# PhySci/MiMG/CaSB M178

## Homework 5

**Due: 11/07/23 at 12:00PM PDT**

**Notes:** This homework involves performing simulations of bacterial chemotaxis we've been discussing in the last two class meetings. In the same page on BruinLearn where you obtained this document, you will also find a file called "HW5_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**. You can upload your Jupyter file (which should be a .ipynb file). Make sure you upload your Jupyter notebook by the due date/time (11/07/23 at 12:00PM PDT).

**Problems**

In class, we talked about bacterial chemotaxis. Chemotactic signaling utilizes a special "Two Component" kinase system involving a phosphotransferase, the "Histidine Kinase" (HK), and its substrate, the "Response Regulator" (RR). One fascinating aspect of these systems is that the HK can serve to both phosphorylate and dephosphorylate the RR substrate. In this homework, we will model this system using the following reactions:

1. HK is phosphorylated to form HKp with rate k_a (first order reaction)
2. HKp is dephosphorylated to form HK with rate k_u (first order reaction)
3. HKp binds with RR to form HKpRR with rate k_p (second order reaction)
4. The complex HKpRR can dissociate into HKp and RR with rate k_m (first order reaction)
5. HKpRR converts into HK plus RRp with rate k_cat (first order reaction) [Note: this is the actual phosphotransferase step!]
6. HK also binds with RRp to form HKRRp with rate k_p (second order reaction)
7. The complex HKRRp can dissociate into HK and RRp with rate k_m (first order reaction)
8. HKRRp converts into HK and RR with rate k_cat (first order reaction) [Note: this is the dephosphorylation step!]

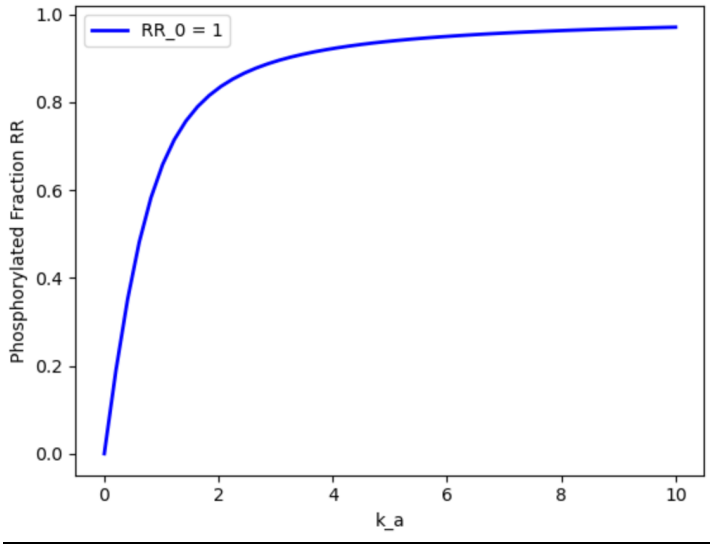
In Two Component systems, the incoming signal modulates the value of k_a (i.e. activating signals increase the value of k_a).

**1. (20 points)** First, write down the change equations for the model described:

HK_prime = -(HK)*(k_a + (k_p)*(RRp)) + (k_u)*(HKp) + (k_cat)*(HKpRR) + (k_m + k_cat)*(HKRRp)
HKp_prime = (k_a)*(HK) - (HKp)*(k_u + (k_p)*(RR)) + (k_m)*(HKpRR)
RR_prime = -(k_p)*(HKp)*(RR) + (k_m)*(HKpRR) + (k_cat)*(HKRRp)
RRp_prime = (k_cat)*(HKpRR) - (k_p)*(HK)*(RRp) + (k_m)*(HKRRp)
HKpRR_prime = (k_p)*(HKp)*(RR) - (k_m + k_cat)*(HKpRR)
HKRRp_prime = (k_p)*(HK)*(RRp) - (k_m + k_cat)*(HKRRp)

**In the section of code called "two component model" implement the change equations to stimulate the model.**

In the next section of code, "STEADY-STATE responses of two component model PART 1", the initial values and parameters we will use for simulation are first set. Note we initialize the phosphorylated and complex species to zero. Here, we will vary the value of the parameter k_a (reflecting the activating signal) and plot the resulting steady state concentration of RRp divided by the initial concentration of RR. Note that the current initial concentration of RR is set to 1 and the value of k_a will be varied from 0 to 10. Run this section of code and paste the resulting graph below:

