14

◉ Sheet ○ Slides

Done By

Tamer Barakat™

Contributed in the Scientific Correction

Razi Kittaneh™

Contributed in the Grammatical Correction

Mohammed Bio™

Doctor

Diala Abu-Hassan

Protein structure:

We already know that when two amino acids bind, a dipeptide is formed which is considered to be an oligopeptide. When more amino acids are added to the chain, a polypeptide is formed which may be considered as a protein.

A protein may have a lot of possible structures, only a few of them can be functional. **Native conformations** are the 3D structures of properly folded and functional proteins.

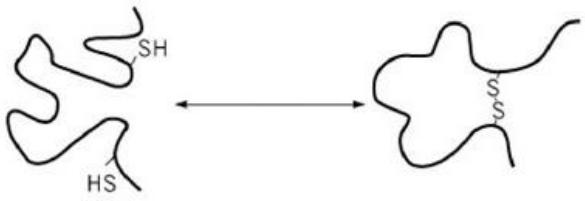
Note: the doctor didn't mention that proteins are polypeptides formed by more than 100 residues as we studied in the last semester.

Levels of organization:

We have four levels of protein organization:

1- **Primary structure:** it's the sequence of amino acids starting from N-terminus toward **C**-terminus.

- Different organisms may have different sequences and numbers of amino acids for the same protein.
- What is the importance of the amino acid sequence?
  This sequence affects the higher levels of organization since it contains the inherited information necessary for protein folding. For more illustration, imagine an amino acid sequence which contains two Cysteine amino acids at positions 5 and 50, these (Cys) will form disulfide bridge in a proper environment (oxidizing environment) by making a huge loop (hence the primary folding).



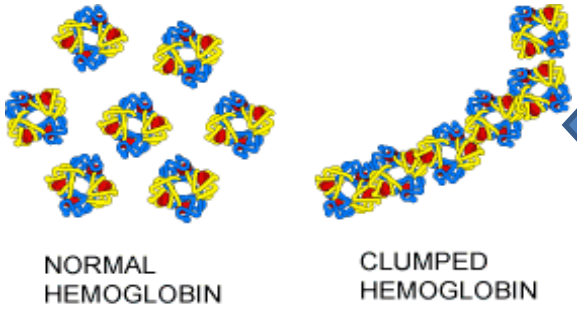
- Another illustration is the ionic interaction between a positive charged amino acid such as lysine with a negative charged one such as glutamic acid. Notice that the distance is an important factor in bonding, if the two charges above are far away, the protein will bend in some specific way to allow the bonding.

Example of primary structure: Leu—Gly—Thr—Val—Arg—Asp—His

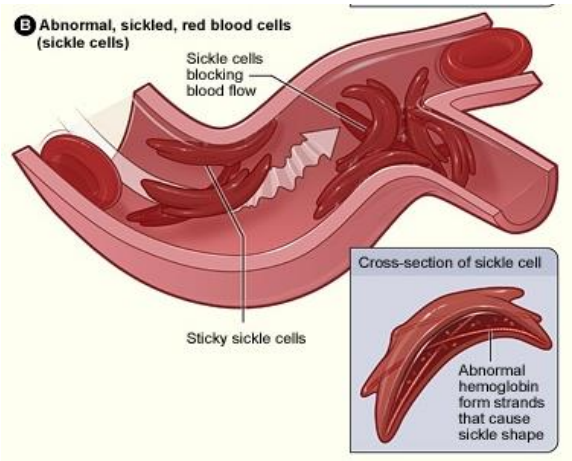
- We conclude that the amino acid sequence determines the structure of the protein, therefore any change in the sequence would result in a change of the protein's structure, which would lead to malfunction as in sickle blood disease.

Sickle blood disease results from DNA mutation, when translation happens, **position 6** in the sequence of **β-globin** is substituted from **Glu** to **Val.**

Remember that Glu is an acidic amino acid while Val is non-polar, this would result in a huge difference of the structure and therefore the function of the protein.



Unlike **Glu**, it would be unstable to have **Val** group in contact with aqueous environments of the cell because it's non-polar, therefore **Val** groups of different **β-globins** will clump together in order to conceal from the environment.

