BioE 135/235 Homework 3

Due Date: Thursday, 3/18/2021, 5:00pm PST to Gradescope
Late work will be deducted 20% for each day it is late.

1. Non-Dimensionalization with a Growing Cell

Consider a protein X that can bind to two different sites upstream of its promoter. Also consider a case where the promoter drives expression of this protein, leading to autoregulation. Assume that the promoter has some basal activity k_0, which is obtained when the promoter is not occupied by X. The activity increases by k_1 when two X molecules are bound. **You may assume that X molecules are either fully occupying both of their binding sites, or that neither site is occupied.** Let's also assume X is stable and does not degrade on its own, but that the cell has a doubling time of T_D. Assume the cell doubles its volume in this time, and then divides into two cells of the same volume with evenly-split contents.

(a) Suppose that the protein X is produced at some known rate $k(t)$. Derive the kinetic equation $\frac{d}{dt}X$ for the system described above. (Hint: how does cell division play into this?)

The easiest way to think about this question would be to—at least initially—ignore $k(t)$ to develop an intuition about the system. We will do so by setting $k(t) = 0$, and observing what happens when no extra X is produced. Consider the case wherein one has an initial concentration of 64 molecules of X vested inside of a single cell. After one doubling time T_D, the cell splits into two, and evenly divides it such that one has 32 molecules of X in each cell. At $t = 2T_D$, we now have 16 molecules of X spread across 4 cells.

In general, we can realize that, every doubling time, we halve the concentration. Thus we have $X(t) = X(0) \cdot \left(\frac{1}{2}\right)^{t/T_D}$, where the $X(0)$ terms gives us our initial concentration, and the $\left(\frac{1}{2}\right)^{t/T_D}$ halves this concentration after each doubling time.

We have found $X(t)$, but we wish to find $\frac{d}{dt}X(t) = X(0) \cdot \left(\frac{1}{2}\right)^{t/T_D} \ln\frac{1}{2}\frac{1}{T_D} = -\frac{\ln 2}{T_D}X(t)$. From here, all that is left is to consider a nonzero production rate $k(t)$, which we can reasonably surmise would be added to the expression to arrive at:

$$\frac{d}{dt}X(t) = k(t) - \frac{\ln 2}{T_D}X(t)$$

(b) Show that $k(t) = \left(k_0 + k_1 \frac{\left(\frac{X}{K_A}\right)^2}{1 + \left(\frac{X}{K_A}\right)^2}\right) P_0$, where P_0 is the total promoter concentration in the system. State any assumptions you make in arriving to this conclusion.

To begin with, we will consider the reactions involved in this system:

$$P \rightarrow P + X (k_0)$$
$$P + 2X \leftrightarrow P : 2X$$
$$P : 2X \rightarrow P : 2X + X (k_0 + k_1)$$

From here, we assume that the second reaction is at equilibrium (with equilibrium constant $E_X = \frac{X^2 \cdot P}{P:2X}$), and define rates for the other two:

$$\nu_1 = k_0 P$$
$$\nu_2 = (k_0 + k_1) P : 2X$$

So that we have, in total,

$$\frac{d}{dt} X = k_0 P + (k_0 + k_1) P : 2X - \frac{\ln 2}{T_D} X$$

Our goal now is to show that the first two terms ($k_0 P + (k_0 + k_1) P : 2X$) are equivalent to the expression shown ($\left(k_0 + k_1 \frac{\left(\frac{X}{K_A}\right)^2}{1 + \left(\frac{X}{K_A}\right)^2} \right) P_0$). To do this, we will convert every instance of P or $P : 2X$ into P_0, the total amount of promoter in the system.

We start by defining $P_0 = P + P : 2X$. We can thus rewrite

$$\begin{aligned} k_0 P + (k_0 + k_1) P : 2X &= k_0 P + k_0 P : 2X + k_1 P : 2X \\ &= k_0 (P + P : 2X) + k_1 P : 2X \\ &= k_0 P_0 + k_1 P : 2X \end{aligned}$$

Now, all that's left is to find $P : 2X$ in terms of P_0; to do this, we will look to our equilibrium constant $E_X = \frac{X^2 \cdot P}{P:2X}$:

$$\begin{aligned} E_X &= \frac{X^2 \cdot P}{P : 2X} \\ \Rightarrow P &= E_X \frac{P : 2X}{X^2} \\ P_0 &= P + P : 2X \\ \Rightarrow P_0 &= P : 2X \left(\frac{E_X}{X^2} + 1 \right) \\ P : 2X &= \frac{1}{\frac{E_X}{X^2} + 1} P_0 \\ &= \frac{\frac{X^2}{E_X}}{1 + \frac{X^2}{E_X}} P_0 \end{aligned}$$

E_X is non-negative by definition, so it has a square root; we can thus write $K_A = \sqrt{E_X}$, and we have

$$P : 2X = \frac{\left(\frac{X}{K_A}\right)^2}{1 + \left(\frac{X}{K_A}\right)^2} P_0$$

Plugging this into our expression from above, we arrive at the intended final result.