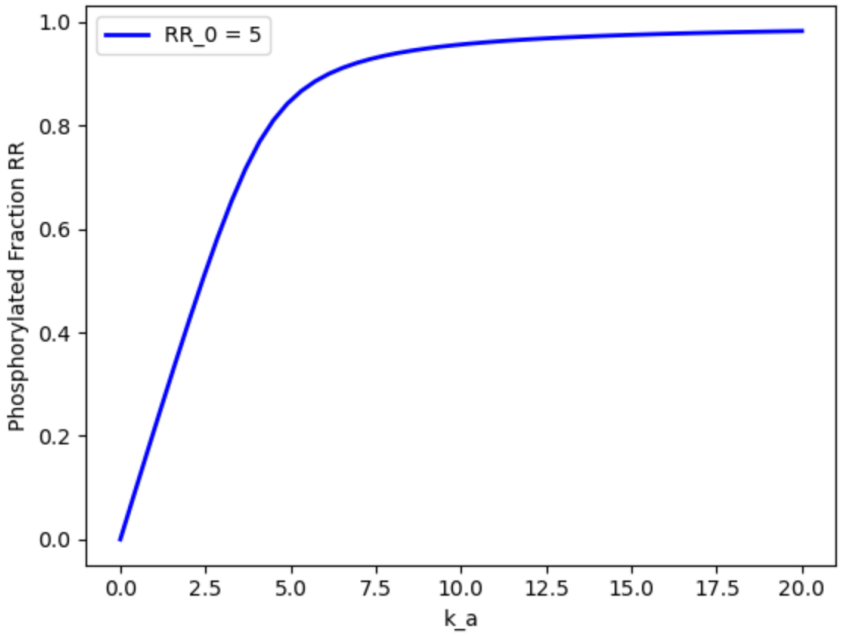


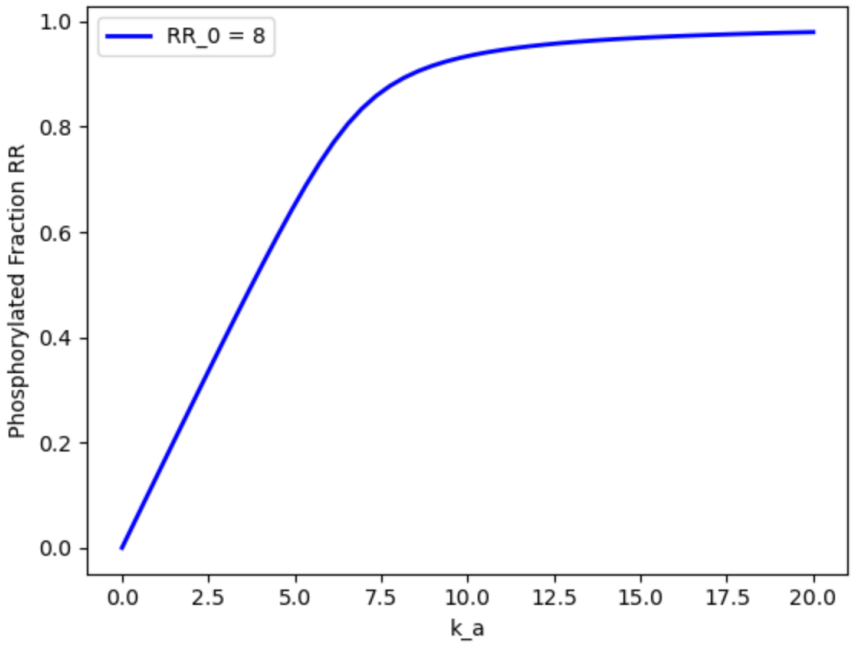
This model is somewhat similar to the PTM cycle models explored in HW1. Relate the species and reactions from our two-component model to the PTM model without synthesis and degradation. Which species/reactions from the PTM model do not have analogous species/reactions in the HK model?

Histidine Kinase (HK) and the Response Regulator (RR) act similarly to substrates in the PTM cycle, where HK can become phosphorylated or dephosphorylated. HKp is like a kinase that transfers a phosphate to RR to create RRp similar to a substrate being modified by a kinase. The HK and RRp interaction is similar to a phosphatase removing a phosphate group to revert RRp to its original state. Both models also don't include synthesis or degradation. HK, RR, RRp are analogous to K, S, and S* in the PTM model, respectively.

The model doesn't include separate kinases and phosphatase enzymes like in PTM cycles since HK performs both functions here. A reaction like the HKp dephosphorylation without a phosphatase differs from the PTM model. In contrast, a standard PTM cycle involves separate enzymes for phosphorylation and dephosphorylation.

