Department of Chemistry at Lehman College City University of New York

Biochemistry CHE 447 (Biochemistry Laboratory) Syllabus, Spring 2019.

Instructor: Cristina C. Clement, Ph.D., Chemistry Department, Lehman College, CUNY; Cell: 347-243-9023.

Email: cclement_us@hotmail.com; cristina.clement@lehman.cuny.edu; ; clement.cristina624@gmail.com

Required textbooks:

Rodney Boyer (2012). *Biochemistry Laboratory: Modern Theory and Techniques; 2nd Edition.* Pearson, Prentice Hall, USA.

Other recommended textbooks:

Alexander J. Ninfa and David P. Ballou (2010). *Fundamental Laboratory Approaches for Biochemistry and Biotechnology; 2nd Edition.* John Wiley & Sons, Inc., USA.

Shawn O. Farell and Lynn E. Taylor (2006). *Experiments in Biochemistry: A Hands-On Approach; 2nd Edition.* THOMSON/Brooks/Cole, USA.

Handouts will be provided for each experiment with details for the experimental design and procedures. In addition, examples of previous lab reports from the students who passed Chem 447 will be posted on blackboard-on line.

CLASS MEETINGS

Tuesday and Thursday, 7:00 – 9:50 pm, Davis Hall room 026

COURSE OBJECTIVE

The primary objective of this course is for students to (1) learn fundamental approaches for experimentally investigating biochemical problems, (2) learn the theoretical foundations for the methods used, and (3) understand the applicability of the biochemical methods to realistic situations.

Topics covered in this course include methods for the isolation, UV-VIS of proteins and nucleic acids, purification, and characterization of proteins and nucleic acids, DNA recombinant technology,

characterization of enzyme kinetics; and manipulation of macromolecular structures from databases using contemporary visualization software.

Laboratory Hazards

Some of the chemicals used in this laboratory are harmful if inhaled or ingested.

 Always wear safety glasses in the Biochemistry Laboratory! Reading eye glasses no longer suffice as suitable safety protection for the eyes.

 Wear suitable clothing in the Biochemistry Laboratory. Sandals and shorts (unless covered by a lab coat) are not permitted in the lab.

 Wear latex gloves when working with dangerous biochemicals.

 Do not allow laboratory chemicals to enter your mouth or small cuts or scratches on your hands. Latex gloves are available for daily use to avoid this problem and to prohibit contamination of laboratory experiments.

 Do not inhale powders or vapors. This is especially important when working with sodium dodecyl sulfate (SDS) powder, concentrated acids/bases, and mixtures of acrylamide and bisacrylamide solutions.

 It is good practice to wash your hands carefully before leaving the laboratory.

Laboratory Notebook Maintenance

 All experimental data, except instrument output, should be recorded in indelible ink in a bound laboratory notebook with pre-printed sequential page numbers.
 Students should sign the notebook on the last page of that day's experiment.
 Do not leave blank pages in a laboratory notebook.
 A lab notebook should include protocols, identification of samples, observations, and data.
 Record data and observations as you obtain or make them. Do not write on scraps of paper with the intention of transferring information to the lab notebook later.
 Do not worry if your notebook is a little messy.
 The recording and organization of a permanent record of laboratory observations is as important a technique to master as any of the experimental methods you learn. The research notebook is a day-by-day record of the progress of experimental work. It should reflect the integrity and honesty of the experimenter as well as the clarity of his or her thought.

