CELL0014 Report Investigation

Exploration of the Genetic Toggle Switch

Report brief:

Demonstrate, discuss and evaluate how different modelling work was utilised to explore and understand the behaviour of the Genetic Toggle Switch.

You should use your own model investigations and results. In your work you should use the model structure and parameter values taken from the later work by Lugange et al.

The suggested word count for the report is 2000-2500 words, however word count penalties will not be applied.

Your report **must** include the following parts:

1. Exploration of Repressor Kinetics used in the Toggle Switch Model

i) investigation of expressed protein level under control of repressor, and the meaning of the model parameters

2. Analysis of the Toggle Switch system

i) demonstration of bistable behaviour

ii) exploration of the relationship between co-operativity of repression and system bistability

iii) Phase space analysis of the system (using simplified reduced model)

3. Investigation of the experimental Toggle Switch system implemented by Lugange et al.

i) simulation of the experimental Toggle Switch using parameters as determined by Lugange et al.

ii) investigation of this system and its behaviour when simulated stochastically and comparison to the deterministic results.

You should read into the related literature of this work and its context, and a strong report write up is will critically evaluate the results and expected to demonstrate a sound understanding of the work completed. For example you would be expected to:

- Demonstrate a good understanding of the models used, e.g. how they are constructed; what the terms relate to; how parameter values have been assigned; relevant assumptions and weaknesses.
- Discuss, explain and evaluate the results in relation to the knowledge of systems modelling principles as covered in the module, e.g. explaining why a reduced model is needed and how the reduction is possible, discussing the different results obtained by deterministic and stochastic methods or how modelling analysis aids experimental lab work.
- Provide insights into the results (e.g. their uses / implications / strengths / weaknesses, or links between your results and other reported work) in the context of the experimental work on the Toggle Switch and Synthetic Biology in general.

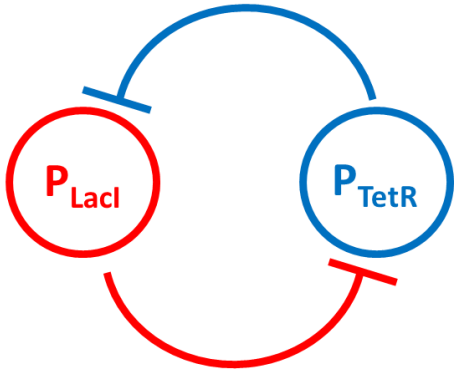
The Genetic Toggle Switch

In this practical we will look at gene regulatory networks in which gene expression is under the control of a repressor (e.g protein P_R) with:

$$\text{rate transcription} \;=\; k_{m_0} + k_m \times \frac{K^n}{P_R^n + K^n}$$

In particular we will be examining the Toggle Switch as described by Gardner et al. This is a synthetic biological gene regulatory circuit that was engineered and demonstrated operating within an E-Coli bacteria.

The experimental work involved inserting an engineered plasmid with genes encoding the transcription factor proteins TetR and LacI, with regulation engineered such that TetR inhibited production of LacI and vice versa:



It was demonstrated that this system could exhibit bistability, so that the system was able to exist in two different stable steady states, with either TetR levels high (and LacI levels low) or LacI levels high (and TetR levels low).

The aim of this investigation is to explore the modelling work associated with the experiment and system.

The following guidance should enable you to work through the required investigation tasks.

Note: In the following models time units are in minutes; concentration units are in copies per cell.

1. Exploration of Repressor Kinetics used in the Toggle Switch Model

MODEL DETAILS

Processes:

Expression of protein P under control of repressor P_R
$\frac{\mathrm{dM}}{\mathrm{d}t}$ production $= \quad k_{m_0} + k_m \times \frac{K^n}{P_R^n + K^n}$
$\frac{\mathrm{dM}}{\mathrm{d}t}$ loss $= \ k_{dm} \times M$
$\frac{\mathrm{dP}}{\mathrm{d}t}$ production $= \quad k_p \times M$
$\frac{\mathrm{dP}}{\mathrm{d}t}$ loss $= \ k_{dp} \times P$

