Appendices

A. How Do I Prefer to Learn?

B. Guidelines for Scientific Papers/Lab Reports

C. Hints for your Oral Presentations

D. Amino Acids: Their Properties & Structures

E. The Periodic Table

F. Some Experimental Approaches & Techniques

G. Study Questions for the Final Exam

H. Laboratory Safety

Copyright ©2000-2014, Department of Biology, Davidson College, Davidson NC 28035

Appendix A: How Do I Prefer to Learn?

This questionnaire is a quick way to begin to identify your preferences for the intake and output of ideas and information. Take this quiz by selecting the *single answer* that best explains your preference and circle the letter. *(Note: the letters on the possible choices are intentionally out of alphabetical order.)*

1. You are about to give directions to a person who is standing with you. She is staying in a hotel in town and wants to visit your house later. She has a rental car. Would you?
   a. draw a map on paper
   b. tell her the directions
   c. write down the directions (without a map)
   d. collect her from the hotel in your car

2. You are not sure whether a word should be spelled 'dependent' or 'dependant'. Do you:?
   c. look it up in the dictionary
   a. see the word in your mind and choose by the way it looks
   b. sound it out in your mind
   d. hand write both versions on paper and choose one

3. You have just received a copy of your itinerary for a world trip. This is of interest to a friend. Would you:?
   b. phone her immediately and tell her about it
   c. send her a copy of the printed itinerary
   a. show her on a map of the world
   d. share what you plan to do at each place you visit

4. You are going to cook something as a special treat for your family. Do you:?
   d. cook something familiar without the need for instructions
   a. thumb through the cookbook looking for ideas from the pictures
   c. refer to a specific cookbook where there is a good recipe
   b. call a friend who's a good cook and ask for suggestions

5. A group of tourists have been assigned to you to find out about wildlife reserves or parks. Would you:?
   d. drive them to a wildlife reserve or park
   a. show them slides and photographs
   c. give them pamphlets or a book on wildlife reserves or parks
   b. give them a talk on wildlife reserves or parks

6. You are about to purchase a new stereo. Other than price, what would most influence your decision?
   b. the salesperson telling you what you want to know
   c. reading the details about it
   d. playing with the controls and listening to it
   a. it looks really smart and fashionable

7. Recall a time when you learned how to do something like playing a new board game. Try to avoid choosing a very physical skill such as riding a bike. How did you learn best? By:
   a. visual clues - pictures, diagrams, charts
   c. written instructions
   b. listening to somebody explaining it
   d. doing it or trying it

8. You have an eye problem. Would you prefer that the doctor:?
   b. tell you what is wrong
   a. show you a diagram of what is wrong
   d. use a model to show you what is wrong
   c. give you a pamphlet to read about the condition

9. You are about to learn to use a new program on a computer. Would you:?
   d. sit down at the keyboard and begin to experiment with the program's features
   c. read the manual which comes with the program
   b. telephone a friend and ask questions about it
   a. consult a flowchart of the program's features

10. You are staying in a hotel and have a rental car. You would like to visit friends whose address/location you do not know. Would you like them to:?
   a. draw you a map on paper
   b. tell you the directions
   c. write down the directions (without a map)
   d. collect you from the hotel in their car

11. Apart from the price, what would most influence your decision to buy a particular text book?
   d. you have used a copy before
   b. a friend talking about it
   c. quickly reading parts of it
   a. the way it looks is appealing

12. A new movie has arrived in town. What would most influence your decision to go (or not go)?
   b. you heard a radio review about it
   c. you read a review about it
   a. you saw an ad or preview of it
   d. you saw a colleague demonstrate a funny scene

13. Do you prefer a lecturer or teacher who likes to use:?
   c. a textbook, handouts, readings
   a. flow diagrams, charts, slides
   d. field trips, labs, practical sessions
   b. discussion, guest speakers

Now that you have completed this questionnaire now tally the number of your responses that were a, b, c, and d in the table below.

	a (Visual**)**	**b (A**ural**)**	**c (R**ead/Write**)**	**d (K**inesthetic**)**
Total				

Source: Neil Fleming (2001-2006) VARK, a Guide to Learning Styles
www.vark-learn.com

Study Practices Keyed to Learning Preferences

Knowledge of your preferences can help you develop more effective learning strategies.

	In Class	When Studying	For Exams
V Visual	• underline, bracket • use different colors • use symbols, charts, arrangements on a page • attend to illustrations, photographs, diagrams	• use "In Class" behaviors • reconstruct images in different ways • draw concept maps • create visual metaphors • replace words with symbols and initials	• recall the 'pictures of the pages' • draw, use diagrams where appropriate • redraw concept maps • redraw charts • practice turning visuals back into words
A Aural	• attend lectures & tutorials • discuss topics with students • explain new ideas to other people • use a tape recorder • describe overheads, pictures, and visuals to somebody not there • leave space in notes for later recall and "filling in"	• may take poor notes because prefer to listen • expand on notes • put summarized notes on tape and listen • read summarized notes out loud • explain notes to another "A" person • create aural mnemonics	• mentally listen to rehearsed answers and write them down • verbally recite answers • recall aural mnemonics
R Reading/ Writing	• use lists, headings • use dictionaries and definitions • use handouts & textbooks • read • use lecture notes	• write out definitions • reread notes silently • rewrite ideas into other words • organize diagrams into statements • practice writing answers to old exam questions	• write out lists • recall headings to prompt memory • write paragraphs, beginnings, endings • write out practice answers
K Kinesthetic	• use all your senses • go to lab, take field trips • use trial-and-error methods • listen to real-life examples • use hands-on approach • attend to demonstrations • attend to gestures of emphasis • attend to teacher "acting out" concepts	• may take notes poorly because topics do not seem relevant • put examples in note summaries • "act out" concepts and processes • talk about notes with "K" person • connect material to personal experience • create real-world examples	• construct personal examples • apply practical knowledge to problems • mentally reconstruct lab/field activities • mentally reconstruct demonstrations

Source: Neil Fleming (2001-2006) The VARK Helpsheets www.vark-learn.com.english/page.asp?p=helpsheets

Appendix B: Guidelines for Scientific Papers/Lab Reports

Note – more information on writing lab reports can be found in *A Short Guide to Writing About Biology* by Jan A. Pechenik, as well as at the LabWrite online resource from NC State (http://www.ncsu.edu/labwrite/).

