Gel Electrophoresis

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Electrophoretic Experiments

- Free Electrophoresis or Moving Boundary Electrophoresis
  - Done in solution with no support Medium
  - No longer widely used due to problems resulting from the formation of convection currents in the solution from heating.
  - Was widely Used as a Structural Probe
- Steady-State Electrophoresis
  - Membrane confined electrophoresis
  - Allows for the calculation of Diffusion Coefficient and macromolecular charge.
- Zonal Electrophoresis
  - Done using a gel as support medium

Table D5.1. *A summary of electrophoretic approaches used in research*

Experimental method	Experimental conditions	Calculated parameter	Analogues in hydrodynamics
Moving boundary electrophoresis	Free electrophoresis	Electrophoretic mobility	Velocity sedimentation
One-dimensional capillary electrophoresis	Free electrophoresis	Electrophoretic mobility	Velocity sedimentation
Steady-state electrophoresis	Membrane confined analytical electrophoresis in zero-field and non-zero field	Diffusion coefficient, charge	Diffusion coefficient using ultracentrifugal boundary-forming cell
Isoelectric focusing in gel	Gradient of pH	Isoelectric point, where mobility is zero	Equilibrium centrifugation in gradient density
Zonal one-dimensional gel electrophoresis	SDS	Separation according to friction properties/mass	Equilibrium centrifugation in gradient density
Zonal two-dimensional gel electrophoresis	Gradient of pH (first dimension) + SDS (second dimension)	Separation according to: charge (first dimension), friction properties/mass (second dimension)	Equilibrium centrifugation in gradient density
Electric birefringence (Kerr effect) (Chapter D6)	Electrical torque	Spectrum of relaxation times	Flow birefringence (Maxwell effect) (Chapter D7)

Moving Boundary Electrophoresis

- Charge is a fundamental property of a macromolecule that is linked to its structure solubility, stability and interactions.
- Therefore the force on a macromolecule with charge Q exposed to an electric field is given by:

$$\mathbf{F} = Q\mathbf{E}$$

- So shortly after the application of the electric field, the particle reaches a steady-state velocity u with the particle moving towards one of the electrodes. At this velocity, friction forces are equal and opposite the applied force.

$$u = QE/f \qquad \text{(D5.2)}$$

where f is translational coefficient of the macromolecule.

Electrophoretic Mobility

The transport of charged particles under the influence of an electric field is called *electrophoretic mobility* (Comment D5.1). The electrophoretic mobility μ can be defined as the velocity per unit field

$$\mu = u/E \qquad \text{(D5.3)}$$

so $$Q = \mu f$$

Stated more commonly as the Huckel Equation

$$\mu = Ze/f \qquad \text{(D5.6)}$$

where the Z is the number of charges, e is the magnitude of the electron charge and f is the frictional coefficient of the charged macromolecule.

- If the particle happens to be spherical, Stoke' Law applies and we can write the electrophoretic mobility coefficient with the translational f in terms of spherical hydrodynamics :

$$\mu = Ze/6\pi\eta_0 R_0 \qquad \text{(D5.7)}$$

where R_0 is the particle's radius and η_0 is the viscosity of the solvent.

Counterion and Ion Atmosphere Effects

- In any aqueous solution there are counterions.
- Since electrophoresis involves the transport of a charged macromolecule, these counterions associate with it and contribute to its net charge.
- In order to weaken the the effects of the counterion pairing on the macromolecule a large amount of electrolyte is introduced in the the solution.
- The electrolyte forms an ion atmosphere around the macromolecule and its associated counterions.

