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INTRODUCTION AND COURSE OUTLINE

CHEMISTRY 477	Sept.-Dec. 2018	
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Objectives

This course is designed to: demonstrate selected biochemical principles; provide an opportunity to learn some advanced biochemical techniques; and familiarize you with the biochemical experimental literature. There is an emphasis on protein structure/function and enzymology. It is important that you continue to develop the ability to analyze data and to critically interpret results. Each experiment comes with assigned readings, either in the course-pack or on reserve at the Vanier library; it is strongly suggested that you read these before arriving in the lab. Additional background reading may also prove useful and is recommended. The weekly tutorials will be used for discussions of your results, for explanations of the techniques being used, for quizzes, and for discussing some selected experimental literature.

Textbook	CHEM 477 Lab Manual, CHEM 477 course pack
Suggested Reading	"Reading Primary Literature", C. M. Gillen,

Lab Attendance: Attendance at the lab will be taken. You are expected to arrive at the lab **on time**. If you miss doing a lab, **5% will be deducted from your final grade** for each lab not attended. Exceptions MAY be granted by the professor under extraordinary circumstances. If a lab session must be missed for a valid reason, you must try to arrange to make up the lab. If that is not possible then results from either your partner or another group may be used *after* approval by either the head demonstrator or the instructor. No excuses will be accepted for a second absence. **If you are absent for more than two labs, whether with a valid reason or not, you will receive a grade of R, meaning you have to repeat the course**.

Pre-Labs

Each week, upon arriving at the lab, you must submit a pre-lab. **If you do not submit a pre-lab you will not be allowed to perform that week's lab; in this case you will get a deduction of 5% from the final grade as if you would have missed that lab**. The pre-lab is in the form of an outline, or flow sheet, of the day's work. It outlines the work you will

do during the lab period, the sequence of the work, and how that work will be divided between you and your partner. This means that you must arrange to confer with your partner in order to plan your work before the beginning of the lab. The pre-lab also includes any calculations (how to prepare a given solution, how to prepare dilutions, etc.) that can be done in advance. For most experiments the pre-lab will be **brief**.

Detailed protocols, recipes, etc. are in the lab manual. **Do not** copy them into your pre-lab! You will need your pre-lab during the lab. Therefore, make a copy of the pre-lab to hand in, and retain one for use during the lab period. ***Do not clutter up your lab notebook with the pre-lab: please do it on a separate piece of paper or a separate notebook***.

At the beginning of every lab period there will be a short pre-lab quiz, no longer than 5 minutes. Pre-lab quiz questions will be related to practical aspects of what you will be doing in the lab that day. A total of 5% of your final grade will be determined from your performance on these quizzes. These quizzes will be open-for Lab notebook (lab manuals are not permitted).

Laboratory Performance Evaluation
Part of your final grade will be determined by your performance in the lab. You will be evaluated on such things as: quality of **your own** data, organizational skills, timely attendance (i.e., not arriving late or leaving early), efficiency, etc. This grade will determined in part by the demonstators and Dr. Chirwa.

Grading scheme

Your performance in the course will be graded according to the following criteria:

Pre-lab quizzes	5%
Laboratory performance evaluation	5%
Laboratory notebook	25%
Tutorial quizzes (6)	15%
Assignments (2)	10%
Final Exam	40%

There will be **no** formal lab reports.

Tutorial quizzes and assignments

There will be 7 quizzes given during the tutorial periods: the best 6 grades will be counted. The quiz topics are shown on the schedule. This means that if you miss a tutorial session for any reason, the grade of zero that will be assigned need not necessarily count in your final quiz grade. If you miss a tutorial it may be possible to "make-up" the quiz at one of the other tutorial sessions: you must obtain the permission of the instructor to do so.

There are two required assignments These assignments will be: 1) an assignment for protein modelling due date to be announced, and 2) an assignment for the stopped-flow experiment, due *one week after* the lab was done during the lab rotation period .

Final Exam

The Final Exam is administered by the university's Examination Office

(http://registrar.concordia.ca/exam/services.html). If you missed the final exam, **you must contact the Examination Office** to arrange to write a deferred exam if they determine that you are eligible to do so. The instructors **WILL NOT** consider **any** requests to write the final exam at an alternate time after the final exam is given to the rest of the class.

