Final project: Analysis of a synthetic oscillatory network by modeling

Lian, Lian

Introduction

The article "A synthetic oscillatory network of transcriptional regulators" presents the design, assembly, and analysis of a synthetic gene regulatory network, termed the "repressilator," within *E.coli*. The repressilator is a genetic circuit composed of three transcriptional repressors — LacI, TetR, and λ cI — that are not part of any natural biological clocks but are well-characterized for their repressive functions. The circuit is conceptualized as a cyclic negative-feedback loop where each repressor protein inhibits the transcription of the subsequent repressor gene in the sequence, thus forming a closed regulatory network. The cyclic negative-feedback loop will produce a predictable oscillatory behavior, which is marked by the periodic induction of green fluorescent protein (GFP) synthesis.

Modeling approach

The model assumes symmetry where all repressors are identical except for their DNA-binding specificities. The kinetics model of transcriptional regulation in the repressilator simplifies into six first-order ordinary differential equations (ODEs), representing three repressors and their corresponding mRNAs:

$$\frac{dm_i}{dt} = -\mathrm{k_m m_i} + \frac{\alpha}{1 + \left(\frac{p_j}{K_M}\right)^n} + \alpha_0, \quad \text{where } \mathrm{k_m} = \frac{\ln(2)}{\mathrm{T_{m,1/2}}}$$

$$\frac{dp_i}{dt} = -\mathrm{k}_p p_i + \beta m_i, \quad \text{where } k_p = \frac{\ln(2)}{T_{p,1/2}}$$

$$(i = LacI, tetR, cI, \quad j = cI, LacI, tetR)$$

Each mRNA's dynamics, m_i, are modeled by a natural degradation term, $-k_m m_i$, a regulated transcription rate, $\frac{\alpha}{1+\left(\frac{p_j}{K_m}\right)^n}$, and a baseline transcription rate, α_0. In natural degradation rate, mRNA decay rate is denoted as k_m which is determined by mRNA half-life, $T_{m,1/2}$. The baseline transcription rate, α_0, is the leaky transcription rate that is independent of repressor binding. The regulated transcription rate models as a Hill function, where n is a Hill coefficient, p_j is the repressor-protein, and K_M quantifying the efficiency and strength of the repressor-protein. The parameters in ODE of m_i are set as $T_{m,1/2} = 2\ min$, $K_M = 40\ monomers/cell$, $n = 2$, $\alpha_0 = 5 \times 10^{-4}\ transcription/second$, and $\alpha = 0.5\ transcription/second$.

The p_i has a more intuitive ODE which contains two parts, the natural degradation rate, $-k_p p_i$, and translation rate, βm_i. The protein decay rate k_p is determined by protein half-life, $T_{p,1/2}$, which is similar to mRNA decay rate. In translation rate, β represents the average translation efficiency. The parameters in ODE of p_i are set as $T_{p,1/2} = 10\ min$, and $\beta = 20\ proteins/mRNA$.

Parameter determination in the repressilator model involved a combination of empirical data and theoretical adjustments to ensure the system's functionality within the desired oscillatory regime. Promoter strength and translation efficiency parameters were set to realistic values known from existing biological data, with promoter strength varying from 5×10^{-4} (repressed state) to 0.5 (fully induced state) transcripts per second, and an average translation efficiency of 20 proteins per transcript. The Hill coefficient was consistently used as n=2, reflecting common cooperative binding effects in transcriptional regulation. To balance the lifetimes of the proteins and mRNA, which critically influence the system's dynamics, specific

genetic modifications were introduced. For example, repressor proteins were tagged with ssrA sequences to decrease their stability, aligning their lifetimes closer to the mRNA's average half-life of about 2 minutes in E. coli. This was done to enhance the natural decay rates to make the oscillations more robust against fluctuations. Additionally, the half-maximal repression concentration, K_M, was set at 40 monomers per cell, a value selected to match the effective concentration needed for repression under the model's conditions.

