University of Guelph - College of Biological Science
Department of Molecular & Cellular Biology

Analytical Biochemistry BIOC*3570

Course Outline: Summer 2017

Course description (Calendar): BIOC*3570 Analytical Biochemistry S,F (3-4) [0.75]

This course covers the tools and techniques by which biological molecules are isolated, separated, identified, and analyzed. Detailed discussion of experimental methods for macromolecule purification and characterization is included.

Prerequisites: (CHEM*2400 or CHEM*2480), BIOC*2580

Teaching team

Instructor: Dr. David Josephy Office: SC2253 ext 53833 djosephy@uoguelph.ca

Office hours: Wednesday, 9:00-10:30 a.m. If that time slot doesn't work for you, please send me an e-mail and we can arrange a mutually convenient time. I welcome receiving your comments and questions by e-mail, and I try to answer queries within one working day.

Laboratory Instructor: Ms. Paula Russell; SC3502 ext. 58763 prussell@uoguelph.ca

Course schedule

***Lectures*: Tues. and Thurs., 10:00 am-11:20 pm, MCKN, Room 226**

The lecture PowerPoint presentations are posted on Courselink.

***Laboratories*: Mon. to Thurs.; 13:30-17:20, SSC 3101**

Attendance for the full time period of each laboratory is compulsory. Laboratory sessions start **during the week of May 15.** Please be prepared. Please see Ms. Russell if you have questions concerning the laboratory.

Learning goals and rationale; course goals and philosophy

Understanding the experimental methods by which biological molecules (especially proteins) are isolated, identified, quantified, and characterized, including some of the underlying theory. Spectroscopic methods and separations science (*e.g.*, chromatography, electrophoresis, centrifugation) are emphasized. We will focus on techniques used in contemporary biochemical research laboratories. The laboratory forms an important component of the learning experience for the course.

Adhering to the *University of Guelph Learning Outcomes,* the course will help to develop the student's "Critical and Creative Thinking", "Literacy" (especially through a term-paper assignment, and encompassing "Information Literacy", "Quantitative Literacy", and "Technological Literacy"), and "Communicating". "Global Understanding" will be enhanced as we consider the historical development of the science and the relationship of biochemistry to other physical and biological sciences.

Learning outcomes

* understand the theory and practice of protein purification, chromatography, electrophoresis, centrifugation, mass spectrometry, and other essential methods in modern molecular bioscience;
* become familiar with the use of biochemical instrumentation, including spectrophotometer, centrifuge, electrophoresis apparatus;
* develop and improve fundamental science laboratory skills, including planning, group work, record-keeping, and trouble-shooting.

An important objective of this course is to empower students to understand and evaluate the primary scientific literature in analytical biochemistry, *i.e.*, to read and analyze research articles (especially, the "Methods" sections). This objective will be supported in several ways. In introductory courses, students are usually shown "contrived" illustrations of techniques - examples that have been simplified for ease of instruction. For example, most textbooks illustrate SDS-PAGE electrophoresis by showing an artist's drawing of a stained gel, rather than a real photograph. In contrast, in this course, the lectures will be illustrated with genuine examples taken from the scientific literature. Second, the problem sets - and the quizzes and exam - will include questions that challenge students to read and interpret published data. Third, the Term Paper assignment (see below) is a critical reading and review of a recently published paper.

Course Resources

Laboratory: **Manual:** can be purchased in room SC3115; see hours posted in SC. **You must provide your own goggles and laboratory notebook.**

Recommended textbook: Principles and Techniques of Biochemistry and Molecular Biology, 7th ed., K. Wilson and J. Walker (Eds), Cambridge Univ. Press, 2010; ISBN 978-0-521-73167-6 (Paperback, 744 pages). Specific readings from the textbook are given on the lecture schedule.

The textbook is a multi-author work, with chapters covering a wide range of techniques, including almost all of the topics that we will be discussing. There are also many chapters that we will not be looking at, *e.g.*, cell culture, microscopy, molecular biology and bioinformatics; but these may be useful to you in some other courses. Overall, the depth of coverage is somewhat limited - the book tends to be broad rather than deep.

You will need to refer to a comprehensive biochemistry textbook. I recommend Nelson and Cox, Lehninger's Principles of Biochemistry, 7th ed, 2017, or Berg, Tymoczko, and Stryer, Biochemistry, 8th ed, 2015.

Several sections of the course require familiarity with basic concepts from physics, including electrical circuits; atomic and nuclear structure; fluid mechanics (Archimedes' Principle; buoyancy; viscous drag). Most of these topics were covered in PHYS*1070 and 1080, but you will probably need to consult a physics textbook from time to time.