Cleanliness

It is important to maintain cleanliness in this laboratory. Even minor impurities on the glassware or on the pipette tip may ruin an otherwise well-done biochemical experiment. For example, using the same pipette tip to transfer two enzymes from their containers into your microcentrifuge tube will most surely contaminate the stock of the second enzyme with the first one and will likely ruin the results for the whole class. You will be working a lot with pipettes that use disposable tips. Discard the tip as soon as you do not need it. They are a lot cheaper than the chemicals that you are working with. Most used plasticware, such as microcentrifuge tubes or Falcon tubes (15 mL, 50 mL size) are 5 for one-time use. Empty all the tubes before discarding them (it's OK to leave less than 50 µL in microcentrifuge tubes). Discard broken glassware and used glass pipets into the red container. Do not discard functional glassware or any parts of the equipment used. If your glassware is visibly dirty, wash it with soap and hot water, otherwise rinse several times with distilled water from the tap, and place on the drying racks. **Do not leave any dishes in the sink**.

On average, the students will perform 2-3 experiments /week (6 hours lab work/week).
The following videos are required for the class:
http://www.bio-rad.com/webroot/web/html/lse/support/tutorial-bradford-assay-wndw.html
http://www.bio-rad.com/webroot/web/html/lse/support/tutorial_micropipet_wndw.html
http://www.bio-rad.com/webroot/web/html/lse/support/tutorial-titration-of-acids-and-bases-wndw.html
http://www.bio-rad.com/webroot/web/html/lse/support/tutorial-sds-page-of-fish-muscle-wndw.html
http://www.bio-rad.com/webroot/web/html/lse/support/tutorial-hic-chromatography-wndw.html

Key Topics and Experimental Work

Weeks 1-2: Check in and Chapters 1- 3&7 from Rodney Boyer's Biochemistry Laboratory.

Required reading: Chapters 1-3 & 7, **Rodney Boyer (2012).** ***Biochemistry Laboratory: Modern Theory and Techniques; 2nd Edition.***

Chapter 1: Introduction to the Biochemistry Laboratory: safety in the laboratory; keeping records and communicating experimental results; using biochemical reagents and solutions; quantitative transfer of liquids; statistical analysis of experimental data.

Chapter 2: Using the computer Internet for research in Biochemistry Lab: Web Sites useful in Biochemistry/Macromolecular structures (PDB and NDB, Expasy Molecular Biology/Proteomics tools/Metabolic pathways (KEGGS and Human Metabolome databases).

Additional databases of interest for the bioinformatics: ***Pharmit*** with ***ChEMBL/MolPort/ZINC*** **databases** (pharmit.csb.pitt.edu) and ***AutoDock Vina*** **molecular docking.**

Chapter3: General Laboratory procedures: pH, Buffers, amino acids titrations.

Experimental work: weeks 1-2

Experiment 1: Biochemical buffers, selection of a biochemical buffer. Preparation of buffers.

Students are required to prepare some biochemical buffers: such as 0.01M Tris-HCl, pH 7.4.

Experiment 2: Titrations of selected amino acids. In this experiment you will titrate 3 known amino acids: one neutral (such as alanine, valine, leucine or others), one acidic (glutamate or aspartate) and one basic (arginine or lysine). You will required to determine their pI, pK acid, pK amine and pK of the side chains and compare your values to the values reported in the literature.

Experiment 3: Identification of all 20 natural amino acids by thin layer chromatography (TLC) and reaction with ninhydrin.

Experimental work: weeks 3-4

Experiment 4: UV-VIS spectroscopy of proteins and nucleic acids.

I)Measurement of protein concentration in solutions using VIS absorption spectroscopy: Bradford and BCA assays (use BSA to construct calibration curve and interpolate the concentration of unknowns).

-Got Protein? Kit 1662900EDU: from Biorad is used for an independent Bradford assay.

II) Use the UV-VIS spectrophotometer Perkin Elmer to acquire the UV spectrum (from 200nm to 340 nm) of bovine serum albumin (BSA) and/or other proteins (lysozyme, carbonic anhydrase, peroxidase, beta-lactoglobulin and others).

1. Determine the protein concentration measurements from UV measurements and construct a calibration curve from the absorption at 280 nm of different known concentrations of BSA or other proteins and use the calibration curve to determine the unknowns concentrations.

