

CHEM 3324: Biochemistry Laboratory

Tu, Th 9:00-11:50am

Summer 2016

INSTRUCTORS: Steven M. Berry; SSB 152C; 726-7087; smberry@d.umn.edu
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TEACHING ASSISTANTS: Shane Wilson, wils1582@d.umn.edu
Office Hours: Monday 11am-1pm in lab (SSB 244)

REQUIRED TEXT: *Modern Experimental Biochemistry 3rd Ed.* by Rodney Boyer (ISBN 0-8053-311-5)

LABORATORY SUPPLIES: Safety Goggles
Bound Laboratory Book (composition)

WEBSITE: <https://ay15.moodle.umn.edu/my/>

COURSE OBJECTIVES: The purpose of this biochemistry laboratory course is to give you hands on experience applying several basic techniques that are commonly utilized in modern biochemistry and molecular biology laboratories. In conjunction with this experience, keeping a quality lab notebook and effectively communicating experimental procedures and results through written reports are also emphasized.

EXPECTATIONS: Students should attend and arrive prepared for every lab. This entails looking over the Moodle site, bringing your lab notebook, and reading the relevant book sections and handouts that pertain to the lab before arriving. In addition, you need to prepare your notebook before you come to lab. This means that if calculations need to be done, such as determining how much protein sample to add to an SDS-PAGE gel, you need to do those in your notebook before you arrive. Three hours will go by very quickly in this biochemistry lab. You should anticipate staying for the full three hours and a lack of preparation or mistakes may necessitate staying longer to complete the lab. We anticipate that most of you will finish most of the labs on time; however, you should be aware of the potential need to stay longer and designate some time after lab for that possibility.

ABSCENCES: Please notify the TA or instructor in advance if you are going to miss a lab to see if you will be excused. Because of the extremely contracted time schedule, there will be no opportunity for make-up labs and it is unlikely that an absence will be excused. Unexcused absences will result in a loss of points.

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|-----------------|--|---------|
| GRADING: | Handout for Exp. 1 | 10 pts |
| | Lab reports (2 x 25 pts, 1 x 40, 1 x 15) | 105 pts |
| | Exp. 2 Rough Draft Checks | 12 pts |
| | Quizzes (5 x 10 pts) | 50 pts |
| | Final Quiz | 20 pts |
| | Lab Participation: | |
| | Notebook Preparation (12 x 3, 2 x 1.5 pts) | 39 pts |
| | Effort | 30 pts |
| | Total: | 266 pts |

No late work will be accepted; however, your TA might be willing to grant a brief extension on a lab report.

QUIZZES (50 points): A quiz will be administered relating to each experiment and usually on the *first week* of the experiment at the beginning of class. If you arrive late, you will receive a zero for the quiz and not allowed to take it at a later time. Your pre-laboratory reading will prepare you for the quiz as well as any discussion your TA provides the

previous week. The assigned readings are listed in the syllabus and the quiz questions will be taken from these materials and white board illustrations or PowerPoints.

FINAL QUIZ (20 points): There will be a comprehensive final quiz the last week of lab.

LABORATORY NOTEBOOKS (39 points): Notebooks are worth ~15% of your final grade. Your notebook should have a **Table of Contents** on the front page that includes page numbers and date of the experiment. The laboratory notebook should have the following sections for each experiment, with clearly marked headings. You should have completed sections 1-4 in your notebook before you arrive at lab (except for the first day). The TA will sign your notebook and assign your "Notebook preparation" grade based on this information. They will also grade sections 5 & 6 from the prior week, so keep your notebooks up to date. It is your responsibility to see that your notebook gets graded each week (sections 1 – 4 are worth 1.5 pts, and sections 5 – 6 are worth the 1.5 pts).

