

This review must **be signed in at my office (Wat 289) BEFORE noon on Monday February 12th**. Refer to the syllabus for information about course policies regarding tests and other written assignments.

- There is no time limit for taking the review except for the final due time. It was designed to be able to be completed in 2 hours and I suggest you use that time as a guideline. To dissuade procrastinators and all-nighters **you MUST BEGIN the review before 10pm Sunday**. Remember to leave time to print and deliver because it isn't 'done' until it is turned in.
- This is a closed-book, closed-note review. Once you have seen any question your review period has begun and you may not talk, listen, read or use other forms of communication to discuss this course.
- This page must be the first page of your answer packet. Fill out this page and attach it to the ones containing your answers. The top of each additional page in the packet should contain only your initials and the page number.
- All answers must be typed and in complete sentences unless otherwise indicated. Any accompanying *graphs or figures may be hand-drawn*.
- **If** a question lists a maximum number of words you must include your response's word count (Microsoft Word/Tools/word count will calculate it for you)
- You may use a calculator for +, -, *, and / only. (no preprogramming) To receive full credit all calculations must be included. Calculations/equations may be hand written and do not need to be sentence form. The *answer to the question* that required the calculation should be in sentence form.
- Be sure to completely answer the question asked. Brevity is encouraged.
- There are 22 questions worth 100pt and 2 bonus questions worth 4pt.

Name: _____

(PRINT)

Signature: _____

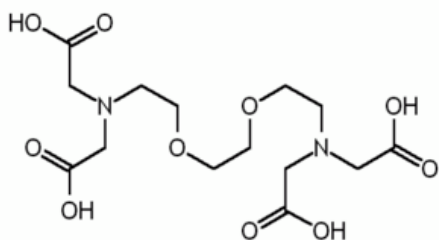
Write out the honor code (found here:

<http://www.davidson.edu/administrative/admission/whydav/honor.html>, 'On my honor...')

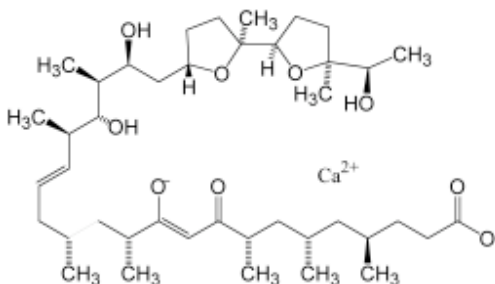
This review was begun at _____ on _____ and completed in _____ hours
Time Day

Carefully read the information given. The questions refer to this background information and its connections with the material covered in Unit 1 and the first three weeks of lab.

In our first unit we have seen calcium used as a second messenger. Two of the tools that scientists have used to study calcium's role as a second messenger are compounds called chelators and ionophores. A chelator is a compound that binds very tightly to an ion. Therefore, a calcium chelator like EGTA, is a compound that binds very tightly to calcium ions. Ionophores are compounds that shuttle ions across membranes. Therefore, ionomycin, a calcium ionophore binds and shuttles calcium across membranes.



Structure of EGTA; MW 468.3g



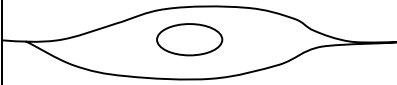
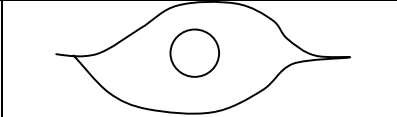


Structure of ionomycin; MW 747.1g

1. What are second messengers? (not a list of examples, a definition of the term) (3pt)
2. Briefly describe three different examples of calcium being used as a second messenger that we covered in Unit I. Your answer should be in the form of 'Calcium is a second messenger in _____ cells where it _____ to result in/cause/promote/inhibit _____. It is also.....' (100 words max.) (6pt)
3. Name two additional examples of molecules used as second messengers. Discuss similarities between calcium and those two additional examples and explain how those similarities help to make the molecules effective messengers. (5pt)

(hypothetical organism) Researchers have recently identified a new multicellular eukaryotic organism and have already determined that it has muscle cells and secretes acetylcholine. You are interested in studying the mechanisms controlling muscle contraction in this new animal. To do so you decide to isolate muscle cells, put them in a buffer that resembles extracellular fluid (so they will live) and examine the effects of acetylcholine and of EGTA.

As stocks you have 5ml of 60mM EGTA; 1ml of 1ng/ml acetylcholine, 50ml of H₂O and all of the balances, tubes, slides and equipment found in a standard laboratory.

