Pharmaceutical Biotechnology
Lecture-3

Excipients Used in Parenteral Formulations of Biotech Product

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- In a protein formulation one finds, apart from the active substance, a number of excipients selected to serve different purposes.
- This process of formulation design should be carried out with great care to ensure therapeutic effectiveness and safe products.
- The nature of the protein (e.g. lability) and its therapeutic use (e.g. multiple injection systems) can make these formulations quite complex in term of excipients profile and technology (freeze-drying, aseptic preparation).

Table 1 components found in parenteral formulations of biotech products

- Active ingredient
- Solubility enhancers
- Anti-adsorption and anti-aggregation agents
- Buffer components
- Preservatives and anti-oxidants
- Lyoprotectants/ cake formers
- Osmotic agents
- Carrier system

All of the above are not necessarily present in one particular protein formulation

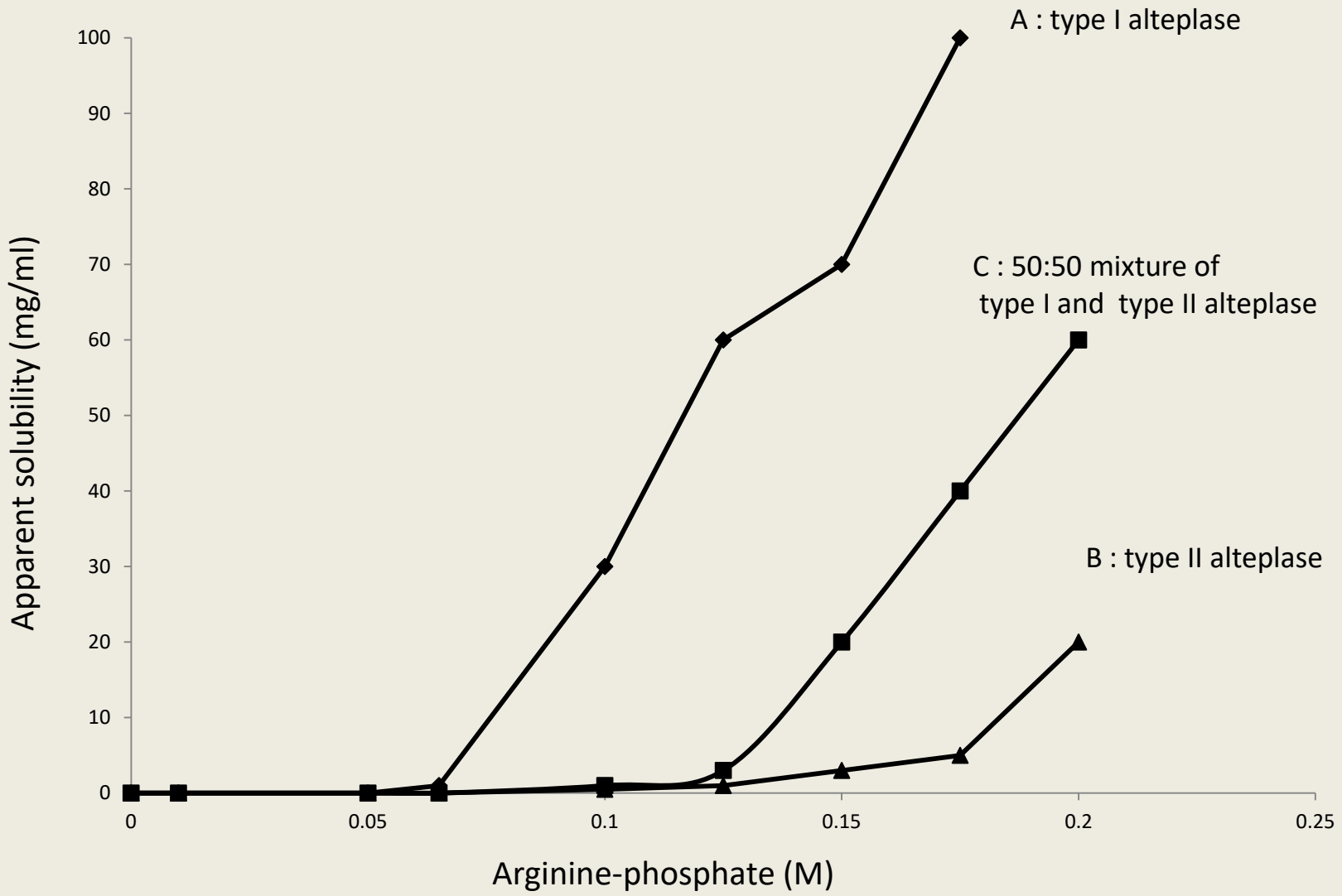
Solubility Enhancers

- Proteins, in particular those that are non-glycosylated, may have a tendency to aggregate and precipitate.
- Approaches that can be used to enhance solubility include

1. the selection of the proper pH and ionic strength conditions
2. the addition of amino acids, such as lysine or arginine (used to solubilize tissue plasminogen activator, t-PA), or
3. surfactants such as sodium dodecylsulfate, to solubilize non-glycosylate IL-2 (interleukin-2) can also help to increase the solubility.

- The mechanism of action of these solubility enhancers depends on the type of enhancer and the protein involved and is not always fully understood.

Figure 2 shows the effect of arginine concentration on the solubility of t-PA (alteplase) at pH 7.2 and 25°C.



- This figure clearly indicates the dramatic effect of this basic amino acid on the apparent solubility of t-PA.
- In the above examples aggregation is physical in nature, i.e. based on hydrophobic and/ or electrostatic interactions between molecules.
- However, aggregation based on the formation of covalent bridges between molecules through disulfide bonds, and ester or amide linkages has been described as well
- In these cases proper conditions should be found to avoid these chemical reactions.

- Tissue plasminogen activator (abbreviated tPA or PLAT) is a protein involved in the breakdown of blood clot.
- As an enzyme, it catalyzes the conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown.
- Because it works on the clotting system, tPA is used in clinical medicine to treat embolic or thrombotic stroke.
- tPA may be manufactured using recombinant biotechnology techniques. tPA created by this way may be referred to as recombinant tissue plasminogen activator (rtPA).

- **Interleukin 2** (**IL-2**) is an interleukin, a type of cytokine signalling molecule in the immune system.
- It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity.

Anti-adsorption and anti-aggregation agents

- Anti-adsorption agents are added to reduce adsorption of the active protein to interfaces.

- Some proteins tend to expose hydrophobic sites, normally present in the core of the native protein structure when an interface is present.

- These 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).

- These adsorbed, partially unfolded protein molecules form aggregates, leave the surface, return to the aqueous phase, form larger aggregates and precipitate.

