Part IB: The Basics -- Introduction to Biomolecules

1. A monocarboxylic acid and its potassium salt were combined in a 2:1 mole ratio and dissolved in water. How might you mathematically define the pH of the resulting solution?
   A. pH = pKa - 0.30
   B. pH = pKa + 0.30
   C. pH = pKa - 0.70
   D. pH = pKa + 0.70
   E. pH = 0.30

2. At what pH's will the average charge on the phosphate species be:
   -0.5, -1.0, -1.5? Use pKa values of 2.12, 7.21, and 12.7 for phosphoric acid.
   A. 2.12, 7.21, 12.7
   B. 2.12, 4.67, 7.21
   C. 4.67, 9.96, 12.7
   D. 7.21, 9.96, 12.7
   E. none of the above

3. One way to define the concentration of a buffer is to add the concentrations of components of the conjugate acid-base pair that produces the buffer. If 25.0 mL of a 0.25 M potassium monohydrogen phosphate was combined with 175 mL of 0.033 M potassium dihydrogen phosphate, what is the

concentration of the resulting buffer? Assume the volumes to be additive.
A. 0.029 M
B. 0.031 M
C. 12.025 M
D. 0.060 M
E. none of the above

4. What impact does dilution have on a buffer?

5. Blood plasma has a pH of 7.4. What is the [H+]/[OH-]?
A. 4.0 x 10-8
B. 2.51
C. 0.16
D. 6.41
E. 0.39

6. A certain insect larvae thrives in small pools deep within a glacier. How might this insect's cell membranes have evolved to function normally in these near-freezing waters?

7. If a small drop of oil and an equal amount of starch are placed on the surface of a glass of water, and if you have starch- and oil-detectors in the bottom of the glass, which will you detect first?

8. If the typical gene in an organism has 35% guanine, 35% cytosine, 15% adenine, and 15% thymine, what kind of environment do you expect the organism to live in? (several answers are possible)

9. Why is the beta anomer of glucose more common than the alpha anomer?

10. Why might you expect high levels of cysteine in proteins from an organism living in a hot spring?

11. Consider a buffer at pH 4.0 Will adding the sodium salt of glutamic acid to this buffer change the pH?

12. Adding either long chain fatty acids or cholesterol to a certain lipid membrane has the same effect. What is the temperature of this membrane relative to its Tm?

13. What roles can acidic amino acids play in protein structure and function? (You should be able to come up with several!)

14. Why does water expand when it freezes, unlike most other compounds?

15. I need to make a Tris buffer at pH 7.5, but all I have is the deprotonated form of Tris. If Tris has a pKa of 8.1, will I need HCl or NaOH to make my buffer?

16. Why do lipids form bilayers?

17. Which amino acids are not chiral?

18. What impact is there likely to be on an organism that cannot regulate the fluidity of its cell membranes?

19. Can you make a buffer out of just HCl and NaOH? If so, what would be its effective pH range?

20. Why is a good buffer one that has a pKa within one pH unit of the target pH?
21. A certain protein is exposed to high levels of BME. Even after the BME is removed by dialysis, only a small portion of the protein is functional. What does this tell you about the protein?
22. What difference in amino acid content might you expect to find between the keratin of a person with straight hair and one with naturally curly hair?
23. Helix-loop-helix proteins involved in DNA binding have arginine and lysine residues on the outer surface of their helices. Why should this be so?
24. A random mutation causes a cysteine codon (UGU) to be replaced by a glycine codon (GGU). Is that apt to have a negative impact on the organism bearing the mutation?
25. When making a buffer, one often dissolves the salts in 80% of the final volume, adjusts the pH, and then brings it to final volume with water. Why not go for the final volume right away and save time?
26. In looking at the sequence of a protein from a wide variety of organisms, you see that an arginine always shows up around the same position in every sequence. In general terms, what is this likely to indicate? More specifically, what are some factors that might explain the conservation of that arginine?
27. If two proteins have the same evolutionary origin, but one functions at pH 6 and one functions at pH 11, what sorts of changes would you expect to find between them in terms of amino acid composition?
28. Our advertising culture tells us that products that are low in saturated fats are better for us than those that are high in saturated fats, e.g., olive oil vs. butter. Suggest a reason why this should be so, given what you know about the properties of fatty acids.
29. Which of the twenty commonly-occurring amino acids will bear charged side-chains if dissolved in a solution at pH 10?
30. Formic acid is a weak acid with a pKa of 3.75. Its name comes from the Latin word "formica" ("ant") because it is very plentiful in the secretions of some species of ants, and is one of the irritants delivered by many stinging ants, bees and wasps. (a) Suppose that I were interested in using formic acid as the basis for a buffer for biochemical investigation. Would it be most useful for creating a buffered solution at pH 2, 4, or 6? (b) If I use formic acid to create 2.00 liters of 50.0 mM buffer at the pH you selected in part (a), please tell me the equilibrium concentrations you'd expect for formic acid and its conjugate base. (c) Suppose I want to make the buffer you described in part (b) by adding either strong acid (6.0 M HCl) or strong base (5.0 M NaOH) to a solution of concentrated formic acid in order to get to the correct pH. I know that I can buy formic acid from Sigma Chemical Corp. as an 88% solution (this works out to about 19 M). Please tell me what volume of 19 M formic acid I should start with, whether I should add HCl or NaOH to get to the target pH, and how many milliliters of strong acid or strong base you predict I would need to add. (302.2003.test1)

