PROTEIN DENATURATION

Introduction:

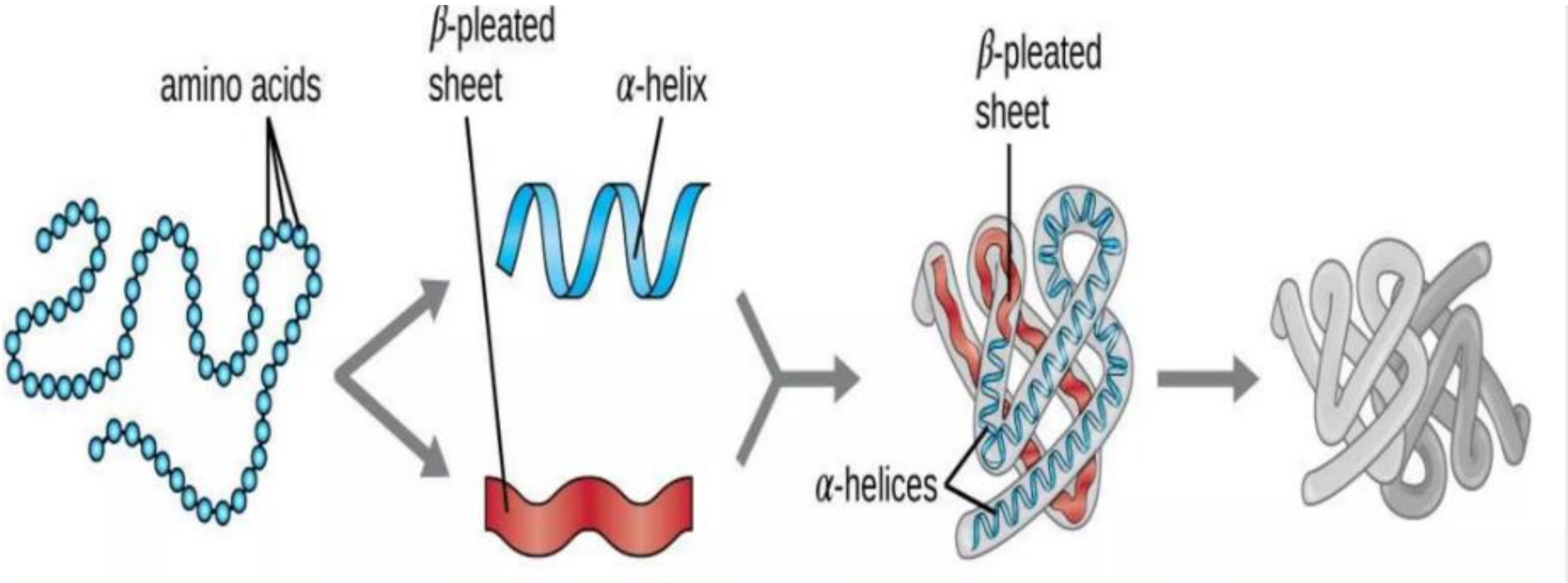
- Denaturation is a process in which a protein loses its native shape due to the disruption of weak chemical bonds and interactions, thereby becoming biologically inactive.

In case of proteins :

- A loss of three-dimensional structure, sufficient to cause loss of function
- Loss of secondary, tertiary and quaternary structure of proteins.
- Change in physical, chemical and biological properties of protein molecules.

Definition:

- Denaturation involves transformation of a well-defined folded structure of a proteins formed under physiological conditions, to an unfolded state under non-physiological conditions is called protein denaturation

