Name: _____
Student #: _____

University of Toronto
Faculty of Applied Science and Engineering
Division of Engineering Science
BME205S Midterm Examination, EX100
Thurs March 9, 2017, 9:00 – 10:30 am
Duration: 90 minutes
Examiner: P. Gilbert

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***. Each is worth 1 mark.
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 is a blank page at the end of the test for rough work.

Last Name: _____

First Name: _____

Student Number: _____

Tutorial section:

[ ]	TUT 01	Wd	09:00	10:00	BA2155
[ ]	TUT 02	Wd	09:00	10:00	GB304
[ ]	TUT 03	Wd	09:00	10:00	BA2165
[ ]	TUT 04	Wd	13:00	14:00	BA2165
[ ]	TUT 05	Tu	13:00	14:00	BA2145
[ ]	TUT 06	Tu	13:00	14:00	BA2139

PART I: Multiple Choice _____ **out of 35**
PART 2: Short Answer Questions

Question	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Marks Available	**5**	**3**	**2**	**3**	**3**	**3**	**7**	**3**	**6**	**5**
Marks Achieved										

PART 2 _____ **out of 40**
TOTAL MARKS _____ **out of 75**

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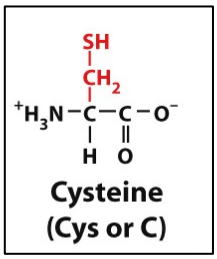
PART 1: Multiple Choice
ANSWERS MUST BE FILLED OUT ON SCANTRON SHEET

1. _____ are _____ lipids that are a major component of cell membranes.

a. Phospholipids, hydrophobic
b. Phospholipids, amphipathic
c. Sphingolipids, hydrophobic
d. Steroids, hydrophobic
e. Steroids, hydrophilic

2. Cysteine, a polar, uncharged amino acid, can form a _____ bond with _____ to create a disulfide bridge.

a. covalent, any polar charged amino acid
b. covalent, another cysteine
c. noncovalent, methionine
d. noncovalent, any polar charged amino acid
e. ionic, another cysteine



Cysteine
(Cys or C)

3. Two highly electronegative atoms often present in biological molecules are ____ and ____.

a. O, N
b. O, C
c. O, Na
d. C, N
e. C, Na

4. Which reaction below might be a suitable coupled reaction for the forward reaction of A + B <—> C + D (ΔG = -8.7 kcal/mole)?

a. E + F <—> G + H (ΔG = -5.4 kcal/mole)
b. B + F <—> G + H (ΔG = -9.4 kcal/mole)
c. C + F <—> G + H (ΔG = +6.7 kcal/mole)
d. C + F <—> G + H (ΔG = +9.7 kcal/mole)
e. A + F <—> G + H (ΔG = +10.2 kcal/mole)

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5. Which of the following is a function of membranes?

a. compartmentalization
b. selectively permeable barrier
c. mediates intercellular interactions
d. helps cells respond to external stimuli
e. All of these are correct.

6. Unsaturated fats are _____ molecules comprised of a glycerol linked by ester bonds to _____ fatty acids and are _____ at room temperature.

a. nonpolar, three, liquid
b. polar, three, liquid
c. nonpolar, two, solid
d. polar, three, solid
e. nonpolar, three, solid

7. Cells store electrical potential energy by forming a concentration gradient of

a. glucose across a membrane
b. ions across a membrane
c. ions across the cytoplasm
d. ATP across the cytoplasm

8. Which of the following volumes would you use to prepare exactly 600 µL of a 1X Fluorescein dye working solution given 40 µL of a 30X stock solution?

a. 40 µL of 30X stock solution + 1160 µL H_2O
b. 20 µL of 30X stock solution + 600 µL H_2O
c. 20 µL of 30X stock solution + 580 µL H_2O
d. 40 µL of 30X stock solution + 560 µL H_2O

9. 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

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10. What is appropriate clothing for laboratory work in the MB325 teaching lab?

a. Comfortable shoes, loose clothing
b. Long pants, long hair tied back, close-toed shoes
c. Old clothing
d. Sandals, shorts, cool clothing
e. Any clothing is appropriate for the lab

11. 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.

12. 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 all remaining proteins from the biomembranes for further investigation.

