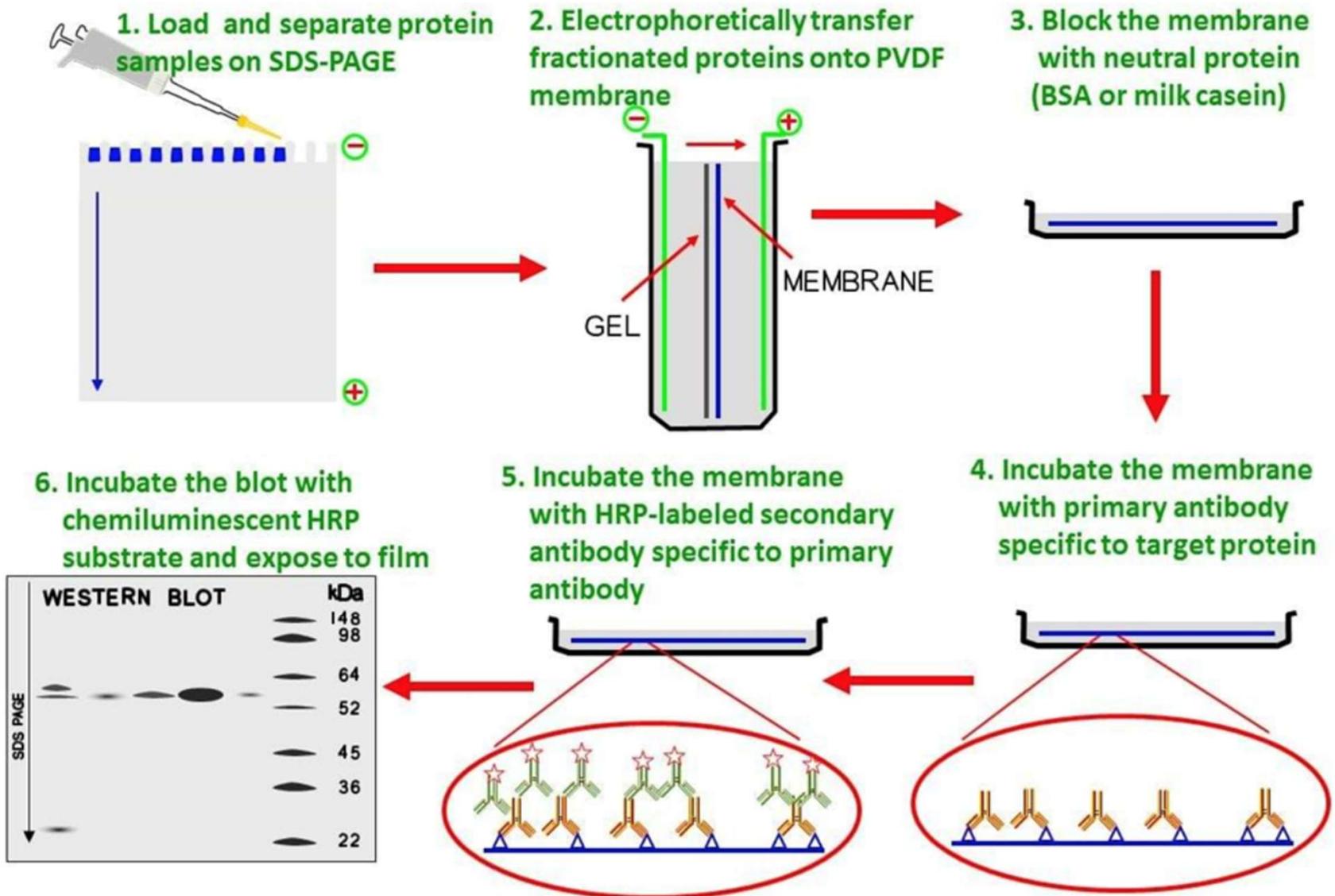


# Western Blot

Detecting and analysing proteins

*Presented by: Maydelene Liew*

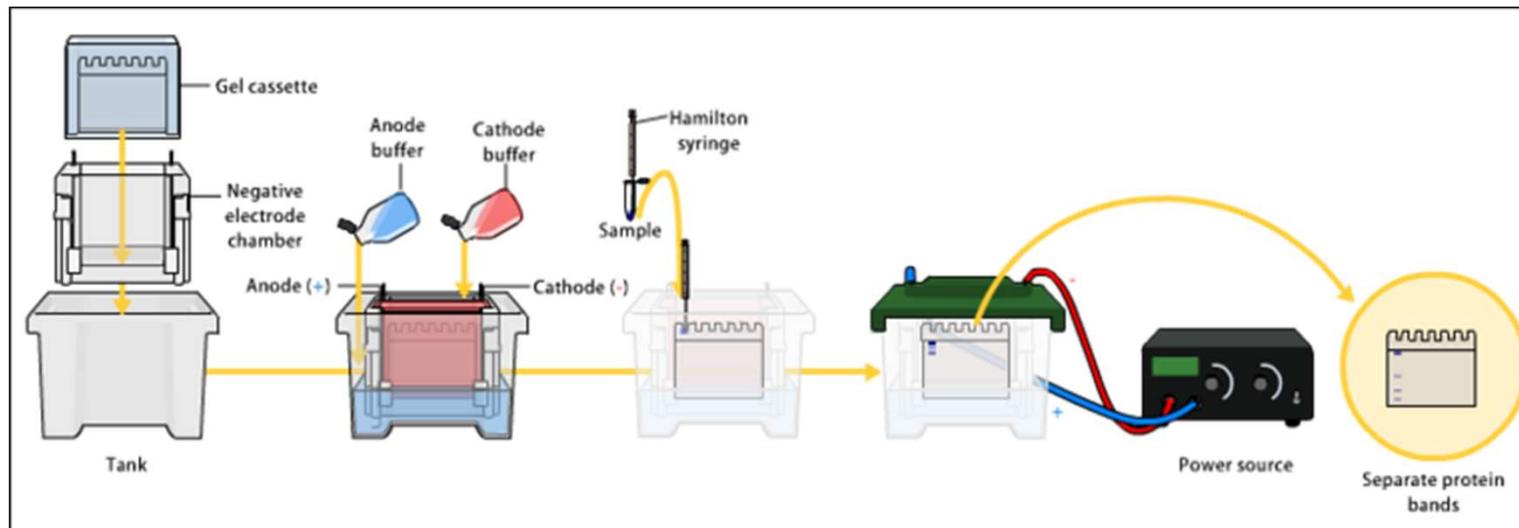


# Extraction of protein

- Protein is extracted from cell by mechanical or chemical lysis of cell → tissue preparation
- Protease inhibitor
- When sufficient amount of protein sample is obtained, it is diluted in loading buffer containing glycerol which helps to sink the sample in well.
- Tracking dye (bromothymol blue) also added in

