

**ADVANCED BIOCHEMISTRY LABORATORY Syllabus**  
**Laboratory Manual for Biochemistry and Molecular Biology 471**  
2020 Edition<sup>1</sup>

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## 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
Graciellou Klinger	313 BCH	(517) 432-3668	klingerg@msu.edu
Samantha Heiler			heilers1@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*, 2<sup>nd</sup> ed., J. Wiley & Sons, Inc., 1976.
- Day, R.A., *How to Write and Publish a Scientific Paper*, 6<sup>th</sup> or 7<sup>th</sup> ed. Oryx Press, 2006.

Recommended to purchase:

- Boyer, R.F., *Modern Experimental Biochemistry*, 3<sup>rd</sup> ed., Addison Wesley Pub. Co., 2000.
- Voet, D. and Voet, J.G., *Biochemistry*, 2<sup>nd</sup> 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*, 1<sup>st</sup> 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*, 3<sup>rd</sup> ed., Clarendon Press, Oxford, 1986.
- Sax, N.I., *Dangerous Properties of Industrial Materials*, 4<sup>th</sup> or 5<sup>th</sup> ed., Van Nostrand Reinhold Co., 1975 (4<sup>th</sup>), 1979 (5<sup>th</sup>)
- 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:
  - o quantitative laboratory skills.
  - o the ability to keep a clear and complete lab notebook.
  - o the ability to analyze and interpret laboratory data.
  - o 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	<b>Unit A</b> - purity of light & wavelength accuracy	
3	Jan. 21 - 24	<b>Unit A</b> - absorbance accuracy & linearity, recorder use	
4	Jan. 28 - 31	<b>Unit B</b> - buffer preparation (titration curve); pH measurements	Jan. 28 - 31: Notebooks** Unit A
5	Feb. 4 - 7	<b>Unit B</b> - effects of salt & dilution on pH of a buffer	
6	Feb. 11 - 14	<b>Unit C</b> – AAT enzyme assays and Lowry protein assay	Feb. 11 - 14: Notebooks** Unit B
7	Feb. 18 - 21	<b>Unit D</b> – AAT heat denaturation and AAT assays	Feb. 18 - 21: Notebooks** Unit C
8	Feb. 25 - 28	<b>Unit D</b> – large-scale $(\text{NH}_4)_2\text{SO}_4$ precipitation and AAT assays	Feb. 24: Report I* Units A - C
	Mar. 2 - 6	Spring Break	
9	Mar. 10 - 13	<b>Unit D</b> - 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	<b>Unit D</b> - SDS-PAGE of AAT fractions <b>Unit E</b> - buffer/ethanol preparation, $\gamma$ ADH assay testing	
11	Mar. 24 - 27	<b>Unit E</b> - [ $\gamma$ ADH], [ $\text{NAD}^+$ ], $\gamma$ ADH stability	Mar. 24 - 27: Notebooks** Unit D (Days 3 & 4)
12	Mar. 31 - Apr. 3	<b>Unit E</b> - approximate $K_m$ & $V_{max}$ , stability	Mar 30: Report II* Unit D
13	Apr. 7 - 10	<b>Unit E</b> - precise valid range, stability	Apr. 7 - 10: Notebooks** Unit E (Days 0 - 2)
14	Apr. 14 - 17	<b>Unit E</b> - 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 <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> fractionation)
February 24	March 9	Unit D – Day 3 (CM-sephadex, Lowry)
March 9	March 16	Unit D – Day 4 (SDS-PAGE, $\gamma$ ADH buffers)
March 16	March 23	Unit E – Day 1 ([ $\gamma$ ADH], [NAD <sup>+</sup> ], $\gamma$ ADH stability)
March 23	March 30	Unit E – Day 2 (Approximate $K_m$ & $V_{max}$ )
March 30	April 6	Unit E – Day 3 ( $\gamma$ ADH 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. <i>Experimental Biochemistry Supplement</i> , Chapter 1 & 3. 2. Segel, I.H. <i>Biochemical Calculations</i> , 2 <sup>nd</sup> ed., pp. 324 - 333
Jan. 13	Spectrophotometry (continued)	
Jan. 27	Buffers, activity, and related subjects	1. <i>Experimental Biochemistry Supplement</i> , Chapter 2. 2. Segel, I.H., <i>Biochemical Calculations</i> , 2 <sup>nd</sup> ed. pp. 1-83.
Feb. 3	Buffers (continued)	
Feb. 10	Assay of enzymes & protein	1. <i>Experimental Biochemistry Supplement</i> , Chapter 4. 2. Segel, I.H., <i>Bioch. Calcs.</i> , 2 <sup>nd</sup> ed. pp. 208-214, 291-92, 333-46.
Feb. 17	Protein purification	1. <i>Experimental Biochemistry Supplement</i> , Chapter 7. 2. Segel, I.H., <i>Bioch. Calcs.</i> , 2 <sup>nd</sup> ed. pp. 287-290
Feb 24	Protein purification	1. <i>Experimental Biochemistry Supplement</i> , Chapter 5.
Mar. 9, 16	Chromatography SDS-PAGE	1. <i>Experimental Biochemistry Supplement</i> , Chapter 6.
Mar. 23, 30 April 6, 13	Chemical and Enzyme Kinetics	1. <i>Experimental Biochemistry Supplement</i> , Chapter 8. 2. Segel, I.H., <i>Biochemical Calculations</i> , 2 <sup>nd</sup> 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%
<u>Pre-lab problems</u>	<u>10%</u>
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.