Exploring Bistability and Stochastic Influence in the Toggle Switch Gene Regulatory Circuit

I. Introduction

The Toggle Switch is a well-characterized synthetic gene regulatory circuit operational in *Escherichia coli* (Gardner, Cantor and Collins, 2000). It functions through the mutual repression of two transcription factors, TetR and LacI, which are produced from an introduced plasmid. This arrangement permits the system to achieve bistability, wherein it can stabilize in either of two distinct gene expression states: one with elevated TetR and suppressed LacI levels, or conversely, elevated LacI and suppressed TetR levels.

The focus of this investigation is to analyse the model associated with the Toggle Switch's operation in *E. coli*. The model employs rate equations to detail mRNA and protein production and degradation, with parameters defined to reflect basal transcription (km0), maximal transcription (km), repressor dissociation (K), cooperative binding (n), and degradation (kdm for mRNA and kdp for protein). These parameters dictate the kinetic responses of the system under various repressor protein concentrations (Pr).

The behaviour of the system is modelled by a set of ordinary differential equations (ODEs) which resolve the time derivatives of mRNA and protein concentrations (dM/dt and dP/dt). These derivatives are computed by balancing the production and loss rates, incorporating the nonlinear effects conferred by the repressor proteins through the term (K/Pr + K)^n, which describes the transcription rate as a function of repressor concentration, with n reflecting the cooperative binding to the DNA.

For a realistic model of the Toggle Switch, parameter values have been selected based on the assumption that both repressors have comparable rate constants and repression strength:

- Basal transcription rate, km0 = 0.01
- Maximum transcription rate, km = 5
- Repressor dissociation constant, K = 500
- Hill coefficient, n = 2

- mRNA degradation rate, kdm = 0.1386
- Translation rate, kp = 1.2
- Protein degradation rate, kdp = 0.0165

These parameters form the basis for the Toggle Switch simulation, allowing us to examine its time-dependent behaviour. Initial deterministic simulations aimed to establish mRNA and protein expression levels under different conditions of repression and initial concentrations. This was followed by stochastic simulations that employed parameters from Lugagne et al. (2017). These simulations assessed the distribution of molecular concentrations across multiple runs to evaluate the system's stability and response to stochastic fluctuations, supporting the model's predictive strength within the stochastic nature of biological systems.

II. Exploration of the model

i) Model Simulation and Analysis

Simulations were conducted to ascertain the dynamic behaviour of mRNA and protein expression under varying repressor protein (Pr) concentrations. Two scenarios were evaluated: one in which Pr was absent (Pr=0), and another in which Pr was present at elevated levels (Pr=3000). The simulations extended over a period of 1000 minutes to allow the system to potentially reach a steady-state from initial mRNA and protein levels of zero.

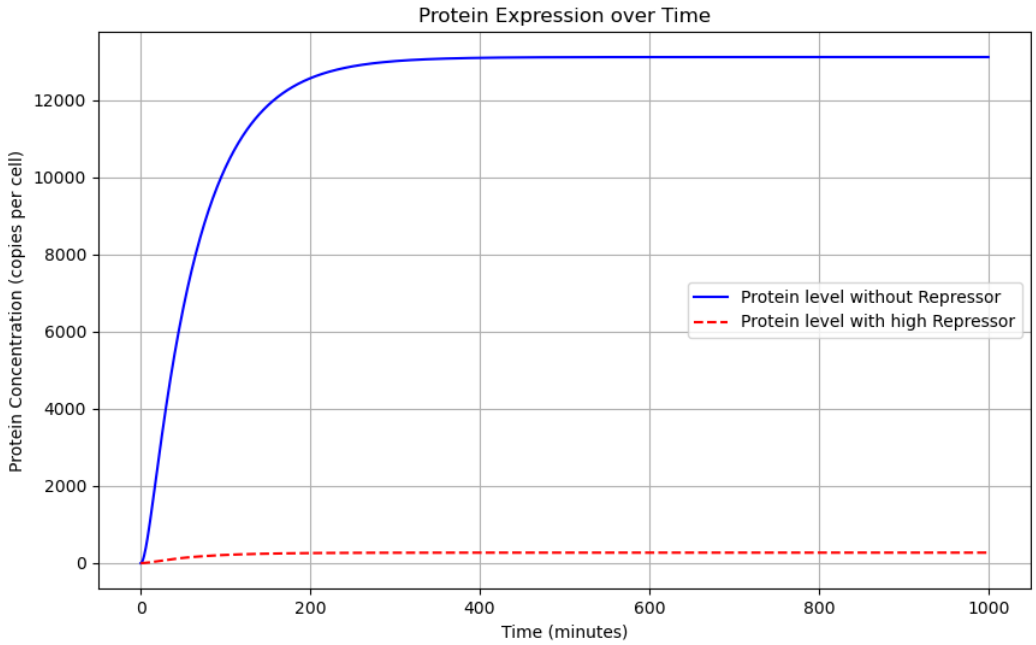

Figure 1: Protein Expression in the absence/presence of high levels of repressor.

As demonstrated in Figure 1, in the absence of the repressor protein (*Pr*=0), the protein level rises and reaches a steady state, indicating a high level of expression in the absence of repression. In conditions of high repressor level (*Pr*=3000), the protein level increases much more modestly and reaches a lower, near-zero protein concentration steady state, demonstrating the repressive effect of *Pr* on protein expression.

Variations in the Hill coefficient (n) were examined to determine their effect on protein expression profiles.

