

Protein Structure

Overview

In this lab session, you will explore protein structure using two different approaches:

- **2-dimensional simulation:** you will use the Protein Investigator to construct proteins and see how they fold in 2-dimensions. Although real proteins are not 2-dimensional, this software allows you to directly observe the effects on structure of changes in amino acid sequence.
- **3-dimensional visualization:** you will use Jmol to explore the structure of a real protein, lysozyme in all its 3-dimensional glory. However, you will not be able to edit the structure and observe the resulting changes.

Part I: 2-d simulation with the Protein Investigator

Objectives

- To learn more about the interactions which govern protein structure.
- To test hypotheses regarding protein structure and function.
- To design proteins with specific shapes.

Introduction

In this lab, you will get an opportunity to interactively explore protein structure and function in a simplified system. This is a highly-simplified model of protein folding. It is not intended to predict the correct structures of any proteins; it is designed to illustrate the major principles involved in that process. The important features of proteins that this software retains are as follows:

- Amino acids have side-chains of varying hydrophobicity, charge, and hydrogen bonding capacity.
- The amino acids are connected in an un-branched chain that can bend.
- Hydrophobic amino acids will tend to avoid the water that surrounds the protein; hydrophilic amino acids will bind to the water.
- Amino acids that can form hydrogen bonds will tend to form hydrogen bonds if they can.
- Positively-charged amino acids will tend to form ionic bonds with negatively-charged amino acids if they can.
- Like-charged amino acids will repel each other if they can.

- Ionic interactions are stronger than hydrogen bonds, which are stronger than hydrophobic interactions.

Even though this software provides some important insights into protein folding, you should always keep in mind that this is an approximation. The most important "gotcha's" to be aware of are:

- This program folds proteins in 2-dimensions only.
- This program treats all amino acids as equal-sized circles.
- This program folds the protein based on the interactions between the side chains only.
- This program does not model secondary or quaternary structure.
- This program assumes that all side chains with hydrogen bonding capability can bond with each other.

These simplifications are necessary for two reasons. The first is technical: it turns out to be extremely difficult to predict the full 3-d folded structure of a protein given only its amino acid sequence. As of the writing of this lab manual, it takes a super-computer several days to predict the fully-folded shape of even a small protein like lysozyme. Even then, the predictions don't always match known structures. Given the computers we have in the Bio 111 labs, it might take years....

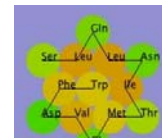
The second reason is educational. As you saw in the lysozyme lab, proteins are complex 3-dimensional molecules. It can be hard to find your way around when inside one. Likewise, it would be very difficult to visually compare two protein molecules to observe the effects of changes to their amino acid sequence. It would be easy to miss the forest (the forces that control protein structure) for the trees (the tiny details of the structures).

For these reasons, we will use this simplification. It retains the properties of amino acids that are important in Bio 111 while being simple and fast.

One other note: this software is under development. Please be patient and gentle with it and report any bugs and/or suggestions to your TA. You may also want to save your History List from time to time to protect against crashes (see page 6 for how to do this).

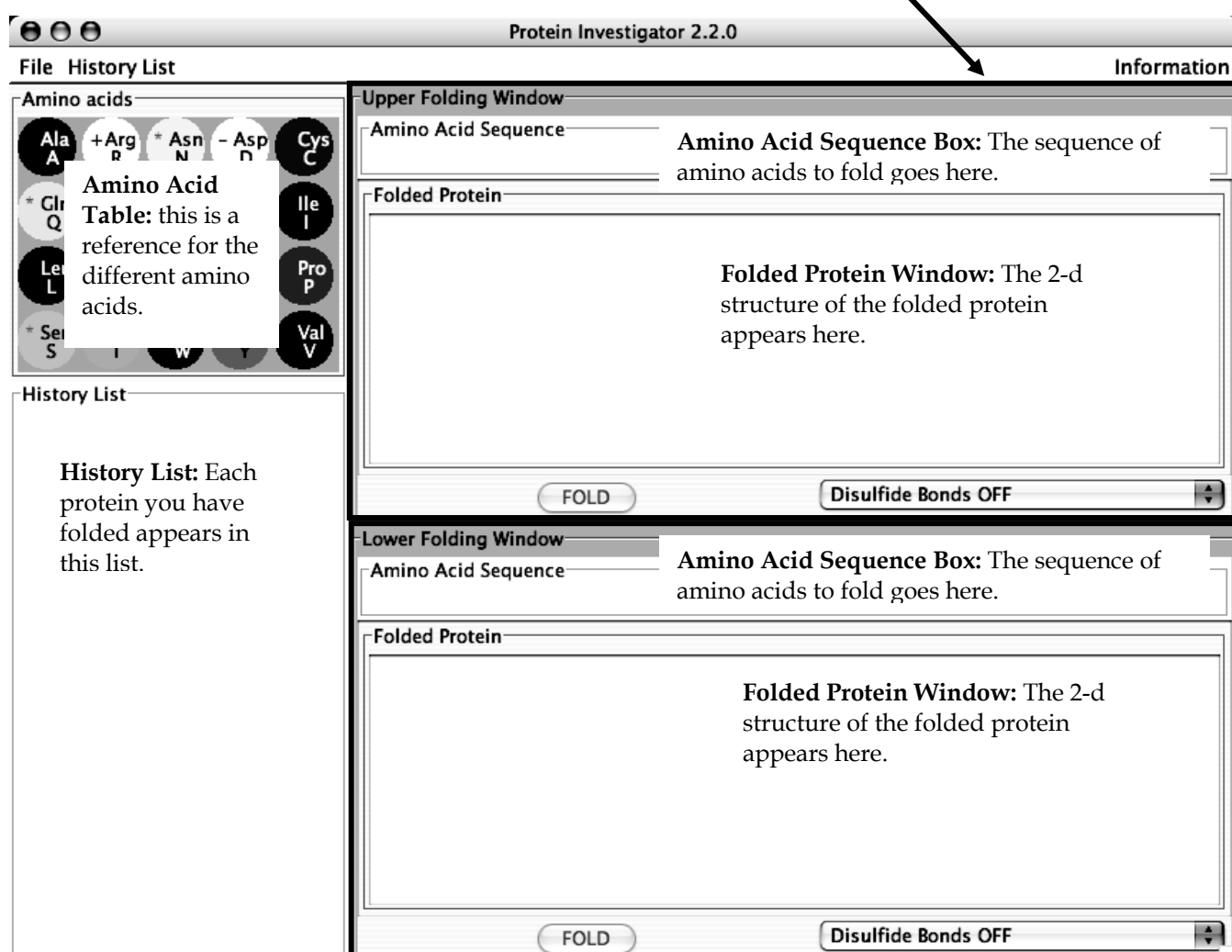
Procedure

1) Start the Protein Investigator. Click on the ProtInv icon in the Dock. It looks like this:



2) ProtInv should start. You should click in the lower right corner and drag the window so that it fills the screen. You will see this:

This is the **Upper Folding Window**.



