INSTRUCTIONS

This is a 1 week long, open-book, take-home exam consisting of 4 multipart questions. We will put a pdf version of the exam on the website (in the lecture note section) in case anyone loses the hard copy.

Please read and follow these instructions carefully.

- ***PLEASE DO NOT DISCUSS THE QUESTIONS OR ANSWERS WITH ANYONE.*** *You are expected to generate the answers on your own with open access to any written resources. Unlike with the proposal, discussion with classmates, instructors, labmates, or anyone else is not allowed. If you need clarifications or have questions, email jais@u.washington.edu and bthielen@u.washington.edu. Jais will answer your questions by emailing the whole class, so that everyone has access to the same information.* ***We request that you email us your questions rather than asking us about in person, so that we can share the answer or discussion with the entire class.***

- ***Please read the questions carefully****. It is very easy to generate the wrong answer because you did not read the question carefully. Approach the answers logically; and don't make them harder than they are. Note that there are sometimes multiple correct answers to these questions.*

- ***You should not need to go to the primary literature in order to answer the questions.*** *The questions only require an understanding of textbook material, lecture notes, and assigned papers as well as discussions and issues in class. These questions emphasize integrating data and experimental design with your knowledge of material covered in class. For some parts of the exam a quick but brief literature search could make you more confident of your answer. But if you find yourself spending hours searching the literature, you are likely on the wrong track.*

- ***Answers should be typed, 12-point, single-spaced, and printed****. You do not need to include the questions; just label your answers 1A, 1B, 1C, etc. Approximate length for each answer is specified to help guide you with your answer. However, note that less is generally better, so be concise! Diagrams are encouraged and in some cases required, and can be handwritten or digital, but must be legible.*

- ***Instead of putting your name on the examination, use the last four digits of your student identification number.*** *This will allow us to grade your examinations blindly to ensure fairness.*

- ***The Last 4 digits of your student ID number should be on ALL pages, and pages should be numbered. Do NOT put your name on your exam.***

- *The mid-term is due on Thursday February 5th, at the START of class.* ***Please turn in a typed hard copy of the answers. Do not email your exams to us.***

- *The total number of points in this exam = 100 (30% of final grade).*

Midterm Question 1 (25 points)

Your lab is studying a new herpesvirus. By means of a screen, you recently found that viral gene P is necessary for your virus to replicate *in vivo* in an animal model but is dispensable in cell culture, and you wish to further characterize this gene. When you examine the predicted amino acid sequence, you observe two hydrophobic regions (2 and 4, see Q1 Fig. 1, length of segments are drawn to scale). To understand the function of the protein, you first want to determine its localization in the cell.

Q1 Fig. 1:

1A) Describe an experiment that you could perform to determine the localization of P in the cell, including a description of important controls. Assume you have successfully cloned the gene for P and you have a rabbit polyclonal antiserum that recognizes the P protein (One paragraph, 5 points)

Your preliminary studies suggest that the P protein is expressed in the ER. You examine the sequence and find a motif that you hypothesize may be important for its localization to the ER.

1B) What motif would you look for and how would you predict it would function? (One or two sentences, 2 points)

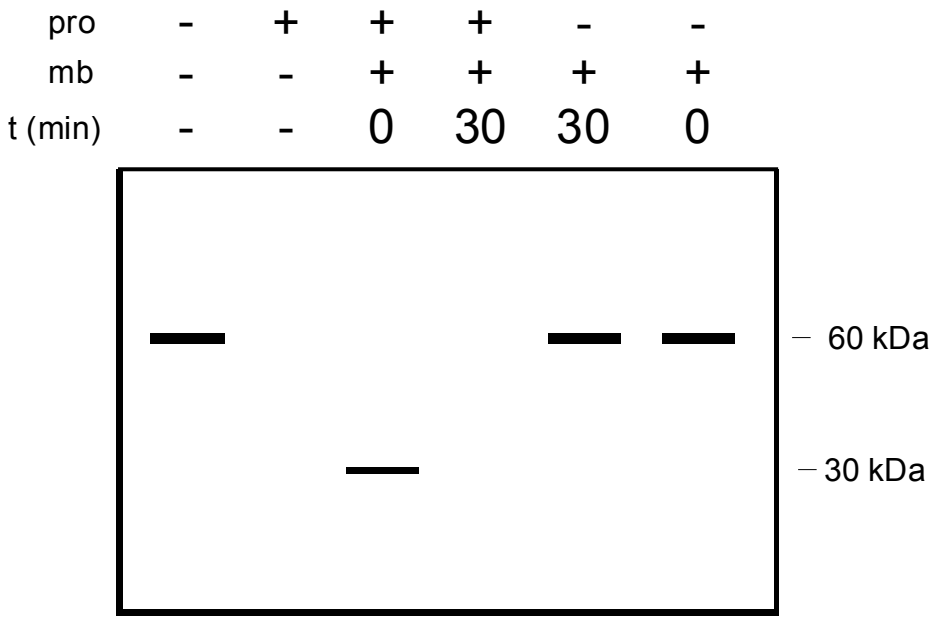
1C) How would you test whether this motif is *necessary* for ER localization? Describe an experiment you would perform and how you would interpret your findings (One paragraph, 4 points)

