Spring 2-1-2022

BCH 486.01: Biochemistry Research Lab

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BCH 486 – Biochemistry Research Laboratory – Spring 2022

Lab hours: MW 3:00 – 5:50 in ISB008 – note some flexibility may be required.

Instructor: Kimberly Stanek, Chem 206, kimberly.stanek@umontana.edu
Office Hours: TTh 9:00-11:00 am or by appointment.

TA: Thomas Bisom, thomas.bisom@umconnect.umt.edu

Course Goals

This course teaches molecular biology and protein biochemistry techniques as part of an integrated research project. This format breaks with the traditional model of undergraduate lab courses (performing discreet, unrelated experiments) and instead provides a research-like experience while providing a solid foundation in standard biochemical techniques. In this way, students will experience the fundamental process of scientific research.

The goal is to understand how substitutions in primary structure (amino acid sequence) affect the biochemical properties of a protein. Each student will prepare a protein from a mutant construct they themselves have built. The first half of the course comprises many standard protein engineering methods that are used widely in research labs, including PCR-based site-directed mutagenesis, preparation of plasmid DNA, DNA sequencing, heterologous expression of a protein in bacteria, protein purification and characterization. The latter part of the course includes more advanced techniques including protein characterization by spectroscopic and thermodynamic techniques as well as mass spectrometry and X-ray crystallography.

Lectures will be integrated into the lab time and will cover both the principles behind the daily experiments as well as the technical details. Should a particular experiment fail for a student, backup supplies will be available for students to continue their projects. The order of experiments described in the syllabus may need to be altered from time to time to accommodate equipment or reagent availability. As much as possible, students will work individually rather than in pairs.

Students will also read and analyze an article from the biochemistry literature related to their project. This article will serve as a template for describing their own research efforts. They will, through a series of written exercises, describe their work in the typical format used in scientific journals. *This course will count for 2/3 of the 'W' upper division writing requirement for majors associated with the Division of Biological Sciences or the Biochemistry program.*

Grading

Attendance is mandatory. Students will be allowed to miss only one class per semester and must arrange to make up the work. Any additional classes that are missed without a validated excuse will lower the student's grade (one +\- letter grade per missed class).

Summary of Article from Current Literature: 10% of final grade. There will be one journal article assigned that will be discussed in class; students will then write a 2-to-3-page summary of the article. The first draft will be worth 6 points and will be graded for both content and writing style and then returned to the student for revision. The revision will be worth an additional 4 points for a total of 10 points.

Write-ups of Research Results: 30%. Students will be asked to hand in three written assignments on their research results (double spaced), each counting toward the final grade. The first two will be edited and handed back for revision. For these, the grade will be based on the revised assignment, which will be due one week after it is returned. The assignments will cover the main parts of a scientific manuscript: (1) Introduction, (2) Methods, and (3) Results and Discussion. The article summarized in the previous assignment will serve as a stylistic template. Each student will write:

1. Introduction describing the protein being investigated for this course and the reason the particular mutant was selected. Worth 10 points (10%) after revision
2. A Methods Paper describing the methods used to make and characterize the protein variant. Worth 10 points (10%) after revision
3. Summary Paper containing the Introduction, Methods, Results and Discussion sections. The Results and Discussion will describe the characterization of the protein variant and discussion of the results of class and individually-designed experiments. Class data will be available to all students. Worth 10 points (10%)

Laboratory Notebooks: 10%. Students will be asked to keep a research-type laboratory notebook that will be checked 3x per semester. Students will be provided with specific examples of excellent and poor notebooks during class; these will be available throughout the semester. The notebooks will be graded and returned, with notations in the first round of grading. While students are not asked to re-write these sections, they are expected to adapt their writing styles in future weeks to include instructor comments and to conform with the notebook guidelines discussed during class. The grade will be assigned based on the last review. The goal is to learn the art of keeping a research notebook:

a. to write clearly and with enough detail that someone will be able to reproduce your experiments, or to adapt your procedure to another problem
b. to describe your results both quantitatively and qualitatively – if something didn't work very well, what went wrong? What would you modify for the next time?
c. to show the connection from the results of one day's experiments to the next.

Homework Assignments: 20%. Students will be given two take-home homework assignments covering the theory and/or interpretation of the techniques used in class.

Bioethics Case Study: 10%. Students will prepare a one-to-two-page (double-spaced) report on a case involving ethics in research and present on their case study during lecture.

Final Presentation: 20%. During finals week each student will do a short (15 minute) individual oral presentation using a program like PowerPoint. The presentation will summarize the project – the background, methods, results and discussion. The presentations will be open to the public (you may invite guests). The times for presentations will be scheduled to accommodate other commitments you may have.

Graduate Increment. Graduate students who take the course will be expected to fulfill the requirements listed above and to perform one additional assignment. Graduate students will (a) write a 5 page critique of a relevant paper chosen with input from the instructor and (b) propose a novel experiment or set of experiments that is suggested from the results of that paper. This assignment will count for 10% of the grade; for graduate students the notebook grade will count for 5% and the final presentation for 15% of the final grade.

Note: If you are taking the course for a non-traditional grade (credit/no credit), university policy states that a "CR" grade is given in lieu of A through D- grade; an "NCR" grade is given in lieu of an F grade. Typically, CR/NCR grades cannot be used for major credit.

