BE 150 Spring 2018
Homework #8
Due at the start of lecture, May 30, 2018.

Problem 8.1 (Cytokines and homeostasis, 40 pts)**.**
In this problem, we will investigate the control of cell proliferation by cytokines in paradoxical regulation. In doing the analysis, you will learn some useful techniques for analyzing dynamical systems.

Recall the general diagram from lecture, shown in Fig. 1.

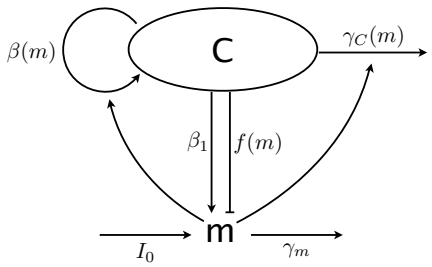

Figure 1: Diagram for a paradoxical regulation of cell proliferation by cytokines. The cytokine, m, has a basal production rate of I_0 from other cells. The cell of interest, C, also secretes cytokines at a rate β_1. The cytokine regulates the proliferation of cells, described by $\beta(m)$. The cells die with rate $\gamma_c(m)$, also regulated by the cytokine. The cytokine degrades with a rate γ_m. Finally, the cell takes in cytokine (thereby clearing it) at a rate $f(m)$.

We can write differential equations to describe the dynamics of the concentration of cells, C, and of cytokine, m.

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \beta_c(m)\,C - \gamma_c(m)\,C, \tag{8.1}$$

$$\frac{\mathrm{d}m}{\mathrm{d}t} = I_0 + \beta_1 C - \alpha_0 C f(m) - \gamma_m(m). \tag{8.2}$$

For simplicity, we will take $\gamma_m(m) \to \gamma_m m$, as we have done thus far this term. We will take

$$f(m) = \frac{(m/k)^n}{1+(m/k)^n}, \tag{8.3}$$

a Hill function. Experimentally, $n \approx 1.7$, and for simplicity, we will take $n = 2$. Also in accordance with experimental measurement, we take $\gamma_c(m)$ to be a linear function of m,

$$\gamma_c(m) \to \gamma_c + \alpha_c m. \tag{8.4}$$

Finally, we also write $\beta_c(m)$ as a Hill function,

$$\beta_c(m) = \beta_0 f(m). \tag{8.5}$$

It has the same form as $f(m)$ since the $\alpha_0 C f(m)$ term in the m-dynamics describes uptake of m by cells, and the $\beta_c(m)$ term in the C-dynamics described cytokine mediated growth, dependent on the uptake rate. Thus, our system of ODEs is

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \beta_0 \frac{C\,(m/k)^2}{1+(m/k)^2} - \gamma_c C - \alpha_c m C, \tag{8.6}$$

$$\frac{\mathrm{d}m}{\mathrm{d}t} = I_0 + \beta_1 C - \alpha_0 \frac{C\,(m/k)^2}{1+(m/k)^2} - \gamma_m m. \tag{8.7}$$

This is the dynamical system we will analyze in the problem.

a) Show that the equations can be nondimensionalized to go from eight parameters to five. Specifically, show that the equations may be written, with appropriate re-definition of variables and parameters, as

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \beta_0 \frac{m^2 C}{1+m^2} - \gamma C - \alpha_c m C \tag{8.8}$$

$$\frac{\mathrm{d}m}{\mathrm{d}t} = I_0 + C - \alpha_0 \frac{m^2 C}{1+m^2} - m. \tag{8.9}$$

Henceforth, we will be working with these dimensionless variables and parameters, e.g., m refers to the redefined dimensionless cytokine concentration.

b) We will now find the nullclines for equation (8.8). These are simply the curves in the C-m plane for which $\mathrm{d}C/\mathrm{d}t = 0$.

   i) Show that there is always a steady state with $C = 0$. Discuss why this nullcline is a vertical line in the C-m plane.

   ii) For arbitrary nonzero C, $\mathrm{d}C/\mathrm{d}t$ vanishes for particular values of m. Show that there are either zero or two such values. Demonstrate that a necessary condition[1] for existence of nonzero values of m for which $\mathrm{d}C/\mathrm{d}t = 0$ is $\beta_0 > \gamma$. (*Hint*: It may help to write the equation for $\mathrm{d}C/\mathrm{d}t = 0$ as a cubic polynomial.) What does this mean physically? Describe why the nullclines for nonzero C are horizontal lines in the C-m plane.

c) Now we will find the nullcline for equation (8.9). The nullcline may be written as $C(m)$, that is, C as a function of m. Derive this function. For what values of m is the function defined (bearing in mind that $m, C \geq 0$)?

