A survey of proteomics methods for classroom and laboratory teaching

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Proteomics is one of the most important new fields of study in the postgenomic era. In order to bring the study of proteomics into the classroom and laboratory, we have been working on several related projects that explore the potential of proteomics in a teaching environment. Two of the projects involve the molecular visualization software VMD and Chime. Both programs are freely available and utilize three-dimensional protein structure data deposited at the Brookhaven Protein Databank (http://www.pdb.org). We have written user's guides for each, and have created a template web page that can be used by students to script their own Chime-based web pages. For the third project, we attempted to streamline a procedure for crystallizing lysozyme from hen egg whites, using the vapor diffusion method of protein crystallization. As a result, we have written a detailed crystallization protocol for lysozyme, which explores the effects of boiling, freezing, differing solution contents, and adding reagents, on the success of crystallization. This protocol includes a brief history of the applications of crystallization, suggested background readings, and a teacher's manual. It is intended that these projects will be the foundation for group investigations and laboratory projects in future biology courses.

Introduction

A proteome is the measurement of all the proteins in a cell at a given time. Since the ability to readily obtain genomic information is no longer a barrier, one of the major challenges that biology faces now is understanding and describing proteomes. Proteomics is a relatively new field that has arisen to address this, and its ultimate goal is to understand the functional role of all proteins. Therefore, the development of high-throughput methods for isolating, identifying, and characterizing proteins has been necessary (Campbell, 2003).

One of the most crucial methods by which biologists gain information about the shape, function, and character of proteins is through three-dimensional molecular visualization (Campbell, 2003). Interestingly enough, the methods that give us the ability to observe proteins' three-dimensional shapes have been around for a fairly long time. Crystallization and crystallography, two different techniques that go hand in hand, have been used to determine three-dimensional structure in proteins since 1934. In 1934, Bernal and Hodgkin were the first to discover that protein crystals surrounded by their mother liquor give better diffraction patterns than dried crystals (Tulinsky, 1999). X-ray crystallography allows us to determine the three dimensional shapes of proteins. In performing x-ray crystallography, proteins are subjected to x-ray beams, which results in the beams' diffraction. Since lenses cannot focus x-ray diffraction, computers analyze

the intensity and direction of the beams' dispersal. Then, the computer uses the data to construct an image of the molecule. In the past, the computer's role was performed using rigorous mathematics, and three-dimensional models, rather than computer images, were the primary means of visualizing proteins (Rhodes, 1993).

Before crystallography can be performed, it is necessary to crystallize the protein of interest; merely possessing it in a purified form is not enough. This is because most x-ray beams pass through powder or dissolved protein molecules without being diffracted. Crystals, on the other hand, are very effective because the ordered array of crystallized molecules plainly diffracts the x-ray beams, and the resulting diffraction pattern is intense and easily detectable (Rhodes, 1993).

In order to perform successful crystallography, it is necessary to grow well-ordered crystals using purified protein. Since protein molecules are globular by nature, their ability to arrange themselves into a crystalline lattice is impeded. The problem lies in the fact that their globular shape results in the formation of crevices and channels within the crystal. These crevices and channels are filled with solvent, because crystallization is performed under wet conditions. Usually, noncovalent bonds, which are necessary for holding the crystalline lattice together, must be formed through several layers of solvent molecules. This seriously weakens the strength of the bond, and as a result, the protein crystal is much more fragile than a crystal formed by, say, sodium chloride (Rhodes, 1993).

Once a well-ordered crystal has been shot with an x-ray beam and a computer analyzes the diffraction pattern, researchers can use the data to produce three-dimensional computer images called PDB files. PDB files can be run by a number of programs, such as Chime-based Java applets, RasMol, and VMD. PDB files are stored in a large depository called the Brookhaven Protein Databank. These files are freely available for download and constitute the most comprehensive collection of three-dimensional molecular data available (Berman, et al., 2000).

The goal of our study has been to explore the possibility of bringing proteomics into the classroom by utilizing both computer and bench-based approaches to learning. Over the course of this semester, we developed a template frameset for the production of Chime-based tutorials, along with an accompanying manual. Chime-tutorials are simple framed or unframed web pages which allow the viewer to automatically orient three-dimensional representations of proteins in various ways by clicking buttons which are embedded into informative text. This Chime-tutorial "kit" allows students to jumpstart their own production of unique Chime tutorial web pages. They are encouraged to find their own molecule at the Brookhaven Protein Databank. The manual provides detailed instructions on how to manipulate the three-dimensional molecule in a number of ways, so that different features of the protein may be highlighted. Thus, the student is available to customize this template to a large degree.

The second project of the study was to attempt to optimize the program Visual Molecular Display, or VMD, designed by the Theoretical Biophyisics group at the

University of Illinois and the Beckman Institute. This program is compatible with a number of different file types, one of which is the PDB format. The strength of this program lies in its ability to project stereoscopic displays that presents the viewer with a truly three-dimensional image of the molecule that seems to float in mid-air. Due to the amount of RAM required to produce the three-dimensional image, VMD can be quite cumbersome. We attempted to determine which parameters to tweak for maximum performance, and in doing so developed a manual that gives suggestions for both optimization and general use. Furthermore, some preset images have been saved and are available on the Davidson Biology Network (www.bio.davidson.edu/courses/genomics/lab/proteomics/john.html), which provide the user with examples of interesting ways in which VMD can be used.

Finally, we developed a laboratory protocol for the crystallization of lysozyme. Lysozyme is particularly conducive to a laboratory instruction course because of its tendency to crystallize, even after being subject to boiling or foreign reagents. Protein crystallization is notoriously difficult, evidenced by the fact that the Syrrx Corporation's crystallization robot, Agincourt, has performed over 5 million crystallization experiments to date and only successfully crystallized 300 proteins (Syrrx, Inc., 2003). Therefore, it is important to have a protein that is hardy, crystallizes relatively quickly (some crystallizations take over a month), and readily available.

This paper includes the three manuals produced over the course of the semester, beginning with the crystallization protocol, followed by the Chime tutorial manual, and the VMD manual. The crystallization protocol is first because it represents the order in which three-dimensional visualization develops: before a protein's three-dimensional coordinates can be deposited at the Brookhaven Protein Databank, it must be crystallized and subject to x-ray in order to determine a diffraction pattern. Although we did not address crystallography this semester, it is hoped that in the future, the diffraction patterns of lysozyme crystals developed by this lab will be read and used to produce PDB files.

Chapter 1: Protocol for the Crystallization of Lysozyme

Introduction

The following is a question-based introduction to the world of protein crystallization. Due to the inefficiency inherent in crystallizing a protein from scratch, robots carry out a great number of the crystallizations performed today. This is mainly because robots are highly accurate, yield data in a high-throughput fashion, and do not complain when asked to perform hundreds upon hundreds of experiments, the majority of which probably will not work. On the other hand, such a tendency towards failure can be frustrating and discouraging for a researcher.   Researchers do still attempt crystallization by hand, whether it is for a tried-and-true protein such as lysozyme, or a protein that has never been crystallized before. Therefore, it is important to learn about crystallization, because it is an important step in determining the three-dimensional structure of proteins, something that is of extreme importance to the field of proteomics.   Furthermore, since the crystallization method employed by robots is based on the same principles as crystallization performed by hand, to a large extent we are gaining insight into the basis of a type of high-throughput technology. Finally, few lab experiments yield results which are as aesthetic as those obtained by crystallization. For this reason, crystallization can be very rewarding.

Materials and basic theory

Equipment needed:	*Reagents needed*:
Crystallization wells	Sodium Acetate
Pipettemen	Acetic Acid
Dissecting microscope	Sodium Chloride
Clear packing tape and scissors	H_2O