This is the **Lower Folding Window**.

Using ProtInv

There are some important parts of the program that you should know about:

- **Menus:** there are three menus at the top of the ProtInv window:
 - **File:** this has two items:
 - **Print:** (not used in lab) this prints the amino acid sequences and structures of the proteins in the upper and lower Folding Windows.
 - **Quit:** this quits the program.
- **Menus, continued:**
 - **History List:** this has five items related to the History List:
 - **Save:** this saves the current History List to a .histlist file. You will need to provide a name for this file.
 - **Save As...:** this saves the current History List to a .histlist file you name.
 - **Load:** this clears the History List and replaces it with the contents of a .histlist file you have selected.
 - **Clear:** this deletes all the entries in the History List. This is not un-doable.
 - **Save as Web Page...** This saves all the entries in the **History List** as a set of files in a folder you name; this set of files can be viewed as a web page with a picture and the amino acid sequence of each item.
 - **Information:** this has 2 items:
 - **Help:** the help file with instructions on how to use ProtInv.
 - **About:** some information from the authors of ProtInv.
- **History List Items:** if you double-click on one of the proteins in the **History List**, you will get a menu with these choices:
 - **Send to Upper Panel:** this moves the selected protein from the **History List** to the **Upper Folding Window** so that you can examine it in detail, edit it, etc.
 - **Send to Lower Panel:** this moves the selected protein from the **History List** to the **Lower Folding Window** so that you can examine it in detail, edit it, etc.
 - **Add Notes...:** when you leave the cursor over a protein in the **History List**, a window pops up showing its amino acid sequence; this item allows you to edit this information and add notes.
 - **Delete from History List:** this removes the item from the **History List**; it is not un-doable.
- **Disulfide Bond Controls:** each **Folding Window** has a menu to select whether or not disulfide bonds will form or not. Disulfide bonds form between the side chains of cysteines only under certain conditions. Typically, they do not form inside cells and only form in fluids like blood plasma, etc. These disulfide bonds are covalent bonds and are typically stronger than ionic bonds; if present, they are shown in yellow on the folded protein. We will not be using these in Bio 111;

you should always leave **Disulfide Bonds OFF**. (You can play with this feature if you are curious).

Note: this program is still under development and it may tend to crash. When it does, you will lose all the proteins in your **History List**. To protect yourself against this, you should:

- take notes on the proteins you've folded (write down the amino acid sequences and sketch their shapes)
- regularly save your history list to a file on the desktop; here's how:
 1. Choose **Save** from the **History List** menu.
 2. Select the Desktop from the list of files/folders that appears.
 3. Type in a descriptive name for your history list. If you forget to add the .histlist extension, the program will add it for you.
 4. Click "Save". Your **History List** will now be saved to this file whenever you select **Save** from the **History List** menu.
- you can re-load a saved **History List** like this:
 1. Choose **Load** from the **History List** menu.
 2. Select the Desktop (or wherever you saved the .histlist file)
 3. Select your .histlist file.
 4. Click "Open". The **History List** will now be replaced by the contents of the file you selected.

In most of the next parts, we will ask you to predict what you think will happen before you actually try it. Although you can easily cheat and skip this step, we *strongly* suggest that you take the time to make a prediction. This will give you a better chance to apply and test your knowledge and will prepare you better for the exams on this material.

The One-Letter Amino Acid Code

Amino acids have full names as well as three-letter abbreviations. There is an even shorter abbreviation for amino acids that uses only one letter for each. Because of its simplicity, you will use this code to *type* protein sequences into ProtInv. This one letter code is used in more advanced biology classes, so it is worth familiarizing yourself with it. Because several amino acids start with the same letter, some of the single letter codes are a little obscure; the mnemonics in the table below will help you learn them. In ProtInv, you *type* the single letter code but the program *shows* you the three-letter code for easier reading. You can use this table or the **Amino Acid Table** in ProtInv for reference.

<u>Amino Acid</u>	<u>3-letter code</u>	<u>1-letter code</u>	<u>Mnemonic</u>
Alanine	Ala	A	<u>A</u> lanine
Arginine	Arg	R	a <u>R</u> ginine
Asparagine	Asn	N	aspara <u>giN</u> e
Aspartic acid	Asp	D	aspar <u>D</u> ic acid
Cystine	Cys	C	<u>C</u> ystine
Glutamine	Gln	Q	<u>Q</u> -tamine
Glutamic Acid	Glu	E	glu-t <u>E</u> -amic acid
Glycine	Gly	G	<u>G</u> lycine
Histidine	His	H	<u>H</u> istidine
Isoleucine	Ile	I	<u>I</u> soleucine
Leucine	Leu	L	<u>L</u> eucine
Lysine	Lys	K	lys <u>i</u> n <u>K</u>
Methionine	Met	M	<u>M</u> ethionine
Phenylalanine	Phe	F	<u>F</u> enylalanine
Proline	Pro	P	<u>P</u> roline
Serine	Ser	S	<u>S</u> erine
Threonine	Thr	T	<u>T</u> hreonine
Tryptophan	Trp	W	t <u>W</u> ptophan
Tyrosine	Tyr	Y	t <u>Y</u> rosine
Valine	Val	V	<u>V</u> aline

The Amino Acid Table

In addition to showing the one-letter and three-letter codes for each amino acid, the Amino Acid Table in ProtInv shows more information about each amino acid:

- The circles are colored to indicate the relative hydrophobicities of the side chains. The most hydrophilic are white; the most hydrophobic are black; gray amino acids are intermediate.
- Amino acids with (+) -charged side chains have a small blue “+” sign on their symbol; those with (-)-charged side chains have a small red “-” sign.

- Amino acids with uncharged side chains that can form hydrogen bonds have a "*" on their symbol.

