ADVANCED BIOCHEMISTRY LABORATORY

Laboratory Manual for Biochemistry and Molecular Biology 471

2020 Edition[1]

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ADVANCED BIOCHEMISTRY LABORATORY SUPPLEMENT

2020 Edition[1]

(Following Experimental Unit E)

INTRODUCTION

Instructors

Faculty & Staff	Office	Phone	Office Hours*
Neil R. Bowlby	117 Biochemistry	353-8546	10 - 12 noon Mon.
R. Michael Garavito	513 Biochemistry	355-9724	3 – 4:00 p.m. Mon. & Wed.
Min-Hao Kuo**	401A Biochemistry	355-0163	3 – 5 p.m. Mon.
Allan TerBush	113 Biochemistry	355-3971	10 - 12 noon Mon.

Teaching Assistants	Office	Phone	Email
Caitlyn Herr	6105 BPS	(517) 884-5369	vangeld6@msu.edu
Gracielou Klinger	313 BCH	(517) 432-3668	klingerg@msu.edu

*students are welcome to come at other times, but should make an appointment.
**course coordinator

Books Used in Biochemistry and Molecular Biology 471

Texts

Required to be purchased by student:

- *Advanced Biochemistry Laboratory*, 2020 ed. (this lab manual)
- *Advanced Biochemistry Laboratory Supplement*, 2020 ed. (Part of this lab manual)
- Segel, I.H., *Biochemical Calculations*, 2nd ed., J. Wiley & Sons, Inc., 1976.
- Day, R.A., *How to Write and Publish a Scientific Paper*, 6th or 7th ed. Oryx Press, 2006.

Recommended to purchase:

- Boyer, R.F., *Modern Experimental Biochemistry*, 3rd ed., Addison Wesley Pub. Co., 2000.
- Voet, D. and Voet, J.G., *Biochemistry*, 2nd edition, J. Wiley & Sons, N.Y., N.Y., 1995.

Available in the Teaching Labs and Room 105 Biochemistry:

- Cooper, T.G., *The Tools of Biochemistry*, 1st ed., Wiley-Interscience, 1977.
- Various authors, textbooks and other reference material on laboratory techniques.

Handbooks

- *Handbook of Chemistry & Physics*, CRC Press (various editions, a new edition is published each year)
- *The Merck Index*, Merck & Co. (various editions)
- Dawson, R.M., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research*, 3rd ed., Clarendon Press, Oxford, 1986.
- Sax, N.I., *Dangerous Properties of Industrial Materials*, 4th or 5th ed., Van Nostrand Reinhold Co., 1975 (4th), 1979 (5th)
- Rayburn, S.R., *The Foundations of Laboratory Safety*, Springer-Verlag, 1990.

Objectives

The subject areas for the course are weak acids and bases, spectrophotometry, protein purification, and enzyme activity assays. The objectives for the course are for students to:

- Develop the ability to design logical experiments given specific experimental objectives but only general procedures, perform these experiments successfully and independently, properly interpret the data, and clearly present the data in writing.
- Learn foundational biochemical laboratory methodology and techniques.
- Further develop:
    - quantitative laboratory skills.
    - the ability to keep a clear and complete lab notebook.
    - the ability to analyze and interpret laboratory data.
    - skill in writing scientific laboratory reports.
- Understand the concepts on which the laboratory experiments are based and related concepts (largely covered in lecture and homework problems).

Organization of Course

The course consists of one 50-minute lecture/discussion period and one 5-hour laboratory period per week. A pre-lab session will be held in Rm. 111 Biochemistry starting at 12:40 p.m. of each scheduled lab period. The course will have a semi-open lab format; students may leave and return to the lab rooms at any time. The labs (113 & 117 Biochemistry) will be open Tuesday through Friday from 12:30 to 5:30 p.m., and students may work only on their scheduled lab day.

