14

BIOCHEMISTRY

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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In the previous lecture, we talked about the four different types of secondary structures that polypeptides can be folded into, which are:

1. Alpha helix
2. Beta-pleated sheet
3. Turns
4. Loops

VERY IMPORTANT NOTE:

Secondary structures are stabilized by **Hydrogen bonds** that form between the groups of the peptide bond (Between one carbonyl group and an amide group), and **not between R groups**.

Super-secondary Structures

They are regions in proteins that contain an ordered organization of secondary structures.

There are at least two types:

1. Motifs
2. Domains

What is common between Motifs and Domains?

- Both are made of multiple secondary structures (a combination of them), and can be made up of either the same or different secondary structures (alpha helices, beta sheets, loops, and turns).

First: Motifs (Modules)

- A motif is a repetitive supersecondary structure, which can often be repeated and organized into larger motifs. (ex: Helix- Loop- Helix- Loop)

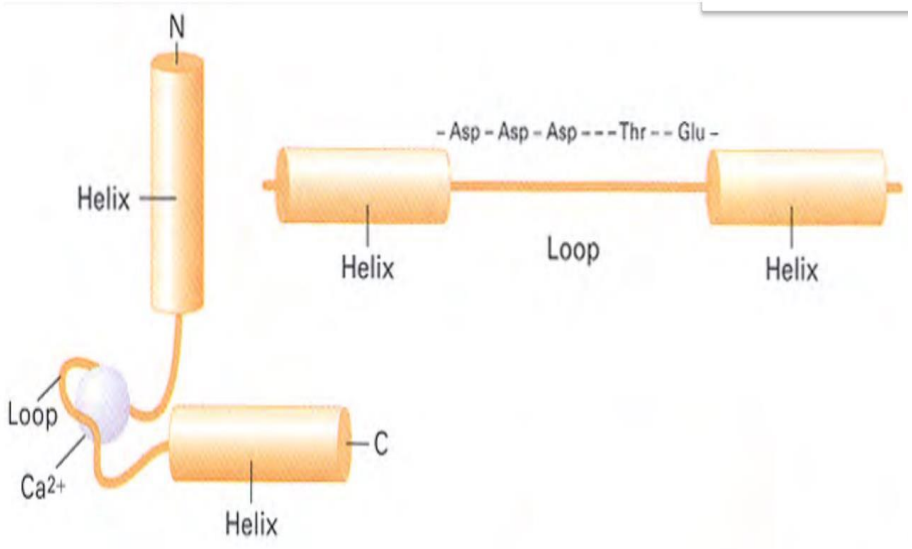
- The specific groupings of secondary structures are consecutive, which means there is no interruption between them.

- It usually constitutes a small portion of a protein (typically less than 20 amino acids, and could be a bit longer- up to 30 amino acids)

- They are **Structural**, and are NOT **Functional**. In general, motifs may provide us with information about **the folding of proteins**, but not **the biological function** of the protein.

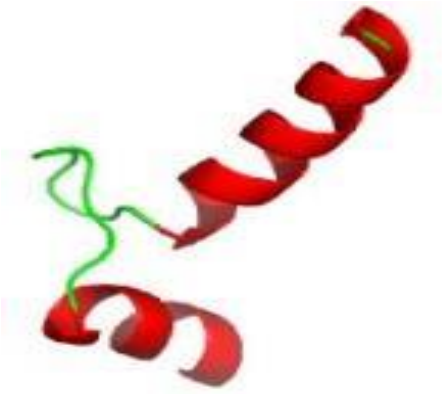
How does it form this consecutive order of secondary structures?

For example, in the figure shown here, one region of the amino acid sequence forms a helix, and the next region forms a loop. The region following the loop forms another helix, then a loop, and so on..

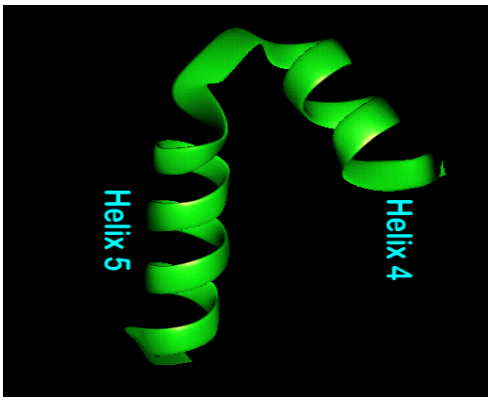


Examples of motifs:

1. **Helix-loop-helix:** It is found in many proteins that bind DNA. It is characterized by two α- helices connected by a loop.
2. **Helix-turn-helix:** It is a structural motif capable of binding DNA. It is composed of two α helices joined by a short strand of amino acids