Laboratory notebook

Every time you come into the lab, you must bring your **bound notebook** for use in the lab. The purpose of the notebook is to document the work that you do in the lab. Notes should always be taken **directly** in the notebook, using a pen. Do not use pencil; do not use a pen with water soluble ink! Do not use white-out. Above all, do not write on another piece of paper and then "recopy" into your notebook (this also means, do not write up your lab on the computer at home and paste it into your lab notebook). The lab notebook should not be used for anything other than your CHEM 477 labs: if you wish to keep tutorial notes in the same book, do so in the last quarter of the book-don't mix tutorial notes up with the labs. The lab notebook may be used during quizzes and during the Final Exam, so this is another incentive to keep it neat and complete. All lab notebooks **must** be handed in at the Final Exam: failure to do so will result in an incomplete ("INC") notation with one letter grade lower until the notebook is received.

For each week, the notebook entry will start with the title and the date, followed by a brief (one or two lines) statement of the purpose of the lab. During the lab, you will record in the notebook any modifications to the experiment that are announced during the lab period and the details of your work, including the data. You do not copy the lab manual into your notebook; however, when you record data, you should identify the section of the experiment. Graphs, printouts and other data must be attached (stapled, glued or taped) into the notebook. Each graph must be separate to facilitate effective presentation of the data. At the end of each experiment include a **brief** section summarizing your conclusions, and answer all questions that were posed in the lab manual. There should be enough information in the notebook so that, 6 months or a year later, you could look at your notebook and understand the data and how you obtained it. Part of your grade will depend on how well you keep your notebook. **If you don't follow these instructions you will lose points!**

It is important that you keep an up-to-date notebook, in the format specified above. Each week, it is expected that the notebook include the completed write-up for the previous week's lab. Notebooks will be checked during the lab period on a random basis and a grade will be assigned. We will be checking that all sections (date, purpose, results, and conclusions) of an experimental write-up are present and complete, and grading four or five specific points. **If we ask to see your notebook and you do not have it, you will be assigned a grade of 0 for that check.** Furthermore, if you have your notebook but did not complete your write-up for that week you may also be assigned a grade of 0 for that check.

Grading of lab notebooks

The grades for lab notebooks will be based on:

1. The quality of your lab work - were you able to complete the experiment? Are your data of reasonable quality?

2. Your presentation of data - are the tables and figures presented in a neat and appropriate manner? Do you follow the format specified below?

3. Your calculations - are they correct? Have they all been done? Are the units indicated?

4. Your conclusions: do they summarize what you observed?

5. Have you compared your results to what is in the scientific literature? For example, if you determine an extinction coefficient experimentally, does it agree with what is in the literature?

The balance of these factors will vary from experiment to experiment; some experiments require a lot of calculations and some require relatively few. We will not grade everything every time, but at one time or another we will be looking for all of these things.

SOME GUIDELINES FOR DATA PRESENTATION

TABLES

The table should have a descriptive title.
Data of the same nature are presented in a column (not a row).
Every column is identified with a heading
Units are indicated in the heading for each column
The number of significant figures corresponds to the precision of the measurement
The legend gives information to make the table understandable without referring to the text of the report (or the lab manual)

FIGURES

The figure should have a descriptive title
The axes are clearly labeled.
Units are indicated and axes are properly subdivided
The legend gives information necessary to understand the figure without referring to the text.

For examples of good tables and figures, look at any article in *The Journal of Biological Chemistry* or in *Biochemistry*.

Suggested Readings

There are three types of material you will find useful to read for these experiments:

1. Background on the techniques or the particular enzyme: consult any enzymology or experimental biochemistry text, including the course-pack and the books on reserve.
2. The journal articles on which the experiments were based.
3. Other journal articles, illustrating the use of these techniques to study other enzymes.