3) Consider the protein arg-arg-arg-arg.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Upper Folding Window** to activate it. You should see a blinking cursor appear.
- Type "RRRR" (without quotes). This is the single-letter code for four arginines.
- You should see "Arg Arg Arg Arg" in the **Amino Acid Sequence Box**. Note that ProtInv shows the three letter code for easy reading. Note also that the border of the **Upper Folding Window** turns pink - this indicates that the protein sequence in the **Sequence Box** has not yet been folded and therefore, the sequences in the **Sequence Box** and the **Folding Window** are no longer the same.
- Click the "FOLD" button at the bottom of the **Upper Folding Window**. Wait a few seconds for the computer to do the calculations.
- You will see the folded protein in the **Folded Protein Window**. You will also see a smaller version of the protein appear in a little box in the **History List**. The border of the **Upper Folding Window** will turn gray to indicate that the sequences in the **Sequence Box** and **Folded Protein** are now the same.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

4) Consider the protein phe-phe-phe-phe.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Lower Folding Window** to activate it. You should see a blinking cursor appear.
- Type "FFFF" (without quotes); this is the one-letter code for four phenylalanines.
- you should see "Phe Phe Phe Phe" in the **Amino Acid Sequence Box**.
- Click the "**FOLD**" button in the **Lower Folding Window** and wait a few seconds.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't, and draw it correctly.

5) Consider the protein phe-arg-arg-arg-arg-phe.

a) Based on the simplifications described previously, predict how this protein would fold. Sketch your predicted folded protein below.

b) Construct the protein.

- Click in the **Amino Acid Sequence Box** of the **Lower Folding Window** to activate it. You should see a blinking cursor appear.
- You can build this protein by selecting the entire protein sequence, hitting delete or backspace to clear the text, and then typing "FRRRRF".
- Alternatively, you can edit the existing sequence as described below:
 - Click to put the cursor anywhere in the third "Phe".
 - Hit delete or backspace twice; it should delete two of the Phe's to give you "Phe Phe" with the cursor in the middle. Notice that hitting the delete key deletes an entire amino acid (not just one letter of its name as you might expect).
 - Type "RRRR".
 - The sequence should now read "Phe Arg Arg Arg Arg Phe"
- Click "**FOLD**" and wait a few seconds.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

6) Consider the protein ile-phe-met-gln-ser-arg-thr-asp-ala-ala. (IFMQSRTDAA)

a) Based on the simplifications described previously, predict how this protein would fold. Roughly sketch your predicted folded protein below.

b) Construct the protein. You should clear one of the **Polypeptide Windows** and start over.

c) Does the actual folded shape match your prediction? If not, make sure you understand why it didn't and draw it correctly.

d) Suppose a mutation in the gene for this protein caused the Arg in the middle (amino acid #6) to be replaced by a Glu. How might this effect the shape of the protein? Sketch your prediction below.

e) Make the mutant protein, either by editing the sequence (you can move the cursor to the right of any amino acid to delete it with the backspace key and then type in the new one) or by clearing and starting over. The mutant protein is: ile-phe-met-gln-ser-glu-thr-asp-ala-ala (IFMQSETDAA). Click "FOLD". Does the actual folded shape match your prediction? If not, make sure that you understand why it didn't.

You can easily compare two different proteins by clicking on the inactive **Folding Window** then double-clicking on the protein you want to see in the **History List**. It will appear in the **Folding Window** that you just activated.

f) Consider a different mutation, one that causes the same Arg (#6) to be replaced by an Ile. Follow the same procedure as for the mutation in part (e). The mutant protein is: ile-phe-met-gln-ser-ile-thr-asp-ala-ala (IFMQSITDAA). You may find it useful to have the normal protein in one **Folding Window** and the mutant protein in the other **Folding Window** for comparison purposes (select it in the **History List** and click one of the ">Upper" or ">Lower" buttons as appropriate).

g) Starting from the original protein from step (6a), make a mutation that has **little or no effect** on the shape of the protein. Explain why this mutation has such a small effect.

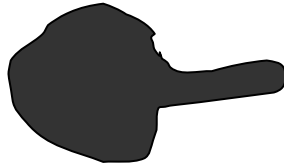
h) Starting from the original protein from step (6a), make a mutation that has **a large effect** on the shape of the protein. Explain why this mutation has such a large effect.

7) Design a protein where an **ionic bond** is critical its shape. Hint: make sure the two amino acids in the ionic bond are separated by at least a few amino acids in the chain. Demonstrate the importance of this ionic bond by mutating one of the amino acids. Have your TA check off that you have shown and explained the role of this ionic bond in your protein. Write the amino acid sequences below:

Normal Protein_____

Mutant Protein_____

8) Design and construct a protein of at least 6 amino acids that has approximately the following shape:



Amino acid sequence _____

You will need to explain to your TA why your protein has this shape as part of your check-off.

Part II: 3-d visualization with Jmol

Objectives:

In the Protein Investigator lab, you modeled proteins as 2-dimensional structures composed of simple circular amino acids. Real proteins are 3-dimensional and amino acids have complex and very different structures. In this lab you will work in groups to map out the primary and tertiary structure of a small protein, the enzyme lysozyme. This will be an application and a demonstration of the principles discussed in lecture and an illustration of what a real protein looks like.

Introduction:

This problem deals with the structure of the protein lysozyme. Lysozyme is a single polypeptide chain 129 amino acids long.

Lysozyme is found in many organisms. It is an enzyme that specifically breaks down the cell wall of certain types of bacteria. Lysozyme binds to the cell wall molecule (called the substrate) and breaks one of the covalent bonds that hold this molecule together. Breaking down the cell walls makes the bacteria fragile and they rapidly burst open and die. Lysozyme is thus an example of the non-specific immune system. The lysozyme you will be looking at was obtained from hen's egg whites; it protects the developing chick from bacterial infection.

In order to look at the structure of lysozyme, we will use the molecular viewing program Jmol. Jmol allows the user to rotate a 2-dimensional representation of a 3-dimensional molecule, as well as highlight particular regions and groups.

Procedure:

Part I: Anatomy of Lysozyme

1) You will work in groups of three people per computer. You may want to take turns using the computer.

2) Your TA will explain how to locate and open the software and exercises for this part.