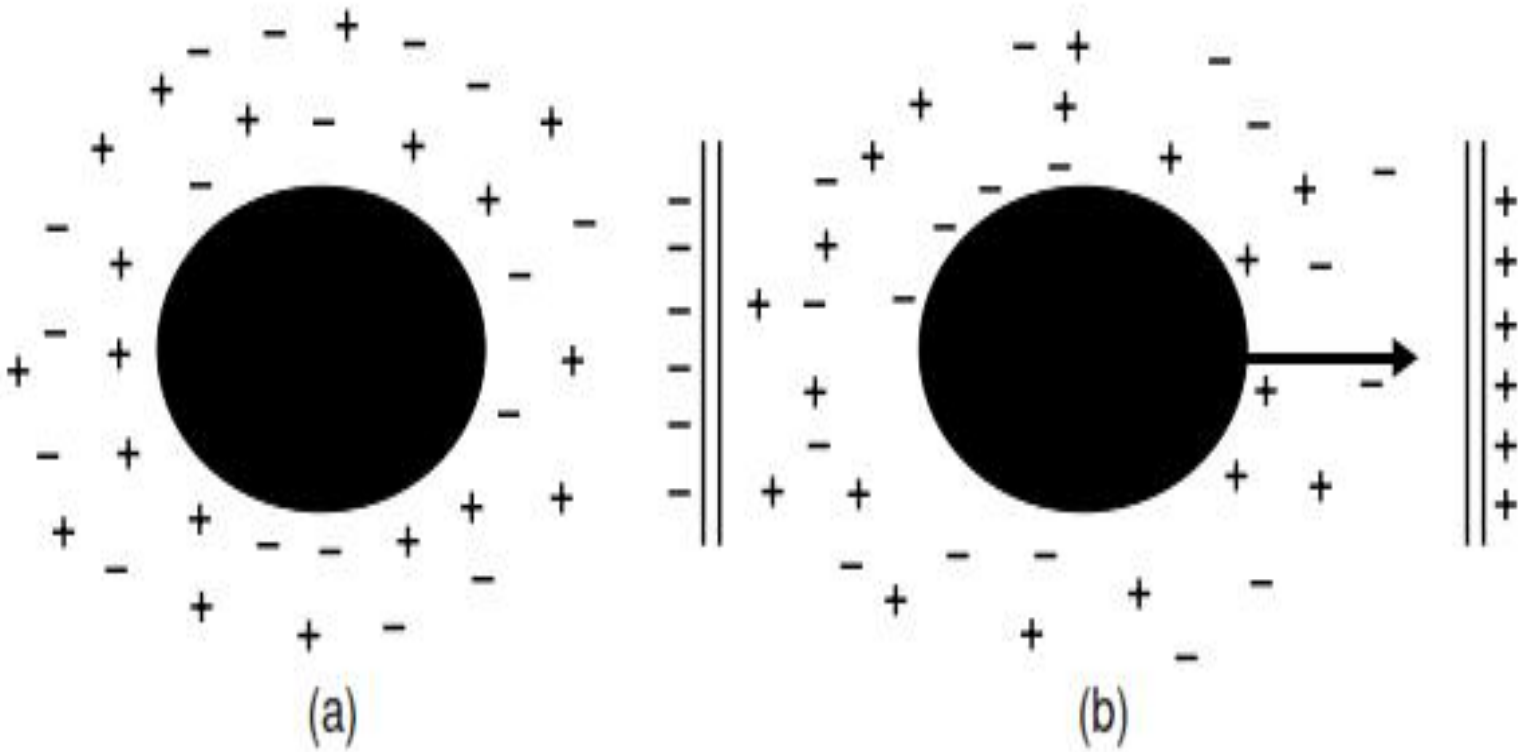


Fig. D5.1 Macromolecules in electric field. (a) In the absence of an electric field, the small ions form an ion atmosphere around the macromolecules in which ions of opposite charge to that of the macromolecules predominate. (b) In the presence of an electric field, the ion atmosphere is distorted by the field and by the motion of the macromolecules. (After Cantor and Shimmel, 1980.)

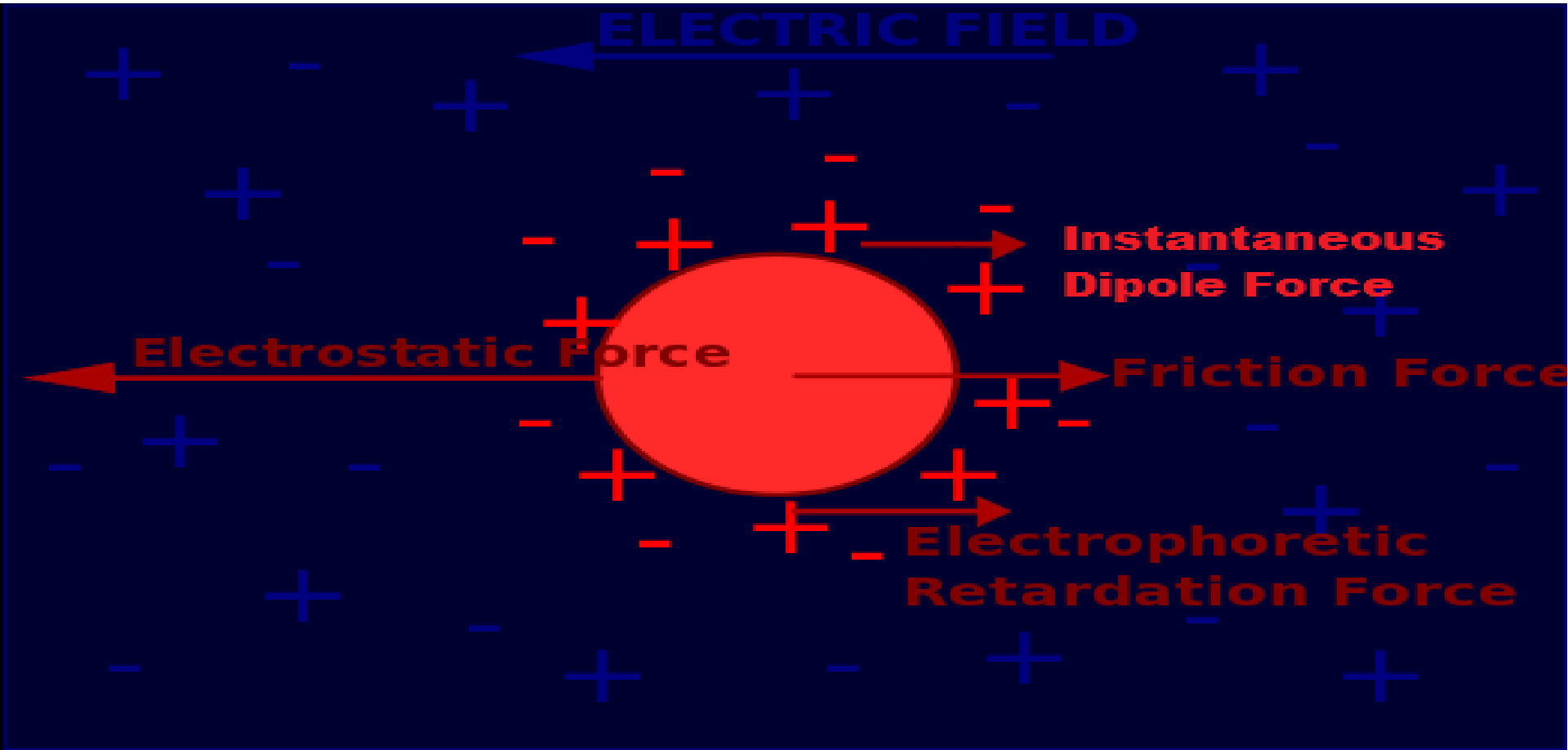
What really happens...

- Consequently a realistic description of the electrophoretic mobility of any macromolecule must take into account the effects of:

1. the electric field on the charge Q of the molecule
2. its associated counterions
3. the ion atmosphere surrounding it.

