Name Matthew Feng Rec. Section R27 TA SAPPINGTON

7.012 Fall 2018: Problem Set 6

Due: Wed 11/28/2018

The solutions to these problems must be submitted electronically to your TA through the 7.012 Stellar site. All submissions must be received before 9:50 AM on November 28, 2018. Check your file to ensure it was successfully submitted. Only the material that is received prior to the deadline will be graded, no additional material will be accepted after the deadline.

Question 1 Cell Structure (2 points)

A. Name two differences between the cell structure of a eukaryotic cell and the cell structure of a prokaryotic cell.

Eukaryotic cells have nuclei & compartmentalized organelles.

B. How do cell structure features of prokaryotic cells facilitate the coupling of transcription and translation whereas eukaryotic cell structure features do not allow for coupling?

Prokaryotes do not have a nucleus, and thus can have translation occur simultaneously with translation.

C. Name one function of the cell membrane.

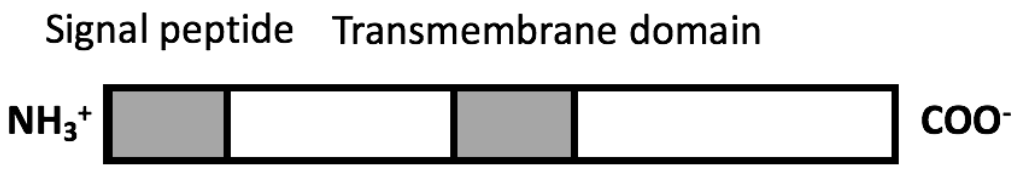
Regulates what leaves & enters the cell.

D. Why is there a limit to how big a cell can get?

The surface area of the cell membrane cannot input enough material to sustain the increased volume of the cells

Question 2 Cell Structure and Protein Location (3 points)

From the following elements provided in the protein below, answer the questions.



A. From the amino acids listed, circle the ones you would expect to find in the transmembrane domain: Leu, Glu, Ala, Arg, Val, Phe, Asp

B. Where in the cell would you expect to find this protein?

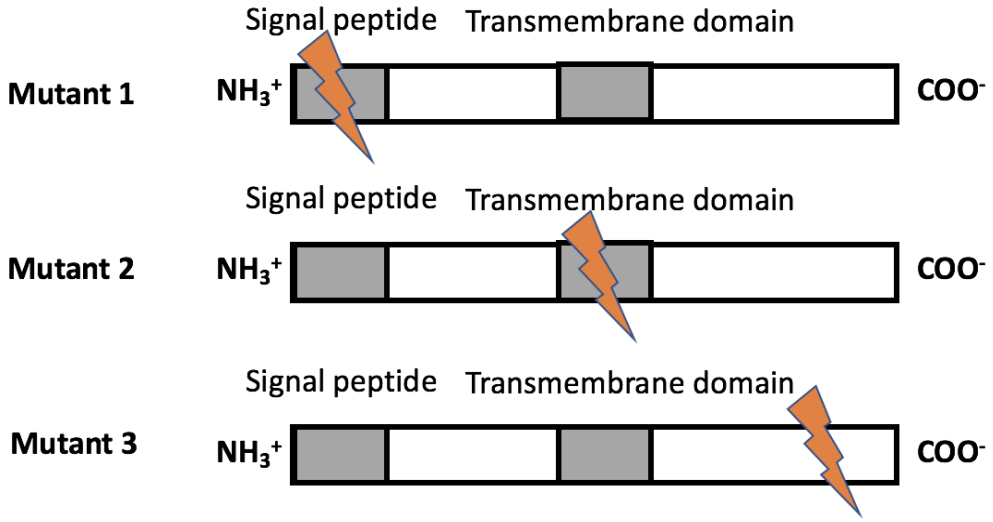
In the cell membrane.