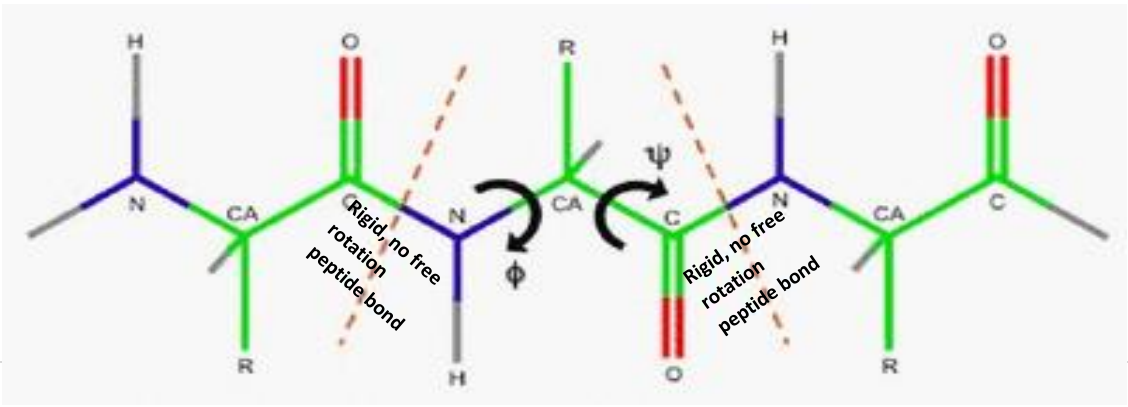


This accumulation would result in shifting the shape of the cell from **Biconcave disk → sickle cell shape**.

The importance of the Biconcave disk shape was its flexibility, since it had two concaves it could easily bend like a sandwich to enter small capillaries for gas exchanging. However, the sickle shape of the cell is rigid because of the clumping of the proteins.

2- Secondary structure: it's the structure resulted from non-covalent interactions of backbone atoms, the most common secondary structures are **α helix** and **β pleated sheet**

the secondary structure results from the folding around two bonds:
Phi → between the alpha carbon and the amine group
Psi → between alpha carbon and the carbonyl group

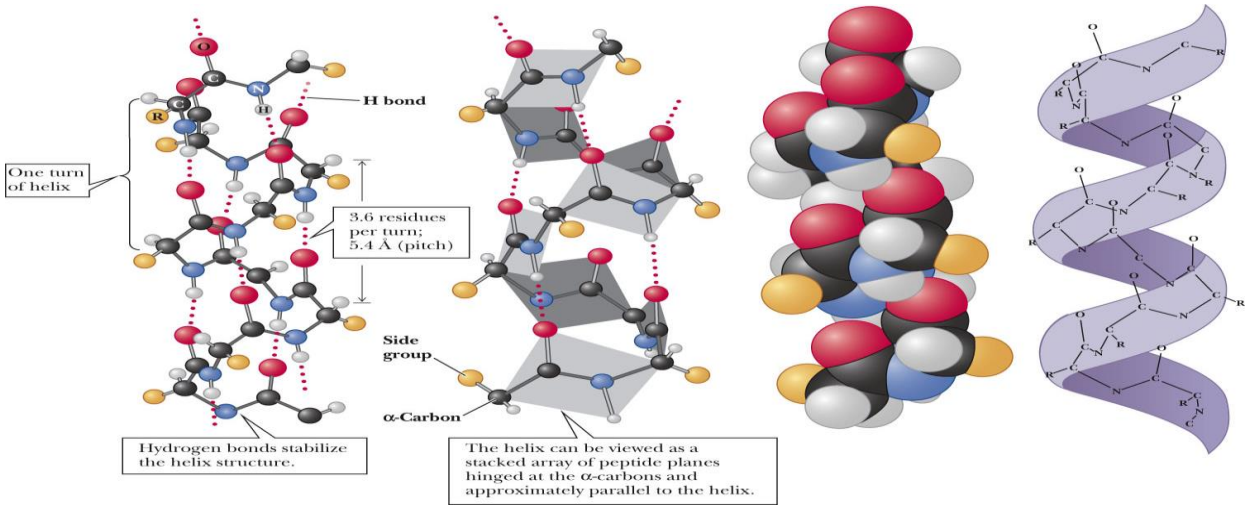


Now, lets study the common secondary structures:

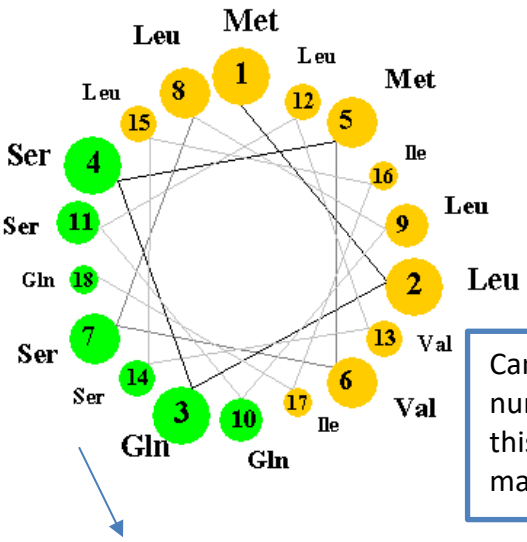
A- **α Helix**: it's helical in shape, hollow in the middle, it can have different lengths, sizes and orientations.

