify the model, we make the assumption that mRNA levels are at a quasi-steady state (QSSA) and so $\frac{dM}{dt} = 0$. This can reduce the processes to the following processes.

### 3.4.1 Expression of LacI protein ($P_L$)

$$\frac{dP_L}{dt}\text{production} = v_{p_0 L} + v_{p_L} \times \frac{K_T^n T}{P_T^n T + K_T^n T}$$

$$\frac{dP_L}{dt}\text{loss} = v_{dp_L} \times P_L$$

### 3.4.2 Expression of TetR protein ($P_T$)

$$\frac{dP_T}{dt}\text{production} = v_{p_0 T} + v_{p_T} \times \frac{K_L^n L}{P_L^n L + K_L^n L}$$

$$\frac{dP_T}{dt}\text{loss} = v_{dp_T} \times P_T$$

### 3.4.3 ODE Rate Equations

$$\frac{dP_L}{dt} = \frac{dP_L}{dt}\text{production} - \frac{dP_L}{dt}\text{loss}$$
$$\frac{dP_T}{dt} = \frac{dP_T}{dt}\text{production} - \frac{dP_T}{dt}\text{loss}$$

### 3.4.4 Phase plots

The phase plots below show two steady states thus establishing bistable behaviour as well as mapping the unstable steady state which can only be observed when the initial parameters for both proteins (LacI($P_L$) and TetR($P_T$)) and mRNA ($M_L$ and $M_P$)are equal.

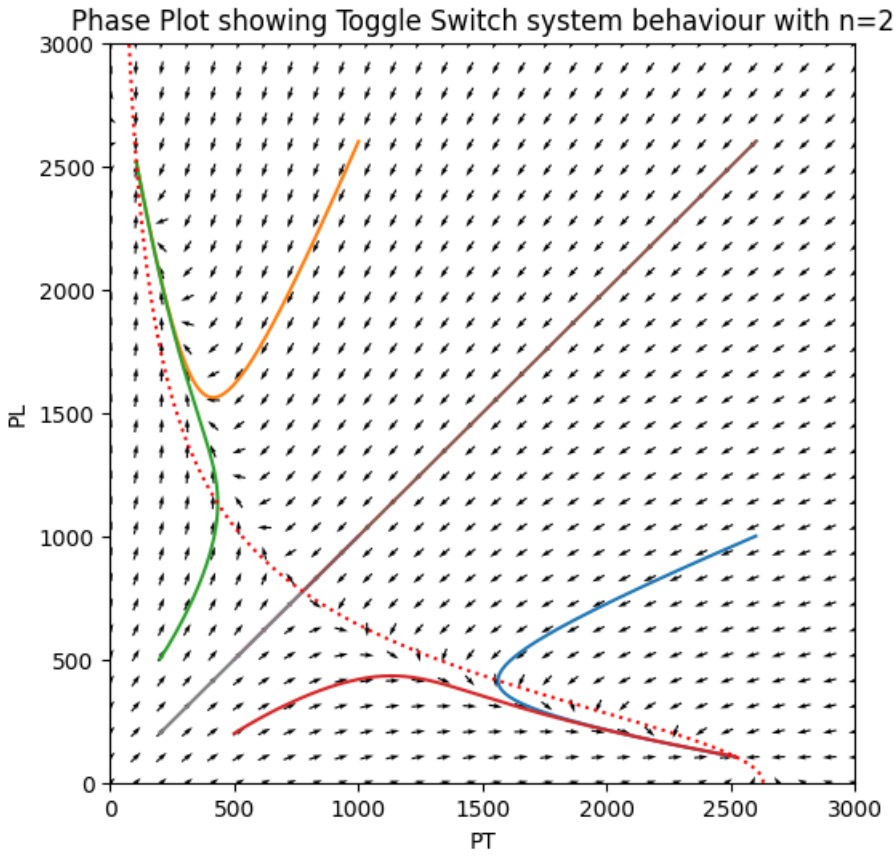


Figure 9: Phase plot showing Toggle Switch system behaviour with $n = 2$

Any small deviation from the initial symmetric state can amplify leading to a positive feedback loop which drives the system away from its steady state.

