

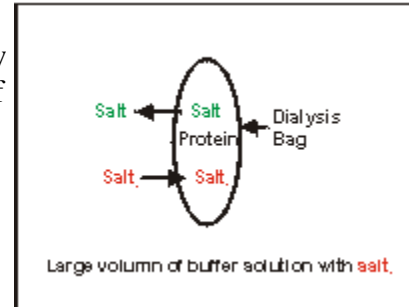




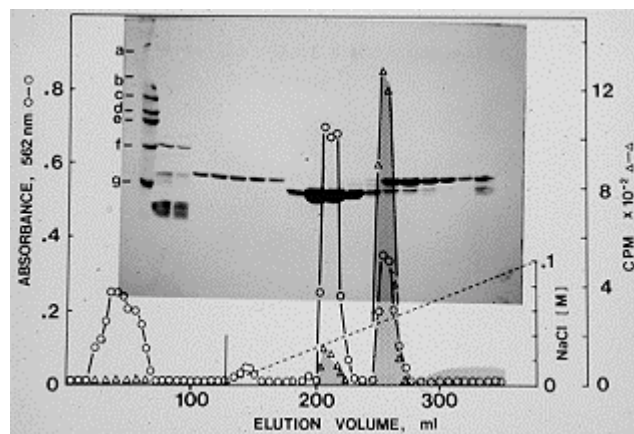
- Such a calibration curve can then be used to estimate the molecular weight of an unknown protein.

- **Dialysis/Ultrafiltration**

- Semipermeable membranes are available, which allow passage of small molecules but exclude the passage of proteins. Sacs made of such material allow the salt and buffer components of a protein solution to be changed to another buffer



- **Practical Example**



- This figure illustrates several of the techniques discussed above. It is taken from "Isolation, Characterization, and cDNA Sequence of Two Fatty Acid-Binding Proteins from the Midgut of *Manduca sexta* Larvae". A. F. Smith, K. Tsuchida, E. Hanneman, T. C., Suzuki, and M. A. Wells, *J. Biol. Chem.* **267**, 380-384 (1992).
- This is the elution profile from an anion exchange resin (binds negatively charged proteins). The proteins were eluted by increasing the NaCl concentration in the eluting buffer.
- Total protein was measured by determining the absorbance at 280 nm.
- In order to follow the fatty acid-binding proteins, they were labeled by binding radioactive fatty acids (CPM=counts per minute - gray shading).
- The purity of each peak was assessed using SDS-PAGE (insert).
- There are two, nearly pure, proteins that bind fatty acids.
- The two proteins were obtained in pure form following one additional step.

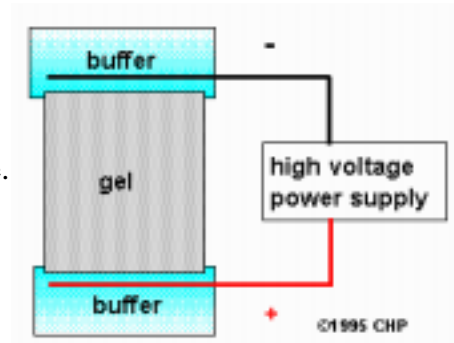
## Protein Characterization

- **Electrophoresis**

- In an electric field a protein or other charged macromolecule will move with a velocity that depends directly on the charge on the macromolecule and inversely on its size and shape.

- **Gel electrophoresis** is carried out in some supporting media, usually polyacrylamide or agarose, which has pores of sufficient size to allow passage of the macromolecule.

- The proteins in the gel are easily stained for detection purposes.
- Because the net charge on a protein and its molecular weight are characteristic properties of a protein, electrophoresis is a powerful method for characterizing the purity of a protein preparation.



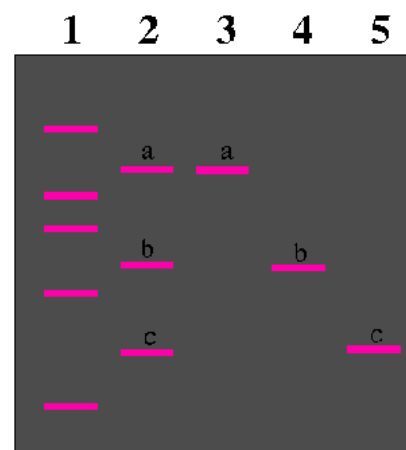
- **SDS-PAGE** (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) is a variant of electrophoresis in which the buffers contain SDS, a detergent that binds to proteins.

- Most proteins bind SDS at a constant ratio, about 1 SDS for every 2 amino acids.
- The large negative charge resulting from the bound SDS masks the native charge on the protein, so that all proteins have essentially the same charge to mass ratio.
- This means that the rate of movement in the electric field depends only on the molecular weight.
- In addition, the SDS causes all proteins to adopt a random-coil structure, which means that shape does not effect movement through the gel.
- Thus SDS-PAGE is very useful method for determining the molecular weight of a protein.