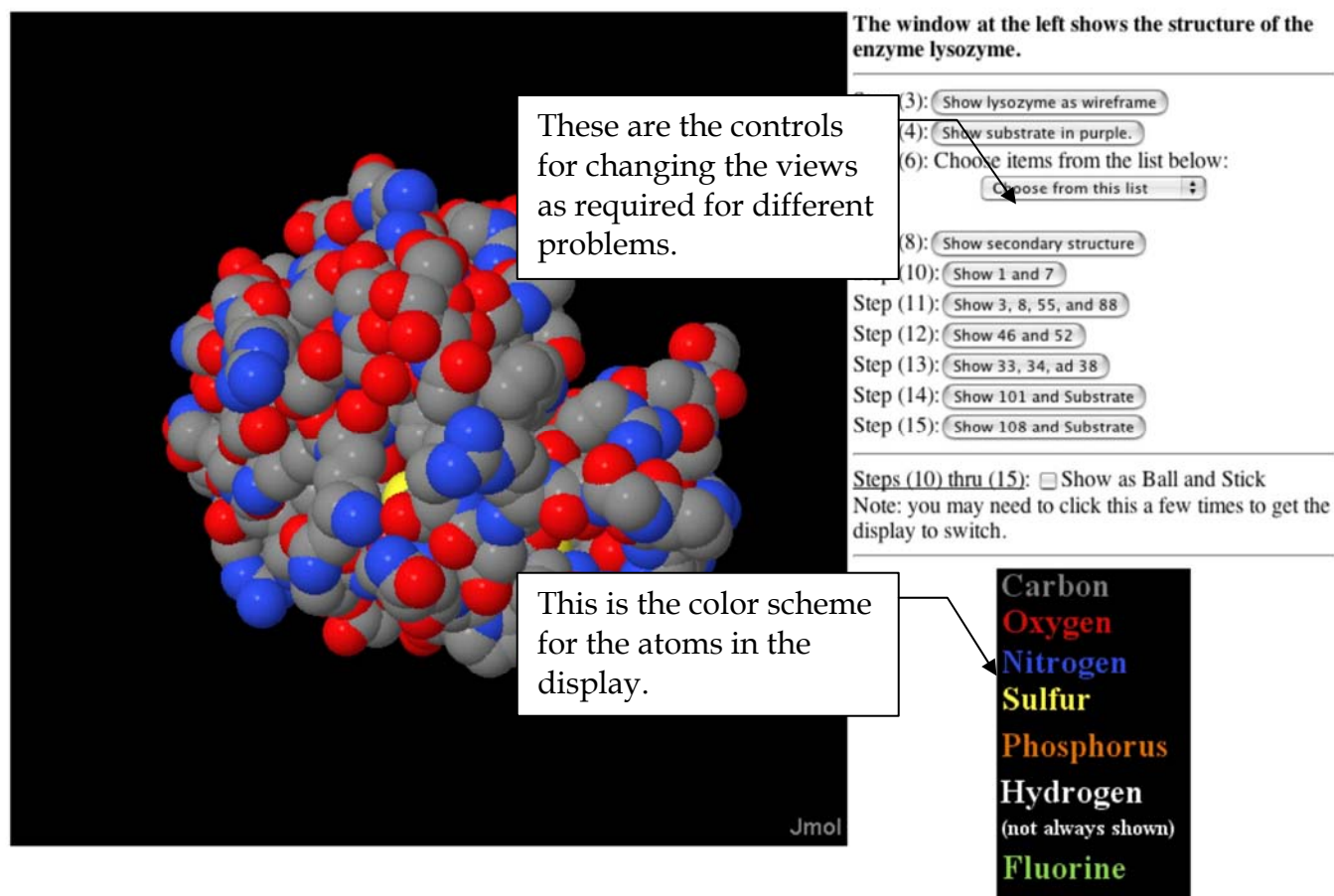
Once you have the program open you should see this:

This shows the molecule under study. In this window, you can:

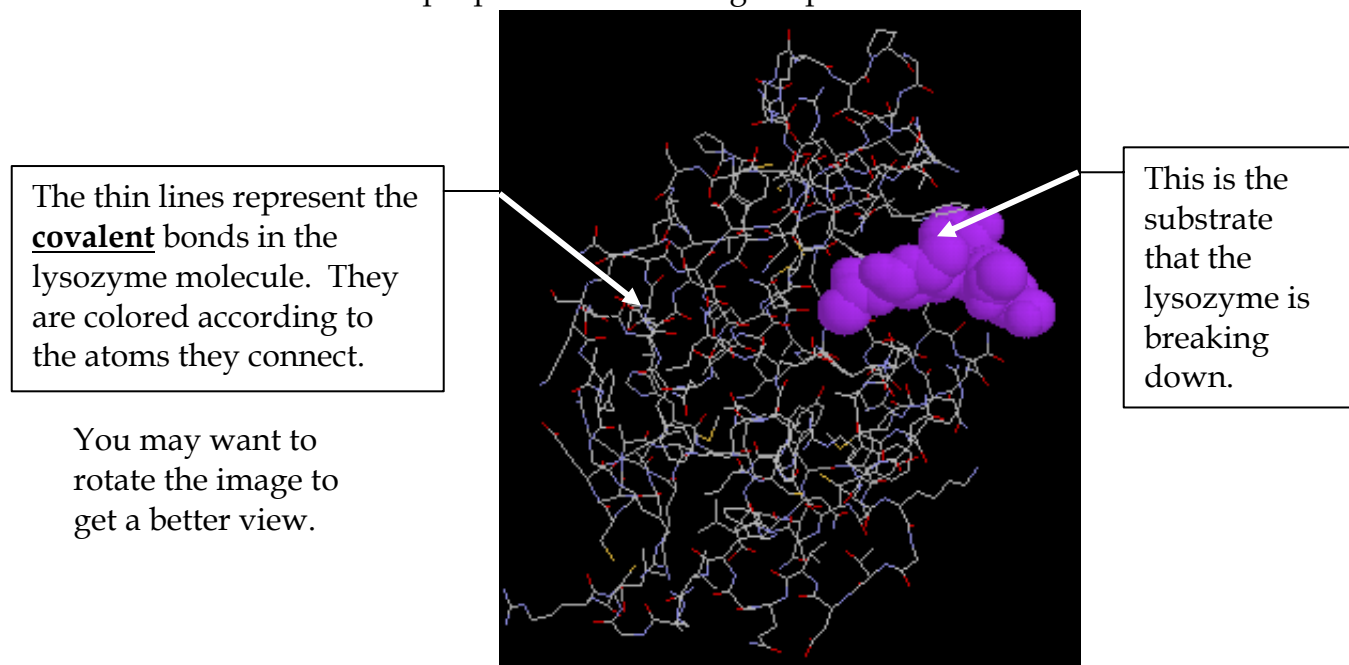
- rotate the molecule by holding the mouse button down while dragging the cursor in the window.
- Zoom in or out by holding the shift key while dragging up or down.
- Identify atoms in the molecule by clicking on them. Information on the last atom clicked on can be found in Safari's "Status Bar" located at the bottom of Safari's

window. For example: `[ASN]77.OD1 #608` Indicates that you have clicked on amino acid #77, which is an asparagine (Asn). The "O" in "OD1" means that you hit an oxygen.

