The Protein Laboratory

Session Slides with Notes

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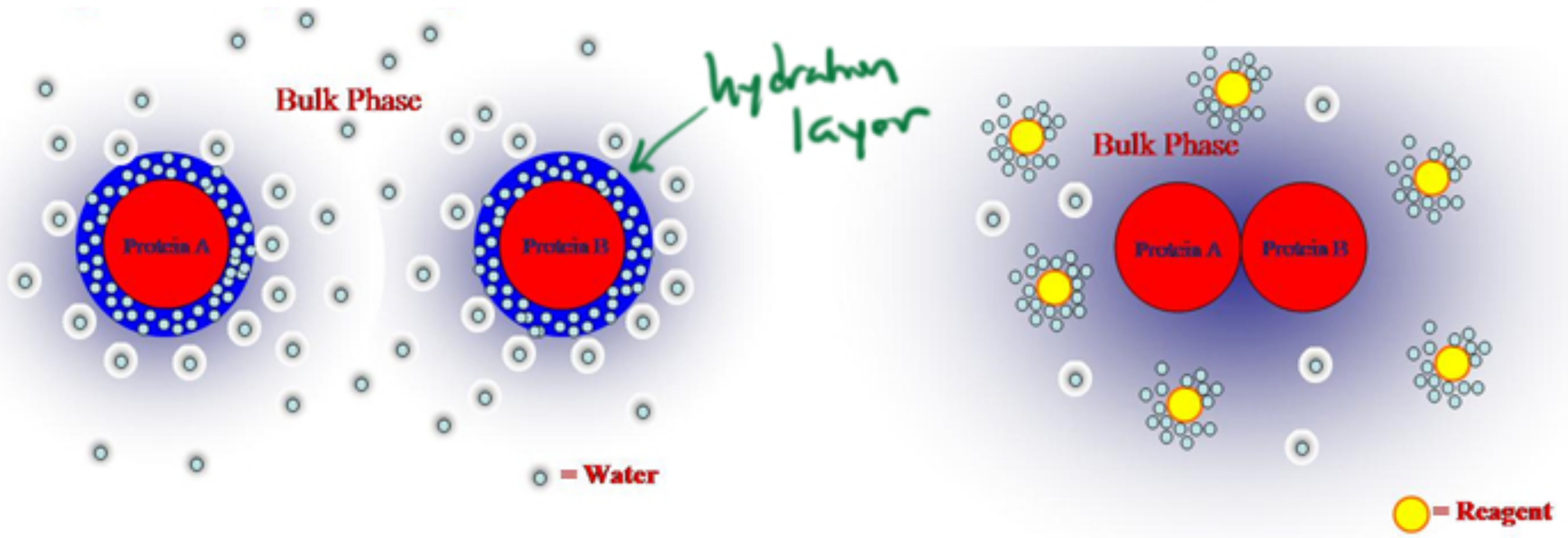
The Protein Lab

- separation & purification
- preparative or analytical

Centrifugation

- low speed - nuclei
- medium mitochondria ribosomes
- higher speed membranes

Protein Precipitation



Hofmeister Series

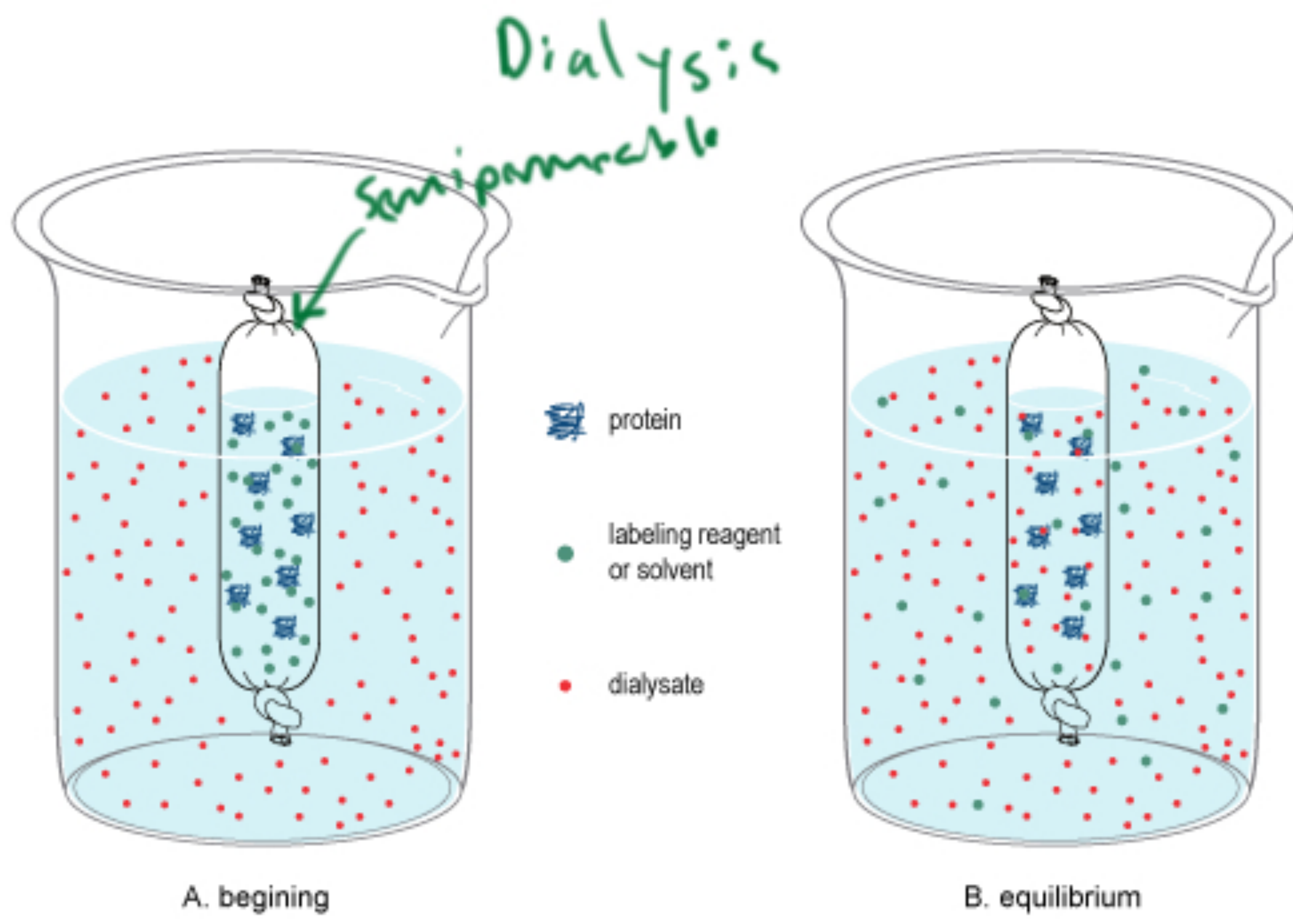
$$F^- \approx SO_4^{2-} > HPO_4^{2-} > \text{acetate} > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$$

