Purifying Proteins
Molecular Cell Biology

Source- Cooper

Submitted BY

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References

- Nelson and Cox, Lehninger's Principles of Biochemistry, 3rd edition, pp. 130-137.
- Wikipedia: en.wikipedia.org/wiki/Protein_purification
- Lecture Presentation by Dr. Chrisopher Fraser: mcb.berkeley.edu/courses/mcb102/

Why purify proteins?

1. Protein research: Understanding protein structure and function requires the study of individual proteins.

2. Protein Drugs: Global market worth $47.4 billion

(Jan 2007; Business Communications Company, Inc)

Insulin

Growth Hormone

Erythropoietin (EPO)

Interferon

Herceptin

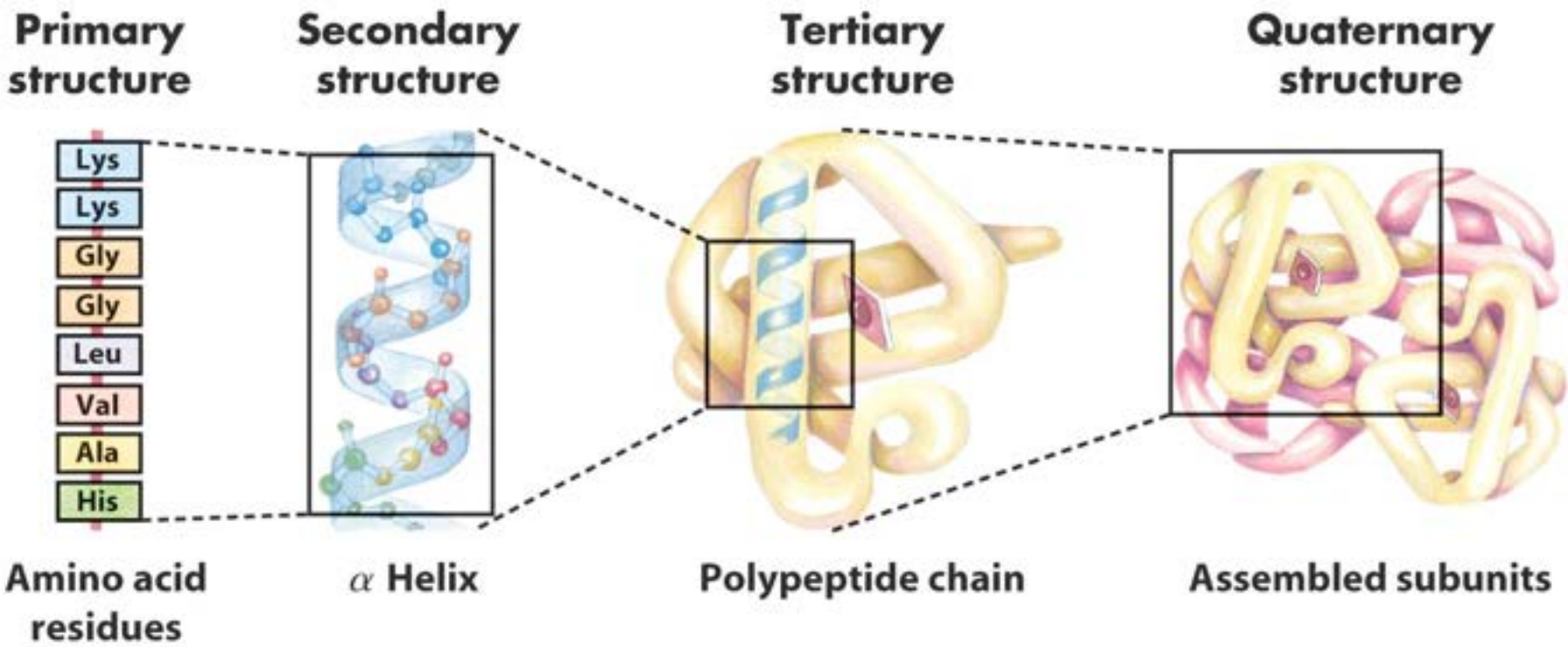
Always have an assay for your protein of interest!

- Enzymes can be measured by reactions they catalyze.

- Measure biological effects (hormones etc)

- Immunochemical technique (antibodies that possess enzyme linked assay)

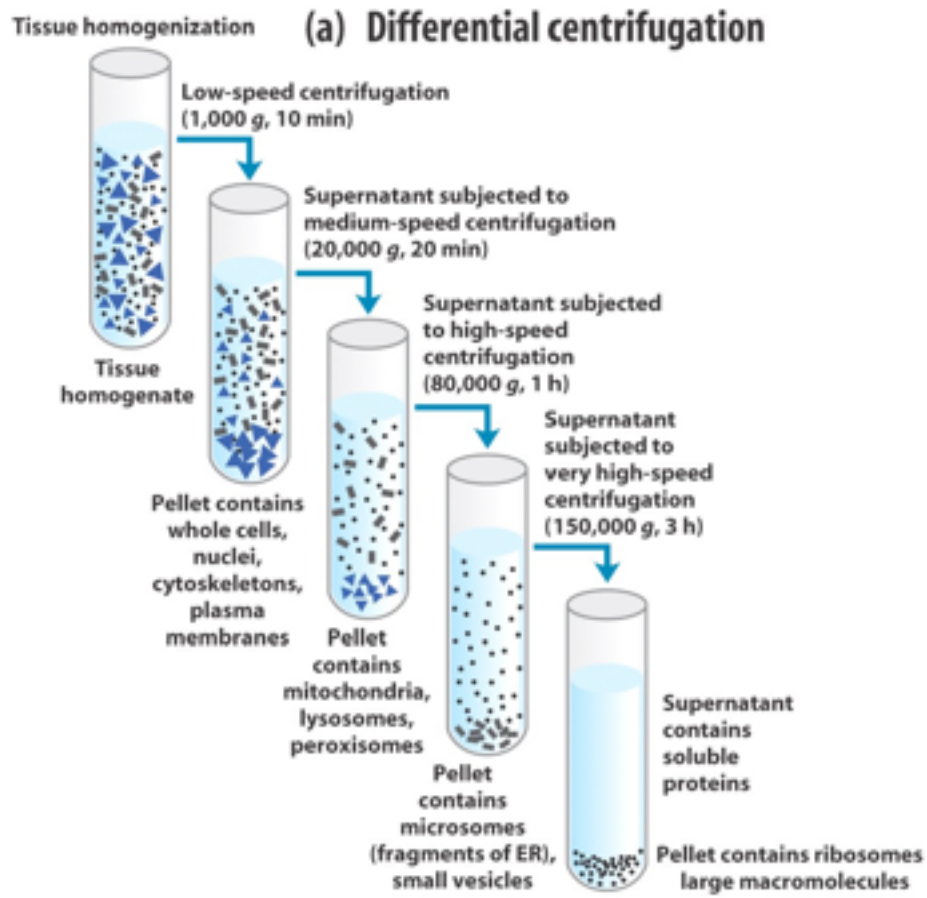
Levels of protein structure



- Preserve the structure during purification
- Consider that the structure may be lost
- Activity assay a good test

First steps…

1. Biological Source: Use a tissue/cell type that contains large amounts of the target protein.
2. Lyse cells: separate cell homogenate into fractions:



Prevent Proteolysis and Keep the Protein Stable

- Cold temperature
  - Also inhibits growth of bacteria
- Chemicals that Inhibit Proteases
  - EDTA to inhibit Ca++ dependent proteases
- Test a variety of buffer conditions
  - Find one where enzyme activity remains constant over time

Purification techniques

All purification techniques utilize different properties of proteins:

Solubility (function of salt, pH, temperature)

