Non-Covalent Mechanisms of Protein Instability

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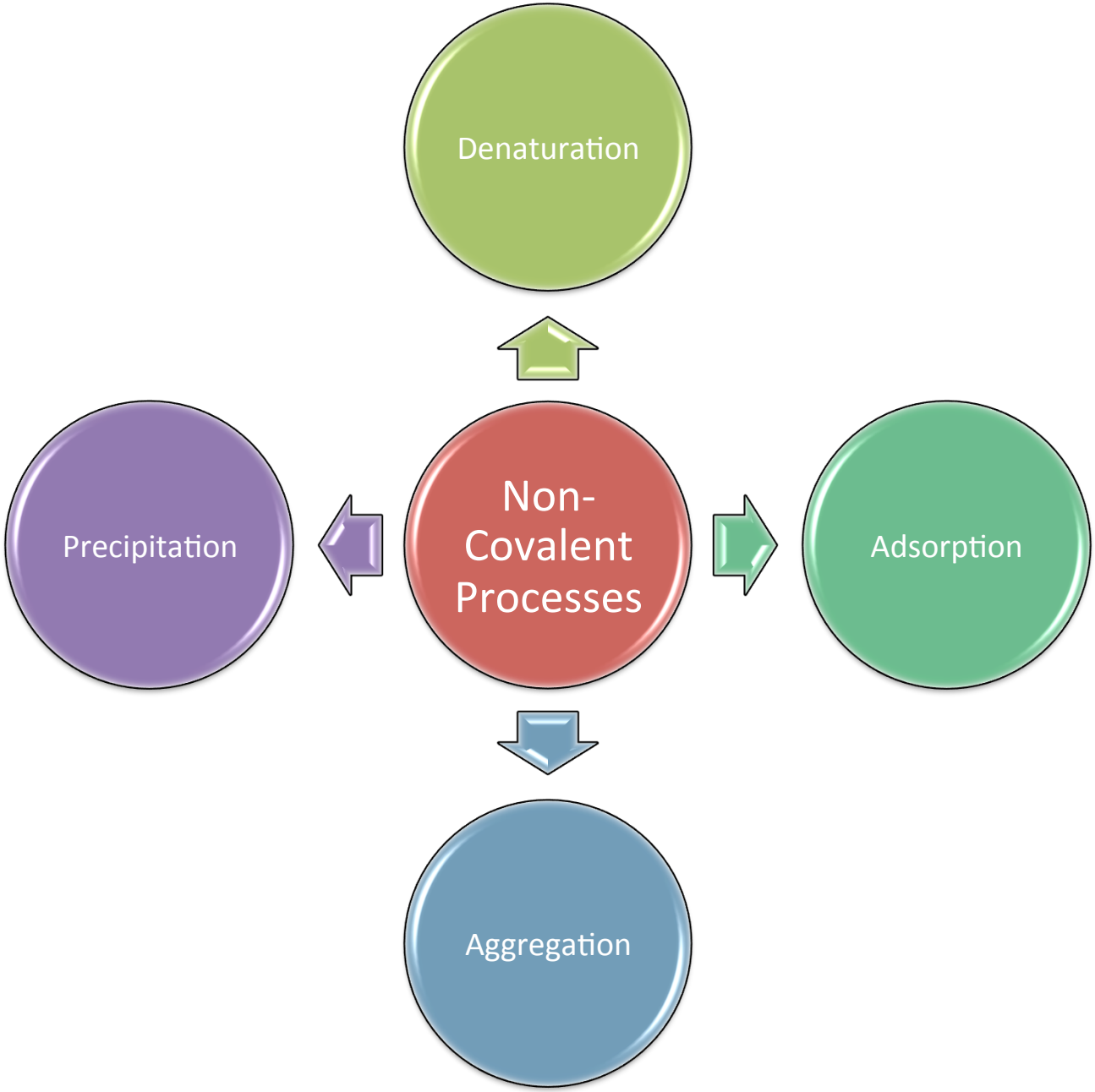
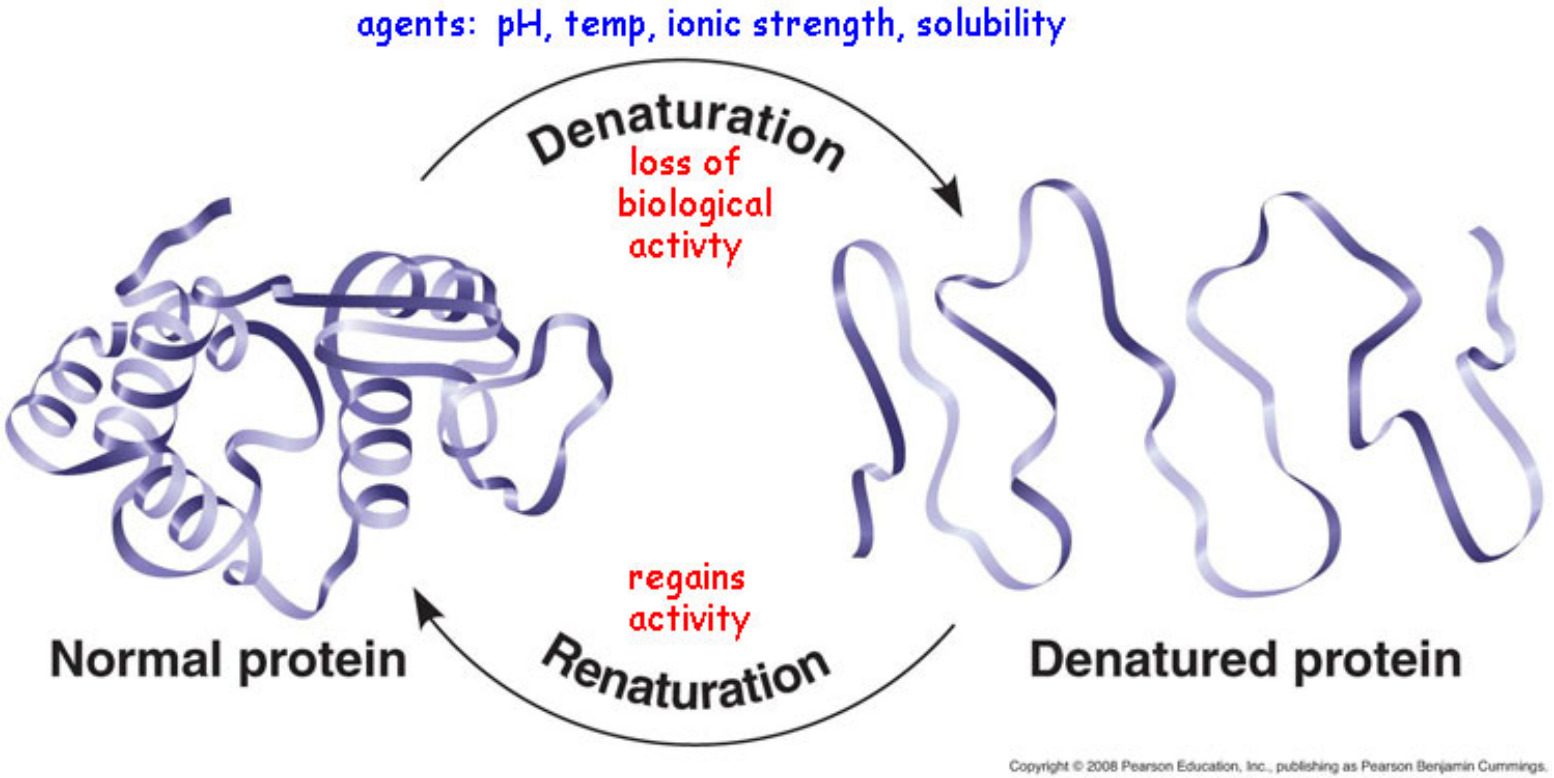
Objectives of this lecture