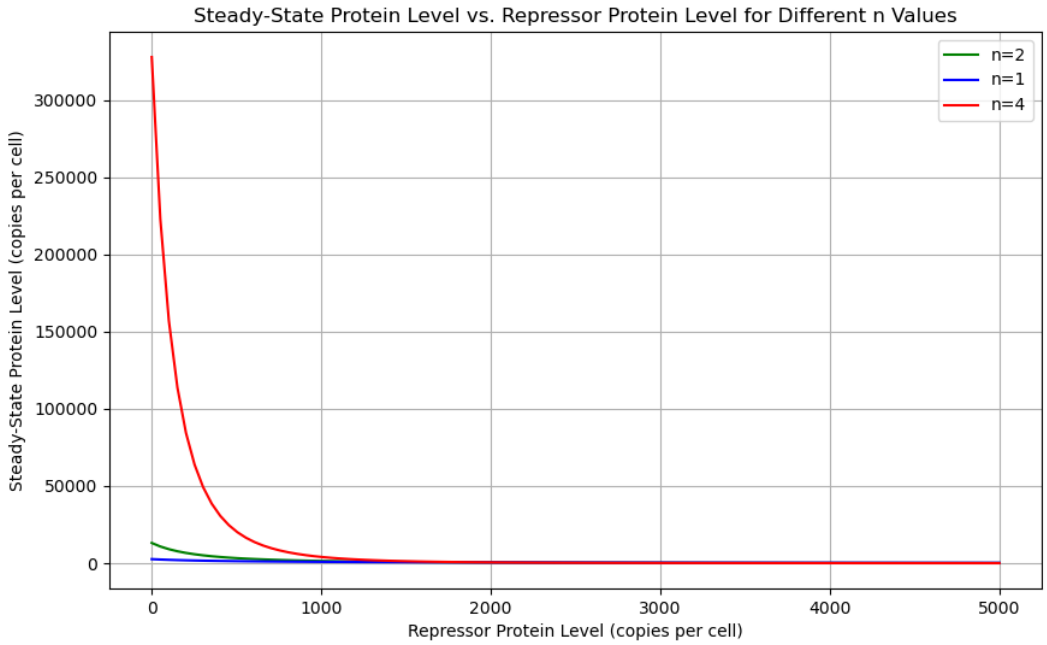

Figure 2: Protein steady-state levels under different values of the Hill coefficient (n).

Figure 2 depicts the results for n values of 1, 2, and 4 These results indicate differing regulatory responses based on system sensitivity to the repressor protein:

- With $n = 1$, the repressor and steady-state protein concentration show a more linear relationship, indicating a proportional response to repression.
- $n = 2$ demonstrates a sharper transition, indicative of cooperative binding where the effect of the repressor is more pronounced once a certain threshold is reached.
- $n = 4$ demonstrates an even more steep transition, suggesting a high sensitivity to the repressor. In this case, small increases in the repressor concentration can lead to significant reductions in protein expression, highlighting a highly cooperative binding behaviour.

We further explored the combined effects of both repressor levels and cooperativity on the steady-state protein levels.

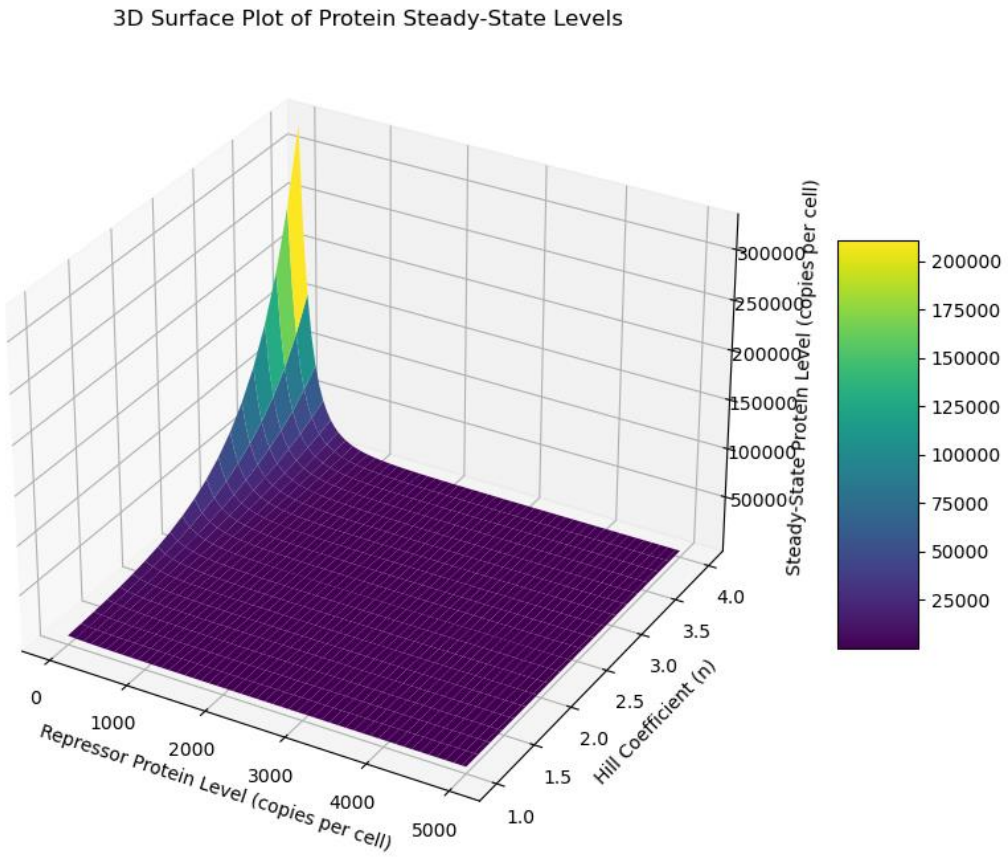

Figure 3: 3D Surface plot of protein steady-state levels under different repressor protein levels and co-operativity values (n).

As demonstrated in Figure 3, higher repressor levels (x-axis) generally lead to lower steady-state protein concentrations (z-axis), demonstrating the repressor's inhibitory effect. Additionally, an increase in the Hill coefficient (*n*, y-axis), reflecting the degree of cooperativity in the system, tends to amplify this effect, particularly at higher repressor concentrations, resulting in even lower protein levels for the same amount of repressor.

ii) Model Analysis

Following the demonstration of the system's response to different repressor levels and varying hill coefficients, we determined whether the Toggle Switch can express bistability at two different states (TetR high, LacI low & TetR low, LacI high) as described by Gardner et al.

