# PhySci/MiMG/CaSB M178

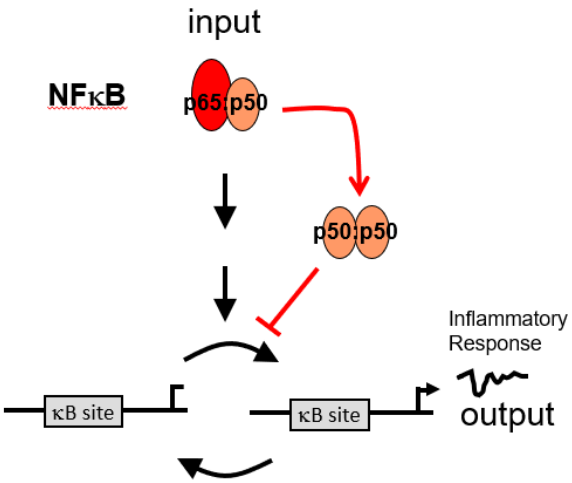
## Homework 8

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

**Notes:** This homework involves performing simulations of the history-dependent signaling systems we've been discussing in the last two class meetings.

**Problems**

In class, we have talked about the NFKB signaling pathway that mediates inflammatory responses. This past week we focused on the following incoherent feedforward loop:




In the first model for this homework, we will explore the effect of this motif on inflammatory responses following repetitive activation of NFKB (p65:p50).

We will start with the simple model of gene expression that we had from Homework 7. In our model, the input NFKB (p65:p50), binds to the promoter region of a gene and converts it from an inactive to active state. In this active state, transcription can occur, initiating an inflammatory response program. Hence in our investigation, we will take the amount of active promoter as a proxy measure for the degree of inflammatory response, the output of our model. Let *NFKB* denote the input signal, whose value can change over time.

One of the effects of activation of the promoter with NFKB is the synthesis of p50:p50 dimer. Much like in Homework 6, this synthesis takes time and hence this is a "delayed reaction". Hence in the model, the amount of p50:p50 dimer 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).

Finally, p50:p50 dimer inhibits the conversion of the inactive promoter site to its active form. To include this in the model, the effective rate of activation of the promoter region is a function of the abundance of both NFKB and p50:p50 dimer, as well as their binding affinities for the promoter region. We include this information in a Hill Equation:

$$H\_n(t) = \frac{NFKB(t)}{NFKB(t) + K_{d1}}$$
$$H\_p(t) = \frac{p50(t)}{p50(t) + K_{d2}}$$
$$H(t) = H\_n(t) * (1 - H\_p(t))$$

*H_n* and *H_p* describe the proportion of binding sites that are occupied by NFKB and p50:p50 dimer respectively. Since p50:p50 dimer binding is inhibitory, the proportion of unbound sites (*1- H_p(t)*), determines the rate of promoter activation. Hence if $k_a$ is the maximum rate of promoter activation, the effective rate dependent on NFKB and p50:p50 is given by the product $k_a * H(t)$.

In summary we have the following reactions:

| Reactions | Description |
|---|---|
| $pr \xrightarrow{k\_a * H} pr\_a$ | Activation of the promoter region |
| $pr\_a \xrightarrow{k\_d} pr$ | Deactivation of the promoter region |
| $pr\_a\_tau \xrightarrow{k\_syn} pr\_a\_tau + p50$ | Synthesis of p50:p50 dimer (delayed reaction) |
| $p50 \xrightarrow{k\_deg}$ | Degradation of p50:p50 dimer |

**1. (20 points)** First write down the change equations for the model described above. For the p50 synthesis term, make sure to write it in terms of the delayed variable, pr_a_tau.

pr_prime = -k_a*H*pr + k_d*pr_a
pr_a_prime = k_a*H*pr - k_d*pr_a
p50_prime = k_syn*pr_a_tau - k_deg*p50

**In the section of code called "NFKB-p50 model" implement the change equations to simulate the model.** Note how the code defines the value of the delayed variable, pr_a_tau, and the Hill Equation (H); use these values in your implementation. Once

you have defined your model equations, run the cell containing the model code as well as the following cell titled "delay helper functions". These functions are used to look up the old values of delayed variables as needed by the model.

Now we will simulate the model in the section of code called "Simulation of NFKB-p50:p50 model". In this section, we have defined our initial conditions and default parameter values which are also listed below for your reference.

| Parameter | Value |
|---|---|
| Kd1 | 0.1 |
| Kd2 | 0.1 |
| k_a | 0.2 $min^{-1}$ |
| k_d | 0.05 $min^{-1}$ |
| k_syn | 0.05 $min^{-1}$ |
| k_deg | 0.01 $min^{-1}$ |
| tau | 25 min |

Below the initial conditions and parameter definitions is a section called "NFKB activation". For the simulations in this homework, the profile of activation for all model inputs will be a box function (identical to that of IKK activation from HW 6 and TF from HW 7). Unlike the prior homeworks, we have the possibility of two successive activations. The first activation period is referred to priming and the second activation period is called the challenge. Hence to describe the temporal dynamics of NFKB, we have to specify a first on time and off time (NFKB_on1_time, and NFKB_off1_time) and a second on time and off time time (NFKB_on2_time, and NFKB_off2_time). We assume the amplitude of both activations are equivalent (NFKB_amplitude). By default, NFKB_on1_time = 0, NFKB_off1_time = 60, NFKB_amplitude = 1, and there is no challenge (the remaining activation parameters are set to zero).

<u>Run the section of code for "Simulation of NFKB-p50:p50 model" and the section of code for "Checking Model Implementation".</u> 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 to the first few decimal places, double check your change equations and code before proceeding.