Most commonly, the goal of crystallization is to produce a well-ordered crystal that is lacking in contaminants and large enough to provide a diffraction pattern when hit with x-ray. This diffraction pattern can then be analyzed to discern the protein's three-dimensional structure. Although necessary reagents differ from protein to protein, the general process by which crystallization occurs, called vapor diffusion, is the same. In vapor diffusion, a droplet containing purified protein, buffer, and precipitant is allowed to equilibrate with a larger reservoir containing similar buffers and precipitants in higher concentrations. Initially, the droplet of protein solution contains an insufficient concentration of precipitant for crystallization, but as water vaporizes from the drop and transfers to the reservoir, the precipitant concentration increases to a level optimal for crystallization. Since the system is in equilibrium, these optimum conditions are maintained until the crystallization is complete (Rhodes, 1993; McRee, 1993). The system is airtight, and the protein solution is elevated, and the droplet either hangs from the chamber's ceiling or sits in a pedestal above the reservoir. In our experiments, we will be using crystallization wells that utilize the sitting drop method (Figs. 1-3).
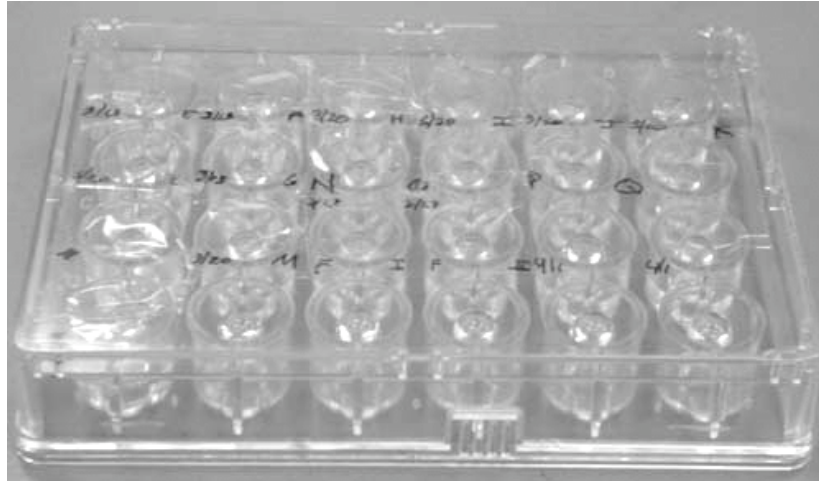
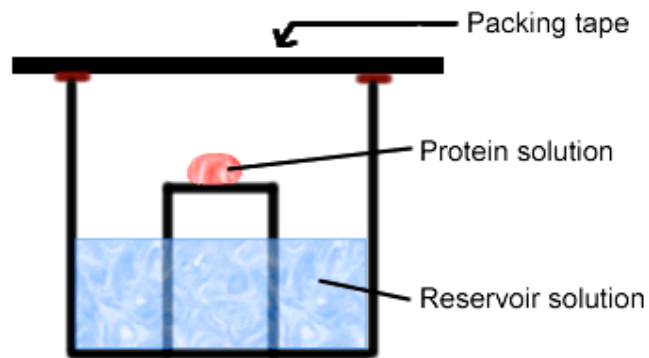**Figure 1. A 24-well crystallization tray.** Photo by John M. Kogoy

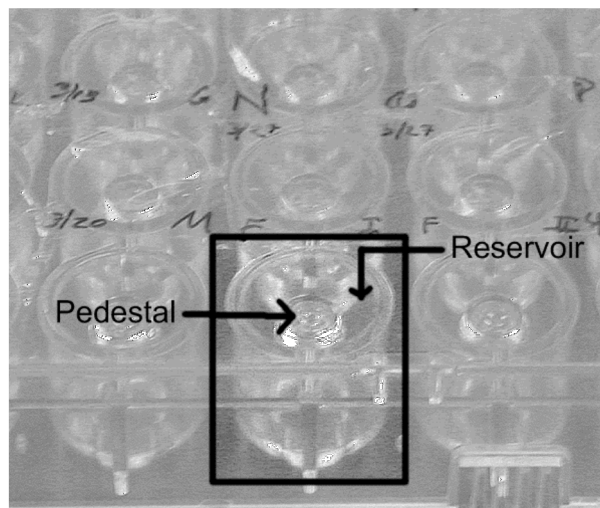**Figure 2. A schematic of a well and its components.**

Figure 3. A close-up of an actual well. Photo by John M. Kogoy

Mixing the Reagents

Solution 1. Make 10X (1M) stock of NaOAc, pH 4.8
    a. Weight out NaOAc
    b. Dissolve in about _ total volume H_2O
    c. pH to 4.8 using acetic acid
    d. Bring up final volume so that the final solution is 1M.
Solution 2. Make reservoir solution (8% w/v NaCl, 0.1 M NaOAc, pH 4.8)
    a. To make 10 mL, weight out 0.8 g NaCl
    b. Add 1 mL of 10X stock solution 1.
    c. Bring volume up to 10 mL with H_2O.
Solution 3. Make protein solution (50 mg/mL lysozyme, 0.1 M NaOAc, pH 4.8)
    a. First, make solution by dissolving 10X stock in H_2O to yield 1X solution.
    b. Weigh out lysozyme.
    c. Add 1X protein solution so that final solution is 50 mg/ml
    d. Store in the freezer.

Procedures

Important: When pipetting 7.5 _L portions of protein and reservoir solution onto the pedestals, be extremely careful to avoid bubble formation. This often occurs when air is blown out of the pipette. Avoid making bubbles, even if it means not dispensing all 7.5 _L of solution.

I. *Control Conditions for Crystallization of Lysozyme*:
Can we use this simple procedure to grow lysozyme crystals?

1. Select crystallization well.
2. Load reservoir with 600 mL reservoir solution.
3. Load pedestal with 7.5 _L reservoir solution.
4. Load pedestal with 7.5 _L protein solution.
5. Cover well with packing tape so that it is airtight.
6. Periodically check for crystals over the next 24 hours.

II. *The Effects of Boiling on Lysozyme Crystallization*
Will boiling denature the protein and thus impede crystallization?

1. Take 50 _L protein solution aliquot. Transfer to microfuge tube.
2. Heat microfuge tube for 3 minutes at 95^oC.
3. Remove microfuge tube from heat, allow it to cool.
4. Select crystallization well.
5. Load reservoir with 600 mL reservoir solution.

6. Load pedestal with 7.5 _L reservoir solution.
7. Load pedestal with 7.5 _L of the boiled protein solution.
8. Seal off well with packing tape, and label.

9. Can you make any immediate observations?

_____

_____

10. Do you think crystals will form? Y/N
11. Periodically check for crystal formation over the next 24 hours.
12. Perform part I, steps 1-6 again as a control experiment.

III. *The Effects of Repeated/Freezing Thawing on Lysozyme Crystallization*
In order to avoid mixing a fresh batch of protein solution every time we perform a crystallize experiment, we freeze our protein solution for later use. Does freezing and thawing a protein repeatedly have any effect on its ability to crystallize?

1. Transfer 5 50 _L aliquots of protein solution to five microfuge tubes. Label them 1-5.
2. Freeze all 5 tubes.
3. Remove all tubes from the freezer. Allow them to thaw. Return tubes 2-5 to the freezer.
4. Repeat until each tube has been frozen and thawed as many times as its number denotes (e.g. tube 1 has been frozen and thawed once, tube five has been frozen and thawed five times).
5. Select a well.
6. Load reservoir with 600 mL reservoir solution.
7. Load pedestal with 7.5 _L reservoir solution.
8. Load pedestal with 7.5 _L of the f/t protein solution.
9. Seal off the well with packing tape and label.
10. Set up crystallization experiments in duplicate for each condition (e.g. perform steps 5-8 twice for each microfuge tube.
11. Perform part I, steps 1-6 again as a control experiment.

IV. *The Effects of a Foreign Reagent on Lysozyme Crystallization*
The presence of an ion or foreign reagent can have positive, negative, or neutral effects on crystallization. For instance, for quite some time, scientists were confounded as to why insulin would crystallize some times, and not others. Finally, they realized that the samples that would crystallize had been collected in galvanized containers. They made the connection that the presence of zinc must be necessary for the crystallization of lysozyme. What do you think will happen if we add a tiny amount of bromophenol blue to our pedestal solution?

1. Prepare a 1% w/v solution of bromophenol blue
2. Add 0.5 _L bromophenol blue (1% w/v) to protein solution
3. Load reservoir with 600 mL reservoir solution.
4. Load pedestal with 7.5 _L reservoir solution.

5. Load pedestal with 7.5 _L of the protein + bromophenol blue solution.
6. Seal off the well with packing tape and label.
7. Perform part I, steps 1-6 again as a control experiment.

V. *The Effects of a Different Reservoir Solution on Lysozyme Crystallization*
When crystallizing a protein for the first time, no one really knows what the optimum reagents will be. In fact, it is difficult to predict whether a set of reagents will cause a protein to crystallize at all. In order to boost the efficiency of "pioneer" crystallization experiments, there are companies who produce "crystallization kits." These kits contain many different pre-mixed reagents, so that the researcher can subject their protein to each reagent, in what is known as the "shotgun" method to crystallization. If any of the solutions yields crystals, then the researcher take note of the particular reagents and exclusively use them for their experiments. Here, we will set up an experiment to test the effectiveness of several different solutions on lysozyme crystallization. Which, other than the control, do you expect to be the most effective? Perform each experiment in duplicate.

Control:
1. Select crystallization well.
2. Load reservoir with 600 mL reservoir solution (8% w/v NaCl, 0.1 M NaOAc, pH 4.8).
3. Load pedestal with 7.5 _L reservoir solution (8% w/v NaCl, 0.1 M NaOAc, pH 4.8).
4. Load pedestal with 7.5 _L protein solution (50 mg/mL lysozyme, 0.1 M NaOAc, pH 4.8).
5. Cover well with packing tape so that it is airtight.
6. Periodically check for crystals over the next 24 hours.

