Protein Characterization

Lecture Five

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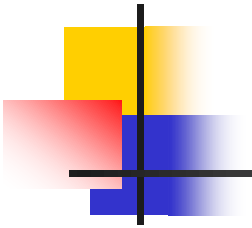
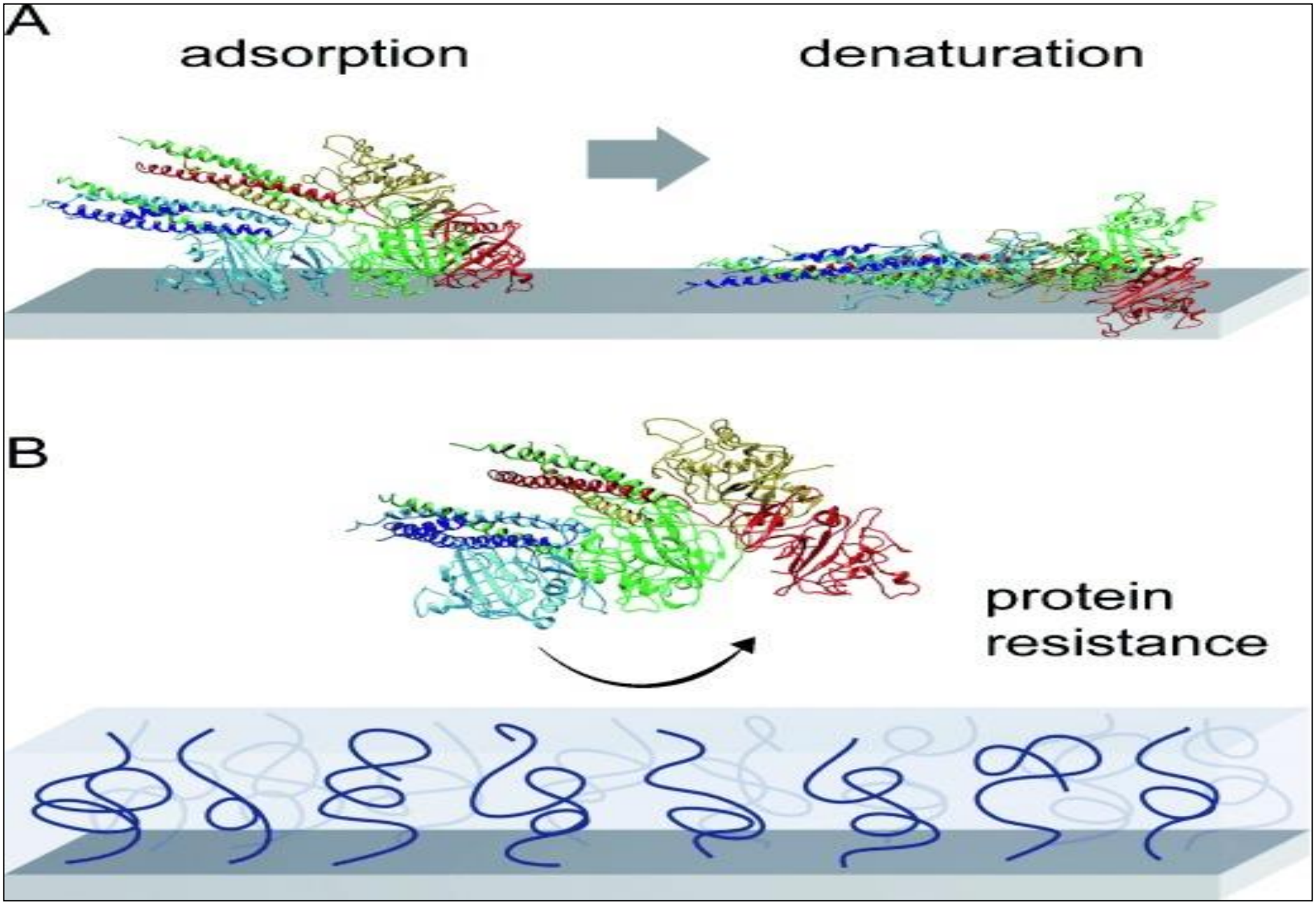
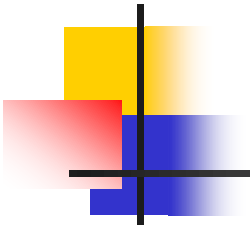
- The extent and reversibility of protein adsorption are dependent on:
  - The conformational state of the protein
  - The pH and ionic strength of the solution
  - The nature of the exposed surface
  - Time of exposure.



