PhySci/MiMG/CaSB M178

Homework 6

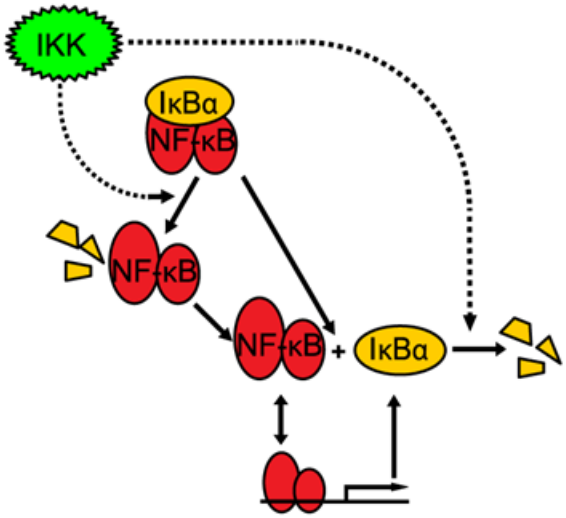
Due: 11/14/23 at 12:00PM PDT

Notes: This homework involves performing simulations of negative feedback we've been discussing in the last two class meetings. In the same folder on BruinLearn where you obtained this document, you will also find a file called "HW6_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 "Homework" section on the left-hand side of the course CCLE website. There you will see an assignment entitled "Homework 6 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 (11/14/23 at 12:00PM PDT).

Problems

In class, we talked about NFκB signalling:



Longo et al. 2013, PLOS *Comput. Biol.*
(https://doi.org/10.1371/journal.pcbi.1003112)

As a reminder, IκBα is a negative regulator of NFκB that is degraded with the activation of IKK, allowing NFκB to translocate into the nucleus and fulfill its role as a transcription factor. NFκB activation leads to downstream transcription of IκBα creating a feedback loop. In summary we have the following reactions:

Reactions	**Description**
$NF\kappa B + I\kappa B\alpha \xrightarrow{k\_a} NF\kappa B{:}I\kappa B\alpha$	Association of NFκB with IκBα
$NF\kappa B{:}I\kappa B\alpha \xrightarrow{k\_d} NF\kappa B + I\kappa B\alpha$	Dissociation of NFκB with IκBα
$IKK + NF\kappa B{:}I\kappa B\alpha \xrightarrow{r} IKK + NF\kappa B$	IKK-mediated degradation of IκBα bound to NFκB
$IKK + I\kappa B\alpha \xrightarrow{r} IKK$	IKK-mediated degradation of IκBα
$I\kappa B\alpha \xrightarrow{g}$	Constitutive degradation of IκBα
$NF\kappa B + \ prI\kappa B\alpha \xrightarrow{f\_a} NF\kappa B{:}prI\kappa B\alpha$	NFκB binding to IκBα promoter
$NF\kappa B{:}prI\kappa B\alpha \xrightarrow{f\_d} NF\kappa B + \ prI\kappa B\alpha$	NFκB unbinding to IκBα promoter
$prI\kappa B\alpha \xrightarrow{a} prI\kappa B\alpha \ + \ I\kappa B\alpha$	Constitutive synthesis of IκBα (delayed reaction)
$NF\kappa B{:}prI\kappa B\alpha \xrightarrow{b} NF\kappa B{:}prI\kappa B\alpha \ + \ I\kappa B\alpha$	Induced synthesis of IκBα (delayed reaction)

The synthesis of IκBα are "delayed reactions" because the cellular processes that compose protein synthesis (transcription, translation, nuclear import/export, etc.) take time. Hence in the model, the amount of IκBα produced at time *t* is dependent on the amount of active promoter present at time *t*-τ, where τ is the delay variable (i.e. the amount of time required for protein synthesis). We call such models "delay differential equations" (DDE). We can add the "_τ" ending to variable names to indicate that the reaction depends on the concentration of the species "τ" units of time in the past, thus we can rewrite our IκBα synthesis reactions as the following:

$prI\kappa B\alpha\_\tau \xrightarrow{a} prI\kappa B\alpha\_\tau \ + \ I\kappa B\alpha$	Constitutive synthesis of IκBα
$NF\kappa B{:}prI\kappa B\alpha\_\tau \xrightarrow{b} NF\kappa B{:}prI\kappa B\alpha\_\tau \ + \ I\kappa B\alpha$	Induced synthesis of IκBα

1. (20 points) First write down the change equations for the model described above. For the IκBα synthesis terms, write them in terms of the delayed variables, prIκBα_tau and NFκB:prIκBα_tau.

NFκB: -k_a*NFKB*IKBa +k_d*NFKB_IKBa +r*NFKB_IKBa*IKK -f_a*NFKB*prIKBa +f_d*NFKB_prIKBa

IκBα: -k_a*NFKB*IKBa +k_d*NFKB_IKBa -r*IKBa*IKK -g*IKBa +a*prIKBa_tau +b*NFKB_prIKBa_tau

NFκB: IκBα: +k_a*NFKB*IKBa - k_d*NFKB_IKBa -r*NFKB_IKBa*IKK

prIκBα: -f_a*NFKB*prIKBa +f_d*NFKB_prIKBa -a*prIKBa_tau +a*prIKBa_tau

NFκB: prIκBα: +f_a*NFKB*prIKBa -f_d*NFKB_prIKBa -b*NFKB_prIKBa_tau +b*NFKB_prIKBa_tau

In the section of code called "NFKB - IKBa model" implement the change equations to simulate the model. Note that the values of prIκBα_tau and NFκB:prIκBα_tau are calculated within the model code in the section "delayed variables". You should use these values in your change equations implementation.

Once you have defined your model equations, run the cell containing the model code and the following cell titled "delay helper functions". These functions are used to look up the old values of prIκBα and NFκB:prIκBα needed by the model.

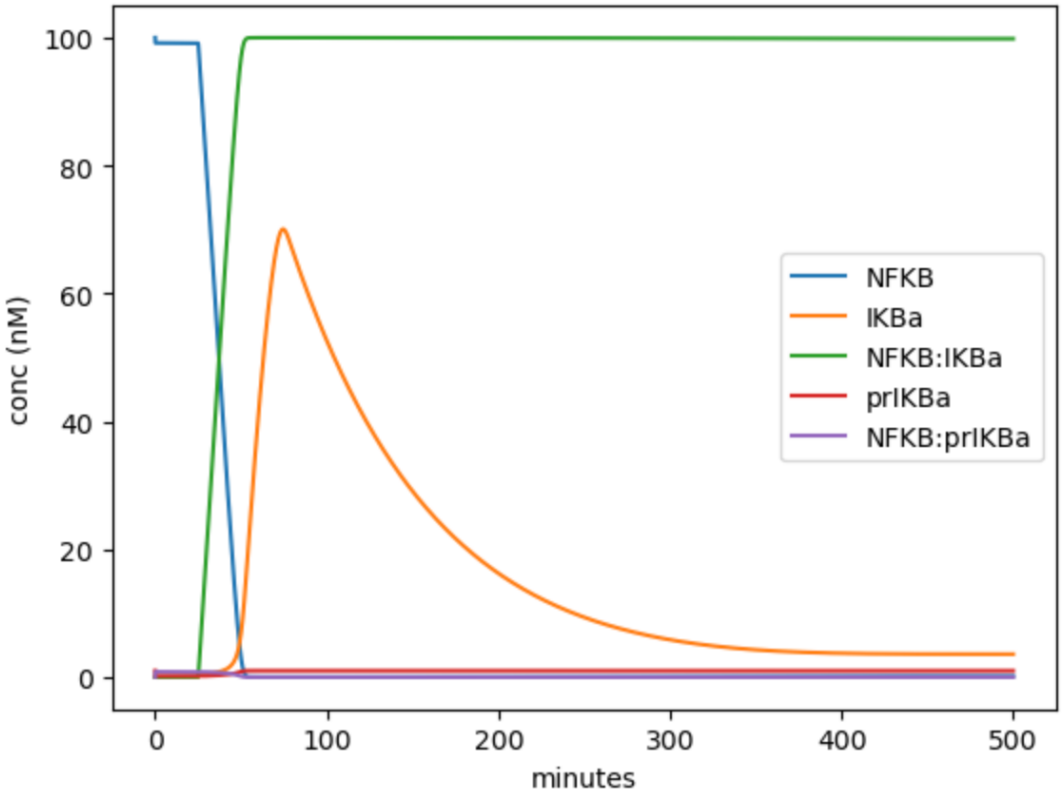
Before we can simulate NFKB responses to IKK activation, we have to find the steady state values of the model species to use as initial conditions for downstream simulations. In the section, "Steady State Simulation", we will find the steady state values given some constraints. First, we assume there is a total of 100 nM of NFκB and "1 nM" of prIκBα (we approximate prIκBα as a continuous variable here, although in reality it can only take on nonnegative integer values). All other species are set to zero for now and this is implemented under "initial condition constraints". Below that we specify the model parameters. Note that all units are nM, $(nM*min)^{-1}$, or min^{-1}. Finally, we specify the parameters of IKK activation (IKK_on_time, IKK_off_time, IKK_amplitude), which are all set to zero to obtain the desired steady state result.