By the end of this lecture you will be able to:

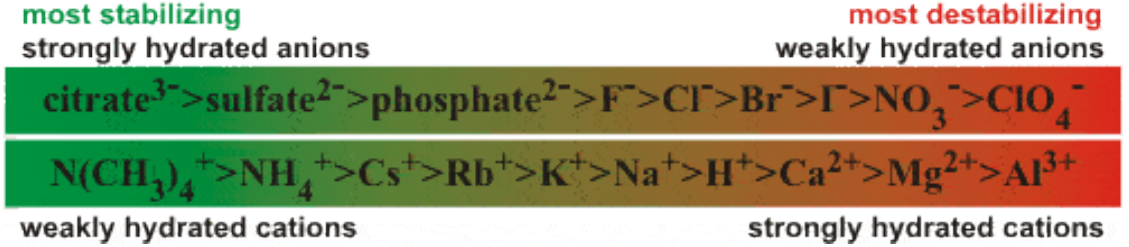
1. Describe the challenges in pharmaceutical proteins production
2. Distinguish between the different mechanisms of protein instability
3. Predict the mechanism of degradation from peptide primary structure

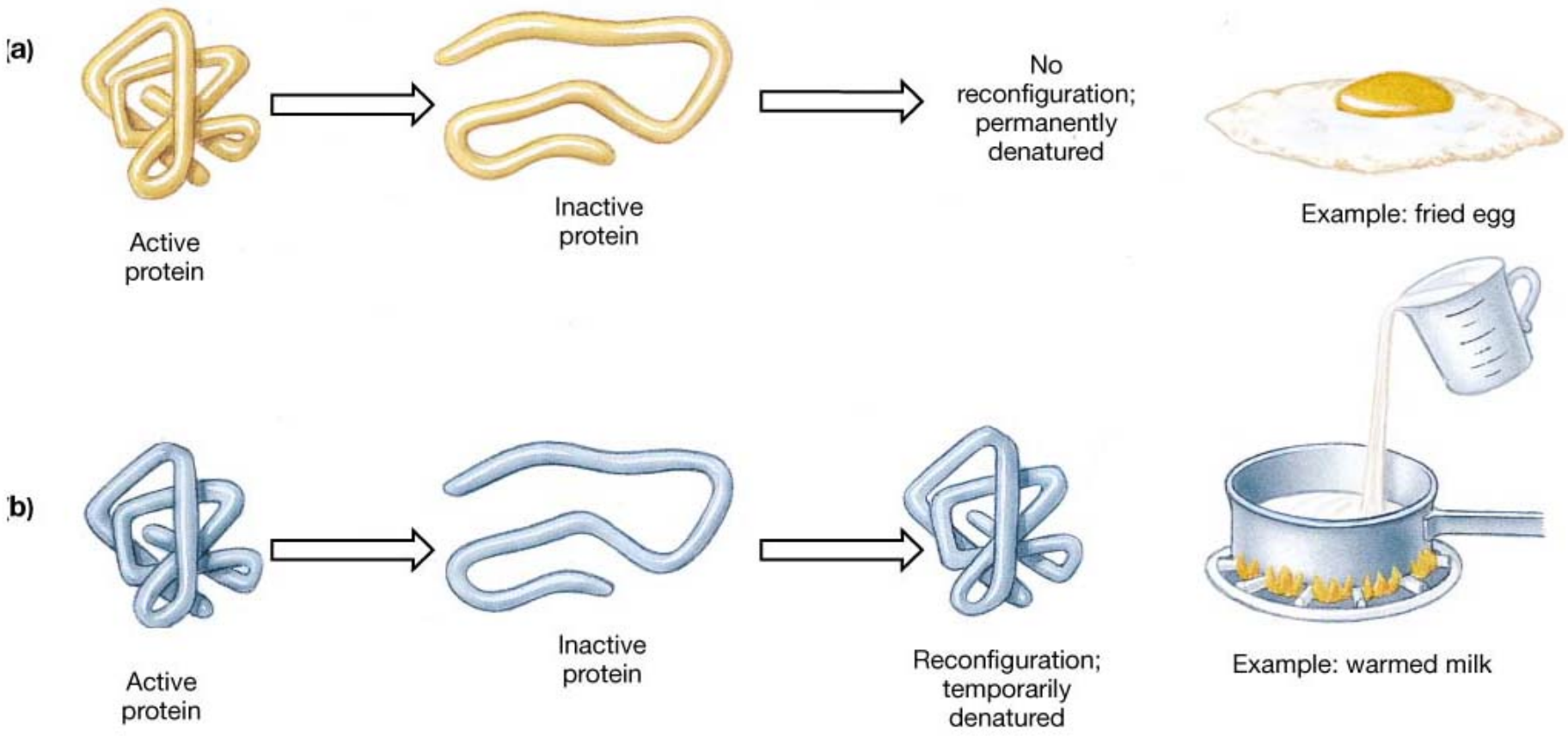

Denaturation
Precipitation
Non-Covalent Processes
Adsorption
Aggregation

Denaturation (unfolding)



