CALIFORNIA INSTITUTE OF TECHNOLOGY
Biology and Biological Engineering (BBE)

BE 150

M. Elowitz and R. M. Murray
Spring 2014

Problem Set #2

Issued: 9 April 2014
Due: 16 April 2014

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.

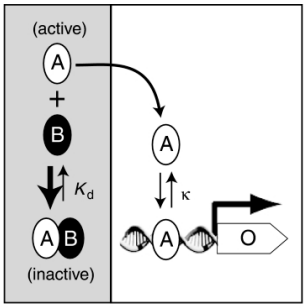
   (a) Download "I1FFL.sbproj" from the class website and use the parameter scanning capability in Simbiology to explore the possible dynamics of this systems.

   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 the amount of unsequestered A in terms of A_{tot}, B_{tot}, and K_d. Assume any A that is *not* bound to B to be *unsequestered*.

   (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.)

(f) Describe a possible role of protein sequestration in a biological circuit. (Take a look at Buchler *et al*, 2009 for some examples).

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Bi 250b

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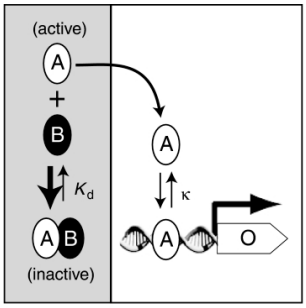
   (b) Analyze a set of genes $\mathrm{Z}_1, \mathrm{Z}_2, \ldots, \mathrm{Z}_\mathrm{n}$, all regulated by the same X and Y in I1-FFLs. Design thresholds such that the genes are turned ON in the rising phase of the pulse in a certain temporal order and turned OFF in the declining phase of the pulse with the same order.

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

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) 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.)

(e) Describe a possible role of protein sequestration in a biological circuit. (Take a look at Buchler *et al*, 2009 for some examples).