Supplemental Files A Course syllabus

NE- Introduction to Cellular and Molecular Biology

Course Instructor/Lab Director:
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Required Text: Essential Cell Biology, 4th edition (2013), by Alberts et al., (Garland Science Publisher).

Course description: This course is an introductory course on the cellular, molecular and developmental biology of mammalian (eukaryotic) cells. It covers the basis of the mechanisms that regulate cell viability and functions, which include cell division (mitosis) and death (apoptosis), DNA replication, RNA transcription and protein translation, intracellular organelles and membrane trafficking; systems of cell communication such as signal transduction, action potentials, active and passive transport. The textbook Essential Cell Biology is central to the development of this part of the course. During the course, students will also approach how basic concepts of cell and molecular biology may apply to the study of neurodegenerative diseases, in particular in Alzheimer's disease. How proteins can become toxic and potentially lead to neuronal death? What turns a molecular mechanism from physiologic to potentially dangerous to the neuron? This approach will be supported by the reading of scientific articles inherent to these topics, both in class and at home (assignments).

Learning Goals:

- To gain a broad perspective on the mechanisms that regulate cell function and life.
- To apply in the lab concepts of molecular and cellular biology learned in the lecture.
- To learn the importance to connect information in order to develop a broad view on a complex situation (cell function or a particular molecular mechanism).
- To experience a research approach in the field of health/life science.
- To develop the ability to read scientific literature.
- To understand how research articles impact our knowledge, stimulate our critical thinking and are necessary to the advancement of biomedical research.
- To develop the capability to identify specific aspects of molecular mechanisms crucial to determine a physiologic versus a pathologic scenario (a point mutations can change a protein's localization and function).
- To understand how proteins are the effectors of cell functions and how we can study them.
- To understand and apply specific techniques for the study of distinct molecules and their modifications.

Grading:

Lecture and lab components of the course will independently contribute to your final grade. The lecture component will contribute 60% of your final grade, whereas the Lab component the remaining 40%.

Scores will be divided as follows:
-*Lecture exams (2 midterms and one final):* 20% each exam (total 60%).
-*Pre-Lab Quiz:* 10%.

-Lab home assignments (answering questions on 3 assigned readings, and writing a report of your lab project in the form of a research article): 30%.

Importantly, STUDENTS MUST EARN A PASSING GRADE **IN BOTH** THE LECTURE AND LAB COMPONENTS OF NE TO EARN A PASSING GRADE FOR THE COURSE. Lecture exams will be administered during regular class hours, and are based on answering both multiple choice, true-false and short answers.

Lecture schedule

Week 1. No Lab.
Introduction to the course: learning objectives and approaches. Basics concepts of cell and molecular biology: the eukaryotic cell, intracellular organelles and their function. *From the book:* Brief description of cellular components, DNA, proteins and lipids (Chapter 1). *What we'll be doing in the lab:* Description of lab project, rationale and experimental approach. Introductory concepts on protein cloning and expression. DNA amplification by Polymerase Chain Reaction and generation of cDNA.

Week 2.
What we'll be doing in the lab. Rationale and Background: the Beta secretase BACE, the amyloid Precursor protein APP and Alzheimer disease. Assignments.
From the book: Chemical Bonds (Chapter 2). Protein Structure and function (Chapter 4).

Week 3.
What we'll be doing in the lab: Description of a mammalian expression vector (plasmid). How to correctly place amplified cDNA (insert) into a vector. Restriction enzymes and DNA digestion. Hints on protein misfolding in neurodegenerative diseases.
From the book: DNA structure and Chromosome (Chapter 5). DNA Replication (Chapter 6).

Week 4.
What we'll be doing in the lab: How to correctly place amplified cDNA (insert) into a vector. DNA Ligation. Preparation of samples for DNA ligation.
From the book: From DNA to protein: transcription and translation (Chapter 7).

