Protein Structure[1]

Model 1. At its simplest level, protein structure is a chain of amino acid residues joined by *peptide bonds*[2]. We can think of a protein chain as a *peptide backbone* from which project the different side chains of the amino acid residues. In Figure 6.1 below, the *peptide backbone* is shown in blue.

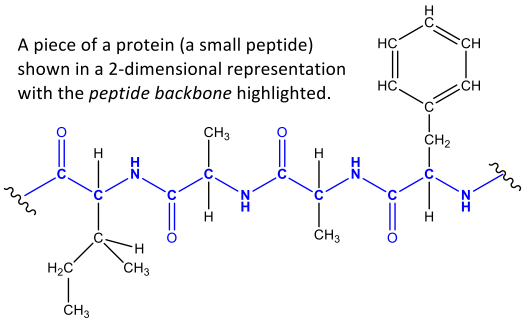


Figure 6.1. A region of protein sequence. The peptide backbone is shown in blue. The side chains of (L > R) isoleucine, alanine, alanine and phenylalanine are in black.

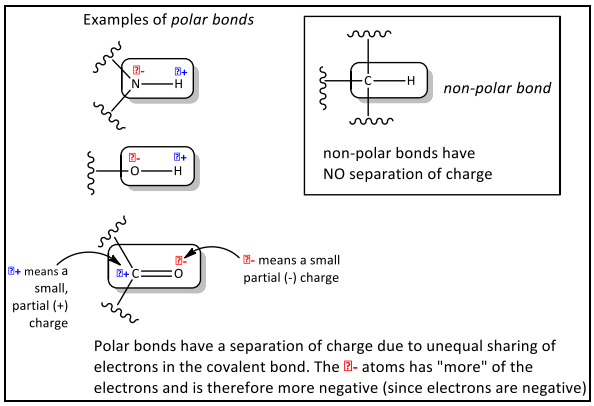
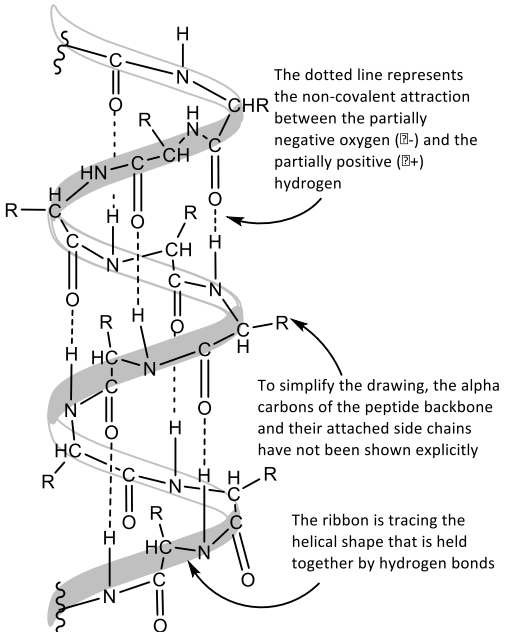


Figure 6.2. Examples of polar and non-polar bonds



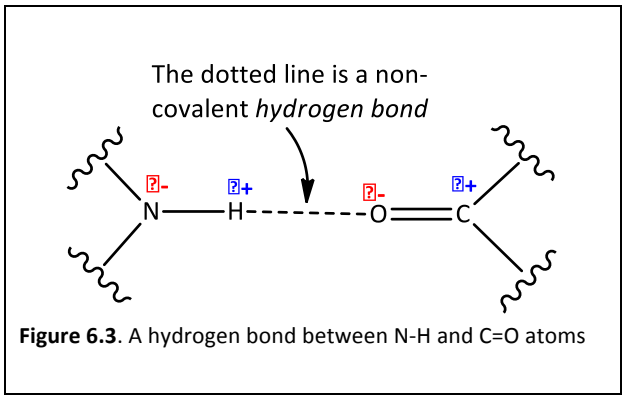


Figure 6.3. A hydrogen bond between N-H and C=O atoms

Figure 6.4. The N-H and C=O bonds of the *peptide backbone* engaging in non-covalent hydrogen bonds.