- As an example, the proposed mechanism for aggregation of insulin in aqueous media through contact with a hydrophobic surface (or water-air interface) is presented in Figure 2.

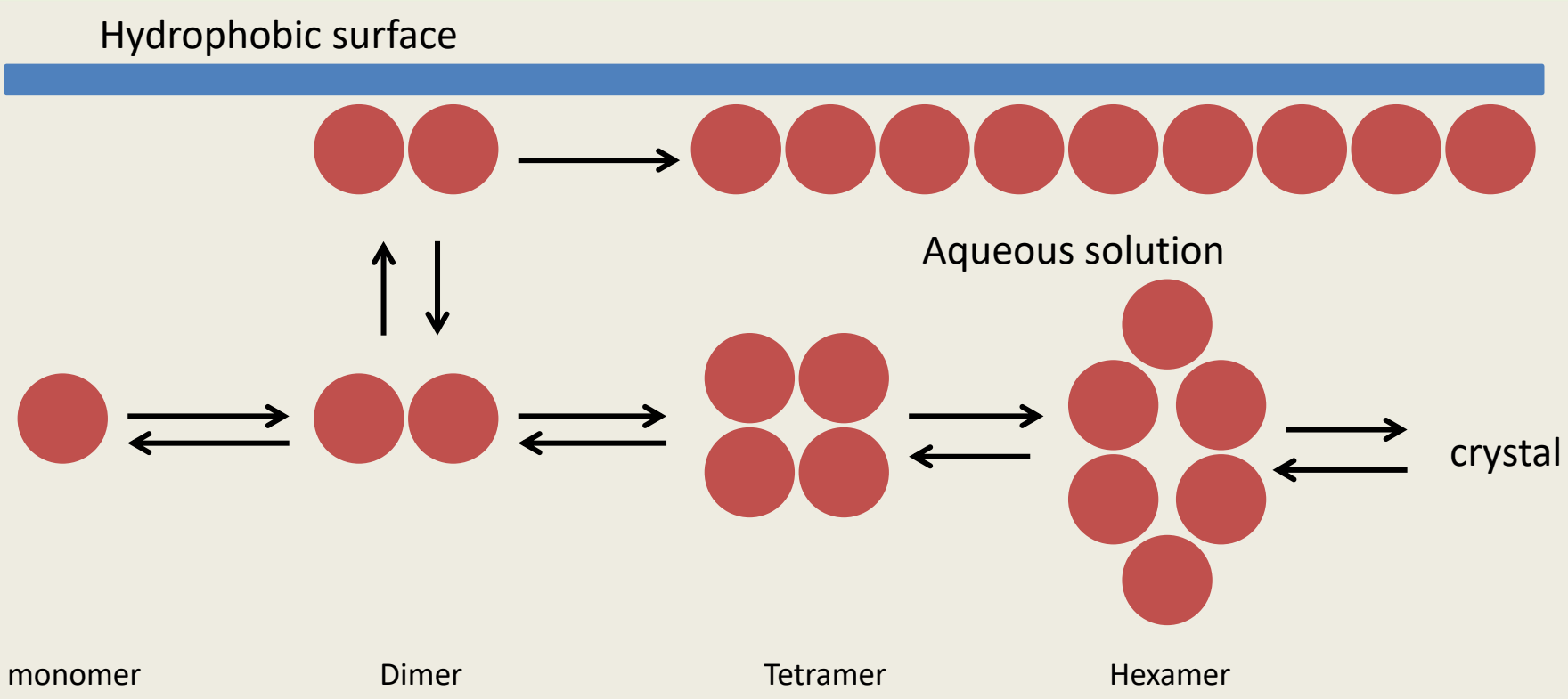


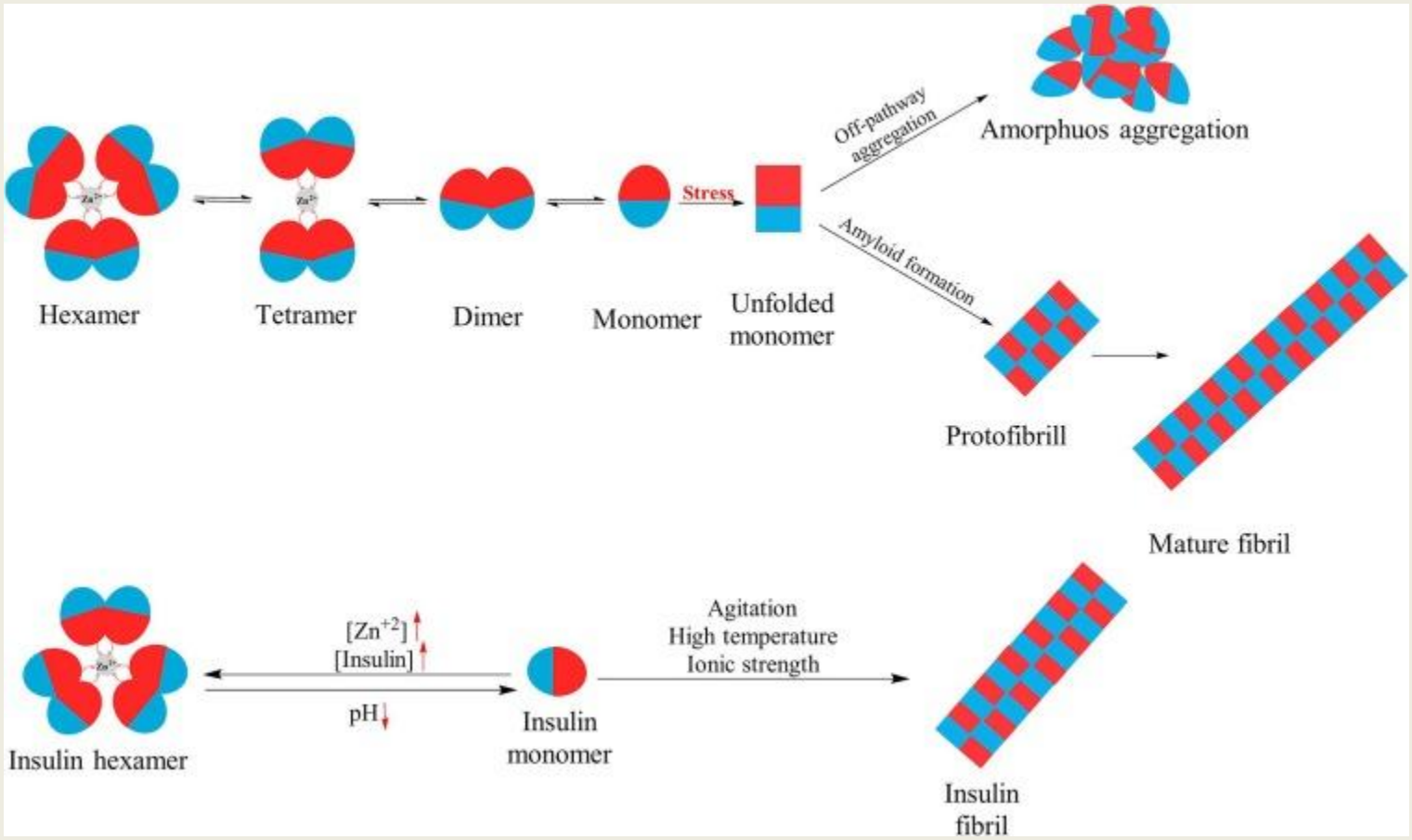
Figure 2 Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film

