Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 21:

Some Basic Principles of Electrophoresis

28 March 2023

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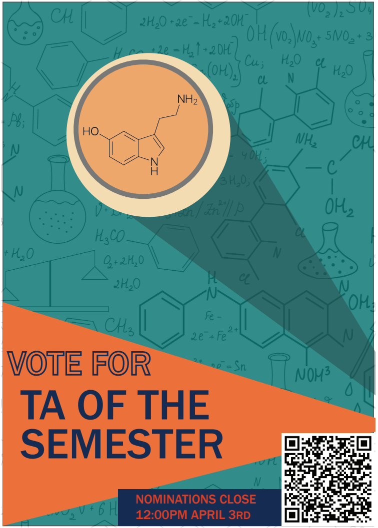
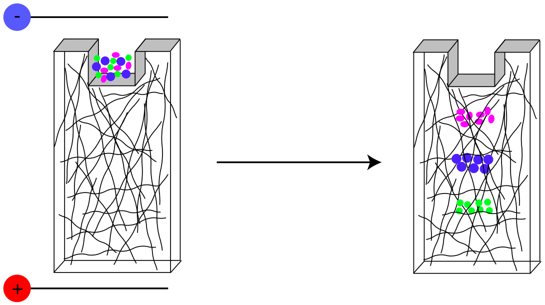
TA of the Semester: Nominate your TA!



Outline of Experiment 5

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

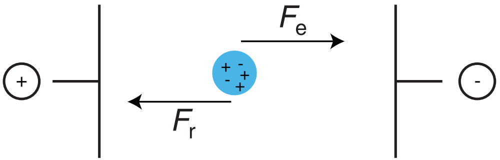
Electrophoresis Through a Gel



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, a dimensionless number

  $e =$ unit of electric charge: 1.6×10^{-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"?

A) $< 1\,\text{sec}$

B) $\approx 1\,\text{sec}$

C) $\approx 1\,\text{min}$

D) $\approx 1\,\text{hr}$

E) $> 1\,\text{hr}$

All answers count for now.

Some Numbers for the Electrophoretic Force

Units of force:

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

Electrophoretic force

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

Assume:

$$z = \text{average net charge} = 10$$
$$E = \text{electric field strength} = 200\,\text{V}/\text{m} = 200\,\text{N}/\text{C}$$

Force:

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

Some Numbers for the Frictional Force

Resistive force:

$$F_\mathrm{r} = v \cdot f$$

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:

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

For a smallish protein:

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

Frictional coefficient:

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

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 satisfied. (F_e is fixed by charge of the molecule and electric field.)

- Solution:

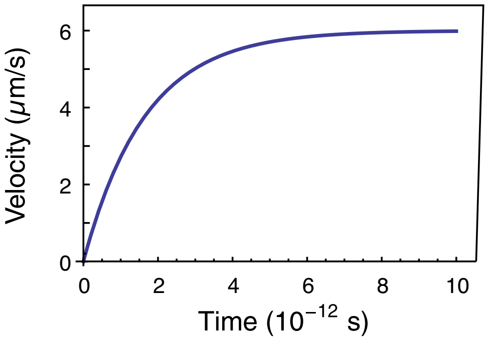
$$v(t) = \frac{F_e}{f}(1 - e^{-t \cdot f/m})$$

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

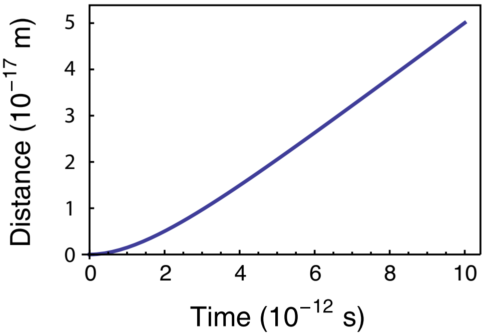
$$m = \text{mass, assume } m = 50,000\,\text{g/mol} = 8.3 \times 10^{-23}\,\text{kg/molecule}$$

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?

