Protein Characterization (Stability)

Lecture Four

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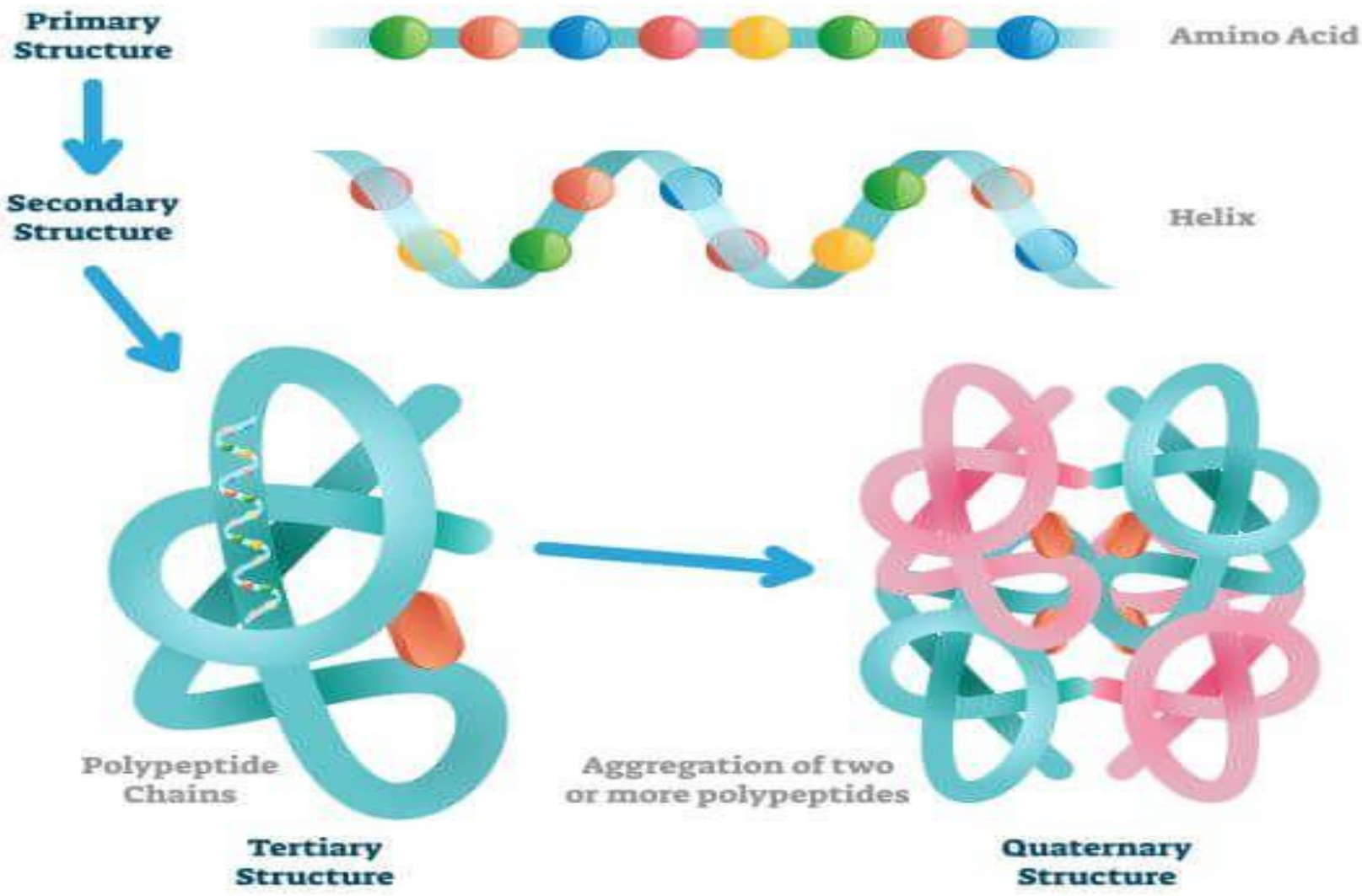
- Proteins are inherently unstable molecules, and their degradation profile can be quite complex.
- Pharmacists involved in compounding of biologically active proteins will be interested in their: Stabilization, formulation and delivery.
- Stability (physical and chemical) generally depend on : pH, temperature, agitation and the overall environment of container.

