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

$$(f_m, f_B, f_P, f_A, f_L) = \big(A,\ M,\ M,\ A \vee (L \wedge B),\ P \vee (L \wedge \overline{B})\big)$$

   (a) What other assumptions are made in this model? (E.g., presence or absense of extracellular sugars, time-steps, degredation, etc.)

   (b) As we saw in class, the dynamics do not accurately reflect the behavior of the biological system it is meant to model. Therefore, something is wrong. Four of these functions are reasonable; justify each one in a single well-written sentence.

   (c) Explain why one of these functions does not accurately reflect the underlying biology and/or the model assumptions. Propose a modification, aimed at eliminating the biologically infeasible fixed point, $(0, 0, 0, 1, 0)$. Give a rationale for your modification and specify the biological mechanism or model assumptions that justify the change.

   (d) Use Macaulay2 to convert these Boolean functions into polynomials over $\mathbb{F}_2 = \{0, 1\}$. Then find the fixed points using a Gröbner basis of a particular polynomial ideal.

   (e) Draw the wiring diagram of the old and new Boolean model.

   (f) Use the BoolNet package in RStudio to find the attractors and plot the state space. Include a print-out or screenshot.

   (g) Repeat the previous step, but with Cyclone.

   (h) Justify why the long-term behavior of the system (fixed points) agrees with what we should expect biologically.

2. **Fixed points**. The following is a model of the tryptophanase (*tna*) operon in E. coli:

$$\begin{array}{ll} f_A = M \wedge \overline{\gamma} & f_W = \omega_e \wedge B \\ f_B = M & f_{W_m} = (\omega_{em} \wedge B) \vee \omega_e \vee W \\ f_C = \overline{\gamma} & f_\gamma = \gamma \\ f_M = C \wedge \overline{P} & f_{\omega_e} = \omega_e \\ f_P = \overline{W} \wedge \overline{W}_m & f_{\omega_{em}} = \omega_{em} \end{array}$$

   The parameters are encoded by "frozen" variables: γ is glucose, ω_e is high levels of extracellular tryptophan, and ω_{em} represents (at least) medium levels.

   (a) Use Cyclone to find the attractors. Code is provided on the course website.

   (b) Fix $(\gamma, \omega_e, \omega_{em}) = (0, 0, 1)$ as constants to get a 7-variable model, which assumes no glucose and medium levels of extracellular tryptophan. Use BoolNet to find the attactors under an asynchronous update, and summarize your findings. This can be done with the command

```
> getAttractors(tnaModel,type="asynchronous")
```

   How does this compare to the synchronous state space?

   (c) Explain how/why this model exhibits bistability.

3. **Decay and degredation**. 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:

$$\begin{aligned}
f_M &= A & f_{B^\downarrow} &= \overline{M} \wedge B \\
f_B &= M \vee \left(B \wedge \overline{B}_1^\downarrow\right) & f_A &= (B \wedge L_m) \vee L
\end{aligned}$$

What is a reasonble assumption of the approximate timestep assumed by this model? (There are multiple feasible solutions, but you need to justify your answer.)

(c) Find the fixed points of this model using computational algebra. Use the variable order $(M, B, B_1^\downarrow, A) = (x_1, x_2, x_3, x_4)$, and include your code from Macaulay2.

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

4. **Reduction**. Consider the following Boolean network model of the *lac* operon.

$$\begin{aligned}
f_1 &= x_4 \wedge \overline{x}_5 \wedge \overline{x}_6 & f_8 &= x_9 \vee x_{10} \\
f_2 &= x_1 & f_9 &= x_3 \wedge \overline{G}_e \wedge L_e \\
f_3 &= x_1 & f_{10} &= \overline{G}_e \wedge \big(L_e \vee (x_3 \wedge L_{em})\big) \\
f_4 &= \overline{G}_e & f_{G_e} &= G_e \\
f_5 &= \overline{x}_7 \wedge \overline{x_8} & f_{L_e} &= L_e \\
f_6 &= x_5 \vee (\overline{x}_7 \wedge \overline{x}_8) & f_{L_{em}} &= L_{em} \\
f_7 &= x_2 \wedge x_9
\end{aligned}$$

Here, the variables represent

$$(x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9, x_{10}) = (M, B, P, C, R, R_m, A, A_m, L, L_m).$$

(a) Use Macaulay2 to reduce this Boolean network, as much as possible.

(b) Draw the wiring diagram of the reduced network. Find its fixed point(s) and use these to determine the fixed point(s) of the original network by back-substitution.