- Hi or low pH (>10 , <3)
- Hi or low temp (>60°C , <0°C)
- Organic solvents and Urea
- Salts (Hofmeister Series)
- Interaction with surface (also in adsorption)





- Proteins are in equilibrium between two states: native and unfolded
- Usually reversible process
- Hydrophobic a.a. hide in the core
- Hydrophilic a.a. form a coat

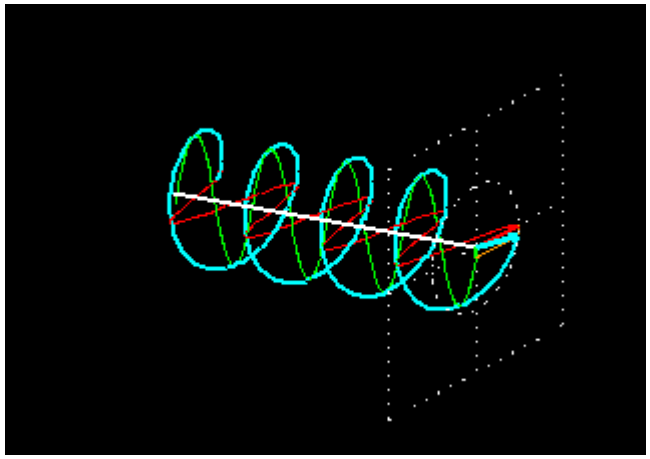
How to study denaturation?

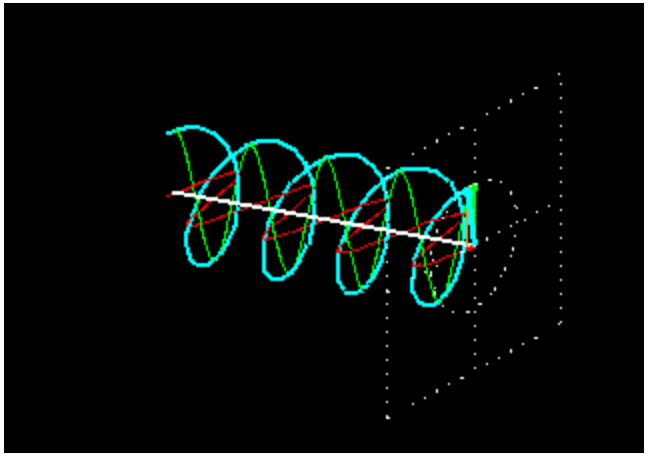
- Denaturation occurs both *in vitro* and *in vivo*

1. Circular dichroism
2. Fluorescence spectroscopy
3. NMR spectroscopy

Circular dichroism (CD)

- Part of UV absorption spectroscopy
- Determines secondary structures of proteins
- Excellent to study changes in protein conformation

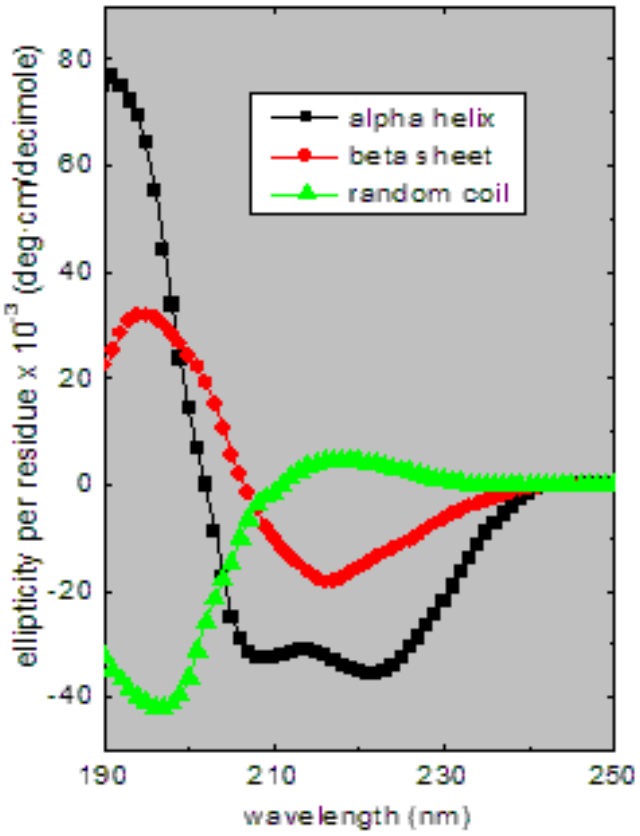




http://www.enzim.hu/~szia/cddemo/edemo0.htm

Circular dichroism

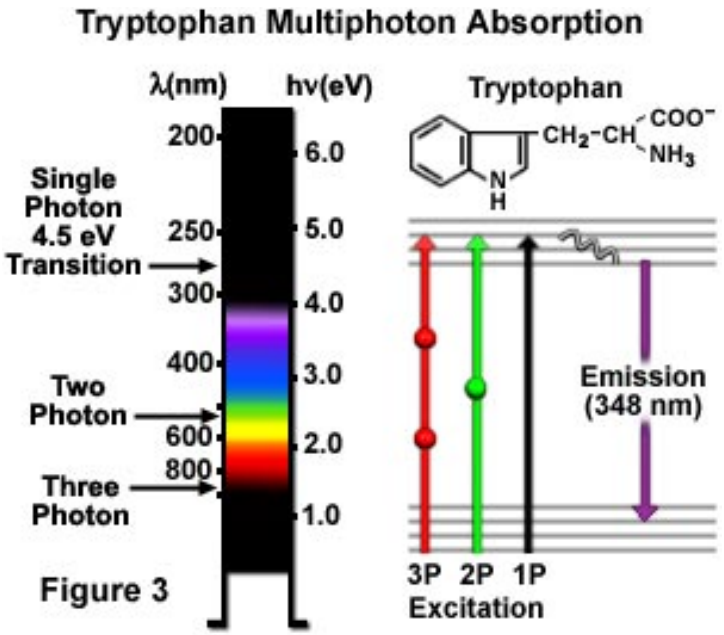
- CD spectrum reflects the structure of:
  - α-helices
  - β-sheets
  - Random coils



