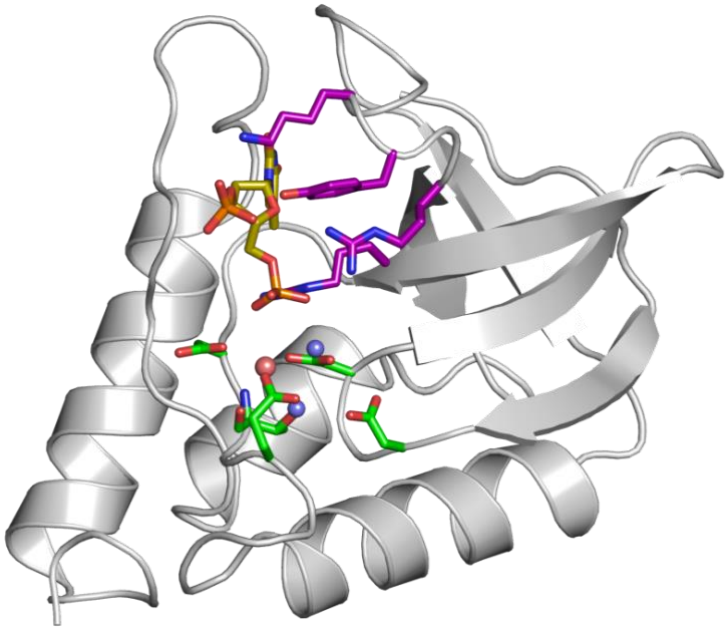
Protein Engineering and Biochemistry Laboratory (PEBL)



Biophysics 250.253

Spring 2022

Department of Biophysics
Johns Hopkins University, Baltimore MD

I. Course information at a glance

Instructors: Dr. Jaime Sorenson (DrJaime@jhu.edu) Office hours posted on Blackboard
Dr. Aaron Robinson (aaronrob@jhu.edu) Office hours posted on Blackboard

Day	Time	Section		TA	Email
Mon	1:30-5:30	Afternoon	01	Iryna Chelepis	ichelep1@jhu.edu
Tue	1:30-5:30	Afternoon	02	Jingzhou Hao	jhao10@jhu.edu
Tue	6:00-10:00	Evening	07	Sushil Pangeni	spangen1@jhu.edu
Wed	6:00-10:00	Evening	08	Tiantian Shang	tshang1@jhu.edu
Thu	6:00-10:00	Evening	09	Qilan Wei	qwei9@jhu.edu
Fri	1:30-5:30	Afternoon	05	Meera Joshi	mjoshi9@jhu.edu

Classrooms: Biophysics Labs in the UTL (G96 and G98)

Grades: Final grades are based on quizzes, worksheet/writing assignments, in-class activities, and a final presentation (details below)

$\geq 90\% = A$; $\geq 80\% = B$; $\geq 70\% = C$; $\geq 60\% = D$; $< 60\% = F$

plusses and minuses will also be used

Credits: 3; W

Textbooks: None

Prerequisites: None, but introductory chemistry or biology is recommended

TA Help Room: Mon-Fri, 6 - 7:30p in UTL G89

Learning outcomes:

As a student in this class, you will be able to experience the thrill of research and discovery. While the research you perform is more rigidly structured than in a faculty laboratory, you will build basic skills necessary to succeed in an independent research project. By the end of the semester you should be able to:

- Perform basic biochemistry laboratory techniques to collect and analyze data and interpret the results in a rigorous and reproducible manner.
- Organize logical written and oral arguments using clear, concise, and informative language to describe your research and its ethical implications.
- Describe fundamental aspects of protein science and the molecular basis of evolution and disease.
- Explain the differences between quantitative and phenomenological research.
- Develop a research plan that involves (a) identifying a question, (b) motivating the investigation, (c) formulating a testable hypothesis, (d) testing the hypothesis using known principles and data, (e) developing arguments that support or reject the hypothesis, (f) amending the hypothesis to conform to evidence while imagining alternate interpretations, (g) describing the implications of the work in the broadest context possible.
- Work well with a team and identify and deploy strategies for productive discussion and resolution of disagreements.

