

# Protein Structure

## Goals

- To explore protein 3-d structure
- To learn about the non-covalent interactions that govern protein structure
- To start learning about enzymes

## Overview

In this lab session, you will explore protein structure using the 3-d visualization tool called jsMol that you used in the warm-up problems.

You will explore the protein amylase. Amylase is an enzyme which you'll learn more about later in the course and use in a later lab. Amylase is involved in the digestion of starch in your diet and is present in saliva and secretions of the pancreas. Amylase binds to starch molecules (the "substrate") and breaks certain covalent bonds in these starch molecules. The resulting products are then further digested by your body.

In the warm-ups, buttons on the web page "told" jsMol how to change its display to show you what you needed to see. In this lab, you will control jsMol directly using typed commands. This means that you will need to learn the jsMol command language. It is a little tricky at first, but easy to use and very powerful once you get used to it.

## Procedure

### Part I: Learning to control jsMol

1) Go to the lab link in the BlackBoard learn course component for this lab and click the "Protein Structure Exercises" link. You should

The structure of the enzyme Amylase

The window at the left shows the structure of the enzyme amylase.

Reset view Show console

Carbon  
Oxygen  
Nitrogen  
Sulfur  
Phosphorus  
Hydrogen  
(not always shown)  
Fluorine

Remember that you can find out which atom you are looking at by leaving the cursor over that atom for a little while until a window pops up. You can interpret the window this way:

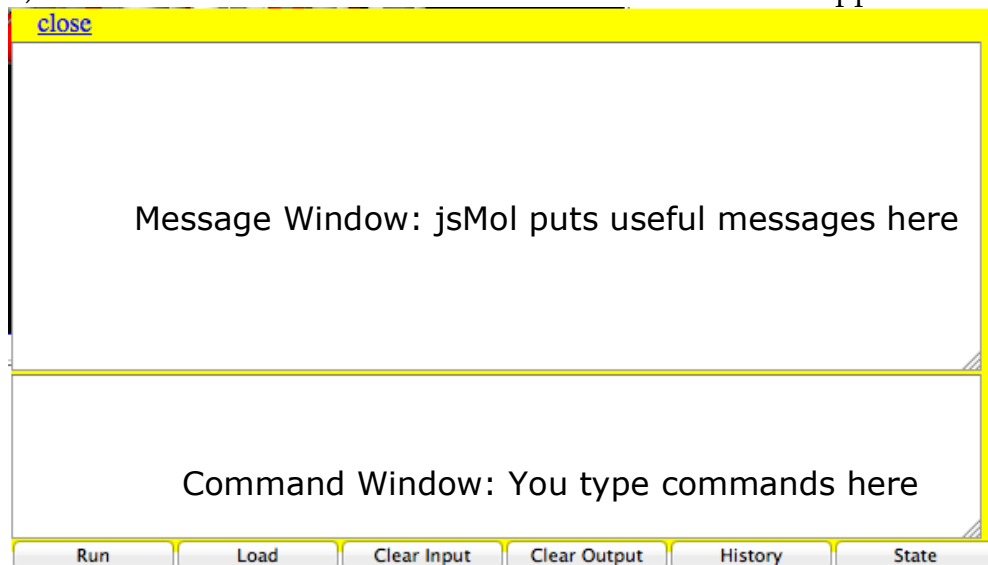
Reading Atom Information

SER 100 #766

[SER]100.O #766

You have selected atom number 766  
It is part of amino acid number 100  
which is SERine

2) Click the “Show console” button and the “console will appear”:



3) Click in the bottom window of the console – the “Command window” – and type your commands there.

A few notes here:

- We’ll show commands in `this font`
- Commands must be entered exactly. Be careful about:
  - Leaving spaces between words
  - Capitalizing or not capitalizing
  - spelling
- Don’t forget to hit “return” or “enter” after each command.
- jsMol typically replies to each command with an informative message in the Message Window. For example, if you select something, it will tell you how many atoms you’ve selected. If this number is zero, you didn’t select anything, and probably made a mistake.

