

ADVANCED BIOCHEMISTRY LABORATORY
Laboratory Manual for Biochemistry and Molecular Biology 471
2018 Edition¹

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ADVANCED BIOCHEMISTRY LABORATORY SUPPLEMENT
2018 Edition¹

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I. INTRODUCTION

A. Instructors

Faculty & Staff	Office	Phone	Office Hours*
Neil R. Bowlby	113 Biochemistry	353-8546	10 - 12 noon Mon.
R. Michael Garavito**	513 Biochemistry	355-9724	10 - 11 a.m. Mon. & Fri.
Min-Hao Kuo	401A Biochemistry	355-0163	3 – 5 p.m. Mon.

Teaching Assistants	Office	Phone	Email
Jiaying Chen	502C Biochemistry	517-940-1981	chenj108@msu.edu
Greg Ciesielski	319 Biochemistry	517-353-4918	glc@msu.edu
Kody Duhl	209 Biochemistry	517-884-8964	duhlkody@msu.edu
Breanna Wydra	459 Giltner Hall	517-432-5439	wydrabre@msu.edu

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

**course coordinator

B. Books Used in Biochemistry and Molecular Biology 471

1. Texts

a. Required to be purchased by student:

- (1) *Advanced Biochemistry Laboratory*, 2017 ed. (this lab manual)
- (2) *Advanced Biochemistry Laboratory Supplement*, 2017 ed. (Part of this lab manual)
- (3) Segel, I.H., *Biochemical Calculations*, 2nd ed., J. Wiley & Sons, Inc., 1976.
- (4) Day, R.A., *How to Write and Publish a Scientific Paper*, 6th or 7th ed. Oryx Press, 2006.

b. Recommended to purchase:

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

2. Handbooks:

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

C. Objectives

The subject areas for the course are weak acids and bases, spectrophotometry, organelles and lipids, and enzymes and other proteins. The objectives for the course are for students to:

1. 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.
2. Learn modern biochemical laboratory methodology and techniques.
3. Further develop:
 - a. quantitative laboratory skills.
 - b. the ability to keep a clear and complete lab notebook.
 - c. the ability to treat laboratory data.
 - d. skill in writing scientific laboratory reports.
4. Understand the concepts on which the laboratory experiments are based and related concepts (largely covered in lecture and homework problems).

D. 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.

Students work in groups of two on Unit C and individually in Units A, B, and D. Students work in pairs only on the experimental (data acquisition) portion of Unit C; notebook preparation, data analysis/post-lab notebook entries and lab reports are to be done individually. **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.**

E. 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.

F. Computer Resources

The Biochemistry Computer Room is located in Room 105 (in the Undergraduate Office) of the Biochemistry Building. This room contains four computers that have Windows and Microsoft Office installed and are connected to several multifunction printers. These computers have SIMZYME and Hyperbolic Regression as well as other 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, however, these computers do not have internet access.

We will be using the Desire2Learn (D2L) system for posting announcements and course materials as well as for submission of text-only laboratory reports. Pre-lab problem sets will be available on LON-CAPA according to the schedule posted in G.2. (Page 5)

G. Schedule and Dates for Experimental Work, Notebooks and Lab Reports, Pre-lab problem sets, Discussion Sections and readings, and Final Exam

1. Experimental work, notebook and laboratory report due dates

Lab Period	Date	Experimental Work	Due Dates
1	Jan. 9 - 12	Lab introduction; check in; safety tour; student practice with spectrophotometers, including recorder set-up and operation	
2	Jan. 16 - 19	Unit A - purity of light, wavelength accuracy, absorbance accuracy	
3	Jan. 23 - 26	Unit A - absorbance linearity, recorder use	
4	Jan. 30 - Feb. 2	Unit B - buffer preparation (titration curve); pH measurements	
5	Feb. 6 - 9	Unit B - effects of salt & dilution on pH of a buffer	
6	Feb. 13 - 16	Unit C – AAT enzyme assays and Lowry protein assay	Feb. 12: Notebooks** Units A-B
7	Feb. 20 - 23	Unit C – AAT heat denaturation and AAT assays	Feb. 19: Report I* Units A-B
8	Feb. 27 - Mar. 2	Unit C – large-scale (NH ₄) ₂ SO ₄ precipitation and AAT assays	Feb. 26: Notebooks - Unit C (Days 1 & 2)**
	Mar. 5 - 9	Spring Break	
9	Mar. 13 - 16	Unit C - CM-sephadex column chromatography - Lowry protein assay of AAT fractions	
10	Mar. 20 - 23	Unit C - SDS-PAGE of AAT fractions Unit D - buffer/ethanol preparation, yADH assay testing	
11	Mar. 27 - 30	Unit D - [yADH], [NAD ⁺], yADH stability	Mar. 26: Notebooks - Unit C (Days 3-5)**
12	Apr. 3 - 6	Unit D - approximate K _m & V _{max} , stability	April 2: Report II - Unit C*
13	Apr. 10 - 13	Unit D - precise valid range, stability	
14	Apr. 17 - 20	Unit D - precise K _m & V _{max} and inhibition, stability	
15	Apr. 24 - 27	Lab check-out	Apr. 23: Notebooks** Apr. 27: Report III* Unit D
Final Exam	Monday, April 30, 12:45 - 2:45 p.m. Location to be announced.		