Syllabus is subject to change at the discretion of the instructor as well as at the whims of science.

II. Introduction

Proteins are amazing biomolecules that we still do not fully understand, that we cannot mimic with man-made materials. Their three-dimensional shapes are determined by their chemical composition; these shapes in turn determine their biological function. They are the workhorses of nature that perform or control most of the biochemical processes that keep cells alive. The central questions that this course examines concern the sometimes knowable, sometimes inconclusive relationship between the structures of proteins, their function, their physical properties, and how these depend on the environmental variables which affect them (temperature, pressure, pH, etc.). The scientific and technological advances over the last few decades have given the scientific community the tools needed to not only observe proteins and the genes that encode them, but also to directly manipulate them.

We now know the complete chemical sequence of DNA of many species, including *Homo sapiens*. This means that we have complete information about the chemical composition of all the proteins in all of our cells, yet the subtle relationship between the information stored in genes and the properties of the proteins they code for is frequently overlooked and misunderstood. Understanding this relationship is central to your success in this course, but it is also the gateway to understanding the molecular basis of evolution and medicine. This relationship is targeted by personalized medicine and the biotechnology industry to engineer novel proteins for specific medical, fabrication, and agricultural purposes. Our ability to selectively modify the genes that code for proteins also raises critical ethical issues that we will discuss. Ultimately, by creating and characterizing a protein this semester in PEBL, we hope you come to understand and appreciate the rippling effects that even subtle changes to a gene can have on a protein. Life itself is balanced on just a knife's edge and small changes in a protein can dramatically alter its structure and function.

PEBL is a large-scale research project that examines structure-function relationships in proteins. Moreover, PEBL is ***an inquiry-based course*** in which the outcomes of the experiments you will perform are not predetermined. You will engineer and study proteins that have never been studied before and analyze experiments to characterize your protein for the first time. The most concrete goal of the course is to understand why the changes you engineered into the protein led to the outcomes you measured. How your discoveries can be interpreted in terms of the influence a protein's structure has over its physical and functional properties, and how this explains evolution and disease at the molecular level. PEBL is ***a hypothesis-driven course*** because the specific experiments you will perform, the experiments that your section will perform this semester, and the experiments that will be performed collectively by many sections over several semesters, test and evaluate specific hypotheses about structure-function relationships in proteins.

PEBL is divided into three parts. In **UNIT I** you will learn fundamental protein science, with special emphasis on the physical properties of proteins (structure, stability, solubility, interactions, function) and the non-covalent forces that govern them. You will learn to use molecular graphics software to examine the three-dimensional structure of a protein in detail. In parallel, you will also learn how to handle the basic equipment and fundamental techniques used in biochemistry and biophysics laboratories; how to manipulate solids and liquids and how to use simple dimensional analysis and other conversion tasks for the preparation of mixtures and solutions. Using this knowledge, you will formulate a hypothesis that attempts to predict how the substitution of one amino acid for another will affect the structure and properties of your protein.

In **UNIT II** you will learn state-of-the-art lab techniques (recombinant DNA technology) for selective modification of DNA to engineer an altered protein as well as standard purification methods to isolate the protein of interest. In **UNIT III** you will perform experiments to characterize functional, physical and structural properties of your protein and evaluate your hypothesis.

There are multiple sections of PEBL are taught every semester. Each section will be broken up into 6 teams of 4 students each. For logistical reasons, each student is assigned a PEBL ID that is the section number, team number (1-6), then student letter (A-D) (e.g. 8-4C for a student in section 8 who is part of Team 4). Each team of 4 will work with one protein. Some of the work is done individually, some as a pair (2 students) and some as a team of 4. (As you might have gathered, patience and collaboration are fundamental to your experience in this course). The proteins studied in each section are all different. Thus, in a single semester of PEBL we can collectively study dozens of new proteins.

