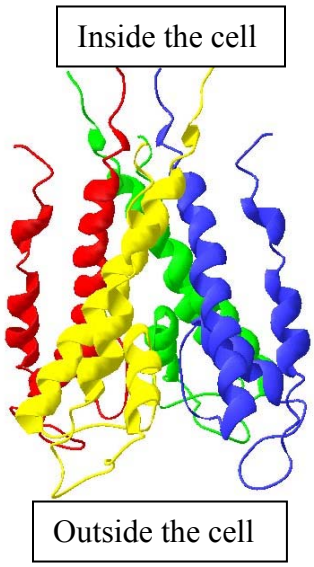
Name:_____ TA:_____

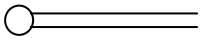
Question 1 (36 points)

Below is a ribbon representation of the K^+ channel, a membrane spanning protein made up of four copies of a single polypeptide. The K^+ channel allows K^+ ions to be shuttled through the membrane.



a) What protein secondary structure is part of the K^+ channel protein as shown above?

b) Does the K^+ channel have quaternary structure? If yes, describe it.

c) Using  as a schematic of a phospholipid, draw a cross-section of the membrane around the K^+ channel shown above.

d) What type(s) of amino acids do you expect to find on the K^+ channel polypeptides

i) next to the tails of the membrane lipids? (Circle all that apply)

Polar Nonpolar Positively charged Negatively charged

Why?

ii) next to the heads of the membrane lipids? (Circle all that apply)

Polar Nonpolar Positively charged Negatively charged

Why?

Name:_____ TA:_____

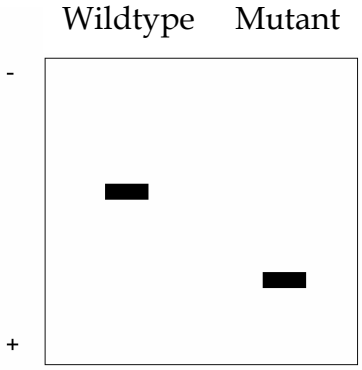
Question 1, continued

e) If you were trying to estimate the volume occupied by this protein, would the picture above provide all the information you need? Why or why not?

The positively charged K^+ ion is a very small soluble molecule.

f) Explain why K^+ cannot come across the membrane without a channel protein.

You isolate a mutant of the K^+ channel that transports less K^+ than normal. You run both the wild-type and mutant proteins on a denaturing gel and get the following result:



g) From the gel data we can conclude that (circle all that apply):

i) Each subunit of the mutant protein is shorter than / the same length as / longer than in the wild-type protein.

Justify your answer(s)

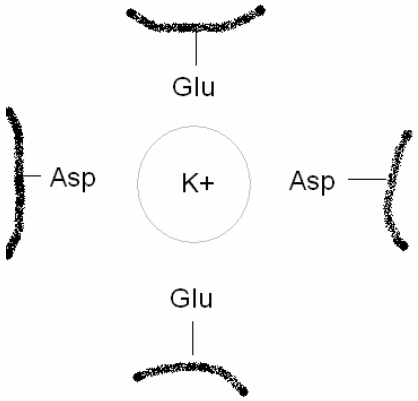
ii) The mutant and wild-type proteins could differ in their primary / secondary / tertiary structure.

Justify your answer(s)

Name:_____ TA:_____

Question 1, continued

The K^+ channel has several binding pockets in which K^+ ions may associate. Below is an image of one of the binding pockets of the K^+ channel shown from above.



h) Circle the strongest type of interaction that exists between the K^+ ion and the Glu residues.

Covalent bond Van der Waals Hydrogen bond Ionic bond

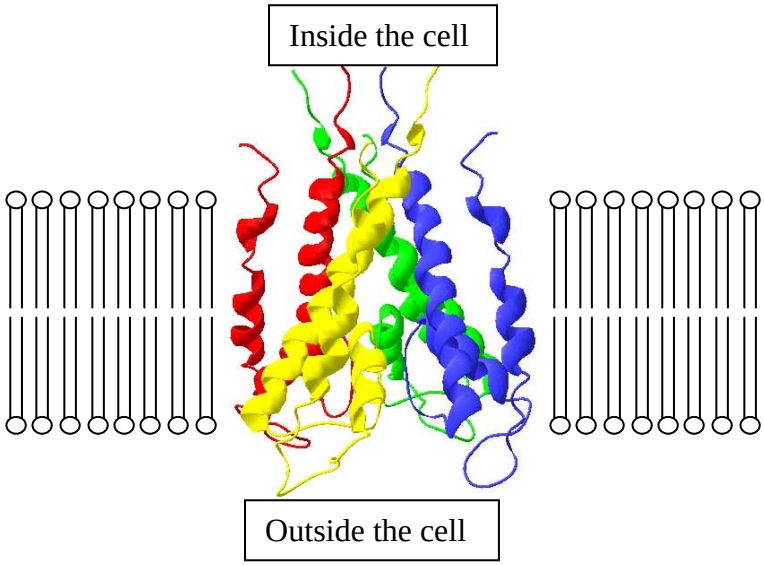
i) You isolate a series of mutant K^+ channel proteins where the two Asp residues have been replaced by amino acid X (see table below). For <u>each</u> X, indicate whether K^+ binding in the resulting pocket will be stronger, weaker, or the same and give a brief explanation of your choice.

X	Interaction (choose one)	Explain why
Asn	stronger weaker same	
Leu	stronger weaker same	
Phe	stronger weaker same	

j) Suppose you isolate another mutant that has four Glu residues instead of two Asp and two Glu residues in the pocket above. You find that the mutant has decreased K^+ transport. Explain this result.