PEG 600/Citrate Reagent:
1. Select crystallization well.
2. Load reservoir with 600 mL reservoir solution (40% v/v PEG 600, 100 mM citrate).
3. Load pedestal with 7.5 _L reservoir solution (40% v/v PEG 600, 100 mM citrate).
4. Load pedestal with 7.5 _L protein solution (50 mg/mL lysozyme, 0.1 M NaOAc, pH 4.8).
5. Cover well with packing tape so that it is airtight.
6. Periodically check for crystals over the next 24 hours.

PEG 300/Acetate/NaCl Reagent:
1. Select crystallization well.
2. Load reservoir with 600 mL reservoir solution (40% w/v PEG 300, 100 mM acetate, 200 mM NaCl).
3. Load pedestal with 7.5 _L reservoir solution (40% w/v PEG 300, 100 mM acetate, 200 mM NaCl).
4. Load pedestal with 7.5 _L protein solution (50 mg/mL lysozyme, 0.1 M NaOAc, pH 4.8).
5. Cover well with packing tape so that it is airtight.
6. Periodically check for crystals over the next 24 hours.

Propanol/Acetate Reagent:

1. Select crystallization well.
2. Load reservoir with 600 mL reservoir solution (35% v/v 2-propanol, 100 mM acetate).
3. Load pedestal with 7.5 _L reservoir solution (35% v/v 2-propanol, 100 mM acetate).
4. Load pedestal with 7.5 _L protein solution (50 mg/mL lysozyme, 0.1 M NaOAc, pH 4.8).
5. Cover well with packing tape so that it is airtight.
6. Periodically check for crystals over the next 24 hours.

# Chapter two: Teacher's Manual for Lysozyme Crystallization

The purpose of this manual is to let you know what to expect regarding each experiment, as well as give pictures of crystals from each example.
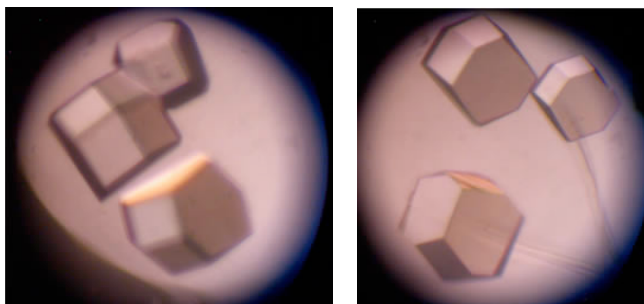
Various pictures of crystals

Below are some of the different crystal formations we have observed (Photos by John M. Kogoy):
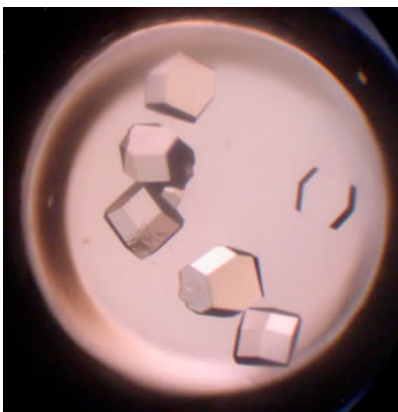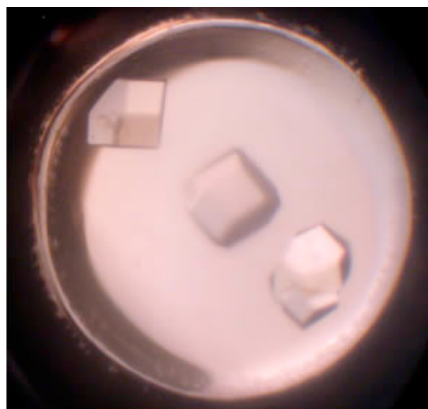

1. Crystals after 5 minutes in 45° C heat (2X magnification).
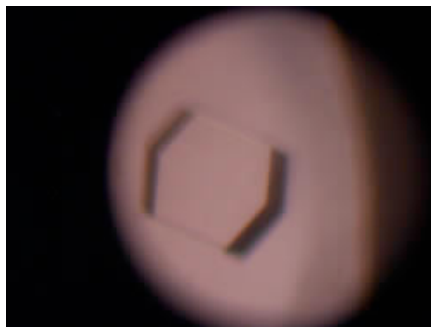
2. Detail of crystal from above (8X magnification)

**3. Magnification of large crystals from control experiments (8X).**

4. Large crystals grown in control conditions (2X).

Basic crystallization of lysozyme

These conditions never failed to form crystals for me. Therefore, crystals should be expected to form, and these conditions can be used as a control for the proceeding experiments. You might have the students attempt to form crystals by replacing the reservoir solution with water as a control for this experiment itself. I never tried it, but I am assuming it will not work because the vapor diffusion should be reversed.

For these conditions, we have found that crystal formation can occur in anywhere from 7 hours to over 20 hours. It is impossible to predict how quickly crystallization will occur, from my experience, but we can say it should be within 24 hours. Usually 3-5 fairly large crystals will form.

For an example of some large crystals grown in control conditions, please see **4** above. Here is a picture of a unique situation where only one large crystal formed:
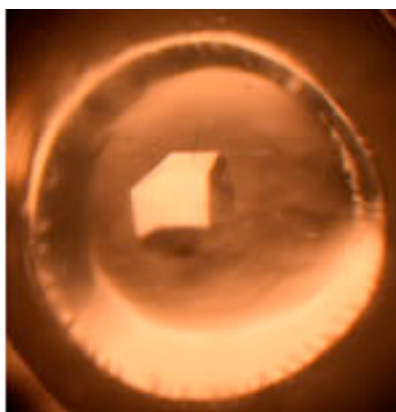
5. Large crystal grown under control conditions.

The Effects of Boiling on Lysozyme Crystallization

The first thing students should notice upon performing this procedure is that boiling causes the formation of a precipitate. Contrary to what one might expect, however, crystals will still form. This experiment was repeated six times. Each time, a precipitate formed, but crystals still grew in the midst of the precipitate on every occasion. It has been speculated that the precipitate is either an impurity that is falling out upon boiling,

or that some but not all the lysozyme is precipitating. Usually more smaller crystals form, and they are not as sharp as crystals grown under other conditions.

6. Crystals formed after 3 minutes of boiling at 95º C

The Effects of Repeated/Freezing Thawing on Lysozyme Crystallization
This experiment was performed to see if the repeated freezing and thawing that the protein solution is subjected to has any effect on its ability to crystallize. This experiment was performed with each condition in duplicate, and crystals formed for all conditions. In spite of one's possible expectations, there was no observable difference between the control crystals and the crystals that formed in the experimental conditions. The teacher may ask the students to conclude whether or not they conclude that freezing and thawing has an observable effect on crystal growth, and if they feel that this justifies storage by freezing, rather than making a fresh protein solution each time.
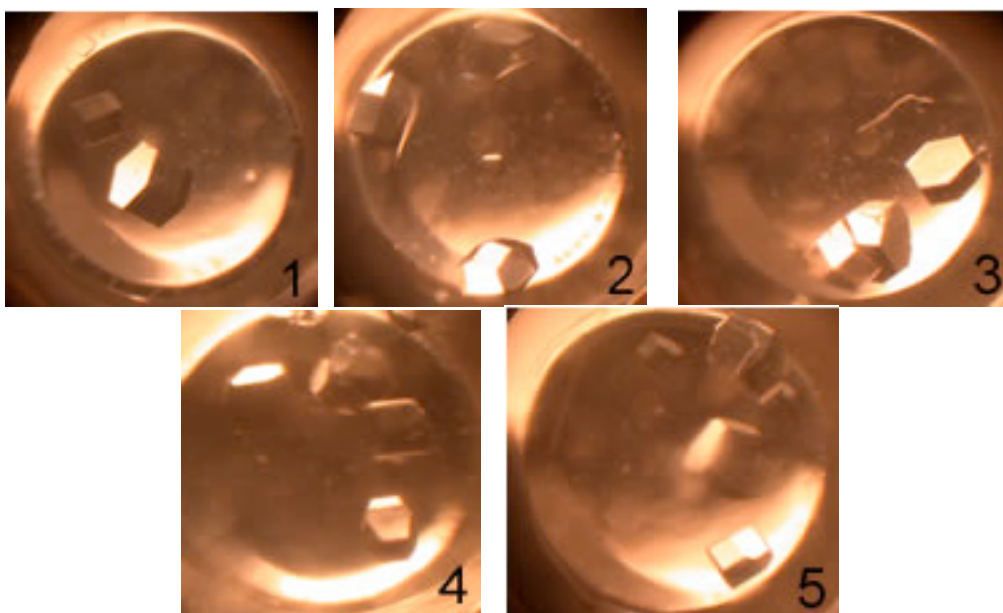
7. Crystals after increasing freezing and thawing. In each case, the number of crystals present was within the same observed range as the crystals I grew under control conditions.