Writing a laboratory report is like writing an original research paper. Scientific papers are usually written in a format with the following sections:

Title
Abstract
Introduction
Methods
Results
Discussion
References
Figures

Abstract

An abstract is a single paragraph summary of your experiment. Like a paper (or lab report), an abstract should contain an introduction, methods, results, and conclusion. Every scientific paper has an abstract at the beginning to let the reader know what the paper is about and to make an informed decision whether the entire paper is worth reading. Abstracts also are printed in reference books and available on line where the whole article does not appear, and are used to decide which articles you need to obtain. A third use of abstracts is to summarize the work you will be presenting at a meeting, so people will know if they should come to see your complete presentation. Thus the abstract is absolutely critical and requires very careful thought in the writing process. FYI most scientific journals limit abstracts to 150-500 words.

Guidelines for writing good abstracts: Revise, revise, revise. The Abstract should be clearly and concisely written. Try to address each of the questions below (under ABSTRACT). Use plain English whenever you can, active voice when you can, and use simple sentences. It is not necessary to refer to any literature (if you do, list the references below the abstract). State only your most important conclusion(s). Remember, the abstract will likely be the only portion of your report that most people read. Make sure it is well written.

1. Title: The title should indicate the question you investigated, or the method, if that is important. Example: Effect of Owner Education Level on Number of Cats per Household.

2. Author(s) and address(es). Example: Mary Darwin, Polly Merase, and John D. Helix, Biology Department, Davidson College, Davidson, NC 28036.

3. What is the general topic you were investigating and why is it important? One to two sentences. Example: Education level may affect choices people make about their personal lives and habits.

4. What are the specific questions you are addressing with this project? The abstract should not include your complete methods. Provide a one or two sentence overview. Example: We investigated the relationship between education level and the number of cats per household for residents of a small town.

5. How did you do this experiment? For a single paragraph abstract, one or two sentences are needed. You are not trying to be complete, just give a general idea of how you did it. Example: The residents of a small town in North Carolina were polled as to the number of years of education for adults in households and the number of cats associated with the household.

6. What did you observe? One sentence should be enough: state only your main point(s). Example: Adults with either low education levels (0-10 years of school) and those with high education levels (more than 16 years of school) had significantly more cats per household than those with intermediate education levels (11-16 years of school). Include your most important data (mean values, standard deviations, number of samples you studied, *etc.)* that influenced your conclusion.

7. What did you find out about the general topic or question (see #3 above)? One sentence, 2-3 sentences for a longer abstract. Example: We concluded that education level can affect choices not directly associated with academic pursuits.

Here is the final abstract from the example above:

Education Level is Associated with the Number of Cats per Household

Anna Author and Aaron Associate
Biology Department, Davidson College, Davidson, NC 28035

Education level may affect choices people make about their personal lives and habits. We investigated the relationship between education level and the number of cats per household for residents of a small town. 156 adult residents of a small town in North Carolina were polled as to the number of years of education for adults in households and the number of cats associated with the household. Adults with either low education levels (0-10 years of school) and those with high education levels (more than 16 years of school) had significantly more cats per household than those with intermediate education levels (11-16 years of school) when analyzed by the statistical test ANOVA, ($p<0.005$). This finding is highlighted by noting that those people with high or low education levels were more likely to have four or more cats (23%) than those people with intermediate education (4%). We concluded that education level directly affects whether a household will include pet cats.

With the method outlined above, you should be able to produce a good abstract in less than an hour. If you haven't clearly and carefully thought through what you did in the experiment, writing the abstract should help you do so. It is shorter than a lab report, but includes most important points. (For your information, the study and abstract above was invented for this lab and does not reflect an authentic study.) Also, consult the posters on display in Watson and Dana.

Introduction

The introduction should explain why the work was done. What were the objectives of the research? How does the research help to fill a hole in our knowledge? The introduction should include a clear statement of the problem or question to be addressed in the experiment. It is always helpful to put this question into some context by stating why this question needs to be answered or why you found this question to be particularly interesting. Any background material that is particularly relevant to the question should be included in this section.

Methods

The methods section tells how the work was done. It should NOT be a simple list of the materials used. What procedures were followed? What research materials were used: the organism, special chemicals, instruments? In some of the experiments you will be doing, many of the procedures are given in great detail in the handouts. It is not necessary to retype these verbatim, but rather summarize the critical steps. The most important feature of this section should be to include enough detail in your description of how your experiment was set up and run so that anyone reading the methods could repeat your experiment. Do not write your methods section as a step-by-step protocol. Write it as descriptive summary of your lab procedures in paragraph form. Include critical information such as the concentration of the reagents you used. Do not include superfluous information that does not affect the outcome of your study (such as what well B2 or A11 contained).

Results

The "Results" section explains in words what you found, the data that you generated, explained succinctly in the body of the report and presented in detail as tables or graphs. The results section should be written so that any college student could read the text to learn what you have done. The text should give the reader a clear idea of the major trends in your data. A reader should have enough information so that s/he could draw the figures (generally) based on your written description of your data in the results section. For example, you might use a paragraph to explain what is seen on a particular graph; "When we soaked the enzyme in sulfuric acid, we observed no change in absorbance (Table 1)" Do not make the common mistake of writing, "We performed the experiment, see figures 1-4." That is too brief and does not describe what you have done or the results you obtained. When stating your results in the body of the text, refer to your graphs and tables. Do not attempt to discuss the interpretation of your data in the results section - explanations should be included in the discussion section. Each table and figure should be numbered sequentially for easy reference in the text, and all figures must have a brief description called a legend, which provides the reader enough information to know what you did to produce the data (even without reading the rest of your manuscript).

