# CaSB M178
# Homework 4

Due: 10/25/22 at 12:00PM PDT

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

## Problems

In class, we talked about the caspase cascade. We initially considered a simple model in which the amount of caspase 8 in the model was fixed. Caspase 8 cleaves pro-caspase 3 to generate the active caspase 3 species. In this problem we will extend the model to include the saturation of caspase 8. To do this, we will need to model the formation of the complex of caspase 8 and pro-caspase 3.

$$C8 + proC3 \xrightarrow{k\_a} C8proC3$$
$$C8proC3 \xrightarrow{k\_d} C8 + proC3$$

As in the model from class we still have the conversion of pro-caspase 3 to caspase 3 via caspase 8, as well as the synthesis of pro-caspase 3, and the degradation of pro-caspase and caspase 3:

$$C8proC3 \xrightarrow{k_c} C8 + C3$$
$$\xrightarrow{Q} proC3$$

$$proC3 \xrightarrow{\delta}$$
$$C3 \xrightarrow{\delta}$$
$$C8proC3 \xrightarrow{\delta} C8$$

We do not model the activation, synthesis, nor degradation of caspase 8 here.

**1) (20 points)** First write down the change equations for this new caspase model. MAKE SURE that you write down equations not just for C8 and the C8proC3 complex, but ALL the species.

C8_prime = -k_a*C8*proC3 + k_d*C8proC3 + k_c*C8proC3 + delta*C8proC3
proC3_prime = -k_a*proC3*C8 + k_d*C8proC3 + Q - delta*proC3
C8proC3_prime =k_a*proC3*C8 -k_d*C8proC3 - k_c*C8proC3 -delta*C8proC3
C3_prime = k_c*C8proC3 - delta*C3

**In the section of code called "initial caspase model" implement the change equations to reflect the new model. Make sure you implement all the equations written above!**

In the next section of code, "INITIAL CONCENTRATIONS & PARAMETERS", the values we will use for simulation are set, except the value of Q, the synthesis of rate of pro-caspase 3.

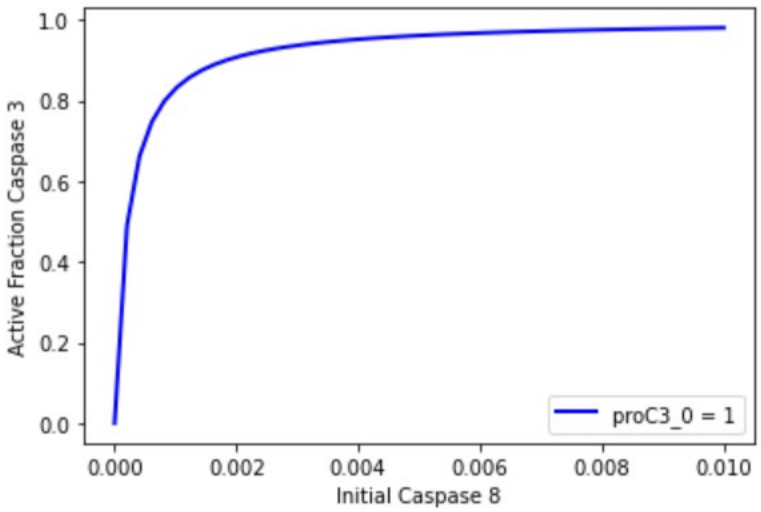
To calculate Q, first imagine there was no caspase 8 in the cell, and so no pro-caspase 3 can be converted to active caspase 3. Note that we have set the pro-caspase initial concentration (called proC3_0 ) to a certain value. Now, we want to set the value of Q so that the amount of proC3 would not change over time in this case were there is no caspase 8 in the cell. In other words, we want the amount of caspase 3 to already be at steady state.

We can write down an equation for Q so that we guarantee that the steady state will always just be proC3_0. Write that equation below. NOTE: you should not use the current *numerical value* of proC3_0 in this equation. You should just use variables (like delta, proC3_0, etc.) in your equation.

The equation for Q is = delta* proC3_0.