Result

Based on the simulation results for the repressilator system using deterministic modeling, the system exhibits the characteristic oscillatory behavior that aligns with the theoretical expectations of a synthetic genetic oscillator. In both mRNA and protein concentration plots, we observe periodic fluctuations indicative of negative feedback and the delayed response inherent in gene expression (Figure 1).

These dynamics can be explained by the repressilator's design, where each gene represses the next, and the last represses the first, creating a feedback loop. The consistent oscillatory behavior across the simulated time indicates that the designed parameters—such as the degradation rates, transcriptional and translational efficiency, and Hill coefficients—are within biologically plausible ranges that facilitate the network's intended function as a genetic clock.

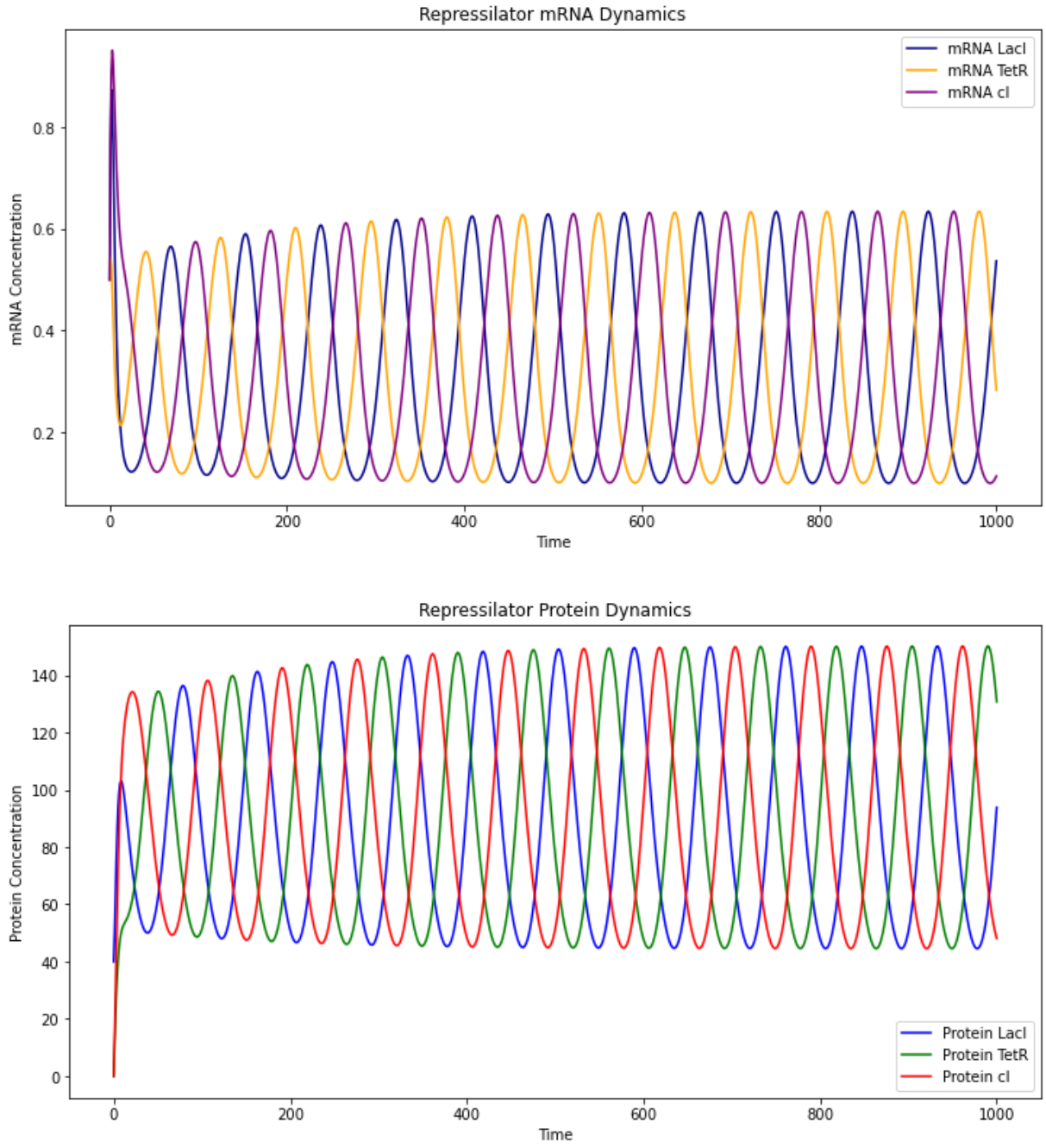

Figure 1. Oscillations in the levels of the three mRNA, and the three repressor proteins. The initial condition set is $m_{lacI} = m_{tetR} = m_{cI} = 0.5$, $\mathrm{p_{lacI}} = 40$, and $p_{tetR} = p_{cI} = 0$.

Discussion of the model

The repressilator model provided several intriguing insights into synthetic biological networks, particularly highlighting the dynamic behaviors that can emerge from simple negative feedback loops. By constructing and analyzing this synthetic oscillatory network in Escherichia coli, the researchers demonstrated that even a minimalistic system composed of three repressors can produce stable, sustained oscillations in protein concentrations over time. The model's use extended beyond demonstrating oscillatory behavior; it also helped identify the critical parameters that influence the stability and robustness of these oscillations. For example, the analysis revealed that the balance between mRNA and protein decay rates is crucial, with more similar decay rates favoring oscillation.

