

Chemistry 477/ 677

Preparation and Analysis of Proteins and Nucleic Acids

Instructor : [Mark Braiman](#)

Office: CST 3-006

Office hours: Th 12-2/By appointment

Lab: CST 0-024

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Class meeting days: M 2:15-3:15, W 12:45-4:45

Location: LSC 207, except as indicated below.

Prerequisites: CHE107 or CHE129 or BIO 306, CHE474 or BCM475 (or permission of instructor).

Textbook: No purchase required; this website has all required material

TAs : **Christopher Saez,**

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Main course readings are under "Lab Protocols etc". Additional readings are available under "Other Info and Resources" tab.

Course Calendar tool has due dates of labs & prelabs.

Grading policy: 20% in-lab performance and results; 50% written lab reports; 30% prelab questions. A course grade of "A" requires an average above 90.

Failure to complete and/or turn in a report for 3 or more labs will automatically result in a grade of F. 2 missing labs will result in a grade of D at best (or F if quality of remaining work is inadequate).

Date, Day	Planned Activities
Jan 18, M	<i>Martin Luther King Jr. Day--No class</i>
Jan 20, W	1. Class organization; (12:45-1:30); Prelab lecture for Labs 1 and 2 (1:30-2:30). Web-based tools for analyzing protein and DNA primary sequences. (2-4:45) Will meet in Chemistry Dept. Learning Center 2, LSC 215.
Jan 25, M	<u>2 A. Polymerase chain reaction (PCR) of a human gene from buccal saliva.</u>
Jan 27, W	2 B. Agarose gel electrophoretic analysis of PCR products.
Feb 1, M	Prelab 3 lecture/discussion.
Feb 3, W	3 A. Site-directed mutagenesis of proteorhodopsin . Preparation of mutant plasmid using thermal cycling mutagenesis kit.
Feb 8, M	3 B. Site-directed mutagenesis of proteorhodopsin. (cont.) <i>DpnI</i> restriction digest; agarose gel electrophoresis; and transformation of

	competent cells. You may come to LSC 207 on Thursday 2-2:30 or 4-5:45 p.m. to select colonies and begin 5-mL cultures
Feb. 10, W	3 C. Site-directed mutagenesis of proteorhodopsin. (cont.) Plasmid miniprep.
Feb 15, M	3 D. Site-directed mutagenesis of proteorhodopsin. (cont.) Transformation of competent <i>UT5600 E. coli</i> cells with mutated plasmid.
Feb 17, W	4 A. Expression of a site-directed mutant in <i>E. coli</i> . Prelab 4 lecture (optimization of strain and culture conditions). Start of slow (2-day) growth of <i>E. coli</i> colonies in 1-mL cultures.
Feb 22, M	4 B. Expression of a site-directed mutant in <i>E. coli</i> . Growth of 50-mL cultures; Induction with arabinose; addition of retinal.
Feb 24, W	4 C. Expression of a site-directed mutant in <i>E. coli</i> . Addition of lysis buffer to cells; Prelab 5 lecture.
Feb 29, M	4 D. Preparation of cleared lysate by centrifugation; spectrophotometric assay of pR . 5A. Proteorhodopsin purification Citrate additions
Mar 2, W	5 B. Proteorhodopsin purification (cont.). First citrate precipitation and re-solubilization: assay of purity using UV/visible spectrophotometer..
Mar 7, M	5B. Proteorhodopsin purification (cont.) Additional citrate precipitation(s) and re-solubilization. Assays of purity using UV/visible spectrophotometer.
Mar 9, W	5B. Proteorhodopsin purification (cont.) Additional citrate precipitation(s) and re-solubilization. Assays of purity using UV/visible spectrophotometer.
Mar 14 & 16	Spring Break; No class
Mar 21, M	5C. Nickel-NTA column for purifying 6x-His-tagged proteorhodopsin. Begin dialysis of eluted sample against imidazole-free buffer.
Mar 23, W	5C. Final precipitation and re-solubilization of purified pR . Final assays of purity using UV/visible spectrophotometer. Prelab 6 lecture, 3:45-4:45
Mar 28, M	6A . Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins . Casting the gel
Mar 30, W	6 B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins. Loading, running and fixing the gel
Apr 4, M	6 E. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins . Staining and destaining the gel
Apr 6, W	7A. Preparation of an RNA for NMR analysis. Transcription setups. 12:45-2:30. 2-D NMR spectroscopy in the lab -- an introduction (Prelab 7 Lecture/Discussion). LSC 215
Apr 11, M	7 B. Preparation of an RNA for NMR analysis. Pouring of PAGE gel. (Prelab Questions Due)
Apr 13, W	7 C. Analysis of RNA transcripts by urea-PAGE. .
Apr 18, M	8A. Growth of crystals of water-soluble and membrane proteins. Prelab 8 lecture.
Apr 20, W	8A. Growth of crystals of water-soluble and membrane proteins.

Apr 25, M	8A. (cont). Growth of crystals of water-soluble and membrane proteins.
Apr 27, W	(Tentative date; no lab report). Visit to Macromolecular crystallography facility at Cornell High-Energy Synchrotron Source (Mac-CHESS , Marian Szebenyi, contact. Click HERE for a map.
May 2, M	9A Characterization of proteorhodopsin mutants prepared in an earlier lab. pH titration with UV/vis measurements
May 4, W	Reading Day (Available as a makeup day for certain missed experiments).

Accommodations for Students with Disabilities. [Students who are in need of disability-related academic accommodations must register with the Office of Disability Services \(ODS\), 804 University Avenue, Room 309, 315-443-4498. Students with authorized disability-related accommodations should provide a current Accommodation Authorization Letter from ODS to the instructor and review those accommodations with the instructor. Accommodations, such as exam administration, are not provided retroactively; therefore, planning for accommodations as early as possible is necessary. For further information, see the ODS website, \[Office of Disability Services\]\(#\).](#)

Academic Integrity Policy: [The Syracuse University Academic Integrity Policy holds students accountable for the integrity of the work they submit. Students should be familiar with the Policy and know that it is their responsibility to learn about instructor and general academic expectations with regard to proper citation of sources in written work. The policy also governs the integrity of work submitted in exams and assignments as well as the veracity of signatures on attendance sheets and other verifications of participation in class activities. Serious sanctions can result from academic dishonesty of any sort.](#)

For more information and the complete policy, see <http://academicintegrity.syr.edu>

Faith-based Observances: [SU's religious observances policy, found at \[http://supolicies.syr.edu/emp_ben/religious_observance.htm\]\(http://supolicies.syr.edu/emp_ben/religious_observance.htm\), recognizes the diversity of faiths represented among the campus community and protects the rights of students, faculty, and staff to observe religious holy days according to their tradition. Under the policy, BCM/CHE 477/677 students will be provided an opportunity to make up any examination, study, or work requirements that may be missed due to a religious observance, provided they notify the instructor *by email before the end of the second week of classes \(i.e. by Jan. 29\)*. An online notification process is available through MySlice/Student Services/Enrollment/My Religious Observances from the first day of class until the end of the second week of class.](#)

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