Helix-Loop-Helix



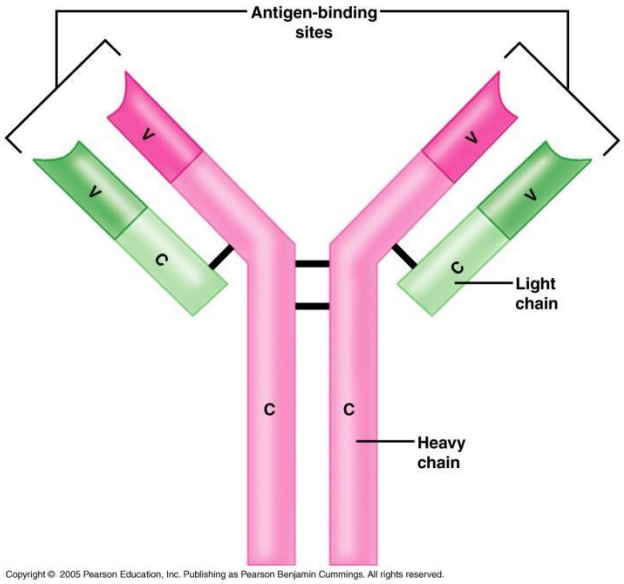
Helix-Turn-Helix

Immunoglobulin module or fold- A more complex motif:

The immunoglobulin fold or module enables interaction with molecules of various structures and sizes (such as in the Antibody-Antigen interactions)

Antibodies are Immunoglobulins

Antibodies: Molecules produced by B cells to recognize and remove certain antigens from our bodies.



The figure on the right shows how the antibody is made up of **four** polypeptide chains:

1. Two identical **light** chain polypeptides
2. Two identical **Heavy** chain polypeptides

Each of these polypeptide chains has a
C Region (Constant region) and a **V Region (Variable region)**

- The Variable region varies from one antibody to another and it found at the tip of the antibody. (The V region of each chain is a motif)

- The Constant region is constant/the same in all antibodies.

V regions are needed for Antigen Recognition. How?

1. The V region recognizes the antigen
2. Interactions occur between the antibody and the antigen at the V region.

Note: The two variable regions of the two light chains are **IDENTICAL**.
The two variable regions of the two heavy chains are **IDENTICAL** .
HOWEVER, the variable region of the heavy chain **IS NOT THE SAME** as the variable region of the light chain.

Thus the types of motifs that we have learnt are:

1. Simple (ex: alpha helix-loop-alpha helix, helix-turn-helix)
2. Complex (Immunoglobulin modules - V regions)

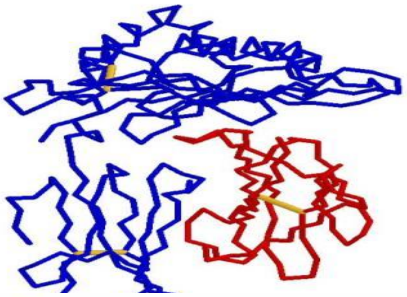
Tertiary Structure

- The overall conformation of a polypeptide chain
- The three-dimensional arrangement of all the amino acids residues
- The spatial arrangement of amino acid residues that are far apart in the sequence
- Determine where amino acids exist and at what angles (3D structure).

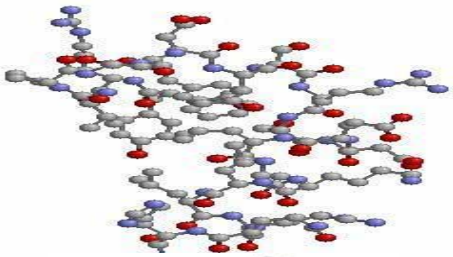
How to look at proteins

There are different ways through which you can illustrate Tertiary structures:

1. **Trace Structure**: Shows what the backbone looks like but not the side groups.

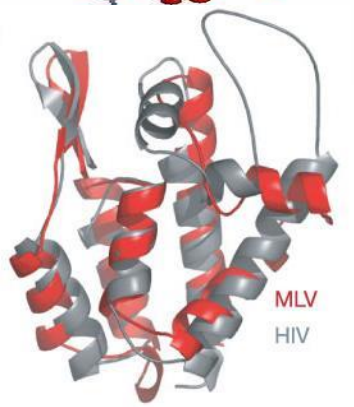


2. **Ball and Stick structure:** Shows the atoms and also shows the backbone (What they usually show you in laboratories)



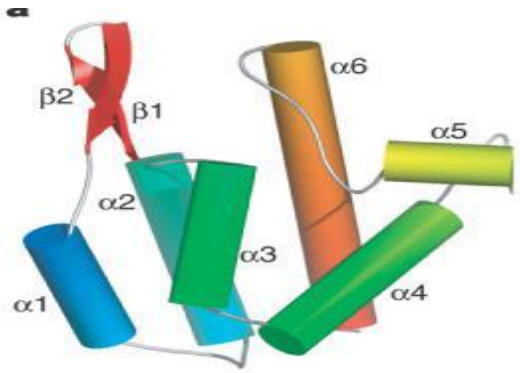
3. **Ribbon Structure:**

- Shows the secondary structures:
- Alpha helix- Represented by ribbons
- Beta Strands- Represented by arrows



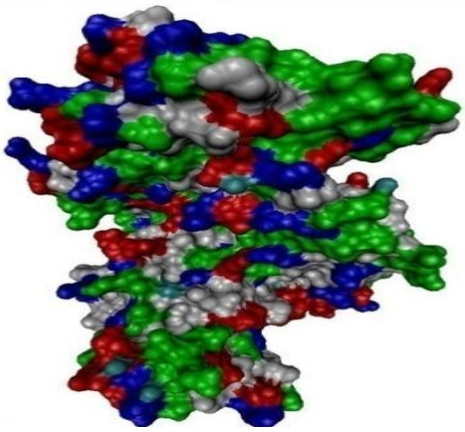
4. **Cylinder Structure:**

- Also shows secondary structures
- Alpha helix- Represented by cylinders
- Beta Strands- Represented by arrows



