Designed oscillations

Exercise 5, BNF079, 2006

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1 Introduction

Construction of small regulatory networks directly within cells, has been made possible in recent years. These systems are typically built within *E. coli* and a main benefit is that analysis of biological parameters, as well as dynamics, is more feasible.

The goal of this exercise is to build a mathematical model of such a network; a three component repressing network, termed the repressilator. It is constructed with three genes that are asymmetrically repressing each other, where gene x_1 represses gene x_2, x_2 represses x_3, and x_3 represses x_1, forming a three node ring. In the process of building the model for this network, we will also analyze the smaller versions of repressor networks, with an one node auto-repressor (gene x_1 represses itself), and a two node repressor network, where x_1 represses x_2, and x_2 represses x_1.

2 Model

We will use a Hill-type of equation to describe the regulatory repression. The gene regulation also has a "leakage" term, which is modeled as a constant production, and results in that the repressor cannot turn off the gene completely. In addition to this there is a simple degradation term, resulting in a general system of equation(s) for gene(s) x_i

$$\frac{dx_i}{dt} = \frac{V_i K_i^{n_i}}{K_i^{n_i} + x_{i\oplus 1}^{n_i}} + k_i - d_i x_i \tag{1}$$

where the parameter V_i is the transcription rate for unbound DNA, K_i is the Hill constant, related to the repressor concentration leading to half of the maximal production rate. The Hill coefficient, n_i, sets the steepness of the function. Finally k_i is the leakage rate, and d_i is the degradation rate. The $\oplus$ is a normal plus but modulated at the largest i value to be 1 (i.e. $(i, i \oplus 1)$ equals (1,1) for a single gene system, (1,2) and (2,1) for a two-gene system, and (1,2), (2,3), and (3,1) for the three-gene case).

3 Exercises

The exercises will be including some analytical work, simulations, and analysis from plotting. It is assumed that you have some basic knowledge about plotting using *gnuplot*. Otherwise you can find some basic commands in appendix A. For the plotting, you will be given some initial example files, which can be used to create postscript output files. The simulations will be done using the perl simulator created in the previous computer exercise, where initial concentrations and the derivatives subroutine needs to be updated.

3.1 One gene auto-repression

First, we will investigate the single gene auto-repressor ($x_i = x$). For simplicity, we set some of the parameters to some specific values ($d = n = 1$, and $k = 0$), leading to a Michaelis-Menten formalism, similar to what has been discussed in the lectures

$$\frac{dx}{dt} = \frac{VK}{K+x} - x. \tag{2}$$

Exercise 1. Open the file *repressor1.gnplt* where some initial plotting commands are given. Plot dx/dt as a function of x for $K = 1, V = 1$ in gnuplot. Find the fixed points, and determine the stability. (Note that x is a concentration and always non-negative.)

From the plot in Exercise 1 it is possible to approximate the dynamics $x(t)$ from different initial values $x(0)$. Try to do this for yourself, before we head off to actually simulate the dynamics.

Exercise 2. Implement a derivatives function to your simulator for Equation 2. Use the parameter values from Exercise 1, and set the initial x to zero. Run a simulation from $t = 0$ to $t = 100$ and plot the dynamics, $x(t)$. Try also some other initial values. What happens?

Exercise 3. Now, use different values for K (e.g. 0.1,1,10). Plot dx/dt as a function of x (as in Exercise 1) for all values of K in the same plot. Does the general system behavior change? Also run the simulator for the other K values and plot the dynamics from an initial value $x = 0$ (in a single plot).

Exercise 4. As discussed during the lectures, it might be more interesting to compare different values of K, in a setting where the equilibrium concentration is constant. Assuming an equilibrium value of 1, the fixed-point equation leads to the relation $V = (K+1)/K$ for the V and K parameters (do you agree?). Plot the dynamics ($x(t)$) for different values of K, using values of V as just defined. How does the K parameter change the dynamics?

3.2 A two gene repressor network, the bistable switch

Now it is time to add a gene and look at the two-gene repressor network we discussed in the lectures. Again, some of the parameters will be fixed during the simulations ($K = d = 1$ and $k = 0$, for both proteins), resulting in the equations

$$\frac{dx_1}{dt} = \frac{V_1}{1 + x_2^{n_1}} - x_1 \tag{3}$$

$$\frac{dx_2}{dt} = \frac{V_2}{1 + x_1^{n_2}} - x_2 \tag{4}$$

Before doing the following exercises it is advisable to copy *repressor1.gnplt* into *repressor2.gnplt*.

