Problem Set #1
7.06 - Spring 2015

Name _____

Section _____

Question 1
You are using fluorescence microscopy to image the localization of proteins within a cell.

A. Name 2 advantages of localizing a protein using a GFP fusion compared to an antibody against that protein?

Can image in live cells
Can conduct time-lapse imaging
Can genetically encode the protein
Don't need to fix or permeabilize the cell

B. Name 2 advantages of localizing a protein using an antibody against that protein compared to a GFP fusion?

Can detect the endogenous protein
Tag doesn't disrupt the function of the protein
Can co-localize more potential proteins based on fluorophores
Can detect less abundant proteins (signal amplification, nature of fluorophores)

C. You test the protein localization using both the GFP fusion and the specific antibodies. However, you find that the GFP fusion shows diffuse localization, whereas the anti-protein antibodies display microtubule localization. You advisor suggests that the localization for the GFP fusion is incorrect. Why might this be the case? How would you test this?

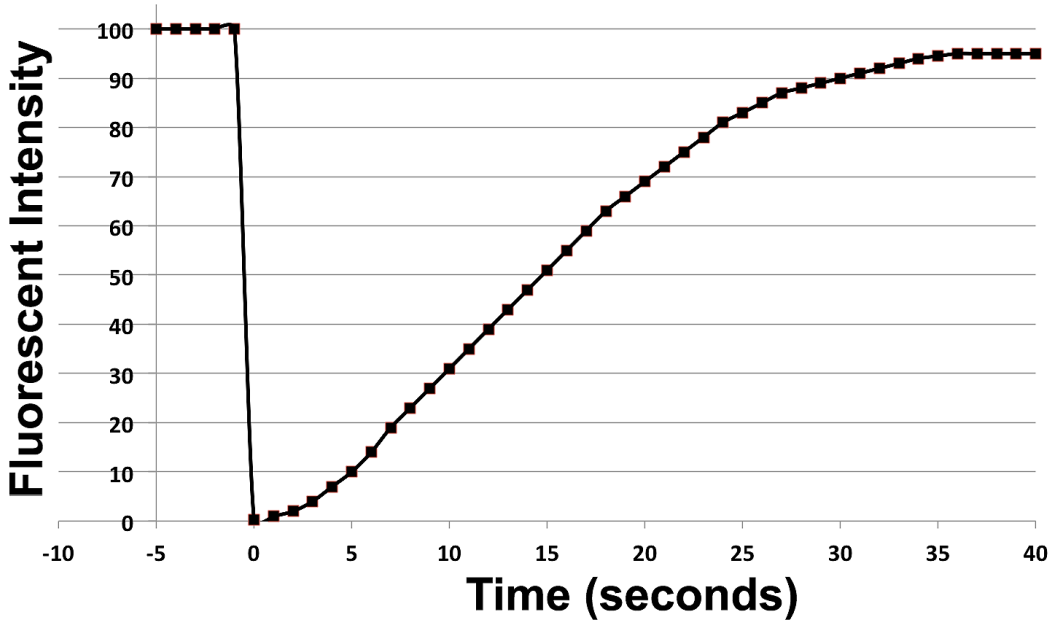
The presence of the tag may be interfering with the function of the protein (targeting, interactions, folding, etc.). You could try a smaller tag, or put this on a different end

D. You next optimize a fluorescent fusion protein to test the localization of a viral coat protein. Using this fusion, you are able to image viral particles within a cell. You want to use fluorescence microscopy to count the number of virus particles infecting a cell. Your advisor suggests that it will be much harder to correctly determine this number when there are a large number of viral particles compared to a small number. Why might this be the case? Explain your reasoning.

There is a resolution limit for light microscopy of about 200 nm (R=wavelength/2). A viral particle is about 100 nm. At low numbers of particles, they will be well spaced. As the number of particles increases, it will be hard to determine whether a spot corresponds to one or two particles.

Question 2

You next want to use fluorescent recovery after photobleaching (FRAP) to analyze the dynamics of a plasma membrane-localized Type I protein using a GFP fusion (with the GFP at the C-terminus). You use a laser to bleach a defined area of the plasma membrane. You measure the intensity of the GFP fluorescence in the bleached area before and after photobleaching. Here is the graph that you obtain:



A. Based on this graph, what is the half-life for recovery of this protein?

~15 seconds

B. What molecular event(s) must occur for the tagged protein to observe fluorescent recovery after photobleaching for this experiment?

Invisible/bleached protein must leave the bleached area.
Fluorescent protein must enter this area.

You next test the recovery of the GFP after several different perturbations. In each case, indicate whether the turnover would be slower, the same, or faster. Note that only ***significant*** changes would be detected. Briefly explain your reasoning.

C. Your cells have reduced levels of Cholesterol. The presence of Cholesterol decreases membrane fluidity.

Turnover would be faster. Proteins would diffuse more rapidly in the membrane.

