BE 159 Winter 2019
Homework #3
Due at the start of class, February 13, 2019

Problem 3.1 (A simple genetic oscillator with coupling)**.**
This problem was inspired by Julian Lewis's 2003 paper entitled "Autoinhibition with Transcriptional Delay: A Simple Mechanism for the Zebrafish Somitogenesis Oscillator." In our discussion of the Soroldoni, et al. paper and in the associated lecture, we did not discuss how the genetic oscillator may work, opting instead to discuss the (very important) Delta-Notch pathway for signaling between neighboring cells. It has been postulated that the oscillations in the zebrafish presomitic mesoderm come from a very simple genetic circuit. In particular, two hairy/E(spl)-related (her) genes, *her1* and *her7*, show oscillations. Interestingly, the protein product of these genes inhibit the expression of the genes themselves. So, a simple genetic circuit arises, in which a *her* gene is autoinhibited. Furthermore, active Notch protein represses expression of Delta in the same cell via the *her* genes. In this problem, we will model the core oscillator made up of the autoinhibitory *her* circuit (show in in black in Fig. 1) and the coupling of oscillators in neighboring cells by Delta-Notch signaling.

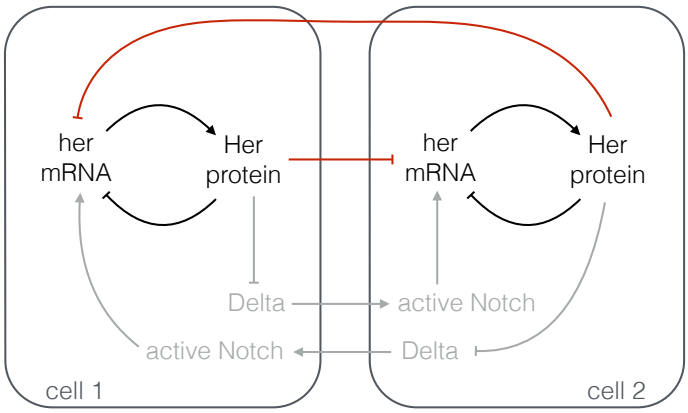

Figure 1: Self-regulation of *her* genes. Her protein represses transcription of her mRNA.

In our analysis, we will neglect the multi-step process of Delta-Notch signaling and the ensuing repression of expression of *her* (depicted in gray in Fig. 1) and instead model it as direct repression of *her* expression in a cell due to Delta in its neighbor (depicted in red in Fig. 1). Most cells in the PSM have many neighbors, all of which contribute to the dynamics, but we will consider only two cells for illustration and for simplicity.

a) As usual, we will describe the dynamics of this circuit with differential equations. Let m_1 be the number of her mRNA molecules in cell 1, and let p_1 be the number of Her protein molecules in cell 1. The variables m_2 and p_2 are similarly defined. Explain in words why the following differential equations

are reasonable choices to model the genetic circuit in Fig. 1.

$$\frac{\mathrm{d}p_1}{\mathrm{d}t} = \beta_p m_1 - \alpha_p p_1, \tag{3.1}$$

$$\frac{\mathrm{d}m_1}{\mathrm{d}t} = \beta_m f(p_1) g(p_2) - \alpha_m m_1, \tag{3.2}$$

$$\frac{\mathrm{d}p_2}{\mathrm{d}t} = \beta_p m_2 - \alpha_p p_2, \tag{3.3}$$

$$\frac{\mathrm{d}m_2}{\mathrm{d}t} = \beta_m f(p_2) g(p_1) - \alpha_m m_2, \tag{3.4}$$

where the Greek parameters are all positive constants and $f(p)$ and $g(p)$ are arbitrary dimensionless decreasing functions.

b) We will first consider a single *her* oscillator alone with no coupling to neighboring cells; i.e., we take $g(p) =$ constant. Prove that this system cannot have oscillations, regardless of what $f(p)$ is. *Hint*: You can use a consequence of the Bendixson-Dulac theorem, which states that the dynamical system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = P(x,y) \tag{3.5}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = Q(x,y) \tag{3.6}$$

has no oscillatory solutions if the quantity

$$\frac{\partial P}{\partial x} + \frac{\partial Q}{\partial y} \tag{3.7}$$

always has the same sign.

c) The repression of expression of *her1* is accomplished by a dimer of Her proteins. Given this, why might the following function be a reasonable choice for $f(p)$?

$$f(p) = \frac{k_c^2}{k_c^2 + p^2}. \tag{3.8}$$

d) As you may have seen if you did the supplementary reading for the Soroldoni paper, delay of regulation by genetic circuits can play a major role in the transmission of signals from one cell to the next. Naturally, they can also play a role in the timing of gene regulation within individual cells. It stands to reason that the amount of mRNA does not immediately affect the rate of production of protein. The mRNA must first be transported out of the nucleus and then be processed for translation. So, we assign a time delay τ_p to this process.

