Class Meeting Time
Lecture: Attended by all students
Tuesdays and Thursdays, 11:30 am-12:20 pm

Lab: Students attend one lab section
Section 001 Tuesdays at 1:30-5:30 pm
Section 002 Thursdays at 1:30-5:30 pm

Prerequisites
Successful completion of CHM 309 Biochemistry I.

“The Biochemistry/Molecular Biology (BMB) teaching laboratory course has become a prominent and essential component in the training of undergraduate students for careers related to the molecular life sciences. These students must acquire extensive experience working with biomolecules and biological processes in the laboratory and a formal lab course is usually the best, first step to that experience. The laboratory provides students with the skills needed for future research participation at the undergraduate and graduate level, and for jobs in the biotechnical and pharmaceutical industries. In addition, a lab experience is an asset for those science majors preparing for careers in law and business which may be related, but outside the realm of the basic sciences (patent law, pharmaceutical sales, etc.).”

Course Goals
In this course, students will obtain expertise in the use of laboratory techniques commonly used in biochemistry and molecular biology. For each experiment, you will learn a new laboratory technique, use that technique to collect reproducible data, use your data to calculate specific results and use your biochemical knowledge to interpret and explain your results. In so doing, you will learn how to evaluate scientific data and to think critically. You will learn how to keep a laboratory notebook that will be useful to yourself and to others. You will learn how to communicate your results by writing a journal-style final paper that summarizes your work in the course.

Rather than performing experiments with known outcomes, this course will use an inquiry-based approach and will be run as if our class is a research laboratory. A course organized as a research project is referred to as a “CURE” (Course-based Undergraduate Research Experience). A CURE is defined as a novel form of laboratory instruction that provides students with hands-on experience performing original research in a laboratory setting. By using a CURE, rather than a traditional approach, students carry out experiments that have never been done before (rather than repeating investigations in which outcomes are known). In this way, each student generates new knowledge that contributes to the scientific community.

Course project
Each student will construct, using the technique of site-directed mutagenesis, a mutant of the carbonic anhydrory enzyme from *Haemophilis influenzae*. Students will examine the three-dimensional structure of the enzyme and choose at least one surface residue to mutate to a His
residue. His residues serve as a “chromatographic handle” to bind to a Ni$^{2+}$ affinity column. All mutants should retain enzymatic activity.

**Required Materials**
We will provide all students with a printed, three hole-punched lab manuals, so please purchase a three-ring binder to store this manual. Bring your laptop, a calculator, and a flash drive with you to lab each week. Lab coats and eye protection will be provided, but you might want to buy your own safety glasses which can be obtained inexpensively on Amazon.

**Software**
Please download these two programs onto your laptop:

- **ApE**: A plasmid Editor (free)
- **PyMol**: The educational version is free; we have the paid version (with additional features); the license file to access the paid version will be provided to you.

**Safety**
Safety must be the foremost concern in any teaching or research laboratory. To help ensure your safety and the safety of those working around you, the Department of Chemistry and Biochemistry has adopted a set of regulations that students are expected to follow. Students must complete an online training course each year and sign the laboratory Standard Operating Procedure (SOP). Students must complete “After Hours” forms to obtain access to the laboratory outside of our normally scheduled laboratory period.

**Lab Preparation**
You should also come to the laboratory period having read the appropriate laboratory protocols, and with your notebook prepared.

**Laboratory Notebooks**
In this course you will keep a laboratory notebook. In all branches of science, it is extremely important to keep a complete and accurate record of experimental work. The purpose of a laboratory notebook is to allow anyone with some biochemical knowledge to understand exactly what you did. You need to record the information in sufficient detail to be able to repeat it, and you must be able to understand exactly what your results were. You will need good notes to be able to write your final paper. You may also find that as your understanding of biochemistry improves as the course progresses, your notebook may allow you to figure out why some parts of your earlier experiments did not work as expected and this can provide a means for performing successful laboratory work in the future.

**Biochemistry laboratory—a Writing Intensive II experience**
As a course that meets the requirement for the Writing Intensive II Proficiency of the College’s Core Curriculum, there are several objectives that must be met, including (1) the requirement for a variety of writing assignments, (2) the opportunity for ample practice in writing outlines, revising drafts, and editing, (3) the development of student ability to write insightful and well-organized essays, (4) the development of student ability to use various stylistic techniques, and (5) the requirement for the proper use and correct citation of sources. In this course, we will meet the requirements by focusing on each one of the five sections of a scientific paper. We will spend class time discussing how the
section is to be written, then each student will write a draft of that particular section. The instructor will critique the drafts and discuss them with you before you turn in your final paper.