For parts of Unit A and all of Unit D, students will work in groups of two. Each student will work individually in Units B, C, and E. When working in pairs, partners only collaborate on the experimental (data acquisition) portions of the labs; all notebook preparation, data analysis/post-lab notebook entries and lab reports are to be done individually. Unit E is considered a practical examination for the course. **Students are expected to perform experimental work each lab period. Except in cases of excused absence, students who do not participate in data collection during the lab period are not entitled to the data collected by others.**

Ethics

Ethical behavior and academic integrity in this course are expected and assumed. The consequences can be quite serious if these are not practiced. We will teach you accepted standards of intellectual honesty with respect to the performance of experiments, the processing of data, and the reporting of results. We expect you to follow these standards. Particularly be aware of when you must work independently. Credit will not be given to work obtained through collaboration when independent effort is required.

Computer Resources

The Biochemistry Computer Room is located in Room 105 (in the Undergraduate Office) of the Biochemistry Building. This room contains five computers that have Windows and Microsoft Office installed and are connected to multifunction printers. These computers have programs useful to BMB 471 students. All computers are available for your use when the office is open, normally between 8:00 a.m. and 5:00 p.m., Monday-Friday. In addition, the Teaching Lab has computers in the lab that may be used during scheduled laboratory hours.

We will be using the Desire2Learn (D2L) system for posting announcements and course materials as well as for pre-lab problem sets (according to the schedule posted on Page 5), and submission of a digital copy of your laboratory reports for Units D and E.

Course Schedule & Due Dates

Experimental work, notebook and laboratory report due dates

Lab Period	Date	Experimental Work	Due Dates
1	Jan. 7 - 10	Lab introduction; check in; safety tour; student practice with spectrophotometers, including recorder set-up and operation	
2	Jan. 14 - 17	**Unit A** - purity of light & wavelength accuracy	
3	Jan. 21 - 24	**Unit A** - absorbance accuracy & linearity, recorder use	
4	Jan. 28 - 31	**Unit B** - buffer preparation (titration curve); pH measurements	Jan. 28 - 31: Notebooks** Unit A
5	Feb. 4 - 7	**Unit B** - effects of salt & dilution on pH of a buffer	
6	Feb. 11 - 14	**Unit C** – AAT enzyme assays and Lowry protein assay	Feb. 11 - 14: Notebooks** Unit B
7	Feb. 18 - 21	**Unit D** – AAT heat denaturation and AAT assays	Feb. 18 - 21: Notebooks** Unit C
8	Feb. 25 - 28	**Unit D** – large-scale $(NH_4)_2SO_4$ precipitation and AAT assays	Feb. 24: Report I* Units A - C
	Mar. 2 - 6	Spring Break	
9	Mar. 10 - 13	**Unit D** - CM-sephadex column chromatography - AAT and Lowry protein assay of AAT fractions	Mar. 10 - 13: Notebooks** Unit D (Days 1 & 2)
10	Mar. 17 - 20	**Unit D** - SDS-PAGE of AAT fractions **Unit E** - buffer/ethanol preparation, yADH assay testing	
11	Mar. 24 - 27	**Unit E** - [yADH], [NAD^+], yADH stability	Mar. 24 - 27: Notebooks** Unit D (Days 3 & 4)
12	Mar. 31 - Apr. 3	**Unit E** - approximate K_m & V_{max}, stability	Mar 30: Report II* Unit D
13	Apr. 7 - 10	**Unit E -** precise valid range, stability	Apr. 7 - 10: Notebooks** Unit E (Days 0 - 2)
14	Apr. 14 - 17	**Unit E** - precise K_m & V_{max} and inhibition, stability	
15	Apr. 21 - 24	Lab check-out	Apr. 20: Notebooks** Unit E (Days 3 & 4) Apr. 24: Report III* Unit E

*submit reports by placing them in the wooden "Assignment Box" in Room 113, BCH Bldg.

REPORTS ARE DUE BY 12:00 p.m. - LATE REPORTS RECEIVE NO CREDIT

**lab notebooks must be submitted by the end of your lab period to be graded and available for return during labs within two business days (Sec. 1: Thur., Sec. 2: Fri., Sec. 3 & 4: Mon.).