Run the section of code for "Steady State Simulation". The ODE solving looks a bit different from past homework, since here we need to solve the ODE system in pieces to call on past values of the delayed variables. **Because of this, the code will take a bit longer to run than in the past; be patient!** The last line of code in this section saves the final entry of the solution (steady_state) to be used as initial conditions for downstream simulations with IKK activation.

Run the section of code for "Checking Model Implementation". Check to see that the values on the right (from simulation of your model implementation) match the values on the left (from simulation of the correct model implementation). If the values don't match, double check your change equations and code before proceeding.

Run the section of code called "Plot Dynamics" and paste your graph here. State whether your solution appears to reach steady state. If so, we can use the

saved steady state value. If not, increase the value of the variable *iterations* (default = 20) in the steady state simulation section. Rerun the steady state simulation and plotting until satisfied with the steady state result.

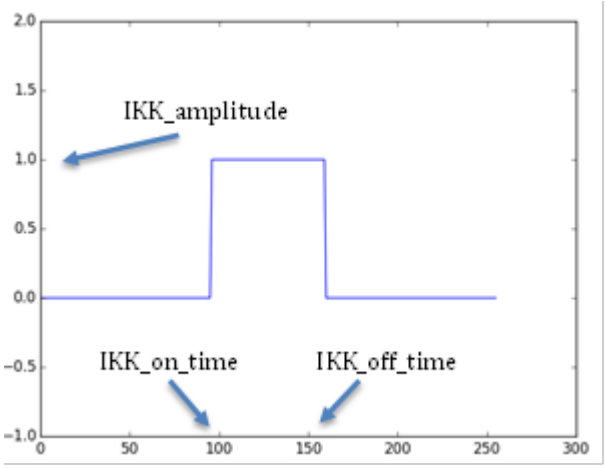


When the number of iterations is set to 20, the solution **appears to reach steady state** because the concentration of each species is unchanging over time. There is a constant horizontal line for each species that doesn't oscillate or change in the long run.

