SDS-PAGE
Bradford

- Quantitative + qualitative assessment of purity/concentration
- Total protein

1. SDS-PAGE

Sodium Dodecyl sulfate - Polyacrylamide Gel Electrophoresis

SDS-PAGE

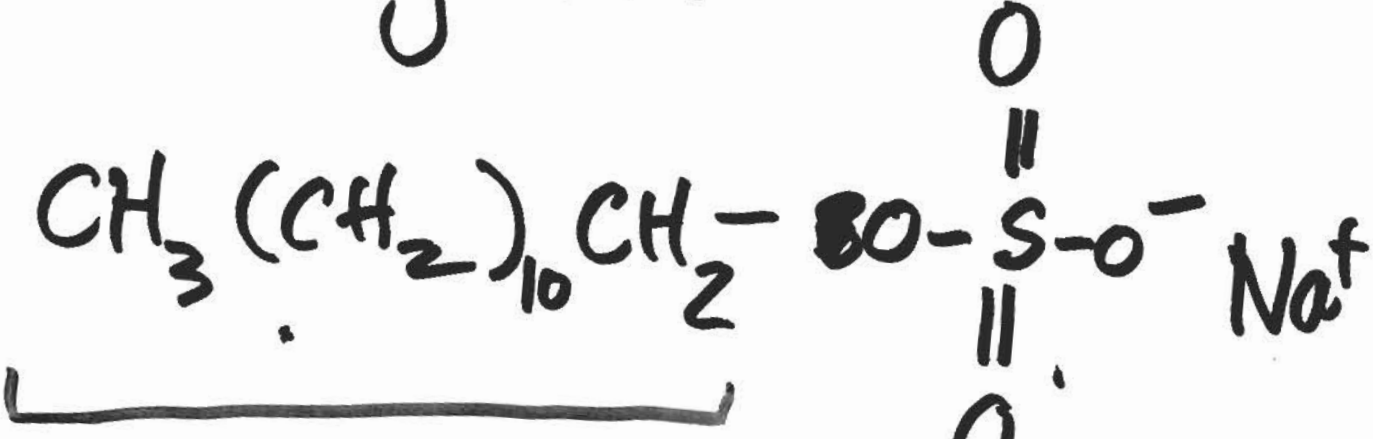
→ Separate proteins according to size

ONLY

(shape, charge, 4^{o} structure has no effect)

