Chemistry 139/422/622 Spring 2015
Honors General Chemistry and Advanced Inorganic Laboratory
Section 1: T 2:00 – 5:00pm, Section 2: W 2:15-5:15pm

Laboratory: **101 Life Science Complex (LSC)**

Instructors

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General: For the Tuesday sessions, section 1 will be held on Tuesday January 13th at **2:00 pm**. For the Wednesday session (section 2), it will be January 14th at **2:15 pm**. (PLEASE NOTE THE EXACT START TIME). No laboratory manual needs to be purchased, but everyone will need a laboratory notebook. The laboratory notebook must be bound but does not need to provide duplicate copies. It can be purchased at the bookstore or Folletts. Lab Coats, gloves and Lab glasses will be provided.

Grading:

Chem 139/Chem 422/622	
Work Plan	20%
Final Report	20%
Lab Technique/Effort	20%
Notebook	20%
Final Presentation	20%
	100%

We will be forming groups (based on class make-up) consisting of 2-4 students who will be working together throughout the semester. Each group will be working on individual laboratory project. Project outlines and ideas will be provided and then the class will begin with literature research, where research teams need to find the information necessary to conduct "their research project". Each group will work out a plan how to approach this project and conduct the research. This proposal will be shared with the instructors. The instructors will discuss the proposal with each group, making suggestions for improvement. Research teams will submit a revised version of

their research plan. The finalized research plan will again be discussed with the instructors, before the laboratory work can begin.

The teaching assistants and instructor will discuss the research goals and strategy with each group, suggesting changes if necessary, and providing help where necessary. When research proposal is written and approved each group will carry out research in the lab using a variety of laboratory techniques, usually not accessible to undergraduate students. We have six projects this year, four of them involve making short 7-residue peptides and two other projects are centered around proteins. All groups working with proteins will learn how to conduct polymerase chain reaction, site directed mutagenesis, protein expression and purification. Peptide projects will teach solid-state synthesis, MALDI-TOF, HPLC purification. We will also utilize various spectroscopy methods, e.g., NMR, IR, UV-Vis spectroscopy, CD, ICP and learn how to use plate reader with fluorescence and absorbance readout.

Each student is required to keep a laboratory notebook. A laboratory manual is not necessary. At the end of the semester each group will write a report about their findings and also present their results in form of a short seminar talk to the instructors, teaching assistants and fellow students.

Schedule:

Date	What?	Where?
January 13/14	General intro, lab equipment, safety, library introduction, lab notebooks, groups will be formed, projects will be given out.	LSB 102
January 20/21	literature search (bring a laptop if you have one)	LSB 101
January 27/28	1st version proposal due to instructors. Discussion with instructors about possible improvements.	LSB 101

Note: between Jan 20/21 and January 27/28 groups must meet themselves to improve proposal

February 3/4	Revised version of proposal will be discussed with instructors, necessary chemicals and glassware will be ordered; lab intro Please send your proposals by email to Olga a day before your class.	LSB 101
February 10/11	Lab work begins on individual projects	CST 2-042/2-026
February 17/18	Lab work	CST 2-042/2-026
February 24/25	Lab work	CST 2-042/2-026
March 3/4	Lab work	CST 2-042/2-026
March 10/11	*Spring break- no labs*	
March 17/18	Lab work	CST 2-042/2-026
March 24/25	Lab work	CST 2-042/2-026
March31/April1	Lab work	CST 2-042/2-026
April 7/8	Lab work	CST 2-042/2-026
April 14/15	Lab work + interpret data and tidy-up lab	CST 2-042/2-026
April 21/22	Groups meet with instructors to finalize talk and paper	CST 2-042/2-026
April 28th Section 1	Final Talks : Presentation, 2.00 pm Room to be announced	
April 29th Section 2	Final Talks : Presentations, 2.15 pm Room to be an announced.	

The Work Plan

Each group is required to submit a work plan. The work plan will mainly consist of the strategy research teams are planning to use for the successful completion of their research projects.

During the first class session groups will be formed, each group will be given a research project. There will be also a short intro into lab safety and other general procedures.

The second lab session will be held in the lab as well but will involve literature searching. The instructors will help you and make sure you will be leaving with all the necessary materials. You and your group should then meet and assemble the work plan.