An unstable steady state represents a steady state which is sensitive to small changes. Figure 10 shows how a slight deviation in the initial symmetric weights have caused a drastic change in the system.

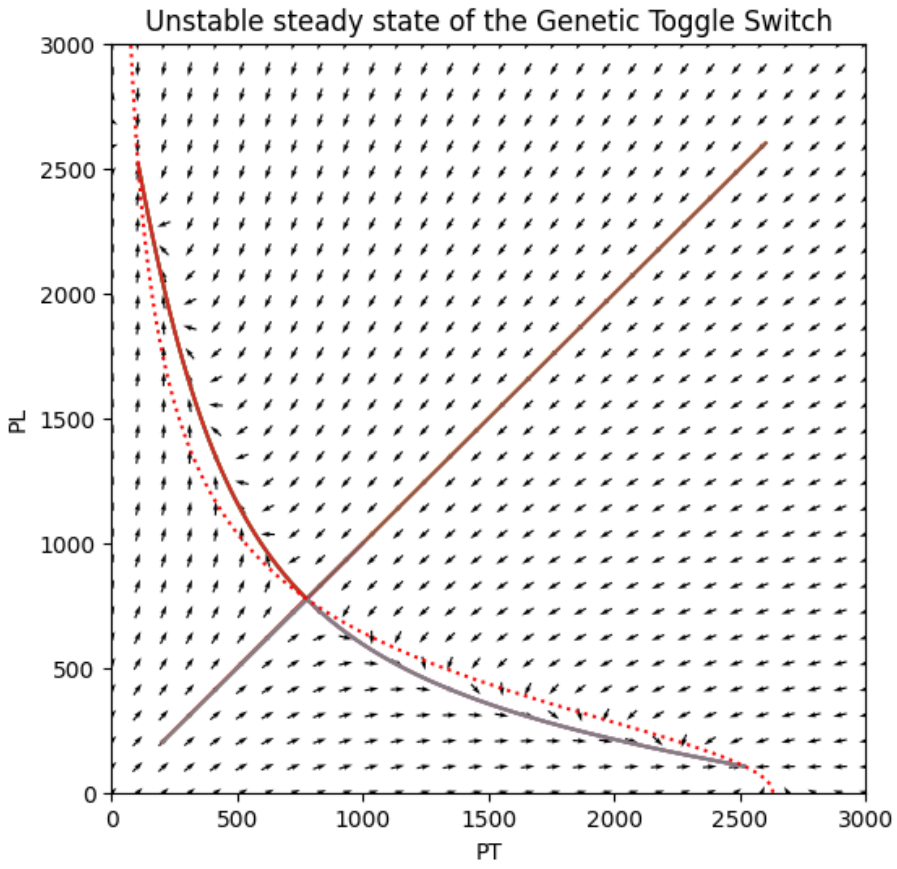


Figure 10: Phase plot showing the unstable steady state deviation when initial parameters are not initially symmetric

As mentioned earlier, when the Hill coefficient $n = 1$, the system exhibits a single equilibrium and this can be visualised in a phase plot as all 4 lies converge at a single point.