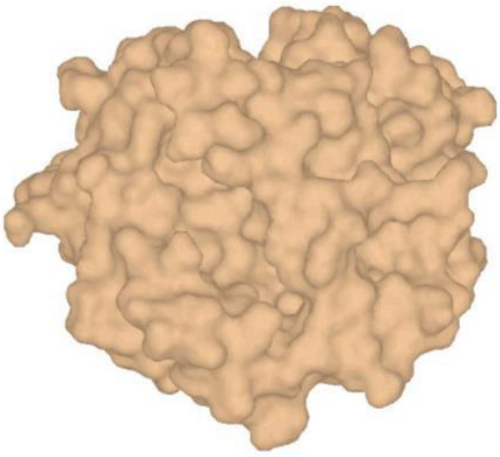
5. **Space filling** structure**:**

- The backbone isn't shown
- Each atom is represented by a large ball
- **Why is it called space filling?** Because these large balls leave no space, it shows the 3D volume of the atoms.



6. **Protein surface map:**

- Computerized
- Only shows what the protein looks like on the surface and not the inside.



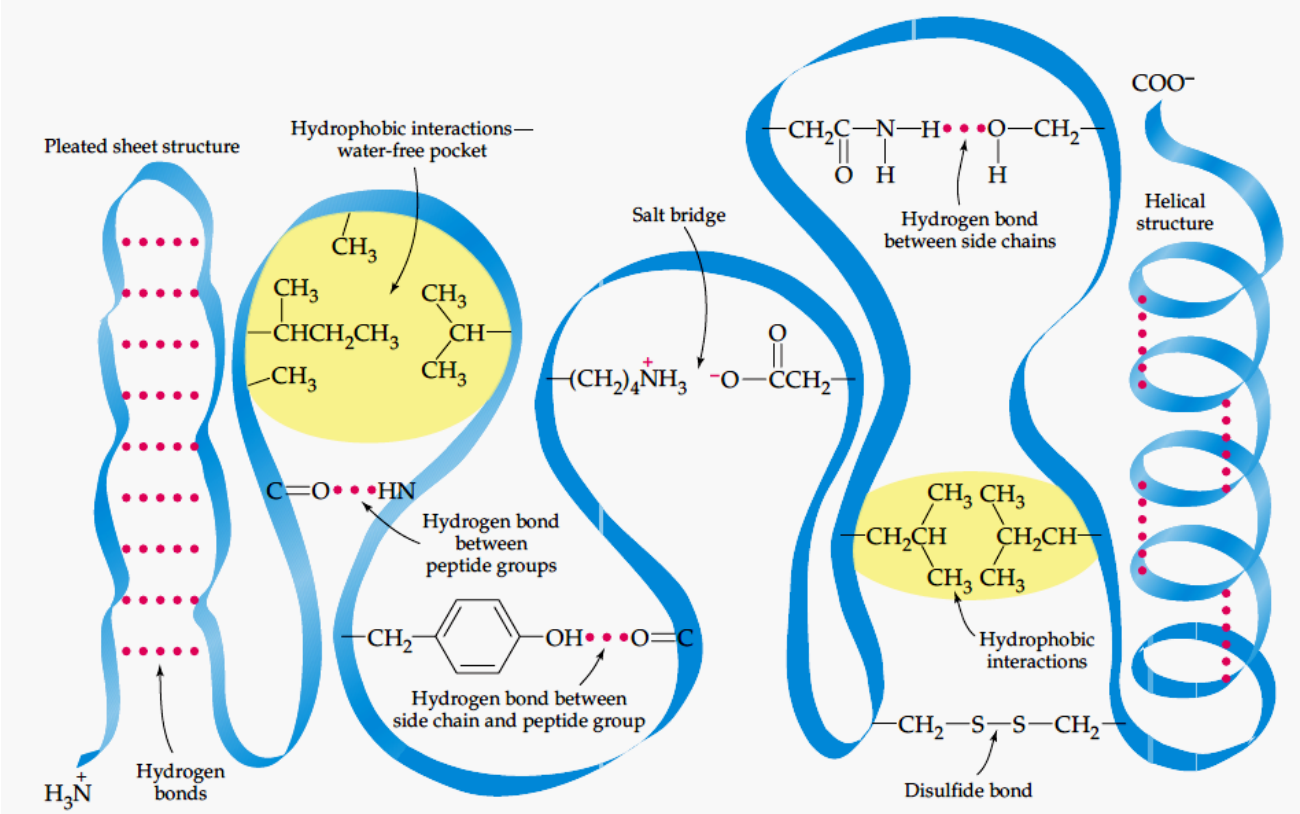
Why is knowing or predicting the shape or structure of a protein important?

This helps scientists know how to fit a molecule into a protein (like for designing and inventing drugs). How?

1. They identify the cause of a certain disease.
2. They identify the gene.
3. Translate codons to amino acids.
4. Look at or take note of the arrangement or sequence of amino acids.
5. Do bioinformatics/ use computerized models or crystallography to show how or what the protein looks like.
6. They design a certain drug and predict a certain shape that could fit into the protein perfectly.

