PhySci/MiMG/CaSB M178

Homework 3

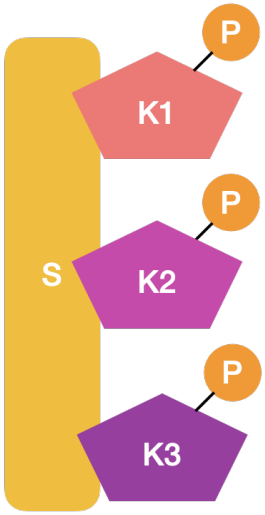
Due: 10/24/23 at 12:00PM PDT

Notes: This homework involves performing simulations of the Scaffold Proteins we've been discussing in the last two class meetings. In the assignment on BruinLearn where you obtained this document, you will also find a file called "HW3_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 3 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 (10/24/23 at 12:00PM PDT).

Problems

In class, we talked about scaffolding proteins. Consider the following scaffold protein example that accommodates three different kinase species:



Like in last week's homework, upon a receptor binding event at the cell surface, the first kinase species, K_1, is changed (phosphorylated) to its active form, K_1p (not modeled here). Note that, for this homework, we are calling the phosphorylated version of any given kinase K_ip rather than K_i^* as we did in our

previous homework problems, but that does not effect the model or our analysis in any way. This active kinase species can bind the scaffold protein, S, and then can phosphorylate the next kinase species, K_2, if present on the scaffold protein as well. K_2p can then activate K_3 if both are bound to the scaffold protein. We will assume all kinase species (whether bound to the scaffold or not) undergo a first-order dephosphorylation at the same rate.

We have the following monomer species in our model: $S, K_1p, K_2, K_2p, K_3, K_3p$.

1) (20 points) First write down the number of dimers, trimers, and tetramers in this model given unordered, independent binding.

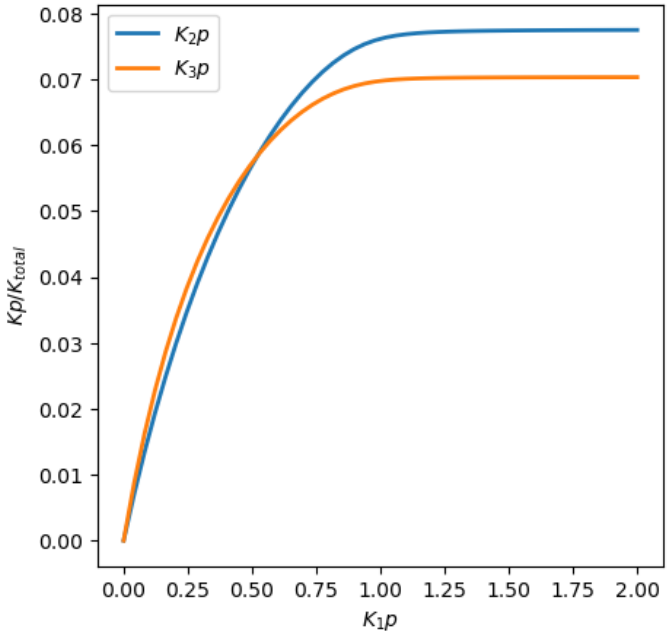
5 Dimers: SK_1p, SK_2p, SK_3p, SK_2, SK_3
8 Trimers: SK_1pK_2, SK_1pK_2p, SK_1pK_3, SK_1pK_3p, SK_2K_3, SK_2K_3p, SK_2pK_3, SK_2pK_3p
4 Tetramers: $SK_1pK_2pK_3p$, $SK_1pK_2K_3$, $SK_1pK_2pK_3$, $SK_1pK_2K_3p$

The unordered scaffold model with three kinases has been implemented for you in the section "scaffold model". We will explore the amount of output, K_3p, present at steady state for varying values of input, K_1p, under the section "STEADY-STATE responses of scaffold model".

Note that the parameters are set at the beginning of this section of code as well as the initial conditions. The rates of association and dissociation of the kinases on to and off the scaffold protein are all $10\mu M^{-1}s^{-1}$ and $0.1s^{-1}$, except the association of the third kinase species (in either its phosphorylated or dephosphorylated form) has a rate of $1\mu M^{-1}s^{-1}$ (k_a3). All of the downstream kinases are in the inactive form at the start and the scaffold protein concentration is equal to the kinase concentrations ($1\mu M$), except the third kinase species is ten times greater in concentration. The differences in rates for the third kinase species allow for more free K_3p to be present at steady state. That kinase must be free from the scaffold to act downstream in the nucleus.