Figures & Tables

Data that have been collected need to be presented clearly and succinctly. As a result, two forms of presentation are most commonly used in scientific papers: figures and tables. Which method to use depends somewhat on the data, but in general anything that can be displayed pictorially (*e.g.,* a graph or diagram) is usually more desirable than a table, because the reader can immediately see the trends in the data. In the paper itself, diagrams, photographs, and graphs are all referred to as "Figures", and are numbered sequentially in the order of presentation (Figure 1, Figure 2, *etc.).* Tables also are numbered sequentially in order of presentation. Although figures and tables often are placed directly into the middle of scientific papers, you may include figures and tables within the text of your report or at the end of your report.

Graphs

Graphs can be made using a graphing program such as Excel. Remember to label each axis, including units of measurement, and clearly identify the data you are displaying (*e.g.,* label each line in a graph). In addition, every graph must have a short description (legend) below it to tell the reader some basic information about that data and the way it was obtained. The legend starts with the figure number, followed by a one-sentence title. The text of the legend should be a one short paragraph. At right is an example of a graphic figure with legend:

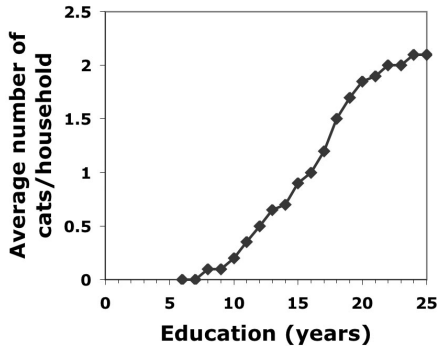


Figure 1. Cat ownership is directly related to educational level. 156 Davidson, NC adult residents were surveyed to determine their education level and the number of cats in the household.

This graph was made using Excel. Notice how the axes are labeled, and the figure is numbered and titled (bold type) and the format is very simple, clear, and the data is obvious (avoid the temptation to add extra grid lines, 3D features, shadows, backgrounds, *etc.* Such "chartjunk" distracts your audience from the data, your main means of conveying scientific evidence. The legend (paragraph below or next to the graph) explains how the data were obtained. You can look at any scientific paper for examples of legends. Note that all figures in your textbook also have legends.

Tables

Tables should be made using the same principles outlined for graphs, though the format is different. Tables can be created with Word or Excel. Tables are numbered, but this number usually appears at the top of the table. The title usually follows the table number:

Table 2. **Number of pet cats and education levels for Davidson, NC residents**

Subject's Initials	Years of Formal Education	# of pet cats
AN	5	0
CD	12	1
CJL	22	2
CGM	9	1
ABH	7	0

Tables generally do not contain legends. Often, though, footnotes are included under a table to provide explanatory information. Of course, all column headings should be clearly labeled to describe the data listed below them.

When preparing your data for a presentation, think about the most effective way of showing your data to the audience. Some information can be conveyed most effectively in a table. Other information can be conveyed most effectively in a figure. If you do decide to use a figure, then consider what type of figure will be most effective. In general figures are more effective than tables. When creating graphs you should also think carefully about what type of graph (X-Y, bar, pie, *etc.*) best conveys your results. Always make your figures and tables as simple and clear as possible. Do not make your reader work hard to understand your point.

Discussion

The "Discussion" section typically includes your appraisal of what your research means, including its success in meeting the objectives stated in the introduction, and its significance in advancing your knowledge of the subject. This section also is the place to explain discrepancies or difficulties with experiments, as well as suggestions for future work. For example, if you had known initially what you know now, how might you have changed your experiments? Most importantly, the Discussion provides an opportunity to compare your results with those of others. What previous information exists that is relevant to your research? Do your results support or supplement that information? Once again, when providing your interpretation of the data, direct the reader to specific tables and graphs to prove your point.

References

Finally, it is important to place your work in perspective with the published work of other scientists. We will not have much opportunity to use references in Bio 111, but references are an important component of any report. Scientific journals usually require specific reference formats. We will discuss the preferred format for your reports. (If your instructor does not recommend a specific citation style, pick a style and use it consistently.) For more information on citing references and academic integrity please consult the biology department's statement on plagiarism at: www.bio.davidson.edu/dept/plagiarism.html

> . NEWS ITEM: If you are under the impression that the research you do is unimportant, then take a lesson from Emily Rosa. Emily published her research results in *JAMA - the Journal of the American Medical Association*. She conducted her research while in the fourth grade! She was curious whether there was any validity to a new form of alternative medical therapy called "touch therapy". She and her mom, a nurse, conducted an experiment that Emily designed. The end result demonstrated that touch therapy was not able to discern as much information as the practitioners claim. (*JAMA*. 279:1005-10).

Appendix C: Hints for Your Oral Presentations

Oral presentations are an important means of communicating scientific information. Oral presentations often are used to present experimental findings at colleges and universities (where they also are known as seminars), and at scientific meetings. Therefore, it is important that you gain experience with this presentation format.

Davidson's science departments each host several seminars each semester. Attending seminars is an excellent method to prepare for your own oral presentations. You will see how different scientists communicate their experimental results (some better than others).

Your instructor realizes speaking in front of a group can be uncomfortable, and it is especially hard the first time. You will make some mistakes - that's part of the learning process. Please realize that any questions that you are asked by your classmates or instructor are not meant to be taken personally. So, don't be afraid of questions - they are intended to further our understanding of your scientific investigation. The best preparation for presentations is to understand what you did, especially why you set the experiment up the way you did in order to answer a specific scientific question. Asking questions of other scientists is also an essential skill for you to develop.

Each group will give an oral presentation about their experiment. The presentation should be organized in a manner similar to your scientific reports, with general categories such as: Introduction, Material and Methods, Results, and Discussion/Conclusion. Your lab group is welcome to divide up the speaking responsibilities as you like as long as the division is equal between all students. The most common division of speaking duties for a group of four has each person presenting one of the following sections:

1) The Introduction can include aspects such as background information, the reasons for doing the experiment, and your hypothesis/experimental question. Your words should make connections to concepts discussed in lab and/or lecture.

2) The Methods section should include your experimental design, where you describe the samples you are testing and the controls you have incorporated into the experiment. In addition, you can do a very brief overview of the major procedures you performed. Remember to consider your audience: all the groups did a basic enzyme laboratory, so there is no need to repeat "standard" protocols. Include procedures that are different from the standard protocol, and be sure to present enough of your protocol so that everyone is clear as to exactly what you did.