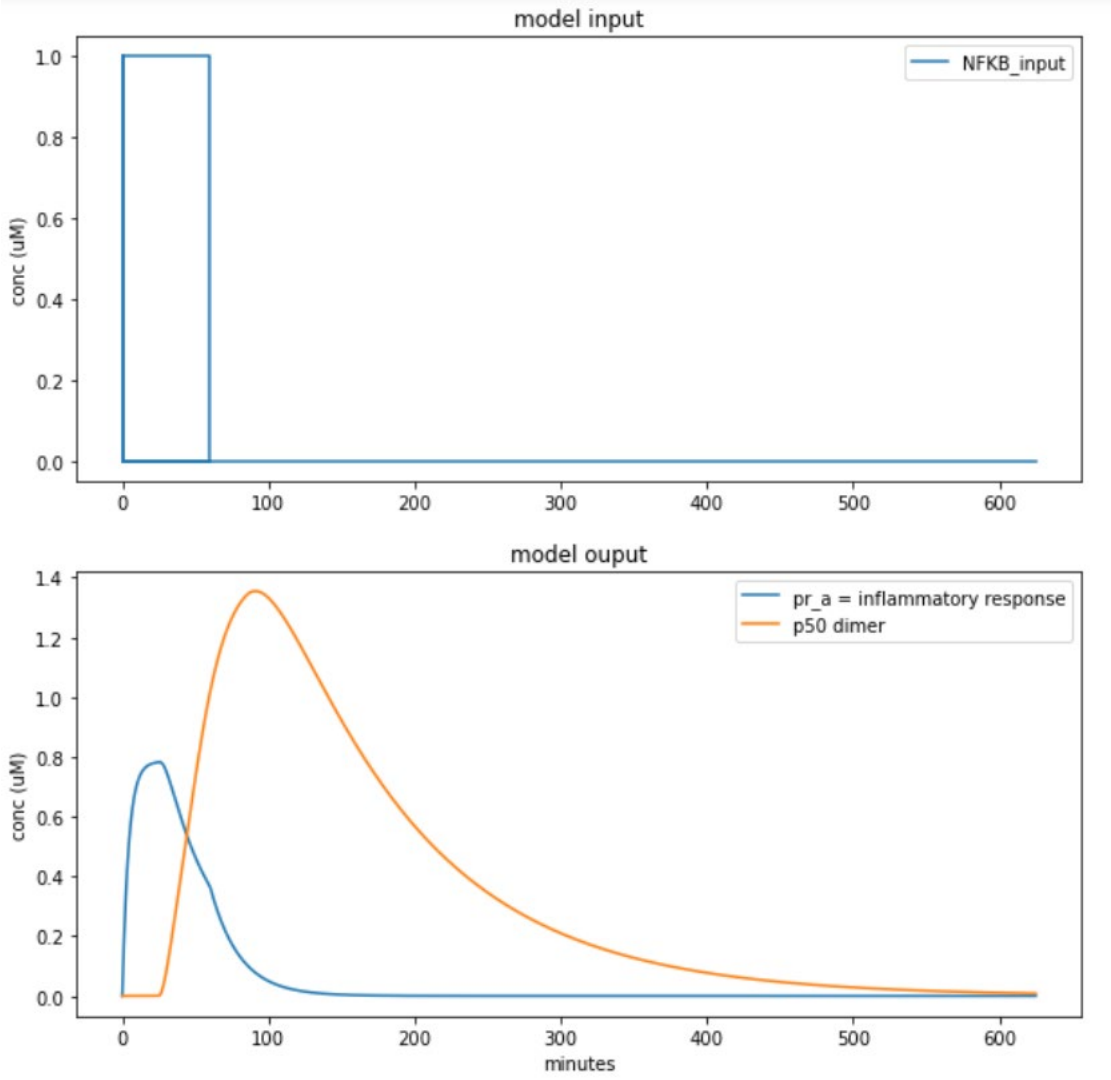
**They match!**

```
Checks on Model Implementation:
For each of the following lines ensure that your value on the right matches that on the left
0.5358304341185932 0.5358304341185932
0.46416956588140657 0.46416956588140657
0.7302990673189521 0.7302990673189521
```

**Run the section of code called "Plot Dynamics" and paste your graph here.** Can you identify from the "model output" (bottom) plot the value of tau? How about the value of NFKB_off1_time?

The tau (time delay) should be 20 minutes because the reaction or response begins with a delay elapsed time of 20 minutes between the inflammatory response and the p50 dimer response.

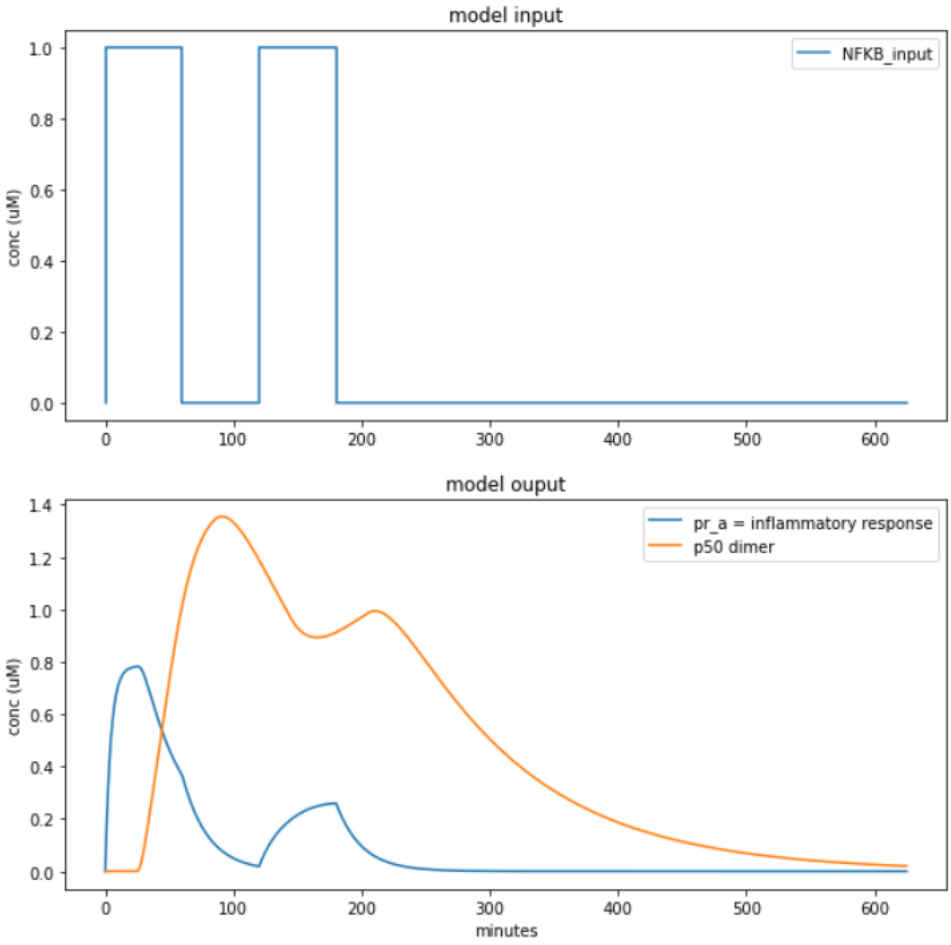
The value of NFKB_off1_time should be 60 minutes to begin with, and with every iteration is subtracted by tau.



We will know add a challenge activation. **Modify "Simulation of NFKB-p50:p50 model" such that NFKB_on2_time = 120 and NFKB_off2_time = 180. Rerun the simulation and plotting cell. Paste your resulting graph here.** Describe the profile of inflammatory response over time. Is the response to the challenge activation different from the priming activation? If so, why?