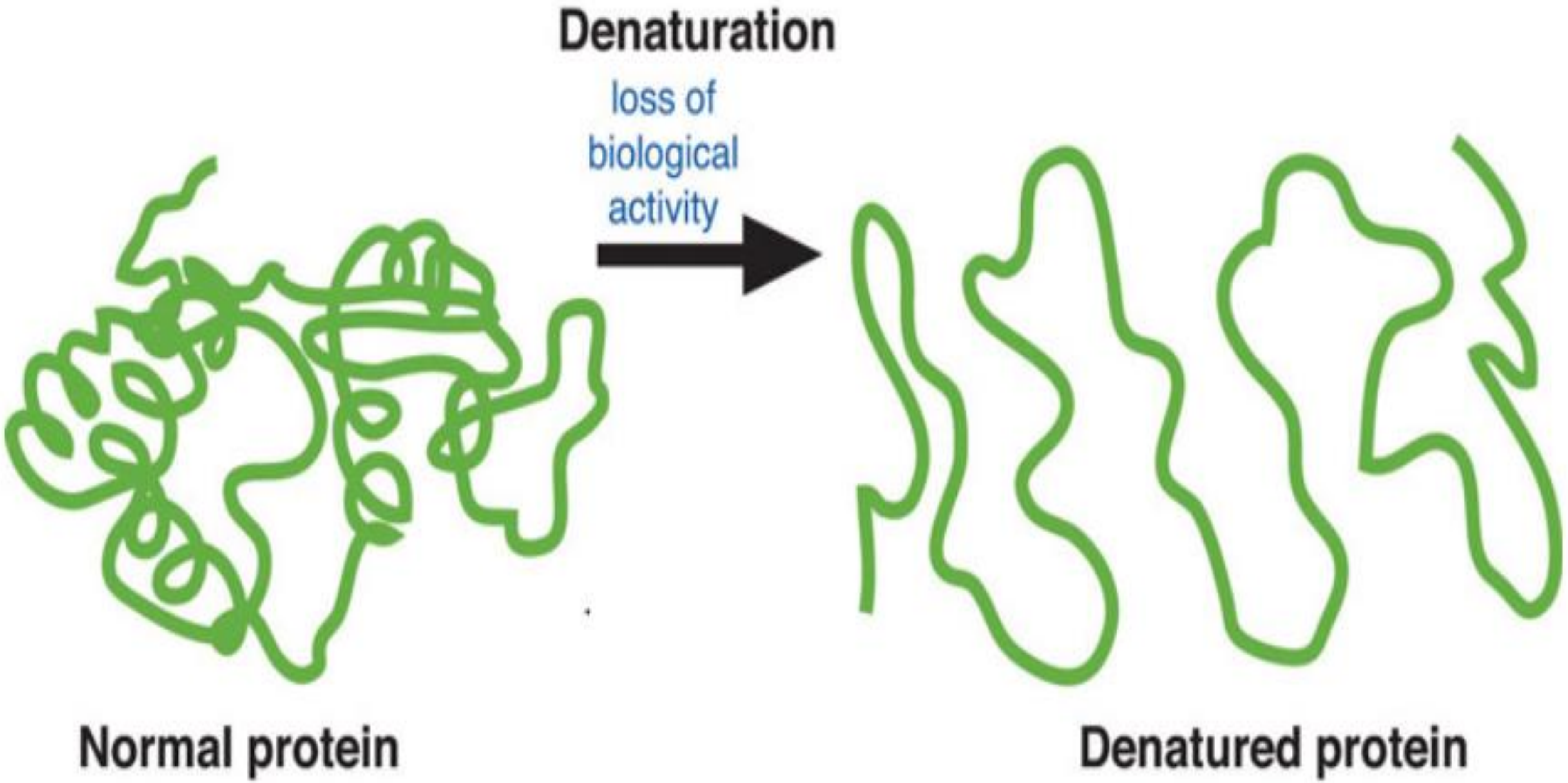


Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

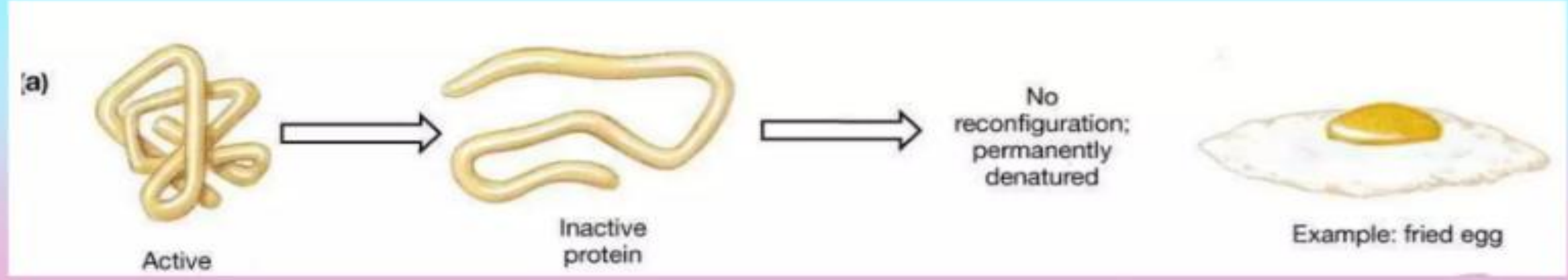


Denaturation of Proteins

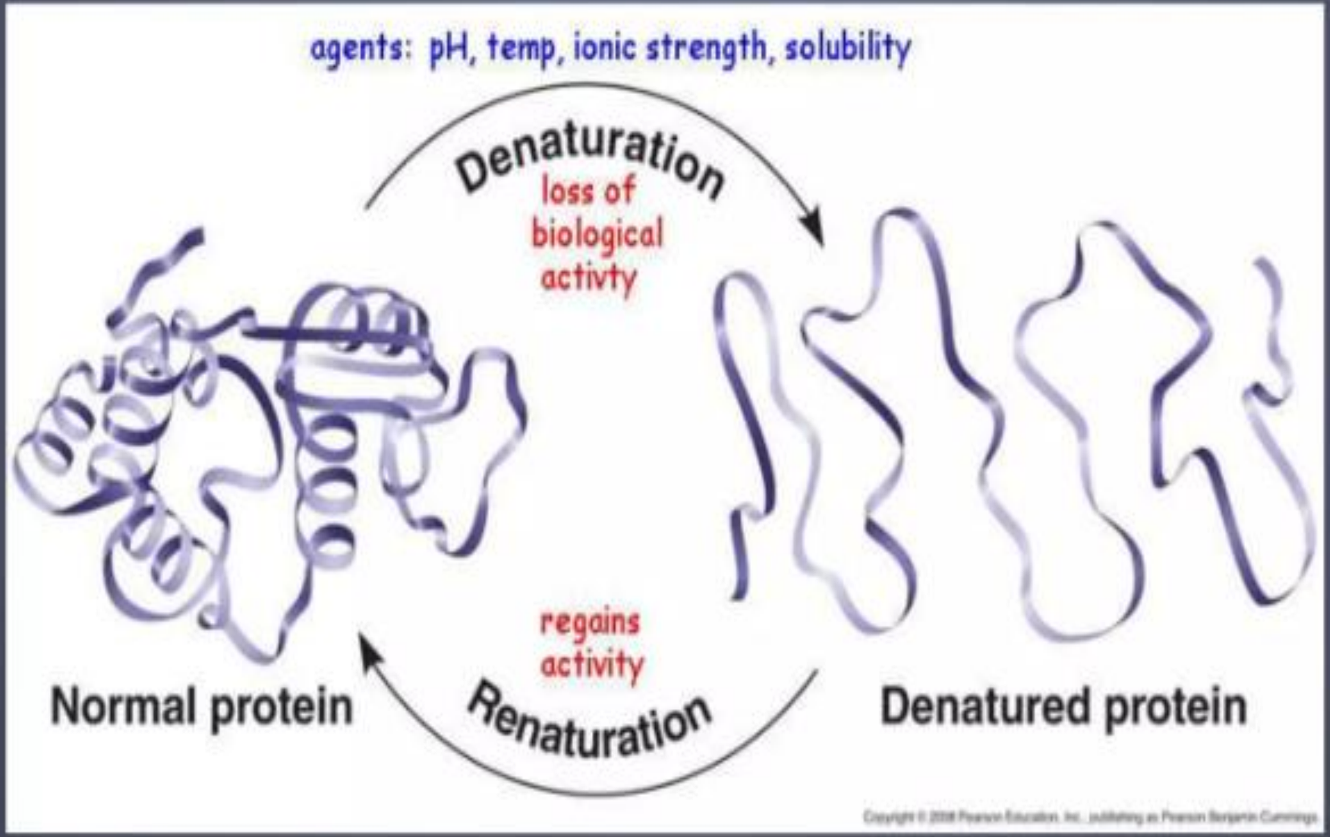
Mechanism of protein denaturation:

- Unfolding of native proteins occurs at both the temperatures at higher temperature denaturation occurs means it is called heat denaturation or thermal denaturation and if denaturation occurs at lower temperature then it is called cold denaturation.

- In both the cases there is breakage of hydrogen bonds, disulfide bonds, hydrophobic interactions, vanderwalls forces but there is no breakage of peptide bonds during denaturation.



- The denatured state does not necessarily equate with complete unfolding of the protein and randomization of conformation.
- Under most conditions, denatured proteins exist in a set of partially folded states that are poorly understood.



Causes of protein denaturation:

- Denaturation occurs when proteins are exposed to an extreme environment conditions such as high level of salt, higher level of acidity, higher temperature etc.

- Because of these extreme conditions the function of the proteins alters due to deformities along their bonds and can be temporarily or permanently denatured.

- **Agents causing denaturation:** There are various agents which causes denaturation of proteins, some of them are as follows:

Physical agents:

- Heat
- Violent shaking or agitation
- Hydrostatic pressure
- UV radiation

Chemical agents:

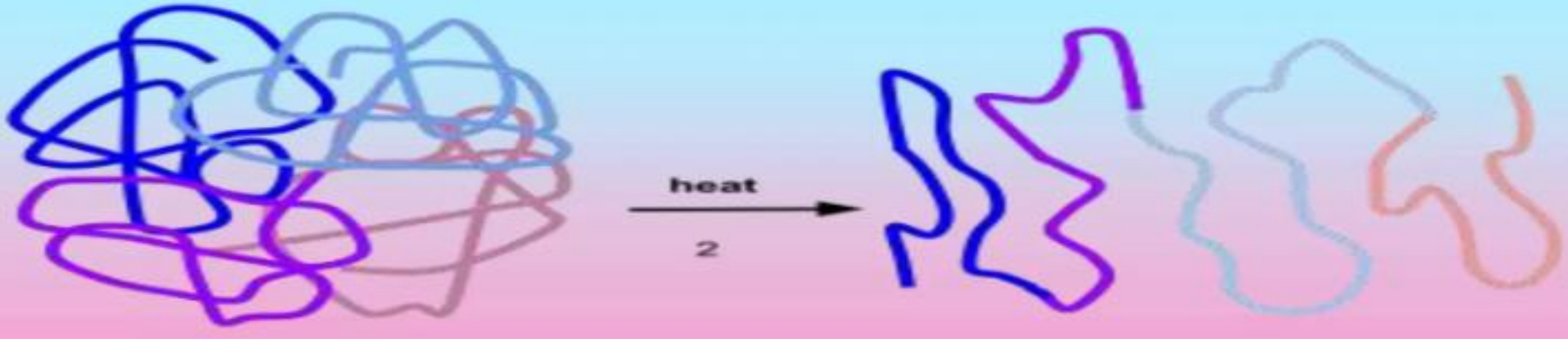
- Acids and alkalis
- Organic solvents
- Salts of heavy metals
- Chaotropic agents
- Detergents
- Altered pH

Denaturation by heat:

- Most proteins can be denatured by heat, which affects the weak interactions in a protein (primarily hydrogen bonds) in a complex manner.