[1] At the discretion of the instructor, this activity could accompany the activities on Eggs and/or Meats.
[2] For a lesson on amino acids and peptide bonds see Activity 5

Proteins also contain *polar* and *non-polar* bonds. When two atoms are joined by a polar bond, one of the atoms has a partially negative (δ-) charge, while the other atom has a partially positive (δ+) charge. The charge comes from an unequal sharing of the electrons in the conjoining covalent bond. These partially charged atoms can form *weak, non-covalent* attractions that hold the two atoms near one another in physical space. If the *weak, non-covalent* attractions involve a partially positive (δ+) hydrogen atom – then the attraction is called a *hydrogen bond*.

In proteins, the N—H and C=O bonds of the peptide backbone can form *hydrogen bonds* with one another. These hydrogen bonds can stabilize 3-dimensional arrangements of amino acids residues in what is called *secondary structure.* Figure 3 is an example of *secondary structure* called an *alpha (α) helix*. The *primary sequence*[3] of amino acids determines whether or not it will fold into an α-helix or another type of *secondary structure*.

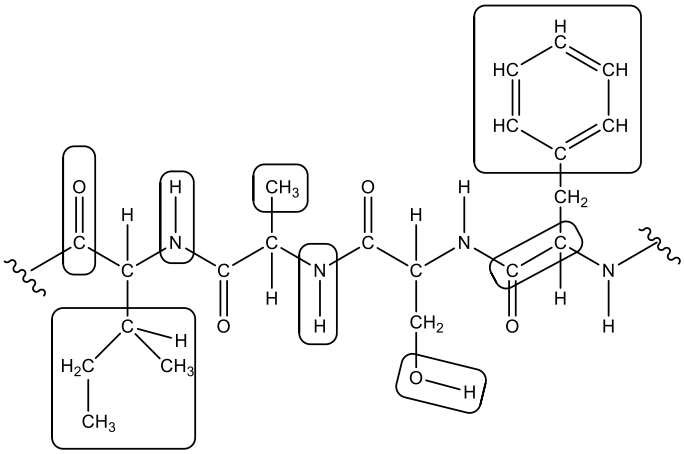
Questions:

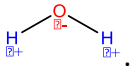
1. Why can hydrogen bonds form between the N—H and the C=O of the peptide backbone but not between the alpha carbon and its hydrogen (C—H)?

2. Draw in the partial negative (δ-) and partial positive (δ+) charges on the atoms of Figure 3 that are engaged in *hydrogen bonding*.

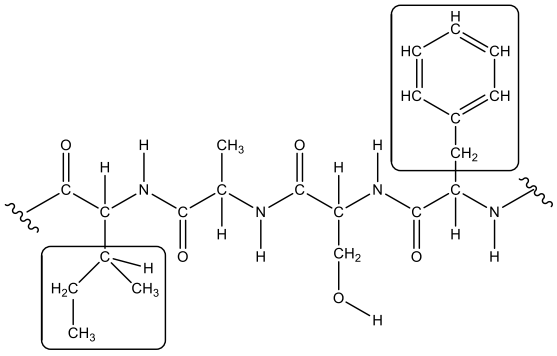
[3] See Activity 5 for a lesson on primary sequence

