Problem Set #2
7.06 - Spring 2015

Name _____

Section _____

Question 1
You are working on the use of ion channels to send an action potential in neurons. Describe the consequences of each of the perturbations listed below on local/cellular ion levels and the ability to send an action potential. *Briefly* explain your reasoning.

A. You have a mutant for the voltage-gated Na^+ ion channel that opens for a much shorter period of time.

Less Na+ internalized. May not depolarize adjacent regions of the membrane. Thus, would prevent the ability to send the action potential.

B. You have a mutant for the voltage-gated Na^+ ion channel that has a shorter refractory period.

Would open again more quickly. May send the signal in both directions because could be reactivated by the downstream channel. Would also mess up overall ability to send a signal.

C. You significantly decrease the extracellular levels of Na^+.

This would reduce the ability of the channel to internalize Na+ when it opens. Wouldn't be able to depolarize the membrane or send a signal.

D. The expression of the voltage-gated Na^+ ion channel is reduced such that there is 1/4 the amount of molecules that are normally present.

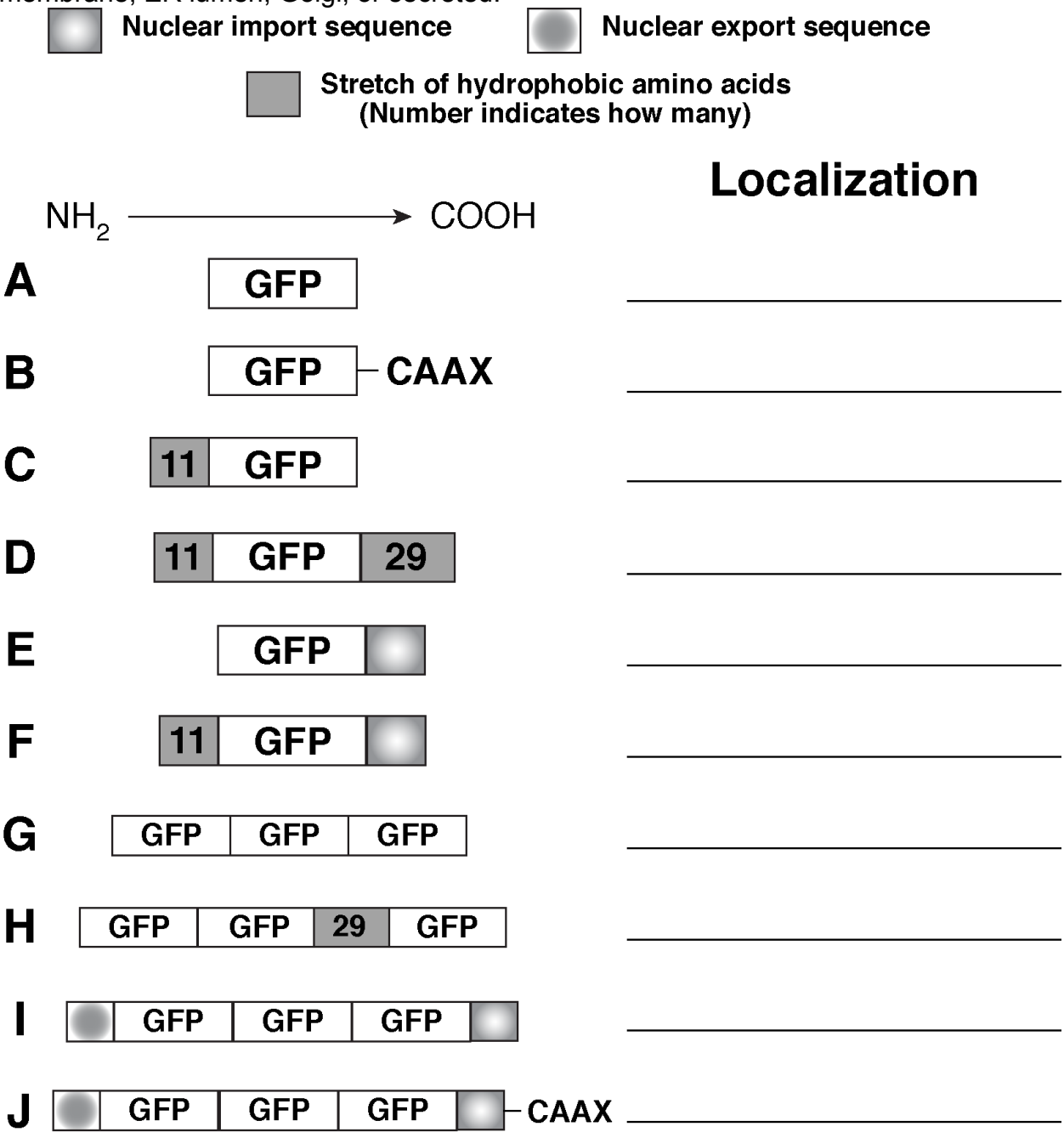
Molecules would be much farther apart on the cell surface. This would make them less susceptible to the membrane depolarization that occurs when you open a channel. Would reduce the ability to send an action potential.

