PhySci/MiMG/CaSB M178

Homework 2

Due: 10/11/22 at 12:00PM PDT

Notes: This homework involves performing simulations of the Kinase Cascade we've been discussing in the last two class meetings.

Problems

In class, we talked about a kinase cascade, such as the MAPK signaling pathway. Here is an example model with three layers:

$$K_1^* + K_2 \xrightarrow{k_a} K_1^* + K_2^*$$
$$K_2^* + K_3 \xrightarrow{k_a} K_2^* + K_3^*$$
$$K_3^* + K_4 \xrightarrow{k_a} K_3^* + K_4^*$$

Upon a receptor binding event at the cell surface, the first kinase species, K_1, is changed (phosphorylated) to its active form, K_1^* (not modeled here). This active kinase species then activates the next kinase species, K_2, and so on.

The active kinase species also experience deactivation (dephosphorylation) modeled as follows:

$$K_2^* \xrightarrow{k_u} K_2$$
$$K_3^* \xrightarrow{k_u} K_3$$
$$K_4^* \xrightarrow{k_u} K_4$$

We can write the change equations like we discussed in class for this system:

$$K_2' = -k_a K_1^* K_2 + k_u K_2^*$$
$$K_2^{*\prime} = k_a K_1^* K_2 - k_u K_2^*$$
$$K_3' = -k_a K_2^* K_3 + k_u K_3^*$$
$$K_3^{*\prime} = k_a K_2^* K_3 - k_u K_3^*$$
$$K_4' = -k_a K_3^* K_4 + k_u K_4^*$$
$$K_4^{*\prime} = k_a K_3^* K_4 - k_u K_4^*$$

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 under "3 layer model". You will need to implement the "4 layer model" and the "5 layer model".

First write down the new change equations that need to be added to the "3 layer model" to describe the "4 layer model". This model has the additional reactions:

$$K_4^* + K_5 \xrightarrow{k_a} K_4^* + K_5^*$$
$$K_5^* \xrightarrow{k_u} K_5$$

write the change equations for these additional reactions here:

K_fiveprime = -ka * K_five * K_fourstar + ku * K_fivestar
K_fivestarprime = -ku* K_fivestar + ka * K_five * K_fourstar

Add these change equations to the Jupyter notebook to complete the "4 layer model".

Second write down the new change equations that need to be added to the "4 layer model" to describe the "5 layer model". This model has the additional reactions:

$$K_5^* + K_6 \xrightarrow{k_a} K_5^* + K_6^*$$
$$K_6^* \xrightarrow{k_u} K_6$$

write the change equations for these additional reactions here:

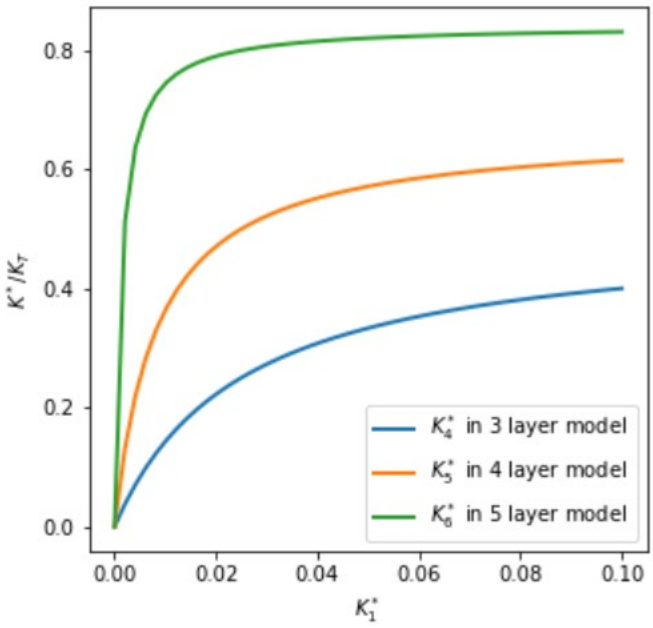
K5_prime = -ka * K_five * K_fourstar + ku * K_fivestar
K5star_prime = -ku* K_fivestar + ka * K_five * K_fourstar
K6_prime = - ka * K6 * K_fivestar + ku* K_sixstar
K6star_prime = -ku* K_sixstar + ka * K6 * K_fivestar

Add these changes equations to the Jupyter notebook to complete the "5 layer model".
You will see that below there is a section called "STEADY-STATE responses". Here we will vary the amount of K_1^*, the activating signal, in the system and observe how it changes the steady state amount of final kinase activation for our different sized cascades.