Some other useful books are listed below:

Laboratory-oriented textbooks

R.F. Boyer, *Biochemistry Laboratory*: *Modern Theory and Techniques,* Benjamin-Cummings, 2006 (QD415.5.B69)

R.R. Burgess and M.P. Deutscher (Eds), *Guide to Protein Purification*, 2nd ed, Methods in Enzymology, vol. 463, Academic Press, 2009. An authoritative collection of review articles on all aspects of the title subject. ISBN 978-0123749789

J.-C.Janson (Ed), *Protein Purification: Principles, High Resolution Methods, and Applications*, 3rd ed., Wiley, 2011 ISBN 978-0471746614

D.J. Holme and H. Peck, *Analytical Biochemistry*, 3rd ed., Longman, 1998 (QP 519.7.H64). This book has some good practical information on laboratory techniques, but the theoretical coverage is weak.

A.J. Ninfa, D.P. Ballou, and M. Benore, *Fundamental Laboratory Approaches for Biochemistry and Biotechnology*, 2nd ed. Wiley, 2008. ISBN 978-047008766-4

R. Reed, D. Holmes, J. Weyers, and A. Jones, *Practical Skills in Biomolecular Sciences*, 5th ed., Pearson, 2016; 556 pages. ISBN 978-1-292-10073-9

R. Switzer and L. Garrity, *Experimental Biochemistry*, 3rd ed., W.H. Freeman, 1999 (QP519.S95) Good coverage of protein purification, radioisotopes, electrophoresis, immunological methods. Nothing on fluorescence or centrifugation.

Theoretically-oriented textbooks

D. Freifelder, *Physical Biochemistry*, 2nd ed., W.H. Freeman, 1982 (QH 345.F72) This is an excellent reference text, especially with respect to the theory of spectroscopy, centrifugation, electrophoresis, and other biophysical techniques. The text provides detailed coverage of specialized topics that are rarely found in other books, such as the determination of partial molar volumes of proteins; but it is now badly out-of-date.

A. Hofmann, A. Simon, T. Grkovic, and M. Jones, *Methods of Molecular Analysis in the Life Sciences*, Cambridge Univ. Press, 2014. Clear, concise introductions to theory and physical principles; strong on spectroscopy.

S.R. Mikkelsen and E. Cortón, *Bioanalytical Chemistry,* 2nd ed, Wiley, 2016 (QP519.7.M54) Mikkelsen is a chemistry professor at Univ. of Waterloo. Good coverage of electrophoresis, but little spectroscopy.

K.E. van Holde, W.C. Johnson, P.S. Ho, *Principles of Physical Biochemistry*, 2nd ed., Pearson/ Prentice-Hall, 2006 (QP 517.P49) Strong on spectroscopic methods and thermodynamics.

Course Content: A detailed lecture schedule for the course is given at the end of ths document.

Course Prerequisites: Introductory Biochemistry is a prerequisite for this course. The following aspects of the subject are important background; familiarity with them will be *assumed* and may be examined: basic aspects of protein and nucleic acid structure, **including the structures of the amino acids (and their one-letter codes)**; basic enzymology. Please review this material carefully, especially if some time has passed since you took BIOC*2580.

Method of Assessment:

Lecture component			**Lab component**	
Quiz 1 **June 22**	15		Notebook	7
Quiz 2 **July 18**	15		Report 1	14
Term paper **July 25**	10		Report 2	14
Final exam	25		*sub-total*	*35*
sub-total	*65*		**total**	**100**

Students must pass (mark of 50% or better) both the laboratory component (35%) and the lecture 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. The quizzes will be held in class - see Lecture Schedule for dates. The final exam. will cover the entire course, with emphasis on the material covered after the second quiz. Exercises (problem assignments) will be posted on Courselink, but will not be graded. Detailed instructions regarding the term paper, which will be a critical review of a recent journal article, will be distributed separately.

Course and University Policies

Students are expected to attend the lectures and labs. Only valid excuses (medical or compassionate) will prevent a grade of zero for any missed lab., lab. quiz, or examination. Documentation may be required. There will be no "make-up" exams. College policy precludes changes to the grading scheme for individual students, except in case of illness. Every student must bring his/ her identification card to the quizzes and exam. All exams must be written in ink. No additions must be made after return of the paper. Papers may be returned to the instructor for correction of grading errors, only within one week of the return of the graded papers. The instructor may refuse to re-grade a paper, at his discretion. The use of stored programs or stored alphanumeric information on calculators, during examinations or tests, is not allowed.