Model equations:

$$\frac{\mathrm{dM}}{\mathrm{d}t} = \frac{\mathrm{dM}}{\mathrm{d}t}\ \text{production} - \frac{\mathrm{dM}}{\mathrm{d}t}\ \text{loss}$$

$$\frac{\mathrm{dP}}{\mathrm{d}t} = \frac{\mathrm{dP}}{\mathrm{d}t}\ \text{production} - \frac{\mathrm{dP}}{\mathrm{d}t}\ \text{loss}$$

Suggested default parameter values:

These are illustrative values for realistic model of expression

k_{m0}	0.01
k_m	5
K	500
n	2
k_{dm}	0.1386
k_p	1.2
k_{dp}	0.0165

Suggested steps:

Run a simulation of the system showing how expressed mRNA and protein levels evolve (starting from initial zero mRNA/protein) to a steady state when:

i) Repressor protein Pr is absent (i.e. Pr = 0)
ii) Repressor protein Pr is at high levels (e.g. Pr = 3000)

TEMPLATE: task_1i_template.ipynb

FIGURE(S): Protein Expression in absence / presence of repressor

Make a plot that summarises the relationship between expressed steady state protein level (y-axis), vs level of repressor protein (x-axis).

TEMPLATE:

```
Pr_vals = np.arange(0,5000,10)

P_ss_vals_n2 = []

for Pr in Pr_vals:

    km0 =
    km =
    K =
    n =
    kdm =
    kp =
    kdp =
    params = [km0, km, K, n, kdm, kp, kdp]

    M0 = 0
    P0 = 0
    # Pr = 0
    s0 = [ 0, 0, Pr ]

    t_start = 0
    t_end = 1000
    t_obs = np.arange(t_start, t_end+0.1, 1)

    s_obs = odeint(sdot, s0, t_obs, args=(params,))

    M_obs = s_obs[:,0]
    P_obs = s_obs[:,1]

    P_ss_vals_n2.append(P_obs[-1])

fig = plt.figure()
ax = fig.add_subplot()
ax.plot(Pr_vals, P_ss_vals_n2)
```

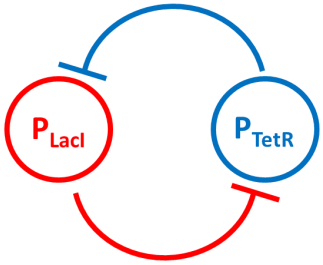
Show how the n-value effects the steady state value reached by adding overlays for different values of n. You can do this by copying the code template section but updating the n value and storing the results into **`P_ss_vals_n1`** and **`P_ss_vals_n4`**.

You can then plot **`P_ss_vals_n1`**, **`P_ss_vals_n2`** and **`P_ss_vals_n4`** against **`Pr_vals`** to make an overlay plot showing the results for n=1, 2 and 4.

FIGURE: Protein steady state levels under different repressor levels and co-operativity

2. Analysis of the Toggle Switch system

MODEL DETAILS (for 2i)



System Variables:

M_L number of LacI RNA molecules

P_L number of LacI protein molecules

M_T number of TetR mRNA copies

P_T number of TetR protein copies

Processes:

Expression of LacI protein (P_L)	**Expression of TetR protein (P_T)**
$\frac{\mathrm{d}M_L}{\mathrm{d}t}$ production $= k_{m_{0L}} + k_{m_L} \times \frac{K_T^{n_T}}{P_T^{n_T} + K_T^{n_T}}$	$\frac{\mathrm{d}M_T}{\mathrm{d}t}$ production $= k_{m_{0T}} + k_{m_T} \times \frac{K_L^{n_L}}{P_L^{n_L} + K_L^{n_L}}$
$\frac{\mathrm{d}M_L}{\mathrm{d}t}$ loss $= k_{dm_L} \times M_L$	$\frac{\mathrm{d}M_T}{\mathrm{d}t}$ loss $= k_{dm_T} \times M_T$
$\frac{\mathrm{d}P_L}{\mathrm{d}t}$ production $= k_{p_L} \times M_L$	$\frac{\mathrm{d}P_T}{\mathrm{d}t}$ production $= k_{p_T} \times M_T$
$\frac{\mathrm{d}P_L}{\mathrm{d}t}$ loss $= k_{dp_L} \times P_L$	$\frac{\mathrm{d}P_T}{\mathrm{d}t}$ loss $= k_{dp_T} \times P_T$