Publication on Digital Commons
Students who complete excellent work will be invited by the instructor to publish their final papers on Providence College’s Digital Commons, an open-access publishing system for research, scholarship and creative expressions by Providence College faculty and students in all media and formats. Scholarship and creative expressions included within Digital Commons at Providence College are required to be reviewed. Papers published on Digital Commons can be accessed by scholars across the country and around the world.

The Phillips Memorial Library Undergraduate Craft of Research Prize
This prize recognizes excellence in research and use of library resources by the undergraduate community at Providence College. You will consult library resources when writing your final paper. In addition, we will provide you with original and “historical” papers that were not available at our library but were obtained through the College’s generous interlibrary loan program.

Winners will be selected by a committee of librarians and faculty. Prizes will be awarded for three winners, and two honorable mentions. The deadline is 15 January of every year and winners are announced in April. All currently enrolled are welcome to submit. Submissions must be at least seven pages in length and can be on any topic: http://providence.libguides.com/research_prize

If you think you might be interested in submitting your final paper for consideration for a prize, you will be required to submit:
• A 500-700-word essay describing your research process including specific sources–primary sources, databases, journals, websites, books, eBooks, videos, etc., used and strategies employed.
• An annotated bibliography with at least 10 annotations.

Prizes will be awarded based on the following criteria:
• Ability to select and evaluate appropriate source material from the Phillips Memorial Library’s databases, the Helin catalog and InRhode, and integrate it into an effective research paper.
• Originality of topic
• Writing and communication skills in presenting a thesis and supporting it with effective research and language.

Expected Academic Conduct
The Providence College Bulletin states, “Acts of academic dishonesty (plagiarism, collusion, cheating, etc.) are subject to an appropriate penalty.” Acts of academic dishonesty must be reported by instructors to the Dean. Penalties will be determined by the instructor and will vary in severity based on the nature of the misconduct. Examples of academic honesty include (but are not limited to): falsifying data, recording another student’s data without attribution, using print or online information in your paper without attribution, sharing electronic files with other students in the class or students who have previously completed the course, or using another student’s paper from a previous year as the basis for writing your own paper. When an experiment is done with a partner you may consult with the partner before preparing the paper to ensure that both partners have the same data or if you get stuck on calculations. Even if an experiment was performed individually, verbal consultation with a colleague is allowed. However, each person must hand in an individually prepared paper. The tables, graphs, discussion, etc. must be unique to your paper. The final paper must represent the student’s own work.
Reading a Research Paper: The “Methods and Logic” Approach

In this course, we will be writing our own research papers, and one of our goals will be to connect what we’ve discovered in the laboratory to the results of other researchers who came before us. One of the ways to learn how to write a research paper is to read the work of others. Reading a research paper requires training and practice, and initially can be daunting, so in order to make the task more manageable, we will use the “Methods and Logic” approach to reading a research paper.

Methods and Logic
Using this approach, we will explore the techniques and experimental design in each figure in the paper. We will integrate the conclusions from the data presented in each figure, and in so doing, we will see how the figures in a paper constitute the author’s narrative of a particular scientific story.

I. Introducing the Scientific Research Paper
   A. What kind of a paper is it?
      1. Research (primary) literature: original research that reports data and mechanism.
         a. Full-length paper–5-7+ figures
         b. Notes/short communications–2-3 figures
         c. Brevia–1 figure
      2. Review (secondary) literature: reviews synthesize data from many published papers on a particular topic. Review articles summarize the current state of research in a field. and are useful gateways for those who are not experts in the field.
   B. Identify the parts of a research paper (not all will be labeled):
      1. Title and author list
      2. Abstract
      3. Introduction
      4. Materials and Methods (sometimes presented at the end of the paper)
      5. Results
      6. Discussion (sometimes the Results and Discussion are combined).

II. Reading the Scientific Research Paper
   A. Identifying the main point of the research paper: Underline the main point in the abstract and in the last paragraph of the Introduction.
   B. Identifying the narrative of the research paper
      1. For each figure, identify the experimental method used. Research the method on your own if you are unfamiliar with the technique.
      2. For each figure, identify the purpose/logic of the experimental method. What question is being asked, and why are the researchers asking this question?
      3. Identify and distinguish between positive and negative controls, and experimental trials. Some figures with descriptive/observational data are not experimental trials and do not have controls.
      4. Underline the sentence(s) in the Results section that describe(s) each figure.
      5. Determine how one figure leads to the next figure in the logic of the narrative.
   C. Conclusions and future directions
      1. Identify the most important figure in the paper.
      2. Underline the main point in the Discussion section that summarizes the narrative.
      3. Underline one sentence in the Discussion section that describes a future experiment that the authors propose to do, then propose a future experiment that you would do.
The purpose of the laboratory notebook

In this course you will be required to keep a detailed laboratory notebook. In all branches of science, it is extremely important to keep a complete and accurate record of all of your experimental work. In a scientific laboratory, chemists keep notebooks in which all data, observations, results and calculations are recorded. A complete notebook will be critically important for writing your final paper.

