

BIOC23H3: Practical Approaches to Biochemistry

Course Outline

Winter 2021

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Lecture will be synchronous on line

Laboratories will be in-person with laboratory class size set at 9 per Teaching Assistant and with very strict social distancing protocols and PPE requirements. We will provide an overview as a separate document.

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 in person laboratory course with a 2-hour lecture component which will be a synchronous virtual meeting. The emphasis is on the laboratory component, therefore work in the laboratory and laboratory-based material on final will represent **greater than 55% of your grade.**

During the synchronous 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 e-text and the lecture notes before class.** A number of lectures will involve the use of **extensive break out groups with students solving problems to help improve understanding.** 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 and evaluate the advantages of each
- Compare and contrast experimental design to formulate hypotheses

- Formulate hypotheses and develop strategies to investigate them.
- Create effective data collection protocols
- Explain the importance of reproducible data
- Create scientific experiments through thoughtful design and understand how to construct a research budget
- Evaluate data collected and present in an appropriate manner in an oral or written presentation and create appropriate hypotheses
- Generate appropriate conclusions from data interpretation

Communication

I will hold virtual office hours on Monday following class from 2 to 3 pm. I will also be available in person by appointment during the laboratory sessions. If you need to speak to me privately we can arrange a video chat.

I will answer email between 9 and 5 pm Monday through Friday. **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:

Monday 2 to 3 pm on line following the lecture.

- 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. It is available as an ebook through the bookstore or through Pearson publishing.

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) - *"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 (your laboratory is a level one Biosafety laboratory): In the era of COVID you will be given a more detailed Guidelines but below are general guidelines for all laboratories and are absolutely required- if you arrive for lab one without your PPE or permanent marker you will not be permitted entry.

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** or **plexiglass face shield** for all labs which must remain on throughout the lab.
3. **a permanent black marker** (Sanford:sharpie fine point)- we will not supply markers
4. **a book for recording your work** (your log book). This book can be hard or soft cover, or a binder. For your own use only.
5. **A bag to place your lab count in** used to bring your coat to lab and place

your coat in after laboratory completion

In the laboratory General Rules COVID RULES UNDER SEPARATE COVER

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. You may not touch or handle in any way your cell phone
2. **NO food or drink can be left outside of the laboratory**
3. **Please do not bring** coats, hats, etc. into the laboratory if at all possible.
4. **You must Always wear a lab coat** (done up) in the laboratory with the sleeves rolled down and **closed shoes**
5. Wear gloves and safety glasses for the entire laboratory
6. Keep paper, pencils, fingers, etc. out of your mouth. Share no writing implements
7. At the beginning and end of the laboratory, follow procedures outlined for you in detail on a separate document
8. Discard chemical waste in appropriate containers
9. Discard bacterial culture material into autoclave bags and wipe benches with alcohol
10. Discard pipets point-down , in the upright plastic pipet holders. Make sure the pipet tips are covered with disinfectant.
11. Place all *test tubes* containing living cells in the designated racks/pans;
12. discard pipet tips in the appropriately marked container
13. **Wash hands thoroughly with soap and water on entry and exit from the lab.**
14. When leaving the lab, **wash your hands** and REMOVE YOUR LAB COA. 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 prior to the lecture.

Laboratories:

- **attendance in labs is mandatory.** If you miss a lab you must provide a **self-declaration or other acceptable reason cleared by Dr. Brunt. Self-Declaration 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 make up labs. **Each unexcused missed lab will result in a loss of 5% of your grade and will prohibit handing in lab work.**

- **You can self declare for two laboratories only (we only have 8 wet lab sessions so this is 25% of the wet labs) -**
- **If you miss 3 laboratories (regardless of the reason you will not be given credit for the lab component of the course (and you will not pass the course and will be asked to leave the course as this is a laboratory based course. If you are seriously ill or diagnosed with COVID. I will address those individually so contact me immediately on diagnosis.**
- 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 a flow chart/prelaboratory and posted it to Quercus in the assignment section. Have a copy of the flow chart in your lab note book and keep complete notes in your personal lab book. Complete the on- line weekly lab assignment.**

