Quantitative Molecular Biology
Problem Set #4
due: Friday March 5, 2021

1. **Amplitude of gene regulation:** A promoter is controlled by an activator A which binds to a single operator site O_A upstream of the core promoter. The effective dissociation constant of A with its operator is $K_A = 80$ nM, and the interaction between the operator-bound activator and the RNAp is described by $\omega = 15$. The promoter drives a gene encoding a polypeptide X that is a subunit of a dimeric protein X_2. The dimer dissociation constant of X_2 is $\frac{k_f}{k_b} = \kappa = 10$ nM, where k_f and k_b are the forwards and backwards rates of dimerization. Furthermore, the monomer X, dimer X_2 and mRNA m can degrade at rates β_X, β_{X_2}, and β_m respectively. Lastly, the mRNA synthesis rate and protein translation rate are given by α_m and α_p respectively. This situation is cartooned in Fig. 1.

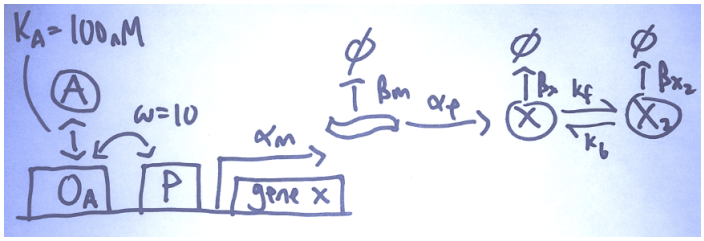

Figure 1: Schematic for Q #1

In the following questions, consider the change in activator concentration to be from 1 nM to 1000 nM:

(a) Fold-change of the mRNA level for the protein X.

(b) Fold-change in the cellular concentration of X_2, assuming that in the absence of the activator, the polypeptide synthesis rate $\alpha_p\alpha_m/\beta_m$ is 1/min, and the proteins are diluted by cell growth only (with a doubling time of 60 min). You may assume that the kinetics of dimer association and dissociation occur at much faster time scales.

(c) Fold-change in the cellular concentration of X_2 if the monomers are degraded with a half-life of 5 min while the dimers are not specifically degraded.

2. **Genetic toggle switch:** Suppose there are two genes r_1 and r_2 on the chromosome, under the control of promoters P_1 and P_2 respectively. r_1 encodes a repressor R_1, which binds to the promoter P_2 and interferes with the transcription of r_2. Similarly, r_2 encodes a repressor R_2, which binds to the promoter P_1 and interferes with the transcription of r_1. The binding of the repressors to their respective sites are characterized by the same Hill coefficient, $n = 2$, and the same dissociation constant K. Both repressors are stable, i.e., not subjected to proteolysis. The basal gene expression activities are such that in the absence of R_1, the steady state level of R_2 is R_{20}, and in the absence of R_2, the steady state level of R_1 is R_{10} at the doubling rate of one per hour. For simplicity, you may assume there is no leakage if the promoters are highly repressed.

(a) Perform a linear stability analysis and derive the criterion for instability in terms of the sensitivities of the promoter functions, $\mathcal{G}_1(R)$ and $\mathcal{G}_2(R)$, defined as $s_i = \frac{\partial \ln \mathcal{G}_i}{\partial \ln R}$ for $i = 1, 2$ evaluated at the fixed point R_1^* and R_2^*.

(b) Assuming that the unstable fixed point is located on the nontrivial branch of the regulation functions, i.e., assuming that $\mathcal{G}_1 = (R_2/K)^{-2}$ and $\mathcal{G}_2 = (R_1/K)^{-2}$, estimate the approximate parameter regime for which the system is expected to exhibit bistability. Plot the regime of bistability in the space $(R_{10}/K, R_{20}/K)$.

(c) Re-plot the above phase diagram (i.e., regime of bistabilty) for cells growing in media with 3x faster and 3x slower growth rate. Indicate on the phase diagram the regime for which bistability is expected to exist for all these growth media. Repeat the above analysis for the autoactivator discussed in class, and find the regime of phase space for which bistability exists in media with 3x faster and 3x slower growth rates. Comment on the robustness of behaviors for the two different designs.

3. **Threshold response of an inducible auto-activator** A transcription factor (TF) A activates the transcription of a gene g, which codes for a protein G. As a result the rate with which G is produced depends on the concentration [A] of A as $\alpha\, \mathcal{G}([\mathrm{A}])$. Here α is the maximum protein synthesis rate, and $\mathcal{G}([\mathrm{A}])$ is the regulation function

$$\mathcal{G}([\mathrm{A}]) = \frac{\epsilon + [\mathrm{A}]/K}{1 + [\mathrm{A}]/K}$$

where K is the equilibrium dissociation constant for the TF-DNA binding and ϵ represents a basal leakage term. The protein G is degraded at a rate β. `[For simplicity assume the volume of cell is constant (= 1 in suitable unit), so that you don't need to distinguish between protein concentration and the number of proteins per cell.]`

(a) Write down the deterministic rate equation describing the dynamics of the product $[G]$. What is the equilibrium value of $[G]$ given $[A]$?

(b) Now consider the case where $[G]$ actually activates its *own* gene (so that A and G are now *one and the same protein*). Write down the differential equation for the concentration $[G]$ and calculate its equilibrium value.

(c) Next we assume that G can activate the transcription of its own gene only if it is bound to some small molecule L (a ligand). We refer to the concentration of G not bound to L as [G], to the concentration of G bound to a ligand as $[G_L]$, and call the total concentration $[G_{tot}] = [G] + [G_L]$.

- What is the equilibrium value of $[G_{tot}]$ if $[L] = 0$?
- What is it if $[L]$ is very large?
- Explain in words why this circuit can be considered a cellular sensor for the concentration of the ligand.

(d) We define $r = [G_L]/[G_{tot}]$, i.e., r is the fraction of G proteins bound to a ligand.

- Write down the differential equation for $[G_{tot}]$.

- Assume for simplicity that ϵ is negligible. What is the equilibrium concentration of $[G_{tot}]$ as a function of r? Sketch a plot of $[G_{tot}]$ as a function of r (You can assume that $K < \alpha/\beta$). Some reasonable parameters would be $\alpha/\beta \approx 90$ nM and $K \approx 30$ nM.
- Explain in words why the behavior of the circuit as a function of the ligand concentration can be called a "threshold response".
- Now assume that ϵ is not negligible, but nevertheless small: ϵ=0.05. Sketch another plot of $[G_{tot}]$ as a function of r. If a strict threshold response is desired, what value should ϵ have?