Question 1a) You are studying a transmembrane protein Tmp1. Your PI wants you to raise a monoclonal antibody against the predicted extracellular domain. Describe how you would do this.

1b) You now have a monoclonal antibody against Tmp1. Describe other tools you need for localization or Western blotting studies.

1c) Describe how you could use this antibody to show that epitope in your predicted extracellular domain is indeed extracellular.

1d) What is another method you could have used to determine the localization of Tmp1 without the use of antibodies? What is a possible caveat to the use of this technique?
1c) How could you use this alternate technique to assess the mobility of Tmp1 in the membrane? Describe the possible results and their interpretation.

**Question 2)** What are the differences between primary cells and cell lines? What are the advantages and disadvantages of each?

**Question 3a)** There are a set of enzymes called phospholipases, which cleave phospholipids at specific bonds. What are some of their **in vivo** functions?

3b) Describe an experiment in which phospholipases can be used as analytical tools to determine if a specific phospholipid is asymmetrically distributed in the two leaflets of the plasma membrane.

3c) What are some examples of phospholipids that are asymmetrically localized?

3d) What are reasons for this asymmetry?
**Question 4a**)
Your lab has produced a mouse which is a knockout for the LNO gene. This gene is a known receptor for the SVU growth factor. This is an important receptor during development and loss of this gene causes embryonic lethality in your mouse. The embryos do survive for 14 days in utero. In order to study the loss of this gene, you decide to create a cell line from the mouse embryos. How would you do this?

4b)
You want to determine the specific binding of the SVU to the LNO receptor to find the Kd of the receptor and the number of receptors per cell. How would you do this? What would be your controls?

4c)
Surprisingly, you get similar binding curves for cell lines expressing the LNO receptor and the cell line from your knockout mouse. You are worried your mouse was not really a protein null! How could you test for presence of the LNO receptor in the knockout cell line?

4d)
Results from the previous experiment show that there is no expression of the LNO receptor in your cell line and you conclude there must be another receptor that binds the SVU ligand. How could you identify this receptor?

4e)
You find one novel receptor LOCI also binds the SVU growth factor. You want to perform a binding assay but do not have a similar cell line which does not express this receptor. How would you perform this binding assay?
4f) You find the Kd of the LOCI receptor is 1 \times 10^{-10}. Embryonic mice usually have a concentration of 1 \times 10^{-8} M. What percentage of receptors is bound under normal conditions?

**Question 5a) 2.** You are studying the effects of various mutations on the ACGP receptor, which, when bound to epinephrine, leads to an increase in intracellular levels of cAMP (as compared to the low basal levels of cAMP in cells not exposed to epinephrine). How would the mutations listed in parts a) – c) affect cAMP concentration?

Indicate whether the mutations will cause levels of cAMP to rise normally in response to epinephrine, will cause constitutively high levels of cAMP, or will cause constitutively low levels of cAMP. Explain your answers.

5a) A mutation in the Gs\_ subunit that inhibits nucleotide exchange

5b) A mutation in the G_ subunit that increases its affinity for the Gs\_ subunit in its GTP bound state

5c) A mutation in Gs\_ that prevents GTP hydrolysis

5d) Increase in cAMP in these cells causes an increase in the production of growth hormone M. How would each of the mutations described in parts a) – c) affect levels of hormone M in liver cells?