http://www.ap-lab.com/circular_dichroism.htm

Fluorescence spectroscopy

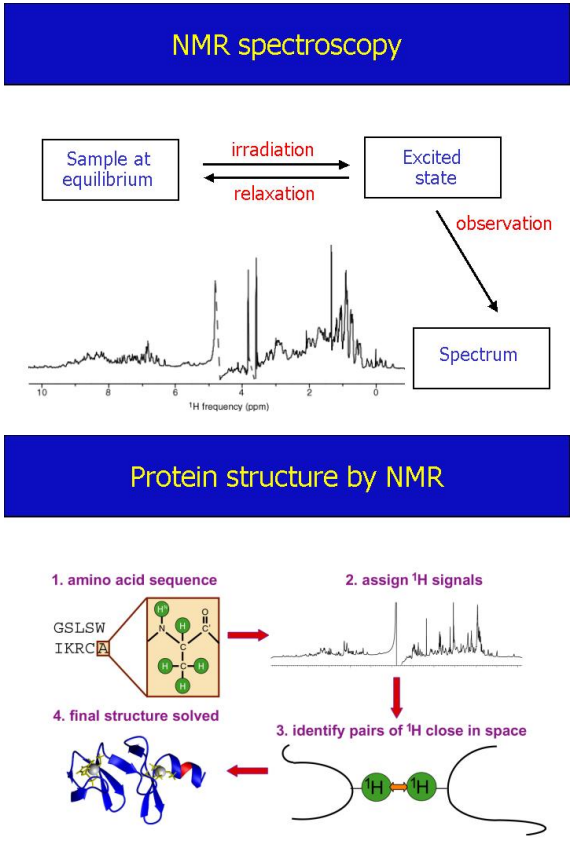
- Emissions spectroscopy
- Monitors quenching of tryptophan
- Denaturation changes the conformation of proteins leading spatial rearrangement of a.a.
- Trp exposure to electromagnetic beam is changed i.e. change in emission detected



http://micro.magnet.fsu.edu/primer/techniques/fluorescence

NMR spectroscopy

- Gives signals in response to proton spinning
- Each proton in a spatial conformation gives different spin i.e. different signal
- Upon exposure to magnetic field , protons spinning is changed to give different peaks
- Each peak represent a single proton which could be identified
- 2D and 3D NMR is available



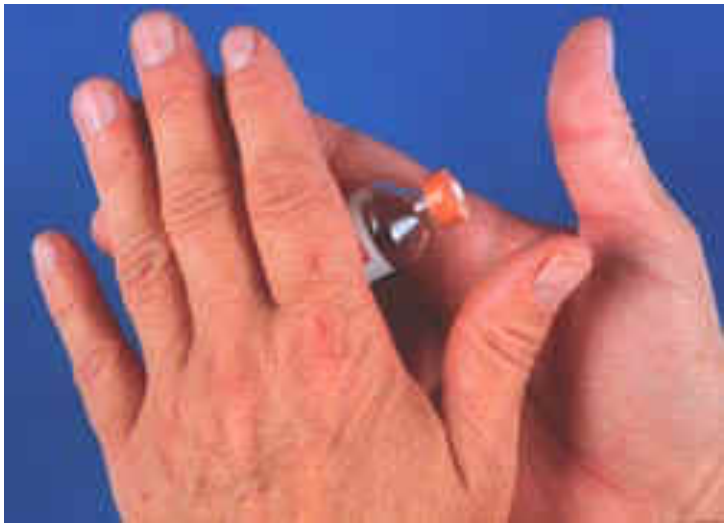
http://www.science.org.au/sats2004/mackay.htm

Aggregation

- Partially unfolded proteins tends to accumulate
- Aggregated forms are more stable thermodynamically
- Aggregates might remain soluble or precipitate
- Adsorption enhances aggregation phenomenon
- Aggregation occurs *in vitro* and *in vivo*

Aggregation

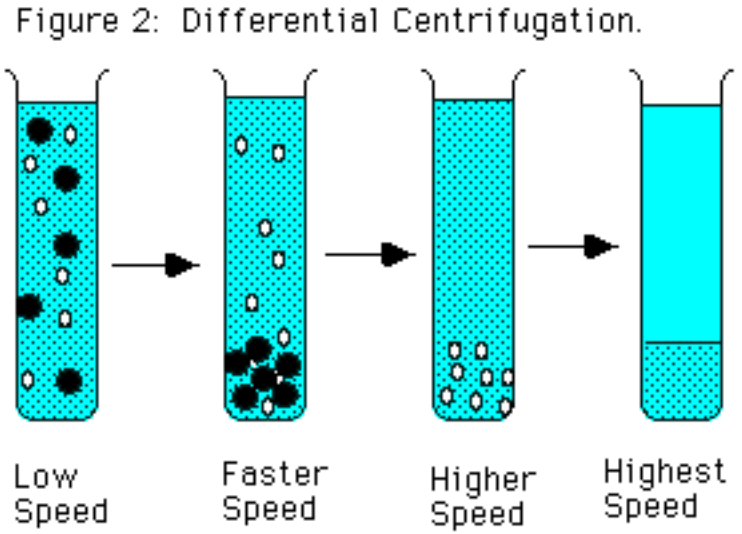
- Physical interactions:
  - Hydrophobic interaction
- Chemical interaction:
  - Disulfide bond formation
- Mechanical agitation
  - Strong shaking