Pre-lab problem sets

A short problem set will be available through D2L on Mondays. The problems will test your understanding of the experiments and prepare you for pre-lab and post-lab calculations/data analysis for experiments performed the following week. The assignments are due **before the scheduled start of the following week's lecture (12:40 p.m.)** on Mondays. The schedule for distribution and due dates of the problems is as follows:

Post Date	Due Date	Material Covered
January 6	January 13	Unit A – Day 1
January 13	January 20	Unit A – Day 2
January 20	January 28	Unit B – Day 1
January 27	February 3	Unit B – Day 2
February 3	February 10	Unit C – Enzyme and protein assays
February 10	February 17	Unit D – Day 1 (Heat denaturation)
February 17	February 24	Unit D – Day 2 ($(NH_4)_2SO_4$ fractionation)
February 24	March 9	Unit D – Day 3 (CM-sephadex, Lowry)
March 9	March 16	Unit D – Day 4 (SDS-PAGE, yADH buffers)
March 16	March 23	Unit E – Day 1 ([yADH], [NAD^+], yADH stability)
March 23	March 30	Unit E – Day 2 (Approximate K_m & V_{max})
March 30	April 6	Unit E – Day 3 (yADH Valid Range)
April 6	April 13	Unit E – Day 4 (Precise K_m & V_{max}, Inhibition)

Discussion section schedule and readings

(Mondays, 12:40 - 1:30 p.m., 101 Biochemistry)

Date	Subject	Reading
Jan. 6	Spectrophotometry, including Beer's and Lambert's laws	1. *Experimental Biochemistry Supplement,* Chapter 1 & 3. 2. Segel, I.H. *Biochemical Calculations*, 2nd ed., pp. 324 - 333
Jan. 13	Spectrophotometry (continued)	
Jan. 27	Buffers, activity, and related subjects	1. *Experimental Biochemistry Supplement,* Chapter 2. 2. Segel, I.H., *Biochemical Calculations*, 2nd ed. pp. 1-83.
Feb. 3	Buffers (continued)	
Feb. 10	Assay of enzymes & protein	1. *Experimental Biochemistry Supplement,* Chapter 4. 2. Segel, I.H., *Bioch. Calcs.*, 2nd ed. pp. 208-214, 291-92, 333-46.
Feb. 17	Protein purification	1. *Experimental Biochemistry Supplement,* Chapter 7. 2. Segel, I.H., *Bioch. Calcs.*, 2nd ed. pp. 287-290
Feb 24 Mar. 9, 16	Protein purification Chromatography SDS-PAGE	1. *Experimental Biochemistry Supplement,* Chapter 5. 1. *Experimental Biochemistry Supplement,* Chapter 6.
Mar. 23, 30 April 6, 13	Chemical and Enzyme Kinetics	1. *Experimental Biochemistry Supplement*, Chapter 8. 2. Segel, I.H., *Biochemical Calculations*, 2nd ed., pp. 208-212 (top), 214-219, 221 (bottom)-236 plus problem 4.9 on page 237, 246-250, 252.

Final Exam

Monday, April 27, 12:45 - 2:45 p.m. Location to be announced.

Some of the main course goals are for you to be able to take laboratory data, analyze and interpret it for meaningful biochemical conclusions. Therefore, the final exam will focus on your understanding of the quantitative and conceptual aspects of the experimental work, the material covered in the discussion (lecture) periods and the assigned readings, including pre-lab problems. Problems of the type encountered in the experimental work and in the pre-lab problems may be on the exams. You should also expect some short-answer questions focusing on the theory of your experiments and the molecular interactions involved in them.

Bring a calculator and transparent ruler to the final exam.

Grading

Grades will be based on the following items and approximate proportions of total points:

Laboratory notebook	40%
Laboratory reports	30%
Final Exam	20%
Pre-lab problems	10%
Total	100%

Students earning 90% or more of the total points will receive a grade of 4.0. The dividing line between 2.5 and 2.0 grades will be approximately 70% of the total points. The range between 90% and 70% will be divided into approximately equal parts for assigning grades of 3.5, 3.0, and 2.5. The dividing line between 1.0 and 0.0 grades will be about 55%. A passing grade will be awarded to any student who completes and submits all Problem Sets, Lab Reports and Notebooks on time, participates in all laboratory sessions (except for excused absences), and earns at least 50% of the points.