1. Date
2. Title of the experiment
3. Purpose and importance of the experiment
4. Materials and Methods – amounts of reagents and equipment needed for the experiment and the description of the procedure required to complete the experiment including any modifications. No need to write the entire protocol here, just summarize the main techniques, reagents etc. This section **should be fairly short (5 – 10 incomplete sentences, may be in bullet points).**
5. Observations/Results/Discussion – should contain the data you collected when performing the experiment including a description of any changes or deviations from the protocol or any calculations if applicable. Record things such as the actual pH of the buffer your TA made; it may be 7.05 instead of 7.00 like the protocol suggested. Record the actual reagents you use. You will also include printouts of any graphs or spectra.
6. Conclusions – your final analysis of the data – just a couple of sentence summary, nothing too detailed.

EFFORT GRADE (30 points): This is more than 10% of your grade and will be a reflection of your individual group participation, engaging in the material, attendance and timeliness, lab technique, and cleaning up after the lab. It is possible to lose all 30 points for being late more than once, or slacking off one day while your partner does this work even if you have fantastic technique the rest of the semester. You must excel in all these terms consistently, not just one or two to earn effort points.

LABORATORY REPORTS (105 points): Written lab reports are expected after each experiment has been completed. A grading rubric is available on the Moodle site and should be printed and attached to each lab report if the instructor asks for a hard copy. However, if lab reports are submitted on-line through the Moodle website, then attachment of the rubric is not necessary. Grades will be based on completeness, clarity, style, and format, with a particular emphasis on the **Results & Discussion** and **Conclusions** sections. The lab reports should be written individually, not as a group. Written material should be your own; however your group may work on your figures, tables and images together. For example you and your partner may want to work together on calculations, or cropping and labeling the lanes on your gel which would result in two identical looking gel images. That is just fine so long as your summary and interpretation of the gel are in your own words.

For more information on the content of the lab report, please see the report writing resources available on Moodle and follow the standard American Chemical Society guidelines outline in the "ACS Report Writing" document provided on the course Moodle site. You will be rigorously graded on the quality of your writing as well as the organization and content.

SAFETY: Each experiment will have safety notes associate with it. Your TA will go over them before you start working each week. However, you must familiarize yourself with them *before* the lab period as they may show up on the quiz. These are some general guidelines to follow:

1. Lab attire. Although it may actually warm up before the end of the semester, you CANNOT work in the lab while wearing tank tops, sandals or shorts/skirts of any length, nor have an exposed midriff. You must wear clothing that covers and protects you should spills occur. Closed toe shoes are especially important due to the potential hazards of dropping glassware. If you show up to lab dressed inappropriately, you will be asked to leave and change your clothing/shoes. This is a requirement of OSHA, the University, the UMD Chemistry & Biochemistry Department as well as the instructor. There will be no makeup time given for the changing of clothes/shoes.
2. **Always use gloves in the lab.** This protects your experiments from you and you from your experiments. If availability of gloves in your size is getting low or is unavailable, please ask the TA.
3. Take note of the location of the eyewash and shower stations in case of emergency. If you wear contacts, please switch to glasses if you have them and EVERYONE should wear goggles to protect their eyes from splashes. There may be some weeks where the TA will let you know that goggles are not needed such as the weeks where we will be doing computer work. Plan on bringing goggles each week and only take them off if told it is alright to do so.
4. Do not leave the lab if you splash something in your eye or on your clothes. Inform the TA immediately so that you can receive the necessary attention. In general a splash to the eye requires 20 minutes of washing at the eye wash sink. A splash on the skin requires 15-20 minutes at the sink or shower.
5. Be aware of the chemicals you are using. We will be using neurotoxins (acrylamide), strong acids and bases, materials that may be toxic if inhaled or spilled on the skin, and materials that can scratch or damage the eye. Please be considerate and clean up after any spills/contamination so that others who use this lab do not need to guess what toxins, etc. you left behind.
6. Immediately report to the TA any missing items, broken items, potentially contaminated reagents, potentially dangerous situations, etc.
7. Always clean up after yourself. This is **your** responsibility—not the TA's. If you are using biological material, clean the bench with 70% ethanol. Wash the bench with water if you may have spilled acid, etc. Never leave anything behind that could hurt someone else if they touch it with bare hands and/or rub their eyes.

