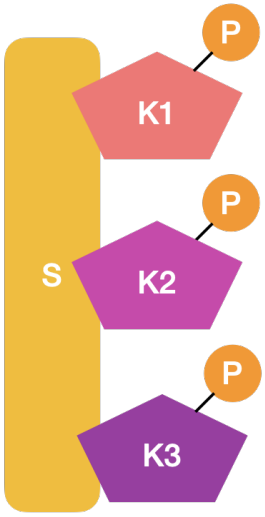
PhySci/MiMG/CaSB M178

Homework 3

Due: 10/18/22 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). 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.

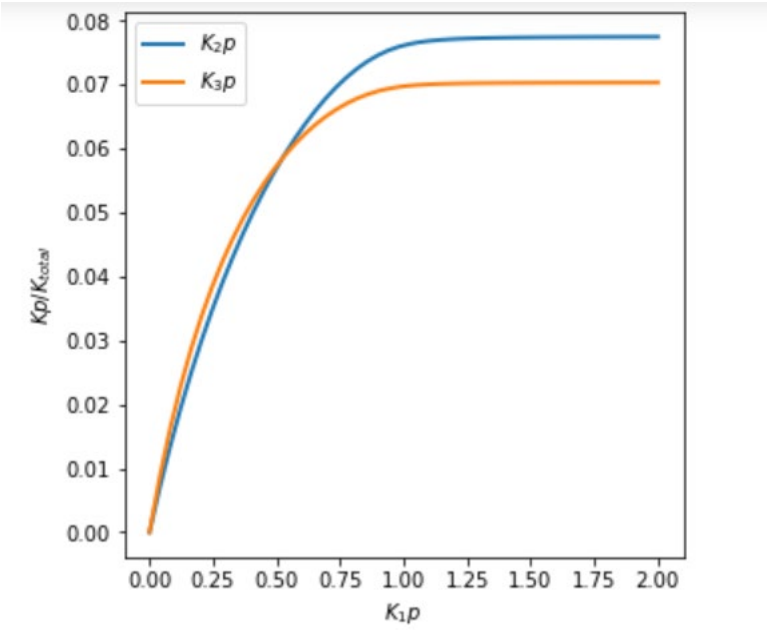
Dimers: 5 dimers (S K1p) (S K2) (S K2p) (S K3) (S K3p)
Trimers: 8 trimers (SK1pK2) (SK1pK2p) (SK1pK3) (SK1pK3p) (SK2K3) (SK2pK3) (SK2K3p) (SK2pK3p)
Tetramers: 4 tetramers (SK1pK2K3) (SK1pK2pK3) (SK1pK2K3p) (SK1pK2pK3p)

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 (1μM), 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. The kinase must be free from the scaffold to act downstream in the nucleus.

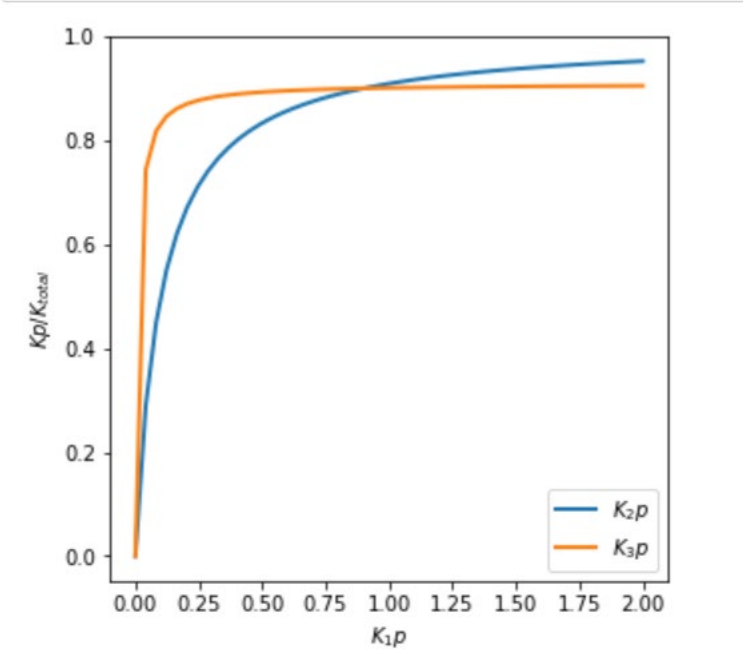
This section of the code then changes the initial value of K_1p , from 0 to 2 (2 times its default value of 1). It then runs a simulation of the scaffold model with those parameters, and saves the result of the final time point. After doing this, it generates a plot of the steady-state value of $K_3p/K_{3\ total}$ 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 <u>paste the resulting graph below:</u>