- Poly(oxyethylene oxide)=(Teflon®) is quite effective at reducing protein adsorption and preventing its denaturation at the surface.
- The adsorption can be decreased by **Siliconization**, soaking or rinsing of the glass vials in a silicon solution or emulsion. Then the drained containers should be placed in an oven at about 250°C for 5-6 hours.
- Albumin can be used as anti-adhesion agent (by competition on the binding sites)


A
adsorption
denaturation
B
protein
resistance



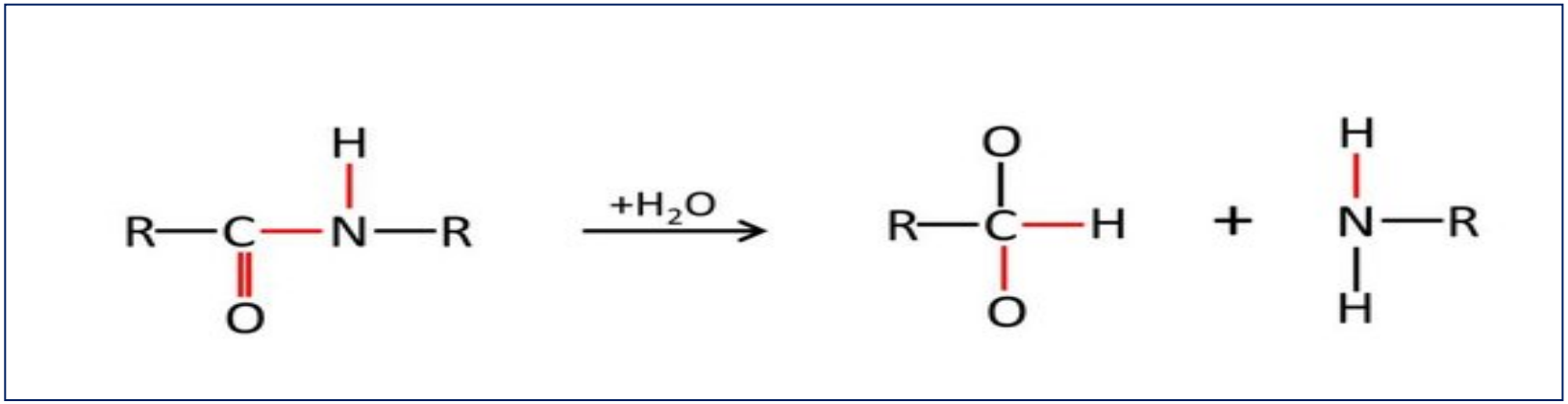
- Surfactants like poly-sorbate 20 or 80 can also prevent adhesion to interfaces and ppt., these molecules readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups to the aqueous phase.

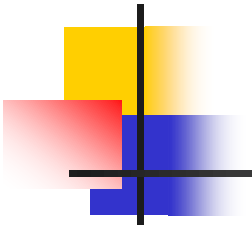
Chemical instability

- Hydrolysis
- Deamidation
- Oxidation
- Racemization
- Disulfide exchange
- Maillard reaction

Hydrolysis

- Proteolysis is the hydrolysis of the peptide bond between amino acids in a peptide or protein.
- At an extreme pH and temperature, the peptide bond can undergo rapid proteolysis resulting in protein degradation and/or fragmentation.

