CHEMICAL BIOLOGY LABORATORY:

FLUORESCENT BIOMOLECULE DESIGN AND APPLICATIONS

UNIVERSITY OF NEBRASKA - LINCOLN

CHEM437/837 SPRING 2023

CLASS MEETS: WF 12:30 PM – 3:20 PM HAH 305

INSTRUCTOR

Dr. Catherine Eichhorn
Assistant Professor of Chemistry

HOW TO REACH DR. EICHHORN

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Student drop-in hours: T 11-12 noon HAH 723
or by appointment

TA

Vishakha Jayasekera
Graduate Student

HOW TO REACH VISHAKHA

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COURSE DESCRIPTION

This course integrates chemical synthesis, biomolecule structure and ligand recognition, and biological applications to advance understanding of the rational design of fluorescent biomolecules. In this course, you will be performing novel research and forming your own hypotheses. Like research in the real world, experiments may fail and/or you may have negative results – that is ok! Your work in this class will lead to generating new knowledge in the areas of fluorogen synthesis, RNA aptamer design, RNA-small molecule interactions, and more. Your contributions may result in authorship in peer-reviewed publications.

LEARNING OBJECTIVES

01 To develop skills in hypothesis generation and experimental design
02 To expand skills in organic synthesis and characterization
03 To synthesize DNA templates and RNA samples
04 To evaluate RNA-ligand binding using high-throughput assays

DETAILED COURSE INFORMATION IN CANVAS UNDER CLASS ESSENTIALS

REQUIRED RESOURCES

1. Lab manual (electronic google document)

2. Truong L and Ferre-D'Amare AR (2019) From fluorescent proteins to fluorogenic RNAs: Tools for imaging cellular macromolecules. *Protein Sci*, **28**, 1374-1386.

3. Chen X, Zhang D, Su N, Bao B, Xie X, Zuo F, Yang L, Wang H, Jiang L, Lin Q, Fang,M, Li N, Hua X, Chen Z, Bao C, Xu J, Du W, Zhang L, Zhao Y, Zhu L, Loscalzo J and Yang Y (2019) Visualizing RNA dynamics in live cells with bright and stable fluorescent RNAs. *Nat Biotechnol*, **37**, 1287-1293.

4. Huang K, Chen X, Li C, Song Q, Li H, Zhu L, Yang Y, Ren A. (2021) Structure-based investigation of fluorogenic Pepper aptamer. *Nat Chem Biol*, **17**, 1289-1295.

COURSE POLICIES

ACCESSIBILITY

If you need any accessibility-based accommodations, please let me know so appropriate arrangements can be made. See the University Policy page on Canvas under Class Essentials for more information. If you have trouble acquiring the resources needed for the class, especially textbook and computer, please let me know immediately so accommodations can be made. The UNL Library has laptops to check out (https://libraries.unl.edu/laptops).

ACADEMIC INTEGRITY

Your intellectual growth depends on responsibility, honesty, and doing your own work. Presenting the work of others as your own by taking ideas from others (plagiarism) or copying other's work is dishonest, hurts your reputation and credibility, and will result in a failing grade on the assignment and potentially disciplinary action. See the University Policy page on Canvas under Class Essentials for more information.

HEALTH AND WELL-BEING

These are not normal times. Please be kind to yourself, and to others. If you are struggling, please reach out for help. See the University Policy on Counseling and psychological services for more information. *Face coverings:* If you have a documented medical need for face coverings in class, please notify me immediately so accommodations can be made.

ATTENDANCE

If you do not feel well **DO NOT** come to lab. Notify me if you are sick and unable to come to lab to arrange for a make-up lab. Students who are sick or who are engaging in self-quarantine in accordance with guidance from the Lincoln-Lancaster County Health Department or their health care professional should not physically attend in-person classes.

DUE DATES

Life happens! Assignment due dates are designed to help you progress through the course while engaging in deep learning. ***Deadline extensions may be permitted on a case-by-case basis.*** Please contact the instructor as soon as possible if you anticipate missing critical deadlines.
Late policy: Assignments turned in one week late will have a 10% penalty, two weeks late will have a 20% penalty. Exceptions to this policy may be granted on a case by case basis.

INSTRUCTIONAL CONTINUITY

If in-person classes are canceled, you will be notified of the instructional continuity plan for this class by Canvas announcement and/or email.