In HW 1, we explored how saturation affects the steady state amount of modified substrate as a function of initial kinase concentration by changing the amount of initial substrate. Rerun the section of code **at least twice** with increased values for RR_0 and paste the resulting graphs below:

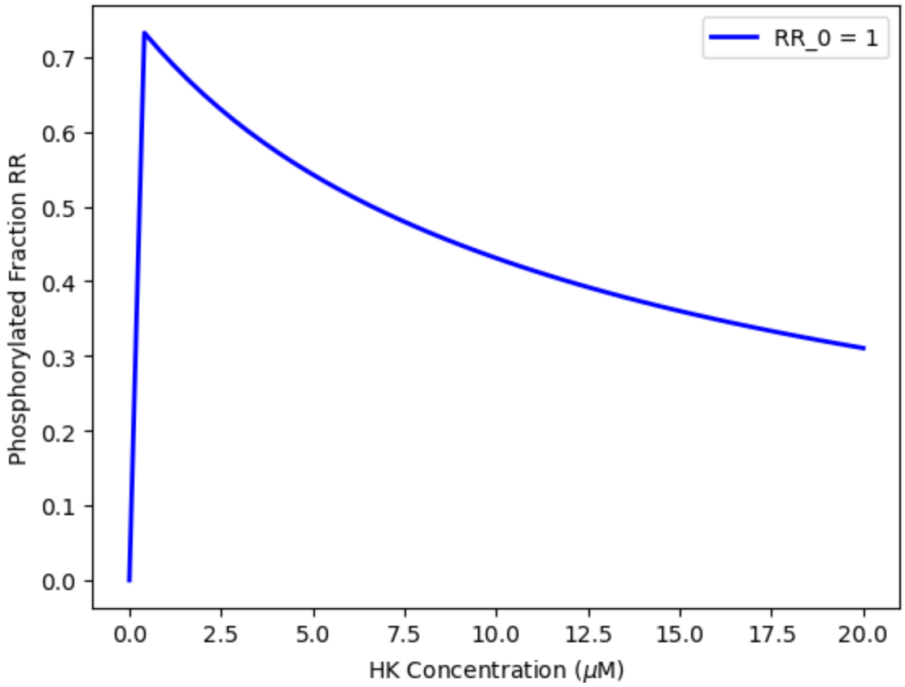





How does saturation of the histidine kinase affect the value of k_a needed to achieve the maximum steady state phosphorylated fraction of response regulator?

Increasing the saturation of the histidine kinase results in a higher k_a value required to reach the maximum steady-state phosphorylated fraction of the response regulator. This is because as the HK becomes more saturated, the graph begins to plateau, showing minimal increases in response as k_a is increased. So, a larger k_a value is needed to reach the maximum response as shown in the graph.

**2) (10 points)** In HW 1, we also explored the steady state amount of modified substrate as a function of initial kinase concentration. We will now similarly explore the steady state concentration of phosphorylated RR as function of initial HK concentration in the section titled "STEADY-STATE responses of two component model PART2". In this section we will vary the initial concentration of HK from 0 to 10 and plot the resulting steady state concentration of RRp divided by the initial concentration of RR. Run this section of code and paste the resulting graph below:




**How does this compare to the plot of steady state modified substrate versus kinase concentration for the PTM cycle? Can you explain why the differences emerge if any?**

This plot reaches its maximum phosphorylated fraction of RR quickly as the HK concentration increases, but only for a small range of HK concentration values. As you increase the HK concentration, the phosphorylated fraction of RR gradually decreases with a smooth curve-like plot shape.

In the plot of steady state modified substrate vs kinase concentration for the PTM cycle, there is a gradual smooth increase in fraction of steady-state modified substrate as the kinase concentration increases and it appears to gradually level off, but never decreases.

This difference is likely due to HK's dual function in the chemotaxis model. Since HK phosphorylates RR and dephosphorylates RRp, at low HK levels, phosphorylation dominates and leads to an initial increase in RRp. As HK concentration increases, dephosphorylation dominates and reduces the fraction of RRp. This could explain why there is a rise and then a fall in the fraction of phosphorylated RR. In the PTM cycle, there's distinct kinase and phosphatase enzymes so increasing kinase concentrations steadily increases the fraction of phosphorylated substrate and causes it to plateau since there's no increase in dephosphorylation activity when there's more kinase.

**3) (25 points)** Next, we will implement a model for the Two Component signaling system that is part of the chemotactic signaling network in bacteria. In this model, the HK species is called CheA and the RR species is called CheY. There is also a separate phosphatase enzyme called CheZ. It dephosphorylates CheY instead of CheA acting as the phosphatase. We will model this system with the following reactions:

1. CheA is phosphorylated to form CheAp with rate k_a (first order reaction)
2. CheAp is dephosphorylated to form CheA with rate k_u (first order reaction)
3. CheAp binds with CheY to form CheApCheY with rate k_p (second order reaction)
4. The complex CheApCheY can dissociate into CheAp and CheY with rate k_m (first order reaction)
5. CheApCheY converts into CheA and CheYp with rate k_cat (first order reaction)
6. CheZ binds with CheYp to form CheZCheYp with rate k_p (second order reaction)
7. The complex CheZCheYp can dissociate into CheZ and CheYp with rate k_m (first order reaction)
8. CheZCheYp converts into CheZ and CheY with rate k_cat (first order reaction)

