SYLLABUS (03-232)

Course Description: This course introduces the application of biochemistry to biotechnology with emphasis on the quantitative analysis and interpretation of the biochemical data and introduce principals that are essential to understand complex biological processes.

The course begins with a presentation of the functional properties of amino acids, nucleotides, lipids, and sugars. This is followed by a discussion of the structural and thermodynamic aspects of the organization of these molecules into higher-order structures, such as proteins, nucleic acids, and membranes. The kinetics and thermodynamics of protein-ligand interactions are discussed for non-cooperative, cooperative, and allosteric binding events. The use of mechanistic and kinetic information in enzyme characterization and drug discovery are discussed. Topics pertinent to biotechnology include: antibody production and use, energy production in biochemical systems, expression of recombinant proteins, and methods of protein purification and characterization. The course is an alternate to 03-231. (Spring: 9 units; 3 hrs. lec.)

Instructor: Dr. Gordon Rule rule@andrew.cmu.edu Office 3183 – Office hours are best by appointment.

Prerequisite, or co-requisite: 09-217 (Organic Chemistry I), 09-217 (Chem II) or 06-221 (Thermodynamics). To be successful the student should have a solid understanding of the major concepts from introductory chemistry. The requirement for organic chemistry (09-217) can be waived with additional readings, contact instructor.

Recommended Supplemental Text: Lehninger Principles of Biochemistry. Nelson & Cox. However, almost any introductory biochemistry text would be a useful reference text.

Course Materials: The OLI course page can be found at: *http://oli.web.cmu.edu* (link *via* Canvas). In addition to the on-line materials, additional materials will be distributed in lecture. The OLI course key is BC-F18.

Suggested reference text is: Lehninger: Principles of Biochemistry, Nelson & Cox. Freeman.

Other Course Resources: In-class and final exams, and solution keys, from previous years are available as a source of practice problems. It is best to work through these problems without consulting the solution. Also keep in mind that the course emphasis changes from year to year and more, or less, detail may be required in response to a similar question on this year's exams. Always consult current course materials to gauge the depth of knowledge required for this offering of the course.

Recitations are designed to provide additional practice in problem solving with guidance from the professor. Solutions to recitation problems are posted and can be consulted as an aid for the weekly problem sets. The exception are problems in recitation that are continued in the problem set.

Tips for success:

1. Do the OLI bonus quizzes, attend class, take notes, review your notes – do this for *every* class.
2. Attend recitation, this is a great opportunity to get one-on-one assistance in problem solving.
3. Do the homework – similar questions will appear on exams.
4. Spend extra time learning the course material from the first 5 weeks – this material is central to understanding the entire course.
5. Work ahead so that you don't get behind – the OLI material is always available.
6. Don't be shy – ask the instructor or teaching assistant for help, inside and outside of class.

Central Concepts for the Course:

1. Constituent parts -- Higher order structures (carbohydrates, proteins, lipids, nucleic acids)
2. Protein-ligand interactions:
    - Direct function (e.g. oxygen binding, immunoglobulins)
    - Regulation (Gene regulation)
    - Enzymology (Regulation of metabolism)
3. Regulation:
    - Energy production (glycolysis, TCA cycle, Oxidative Phosphorylation)
    - Energy consumption (biosynthetic pathways)
    - Template directed synthesis (DNA, RNA, protein)
4. Recombinant DNA methods

Course Goals: The overall goal of this course is for the student to gain a fundamental working knowledge of biochemistry and to appreciate the impact of this field on modern biotechnology.

Assessed Learning Objectives:

1. Know the structures and predict the chemical reactions of key functional groups in biological molecules such as amino acids, nucleotides, lipids (fatty acids, triglycerides, waxes, phospholipids), and carbohydrates (mono-, di-, polysaccharides).
   -identify hydrogen bond donors and acceptors, ionizable groups, non-polar groups
   -draw polypeptide of any given sequence
   -draw a phospholipid, wax, triglyceride
   -draw and name disaccharides/polysaccharides
   -draw a polynucleotide, distinguish purine from pyrimidine bases
2. Understand the importance of pH in biological processes
   -Formulate a buffer given the pH of the solution and a list of buffers with known pKa values.
   -Be able to predict how the electrostatic environment alters the pKa of functional groups.
3. Explain the basic relationship between thermodynamics and protein structure at the level of secondary and tertiary structure.
   -Predict how changes in sequence lead to changes in structure.
   -Obtain thermodynamic parameters (enthalpy and entropy) from thermal melting curves
   -Predict fraction unfolded from thermodynamic parameters.
4. Protein Structure Determination
   -Determine primary sequence using Edman degradation
   -Determine tertiary structure by fitting electron density maps
   -Determine quaternary structure using size exclusion chromatography and SDS-PAGE
5. Apply the principles of protein separation to design a multi-step purification scheme.
   -Describe principles of separation by solubility, ion exchange chromatography, size exclusion chromatography, affinity chromatography
   -Be able to use specific activity to optimize purification scheme.
6. Students should be able to explain the relationship between the structure of a protein-ligand complex and the binding affinity and be able to predict how changes in the protein or ligand affect affinity.
   -Identification of favorable and unfavorable interactions in protein-ligand complexes.
   -Analysis of equilibrium dialysis or spectrophotometric data to obtain fractional saturation
   -Use binding curve or Hill plot to obtain K_D
   -Use Hill plot to obtain Hill coefficient, interpret Hill coefficient in terms of molecular distribution of bound ligands.
7. Know the meaning of enzyme kinetic parameters (k_{cat}, K_m) and inhibition constants (K_I). Determine these parameters from steady-state enzyme data, and distinguish the type of inhibition (competitive, mixed type) from experimental data.
   -Understand importance of steady-state approximation in kinetics measurements
   -Convert experimental data to reaction velocities
   -Use velocity curve or double reciprocal plot to obtain kinetic parameters
   -Use double reciprocal plots to obtain binding constants of inhibitors
   -Redesign of inhibitors in response to changes in their binding site
8. Explain the role of functional groups in catalysis for a select group of enzymes. Effect of pH on kinetic parameters.
   -serine proteases, aspartyl proteases, dehydrogenase
9. Students should be able to apply the concept of allosteric effects to metabolic regulation.
   -Control of glucose metabolism by allosteric regulation by ATP, ADP, AMP, F26P
   -Control of glycogen metabolism by hormonal control (insulin, glucagon) of enzyme phosphorylation.
10. Explain emerging properties of biological membranes.
    - Distinguish between passive and active transport, symport and antiport.
    - Understand selectivity of ion channels, e.g. K channel.