Shape determining forces

- Electrostatic interactions within a protein are called Salt Bridges
- Electrostatic interactions exist between 2/3 atoms.
- Secondary structures are stabilized by H bonds between backbone molecules
- Tertiary structure: Non-covalent interactions between R Groups such as hydrophobic interactions, hydrogen bonds, and salt bridges. The exception is the disulfide bond which is a covalent bond.



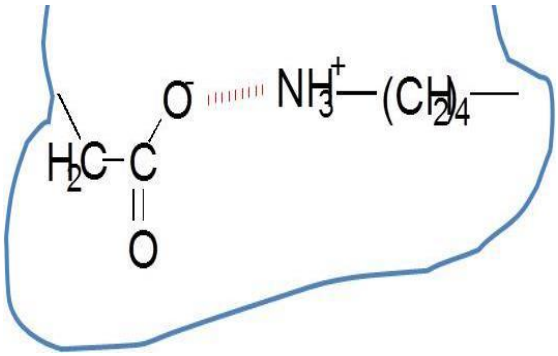
Non-covalent interactions

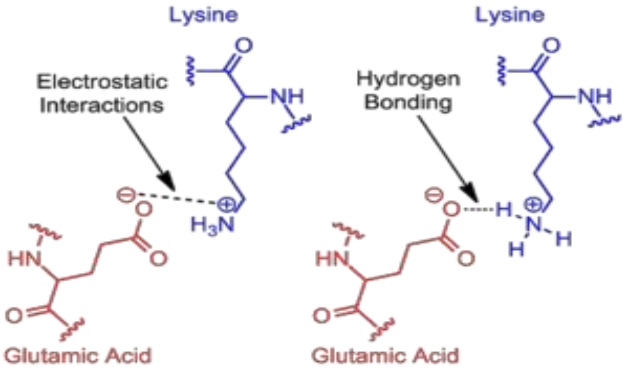
Hydrogen bonds occur not only within and between polypeptide chains but with the surrounding aqueous medium.

Charge-charge interactions (salt bridges) occur between oppositely charged R-groups of amino acids.

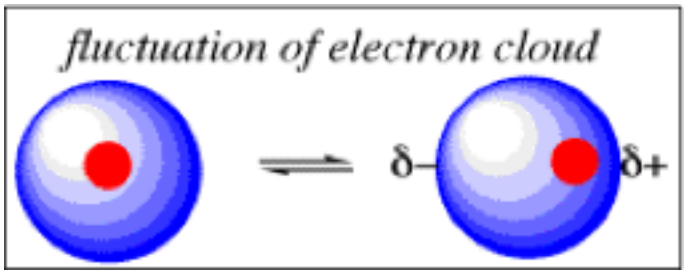
• Charge-dipole interactions form between charged R groups with the partial charges of water.

The same charged group can form either hydrogen bonding or electrostatic interactions
What does this mean?
If you have two charged groups near each other like Lysine and glutamate, they can form hydrogen bonds (between the oxygen and the hydrogen atoms) or electrostatic interactions (between charges).



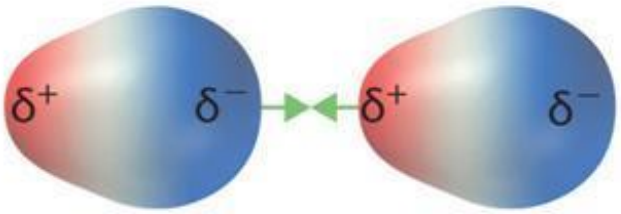


Van der Waals Attractions

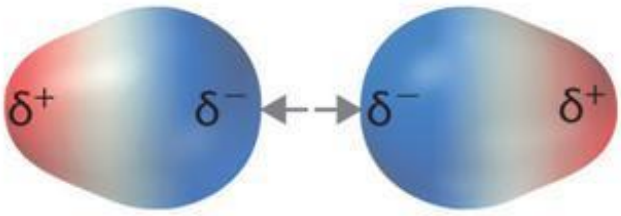


There are both attractive and repulsive van der Waals forces that control protein folding.

Although van der Waals forces are extremely weak, they are significant because there are so many of them in large protein molecules.

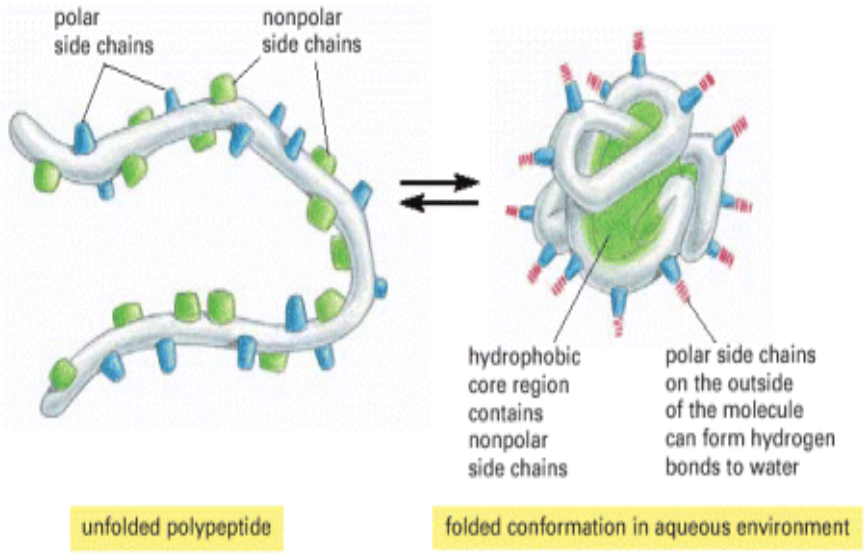


(b) Attraction



(d) Repulsion

Hydrophobic interactions



<u>They may be the most important in determining protein structure</u>

Why?