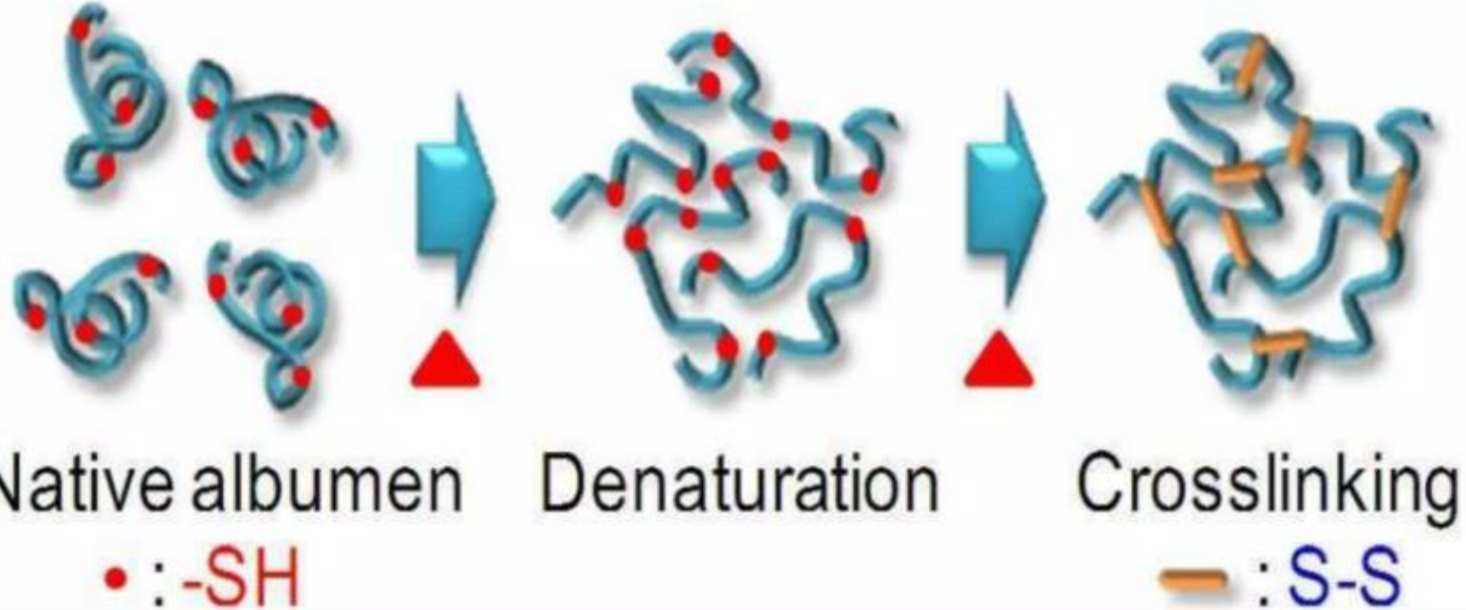
- If the temperature is increased slowly, a protein's conformation generally remains intact until an abrupt loss of structure and function occurs over a narrow temperature range.

- During cooking, this stress causes denaturation which is typically as heat and ultimately proteins gets coagulated.



(a) Denaturation of egg protein:

(b) Protein Thermal Irreversible Denaturation



- As higher temperatures can cause irreversible denaturation of proteins, and when a cell is exposed to high temperatures, several types of molecular chaperones swing into action for this reason, these chaperones are also called **heat-shock proteins** (HSPs).

Denaturation by violent shaking:

- Agitation also denatures protein.
- We see this clearly in the whipping of egg whites.
- The constant churning of milk or cream creates foam from various proteins which also causes denaturation of proteins.
- Denaturation of milk protein occurs during whipping or beating of ice-cream mix during ice-cream manufacturing.

Coagulation of milk proteins

Denaturation of proteins by hydrostatic pressure:

- Proteins undergo dissociation and unfolding by pressure mostly because the final states are more hydrated, have fewer non-hydrated cavities and therefore, occupy smaller volumes.

- For a typical case of protein denaturation, pressure will shift the equilibrium of the reactants.

$$\mathbf{PN} + nH_2O \xrightarrow{\text{5000-10,000 atm}} \mathbf{PD}(H_2O)_n$$

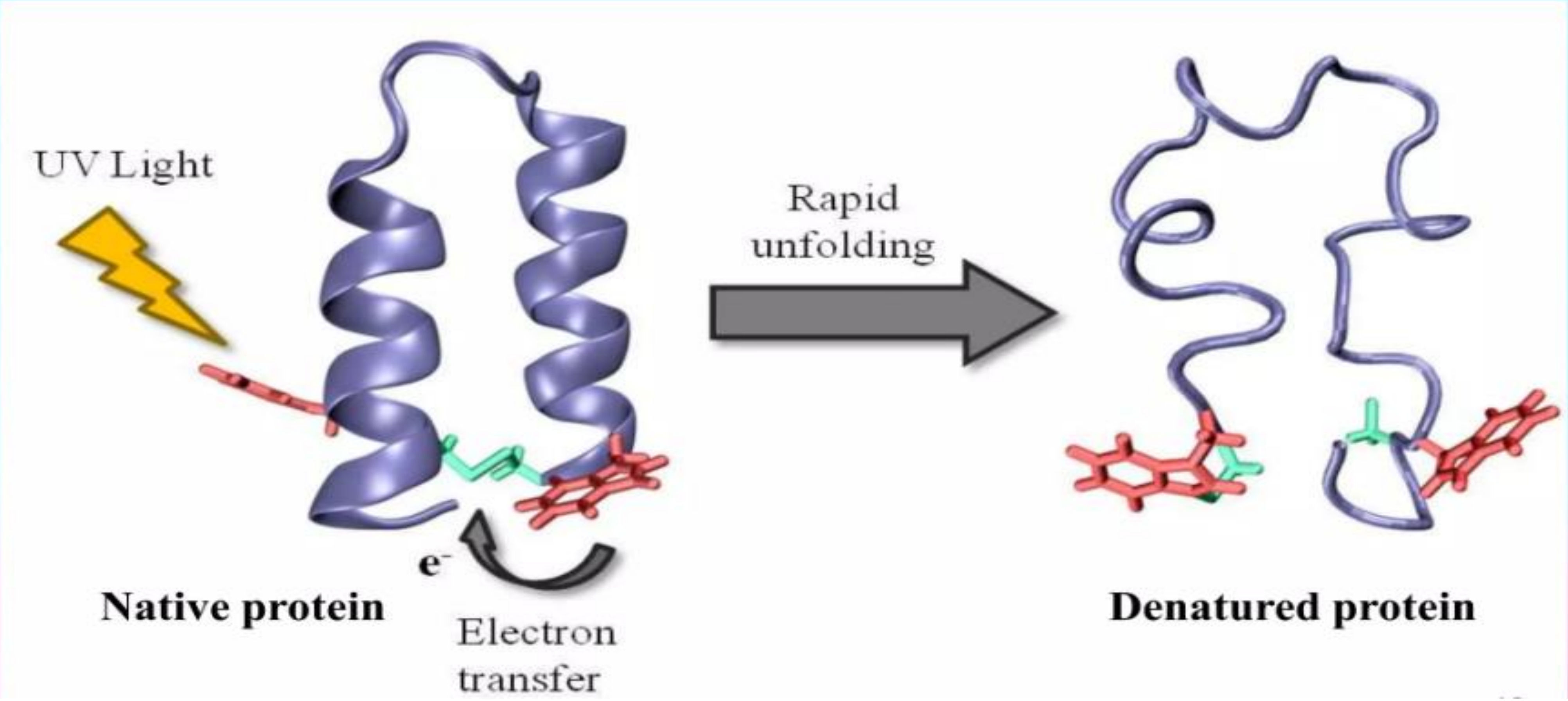
  where PN and PD are native and denatured proteins.

- The decrease in volume is due to hydration of newly exposed nonpolar and polar residues as well as to the loss of free volume arising from packing defects in the folded structure.

- High pressure has been used to assess the underlying mechanisms of protein misfolding and aggregation.

Denaturation by UV radiation:

- UV radiation supplies kinetic energy to protein molecules, causing their atoms to vibrate more rapidly and disrupting the relatively weak hydrogen bonding and dispersion forces of protein molecules.