Note: Reports (both a printed copy and the electronic submission of completed template) are **due by 12:00 p.m.** on the day listed in the Schedule. **Reports submitted after the due time/date will not receive any credit**. Put reports II and III, unstapled, in a large envelope **with your name and group number on the outside** and place the envelope in the wooden "Assignment Box" in Room 113. Submit the "completed template" electronic copy of your report to the D2L dropbox for your section **by 12:00 p.m.** on the day listed in the Schedule.

Notebooks are **due before you leave lab (except the last grading; see schedule)** on the day listed in the schedule and will be available for pick-up two days after submission (Sections 3 & 4 notebooks will be available for pick-up first thing on Monday morning). Late notebooks will incur a **40 points per day penalty** and may not be available for pick up until the following Monday. Notebooks submitted more than 2 days late will receive no credit, but will be corrected, and will be available for pick-up as soon as they are graded.

Items Provided to Each Student Group

Each student pair will be provided with the following materials:

- Two reusable plastic Pasteur pipettes
- Labeling tape
- One styrofoam cuvette holder with plastic cuvettes - keep these clean (rinse at least four times with distilled water, shake out, and allow to dry after each day of use). Make certain they are matched within ± 1% before using with each other. Otherwise, apply a correction factor.
- Two boxes of yellow tips (one box per person) for use with the P-20 and P-200 Pipetmen, and one box of blue tips (shared by the group members) for use with the

P-1000 Pipetman. Additional yellow and blue tips will be available on the front bench to refill your boxes but **you should be conservative in your use of pipet tips**. We do not do any sterile work in this course, so a new pipet tip is not needed for every pipetting.

The following items will be in the drawers shared among sections:

- Four Sharpie marking pens (2 large, 2 small)
- Two sets of 3 spatulas (2 scoopula, 2 regular, 2 small)
- Five magnetic stir bars - two large, two small, one jumbo
- Four glass stirring rods - two large, two small
- 2 sets of Pipetters (P-20, P-200, P-1000); Pi-Pumps (2 green, 2 blue)
- Two each 10, 25, 50, 100, 200, 250 mL volumetric flask
- Two each: 400, 250, 30 mL glass beaker; 250, 125 mL Erlenmyer flask
- four each 50, 25 mL Erlenmyer flask
- six each: 50, 100 mL plastic beaker
- Two each: 25, 50, 100 mL graduated cylinder
- Six 25 mL plastic snap-cap containers

(All items used from the shared bench drawer must be cleaned and returned to the shared drawer at the end of lab)

The following equipment is continuously available in the laboratory **(do not put any of this in your lockers. Remove tape, rinse and place on the "dirty dish cart" after use)**:

- Beakers: 600, 100, 50 mL
- Erlenmeyer flasks: 300, 500 mL
- Funnels: various sizes
- Test tubes (in drawers): two sizes (18 x 150 mm [20 mL max.], 13 x 100 mm [7 mL max.])
- Pipettes (in drawers):
    - 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mL Mohr pipettes
    - 2.0, 5.0, 10.0, and 25.0 mL serological (blow-out) pipettes
    - 1.0, 2.0, 5.0, 10.0, 20.0, 25.0 mL volumetric pipettes
- Magnetic stirring motors and vortex mixers
- Ice buckets
- Water bottles for de-ionized water (fill from de-ionized water taps)

Each student pair will be assigned a laboratory drawer, with a lock, for storage. A plastic bin is also provided. Use this bin for storage of reagents in the cold room from one laboratory period to the next. Label the bin with your group member names printed on a small piece of tape. The combination for the lock is on the three-part paper ticket on the front of the lower drawer. When opening the lock, dial the combination precisely and open the lock by pulling gently on the lock. Fill out the paper ticket and place the top part back in the metal frame on the drawer. Tape the middle part, which contains the combinations to your drawer and the drawer containing the set of shared equipment, into your notebook. Sign the bottom portion after you have checked that all items listed above are in the pan and shared drawer, and return the bottom part of the paper ticket to an instructor or TA. All items are to be turned in at the end of the semester, and tape should be removed from the drawer and pan at that time.