ASSIGNMENTS & GRADING

LAB NOTEBOOK — 100 POINTS

It is essential to document a complete recording of all experimental conditions and observations. At the end of each class period your lab notebook will be submitted for grading. Grading will be based on legibility, complete documentation of procedures (and deviations), documentation of observations, and inclusion of data and analysis (e.g. reporting yield, NMR spectrum, picture of gel)

PRE-LAB/POST-LAB — 200 POINTS

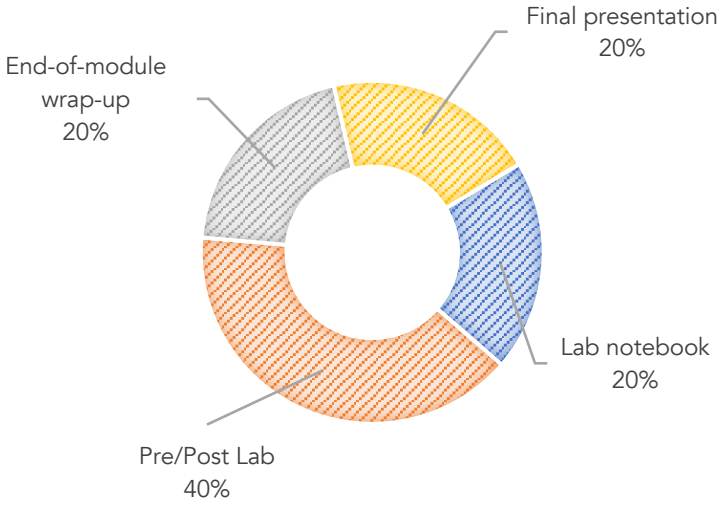
Several experiments require a pre-lab assignment to be completed and submitted through Canvas. These assignments are designed to prompt you to read through the procedure, understand the purpose and expected outcomes of the experiment, and form hypotheses. Post-lab assignments will be turning in data (e.g. NMR spectrum, spreadsheet of data points).

END-OF-MODULE WRAP-UP — 100 POINTS

At the end of each module, you will give a short (5-10 minute) presentation summarizing your data and results. Grading will be based on demonstrating experimental understanding, data interpretation, organization, and participation in group discussion.

FINAL PRESENTATION — 100 POINTS
REQUIRED FOR 837 | EXTRA CREDIT FOR 437

At the end of the semester, you will give a presentation that is a combination summary of results and proposal. This presentation will combine your end-of-module summaries and ask you to propose new experiments to move the project forward.



POINTS	PERCENT	GRADE
485-500	97.0+	A+
465-484	93.0+	A
450-464	90.0+	A-
435-449	87.0+	B+
415-434	83.0+	B
400-414	80.0+	B-
385-399	77.0+	C+
365-384	73.0+	C
350-364	70.0+	C-
335-349	67.0+	D+
315-334	63.0+	D
300-314	60.0+	D-
<300	<60.0	F

LET ME KNOW IF YOU HAVE ANY QUESTIONS!

COURSE SCHEDULE

JAN 25
Introduction and orientation

MODULE 1

JAN 27
Lab 1: RNA folding and structure

FEB 1
Lab 2: Pymol tutorial, RNA-ligand interactions

FEB 3
Lab 3: RNA construct design

FEB 8
Module 1 wrap up discussion

MODULE 2

FEB 10
Lab 4: HBC synthesis

FEB 15
Lab 5: HBC purification

FEB 17
Lab 6: HBC purification part 2

FEB 22
Lab 7: HBC characterization: NMR

FEB 24
Lab 8: HBC characterization: MS

MAR 1
Lab 9: HBC characterization: HPLC

MAR 3
Module 2 wrap up discussion

MODULE 3

MAR 8
Lab 10: Micropipettes and calibration

MAR 10
Lab 11: Polymerase Chain Reaction

MAR 13-20
SPRING BREAK, NO CLASS

MAR 22
Lab 12: PCR purification

MAR 24
Lab 13: RNA transcription

MAR 29
Lab 14: RNA analysis: denaturing PAGE

MAR 31
Lab 15: RNA purification

MODULE 3

APR 5
Lab 16: RNA characterization: native PAGE

APR 7
Module 3 wrap-up discussion

MODULE 4

APR 12
Lab 17: Intro to high-throughput experiments

APR 14
Lab 18: Control plate reader assay part 1: cations

APR 19
Lab 19: Data fitting and programming basics

APR 21
Data fitting and programming basics

APR 26
Lab 20: Control plate reader assay part 2: ligand

APR 28
Lab 21: Sample plate reader assay part 3: cations

MAY 3
Lab 22: Sample plate reader assay part 4: ligand

MAY 5
Module 4 wrap-up discussion

MAY 10
Student presentations

MAY 12
Student presentations

LAST CLASS