When translation occurs, and the protein comes out of the ribosome, amino acids show up in the cytosol. If the amino acids are nonpolar and the cytosol IS in fact polar, then these amino acids will hide from the water (Polar environment).
Polar side chains are therefore exposed on the surface while the hydrophobic side chains are hidden.

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

Glutamate (polar) must be on the cell surface and exposed

Valine (nonpolar) must be hidden and directed away from the polar environment.

In sickle cell anemia when Glutamate is replaced by Valine (Substitution), the structure of the protein is changed completely, since the arrangement of these amino acids has been changed and disrupted due to different interactions forming. Changing from a polar to a nonpolar amino acid has a huge effect on the protein structure.

Can polar amino acids be found in the interior??

YES. HOW?

- For example, when polar amino acids are found in the interior of a protein, they form hydrogen bonds to other amino acids or to the polypeptide backbone.
- This is to ensure that they are surrounded by compatible amino acids to prevent a build-up or great degree of repulsion.

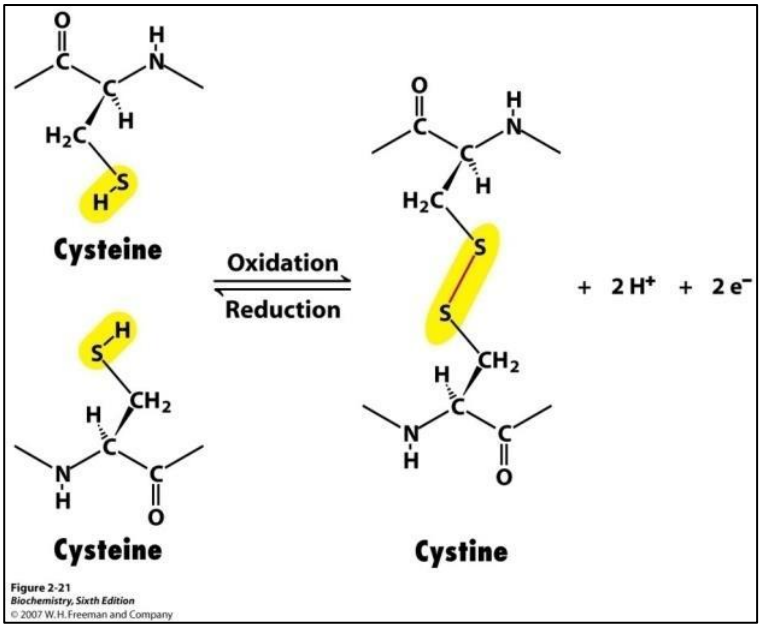
However, In order for this to work, the amino acids **MUST** have a **functional** role. **(Like enzymes and their active sites, where you can have LYSINE Or HISTIDINE perform their function)**

Stabilizing factors:

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

1. **Disulfide bonds**
2. **Metal ions**

- **Disulfide bonds**
  - The side chain of cysteine contains a reactive sulfhydryl group (—SH), which can oxidize to form a disulfide bond (—S—S—) to a second cysteine.
  - The crosslinking of two cysteines forms a new amino acid, called cystine.

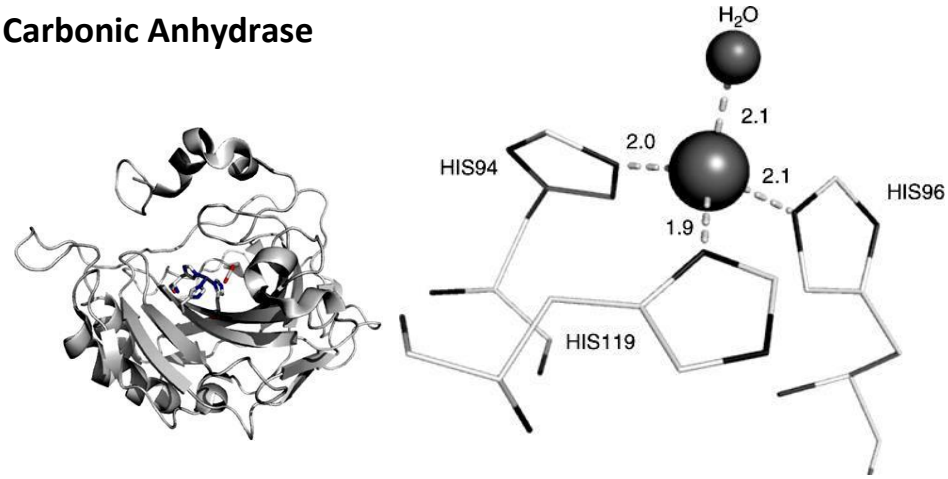


- **Metal Ions** (ex: Zinc)