$$NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+} > \text{guanidinium}$$

← Chaotropic

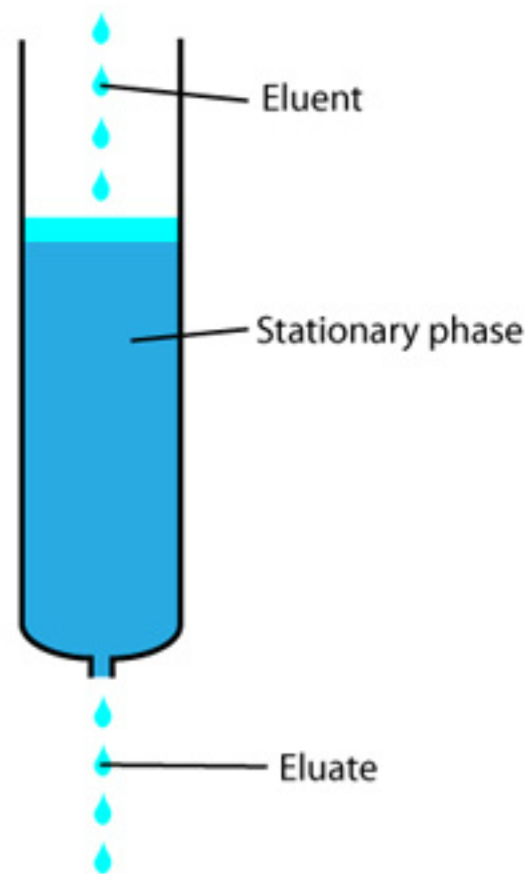
- enthalpy of hydration
- → entropic penalty

Na^+


Dialysis
Semipermeable
protein
labeling reagent
or solvent
dialysate
A. begining
B. equilibrium

Chromatography

- mobile phase and a stationary phase



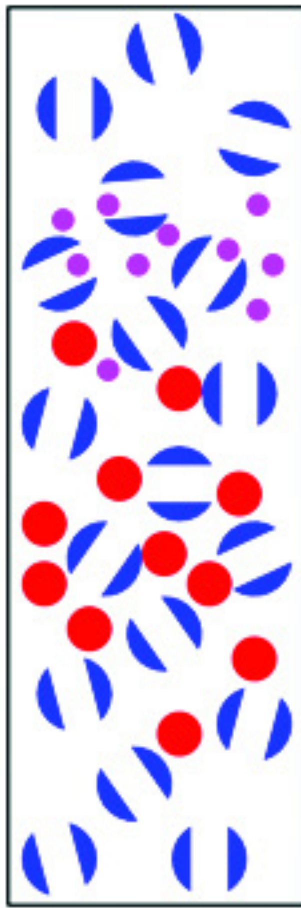
- Column chromatography
  - size exclusion (gel filtration)
  - ion exchange
  - affinity chromatography
- TLC - thin layer and paper

  TLC - silicate stationary phase (hydrophilic)