General Policies

University policies on drops, adds, changes of grade option, or change to audit status will be strictly enforced in this course. These policies are described in the current catalog. The 15th day of the semester (February 7th 5:00 pm) is the last day to withdraw with a refund and no "W" on the transcript. The 45th day of the semester (March 29th by 5:00 pm) is the last day to withdraw with a W and without the Dean's signature and documentation of extenuating circumstances. After the 45th day a "WP" or a "WF" will appear on the transcript. Approval to withdraw at this point is not automatic. Students cannot drop classes after the last day of classes (May 6th at 5:00 pm).

In working through assignments, students are encouraged to work together to solve problems, to share information or resources, and to test each other's understanding of the material. These are all acceptable forms of collaboration. However, the written work that each student turns in must be his or her own. Only in this way can faculty judge individual understanding of concepts or information. A good rule of thumb for students to follow is to work together up to the point of committing words to paper. At that stage, each student must work independently. A second key guideline is that once a student has written an out-of-class assignment, it must not be shown to or discussed with another student in the course. Assignments from two or more students that have significant overlap, in the professional judgment of the faculty member, will be regarded as reflecting a violation of the expectation that students turn in independent assignments.

Please note that direct copying of sentences from any published source without proper citation is considered plagiarism. THIS INCLUDES THE INTERNET. Additionally, excessive quoting is generally unacceptable in the sciences. Be sure to put the information in your own words and be aware that the instructor will check literary and Internet resources. Violations will be dealt with according to the Student Conduct Code. *All students must practice academic honesty. Academic misconduct is subject to an academic penalty by the course instructor and/or a disciplinary sanction by the University. All students need to be familiar with the Student Conduct Code:* http://www.umt.edu/student-affairs/dean-of-students/default.php

Special Accommodations

If you are registered with the Office of Disability Equity (http://www.umt.edu/accessibility/) and require special accommodations, or if a class period conflicts with service requirements such as jury duty, military/national guard, or falls on a religious holiday and you wish to reschedule, please contact Dr. Stanek.

Tentative Schedule

Date	Experiment
Week 1	
Jan 17	NO CLASS – **Martin Luther King Day**
Jan 19	Introduction to the class and cytochrome *c* – select variants Review article on cytochrome *c* function handed out Instructions for keeping a laboratory notebook
Week 2	
Jan 24	Discussion of review article **Lab 1:** Using Pipets (practice) **Lab 2:** Measuring DNA concentrations (primers)
Jan 26	**Lab 3:** QuikChange Site Directed Mutagenesis Understanding PCR basics and primer design (**Homework Assignment 1**)
Week 3	
Jan 31	**Lab 4:** Agarose Gel Electrophoresis (AGE) analysis **Lab 5A:** Pouring plates
Feb 2	**Lab 5B:** Transformation (Culture growth for plasmid minipreps) (**Homework Assignment 1 is due**)
Week 4	
Feb 7	**Lab 6:** Plasmid mini-preps **Lab 7:** Plasmid DNA Concentration Determination **DNA sent out for sequencing**
Feb 9	**Lab 8:** DNA Sequencing Analysis using Databases **Hand-in Lab Notebooks for first check**
Week 5	
Feb 14	**Discussion on Bioethics** **Selection of Bioethics Case Studies**
Feb 16	**Lab 9:** Structure Analysis by Computer Visualization Software **Article from current literature handed out**

Date	Experiment
Week 6	
Feb 21	NO CLASS – **President's Day**
Feb 23	**Discussion on article from current literature** **Bioethics Presentations**
Week 7	
Feb 28	**Lab 10A:** Transformation for protein expression **Lab 10B:** Making liquid growth media
Mar 2	**Lab 11A:** Inoculation of 1 L cultures **Discussion about writing Introduction** **First draft of article summary due**
Week 8	
Mar 7	**Lab 11B:** Cell Lysis and setup ammonium sulfate precipitation
Mar 9	**Lab 11B:** Finish ammonium sulfate precipitation and setup dialysis **First draft of article summary handed back** **Hand in Lab Notebooks for second check**
Week 9	
Mar 14	**Lab 11C:** Purification by cation exchange chromatography
Mar 16	**Lab 11D:** Concentrating a protein and buffer exchange by ultrafiltration. **Second Draft of article summary due**
Week 10	
Mar 21-25	Spring break…. ☺
Week 11	
Mar 28	**Lab 11E:** Determination of protein concentration, yield and degree of oxidation by UV-Vis Spectroscopy **First draft of Introduction due**
Mar 30	**Lab 12:** Purity determination and mass estimation by SDS/PAGE. Mass determination by Matrix-assisted Laser Desorption Time-of-flight (MALDI-TOF) Mass Spectrometry of cytochrome *c*. **Homework assignment 2 handed out**

Date	Experiment
Week 12	
Apr 4	**Lab 13:** X-ray crystallography setup and facility tour **First draft of Introduction handed back**
Apr 6	**Lab 14A:** Redox potential measurement **First draft of Methods due**
Week 13	
Apr 11	**Lab 14B:** Analysis of redox potential data **Second draft of Introduction due**
Apr 10	Discussion of possible individual experiments. Defining a hypothesis and proposing an experimental approach. **Discussion of Summary Paper** **First draft of Methods handed back** **Homework assignment 2 due**
Week 14	
Apr 15	Individual experiments **Second draft of Introduction handed back**
Apr 17	Individual experiments/Analysis of data **Second draft of Methods due** **Hand in lab notebooks for final check**
Week 15	
Apr 22	Analysis of data **Second draft of Methods handed back**
Apr 24	Analysis of data/preparation of presentations
Week 16	
April 29 – May 3	Individual presentations (15 minutes each, to be scheduled) **Summary papers due at time of presentation**