3) The Results should be a clear and concise display and explanation of your data. Your data should be distilled down to the important facts, and not necessarily every piece of data you collected. However, don't make the mistake of showing a figure and saying, "This is what we got." and then sitting saying nothing else. Walk us through the figure. Point out important parts of each figure. Make certain that your "take-home message" is stated very clearly and emphasized.

4) Finally, the Discussion will be your interpretation of your results. What do your data mean? Discuss whether your data support your hypotheses. Do you have reason to believe your data were inaccurate? What would you do next time to investigate the problem further? What follow-up experiments could you perform as a result of your data? Do not blame "human error" unless you can describe the specific nature of a particular error that your group made.

Your group's presentation should last no more than 10-15 minutes, because there must be time for questions and discussion with the rest of the class afterward. Each person in your group must speak during the presentation. The use of visual aids is very important. If you use the document camera you need to print very small figures.

In preparing your presentation, you may find it helpful to keep the following questions in mind:

1. Do you clearly state the question(s) you are trying to answer?
2. Is it clear what you did to try and answer your question?
3. Do you explain your results, especially inconsistent or unexpected results?
4. Do you convey why you did the different conditions in your experiment?
5. Did you explain what your data mean? Can you answer the question from number 1 above?

Your group will be critiqued in two ways. First, your classmates will review your presentation. Your classmates will not grade you - these comments are to help you. Each person will review every group by responding to the following two questions:

1) What were the strengths of this group?

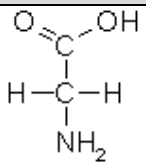
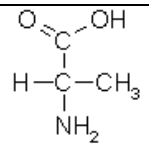
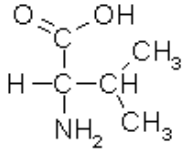
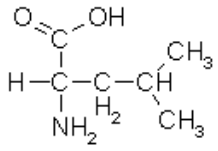
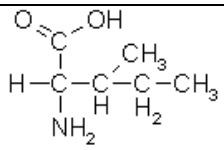
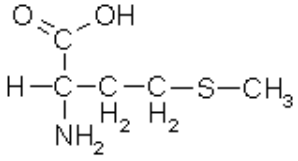
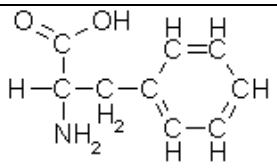
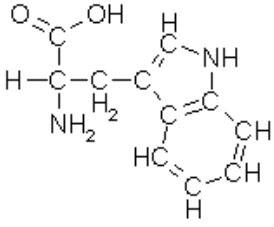
2) What improvements could be made by this group?

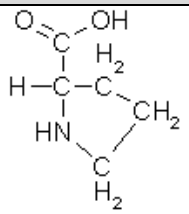
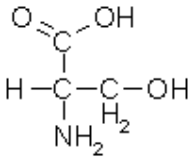
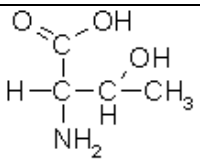
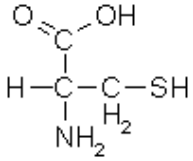
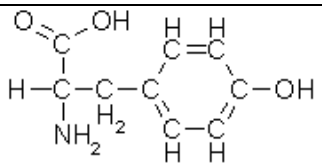
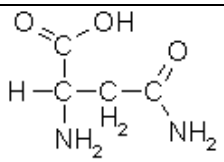
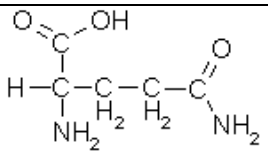
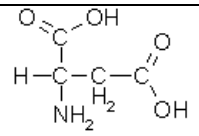
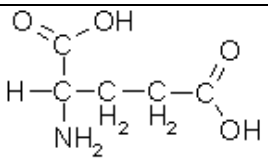
When making comments about the presentation of others, keep in mind the five questions listed above, as well as other things such as whether the group was organized, if everyone participated, if their conclusions were valid, *etc.* These comments are meant to be helpful suggestions and not a slap in the face.

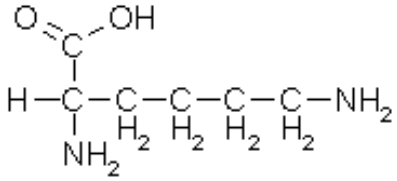
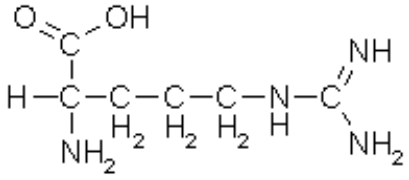
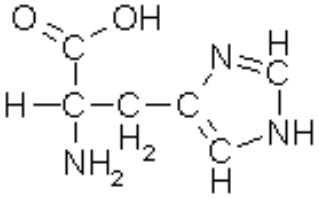
Your instructor will be interested in similar categories, especially how clearly you present your material, whether you display understanding of what you did and why you did it, and if the data support your conclusions. You will receive a group grade, but the most important aspect of this exercise is to become comfortable talking in front of a group and to enjoy your presentation.

