Quantitative Microiology
Problem Set #3 (amended)
due: Thursday Mar 2, 2023

[The first two problems are on Lecture #9 and can be attempted right away. Problem #3 and #4 are on Lecture #10. Problem #5 is on Lecture #12.]

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 $K_A = 100$ nM, and the interaction between the operator-bound activator and the RNAp is described by $\omega = 10$. 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 $\frac{k_b}{k_f} = \kappa = 10$ 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 are degraded at rate β_X, β_{X_2}, and β_m respectively. Lastly, the transcription initiation rate and translation initiation rate is given by α_m and α_p, respectively. This situation is cartooned in Fig. 1.

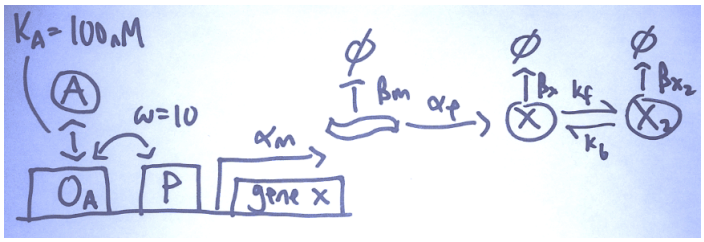

Figure 1: Schematic for Q #1

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 of the gene encoding 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. [Note that both the monomers and dimers are still diluted given that the cells are growing with a doubling time of 60 min.]

2. **Cross-talk in sRNA-mediated gene silencing:** In this problem, we consider the effect of a small RNA (S) negatively regulating the expression of two genes (A and B), through binding to each mRNA species and co-degradation with the bound mRNA as described in class. The transcription initiation rate of the small RNA is denoted by α_S, and those of the two target genes are denoted as α_A and α_B. Let the binding rate of the small RNA with mRNA A be k_A and that with mRNA B be k_B. Let the turnover rate of each mRNA species in the absence of the small RNA be β_A and β_B, and let the turnover rate of the

small RNA in the absence of any target mRNA be β_S. The turnover rates βs and the binding rates ks are fixed genetically by sequence features of the mRNAs and the sRNA. In contrast, the transcription initiation rates α can be varied by controlling the activities of the respective promoters via transcription factors.

Let us first perform a qualitative analysis of this system.

(a) If gene B is not expressed, sketch the threshold-linear response of the concentration of the mRNA A m_A on the transcription rate α_A, for a fixed sRNA transcription rate α_S.

(b) Repeat the sketch of part (a) for $\alpha_B = 0.5 \times \alpha_S$ and for $\alpha_B = 2 \times \alpha_S$ if the small RNA has much higher affinity for mRNA B than mRNA A, i.e., $k_B \gg k_A$.

(c) Explain in words the effect of gene B on gene A as obtained above. What would you expect to happen if the small RNA has a much higher affinity for mRNA A rather than mRNA B.

Now let us work out the above quantitatively.

(d) Write down the set of ODEs describing changes in the concentrations of the mRNAs, m_A and m_B, and the concentration of the small RNA s. In the steady state where d/dt of all 3 species are zero, write down the algebraic equations coupling the 3 concentrations.

(e) Let $\beta_A = \beta_B = 0.1$ min^{-1}, and $\beta_S = 0.02$ min^{-1}. Let $k_A = 0.01$ nM^{-1}min^{-1} and $k_B = 0.05$ nM^{-1}min^{-1}. Solve the above equations numerically to generate a plot of m_A against α_A for various levels of α_B: 0, 0.2 nM/min, 0.4 nM/min, 0.6 nM/min, 0.8 nM/min, 1.0 nM/min, with $alpha_S$ fixed at 0.5 nM/min.
[Hint: First, from the steady-state condition obtain an inverse relation between m_A and s, and between m_B and s. Next, exploit the co-degradation condition to obtain a linear constraint on the concentrations m_A, m_B, and s. Eliminate m_A and m_B to obtain a cubic equation for s in terms of the systems parameters (αs, βs, etc). Use the exact solution of the cubic equation to solve for the value of s for any parameter values.]