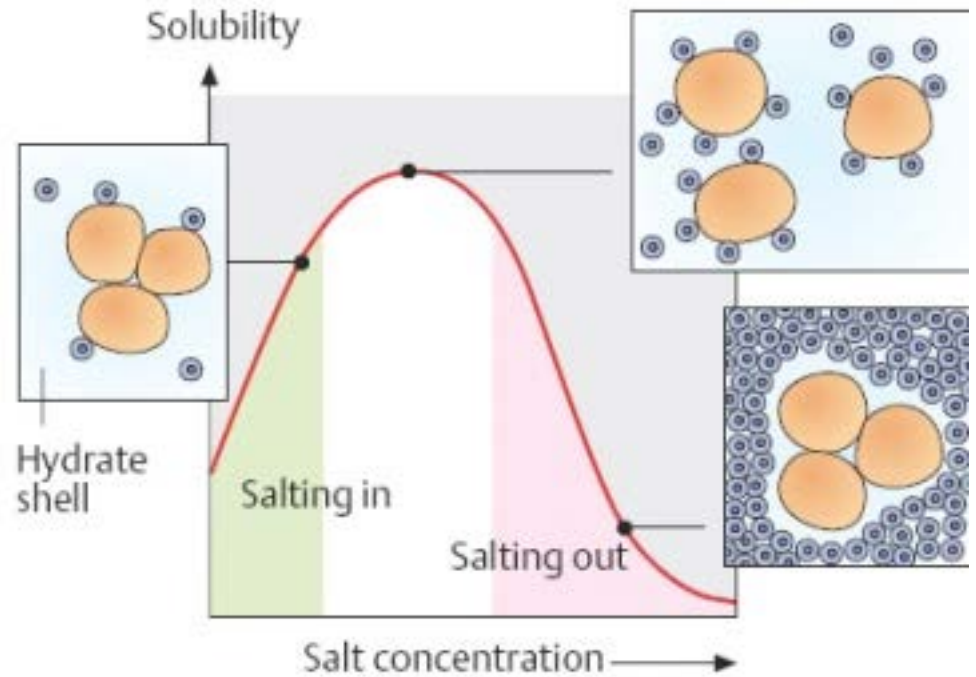
Charge

Size

Binding properties (Ligands)

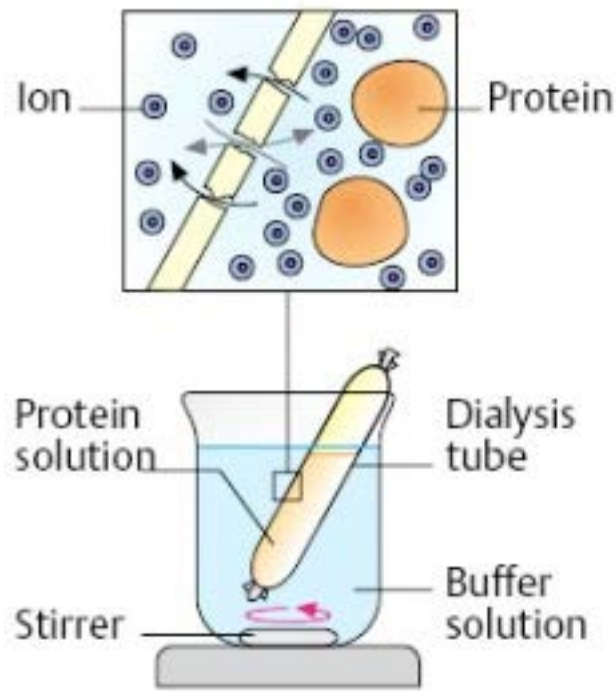
Ammonium Sulfate Precipitation

- Very high ionic strength - Proteins precipitate - “Salting Out”
- Modest Purification but Also Useful to Concentrate the Sample

