Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 23:

SDS Gel Electrophoresis and Stacking Gels

3 April 2018

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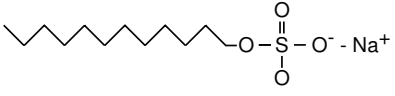
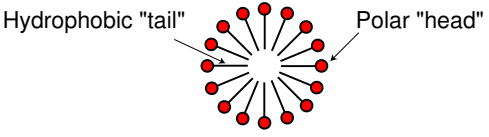
SDS - Sodium Dodecyl Sulfate

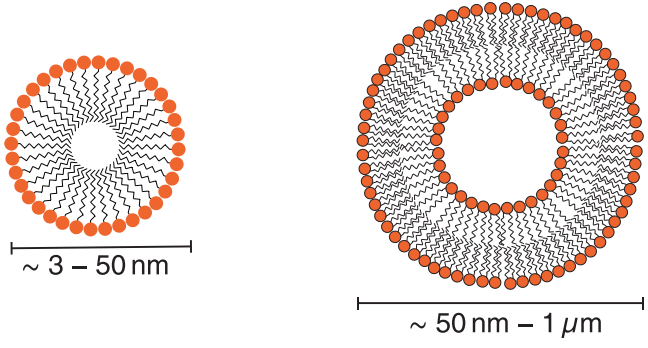


- Also called lauryl sulfate
- A common ingredient of shampoos
- Forms micelles in water



Micelles are three-dimensional, *i.e.,* roughly spherical.

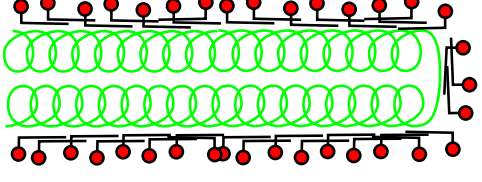
Micelles Versus Vesicles



- Micelles are formed by detergents and soaps; vesicles are formed by phospholipids.
- Micelles are made up of a single shell detergent or soap molecules; vesicles are made up of lipid bilayers.
- Micelles are generally much smaller than vesicles.
- Different shapes and sizes of the micelles and vesicles reflect the different shapes of detergents and soaps (~conical) and phospholipids (~cylindrical).

SDS Denatures Proteins and Binds to Them

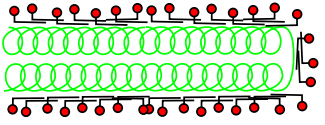
- Most proteins bind SDS at a constant ratio:
  $\approx$ 1.4 g SDS per g protein.
- Complexes are rod shaped.
- Polypeptides form α-helical structures in SDS.
- A possible structure of SDS-protein complexes:



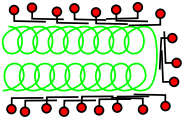
Clicker #1

Which will have the higher electrophoretic mobility, in the absence of a gel?

1. A large protein with SDS bound:



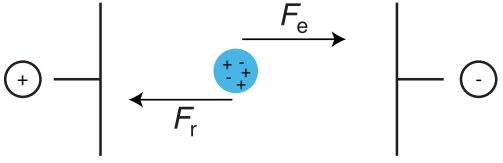
2. A small protein with SDS bound:



3. They will have the same mobility.

All answers count (for now)!

Electrophoresis in the Absence of a Gel



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

  $z =$ average net charge, a dimensionless number

  $e =$ unit of electric charge: 1.6×10^{-19} coulomb (C)

  $E =$ electric field strength, proportional to voltage: units of V/m

- 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 and Net Charge for SDS-Protein Complexes

- 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.

Free Electrophoretic Mobilities of SDS-Protein Complexes

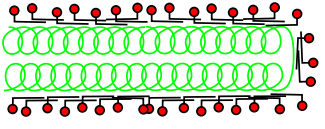
$$M_0 = \frac{z \cdot e}{f} = \frac{C_z \cdot MW \cdot e}{C_f \cdot MW}$$

$$M_0 = \frac{C_z \cdot e}{C_f}$$

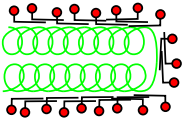
Clicker Question #2

Which will have the higher electrophoretic mobility, in the absence 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!

