Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 22:

Non-Denaturing and SDS Gel Electrophoresis

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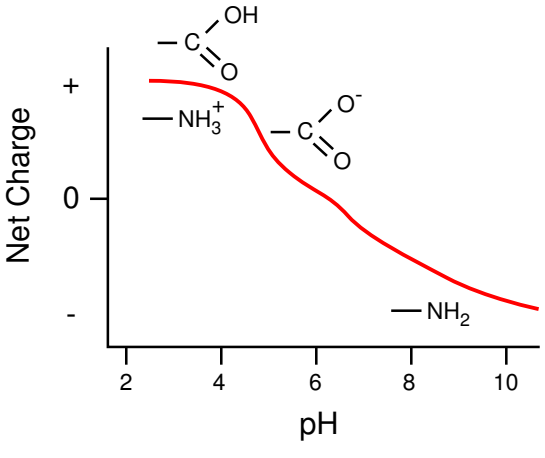
Factors That Influence Mobilities in Non-Denaturing Gels

1. Net charge of protein
   - Amino acid sequence (relative number of acidic and basic residues)
   - Solution pH
   - Three dimensional structure (can influence pK_as and interactions with ions.)
2. Size and shape of protein
3. Concentration and degree of cross-linking in gel
   - Gel will generally reduce mobilities of proteins, relative to their free mobilities.
   - Larger molecules will be affected by the gel more than smaller ones.
   - Composition of the gel can be manipulated to fractionate molecules of different sizes.

- Conditions often have to be optimized for a particular protein.

Effects of pH on Protein Net Charge



- Curve represents a population average! (or a time average)
- Shape of curve will depend on amino acid sequence and structure of a particular protein.
- For each protein, there is a pH at which positive and negative charges are balanced and the molecules have an average net charge of 0. This is the isoelectric point, pI.

pK_a Values of Ionizable Groups in Proteins

Group	In peptides	Avg. in proteins	Low in proteins	High in proteins
Asp	3.9	3.5±1.2	0.5	9.2
Glu	4.3	4.2±0.9	2.1	8.8
His	6.5	6.6±1.0	2.4	9.2
Cys	8.6	6.8±2.7	2.5	11.1
Tyr	9.8	10.3±1.2	6.1	12.1
Lys	10.4	10.5±1.1	5.7	12.1
C-term	3.7	3.3±0.8	2.4	5.9
N-term	8.0	7.7±0.5	6.8	9.1

Grimsley, G. R., Scholtz, J. M. & Pace, C. N. (2008). A summary of the measured pK values of the ionizable groups in folded proteins. *Protein Sci.*, 18, 247–251. http://dx.doi.org/10.1002/pro.19

Clicker Question #1

For a protein containing:

3 Asp residues
6 Glu residues
4 His residues
6 Lys residues

At what pH will the mobility be greatest?

A) pH 2

B) pH 4

C) pH 6

D) pH 8

E) pH 10

All answers count for now.

Clicker Question #2

For a protein containing:

3 Asp residues
6 Glu residues
4 His residues
6 Lys residues

At what pH will the mobility be smallest?

A) pH 2

B) pH 4

C) pH 6

D) pH 8

E) pH 10

Potentially Charged Residues in RNAse A

- Potentially negatively charged:
  - Terminal carboxyl group: 1
  - Aspartic acid: 5
  - Glutamic acid: 5
- Potentially positively charged:
  - Terminal amino group: 1
  - Arginine: 4
  - Lysine: 10
  - Histidine: 4
- Only present in RCM form:
  - Carboxymethylated Cys: 8

- For native and RCAM forms:
  - Calculated isoelectric point: 8.6
  - Expected charge at pH 7: $+4$
- For RCM form:
  - Calculated isoelectric point: 5.6
  - Expected charge at pH 7: -4

Conditions for Electrophoresis of Ribonuclease A

1. pH 4.4
   - All forms of RNAse A have a net positive charge.
   - Buffered with β-alanine and acetate.

2. All molecules migrate towards the negative electrode (cathode)

3. Gel is composed of 12% acrylamide, 0.032% bisacrylamide (cross-linker)

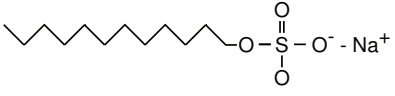
   (A relatively high concentration for a relatively small protein)

Warning!