The structure of the enzyme Lysozyme



4) To see the lysozyme molecule and the substrate it is binding to, click the button marked "Show Substrate in purple". You should get a picture like this:



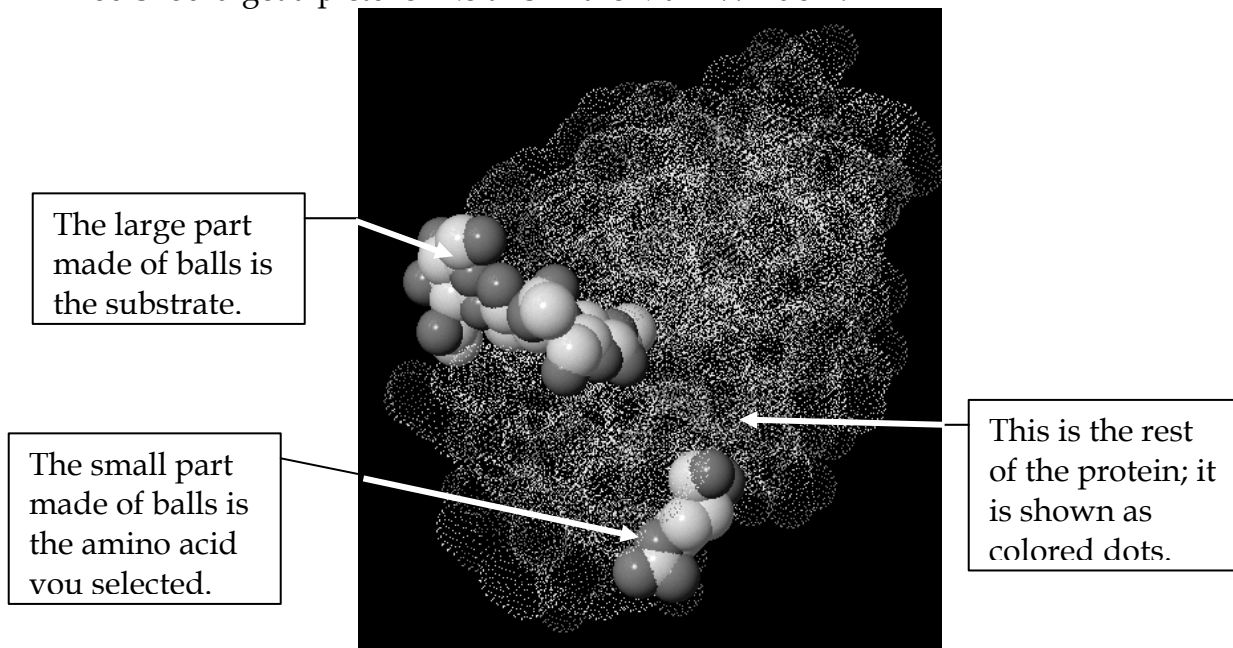
5) There are 129 amino acids in the lysozyme protein chain; not all of them are interesting from our point of view. Each lab group will be responsible for analyzing one set of 5 consecutive amino acids. Your TA will tell you the 5 amino acids that you are responsible for:

Side-chain Location	Interior (In)					
	Exterior (Out)					
	Substrate-Binding (Sub)					
	Can't Tell (?)					
Side-chain type	Hydrophobic ('phobic)					
	Hydrophilic ('philic)					
	Amino Acid Number					

Your assignment is to complete this chart for the amino acids assigned to you by your TA.

6) The first task is to determine the location of each amino acid. Is it inside the protein, on the surface, in the substrate binding area, or you can't tell for sure. To do this, you must select each amino acid in turn and look at its position. To select amino acid #45, you pick "show 45 and substrate" from the menu for part (6).

You should get a picture like this in the Main Window:



Rotate the protein around until you can get a good look at where the amino acid is located. The dots show you where the atoms from the rest of the protein are; if you can't see your amino acid without looking through some dots, that means that your amino acid is inside the protein.

In this case, the amino acid is on the surface of the protein, so you would check in the box in the "Outside Protein" row above the "Amino Acid" #45. Be sure to look at where the **side chain** is located; you can tell the side chain from the backbone because the backbone parts are connected to the rest of the protein by thin blue lines.

Surface of protein						
Unsure						
Inside Protein						
Substrate Binding						
Amino Acid #	43	44	45	46	47	48

If the amino acid were on the inside, you'd check the "Inside Protein" box.

If the amino acid were in the cleft that the substrate fits into, you'd check the "Substrate Binding" box.

For some amino acids, it will be difficult to tell for sure where it is, in that case, check "Unsure".

Do this for each amino acid that your TA assigned.

7) The next task is to determine whether the side chains of the amino acids in your set are hydrophobic or hydrophilic. Shown below is a list of the amino acid sequence of lysozyme. The amino acids are listed in order from # 1 (the amino terminus) to # 129 (the carboxyl terminus). Look up each amino acid in your set and, using the sheet of amino acid structures on the last page of this handout, determine if each is hydrophobic or hydrophilic. Check the appropriate box on your sheet.

1					5					10					15					20
LYS	VAL	PHE	GLY	ARG	CYS	GLU	LEU	ALA	ALA	ALA	MET	LYS	ARG	HIS	GLY	LEU	ASP	ASN	TYR	
21					25					30					35					40
ARG	GLY	TYR	SER	LEU	GLY	ASN	TRP	VAL	CYS	ALA	ALA	LYS	PHE	GLU	SER	ASN	PHE	ASN	THR	
41					45					50					55					60
GLN	ALA	THR	ASN	ARG	ASN	THR	ASP	GLY	SER	THR	ASP	TYR	GLY	ILE	LEU	GLN	ILE	ASN	SER	
61					65					70					75					80
ARG	TRP	TRP	CYS	ASN	ASP	GLY	ARG	THR	PRO	GLY	SER	ARG	ASN	LEU	CYS	ASN	ILE	PRO	CYS	
81					85					90					95					100
SER	ALA	LEU	LEU	SER	SER	ASP	ILE	THR	ALA	SER	VAL	ASN	CYS	ALA	LYS	LYS	ILE	VAL	SER	
101					105					110					115					120
ASP	GLY	ASN	GLY	MET	ASN	ALA	TRP	VAL	ALA	TRP	ARG	ASN	ARG	CYS	LYS	GLY	THR	ASP	VAL	
121					125					129										
GLN	ALA	TRP	ILE	ARG	GLY	CYS	ARG	LEU												

8) Once all groups have finished, you will pool your data. Enter into the “Lysozyme” application as demonstrated by your TA. As a class, answer the following questions:

a) Is there a correlation between whether an amino acid is found on the inside or outside of the protein and its hydrophobicity/hydrophilicity? Count up the class results and fill on the table below. Does this make sense in terms of what we know about chemistry?

