**MBL Gene Regulatory Networks class**

**Exploratory exercises**

In the following instruction notes, text in the Calibri font indicates the name of a webpage or an item on a webpage, or a term being used in a particular manner in the context of these exercises.

These exercises will continue to be available at [http://CRdata.org](http://CRdata.org) after the course so you can continue to explore them at leisure. Use the course time to explore those issues that you feel you have not fully understood and would like to discuss with me.

To start:

- Go to CRdata.org and Register as a user.
- After registration, log in to CRdata then go to My Account (button at the top right of the Home page) and set your Default Queue to 'Public' (i.e. the free service provided by CRdata for small jobs).
- Explore the User Guide, or watch some of the videos on the “Take a Tour” page to get familiar with CRdata.org.

In CRdata’s R Scripts tab, directory Public (under the column heading: Script type) contains a sets of laboratory tagged with the label: MBL. Search for the tag or simply click “view all records” and search visually.

Feel free to view the contents of each file by clicking “view” in the R Scripts tab.

To run a script, go to the Run analyses tab, then click on Add New Job – Wizard. You will be presented with a directory listing from which you can choose the appropriate script. Next, you will be presented with a number of dialogue boxes and choices.

Give a unique name to the run (so that you can find it in the Run Analyses directory later). Click the link “Help with chosen script...” to read a brief description of what the script does. For the inputs to the script, CRdata provides you with brief descriptions and recommended values (which you can change).

**Two useful short cuts:**

1. To run the same script multiple times with different inputs/parameters, you can replicate an earlier run by clicking “Clone” and then change the run settings as desired.

2. Instead of clicking Add new Job – Wizard, try Add New Job – Manual. As long as you remember at least part of the name of the script you want to run, CRdata will fill out the rest, making this a quicker way to launch new jobs.
**Kinetic models of GRNs,** you will find three sets of simulation scripts in the Public directory.

1. The script Laslo et al Mac vs Neutrophil cell fate switch performs simple a time course simulation of the model described in the Supplementary Materials of Laslo et al, Cell 2006, 126:755–766 (see equations S2a, S2b, page 17). The equations are:

\[
\frac{d(PU.1)}{dt} = \frac{e_p}{1 + Gfi^{nr}} - PU.1
\]

\[
\frac{d(Egr)}{dt} = \left( \frac{\alpha.PU.1}{1 + PU.1} \right) \left( \frac{1}{1 + Gfi^{nr}} \right) - Egr
\]

\[
\frac{d(Gfi)}{dt} = \left( \frac{\alpha.CEBP}{1 + CEBP} \right) \left( \frac{1}{1 + Egr^{nr}} \right) - Gfi
\]

Here C/EBP\(\alpha\) is held constant (at a user specified value, \(e_c\)) throughout the simulation. PU.1 starts low and is then switched to a high value. You can choose the values and timing of PU.1 activity when you launch a new simulation run.

At the default parameter settings, the early period of the simulation (during which both C/EBP\(\alpha\) and PU.1 are expressed at low levels) corresponds to the ‘primed’ state discussed in Laslo et al.

Change the level of C/EBP\(\alpha\) from 0.1 to 0.5. You will note that the system no longer switches to (Egr > Gfi) when PU.1 is increased to 2. Under these conditions, what do you need to increase PU.1 to in order to switch the state of system from (Gfi > Egr) to (Gfi < Egr)?

Explore how combinations of C/EBP\(\alpha\) and PU.1 expression levels and kinetic parameter values affect the behavior of the model.

2. To better understand the model of Laslo et al, I recommend you explore the much simpler mutual repression model of Cherry and Adler (J. theor. Biol. (2000) 203, 117 133). There are 4 scripts.

- Cherry & Adler mutual repression GRN time-course simulation
- Cherry & Adler - parameter requirements for bistability
- Cherry & Adler steady states trajectories
- Mutual Repression On-Off Thresholds (Cherry&Adler)
Start with the Cherry & Adler mutual repression GRN time-course simulation script. You can choose the initial conditions and parameter values and see how they affect the kinetic and steady state behavior of the system. The equations are:

\[
\frac{dx}{dt} = \frac{k_1}{1 + y^n} - \mu_1 x
\]

\[
\frac{dy}{dt} = \frac{k_2}{1 + x^n} - \mu_2 y
\]

You will note that for some parameter combinations, the above system is NOT bistable (it has only one stable steady state). The script Cherry & Adler - parameter requirements for bistability visualizes the parameter ranges which result in bistability (as described in the paper and my lecture). Using the output from this script, choose parameter combinations that you would expect to produce bistable and monostable systems, then test your hypotheses by running time course simulations with these parameter values.

The script Cherry & Adler steady states trajectories generates a summary plot that shows how the system behaves starting from a wide range of initial conditions. Explore how the system behavior changes with different parameter choices. For example, what happens when you change the cooperativity parameter “n” to 1?

As discussed in the lecture, bistable systems (such as this model) exhibit differing turning-on and turning-off thresholds (hysteresis). The script Mutual Repression On-Off Thresholds (Cherry&Adler) demonstrates this property when run with the default parameter settings. Change k1 and k2 to 10 to see another interesting behavior (refer to my lecture slides for explanation).

3. An intriguing alternative model of how the steady states of mutually repressing genes may be switched is presented in Saka & Smith, BMC Developmental Biology 2007, 7:47. Here the mutually repressing genes are both downstream of a single regulator. Saka & Smith were particularly interested in the case where the shared regulator is a morphogen, but the model is general and the shared input (called M in the model) could just as easily be a cytokine or other signal.

The equations are:
\[
\frac{dA}{dt} = \frac{k_a}{1 + B^\beta} \cdot \frac{M^\mu}{1 + M^\mu} - k_{d_a} \cdot A
\]

\[
\frac{dB}{dt} = \frac{k_b}{1 + A^\alpha} \cdot \frac{M^\mu}{1 + M^\mu} - k_{d_b} \cdot B
\]

Note: For simplicity, Saka & Smith set \(k_{d_a} = k_{d_b} = 1\). The steady state effects of changing \(k_{d_a}\) and \(k_{d_b}\) can be mimicked by changing \(k_a\) and \(k_b\) in the opposite direction.

There are four scripts for you to explore:

- **Saka & Smith bistable morphogen detector model**
  This script performs a time-course simulation

- **Saka & Smith model, plot trajectories & nullclines**
  This script plots the trajectories from a variety of initial states to steady state. It also plots the steady state loci of the two genes so that you can verify the locations of the system steady states (crossing points of the 2 loci).

- **Saka & Smith mutual repression GRN, scan M**
  This script varies the common input M across a user-specified range of values and plots the resulting steady states. With the default parameter settings, you should see the switch flip at about \(M=1.3\).

- **Saka & Smith morphogen-driven bistable array of cells**
  This script is essentially the same as preceding script, except that \(M\) is assumed to be a diffusible morphogen whose concentration is distributed exponentially across an array of cells. The simulation results are presented as both a graph and also a 2D plot mimicking a Fluorescence In-Situ Hybridization experiment.

How is it possible for a single input \((M)\) to result in \((\text{activity of } A) > (\text{activity of } B)\) at one concentration and \((\text{activity of } A) < (\text{activity of } B)\) at another concentration?