Appendix D: Amino Acids: Their Properties & Structures

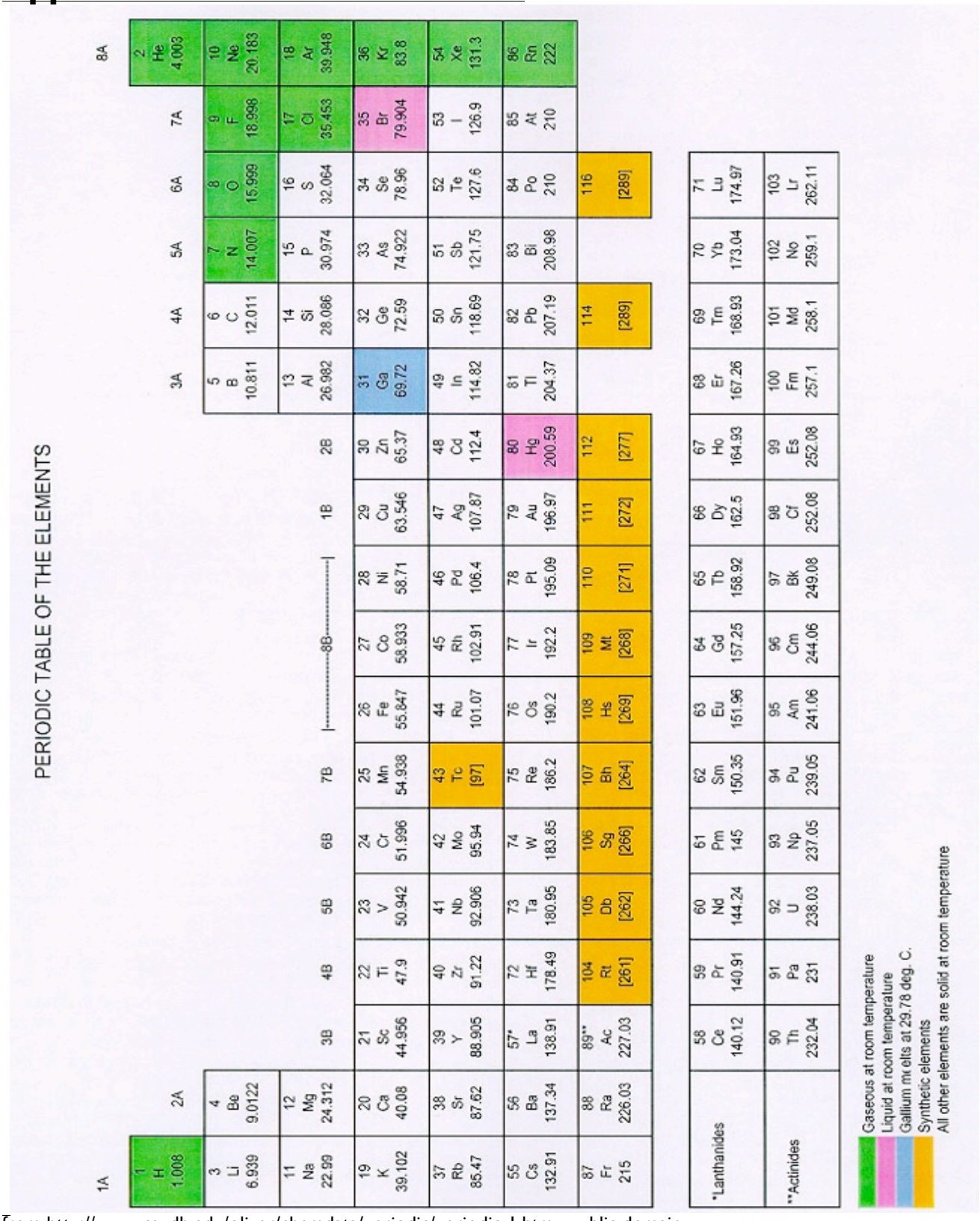
Also at: Table 3.1 (page 43), Fig. 14.6 (p. 299), and www.bio.davidson.edu/people/macampbell/geneticode.html

Name	3 letter code	1 letter code	Codon(s)	Side Chain Charge Hydrophobic/philic Other comment(s)	Structure
glycine	gly	G	GGU GGC GGA GGG	neutral hydrophobic nonpolar sidechain	
alanine	ala	A	GCU GCC GCA GCG	neutral hydrophobic nonpolar sidechain	
valine	val	V	GUU GUC GUA GUG	neutral hydrophobic nonpolar sidechain	
leucine	leu	L	UUA UUG CUU CUC CUA CUG	neutral hydrophobic nonpolar sidechain	
isoleucine	ile	I	AUU AUC AUA	neutral hydrophobic nonpolar sidechain	
methionine	met	M	AUG (start)	neutral hydrophobic nonpolar sidechain contains sulfur	
phenylalanine	phe	F	UUU UUC	neutral hydrophobic nonpolar sidechain	
tryptophan	trp	W	UGG	neutral hydrophobic nonpolar sidechain	

Name	Three letter code	One letter code	Codon(s)	Side Chain Charge Hydrophobic/philic Other comment(s)	Structure
proline	pro	P	CCU CCC CCA CCG	neutral hydrophobic nonpolar side chain	
serine	ser	S	UCU UCC UCA UCG AGU AGC	neutral hydrophilic polar side chain can be phosphorylated	
threonine	thr	T	ACU ACC ACA ACG	neutral hydrophilic polar side chain can be phosphorylated	
cysteine	cys	C	UGU UGC	neutral hydrophilic polar side chain contains sulfur	
tyrosine	tyr	Y	UAU UAC	neutral hydrophilic polar side chain can be phosphorylated	
asparagine	asn	N	AAU AAC	neutral hydrophilic polar side chain	
glutamine	gln	Q	CAA CAG	neutral hydrophilic polar side chain	
aspartic acid	asp	D	GAU GAC	negatively charged hydrophilic	
glutamic acid	glu	E	GAA GAG	negatively charged hydrophilic	

Name	Three letter code	One letter code	Codon(s)	Side Chain Charge Hydrophobic/philic Other comment(s)	Structure
lysine	lys	K	AAA AAG	positively charged hydrophilic	
arginine	arg	R	CGU CGC CGA CGG AGA AGG	positively charged hydrophilic	
histidine	his	H	CAU CAC	positively charged hydrophilic	
--	--	--	UAA UAG UGA (stop)	--	--

Appendix E: The Periodic Table



from http://www.csudh.edu/oliver/chemdata/periodic/periodic-1.htm , public domain

Appendix F: Some Experimental Approaches & Techniques

The approaches and methods used to investigate the biology of cells and their communication processes are numerous and most are beyond the scope of this course. However, as a starting point, we will introduce a few basic methods upon which many others are based.

