SUGGESTIONS FOR EXTENSION WORK

Extensions marked with a * should be completed by students aiming for a 1st class grade.

Extensions marked with a ** should be completed by students aiming for a strong 1st class grade.

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

SUGGESTED EXTENSIONS

- (Maths derivation) Work through the nullcline analysis that allows calculation of steady state mRNA and protein levels from the model parameter values (i.e. without simulation).

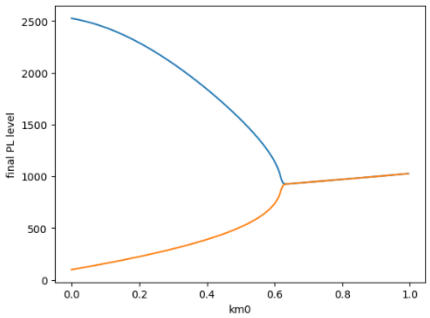
- *(Code extension) You have been asked to make a plot showing how the repression characteristics depend on parameter n. You might repeat this to examine how they depend on k_{m_0}.

2. Analysis of the Toggle Switch system

SUGGESTED EXTENSIONS

- *(Code extension) The Toggle Switch system actually has three possible steady states. For the model parameters suggested the third steady state can be found by starting the system in a balanced state (so initial levels of mRNA for TetR and LacI are equal, and initial protein levels for TetR and LacI are equal). Run a simulation to identify this steady state, and demonstrate that this steady state is unstable) e.g. by starting a simulation close to the steady state and checking it moves away.

- *(Code extension) Repeat the bifurcation analysis but with relation to the parameters $k_{m_{0L}}$ and $k_{m_{0T}}$. You should be able to reproduce something similar to the plot below:



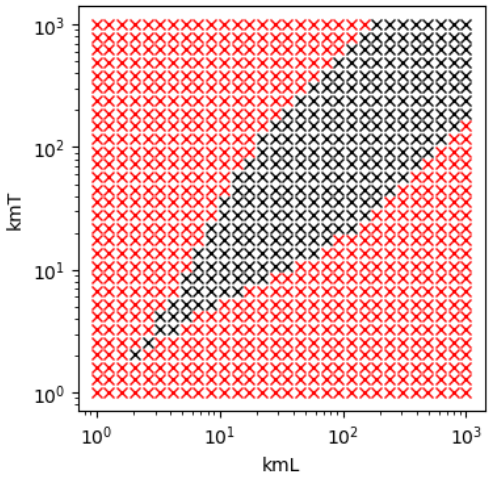
- (Maths derivation) Work through the steps involved in the model reduction, and hence relate the parameters used in the full and reduced models.

- (Code extension) The separatrix is defined as the boundary on the phase plot that separating the regions that end in each steady state (e.g. see the marked separatrix on fig 2a of the original Toggle Switch paper). Through consideration of the model, determine and mark on the separatrix boundary onto your phase plot.

- *(Code extension). In the task you are asked to produce a phase plot that illustrates the loss of bistability when n=1. Repeat this to illustrate the loss of bistability associated with a change to the $k_{m_{0L}}$ and $k_{m_{0T}}$ values.

- **(Code extension). Figure 2b and 2c in the original Toggle Switch paper look at how different promoter strengths (regulating transcription rate) affect bistable behaviour. Perform a similar analysis using our model. You should be able to produce a plot similar to below. Here each point examines the result of a simulation with particular kmL and kmT values, and a black marker indicates bistability, vs a red marker if no bistability was found. You may wish to illustrate using a phsae plot, which changing transcription rate affects bistability of the system.

TEMPLATE: extension_2D_bifurcation_analysis_template.ipynb



(This analysis can be repeated with different n values to reproduce analysis similar to Fig 2d in the original Toggle Switch paper).

