BCEM 393 Introduction to Biochemistry Course Outcomes

By the end of this course, you should be able to:

- compare and contrast the roles of van der Waals forces, charge-charge interactions, hydrogen bonds, and hydrophobic interactions in protein and macromolecular structure and indicate how the roles of these forces differ from those of covalent bonds;
- describe the role of buffers in biological systems, and select and prepare the best buffer when given specific conditions;
- describe the structures and the physicochemical properties of the 20 amino acids, carbohydrates (monosaccharides, disaccharides and polysaccharides), lipids (fatty acids, triglycerides and glycerophospholipids) and nucleic acids (DNA and RNA);
- distinguish between the four levels of protein structure and identify the highest level of structure for a given protein;
- contrast the function of myoglobin and hemoglobin using differences in protein structure;
- list, discuss, use and evaluate the major techniques used in separating proteins, including ammonium sulphate precipitation, column chromatography, and SDS-PAGE;
- describe and experimentally examine how enzymes catalyze reactions, and how inhibitors and allosteric regulators can affect their function using the principles of protein structure and Michaelis-Menten kinetics;
- formulate a hypothesis and generate a written research proposal to investigate the effects of mutations on protein structure and function;
- distinguish between aerobic and anaerobic carbohydrate metabolism, and describe the reactants and products, the reaction purpose(s), the conditions under which they occur, and their regulatory mechanisms; and
- work effectively in diverse teams and provide constructive peer feedback to teammates.

Prepared by Isabelle Barrette-Ng for BCEM 393

Team Application Activity – Week of February 4, 2019

Team number: _____

Names of teammates PRESENT:

_____ _____

_____ _____

_____ _____

Name(s) of teammate(s) ABSENT:

Case Study #1: Effects of lipids on Na^+/K^+-ATPase

Part A: Certain lipids have been discovered to bind to and either activate or inhibit the Na^+/K^+-ATPase. The phospholipid PE with 18:0 and 24:2 fatty acyl chains is particularly stimulatory, whereas PC with 20:0 fatty acyl chains is particularly inhibitory. Although it is not completely understood at present, cells appear to regulate the synthesis and localization of these and other specific lipid species to affect the activity of the ATPase and resting potential of the membrane.

1. Compare and contrast the physical properties of PE with 18:0 and 24:2 fatty acyl chains versus PC with 20:0 fatty acyl chains. **(4 marks)**

2. Based on PE's ability to specifically bind to and stimulate the Na^+/K^+-ATPase, predict whether the concentrations of **intracellular** Na^+ and K^+ are expected to change in neuronal cells where PE 18:0 24:2 is produced at higher levels. CIRCLE your answer and briefly explain your reasoning. **(3 marks)**

(a) Concentrations of Na^+ and K^+ don't change
(b) $[Na^+]$ goes down and $[K^+]$ remains the same
(c) Concentrations of Na^+ and K^+ both go up
(d) $[Na^+]$ goes down and $[K^+]$ goes up

Reasoning (please restrict your answer to the lines below):

_____

_____

_____

3. Predict whether you expect the transmembrane potential to change in a cell where PE 18:0 24:2 was expressed at high levels. CIRCLE your answer and briefly explain your reasoning. **(3 marks)**

(a) There will be no change in the transmembrane potential.
(b) The transmembrane potential will become less negative.
(c) The transmembrane potential will become more negative.
(d) The transmembrane potential will become positive.

Reasoning (please restrict your answer to the lines below):

_____

_____

_____

Case study #2: Scorpion venom peptides and voltage-gated sodium ion channels

Peptides in the venom from bark scorpions in the genus *Centruroides* bind quite specifically to the NaV1.7 voltage-gated sodium channels that are particularly abundant in peripheral neurons that specialize in sensing pain (nociceptors) in many mammals including mice and humans.

Some of the peptides in the venom appear to promote the open state of NaV1.7, thus triggering an increased frequency of action potentials in nociceptor neurons, which in turn leads to the sensation of pain and triggering of inflammation as responses.

