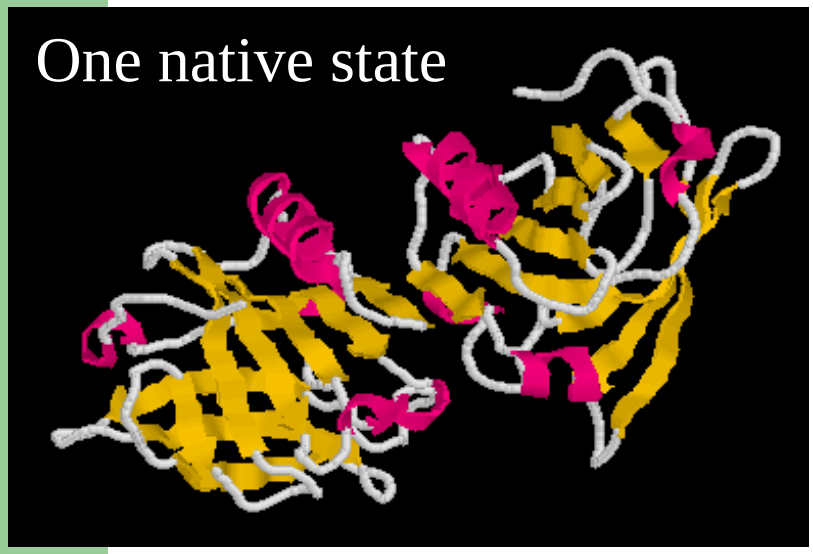
Protein Denaturation

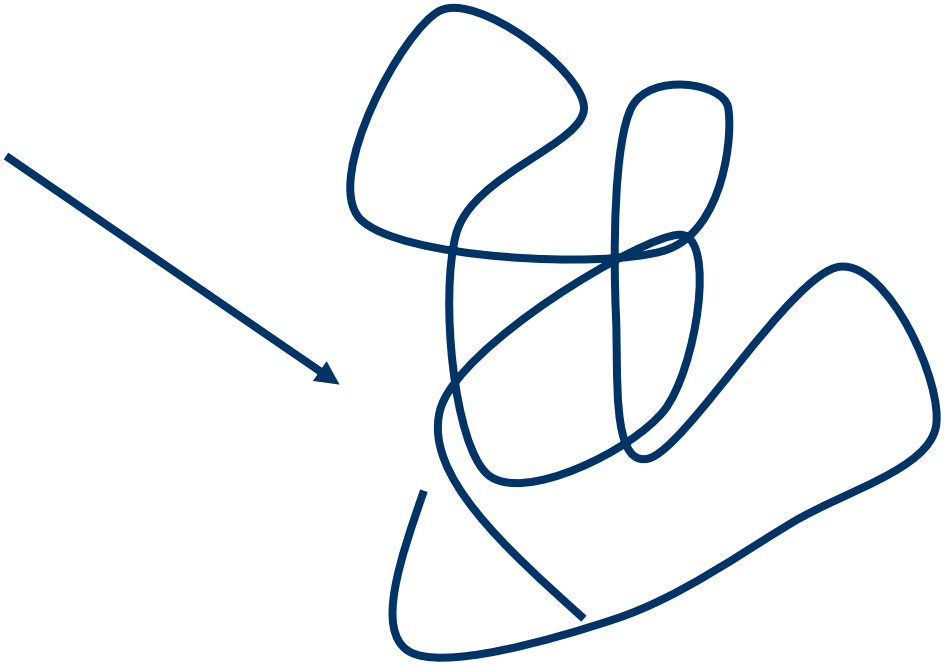
- Any modification in conformation not accompanied by rupture of peptide bonds
- Ultimate step might correspond to a totally unfolded polypeptide structure
- Reversible or irreversible

Effects of Denaturation

- Decreased solubility
- Altered water binding capacity
- Loss of biological activity
- Destruction of toxins
- Improved digestibility
- Increased intrinsic viscosity
- Inability to crystallize

Denaturation





Many denatured states

Physical Agents

Thermal Denaturation

- Rate of denaturation depends on the temperature
- As T is increased
  - Affect interactions of tertiary structure
  - Increased flexibility $\longrightarrow$ reversible
  - H-bonds begin to break $\longrightarrow$ water interaction
  - Increased water binding
  - Increased viscosity of solution
  - Structures different from native protein

Thermal Denaturation

- Upon cooling
  - Aggregation
  - Loss of solubility
- Water content affects heat denaturation
- Other consequences
  - Splitting of disulfide bonds
  - Chemical alterations of amino residues
  - Inter- or Intra- crosslinks

Effect of Cold Temperatures

- Can result in denaturation
  - Gliadins, egg and milk proteins
- Remain active
  - Some lipases and oxidases
  - Release from sub-cellular compartments
- Proteins with high hydrophobic/polar amino residues and structures dependent on hydrophobic interactions

Interfaces

- Liquid-air or Liquid-liquid interfaces
- If allowed at interfaces
  - Unfold
- Depends on
  - Rigidity of the 3-D structure
  - Number and location of hydrophobic groups
  - Accelerated if applied energy to cause shear
- Reversible?