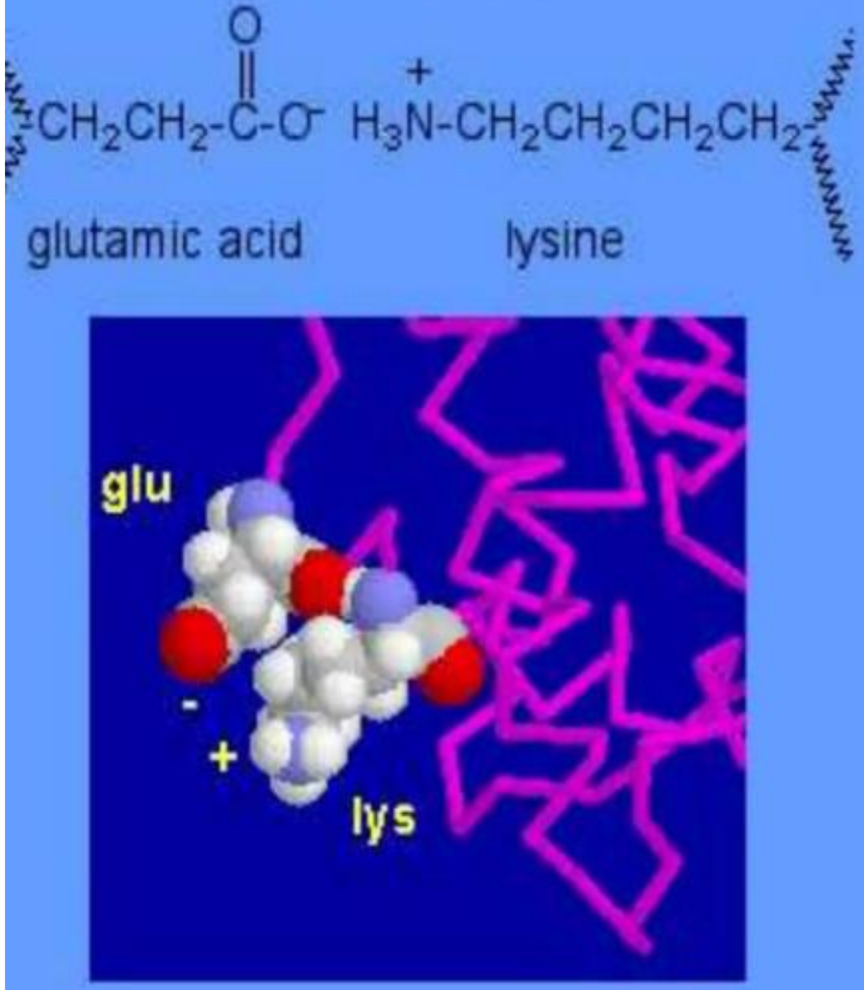
Chemical agents:

Denatured by Acids and alkalis:

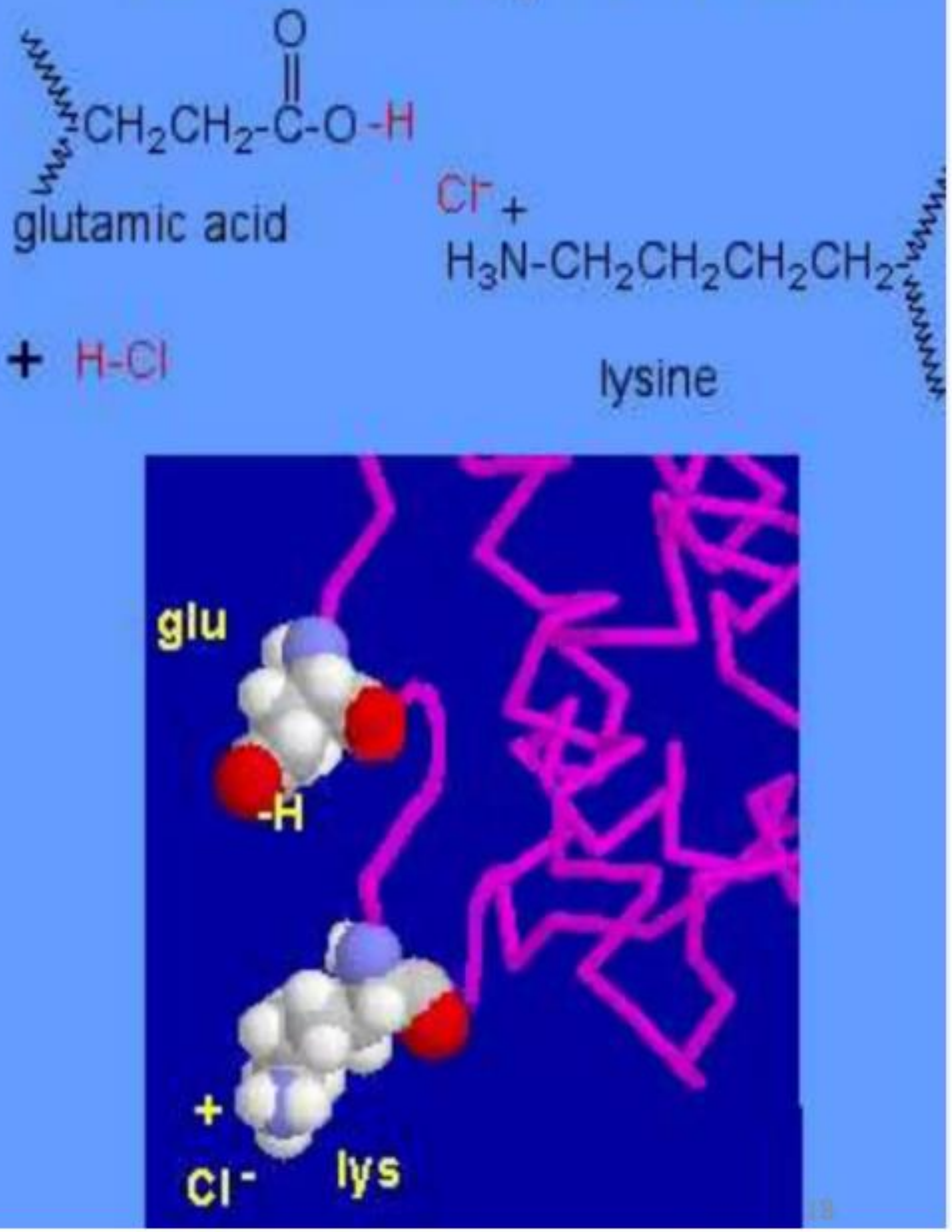
- Acids and bases disrupt salt bridges held together by ionic charges.
- Double replacement reaction occurs where the positive and negative ions in the salt change partners with the positive and negative ions in the new acid or base added.
- This reaction occurs in the digestive system, when the acidic gastric juices cause the curdling (coagulating) of milk.

• Strong Acids or Bases	salt formation; disruption of hydrogen bonds. (skin blisters and burns, protein precipitation.)

Tertiary Structure - Salt Bridges



Denaturation by Acid or Base



Acidic protein denaturants include:

- Acetic acid
- Trichloroacetic acid 12% in water
- Sulfosalicylic acid

Basic protein denaturants include:

- Sodium bicarbonate
- Sodium sulphate

Denaturation by organic solvents:

- Proteins can also be denatured by using organic solvents such as ether, alcohol, acetone, diethyl ether etc. These added alcohol disrupts the hydrogen bond between protein molecules and new hydrogen bonds are formed instead between the new alcohol molecule and the protein side chains.

Chaotropic agents:

- A chaotropic agent is a molecule in water solution that can disrupt the hydrogen bonding network between water molecules.

- This has an effect in the stability of the native state of other molecules in the solution, mainly macromolecules (proteins, nucleic acids) by weakening the hydrophobic effect.

