PURDUE
UNIVERSITY

DEPARTMENT OF BIOCHEMISTRY

BCHM 62000 – Protein Mass Spectrometry and Proteomics
Spring, 2016

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COURSE OBJECTIVES

This is a two credit course. The goals of this course are to introduce students to 1) basic principles of mass spectrometry, 2) common instruments used for protein mass spectrometry, 3) the most common applications of protein mass spectrometry in biological research, 4) current approaches to quantitative protein mass spectrometry and their use in proteomic studies 5) the skills necessary to analyze mass spec data from a variety of experiment types including the ability to use and understand common database search programs, and 6) contemporary issues associated with large-scale proteomics experiments (including technical challenges and limitations) culminating in the ability to design appropriate experiments to answer a specific proteomic question.

LEARNING OUTCOMES

BCHM 62000 students will be knowledgeable in practical skills associated with mass spectral analysis of biological molecules, with a heavy emphasis on proteins.

BCHM 62000 students will understand the basics of experimental design for the mass spectral analysis of proteins and other biological molecules.

BCHM 62000 students will acquire an appreciation for current instrumentation and methods commonly used in protein mass spectrometry and the advantages and disadvantages of each.

BCHM 62000 students will be able to read and critically evaluate primary literature pertaining to proteomic studies.

TEXTBOOK

There is no textbook for this course. Assigned reading material will be provided via the course Blackboard page (see below).

TIME AND PLACE

Tuesday and Thursday 10:30-11:20, BCHM 102

Attendance policy:
Please let me know ahead of time if you will have to miss a class. Since this is a small class and there will be extensive discussion and class participation at times, and because the majority of exam material will come from the class lectures, it is important for students to attend each class. I will allow two unexcused absences, and then each additional unexcused absence will result in a deduction of 5 points from the final grade. Excused absences must be requested ahead of time and may require official documentation at the discretion of the instructor verifying why the class must be missed.

BLACKBOARD

The course syllabus, PowerPoint lecture files, assigned reading material, answer keys for homework sets and the mid-term exam, and any additional course information will be available on the course Blackboard webpage.

ASSESSMENT

Exams:
There will be one mid-term exam on Thursday, March 10.
The second exam will be a take home exam given out a couple weeks before the semester ends and due by then end of the day on Friday, April 22. It will be partially cumulative but will focus mostly on material from the second half of the class.

Homework Sets:
Because a major goal of this course is to provide students with the skills necessary to analyze mass spectrometric data for a variety of experiment types, there will be 4 homework problem sets during the semester given out at 2-3 week intervals that give students a chance to practice data analysis as well as solidify their understanding of basic principles and instrumentation. Students will have one week to complete each set.

Grading Policy:
- Mid-term and final exam will each count for 35% of the final grade.
- Homework sets collectively will count for 25% of the final grade.
- Class participation is expected and the final 5% of the grade will be depend on the extent of class participation with emphasis on the final two weeks that involve discussion of proteomics literature.

The following grading scale will be used:

92-100 = A
90-91 = A-
88-89 = B+
82-87 = B
80-81 = B-
78-79 = C+
72-77 = C
70-71 = C-
60-69 = D
below 60 = F

I reserve the right to apply grading curves to any of the tests and homework sets but there will not automatically be a defined curve.

Missing a homework assignment will result in a grade of 0 being recorded unless documented justification is presented.

If you have any disagreements with the way any of your assignments have been graded, please make an appointment to see the instructor.

Requests for re-grades must be submitted no later than the end of the second class period after the grade is received.

EXTRA CREDIT

There will be no opportunity for extra credit.

OBTAINING EXTRA HELP

The instructor will be available to answer questions immediately after class or by appointment (arranged in class or by e-mail). Alternatively, you can submit questions by e-mail.

ACADEMIC MISCONDUCT

Academic misconduct of any kind will not be tolerated in any course offered by the Department of Biochemistry. Information on Purdue's policies with regard to academic misconduct can be found at http://www.purdue.edu/ODOS/osrr/integrity.htm.

To provide you with an unambiguous definition of academic misconduct, the following text has been excerpted from "Academic Integrity: A Guide for Students", written by Stephen Akers, Ph.D., Executive Associate Dean of Students (1995, Revised 1999, 2003), and published by the Office of the Dean of Students in cooperation with Purdue Student Government, Schleman Hall of Student Services, Room 207, 475 Stadium Mall Drive West Lafayette, IN 47907-2050.

"Purdue prohibits "dishonesty in connection with any University activity. Cheating, plagiarism, or knowingly furnishing false information to the University are

examples of dishonesty." [Part 5, Section III-B-2-a, *University Regulations*] Furthermore, the University Senate has stipulated that "the commitment of acts of cheating, lying, and deceit in any of their diverse forms (such as the use of substitutes for taking examinations, the use of illegal cribs, plagiarism, and copying during examinations) is dishonest and must not be tolerated. Moreover, knowingly to aid and abet, directly or indirectly, other parties in committing dishonest acts is in itself dishonest." [University Senate Document 72-18, December 15, 1972]

More specifically, the following are a few examples of academic dishonesty which have been discovered at Purdue University.