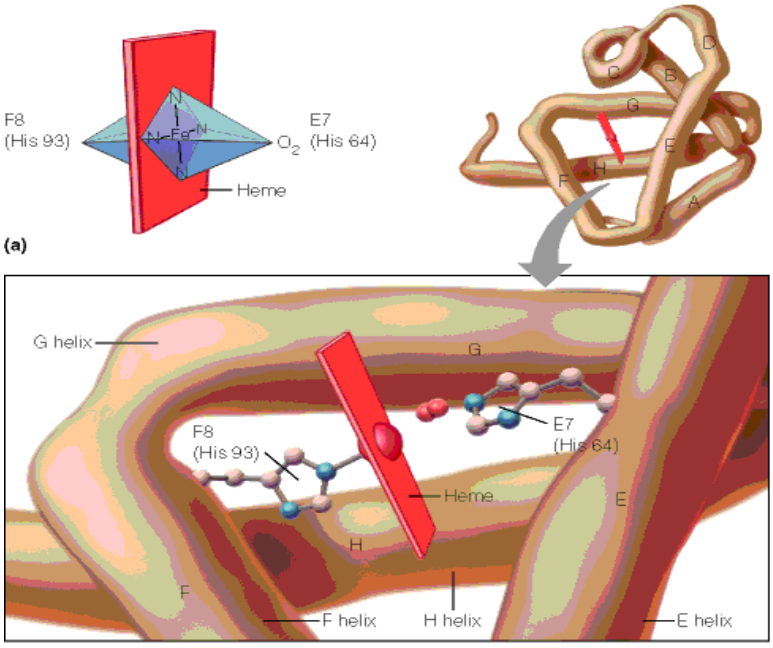
  Several proteins can be complexed to a single metal ion that can stabilize protein structure by forming:

  - **Covalent interaction (myoglobin) < And hemoglobin >:** Both have heme groups. Heme has iron (Fe) in the middle. The heme serves as a sort of rigid stick that prevents the heme structure from being bent, stabilizing it.
  - **Salt bridges (carbonic anhydrase):** This allows for electrostatic interactions b/t the metal ion and amino acids.

Carbonic Anhydrase



Myoglobin



A Domain

- Larger than motifs.
- Part of the tertiary structure of a protein
- Form the 3D structure
- They help us predict the function of a protein
- Is a compactly folded region of polypeptide found in proteins with similar function and/or structure.
- Domains with similar conformations are associated with a particular function.
- A structural domain may consist of 100– 200 amino acid residues in various combinations of α helices, β sheets, turns, and random coils.

- They fold independently of the rest of the protein: If you cut a domain it still maintains its structure and function.
- Domains may also be defined in functional terms:
    - enzymatic activity
    - binding ability (e.g., a DNA-binding domain)

Difference between motifs and domains:

Domains: The components of the domain (such as alpha helices and beta sheets) are far from each other in the primary structure. However, once the three dimensional structure forms and the protein folds they will become near each other.

Motifs: The components occur consecutively (one after the other) in terms of primary structure.

Say we have a polypeptide,

- One part of it forms an alpha helix, another part or region far from it forms an alpha helix, and at the end, forms another alpha helix
- When the protein folds, these alpha helices (which represent the secondary structure) come together and form the 3D structure of that domain.

Properties of proteins (Denaturation and Renaturation)

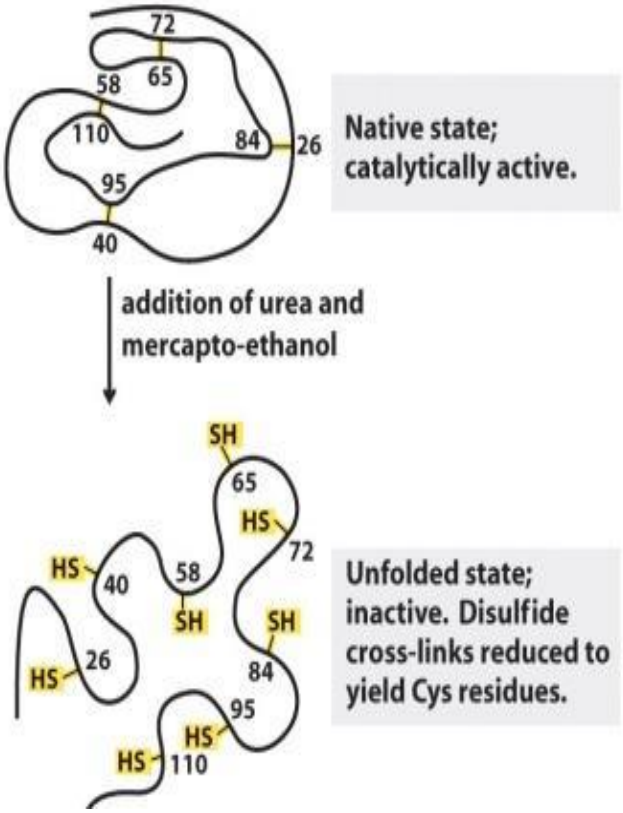
Denaturation

What is denaturation?

