CH 343: Experimental Biochemistry Laboratory
Spring 2013 Syllabus

GENERAL INFORMATION

Instructor: Dr. Kelly Sheppard
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Office Hours:

Lab Section: Tuesday 4-Hour Time Slot

Location: Dana Science Center XYZ

Course Description: A project driven laboratory course to provide advanced training in experimental biochemistry. The course focuses on the isolation, purification, manipulation, and characterization of biological macromolecules, in particular proteins, in an investigative context. Students will be asked to apply previously learned biochemical knowledge and skills to answer an open question in biochemistry while also learning new laboratory techniques. Emphasis is placed on the proper collection, interpretation, synthesis, and presentation of *in vitro* and *in silico* results.

Student Learning Goals: Biochemistry is an experimental science focused on understanding the chemistry of life, in particular the structure and function of biological macromolecules. Biochemists take a reductionist approach to study biological macromolecules, purifying them from the rest of the cellular content. A reductionist approach, while powerful, does have limitations. Biochemical results must be placed in the proper context (life). Biochemists thus rely on the disciplines of biology (*e.g.*, genetics, physiology, cell biology, botany, microbiology, molecular biology, ecology, and evolutionary biology) to properly critique, analyze, and discuss biochemical results. This course is designed to teach you in a hands-on manner how biochemists carryout their studies in a post-genomics era.

By the end of the semester, students should be able to:

1. Use available genomic databases and sequence data to develop a hypothesis as to the function of a biological macromolecule in an organism.
2. Read, understand, and critique the primary literature related to a biochemical topic discussed.
3. Place *in vitro* results into the proper cellular context.
4. Understand the reductionist approach of biochemistry, and its strengths and limitations.

5. Apply previous biochemical skill sets to learn and understand new laboratory techniques.
6. Think critically and creatively about biochemical topics.
7. Analyze and critique scientific data generated.
8. Interact effectively with others in a collaborative fashion.
9. Synthesize previous work found in the primary literature, including *in silico* and *in vivo* studies, with data generated to place the results in a broader context and propose future experiments.
10. Communicate effectively about biochemistry (both in oral and written formats).
11. Understand how advances in molecular biology revolutionized biochemistry especially in a post-genomic era.
12. Understand science is an investigative and iterative human process to better comprehend the natural world.
13. Take responsibility for learning and engaging in a scientific project.

Blackboard: Lab protocols, assignment guidelines, grading rubrics, and other helpful documents will be uploaded to Blackboard. http://learn.skidmore.edu

LABORATORY COURSE POLICIES

Attendance: Attendance at all laboratory sessions is mandatory. Attendance means arriving on time to section, prepared, dressed appropriately, and ready to carryout experiments in a safe manner. Labs that are missed cannot be made up. Unexcused absences from the laboratory section are grounds for failure. Absences that are a result of a medical or personal emergency will be accommodated at the discretion of your instructor.

Academic Honesty: To truly learn and develop your skills, you must complete your own work in an honest fashion. Accordingly in this course, you are bound by all the academic standards detailed in the Skidmore Honor Code. This includes but is not limited to weekly written assignments, laboratory reports, and data collection. Fabricating and/or altering data obtained in the laboratory is a serious violation not only to the Skidmore community but also the wider scientific community, and will not be tolerated. While experiments in the laboratory course are many times collaborative, the written assignments (*e.g.,* weekly assignments and lab reports) are not. Each student is thus responsible for producing his/her own original work in the course. All work must be properly cited. Students caught violating the Honor Code will receive a zero for the assignment and will be referred to the Office of Academic

Advising. I reserve the right to Fail any student caught violating the Honor Code.

Accommodations: If you are a student with a disability, please inform me and provide me your semester memo of accommodations as soon as you obtain it. If you think that you need accommodations but do not yet have them, please contact the Coordinator for Students with Disabilities in the Office of Student Academic Services. It is your responsibility to provide this document to me in a timely manner.

Lab equipment: Lab groups will be assigned a drawer in Dana 201 containing glass-ware and other equipment on the first day of lab. Said glass-ware and equipment in your assigned drawer will be checked again on the last day of lab. Additional equipment will be assigned as needed and will be returned at the end of that day's lab.