SDS

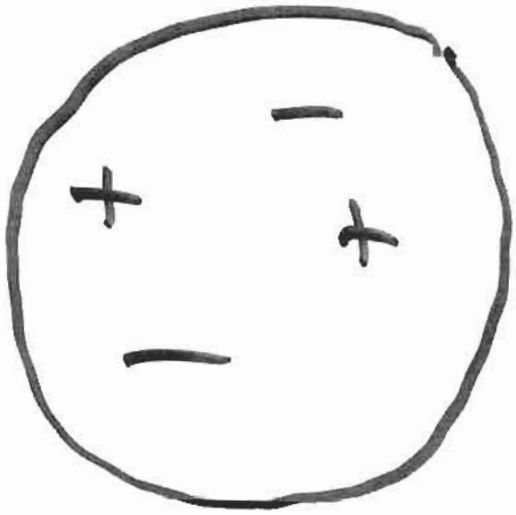
Detergent



Purpose → denature structure of protein

→ charge to mass ratio of proteins is made CONSTANT

charge to mass ratio

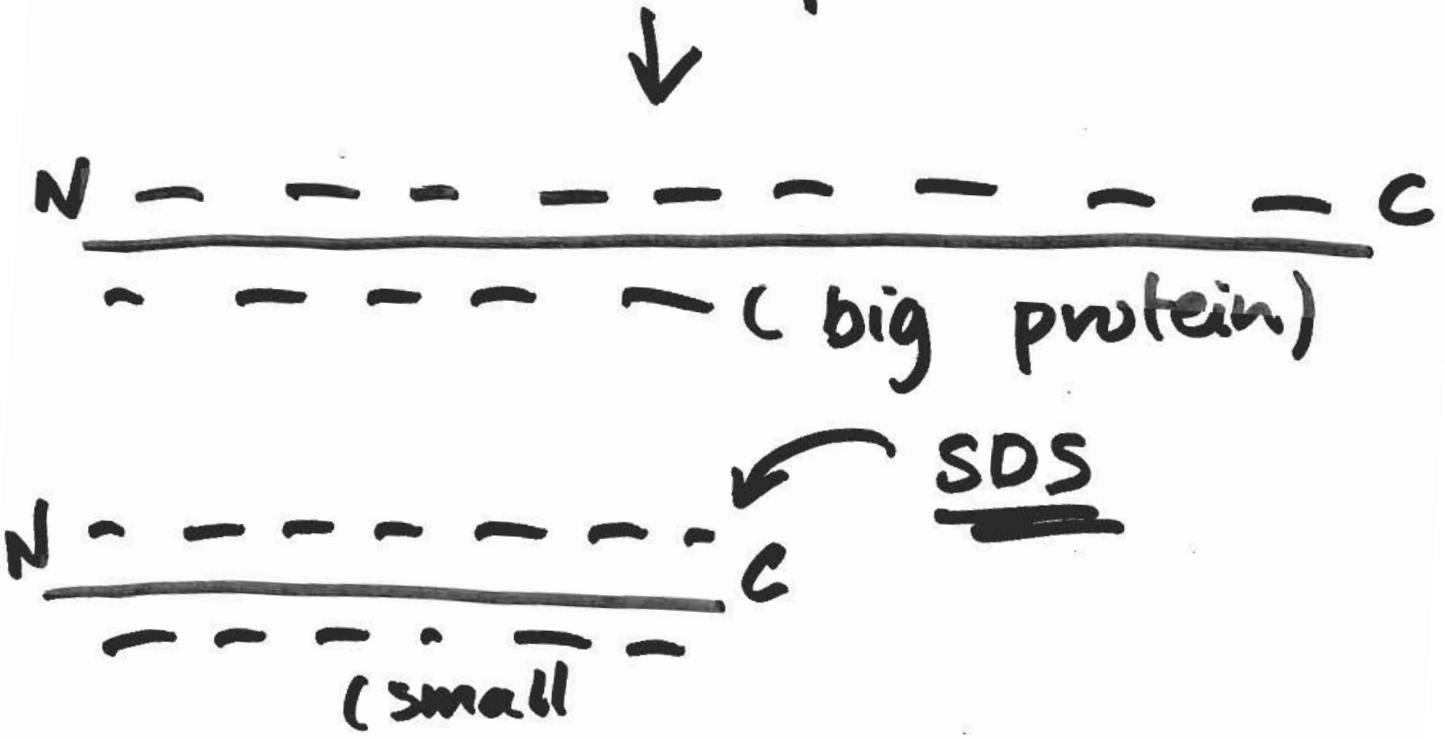


big globular protein

small elongated protein

↓

Denature protein



1 SDS molecule / 2 amino acids.

- same shape
- same charge : mass ratio

- Separate based on size

2X sample buffer

1. SDS
2. β-Mercaptoethanol (BME)
   reducing agent
   - Break disulfide bonds (ALL)
3. Glycerol
   - increases density of sample so that it doesn't float away

