PhySci/MiMG/CaSB M178

Homework 2

Due: 10/17/23 at 12:00PM PDT

Notes: This homework involves performing simulations of the Kinase Cascade we've been discussing in the last two class meetings. In the same assignment on BruinLearn where you obtained this document, you will also find a file called "HW2_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 BruinLearn**; to do so, navigate to the "Assignments" section on the left-hand side of the course BruinLearn website. There you will see an assignment entitled "Homework 2." You can upload your Jupyter file (which should be a .ipynb file). Make sure you upload your Jupyter notebook by the due date/time (10/17/23 at 12:00PM PDT).

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:

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

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:

$$
\begin{aligned}
K_4^* + K_5 &\xrightarrow{k_a} K_4^* + K_5^* \\
K_5^* &\xrightarrow{k_u} K_5
\end{aligned}
$$

write the change equations for these additional reactions here:

$K_5' = -K_5 \cdot K_4^* \cdot k_a + K_5^* \cdot k_u$

$K_5^{*\prime} = +K_5 \cdot K_4^* \cdot k_a - K_5^* \cdot k_u$

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:

$$
\begin{aligned}
K_5^* + K_6 &\xrightarrow{k_a} K_5^* + K_6^* \\
K_6^* &\xrightarrow{k_u} K_6
\end{aligned}
$$

write the change equations for these additional reactions here:

$K_6' = -K_6 \cdot K_5^* \cdot k_a + K_6^* \cdot k_u$

$K_6^{*\prime} = +K_6 \cdot K_5^* \cdot k_a - K_6^* \cdot k_u$

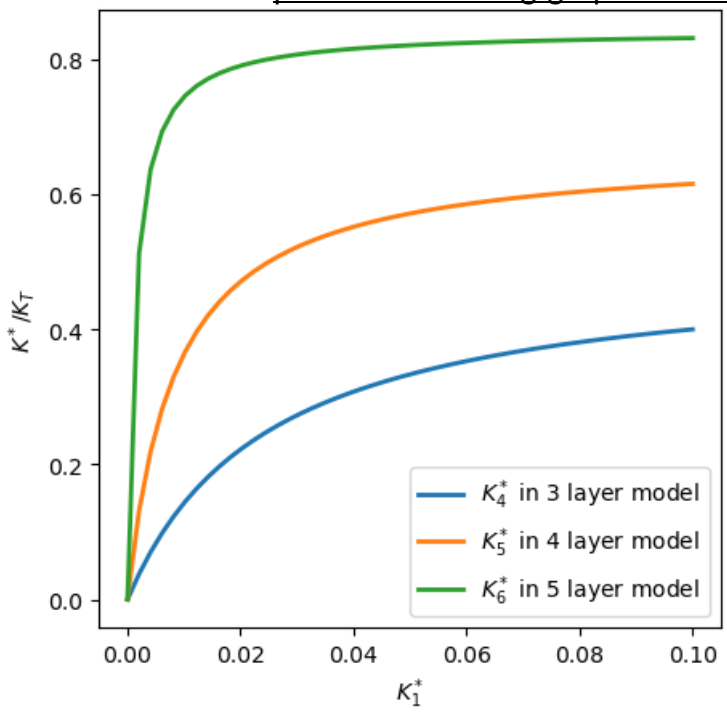
Add these changes equations to the Jupyter notebook to complete the "5 layer model".

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:



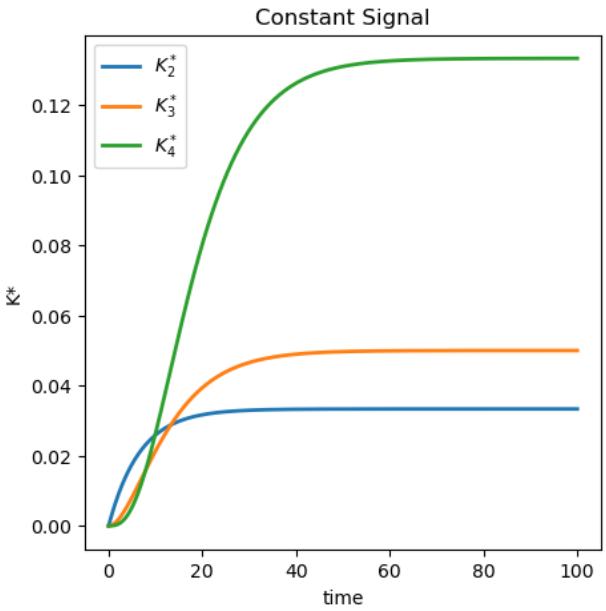
How does the amount of K_1^*, the activating signal, that is needed to achieve maximum activation of the final level kinase vary with the number of levels in the cascade? How might then a kinase cascade provide "signal amplification"?