- The helix has an average of 3.6 amino acids *per* turn, which means that a single turn has 3 amino acids and 60% of the fourth.
- If the structure is hollow from the inside, what did stabilize the helix? It's the H-bonds between successive turns
- The pitch of the helix (the linear distance between corresponding points on successive turns) is 5.4 Å (1 Å = 10^{-10} m). This distance also corresponds to the linear H-bonds, which makes the structure rigid.
- Since the turn contains 3.6 amino acids and the turns height is about 5.4 A

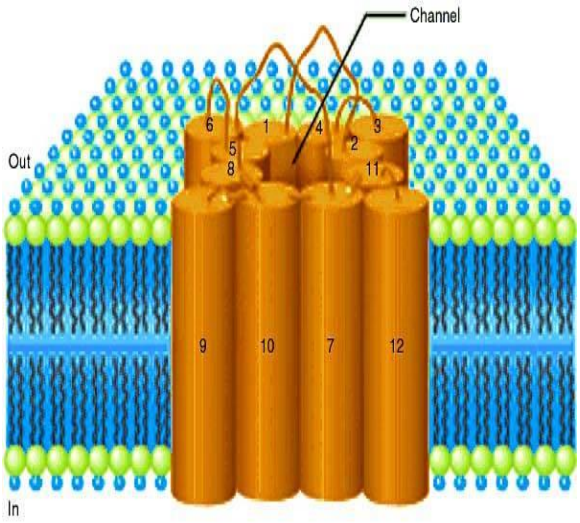
$$\frac{5.4}{3.6} = 1.5\ A\ or\ .15\ nm\ height\ per\ aminoacid$$



- Would you expect the R groups to be inside the helix?
  No, The R groups would orient themselves outside the helix to prevent steric hindrance and other disturbing factors. However, if two tryptophan molecules for example are in sequence, there would be no space for the 2 bulky side chains and the helix would be disturbed. Same thing would happen if we have the same charge in 2 successive amino acids, repulsion would destroy the Helix**.**
- These are some amino acids that disturb the alpha helix:
  1. Glycine; it's too small.
  2. Proline; it can't form a H-bond with the following turn so the helix is disturbed, also it's a **Psi** bond and can't rotate due to the penta-ring structure.
  3. Amino acids with same charges are close to each other; due to repulsion.
  4. Amino acids with branched side chains at β carbon (Val, Thr, Ile); due to steric hindrance.



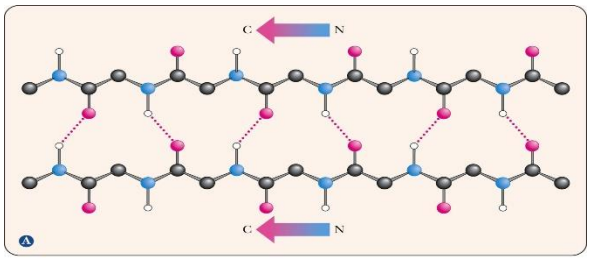
Can you figure out the number of helixes in this figure? I think it may be 3!



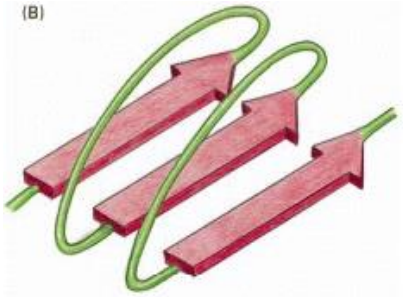
The picture illustrates an alpha helix (from above) that is part of a channel protein, remember that the side chains are always outside the helix.
We need the side chains to be polar at areas in contact with the ions (interior of the channel) and non-polar at areas in contact with the fatty acid's tails.

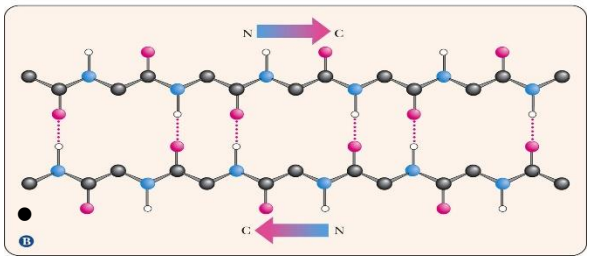
B- **β pleated sheets**: They are composed of two or more straight chains (β strands) that are hydrogen bonded side by side, don't forget that all shapes of secondary structure rely on the interactions of the backbones.

- Keep in mind that the 3D structrue of β pleated sheet isn't flat but zigzaging due to the rotations around Phi and Psi bonds.
- There are two types of β pleated sheet; parrallel and anti-parallel:

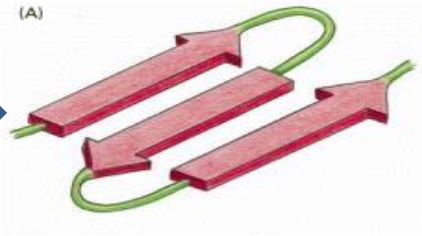


parallel





Anti-parallel



- Notice that the N-terminus and the C-terminus are responsible for these two types. If the loop between the sheets is small, the sheets would have anti-parallel orientation, with its N-terminus and C-terminus alternating positions. Parallel orientation has big loops and parallel N/C- terminus
- a protein may contain one or both types of beta sheets.
- β sheets can be formed between many strands, typically 4 or 5, but might be formed between 10 or more.