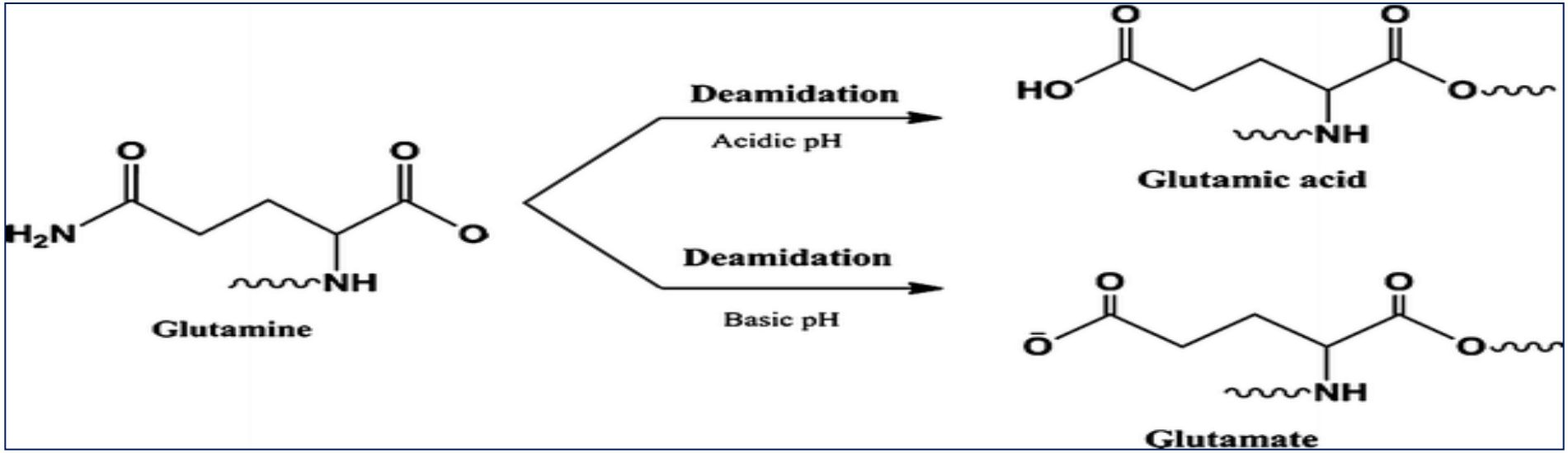




- Several therapeutic proteins are known to degrade through hydrolysis.
- These include luteinizing hormone releasing hormone (LHRH), macrophage colony stimulating factor (M-CSF), human growth hormone, and vasoactive intestinal peptide (VIP).

Deamidation

- Is one of the main chemical degradation pathways of proteins, in which the side chain linkage in a glutamine (Gln) or asparagine (Asn) residues is hydrolyzed to form a carboxylic acid.
- The deamidation of Asn and Gln residues of proteins is an acid and base-catalyzed hydrolysis reaction, which can occur rapidly under physiological conditions.



- Solution pH optimization and lyophilization (not almost) are frequently used to minimize deamidation in proteins.
- In some cases, protein engineering to replace Asn residue with Serine can be used if it does not affect protein conformation and biological activity.

Oxidation

- Oxidation is one of the major causes of chemical degradation in proteins and peptides.
- Some functional groups in proteins that can undergo oxidation such as:

SH- in cysteine, imidazole in histidine and phenol in tyrosine.