<u>Side-chain</u>	<u>Location</u>	
	<u>Inside</u>	<u>Outside</u>
Hydrophilic		
Hydrophobic		

b) The side chains of the amino acids in the substrate binding pocket are clustered together in the tertiary structure of the protein; are they close together in the primary structure as well?

c) The side chains of amino acids 6 and 124 are very close in the folded protein, but very far apart along the backbone. How is this possible?

Part II: Side-chain interactions

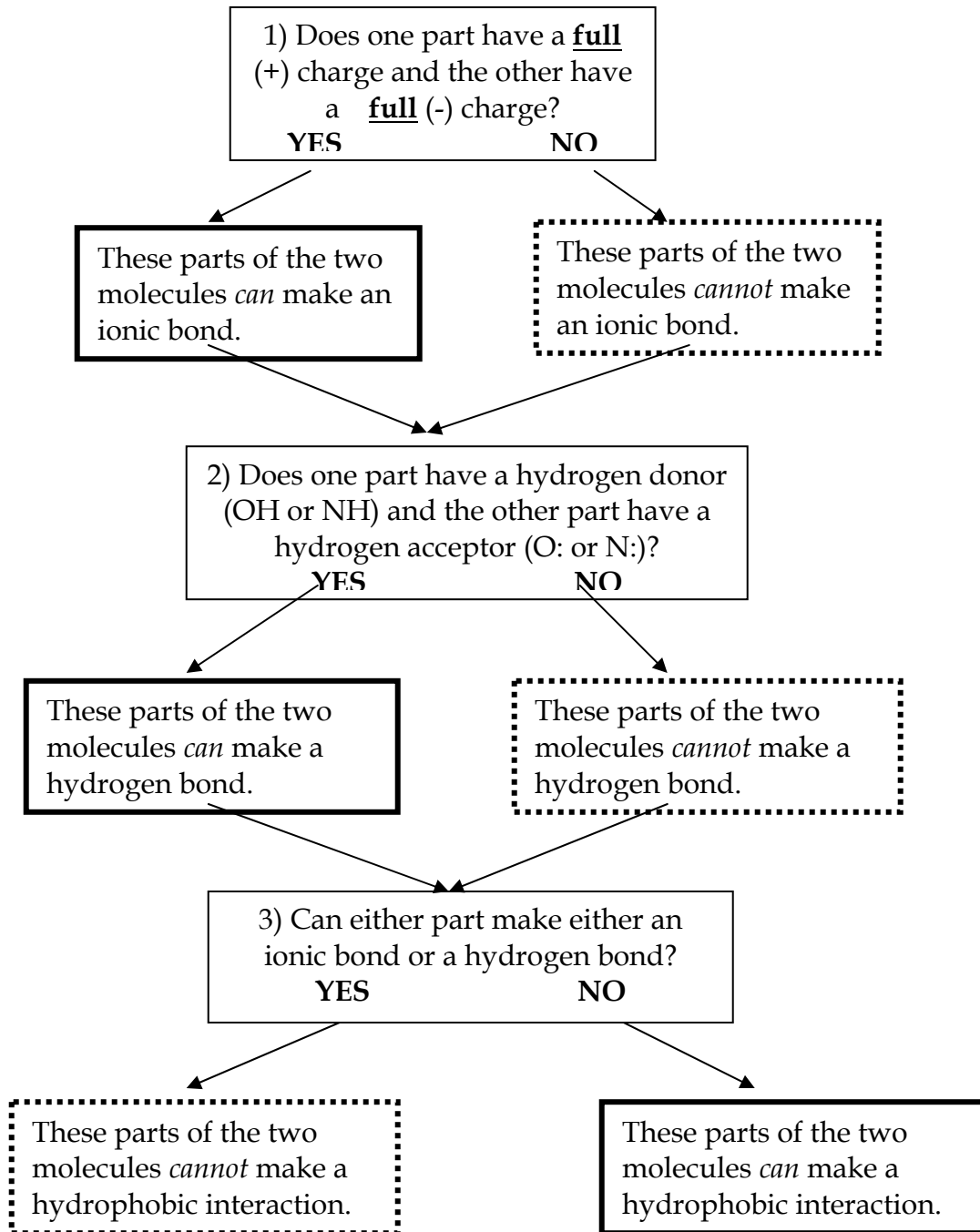
In this part of the lab, you will examine specific interactions between different side chains of the protein and between side chains of the protein and the substrate.

Part II (a) Interactions between side chains in the protein.

Notes:

- For each of these questions, simply click the button for that part.
- You may find it useful to switch back and forth between the two views:
 - “Ball and stick” - this shows atoms as small balls; the **covalent** bonds connecting them are shown as rods. This is useful for finding out which atoms are covalently bonded and which are not.
 - “Spacefill” - this shows atoms as large spheres at approximately their appropriate sizes. This is useful for figuring out which atoms are near each other.
- The program shows the **covalent** bonds, your job is to infer the presence of various non-covalent interactions using your knowledge of structure and bonding. The chart on the next page outlines this process:

How to decide about non-covalent bonds:



(9) Amino acid #1 is lysine; amino acid #7 is glutamic acid.

a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?

b) Which of these interactions is the strongest?

c) Highlight these two amino acids in the structure of lysozyme. (Click “select 1 or 7”).

Which part(s) of their side chains are closest together (middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I).

d) Draw the structure of the parts of the side chains that are closest together and indicate the bond you named in part (b). Use a thick line to show where the atoms you’ve drawn are covalently bonded to the rest of the molecule.

(10) Use jMol to find other ionic bonds in lysozyme

Researchers often use tools like jmol to find particular types of interactions. If you click the button marked "Show Charged Side Chains", the side chain atoms of all the (+)-charged (arg, his, and lys) and the (-)-charged (asp and glu) side chains. Using this view, try to find as many possible ionic bonds as you can. You may want to switch back and forth between spacefill and ball and stick views as well.