2. Molar extinction coefficient determination for BSA from experimental UV measurements. Use the molar extinction coefficient to determine the concentration of an unknown sample of BSA and other proteins. Compare the molar extinction coefficients experimentally determined with the one determined from the amino acid sequence using the “Proteomics” server at the Expasy database.

II) UV spectroscopy of nucleic acids:

- Record the UV-scan of different nucleoside triphosphates (NTPs and dNTPs), nucleotide diphosphates (NDP), monophosphates (NMP) between 200nm-340 nm and use the Perkin Elmer software to assign the UV-maxima.

-Determination the molar extinction coefficients of different dNTPs/NTPs/NDP/NMP.

Weeks 5-6:

A) Purification and analysis of biomolecules by different chromatographic methods.

B) Characterization of proteins and nucleic acids by gel electrophoresis.

Theoretical background: Partition versus Adsorption Chromatography; Column Chromatography: The theory of electrophoresis; Methods and practical aspects of electrophoresis. Immuno-adsorption, immune-elctrophoresis and enzyme-linked immunoassays (ELISA).

Required reading: Chapters 5, 6 &9, **Rodney Boyer (2012).** ***Biochemistry Laboratory: Modern Theory and Techniques; 2nd Edition.***

Experimental work: Practical Aspects of Column Chromatography:

I) Gel-exclusion Chromatography:

1. Size Exclusion Chromatography **Kit 1660008EDU-Biorad**: first experiment.
2. Purification & Size Determination of Blue & Green Fluorescent Proteins by Gel-Filtration Chromatography, (Edvotek kit# 255 and handouts)-second experiment.

II) Ion Exchange chromatography (IEC):

Purification of the Restriction Enzyme *Eco* RI using ion exchange chromatography (Edvotek kit #302 and handouts)

II) SDS-PAGE electrophoresis for proteins identification.

SDS-PAGE analysis of the protein fractions from *EcoRI* enzyme experiment and from the green/blue/ fluorescence proteins purified by gel filtration. Protein staining in gels using Coomassie /Colloidal Blue and/or silver staining methods.

I**V) Agarose gel electrophoresis for nucleic acid identification.**

Analyze the activity of the purified EcoRI enzyme using agarose gel electrophoresis of lambda DNA digestion products (agarose gel electrophoresis and ethidium bromide/methylene blue staining of the nucleic acids bands).

Week 7: immunochemical technologies.

Practical aspects of immunochemical methods: Enzyme linked immunosorbent technologies (ELISA):

1. **ELISA Immuno Explorer™ Kit 1662400EDU: Biorad-1st experiment.**
2. **Quantitative ELISA. Cat. #278 Edvotek -2nd experiment.**

Weeks 8-10: Introduction to protein expression, purification and enzymology: Theoretical background: Theory of enzyme action, Michaelis-Menten kinetics, enzyme inhibition.

Required reading: Chapters 8, Rodney Boyer (2012). ***Biochemistry Laboratory: Modern Theory and Techniques; 2nd Edition and handouts.***

Experiment 1: Protein Expression and Purification Series – Hand-packed Purification Process #1665045EDU: Expression, purification and **enzyme kinetics of dihydrofolate reductase (DHFR).**

Experiment 2: Understanding catalytic activity of enzymes and optimum temperature/pH.

- ***2.1.*** **Biofuel Enzyme Kit, # 1665035EDU**
- ***2.2.*** **Recombinant beta-lactamase from MTB: enzyme kinetics and inhibition.**

(I) Comparative proteomics (I): Protein Profiling module: (Biorad kit 166-2700EDU).
-Protein extraction from muscle of different fishes.
-Electrophoresis: SDS-PAGE and agarose gel electrophoresis for profiling the proteins extracted from muscle.
-Gel scanning and bioinformatics introduction for the creation of cladograms.
(II) Comparative proteomics (II): WESTERN BLOT module (Biorad kit 166-2800EDU).
-Protein extraction from muscle.
-Electrophoresis: SDS-PAGE.
-Western blotting and immune-detection of the myosin light chains.