PROTEIN STRUCTURE



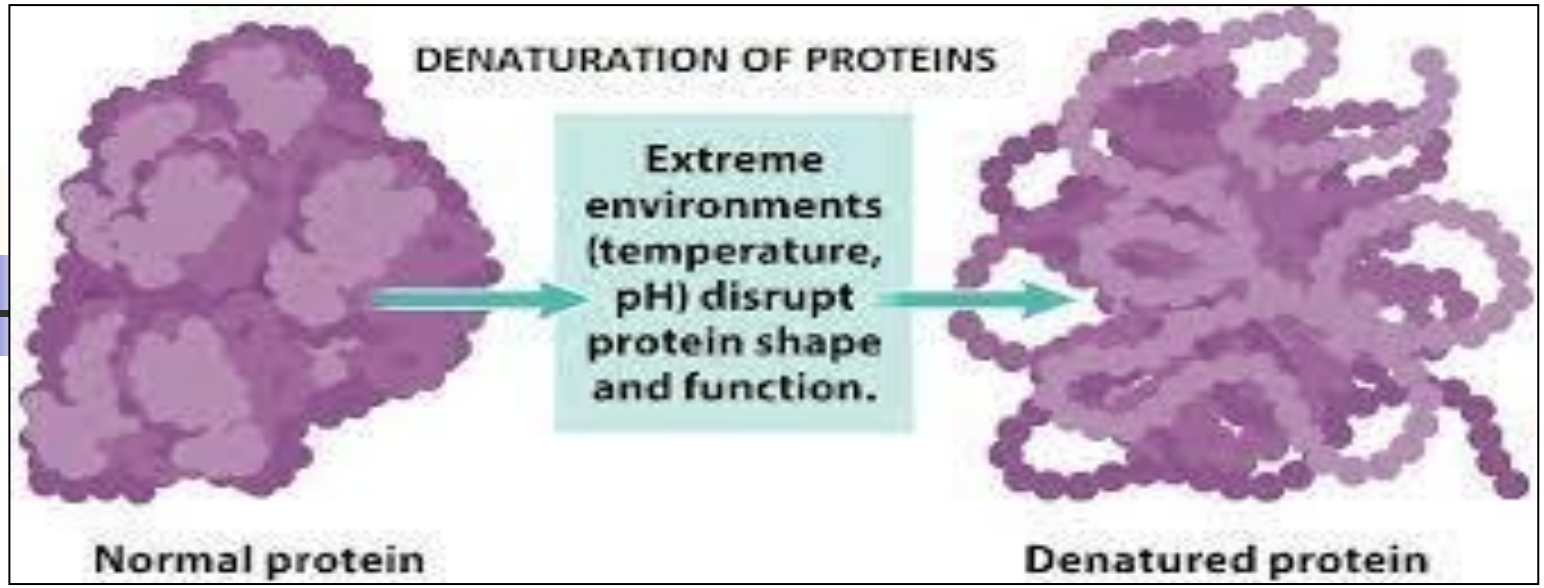
Physical instability

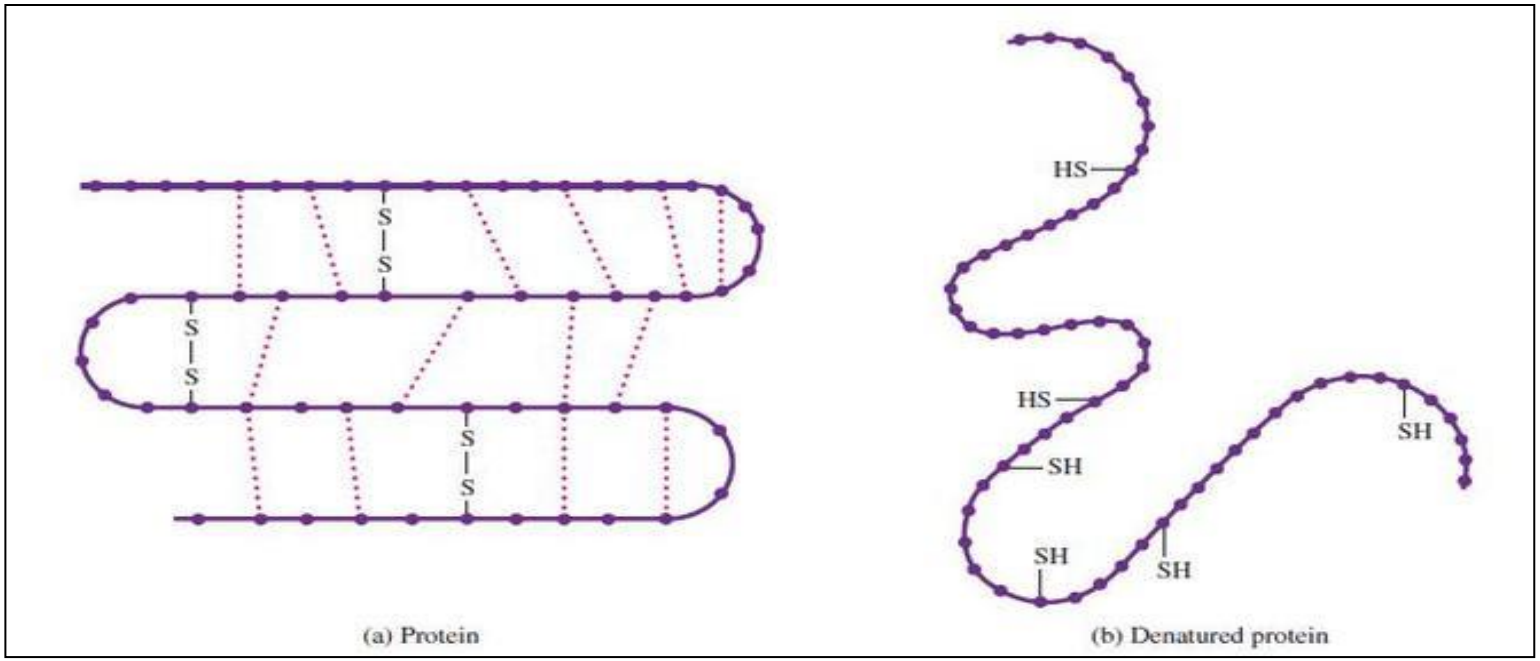
- Protein denaturation by a change in higher order folding or conformation can lead to aggregation, precipitation, and/or adsorption to the surface. Then, we have:

1) Denaturation
2) Aggregation and precipitation
3) Surface adsorption

Denaturation

- Protein denaturation refers to disruption of **the tertiary and secondary structure** of a protein or peptide.
- It can be caused by (**heating, cooling, freezing, extremes of pH, and contact with organic chemicals**).
