RESEARCH

Introduction to Focus Areas in Bioinformatics Project Week 8

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Abstract

Goal of the project: To model and simulate the 'Repressilator' Model from MB Elowitz and S Leibler with the software Copasi.

Main results of the project: We could simulate the models of the paper. The results for the stochastic model were not as constant as in the original case.

Personal key learnings:
Sina: I learned about working with Copasi.
Christina: Understanding of the network designs.
Swetha: I learned about deterministic method in COPASI.
Gokul: Understood the biology of COPASI results and ODE models.

Estimation of the time:
Sina: 6 hours
Christina: 6 hours
Swetha : 6 hours
Gokul: 6 hours

Project evaluation: 2

Number of words: 1348

1 Biological background

The interactions of biomolecules in intracellular networks are fundamental for the function of living cells. One example for such a network would be an oscillating network for *Escherichia coli*, which is built out of the three repressor systems. The protein LacI inhibits the gene TetR, which protein inhibits δ cI. By inhibiting the expression of LacI, cI completes the cycle.

However, the designs of these molecular networks are hard to understand through approaches like a quantitative analysis. To get a deeper insight into the behaviors of the cells and the design of the networks, they can be simulated through synthetic networks. [1]

2 Mathematical model

The model was simulated with two different methods, a deterministic, continuous approach and a stochastic, discrete approach.

The goal of the network designs is to identify dynamic behaviors and regulate the parameters to get permanent oscillations since there is not enough information on the network to do an exact description of the system.

As described by Elowitz and Leibler, only the symmetrical case was considered, meaning that all three repressors have identical characteristics except for the DNA binding.

The kinetics of the system are described by the following differential equations, where $i = LacI, TetR, cI$ and $j = cI, LacI, TetR$.

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1 - p_j^n} + \alpha_0$$
$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

The variable p_i denotes the repressor concentrations, while m_i stands for the corresponding mRNA concentrations. α describes the number of protein copies produced per cell for the absence (α_0) and the presence ($\alpha + \alpha_0$) of repressors, β the ratio between the protein decay rate and the mRNA decay rate, and n is a Hill coefficient.

It is assumed that the stochastic character of such interactions is important in biochemical and genetic networks. Due to this fact, an stochastic approximation was done according to the description and the differential equations mentioned above. [1]

For the stochastic approach, we utilized the method described by Gibson and Bruck in 2000 [2].

3 Results

Converting the model into a set of ordinary differential equations is one possible mathematical conclusion. The particle numbers of the species in the model are the equation's variables. The differential equation's right-hand side is built as follows: Particle numbers are transformed to concentrations by dividing by compartment capacity and taking into account the unit for substance amounts. The reaction fluxes are calculated using these concentrations. The kinetic functions specified in COPASI produce a value that is either a concentration rate for single compartment reactions or an amount of substance rate for multi-compartment reactions. The value of the kinetic function is calculated by the compartment volume for single compartment reactions; kinetics for multi-compartment reactions are presumed to have already been given in terms of the concentration of the substance per time. We applied different sets of duration and time intervals for our model to understand the kinetics simulation between LacI-protein and TetR-protein.

We plotted the time courses for all variables as seen in Figure 1. Additionally, by suppressing the output for 250 minutes, the phase plane plot in Figure 2 compares the TetR-protein to the LacI-protein.

The model was then tested with different initial conditions. First, we changed the initial concentrations of each protein by amplifying the value of each on their own and every combination out of two. It is noticeable, that these changes only influence the first periods of the simulation. After about 300 minutes, the curves are similar to the standard curves again. Much more interesting results were generated by changing one of the mRNA concentrations. The corresponding protein was expressed differently and the amplitudes of all curves changed. More initial LacI