Laboratory Safety

For the welfare of fellow students, and for your own well-being, each student is expected to follow a set of accepted laboratory precautions. To ensure that each student understands these guidelines, he/she is expected to sign a consent form. The form will be provided on the first day of class. In the lab:

- **Protective eyewear must be worn at all times** (safety glasses are acceptable); **chemical splash goggles (that you must bring to lab) must be worn when working with hazardous chemicals** (Units B and E). **No exceptions to this policy will be made.**
- Gloves must be worn when working with hazardous chemicals (Units B and D). We will supply suitable gloves for this purpose.
- Do not eat, drink, or apply cosmetics at any time while in the laboratory. **Do not place food wrappers, beverage cups, or other food-related waste in the trash cans in the lab.**
- Mouth pipetting is not permitted.
- Do not sit on the lab benches.
- Use chemicals with high vapor pressure only in the hood.
- Handle and dispose of hazardous chemicals properly. Disposal containers are provided.
- When chemicals are spilled they should be wiped or swept up or both as soon as possible. If the spillage is large, immediately notify an instructor. Sponges and brushes are provided and should be used to keep your work area clean.
- Be aware of objects that can burn or give electrical shocks.
- Do not use open flames near flammable chemicals.
- In general, be alert to possible hazards from chemicals, glassware, electrical connections, and flammable solvents. Read labels and observe suggested precautions.
- Sweep up broken glassware as soon as possible and deposit it in the labeled container set aside for this purpose.
- Never work alone.
- All solutions that you prepare must be labeled with date, your last name, and the type and concentration of the reagent. Unmarked solutions will be disposed.
- Wipe your lab bench with a damp sponge at the end of each lab period.
- Be familiar with location and use of safety items — location of safety showers, eyewash stations, fire blankets, fire extinguishers, medical kit. Review the lab floor plan and evacuation routes on page 11 before coming to the first lab period.

General Laboratory Rules

- Used glassware from the glassware cabinets must be rinsed with tap water and placed on the designated cart after all tape has been removed. Ink writing on this glassware need not be removed. Glassware from the shared bench drawers must be rinsed with tap water, then rinsed several times with de-ionized water and returned to the shared drawer.
- Items stored in the cold room or freezer boxes should be removed and disposed when they are no longer needed.
- The instructors will make every effort to keep equipment in good working order. It is your responsibility to read and understand instructions for the use of equipment. Do not turn on an instrument until you have done this. Do not hesitate to ask questions of your instructor after you have read the instructions. If any equipment malfunction is noted, report this immediately to an instructor.
- **Be considerate of others.** Clean up after yourself after using any piece of equipment. All equipment, especially balances, should be left ready for immediate use by another student.

In Case of an Accident

- Report all injuries, even minor ones, to an instructor immediately.

- In case of even minor laboratory accidents, you should go to Olin Health Center for treatment. Your instructor will provide an accident report form; a copy of this form must be taken to the Center.
- For any chemicals splashed in the eye, hold the eye open and flush immediately with cold water by using the eye wash. Flush for at least 5 minutes and call for help from an instructor.
- For chemicals spilled on the skin or splashed into the mouth, again, flush with large amounts of cold water for 5 or more minutes. Call for an instructor.
- For severe bleeding, apply pressure and call for help from an instructor.
- For burns, flush with cold water and contact an instructor.
- Note: Injury to students in the laboratory is not covered by University insurance. Students must provide their own insurance coverage.

Recycling

Containers are provided in Room 113 & 117 for recycling white office paper and mixed or colored paper. Please use these for disposing of paper. Unused photocopies or pages from the printer go in the white office paper bin. Unused chart paper goes in the white paper bin. Newspapers should be placed in the “Mixed paper” recycling container or the newspaper recycling container in Room 113. The printers contain "once-used" paper and are intended to be used to produce "rough-draft" hardcopies. When finished with these printouts, place them in the white office paper recycling bin. If you need blank paper for printing, see Dr. Bowlby.

Academic Honesty

Academic honesty is central to the educational process and acts of academic dishonesty are serious offenses within the University community. **Suspension from the University could be the consequence for acts of academic dishonesty**.

