Teaching Protein Purification and Characterization Techniques

A Student-Initiated, Project-Oriented Biochemistry Laboratory Course

Gina MacDonald
Department of Chemistry, James Madison University, Harrisonburg, VA 22807; *macdongx@jmu.edu

For many years anecdotal evidence has suggested that undergraduate research experiences are key components to retaining the next generation of scientists. Formal assessment studies have substantiated this view and highlighted that these experiences provide unique benefits that are not found in other chemistry courses (for some examples see ref *1–4*). The recent assessment studies clearly indicate that bringing research experiences to a larger number of undergraduates may be a key determinant as we work to increase the number of science students *(1–4).* Before formal assessment studies were available, educators believed that research-like experiences in undergraduate laboratories would help retain students and help prepare them for teaching, graduate school, and the workforce. Funding agencies and educators are interested in bringing the excitement of research to a larger, more diverse population of students. In response to this interest, efforts to modify teaching laboratories have led to the development of project-oriented chemistry and biochemistry labs that infuse traditional laboratories with a flavor of research. These efforts are especially crucial for the large numbers of students who are not able to participate in the traditional mentored undergraduate research experience.

This university has some history of integrating research experiences into science laboratories *(5, 6).* The biochemistry laboratory is a two-credit lab that is scheduled for four hours per week and is typically populated with six to twelve third-year or fourth-year chemistry, biology, and biotechnology majors. The first semester of biochemistry lecture is a prerequisite for the laboratory and typically covers the basic principles of protein purification. The required laboratory text *(7)* contains detailed information about biochemical techniques. I have taught the laboratory for many of the last eleven years and have gradually modified the laboratory such that is now almost entirely project-oriented and student-driven. My initial reasons for changing the laboratory included expanding students' interest in biochemistry, enhancing critical thinking skills, and ensuring that the students learn to think about the theory behind the biochemical techniques used to purify and characterize proteins. The laboratory is now based on student-initiated projects and is briefly described as a highlight in the Council Undergraduate Research's publication entitled: Developing and Sustaining a Research-Supportive Curriculum: A Compendium of Successful Practices *(8).* Here I present a detailed description of the most updated version of this laboratory in hopes that others may use the ideas to more fully integrate research and teaching at their home institutions.

There are numerous, successful project-oriented laboratories that focus on the purification or characterization of a single protein *(9–17).* Even with previous successes obtained in project-based laboratories, many educators are reluctant to abandon all aspects of a traditional laboratory. The biochemistry laboratory described here lacks formal structure and traditional laboratory experiences. However, if desired, one or more standard experiments may be used to acquaint students with some basic calculations and techniques such as determination of protein concentration and gel electrophoresis. The timeline for the purification projects is driven solely by the syllabus deadlines and student motivation. The difference between this laboratory and most of the previously described project-oriented laboratories *(9–17)* centers on the idea that each small group of students chooses their own protein for purification. The instructor(s) may suggest proteins from ongoing independent research projects or proteins that have a good chance at successful purifications. Proteins such as pyruvate kinase or lactate dehydrogenase have worked well for some student groups. The students are informed that any protein that is naturally abundant and can be purified from *E. coli* or something easily obtained from the grocery store or slaughterhouse may be suitable for purification. Instructor consultations should ensure that students choose a purification that involves centrifugation and column chromatography. The students are directed to use an appropriate library research database such as PubMed, Scopus, or SciFinder Scholar to search for protein purification papers. The students must search the literature to identify a protein of interest (or a purification procedure for the protein associated with their research interests) and then come up with a list of supplies and an outline of the purification procedure. Substitutions of column resins may be suggested based on budgets and availability. Once the students have completed their plans they begin calculations and proceed to make buffers. Subsequently, the students "jump in" to their protein purifications. Over the semester the students learn all of the techniques described in List 1. Once the semester is in full swing experiments and equipment time may need to be thoughtfully scheduled from week to week. Minimum equipment needs should include a UV–vis spectrophotometer and a gel-electrophoresis setup for every two groups, a pH meter, a centrifuge, and a variety of glass columns for chromatography.