  mobile phase - most often hexane

  - HPLC

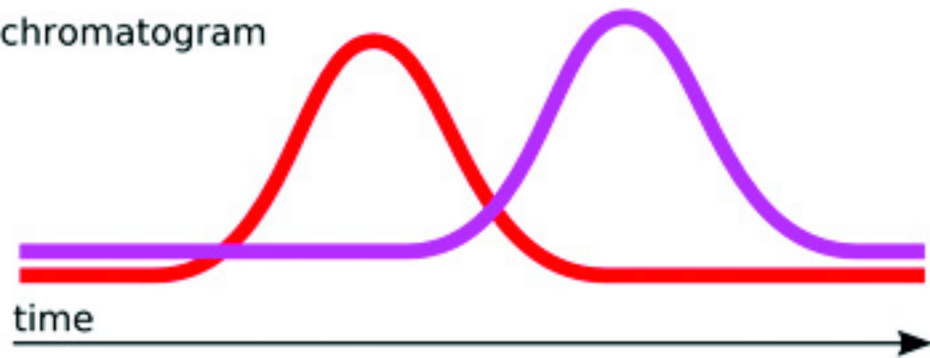
size exclusion chromatography



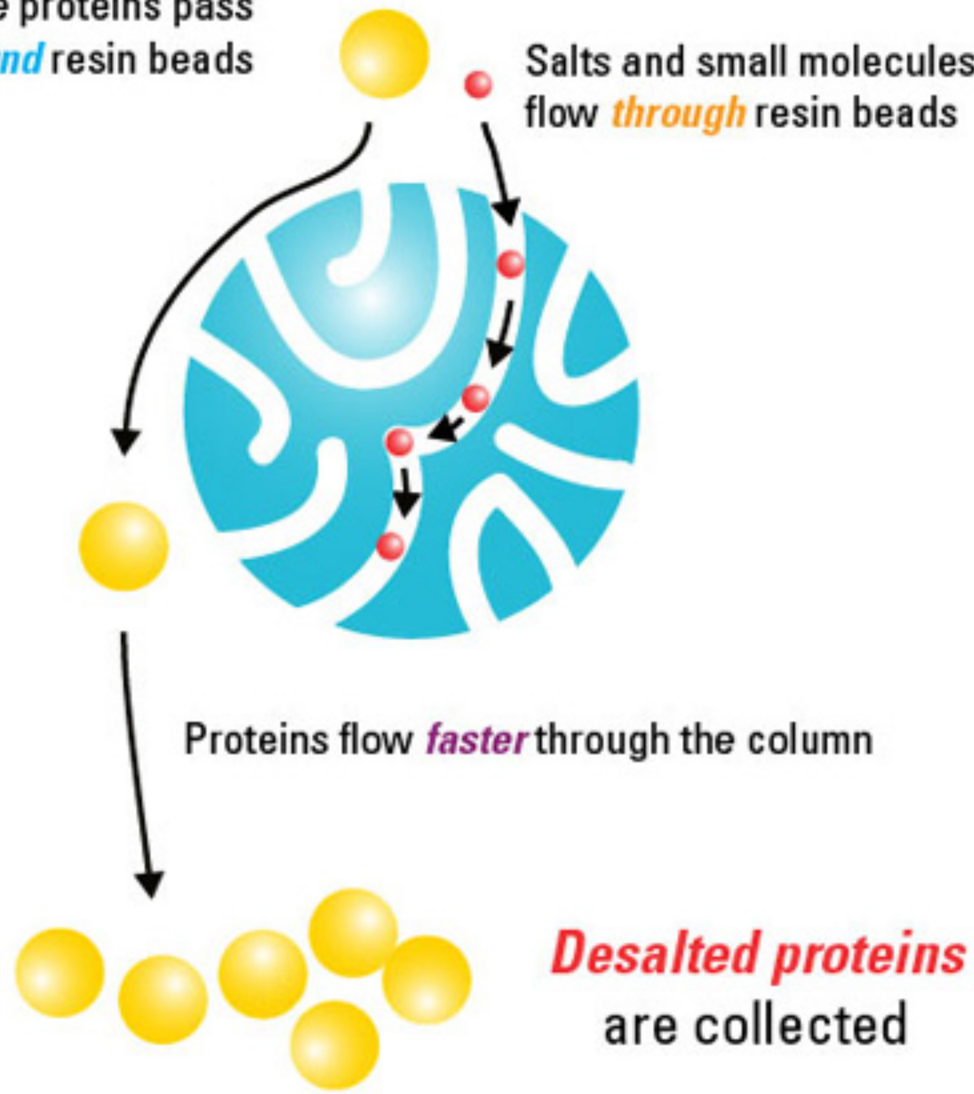
flow

Large particles cannot enter gel and are excluded. They have less volume to traverse and elute sooner.

Small particles can enter gel and have more volume to traverse. They elute later.

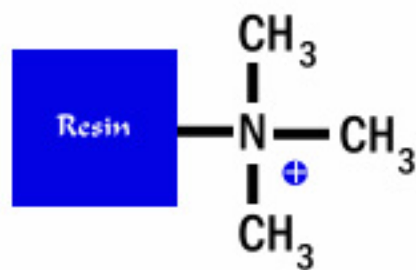


Large samples have a lower retention time.

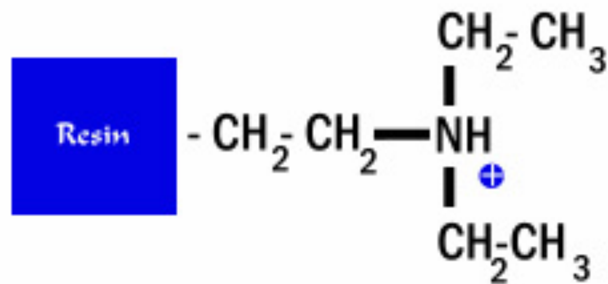


Sample proteins pass around resin beads
Salts and small molecules flow through resin beads
Proteins flow faster through the column
Desalted proteins are collected

ion exchange



Q-anion exchanger



DEAE-anion exchanger

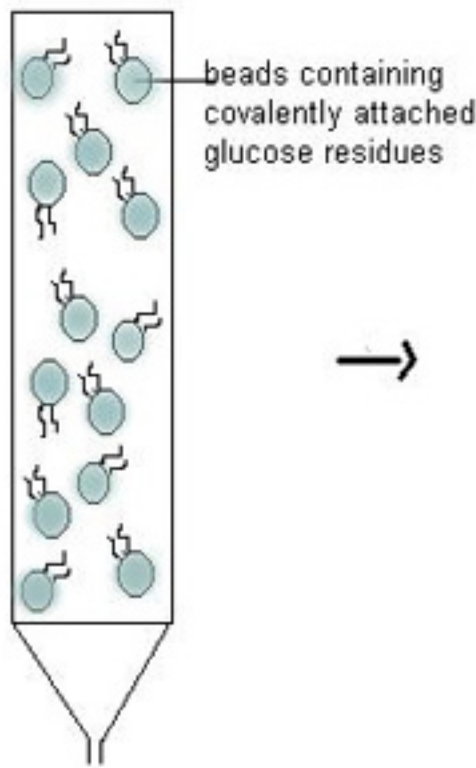
an anion exchange resin is positively charged