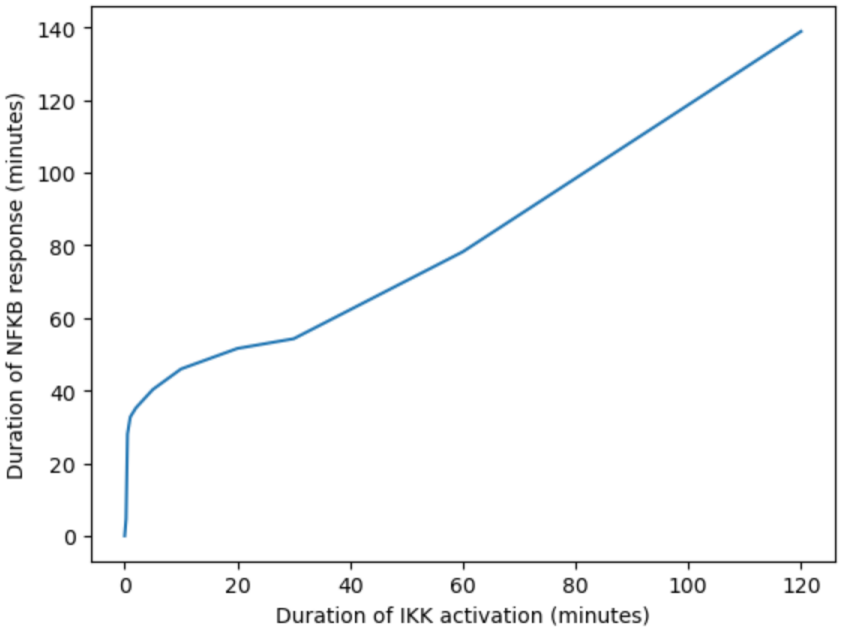
What's the dominant form of NFκB at steady state (free or bound to IκBα)?

The dominant form of NFkB at steady state is NFkB:IKBa, which has the highest species concentration of 100 nM at steady state while free NFkB has a steady state concentration of less than 10 nM at steady state. **The dominant form of NFkB at steady state is bound to IKBa.**

2. (20 points) Now we will simulate NFκB responses to IKK activation. In these simulations, the profile of IKK activation will look like a box function:



Run the section of code "NFKB responses to IKK activation" to define our model simulations. We are just putting the code that solves for our model inside of a function call so we can more easily reuse this piece of code. **Next, we will run the section of code called "Duration of NFKB response versus IKK duration".** For these simulations we will activate IKK at time = 0 (IKK_on_time = 0) and set its amplitude to 10, a high value. We will then vary the duration of IKK activation by changing IKK_off_time, all the way from 15 second (0.25 minutes) to 2 hours (120 minutes). For each IKK activation profile, the simulation will run and the code then finds the amount of time the NFκB response is above a specified threshold (NFKB_threshold = 1 by default). **Run the section of code "Plot IKK activation versus NFKB response duration" and paste the resulting graph here.**



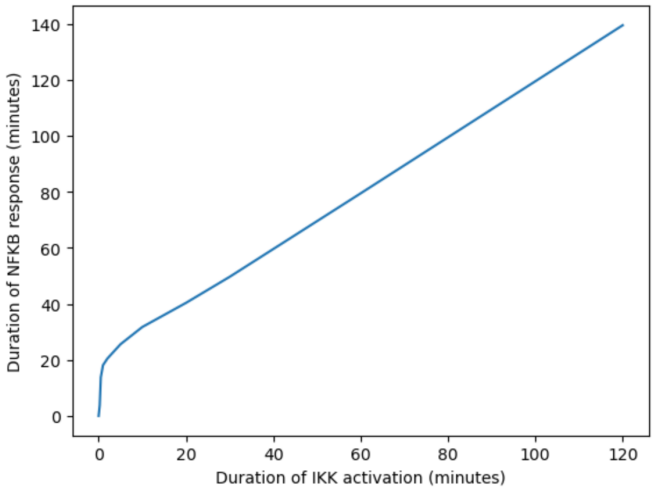
How does NFKB response duration vary with the duration of IKK activation? Can you mechanistically explain the difference in behavior for low values of IKK activation duration versus high values?