Signed written comments on the teaching performance of the lecturer may be sent to the Chair, Department of Molecular and Cellular Biology, at any time (a copy will be made available to the instructor after submission of final grades).

When You Cannot Meet a Course Requirement: When you find yourself unable to meet an in-course requirement because of illness or compassionate reasons, please advise the course instructor (or designated person, such as a teaching assistant) in writing, with your name, id#, and e-mail contact, and be prepared to provide supporting documentation. See the undergraduate calendar for information on regulations and procedures for Academic Consideration.

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 : Student Accessibility Services as soon as possible. For more information: 519-824-4120 ext. 56208 or email: accessibility@uoguelph.ca.

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. Please note: Whether or not a student intended to commit academic misconduct is not relevant for a finding of guilt. Hurried or careless submission of assignments does not excuse students from responsibility for verifying the academic integrity of their work before submitting it. 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.

E-mail Communication
As per university regulations, all students are required to check their <uoguelph.ca> e-mail account regularly: e-mail is the official route of communication between the University and its students.

Drop Date
The last date to drop one-semester courses, without academic penalty, is the 40th class day. To confirm the actual date please see the schedule of dates in the Undergraduate Calendar. For regulations and procedures for Dropping Courses, see the Undergraduate Calendar.

Copies of out-of-class assignments
Keep paper and/or other reliable back-up copies of all out-of-class assignments: you may be asked to resubmit work at any time.

Recording of Materials
Presentations which are made in relation to course work - including lectures - cannot be recorded or copied without the express written permission, obtained in advance, of the instructor. Material recorded with permission is restricted to use for that course, unless further permission is granted.

Campus Resources

The Academic Calendar is the source of information about the University of Guelph's procedures, policies and regulations which apply to undergraduate, graduate and diploma programs. If you are concerned about any aspect of your academic program, make an appointment with a program counsellor in your degree program.

If you are struggling to succeed academically: There are numerous academic resources offered by the Learning Commons including, Supported Learning Groups for a variety of courses, workshops related to time management, taking multiple choice exams, and general study skills. You can also set up individualized appointments with a learning specialist.

If you are struggling with personal or health issues: Counselling services offers individualized appointments to help students work through personal struggles that may be impacting their academic performance.

- Student Health Services is located on campus and is available to provide medical attention.

- For support related to stress and anxiety, besides Health Services and Counselling Services, Kathy Somers runs training workshops and one-on-one sessions related to stress management and high performance situations.

If you have a documented disability or think you may have a disability: Student Accessibility Services provides services and support for students with a documented learning or physical disability. They can also provide information about how to be tested for a learning disability. For more information, including how to register with the centre, please see:
https://wellness.uoguelph.ca/accessibility/

Analytical Biochemistry BIOC*3570
Term Paper Assignment; Summer 2017

Before a scientific paper can be published in a reputable journal, the manuscript must be evaluated by two or more "referees", independent experts who assess the manuscript's quality and provide their recommendations to the journal's Editor. Each journal maintains an Editorial Board of trusted reviewers. The major criteria for judging manuscript submissions are *scientific quality*, *editorial quality*, *originality*, and *significance to the field of study*.

Scientific quality: Have the researchers chosen appropriate biological systems to study? Did they choose appropriate techniques with which to test their scientific hypothesis? Were the experiments designed and performed correctly? Were the necessary controls performed? Were the right instruments used? Were the data - spectra, counts, assays, images - presented clearly and analyzed appropriately? Were valid statistical tests performed? Overall: have the authors established their claims?

Editorial quality: Is the writing clear, concise, and readable? Does the organization of the paper (*e.g.*, the titles and order of the sections) conform to the journal's guidelines? Are the Figures and Tables designed and organized well*?* Are the appropriate references cited?

Originality: Are the methods and findings new? Or does this study merely repeat work that has already been published - perhaps with a few minor changes, such as repeating in one organ/ species/ cell line/ etc. work that has previously been done in another?

Significance: Is this a new finding that greatly changes our understanding of the subject? If a new technique or assay is described, is it one that other scientists will be eager to apply? Putting it simply, if one of our readers picks up this issue of the journal, will he or she want to read this paper?

Reviewing manuscripts is considered to be an important duty of a professional scientist. It allows a scientist to participate directly in influencing the direction of a field of research, protecting the literature against the publication of erroneous or duplicative studies, and, where appropriate, advocating for the publication of important work that might otherwise go unrecognized. (Some great manuscripts have been rejected! A famous instance is the work of Berson and Yalow describing the radioimmunoassay, work which subsequently won them the 1977 Nobel Prize in Physiology or Medicine. Their first manuscript describing the technique was rejected by the prestigious *Journal of Clinical Investigation*, which told them "The data you present are indeed suggestive, but any more positive claim seems unjustifiable at present." Happily, if a manuscript is rejected by one journal, you can always submit it to another!)