Answers for part IB

1. A

2. B
3. D
4. The pH of a buffer is determined by the mole ratio of the components in an acid/base conjugate pair and the pKa involving those two components. The major impact of dilution is to reduce the buffering capacity of a specific buffer volume.
5. C. There are two ways to approach this. First, a pH of 7.4 is very close to the neutral pH (in water) of 7.0. That means that you will have very nearly equal concentrations of protons and hydroxide ions. So the answer will have to be quite close to 1.0. (Note that at pH 8, [H+]=10^-8 and [OH-]=10^-6, so [H+]/[OH-]=0.01) That leaves B, C, D, or E as options. Moreover, this pH is slightly on the basic side, so that means that there must be more hydroxide ions than hydronium ions, right? So that rules out answers B and D. OK, we're down to 2 options, 0.16 or 0.39. Those are both pretty close to, but less than, one, as expected for a slightly basic solution. Unless you're very good with logarithms, you may not be able to get much further than this without a calculator, but at least now you have a good logical check on the answer you get from your calculator....Second, then, is the rigorous mathematical approach. pH of 7.4 gets you a hydronium ion concentration of 3.98x10^-8 (answer A). From that, you should be able to find [OH-] (from Kw), and then take the ratio of the two to find that the correct answer is indeed C, [H+]/[OH-] = 0.16.
6. I would expect lipid composition to be altered in favor of greater membrane fluidity, perhaps involving shorter FA chains or greater levels of unsaturation.
7. I would expect to detect the starch first, as the oil should be minimally miscible with the water.
8. The relatively high percentage of G's and C's suggests that this organism requires high levels of hydrogen bonds to stabilize the DNA duplex. Reasons for this might include a high ambient temperature or high intracellular salt concentration.
9. It is more common because it is thermodynamically favored. That is to say that it is more stable, because it experiences less steric hindrance between adjacent hydroxyl groups.
10. Hot-spring organisms must have evolved ways to stabilize their proteins to prevent denaturation at temperatures that would be fatal to many other organisms. Although many strategies have been explored evolutionarily, one route to greater protein stability involves a higher number of disulfide bonds, and thus a higher composition of cysteines than would be found in the homologous proteins of organisms living at cooler temperatures.
11. The sodium salt of glutamic acid contains the conjugate base of a weak acid and is thus expected to be basic. At pH 4, roughly 50% of the glutamate carboxyl groups should become protonated. So yes, it should change the pH, though it is unlikely to change it by much.
12. Long-chain fatty acids tend to increase inter-chain interactions and thus decrease membrane fluidity. Below the Tm, cholesterol tends to increase membrane fluidity, and above the Tm, it decreases fluidity. Thus if adding either long-chain FA or cholesterol have the same effect, we must be above the Tm.
13. Oh my! Let me count the ways...
14. Water molecules are capable of participating in four hydrogen bonds (one per H, one per non-bonding e-pair on the O) per molecule. In liquid water, not all of these potential interactions are formed due to thermal motion, and as a consequence, the water molecules can actually pack more closely together. When water freezes, molecular motion slows down and nearly all of the H-bonds can be formed, imposing a strict order on the population of water molecules and forcing them to unpack a bit in the process.
15. pH 7.5 is below the pKa of Tris. Thus, starting only with the deprotonated form, I need to protonate more than half of the molecules (about 75%) to get to the proper [conj. base]/[conj. acid] ratio. To protonate a molecule, I must add an acid, in this case HCl.
16. Lipids are amphipathic -- they have hydrophilic and hydrophobic portions. To optimize the entropy of the

solvent, therefore, a collection of lipid molecules in aqueous solution is oriented to present the hydophilic portions to the solvent and the hydrophobic portions towards each other. WIth the right lipid concentration (and a few other factors), this naturally leads to the formation of bilayer vescicles.