3. Shown below is a drawing of a peptide. Label each box as containing polar or non-polar bond(s).

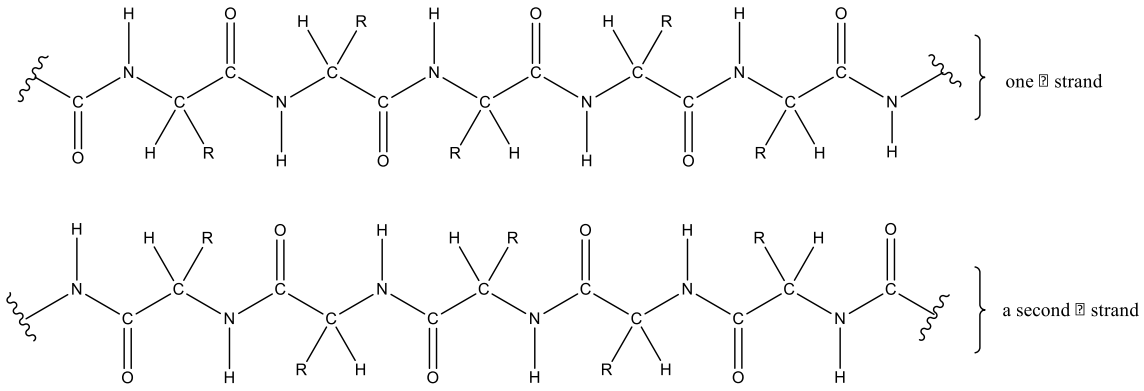


4. Water is another molecule with polar bonds (O—H)  .
    a. Using the structure below, show how water would interact through at least three different *non-covalent hydrogen bonds* with the peptide. Draw in the partial negative (δ-) and partial positive (δ+) charges. These parts of the structure are called *hydrophilic* (literally, "loves water")

    b. Explain why the areas of the structure that are boxed are called *hydrophobic* (literally, "fears water") – these areas of the structure do *not* interact with water. Why?



5. Beta (β) sheets are another type of *secondary structure* found in proteins. Two or more β strands of the protein chain are held next to one another by *hydrogen bonds*. Using the image below, draw in the *hydrogen bonds* that are holding these two chains of amino acids in a β-sheet.



Model 2. Proteins are *amphiphilic* molecules - they have both *polar* (*hydrophilic* = "loves water") and non-polar (*hydrophobic* = "fears water") parts. In nature, we find proteins in water based environments. Because not all parts of the protein love the water (some parts are *hydrophobic*) the protein *folds* in a 3-dimensional way that buries the *hydrophobic* parts on the inside of the structure, and exposes the *hydrophilic* parts to the outside, where they can interact with the watery environment.

Key Concept

Most proteins fold into 3-dimensional structures made of α-helices, β-sheets and loops each held together by hydrogen bonds. The protein folds in order to hide hydrophobic parts and expose hydrophilic parts to water.

Modern biochemistry has allowed scientists to "see" how proteins fold at a molecular level. What they found was quite beautiful (see Figure 6.5).

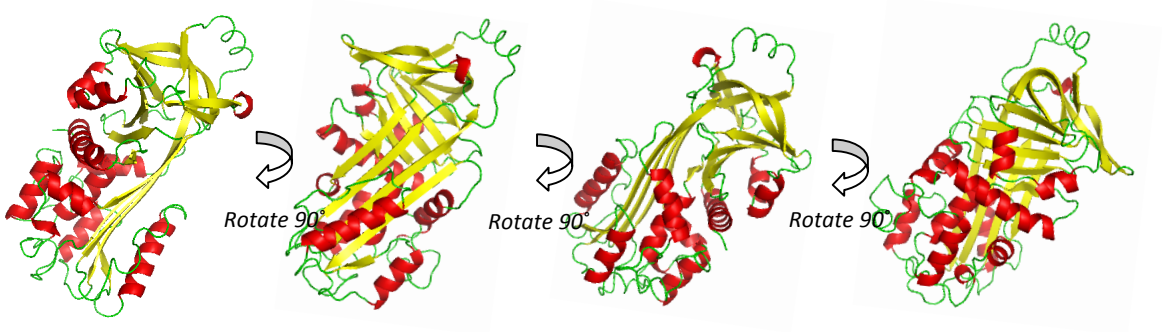
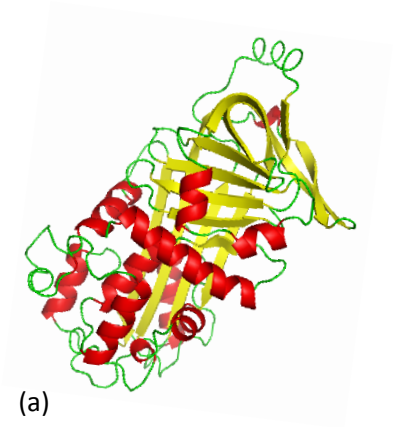


Figure 6.5. The protein ovalbumin (from chicken egg white) as viewed rotating around a vertical axis[4].

But how can Figure 6.5 be depicting a protein? There are no visible amino acids or peptide bonds, just twirly ribbons and curvy arrows. What are these images depicting anyway?

From Figures 6.5 and 6.6, we can see that the protein ovalbumin has many α-helices and β-sheets all *folded* on top of and around each other to make a *globular* structure. In fact, when you fill in all the atoms using a type of *space filling* representation, we can see the lumpy, 3-dimensional *globular* looking protein (Figure 6.7). The α-helices, β-sheets and loops also interact *with each other* by hydrogen bonds.