- For example, a chaotropic agent reduces the amount of order in the structure of a protein formed by water molecules, both in the bulk and the hydration shells around hydrophobic amino acids, and may cause its denaturation.

Chaotropic agents include:

- Urea competition for hydrogen bonds. (precipitation of soluble proteins.)

- Urea 6–8 mol/l
- Guanidinium chloride 6 mol/l

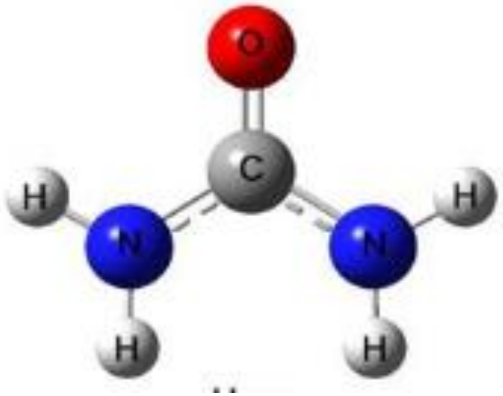
Urea - the denaturant

Uncharged – implicates its interaction with hydrophobic groups of proteins

Originally thought to act in a similar manner as GdnHCl, but was found to denature proteins via a separate mechanism[6].

Forms non-covalent interactions with peptide backbone, stabilizing non-native (i.e. unfolding intermediates) structures (direct effect)[7].

Disrupts water-water interactions, making water more able to solvate hydrophobic groups, allowing water to compete with intra-protein interactions (indirect effect)[7].

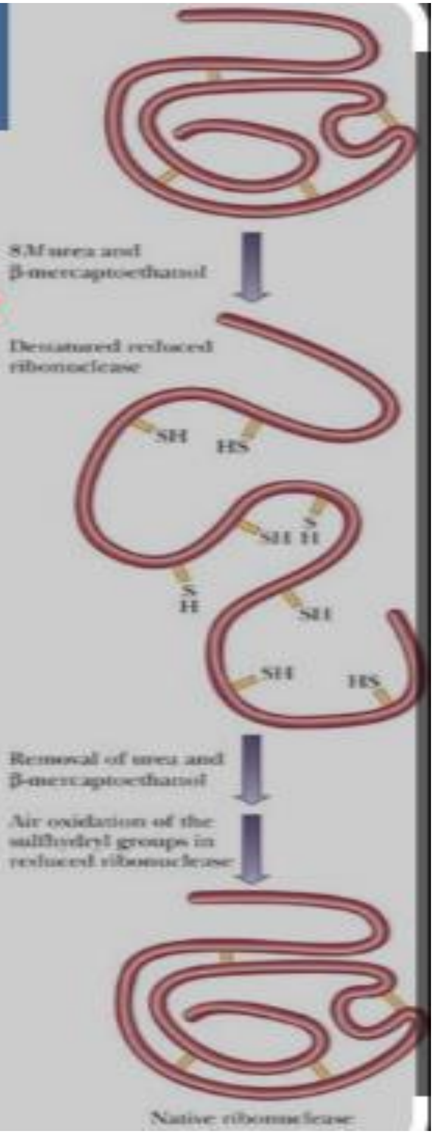


REDUCING AGENT

Denaturation

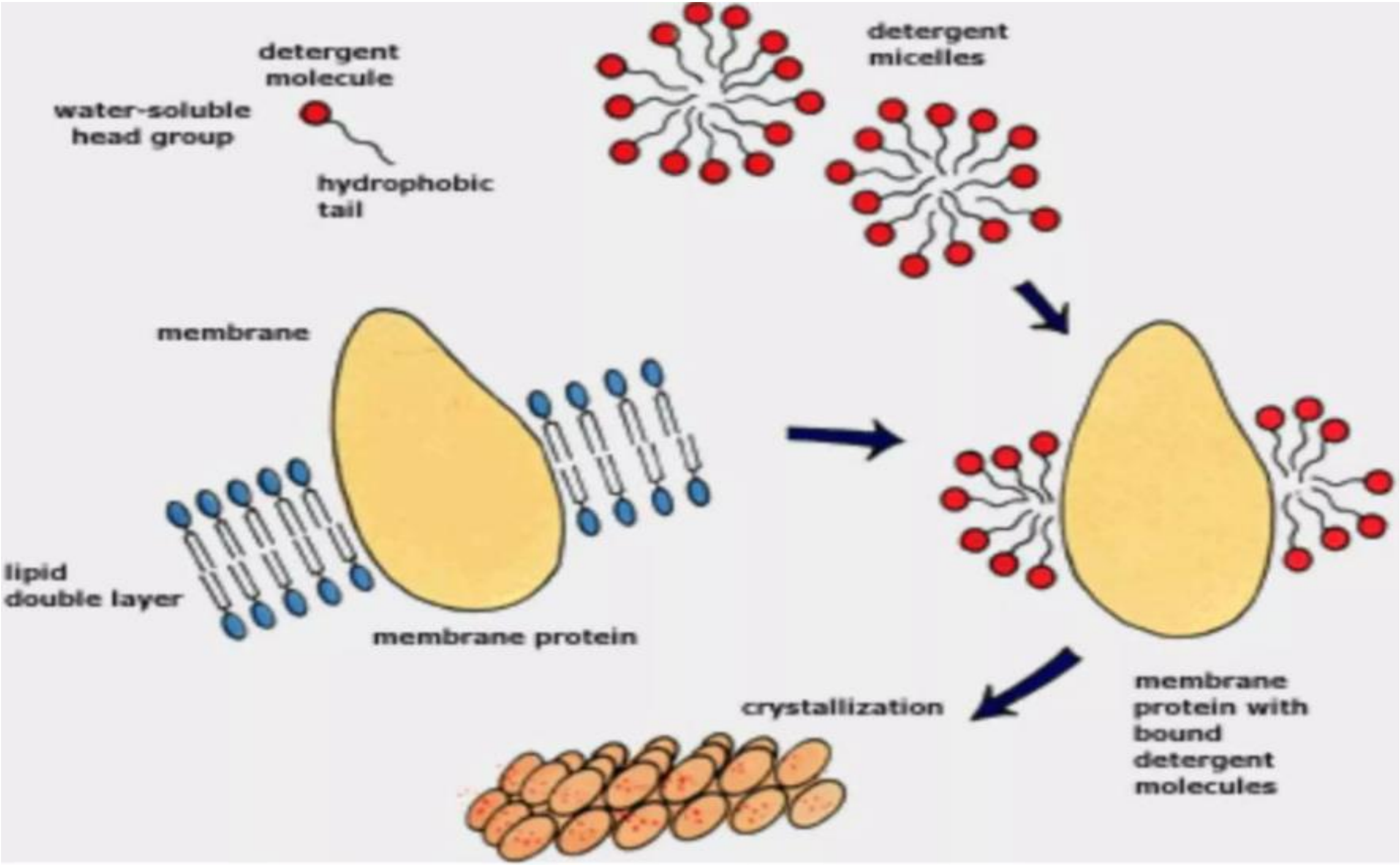
- β-mercaptoethanol example of reversible denaturation.

- β-mercaptoethanol reduced the disulfide bridges of protein ∴ the unfolding of 3° structure,
- the removal of β-mercaptoethanol will cause the oxidation of SH group to form disulfide bridges again and the 3° structure is recovered.

