Out-of-Class Questions

Article: M. Dittrich and S. Sibler, "Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration, and infrared spectroscopy," *J. Coll. Int. Sci.* **2005**, *286*, 487-495.

Note that you can skip Sections 2.6 and 3.5 because we will not be discussing the infrared spectroscopy results.

1. Because this article deals with a microbiological and geochemical application, you need to familiarize yourself with a few terms. Match the following terms with their definitions/descriptions.

_____ autotropic	_____ peptidoglycan
_____ oligotrophic	_____ picocyanobacteria
_____ pelagic	_____ picoplankton
_____ calcite	_____ *Synechococcus*
_____ electrophoretic mobility	_____ phycocyanin
_____ zeta potential	_____ phycoerythrin

A) "self-feeding" using energy from light or chemical reactions

B) the most stable crystal form of $CaCO_3$

C) used to describe living systems in open water

D) a protein complex in blue-green algae

E) a protein complex in red algae

F) the polymer that forms the bacterial cell wall and is composed of sugars and proteins

G) the smallest organisms in the water column that are incapable of swimming against current

H) a general name for photosynthetic bacteria with diameters < 3 μm

I) a specific genus of photosynthetic bacteria commonly found in marine environments

J) capable of living in a low-nutrient environment

K) the electric potential difference between the surface of a particle and the medium around it that arises from net electrical charge on the particle's surface

L) a measure of how much an electric field affects the migration of a particle

2. In the abstract of the article, mark which sentences correspond to (1) background information and significance, (2) methodological details, (3) results, and (4) conclusions.

3. In your own words, describe why the surface chemistry of picocyanobacteria is an important area of research.

4. Research and summarize the major difference between Gram negative and Gram positive bacteria. How might this difference affect the surface properties of these two types of cells?

5. Re-read the final paragraphs of the introduction. What specific question/problem is being addressed in this article?

6. In Section 2.5, the authors state that the titration data will be plotted with $-\log[H^+]$ on the x-axis and $C_A-C_B-[H^+]+K_w/[H^+]$ on the y-axis. Assuming that $\gamma = 1$ (i.e., that activity is equal to concentration), fill in the blanks below.

    a. $-\log[H^+]$ = _____

    b. $K_w/[H^+]$ = _____

7. In their modeling of the data, what assumption do the authors make about surface charge on the bacteria?

8. On Figure 1, circle the isoelectric point of the bacteria. How do you know that this is the isoelectric point?

9. Based on their modeling results, the authors identify three separate pK_a values associated with the picocyanobacteria surface. Fill in the table below to match the range of fitted pK_a values with the most likely corresponding functional group.

Approximate Fitted pK_a Value	**Corresponding Functional Group**
~5	
~6.5	
	amine

10. The inflection points at the three pK_a values are very weak, making them impossible to identify accurately without modeling. What explanation do the authors give for the weakness of these inflection points?

11. Note that generally the conclusion section of an article should not just summarize the paper. Instead, the conclusion might address (1) questions that remain to be answered about the data, (2) potential future experiments, (3) limitations of the work, and/or (4) the broader significance of the results. Give an example of one of these from the conclusion of this paper.

In-Class Questions

1. Prior to the titrations, the authors washed the cells in a solution of 1 mM EDTA and then resuspended them in $NaNO_3$. Both the $NaNO_3$ and the NaOH used in the titration experiments were degassed with N_2 before use. What was the purpose of each of these steps, and why were they necessary?

2. When interpreting Figures 2-4, it will be helpful to consider how these plots differ from typical plots of titrations.
   a. Have we typically plotted pH on the x- or y-axis? How does that compare to these plots?
   b. What have we plotted on the other axis? How is that different from what is plotted here?
   c. We know that ultimately the charges must balance, so where is this "excess" $[H^+]$ coming from? What is being deprotonated to release these hydrogen ions?
   d. How do the bacteria contribute to the buffering of the system, i.e. how is this possible?

3. For the third pK_a value (pK_3 in the manuscript), the authors state that either amine or hydroxyl functionality could give rise to the observed pK_a value, but they conclude based on the zeta potential measurements that this pK_a corresponds to amine groups on the cell surface. Sketch the protonation reactions for a generic amine and a generic hydroxyl group in aqueous solution. Use your sketches to explain the authors' reasoning.

4. A related article (*Aquat. Sci.*, **2004**, *66*, 19-26) critiques studies like this one, in which a cell suspension is titrated. In the related article, Claessens *et al*. argue that because cells are dynamic, living systems, they respond differently to titrant than a chemical solution of weak acid or weak base would respond. For example, in addition to the chemical process of protonation or deprotonation, cells may also respond with metabolic activity or biochemical reactions, including pumping of protons across the cell membrane, unfolding of cell wall proteins, cell lysis, etc. As a result, Claessens *et al.* suggest that titration data does not necessarily just reflect the acid-base surface chemistry of bacterial assemblies. If you were a program officer at a funding agency, would you provide financial support for further studies like the one you read? Consider the authors' purpose, as you described it in out-of-class questions 3 and 5, and justify your answer.