use with a gradient elution buffer

such as 0.01 M NaCl ⟶ 1.0 M

affinity chromatography

+



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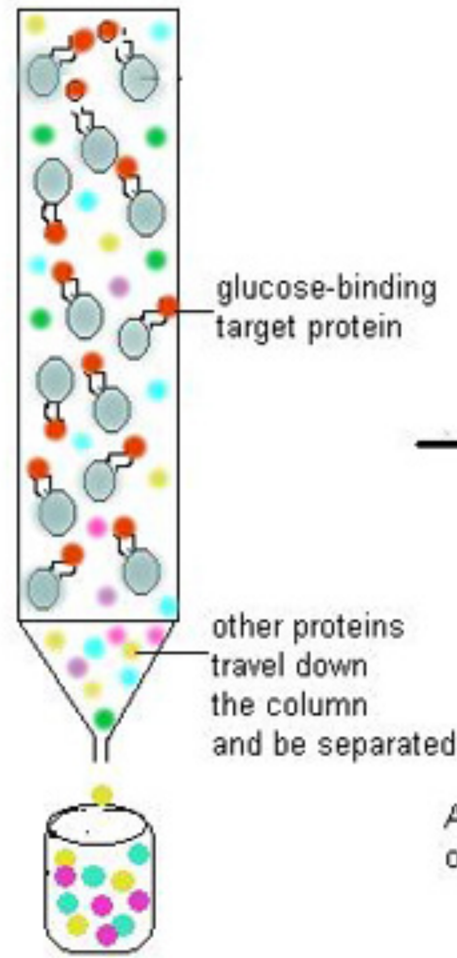

