

Name: _____

7.06 Cell Biology QUIZ #1

This is an **open book** exam, and you are allowed access to books and notes, but not computers or any other types of electronic devices.

Please write your answers to questions in **pen** (not pencil) in the space allotted.

Please write only on the **FRONT SIDE** of each sheet.

And be sure to put your **name on each page** in case they become separated.

There are **10 pages** to the quiz, make sure you have a complete copy!

Remember that we will Xerox all of the quizzes.

Good Luck!

Question 1. 30 pts _____

Question 2. 20 pts _____

Question 3. 30 pts _____

Question 4. 20 pts _____

Question 1 (30 points)

Name: _____

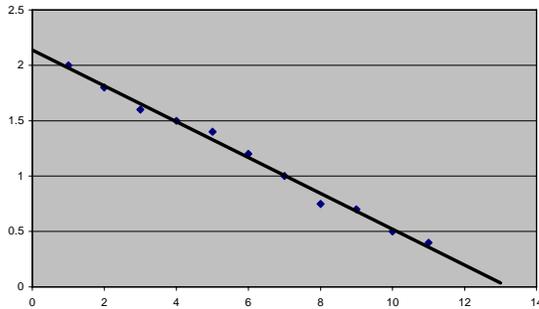
You are studying activated T helper cells in a mouse model system. T helper cells are a sub-group of lymphocytes that play an important role in establishing and maximizing the capabilities of the immune system without themselves having any cytotoxic or phagocytic activity. Your lab studies small molecules secreted by T helper cells that activate Natural Killer (NK) cells.

A (2 points). You identify a small glycoprotein Interleukin-79 that is secreted by T helper cells, and seems to activate NK cells. This glycoprotein is particularly highly expressed in a subset of T helper cells which you have purified from primary mouse serum. Why isn't this primary cell line suitable for long term study?

B (8 points). You now have found the means to generate an immortal T helper cell line by transformation with a tumor cell virus. You want to find the affinity of Interleukin-79 for its *unknown* receptor on NK cells. Describe how you would perform a binding experiment to determine this. Include a description of how you would control for nonspecific binding. (Assume 1:1 binding ratio of Interleukin -79 to its receptor on NK cells)

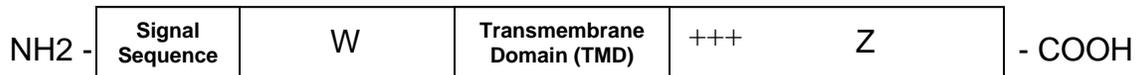
Name: _____

C (4 points). You perform a binding assay and plot your data on a Scatchard plot, which is presented below. Label the x and y axes in terms of [R], [L] and [RL], where R is the receptor, L is the ligand and RL is the receptor-ligand complex. Also, indicate how you would determine the binding constant, K, and the number of ligand binding sites, remembering the equation $y = mx + b$.



D (4 points). You isolate a cDNA that you think might encode your receptor. You transfect this into a cell line that does not express immune cell receptors and see no binding. Explain how the composition of receptor in terms of its subunit structure might affect ligand recognition. What experiment might you use to prove your contention?

E (4 points). You obtain a cDNA which encodes the main subunit of the Interleukin-79 receptor. You analyze this sequence and obtain the information below (a + sign indicates a positively charged amino acid):



- 1) What class of membrane protein is this receptor?
- 2) Name amino acids that you may find in the TMD.
- 3) Label W and Z as intracellular or extracellular.

Name: _____

F (5 points). How would you experimentally verify your predicted topology for the Interleukin-79 receptor? Make use of epitope tags in your experimental strategy. Hint: be specific on how you establish cytoplasmic exposure of domains W or Z.

G (3 points). You create a N terminally GFP tagged version of the Interleukin -79 receptor. Using this construct, you then make a series of deletion mutants:

- A) A deletion of the signal sequence
- B) A deletion of the transmembrane domain
- C) A deletion of the fifteen C-terminal amino acids.

However, you dropped the tubes before you labeled them! You have decided to transfect these mutants into cells to determine their fate. Match the above mutant with the localization patterns below.

