Name: _____

Student #: _____

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University of Toronto

Faculty of Applied Science and Engineering

Division of Engineering Science

Midterm Examination

BME205S

Thurs Mar 26, 2015, 9:00 – 10:50 am

Duration: 110 minutes

Examiner: P. Gilbert

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1. No cell phones are allowed.
2. Type A: Closed book examination, no aids permitted.
3. Part 1 Multiple Choice Questions. ***Mark multiple choice answers on Scantron***.
4. Part 2 Questions have the mark available in the square brackets []; each portion of a question also shows how many marks are allocated to it. ***Each question has a strict sentence limit restriction, each sentence written above the limit will be deducted half a mark***.
5. There are two extra blank pages at the end of the test for rough work.

Last Name: _____

First Name: _____

Student Number: _____

Tutorial section:

[   ]   TUT 01   1   Tue   13:00   14:00   RS412

[   ]   TUT 02   1   Tue   13:00   14:00   BA2175

[   ]   TUT 03   1   Thu   16:00   17:00   RS412

[   ]   TUT 04   1   Thu   16:00   17:00   BA2185

[   ]   TUT 05   1   Fri   12:00   13:00   RS412

[   ]   TUT 06   1   Fri   12:00   13:00   BA2175

[   ]   TUT 07   1   Fri   10:00   11:00   RS412

[   ]   TUT 08   1   Fri   10:00   11:00   BA2195

PART I: Multiple Choice _____ **out of 40**

PART 2: Short Answer Questions

Question	1	2	3	4	5	6	7	8
Marks Available	2	4	3	3	3	4	4	7
Marks Achieved								

PART 2 _____ **out of 30**

TOTAL MARKS _____ **out of 70**

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PART 1: Multiple Choice
ANSWERS TO BE FILLED OUT ON SCANTRON SHEET

1) Which of the following is NOT true of the sodium/potassium pump of animal cells?

    a) It undergoes conformational changes
    b) It uses the energy from ATP
    c) It creates a membrane potential
    d) It serves as an example of a facilitated diffusion transport system

2) Cells are very small in size regardless of whether they are isolated from a large organism, like a human, or a small one, such as a mouse. The reason(s) for this is(are) that _____

    a) diffusion becomes less efficient as cell area increases
    b) tissues made from many small cells are more robust than a tissue made from a few big cells
    c) the surface area of the cell becomes limiting as cells increase in volume
    d) (a), (b), and (c) are all reasons why cells are very small in size
    e) (a) and (c), but not (b), are reasons why cells are very small in size

3) What kind of noncovalent interaction is typified by interactions between two molecules that are so close together that they can experience weak attractive forces bonding them together?

    a) Hydrogen bonds
    b) Ionic bonds
    c) Van der Waal forces
    d) Hydrophobic interactions
    e) Polar covalent bonds

4) Given the equation $\Delta G = \Delta H - T\Delta S$, which set of conditions would result in a reaction that is unambiguously nonspontaneous?

    a) entropy decreases and the reaction is exothermic
    b) entropy stays the same and there is no change in enthalpy
    c) entropy increases and the reaction is exothermic
    d) entropy decreases and the reaction is endothermic
    e) entropy increases and the reaction is endothermic

5) Tristearate is a type of animal fat that is solid at room temperature and is most likely a/an _____ fat. Linseed oil is a type of vegetable oil that is a liquid at room temperature and is most likely a/an _____ fat.

    a) Trans, unsaturated
    b) Unsaturated, saturated
    c) Saturated, unsaturated
    d) Saturated, trans
    e) Trans, saturated

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6) Glycolysis and gluconeogenesis are the _____ and _____ pathways of glucose metabolism, respectively. Although the two pathways may have steps in common, the two pathways utilize different _____ to catalyze some chemical reactions.

    a) catabolic, anabolic, enzymes
    b) catabolic, anabolic, temperatures
    c) anabolic, catabolic, temperatures
    d) anabolic, catabolic, enzymes

7) Which statement is FALSE?

    a) Cell volume can be altered by solute concentration differences.
    b) Osmosis is the diffusion of water through a semipermeable membrane.
    c) Animal cells shrink in size when they are placed in a hypertonic solution.
    d) An animal cell placed in a hypotonic solution will shrink.

