Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
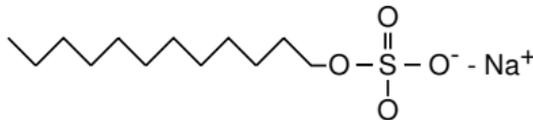Spring 2017

Lecture 21:

SDS Gel Electrophoresis

30 March 2017
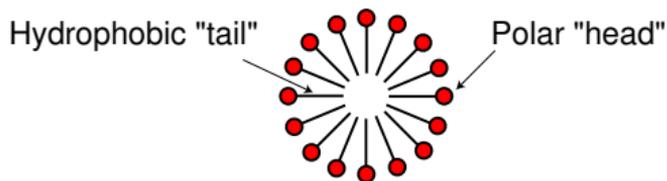©David P. Goldenberg
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SDS - Sodium Dodecyl Sulfate

- Also called lauryl sulfate

- A common ingredient of shampoos

- Forms micelles in water

Hydrophobic "tail"   Polar "head"

Micelles are three-dimensional, *i.e.,* roughly spherical.
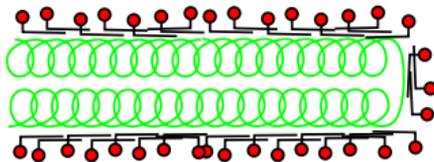
SDS Denatures Proteins and Binds to Them

- Most proteins bind SDS at a constant ratio:
  $\approx$ 1.4 g SDS per g protein.

- Complexes are rod shaped.

- Polypeptides form α-helical structures in SDS.
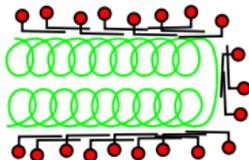
- A possible structure of SDS-protein complexes:
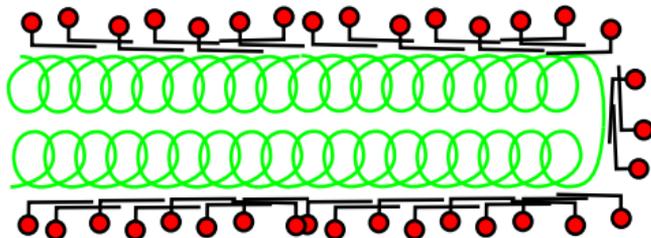
Which will have the higher electrophoretic mobility, in the <u>absence</u> of a gel?

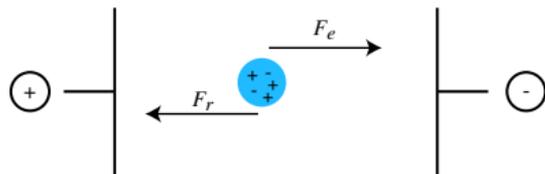1 A large protein with SDS bound:

2 A small protein with SDS bound:

3 They will have the same mobility.

Electrophoresis in the Absence of a Gel

- Electromotive force: $F_e = z \cdot e \cdot E$

    z = average charge

    e = unit of electric charge, a constant

    E = electric field strength, proportional to voltage

- Resistive force: $F_r = v \cdot f$

    v = velocity

    f = frictional coefficient, a molecular property

- Molecule accelerates until $F_r = F_e$

Velocity when $F_r = F_e$

- Electrophoretic and frictional forces:

$$F_e = z \cdot e \cdot E$$
$$F_r = v \cdot f$$

- When the forces are equal:

$$v \cdot f = z \cdot e \cdot E$$
$$v = \frac{z \cdot e \cdot E}{f}$$

- Define free mobility (mobility in absence of gel) as the velocity normalized by electric field:

$$M_0 = \frac{v}{E} = \frac{z \cdot e}{f}$$

- M_0 should be independent of applied voltage.

- Frictional coefficient
  - Frictional coefficient for rod-shaped molecule:

    $f \propto$ Rod Length

    $f \propto$ Molecular Weight

    $f = C_f \cdot MW$

  - C_f is a constant for SDS-protein complexes.

- Net charge
  - Charges from SDS generally overwhelm intrinsic charge of polypeptides.
  - Since proteins bind a constant amount of SDS per g:

    $z \propto$ Molecular Weight

    $z = C_z \cdot MW$

  - C_z is a constant for SDS-protein complexes.
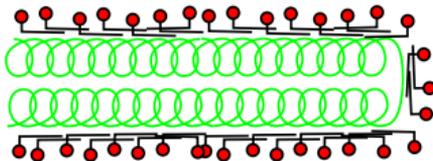
$$M_0 = \frac{z \cdot e}{f} = \frac{C_z \cdot MW \cdot e}{C_f \cdot MW}$$
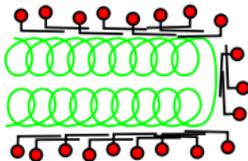
$$M_0 = \frac{C_z \cdot e}{C_f}$$

Which will have the higher electrophoretic mobility, in the <u>absence</u> of a gel?

