1. A bifunctional component provides a robust input-output relationship. (This is a review of lecture on robustness by bi-functional components). The receptor $X$ catalyzes two opposing reactions: phosphorylating $Y$ at one catalytic site on the receptor, and de-phosphorylating $Y$ at a different catalytic site. Thus, the opposing kinase and phosphatase activities are rolled up into the same protein, instead of being separated on two different proteins. One way to solve this example is to write down several mass-action equations for the system, and find their fixed-point. An easier way to obtain the input–output relations presents itself when we view the system as a black box that breaks down ATP and releases phosphoryl groups back to the cytoplasm as inorganic phosphate (Pi). Consider the fluxes of phosphoryl into and out of the system.

   a. Calculate the rate at which phosphoryl, $p$, enters the box, $J_{in}$, and exits $J_{out}$. Explain why $J_{in}$ depends only on $X_0$ and $J_{out}$ on $X_0$ and $Y_p$.
   b. Show that $Y_p = v_k(s)/v_p(s)$ at steady state provided there is enough protein $Y$.
      Explain why this resulting output level $Y_p$ is robust to $X$ and $Y$ protein levels.
   c. What happens if total $Y_T$ protein ($Y_0 + Y_p$) is below $Y_p$ for a given value of $s$?

2. Spontaneous dephosphorylation leads to small loss of robustness. In the EnvZ-OmpR circuit, $Y_p$ can be spontaneously dephosphorylated without the action of $X$. The half-life of $Y_p$ due to this reaction is ~90 minutes, compared to half-life of $Y_p$ of seconds due to the dephosphorylation catalyzed by $X$.

   a. Write an equation for $Y_p$ dynamics assuming the two-component mechanism also has a reaction of spontaneous dephosphorylation at rate $\epsilon$.
   b. Use the black box approach to calculate the steady-state level of $Y_p$.
   c. Explain why robustness is lost only to order $\epsilon$. 
3. Use the black box to analyze a circuit that has an additional phosphor-transfer step (in bold). Here $Z_p$ passes the phosphoryl to $Y_0$

a. What is $Y_p$ at steady state?

b. Is it robust? Assume there are a lot of Z and Y proteins.

\[ Z_p + Y_0 \xrightarrow{v_t(s)} Z_0 + Y_p \]

4. **The chemotaxis protein circuit.** We learned in class how attractant lowers the probability that motors turn CW and generate tumbling. Consider here other input alternatives.

   a. Repellent. Explain how the chemotaxis circuit responds to a step of repellent.
   Repellent binding increases receptor activity.

   b. Reduction in attractant: Suppose attractant is removed in a step like manner. What is the response of the cells?

5. **Sensory adaptation.** Explain how the idea of exact adaptation applies to human senses: vision, hearing and smell. Are there senses which do not show exact adaptation? What might be the reason that some senses do and others do not show exact adaptation?

6. **Robust adaptation and Integral feedback.** The mapping of the Barkai-Leibler model and integral feedback is easiest to see from the equation.

\[ \frac{dm}{dt} = v_B (X_{st}^* - X^*) \]

The model becomes simpler when one uses the receptor binding constant $K$ as a variable instead of $m$.

a. Use the relations

\[ X^* = \frac{x_T}{1+(s/k)^n}, \quad K = K_0 e^{ym} \]

to show that the equation for $K$ is:

\[ \frac{dK}{dt} = c K (a_{st} - a), \quad a = \frac{1}{1+(s/k)^n} \]

where $a = X^* / X_T$

b. Simulate and plot the response when (i) $s = 1$ for a long time and then goes in a step-like way to $s = 2$, (ii) $s = 2$ goes to $s = 4$, and (iii) $s=4$ goes to $s=2$. Choose $n = 6$, $a_{st} = 1/4$, $c = 4$, and $k(0) = 1/3 \approx 0.33$. 

\[ \text{Simulations} \]
c. Do you see adaptation?