Write the change equations for this new model below:

CheA_prime = -k_a*CheA +k_u*CheAp +k_cat*CheApCheY

CheAp_prime = +k_a*CheA -k_u*CheAp -k_p*CheAp*CheY +k_m*CheApCheY

CheY_prime = -k_p*CheAp*CheY +k_m*CheApCheY +k_cat*CheZCheYp

CheYp_prime = +k_cat*CheApCheY -k_p*CheZ*CheYp +k_m*CheZCheYp

CheZ_prime = -k_p*CheZ*CheYp +k_m*CheZCheYp +k_cat*CheZCheYp

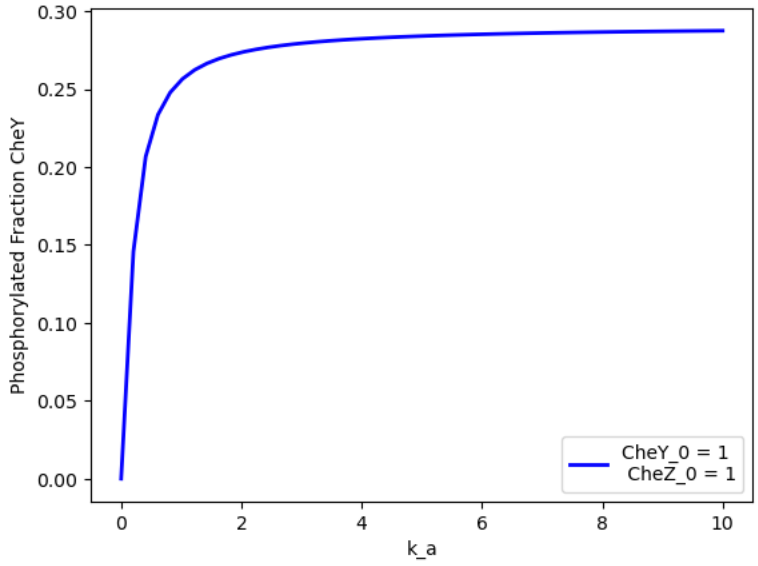
CheApCheY_prime = +k_p*CheAp*CheY -k_m*CheApCheY -k_cat*CheApCheY

CheZCheYp_prime = +k_p*CheZ*CheYp -k_m*CheZCheYp -k_cat*CheZCheYp

**Now add these change equations to the section of code called "chemotaxis model".**

We will once again explore steady state responses of the model in the section "STEADY-STATE responses of chemotaxis model". Note we initialize the phosphorylated and complex species to zero. We will again vary the value of

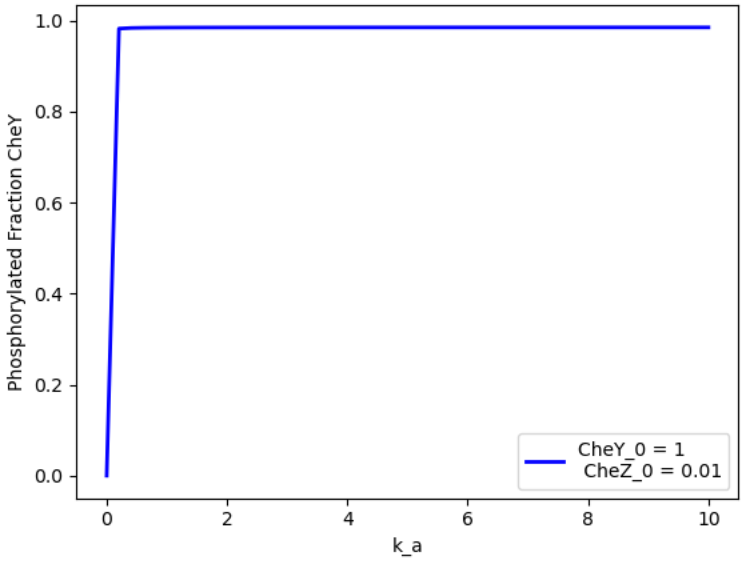
the parameter k_a (reflecting the activating signal) and plot the resulting steady state concentration of CheYp divided by the initial concentration of CheY. Note that the current initial concentration of CheY is set to 1 and the value of k_a will be varied from 0 to 10. Run this section of code and paste the resulting graph below:

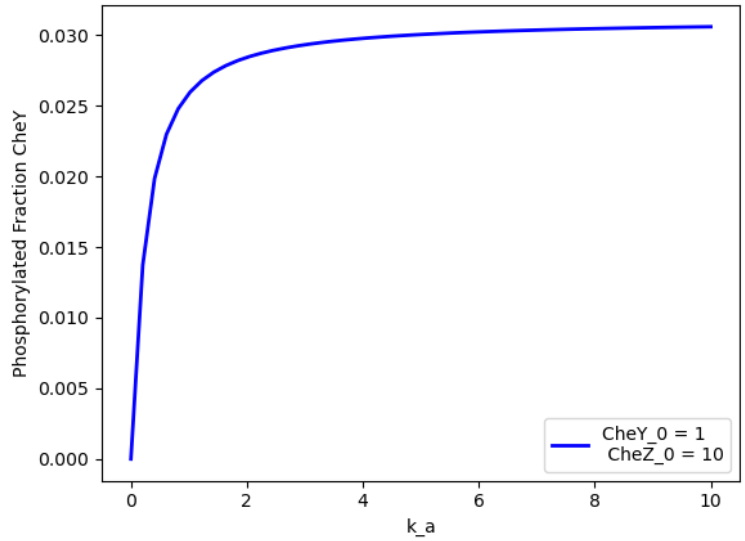



How does this result compare to your initial graph from problem 1?

This result is similar to the initial graph from problem 1 where both show a plot that increases over time as the k_a value increases, but then levels off as the fraction of phosphorylated RR/CheY becomes saturated. It is important to note that this plot has a sharper initial increase in phosphorylated fraction of CheY before it begins to gradually level off. However, the scale of the y-axis is noticeably smaller in magnitude as it only ranges from 0 to 0.30, while the y-axis scale from problem 1 ranges from 0 – 1.0. The overall shape of the curve in both graphs are similar, but the scale of the graphs differ.

Now let's rerun this section of code with some initial conditions altered. First, let's focus on the phosphatase, CheZ. Choose **two different values** for CheZ_0, and paste the resulting graphs below. [Note: try one value that is smaller than the default value you had, and one that is larger].



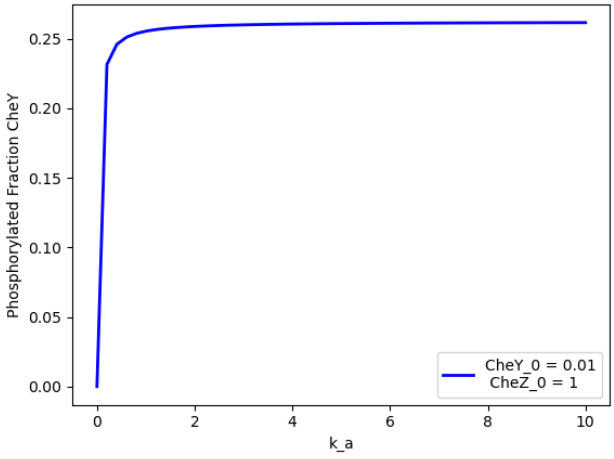



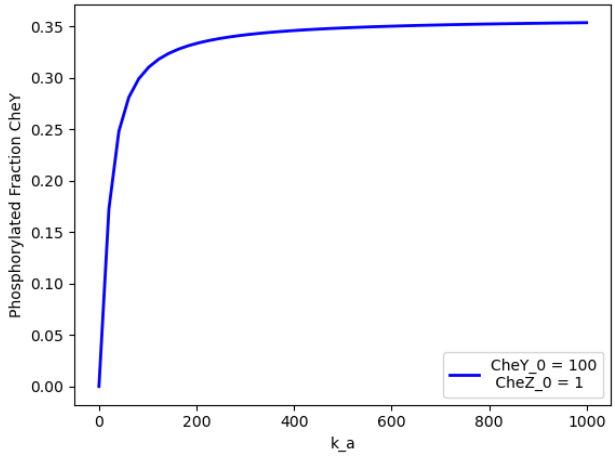

How does modifying the initial amount of CheZ affect the steady-state response of CheY?

Modifying the initial amount of CheZ changes how quickly CheY saturates and the amount it saturates to. If you decrease the initial amount of CheZ, the phosphorylated fraction of CheY saturates quickly where there's a sharper increase and it reaches its maximum quicker, and also has a higher saturation value. As you increase the initial amount of CheZ, the maximum phosphorylated fraction of CheY decreases. You can see that when the initial CheZ amount is increased by a factor of 10, the fraction of phosphorylated CheY is roughly 10 times smaller.