Week 5.
What we'll be doing in the lab: Amplification of plasmid cloned DNA in E. Coli, Transformation. Selection of bacterial colonies.
From the book: Control of gene expression (Chapter 8).

Week 6. NO LAB
From the book: Analyzing genes and genome: Techniques to identify and study mRNA, DNA, and proteins (Chapter 10).
Recap. Q&A for first midterm exam.

Week 7.
What we'll be doing in the lab: Extraction of plasmid DNA from bacteria and verification of the presence of cDNA in the vector. **Midterm Exam.**
From the book: Membrane structure (Chapter 11).

Week 8.
What we'll be doing in the lab: In vitro transcription and translation.
From the book: Membrane transport (Chapter 12). Intracellular organelles and protein trafficking part 1 (Chapter 15).

Week 9.
What we'll be doing in the lab: BACE expression in mammalian cells/transfection. Protein separation in SDS-PAGE.
From the book: Intracellular organelle and protein trafficking part 2 (Chapter 15).

Week 10.
What we'll be doing in the lab: Western Blot: Identification of BACE using specific antibodies. Preparation of cell lysates from cells overexpressing AD related APP, and overexpressing the cloned BACE mutants. Evaluation of protein concentration.
From the book: Mitochondria, Energy and Metabolism (Parts of Chapters 13,14).
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Week 11.
What we'll be doing in the lab: separation of proteins in SDS-PAGE and transfer to nitrocellulose.
From the book: Cell signaling (Chapter 16).
Recap. Q&A for second Midterm.

Week 12.
What we'll be doing in the lab: Western Blot (WB) of BACE and APP, and evaluation of APP processing. Immunocytochemistry: fixing living cells and staining proteins of interest with specific antibodies.
Second Midterm Exam.
From the book: Cytoskeleton. (Chapter 17). Neuronal cytoskeleton: the case of tau.

Week 13.
What we'll be doing in the lab: Evaluation of BACE intracellular localization/trafficking by means of immunocytochemistry: analysis using a fluorescence microscope. Different effects of BACE mutants. Data analysis of BACE and APP Western Blot from transfected cells.
From the book: Mitosis/cell division. Control of cell growth: Apoptosis and cell death (Chapter 18). How apoptosis could regulate BACE stabilization in Alzheimer's disease.

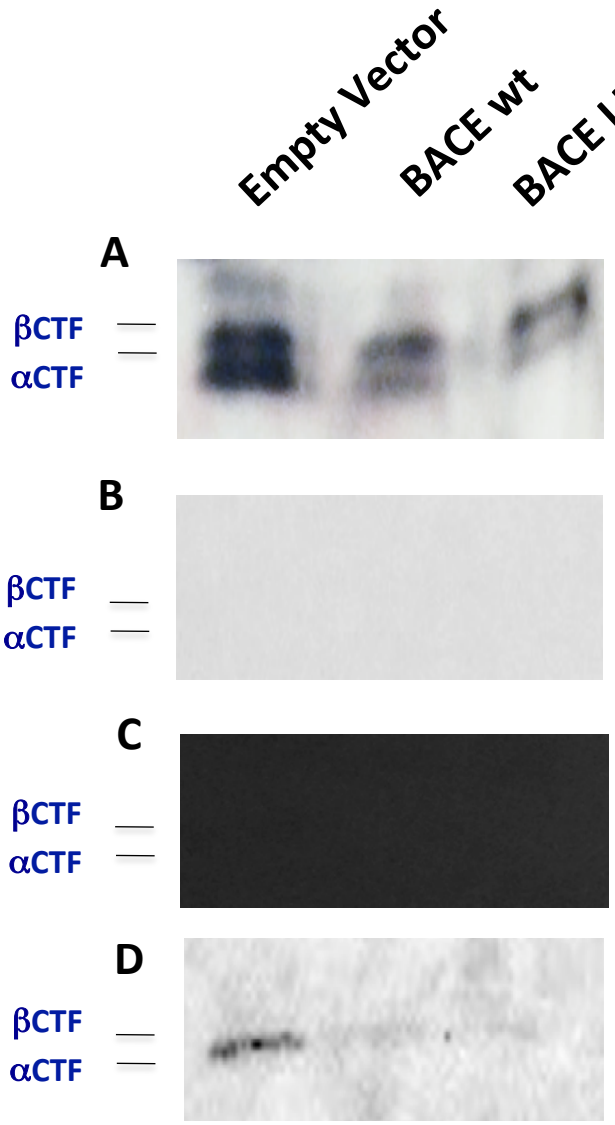
Week 14. No Lab
From the book: Meiosis (Chapter 19).
Recap on the Assignment: Discussion of the lab project.