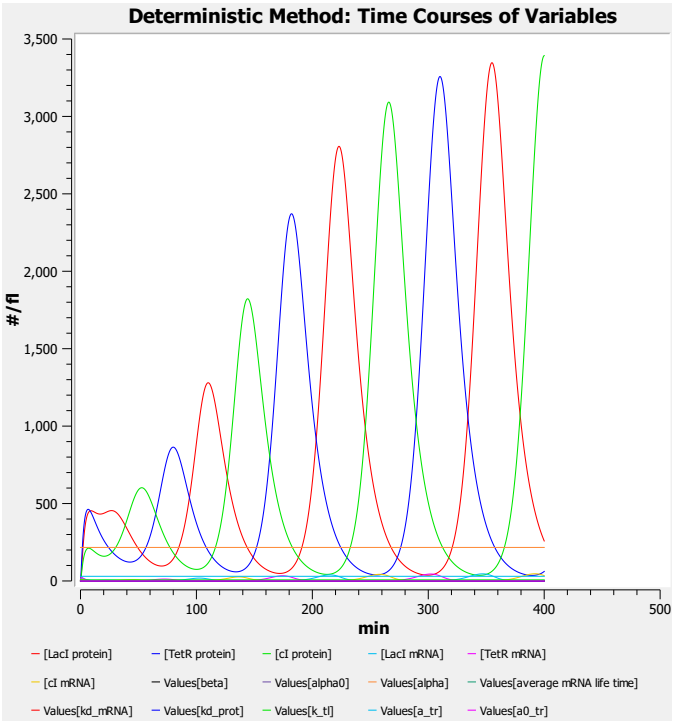

Figure 1: **Time course** This shows the time course for default variables in deterministic model.

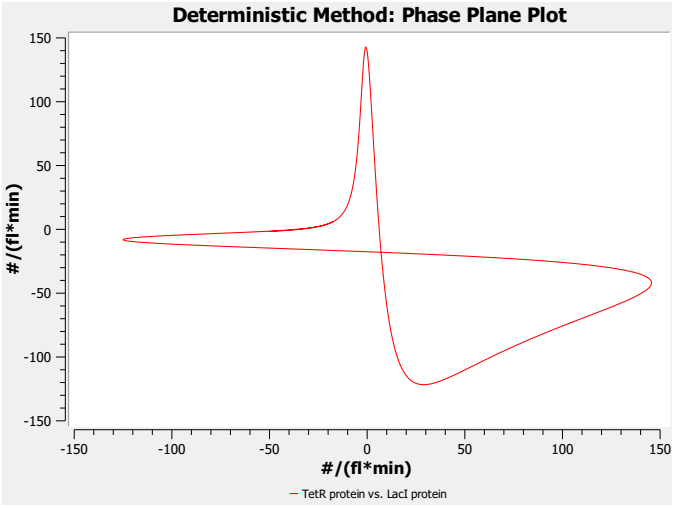

Figure 2: **Phase Plane** This shows the Phase Plane for the deterministic model.

mRNA leads to higher overall amplitudes, while increased cI mRNA leads to lower amplitudes. High values for TetR mRNA resulted in irregular amplitudes.

There occurred other notable changes for variables. For instance, a short half life for proteins drastically increases the frequency of the curve, on the contrary, a long

half life dissolves the periodic nature of the curves. Changes in mRNA half life had similar effects.

These effects could be used to find parameters for a steady state. A strong increase, from the initial two to 1000, in half life of mRNA created a steady state for all species. The same can be said for the protein half life, where an increase from 20 to 10,000 gives similar results. A great increase of the transcription from a fully repressed promotor in transcripts per second and promotor (tps_repr) could lead to a steady state as well. Here the given value of 0.000005 was increased to 1.

Additionally, other kinetic variables were changed to see their influence on the curves. The translation efficiency proportionally altered the frequency of the peaks. Therefore, a low efficiency also leads to an apparent steady state. A high transcription from an active promotor leads to a rising amplitude over the time course simulation.