You expose the muscle cells to the following conditions for 5min and then view them under the microscope. You include a scale diagram of the cells you saw in your notebook. All assays include buffer to bring them to a final volume of 200 μ l.

	60mM EGTA	1ng/ml acetylcholine	Representative cell	Assay	60mM EGTA	1ng/ml acetylcholine	Iono-mycin
1	10 μ l	3 μ l		5	10 μ l	3 μ l	2 μ l
2	0 μ l	3 μ l		6	0 μ l	3 μ l	2 μ l
3	10 μ l	0 μ l		7	10 μ l	0 μ l	2 μ l
4	0 μ l	0 μ l		8	0 μ l	0 μ l	2 μ l

For questions 11 and 12

- What is the molar concentration of EGTA in assay #1? (3pt)
- What is the concentration of EGTA in assay #1 in % w/v ? (3pt)
- Acetylcholine binds muscle cells through non-covalent interactions. Explain why this type of interaction between acetylcholine and the cell is important in correct muscle function. (4pt)
- With what type of muscle cell macromolecule does acetylcholine interact? (2pt)
- Of the four types of biological macromolecules that we have discussed—which type does not form covalently bonded polymers? What type of bonds does this macromolecule form between multiple copies and why is the non-covalent nature important to the roles that macromolecule plays in the cell. (It may help to define polymer and discuss what the cell would or would not be able to do if this macromolecule *did* form covalently bonded polymers) (5pt)

Bonus: Name and draw the monomeric form of one of the four major types of biological macromolecules. (2pt)

9. Consider the scale images of cells in the table above. Briefly *describe* what happened in **assay #2** that made that cell look the way it does. (50word max—looking for general not details) 4pt

10. Propose an explanation for why including EGTA in the assays has the effect it does. In your response include what EGTA is, and aspects of its chemical structure and the structure of the muscle cell it interacts with that could explain the effect seen. (4pt)

In a second experiment the assays were repeated but ionomycin was added to each assay. (see assay conditions in the table above that have bracket under them)

11. If the cells in **assay 6** and **assay 8** were viewed under a microscope what would you expect them to look like? Explain your reasoning (you may include a sketch but the reasoning must be included in the text) (6pt)

12. If the membrane potential of the cells in **assay 8** and **assay 4** were compared would you predict that would assay 8's cells would be higher, lower or the same. Explain your reasoning. (5pt)

Bonus: Describe how you would make 15ml of 4mM ionomycin. (2pt)

13. Neurons are 'excitable cells' whose membrane potential is capable of changing. Discuss two types of channels that are involved in changing the membrane potential. Provide the channels' names, what controls their opening and how opening each channel affects the membrane potential (does it increase/decrease as a result). (8pt)

14. We have discussed a number of examples where ATP and its derivatives are involved in modulating enzyme activity. Discuss two of them mentioning what the effected enzyme is and how interaction with ATP or its derivatives changes that enzyme's activity. (8pt)

15. G_{ap} is known as a 'molecular clock', why? What does it 'time'? (3pt)

Since there never seems to be enough time—you become interested in studying G_{ap} using spectrophotometry. In particular you wish to study the effect of increasing substrate concentration on G_{ap} 's rate of reaction.

16. Enzymatic reactions include E, S, and P. Define those terms in light of the reaction catalyzed by G_{ap} . (2pt)

17. Describe how you would set up an experiment studying the effect of increasing substrate concentration on this reactions rate. Explain what component would you follow (and why) and how you could use spectrophotometry to 'watch' the reaction proceed? (8pt)

18. Predict the effect increasing substrate concentration will have on the rate of reaction. Draw a well-labeled graph (hand-drawn) and explain your prediction in words. (5pt)

19. G_{ap} was discussed in our 'egg fertilization' example. Given your prediction above, what do you think would happen to the egg if G_{ap} 's substrate levels were artificially increased (significantly) just as the sperm contacted the plasma membrane? (4pt)

20. We have talked repeatedly about communication pathways being able to be 'reset to normal'. Why is this so important? Use the liver's response to epinephrine as the focus of your response. (note the question asks why resetting is important—not how resetting happens) (4pt)

21. Stress 'causes' both your heart and your liver to be exposed to epinephrine and both cells interact with the epinephrine but your liver cells release glucose while your heart muscle cells do not. Explain why this is the case. (100 word max) (4pt)

22. Adenylyl cyclase molecules are activated as part of the cascade in the liver. Since adenylyl cyclases are enzymes they can catalyze thousands of reactions in seconds. How does the cell deal with getting rid of all of the products of this reaction? (4pt)