Actual Force Diagram

- In actuality there are 4 forces acting on a macromole during free electrophoresis



A More Realistic Model involving Effective Charge

- Therefore, a more complete computation of the macromolecule velocity u is of the form

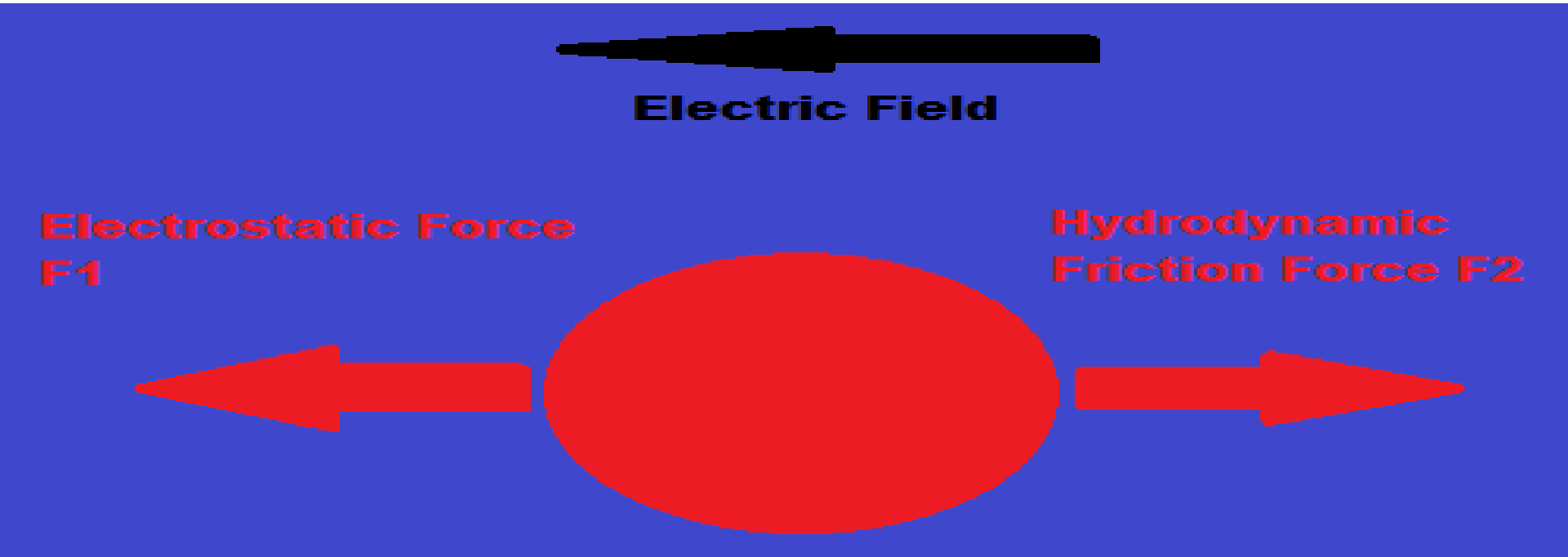
$$u = Q_{eff} * E/f$$

- However, there are no experimental methods to date to determine the effective charge independently of other macroion properties
  - Since it is very difficult to determine the effective charge Q_{eff} of a macromolecule in solution we will only be concerned with the idealized case of only 2 forces acting on the

“Simplified” Force Diagram

Forces of Interest:

1. Electrostatic force resulting from application of the electric field to the macromolecule.
2. The Hydrodynamic friction force associated with the the macromolecular flow in solution



Steady-state electrophoresis (SSE)

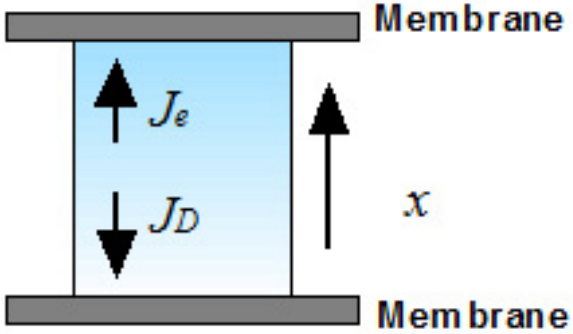
-
- In SSE macroions are trapped in a small chamber whose top and bottom are sealed with semipermeable membranes.

- An Electric Field is applied along the chamber so that the macroions crowd up against one of the membranes

SSE Continued

- Diffusion produces a macroion flux in the opposite direction of the applied electric field.
- When steady state is reached, the flux due to electrophoresis and the flux due to diffusion are balanced.

$$J_e = J_D$$



- Therefore in SSE both the fluxes and the forces are balanced. At any point x in the cell, the flux due to electrophoresis J_{eff} is

$$C \times u'$$

where C is the concentration of macroions at x and u' is their velocity. the Flux Jeff is the effective flux resulting from all of the forces F_1, F_2, F_3, F_4. since:

$$f_{eff}/u' = QE$$

Similarly $u' = QE/f_{eff}$

where f_{eff} is the frictional coefficient produces by the forces F_1, F_2, F_3, F_4

- Recalling that J_{eff} is C x u' we can write:

$$J_{eff}=(QE/f_{eff})C$$

- Further, Recalling Fick's first Law…

- and that Concentration Gradient produce flux due to Diffusion so:

$$dC/dt = Dd^2C/dx^2 \qquad \text{(D3.7)}$$

- At steady state $J_{eff}+ J_D=0$ hence adding J_D to J_{eff} and setting them equal to 0 yields:

$$(QE/f_{eff})C=DdC/dx$$

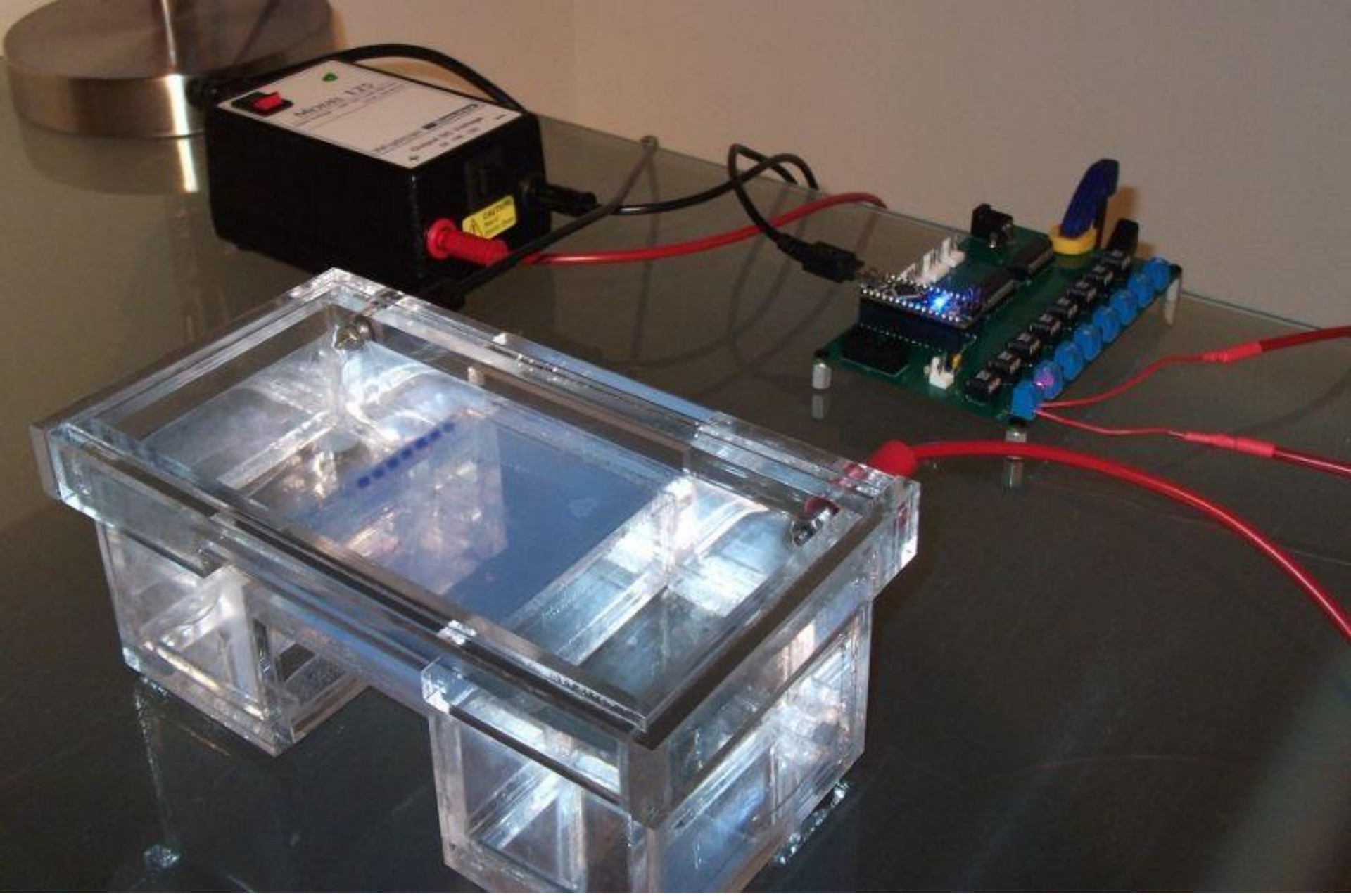
- The solution to $(QE/f_{eff})C=DdC/dx$ is

$$C = C_0 \exp\left[\sigma(x - x_0)\right] \qquad \text{(D5.13)}$$

where C_0 is the concentration at a reference point x_0 and the exponent $\sigma \equiv QE/f_{\text{eff}}D_{\text{eff}}$ includes D_{eff}, the diffusion coefficient in the presence of the electric field. Now $Q = \sigma f_{\text{eff}}D_{\text{eff}}/E$. Assuming that the relation $f_{\text{eff}} = kT/D_{\text{eff}}$ is valid we have

$$Q = \sigma kT/E \qquad \text{(D5.14)}$$

Ultimately $Q=\mu f$ provides a simple way of determining the effective charge of the molecule directly from experimental measurements sigma, E and T unlike mobility measurements which also require knowledge of f_{eff} or D_{eff}