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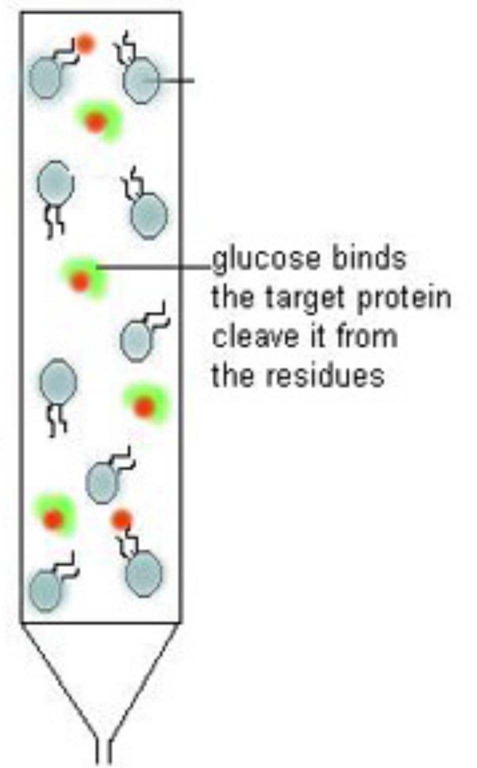
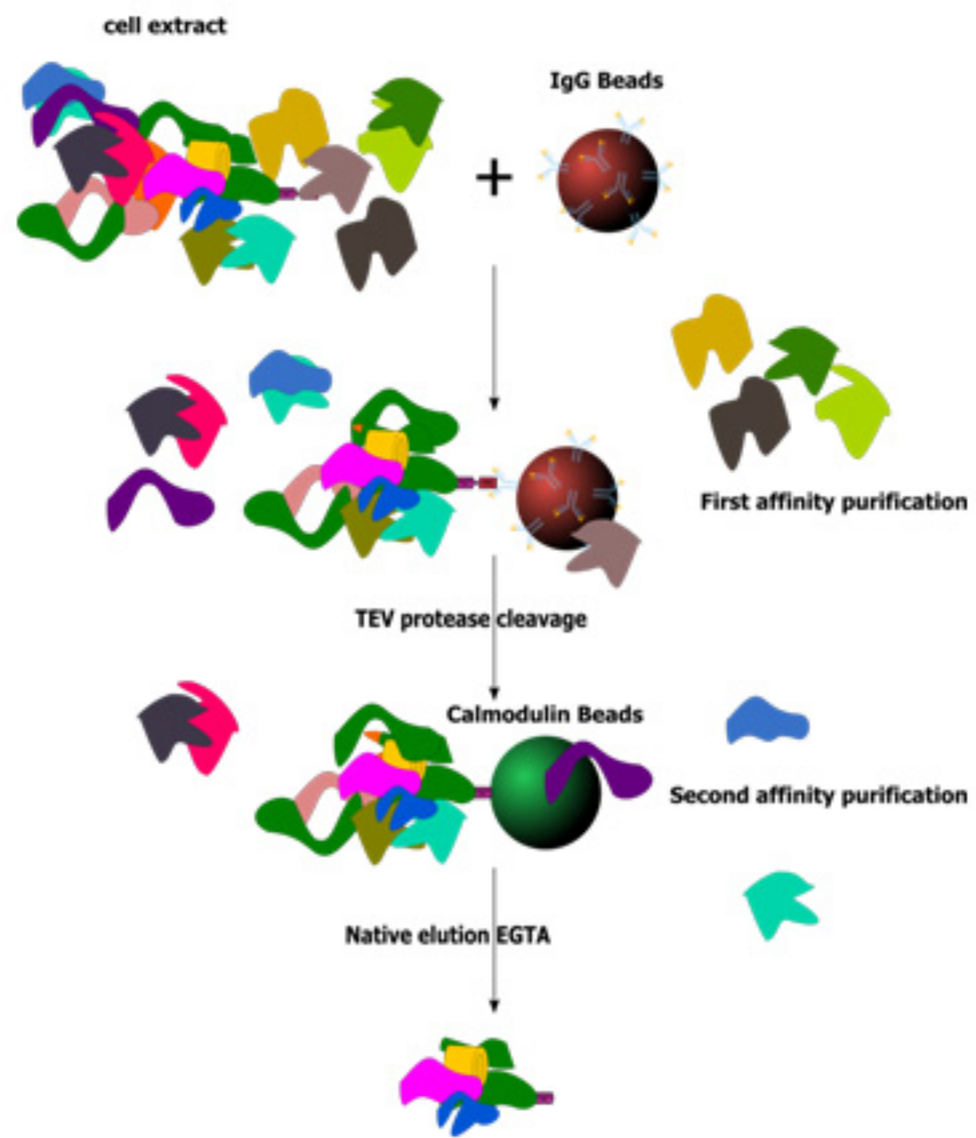
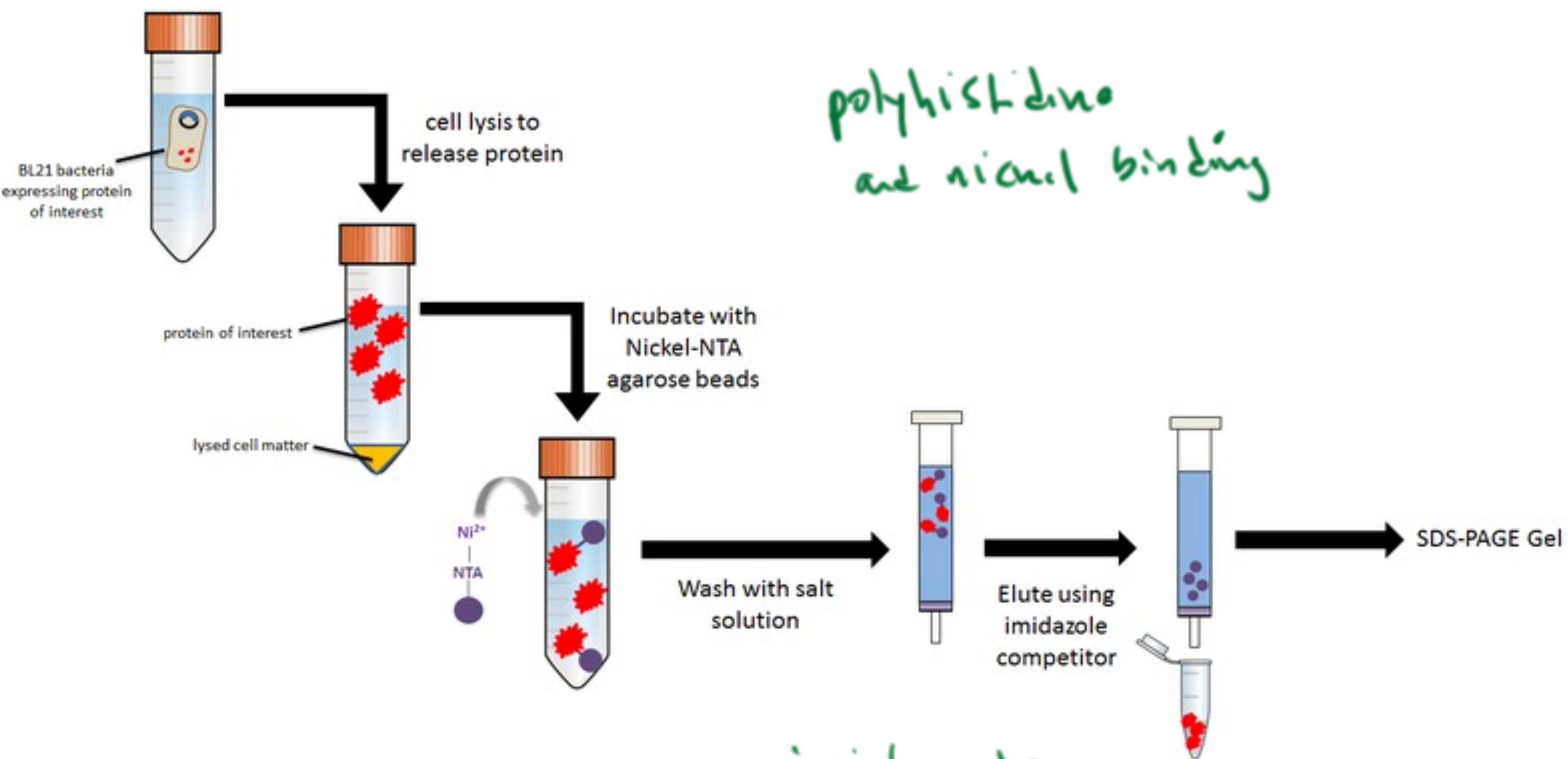