**Enter your expression for the value of Q in the code.**

Finally in the section of code called "STEADY-STATE responses of initial caspase model", we will vary the initial concentration of C8 and plot the resulting steady state concentration of C3 divided by the initial concentration of proC3. Note that the current initial concentration of proC3 is set to 1 and the initial concentration of C8 will be varied from 0 to 0.01. **Run this section of code and paste the resulting graph below:**




This model is similar PTM cycle models explored in HW1. Relate the species and reactions from our caspase model to the PTM model with synthesis and degradation. Which species/reactions from the PTM model do not have analogous species/reactions in the caspase model?

In the reaction of C8 interacting with proC3 to bind to become C3 and C8, the C8 is acting like a kinase. The species/reaction from the PTM that do not have analogous species/reactions in the caspase model is that there is no degradation in the caspase modeling where active C3 and C8 will stay active, and it is irreversible.

ka

PC3 + C8 → C3 + C8

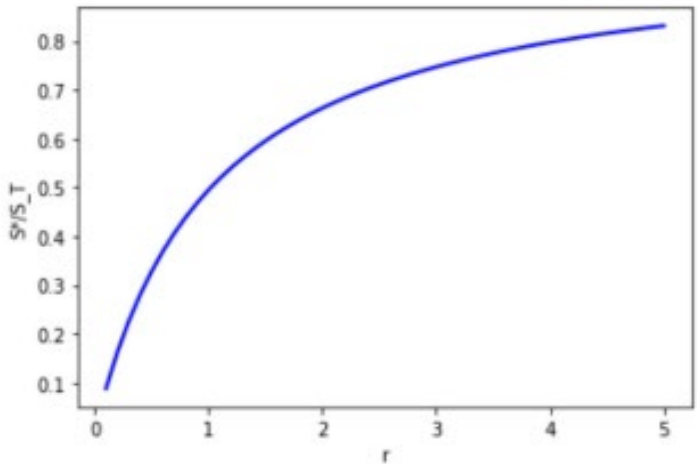
C3' = kaC8 * PC3

pC3' = -ka* C8 * PC3

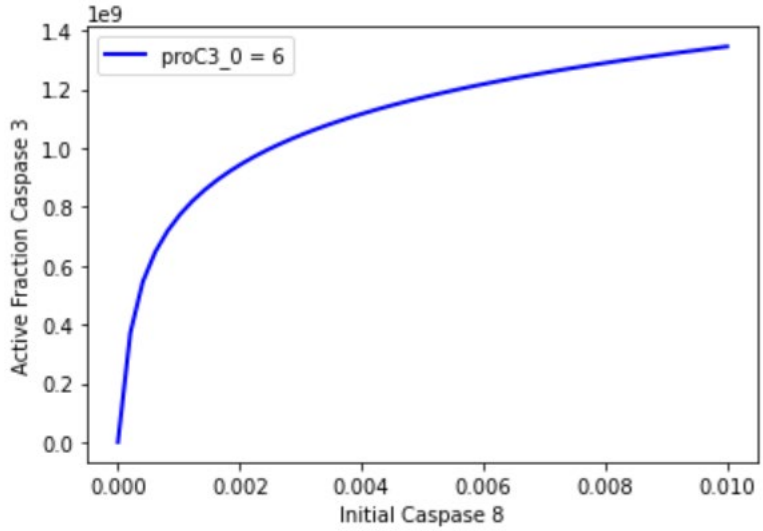
In HW 1, we explored the effect of saturation on the steady state amount of modified substrate as a function of initial kinase concentration by changing the initial concentration of substrate. **Increase the initial concentration of the substrate species in the caspase model at least twice to explore the effect of saturation. Make sure you increase the concentration *significantly*. Rerun the code and the paste your resulting graphs.** How do the results compare to HW1? Can you explain why differences emerge, if any?