LABORATORY SAFETY

Safety Training: All students are required to attend and pass a mandatory safety training session conducted by Lorretta Greenholtz, Skidmore College Academic Safety Officer. Please refer to the *Skidmore College Laboratory Safety Guidelines for Students* as these safety regulations will be in effect for the course. In addition, students must complete the online Advanced Safety Training.

Safety Policy: Safety goggles and lab coats (available in the bookstore) must be worn at all times in the laboratory, unless otherwise instructed, along with closed toe shoes and long pants. All other safety instructions from your instructor must be followed. Eating and drinking in laboratory space is prohibited. In case of an accident or injury, no matter how minor it seems, please notify me immediately. Waste should be appropriately disposed of.

ASSIGNMENTS

Weekly Assignments (due at the start of lab the following week):

You will have weekly assignments in the course focused on getting you to think more deeply about your project and preparing you to write a laboratory report due at the end of the semester (see below). Feedback on the assignments should be incorporated in your final laboratory report. Listed below are the assignments for each week of the course. Details about each individual assignment will be discussed in class.

Week 1: Hypothesis regarding the discriminating nature of the bacterial AspRS you will be characterizing based on bacterial genomic content.
Week 2: Critical review of one of the research articles you have read related to the project (not including articles listed below).
Week 3: Write experimental procedures and results sections for weeks 1-3.
Week 4: Bibliography of articles read to date related to the project.

Week 5: Write experimental procedures and results sections for weeks 4 & 5.
Week 6: Critical review of one an additional research article you have read related to the project (not including articles listed below).
Week 7: Write experimental procedures and results sections for weeks 6 & 7.
Week 8: Critical review of one an additional research article you have read related to the project (not including articles listed below).
Week 9: Write experimental procedures, and results sections for weeks 8 & 9.
Week 10: Critical review of one an additional research article you have read related to the project (not including articles listed below).
Week 11: Write experimental procedures and results sections for weeks 10 & 11.
Week 12: Updated bibliography of articles read to date related to the project.
Week 13: Write experimental procedures and results sections for weeks 12 & 13.

Laboratory Report (due at the start of the final for the course):
A written laboratory report on your semester's work in this class will serve as the take home portion of the final in this course and will be due at the start of designated final exam slot for the course. The laboratory report serves as an evaluation of your understanding of the project, your ability to synthesize your results into a broader context, your ability to think critically and creatively about your project and future experiments, and ability to communicate biochemistry in a written form. The report should be written in the format of a manuscript submitted to the *Journal of Biological Chemistry* with a title, abstract, introduction, experimental procedures, results, discussion, acknowledgements, references, and if appropriate supplemental information. Please refer to the laboratory report guidelines and laboratory report grading rubric for more specifics.

Seminar Presentation (during the final for the course):
A seminar presentation with your lab partner will serve as the in-class portion of the final in this course. Presentations will be during the designated final exam slot for the class. The seminar presentation serves as an evaluation of your ability to present your research orally in a collaborative fashion as well as an additional assessment of your understanding of the project, and ability to think critically and creatively about it. Please refer to the seminar presentation guidelines and presentation grading rubric for more specifics.

GRADING
Your evaluation in this course will consist of 13 weekly assignments, a laboratory report, a seminar presentation, and assessment of your laboratory notebook as well as your laboratory technique/participation.

Component	Percent of final grade
Weekly assignments (13 total)	2.5 % each, 32.5 % overall
Notebook	8.0 % overall
Laboratory technique/participation	5.5 % overall
Laboratory report	27.0 % overall
Presentation	27.0 % overall

READING ASSIGNMENTS

The laboratory protocols for this course will be posted on Blackboard. You are expected to read and think critically about the protocols before coming to class. In addition, you are expected to read research and review articles related to the project. A list of suggested readings is detailed below. You are expected to find additional articles to read. Pubmed (http://www.pubmed.gov) is a good place to search for biochemical articles.

Suggested articles:

1. Ibba, M., Becker, H.D., Stathopoulos, C., Tumbula, D.L. and Söll, D. (2000) The adaptor hypothesis revisited. *Trends in Biochem. Sci.*, **25**, 311-316.