In addition to the techniques in List 1, the students are required to determine the protein concentration and activity of each of their purification steps so that they are able to generate a complete protein purification table. The final "most purified"

List 1. Techniques and Performance Objectives for the Biochemistry Laboratory

Search and utilize the primary literature
Perform calculations and make buffers
Perform centrifugation
Perform column chromatography
Perform SDS gel electrophoresis
Perform total protein assays using a UV–vis spectrophotometer
Perform enzyme activity assays
Write a formal (publication form) laboratory report
Peer-review student reports
Prepare and deliver an oral presentation

Table 1. Sample Biochemistry Laboratory Syllabus

Week	Schedule
1	Introduction to the laboratory. Biochemical calculations and project explanation.
2	Lecture on protein purification and characterization, buffers, pH, etc. Meeting with project partners for independent research, discussion, and selection of project. The topic must be approved by the instructors before proceeding. Begin making buffers.
3	Hand in the project's specific aims and procedure to be followed. List of materials necessary for purification. Materials that need to be ordered. Begin making buffers or working on projects.
4–7	Independent projects; draft of introduction and materials and methods to date.
8	Independent projects
9	Spring break
10–11	Independent projects
11	Draft of introduction, materials and methods, results and discussion to date.
12–14	Independent projects; first full draft of project paper due.
15	Peer review of papers due.
16	Group oral presentations; final project paper due.

protein fractions are then used for characterization projects. The characterization may include examining activity as a function of salt concentration or using infrared spectroscopy (IR) or circular dichroism (CD) to monitor solution dependent structures or the unfolding–folding of the protein. The culmination of the laboratory is a final report that is written in the form of a research publication, peer-review of other class members' reports, and group oral presentations of the results obtained during the semester.

Description of the Laboratory Course

Although there is a required text for the laboratory *(7),* much of the student's information is obtained from the primary literature. Based on the experience of students working in groups of two to four per group, the students are typically assigned to groups of three if at all possible. Final grades are assigned on the basis of cumulative scores of required drafts, class participation, final project report, and oral presentation. A typical grading scale is as follows: individual project is 75% and class participation is 25%. The individual project assessment includes notebook, topic approval, specific project aims, a plan of procedure, paper drafts, final paper, peer reviews, and oral presentation. The fact that the grading is based on specific assignments and participation (as opposed to results) allows enough flexibility to ensure students who choose a challenging purification can still earn a high grade. Each student is graded independently on class participation relative to all students in the class. This system ensures that students are rewarded based on their independent contributions as opposed to the entire group's efforts. Table 1 shows the tentative schedule given to the students at the beginning of the semester.

Hazards

The hazards for this lab are dependent on student projects chosen such that the hazardous chemicals will vary from year to year. Premade electrophoresis gels can be purchased to reduce student exposure to acrylamide (a carcinogen and neurotoxin). Sodium dodecyl sulfate (SDS) can irritate the eyes and skin and beta-mercaptoethanol is toxic by inhalation or skin contact. After choosing the necessary chemical reagents students and instructors should handle the buffers and reagents appropriately. Careful instruction about the use of the ultracentrifuge should be given to each student.

Results and Discussion

The sooner the student groups perform their initial literature research and choose their protein, the better their chance of success. Much of the first laboratory period can be dedicated to searching the literature and obtaining immediate feedback about purification papers and ideas from the instructors. Most student groups have identified their protein by the second week of laboratory. Students learn techniques as they progress through the laboratory.

Clear communication and uplifting conversations are necessary throughout the semester as students will encounter multiple problems. Keeping students motivated is especially important for students who have not worked in a research laboratory and who have only been exposed to more typical laboratory experiences that always "work". Students may complete a purification one time and find they have little or no active protein. It is not uncommon that students attempt the purifications at least two times. However, after the initial learning curve the purification will proceed much more rapidly. It is interesting to note that the most learning seems to occur after a technique did not work as expected. The surprising results seem to inspire the most thought about subsequent purification steps. Instructors must be careful to ensure that each member of the group is participating (the participation grade is critical) and that each student has performed all of the techniques. Although the laboratory is scheduled for one afternoon and students are required to show up for at least part of that laboratory period, students often elect to work additional hours. To maximize equipment time, some student groups may decide to work outside scheduled laboratory times if an instructor is in the building and available for questions.

The lack of laboratory structure and the student ownership of their projects led to increased student excitement and participation. Students want to obtain pure protein and start the characterization phase of the project. Interactions between the instructor(s) and students resemble a typical research laboratory

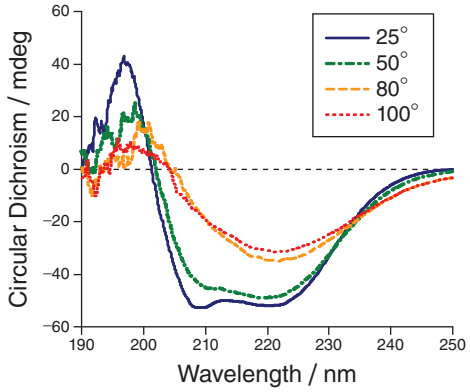

Figure 1. CD spectra of student-purified lactate dehydrogenase at increasing temperatures: 0.7 mg/mL of purified LDH in 50 nm phosphate buffer (pH 7.4) was used to obtain the CD spectra. The figure was taken directly from the student's lab report.

as opposed to a teaching laboratory. Having two instructors in the laboratory allows for more ideas when problems are encountered (this happens daily) and enhanced student–instructor interactions. Requiring initial drafts of the final project report helps to ensure students start their reading and writing in a timely manner and understand the protein they are attempting to purify.