1D) How would you test whether this motif is *sufficient* for ER localization? How would this differ from testing whether it is necessary? (One paragraph, 4 points)

Given its localization to the ER and its role during *in vivo* infection, you hypothesize that the protein may be involved inhibiting translocation of viral peptides across the ER membrane for antigen presentation (more about this later in the course). To study this, you decide to develop an *in vitro* system for studying protein P at membranes. In your first experiment, you use a "cell-free system" to translate P in the presence of ^{35}S methionine and analyze the translated material by SDS-PAGE followed by autoradiography. The results are shown below in Q1 Fig. 2:

Q1 Fig. 2

mb = ER membranes
t (min) = interval between initiation of translation and addition of membranes
pro = protease added at the end of translation reaction



1E) Based on these data, draw a cartoon illustrating the topology of P relative to the ER membrane. Be sure to clearly label both the ER lumen and cytosol in your diagram. (cartoon, 5 points)

1F) In the course of your studies, you generate a form of P that no longer contains the motif important for ER localization. Assuming no other specific sorting signals, where would this version of P localize when expressed in cells and why? Draw a cartoon to illustrate this, again clearly labeling appropriate compartments. (One or two sentences + cartoon, 5 points)

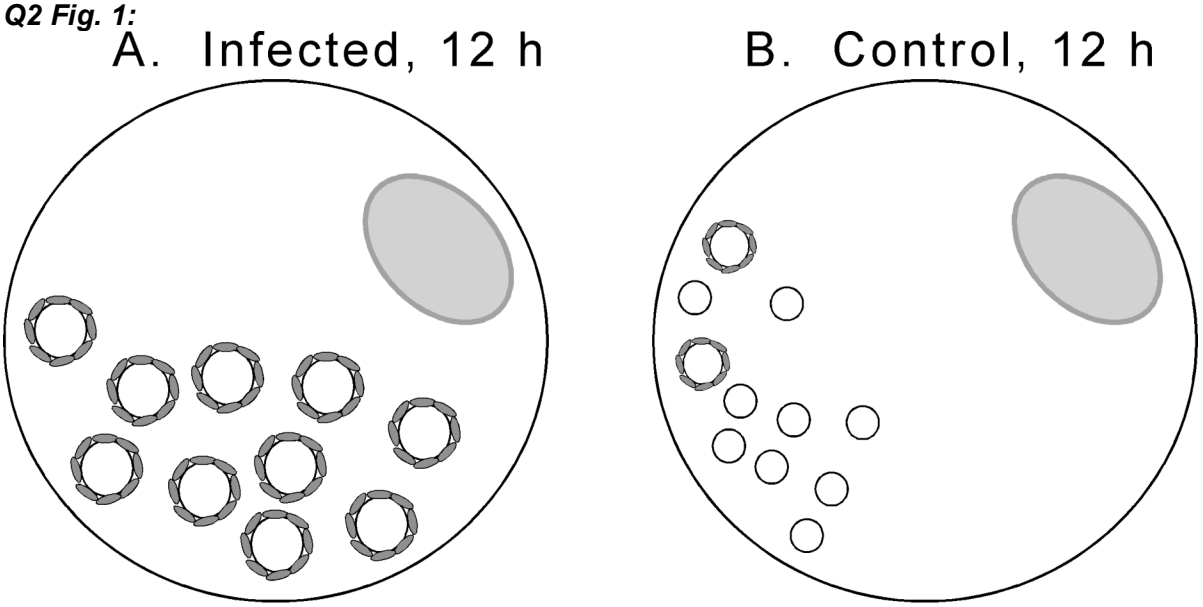
Midterm Question 2 (25 points):

You are a new postdoc in a lab studying a recently identified bacterial pathogen, *Studentis sufferus*. This organism is able to remain hidden inside host cells, eventually causing significant cell death. How the bacterium survives inside the cell is not known; nor is it known how it causes cell death.