Note the initial conditions (shared for the 3, 4, and 5 layer models) are set at the beginning of this section of code such that all of the downstream kinases are in the inactive form at the start. Furthermore, moving down the layers, the total amount of kinase protein doubles.

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_1^*, from 0 to 0.1 (double its default value of 0.05). It then runs a simulation of the 3, 4, and 5 layer models 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 $K_{\#}^*/K_{\#total}$ as a function of the parameter K_1^*. Here # refers to the final kinase in each cascade (# = 4 for the 3-layer model, # = 5 for the 4-layer model, # = 6 for the 5-layer model).

Run this code and paste the resulting graph below:



How does the amount of K_1^*, the activating signal, that is needed to achieve maximum activation of the final level kinase vary with the number of levels in the cascade? How might then a kinase cascade provide "signal amplification"?

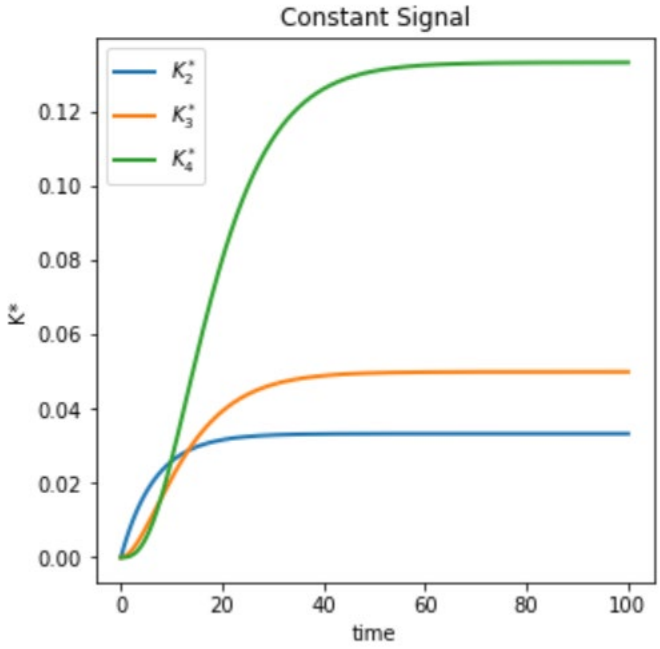
There needs to be a much higher ratio of the $K_{\#}^*/K_{\#total}$ for a n-layer model, as n gets larger and larger, which means the activating signal must be large to support the number of levels in the cascade.

A kinase cascade provides "signal amplification" by having one (or limited amount of) ligand and creating a series of responses instead of one phosphorylating response. This has the benefit for remaining in active stage for much longer than a single layer (for example), and can process more pathways using, say the same original signal.

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 the activation of the different kinases for the three-level model.

We will first look at the dynamics of the 3-layer model defined previously. Notice again, that there is a section to define the initial conditions, which are the same as the previous simulations. Then there is a section to plot kinase activation versus time.

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



How does the time until of maximum activation of each kinase vary with level?

The curves reach maximum activation of each kinase at incremental levels, and the time also increases. For K2*, it takes the least amount of time to reach steady-state or equilibrium, about 20 seconds. For K3*, it takes about 40 seconds to reach steady-state. However, for K4*, it takes about 55 seconds.

In the above simulation the activating signal, K_1^*, is on and constant in value for the entire simulation. Predict how might the maximum activation of the kinases change if the activating signal was transient (in other words, if it was active only for a short time)?

