Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2017

Lecture 18:
Introduction to Electrophoresis and Thiol-disulfide Chemistry

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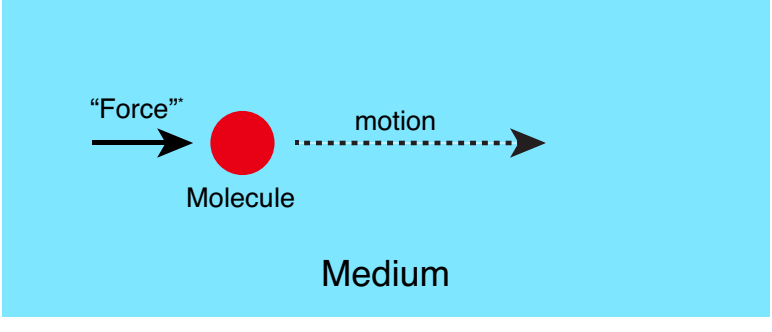
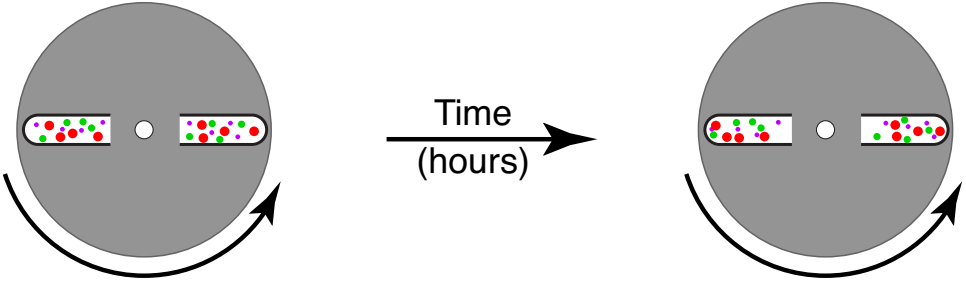
Separation Methods: The General Idea



- Something (a "force"*) causes molecules to move through a medium.
- The rate of motion depends on the strength of the force and the interactions of the molecules with the medium.
- Different kinds of molecules move at different rates, allowing them to be separated.

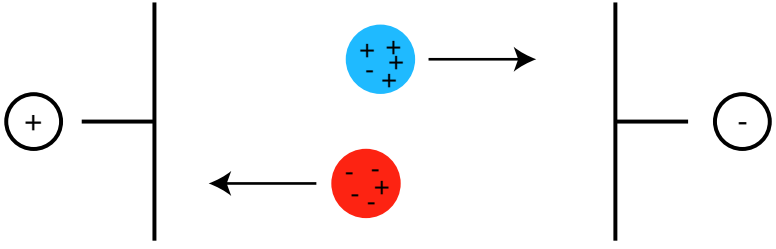
* "Force" is used rather loosely here to describe anything that causes motion of the molecules.

Centrifugation

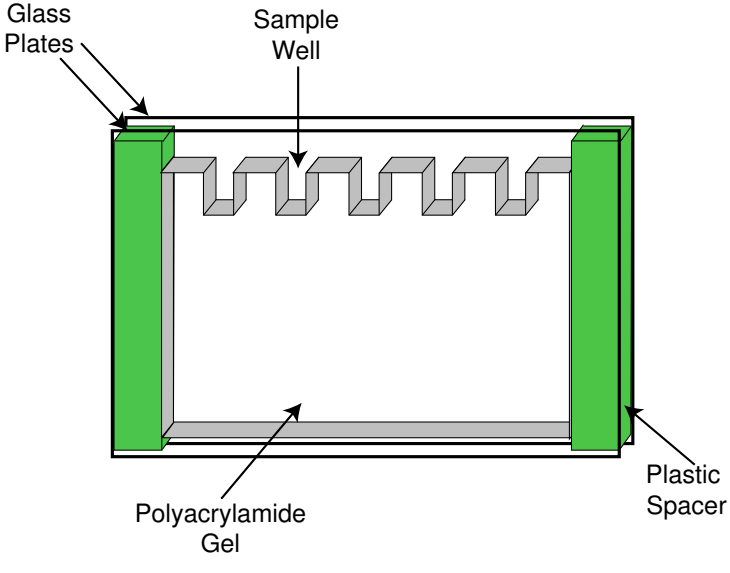


- “Centrifugal force” moves molecules outward from the center of the rotor.
- Rate of motion depends on magnitude of centrifugal force and friction between molecules and solvent.
- Larger molecules move faster than smaller ones, allowing them to be separated. (shape also has an effect)
- An ultracentrifuge: A centrifuge capable of separating ultra-small particles. Invented by Theodor (The) Svedberg in the 1920s and 30s.