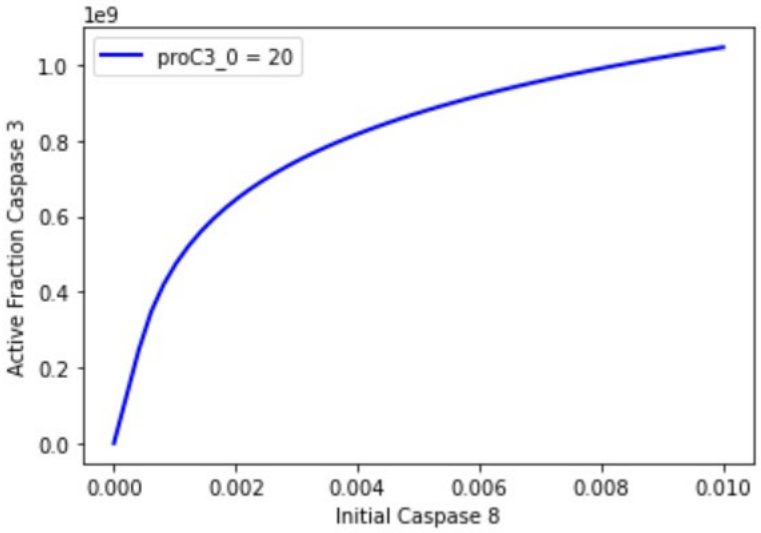
The results are similar to HW1 because kinase and phosphatase reactions have the same behavior as the C3 and C8 activation reactions. However, the difference here is

that we have the issue of synthesis and degradation AS WELL AS the issue of reversible and non-reversible reactions. However, we should note that it is more relevant to use the synthesis/degradation case, since C3 and C8 can only be deactivated by degradation. In biochemical simulations of Michaelis-Menten kinetics, there wouldn't be any saturation because there will most likely be equal amounts of substrate (kinase) and enzyme (phosphatase). These graphs below all show a saturation, whether it be on the S*/Stotal scale or the active fraction of Caspase 3 scale.




^ Homework 1 saturation graph






**2) (20 points)** Next, we will implement a version of the caspase model that includes a receptor activating pro-caspase 8, FLIP binding at the receptor, and positive feedback by caspase 3 in the section "simple caspase model with feedback and FLIP".

In this model we will have the following species: proC8, C8, proC3, C3, R (receptor), F (FLIP), and RF (receptor-FLIP complex). Write the change equations for this model that satisfy the below description and **add these to the section of code.**

a) proC8 and proC3 are synthesized at rate QC8 and QC3 respectively (zeroth order reaction).
b) proC8, C8, proC3, and C3 are all degraded at rate delta (first order reaction).
c) proC8 binds R at rate k_ba and generates C8 (second order reaction). Note that, in contrast to the model in question 1, we are not forming an R-C8 *complex*; rather, R interacts with proC8 and generates active C8 all in one step.
d) proC3 binds C8 at rate k_a and generates C3 (second order reaction). As in step "c" above, we do not form a complex here.
e) C3 binds proC8 at rate k_a and generates C8 (second order reaction). As in step "c" above, we do not form a complex here.
f) F binds R at rate kp to generate RF (second order reaction). Note that, in this case, F and R *are* forming an explicit complex (RF).
g) RF dissociates into F and R at rate km (first order reaction) .

The change equations for this models are:

proC8prime = QC8 - delta*proC8 - k_ba*proC8*R -k_a*C3*proC8

C8prime = k_ba*proC8*R - delta*C8 +k_a*C3*proC8
proC3prime = QC3 - delta*proC3 -k_a*proC3*C8
C3prime = -delta*C3 + k_a*proC3*C8
Rprime = -kp*F*R +km*RF
Fprime = -kp*F*R + km*RF
RFprime = -km*RF + kp*F*R

Which reaction in the above list implements a positive feedback mechanism? Write that specific reaction again below

The reaction in the above list that implements a positive feedback mechanism is the activation of C3 caspase by C8 once C8 receives an incoming signal. Once C8 has received big values of k+.

We will once again explore steady state responses of the model in the section "STEADY-STATE responses of feedback + FLIP model". In particular, we want to simulate how varying the concentration of initial active receptors affects the downstream activation of caspase 3.

Note we once again first define initial conditions and parameter values. All caspases exist only in their non-active state at the beginning of the simulation and the concentration of FLIP is set equal to that of the caspases. The rate of activation of pro-caspase 8 is slower to mimic the "basal" activity of proC8 cross-activation when bound to the receptor (without feedback).