8) Did you know that bowhead whales have an average lifespan of 200 years? Your search for the fountain of youth leads you to evaluate the biomembranes of blood cells obtained from a bowhead whale. To identify the different types of proteins associated with the plasma membrane of the blood cells you first treat the cells with high concentrations of salt to remove the _____ proteins and then you use _____ detergent to extract the remaining proteins in their folded and active form for further investigation.

    a) lipid anchored, ionic
    b) lipid anchored, nonionic
    c) integral membrane, amphipathic
    d) peripheral, ionic
    e) peripheral, nonionic

9) What kind(s) of membrane protein have parts which penetrate into the hydrophobic part of the lipid bilayer?

    a) Integral membrane protein
    b) Lipid anchored membrane protein
    c) Peripheral proteins
    d) Integral membrane proteins and lipid anchored proteins penetrate the hydrophobic bilayer
    e) None of these types of proteins penetrate the hydrophobic bilayer

10) Which reaction below might be a suitable coupled reaction for the forward reaction of A + B <—> C + D (ΔG = +7.1 kcal/mole)?

    a) E + F <—> G + H (ΔG = +11.2 kcal/mole)
    b) E + F <—> G + H (ΔG = +8.7 kcal/mole)
    c) E + F <—> G + H (ΔG = -7.6 kcal/mole)
    d) A + F <—> G + H (ΔG = -5.4 kcal/mole)
    e) E + F <—> G + H (ΔG = +7.1kcal/mole)

11) If a triplet in the coding strand of DNA was 5'-GAC-3', what would the RNA guide be when trying to target it using CRISPR/CAS9?

    a) 3'-CTG-5'
    b) 3'-GUC-5'
    c) 3'-CUG-5'
    d) 3'-GUU-5'

12) Starch, like _____ , is a carbohydrate polymer made exclusively of glucose monomers, but its unique physical properties are due to the _____ arrangement of the polymer chains.

    a) cellulose, branched
    b) glycogen, helical
    c) chitin, unbranched
    d) chitin, helical
    e) glycogen, branched

13) A Western blot is best described by which of the following statements?

    a) A multi-step method to detect a specific protein in its natural subcellular location.
    b) A multi-step method using 2 different antibodies to detect a specific protein in a complex mixture.
    c) A multi-step method using a DNA probe to detect the expression of a specific gene in a complex mixture.
    d) A multi-step method for assaying the expression of a specific gene in its natural subcellular location.

14) Which of the following molecules can easily pass through the cell membrane without the need of transport proteins?

    a) O_2
    b) HCO_3^-
    c) Ca^{2+}
    d) Glucose
    e) ATP

15) As humans age, the function of many tissues declines. Some scientists believe this can be explained by changes in the composition of the circulating blood that feeds all tissues. This is an exciting notion because it suggests that blood may contain a 'youth factor' and if we could figure out what that factor was, then we might be able to extend human life. As part of your summer research, you aim to compile a list of potential youth factors by comparing the protein profile of blood collected from 20 year old and 70 year old volunteers. What scientific method should you use to reveal the identity of all of the proteins contained in each blood sample?

    a) microarray technology
    b) mass spectrometry
    c) yeast 2-hybrid
    d) PolyAcrylamide Gel Electrophoresis (PAGE)
    e) RNA sequencing

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16) Which of the following statements about the glycocalyx coating on the outside surface of the plasma membrane is FALSE?

    a) Mediates cell-cell and cell-substratum interactions
    b) Is formed by lipid groups projecting from the plasma membrane
    c) Binds important regulatory molecules that act on the cell surface
    d) Serves as a barrier
    e) Provides mechanical protection

17) Phospholipids are one of the three types of lipids that make up cell membranes. Phospholipids, like cholesterol and sphingolipids, are _____. In contrast to sphingolipids, phospholipids possess _____ relatively _____ fatty acid chain(s).

    a) amphipathic, two, short
    b) amphipathic, one, long
    c) amphipathic, three, long
    d) nonpolar, one, short
    e) polar, two, short