When there's a low amount of K_1*, there tends to be a lower amount of final level kinase in the system, but as you increase the amount of K_1^*, there tends to be a higher amount of final level kinase in the system up to a certain point until it reaches a maximum amount where adding additional K_1^* doesn't yield more final level kinase. You can see that the curve increases vertically and steeply up to a certain point and then levels off gradually as K_1^* is increased. However, when there's more levels in the cascade, less amount of K_1^* is needed to reach maximum activation of the final level kinase. So, given the same amount of K_1^*, if there's more levels in the cascade, there will be a higher amount of final level kinase in the system. A kinase cascade could provide signal amplification by activating higher level kinases, which in turn activates other higher level kinases, which amplifies a signal through a "multiplier" effect.

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?

As you increase the number of levels, the time until maximum activation of the final kinase increases. You can see that the final kinase of the lowest level reaches its maximum of a certain amount in the shortest time while the final kinase of the highest level takes the longest before its maximum activation of the final kinase levels off.

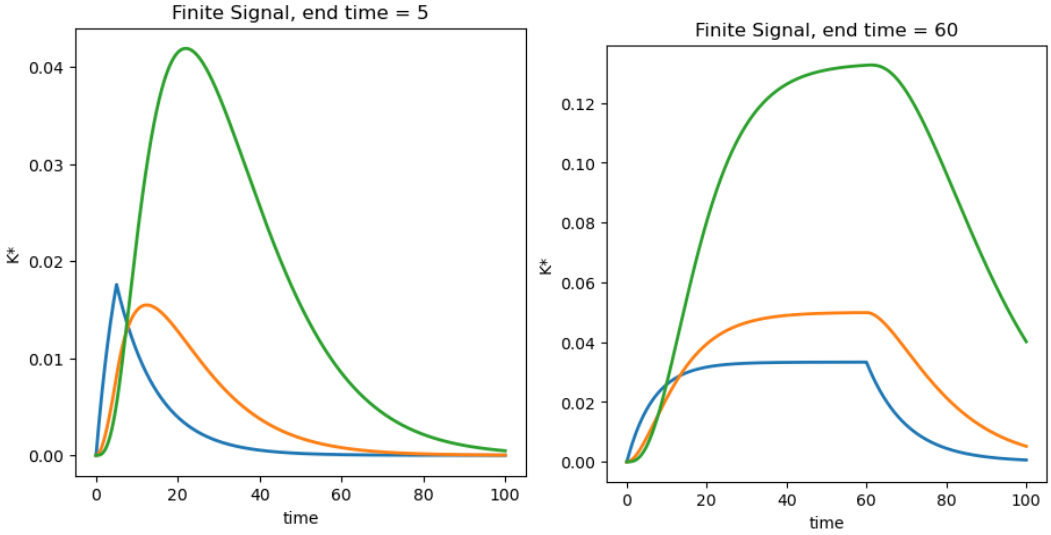
In the above simulation the activating signal, K_1^*, is on and constant in value for the entire simulation. Predict how might the maximum activation of the kinases change if the activating signal was transient (in other words, if it was active only for a short time)?

The maximum activation of the kinases would most likely decrease and will not be near its maximum activation because there isn't a constant activating signal continually activating downstream kinases. The maximum activation of the kinases could still be reached but it would also decrease to 0 over time because there isn't a continuous input signal that keeps the final kinase levels activated.

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?