4. Bromophenol Blue
Dye (Blue)

5. Tris (BUFFER)

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heat @
95°C

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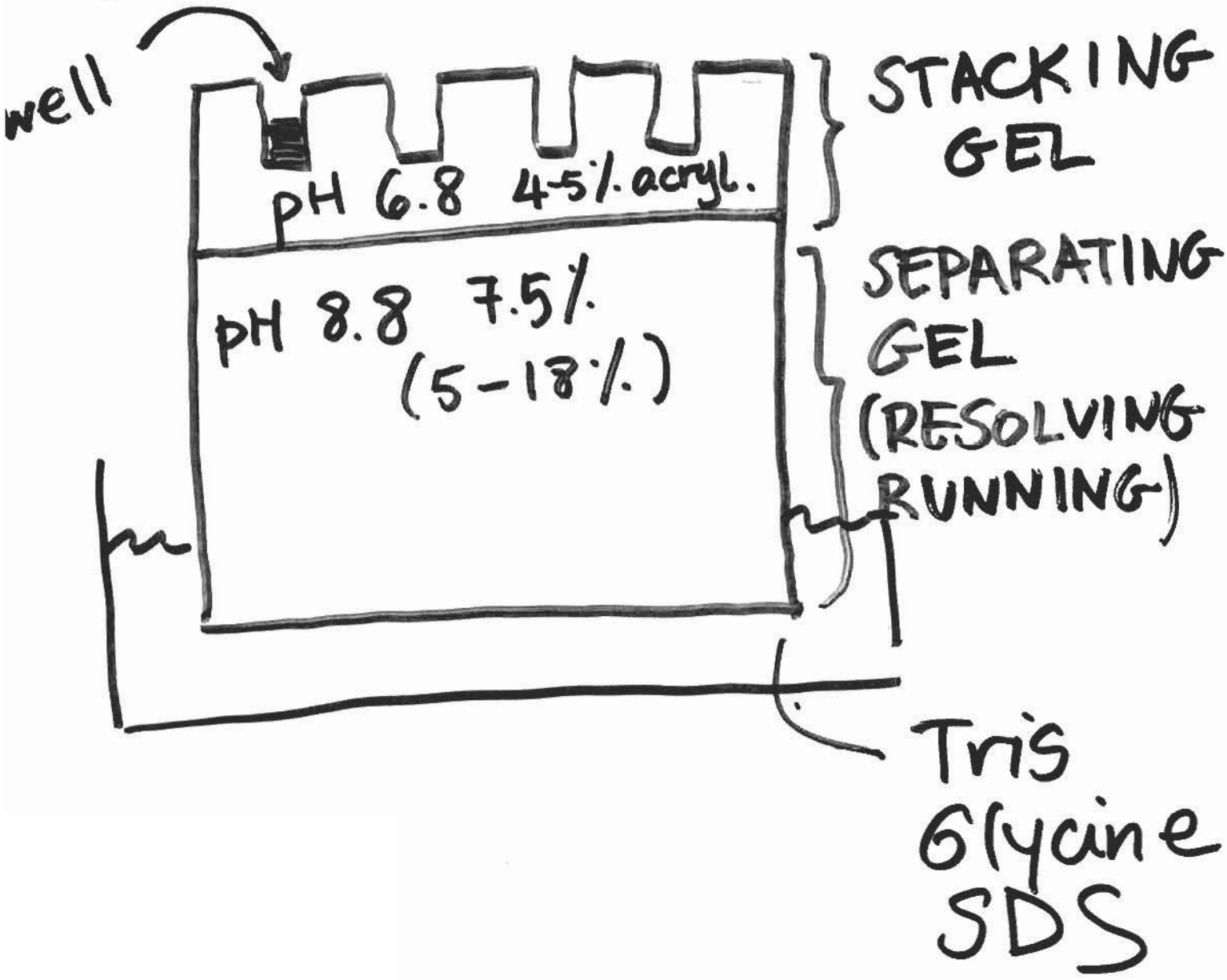
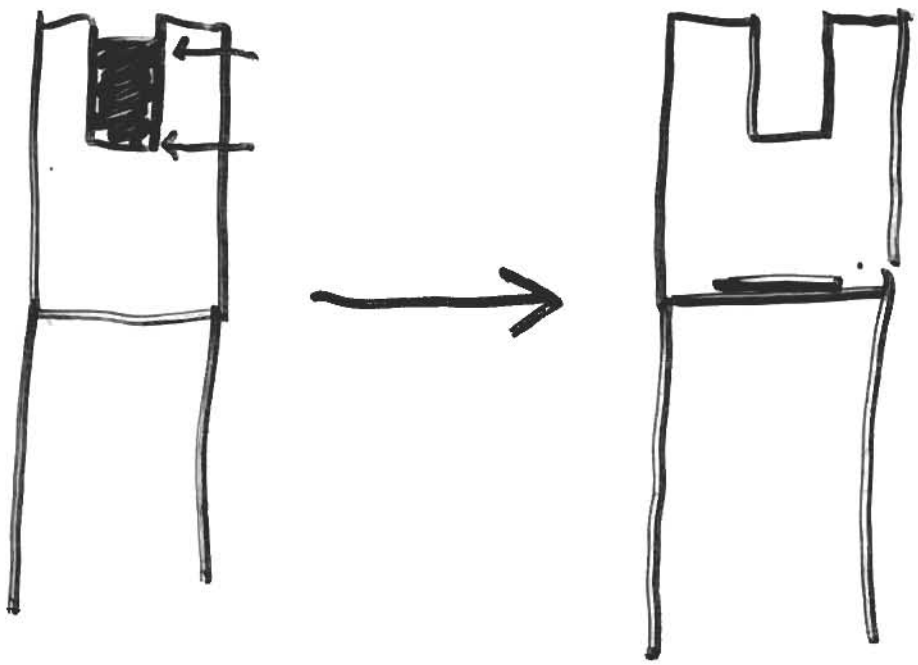
Anatomy of the SDS-PAGE Gel.



Stacking gel



Allows all proteins in sample to start @ the same starting point.

Separating gel

- ↑ % polyacrylamide than stacking gel.
- pH 8.8.

