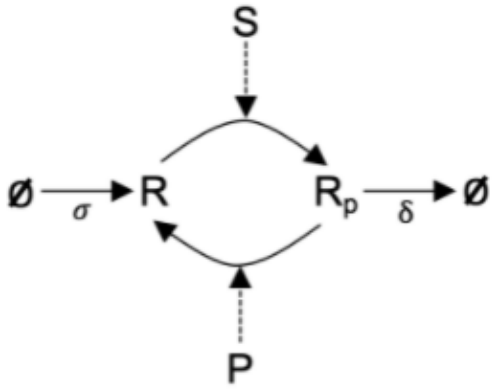
CELLULAR AND SYSTEMS MODELING - EXAM

Student: Shu-Ting Cho (shc167@pitt.edu)

1. *Sniffer.* (25 points) Consider the diagram of a simple molecular circuit shown below where R represents a receptor, R_p its phosphorylated form, and S and P represent phosphorylation and dephosphorylation signals respectively.



The rate constants σ and δ can be taken to be 10 molecules/s and 1/s respectively, and the phosphatase rate constant P can be taken to be 1/s. You can also assume that there is always a basal kinase (phosphorylation) activity such that S is always greater than 0.01/s.

(a) (5 points) Write the differential equations for the time evolution of R and R_p following the conventions from class and assuming that each process depicted is either zeroth or first order.

$$\frac{dR}{dt} = \sigma + P[R_p] - S[R] = 10 + R_p - SR$$

$$\frac{dR_p}{dt} = S[R] - \delta[R_p] - P[R_p] = SR - 2R_p$$

(b) (5 points) Use the fact that $R_t = R + R_p$ to find a differential equation for R_t and use this equation to find the values of R_p and R_t at steady state.

$$\frac{dR_t}{dt} = \frac{dR}{dt} + \frac{dR_p}{dt} = 10 + R_p - SR + SR - 2R_p = 10 - R_p$$

At steady state, let the derivatives to be 0.

$$\frac{dR_t}{dt} = 0 \Rightarrow R_p = 10$$

$$\frac{dR_p}{dt} = SR - 2(10) = 0 \Rightarrow R = \frac{20}{S}$$

$$R_t = R + R_p \Rightarrow R_t = \frac{20}{S} + 10$$

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(c) (5 points) Considering this system as a signal-response network with S as the input and R_p as the output, what is notable about the steady state?

At steady state, R_p is not affected by S, and will always be 10.

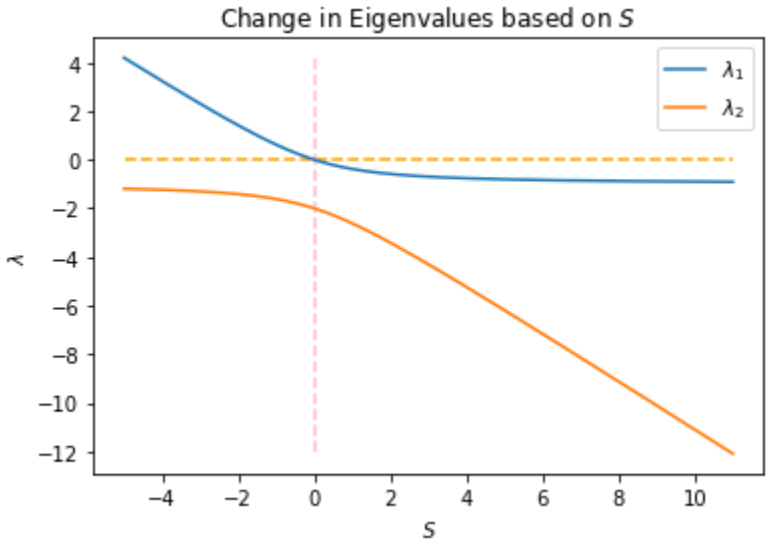
(d) (5 points) Find the Jacobian for this system and use it to determine the stability of the steady state over the full range of S.

$$JM = \begin{bmatrix} -S & 1 \\ S & -2 \end{bmatrix}$$

$$\Rightarrow (-S-\lambda)(-2-\lambda)-S$$

$$= \lambda^2 + (S+2)\lambda + S = 0$$

$$\Rightarrow \lambda = \frac{-S-2\pm\sqrt{S^2+4}}{2}$$



$S \leq 0 \Rightarrow Mixed\ +\ \&\ -\ Real \Rightarrow Unstable\ steady\ state$

$S > 0 \Rightarrow Both\ Real\ \&\ - \Rightarrow Stable\ steady\ state$

Also, in this question, we will only consider S ≥ 0.01/s. Thus, for S in this range, the steady state will always be stable.

