Homework #5
Distributed: Weds. April 29th, 2020
Due: Thurs. May 7th, 2020 by class start

Problem 1: Mitogenic response to EGF binding. (ref.: Knauer, D. J., H. S. Wiley, and Dennis D. Cunningham. Journal of Biological Chemistry 259.9 (1984): 5623-5631)

a) Add a recycling step from the endosomes back to the surface in the scenario that we treated in class. Allow both active (ligand bound) and inactive receptors to be recycled with the same rate constant, k_{rec}. Write out the species balances for the active and inactive receptors at the surface and in the endosomes and find an expression for the total concentration of active receptor as well as the maximum total concentration of active receptor. What impact does this additional process have on the maximum concentration of active receptors? Interpret these results.

Problem 2: The biological oscillator. The oscillator is dynamical element that is ubiquitous in cell biology. One such system is described by the following system of equations, written in dimensionless form:

$$\frac{dc_a}{dt} = -d_a c_a + \frac{r_{oa} + r_a c_a^2}{1 + c_a^2 + c_r^2}$$

$$\frac{dc_r}{dt} = -c_r + \frac{r_{or} + r_r c_a^2}{1 + c_a^2}$$

where c_a and c_r are the concentration of species A and R respectively.

a) State whether A is an activator of, inhibitor of, or has no effect on production of A and/or R. State whether R is an activator of, inhibitor of, or has no effect on production of A and/or R. What is the significance of the parameter d_a (i.e. what type of process does it describe the kinetics of?)? What are the basal rates and maximal rates of A and R production?

b) Find the fixed point(s) of the system by plotting the nullclines for the parameters above. Is the fixed point(s) stable or unstable?

c) Construct a phase portrait of the system in the phase space from $c_a = 0$ to $c_a = 200$ and $c_r = 0$ to $c_r = 100$ for the following parameters:
$r_{or} = 1$
$r_r = 100$
$r_{oa} = 100$
$r_a = 5000$
$d_a = 30$

Include the nullclines on your phase portrait and a representative solution for initial concentrations $c_{ao} = 1$ and $c_{ro} = 10$.

d) Describe how the oscillator works using geometric reasoning (i.e. based on visual analysis of the phase portrait).

e) Plot the solution of the ODE system in the time domain for initial concentrations $c_{ao} = 1$ and $c_{ro} = 10$.

Problem 3: Stability analysis of Collins toggle switch. (Gardner, T. S., C. R. Cantor, and J. J. Collins, 2000, Nature **403**, 520.)

The behavior of the toggle switch and the conditions of bistability can be understood using the following dimensionless model for the network:

$$\frac{du}{dt} = \frac{\alpha}{1+v^n} - u = f(u,v) \qquad (1)$$

$$\frac{dv}{dt} = \frac{\alpha}{1+u^n} - v = g(u,v) \qquad (2)$$

a) Identify each variable or parameter in the model as one of the following:

i. Concentration of a repressor of gene expression (there are two repressors)
ii. Effective rate of synthesis of repressor; lumped parameter that describes the net effect of RNA polymerase binding, open-complex formation, transcript elongation, transcript termination, repressor binding, ribosome binding and polypeptide elongation
iii. Cooperativity of repression
iv. Degradation rate constant for repressor

b) Plot the nullclines (lines for which f(u,v) = 0 or g(u,v) = 0) for the system for $\alpha = 10$ and $n = 1$ **AND** 2. How many steady-state solutions exist for each case? Comment on the influence of the degree of cooperativity.

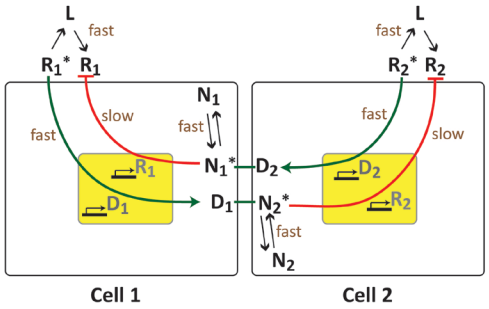
c) To gain intuition for this system, generate a phase portrait for the system and include the nullclines. On your graph, identify the steady states and assess the character of each steady state (stable or unstable?). What is the influence of the degree of cooperativity?

d) Build the Jacobian for the system (for arbitrary α and n) at its steady states and write down the stability criterion. (You can leave your expression in terms of u_s and v_s, the concentrations of u and v at steady-state.) Use the stability criterion to explain the influence of the degree of cooperativity and the rate of synthesis on the stability of the center steady state (i.e., the one with $u = v$).

e) Find the numerical eigenvalues for the center steady state for $\alpha = 10$ and $n = 1$ and 2. How does the change in cooperativity affect the system?

e – bonus challenge) Calculate the value of α ($\alpha_{critical}$) where the system transitions from having one stable point to having two unstable points.

f) Now we will use what we have learned about stability of coupled inhibition in the Collins toggle switch to consider patterning via intercellular signaling under the control of a growth factor, *L*. The particular case we consider models so called "juxtacrine" signaling in which signals are only communicated between adjacent cells via membrane-bound pairs of receptors. A common example of this mechanism is mediated by Notch (*N*) and Delta (*D*) (see Alberts, pp. 893-895). We consider a case inspired by vascular patterning in which it is thought that the activated receptor (*R*) to Vascular Endothelial Growth Factor (VEGF = *L*) up-regulates the expression of Delta when activated by binding VEGF (activated receptor = $R^* = R\text{-}L$). In turn, the binding of Delta to Notch in a neighboring cell down-regulates expression of the receptor. If this coupled set of reactions becomes unstable, the VEGF-R rises in one cell and drops in the neighboring cell; the cell with high VEGF-R then becomes a "tip cell" that attempts to form a vessel sprout.[1] This scenario is depicted in the diagram for a two-cell model of the patterning process.



$$\frac{dR_i^*}{dt} = k_f L R_i - k_r R_i^* \quad (3)$$

$$\frac{dN_i^*}{dt} = k_f^{ND} N_i D_j - k_r^{ND} N_i^* \quad (4)$$

$$\frac{dD_i}{dt} = k_D R_i^* - \gamma_D D_i \quad (5)$$

$$\frac{dR_i}{dt} = \frac{\beta^n}{K^n + N_i^{*n}} - \gamma_R R_i \quad (6)$$

The species balances (3)-(6) are the same for both cells (e.g., $i = 1$ and $j = 2$, or visa versa). Notch is assumed to be present in excess such that N_i = const.

f-1) Assume fast equilibrium for the processes in (3)-(5) to find the "toggle switch" of coupled inhibition between the two cells. You should obtain two equations of the form:

$$\frac{dR_1}{dt} = f(R_1, R_2)$$

$$\frac{dR_2}{dt} = g(R_1, R_2)$$

f-2) Non-dimensionalize your equations in f-1 to find the form above for the Collins toggle switch (eqs 1, 2). Non-dimensionalize by subbing in the following non-dimensional variables, $u=R_1/K$, $v = R_2/K$, $\tau=\gamma_R t$. Comment on the influence that the concentration of ligand has on the stability of the uniform state. What else could you manipulate to drive the system toward the instability?

[1] Jakobsson, Lars, et al. "Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting." Nature cell biology 12.10 (2010): 943-953.