

BIOC23H
Summer 2019
Practical Approaches to Biochemistry
Course outline

Instructor:

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Rm S563A

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The objective of this course is to provide the student with the practical experience in biochemical techniques that are used in industry, research and government laboratories. In addition, we will provide the background necessary to understand and trouble shoot the techniques. The course will expand your knowledge of more advanced techniques. Students will gain experience in experimental design, data analysis and interpretation, with emphasis on scientific writing, oral presentation and critical thinking. Application of biochemical techniques to biological studies will be a major focus. You will be learning skills that are useful in the job market as well as skills that will be useful in graduate studies.

This is a laboratory course with a 2 hour lecture component. The emphasis is on the laboratory component, therefore work in the laboratory and laboratory based material on the midterm and final will **represent over 55% of your grade.**

During **lecture we will do a number of exercises that will apply the material covered in lecture that is applicable to laboratory exercises. You therefore must come prepared for lecture. Read the text and the lecture notes before class.** A number of lectures this year will at times take the form of a reverse classroom with the students solving problems to help improve understanding. I will therefore not always give a detailed lecture. The lectures notes provided are detailed enough for a standalone review and the text provides excellent background and problems to solve.

Learning outcomes:

- Understand the role of biochemical techniques in protein study
- Evaluate the function of an enzyme using appropriate controls
- Compare and contrast types of chromatography, gel electrophoresis and protein purification and protein/protein interaction methods
- Compare and contrast experimental design to formulate hypothesis
- Formulate hypotheses and develop strategies to investigate them.
- Create effective data collection protocols
- Explain the importance of reproducible data

- Evaluate data collected and present in appropriate manner for oral presentation and written research papers
- Generate appropriate conclusions from data interpretation

Communication

The best way to reach me is to drop by and see me (if my door is open I am available, if it is closed it usually means I am not there, but by all means try knocking) . I prefer to meet with you in person to answer your questions. Hence why I have an **open door policy**. **Alternatively come by during my formal office hours**. I encourage you to ask questions about the course material (lecture of lab) in class, in lab or in my office, feel free to drop by to discuss other issues related to your education. If you wish to see me at a specific time outside of office hours then it is best to email me the request for an appointment. I will respond within the day.

I will answer emails when I am on campus. I am on campus generally Monday through Friday. If you send me emails on the weekend, you may not get a response until the following Monday. **Please use U of T account for email and please do not use the email function in Quercus. (I will not answer emails from non-U OF T accounts) and please indicate the course in the subject heading**

General announcements and any material needed for the course will be posted on **Quercus**.

Office hours:

Tuesday 10 am to 11 am and 2:15 to 3 pm
Wed 11 to noon

- If you like come as a group and ask questions as a group

Textbook:

Biochemistry laboratory Modern Theory and Techniques
Boyer R 2012 Publisher Pearson, available in bookstore.

The text is strongly recommended

This book is an excellent resource for theory and provides many practice questions and I highly recommend you purchase this text. If you take any other advanced lab in molecular biology it will also be valuable.

AccessAbility statement:

"Students with diverse learning styles and needs are welcome in this course. In particular, if you have a disability/health consideration that may require accommodations, please feel free to approach me and/or the AccessAbility Services Office as soon as possible.

AccessAbility Services staff (located in Rm SW302, Science Wing) are available by appointment to assess specific needs, provide referrals and arrange appropriate accommodations 416-287-7560 or email ability@utsc.utoronto.ca. The sooner you let us know your needs the quicker we can assist you in achieving your learning goals in this course."

Academic integrity/plagiarism

The University treats cases of cheating and plagiarism very seriously. The University of Toronto's Code of Behaviour on Academic Matters (<http://www.governingcouncil.utoronto.ca/policies/behaveac.htm>) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences.

Potential offences in papers and assignments include using someone else's ideas or words without appropriate acknowledgement, submitting your own work in more than one course without the permission of the instructor, making up sources or facts, obtaining or providing unauthorized assistance on any assignment.

On tests and exams cheating includes using or possessing unauthorized aids, looking at someone else's answers during an exam or test, misrepresenting your identity, or falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes."

<http://academicintegrity.utoronto.ca/>
(<http://www.governingcouncil.utoronto.ca/policies/behaveac.htm>) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences. Potential offences include, but are not limited to:

(source http://www.utsc.utoronto.ca/~vpdean/academic_integrity.html)

In papers and assignments:

- Using someone else's ideas or words without appropriate acknowledgement.
- Submitting your own work in more than one course without the permission of the instructor.
- Making up sources or facts.
- Obtaining or providing unauthorized assistance on any assignment.

On tests and exams:

- Using or possessing unauthorized aids
- Looking at someone else's answers during an exam or test.
- Misrepresenting your identity.

In academic work:

- Falsifying institutional documents or grades.
- Falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes.