The postdoc who worked on this organism before you demonstrated that when *S. sufferus* first contacts a mammalian cell, it uses a type III secretion system to inject the a toxin (Suffero toxin) into the host cell. (David Sherman will be lecturing soon on bacterial secretion mechanisms like type III secretion systems; however, for the purposes of this question you simply need to know that the bacterium makes a syringe like apparatus that allows it to inject bacterial protein effectors into the cytoplasm of the host cell.) After injecting Suffero toxin into the host epithelial cell, the bacterium is endocytosed through a clathrin-mediated pathway.

Your project is to figure out what the Suffero toxin does. You start by using transmission electron microscopy with negative staining to examine mammalian epithelial cells at different times after infection with *S. sufferus*. What you see is shown in Q2 Fig. 1:

Q2 Fig. 1:



You conclude that endocytic vesicles in the infected cell are larger but also morphologically abnormal. You have Suffero toxin encoded on a plasmid, so you decide to transfect cells with vector alone versus Suffero toxin plasmid. You see that Suffero toxin alone gives you the same pattern as in Q2 Fig. 1A (while vector alone gives you the pattern seen in Q2 Fig. 1B). You also have an antiserum to Suffero toxin, so you use that to confirm that the toxin is expressed upon transfection.

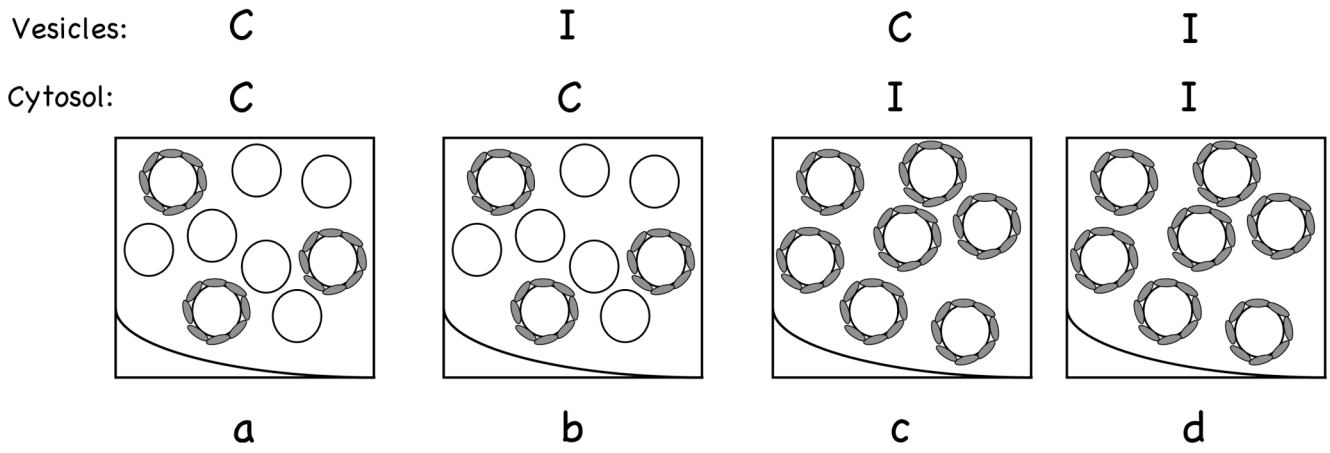
2A) Propose a hypothesis for what Suffero toxin does based on the results of your experiments. (One or two sentences, 3 points).

You perform the following experiment:
You isolate endocytic vesicles from control cells and infected cells. You also isolate cytosol from control cells (C) and infected (I) cells. You mix vesicles from control cells with cytosol from control vs. infected cells; you also incubate vesicles from infected cells with cytosol from control vs. infected cells.

2B) What question will this experiment test? (One sentence, 3 points)

When you perform this experiment, you obtain the results shown in Q2 Fig. 2:

Q2 Fig. 2:



2C) What do you conclude from the data in Q2 Fig. 2? (one short paragraph, 3 points)

2D) Given these findings and assuming that Suffero toxin acts on only ONE factor, state which factor you think Suffero toxin acts on and describe how your model explains the observed results. (one or two paragraphs, 5 points)

2E) Explain how your model for Suffero toxin action explains how S. sufferus evades host defenses (two or three sentences, 3 points).

2F) Describe two additional experiments that will BEST test your model. Both experiments should utilize the general experimental set up described in Fig. 2 and tools that you either know you have or that are expected to be commercially available (i.e. don't propose to use complicated approaches). EXPLAIN EXACTLY HOW YOU WILL INTERPRET YOUR EXPERIMENTS. (Less than half a page, 6 points).