- Val, Thr and Ile are preferable in β sheets. Since the successive amino acids are in trans configuration (R1 is above the sheet, R2 below….) there would be enough space for the branched R groups.
- Proline would disturb the beta sheet because it can't form H-bonds.

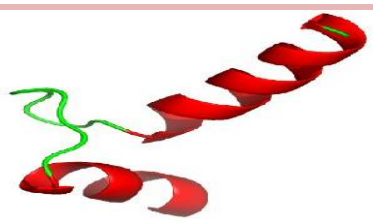
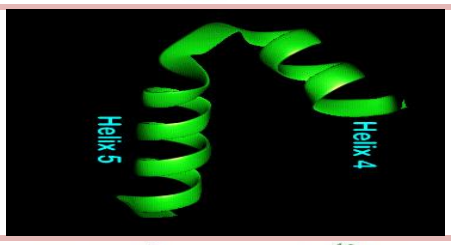
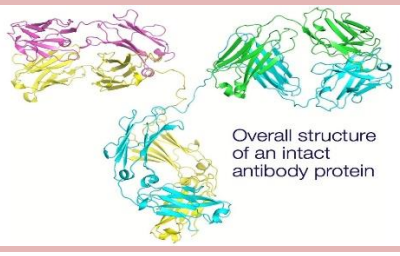
C- **Turns**: they're the most variable type of the secondary structures, they're very important for the functional conformation because they form the most sensitive parts.

- A turn can be found between two helices, two beta sheets or between a helix and a sheet, it is important to give free space between helices (for example) to prevent steric hindrance.
- Turns form the "loops" between parallel beta sheets and anti-parallel ones.
- They are also known as **β turn** or **hairpin bend**.
- The difference between a loop and a turn is the length only, a turn is smaller than a loop.
- Proline is commonly present in turns, due to its shape which reflects a curvature, also glycine is present due to its small size; hence the variability in turns.

Super-secondary structures: They are regions in proteins that contain an ordered organization of secondary structures, they have two types: **motifs** and **domains**

a. **motifs**: is a repetitive super-secondary structure, which can often be repeated and organized into larger motifs in different proteins.

- A small portion of a protein (typically less than 20 amino acids)
- In general, motifs may provide us with information about the folding of proteins, **but it's not related to the biological function.**
- Some examples of motifs are:

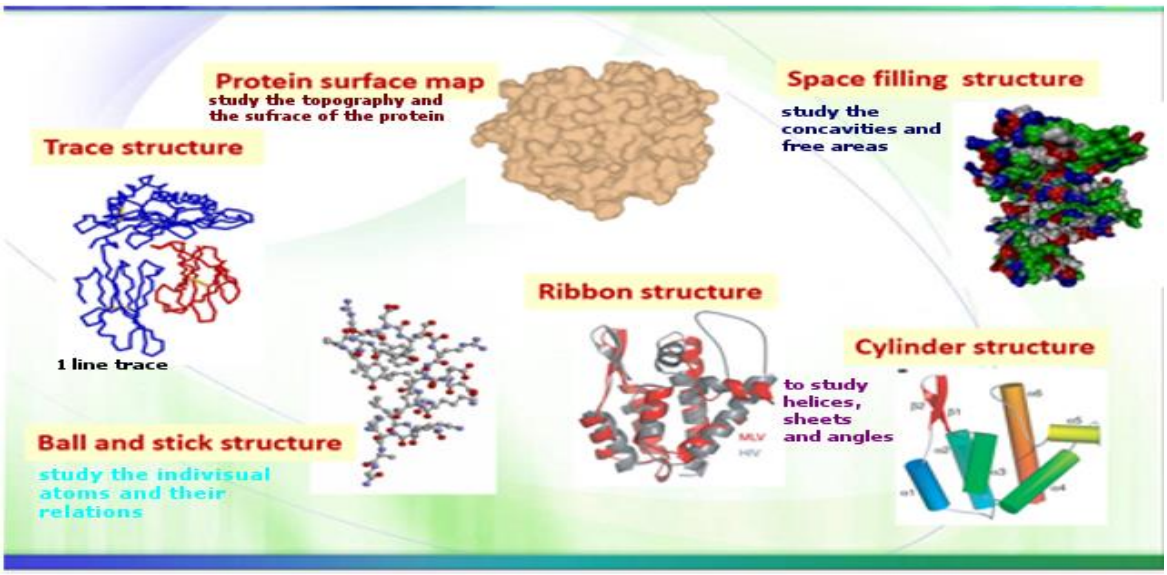
Helix-loop-Helix	Found in proteins that bind DNA and is characterized by two alpha helices connected by a loop	
Helix-turn-Helix	it's a motif capable of binding DNA, its composed of two helices connected by a short strand (a turn).	
Anti-body motif (a more complex one).	The immunoglobulin fold or module that enables interaction with molecules of various structures and sizes.	

- Remember that motifs are not related to function, which means we can find Helix-turn-Helix in a channel and an enzyme.
- Don't forget that a turn is a small loop.

