BIOL-UA 130 ATB Biological Chemistry: Genomes to Molecular Machines

Description
This is an upper-level elective lab course for students majoring in Biology and those seeking to fulfill requirements for entrance into advanced degree programs. Using biochemical and genetic approaches with the yeast *Saccharomyces cerevisiae*, students will characterize a large multisubunit protein complex that modifies chromatin and is involved in gene regulation. *S. cerevisiae* is a unicellular eukaryote better known as baker’s yeast that is a widely used biochemical and genetic model organism. Affinity chromatography will be used to produce purified preparations of wild-type and mutant protein complexes. The purified protein complexes will be compared using a wide variety of biochemical techniques, including Sypro Red-stained SDS-PAGE, western blot, enzymatic assay, and protein interaction assays. Yeast expressing the same mutants will be used in genetic experiments to evaluate the importance of the protein complex in cell growth and gene regulation in the cell.

Room
Silver 603

Time
Monday 12:30pm-1:45pm
Wednesday 12:30-3:15

Instructor
Professor Michael Carrozza
Phone: 212-992-6961
Email: mjc24@nyu.edu

Office Hours
Monday 2:00pm-3:00pm
Silver 602

Prerequisites
Molecular and Cell Biology I & II (BIOL-UA 21 & 22)
Organic Chemistry I & II (CHEM-UA 225 & 226)

Objectives of the course
1. Teach students key biochemical concepts, including amino acid properties, protein structure, spectroscopy, enzyme kinetics, transcription, sugar catabolism, electrophoresis, immunochemistry, protein chromatography, protein interactions, post-translation modifications of proteins and histones, analysis of transcription and chromatin structure.
2. Allow students to experience a laboratory class that closely resembles a real research environment.
3. Convey an appreciation of how the combination of biochemical and genetic approaches provide a more complete understanding of a research topic.
4. Facilitate students’ abilities to read and evaluate scientific literature.
5. Foster improvement of presentation and writing skills with the assistance of the University Writing Tutors Program.

Required Text

Grading

The final grade for the course will be determined as follows:
- Midterm exam: 25%
- Final exam: 25%
- Lab report 1: 15%
- Lab report 2: 15%
- Oral Presentation: 10%
- Laboratory participation: 5%
- Laboratory performance: 5%

Exams The midterm and final exam will cover the material from the first and second half of the course, respectively. The final exam will not be cumulative in the sense that you will not be tested directly on material from the first half of the course. However, some material from the first half will be relevant to understanding the remaining topics in the course. Material on the exam will include introductory material, laboratory procedures, experimental design and interpretation of results.

There will be no make up exams. If you miss an exam for a valid reason, subject to my discretion, your other exam will be counted for 50% of your grade.

Lab reports There will be two writing assignments that will involve describing, in a scientific-journal format, your results and conclusions from data obtained in lab. Writing assignments will be graded based on your ability to convey your scientific hypothesis and findings in a clear, complete and well-organized way. Work on these assignments will begin several weeks before their due dates.

Lab reports are to be written in your own words. You will be submitting your reports through Turnitin. This is a web-based program that compares your writing to work done by other students using Turnitin worldwide and other material available on the internet. Similarities are provided in a report to me. Any instance of obvious plagiarism will result in zero for the report.

In this class, we are fortunate to have help from the Undergraduate Writing Tutors Program. Writing tutors are curious, well-trained peers who provide feedback to their peers on drafts of writing assignments. Their role is to encourage and challenge students to strengthen their writing. Writing tutors are trained to support the aims of the class, learning about the expectations for writing in the class and listening carefully to student writers. While writing tutors are not Teaching Assistants and will not assess papers, they will focus writing conferences with students on questions that generate both clearer writing and stronger thinking about the content. Writing tutors will also look for patterns of grammatical
error in student papers, explaining how students can learn to correct these errors. But the writing tutors' main goals are to help students develop their writing and thinking in response to particular assignments as well as help them to become better writers over the long term.

Students are required to meet outside class with writing tutors. Writing tutors should receive complete drafts from students, not outlines or rough notes, by prearranged due dates. Late submission of drafts to tutors and missed writing conferences are reported to the Professor, who reduces the final grade on the finished version. During conferences the tutors will discuss my comments and provide their own feedback on your drafts. As part of your final drafts, I will expect you to address both the tutor's and my comments on your work.

Failure to attend tutor conferences or submit complete drafts will result in a 10% reduction in the overall score for your report. Failure to submit any drafts on time will result in a 1 point reduction in the overall score of the report for each day late.

