Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 23:

SDS Gels and Stacking Gels

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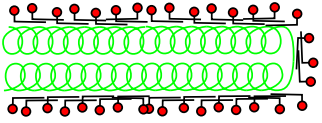
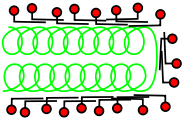
Clicker Question #1

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

A) A large protein with SDS bound:



B) A small protein with SDS bound:



C) They will have the same mobility.

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

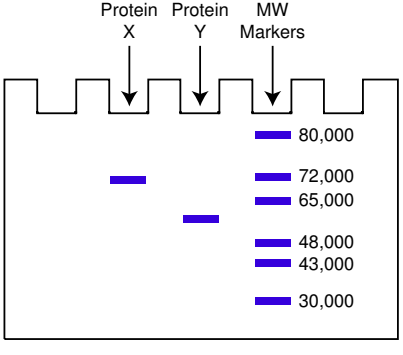
Free Electrophoretic Mobilities of SDS-Protein Complexes

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

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

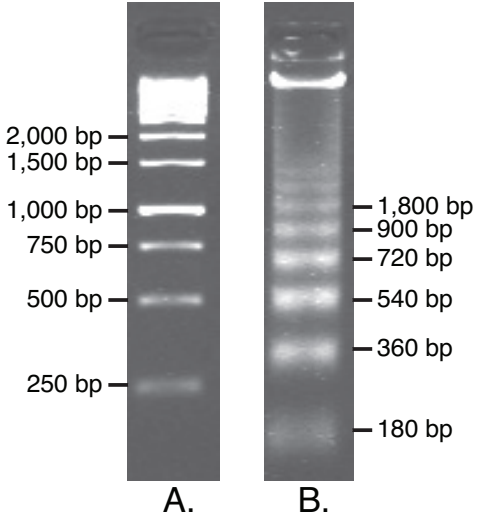
Separation of Proteins on SDS Gels Depends Almost Exclusively on Sieving Effect of the Gel.

- 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?

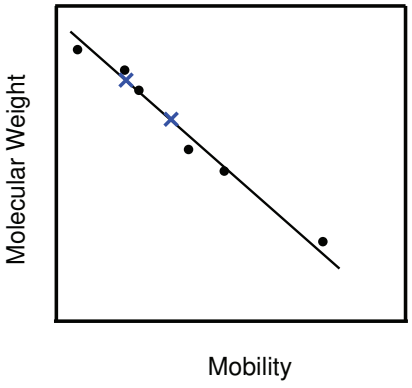
Gel Electrophoresis of DNA Fragments



- 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



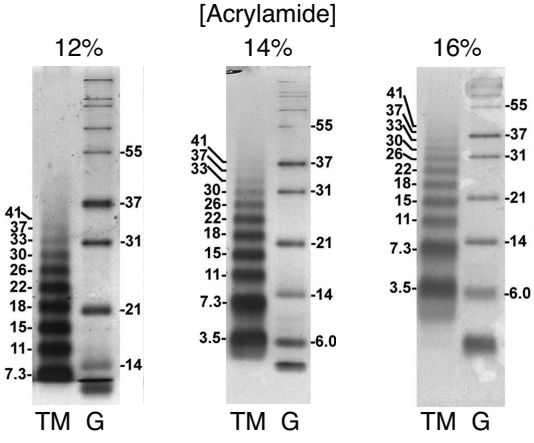
- Measure mobilities of proteins with known molecular weights.
- Fit a curve to data for standards.
- Estimate molecular weights of other proteins from mobilities and empirical calibration curve.
- Do you expect the relationship to be linear?

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.

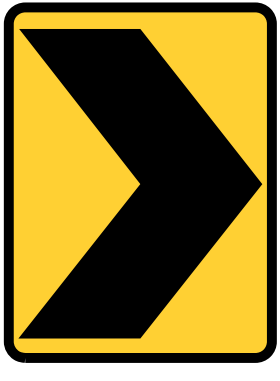
Effects of Acrylamide Concentration on the Mobilities of Globular and Transmembrane Proteins in SDS Gels



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

- 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.

Warning!