Week 15.
From the book: Mendelian inheritance (Chapter 19).
Recap. Q&A for Final Exam.

List of Research Articles for the Assignments
i) Cold Spring Harb Perspect Med. 2012 May;2(5):a006270. Trafficking and proteolytic processing of APP. Haass C, Kaether C, Thinakaran G, Sisodia S. (pages 1-7).

ii) Mol Cell Neurosci. 1999 Dec;14(6):419-27.Identification of a novel aspartic protease (Asp 2) as beta-secretase. Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C, Christie G.
iii) The carboxyl-terminus of BACE contains a sorting signal that regulates BACE trafficking but not the formation of total A(beta). Pastorino L, Ikin AF, Nairn AC, Pursnani A, Buxbaum JD. Mol Cell Neurosci. 2002 Feb;19(2):175-85.
iv) J Neurosci. 2009 Oct 14;29(41):12787-94. The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. Vassar R, Kovacs DM, Yan R, Wong PC. **OPTIONAL.**

Supplemental Files B Addressing Expected and Unexpected Results



Expected outcome

Are the levels of βCTF accumulating in presence of BACE? Why are the levels of αCTF concomitantly decreasing? Are these data consistent with the hypothesis that BACE is the beta secretase, and with the data reported in the literature? From a technical standpoint, which steps were critical in the development of this successful experiment?

Unexpected outcome: Scenario #1

No result: did antibodies work properly? Can you recap the steps of the Western Blot?

Unexpected outcome: Scenario #2

No result: why are bands not visible? Why is the background so high? Can you recap the steps of the Western Blot?

Unexpected outcome: Scenario #3

Only αCTF, but not βCTF is visible. Could this be a problem of the Western Blot technique or could this be due to different overall protein content in each lane? How did your transfer look like before Western BlotB? Was overall protein load comparable?

Figure Legend:
This figure represents possible results from the Western Blot experiment carried out during the semester. Scenarios #1-3 represent common experimental outcomes, different from expected results, that our students might obtain at the end of the semester. Each and every of these scenarios serves as a cue to discuss each steps of the experiment, to identify and discuss potential pitfalls.

For each of the scenarios included in the figure and related to unexpected/unsuccessful results, the Lab Instructor will encourage the students to think about the steps of the experiment, when possible to assess which of the steps might have failed (was the protein concentration in each of their sample in the range needed for a successful Western Blot? Or was it the protein transfer that did not work as visible with an uneven Ponceau staining? Did they forget to carry out some of the steps of the Western Blot?). In this way, students will virtually in their mind repeat the steps of the experiment, recall the rationale of each steps and function of the reagents. This will be a great opportunity for them to reconsider their experiment, approach, rationale and techniques.

A This frame is representative of a successful experiment in which the two C terminal fragments are well separated and detected by means of SDS-PAGE/Western Blot. Normally and in the cell line used for the experiment, both fragments can be visible, with αCTF being more abundant than βCTF (Lane empty vector). The overexpression of forms of BACE wt (wild type) or LL/AA mutant is associated with increased production of βCTF, reducing the levels of αCTF. This is due to the fact that BACE (either wt or LL/AA) acts as the enzyme that cleaves the precursor protein APP generating βCTF. This is strongly supported in the literature and is one of the fundamental steps that lead to Alzheimer's disease pathology. The detection of both a and βCTF and the relative ratio of these fragments secondary to BACE expression prove that the experiment was successful both from a technical and a conceptual point of view. This is typically what students would expect to obtain as the result of their experiment.

