PhySci/MiMG/CaSB M178

Homework 1

Due: 10/10/23 at 12:00PM PDT

Notes: This homework involves performing simulations of the Posttranslational Modification (PTM) cycle we've been discussing in the first two class meetings. In the same folder on CCLE where you obtained this document, you will also find a file called "HW1_template.ipynb" that contains a template Jupyter notebook that you can use as a starting point to complete the questions below. Please modify this notebook and use it as the starting point for answering the following problems.

To submit your homework, please answer the questions below. Note that you will have to paste in several graphs that you generate using the Jupyter notebook. After completing the questions, **save this document as a PDF and upload it to Gradescope**. You **must also upload the Jupyter notebook to CCLE**; to do so, navigate to the "Homework" section on the left-hand side of the course CCLE website. There you will see an assignment entitled "Homework 1 Jupyter submission." You can upload your Jupyter file (which should be a .ipynb file). Make sure you upload your Jupyter notebook by the due date/time (10/5/21 at 12:00PM PDT).

Problems

In class, we talked about a kinase binding and phosphorylating a substrate protein "S." Just to re-iterate, the chemical reactions are:

$$\mathrm{K} + \mathrm{S} \xrightarrow{k_+} \mathrm{KS}$$

$$\mathrm{KS} \xrightarrow{k_-} \mathrm{K} + \mathrm{S}$$

$$\mathrm{KS} \xrightarrow{k_{cat}} \mathrm{K} + \mathrm{S}^*$$

where K and S are the free kinase and substrate, KS is the kinase-substrate complex, and S^* is the phosphorylated substrate. The various *k* values represent the rate parameters for each reaction.

In a posttranslational modification cycle, we also have a phosphatase enzyme, P, that binds to the phosphorylated substrate and undoes the modification. Its chemical reactions are:

$$\mathrm{P} + \mathrm{S}^* \xrightarrow{k_+} \mathrm{PS}^*$$

$$\mathrm{PS}^* \xrightarrow{k_-} \mathrm{P} + \mathrm{S}^*$$

$$\mathrm{PS}^* \xrightarrow{k_{cat}} \mathrm{P} + \mathrm{S}$$

which are very similar to the ones for the kinase, but P binds S* and produces the unmodified substrate S. Note that, for the purposes of this homework, we will use the ***same rate parameters*** for K and P.

In class, we wrote down the change equations for the simple case where there is just a Kinase (i.e. no phosphatase). Please refer to the posted lecture notes if you need to refresh your memory for those change equations. Since the kinase and phosphatase work together, the really interesting situation occurs when we have all three proteins (K, P and S) present in the same system.

For your reference, we have provided the change equations for the system below, which we worked out in class.