Before you can develop a work plan you must sit down and read and understand all pertinent literature. If you know the background, understand how related proteins/peptides were prepared and purified, which properties related molecules had, which pitfalls were involved and which safety precautions were undertaken you will be ready to write your work plan.

The work plan consists of two main parts: First you should write an **introduction,** which should include a brief review of known literature, and a statement how the known literature relates to your project. In the **main part** you should explain how you want to approach your research project. This part is divided in three segments: 1) preparation of peptides/proteins, 2) purification, 3) characterization. Your work plan concludes with a listing of all literature used to assemble the work plan.

1) Preparation of peptides/proteins: Which reagents and solvents are used (make a list), how can they be obtained. Are any of the chemicals dangerous and do you need any special safety precautions? Your work plan should address these points, and you should describe in detail the procedures you are planning to perform. For example if you are doing peptide synthesis make a scheme explaining synthesis, which protecting groups you would be using, how long coupling time would be and at what temperature you are planning to conduct synthesis. If you are doing protein expression, suggest what cell line you would be using and what temperature you would be growing your culture at. For both types of projects suggest how you would estimate purity of your product. This information is available from the literature published on related systems and teaching assistants will help you to decide which approach to choose.

2) Purification: Peptides will be purified using HPLC; specify what gradient you are going to use, what kind of column, make a list of solvents you will need. Proteins will be purified using affinity chromatography and anion chromatography. In case you are preparing apo-protein (no metal bound) you might need to use a chelator during purification to ensure no metal remains bound to the protein.

3) Characterization: This is the most interesting and most challenging part of your project because you will be doing work no one tried before! If you are working with hydrogels explain how you are going to trigger gel formation and how to measure extend of gelation. What problems you may encounter and how to troubleshoot them? If you are studying metallo-fibril catalysis explain how you will assemble fibrils and what concentrations of fibrils and substrate you would use, in what buffer, at what pH and temperature. Here make a list of substrates you would need. For protein project describe how you would study metal binding.

Your work plan will be discussed with your instructors, who will make suggestions on how to improve your plan. After revisions have been included in your plan, you will sit down for another discussion of your plan with your instructors, who make sure all necessary steps have been considered. The instructors will then order all necessary chemicals and equipment with you.

Safety in the Chemical Laboratory -
Accidents DO happen.....

Safety in the laboratory evolves around knowledge and prevention. Knowledge not only includes an understanding of the nature of the chemicals being used and the experiment performed, but it also involves an understanding of how to deal with emergencies. Prevention involves safe practices and above all the use of common sense.

1) Familiarize yourself with the hazards associated with the chemicals being used in the experiment. Do not rely on labels alone. Familiarize yourself with the Materials Data Safety Sheets (MSDS). Know how to use common reference manuals. Know the type and extend of the hazard associated with the material. Merely being afraid of chemicals does not maximize safety. A folder containing relevant MSDS sheets will be available in the laboratory. Also MSDS sheets are readily available on the Internet.

2) Learn to recognize the principles of safe operating condition. For example, high-pressure cylinders should be clamped or otherwise secured. Open flames should not be used to heat flammable liquids.

3) Practice personal safety. Always wear eye protection in the laboratory. Do not wear contact lenses. Eye injuries are extremely serious, but they can be avoided if you keep your goggles on **ALL** the times. Wear adequate shoes, not sandals or slippers. Wear appropriate clothing, no skirts or shorts. Tie long hair back. Lab coats are desirable and are recommended and will be provided in the lab. If you want to buy your own lab coat you can do so in the Medical School Bookstore, which is located in the basement of the building on the corner of Harrison and Irving Ave.

4) Common sense prohibits horse-play or practical jokes. Working alone is not permitted. Common sense says that one should not eat, drink or smoke in the laboratory. Common sense does include learning the location of the safety equipment such as fire extinguishers, eye-wash fountains, fire blankets, and emergency showers. Common sense says that one should know how to use these items. Common sense further says that one should not be afraid or embarrassed to ask how to use these safety items. Common sense also includes the necessity for prior orientation and reading before initiating a laboratory experiment. If you have gloves on do not touch your cell phone, books and personal belongings to avoid contamination.