D. You test the turnover of your protein in a cell that is expressing half the normal concentration of the protein.

There would be no difference. You are looking at half maximal recoverery. In this case, the starting point would have lower fluorescence, but it wouldn't affect diffusion.

E. You grow cells on coverslips coated with a protein that binds to your protein and test turnover in regions of the membrane associated with the coverslip.

This would be slower. Binding would prevent movement of the bleached protein and turnover could not occur as readily.

You next generate a similar fusion for your protein using a variant of GFP termed photoactivatable GFP. This protein is invisible (non-fluorescent) until it is activated by a laser pulse. Using this new fusion protein, you conduct an experiment in which you activate the photoactivatable GFP in a similar sized defined region of the plasma membrane and measure the GFP fluorescence in that region before and afterwards.

F. Draw a graph of what this would look like:

Basically completely opposite of the FRAP graph above.

G. What molecular event(s) must occur for the tagged protein to observe the change in fluorescence indicated in your graph?

Fluorescent protein must move out of the photoactivated area.

For the original GFP fusion, you next conduct photobleaching on other areas of the cell. In each case, the indicated site corresponds to both the area you are photobleaching and the area where you are measuring the fluorescence. What molecular events must occur to see fluorescent recovery in each of these cases?

H. You bleach the entire plasma membrane (but not internal regions of cell).

You must have new trafficking from the ER/Golgi or endosome to the cell surface.

I. You bleach the entire cell.

(Transcription) and translation must occur to synthesize new protein.
Trafficking is not strictly needed to restore cellular fluorescence, but would be needed to obtain the similar staining to the starting point.

Question 3.

A. You have identified some proteins that localize to the plasma membrane. Your advisor wants you to demonstrate that these are *stable* membrane proteins, not just peripherally associated with membranes. At this stage, you have no knowledge of their amino acid sequences. What biochemical perturbation would be required to separate a stable protein from the membrane?

A detergent will remove the protein from the plasma membrane. Both ionic and non-ionic detergents can be used, depending on what you want to do with the protein afterwards.

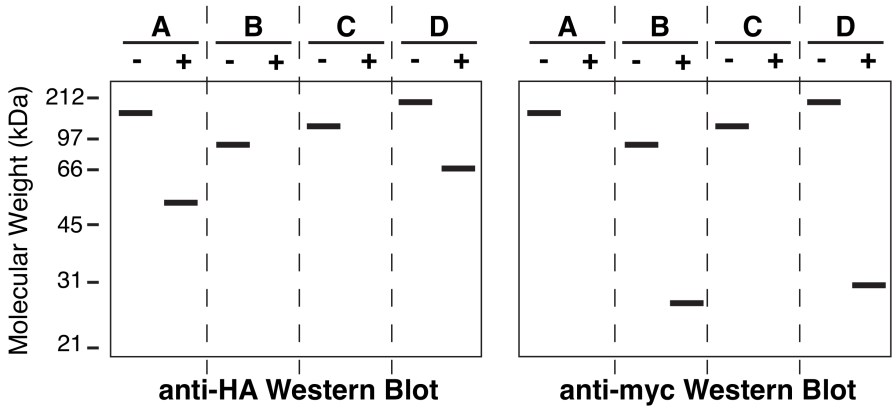
B. For each of the following, indicate the primary mechanism by which each of these molecules enters into a cell. Be as specific as possible.

	Mechanism of Entry
H_2O	*Aquaporin/uniporter channel*
Cholesterol	*Endocytosis/diffusion*
Water	☺
Gleevac (a small molecule kinase inhibitor used to treat cancer)	*Diffusion*
Glucose	*Na+/glucose symporter*
A 15 kDa protein	*endocytosis*

Question 4

You are working on four different plasma membrane proteins (A, B, C, and D). To understand the function of these proteins, you decide to test their orientation in the membrane. In each case, you generate a version of the cDNA where you add an HA epitope tag at the N-terminus and a myc epitope tag at the C-terminus (assume that these tags do not disrupt any signal sequences). You then introduce these cDNAs individually into cells. In each case, you split the cells into two populations. For the first, you generate a protein sample from the complete cell ("-" samples). For the second, you treat the intact cells with a protease (Proteinase K) which will digest all accessible protein ("+" samples). In each case, you run the samples on an SDS-PAGE gel, and probe to detect for either the HA or myc tag by Western blot

A. Based on the Western blots shown below, draw the most likely orientation of each protein.



A

Outside

Inside

B

Outside

Inside

C

Outside

Inside

D

Outside

Inside

B. In each case, based on the topology, indicate its corresponding class of membrane protein next to the diagram.

A. Type II
B. Type I
C. Polytopic (with both sides out)
D. Polytopic (with both sides in)