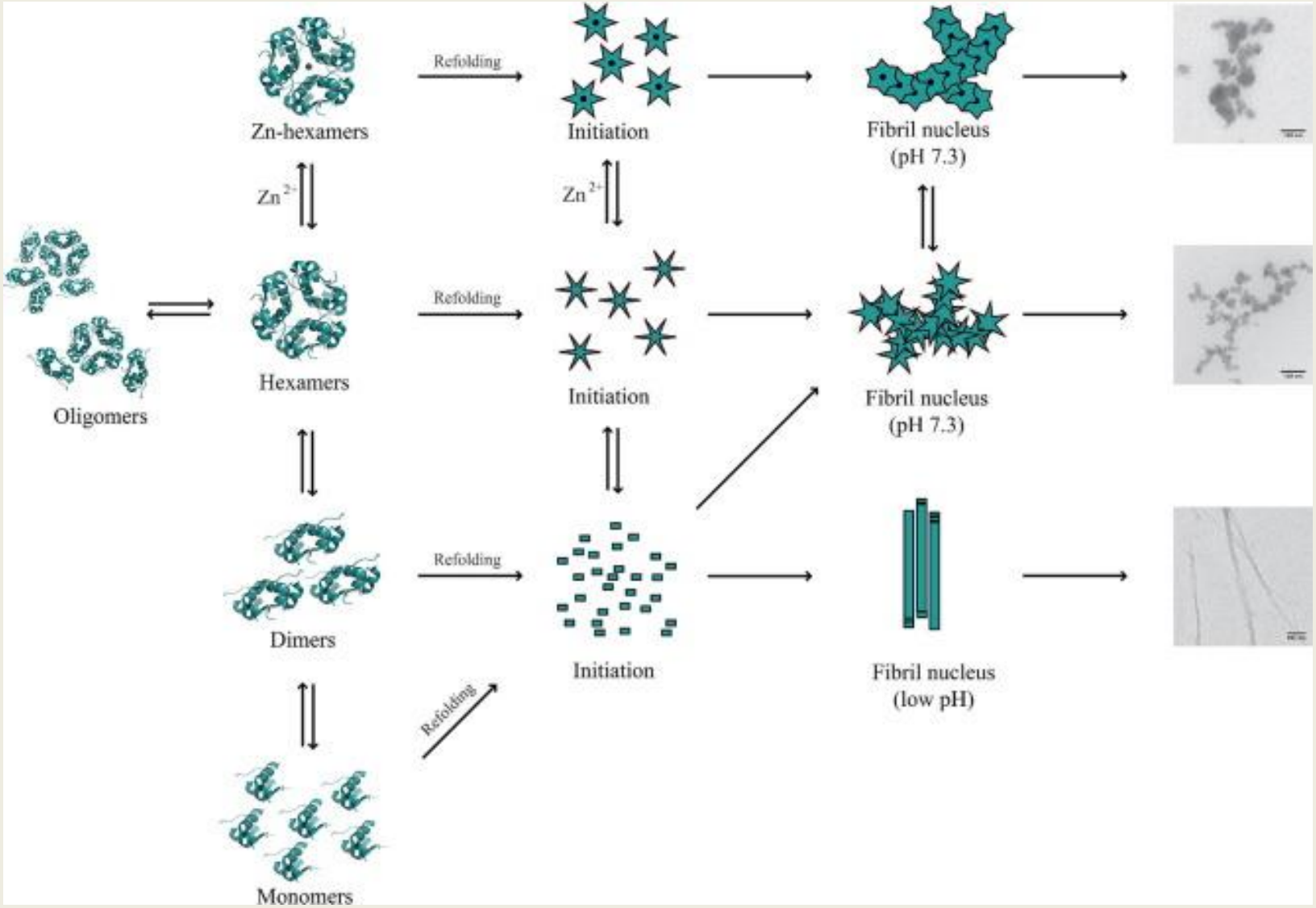
- Native insulin in solution is in an equilibrium state between monomeric, dimeric, tetrameric and hexameric form (cf.Chapter 10).
- The relative abundance of the different aggregation states depends on the pH, insulin concentration, ionic strength and specific excipients (Zn^{2+} and phenol).
- It has been suggested that the dimeric form of insulin adsorbs to hydrophobic interfaces and subsequently forms larger aggregates at the interface.
- This adsorption explains why anti-adhesion agents can also act as anti-aggregation agents.

- Albumin has a strong tendency to adsorb to surfaces and is therefore added in relatively high concentration (e.g. 1%) to protein formulations as an anti-adhesion agent.