[1] As an aside, it can be shown that the necessary and sufficient condition for having two nonzero values of m for which $\mathrm{d}C/\mathrm{d}t = 0$ is $\alpha_c^2\left(\beta_0^2 - 20\beta_0\gamma - 8\gamma^2\right) + 4\gamma(\beta_0-\gamma)^3 - 4\alpha_c^2 > 0$.

d) Now that we have the nullclines, we can compute the fixed points (a.k.a. steady states), which is where these lines cross.

   i) The first fixed point is the one with $C = 0$. What is the value of m at this fixed point?

   ii) For the other (possibly) two fixed points, analytical solutions exist. However, the analytical solution is a mess, and it is easier to consider numerical solutions. The dimensionless parameters used in Hart, et al., *Cell*, **158**, 1022-1032, 2014, which are based on experimental measurements, are: $\beta_0 = 9.78$, $\gamma = 2.14$, $I_0 = 24.67$, $\alpha_c = 1.06$, and $\alpha_0 = 11.21$. Note that these are the **dimensionless** parameters, as defined in equations (8.8) and (8.9). For this parameter set, find the other two fixed points. *Hint*: NumPy's `roots()` function may be useful.

   iii) Plot all of the nullclines for the given set of parameters. Overlay the fixed points as dots.

   iv) In part (b)ii, you already discussed conditions on β_0 and γ that are necessary to get bistability. From your analytical analysis, can you comment on the magnitudes of I_0 and α_0 are are needed to get bistability? What does this mean physically?

e) Plot the separatrix.

f) Finally, overlay the a map of the flow of the dynamical system. Your final plot should look very much like slide 29 of lecture 17.

g) Solve the system of ODEs for the given parameter set starting with an initial condition of C and m just above the separatrix. Do this again with an initial condition just below the separatrix. Plot your temporal profiles of C and m and comment on the results.

h) Play with parameter values and investigate conditions for bistability. You already have much the machinery in place to do this, since you can already computed the fixed points. For bistability, we must have three total fixed points (two in addition to the $C = 0$ fixed point that always exists). You can use this as a criterion to find ranges of parameter values for which bistability exists. With respect to which parameter values is bistability most robust? How about least robust?

Problem 8.2 (Analysis of *cis*-inhibition in the Delta-Notch system, 60 pts, +5 extra credit)**.**
In this problem, we will perform some of the analysis of the Delta-Notch system, including *cis*-interactions, as done in Sprinzak, et al., *Nature*, **465**, 86–90, 2010. This is a great example of how we can use analytical and numerical analysis of a simple circuit to gain insights about its properties.

The dynamics of Delta (D), Notch (N), Nicd (S), and the fluorescent reporter (R) in a given cell are given by the following differential equations.

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \beta_N - \gamma_N N - \frac{ND_{\text{trans}}}{k_t} - \frac{ND}{k_c} \tag{8.10}$$

$$\frac{\mathrm{d}D}{\mathrm{d}t} = \beta_D - \gamma_D D - \frac{N_{\text{trans}}D}{k_t} - \frac{ND}{k_c} \tag{8.11}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{ND_{\text{trans}}}{k_t} - \gamma_S S \tag{8.12}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \beta_R \left(\frac{ND_{\text{trans}}}{\gamma_S k_{RS} k_t}\right)^p - \gamma_R R. \tag{8.13}$$

In these equations, the subscript "trans" is used to denote the total amount of Delta or Notch in contact with the cell of interest, either from neighboring cells or from the plate on which they sit. We have also made approximations about fast dynamics of cleavage of the Notch-Delta complex and subsequent expression of reporter such that the equation for R is decoupled from that of S.

a) We first consider the first experiment in the Sprinzak paper in which the cells were on a plate with a set concentration $D_{\text{trans}} = D_{\text{plate}}$ in the absence of doxycycline. In this case, $N_{\text{trans}} = 0$ and $\beta_D = 0$. Because we are looking at reporter dynamics, we do not need to consider the ODE for S. Solve for the dynamics of the reporter, starting from an initial condition of $R(0) = 0$, $D(0) \equiv D_0 = 200$, and

$$N(0) = \frac{\beta_N}{\gamma_N + D_0/k_c + D_{\text{plate}}/k_t}. \tag{8.14}$$

Use the following parameter values.