- Protein denaturation is often associated with increased hydrophobic surface of a protein.
- In such cases, several protein molecules in solution might self-associate and exclude the solvent. This phenomenon is termed **aggregation**.
- If the aggregates separate from the solution and become visible, the phenomenon is called **protein precipitation**.


DENATURATION OF PROTEINS
Extreme environments (temperature, pH) disrupt protein shape and function.
Normal protein
Denatured protein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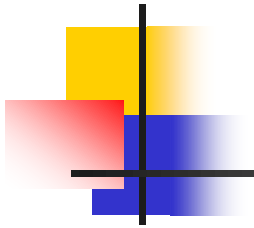
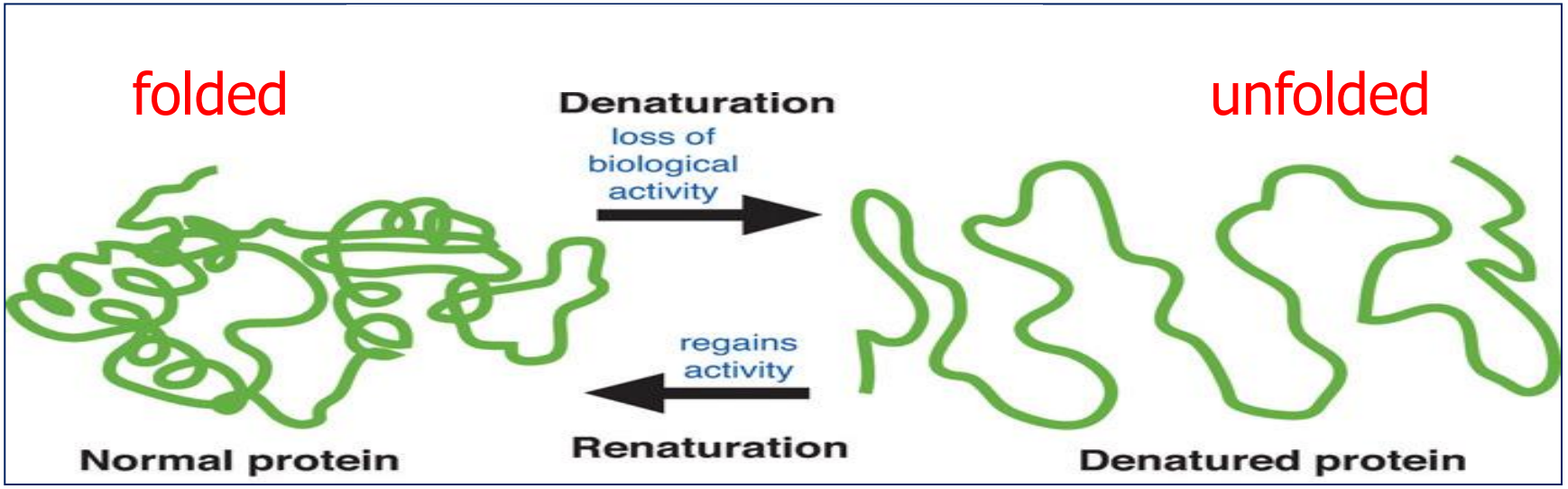
(a) Protein
(b) Denatured protein