The following books are on reserve at the Vanier library:

Enzyme assays : a practical approach / edited by R. Eisenthal and M.J. Danson; QP 601 E5153 2002. *This book contains practical information on designing and using enzyme assays.*

Structure and mechanism in protein science: a guide to enzyme catalysis and protein folding by Alan Fersht; QD 431.25 S85F47 1999. *This is a comprehensive source of information on the study of proteins, especially enzymes, by one of the most prominent scientists in the field. Case studies of some of the enzymes studied in the course are included.*

Enzymes: a practical introduction to structure, mechanism, and data analysis by Robert A. Copeland; QP 601 C753 2000 *Another good source of enzymology information*.

Enzymatic Reaction Mechanisms by P.A. Frey and A.D. Hegeman, QP601 F725 2007. *This book is a useful source of background information on different kinds of enzymes.*

From Enzyme Models to Model Enzymes by A.J. Kirby and F. Hollfelder, QP 601 K57 2009. *This book contains background information on different kinds of enzymes as well as some enzymes covered in class.*

Protein function: a practical approach, edited by T.E. Creighton QP 551 P69583 1997 *This book is old, but provides detailed practical information about some of the techniques used in the course, such as ligand binding, chemical modification and electrophoresis.*

Biophysical Chemistry, 2nd edition, by A. Cooper, QD 476.2 C66 2011. *An easy explanation of the theory behind some of the techniques used in the course.*

Introduction to protein science. Architecture, fuction and genomics, 2nd edition, by A.M. Lesk. QP 551 L465 2010. *The focus of the book is on protein structures and techniques to study them, including bioinformatics and modelling.*

Proteomics. Introduction to Methods and Applications, edited by A. Kraj and J. Silberring; QP 551 P7868513 2008. Good Introduction to Proteomics techniques.

The comprehensive series Methods in Enzymology (over 300 volumes!) is available in the reference section (QP 601 M5), and may be worth consulting from time to time. This resource is also available online:

http://0-www.sciencedirect.com.mercury.concordia.ca/science/journal/00766879

Biophysical Chemistry of Proteins. An introduction to laboratory methods, by E. Buxbaum.
This book is available as an e-book from the Concordia Library. It contains explanations of several of the methods used in the course.

A set of readings is available in the bookstore as a “course pack”. This is required.

For each experiment, you will be given a list of suggested readings, You are expected to read enough background (1 and 2 above) to understand the experiment you are doing. Some "other journal articles" (3, above) may be discussed during tutorials and you will be responsible for them on the final exam. Others you may want to read for your conclusions in the lab notebook, or the assignments.

PLAGIARISM AND OTHER FORMS OF ACADEMIC DISHONESTY:

Your lab notebook writeups and submitted assignments are expected to be written **in your own words**. All written material for credit must be reflecting your individual work; collaborative submissions are not acceptable. When conveying the words/ideas of others in your written material use proper quoting and paraphrasing. Cite all external sources of information related to your quotations and paraphrases. **Any form of cheating, copying, or plagiarism will be reported to the Dean's Office, Faculty of Arts and Science, and appropriate sanctions will be applied.** The academic code of conduct can be found in section 17.10.3 of the academic calendar: *http://www.concordia.ca/academics/undergraduate/calendar/current/17-10.html*

Ignorance of these regulations is no excuse and will not result in a reduced sanction in any case where academic misconduct is detected.

The Department of Chemistry and Biochemistry offers a mini-course on Academic Integrity and the Academic Code of Conduct (CHEM 101). Although it is not a mandatory requirement for this course, you are strongly encouraged to take this mini-course if you haven't done so already. Contact the Chemistry Main Office for further information.

Some standard units and conventions in enzymology

Rate or velocities (v): $v = d[Product]/dt$ or $v = -d[Substrate]/dt$

Rates are expressed as μM/min and occasionally as ΔA/min (although if an extinction coefficient is available (it usually is), concentration changes should be calculated!). Note that V_{max} has the same units as v!

It is often useful to calculate the *specific activity,* which is the activity per mg of protein. For a pure protein, a *turnover number* (k_{cat}) can be calculated by dividing V_{max} by the concentration of active sites. Units are therefore min^{-1} or sec^{-1}. Calculation of a turnover number or specific activity allows comparison of results between enzymes or between different preparations of the same enzyme.