The Effects of a Foreign Reagent on Lysozyme Crystallization

Over the course of my experiment, I found lysozyme to be quite hardy, and for the most part resistant to alterations of experimental conditions. I boiled it, heated it for 3, 5, 7, and 9 minutes at 45°C, froze and thawed it repeatedly, and attempted to crystallize it in completely different reagents. Although some of these, namely boiling and exposure to different reservoir solutions had slight effects in crystal morphology, none had as dramatic of an effect as introducing bromophenol blue into the protein solution. The presence of bromophenol blue caused the formation of hundreds of tiny crystals. The difference is dramatic.
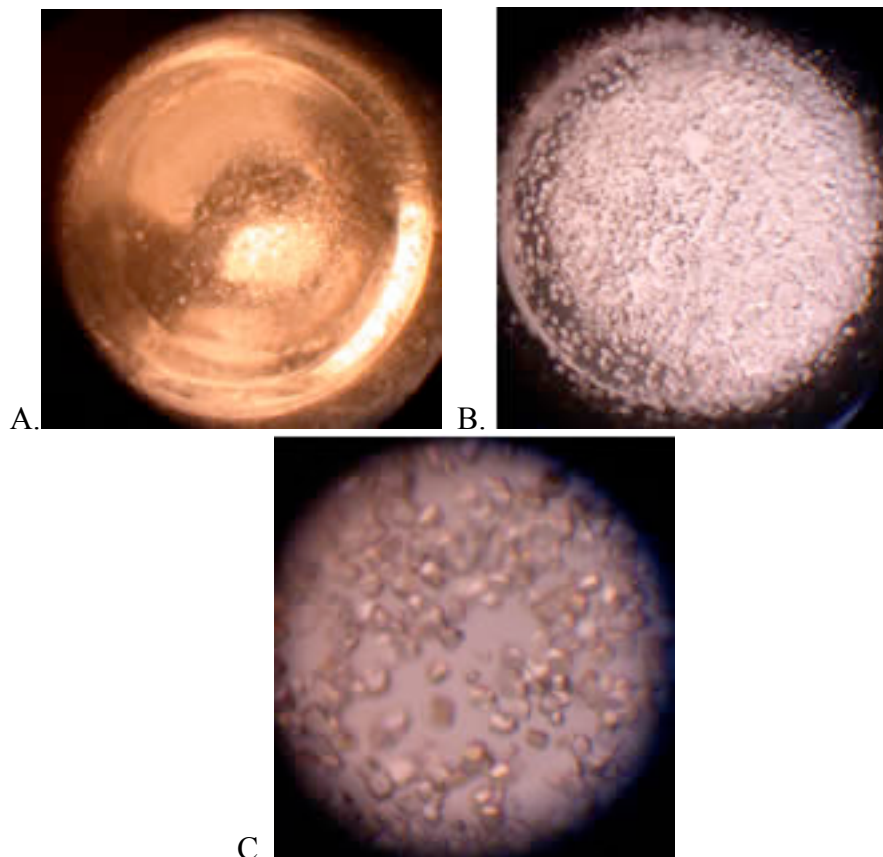

8. A) 2X magnification of crystals formed in the presence of bromophenol blue. B) 2X magnification of crystals grown under control conditions that errantly resemble the crystals grown with bromophenol blue. C. 8X magnification of B.

The Effects of a Different Reservoir Solution on Lysozyme Crystallization

In an attempt to test the hardiness of lysozyme, I subjected it to a number of alternative reagents that came as part of a crystallization kit produced by Emerald BioStructures. I have included three of these reagents because of their interesting results in my own experiment. The first (40% v/v PEG 600, 100 mM citrate) was chosen because it successfully resulted in crystals, in spite of the fact that the reagent ingredients are completely different from the control reagents. The second (40% w/v PEG 300, 100 mM acetate, 200 mM NaCl) was chosen because it resembles the control scenario but does not

result in crystallization.   The third (35% v/v 2-propanol, 100 mM acetate) was chosen because it results in a precipitate. I think this experiment teaches a lesson in the unpredictable nature of protein crystallization, because one would expect the second reagent to effectively crystallize lysozyme, whereas one would have no reason to expect the first reagent.

A.   B.   C.
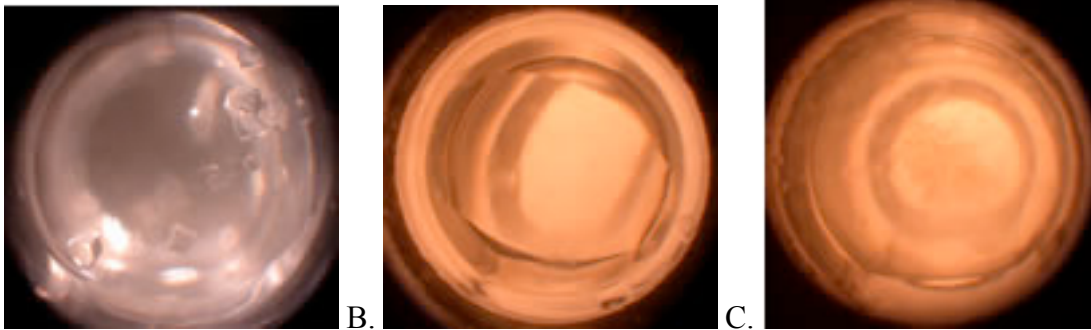
9. The effects of three alternative reagents on lysozyme crystallization. A) A number of crystals have formed using PEG 600/Citrate. Note that they are not as sharp as those formed in control experiments. B) Empty pedestal containing solution but no crystals, using PEG 300/Acetate/NaCl reagent. C) Precipitate formed by 2-Propanol/Acetate solution; no crystals.

Chapter 3: Getting Started With Your Chime Tutorial

Things Needed (included in Chime_Tutorial.zip)

This manual

Your favorite PDB file (this package contains de-oxy hemoglobin as an example).

Template files (templateframe.html, templatebuttons.html, and templateleft.html)

Make sure the Chime plug-in has been installed on your computer. It can be downloaded at http://www.mdlchime.com/chime/.

How to Download a PDB File:

Go to http://www.pdb.org

Use the search tool to search for the protein you want.

If you get hits, the page will show a list that looks like this:

☐ **1A00**	▣▤▣ *Deposited:* **08-Dec-1997** *Exp. Method:* **X-ray Diffraction** *Resolution:* **2.00 Å**	{ EXPLORE }
Title	Hemoglobin (Val β1 Met, Trp β37 Tyr) Mutant	
Classification	Oxygen Transport	
Compound	Mol_Id: 1; Molecule: Deoxyhemoglobin A; Chain: A, B, C, D; Engineered: Synthetic β Globin Gene; Mutation: Chain B, D, V1M, W37Y; Biological_Unit: Tetramer	
☐ **1A01**	▣▤▣ *Deposited:* **08-Dec-1997** *Exp. Method:* **X-ray Diffraction** *Resolution:* **1.80 Å**	{ EXPLORE }
Title	Hemoglobin (Val β1 Met, Trp β37 Ala) Mutant	
Classification	Oxygen Transport	
Compound	Mol_Id: 1; Molecule: Deoxyhemoglobin A; Chain: A, B, C, D; Engineered: Synthetic β Globin Gene; Mutation: Chain B, D, V1M, W37Y; Biological_Unit: Tetramer	

If the protein you want is in this list, click on the red Explore on the right. This will take you to the page for that protein. This page gives you the name of the document that accompanies this PDB file, if available, as well as a brief description of the molecule and what is depicted in the PDB file. On the left you will see a menu, with the items, "summary information, view structure, download display file, etc." If you choose "View Structure" you will be taken to a page that accesses a number of Chime plug-ins. You can use these to preview your protein before you download. To download the protein's PDB file, go to "Download Display File." Scroll down until you see this menu:

Download the Structure File:

Choose from the following file and compression formats:

	file format	
compression	PDB	mmCIF
none	X	X
Unix compressed	X	X
GNU zipped ("gzipped")	X	X
ZIPped	X	X

Mouse over the X under Column "PDB" and Row "none." On a PC, right click and "save target as" to the folder of your choice. You have now downloaded your PDB file and can use it for your Chime tutorial. If you are using a Macintosh, do the following.

Let's Begin:
Make sure all 4 files (1A3N.PDB file + 3 template web pages) are all in one folder. The
file 1A3N (deoxyhemoglobin) has been included as a default to show you an example of
a working chime file. If you want to explore, the 3 web pages are configured to show a
simple tutorial with one working button for deoxyhemoglobin. Familiarizing yourself
with the html for these web pages using "View Source" may be useful in helping you to
become comfortable with way they are organized. On a PC, right click on a page and
select "View Source." This will allow you to view the code for the web page. Skim over
the html in order to see the basic layout and organization of the html code. That way,
when you begin to follow the directions below, you will not be a stranger to the format.
In order to make your own tutorial, you must add your own PDB file to this folder.
These can be downloaded at http://www.pdb.org. The process is described in detail
above.

