

Chemistry 477/ 677

Preparation and Analysis of Proteins and Nucleic Acids

Class web page: <http://syllabus.syr.edu/CHE/mbraiman/CHE477/>

Class meeting days: M2:15-3:15, W 12:45-4:45

Location: LSC 207, except as indicated below.

Prerequisites: CHE107 or 129 AND CHE474 or BIO326 AND BIO575 (or permission of instructor).

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

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[Accommodations for Students with Disabilities](#)

[Additional readings](#).

[Schedule of due dates](#) for labs & prelabs.

Click [here](#) to submit comments anonymously.

Grading policy: 40% in-lab performance and results; 50% written lab reports; 10% 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 12, M	Class organization; Prelab lecture for Labs 1 and 2.
Jan 14, W	1. Web-based tools for analyzing protein and DNA primary sequences . Will meet in Chemistry Dept. Learning Center 2, LSC 215 .
Jan 19, M	<i>Martin Luther King Jr. Day--No class</i>
Jan 21, W	2A. Polymerase chain reaction (PCR) of a human gene from buccal saliva .
Jan 26, M	Prelab 3 lecture/discussion.
Jan 28, W	2B. Agarose gel electrophoretic analysis of PCR products .
Feb. 2, M	3A. Site-directed mutagenesis of proteorhodopsin . Preparation of mutant plasmid using thermal cycling mutagenesis kit.
Feb 4, W	3B. Site-directed mutagenesis of proteorhodopsin . (cont.) <i>DpnI</i> restriction digest; agarose gel electrophoresis; and transformation of competent cells. You may come to CST 0-028 on Thursday afternoon 1-5 p.m. to select colonies and begin 5-mL cultures
Feb 9, M	3C. Site-directed mutagenesis of proteorhodopsin . (cont.) Plasmid miniprep.
Feb 11, W	3D. Site-directed mutagenesis of proteorhodopsin . (cont.) Transformation of competent <i>UT5600 E. coli</i> cells with mutated plasmid.
Feb 16, M	4A. Expression of a site-directed mutant in E. coli . 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 18, W	4B. Expression of a site-directed mutant in E. coli . Growth of 50-mL cultures; Induction with arabinose; addition of retinal.
Feb 23, M	4C. Expression of a site-directed mutant in E. coli . Addition of lysis buffer to cells; Prelab 5 lecture.
Feb 25, W	4D. Preparation of cleared lysate by centrifugation; spectrophotometric assay of pR . 5A. Proteorhodopsin purification Citrate additions
Mar 2, M	5B. Proteorhodopsin purification (cont.) . Additional citrate precipitation(s); Assays of purity using UV/visible spectrophotometer..

Mar 4, W	5C. Nickel-NTA column for purifying 6x-His-tagged proteorhodopsin. Final assays of purity using UV/visible spectrophotometer. Prelab 6 lecture, 3:45-4:45
Mar 9 & 11	<i>Spring Break; No class</i>
Mar 16, M	6 A. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins , Gel casting only.
Mar 18, W	6B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins . Loading, running and fixing the gel.
Mar 23, M	6E. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins , Staining and destaining the gel (Casting gel makeup)
Mar 25, W	6B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins . Loading, running and fixing the gel (Makeup day)
Mar 30, M	6E. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins , Staining and destaining the gel
Apr 1, W	7A. 2-D NMR spectroscopy in the lab -- an introduction (Prelab 7 Lecture/Discussion)
Apr 6, M	7B. Preparation of an RNA for NMR analysis . Pouring of PAGE gel
Apr 8, W	7C. Preparation of an RNA for NMR analysis . Transcription setups. Prelab 8 lecture
Apr 10, M	Analysis of RNA transcript by urea-PAGE.
Apr 12, W	8B. (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)
Apr 17, M	<i>Mayfest; No class</i>
Apr 19, W	8A. Growth of crystals of water-soluble and membrane proteins .
Apr 24, M	9A. Characterization of proteorhodopsin mutants prepared in an earlier lab . pH titration with UV/vis measurements
Apr 26, W	9B. Characterization of proteorhodopsin mutants prepared in an earlier lab . pH titration with UV/vis measurements
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