Question 2

Your lab studies protein trafficking. To test protein targeting, you develop an assay to visualize protein localization in live cells using GFP (a 26 kDa protein). You then express a variety of different fusion proteins listed below.

[this question need some information from the nuclear transport lecture. Basically, a 40 kDa protein can diffuse freely through the nuclear pore, an NLS will target the protein to the nucleus, and an NES will cause it to be exported]

In each case, specify the likely ***primary*** localization of the protein as diffuse throughout the cell, cytoplasmic, nuclear, plasma membrane (specify "transmembrane" or "membrane-associated"), ER membrane, ER lumen, Golgi, or secreted.



A. Diffuse throughout the cell

B. Plasma membrane associated
C. Secreted
D. Plasma membrane (transmembrane)
E. Nuclear
F. Secreted
I. Cytoplasmic
H. Plasma membrane (transmembrane)
I. Diffuse throughout the cell
J. Plasma membrane associated

Question 3
Circle which of the following proteins passes through the Sec61 channel on the way to its final destination.

• a secreted protein ***Yes***

• a soluble protein in the ER lumen ***Yes***

• a nuclear protein ***No***

• a Golgi protein ***Yes***

• a transmembrane protein present on the plasma membrane ***Yes***

• a transmembrane protein present in the outer nuclear membrane ***Yes***

• a cytoplasmic protein ***No***

• a peripheral membrane protein on the cytoplasmic side of the plasma membrane ***No***

• SRP ***No***

• BiP ***Yes***

Question 4
You are working on a secreted protein. Where would this protein localize in each of the following temperature sensitive (conditional) mutants? Answer the questions based on the primary defect associated with the mutation (not secondary consequences).