C. What path would this protein take to get to the where it is supposed to go? Choose from the following:

i) smooth ER -> Golgi -> lysosome
ii) smooth ER -> plasma membrane
iii) rough ER -> Golgi -> plasma membrane
iv) rough ER -> mitochondria -> Golgi

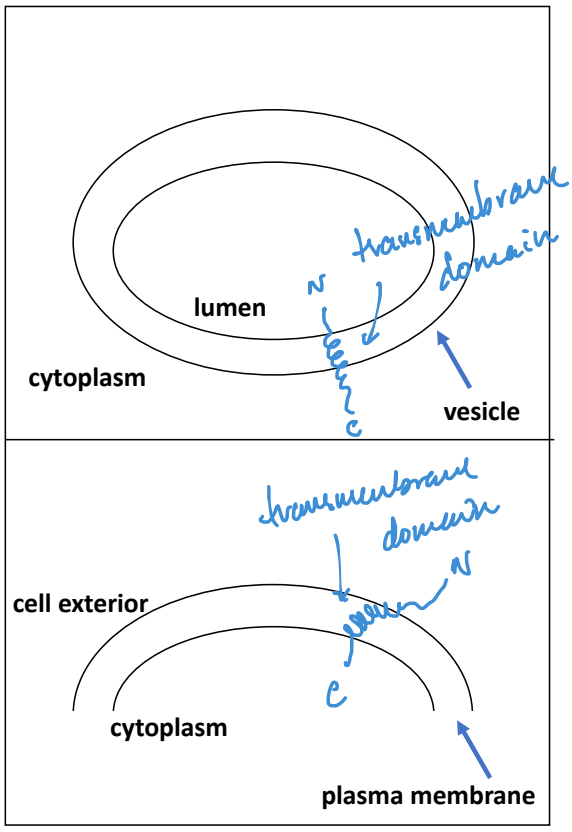
Question 2, continued

D. Where in the cell would you expect to find the following mutants of this protein if the indicated parts are mutated?



Mutant 1: Cytosol
Mutant 2: Cell exterior
Mutant 3: Membrane

Assume the protein from part A goes to the plasma membrane.



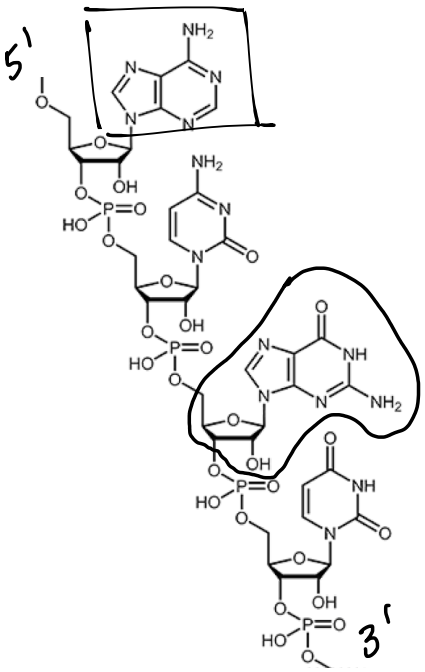
E. Draw the protein (i) in a vesicle in the upper panel and (ii) on the plasma membrane in the lower panel. Indicate N, C termini, and transmembrane domain.

F. When you purify the protein you are surprised that the protein at the plasma membrane is 20 amino acids shorter. What is the reason for this observation?

The signal sequence is cleaved off after translation.

Question 3 Viruses (3 points)

A. Shown below is a few nucleotides of a virus genome.



(i) What type of genome does this virus have? Choose from ds/ss and DNA/RNA. State briefly how you came to that conclusion.

ss RNA 2' carbon has Oxygen, only 1 strand.

(ii) Label the 5' and 3' ends of the molecule shown.

(iii) Put a square around one of the bases.

(iv) Circle one complete nucleotide.

B. If a restriction enzyme is added to the content of a cell, which type(s) of virus from the list below would be affected? Explain for each virus why it is affected or is not affected by the restriction enzyme activity.

- (-)-sense RNA virus
- ds RNA virus
- retrovirus ← affected
- ss DNA virus ← affected
- ds DNA virus ← affected.

retrovirus converts RNA back into DNA, but the DNA is susceptible to being cut by restriction enzyme.

dsDNA, ssDNA virus may have its plasmid cut.

C. In a stretch of 1000 nucleotides of the viral genome, the genes for two proteins A and B are present. Both genes are 700 nucleotides in length. How can they be transcribed from the region of 1000 nucleotides?

Different reading frames could lead to different proteins.