(e) (5 points) Plot the nullclines and fixed point in the $R_p(x)$-$R_t(y)$ plane for S=1 and also plot two trajectories, one starting at the steady state for S=0.1 and one starting from the steady state for S=10. Based on these trajectories and your phaseplane analysis, what can you conclude in general about the response of this system to a change in S?

Fixed point: $(R_p, R_t) = (10, \frac{20}{S} + 10)$.

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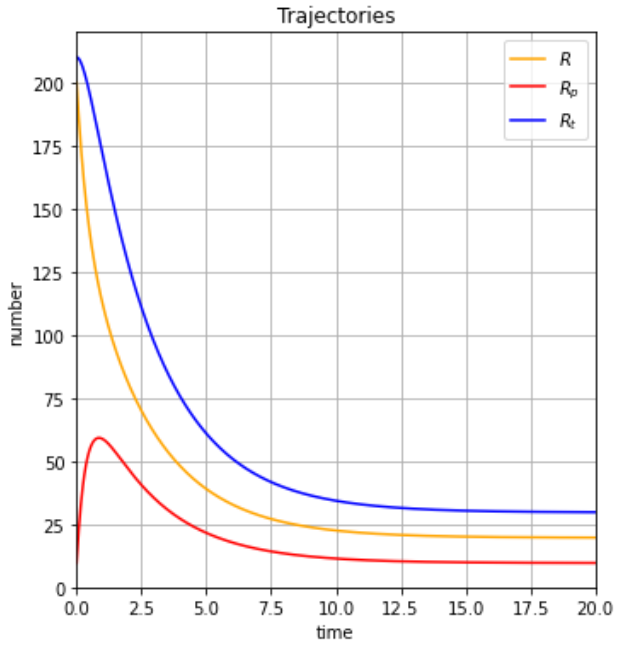
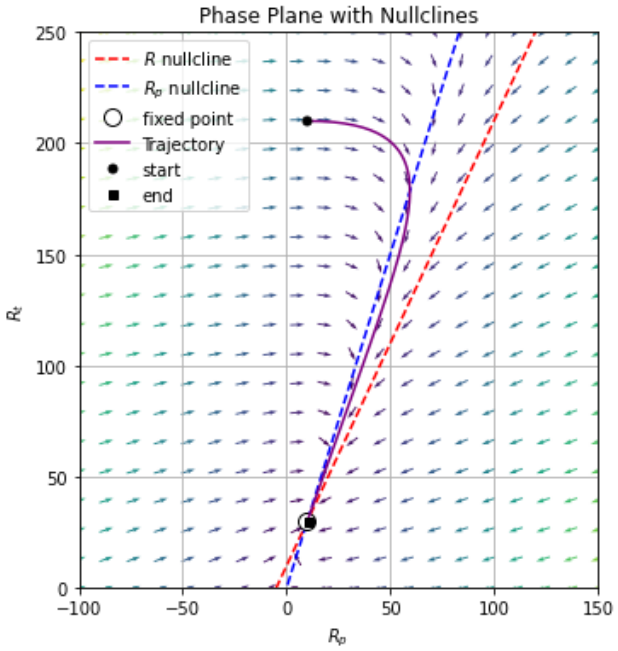
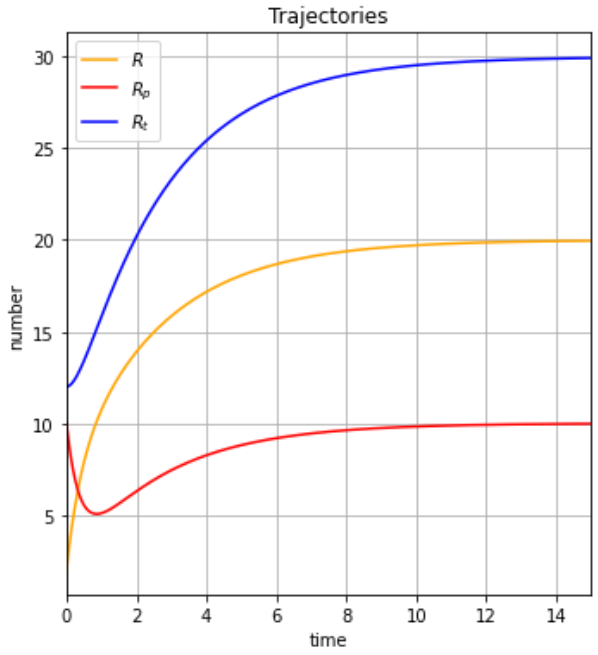
For S=1, $fp(R_p, R_t) = (10, 30)$