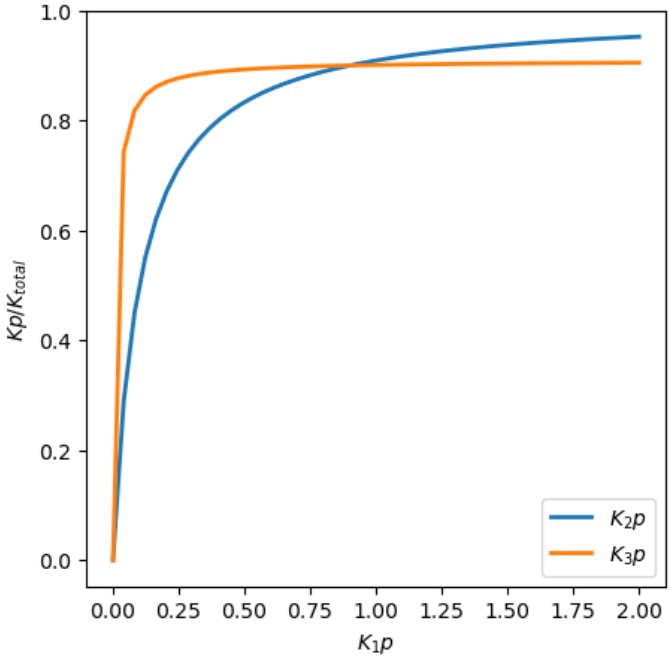
This section of the code then changes the initial value of K_1p , from 0 to 2 (2 times its default value of 1). It then runs a simulation of the scaffold model with those parameters, and saves the result of the final time point. After doing this, it generates a plot of the steady-state value of $K_3p/K_{3\ total}$ as a function of the parameter, K_1p. Note that the code also outputs the value of $K_2p/K_{2\ total}$ for reference.

Run this code and paste the resulting graph below:



Next under the section of code "no scaffold model" a kinase cascade with three kinase species has been implemented similar to the models in last week's homework. Again, in the section "STEADY-STATE responses--no scaffold" we will explore how varying K_1p changes the steady state value of $K_3p/K_{3\ total}$.

Run this code and paste the resulting graph below:



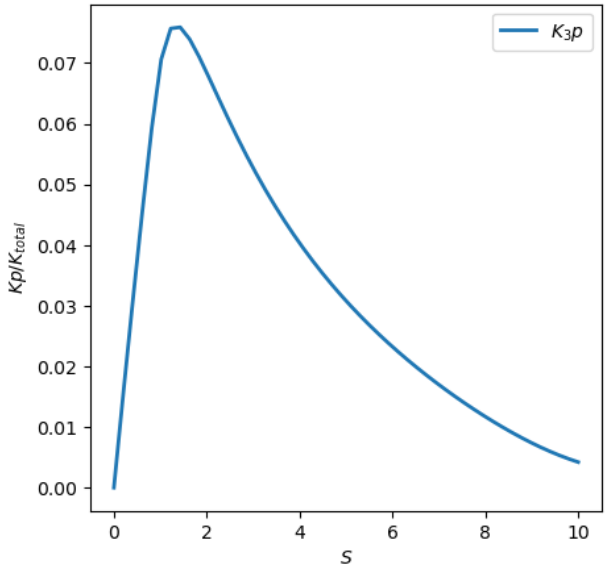
How does the presence of the scaffold protein alter the amount of K_1p needed to achieve maximum activation of the final level kinase? How does the scaffold protein alter the "signal amplification" provided by the cascade (compare the relationship between the K_3p and K_3p curves, and note the *scale* of the y-axis!)?

The presence of the scaffold protein decreases the concentration of K_3 given the same amount of K_1p. For a given K_1p, having a scaffold protein shows a lesser steady-state value of $K_3p/K_{3\,total}$. There's also a much more gradual upward curve with a scaffold protein, but a steeper upward curve where there appears to be a minimum amount of K_1p that results in a significant increase in $K_3p/K_{3\,total}$ when there's no scaffold protein. The scaffold protein decreases the signal amplification provided by the cascade by having a lower steady-state value of $K_3p/K_{3\,total}$ and $K_2p/K_{2\,total}$. The scale of the y-axis in the presence of a scaffold protein shows a maximum of 0.08 compared to a maximum of 1.0 without the presence of a scaffold protein.