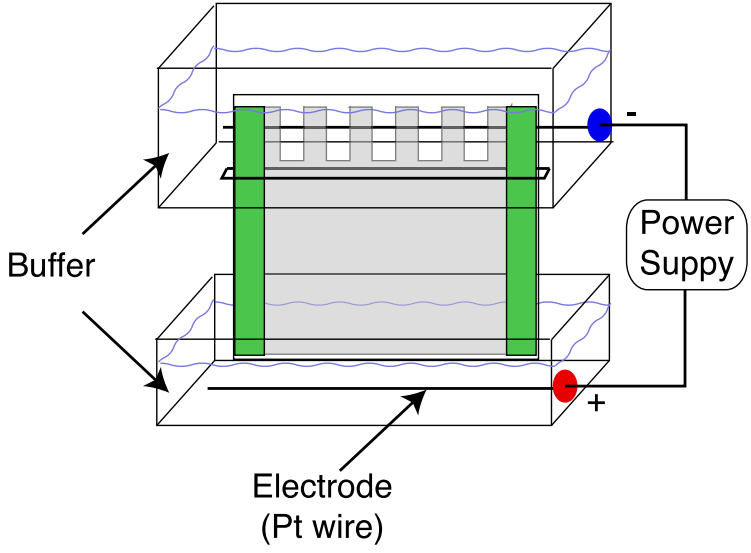
Electrophoresis:
Separation based on movement in an electric field



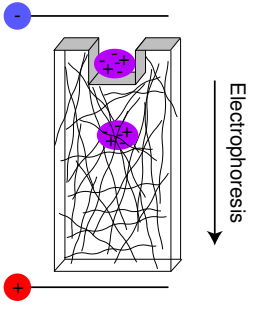
A Gel “Sandwich” for Electrophoresis



Apparatus for Gel Electrophoresis



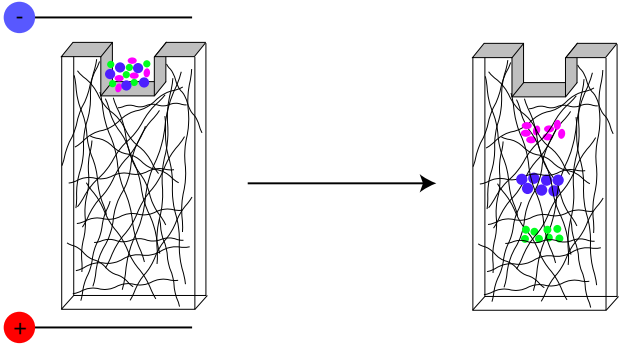
Electrophoresis Through a Gel



Rate of migration through the gel depends on:

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

Separation of Proteins by Electrophoresis

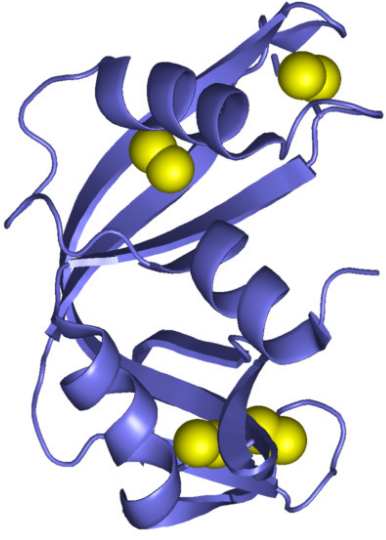


- Proteins with different mobilities migrate as “bands” in the gel.

Two Major Variants of Gel Electrophoresis for Proteins

1. Non-denaturing ("Native") electrophoresis.
   - Carried out in the absence of denaturants, though sometimes relatively low or high pH values are used.
   - Protein migrates through the gel on the basis of its net charge, size, shape and sieving effect of the gel.
2. SDS gel electrophoresis
   - Proteins are denatured in the presence of sodium dodecyl sulfate (SDS), a detergent that denatures proteins and complexes.
   - Mobilities reflect molecular weights of polypeptide chains.
   - Very useful for analyzing complex samples and macromolecular complexes composed of multiple polypeptides (*e.g.,* viruses, organelles, membranes).