-Calculate the energy stored in concentration gradients.

11. Students should be able to apply the thermodynamic principles of direct and indirect coupling to understand free-energy management in metabolic pathways.
    - Calculate Gibbs free energy to determine direction of reaction and maximum possible energy output.
12. Students should be able to describe the basic mechanisms of DNA replication, transcription, and translation and how changes in DNA/RNA sequences affect these processes.
    - Distinguish sequence specific from non-specific protein-nucleic acid interactions.
13. Students should be able to interpret DNA sequencing data and predict the effect of sequence changes on protein structure and function.
    - Convert contemporary Sanger sequencing data to amino acid sequence.
14. Students should be able to design appropriate expression vectors for the expression of recombinant proteins in bacteria under the control of *lac* or T7 expression systems.
    -Design of appropriate PCR primers to amplify DNA segments for insertion into expression vectors.

You will be assessed on exams as follows:

- Short answer type questions that will test you understanding of key concepts in the course.
- Quantitative calculations, for example on how to make a buffer.
- Drawing molecular structures, e.g. chemical structure of protein, nucleic acid.
- Analysis of data, usually graphically, e.g. ligand binding, enzyme kinetics.
- Molecular interaction & design; How to modify interacting species to enhance the level of interaction.

Grading:

[50% or 45% or 40%] Three in-class hour exams
[30% or 35% or 40%] Final Exam (comprehensive)
[10%] Graded quizzes on OLI
[10%] Problem sets
[100%] Total

I will calculate your final grade in three ways, giving different weights to the in-class exams and the final. *Tentative* letter grades are A >90, B>80, C>70, D>50, consistent performance during the course, or exemplary performance on the comprehensive final, can lead to an increase in your letter grade.

Exams: Three exams will be given during the semester. One-half of the grade from the lowest in-class hour exam will be dropped. For example, if the in-class exams are worth 45%, the two best in-class exams will each contribute 18% of your final grade while the worst in-class exam will contribute only 9% towards your final grade. A comprehensive final exam will be given during the traditional final exam period. Make-up exams will be allowed for legitimate reasons that are supported by appropriate documentation. Please schedule make-up exams before scheduled exam dates.

OLI Quizzes will comprise 10% of the final grade. A total of 10 quizzes will be used to determine your grade, the remaining quizzes will be dropped. You have two attempts at each quiz and your best attempt will be used. These are largely multiple-choice questions.

Problem Sets will constitute 10% of the final grade. Problem sets will be assigned on a weekly basis, with the exception of weeks that contain exams. Of the 10 to 12 problem sets in the course, the two with the lowest score will be dropped in calculating the final grade. Problem sets consist of quantitative problems, data analysis, or using computer graphics to view molecular structures to discern inter-molecular interactions. The problem sets are designed to give you practice for the exams. ***Late assignments may be accepted, however the request must be made before the due date.***

Bonus Quizzes can contribute up to 3% of your final grade. There is a OLI based bonus quiz associated with each lecture and a Canvas quiz associated with many recitation sessions. You must complete the lecture bonus quiz by 2 AM the day of lecture. The recitation bonus quizzes can only be completed in recitation.

Academic Integrity, as defined by the university, will be strictly enforced (see https://www.cmu.edu/policies/student-and-student-life/academic-integrity.html). Your submitted problem sets should be entirely your own work. Although you are encouraged to discuss general

approaches to problem solving with your peers, you should not work together when completing problem sets because this will likely lead to students having very similar answers. You should not consult solution keys to problem sets from previous years as these are not available and access to such keys would be unauthorized access to this material.