[4] Images of ovalbumin in Figures 6.5-6.9 were author-generated using PDB: 1UHG (J.Biol.Chem.(2003) **278:** 35524-35530) and PyMol.



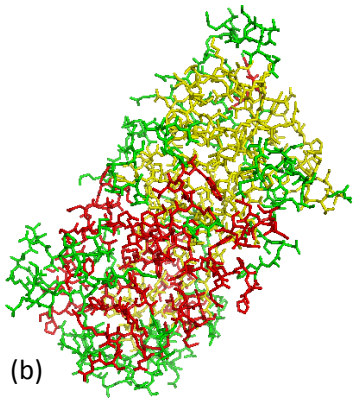


Figure 6.6. The same orientation of the ovalbumin protein molecule represented as a cartoon (a), and then as using *amino acid residues* (b) – the amino acids joined by peptide bonds.

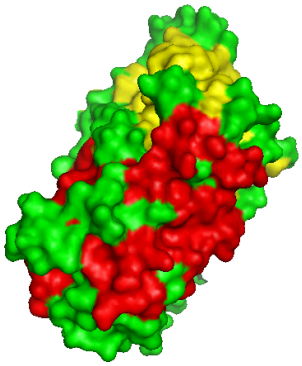


Figure 6.7. A *space filling* representation of ovalbumin. Red are α-helices, yellow are β-sheets and green are loops

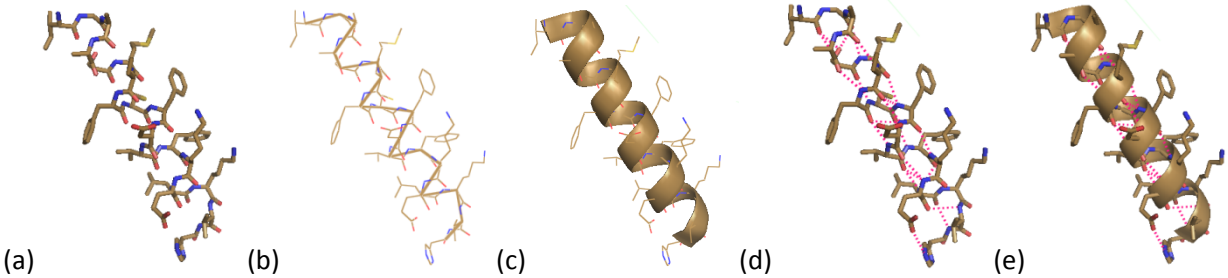


Figure 6.8. A single α-helix from ovalbumin drawn five ways: (a) first as *amino acid residues* - amino acids joined by peptide bonds (carbon = gold, nitrogen = blue, oxygen = red, hydrogens are not shown); then in (b) a thin line is tracing the 3-dimensional pattern of the the peptide bonds between each amino acid (the *peptide backbone*), which makes the helical ribbon shape. In (c), a cartoon ribbon has replaced the thinly traced line. In (d) and (e), we see that hydrogen bonds (pink dashed lines) hold the amino acid residues in this special helical shape.

In the cartoons of Figure 6.5 and Figure 6.6 part (a), a red twirly ribbon is an *α-helix* ("alpha" helix), while a yellow curvy arrow is a β-*strand* ("beta" strand) and many curvy arrows lined up lengthwise next to each other make a β-*sheet*. The squiggly green parts are called *loops*. If we represent a structure of the molecule ovalbumin using the same color scheme as you see in Figures 6.5 and 6.6a, but instead we replace the squiggles, twirly ribbons and curvy arrows with atoms – the structure looks like Figure 6.6b. In Figure 6.6b, those same *helices* (ribbons), *sheets* (arrows) and *loops* are depicted using

amino acid residues (the amino acids joined by peptide bonds). Let's look more closely at the amino acid atoms making a *helix* in Figure 6.8.

In ovalbumin (as with pretty much any protein) some groups of amino acids fold into *α-helices*, some into β-*strands* and some stay as *loops*. The *primary sequence* of the amino acid residues determines which structure they form. Figure 6.8 depicts how an *alpha helix* (for example) is created by an arrangement of *amino acid residues* held together by *hydrogen bonds*. β-*sheet* (2 or more β-strands) and *loop* structures are also held together by *hydrogen bonds* (Figure 6.9).