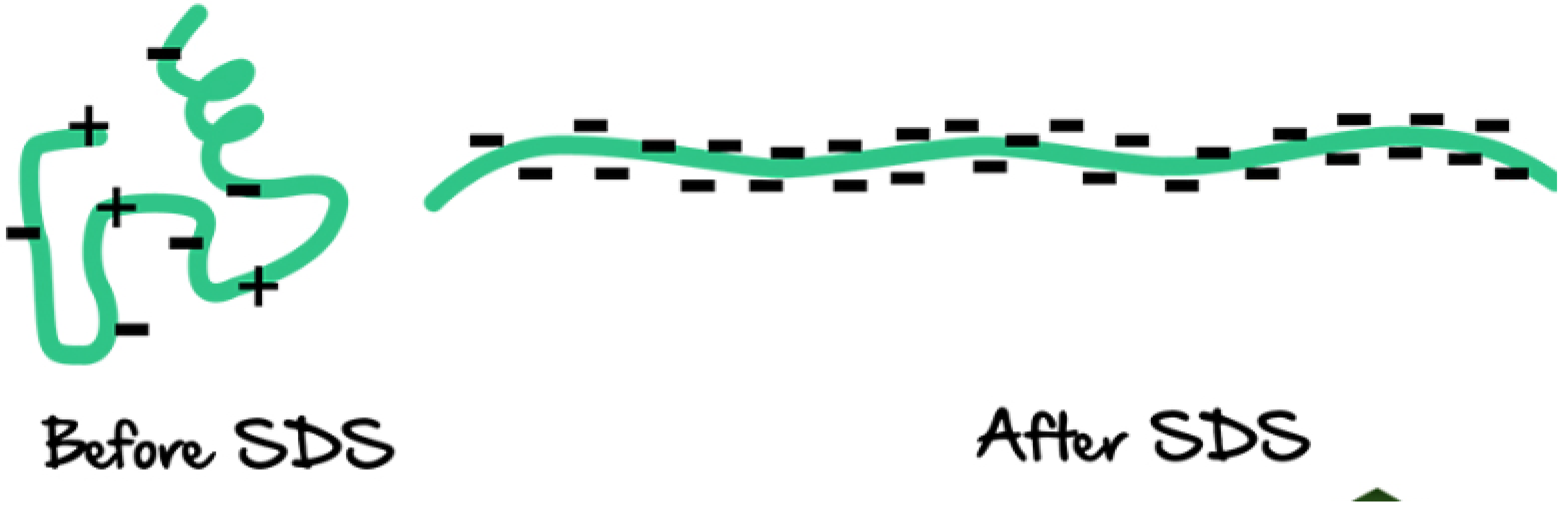


Denaturation of proteins by detergents:

- Detergents are amphipathic in nature having both hydrophobic side and a hydrophilic side (When it dissolves grease, it forms protective bubbles from the water by surrounding grease molecules with the hydrophobic side).
- Proteins have hydrophobic and hydrophilic sides, the detergent is attracted to these and forces the protein apart.
- A protein's 3-D structure is partially created by hydrophobic and hydrophilic interactions to itself, the detergent substitutes this self bonding with detergent-amino acid bonding.
- Furthermore, detergent is a salt and breaks up positive and negative interactions of the 3-D shape as well and denatures the proteins.


detergent molecule
water-soluble head group
hydrophobic tail
detergent micelles
membrane
lipid double layer
membrane protein
crystallization
membrane protein with bound detergent molecules

Protein Denaturation
Using Sodium Dodecyl Sulfate (SDS)



HOW SDS DENATURES PROTEIN

1. Disrupting Hydrophobic Interactions:
SDS is a surfactant with a hydrophilic (water-loving) head and a hydrophobic (water-fearing) tail. In aqueous solution, the hydrophobic tails of SDS molecules insert themselves into the hydrophobic regions of the protein.
2. Unfolding the Protein:
This interaction breaks apart the non-covalent bonds that hold the protein's hydrophobic regions together, leading to the disruption of its three-dimensional structure.
3. Imparting Negative Charge:
SDS molecules then coat the now-unfolded protein with a uniform negative charge.
4. Straightening the Polypeptide Chain:
The repulsive forces between these negative charges cause the polypeptide chain to straighten out and become a linear, unfolded molecule

Denaturation of proteins by altered pH:

- There are ionizable groups in the individual amino acids.
- The rate at which they ionize depends on the group and the pH.
- A high concentration of hydrogen ions (low pH) will result in more groups being protonated.
- Carboxyl groups (aspartic acid, glutamic acid, the carboxy terminus) and phenolic groups are uncharged when protonated. The nitrogen groups (amines on lysine, guanidine of arginine, and imidazole in histidine, etc.) are charged when protonated.
- Charged groups will tend to move towards the surface of the proteins and uncharged groups tend to move inwards.

- **How denaturation occurs at the level of protein structures:**
- Denaturation occurs when that shape is compromised and the molecule can no longer function in its desired capacity.

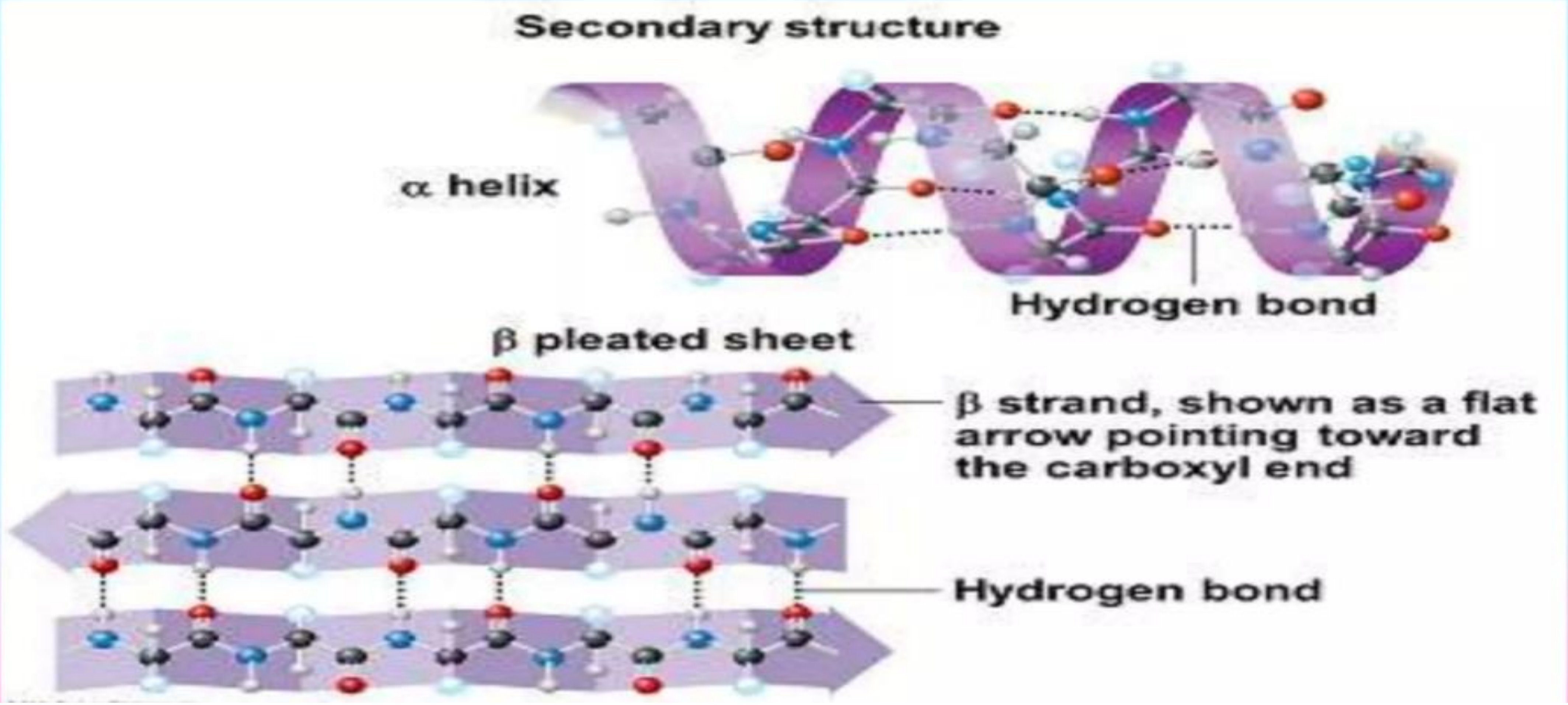
- Proteins may be denatured at the secondary, tertiary and quaternary structural levels but not at the primary structural level.

