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.

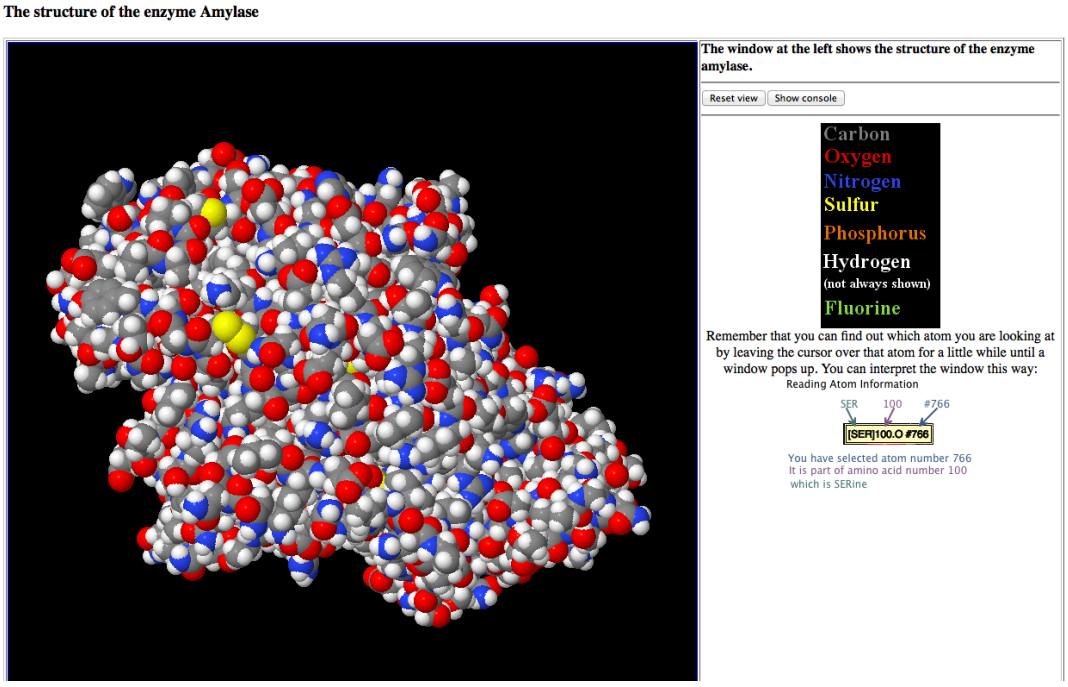
You will explore the protein amylase. Amylase is an enzyme which you'll learn more about later in the course and use in a later lab. Amylase is involved in the digestion of starch in your diet and is present in saliva and secretions of the pancreas. Amylase binds to starch molecules (the "substrate" also referred to as a "ligand") 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 want 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.

