1. Read the article “Characterization of Saxitoxin Binding to Saxiphilin, a Relative of the Transferrin Family That Displays pH-Dependent Ligand Binding?” (Published by Llewellyn and Moczydlowski in Biochemistry, 33, 12312, 1994; copy on the course website). You can ignore the part where binding of metal ions is discussed. Answer the following questions. Each answer is worth 0.5 points. Total 7 points.

1) Cells of which organisms synthesize saxitoxin?

2) Authors state that the physiological function of saxiphilin is unknown. Propose one possible physiological role for this protein in bullfrogs.

3) Summarize the biochemical mechanism of toxicity of saxitoxin.

4) Describe the physiological role of protein lactoferrin

5) Health dangers of tritium (¹H) are well known. Why did scientist have to use tritiated saxitoxin for their studies?

6) Inspect the structure of saxitoxin and explain why would a tritium atom from ³H₂O get into the structure of saxitoxin. Keep in mind that 3H was in the position where it stays long enough to be useful for the binding studies. Which position(s) contain the tritium?

7) Explain what is observed on Figure 2A. Why were some assays done in the presence of 10 μM unlabeled saxitoxin?
8) The binding experiment was performed in with total saxiphilin concentration of 120 ng/mL. What is the molar concentration of saxiphilin in this experiment (M_w of this protein is 90.8 kDa)?

9) Authors state that if saxiphilin had one binding site for saxitoxin, the expected maximal binding $B_{max}$ is 11.0 nmol saxitoxin per mg of saxiphilin. Show calculations to back up this claim.

10) Propose one explanation of why the binding of saxitoxin to saxiphilin is slower at pH 5.99 than at pH 7.42?

11) Based on analysis of its structure, binding data on Figure 4B, and the associated discussion by authors, give the net charge of the form that binds to the saxiphilin at neutral pH.

12) What is the difference between the Arrhenius plots and van’t Hoff plots (this is a general question, not specific to this paper). What information about binding can be calculated from these plots?
13) Based on the data given in the paper, is it likely that the binding of saxitoxin to saxiphilin is largely driven by hydrophobic interactions? Justify.

14) Classic Scientific Astrologer Theodore White forecasts a very strong El Nino for 2010-2011. My crystal ball reveals that a massive red tide arrives to shores of Santa Barbara in early 2010. Worse, by the next summer Lake Cachuma gets contaminated with the organisms that produce saxitoxin, and the level of free toxin raises to dangerous levels in our drinking water. Propose a plan for biochemistry-based personal water purification device that would eliminate saxitoxin from drinking water.

2. As a kid, I occasionally obtained valuable chemicals from dumpsters of a nearby Chemistry Institute. One day I found a bottle with partially damaged label that stated the content to be an acid, and provided its molar concentration. However, it was not clear which acid it was. I took the bottle to school and used the pH meter during our seventh-grade chemistry lab to find out that the proton concentration in the acid numerically matched the molar concentration marked in the bottle. Thinking it is a strong mineral acid, I performed tests with silver nitrate but nothing precipitated. And the acid did not dissolve my mom’s silver engagement ring. Desperate trying to figure out what it was, I tasted it, and it tasted little bit like vinegar! Could it be that the acid I had found was acidic acid (pKa 4.8)? If yes, calculate the pH (or the total (acetic acid + acetate) molar concentration) of the solution. If no, justify your answer with appropriate equations. (3 points)