- Western blotting is a technique for detecting a specific protein in a mixture.

- **Gel electrophoresis and SDS-PAGE electrophoresis are primarily useful as analytical techniques, although they can be used for purification.**

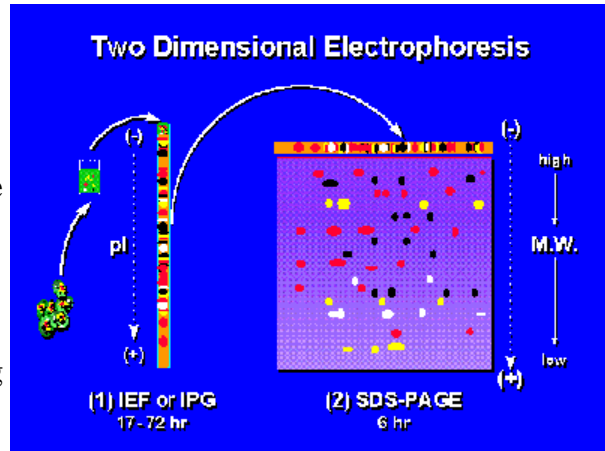
- In this figure, lane 1 would contain standards of known molecular weight, lane 2 a mixture of three unknown proteins and lanes 3-5 the three unknown proteins.
- SDS-PAGE can be used to determine the molecular weight of a protein.
- The molecular weights of the three unknown proteins can be determined from a calibration curve constructed by plotting the log of the molecular weight of the standard proteins vs. the distance traveled in the gel.
- The distance traveled depends on the porosity of the gel. The lower the porosity the further the proteins move.



- Depending on the molecular of the unknown proteins, different porosity gels will need to be used.

- **Isoelectric Focusing**

- In this technique electrophoresis occurs through a stable pH gradient.
- Under these conditions the proteins will migrate in the electric field until they reach a point in the pH gradient where their net charge becomes 0 - **the isoelectric point**.
- The isoelectric point depends on the exact number and type of weak acid amino acid side chains present in the protein. Therefore, isoelectric focusing is a useful purification procedure.
- Often isoelectric focusing is combined with SDS PAGE in two-dimensional electrophoresis.



- **Molecular Weight and Shape** are fundamental physical properties of a protein.

- Estimates of molecular weight can be obtained using **SDS-PAGE** or **gel filtration**, as described above.
- One very useful technique for measuring molecular weight and shape is **centrifugation**.
  - A particle that is subjected to a centrifugal field by being spun in a centrifuge is subjected to a force,  $f = m(1 - \bar{v}\rho)\omega^2 r$ , where **m** is the mass of the mass of the particle, **r** is the distance of the particle from the center of rotation, and  **$\omega$**  is the angular velocity.
  - **$(1 - \bar{v}\rho)$**  is the buoyancy factor which accounts for the fact that particle is buoyed up by the surrounding solvent of density  **$\rho$**  (g/ml).
  - **$\bar{v}$**  is the specific volume of the particle (ml/g) (= 1/density of the particle).
  - If  **$\bar{v} = \rho$**  then the particle will not move.
- The movement of the particle through the solvent is resisted by a frictional coefficient, **f**, that depends on the shape of the particle.
  - The frictional coefficient is an important factor in any transport process, such as centrifugation or gel filtration.
  - A spherical particle has a **f = 1.0**, whereas a cigar-shaped or cylindrically-shaped particle will have **f > 1.0**.
- The movement of any particle under the influence of a centrifugal field is characterized by its sedimentation coefficient, **S**, which is directly proportional to its molecular mass, **M**, and inversely proportional to **f**.

$$S = \frac{M(1 - \bar{v}\rho)}{Nf}$$

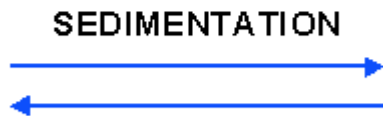
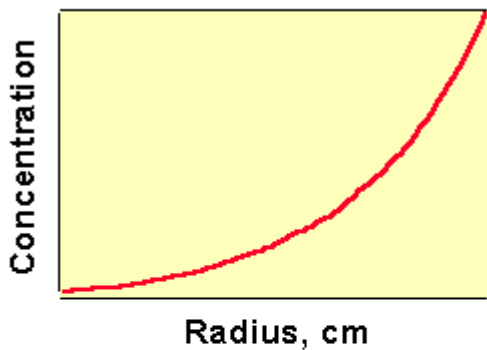
- , where **N** is Avogadro's number.