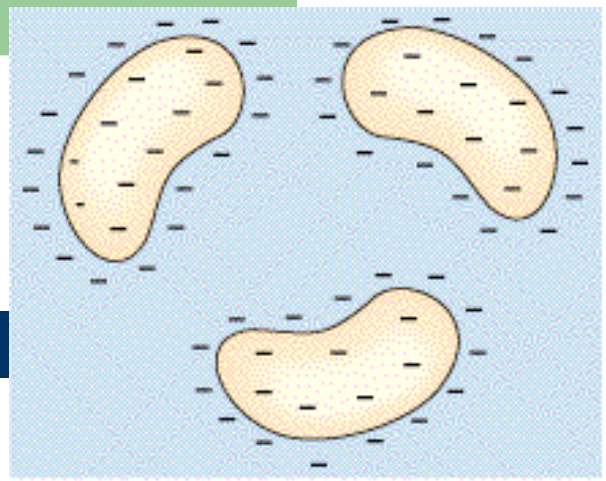
Others

- Mechanical treatments
- Hydrostatic Pressure
- Irradiation

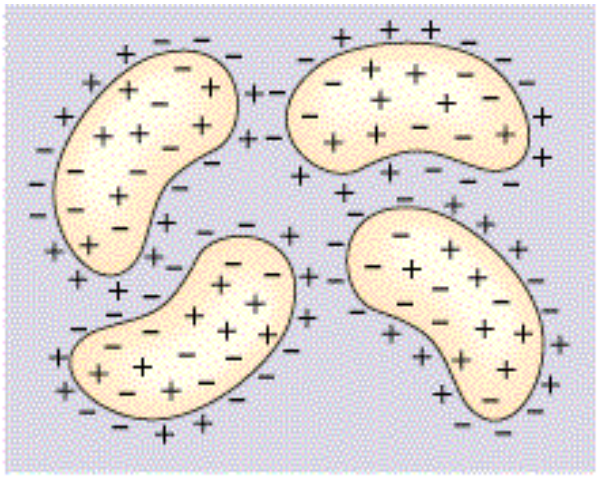
Chemical Agents

Effect of pH

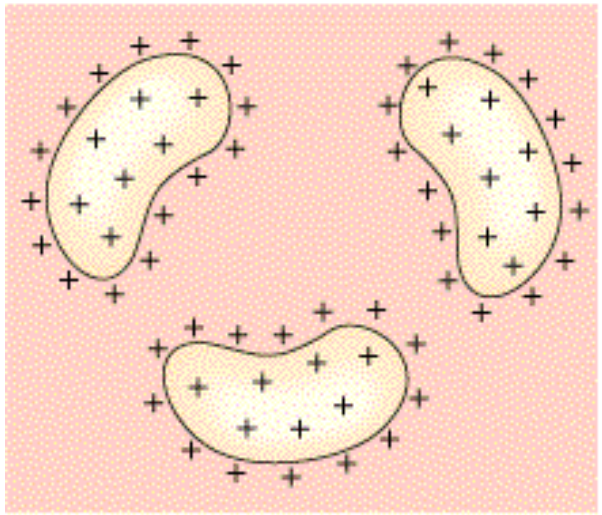
- Proteins @ physiological pH
- pI
- pH <<<< pI
  - Strong acid + T → deamination ….. Hydrolysis peptide bonds
- pH >>>> pI
  - Formation of fibers
  - Chemical modifications (beta elimination of sulfur)
  - Very high pH at elevated T results in peptide hydrolysis



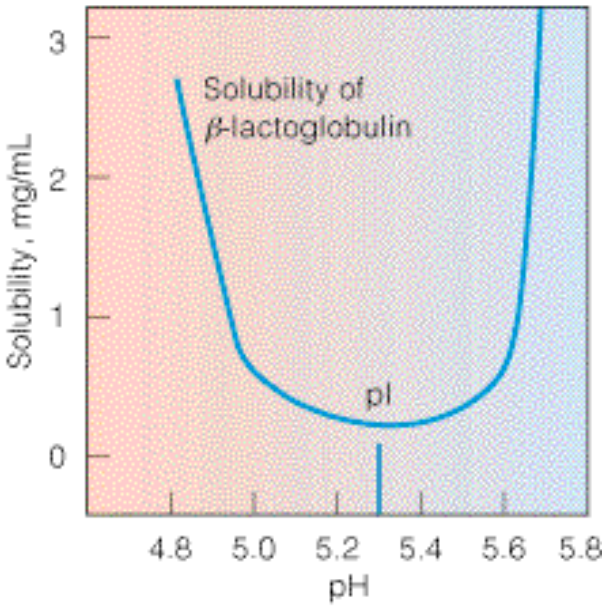
(a) High pH: protein soluble (deprotonated)



(b) Isoelectric point: protein aggregates



(c) Low pH: protein soluble (protonated)