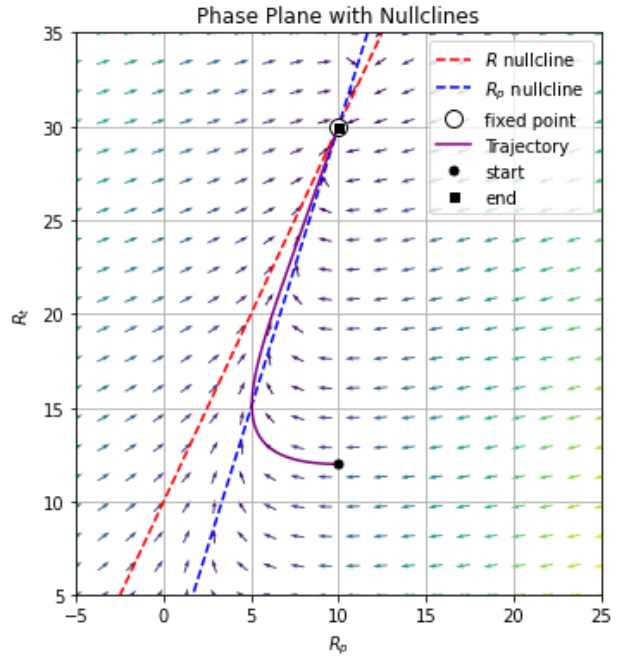
Trajectory starting at the steady state for S=0.1, (R_p, R_t)=(10, 210)





Trajectory starting at the steady state for S=10, (R_p, R_t)=(10, 12)





Based on the analysis, we can see that the fixed point for S=0.1, S=1, or S=10 all have the same R_p value (R_p=10) but different R_t values (R = 20/S). Thus, the higher S is (stronger signal), the more R needed to reach its steady state, but the response (R_p) will remain the same.

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2. *Signaling on a Scaffold.* (25 points) Mitogen-activated protein kinases (MAPKs) couple a diverse array of signals including growth factors, nutrients, osmotic shock and inflammatory factors to a diverse set of cellular responses including gene expression, growth, proliferation, and even cell death. A key step in the signal transduction involve the phosphorylation of a MAP kinase (MAPK) by a MAP kinase kinase (MAPKK). This process often takes place when both the MAPKK enzyme and its MAPK substrate are bound to a third molecule called a scaffold. In this problem we will characterize the strength of signal transduction by computing the amount of scaffold that is bound to both MAPK and MAPKK. To simplify our calculations we will make several assumptions that hold throughout this problem:

1. The scaffold has two binding sites, one of which binds MAPK (K1) exclusively and one of which binds MAPKK (K2) exclusively.
2. The dissociation constants for K1 and K2 to their respective sites on the scaffold have the same value, $K_d=10$ Molecules.
3. Both K1 and K2 are expressed at a level of 1000 Molecules per cell.
4. The signal strength is proportional to the total number of scaffold-K1-K2 complexes present at steady-state.

(a) (5 points) Use Equilibrium Law of Mass Action and mass conservation to determine the fraction of each scaffold site bound to a kinase molecule as a function of the number of scaffold molecules, S.