(c) Now, we will do something referred to as *non-dimensionalization*, so that we take our expression for $\frac{d}{dt} X$, which has units of M/s, and—with appropriate transformations $\overline{X} = f(X)$, $\tau = g(t)$—convert it into a new expression $\frac{d}{d\tau} \overline{X}$ that is unitless.

Using $\overline{X} = \beta_1 X$ and $\tau = \beta_2 t$, show that the whole expression $\frac{dX}{dt}$ can be non-dimensionalized to arrive in the form

$$\frac{d}{d\tau}\overline{X} = \alpha_1 + \alpha_2 \frac{\overline{X}^2}{1+\overline{X}^2} - \overline{X} = p(\overline{X}) - q(\overline{X})$$

What are the $\beta_1, \beta_2, \alpha_1, \alpha_2$ that satisfy this?

Recall the chain rule from calculus:

$$\frac{df}{dt} = \frac{df}{dx}\frac{dx}{dt}$$

In our case, it'll be a bit more complicated, though:

$$\frac{d\overline{X}}{d\tau} = \frac{d\overline{X}}{dX}\frac{dX}{dt}\frac{dt}{d\tau}$$

Note that the X and t terms cancel out, leaving only $\overline{X}$ and τ on the right. From the given expressions, we know that $\frac{d\overline{X}}{dX} = \beta_1$ and $\frac{dt}{d\tau} = \frac{1}{\beta_2}$, so we have:

$$\begin{aligned}
\frac{d\overline{X}}{d\tau} &= \frac{\beta_1}{\beta_2}\frac{dX}{dt} \\
&= \frac{\beta_1}{\beta_2}\left(\left(k_0 + k_1 \frac{\left(\frac{X}{K_A}\right)^2}{1+\left(\frac{X}{K_A}\right)^2}\right) P_0 - \frac{\ln 2}{T_D} X\right) \\
&= \frac{\beta_1}{\beta_2} k_0 P_0 + \frac{\beta_1}{\beta_2} k_1 P_0 \frac{\left(\frac{X}{K_A}\right)^2}{1+\left(\frac{X}{K_A}\right)^2} - \frac{\beta_1}{\beta_2}\frac{\ln 2}{T_D} X \\
&= \frac{\beta_1}{\beta_2} k_0 P_0 + \frac{\beta_1}{\beta_2} k_1 P_0 \frac{\left(\frac{\overline{X}}{\beta_1 K_A}\right)^2}{1+\left(\frac{\overline{X}}{\beta_1 K_A}\right)^2} - \frac{1}{\beta_2}\frac{\ln 2}{T_D} \overline{X}
\end{aligned}$$

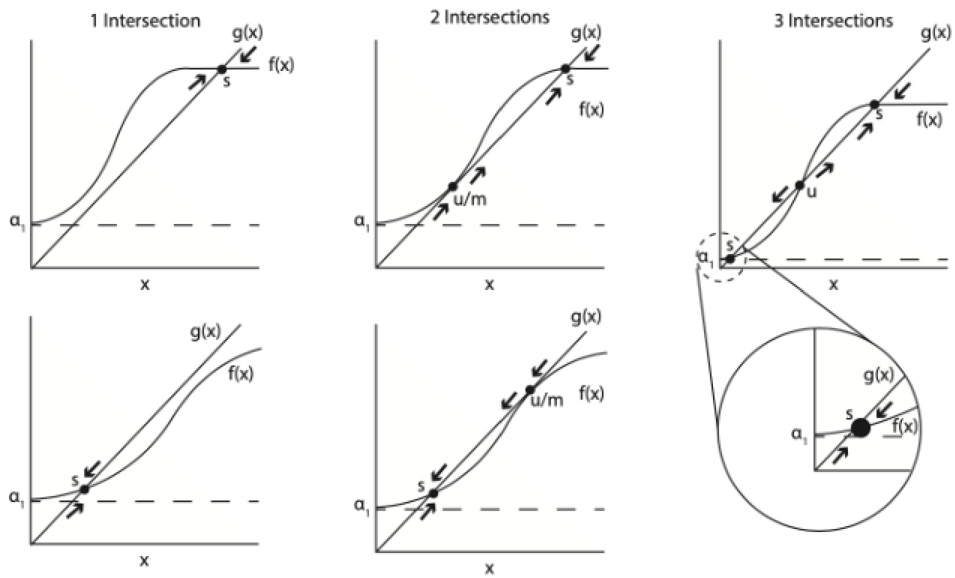
Where the last line is obtained by plugging in $\overline{X} = \frac{1}{\beta_1} X$. All that's left is to choose $\beta_1, \beta_2, \alpha_1, \alpha_2$ to satisfy the original expression. Observe that