1 A large protein with SDS bound:


2 A small protein with SDS bound:

3 They will have the same mobility.

- All SDS-protein complexes should have the same free mobility, irrespective of molecular weight!

Separation of Proteins on SDS Gels Depends Almost Exclusively on Sieving Effect of the Gel.

- Sieving effect depends only on size (since protein/SDS complexes have a common shape).

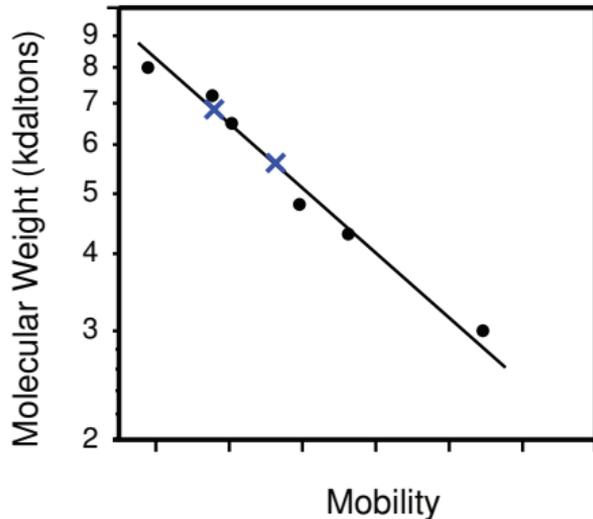- SDS gels can be used to determine molecular weights of polypeptides
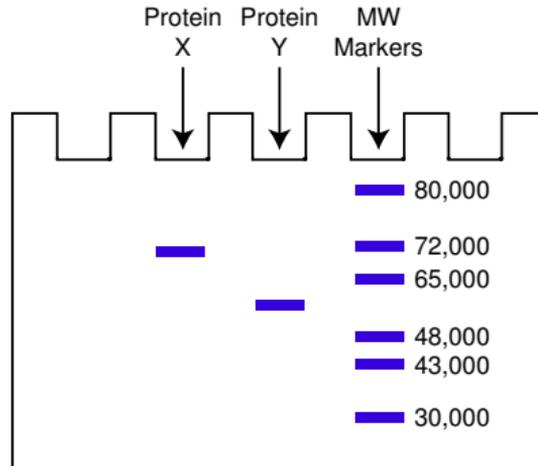
- What other class of molecules is expected to behave this way in electrophoresis?

Gel Electrophoresis of DNA Fragments
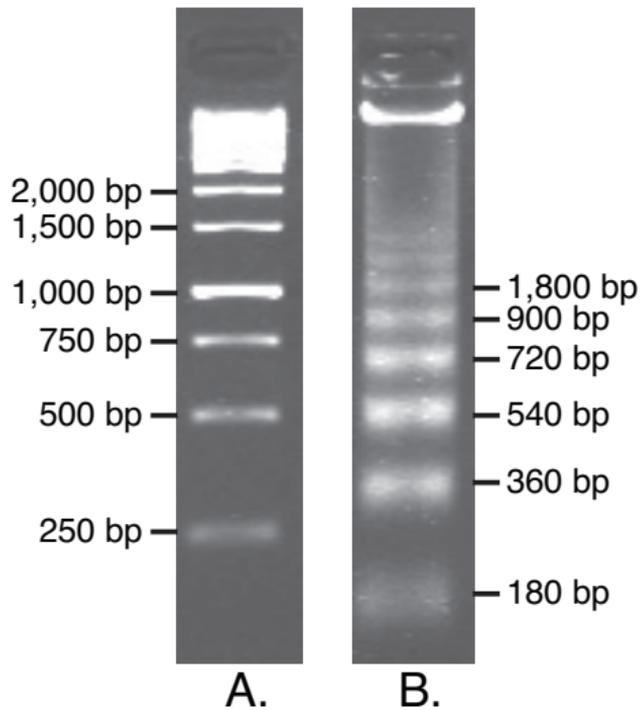
- Electrophoresis through agarose gel.

- DNA stained by binding a fluorescent dye.

- A. Artificial DNA fragments.

- B. DNA fragments generated during cell death (apoptosis).

Lane A markers: 2,000 bp; 1,500 bp; 1,000 bp; 750 bp; 500 bp; 250 bp

Lane B markers: 1,800 bp; 900 bp; 720 bp; 540 bp; 360 bp; 180 bp

A.   B.

https://en.wikipedia.org/wiki/DNA_laddering

Calibration Curve for SDS Gel Electrophoresis

- Measure mobilities of proteins with known molecular weights.
- Fit a line (or curve) to data for standards.
- Estimate molecular weights of other proteins from mobilities and empirical calibration curve.

Cautions Regarding Determination of Molecular Weights by SDS Gel Electrophoresis

1. Molecular weights are for individual polypeptides.

   - Oligomeric proteins or other complexes are dissociated by SDS, and information about their structures or sizes is lost.

2. Deviations from expected mobility are common.

   Possible explanations:
   - Differences in amount of SDS bound to protein.

