**Biochemical Signaling**

Many of the most critical biochemical signaling pathways originate with an extracellular signal being recognized by a GPCR or a RTK. In this activity, we will explore these two signaling pathways in more detail with special emphasis placed on the big picture ideas that you should be comfortable with moving forward.

GPCR

1. What does GPCR stand for?

2. Briefly describe the steps in PKA activation by a GPCR signal. You are encouraged to include a sketch.

3. What do all G proteins do?

4. Predict a catalytic mechanism for the G-protein reaction. Use the image below to get you started.
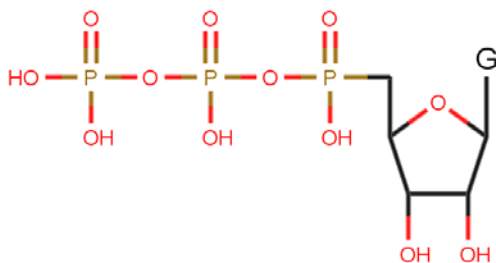
5. Mg^{2+} is required for enzyme function. Propose two ways that Mg^{2+} could be involved in enzyme function.

6. Consider the transition state of your mechanism.
   a. Circle it and comment on how you think the enzyme is able to stabilize the transition state.

   b. Write the rate law for the enzyme catalyzed reaction. Remember that the rate of a reaction is contingent on the slowest step.

   c. Now comment on what part of the rate law is changed by the enzyme catalyzing the reaction.

   d. Now consider the image shown below. This is a GTP mimetic bound to the active site of a G protein. Does this image support your proposal in part a?
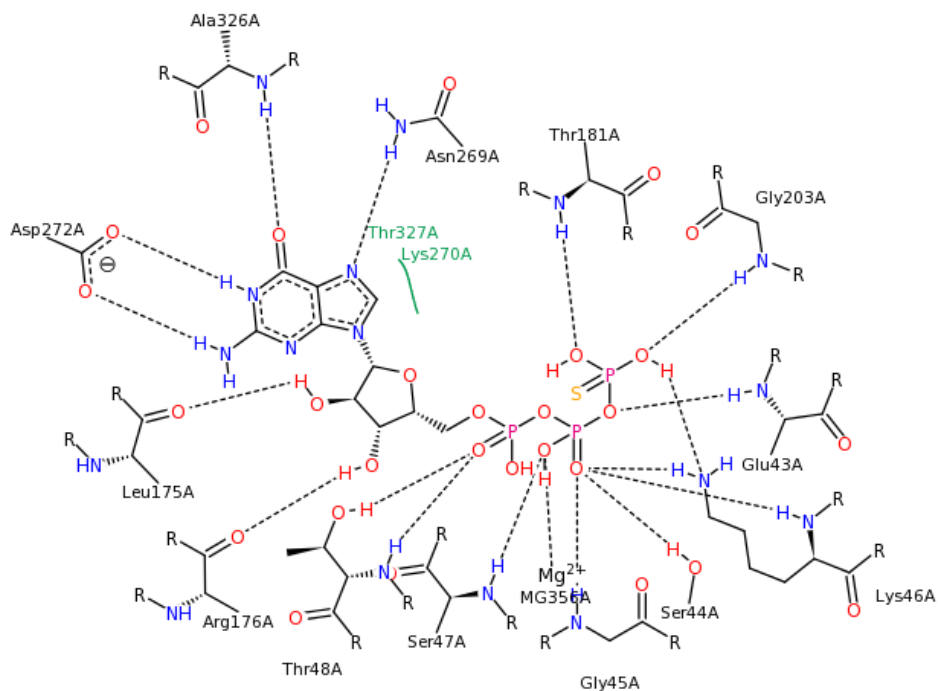
e. Look closely at the image above. How is this molecule different than the normal substrate for the G protein?

f. Think very critically about the mechanism. How does this very small difference in the substrate prevent hydrolysis?

g. Note the position of Mg^{2+}. Based on its position, what role do you think it plays in enzyme function?

h. Zn^{2+} can easily replace Mg^{2+} in the enzyme active site and it yields an inactive enzyme. What type of inhibitor do you think Zn^{2+} is? What effect would this inhibitor have on Km and Vmax?

7. G proteins involved in signaling are notoriously slow. For example, the stimulatory $G\alpha$ from common rice has a k_{cat} of 1.14 s^{-1}.
   a. How many GDP are produced in one minute?

   b. How long does it take to produce on GDP?

   c. Consider the overall sequence of events in GPCR signaling. What happens when GTP is hydrolyzed?

   d. Based on your answer to c, why is slow turnover important in this signaling pathway?

e. One of the ATP hydrolases from humans has a turnover number of 425 s^{-1}.
   i. Is hydrolysis by the ATPase or GTPase faster?
   ii. Which would have a lower activation energy? Explain your answer.

   iii. Based on this, do you think the ATPase is better at stabilizing the transition state during the hydrolysis reaction?
   iv. Consider your answer to 5f. The same modification on ATP does not prevent the enzyme from hydrolyzing the substrate. Justify this observation based on what you have learned in problem 6.