- antibody antigen
- enzyme substrate

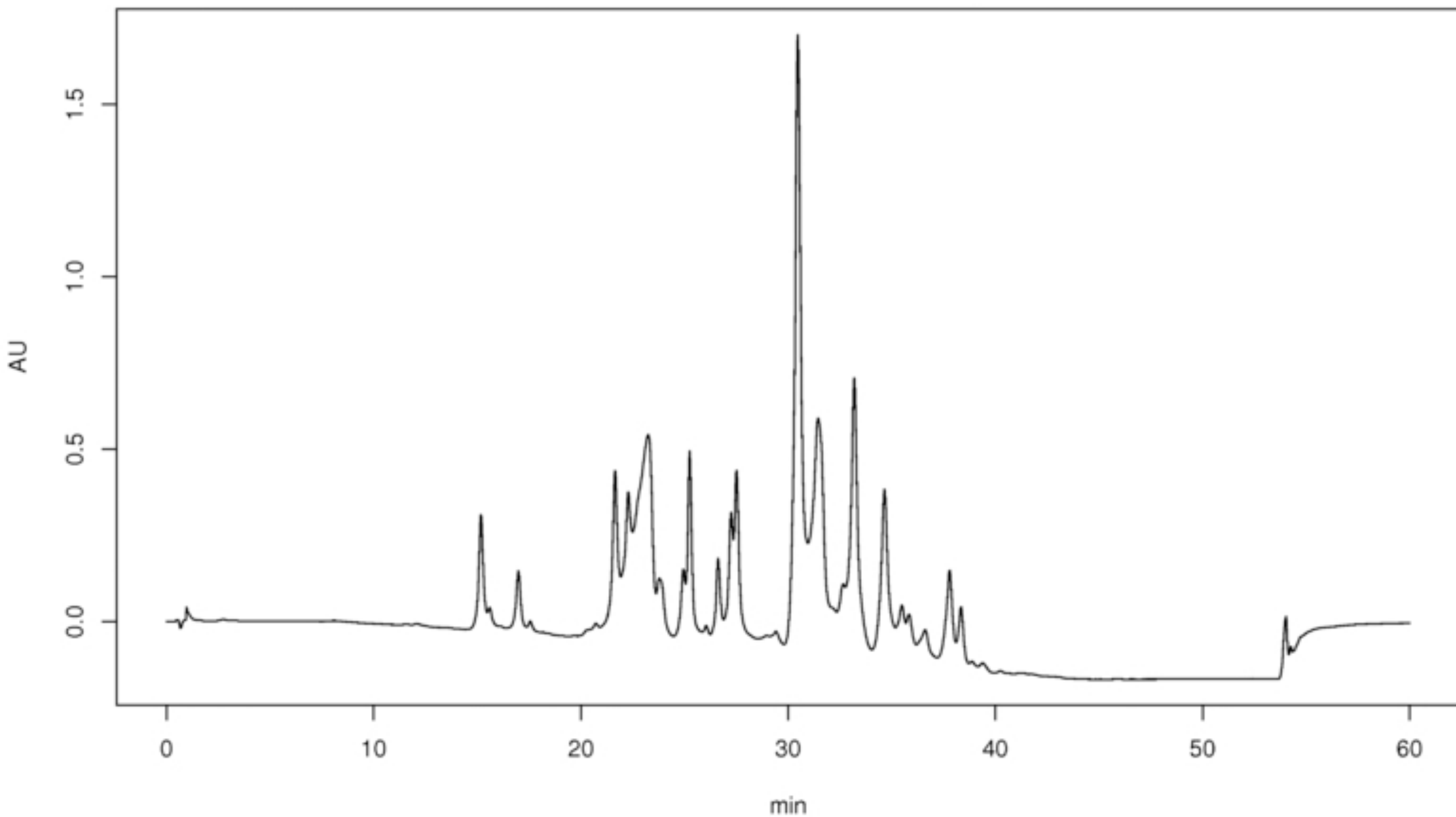
The demonstration of the steps in Affinity Chromatography

- polyhistidine tail AAAAAAAHHHHH
  with nickel coated beads
- biotinylation with avidin coated beads


cell extract
IgG Beads
First affinity purification
TEV protease cleavage
Calmodulin Beads
Second affinity purification
Native elution EGTA