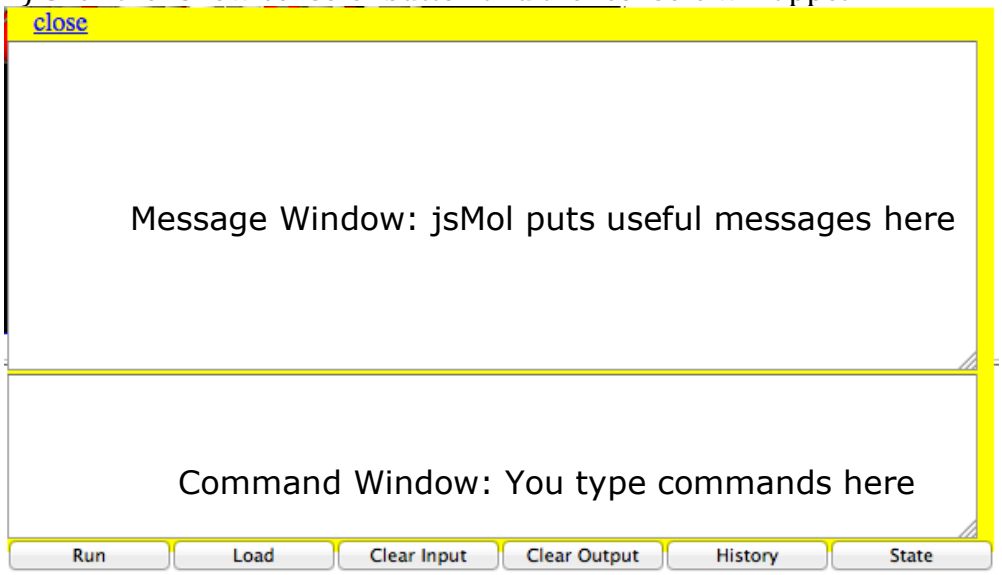
Procedure

<u>Part I: Learning to control jsMol</u>

1) Go to the OLLM for this lab and click the "Protein Structure Exercises" link. You should see this:



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

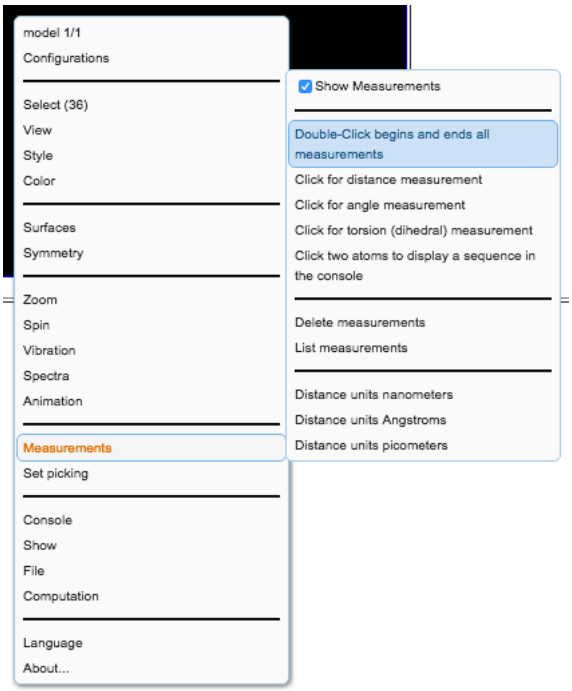
- *Selecting things* (for example):
  - `select 27` – selects amino acid number 27
  - `select ala` – selects all alanines
  - `select ligand` – selects the ligand (aka "the substrate")
  - `select ca` – selects the Calcium ion (Ca^{2+}) that is an important part of the structure
  - `select within (2.0, 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.
  - `center selected` – puts whatever you've selected at the center of the display and makes the selection the center of rotation and zooming. This makes it much easier to zoom in on and explore the part(s) you've selected.

- *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 <u>either</u> in 27 <u>or</u> in 100".
  - `select 27 and 100` – selects no atoms since there are no atoms that are in <u>both</u> 27 <u>and</u> 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 phenylalnine. That is, the nearby neighbors of phenylalnines. Note that other distances can be useful; you should play around to see what works.

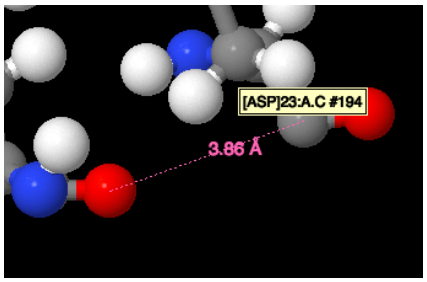
- *Showing things*:
  - `spacefill on` – shows the selected atom(s) as big spheres
  - `spacefill off` – turns off the spacefill view of the selected atom(s)
  - if you want to show the selected atom(s) as "ball and stick", it takes two commands:
    - `spacefill 0.5`
    - `wireframe 0.2`
    - remember 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.

- *Measuring the distance between two atoms*
  - Right-click (or control-click) anywhere on the black part of jsMol; you'll see a menu pop up
    - Choose "Measurements" and then "Double-click begins and ends all measurements"



  - From the same menu, choose "Distance units Angstroms"
  - Double-click on one of the atoms you're interested in.
  - Click on the other atom and the distance will be shown on the screen like this:



    This shows that the distance from the oxygen on the left to the carbon atom in Asp 23 is 3.86 Angstroms.