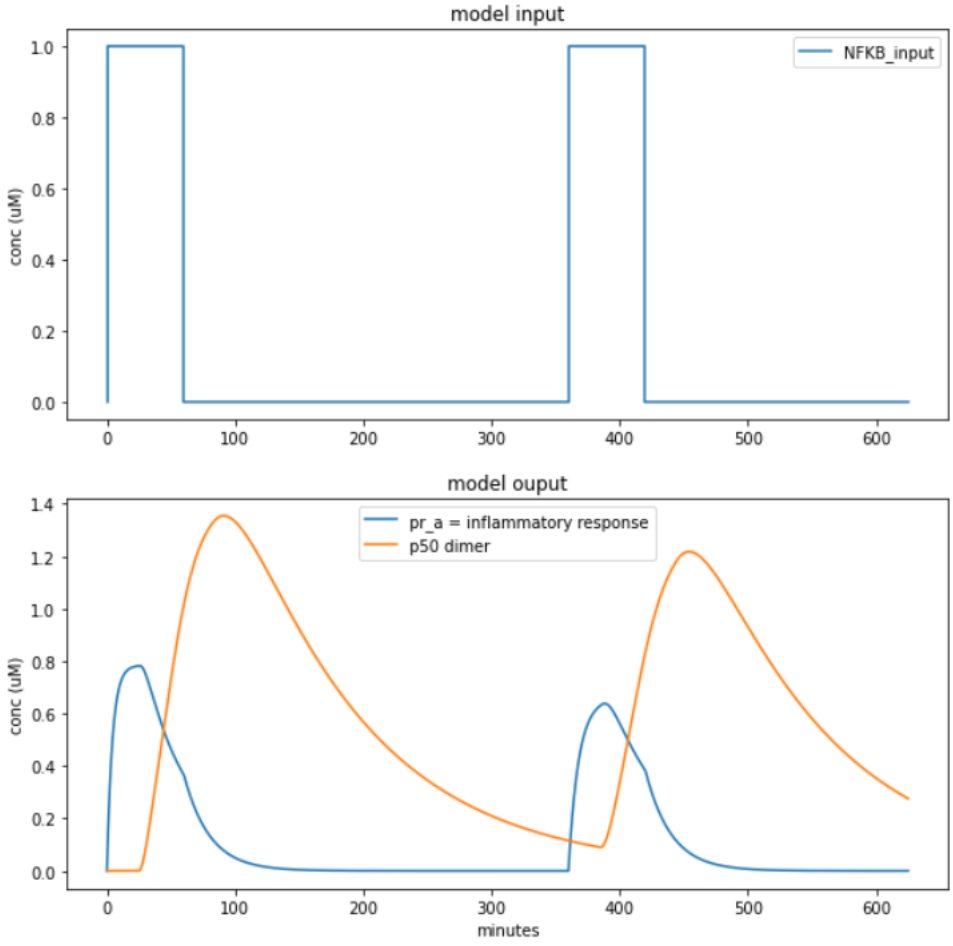
The effect of the second NFKB from 120 to 180 has resulted in a second peak for both pr_a and p50 dimer, with or without time delay. An interesting phenomenon is that they do not happen to peak at the same time, for the p50 dimer, it peaks at 220 minutes, for the pr_a inflammatory response, it peaks at 180 minutes.

The response to the challenge activation is different from the priming activation because the concentration doesn't start at zero anymore, instead, it catches the concentration at the declining trend around 120 minutes. It acts as an impulse, and characterizes a challenge activation.



**Modify "Simulation of NFKB-p50:p50 model" such that NFKB_on2_time = 360 and NFKB_off2_time = 420. Rerun the simulation and plotting cell. Paste your resulting graph here.** Describe the profile of inflammatory response over time. Is the response to the challenge activation more or less like the priming activation compared to the previous simulation? Why?

The response to the challenge activation is more like the priming activation compared to the previous simulation because the amount of time that has elapsed is over 300 minutes and the model concentration has declined to steady state of 0 (uM) which makes the challenge activation become a priming activation, given that the model input is the same concentration of 1 uM.

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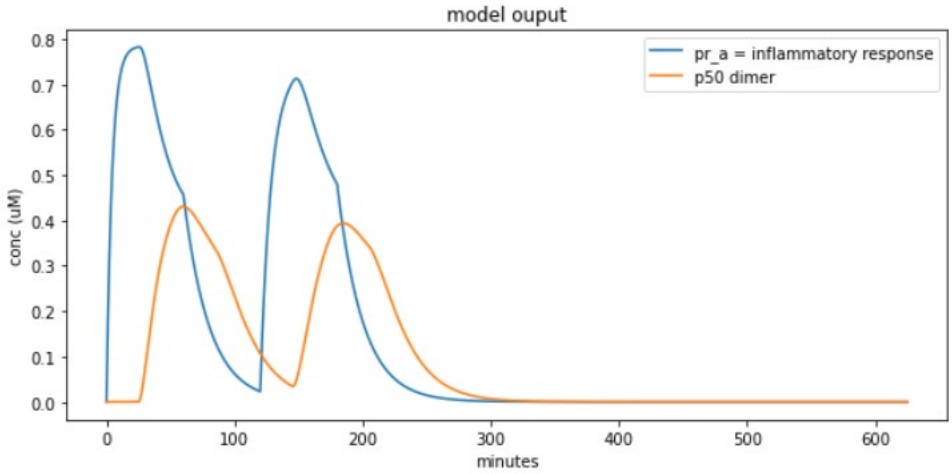
**Reset "Simulation of NFKB-p50:p50 model" such that NFKB_on2_time = 120 and NFKB_off2_time = 180. Modify the value of k_deg until the response to challenge activation looks more like the response to the priming activation. Paste your resulting graph and state the value of k_deg you used.**

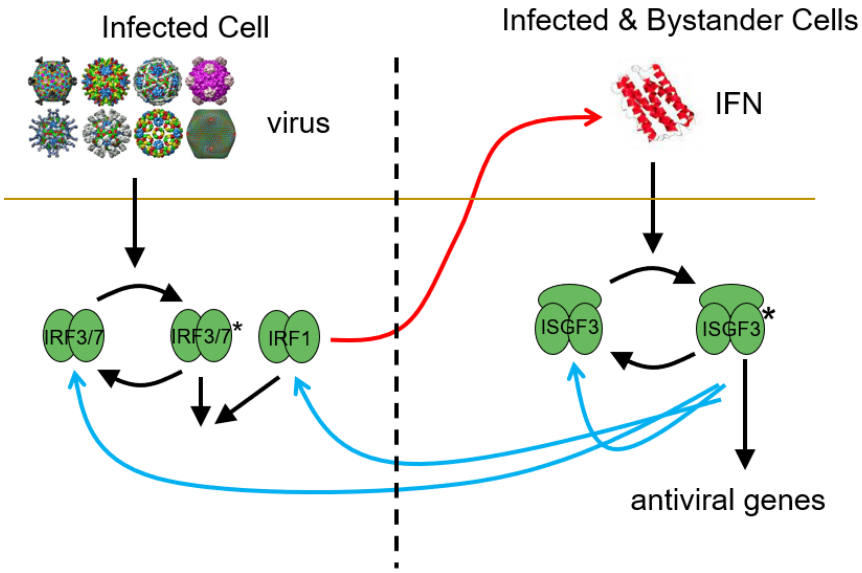



The k_deg I used was 0.09, which means that it didn't have memory after exponential decay. There is no finite time for the concentration to drop to 0.

**How does k_deg control the priming effect?**

K_deg controls the priming effect by degrading the leftover substrates, and when it is greater than the rate of synthesis (k_syn = 0.05), it will quickly drop the pr_a inflammatory response and the p50 dimer in the model output graph. The priming effect is not long-lasting and in the context of immunology, it is meant to avoid septic shock.