Denaturation is the disruption of the native conformation of a protein, the characteristic three- dimensional structure that it attains after synthesis. (it loses its tertiary structure).

What happens?

1. **Breakage** of the noncovalent bonds (which determine the structure of a protein).



2. Complete disruption of tertiary structure is achieved by reduction of the disulfide bonds in a protein .
3. The denatured protein loses its properties such as activity and becomes insoluble

Note: Disulfide bonds are covalent and will not be broken with the methods that remove noncovalent interactions. However, they can be eliminated by reduction through a reducing agent.

REMEMBER that the formation of disulfide bridges is an oxidation reaction

Denaturing agents

1. Heat: disrupts low-energy van der waals forces in proteins.
2. Extremes of pH: causes change in the charge of the protein's amino acid side chains (affecting electrostatic interactions and hydrogen bonds).
3. Detergents: Detergents (Triton X-100 (nonionic, uncharged) and sodium dodecyl sulfate (SDS, anionic, charged)) disrupt the hydrophobic forces.

Anionic: Changes the protein's charge and structure. In the case of SDS, it makes the protein negatively charged.

Nonionic: Does not change the protein's charge, only disrupts the structure.

- **SDS also disrupt electrostatic interactions.**

4. Urea and guanidine hydrochloride disrupt hydrogen bonding and hydrophobic interactions.

- Reducing agents such as β-mercaptoethanol (βME) and dithiothreitol (DTT) both reduce disulfide bonds.

NOTE that:

- If a scientist took ribonuclease and added a type of detergent (denaturing agent) the protein then loses its function
- The protein then loses the the S-S bond (disulfide bond) by using reducing agents.
- He then removes the denaturing agent *only*.
- The S-S bonds (which are also covalent) are not formed again (because the reducing agent is still presents) BUT the noncovalent interactions can reform!
- Ribonuclease's function then returns.

CONCLUSION:

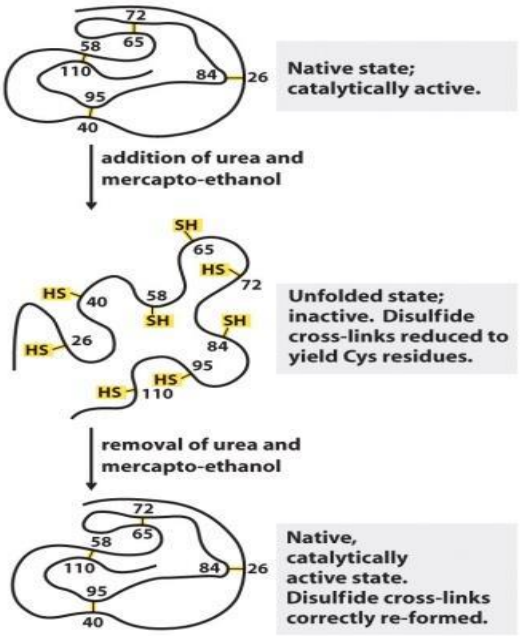
Non covalent bonds are important for the tertiary structure and the protein's function.

However, Covalent bonds (S-S bonds) only support and STABILIZE the structure and are unaffected by denaturing reagents, only REDUCING AGENTS can remove them.

Renaturation

What is renaturation?

- **Renaturation** is the process in which the native conformation of a protein is re-acquired.
- Renaturation can occur quickly and spontaneously and disulfide bonds can re-form correctly so long as both the denaturing and reducing agents are removed.



Factors that determine protein structure

It's all related to the energy between bonds.

The structure that forms is the structure that needs the least amount of energy to form a stable protein. This (the energy needed) is determined by:

- The amino acid sequence (the primary structure), mainly the internal residues. (the most stable sequence of amino acids is favored)
- The proper angles between the amino acids
  - The different sets of weak noncovalent bonds that form between the mainly the R groups.
  - Non-protein molecules.

Can an unfolded protein refold?