$$S' = -k_+ S \cdot K + k_- KS + k_{cat} PS^*$$

$$KS' = k_+ S \cdot K - k_- KS - k_{cat} KS$$

$$S^{*\prime} = -k_+ S^* \cdot P + k_- PS^* + k_{cat} KS$$

$$PS^{*\prime} = k_+ S^* \cdot P - k_- PS^* - k_{cat} PS^*$$

$$K' = -k_+ S \cdot K + k_- KS + k_{cat} KS$$

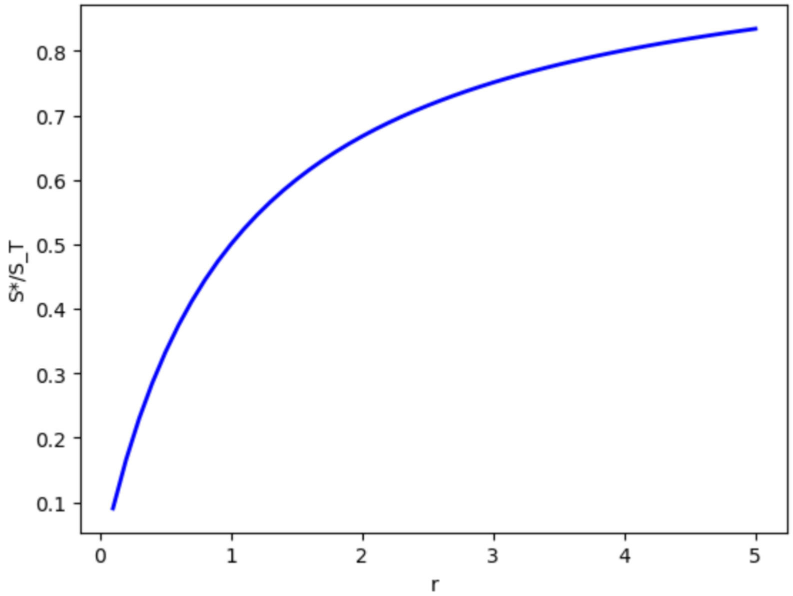
$$P' = -k_+ S^* \cdot P + k_- PS^* + k_{cat} PS^*$$

1) (20 points) In the provided template Jupyter notebook, we have already coded up the change equations for this system. They are towards the top of the template.

You will see that below there is a section called initial conditions. You will need to modify that section to add initial conditions for all six state variables. Note that the **units** of all the state variables are “micro Molar” (μM). You won’t have to explicitly set the units in the template, but it is good to know what they are. Set the initial conditions to: $S(0) = 0.1$, $KS(0) = 0$, $S^*(0) = 0$, $PS^*(0) = 0$ and $P(0) =0.001$. Note that this is an “unsaturated” case.

Below that, there is a section of code that is called “PLOT your steady-state results.” Note that this section of the code changes the initial value of *K* from 0.0001 (10 times less than the phosphatase amount) to 0.005 (five times more than the phosphatase amount). It then runs a really long simulation with those parameters, and saves the result of the final time point. After doing this, it generates a plot of the steady-state value of S^*/S_T as a function of the parameter “*r*”, as described in lecture.

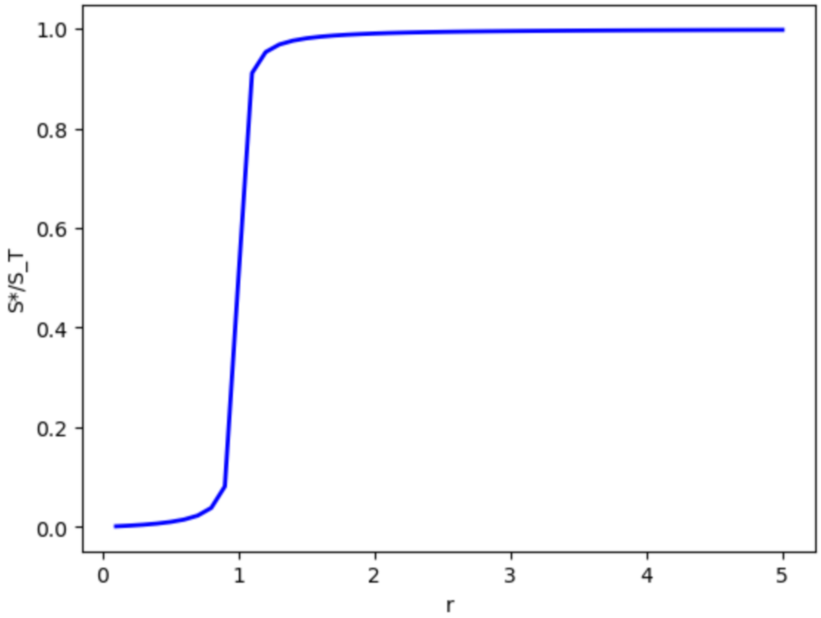
Run this code with your modified change equations, and paste the resulting graph below:



Would you describe this as “switch-like” behavior?

I wouldn't call it "switch-like" behavior. The graph shows a smooth, consistent curve rather than transitioning sharply between steep and less-steep regions. There's no evident "switch" point. Here, there’s a more consistent curve with a change in steepness that’s more uniform and is non-sigmoidal.