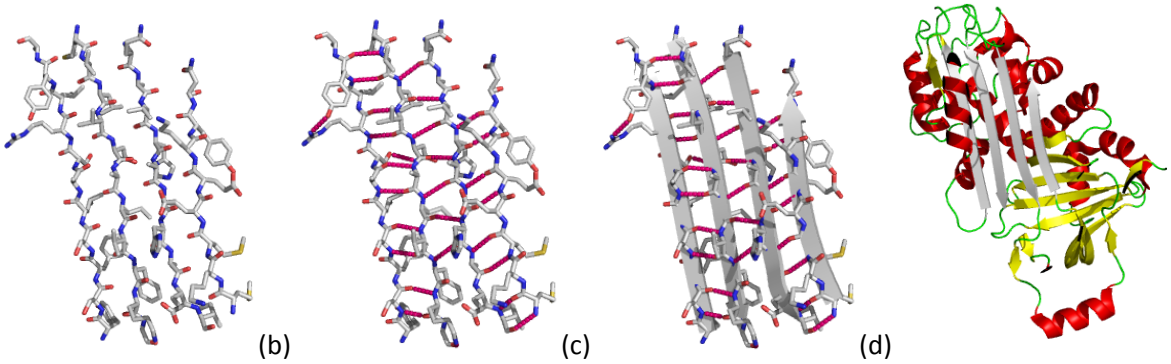


Figure 6.9. A β-sheet from ovalbumin drawn four ways: (a), first as *amino acid residues* - amino acids joined by peptide bonds – gray (carbon), blue (nitrogen), red (oxygen), hydrogens are not shown. then in (b) pink dashes show the *hydrogen bonds* joining the strands. In (c), a cartoon ribbon is superimposed on the previous image. In (d), the ribbon β-sheet is placed within the larger context of the ovalbumin protein.

6. Why do proteins fold into 3-dimensional globular structures made of α-helices, β-sheets and loops? Use the words *polar* and *non-polar* in your answer.

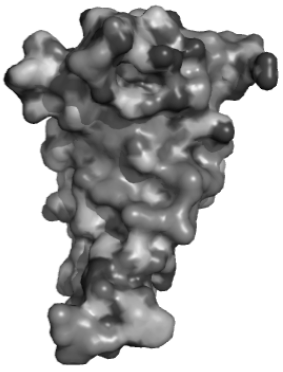
7. Shown below is the structure of the protein avidin – also found in chicken egg white.

[5]

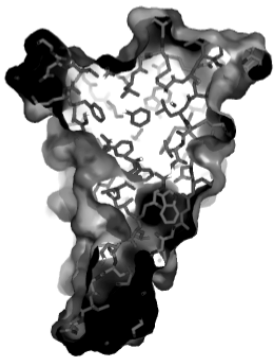
a. Using arrows, identify an α-helix and a β-sheet (a β-sheet is comprised of 2 or more β-strands).

b. What type of secondary structure do you find in avidin *most* often?

8. There are two representations of avidin below. The first (a) shows the overall globular protein in a "space filling" model. The second (b) is a slice right through the middle of avidin – so we can see what the protein looks like on the inside.



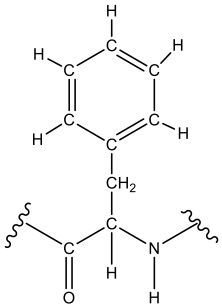
(a)



(b)

[6]

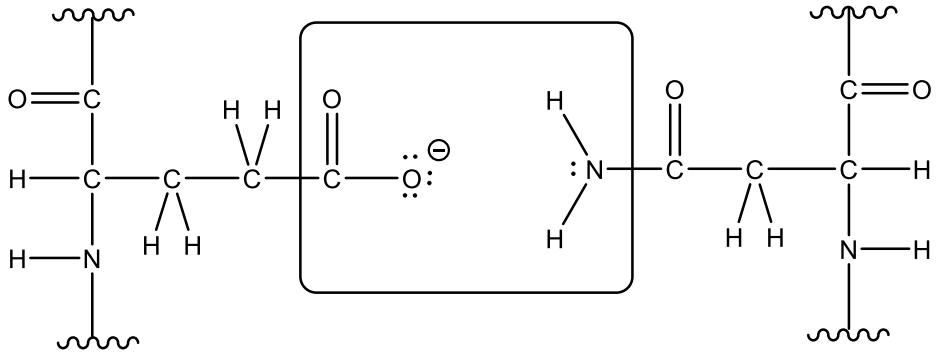
On the interior of avidin, we find several phenylalanine residues. Why?