ODE Rate equations:

$$\frac{\mathrm{d}M_L}{\mathrm{d}t} = \frac{\mathrm{d}M_L}{\mathrm{d}t}\text{ production} - \frac{\mathrm{d}M_L}{\mathrm{d}t}\text{ loss}$$

$$\frac{\mathrm{d}P_L}{\mathrm{d}t} = \frac{\mathrm{d}P_L}{\mathrm{d}t}\text{ production} - \frac{\mathrm{d}P_L}{\mathrm{d}t}\text{ loss}$$

$$\frac{\mathrm{d}M_T}{\mathrm{d}t} = \frac{\mathrm{d}M_T}{\mathrm{d}t}\text{ production} - \frac{\mathrm{d}M_T}{\mathrm{d}t}\text{ loss}$$

$$\frac{\mathrm{d}P_T}{\mathrm{d}t} = \frac{\mathrm{d}P_T}{\mathrm{d}t}\text{ production} - \frac{\mathrm{d}P_T}{\mathrm{d}t}\text{ loss}$$

Suggested default parameter values:

These are illustrative values for realistic model of Toggle Switch in case that both repressors have same characteristics in terms of rate constants / repression strength.

$k_{m_{0L}}$	0.01
k_{m_L}	5
K_T	500
n_T	2
k_{dm_L}	0.1386
k_{P_L}	1.2
k_{dP_L}	0.0165

$k_{m_{0T}}$	0.01
k_{m_T}	5
K_L	500
n_L	2
k_{dm_T}	0.1386
k_{P_T}	1.2
k_{dP_T}	0.0165

Suggested steps (2i)

Code an ODE simulation of the system. Demonstrate the system is bistable by showing it can end in two different steady states (one with TetR high and LaCI low, and one with TetR low and LacI high).

e.g. run with initial conditions:

i) MT = 36, PT = 2600, ML = 0, PL = 0

(starting with TetR expression close to its unrepressed level and no initial expression of LacI)

ii) MT = 0, PT = 0, ML = 36, PL = 2600

(starting with LacI expression close to its unrepressed level and no initial expression of TetR)

TEMPLATE: task_2i_template.ipynb

FIGURE(S): Bistability of the Toggle Switch System

Suggested steps (2ii)

Repeat the simulation but setting the n=1. Hence demonstrate in this case the system is not bistable.

FIGURE(S): Behaviour of Toggle Switch System when n=1

Make a bifurcation plot that explores the minimum value of n required for the model to exhibit bistability. To do this run simulations over a range of n values (e.g. set up **`n_vals`** ranging from 1 to 4 in steps of 0.1).

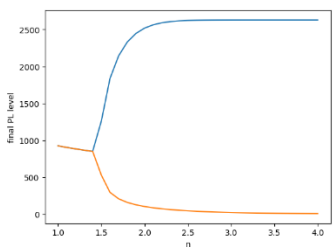
For each n value:

- Update the system parameters i.e. set n_L and n_T to n
- run two simulation starting in i) TetR high ii) LacI high conditions (as above).
  Note run the simulation for 10,000 minutes to try to ensure the system will have moved to its steady state by the simulation end.
- record the final LacI level from the two simulations (e.g. store into **`PL_final_1`** and **`PL_final_2`**).

Make a plot overlaying **`PL_final_1`** and **`PL_final_2`** against **`n_vals`**, to visualise the conditions under which the system is bistable.

FIGURE: Behaviour of Toggle Switch System at different n values

Hint. This should look similar to the plot below



MODEL DETAILS (for 2iii)

To simplify the model we make an assumption that mRNA levels stay close to the levels under which they would be at steady state (such that we assume the condition under dM/dt = 0 in the full model holds). In this case the model equation reduces to the following:

System Variables:

P_L number of LacI protein molecules

P_T number of TetR protein copies

Processes:

Expression of LacI protein (P_L)	**Expression of TetR protein (P_T)**
$\frac{dP_L}{dt}$ production $= v_{p_{0L}} + v_{p_L} \times \frac{K_T^{n_T}}{P_T^{n_T} + K_T^{n_T}}$	$\frac{dP_T}{dt}$ production $= v_{p_{0T}} + v_{p_T} \times \frac{K_L^{n_L}}{P_L^{n_L} + K_L^{n_L}}$
$\frac{dP_L}{dt}$ loss $= v_{dp_L} \times P_L$	$\frac{dP_T}{dt}$ loss $= v_{dp_T} \times P_T$