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

13. 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, temperatures
b. anabolic, catabolic, temperatures
c. catabolic, anabolic, enzymes
d. anabolic, catabolic, enzymes

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

a. HCO_3^-
b. K^+
c. Glucose
d. O_2
e. ATP

15. As humans age, the function of many tissues declines. Some scientists believe there is a genetic basis of longevity. This is an exciting notion because it suggests that if we could figure out which genes support longevity, then we might be able to extend human life. As part of your summer research, you aim to compile a list of potential youth genes by comparing the genetic profile of tissues collected from 20 year old and 100 year old volunteers. What scientific method should you use to reveal the identity of <u>all</u> of the genes expressed in each sample?

a. microarray technology
b. mass spectrometry
c. EMG
d. PolyAcrylamide Gel Electrophoresis (PAGE)
e. RNA sequencing

16. In addition to ATP, what are the end products of glycolysis?

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

17. What must you do each time that you depart the MB325 teaching laboratory?

a. Ask your TA what to do
b. Remove personal protective equipment and wash your hands
c. Leave the MB325 door propped open so that others can enter easily
d. Remove personal protective equipment and leave it on the bench top
e. Collect your personal items and grab a quick drink from the eyewash on your way out

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18. 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

19. 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 yellow 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 yellow 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

20. The building blocks of a nucleotide are _____.

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

21. 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

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22. Gibbs free energy depends on both _____ and _____. An _____ reaction is considered to be spontaneous.

a. Entropy, enthalpy, endergonic
b. Entropy, enthalpy, exergonic
c. Exergonic, endergonic, enthalpy
d. Exergonic, endergonic, entropy
e. Entropy, exergonic, endergonic

23. What gives sugar its highly water soluble property?

a. The glycosidic bonds
b. The ring structure
c. The hydroxyl groups
d. The carbonyl groups
e. The branched structure

24. If the FRAP technique is used to bleach a small zone of fluorescent microtubules in a cell, which of the following is a possible explanation for the recovery of fluorescence in the region of the cell previously bleached?

a. the dynamics of the microtubules turning over in that bleached zone of the cell
b. the growth of new microtubules into the bleached zone
c. movement of microtubules through the bleached zone
d. the dynamics of the microtubules turning over in that bleached zone of the cell and the growth of new microtubules into the bleached zone
e. all of these choices

25. Peptides fold in such a way that the amino acid residues with hydrophobic R-groups are generally

a. at the surface of the peptide
b. dielectrically opposed to other hydrophobic groups
c. in the core of the peptide
d. surrounded by water

26. The integral membrane class of proteins most commonly associates with biomembranes:

a. by insertion of an α-helix structure
b. by insertion of a GPI anchor
c. by interacting with peripheral membrane proteins
d. both (a) and (b) are correct

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27. Which statement is FALSE?

a. Uniporters transport ions or molecules down a concentration gradient
b. ATP-pump transport is not reversible
c. Conformational change in uniporter proteins is fueled by ATP hydrolysis
d. all of the above
e. none of the above

28. Which of the following statements is TRUE?

a. Cholesterol is a phospholipid
b. Due to its small head group, phosphotidylcholine creates membrane curvature
c. Sterols decrease local fluidity
d. Phosphoglycerides are derived from sphingosine.

29. Which of the following statements about electromyography (EMG) are TRUE?

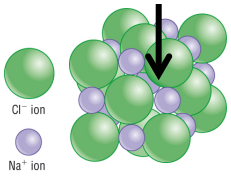
a. EMG records electrical activity produced by muscles
b. EMG electrodes convert electrical signals to mechanical signals
c. Typically, an EMG signals has components between 5-200 Hz
d. EMG signals, like all bipotential signals, do not require pre-processing to remove noise
e. (a) and (c) are both TRUE statements about EMG

30. _____ divide by mitosis, have _____ , and genetic material that is _____.

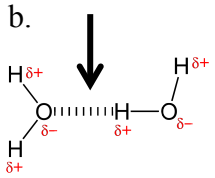
a. Prokaryotes, membrane bound organelles, packaged into a single circular DNA
b. Prokaryotes, flagella, packaged into chromosomes
c. Prokaryotes, a nucleoid region, abundant compared to eukaryotes
d. Eukaryotes, membrane bound organelles, packaged into chromosomes
e. Eukaryotes, a nucleoid region, packaged into a single circular DNA

31. In which of the following images does the arrow point at a covalent bond?

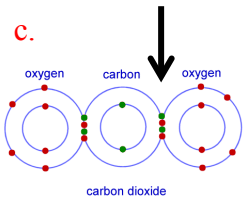
a.



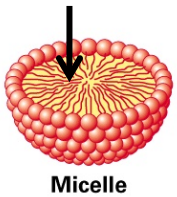
b.



c.



d.