5) Do not rush, do not take shortcuts. If you rush your work at best you will get poor results. At worst, you will be dangerous to yourself and those around you.

6) One should know some general rules for emergencies. The first thought in all chemical spills or splashes is to wash with lots of water. Acid spills should be neutralized with bicarbonate. If the instructor orders the lab evacuated, leave at once. Find out how you would get medical help, if you need it. Report **ALL** accidents to your instructor.

7) Do not perform unauthorized experiments.

8) Report any spill *immediately* to your instructor.

9) Chemical waste is a big problem. Do **NOT** discard any chemical waste in the sink. Use the appropriately labeled waste containers. If you are not sure, ask your instructor. During peptide synthesis collect waste in organic waste container, properly describe on the label what was added to the container, make sure waste you are adding is compatible with what is already in the bottle. Cell culture media need to be bleached before pouring down the sink.

10) Keep your laboratory space clean. This also pertains to the balance area and where chemicals are dispensed. You and your fellow students unknowingly can be burned or exposed to toxic chemicals if

you do not clean up a spill immediately. Keep in mind that you will be performing experiments in a real research lab with graduate students working around you, so please respect people around you.

11) Replace caps on containers immediately after use. An open container is an invitation for a spill. Furthermore, some reagents are very sensitive to moisture, and many decompose if left open.

12) Never heat a closed system. Always provide a vent to avoid an explosion.

Laboratory Notebooks

Laboratory notebooks fulfil a technical and legal need. Learning to keep a good laboratory notebook is an on-going process. Even experienced researchers sometimes find that when it comes to writing a paper, a crucial bit of data has not been recorded. Almost everyone has had the unpleasant experience of repeating experiments because some little detail was not recorded in the lab notebook. For this reason it is important to keep a good laboratory notebook whenever laboratory work is done.

The basic requirements for a laboratory notebook are as follows:

1) Notebooks must be bound.

2) Write with an indelible writing instrument.

3) Writing should be readable and well organized.

Bound notebooks are a must. If a notebook is involved in an important patent litigation, loose-leaf notebooks or spiral bound notebooks will not stand up in court. It is important to enter into the notebook directly. The worst habit one can acquire is to make entries on scraps of paper with the intention of later entry so the notebook can be kept neat. Making entries on scraps of paper will not be tolerated in this class.

Notebook entries must be made with an indelible writing tool. A pencil is not acceptable. If original entries can be erased or otherwise easily modified, it cannot be considered to be sound legal evidence. It is important to use waterproof ink, which does not fade in light. Do not use felt tip pens. Generally, black inks are best.

Entries into a notebook should be always dated. Generally, start off the day's entry with a date. If a preliminary lab report is done, it should be dated with the date the experiment will be performed on. At the conclusion of the lab, once again date your lab notebook and sign your name.

The writing should be readable and well organized. The notebook is not judged for its' beauty, but it is a record which other people should be able to read and understand. The criterion for good organization is that of being able to convey to others what has happened in a clear fashion. If you make an error cross out the error with a single line. It sometimes happens that was thought an error is in fact an important observation. One should be able to read a notebook entry even if it had been crossed out.

Notebook entries should be periodically witnessed. In a class the laboratory instructor periodically looks at the notebooks. In a research lab, the group leader will want to make sure he or she understands your research results. Get in the habit of having your notebook witnessed. The questions that will be asked will help you to improve your notebook keeping technique.

The Laboratory Report

(taken in part from: Z. Szafran, R. M. Pike, M. M. Singh: Microscale Inorganic Chemistry, Wiley and George M. Whitesides, Whiteside's group: writing a paper. *Adv. Mater.* **2004**, *16*, no. 15, 1375-1377)

The full laboratory report consists of several parts:
abstract
introduction
experimental section
Results and discussion
conclusions
acknowledgements
references

The **abstract** is a short (one or two paragraphs) summary of the experimental procedure and results. It is found at the beginning of a laboratory report that it is possible for the reader to get a quick overlook.

The abstract is followed by the **introduction**. The introduction should give a brief summary of what is known in this area of research. Common knowledge that is found in textbooks does not need to be included. The introduction should state concisely the objective of the work and indicate why this work is important.