- 1) The GFP signal is in the cytosol of the cell
- 2) The GFP signal is in the ER lumen
- 3) The GFP signal is associated with the ER membrane

Question 2 (20 Points)

Name: _____

You've prepared two different sets of liposomes, which are small, spherical phospholipid-bilayer enclosed structures with an aqueous interior. One such set of liposomes contains no membrane proteins (set A), while another set contains a variety of transporters and ion channels (set B). You prepare these liposomes such that the interior of the liposome is topologically equivalent to the extracellular space, and the outside is topologically equivalent to the cytoplasm.

A (2 points). What types of molecules can pass into each type of liposome?

B (6 points). Liposomes in set B contain the K^+ channel, which transports K^+ down its concentration gradient. Using a radioactive isotope of K^+ you can monitor movement into and out of the liposomes; you can also prepare liposomes with a variety of K^+ concentrations inside, and can adjust the K^+ concentration outside.

a. Under what conditions will you see radioactive K^+ flow into the liposomes?

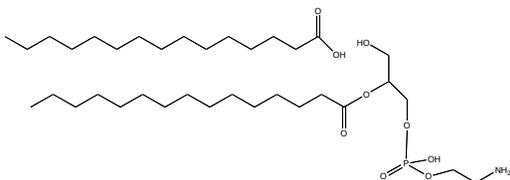
b. Under what conditions will you see radioactive K^+ flow out of the liposomes?

Name: _____

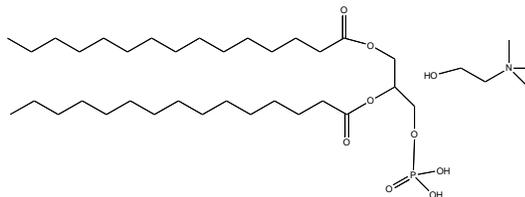
C (8 points). You prepare a different set of liposomes that contain a novel transport protein that uses a Ca^{2+} concentration gradient to drive glucose transport against its concentration gradient. You can use radiolabeled glucose to monitor its movement into, and out of liposomes. Design an experiment to determine whether the transport protein is a symporter or an antiporter **by monitoring the influx of radioactive glucose into liposomes**. Be sure to include the relative concentrations of Ca^{2+} and glucose inside and outside of the liposomes.

D (4 points). You have a set of phospholipases, which cleave the various bonds in phospholipids. Unfortunately the labels have fallen off of the tubes. You want to identify the enzymes each tube by performing enzymatic reaction on membrane preparations and identifying the products by a chromatographic method called High-Powered-Liquid-Chromatography (HPLC). Identification of each product is accomplished by comparison with reference compounds of known structure. Based on the resulting products, what enzymes are in each tube?

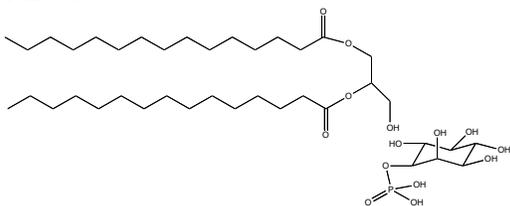
Tube 1:



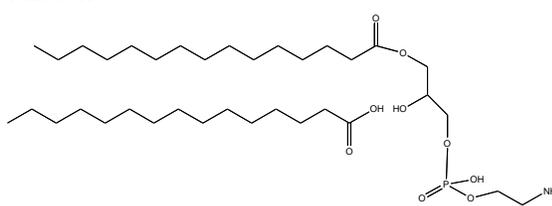
Tube 2:



Tube 3:



Tube 4:



Question 3 (30 Points)

Name: _____

You're studying cellular signaling through G-Protein Coupled Receptors (GPCRs). Specifically you're working on a pair of newly identified GPCRs, GPCR-A and GPCR-B. Each binds the same small ligand, but activates different heterotrimeric G-proteins that act on adenylyl cyclase.

A (3 points). Your grad student mentor explains that both the receptors and the G_{α} and $G_{\beta\gamma}$ subunits of the heterotrimeric G-proteins are membrane associated. You're puzzled, because although you find obvious transmembrane domains in the receptor, you find none in G_{α} or $G_{\beta\gamma}$. How are G_{α} and $G_{\beta\gamma}$ associated with the membrane (be specific)?

B (5 points). You examine the sequences of the seven predicted transmembrane α -helices of GPCR-B. You're surprised to find that there are hydrophilic amino acid residues in these helices. How can you explain this observation?

You find that the binding of ligand has opposite effects on adenylyl cyclase activity for each receptor. GPCR-A causes an increase in adenylyl cyclase activity, while GPCR-B causes a decrease in adenylyl cyclase activity.

