

**Trask**

**Zool 3200: Cell Biology**  
**Exam 4—Part II**  
 2/3/15

Answer each of the following questions in the space provided, explaining your answers when asked to do so; circle the correct answer or answers for each multiple choice question. (32 points)

Although membrane proteins contribute roughly 50% of the total mass of the membrane, there are about 50 times more lipid molecules than there are protein molecules in cellular membranes. Explain this apparent discrepancy. (2 points)

Proteins are typically much larger than lipids, so while these two biological molecules make up equal percentages of cell membranes, they are not equal in number due to the relative small size of lipids.

In an attempt to better understand fluid tonicity and its effect on cells, you attempt to culture cells in a liquid medium with a  $\text{Na}^+$  concentration of 10 millimolar. The intracellular concentration of  $\text{Na}^+$  is also typically around 10 millimolar. In this scenario, what is the voltage across the membrane with respect to  $\text{Na}^+$ ? Given the voltage across the membrane you calculated, if a sodium channel opened in the plasma membrane of these cultured cells, which direction would  $\text{Na}^+$  travel? Explain your answers. (3 points)

$V_m = 61 (\log 10\text{mm}/10\text{mm})$ ;  $V_m = 61 (\log 1)$ ;  $V_m = 61 (0)$ ;  $V_m = 0$

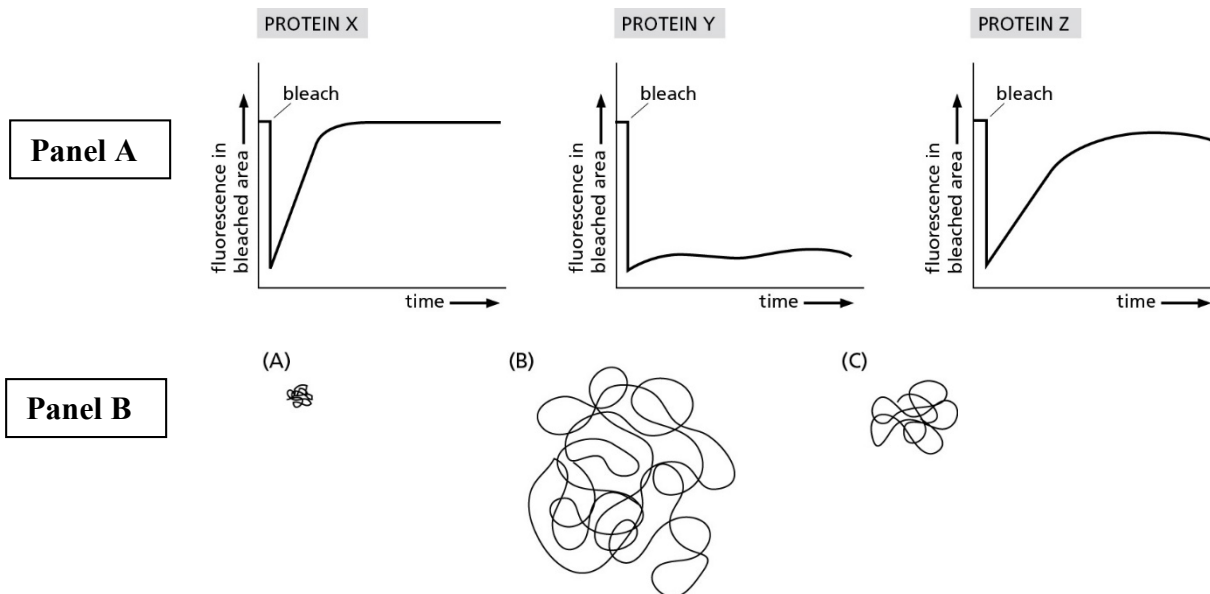
As concentrations are equal on both sides of the membrane,  $V_m$  did not need to be calculated to come to the conclusion that it was 0. At this voltage, if  $\text{Na}^+$  channels opened, the net movement would be zero because the concentrations of  $\text{Na}^+$  outside and inside the cell are equal (per the question), so there is no concentration gradient along which  $\text{Na}^+$  will move. Further, at a  $V_m$  of 0, there is also no electrical gradient (the inside and outside have no voltage across the membrane), so  $\text{Na}^+$  movement will not be motivated in any single direction.