By the way, in a typical good-quality journal, such as *Analytical Biochemistry,* about 60-80% of the submitted manuscripts are rejected - *i.e.*, not published. In the highest-impact journals, such as *Science*, *Nature*, or *Cell*, the rejection rate is more than 90% - and most scientists would never even *submit* a manuscript to one of those journals unless they believed that it represented their very best work.

All of the papers listed below have been published in the journal *Analytical Biochemistry* in the past year. You have been assigned one of the papers at random, based on your student number (see below). *Your assignment is to write a referee report on that paper*. The report should consist of a summary and an evaluation. In the summary, you will write a short informative summary of the contents of the paper, explaining the scientific problem being studied, the experimental approach, and the findings. In the evaluation, you will discuss the scientific quality and novelty of the work and you will scrutinize the authors' interpretation of their findings.

Make sure that you understand all of the concepts that you discuss in your critique. If particular methods are unfamiliar to you, you may need to study other references or textbooks; feel free to discuss things with me. You can assume that the reader is familiar with the methods that we are discussing in the

course, such as electrophoresis, optical spectroscopy, immunoblotting, etc. But *you should explain clearly, in your own words, any unusual or unfamiliar reagents, techniques, or systems*. A simple test for "readability" is to read the paper over to yourself, or to a fellow student: if you, or they, have difficulty following something, then you probably need to clarify that section. Suppose that I were presenting this material to you in class. Would you put up your hand to ask for an explanation? If so, then you probably need to explain the concept in your report.

It is not necessary to re-state the methods and results of the paper in detail and you should not copy any text verbatim. Your summary should *summarize* and *explain* the contents of the paper, in your own words.

Note that an evaluation of a work does not necessarily mean a *demolition*. If you think the paper is excellent, say so - but make sure that you support your rating with a convincing argument.

I hope that you will find this exercise to be useful training in the important skills of independent study, critical analysis, accessing the scientific literature, and writing.

Please focus your evaluation on significant concepts and ideas, rather than on minor matters of formatting, grammar, or typographical errors; these can be listed at the end of the evaluation.

Typesetting: Sometimes, students criticize the layout of the article; e.g., where the figures are placed relative to the corresponding text; the fonts; the sizes of the figures, etc. Note that these features are determined by the publisher's typesetters, and not by the authors.

The term papers will be graded for quality of the scientific analysis and judgement, originality, accuracy, and clarity of writing.

Proof-read your work carefully for grammar and spelling. Do not confuse "it's" and "its"; "there" and "their"; "then" and "than". Note that "*i.e.*" (*id est*) means "that is"; "*e.g.*" (*exempli gratia*) means "for example"; "*et al.*" (*et alia*) means "and others"; "*et*" is not an abbreviation and is not followed by a period.

References: You may need to refer to some other journal articles or books. If so, they should be cited *using the reference style of Analytical Biochemistry*. (Note that *only the first word of the title of a journal article is capitalized* in a literature citation, even if a different style was used in the original article.)

Due date: Tues. July 25, 10:00 a.m. (at the start of the class).

If you submit the assignment by Thurs. July 13, 10:00 a.m., you will receive 2 bonus marks (out of 10).

Suggested length: Approximately 1000 words. Please type your paper, single-sided, double-spaced, in Times Roman 12 point or a similar standard font. Leave standard margins (1" on all sides). Number the pages. Include a cover page (which is not numbered) with your name, student number, and the date of submission. Please staple or bind your paper. All assignments must be submitted on paper.

Frequently asked questions:

1. When I submit my term paper, should I attach a printed copy of the paper that I am reviewing?
   Answer: No.

2. Should I reproduce the Tables or Figures from the paper, in my review?
   Answer: No.

3. I have never written a review before. Can I meet with Prof. Josephy for help and advice?
   Answer: Yes indeed!

4. I really don't understand this assignment! These papers have *already* been reviewed and published, so they must be good! How can there be anything to criticize?
   Answer: You might be surprised to learn how many published papers turn out to be of poor quality - sloppy or inaccurate measurements, missing controls, improper design, or simply papers that are routine work, tedious, and of little significance. Perhaps 50% of published papers are never cited again by anyone - they just sink into well-deserved obscurity!

5. Did Dr. Josephy select these particular papers because each one contains some critical flaw, which we are supposed to discover?
   Answer: No. These papers were chosen simply because they are up-to-date and relevant to the techniques we will be discussing in the course. They are not necessarily examples of particularly good or particularly bad research.