III. General organization of PEBL

Class	Subject	Due before class	Due after class
UNIT I: Formulation of hypothesis			
1	**Protein structure and function:** Introduction to structure and function of biological macromolecules and the PyMOL molecular graphics software. *Basic laboratory technique I*: pipetting and microbalances	N/A	PyMOL Part I Post-Lab: Pipetting Quiz #1
2	**DNA, genes, proteins, and evolution.** Central dogma of biology, genomics, and primer on molecular evolution based on sequence alignment. *Basic laboratory technique II*: UV-VIS spectroscopy to measure concentration	Quiz #2 Pre-Lab Conceptual Questions	PyMOL Part II Post-Lab: Standard curve
3	**Formulation of hypothesis.** *In silico* mutagenesis of the protein and formulation of hypotheses. *Basic laboratory technique III*: pH titration for determination of a pK_a value.	Quiz #3 Pre-Lab Conceptual Questions	Post-Lab: pK_a titration Team Post-Lab: in-silico mutagenesis (u*nlimited resubmissions allowed until Lab 10*)

UNIT II: Protein production			
4	**Site-directed mutagenesis:** Design of primers and use of polymerase chain reaction (PCR) to modify a single amino acid in a protein. Use agarose gel electrophoresis to visualize PCR results.	Quiz #4 Pre-Lab Hypothesis draft	Long Assignment #1: Hypothesis (*one resubmission allowed until Lab 10*)
5	**Bacterial transformation:** Incorporation of exogenous DNA into *E. coli* to create a clone for production of variant proteins	Quiz #5 Pre-Lab Conceptual Questions	Post-Lab: Mutagenesis & transformation
6	**DNA purification & sequencing:** Extraction of DNA for determination of the sequence for the gene of interest. Measurement of plasmid concentration and storage of transformed cells.	Quiz #6 Pre-Lab Conceptual Questions	Post-Lab: DNA purification
7	**Protein production-overexpression & extraction:** Growth of *E. coli* and overexpression of variant protein. Extraction of recombinant protein and initial purification steps based on centrifugation and differential solubility.	Quiz #7 Pre-Lab Conceptual Questions	Post-Lab: DNA sequencing Post-Lab: Protein prep I Amino Acid Quiz
8	**Protein purification- column chromatography and dialysis:** Ion exchange chromatography and dialysis for final purification of recombinant proteins.	Quiz #8 Pre-Lab Conceptual Questions	Post-Lab: Protein prep II
9	**Yield, purity and storage:** UV-VIS spectroscopy, SDS-PAGE and mass spectrometry for determination of yield, purity and molecular weight. Cryogenic storage of protein.	Quiz #9 Pre-Lab Conceptual Questions	Post-Lab: Protein yield & purity

UNIT III: Experimental characterization			
10	**Enzymatic activity and pH effects on stability:** Assay to determine enzymatic activity. Stability and pH sensitivity examined by acid denaturation monitored by Trp fluorescence.	Quiz #10 Pre-Lab	Quick Check: Activity Long Assignment #2a: Activity
11	**Secondary structure by CD and temperature effects on stability:** Circular dichroism at three different pH values to assess secondary (2˚**)** structure. Stability and temperature sensitivity monitored by Trp fluorescence.	Quiz #11 Pre-Lab	Quick Check: Structure Long Assignment #2b: Structure
12	**Thermodynamic stability and ionic strength effects:** Thermodynamic stability and salt sensitivity thereof measured by chemical denaturation monitored by Trp fluorescence.	Quiz #12 Pre-Lab	Post-Lab: Protein denaturation Quick Check: Salt Long Assignment #2c: Salt effects
13	**Presentations**	Draft of slides	Long Assignment #3 outline
14	**Finals week:** no class	Long Assignment #3: Summary, synthesis, and conclusions	

IV. Student responsibilities

Before class

Carefully review the course material for that Lab including readings, protocols, and videos and complete any *Pre-Lab*, *Notebook*, or written assignments, and *Quizzes*. Doing so will ensure that you are prepared and can effectively use the limited lab time for the experiments. Additionally, engage with the discussion board to provide peers with writing feedback and ethical discussions.

During class

Fully engage with the course by participating in discussions about the scientific theory and ethical questions for the lab as well as work with your lab partner(s) to collect experimental data and submit your completed *Notebook.*

After class

Complete all homework assignments (*Post-Lab, Quick Check, Long Assignment*) that are related to that lab.

