

Read: Chapter 2: Bistability in the lactose operon of *Escherichia coli*: A comparison of differential equation and Boolean network models, by R. Robeva, and N. Yildirim. pages 57–73.

1. Recall the model of Stigler and Veliz-Cuba for the *lac* operon, where a global system state is a 9-variable Boolean vector $(M, P, B, C, R, A, A_\ell, L, L_\ell) = (x_1, x_2, \dots, x_9) \in \mathbb{F}_2^9$:

$$\begin{aligned} f_M &= \overline{R} \wedge C \\ f_P &= M & f_B &= M \\ f_C &= \overline{G_e} & f_R &= \overline{A} \wedge \overline{A_\ell} \\ f_A &= L \wedge B & f_{A_\ell} &= A \vee L \vee L_\ell \\ f_L &= \overline{G_e} \wedge P \wedge L_e & f_{L_\ell} &= \overline{G_e} \wedge (L \vee L_e) \end{aligned}$$

Modify this model by removing the variables L_ℓ and A_ℓ , which represent low concentrations of intracellular lactose and glucose, respectively. Use Gröbener bases and Sage to compute the fixed points of this model for each of the four possibilities for (L_e, G_e) , external lactose and glucose. Do the fixed points make sense biologically? Since the state space has size $2^7 = 128$, the Analysis of Dynamic Algebraic Models (ADAM) toolbox, at <http://adam.plantsimlab.org/>, should be able to handle it. Are there any periodic cycles that are not fixed points? Do you notice any shortcomings of this new model?

2. Recall our original Boolean network model for the *lac* operon:

$$\begin{aligned} f_M &= \overline{G_e} \wedge (L \vee L_e) \\ f_E &= M \\ f_L &= \overline{G_e} \wedge ((E \wedge L_e) \vee (L \wedge \overline{E})). \end{aligned}$$

Suppose now that L_e instead stands for external concentration of lactose being at least medium. Introduce a new parameter L_{high} to denote high levels of external lactose.

- (a) Modify the transition functions above to get a model that exhibits bistable behavior for medium lactose concentrations.
 - (b) For the three possible concentration levels of lactose; low: $(L_e, L_{\text{high}}) = (0, 0)$, medium: $(L_e, L_{\text{high}}) = (1, 0)$, and high: $(L_e, L_{\text{high}}) = (1, 1)$, sketch or print out the phase space of this model using ADAM. Clearly show how this model exhibits bistability.
3. So far, we have used designated “old” variables to separate the time scales of dilution and degradation processes from those of synthesis. An alternate to introducing new variables this is to properly modify the transition functions.

Consider a model of the *lac* operon using variables M , B , and A , and parameters L and L_{high} . Suppose that the degradation time for A is much larger than that for M and B . In this case, choosing a proper timestep allows us to neglect the degradation times for M

and B . Instead of introducing an A_{old} variable, we propose the following model, which builds this feature into the function:

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

- (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 (or print) 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?
4. Consider a Boolean model of the *lac* operon, based on five variables: mRNA (M), β -galactosidase (B), lac permease (P), intracellular lactose (L), and allolactose (A), 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) Sketch the state space for this model. Feel free to use ADAM, but let $x_1 = M$, $x_2 = B$, $x_3 = A$, $x_4 = L$, and $x_5 = P$.
- (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 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 $k \geq 2$. (Why?)