

# Proteins are polymers of amino acids

## L- $\alpha$ -AMINO ACIDS

### Nonpolar

Alanine

Glycine

Isoleucine

Leucine

Methionine

Phenylalanine

Proline

Tryptophan

Valine

### Polar

Arginine

Asparagine

Aspartic acid

Cysteine

Glutamic acid

Glutamine

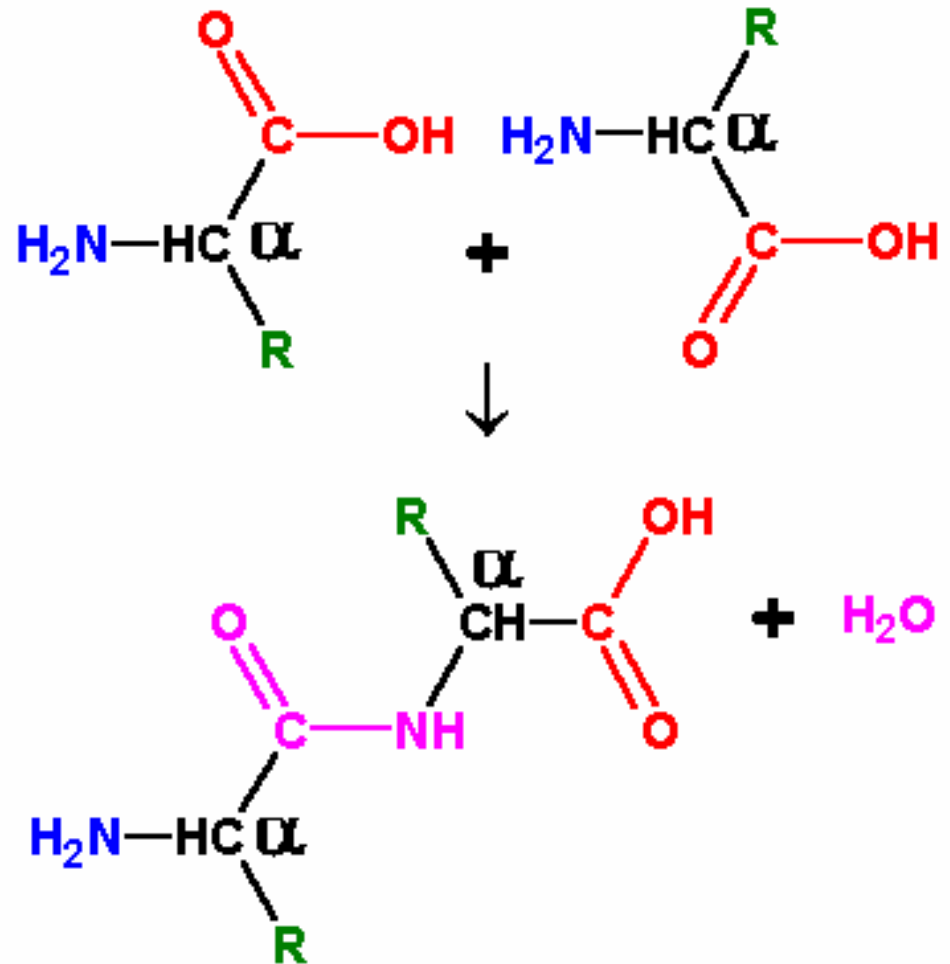
Histidine

Lysine

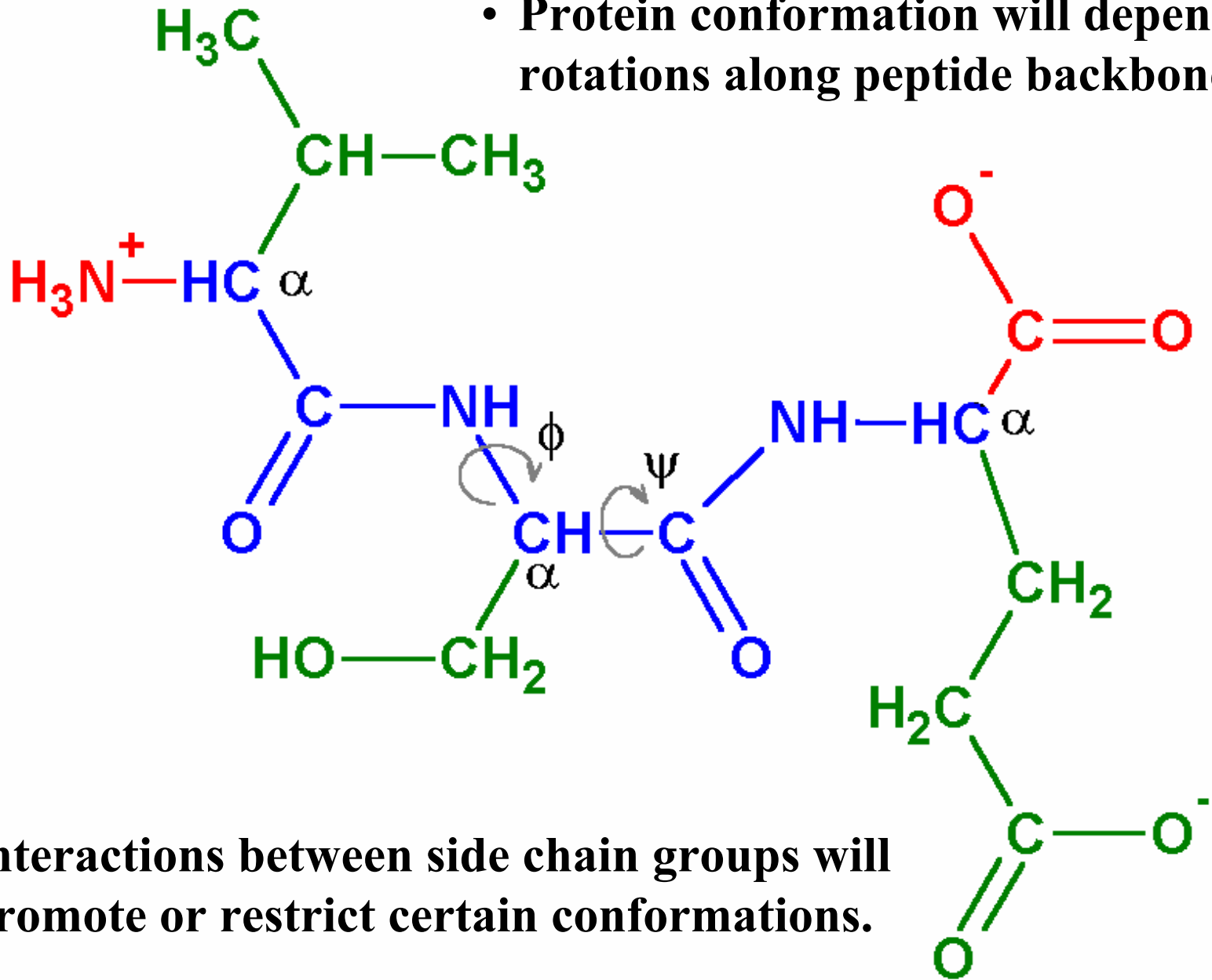
Serine

Threonine

Tyrosine



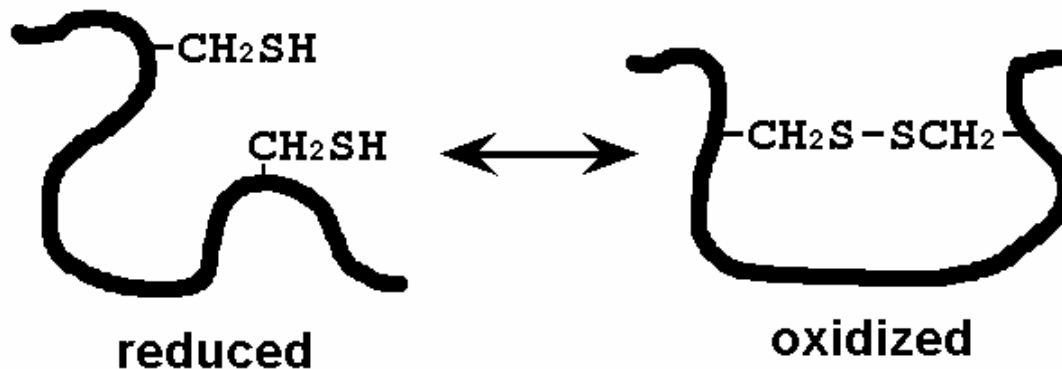
- Protein conformation will depend on rotations along peptide backbone.