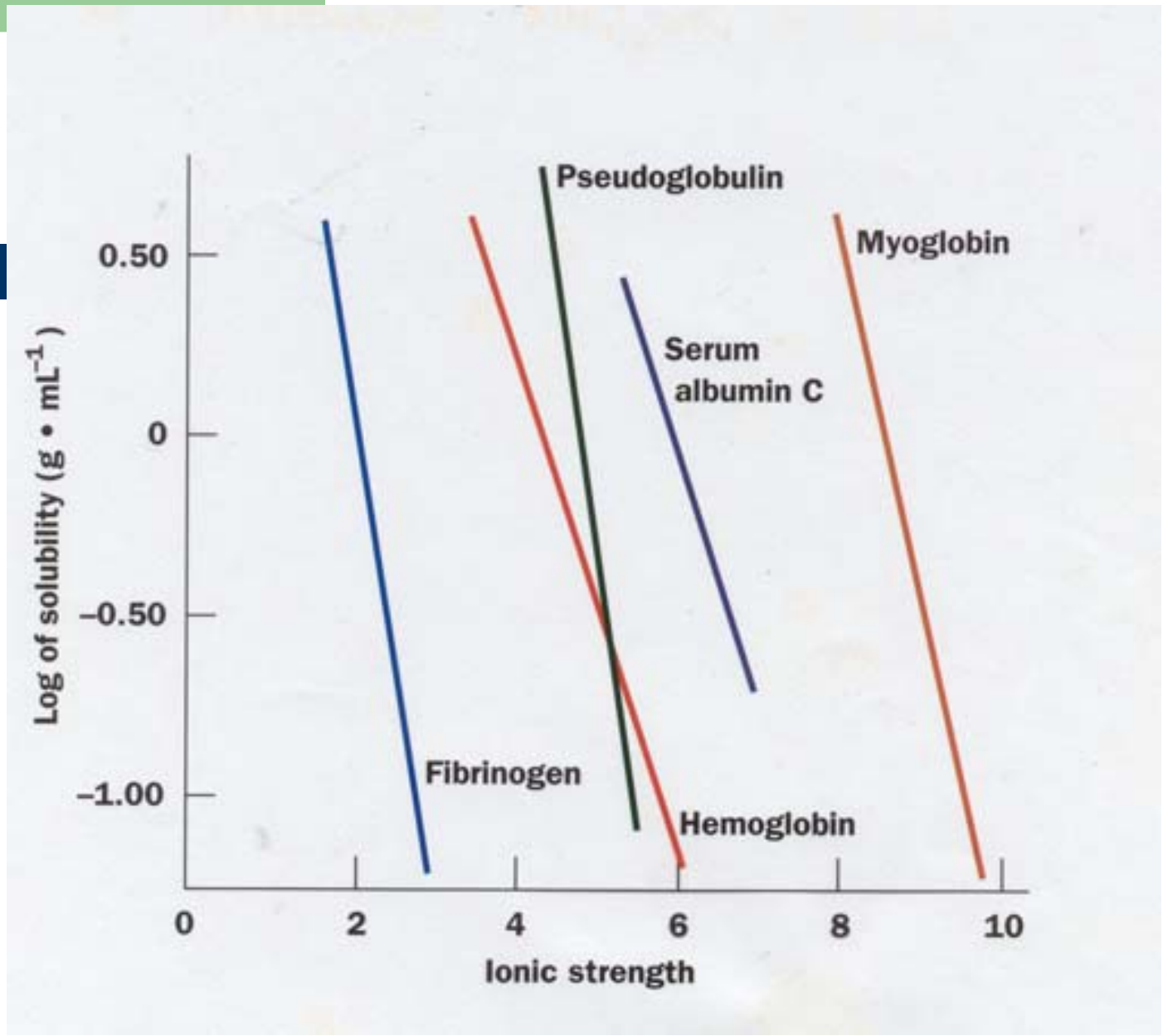
(d) Solubility of β-lactoglobulin

Changes in Dielectric Constant

- Organic solvents
- Apolar organic solvents
  - Penetrate into hydrophobic regions
- Increase electrostatic interaction
- Weaken hydrophobic interactions
- Reversible?

Ionic strength

- Proteins are soluble in dilute salt solutions
- Salts associate with charged groups
- Increased protein hydration
- Salting out
  - Protein gets dehydrated


Pseudoglobulin
Myoglobin
Serum
albumin C
Fibrinogen
Hemoglobin
0.50
0
–0.50
–1.00
Log of solubility (g • mL^{-1})
0
2
4
6
8
10
Ionic strength

Aqueous Organic Compounds

- Urea and Guanidine salts
  - Disrupts H-bonds
  - Decrease hydrophobic interactions
- Surface-active agents (SDS)
  - Disrupt hydrophobic interactions
  - Increase internal repulsive forces
  - Unfold
- Reducing agents
  - Disulfide crosslinks

Crosslinkers

- Tend to lower extent of denaturation
- More difficult to unfold
- ↑ disulfide linkages ↑ stability