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

SUGGESTED EXTENSIONS

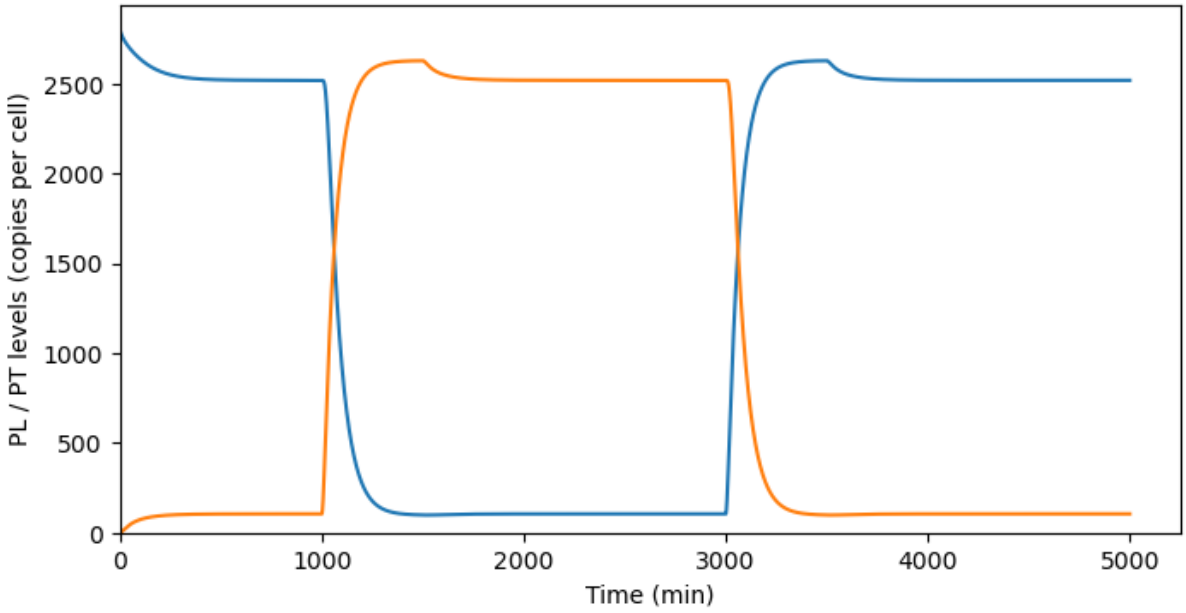
- (Maths/code analysis). Identify the position of the unstable steady state for the system when the parameters are set to those fitted by Lugange et al. One easy-ish way to do this is to plot and locate the position of the nullclines intersection.

- **(Code extension). Marking the Separatrix. For the parameters fitted by Lugange et al it is more challenging to determine the separatrix. However by performing a grid search over initial conditions (using a similar approach to the 2D bifurcation plot code example) and marking on initial PL and PT coordinates that lead to the different steady states in different colours we can indicate the regions on the phase plot that lead to each steady state.

- ***(code extension). Due to the random nature of stochstic simulation, the same initial considtions can lead to different steady states. However through repeated simulation from a grid of starting points it is possible to generate a plot indicating the likelihood of different positions ending in each of the steady states.

ADDITIONAL EXTENSION WORK Control of the Toggle Switch system

One interesting extension is to demonstrate how the Toggle Switch system can be switched between states e.g. to produce a plot like



SIMPLE MODEL

To control the Toggle Switch IPTG and aTC can be used to inhibit the repression by LacI and TetR. We can model this by considering that the P_L and P_T present in the system, may either be free, or bound to IPTG / aTC.

$$P_{L,free} + IPTG \rightleftharpoons P_{L,bound} - IPTG$$

$$P_{T,free} + aTC \rightleftharpoons P_{T,bound} - aTC$$

When bound to IPTG or aTC, the repressors cannot bind to the gene promoter and hence take part in repression.

Experimentalists can change the contentrations of IPTG and aTC in the system to change the amount of P_L and P_L repressor proteins that are free and hence active. This can then be used to shift the Toggle Switch between states.

We can make a simple simulation in which we assume that i) the IPTG and aTC can be added/removed from the system instantaneously, and ii) when added they are at high concentrations are such that the levels of $P_{L,free}$ and $P_{T,free}$ are effectively zero.

