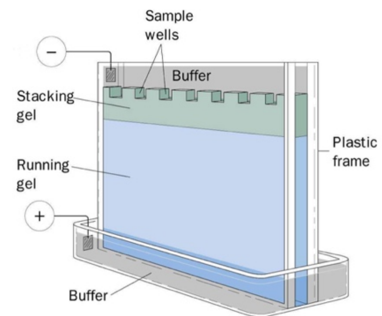
Lecture 7: Protein Analysis

- Electrophoretic analyses → measure relative amounts of proteins, including protein of interest (POI)
    - Native electrophoresis, SDS-PAGE, western blot, 2D GE
- Quantitative assays → measure the total concentration of protein, incl. POI
    - Lowry assay, Bradford assay, Biuret assay, UV spectrophotometry
- Activity assays → measure activity of POI
    - Need to consider the reaction being catalysed, have an analytical procedure to measure the progression of the reaction (e.g. spectrophotometry) and be aware of enzymatic conditions

- Native (vertical) gel electrophoresis:
    - Separates proteins based on size AND charge
    - Uses mild conditions i.e. maintains protein structure
    - Proteins (negatively charged) will migrate downwards, towards the anode
        - Smaller proteins will move further
    - Migration of a protein is defined as:
        - $\mu = {}^{V}/_{E} = {}^{Z}/_{f}$
            - V = velocity of the molecule
            - E = electrical potential (force)
            - Z = net charge of molecule (peptide)
            - f = frictional coefficient



- Denaturing (vertical) gel electrophoresis (SDS-PAGE):
    - Separates proteins based on size by denaturing the proteins and adding an excess of negative charge (SDS)
        - Buffer components: Tris, SDS (sodium dodecyl sulphate), DTT or β-mercaptoethanol (reducing agent to remove disulfide bonds), sucrose/glycerol, bromophenol blue (dye to visualise gel running)
        - Mixture must be boiled at 100°C for 5-10 min

Acrylamide concentration (% w/v)	**6**	**8**	**10**	**12**	**15**
Separation range (kDa)	50–200	30–95	20–80	12–60	10–43

    - Relative molecular weight (Mr) can be estimated by constructing a standard curve of logMr vs relative migration of a known size marker
    - Proteins are visualised on the gel by staining:
        - Coomassie blue → blue dye that binds proteins → detects ~50 ng protein/band