It is important to remember that this process does not employ any sort of web editor such
as Frontpage or Dreamweaver. We will be working with the html itself. If you are new
to html, don't be intimidated. Minimal knowledge of html is necessary.

Now we can get started. Open templateframe.html. You will see a two-frame web page,
with the PDB file in the left frame, and the area for text and buttons in the right frame.
On a PC, go to the "View" pull-down menu. Select "Source." This will open notepad.
On a Macintosh, do the following steps. Here you will see, among a lot of other html, the
following text:

<frameset cols="70%, 30%"><!-- "*,*" means 2 equal-sized columns -->
    <frame src="templateleft.html" name="chimeleft">
    <frame src="templatebuttons.html" name="chimeright">
</frameset>

Basically, this is showing us that contents of the frames are derived from other html files.
This templateframe.html page does not really contain any content, other than these
directions and a title.
You will also notice the following:
<!-- A simple 2-frame page -->
The <!-- *** --> notation allows you to type within the arrows without that information
being displayed on the website. This is useful for making notes to oneself. You will
notice hints and directions have been inserted throughout the source codes of the three
websites using this notation.
You can now close this notepad file.

Editing the Left Frame:
On the PC, right click on the left frame, and select "View Source." On the Macintosh, do
this. The first modification you will learn how to make is changing the page's title. At
the top, find the line
<title>*3D Structure of *A PROTEIN*</title>

Replace *A PROTEIN* with the name of your protein.

Next, you will insert your own PDB file.
Here you will see the following text:
<embed src="1a3n.pdb" align=center bgcolor="#000000"
    width="450" height="450"
    name="myfavorite"></embed></div>

"1a3n.pdb" is the file for deoxy-hemoglobin. Within the quotes, replace 1a3n.pdb with the name of *your* PDB file. Also, notice the line 'name="myfavorite"' It is a good idea to change this to something that relates to the protein whose PDB file you are displaying, for instance, "hemoglobin". Remember what you put down here because you will need it again for the right frame.
Now click "File" and select "Save As". Save the file under a name related to your protein; keep it short if possible. You may also want to add the suffix "left" to the name, for example: "hemoglobinleft.html". This helps with organization but is not required. This window can now be closed.

Editing the Frame Set, Editing the Right Frame, and Adding a script
Bring back the templateframe.html page. Move the mouse over the right frame, and right click on a PC. Select "View Source." Once again, another Notepad window will open, so that you can edit the html. On a Macintosh, you follow these steps.
First, go to "File" and choose "Save As." Once again, name this file *.html. Following the same logic as above, you may want to make it *buttons.html.

Bring the templateframe.html back up. Select the "View" pull-down menu from the top menu. Select "source." Once again, choose "Save As" and name this file in a similar fashion as before.
Now we are going to direct the frameset to the two web pages that will be seen inside the right and left frames.
Remember this from earlier:
<frameset cols="70%, 30%"><!-- "*,*" means 2 equal-sized columns -->
    <frame src="templateleft.html" name="chimeleft">
    <frame src="templatebuttons.html" name="chimeright">
</frameset>

Using the names you designated for your left and right frames, replace
"templateleft.html" and "templatebuttons.html", respectively.
Click "File." And "Save." You can close this notepad. You can also close templateframe.html.
Open the website that designates your frameset.

Mouse over the right frame. Right click, and select "View Source." On a Macintosh, do this.

The default title reads
<title>MY FAVORITE PROTEIN</title>
You can change this to the title of your choice. Slightly lower you will see:

<h2>3D Structure of **A PROTEIN**</h2>

You can change this too. Scroll down a bit, and you will see some notations made using the <!-- * --> format.
After some text, you should also see the following command:

<embed type="application/x-spt" width=13 height=13 button=push target="myfavorite" script="

Go ahead and replace "myfavorite" with the name that I told you to remember on page 17. Remember to leave the quotes. This command directs the button to the chime-plug-in window located in the left frame.
One line down you will see "reset", "slab off", etc. This is the beginning of the script that I wrote for hemoglobin as a demo. Starting with "reset", select this text and scroll down so that you have highlighted all the text between "reset" and "select all". Delete this text. You will now see:

Let's display the molecule as four distinct peptide chains. Click <embed type="application/x-spt" width=13 height=13 button=push target="myfavorite ⇓(you will have changed this by now, hopefully)"
script="

"> here.

You can replace my text ("Let's…Click.") with your own, remembering that what will appear in the text instead of <embed……"> is a little grey button. You will probably want to type something that explains the way your molecule will be depicted in the Chime window after the button is clicked.

Now go back to your frameset and manipulate your Chime protein to the desired depiction. Once you have achieved this, right click on the Chime window. Scroll down to "Edit" and select "Copy Chime Script."

Now go back to the html code for your right frame. Place the cursor between the quotation marks, right click and paste the script into this area. The script is now located within the "embed" command. You are not done yet, though. Scroll back up to the beginning of your script. Remember, that scripts are sometimes very long, so be sure you scroll to beginning of the correct one.

At the beginning of the script, you will see this:

#!rasmol -script
File: \s3ds.
Creator: Chime Version 2.0

set load check on
load pdb "1msw.pdb"
background [0,0,0]
set ambient 60
set specular off

Delete all of this. You must do this every time you create a new button and copy a script. Do not delete the five lines that resemble this:

reset
slab off
rotate z 67
rotate y 49
rotate x 109

These commands are very important. The rotate x, y, and z commands determine the three-dimensional orientation of the molecule. The reset command brings the molecule back to its default three-dimensional orientation so that the old orientation does not carry over. The slab command is there so that slab mode will be turned off as a default. If slab mode is unintentionally on, it may disrupt your image. For more information on slab mode, see page 25.
Click on file and save your work.

Bring back your frameset and refresh the page. Hopefully your page appears with some text and a button. Click on the button to see if the script executes correctly.

Adding More Text and Buttons
Remember you can add a blank line between blocks of text using

You can begin a new paragraph using

When you want to add another button, simply type in the desired text, and copy and paste
<embed type="application/x-spt" width=13 height=13 button=push target="myfavorite" script="

from somewhere else in the html. The skip several lines and add this:

">

followed by any text that you want to occur after the grey button. Then go back to your PDB image, manipulate it to the desired depiction, and copy the Chime script using the

procedure above. Paste the chime script between the quotation marks and remember to delete the top lines that look like this:

#!rasmol -script
File: \s3ds.
Creator: Chime Version 2.0

set load check on
load pdb "1msw.pdb"
background [0,0,0]
set ambient 60
set specular off

Using the procedure described above, you can make a page using any number of combinations of text and buttons.
In addition to the parameters available within the Chime plugin, there are programs available which can assist you in manipulating the PDB file in more advanced ways. One such program is called RASMOL and is available for download at this website: http://www.umass.edu/microbio/rasmol/getras.htm

Hints for Efficient Script-writing

The above instructions are included to introduce you to the creation of chime scripts for chime tutorials, but I will now suggest a method to producing scripts in a more organized and efficient manner, once you get your bearings. Here is a table with some commands you should be familiar with:

Command	Possible values
Select (use this command to specify the target of other commands).	all, DNA, amino, nucleic, ala, cys, aromatic, hetero, etc.
Color	green, red, blue, magenta, etc.
spacefill	on, off, a value such as 100 or 500 (500 having a larger radius)
wireframe	on, off, 100, 399
reset	n/a
hbond	on, off

1) Here is a default script that you can copy and paste into your html source each time you add a new button. Please recognize that the presence of # before a command line denotes that the line will not be read as code. Placing # before a line can allow you to make notes to yourself within the script.

<embed type="application/x-spt" width=13 height=13 button=push target="myfavorite" script="
reset
slab off
rotate z -117

```
rotate y 15
rotate x -64
translate y -2
zoom 140

center all
translate center center
set zoom center true
set bonds off
set axes off
set boundingbox off
set unitcell off
set bondmode and
dots off


# Avoid Colour Problems!
select all
colour bonds none
colour backbone none
colour hbonds none
colour ssbonds none
colour ribbons none
colour white

# Atoms
select all
spacefill off

# Bonds
wireframe 50

# Ribbons
ribbons off

# Backbone
backbone off

# Labels
labels off

# Monitors
monitors off

ssbonds off
hbonds off
```

Definitions

Current Selection
set bondmode and
select all

2) With this default script, you can copy it into the source for your right-hand frame each time you want to add a new button. Edit this script directly, rather than having the Chime plug-in generate your scripts. In this way, you avoid the problem of very long, slow-loading scripts that are cumbersome to edit. You will need to generate scripts from the Chime plug-in to determine the molecule's coordinates. This is process is outlined in step 3.