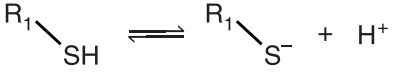
Ribonuclease A: A “Classic” Protein Stabilized by Disulfide Bonds



- Hydrolyzes RNA, much as trypsin hydrolyzes proteins.
- Like trypsin, made in pancreas.
- A favorite protein for chemical, enzymatic and structural studies in the 1950s and 1960s.
  Two Nobel prizes (4 awardees).
- Produced in large quantities (kilogram) by the Armour Meat Packing Company after World War II, and provided free and without constraints to scientists.
- Close relatives are cytotoxic and are being explored as anti-cancer agents.
- Presence of 4 disulfide bonds allows some neat chemical manipulations of the protein.

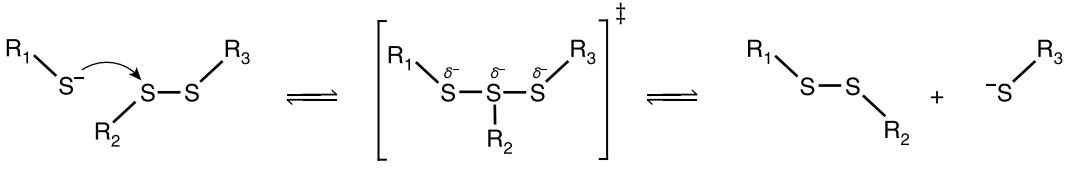
Thiol-disulfide Exchange Chemistry

- Reactive species is the ionized thiol group, a thiolate:



  - Thiol pK_as are about 8–9.
  - Un-ionized thiol is not very reactive.
  - Ionized Cys is the most reactive of all amino-acid side chains.

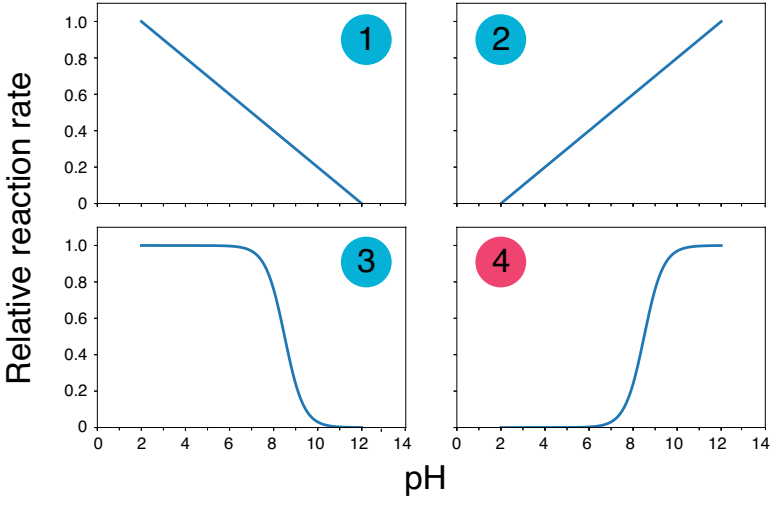
- Exchange reaction:



  - Reaction is an SN2 nucleophilic substitution.
  - For the ionized thiolate, the second-order rate constant is about $20\,s^{-1}M^{-1}$

Clicker Question #1

How does the reaction rate change with pH? (All answers count for now!)

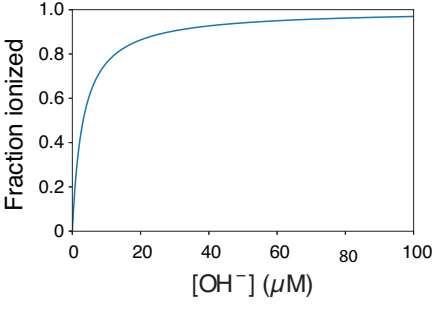


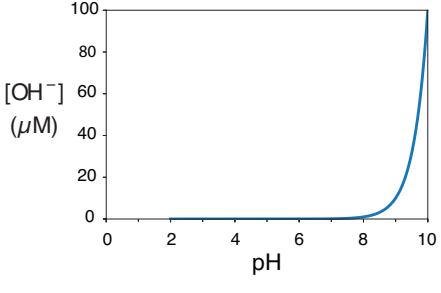
Why Does the Reaction Rate vs. pH Curve Have the Shape it Does?

