Instructor: Alberto A. Rascón, Jr., Ph.D.

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Office hours: T 13:00-15:00 (or by appointment)

Class days/times: W 15:00-17:50
F 13:30-17:20

Classroom: DH 609

Prerequisites: A letter grade of “C” or better in Chem 100W, 130A, 131A; NOTE: prerequisites will be checked.

Co-requisites: Chem 130B or Chem 130C

eCAMPUS Course Page

Copies of the course materials such as the green sheet, major assignment handouts, etc... may be obtained by logging on to Canvas at http://www.sjsu.edu/at/ec/canvas/index.html. You are responsible for regularly checking the course page for updates. NOTE: In case website is not functioning properly, materials and announcements will be emailed directly to the students.

Course Description

Chem 131B is the second semester of a two semester biochemistry laboratory course. Laboratory work associated with intermediate qualitative and quantitative techniques in modern biochemistry. This is a capstone course. A capstone experience requires students to integrate principles, theories, and methods learned in previous courses throughout the major. Students will be working on research projects that will allow analysis, synthesis, and evaluation of learned knowledge and will communicate the results of the projects effectively in a professional manner. This course also contains content that addresses Area R learning objectives.
Student Learning Objectives

SJSU Studies Area R Learning Outcomes addressed by Chem 131B

SLO 1: Students will be able to demonstrate an understanding of the methods and limits of a scientific investigation.

SLO 3: Students will be able to apply a scientific approach to answer questions about the earth and environment.

Course Content Learning Outcomes:

Upon successful completion of this course, students will be able to:

LO(1) apply proper laboratory practices including safety, waste management, and record keeping.

LO(2) use and understand modern biochemical techniques and instruments.

LO(3) plan, design, and execute experiments based on biochemical literature.

LO(4) interpret experimental results and draw reasonable conclusions.

LO(5) communicate effectively through written and oral reports.

BS/BA CHEMISTRY PROGRAM LEARNING OUTCOMES ADDRESSED BY Chem 131B

PLO #5 - Demonstrate understanding of core concepts and to effectively solve problems in biochemistry.

PLO #6 - Answer questions regarding safe practices in the laboratory and chemical safety.

PLO #7 - Demonstrate safe laboratory skills (including proper handling of materials and chemical waste) for particular laboratory experiments.

PLO #9 - Effectively present a scientific paper orally, as per at an American Chemical Society symposium.

PLO #10 - Write a formal scientific laboratory report, using the format and style of an article in a peer-reviewed American Chemical Society journal

Required Text/Materials

Research laboratory notebook with duplicate pages; scientific calculator (equivalent to Ti30) capable of performing linear regression analysis.
Classroom Protocol

Students are expected to arrive on time and attend all classes. Students should be courteous to other students, the instructor and guest instructors. Cell phones should be turned off during class time. NO FOOD OR DRINKS ALLOWED IN THE LABORATORY.

Dropping and Adding

Students are responsible for understanding the policies and procedures about add/drop, grade forgiveness, etc... Refer to the current semester’s Catalog Policies section at http://info.sjsu.edu/static/catalog/policies.html. Add/drop deadlines can be found on the current academic calendar web page located at http://www.sjsu.edu/provost/docs/1314aycalendar.pdf. The Late Drop Policy is available at http://www.sjsu.edu/aars/policies/latedrops/policy/. Students should be aware of the current deadlines and penalties for dropping classes.

Information about the latest changes and news is available at the Advising Hub at http://www.sjsu.edu/advising/.

Assignments and Grading Policy

The course will consist of the following:

1. Midterm Exam (Fri. Mar. 14th) 100 points
2. Laboratory Paper (scientific journal article style) 100 points
3. Assignments (both in and out of class) 100 points
4. Notebook & Reference Binder 50 points
5. Lab Talk & Journal Article Presentation (25 pts ea.) 50 points
6. 1 Final Exam (Fri. May 16th @ 12:15 - 14:30) 100 points

The instructor evaluation is based on technique, performance, lab organization, lab work, comprehension of experiments, safety, attitude, proper use and disposal of chemicals, preparation prior to class, following directions, lab lecture involvement, etc...
Two exams will be given during the semester, a midterm and a final. The midterm will be given during one of the scheduled lab periods. The date is given on the attached schedule. The final will be given during the final exam period and is also noted on the schedule. Exams will cover theory, experimental protocol and data analysis. The content will be a combination of objective, calculations, short answer and short essay questions. Calculators (non-graphing, no memory) are permitted during exams and all exams are closed book.