**2. (20 points)** In class we also talked about the Interferon signaling pathway and responses to viral pathogens. This past week we focused on the positive feedback loops present in the pathway:




We will first focus on simulating the portion of the pathway on the right hand side of this schematic. For this model, the input is interferon, IFN. It stimulates the conversion of ISGF3 into its active form, ISGF3*. ISGF3* activates an antiviral gene response program, and hence we will use it as the output measure from our model. Finally, ISGF3* stimulates the production of more ISGF3. The induced production of ISGF3 is proportional to fraction of ISGF3* bound DNA binding-sites, and we once again use a Hill term to describe this. Additionally, this synthesis is also a delayed reaction, so we use the value of ISGF3* at time $t$-τ (ISGF3*_tau) to calculate the value of the Hill term at time $t$.

$$H(t) = \frac{ISGF3 * \_tau(t)}{ISGF3 * \_tau(t) + K_d}$$

Finally, if $k1\_syn$ represents the basal synthesis rate of ISGF3, the total synthesis rate including that from ISGF3* stimulation is given by $k1\_syn * (1 + H(t))$.

In summary we have the following reaction:

| Reactions | Description |
|---|---|
| $\xrightarrow{k_{1syn*(1+H)}} ISGF3$ | ISGF3 synthesis |
| $ISGF3 \xrightarrow{k_{1deg}}$ | ISGF3 degradation |
| $ISGF3p \xrightarrow{k_{2deg}}$ | ISGF3* degradation |
| $IFN + ISGF3 \xrightarrow{k_a} IFN + ISGF3p$ | IFN mediated activation of ISGF3 |
| $ISGF3p \xrightarrow{k_d} ISGF3$ | Deactivation of ISGF3 |

First write down the change equations for the model described above. For the ISGF3 synthesis term, make sure to write it in terms of Hill term.

ISGF3_prime = k1_syn*(1+H) - k1_deg*ISGF3 - k_a*ISGF3*IFN + k_d*ISGF3p
ISGF3p_prime = -k2_deg*ISGF3p + k_a*IFN*ISGF3 - k_d*ISGF3p

**In the section of code called "simple IFN model" implement the change equations to simulate the model.** Note how the code defines the value of the delayed variable, ISGF3p_tau and uses it in the calculation of the Hill Term (H). Make sure to use the value of H in your implementation. Once you have defined your model equations, run the cell containing the model code as well as the following cell titled "delay helper functions". These functions are used to look up the old values of delayed variables as needed by the model.

Now we will simulate the model in the section of code called "Simulation of simple IFN model". In this section, we have defined our initial conditions and default parameter values which are also listed below for your reference.

| Parameter | Value |
|---|---|
| k1_syn | 0.1 $nM^{*}min^{-1}$ |
| k1_deg | 0.01 $min^{-1}$ |
| k2_deg | 0.01 $min^{-1}$ |
| k_a | 0.2 $nM^{-1}min^{-1}$ |
| k_d | 0.05 $min^{-1}$ |
| Kd | 0.1 |
| tau | 25 min |

Below the initial conditions and parameter definitions is a section called "IFN activation". The definitions are much like that for NFKB activation in problem 1. By default, IFN_on1_time = 0, IFN_off1_time = 10, IFN_amplitude = 1, and there is no challenge (the remaining activation parameters are set to zero).