- Protein denaturation may be reversible and irreversible.
- **Reversible type** can be caused by temperature or exposure to chao-tropic agents, such as **urea and guanidine hydrochloride**.
- These agents interfere with stabilizing intra-molecular non-covalent interactions in proteins, including hydrogen bonding, van der Waals forces, and hydrophobic effects.

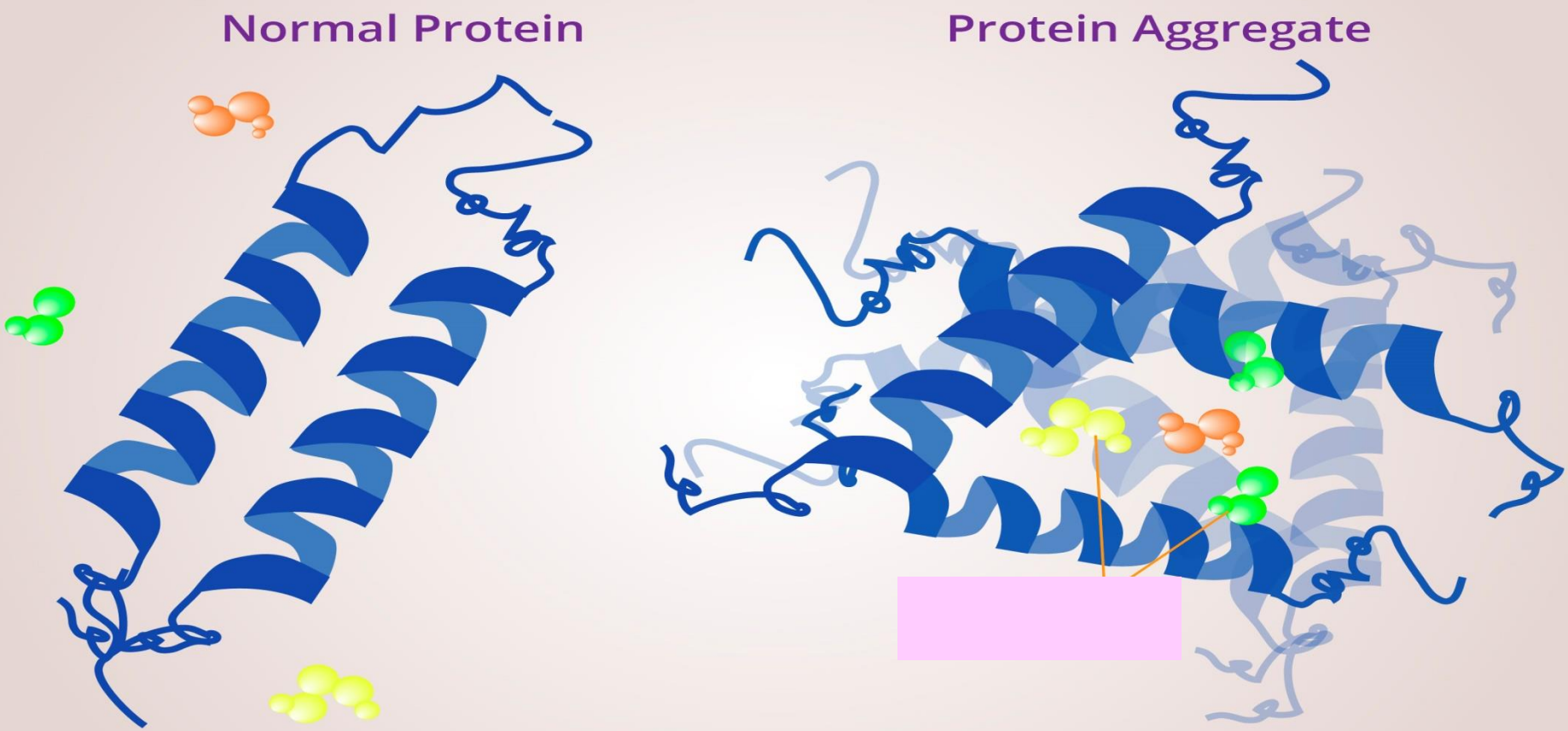
- **Irreversible type** refers to that the unfolding process disrupted the native protein structure to the extent that the native structure cannot be regained simply by changing the denaturing condition (such as temperature).