Assignment of papers: Let *n* = your student ID number. Now, calculate the value of *n* modulus 3. You can use the modulus function in Excel: =MOD(n,3)

If the modulus is ...	**... then your assigned paper is ...**
0	Ponniah et al., Characterization of charge variants of a monoclonal antibody using weak anion exchange chromatography at subunit levels.
1	Firsov et al., Purification and characterization of recombinant supersweet protein thaumatin II from tomato fruit.
2	Robinson et al., Cloning, expression, purification, and characterisation of the HEAT-repeat domain of TOR from the thermophilic eukaryote *Chaetomium thermophilum*

These papers are posted on Courselink.

Appendix 1:

The following guidelines for reviewers are based on the "Information for Referees" for the journal *Nucleic Acids Research*, and they provide some helpful suggestions for writing referee reports.

Referees should approach each manuscript with an impartial and positive but critical mind. Comments must be constructive, clearly identifying the manuscript's strengths and weaknesses, and providing practical suggestions for improvement.

The ideal report should begin with an introductory section placing the paper in a broader context, summarizing the main findings and claims, describing the contribution it makes to the field, and giving the Referee's overall impression of the manuscript. This should be followed by specific comments and suggestions to improve the manuscript, which should be further divided into Major and Minor comments. These may include (major): necessary improvements to the described experiments; errors of design or interpretation; (minor) addition/deletion of references; changes to the text to improve presentation, quality of English, length, etc.

Appendix 2.

I serve as Editor for the journal *Mutation Research*. Here is an example of an actual referee's report for that journal. (The referee recommended Rejection of the manuscript, in this case.)

This example is not intended as a "template" for your assignment. You will be expected to provide a more careful and comprehensive description of the content of the manuscript than is done here, and your report will probably be about twice as long as this example, but it can serve to give you the "flavour" of real referee reports. Notice how the reviewer has highlighted specific problems with the manuscript (identifying the pertinent statement or Figure, in each case) and explains succinctly what each of the problems is. The reviewer's attention is focussed on the design of the experiments and the interpretation of the data.

Review:

In this work, the authors expose a plasmid containing the p53 sequence (presumably human, although this was not directly stated) to BPQ, a by-product of one of the most common polycyclic hydrocarbons found in cigarette smoke. Purified plasmid DNA was incubated with BPQ in the presence of $CuCl_2$ and NADPH, since previous work has demonstrated that BPQ is not mutagenic in their absence. The in vitro mutagenized plasmid was then transformed into yeast cells bearing a reporter for p53 function. The authors tested the effects of mutations in OGG1 and APN1, genes encoding components of the DNA base excision repair pathway, to determine whether mutation frequency is affected by their loss. They conclude that loss of OGG1 results in increased mutation frequencies, while loss of APN1 does not. The biological significance of this result is not clear, given that the exposure of p53 sequences to the mutagen was performed in vitro whereas the repair was performed in yeast cells, which contain an additional AP endonuclease gene.

Unfortunately, the authors do not describe the reporter system they are using in enough detail for readers to follow easily. The assay should be more fully described at the beginning of the Results section.

There are several other significant problems with the work, as follows:

1. The discussion of strand bias is flawed. The authors state that, "there are more mutations on the coding strand compared to the transcribed strand". This statement does not make sense, because although it is possible for damage to be found on one strand or the other, by the time the plasmid is examined, the mutation is present on both strands. It is not possible to ascribe strand specificity to a mutation. It would appear that this conclusion is derived from the observation that there are more guanine substitutions on one strand than the other. If so, this conclusion rests on the assumption that all of the mutations result from oxidized guanine after exposure to the mutagen, which has not been demonstrated.

2. The mutational spectra presented in Figure 3 are of some concern. When examining these types of data, it is important that the sequenced mutants be independently derived. In other words, the transformants from a single transformation may not have arisen independently, but may be derived from the division of a single transformant or a mutation that preexists at low concentration in the plasmid preparation from *E. coli*. The methods are not provided in sufficient detail to determine whether this is so. However, the fact that, in the *ogg1* strain, the same mutation was recovered nine times suggests that they may not be independent.

3. On page 12, the authors state that, "Only 8-oxo-dGuo is likely to occur frequently enough to cause mutations." It is not clear how this conclusion was made. Any type of lesion may lead to mutation, although the frequency is likely to be higher for damage that is more abundant, the frequency of mutations caused by any type of damage will also depend on the efficiency of the respective repair pathways. As a result, it is not possible to directly correlate the amount of each type of damage with the number of mutations that result from that damage.