4. If the resting transmembrane potential of a nociceptor is -70 mV, explain how the opening of NaV1.7 would be expected to affect ion concentrations inside the nociceptor **immediately following the opening**. **(2 marks)**

Sodium ion concentration inside the cell (INCREASES / DECREASES / STAYS THE SAME).

Potassium ion concentration inside the cell (INCREASES / DECREASES / STAYS THE SAME).

5. Briefly explain how these changes in ion concentrations lead to changes in membrane potential which in turn lead to the generation of an action potential. **(2 marks)**

Membrane potential becomes (MORE / LESS) negative, because

_____

_____

6. The grasshopper mouse specifically preys on bark scorpions. When grasshopper mice are occasionally stung by the scorpions, they do not appear to sense pain or to develop inflammatory responses to the scorpion venom. An initial hypothesis proposed to explain this behavior was that the NaV1.7 channels in grasshopper mice differ in sequence and structure from the channels found in other mice and humans. How could a change in sequence and structure help to make the NaV1.7 channels in grasshopper mice insensitive to the peptides in bark scorpion venom? List two specific mechanisms. **(2 marks)**

_____

_____

_____

7. It actually turns out that the initial hypothesis that NaV1.7 channels in grasshopper mice are insensitive to bark scorpion venom is INCORRECT. In fact, the NaV1.7 channels are similar in structure and function to those in other mice and humans, and are similarly affected by the peptides in bark scorpion venom.

Researchers reported in 2013, however, that the NaV1.8 voltage gated sodium channels, which are also abundant in nociceptors, differ in grasshopper mice versus other mice or humans. NaV1.8 channels from grasshopper mice bind to and are strongly **INHIBITED** by peptides from bark scorpion venom, whereas NaV1.8 channels in other mice or humans are not affected by bark scorpion venom.

NaV1.8 is not required for the initiation of action potentials in nociceptors, but these channels allow nociceptors to sustain multiple rounds of action potentials. These multiple rounds are likely responsible for the pain inflicted by a scorpion sting.

Which of the following two statements describes a difference in behavior between NaV1.7 and NaV1.8 channels that would allow NaV1.8 channels to help create repeated action potentials in neurons like nociceptors? CIRCLE your answer and BRIEFLY explain your reasoning. Hint: For sustained pain response, you need a series of rapid, repeated action potentials in the nociceptors. **(3 marks)**

a) NaV1.8 channels stay open for longer after voltage gating than NaV1.7 channels

b) NaV1.8 channels close faster after voltage gating than NaV1.7 channels

Reasoning:

_____

_____

_____

Total: _____ / 19 marks

BIOL 331 W2019 Assignment 2

Team number: _____

Names of teammates PRESENT:

_____ _____

_____ _____

_____ _____

Name(s) of teammate(s) ABSENT:

Background

Defects in the function of mitochondria have been linked to many neurodegenerative diseases, including one of the most common, Parkinson's Disease (PD). Since almost 1% of people over 60 years of age are affected by PD, there has been intensive research into the causes and treatments of PD.

Although most cases of PD arise from a complex interplay of genetic and environmental factors, about 5-10% of PD cases ("familial PD") can be linked to a genetically heritable mutation. Some of these genetically heritable cases of familial PD have been linked to genes and proteins that play key roles in the normal function of mitochondria, providing a molecular link between dysfunctional mitochondria and PD.

Some cases of familial PD are linked to mutations in the protein alpha-synuclein (AS). Some of these mutations lead to the formation of AS aggregates that can accumulate in protein deposits known as Lewy bodies found in the brains of patients with advanced stages of PD.

Aggregated forms of AS also appear to bind to the TOM20 protein, the central protein in the TOM complex in mitochondria, whereas normal AS does not appear to bind to TOM20.