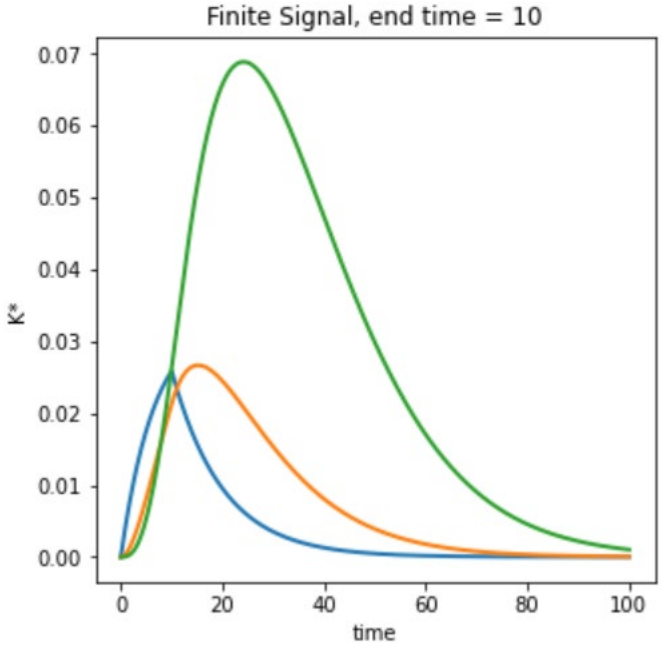
I predict that, if the activating signal was transient, then the maximum activation of the kinases will not exhibit the current plateau behavior, but instead be an all-or-nothing behavior where the threshold is not reached and the K* level will drop to 0.

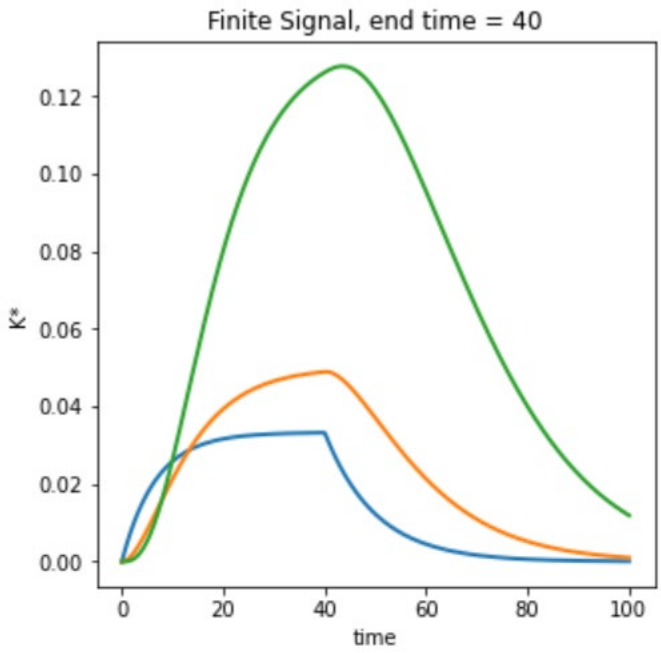
3) (15 points) Underneath the section where you plot the kinase activation over time, the code next defines a new model, "3 layer model with finite signal", where K_1^*, the activating signal, can be turned off after some time; this time is called "end_signal". You will test your prediction by varying the input for the "end_signal" time.

Based on your results above, you will need to choose **2 different** values of the "end_signal" time to use in these simulations. Note that "end_signal" should always be less than the max value of time you use for your time vector "t."

Run the code and paste your graph ("# PLOT dynamics of kinase activation with finite signal") below for the **2 different values** of "end_signal" you chose. Do the simulation results support the prediction you made in your answer to question #2 above?

Given that the max value of time I use for t is 100, I have chosen 40 and 10. The simulation results support the prediction I have made in my answer because signals or levels of K* will drop after finite amount of signal is put into the system.

