CALIFORNIA INSTITUTE OF TECHNOLOGY
Biology and Biological Engineering (BBE)

BE 150

M. Elowitz and R. M. Murray
Spring 2014

Problem Set #3

Issued: 16 April 2013
Due: 23 April 2013

1. (Adaptation in chemotaxis) Consider the reduced order model of chemotaxis shown in Alon, Figure 7.9, for which the reactions can be written as:

$$\begin{aligned}
\text{R1 (Methylation of X):} \quad & \mathrm{X} + \mathrm{R} \rightleftharpoons \mathrm{X{:}R} \longrightarrow \mathrm{X_m} + \mathrm{R} \\
\text{R2 (Demethylation of X}_\mathrm{m}^*\text{):} \quad & \mathrm{X_m^*} + \mathrm{B^p} \rightleftharpoons \mathrm{X_m^*{:}\,B^p} \longrightarrow \mathrm{X} + \mathrm{B^p} \\
\text{R3 (activation/deactivation of X):} \quad & \mathrm{X_m^*} \underset{k^r}{\overset{k^f(L)}{\rightleftharpoons}} \mathrm{X_m}
\end{aligned}$$

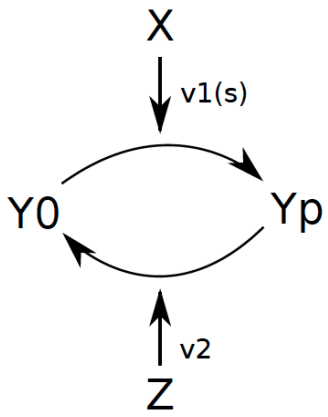
In this model, ligand-binding is approximated by affecting the transition between the active ($\mathrm{X_m^*}$) and inactive states($\mathrm{X_m}$). Furthermore, we assume that the concentration of the demethylase (B) and methylase (R) are constant, and that demethylation can only occur in the active state and methylation can only occur in the inactive state.

   (a) Implement this reduced order model in Simbiology. You can assume Michaelis-Menten kinetics for R1 and R2 and that $k_f(L)$ is a linear function of the ligand concentration, L. Submit a printout of the reactions used, the rate laws, and the parameter values used in the model.

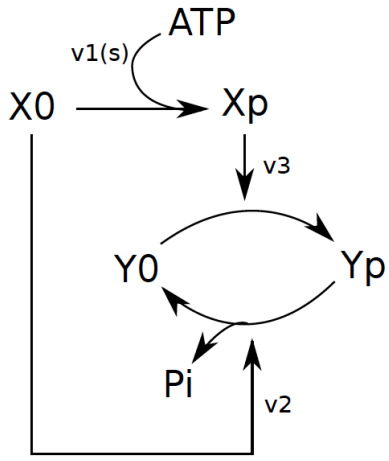
   (b) Assume that $X \gg K_x > 1$, where K_x is the Michaelis constant for CheR. This implies that CheR acts in saturation since there is always enough substrate for the enzyme. Consider the effect of doubling/halving the ligand concentration on the level of active receptor ($\mathrm{X_m^*}$). Plot the response of X_m^* to both increases and decreases in ligand concentration and verify that the circuit adapts to the concentration. (Hint: You can use the event expression in Simbiology to double the ligand concentration at a set time. Be sure to run your simulation long enough to reach steady-state before and after the ligand spike.)

   (c) Now assume that CheR no longer acts in saturation such that the total amount of X is limiting. Plot the time response for increases and decreases in ligand concentration, and comment on how this assumption affects adaptation in the system.

2. (Robustness in signal transduction systems) Consider the following two phosphorylation-based signal transduction systems:



(F1) Separate kinase (X)/phosphatase(Z)



(F2) Single bifunctional kinase/phosphatase (X)

(a) For both systems F1 and F2, identify the kinase and the phosphatase molecule(s). Identify the species that is acting as an auto-kinase in system F2.