**Run the section of code for "Simulation of simple IFN model" and 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 to the first few decimal places, double check your change equations and code before proceeding.

```
Checks on Model Implementation:
For each of the following lines ensure that your value on the right m
atches that on the left
2.8790276213841497 2.8790276403290833
7.120972378615856 7.1209723596709225
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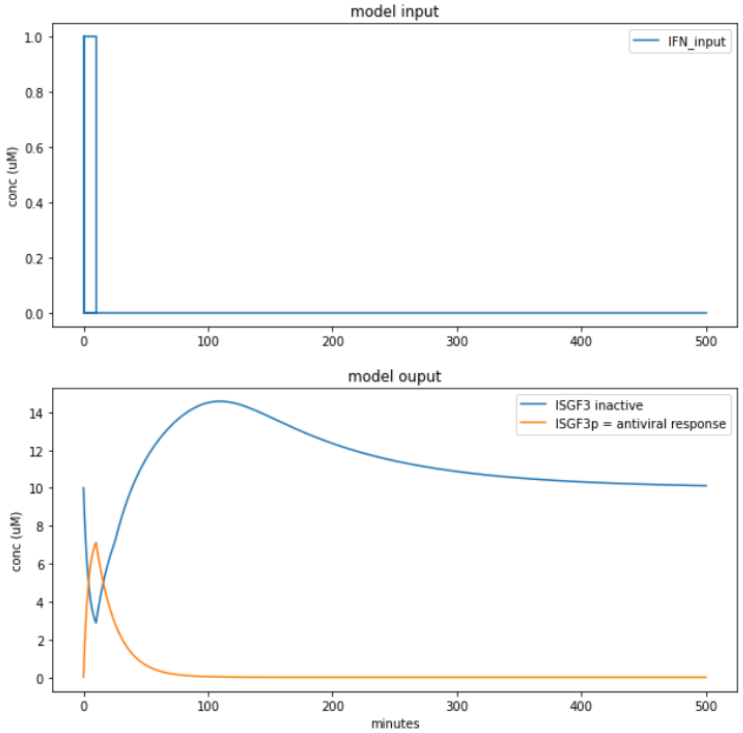
They match!

**Run the section of code called "Plot Dynamics" and paste your graph here.** Can you explain the shape of the trajectories in the "model output" (bottom) plot? Does the model return to its initial steady state?

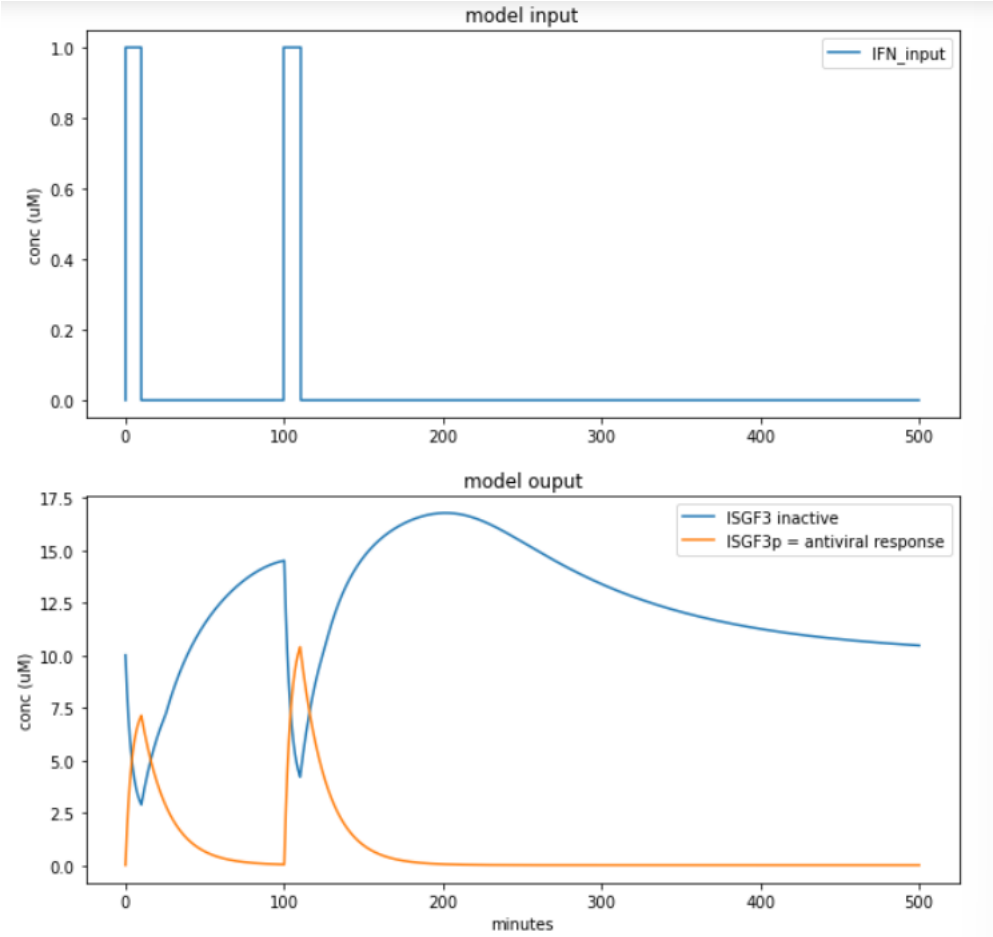
There is an initial dip of the ISGF3 inactive curve from the initial concentration of 10 uM, which rises to a maximum of around 14 uM, then plateaus to steady state by regressing back to 10uM. This is because ISGF3 is initially mainly inactive in the presence of no IFN input. Then, after IFN input is prompted in the system, we see ISGF3 concentration decreasing because it is transforming into ISFG3*, initiating signaling for the antiviral genes to be transcribed. When the IFN input is exhausted, we see both ISGF3 inactive and ISGF3* go back to their original steady state values.




We will know add a challenge activation. **Modify "Simulation of simple IFN model" such that IFN_on2_time = 100 and IFN_off2_time = 110. Rerun the simulation and plotting cell. Paste your resulting graph here.** Describe the profile of antiviral

response over time. Is the response to the challenge activation different from the priming activation? Why or why not?

The response to the challenge activation is different from the priming activation because of the timing of IFN input. When the time elapsed between input is short, like 100 minutes, the ISGF3 positive feedback allows a larger concentration of ISGF3p being in the active state to provide an antiviral response. In this case, we see a 7.5 uM ISGF3p followed by a 10 uM ISGF3p concentration in this challenge activation. This is because the positive feedback loop takes in consideration of an immediate immunology signaling where infected cells and neighboring cells are alerted in a bigger amount if there are multiple IFN inputs. The response is heightened.

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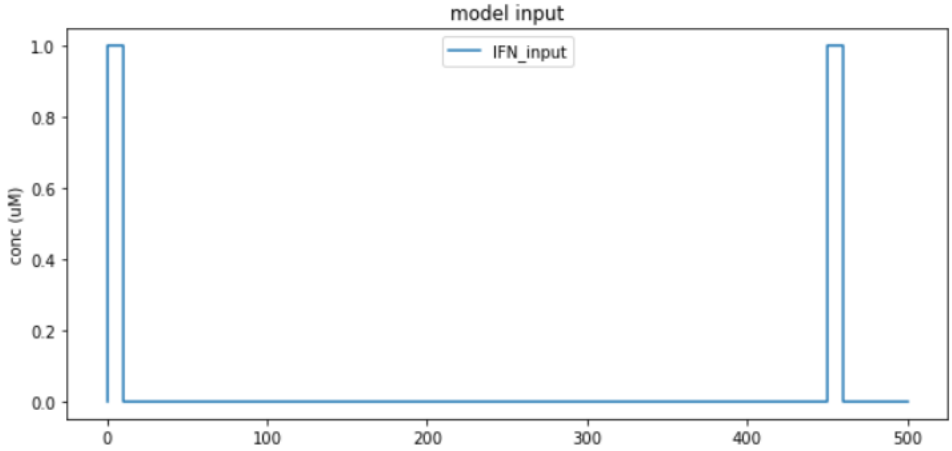
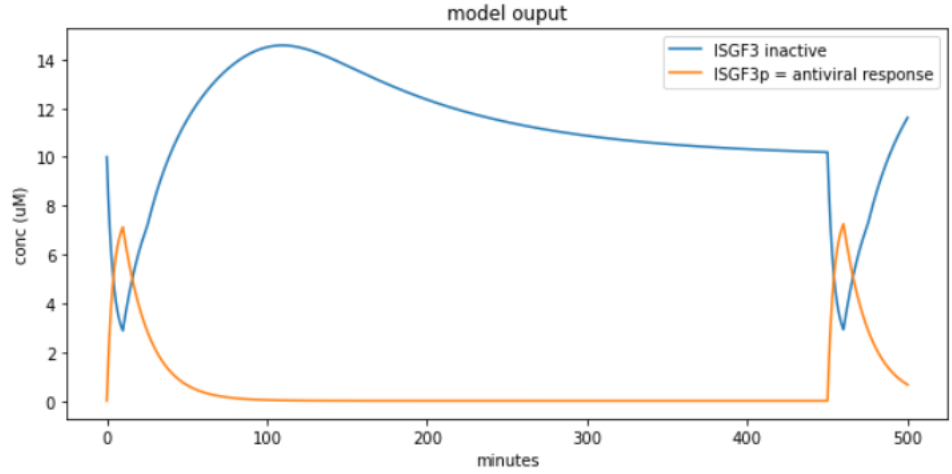
**Now modify "Simulation of simple IFN model" such that IFN_on2_time = 450 and IFN_off2_time = 460. Rerun the simulation and plotting cell. Paste your resulting graph here.** Describe the profile of antiviral response over time. Is the response to the challenge activation different from the priming activation? Why or why not?

This response is delayed for over 400 minutes. When the elapsed time is over a periof of time that is significantly longer so that it is no longer considered sequential, the positive feedback of the system does not take place. Instead, we see the ISGF3 curve regressing to steady state of 10 as usual and the ISGFp curve regressing to 0 as usual, and the second IFN input is similar to the priming activation. So we can say that the response to the challenge activation is not different from the priming activation.