Microscopy -- The Direct Approach

Thanks to the Dutch lens grinders of the 17th century, we can see prokaryotic and eukaryotic cells simply by looking through a microscope. Because most animal cells are clear as are most of the parts of plant cells (only the chloroplasts and chromoplasts are colored), cells usually need a little help in order to be visible through the microscope. Without this help, they would be like small panes of glass -- present, but transparent. Several methods are available. The simplest is staining the cell to make it colored. Other methods allow the microscope to distinguish differences in structures due to their different abilities to diffract light. For example, in **phase contrast microscopy** (we'll see this in lab), some structures will appear dark while others will appear light due to differences in diffracted light. Finally, dyes that fluoresce when excited by light can be used to label organelles and molecular components of cells. These dyes are observed with a **fluorescence microscope** (See Figure 4.3 (Looking at Cells) on page 71 of the textbook for examples).

Even with the best available optics, the light microscope can only magnify about 1500 times. This magnification is enough to allow you to see cells, but not enough to allow a clear view of most organelles and cellular inclusions. For that, you need a source of electromagnetic radiation that has a much shorter wavelength than light. In the 1950s, engineers perfected the **electron microscope** that uses electrons instead of light to produce images. Electron microscopy is described on page 71 of your text and example images are in Figure 4.3 on page 71. The transmission electron microscope (TEM) allows the clear definition of cellular organelles and inclusions (such as cortical granules, synaptic vesicles, microfilaments, *etc.*). Viruses can also be seen with electron microscopy. Using special methods, very large macromolecules can also be visualized (*e.g.,* transport proteins in the cell membrane).

Isolating Living Cells for Experimentation -- Cell Culture

Most plant and animal cells can be kept alive for some time outside the host if they are maintained in conditions that mimic those of the body fluids. Cells are placed in **culture medium,** which is a fluid designed to provide all the nutrients, salts, vitamins, *etc.* that the host normally provides in the right concentrations and at the right pH. If you can get cells to live in cell culture, you can do some pretty fancy experiments on them. For example, if you put muscle cells in culture medium that contains high levels of Ca^{2+}, nothing will happen because the living muscle cell can pump Ca^{2+} out of its cytoplasm as fast as it enters. However, if you then add a **Ca^{2+} ionophore** to the medium (an ionophore will insert itself into the cell membrane and create an artificial ion channel that cannot be closed), the cell will contract. This contraction indicates that high levels of intracellular Ca^{2+} trigger muscle contraction. By this approach, you could determine the concentration of Ca^{2+} necessary to elicit contraction. If you wanted to see that the concentration of an ion had actually changed inside a cell, you might use an **ion-sensitive dye** that will glow in the dark when it selectively binds to its ion.

Focused Reading

- p 325 Figure 15.8 (Separating Fragments of…)
- p 324 "Gel electro…" to end of page

Web Reading

- Gel Electrophoresis Methodology
  www.bio.davidson.edu/courses/Molbio/SDSPAGE/SDSPAGE.html

Isolation of Organelles, Cellular Inclusions, and Other Cell Parts

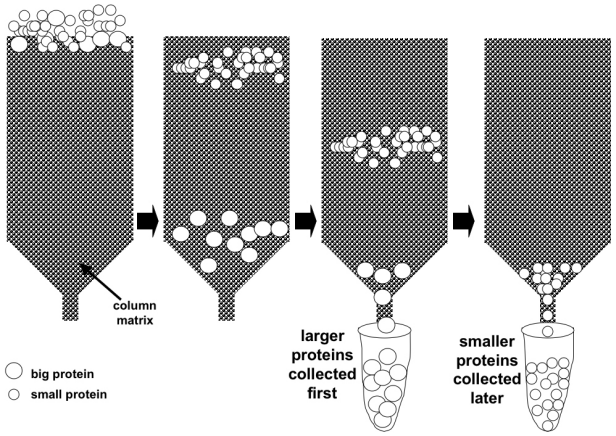
Sometimes it is beneficial to isolate part of a cell for study. For instance, if you are interested in a protein found only in the plasma membrane, it may be helpful to isolate the plasma membrane from the rest of the cell. Or if you are interested in ribosomes, you may wish to isolate them from the

rest of the cell. All these cell parts are called **subcellular fractions** and they can be isolated using a method called **cell fractionation** using a **centrifuge** or an **ultracentrifuge**. A centrifuge 'spins' samples like a washing machine or the machine used to train astronauts. Density gradient centrifugation is used to separate pieces of DNA that have nucleotides that vary slightly in weight.

Isolation of Proteins by Molecular Sieves

Quite frequently, it is necessary to isolate a single protein from a cell. **Gel electrophoresis** is a commonly used method. In gel electrophoresis, cells are homogenized (ground up in a blender) to release all proteins. The cellular proteins are then usually dissolved in a detergent that covers them with negative charge. When these proteins are put in a gel (like a slab of Jello) and a voltage is placed across the gel (one end of the gel is made negative (the cathode) and the other end is made positive (the anode)), the negatively charged proteins move toward the anode. Just like people in a thick forest, the smaller they are, the quicker the proteins can move through the obstacle course of the gel to get to the anode. Thus, the smaller proteins move faster than the larger proteins and the proteins of the cell separate by size or molecular weight. You will run a gel in lab.

If you want to study a protein further after it has been isolated, gel electrophoresis is not such a good method because detergent is very harsh on proteins and frequently destroys their native conformation during the separation process. A better method is one form of **chromatography** in which proteins are poured over a matrix in a glass tube (the tube length can range from two inches to five feet and the diameter from 0.25 inches to three feet.) The proteins are not treated to cover them with negative charge, as in electrophoresis, so they retain their native conformations. The proteins enter the matrix and, this time, the larger proteins get through the matrix first while the matrix retards the movement of smaller proteins so they come out last. This size separation results from the matrix that fills the columns. The matrix is made of small "beads" that contain tiny holes or channels, which the small proteins are small enough to enter, but the large proteins are too big to fit into. The small proteins spend a lot of time wandering around in these channels and it takes them a long time to get through the entire matrix. The large proteins cannot get into the channels so they continue through the tube on the outside of the beads (in the space between the matrix particles). By taking this alternative route, the proteins get to the bottom of the tube rapidly. Thus, the proteins are separated by size and maintain their native conformations and therefore can be used for further study.