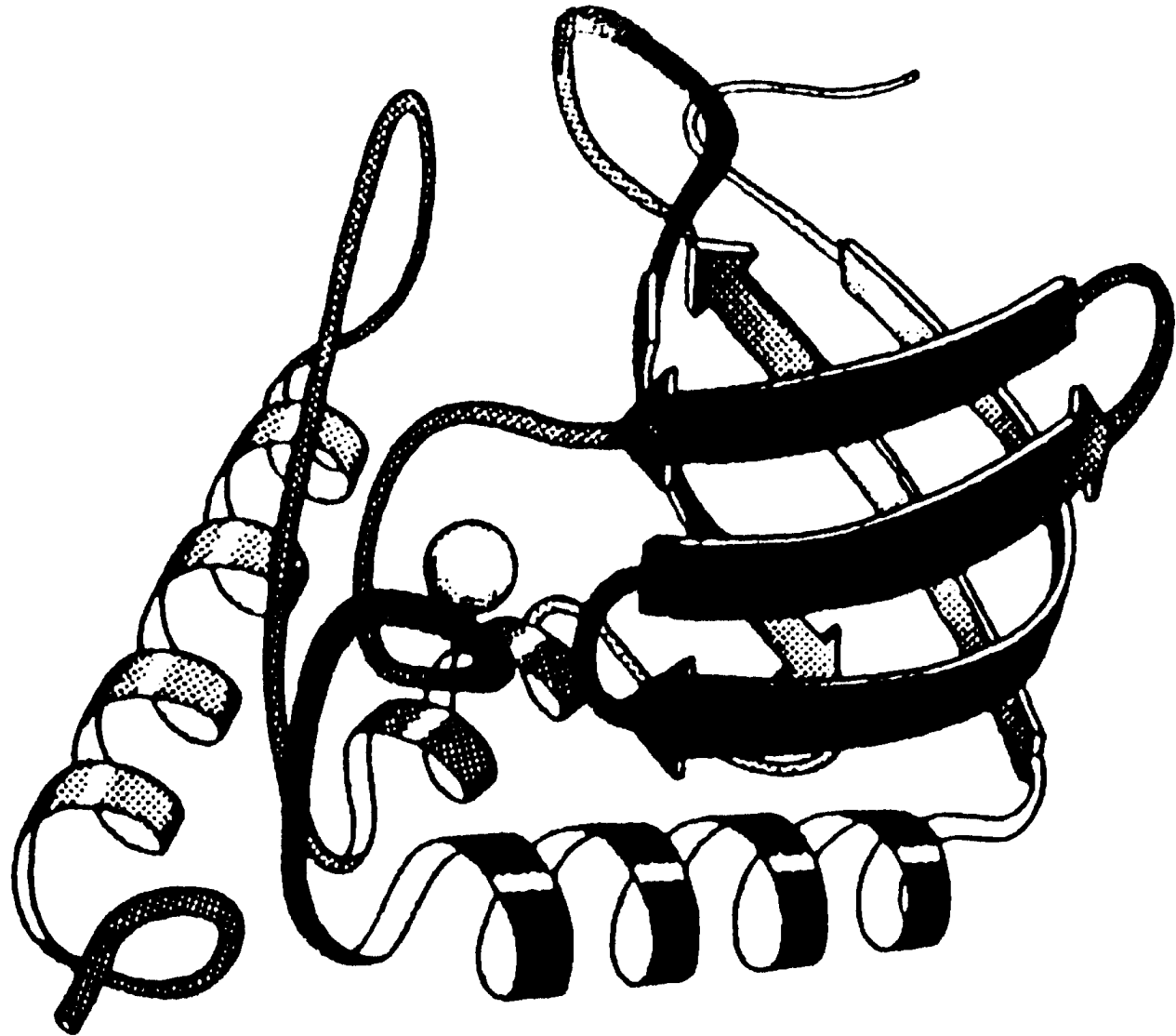
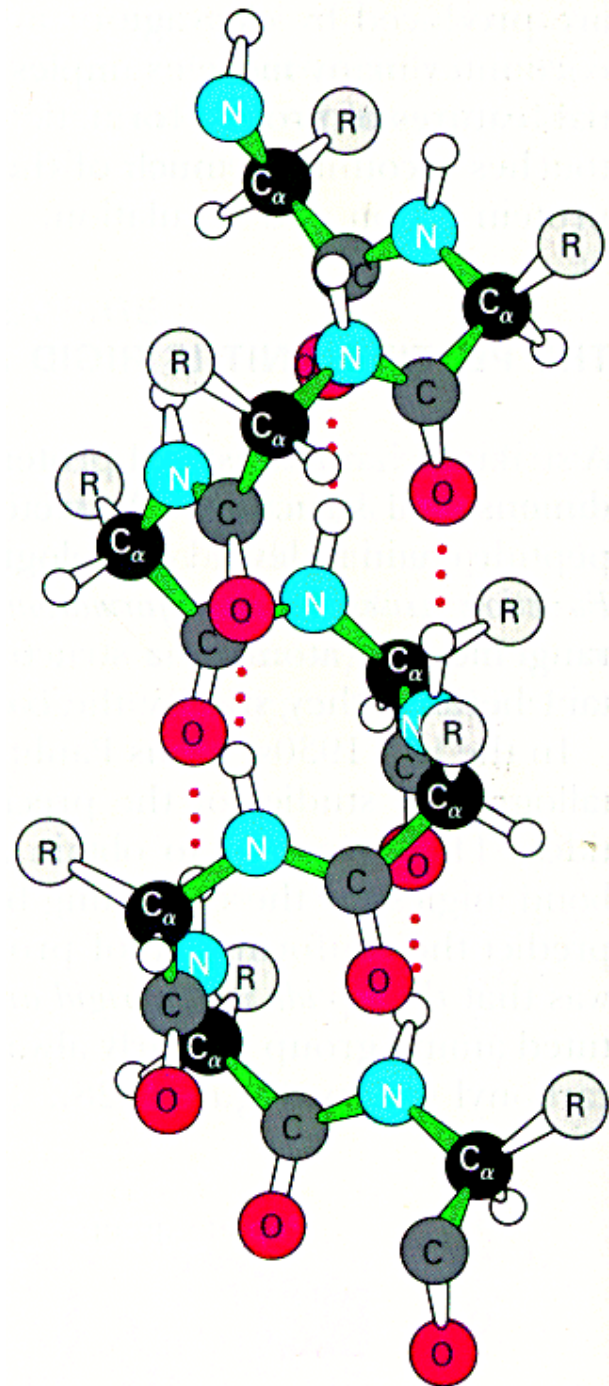
- Interactions between side chain groups will promote or restrict certain conformations.