17. The only non-chiral amino acid is the one with two identical species attached to the alpha-carbon: glycine.
18. An organism incapable of regulating its membrane fluidity would, at the very least, be severly restricted in the range of temperatures at which its cell membranes would function properly.
19. No, HCl and NaOH -- or any strong acid/strong base pair -- will not form a buffer solution. Buffering capacity is critically dependent on the presence of adequate concentrations of a weak acid and its conjugate weak base.
20. As noted in the previous answer, a good buffer needs appreciable amounts of both the conjugate acid and conjugate base that form the buffer system. This will only occur within a range around the pKa (the pH at which [conj. acid] = [conj. base]). Practically speaking, this is the range of +/- 1 pH unit -- which gives a range of 10-fold excess of conjugate acid up through 10-fold excess of conjugate base.
21. This protein can be inferred to be dependent on a certain arrangement of disulfide bonds for proper folding and function. The high concentration of reducing agent has probably broken and/or scrambled the disulfide bonds, thus destroying protein function.
22. The curls in hair are held in place by disulfide bonds. A person with naturally curly hair presumably has a greater number of disulfides in her/his keratin, and this suggests that her/his keratin has a higher percentage of cysteins than the keratin of those of us with straight hair.
23. DNA-binding proteins need to bind to DNA. As the DNA backbone consists of a sugar-phosphate framework, and as the phosphates are negatively charged at neutral pH, positively-charged amino acids such as lysine and arginine make natural DNA-binding "fingers".
24. Replacing the thiol side-chain of a cysteine with the hydrogen of a glycine is NOT a conservative subsitution -- there are changes in size, polarity, and disulfide-bond capability. Especially given the relative rarity of Cys residues in most proteins, this substitution is apt to have deleterious effects.
25. Adjusting the pH of the buffer usually involves adding strong base or strong acid, sometimes in considerable amounts. Thus it is always safer to start out with a smaller volume to ensure that the pH adjustment doesn't cause you to overshoot your target volume and so dilute your buffer. From a practical standpoint, it is also more convenient -- you can dissolve the buffer components in 80% of the water in a beaker on a stir-plate, estimating volume by the crude markings on the beaker, and only when the pH is right, transfer the solution to a more accurate measuring device for final volume adjustment.
26. The recurring presence of this residue at this spot in this protein, despite the ongoing mutational pressure on the gene encoding this protein strongly suggests that it is somehow important to the biological role of the protein for that amino acid to be there. For instance, an arginine might form a salt bridge with a negatively charged amino acid elsewhere in the protein, holding the protein in a functionally important conformation, it might be directly involved in mediating binding of this protein to another molecule (as to a negatively-charged ligand), or it might be directly involved in the catalytic mechanism of an enzyme.
27. Different amino acid sidechains will be protonated and deprotonated at pH 6 and pH 11. Thus, in order to preserve the same structure and function in these two proteins that function in different environments, one might reasonably expect to find amino acid substitutions that preserve protonation state rather than other physical properties. For instance, if it were critical to protein function for a particular amino acid to bear a partial positive charge at a certain position, then one might expect to find a histidine there in the protein at pH 6, but a lysine there at pH 11. (This ignores, of course, differences in geometry between these two amino acids; that would have to be compensated for by changes in the "scaffolding" holding the charged residue at the proper spot.)
28. Saturated fats are more likely to form solid aggregates at physiological temperatures than are unsaturated

fats. Hence, they are more likely to form occlusions ('plaques') in blood vessels than unsaturated fats. (The impacts of different types of fats on human health are complex and in many cases, still poorly understood. Nevertheless, a naively simplistic view like that just presented actually has pretty impressive predictive power.)

29. At pH 10, the following amino acid side chains will be charged: carboxyl groups on aspartic acid and glutamic acid (deprotonated and thus negatively charged), and the basic side chains of lysine and arginine (protonated and thus positively charged).
30. (a) For pH 4. The weak acid used for a buffer should have a pKa within one pH unit of the target pH of the buffer. (b)(c)