

## PROBLEM SOLVING IN BIOCHEMISTRY.

What does this course try to accomplish?

My objective is to create a laboratory-type experience without having the students work in the lab. You wear no goggles or gloves, manipulate no equipment, and never have to worry about doing experiments incorrectly. And, there is no cleanup.

I do the experiments or I provide simulated experiments—data sets for example. We cover the part of a biochemistry lab experience that involves analyzing and interpreting results, reducing data to tables, graphs, and diagrams, and, most of all, dealing with (1) contradictions, (2) confusing results, (3) data that are internally inconsistent, and (4) results that seem to go against what you have previously learned. The course is exactly what its title says—problem solving.

What do you need for the course?

- (a) It is necessary to have a firm grounding in biochemistry and biochem lab, otherwise you will have trouble following some of the things I do or say.
- (b) You **MUST** ask questions. In fact, very few problems I introduce can be solved without a lot of in-class dialogue. If you are shy, get over it. I never criticize or embarrass a student in the classroom. Instead you will be encouraged and congratulated when you ask questions or respond to what I say or your colleagues say. I prefer to joke around and make light of my own limitations more than yours. You will hear a lot about my life's story because professors are programmed to talk and because you can relate better with me if I tell you some of the stupid things I have done in the lab.
- (c) There is no test book (the course is free), but I will suggest you download a fairly long review paper I wrote called, "The art of protein purification." It was published in an open (free) electronic journal called InTech Open. I paid for the site's being free, so you don't have shell out any money. I like humor and satire, so check out Father Guido Sarducci's "The Five Minute University. It comes right up on Google.
- (d) You need to take complete notes and you should look over the notes soon after class is over. Do as I say, not as I did as a student.
- (e) You need to keep a positive attitude. The course is not easy, but once you know the solution to the problem, all that is left is describing what happened and explaining why it happened as it did.

What is a typical class day (or fortnight) like—you are working on a 14 day cycle?

- (a) On TUESDAY, I will give you a chance to ask questions about last Tuesday's experiment and then I will do a new experiment or, more often, describe how the new experiment was originally conducted and I will provide relevant data. After the presentation is over—usually less than 30 min, the new experiment is open for discussion. You **MUST** participate. I guarantee you that the more you speak up in class the better you will do and the easier the course will be. Most of the questions you ask, I will answer directly and honestly. But, once in a while I will give an

ambiguous response—“Well, have you thought of this possibility----? Is this an expected result? Did you understand what actually happened? The most helpful answers will come on Thursday.

- (b) On THURSDAY, I will spend the whole 55 minutes answering questions. If you cannot fill the 55 minutes with questions, I may leave. I have done this before. Near the end of the class period, I hope that everyone now has “the answer.” You may not have a clear understanding, but you should not leave the room unless you know where the solution is headed. As you come closer and closer to the right answer, I will begin encouraging you more than I had been before. I will say things like, “You are absolutely right. You have gotten to the heart of the problem. But, you have left out one detail, a particular set of data. Do you know what data I am referencing? And the dialogue (really polylog—but that sounds like a larval frog) will continue. When someone gets it exactly right and expresses his/her solution to the problem quite clearly, I am likely to shout out, RIGHT! If you are that person, everyone else will be most thankful—you will be a hero.
- (c) On the next TUESDAY, the process repeats—It’s last chance, in class, to ask questions you wish you had asked on Thursday, and we start up a new experiment
- (d) On the next THURSDAY you turn in a report (you have had 14 days to work on the report—assuming you have no other courses or extracurricular activities to get in the way—like working for a living).
- (e) What is required in the report? (1) You will have no methods and materials section. (2) You need to describe the experiment. (3) You need to present the data—all of it. (4) You must reduce the data—charts, graphs, sketches, tables, etc. (5) You need to present your hypothesis as to what happened and why. (6) You need to clear up the contradiction or the mystery or the counter-intuitive aspects of the results. And always, once you present your interpretation, you must defend what you say.

What are the goals of the course?

1. This is like a regular lab course in some respects. The big difference is that almost 100% of your effort will be on 5 areas that involve no hands-on manipulations. These five areas include:
  - (a) data presentations (graphs, tables, data reductions, etc.,
  - (b) interpretations,
  - (c) conclusions,
  - (d) implications of those conclusions, and
  - (e) effective communication via writing.

You will not get your hands (or gloves) wet. There is nothing hands-on for you to do other than taking notes. I will be doing any live experiments. Here you just observe and question what is going on. You question and then you question and then you question. When you finish this, you question others' questions. Once you think "you get it," you proceed with the five steps above (a) through (e). Some of the exercises will involve no live experiments. In these cases, you will be given written material about the experimental set up and you will be presented with raw data. Again, you need to go through the 5 steps I listed.