# Levels of Protein Structure

<b>Primary</b>	Refers to the amino acid sequence and the location of disulfide bonds (i.e., covalent bonds).
<b>Secondary</b>	Refers to interactions between amino acids that are close together (eg., $\alpha$ -helix, $\beta$ -sheet, $\beta$ -turn, random coil).
<b>Tertiary</b>	Refers to interactions between amino acids that are far apart (eg., motifs, domains).
<b>Quaternary</b>	Refers to interactions between two or more polypeptide chains (i.e., protein subunits).



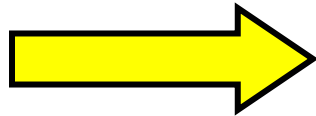
# 2° and 3° Structure



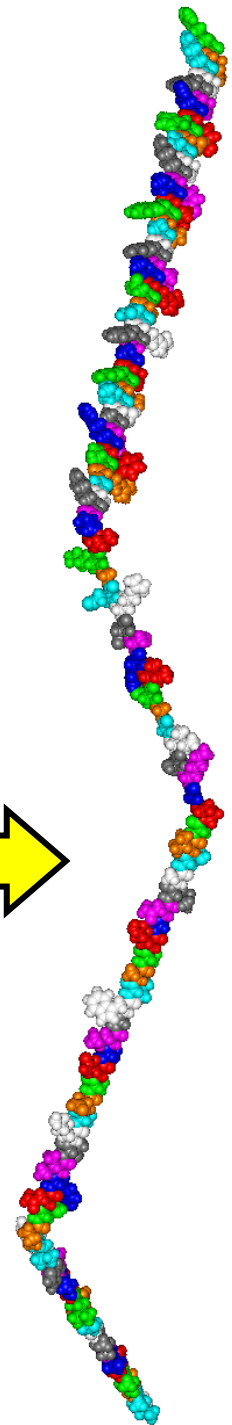
# Protein Denaturation

- denature: loss of structure due to protein unfolding
- unfolding leads to loss of function