Primary structure:

- In primary structure the sequence of amino acids held together by covalent peptide bonds which are not disrupted by the process of denaturation.

Denaturation of proteins at secondary structure level:

In secondary structure level of denaturation, proteins lose all regular repeating units or patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration.



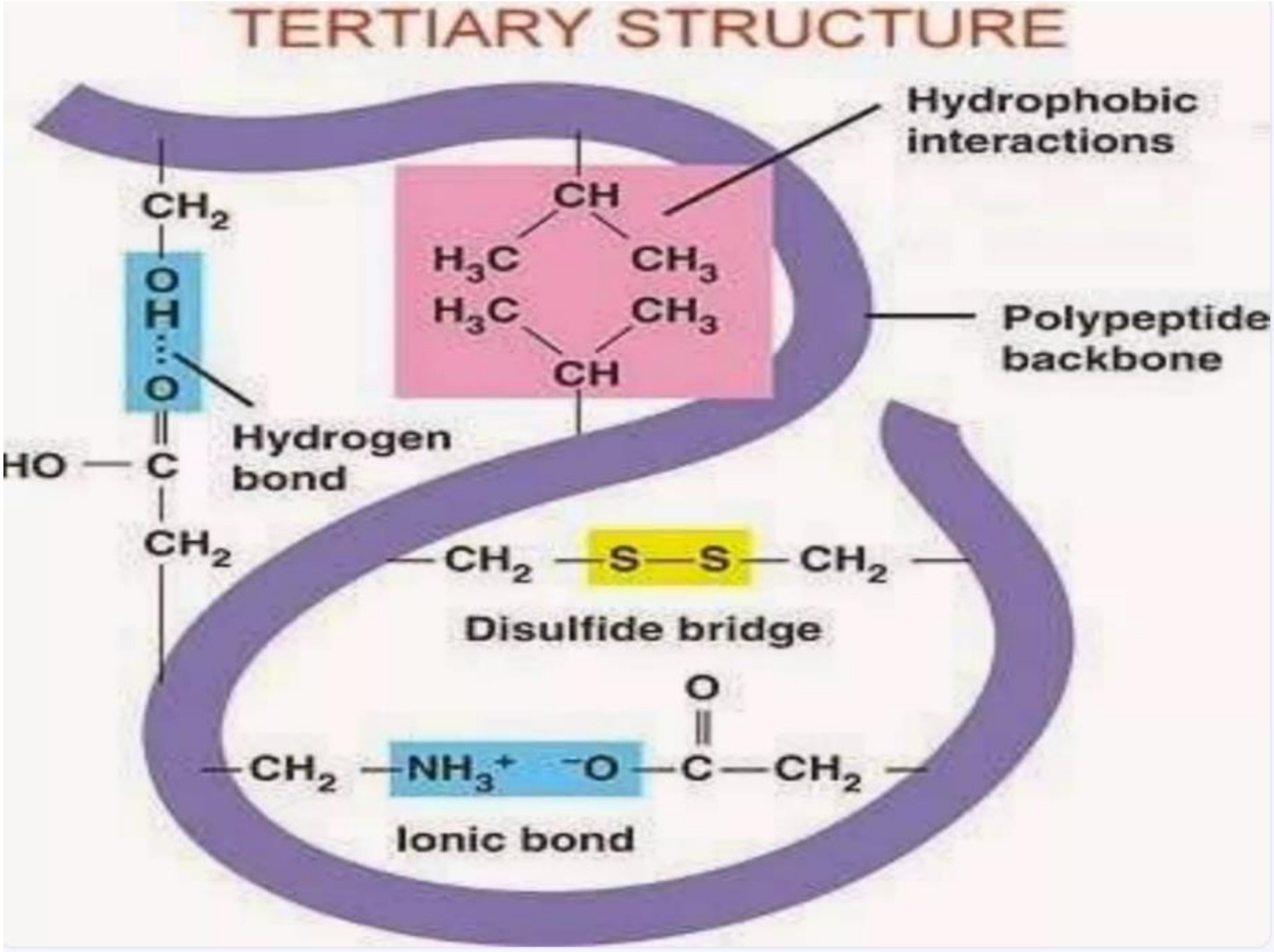
Denaturation of proteins at tertiary structure level:

- In tertiary structure denaturation of proteins involves the disruption of the following bonds

- Covalent interactions between aminoacid side chains(such as disulfide bridges between cysteine groups).

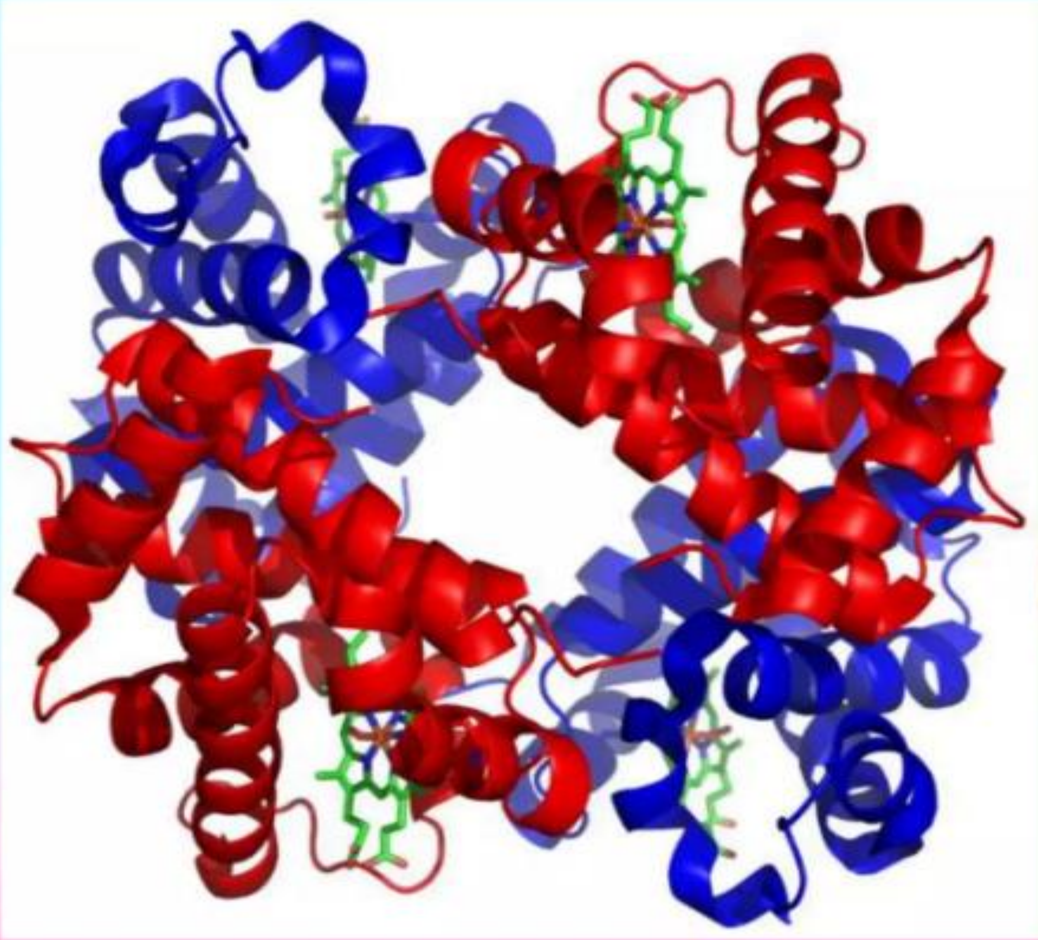
- Non-covalent dipole-dipole interactions between polar amino acids side chains and surounding solvents.