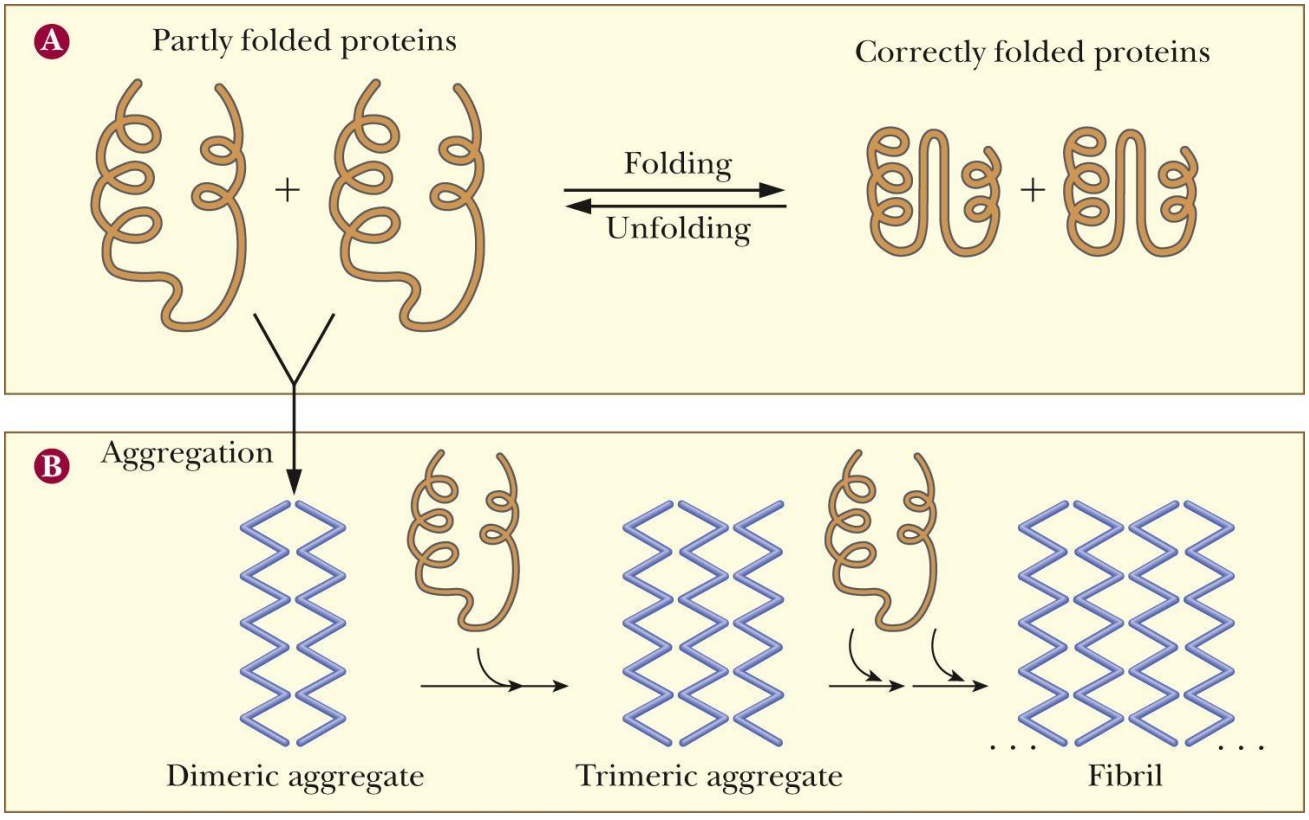
If a protein is unfolded, it can refold to its correct structure placing the S-S bonds in the right orientation (adjacent to each other prior to formation), then the correct S-S bonds are reformed.

This is particularly true for small proteins, and is more difficult for LARGE proteins.

How come? Large proteins need help using **Chaperones.**

The problem of misfolding

- When proteins do not fold correctly, their internal hydrophobic regions become exposed and interact with other hydrophobic regions on other molecules, and form aggregates. The aggregates can then precipitate which causes problems and damages cells and tissues.
- Example: In sickle cell anemia, the change in one amino acid causes the exposure of the hydrophobic region, so clusters are formed to hide from water, forming aggregates. Precipitation occurs in cells and cells get damaged.
- A change in 1 or 2 amino acids/deletion within a gene changes the structure of the protein.
  Example: in sickle cell anemia



When misfolding occurs in cells the cell can either:

1. Denature the protein and reform it

OR

2. Degrade the protein

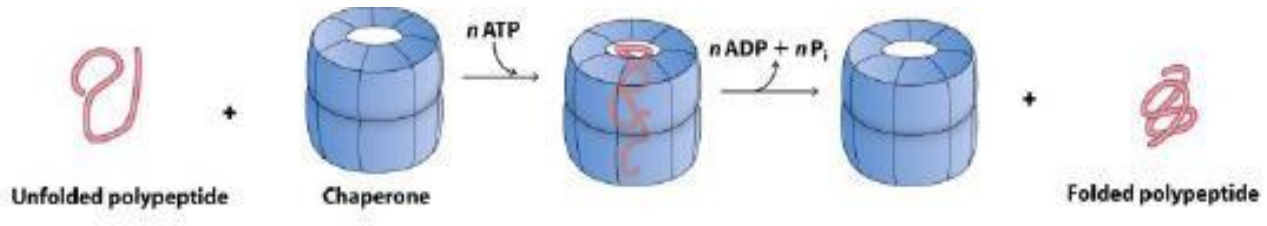
For correct folding, we need:

1. The correct sequence of nucleotides in mRNA
2. The correct formation of peptide bonds between the correct amino acids in translation
3. The correct modification of this protein

Problem solvers: Chaperones

What are chaperones?

- These proteins bind to polypeptide chains and help them fold with the most energetically favorable folding pathway.
- They also prevent the hydrophobic regions in newly synthesized protein chains from associating with each other to form protein aggregates .



1. They help especially when proteins are large and have a lot of hydrophobic regions.

2. These hydrophobic regions cant wait to hide from the hostile polar environment of the cytosol and immediately want to hide.
3. Chaperones bind to these proteins or accompany them.
4. They make the environment friendly for the protein.
5. The position and organization of the amino acids is restored.
6. This prevents the random formation of hydrophobic interactions (and clustering).

Outcome of Protein misfolding

- Partly folded or misfolded polypeptides or fragments may sometimes associate with similar chains to form aggregates.
- Aggregates vary in size from soluble dimers and trimers up to insoluble fibrillar structures (amyloid).
- Both soluble and insoluble aggregates can be toxic to cells.

GOOD LUCK ☺