3) Each time you want to create a new button, you can use the default script from # 1 above as a template of sorts, having pasted it into your source and change its parameters on your own. You should still use the Chime window to manipulate your molecule. Once you have achieved the desired orientation of your molecule, you will want to copy the new Chime script into yet another Notepad window, for no other reason than to copy the coordinates into the default script. The only information from the new script you will need will look something like this:

reset
slab off
rotate z -117
rotate y 14
rotate x -64
translate y -2
zoom 140

This information describes the 3-dimensional orientation of the molecule. Find the corresponding information in your default script and replace it with this new information. You are now ready to change some internal parameters, such as molecule display. As you scroll down your script, you should see these headings, followed by parameters for each one:

Atoms
Bonds
Ribbons
Backbone

I will now describe what each of these headings contains and how to use them to display the molecule in the intended way.

 # Atoms ◊ Under this heading, you can alter the color displayed or determine whether or not you would like to display the molecule as "space-filling" and to what degree. By this

time, you should know that you can type in the "select" command to choose a specific portion of the molecule. For instance, if you would like to color amino acids 1-40 green and 41-60 blue, as well as set spacefill for the entire molecule at 500, you enter the following information under the # Atoms heading:

select 1-40
color green
select 41-60
color blue
select all
spacefill 500

 # Bonds ◊ Under this heading, you determine the degree of "wireframe" displayed for the entire molecule or a portion of it. For instance, if you want wireframe to be set at a relatively thin level for all of the molecule you would type:

select all
wireframe 50

If you would like a subset of the molecule (hypothetically, residues 25-100) to be displayed significantly thicker, type this:

select 25-100
wireframe 200

 # Ribbons ◊ This heading allows you to present the molecule in a ribbon display. If you want to depict all of the molecule, or a portion of the molecule, in a "Ribbons" display, remember to go back and turn off both spacefill and wireframe for that portion of the molecule under the headings above. Let's say you wanted to depict residues 25-40 as ribbons. Do the following:

Go back to #Atoms, and make sure the following settings are present:
select 25-40s
spacefill off

Repeat the process for the #Bonds heading to make sure wireframe is off for residues 25-40.
Now go to the #Ribbons heading and type:

select 25-40
ribbons on

Hopefully you have the hang of it by now. The category #Backbone can be dealt with in the same manner as #Ribbons.

Once you have completed your script, you can paste it into the source for your right-hand frame, save your work, and **refresh the frameset**. It is a common error for a person to make changes, load up their page, forget to refresh, and wonder why their changes are not readily apparent. Click on the button to see if the script executes the way you intended. If it does not, go back into the source for the right hand frame, alter the script, save your work, refresh the browser window for the frameset. Repeat this process until desired action executes.

4) It is also crucial to choose a "reset" orientation for your molecule. In other words, you should select some orientation and depiction of the molecule that you would like the molecule to exhibit upon opening the page. This is not to be confused with the default orientation to which the reset command returns the molecule. Often, the default orientation and the orientation you want to appear on opening the page will be different. This is done by adding the script of your "reset" orientation into the source for the left-hand frame directly after the html that specifies which Chime image is being depicted. Find this line:

<embed src="1msw.pdb" align=center bgcolor="#000000"
width=99% height=80%
ALIGN="BOTTOM"
name="anything"

Directly under this, add:
Script="

">

and paste the "reset" orientation script within the quotation marks.
Furthermore, it is also convenient to have a button that resets the image to this default display.

Tips for Advanced Display

Changing the size of the Chime window plug-in:
In the event that the chime window of this template is too big or too small, you can change the size of the window by changing the following values:
<embed src="1a3n.pdb" align=center bgcolor="#000000"
    width="450" height="450"
    name="myfavorite"></embed></div>
Notice that the values given for height and width are both 450 pixels. These values can be changed according to your application.

Making a portion of a molecule disappear:

There are times where you may want to display only a portion of the molecule, for various reasons. Here is an example of how to do it, which you can apply to your situation as necessary. Let's say you have a 400-residue protein and you only want to depict residues 1-300. In this situation, you must turn off all possible displays for residues 301-400. After you do this, only residues 1-300 will appear.
Under each category (#Atoms, #Bonds, etc.), make sure you select the unwanted residues (eg. select 301-400) and turn off the respective display (eg. "spacefill off," "wireframe off," etc.)

Slab Mode:
Slab mode allows you to peel away layers of the molecule as you zoom or rotate, which allows the viewer to see inside the molecule. This application can be useful for depicting certain structures that would otherwise be obscured by other structures. The easiest way to use slab mode is to manipulate the molecule in the Chime window with slab mode turned on (Right click on the window, highlight "options", click "slab mode.") Once you have the desired orientation, copy the Chime script to get the coordinates, replace the old coordinates with new ones as described above, and turn slab mode on by typing "slab on." On a PC, the Chime script is copied by right-clicking on the molecule, selecting edit, and then selecting "copy Chime script." On a Macintosh, you do this, this, and this.

Adding Labels:
You can add labels to certain residues to inform the viewer what particular residue they are seeing. The best method for labeling one or more residues is to determine an atom number from each of them by left clicking on the residue in an open chime window. The atom number will appear in the lower left hand column of the Internet browser. Let's say you want to label an alanine residue. Find that particular residue in the Chime window. Click on it anywhere to find an atom within that residue. Then go to the #Labels category and add the following (commands in blue, parameter descriptions in black):

labels off (this turns off labels for the rest of the molecule
color labels red (use this command to designate the color of the label)
set fontsize 8 (this command designates font size)
select atomno=# (let # represent the number of the atom within the residue you choose)
label # (let # represent the text you want to appear for your label. This can be anything, although you may want it to say "Alanine 58", for instance)

If you want to add labels to more than one residue, add new select atomno= and label lines for each additional residue. The reason you designate an atom number instead of a residue number is that selecting a residue number results in every atom of the residue being labeled, making it difficult to read.

Making color changes:
In the event that you would want to highlight certain structures by making them change color more than once or blink from yellow to black, etc. you can set the delay between color changes using the following commands, inserted into the script:

select # (let # represent the desired range of amino acids or residues)
color # (let # represent the desired color)
delay {#} (let # represent the desired duration between color changes (eg. 0.5)
color #
delay {#}
repeat as many times as is desired or necessary

Rotation and Other Scripted Movements
If used appropriately, this can add a very aesthetic feeling to your script. Simple movement such as rotation and zooming can be achieved individually or combined together. Movement commands can also be added in sequence to create extended animations.
The movement commands can all be controlled using one simple line:
move 0 0 0 0 0 0 0 0 3 60

Each number following the word "move" represents a possible motion for the molecule. There must always be 10 numbers after the word move. Therefore, it is important to leave the value at zero when that motion is not being used, because if the wrong number of zeroes is not present, the movement will not work.
I will now go through the movements designated by the numbers individually, but you must realize that you can combine the movements according to what you want to show. The # sign represents the number being discussed.

move # 0 0 0 0 0 0 0 3 60
controls rotation around X axis. A (-) value causes the molecule to spin backwards, a (+) causes forward spin. (0-360)

move 0 # 0 0 0 0 0 0 3 60
 # controls rotation around Y axis. (-) value causes left-hand spin, (+) number causes right hand spin. (0-360)
move 0 0 # 0 0 0 0 0 3 60

controls rotation around Z axis (axis pointed at you). (-) value causes left-hand rotation, (+) value causes right-hand rotation (0-360)

move 0 0 0 # 0 0 0 0 3 60
 # controls zoom in/zoom out. (-) value zooms out, (+) value zooms in. (Values may vary per molecule).

move 0 0 0 0 # 0 0 0 3 60
 # controls horizontal translation. (-) value moves molecule left, (+) value moves molecule right.

move 0 0 0 0 0 # 0 0 3 60
 # controls vertical translation. (-) value moves molecule down, (+) value moves molecule up.

move 0 0 0 0 0 0 # 0 3 60

 I have tried this by itself and in conjunction with other parameters and I am unable to determine what it does.

move 0 0 0 0 0 0 0 # 3 60

 # slabs toward you or away from you, depending on (-) or (+) value, respectively. Larger magnitudes make the slab cut deeper.

move 0 0 0 0 0 0 0 0 # 60

 # designates the speed at which this movement set will occur. Larger values result in slower speeds, eg. #=0.5 results in a really quick motion, #=30 results in extremely slow motion.

move 0 0 0 0 0 0 0 0 3 #

 # seems to determine the smoothness of the movement. Based on a few trial runs, I observed that a value of 20 results in fairly choppy movement, whereas a value of 60 gives smooth movement.

It is important to remember that you can use multiple values within one line, eg. the command:

move 0 180 0 50 0 0 0 0 3 60

will cause the molecule to rotate 180 degrees to the right while zooming in..
You can also make sequences of lines to create complex animations. All of the following commands, when added in sequence:

move 0 180 0 50 0 0 0 0 3 60
move 0 -180 0 -50 0 0 0 0 3 60
move 0 60 0 0 50 0 0 0 3 60

will cause the molecule to zoom in while rotating CW, then zoom out while rotating CCW, and finally translate to the right while rotating CW.