→ Allows proteins to separate according to size

Biggest proteins migrate SLOWEST

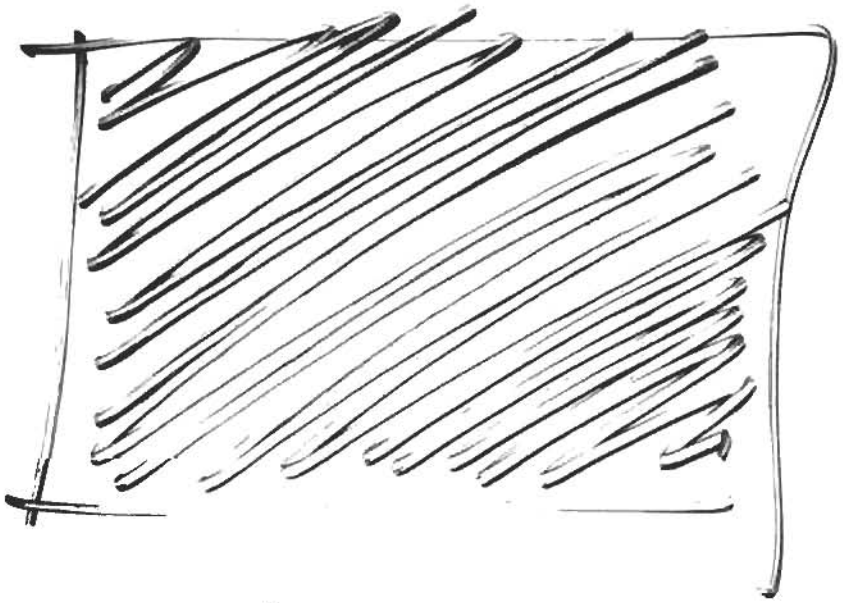
Smallest proteins migrate fastest

Staining + Destaining

Staining solution

- Coomassie Blue (binds non-specifically to proteins
- Acetic Acid
- Methanol

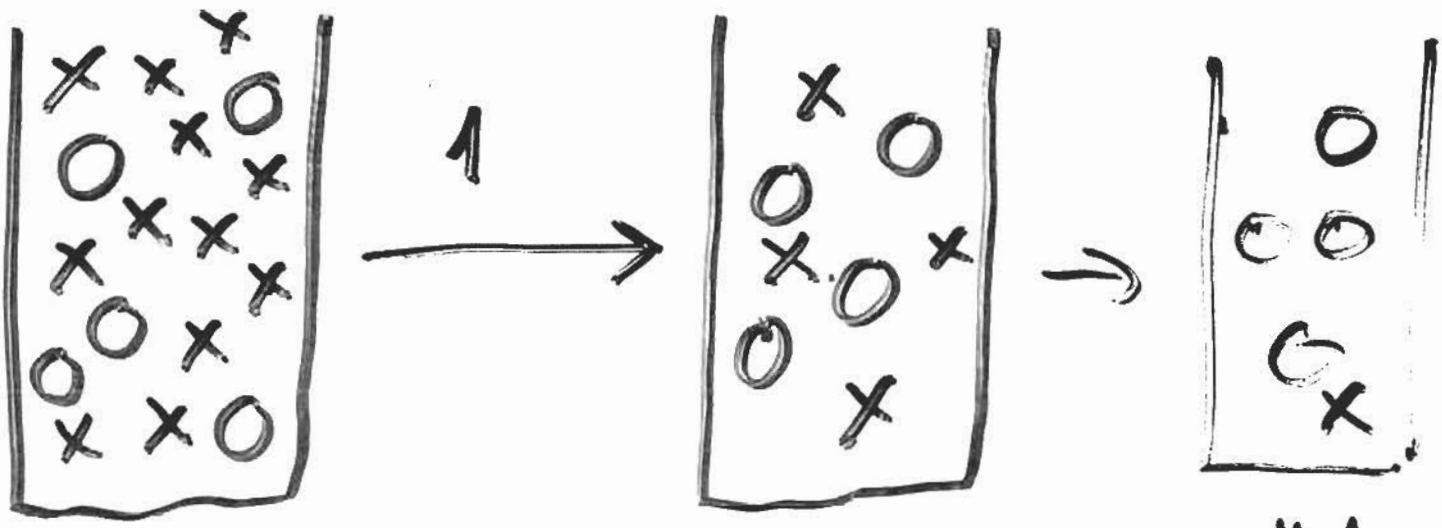
Afterstaining



Destain

- Acetic acid
- Methanol

Specific activity



ON PAGE → 5/17 } SPECIFIC ACTIVITY

BRADFORD

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Specific Activity = $\frac{\text{Total Activity}}{\text{Total amount of protein}} \times 100$

2. Bradford assays

- Measures TOTAL protein in your sample (quantitatively)

→ Allows measurement of purity (specific activity)

- Coommassie Blue

Dye that binds to proteins non-specifically

Bradford reagent
- comes red/brown

+ protein → BLUE

→ Use spec to quantitate

A_{595}, 0.1 → 0.6

BSA Standard Curve