phenylalanine residue

[5] This image was author-generated from the PDB: 1VYO (Chem.Biol. 2006 **13:** 1029) using PyMol.
[6] These images were author-generated from the PDB: 1VYO (Chem.Biol. 2006 **13:** 1029) using PyMol.

9. 3-dimensional protein structure can also be held together by interactions of ionic[7] amino acid side chains. Drawn below are two amino acid side chains – a glutamate and an aparagine. The glutamate is an anion. For the bonds inside the "box", draw in partial positive (δ+) and negative (δ-) charges, then use dotted lines to show any non-covalent attractions between the glutamate and asparagine atoms in the "box."



[7] In this example, the glutamate has an anionic oxygen (negatively charged). The presence of the full charge means this oxygen is *ionic* – rather than *polar*.

Protein structure…unraveled

Model 3. There is more than one way to disrupt unfold or otherwise ruin the *folded* protein structure. Unfolding of a protein is called *denaturation*. *Denaturation* is the process of disrupting the *hydrogen bonds* and other non-covalent interactions holding the pretty α-helices, β-sheets and loops together, and the 3-dimensional *globular* protein structure unravels. When the protein unfolds, all the *hydrophobic* parts that were buried inside the protein are exposed to the watery environment – AH! The hydrophobic parts *hate* the water, and instead they'd like to find another hydrophobic place to be. The exposed hydrophobic parts of a protein join together with exposed hydrophobic parts of other proteins, clumping together in a process called *coagulation*. The *unfolded*, *coagulated* protein is now a large, aggregated complex that can't stay dissolved in the water – so, the coagulated protein *solidifies,* trapping other molecules (such as water or fat) between the unfolded proteins.

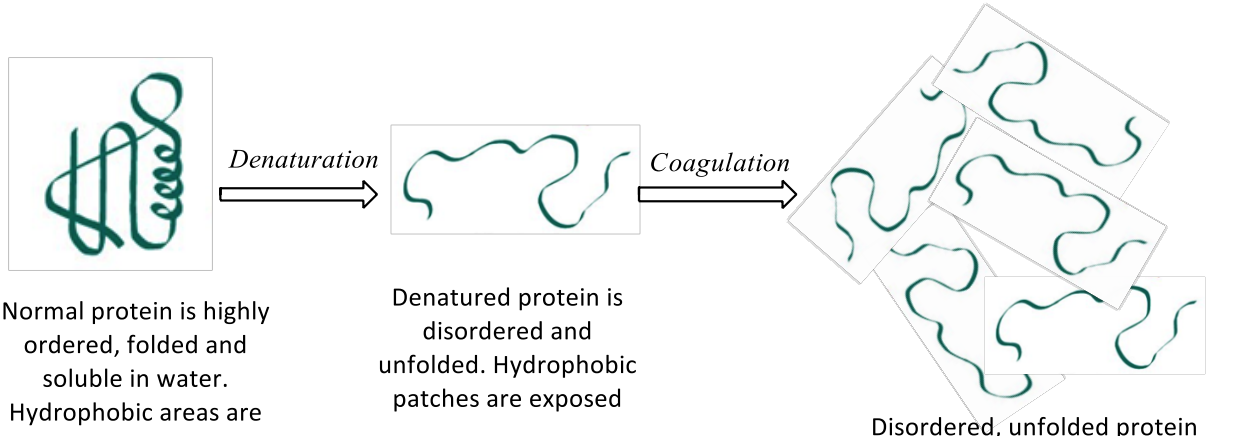


Figure 6.10. Protein Denaturation and coagulation at the molecular level[8]

Heat is one strategy for protein denaturation. When a protein is *denatured* by heat, the heat energy added to the mixture of proteins makes the molecules move around. Add enough energy and the proteins move and groove enough to break all the *non-covalent*

[8] The cartoon images are from Boyer's Concepts in Biochemistry, 3rd edition, p.110, Figure 4.6

interactions holding the protein together, and the 3-dimensional *globular* protein structure unravels. This is the type of denaturation and coagulation we see in the cooking of an egg.