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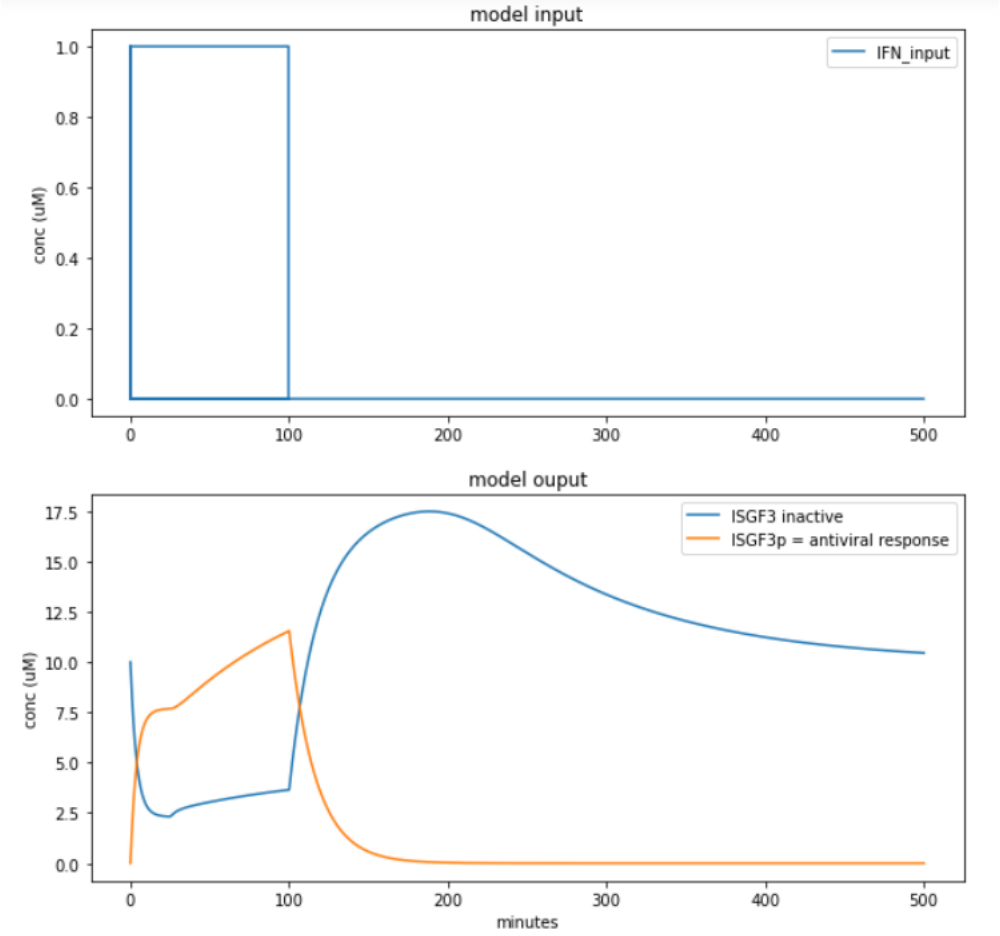
**Set back** IFN_on2_time = 0 IFN_off2_time = 0 i**n the section "Simulation of simple IFN model".** We will now explore the effect of lengthening the priming activation. **Modify "Simulation of simple IFN model" such that IFN_on1_time = 0 and IFN_off1_time = 100. Rerun the simulation and plotting cell. Paste your resulting graph here.**




How does the antiviral response differ from that with only a single 10-minute IFN activation? Why is there difference in the shape of the response?

The input is much longer than 10 minutes this time, elongated to 100 minutes of IFN input. We know that ISGF3 is a transcription factor that controls the proteins to fight virus, so if we have more ISFG3, the more likely our system is to fight off viruses. ISGP3 is converted to ISGP3 via ka and converted back via kd, with the delay tau (with protein synthesis k_syn), after an hour has passed, the pulse is gone. The second pulse comes and when the second pulse comes, there is more ISGF3, therefore giving us a higher concentration. If potentiation lasts longer than tau, there is more ISGF3 available, more gets made, and now can be converted to the phosphorylated form, and so the graph reflects a longer pulse. Finally, even the longer pulse also decline like the previous graphs due to degradation. The main difference here is that a 10 minute IFN input is a pulse, and an elongated signaling will use up the accumulated ISGF3.

**3. (20 points)** We will now simulate the complete IFN model depicted in the above illustration. We now also have a virus, V, that stimulates the conversion of IRF3/7 to

its active form IRF3/7*. IRF3/7* and IRF1 (which is always active) stimulates the production of IFN. Finally, ISGF3* stimulates the synthesis of ISGF3, IRF3/7, and IRF1 (IRF1 unlike the other species has no basal production).

In summary we have the following reaction:

| Reactions | Description |
|---|---|
| $\xrightarrow{k_{1syn*(1+H)}} ISGF3$ | ISGF3 synthesis |
| $\xrightarrow{k_{3syn*(1+H)}} IRF37$ | IRF3/7 synthesis |
| $\xrightarrow{k_{5syn*(H)}} IRF1$ | IRF1 synthesis |
| $ISGF3 \xrightarrow{k_{1deg}}$ | ISGF3 degradation |
| $ISGF3p \xrightarrow{k_{2deg}}$ | ISGF3* degradation |
| $IRF37 \xrightarrow{k_{3deg}}$ | IRF3/7 degradation |
| $IRF37p \xrightarrow{k_{4deg}}$ | IRF3/7* degradation |
| $IRF1 \xrightarrow{k_{5deg}}$ | IRF1 degradation |
| $IFN + ISGF3 \xrightarrow{k_{a1}} IFN + ISGF3p$ | IFN mediated activation of ISGF3 |
| $ISGF3p \xrightarrow{k_{d1}} ISGF3$ | Deactivation of ISGF3 |
| $V + IRF37 \xrightarrow{k_{a2}} V + IRF37p$ | Viral mediated activation of IRF3/7 |
| $IRF37p \xrightarrow{k_{d2}} IRF37$ | Deactivation of IRF3/7* |
| $\xrightarrow{k_{6syn*H_{OR}}} IFN$ | IFN synthesis |
| $IFN \xrightarrow{k_{6deg}}$ | IFN degradation |