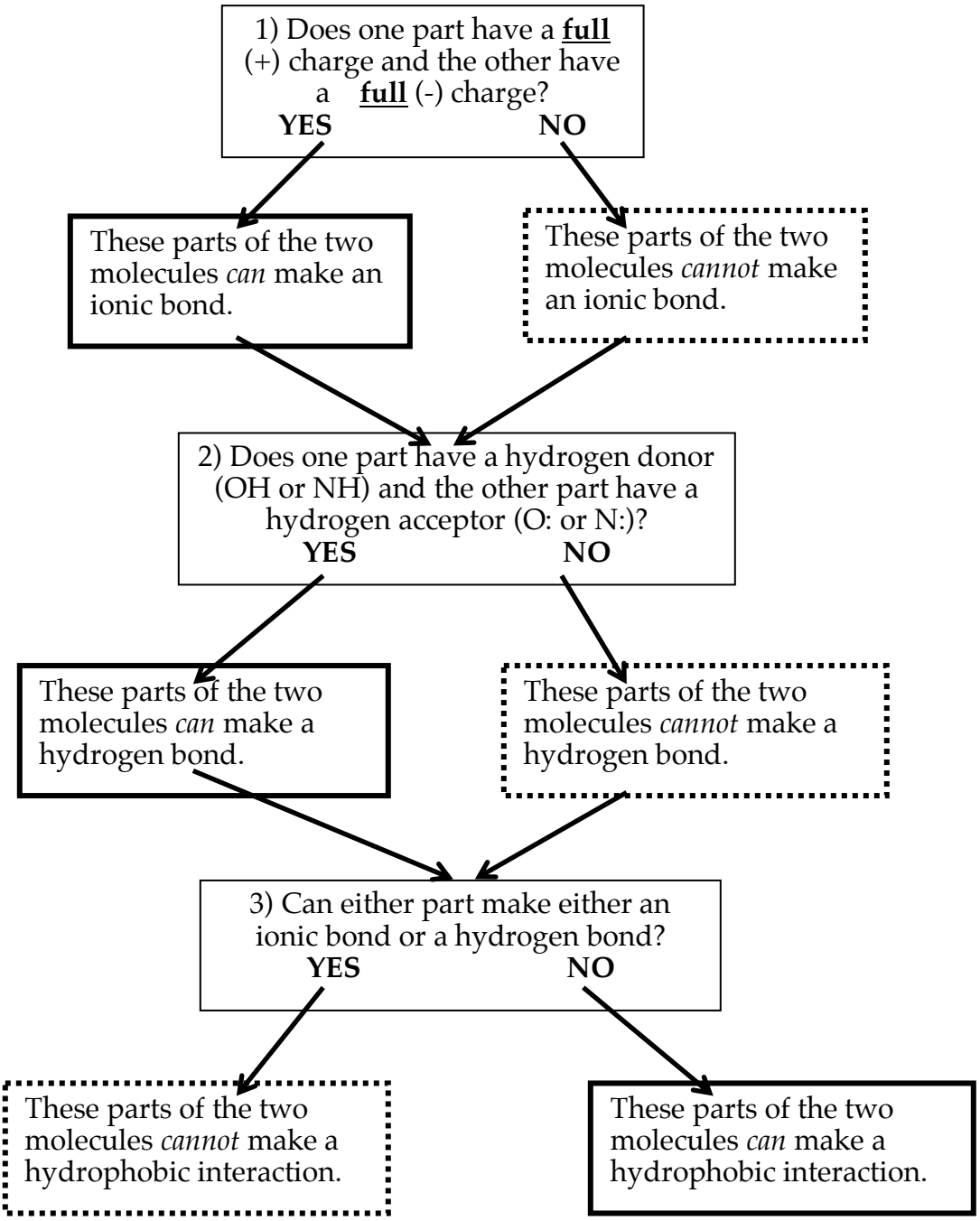
- *Taking a Screenhshot*
  - On the macs, if you hit command-shift-4, you get a little cross-hair cursor. Drag that to select the part of the screen want. A file called "Screen shot…" will appear on the desktop. You can then post this or e-mail it to yourself.