Above all, it is critical that you enter all procedures and data directly into your notebook promptly, that is, while you are conducting the actual work. Your entries must be sufficiently detailed so that you or someone else could conduct any procedure with only the notebook as a guide. Few students (and not that many researchers for that matter) record sufficiently detailed and organized information, so our challenge to you is to be disciplined from the start and keep a good lab notebook. You will need good notes to be able to write your final paper; in addition, as your understanding of biochemistry improves, you may find that you’ll be able to look back at experiments done earlier in the course and you’ll be able to figure out why the experiment didn’t work as expected. It’s also important to note that we usually record a lot more information in a laboratory notebook than we would report in a research paper.

Companies that perform research require their employees to keep proper notebooks. Researchers in the laboratory keep a chronological record of all of the experiments that they have performed, and at the end of the workday, the notebook is signed by the worker and then co-signed by a coworker or supervisor. Such a record is necessary if you or your employer are to keep your rights to your discoveries. Following proper laboratory notebook procedures decreases the possibility of fraud or other scientific misconduct and allows the notebook to be used as evidence in legal proceedings. In cases of disputes, notebook dates are sometimes used to indicate exactly when an experiment was performed. Ownership of patents can therefore be critically dependent on keeping a proper notebook. Instruction in keeping laboratory notebooks is therefore a major part of most laboratory courses.

In this course, your lab notebook will remain accessible to us, so we may consult your procedures for future course work and for possible publication of your work.

Using an electronic lab notebook

We are choosing that you write in an electronic notebook using OneNote. Modern experimental and bioinformatic research produces enormous amounts of data or high-resolution images that are first generated from computers. Thus, it can be easiest to keep all data and records in an electronic format. Electronic notebooks also present the advantage that your instructors can view and grade your lab notebooks without your losing access to them. OneNote was not designed to be used as a laboratory notebook but has many features that makes it attractive to use in the biochemical research lab.
Structuring your electronic notebook

Your lab instructor will prepare your notebook for you, and the notebook will be named after you. You will also have access to the “Example Lab Notebook” pictured on the left. In OneNote, a notebook has sections, each section has pages, and pages have subpages. Your notebook should have three sections: Protocols, Bioinformatics, and Experiments. You may decide to have your class notes in your lab notebook for easy reference, but that is up to you. The Protocol section will have the common procedures you will find in your printed lab notebook and on Sakai. Notice that these are not in your lab notebook automatically – you will have to construct these protocols in a way you understand. The Bioinformatics section will have the DNA and protein sequences, along with any other pertinent information. The Experiments section will have the specific details and observations made every day in the lab.

You will want to use unique labels that you can trace overtime. If two identical plasmids were produced by two different protocols, and you find one is higher purity or concentration, it is important that you can figure out 1) when you produced each and 2) the protocol used in each. Because of this, do not delete anything. It is OK to fail – in a research lab it is expected often. It is not OK to remove a failed experiment and learn nothing from it. It is important to trace protocols, bioinformatics, and experiments over time. We suggest that, for example, an experiment can be named “EX_001_TS_19Jan2021 pHICA plasmid miniprep”, in which EX = experiment, 001 = experiment number, TS = researcher’s initials, 19Jan2021 = entry date, and pHICA plasmid miniprep = title of the experiment. Protocols, bioinformatics, and experiments can be labeled accordingly: experiments = EX, protocols = PR, and bioinformatics = BI. So, if you are performing a plasmid prep of pHICA, your experiment (EX001) will reference the protocol you are following (PR001) to produce the plasmid (BI001). Your finished plasmid can be labeled pHICA, but on the tube include the experiment (EX001) and plasmid (BI001) for easy reference that can be tracked over time.