# Gel electrophoresis

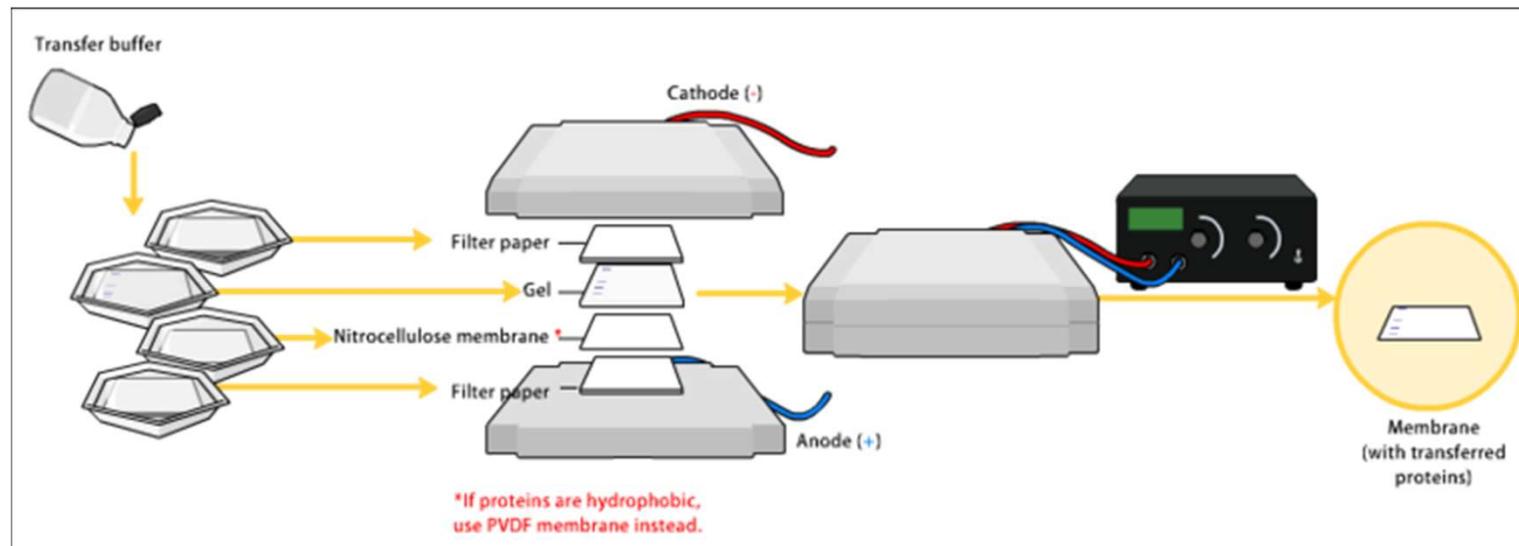
- Proteins separated on basis of:
  - Electric charge,
  - Isoelectric point,
  - Molecular weight, or
  - Combination of these
- SDS-PAGE



Source: [https://en.wikipedia.org/wiki/Western\\_blot#Gel\\_electrophoresis](https://en.wikipedia.org/wiki/Western_blot#Gel_electrophoresis)