The Hill term, *H*, is the same as it was as problem 2, containing the delayed value of ISGF3*. We additionally have the Hill term, $H_{OR}$, to describe the effective synthesis rate of IFN which can be stimulated by IRF3/7* or IRF1:

$$H_{OR}(t) = 1 - \frac{K_{d2}}{IRF3/7 * \_tau + K_{d2}} \frac{K_{d3}}{IRF1\_tau + K_{d3}}$$

We use the Hill equation from Homework 7 to describe a logical "OR" gate and use the delayed values of IRF3/7* and IRF1 to account for the time it takes to synthesis a protein.

First write down the change equations for the model described above, only for the species listed below. Make sure to include the Hill terms in the synthesis terms.

ISGF3_prime = k1_syn*(1+H) - k1_deg*ISGF3 - k_a1*IFN*ISGF3 + k_d1*ISGF3p

ISGF3p_prime = -k2_deg*ISGF3p + k_a1*IFN*ISGF3 - k_d1*ISGF3p

IRF37_prime = k3_syn*(1+H) -k3_deg*IRF37 - k_a2*IRF37*V + k_d2*IRF37p
IRF37p_prime = -k4_deg*IRF37p + k_a2*IRF37*V - k_d2*IRF37p
IRF1_prime = k5_syn*H - k5_deg*IRF1
IFN_prime = k6_syn*H_or - k6_deg*IFN

**In the section of code called "complete IFN model" implement the change equations to simulate the model.** Also include your equations for ISGF3 and ISGF3* from problem 2. Note how the code defines the value of the delayed variables and uses it in the calculations of the Hill Terms. Make sure to use the values of H and H_or in your implementation. Once you have defined your model equations, run the cell containing the model code as well as the following cell titled "delay helper functions". These functions are used to look up the old values of delayed variables as needed by the model.

Now we will simulate the model in the section of code called "Simulation of complete IFN model". In this section, we have defined our initial conditions and default parameter values which are also listed below for your reference (numerical subscripts are eliminated for brevity).

| Parameter | Value |
|---|---|
| k_syn | 0.1 $nM*min^{-1}$ |
| k_deg | 0.01 $min^{-1}$ |
| k_a | 0.2 $nM^{-1}min^{-1}$ |
| k_d | 0.05 $min^{-1}$ |
| Kd | 0.1 |
| tau | 25 min |

Below the initial conditions and parameter definitions is a section called "viral activation". The definitions are again much like NFKB and IFN from the previous problems. By default, V_on1_time = 0, V_off1_time = 10, V_amplitude = 1, and there is no challenge (the remaining activation parameters are set to zero).

**Run the section of code for "Simulation of complete IFN model" and 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 to the first few decimal places, double check your change equations and code before proceeding.

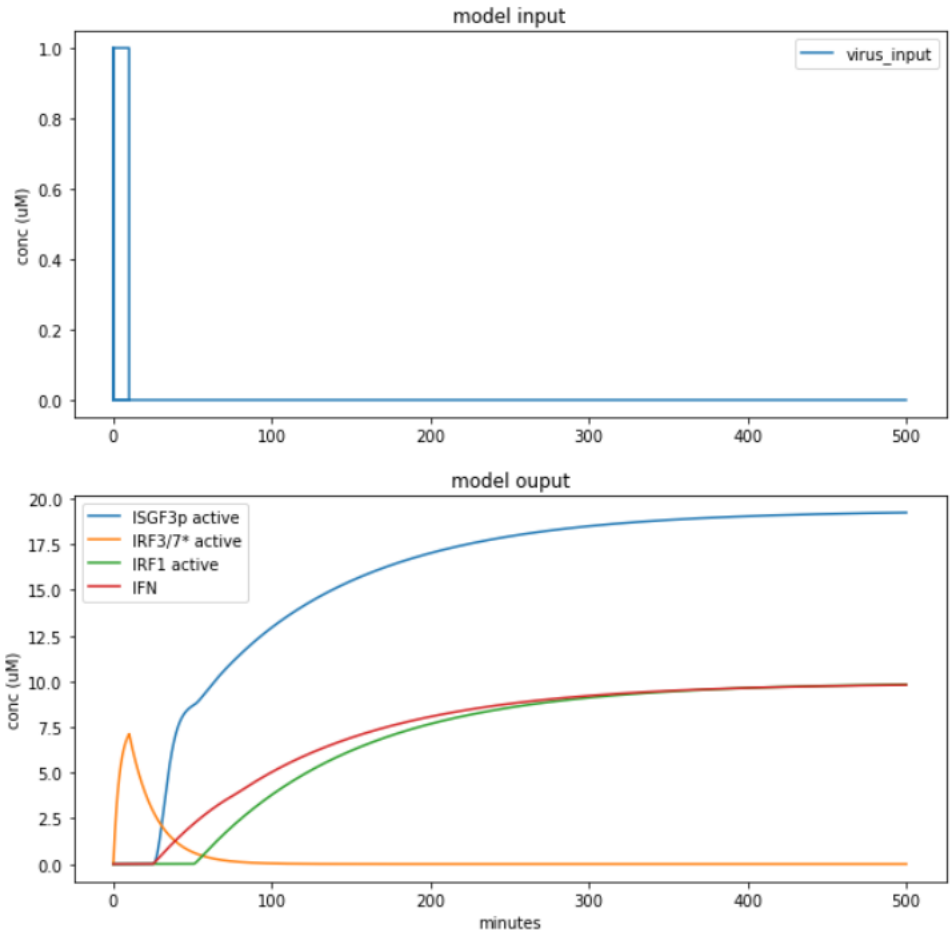
```
Checks on Model Implementation:
For each of the following lines ensure that your value on the right m
atches that on the left
1.3342367233230394 1.3342369722071357
12.957147386427966 12.957148245670236
2.8790276213841497 2.8790276403290833
7.120972378615856 7.1209723596709225
3.794056609229181 3.794056873963138
5.038355452623642 5.038359153346808
```

They match!