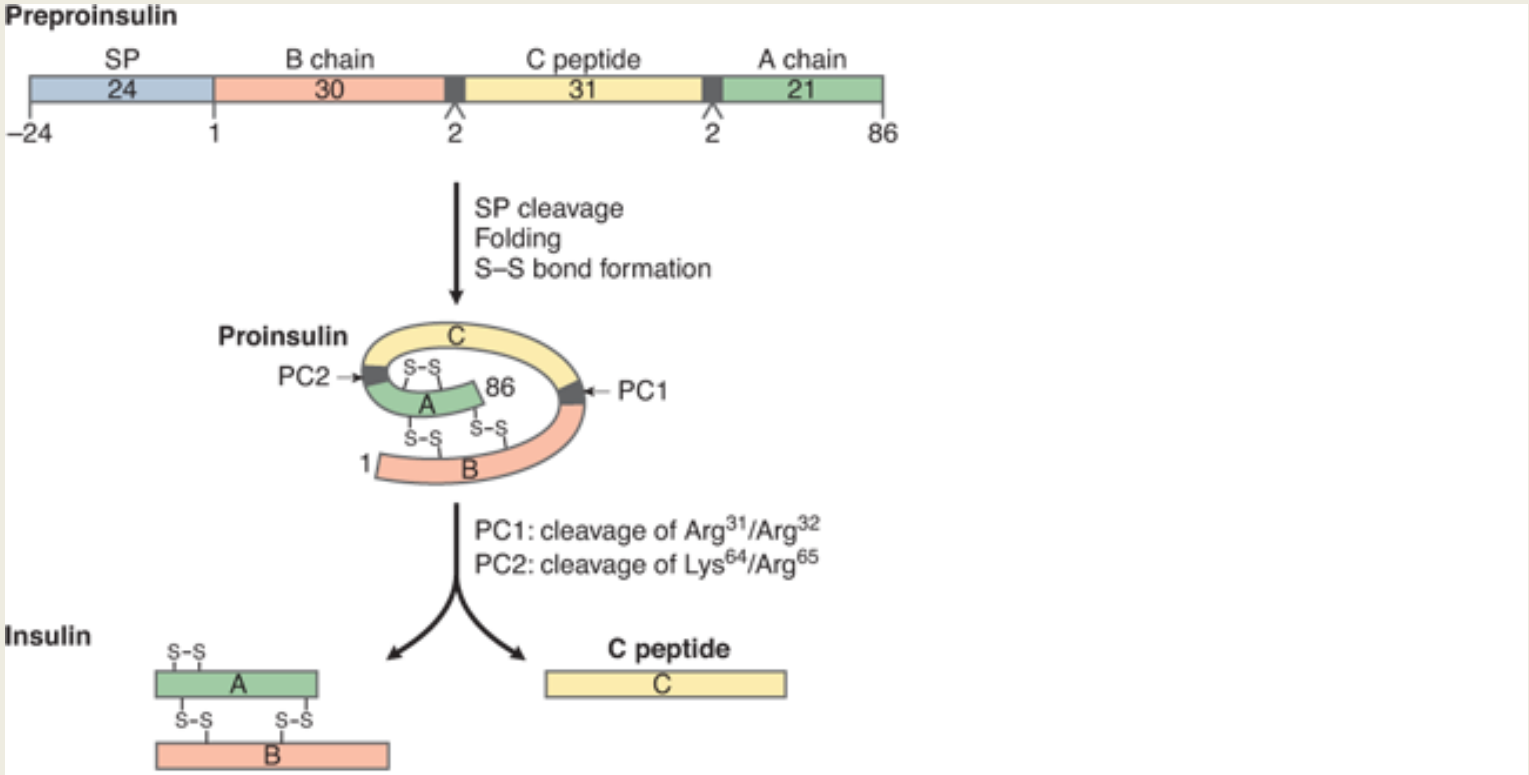
- albumin competes with the therapeutic protein for binding sites and supposedly prevents adhesion of the therapeutically active agent by a combination of its binding tendency and abundant presence.

- Insulin is one of the many proteins that can form fibrillar precipitates (long rod-shaped structures with diameters in the 0.1 µm range).
- Low concentrations of phospholipids and surfactants have been shown to exert a fibrillation-inhibitory effect.
- The selection of the proper pH can also help to prevent this unwanted phenomenon.


Hexamer
Tetramer
Dimer
Monomer
Stress
Unfolded monomer
Off-pathway aggregation
Amorphuos aggregation
Amyloid formation
Protofibrill
Mature fibril
Insulin hexamer
[Zn+2]
[Insulin]
pH
Insulin monomer
Agitation
High temperature
Ionic strength
Insulin fibril

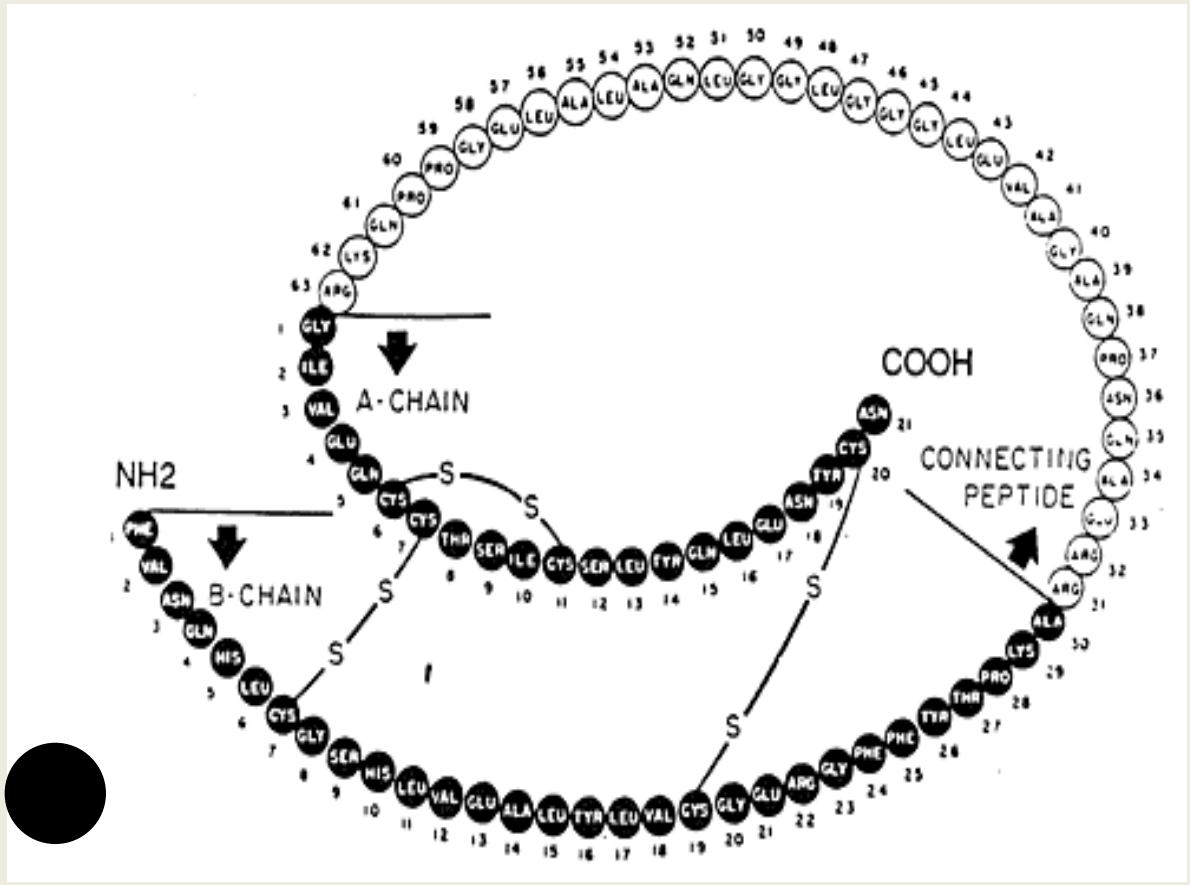

Zn-hexamers
Refolding
Initiation
Fibril nucleus
(pH 7.3)
Zn 2+
Zn 2+
Oligomers
Hexamers
Refolding
Initiation
Fibril nucleus
(pH 7.3)
Dimers
Refolding
Initiation
Fibril nucleus
(low pH)
Refolding
Monomers

- Apart from albumin, surfactants can also prevent adhesion to interfaces and precipitation.
- These molecules readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups phase.