Tips on using an electronic notebook

- OneNote allows you to add the current date and time to add in your experiments. For example, you can write “Began incubation at” and for Macs press “Shift+Command+D” or for PCs press “Alt+Shift+F” to automatically enter the current date and time.
- You can produce links in your pages to your other protocols, experiments, or bioinformatics to easily reference pages. If you right-click a page and select “Copy link to page,” return to another page and Paste the link, this will allow for easy reference. This is demonstrated in the “Example Lab Notebook” by referencing the plasmid and the protocol in the current experiment.
- You can add any pdf, word document, tables from Excel, or slides from PowerPoint into OneNote.
- OneNote performs calculations by typing a function followed by an equal sign. For example, type in “4*12=” and hitting space causes 48 to fill in automatically after the “=”.
- The “To Do” tab is helpful to remind you of steps you need to refer to in a protocol. You can make new tags for whatever purpose suits your needs.
Format: What to enter

**Title and date**: Begin each entry with a title and a date. OneNote will add this to the top of every new page you create. The use of numerals only can cause confusion. In every other part of the world other than the US, the day comes before the month. Thus, in most countries April 5, 2019 would be written as 5/4/19 instead of 4/5/19. When you make your first entries, enter the date, write out the month or abbreviation for the month (e.g., 5 Apr ’19, or April 5, 2019, but not 4/5/19), then the year.

**Objective**: An objective is a sentence or two that summarizes the planned work.

**Introduction**: Write a paragraph in which you expand on the ideas noted in the objective.

**Procedures and Notes**: It helps to distinguish the procedure from the notes by keeping them in different subpages in OneNote. A subpage is simply the box that surrounds text or an image. Clicking on a blank page will generate a subpage. Clicking outside of the subpage produces another subpage. It can help to divide the page in half into two columns. The column on the left should be labeled **Procedure** and the column on the right should be labeled **Notes**. On the left-hand side of the page under **Procedure**, you should write out the procedure that you plan to carry out in the laboratory before you begin the work. On the right half of the page under **Notes** you should **record exactly what you did at each step** (being sure to mention anything that you did that differed from the procedure that you wrote on the left-hand side). In addition, you should record any numerical information, such as the weights of reagents used, absorbance readings, enzyme activities, and buffer concentrations. In addition to the results, record your thoughts on the experiment and how you think it is going. Record your mistakes, and your attempts to rectify them. Record the calculations involved in any type of data analysis, as well as explanations for both what you did and what you think it means. You may add your notes after the procedural steps (one advantage of electronic notebooks), but you will need to set it up in a way that you remember to add this information! In the “Example Lab Notebook,” there is a “To Do” tag that marks where to add necessary information, along with general notes marked with “Important” tags. You may set your lab notebook how you choose, but you must clearly indicate the steps you took, and the necessary observations required to repeat the procedure (volumes, masses, absorbances, etc.).

When using the laboratory instruments, we will be recording and pasting the data sheets produced by the various computer programs directly into OneNote. If you have OneNote on a tablet or a phone, you can take a picture of your setup or results and add it to your notebook (make sure the quality is high enough and add enough detail so others can understand). Some of our experiments will extend over several days, and over several pages in your notebook. Either separate the experiments into different pages or be sure to add date and time marks to indicate when each step is finished. If in doubt, it is best to create a new experiment page, especially if the experiment produces a new product or piece of data. It is also important that someone who is reading your notebook can follow the chronology of your work.

**Summary**: When you have finished your work for the day, summarize what you have accomplished. You do not have to draw conclusions, just indicate what sort of data or observations you collected, samples you saved (and where and how you saved them), or any other relevant information that wraps up the study. For a continuing study you can keep the summary brief. Summaries help support continuity. They indicate where the work left off and how it might resume. It may help to write out what you need to do next, or what you need to prepare for the next experiment.
The nitty-gritty
Keep the following in mind:
• List all incubation times. For short times, list the length of times as in “Incubated for 2 minutes.”
  Longer incubation times should use “clock” time, i.e., “Incubated from 3:10 pm until 3:30 pm.”
  Overnight incubations should specify both times and dates, i.e., “Incubated overnight culture from 8:10 pm on 26 January 2021 until 1:45 pm 27 January 2021.”
• When noting centrifugations, you must absolutely record the relative centrifugal force (rcf, written as # × g). For example, “Centrifuged for 4 minutes at 13,500 × g”. You can include the speed in revolutions per minute (rpm) as well, but the rcf information absolutely must be in your notebook.
• Product inserts must be pasted in your lab notebook when these reagents are used.

Repeated procedures
Always reference the procedure in your experiment. If you change the procedure, make another procedure page in OneNote, no matter how small the detail. It is better to note that you have made a change to the procedure, rather than assume you will remember to do it again next time.