As a test, try the following:

- type: `select all`
- You should see “8087 atoms selected” in the Message Window
- type: `spacefill off`
- The display should go blank (you turned off all the atoms!)
- type: `select 100`
- You should see “14 atoms selected” in the Message Window; you’ve selected amino acid number 100.
- type: `spacefill on`
- You should see one little amino acid in spacefill mode.
- If you leave the cursor over it, a message will pop up telling you that it’s an ASN (Aspargine).
- You can also click on it and that same information will appear in the Message Window.
- Click “reset view” to get back to the starting view.

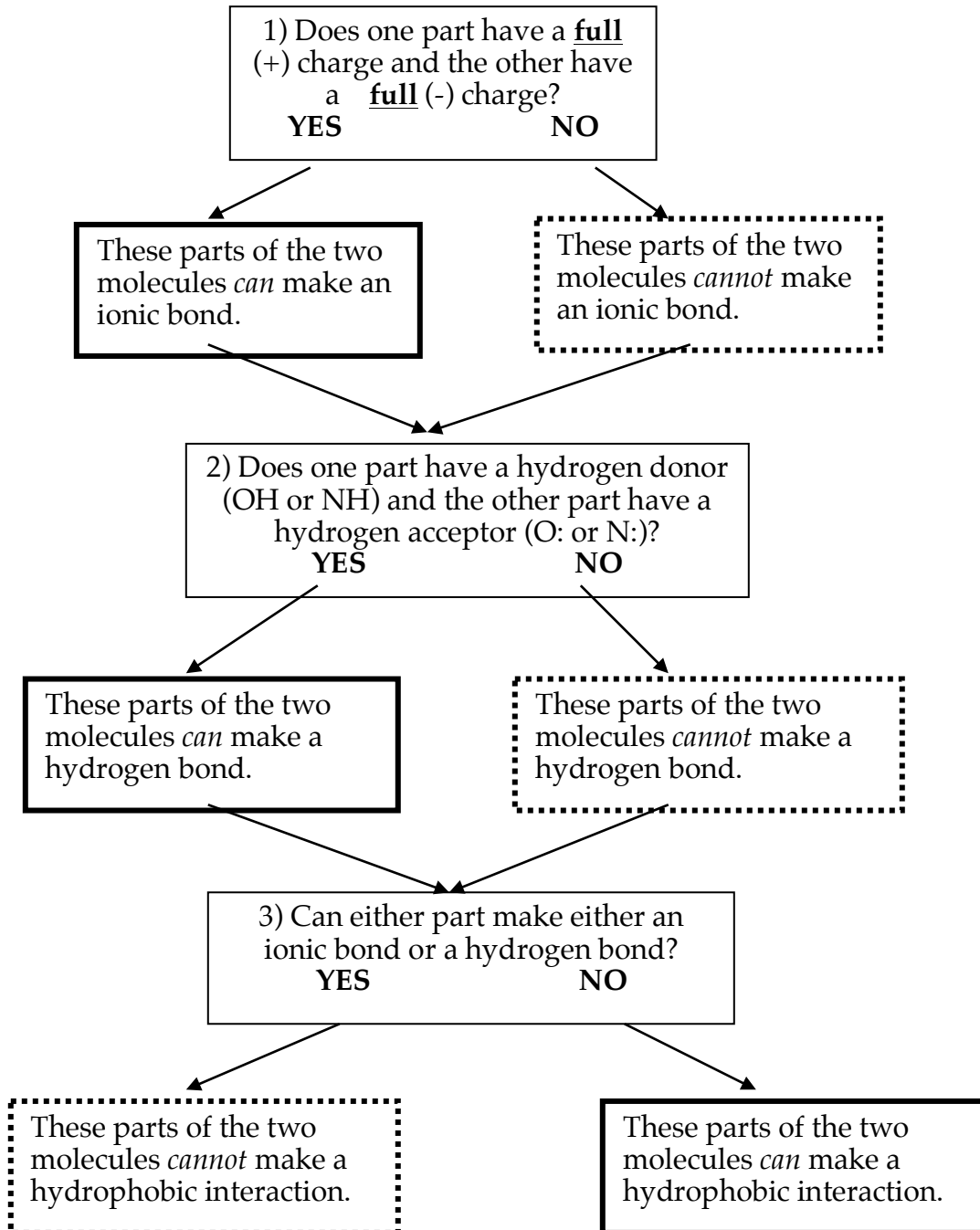
## Some useful commands

- Selecting things (for example):
  - `select 27` – selects amino acid number 27
  - `select ala` – selects all alanines
  - `select ligand` – selects the ligand (aka “the substrate”)
  - `select ca` – selects the Calcium ion ( $\text{Ca}^{2+}$ ) that is an important part of the structure
  - `select within (2.0, phe)` – selects all the atoms that are within 2.0 Angstroms of any of the phenylalanines. An atom is about 1-3 angstroms in diameter.
- Selecting more than one thing. This is a little counter-intuitive unless you are a computer scientist.
  - If you want to select BOTH amino acid 27 AND amino acid 100, you type: `select 27 or 100`. Note that, to jsMol, “or” means “select all the atoms that are either in 27 or in 100”.
  - `select 27 and 100` – selects no atoms since there are no atoms that are in both 27 and 100 at the same time.
  - `select within(2.0, phe) and not phe` – selects all the atoms that are both within 2.0 angstroms of a phenylalanine and not in a phenylalanine. That is, the nearby neighbors of phenylalanines.
- Showing things:
  - `spacefill on` – shows the selected atom(s) as big spheres
  - `spacefill off` – turns off the spacefill view of the selected atom(s)
  - if you want to show the selected atom(s) as “ball and stick”, it takes two commands:
    - `spacefill 0.5`
    - `wireframe 0.2`
    - remember that this shows single, double, and triple bonds as identical rods
  - to turn off “ball and stick” for the selected atom(s), it also takes two commands:
    - `spacefill off`
    - `wireframe off`
  - `dots on` – shows the selected atom(s) as dots
  - `dots off` – turns off the dots on the selected atom(s)
- Coloring things:
  - `color cpk` – colors the selected atom(s) by which atom they are using the scheme shown on the web page.
  - `color blue` – colors the selected atom(s) with the specified color. Some colors that work are red, blue, green, yellow, purple, white; you can try others.