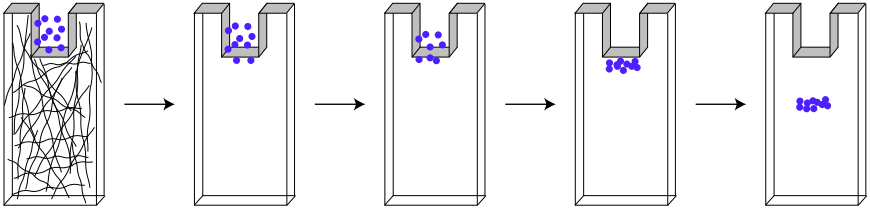


Direction Change

2-part Gels: Stacking and Separating Gels

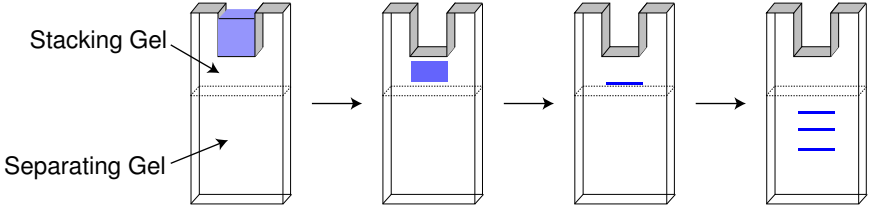
Resolution and Sensitivity Depend on Narrow Protein Bands

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



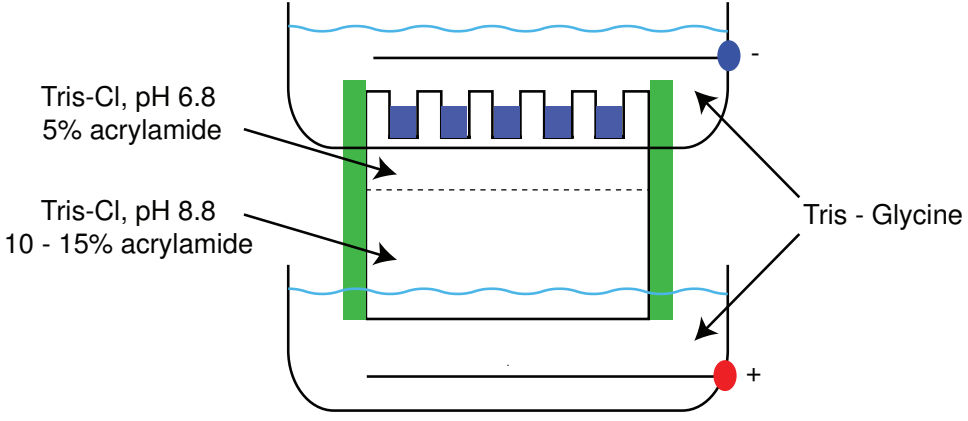
- Final protein bands are often much narrower than the original sample applied in the well.

Stacking and Separation in a Discontinuous Gel

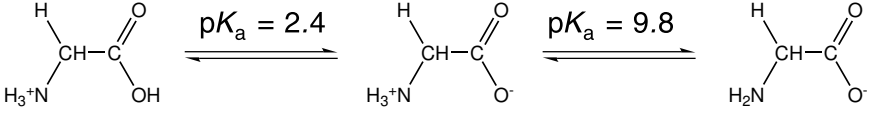


How does it work?