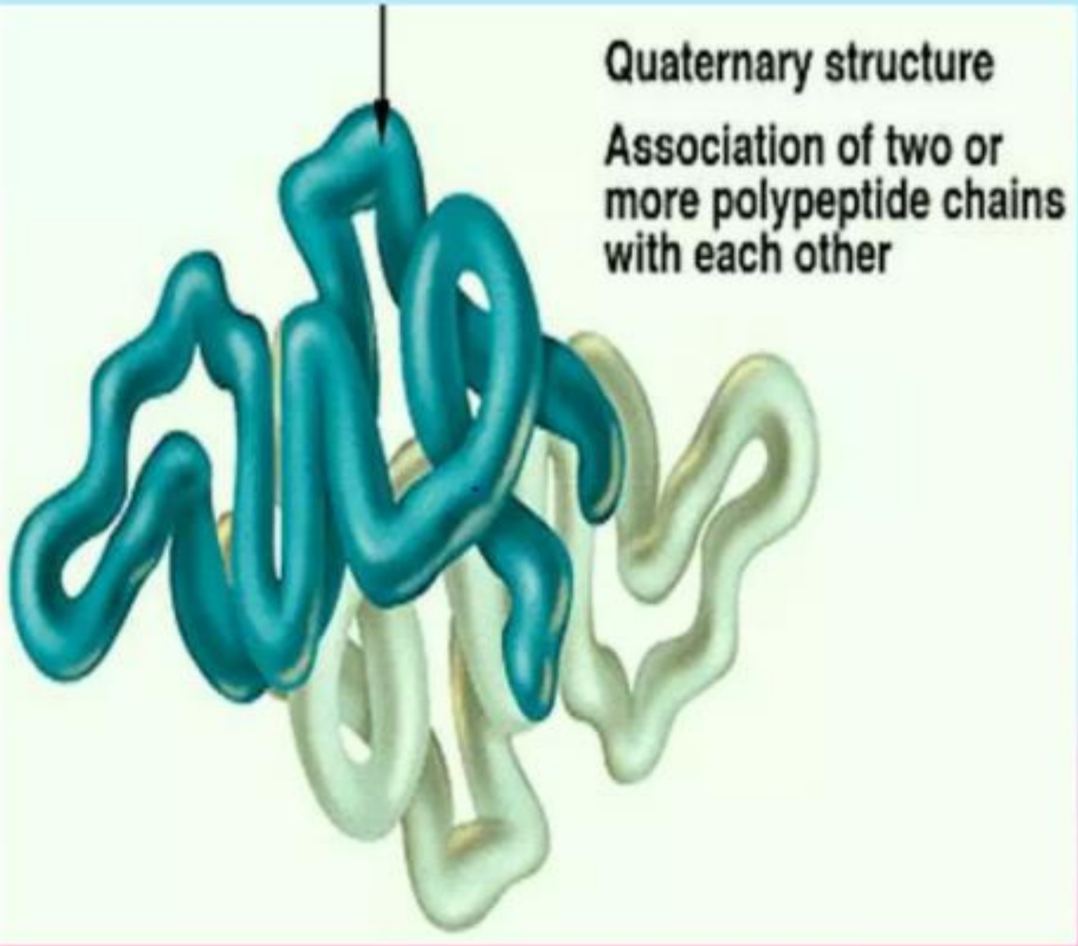
- Vanderwaals (induced dipole) interactions between nonpolar amino acid side chains.


TERTIARY STRUCTURE
Hydrophobic interactions
CH
H3C
CH3
H3C
CH3
CH
Polypeptide backbone
CH2
O
H
O
HO
C
Hydrogen bond
CH2
CH2
S
S
CH2
Disulfide bridge
O
CH2
NH3+
-O
C
CH2
Ionic bond

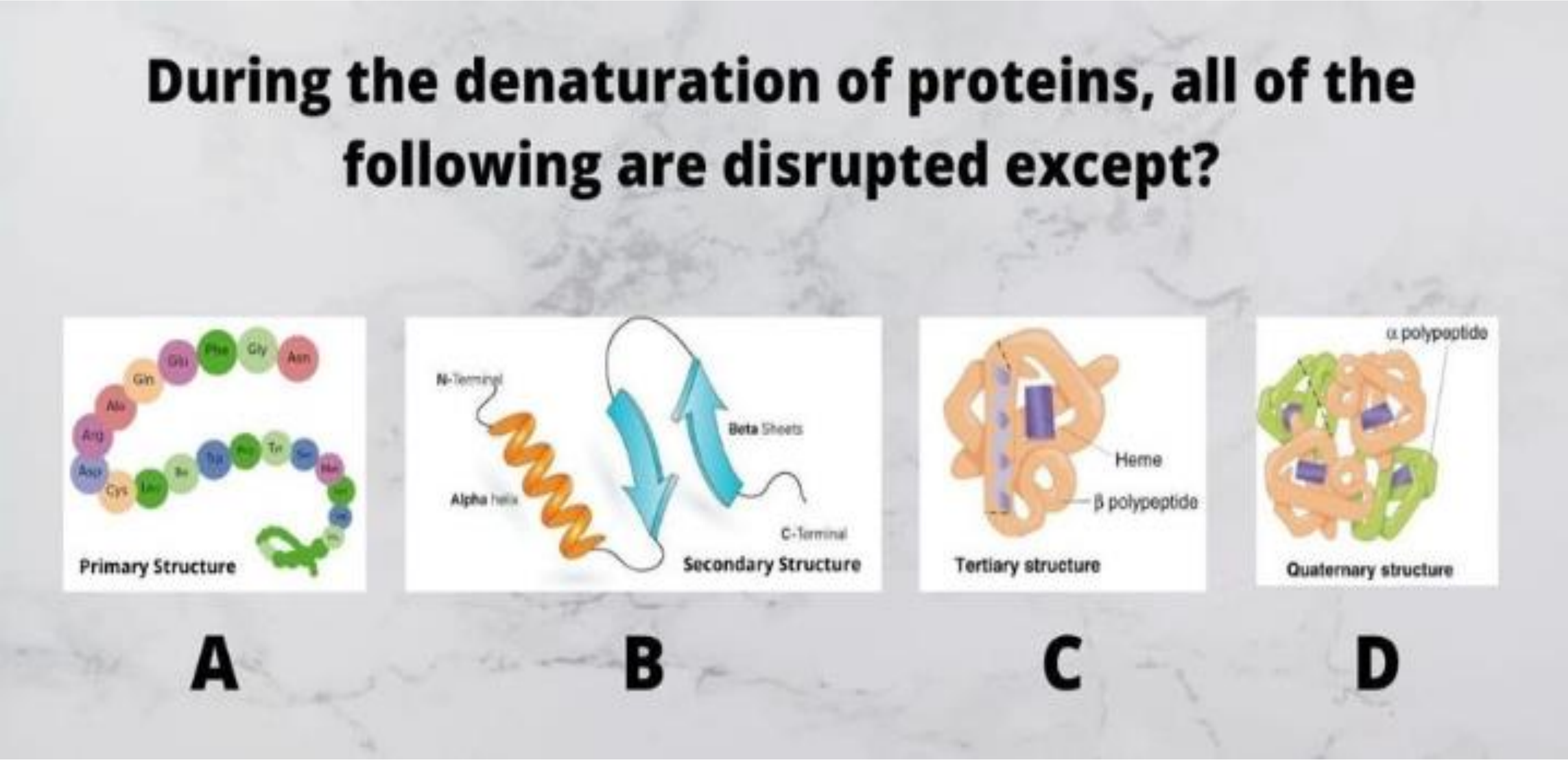
Denaturation of proteins at quaternary structure level:

in quaternary structure of protein denaturation , the protein subunits are dissociated and/or the spatial arrangement of proteins subunit is disrupted.





QUESTION??


During the denaturation of proteins, all of the following are disrupted except?
Primary Structure
N-Terminal
Alpha helix
Beta Sheets
C-Terminal
Secondary Structure
Heme
β polypeptide
Tertiary structure
α polypeptide
Quaternary structure
A
B
C
D