Assume S = the free scaffold molecules without binding to any kinase molecule:

$$Let\ [K1] = [K2] = [K]$$

$$\frac{[S][K]}{[SK]} = 10 \Rightarrow [SK] = \frac{[S][K]}{10}$$

$$\frac{[SK][K]}{[SKK]} = 10 \Rightarrow [SKK] = \frac{[SK][K]}{10} = \frac{[S][K]^2}{100}$$

$$K_{total} = [K] + [SK] + [SKK] = [K] + \frac{[S][K]}{10} + \frac{[S][K]^2}{100} = 1000$$

$$[K] = \frac{-10S-100+\sqrt{(10S+100)^2+400000S}}{2S}$$

Let F = fraction of scaffold bound with K1 = fraction of S bound with K2, 0 ≤ F ≤ 1

$$F = \frac{[SK1]+[SK1K2]}{[S]+[SK1]+[SK2]+[SK1K2]} = \frac{\frac{[S][K]}{10}+\frac{[S][K]^2}{100}}{[S]+2\times\frac{[S][K]}{10}+\frac{[S][K]^2}{100}} = \frac{\frac{[K]}{10}+\frac{[K]^2}{100}}{1+2\times\frac{[K]}{10}+\frac{[K]^2}{100}}$$

$$= \frac{\frac{\frac{-10S-100+\sqrt{(10S+100)^2+400000S}}{2S}}{10}+\frac{\left(\frac{-10S-100+\sqrt{(10S+100)^2+400000S}}{2S}\right)^2}{100}}{1+2\times\frac{\frac{-10S-100+\sqrt{(10S+100)^2+400000S}}{2S}}{10}+\frac{\left(\frac{-10S-100+\sqrt{(10S+100)^2+400000S}}{2S}\right)^2}{100}}$$

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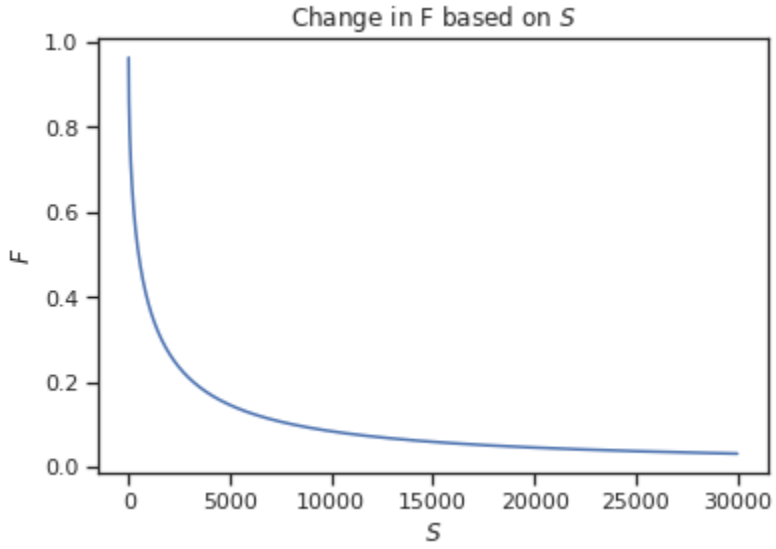
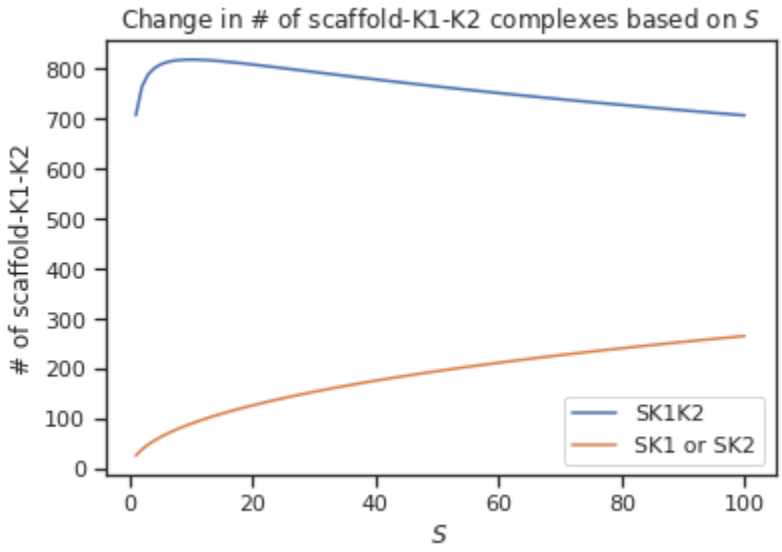


(b) (10 points) Assuming that there is no cooperativity in binding, calculate and plot of the *number* of scaffold-K1-K2 complexes as a function of S. Be sure to plot S over a wide enough range that the full range of behavior of the curve is clear. Explain the essential features of your plot.