Final thoughts
A research project is a journey into the unknown; your laboratory notebook is usually your only guide through this uncertainty. It is a good idea to look over your notebook periodically during the semester and make notes of things that you do not understand, so that you can ask questions before the lab notebooks are due. Do not say “I will remember what this means”; instead, write it down! Do not say “I will remember what I was thinking while I did this”; instead, write it down! Your memory will fade as time progresses, but a notebook will keep your record clean and organized if you meticulously keep it. A great lab notebook will help you draft your final paper, and you will be developing good habits for the future.
In this course, we will write our final laboratory report in the format of a scientific paper. The format we will use will be the *Journal of Biological Chemistry* (www.jbc.org). There are several JBC papers posted on Sakai for you to consult when writing your paper. In addition, you may consult the “Instructions to Authors” on the JBC web site: http://www.jbc.org/site/misc/ifora.xhtml. Note that your final paper should look like a JBC published paper (and not a paper in press). Ten percent of the final paper grade will be assigned in accordance with your ability to follow this requirement.

**Title, Authors**

The title of the paper appears first. The title is a statement written in active voice that succinctly summarizes the most important finding of the paper and should be as short and informative as possible (not to exceed two lines). If acronyms or abbreviations are used, the name/term should be first indicated in full followed by the short form/acronym. Here is an example: “Visualization of polarized membrane Type 1 matrix metalloproteinase (MT1-MMP) activity in live cells by fluorescence resonance energy transfer (FRET) imaging”. A full name is not required for common biochemical abbreviations (e.g., ATP).

Below the title, list yourself as the author of the manuscript. The convention for authors is that the first author is the one who did most of the work; the last author is usually the principal investigator (i.e., the head of the lab); in-between authors are people who made a significant contribution to the manuscript, either in collection of data, data analysis, or interpretation. If you did the experiment largely on your own, you may list yourself as the only student author. The instructors are listed as the last authors, with your section instructor as the final author. If you had a partner or partners, they should also be listed. Minor contributors can be listed in the Acknowledgments section rather than listed as authors. A person listed as an author should not appear in the Acknowledgment section because authorship already acknowledges that person’s contributions.

**Affiliation**

If you are a biochemistry major, list your affiliation as the Department of Chemistry and Biochemistry, Providence College, Providence, RI 02918. Biology majors will list their affiliation as the Department of Biology. If the authors have different affiliations, you will need to indicate the affiliation of each author. You can use funny symbols, as the figure shows below, or you can use footnotes. If all the authors have the same affiliation, footnotes are not needed.

![Figure 1](image-url)

**Figure 1:** This example is taken from paper that appeared in the 10 May 2019 issue of the *Journal of Biological Chemistry*. Note that the title is in the form of an active-voice sentence that summarizes the most important finding of the work. The submission date is listed below the title. The authors and their affiliations are listed. Footnotes are required because all the authors do not have the same affiliation. If all the authors have the same affiliation, footnotes are not needed.
**Abstract**
A well-prepared abstract should enable the reader to identify the basic content of the document quickly and accurately and to decide its relevance to the reader’s interests. Having said this, the abstract should not be dominated by background or introductory material. The abstract, together with the title, must be self-contained as it is often published separately from the paper in abstracting services. Organizationally, the abstract is written as an inverse pyramid beginning with the principal objective and scope of the investigation where these are not obvious from the title. The procedure is described briefly but should not include details of the methods employed unless the study is primarily concerned with methods. The main results are reported. The abstract ends with a succinct statement of the main research finding of the paper. The abstract should be written in the active voice in short and concise sentences. In-depth analysis of the results should not appear in the abstract. Error analysis should also not be presented in the abstract. Omit all references to the literature and to tables or figures and omit obscure abbreviations and acronyms even though they may be defined in main body of the paper. Limit your abstract to 250 words. A sample abstract is shown in Figure 2.