NFkB response duration increases as the IKK activation duration increases. When there are low values of IKK activation, there appears to be a non-linear and rapid increase in NFkB response duration as IKK activation duration increases. However, at relatively high values of IKK activation duration (>40 minutes), there is a relatively linear increase in NFkB response duration as a result of increased IKK activation duration.

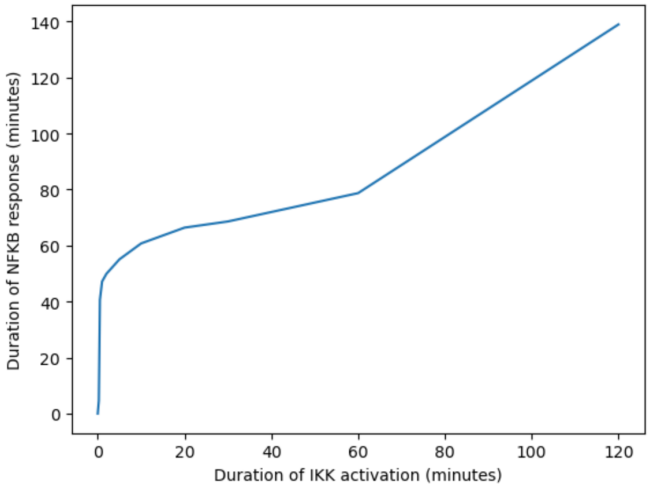
This difference could be due to the fact that the NFkB signaling pathway has multiple steps, which could add a delay. At lower levels of IKK activation duration, NFkB is likely rapidly released from IKBa due to the negative feedback loop not being activated yet. IKBa is less likely to be degraded and more likely to be bound to NFkB so the NFKB response hasn't caught up yet. As IKK activation persists and IKK values are high, NFkB release can translocate into the nucleus and could result in more IKK production, which then inhibits NFKB and creates a negative feedback loop. Eventually, there will be an equilibrium, where the feedback mechanisms catch up with the initial stimulus, which could explain the linear relationship.

Now we will rerun this entire exercise with a different value for the delay parameter. We will try the delay parameter, tau, set to 10 minutes and 40 minutes. Return to the section of code called "Steady State Simulation". Change the value of tau accordingly and rerun the section of code. Next rerun the section of code "Plot Dynamics" and once again ensure steady state is reached. Then rerun the section of code "NFKB responses to IKK activation" and "Duration of NFKB response versus IKK duration". Finally, plot the result from the section "Plot IKK activation versus NFKB response duration". Paste your resulting graph here and repeat the entire process for the other value of tau.

tau = 10 minutes:



tau = 40 minutes:



How does the relationship between duration of IKK activation and NFKB response duration change with different values for the delay parameter, tau?

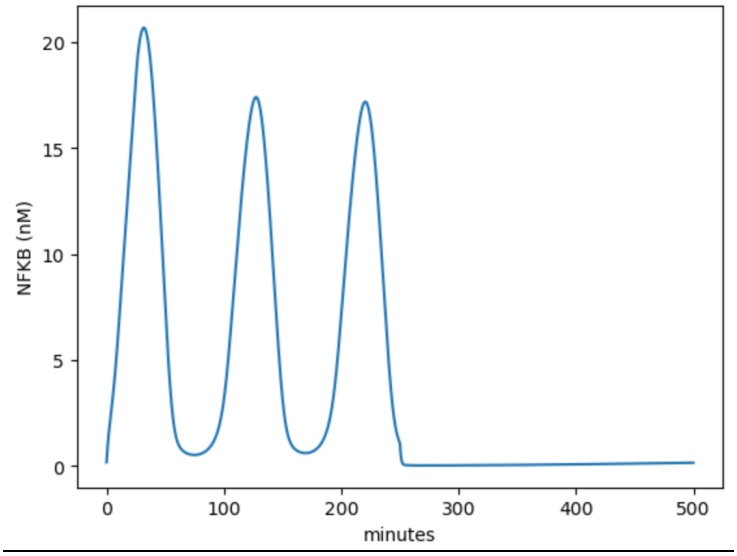
When tau is a lower value, the relationship between IKK activation and NFKB shows a response curve that's also steep initially but transitions to a linear phase earlier, suggesting that the negative feedback mechanism takes less time to activate with a smaller delay of tau. After 5 minutes, the response curve has a stable linear relationship between NFKB response duration and IKK activation duration.
With a higher tau, the initial response is more steep and reaches a higher duration of NFKB response at a low duration of IKK activation. The feedback loop then takes longer to kick in, leading to a longer period in which the response curve gradually increases and has a "curve" shape plateau from around 5-60 minutes. After 60 minutes, the response curve appears to show a