**Laboratory Notebook**  You are expected to keep notebook of work in the lab. I will on several occasions collect notebooks and inspect your notekeeping to insures that you are maintaining notes in your labwork. Proper maintenance of your notebook will be reflected in your laboratory performance score.

**Oral presentations**  During the second half of the course, we will take time during recitation and lab to listen to presentations by each student in the course. You will be graded based on your ability to organize and to articulate your research to the audience.

**Laboratory participation**  This portion of your grade will be based on your participation in laboratory discussions, attendance and contributing to your group's research progress.

**Laboratory performance**  This portion of the grade is based on how well you conduct yourself in the laboratory. This will include how you handle equipment, adhere to the safety regulations, record your data and observations, and conduct your experiments.

**Attendance**

You should make every effort to attend the recitation and lab. Absences without a valid excuse will result in a deduction in your laboratory participation score (½ – 1 point for each absence). Keep in mind that based on the design of this course, repeated absences will interfere with your ability to conduct experiments and this will negatively impact the quality of your written and oral assignments. Any absences due to illness should be accompanied with a note from a doctor. In the event that you are unable to attend you should contact me and explain your circumstances and communicate with your lab partners about missed material and status of your projects.

**Schedule (subject to change)**

**Week**

1. **Recitation:**  Model organisms for genetics and biochemistry (Paper 1)

   Biochemical reagents and solutions (Lab manual pp. 14-17, 53-64)
Lab: Preparing solutions
   Pipetting (Lab manual pp 18-22) (Handouts)

2. Recitation: Chromatin structure

   The Role of HATs in regulating transcription within chromatin (Paper 2)

   Preparing Cell extracts (Lab manual pp. 307-314)

Lab: Whole cell extract preparation/ Centrifugation (glass bead breakage; salt extraction; high speed centrifugation)

   Preparation of a cell extract from yeast (handout)

3. Recitation: Amino acid properties and protein structure
   (Paper 4)

   Centrifugation
   (Lab manual Ch. 4 95-112)

   Chromatographic separation of proteins
   (Lab manual Ch. 5 115-118, 122-140, 152-157)

   Tandem Affinity purification of proteins (Paper 3)

Lab: Immunoglobin affinity purification (Modified TAP purification handout)

4. Lab: Calmodulin affinity purification (Modified TAP purification handout)

5. Recitation: Electrophoretic separation of macromolecules and Immunochemistry

   Separation of protein (SDS-PAGE) and western blot analysis of proteins
   (Lab manual Ch. 6 pp. 165-176, pp. 194-198)
   (Paper 5)

Lab: SDS-PAGE of complexes and Sypro Red staining (Handout)

   Notebook review

6. Recitation: Spectroscopic Analysis of Biomolecules/Enzyme Kinetics

   Assaying for enzymatic activity (Lab manual Ch 8; Ch. 7 spectrophotometric analysis of proteins)
Lab report Introduction Draft due

Lab: Assay of Acetyltransferase enzymatic activity (Handout)
    Bradford assay (Lab manual Ch. 69-70)

7. Recitation: Exam I

Lab: Western blot analysis of Acetyltransferase assay

Lab report 1 draft due

8. Recitation: Spring Break

Lab: Spring Break

9. Recitation: Paper discussion

Lab: Develop western blot and analyze data

10. Recitation: Protein interactions
    (Paper 6)

Lab: Protein-Protein Pulldown SAGA complex interaction with Gal4 transcription factor
    (Paper 7) (Handout)

Lab report 1 final draft due

11. Recitation: Paper discussion

Student presentations

Lab: Develop pulldown western and analyze data

12. Recitation: Catabolism of sugars/The galactose regulon
    (Paper 8)

Student presentations

Lab: Growth of SAGA mutants using galactose as sole carbon source (Handout)

Notebook review
13. Recitation:  *Approaches to analyzing transcription*

   Reporter assays for measuring transcription in the cell

   Basic statistics of experimental data (review Ch.1 pp. 23-30)

   **Lab:** Measuring effect of mutants on a galactose inducible LacZ reporter (Paper 16)

   Preparartion of LacZ extracts and Bradford assay

14. Recitation:  *Isotopes in biochemistry experiments*

   **Lab:** Measuring effect of mutants on a galactose inducible LacZ reporter (part 2)

   Measurement of LacZ activity

   **Lab report 2 first draft due**

15. Recitation  Paper Discussion

   **Lab:** Analysis of LacZ data

16. Recitation  Final exam

   **Lab report 2 final draft due**

**Papers**