You might want to play with this a little before you go further.

**Reminder:** 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:



## Part II: Interactions

Your TA will assign your group one of these tasks:

### a) Hydrogen Bond or Ionic Bond?

The list below shows pairs of amino acids that are known to interact via hydrogen bonds or ionic bonds.

280, 258	20, 23	30, 27	48, 60	101, 100
138, 124	250, 232	284, 279	322, 485	386, 340
387, 27	456, 393	493, 396	336, 12	322, 485

Your TA will assign your group one of these pairs. Your task is to:

- 1) Set up jsMol to show those two amino acids.
- 2) Determine the strongest possible interaction given their structures and their relative orientation: hydrogen bond or ionic bond?
- 3) Present your findings to the class:
  - a) On the projection screen, set up a view to show your amino acids.
  - b) On the whiteboard next to the screen, draw the interacting parts of your two amino acids and indicate the bond from (2).
  - c) Explain to the class how you found the bond and determined which type it was.

### b) Interaction with substrate

The list below gives several amino acids that interact with the substrate.

63    101    200    201    233    299    300    305

Your TA will assign your group one of these amino acids. Your task is to:

- 1) Set up jsMol to show your amino acid and the substrate ("ligand").
- 2) Determine the strongest possible interaction given their structures and their relative orientation.
- 3) Present your findings to the class:
  - a) On the projection screen, set up a view to show your amino acids.
  - b) On the whiteboard next to the screen, draw the interacting parts of your amino acid and the substrate and indicate the bond from (2).
  - c) Explain to the class how you found the bond and determined which type it was.

### c) Interaction with the Calcium ion

There is a calcium ion that is an essential part of the structure of amylase. The calcium ion has a positive charge and it interacts with a nearby negatively-charged amino acid. Your task is to:

- 1) Set up jsMol to show the calcium ion.
- 2) Find the amino acid that interacts with the calcium ion via an ionic bond.
- 3) Present your findings to the class:
  - a) On the projection screen, set up a view to show your amino acid and the calcium ion.
  - b) On the whiteboard next to the screen, draw the interacting parts of your amino acid and the calcium ion and indicate the ionic bond.
  - c) Explain to the class how you found the amino acid that interacts with the calcium ion.