$$\begin{aligned}
\beta_1 &= K_A^{-1} \\
\beta_2 &= \frac{\ln 2}{T_D} \\
\alpha_1 &= \frac{\beta_1}{\beta_2} k_0 P_0 \\
&= \frac{k_0 P_0 T_D}{K_A \ln 2} \\
\alpha_2 &= \frac{\beta_1}{\beta_2} k_1 P_0 \\
&= \frac{k_1 P_0 T_D}{K_A \ln 2}
\end{aligned}$$

Satisfy these requirements.

(d) The steady states of our system will occur when $p(x) = q(x)$. The exact type of steady state will depend on the values of α_1 and α_2. For fixed α_1, α_2 it is given that there will be either 1, 2, or 3 steady states; qualitatively sketch the graphs that may lead to these amounts of steady states. What is the stability of each of these steady states in each of these cases?

1 and 2 intersection cases had multiple possible correct answers:



For the case of 1 intersection, we see that the steady state is stable because any deviation from the steady state results in convergence back to the steady state. Specifically, when the positive deviation in $q(x)$ (i.e. degradation) is greater than $p(x)$ (i.e. generation), x decreases. Similarly, when the negative deviation in $q(x)$ is less than $p(x)$, x moves in the positive direction.

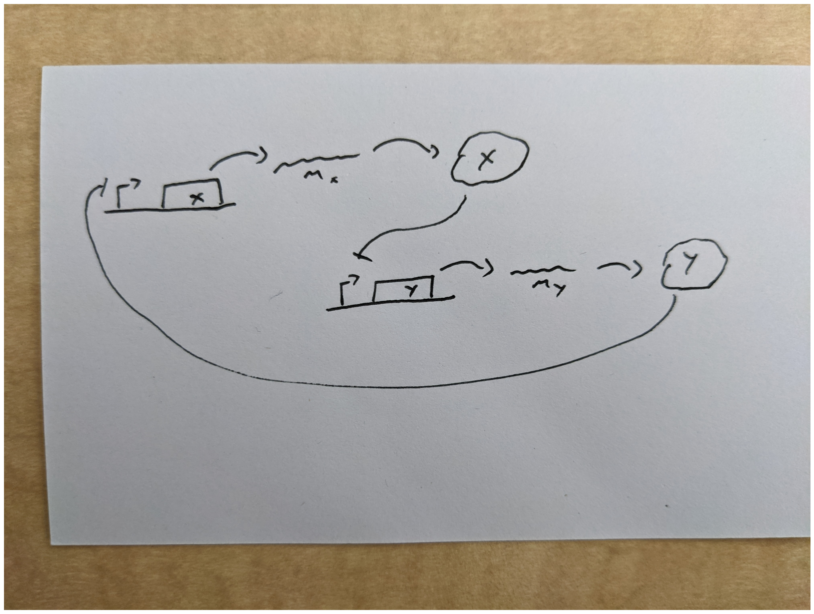
For the case of 2 intersections, we see that one steady state is stable (i.e. $q(x) > p(x)$ for positive deviations from the steady state, $p(x) > q(x)$ for negative deviations from the steady state) while the other steady state is a mixed/unstable steady state (i.e. a deviation in one direction leads back to the steady state while deviation in the other direction leads the system away from the steady state).

For the case of 3 intersections, we see that the two external steady states are stable while the internal steady state is unstable. At the internal steady state we see that a positive deviation in x leads to $p(x) > q(x)$, which tends towards the higher external steady state. Similarly, a negative deviation from this internal steady state leads to a $q(x) > p(x)$, which tends towards the lower external steady state.