Now, go back to the initial conditions and modify the initial conditions so *S*(0) = 1,000, and re-run the code that calculates and plots the S^*/S_T vs. *r* graph. Note that this is the "saturated" case. Paste your graph below:



Would you describe this as "switch-like" behavior?

Yes, because there is an s-shaped sigmoidal curve now on the graph now, which is evident of "switch-like" behavior. There's a rapid transition from one state to another when r is roughly 1. The S^*/S_T rapidly increases from ~0.1 to near 1 with a minimal increase in r. There is a region on the curve where the slope is extremely steep and sensitive.

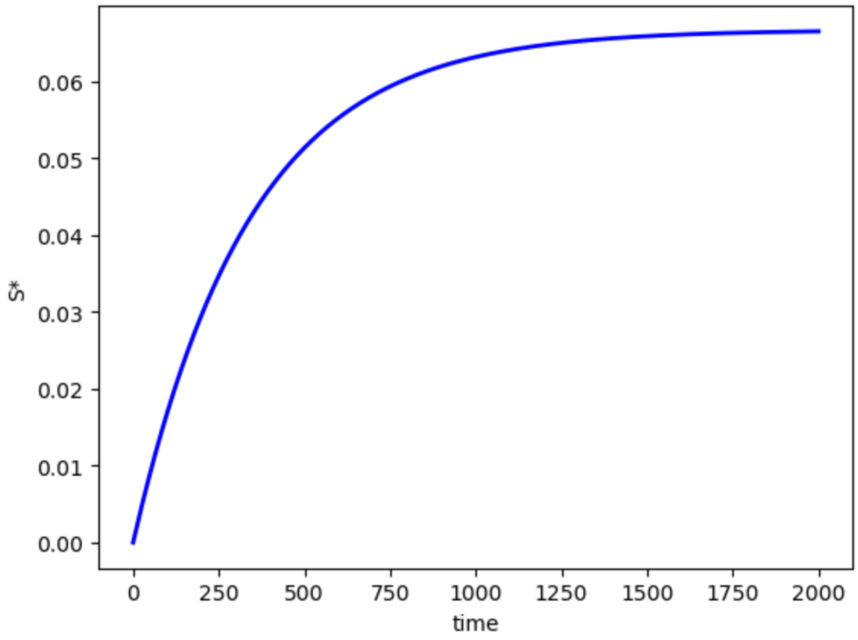
2) (15 points) Underneath the section where you plot the "steady-state behavior," there is a section called "dynamics." This is where we will look at the time series of phosphorylation for two different scenarios described above.

Let's first consider the unsaturated case. Notice that there is a section to define the initial conditions. To start, let's use the following values: *S*(0) = 0.1, *KS*(0) = 0, *S**(0) = 0, *PS**(0) = 0, *K*(0) = 0.002 and *P*(0) =0.001.

Given these initial conditions, what value of the parameter "*r*" are we simulating here?

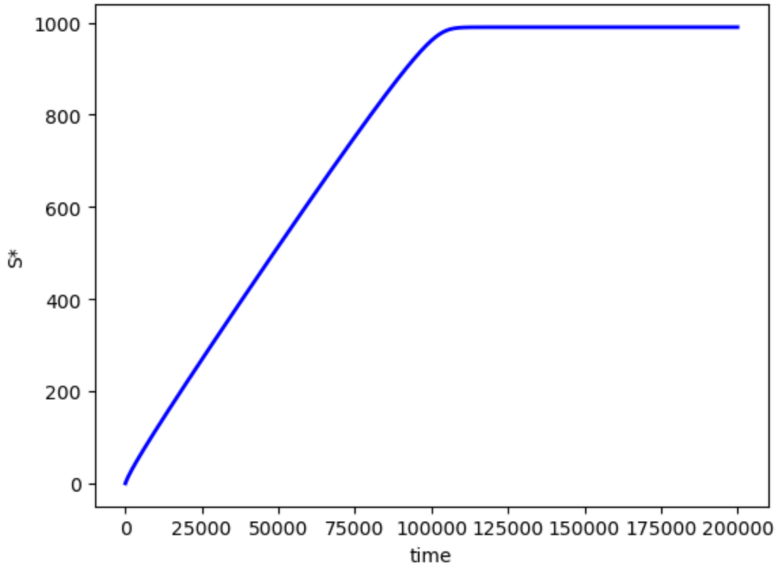
We are simulating the ratio of the kinase divided by the phosphatase reacting. r = Kcat*K/k_{cat}*P = K/P = 0.002/0.001 = 2. Thus, r = 2 is the parameter value we're simulating here.