2) (15 points) Underneath the section where you plotted the "steady-state behavior," there is a section called "Prozone Effect." This is where we will examine the prozone effect or combinatorial inhibition discussed in class.

We will use the unordered scaffold model as defined previously. Notice again, that there is a section to define the parameters and initial conditions, which are the same as the previous simulations. Now there is a section that will vary the concentration of the scaffold protein, S, from 0 to 10 (ten times its default values of 1) and then plot the steady state value of $K_3p/K_{3\,total}$ of the resulting simulations.

Run the code, and paste the graph ("PLOT dynamics of kinase activation") below:



Explain in your own words what causes the initial rise in $K_3p/K_{3\,total}$. Now explain what causes the decline in $K_3p/K_{3\,total}$ as S further increases.

The initial rise in $K_3p/K_{3\,total}$ is caused by having an excess of K_3 within the cell compared to the available scaffold proteins to bind to. This means plenty of K_3 have available scaffold proteins to bind to, leading to an increase in successful bindings and subsequent reactions. As you increase the number of scaffold proteins, there's an increase in successful bindings and more reactions can take place. However, at a certain point, there's going to be too many scaffold proteins and too little substrates so fewer substrate molecules are available to bind and catalyze subsequent reactions. As a result, the $K_3p/K_{3\,total}$ decreases as S concentration continues to increase. **Basically, the kinases are spread too thinly among the competing scaffold proteins.**

What value of scaffold protein gives a maximal response? Predict how might this value might change if the kinase concentrations were smaller/larger? (Note: you can check your prediction by altering the initial conditions (K1p, K2)).

There is a maximal response around a scaffold protein concentration of 1.25 μM. I predict that if kinase concentrations were larger, then the maximal response would occur when there's a larger scaffold protein concentration so the peak would be further right, and if kinase concentrations were smaller, the maximal response would occur when there's a smaller scaffold protein concentration so the peak would be further left.

3) (15 points) Thus far we have considered an unordered binding model. We will now consider an ordered binding model in the section "Ordered Binding". In this model we still have a scaffold protein, S, and three kinase species. However, binding now occurs with the following rules:

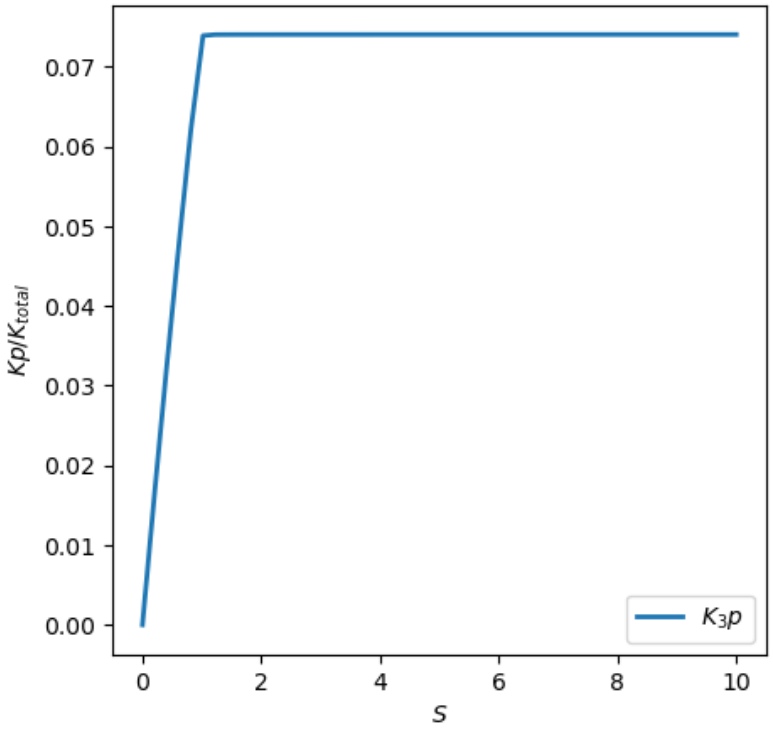
1. Only K_1p can bind to S
2. Only K_2 or K_2p can bind to SK_1p
3. K_3 and K_3p can only bind SK_1pK_2p (at a reduced rate like the previous model)
4. Only K_3 and K_3p can unbind $SK_1pK_2pK_3$ and $SK_1pK_2pK_3p$ respectively
5. Only K_2 and K_2p can unbind to SK_1pK_2 and SK_1pK_2p respectively
6. K_1p can unbind from SK_1p
7. K_1p can phosphorylate K_2 if both species are bound to the scaffold (SK_1pK_2)
8. K_2p can phosphorylate K_3 if both species are bound to the scaffold ($SK_1pK_2pK_3$)
9. K_2p and K_3p can both dephosphorylate when unbound from the scaffold. They can also dephosphorylate when bound to the scaffold, except K_2p if K_3 or K_3p is also bound to the scaffold.
10. All phosphorylation and dephosphorylation reactions are first-order with rates k_p and k_u respectively
11. Association/binding events all occur with rate k_a and dissociation/unbinding events all occur with rate k_d, except K_3 and K_3p associate with the scaffold with reduced rate k_a3