Other responsibilities

- Attend your registered section only. Due to the unique structure of the course, make-up labs are not possible except in rare cases.
- Check the Blackboard course site daily.
- Inform the instructor via email about any planned scheduling conflicts as early as possible, preferably the beginning of the semester, so that adjustments can be discussed. Inform the instructor via email about any unplanned conflicts (e.g. illness) as soon as possible. See "Absences and Extensions" below for more details.
- Absolutely no food, drink, or anything in your mouth in the lab.
- Playing music, non-lab videos, and use of headphones is never allowed unless express permission is granted by the instructor.
- Dress code must be followed strictly for safety reasons. Failure to adhere to the dress code or any safety protocols is grounds for dismissal from the lab and potential deduction of your final course grade.
    - No open-toe shoes
    - Masks covering your nose, mouth, and chin must be worn securely at all times
    - Lab coats (provided or your own) must be worn at all times
    - Lab gloves (provided) must be worn at all times while working with lab reagents or solutions
    - Eye protection (provided or your own) must be worn when performing certain experiments
    - Loose hair and clothing (e.g. scarves, hoodie ties) must be tied back when using open flames or any instrument that has a motor or outer spinning parts

V. Assignments and grading

Grades and regrades

Grades will be reported on Blackboard and Gradescope. Please check for accuracy. Any requests for grade reviews must be made within 7 days from when the grade is posted. A request must be made via the Google form, one assignment request per form submission. Regrade requests made via email or to a TA will not be considered. Regrades are within the purview of the instructor only. If granted, the whole assignment will be assessed anew. The final grade is subject to increase or decrease; this grade stands as final.

A regrade is different than a resubmission. Specific assignments like the team in-silico mutagenesis or Long Assignment #1 have optional resubmissions after the initial deadline where the original work may be replaced with another version. These do not require a request form as the resubmission option is already available and unique to only those designated assignments.

10%	Pre-Lab & In-Lab/Notebook
5%	Conceptual Questions
10%	Quizzes
25%	Post-Lab
10%	Long Assignment #1
20%	Long Assignment #2
15%	Long Assignment #3
5%	Presentation

Absences and extensions
Students are expected to arrive on time, however this may not always happen. If there is an event that prevents this, it is the student's responsibility to contact the instructor by email as early as possible. The instructor may mark the absence as "Unexcused" (e.g. slept through alarm) or "Excused" (e.g. medical or family emergency). Any assignments missed as a result of unexcused absences will receive no credit. Excused absences are exempted from associated *In-Lab* and *Post-Lab Assignment*s. Excused absences are not exempted from completing *Quizzes*, *Conceptual Questions*, *Pre-Lab,* or *Long Assignments,* though these deadlines may be adjusted on a case-by-case basis.

When requesting an extension, contact the instructor via email as soon as possible prior to the initial deadline. Only the instructor has the ability to grant extensions and determine the new deadline; the TA has no influence over extensions. Unless an extension has been requested and granted prior to the assignment deadline, deductions will be applied.

Late work
It is the student's responsibility to ensure assignment submissions are completed successfully; both Turnitin and Gradescope provide confirmation messages. Any work that has not been submitted successfully by the assignment deadline may be subject to deductions. Due to the nature of the *Quizzes*, *Pre-Lab Assignments*, and *Conceptual Questions*, late submissions will not be accepted and will receive no credit. Late *Post-Lab* and *Long Assignments* will not receive full credit; the later the assignment, the less credit received (typically 10% per day). **Assignments turned in more than 4 days past the due date will receive no credit**.

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Pre-Lab (5%) and Quiz (10%)
These assignments are designed to ensure that you come to class prepared to ask questions and discuss the finer aspects of the scientific concepts. These assignments should be submitted online before class starts unless an extension has been granted by the instructor. Late submissions will not be accepted and will receive no credit. *Pre-Lab* assignments are submitted to Gradescope are meant to prepare you for the activities of the day by including questions about basic lab concepts, calculations, and preparing data collection tables. These are graded on effort/completion, not correctness.