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 purpose of a scaffold protein is to facilitate signal transduction by bringing/ anchoring together multiple signaling components. In the presence of the scaffold protein, the amount of K1p needed to achieve maximum activation decreases, using K2p as reference. For example, in the two graphs above, the amount of K1p needed to achieve maximum activation without scaffold proteins is about 1.00 for final level kinase K3p, but only ~0.13 with scaffold proteins.

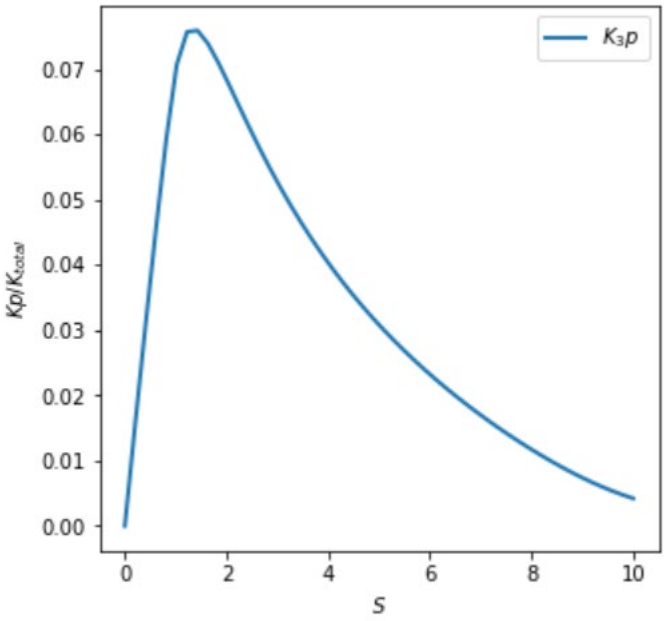
The signal amplification provided by the cascade between the K3p curves is that the Kp/Ktotal ratio has risen to ~ 0.9, which is more than 10 times the ratio for non-scaffold protein interaction, about 0.07. This high specificity cascade phenomenon

shows that all kinases' in the phosphorylated state is transmitting a signal downstream, much more than in the non-scaffold protein scenario.

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 cause for the initial rise in K3p/K3total is because the amount of scaffold proteins has not been saturated by the number of kinases available in the system yet. However, at around concentration of around 2, the formation of phosphorylated/unphosphorylated kinases and the scaffold protein reached a decreasing rate. This is because too high concentrations of assays such as the scaffold protein give rise to interference, and few or no kinase are able to bind to more than one scaffold protein--- free kinases is in competition to docked kinases. The slope of