B In Scenario #1, the Western Blot shows the same background as in frame A, however proteins of interest are not detected. This could be due to a series of technical factors, including malfunctioning of reagents, insufficient protein level in each of the lanes, unsuccessful protein transfer (that could be assessed by Ponceau staining of the membrane before the use of the antibody-data not shown), and other reasons.

C In Scenario #2, the Western Blot shows incredibly high background. This is normally caused by inappropriate execution or use of the reagents in the Western Blot, for example this can happen when the blocking step was carried out inappropriately or not at all, or when washing between antibodies was ineffective.

D In Scenario #3, the Western Blot technically worked (one of the two fragments are visible), however we can detect only αCTF but not βCTF. This could be secondary to overall low protein amount loaded in each well, or to inefficient protein transfer.

Supplemental Files C Content and Lab Skills Assessment Questions

For copyright purposes, only questions developed by the authors are presented here.
Notes: Questions were multiple choice with one correct answer. For analysis, correct responses were graded as "1" and incorrect responses were graded as "0".

Molecular biology preassessment

We are interested in your current level of understanding of some biological concepts. This will not be part of your grade. This assessment allows us to understand what you know at the beginning of the course so we can gauge what you learn from this course.

Note: The questions below are from previously published work, thus the questions are not reproduced here. A copy of the original survey can be requested directly from the author at Meredith.m.thompson@gmail.com.

Part 1: Concepts
1. Question about polar and non polar regions and what happens when they dissolve in water.

2. Mutations that lead to misfolding of proteins in Alzheimer's Disease.

3. Type of bonding between two non polar amino acids.

4. Protein folding in alpha helices and beta pleated sheets.

5. Fitting molecules of saturated and unsaturated fat.

6. Comparing monosaccharides and RNA to other macromolecules.

7. The main features of DNA

8. Contrasting RNA and DNA

9. Commonality between DNA and RNA

10. ADP becoming ATP

11. Role of oxygen in the process of oxidation of glucose

12. Makeup of chromosome before mitosis

13. Number of molecules of DNA present in nucleus during anaphase

14. Phase in which DNA is replicated

15. Sperm genotypes of two cells

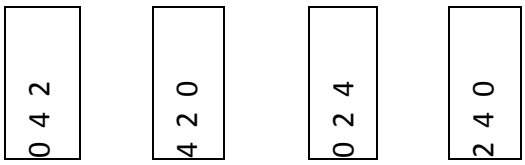
16. Chromosome make up of a cell that has not crossed over

Part 2: Laboratory skills and techniques

17. You have to add a volume of 45µL to your sample. Which micropipettor would you choose?
A) 1000 µL
B) 200 µL
C) 20 µL
D) 2 µL

18. You've selected a 1000µL micropipettor to add a volume of 420µL to your tube. The numbers on the micropipettor window should read:

a. b. c. d.



19. Which technique would you choose to introduce proteins into cells?
A) gel electrophoresis
B) recombinant DNA
C) immunoblotting
D) fluorescence microscopy

20. Which technique would you choose to separate DNA or proteins by size?
A) gel electrophoresis
B) recombinant DNA
C) immunoblotting
D) fluorescence microscopy

21. Which technique would you choose to identify which proteins are present in the sample?
A) gel electrophoresis
B) recombinant DNA
C) immunoblotting
D) fluorescence microscopy

22. Which technique would you choose to determine where proteins localize in cells?

A) gel electrophoresis followed by immunoblotting
B) transfection with GFP fusion plasmid
C) gel electrophoresis followed by Coomasse Stain
D) transfection with GFP fusion plasmid followed by immunoblotting