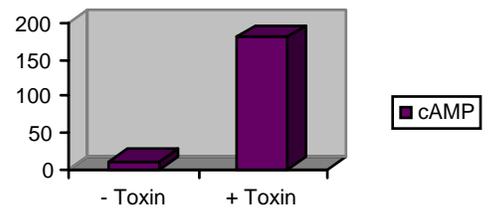
C (2 points). Based on the above stated effects on adenylyl cyclase activity, what type of G-protein is activated by each receptor?

Name: _____

D (6 points). You have a cell line that expresses both GPCR-A, GPCR-B, the corresponding G-proteins, and adenylyl cyclase. There is a basal level of adenylyl cyclase activity that produces a baseline cAMP concentration. Your project is to characterize a series of mutations in these components. Will each mutation increase or decrease the intracellular levels of cAMP upon ligand addition (remember, both GPCR-A and GPCR-B bind the same ligand)?

A mutation in GPCR-A that prevents G-protein activation?	
A mutation in GPCR-B that prevents G-protein activation?	
A mutation in G_s that prevents release of bound GDP.	
A mutation in G_i that prevents release of bound GDP.	
A mutation in G_s that prevents GTP hydrolysis.	
A mutation in G_i that prevents GTP hydrolysis.	

E (7 points). A colleague studying a pathogenic bacterium has discovered that it secretes a toxin that interferes with signaling in response to your ligand. She wants to collaborate with you in determining how this toxin acts. You do a preliminary experiment in which you look at intracellular cAMP levels in untreated and toxin treated cells. The data is presented at right.



How does the toxin affect adenylyl cyclase activity?
Propose two models that explain how the toxin might act based on this data.

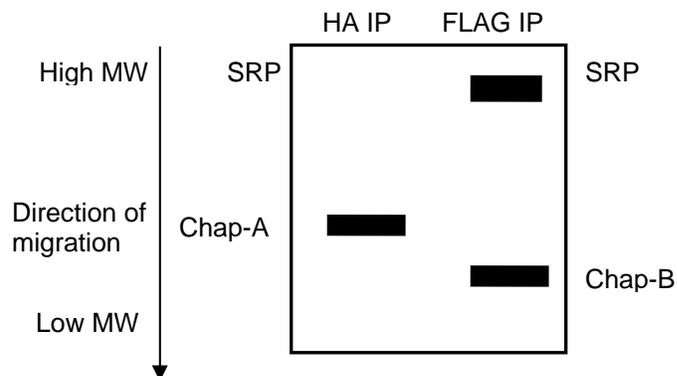
Name: _____

F (7 points). Using cell lines lacking either your G_s or G_i of interest, propose an experiment that might help you differentiate between these two models. You also have an assay for intracellular cAMP and purified toxin.

Question 4 (20 points)

You are studying chaperone proteins associated with the Signal Recognition Partical (SRP). You have an antibody specific to a known SRP protein, and you have epitope-tagged versions of chaperone-A (Chap-A) and chaperone-B (Chap-B), which you think might associate with the SRP. Recall that all eukaryotic cells that synthesize proteins possess a functioning ER as well as SRP. You simultaneously transfected epitope tagged versions of Chap-A-HA and Chap-B-FLAG into an SRP expressing cell line.

A (6 points). You perform **immunoprecipitations (IPs) with antibodies to your epitope tags**, and then perform an immunoblot for SRP and your two chaperone proteins on these samples. Remember that IP is a powerful technique for quickly purifying specific proteins and their binding partners away from other cellular proteins. The SDS-PAGE/immunoblot data is presented at right.



- 1) Does Chap-A interact with SRP?
- 2) Does Chap-B interact with SRP?

Name: _____

B (5 points). Can the results above prove that SRP directly interacts with the either Chap-A or Chap-B? Briefly state why or why not.

C (5 points). You perform immunofluorescence to determine the cellular localization of SRP, Chap-A and Chap-B. You find that SRP and Chap-B are localized in the cytosol, while Chap-A is localized to the nucleus. Are these results consistent with your immunoprecipitation data?

D (4 points). You discover a third chaperone protein, Chaperone-C (Chap-C). You find that this protein does not interact with SRP. In your **immunoprecipitation experiment you used an antibody to SRP** that was bound to a column. After washing, where would Chap-C be? In association with the column, or in the flow-through?