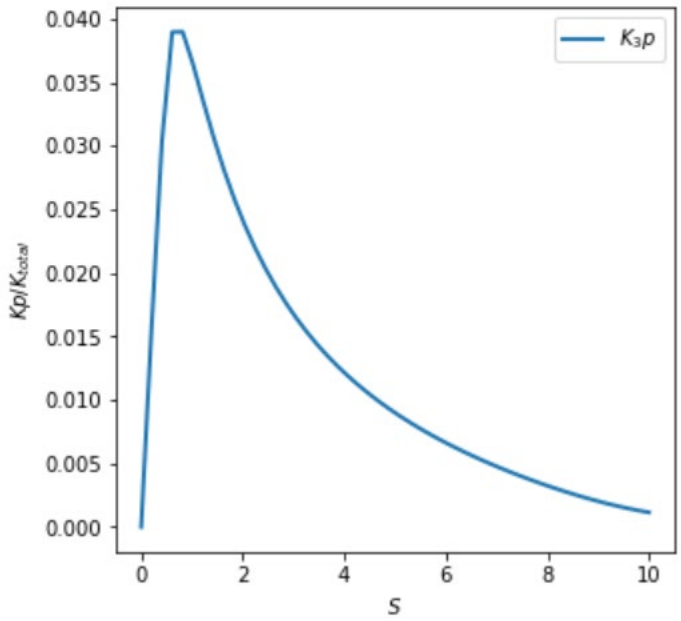
the “prozone” is definitely in a magnitude that is smaller than the increase, but it is significant in that falsely low signal transduction is happening.

The “inhibition” in combinatorial inhibition is this blockage of signal because of excess scaffold protein, and it is decreasing at an exponential rate, hence, “combinatorial”.

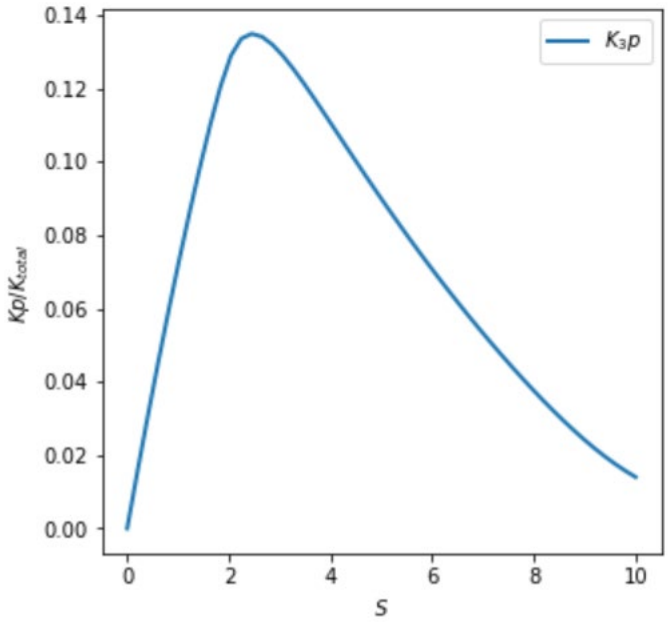
What value of scaffold protein gives a maximal response? Predict how might this value might change if the kinase concentrations were smaller/larger? (Note: you can check your prediction by altering the initial conditions (K1p, K2)).

The value of scaffold protein that gives a maximal response is about ~1.8 or ~1.9 concentration of scaffold protein for an IC of K1p =1 and K2 = 1.

If the kinase concentrations were smaller, for example, K1p = 0.5 and K2 = 0.5. I predict that it will take even less concentration of scaffold proteins to reach the maximum saturation protein for the prozone effect to take place.



If the kinase concentrations were larger, for example, K1p = 2 and K2 = 2. I predict that the corresponding concentration for scaffold proteins increases also, because of the substrate-ligand relationship similar to this scaffold-kinase relationship. It is also worth noting that the Kp/Ktotal ratio is also much higher when the kinase concentration increases to induce more signal transduction.



