BIOC*3570 Analytical Biochemistry Summer 2016

Instructor: **Dr. M. Brauer**
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Lectures: Tue. 10:00 a.m. - 11:20 p.m., McKn 225.
Thur. 10:00 a.m. - 11:20 p.m., McKn 225.

Office hours: Tuesday & Thursday after class, by appointment or e-mail.

Grade Assessment:

Midterm exams (in class)	30%
Laboratory	35%
Final examination (cumulative)	35%
	100%

Students must pass (mark of 50% or better) **both** the laboratory component (35%) **and** the theory component (65%) to obtain a final passing mark in the course. In cases where this standard is not reached, the final mark assigned will be either the mark calculated as given above or 47%, whichever is *less*. College policy precludes changes to the marking scheme for individual students, except in case of illness.

Objectives:

A study of the tools and techniques by which biological molecules are isolated, separated, quantitated, characterized and manipulated. Proteins and nucleic acids will be emphasized. We will focus on the major techniques which are currently used in biochemical research laboratories.

Prerequisites:

CHEM*2400 or 2480, BIOC*2580. Introductory biochemistry is a prerequisite for this course. The following aspects of the subject are important background, and familiarity with them will be assumed: basic aspects of protein and nucleic acid structure, including structures of all amino acids and nucleotides; flow of genetic information; basic enzymology. Please take some time to review this material carefully, especially if some time has passed since you took intro. biochemistry.
If you have not taken a molecular biology course before, you should read the relevant chapters on DNA and RNA: Molecules of Heredity and Flow of Genetic Information in Stryer, Lehninger or another textbook before week 5.

Method of Presentation:

In addition to lectures, we will have tutorials/problem sessions, as indicated in the detailed outline. Problem assignments will be given out regularly; solutions will be discussed in the tutorial sessions, and made available at the library Reserve desk.
To obtain benefit from these exercises, it is essential that you attempt the problems on your own before attending the tutorial session.

Policy on Missed Examinations:

Only valid medical or compassionate reason will prevent a grade of zero for any missed examination. It is the student's responsibility to obtain the necessary documentation from Medical or Psychological Services or the Director of Student Affairs. *Make-up tests will not be given.*

Course Evaluation:

As part of the faculty evaluation process in the Department of Molecular and Cellular Biology, students are reminded that written comments on the teaching performance of the lecturer may be sent to the Chair at any time. Such letters must be signed; a copy, with the signature removed, will be made available to the instructor. Your comments and feedback are always appreciated.

Textbooks:

1. **Recommended** (not required) texts: *Principles and Techniques of Biochemistry and Molecular Biology*, by **K. Wilson and J. Walker**, 7th edition, **2011**. Also good texts: *Biochemistry Lab*: *Modern Theory and Techniques*, by R. Boyer, 2nd edition, 2011. *Fundamental Laboratory Approaches for Biochemistry and Biotechnology*, 2nd edition, by A. Ninfa & D. Ballou, 2010, *Bioanalytical Chemistry*, by Mikkelsen & Corton, 2004 are of limited value for this course.
2. Recommended General Biochemistry Texts: the latest editions of the texts by Lehninger *et al.* (6th edition, 2013), Voet & Voet (5nd edition, 2016), J. Berg, J. Tymoczko and L. Stryer (7th edition, 2014), and Mathews, Van Holde and Ahern (2013) are among the best.

Web Resources:
www.youtube.com search: enzyme techniques, electrophoresis molecular biology, recombinant, mass spectrometry, proteomics, fluorescence. Google Scholar, scirus.com, sciencedirect.com (look for Methods in Enzymology), ncbi.nlm.gov/pubmed, etc.