Potential problems with complicated molecule depictions (and suggestions on how to fix them):
Oftentimes, after copying a script into your html and inserting a button into the right frame, you will notice that a) the script is very long and b) it takes longer after clicking the button for the chime window to depict the molecule in the proper orientation. Here is an example:
Imagine that you have a Chime file of a protein consisting of two distinct chains, A and B. Let's say you select chain A and display it with the "spacefilling" option. Likewise, you select chain B and display it as "wireframe." If you copy the chime script into notepad after the
<embed type="application/x-spt" width=13 height=13 button=push target="myfavorite" script="

and before the

">

you will notice that this script is very large (sometimes several thousand lines long!). This lengthy script makes it not only cumbersome to navigate around your html code but also results in the plug-in taking a long time to load your chosen orientation. You can imagine with larger proteins that this can become quite a nuisance. This situation can be remedied, although it is often on a case-by-case basis.
Here is an example (you may want to try this for practice):
Let's say you want to depict your protein as mostly wireframe, but you wish to depict all of the alanine residues as spacefilling. Using the menus and options available to you by right-clicking the Chime window, you succeed in capturing this display. Now you want to create a button in your right frame that will orient the molecule in this manner, so you copy and paste this command:
 <embed type="application/x-spt" width=13 height=13 button=push target="myfavorite" script="

followed by ">

Now, on PC, you right click on the Chime window and "Copy Chime Script." On a Macintosh you do this. You now paste your script between the quotation marks, but the script is really long! How can you clean this up?
Look for this text or something similar:

Atoms
colour atoms cpk
select all
spacefill off
select (atomno>=29) and (atomno<=33)
spacefill on
select (atomno>=82) and (atomno<=91)
spacefill on
select (atomno>=130) and (atomno<=134)

you will notice that the "select (atomno>130 etc…" command goes on for quite some time. Beginning with "select all" you can delete everything from there until the next # sign, which usually says "#bonds"
Once you have deleted this, you can simply replace it with

select ala
spacefill on

and have the same effect as the previous string of commands. Now, suppose you want to color the alanine residues green. Simply type

color green

directly after "spacefill on". Hopefully with practice, your ability to manipulate chime scripts will become more intuitive. One way to do this is to download RASMOL and familiarize yourself with the various Chime commands. RASMOL is available for the Macintosh. There is also a version for the PC, called RASWIN.

Color Codes[1]:
These are the available colors on Chime and their corresponding numerical codes:

blue [0,0,256]
black [0,0,0]
cyan [0,255,255]
green [0,255,0]
greenblue [46,139,87]
magenta [255,0,255]
orange [255,165,0]
purple [160,32,240]
red [255,0,0]
redorange [255,69,0]
violet [238,130,238]
white [255,255,255]
yellow [255,255,0]

Adding Links:
Since you may be used to making links in Dreamweaver, where the process is fairly uncomplicated, you may initially be intimidated by the thought of making a link using html tags. However, here is a simple way to do it. Copy and paste this code into your own source code where you want the link to appear and alter it to your liking:

<p>My Home Page</p>

"home.html" can be changed to anything, for instance: "http://www.yahoo.com." The abbreviated "home.html" is a "relative link." When you are linking to a page that is in the same folder as the page you are linking from, the full URL is not necessary. The target="_parent" notation is important because it guarantees that the new page will open in the full window, rather than only in the frame where the link is located. Finally, "My Home Page" should be replaced with whatever text you would like to actually appear. This is the text that people will click on to access the link. If you do not want the link to be its own paragraph then remove the <p> and </p> notation. This will allow you to put the link within your own text.

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[1] Sayle, Roger. "RasMol V2.4 Help File." *RasMol v2.4*. Copyright 1995. Download RasMol at http://www.umass.edu/microbio/rasmol/getras.htm

Good luck and <u>remember to save your work</u>!!!!!

Chapter 4: A Brief Tutorial on VMD

VMD is a freely downloadable molecular visualization program designed by the Theoretical Biophysics Group at the University of Illinois and Beckman Institute. It can be downloaded at http://www.ks.uiuc.edu/Research/vmd/. One of the program's strengths is the ability to present molecules using stereoscopic display. This requires two important pieces of hardware: a monitor capable of stereo display, as well as visualization glasses such as CrystalEyes. However, especially with larger molecules, the program tends to get bogged down when running in 3D mode. It is the goal of this manual to give the reader a few hints to help optimize VMD's performance, as well as provide a basis for utilizing VMD's various capabilities.
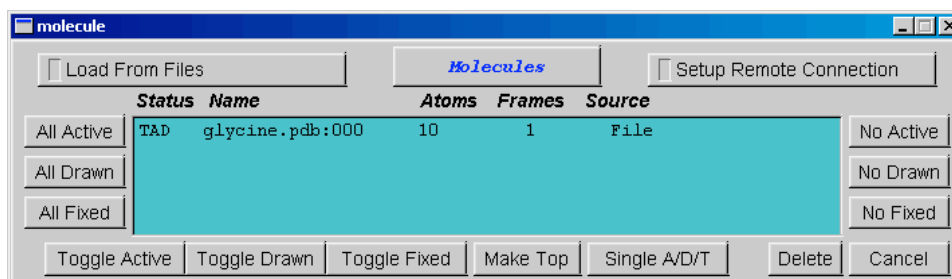
Getting Started

After opening the VMD Program, you will notice that three windows open: a visualization window, the main menu, and a command window.
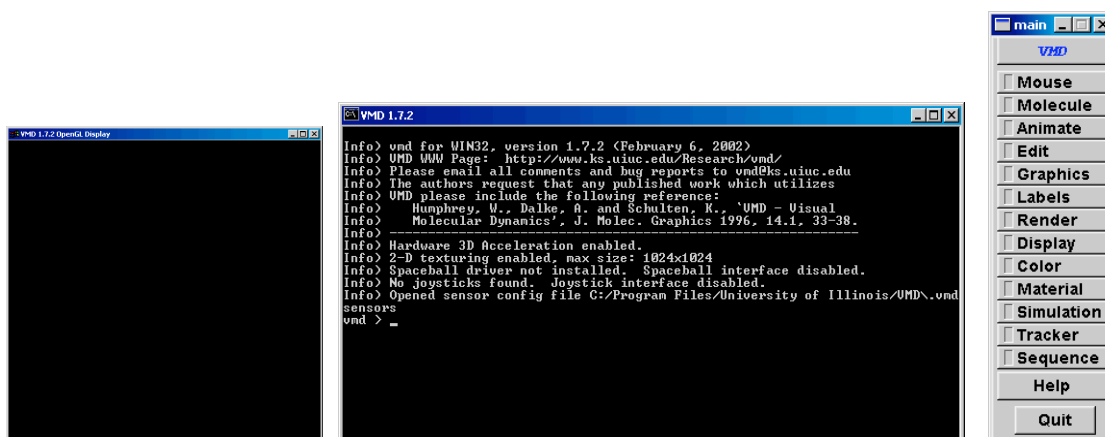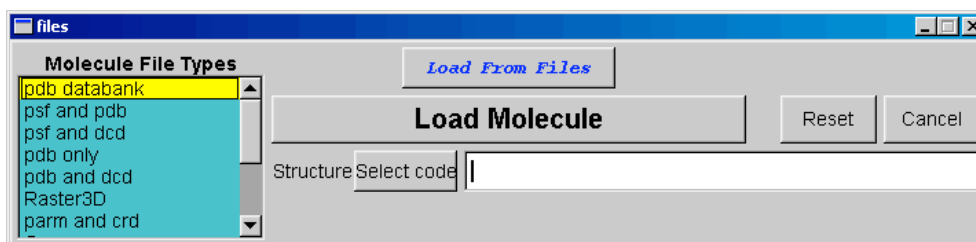

The visualization window, command window, and main menu (l-r).

To open a PDB molecule, go to the main menu. Click on Molecule. The Molecule window will open.

Now click Load From Files in the upper left hand corner. This opens the Files window.

From the list labeled Molecule File Types, choose PDB only. Now click Select pdb. This allows you to choose a pdb file on your hard drive. If you do not have the pdb file on your hard drive but you know the 4-character identifier (for instance, hemoglobin in 1A3N), then you can download the pdb file directly off the http://www.pdb.org database, provided you have an active internet connection. To do this, in the Molecule File Types list on the files window, select pdb databank. In the space provided type the 4-character identifier and click Load Molecule.

Remember, prior to loading another molecule, remove the previous molecule by going to the molecule window. Highlight the previous molecule and click Delete, otherwise VMD will open both molecules, which might slow the program down to the point of crashing.