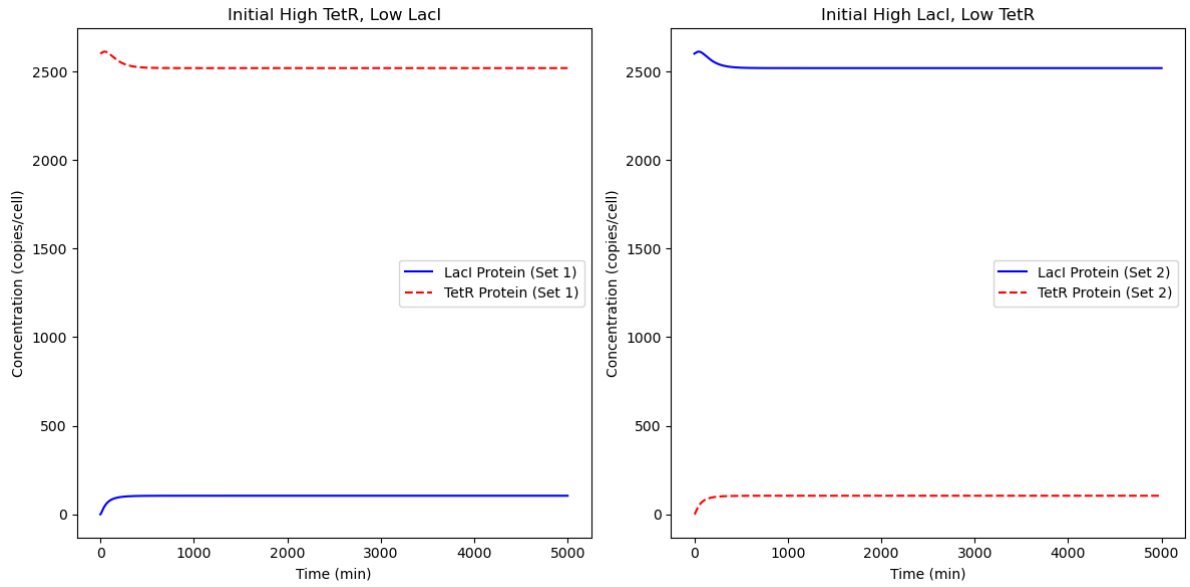

Figure 4: Bistability of the Toggle Switch system described by Gardner et al.

In Figure 4, with both initial High TetR, Low LacI and initial High LacI, Low TetR the system maintains a consistent state over a prolonged period (5000 minutes), which supports the hypothesis of bistability for both sets of initial conditions.

We further explored the Toggle Switch System by examining its behaviour under a non-cooperative model, setting the cooperativity factor n=1. In this simplified scenario, we anticipated that the system could maintain different stable states, influenced by the initial conditions.

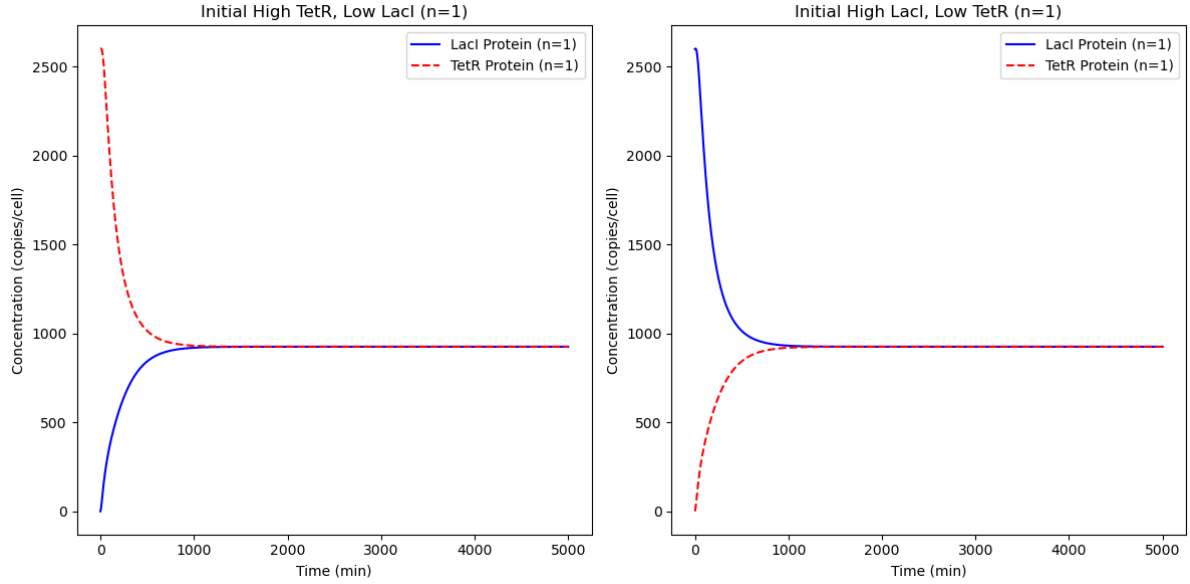

Figure 5: Behaviour of the Toggle Switch System when n = 1.

The plots in Figure 5 present a monostable system where both proteins' concentrations converge to the same equilibrium point, regardless of their initial levels. robust bistability of the Toggle Switch system is compromised when cooperativity is absent. These findings illustrate the importance of cooperativity (n>1) in achieving bistability of the Toggle Switch System.