In summary these are then the possible species in the model: $S, K_1p,\ K_2p, K_2, K_3, K_3p,\ SK_1p,\ SK_1pK_2,\ SK_1pK_2p,\ SK_1pK2_pK_3,\ SK_1pK2_pK_3p$.

Write the change equations for all species in the model and add them to the code section "Ordered Binding" where it says fill in your change equations.

$$S' = -K_1p \cdot k_a + SK_1p \cdot k_d$$
$$K_1p' = -SK_1p \cdot k_a + SK_1p \cdot k_d$$
$$K_2' = -SK_1pK_2 \cdot k_a + SK_1pK_2 \cdot k_d + K_2p \cdot k_u$$
$$K_2p' = -SK_1pK_2p \cdot k_a + SK_1pK_2p \cdot k_d + K_2p \cdot k_u$$
$$K_3' = -SK_1pK_2pK_3 \cdot k_{a3} + SK_1pK_2pK_3 \cdot k_d + K_3p \cdot k_u$$
$$K_3p' = -SK_1pK_2pK_3p \cdot k_{a3} + SK_1pK_2pK_3p \cdot k_d - K_3p \cdot k_u$$
$$SK_1p' = -SK_1pK_2 \cdot k_a - SK_1pK_2p \cdot k_a + SK_1pK_2 \cdot k_d + SK_1pK_2p \cdot k_d$$
$$SK_1pK_2' = +SK_1pK_2 \cdot k_a - SK_1pK_2 \cdot k_d - SK_1pK_2 \cdot k_p + SK_1pK_2p \cdot k_u$$
$$SK_1pK_2p' = +SK_1pK_2p \cdot k_a - SK_1pK_2p \cdot k_d + SK_1pK_2pK_3 \cdot k_d + SK_1pK_2pK_3p \cdot k_d + SK_1pK_2p \cdot k_p - SK_1pK_2p \cdot k_u$$
$$SK_1pK_2pK_3' = +SK_1pK_2pK_3 \cdot k_{a3} - SK_1pK_2pK_3 \cdot k_d - SK_1pK_2pK_3 \cdot k_p + SK_1pK_2pK_3p \cdot k_u$$
$$SK_1pK_2pK_3p' = +SK_1pK_2pK_3p \cdot k_{a3} - SK_1pK_2pK_3p \cdot k_d + SK_1pK_2pK_3 \cdot k_p - SK_1pK_2pK_3p \cdot k_u$$

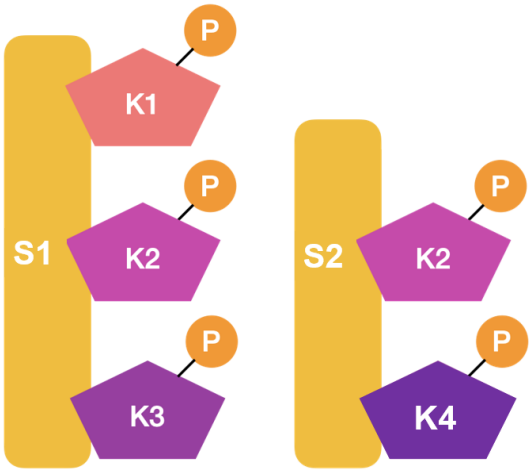
We will again examine the steady-state prozone effect as we did in question 2, but now for our new model. The next section of code again varies the concentration of the scaffold protein, S, and reports the output signal $K_3p/K_{3\ total}$. Run the code in the section "Prozone effect--Ordered binding" below the ordered model and paste your graph.



How do the results differ from the unordered binding case? Why might this be?