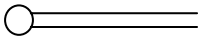
Question 1 (36 points)

Below is a ribbon representation of the K^+ channel, a membrane spanning protein made up of four copies of a single polypeptide. The K^+ channel allows K^+ ions to be shuttled through the membrane.



a) What protein secondary structure is part of the K^+ channel protein as shown above?
K^+ channel contains only a-helices.

b) Does the K^+ channel have quaternary structure? If yes, describe it.
Yes, the channel has quarternary structure. It consists of four interacting polypeptides. It appears that each polypeptide interacts with its two neighboring polypeptides, such that the overall shape of the channel is a barrel.

c) Using  as a schematic of a phospholipid, draw a cross-section of the membrane around the K^+ channel shown above.

d) What type(s) of amino acids do you expect to find on the K^+ channel polypeptides

i) next to the tails of the membrane lipids? (Circle all that apply)

Polar (Nonpolar) Positively charged Negatively charged

Why?
The tails of the membrane lipids are hydrophobic, and it is energetically favorable for the protein to have hydrophobic amino acids in the hydrophobic environment.

ii) next to the heads of the membrane lipids? (Circle all that apply)

(Polar) Nonpolar (Positively charged) Negatively charged

Why?
The heads of the membrane lipids have negatively charged phosphate groups, and it is energetically favorable for the protein to have positively charged amino acids that can form ionic bonds, or polar amino acids that can form hydrogen bonds in that environment.

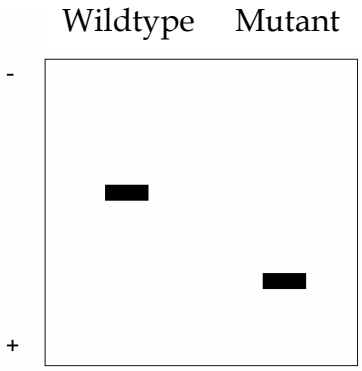
Question 1, continued

e) If you were trying to estimate the volume occupied by this protein, would the picture above provide all the information you need? Why or why not?
No, the picture above is of a ribbon representation, i.e. backbone trace. It does not show any of the sidechains, and thus does not give us a clear picture of how much space the protein occupies. In addition, for the atoms in the backbone, the vdW radii are not shown.

The positively charged K^+ ion is a very small soluble molecule.

f) Explain why K^+ cannot come across the membrane without a channel protein.
The tails of the membrane lipids are hydrophobic. It is not energetically favorable for a charged molecule, such as K^+ to be in a hydrophobic environment. Thus, K^+ by itself can not come across the membrane.

You isolate a mutant of the K^+ channel that transports less K^+ than normal. You run both the wild-type and mutant proteins on a denaturing gel and get the following result:



g) From the gel data we can conclude that (circle all that apply):

i) Each subunit of the mutant protein is (shorter than / the same length as / longer than) in the wild-type protein.

Justify your answer(s)

The mutant protein shows only one band on the gel, meaning that it consists of only one type of subunit. Denaturing gel separates protein fragments based on their size, with shorter fragments traveling further. Since the mutant subunit travels farther on the gel, we conclude that the mutant subunits are shorter than the wild type ones.

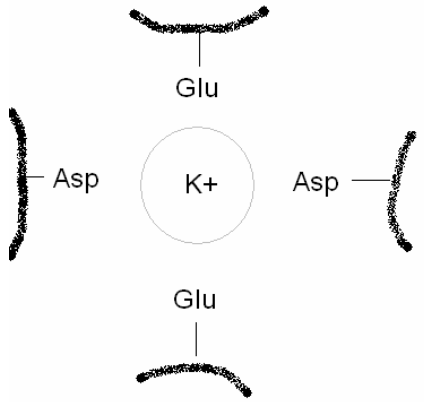
ii) The mutant and wild-type proteins could differ in their (primary / secondary / tertiary) structure.

Justify your answer(s)

Because the mutant subunits are shorter than wild-type subunits, it is clear that the primary structure is affected. If the lost fragment is of significant length, tertiary structure will most likely be affected too. And depending on where the fragment the lost in the mutant subunit is located in the wild-type protein, it is possible that the secondary structure would also be altered.

Question 1, continued

The K^+ channel has several binding pockets in which K^+ ions may associate. Below is an image of one of the binding pockets of the K^+ channel shown from above.



h) Circle the strongest type of interaction that exists between the K^+ ion and the Glu residues.

Covalent bond Van der Waals Hydrogen bond (Ionic bond)

i) You isolate a series of mutant K^+ channel proteins where the two Asp residues have been replaced by amino acid X (see table below). For <u>each</u> X, indicate whether K^+ binding in the resulting pocket will be stronger, weaker, or the same and give a brief explanation of your choice.

X	Interaction (choose one)	Explain why
Asn	stronger (weaker) same	*Same size, weaker interaction – H-bond instead of ionic bond*
Leu	stronger (weaker) same	*Approximately same size, weaker interaction – vdW instead of ionic bond*
Phe	stronger (weaker) same	*Weaker interaction – vdW instead of ionic bond. Phe is also significantly larger than Asp, so steric hindrance is likely*

j) Suppose you isolate another mutant that has four Glu residues instead of two Asp and two Glu residues in the pocket above. You find that the mutant has decreased K^+ transport. Explain this result.
Glu is a negatively charged amino acid, just like Asp. Therefore, mode of interaction, i.e. ionic bond would be preserved. The only difference between Glu and Asp is the extra CH_2 group in Glu. Since the function of the protein is impaired, we conclude that it is harder for the K^+ ion to fit into the new, smaller binding pocket.