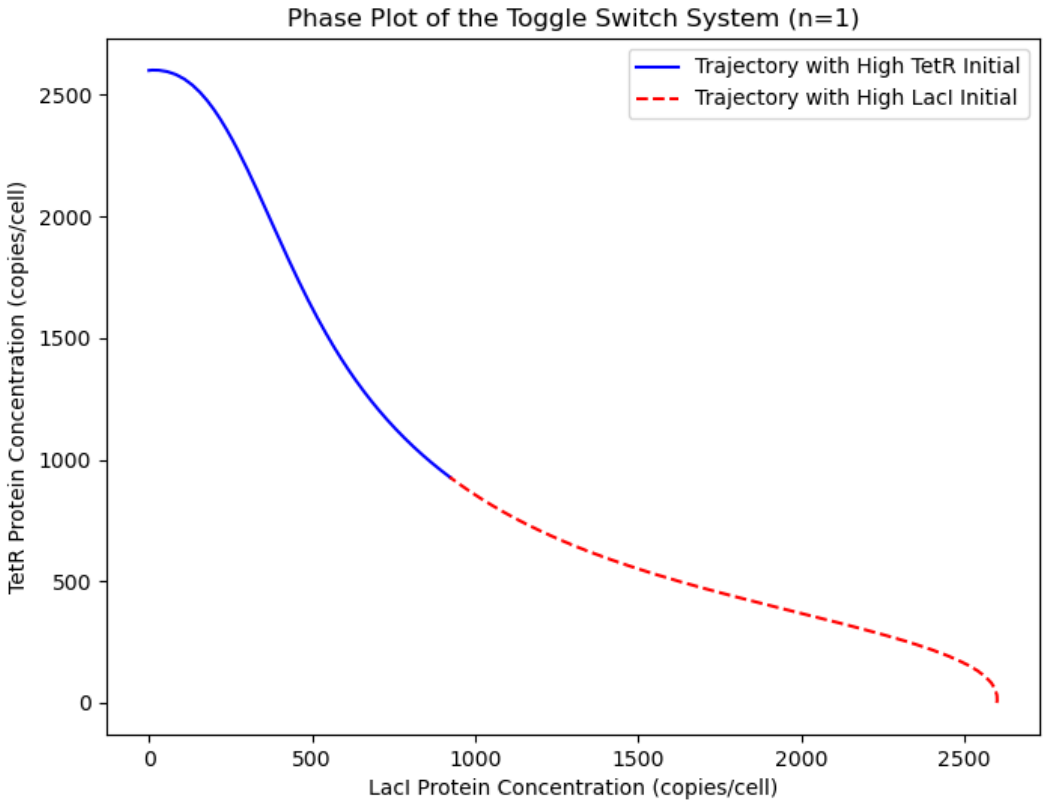

Figure 6: Phase plot of the Toggle Switch System when n = 1.

The phase plot in Figure 6 shows that, for $n = 1$, regardless of whether the initial condition is high TetR or high LacI, the system converges to a single steady state where the LacI and TetR protein concentrations are at equilibrium. The plot indicates that the system is not sensitive to initial conditions for $n = 1$, and therefore lacks the memory property that is typical in a bistable switch.

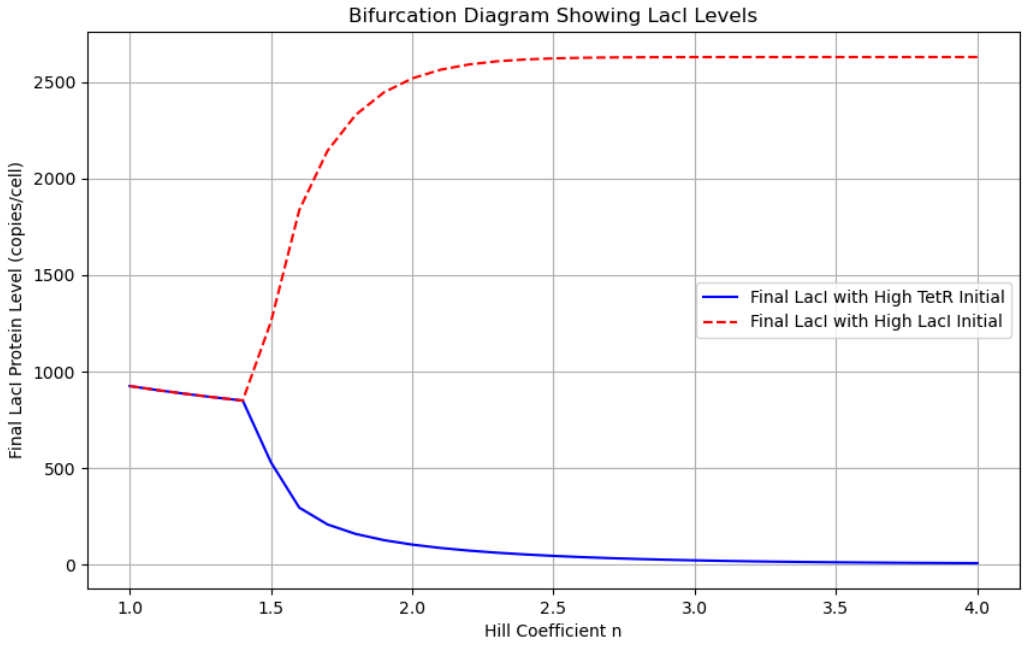

Figure 7: Bifurcation diagram showing the behaviour of the Toggle Switch System at different n values.

The emergence of bistability as a function of the Hill coefficient is presented in Figure 7. For lower values of *n*, the system exhibits monostable behaviour, indicated by the convergence of final LacI protein levels irrespective of the initial conditions. As *n* increases beyond a critical value, the system transitions to a bistable regime, where two distinct steady-state levels of LacI are achievable, depending on whether the initial condition is high in TetR or high in LacI. This bifurcation diagram underscores the sensitivity of the Toggle Switch system to the cooperativity factor *n*, highlighting the range over which bistability is observed and confirming the dependence of the system's final state on initial conditions within this bistable range.

We created phase plots with nullclines and a direction field for n=2 and n=1 to provide further insight into the system's stability in conditions of cooperativity versus no cooperativity.