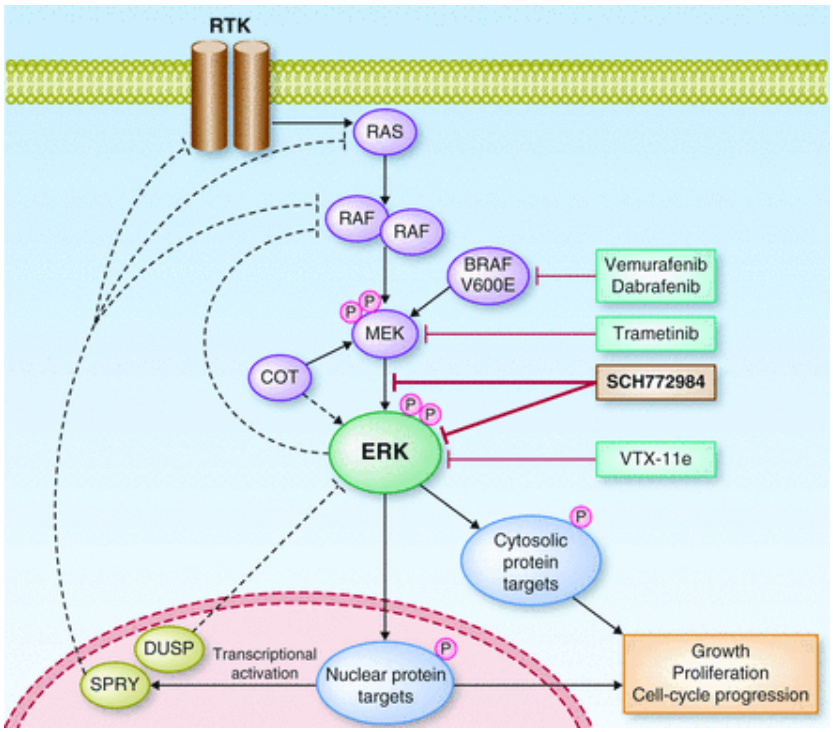




As kinase cascades contain more and more layers, how might the activating signal need to be modified in order to achieve maximum activation of the final kinase in the cascade?

As kinase cascade contain more and more layers, the activating signal needs to be modified in such a way that there is more activating signal so that it does not get depleted when the cascading effect propagates to the final kinase. Another potential way to modify is to elongate the end time because the n layers follow a dramatic increase following the n-1 layer, longer persistence or longer end time will allow the K* to be as high as possible (peak value) for the final kinase. For example, the end time of 40 had K4*'s trend to be 0.05 higher than end time of 10. In this way, the cascade will be able to achieve maximum activation of the final kinase in the cascade.

4) (15 points) The MAPK signaling pathway is overactivated in several cancers. Inhibitors of kinases in the pathway have been developed as pharmacologic therapeutics. Trametinib was the first MEK inhibitor developed and is used to treat melanoma patients with BRAF V600E mutations.



Nissan MH, Rosen N, Solit DB. ERK Pathway Inhibitors: How Low Should We Go? *Cancer Discovery*. 2013;3(7):719-721. doi:10.1158/2159-8290.cd-13-0245

Aligning the MAPK pathway with our 3-layer model above, RAS is the activating signal, K_1^*, RAF is K_2, MEK is K_3, and ERK is K_4. We want to model the effect of Trametinib administration on ERK activation and will do so by adding the following reaction:

$$K_3^* + T \overset{k_i}{\rightarrow} K_3^* T$$

The complex $K_3^* T$ sequesters the active K_3^* complex and prevents it from activating K_4.

Write the modified change equation for K_3^* to account for this additional reaction:

K3star_prime = k_a*K2star*K3 - k_u*K3star – k_i * K3star * T + k_d* K3starT

There is a section of code called "3 layer model with kinase inhibitor" that defines the new model. Add your modified change equation to the code.