Parameter	Value	Units
γ_N	0.08	hours^{-1}
γ_D	0.08	hours^{-1}
γ_R	0.01	hours^{-1}
γ_S	0.1	hours^{-1}
k_t	2	plate concentration units $\cdot$ hours
k_c	0.2	relative fluorescent units $\cdot$ hours
k_{RS}	1500	relative fluorescent units $\cdot$ hours
β_N	1	relative fluorescent units/hour
β_R	1.25×10^8	relative fluorescent units/hour
p	2	dimensionless

Note that we have set $\gamma_N = \gamma_D$, and we will assume this to be the case for this entire problem. Calculate a curve for each value of $D_{\text{plate}} = \{0.063, 0.084, 0.11, 0.15, 0.20, 0.26, 0.35, 0.46, 0.62, 0.82, 1.1, 1.4\}$. Plot all of these curves. Your plot should look a lot like Fig. 3h of the Sprinzak paper.

b) Now consider two cells in contact with each other. They have the same Notch production rate, β_N, but they have different Delta production rates, $\beta_D^{(1)}$ and $\beta_D^{(2)}$. Our goal is to reproduce Fig. 4b of the Sprinzak paper, so we do not need to consider reporter dynamics.

i) Recalling the definition of signal amplification given in lecture, show that the signal amplification is

$$\text{amplification} = \frac{1 - D_2 N_1 / D_1 N_2}{1 - \beta_D^{(2)} / \beta_D^{(1)}}. \tag{8.15}$$

ii) Nondimensionalize equations (8.10) and (8.11), assuming $\gamma_N = \gamma_D$, to get

$$\frac{\mathrm{d}n_1}{\mathrm{d}t} = \tilde{\beta}_N - n_1 - \kappa d_1 n_1 - d_2 n_1 \tag{8.16}$$

$$\frac{\mathrm{d}n_2}{\mathrm{d}t} = \tilde{\beta}_N - n_2 - \kappa d_2 n_2 - d_1 n_2 \tag{8.17}$$

$$\frac{\mathrm{d}d_1}{\mathrm{d}t} = \tilde{\beta}_D^{(1)} - d_1 - \kappa d_1 n_1 - d_1 n_2 \tag{8.18}$$

$$\frac{\mathrm{d}d_2}{\mathrm{d}t} = \tilde{\beta}_D^{(2)} - d_2 - \kappa d_2 n_2 - d_2 n_1. \tag{8.19}$$

iii) Show that a homogeneous steady state $n_1 = n_2 \equiv n_0$ and $d_1 = d_2 \equiv d_0$ only exists when $\beta_D^{(1)} = \beta_D^{(2)}$. Why is this fact pertinent when considering the states of these neighboring cells?

iv) Equations (8.16)-(8.19) have a unique steady state. Why is it important to know this fact? *5 points extra credit*: Prove the existence and uniqueness of the steady state. This is not easy, so only do it if you have time.

v) Show that the steady state is linearly stable. Why is this an important fact? *Hint:* Use the following two facts. 1) A square matrix A has the same eigenvalues as its transpose, A^T. 2) The Gerschgorin Circle Theorem states that for an $n \times n$ matrix A with entries a_{ij}, every (potentially complex) eigenvalue λ satisfies

$$|\lambda - a_{ii}| \leq \sum_{j \neq i} |a_{ij}| \tag{8.20}$$

for at least one i. This gives the regions in the complex plane where the eigenvalues may lie.

vi) Numerically compute the steady state of the system and use it to reproduce Fig. 4b in the Sprinzak paper. Use $\beta_D^{(2)} = 1.35\beta_D^{(1)}$ and the following parameters.

Parameter	Value	Units
γ_N	0.1	hours^{-1}
γ_D	0.1	hours^{-1}
γ_S	1	hours^{-1}
k_t	10	effective plate concentration units $\cdot$ hours
k_c	$\{0.5, 1, 10\}$	relative fluorescent units $\cdot$ hours
β_N	20	relative fluorescent units/hour