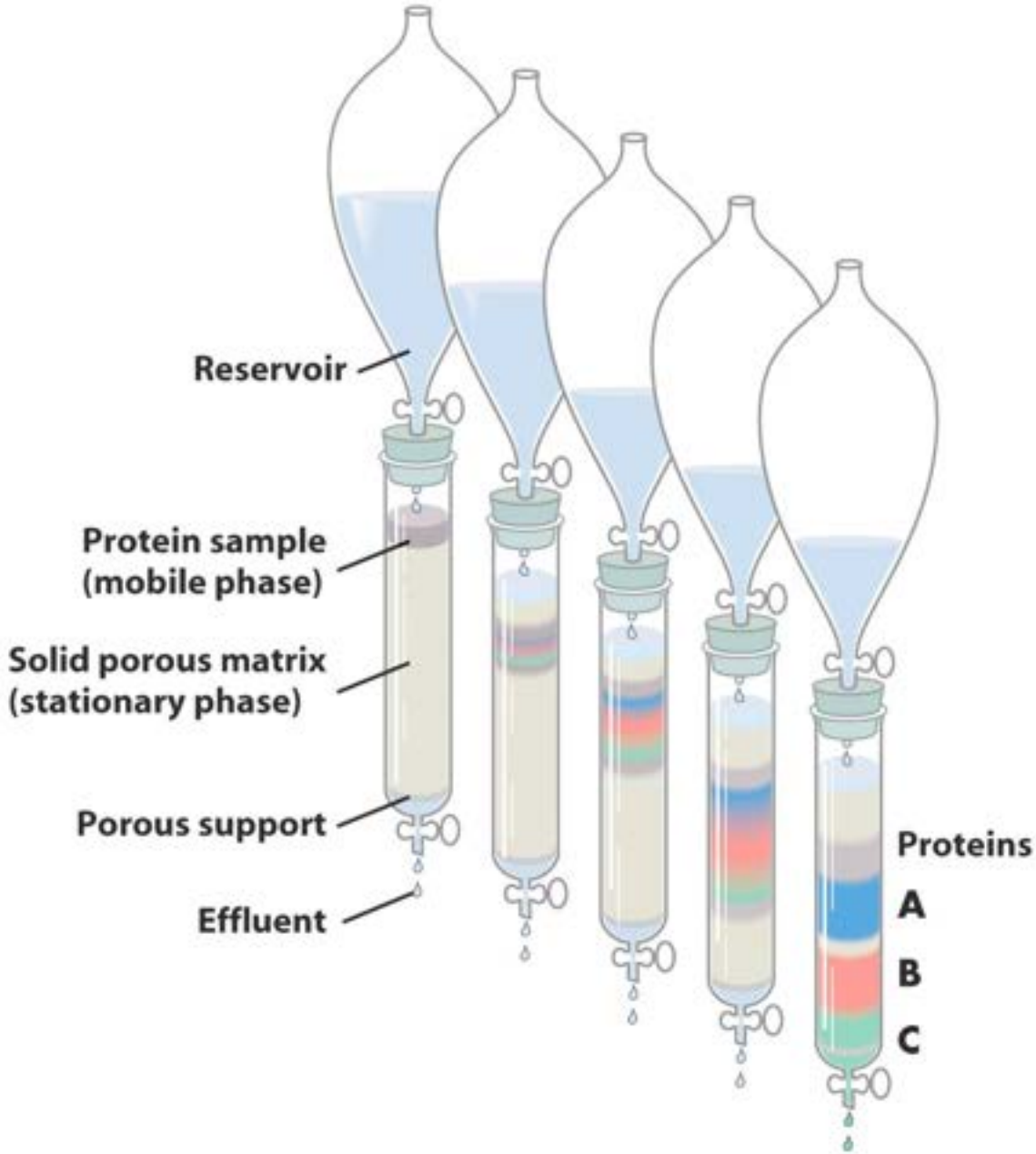


Dialysis to Change Solution Conditions

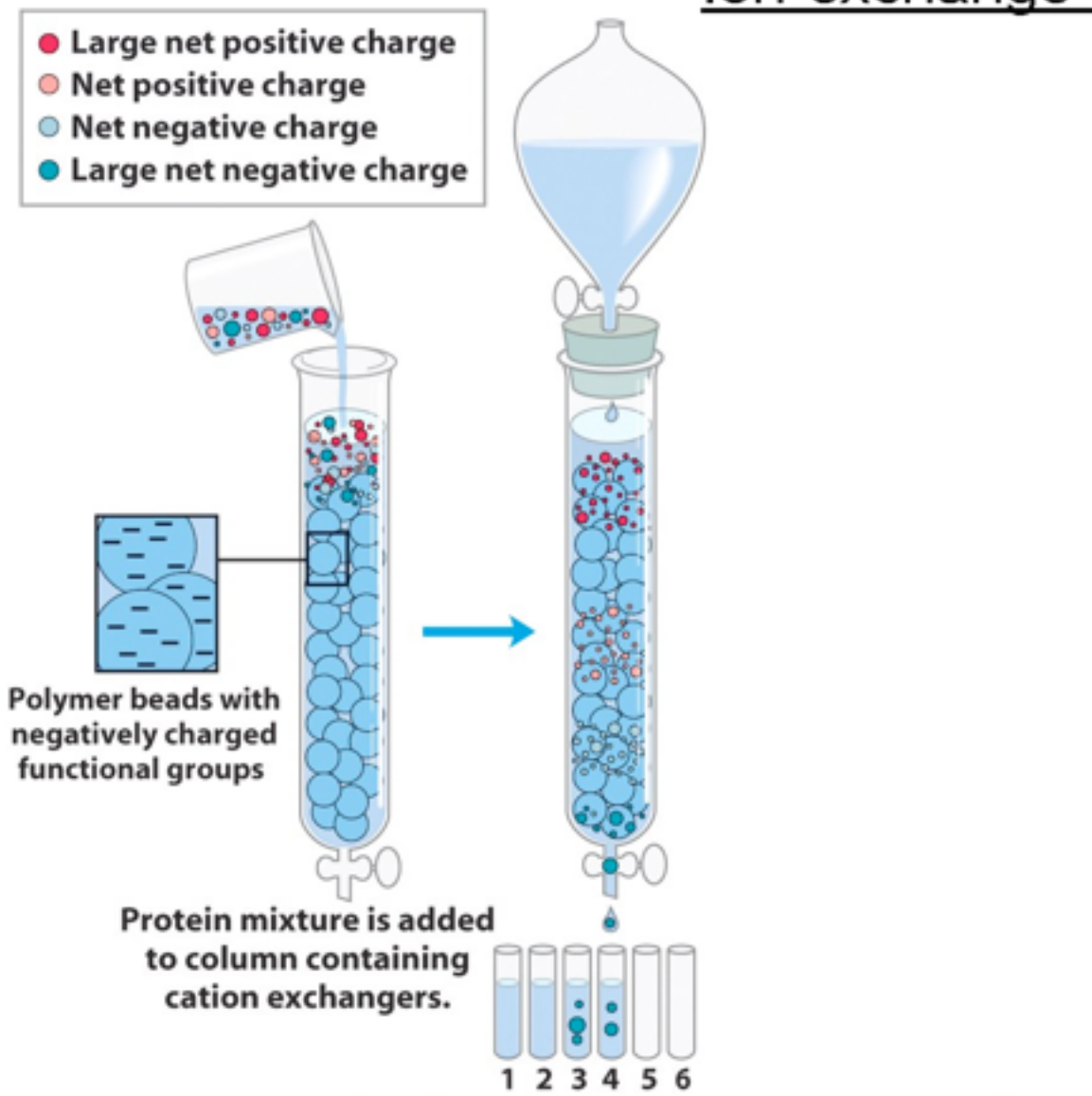
- For Various Steps, often want protein mixture to be in a certain buffer
- Dialyse the sample against a semi-permeable membrane



Column Chromatography: Principles

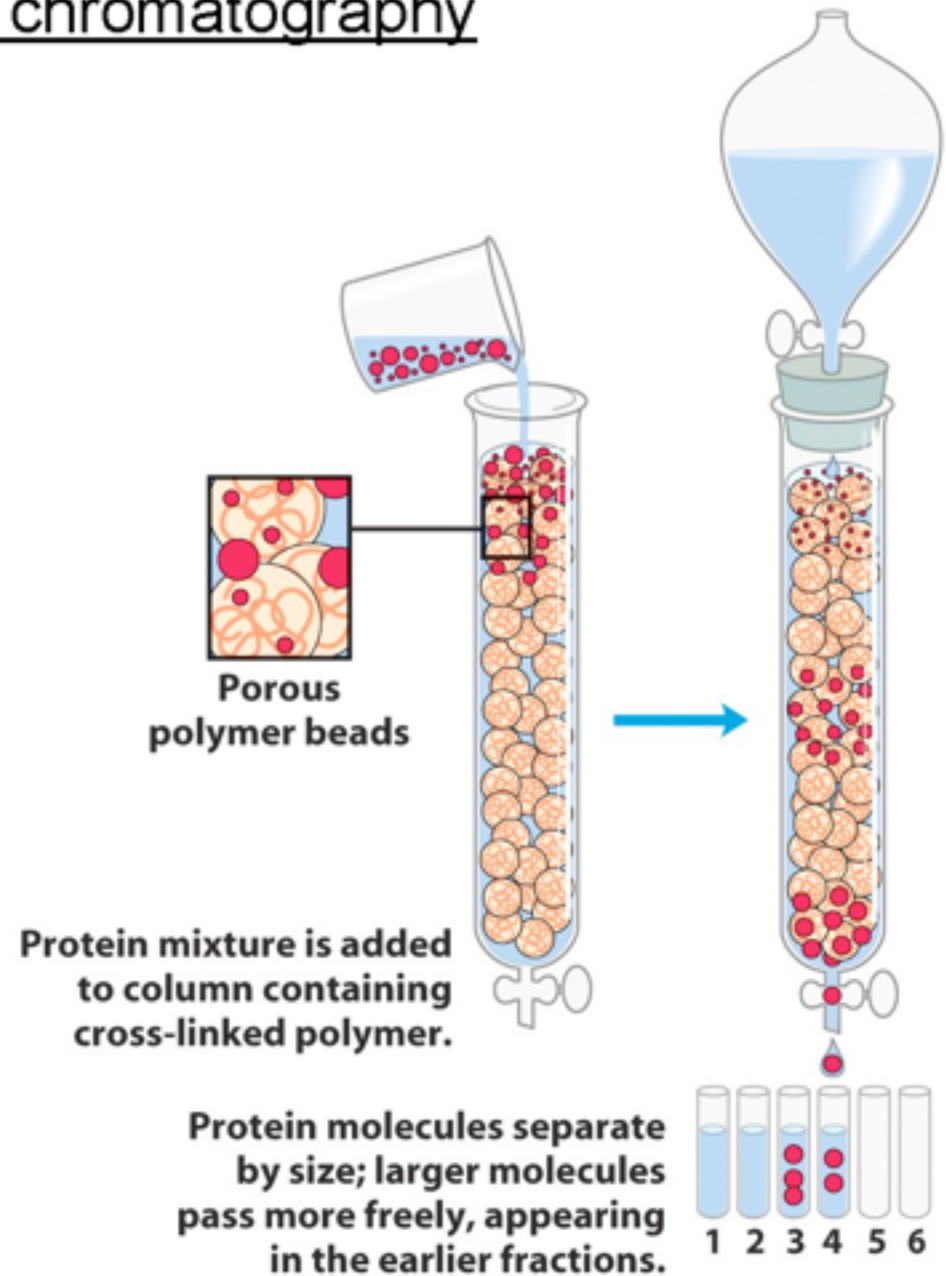


Ion-exchange chromatography



Proteins move through the column at rates determined by their net charge at the pH being used. With cation exchangers, proteins with a more negative net charge move faster and elute earlier.