Moreover, the model underscored the impact of stochastic fluctuations inherent in gene expression, which can introduce noise and variability in the oscillations. This observation led to further experiments that confirmed the model's predictions, demonstrating the repressilator's sensitivity to molecular noise and the potential need for additional regulatory mechanisms to mitigate these effects. The realization that both positive and negative feedback loops might be necessary to create more stable oscillations paves the way for designing more complex and reliable synthetic networks that can better mimic natural biological systems.

In summary, the repressilator model not only illustrated the feasibility of constructing a synthetic gene network capable of complex dynamic behavior but also provided a foundational framework for understanding and designing other artificial regulatory networks with predictable and tunable properties.

Extension analysis of model:

The oscillation behavior of this feedback loop depends on the symmetry of initial conditions. The symmetry of initial condition here indicates the identical initial condition of each mRNAs or proteins. In our model, all three repressors are considered in same first-order differential equations except for their DNA-binding specificities. The symmetrical initial conditions will lead the system deviate from oscillation behavior, because no inherent delay or asymmetry in the system drive oscillations (Figure 2).

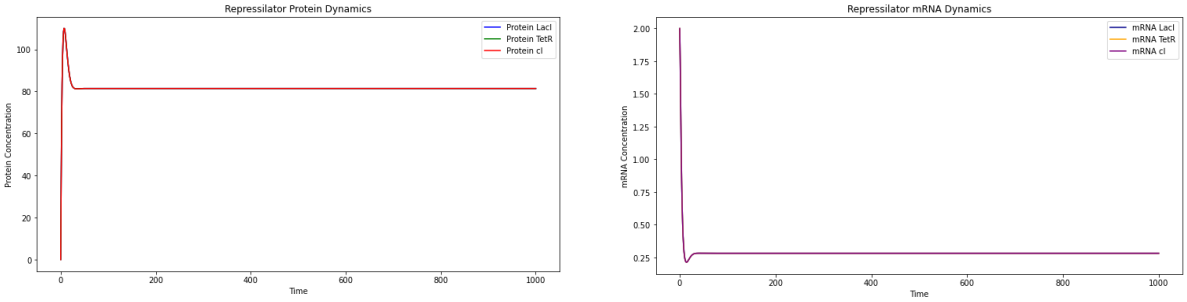

Figure 2. mRNAs and repressor-proteins behavior under symmetrical initial condition. The initial condition set is $m_{lacI} = m_{tetR} = m_{cI} = 2$, and $p_{tetR} = p_{cI} = p_{\mathrm{lacI}} = 0$.