Spring 2-1-2018

BCH 486.01: Biochemistry Research Lab

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BCH 486 – Biochemistry Research Laboratory – Spring 2018

Lab hours: MW 3:00 – 5:50 in ISB008 – note some flexibility may be required.
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Office Hours: 11-12 T and Th or by appointment.
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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 an integrated 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 full 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 up a 2-

page summary of that 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 (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 scientific writing: (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. 1-page Introduction describing the protein being investigated for this course and the reason the particular mutant was selected. Worth 10 points (10%) after revision
2. 2-page Methods Paper describing the methods used to make the mutant, and then a longer discussion of one of the techniques used in class (each student will select a unique method) Worth 10 points (10%) after revision
3. Summary Paper containing the Introduction, Methods, Results and Discussion sections (2 – 4 pages). The focus of this paper will be the last experiment of the semester, which will be designed by the student to probe a property of the protein variant. Assistance with protocol development will be available before the assignment is due. 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, usually with copious 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.

Mini-Research Proposal: 10%. Students will prepare a one to two page (double-spaced) research proposal with a focused hypothesis and an outline of the experimental approach (an Aim) to be used to test the hypothesis.

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.

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. Students should specifically note that:

- The 15th day of the semester (Friday, Feb. 9, 5:00 pm) is the last day to withdraw with a refund and no "W" on the transcript.
- The 45th day of the semester (Monday, April 2, 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 (Friday, May 4)

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.

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. The Code is available for review online: http://www.umt.edu/vpesa/Dean%20of%20Students/default.php.

Special accommodations: If you are registered with Disability Student Services (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. Bowler.

Tentative Schedule

Date	Experiment
Jan 22	Introduction to the class and cytochrome *c* – select variants Review article on cytochrome *c* function handed out Instructions for keeping a laboratory notebook
Jan 24	Discussion of review article **Lab 1:** Using Pipets (practice) **Lab 2:** Measuring DNA concentrations (primers)
Jan 29	**Lab 3:** QuikChange Site Directed Mutagenesis Understanding PCR basics and primer design (**Homework Assignment 1**)
Jan 31	**Lab 4:** Agarose Gel Electrophoresis (AGE) analysis **Lab 5A:** Pouring plates
Feb 5	**Lab 5B:** Transformation (Culture growth for plasmid minipreps) **Homework Assignment 1 is due**)
Feb 7	**Lab 6:** Plasmid mini-preps **Hand-in Lab Notebooks for first check** **Article from current literature handed out**
Feb 12	**Lab 7A:** Plasmid DNA Concentration Determination **Lab 7B:** Preparation of DNA Sequencing Samples
Feb 14	**Lab 8:** DNA Sequencing Analysis using Databases **Discussion of article from current literature**
Feb 19	NO CLASS – **President's Day**

Date	Experiment
Feb 21	**Lab 9:** Making media/Transformation for protein expression
	First draft of article summary due
Feb 26 – 28	**Lab 10:** Large scale growth (done in groups over the entire week)
	First draft of article summary handed back
March 5	**Lab 11 A:** Buffer prep and cell lysis, ammonium sulfate precipitation
March 7	**Lab 11B:** Dialysis set up
	Second draft of article summary is due
	Discussion about writing Introduction
March 12	**Lab 11C:** CM-sepharose chromatography
March 14	**Lab 11 D:** Protein concentration and buffer exchange by ultrafiltration.
	Hand-in Lab Notebooks for second check
	First Draft of Introduction is due
March 19	Concentration determination by UV-Vis; purity determination and mass estimation by SDS/PAGE. Matrix-assisted Laser Desorption Time-of-flight (MALDI-TOF) Mass Spectrometry of cytochrome *c*.
March 21	Protein Determination by BioRad Dye Assay
	(**Homework Assignment 2**)
	First Draft of Introduction handed back
March 26-30	Spring break…. ☺
April 2	**Lab 12:** X-ray crystallography setup and facility tour
	(**Discussion of Methods Paper**)
April 4	**Lab 13:** Structure Analysis by Computer Visualization Software
	(**Homework Assignment 2 is due**)
	Second Draft of Introduction is due
April 9	**Lab 14A:** Redox potential measurement
	(**First Draft of Methods Paper is due**)
April 11	**Lab 14B:** Analysis of redox potential data
Date	**Experiment**
April 16	Discussion of possible individual experiments. Defining a hypothesis and proposing an experimental approach.

	(**First Draft of Methods paper is handed back**)
April 18	Detailed experimental design period
April 23	Individual experiments
	(**Second Draft of Methods paper is due**)
	(**Mini-research proposal is due**)
April 25	Individual experiments
April 30	Analysis of data and preparation of presentations
May 2	Preparation of presentations
May 7 – 9	Finals: Individual presentations (15 minutes each, to be scheduled)
	(**Summary paper is handed in at time of presentation**)