23. Green fluorescent protein, or GFP, is an important visualization tool. The method scientists use to "tag" GFP to a protein of interest is
A) insert the gene for protein of interest into plasmid adjacent to gene for GFP
B) transfect cells with a GFP plasmid and a plasmid with your gene of interest
C) conduct immunoblotting for protein of interest
D) make cell lysates from GFP transfected cells

24. An immunoblot assay uses the following biological molecule
A) lipids
B) carbohydrates
C) antibodies
D) receptors

Part 3: Goals and demographic questions

25. What are your learning goals for this course? (Blank space is removed here for brevity)

26. Gender (circle one): male female

27. Please select one or more of the following racial categories to describe yourself:
_ American Indian or Alaska Native
_ Asian
_ Black or African American
_ Native Hawaiian or Pacific Islander
_ White
_ Prefer not to respond

Do you consider yourself to be Hispanic or Latino?
__Yes
__No

28. Current projected date of graduation: _____

29. Current or intended major:_____

30. After I graduate from college, I intend to (check one):

__ Continue onto graduate school for a (circle one) Masters PhD
__ Continue onto medical school for a (circle one) MD MD/PhD

__ Find a job
__Other (please explain)

_____

31. Comments or questions for us?

Results for BI and NE for content questions

Table A. Percentage correct for pre and post content questions for NE and BI

	NE pre	NE post		BI pre	BI post
RNADNA	6%	6%	RNADNA	92%	3%
mix	30%	22%	mix	5%	96%
model	25%	30%	model	25%	35%
DNA	22%	41%	DNA	4%	6%
poly	67%	52%	poly	5%	6%
polar	55%	59%	polar	72%	80%
germ	63%	77%	germ	5%	5%
photo	58%	80%	photo	32%	45%
addvol	76%	82%	addvol	5%	4%
RNA	60%	83%	RNA	60%	78%
mit	6%	91%	mit	28%	68%

Supplemental Files D Figure Assignment

Figure Assignment: Organizing the Figures of the Research Article

Objective:

This assignment is designed to make the student elaborate on the logical thread that is followed when experiments are presented in research articles. The idea is to help them develop their critical ability to analyze a research article, linking the results reported and identifying the key experiments that support the major findings.

Rationale and Design

Students will be provided of the sole title of the article (normally stating the major goal of the manuscript), and of all the figures and figure legends. However, they will not have any information relative to the sequence of the figures in the manuscript (figures will be in a random order), and they won't have access to the full text of the manuscript.

Based on the title and the data represented in each figure, we ask the students to "put the figures in the right order" and to explain the rationale of their choices. Which is figure 1? Which is figure 2? Which is the last figure of the manuscript? Because the figure legends report a brief title descriptive of the outcome of the experiment shown, we think that they will have the tools needed to analyze the manuscript using only the figures. In the assignment, the figure title is reported as the title of each slide.

Normally, in a research article data are presented following a specific logical thread: "key experiments", those ones that prove the authors' hypothesis (and whose outcome is acknowledged in the title) are normally shown last. These experiments are normally showing the functionality of molecules or processes inside the cell, and their relevance in a certain biological scenario. All the experiments that contributed knowledge and generated the questions leading to such key experiments are considered as building blocks and are shown first. They are normally identified as those experiments that allow the "characterization" of the biological system employed.

By asking the students to place the figures in the right order, we will ask them to identify and distinguish among the experiments proposed which one contributed to the key findings and which ones allowed the characterization of the system employed. In this way, we hope that they can understand the logical thread at the basis of a scientific article, and how knowledge is built in scientific research.