**Run the section of code called "Plot Dynamics" and paste your graph here.** Can you explain the shape of the trajectories in the "model output" (bottom) plot? Does the model return to its initial steady state?

The model's trends are that there is a gradual increase in ISGF3 active concentration, IRF1 active concentration, and IFN concentration; there is an impulse peak for IRF3/7* active concentration at around 15 minutes at a concentration of 7.5 uM. This is because IRF3/7* active activates IFN signaling, which we did in previous questions. The IFN signaling activates ISGF3 phosphorylation (from ISGF3 to ISGF3*), so we see the blue and red curve taking off together. The green curve, IRF1 active has a time delay of about 25 minutes.

With the given time frame of 500 minutes, all IRF3/7 active regress to zero because the viral signal is gone and IRF3/7* returns to IRF3/7. IRF1 active and IFN plateaus at 10uM concentration because they are needed to keep ISGF3 activity going. And indeed, ISGF3 activity is high and plateaus at a 20 uM concentration.

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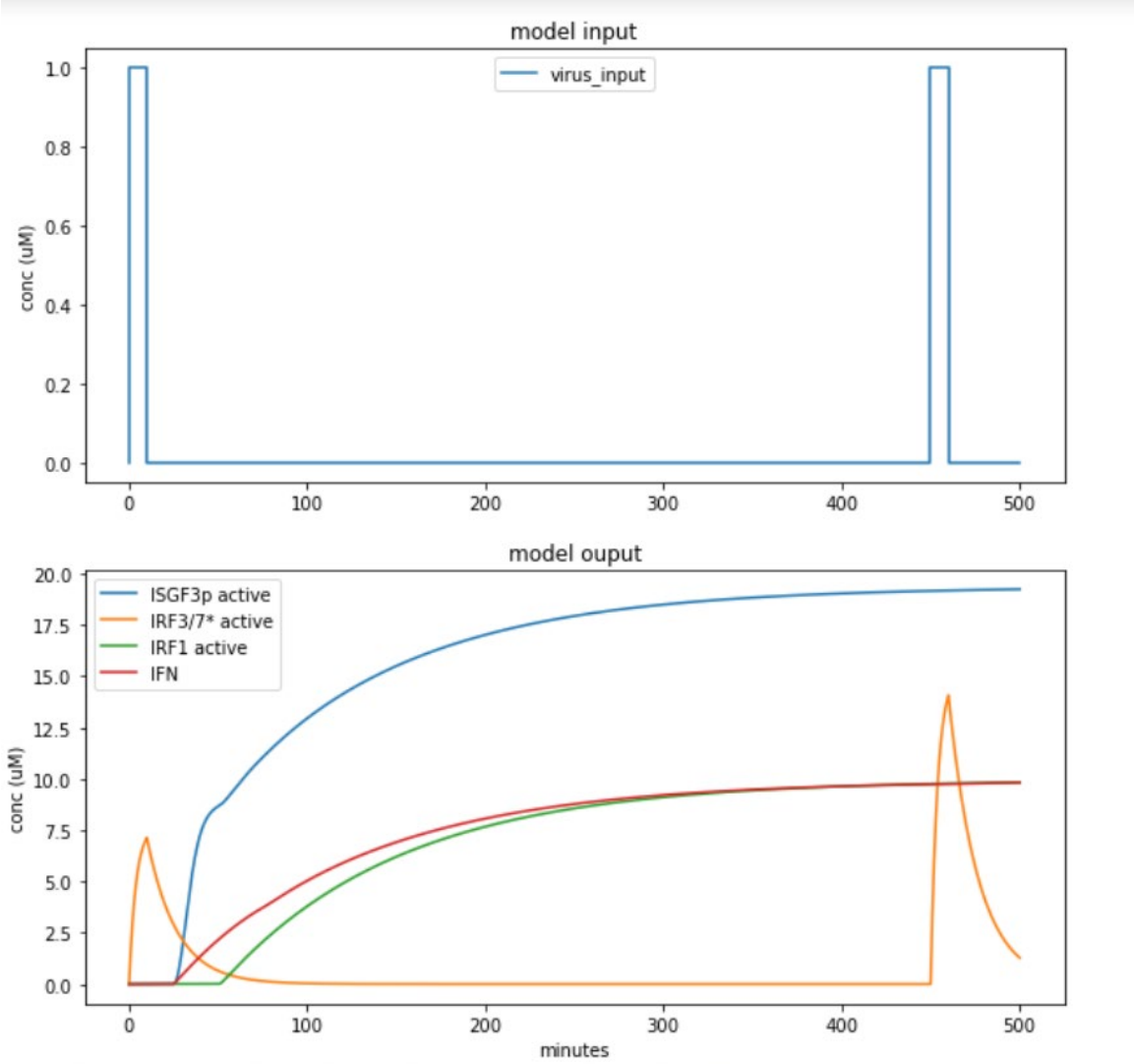
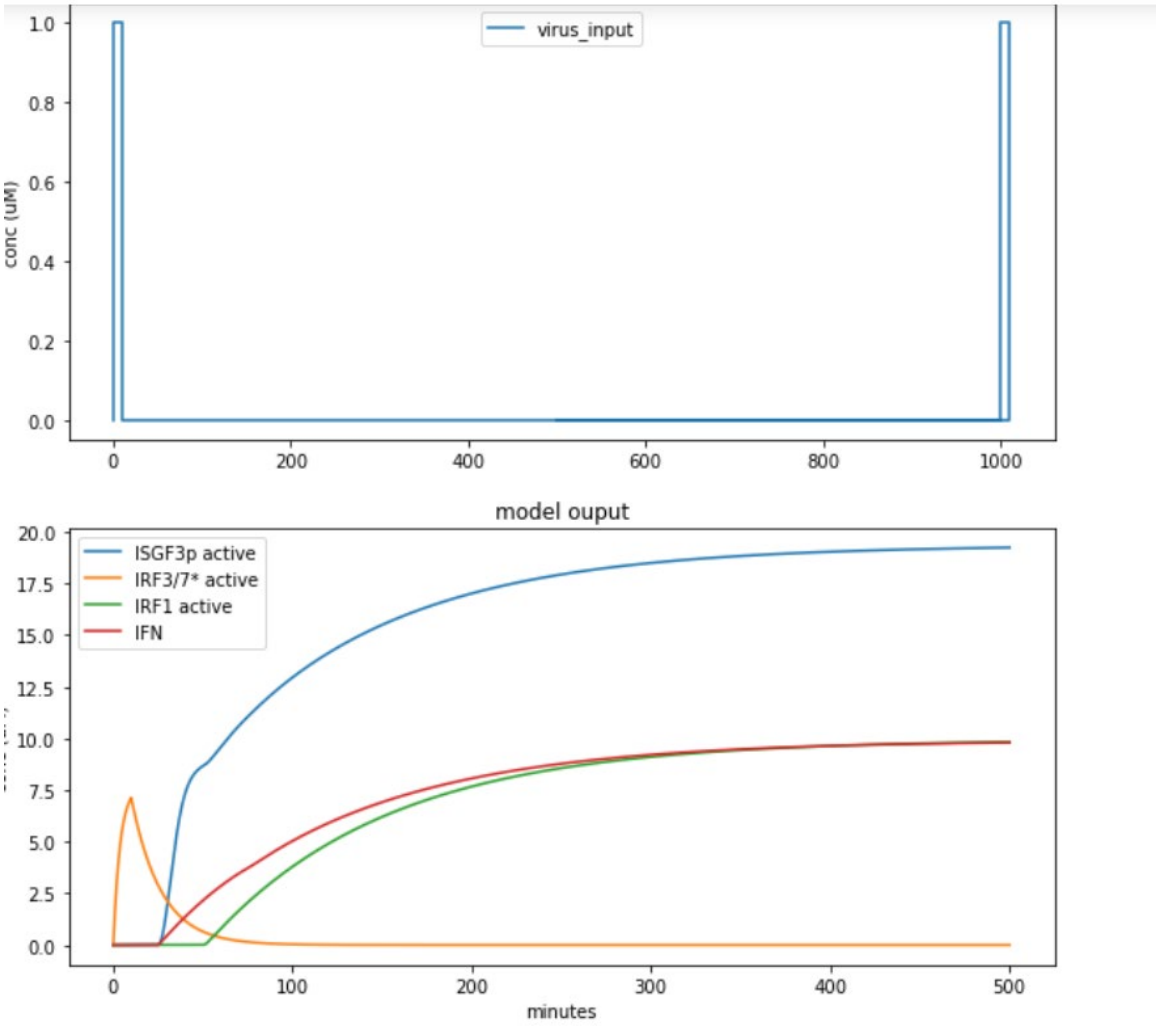
We will know add a challenge activation. **Modify "Simulation of complete IFN model" such that V_on2_time = 450 and V_off2_time = 460. Rerun the simulation and plotting cell. Paste your resulting graph here.** Is the response to the challenge activation different from the priming activation? Why or why not?

The response to the challenge activation is different from the priming activation because the time elapsed since the priming activation is not shorter than the time delay tau, used for protein synthesis. Therefore, it is worthy to note that the amount of active IR3/7* concentration is heightened to twice previously (15 uM rather than 7.5 uM). This implies that there is memory in the system, so more protection against viral infections will be granted.




Suppose instead the timing of the challenge activation was further delayed (i.e. V_on2 time = 1000, V_off2 time = 1010). Predict what the response of ISF3/7* would look like to the challenge.

To the challenge activation, in a case where the time interval is much longer, I predict that there would not be another peak (sudden increase of IRF3/7*). I predict that IRF3/7* active will deplete itself and return to a steady state of 0 around 100 minutes. I foresee that all other curves will be the same.




How is the priming in this model different from that in the previous two problems? Can you think of scenarios where these different types of "memory" could be useful to a cell?

Priming in this model is different by that even after a really long time, for up to 400 minutes, the system can still detect increases in IRF3/7* active concentration. Whereas previously in question 2, with the same input over 400 minutes it would not have a positive feedback anymore. It is not a situation where time delay is playing a big part on the priming, so it is different from question 1 also. Scenarios of which where these different types of memory could be useful is when T cells and B cells are targeting virulent species, and that even over a long period of non-exposure, they are able to recognize the species.