Size-exclusion chromatography



Affinity chromatography

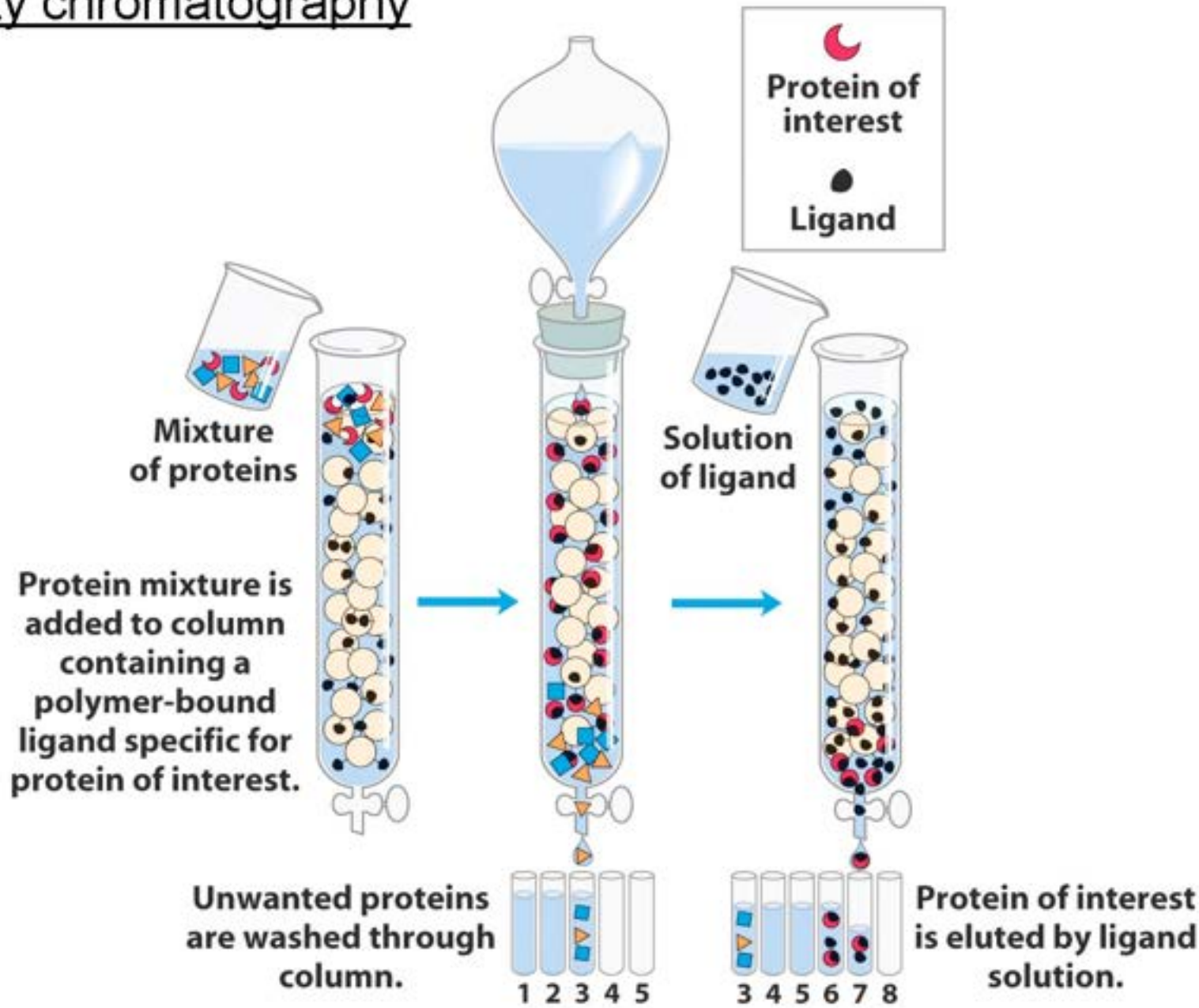
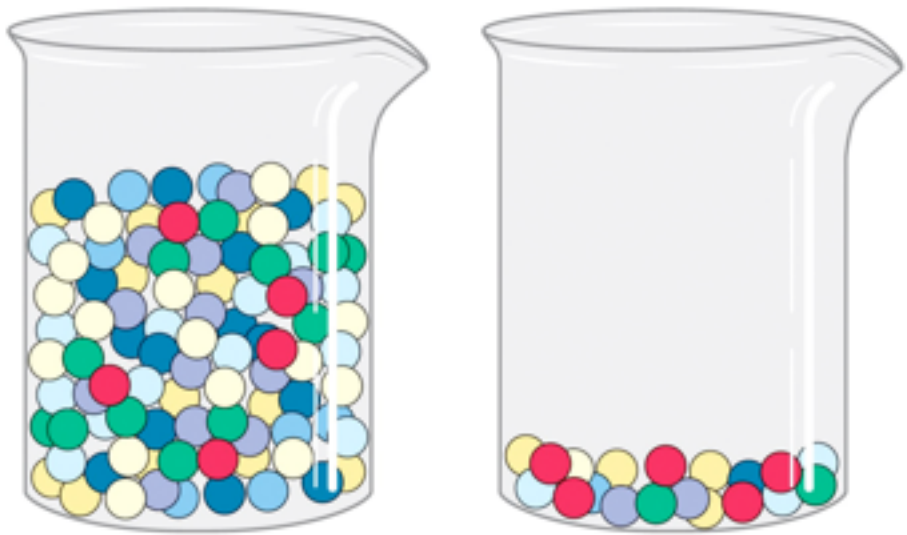


TABLE 3–5 A Purification Table for a Hypothetical Enzyme

Procedure or step	*Fraction volume (ml)*	*Total protein (mg)*	*Activity (units)*	*Specific activity (units/mg)*
1. Crude cellular extract	1,400	10,000	100,000	10
2. Precipitation with ammonium sulfate	280	3,000	96,000	32
3. Ion-exchange chromatography	90	400	80,000	200
4. Size-exclusion chromatography	80	100	60,000	600
5. Affinity chromatography	6	3	45,000	15,000

Note: All data represent the status of the sample *after* the designated procedure has been carried out. Activity and specific activity are defined on page 94.

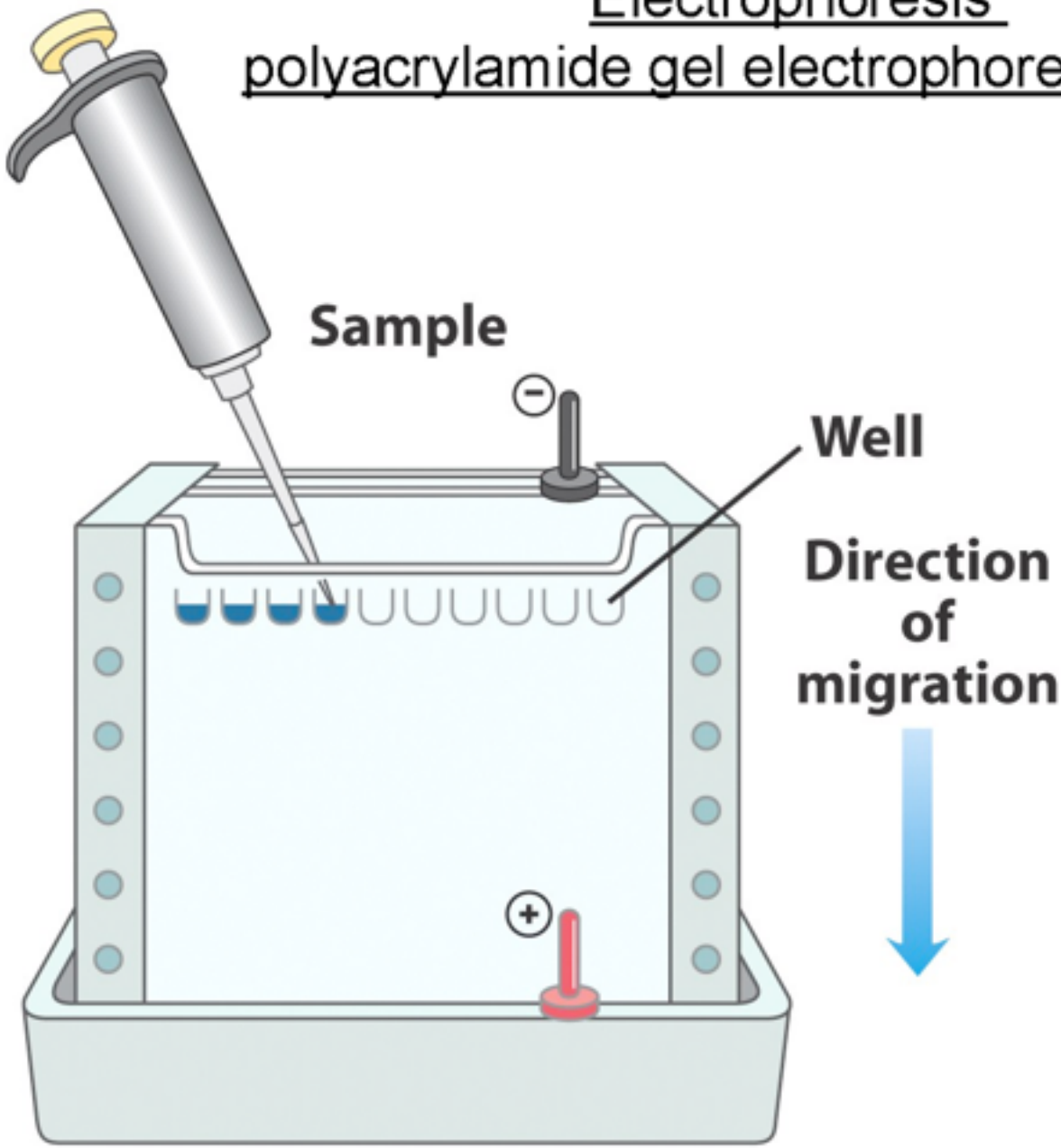


Specific activity=

enzyme activity / amount of protein (mg)