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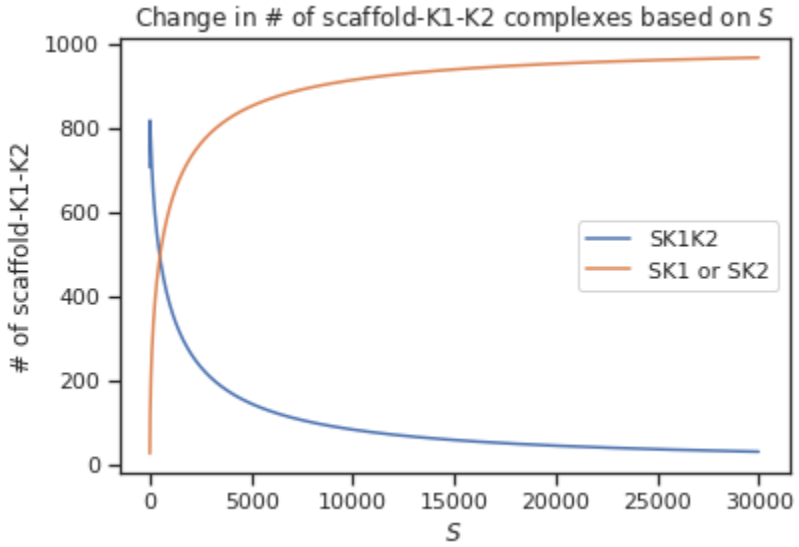

For S <10, number of scaffold-K1-K2 complexes increases rapidly with increasing S and reaches its maximum value 819.0024875775821.
For S ≥ 10, number of scaffold-K1-K2 complexes drops exponentially with increasing S. This indicates that, if there is too much scaffolds molecules in the system, they will compete for binding K1 and K2, which resulted in the decrease of the number of scaffold-K1-K2 complexes in the system and the increase of the number of scaffold-K1 and scaffold-K2.

(c) (5 points) Based on your findings, would it make sense for a cell to use an increase in the scaffold concentration as a mechanism to amplify signaling through the MAPK pathway? Explain your reasoning.

No, if there are too much scaffolds molecules in the system, the probability of scaffolds binding with two kinases at the same time will be lower than the probability of scaffolds binding with only one kinase. This is because the scaffolds will compete with each other to bind with the kinases. The cell should increase the number of K1, K2 molecules to obtain more scaffold-K1-K2 complexes more efficiently.

(d) (5 points) Show how you would set up the calculation to include the effect of cooperativity in the kinases binding to S. Do you expect the effect of cooperativity to be more important at low concentration or high concentration?

Let $K1_d = \frac{[S][K1]}{[SK1]}$, $K2_d = \frac{[SK1][K2]}{[SK1K2]}$, $K2_d > K1_d$

This means once the first ligand is bound to the scaffold, it will be easier for the second ligand to bind. The effect of cooperativity is more important at low concentration since it can increase the chance of another ligand binding.

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3. *Stochastic Antagonism.* (35 points) In this problem we consider a simple network of two interacting proteins X and Y. The five reactions in the basic network are as follows:

$$\emptyset \xrightarrow{\lambda_X} X \xrightarrow{1} \emptyset$$
$$\emptyset \xrightarrow{\lambda_Y} Y \xrightarrow{1} \emptyset$$
$$X + Y \xrightarrow{c} XY \xrightarrow{1} \emptyset$$

Protein X is a transcription factor that acts as a repressor for the expression of a third protein Z, which we will add to the model later. Protein Y binds to X to form a complex XY that is transcriptionally inactive. Each of the reactions above proceeds by mass action kinetics. Protein loss is assumed to occur at the same rate for each protein component and this rate is set to 1. In these units the production rates λ_X and λ_Y can be taken to be 50 molecules and 30 molecules respectively, and c is 50 /molecule.

(a) (5 points) If dilution is the only mechanism for protein concentration loss, what biological process is the time unit a measure of?

If the volume of the cell is increasing, the protein concentration will decrease even if there is no clearance (degradation). The time unit is a measure of the growth rate of the cell.