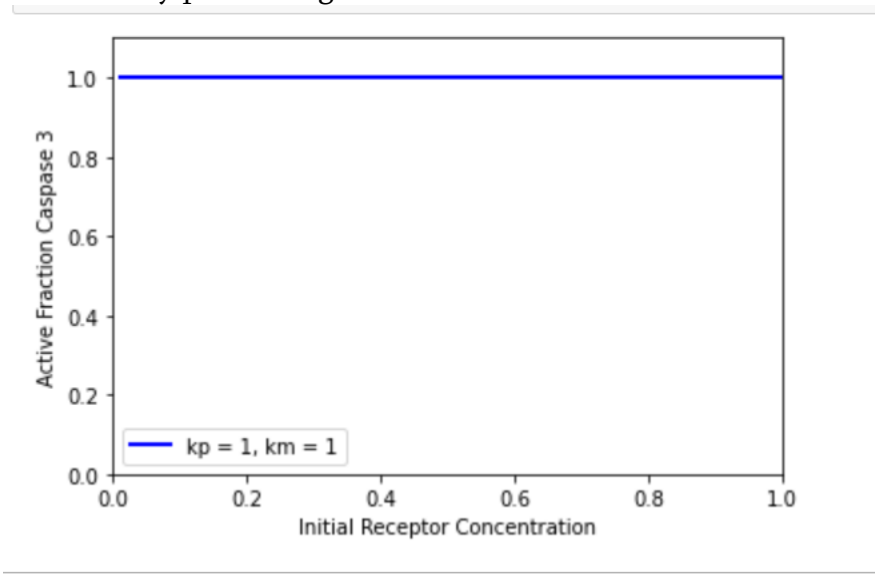
Run this section of code and paste the resulting graph. If complete activation of C3 leads to cell death, how does the fate of the cell vary with receptor concentration? Is this result reasonable for a cell? Why or why not?

If the complete activation of C3 leads to cell death, the fate of the cell will vary with receptor concentration because the higher the receptor concentration, we will see more active fraction of Caspase 3. Given that we know this is a steady state that has feedback, recalling from the lecture, the active C8 gets degraded and the only way to replace them is feedback. This leads to C3 levels increasing, which allows for increasing active C8 levels, leading to full activation of C3.

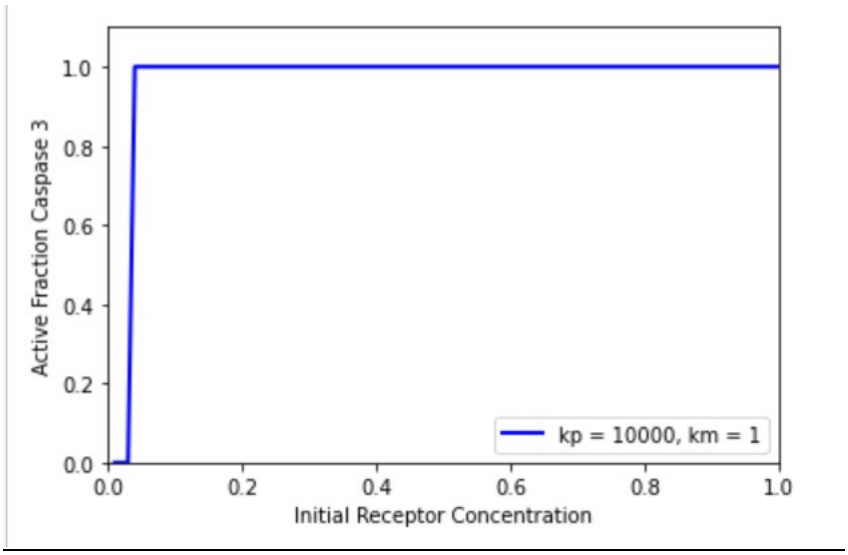
The role of the receptor is to buffer the cell for stochastic dynamical inputs or "random" molecule of C3 that goes into the system, for that molecule to not lead to the death of the cell. Therefore, the receptor concentration here, for a kp = 1 and km