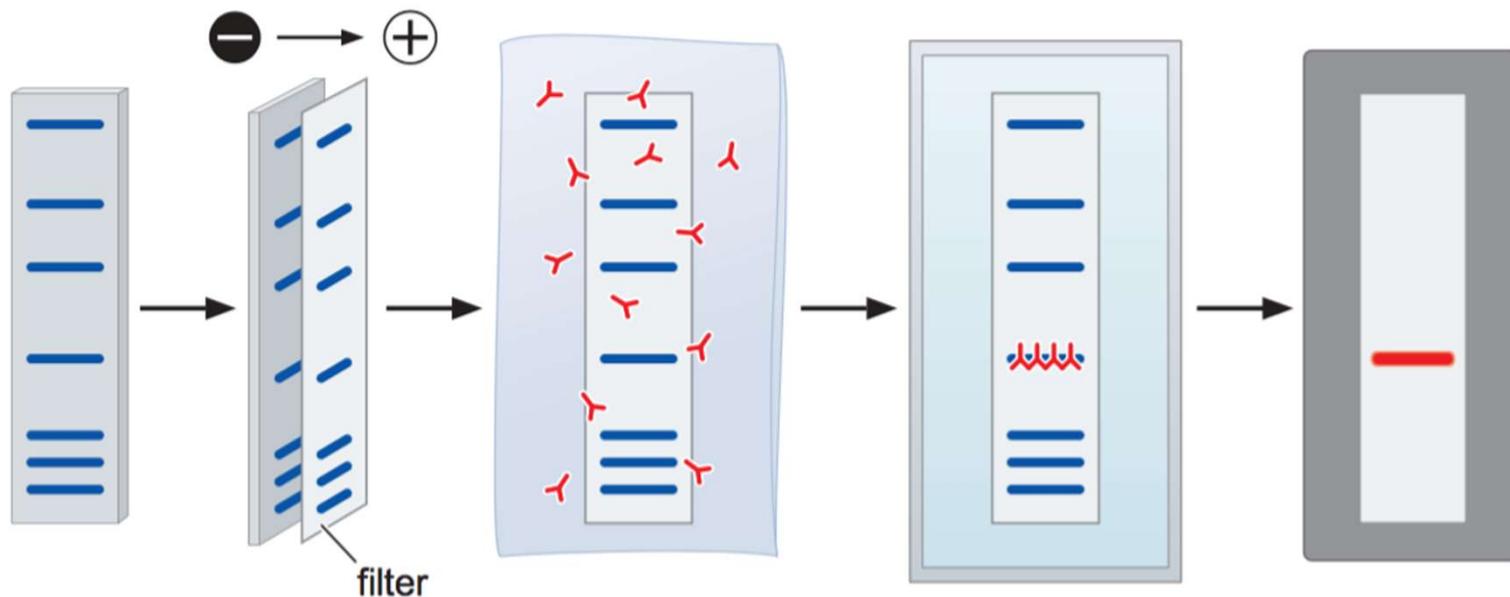
# Transfer – blotting to membrane

- Membranes with high affinity for proteins
  - Nitrocellulose
  - PVDF (Polyvinylidene difluoride)
- Capillary action
- Electroblotting → faster & more efficient



