Name: _____________________________

The following comprehensive examination is based on the article:


Put your name only on the cover page of the exam. Make sure the exam number on all following pages matches the number on the cover page.

You will be given a fresh copy of the article to use as a reference during the exam. You will have three hours to answer the questions. Read each question carefully and think about your response before writing your answer. Be sure to attempt all problems.

Please answer all the questions in the space provided. If you need more room, please ask for additional paper (be sure to note on your exam that the answer continues on an additional page and be sure that any additional pages are clearly marked with the appropriate problem number). Write legibly and be sure that your answers are dark enough so that your exam may be photocopied for grading.

Good Luck!!!!
1) In the first paragraph of the introduction, the author’s describe the overall structure of BoNTs.

   a) Based on this description, draw a “cartoon” representing the three subunits. Clearly label each subunit, the location in an affected cell, and the proposed function. Include any important structural features.

   b) Why is the author’s description of three subunits not entirely correct when characterizing the BoNTs? What would be a better description?

2) In the first paragraph of the article, the authors describe how BoNT acts to prevent the release of acetylcholine. Draw a “cartoon” of a typical chemical synapse and show the roles of the HC, LC, SNAP-25, the vesicle and acetylcholine as described in this paper. Your diagram should be numbered so that the sequence of events is clear. Be sure to annotate your diagram to show why BoNT is toxic.
3) The authors state (pg 2399) that the crystal structure of BoNT/A “…supports a model in which the H223, H227 and E262 of the HEXXH motif directly coordinate the zinc, and E224 coordinates a water molecule as the fourth ligand.” Draw a structural diagram corresponding to this description. Show all atoms in your diagram (you may omit hydrogen atoms on carbons). Be sure to show the peptide backbone of HEXXH and any charges. Make the geometry around the zinc atom clear. Where necessary, use R to indicate unspecified side chains.

4) The authors mutate E224 to either Asp or Gln.

a) Draw all three amino acids.

b) Would you consider the substitutions E224D and E224Q to be conservative, somewhat conservative, or a very large change? To support each of your answers, explain your rationale for each substitution.
5) Diagram and label the process of PCR. There are a number of variations in the steps of this technique; your diagram should contain the essential events and generic components of any PCR experiment. How is this process utilized in this paper?

6) Diagram the process of dialysis, showing a starting condition and the final state. Annotate as necessary to explain what is happening.
7) In the methods section, the authors utilize a number of different techniques to quench reactions. For the following two situations, explain how each buffer quenches the process, giving specific molecules involved.

a) Explain how a SDS buffer quenches the endopeptidase assay.

b) Explain how a DTT buffer quenches the binding between BoNT/A LC and SNAP-25.

8a) What structural feature of a peptide gives rise to circular dichroism?

b) The authors present CD spectra in Figure 3 and use these data to support their claim that the conformational structure has not been changed by site mutation. Although their claim may be correct, what additional CD spectra should have been measured or presented to convince a critical reviewer?
9) a) Define $k_{\text{cat}}$ and $K_M$

b) What does it mean when $K_M$ is unchanged between the wild type and the mutant, but $k_{\text{cat}}$ is altered?

c) Once the authors found that $k_{\text{cat}}$ was altered, why was it important for the authors to determine the value for $K_M$? What is the significance of the $K_M$ determination for E224D?

10) In Figure 2, there are two lines with four data points on each line.

a) Describe what experiment they used to collect the data to create these plots.

b) Recreate Figure 2 below, but draw an additional line that would indicate a mutant form of botulinium toxin that is more active than the wild-type. Label all aspects of your graph and briefly explain your rationale for the new data line.
11) In the first paragraph of the results, the author’s talk about data not provided. What would Figure 1 look like if over time cleavage **began** to occur in the E224D experiment? Draw data for 1 hr, 5 hr and 10 hr.

12) Consider the isothermal titration calorimetry (ITC) experiments.

a) What is the role of EDTA in the preparation of apo-LC’s? Illustrate how it works with a structural diagram.

b) ΔH is found to be endothermic for the binding of Zn^{2+} to the apo-proteins, yet the affinity constants K are found to be quite large. Explain how this can be.

c) In the upper panel of Figure 7, why is power added to the sample with each injection?

d) In general, what aspect of the lower panel of Figure 7 allows the authors to calculate the mole ratio of Zn^{2+} to protein (the n value in Table 4)?
13) Regarding the putative reaction mechanism (Figure 8) and its associated discussion:

a) Would you expect there to be a pH dependence on zinc binding? If there were a dependence which amino acids would effect this binding?

b) Figure 8, while labeled as a “mechanism”, strains the very definition of the term, as it attempts to show too much on one diagram. It is more reasonable to break the mechanism into four stages:

1. active site containing only water and Zn$^{2+}$ (no substrate)
2. bound substrate and water, poised for nucleophilic attack
3. tetrahedral intermediate, ready to break down
4. broken peptide bond (the products)

Draw the four stages described above using Figure 8 and the description of the reaction, which begins at the very end of pg. 2404, as your guide. To simplify matters, show only the relevant peptide bond (stick an R on either end of it) and the protein side chains involved in the chemical step of catalysis (as opposed to binding; side chains coordinating to Zn$^{2+}$ may be shown as in Figure 8, but other catalytic side chains should be drawn out fully). Add arrows for electron migrations to your mechanism.
14) The authors examined Trp fluorescence to see if the global structure was altered by the mutations.

a) Will does not necessarily tell you anything about the integrity of the active site. Why not?

b) What properties of FITC and IADEANS make these molecules useful to the authors? Why did the authors use the combinations of substrate/enzyme mutant/wt that are listed in Table 2?

15) Looking at Figure 6:

a) What is the relevance of $K_d$ and how does that compare to $K_M$? Write an expression for $K_d$.

b) What type of information does a Scatchard plot provide? What is the significance of the linearity of the plot in Figure 6B?
16) a) In Table 3, they list a zinc binding ratio for the E198D mutant. What is wrong with the report of this mutation? In your answer correct this table.

b) They mention in the results section that the E224Q mutant binds zinc just as well as wild type. They do not include data for the Asp mutation. Why do you think the authors failed to mention the results of the E224D?

c) The wild-type enzyme had a zinc/E224 distance of 5.1Å. They argue that since Asp is one carbon shorter the zinc/D224 distance would be 6.5 Å. Is there anything wrong with this assumption? If the E224D mutant bound zinc as well as wild-type, would that support or refute the authors conclusion? How would one explain the lower activity?
17) Mutations of BoNT/A LC were made by replacing E224 with Gln or Asp to modify the catalytic activity of the toxin. Imagine replacing E224 with Ser or Thr and speculate what type of activity you would expect in these new forms of the toxin. Explain your rationale.

18a) State the main conclusion of this paper succinctly, in your own words. Give three pieces of evidence that most directly support the main conclusion.

b) Do you agree with the conclusions in this paper? Are there other interpretations? Clearly support your answer.