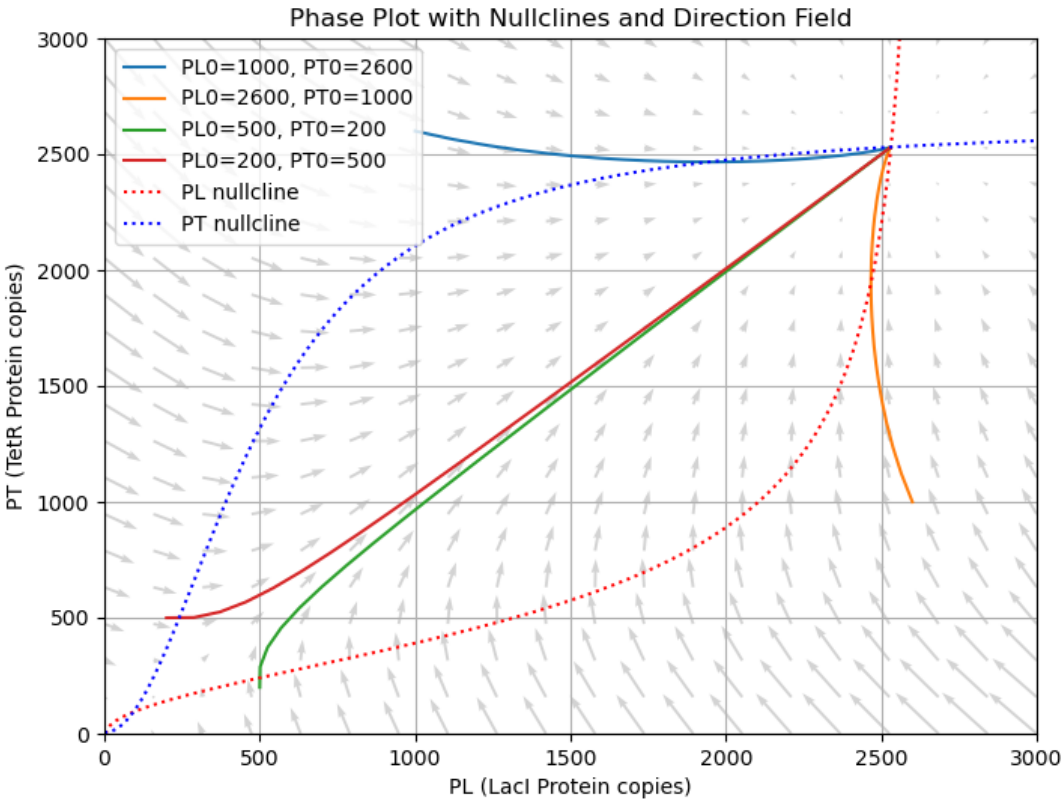

Figure 8: Phase plot showing the Toggle Switch behaviour with n = 2.

With a degree of cooperativity where nL = nT > 1, (as illustrated in Figure 8), the system's nullclines intersect at two distinct points. These intersections correspond to the stable steady states of the Toggle Switch system. The trajectories shown on the plot indicate the path the system will follow from various initial conditions, converging on these stable points. Such dynamics are indicative of a bistable system, which can maintain either of two stable states over time.

Cooperativity is essential for this nonlinear behaviour. When $n>1$, the repressive effect of the protein on gene expression is not merely a proportional response to its concentration. Instead, there's an amplified effect—once the repressor concentration surpasses a critical threshold, the gene expression is significantly repressed. This nonlinear response, facilitated by cooperativity, allows the system to stabilize in one of two states: either 'high LacI/low TetR' or

'low LacI/high TetR,' thereby exhibiting the characteristic of bistability.

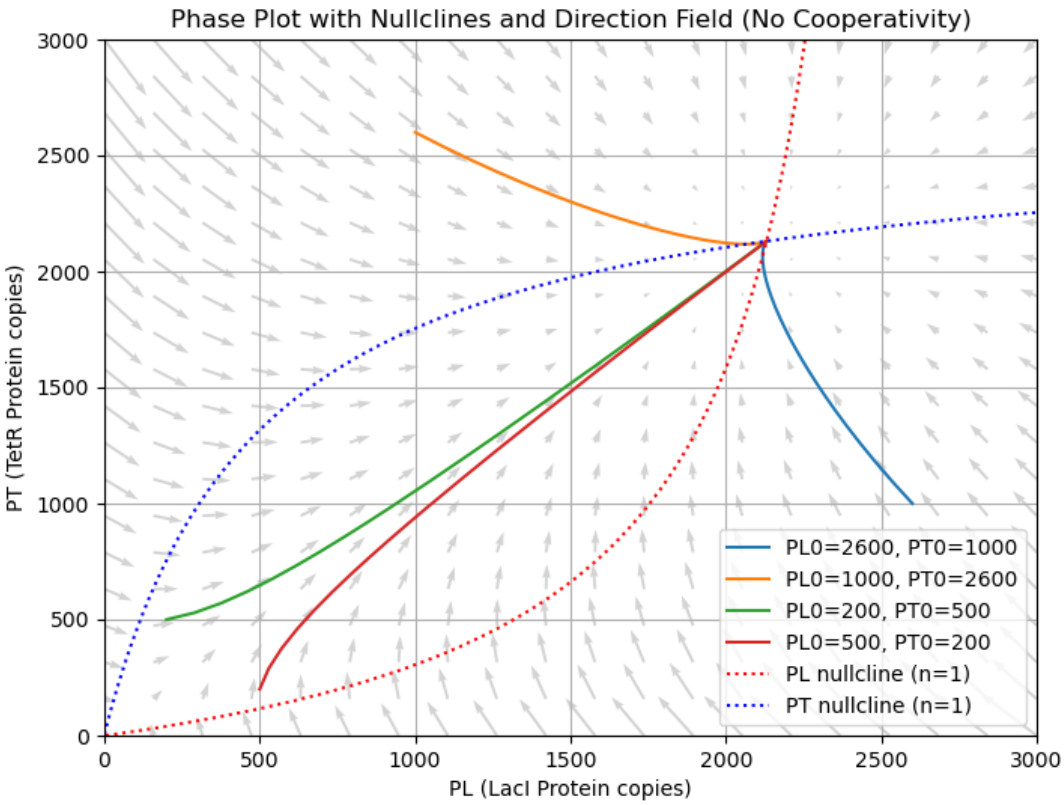

Figure 9: Phase plot showing Toggle Switch system behaviour with n = 1.