(b) For system F1 show that, at steady-state, $Y_p = Y_T \frac{v_1(s)}{v_1(s)+Zv_2/X}$, where $Y_T = Y_o + Y_p$. Note that $v_1(s)$ means that the phosphorylation rate v_1 is a *function* of the input signal s, NOT that v_1 is multiplied by s. Assume a simple mass action model with no intermediate complexes $[X_pY_o], [X_oY_p]$.

(c) Assume that $v_1(s) = v_{10}\frac{s}{s+K}$. What is the concentration of signal s that results in 50% of the maximal Y_p concentration, denoted s_{50}? (Hint: First find an expression for the maximum Y_p, then solve for s_{50}. Don't be afraid of algebra)

(d) Now look at system F2. Find the ODEs for dX_o/dt and dY_p/dt in terms of X_o, X_T, Y_T, Y_p. Use the conservation equations $X_T = X_o + X_p$ and $Y_T = Y_o + Y_p$. Assume that ATP is in excess and therefore its concentration is a constant that is absorbed into $v_1(s)$ (i.e. you don't have to include it as a specific species).

(e) Using the two ODEs you found, find the steady state expression for Y_p. Then, like before, assume $v_1(s) = v_{10}\frac{s}{s+K}$ and solve for s_{50}. What do you notice about this new expression for s_{50}? How does it differ from the expression for s_{50} that you derived for system F1?

(f) Generate plots to show how s_{50} for F1 and F2 changes as a function of X, Z, and Y_T. (Pick 3 values for each to illustrate). Compare your results for F1 versus F2.

(g) Bonus question! Consider the "black box" approach used to analyze the robust model in lecture (F2). This approach assumes that the rate at which ATP is being used during phosphorylation is equal to the rate at which phosphates (p_i) are coming off of as Y_p is being dephosphorylated into Y_o. This assumption allows us to reach the conclusions that you derived in this problem. Now assume that $X_o + Y_p -> X_o + Y_o + p_i$ is not the *only* reaction that dephosphorylates Y_p. For instance, add a term for the spontaneous dephosphorylation of Y_p. How does this change the robustness of the circuit?

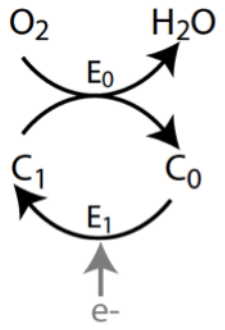


Figure 1: Simple model of the mitochondria ETC

3. (Co-substrate compensation, *Kueh et al, 2013*) Consider a simple model of the mitochondrial electron transport chain (ETC) consisting of a single electron carrier and two reactions that catalyze its oxidation and reduction:

   (a) Write down an ODE model for this enzyme network. Assume that the rate of oxygen reduction by enzyme E_0 is hyperbolic with respect to oxygen concentration with a half-maximal value K_0 i.e. $v_0 \sim [O_2]/([O_2] + K_0)$. For simplicity, also assume that the rate of carrier reduction is first order with respect to the carrier concentration.

   (b) Simulate the response of the system to a 5-fold drop in oxygen concentration from a value greater than K_0 to one lower than K_0, for one chosen set of parameters. Does the system maintain a constant oxygen consumption rate after the drop in oxygen levels? If not, repeat these simulations for a different set of parameters to identify a set of parameters where rate constancy is maintained.

   (c) Solve for the steady-state oxygen consumption rate as a function of oxygen concentration. Using this expression, derive an analytical expression for the oxygen concentration at which oxygen consumption rate is half-maximal (K_m).

   (d) Now, consider the regime where the maximal rate of enzyme E_0 is much greater than that of E_1 . Show that, in this regime:

      i. The model recapitulates the Chance relationship (B Chance, J Gen Phys. 1965), which states that the K_m scales linearly with the maximal rate of electron transfer, and inversely with the reaction rate constant for oxygen reduction.

      ii. The system implements integral feedback. Specifically, show that the time integral of the difference between an enzymes operational velocity and its steady-state velocity is conveyed to the enzyme by the levels of reduced carrier.