Hint: it will be easier to look for patterns of color. In general, which atoms have a (+) charge and which have a (-) charge? Look for (+)'s near (-)'s.

You may well ask, "How far apart can they be and still be ionic bonded?" The answer is no farther than one spacefill atom's diameter (the attraction decreases with the square of the distance).

List possible ionic bonded pairs below (for example, "arg₂₃₄₅ - glu₂₂₃₄"):

(11) Amino acid #3 is phenylalanine; amino acid #8 is leucine; amino acid # 55 is isoleucine; amino acid #88 is isoleucine.

a) Given their structures, what type(s) of interactions are *possible* between the side chains of these amino acids?

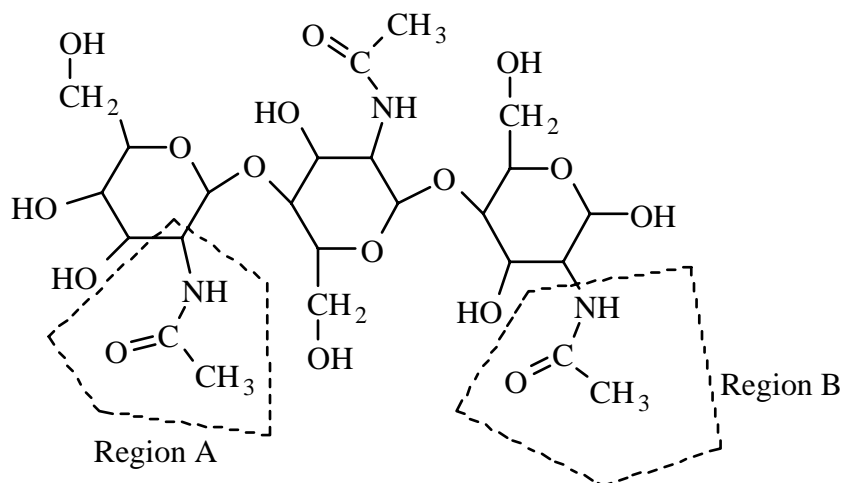
b) Which of these interactions is the strongest?

c) Highlight these amino acids in the structure of lysozyme. (Click “Show 3, 8, 55, and 88”). Which part(s) of their side chains are closest together (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of section I).

d) Are the locations of these side chains relative to one another consistent with the bond you named in part (b)? Why or why not?.

Part II (b): Interactions between side chains and the substrate.

The structure of the substrate is shown below, with two important regions circled.



(12) Amino acid #101 is aspartic acid; its side chain interacts with region A of the substrate.

a) Given their structures, what type(s) of interactions are *possible* between the side chain and this part of the substrate?

b) Which of these interactions is the strongest?

c) Highlight the amino acid and the substrate in the structure of lysozyme. (Click "Show 101 and substrate"). Which part of the side chain is closest to the substrate (backbone, middle of side chain, tip of side chain, etc.)? (Note that you can click on atoms to find out what side chain they are part of as in part (8) of Section I).

d) Draw the structure of the parts of the side chain and the substrate that are closest together and indicate the bond you named in part (b). Use a thick line to show where the atoms you've drawn are covalently bonded to the rest of the molecule.

Additional Questions

Depending on time, try to answer some or all of the following. Check with your TA for guidance.

(A) (For each question (9), (11) and (12), answer the following questions:

In this question, you described an interaction between parts of the lysozyme molecule or between the lysozyme molecule and the substrate. Strictly speaking, this is a *hypothesis*; based on the structural data, you are *hypothesizing* that the interaction exists and is important to the protein. You could test this hypothesis by changing one of the amino acids involved in this interaction and observing the effect of this change on the ability of the resulting enzyme to break down the substrate.

a) Give an amino acid change that would be likely to abolish the interaction you described in lab. For example, if you thought that serine 27 and threonine 89 interacted via a hydrogen bond between their side chains, one possible change that would abolish this hydrogen bond would be to change serine 27 to an alanine. Your answer would read "To eliminate the hydrogen bond between serine 27 and threonine 89, I would change serine 27 to alanine."

b) Explain why this change would be expected to abolish the interaction. In the example given, you'd say something like, "The -OH of serine 27 forms a hydrogen bond with the -OH of threonine 89. If I changed serine 27 to alanine, the alanine can no longer form a hydrogen bond (since the H's on the -CH₃ of alanine are not "special hydrogens"), the hydrogen bond between the side chains of 27 and 89 will be abolished."

(B) Design a hypothetical binding site.

(B1) Pick a molecule from Campbell. It can be any molecule from anywhere in the book, but it must be found in Campbell. Hints: don't pick one that is too large or it will be hard to draw. You should also choose a molecule that is capable of making at least one H-bond, ionic bond, and hydrophobic interaction. Give the name of the molecule and the figure in Campbell where you found it.

(B2) Draw the molecule. Circle one hydrophobic part and one hydrophilic part. Clearly indicate which is hydrophobic and which is hydrophilic.

(B3) Draw the molecule again, this time surrounded by a simple binding site consisting of 3 amino acids of your choosing. Choose your amino acids so that:

i) The side chain of one amino acid interacts with your molecule via a hydrogen bond.

ii) The side chain of another amino acid interacts with your molecule via an ionic bond.

iii) The side chain of the last amino acid interacts with your molecule via a hydrophobic interaction.

Be sure to clearly indicate the nature of each interaction.

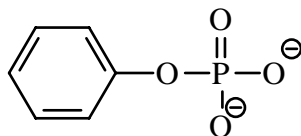
B4) Choose one of the amino acids from part B3.

i) Describe a mutation that would alter the amino acid sequence of your protein so that that amino acid would be different. **Note:** mutations only effect one amino acid (there are exceptions that we will learn about later) **and** mutations can only result in the replacement of one amino acid by another (for example, cystine to serine); mutations **never** alter the structure of an amino acid (for example having cystine lose its SH group since the resulting molecule is not one of the 20 amino acids on the chart).