http://www.vetmed.wsu.edu

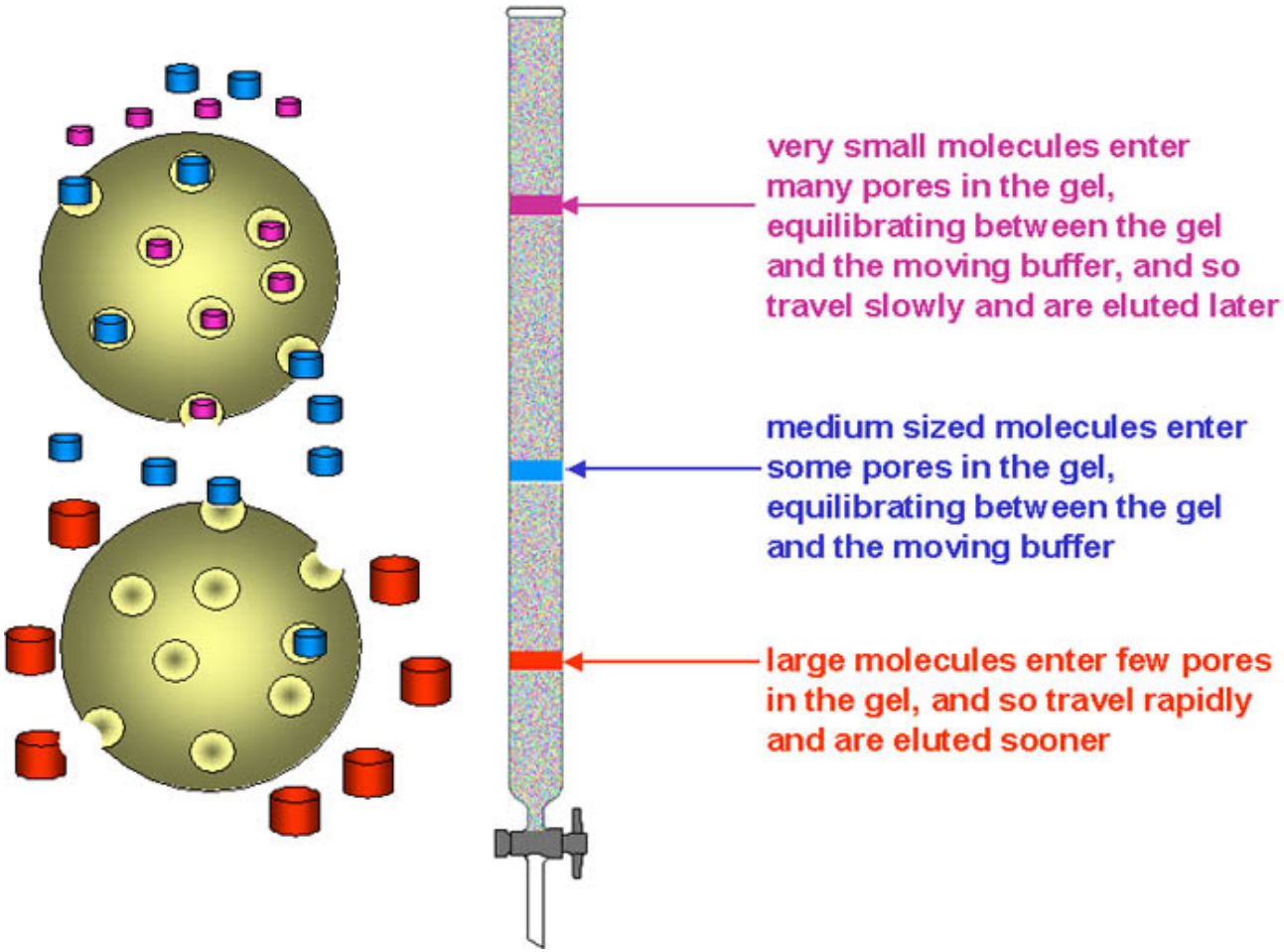
Aggregation

Centrifugation

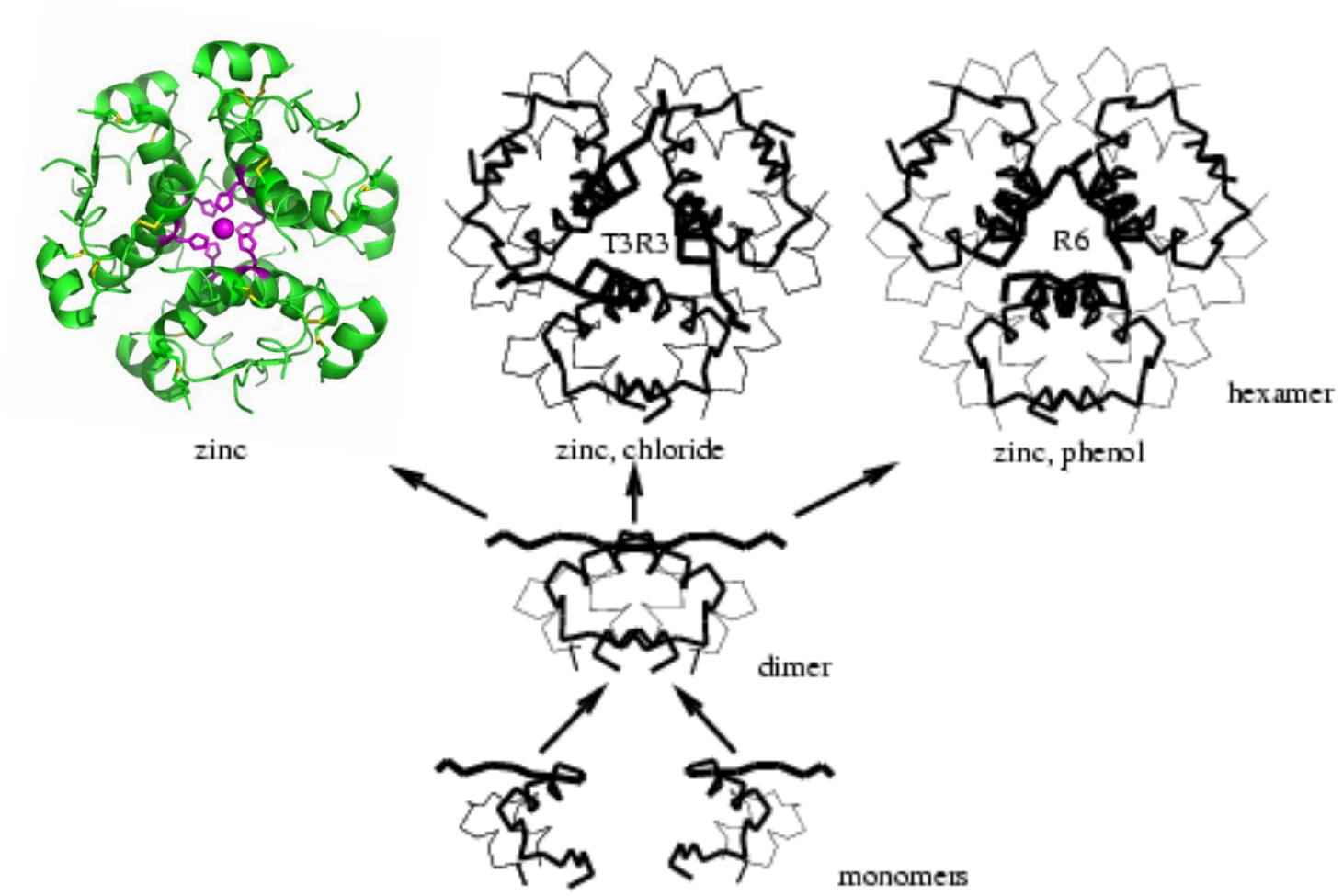


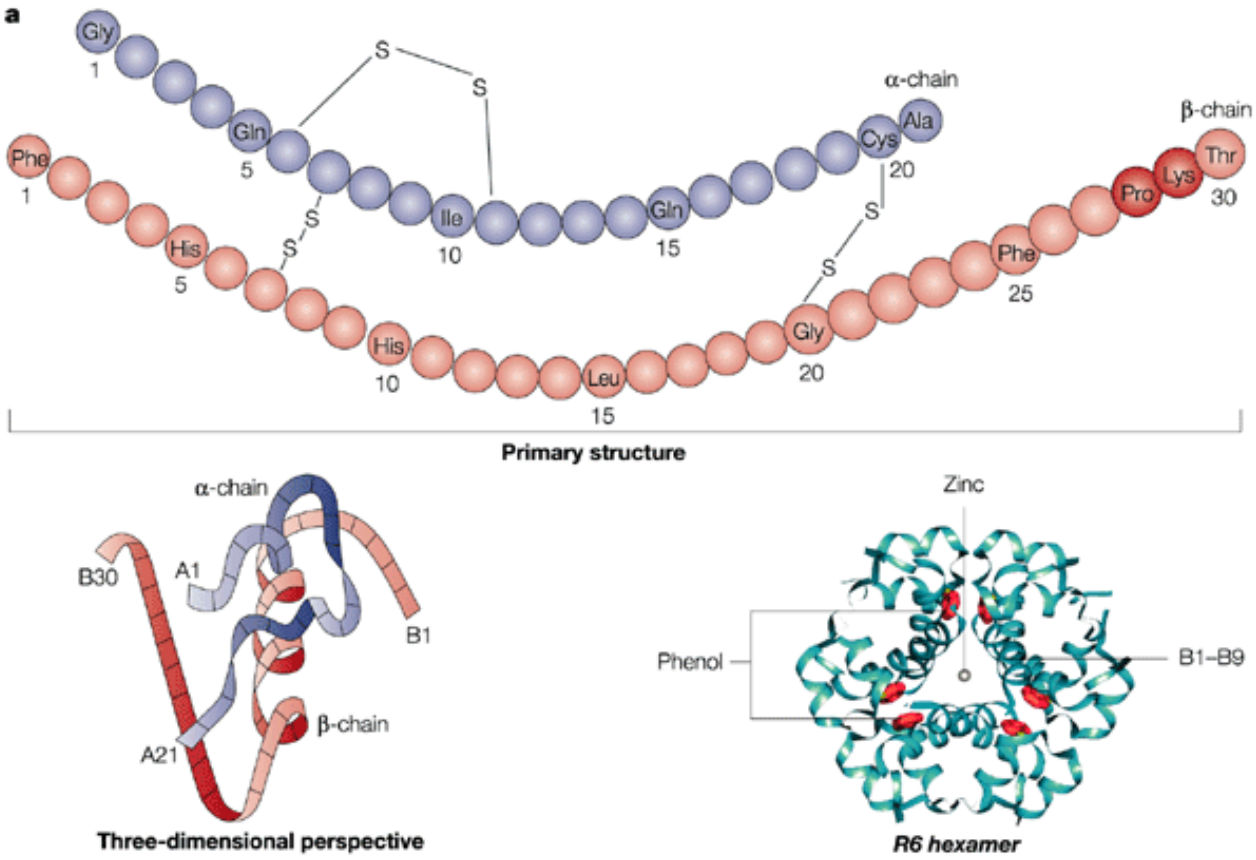