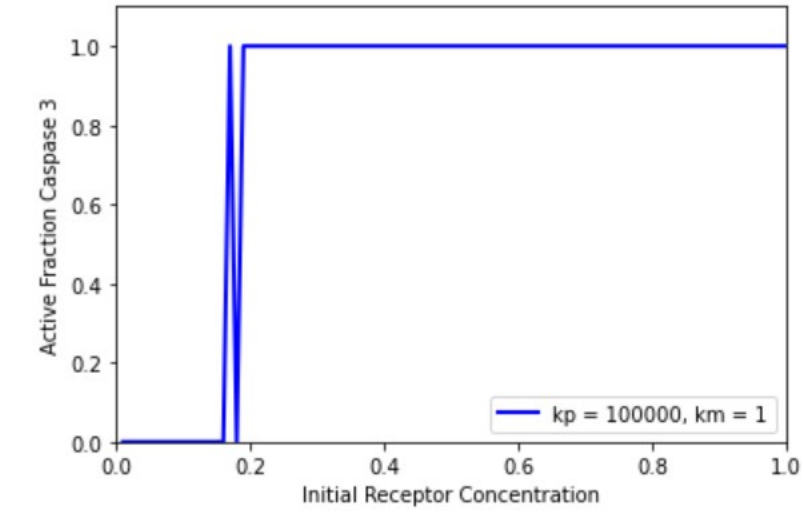
= 1, is not a factor to cell fate. However, if the binding affinity is stronger, we will see a delay or a sharp change followed by activation.

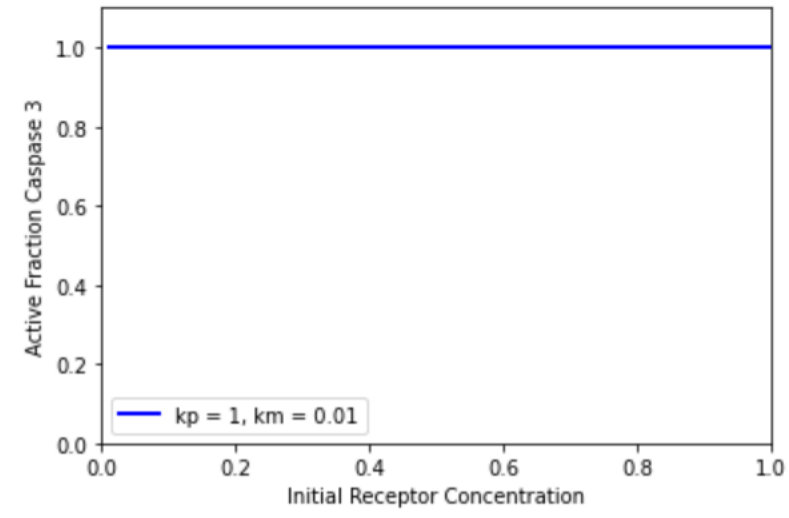
Cell death is activated independent of the receptor concentration, and this is not reasonable because receptors' functions are to buffer the cell death phenomenon, but it is not fully performing this function.



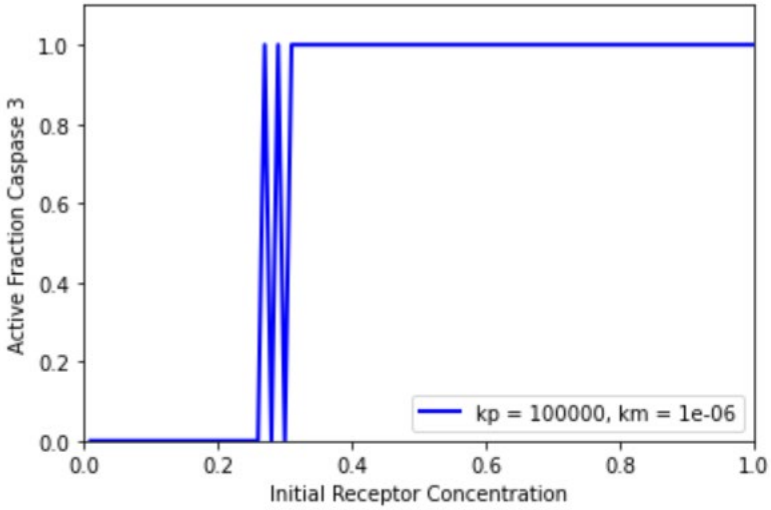

Let's now see if we can suppress activation of the caspase cascade by modifying parameters values from reactions involving FLIP. **Modify the values of kp and km and rerun this section of code until the plot generated differs from the previous result. Try making the binding affinity stronger; to do this, you should increase kp or decrease km. Try *three* different values Paste this final graphs here:**


Active Fraction Caspase 3
Initial Receptor Concentration
kp = 10000, km = 1
0.0
0.2
0.4
0.6
0.8
1.0


Active Fraction Caspase 3
Initial Receptor Concentration
kp = 100000, km = 1
0.0
0.2
0.4
0.6
0.8
1.0


Active Fraction Caspase 3
Initial Receptor Concentration
kp = 1, km = 0.01
0.0
0.2
0.4
0.6
0.8
1.0

and an additional one when I increased kp and decreased km




Did your changes make any difference for the behavior of the system? Are any of these cases more reasonable as a model for cell death?