Manipulating Molecules

Once you have loaded a molecule, you can explore its structure using the various display options that VMD provides. In the display window you can use the mouse to drag the molecule to different orientations. There are three possible mouse actions that can be very useful. These actions can be toggled using the R, S, and T keys on your keyboard.

Key	Action
R	Rotation
T	Translation
S	Scaling

By left-clicking with the rotation action, you can rotate the molecule along the X, Y, and Z axes. Right clicking allows you to rotate the molecule laterally, which can be very useful sometimes. By dragging the molecule and letting go quickly, you can set the molecule into a spin. The translation action simply allows you to drag the molecule

along the X and Y axes without disrupting its orientation. Finally, scaling allows you to zoom in and out.

The Graphics Window

One way to produce interesting and informative molecular visualizations is to change the drawing method by which the molecule is displayed. These are available in the Graphics window, accessed by clicking Graphics on the Main Menu.
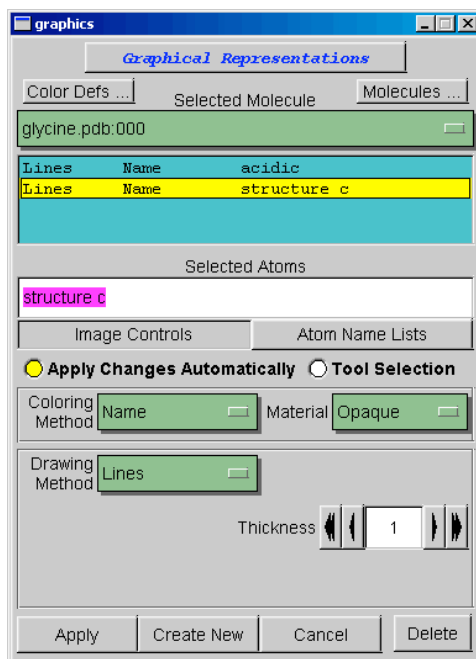
The various types can be toggled using the Drawing Method menu. These include: VDW, CPK, bonds, ribbon, licorice, etc. A word of warning: depending on the size of your molecule, some of these displays might result in extremely slow performance. Quick and dependable drawing methods: lines, points, and tubes.
Potentially difficult drawing methods: CPK, surf, and licorice.
One way to avoid an accidental crash is to deactivate the Apply Changes Automatically option (yellow = activitated). When this feature is activated, the feature you choose is applied automatically. Some drawing modes such as spacefill, are very exhausting on computers. Accidentily clicking "spacefill" for a large protein can overload the system and crash it. Canceling "Apply Changes Automatically" would prevent such an accident. In order to select portions of the molecule you are looking at, a number of commands can be typed in to the Selected Atoms box. A menu of commands is also available by clicking Atom Name Lists.
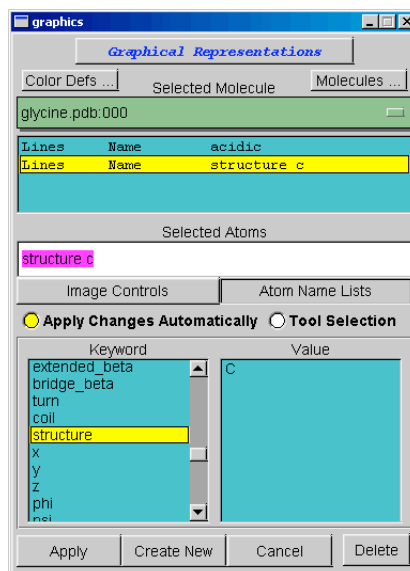
Here you can simply click on the set of atoms you wish to select. Often you will want to display different groups of atoms using different drawing methods. For example, if you want to display one chain using the lines method and another group using CPK (which is very similar to ball-and-sticks), click Create New. This will bring another item into the Graphics menu. Your two items can now be altered independently, by highlighting the item of interest and choosing its drawing method.

In addition to drawing method, another useful option is Coloring Method. You can use this to add a variety of color schemes to your molecule.

Saving Your Molecule's State

This option seems to be less than convenient, given that save states can only be saved to the desktop. At any rate, it is very simple to save the graphical display you have chosen for your molecule. In the command window simply type

save_state *

* being the name you wish to give your save state. This will save the state to your desktop. There is no way to direct where the file saves, because the program defaults to the desktop. Obviously, most people do not like to store a bunch of loose files on their desktop. Your save state files can be moved to another folder, but, should you choose to load them again, you must move them back to the desktop. It seems like there should be a way to choose the target directory of your save states, but I have yet to find it.

Loading A Saved State

In the command window, type

play *

Let * the name of your save state. Remember that the save state must be located on the desktop for this to work. Another caveat: if loading your save state is the first thing you do when you run VMD, the program will put the molecule into a spin once it is loaded. Subsequent loads will not have the spin. If VMD spins your molecule, simply reload it.

Hints for Optimization

In order to gain better performance using VMD, there are a few options that can be tweaked. First, the program runs faster when Stereo Mode is turned off. To do this, click Display on the main menu, which opens the Display window. Click the Stereo list, and select Off. This will allow you to manipulate your molecule into an appropriate view. Then when you wish to resume the 3D effects, turn Stereo back on to CrystalEyes or whatever your Stereo Mode may be. There are several other options that can be toggled on this same Display menu which boost performance. Find the Detail and Alt. Detail lists. For Alt detail, you may want to set this to Points. This causes your molecule to shift to a simpler "points" display while rotating, so the program does not have to do as many calculations with rotating a more complicated display. The Detail list allows you to choose the display that is given when the molecule is not being actively rotated. The default is wireframe. Another nice option is Full, which depicts the molecules with very pretty, metallic looking surfaces. This option runs surprising fast when used in conjunction with the Points setting in Alt Detail. The values seen in the panel below for Near Clip, Far Clip, etc. are values that I have found provide an optimized three-dimensional display. I recommend altering the default settings to these numbers for enhanced viewing of the molecule.
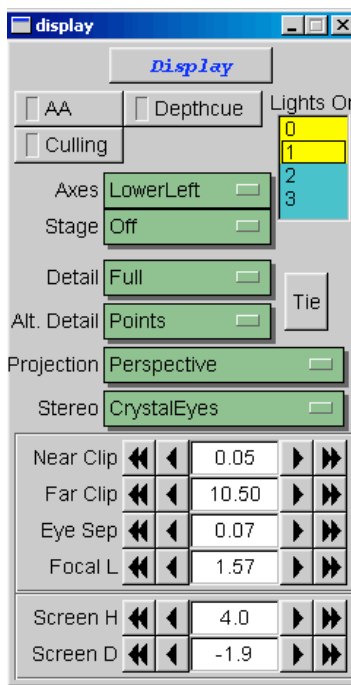
By now, your VMD settings should be optimized.

Appendix 1: Protein Crystallization Worksheet

Use these tables for book-keeping purposes, and to record your observations.

I. *Basic Crystallization of Lysozyme*:

Date	Well Coordinates	Contents	Crystals? (Y/N)	If Y, When?	Ppt? (Y/N)	Observations

II. *The Effects of Boiling on Lysozyme Crystallization*:

Date	Well Coordinates	Contents	Crystals? (Y/N)	If Y, When?	Ppt? (Y/N)	Observations

III. *The Effects of Repeated/Freezing Thawing on Lysozyme Crystallization*:

Date	Well Coordinates	Contents	Crystals? (Y/N)	If Y, When?	Ppt? (Y/N)	Observations

IV. *The Effects of a Foreign Reagent on Lysozyme Crystallization*:

Date	Well Coordinates	Contents	Crystals? (Y/N)	If Y, When?	Ppt? (Y/N)	Observations

V. *The Effects of a Different Reservoir Solution on Lysozyme Crystallization*:

Date	Well Coordinates	Contents	Crystals? (Y/N)	If Y, When?	Ppt? (Y/N)	Observations

Acknowledgments:

References with annotations for good crystallization readings:

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Branden, Carl and John Tooze. Introduction to Protein Structure. New York: Garland,
     1999 (pp. 374-376).

Branden and Tooze give a very concise, generalized look at crystallization's place in the process of isolating a protein and determining its molecular structure.

Campbell, A. Malcolm and Laurie J. Heyer. Discovering Genomics, Proteomics, and
     Bioinformatics. New York: Benjamin Cummings, 2003. pp. 161-204.

McRee, Duncan E. Practical Protein Crystallography. San Diego: Academic Press, 1993.
     (pp. 1-23).

The first chapter and part of the second chapter of this book give a detailed description of various crystallization techniques, as well as good schematic drawings of crystallization apparatus. The strength of this reading lies in the figures.

Rhodes, Gale. Crystallography Made Crystal Clear. San Diego: Academic Press,
     1993. (pp. 8-10, 29-38).

Pages 29-38 give the best description I have found of the crystallization process. It is easy to understand and well written. Pages 35-37 give an excellent explanation of vapor diffusion. Rhodes also goes into detail about crystallization kinetics (pages 28-39).

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