Protein Purification

1) Salt fractionation

proteins are poorly soluble in pure water

Salt in : increase salt to increase solubility by increasing electrostatic and H-bonds

Salt out : Very high concentration of ions, so less water hydrates proteins ⇝ proteins aggregates and form hydrophobic interaction ⇝ precipitation

2) Dialysis

Separation of protein sample through a semi-permeable membrane

3) Chromatography ⇝ uses a stationary phase of beads

Gel filtration

(Size exclusion chromatography)
(Molecular sieve chromatography)

separate according to size using porous beads

Small proteins are included into the beads and precipitate later

Larger proteins are excluded from the beads and precipitate faster

Ion Exchange Chromatography

separation according to charge

⊕ → Basic → ↑ pI

⊖ → acidic → ↓ pI

Anionic exchange

use ⊕ beads

Elution order ⊕ then ⊖

Cationic exchange

use ⊖ beads

Elution order ⊖ then ⊕

Affinity chromatography

use molecules (chemical or antibodies) that bind specific protein with high affinity

☆ Example : Concanavalin A purified by Glucose

4) Gel electrophoresis ⇝ Gel = polyacrylamide

SDS-PAGE

SDS is used to :

1) denature and solubilize proteins
2) Give proteins uniform ⊖ charges

Reducing agent
- → β-mercaptoethanol
  dithiothreitol
- → Reduce disulfide bonds

☆ It seperates according to size

Small = faster , large = slower

Isoelectric focusing

☆ uses a gel with pH gradeint

So separate according to charge

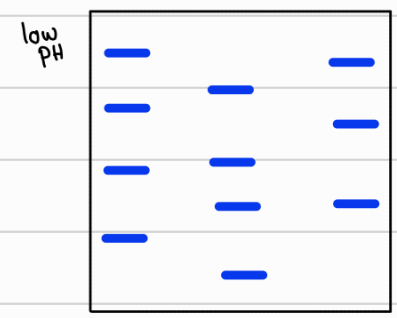
☆ a protein stops where pH = pI

2D - PAGE

separate according to charge and size

Isoelectric focusing → charge

SDS PAGE → size



5) Immunoblotting (western blotting)

SDS-PAGE then add antibodies

Primary antibody ⇝ bind the needed protein

Secondary antibody ⇝ bind the primary antibody and has an enzyme on its Fc

6) ELISA ⇝ detect protein and its amount

more rapid, convenient and sensitive

Capture antibody ⇝ bind the needed protein

↳ bound to a solid surface

Secondary antibody ⇝ bind the needed protein

Protein sequencing

1) Edman degradation ⇝ uses phenyl isothiocyanate (PITC) which reacts with the N-terminus

↳ can be used only for short fragments (50 amino acids)

2) Cleavage method

↳ used for the whole protein

A) chemical digestion

↳ uses Cyanogen bromide (CNBr) ⇝ cleave on C-terminus of Met

B) Endopeptidase

☆ Trypsin ⇝ cleave on C-terminus of Arg, Lys

☆ Chemotrypsin ⇝ cleave on C-terminus of Phe, Tyr, Trp

☆ Elastase ⇝ cleave on C-terminus of Ala, Gly, Ser, Val

☆ Pepsin ⇝ cleave on N-terminus of Leu, Phe, Tyr, Trp

→ Not next to Proline

C) Exopeptidase

↳ Amino peptidase ⇝ cleave on the N-terminus

↳ Carboxy peptidase ⇝ Cleave on the C-terminus

→ ends

Determination of 3D structure

1) Crystallography

↳ involves removing water

2) Nuclear magnetic Resonance (NMR) Spectroscopy

↳ without removing water (in solution)

↳ detect the dynamic and conformational changes