*The idea behind **motifs** is to **abbreviate**, for instance, instead of saying **four alpha** helices we can say **two Helix-loop-Helix** and so on.*

3- Tertiary structure: it's the final shape of the peptide chain, with the interactions between R groups and with all modifications.

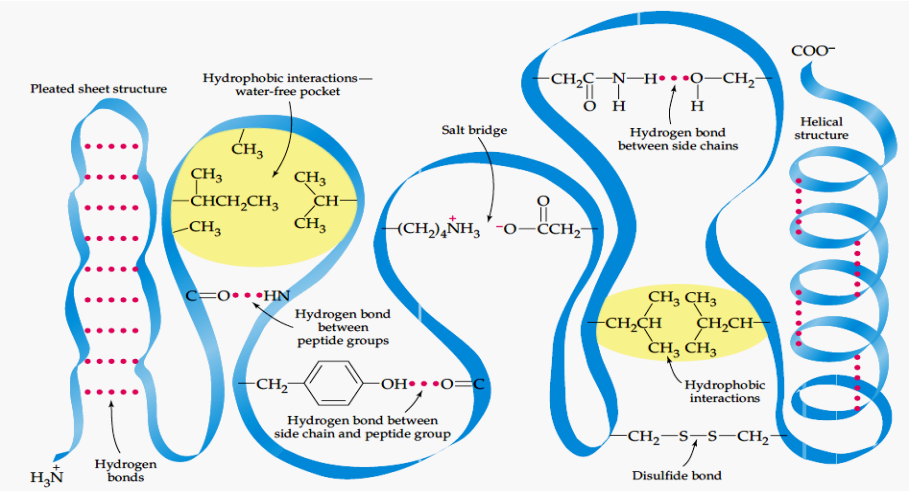
- The difference between the secondary and tertiary structures: from secondary structure I may know that my protein contains three alpha helices, but I don't know their orientation in 3D space, are they parallel? Perpendicular? This orientation depends on the side chains, which is the tertiary structure.
- The only covalent interaction in tertiary structure is disulfide bridges, the other interactions are all non-covalent.
- We can illustrate proteins by different ways according to the purpose of our study, check the picture.



- As mentioned earlier, the tertiary structure takes the interactions of the side chain in consideration.

Try to point out each of the following interactions in the picture:

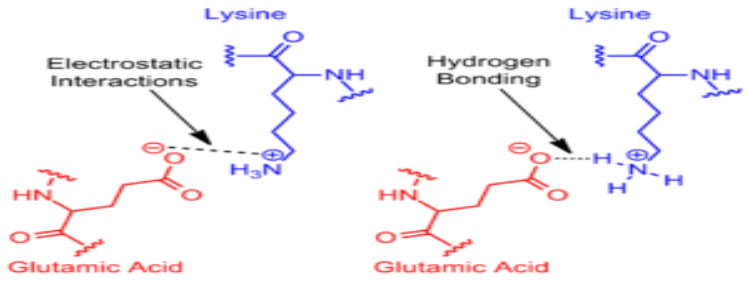
1- **Covalent** disulfide bridge
2- H-bonds
3- Hydrophobic interactions



Let's study the non-covalent interactions with more details:

Remember: the interactions can happen backbone-backbone, which contributes to secondary structure, backbone to sidechain and sidechain to sidechain (contributes to tertiary structure).

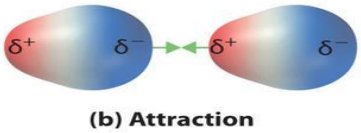
- Hydrogen bonds does **not** only occur within and between polypeptide chains but with the surrounding aqueous medium.
- Charge-charge interactions (salt bridges) occur between oppositely charged R-groups of amino acids.
- Charge-dipole interactions form between charged R groups with the partial charges of water.



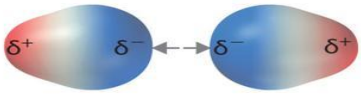
Remember: The same charged group can form either hydrogen bonding or electrostatic interactions

- Van Der Waals interactions can be both attractive and repulsive as we studied before, although they are weak, they're very important because they are present excessively in big proteins.

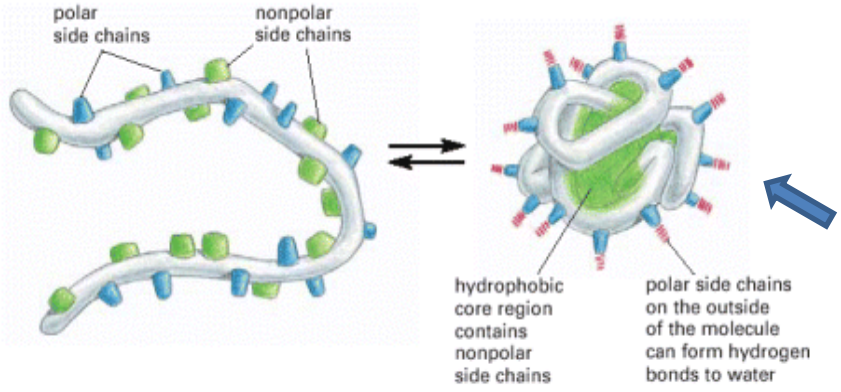
(Remember that Van Der Waals interactions occur between all atoms)