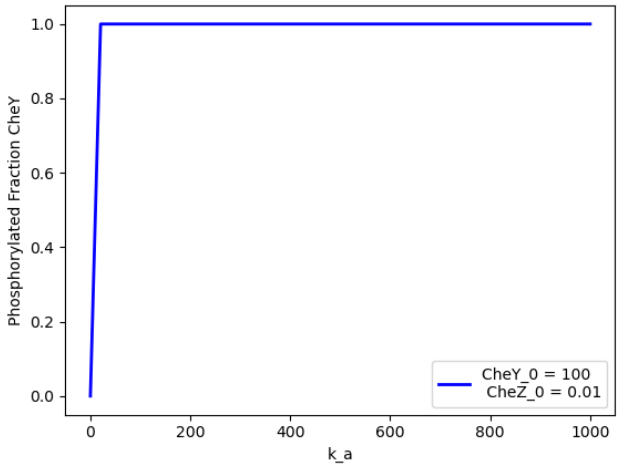
Reset the initial conditions for CheZ to its default value. Now we will look at how changing the total amount of CheY affects the system. As above, **choose two**

**new values** CheY_0 and paste the resulting graphs below: [Note: we suggest increasing CheY levels, as this will *increase* saturation!]




Now, as a final exploration, keep CheY_0 at one of the larger values you chose, and try changing CheZ_0 to the **smaller** value that you tried above (in other words, *increase* CheY_0 from the default value, and *decrease* CheZ_0). Paste the resulting graph below:



Overall, how do these results compare now to the results from problem 1? Does increasing CheY always have the same effect? How does changing the phosphatase levels affect the behavior. Overall, how does the behavior of this system compare to the original PTM cycle model results from HW1?

Comparing to the results from problem 1, the results in this problem shows a sharp increase to a maximum level of phosphorylated CheY, which then remains constant regardless of further increase in k_a. However, the results from problem 1 show a smooth, saturating curve as k_a increases and reaches a plateau.

Increasing only CheY will increase the value of k_a required for the phosphorylated fraction of CheY to reach its maximum steady state value. Increasing CheY_0 will also generally increase the maximum steady state value of phosphorylated fraction of CheY.

Increasing CheY_0 and decreasing CheZ_0 increased the maximum steady state,but decreased the k_a value required to reach the maximum steady state of phosphorylated fraction of CheY.

In the original PTM model, increasing S_0 resulted in an increase in the maximum steady state of the phosphorylated fraction of S, while simultaneously reducing the value of k_a needed to reach the maximum steady state.

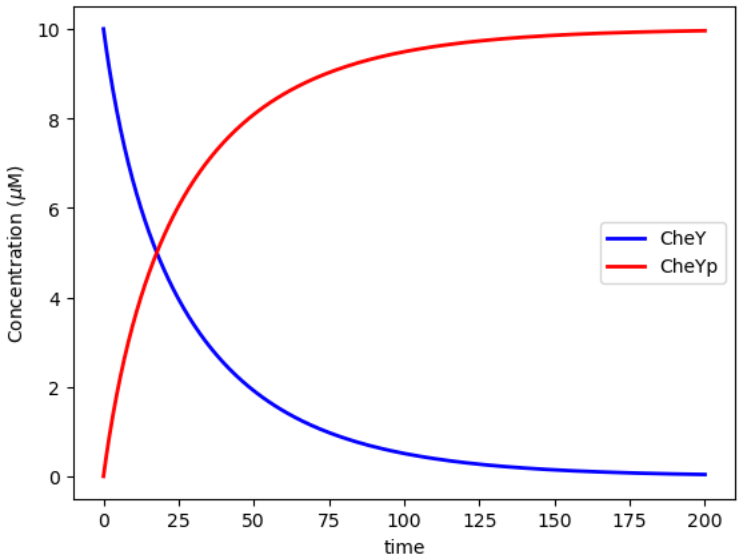
This reflects how biological systems use enzyme-substrate balances to adapt to environmental stimuli.

**4) (20 points)** Next, we will implement a simple model for perfect adaptation. The model consists of the following two change equations:

$$CheY' = -V_{max}^{A} + V_{max}^{Z}\frac{CheYp}{K_z + CheYp}$$
$$CheYp' = V_{max}^{A} - V_{max}^{Z}\frac{CheYp}{K_z + CheYp}$$

In this model, we assume that CheA is phosphorylated and operating at its maximum velocity, $V_{max}^{A}$, to phosphorylate CheY. CheZ, which dephosphorylates CheYp is not; its rate is dependent on the amount of $CheYp$ present. The maximum velocity of CheA, $V_{max}^{A}$, is less than the maximum velocity of CheZ, $V_{max}^{Z}$, for this model.