For the ordered binding case, there's a steeper and more linear increasing response as S increases, up to a certain point. Also, after a certain concentration of S, $K_3p/K_{3\ total}$ doesn't decrease, but instead stays constant indefinitely as S continues to increase. There also appears to be a lower maximum $K_3p/K_{3\ total}$ when there's ordered binding at 0.0725, compared to 0.0750 when there's unordered binding. There is a linear line possibly because as S increases, more molecules can bind in the required order, leading to a proportional increase in phosphorylation. This is because each binding event is dependent on the previous one. There's a constant $K_3p/K_{3\ total}$ at high S concentrations because kinases must bind in order before the next kinase can be activated and if the concentration of the scaffold protein becomes too high, it can saturate all available binding sites with non-phosphorylated molecules without allowing other kinases to bind and activate.

4) (15 points) We finally explore a model of crosstalk in the section called "crosstalk scaffold model". Here we have the same unordered binding model as before, but now we have an additional scaffold protein, S_2 (we will call the original scaffold protein S_1 now). S_1 participates in all the same reactions as it did in the above unordered model, but now the second kinase species, K_2, can also bind to S_2. S_2 also accommodates an additional kinase species, K_4. Again K_2 *and* K_4 associate to S_2 in an unordered manner, in either their phosphorylated or dephosphorylated form. In its phosphorylated form, K_2p, phosphorylates K_4 when both are bound to S_2. The scaffold proteins and kinases in our model can be summarized:



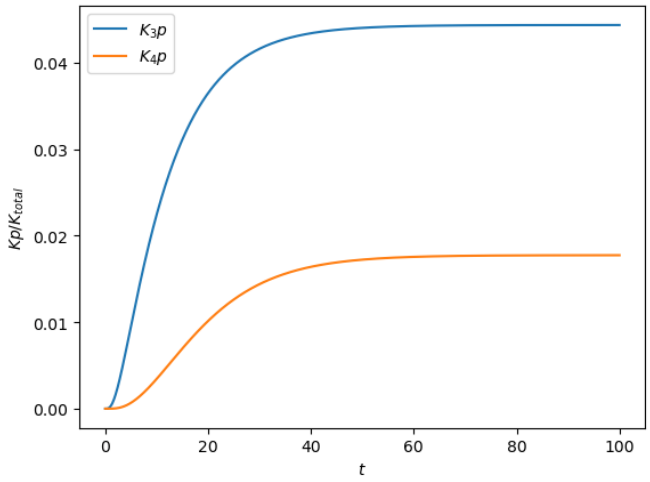
First write down the number of new dimers and trimers (containing S_2) in this model given unordered, independent binding.

4 New Dimers: S_2K_2, S_2K_2p, SK_2K_4, SK_2K_4p
4 New Trimers: $S_2K_2K_4$, $S_2K_2pK_4$, $S_2K_2pK_4p$, $S_2K_2K_4p$

In the model code we have provided the change equations for the new species. However, **we have not updated the change equations for K_2 and K_2p that will need to account now for binding events involving S_2 as well**. What new terms need to be added to these change equations? Modify the model code to implement these updates (there is a comment in the code indicating where these changes need to be made).

Additional K_2' Terms $= -S_2K_2 \cdot k_a + S_2K_2p \cdot k_d - S_2K_2K_4 \cdot k_a - S_2K_2K_4p \cdot k_a + S_2K_2K_4 \cdot k_d + S_2K_2K_4p \cdot k_d$
Additional K_2p' Terms $= -S_2K_2p \cdot k_a + S_2K_2p \cdot k_d - S_2K_2pK_4 \cdot k_a - S_2K_2K_4p \cdot k_a + S_2K_2pK_4 \cdot k_d + S_2K_2pK_4p \cdot k_d$

We will now compare the activation over time of K_3p vs K_4p in the section of code titled "Dynamics of Crosstalk". Similar to K_3, we impose K_4 has a higher initial concentration and that K_4 and K_4p have weaker affinity (reduced k_a4) for S_2. S_2 has the same initial concentration as the other kinases. All other parameters and initial conditions are as defined previously. Run the code in this section and paste your graph.



How does maximum activation and timing of activation of K_4p compare to that of K_3p? Suppose there are two downstream targets, A that depends only on K_3p, and B that depends both on K_3p and K_4p. How might the activation of A and B differ if the activating signal K_1p is quite transient (short)?

K_3p has a higher maximum activation than K_4p by more than twofold and both phosphorylated kinases reach maximum activation around the same time of t=40 and t=45. For a transient signal K_1p, the activation of A would be greater than the activation of B, but both will most likely not reach the maximum activation because a transient signal isn't continuous so the signal produced will likely die off.