In this assignment, the only parts of the research article that we hand out are:
-The title
-The figures with the figure legend, in a random order
-A description of some of the reagents used in the study

We do not hand out:
-The full text of the article
-The number of the figures

With this approach, we hope to engage the students' ability to distinguish the relevance of each single experiment employed in the article, and how it contributes to the development of a scientific project.
Of note, the article we use nicely overlaps with our lab project, both from a conceptual point of view and from a technical perspective (we employ in the lab similar approaches, and the same recombinant DNA for BACE), therefore stimulating the students to think logically about this research article is also beneficial to their understanding/critical thinking of the lab project.

Pre- and post- assignment
We designed two assignments: a "pre-assignment" was taken by the students around week 8 of class and was based on the reorganization of all the figures of the research article "BACE is degraded via the lysosomal pathway", Koh YH[1], von Arnim CA, Hyman BT, Tanzi RE, Tesco G. J Biol Chem. 2005 Sep 16;280(37):32499-504. A post-assignment was designed for a bonus question offered during the final exam at the end of the course. In this case, students had to re-organize only part of the figures from the article Intracellular itinerary of internalized b-secretase, BACE1, and its potential impact on b-amyloid peptide biogenesis.Chia PZ, Toh WH, Sharples R, Gasnereau I, Hill AF, and Gleeson PA. Traffic. 2013 Sep; 14(9): 997-1013.

1-Evaluation of the pre-assignment:
The article by Koh et al that we used for this assignment studies BACE degradation pathway and is essentially divided into three parts:

1- The first part focuses on the use of pharmacological treatments to understand which could be the pathway responsible for BACE degradation. Experiments shown in *Figure 1* use the same approach and rationale of the experiments shown in *Figure 2*, with the only difference of the cell model employed. For this reason, we consider these figures comparable in terms of the order they should be arranged with.

2-The second part focuses on the study of post-translational modification of BACE, as a result of the use of specific pharmacologic treatments that were selected based on the two previous experiments. *Figures 3 and 4* show these modifications (maturation and shedding/secretion). Because these experiments could be independent on each other, we consider these figures comparable in terms of the order they should be arranged with. We decided not to include these Figures in the evaluation of the assignment, since these experiments covered aspects of protein modifications that we did not discuss in class.

3-The third part focuses on the study of BACE intracellular localization, also depending on the use of specific pharmacological approaches, already studied in previous figures. Experiments are shown in *Figures 5 and 6* and employ approaches of immunocytochemistry, using specific organelle markers and BACE antibodies. Because pharmacological regulation shown in *Figures 1*

and 2 helped understand the specific intracellular organelle where BACE could be degraded, therefore where BACE could be found in the cell, it is a rationale approach to show these figures last. Because these last two experiments could be independent on each other, we consider these figures comparable in terms of the order in which they should be arranged.

2-Evaluation of the post-assignment

We offered this assignment as part the Final Exam in the form of a Bonus Question. The article by Chia et al that we used focuses on the study of BACE intracellular localization (trafficking) as a determinant factor that can directly influence BACE activity on the specific substrate Amyloid Precursor Protein (APP) causing Alzheimer's Disease. We chose this article because it is closely linked to the topic of the lab project and therefore the students had at this point all the tools to understand the significance of the experiments shown. In facts, the lab project focused on the molecular and cell biology of the protein BACE, focusing on the generation of recombinant BACE and on the analysis of BACE enzymatic activity and localization. In addition, this study gives the opportunity to learn how the application of the concepts and approaches covered in the lab project help reach a deeper understanding of the molecular biology of this disease.

Given the time limitation due to the exam hours, we decided to include in this assignment only three out of the nine figures shown in the article. We chose Figures 1, 6 and 2 which we renamed on the exam as X, Y and Z, respectively.
Figure 1 (X) analyzes the intracellular distribution of BACE, without discriminating the pool of newly synthesized BACE from the pool of BACE recycling from the plasma membrane. Figure 6 (Y) excludes the analysis of the localization of newly synthesized BACE, but focuses solely on the intracellular localization of the pool of BACE internalizing from the plasma membrane into the endocytic compartment, which is relevant to its toxic function in Alzheimer's disease (AD). Figure 2 (Z) focuses on the analysis of the pool of BACE that internalizes to non-endocytic compartments, which might not be relevant to its toxic function in Alzheimer's disease.
We also provided the students with a short description about the function of some of the proteins/intracellular markers studied in these figures, which had never been covered during class or lab hours (this was meant to fill in the missing information, to facilitate their understanding of the figures).

