SYLLABUS
Biochemistry 486 Spring 2009

Text: none – notes and reference materials to be provided by instructor

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TA: Travis Danielson

Summary

The purpose of this course is to teach current molecular biology and protein biochemistry techniques as an integrated part of a research project suitable for publication. In this way, not only will the student become familiar with the technical aspects of techniques such as PCR, cloning, and protein purification, but he or she will experience the fundamental process of scientific research. The course format will break with the traditional model of undergraduate lab courses (performing discreet, unrelated experiments) and instead provide a research-like experience where the semester is devoted to the achievement of one long-term goal. 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, backup supplies will be available to enable the student to continue with the scheduled research.

The main goal is to understand how mutations in amino acid sequence may affect the biochemical properties of a protein. Each student will prepare a mutant of azurin, a blue copper protein that is involved in electron transfer during bacterial respiration. The mutants will be purified and characterized by a variety of spectroscopic techniques. Students will gain experience in recombinant protein expression in bacteria, site-directed mutagenesis, PCR techniques, protein purification, and protein characterization, including several types of spectroscopy.

Students will read an article from current biochemistry literature that is related to their projects. 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. Pending university approval, 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 related 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, each worth 10% of 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) Abstract/Introduction, (2) Methods, and (3) Results and Conclusions. The article summarized in the previous assignment will serve as a stylistic template. Each student will write:

1. 1-page Abstract Paper describing the protein being investigated and the reason the particular mutant was selected. Worth 5 points after revision
2. 2-page Methods Paper describing one of the techniques used in class (each student will have a unique method) Worth 10 points after revision
3. 4-Page Summary Paper containing Abstract, Methods, Results and Conclusions sections. The focus of this paper will be the last experiment of the semester, which will be designed by the student to probe the stability of the mutant protein. Assistance with protocol development will be available before the assignment is due. Worth 15 points

Laboratory Notebooks: 10%
Students will be asked to keep a lab notebook that will be checked every three weeks (4x 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 few rounds 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 given out during class. The grade will be assigned based on the last two reviews. The goal is to learn the art of keeping a research notebook:

a. to write clearly and with enough detail that someone would be able to reproduce your experiments, or 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: 30%
Students will be given three short-answer (but difficult) take-home homework assignments (10% each) covering the theory and/or interpretation of the techniques used in class.

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 describe the project – the background, experiments, results, and conclusions. The presentations will be open to all UM faculty and staff.

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 after the 30th day of the semester, such changes are NOT automatically approved. They may be requested by petition, but the petition MUST be accompanied by documentation of extenuating circumstances. Requests to drop a course or change the grade basis to benefit a student's grade point average will not be approved.

Note:
• If you are taking the course for a non-traditional grade (credit/no credit), a recent change in 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. From the 15th day of classes to the 30th day, changing from traditional grade to credit/no credit, or the reverse, requires the use of an add/drop slip. No changes are allowed after the 30th day.
• After the 15th day of classes, students will not be able to change from a grade to audit status, under any circumstances.

The use of any external device (electronic devices such as calculators, translators, etc) for quizzes and exams requires the advanced approval of the instructor.

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. Those 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. 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 at http://www.umt.edu/SA/VPSA/index.cfm/page/1321.

Special accommodations: If you are registered with Disability Student Services and require special accommodations, please contact Dr. McGuirl to make arrangements. If an evening class period falls on a religious holiday and you wish to reschedule, please contact Dr. McGuirl.

BIOC 486  Biochemistry Research Lab

<table>
<thead>
<tr>
<th>DATE</th>
<th>EXPERIMENTS</th>
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<tbody>
<tr>
<td>Jan 27</td>
<td>Introduction to the class and the azurin protein</td>
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<td>Instructions for pipet use</td>
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<td>Jan 29</td>
<td>Using Pipets (practice)</td>
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<td>Understanding PCR basics and primer design</td>
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<td>Measuring DNA concentration (primers)</td>
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<td>Feb 3</td>
<td>Making mutants of the azu (azurin) gene - QuikChange</td>
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<td>Feb 5</td>
<td>Agarose Gel Electrophoresis (AGE) analysis</td>
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<td>Feb 10</td>
<td>Transformation, pouring plates</td>
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<td>Feb 12</td>
<td>Culture growth for plasmid minipreps</td>
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<td>Plasmid minipreps</td>
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<td>Feb 17</td>
<td>Plasmid DNA Concentration Determination, DNA</td>
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<td>Sequencing (1)</td>
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Feb 19  DNA Sequencing Analysis (2)
      DNA Database Tutorial (I)

