AAPT 2012 Workshop W30: Biologically rich electrostatics for life science students
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Notes about this problem: The version below is designed for students to do in a setting where they can work in groups with instructor support. I haven't tested this approach with students yet. In the past I have used it as a homework problem and this version is provided on the next page.

The phosopholipid molecule PIP_2 (phosphatidylinositol 4,5-bisphosphate) is an important constituent of eukaryotic cell membranes. Its hydrophilic head group has a greater negative charge (typically -4e at physiological pH) than most of the other phospholipids present in eukaryotic cell membranes. PIP_2 makes up only a small fraction of the membrane (typically ~1 mol%), but in spite of this low concentration, it is known to form clusters with multiple PIP_2 molecules that are thought to be important in cell signaling. Understanding why these clusters form is an area of ongoing research.

Recent research[1] provides evidence for an electrostatic role in clustering, in which Ca^{2+} ions hold the PIP_2 molecules together. The details of how Ca^{2+} ions and PIP_2 molecules are arranged in clusters are not yet known. It is also known that PIP_2 molecules can form clusters with Mg^{2+}, but the resulting clusters are smaller; PIP_2 clusters do not form with singly charged ions such as Na^+.

(a) Design a simple model of such a cluster including five PIP_2 molecules, each with charge -4e, and four Ca^{2+} ions, and show that electric forces hold this cluster together rather than pushing it apart.

Some hints:
• Arrange the molecules and ions symmetrically. This is more biologically plausible and will make your calculation of the relevant electric forces easier.
• Before you start calculating forces: Is it plausible that your model will hold together? What is the direction of the total electric force on each ion? Check with an instructor if you are unsure.
• Again before you start calculating, identify the simplest way (with the least calculation) to convince someone else that the cluster holds together. Check with your instructor if you are unsure.
• In your calculation you will add up many terms, each of which corresponds to a component of an electric force. Each term can be written as a number and a unit vector multiplied by ke^2/s^2 , where *e* is the charge of the electron, *k* is the constant in the electric force law, and *s* is a distance that you define in your model. Take advantage of this common factor.
• Organize your calculation neatly in a table or spreadsheet; this will help you avoid mistakes and will make it easy to repeat it with small changes for part (b).

(b) Would your answer to (b) change if the Ca^{2+} ions were replaced by Na^+ ions? (Hint: You should be able to make small changes to your calculation in (a) to answer this.)

(c) Now consider the difference between clusters formed with Ca^{2+} ions and those formed with Mg^{2+} ions. Approximating the ions and molecules as spherical, the radius of a PIP_2 headgroup is approximately 0.4 nm (4 Å) and the radius of a Ca^{2+} ion is approximately 0.1 nm (1 Å).
Although Mg^{2+} ions and Ca^{2+} ions are very similar in size, in clusters the Mg^{2+} ions are surrounded by water molecules, increasing their radii to approximately 0.4 nm.
Given this information, suggest an explanation for why fewer PIP_2 molecules cluster together in the presence of Mg^{2+} than in the presence of Ca^{2+}.

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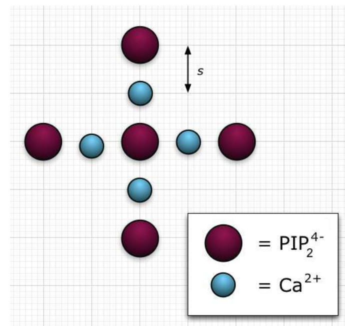
[1] Ellenbroek, Wang, Christian, Disscher, Janmey, and Liu, accepted to Biophysical Journal, 2011; Wang, Collins, Guo, Smith-Dupont, Gai, Svitkina, and Janmey, 2011.

Homework version:

The phosopholipid molecule PIP_2 (phosphatidylinositol 4,5-bisphosphate) is an important constituent of eukaryotic cell membranes. Its hydrophilic head group has a greater negative charge (typically -4e at physiological pH) than most of the other phospholipids present in eukaryotic cell membranes. PIP_2 makes up only a small fraction of the membrane (typically ~1 mol%), but in spite of this low concentration, it is known to form clusters with multiple PIP_2 molecules that are thought to be important in cell signaling. Understanding why these clusters form is an area of ongoing research.

Recent research[2] provides evidence for an electrostatic role in clustering, in which Ca^{2+} ions hold the PIP_2 molecules together. The details of how Ca^{2+} ions and PIP_2 molecules are arranged in clusters are not yet known. It is also known that PIP_2 molecules can form clusters with Mg^{2+}, but the resulting clusters are smaller; PIP_2 clusters do not form with singly charged ions such as Na^+.



In this problem we'll consider a highly simplified model to give a feel for how the electrostatic interactions in this system work. Let's model the cluster as five PIP_2 molecules, each with a headgroup charge of -4e, and four Ca^{2+} ions arranged on a square grid with grid spacing s, as shown.

(a) What is the total electric force on the central PIP_2? Explain briefly.

(b) To determine whether electric forces hold this charge arrangement together or push it apart, we only need to calculate the total electric force on one of the outer four PIP_2 molecules. Explain briefly why.