18) Which of the following amino acids can form disulfide bridges?

    a) Glycine
    b) Methionine
    c) Cysteine
    d) Proline
    e) Lysine

19) _____ pass through biomembranes such that their _____ regions form functional domains outside the bilayer while their _____ region penetrates through the membrane.

    a) Peripheral membrane protein, hydrophobic, hydrophilic
    b) Lipid anchored proteins, hydrophilic, hydrophobic
    c) Lipid anchored proteins, hydrophobic, hydrophilic
    d) Integral membrane proteins, hydrophobic, hydrophilic
    e) Integral membrane proteins, hydrophilic, hydrophobic

20) In addition to ATP, what are the end products of glycolysis?

    a) CO_2 and H_2O
    b) CO_2 and pyruvate
    c) Pyruvate and NADH
    d) CO_2, NADH, and $FADH_2$
    e) Pyruvate and H_2O

21) Ionic bonds are _____.

a) strengthened in the presence of water
b) weak bonds that are more easily broken and reformed, when compared to covalent bonds
c) important to maintaining the double helix structure of DNA
d) a type of covalent bond
e) (b) and (c) are accurate statements about ionic bonds

22) Which of the following statements is NOT CORRECT?

a) The subunits of DNA and RNA are nucleotides
b) Nucleotides contain a base linked to the 5' position of deoxyribose or ribose.
c) Nucleotides have a phosphate group attached through a phosphodiester bond.
d) DNA holds the genetic information in all cellular organisms and some viruses
e) RNA is the genetic material in some viruses.

23) Woo hoo! In your 4th year thesis studies you identify a new protein that can differentiate embryonic stem cells into hair cells, which you name WRWLF. As you are daydreaming about how to commercialize this exciting finding into a useful product, your advisor walks by and reminds you that now you need to figure out how it is that WRWLF makes hair cells. You vaguely recall a technique you learned about in BME205 called _____ that you can use to identify all of the proteins that physically interact with your protein of interest (e.g. WRWLF). Yes, that's what you'll do!

a) RNA sequencing
b) PolyAcrylamide Gel Electrophoresis (PAGE)
c) mass spectrometry
d) yeast 2-hybrid
e) microarray technology

24) A mutation caused an unknown structural change in the Iron (Fe)-containing protein component of the electron transport chain. Although the proteins were still found to be in the mitochondrial membrane as usual, oxidative phosphorylation was completely inhibited. Predict the mechanism(s) of how the mutation shut down the electron transport chain.

a) Electrons would be unable to channel through the electron transport chain, and therefore no oxidative phosphorylation would occur.
b) A proton gradient would not be established, therefore no transition of energy between the nutrient and the production of ATP.
c) The mutation directly affects both the establishment of a proton gradient and electron movement through the electron transport chain, so that both processes do not happen.
d) None of the above.

25) The electron transport chain:

a) Is coupled to proton pumping
b) Produces conformational changes in transport chain complexes
c) Generates a series of reduction/oxidation (redox) reactions
d) Is mediated by complexes containing prosthetic groups
e) All of these statements describe the electron transport chain

26) As part of your 4th year thesis project, you are evaluating the integral membrane protein PLP that is localized to the plasma membrane. You labeled the polar region of the protein that faces the outside of the cell with green fluorescent dye and now you plan to use FRAP to quantify the rate of movement of your protein within the plasma membrane. As a control, you label a second protein (NTRL) with a red fluorescent dye, which you previously characterized using FRAP and found to be very mobile. You look at your dual-labeled cell to find that, to your surprise, PLP, labeled with the green dye, moves much more slowly than NTRL labeled in red. What could account for the reduced mobility of PLP?

a) you accidentally performed your experiment at 30°C rather than 37°C
b) attachment to cytoskeletal mesh in the interior of the cell
c) localized to a region of the plasma membrane with higher than average amounts of cholesterol
d) (a), (b), and (c) could all account for the slow movement of PLP
e) (b) and (c), but not (a), could account for the slow movement of PLP

27) Proteins are often composed of two or more distinct modules that fold up independently of one another. They often represent parts of a protein that function in a semi-independent manner. These modules are called _____.