Electrophoresis

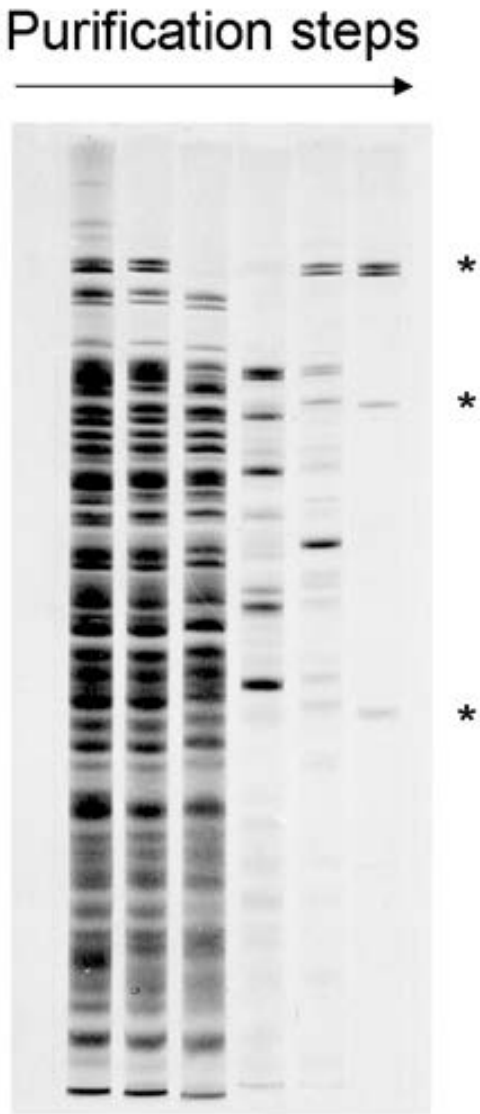
polyacrylamide gel electrophoresis (PAGE)



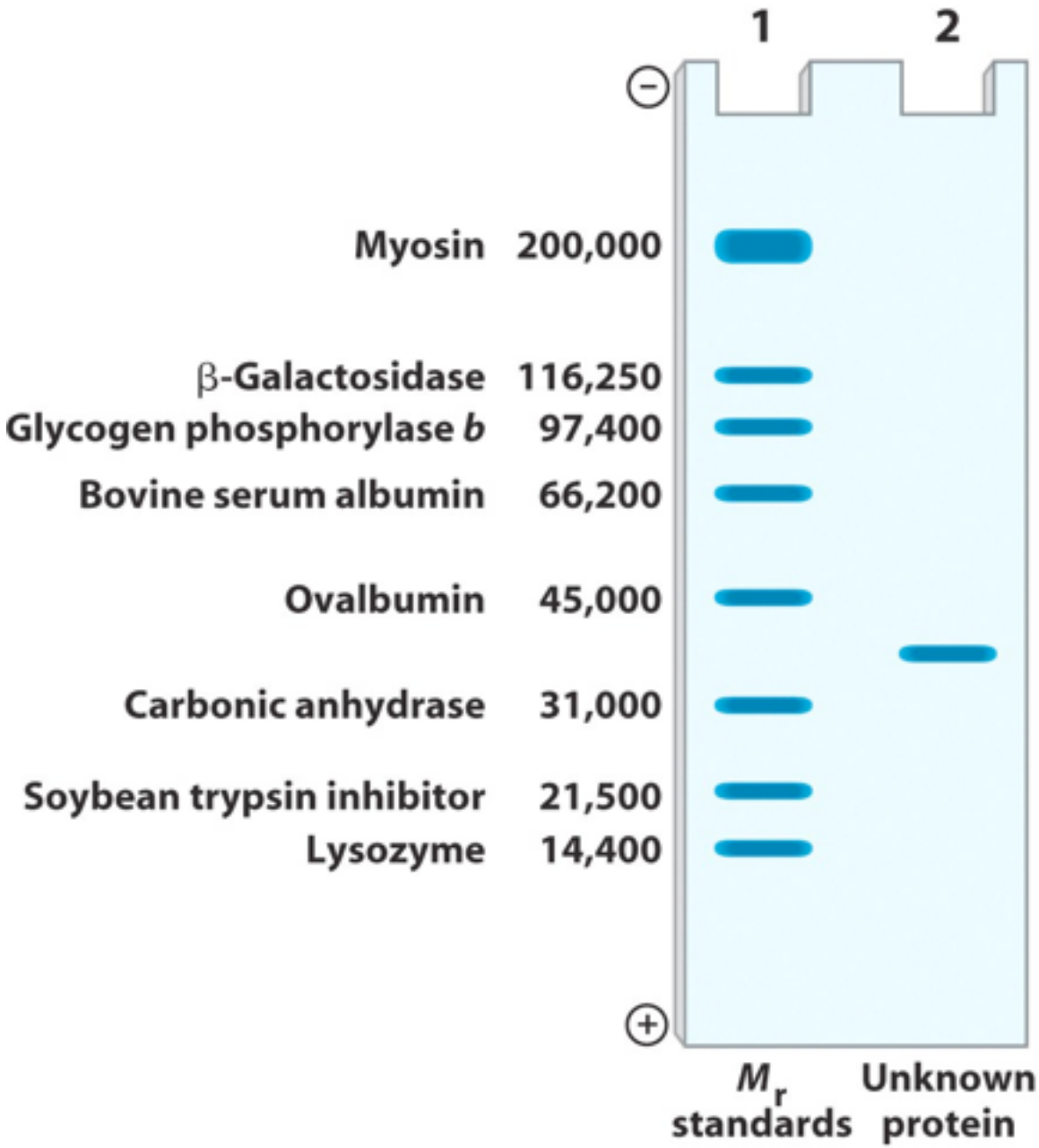
Using SDS-PAGE to Assess Purity

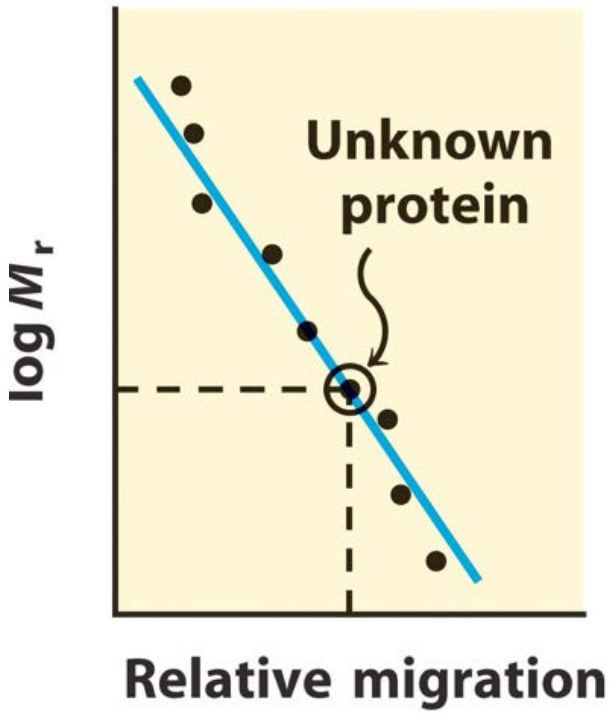
Separate the Polypeptides

Migrate
Based on Mass



Determining Mass of Polypeptide





Isoelectric focusing electrophoresis

(Depends on pI of protein)