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. This will be used to write up your weekly laboratory assignments and lab reports. 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. We will not be grading the lab books due to COVID but they will be need for your success in your assignments and for success in the comprehensive final take home exam**

Grade Breakdown

Final take home exam which will be an cumulative essay exam based on all of the lecture and laboratory material TBA in final exam period

34%

prelabs with flow chart for the six laboratory modules
Technical lab performance

6%

4%

small weekly laboratory assignments due after the laboratory for a number of laboratory sessions-percentages will change with the particular week – see Quercus

10%

One-minute writes/case studies/reflective writing in lecture (occur randomly, must complete a minimum of 80% in lecture) you may opt out and have it moved to the final exam

6%

Research proposal: Due March 30 see Quercus **14.5%**
Concept map (2%) is part of this as is an annotated bibliography (2%) and will be due prior to the final assignment is due. This is a major assignment and the final paper is work 10.5%

Laboratory Major Assignments: **25.5 %**

- 1. mitochondrial isolation: (3.5 (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, (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 for major laboratory assignments:**
 - Mitochondrial isolation: Due week of Jan 25 see Quercus for your lab due date
 - formal report 1: IgG: electronically week of March 8 see Quercus
 - formal report 2: GST analysis electronically week of March 23, 2020 see Quercus
 - assignment: alkaline phosphatase due the week of April 5 see Quercus.

Turnitin:

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Turnitin.com service are described on the Turnitin.com web site”.

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 2021

Lab week date

Jan 11: Week 1: on line module

Experiment 1: differential centrifugation/ mitochondrial isolation, go over data before you leave the laboratory. Post prelab prior to laboratory see Quercus. The entire lab will be evaluated and you will do the spectrophotometer assay week 2.

Jan 18: week 2 (in person labs begin)

Experiment 1: spectrophotometer assay. Assignment due in a week see Quercus

Experiment 2: Ion exchange chromatography. Post Prelab prior to laboratory see Quercus

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

relations in your lab book and do class discussion

Work on reflective practice and start working on your thoughts on your research proposal- you can have a discussion with your TA or myself when I come around to class-this is a start to think about it

On- line lab assignment due after lab- see Quercus

Jan 25: Week 3:

Finish experiment 2: thin layer chromatography

Experiment 3: gel permeation

In class reflective practice or other small assignment due on Quercus see assignments on Quercus

Discuss research proposals with your TAs during your down times

Feb 1: Week 4

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

- **write a figure legend for the thin layer chromatography result and do an in class discussion. Post your final figure legend to Quercus as your assignment**

Feb 8: Week 5:

Experiment 4 part 2: IgG: DEAE Sephadex, protein A and protein quantification

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

Assignment is your standard curve and quantification of your samples- see Quercus

Week of Feb 15 no lab reading week

Feb 22 Week 6:

Experiment 4 part 3: IgG: SDS gel electrophoresis and staining (picture posted by the following day on Quercus). **Make gel figure for your presentation and write figure legend and submit as your assignment on Quercus- this is your preparation for your presentation in class next week for the IgG gel picture**

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

March 1: Week 7

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

Experiment 4: discussion of gel IgG data by each pair:- **Please send the PowerPoint by email to your TA prior to lab** so your TA can put the files on the computer. You will present per pair standing on the opposite side of the screen. Then a classroom discussion on the figure legend submitted.

March 8: Week 8

Experiment 5 part 3: GST fusion protein: immunoblot with GST antibody/
development-CNS, data returned via Quercus.

All labs for the rest of the year are on line synchronous

March 15: Week 9- labs will be synchronous virtual no in person attendance

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

Complete class discussion and break out groups. By the end of this lab you will have your figures labeled and major concepts developed for your lab report due next week.

March 22: Week 10- lab will be virtual synchronous

Experiment 6: enzyme kinetics: alkaline phosphatase- post prelab on Quercus

No further labs you will submit your remaining assignments as indicated on Quercus