a) protein motifs
b) functionals
c) dominoes
d) domains

28) Where in a myelinated axon are nearly all of the ion channels concentrated?

a) nodes of Ranvier
b) dendrites
c) the cell body
d) axon terminals
e) neuron nucleus

29) Collagen 1 consists of _____ chains arranged in a _____.

a) 2, double helix
b) 3, double helix
c) 3, triple helix
d) 3, triple lattice
e) 2, triple helix

30) Experimental evidence strongly suggests that the bent conformation of an integrin is _____ and therefore unable to bind its ligand.

    a) loose
    b) inactive
    c) tight
    d) active
    e) poised

31) The citric acid cycle (aka TCA cycle) primarily takes place in the:

    a) cytoplasm
    b) intermembrane domain of mitochondria
    c) matrix domain of the mitochondria
    d) lysosome
    e) nucleus

32) You are a marine biologist who randomly samples the ocean waters to gain a sense of how the ecosystem changes over time. A tiny sample of water contains many different types of microbes. Being the curious scientist that you are, you want to identify all of the organisms in your water sample. Since many of the organisms could be closely related, it is important to know the identity of all of the genes that are expressed in your sample of water. What experimental technique should you use?

    a) mass spectrometry
    b) PolyAcrylamide Gel Electrophoresis (PAGE)
    c) yeast 2-hybrid
    d) RNA sequencing
    e) microarray technology

33) Which of the following statements about the proton-motive force is FALSE?

    a) It is a combination of a proton concentration gradient and the membrane potential
    b) It is an anaerobic process
    c) It is necessary for ATP generation by the ATP synthase
    d) It lends (a synonym for 'lends' is 'leads') to the pumping of protons into the intermembrane space
    e) It is achieved by the electron transport chain

34) The building blocks of a nucleotide are _____.

    a) a pentose sugar, a phosphate group and a nitrogenous base
    b) a pentose sugar, a phosphate group and an amino acid
    c) a pentose sugar and a phosphate group
    d) a pentose sugar and a nitrogenous base
    e) a phosphate group and a nitrogenous base

35) Which of the following statements about the basement membrane is FALSE?

    a) Functions as a sticky surface for cells to migrate on
    b) Is a barrier to macromolecules
    c) Contains Collagen IV, a fibrillar member of the Collagen family
    d) Is a continuous layer that is important for establishing skin cell polarity
    e) Often separates two distinct regions of a tissue

36) Covalent bonds between which of the following pairs of atoms are relatively nonpolar?
    a) C–C
    b) C–H
    c) O–H
    d) a and b
    e) b and c

37) Which of the following volumes would you use to prepare only 500 µL of a 1X Fluorescein dye working solution given 40 µL of a 25X stock solution?

    a) 40 µL of 25X stock solution + 1000 µL H_2O
    b) 20 µL of 25X stock solution + 480 µL H_2O
    c) 20 µL of 25X stock solution + 500 µL H_2O
    d) 40 µL of 25X stock solution + 460 µL H_2O

38) _____ is induced by _____ binding to the cytoplasmic tails of integrin α and β chains and causing a conformational change that activates integrins and allows them to bind to the extracellular matrix.

    a) Outside-in signalling; talin
    b) Inside-out signalling; talin
    c) Outside-in signalling; fibronectin
    d) Inside-out signalling; fibronectin

39) Cells store electrical potential energy by forming a concentration gradient of
    a) glucose across a membrane
    b) ATP across the cytoplasm
    c) ions across a membrane
    d) ions across the cytoplasm

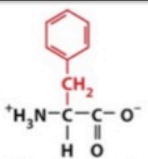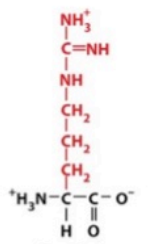40) Which of the following statements about proteoglycans is FALSE?

    a) Enable joints to resist compressive forces
    b) Its positively charged glycosaminoglycan (GAG) groups aid in attracting water
    c) Contains glycosaminoglycan (GAG) groups with a repeating disaccharide structure
    d) Has the unique property of being able to form a porous, hydrated gel