### d) Hydrophobic Interactions

Your group's task is to find and convincingly demonstrate a hydrophobic interaction. That is:

- 1) Use jsMol to find a hydrophobic interaction.
- 2) Present your findings to the class:
  - a) On the projection screen, set up a view to show your amino acids from (1).
  - b) On the whiteboard next to the screen, draw the interacting parts of your amino acids.
  - c) Explain to the class how you found the amino acids.

Your group will receive a 20-point checkoff for successfully and thoroughly completing this part.

## Lab Report

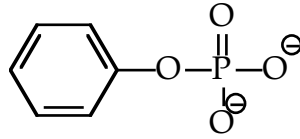
Your lab report must consist of the following:

Design a hypothetical binding site.

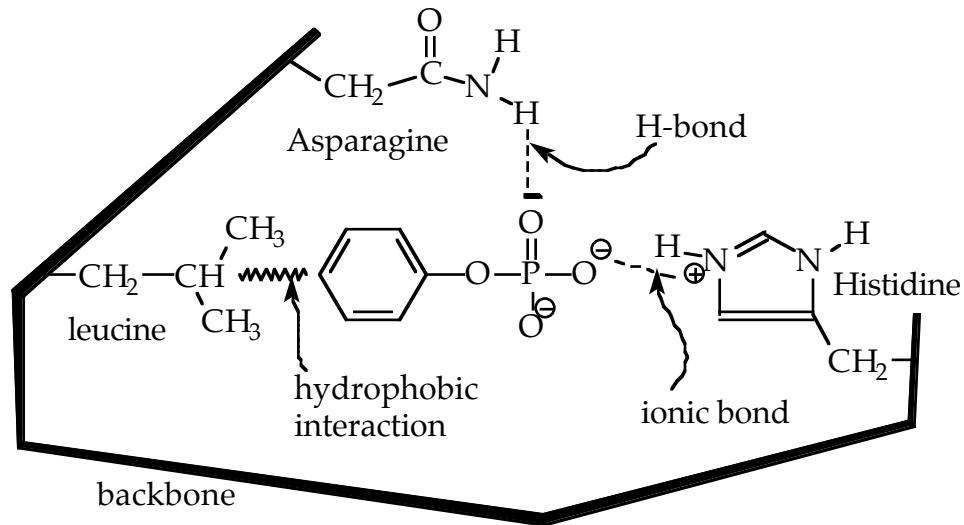
- (1) Pick a molecule from somewhere in the course materials. It can be an amino acid.  
Hints: don't pick one that is too large or it will be hard to draw. You should also choose a molecule that is capable of making at least one H-bond, ionic bond, and hydrophobic interaction. Give the name of the molecule and the part of the course materials where you found it.
- (2) Draw the molecule. Circle one hydrophobic part and one hydrophilic part. Clearly indicate which is hydrophobic and which is hydrophilic.
- (3) 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.
- (4) Choose one of the amino acids from part B3.
  - i) Describe an alteration that would alter the amino acid sequence of your protein so that that amino acid would be different. **Note:** these alterations only effect one amino acid (there are exceptions that we will learn about later) **and** these alterations can only result in the replacement of one amino acid by another (for example, cystine to serine); these alterations **never** alter the structure of an amino acid (for example having cystine lose it's SH group since the resulting molecule is not one of the 20 amino acids on the chart).
  - ii) Describe the effect of that alteration 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.

(1) Phenyl phosphate.



(2)



- (3)
- The side chain of the asparagine interacts with the O via a hydrogen bond as shown above.
  - The side chain of the histidine interacts with the  $O^{-}$  via an ionic bond as shown above.
  - The side chain of the leucine interacts with the carbon ring via a hydrophobic interaction as shown above.

