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) 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 $Z_1, Z_2, ..., Z_n$, all regulated by the same X and Y in I1-FFLs. Design thresholds such that the genes are turned ON in the rising phase of the pulse in a certain temporal order and turned OFF in the declining phase of the pulse with the same order.

   c) Design thresholds such that the turn-OFF order is opposite the turn-ON order. Plot the resulting dynamics.

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) Find an expression for $A$, the amount of free protein in terms of $A_{\text{TOTAL}}$, $B_{\text{TOTAL}}$, and $K_D$.

   b) 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, find an expression for $A$ when $A_{\text{TOTAL}} < B_{\text{TOTAL}}$ and when $A_{\text{TOTAL}} > B_{\text{TOTAL}}$.

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

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

e) Fit the graphs to the following Hill Function:

\[
O = O_{basal} + O_{activated} \frac{A^{n_H}_T}{A^{n_H}_T + K_H^{n_H}}
\]

What are the Hill-coefficients \((n_H)\) for each concentration of \( B_{TOTAL} \)? (Hint: The cftool, toolkit in MATLAB may be helpful. The fitting algorithm is highly dependent on the initial value for the terms to be fitted)

f) Describe a possible role of protein sequestration in a biological circuit.

g) 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?

3. Two component systems, (based on Shinar, et al., 2007, doi: 10.1073/pnas.0706792104)

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

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

\( s \) 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 ODE model for the circuit and find an equilibrium expression for the output \( X_p \) as a function of the input \( s \) and total protein \( X_T \).
b) Plot the output as a function of the equilibrium constant of the chemical reaction for different values of total protein $X_T = 0.8, 1, 1.2$ where $k = 2$ and $k(s) = 5s$. Comment on the circuit’s robustness with respect to varying protein concentration $X_T$.

Consider a two component phosphorylation system:

\[
\begin{align*}
X & \xrightarrow{k(s)} X_p, \quad \text{Autophosphorylation} \\
X_p + Y & \xrightleftharpoons{k_1, k'_1} X + Y_p, \quad \text{Phosphotransfer step} \\
X + Y_p & \xrightarrow{k, k'} XY_p \xrightarrow{k_d} X + Y + P_i, \quad \text{Dephosphorylation}
\end{align*}
\]

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

c) Express the input and output fluxes of phosphoryl groups into and out of this system. The input flux, $J_i$, can be defined as the rate of phosphorylation of $X$. The output flux $J_o$ can be defined as the rate of dephosphorylation of $X$.

d) Find an equilibrium expression for the output $Y_p$ as a function of the input $s$ and other rate constants. (Hint: Consider the steady state level of $XY_p$ and the relationship of input and output flux at steady state.)

e) Plot the output $Y_p$ as a function of the input $s$ for $k(s) = 5s$, for different values of total protein. Comment on the circuit’s robustness with respect to varying concentrations of total protein.