imidazole -
histidine side chain



HPLC - high performance liquid chromatography

- often using a gradient such as 100% acetonitrile → 100% water

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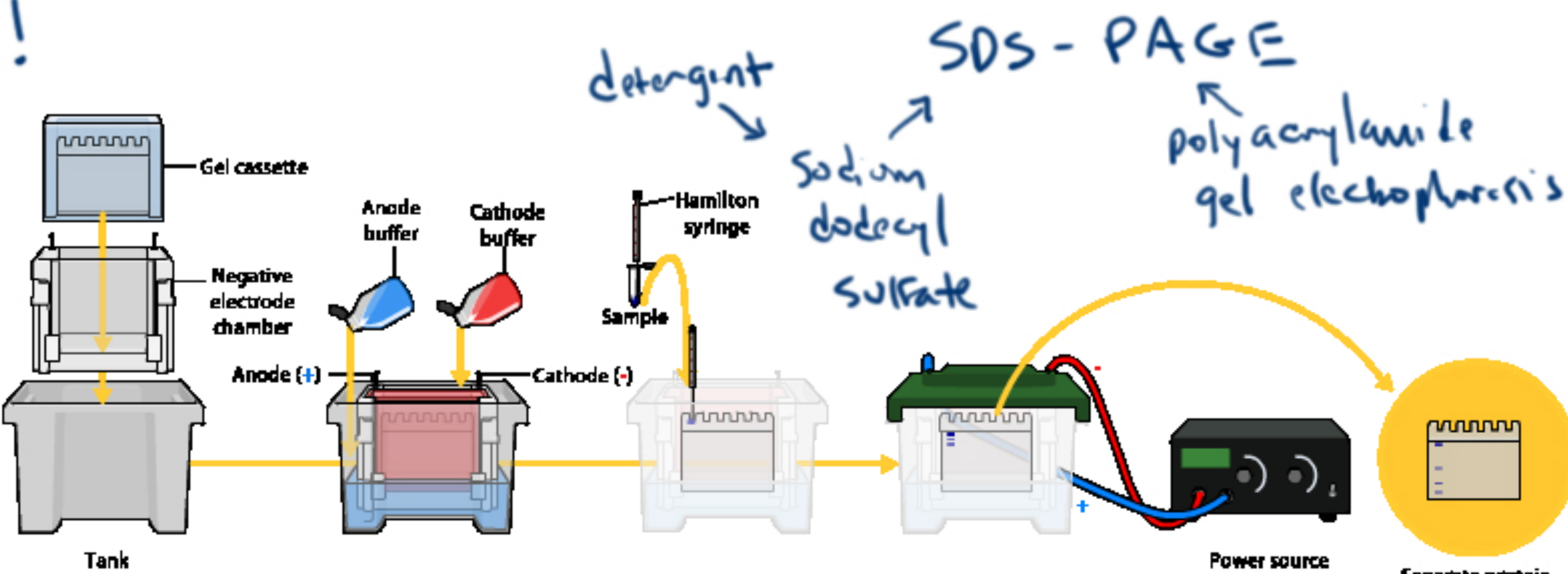
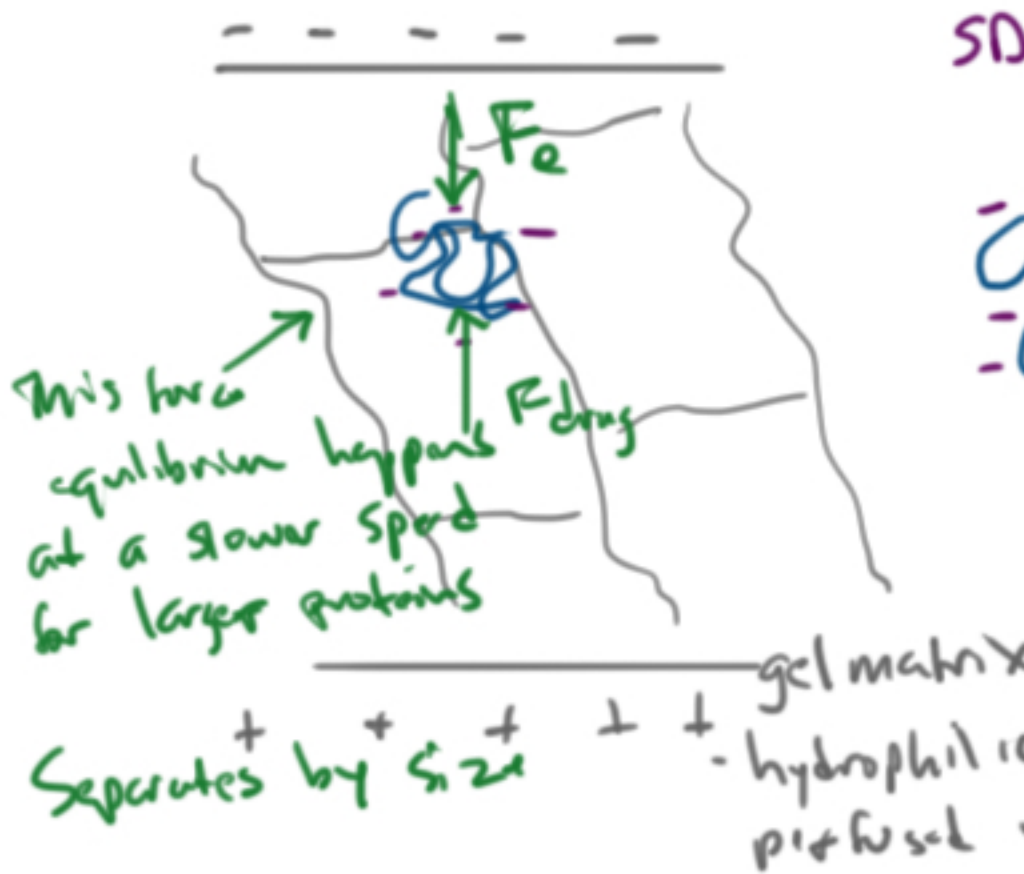
SDS - PAGE

Sodium dodecyl sulfate (detergent) → SDS

polyacrylamide gel electrophoresis → PAGE





Separates by size

gel matrix - hydrophilic mesh perfused with buffer ions.

SDS - denatures and disrupts noncovalent interactions

SDS imparts a uniform charge to mass ratio

- denaturing gel - with SDS
- native gel - without SDS
- ALSO - reducing gel uses β mercaptoethanol in the loading buffer