In the absence of cooperativity, as depicted in Figure 9 where $n_L = n_T = 1$, the nullclines intersect at a singular point, suggesting that the Toggle Switch system has only one stable steady state. This intersection point reflects a monostable system behaviour, where the dynamics, irrespective of the starting concentrations of LacI and TetR, converge to this one attractor. This contrasts with a bistable system, which requires nonlinearity introduced by cooperativity to maintain multiple stable states.

When the Hill coefficient n equals 1, the repression exerted by a repressor protein on gene expression is strictly proportional to its concentration. This proportional relationship, which lacks the threshold effects seen in cooperative binding, typically cannot support the complex dynamics necessary for bistability. Instead, the system will naturally gravitate toward the equilibrium point where the rates of protein production and degradation are balanced.

iii) Empirical Model Evaluation

To validate our theoretical Toggle Switch model, we used experimentally derived parameters from actual biological systems obtained in a study by Lugagne et al.

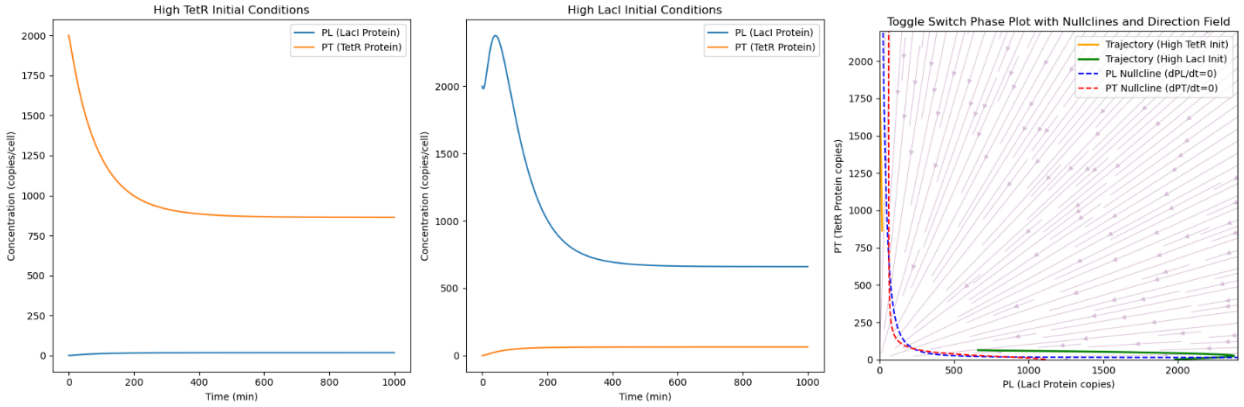

Figure 10: Behaviour of the Toggle Switch system using the parameter values of Lugagne et al. (Lugagne et al., 2017).

As with the theoretical model, the TetR level decreases and the LacI level increases and stabilizes, and vice versa. This aligns with the theoretical prediction of bistability and the system's ability to switch states given a high initial concentration of TetR.

Further model simulation was conducted using stochastic methods to explore the Toggle Switch system's robustness to biological noise. This uses the estimates of parameter values and initial conditions from a Toggle Switch System implemented by Lugagne et al. (Lugagne *et al.*, 2017) which have been chosen to provide an initial estimate for parameter values in the real system.

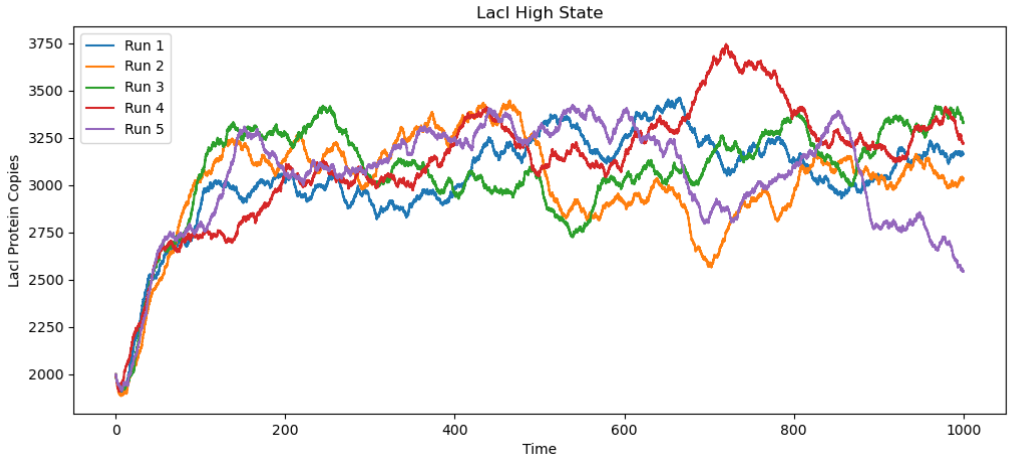

Figure 11: Behaviour of the Toggle Switch system when simulated using stochastic methods (LacI high state), over a duration of 1000 minutes.

The results, as depicted in Figure 11, exhibit multiple stochastic trajectories, each representing a potential outcome within the LacI high state. Despite the inherent randomness introduced in these simulations, the concentration of LacI protein for each run displayed consistent fluctuations around a high steady-state level, suggesting the robustness of the LacI-dominant state against stochastic perturbations. This behaviour validates the bistability of the Toggle Switch system, indicating its capacity to maintain a high LacI state across a range of stochastic scenarios.

