1. Analyze the biomolecular feed-forward loop pictured. This motif contains an “OR gate,” which means that Z is transcribed when either the concentration of X is above some threshold $K_{xz}$ or the concentration of Y is above some threshold $K_{yz}$ (or both).

   a. Make a sketch of the system showing the biomolecular components and the control interactions.
   b. Write model equations to represent the amount of proteins X, Y, and Z in the system. Explain any assumptions.
   c. Assuming that the activating signal $S_y$ is always present, show plots of the protein concentrations over time as $S_x$ is turned on and later turned off.
   d. Find an expression for the amount of time between the step change in X (on or off) and the beginning or ending of transcription of Z in response.
   e. What could be the biological function of this motif?

2. Let’s test the accuracy of the logic function assumption.

   a. Create a numerical simulation using Matlab functions or Simulink of the biomolecular feed-forward loop motif (coherent type 1) with AND logic, depicted at the right. Use initial concentrations $Y = Z = 0$ and $X = 1$. Turn $S_x$ on at $t = 0$ and off at $t = 10$, and assume $S_y$ is always present (activation/deactivation of X and Y via the signals $S_x$ and $S_y$ is practically instantaneous). For simplicity, use $\beta_x = \beta_y = 1$, $\alpha_y = \alpha_z = 1$, and $K_{xy} = K_{xz} = K_{yz} = 0.5$.
   b. Plot the concentrations of Y and Z over time. Compare results using the logic function assumption for transcription activation versus a Hill function activity model of order 2, i.e.,

   transcription rate of $Y = \frac{\beta_x(X / K_{xy})^2}{1 + (X / K_{xy})^2}$ and

   transcription rate of $Z = \frac{\beta_x(XY / K_{xz}K_{yz})^2}{1 + (X / K_{xz})^2 + (Y / K_{yz})^2}$.

   c. What is the delay in the response of Z to $S_x$ ($t_{ON}$ and $t_{OFF}$) in each case? Note that the logic function case should match the analytical solution.
   d. Is the logic function assumption valid?
3. [Adapted from Alon 4.4] *The diamond motif.* The four-node diamond pattern occurs when X regulates Y and Z and both Y and Z regulate gene W.

   a. How does the mean number of diamonds scale with network size in random networks?

   b. What are the distinct types of sign combinations of the diamond (where each edge is either activation + or repression -)? How many of these are coherent?

4. [Adapted from Alon 5.4] What are the topological generalizations of the diamond pattern based on duplication of a single node and all of its edges? How are these different from DORs? Interestingly, most of the five-node generalizations of the diamond are network motifs observed in the neuronal network of *C. elegans.*

5. [Alon 6.3] Design a multi-layer perceptron with two input nodes, one output node, and as many intermediate layers as needed, whose output has a region of activation in the shape of a triangle in the middle of the $X_1$-$X_2$ plane.

6. [Optional for additional frequency response and Matlab practice] Work through Bequette’s Module 6. Complete Problem 3, showing how you obtain your results including the required plots to obtain your answers and check your work.