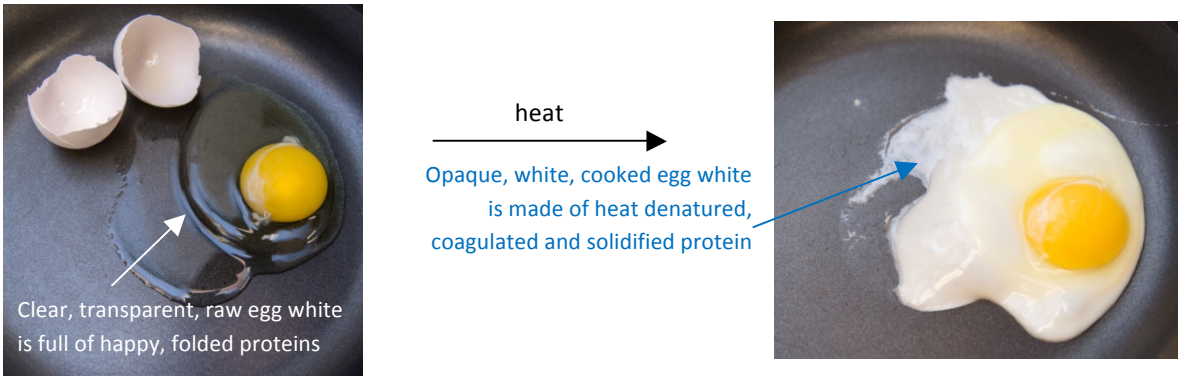


Figure 6.11. Protein denaturation and coagulation caused by heat – as seen with the unaided eye. *Photos by Bill Keller*

Acidic conditions are another way to *denature* protein. Some amino acid side chains react with the protons (H^+) in acid. Amino acid residues like lysine, aspartate or glutamate have side chains that gain or lose a proton (H^+) depending on the pH– these atoms are said to be *protonated* (gain of H^+) or *de-protonated* (loss of H^+).[9] Changing the charge disrupts non-covalent interactions between amino acid residues.

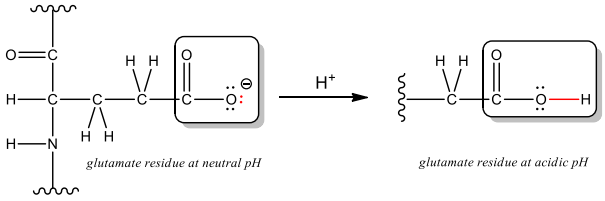
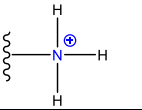
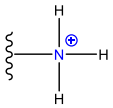
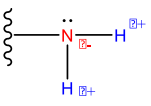
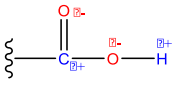
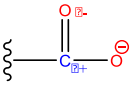
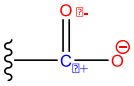


Figure 6.12. The side chain of the amino acid residue glutamate after *protonation* under acidic conditions.

Table 6.1. Amino acid side chains and pH

Very Acidic (< pH 4.5)	Mildy acidic to mildly alkaline (pH 4.5-10)	Very alkaline (pH > 10)
		
		

[9] For a lesson on pH, acids, protonation and deprotonation – see Activity 8.

