Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2017

Lecture 19:
Some Basic Principles of Electrophoresis

23 March 2017

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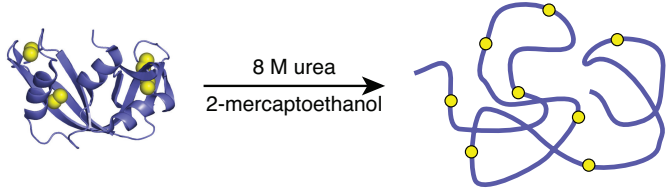
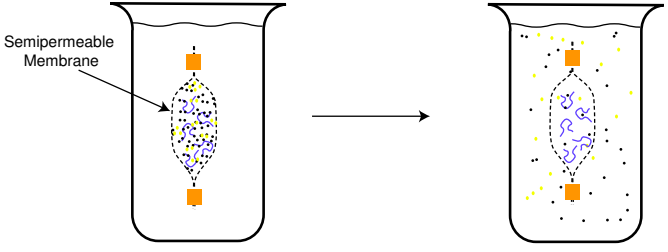
The Anfinsen Experiment

- Unfolding and reduction of RNAse A:



- Removal of urea and 2-mercaptoethanol by dialysis in the presence of O_2:



- Recovery of active RNAse A!

Reformation of Disulfides Can be Prevented by Alkylating the Cys Thiols

- Reaction with iodoacetamide

Protein thiol | Iodoacetamide | Carboxyamidomethylated protein thiol

- Reaction with iodoacetic acid

Protein thiol | Iodoacetic acid | Carboxymethylated protein thiol

- Reactions are essentially irreversible.
- Reactive species is ionized thiol (thiolate).

Three Forms of RNase A for Electrophoresis Experiment

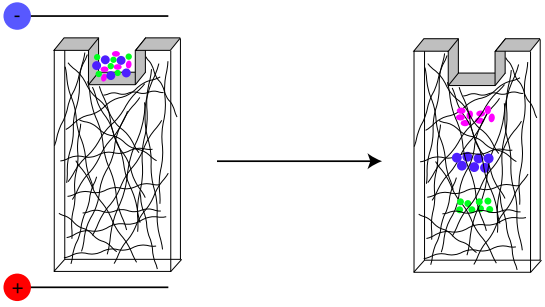
1. Native RNAse A (N). Compact, net positive charge.
2. Reduced and carboxyamidomethylated (RCAM). Less compact than native, same net charge as native.
3. Reduced and carboxymethylated (RCM). Less compact than native, decreased positive charge.

- How will they behave upon electrophoresis?

Outline of Experiment 5

- Day 1:
  1. Preparation of RCAM and RCM RNase
- Day 2:
  1. Non-denaturing gel electrophoresis of N, RCAM and RCM RNase
  2. Trypsin treatment of RNase A forms
- Day 3:
  1. SDS gel electrophoresis trypsin-treated RNase.
  2. Image capture of non-denaturing gel
- Day 3+1 (first day of experiment 6):
  1. Image capture and quantitation of SDS gel

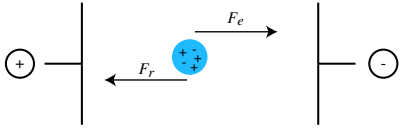
Electrophoresis Through a Gel



Rate of migration through the gel depends on:

- Net charge of protein
- Size and shape of protein.
- Density of gel matrix

Electrophoresis in the Absence of a Gel



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

  z = average net 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 $F_r = F_e$

Clicker Question #1

How long does it take an electrophoresing molecule to reach "terminal velocity"?

1. $< 1\,\text{sec}$
2. $\approx 1\,\text{sec}$
3. $\approx 1\,\text{min}$
4. $\approx 1\,\text{hr}$
5. $> 1\,\text{hr}$

All answers count for now.

