**YOUR NAME:**_____

**YOUR STUDENT NUMBER:** _____

**COURSE: BLG311 Cell Biology**

**October 5th, 2016**

INSTRUCTOR: Dr. Costin Antonescu

# VERSION A

## BEFORE PROCEEDING ANY FURTHER, MAKE SURE TO ENTER YOUR TEST VERSION ON THE SCANNING SHEET!

You have **90 MINUTES** to complete this examination

Part A: 18 multiple-choice questions each worth 2 points. Total 36 marks

Part B: 6 short-answer questions. Total 34 marks

Total possible test points = 70

**There are 6 pages (including this one) in this examination.**

**At the end of the examination, you must return:**

**1) This question sheet including all its pages**

**2) Your completed bubble sheet with your answers to Part A**

**3) Your completed exam booklet with your answers to Part B.**

Make sure to follow the instructions given for completing the scanning sheet.

## FAILURE TO PROPERLY FOLLOW INSTRUCTIONS FOR COMPLETING THE BUBBLE SHEET WILL RESULT IN A 10% PENALTY ON THIS EXAM

**Good luck!**

## Part A: Multiple Choice (18 questions, 36 total marks)

Answer EACH of the following questions by filling in the corresponding answer on the bubble sheet. There is only ONE correct answer per question. Each question is worth 2 marks. Any answers indicated on this question sheet will not be graded; only the bubble sheet will be graded for this section. You will not be given additional time to copy your answers from this sheet to the bubble sheet.

1. When considering only a reaction that forms a large macromolecule such as DNA and proteins from smaller molecules, such as nucleotides and amino acids, respectively (and not any other "coupled" reactions):"

(A) this is always favorable since it involves an increase in enthalpy and decrease in entropy.
(B) this is often unfavourable since this involves a decrease in entropy and may also have an increase in enthalpy.
(C) this is always favorable since it involves a decrease in enthalpy and a decrease in entropy.
(D) this is always unfavourable since it involves an increase in entropy and an increase in enthalpy.
(E) none of the above

2. Which of the following pairings of a membrane-bound organelle and its function is INCORRECT:

(A) Golgi Apparatus and degradation of large macromolecules.
(B) Endosomes and exchange of materials with the extracellular environment.
(C) Endoplasmic reticulum and synthesis of large macromolecules such as certain proteins.
(D) Peroxisomes and oxidation-reduction reactions.
(E) Mitochondria and oxidation (for catabolism).

3. The modern eukaryotic cell likely evolved in three distinct and successive steps. Which of the following is the likely order of these events?

(A) Membrane involution, followed by chloroplast evolution, followed by mitochondria evolution
(B) Membrane involution, followed by concurrent chloroplast and mitochondria evolution.
(C) Chloroplast evolution, was followed by mitochondria evolution, followed by membrane involution.
(D)Membrane involution, followed by mitochondria evolution, followed by chloroplast evolution.
(E) Membrane involution, chloroplast evolution, and mitochondria evolution all occurred concurrently.

4. The Last Universal Common Ancestor would have had each of the following EXCEPT:

(A) the ability to synthesize specific proteins using information within DNA and RNA.
(B) the ability of synthesize lipids to generate cell membranes.
(C) ribosomes.
(D) lysosomes for the degradation of larger macromolecules.
(E) complex metabolic pathways.

5. GTPase-activating proteins (GAPs) are:

(A) Proteins that stimulate the phosphorylation of GDP back to GTP to activate GTPase signals.
(B) Proteins that displace GTP and permit GDP to bind to GTPases, thus activating GTPase signals.
(C) Proteins that stimulate the exchange of ADP for ATP binding by GTPases
(D) Proteins that stimulate GTP hydrolysis by GTPases to inactivate GTPase signals.
(E) Proteins that displace GDP and permit GTP to bind to GTPases

6. PP2A is a phosphatase that has Akt as a substrate. Which of the following best describes the properties and behaviour of a cell with a mutation in the gene encoding PP2A that causes PP2A to be completely inactive? In the cell with mutated (inactive) PP2A:

(A) Akt would be primarily dephosphorylated and inactive, and would thus signal for enhanced cell growth and survival.
(B) Akt would be primarily dephosphorylated and active, and would thus NOT signal for enhanced cell growth and survival.
(C) Akt would be primarily phosphorylated and active, and would thus signal for enhanced cell growth and survival.
(D) Akt would be primarily phosphorylated and inactive, and would thus NOT signal for enhanced cell growth and survival.
(E) none of the above.