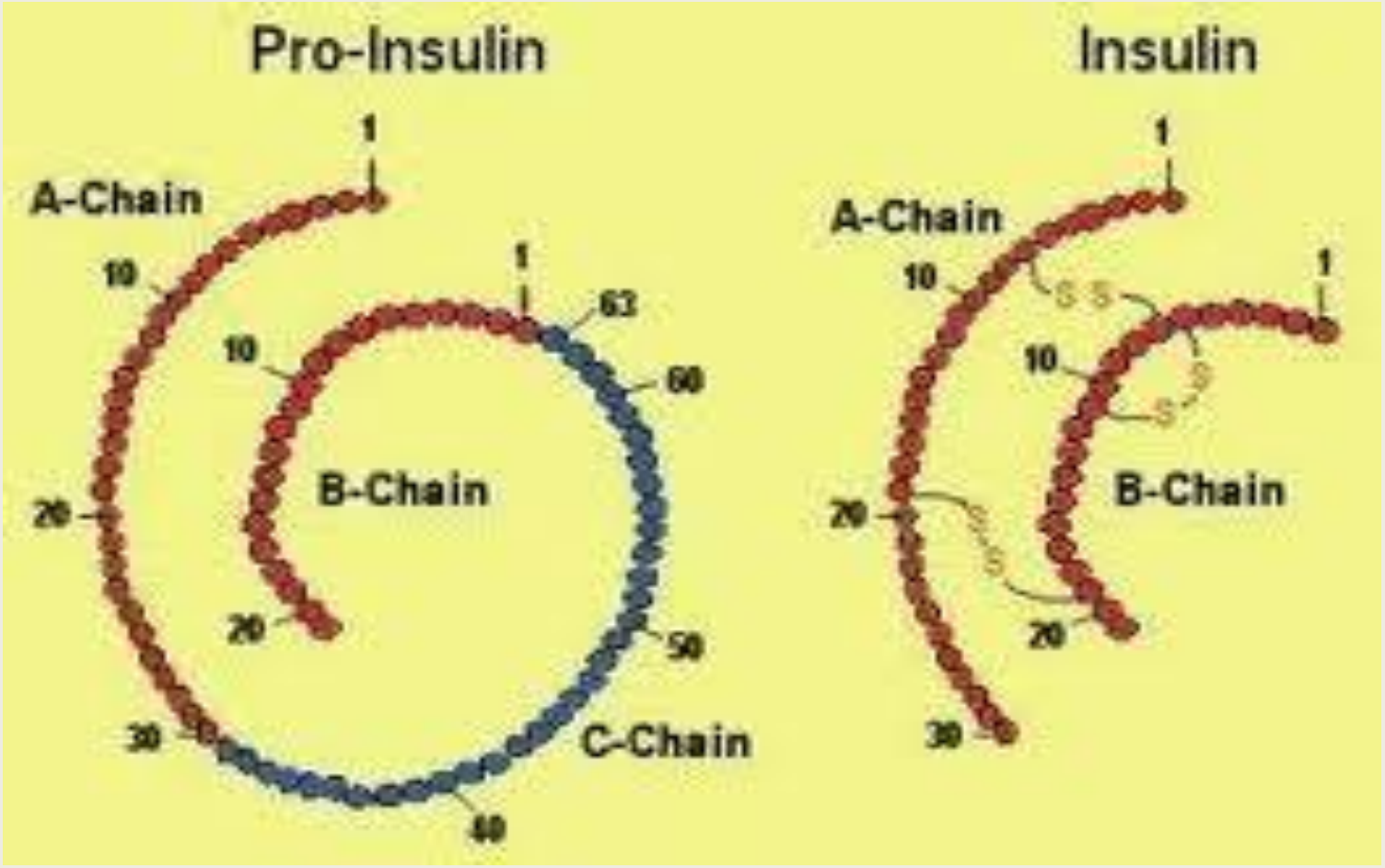
Source: L. L. Brunton, B. A. Chabner, B. C. Knollmann: Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12ed.
www.accesspharmacy.com

Insulin structure



Amino acids

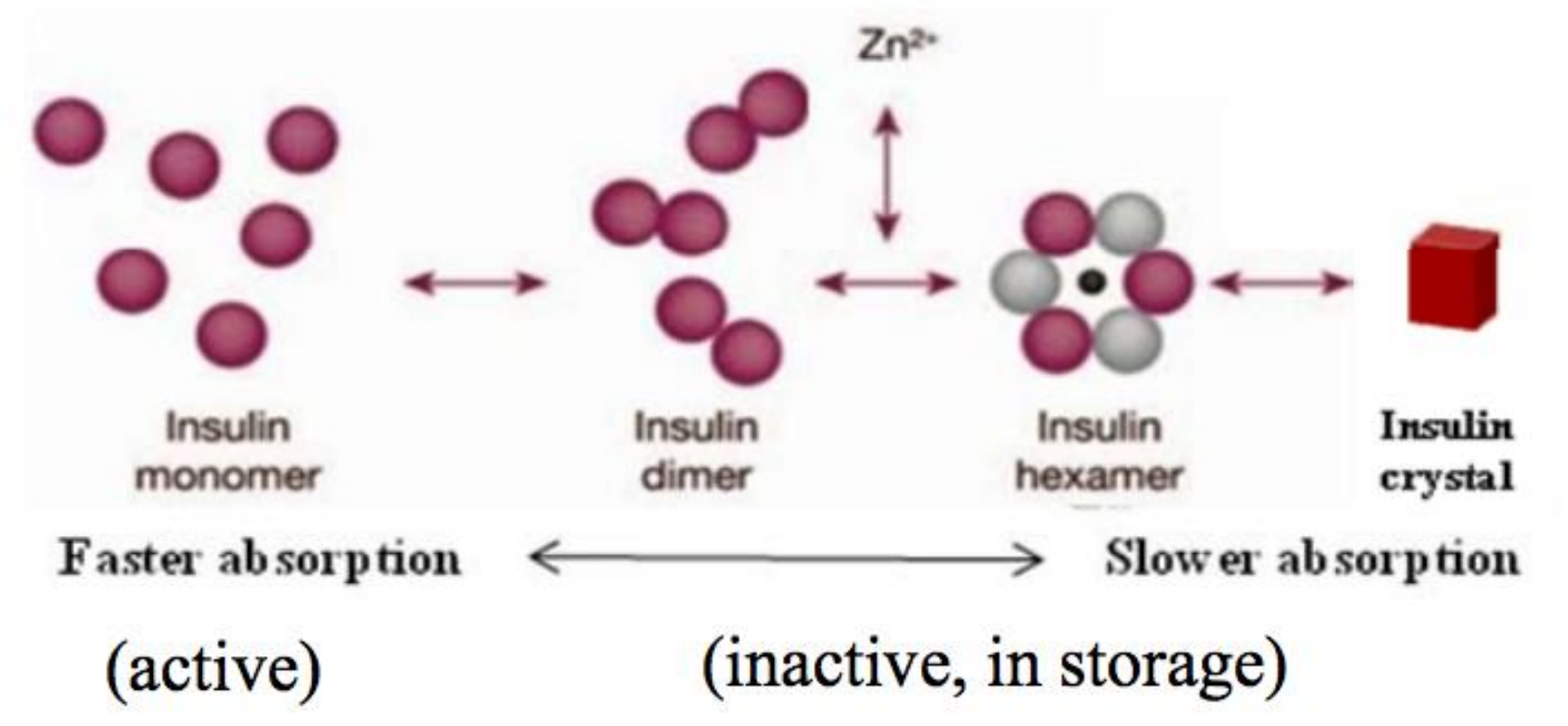
http://www.endotext.org/diabetes/diabetes1/diabetes1.html