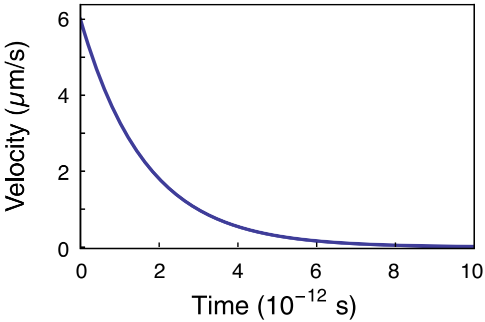
A) The protein keeps coasting.

B) The protein decelerates in about a second.

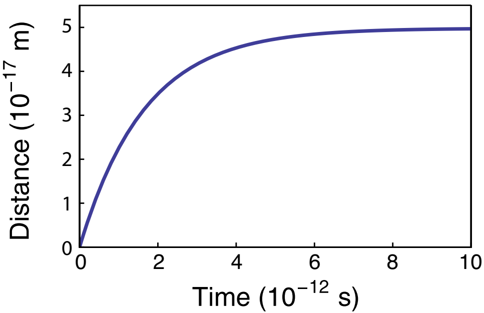
C) The protein stops almost instantly.

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

Spring 2024

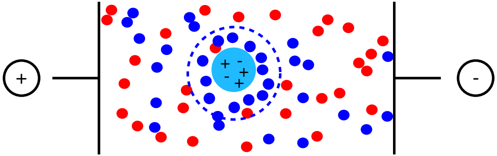
- Probability
- Diffusion and random walks
- Energy and thermodynamics
- Molecular motors
- And more!

- Satisfies University Quantitative Intensive (QI) requirement

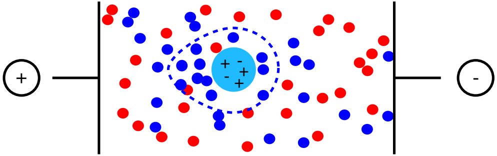
http://goldenberg.biology.utah.edu/courses/biol3550/

A More Realistic Description of Electrophoresis

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

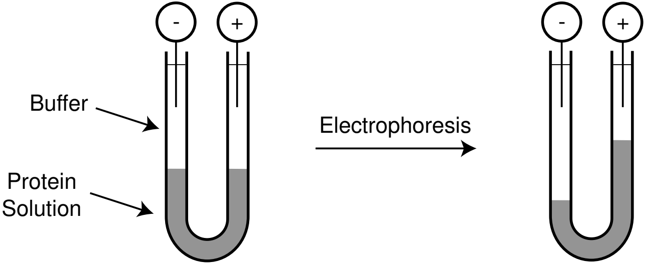


- The cloud is distorted as soon as the particle begins to migrate.



- Describing these effects quantitatively is very difficult.

The Tiselius Free-Boundary Electrophoresis Apparatus



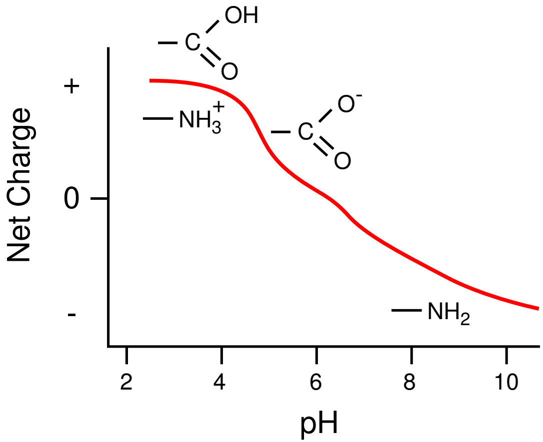
- Movement of solution boundaries is detected optically as electrophoresis progresses.
- Allows measurement of electrophoretic mobilities in free solution.
- Electrophoresis through gels is easier, cheaper and more useful!

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 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:
  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