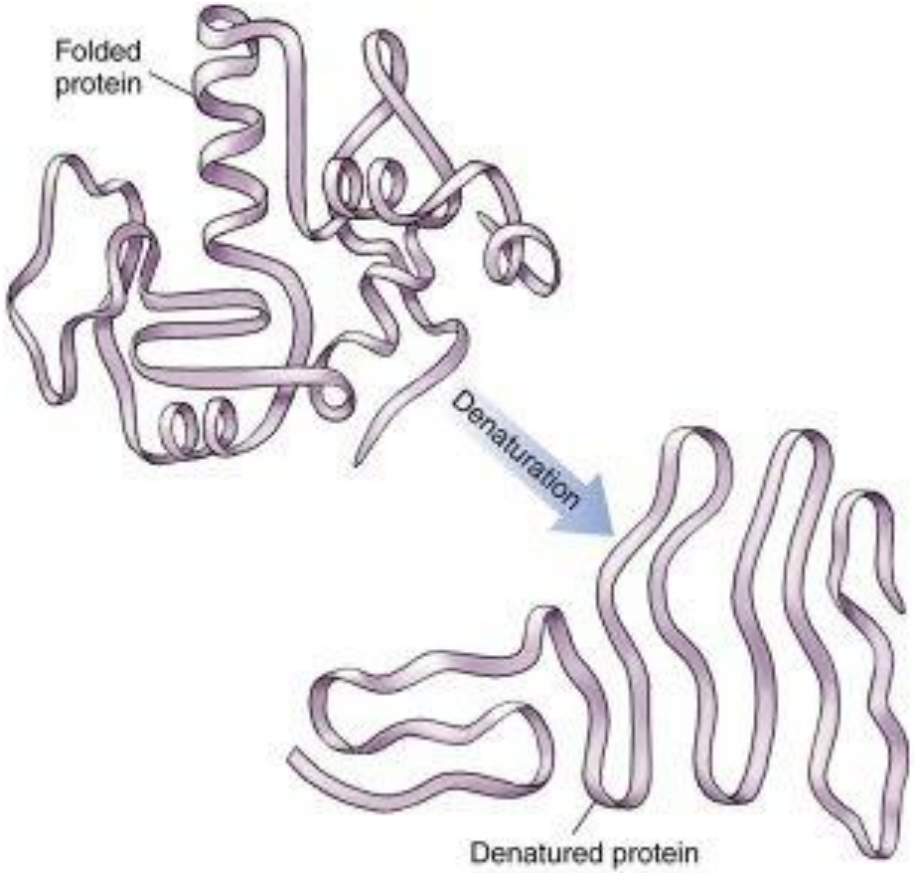
Denaturation and renaturation of proteins

- Higher levels of protein structure are formed without covalent bonds. Therefore, they are not as stable as peptide covalent bonds which make protein primary structure
- Under external stress like, temperature or action of certain chemical compounds the protein 3-dimentional structure becomes even less stable and can unravel


Folded
protein
Denaturation
Denatured protein

How denaturation occurs at levels of protein structure

- In **quaternary structure** denaturation, protein sub-units are dissociated and/or the spatial arrangement of protein subunits is disrupted.
- **Tertiary structure** denaturation involves the disruption of:

i. Covalent interactions between amino acid side-chains (such as disulfide bridges between cysteine groups)
ii. Non-covalent dipole-dipole interactions between polar amino acid side-chains (and the surrounding solvent)

- In **secondary structure** denaturation, proteins lose all regular repeating patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration.
- **Primary structure**, such as the sequence of amino acids held together by covalent peptide bonds, is **not disrupted** by denaturation.

Denaturation usually disrupts the function of a protein.