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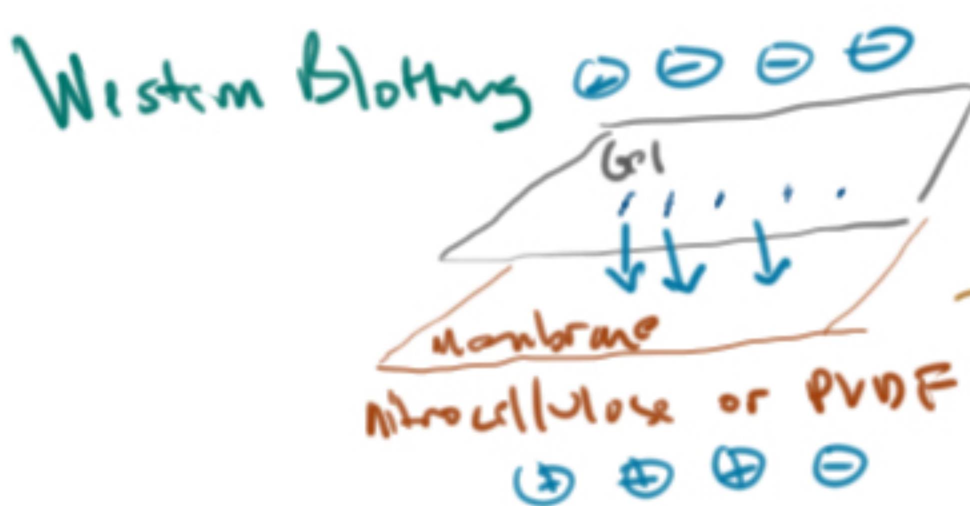
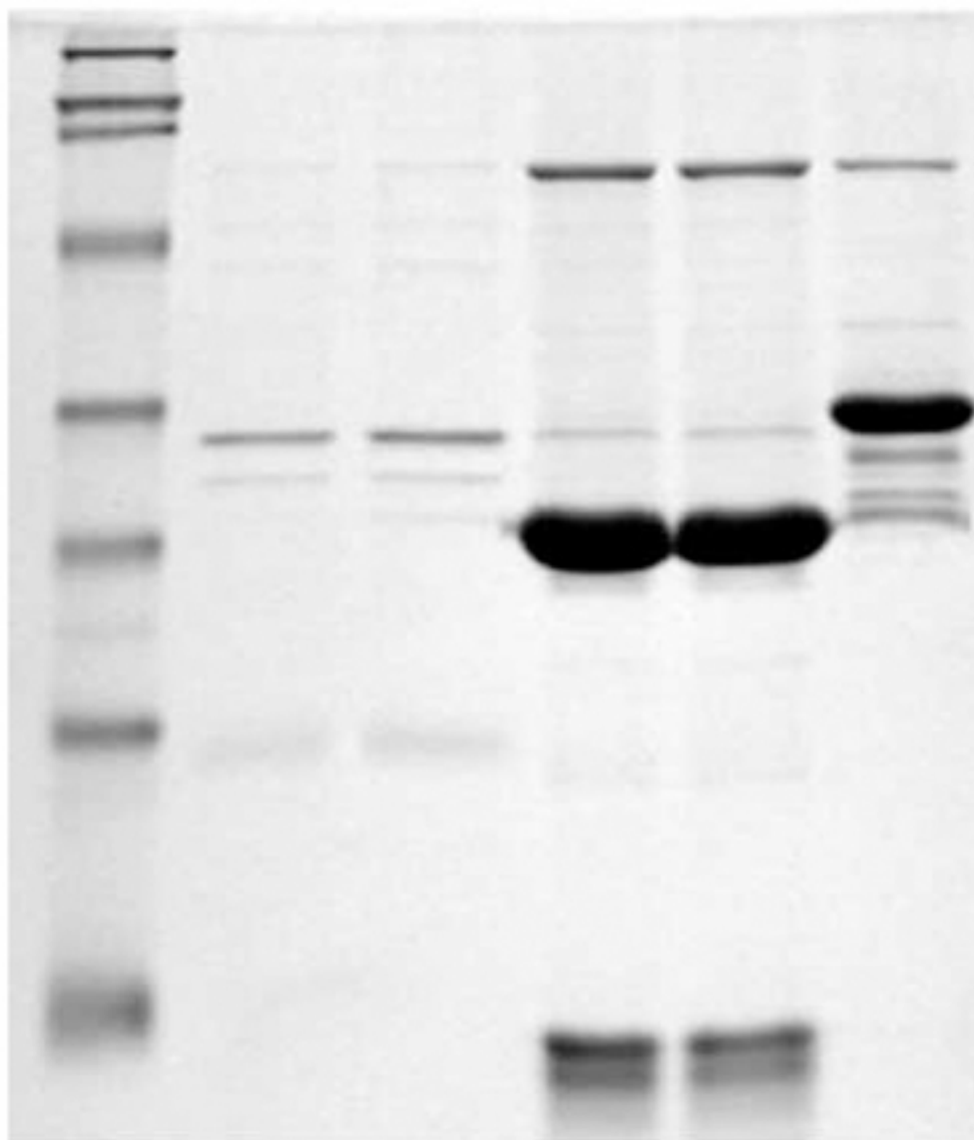
Post electrophoresis - staining or sample recovery - crush and soak, electroelution

Blotting -
- Western - Protein
- Southern - DNA
- Northern - RNA

Western Blotting





1 Dalton = 1 amu

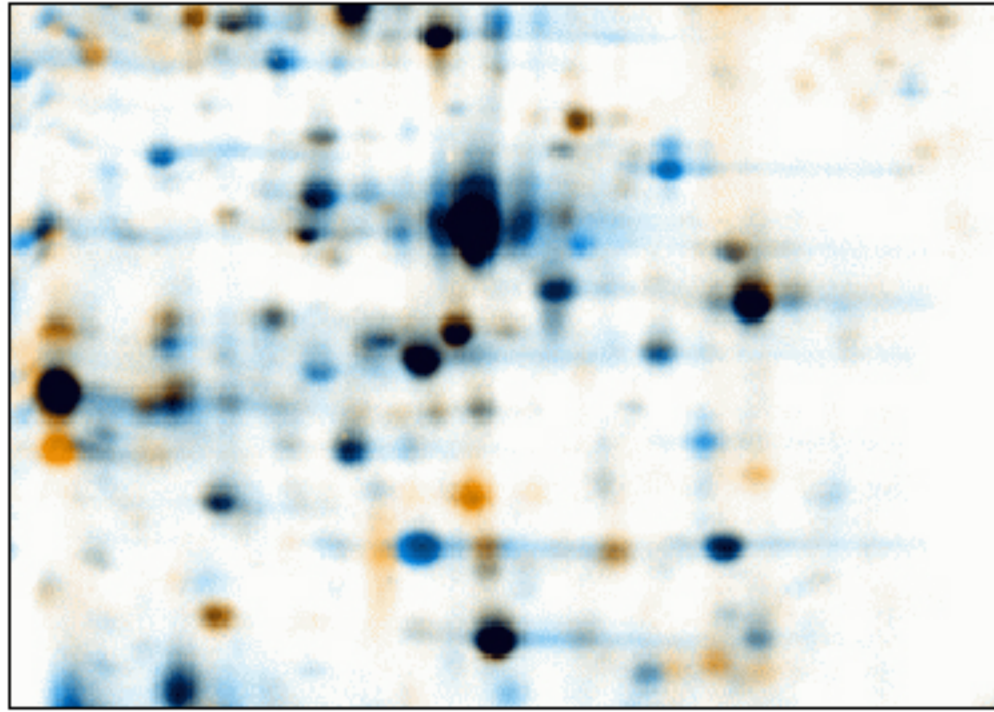
1 kD = 1000 Daltons

1 kD ~ 9 residues

isoelectric focusing

low pH (+) DEDE.. . . . . KRKR- (−) high pH

2nd dimension normal electrophoresis ↓



Two Dimensional Electrophoresis

(actually two 2-D gels superimposed one stained blue, one stained orange)

Protein Mass Spec