Quizzes are completed online in Blackboard and marked as either "Satisfactory" (≥ 85%) or "Unsatisfactory" (< 85%). Except for Quiz #1, each lab quiz covers concepts related to that lab's experiments and has a 15-minute time limit with a maximum of 3 attempts. The amino acid quiz (unlimited attempts until the deadline) covers the names, structures, and properties of the 20 amino acids. Quiz #1 covers the information in this syllabus and you have unlimited time and attempts to complete is by the deadline. Your final quiz grade is determined by the number of Satisfactory scores received.

Final letter grade	With a 100% for Quiz 1	With < 100% for Quiz 1
A	≥ 11 Satisfactory	---
B	≥ 9 Satisfactory	≥ 11 Satisfactory
C	≥ 7 Satisfactory	≥ 9 Satisfactory
D	≥ 5 Satisfactory	≥ 7 Satisfactory
F	< 5 Satisfactory	< 7 Satisfactory

Notebook (5%)

The *Notebook* is an essential part of any research project and contains the record of what happened during the course of the experiment. Prior to class you will prepare your online lab notebook with a brief purpose statement and outline of the wet lab protocols you need to use for the day to ensure your understanding of the tasks at hand. While in class, all experimental work is to be recorded in the notebook including observations, calculated or measured values, and a *brief* reflection on the experiments. Your notebook will be graded at the end of class as Satisfactory or Unsatisfactory. There are 14 notebook entries and your final notebook grade is determined by the number of Satisfactory scores received.

Final letter grade	Minimum scores needed
A	≥ 13 Satisfactory
B	≥ 11 Satisfactory
C	≥ 9 Satisfactory
D	≥ 7 Satisfactory
F	< 7 Satisfactory

Post-Lab (25%)

Most labs have an associated *Post-Lab* worksheet that is to be submitted to Gradescope by midnight 2 days after the relevant class (e.g. Monday lab deadline is 11:59 pm on Wednesday"). In these assignments you report and reflect on the observations you made in lab and answer questions concerning the preparatory material.

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Because PEBL is an inquiry-based course, and writing is central to the process of inquiry, it is also a writing-enriched course. Writing is useful as a tool for learning as it tests both how well you understand complex concepts and helps you recognize the limits of your understanding. To this end, writing concisely, with clarity, precision, logical structure, and elegance, is an invaluable skill, regardless of the path you choose to follow. Writing in PEBL takes many forms:

Conceptual Questions (5%)

Conceptual Questions are designed to help you think beyond what is presented in the preparatory reading and to practice clear and concise written communication. These exercises are designed to help you distill ideas and think through a question or problem. Initial answers are posted to the Blackboard discussion boards at least 1 day prior to class and you will discuss each other's writing assignments initially via the discussion boards. Final versions of your work are submitted to Gradescope prior to the start of class. As these discussions frequently extend into class, late submissions will not be accepted. There are 21 questions and discussion boards that are graded as Satisfactory or Unsatisfactory. Your final Conceptual Questions grade is determined by the number of Satisfactory scores received.

Final letter grade	Minimum scores needed
A	≥ 19 Satisfactory
B	≥ 16 Satisfactory
C	≥ 13 Satisfactory
D	≥ 10 Satisfactory
F	< 10 Satisfactory

Long Assignment #1—Hypothesis (10%)
In this short writing assignment, you will articulate (a) the questions and motivation behind the research PEBL is undertaking; (b) your protein's role in the specific research question for the semester, and (c) you will propose a hypothesis (*a speculative yet reasonable projection*) that summarizes the expected outcomes of your characterization experiments. LA #1 will be peer-reviewed in Meeting #4 and submitted to Turnitin in lieu of a *Post-Lab* within 3 days after Meeting #4. You will receive extensive feedback and one opportunity to resubmit the assignment before Meeting #10; the last submitted version will be graded. This assignment serves as your formalized hypothesis for your protein and summary of the course goals for the semester.

Long Assignment #2—Experimental Outcomes & Preliminary Evaluation (20%)
In Unit III of the course you will perform experiments to test your hypothesis and allow you to examine six different functional or physical aspects of your protein. Long Assignment #2 consists of short summaries and provisional evaluations of the hypothesis for your experiments. LA #2 consists of 3 parts (Lab #10, #11 and #12), each with a Quick Check typically due 1 day after class (graded on completion) to help you organize your ideas logically and receive pointed feedback. These written assignments are due 3 days after the associated Quick Check.