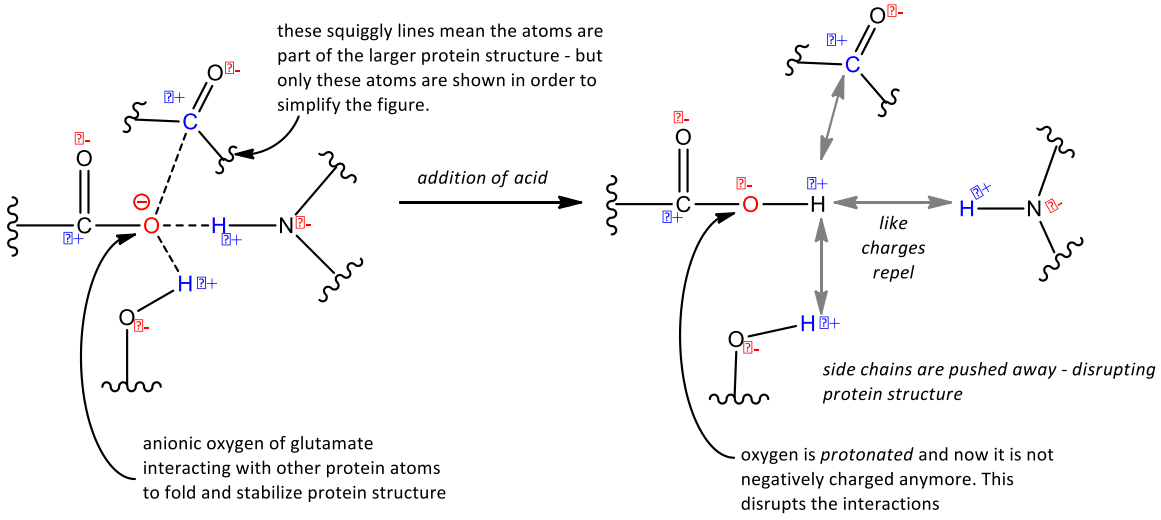
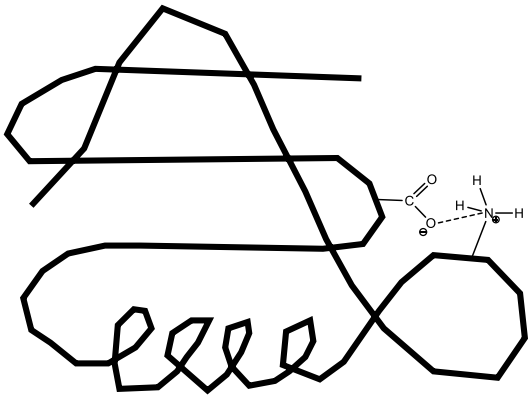


Figure 6.13. Changing the pH changes the charges on some amino acid side chains. If these side chains are found within a protein structure, the changes in charge impact interactions between side chains and therefore the overall protein structure.



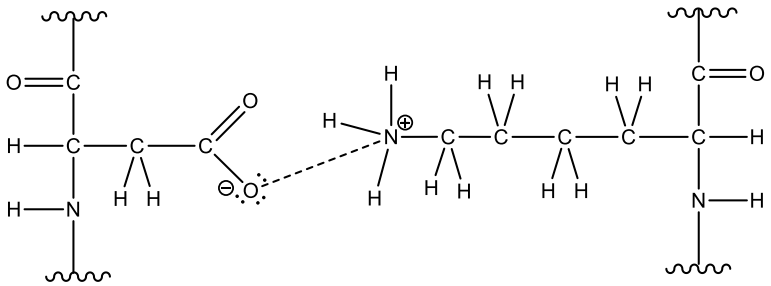
Acid *denaturation* unfolds the *globular* protein structure, causing exposure of hydrophobic areas and coagulation as shown in Figure X.X. The disruption of *non-covalent* attractions between charged or partially charged atoms in amino acids weakens the folded structure until the protein unravels. The process of turning milk into cheese or yogurt can be accomplished by acid *denaturation* of the milk protein, casein.

Figure 6.14. A cartoon showing charged amino acid side chains stabilizing a protein fold.

Questions

10. A hard-boiled egg is prepared by heating raw eggs in their shells in a pot of boiling water. Explain why a raw egg will release its gooey, slimy contents when cracked, and yet a hard-boiled egg looks like this…? Use the words *denaturation* and *coagulation* in your answer.

11. At the neutral pH of milk (pH 6.5-6.9), the side chains of lysine and aspartate can interact as shown below:



    a. What is the nature of this interaction (the dotted line)? (hint: it is *not* a hydrogen bond)

    b. Under acidic conditions (such as those in the curdling of milk), what happens to this non-covalent interaction? Draw on the figure and show any changes to the charged amino acid residues.

c. Draw a cartoon to explain how disruption of interactions between amino acid atoms leads to unfolding (denaturation) of the protein structure.