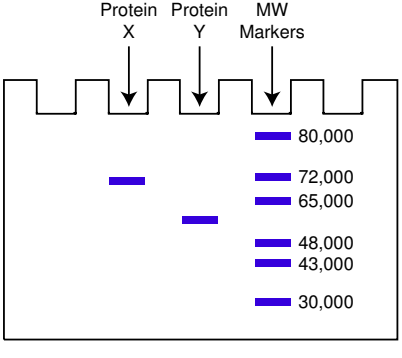
Free Electrophoretic Mobilities of SDS-Protein Complexes

$$M_0 = \frac{z \cdot e}{f} = \frac{C_z \cdot MW \cdot e}{C_f \cdot MW}$$

$$M_0 = \frac{C_z \cdot e}{C_f}$$

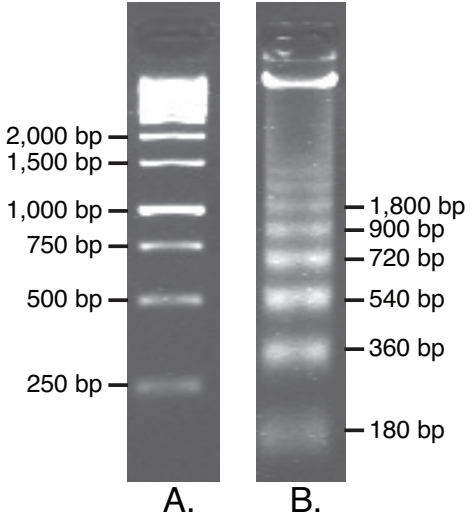
Separation of Proteins on SDS Gels Depends Almost Exclusively on Sieving Effect of the Gel.

- 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?

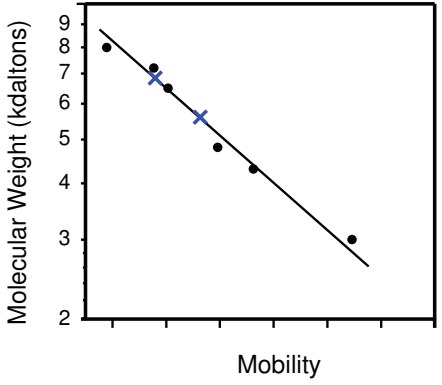
Gel Electrophoresis of DNA Fragments



- Electrophoresis through agarose gel.
- DNA stained by binding a fluorescent dye.
- A. Artificial DNA fragments.
- B. DNA fragments generated during programmed cell death (apoptosis).

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

Calibration Curve for SDS Gel Electrophoresis



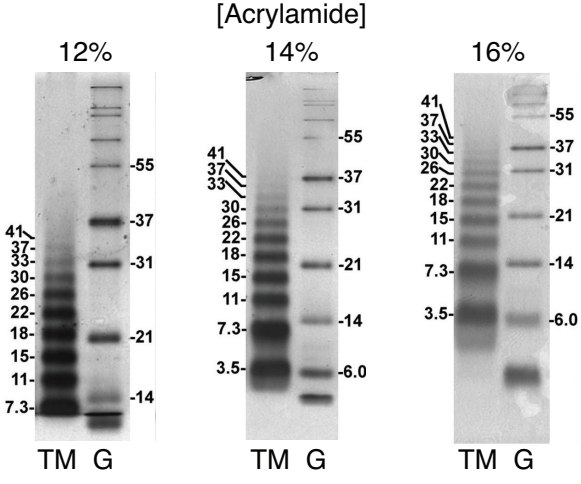
- Measure mobilities of proteins with known molecular weights.
- Fit a line (or curve) to data for standards.
- Estimate molecular weights of other proteins from mobilities and empirical calibration curve.

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

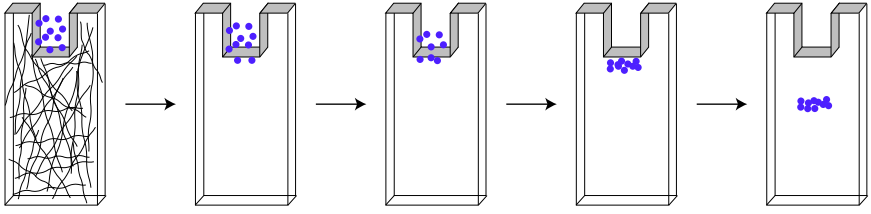


- TM: Artificial mimics of transmembrane proteins.
- G: “Normal” globular proteins.
- Numbers indicate molecular weights, in kdalton.
- Mobilities decrease as acrylamide concentration is increased.
- Separation of smaller proteins increases as acrylamide concentration is increased.
- Separation of larger proteins is better at low acrylamide concentrations.
- Relative mobilities of TM and globular proteins change with acrylamide concentration.