(f) Repeat part (e) with $k_A = 0.05$ nM^{-1}min^{-1} and $k_B = 0.01$ nM^{-1}min^{-1}.

3. **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. Both repressors are stable, i.e., not subjected to proteolysis.

(a) Write down two coupled ODEs describing the dynamics of the concentrations of the two repressors, R_1 and R_2, as specified by this genetic circuit for cells growing exponentially at rate λ. Let the rate of synthesis of R_1 be $\alpha_{10} \cdot \mathcal{G}_1(R_2)$ and the rate of synthesis of R_2 be $\alpha_{20} \cdot \mathcal{G}_2(R_1)$, where the repression functions $\mathcal{G}_1$ and $\mathcal{G}_2$ are defined relative to their respective maximum, such that α_{10} is the synthesis rate of R_1 in the absence of R_2 and α_{20} is the synthesis rate of R_2 in the absence of R_1.

(b) You are given 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}. Write down the two conditions

satisfied by the steady state solutions R_1^* and R_2^* in terms of $\mathcal{G}_{1,2}$ and R_{10}, R_{20}. Suppose the binding of repressor R_i to its respective site is described by the same dissociation constant K and with the same Hill coefficient $n = 2$. Further suppose there is no leaky transcription when the promoters are highly repressed. Write down the two algebraic equations satisfied by R_1^* and R_2^*.

(c) Solve the above system graphically: make log-log plot of the relation between R_1^* and R_2^* corresponding to the condition $dR_1/dt = 0$; make another log-log plot of the relation between R_1^* and R_2^* corresponding to the condition $dR_2/dt = 0$. How would you combine the two conditions together graphically given that R_1^* and R_2^* satisfy both conditions? Make a sketch for each of the following three scenarios: (i) one solution only for $R_1^* \approx R_{10}$ and small R_2^*; (ii) one solution only for R_{20}^* and small R_1^*; (iii) three solutions including a nontrivial one with both R_1^* and R_2^* not small. In the latter case, explain qualitatively the stability of each solution and note the one giving "bistability".

(d) Approximating the functions $\mathcal{G}_{1,2}$ by piece-wise linear functions in log-log space (as described in class) to estimate the approximate parameter regime for which the system is expected to exhibit bistability. Plot the "phase diagram", i.e., the regime of bistability, in the space $(R_{10}/K, R_{20}/K)$.

(e) For the same cells growing at a different rate (due to different growth media), what parameters in the phase diagram are changed? Re-plot the phase diagram 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 three growth media. Compare the above to the auto-activator discussed in class and comment on the robustness of behaviors for the two different designs. [You can just take the phase boundaries derived in class for the auto-activator and sketch qualitatively what would happen if the growth rate is increased or decreased 3x.]

You do not need to attempt the last part of this problem (below) if you have not had a course covering linear stability analysis. However, the only math you need is ODE and linear algebra, and you can teach yourself this very important analysis tool by following the procedure outlined below.

(f) Consider the general system as formulated in part (a), it is possible to derive analytically the criterion for the stability of any fixed point. Suppose there is a steady state given by the fixed point (R_1^*, R_2^*). Perform a linear stability analysis as follows:

- Write down the two conditions satisfied by R_1^* and R_2^* for general repression functions $\mathcal{G}_1(R_2)$ and $\mathcal{G}_2(R_1)$, by setting dR_1/dt and dR_2/dt to zero.
- For small deviation from the fixed point, i.e., for $R_1(t) = R_1^* + \delta R_1(t)$ and $R_2(t) = R_2^* + \delta R_2(t)$ where $|\delta R_{1,2}| \ll R_{1,2}^*$, substitute $R_{1,2}(t)$ into the ODEs in (a) and expand to leading order in $\delta R_{1,2}$. You can write the resulting ODEs for $\delta R_{1,2}$ in the matrix form

$$\frac{d}{dt}\begin{bmatrix}\delta R_1(t)\\ \delta R_2(t)\end{bmatrix} = \begin{bmatrix}M_{1,1} & M_{1,2}\\ M_{2,1} & M_{2,2}\end{bmatrix} \cdot \begin{bmatrix}\delta R_1(t)\\ \delta R_2(t)\end{bmatrix} \tag{1}$$

  where $M_{i,j}$ are the elements of the matrix $\mathcal{M}$. By using the steady state conditions worked out above, you can express the matrix elements $M_{i,j}$ in terms of the time scale λ and the sensitivities, $s_1 \equiv \frac{d\ln \mathcal{G}_2}{d\ln R_1}$ and $s_2 \equiv \frac{d\ln \mathcal{G}_1}{d\ln R_2}$, evaluated at the fixed point R_1^* and R_2^*.