2. From my experience, the easiest part of research is conducting the experiment. The hardest parts are coming up with a testable hypothesis, planning the experiment that directs the testing, and, finally, interpreting the results.
3. You will be interpreting the results

3. You will be asked to come up with a plausible and testable hypothesis, however, without the ability to perform further hands-on testing, you can only propose the hypothesis and you can only say how you would test that hypothesis.

Let's say I tell you that an enzyme's activity was lost when the crude tissue containing that enzyme was homogenized. You could hypothesize that the enzyme has an active site sulfhydryl group that has been oxidized upon vigorous blending in air. Your test of that hypothesis might be to alter the protocol as follows:

- (a) Purge the buffer with nitrogen and homogenize the tissue in a sealed blender, or
- (b) Perform the homogenization in the presence of mercaptoethanol, or
- (c) Add mercaptoethanol to the un-reduced homogenate and hope that the activity will recover.

Alternatively, you might suggest that the enzyme underwent surface denaturation caused by the generation of a huge number of air/water interfaces caused by vigorous blending. Or, you might suggest that the enzyme is so hydrophobic that it precipitates in water.

4. By the time the course is over, you are expected to:

- (a) Understand how to create meaningful and understandable graphs, tables, charts, spectral profiles, and other forms of reduced data.
- (b) Interpret experimental results
- (c) Formulate relevant and useful hypotheses
- (d) Describe how your hypothesis could be tested
- (e) Feel comfortable asking questions even if you fear personal embarrassment
- (f) Improve your scientific writing skills
- (g) Form a better idea of the profession you want to pursue

## EXERCISE # 1

Initial information

Absorbance (optical density) =  $ECl$  where  $E$  = molar extinction coefficient,  $C$  = molar concentration and

$l$  = optical path length. So,  $E = OD/Cl = OD/C$  as pathlength is usually 1.0 cm. In biochemistry we often use a different form of extinction coefficient based not on molarity but on concentration in mg/ml.

Figure 1 shows the absorption spectrum of a 99% pure sample of recombinant Jellyfish GFP from *Aequorea Victoria*.

Figure 2 shows the ratio of  $A_{475}/A_{397}$  as a function of log protein concentration in mg/ml. The extinction coefficient of this protein at 276 nm is very close to 1.0. So, based on this number, the protein concentration is  $0.846/1.0 = 0.846$ . The established extinction coefficient at 397 nm is 1.25, so, based on this value, protein concentration is  $0.993/1.25 = 0.794$ . Don't be surprised that the two calculations do not agree. There are reasons that you might be able to deduce later.

MORE DATA AND IMPORTANT QUESTIONS (not necessarily in the most logical order):

1. Most proteins have spectra with peaks in the vicinity of 280 nm. While the UV-Vis spectrophotometer shows the UV peak in Fig. 1 to be centered at 280 nm, this peak is really at 276 nm. What does a peak at 276 nm, with a weak shoulder at 290 nm, tell you about the amino acid composition of this protein? What does the scalloped appearance to the left of the 276 nm peak tell you about amino acid composition?
2. The chromophore of every known GFP is covalently bound in the center of a fairly rigid beta can, that beta-can being formed by 11 beta strands that wrap around the central region of the protein like stripes on a barber pole.
3. The chromophore of Aequorea GFP has two absorbance peaks, one at 397 nm and another at 475 nm. The wavelength positions and relative intensities of these peaks are invariant at pH values from about 6.5 to 11.5.
4. The absorbance (excitation) bands at 397 nm and 475 nm come from a single chromophore that oscillates between two electronic forms.
5. How was it possible for me to acquire such precise data over a 340,000-fold range in protein concentration (as shown in Fig. 2)
6. Why is the relationship between absorbance ratio and protein concentration semi-logarithmic? The answers to questions 5 & 6 are central to the solution of this problem.
7. Under the same conditions as those for Aequorea GFP (as shown in Fig. 1) the green fluorescent proteins (from the sea pansy *Renilla reniformis* and from a variety of other sea pansies and sea pens) have chromophore absorption maxima at 470 nm and 498 nm.
8. There is no change in peak position or ratios of peak heights for any of these GFPs.

ASK ALL THE QUESTIONS YOU CAN THINK OF THIS PERIOD. ON THURSDAY (55 MIN), YOU WILL BE ABLE TO ASK QUESTIONS NON-STOP. Q & A IS ALL THAT WE DO ON THURSDAYS. NEXT TUESDAY WE WILL HAVE A NEW PROBLEM. REPORTS ON TODAY'S PROBLEM ARE DUE A WEEK FROM THIS COMING THURSDAY.

**\*\*Note: This syllabus/schedule is subject to change. You will be notified of changes before or in the class.**