The line below represents a protein with its amino (N) and carboxyl (C) termini identified. Draw rectangles on the line to represent hydrophobic sequences as they would necessarily be arranged if this protein were synthesized at the ER as a 7-pass transmembrane protein with its amino terminus in the ER lumen and its carboxyl terminus in the cytoplasm. (2 points)



The relative mobility of a population of molecules along the surface of a living cell can be estimated by fluorescently labeling the molecules of interest, bleaching the label in one small area, and then

measuring the speed of signal recovery as molecules migrate back into the bleached area. This technique is called ‘Fluorescence Recovery after Photobleaching’ (FRAP). FRAP data for the mobility of three different proteins (X, Y, and Z) are shown in the top panel (A) in the figure below. Separately, single-particle tracking (SPT) data, which follows the movement of a single fluorescently-labeled protein, was collected for each of these same proteins, as shown [in random order] in bottom panel (B).



The SPT data is shown in panel B are presented in a random order. Using the FRAP data presented in panel A, assign each SPT profile (A, B, or C) with one of the membrane proteins to complete the table below. (3 points) **See figure 11-34 for an explanation of how the table was completed.**

Membrane Protein	SPT profile
<b>Protein X</b>	<b>B</b>
<b>Protein Y</b>	<b>A</b>
<b>Protein Z</b>	<b>C</b>

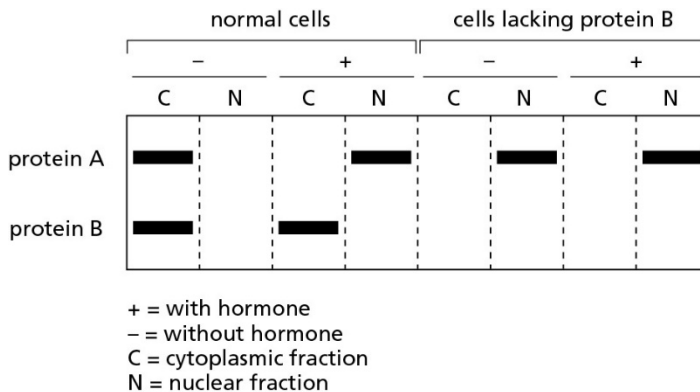
It is important to remember that in each of these experiments, the results reflect a real, physical difference in the way in which these proteins are situated in the plasma membrane. Provide a plausible explanation for the mobility differences observed between the three proteins X, Y, and Z. (3 points)

**Based upon the FRAP and SPT data, protein X is freely able to move in the membrane. There is no limitation upon its ability to diffuse.**

**Protein Y, on the other hand, is not able to move—hardly at all. Sometimes, protein mobility within the membrane is significantly limited due to protein association with other ‘anchoring’ proteins; thus, membrane protein mobility is limited when it is anchored to a cytoplasmic protein network (e.g., the cell cortex) or when it is anchored to an extracellular protein network (e.g., a protein on an adjacent cell or a protein in the extracellular matrix).**

**Protein Z has some movement, but there seems to also be some restriction on mobility. The things that restrict mobility within a membrane are the same things that influence membrane fluidity—namely, the presence of high concentrations of cholesterol, and/or of long-chain or saturated fatty acids in the phospholipids.**

A gene regulatory protein, A, contains a typical nuclear localization signal but surprisingly is usually found in the cytosol. When the cell is exposed to hormones, protein A moves from the cytosol into the nucleus, where it turns on genes involved in cell division. When you purify protein A from cells that have not been treated with hormones, you find that protein B is always complexed with it. To determine the function of protein B, you engineer cells lacking the gene for protein B. You compare normal and defective cells by using differential centrifugation to separate the nuclear fraction from the cytoplasmic fraction, and then separating the proteins in these fractions by gel electrophoresis. You identify the presence of protein A and protein B by looking for their characteristic bands on the gel. The gel you run is shown in the figure below. On the basis of these results, what is the function of protein B? Explain your conclusion and propose a mechanism for how protein B works. (4 points)



Protein B functions to sequester protein A in the cytoplasm. It is likely that protein B is a very large protein without a nuclear localization signal such that it cannot fit through the nuclear pore nor can it be actively transported there. When a hormone enters the cell (or signals from the hormone enter the cell), the hormone binds to one of the two proteins. In either case, hormone binding would be predicted to alter the shape(s) of

protein A and/or B such that the two proteins are unable to associate with one another. IN this way, protein B will 'let go' of protein A. Free protein A is either small enough to move freely through the nuclear pore and enter the nucleus, or it has a nuclear localization signal (positively-charge sequence or patch) that is exposed upon liberation from protein B. In either case, protein A can move to the nucleus in the presence of hormone.