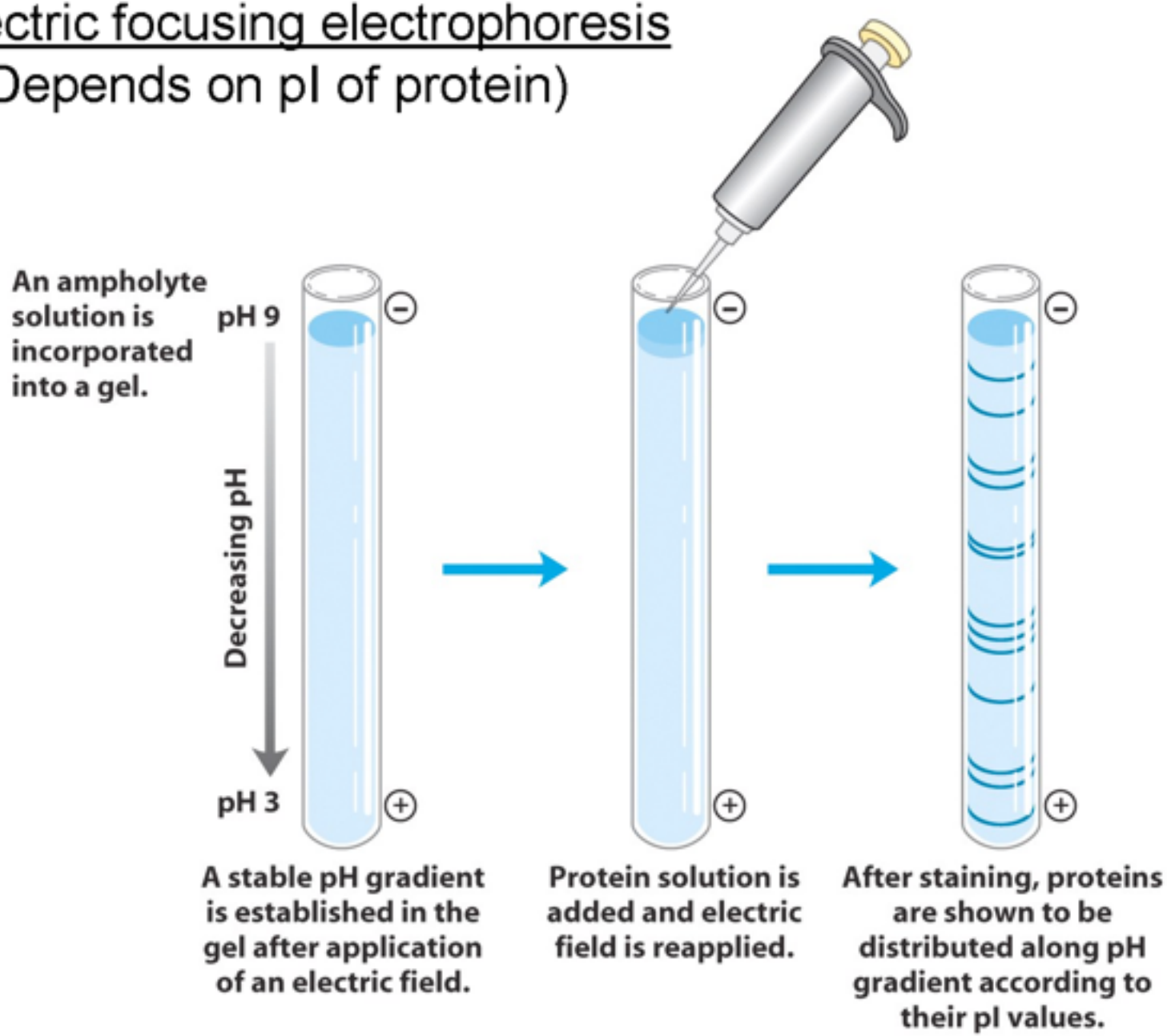
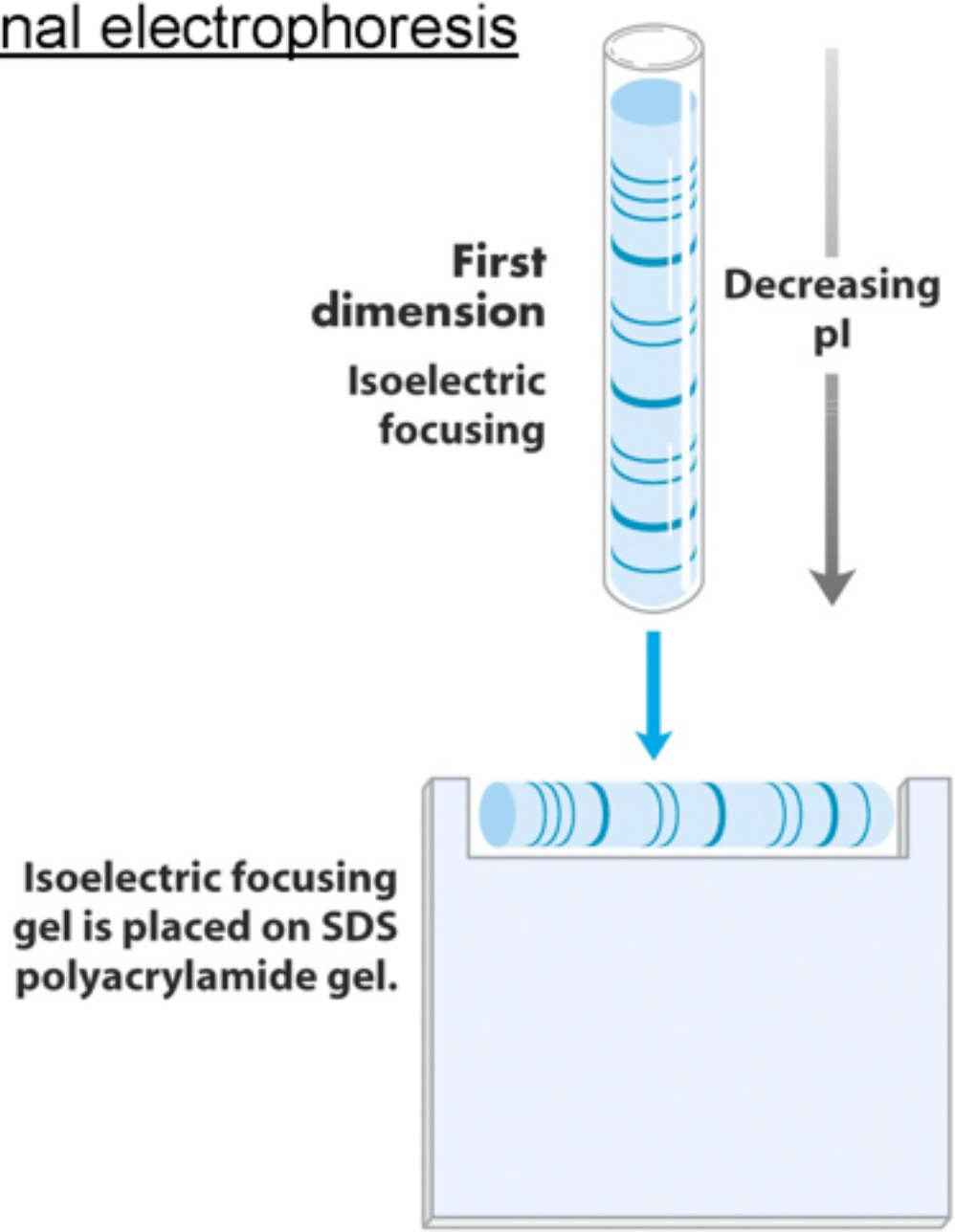
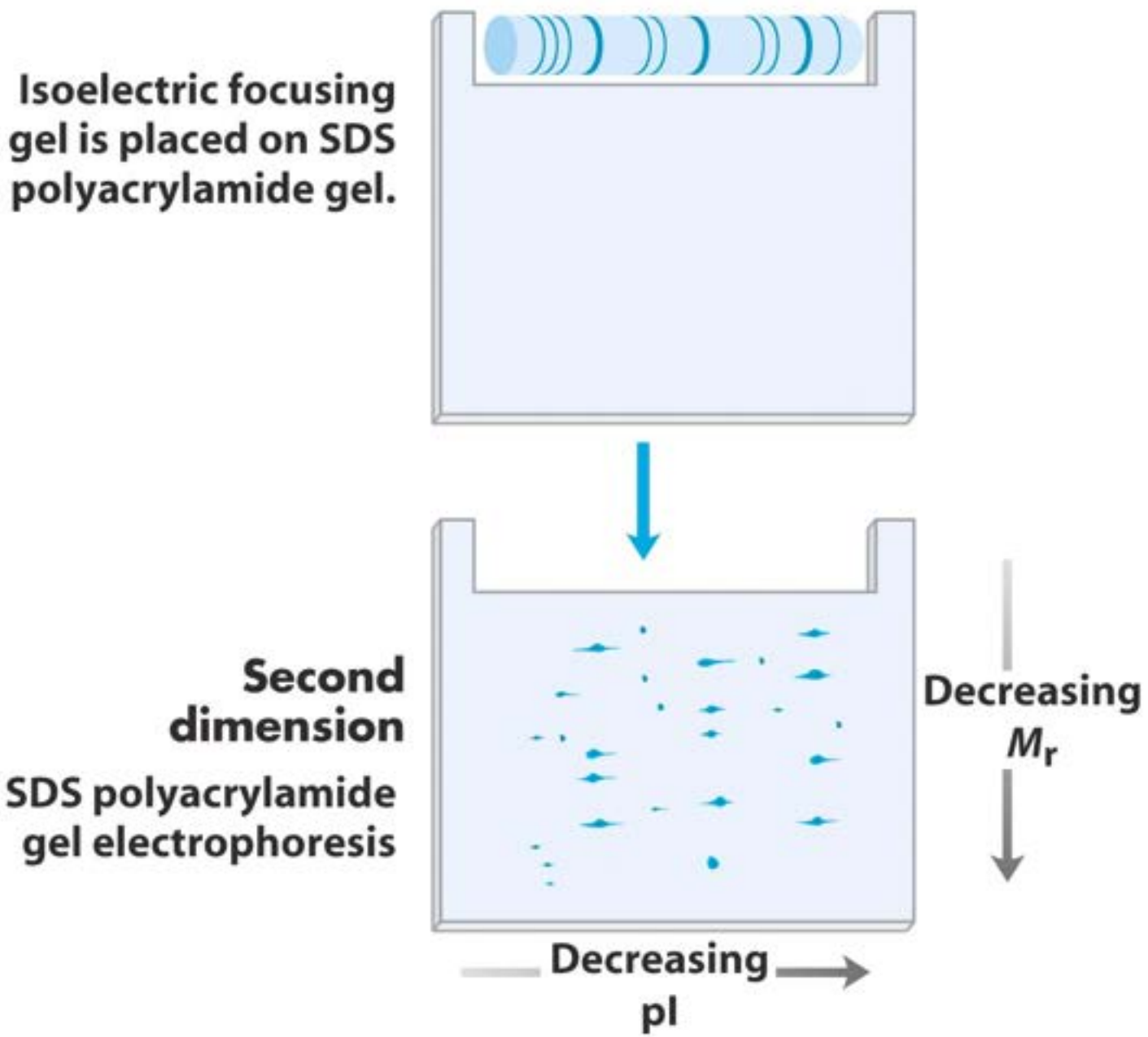