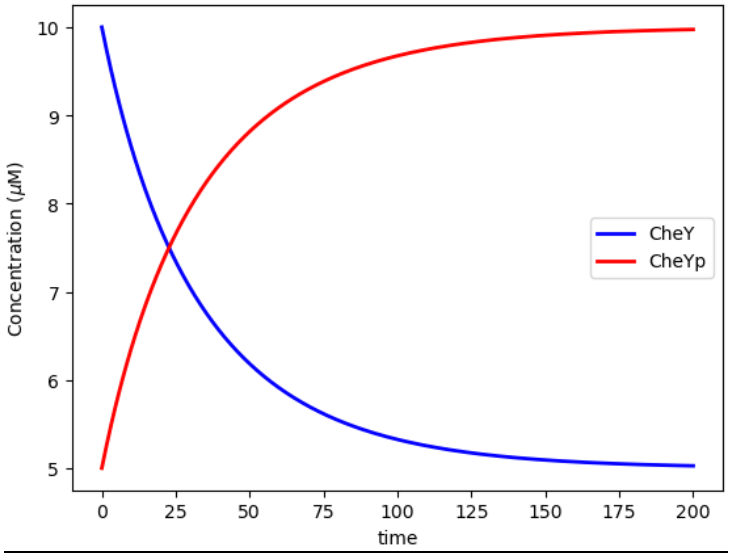
We have implemented this mode in the section called perfect adaptation
We will explore this model in the section "Responses of perfect adaptation model". This section first implements the initial conditions and parameters for this model. By default, Chey_0 is set to 10 and CheYp_0 is set to 0. Va (the maximum velocity of CheA) is set to 0.5, Vz (the maximum velocity of CheZ) is set to 1, and Kz is set to 10. <u>Run this section of code to simulate the perfect adaptation model for this set of initial condition and paste the resulting graph here:</u>



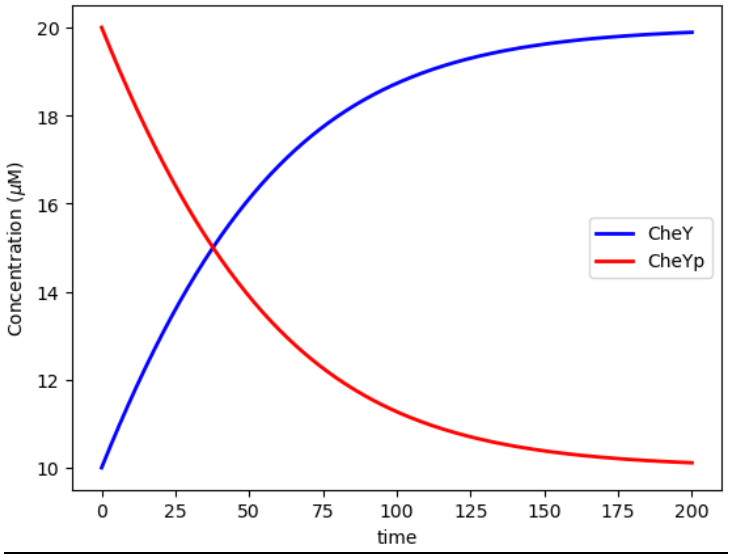
What is the steady state value of CheYp?

The steady state value of CheYp = 10μM

Rerun the code twice. In the first case, set an initial value of CheYp **below** the steady state value you found. In the second case, set an initial value of CheYp **above** this steady-state value. Paste the resulting graphs below:




CheYp_0 = 5μM




CheYp_0 = 20μM

What are the steady state values of CheYp now?

CheYp=5μM below steady state value: 10μM
CheYp=20μM, above steady state value: 10μM

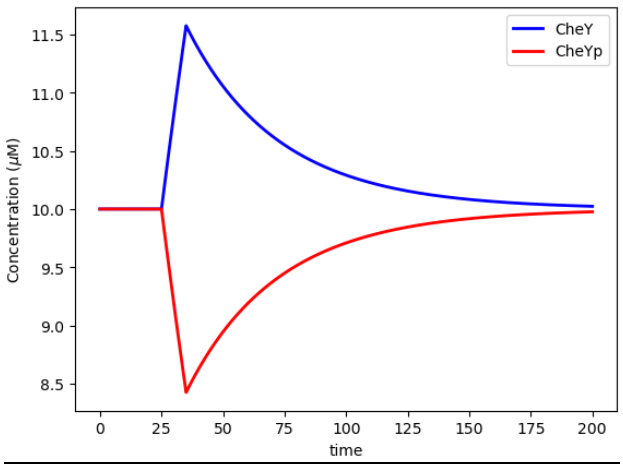
Explain how this model demonstrates perfect adaptation. Why is this important for chemotaxis?

There is perfect adaptation because despite whatever initial concentration of CheYp we have, whether it's below or above the steady state value, the long-term concentration of CheYp will converge to the same steady-state value of CheYp of 10μM. Whenever there is a disturbance, the same steady state level of CheYp will be reached in the long run.

Perfect adaptation is important because chemotaxis can reset its sensory system after a response to a stimulus. Despite changes in chemical concentrations, the chemotaxis signaling pathway can always return to pre-stimulus activity level. This allows chemotaxis to remain sensitive to new changes in chemical gradients cand can continue to move toward more favorable environments (or away from harmful ones). Being able to return to steady state after a temporary response allows chemotaxis to make time comparisons of concentration changes in their environments, which helps them navigate efficiently involving knowing when to stop.

