Problem Set #1
361/562, Spring 2024
DUE: Friday, February 2, 2024, by midnight

You may consult with your classmates and TAs, but all scripts and answers must be your own. We will look at the scripts and answers closely 🕵️.

Hand in you exported livescript file (as a pdf). See the skeleton code, which is also available.

A Protein Phosphorylation Switch regulated by Phosphatases
[15 points for 361 (undergraduates), 18 pts for 562 (grad students)]

Phosphorylation can lead to switching of an enzyme from OFF to ON states if the enzyme is a kinase that phosphorylates itself in a positive feedback loop. Interestingly, this system can be turned on by changing the level of dephosphorylation (by phosphatases): if phosphatase activity is low, the system is ON and if phosphatase activity is high, the system is OFF. One signaling mechanism where this happens is through protein tyrosine phosphatases (Tonks 2013, Protein tyrosine phosphatases – from housekeeping enzymes to master regulators of signal transduction. FEBS J 280, 346–378). It is not necessary to read this paper, though.

Auto-phosphorylating proteins are discussed in the notes to Class 2 (read over them). In this problem, x is a kinase, which is a protein that can phosphorylate proteins, including itself. There is also a phosphatase, which can take the phosphate groups off. The fraction of molecules that are phosphorylated, x, is governed by the following equation:

$$\frac{dx}{dt} = \dot{x} = f(x) = K\left(\frac{x^n}{x_{\mathrm{M}}^n + x^n} + 0.1\right)(1 - x) - Px \qquad \text{(Equation 1)}$$

(i) $K = 1$ is the maximum kinase activity. Note that only non-phosphorylated protein can be phosphorylated (hence the $1 - x$ term).
(ii) $n = 6$ is the cooperativity.
(iii) $x_M = 0.2$ the value of x where phosphorylation is 50% max.
(iv) 0.1 is the constitutive or basal phosphorylation activity.
(v) $P = 2$ is the phosphatase activity.

Note also that because x is the fraction of proteins phosphorylated, it is nonsensical to have $x > 1$.

In this problem, you will use Matlab to make a plot of the fixed points of the system as the decay rate, P, varies. You will make this plot in two ways, as outlined below. Skeleton code is available - use it.

(a)

(i) [2 pts] Plot the phosphorylation and dephosphorylation rates as functions for $x \in [0,1]$ and for $P = 2$.

(ii) [2 pts] Plot the phosphorylation and dephosphorylation rates as functions for $x \in [0,1]$ and for P with several values between 0.5 and 5 (increment 0.5).

(iii) [1 pt] What are the values of P for which the number of fixed points changes?

(b) Note that in this system it is difficult to solve for the fixed points (x^*) for which $\dot{x}(x^*) = 0$ as a function of P because there are multiple fixed points for single values of P so x^* is not a "function" of P. However, it's actually quite simple to solve for P as a function of the fixed-point value, x^*.

(i) [1 pt] Solve for $P(x^*)$ by hand in the functional form by rearranging the equation. This will be the basis for one of the lines of code.

(ii) [2 pts] Plot x^* on the *y*-axis against P on the *x*-axis, with the P-axis ranging from 0.5 to 5.

(c) Suppose one starts with $P = 0.5$, and then slowly increases P to 5, on a timescale much longer than the equilibration time of the system, before returning P slowly to 0.5. That is, we are thinking of P being a function of time, $P(T)$ where T is changing very slowly.

(i) [1 pt] Please add arrows to the plot in (bii) to show the approximate trajectory of the fixed point $x^*(T)$, which changes slowly as P changes. Arrows are a pain in MATLAB.

(ii) [1 pt] What are the approximate critical values of P at which the system undergoes fast transitions?

(iii) [1 pt] Describe in words how this plot shows hysteresis for this system.

(d) (i) [2pts] Fixed points for this system can be found numerically as well as analytically. Stable fixed points can be found by allowing the system to evolve forward in time. Set up an array of starting points (*x*), about 20 of them between 0 and 1, then let the system evolve for a sufficient amount of time (i.e., a long time! – you will have to experiment to find out how long). Do this for a specific value of $P \in [0.5,5]$. Now plot all the end points of the trajectories on the *y*-axis, against P on the x-axis. Repeat for many values of $P \in [0.5,5]$ (increments of 0.1). Use ODE45.

(ii) [1pt] To find unstable fixed points, you can try an alternate method, in which you evolve the system backwards in time: from initial conditions, use $-\dot{x}$ to evolve the system to a steady state, which will be your unstable fixed point. *Important*: there are not always unstable fixed points to converge on, and the system will diverge if there isn't one; you will want to choose this reverse integration only in certain conditions (i.e. certain values of P and certain values of initial conditions, *x*), based on values you find for the stable fixed points. Now, color code your plot for stable (green) vs. unstable (red) fixed points. They should lie directly on the analytical solution you found in (b).

(iii) [1 pt] Why are some points not exactly on the line?

Your final plot for this question should be the analytical solution from (c) with a set of green (stable) and red (unstable) points plotted on top of it, along with the answers to the questions.

(e) [3 pts, *required for students enrolled in 562 (graduate students)]*: An alternate method to plot fixed points as a function of P would be to find where $\dot{x} = f(x, P) = 0$. To plot this, use the command `meshgrid` to make arrays of values of *x* and P. Then compute the value $\dot{x}$ as a function of those two arrays and plot the surface (using the command `surf)`. You can then use the function `contour` to plot the line where that function is 0. Please hand in this plot of the fixed points for $0 \leq x \leq 1$, and $0.5 \leq P \leq 5$.