TABLE 3–6 The Isoelectric Points of Some Proteins

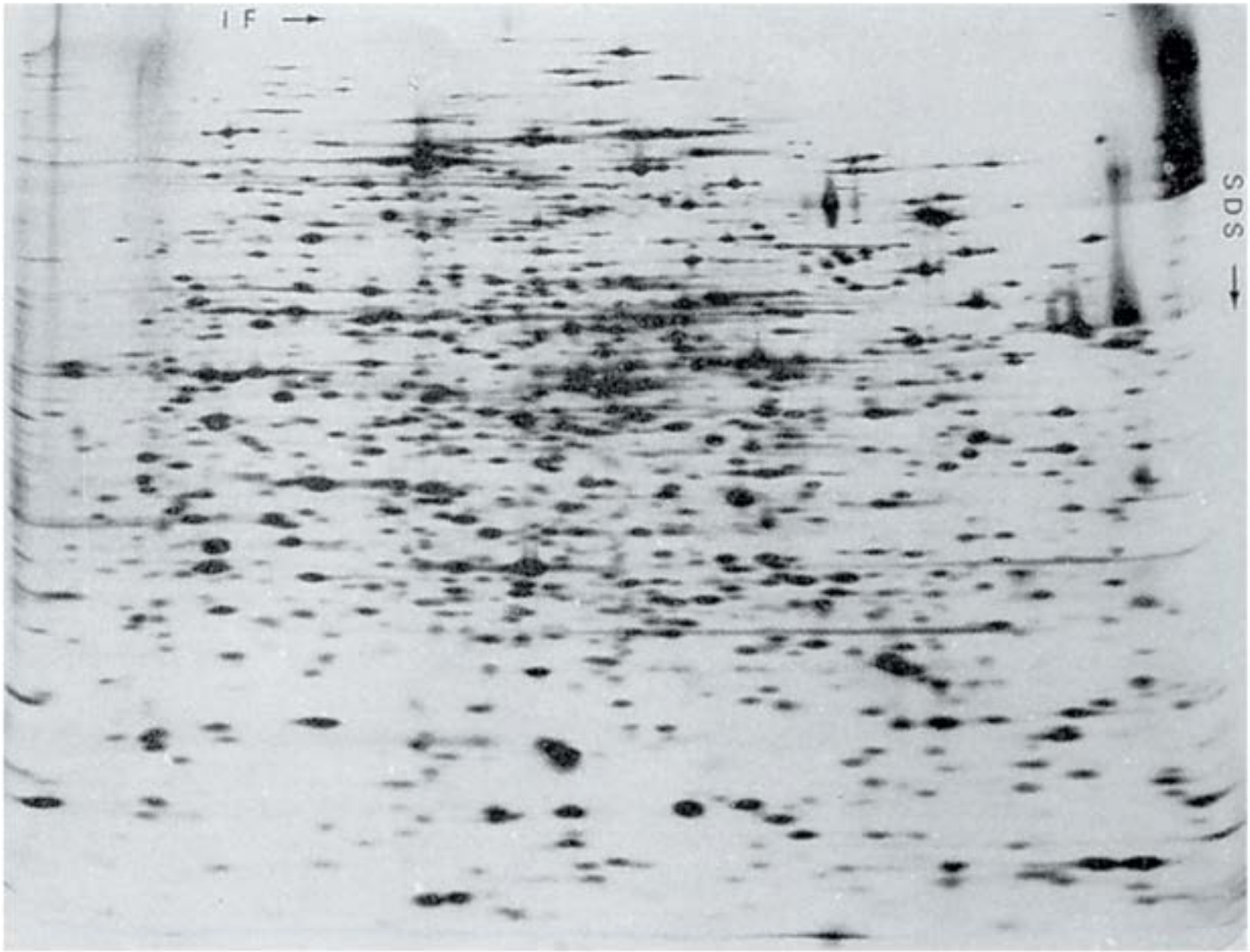
Protein	*pI*
Pepsin	<1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