Aggregation

Size Exclusion Chromatography

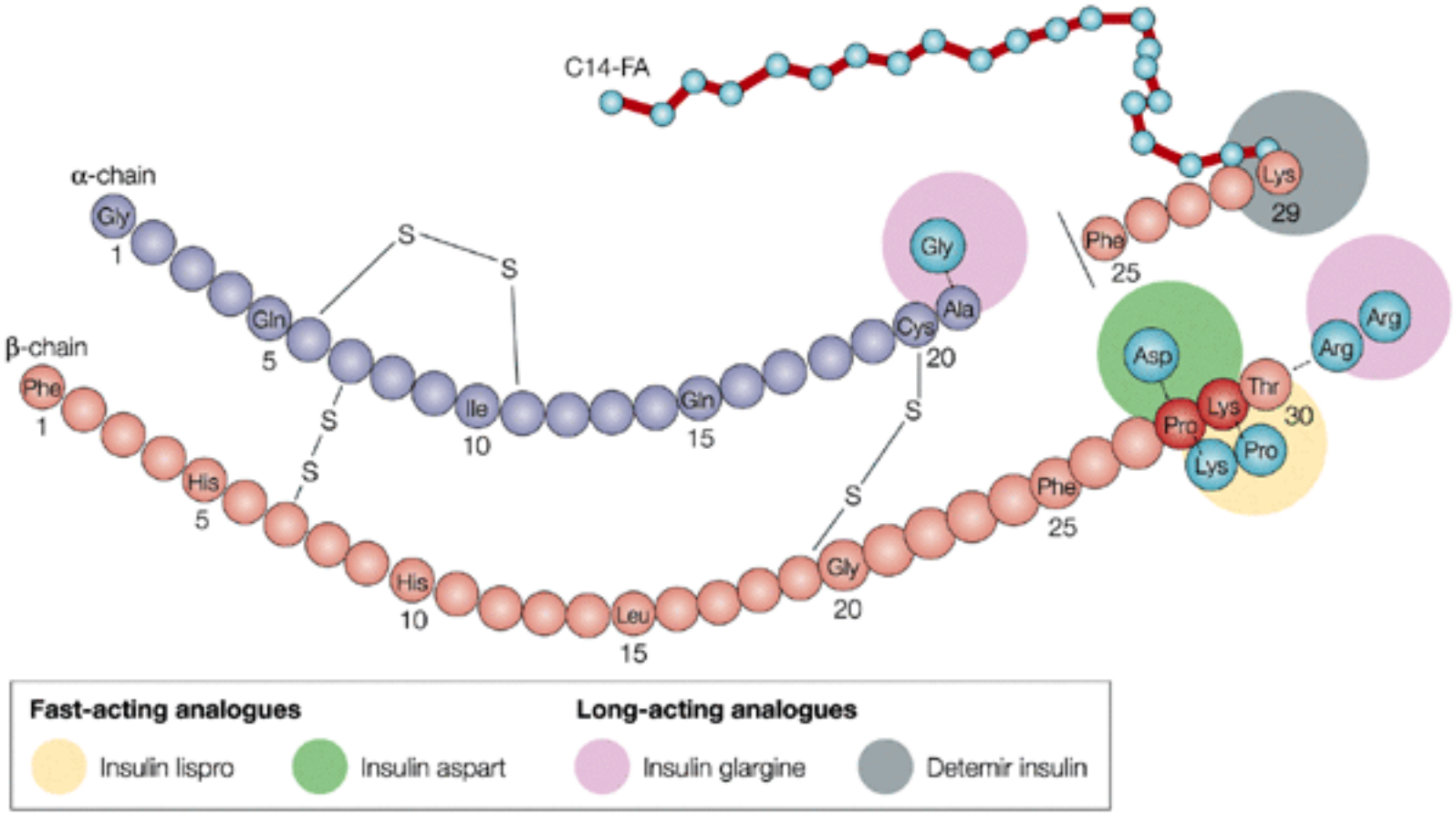


Insulin Aggregates



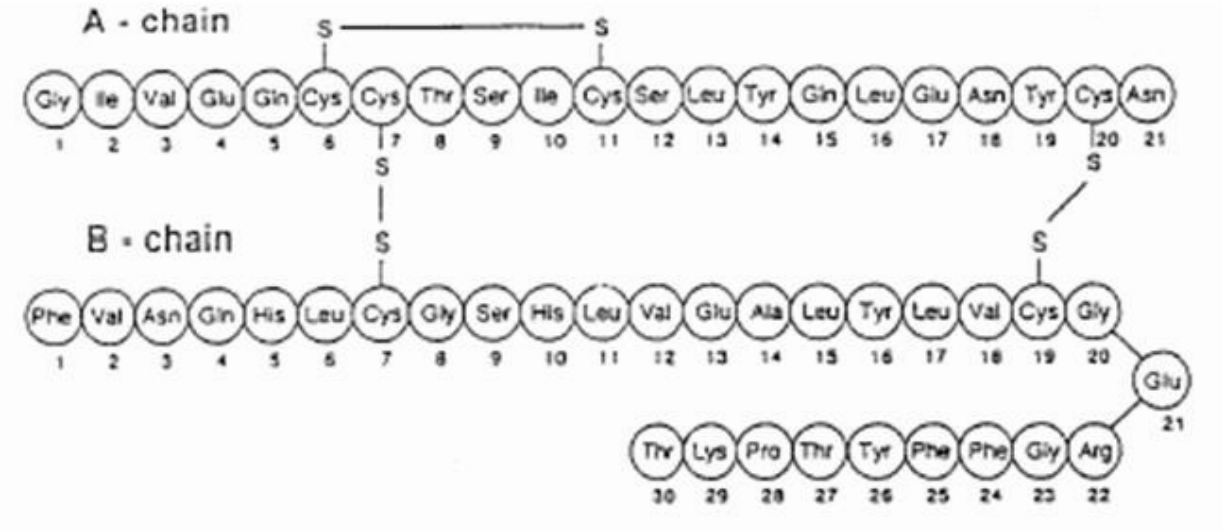

a
Gly
1
Gln
5
S
S
α-chain
Ala
Cys
20
β-chain
Thr
Lys
Pro
30
Phe
1
His
5
S
S
Ile
10
Gln
15
S
S
Phe
25
His
10
Leu
15
Gly
20
Primary structure
α-chain
B30
A1
B1
β-chain
A21
Three-dimensional perspective
Zinc
Phenol
B1–B9
R6 hexamer