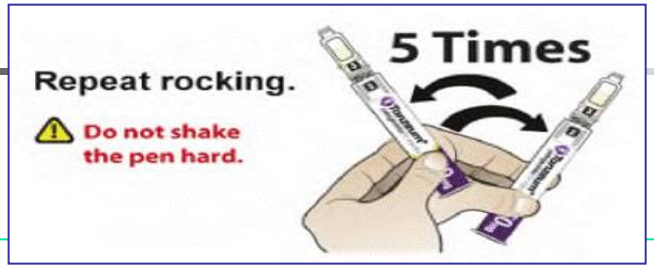
Aggregation and precipitation

- Aggregation of proteins refers to irreversible interaction and **clustering** of two or more protein molecules.
- Protein aggregates may be (soluble or insoluble).
- Protein aggregation is driven by the unfolding process, which exposes the interior hydrophobic region to the solvents, usually water, leading to thermodynamically unfavorable surroundings of the hydrophobic protein.
- This drives intermolecular interactions (hydrophobic or electrostatic) between exposed hydrophobic regions of different protein molecules, leading to association and, thus, aggregation.

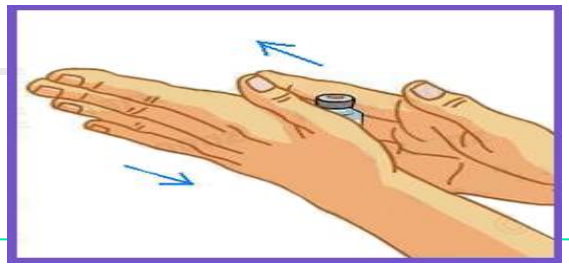
Protein Aggregate



Several factors may lead to protein aggregation:



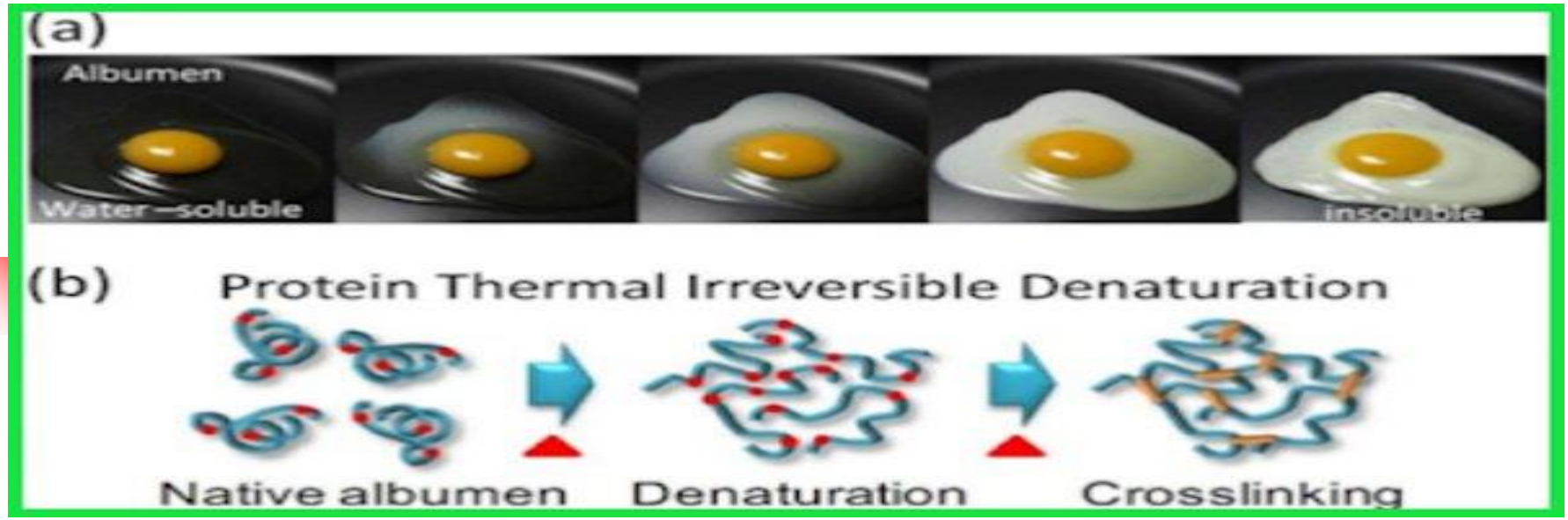
Right Way



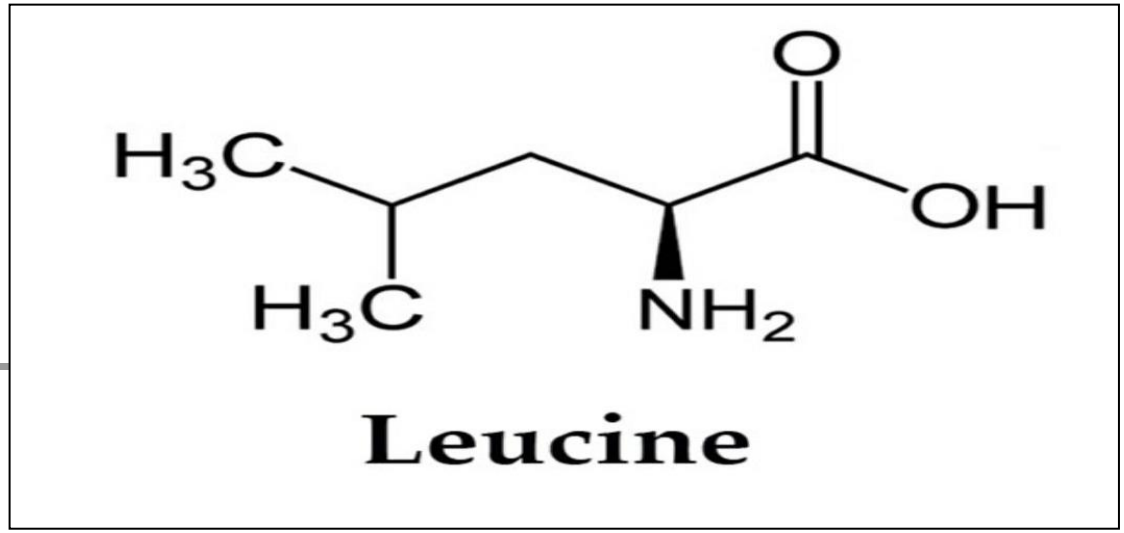
For example:

- **Shear forces:** Shearing and shaking of protein solutions during formulation and shipment.
- **Temperature:** An increase in temperature results in greater flexibility (unfolding) of proteins and an increased tendency to form aggregates.
- **Ionic strength:** An increase in the ionic strength may lead to neutralization of the surface charge of the protein molecules.

- **pH:** Charge neutralization and subsequent aggregation can also occur when the pH of the solution approaches the isoelectric point of the protein.
- **Moisture:** An optimal residual moisture level is required to maintain stability of lyophilized protein formulations, the absence of which may lead to protein aggregation.



- Insoluble protein aggregates are visually evident **(the protein precipitation)** which is a macroscopic process producing a visible change of the protein solution, such as turbidity/clouding of the solution or formation of visible particulates (containing more stronger interaction forces may be covalent).
- May be caused by salting out and isoelectric ppt.



How decrease this instability??

- Leucine (amino acid) can be used as anti-aggregation.
- Low concentration of phospholipids and surfactants used for decreasing of insulin ppt. (or called fibrillation).

