Read: Chapter 2.3–2.7: Bistability in the lactose operon on *Escherichia coli*: A comparison of differential equation and Boolean network models. By R. Robeva and N. Yildirim, pages 47–73.

1. Consider a Boolean model of the *lac* operon, based on five variables: mRNA (M), β-galactosidase (B), allolactose (A), intracellular lactose (L), and *lac* permease (P), and the following transition functions:

$$\begin{aligned} f_M &= A \\ f_B &= M \\ f_A &= A \vee (L \wedge B) \\ f_L &= P \vee (L \wedge \overline{B}) \\ f_P &= M \end{aligned}$$

   This model does not have any parameters – it assumes that extracellular lactose is always available and extracellular glucose is always unavailable, and thus it is only able to describe the behavior of the system under the conditions.

   (a) Sketch the wiring diagram for this model.

   (b) Print out the state space for this model using ADAM.

   (c) There are 3 fixed points: $(0,0,0,0,0)$, $(1,1,1,1,1)$, and $(0,0,0,1,0)$. Give a biological interpretation of the first two.

   (d) Explain why the fixed point $(0,0,0,1,0)$ does not make sense biologically.

   (e) Since the dynamics do not accurately reflect the behavior of the biological system it is meant to model, something is wrong. For each function, decide if it accurately reflects the underlying biology and/or the model assumptions.

   (f) Propose a modification of the transition functions aimed at eliminating the biologically infeasible fixed point. Give the rationale for your modification and specify the biological mechanism or model assumptions that justify the change.

   (g) Draw the wiring diagram and print and state space of your modified model. Use the ADAM software.

   (h) Analyze your model. How many fixed points are there? Do they all correspond to biologically realistic situations? Note that there should be no limit cycles of size $k \geq 2$.

2. Recall the 3-variable ODE model of the *lac* operon proposed by Yildirim and Mackey in 2004, where $M(t)$ = mRNA, $B(t)$ = β-galactosidase, and $A(t)$ = allolactose (concentrations), respectively.

$$\begin{aligned} \frac{dM}{dt} &= \alpha_M \frac{1 + K_1(e^{-\mu\tau_M} A_{\tau_M})^n}{K + K_1(e^{-\mu\tau_M} A_{\tau_M})^n} - \widetilde{\gamma_M} M \\ \frac{dB}{dt} &= \alpha_B e^{-\mu\tau_B} M_{\tau_B} - \widetilde{\gamma_B} B \\ \frac{dA}{dt} &= \alpha_A B \frac{L}{K_L + L} - \beta_A B \frac{A}{K_A + A} - \widetilde{\gamma_A} A \end{aligned}$$

Suppose the exponential decay constants are estimated from the literature to be $\widetilde{\gamma_M} = .441$, $\widetilde{\gamma_B} = .031$, and $\widetilde{\gamma_A} = .55$.

(a) Compute the half life for M, B, and A.

(b) Justify the following Boolean model by explaining the logical expression defining each transition function:

$$f_M = A \qquad f_{B_{\text{old}}} = \overline{M} \wedge B$$
$$f_B = M \vee \left(B \wedge \overline{B_{\text{old}}}\right) \qquad f_A = (B \wedge L) \vee L_{\text{high}}$$

What approximate timestep is assumed by this model?

(c) Assuming $(M, B, B_{\text{old}}, A) = (x_1, x_2, x_3, x_4)$, write the polynomial form of the Boolean model above. The easiest way to do this is to go into Sage and type the following commands (hit shift-enter after each one):

```
%default_mode macaulay2
R = ZZ/2[x1,x2,x3,x4] / ideal(x1^2-x1, x2^2-x2, x3^2-x3, x4^2-x4);
RingElement | RingElement :=(x,y)->x+y+x*y;
RingElement & RingElement :=(x,y)->x*y;
```

Now, if you want to store $f = (x_1 \vee x_2) \wedge x_3$, you can enter `f=(x1 | x2) & x3`. Typing `f` will give the polynomial form of f.

(d) Open a new tab of Sage (so you aren't in the Macaulay2 environment), and find the fixed points of the Boolean network by solving $\{f_1 + x_1 = 0, \ldots, f_4 + x_4 = 0\}$.

(e) Does this model exhibit bistability? Justify your answer.

3. So far, we have used designated "old" variables to separate the time scales of dilution and degradation processes from those of synthesis. Alternately, this can be done implicitly through the logical assumptions built into the transition functions. For example, if we choose to work with $\gamma_A = 1.8 \times 10^{-4}$, then the degradation time for A will be slower than both M and B. Instead of introducing an A_{old} variable, we propose the following model, which builds this feature into the function:

$$f_M = A,$$
$$f_B = M,$$
$$f_A = (B \wedge L) \vee L_{\text{high}} \vee (A \wedge \overline{B}).$$

(a) Justify the three equations in this model, and why this captures the delay in the degradation of A. Your answer should be clear and convincing.

(b) Use the ADAM software to sketch the phase space of this model for the three levels of lactose concentration: low, medium and high.

(c) Does this model exhibit bistability? Why or why not?