CALIFORNIA INSTITUTE OF TECHNOLOGY
Biology and Biological Engineering (BBE)

BE 150

M. Elowitz and R. M. Murray
Winter 2013

Problem Set #2

Issued: 16 Jan 2013
Due: 23 Jan 2013

1. (Shaping pulses; based on Alon 4.6) 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.

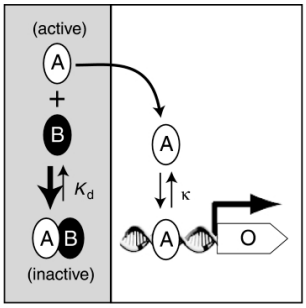
   (a) Download "I1FFL.sbproj" from the class website and use the parameter scanning capability in Simbiology to explore the possible dynamics of this systems.

   How does the pulse shape generated by the I1-FFL depend on the thresholds K_{xz}, K_{xy}, and K_{yz}, and on β, 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 $\mathrm{Z}_1, \mathrm{Z}_2, \ldots, \mathrm{Z}_\mathrm{n}$, 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. (Protein sequestration and ultrasensitivity; based on Buchler *et al.*, MSB 5:272, 2009) Consider the circuit below. In the circuit, A is a transcriptional activator that binds to a single DNA site with dissociation constant κ. 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_{tot}, B_{tot}, 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 $A_{\mathrm{tot}} < B_{\mathrm{tot}}$ and when $A_{\mathrm{tot}} > B_{\mathrm{tot}}$.

(c) Write an expression for the rate of change of O in terms of A, κ, the basal transcription rate (β_0), the activated transcription rate (β), and the degradation rate (γ). 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_{tot} from 0 nM to 10 uM for the following values of B_{tot}: 0 nM, 500 nM, 5000 nM. Use the following constants:

$$\frac{\beta_0}{\gamma} = 1\text{nM} \qquad \frac{\beta}{\gamma} = 100\text{nM} \qquad \kappa = 100\text{nM} \qquad K_d = 1\text{nM}$$

(e) Fit the graphs to the following Hill Function:

$$O = O_{\text{basal}} + O_{\text{activated}} \frac{A_{\text{tot}}^{n_H}}{A_{\text{tot}}^{n_H} + K_H^{n_H}}$$

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

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 $\mathrm{X^P}$ and an inactive state X:

$$\mathrm{X} \underset{k}{\overset{k(s)}{\rightleftharpoons}} X_p$$

The variable s represents an input signal to the system that affects the activation rate constant $k(s)$. $\mathrm{X^P}$ is the output of the system.

(a) Write down an ODE model for the circuit and find an equilibrium expression for the output $\mathrm{X^P}$ as a function of the input s and total protein X_{tot}.

(b) Plot the output as a function of the equilibrium constant of the chemical reaction for different values of total protein $X_{\text{tot}} = 0.8, 1, 1.2$ where $k = 2$ and $k(s) = 5s$. Comment on the circuit's robustness with respect to varying protein concentration X_{tot}.

Next consider a two component phosphorylation system:

$$\begin{aligned}
&\text{Autophosphorylation :} && \mathrm{X} \xrightarrow{k(s)} \mathrm{X^P},\\
&\text{Phosphotransfer step :} && \mathrm{X^P + Y} \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} \mathrm{X + Y^P},\\
&\text{Dephosphorylation :} && \mathrm{X + Y^P} \underset{k'}{\overset{k}{\rightleftharpoons}} \mathrm{XY^P} \xrightarrow{k_d} \mathrm{X + Y + P_i}
\end{aligned}$$

where s is an input signal to the system that affects the rate of autophosphorylation $k(s)$ and $\mathrm{Y^P}$ is the output of the system. (Note that the kinase X is bifunctional: it participates in both phosophrylation and dephosphorylation of Y.)

(c) Express the input and ouput 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 $\mathrm{XY^p}$ 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 input/output relationship (linear, ultrasensitive, etc) as compared with the system in parts (a) and (b). Would the same results occur if there was a separate phosphotase instead of the bifunctional kinase X?

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Bi 250b

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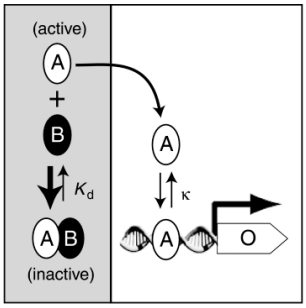

   (a) We assume that A and B bind stoichiometrically, that is, that the formation of the heterodimer is greatly favored over free A. Write down a mathematical relation that reflects this. With this assumption, qualitatively describe what happens in the case when there is less A than B. What about if there is more A than B?

   (b) Write an expression for the rate of change of O in terms of A, κ, the basal transcription rate (β_0), the activated transcription rate (β), and the degradation rate (γ). 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_{tot}, B_{tot}, and K_d. We get that:

$$A = \frac{1}{2}\left(A_{\text{tot}} - B_{\text{tot}} - K_d + \sqrt{(A_{\text{tot}} - B_{\text{tot}} - K_d)^2 + 4A_{\text{tot}} \cdot K_d}\right)$$

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

$$\frac{\beta_0}{\gamma} = 1\text{nM} \qquad \frac{\beta}{\gamma} = 100\text{nM} \qquad \kappa = 100\text{nM} \qquad K_d = 1\text{nM}$$

(d) One possible use of molelcular sequestration to achieve ultrasensitivity is in the context of small RNAs. Discuss how ultrasensitivy could be useful in this context. (Hint: try looking up E. Levine et al, PLoS Biology, 2007.)

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 $\mathrm{X^P}$ and an inactive state X:

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\text{Dephosphorylation}: &\quad \mathrm{X} + \mathrm{Y^P} \underset{k'}{\overset{k}{\rightleftharpoons}} \mathrm{XY^P} \xrightarrow{k_d} \mathrm{X} + \mathrm{Y} + \mathrm{P_i}
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where s is an input signal to the system that affects the rate of autophosphorylation $k(s)$ and $\mathrm{Y^P}$ is the output of the system. (Note that the kinase X is bifunctional: it participates in both phosophrylation and dephosphorylation of Y.)

(c) Using the results in Shinar *et al.*, comment on the input/output relationship (linear, ultrasensitive, etc) as compared with the system in parts (a) and (b). Would the same results occur if there was a separate phosphotase instead of the bifunctional kinase X?