A. A *sec61* temperature sensitive mutant at the permissive temperature of 25°C.

Secreted

B. A *sec61* temperature sensitive mutant at the restrictive temperature of 37°C.

Cytoplasmic

C. A *sec61* temperature sensitive mutant at the semi-permissive temperature of 30°C.

Some secreted, some cytoplasmic

D. A COPII mutant at the restrictive temperature.

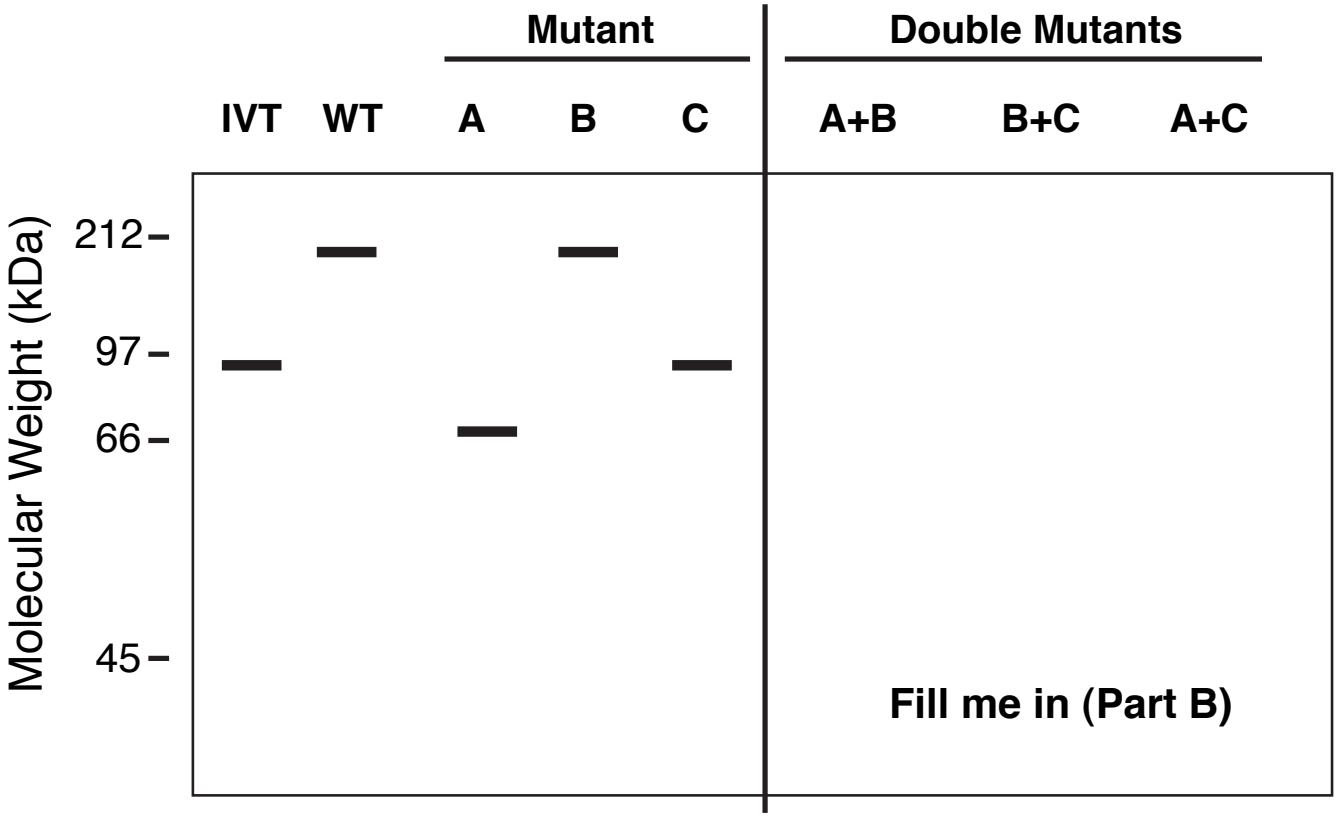
ER

E. A mutant for the plasma membrane localized T-SNARE at the restrictive temperature.

In vesicles near plasma membrane (can't fuse to membrane)

Question 5

You are working on a plasma membrane protein with a classic "Blobel"-type signal sequence. This protein is extensively modified in the Golgi to add sugar groups. You have an antibody against this protein that you use to conduct Western blots of either in vitro translated protein (IVT) or of SDS solubilized wild type cells. You also test a range of different conditional hypomorphic mutants in the secretory pathway (you grow these cells at the restrictive temperature). You find that these show these different behaviors (A, B, and C).



A. Based on this gel, which class of mutants would most likely correspond to a defect in each of the proteins listed below? Each class can be used more than once or not at all. Answer based on the primary defect associated with that protein.

COPI – ***Mutant class B***

COPII- ***Mutant class A***

Sec61 - ***Mutant class C***

ARF – **Mutant class B**

A plasma membrane T-Snare- ***Mutant class B***

B. On the gel above, draw the expected behavior for the three double mutant combinations.

A+B – looks like A
B+C – looks like C
A+C – looks like C