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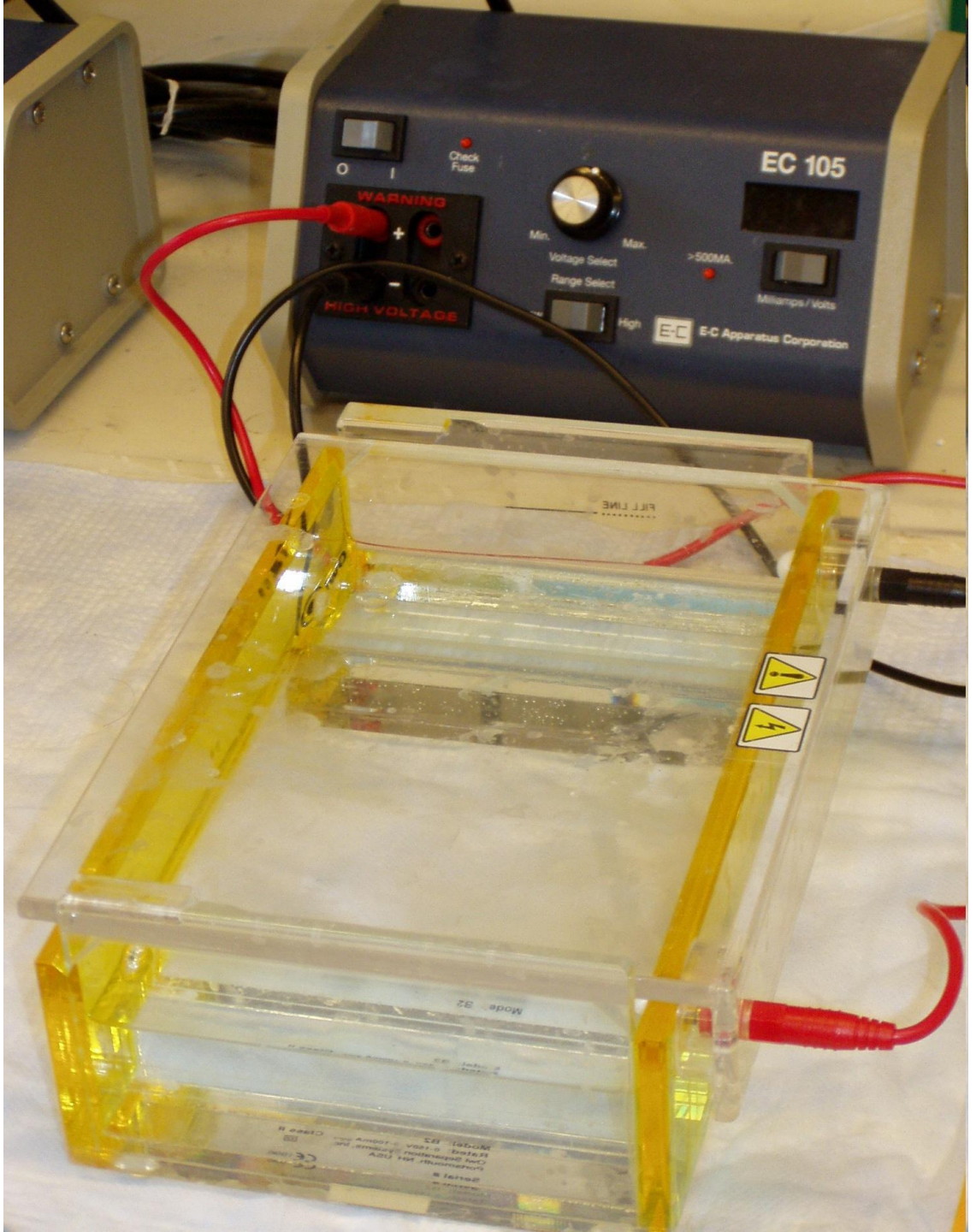
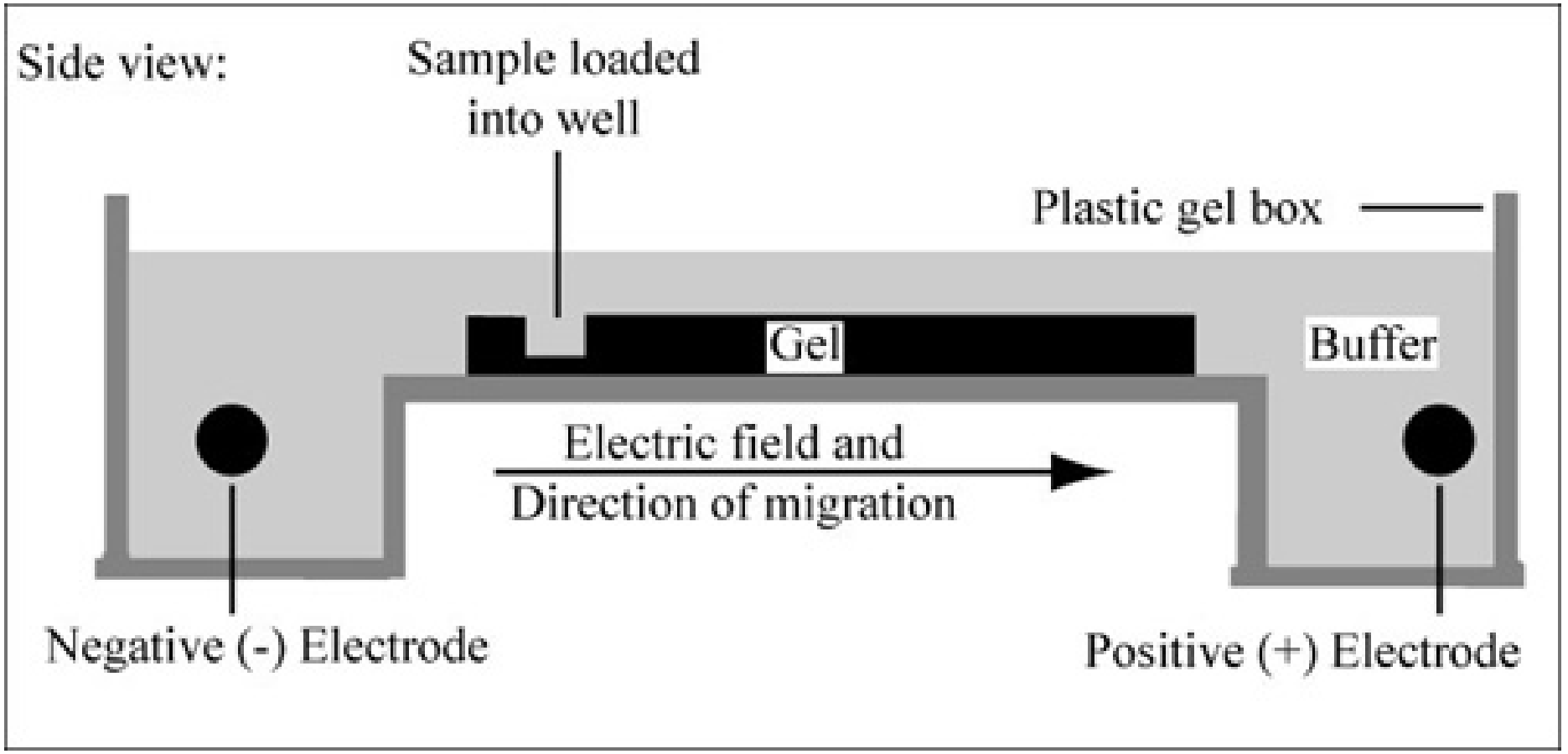