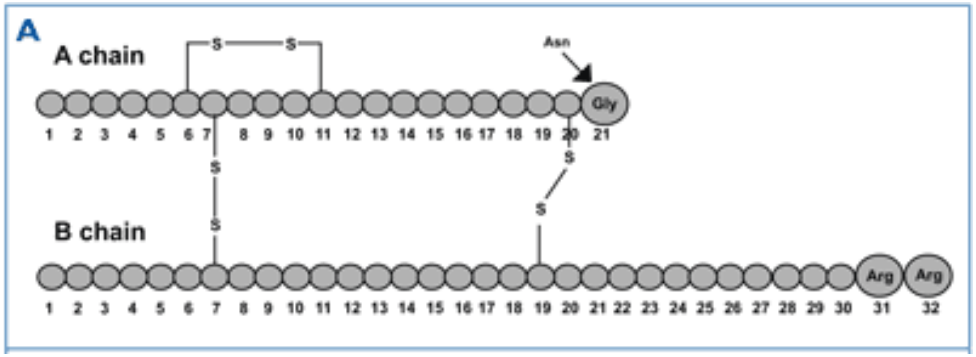
Types of Insulin

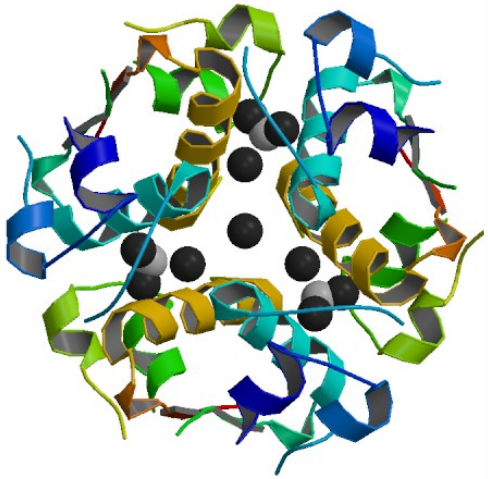


Nature Reviews | Drug Discovery

Insulin Glargine







Insulin Aggregation

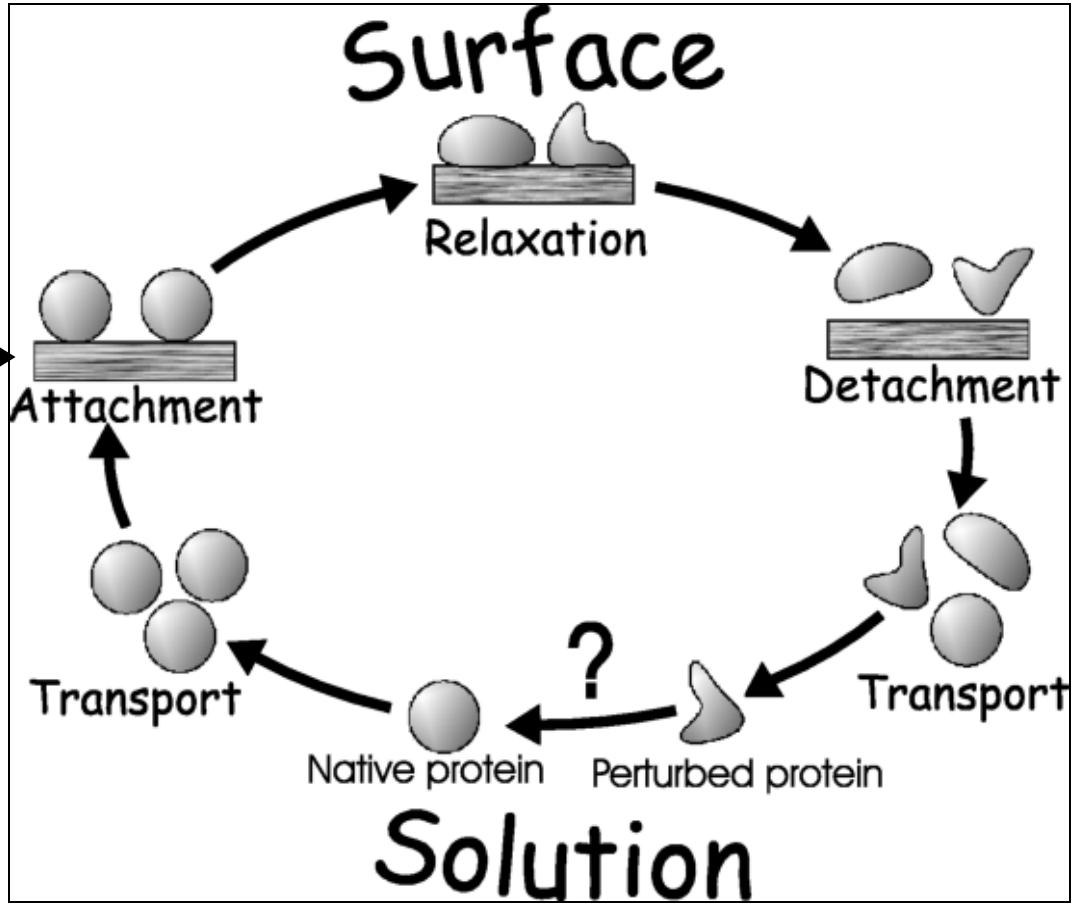
- The tendency of insulin to form aggregates causes problems in the diabetic patient, because of the slow release of insulin from the injection site.
- Genetically engineered insulins are therefore being produced which are not aggregated after injection and therefore quickly released into the blood stream.

Precipitation

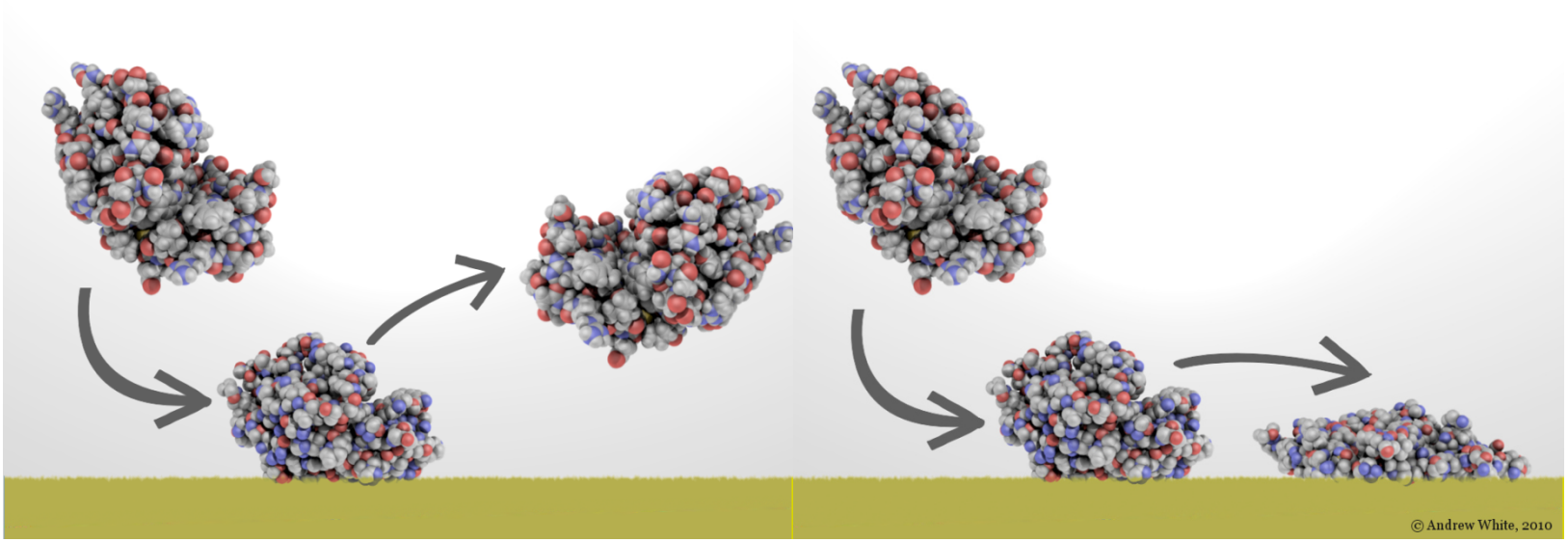
- Consequence of aggregation
- Large aggregates are no more soluble
- Might occur along with denaturation
- Adsorption could induce precipitation
- Stabilizing salts, pH, protein concentration, zinc …
- Detected by "Turbidity Measurment" i.e. absorbance at 600 nm

Adsorption

Oil/Water
Air/Water
Solid/Water



Adsorption



- **Hydrophobic**
- **Electrostatic**

Now you are able to:

- ✓ Describe the challenges in pharmaceutical proteins production
- ✓ Distinguish between the different mechanisms of protein instability
- ✓ Predict the mechanism of degradation from peptide primary structure