Similarly to the pre-assignment, we ask the students to "put the figures in the right order" and to explain the rationale of their choices. For the ordering of the figures, we gave a number of possibilities to choose from (they could choose only one possibility):

I. **X, Y, Z**
II. **X, Z, Y**
III. **Y, Z, X**
IV. **Y, X, Z**
V. **Z, Y, X**

For the explanation of the rationale, we specified that:

- They should not limit to list one more time the order of the figures, rather spend some time explaining the reasons why they chose that specific order.
- In addition, they should not just rephrase the figure caption in their explanation, as we would rather want to know how they interpreted it.

Evaluation of the post-assignment.
The correct ordering of the figures is X, Z, Y. From a scientific standpoint, the rationale of such order is the following:

a. When studying the trafficking of a protein from the plasma membrane into intracellular organelles by means of immunocytochemistry, one first needs to know where the protein can be found at the steady state (in any given moment of the trafficking process, therefore including both the pool of newly synthesized protein and the pool of recycling protein). This information in provided in Figure 1 (X).
b. Once it is clear where the protein localizes in the cell, one can move forward to the application of specific experimental protocols that can allow to discriminate the pool of recycling protein from the pool of newly synthesized protein. This information is provided in Figures 2 (Z) and 6 (Y). The rationale for choosing Y and not Z as the last one is in the fact that the experiment provided in Y is based on the outcome of the experiment described in Z, and therefore the information provided in figure Z must be preliminary to the experiment described in figure Y.

Although these are logical steps, we do recognize that our students might not fully understand the significance of each intracellular organelle studied in these figures in determining BACE toxic activity in AD, and therefore might not understand why Z should come before Y. Hence, when evaluating this bonus question, it was mostly important for us that they had chosen X (Fig 1) to precede either Y or Z. Most importantly, we evaluated that they did not placed X as last. For this reason, when evaluating the order of the figures, we gave partial credit to option I and IV, full credit to option II, zero credit to options III and V.

Students' answers were graded based on two factors: i) order of the figures; ii) rationale of their choice. Grading criteria are summarized below:

a) Grading the order of the figures

i) **Check+:** need both Figures 1 and 2 (regardless of the order) before Figures 5 and 6 (regardless of the order).
ii) **Check:** Need at least Figure 1 or 2 before Figure 5 or 6.
iii) **Check-**: both Figures 1 and 2 (regardless of the order) placed after Figures 5 and 6 (regardless of the order).

b) Grading the Rationale

If Check-:

i) They tend to give the explanation for their choice, but do not link the findings together.
ii) They may have understood, but are unable to put concepts into words.

iii) They may have not understood anything.
iv) They try to explain only with “empty” words (repeating the same words, without explaining their meaning or the meaning of the experiment).
v) They may have not understood the need to use pharmacological treatment (Figures 1 and 2) before investigating BACE intracellular localization (Figures 5 and 6), and the goal of this research article.

If Check:

i) They may have understood some of the experiments but not all of them.
ii) They may have not understood the need to use pharmacological treatment (Figures 1 and 2) before investigating BACE intracellular localization (Figures 5 and 6), but they may have understood the goal of this research article.
iii) They may have understood the need to use pharmacological treatment (Figures 1 and 2) before investigating BACE intracellular localization (Figures 5 and 6), but they have being unable to link the experiemtns/Figures, or have not understood the significance of some experiments.