Heat denaturation

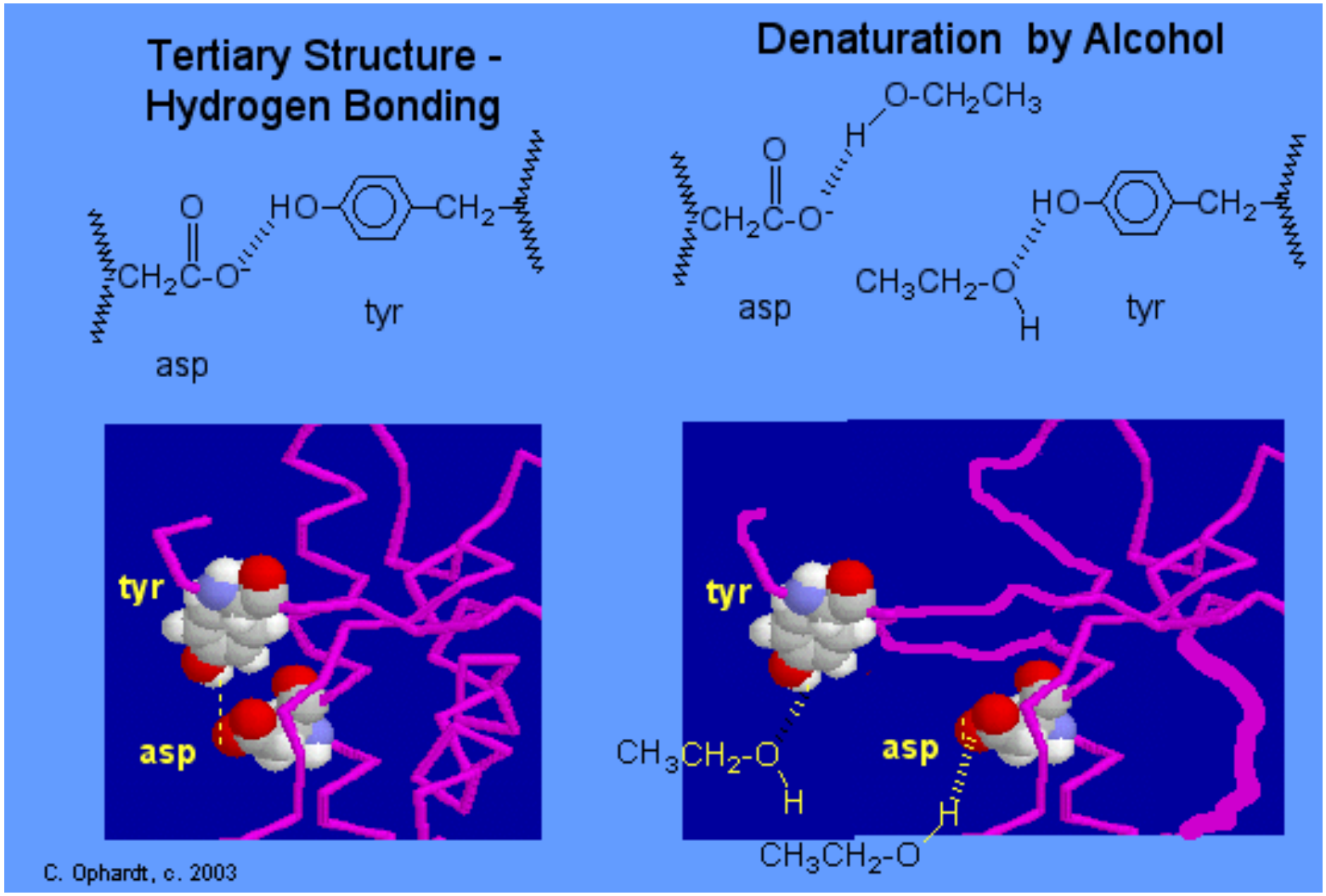
- **Heat** can be used to disrupt hydrogen bonds and non-polar hydrophobic interactions. This occurs because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted. The proteins in eggs denature and coagulate during cooking. Other foods are cooked to denature the proteins to make it easier for enzymes to digest them. Medical supplies and instruments are sterilized by heating to denature proteins in bacteria and thus destroy the bacteria.

Alcohol Disrupts Hydrogen Bonding:

- Hydrogen bonding occurs between amide groups in the secondary protein structure. Hydrogen bonding between "side chains" occurs in tertiary protein structure in a variety of amino acid combinations. All of these are disrupted by the addition of another alcohol.

- A 70% alcohol solution is used as a disinfectant on the skin. This concentration of alcohol is able to penetrate the bacterial cell wall and denature the proteins and enzymes inside of the cell. Alcohol denatures proteins by disrupting the side chain intramolecular hydrogen bonding. New hydrogen bonds are formed instead between the new alcohol molecule and the protein side chains

Alcohol Disrupts Hydrogen Bonding:

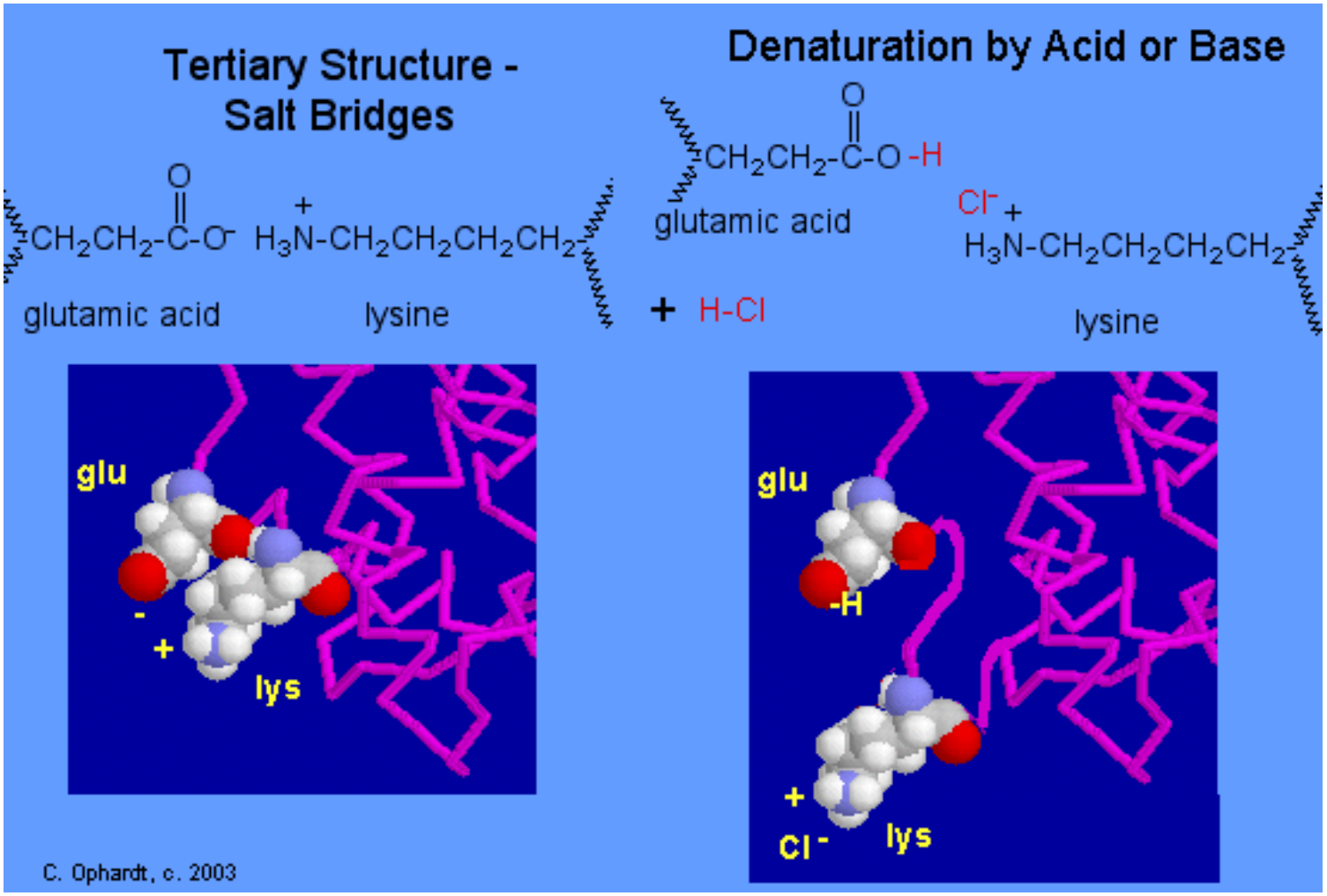


Acids and Bases Disrupt Salt Bridges:

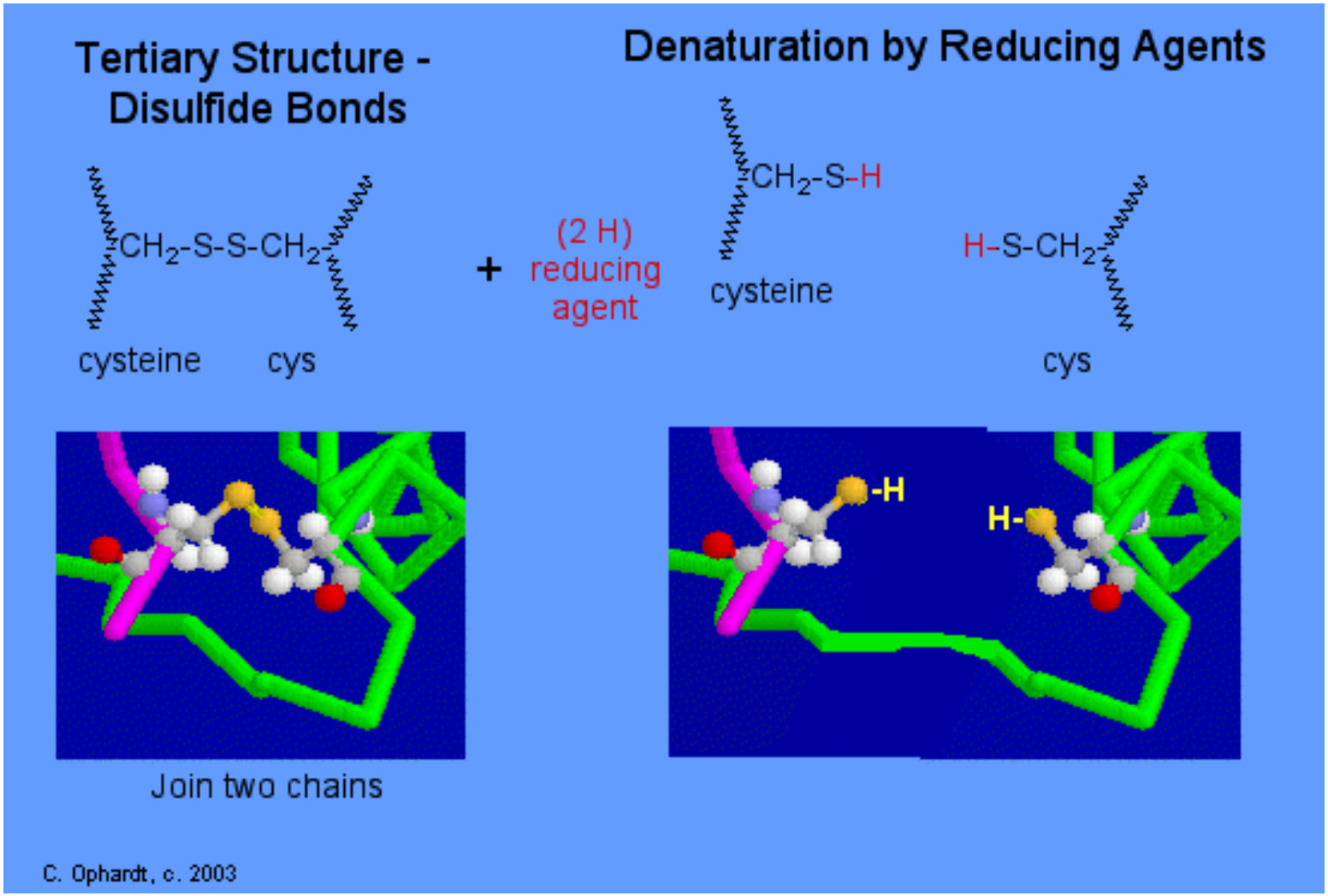
- **Salt bridges** result from the neutralization of an acid and amine on side chains. The final interaction is **ionic** between the positive ammonium group and the negative acid group.

- As might be expected, **acids and bases disrupt salt bridges** held together by ionic charges. A type of 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.

Acids and Bases Disrupt Salt Bridges:

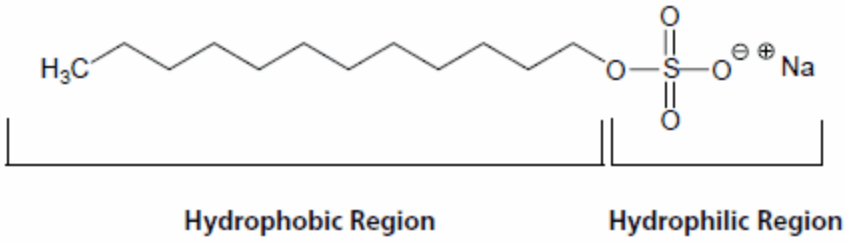


Disruption of disulphide bonds by reducing agents



Detergents disrupt hydrophobic interactions

- Detergents are used in biomedical laboratories for the disruption of cell membranes (cell lysis) and the release of intracellular materials in a soluble form. Detergents break the protein-protein, protein-lipid and lipid-lipid associations, denature proteins.
- Detergents are molecules that contain both hydrophobic groups (their tails) and hydrophilic groups (their heads)



A detergent – Sodium dodecyl sulphate (SDS)