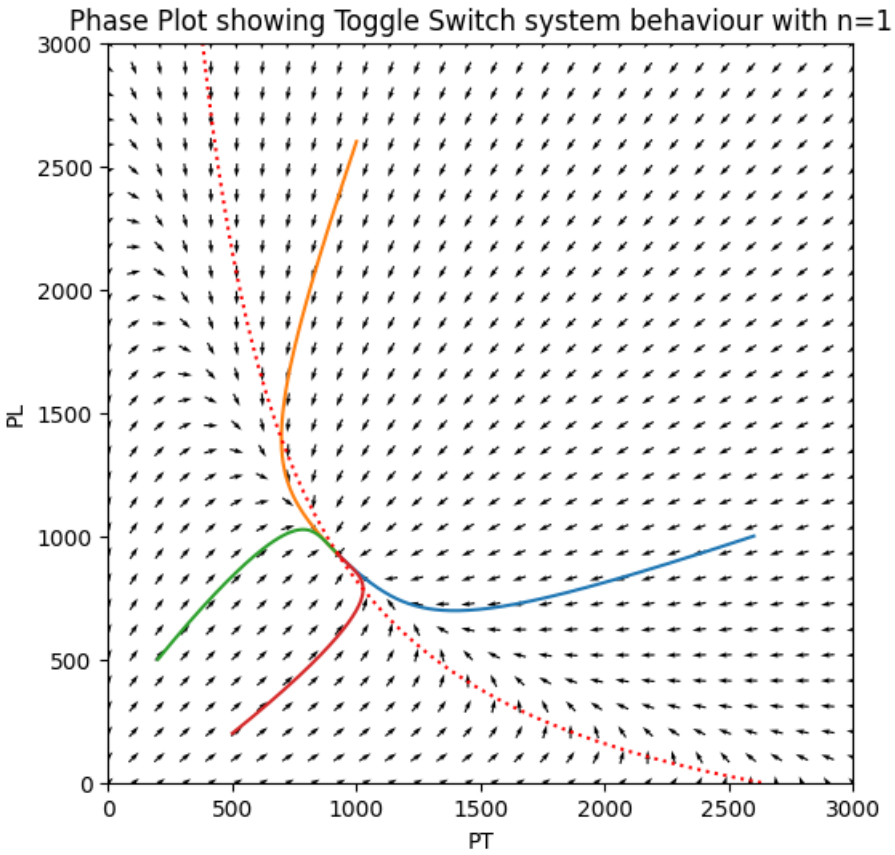


Figure 11: Phase Plot showing Toggle Switch system behaviour with $n = 1$

### 3.4.5 Bifurcation analysis

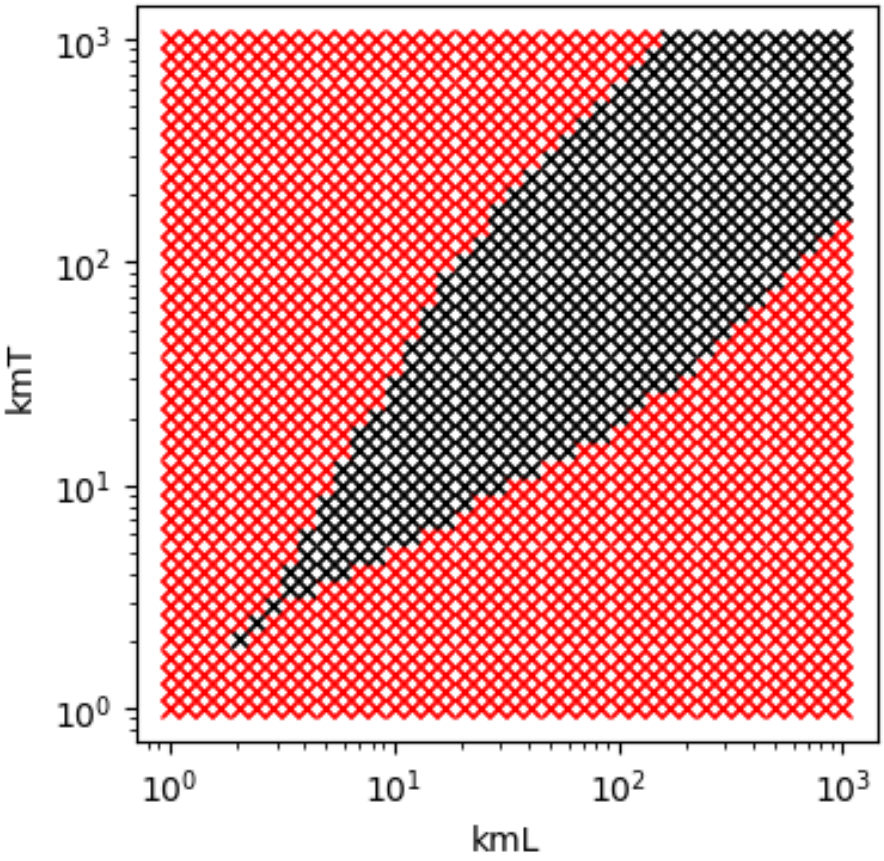


Figure 12: Bifurcation analysis at differing $k_m$ values

# 4 Investigation of the experimental Toggle Switch system implemented by Lugagne et al.

Lugagne and colleagues showed that the bistable genetic toggle switch, first established by Gardner and Collins, can be maintained near the unstable steady state position for long periods of time. Figure 12 shows the previously established bistability of the genetic toggle switch system as well as the unstable steady state using the parameter values of Lugagne et al. (INSERT CITATION)