In this case we can add code to our model function to simulate periods in which IPGT and aTC are applied e.g.

```
ML, PL, MT, PT= s
km0L, kmL, KL, nL, kdmL, kpL, kdpL, km0T, kmT, KT, nT, kdmT, kpT, kdpT  = params

# simulates saturation by IPGT between 300 and 800
if 1000 < t < 1500:
    # at high IPGT levels PL_free ~ 0
    PL_free = 0
else:
    # assume no IGPT so all PL unbound
    PL_free = PL

# simulates saturation by aTC between 1300 and 1800
if 3000 < t < 3500:
    # at high aTC levels PT_free ~ 0
    PT_free = 0
else:
    # assume no aTC so all PT unbound
    PT_free = PT
```

where in our rate expressions transcription rate now is controlled by the level of unbound repressor $P_{L,free}$ and $P_{T,free}$

Expression of LacI protein (P_L)	**Expression of TetR protein (P_T)**
$\frac{\mathrm{d}M_L}{\mathrm{d}t}$ prod.$= \quad k_{m_{0L}} + k_{m_L} \times \frac{K_T^{n_T}}{P_{T,free}^{n_T} + K_T^{n_T}}$	$\frac{\mathrm{d}M_T}{\mathrm{d}t}$ prod.$= \; k_{m_{0T}} + \; k_{m_T} \times \frac{K_L^{n_L}}{P_{L,free}^{n_L} + K_L^{n_L}}$

FULL MODEL

The simple model above allows us to demonstrate switching but for a more realistic model of the experimental system we need to take into account the fact that the repressors exist in a mixture of free and bound states, and that LacI and aTC take time to diffuse from the external environment into the cell.

The paper by Lugange et at. includes a model that includes these details. In this case the level of $P_{L,free}$ and $P_{T,free}$ are calculated by expressions:

$$P_{L,free} = P_L \times \frac{K_{IPTG}^{n_{IPTG}}}{[IPTG] + K_{IPTG}^{n_{IPTG}}}$$

$$P_{T,free} = P_T \times \frac{K_{aTC}^{n_{aTC}}}{[aTC] + K_{aTC}^{n_{aTC}}}$$

The cellular concentrations [IPTG] and [aTC] are added as system variables, with rate equations:

$$\frac{\mathrm{d}[IPTG]}{\mathrm{d}t} = k_{d1}([IPTG]_{ext} - [\mathrm{IPTG}]) \quad \text{if} \quad [IPTG]_{ext} > [\mathrm{IPTG}]$$

$$\frac{\mathrm{d}[IPTG]}{\mathrm{d}t} = k_{d2}([IPTG]_{ext} - [\mathrm{IPTG}]) \quad \text{if} \quad [IPTG]_{ext} \leq [\mathrm{IPTG}]$$

$$\frac{\mathrm{d}[aTC]}{\mathrm{d}t} = k_{d3}([aTC]_{ext} - [aTC]) \quad \text{if} \quad [aTC]_{ext} > [aTC]$$

$$\frac{\mathrm{d}[aTC]}{\mathrm{d}t} = k_{d4}([aTC]_{ext} - [aTC]) \quad \text{if} \quad [aTC]_{ext} \leq [aTC]$$

Where the values fitted by Lugange et al for binding characteristics for IPTG and aTC are:

$K_{IPTG} = 0.0906$ $n_{IPTG} = 2$ $K_{aTC} = 11.6531$ $n_{aTC} = 2$

With diffusion constants: $k_{d1} = 2183.2$ $k_{d2} = 540$

$k_{d3} = 369.6$ $k_{d4} = 3000$

Note there are four values because diffusion rate was found to depend on i) molecule type and ii) if net flow is inward or outwards

Suggested code structure for the model function incorporating these modifications, and with a section that varies the external IPTG and aTC levels over time is provided below:

```
ML, PL, MT, PT, IPTG, aTC = s
km0L, kmL, KL, nL, kdmL, kpL, kdpL, km0T, kmT, KT, nT, kdmT, kpT, kdpT, K_IPTG, n_IPTG,  K_aTC, n_aTC, kd1, kd2, kd3, kd4 = params

IPTG_ext = 0
aTC_ext = 0

if 1000 < t < 1500:
     IPTG_ext = 5

if 3000 < t < 3500:
    aTC_ext = 200

PL_free =
PT_free =

if IPTG_ext > IPTG:
    d_IPTG = (IPTG_ext - IPTG)/kd1
else:
    d_IPTG = (IPTG_ext - IPTG)/kd2

if aTC_ext > aTC:
    d_aTC = (aTC_ext - aTC)/kd3
else:
    d_aTC = (aTC_ext - aTC)/kd4
```

When you have this code working you can potentially investigate reproducing some of the additional results reported in the paper by Lugange et al. e.g. driving the applied external IPTG and aTC levels in such a way to maintain the system close to its unstable steady state.

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