(b) Attraction



(d) Repulsion



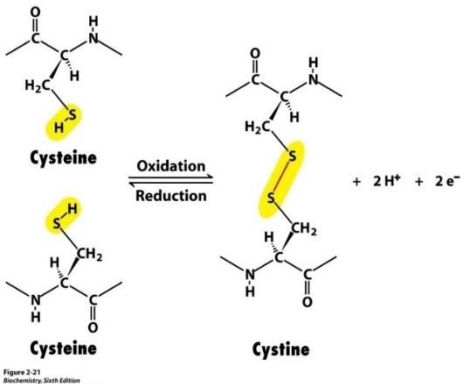
A system is more thermodynamically (energetically) stable when hydrophobic groups are clustered together rather than extended into the aqueous surroundings. *(slides)*

In the picture above the hydrophilic side chains oriented themselves to the surface of the protein, but can they orient themselves to the interior? Yes, when the interior is hydrophilic as in ionic channels.

- There are two forces that do not determine the three-dimensional structure of proteins, but stabilize these structures:
  Disulfide bonds & metal ions

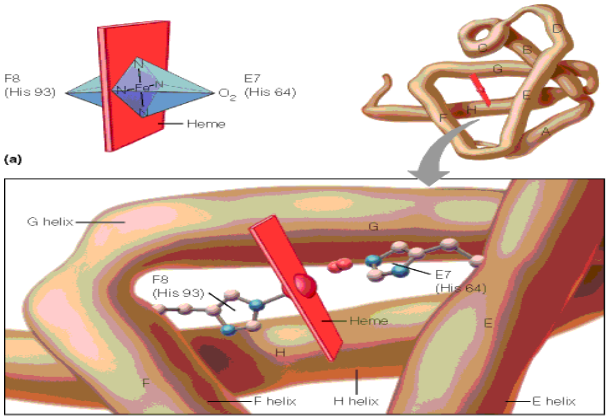
Disulfide bonds: Covalent bonds, relatively stronger than non-covalent ones.

- When the reactive sulfhydryl group is oxidized in Cysteine amino acid, it makes a disulfide bond with another Cysteine, the overall two oxidized cysteine molecules are called Cystine.



Metal ions: Several proteins can be *complexed to a* single metal ion that can stabilize protein structure by forming:

- Covalent interactions (myoglobin)
- Salt bridges (carbonic anhydrase)



- Myoglobin and hemoglobin both contain heme groups, which in turn contains the metal ion.
  Note: the doctor said that salt bridges are sulfide bridges, but the internet doesn't agree…

Domains *(from slides)*:

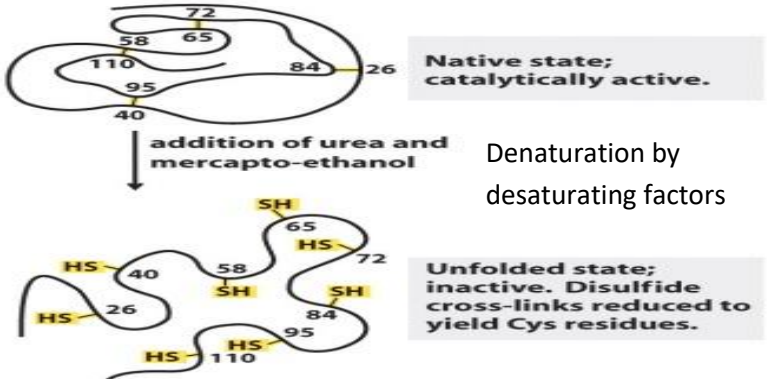
- A domain is a compactly folded region of a polypeptide found in proteins with similar function and/or structure (they are **related** to function unlike **motifs**).
- Domains with similar conformations are associated with the **particular function**.
- A structural domain may consist of 100–200 residues in various combinations of α helices, β sheets, turns, and random coils.
- They fold independently **of** the rest of the protein.
- Domains may also be defined in functional terms
  - enzymatic activity
  - binding ability (e.g., a DNA-binding domain)
- We can predict the function of the protein from certain domains, for instance, a protein that has a kinase domain must have enzymatic activity.