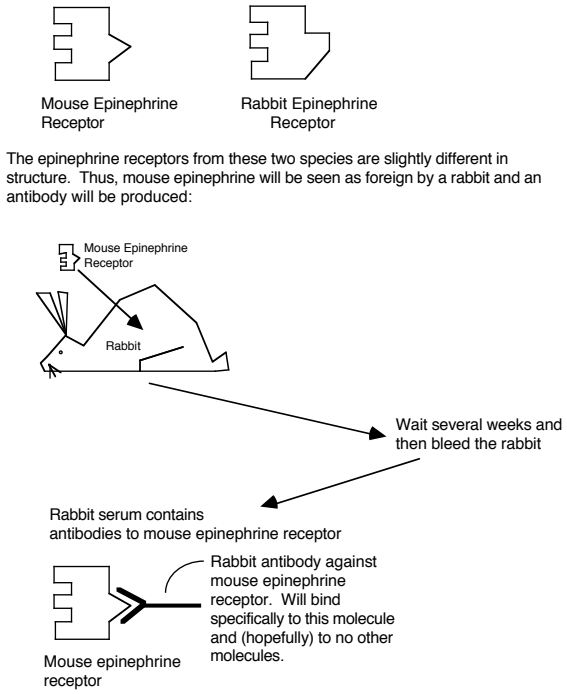


Focused Reading

- p 885-887 "Different antibodies…" to "42.5 recap"
- p 887 Figure 42.1 (Creating Hybridomas….)

Identification of Proteins with Antibodies (Abs)

Since proteins do most of the meaningful work of living creatures, it is extremely important to biologists to be able to isolate and identify individual proteins. Proteins can be isolated in a number of ways. One commonly used method involves the use of antibodies that bind to proteins with great specificity. When a foreign protein is injected into an animal (*e.g.*, rabbit albumin into a mouse or goat insulin into a rabbit) the animal's immune system recognizes this foreign protein and interprets it as a microbial invader. (The immune system recognizes foreign molecular shapes whether they are harmful or not. Thus, you can get allergic reactions (an immune response) to pollen even though pollen can't harm you). This immune response to the foreign molecule produces **antibodies** (which are proteins) that bind specifically to the foreign protein (called an **antigen**.) Antibodies have active sites, like enzymes, and the antigen is the **ligand** that binds at the active site. The production of antibodies for research is diagrammed below:



So, you can raise these specific antibodies against a protein you might be interested in studying and use the antibody as a probe for that protein since it will bind specifically to that protein and no other. You can probe for proteins ***in situ,*** which means that they are still in their normal location within the intact cell. The identification of proteins *in situ* using antibodies is called **immunocytochemistry** or **immunohistochemistry**. You can also remove the proteins from the cell, separate them by electrophoresis (see above), and then apply the antibody probe for the protein. This method is called an **immunoblot** (or a western blot in the vernacular).

Identification of Specific Proteins Through the Use of Radiolabelled Ligands

This method uses radioactivity to identify specific proteins. While there are many variations on this method, the basic idea is this. You buy or synthesize a ligand that contains a radioactive element. For instance, if you wanted to study the acetylcholine receptor, you would obtain **radiolabelled** acetylcholine. This acetylcholine could contain radioactive hydrogens (called **tritium**) or radioactive carbon (^{14}C) or an additional radioactive element (such as iodine - ^{125}I) could be added. These radioactive elements are **isotopes** of the non-radioactive elements. Isotopes are described on page 18 of your text.

These **radioligands** (in this case, radiolabelled acetylcholine) can be bound to various kinds of cells to determine whether they bear the ligand's receptor. For instance, if you wanted to know if liver cells have acetylcholine receptors in their membranes, you would incubate radiolabelled acetylcholine with liver cells. If the liver cells bind the ligand (*i.e.,* if the cells become radioactive), then you can assume (if your experiment is properly controlled), that the liver cells are radioactive because they bound the radioactive ligand. You can also use this procedure to determine **the concentration or density** of a receptor in a membrane. Therefore, you can use this method to see if receptor densities change over time as you subject the cell to various treatments.

Drs. Candice Pert and Sol Snyder used this method in order to identify the receptors in the brain that bind (and respond to) opiates such as heroine. Through the use of this method (and others), we now know that we make internal or **endogenous** opiates called **endorphins** that reduce pain and may have other beneficial effects.

Molecular Models and Computer Graphics

One of the most exciting new methods in biology is the ability to build fairly accurate, complex three-dimensional models of proteins based on computer analysis of data obtained by **x-ray crystallography**. Because it is difficult to crystallize many important molecules, their 3-D structure at the atomic level (in their native conformation) remains illusive. However, if we learn enough about how amino acid sequence translates into 3-D structure, we may be able to predict (or teach a computer how to predict) the 3-D structure of a protein from its primary amino acid sequence. Because the amino acid sequence of proteins is becoming much easier to obtain (through the remarkable progress being made in molecular biology), determining 3-D protein structures is a tremendously important breakthrough and would give us new worlds of information about how living things function.

Focused Reading

- p 320-322 "Mutations can.." to " Mutations have…"

- p 292-294 "Experiments…" to "14.1 recap"
- p 293 Figure 14.1 (One Gene, One Enzyme)

Web Reading

- Movie of Microinjection
  www.bio.davidson.edu/misc/movies/injectionb.mov

Use of Genetic Mutants

Because mutations are changes in the DNA that can alter the activity of one protein, they can be used to identify the protein responsible for a specific function. For instance, scientists have used genetic mutants to study the process of membrane traffic in the cell. Using mutant yeast, investigators have identified several mutant strains that each have one important protein altered. For instance, let's say Mutant strain #1 is missing Protein #1. Investigators find that this mutant strain cannot transport protein from the ER to the Golgi. Mutant #2 is missing Protein #2. This mutant strain cannot transport protein from the Golgi to the secretory vesicle. Thus, by identifying the protein that is missing and correlating it with the functional deficit in the cell, investigators can determine the proteins that are responsible for each step in a biological process. We will use genetic mutants to screen compounds to see if they are mutagens. We will perform this experiment in lab (the Ames test) later in the semester.