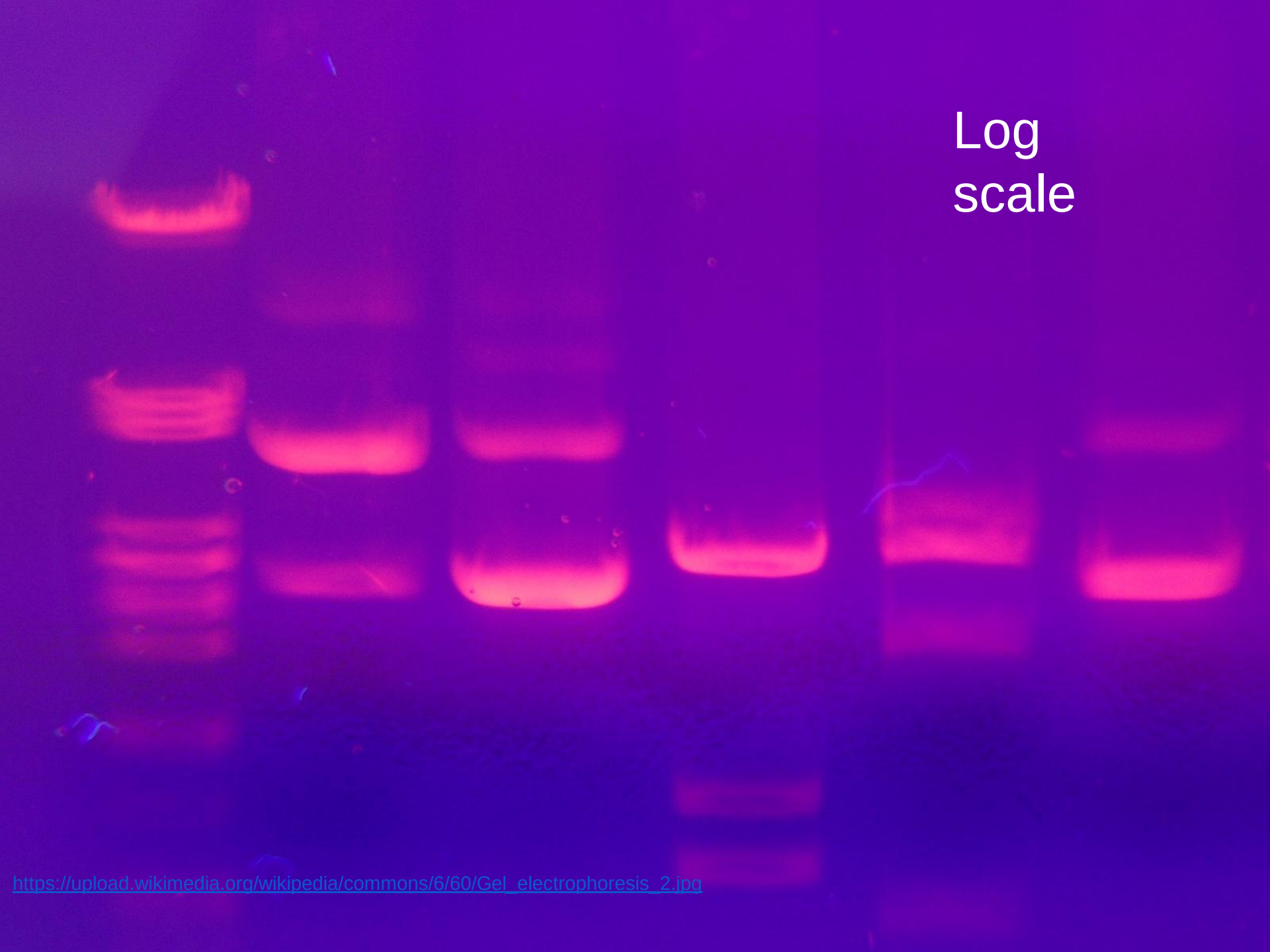
https://upload.wikimedia.org/wikipedia/commons/a/a6/Gel_electrophoresis_apparatus.JPG