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32. Which of the following statements about enzymes is (are) TRUE?

a. Enzymes are present in cells in small quantities
b. Enzymes supply energy to a reaction thereby allowing an unfavourable reaction to become favourable
c. Enzymes can catalyze the reaction of many distinct substrates
d. Enzymes are permanently altered during a reaction and for this reason cannot catalyze subsequent reactions

33. Cholesterol in a biomembrane serves to _____ and _____ membrane thickness by binding to phospholipid tail groups.

a. decrease local fluidity, increase
b. decrease local fluidity, decrease
c. increase local fluidity, increase
d. reduce rigidity, decrease

34. Why are frog oocytes often used to study ion channels?

a. When injected with mRNA encoding a channel of interest, frog oocytes faithfully express channel proteins in the plasma membrane
b. Frog oocytes have few endogenous channel proteins making it easier to study the exogenously introduced ion channel of interest.
c. Frog oocytes are quite large, which makes patch clamp studies more feasible.
d. (a) and (b), but not (c) are reasons that frog oocytes are used to study ion channel
e. (a), (b), and (c) are all reasons that frog oocytes are used to study ion channels

35. Which of the following statements about the movement of substances across cell membranes is (are) TRUE?

a. The Na+/K+ ATPase is classified as 'active transport' because the pump must undergo a conformational change in order for the ions to pass through the membrane.
b. Na+ is generally maintained at a higher concentration outside of the cell relative to the concentration inside the cell.
c. Movement of ions down a concentration gradient requires an input of energy.
d. Glucose readily diffuses across biomembranes.
e. Only in manmade systems do we ever find charge separation such that the inside of the cell is negative relative to the outside.

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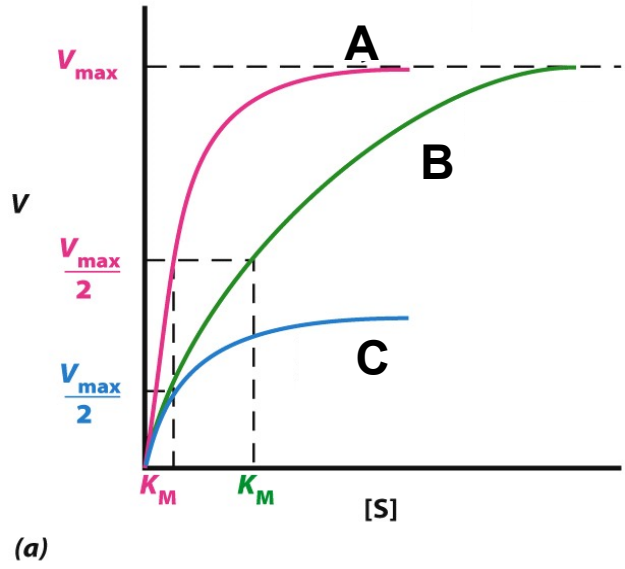
PART 2: SHORT ANSWER QUESTIONS

1. [5 marks] Enzymes are biological catalysts. Below is a chart showing the kinetics of a reaction catalyzed by an enzyme. The three curves correspond to an uninhibited (control) reaction, or reactions treated with a competitive or non-competitive enzyme inhibitor.



(a)

a. Indicate which line corresponds to which treatment condition [1 mark].
A. Uninhibited B. +Competitive inhibitor C. +Non-competitive inhibitor
There are no partial marks for this question.

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**].

c. 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

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2. [3 marks] You want to test the effect of putrescine on your stem cell cultures since it is thought to be important for cell division. The instructions provided by the manufacturer instruct you to dissolve the putrescine powder in water to a concentration of 400 mM and to use it at a final concentration of 100 µM. If the molecular weight of putrescine is 161.07 g/mol, how much water do you need to use to dissolve 25 mg of putrescine powder to create your stock [1.5 marks]? And how much of the stock would you need to use in your 10 ml culture [1.5 marks]? You must show your work for full marks. Note: Putrescine is super stinky so be sure that your calculations are correct before opening that bottle!

(400 mM) x (volume) x (161.07 g/mol) = 25 mg → volume = 388 µl
∴ dissolve 25 mg putrescine in 388 µl water [1.5 marks]

(400 mM) x (volume) = (100 µM) x (10 ml) → volume = 2.5 µl
∴ add 2.5 µl of stock putrescine to 10 ml culture [1.5 marks]

3. [2 marks] A particular enzyme acts on substrate A to produce product B. Compound C is an allosteric activator of the enzyme that enhances the enzymatic activity without binding to the active site. With genetic techniques, the valine at position 57 in the enzyme was mutated. The mutated and unmutated proteins were purified and their activities were measured in a standard assay system, which included 10 mol A/ml of reaction mixture (a saturating level of A), in the presence and absence of C. The assay results are presented below.