Microinjection

There is a very difficult, and labor intensive method to place a molecule of interest inside a particular cell and this method is called **microinjection**. As the name implies, you take a very small needle, usually made of a glass tube that has been heated and pulled to a very fine point, attach the needle to a syringe, and inject a cell with a very small volume of solution that contains your molecule of interest. DNA, RNA, antibodies, fluorescent dyes, and purified proteins can all be injected into cells to see how the cell reacts to the microinjected molecule.

Study Questions:

1. Be able to describe each of the techniques outlined above.

2. If you had all of these methods available to you in the lab, how would you go about answering the following questions? Note: Just because a method is available does not mean it is the best approach to the problem. In each case, choose the method or methods that you think provide the most efficient route to an interesting and substantive answer:

3. Do plant cells use cAMP second messenger systems?

4. Is Ca^{2+} involved as an intracellular messenger in the secretion of saliva from the salivary glands?

5. The microfilaments (actin and myosin) in vertebrate muscle cells are aligned in organized units which produce contraction as described by the sliding filament theory (outlined above). Are the microfilaments of the muscle cells of insects aligned in the same manner?

6. Some forms of breast cancer are stimulated by estrogen (a female sex hormone). Do these breast cancer cells have a higher concentration of estrogen receptors than normal breast cells?

7. Plant cells secrete the cell wall. Is the secretion of the cell wall constitutive or regulated?

8. What proteins mediate each of the steps that lead from ligand binding to cell division in fat cells?

9. Plants and animals both use the inositol triphosphate second messenger system which requires the use of phospholipase C. Is the phospholipase C used by plant cells similar in molecular weight and three-dimensional structure to the phospholipase C used by animal cells?

10. Does the Ca^{2+}pump in the SER membrane have the same molecular weight as the Ca^{2+} pump in the plasma membrane?

Appendix G: Study Questions for the Final Exam

1. Describe the common themes found in cellular communication (*e.g.,* the roles of calcium, ion gradients, phosphorylation, ligand binding to receptors, etc.). You do not have to know each enzyme in every pathway that we studied though specific examples used correctly will enhance your answer.

2. Explain the concepts of:
   - signal transduction
   - receptor-ligand interactions
   - amplification of the message
   - second messenger
   - turning off a cellular signal

3. Be able to interpret a pedigree; predict the outcome of a Mendelian cross; predict the probability of a certain genotype if you are given the phenotypes of the parents; know the major steps of mitosis and meiosis and how the two types of nuclear division differ, on the macro scale.

4. Understand the big picture of gene expression. Do **not** focus on every component (*e.g.,* single-strand binding protein), but be able to explain the major events, especially in regards to cancer, AIDS, and transgenics.

5. Know what is consumed and produced in the following: (Do **not** worry about step-by-step details, but focus on the overall process of each. This is not the same as saying to memorize the overall equation - be able to follow the energy in a general sense.)
   - photosynthesis (light & dark reactions)
   - cellular respiration
   - fermentation
   - chemiosmosis

6. Be able to interpret a Southern blot or a DNA gel.

7. Be very familiar with the Ames test and PCR experiments that you conducted in lab. This includes the theoretical aspects as well as the logistical ones (hint: be able to name the parts on the 3D model of DNA).

For example, here is a particular question that requires you to use what you have learned in different sections. Human eggs secrete a protein that binds to a receptor located in the middle piece of the sperm tail. These receptors, which resemble the odor receptors in your nose, help the sperm "smell" the egg and swim towards it. Design a contraceptive that uses this aspect of a sperm's ability to locate an egg.

Appendix H: Laboratory Safety

General Safety Rules

- Work in a laboratory only during regular, assigned period when an instructor is present, unless specific authorization has been given by the instructor to work in the laboratory at other times.
- Read carefully and observe fully all laboratory instructions. In case there is any doubt about any procedure, check with your instructor.
- Learn the location and proper use of emergency showers, fire extinguishers, and eye wash stations.
- Avoid inhaling chemical vapors or gases. Use fume hoods for hazardous materials.
- Immediately wash off and chemicals spilled on the skin with lots of water. In case of a serious spill, remove contaminated clothing immediately and flush affected area with lots of water.
- Do not eat, smoke, or drink in the laboratory. Do not bring any food items into the laboratory.
- Do not leave experiments in progress unattended without authorization.
- Keep working areas neat and clean at all times.
- Report all accidents to the instructor immediately.

Personal Protective Equipment

- All persons working with hazardous chemicals should wear gloves.
- All persons working with chemicals that could be splashed in the eyes are required to wear safety goggles or glasses.
- Contact lenses should not be worn in lab when hazardous chemicals or vapors are being used.
- Because of the danger of broken glassware or spilled chemicals, covered shoes should be worn in the laboratory. (No types of open toe shoes are permitted in labs.)

Chemical Safety

- Almost every chemical, whether solid, liquid or gaseous, is poisonous to the human body to some degree. Always use proper caution when handling chemicals.
- Consult a physician if you are pregnant or have any other medical condition that might render you susceptible to exposure to the chemicals used in this laboratory.
- When handling chemicals, keep your hands away from your face, eyes and body until your hands have been washed thoroughly.
- Do not taste any chemical. Label every container so items can be identified.
- When diluting acids, ALWAYS POUR ACID INTO WATER SLOWLY.
- Do not pipet anything into the mouth.

Waste Disposal

- Always treat laboratory glassware as if it were fragile. If glassware breaks, do not pick it up with your hands. Use a broom and dustpan, then place pieces in the cardboard box labeled "Glass Disposal Box."
- Do not pour any chemicals down the drain. The instructor will advise you on the proper waste containers.
- Discarded animals parts must be placed in a red cardboard "Biohazardous Waste" box.
- Discarded sharp items including scalpels, dissecting pins, probes, and needles must be placed in a red plastic "Sharps Box."

Safety Agreement

Biology 111

I have read the Lab Safety Rules and procedures for the prevention of injuries in the laboratory, and I will observe them in my lab work.

INSTRUCTOR'S NAME:

STUDENT'S NAME (PRINT)

STUDENT'S SIGNATURE

DATE