Rath, A., Cunningham, F. & Deber, C. M. (2013). *Proc. Natl. Acad. Sci., USA*, 110, 15668–15673.
http://dx.doi.org/10.1073/pnas.1311305110

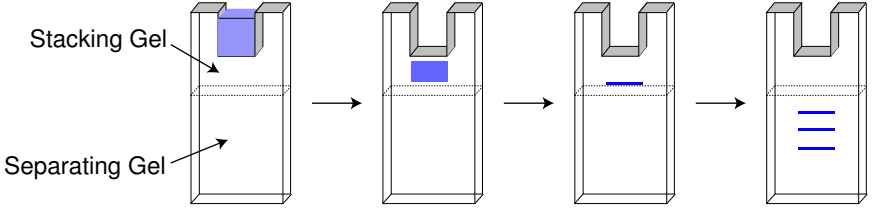
Resolution and Sensitivity Depend on Narrow Protein Bands

- Natural “stacking” of protein molecules as they enter a gel:



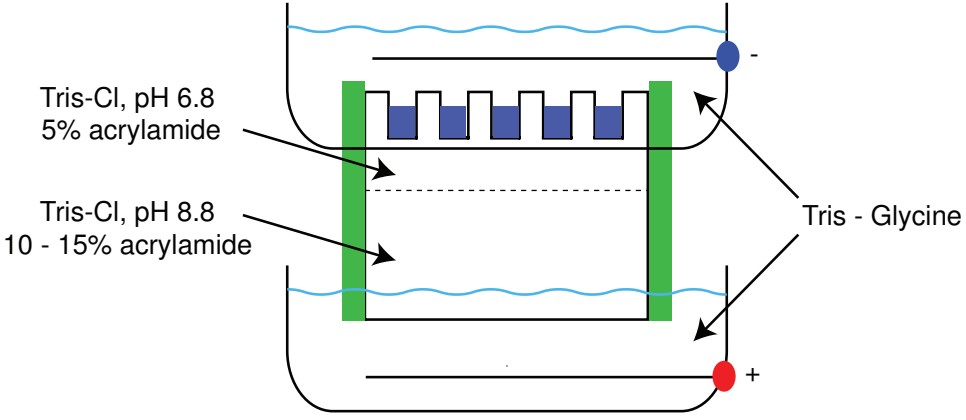
- Final protein bands are often much narrower than the original sample applied in the well.

Stacking and Separation in a Discontinuous Gel

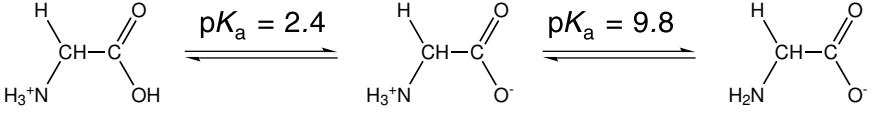


How does it work?

Buffer Compositions Control Stacking and Separation

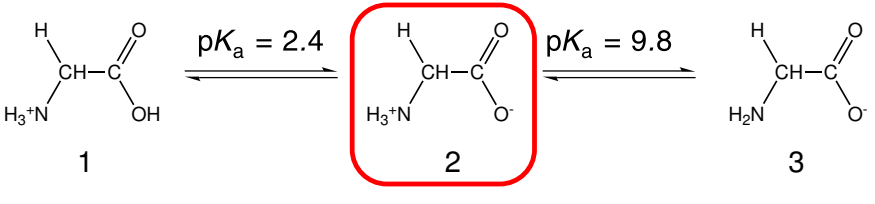


Glycine Ionization Equilibria



Clicker Question #3

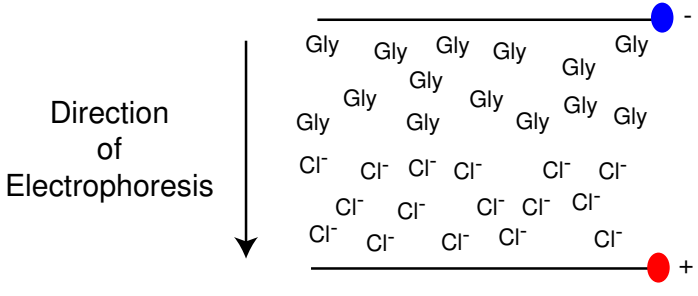
Which form of glycine will predominate in the stacking gel (pH 6.8)?