You have recently discovered a drug that blocks the ability of Ran to exchange GDP for GTP. What is the most likely effect of this drug on nuclear transport? Explain your answer. (2 points)

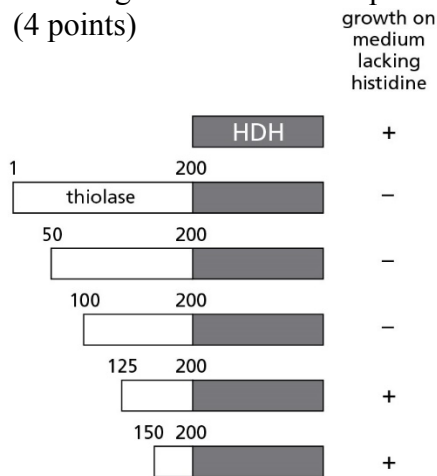
If GDP is unable to be 'exchanged' for GTP, Ran will continually be bound to GDP. In this state, Ran will be unable to bind to the nuclear export receptor, so no cargo would ever be able to be picked up in the nucleus, nor would it be able to leave the nucleus—export would be halted. Similarly, nuclear import would be significantly impaired because while the import receptor would bind to cargo in the cytoplasm and would be able to be transported to the nucleus, the absence of Ran-GTP would preclude cargo delivery; the import receptor would be able to bring cargo to the nucleus, but would not be able to let go of it once there.

You have introduced high concentrations of a non-hydrolyzable form of GTP into the cytoplasm of cells such that it is 10x more concentrated than normal cytoplasmic GTP. What is the most likely effect of this treatment on nuclear transport? Explain your answer. (2 points)

In the presence of a non-hydrolyzable form of GTP, Ran would be in the GTP-bound state continuously. In this case, the export receptor would be able to bind cargo and, in fact, be able to leave the nucleus with it. Once in the cytoplasm, however, the export receptor would not be able to 'let go' of the cargo, as hydrolysis results in release of both Ran and the exported cargo. With respect to import, the import receptor would be able to bind to cytoplasmic cargo, import it and, in fact, release it in the nucleus when Ran-GTP binds. The import receptor + Ran-GTP (no cargo) would leave the nucleus, but without hydrolysis, would not be able to release Ran and would not be able to bind a 'second' cargo. Import would therefore be halted after the 'first trip' into the nucleus.

You are trying to identify the peroxisomal-targeting sequence in the thiolase enzyme, an enzyme that normally resides in the peroxisome. For peroxisomal targeting, you know that thiolase has a signal sequence rather than a signal patch is used to target the enzyme for import into the peroxisome. To identify the targeting sequence, you create a set of hybrid genes that encode fusion proteins containing part of the thiolase protein fused to another protein, histidinol dehydrogenase (HDH). HDH is a cytosolic enzyme required for the synthesis of the amino acid histidine and cannot function if it is localized in the peroxisome due to the conditions in that organelle. You genetically engineer a series of cells to express these fusion proteins instead of their own versions of these enzymes. If the fusion proteins are imported into the peroxisome, the HDH portion of the protein cannot function and the cells cannot grow in a medium lacking histidine. You obtain the results shown in the figure below. What region of the thiolase protein contains the peroxisomal targeting sequence? Explain your answer.

(4 points)



The numbers above each protein indicate the amino acids from thiolase present in the hybrid protein.  
 + indicates growth on medium lacking histidine;  
 - indicates lack of growth on medium lacking histidine.

