Name _____

# 7.06 Cell Biology
# Exam #1

This is a closed book exam. You are allowed only two pages of notes, but not computers or any other types of electronic devices.

Please write your answers to the questions in pen (not pencil) in the space provided.

Please write only on the FRONT SIDE of each sheet.

Be sure to put your first and last name on each page in case they become separated.

There are **9** pages to the exam. Make sure that you have a complete copy.

Remember that we will photocopy all of the exams before returning them to you.

**Good luck.**

Question 1. 12 points

Question 2. 12 points

Question 3. 14 points

Question 4. 10 points

Question 5. 14 points

Question 6. 38 points

**Total. 100 points**

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**Question 1 (12 points)**
Green Fluorescent Protein (GFP) is a powerful tool for cell biology. Each of the following is a real variation on GFP that someone has developed. You have generated fusions between these GFP variants and a protein (or proteins) of interest. Briefly describe the nature of the protein's interactions, localization, or other aspects of its properties or behavior that you would be able to test using each variant.

A. In addition to traditional GFP, you have a version of GFP in which the emission and excitation spectra are altered to cause this to appear as a Red Fluorescent Protein. (3 pts)

B. A version of GFP in which a pulse of light at a specific wavelength will convert its emission and excitation spectra from green to red (photoconvertible). (3 pts)

C. A version of GFP that possesses a very slow maturation time for achieving its fluorescence. The protein is produced and folded rapidly, but it takes >20 hours for it to display fluorescent behavior. (3 pts)

D. A version of GFP in which the coding sequence is split into two separate proteins. Neither portion is fluorescence on its own, and they also have low affinity for each other such that they will not associate when expressed at normal levels. However, when the two halves are brought together artificially in close proximity (within 10 Å), they will reform GFP and display fluorescence. (3 pts)

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**Question 2 (12 points)**
In class, we discussed various ways that molecules can cross a membrane. These include channels, transporters, and the nuclear pore, both of which effectively generate a specific and regulated "hole" in the membrane. One key property of both pumps/channels/transporters and the nuclear pore is the directionality of transport. *Below, briefly explain your answers in each case*.

**A.** Provide an example of a situation in which a **pump, channel or transporter** would allow a molecule to pass based on the relative concentrations of that molecule on either side of the membrane. (3 pts)

**B.** Under what conditions (if any) would the **nuclear pore** allow a protein to pass based on the relative concentrations of that molecule on either side of the membrane. (3 pts)

**C.** Provide an example of a situation in which a **pump, channel or transporter** would allow a molecule to pass such that the molecule displayed a highly increased concentration following its transport. (3 pts)

**D.** Under what conditions (if any) would the **nuclear pore** allow a protein to pass such that the molecule displayed a highly increased concentration following its transport. (3 pts)

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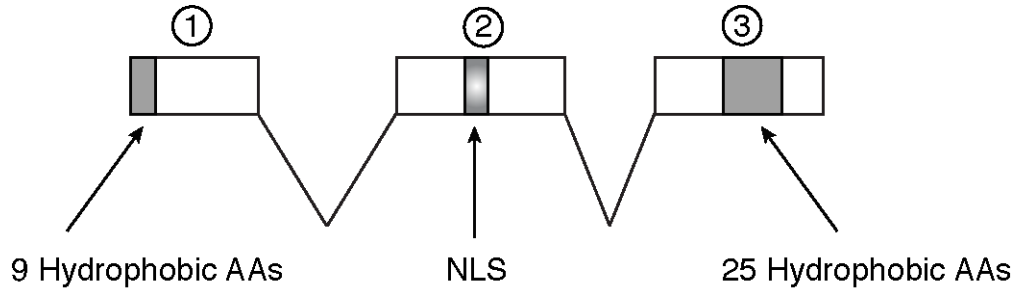
**Question 3 (14 points)**

You are working on a protein that is alternatively spliced. The diagram below shows the three exons (and spliced out introns) for the full-length mRNA (with exons 1, 2, and 3). However, there are multiple versions of this protein that exist with different combinations of these exons. For isoforms lacking exon 1, there are internal start sites that will allow the protein to be produced.




For each of the spliced version listed below, indicate the likely localization of the protein. For membrane proteins, also indicate whether these are Type I or Type II proteins. (2 pts each)

**A.** Exon 1

**B.** Exon 2

**C.** Exon 3

**D.** Exon 1 + 2

**E.** Exon 2 + 3

**F.** Exon 1 + 3

**G.** Exon 1 + 2 + 3

Name _____

**Question 4 (10 points)**
You are using Fluorescence Recovery After Photobleaching (FRAP) experiments to test the turnover of different membrane proteins.