- *Clearing the screen*
  - `select all`
  - `spacefill off`

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

How to decide about non-covalent bonds:

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 below outlines this process:



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	
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

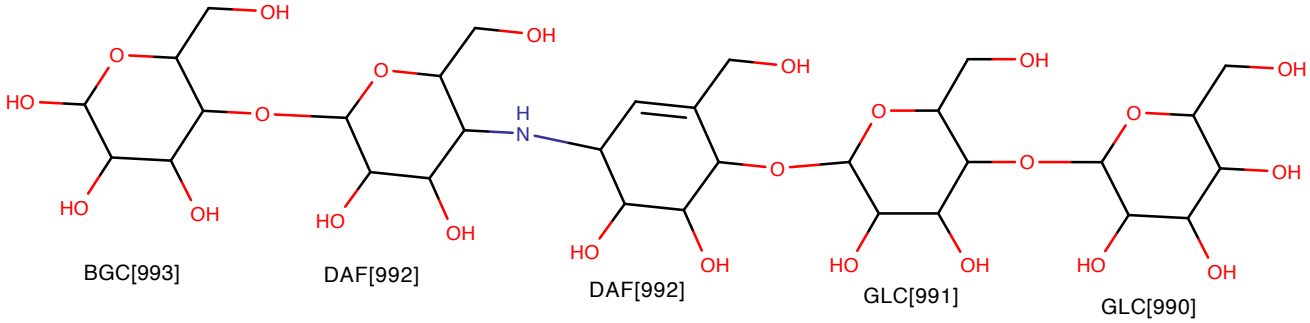
The list below gives several amino acids that interact with the substrate.

63 200 201 233 299 300 305

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"). jsMol calls the ligand "BGC" "DAF" and "GLC" depending on what you click on.

The ligand's structure is shown below. It is a structural analog of starch that cannot be cut by amylase – it is a common technique of crystallographers to use non-reactable substrate analogs because if it were cut, it wouldn't stay at the active site! Note that the middle sugar "DAF[992]" does not have the same structure as the other parts of the molecule – this is the part that cannot be cleaved by amylase.



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. Note that jsMol shows this as a covalent bond even though it shouldn't. Note also that ionic bonds can be 2-4 angstroms long.

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. Hint: you can select all the phenylalanines in the protein by typing "`select phe`"

2) Present your findings to the class:
   a) On the projection screen, set up a view to show your amino acids from (d1).
   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:

Choose two of the interactions from **Part II** (a), (b), (c), or (d). Note that you must choose from two **different** parts – that is, for example, one from (a) and one from (d). For **each** of the two interactions, do **all** of the following:

i) Name the type of interaction (hydrogen bond, ionic bond, interaction with substrate, interaction with calcium ion, or hydrophobic interaction).

ii) Give a screenshot from jsMol that **clearly** shows the interaction. Note that the command: set background white can sometimes make it easier to get a clear screenshot.

iii) A drawing (hand drawings are OK as long as they are **clear and legible**) showing the chemical structures of the parts that are highlighted in the screenshot in (ii). Your drawing should also indicate the interaction from (i) with a dashed line or some other useful symbol.

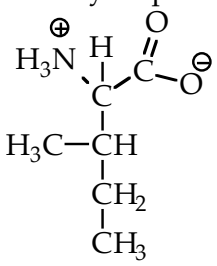
iv) A brief explanation of how you found the interaction and knew what type it was.

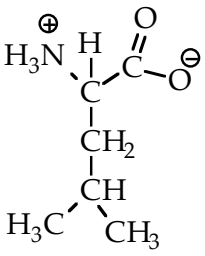
Your lab report should include 2 complete sets of interaction name, screenshot, drawing, and explanation – one for each of the interactions you have chosen.