PART 2: Short Answer Questions

(1) [**2 marks**] Fill out the missing information in the chart below:

Amino Acid	Type of Amino Acid	Type of Side Chain Interaction
Phenylalanine (Phe or F)		
Arginine (Arg or R)		

Amino acid types: polar charged, polar uncharged, nonpolar, other
Side chain interaction types: covalent bonds, ionic bonds, H-bonds, hydrophobic & van der Waals interactions

Phenylalanine – Non Polar Amino Acid [**0.5 mark**]; Hydrophobic & van der Waals [**0.5 mark**]
Arginine – Polar Charged Amino Acid [**0.5 mark**]; ionic bonds [**0.5 mark**]

(2) [**4 marks**] You are testing the effect of a new drug on your cell culture. You name the drug Crixus. Crixus has a molecular weight of 348.54 g/mol. You decide that it is better to prepare a 10X stock solution of Crixus, store it in small aliquots and add it to your 10mL culture media to obtain a final concentration of 175mM. How much Crixus should be added to make 10mL of stock solution [**2 marks**]? How much of the stock solution should you add to your 10mL culture media when you use it [**2 marks**]?

Since it's a 10X stock, the concentration of the stock solution should be 10*175 mM = 1.75 M

Amount of Crixus required in stock in mol:

1.75 mol/L x $(1 \times 10^{-1}$ L$) = 0.175$ mol

Amount of Crixus required in stock in grams:
0.175 mol x $(348.54$ g/mol$) =$ **60.994 grams** (or rounded to 61 grams) [**2 marks**]

You should add 10 mL/10 = **1000 µL** of stock solution [**1.5 marks**] to 9 mL of your culture media [**0.5 mark**].

(3) [**3 marks**] You perform a voltage clamp experiment by clamping a patch of membrane and measuring the corresponding current that travels through the single ion channel within your patch. For voltage levels of -20mV, 10mV, 60mV, 120mV, you measure current levels of -1.92pA, -1.2pA, 0pA, and 1.44pA, respectively.

a) Calculate the conductance of the ion channel within your patch. [**1 marks**]
Conductance = Slope of I-V plot = 0.024 pA/mV = 24 picoSiemens

b) In 3 sentences or less, what is the value of the Nernst potential according to your measurements, and how is it determined experimentally from the data given? [**2 mark**]
The Nernst potential is the electrical potential at which the net flow of current through the ion channel is zero [**1 mark**]. Therefore, from the above measurements, the Nernst potential is 60mV [**1 mark**].

(4) [**3 marks**] As part of your BME205 lab you are expected to transfer 10ul of a 10X stock of blue dye into an eppendorf tube containing 90ul of water using your micropipettor. Listed in the chart below is each of the steps necessary to pipette a solution from one vessel to another. Indicate the correct order of each step by labeling the chart (e.g. Step 1, Step 2, Step 3, Step 4, and so on).

Order	Steps of micropipette use
Step 2	Press and hold the plunger at the first stop
Step 5	Transfer tip with dye to tube containing water
Step 1	Set the volume to 10µl and load the tip
Step 6	Press and hold the plunger at the second stop
Step 3	Insert the tip in the 10X stock of blue dye
Step 4	Gently release the plunger to draw up liquid

(5) [**3 marks**] You've identified a new species of mouse and decide to isolate cardiac cells and characterize properties of the plasma membrane. Your measurements indicate that the mouse cardiac cell membrane exhibits high stability and a reduced permeability similar to that in most mammals. In two or less sentences, what membrane component is responsible for these traits and how does it do it?

Cholesterol [**2 marks**] alters the way hydrocarbon chains pack together [**1 mark**] and is responsible for these traits.