**Folded**



**Unfolded**



# Factors Affecting Protein Stability

<b>Factor</b>	<b>Possible Remedies</b>
<b>temperature</b>	<b>Avoid high temperatures. Keep solutions on ice.</b>
<b>freeze-thaw</b>	<b>Determine effects of freezing. Include glycerol in buffers. Store in aliquots.</b>
<b>physical denaturation</b>	<b>Do not shake, vortex or stir vigorously. (Protein solutions should not foam.)</b>
<b>solution effects</b>	<b>Mimic cellular environment: neutral pH, ionic composition, etc.</b>
<b>dilution effects</b>	<b>Maintain protein concentrations &gt; 1 mg/ml as much as possible.</b>
<b>oxidation</b>	<b>Include 0.1-1 mM DTT (or <math>\beta</math>-ME) in buffers.</b>
<b>heavy metals</b>	<b>Include 1-10 mM EDTA in buffers.</b>
<b>microbial growth</b>	<b>Use sterile solutions, include anti-microbials, and/or freeze.</b>
<b>proteases</b>	<b>Include protease inhibitors. Keep on ice.</b>

# Measuring Protein

- **Specific Proteins**
  - assay based on biological activity (eg., enzyme, ligand binding, etc.)
  - immunoassay/antibodies
  - 'band' on gel
- **Total Protein**
  - direct: UV spectrophotometry
  - indirect: eg., dye binding (Bradford)

# UV Absorption

- $A_{\max}$  of Tyr and Trp  $\sim$  280 nm
- Tyr and Trp distribution  $\sim$  constant
  - $A_{280}$  of 1.0  $\cong$  1 mg/ml protein
  - sensitivity  $\sim$  5-10  $\mu$ g/ml
- sample recovery is possible
- interfering substances (eg., nucleic acids have  $A_{\max}$  of 260 nm
  - correction factors possible
  - eg., mg/ml protein =  $(A_{235} - A_{280})/2.51$

# Bradford (Coomassie-blue G-250)

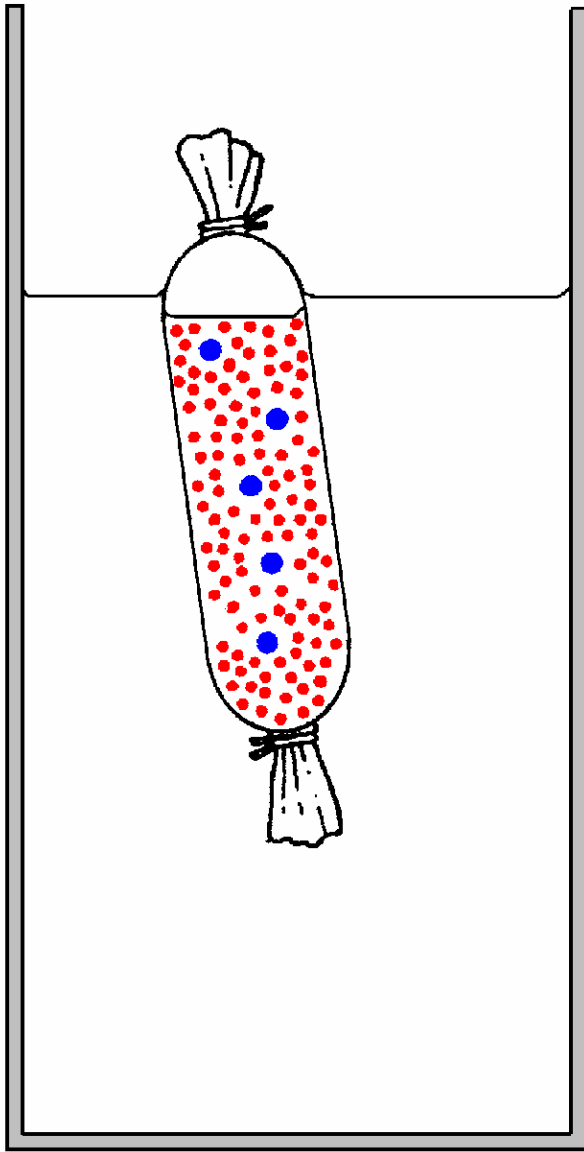
- $A_{\max}$  of CB G-250 shifts from 465 to 595 nm when bound to protein
  - dye reacts primarily with Arg
  - lesser extent with His, Lys, Tyr, Trp, Phe
- sensitivity is 1-100  $\mu\text{g/ml}$  depending on circumstances
- single step and few interfering substances
- protein concentration extrapolated from standard curve
- sample not recoverable