2. Bistable Circuit Analysis

Consider a system of two genes, encoding two proteins X and Y. Suppose n_X molecules of X work together to cooperatively repress Y's promoter, and n_Y molecules of Y work together to cooperatively repress X's promoter. Neither protein is stable, and each degrades at a rate k_{dp}.

(a) Draw the schematic gene circuit that represents this system.



(b) Assume that gene X is present in the same quantity as gene Y, with $[Promoter_X]_0 = [Promoter_Y]_0 = [Promoter]_0$. Show that this system may then be expressed as:

$$\frac{d}{dt}X = k_p \frac{1}{1+\left(\frac{Y}{K_Y}\right)^{n_Y}} - k_{dp}X$$
$$\frac{d}{dt}Y = k_p \frac{1}{1+\left(\frac{X}{K_X}\right)^{n_X}} - k_{dp}Y$$

For $k_p := \frac{k_{tr}k_{tx}}{k_{dm}}[Promoter]_0$, and for K_X and K_Y adequately defined. State all assumptions made. How do K_X and K_Y relate to the equilibrium binding constants $\frac{[Promoter_Y][X]^{n_X}}{[Promoter_Y:X_{n_X}]}$ and $\frac{[Promoter_X][Y]^{n_Y}}{[Promoter_X:Y_{n_Y}]}$? (Hint: As written, they are **not** the same; consider reviewing the assumptions made in Homework 2 Questions 1(b) and 1(c) for guidance.)

First, consider the reactions involved in this system:

$$P_X \to P_X + X(k_{prod} = \frac{k_{tx}k_{tl}}{k_{dm}})$$
$$P_Y \to P_Y + Y(k_{prod} = \frac{k_{tx}k_{tl}}{k_{dm}})$$
$$P_X + n_Y Y \leftrightarrow P_X : n_Y Y$$
$$P_Y + n_X X \leftrightarrow P_Y : n_X X$$
$$X \to \emptyset(k_{dp})$$
$$Y \to \emptyset(k_{dp})$$

We will preemptively define equilibrium constants E_X and E_Y as we did in the previous question, so that we have:

$$E_X = \frac{P_Y \cdot X^{n_X}}{P_Y : n_X X}$$
$$E_Y = \frac{P_X \cdot Y^{n_Y}}{P_X : n_Y Y}$$

From the reactions above, we can construct our rate equations:

$$\frac{d}{dt}X = k_{prod}P_X - k_{dp}X$$
$$\frac{d}{dt}Y = k_{prod}P_Y - k_{dp}Y$$

Looking at the equation for $\frac{d}{dt}X$ given in the prompt, it becomes evident that we want to show that our first term $k_{prod}P_X$ is equivalent to their first term $k_p\frac{1}{1+\left(\frac{Y}{K_Y}\right)^{n_Y}} = k_{prod}P_0\frac{1}{1+\left(\frac{Y}{K_Y}\right)^{n_Y}}$. To do this, we will put P_X in terms of $P_{X0} = P_0$:

$$\begin{aligned} P_0 &= P_X + P_X : n_Y Y \\ &= P_X + P_X\left(\frac{Y^{n_Y}}{E_Y}\right) \\ \Rightarrow P_X &= P_0\frac{1}{1+\frac{Y^{n_Y}}{E_Y}} \end{aligned}$$

Let $K_Y^{n_Y} = E_Y$, and we have the desired expression. We can perform a similar analysis on the expression for $\frac{d}{dt}Y$, and we will arrive to similar results.

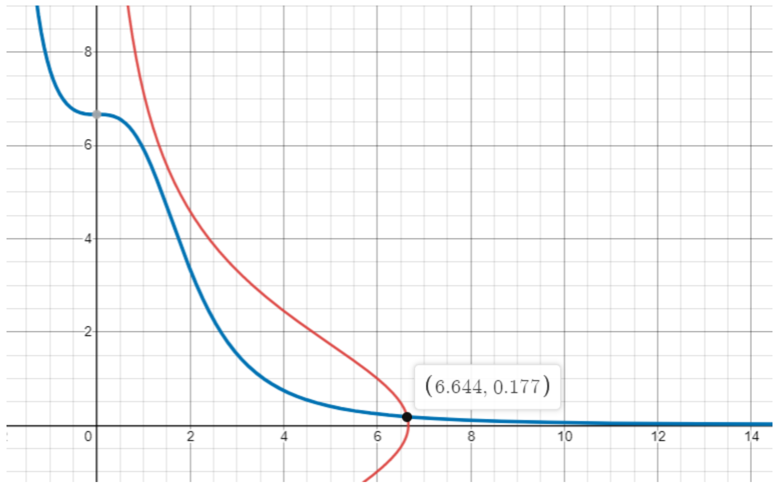
(c) Assuming $Y \ll K_Y$, determine an expression for the steady-state values X_{ss} and Y_{ss}.