Exercise 5. Create a derivatives function for the model described in Equations 3 and 4. Use $n_1 = n_2 = 1$, and V values close to two (but maybe not exactly equal), and set the initial x_i values to zero. Run a simulation using the simulator program, and plot the dynamics, $x_i(t)$ for both proteins. What happens?

We will now start analyzing the model further.

Exercise 6. Find the null-clines for both proteins ($dx_i/dt = 0$). Write both in the form $x_2 = f(x_1, p)$, where $f(x_1, p)$ is a function of x_1, and parameters. Plot these functions using gnuplot, and try to analyze the behavior in different regions and stability of fixed points. Plot also x_2 as a function of x_1 from the data file generated in Exercise 5 in the same plot. Is the dynamics expected?

Exercise 7. Now, change the initial configurations and run simulations with (x_1, x_2) equal to (0,2),(2,0),(2,2), as well as the (0,0) case from Exercise 5. Plot the output in the x_2 vs. x_1 plot (including the null-clines). What happens?

Finally we will see what happens if we increase the value of the n_i parameter.

Exercise 8. Change the n_i parameters for both proteins to the value three. Redo Exercise 6, i.e. plot the null-clines together with simulations for initial values (x_1, x_2) equal to (0,0),(0,2),(2,0),(2,2) and possibly some additional points, in a x_1x_2-plot. Is the term "bistable switch" a relevant description of the dynamics?

3.3 A three gene repressor network, the repressilator

We are now ready to add another protein, resulting in a three gene repressor network termed the repressilator. The model was presented in a paper by Elowitz et. al., where they studied the system both *in vivo*—by introducing a small genetical network in E.coli—and *in silico*—by performing simulations simular to what you will do in the remaining part of this exercise. The original model included both mRNA and proteins in the dynamics, but here we will simplify matters by only considering mRNA. This results in the following three equations.

$$\frac{dx_1}{dt} = \frac{V_1}{K_1 + x_2^{n_1}} + k_1 - x_1 \tag{5}$$

$$\frac{dx_2}{dt} = \frac{V_2}{K_2 + x_3^{n_2}} + k_2 - x_2 \tag{6}$$

$$\frac{dx_3}{dt} = \frac{V_3}{K_3 + x_1^{n_3}} + k_3 - x_3 \tag{7}$$

Hopefully, you are somewhat acquainted with the software/plotting environment now, and the exercises will be formulated in a more general fashion.

Exercise 9. Make a repressilator model from Equations 5-7, where a Michaelis-Menten formalism is used. Simulate for a number of different initial conditions, and analyze the dynamics by plotting $x_i(t)$ and phase planes (x_i versus x_j).

Exercise 10. Try to find an oscillatory behavior by adjusting the parameters. Plot the result. What is typically needed to get oscillations? Can you tune the period of the oscillations?

Exercise 11. Adjust the leakiness parameter in an oscillating system ($k_i > 0$). What does this parameter do to the dynamics?

4 Additional exercises

4.1 Repressilator for populations of cells

Exercise 12. Create a model with two cells with a repressilator model in each cell (6 molecules). Use parameters from Exercise 10, and somewhat different initial concentrations in the two cells. Explain what happens?

Exercise 13. Add diffusion of one of the proteins (e.g. x_1). What happens? Assuming x_1 and x_4 represents the same protein in the two cells diffusion is defined by the derivative contribution

$$\frac{dx_1}{dt} = -\frac{dx_4}{dt} = D(x_4 - x_1) \tag{8}$$

where D is the diffusion constant.

4.2 Full repressilator model

Exercise 14. Implement the full model given in Elowitz *et.al.*, and investigate the dynamics. What can be the benefit of this model compared to our previous more simplistic version?

A Gnuplot

Typically, it is easier to save all plot commands within a file and then use *gnuplot file* to execute the plotting commands. Here follows some examples of useful plotting commands in gnuplot.

Setting a postscript file as output:

```
set term postscript
set out ‘‘file.ps’’
```

Defining and plotting a function:

```
f(x,a) = x+a
plot f(x,1) title ‘‘a=1’’, f(x,2) title ‘‘a=2’’
```

Plotting columns in a file (columns 2 and 3 in file *file.data*):

```
plot ‘‘file.data’’ using 2:3
```

Setting ranges and labels for the axes:

```
set xrange [0:1]
```

```
set yrange [0:*]}
```

(only lower bound set)

```
set xlabel ‘‘time’’
```