D. What is the function of reverse transcriptase?

Converts RNA back into DNA.

Question 4 Microbes (4 points)
In 2012, an enzyme was discovered that appeared to be prominent in the gut microbiomes of people living in Japan. To learn more about the gene encoding this enzyme and its function, we will be using Basic Local Alignment Search Tool (BLAST), a free, online tool that searches for regions of similarity between biological sequences. We will use this tool to discover which organism this gene is found in and to find similar genes in other organisms. In order to investigate these questions, follow these directions:

· Go to the BLAST website https://blast.ncbi.nlm.nih.gov/Blast.cgi and click on "Protein BLAST".

· Copy and paste the amino acid sequence provided below into the text box for sequences.

· Scroll down and hit "BLAST" and open results in new window.

· Results will indicate all genes that share sequence similarity with this amino acid sequence.

MRKTVLYLSAASLFLSSYTLKNDKEYSLAEEHIKNLPEAPEGYKWVVNEDYTDEFNGKRLNAAKWHAKSPYWTNGRP
PATFKAENVSVKKGCLRIINTVLSPTEGLDGKPGDKYRLAGGAVASVKNQAHYGYYETRMKASLTTMSSTFWLSNRP
VMKEIMKGGKKIKTWSSQELDIIETMGIIRSVNPDNPWNKTWNMQMNSNTHYWYQEQGGKRTDNTAKRSDVVSYMT
DPSAEDFHTYGCWWVDANTVKFYYDGKYMYTIKPTTKYTDTPFDRPMFIHIVTETYDWEKQVPTAEDLKDKDKSTTY
YDWVRAYKLVPIEE

A. Which organism does this sequence belong to and what type of enzyme is it?

Bacteroides Plebius, β-porphyranase-

B. Which organisms express similar enzymes (list the top 3), and what is the percent identity between each enzyme and our target sequence? Percent identity is indicated in "Ident" column.

Bacteriodes uniformis, 99%
Rhodopirellula sp. SWK7 55%
Rhodopirellula salletina 53%

C. Scientists often use E values as an alignment score (a metric of how similar two sequences are). Observing E values for your results, would you expect two sequences with an E value of 10^{-100} to be more or less similar than two sequences with an E-value of 10^{-50}? What does an E value of 0 mean?

E-value of 10^{-100} is more similar than E-value of 10^{-50}. E-value of 0 means there's an exact match.

D. Look at the top 3 organisms with enzymes similar to our enzyme of interest. Research where these species are found.

Bacteroides uniformis: Human Gastrointestinal Tract

R. salletina: European marine waters.

R. sp. SWK7. North Sea coastal sediment.

Question 4, continued

E. Our enzyme of interest is able to metabolize unique carbohydrates found in red algae, the most commonly consumed seaweed by humans. Hypothesize why people living in Japan might have gut microbes that can degrade these carbohydrates? From a genetic perspective, how might this gut microbe have obtained the gene encoding this unique enzyme?

Japanese people often eat sushi/seaweed as part of their diet, and thus benefit from the ability to degrade the carbohydrates. The gut microbes may have obtained the gene via horizontal gene transfer.

Question 5 Microbes (2 points)

An MIT student is fighting a Streptococcus pyogenes infection resulting in strep throat. This student's doctor first tried treating her with the antibiotic clarithromycin, but it appears that this strain of S. pyogenes might be resistant to this antibiotic as the student's health is not improving with treatment.

A. Clarithromycin targets the ribosome, thus inhibiting protein translation. How might a strain of S. pyogenes become resistant to this antibiotic?

S. pyogenes that contained mutation & survived the antibiotic reproduced.

B. The doctor decides to culture the strain of S. pyogenes that is making the student sick. She plates the strain on three agar plates below.

Agar plate #1:
- 15 g Bacto agar
- 1 L LB media

Agar plate #2:
- 15 g Bacto agar
- 1 L LB media
- 100 µg/mL ampicillin

Agar plate #3:
- 15 g Bacto agar
- 1 L LB media
- 100 µg/mL cephalexin

i. If the doctor doesn't see colonies on any plate, can it be concluded that the strain is susceptible or resistant to ampicillin and cephalexin? Explain your answer.