(A) The TOM20 gene is in the _____ ; the TOM20 protein is in the _____. CIRCLE 2 choices. **(2 marks)**

Nucleus	Outer mitochondrial membrane
Mitochondrial genome	Inner mitochondrial membrane
	Intermembrane space
	Mitochondrial matrix

(B) To import proteins from the cytoplasm and into the matrix of the mitochondrion, which of the following proteins or protein complexes are needed? CIRCLE all correct choices below. **(5 marks total; 1 mark deducted for each incorrect choice)**

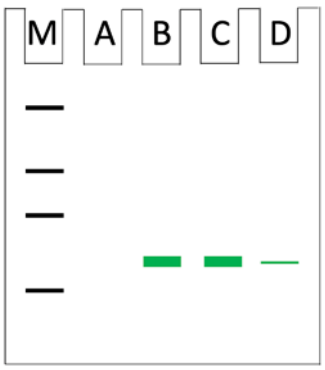
Cytoplasmic chaperones (e.g., Hsp70)
Matrix chaperones (e.g., mtHsp70)
Signal recognition particle
TIM22
TIM23
TOM complex
Mitochondrial Processing Peptidase

(C) Once the misfolded form of AS binds to TOM20, it appears to impair mitochondrial function in a number of ways. Eventually, the impairment of mitochondrial function becomes so severe that the process of mitophagy (*i.e.*, autophagy of mitochondria) occurs. For mitophagy to occur, mitochondria are transported along microtubules in the cell and eventually fuse with lysosomes. To investigate how mitochondria are transported in the cell, you have available two highly specific enzyme inhibitors (1) EHNA, which specifically inhibits the dynein motor ATPase, and (2) AC13, which specifically inhibits the kinesin motor ATPase.

In treatment A, you add EHNA to neuronal cells from a PD patient, isolate the lysosomes by differential centrifugation, and separate the lysosomal proteins by SDS-PAGE. Finally, you perform a Western blot with a fluorescently labeled antibody specific for the mitochondrial transporter receptor TOM20.

In treatment B, you perform the same set of procedures after adding AC13 to PD neuronal cells.
In the control treatment C, you perform the same set of procedures for untreated PD neuronal cells.
In a second control treatment D, you perform the same set of procedures for normal neuronal cells.

The results of the Western blot are shown below. Based on these data, CIRCLE the appropriate choices for Questions C1 and C2:



Western Blot
(anti-TOM20)

C1. These results indicate that _____ is / are important for mitophagy. **(1 mark)**

DYNEIN KINESIN NEITHER MOTOR PROTEIN

C2. These results indicate that mitophagy is _____ in PD vs. normal cells. **(1 mark)**

INCREASED DECREASED THE SAME

C3. Briefly explain your reasoning for your answer to question C1. **(2 marks)**

C4. Briefly explain your reasoning for your answer to question C2. **(2 marks)**

(D) Levels of the mannose-6-phosphate receptor (MPR) are dramatically LOWER in the neurons from PD patients. CIRCLE your prediction of how a reduction in the levels of MPR would be expected to affect the levels of AS in PD patient neurons. Provide a brief explanation supporting your prediction. **(1 mark for prediction; 3 marks for explanation)**

Decreased MPR leads to a DECREASE in AS aggregates in PD neurons

Decreased MPR leads to an INCREASE in AS aggregates in PD neurons

Explanation:

(E) The destabilization of microtubules in neurons is common in many neurodegenerative diseases, including PD. An exciting, novel approach to treating these diseases is the administration of drugs known to specifically stabilize the interactions between the tubulin subunits that form microtubules.

Taxol or paclitaxel from the Pacific Yew tree is widely used to treat aggressive forms of cancer by stabilizing the interactions between the subunits of tubulin that form microtubules. Based on your understanding of dynamic instability, CIRCLE one or more of the following effects ONLY IF the effect is consistent with the observation that taxol stabilizes the interactions between tubulin subunits. **(1 mark)**

I. Growth at the "+" end is inhibited by taxol
II. Growth at the "-" end is inhibited by taxol
III. Disassembly of the microtuble at the "+" end is inhibited by taxol
IV. Disassembly of the microtubule at the "-" end is inhibited by taxol

Briefly explain your choice **(3 marks)**:

(F) Briefly explain how the effect(s) you chose in (E) help(s) to make taxol an effective drug for stabilizing the microtubules in neurodegenerative diseases like PD. **(3 marks)**

Total: _____ / 24 marks