

Biological Chemistry Laboratory  
Biology 3515/Chemistry 3515  
Spring 2018

Lecture 21:

Some Basic Principles of Electrophoresis

27 March 2018

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University of Utah

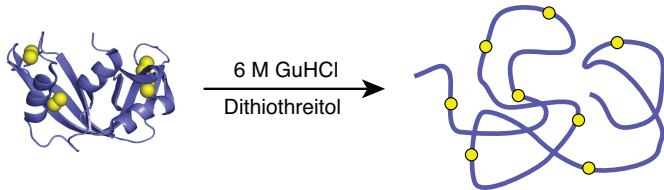
goldenberg@biology.utah.edu

# 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

# Reduction and Alkylation of Ribonuclease A

- Unfolding and reduction of RNase A:



- Carboxyamidomethylation with iodoacetamide



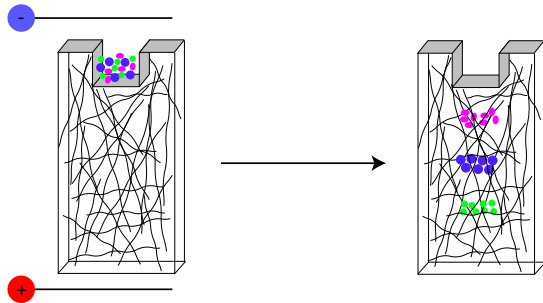
- Carboxymethylation with iodoacetic acid



# 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?

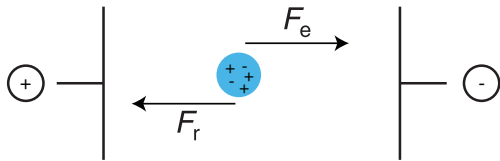
# Electrophoresis Through a Gel



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, a dimensionless number

$e$  = unit of electric charge:  $1.6 \times 10^{-19}$  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  $F_r = F_e$

## Clicker Question #1

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

1 < 1 sec

2  $\approx$  1 sec

3  $\approx$  1 min

4  $\approx$  1 hr

5 > 1 hr

All answers count for now.

# Some Numbers for the Electrophoretic Force

Units of force:

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

Electrophoretic force

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

Assume:

$$\begin{aligned} z &= \text{net charge} = 10 \\ E &= \text{electric field strength} = 200 \text{ V/m} = 200 \text{ N/C} \end{aligned}$$

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:

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

$\eta$  = 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:

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

Frictional coefficient:

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

## When Forces are Balanced:

- $F_e = F_r = v \cdot f$

$$v = \frac{F_e}{f}$$

$$= \frac{3 \times 10^{-16} \text{ Kg} \cdot \text{m} \cdot \text{s}^{-2}}{5 \times 10^{-11} \text{ Kg} \cdot \text{s}^{-1}}$$

$$= 6 \times 10^{-6} \text{ m} \cdot \text{s}^{-1}$$

- Time to move 5 cm:

$$0.05 \text{ m} \div 6 \times 10^{-6} \text{ m} \cdot \text{s}^{-1} \approx 8,000 \text{ s} \approx 2 \text{ h}$$

# How long does it take to accelerate to terminal velocity?

- A differential equation:

$$F = ma = m \frac{dv}{dt}$$

$$F = F_e - vf$$

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

Solution is a function describing velocity as a function of time,  $v(t)$ , for which this equation is valid. ( $F_e$  is fixed by charge of the molecule and electric field.)

- Solution:

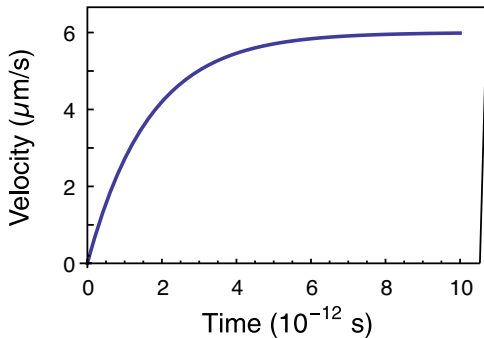
$$v(t) = \frac{F_e}{f} (1 - e^{-t \cdot f/m})$$