Before setting up your experiment in Fig. 2, you performed some controls to make sure your purified endosomes and lysates were functioning properly. In one of these experiments, you added a large excess of a non-hydrolyzable analog of GTP. This analog binds properly, but cannot be hydrolyzed to GDP because of a modification in its structure.

2G) How would you expect control vesicles incubated in control lysate to look after you incubate with the non-hydrolyzable GTP analog? (One sentence, 2 points)

Midterm Question 3 (35 points):

You are studying trafficking in cells infected by a newly identified viral pathogen that infects T cells. You have just obtained antisera against four of the proteins encoded in this virus, W, X, Y, and Z.

Your student validates the antisera. When he infects cells with the virus and performs indirect immunofluorescence on cells at 48 hours using a primary antibody against W, he finds Protein W localized at the plasma membrane. Your student makes tagged constructs and examines their localization. When he puts either an HA or myc tag on the N terminal end of Protein W and expresses tagged W in the context of the whole viral genome, he finds W in the cytoplasm. However, when he puts either an HA or myc tag on the C-terminus of protein W and expresses that in the context of the whole viral genome, proper plasma membrane localization occurs. Your student concludes that the protein must be an integral membrane protein.

3A) What was the student's reasoning in coming to this conclusion based on his experimental findings? (One paragraph, 3 points)

To see if his conclusion makes sense, you go back to the sequence for protein W predicted from the cDNA and observe that the first 15 amino acids starting with the initiating methionine are predicted to be:

MGARKKRLNSKVIRD

3B) Based on the sequence information, do you agree with your student's conclusion? What information in the sequence supports your answer? (One paragraph, 4 points)

To help resolve this controversy, you ask your student to make two additional constructs. The first consists of the first 10 amino acids (shown above) fused onto the N terminus of GFP. Your student finds that adding these 10 amino acids alone to GFP causes GFP to localize to the plasma membrane.

3C) Given these data and the sequence of the N terminus, what is your hypothesis about how protein W traffics to the membrane (be specific)? What aspects of the data support your hypothesis? (On paragraph, 5 points)

3D) You ask your student to make one more mutant to more definitively test your hypothesis? What is the mutant you want him to make? (One sentence, 4 points)

Until now, your student has always expressed W along with all the whole virus. Interestingly, when he expresses W alone without any tag, he finds it localized in the cytoplasm. He hypothesizes that another viral protein is needed for plasma membrane targeting. So he systematically tests this hypothesis using constructs with deletions and demonstrates that expression of X is required to target W to the plasma membrane.

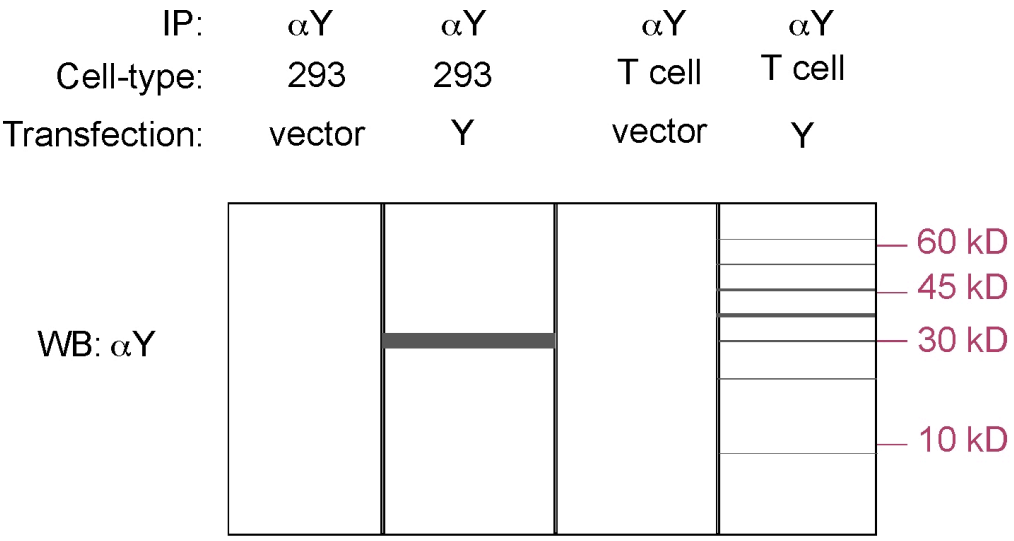
3E) What is your hypothesis for how X changes that topology of W, based on these data? (One paragraph, 5 points)

Another student in your lab is studying protein Y. She finds that when protein Y is expressed in a 293 cells (epithelial-derived human kidney cell), it is localized to the ER by indirect

immunofluorescence with an anti-Y antibody. However, when it is expressed in a human T cell, it is localized primarily in the cytoplasm.

