## Bioen 485/585 Lab 3 2015: Nonlinear Analysis

In the paper you read for Tuesday, "Construction of a genetic toggle switch in Escherichia coli" by Gardner, et al, the following model was proposed for the genetic toggle switch.

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$

Where u and v are the concentrations of two proteins that repress each others' expression. Today you will reproduce many of their theoretical and computational results.

1. Stability Analysis
    a. **(5 points)** Solve for the nullclines and equilibrium points and plot them on a phase plate plot. For this, assume that the cooperativity is 2 for each gene ($\beta = \gamma = 2$), and that production rates are $\alpha_1$ = 3.5, $\alpha_2$= 4, *(Hints: label your axis and then make sure you are plotting u and v on the proper axes for all the different things you are adding to this plot! You can use analytic manipulations to get an equation that sets a polynomial expression for either u or v to zero. You can always solve this numerically. Don't include non-physiological values of u and v.)*
    b. **(5 points)** Determine if this is a bistable switch using stability analysis and interpreting your results. (*Hints: how do you determine stability of an equilibrium point in a nonlinear system? A bistable switch is like a light switch – depending on where you move the switch, it will go to one of two positions and stay there. Maybe you can get it balanced exactly in the middle too. Remember to show your work.)*
2. Phase plane analysis.
    a. (**10 points**) Illustrate the bistability by plotting trajectories on a phase plane plot and interpreting the results. (*Hints: you can graph the trajectories of the system starting with an array of initial conditions, coloring each trajectory differently depending on which equilibrium point it finds. You can use embedded for-loops in u and v. Be sure that you are running the trajectories long enough to reach steady state. To identify which equilibrium point automatically, you can use an if-command on the final value of each trajectory. Now, do you see the separatrix and the basins of attraction, and how does this relate to bistability?)*
    b. (**Extra Credit 2 points**) Plot the eigenvectors on this or a similar plot, and interpret them. (*Hints: For each equilibrium point, plot each eigenvalue/eigenvector combination by making a line centered at the equilibrium point with the length of the eigenvalue and direction of the eigenvector, and color it black or red depending on the sign of the eigenvalue. This will require plotting lines through each equilibrium point with the slope of the corresponding eigenvectors. If you don't understand what these tell you, you might also try to plot a few trajectories.*
3. Bifurcation Analysis. (**10 points**) Reproduce the bifurcation analysis shown in Gardner figure 2, panels a, b, and c, with $\beta = \gamma = 2$, and interpret your analysis. Is this a fold, transcritical, pitchfork of hopf bifurcation, or none of these? *Hints: You have already plotted something similar to panels a and b in problem 1, so just figure out what to change. You should*

*be able to plot the lines in panel c for $\beta = \gamma = 2$. They never derive their equation, or explain how they obtained it. Indeed, since they don't include labels in their axes, they don't give the equation, just the slopes of the lines. Unless you want to derive this analytically, you should verify it numerically, by choosing points $(\alpha_1, \alpha_2)$ suspected to be on both sides of each of the lines, determine whether each point makes a monostable or bistable switch, and if monostable, which type, and plotting the points on the graph with a color coding to indicate this behavior. You want to do this as automatically and as efficiently as possible, so chose the fastest tools from the previous steps. You should not have to run any simulations or plot any trajectories for this part, but unless you are more clever than I, you will have to do some numeric calculations.)*

4. Model Building. For the next problem, we will work with the following information and assumptions.
    - Let $x_1$ and $x_2$ be the concentration of monomeric proteins 1 and 2 respectively.
    - Both monomeric proteins are degraded with the same rate constant $k_d$ (1/sec).
    - The transcription and translation of proteins 1 and 2 are grouped into a single rate constant for production $k_1$ and $k_2$ (Molar/sec) respectively, times the fraction of active promoter, which we call $A_1$ and $A_2$ respectively. Thus, proteins 1 and 2 are made at a rate $k_1 A_1$ and $k_2 A_2$ respectively.
    - Both proteins form multimeric complexes of $N_1$ and $N_2$ monomers, but their affinity for each other is sufficiently low that most of x1 and x2 are in monomeric form, and we do not consider loss of monomers to multimers when we write the equations for $dx_1/dt$ and $dx_2/dt$.
    - These complexes are called repressors (because they repress transcription), and the concentrations of $r_1$ and $r_2$ are assumed to be
    $$r_1 = \frac{(x_1)^{N_1}}{(K_{D1})^{N_1-1}}, r_2 = \frac{(x_2)^{N_2}}{(K_{D2})^{N_2-1}}$$
    where $K_{D1}$ and $K_{D2}$ are the dissociation constants for multimerization (in Molar). (Thus $r_1 = x_1$ if $N_1 = 1$, and $r_1 = x_1^2/K_{D1}$ if $N_1 = 2$.)
    - The fraction of active promoters are assumed to be
    $$A_1 = \frac{K_{P2}}{r_2 + K_{P2}}, A_2 = \frac{K_{P1}}{r_1 + K_{P1}}$$
    where $K_{P1}$ and $K_{P2}$ (Molar) are the dissociation constants for the repressors $r_1$ and $r_2$ to bind to the promoter for proteins 2 and 1, respectively. That is, each protein forms a repressor that blocks the synthesis of the other.

    Given all of these constraints, we can write the differential equations for $dx_1/dt$ and $dx_2/dt$ in terms of 9 parameters: $k_d, k_1, k_2, K_{D1}, K_{D2}, K_{P1}, K_{P2}, N_1, N_2$. We know that the rate of change is the rate of production minus the rate of degradation. $r_1, r_2, A_1 and\ A_2$ are intermediate variables that need to be removed, which will leave $x_1, x_2$ as variables. We begin with

$$\frac{dx_1}{dt} = k_1 A_1 - k_d x_1$$

We can then substitute the expressions for r and A to obtain the following:

$$\frac{dx_1}{dt} = \frac{K_{P2}}{\left(\frac{x_2^{N2}}{K_{D2}^{N2-1}}\right) + K_{P2}} \cdot \; k_1 - k_d x_1$$

We then simplify the fractional term preceding k1. The resulting expression represents the efficiency of the translation process represented by k1 and is bounded in [0,1]. This term is called a Hill function in biochemical modeling.

$$\frac{dx_1}{dt} = \frac{1}{\left(\frac{1}{K_{D2}^{N2-1}K_{P2}}\right) x_2^{N2} + 1} \cdot \; k_1 - k_d x_1$$

By symmetry, we have

$$\frac{dx_2}{dt} = \frac{1}{\left(\frac{1}{K_{D1}^{N1-1}K_{P1}}\right) x_1^{N1} + 1} \cdot \; k_2 - k_d x_2$$

a. **(10 points)** Nondimensionalize this model to produce the differential equations provided at the top of the lab. (*Hint: this means you must define u and v in terms of x1 and x2, and define the nondimensional parameters* $\alpha_1, \alpha_2, \beta, \gamma$ *in terms of the original 9 parameters.*