	Enzyme activity (nmol B produced/(min)(mg enzyme M))	
Mutants	**No C added**	**1mM C added**
None	10.3	41.4
Val 57 → Ser 57	10.5	30.2
Val 57 → Glu 57	10.2	11.1
Val 57 → Leu 57	10.1	49.5

2a. In one sentence, what mutation has the greatest effect on the activity of the enzyme [1 mark] and why [1 mark]?

Glu has the greatest effect because its chemical properties are most dissimilar to Val.
Marking scheme:
+1 mark for Glu
+1 mark for chemistry most dissimilar to Val.

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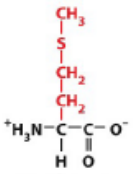
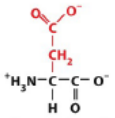
4. [3 marks]
4a. Fill out the missing information in the chart below [2 marks]:

Amino Acid	Amino Acid Type	Side Chain Interactions
 Methionine (Met or M)	Nonpolar	Van der waals
 Aspartic acid (Asp or D)	Polar charged	Ionic

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]

4b. Which of the amino acids in the chart above would be most likely to be found on the outside portion f a cytoplasmic protein and why do you think this [1 mark]? Aspartic acid [0.5 marks] because it is charged and would be more compatible with the water containing cytosolic environment [0.5 marks].

5. [3 marks] Compare the rate of lateral diffusion of a phospholipid with the rate of phospholipid 'flip-flop'[1 mark]? What is the reason for the rate differences [2 marks]? Limit your answer to 2-3 sentences.

Phospholipids lateral movement within a membrane layer is quite rapid compared with phospholipid 'flip-flop' from the outer to inner membrane (or vice versa) [1 mark]. In lateral diffusion phospholipid polar head groups maintain association with other polar head groups and fatty acyl tail groups remaining associated with other non-polar groups [1 mark]. In the case of 'flip-flop' the polar head group must traverse the lipid bilayer and this is quite energetically unfavourable [1 mark].

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6. [**3 marks**] Which of the following tripeptides would be most likely to be soluble in an aqueous solvent like water: N - phenylalanine - alanine - glutamine - C, or N - leucine - alanine - lysine - C, or N - proline - phenylalanine - leucine - C, or N - arginine - lysine – aspartate - C, or N - glutamate - aspartate - serine - C? Explain your answer in three or fewer sentences.

Solution: N – arginine – lysine - aspartate - C would be most soluble in a hydrophilic solvent [1 mark]. All three amino acids are classed as polar charged amino acids and could be soluble in water [1 mark]. In the other tripeptides, at least one of the amino acids belongs to the nonpolar charged class [1 mark].

7. [**7 marks**] Alpha-5 integrin is a single pass transmembrane receptor protein, which means that only one segment of the protein is embedded in the plasma membrane of the cell. The part of the protein that is N-terminal to the transmembrane segment is on the outside of the cell and the part that is C-terminal is on the inside.

1a. The transmembrane segment adopts the secondary structure of an alpha helix. In the structure of an alpha helix, there are 3.6 amino acids per turn and each turn is the 5.4 Å in length. Given a plasma membrane thickness of 42 Å, how many amino acids are in the transmembrane segment [1 mark]?

(42/5.4)*3.6 = 28 amino acids
Marking scheme:
1 marks for above solution
0 marks otherwise

1b. Below is a segment of the amino acid sequence of alpha-5 integrin containing the transmembrane segment. Circle the amino acids that are most likely the transmembrane segment [2 marks]. In three sentences or less, state the reason for your selection [2 marks].

QVTTLDVHVC**DVPPGILGGAILILALLLILILLLLLFLR**DCEGTVNNCMKAGIVAAGLRRTVV
KEPLLPPDDDT

Looked for a sequence with consecutive amino acids with **hydrophobic** R-groups around 28 aa long (in this case 26 aa) that interact with **the fatty acyl tails of the phospholipids**. The transmembrane space also includes **hydrophilic amino acids** flanking the hydrophobic portion (at the ends) that interact with the **hydrophilic head group of the phospholipids**. The selected sequence is therefore 28 amino acids long, with one hydrophilic amino acid on each side (flanking) the 26 hydrophobic amino acids.