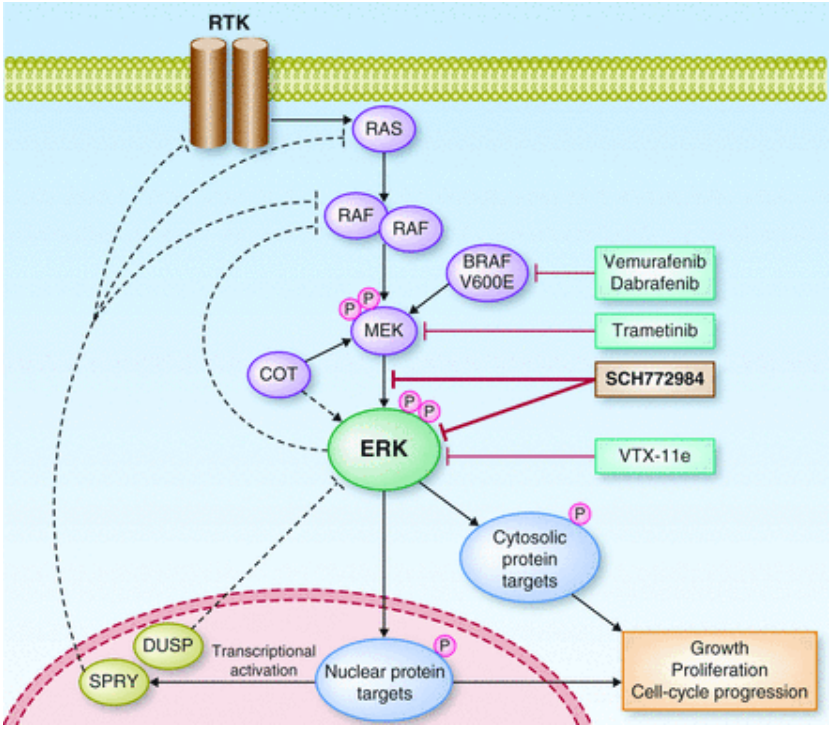


Yes, the simulations results do support my prediction because over time, the kinase level decreases and returns to 0. It also doesn't reach the maximum activation level when the finite signal time is short.

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?

You'll need more activating signal or longer duration of the activating signal in order to achieve maximum activation of the final kinase because there's more layers for the signal to have to amplify to.

3) (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:

$$K_3^{*\prime} = k_a K_2^* K_3 - k_u K_3^* - T \cdot K_3^* \cdot k_i + K_3^* T \cdot k_d$$

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.

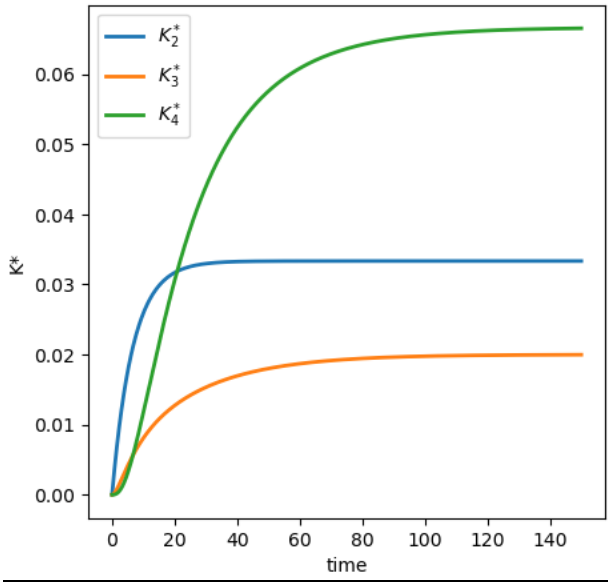
$$K_3^*T \xrightarrow{k_d} K_3^* + T$$

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

$$K_3^*T' = T \cdot K_3^* \cdot k_i - K_3^*T \cdot k_d$$

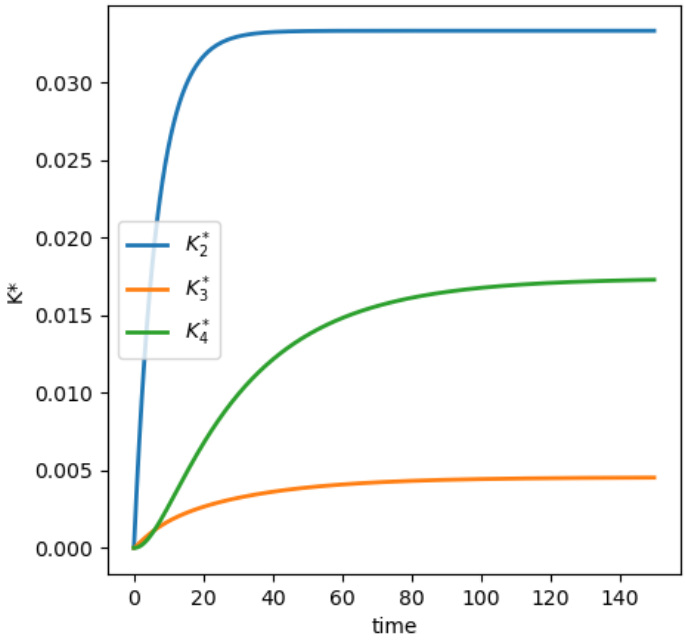
We will assume the drug is highly abundant and concentration remains constant throughout the simulation. Below the ode model code, we set the parameters and initial conditions. Notice that we initialize K_3^*T to zero at the start of the simulation.

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



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).

Drug Concentration (T) = 4



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?

Increasing the drug concentration results in a higher amount of K_2^*, but a lower amount of K_3^* and K_4^*. There is now also a different order of highest amount of final kinase level where K_2^* has the highest maximum amount, followed by K_4^*, then K_3^*. It also appears that K_4^* takes slightly more time to reach its maximum amount.