Weeks 11-12: Molecular Biology: Recombinant DNA technology

Theoretical background: Recombinant DNA technologies.

Required reading: Chapters 9&10, Rodney Boyer (2012). ***Biochemistry Laboratory: Modern Theory and Techniques; 2nd Edition.***

Experimental work:

1. Restriction Digestion and Analysis of Lambda DNA Kit **1660002EDU Biorad.**
2. Forensic DNA Fingerprinting Kit **1660007EDU**
3. Mitochondrial DNA Analysis Using PCR, Cat#332 Edvotek.

Week 13: Final review of all experiments, bioinformatics and check-out.

Methods of Evaluation:

Students are evaluated on their attendance, attitude, and participation in laboratory discussions, research and lab reports. Participation includes reading and evaluation of assigned literature, planning and execution of experiments, analysis of the resulting data, and preparation of oral or written reports.

Required presentations of reports

The purpose of the laboratory report is to communicate experimental work in writing. The educational goal is to help students learn and practice expressing their ideas and describing their work in a professional manner. The requirements for the structure of the laboratory report are similar to those for peer-reviewed scientific literature:

Students are required to present the reports as "*Journal of Biological Chemistry"*-type of written paper.

Requirements: CD/emailed/electronic back-up of all reports; Organic-chemistry notebooks like.

All reports will be sent to:

cclement_us@hotmail.com; cristina.clement@lehman.cuny.edu;
clement.cristina624@gmail.com

Paper Format:

Abstract: maximum 1 page

Introduction (1-2 pages): Methodology theory: physical-chemical background of the specific method you have used; use all hand-outs, other resources, books of analytical chemistry and biochemistry; use diagrams, pictures of the instrumental set-up, all chemical reactions described in detail, use the pictures and presentations from different companies which provided you the instrumentation for the specific method you described.

Materials and Methods (3-4 pages): present all details of calculation, mathematical treatment of data, the real experimental procedures you have used for that specific lab; use pictures, diagrams, mathematical formulas to show all the data (all calculations for concentrations, all solvents and buffers used in chromatography, all the details related to spectroscopic measurements, details of sample preparation and sample analysis by SDS-PAGE, agarose gel electrophoresis for nucleic acids, all details related to the elution methods used in ion exchange chromatography and gel-filtration, etc). Describe details of enzyme kinetics for each enzymatic assay, calculation of km, vmax, kcat and Ki (inhibitory constant).

Results (unlimited number of pages): Show all results as graphs, pictures, tables; all UV-scans, all extinction coefficient determination, all gel-photo documentations properly labeled, the chromatograms showing the A (595 nm) =f (fraction number), as described by each handout accompanying the experiment.

Discussions and Conclusions (unlimited number of pages): Discuss the results carefully, focusing on the analytical part of the method, explaining details of the findings related to molar extinction coefficients, assess the success of the chromatographic method by inspection of the SDS-PAGE gels and the output of the chromatograms; or in the case of the nucleic acids, describe the success of the restriction analysis using the required photo documentation of the agarose gel stained with ethidium bromide or methylene blue. Describe the type of inhibition you observed with provided compounds in the case of enzyme inhibition. Describe the advantages of the immunochemical methods vs classical methods for analyzing the proteins and protein-protein interactions. Describe the selectivity and the sensitivity of the new recombinant DNA technologies applied to the understanding of molecular pathology. Provide details of all the bioinformatics resources used from the available internet websites.

Grading

Your grade will be based on the number of points you earn out of 1000 total points. There are 10 lab write-ups worth 100 points each.

Grade Assignments:

100%-93% = A; 92%-88% =A-; 87%-83% = B+; 82-80% = B 79%-75% = B-; 74%-70% = C+; 69%-65% = C; 64%-60% =C-; 59%-55% = D; Below 55% = F