Two-dimensional electrophoresis



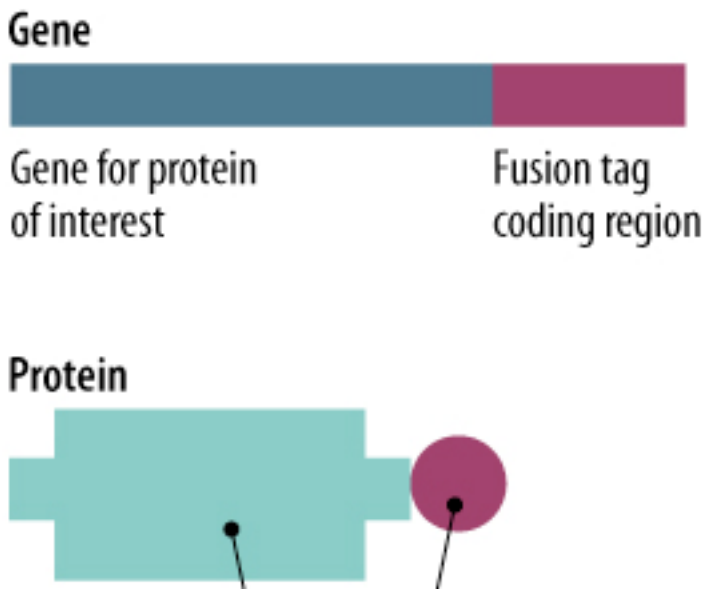

Isoelectric focusing gel is placed on SDS polyacrylamide gel.
Second dimension
SDS polyacrylamide gel electrophoresis
Decreasing M_r
Decreasing pI

Typical 2-D Gel from Whole Cell or Tissue Sample



Affinity-tag Purification

- Use Recombinant DNA Technology to Create a Fusion Protein containing the Tag and Your Favorite Protein (YFP).



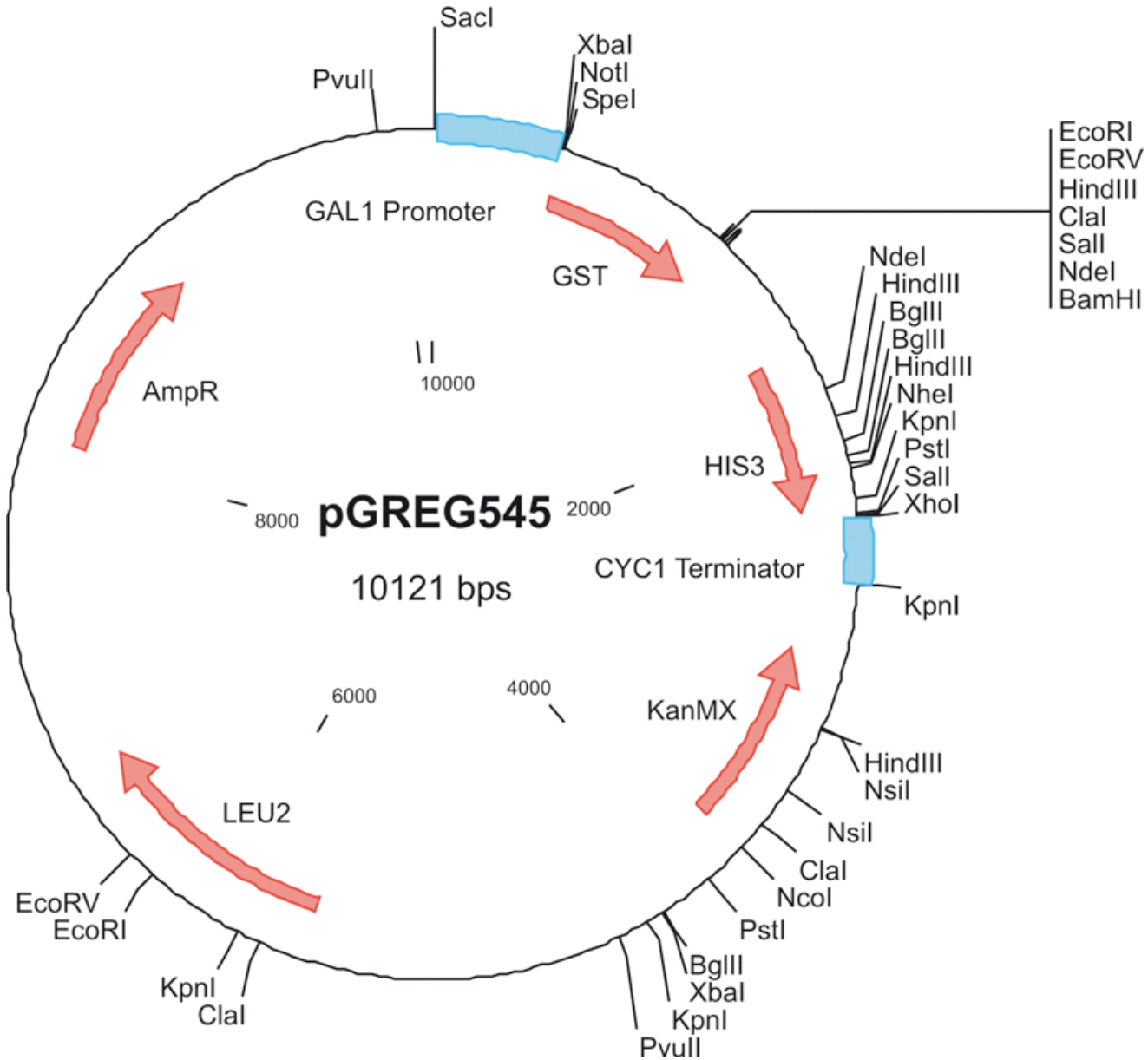
Affinity-tag Purification

- Optional: Place a very specific (i.e. rate) proteolytic cleavage site between the Tag and YFP.
  - TEV Protease: ENLYFQG
  - PreScission Protease: LEVLFQ/GP
- Express in bacteria, yeast, insect or mammalian cells

Advantages and Disadvantages of Protein Expression Systems

- www.genwaybio.com/gw_file.php?fid=6033
- Bacteria: Large amounts of protein, Easy to grow. No post-translational modifications. Low cost.
- Yeast: Intermediate Ease of Growth and Yield of Protein. Some modifications: Lipid, Carbohydrate. Low cost.
- Insect Cells: Intermediate Ease of Growth and Yield of Protein. Baculovirus. High cost.
- Mammalian Cells: Full range of modifications. Poor yield, hard to grow. High cost.

Example of a GST Expression Plasmid for Yeast



Your Protein Goes Here

Affinity Tags Used for Purification of Recombinant Proteins

- GST: Glutathione-S-Transferase. Purify w/ column of glutathione. Elute w/ same.
- MBP: Maltose-Binding Protein. Purify w/ column of amylose. Elute w/ maltose.
- 6xHis: Poly-Histidine. Purify w/ metal (Nickel or Cobalt) resins. Elute w/ imidazole or low pH.

Antibody-based Purification

- Fuse a short Peptide to Your Favorite Protein
  - HA: YPYDVPDYA
  - Myc: EQKLISEEDL
  - FLAG: DYKDDDDK
- Purify w/ affinity beads containing a monoclonal antibody that binds tightly to the peptide
- Elute w/ peptide

TAP: Tandem Affinity Purification

- Two tags - Protein A and Calmodulin-binding peptide, separated by a proteolytic cleavage site.
  - Purify on IgG beads first.
  - Cleave with highly specific protease (TEV) to elute
  - Purify on Calmodulin beads. Elute w/ EGTA.

Concentrating the Purified Protein

- Often Need to Increase Protein Concentration at End of Protocol
- Dialyze into water +/- sucrose. Lyophilize (freeze-dry).
- Ultrafiltration. Force through a semi-permeable membrane.
- Ion-exchange chromatography column w/ large step increase in salt.

Determining Protein Concentration

- Absorbance (ultraviolet): 280 nm. Depends on number of Trp, Tyr and Phe residues in the protein.
  - Calculate / Predict extinction coefficient
  - Lower wavelength (230 or 205 nm) alternative
- Colored Dye Absorbance
  - Lowry, Biuret, Bradford Assays
  - Absorb at Higher Wavelength (e.g. 595 nm)