# Differential Protein Solubility

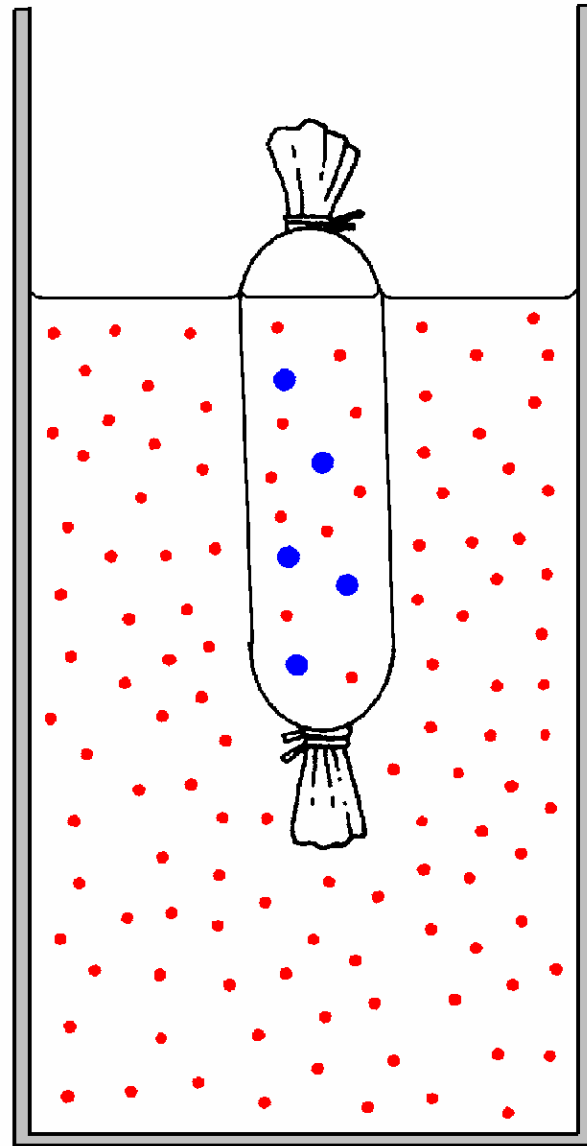
- individual proteins can be separated based on different physical and chemical properties
- common techniques:
  - differential solubility →
    - salt,  $(\text{NH}_4)_2\text{SO}_4$
    - solvents (acetone)
    - acidic pH
    - high temperature
  - chromatography
  - electrophoresis
- salting-out effect
  - as [salt] ↑ less  $\text{H}_2\text{O}$  is available for hydration of protein
  - proteins will aggregate, or precipitate, according to their hydrophobicity

# Procedure for $(\text{NH}_4)_2\text{SO}_4$ Precipitation

- slowly add  $(\text{NH}_4)_2\text{SO}_4$  to desired concentration
- continue stirring until equilibrium is reached
- collect precipitated protein by centrifugation
- dissolve protein in appropriate buffer
- subject to dialysis to remove excess salt if necessary
  
- 2-step procedure
  - discard first pellet
  - add more  $(\text{NH}_4)_2\text{SO}_4$  to supernatant



**Start Dialysis**



**At Equilibrium**