Surface adsorption

- The adsorption of proteins and peptides to the surfaces of the intermediate container and filter results from protein surface interaction with nonpolar surfaces.
- This can cause proteins to expose their hydrophobic interior, leading to adherence or adsorption to the surfaces of the containers.
- Alterations in the pH and ionic strength of the media can significantly enhance or reduce the protein's tendency to adsorb.
- Protein adsorption to neutral or slightly charged surface is greatest at its isoelectric point.

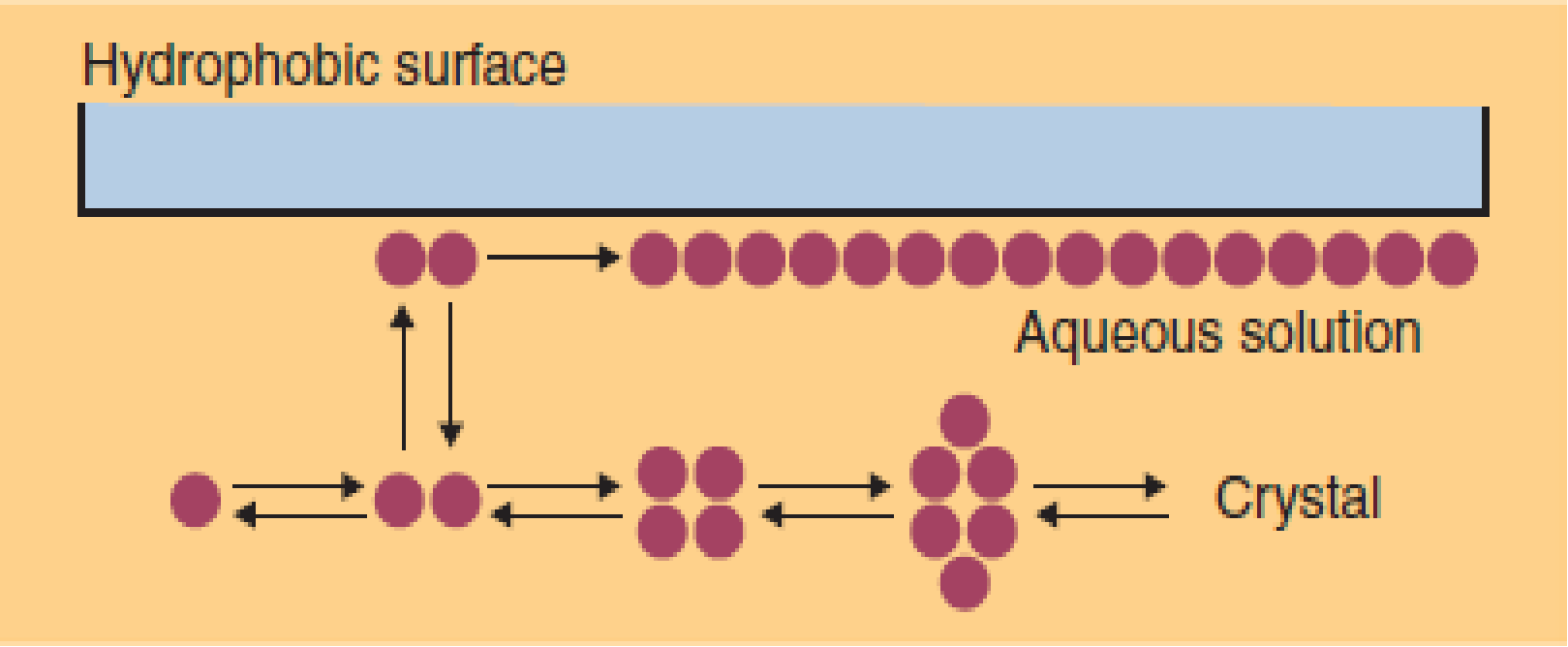
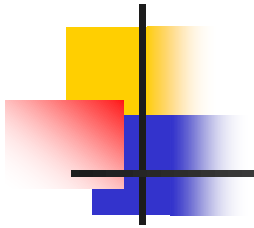


Figure 3 Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein films. Each circle represents a monomeric insulin molecule. *Source*: Adapted from Thurow and Geisen,



- The interfaces can be water/air, water/container wall or interfaces formed between the aqueous phase and utensils used to administer the drug (e.g. catheter, needle).
- Surface adsorption can be substantial when the initial concentration of the protein in solution is low, leading to high proportion of loss of drug.