Buffer Compositions Control Stacking and Separation

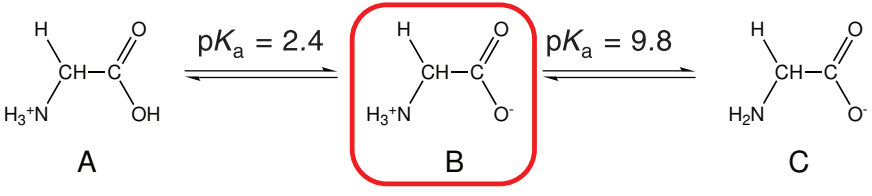


Glycine Ionization Equilibria



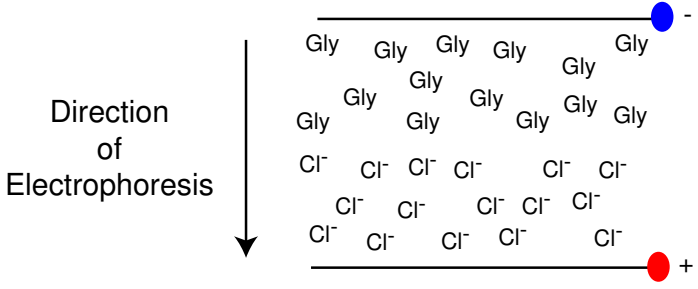
Clicker Question #2

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



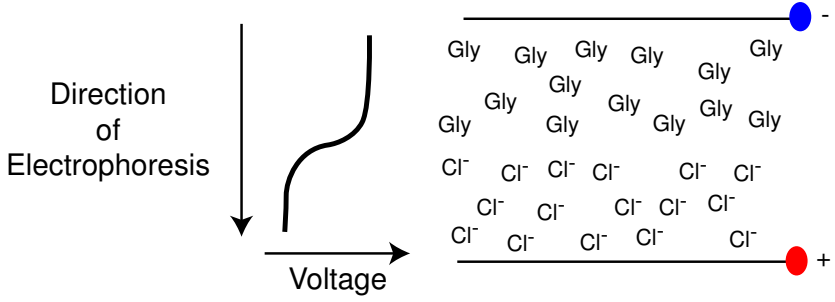
- ≈ 0.1% of glycine is negatively charged.
- Average electrophoretic mobility is very low.

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.

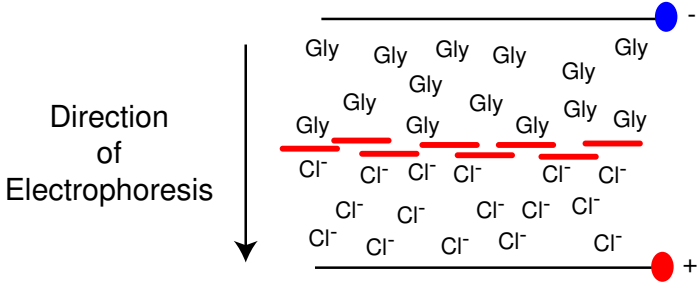
Voltage Gradient Sharpens the Ion Boundary



- 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.

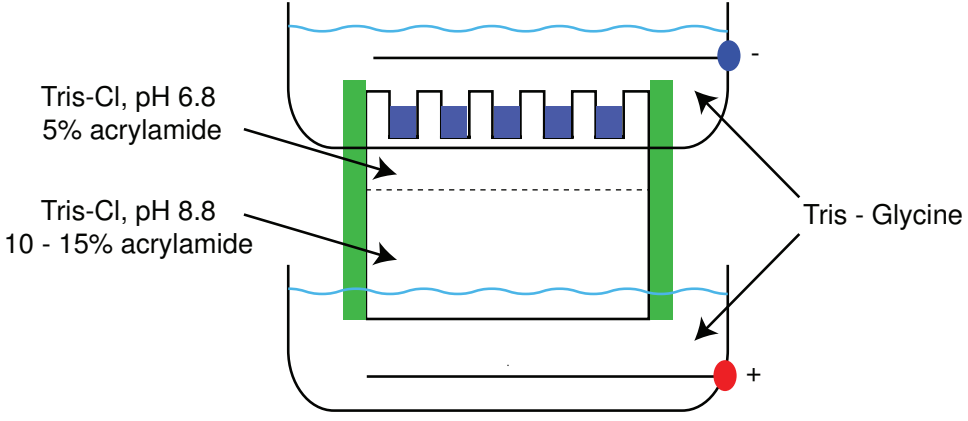
What Happens to the Protein?

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



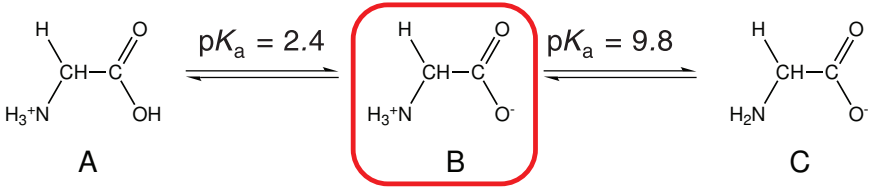
- Proteins form a very tight band.

Buffer Compositions Control Stacking and Separation



Clicker Question #3

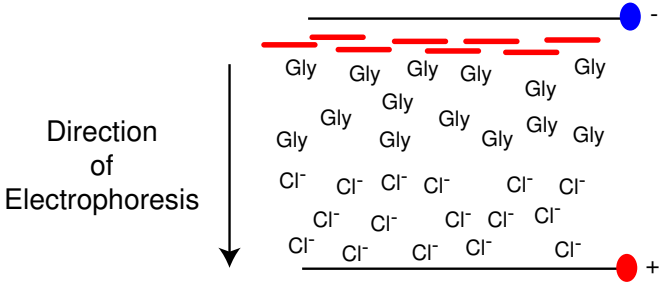
Which form of glycine will predominate in the separating gel (pH 8.8)?