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

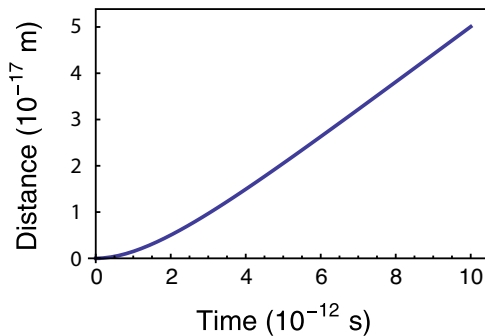
$m = \text{mass}$ , assume  $m = 50,000 \text{ g/mol} = 8.3 \times 10^{-23} \text{ Kg/molecule}$

# How long does it take to accelerate to terminal velocity?

- Velocity as a function of time:



- Distance as a function of time:



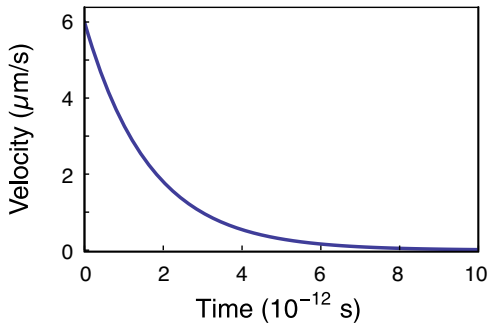
## Clicker Question #2

What happens if we turn off the electric field?

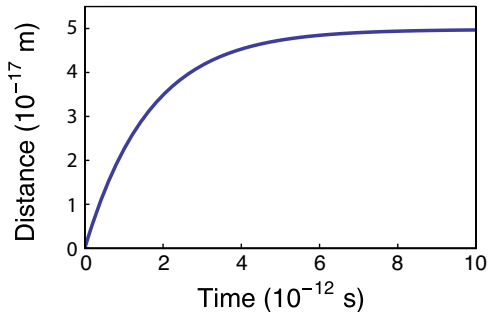
- 1 The protein keeps coasting.
- 2 The protein decelerates in about a second.
- 3 The protein stops almost instantly.

# 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

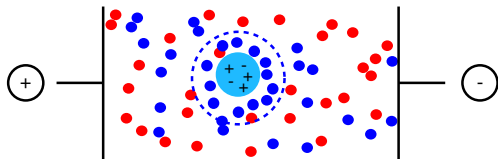
Biology 3550  
Physical Principles in Biology  
Fall 2018

- Diffusion and random walks
- Energy and thermodynamics
- Molecular motors
- And more!
  
- Satisfies University Quantitative Intensive (QI) requirement

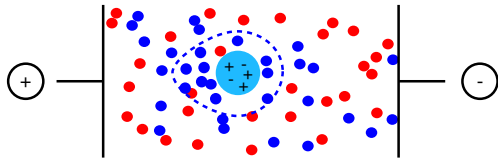
<http://courses.biology.utah.edu/goldenberg/biol3550/>

# A More Realistic Description of Electrophoresis

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



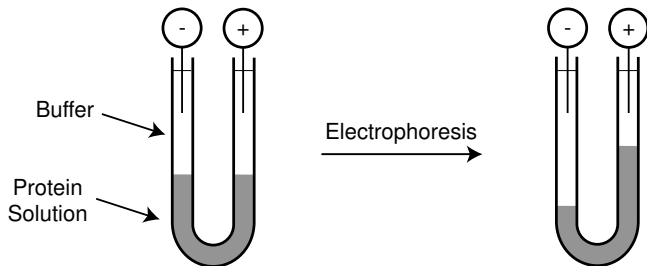
- The cloud is distorted as soon as the particle begins to migrate.



- 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  $pK_a$ s)

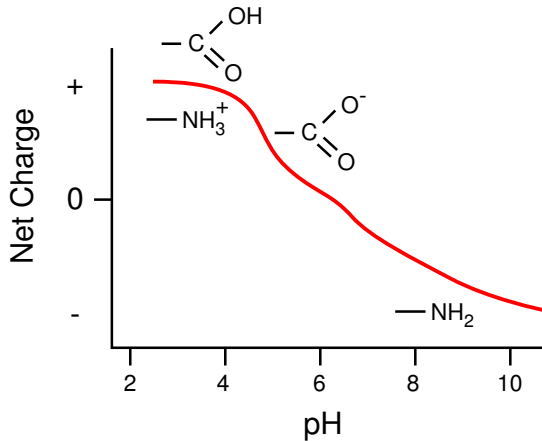
## 2 Size and shape of protein

## 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.