Make-up midterms will NOT be given. The final exam must be taken to pass the class.

Letter grades for the course are A(+/-), B(+/-), C(+), No Credit. Letter grades will be based on a total percentage for the class as follows: 90s=A, 80s=B, 70s=C, etc..., where appropriate “+” and “-” signs are added (as an example: 80-82=B-, 83-86=B, 87-89=B+). There is NO extra credit or extra assignments during or after the semester is over, so please don’t ask.

**Laboratory Notebook**

It is imperative that all experimental data are recorded in the laboratory notebook and that this information is kept up-to-date. Never depend on your memory to record such data; you will forget it if it is not written down. Notebook entries should be clear and concise. Entries should be neat enough and annotated so that the experimental notes and data can be read and understood by others. Your notebook will be graded on these criteria.

The laboratory notebook does not have to look like a final report! Do NOT use scratch paper for experimental notes and data so that you can neatly transfer such into the notebook at a later time. It is quite acceptable to cross out information (with a single line) and rewrite it. Further, in professional settings, the notebook is the primary document verifying your intellectual property. Establishing good notebook habits now will prepare you for your career.

You will use the Laboratory Notebook with duplicate pages. The duplicate pages will be removed from the notebook and turned into the instructor at the end of each laboratory period.

**Laboratory Report**

A report of all laboratory work will be required in the form of a scientific journal article. This is to be completed outside of the lab period. The required content and format will be explained in class. Although the experiments may be performed in groups, all interpretations must be your own.

Deadlines for submission of sections (e.g. Introduction, etc…) throughout the semester and final paper will be announced or posted on Canvas. Failure to submit a report by the specified deadline will automatically lower your grade.

**NOTE:** Papers are due at the beginning of the laboratory period on the particular date. Reports submitted on the due date but later than the beginning of class are considered late and subject to 5% point reduction for every 30 min. Details regarding the format of the paper will be available on Canvas and discussed periodically.
Oral Presentations

Two oral presentations are required. One presentation is a “lab talk” covering background and work done towards the laboratory project (will be presented near the end of the semester). The other presentation is a “journal article” presentation. The journal article must be approved by the instructor and the approved journal article must be emailed to the instructor at least two weeks in advance of the presentation. The selected article should be a recent primary article (published within the last 5 years) and closely related to the laboratory project. The required content and format will be explained in class.

University Policies

Academic Integrity

Your commitment as a student to learning is evident by your enrollment at San Jose State University. The University’s Academic Integrity policy, located at http://info.sjsu.edu/static/catalog/integrity.html, requires you to be honest in all your academic course work. Faculty members are required to report all infractions to the office of Student Conduct and Ethical Development. The Student Conduct & Ethical Development | San Jose State University is available at http://www.sjsu.edu/studentconduct/.

Instances of academic dishonesty will not be tolerated. Cheating on exams or plagiarism (presenting the work of another as your own, or the use of another person’s ideas without giving proper credit) will result in a failing grade and sanctions by the University. For this class, all assignments are to be completed by the individual student unless otherwise specified. If you would like to include your assignment or any material you have submitted, or plan to submit for another class, please note that SJSU’s Academic Policy S07-2 requires approval of instructors.

Campus Policy in Compliance with the American Disabilities Act

If you need course adaptations or accommodations because of a disability, or if you need to make special arrangements in case the building must be evacuated, please make an appointment with me as soon as possible, or see me during office hours. Presidential Directive 97-03 requires that students with disabilities requesting accommodations must register with the Accessible Education Center at http://www.sjsu.edu/aec/ to establish a record of their disability.

Chemical Safety

Chem 120S is a required course for all Chemistry majors and minors and a prerequisite for all Chem 180/298 research.