(6) [**4 marks**] Consider the following questions about the effect of enzymes on chemical reactions:

a) In 2 sentences or less, describe <u>two</u> of the mechanisms by which enzymes can accelerate reactions. [**2 marks**]
i. Substrate Orientation – by bringing multiple substrates together in correct orientation
ii. Changing substrate reactivity: alters chemical properties (e.g. charge) of substrate by amino acid side chains in active site
iii. Inducing strain in substrate – changes conformation of substrate to bring closer to that of transition state

b) In 3 sentences or less, explain the two main differences between competitive and noncompetitive enzyme inhibitors. [**2 marks**]
i. Competitive – compete for active sites [**0.5 mark**], can be overcome with high substrate-to-inhibitor ratios [**0.5 mark**].
ii. Non competitive – binds to sites other than active sites [**0.5 mark**], cannot be overcome with high substrate-to-inhibitor ratios [**0.5 mark**].

(7) [**4 marks**] As a summer student, you aimed to determine whether the small peptide called FGF2 activates the ERK1/2 protein in insulin secreting beta-cells. ERK1/2 is considered 'activated' when it is phosphorylated. This phosphorylation can be detected in a Western blot using a phosphorylation-specific antibody (pERK1/2) and comparing cells in the absence (-ve) and presence (+ve) of FGF2. Shown below are scan of an actual Western blot.

a) Based on the western blot image, does FGF2 activate ERK1/2 in these cells? In one/two sentences, describe how you can tell [**2 marks**]. Yes [**1 mark**], FGF2 activates ERK1/2 by increasing causing its phosphorylation. We can tell that because the density/darkness of the pERK1/2 western blot band increases [**1 mark**], with no increase in ERK1/2 and at the appropriate molecular weight (not needed for full marks).

b) We also probed this same blot for total amount of ERK1/2 ("ERK1/2"). Does the total amount of ERK1/2 increase with treatment by FGF2? If you were told that the cells were treated with FGF2 for only 10 min, what would the major reason be for any increase in total amount of ERK1/2? In one/two sentences describe why [**2 marks**].
No, the total amount of ERK1/2 does not increase. [**1 mark**]
The major reason for an increase or decrease would be due to loading errors [**1 mark**] as 10 minutes is not enough time to significantly increase/decrease protein level by translation (this last part is not required for full mark).

(8) [**7 marks**] As a summer student you are working alongside a grad student to design a mouse model to research Vlahos Syndrome – a disease that affects the potassium ion channels of all of the cells in the organism. In previous studies the lab determined that this disease is caused by a genetic mutation in the *JMC1* gene.
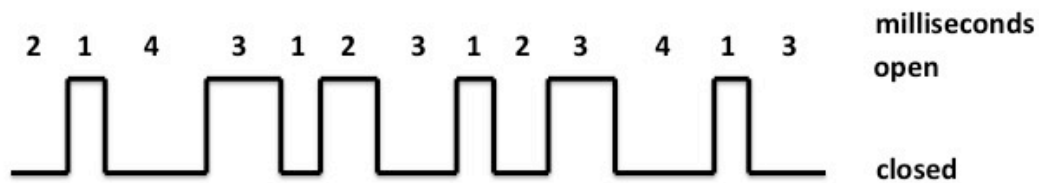
a) The first step in creating your transgenic mouse that models Vlahos syndrome is to introduce the mutation from the human disease into the *JMC1* gene of the mouse. Name a strategy you can use to edit the mouse genome so that the disease mutation is incorporated into the *JMC1* gene [**1 mark**]. The CRISPR/Cas9 system

b) Being the savvy summer student you are; you decide that you want to determine the total number of potassium channels present in the cell membrane of the harvested cells from your mouse model. Below is a

sample ion channel recording of a single channel performed at a voltage clamp of -70 mV. Determine the channel's probability of being open at -70 mV. Be sure to show your work for full marks [**4 marks**].

[**1 mark** for demonstrating that they quantified time spent open & close]
time spent open = 11 seconds
time spent close = 19 seconds

probability of being open → 11/30 [**1 mark** for correct dividing time open by total recording time and **1 mark** for correct answer] = 0.367 or 36.7% [**0.5 mark** for correct method to calculate probability and **0.5 mark** for correct answer]

c) Determine the number of potassium channels present in your cell membrane if the single channel current is 1/600 the magnitude of the total current of the cell [**2 marks**]. (Hint: I = i x n x p)
Probability from above → 0.367
I/i = n x p
600 = n x p
600 / 0.367 = n
n = ~1635 ion channels