BCH 486 – Biochemistry Research Laboratory – Spring 2020

**Lab hours:** MW 3:00 – 5:50 in ISB008 – note some flexibility may be required.
**Instructor:** Klára Briknarová, CHEM 111 (aka Mouse House), 406-243-4408, klara.briknarova@umontana.edu
**Office Hours:** TR 12-1 or by appointment.
**TA:** Precy Nepomuceno, preciousann.nepomuceno@umontana.edu

**Course Goals:** This course teaches molecular biology and protein biochemistry techniques as part of an integrated research project. This format differs from the traditional model of undergraduate lab courses (performing discreet, unrelated experiments) and instead provides a solid foundation in standard biochemical techniques in the context of a semester-long research-like experience.

The goal is to understand how substitutions in primary structure (amino acid sequence) affect the properties of a protein. Each student will prepare and characterize a mutant protein using DNA into which they themselves will have introduced the mutation. To accomplish this, the students will use many standard molecular biology and protein biochemistry methods: they will perform PCR-based site-directed mutagenesis, isolate plasmid DNA, analyze results of DNA sequencing, express the protein in bacteria, purify the protein by affinity and size-exclusion chromatography, and finally characterize the protein by NMR spectroscopy. In addition, students will learn to visualize and analyze protein structures using the Pymol molecular visualization system (for free Pymol for educational use, see [https://pymol.org/edu/?q=educational/](https://pymol.org/edu/?q=educational/)).

**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 materials will be provided so that the student can continue with his/her project. 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 and will describe their own research efforts in a format used in scientific journals. In addition, students will prepare a bioethics case study report. **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. Students will write a 2 to 3 page summary of a research article. The first draft will be worth 6% 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% for a total of 10%.


Presentation of a bioethics case study and an accompanying written report: 10%.

Write-ups of Research Results: 25%. Students will write a paper describing and discussing their results. The paper will also contain Introduction and Methods sections. Class data will be available to all students. The article summarized in the first assignment will serve as a stylistic template.

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 to 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%.

Quizzes: 5%.

Final Presentation: 20%. At the end of the semester, each student will do a short (20-25 minute) individual oral presentation using a program like PowerPoint. The presentation will summarize the project – the background, methods, results and discussion.
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 (Monday, February 3, 5:00 pm) is the last day to withdraw with a refund and no "W" on the transcript.
- The 45th day of the semester (Tuesday, March 24, 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 1, 5:00 pm)

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/student-affairs/dean-of-students/default.php](http://www.umt.edu/student-affairs/dean-of-students/default.php)

Special accommodations: If you are registered with Disability Student Services ([http://www.umt.edu/accessibility/](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. Briknarová.
### Tentative Schedule

#### Week 1
- Jan 13: Introduction
- Jan 15: Pipetting and primer design

#### Week 2
- Jan 20: NO CLASS — **Martin Luther King Day**
- Jan 22: Pymol

#### Week 3
- Jan 27: PCR mutagenesis
- Jan 29: Agarose gel electrophoresis

#### Week 4
- Feb 3: Transformation
- Feb 5: Plasmid mini-preps

#### Week 5
- Feb 10: Ethics in research
- Feb 12: Analysis of DNA sequencing results

#### Week 6
- Feb 17: NO CLASS — **President’s Day**
- Feb 19: No class

#### Week 7
- Feb 24: Bioethics case study presentations I
- Feb 26: Bioethics case study presentations II

#### Week 8
- March 2: Transformation and expression I
  - M Transform
  - T 1 mL & 50 mL cultures
  - W 1 L culture, induction
  - R harvest
- March 4: Transformation and expression II
  - W Transform
  - R 1 mL & 50 mL cultures
  - F 1 L culture, induction
  - Saturday harvest
Week 9
March 9 SDS-PAGE
March 11 Overview of protein purification, Sparky?
March 16-20 Spring break.... 😊

Week 10
March 23 Purification I
M lysis, load lysate on affinity column
T collect the protein after TEV protease cleavage
T/W concentrate protein for size-exclusion chromatography (SEC),
SEC, concentrate protein for NMR, NMR
elute and regenerate affinity column!

March 25 Purification II
W lysis, load lysate on affinity column
R collect the protein after TEV protease cleavage
R/F concentrate protein for size-exclusion chromatography (SEC),
SEC, concentrate protein for NMR, NMR
elute and regenerate affinity column!

Week 11
March 30 Purification III
April 1 Purification IV

Week 12
April 6 Purification V
April 8 Purification VI

Week 13
April 13 SDS-PAGE and analysis of data
April 15 Analysis of data

Week 14
April 20 Analysis of data/preparation of presentations
April 22 Analysis of data/preparation of presentations

Week 15
April 27 Presentations I
April 29 Presentations II