- substituting on an exam for another student
- substituting in a course for another student
- paying someone else to write a paper and submitting it as one's own work
- giving or receiving answers by use of signals during an exam
- copying with or without the other person's knowledge during an exam
- doing class assignments for someone else
- plagiarizing published material, class assignments, or lab reports
- turning in a paper that has been purchased from a commercial research firm or obtained from the internet
- padding items of a bibliography
- obtaining an unauthorized copy of a test in advance of its scheduled administration
- using unauthorized notes during an exam
- collaborating with other students on assignments when it is not allowed
- obtaining a test from the exam site, completing and submitting it later
- altering answers on a scored test and submitting it for a regrade
- accessing and altering grade records
- stealing class assignments from other students and submitting them as one's own
- fabricating data
- destroying or stealing the work of other students

Plagiarism is a special kind of academic dishonesty in which one person steals another person's ideas or words and falsely presents them as the plagiarist's own product. This is most likely to occur in the following ways:

- using the exact language of someone else without the use of quotation marks and without giving proper credit to the author
- presenting the sequence of ideas or arranging the material of someone else even though such is expressed in one's own words, without giving appropriate acknowledgment
- submitting a document written by someone else but representing it as one's own

EMERGENCY PREPAREDNESS

In the event of a major campus emergency, course requirements, deadlines and grading percentages are subject to changes that may be necessitated by a revised semester calendar or other circumstances. To get information about changes in this course consult the class Blackboard site or e-mail or phone the instructor.

ON-LINE COURSE EVALUATIONS

During the last two weeks of the semester, you will be provided an opportunity to evaluate this course and your instructor(s). To this end, Purdue has transitioned to online course evaluations. On Monday of the fifteenth week of classes, you will receive an official email from evaluation administrators with a link to the online evaluation site. You will have two weeks to complete this evaluation. Your participation in this evaluation is an integral part of this course. Your feedback is vital to improving education at Purdue University. I strongly urge you to participate in the evaluation system.

LECTURE SCHEDULE

CLASS 1 – Tuesday, January 12
Introduction; course outline and goals; the Syllabus;
Defining proteome and proteomics; What is a mass spectrometer?; Brief history of mass spectrometry (MS); Proteomic resources

CLASS 2 – Thursday, January 14
Understanding the mass spectrum; basic principles of MS

CLASS 3 – Tuesday, January 19
Review of basic MS principles with practice problems; MS instrumentation: ionization sources and mass analyzers

CLASS 4 – Thursday, January 21
MS instrumentation – tandem MS configurations and introduction to ion fragmentation

CLASS 5 – Tuesday, January 26
Peptide ion fragmentation and generating peptides for MS analysis

CLASS 6 – Thursday, January 28
Protein MS applications – identifying unknown proteins by peptide mass fingerprinting; An introduction to database search algorithms

CLASS 7 – Tuesday, February 2
Using Mascot to conduct peptide mass fingerprinting analyses with real data

CLASS 8 – Thursday, February 4
Protein MS applications – de novo sequencing of peptides from fragment ion spectra obtained by tandem MS; Homology searching

CLASS 9 – Tuesday, February 9
Protein MS applications – identifying unknown proteins from tandem MS data by database search algorithms

CLASS 10 – Thursday, February 11
Using Mascot to conduct simple tandem MS protein ID analyses with real data

CLASS 11 – Tuesday, February 16
Protein MS applications - identification of post-translational modifications from tandem MS data

CLASS 12 – Thursday, February 18
Protein MS applications – identification of post-translational modifications using specialized analyses, focusing on protein phosphorylation

CLASS 13 – Tuesday, February 23
Challenges with PTM mapping; Tools for scoring PTM assignments; Practice with manual analysis of tandem MS data for PTM mapping

CLASS 14 – Thursday, February 25
Quantitative MS – Absolute and relative quantification of biomolecules using stable isotope labeling (Dr. Aryal)

CLASS 15 – Tuesday, March 1
Quantitative MS - Label-free approaches to quantification of biomolecules (Dr. Aryal)

CLASS 16 – Thursday, March 3
Instrument demonstration day at Purdue Proteomics Facility (Dr. Aryal)

CLASS 17 – Tuesday March 8
Summary of quantitative MS techniques; Question and answer session for mid-term exam with additional practice problems

CLASS 18 – Thursday, March 10
Mid-term exam

CLASS 19 – Tuesday, March 22
Dealing with complex samples – protein and peptide separation technologies

CLASS 20 – Thursday, March 24
Coupling liquid chromatography and MS (LC-MS)

CLASS 21 – Tuesday, March 29
Sample preparation for proteomics and protein MS (Dr. Aryal)

CLASS 22 – Thursday, March 31
Database searching with large LC-MS datasets (Dr. Aryal)

CLASS 23 – Tuesday, April 5
Tools for analysis and interpretation of large-scale proteomics data, including quantitative analyses (Dr. Aryal) - MaxQuant

CLASS 24 – Thursday, April 7
Bioinformatics tools: extracting biological meaning from proteomic experiments (Dr. Aryal)

CLASS 25 – Tuesday, April 12
Summary of common proteomic workflows; practical considerations for designing proteomic experiments; Overview of top-down proteomics; Specialized MS applications; Examples of protein MS as an analytical tool

CLASS 26 – Thursday, April 14
The future of proteomics: upcoming technologies, challenges to overcome in the field, new and improved applications; Alternative experimental approaches for proteomics

CLASS 27 – Tuesday, April 19
Critical analysis of proteomics research literature: protein profiling

CLASS 28 – Thursday, April 21
Critical analysis of proteomics research literature: comparative expression analysis

CLASS 29 – Tuesday, April 26
Critical analysis of proteomics research literature: protein interaction networks

CLASS 30 – Thursday, April 28
Critical analysis of proteomics research literature: identification of post-translational modifications