stable linear relationship between NFKB response duration and IKK activation duration.

3. (20 points) Finally we will simulate NFκB responses to changing amplitudes of IKK activation. For these simulations, we will maintain a constant duration of IKK activation, 250 minutes (IKK_on_time = 0, IKK_off_time = 250). In the section titled "NFKB response versus amplitude of IKK activation (1)" you can vary the amplitude of IKK activation (final argument of function call NFKB_response, default value = 1).

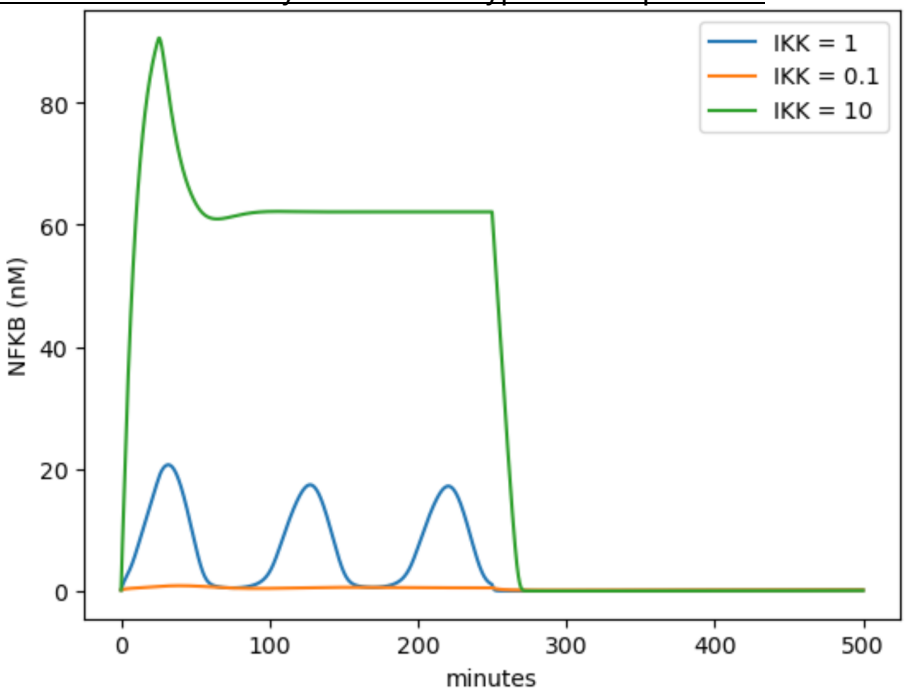
Before running this section of code, reset the delay parameter, tau, to 25 minutes. Rerun the "Steady State Simulation" and "NFKB responses to IKK activation" sections so the change takes effect.

Now run the code with the default amplitude of 1 and paste the resulting graph. Describe the activation dynamics of NFκB.