ACCESS FOR STUDENTS WITH DISABILITIES: Individuals who have any disability or physical condition (such as pregnancy or allergy), which might affect their ability to perform in this class are encouraged to inform the instructor at the start of the semester. Adaptation of the methods, materials or testing may be made as required for equitable participation. This publication/material is available in alternate formats to individuals with disabilities upon request. Please contact Penny Cragun, Disability Services and Resources Center (726–8727).

STUDENT ACADEMIC INTEGRITY POLICY: “Academic dishonesty tarnishes UMD's reputation and discredits the accomplishments of students. UMD is committed to providing students every possible opportunity to grow in mind and spirit. This pledge can only be redeemed in an environment of trust, honesty and fairness. As a result academic dishonesty is regarded as a serious offense by all members of the academic community. In keeping with this ideal, this course will adhere to UMD's Student Academic Integrity Policy, which can be found at: <http://www.d.umn.edu/vcaa/StudentAcademicIntegrity.html>. This policy sanctions students engaging in academic dishonesty with penalties up to and including expulsion from the university for repeat offenders.”

UNIVERSITY STUDENT CONDUCT CODE: Disruptive classroom behavior that substantially or repeatedly interrupts either the instructor's ability to teach, or student learning is prohibited. Disruptive behavior includes inappropriate use of technology in the classroom. Examples include ringing cell phones, text-messaging, watching videos, playing computer games, doing email, or surfing the Internet on your computer, instead of note-taking or other instructor-sanctioned activities. This code will be enforced and can be found at: http://regents.umn.edu/sites/default/files/policies/Student_Conduct_Code.pdf.

SCHEDULE FOR LABORATORY EXPERIMENTS

| Experiment | Date | Description | Quiz | Report Due | Readings |
|---|--------|--|------------------------|--|--|
| Check in Exp. 1: Intro to pipetting | May 10 | Safety / Syllabus Pipetting & UV-Vis Spectrophotometry | | | Boyer: pp 3-10, 16-25, and handouts |
| Exp. 2: Isolation and Characterization of bovine milk α -lactalbumin | May 12 | 1) Purification of milk protein 2) Pouring an SDS-PAGE gel | Quiz 1 (10 pts) | Exp. 1 Handout (10 pts) | Boyer: pp 41-45, 111-121, 133-135, 257-277 |
| | May 17 | Size exclusion chromatography | Quiz 2 (10 pts) | Exp. 2 Intro only (4 pts) | |
| | May 19 | 1) Affinity chromatography 2) Bradford assay | | Exp. 2 Methods only (4 pts) | |
| | May 24 | 1) Running of SDS PAGE | | Exp. 2 Results (no gel) (4 pts) | |
| Exp. 3: Identification of serum glycoproteins | May 26 | 1) Running of SDS PAGE 2) Western blot – transfer | | | Boyer: pp 136-138, 321-331 |
| | May 31 | Western blot – develop | Quiz 3 (10 pts) | Exp. 2 (25 pts) | |
| Exp. 4a: Bioinformatics and molecular modeling of tyrosinase structure | Jun 2 | Utilization of online databases and resources, web based sequence analysis, and molecular modeling tools | | | Boyer: pp 211-225 and handouts |
| | Jun 7 | PyMOL | | Exp. 3 (25 pts) | |
| Exp. 4b: Kinetic analysis of tyrosinase | Jun 9 | Tyrosinase enzyme kinetics: Determination of substrate K_m and V_{max} | Quiz 4 (10 pts) | | Boyer: pp 141-157, 279-301 |
| | Jun 14 | Tyrosinase enzyme inhibition kinetics: Determination of substrate K_m and V_{max} in the presence of inhibitors | | | |
| Exp. 5: Restriction mapping of plasmid DNA | Jun 16 | 1) Restriction digest of DNA 2) Agarose gel analysis | | Exp. 4 (40 pts) | Boyer: pp 111-113, 122-127, and handouts |
| | Jun 21 | 1) Restriction digest of unknown DNA 2) Agarose gel analysis | Quiz 5 (10 pts) | | |
| Checkout | Jun 23 | Check out of lab drawers | Final Quiz (20 pts) | Exp. 5 Analysis and pre-lab only (15 pts) | |