On Reserve:
1. A binder containing reprints and information for the laboratory exercises.
2. Copies of *Biochemistry Lab*: *Modern Theory and Techniques*, by R. Boyer, 2006, *Fundamental Laboratory Approaches for Biochemistry and Biotechnology*, by A. Ninfa and D. Ballou, 2007, *Bioanalytical Chemistry*, by Mikkelsen and Corton, 2004, *Experimental Biochemistry*, by Switzer and Garrity, 1999, *Analytical Biochemistry*, 2nd edition, by D.J. Holme and H. Peck, Longman, 1993, and Stryer (4th edition).
3. The following additional texts, which you will find useful: *Physical Biochemistry* (2nd edition, 1982) D. Freifelder (QH 345.F72). This is a particularly good reference text for spectroscopy, centrifugation, electrophoresis, and other physical techniques.

Laboratory:

SC3101 1:30 - 5:20 pm.
Labs start on the first week with Experiment 1: please be prepared. You will need to read the lab manual and also do the required calculations for protein dilutions. Please see your demonstrator, or lab coordinator Dr. Paula Russel (SC 3115, ext 58220) if you have lab questions.
Attendance at all laboratory periods is mandatory. If a lab (and associated lab quiz) is missed, medical or compassionate documentation must be give to the lab demonstrator as early as possible. If this documentation is not received, a mark of zero will be given for that lab and/or quiz. The laboratory portion of the course is worth 35% of the course grade. This 35% consists of three lab reports worth 9% each, lab performance and notebook worth and lab tests (7 in total) worth the remainder (see lab manual). Lab reports will be handed in to the demonstrator at the time specified by the demonstrator. Late reports will be penalized at the rate of 10% per day. Graded reports and notebooks are kept by the demonstrator at the end of the semester.

Laboratory Manual: Available at course-orientation meeting.
Students should provide their own safety goggles, 3-ring binder, and laboratory notebook.

Accessibility:
The University of Guelph is committed to creating a barrier-free environment. Providing services for students is a shared responsibility among students, faculty and administrators. This relationship is based on respect of individual rights, the dignity of the individual and the University community's shared commitment to an open and supportive learning environment. Students requiring service or accommodation, whether due to an identified, ongoing disability or a short-term disability should contact the Centre for Students with Disabilities as soon as possible. For more information, contact CSD at 519-824-4120 ext. 56208 or email csd@uoguelph.ca or see the website: http://www.csd.uoguelph.ca/csd/

Academic Misconduct:
The University of Guelph is committed to upholding the highest standards of academic integrity and it is the responsibility of all members of the University community - faculty, staff, and students - to be aware of what constitutes academic misconduct and to do as much as possible to prevent academic offences from occurring. University of Guelph students have the responsibility of abiding by the University's policy on academic misconduct regardless of their location of study; faculty, staff and students have the responsibility of supporting an environment that discourages misconduct. Students need to remain aware that instructors have access to and the right to use electronic and other means of detection.

Students who are in any doubt as to whether an action on their part could be construed as an academic offence should consult with a faculty member or faculty advisor.

The Academic Misconduct Policy is detailed in the Undergraduate Calendar: http://www.uoguelph.ca/registrar/calendars/undergraduate/current/c08/c08-amisconduct.shtml

Learning Objectives:

1. Students will learn modern and classical methods to characterize protein structure and function, including UV-Vis spectroscopy, enzyme assays, peptide sequencing, and mass-spectrometry.

2. Students will learn how to purify proteins from a biological source, using ion-exchange, gel-exclusion and affinity chromatography, electrophoresis, centrifugation and immunological methods.

3. Basic comprehension of chemical principles of acid-base equilibria, dissociation constants and binding specificity will be reinforced and expanded.

4. Students will learn how to characterize, isolate, purify, amplify and modify RNA and DNA. This will include up-to-date PCR methods, recombinant DNA methods in procaryotic and eucaryotic systems, and use of reporter genes.

5. The laboratory will provide hands-on experience in applying concepts covered in lecture, and in scientific reporting of the results obtained.

