

Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

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_a s and interactions with ions.)

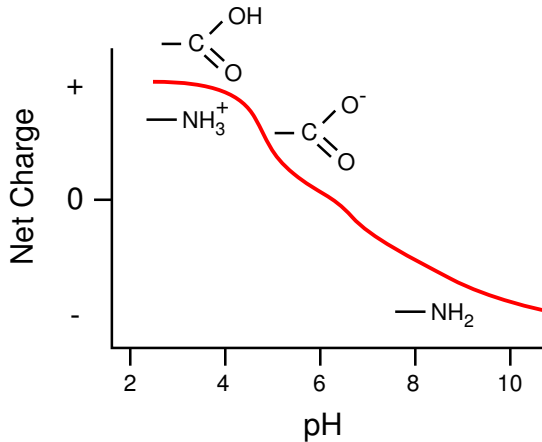
2. Size and shape of protein

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

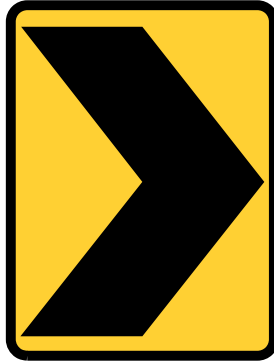
■ For RCM form:

- 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)

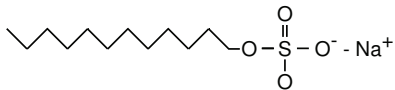
Warning!



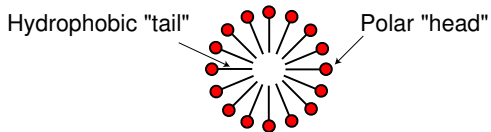
Direction Change

SDS Gel Electrophoresis

SDS - Sodium Dodecyl Sulfate



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

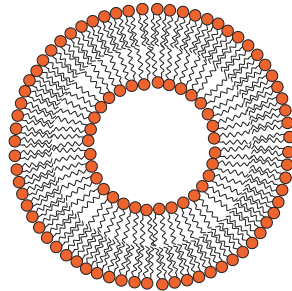


Micelles are three-dimensional, *i.e.*, roughly spherical.

Micelles Versus Vesicles



~ 3 – 50 nm

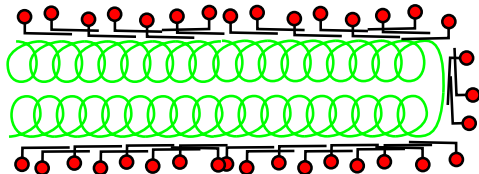


~ 50 nm – 1 μ m

- 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 (\sim conical) and phospholipids (\sim cylindrical).

SDS Denatures Proteins and Binds to Them

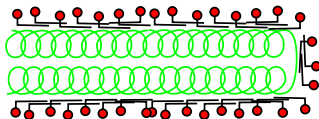
- Most proteins bind SDS at a constant ratio:
 ≈ 1.4 g SDS per g protein.
- Complexes are rod shaped.
- Polypeptides form α -helical structures in SDS.
- A possible structure of SDS-protein complexes:



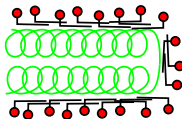
Clicker Question #3

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

A) A large protein with SDS bound:



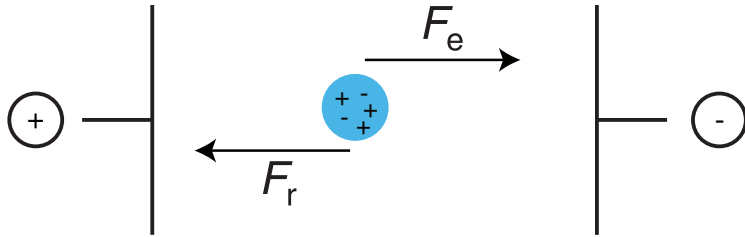
B) A small protein with SDS bound:



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:

$$f \propto \text{Rod Length}$$

$$f \propto \text{Molecular Weight}$$

$$f = C_f \cdot MW$$

- 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:

$$z \propto \text{Molecular Weight}$$

$$z = C_z \cdot MW$$

- C_z is a constant for SDS-protein complexes.

Free Electrophoretic Mobilities of SDS-Protein Complexes

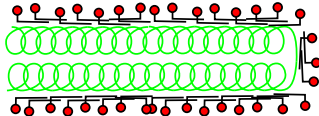
$$M_0 = \frac{z \cdot e}{f} = \frac{C_z \cdot MW \cdot e}{C_f \cdot MW}$$

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

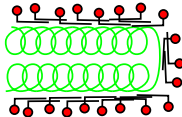
Clicker Question #4

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!

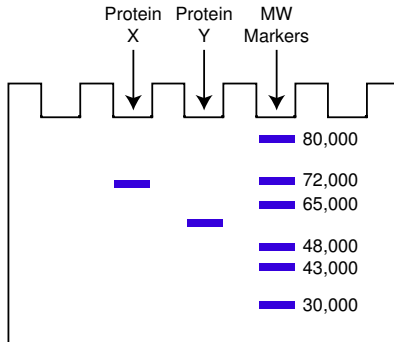
Free Electrophoretic Mobilities of SDS-Protein Complexes

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

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

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?