The region between amino acids 100 and 125 are *involved* in the targeting of thiolase to the peroxisome. When these amino acids are deleted, the thiolase-HDH protein cannot be targeted to the peroxisome. It therefore remains in the cytoplasm, thereby allowing HDH to function such that the cells remain viable even in the absence of exogenous (medium-derived) histidine. As an aside, this experiment verifies that some or all of the 100-125 amino acid sequence is *necessary* for peroxisomal targeting, but it does not address whether these amino acids are *sufficient* to target proteins to the peroxisome—to do this, the 25 amino acid sequence could be added to a cytoplasmic protein whose location could be assessed. If adding this 100-125 sequence results in localization of a cytoplasmic protein to the peroxisome, then the sequence is, indeed, *sufficient* for peroxisomal targeting.

A v-Rab protein on the membrane of vesicles targeted to the lysosome bind to a t-Rab protein on the lysosomal membrane prior to vesicular fusion. You have mutated the gene for this v-Rab such that it now binds to a t-Rab on the cytosolic side of the plasma membrane. Where will the contents of vesicles with this mutant v-Rab be found? (2 points)

The contents will remain in the vesicle because while the v-rab will inappropriately target the lysosomal contents to the plasma membrane, it will be docked there but not be able to fuse. Fusion of the vesicle depends upon a proper association between a v-SNARE and a t-SNARE on the correct target membrane. The question did not mention simultaneous mutation of the v- or t- SNARE, so the vesicle would dock at the plasma membrane, but remain there unfused. The simple answer, the contents will be in a vesicle docked at the plasma membrane.

The lab you work in has discovered a previously unidentified extracellular signal molecule called X, a 75,000-dalton protein. You add purified protein X to different types of cells to determine its effect(s). When you add protein X to heart muscle cells, you observe an increase in cell contraction. When you add it to fibroblasts, they undergo cell division. When you add it to nerve cells, they die. When you add it to glial cells, you do not see any effect on cell division or survival. How might these observations be explained? (2 points)

The differences in response (or lack thereof) are easily explained by the presence/absence of a receptor for protein X, by the TYPE of protein X (different receptors yield different responses), and by the intracellular cascade(s) that occur after receptor binding. (i.e., even with the same receptor, different intracellular cascades can produce different cellular responses).

When the neurotransmitter acetylcholine is applied to skeletal muscle cells, it binds the acetylcholine receptor and causes the muscle cells to contract. Succinylcholine, which is a chemical analog of acetylcholine, binds to the acetylcholine receptor on skeletal muscle cells but causes the muscle cells to relax; it is therefore often used by surgeons as a muscle relaxant. Propose a model for why succinylcholine causes muscle relaxation. What might be the mechanism to explain the different activities of acetylcholine and succinylcholine on the acetylcholine receptor? (2 points)

Given that Succinylcholine is a chemical analog of acetylcholine (Ach), they likely have very similar structures. This is supported by the fact that both molecules bind to the same (Ach) receptor. The most likely explanation is that Ach binding to its receptor produces a cascade (or other response) that results in muscle cell contraction while Succinylcholine binding to the same receptor interferes with Ach binding; further, Succinylcholine binding to the receptor does not induce a receptor shape-change that results in intracellular signaling. This blocking of Ach-induced contraction results in muscle cell relaxation (i.e., the absence of contraction—no signal to contract). Simply put, Succinylcholine is a competitive inhibitor of Ach binding.

Two cell lines, A and B, both survive but do not divide when they are cultured in vitro with a medium containing serum. Factor F is a protein that is known to stimulate cell division in cell line A. Cell line A produces a receptor protein (R) that cell line B does not produce. To test the role of receptor R, you introduce this receptor protein into cell line B, using recombinant DNA techniques. You then test all of your various cell lines in the presence of serum for their response to factor F, with the results summarized in the table below.

Cell Line	Factor F Added	Response
A	-	No division
A	+	Division
B	-	No Division
B	+	No Division
B + Receptor R	-	Division
B + Receptor R	+	Division

Which of the following cannot be concluded from your results above? (1 point)

- a.) Binding of factor F to its receptor is required for proliferation of cell line A.
- b.) Receptor R binds to factor F to induce cell proliferation in cell line A.
- c.) Cell line A expresses a receptor for factor F.
- d.) Factor F is not required for proliferation in cell line B.