Emergencies and Evacuations

If you hear a continuously sounding alarm, or are told to evacuate by Emergency Coordinators (colored badge identification), walk quickly to the nearest stairway (end of each hall). Take your personal belongings, as you may not be allowed to immediately return. Follow instructions of Emergency Coordinators. Be quiet so you can hear. Once outside, move away from the building. Do not return to the building unless the Police or Emergency Coordinators announce that you may.
Laboratory Safety

You should read the safety section of the SJSU Catalog under the Chemistry Department. Note in particular: “Failure to comply with proper procedures and prescribed safety cautions shall subject the student to disciplinary action. 1) Any student who engages in unauthorized experimentation or who seriously disregards safety, thereby endangering self or others shall be withdrawn immediately from the class with a grade of F. 2) Any student who shows persistent disregard for safety may have his/her grade lowered, and may risk being withdrawn with a final grade of F.”

**NOTE:** A safety quiz will be given during the second day of class and must be passed with a grade of 80% or better. The quiz will be based on the SJSU Chemistry Department Safety Rules found at the following website: http://www.sjsu.edu/chemistry/docs/Safety_Sheet_IIC.pdf. Signed Safety slips (found at the end of the PDF) shall be returned on the second day of class.

Student Technology Resources

Computer labs for student use are available in the Academic Success Center located on the 1st floor of Clark Hall and on the 2nd floor of the Student Union. Additional computer labs may be available in your department/college. Computers are also available in the Martin Luther King Library. A wide variety of audio-visual equipment is available for student checkout from Media Services located in IRC 112. These items include digital and VHS camcorders, VHS and Beta video players, 16 mm, slide, overhead, DVD, CD, and audiotape players, sound systems, wireless microphones, projection screens and monitors.

Peer Connections

Peer connections is located in Room 600 in the Student Services Center. It is designed to assist students in the development of their full academic potential and to motivate them to become self-directed learners. The center provides support services, such as skills assessment, individual or group tutorials, subject advising, learning assistance, summer academic preparation and basic skills development. The Peer Connections | San Jose State University is located at http://peerconnections.sjsu.edu.

SJSU Writing Center/Plagiarism

The SJSU Writing Center is located in Room 126 in Clark Hall. It is staffed by professional instructors and upper-division or graduate-level writing specialists from each of the seven SJSU colleges. Our writing specialists have met a rigorous GPA requirement, and they are well trained to assist all students at all levels within all disciplines to become better writers. The Writing Center is located at http://www.sjsu.edu/writingcenter/. See SJSU plagiarism policy, interactive tutorial and the relevant quiz (http://tutorials.sjlibrary.org/tutorial/plagiarism/index.htm). **NOTE:** No editing of assignments by others.
Course Outline
(schedule may deviate slightly as time dictates)

Important note: Many of the experiments in this lab will require you to work outside of the scheduled class period, so please plan ahead!!

Course Overview and Introduction - First Day of Class

- Course expectations
  - Lab Notebooks and Lab Research Paper Format
  - Mosquito Protease Project

Phase I - DNA Cloning of Mosquito Protease Genes

- Recombinant DNA technology
- Differences between expression vectors
  - Tags vs native proteins
- Primer design and Polymerase Chain Reaction (PCR)
- Restriction enzymes, Phosphatase, and DNA ligase
- DNA gel electrophoresis
- Transformation of vector and newly designed plasmids
- Plasmid/Vector DNA isolation
- DNA sequencing and analysis

Phase II - Mutagenesis of Mosquito Protease Genes

- Bacterial transformation of mosquito protease plasmid stock
  - Zymogen Forms of AaET, AaSPVI, AaSPVII, and AaLT
- QuikChange Mutagenesis Strategy for Arginine to Alanine mutation

Phase III - Bacterial Protein Expression

- Bacterial standard growth conditions
- Differences between commercially available bacterial competent cells
- Cell induction/lac operon system - IPTG vs. allolactose
- Protein gel electrophoresis
  - MW standards, MW and isoelectric point (pI) of mosquito proteases
- Soluble vs insoluble protein expression
Phase IV - Protein Purification

- Soluble or insoluble protein purification
  - His$_6$-tagged affinity purification
  - Buffers and solutions for Ni$^{2+}$ columns
  - Second or third-step ion exchange columns for further purification

Phase V - Auto-activation/Activity Assays of Mosquito Proteases

- Auto-activation buffer conditions
  - Zymogen form of proteases
- Trypsin and other protease substrates
  - Enzyme kinetics and inhibitor assays
    - Michaelis-Menten and Lineweaver-Burke (or Double Reciprocal) plots