(b) (5 points) Compute steady state levels of X, Y, and XY for the model parameters given above. How do the steady state levels of X and Y compare to what their levels would be in the absence of complexation? How would you describe Y's regulation of X?

$$\frac{dX}{dt} = 50 - [X] - 50[X][Y]$$
$$\frac{dY}{dt} = 30 - [Y] - 50[X][Y]$$
$$\frac{dXY}{dt} = 50[X][Y] - [XY]$$

At steady state, let the derivatives to be 0.

$$[X] = \frac{999+\sqrt{999^2+4\times50\times50}}{2\times50} = 20.0299253$$
$$[Y] = [X] - 20 = 20.0299253 - 20 = 0.0299253$$
$$[XY] = 50[X][Y] = 29.97007470147267$$

If there is no complexation for X+Y, the differential equations will be:

$$\frac{dX}{dt} = 50 - [X], \frac{dY}{dt} = 30 - [Y]$$
$$\Rightarrow [X] = 50,\ [Y] = 30$$

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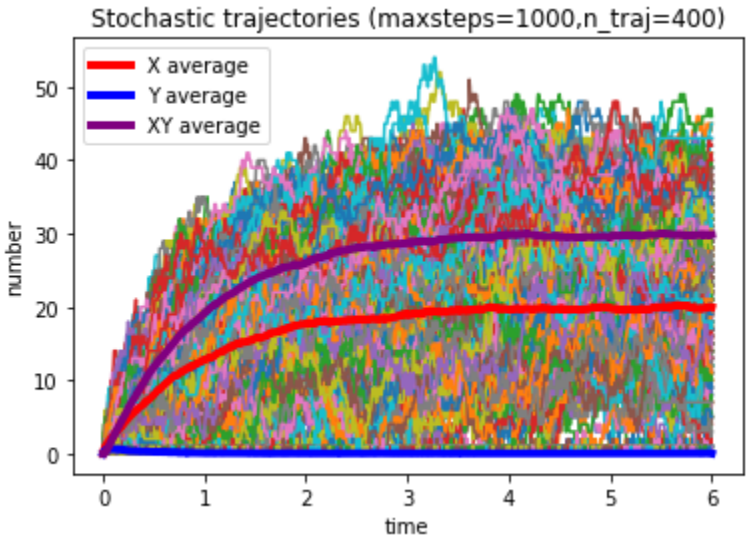
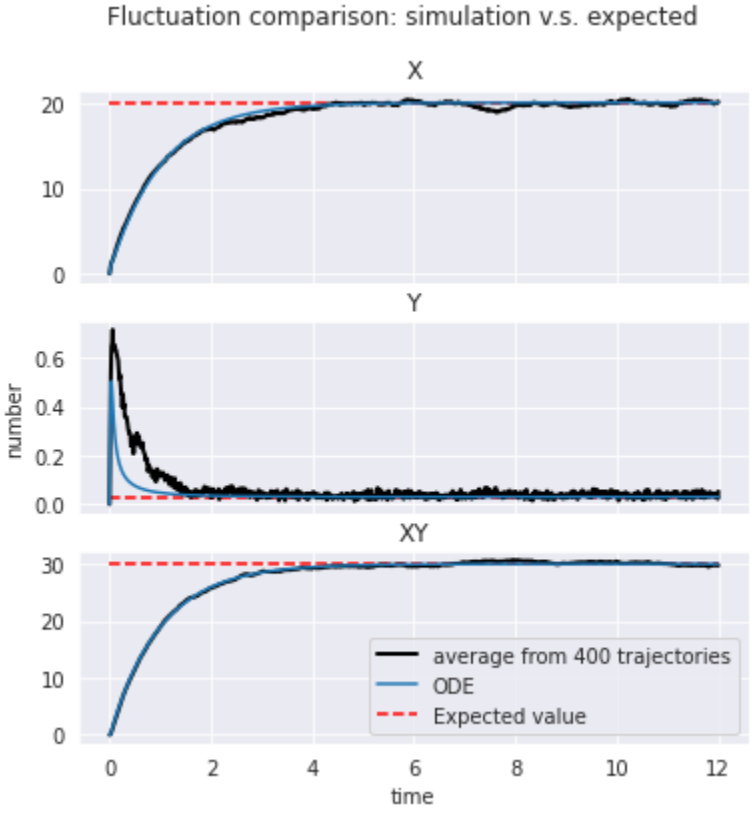
Y regulates the level of X by binding to X to form XY complex and decrease the free X in the cell (or organelle) at the steady state.