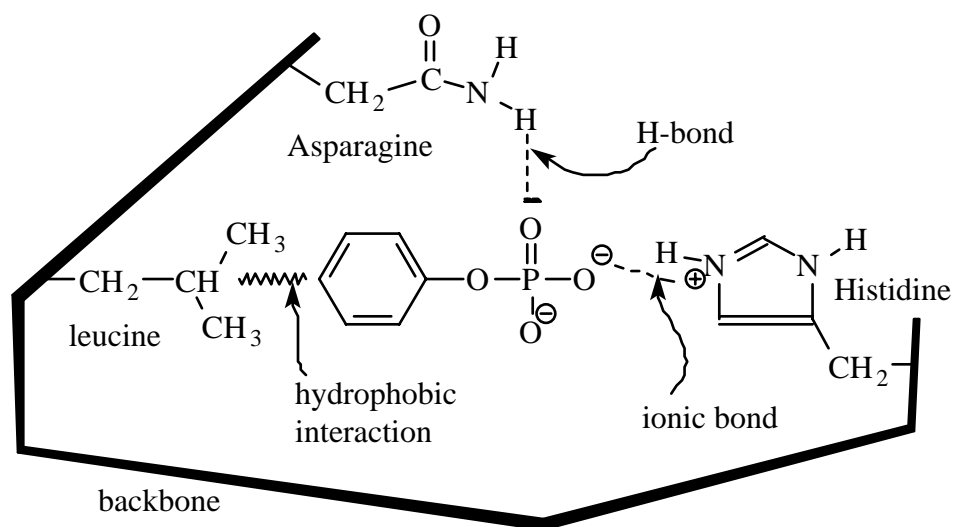
ii) Describe the effect of that mutation on the binding of your molecule and explain your reasoning.

An example is shown below. Note that the molecule used in this example is not found in Campbell.

(B1) Phenyl phosphate.



(B2)



(B3) i) The side chain of the asparagine interacts with the O via a hydrogen bond as shown above.

ii) The side chain of the histidine interacts with the O⁻ via an ionic bond as shown above.

iii) The side chain of the leucine interacts with the carbon ring via a hydrophobic interaction as shown above.

(B4) A mutation which changed the histidine to a glutamic acid would reduce the strength of binding of phenyl phosphate to my enzyme. This is because now there would be a (-) charge on the glutamic acid right next to the (-) charge on the phosphate - since like charges repel, the phenyl phosphate would be repelled out of the binding site.

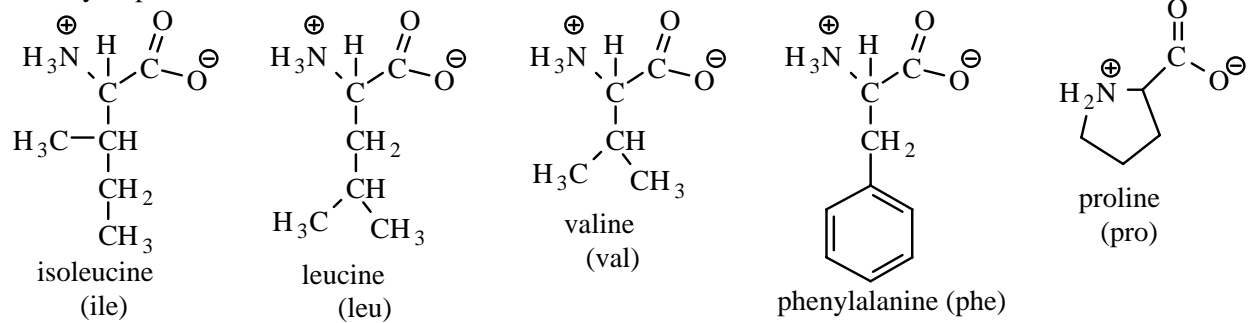
Check-off

Your TA will let you know exactly what you need to complete in lab today to receive check-off credit.

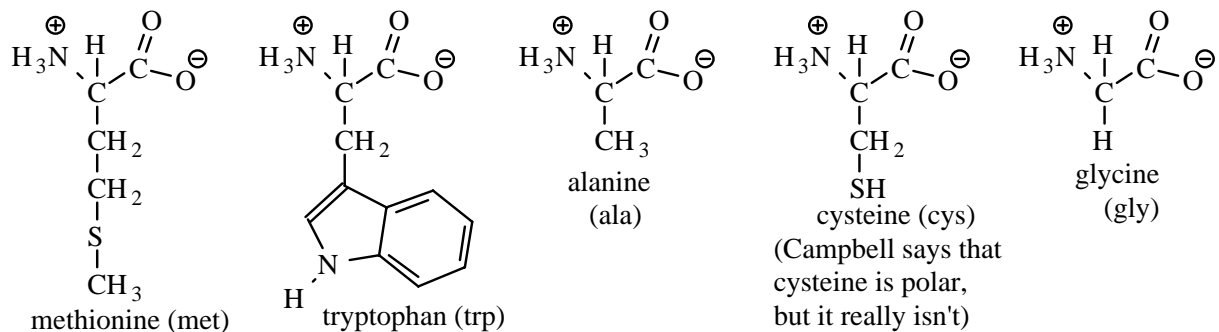
Amino Acids

Non-polar

most hydrophobic



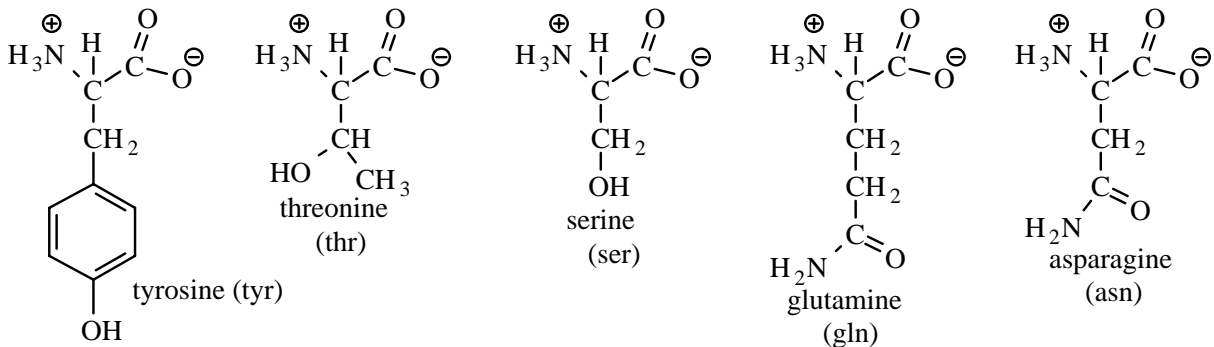
least hydrophobic (but still non-polar)



Polar (but uncharged)

least hydrophilic

most hydrophilic



Charged

least hydrophilic

(but still very hydrophilic)

most hydrophilic