The human insulin protein is composed of 51 amino acids , and has a molecular mass of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by disulfide bonds.

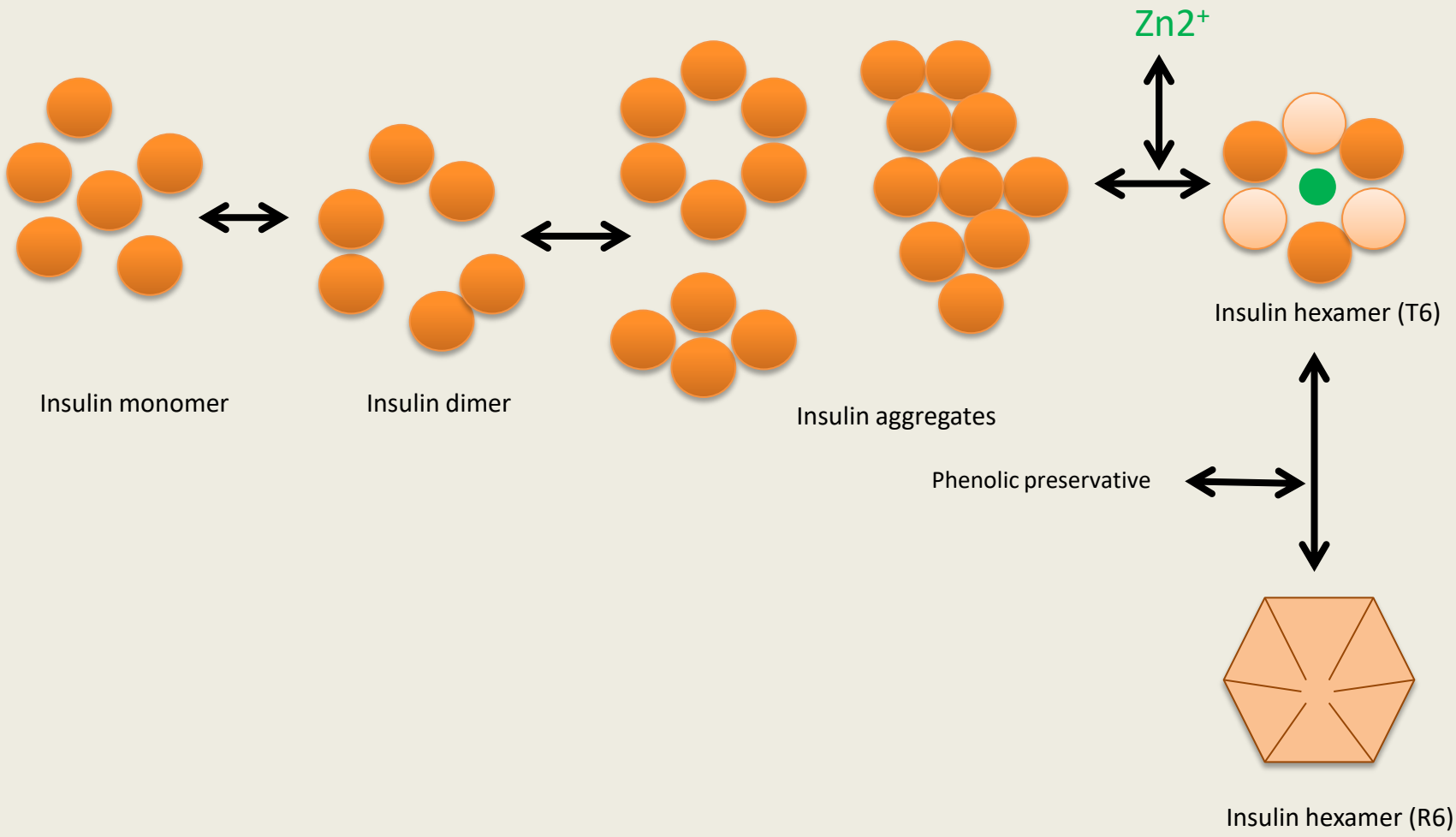
- Insulin has as isoelectric point (PI) of 5.3 in the denatured state; thus, the insulin molecule is negatively charged at neutral pH.
- This net negative charge-state of insulin has been used in formulation development.
- In addition to the net charge on insulin, another important intrinsic property of the molecule is its ability to readily associate into diamer and higher order state.
- The deriving force for dimerization appears to be the formation of favorable hydrophobic interactions at the C-terminus of the B-chain