1 2 3

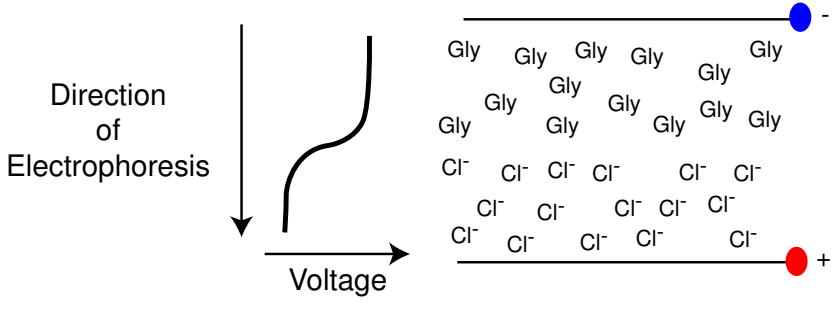
- ≈ 0.1% of glycine is negatively charged.
- Average electrophoretic mobility is very low.

Formation of an Ion Front



- Cl^- has much higher mobility than Gly:
  Electrical resistance (R) is lower in the Cl^- region.
- The two regions are in series electrically:
  Electric current (I) must be the same in both.
- Ohm's law: $V = I \cdot R$:
  The voltage is higher in the Gly region.

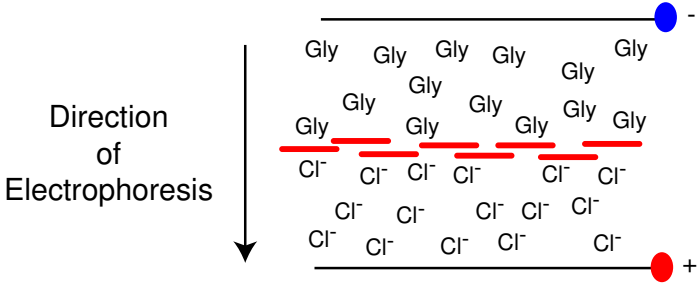
Voltage Gradient Sharpens the Ion Boundary



- If a Gly molecule diffuses ahead into Cl^- region, it experiences a lower voltage and slows down.
- If a Cl^- ion diffuses back into the Gly region, it experiences a higher voltage and speeds up until it reaches the boundary.
- Ion boundary and voltage gradient become progressively sharper.

What Happens to the Protein?

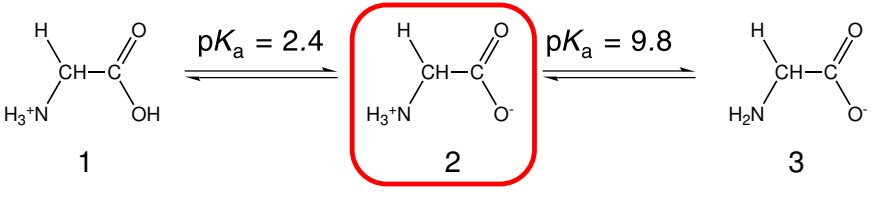
- SDS-protein complexes have mobilities between those of Gly and Cl^-
- Proteins are trapped between fast Cl^- ions and slow glycine



- Proteins form a very tight band.

Clicker Question #4

Which form of glycine will predominate in the separating gel (pH 8.8)?

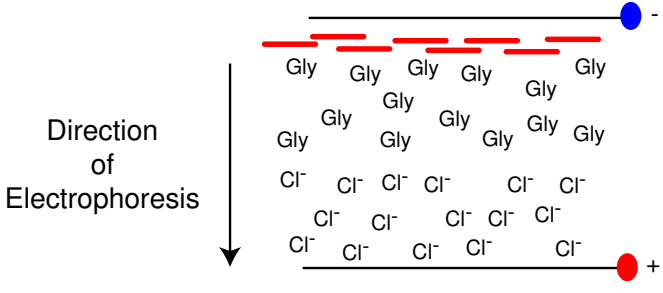


1 2 3

- ≈ 10% of glycine is negatively charged.
- Average glycine electrophoretic mobility much higher than at pH 6.8.

In Separating Gel:

- Gly mobility increases, becomes greater than SDS-protein mobility, but still slower than Cl^-.



- SDS-protein complexes are no longer trapped in a sharp voltage gradient.
- SDS-protein complexes are separated on basis of molecular weight.

Stacking and Separation in a Discontinuous Gel