**A.** You first test the turnover of a plasma membrane-localized transmembrane protein (in which you have added a GFP tag to its C-terminus) by bleaching a portion of the plasma membrane. For the wild type protein, you find the t½ for recovery is 3 minutes. You next remove the first 10 amino acids of the proteins (leaving the start codon), which contain the sequence "RLIVLMIIVL". It still localizes to the plasma membrane, but you find instead that its t½ for recovery is 20 seconds. Provide a model that explains the change in fluorescence recovery and the effect of the N-terminal deletion on the properties and behavior of the protein. (4 pts)

**B.** You next test the recovery of a protein that is localized to the ER lumen. You develop a laser bleaching protocol that allows you to specifically bleach the entire ER. Following the photobleaching, the t½ for recovery for ER fluorescence is 45 minutes.

• If you were to mutate (eliminate) the KDEL receptor, would the turnover of your protein be faster, slower, or unchanged? Assume that the KDEL receptor is permanently inactivated at the precise time of your photobleaching. ***Briefly*** explain your reasoning. (3 pts)

• If you were to mutate (eliminate) Sec61, would the turnover of your protein be faster, slower, or unchanged? Assume that Sec61 is permanently inactivated at the precise time of your photobleaching. ***Briefly*** explain your reasoning. (3 pts)

Name _____

**Question 5 (14 points)**

You are studying viral infection of mammalian cells, focusing on RNA viruses. For these viruses to infect a host cell and replicate, a key step is for the viral RNA to enter the host cytoplasm. An intact viral particle contains a protein "capsid" (shell) that is approximately 100 nm in diameter, additional associated proteins, as well as the viral RNA.

**A.** What types of molecules are able to cross the plasma membrane on their own? (2 pts)

You are testing two different viruses (Virus A and Virus B). In each case, you infect the host cell with the virus. After a brief incubation, you then treat cells with the protease trypsin, wash the cells, and then lyse the cells with detergent. For both viruses, you are able to detect the viral RNA in the cell lysate following these steps.

**B.** For Virus A, following these steps, you are able to detect the viral capsid proteins by Western blot of the cell extract. Propose a model by which the virus and the viral RNA is able to enter the host cell. (4 pts)

**C.** For this virus, you find that viral proteins are required for host cell entry and the cell type specificity of its infection. Explain why this is the case. (4 pts)

**D.** For Virus B, following similar steps, you are able to detect the viral RNA, but you are UNABLE to detect any viral proteins by Western blot of the cell extract. However, despite the fact that you do not detect viral proteins in Western blots of cell extracts, you find that entry of the viral RNA requires the function of the capsid-associated viral proteins. Propose a model by which the viral RNA is able to enter into the host cell. (4 pts)

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**Question 6 (38 points)**
You are working to define the order of events during vesicle trafficking in the secretory pathway. For these experiments, you are working in a cell type that is highly secretory such that the majority of the newly synthesized proteins are destined for secretion from the cell.

You have developed a protocol for differential centrifugation that allows you to generate purified populations of nuclei, ER, Golgi, plasma membrane, and vesicles, as well as a cytoplasmic (membrane-free) fraction, and the extracellular media.

**A.** You have obtained a source of radioactive amino acids that you can "feed" to the cells. Following your differential centrifugation procedure, you detect can radioactivity in each fraction. Using this as a tool, describe how you could define the general order of the secretory pathway. At this stage, you do not have access to specific mutants. (4 pts)

**B.** Is this assay a "population-based" assay, a "single-cell" assay, or "single-molecule" assay? ***Briefly*** explain your answer. (2 pts)

**C.** You next isolate a series of mutants in this cell line. In each mutant below, where (if anywhere) would you see an ***accumulation*** of the labeled amino acids relative to control cells? Specify, cytoplasm, nuclei, ER, Golgi, vesicles, plasma membrane, or extracellular (i.e. secreted). Assume that these mutants act relatively fast (i.e., you are looking at primary defect, not secondary consequences). Unless otherwise indicated, assume that the mutant is a loss of function (i.e., you are looking at a temperature sensitive at the restrictive temperature).
(2 pts each)

• A Sar1 mutant in GTP trapped state (the GTPase responsible for ER to Golgi transport)

• A Sar1 mutant in GDP trapped state

• BiP

Name _____

• SRP

• Sec61

• KDEL receptor

• Plasma membrane t-snare

• v-snare for targeting to the plasma membrane

• The Ran GEF (also called RCC1)

**D.** You have isolated three different mutants in the secretory pathway. Mutant A shows an accumulation of amino acids in the Golgi. Mutant B and Mutant C both show an accumulation of labeled amino acids in vesicles. However, in the double mutants, Mutant A + Mutant B displays an accumulation of labeled amino acids in vesicles whereas Mutant A + Mutant C displays an accumulation in the Golgi. What does these data suggest about the potential role of each protein and the order of these proteins within the secretory pathway? (6 pts)

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**E.** For each of the following cellular trafficking events, indicate whether this event is reversible or irreversible. In either case, indicate what ensures the directionality of this event. (2 pts each)

• Nuclear import

• Trafficking from the ER to the Golgi

• Insertion and translocation of a protein into the ER

• Vesicle coat formation