Run the code, and paste the resulting graph ("PLOT your dynamics") below:



Now, consider the saturated case, and modify the initial conditions to: *S*(0) = 1000, *KS*(0) = 0, *S**(0) = 0, *PS**(0) = 0, *K*(0) = 0.002 and *P*(0) =0.001.

Run the code, and paste the resulting graph below:



Is there any difference in the two cases (i.e. saturated and unsaturated)? How long does it take each case to achieve steady state [NOTE: you may need to extend the time you are simulating to see the system achieve steady-state in some cases]? How might this influence the physiological relevance of the steady-state response graphs that you plotted in your response to question 1?

The difference in saturated and unsaturated is that the graph of unsaturated shows a steady curve that increases in S*, while in the saturated case the graph appears to be a linear line that increases in S* in the given time frame of 2000s. The S* value for saturated is also much lower and the graph taper off at S* ≈ 0.07. The S* value for saturated reaches 30 in the same given time frame and doesn’t appear to taper off. Saturated takes approximately 1.16 days to achieve steady state, while unsaturated takes approximately just over 33 minutes to achieve steady state. This model may not accurately represent fast-acting processes like nerve signaling so it’s likely better suited for capturing long-term phenomena like immune responses. With the graphs from question 1, reaching a steady state could take a considerable amount of time, making the model not as relevant for real-world physiological situations, which can be time-dependent.

3) (15 points) One of the more unrealistic things about the PTM cycle model described above is that there is no synthesis and degradation of the substrate protein. In real cells, proteins experience *turnover*; they are not just made one time by the cell and then sit around forever. Real proteins can get damaged, unfold, or for other reasons “go bad.” So, the cell constantly degrades proteins to keep damaged proteins from causing problems, and to make up for this, it is also constantly making proteins to make up for the proteins that are lost.

To illustrate how we might model this turnover process, consider some generic protein in the cell called “Z.” Let’s say the cell makes this protein at a constant rate *Q* and degrades it at a per-molecule rate of *δ*. We can write this as chemical reactions in the following way:

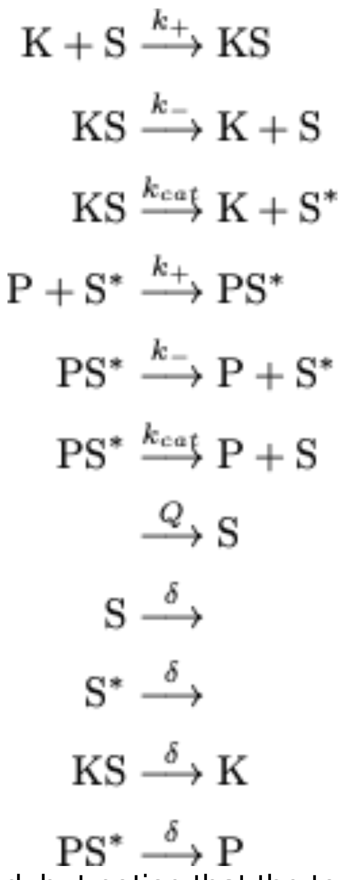
$$\xrightarrow{Q} \mathrm{Z}$$

$$\mathrm{Z} \xrightarrow{\delta}$$

The resulting change equation would be:

$$Z' = Q - \delta Z$$

Let's make a model of the PTM cycle where the substrate is synthesized and degraded. To do so, use the following system of chemical reactions:



$$\mathrm{K} + \mathrm{S} \xrightarrow{k_+} \mathrm{KS}$$
$$\mathrm{KS} \xrightarrow{k_-} \mathrm{K} + \mathrm{S}$$
$$\mathrm{KS} \xrightarrow{k_{cat}} \mathrm{K} + \mathrm{S}^*$$
$$\mathrm{P} + \mathrm{S}^* \xrightarrow{k_+} \mathrm{PS}^*$$
$$\mathrm{PS}^* \xrightarrow{k_-} \mathrm{P} + \mathrm{S}^*$$
$$\mathrm{PS}^* \xrightarrow{k_{cat}} \mathrm{P} + \mathrm{S}$$
$$\xrightarrow{Q} \mathrm{S}$$
$$\mathrm{S} \xrightarrow{\delta}$$
$$\mathrm{S}^* \xrightarrow{\delta}$$
$$\mathrm{KS} \xrightarrow{\delta} \mathrm{K}$$
$$\mathrm{PS}^* \xrightarrow{\delta} \mathrm{P}$$

I know this looks complicated, but notice that the top six reactions are just the standard PTM loop.

Write out the modified change equations below:

(note that you should have an equation for S′, KS′, S*′, PS*′, K′ and P′)

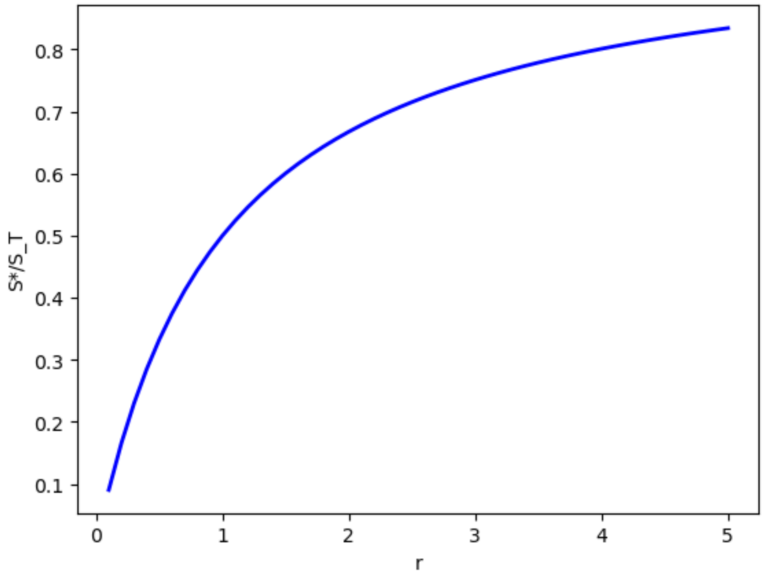
$$S' = -K \cdot S \cdot k_+ + KS \cdot k_- + PS^* \cdot k_{cat} + Q - S \cdot \delta$$
$$KS' = +K \cdot S \cdot k_+ - KS \cdot k_- - KS \cdot k_{cat} - KS \cdot \delta$$
$$S^{*\prime} = +KS \cdot k_{cat} - P \cdot S^* \cdot k_+ + PS^* \cdot k_- - S^* \cdot \delta$$
$$PS^{*\prime} = +P \cdot S^* \cdot k_+ - PS^* \cdot k_- - PS^* \cdot k_{cat} - PS^* \cdot \delta$$
$$K' = -K \cdot S \cdot k_+ + KS \cdot k_- + KS \cdot k_{cat} + KS \cdot \delta$$
$$P' = -P \cdot S^* \cdot k_+ + PS^* \cdot k_- + PS^* \cdot k_{cat} + PS^* \cdot \delta$$

To simulate this system, look below where you were working in the Jupyter notebook template for the section labeled “Second model: includes synthesis and degredation.” (Note that you can copy-and paste what you write there to answer the question above!).