8. Now think about Adenylate Cyclase (AC).
   a. This enzyme converts ATP to 3'5' cyclic-AMP (cAMP). Propose a mechanism for this enzyme catalyzed reaction. Make sure to consider how the enzyme may be able to activate the nucleophile.

   b. Consider the inhibitor of AC shown below. Clearly explain why this inhibitor is not reactive.
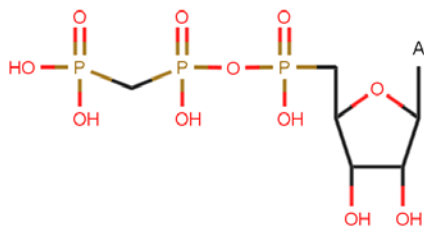
c. Is this a competitive or uncompetitive inhibitor? Explain your answer. How would it influence Km and Vmax?

RTK

9. What does RTK stand for?

10. There are a very wide variety of RTK in your body. What features do each have in common?

11. What does "autophosphorylation" mean and why is it relevant to RTKs?

12. RTK are allosteric enzymes. Signal binding on the outside of the membrane causes PTK domain on the inside to become active. Discuss this process in the context of the "R" and "T" states that we learned about in other allosteric proteins.

13. Grb2 is an adaptor protein that has two different recognition domains; an SH2 domain that specifically binds to phosphotyrosines and an SH3 domain that binds to proline rich sequences. The SH3 domain latches on to a protein called SOS and anchors it close to the membrane.
    a. Why is it anchored close to the membrane? Hint: What else is Grb2 interacting with that keeps it membrane associated?

    b. SOS is known as a nucleotide exchange factor. It's role in this signaling pathway is the promote the exchange of GDP for GTP in the monomeric G-protein, Ras. Based on this, what reaction do you think Ras catalyzes?

c. Believe it or not, Ras is even worse of an enzyme than $G\alpha$ with a k_{cat} of 0.0005 s^{-1}. If the catalytic efficiency of Ras is 0.5 mM^{-1}s^{-1}, determine the dissociation constant for GTP.

d. This very poor turnover number can be explained when considering the active site (below). In this image, the green protein (top and left side) is Ras and the blue protein (bottom right corner) is a G-protein Activating Protein (GAP). GDP is shown in white, AlF$_3$ is shown in purple and green, and water molecules are red spheres; together ADP, AlF$_3$, and H$_2$O mimic the transition state of ATP hydrolysis.
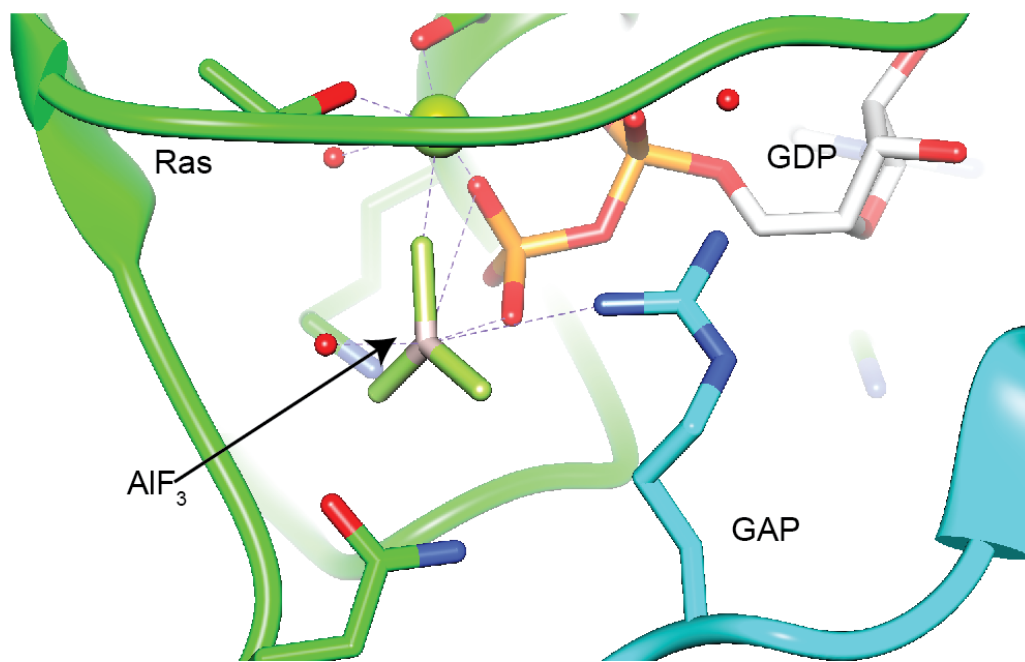
i. Why is the AlF$_3$, H$_2$O, GDP combo a good estimation of the transition state structure? Perhaps drawing what the transition state would help you answer this question.

ii. What do you notice about the interactions between the transition state atoms and Ras?

iii. Why do you think the GAP is able to accelerate the rate of GTP hydrolysis?