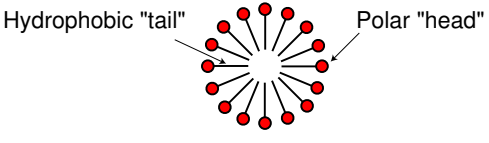
Direction Change

SDS Gel Electrophoresis

SDS - Sodium Dodecyl Sulfate

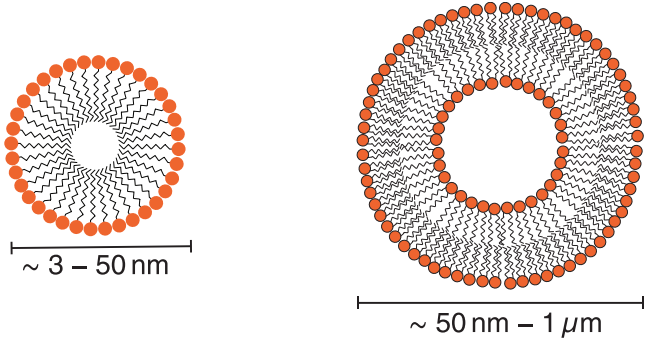


- Also called lauryl sulfate
- A common ingredient of shampoos
- Forms micelles in water



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

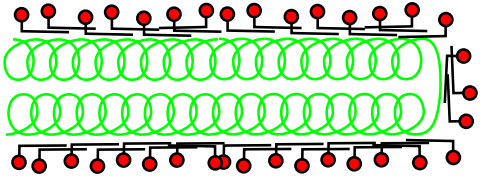
Micelles Versus Vesicles



- Micelles are formed by detergents and soaps; vesicles are formed by phospholipids.
- Micelles are made up of a single shell of 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

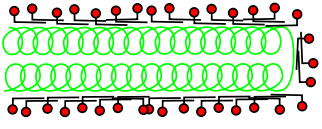
- Most proteins bind SDS at a constant ratio:
  $\approx$ 1.4 g SDS per g protein.
- Complexes are rod shaped.
- Polypeptides form α-helical structures in SDS.
- A possible structure of SDS-protein complexes:



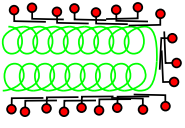
Clicker Question #3

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

A) A large protein with SDS bound:



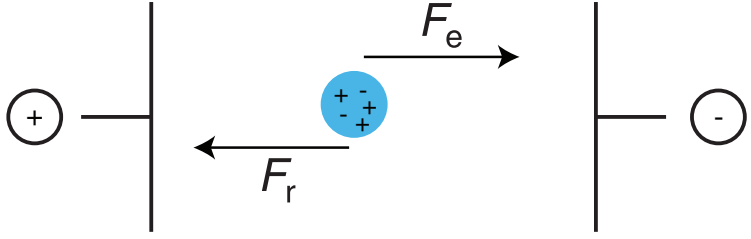
B) A small protein with SDS bound:



C) They will have the same mobility.

All answers count (for now)!

Electrophoresis in the Absence of a Gel



- Electromotive force: $F_e = z \cdot e \cdot E$
- Resistive force: $F_r = v \cdot f$
- 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

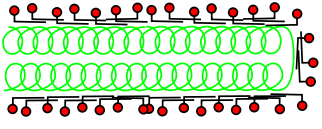
$$M_0 = \frac{z \cdot e}{f} = \frac{C_z \cdot MW \cdot e}{C_f \cdot MW}$$

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

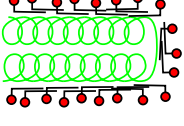
Clicker Question #4

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!

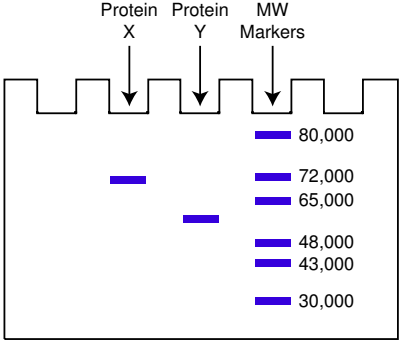
Free Electrophoretic Mobilities of SDS-Protein Complexes

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

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

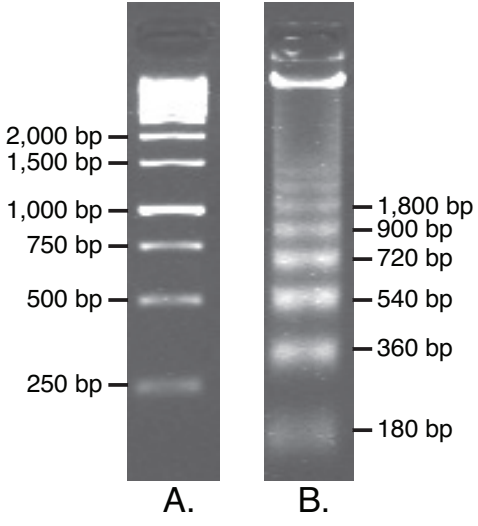
Separation of Proteins on SDS Gels Depends Almost Exclusively on Sieving Effect of the Gel.

- 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?

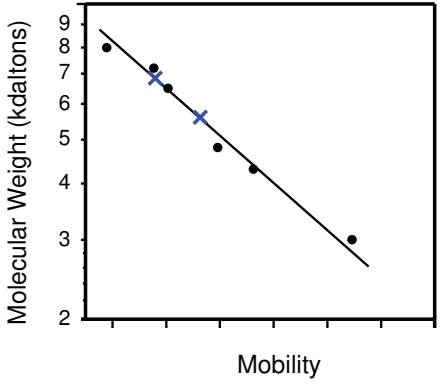
Gel Electrophoresis of DNA Fragments



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