- The reaction rate is proportional to the fraction of molecules in which the thiol is ionized.

$$f_{\text{ion}} = \frac{[\text{R}-\text{S}^-]}{[\text{R}-\text{S}^-] + [\text{R}-\text{SH}]}$$

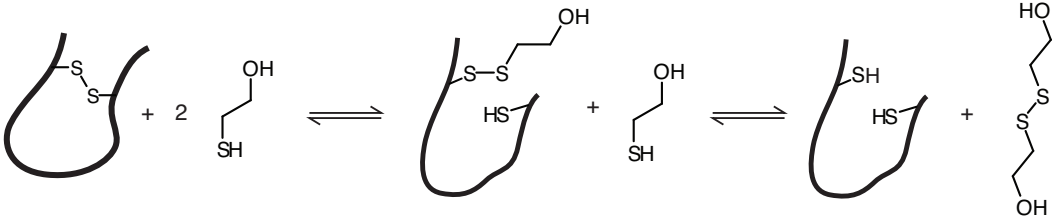
- The fraction ionized increases as the pH increases, and as the concentration of OH^- increases.
- The concentration OH^- increases in proportion to 10^{pH}
- The two curves combine to give a curve that represents the fraction ionized as a function of pH.



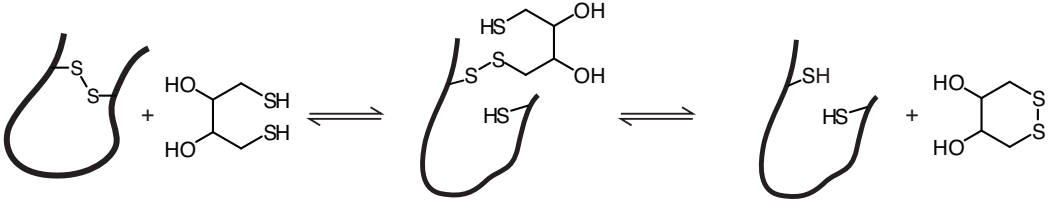


Reduction of Protein Disulfides by Thiol-Disulfide Exchange

- By 2-mercaptoethanol (β-mercaptoethanol, BME)



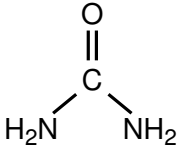
- With dithiothreitol (DTT, Cleland's reagent)



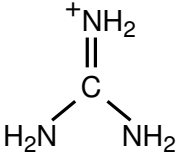
- Which reaction is faster, at equal reagent concentrations?

Reduction of Disulfides in RNAse A

- Rate is much higher in presence of strong denaturants, such as 8 M urea or 6 M GuHCl (guanidinium chloride).



Urea

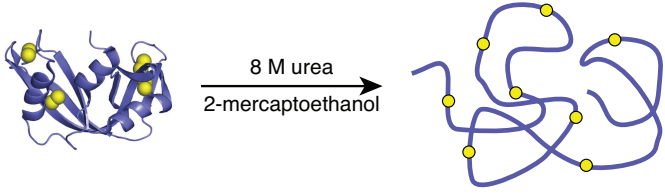


Guanidinium

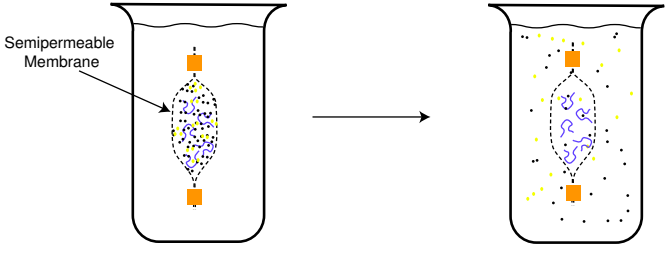
- Urea and GuHCl destabilize folded proteins. Why?
  Probably by weakening the hydrophobic effect. ~~Probably by weakening the hydrophobic effect~~
  Probably by interacting with the polypeptide backbone (as of 2017).

The Anfinsen Experiment

- Unfolding and reduction of RNAse A:



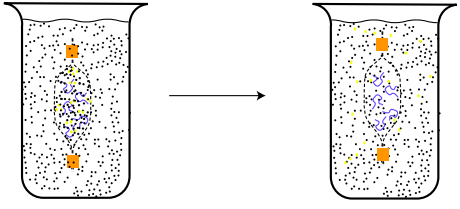
- Removal of urea and 2-mercaptoethanol by dialysis in the presence of O_2:



- Recovery of active RNAse A!

Anfinsen Experiment: Part II

- Reduce and unfold RNAse A, as before.
- Remove 2-mercaptoethanol and form disulfides, without removing urea.



8 M urea in the dialysis buffer.

- Recover only about 1% RNAse A activity.
- Conclusions:
  - Information to specify the native structure is contained within the amino acid sequence and its interactions with solvent.
  - Disulfides and non-covalent interactions act together to stabilize the native structure.
- Nobel Prize in Chemistry to Christian B. Anfinsen, 1972.