Techniques in protein biochemistry

BIOS 10016

23 June 2025

Objectives

- Know all definitions
- Describe the importance of assays and differentiate between direct and indirect methods
- Calculate specific activity, percent yield and purification level
- Describe how assays and PAGE can be used to assess protein purity.
- Describe the principles of PAGE and SDS-PAGE
- Describe the theory, principles, conditions required and the results of the different purification procedures discussed:
  - Size-exclusion chromatography
  - Ion exchange chromatography
  - Affinity Purification

Laboratory techniques

Protein purification

Requires:

- A source (natural or genetically engineered)
- A method to assess purity (activity assays, gel-based)

Direct activity assay

Test used to measure the enzymatic activity of a specific enzyme by directly detecting the change in a substrate or the formation of a product as the reaction occurs.

activity $\frac{\text{amount}}{\text{time}}$

standard enzyme activity, U $\frac{\mu}{\text{min}}$

specific activity $\frac{\text{enzyme activity (Units)}}{\text{mg of total protein}}$, a measure of purity (as mg of total protein decreases, activity increases, considering the amount of protein of interest remains the same)

Percent yield (how much activity is left after performing a certain step relative to original sample):

$$\text{Percent yield} = \frac{\text{remaining total activity}}{\text{initial total activity}} \times 100\% \tag{1}$$

Purification level (how much purer the sample is after a certain step relative to original sample):

$$\text{Purification level} = \frac{\text{specific activity}}{\text{initial specific activity}} \times 100\% \tag{2}$$

***P*oly*A*crylamide *G*el *E*lectrophoresis (PAGE)**

- Matrix is **polyacrylamide** that has holes and pores, changing how quickly the material can pass through in response to electric field. Bigger molecules move much slower
- Separates proteins based on **charge and size**
- **Cathode** (negative) attracts **positive** ions
- **Anode** (positive) attracts **negative** ions
- Proteins migrate towards electrode of opposite charge

*S*odium *D*odecyl *S*ulfate-PAGE (SDS-PAGE)

- SDS (negatively charged detergent) binds tightly to proteins (1.4 g SSDS/1 g protein)
- All proteins become very negatively charged and separation depends only on size
- 3° and 4° structure disrupted (denatured)
- Often reducing conditions used:
  - DTT
  - β-Mercaptoethanol (β-ME)
- $\implies$ disulfide bonds broken
- Protein mobility $\propto \dfrac{1}{\log(\text{molecular weight})}$
- Smears indicate impurity; isolated proteins indicate purity

Chromatography

Gel filtration/Size exclusion column chromatography

- Separated based on size
- Presence of a gel matrix with holes and gel beads attached
- Proteins elute (come out) in order of decreasing molecular weight, shown by a peak in the graph
- Larger molecules elute first (excluded from pores) because they interact less with the gel beads
- Smaller molecules elute later (enter pores) because they interact more with the gel beads
- Time of elution $\propto \dfrac{1}{\log(\text{molecular weight})}$

Ion exchange chromatography

Anion exchange chromatography The **resin** is **positively charged**, the **protein** to be isolated is **negatively charged**.

Cation exchange chromatography The **resin** is **negatively charged**, the **protein** to be isolated is **positively charged**.

- pH > pI $\implies$ negative proteins
- pH < pI $\implies$ positive proteins
- More charges $\rightarrow$ higher affinity

- Once charged molecules are stuck to the resin, elution is done by changing pH or salt concentration in the buffer