My changes made a difference in the sense that the activation was delayed for lower initial receptor concentrations (see graph kp = 100000, km = 1). In that graph, only concentrations higher than 0.2 reached active fraction caspase 3 of 1. This is a reasonable model for cell death because if the binding affinity is strong, R can not activate C8, which then takes a little longer to activate C3.

This also was discussed in lecture 8, where at low R levels, FLIP binds, thereby preventing C8 activation.

**3) (20 points)** Underneath the section where you plotted the steady state responses for the FLIP model, there is a section called "STEADY-STATE responses of feedback + irreversible FLIP model". In this section, we will now assume that binding of FLIP to the receptor is ***irreversible***. We will implement this using the same model as before ("caspase_FLIP_model") and modifying a single parameter value.

What parameter value do we change to simulate *irreversible* binding of FLIP to the receptor? What value do we set it to?

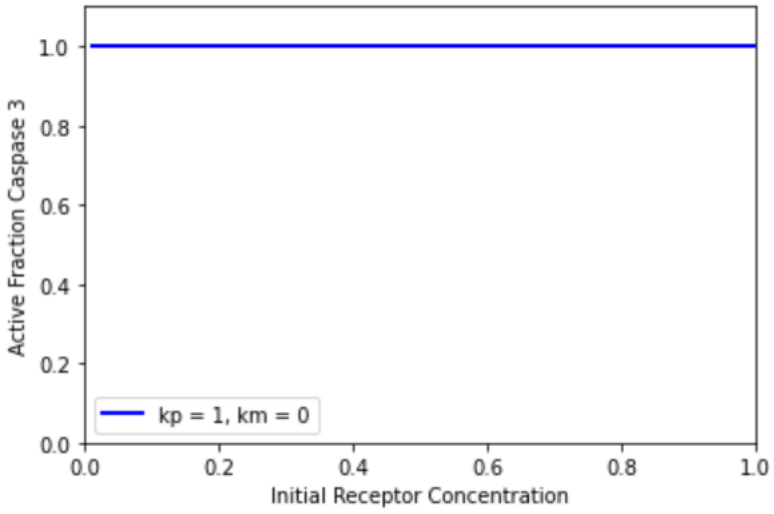
The parameter value that we change to simulate irreversible bind of FLIP to the receptor is k_m, we set this value to 0. This is because the RF complex does not disassociate into FLIP and receptor anymore.

**Implement this change into this section of model code under the "#parameters" section.**

K_m = 0

The remaining parameters are will remain unaltered to keep them in a biologically reasonable range. Does irreversible FLIP binding alone alter C3 activation? To answer this question, go BACK to the "STEADY-STATE responses of feedback + FLIP model" code above, make this change to the one parameter you identified above, and re-run the code. Does anything change?