Students should be familiar with General Student Regulation 1.00 on Scholarship and Grades (see below), and with the all-University policy on Integrity of Scholarship and Grades. In addition, it is important that students clearly understand the specific expectations of their individual instructors with regard to this important matter. The process for adjudicating cases of academic dishonesty is outlined in Section 2.4 of Academic Freedom for Students at Michigan State University, pp. 45-62.

1.00 PROTECTION OF SCHOLARSHIP AND GRADES

The principles of truth and honesty are fundamental to the educational process and the academic integrity of the University; therefore, no student shall:

1.01 **claim or submit the academic work of another as one's own**.

1.02 **procure, provide, accept or use any materials containing questions or answers to any examination or assignment without proper authorization**.

1.03 **complete or attempt to complete any assignment or examination for another individual without proper authorization**.

1.04 **allow any examination or assignment to be completed for oneself, in part or in total, by another without proper authorization.**

1.05 **alter, tamper with, appropriate, destroy or otherwise interfere with the research, resources, or other academic work of another person.**

1.06 **fabricate or falsify data or results.**

INTEGRITY OF SCHOLARSHIP AND GRADES (All-University Policy)

The following statement of University policy was approved by the Academic Council and the Academic Senate, and serves as the definitive statement of principle and procedure to be used in instances of academic dishonesty.

1. The principles of truth and honesty are recognized as fundamental to a community of scholars. The University expects both instructors and students to honor these principles and, in so doing, to protect the validity of University education and grades. Practices that maintain the integrity of scholarship and grades include providing accurate information for academic and admission records, adherence to unit-approved professional standards and honor codes, **and completion of original academic work by the student to whom it is assigned, without unauthorized aid of any kind.** To encourage adherence to the principles of truth and honesty, instructors should exercise care in planning and supervising academic work.
2. **If an instructor alleges a student has committed an act of academic misconduct, the instructor is responsible for taking appropriate action.** Depending on the instructor's judgment of a specific instance, the instructor may give the student a penalty grade. A penalty grade may be a reduced score or grade for the assignment or a reduced grade for the course.
3. **When an instructor gives an undergraduate or graduate student a penalty grade for academic misconduct, the instructor must provide a written description of the details of the academic misconduct to the student and to the student's academic dean.** The student's academic dean will add the written description to the student's academic record, where it will remain, unless the student successfully grieves the allegation.
4. In notifying the student's academic dean of the student's act of academic misconduct, the instructor may request the student's academic dean to initiate an academic disciplinary hearing to impose sanctions in addition to, or other than, a penalty grade.
5. When in the judgment of the student's academic dean, a sanction in addition to, or other than, a penalty grade is warranted (e.g., dismissal from a unit or program), the dean may call for an academic disciplinary hearing. In calling for an academic disciplinary hearing, the student's academic dean may act independently or in response to a request by the instructor.
6. An undergraduate student accused of academic misconduct may request an academic grievance hearing to contest a penalty grade based on a charge of academic misconduct before the University Academic Integrity Hearing Board.....In cases involving academic misconduct, no student may be dismissed from a program of study without an academic disciplinary hearing.
7. On the first offense of academic misconduct, the student must attend an educational program on academic integrity and academic misconduct provided by the Associate Provost for Undergraduate Education and Dean of Undergraduate Studies for undergraduate students and the Dean of The Graduate School for graduate students.

For more information, refer to: **Academic Freedom for Students at Michigan State University**.