**5) (10 points)** Finally we will explore the effects of adding a transient spike of CheYp on the results of the perfect adaptation model in the section "perfect adaptation model with transient spike". In this model, when the time in the simulation is greater than "t_on" but less than "t_off", an additional amount of CheYp will be added to the model, called "spike".

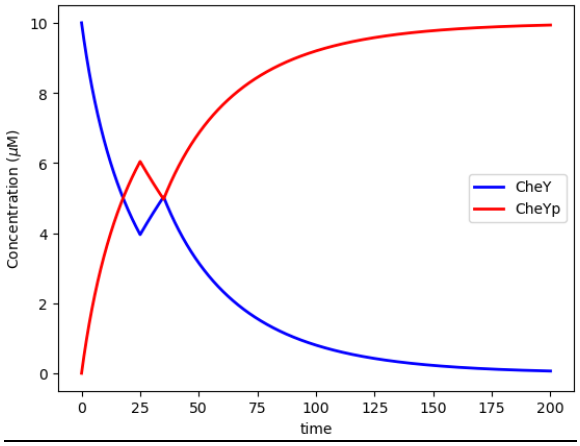
We will explore this model in the section "Responses of perfect adaptation model with spike". This section first implements the initial conditions and parameters for this model. By default, Chey_0 is set to 10.05 and CheYp_0 is also set now to 10. We additionally have a parameters section to implement the transient spike in CheYp. Run this section of code to simulate the perfect adaptation model with CheYp spike for this set of initial condition/parameters and paste the resulting graph here:



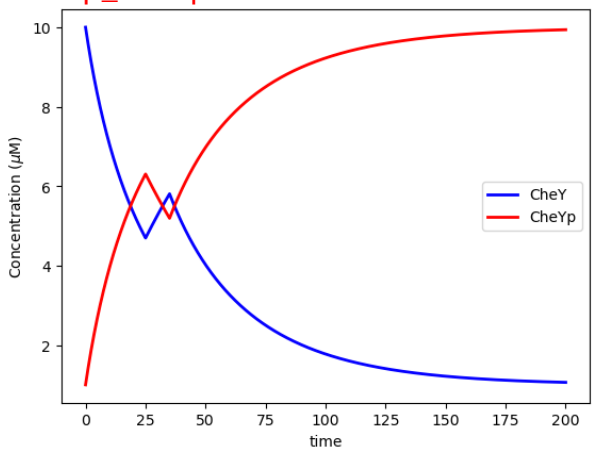
What happens to the value of CheYp once the spike is initiated and after it's turned off?

The value of CheYp decreases when the spike is initiated, but then gradually increases after the spike is turned off.

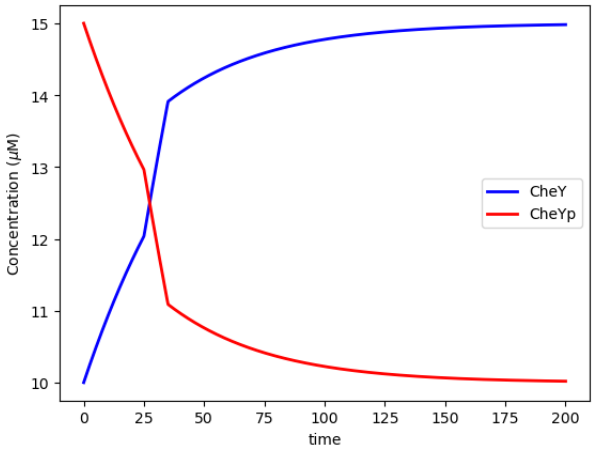
Rerun the code with an altered initial condition for CheYp and see how the trajectory changes? Paste your graph below. Do you still end up with the same steady state value? Based on your results, does this model exhibit perfect adaptation after exposure to a stimulus?




CheYp_0 = 0μM




CheYp_0 = 1μM




CheYp_0 = 15μM

When the initial amount of CheYp is decreased, the concentration of CheYp begins to increase toward the steady state value of 10μM, then the concentration of CheYp decreases once the spike is activated, and finally it begins to increase again toward the steady state value of 10μM after the spike is turned off. The CheYp concentration ends up at 10μM in the long run.

When the initial concentration of CheYp is increased, the concentration of CheYp begins to decreases toward the steady state value of 10μM, then the concentration of CheYp decreases even more once the spike is activated, and finally it decreases at a slower and more gradual rate once the spike is deactivated. Over time, the CheYp concentration is at 10μM.

Based on these results, I can conclude that this model does exhibit perfect adaptation because when after a disturbance in the system, the CheYp concentration will still end up at the steady-state value in the long-run. This mirrors the ability of chemotaxis to adapt to varying levels of stimuli regardless of initial conditions.