Amounts of enzyme are usually expressed as *Units* (U). One Unit is usually defined as the amount of enzyme catalyzing the conversion of 1 μmol of substrate to product in 1 minute at 25°C. If an enzyme has a low turnover number, then the term milliUnit (mU) can be used (1/1000 of a Unit).

Biochemistry Web Resources

The World Wide Web (otherwise known as the Web or WWW) has many useful resources for biochemistry and molecular biology. You will be referred to some of these Web resources for certain experiments during the course. Below, I have listed more general resources, and collections of biochemistry-related links maintained by others, that contain material likely to be useful throughout the course. It would be a good idea to access some of these sites early on, and become familiar with what they offer.

There is a CHEM 477 course website that can be accessed through the university website: the web address will be announced in class. This is where you will be able to find class notes, assignments, supplements to the lab manual, and your grades.

A site that many biochemists visit often is the one at http://ca.expasy.org/. This site provides access to many useful tools for the biochemist, as well as links to other sites. Another excellent starting place is: http://web.archive.org/web/20110721205913/http://mcb.harvard.edu/BioLinks.html. Here you can find links to an incredible number of useful sites, including universities, journals, databases, companies, and scientific societies.

An indispensable site for searching the recent biochemistry literature is PubMed http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed.
In addition to allowing you to search the biochemistry literature, PubMed also provides abstracts of papers and links to the journals themselves. Concordia's library (http://library.concordia.ca) has electronic subscriptions to many of the major biochemical and chemical journals, which you may access via CLUES using your Concordia computer account. In addition, many journals now provide free electronic access to articles 6 months to a year old, or older.

Departmental home pages are a useful source of information about biochemistry and molecular biology research going on at Concordia.

Chem/Biochem	http://chem.concordia.ca
Biology	http://biology.concordia.ca

The following sites are useful resources especially for proteins and enzymes:

http://www.rcsb.org/pdb/ Database of protein structures. Here you can download coordinates of protein structures for visualization.

http://www.uniprot.org/ A comprehensive database of protein information.

http://www.proteinsociety.org/ The Protein Society publishes the journal *Protein Science*, and maintains a number of links that are of interest to protein chemists

http://www.ncbi.nlm.nih.gov/Genbank/index.html Genbank, one of the main repositories of nucleotide and protein sequence information

https://www.ebi.ac.uk Bioinformatics resources, including many useful tutorials. Much useful information about protein structures and genomics.

http://ca.expasy.org/tools/protparam.html A suite of useful tools for analyzing protein properties based on primary amino acid sequence input.

Useful addresses for searching for chemical information - product safety, availability, protocols, etc., include:

http://portal.acs.org/portal/acs/corg/content American Chemical Society

http://www.cas.org Chemical Abstracts Service

http://www.cambridgesoft.com/support/ProductHomePage.aspx?KBCatID=119 Database and Internet Searching Tool for information about chemicals

Many chemical companies have on-line catalogues, as well as MSDS sheets, protocols, technical information, etc.

http://www.sigmaaldrich.com/canada-english.html

http://www.bio-rad.com

https://www.fishersci.ca/Default.aspx

http://www.roche-applied-science.com/

http://www.gelifesciences.com/

Some more general sources of information about science include:

http://www.nature.com *Nature*

http://www.sciencemag.org *Science*

> These sites do not allow you to read all the articles, unless you are a subscriber. However, there are some features you can read, including some online features that are not in the print version of the journal.

http://sciencecareers.sciencemag.org/ Information about careers in science

Downloading PDB Structure Files

1. Go to http://www.rcsb.org/pdb

2. *If you know* the PDB ID of the protein, enter it and click on *Search*. *If you do not know* the PDB ID, enter the name of the protein in the search field in the upper-centre of the main page.Hit the *Go* button. Select the appropriate entry.

Read the summary information, write down anything of interest and click on *Download Files* in the right-hand panel of the website. This will expand a menu of download options. Click on 'Download Files', then 'PDB Format'. This will result in your web browser automatically downloading the associated PDB file, with the filename as ####.pdb, where '####' is the appropriate PDB code.