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:

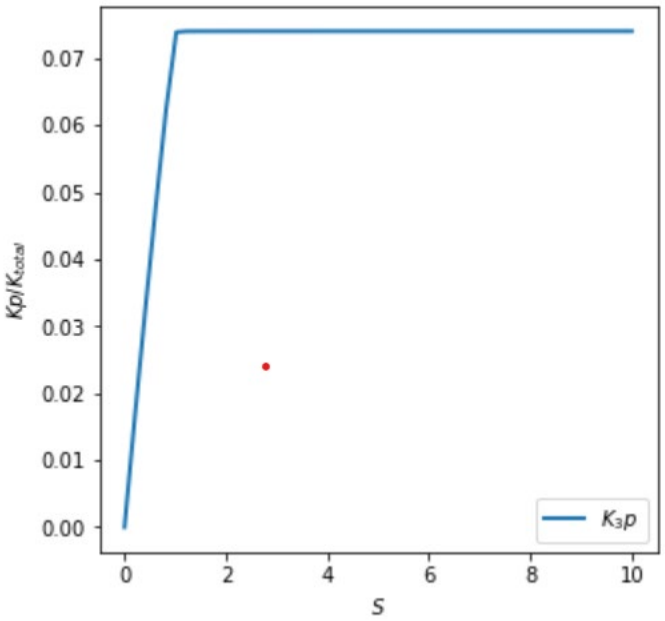
1. Only K_1p can bind to S
2. Only K_2 or K_2p can bind to SK_1p
3. K_3 and K_3p can only bind SK_1pK_2p (at a reduced rate like the previous model)
4. Only K_3 and K_3p can unbind $SK_1pK_2pK_3$ and $SK_1pK_2pK_3p$ respectively
5. Only K_2 and K_2p can unbind to SK_1pK_2 and SK_1pK_2p respectively
6. 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: $S, K_1p,\ K_2p,\ K_3,\ K_3p,$ $SK_1p,\ SK_1pK_2,\ SK_1pK_2p,\ SK_1pK2_pK_3,\ SK_1pK2_pK_3p$.