No, because it didn't grow on the plate w/ only growth media.

ii. If colonies grow on plate 1 and 2, but not plate 3, what conclusion can you draw?

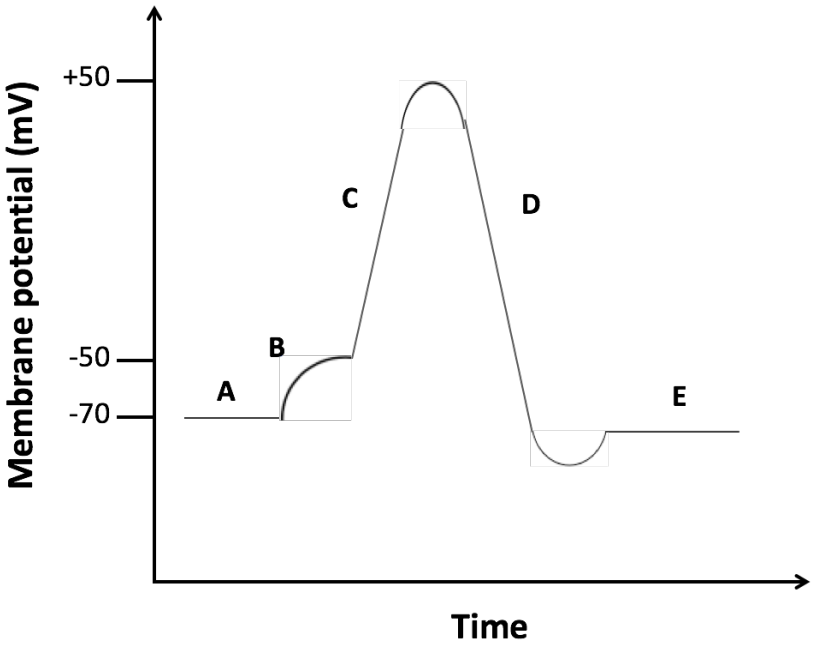
The colony is resistant to ampicilin but susceptible to cephalexin.

Question 6 Neurobiology (2 points)

A. What is meant by saying "an action potential is binary"?

Action potential is all or nothing — can't have part of an AP.

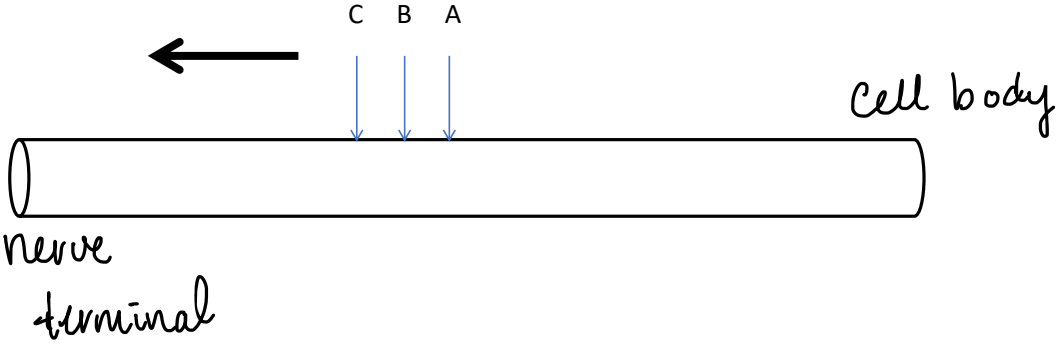
B. Consider the action potential graph below and fill in the table:



	Where on the graph does the protein function? (A-E)	Which way do the ions move? (In or out of cell)
Resting K^+ channels	A, B, C, D, E (always function)	K^+ out
Voltage-gated Na^+ channels	C	Na^+ in
Voltage-gated K^+ channels	D	K^+ out

Question 7 Neurobiology (1 points)

A. If an action potential is moving in the direction shown, mark the location of the cell body and the nerve terminal on the figure.