At steady state, $\frac{d}{dt}X = \frac{d}{dt}Y = 0$. Furthermore, if $Y \ll K_Y$, then $\frac{Y}{K_Y} \approx 0$. Therefore:

$$\begin{aligned} \frac{d}{dt}X = 0 &= k_p\frac{1}{1+\left(\frac{Y_{ss}}{K_Y}\right)^{n_Y}} - k_{dp}X_{ss} \\ &\approx k_p\frac{1}{1+0} - k_{dp}X_{ss} \\ \Rightarrow X_{ss} &= \frac{k_p}{k_{dp}} \\ \frac{d}{dt}Y = 0 &= k_p\frac{1}{1+\left(\frac{X_{ss}}{K_X}\right)^{n_X}} - k_{dp}Y_{ss} \\ \Rightarrow Y_{ss} &= \frac{k_p}{k_{dp}}\frac{1}{1+\left(\frac{k_p}{k_{dp}K_X}\right)^{n_X}} \end{aligned}$$

(d) Let $n_X = 3, n_Y = 2, K_X = 2, K_Y = 3, k_p = 1$, and $k_{dp} = 0.15$, with units adequately defined for each. Numerically determine the steady state(s) of this system. Which of the points is/are stable?

We are interested in the steady states of the system, so we will set $\frac{d}{dt}X = \frac{d}{dt}Y = 0$ and consider the functions that result from this assertion. Plotting both of these expressions on the xy-plane via Desmos, we arrive at the following graph:



Where the red line traces out all the (x, y) pairs such that $\frac{d}{dt}X = 0$, and the blue line traces out all the (x, y) pairs such that $\frac{d}{dt}Y = 0$. The two intersect at one point: $(x, y) = (6.644, 0.177)$. This is thus the only steady state of the system, as it satisfies both $\frac{d}{dt}X = 0$ and $\frac{d}{dt}Y = 0$.

From here, we can calculate the stability of this point by looking at the Jacobian. Let $\vec{x} = \begin{bmatrix} X(t) \\ Y(t) \end{bmatrix}$, so that $\frac{d}{dt}\vec{x} = \begin{bmatrix} f_1(\vec{x}) \\ f_2(\vec{x}) \end{bmatrix} = \begin{bmatrix} k_p \frac{1}{1+\left(\frac{Y}{K_Y}\right)^{n_Y}} - k_{dp}X \\ k_p \frac{1}{1+\left(\frac{X}{K_X}\right)^{n_X}} - k_{dp}Y \end{bmatrix}$.

The Jacobian, then, looks like:

$$\begin{aligned} Df(\vec{x}) &= \begin{bmatrix} \frac{d}{dX} f_1 & \frac{d}{dY} f_1 \\ \frac{d}{dX} f_2 & \frac{d}{dY} f_2 \end{bmatrix} \\ &= \begin{bmatrix} -k_{dp} & -k_p \frac{n_Y \left(\frac{Y}{K_Y}\right)^{n_Y - 1}}{\left(1+\left(\frac{Y}{K_Y}\right)^{n_Y}\right)^2} \\ -k_p \frac{n_X \left(\frac{X}{K_X}\right)^{n_X - 1}}{\left(1+\left(\frac{X}{K_X}\right)^{n_X}\right)^2} & -k_{dp} \end{bmatrix} \end{aligned}$$

Plugging everything in and evaluating at our steady state, we have:

$$Df(\begin{bmatrix} 6.644 \\ 0.177 \end{bmatrix}) = \begin{bmatrix} -0.15 & -0.117 \\ -0.023 & -0.15 \end{bmatrix}$$

To determine stability, we must determine whether or not the eigenvalues of this matrix all strictly negative. To this end, we find that

$$\lambda_{1,2}(Df(\begin{bmatrix} 6.644 \\ 0.177 \end{bmatrix})) = \frac{-0.3 \pm \sqrt{0.010764}}{2}$$

Both of which are strictly negative. And so, our solution is stable.