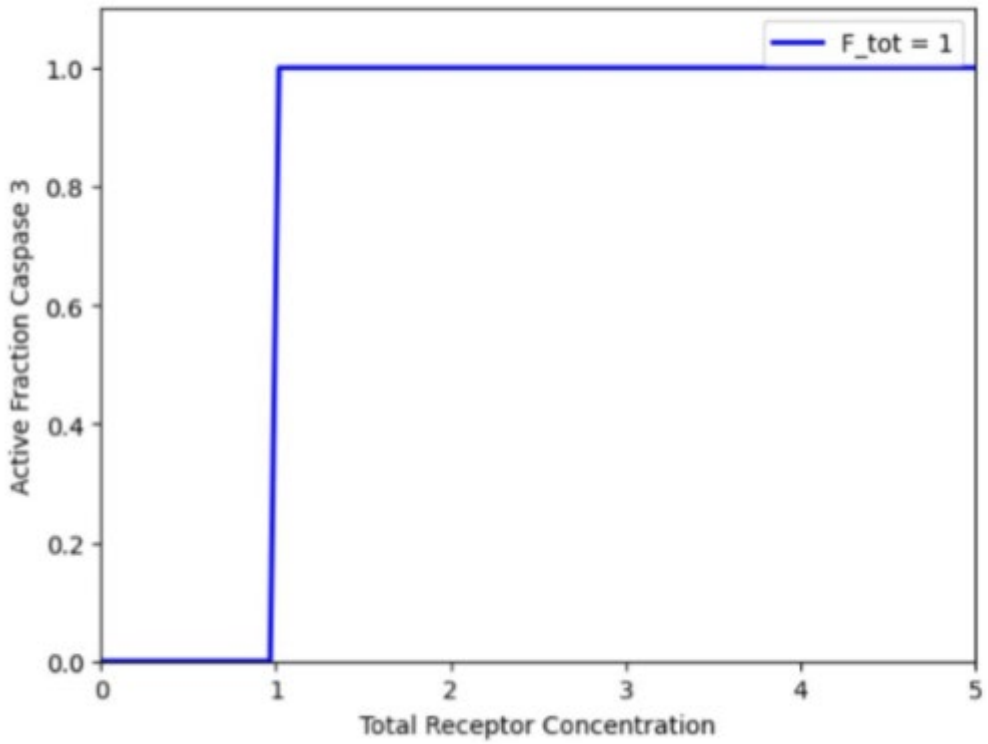
When I change the k_m parameter to 0, now it shows me that everything is constant at 1. So it does change in the sense that there is not even a slight linearity trend, but this change does not affect C3 activation in the sense that there is enough active fraction of caspase to be statistically significant.




We will now assume that FLIP is bound to the receptor prior to the start of the simulation. If the total amount of FLIP (F_tot) is greater than or equal to the total amount of receptors (R_tot), than all receptors are initialized to the receptor-FLIP complex (RF_0 = R_tot), R_0 = 0, and F_0 is equal to amount of FLIP in excess of R_tot. If F_tot is less than R_tot, than all FLIP is initialized to the receptor-FLIP

complex (RF_0 = F_tot), F_0 = 0, and R_0 is equal to amount of receptors in excess of FLIP.

**Implement these rules for the initial conditions utilizing the if/then statement inside of the for loop. Run the section of code and paste the resulting graph.**




Explain in your own words what is happening in the model as the total receptor concentration surpasses the total FLIP concentration.

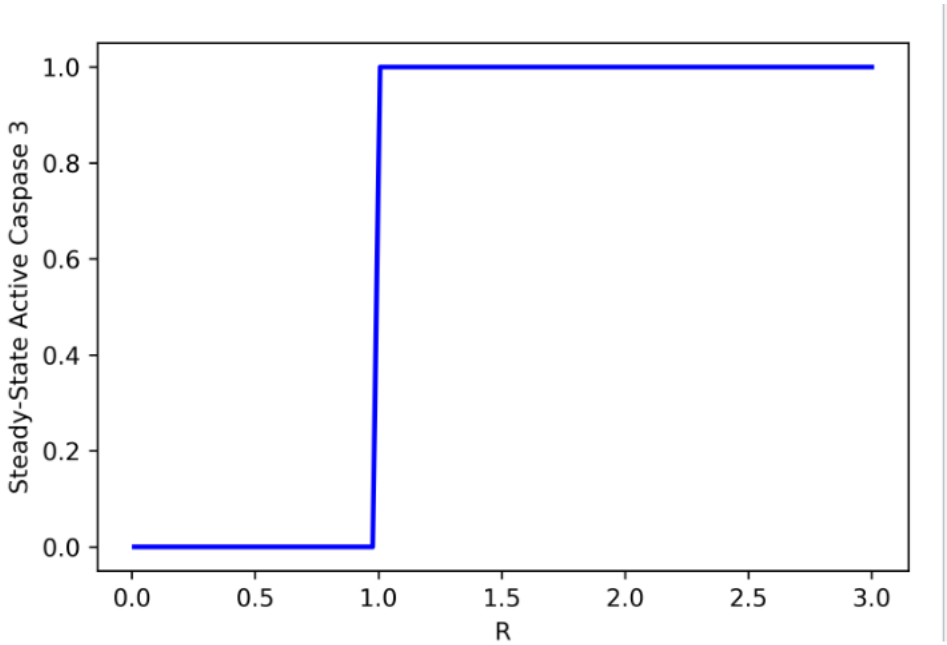
```
### fill in the following lines
if (F_tot >= R_tot):
    RF_0 = R_tot
    R_0 = 0
    F_0 = F_tot - R_tot
###

#This is the case where there are more receptors in
### fill in the following lines
else:
    RF_0 = F_tot
    R_0 = R_tot - F_tot
    F_0 = 0
###
```