Marking scheme:
+ 2 mark for selecting the exact region, +1 for mostly correct
+ 1 marks for mentioning fatty acyl tails interact with hydrophobic R-groups (or amino acids)
+ 1 marks for mentioning the hydrophilic head group of the phospholipid interact with hydrophilic aa

1c. What is the membrane thickness due to the fatty acyl tails of one phospholipid [2 marks]?
Recognition that 26 hydrophobic aa acids interact with the phospholipid tails – which works to [1 mark]:
If x/5.4*3.6 = 26, then x = 39 A

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This thickness is the phospholipid bilayer, so one phospholipid tail would add 39/2 = ~19.5A [1 mark]

8. [3 marks] The teaching assistant in the lab gives you a flask containing 50 ml of culture media containing non-adherent cells. She tells you that these cells grow very slow and challenges you to make them grow faster. To test the effects of different reagents that may speed up cell growth, you portion the 50 mL of cells into 5 equal sized portions. Upon searching through the lab fridge you find a nondescript vial simply labeled 'U_Bolt Potion, 2000X'. You decide to test the effects of U_Bolt Potion on one of the culture portions. How much of U_Bolt Potion would you need to add to the cell culture portion [3 marks]? You must show your work for full marks.

volume of split cultures: 10 ml each or 10,000 µl [1 marks]
add U_Bolt Potion to final concentration of 1X; 10,000 µl / 2000 = 5 µl [2 marks]
∴ add 5 µl U_Bolt Potion to the culture

9. [6 marks] Consider the following questions about SDS-PAGE, a variant of western blotting.

9a. While preparing a protein sample for SDS-PAGE the protein is loaded into a loading buffer containing ß-mercaptoethanol (BME) and sodium dodecyl sulfate (SDS). Explain the role of BME [2 marks] and SDS [2 marks] in this process in **2-3 sentences**.

The role of BME is to cleave/reduce disulphide bonds [1 mark] to disrupt the protein structure [1 mark]. SDS is negatively charged and binds to the denatured proteins [1mark]. It attributes an overall negative charge to the proteins in the sample and allows them to be pulled towards the anode [1mark].

9b. After completing your western blot you realize there are no protein bands visible on your blot. In **2-3 sentences**, provide <u>one</u> potential cause that may have happened during the isolation of proteins from cells [1 mark] and <u>one</u> potential cause that may have happened while transferring the proteins onto a membrane [1 mark].

Isolating proteins from cells [1 mark]:
The cell lysis buffer was very weak and did not properly lyse the cell sample OR
The cell sample did not express the protein of interest.

Transferring proteins onto a membrane [1 mark]
The gel and membrane were not clamped together, causing them to separate OR
The electrical field was applied in the wrong direction.

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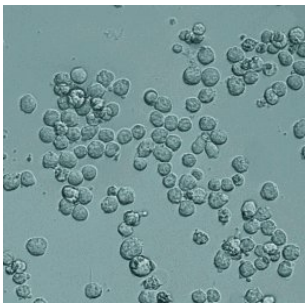
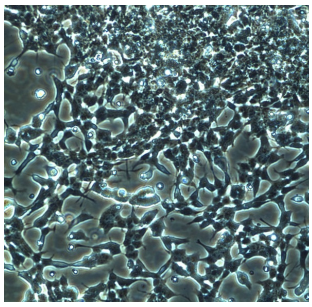
10. [**5 marks**] Your Lab TA hands you two flasks each with 10 mL of culture media containing cells. She tells you to transfer each flask of cells into a 10 cm tissue culture plastic dish, to place the dishes in the incubator overnight, and to acquire representative images the next day. Below are the light microscope images you captured.



Flask 1



Flask 2

10a. Based on the images you took, which flask contained non-adherent cells and which contained adherent cell [0.5 mark]? How do you know [1 mark]? Flask 1 = non-adherent; Flask 2 = adherent [0.5 mark]. The cells from flask 1 are very round in morphology, while the cells from flask 2 appear to have attached to the tissue culture plate and spread out [1 mark].

10b. Your TA then asks you to quantify the viability of the cells you plated. Before she tells you what to do, you suggest a method you learned in the BME205 Microscopy lab. What dye do you suggest staining the cells with [1 mark]? How does this dye allow you to assess viability [1 mark]? Tryan blue dye [1 mark]. The plasma membrane of live cells excludes the dye, but the membrane of dead cells is permeable allowing the dye to enter the cell and stain the cell a blue colour [1 mark].