**Figure 2:** The abstract from the paper cited in Figure 1. Note that all of the elements described above are present.

**Introduction**
The Introduction gives the reader the necessary information to understand the rationale and the conclusions of the experiments described in the paper. It is an extended version of the abstract. Thus, it is written as an inverse pyramid beginning with a paragraph that gives the reader the necessary background information for the project for a reader unfamiliar with the topic. This is followed by a paragraph that explains the rationale for the experiment: Given what was known about the science, why did you decide to do the experiments that you did? The final paragraph is a summary of the main research findings of the paper. The reader should be able to explain the entire paper to another scientist by simply reading the last paragraph of the Introduction. The last paragraph of the introduction should be what I like to call a “launch” paragraph that directs the reader’s attention to your specific investigation and is often begun with “Here we present....”
In articles focusing on enzymes – as your paper will do – the Introduction should include at the first mention of the name of the enzyme the Enzyme Commission1 (EC number) in parentheses. Abbreviations should be given, and the reaction should be given as a balanced chemical equation or described in words. Note how the following authors (JBC 277:3698) meet these requirements: “NAD is synthesized via a multi-step de novo pathway or via a pyridine salvage pathway. The enzyme nicotinic acid mononucleotide (NaMN) adenylyltransferase (AT, EC 2.7.7.18) sits at the convergence of these two pathways.” Later in the paper, the authors present a figure with the structures of the reactants and products synthesized by the AT enzyme.

Results
In this section, you should present the data you collected without interpretation. This usually takes the form of figures and tables, with brief accompanying explanations of the data. All figures and tables should be numbered and titled. Figure captions are positioned below the figures, while table captions appear above the tables. Figures are usually elution profiles, graphs (generated in Excel), gels or blots; tables may be generated either in Word or in Excel. A set of instructions on the use of Excel may be found on Sakai.

Data should be presented in the order in which it was collected unless there is a compelling reason not to do this. Present all data that pertain to your project, even data collected by a lab partner or classmate. The contribution of your partner or classmate can be acknowledged by joint authorship or in the Acknowledgments section.

A few notes about graphs:
• Graph titles should be informative and should not be of the “y vs x” variety. Titles should be presented in the figure caption and should not appear at the top of the graph, as you may have done in previous lab courses.
• Use Greek letters correctly and as necessary.
• Use subscripts and superscripts correctly and as necessary.
• Fit data to a linear trend line only when it is appropriate to do so. If data do conform to a straight line, you should carry out a linear regression analysis and report the error in the slope. Recall that the slope of a linear plot contains information and will almost always contain units. Display the equation of the line and the R2 value on the plot and be sure that these values contain at least three significant figures.

Discussion
When writing this section, keep in mind that scientists, when reading published papers, often do not have the time to read papers in their entirety. The number of published papers is staggering, and it is impossible to read them all. Of course, you would carefully read papers published in your field of interest, but if the paper is tangentially related to your field, you might only read the Abstract and the Discussion. Therefore, you should begin the Discussion section by summarizing the goal of the project and the work that you accomplished. Then you will move on to discuss your results and their significance. What biochemical principles have been established or reinforced? What generalizations can be drawn? How do your findings compare to the findings of others or to expectations based on

1The Enzyme Commission web site can be found here: http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html#recommend%00
previous work? If a thorough understanding of the results of others is required for the reader to understand your results, you should describe the previous work in detail and provide appropriate references. Are there any theoretical/practical implications of your work? When you address these questions, it is crucial that your discussion rests firmly on the evidence presented in the Results section. Do not use the words “prove” or “demonstrate”, but rather “suggest”, as in “the data suggest” a certain conclusion. Continually refer to your results, and be quantitative (i.e., list values and percentage changes rather than using the terms “increase” or decrease”, for example). Most importantly, do not extend your conclusions beyond those which are directly supported by your results. Speculation has its place but should not form the bulk of the Discussion section. Error analysis can also form part of the discussion section, but any errors should be reasonable and specific. You are not allowed to blame the equipment, and please do not use the phrase “human error”—you are assumed to be competent. (Of course, if you know you made a mistake, you could describe the mistake in the Discussion and explain how the mistake affected the outcome of the experiment.) End the Discussion section with a summary or conclusion regarding the significance of the work, followed by suggestions for future study.

Experimental procedures
In paragraph form, you should describe your investigation in sufficient detail that a student of your educational background would be able to repeat the investigation and reproduce your results. Each type of procedure should be written as a separate paragraph. At the same time, you should avoid excessive detail that someone familiar with basic laboratory techniques would know. Review other JBC articles to see the level of detail required. Your notebook will include a detailed procedure of the PCR, while your paper will not include such detail. This section should be written in past tense, passive voice. Do not include figures or tables in this section. Do not include any data or results. If the procedure you have followed has been published elsewhere, you may list a reference along with your modifications, if any, to the published procedure. Use subheadings for different procedures. Look at the sample journal articles and note conventions, for example, centrifugation runs are reported as × g and not as rpm.

When describing your experimental methods, you should also list the sources of the materials and equipment that you used. This is best done parenthetically as shown in this recent paper on C. elegans; the investigators purchased a buffer from Invitrogen and a homogenizer and tubes from MP Biomedicals: “Briefly, worms were resuspended in TRIzol (Invitrogen) reagent followed by lysis using a Fast-Prep-24 (MP Biomedicals) homogenizer in Lysing Matrix Tubes (MP Biomedicals).