   (e) What are the main conclusions that you were able to reach from this simple toy model of the ETC?

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Bi 250b

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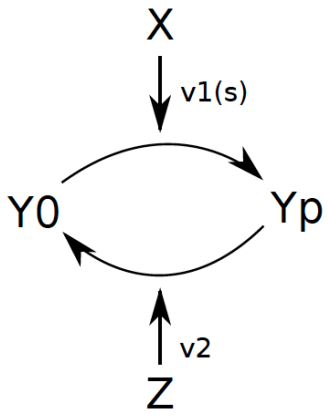

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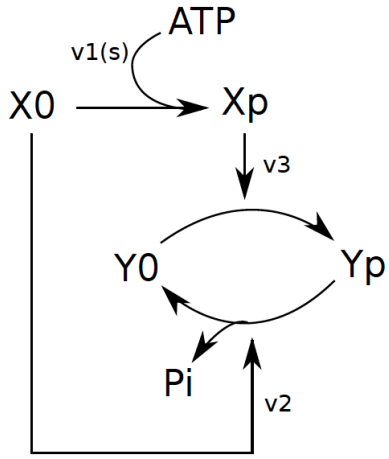


(F2) Single bifunctional kinase/phosphatase (X)

(a) For both systems F1 and F2, identify the kinase and the phosphatase molecule(s). Identify the species that is acting as an auto-kinase in system F2.

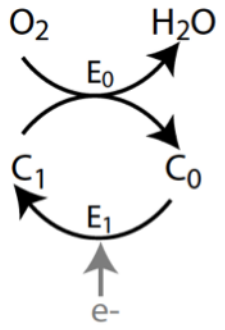
(b) When system F1 is at steady state, $Y_p = Y_T \frac{v_1(s)}{v_1(s)+Zv_2/X}$. Describe the different terms in this equation. How does Y_p change as $v_1(s)$ increases?

(c) Now look at system F2. Write out the ODEs for dX_o/dt and dY_p/dt in terms of X_o, X_T, Y_T, Y_p. Use the conservation equations $X_T = X_o + X_p$ and $Y_T = Y_o + Y_p$. Assume that ATP is in excess and therefore its concentration is a constant that is absorbed into $v_1(s)$ (i.e. you don't have to include it as a specific species).

(d) Using the two ODEs you found, find the steady state expression for Y_p.

(e) Graph the two expressions for Y_p of F1 and F2 in Matlab for $Y_T = 10, 50, \text{and} 100$. Use Z = 10, X = 10, $v_{10} = 100$, $v_2 = 1$, k = 50, s = [0:1:100], and $v_1 = v_{10}.*s./(s+k)$. Identify which system is *robust* and which is *fine-tuned* and explain why.

(f) Bonus question! Consider the "black box" approach used to analyze the robust model in lecture (F2). This approach assumes that the rate at which ATP is being used during phosphorylation is equal to the rate at which phosphates (p_i) are coming off of as Y_p is being dephosphorylated into Y_o. This assumption allows us to reach the conclusions that you derived in this problem. Now assume that $X_o + Y_p -> X_o + Y_o + p_i$ is not the *only* reaction that dephosphorylates Y_p. For instance, add a term for the spontaneous dephosphorylation of Y_p. How does this change the robustness of the circuit?



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   (b) Simulate the response of the system to a 5-fold drop in oxygen concentration from a value greater than K_0 to one lower than K_0, for one chosen set of parameters. Does the system maintain a constant oxygen consumption rate after the drop in oxygen levels? If not, repeat these simulations for a different set of parameters to identify a set of parameters where rate constancy is maintained.

   (c) Solve for the steady-state oxygen consumption rate as a function of oxygen concentration. Using this expression, derive an analytical expression for the oxygen concentration at which oxygen consumption rate is half-maximal (K_m).

   (d) Comment on the significance of co-substrate compensation in the electron transport chain.