10c. Ultimately to assess viability you will need to calculate the % total cells that are alive in each field of view. You find that it rather difficult to discern how many cells are in Flask 2, but you remember another dye that you used in BME205 microscopy lab that could help out. What is the dye [0.5 mark]? How will it help you to count the total number of cells [1 mark]. Fast Blast DNA stain [0.5 marks] will allow you to easily visualize the circular nucleus of each cell in the Flask 2 images [1 mark].

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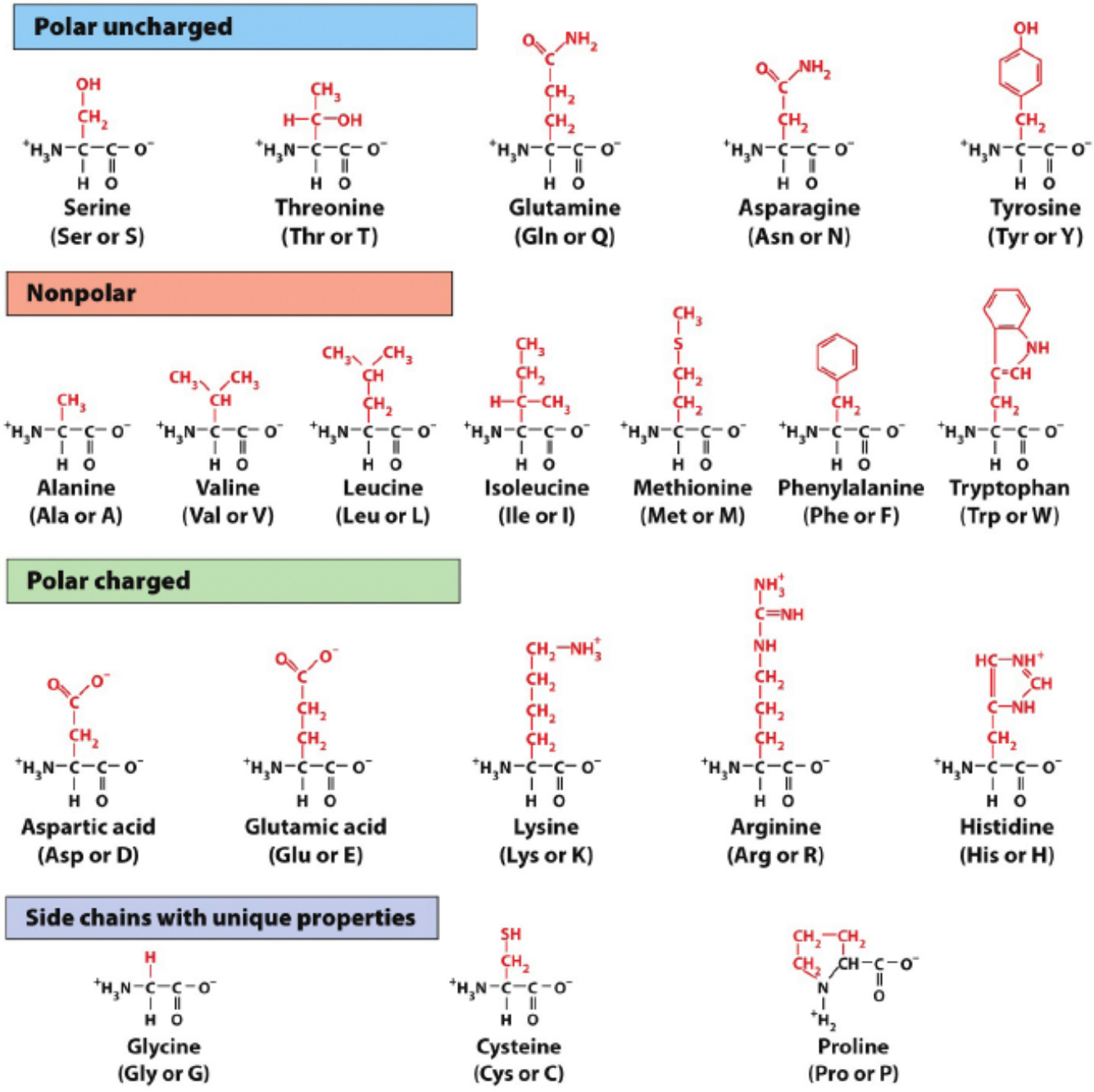
Student #: _____

Resource



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