Acknowledgments
In this section you may acknowledge faculty, students, staff, and anyone else who assisted you in the preparation of your manuscript. Persons cited in the Acknowledgment section are not listed as authors.

References
Please use the JBC format when citing references and use numbers throughout the text. Examples are given on the journal web site at [http://www.jbc.org/site/misc/ifora.xhtml#_References](http://www.jbc.org/site/misc/ifora.xhtml#_References). The references will be listed in the order of their citation. In the references section, the complete reference should be given. Include the following: all the authors listed on the publication (or on the chapter if citing a book); the title of the paper (or chapter if citing a book); the name of the journal.
(or book); editors if a book is cited; volume number; complete pagination (first and last page of the work cited); and year of publication. List only references that you yourself have consulted; do not list references contained in the laboratory manual unless you yourself consulted that reference. It is likely that you will have consulted your lecture and laboratory texts when preparing your manuscript; these references should be cited. On-line resources must be cited as well. **Your final paper must contain a minimum of ten references.**

**A final note—be professional**

- The entire paper should be entirely word-processed; use the Equation Editor for equations and ChemDraw for chemical structures.
- Use zeroes before decimals, i.e., 0.1, not .1.
- Use the proper notation, i.e., use the Greek letter μ and not u.
- Use equilibrium arrows (⇌) when appropriate and do not substitute resonance arrows (↔), which have a different chemical meaning. If your Word program does not have the equilibrium arrows you can obtain the Chemistry True-type fonts or use the equilibrium arrows from the Equation Editor.
- Express numbers in scientific notation correctly: 1.0 × 10⁻⁴ is correct; 1.0E-4 is not, nor is 1.0 × 10⁻⁴.
- To a chemist, brackets [ ] mean “molar concentration”. If this is not what you mean, use parentheses.
- Regarding significant figures, three SF is about right for this laboratory; be sure to format spreadsheets and carry out calculations with due regard for significant figures.
- Avoid use of the terms “create” and “prove”!
- Please submit your document to a grammar/spell check before handing it in.

**Rough drafts**

To ensure that your paper is of the highest quality, you will be required to submit rough drafts of different sections of the paper throughout the semester. Credit is awarded simply for turning in the rough draft; the drafts will not be graded. You will receive feedback on the rough draft so that you may improve the quality of your writing. We will also occasionally devote class time to working on the final paper so that you may ask questions of the instructor as you conduct your work. Rough drafts will be required for the following sections:

**Introduction:** One rough draft, 2% of the grade  
**Experimental Procedures:** Four rough drafts throughout the semester, 1% of the grade each  
**Results:** One rough draft, 2% of the grade  
**Discussion:** One rough draft, 2% of the grade

**Grading**

50% Laboratory notebook  
10% Final paper rough drafts  
40% Final paper (8-12 pages in length)
**Schedule of Class Activities and Experiments**

**NOTE:** Since our biochemistry laboratory course takes the form of a research project, it’s possible that an experiment that you do will not work the first time. That’s okay! It’s happened to all of us, including your professors! When presented with the results of a failed experiment, it’s important to check your notebook and talk with your instructor and trouble-shoot what went wrong and learn what to do differently the next time. The laboratory schedule is show below and note that there is time built into the schedule for students who might need to repeat an experiment.

<table>
<thead>
<tr>
<th>Week</th>
<th>Lecture</th>
<th>Laboratory</th>
</tr>
</thead>
</table>
| 1    | Meeting 1:  
• Introduction to the course  
• Pre-CURE survey  
• Safety training and laboratory SOP  
• Effective use of micropipets  
Meeting 2:  
• Methods and Logic reading of Hoffman (2014), et al. | Lab session 1:  
• Students will consult with the instructor and each other to use PyMOL to design HICA mutants.  
• Please bring your laptops to lab, with the PyMOL program installed. (If you’re having trouble, we will help.) |
| 2    | Meeting 3:  
• Introduction to PCR  
• Designing your PCR primers  
**DEADLINE:** Primer order will be placed at the end of Week 2.  
Meeting 4:  
• Plasmid mini-prep: Theory and Practice  
• Restriction enzymes: Theory and Practice | Lab session 2:  
• pHICA plasmid miniprep (**NOTE:** Students will need to come to lab the night before to start cultures.)  
• Restriction digest  
• Analysis by agarose gel electrophoresis |
| 3    | Meeting 5:  
• Post-lab discussion: Restriction digest analysis  
• PCR: Theory and Practice  
Meeting 6:  
• Preparation for the first round of PCR | Lab session 3:  
• First round of PCR  
• Gel electrophoresis  
• Isolation of product from the gel  
**NOTE:** If no product is observed, students may set up a second attempt at the first round of PCR which can run overnight. |
| 4    | Meeting 7:  
• Post-lab discussion: Results of the first round of PCR  
Meeting 8:  
• Preparation for the second round of PCR  
• Third round of PCR: Theory and Practice | Lab session 4:  
• Second round of PCR  
• Gel electrophoresis  
• Isolation of product from gel  
**NOTE:** If you did a second attempt on the first round of PCR, you should run a gel, cut out the band and then set up the second round of PCR to go overnight. |
### Meeting 9:
- Post-lab discussion: Results of the second round of PCR, review procedure for third round of PCR
- Writing an “Experimental Procedures” section of a journal-style paper