If Check +

i) Nice understanding of the flow of the paper.
ii) They may have understood the need to use pharmacological treatment (Figures 1 and 2) before investigating BACE intracellular localization (Figures 5 and 6) and the goal of this research article.
iii) Understood the concept of BACE degradation in specific, identified organelles and how to assess it.
iv) Sometimes assigned also to students who gave the wrong order of the figures, but gave a wonderful explanation of their rationale, showing that they were able to use their knowledge and their understanding to contribute critical thinking, that supported the rationale of this study.

Supplemental Files E Sample Figure Responses
Full credit (10 points)

Question text: Three figures were selected from the following paper:
Intracellular itinerary of internalized beta-secretase, BACE1, and its potential impact on beta-amyloid peptide biogenesis.

Chia, Pei Zhi Cheryl, et al. "Intracellular Itinerary of Internalised β-Secretase, BACE1, and Its Potential Impact on β-Amyloid Peptide Biogenesis." *Traffic* 14.9 (2013): 997-1013.

Example 1: Full credit (10 points)

Which do you think is the order of the figures as they are presented in the manuscript?

4/4

i) X, Y, Z
ii) X, Z, Y
iii) Y, Z, X
iv) Y, X, Z
v) Z, Y, X

Explain your rationale for the order of each figure. Please don't limit to list one more time the order of the figures, rather spend some time explaining the reasons why you chose that specific order. In addition, please don't limit to rephrase the figure caption in your explanation. We don't only want to know what the figure caption says in your own-words - we want you to interpret it.

6/6.

Because first the researchers needed to do a steady-state distribution of BACE1 in different cell types to understand the rates of internalization, before they could test other factors such as recycling of the endosomes. They also have to check the effect of BACE before testing for the internalised APP in endosomes. the BACE markers are distributed in BACE → check BACE activity/internalization → then check APP with the same markers.

Example 2: Partial credit (7 points)

Which do you think is the order of the figures as they are presented in the manuscript?

i) X, Y, Z
ii) X, Z, Y
iii) Y, Z, X
iv) Y, X, Z
v) Z, Y, X

2/4 scantron?

Explain your rationale for the order of each figure. Please don't limit to list one more time the order of the figures, rather spend some time explaining the reasons why you chose that specific order. In addition, please don't limit to rephrase the figure caption in your explanation. We don't only want to know what the figure caption says in your own-words - we want you to interpret it.

1) Because the study is about internalization of beta secretase and its impact of Amyloid peptide biogenesis, I felt like introducing APP, the precursor for beta secretase, should be the first thing that happens, and that visualizing the localization of APP itself might helps give us more information about why/why not BACE internalizes in a certain way (Image Y). 2/2

2) The next image (X) appears to be the next level up in complexity, because now we are dealing with BACE1 our subject of interest and are looking into the localization of BACE (using BACE1 antibodies). 2/2

3) The last image, I think is image Z, it looks like it is aiming to explore what happens AFTER internalization and says that it transits the juxtanuclear recycling endosomes and the graphs make it seem like the recycling endosomes play more of a role in THIS stage than the other stages. 1/2

Example 3: Partial credit (2 points)

Which do you think is the order of the figures as they are presented in the manuscript?

0/4

i) X, Y, Z
ii) X, Z, Y
iii) Y, Z, X
iv) Y, X, Z
v) Z, Y, X

Explain your rationale for the order of each figure. Please don't limit to list one more time the order of the figures, rather spend some time explaining the reasons why you chose that specific order. In addition, please don't limit to rephrase the figure caption in your explanation. We don't only want to know what the figure caption says in your own-words - we want you to interpret it.

2/6

Figure Y explains what happens to BACE right after it is internalized. Then, figure Z explains that it then goes to the recycling endosomes after internalization. Finally figure X explains that the amount of BACE stays stable with this mechanism. This paper tries to explain where BACE goes and how it is moved w/in a cell after it is internalized. So, the figures should be in the same order as where BACE goes after internalized chronologically, w/ the final figure saying that after all of this transport, the amount of BACE synthesized & degraded remains relatively stable.