- Find the eigenvalues of the matrix $\mathcal{M}$. Negative eigenvalue implies that small deviation *decreases* in time, i.e., the fixed point is stable, while posiive eigenvalue implies that small deviation *decreases* in time, i.e., the fixed point is unstable. The criterion for instability is if either of the eigenvalue is positive.
- Find the stability criterion in terms of s_1 and s_2, and explain what this means in term of the graphical solution constructed above. Compare this solution to the one discussed in class for the auto-activator and explain why it is more stable.

4. **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?

5. **Uptake vs de novo synthesis of amino acid.** An amino acid a can be taken up by the transporter T if it is available in the environment or it can be synthesized *de novo* by a series of enzymes collectively referred to as E. It is advantageous for the cell to synthesize the transporter instead of the enzyme E when a is available from the environment because synthesizing E costs a lot more than synthesizing T. In this problem, you will investigate a simple regulatory strategy that allows the cell to synthesize the enzyme E only when needed, i.e., when the external concentration of amino acid $[a]_{\text{ext}}$ is low.

   Let the specific rate of uptake by the transporter be k_T, and specific rate of synthesis by the enzyme be k_E. Then changes in the cellular concentration of the amino acid, $[a]$, can be described by

$$\frac{d[a]}{dt} = k_T \cdot [T] + k_E \cdot [E] - j, \tag{2}$$

   where j is the flux of a demanded by cell growth and is proportional to the growth rate. The uptake rate k_T itself depends on the external concentration of a as will be specified below.

   (a) Both the transporter and enzyme concentrations are regulated by a common regulatory scheme called "end-product inhibition", with

$$[T] = T_0 \frac{1 + ([a]/K_T)^n}{1 + f \cdot ([a]/K_T)^n}, \tag{3}$$

   and

$$[E] = E_0 \frac{1 + ([a]/K_E)^n}{1 + f \cdot ([a]/K_E)^n}. \tag{4}$$

   For simplicity, we use here regulatory functions with the same capacity $f \gg 1$ and Hill coefficient $n \to \infty$. Sketch (by hand) the dependence of $[T]$ and $[E]$ on $[a]$ and explain the meanings of the parameters T_0, K_T, E_0, and K_E.

   (b) For $K_T < K_E$, sketch the dependence of the concentrations $[T]$, $[E]$, and $[a]$ on the flux demand, j. (You can take the steady state where $d[a]/dt = 0$.) Find an expression for the critical value of the flux at which the enzyme E is 'turned on'? How does this critical value depend on the values of K_T, K_E, and f? What happens for $K_E < K_T$?

   (c) In part (b) above, you will notice that there is no solution for very small and very large values of j. Write down the lower and upper limit in terms of the model parameters, and explain what "goes wrong" in each case. Show how the problem at small-j end disappears if n is taken to be finite.

   (d) The specific uptake rate depends on the external concentration of a via the standard Michaelis-Menton form,

$$k_T = k_{T,max} \frac{[a]_{\text{ext}}}{[a]_{\text{ext}} + K_a}, \tag{5}$$

   where $k_{T,max}$ is the maximal uptake rate for saturating concentration of a, and K_a is the half-saturation constant for the transporter. Sketch the dependence of the concentrations $[T]$, $[E]$, and $[a]$ on the external concentration $[a]_{\text{ext}}$ for a fixed value of

the demand flux j. What is the critical concentration of external a below which the enzyme E is 'turned on'? Describe or sketch qualitatively how the dependences of $[T]$, $[E]$, and $[a]$ on $[a]_{\text{ext}}$ would change if n has a finite value, e.g., $n = 2$?