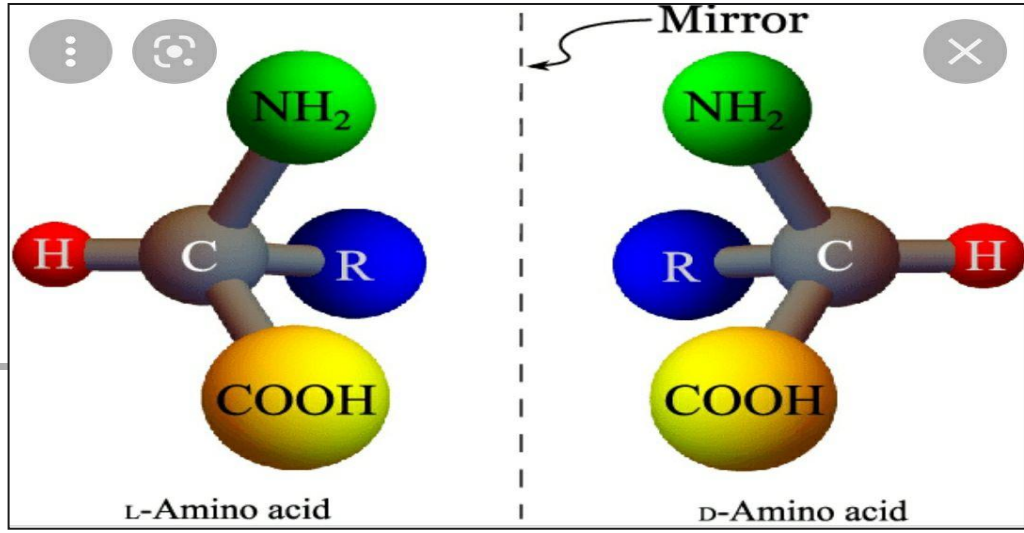
Factors that increase oxidative degradation in proteins include:

1) Atmospheric oxygen
2) Peroxides, such as hydrogen peroxide (at neutral or slightly alkaline pH). The source of peroxides in formulation is often the hydrophilic polymeric excipients used.
3) Metal contaminants (e.g., Fe^{2+}/Fe^{3+} and Cu^{+}/Cu^{2+}), light, acid/base, and free radicals (Catalysts of oxidation).
4) Solution pH, nature of buffers, presence of metal ions and metal chelators, and neighboring amino acid residues of susceptible amino acids influence oxidation in solution.

To decrease that:

- Low temperature storage or refrigeration to reduce reaction rates.
- Protection from light.
- pH optimization.
- Reduction of oxygen exposure.
- Use of antioxidants and chelating agents. Antioxidants terminate free radical reactions. Chelating agents sequester free metals, such as iron and copper from the formulations.
- Lyophilization.
- Certain sugars might prevent or minimize protein oxidation by complexation with metal ions or hydrogen bonding on the protein surface to preserve its native conformation.