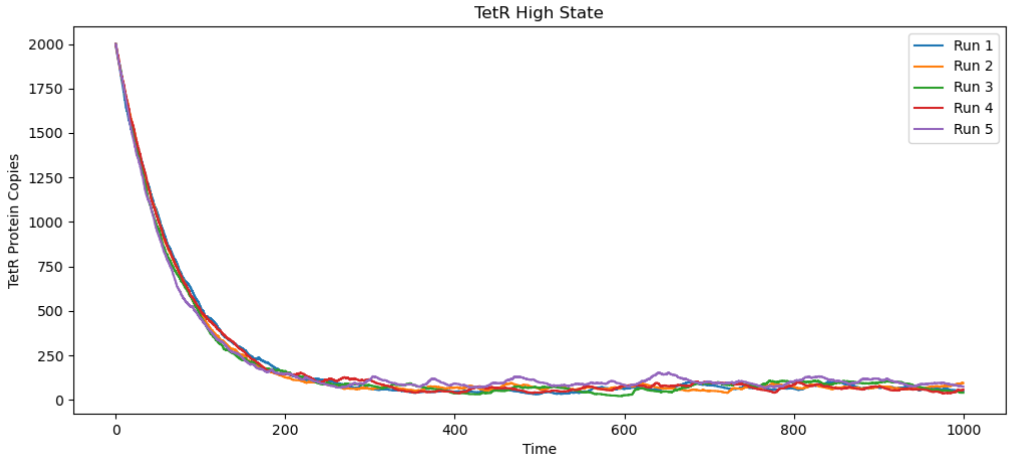

Figure 12: Behaviour of the Toggle Switch system when simulated using stochastic methods (TetR high state), over a duration of 1000 minutes.

Figure 11 demonstrates the stability of the low TetR state when starting from high TetR conditions. The absence of large fluctuations or transitions back to high TetR levels suggests that, in this model, once the TetR concentration decreases, it remains low, potentially due to the influence of LacI, which is not actively repressed and therefore can stabilize the system in the alternate state.

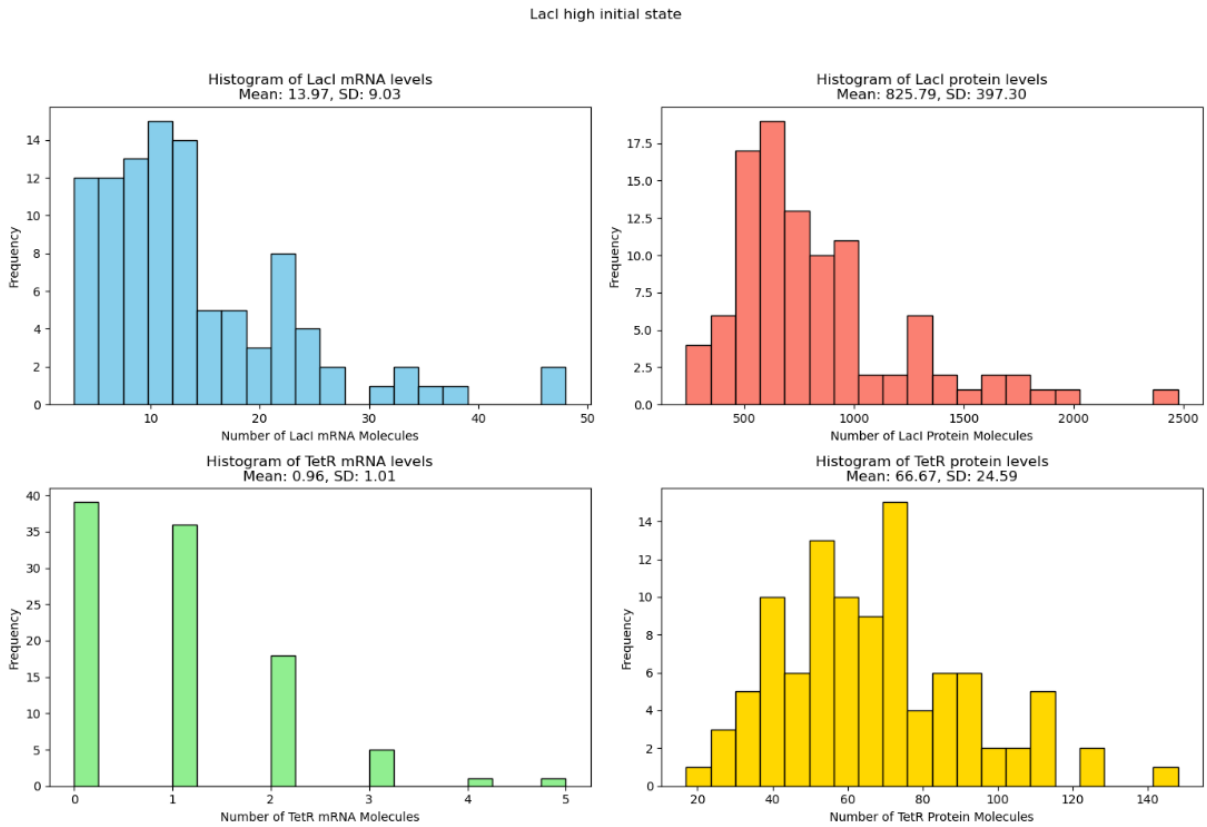

Figure 13: Distributions of mRNA and protein levels when simulated using stochastic methods (LacI high state, 100 runs).

Stochastic simulation of the LacI high state produced left-leaning histograms (Figure 13). The LacI mRNA and protein levels have higher means compared to the levels of TetR, consistent with the LacI high initial state. In addition, there are a relatively low number of TetR mRNA and protein molecules, with a tight distribution around zero. This is indicative of effective LacI repression.