In general, the introduction should have these elements:

- The *objectives* of the work
- *Justification*: why the work is important?
- *Background*: who else has done what? How?
- *Guidance to the reader*: what are the interesting points of this research? What strategy did you use?
- *Summary/conclusion*: what should the reader expect as conclusion?

The **experimental** section should begin with a listing of the chemicals used, and where they were obtained form, what the purity was, and if any further purification steps where undertaken. The manufacturer and model number of all equipment used should be given. The manner in which spectra where recorded should be included. For example: "IR spectra where recorded as Nujol mull between KBr plates on a Perkin Elmer Paragon 2000 spectrometer". Write down how exactly you performed an experiment (concentrations, pH, what buffer you used, did you have to make solutions fresh, at what temperature experiment was performed), in which order certain steps where performed.

The **results and discussion** sections are usually combined. This section should list all raw data (in the form obtained), including a short narrative how the data were recorded. Cover these points:

- Synthesis (or expression)
- Characterization of products
- Results (rate constants, binding affinity..)

In the **discussion section** data should be discussed and evaluated, and if applicable compared to known analogs. Do not try to fit the results you think should be obtained. Let the data speak for themselves, and evaluate the data fairly, even if your data seem to contradict the theory. Many times in history great scientific opportunities were missed because the experimentalist skewed the data to fit his/her perceptions. A discussion of possible sources for errors should also be included in this section.

The results should be summarized and conclusions drawn in the **conclusion section**. In situations where more than one explanation is possible, the different arguments should be weighted against each other. It should add a new, higher level of analysis (as compared to results), and should indicate explicitly the significance of the work.

Any help that was provided to you during the course of your experiment should be acknowledged in the **acknowledgment section**.

All materials that were used in writing the lab report, or used to gather background information should be listed in the **reference section**.

Journal articles are commonly cited the following way:

Smaith, A. B.; Loomis, K. B.; *J. Am. Chem. Soc*. **2000**, *239*, 4593- 4599.
Author(s), *Journal name*, **year**, *volume*, pages

References need to be consecutively numbered, the numbers will be included in the main body of your lab report in upper case. For example: As extensively shown in previous work,[1].....
Failure to reference material obtained elsewhere constitutes plagiarism. Even if an idea from elsewhere is put into your own words, rather than being directly copied or quoted it must be referenced. If you have EndNote software it will help you to organize your citations.

Criteria for grading:

1. **Notebook**: General
   - Description of experiments
   - Flow chart of synthetic procedure (for peptides)
   - Labeled sample and spectra
   - Neatness
   - Organization

2. **Technique**:
   - Safety, clean up, neatness
   - Efficiency
   - Instrument operation
   - Appropriate condition for synthesis
   - Purifications

3. **Data and Results**:
   - Yield of product
   - Characterization
   - Observations throughout lab work

4. **Analysis and Discussion**:
   - Interpretation of results
   - Possible sources of error
   - Indications of error and alternate plans
   - Plans for work based on current information

Work Plan Grading Scheme

Abstract
- Hypothesis
- Statement of studies to be done is clear
- Statement of studies to be done is focused

Introduction
- Choice of relevant articles
- Understanding of articles
- Description of relevance of the proposed studies

Main part
- Specific procedures for the experiments to be conducted
- Discussion of possible hazards
- Choice of appropriate characterization methods and rationale for choice
- Consideration of other, unwanted outcomes and fallback plan

Process of work plan development

- Timely submission of all previous versions (numbered versions)
- Synopsis of critique of previous versions
- Description of changes in response to critique and/or rebuttal of critique (point-by-point)

Final Lab Report Grading Scheme

Abstract

- Accuracy and clarity
- All results are given
- Format (scientific journal)
- Correlation with data obtained

Introduction

- Background (careful and enough study for previous works)
- Importance of work
- Outline of paper
- Organization

Experimental

- Source of materials
- Synthesis/protein expression
- Characterization of products
- Methods and procedures

Figures

- Clarity of figure
- Figure caption should provide enough explanation without main text of the paper

Results and discussion

- Data
- Importance of data
- Organization
- Concise and correct interpretation of data
- Correlation with previous work
- New finding (comparison with previous work)
- Source of error
- Solutions to the source of error
- Learning experience
- Suggestions for future work (in following years)

Conclusion

- Correlation of results with purpose of work

Citations

- Correct citation and format