You ask her to repeat the experiment, denature the lysate, and immunoprecipitate Y with anti Y antibody from the denatured, diluted cell lysate. She separates proteins the eluted proteins by SDS-PAGE, transfers to nitrocellulose, and western blots with anti-Y antibody expecting to see the 30 kD Y protein. Q3Fig. 1 shows the result of that experiment.

Q3 Fig. 1:

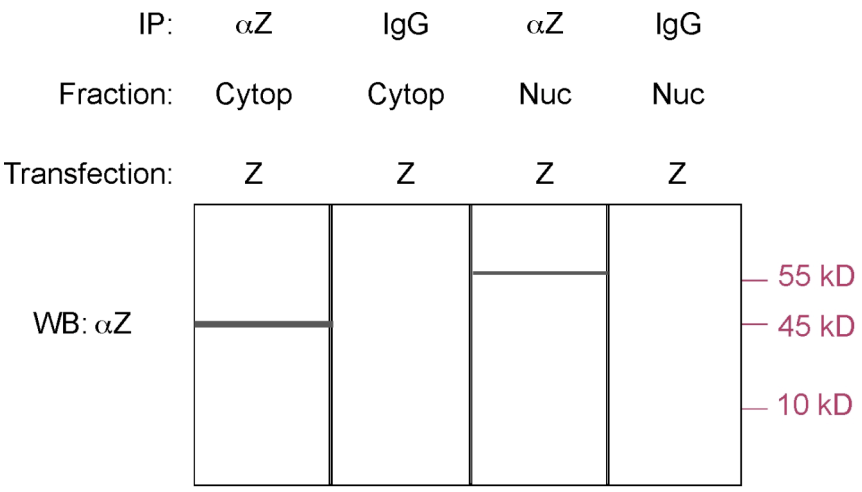


3F) Taking into account what is known about mechanisms for how viruses evade hosts, generate a hypothesis for how Y is trafficked to the cytoplasm in T cells. Your hypothesis should take into account all the data shown in Q3Fig. 1. (One paragraph, 6 points)

3G) Speculate on why the Protein Y might undergo the trafficking that you described in your answer to 3F (i.e. what might be going on that could be beneficial to the virus?). (One paragraph, 3 points)

This student is also studying Protein Z. When Z is deleted from the virus, no viral replication occurs, so Z appears to be essential. Z is present in the cytoplasm and in the nucleus. Your student transfects Z into 293 cells, separates nuclei and cells by centrifugation, denatures each fraction, and performs an immunoprecipitation on each fraction using anti-Z antibody or normal rabbit IgG. Q3Fig. 2 shows the result of her experiment. In addition, she demonstrates in a separate experiment that the N-terminal half of protein Z localizes to the nucleus and cytoplasm, as is the case for wild-type Z, but the C-terminal half is localized only to the cytoplasm.

Q3Fig. 2:



3H) Present a hypothesis for trafficking of Protein Z into the nucleus that takes account of all the data for Protein Z and known mechanisms of nuclear trafficking.
(One paragraph, 5 points)

Midterm Question 4 (15 points):

You have a friend who is studying RNA expression of the virus described in Question 3. The virus makes 4 spliced transcripts for expression of early genes, and one unspliced full-length genomic RNA that is used for expression of late genes (which are not encoded on the spliced transcripts). Your friend tells you about three new pieces of data:

1) He has discovered that the unspliced transcript is not expressed if the 100 bp at the 3 prime end of the full length RNA (called region A) is deleted.

2) He also encoded stop codons into various viral genes and discovered that expression of one early protein, E1, is required for expression of all the late proteins.

3) Leptomycin B has no effect on expression of either early or late viral genes.

4A) What controls do you want to make sure your friend has done when he tells you the results of the Leptomycin B experiment? (One or two sentences, 5 points).

4B) Describe a model for export of spliced and unspliced RNA for this virus. Your model should be the simplest one that is consistent with current thinking about RNA export and also explains all of the data. For all trafficking events in your model, state whether you hypothesize that a specific trafficking pathway is used and what your evidence is for the putative pathway. If you lack enough information to speculate about a pathway, indicate that as well. (One or two paragraphs, 5 points).

4C) What is the ONE MOST DEFINITIVE experiment that your friend could perform to identify (or confirm) which nuclear export pathway is used by the unspliced genomic RNA? (One or two sentences, 5 points)