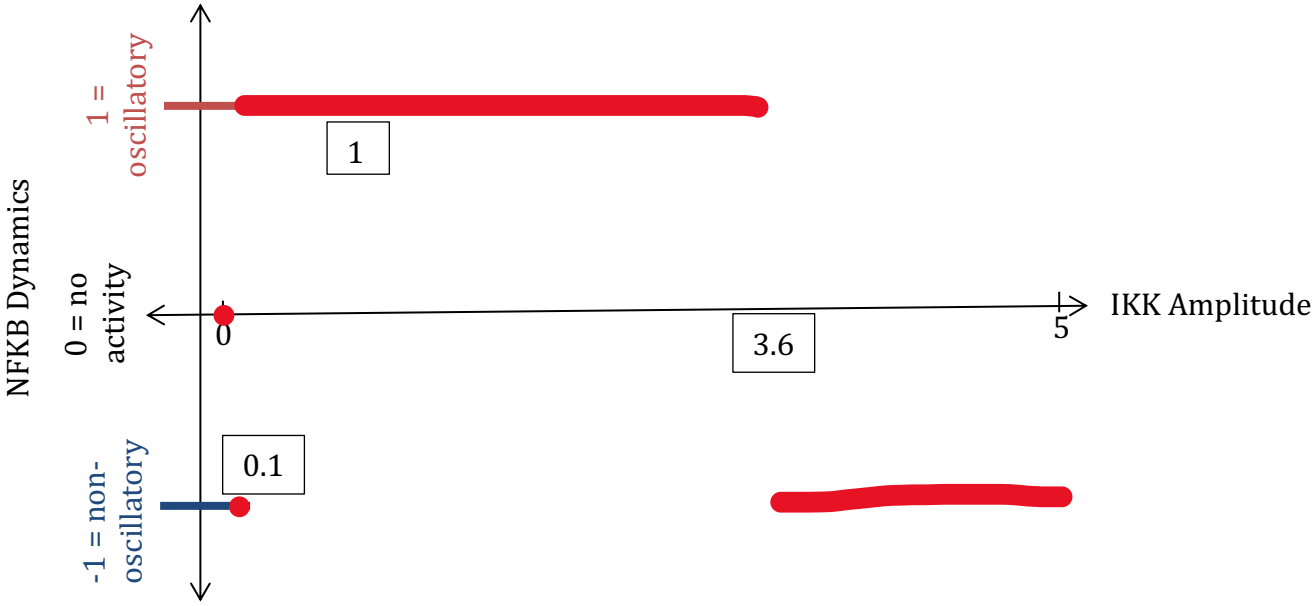
There is oscillatory behavior of activation of NFkB because while the IKK activation is constant from 0-250 minutes, NFKB concentration initially increases to a concentration about 17 nM, then decreases to near 0 nM as time progresses. After, the NFkB concentration levels off after IKK activation ends.

Run the section of code "NFKB response versus amplitude of IKK activation (2)" and paste the resulting graph. This plot includes NFκB responses to IKK amplitudes of 0.1, 1, and 10 on the same plot. How might you describe and classify these three types of responses?



IKK = 0.1: No activity and little to none NFkB response.
IKK = 1: Oscillatory behavior present.
IKK = 10: Non-Oscillatory behavior, reaches an amplitude of 10 with a sharp initial increase in NFkB concentration, but proceeds to a short decline, then a plateau up until ~270 minutes, and finally a sharp decline to no activity.

Rerun the section of code "NFKB response versus amplitude of IKK activation (1)" with different values of IKK amplitude. Start at 0 and increase IKK Amplitude. Find the minimum value needed to obtain oscillations (to the nearest tenth). Continue to increase IKK Amplitude and find the minimum value needed to eliminate the oscillations (to the nearest integer). **Draw your results onto the graph below** where the x-axis is IKK amplitude (from 0 to 5) and the y-axis is NFKB response behavior (let 1 = oscillatory, -1 = non-oscillatory, 0 = no activity). For each IKK amplitude value you test, record the observed behavior. **Make sure to mark the two critical points described above.**



Minimum value needed to obtain oscillations: 0.2
Minimum value needed to eliminate the oscillations: 3.6

There is no oscillation at IKK = 0.0 - 0.1 and 3.6 - 5.0
There is oscillation at IKK = 0.2 - 3.5