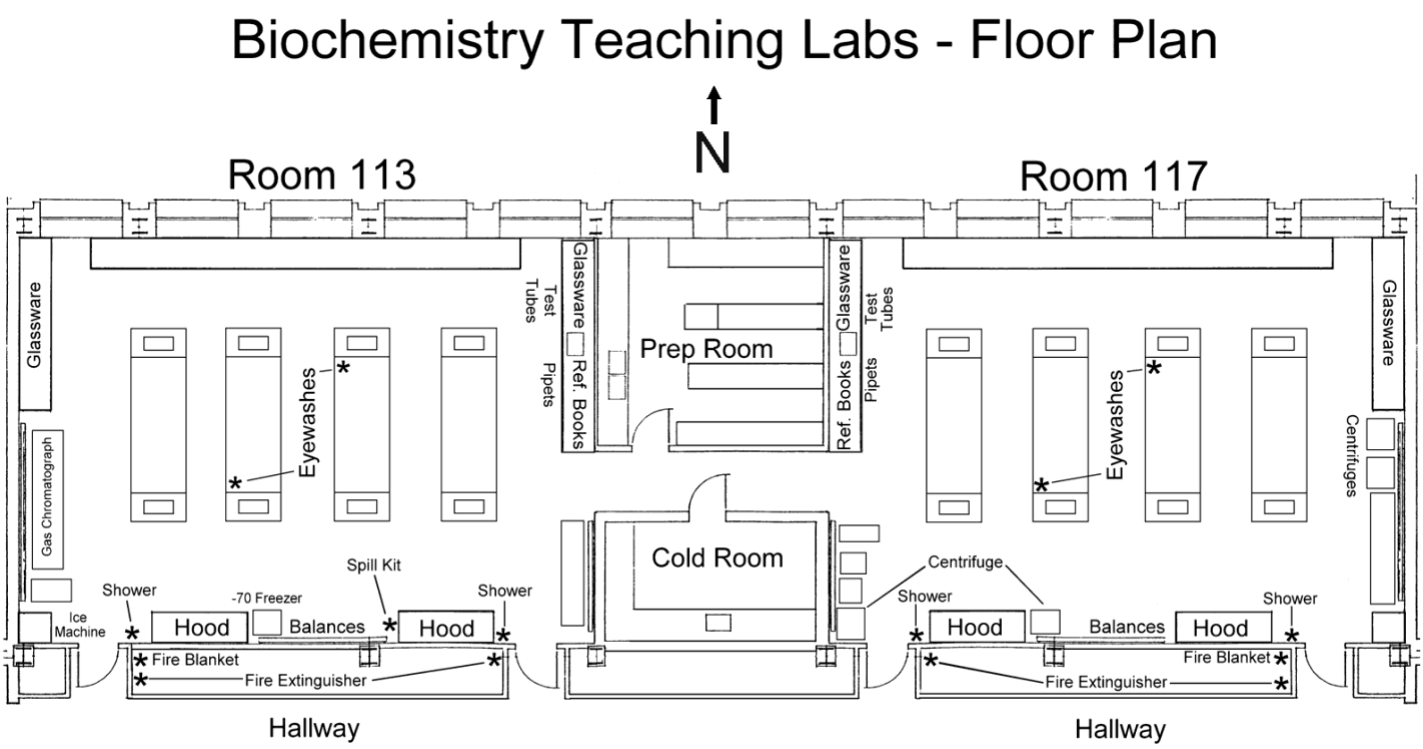
ADDITIONAL COMMENTS ON ETHICS IN BMB471:

Ethical behavior and academic integrity in this course are expected and assumed. Deviations from either will have serious consequences. We will teach you accepted standards of intellectual honesty with respect to the performance of experiments, the processing of data, and the reporting of results. We expect you to follow these standards.

Each student is to work completely independently in the processing of their data, as well as in the writing of the reports. Students are welcome to discuss their data with each other, with instructors, or with others to achieve a full understanding of them before processing them. The exception to open discussion: ***In the units where a coworker collects some of your data, you may not discuss the processing of the data or the preparation of the report with your coworker in any way.*** Discussions about the data and experiment with anyone else are still encouraged.

Plagiarism of any kind will not be tolerated. Penalties for plagiarism will range from ***a severe point deduction to a score of zero*** for the assignment, and as such will be regarded as a ***"penalty grade"***. In accordance with Item #3 above, a report will be forwarded to the academic dean, which will become a part of the student's academic record. In addition, ***falsification or fabrication of any laboratory data*** will result in a failing grade in the class and a report will be forwarded to the academic dean.

Teaching Labs Floor Plan



Emergency Evacuation Instructions

In Case of an Emergency

Fire: Exit room and go left (East) to EXIT and move away from building

Tornado: Exit room and go left (East) and downstairs to middle of building