ODE Rate equations:

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

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

Parameter values (chosen to match full model):

$v_{p_{0L}} = 0.0866$	$v_{p_L} = 43.3$	$v_{p_{0T}} = 0.0866$	$v_{p_T} = 43.3$

$v_{dp_L} = 0.0165$	$v_{dp_T} = 0.0165$

$K_L = 500$	$K_T = 500$
$n_L = 2$	$n_T = 2$

Suggested steps (2iii)

Code the reduced ODE model of the Toggle Switch and run simulations with initial conditions:

i) PT = 2600, PL = 1000 ii) PT = 1000, PL = 2600

iii) PT = 200, PL = 500 iv) PT = 500, PL = 200

Plot the four trajectories on a phase plot of PL (x-axis) and PT (y-axis) showing the direction field

TEMPLATE: task_2iii_template.ipynb

To understand the behaviour of the system it is useful to add on the nullcines. The first step is to analyse the model equations for $\frac{dP_L}{dt}$. Through this you should be able to write and rearrange the nullcline condition into:

P_L = (some expression involving $v_{p_{0L}}$ v_{p_L} v_{dp_L} P_T n_T K_T)

This relationship defines the P_L nullcline. In this case the above expression defines a curved line on the phase plot, and allows us to calculate the P_L value for a given P_T value.

Therefore to draw the nullcline we can generate a range of P_T values, and calculate the corresponding nullcline P_L values then plot these onto the phase plot i.e.

```
# draw PL nullcline
PL_nullcline_PT_vals = np.arange(0,3000,1)
PL_nullcline_PL_vals = []

for PT in PL_nullcline_PT_vals:
    PL = ...
    PL_nullcline_PL_vals.append(PL)

ax.plot(PL_nullcline_PL_vals, PL_nullcline_PT_vals, 'r:')
```

Use this method to add both the PL and PT nullclines to the phase plot.

(Hint for the PT nullcline you will find an expression starting PT = , then repeat the code to take a set of values in PT_nullcline_PL_vals and use the expression to calculate PT_nullcline_PT_vals).

FIGURE: Phase Plot showing Toggle Switch system behaviour with n=2

When you have completed this plot, copy your code and rerun it with no co-operativity i.e. with $n_L = n_T = 1$. This should help illustrate why the n values used help determine whether the system is bistable or not.

FIGURE: Phase Plot showing Toggle Switch system behaviour with n=1

3. Investigation of the experimental Toggle Switch system implemented by Lugange et al.

MODEL DETAILS

Lugange et al used the Toggle Switch model and fitted the following parameter values to their experimental results.

$k_{m_{0L}}$	0.032
k_{m_L}	8.30
K_T	30
n_T	2
k_{dm_L}	0.1386
k_{P_L}	0.9726

k_{dP_L}	0.0165
$k_{m_{0T}}$	0.119
k_{m_T}	2.06
K_L	31.94
n_L	2
k_{dm_T}	0.1386
k_{P_T}	1.170
k_{dP_T}	0.0165

Suggested steps (3i)

Code and run the Toggle Switch model using the Lugange et al. parameter values. Make plots to illustrate the bistable behaviour, this might include plots of expression vs time, and/or a phase plot of the system.

FIGURE(S): Behaviour of the Toggle Switch system using the parameter values of Lugange et al.

Suggested steps (3ii)

TEMPLATE: task_3ii_template.ipynb

Code and run a stochastic version of the Toggle Switch system. Create plots (behaviour vs time) that overlay (a few) runs in i) the LacI high state ii) the TetR high state.

FIGURE(S): Behaviour of the Toggle Switch when simulated using stochastic methods.

Run two sets of stochastic simulations (e.g. >100 runs) starting in in i) the LacI high state ii) the TetR high state. Make histograms (and note the summary statistics such as mean and standard deviation) of the mRNA and protein levels at the end of the simulation.

FIGURE(S): Distributions of mRNA and protein levels when simulated using stochastic methods.