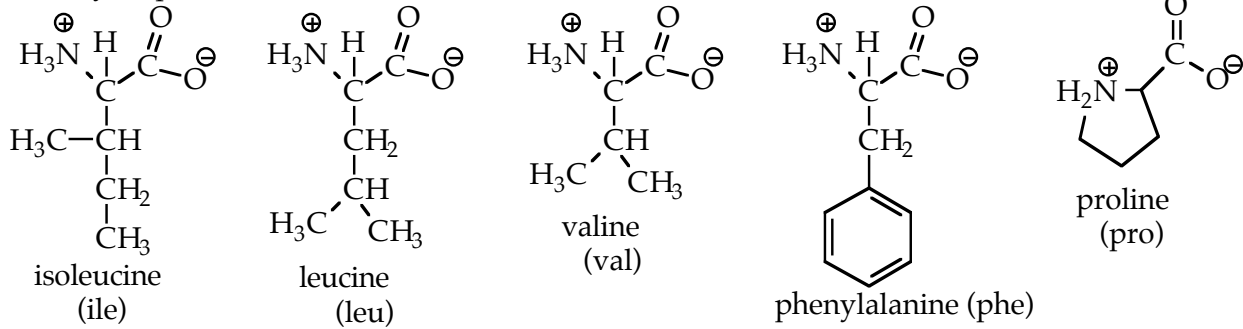
(4) An alteration which changed the histidine to a glutamic acid would reduce the strength of binding of phenyl phosphate to my enzyme. This is because now there would be a  $(-)$  charge on the glutamic acid right next to the  $(-)$  charge on the phosphate - since like charges repel, the phenyl phosphate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



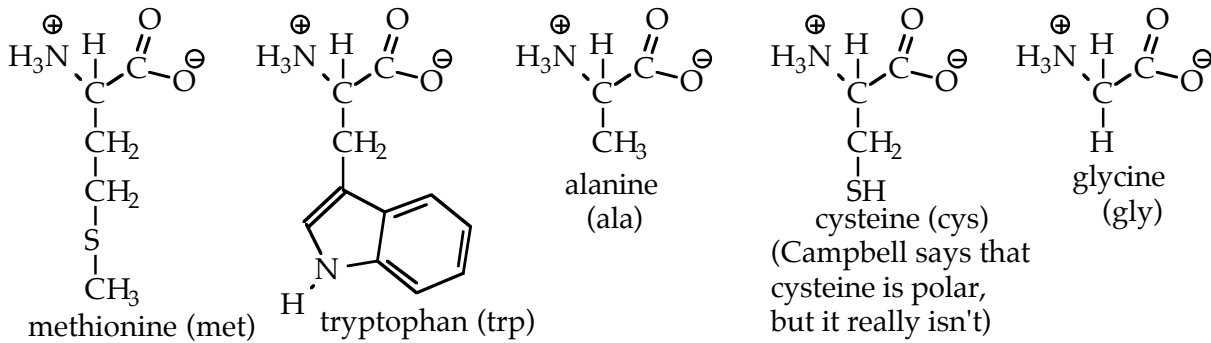
# Amino Acids

## Non-polar

most hydrophobic  $\longrightarrow$



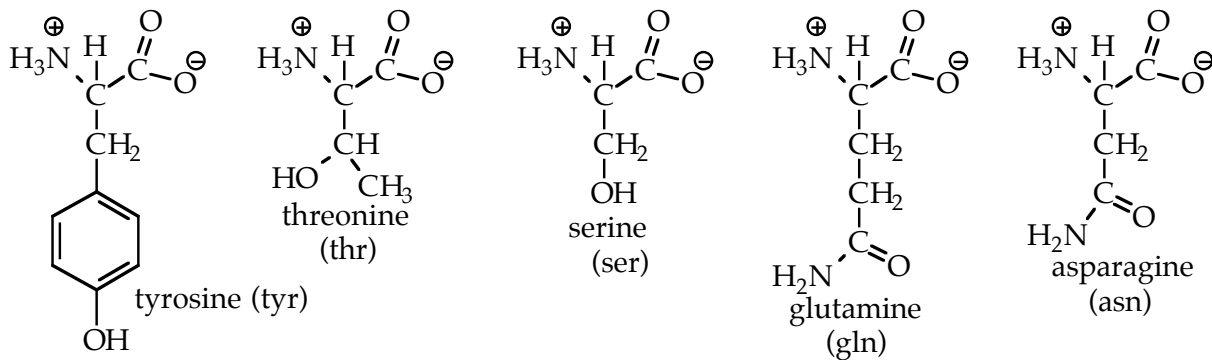
$\longrightarrow$  least hydrophobic (but still non-polar)



## Polar (but uncharged)

least hydrophilic  $\longrightarrow$

most hydrophilic



## Charged (but still very hydrophilic)

least hydrophilic  $\longrightarrow$

most hydrophilic