Long Assignment #3 (15%) and Presentation (5%)
This final assignments build on Long Assignments #1 and #2 to describe, in full, your process of inquiry over the course the semester. The purpose of LA #3 is for you to (a) evaluate the outcomes of your characterization experiments, (b) evaluate the class research project, (c) examine the validity of your hypotheses against the data collected, and (d) examine implications of and for the measured outcomes. Each team will present a 10-minute oral presentation in Meeting #13 summarizing their experimental outcomes and individual hypothesis evaluations. The goals of this exercise are to help you prepare for LA #3 and practice how to communicate research to a diverse audience. LA #3 is a longer writing assignment that will be produced in two parts. You will turn in an outline (graded on completion) within 3 days after Meeting #13 which will receive general feedback, and the final assignment during finals period (specified in the assignment). Please note: outlines are required; **if you fail to submit a completed outline, your final LA #3 grade will be lowered by 1 letter**.

VII. Code of Ethics and Academic Integrity

Academic integrity is central to the education mission of Johns Hopkins University. During the labs you will work individually, in pairs, or as part of a team of four students, and we encourage, even celebrate collaborative efforts in many instances. But please note that all graded (and required but ungraded) work – including all quizzes, pre-labs, post-labs and Long Assignments– are to be completed ***entirely*** on your own, without any form of collaboration with your peers (past or present), team members, or pair partner unless otherwise specified. Copying someone else's work of any kind, from borrowing a figure to copying sections of text, is unacceptable and will result in an ethics violation investigation. Both the person doing the copying and the person who willfully allows to be copied from will be penalized. **You are required** to complete the "Avoiding Plagiarism" course found at https://guides.library.jhu.edu/avoidingplagiarism and submit the certificate of completion.

Violations of University policy on academic integrity range, and can result in failure on the assignment, in the course as a whole, or in suspension or expulsion from Johns Hopkins University. To ensure you understand the severity of possible consequences, you will be asked to sign a document on the first class which outlines our position on ethics and academic integrity in PEBL. *Any* violation of conduct will be reported to the Ethics Board, and if you are found to be guilty, the violation will be reported in your applications to medical school, law school, and graduate school, and to some employers. We take academic integrity seriously, and so should you.

For additional information, please read the *Academic Ethics for Undergraduates* guide located at https://studentaffairs.jhu.edu/policies-guidelines/undergrad-ethics.

VIII. Accommodations

Johns Hopkins University values diversity and inclusion. We are committed to providing welcoming, equitable, and accessible educational experiences for all students. Students with disabilities (including those with psychological conditions, medical conditions and temporary disabilities) can request accommodations for this course by providing their instructor with an Accommodation Letter issued by Student Disability Services (located at 101 Shaffer Hall; 410-516-4720; studentdisabilityservices@jhu.edu; https://studentaffairs.jhu.edu/disabilities/). Please request accommodations for this course as early as possible to provide time for effective communication and arrangements. Students submitting their letter later in the semester are still eligible to receive approved accommodations for the remainder of the course, but there could be a delay in accommodations being implemented. If an event occurs during the semester (e.g. concussion, broken bones) which requires special accommodations, you must report this to the instructor as soon as possible.

IX. Additional resources

Anxiety, stress, and mental health

If you are struggling with anxiety, stress, depression or other mental health related concerns, please consider visiting the JHU Counseling Center. If you are concerned about a friend, please encourage that person to seek out their services. The Counseling Center is located at 3003 North Charles Street in Suite S-200 and can be reached at 410-516-8278 and online at http://studentaffairs.jhu.edu/counselingcenter/.

Writing Center and Academic Support

If you are looking to improve your English writing skills or strengthen your study skills, the JHU Writing Center and the Office of Academic Support have tutoring, programs, and additional resources available for students. The Writing Center is located in Gilman Hall 230 and online at https://krieger.jhu.edu/writingcenter/ and the Office of Academic Support is online at https://academicsupport.jhu.edu/.