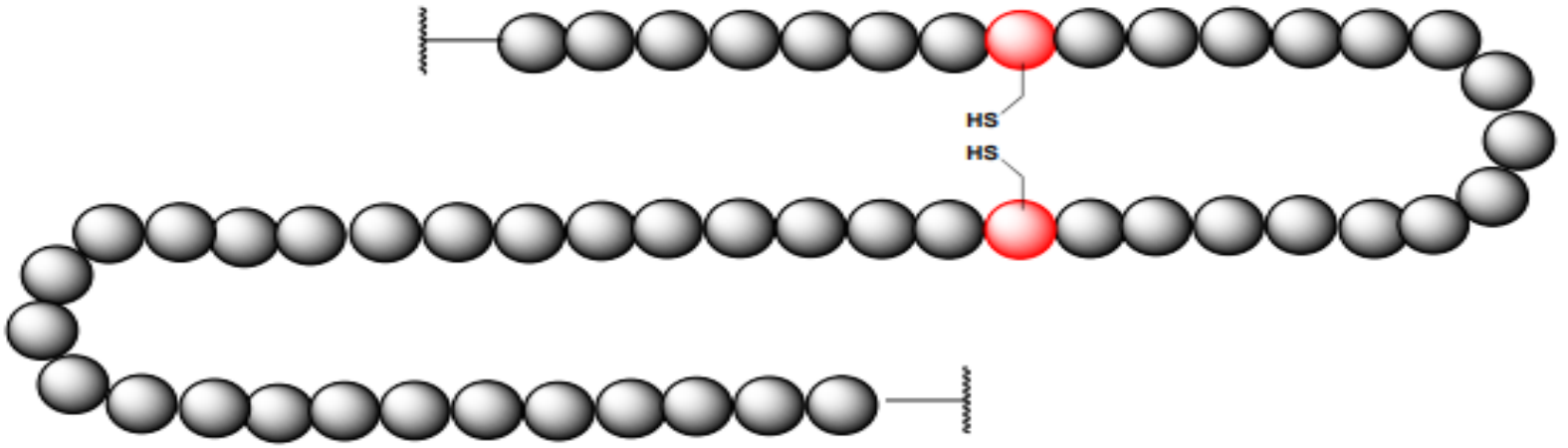
Racemization



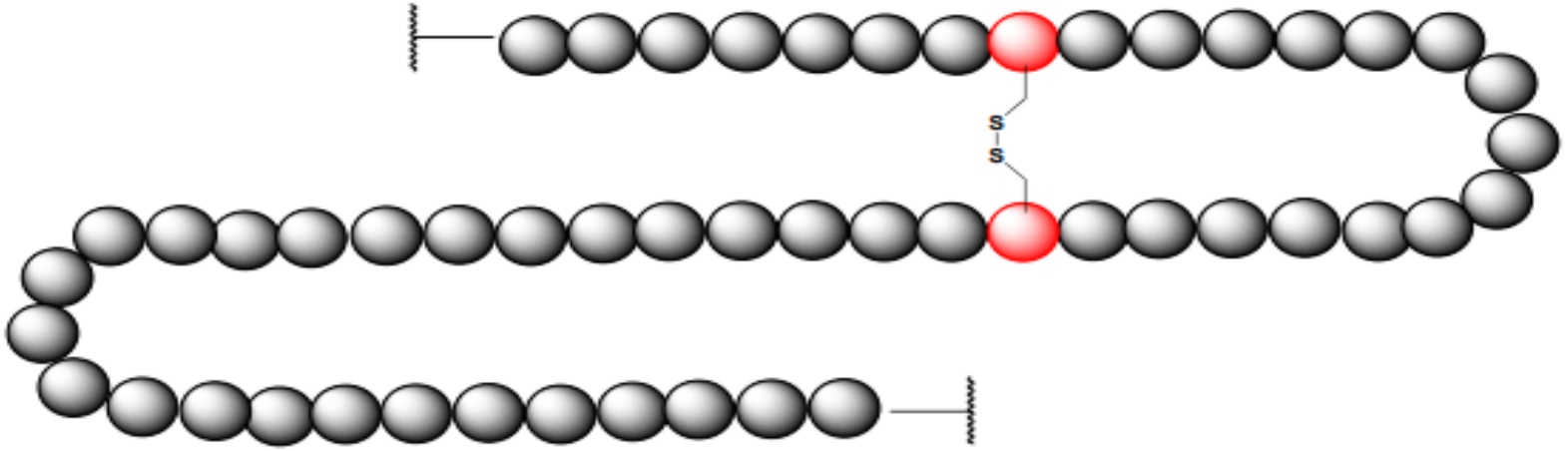
- Racemization can affect protein conformation.
- All amino acid residues except glycine are chiral at the carbon atom bearing the side chain and are subject to base-catalyzed racemization.
- The rate of racemization depends on the particular amino acids, and is influenced by temperature, pH, ionic strength, and metal ion chelation.
- Aspartic acid and serine residues are the most prone to racemization.