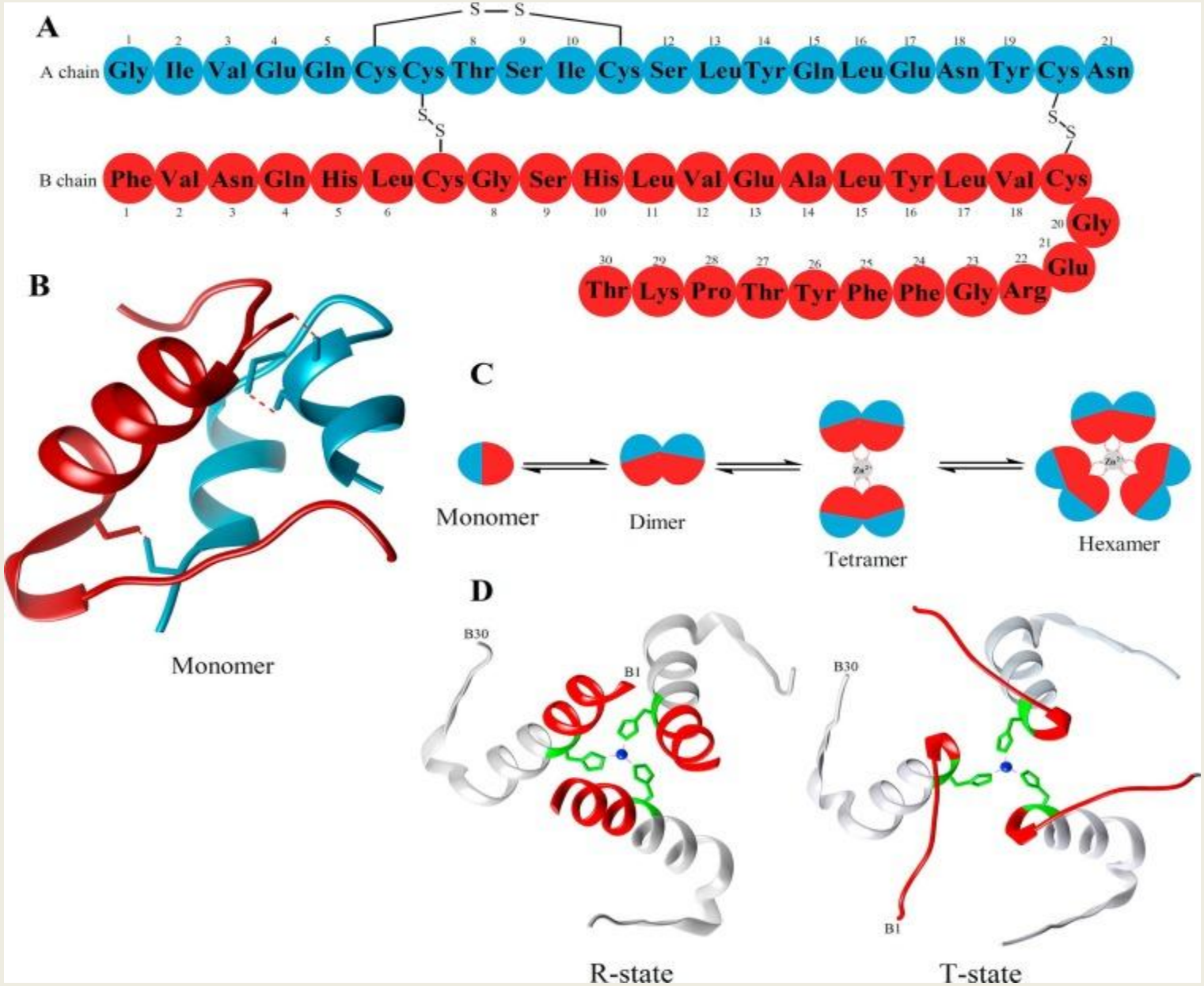
- Insulin can associate into discrete hexameric complexes in the presence of various divalent metal ions, such as zinc at 0.33 g-atom/ monomer, where each zinc ion (a total of two) is coordinated by His^{B10} residue from three monomers.
- The ability to form discrete hexamers in the presence of zinc has been used to develop therapeutically useful formulation of insulin.


Zn2+
Insulin monomer
Insulin dimer
Insulin hexamer
Insulin crystal
Faster absorption
Slower absorption
(active)
(inactive, in storage)

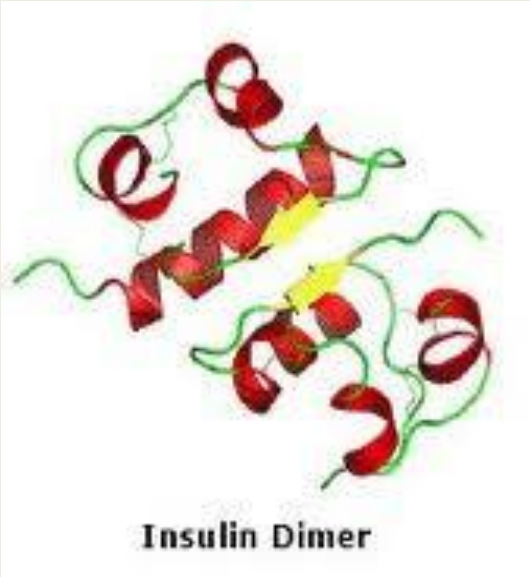
- Commercial insulin preparations also contain phenolic excipients (e.g., phenol, m-cresol, or methyl-paraben) as anti microbial agents.
- These phenolic species also bind to specific sites on insulin hexamers, causing a conformationl change that increases the chemical stability of insulin in commercial preparations. (This reduce high-molecular-weight polymer formation)

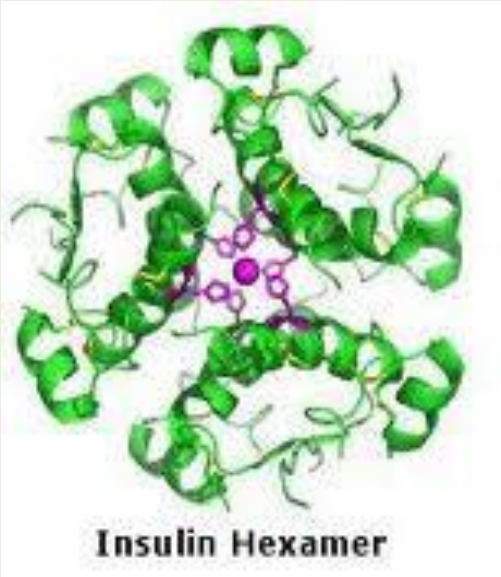
Schematic representation of insulin association in presence of zinc and phenolic antimicrobial preservatives