Submitted work may be requested to be submitted turnitin

- *"Normally, students will be required to submit their course essays to Turnitin.com for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the Turnitin.com reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of the Turnitin.com service are described on the Turnitin.com web site".*

Intellectual Property (CTSI) - <http://teaching.utoronto.ca/teaching-support/course-design/developing-a-syllabus/> - *"Recording or photographing any aspect of a university course - lecture, tutorial, seminar, lab, studio, practice session, field trip etc. – without prior approval of all involved and with written approval from the instructor is not permitted. For further information on University policies, please refer to the following links for details*

For the laboratory:

Students require:

1. **lab coat** (no exceptions) and closed toed shoes. You will be asked to leave if you come with inappropriate attire and no lab coat: **this will also lead to a loss in associated marks**
2. safety glasses for most labs
3. a permanent black marker (Sanford :sharpie fine point)
4. a book for recording your work (your log book). This book can be hard or soft cover, or a binder.

Safety (your laboratory is a level one Biosafety laboratory)

1. **Eating, drinking, chewing gum and use of cell phones is prohibited in the laboratory** Breaking this rule will lead to expulsion from the laboratory and loss of marks associated with the laboratory for which you have been asked to leave
2. **Please do not bring** coats, hats, etc. into the laboratory.
3. **You must Always wear a lab coat** (done up) in the laboratory with the sleeves rolled down and **closed shoes**
4. Wear gloves or safety glasses when instructed to do so.
5. Keep paper, pencils, fingers, etc. out of your mouth.
6. At the beginning and end of the laboratory, follow procedure 13
7. Discard chemical waste in appropriate containers
8. Discard bacterial culture material into autoclave bags and wipe benches with alcohol
9. Discard pipets point-down, in the upright plastic pipet holders. Make sure the pipet tips are covered with disinfectant.
10. Place all *test tubes* containing living cells in the designated racks/pans;
11. discard pipet tips in the appropriately marked container
12. **Wash hands thoroughly with soap and water** once or twice during the lab, at any time you come in contact with chemicals/live cells and **also just before leaving the laboratory.**
13. When leaving the lab, **Wash your hands** and REMOVE YOUR LAB COAT. DO NOT UNDER ANY CIRCUMSTANCES wear your lab coat in any public area.

Course schedule:

Lectures:

BIOC23 is a laboratory course. The material covered will relate to the **laboratory techniques carried out throughout the course.** It will include discussion of methodologies, theory behind the methodology and relevant applications for the particular techniques used. I will also cover the data obtained from the labs carried out. I will address specific aspects of the protocols in each lecture. Lecture gives you a chance to also ask questions regarding the theory behind the labs you will be conducting as well as how these techniques can be applied to various biological questions. Therefore come with questions. Lecture material will be posted by the day of the lecture.

Laboratories :

- **attendance in labs is mandatory.** If you miss a lab you must provide a **UTSC medical certificate/self declaration or other acceptable reason cleared by Dr. Brunt. Documentation must be submitted to Dr. Brunt and Jennifer Campbell** to be excused from the lab and to be able to hand in any assignments related to that lab. There is no possibility to makeup labs. **Each unexcused missed lab will result in a loss of 5%**

of your grade. If you miss more than 2 laboratories regardless of the reason you will not be given credit for the lab component of the course (49% of your grade and you will not pass the course and will be asked to leave the course as this is a laboratory based course.

- Laboratories are posted on Quercus a week before the lab is scheduled to be carried out.

It is your responsibility to come prepared each week to carry out your laboratory. **To have completed in your lab note book an introduction** to each lab (a paragraph) explaining the purpose and to have written out your own **flow chart** (this is what you should refer to when doing the lab, not your lab outline. **Students that insist on using the lab outline in lab rather than their flow with have their performance grade significantly impacted**). When carrying out experiments you must take complete notes. Therefore, as you do the experiment you will **“log it”**. This means writing down in detail what you did, what you observed, times of incubations. You will have **data analysis** (including standard curve construction) **summary / conclusion**, where you analyze the data, do any graphs etc that are required. To ensure you are prepared for the lab there will also be **quizzes** before each major lab topics which will test whether you have adequately prepared for the lab

- The use of a laboratory log book is protocol in any laboratory setting (work or research) and **learning how to properly document data and experiments is critical and therefore you will be rewarded with grades for doing this**
 - Equipment in the lab is expensive. You are responsible for your equipment. Accidents can happen but most “accidents” are avoidable with proper preparation and attention to the task at hand

Grade Breakdown

Midterm: June 27th in class

(multiple choice , short answer, short and long essay)

14.5%

- You must contact me within 48 Hours of missing the midterm and provide me with the UTSC medical certificate filled in by your doctor to be able to write midterm. Please provide copy to Jennifer Campbell in the Biological Sciences administrative office
- – <http://www.utoronto.ca/ctl/sites/utoronto.ca.ctl/files/resource-files/Verification-of-Illness-or-Injury-form-Jan-22-2013.pdf>
-

Final exam TBA in final exam period (multiple choice , short answer, short essay) (cumulative) 3 hrs

32%

Date of Quizzes (6 X 0.25%)