(c) (5 points) Compute stochastic trajectories of this system and compare the fluctuations in X, Y, and XY to the fluctuations expected for the same steady state expression levels resulting from a simple birth-death process.





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At the steady-state:

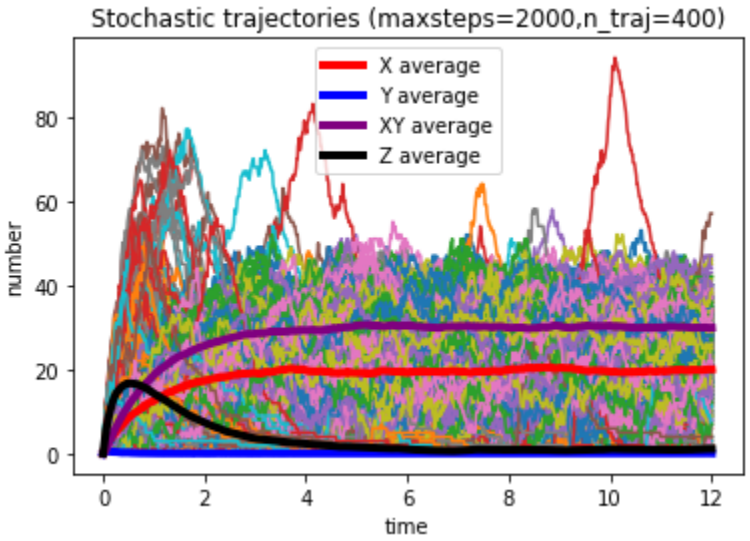
	Mean from simulation (1000000 steps)	Expected value
X	20.029925300028882	20.0299253
Y	0.029925299999958025	0.0299253
XY	29.97007470154787	29.97007470147267

For the remainder of this problem we will consider an additional component Z, whose production is regulated in an all-or-none fashion by X:

$$\emptyset \xrightarrow{f(X)} Z \xrightarrow{1} \emptyset,$$

where $f(X) = \lambda_Z \Theta(X < 4)$ and Θ Is the Heaviside function, which evaluates to 1 if its argument is True and 0 is its false. Take λ_Z to be 1000 molecules.

(d) (5 points) Compare the observed probability that $X < 4$ from your stochastic trajectory with the expected probability from a simple birth-death process with the same steady state level of X. Is this result consistent with your findings in (c)?



Observed probability from 2000 steps, 400 traj, x0 as zeros:
P(X < 4): 0.00643
Expected probability, assum the amount of X is poisson distributed with mean = 19.86191
CDF(X<4)=1.8950954922368243e-05
Yes it is consistent with the findings in (c). X mean is around the same expected value.

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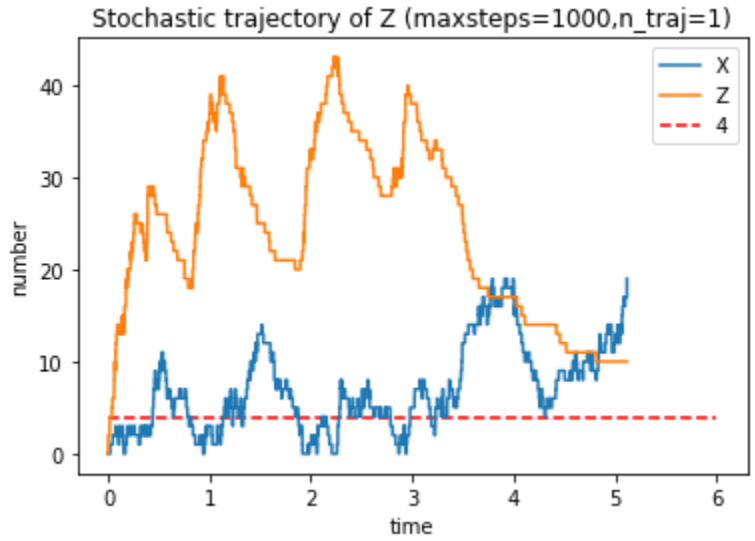
(e) (5 points) Compute a stochastic trajectory of the system of about 1000 time units and plot the amount of Z vs. time. Describe your observations.



The amount of Z increases in the beginning then decreases once X is higher than 4, and then increases again when the amount of X is below 4. This example trajectory shows that the expression level of Z is regulated by the amount of its repressor X.