Disulfide exchange

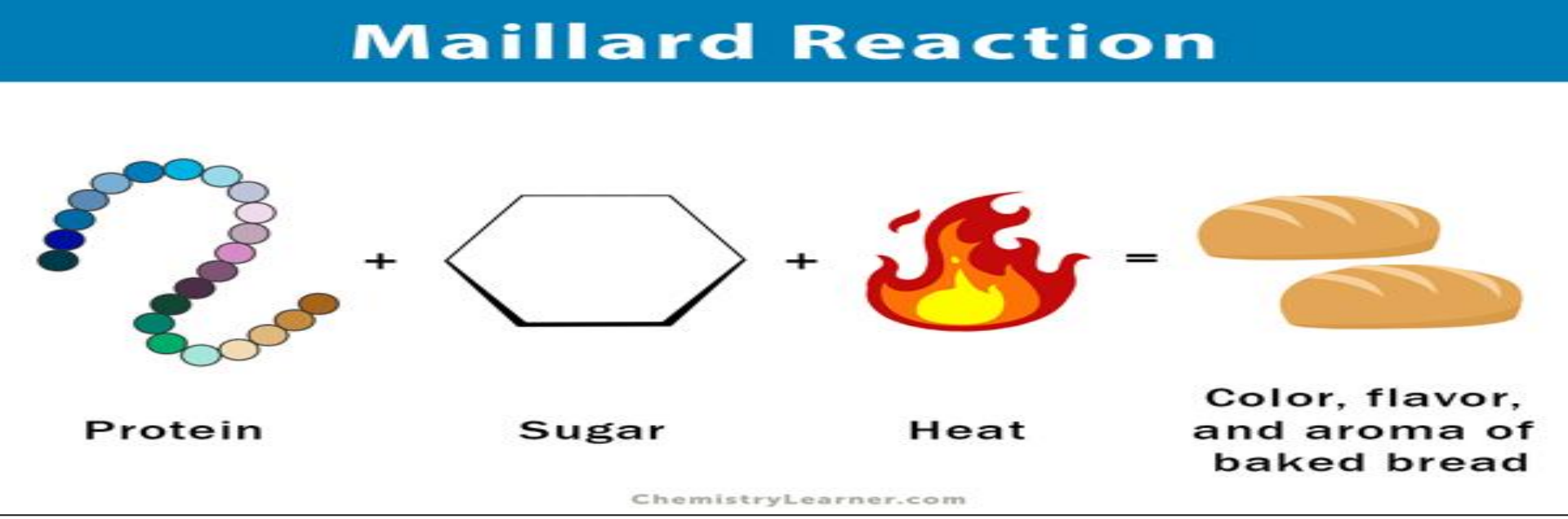
- Disulfide bonds provide covalent structural stabilization in proteins.
- Cleavage and subsequent rearrangement of these bonds can alter the tertiary structure, thereby affecting protein conformation, stability, and biological activity.
- Disulfide exchange is catalyzed by thiols, which can arise by initial hydrolytic exchange of disulfide, or β-elimination in neutral or alkaline media.
- **How avoid it??**



reduced protein (free cysteines)



oxidized protein (disulfide)



Maillard reaction

- The use or presence as impurities, of reducing sugars (e.g., glucose, lactose, fructose, maltose, xylose) in a protein formulation can result in the Maillard "browning reaction," which involves non-enzymatic glycation of the protein at the basic protein residues such as lysine, arginine, asparagine, and glutamine.

- It could be minimized or prevented by removing reactive substrate (reducing sugars), pH adjustment, chelation of trace metals, use of antioxidant, reducing water content and storage at low temperatures.

Antigenicity & Immunogenicity

- The ability of a protein to generate an immune response, triggering the production of antibodies, is referred to as immunogenicity.
- Immunogenicity of a protein indicates that its first administration did not elicit an immune response since the protein was not recognized as an antigen. However, repeated protein administration led to the formation of antibodies, causing an immune reaction.

❑Immunogenicity is the ability to induce a humoral and/or cell-mediated immune response.

B cells + antigen ⟶ effector B cells + memory B cells

T cells + antigen ⟶ effector T cells + memory T cells

❑Antigenicity is the ability to combine specifically with the final products of the **immune response** (i.e. secreted antibodies and/or surface receptors on T-cells).

- Each immunogenic protein is antigenic, but the reverse is not true.
- Antigenicity refers to the ability of specific sites (epitopes) on the protein to recognize antibodies in the host immune system.
- Thus, the first administration of an antigenic protein would lead to an immune reaction.
- While proteins made in a particular organism are recognized by the immune system as "self" protein and normally do not elicit an immune response, misfolded or denatured forms of "self" proteins may be immunogenic.