Electronic Equipment: You are free to use laptops in class, but note that several studies have clearly shown that hand-written notetaking is superior to taking notes on a computer.

Recording of Lectures: This is permitted if the recording will only be used by students within the course.

Regrading: Requests for regrading of problems sets need to be submitted within one week from when they were returned. The time limit for regrading in-class exams and the final is within one year from the end of the course. It is the student's responsibility to retain exams for re-grading purposes. All re-grading requests should be accompanied with a short note that describes the grading issue.

An Invitation to Students with Learning Disabilities: Carnegie Mellon University is committed to providing reasonable accommodations for all persons with disabilities. To access accommodation services you are expected to initiate the request and submit a Voluntary Disclosure of Disability Form to the office of Health & Wellness or CaPS-Q. In order to receive services/accommodations, verification of a disability is required as recommended in writing by a doctor, licensed psychologist or psycho-educational specialist.

If you have a disability and require accommodations, please contact Amie Rollins, Director of Health and Wellness at amier@andrew.cmu.edu or Dr. Salaha Khan, Psychologist Counseling and Psychological Services at salahak@qatar.cmu.edu. The office of Health & Wellness, CaPS-Q and Office of Disability Resources in Pittsburgh will review the information you provide. All information will be considered confidential and only released to appropriate persons on a need to know basis.

If you have an accommodations letter from the Disability Resources office, you are encouraged to discuss your accommodations and needs with Catherine Getchell <getchell@cmu.edu>, as early in the semester as possible. She will work with you to ensure that accommodations are provided as appropriate.

Once the accommodations have been approved, you will be issued a Summary of Accommodations Memorandum documenting the disability and describing the accommodation. You are responsible for providing the Memorandum to me after you receive it.

For more information on policies and procedures, please visit:
https://scotty.qatar.cmu.edu/qword/student-affairs/office-of-health-and-wellness/assistance-for-individuals-with-disabilities/

Please take care of yourself. *Do your best to maintain a healthy lifestyle this semester by eating well, exercising, getting enough sleep and taking some time to relax. This will help you achieve your goals and cope with stress. All of us benefit from support during times of struggle. You are not alone. Asking for support sooner rather than later is usually beneficial. There are many helpful resources available on campus and an important part of the college experience is learning how to ask for help:*

- Student Affairs 24/7 Emergency Line: +974 5554 7913
- Counseling and Psychological Services-Qatar (CaPS-Q): +974 4454 8525
- Health and Wellness Office: +974 4454 8680 or book an appointment via Health Connect
- Student Life Office: +974 4454 8545

Please feel comfortable approaching me in requesting extensions for problem sets and to adjust exam schedules depending on demands in your other courses or other issues in your life.

If you or anyone you know experiences any academic stress, difficult life events, or feelings like anxiety or depression, we strongly encourage you to seek support. Counseling and Psychological Services (CaPS-Q) is here to help: call 4454 8525 or make an appointment to see the counselor by emailing student-counselling@qatar.cmu.edu . Consider reaching out to a friend, faculty or family member you trust for help.

If you or someone you know is feeling suicidal or in danger of self-harm, call someone immediately, day or night at 5554 7913. If the situation is life threatening, please call 999

Tentative Lecture Schedule:

Lecture Number	Topic	
1	Review of chemical structure and bonding, functional groups	
2	Molecular forces, water structure, hydrogen bonding	
3	pH equilibrium and environmental effects on pKa	
4	Buffers	
5	Amino acids, structure and properties	
6	Primary structure of proteins	
7	Secondary structure	
8	Tertiary structure	
9	Thermodynamic stability of proteins	
10	Immunoglobulins and their uses	
11	Ligand binding – theory and measurement	
12	Oxygen transport and cooperative binding	
13	Allosteric effects	
14	Quantitative analysis of cooperative binding – Hill plot	
15	Introduction to enzymes	
16	Serine proteases	
17	Theory of steady-state enzyme kinetics	
18	Inhibitors: suicide, competitive, mix-type (un- and non-competitive)	
19	Analysis of inhibition – type of inhibitor and binding affinity	
20	Retroviruses (HIV) and drug design	
21	Protein purification	
22	Protein structure determination (Subunit composition, X-ray diffraction)	
23	Carbohydrates I	
24	Carbohydrates II	
25	Lipids	
26	Biological membranes	
27	Introduction to metabolism	
28	Biochemical energetics (group transfer, redox, Gibbs free energy)	
29	Glycolysis	
30	Gluconeogenesis, metabolic regulation	
31	Regulation of Glycogen/glucose metabolism	
32	Citric acid cycle	
33	Fatty acid oxidation, electron transport, ATP synthesis	
34	Nucleic acids	Capstone theme: How to express a mutant HIV protease in E. coli, determine its DNA sequence, and design modified inhibitors that are effective against the altered HIV protease.
35	DNA stability, protein-DNA interactions, polymerases	
36	Polymerase chain reaction (PCR)	
37	DNA sequencing, expression vectors	
38	DNA transcription, lac operon, tRNA & ribosome structure	
39	Translation, protein export, T7 expression system.	