7. Which of the following about specific macromolecules is FALSE?

(A) Proteins are formed by condensation reactions between amino acids.
(B) RNA (ribonucleic acid) is formed by condensation reaction between nucleotides.
(C) Cellulose is formed by condensation reactions between specific simple sugars.
(D) The bond formed between sugar subunits within a polysaccharide is called a peptide bond.
(E) None of the above.

8. Which of the following statements about the secondary and tertiary structure of proteins is FALSE?

(A) Hydrogen bonds are important for the formation of secondary structure in proteins.
(B) $\alpha$-helicies and $\beta$-sheets are examples of secondary structures.
(C) Secondary structure of a protein contribute to protein function.
(D) Tertiary structure does not contribute to the function of a protein.
(E) none of the above

9. ALL chemical reactions that have a negative $\Delta S$ are

(A) spontaneous for the formation of products over substrates.
(B) exergonic.
(C) favourable.
(D) all of the above
(E) none of the above.

10. Which condition below would produce the BEST resolution:

(A) 400 nm light and a numerical aperture of 1.50
(B) 475 nm light and a numerical aperture of 1.45
(C) 620 nm light and a numerical aperture of 1.25
(D) 450 nm light and a numerical aperture of 1.32
(E) 515 nm light and a numerical aperture of 1.28

11. Which methods has as a major limitation that it can only allow delivery of DNA plasmids to a few cells at a time (as opposed to thousands of cells simultaneously)?

(A) Microinjection
(B) Electroporation
(C) Carrier-Vesicle Fusion
(D) Ballistic transformation
(E) All of the above.

12. Super-resolution microscopy techniques such as Stochastic optical reconstruction microscopy (STORM) and Photoactivated localization microscopy (PALM) involve:

(A) angular illumination so that diffraction of light is reduced by a factor of 9.5-10.8 as it passes through a sample.
(B) the illumination of all fluorophores in a sample at the same time, then software analysis to dissect the individual positions of all fluorophores at once.
(C) the illumination of only a small percentage of all fluorophores in a sample at a time.
(D) technological advances in objective optics that allow new objectives (e.g. Zephyr and DiConTra) that have numerical aperture values >3.5.
(E) illuminating regular fluorescent molecules like Rhodamine, BODIPY or GFP with electron beams, which can be focused at higher resolution than light in the visible range.

13. Fluorescence is a phenomenon that involves

(A) absorbance of light of a particular wavelength and formation of a new covalent bond.
(B) absorbance of light of a particular wavelength and nearly instantaneous emission of light of a lower (shorter) wavelength.
(C) conversion of energy released by a chemical reaction into light emission.
(D) absorbance of light of a particular wavelength followed by near instantaneous emission of directed electrons.
(E) none of the above.

14. Which of the following methods allows detection of the location of a specific protein within a cell, using electron microscopy?

(A) Labeling of samples with uranium acetate, followed by transmission electron microscopy.
(B) Immunoelectron microscopy, which involves labeling with antibodies conjugated to gold particles.
(C) Cryoelectron microscopy, a form of electron microscopy that does not involve sample labeling.
(D) Scanning electron microscopy.
(E) Negative staining followed by transmission electron microscopy.

15. Confocal microscopy

(A) uses computer software to reconstruct a sharper image, after a series of images of the same region of the same are taken, each focused on a slightly different positiomn.
(B) is a form of computed tomography (CT), which requires the repeated acquisition of images of a sample at different tilts, and thus allowing reconstruction of a 3D model of the sample.
(C) uses coherent and polarized light to illuminate a sample to allow detection of objects by phase shifting and interference.
(D) requires sample preparation to label specific macromolecules (such as a specific protein) with gold-conjugated antibodies.
(E) uses a pinhole to restrict the majority of out-of-focus light from reaching the image detector.