   - Residual structure of polypeptide after binding SDS.

   - Intrinsic charge of protein is not insignificant.

Effects of Acrylamide Concentration on the Mobilities of Globular and Transmembrane Proteins in SDS Gels
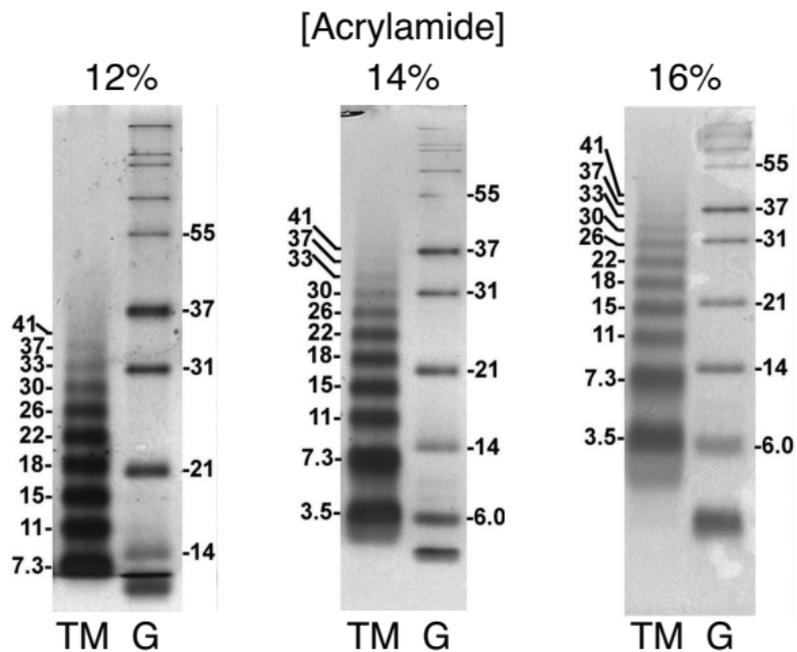
[Acrylamide]

- TM: Artificial mimics of transmembrane proteins.
- G: "Normal" globular proteins.
- Numbers indicate molecular weights, in kdalton.
- Mobilities decrease as acrylamide concentration is increased.
- Separation of smaller proteins increases as acrylamide concentration is increased.
- Separation of larger proteins is better at low acrylamide concentrations.
- Relative mobilities of TM and globular proteins change with acrylamide concentration.
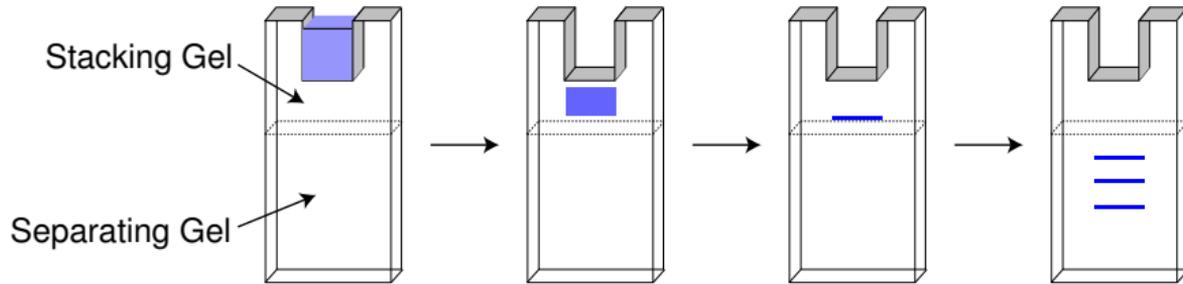
- Natural "stacking" of protein molecules as they enter a gel:
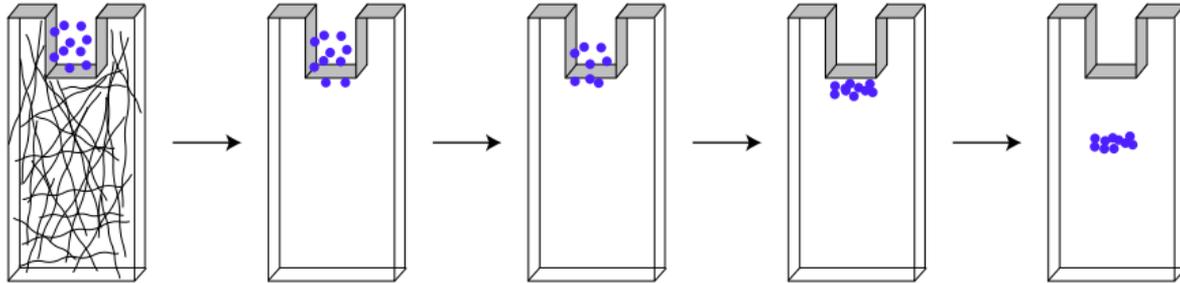
- Final protein bands are often much narrower than the original sample applied in the well.

Stacking and Separation in a Discontinuous Gel

Stacking Gel

Separating Gel

How does it work?