Some Numbers for the Electrophoretic Force

Units of force:

$$F = \text{mass} \times \text{acceleration} = \text{mass} \times \Delta\text{velocity/time}$$
$$= \text{kg} \cdot (\text{m/s})/\text{s} = \text{kg} \cdot \text{m} \cdot \text{s}^{-2}$$
$$= 1\,\text{newton} = 1\,\text{N}$$

Electrophoretic force

$$F_e = z \cdot e \cdot E$$
$$e = 1.6{\times}10^{-19}\,\text{coulomb(C)}$$

Assume:

$$z = \text{net charge} = 10$$
$$E = \text{electric field strength} = 200\,\text{Vm}^{-1} = 200\,\text{N/C}$$

Force:

$$F_e = z \cdot e \cdot E \approx 3{\times}10^{-16}\,\text{N} = 3{\times}10^{-16}\,\text{kg} \cdot \text{m} \cdot \text{s}^{-2}$$

Some Numbers for the Frictional Force

Resistive force:

$$F_r = v \cdot f$$

The frictional coefficient for a spherical particle in a viscous fluid:

$$f = 6\pi\eta r \qquad \text{(Stokes' equation)}$$

$\eta =$ viscosity

$r =$ radius

For water at 20°C:

$$\eta \approx 1\,\text{cP} = 10^{-3}\,\text{Kg} \cdot \text{m}^{-1}\text{s}^{-1}$$

For a smallish protein:

$$r = 25\,\text{Å} = 2.5{\times}10^{-9}\,\text{m}$$

Frictional coefficient:

$$f = 6\pi\eta r \approx 5{\times}10^{-11}\,\text{Kg} \cdot \text{s}^{-1}$$

When Forces are Balanced:

- $F_e = F_r = v \cdot f$

$$
\begin{aligned}
v &= \frac{F_e}{f} \\
&= \frac{3\times10^{-16}\,\mathrm{Kg}\cdot\mathrm{m}\cdot\mathrm{s}^{-2}}{5\times10^{-11}\,\mathrm{Kg}\cdot\mathrm{s}^{-1}} \\
&= 6\times10^{-6}\,\mathrm{m}\cdot\mathrm{s}^{-1}
\end{aligned}
$$

- Time to move 5 cm:

$$0.05\,\mathrm{m} \div 6\times10^{-6}\,\mathrm{m}\cdot\mathrm{s}^{-1} \approx 8,000\,\mathrm{s} \approx 2\,\mathrm{h}$$

How long does it take to accelerate to terminal velocity?

- A differential equation:

$$F = ma$$

$$F = F_e - vf = m\frac{dv}{dt}$$

Solution is a function describing velocity as a function of time $v(t)$ for which this equation is valid.

- Solution:

$$v = \frac{F_e}{f}(1 - e^{-t \cdot f/m})$$

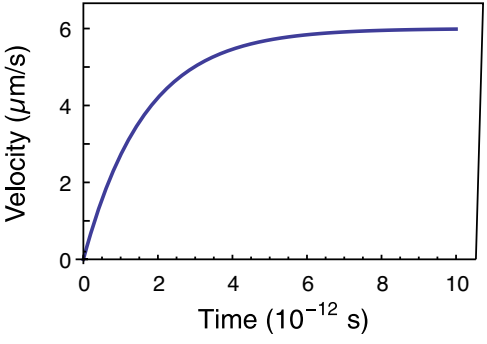
$$x = \text{distance} = \int_{t=0}^{t=t} v dt = \frac{F_e\left(\left(e^{-t \cdot f/m} - 1\right) m + t \cdot f\right)}{f^2}$$

$m = \text{mass}$

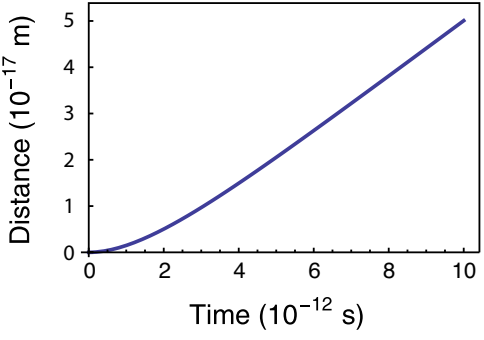
- Assume $m = 50,000\,\text{g/mol} = 8.3{\times}10^{-23}\,\text{Kg/molecule}$

How long does it take to accelerate to terminal velocity?