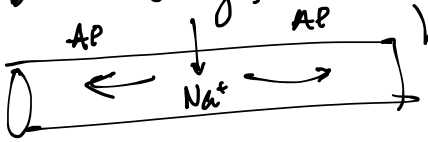
Question 7, continued

B. How is an action potential able to move in a uniform direction? Explain.

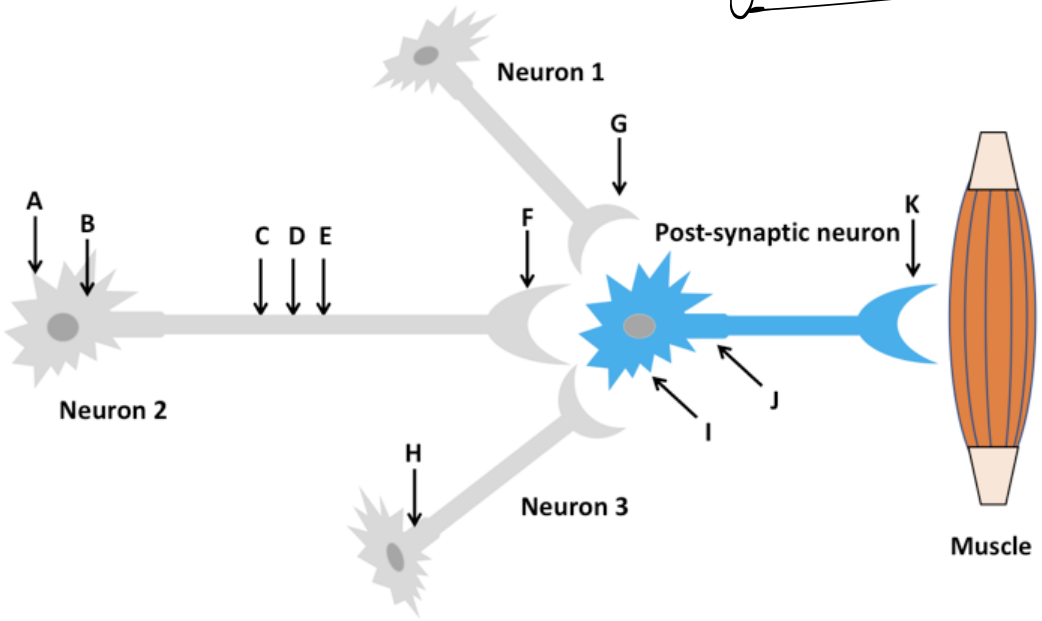
A refractory period enforces that only downstream voltage gated Na^+ channels open in response to depolarization.

C. If the axon is cut out and an electrode is inserted into position B and a potential difference is applied or if Na^+ ions are injected, which way will the action potential move?

The action potential will move both ways, since neither side has refractory period.



Question 8 Neurobiology (2 points)



A. Indicate where you can find the following in the cells shown in the diagram (A-K):

	Location
axon	C, D, E, F, G, H, J, K
dendrite	A, I
Schwann cells	C, D, E
acetylcholine filled vesicles	K
neuromuscular junction	K
calcium channels	F, G, K
axon hillock	J, H

Question 8, continued
B. Predict the response at the muscle under the following conditions 1-5, by circling those conditions where contraction will occur.

	Neuron 1	Neuron 2	Neuron 3	Neuromuscular junction
1	Cl^- influx	Na^+ influx	Na^+ influx	
2	Glycine release	Glutamate release	Glycine release	
3	Na^+ influx			Calcium channel blocker
4	Glycine release	Na^+ influx	Glutamate release	acetylcholine esterase inhibitor
5	Na^+ influx	Glutamate release		Ca^{+2} dependent protein kinase inhibitor

Question 9 Neurobiology (1 point)
A. Venomous snakes such as mambas and cobras have a neurotoxin that binds and inactivates acetylcholine receptors in the neuromuscular junction. What is the effect of the venom on muscle contraction? Explain your answer.

ACh is the neurotransmitter that signals contraction, so inactivating the receptors means there is NO muscle contraction.

B. Nerve agents like sarin inhibit acetylcholinesterase. What is the effect of sarin on muscle contraction? Explain your answer.

The muscle would stay contracted for extended periods, since the breakdown of ACh is slowed.

C. Which of the cases A or B can be treated with amlodipine, a calcium channel blocker? Explain your answer.

Case B; blocking Ca^{2+} channels would reduce ACh levels, leading to relaxation of muscles.