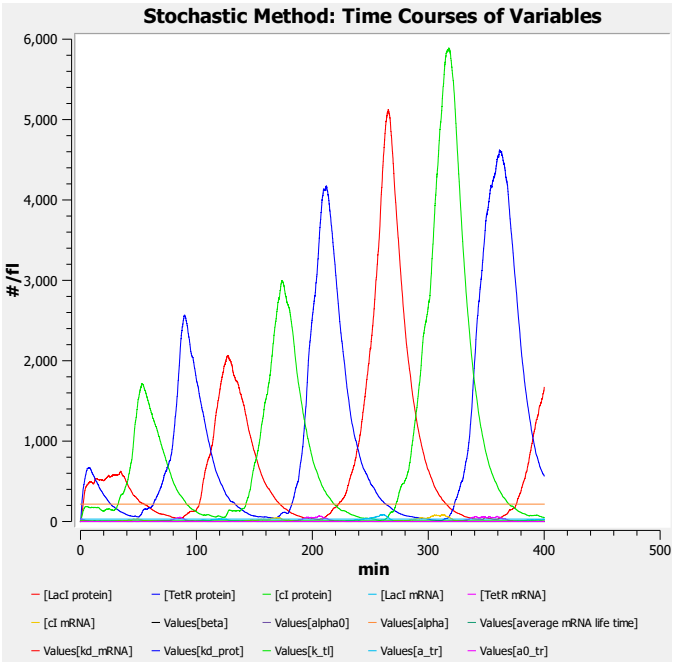

Figure 3: **Time Course** This shows the time courses for all variables in the stochastic model.

Then, the model was simulated with a stochastic approach. To do so, the method described by Gibson and Bruck [2] integrated in Copasi [3] was utilized.

We applied the method to the model for a duration of 400 minutes. The intervals were about 0.0233 minutes long, resulting in 17144 intervals overall. With the standard settings, the time course for each variable visible in Figure 3 was generated. It can be seen, that the protein concentrations rise and fall alternating and periodic, but less regularly than the curves of the deterministic method. The comparison between TetR-protein and the LacI-protein is visible in a phase plane (Figure 4). This curve is less smooth.

When testing with different parameters it was noticeable, that the stochastic model acted very similar to the deterministic one.

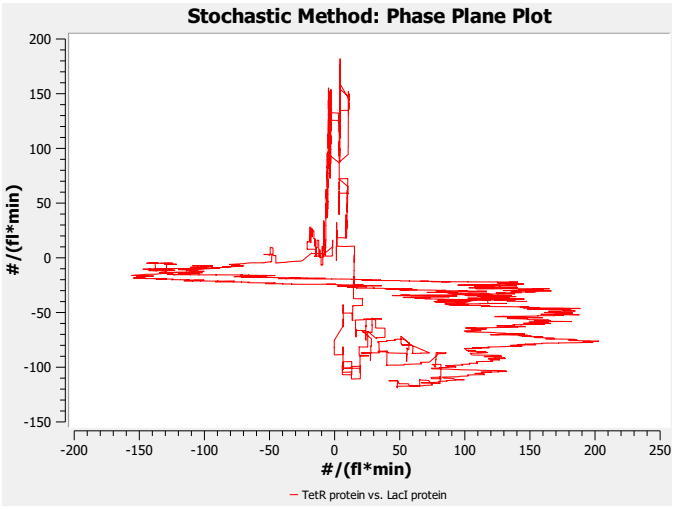

Figure 4: **Phase Plane** This shows the phase plane for the stochastic model.

4 Discussion

4.1 Discussion I

Lac repressor (LacI) is a DNA-binding protein that regulates the expression of genes that code for specific proteins in the lactose metabolism in bacteria. When lactose is not exposed to the cell, these genes are silenced, ensuring that perhaps the bacterial population only allocates energy in the development of mechanisms required for lactose absorption and metabolism when lactose is present whereas the tetracycline repressor (TetR) suppresses gene expression in bacteria by binding regulatory sequences (TetO) found upstream of the tetracycline resistance gene. The system in this work is a negative feedback resulting in oscillations in the time course graph, for the default kinetic parameters. As the duration and time intervals are increased by the order of 10^n like it is shown in the results, the number of oscillations per unit time in concentration, volume, and global quantity values plot increases eventually.

4.2 Discussion II

The fundamental premise of the boolean model is that the regulators or genes work like a switch (On or Off). In last week's project, a node in a model is a basic boolean function that represents the state of a regulator / gene / protein at each level that is either activated or inhibited. In case of a quantitative ODE model, the same node is defined by an Ordinary Differential Equation. The ODE model gives information about the time evolution of concentrations, volume, and global quantities of each molecular species. We can change the kinetic parameters of each molecular species, duration, and intervals of time evolutions until it reaches a steady state.

Competing interests
The authors declare that they have no competing interests.

Author's contributions
Sina Glöckner applied the two methods to the model. Gokul Thothathri wrote the Discussion. Swetha Rose Maliyakal Sebastian applied the deterministic method to the model. Christina Kirschbaum wrote about the Biological Background and the Mathematical model.

References

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