- Velocity as a function of time:



- Distance as a function of time:



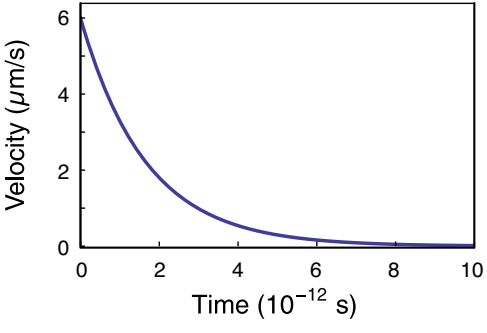
Clicker Question #2

What happens if we turn off the electric field?

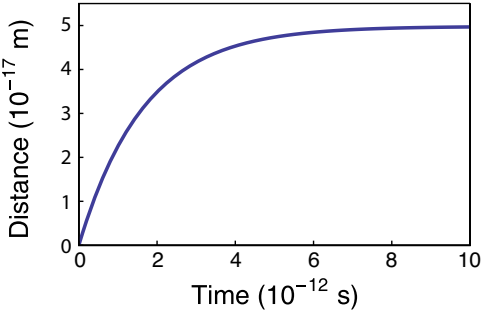
1. The protein keeps coasting.
2. The protein decelerates in about a second.
3. The protein stops almost instantly.

How long does it take to decelerate?

- Velocity as a function of time:



- Distance as a function of time:



- There's no coasting in biochemistry!

Shameless Plug for Another Class

Biology 3550

Physical Principles in Biology

Fall 2017

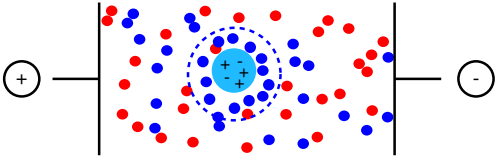
- Diffusion and random walks
- Energy and thermodynamics
- Molecular motors
- And more!

- Satisfies University Quantitative Intensive (QI) requirement

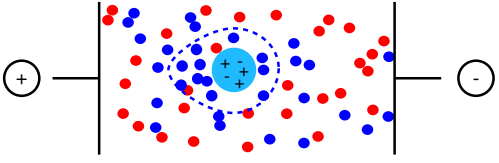
`http://courses.biology.utah.edu/goldenberg/biol3550/`

A More Realistic Description of Electrophoresis

- A particle with a net electric charge attracts a “cloud” of counterions.

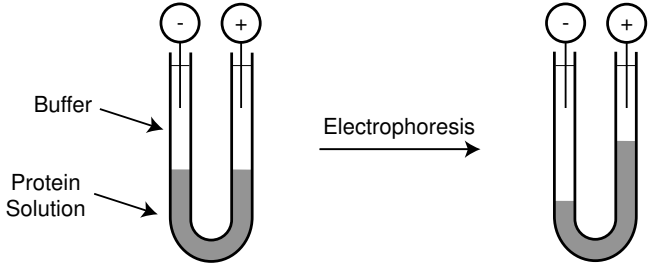


- The cloud is distorted as soon as the particle begins to migrate.



- Describing these effects quantitatively is very difficult.

The Tiselius Free-Boundary Electrophoresis Apparatus



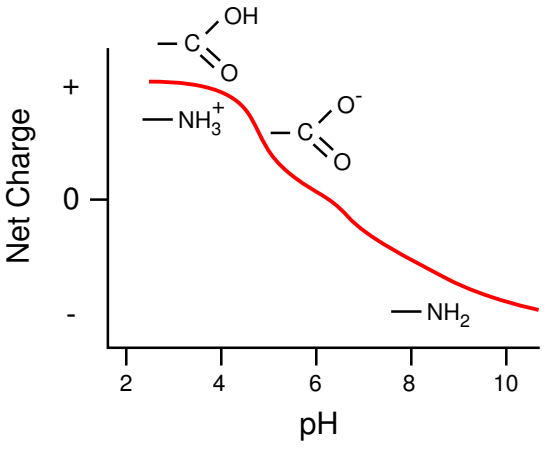
- Movement of solution boundaries is detected optically as electrophoresis progresses.
- Allows measurement of electrophoretic mobilities in free solution.
- Electrophoresis through gels is easier, cheaper and more useful!

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)
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



- 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 molecule has no net charge. This is the isoelectric point, pI.