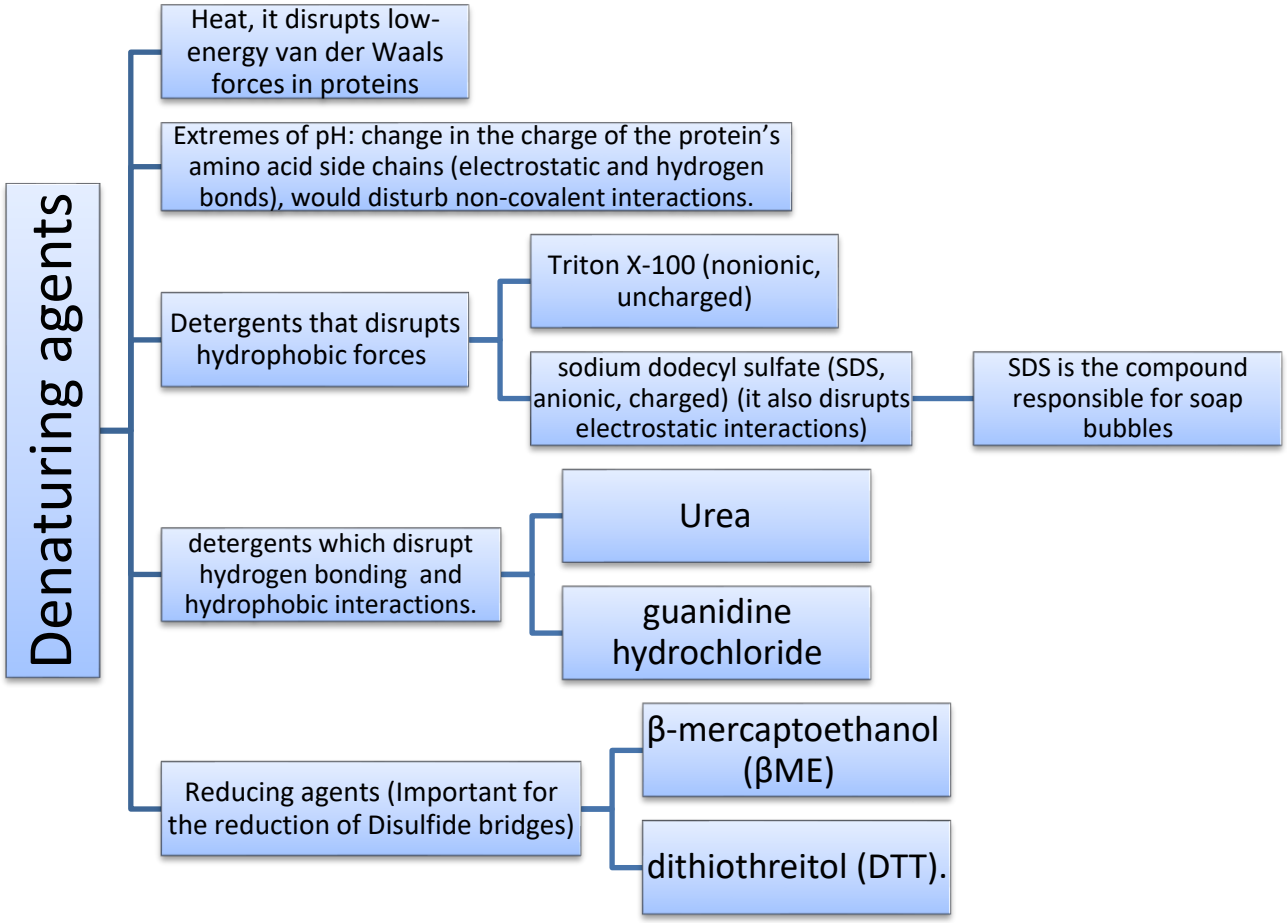
Properties of Proteins:

a. **Denaturation:** is the disruption of the *native conformation* of a protein, the characteristic three-dimensional structure that it attains after synthesis.

- Denaturation involves the breaking of the noncovalent bonds which determines the structure of a protein, *NOT the peptide bonds.*
- Complete disruption of tertiary structure is achieved by reduction of the disulfide bonds in a protein

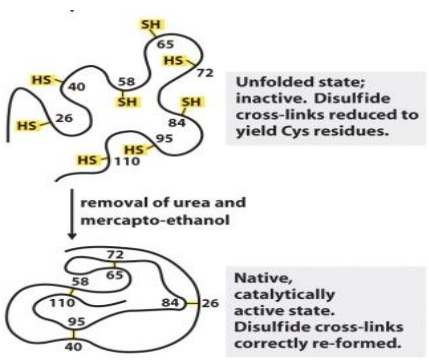
- Generally, the denatured protein will lose its properties such as activity and become insoluble.
- A great example of denaturation process is what happens when you fry "heat" an egg, the egg will harden, and a whitish color appears, this is due to the denaturation of the proteins of an egg.