Trametinib binding is in fact reversible, so we additionally have the following reaction.

$$K_3^*T \xrightarrow{k_d} K_3^* + T$$

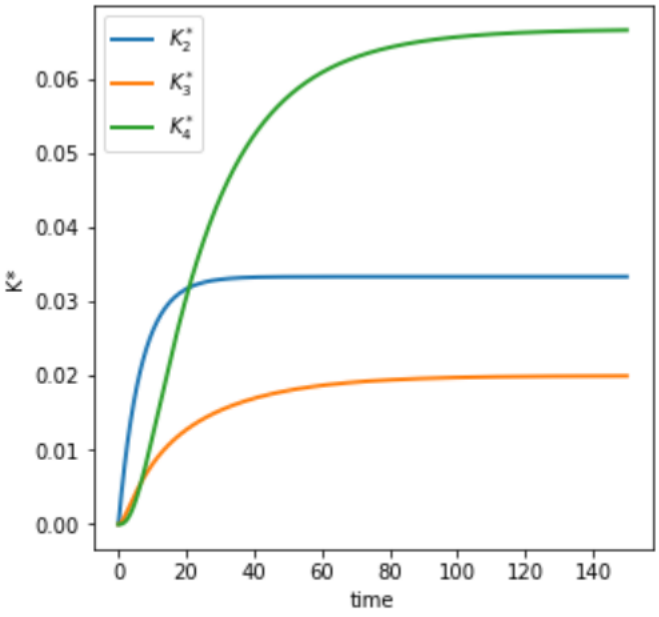
Write the change equation for K_3^*T and add it to the model code as well:

K3starT_prime = k_i * K3star * T – k_d* K3starT

We will assume the drug is highly abundant and concentration remains constant throughout the simulation. Below the ode model code, we set the parameters and initial conditions. Notice that we initialize K_3^*T to zero at the start of the simulation.

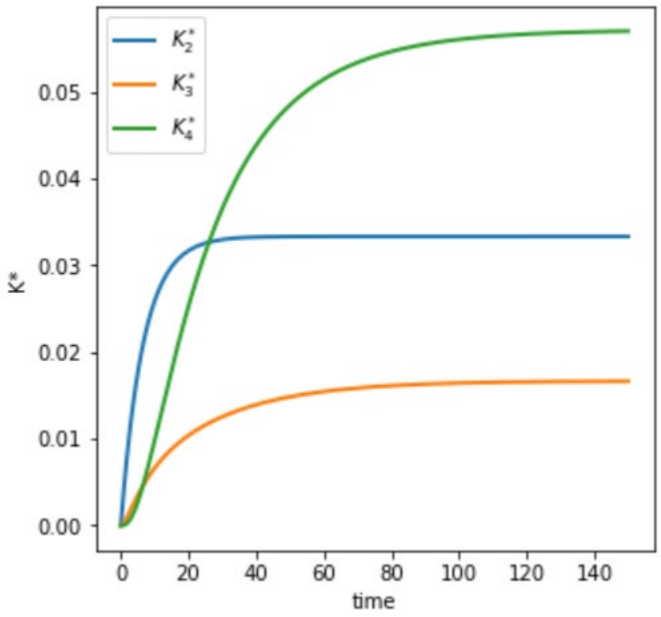
Run the code and paste your graph (# PLOT dynamics of kinase activation with inhibitor.") below for a drug concentration of 0.6.

```
<matplotlib.legend.Legend at 0x136c1656550>
```



Choose an increased value of drug concentration and re-run the code. Paste your graph for this increased value of the drug concentration below (indicate how much drug you chose to add here).

T = 0.8



Compare your two graphs with T present to the one you obtained with no drug (i.e. the answer to question #2 above). How does increasing the drug concentration alter the timing and amplitude of K_4 activation?

When comparing my two graphs with T present, we notice that the K2* trend have been static all across, regardless of drug concentration--- this is the trendline that reaches 0.03 as the equilibrium or steady-state. Since we know that K3* is actively being sequestered from high concentration of the drug, then the higher concentration of drug, the lower the amplitude of K* the K4* activation trendline is going to be.