Detergents

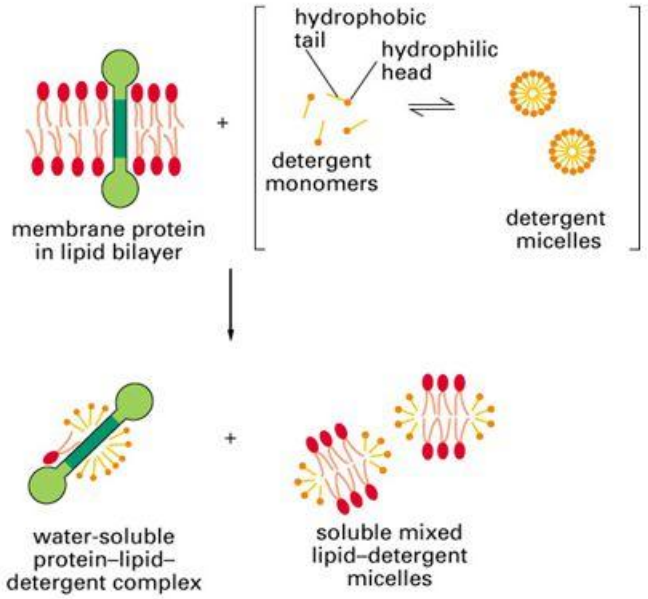
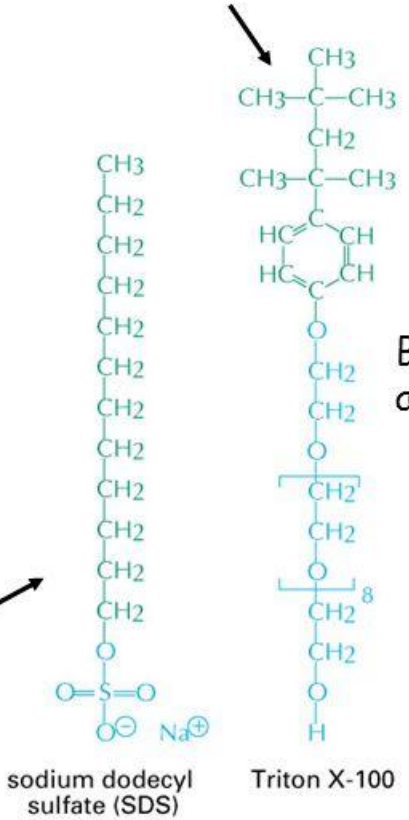


Figure 10–24. Molecular Biology of the Cell, 4th Edition.

Mild nonionic detergent that dissolves membranes without unfolding proteins.

Ionic detergent that dissolves membranes and unfolds proteins.



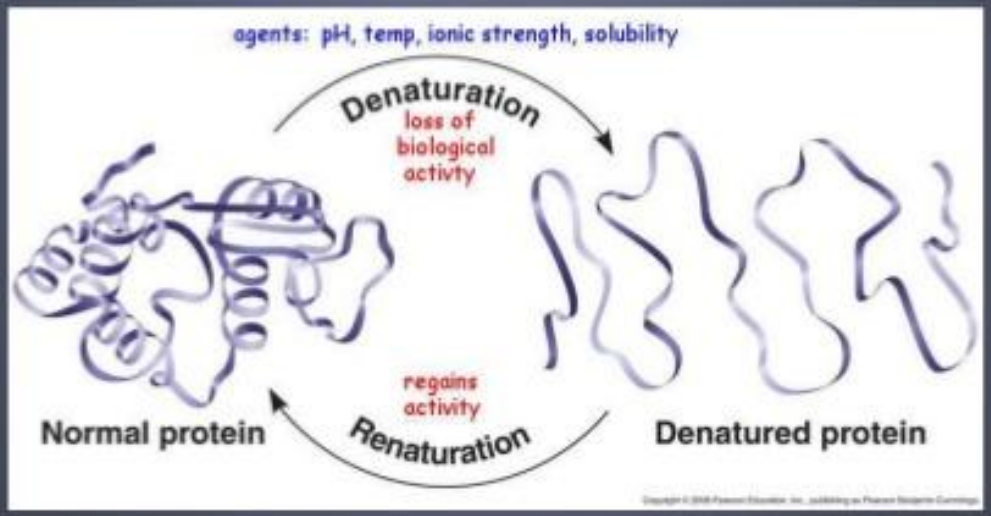
Both are amphipathic

Figure 10–25. Molecular Biology of the Cell, 4th Edition.

Renaturation

- In many cases, denaturation is reversible (the proteins can regain their native state when the denaturing influence is removed). This process is called renaturation. It could be complete or partial.
- Renaturation can completely or partially restore the protein function lost because of 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.



Renaturation is similar to protein folding during protein biosynthesis