Similarly, the protein cannot immediately regulate expression of the mRNA, as it must enter the nucleus and bind to the appropriate operator. So, we assign a time delay τ_m to this process. Finally, there is a time delay τ_d associated with *her* repression due to Delta-Notch signaling. For simplicity, we will take $\tau_p \approx 0$, thereby only considering time delays in repression. Because it is of interest in analysis of coupling, we will also assume that τ_m for two different cells need not be equal. We therefore have a system of *delayed differential equations*,

$$\frac{\mathrm{d}p_1(t)}{\mathrm{d}t} = \beta_p m_1(t) - \alpha_p p_1(t), \tag{3.9}$$

$$\frac{\mathrm{d}m_1(t)}{\mathrm{d}t} = \beta_m f(p_1(t-\tau_{m,1}))g(p_2(t-\tau_d)) - \alpha_m m_1(t), \tag{3.10}$$

$$\frac{\mathrm{d}p_2(t)}{\mathrm{d}t} = \beta_p m_2(t) - \alpha_p p_2(t), \tag{3.11}$$

$$\frac{\mathrm{d}m_2(t)}{\mathrm{d}t} = \beta_m f(p_2(t-\tau_{m,2}))g(p_1(t-\tau_d)) - \alpha_m m_2, \tag{3.12}$$

where the time dependence on each variable is now explicit. Going forward, we will use

$$g(p) = \frac{k_t}{k_t + p}. \tag{3.13}$$

Nondimensionalize equations (3.9) through (3.12), using equation (3.8) for $f(p)$ and equation (3.13) for $g(p)$, to get

$$\frac{1}{\gamma_p}\frac{\mathrm{d}\tilde{p}_1}{\mathrm{d}\tilde{t}} = \beta\, \tilde{m}_1(\tilde{t}) - \tilde{p}_1(\tilde{t}), \tag{3.14}$$

$$\frac{1}{\gamma_m}\frac{\mathrm{d}\tilde{m}_1}{\mathrm{d}\tilde{t}} = \frac{1}{\left(1 + \left(\tilde{p}_1(\tilde{t}-1)\right)^2\right)\left(1 + \kappa\tilde{p}_2(\tilde{t}-\tau)\right)} - \tilde{m}_1(\tilde{t}), \tag{3.15}$$

$$\frac{1}{\gamma_p}\frac{\mathrm{d}\tilde{p}_2}{\mathrm{d}\tilde{t}} = \beta\, \tilde{m}_2(\tilde{t}) - \tilde{p}_2(\tilde{t}), \tag{3.16}$$

$$\frac{1}{\gamma_m}\frac{\mathrm{d}\tilde{m}_2}{\mathrm{d}\tilde{t}} = \frac{1}{\left(1 + \left(\tilde{p}_2(\tilde{t}-\tau_{12})\right)^2\right)\left(1 + \kappa\tilde{p}_1(\tilde{t}-\tau)\right)} - \tilde{m}_2(\tilde{t}), \tag{3.17}$$

where γ_p, γ_m, β, κ, τ, and τ_{12} are dimensionless constants. Be sure to write expressions for these constants. Give a physical meaning for γ_p and γ_m. Note that we have reduced the number of parameters from nine to six. Henceforth, as you are working through the problem, you can drop the tildes for notational convenience.

e) We have now conveniently nondimensionalized the governing equations, and we return for a moment to analyze the case of a single oscillator ($\kappa = 0$). If γ_p and γ_m are very large, the left hand sides of the dimensionless dynamical equations are close to zero. Thus, if there are two solutions for the steady states of equations (3.14) and (3.15), the system can oscillate between the two steady states. Show that two steady states exist for $\beta > 2$, but not otherwise. *Hint*: When working out the steady states, consider $\tilde{p}(t) = \tilde{p}_b$ and $\tilde{p}(t-1) = \tilde{p}_a$, with similar definitions for $\tilde{m}_a$ and $\tilde{m}_b$.