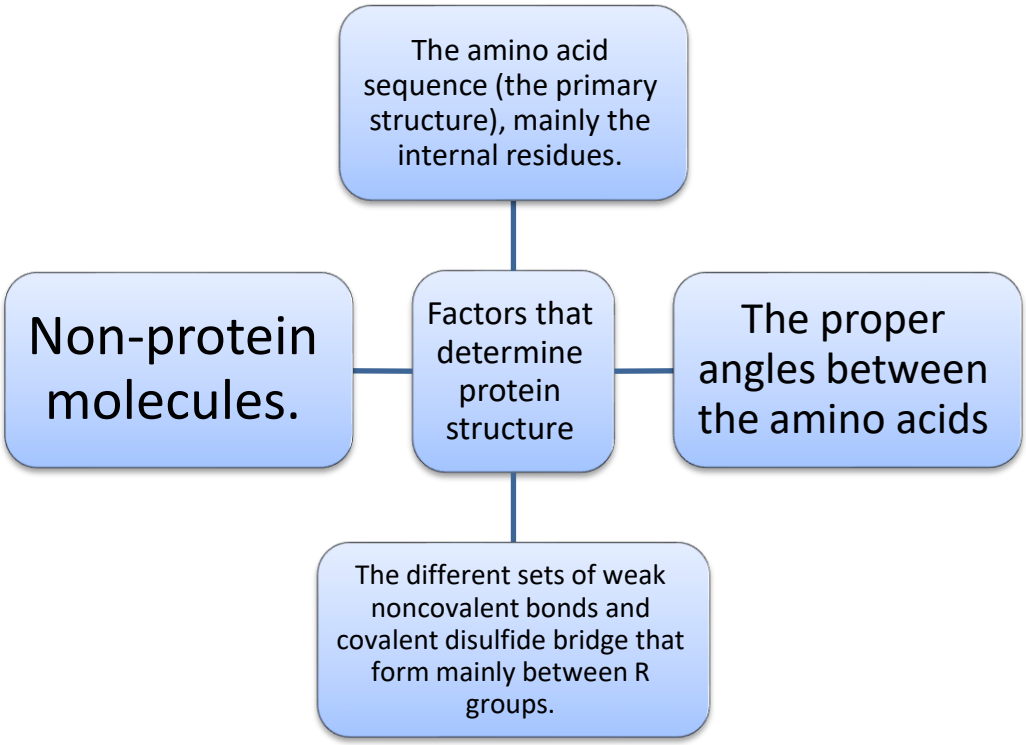


b. Renaturation: is the process in which the native conformation of a protein is re-acquired when the circumstances are back to normal.

- Renaturation occurs quickly and spontaneously, However the problem with renaturation is that the non-covalent bonds may form a new 3D structure due to new bonds being formed.



– The least amount of energy needed to stabilize a protein is determined by *(from Slides)*:



Test your understanding

These questions may include information from previous lectures
There may be more than 1 correct answer

1- Which of the following amino acids have a second chiral carbon?

A- Glu B- Thr C- Ser D- Ile E- Pro

2- How many non-polar amino acids are present in the following peptide?

Glu-Thr-Val-Asp-Ile-Ser-Ala

A- Three B- five C- one D- two E- four

3- Which of the following amino acids is/are in zwitterion form when pH=7?

The PkR of Glu=4.1 His=6 Arg=12.5 Asp=4.0

A- Glu B- Leu C- His D- Arg E- Asp

4- What are the different amino acids between oxytocin and vasopressin?

A- Oxytocin has 3 Leu and 8 Ile
B- Oxytocin has 3 Ile and 8 Leu
C- Oxytocin has 3 Phe and 8 Arg
D- Oxytocin has 3 Arg and 8 Phe
E- Vasopressin has 3 Arg and 8 Phe

5- Why would a glass of milk help you sleep at night?

A- High concentration of tyrosine would increase dopamine which relaxes the brain.

B- High concentration of glutamate would increase GABA which is an inhibitory NT

C- High concentration of Arginine would increase nitric oxide concentration

D- High concentration of tryptophan would increase serotonin and melatonin

6- Which of the following would help you to get a better mark in your next exam?

A- A glass of milk before the exam to increase tryptophan

B- A piece of cheese before the exam to increase tyrosine

C- Mansaf before the exam because it's delicious

D- A piece of cheese before the exam to increase tyramine

7- Which of the following is a definition for the secondary structure?

A- The three-dimensional arrangement of all atoms

B- The order of amino acid residues in the polypeptide chain

C- The interaction between subunits in proteins that consist of more than one polypeptide chain

D- The hydrogen-bonded arrangement of the polypeptide backbone.

Can you figure out the concepts of the other definitions?

8- Which of the following is not affected by denaturation process?

A- Primary structure B- Secondary structure C- Tertiary structure D- quaternary structure

Answers

1- *B+D, because they have 4 different groups at the beta carbon*
2- *A, Val, Ile and Ala are hydrophobic*
3- *B+C just think imagine their structures and compare the pK^Rs with the pH*
4- *B, memorize it*
5- *D, serotonin relaxes the brain and melatonin is good for sleep cycle*
6- *D, tyramine mimics epinephrine and binds the its receptor*
7- *D*
8- *A, because denaturation affects non-covalent bonds only*

Critical thinking

These questions have nothing to do with the exam material, they are just to test your cogitation pathways.

1. Aspartame is 200 times sweeter than normal sugar sucrose, but how did we measure that? Is there a machine that "tastes sweetness"? or is it a person who gave their opinion?
2. In histidine titration curve, the R group "imidazole" gives its proton before the amine backbone, however we studied in organic chemistry that the sequence of basicity of amines is secondary> tertiary> primary, so why is the imidazole the (tertiary amino acid) more acidic than the primary backbone amine?

Search for your answers. ***(A TYPICAL CLIFFHANGER BY TAMER)***