### Lab session 5:
- Third round of PCR

**NOTE:** This laboratory period can be used as a “catch-up” period to complete the first and/or second rounds of PCR, and the third round can be set up to run overnight.

### Meeting 10:
- Writing workshop: Students will be provided with in-class time to work on their Experimental Procedures section.

### Lab session 6:
- Dpn I treatment
- Transformation and plating

**NOTE:** The next day, check your plates for colonies, photograph your plate, parafilm it and store it at 4°C for next week.

### Meeting 11:
- Post-lab discussion: Results of the third round of PCR.
- Continuing discussion: Writing an “Experimental Procedures” section of a journal style paper.

### Meeting 12:
- Transformation: Theory and Practice

### Lab session 7:
- pHICA mutant plasmid miniprep (**NOTE:** Students will need to come to lab the night before to start cultures.)
- Preparation of glycerol stocks to store at -80°C.
- Preparation of plasmids for sequencing (**NOTE:** Plasmids will be sent out for sequencing at the end of the laboratory period.)

### Meeting 13:
- Writing a “Results” section of a journal-style paper

### Meeting 14:
- Sanger sequencing: Theory and Practice
- Discussion of sequencing requirements and proper sample preparation

### Lab session 8:
- Transformation of Xjb cells with mutant plasmids
- Plating

**NOTE:** If you have sufficient plasmid left from Week 7, you can proceed directly to the transformation step. If not, you’ll need to start a culture using your glycerol stock the night before and carry out a plasmid miniprep prior to transformation.

**NOTE:** The next day, check your plates for colonies, photograph your plate, parafilm it and store it at 4°C for next week.
<table>
<thead>
<tr>
<th>Meeting</th>
<th>Topics</th>
</tr>
</thead>
</table>
| **9**  | Meeting 17:  
• Writing an “Introduction” section of a journal-style paper  
Meeting 18:  
• Centrifugation: Theory and Practice  
• Dialysis: Theory and Practice  
**Lab session 9:**  
• Overexpression of mutant HICA protein  
• Cell lysis and protein recovery  
• Dialysis |
| **10** | Meeting 19:  
• Methods and Logic reading of Rowlett on the structure and catalytic mechanisms of β-carbonic anhydrases  
Meeting 20:  
• Writing workshop: Students will be provided with in-class time to work on their Introduction section.  
**Lab session 10:**  
• Cell lysis and protein recovery  
• Dialysis |
| **11** | Meeting 21:  
• ACS Exam in Biochemistry  
Meeting 22:  
• Affinity chromatography: Theory and Practice  
**Lab session 11:**  
• Ni-NTA affinity chromatography of mutant HICA proteins from cell lysate |
| **12** | Meeting 23:  
• Protein assays: Theory and practice  
• We will investigate these concepts by completing a Beer’s Law POGIL activity.  
Meeting 24:  
• Continuation of Beer’s Law POGIL activity.  
**Lab session 12:**  
• Protein assay: The BCA Assay  
• Analysis of data and calculation of protein concentration |
| **13** | Meeting 25:  
• Writing a “Discussion” section of a journal-style paper  
Meeting 26:  
• SDS-PAGE: Theory and Practice  
**Lab session 13:**  
• SDS PAGE of chromatography results |
| **14** | Meeting 27  
• Writing an “Abstract” section of journal-style paper.  
Meeting 28:  
• Gel-filtration POGIL activity  
**Lab session 14:**  
• Course evaluations  
• Post-CURE survey  
• Purification of a selected mutant on gel filtration (sizing) column |