http://ocw.mit.edu/courses/biological-engineering/20-109-laboratory-fundamentals-in-biological-engineering-fall-2007/labs/mod1_2_photo.jpg



https://upload.wikimedia.org/wikipedia/commons/6/60/Gel_electrophoresis_2.jpg

Free Solution vs Mechanical Support

Free = bad

- convection currents
- diffusion

Early porous mechanical supports

- filter paper and cellulose acetate strips
- small molecules

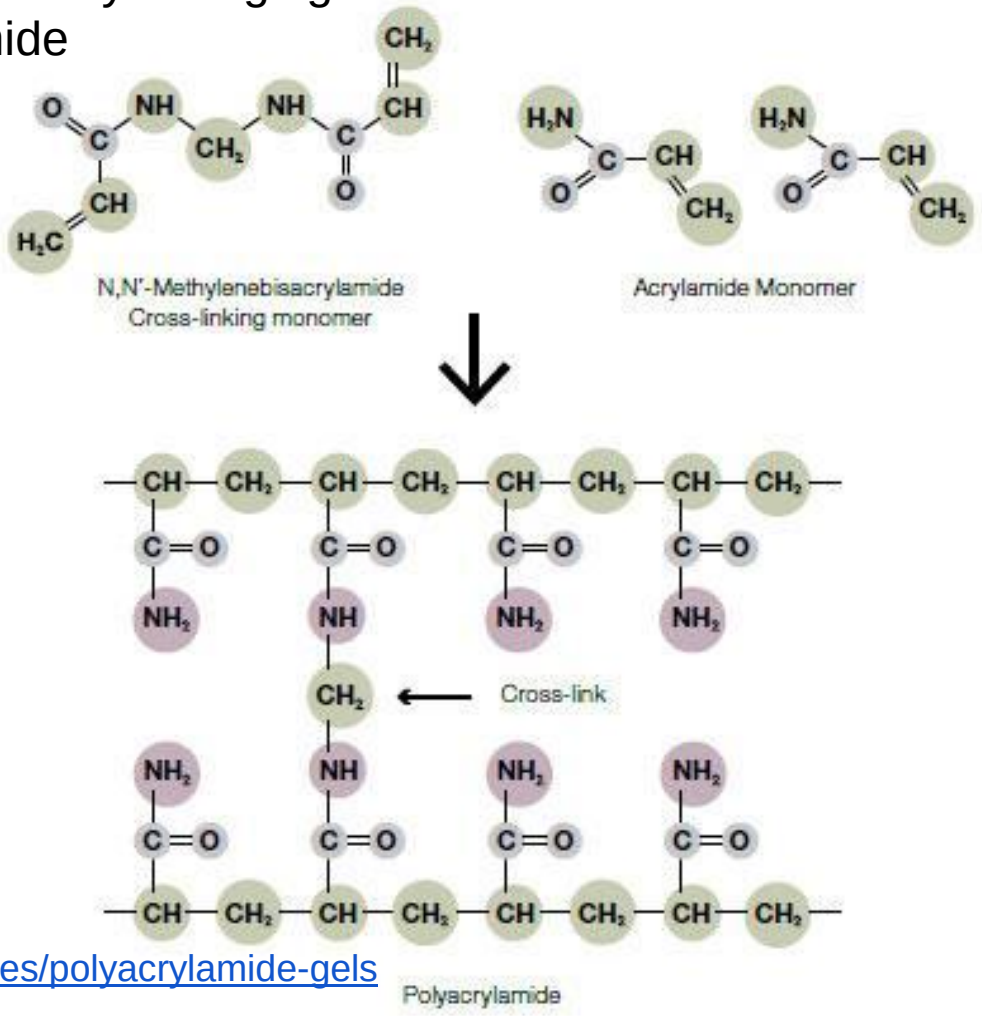
Gels = Better

Agarose Gel

- linear polysaccharide from certain types of seaweed
- easy to make:
  - mix agarose w/buffer
  - boil
  - pour and let sit
  - celebrate
- good for separating large amounts DNA by length (50-20,000 bp length wikipedia)

Polyacrylamide (PAGE)

- polymerisation of acrylamide monomers in the presence of small amounts of comonomer (bisacrylamide)
- the pore size in the gel can be varied by changing the concentration of both the acrylamide and the bisacrylamide
- 5 to 2,000 kDa
- “Polyacrylamide is ideal for protein separations because it is chemically inert, electrically neutral, hydrophilic, and transparent for optical detection at wavelengths greater than 250 nm. Additionally, the matrix does not interact with the solutes and has a low affinity for common protein stains.”



http://www.bio-rad.com/en-us/applications-technologies/polyacrylamide-gels

SDS-PAGE

Sodium dodecyl sulfate (SDS)

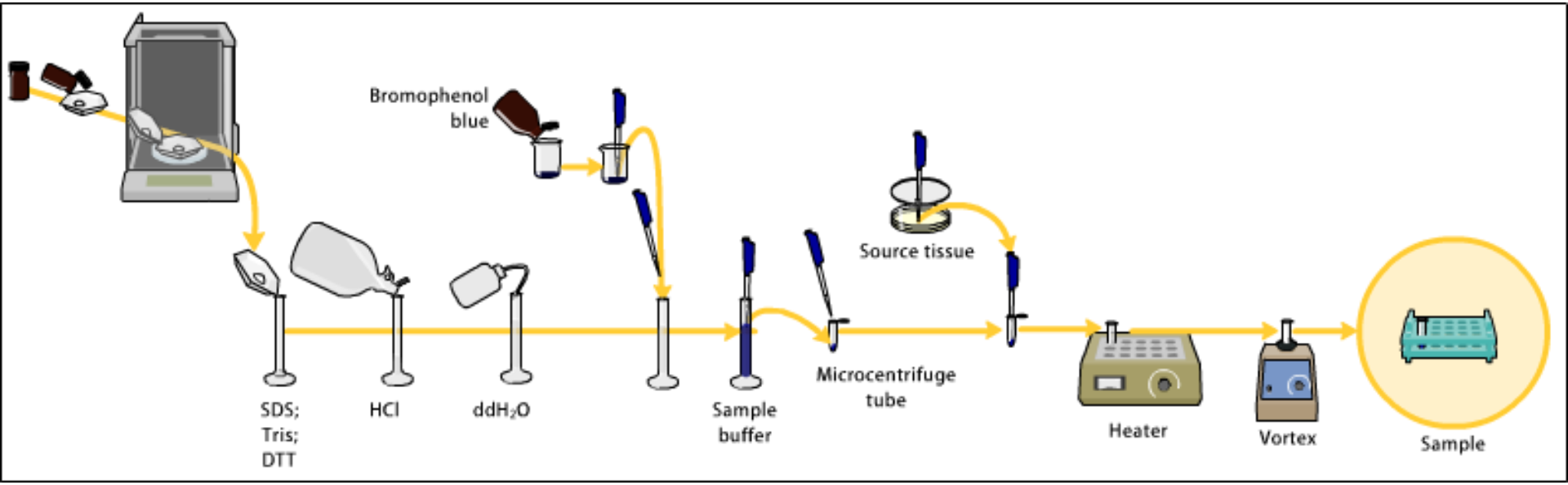
- linearize proteins (reduces to primary structure)
- imparts a negative charge (even distribution of charge per unit mass) → separation by mass
- denatured protein is a rod-shaped structure with negative SDS molecules attached