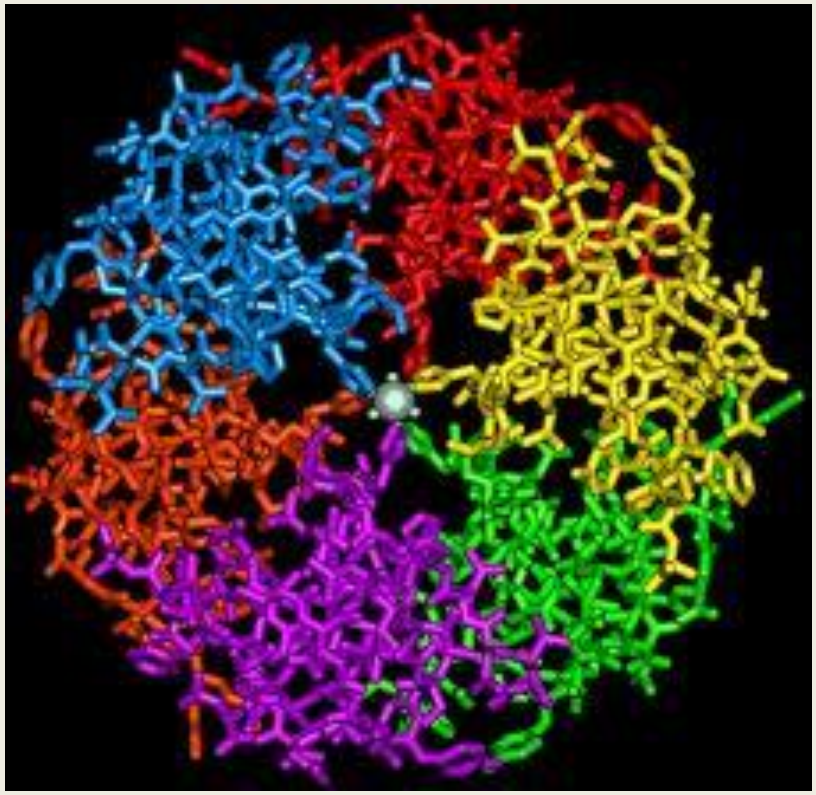

A
S — S
A chain
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
B chain
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu
Thr Lys Pro Thr Tyr Phe Phe Gly Arg
B
Monomer
C
Monomer
Dimer
Tetramer
Hexamer
D
B30
B1
R-state
T-state

T-state dimer and hexamer





Schematic representation of insulin association in presence of zinc and phenolic antimicrobial preservatives



- In addition to the presence of zinc and phenolic preservatives, modern insulin formulation may contain an isotonicty agent (glycerol or NaCl) and/or a physiologic buffer (sodium phosphate).
- The former is used to minimize the subcutaneous tissue damage and pine on injection.
- The latter is present to minimize pH drift in some pH-sensitive formulations.

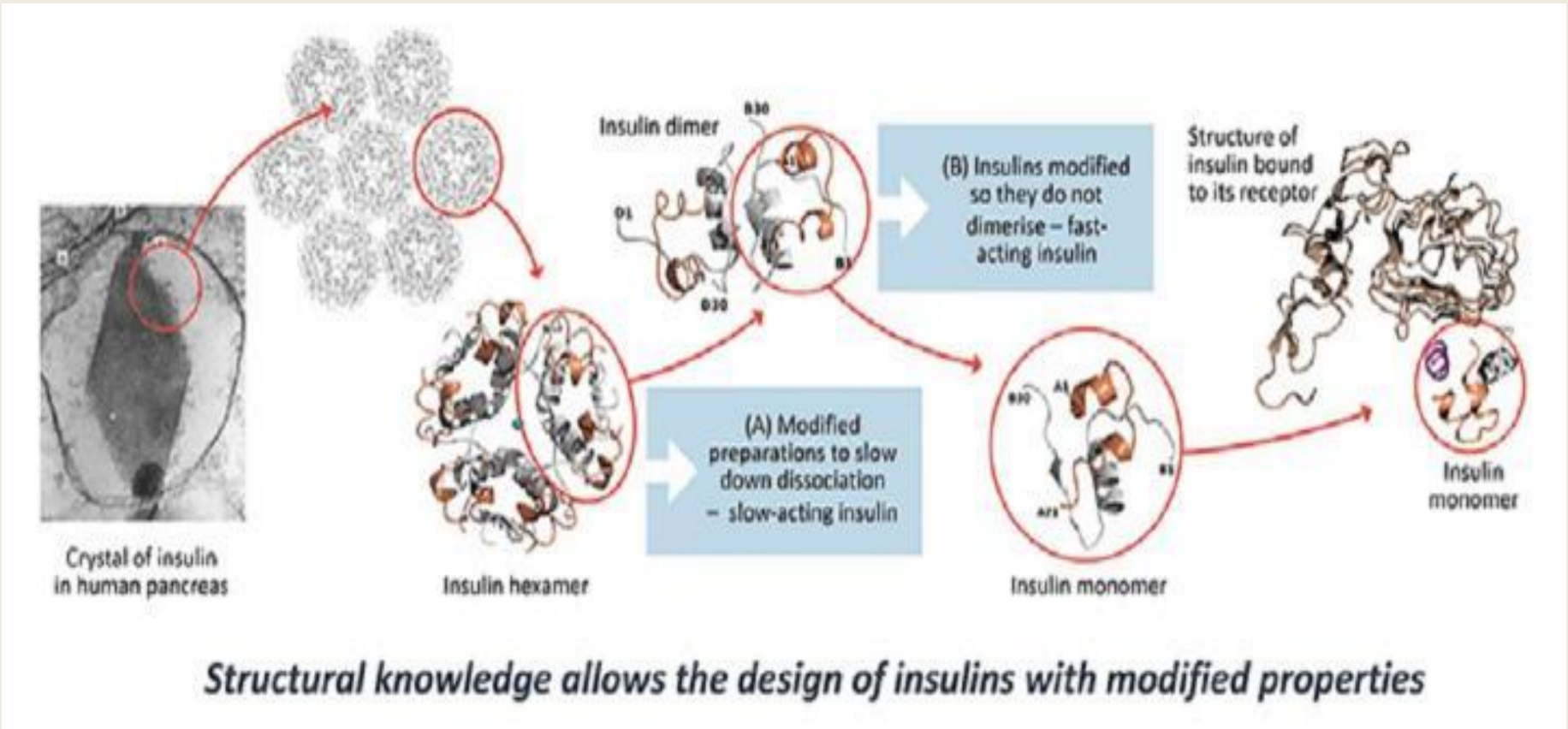
Short and long-acting insulins

Naturally occurring insulin is stored in the pancreas in crystals which are made up of three linked pairs of insulin molecules connected to zinc ions.

The response to insulin depends on how fast these aggregates break up into individual insulin molecules in the blood. From 1984 to 2000, YSBL engaged in a major collaboration on recombinant, novel modified insulins with Novo Nordisk A/S (www.novonordisk.com).

The structures of these insulins provided a detailed understanding of the nature of insulin aggregation.

In this way, one type of insulin was discovered that does not aggregate while another was found in which the aggregates are particularly stable. In combination, these provide the diabetic with both fast-acting and slow-acting insulin.



For further information on our research in this area please see the [York Structural Biology Laboratory] page.