- **Ultracentrifugation is used in two ways to characterize proteins**

In **sedimentation equilibrium** experiments, the centrifuge is operated at a relative low speed so that the forces of sedimentation and diffusion balance and the protein distributes in the centrifuge cell in a manner proportional to its molecular weight.

In **sedimentation velocity** experiments, the centrifuge is operated at maximal speed, which causes the protein to sediment to the bottom of the tube. The rate at which the boundary moves gives  $S$ , which when combined with  $M$  gives  $f$ , a measure of the shape of the protein.

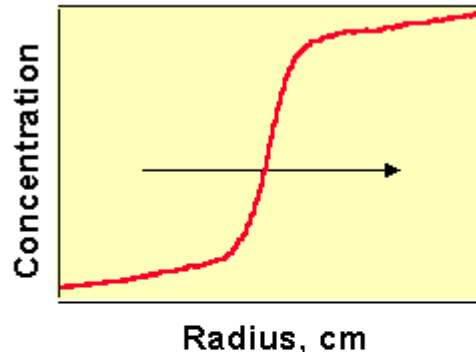
**SEDIMENTATION EQUILIBRIUM  
THERMODYNAMICS**



No Net Transport -

**MOLECULAR WEIGHT**

**SEDIMENTATION VELOCITY  
HYDRODYNAMICS**



Net Transport -

**SHAPE**

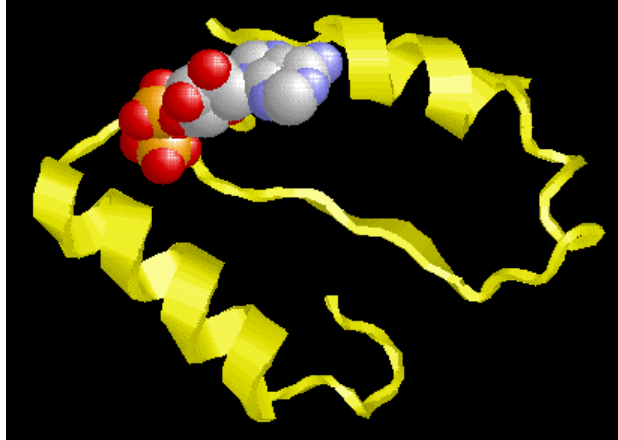
**MOLECULAR WEIGHT**

**Three Dimensional Structure**

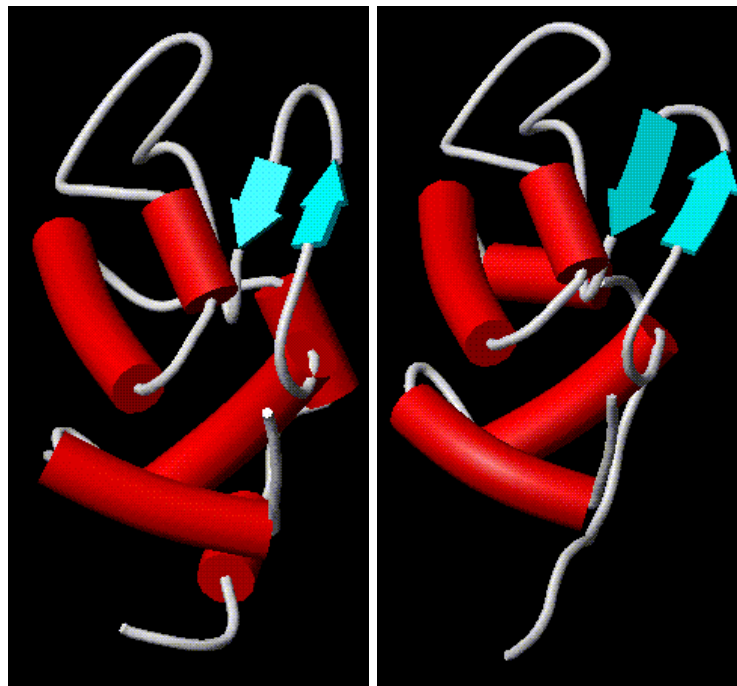
- Whenever possible, it is highly desirable to obtain the three dimensional structure of a protein.
- Most often, this is done by X-ray crystallography, although NMR can be used, especially with small proteins.
- It is impressive to note that more than 10,000 structures have been determined, most in the last decade, as new, more powerful instruments have become available.

## Structural Homology

- In addition to sequence homology for proteins with identical functions from different organisms, there are often domains in a protein that are conserved. For example, most proteins that bind nucleotides, such as ADP, have a common nucleotide-binding motif.



- There are even a few cases in which proteins with entirely different functions have very similar three-dimensional structures, as shown below for lysozyme, an enzyme, and  $\alpha$ -lactalbumin, a milk protein.



Lysozyme

$\alpha$ -Lactalbumin