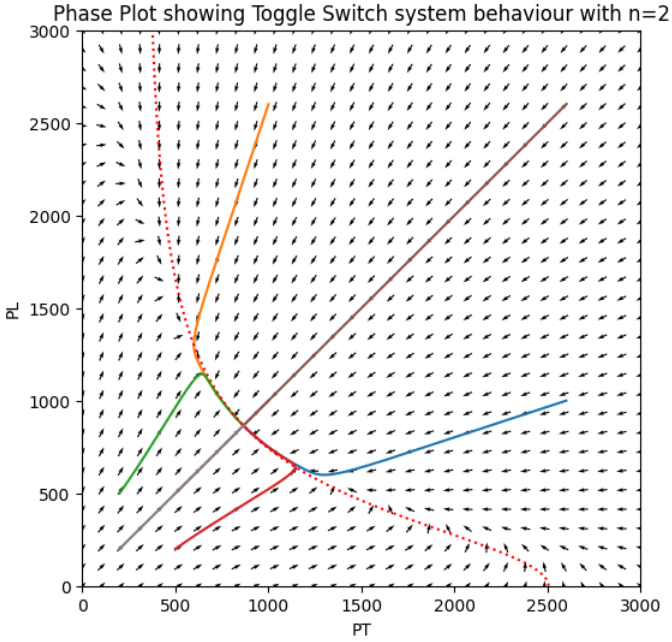


Figure 13: Behaviour of the Toggle Switch system using the parameter values of Lugagne et al.

## 4.1 Stochastic simulations

### 4.1.1 Initial state: high LacI

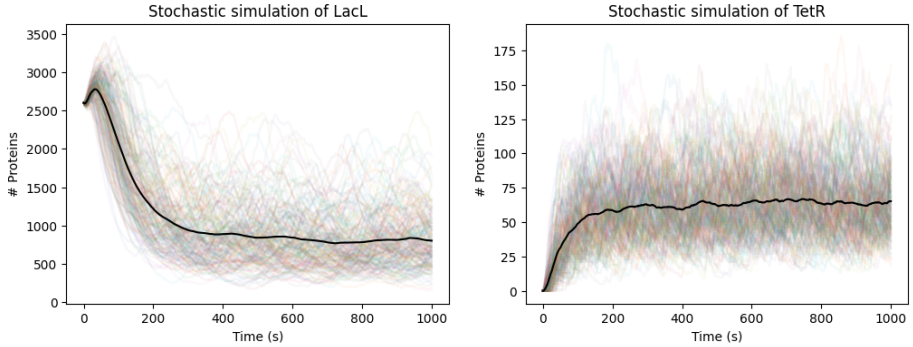


Figure 14: Behaviour of the Toggle Switch when simulated using stochastic methods with LacI high state