Beta-mercaptoethanol breaks disulfide bonds

Urea used to break down nucleic acids

Preparing the sample

Heating and reducing agents may be added to help with the denaturation process



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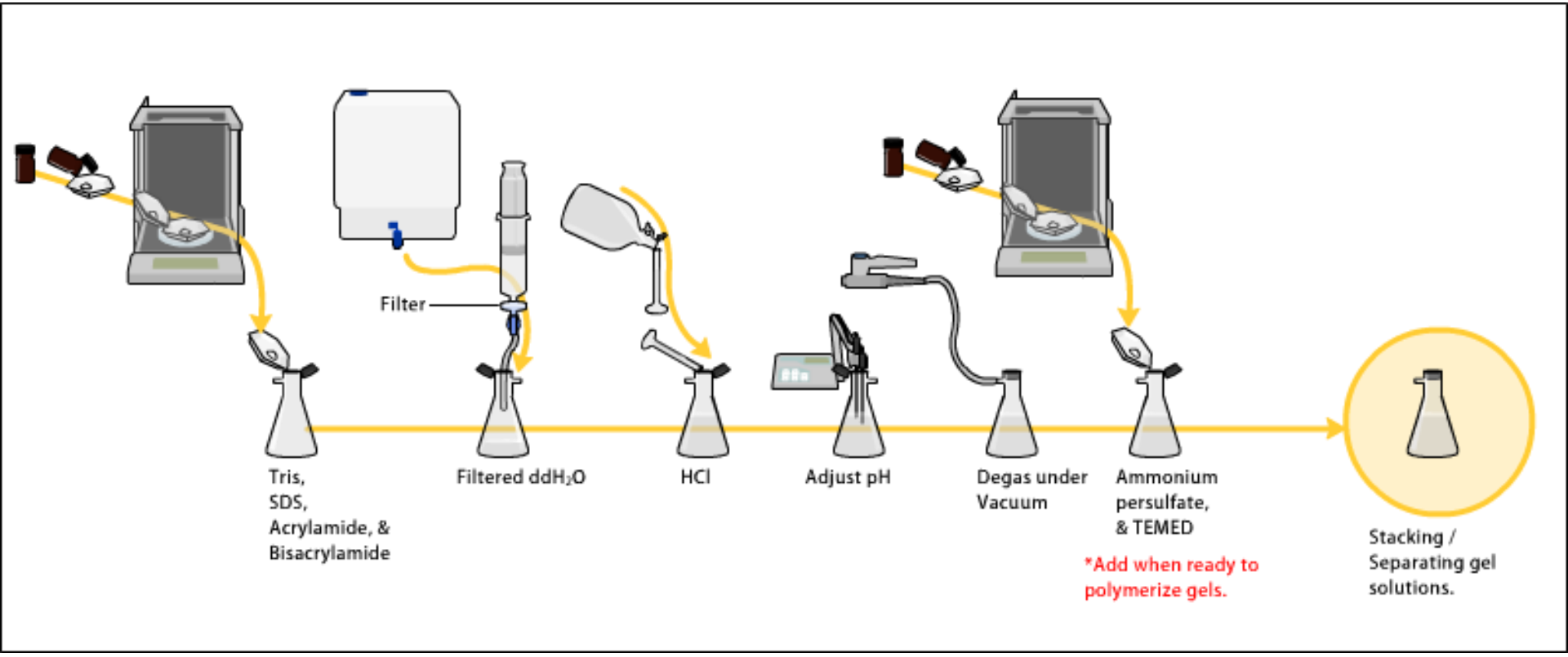
Preparing the acrylamide gel

The acrylamide concentration of the gel can also be varied, generally in the range from 5% to 25%. Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages are needed to resolve smaller proteins.



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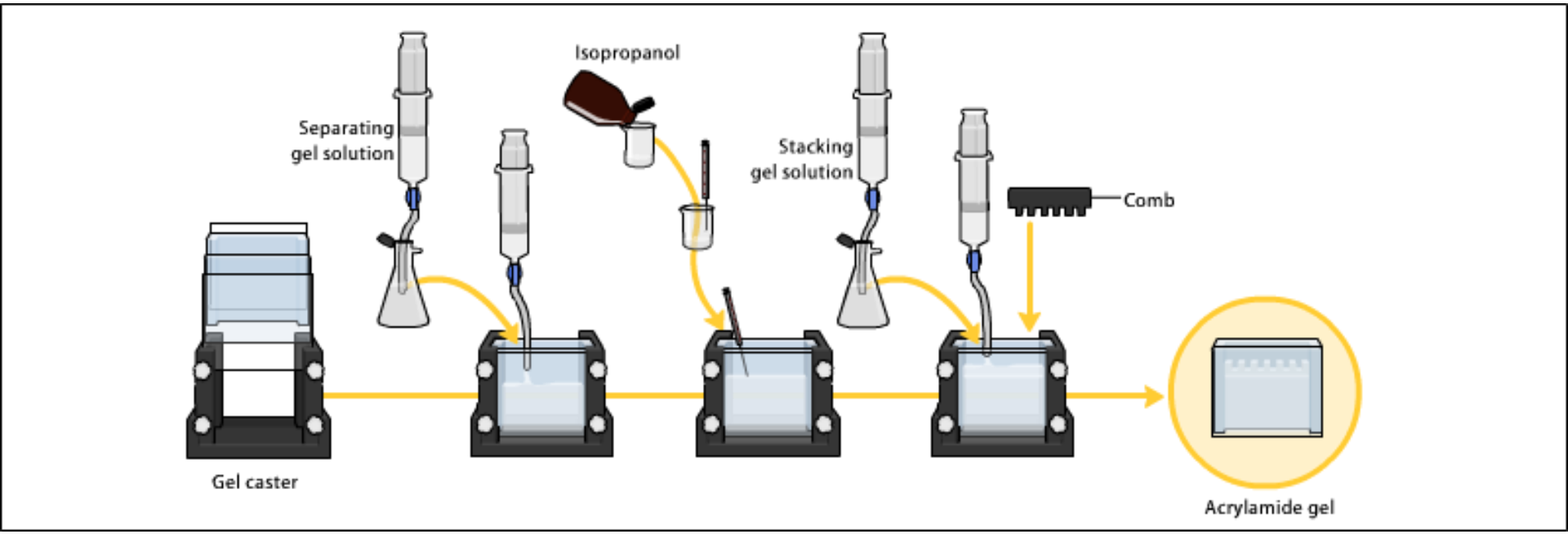
Casting the gel



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Stains

silver stain

Coomassie Brilliant Blue (need to destain polyacrylamide gel w/acetic acid)

Ethidium bromide - fluoresce for nucleic acids under UV light

Applications

Paternity test

Crime forensics

Determining size of protein

Determining length of nucleic acid (base pair length)