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_prime = -k_a * K1p * S   +   k_d * SK1p
   K1p_prime = -k_a * K1p * S   +  k_d * SK1p
   K2_prime = -k_a * K2 * SK1p  +  k_d*SK1pK2 + k_u * K2p
   K2p_prime = -k_a * K2p * SK1p + k_d * SK1pK2p - k_u*K2p
   K3_prime = -k_a3 * K3 * SK1pK2p + k_d * SK1pK2pK3 + k_u*K3p
   K3p_prime = -k_a3 * K3p * SK1pK2p + k_d * SK1pK2pK3p -k_u*K3p
   SK1p_prime = (k_a * K1p * S ) - (k_a * SK1p * K2)  - (k_a * SK1p * K2p)  + (k_d *
SK1pK2)  + (k_d * SK1pK2p)  - (k_d * SK1p)
   SK1pK2_prime = k_a * SK1p * K2 - k_d * SK1pK2 - k_p * SK1pK2 + k_u *SK1pK2p
   SK1pK2p_prime = (k_a * SK1p * K2p) - (k_a3 * SK1pK2p * K3) - (k_a3 * SK1pK2p *
K3p) + (k_d * SK1pK2pK3) + (k_d *SK1pK2pK3p) - (k_d * SK1pK2p) + (k_p * SK1pK2)
- k_u *SK1pK2p
   SK1pK2pK3_prime = (k_a3 * K3 * SK1pK2p) - (k_d* SK1pK2pK3) - (k_p *
SK1pK2pK3) + (k_u * SK1pK2pK3p)
   SK1pK2pK3p_prime = (k_a3 * K3p * SK1pK2p) - (k_d * SK1pK2pK3p)  + (k_p *
SK1pK2pK3) -(k_u * SK1pK2pK3p)
```

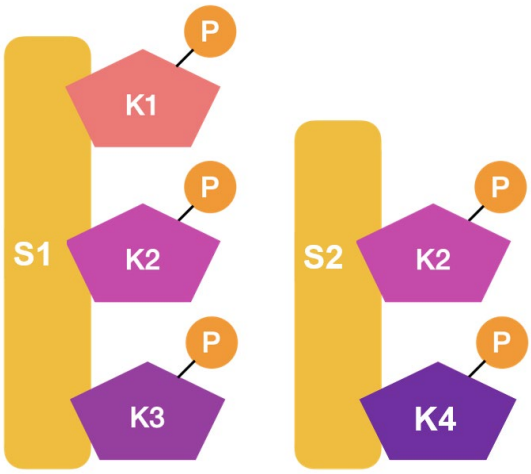
We will again examine the steady-state prozone effect as we did in question 2, but now for our new model. The next section of code again varies the concentration of the scaffold protein, S, and reports the output signal $K_3p/K_{3\ total}$. Run the code in the section "Prozone effect--Ordered binding"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



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

The results differ from the unordered binding case in that there is no "hook" behavior after the anchor point (if there even is an anchor point) in the graph above. It shows that the Kp/Ktotal ratio is about 0.075, and that the trend plateaus and reaches stability or equilibrium point. This is because there is no more combinatorial inhibition and that there is a sequential order to kinase binding to the scaffolds, and the kinase-scaffold interactions can go to completion and stabilize thereafter. Ordered models can only fall apart in one way, and it is much simpler.

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.

The number of new dimers and trimers are:

Dimers: 4 dimers (S2K2), (S2K2p), (S2K4), (S2K4p)
Trimers: 4 trimers (S2K2K4), (S2K2pK4), (S2K2K4p), (S2K2K4p)

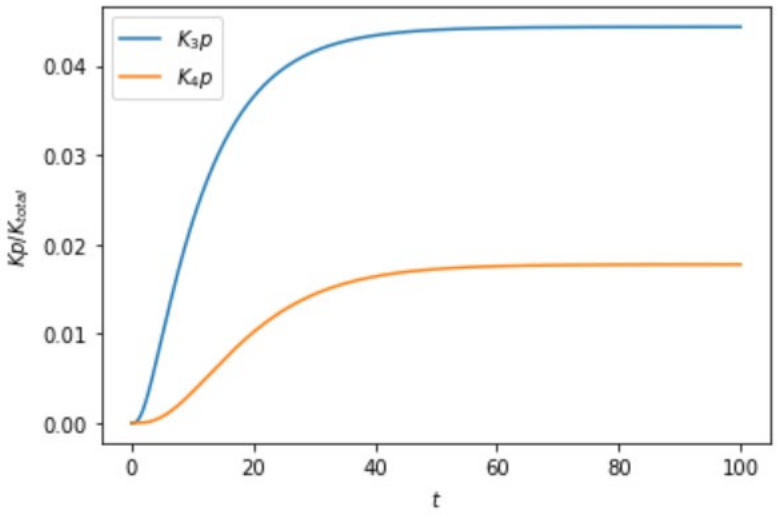
In the model code we have provided the change equations for the new species. However, **we have not updated the change equations for K_2 and K_2p that will need to account now for binding events involving S_2 as well**. What new terms need to be added to these change equations? Modify the model code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

The new terms that need to be added to these change equations are K2' and K2p'

-k_a*S2*K2 - k_a*S2K4*K2 - k_a*S2K4p*K2 + k_d*S2K2 + k_d*S2K2K4 + k_d*S2K2K4p
#added to K2'

-k_a*S2*K2p -k_a*S2K4*K2p -k_a*S2K4p*K2p + k_d*S2K2p + k_d*S2K2pK4 + k_d * S2K2pK4p
#added to K2p'

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)?

The maximum activation of K4p is much lower than the maximum activation of K3p (with a very different kp/ktotal ratio), however, the timing of activation is fairly close in proportion to each other. Whereas K4p has a max activation of 0.015, K3p has a max activation of 0.045. Both timing of activation happens around 40 to 45 seconds.

If there are two downstream targets, then the activation of A might be stronger because K4p's maximum activation is much lower for B to be dependent on. Should it be ordered binding, where K3p enables the binding of K4p, then the activation of B will take place much later than activation of A. Since K1p will stop signaling in a short period of time, by the time the signal reaches K4p, there may not be enough signal to cause a maximum response.