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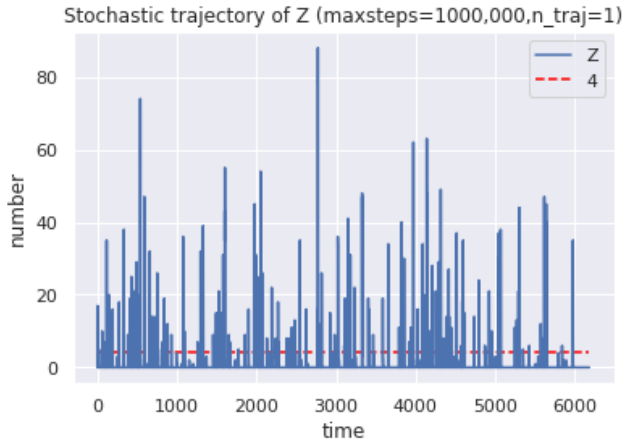
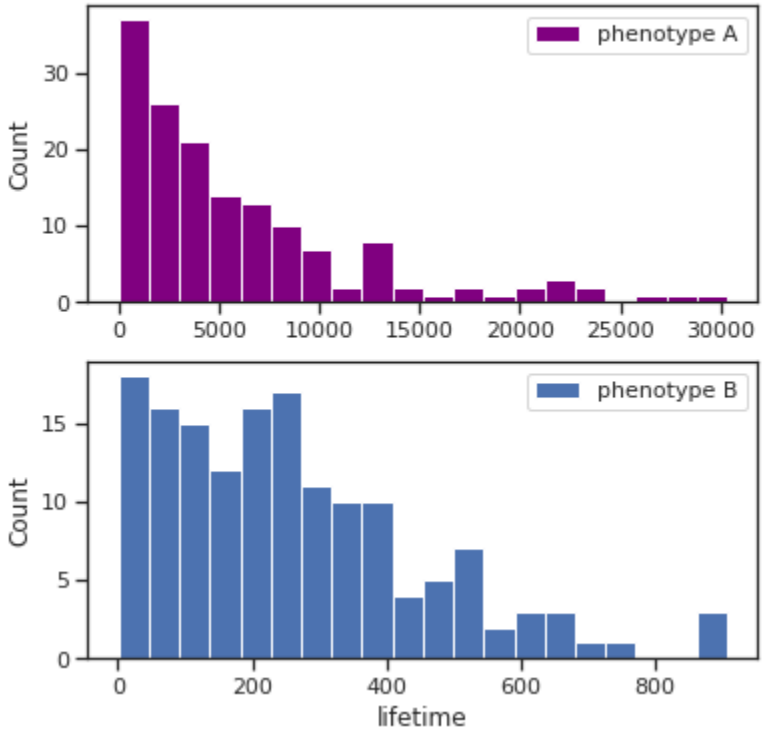
(f) (5 points) Suppose that Z is itself a transcription factor that induces expression of genes that switches the cell to a different phenotype. We will refer to the phenotype with low Z expression as A and the phenotype with high Z expression as B. Assume that phenotype B persists as long as $Z > 4$. Run a trajectory of at least 20,000 time units and determine the distribution of lifetimes for each phenotype. Plot the resulting distributions and compute their relevant parameters.



Distributions of lifetimes for phenotypes (from 1000000 time units)



The average lifetime for phenotype A (Z≤4) is 6022.357142857143
The average lifetime for phenotype B (Z>4) is 259.46753246753246

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(g) (5 points) Discuss the biological implications of your observations. How would you characterize the biological processes underlying the switch from phenotype A to B and the switch from B to A respectively?

The expression level of Z is regulated and is switching between highly expressed and no expressed based on the level of X. Whenever X > 4, Z will start to decrease and switches the cell to phenotype A. Since the average of the amount of X is around 20 molecules and P(X<4) is around 0.00643, the cell would be at phenotype A for most of the time. For a very small probability, the level of X can be lower than 4, the expression of Z will be turned on and switching the cell to phenotype B. Thus, base on the distribution, we can see that the lifetime of phenotype A is overall longer than the lifetime of phenotype B.

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4. *Work-life balance.* (15 points) Write a short description of something fun you did this weekend.

Me and my labmates went to Dave & Buster's on Saturday. It was super fun and we all had a great time!