(c) The strength of the total electric force on the top PIP_2 is a number multiplied by ke^2/s^2. Explain why. (You can use dimensional analysis, consider each individual force, or take another approach.)

(d) Find the strength (as a multiple of ke^2/s^2) and direction of the total force on the top PIP_2 molecule. Does this force hold the cluster together?

This calculation involves summing many terms and can be really time-consuming if you don't do it neatly and efficiently. Here is some guidance on how to do it efficiently.

- Number the ions and molecules so that you have an easy way to keep track of the terms.
- Write separate sums for the horizontal and vertical components of the total force.
- Use symmetry to identify any terms in your sums that are equal or that cancel.
- Organize the terms in a table or spreadsheet. This will make it easy to do the arithmetic and to modify your calculation to answer part (d).
- Define a single symbol to represent a common factor, in order to save writing.

(e) Would your answer to (b) change if the Ca^{2+} ions were replaced by Na^+ ions? (Hint: If you have a nicely organized calculation from the previous part, you should be able to substitute the charge of Na^+ for the charge of Ca^{2+} without too much effort.)

(f) Now consider the difference between Ca^{2+} ions and Mg^{2+} ions. Approximating the ions and molecules as spherical, the radius of a PIP_2 headgroup is about 0.4 nm (4 Å) and the radius of a Ca^{2+} ion is about 0.1 nm (1 Å). Although Mg^{2+} ions and Ca^{2+} ions are very similar in size, in clusters the Mg^{2+} ions are surrounded by water molecules, increasing their radii to about 0.4 nm.

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Electrostatics in salt solution: Starting by developing a model

As you may have learned in a biology or chemistry class, DNA is a long, thin molecule consisting of two strands wrapped around each other in a double helix. When DNA is dissolved in water, the nucleic acids ("bases") each lose a proton (H+). The nucleic acids are attached to each strand with a spacing of approximately 0.33 nm, so when the protons are lost, each strand of the DNA becomes highly negatively charged, with a charge of –e every 0.33 nm.

Suppose you are in a biochemistry lab and you can measure whether a solution of DNA remains double-stranded or separates into individual single strands. You make the following observations:

(1) If you dissolve DNA in pure water, it comes apart into individual strands.
(2) If you dissolve DNA in water that also has dissolved salt (Na^+Cl^-) in it, matching normal physiological conditions, it remains double-stranded.

With your group, come up with a suggested explanation for these observations. As part of your explanation draw sketches representing how the negatively charged DNA strands, the protons lost from the DNA, and (in case 2) the salt ions are arranged in the water.

<u>Thinking about Screening in Salt Solution</u>

Consider (negatively charged) DNA in salt solution (the salt solution includes equal numbers of positive and negative small ions in addition to the DNA), as illustrated in the diagram below taken from Phillips, Kondev, and Theriot, Physical Biology of the Cell (Garland Scientific, 2008).

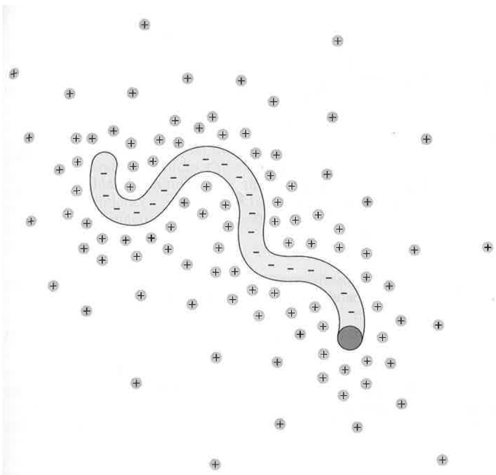

Figure 9.14 DNA in an ionic solution. The schematic shows the large negative charge density on the DNA molecule and the positive counterions in the surrounding solution.

1. **Why don't positive ions attach all along the length of the DNA until the DNA is electrically neutral? What factor/force is opposing that process?**

2. **Outside the "cloud" of positively charged ions, is the electric field magnitude smaller or larger than it would be if not for the positive ions? Why?**

3. **T**he positive cloud of ions surrounding the negatively charged DNA can be approximated as extending a distance λ_D, the "Debye length." Under physiological conditions this length is about 1 nm. **Explain qualitatively why the Debye length λ_D increases with temperature.**

4. **Explain qualitatively why the Debye length λ_D decreases with increasing salt concentration.**

5. **Explain qualitatively why the Debye length λ_D decreases as the charge of the positive ions increases.**

6. **DNA is highly negatively charged when dissolved in water (because H+ ions easily detach from it in water), whether pure water or salt solution. It is physiologically critical for DNA to normally exist in a double helical form. Does the presence of the ions in solution make it harder or easier for two strands of DNA to remain in the double-stranded form, rather than separating into single strands?** Why? (Hint: the picture above is not to scale; the Debye length is only about 1 nm so many ions are clustered very closely in around each of the strands of the backbone.)

This activity in this format was developed initially by Ben Geller and the University of Maryland PERG/BERG after discussions with Catherine Crouch.