Almost all students have been successful in obtaining active protein with varying levels of purification. Examples of proteins that the students have chosen to purify over the years include myoglobin, lactate dehydrogenase, pyruvate kinase, mungoin protease inhibitor, α-glucosidase, rubisco, RecA, and phosphoglycerate kinase. The design of this laboratory can be used to integrate education and research. Some students may be interested in a protein related to their independent research. However, students will often choose proteins based solely on personal interests or the perceived ease of purification. If students choose a protein that is easily purified they are expected to have a larger portion of the project dedicated to characterization. However, some students will choose proteins that are quite difficult to purify and will have minimal characterization. It should be noted that many purifications will not result in a single band on a SDS gel and that students may end up characterizing a preparation that is not pure. However, in this case students will often times study activity as a function of salt or temperature instead of using IR or CD to study the protein structure. Figure 1 shows the CD spectra[1] obtained on bovine heart lactate dehydrogenase (LDH) and presented in a formal lab report. The protein was purified by students using centrifugation and a combination of column chromatographic techniques.

Summary

Although there are different degrees of purification success, all students learn the standard biochemical techniques while learning how to work in groups, write formal reports, review other students' reports, and present their results to a group of their peers. The lab teaches students how to use the primary literature and increases their problem-solving skills. Although the student grades are not totally dependent on the purity of the protein, the students work hard to purify and characterize their protein. The most important benefit of the lab is that it enhances the interest of students and instructors and works toward increasing students' understanding of biochemistry and the scientific process.

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Note

1. The CD spectrometer was recently obtained through a NSF-MRI grant and has allowed the students to characterize the overall structure and the folding–unfolding of their purified proteins.

Literature Cited

1. Seymour, E.; Hunter, A.-B.; Laursen, S. L.; Deantoni, T. *Science Educ.* **2004,** *88,* 493–534.
2. Hathaway, R. S.; Nagda, B. A.; Gregerman, S. R. *J. College Student Develop.* **2002,** *43,* 614–631.
3. Nagda, B. A.; Gregerman, S. R.; Jonides, J.; von Hippel, W. V.; Lerner, J. S. *Rev. Higher Educ.* **1998,** *22,* 55–72.
4. Lopatto, D. *Cell Biol. Educ.* **2004,** *3,* 270–277.
5. Amenta, D. S.; Mosbo, J. A. *J. Chem. Educ.* **1994,** *71,* 661–664.
6. Monroe, J. D.; Knight, I. T. *CUR Quarterly* **1996,** *16,* 109–114.
7. Robyt, J. F.; White, B. J. *Biochemical Techniques Theory and Practice;* Waveland Press, Inc: Prospect Heights, IL, 1990.
8. MacDonald, G. Best Practices for a Research-Supportive Curriculum. In *Developing and Sustaining a Research-Supportive Curriculum: A Compendium of Successful Practices;* Karukstis, K. K., Elgren, T. E., Eds.; CUR Publications: Washington, DC, 2007.
9. Craig, P. A. *J. Chem. Educ.* **1999,** *76,* 1130–1135.
10. Deal, S. T.; Hurst, M. O. *J. Chem. Educ.* **1997,** *74,* 241–242.
11. Brockman, M.; Ordman, A. B.; Campbell, A. M. *J. Chem. Educ.* **1996,** *73,* 542–543.
12. Stahelin, R. V.; Forslund, R. E.; Wink, D. J.; Cho, W. *Biochem. Mol. Biol. Educ.* **2003,** *31,* 106–112.
13. Wolfson, A. J.; Hall, M. L.; Branham, T. R. *J. Chem. Educ.* **1996,** *73,* 1026–1029.
14. Harman, J. G.; Anderson, J. A.; Nakashima, R. A.; Shaw, R. W. *J. Chem. Educ.* **1995,** *72,* 641–645.
15. Markwell, J. *J. Chem. Educ.* **1993,** *70,* 1018–1019.
16. Russo, S.; Gentile, L. *J. Chem. Educ.* **2006,** *83,* 1850–1852.
17. Vincent, J. B.; Woski, S. A. *J. Chem. Educ.* **2005,** *82,* 1211–1214.

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