We will be looking at the steady-state response of this for the two different conditions that we've been discussing. Doing this is a little more complicated than the case without synthesis and degradation. Note that the rate parameters k_+, k_-, and k_{cat} don't change here. We have to add the parameter δ, which has already been done for you, and that won't change between the saturated and unsaturated cases. But the value of the parameter *Q* will change, so be sure to watch for that!

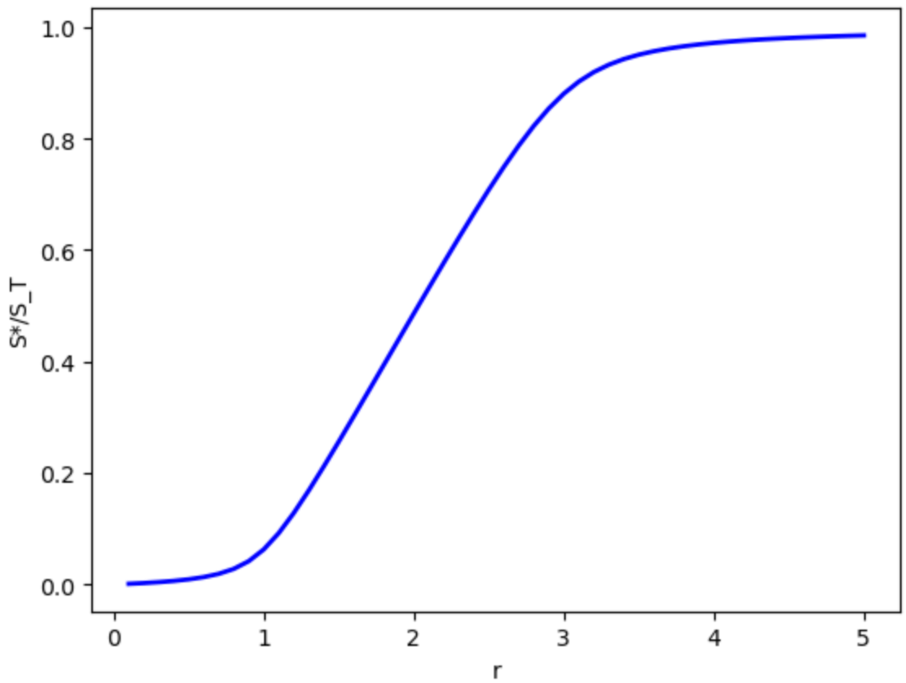
Focus first on the unsaturated case. You will see that again there is code in the template for generating the steady-state response curve. First, run that code with the following initial conditions: $S(0) = 0.1$, $KS(0) = 0$, $S^*(0) = 0$, $PS^*(0) = 0$, $K(0) = 0.001$ and $P(0) =0.001$ (these are the default values in the template). We will also set *Q* to be $\delta^*S(0)$. Run the code for this case.

Paste your unsaturated steady-state response graph below:



Now let's run the saturated case. Here, set the initial conditions to: $S(0) = 1000$, $KS(0) = 0$, $S^*(0) = 0$, $PS^*(0) = 0$, $K(0) = 0.001$ and $P(0) =0.001$, and set *Q* to $\delta^*S(0)$. Now run the code again.

Paste your saturated steady-state response graph below:



How does the *unsaturated* result compare to your answer to question 1 for the unsaturated case? Does this look more or less switch-like? How does the *saturated* result compare; is it more or less switch-like? Given your findings, how physiologically relevant is the emergence of extreme switch-like behavior in the traditional PTM cycle likely to be for substrates that experience turnover?

The unsaturated result is nearly identical to the unsaturated case in question 1 in that it doesn't show an apparent sigmoidal curve and is not “switch-like.” In contrast, the saturated case appears less "switch-like" than the original saturated case in question 1. With these observations, extreme switch-like behavior in the traditional PTM cycle may not be highly relevant physiologically for substrates with turnover. The lack of a pronounced switch-like behavior suggests that the system could be more graded or gradual in its response, rather than acting like an on/off switch.