16. Which of the following about cryoelectron microscopy is FALSE? Cryoelectron microscopy

(A) is useful for imaging of an aqueous suspension of large particles (e.g. ribosomes).
(B) does not require any fixation, drying or staining.
(C) requires labeling with cryogenically preserved uranium-labelled antibodies.
(D) involves analysis of thousands of images and computer-based image reconstruction.
(E) involves imaging in a vacuum, as well as at very cold temperatures (-160 degrees Celsius).

17. Deep etching is a technique that specifically involves:

(A) labeling of macromolecules placed on a thin film of carbon with a heavy metal.
(B) flash freezing of biological samples (e.g.) cells with results in sample fracture.
(C) scratching the top of a cell with a metal cantilever to expose the cellular interior.
(D) fixation and embedding for silver nitrate labeling of neurons.
(E) forming small temporary holes in the plasma membrane to allow delivery of DNA into the cell.

18. Which statement about the green fluorescent protein (GFP) is TRUE:

(A) The gene for GFP was isolated from a coral.
(B) There are alleles/mutants of GFP that emit other colours.
(C) GFP can be used to visualize proteins by electron microscopy.
(D) GFP is excited by green light.
(E) GFP fusion to proteins of interest occurs non-covalently.

## **Part B: Written Answer Questions** (6 questions, 34 total marks)

Answer ALL of the following questions in the examination booklet provided. Answers written on the question sheet will not be marked. The marks allotted to each question are indicated below.

1. Provide a brief (1-2 sentence) explanation of each of the following (2 marks each, **10 marks total**)

(i) osmium tetroxide

- used for electron microscopy to stabilize membranes or proteins

(ii) dichroic mirror

- mirror used in fluorescence microscopy that reflects light of 1 wavelength and transmits light of a different wavelength

(iii) microscope condenser

- microscope lens that focuses light ONTO the sample

(iv) anabolism

- either:
  - reactions that are endergonic
  - reactions that decrease disorder and/or "store" energy in bonds)
- these are not spontaneous on their own

(v) FM4-64

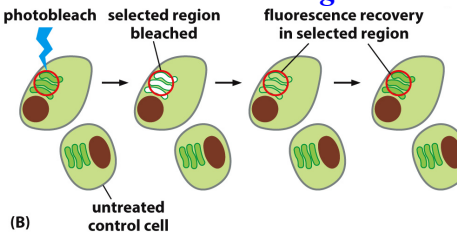
- fluorescent dye that inserts/intercalates into membranes to label membranes

2. Name a fluorescence microscopy method that is useful for measurement of protein movement in cell (e.g. for measurement of diffusion), and provide a description of how it works. Include in your answer any specific fluorophore properties for this method, as well as any unique illumination configuration required. (**6 marks**)

2 possible answers (they only need to provide one of these, and can only get 6/6 total marks):

1) Fluorescence recovery after photobleaching or FRAP
   - this can be done with most fluorescent proteins or dyes, which start off as fluorescent
   - this is used to study protein dynamics as follows:
     - the fluorescent protein or dye (bound to a protein under study) is initially found in a large area of the cell
     - the bleaching light (high intensity) is directed to a small region of the cell, which causes the fluorophores in that region to be permanently inactivated.
     - tracking fluorescence recovery in the photobleached region informs about the dynamics (movement) of non-bleached fluorophores arriving there from somewhere else in the cell

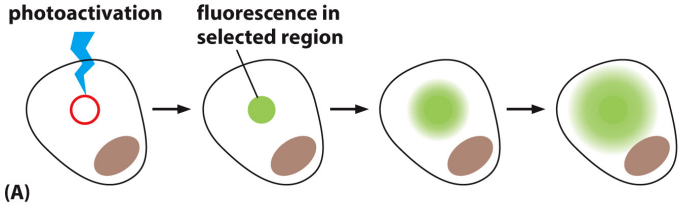
***Note: Diagram below is not required, it here for reference only***




2) Photoactivatable fluorophores (e.g. GFP)