1.5%

Week 1 (differential centrifugation/ mitochondrial isolation (May 9, 2019)

week2 (ion exchange) (May 16, 2019)

week 3: (gel permeation) (May 23, 2019)

week 4-6: (IgG) (May 30 2019)
week 6-8 (GST/Western) (June 13, 2019)
week 10 (alkaline phosphatase) (July 18, 2019)

One-minute writes/case studies/reflective writing in lab and lecture summaries/data presentation in lab/concept maps (occur randomly, must complete a minimum of 80% in lecture and all assignments in lab including oral presentations for full credit)

lecture	5%
lab	7%

Lab performance: 10%

Preparation

Laboratory log/ summary/data analysis

Technical performance

Your book will be initialed each week and collected at the end. This is a cumulative process if you do not keep you book up to date this will be reflected in your grade. You may be asked to do small Pass/Fail assignments during class that may be used in the determination of you performance grade

Research proposal: 6%

Concept map due June 6 in lab, proposal due June 24 by 11:59 online

max 3 page (double space and 12 point)

Lab assignments: 24 %

- 1. mitochondrial isolation: (2.5%)** (maximum 2 page double spaced not including figures, legends, tables, references)
- 2. formal report 1: IgG (7.5%)** (maximum 5 double spaced pages of text not including figures/figure legends/ reference page)
- 3. formal report 2: GST and western (9.5%)** (maximum 7 pages of double spaced pages of text not including figures/figure legends/ reference page)
- 4. Alkaline phosphatase, (4.5%)** will not require materials and methods requires brief introduction, graphs/with appropriate legends and data analysis (**maximum 1 pages double spaced** of text not including graphs, legends or references)

- the content required for each assignment will be explained during the appropriate lecture and laboratory class. It is also addressed at the end of each laboratory exercise that is posted.
- **Due dates:**
 - Mitochondrial isolation : May 16, 2019 in lab
 - formal report 1: IgG: electronically by Sunday July 7 11:59 pm
 - formal report 2: GST analysis electronically Wed July 24 at 11:59 pm

- assignment: alkaline phosphatase In your lab period August 1, 2019
- formal reports IgG and GST and research proposal will be submitted electronically for marking to Quercus

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Lecture schedule (material will be posted a week in advance. Much of the lecture material will be done using examples, therefore I will not cover the material in detail for all lectures. You are responsible for preparing for the lecture so we can try to use example questions to address the topics

(I have given topic numbers rather than dates). Lectures relevant to the particular lab will occur either the week before or the week of the laboratory. Some topics will overlap multiple weeks. All lab procedures will be reviewed in the lecture.

Topic 1: pH and buffers (will be posted, and you are responsible for the material, I will not cover in class)

Topic 2: cell fractionation/protein isolation/ purification

Topic 3: chromatography

Topic 4: protein quantification and gel electrophoresis

Topic 5: Immunochemistry/ western analysis

Topic 6: enzyme kinetics

Topic 7: Mass spectrophotometry and information science : use in protein identification, related protein technologies

Topic 8 Use of isotopes and new technologies in non-radioactive monitoring in biochemical studies

Laboratory schedule for 2019

Lab date

May 9 : Week 1:

Experiment 1: differential centrifugation/ mitochondrial isolation, go over data before you leave the laboratory

May 16: week 2

Experiment 2: Ion exchange chromatography

In class reflective practice and construct a proper table for column sample elutions and do class discussion

Work on concept map of research proposal

May 23: Week 3:

Finish experiment 2: thin layer chromatography

Experiment 3: gel permeation

In class reflective practice

Discuss research proposals with your TA

May 30: Week 4

Experiment 4 part 1: IgG: salting out via ammonium sulfate/dialysis

- write a figure legend for the thin layer chromatography per bench and do group critique and class discussion

June 6: Week 5:

Experiment 4 part 2: IgG: DEAE Sephdex and protein quantification

-finish protein quantification table and determine volume to load on the gel

Hand in concept map in lab

June 13 Week 6:

Experiment 4 part 3: IgG: SDS gel electrophoresis and staining (picture posted by the following day).

Experiment 5 part 1: Purification of GST fusion proteins from *E.coli* (isolation and affinity chromatography)

Reading week : no lab June 20

June 27 : Week 7

Experiment 5 part 2: GST fusion protein: gel electrophoresis and Western transfer

Experiment 4: discussion of gel IgG data by each bench: then a classroom discussion, one-minute write to summarize the data, go through a example figure legend: do in pairs and critique

July 4: Week 8

Experiment 5 part 3: GST fusion protein: immunoblot with GST antibody/dark room, data returned via blackboard. During wait period work on and discuss your concept map for the research proposal with your TA if you have questions (you may want to revise it based on my comments and your TAs)

July 11: Week 9

Experiment 5: group PowerPoint presentation: full analysis of GST presented,

July 18: Week 10

Experiment 6: enzyme kinetics: alkaline phosphatase

July 25: Week 11

Data analysis of enzyme kinetics, in class discussion/presentation of data. I highly recommend you do this in class as you have four hours and could complete most of the work

August 1: Week 12

No lab: Alkaline phosphatase assignment due along with your note books at a time to be arranged with your TA in your lab period