f) We have demonstrated that with time delay, we can get oscillations from a self-repressing gene. In fact, the time delay is crucial for the oscillation. Now, let's see the oscillations! Numerically solve equations (3.14) and (3.15) for various values of the parameters γ_p, γ_m, and β. For simplicity, assume $\gamma_p = \gamma_m$. For your initial conditions, assume that mRNA and protein are both absent and then *her1* is suddenly available for transcription at time $t = 0$. Plot your results and comment on them. In particular, what must be true of the magnitude of γ_m and γ_p in order to get oscillations, and what does this mean physically? *Hint:* You do not need to do any fancy integration techniques for these DDEs. You can just use simple Euler time stepping. I wrote a Python script to do this, and it appears at the end of this problem statement. You can use it, or use it as a basis for your own code in whatever language you like.

g) We will now investigate how coupling serves to bring the oscillators into synchrony. We will consider $\tau_{12} \neq 1$, which means that the oscillators in the respective cells have inherently different periods, so they should be out of phase without coupling. We will numerically solve equations (3.14) through (3.17) with nonzero κ. For this, we will take $\gamma_p = \gamma_m = 20$, $\beta = 3$, $\kappa = 1$, and $\tau = 1.25$. What do the latter two choices mean physically?

For your initial conditions, assume both cells are completely absent of *her* mRNA and protein and the *her* gene suddenly become transcriptionally active at time $t = 0$. Investigate how coupling brings the oscillators into phase by numerically integrating equations (3.14) through (3.17) for various values of τ_{12}.

```python
import numpy as np

import bokeh.layouts
import bokeh.plotting
import bokeh.io

# Useful functions for integration
def f(p):
    return 1.0 / (1.0 + p**2)

def dp_dt(m, p, gamma_p, beta):
    return gamma_p * (beta * m - p)

def dm_dt(m, p, gamma_m):
    return gamma_m * (f(p) - m)

# Function to perform solution
def solve_her_one_oscillator(
        gamma_p=20.0, gamma_m=20.0, beta=3.0, dt=0.001, t_stop=30.0):

    # Number of indices to go back for time unit (useful for delays)
    i_time = int(1 / dt)

    # Time points (start -1 time unit so we can handle delays)
    t = np.linspace(-1, t_stop, int((1 + t_stop) / dt))

    # Initialize output arrays
    m = np.zeros_like(t)
    p = np.zeros_like(t)

    # Do Euler stepping
    for i in range(i_time, len(t)-1):
        m[i+1] = m[i] + dt * dm_dt(m[i], p[i - i_time], gamma_m)
        p[i+1] = p[i] + dt * dp_dt(m[i], p[i], gamma_p, beta)

    return t, m, p


if __name__ == '__main__':
    # Run the calculations for small and large gamma
    t, m_small, p_small = solve_her_one_oscillator(gamma_p=2.0,
                                                   gamma_m=2.0,
                                                   t_stop=25.0)
    t, m_large, p_large = solve_her_one_oscillator(gamma_p=20.0,
                                                   gamma_m=20.0,
                                                   t_stop=25.0)

    # Set up plots
    p1, p2 = [bokeh.plotting.figure(plot_height=200,
                                    plot_width=450,
                                    x_axis_label='dimensionless time',
```

```
                                y_axis_label='m, p',
                                x_range=[t.min(), t.max()])
                for _ in [1, 2]]
    p1.x_range = p2.x_range
    p1.y_range = p2.y_range
    p1.title.text = 'Single oscillator, γp = 2, γm = 2, β = 3'
    p2.title.text = 'Single oscillator, γp = 20, γm = 20, β = 3'

    # Add glyphs
    p1.line(t, m_small, line_width=2, line_color='dodgerblue',
            legend='m')
    p1.line(t, p_small, line_width=2, line_color='tomato',
            legend='p')
    p2.line(t, m_large, line_width=2, line_color='dodgerblue')
    p2.line(t, p_large, line_width=2, line_color='tomato')

    # Display
    bokeh.plotting.output_file('single_oscillator.html')
    bokeh.io.show(bokeh.layouts.gridplot([p1, p2], ncols=2))
```

her_circuit_single_oscillator.py