- the molecule (e.g. PA-GFP) has a special property in that it is made (or initially present) in a non-fluorescent state. In other words, excitation light is not absorbed and emission light is not emitted (yet)
- photoactivation requires that molecule first be subject to light of a THIRD wavelength of light (e.g. UV light for GFP), which permanently renders the molecule fluorescent
- this is used to study protein dynamics as follows:
    - the PA-GFP (bound to a protein under study) is initially found in a large area of the cell
    - the photoactivating light is directed to a small region of the cell
    - then, tracking fluorescence of the activated PA-GFP over the whole cell informs about that molecule's dynamics

***Note: Diagram below is not required, it here for reference only***

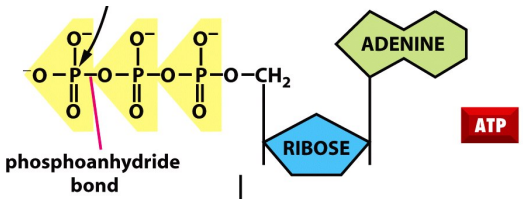



3. What is the difference between resolution and magnification? Briefly explain your answer (**2 marks**).

- **magnification** is apparent "visual" increase in the size of an object
- **resolution** is "visual" separation of the individual components of an object, which previously appeared as one

4. Describe how adenosine triphosphate (ATP) is used within cells to ensure that otherwise unfavourable reactions are able to occur. Include in your answer a description of the structure of ATP (you do not need to draw it in detail, just describe the part that is important for energetic considerations), the name of the bond that provides energy when hydrolyzed, and in general terms how ATP can be used to make other reactions more thermodynamically favourable. (**4 marks**)

***Note: Diagram below is not required, it here for reference only***

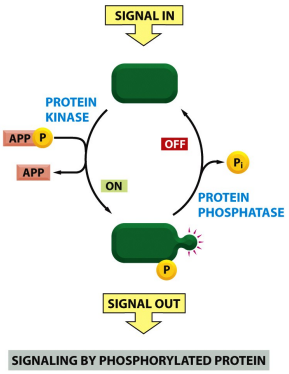



- ATP has three major chemical components: adenine, ribose and three phosphate groups
- the name of the bond that provides energy when hydrolyzed is the phosphoanhydride bond
- the hydrolysis of this bond is highly exergonic (spontaneous) (
- the hydrolysis of this bond is coupled in some way to another chemical reaction, the latter which on its own is endergonic (not spontaneous), the overall reaction (2 coupled reactions) can become favourable

5. Describe how phosphorylation and dephosphorylation control the function of a protein. Include in your answer the names of the enzymes responsible for phosphorylation and dephosphorylation, a description of the nature of the biochemical modification of proteins resulting from phosphorylation, and the effect that this form of regulation can have on a protein. (**6 marks**)

- Phosphorylation is the covalent attachment of a phosphate to a specific amino acid side chain of a protein
- (**BONUS**: amino acid side chain is serine, threonine or tyrosine, they only need to name one of these for the bonus)
-dephosphorylation is the reverse reaction of the above (removal of the phosphate)
- kinase is responsible for phosphorylation
- phosphatase is responsible for dephosphorylation
-phosphoryaltion causes a slight change in protein conformation or shape which can alter a protein's function (ie activation or inactivation)

***Note: Diagram below is not required, it here for reference only***



6. You are working in a cell biology laboratory. You are using a fluorescence microscopy to examine some cells that express your favourite protein fused to green fluorescent protein (GFP). When examining these cells with a fluorescent microscope, you observe something strange. You look at the microscope and you discover that someone has replaced the correct emission and excitation filters that allow observation of GFP with ones that are designed for observation of red fluorescent protein (RFP). What would you observe when examining your cells expressing a GFP-labeled protein with the excitation and emission filters designed for observation of RFP? You should include in your answer the function of emission and excitation filters, whether this will be effective and an explanation of your answer. (**6 marks**)

-No, using the excitation and emission filters for RFP would not work to see GFP

- Excitation filter: eliminates all light other than a narrow range required for excitation of a fluorophore
- emission filter: eliminates all light other than a narrow range that is specifically emitted by a fluorophore
- Using an excitation/emission filter combination for RFP would not allow you to see GFP fluorescence because:
    - the RFP excitation filter would cause illumination of the sample with light other than blue, so there would be little illumination
    - the RFP emission filter would cause any green fluorescent light emitted light from the GFP to be blocked from passing through the filter (since the RFP filter is not transparent to green light)