CLASS / DATE		TOPICS (**APPROXIMATE** TIME LINES)
1	MAY 12	**I. UV-VIS SPECTROSCOPY.** [COURSE ORIENTATION.] A. UV-VISIBLE SPECTROSCOPY. BEER'S LAW AND ABSORBANCE.
2	17	B. CHROMOPHORES. UV-VISIBLE SPECTROSCOPY OF BIOMOLECULES.
3	19	C. INSTRUMENTATION. APPLICATIONS.
4	24	**II. PROTEIN METHODS.** A. REVIEW OF PROTEIN STRUCTURE AND PROPERTIES. *TUTORIAL #1. SPECTROSCOPY.*
5	26	B. GENERAL STRATEGIES FOR PROTEIN PURIFICATION. 1. CELL DISRUPTION. 2. BULK METHODS FOR PROTEIN FRACTIONATION.
6	31	C. ION EXCHANGE CHROMATOGRAPHY & PH / CHARGE. D. GEL FILTRATION CHROMATOGRAPHY AND COLUMN MATRIX.
7	JUNE 2	E. AFFINITY CHROMATOGRAPHY & BINDING CONSTANTS. F. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) OF BIOMOLECULES.
8	7	G. IMMUNOLOGICAL METHODS. ANTIBODIES AND THE IMMUNE RESPONSE. IMMUNOPRECIPITATION. *TUTORIAL #2. CHROMATOGRAPHY.*
9	9	H. ELECTROPHORESIS. 1. INTRODUCTION. 2. PAPER AND GEL ELECTROPHORESIS. 3. SDS-PAGE AND NATIVE GELS. 4. PROTEIN STAINING METHODS. 5. WESTERN BLOTTING.
10	14	**MIDTERM EXAM #1.**
11	16	6. DISC ELECTROPHORESIS. 7. ISOELECTRIC FOCUSING. 8. 2-D SDS-PAGE/IEF. *TUTORIAL #3 - ELECTRO.*
		12

13	23	*J. MASS SPECTROSCOPY* OF PROTEINS.
14	28	**III. DNA / RNA METHODS.** A. REVIEW OF DNA AND RNA STRUCTURE. DENATURATION. RENATURATION AND HYBRIDIZATION.
15	30	B. ELECTROPHORESIS OF NUCLEIC ACIDS. C. RESTRICTION ENDONUCLEASES. (JULY 1 HOLIDAY)
16	JULY 5	D. SOUTHERN AND NORTHERN BLOTTING. RFLPS. E. DNA SEQUENCING: SANGER DIDEOXY METHOD.
17	7	F. THE POLYMERASE CHAIN REACTION. G. GENE CLONING STRATEGIES - 1. CHOICE OF TARGET DNA. *GENOMIC VS. CDNA. TUTORIAL* # 5 - NUCLEIC ACIDS, RESTRICTION ENDONUCLEASES AND SEQUENCING.
18	12	**MIDTERM EXAM #2.**
19	14	2. PLASMID CLONING. CUT, RESEAL, TRANSFORM, SELECT, EXPRESS PROTEIN, PURIFY PROTEIN.
20	19	3. OTHER CLONING STRATEGIES - EUCARYOTIC VECTORS.
21	21	H. SITE DIRECTED MUTAGENESIS. I. DNA CHIPS AND GENOMICS / TRANSCRIPTOMICS.
22	26	**IV. GENERAL METHODS.** A. FLUORESCENCE SPECTROSCOPY. PRINCIPLES. APPLICATIONS TO BIOLOGICAL MACROMOLECULES.
23	28	*TUTORIAL* #6. B. CENTRIFUGATION. 1. SEDIMENTATION COEFFICIENT. ULTRACENTRIFUGATION.
24	AUG 2	2. DENSITY GRADIENT CENTRIFUGATION. ISOPYCNIC CENTRIFUGATION. *TUTORIAL #7 - CENTRIFUGATION.*