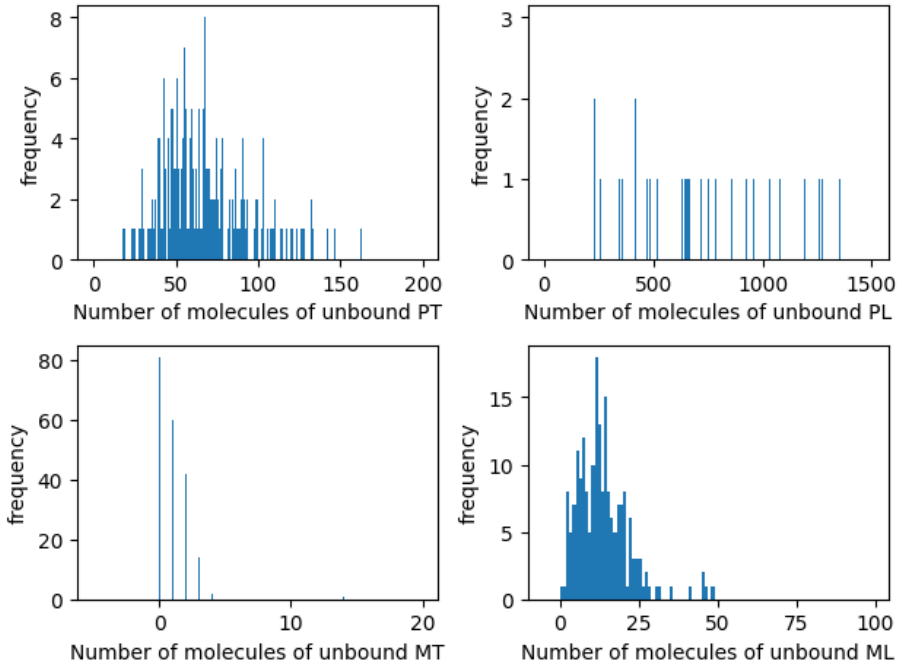


Figure 15: Distributions of mRNA and protein levels when simulated using stochastic methods with high LacI state

### 4.1.2 Initial state: high TetR

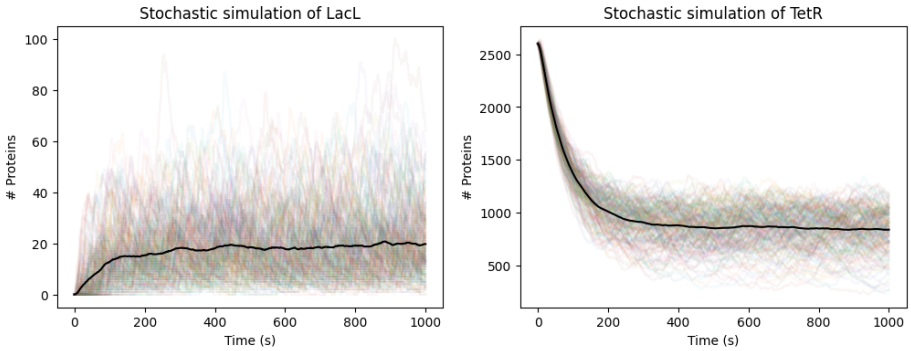


Figure 16: Behaviour of the Toggle Switch when simulated using stochastic methods with TetR high state

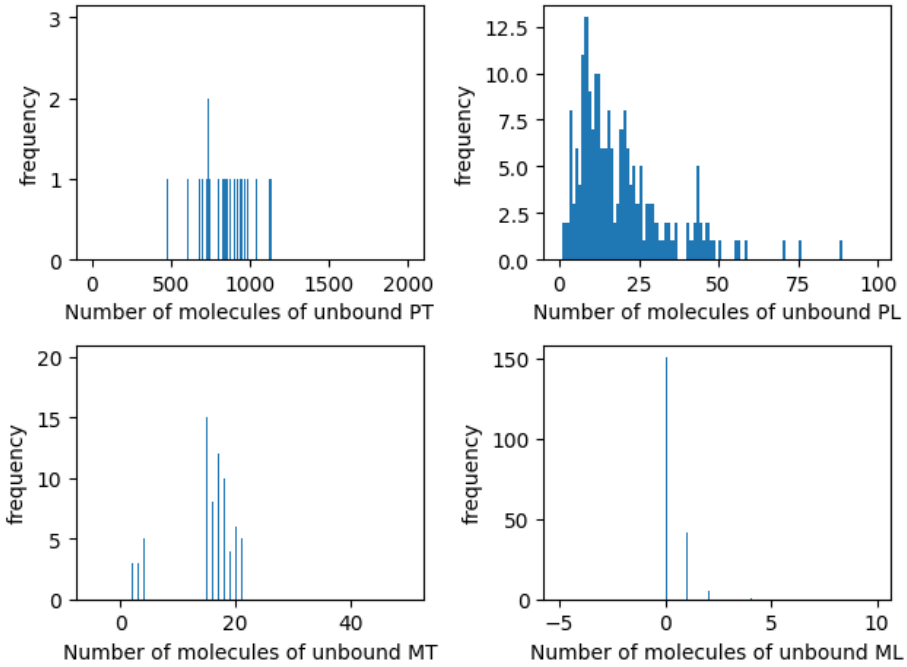


Figure 17: Distributions of mRNA and protein levels when simulated using stochastic methods with high TetR state