Feb 24  Transformation by Electroporation
      DNA database Tutorial (II)

Feb 26  Culture Growth and Small Scale Induction

March 3  SDS/PAGE analysis, start of large scale growth

March 5  Large scale growth (done in groups over W/Th/F)

Mar 10  Western Blot (1)

Mar 12  Western blot (2), buffer prep and dialysis

Mar 17  Protein Purification (I)

Mar 19  Protein Purification (II)

March 24  SDS/PAGE

March 26  X-ray Crystallography SetUp

Spring break…. 😊

April 7  Characterization of Cu(II)-protein by UV/Vis Spectroscopy

April 9  Protein Determination by BioRad Dye Assay

April 14  BQ copper Assay

April 16  Stoichiometry Data Analysis

April 21  Mass Spectrometry

April 23- May 5  Individual experiments: protein stability tested by (a) circular
      dichroism, (b) fluorescence, or (c) ANS dye binding using denaturation by (d)
      heat (e) guanidine HCl  or (f) pH.

May 5  X-ray Crystallography and Structure Analysis by
      Computation

May 7  Computer Lab: Preparation of presentations
Finals Week: Presentations due (individual, 15 minutes each, to be scheduled)
Directions for Writing a Summary of an Article from Current Literature:
10% of final grade.

Select one of the following three articles:


Write a 2-page (double spaced) 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.

Guidelines for writing the summary:
This should look somewhat like an expanded version of the article abstract. You should explain the question being asked/problem being addressed and why it’s important. Then summarize the results (e.g., the authors used technique x to investigate protein y. Their results indicate that…) Then summarize the authors’ conclusions. Optional: If you wish to critique the experimental details, results or conclusions, you may do so. However, this is not required – not everyone will have sufficient background to do so.

Some more detailed instructions
1. BACKGROUND
Include everything we need to know to understand the work and its interpretation:
   • define important terms
   • protein/gene names & functions you will refer to often
   • provide physiological context and "big picture" importance of the problem, e.g. what are the established biological functions of the protein, or why are disulfide bonds important to protein structure?
   • medical relevance, if there is one

   Most importantly, you need to carefully identify the gap in our knowledge that this work is attempting to fill.
   • What is already known about this particular problem?
   • What has already been done (important previous papers)?
   • What features of the problem are currently controversial or unaddressed?
   • What do you personally find neat/interesting/puzzling about this system or question?

2. EXPERIMENTAL
When reviewing a paper, going through the paper figure by figure is a good idea. Give a summary of what the figure tells us – make a short conclusion from each figure! You will need to describe any specialized methods and protocols, but you do not have to become an expert in the techniques – we will review this in class. Tell us what the authors wanted to know, what was done, and what was concluded from each experiment, in line with these points:

- 1-2 line summary of result of experiment as it relates to the problem.
  Every experiment had a motivation that needs to be clear--don't say "next they did a blah blah assay"; instead say "next they wanted to see if blah was true, so they tested this using a blah blah assay"
- Know the basics of each technique used
- State conclusion(s) before introducing the next experiment/figure

As you grow in your scientific prowess, you will want to point out where you disagree or what they should have concluded from each experiment as you go and also what controls would have made their conclusion stronger. However, it is okay to omit this unless you are a graduate student enrolled in the course.

3. CONCLUSIONS/DISCUSSION

Reiterate major conclusions. How has this work/paper changed the way we think about the problem (with reference to the physiological context and "big picture" presented at the beginning)? Feel free to insert your personal opinions of the paper in these categories:

- How novel was it? (novel is good)
- Strength of the conclusions
- Does it really fill the intended gap in knowledge?
- Magnitude of the impact of paper on field
- Does their model make good biochemical sense?

Discuss any interesting questions, puzzles or paradoxes brought up by your/the authors' work.