Biological Chemistry 395: Chemistry & Biology of Nucleic Acids (Lab)

Lab Overview:

This lab is set up as a series of related projects designed to expose you to chemical and biological techniques used in the design, analysis and application of nucleic-acid based sensors. Each project is sub-divided into parts by technique, and within each technique you will have some latitude to explore areas of particular interest. You will work in groups of two, and the first lecture/lab will largely be spent researching and picking micro-RNA targets of interest, and designing simple probes that you will purify, characterize, and use throughout the semester. The lab is divided into three, unequal, portions.

The first part of the lab will focus on the chemistry of synthetic nucleic acids, and chemical techniques for analysis. These techniques will be introduced through the design, purification, characterization and testing of a probe for detecting a specific micro-RNA. This part of the lab will last for the first 8 weeks of the semester (until Fall Break), and consist mainly of spectroscopic techniques. By the end of this portion of the lab, you will have a good idea of the effectiveness and specificity of your designed miRNA sensor.

The second part of the lab will focus on live cell techniques. It will include basic human tissue culture as an introduction, followed by transfection of your probes and microscopy-based analysis of their *in vitro* effectiveness. It will culminate in the harvesting of total cellular RNA just before Thanksgiving break.

The final part of the lab will be individual projects to analyze the total cellular RNA by a variety of available techniques. Each group will focus on comparisons between the earlier *in vitro* effectiveness of their sensors, and some *ex vivo* test of the utility.

Lab Expectations & Grading:

For the lab to run smoothly and safely, it is extremely important that everyone come to lab prepared and having read and studied any necessary background material. The lab will make up 25% of your course grade, broken up as described below.

Everyone will be required to take (and pass) the "Training for Investigators, Staff and Students Handling Biohazards" course via the CITI Program (https://www.citiprogram.org). Instructions for registering can be found here:

https://support.citiprogram.org/customer/portal/articles/163300-how-do-i-enroll-in-a-citi-course-for-the-first-time-

This course will introduce you to the basics of Biosafety and dealing with Biological Hazards, and will be supplemented by in-lab training as we encounter specific hazards over the course of projects.

Lab Notebooks (5%)

Keeping a thorough and well-organized lab notebook is a fundamental skill in the bench sciences, and is one of the most important underpinnings of good research. We will be using electronic lab notebooks through LabArchives to facilitate course-wide access to each other's findings. Each group will be working with different targets in parallel, and it will be useful for you to be able to see the results of other probe/target combinations as the course progresses. The lab manual includes a discussion of proper lab-notebook habits, and you will be graded on accuracy of your record keeping, clarity of your notes/data, and organization. Your lab notebook should be something that you use to look back on data, but is also easily accessible to another student or researcher.

You will be expected to keep your lab notebook up to date- summaries of the experiments before lab, results during lab, and any notes or discussion that come up after lab, including analysis of data.

Your lab notebook grade will also incorporate your preparedness for lab, as well as your degree of participation in your group projects and any issues with safety or lab hygiene during the semester.

Summary Reports (10%)

You will each write summary reports at the end of parts 1 & 2 in the format of a JACS Communication. It should succinctly outline the purpose of the project, your experimental design, and your findings. The summary reports will be due two weeks after the end of the project: November 4th for Report 1, and December 9th for Report 2. I will be happy to read and provide feedback on your drafts if they are given to me at least 1 week before the due date.

Final Presentation (5%)

Following your final group projects, we will meet during Finals Week for each group to give a short (20-25 minute) presentation on what you chose for your *ex vivo* RNA analysis, and what you found. These talks will take the format of a traditional oral conference presentation, with time for questions after.

Tentative Fall 2016 Lab Schedule

Week	Lab Dates	Lab Projects	Assignments
1	26-Aug	Introduction, Target Selection	
2	2-Sep	Buffer Preparation Experimental Design	
3	9-Sep	Purification, Concentration Determination Sample Preparation	
4	16-Sep	Melting Curves	
5	23-Sep	Melting Curves	
6	30-Sep	Fluorescence Titrations	
7	7-Oct	Fluorescence Titrations	
8	14-Oct	Wrap-Up, Discussion of Probe Effectiveness	
9	21-Oct	Fall Break	Fall Break
10	28-Oct	Introduction to Cell Culture Techniques	
11	4-Nov	Transfections/Imaging	Summary Report 1
12	11-Nov	Transfections/Imaging	
13	18-Nov	RNA Extraction	
14	25-Nov	Thanksgiving Break	Thanksgiving Break
15	2-Dec	Ex-Vivo Analysis of RNA Group Projects	
16	9-Dec	Ex-Vivo Analysis of RNA Group Projects	Summary Report 2