When the total receptor concentration surpasses the total FLIP concentration, then all of FLIP is being used. This means that the amount of RF complex is equal to the FLIP concentration. So we know that when receptor concentration is lower than FLIP concentrations, all the receptors are going to get used up. The RF complex is limited by the R concentration, and whatever is left for FLIP is subject to how much Receptors are used up.

Otherwise, if there are more receptors, then there isn't a depletion problem. The total RF complex concentration is determined by F concentration. We use up R instead, and watch F go to 0.

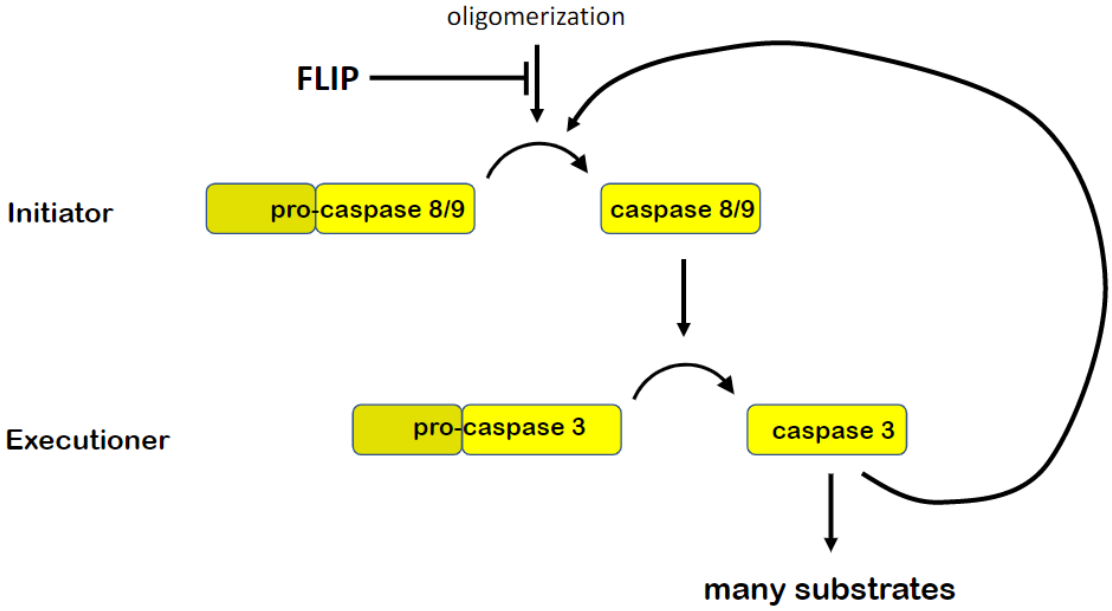
In this model, how does FLIP concentration control C3 activation as a function of receptor concentration?

From what I learned in class, I expected the curve to be a sigmoidal curve like this clicker question.




The FLIP concentration is controlling C3 activation by acting as an inhibitor-type molecule. The more C3, the more FLIP is made in the system, and the initiator (procaspase 8 can't make any more caspase 8). Higher FLIP concentration also means we are depleting the amount of R concentration in the system, and so we notice this delay. Until there are higher amounts of R concentration, no increase in the fraction of caspase 3.

Here was the slide from lecture:



Is the modeled behavior a reasonable for the cell? Why or why not? Do you think that the *assumptions* we need to make here are reasonable?

This model is reasonable for the cell because we are seeing a sigmoidal curve that is signifying that we have a switch-like behavior. For until we have ensured that there isn't a random molecule of caspase 3 in the system, the cell-death signaling will be activated. FLIP is a part of this process. The assumptions made here are that there is going to be, more often than not, an unbalanced amount of substrates and enzyme (FLIP, Receptor). We are assuming that all that do not get consumed will be leftover, and this is a very reasonable assumption.