2. Sheppard, K., Yuan, J., Hohn, M.J., Jester, B., Devine, K.M. and Söll, D. (2008) From one amino acid to another: tRNA-dependent amino acid biosynthesis. *Nucleic Acids Res.*, **36**, 1813-1825.

3. Becker, H.D. and Kern, D. (1998) *Thermus thermophilus*: a link in evolution of the tRNA-dependent amino acid amidation pathways. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 12832-12837.

4. Becker, H.D., Reinbolt, J., Kreutzer, R., Giegé, R. and Kern, D. (1997) Existence of two distinct aspartyl-tRNA synthetases in *Thermus thermophilus*. Structural and biochemical properties of the two enzymes. *Biochemistry*, **36**, 8785-8797.

5. Min, B., Pelaschier, J.T., Graham, D.E., Tumbula-Hansen, D. and Söll, D. (2002) Transfer RNA-dependent amino acid biosynthesis: an essential route to asparagine formation. *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 2678-2683.

6. Saad, N.Y., Schiel, B., Braye, M., Heap, J.T., Minton, N.P., Durre, P., and Becker, H.D. (2012) Riboswitch (T-Box)-mediated control of tRNA-dependent amidation in *Clostridium acetobutylicum* rationalizes gene and pathway redundancy for asparagine and asparaginyl-$tRNA^{Asn}$ synthesis. *J. Biol. Chem.*, doi:10.1074/jbc.M1111.332304.

7. Sheppard, K., Akochy, P.M., Salazar, J.C. and Söll, D. (2007) The *Helicobacter pylori* amidotransferase GatCAB is equally efficient in glutamine-dependent transamidation of Asp-$tRNA^{Asn}$ and Glu-$tRNA^{Gln}$. *J. Biol. Chem.*, **282**, 11866-11873.

8. Bailly, M., Blaise, M., Lorber, B., Becker, H.D. and Kern, D. (2007) The transamidosome: a dynamic ribonucleoprotein particle dedicated to prokaryotic tRNA-dependent asparagine biosynthesis. *Molecular Cell*, **28**, 228-239.

9. Blaise, M., Bailly, M., Frechin, M., Behrens, M.A., Fischer, F., Oliveira, C.L., Becker, H.D., Pedersen, J.S., Thirup, S. and Kern, D. (2010) Crystal structure of a transfer-ribonucleoprotein particle that promotes asparagine formation. *EMBO J.*, **29**, 3118-3129.

Reference Textbook: *Fundamentals of Biochemistry*, 4th ed., ISBN # 978-0-470-54784-7
Voet, Voet and Pratt; John Wiley and Sons, Inc. 2012.

LABORATORY SCHEDULE

Week	Week of	
1	01/21	Overview of course and project, sequence & genomic database search, primer design to amplify *aspS* gene, PCR of tRNA gene using stock M13 primers
2	01/28	PCR of *aspS* gene, *in vitro* transcription of tRNA, literature search (AspRS)
3	02/04	PAGE of *in vitro* transcription reactions, agarose gel electrophoresis of PCR, digestion of PCR and pET28a vector (if no PCR product, redo PCR – may have to do outside of scheduled lab time)
4	02/11	Gel electrophoresis of digested DNA samples, ligation of *aspS* into pET28a, transformation into *E. coli* DH5alpha strain, additional literature search (organism)
5	02/18	Plasmid isolation and digestion (send for sequencing), new round of *in vitro* transcription, media preparation
6	02/25	Check sequence against database, transformation of plasmid into *E. coli* BL21 strain for protein overproduction, tRNA purification
7	03/04	Protein overproduction and purification, discussion of project
	03/11	Spring Break
8	03/18	SDS-PAGE and Western Blot, buffer and solution preparation
9	03/25	Activity assays
10	04/01	Kinetic or gel shift studies I (half the lab kinetic studies, other half gel shift)
11	04/08	Kinetic or gel studies II (continuation from week 10)
12	04/15	Gel shift or kinetic studies I (half the lab gel shift studies, other half kinetics)
13	04/22	Gel shift or kinetic studies II (continuation from week 12)
14	04/29	Loose ends, clean-up, discussion of project
Final	TBD	Presentations