As educators, we are continually designing new methods and procedures to enhance learning. During this process, good ideas are frequently generated and tested, but the extent of such activities may not be adequate for a full manuscript. Nonetheless, the ideas may be quite beneficial in improving the teaching and learning of physiology. Illuminations is a column designed to facilitate the sharing of these ideas (illuminations). The format of the submissions is quite simple: a succinct description of about one or two double-spaced pages (less title and authorship) of something you have used for the classroom, teaching, laboratory, conference room, etc. You may include one or two simple figures or references. Submit ideas for inclusion in Illuminations directly to the Associate Editor in charge, Stephen DiCarlo (sdicarlo@med.wayne.edu).

“Challenge” Questions to Enhance Laboratory Experience and Student Skills: an Example

I teach a section of a mandatory, second-year undergraduate laboratory course: Scientific Methods in Biology. The course deals with experimental design, instrumentation, the evaluation of experimental data, and scientific writing. One experimental approach I have developed within the course quantifies light-dependent proton translocation across the thylakoid membranes of isolated chloroplasts. Experimentally, this involves measuring the pH increase in a solution in which chloroplasts are suspended using a pH electrode attached to data-acquisition software. Energy from absorbed photons generates a flow of electrons from water through a series of complexes associated with the thylakoid membrane to a final electron acceptor. In one step, electrons are accepted by plastoquinones that consequently become reduced. As the plastoquinones become reduced, they also accept protons from the outside of the membrane. When the plastoquinones become oxidized by passing electrons on to the next acceptor, they deposit the protons on the inside of the membrane. Consequently, protons are translocated from the outside to the inside of the thylakoids, and the decline in proton concentration in the bathing solution can be measured as an increase in pH. A variety of different treatments can be investigated using this method.

After students have done experimental work with this method, I present them with a “challenge” question designed to give them experience in thinking logically and applying information learned in introductory chemistry in a biological context. The question is as follows:

If the pH of the solution in which chloroplasts are suspended changes from 7.36 to 7.67 during a period of illumination, what assumption must be made and then requires the students to make a series of calculations associated with hydrogen ion concentration and volume. Finally, I make sure that they know my e-mail address.

I tell them that I haven’t given them all of the information they need to answer the question and that they will need to make one reasonable assumption in reaching the solution. The problem makes the students think about what other information is required and what assumption must be made and then requires them to make a series of calculations associated with hydrogen ion concentration and volume. Finally, I make sure that they know my e-mail address.

The missing information is the internal volume of the thylakoids. When they tell me this, I give them a published value of 10 µl/mg chlorophyll (2). The assumption that needs to be made is that, prior to illumination, the pH values on both sides of the membrane are equal. The explained solution to the problem is as follows:

We begin with isolated thylakoids containing 3 mg of chlorophyll suspended in 10 ml of solution. The published internal thylakoid volume is 10 µl/mg chlorophyll.

The internal thylakoid volume is 3 mg × 10 µl = 30 µl.

If the pH of the solution changes from 7.36 to 7.67 during a period of illumination, its original proton concentration is as follows:

\[ [H^+] = 10^{-pH} = 10^{-7.36} = 4.37 \times 10^{-8} \text{ mol protons/}l \]

Its final proton concentration is \(10^{-7.67} = 2.14 \times 10^{-8} \text{ mol protons/}l\).

\[ 4.37 \times 10^{-8} \text{ mol protons/}l - 2.14 \times 10^{-8} \text{ mol protons/}l = 2.23 \times 10^{-8} \text{ mol protons/}l \text{ translocated into the thylakoids.} \]

The volume of the reaction mixture is 10 ml (=0.01 liters);

\[ 2.23 \times 10^{-8} \times 0.01 = 2.23 \times 10^{-10} \text{ mol of protons have been translocated from 10 ml of reaction mixture into 30 µl of thylakoid lumen.} \]

If we assume that the original internal pH is in equilibrium with the original external pH, then the internal pH starts at 7.36, which equals \(4.37 \times 10^{-8} \text{ mol protons/}l\).

Since \(30 \mu l = 3 \times 10^{-5} l\), \(4.37 \times 10^{-8} \text{ mol protons/}l \times 3 \times 10^{-5} = 1.31 \times 10^{-12} \text{ mol protons/30} \mu l\).

\(1.31 \times 10^{-12} \text{ mol protons/30} \mu l + 2.23 \times 10^{-10} \text{ mol of protons translocated into the thylakoids} = 2.24 \times 10^{-10} \text{ mol protons/30} \mu l \text{ of thylakoid lumen.} \)

\(2.24 \times 10^{-10} \text{ mol protons/30} \mu l \div 30 = 7.47 \times 10^{-12} \text{ mol protons/}l \times 1 \times 10^6 \mu l/1 = 7.47 \times 10^{-6} \text{ mol protons/}l. \)

\[ \text{pH} = -\log 7.47 \times 10^{-6} = \text{pH} 5.13. \]

The internal pH declines from 7.36 to 5.13 as a result of proton translocation during the period of illumination.

Clearly, the solution presented here is a bit simplistic. It ignores, for example, proton buffering power inside and outside of the membrane and so underestimates the magnitude of proton translocation. If, however, the question is regarded as an “order of magnitude” (or Fermi) estimate, it has considerable pedagogical value. “Fermi estimates encourage students to reason creatively with approximate quantities and uncertain information” (3).

In discussion with students who have accepted this challenge question, I was pleasantly surprised to hear that it was “fun” or “exciting.” All reported that their understanding of the process that we had measured in the laboratory was enhanced by thinking through the problem, and many were surprised by the magnitude of the proton gradient that they calculated. Students who collaborated on the problem commented that it was a good stimulus for reciprocal peer teaching. Some seemed to feel empowered, or that their
confidence had been enhanced, by integrating logic, their knowledge of physical chemistry, making appropriate assumptions, and thinking about what other information was required to find a solution.

In its Recommended Curriculum for Programs in Biochemistry and Molecular Biology, the American Society for Biochemistry and Molecular Biology includes a list of skills that biochemistry and molecular biology students should obtain by the time they have finished their undergraduate program (1). These include the following:

- The ability to dissect a problem into its key features.
- The ability to think in an integrated manner.

Challenge questions like the one outlined here contribute to the development of these desirable skills.

REFERENCES


Rob L. Dean
Biology Department
University of Western Ontario
London, ON, Canada N6A 5B7
E-mail: rdean1@uwo.ca
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