HW 6: Synthetic transcriptional circuits.

(Dated: November 15, 2010)

1. PREVIEW OF FINAL PROJECT.

(a) (5 points). Find a synthetic biology paper that you find interesting and, in two paragraphs, explain (i) what the paper is about and (ii) why you picked it (why it is interesting). Use your own words rather than copying the abstract. Pick a different paper from last time and try to look at something that is thematically different. You will be able to change topic for the final project but this is a good opportunity to start looking around. Use pubmed or Google Scholar to look up papers.

(b) (5 points). Pick a second paper from the list of references in the paper you have chosen in (a) and, in one paragraph, explain how the two papers are related.

2. PROTEIN MODULES.

(a) (10 points) Go to the NCBI database http://www.ncbi.nlm.nih.gov/ and find the protein sequences for the E. coli EnvZ protein and for the Bacteriaphytochrome Cph1 from Synechocystis. Both of these proteins are sensors that are part of a two-component system. Use the Smart protein domain tool http://smart.embl-heidelberg.de/ to identify conserved functional sub-domains within these proteins. Which domains are found in both proteins, what are their functions and where are they located in the protein sequence (give position of first and last amino acid).

(b) (5 points) In what amino acid position would you cut and recombine the two sensors in order to obtain the synthetic light sensor discussed in class?

3. ENZYME CASCADES.

(15 points). Consider the following two-step cascade of enzymatic reactions:

$$E_2 + nE_1^* \xrightarrow{k} E_2^* + nE_1^*, \quad E_2^* \xrightarrow{1} E_2, \tag{1}$$

$$E_3 + nE_2^* \xrightarrow{k} E_3^* + nE_2^*, \quad E_3^* \xrightarrow{1} E_3. \tag{2}$$

In this example E_i could be a kinase in an inactive state while E_i^* is the active (phosphorylated) kinase. Conservation of mass implies that $E_i^{tot} = E_i^* + E_i$ $(i = 2, 3)$. Find the steady state solution for the amount of E_3^* as a function of E_1^*. Then, plot E_3^* and E_2^* as a function of E_1^* (the input). Use $E_i^{tot} = 10$ and $k = 1$. Show plots for $n = 1$ and $n = 5$.

4. ZERO ORDER ULTRASENSITVITY REVISITED.

In class we consider the a simple reaction network where a substrate y is phosporylated by the kinase E_1 to make y^*, while the reverse reaction (dephosphorylation of y^*) is catalyzed by the phosphatase E_2.

(a) (5 points). Following the approach we used in class for similar systems, solve for the steady state value of y^* as a function of E_2/E_1. Plot the result keeping either of the enzymes fixed and convince yourself that there is no ultrasensitivity in this system.

(b) (5 points). In the models discussed in class, we have assumed that an enzymatic reaction can be described by $E + y \xrightarrow{k} E + y^*$ which results in $\dot{y^*} = kEy$. This result is different from Michaelis Menten model which yields

$$\dot{y^*} = \frac{v_{max} y}{K_M + y}. \tag{3}$$

Find the equations from which this model is derived (using our notation). You don't need to copy the whole derivation, just provide the starting point and see how it differs from the simper model $E + y \xrightarrow{k} E + y^*$. Then,

express v_{max} and K_M in terms of the underlying variables of the model, and explain what the meaning of these two variables is. Finally, in what regime does the Michaelis Menten model agree with the simpler model?

(c) (5 extra points). Return to the problem stated in (a) but now assume that E_1 and E_2 are Michaelis Menten enzymes. Write the equation of motion for $\dot{y^*}$ and assume steady state. Solve the resulting quadratic equation for y^* (again, use conservation of mass) and show that you now get ultrasensitivity. Assume that the K_M for both enzymes is the same and normalize all concentrations to y_{tot} for simplicity. (The result can be found in the original paper by Goldbeter and Koshland, PNAS **78**, 6840 (1981).)