Final

Problem Set 4
MB&B/MCDB 361/562
Spring 2024
DUE: Monday, April 5, 2024, by midnight

You may consult with the TAs, but all scripts and answers must be your own. We will inspect the scripts and answers closely. Please do this on your own!! Hand in a pdf file with the written answers along with the MATLAB code.

You are going to explore how the number of molecules in an oscillating circuit affects the quality of the oscillations.

This is a classic paper on switching and oscillations in biochemical networks.

> *Tyson, J., Chen, K., Novak, B.* (2003). **Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell.** Current opinion in cell biology 15(2), 221 - 231.

We are going to choose oscillator 2c from Figure 2: the substrate depletion oscillator.

Question 1. (10 points total) Using the equations and parameters from Box 1, solve the dynamical equations for the hidden variable X and response R. Note that the function $G(R)$ is an interesting alternative to the Hill equation to get steep dependence on R; but for all intents and purposes it simply acts like a Hill equation to produce "ultra-sensitivity".

(i) (4 points) Choose a signal concentration (S) in the middle of the range in Figure 2c (right), solve with ODE45, and plot $R(t)$ for 0 to 200 s. See the skeletal code in: PS4_skeleton.mlx.
(ii) (4 points) Choose two other values of S: one outside the range on the low side and one on the high side. Solve the equations and plot $R(t)$.
(iii) (2 points) In a sentence summarize your findings.

Question 2. (20 points total) Use the Gillespie algorithm to solve the stochastic equation with $S = 0.25$ μM with different numbers of molecules. This is going to be done by changing the cell volume (`p.volume`) from 100 μm^3 (a small blood cell), 10 μm^3 (small yeast) and 1 μm^3 (*E. coli*), 0.1 μm^3 (small bacterium), 0.01 μm^3 (micoplasma, the small known genus of bacteria).

A few preliminaries
(i) (1 points) Calculate how many molecules there are per μm^3 at 1 μM concentration using the Avogrado constant N =6.022 x 10^{23} molecules/mole. Hint: Note that 1 μm^3 = 10^{-15} liter and μM = 10^{-6} moles/liter.

(ii) (4 points) Propensities and stoichiometries.

There are **three** independent reactions: X → X+1, X → R, R → R-1

(**Important point that was left out the lectures**: In the differential equations below the highlighted terms correspond to the same reaction: it is the same arrow in the diagram.

$$\dot{X} = k_1 S - (k'_0 + k_0 E_P(R))X$$

$$\dot{R} = (k'_0 + k_0 E_P(R))X - k_2 R$$

Thus, the sub-reactions X→X-1 (top line) and R→R+1 (bottom line) and really one reaction X → R. This is important because the subtraction of X and the addition of Y are coupled (you can't have one without the other). Also, we only want to count it once when the calculate the probabilities of the reactions occurring).

Calculate the propensities of the three reactions:

	X → X+1	X → R	R → R-1
Propensity	...	...	...

Write it as a 1x3 (row x column) matrix (which you will need in the code).

Calculate the stoichiometries:

Stoichiometry			
X	...	...	...
R	...	...	...

Write it as a 2x3 (row x column) matrix (which you will need in the code)

Note: In the code, S, X and R will be in units of numbers (and not μM). The conversion will be done in the code (not here).

(iii) (12 points) Use the Gillespie algorithm (`gillespie.m`) to solve a discrete version of the equations with the default value of $S = 0.25$ μM and the other parameters from the Tyson and Novak paper. For the stochastic simulation, S needs to be converted to number by multiplying by the parameter `p.uM2num`. Use the skeleton code (`PS4_skeleton.mls`). Start with a large volume (100 μm^3). Then lower the volume to 10 μm^3, 1 μm^3, 0.1 μm^3 and then 0.01 μm^3.

The program `GillespieGeneral.mlx` is a general guide to using the Gillespie algorithm. It solves the transcription-translation problem but shows you how to set up the variables, the propensities and the stoichiometries.

For each volume, superimpose on the same graph:
(a) the ODE solution (in μM)
(b) three individual replicates of the stochastic output response (R) in μM (you have to divide by `p.uM2num` to convert numbers to μM).
(c) the average of all the replicates (between 3 and 100 depending on the total number of molecules because fewer replicates are need for high numbers and the higher the number the long the computational time).

(iv) (3 points) How do the oscillations change qualitatively as the number of molecules changes (amplitude, accuracy of the frequency, variation from replicate to replicate)? Is there a minimum number to get oscillations or synchronization? No more than 300 words in the `mlx` file. Hand in the `pdf`.

(v) BONUS (5 points) For graduate students, bonus for undergraduates. What happens when you are near the critical signal (i.e., in the oscillation region of signals but near the critical value). Explore using simulations and describe your findings.