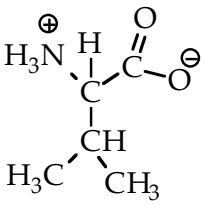
Amino Acids

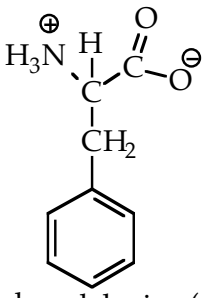
Non-polar

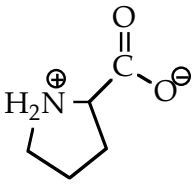
most hydrophobic →


isoleucine (ile)

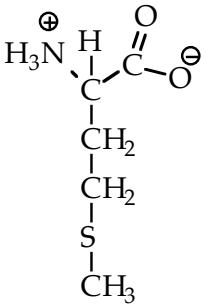

leucine (leu)

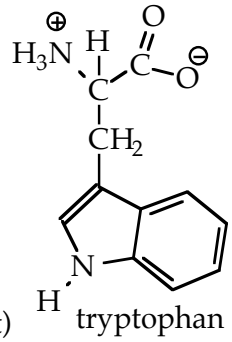

valine (val)

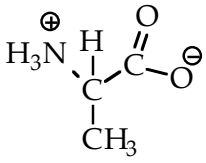

phenylalanine (phe)

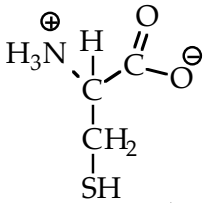

proline (pro)

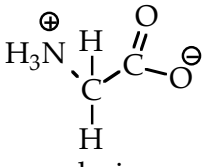
→ least hydrophobic (but still non-polar)


methionine (met)


tryptophan (trp)

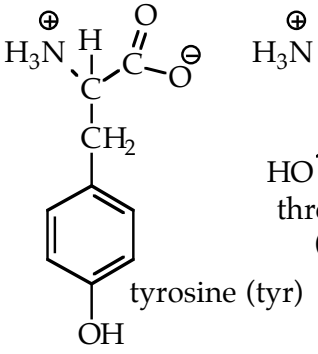

alanine (ala)

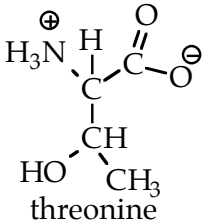

cysteine (cys)
(Campbell says that cysteine is polar, but it really isn't)

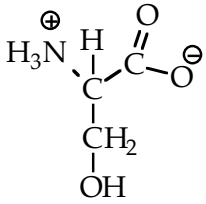

glycine (gly)

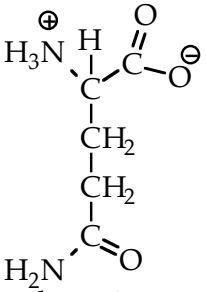
Polar (but uncharged)

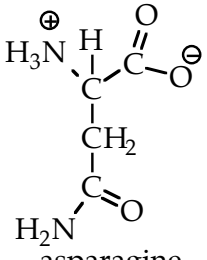
least hydrophilic → most hydrophilic


tyrosine (tyr)


threonine (thr)

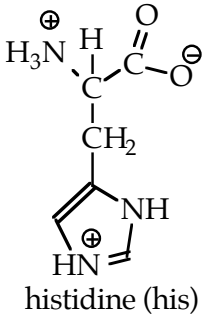

serine (ser)

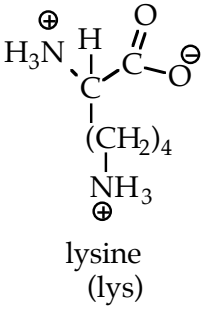

glutamine (gln)

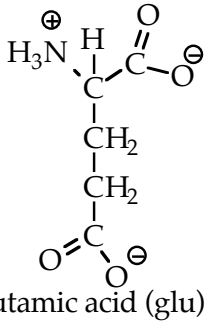

asparagine (asn)

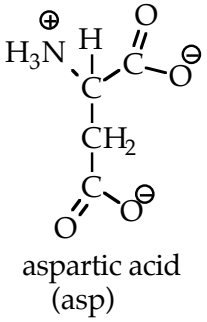
Charged

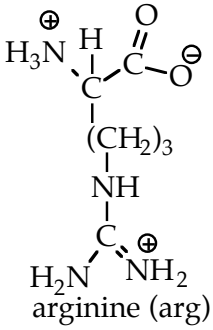
least hydrophilic (but still very hydrophilic) → most hydrophilic


histidine (his)


lysine (lys)


glutamic acid (glu)


aspartic acid (asp)


arginine (arg)