1. (Based on Alon 4.6) **Shaping the pulse.** Consider a situation where X in an I1-FFL begins to be produced at time \( t = 0 \), so that the level of protein X gradually increases. The input signal \( S_x \) and \( S_y \) are present throughout.

a) Download “plot.m” and “prob1.m” from the class website and place them in the same folder. Modify “plot.m” to specify values for \( K_{xz} \), \( K_{xy} \), \( K_{yz} \), and \( \beta \) and run the script. (Note: Script will not run unless 3 values for each parameter have been specified). Study the graphs for different values of parameters.

How does the pulse shape generated by the I1-FFL depend on the thresholds \( K_{xz} \), \( K_{xy} \), and \( K_{yz} \), and on \( \beta \), the production rate of protein X? (i.e. How does increasing or decreasing these parameters change the height or position of the pulse peak, the slope of the rise of the pulse, etc?) Explain qualitatively in a biological context why you would expect changing the parameters to have this effect.

b) Analyze a set of genes \( Z_1, Z_2, \ldots, Z_n \), all regulated by the same X and Y in I1-FFLs. Describe the relationship of the thresholds, \( K_{xz} \), \( K_{xy} \), and \( K_{yz} \), such that the genes are turned ON in the rising phase of the pulse in a certain temporal order (i.e. \( Z_1 \) is turned on, then \( Z_2 \) then \( Z_3 \ldots \)) and turned OFF in the declining phase of the pulse with the same order.

c) What would the relationship be if the turn-OFF order is opposite the turn-ON order?

2. (based on Buchler, et al., 2009, MSB 5:272) **Protein Sequestration and Ultrasensitivity.**

Consider the circuit below. In the circuit, \( A \) is a transcriptional activator that binds to a single DNA site with dissociation constant, \( \kappa \). \( A \) activates \( O \) in a non-cooperative, Michaelis-Menton fashion. \( B \) can bind to \( A \) with a dissociation constant of \( K_D \), rendering \( A \) inactive.

a) We assume that \( A \) and \( B \) bind stoichiometrically, that is, that the formation of the heterodimer is greatly favored over free \( A \). Write down a mathematical relation that reflects this. With this assumption, qualitatively describe what happens in the case when there is less \( A \) than \( B \). What about if there is more \( A \) than \( B \)?
b) Write an expression for the rate of change of \( O \) in terms of \( A, \kappa \), the basal transcription rate (\( \beta_0 \)), the activated transcription rate (\( \beta \)), and the degradation rate (\( \gamma \)). What is the steady-state value of \( O \) (\( O_{ss} \)) in terms of these constants and \( A_{ss} \)?

c) By using mass conservation, we can find an expression for the amount of free \( A \) in terms of \( A_{TOTAL} \), \( B_{TOTAL} \), and \( K_D \). We get that:

\[
A = \frac{1}{2} \left( A_{TOTAL} - B_{TOTAL} - K_D + \sqrt{(A_{TOTAL} - B_{TOTAL} - K_D)^2 + 4A_{TOTAL} \cdot K_D} \right)
\]

Plot the concentration of \( O \) at steady-state as a function of \( A_{TOTAL} \) from 0 nM to 10 nM for the following values of \( B_{TOTAL} \): 0 nM, 500 nM, 5000 nM. Use the following constants:

\[
\begin{align*}
\beta_0 &= 1 \text{ nM} \\
\beta &= 100 \text{ nM} \\
\kappa &= 100 \text{ nM} \\
K_D &= 1 \text{ nM}
\end{align*}
\]

d) Describe qualitatively the effect of increasing \( B \) regarding the sensitivity of the system. Describe a possible role of protein sequestration in a biological circuit.

e) In order to test their mathematical hypothesis, Buchler and colleagues implemented the circuit shown below. RFP was fused to CEBP\( \alpha \), a transcriptional activator which drove the production of YFP. YFP and RFP levels could then be monitored with flow-cytometry. What species do the YFP and RFP levels relate to in the hypothetical system?


Consider a protein \( X \) that undergoes transitions between an active state \( X_p \) and an inactive state \( X \).

\[
X \quad \xrightarrow{k(s)} \quad X_p
\]

\( s \) is an input signal to the system that affects the activation rate constant \( k(s) \), \( X_p \) is the output of the system.
a) Write down an expression for the change in $X_p$ in terms of the activation rate constant $k(s)$, deactivation constant $k$ and inactive protein $X$.

b) Find the steady state of the expression found in a). Express the active protein $X_p$ as a function of the activation rate constant $k(s)$, deactivation constant $k$ and total amount of protein $X_T = X_p + X$.

c) Plot the output as a function of the input $s$ found in b), for different values of $X_T = 0.8, 1, 1.2$ where $k = 2$ and $k(s) = 5s$. Comment on the circuit’s robustness with respect to varying protein concentrations by using part b) to reason about it.

Consider a two component phosphorylation system:

$X \xrightarrow{k(s)} X_p$, *Autophosphorylation*

$X_p + Y \xrightarrow{k_1/k_1'} X + Y_p$, *Phosphotransfer step*

$X + Y_p \xrightarrow{k/k'} XY_p \xrightarrow{k_d} X + Y + P_i$, *Dephosphorylation*

$s$ is an input signal to the system that affects the rate of autophosphorylation, $k_a(s), Y_p$ is the output of the system.

d) *phosphoMainIO.m* plots the output $Y_p$ as a function of the input $s$. Run *phosphoMainIO.m* and comment on the relation between the input $s$ and the output $Y_p$ and compare it to the simple phosphorylation case.