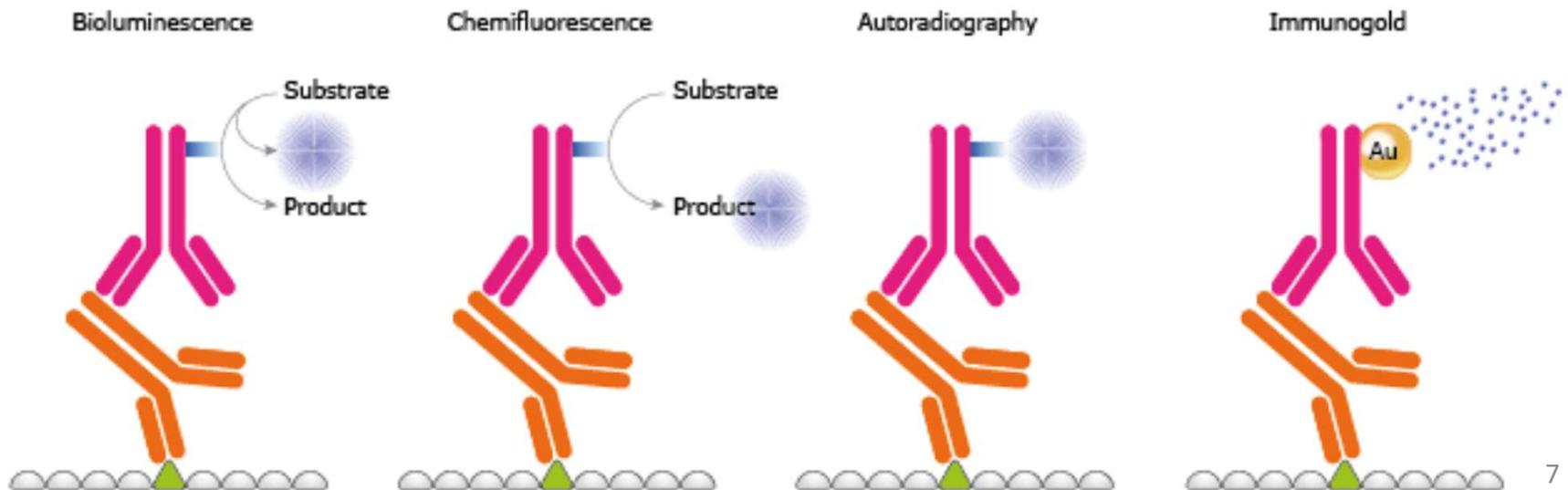
# Antibody probing

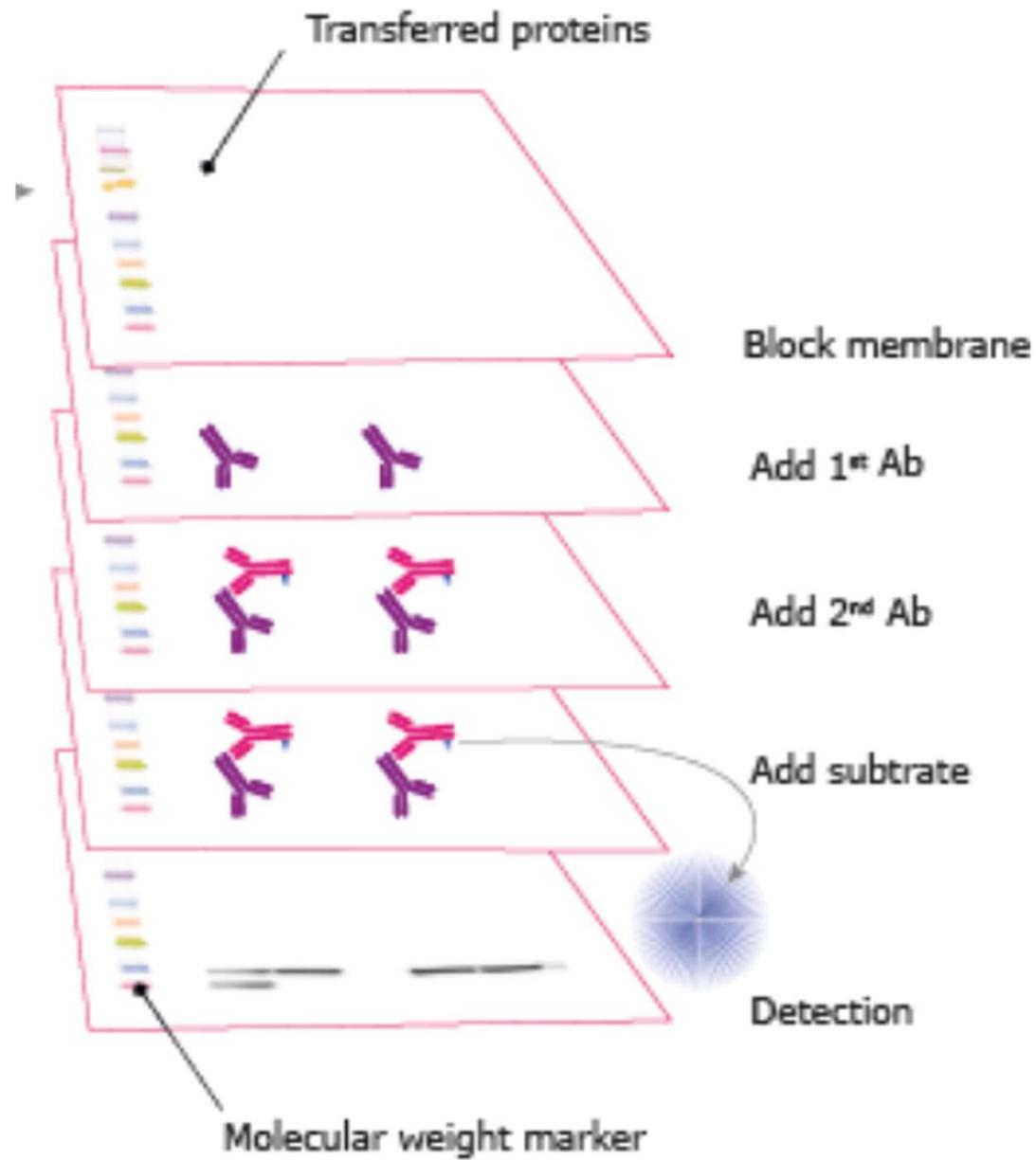
- Blocking
- Binding of primary antibody to specific protein
- Binding of secondary antibody to primary antibody



# Detection & imaging

- Labelling of secondary antibody:
  - enzymes,
  - fluorophores,
  - biotinylation,
  - gold-conjugation,
  - Radioisotopes





# Analysis

- Typical qualitative analysis:
  - Presence – confirmed by positive result
  - Amount – visual inspection
  - Size – comparison with marker
- Possible quantitative analysis with developments in molecular biotechnology

# References

- <https://www.proteinatlas.org/learn/method/western+blot>
- <http://www.onlinebiologynotes.com/western-blotting-technique-principle-procedure-application/>
- [https://en.wikipedia.org/wiki/Western\\_blot](https://en.wikipedia.org/wiki/Western_blot)