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

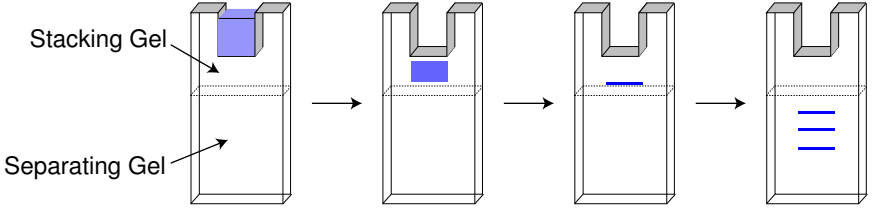
In Separating Gel:

- 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.

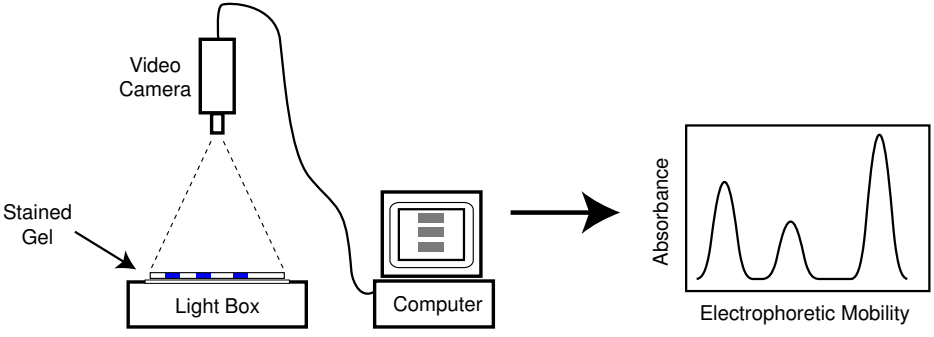
Stacking and Separation in a Discontinuous Gel



Staining and Destaining Protocols (updated)

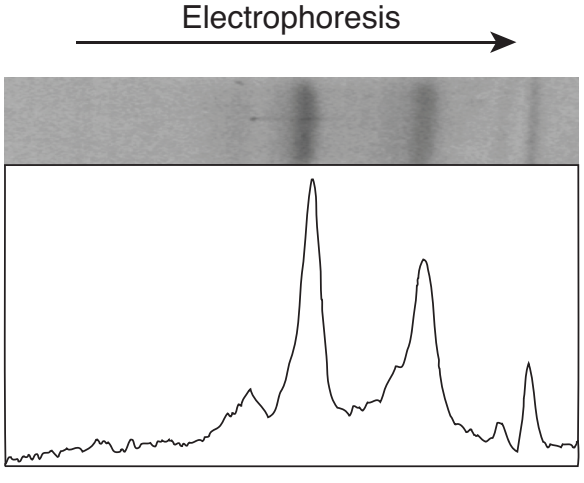
- Non-denaturing gels:
  1. Stain the gel in Coomassie-blue solution containing 10% Trichloroacetic acid and 10% 5-sulfosalicylic acid.
  2. After one day, transfer the gel to destain solution containing 5% methanol and 7.5% acetic acid.
     - Add a KimWipe to the box to absorb the dye.
  3. The day before the first day of the chromatography experiment, check on the gel and transfer to fresh destain solution if needed.
- SDS gels:
  1. Stain the gel in Coomassie-blue solution containing 50% methanol and 7.5% acetic acid.
  2. After one day, transfer the gel to destain solution containing 50% methanol and 7.5% acetic acid.
  3. After one–two hours, transfer the gel to destain solution containing 5% methanol and 7.5% acetic acid.
     - Add a KimWipe to the box to absorb the dye.
  4. The day before the first day of the chromatography experiment, check on the gel and transfer to fresh destain solution if needed.

Video Densitometry of Gels



- Lightbox and camera function as a position-specific spectrophotometer.

Scans of SDS Gel Lanes



- Integrals of peaks are proportional to protein concentrations.
- Have to define limits of individual peaks and baseline.