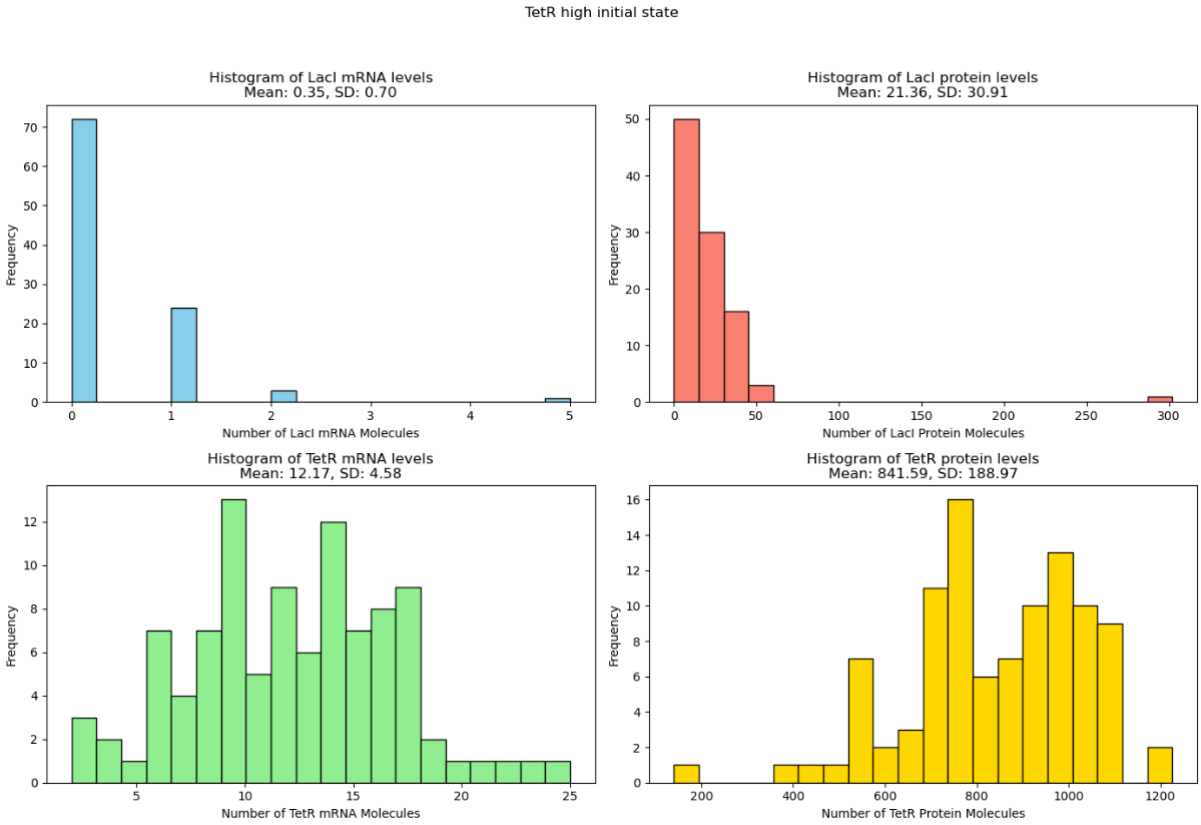

Figure 14: Distributions of mRNA and protein levels when simulated using stochastic methods (TetR high state, 100 runs).

The distributions of the TetR high initial state (Figure 14) suggest that it experiences repression of LacI (both mRNA and protein), consistent with the behaviour of the Toggle Switch. As with TetR proteins in the LacI initial state LacI persists at a low level, suggesting that these protein molecules may persist in the cell. In most high TetR simulations, TetR protein levels persist at high levels, but with a much higher level of variability than the simulations of LacI in the high LacI state.

III. Discussion and Summary

In agreement with the initial demonstrations by Gardner et al., our deterministic simulations confirmed the bistability of the model. The Toggle Switch has been posited as a model system for studying bistability in gene expression (Gardner, Cantor and Collins, 2000). Bistability is a crucial feature of cellular decision-making processes, allowing for the switch-like response necessary for differentiation, signal processing, and development in biological systems (Pomerening, 2008; Liu *et al.*, 2011). In synthetic biology, Bistability is vital where switch-like

control over gene expression is desired (Tiwari *et al.*, 2011). The introduction of non-cooperative dynamics (n=1) into the model revealed the dependence on cooperative binding for maintaining bistability, as without cooperativity, the model predicted a convergence towards a monostable state regardless of initial conditions. These observations are consistent with the established understanding that cooperative binding is a prerequisite for bistability in regulatory circuits (Cherry and Adler, 2000; Gardner, Cantor and Collins, 2000; Warren and ten Wolde, 2004).

The phase plots with nullclines for n=2 and n=1 further illustrated the system's behaviour, offering a visual representation of the trajectories converging to stable points under cooperative conditions and a singular attractor in the absence of cooperativity. These findings align with theoretical predictions and underscore the sensitivity of the system to the Hill coefficient, an important parameter defining the nonlinearity and feedback strength within the gene regulatory network (Kim, White and Winfree, 2006).

The stochastic simulations aligned with the empirical data from Lugagne et al., supporting the theoretical model's capability to replicate biological phenomena. Results indicated the maintenance of bistable states in the presence of stochasticity, supporting the model's robustness. Distributions of mRNA and protein levels showed a tendency for lower values in both the LacI and TetR high states across multiple simulations. It must be noted, however, that real biological systems may present complexities beyond the scope of this model. External factors such as environmental variability and intrinsic molecular noise, along with unmodeled cellular interactions, could impact system dynamics in vivo (Gonze *et al.*, 2018).

The data indicate a sustained bistability amidst stochastic fluctuations, highlighting the Toggle Switch's stability in the inherently variable cellular environment. Moreover, the system's interaction with noise hints at the possibility of stochastic resonance, where a certain level of noise could enhance the system’s responsiveness to external stimuli—a feature that could be beneficial for refining synthetic gene circuits or in developing therapies that require precise control over gene expression dynamics.

The study did not examine metastable or intermediate states; however, the observed durability of bistable states in stochastic conditions observed here suggests that the system is less prone to such transient states within the parameter ranges explored. Metastable states, often associated with gradual transitions in biological processes, remain an area for future investigation, particularly in the context of cellular differentiation and signal processing (Díaz-Hernández and Santillán, 2010; Lugagne *et al.*, 2017).

Future work should continue to focus on direct empirical observations of the Toggle Switch within cellular environments to evaluate its performance under real biological conditions. Additionally, the exploration of the effects of varying individual rate constants and other parameters on the system's robustness could offer further insights into the design principles necessary for constructing stable and reliable synthetic gene circuits.

In summary, the current study provides computational evidence for the system's stability and responsiveness under a range of conditions, both deterministic and stochastic. These findings enhance our understanding of the operational parameters that govern bistable behaviour in synthetic biology applications. The robustness of the system's bistable states to stochastic perturbations has implications for the design and application of synthetic regulatory networks in complex biological environments.

Word count: 2661

IV. References

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