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

REPORTS ARE DUE BY 2:00 p.m. - LATE REPORTS RECEIVE NO CREDIT

**lab notebooks must be submitted by 9:00 a.m. to be graded and returned by 12:40 of that day.

2. Pre-lab problem sets

A short problem set will be available through LON-CAPA on Mondays. The problems will test your understanding of the experiments and the processing of the data collected 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 8	January 15	Unit A – Day 1
January 15	January 22	Unit A – Day 2
January 22	January 29	Unit B – Day 1
January 29	February 5	Unit B – Day 2
February 5	February 12	Unit C – Day 1 (AAT assay)
February 12	February 19	Unit C – Day 2 (Heat denaturation)
February 19	February 26	Unit C – Day 3 ((NH ₄) ₂ SO ₄ fractionation)
February 26	March 12	Unit C – Day 4 (CM-sephadex, Lowry)
March 12	March 19	Unit C – Day 5 (SDS-PAGE, yADH buffers)
March 19	March 26	Unit D – Day 1 (yADH assays)
March 26	April 2	Unit D – Day 2 (Approximate K_m & V_{max})
April 2	April 9	Unit D – Day 3 (Valid Range)
April 9	April 16	Unit D – Day 4 (Precise K_m/V_{max} , Inhibition)

3. Discussion section schedule and readings.

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

Date	Subject	Reading
Jan. 8	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 nd ed., pp. 324 - 333
Jan. 22	Spectrophotometry (continued)	
Jan. 29	Buffers, activity, and related subjects	1. <i>Experimental Biochemistry Supplement</i> , Chapter 2. 2. Segel, I.H., <i>Biochemical Calculations</i> , 2 nd ed. pp. 1-83.
Feb. 5	Buffers (continued)	
Feb. 12, 19	Assay of enzymes Protein purification	1. <i>Experimental Biochemistry Supplement</i> , Chapters 4 & 7. 2. Segel, I.H., <i>Bioch. Calcs.</i> , 2 nd ed. pp. 208-214, 291-92, 341-46.
Feb 26	Protein purification	1. <i>Experimental Biochemistry Supplement</i> , Chapters 5 & 7. 2. Segel, I.H., <i>Bioch. Calcs.</i> , 2 nd ed. pp. 287-290, 333-341.
Mar. 12, 19	Chromatography SDS-PAGE/Protein Assays	1. <i>Experimental Biochemistry Supplement</i> , Chapter 6.
Mar. 26 April 2, 9, 16	Chemical and Enzyme Kinetics	1. <i>Experimental Biochemistry Supplement</i> , Chapter 8. 2. Segel, I.H., <i>Biochemical Calculations</i> , 2 nd ed., pp. 208-212 (top), 214-219, 221 (bottom)-236 plus problem 4.9 on page 237, 246-250, 252.

4. Final Exam: Monday, April 30, 12:45 - 2:45 p.m. Location to be announced.

The exam will test 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. Other questions will involve a narrative response.

Bring a calculator and transparent ruler to the final exam.

H. Grading

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

Laboratory reports:	I	200
	II	400
	III	600
Laboratory notebook		~ 800
Pre-lab problems		~ 300 (LON-CAPA)
<u>Final Exam</u>		400
Total		~2600

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 2:00 p.m.** on the day listed in the Schedule. **Reports submitted after the due time/date will not receive any credit.** Put all reports, 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 2:00 p.m.** on the day listed in the Schedule.

Notebooks are **due at 9:00 a.m.** on the day listed in the schedule and those submitted on time will be available for pick-up after lecture the same day. Late notebooks will incur a **20 point per hour penalty** and may not be available for pick up until the following day. Notebooks submitted after 2:00 p.m. will receive no credit, but will be corrected, and will be available for pick-up the following day.