BIOCH 755: Biochemistry I Fall 2015

9. Protein Purification and Sequencing

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5.2 Protein Purification and Analysis

- **Key Concepts 5.2**
  - Environmental conditions such as pH and temperature affect a protein's stability during purification.
  - An assay based on a protein's chemical or binding properties may be used to quantify a protein during purification.
  - Fractionation procedures take advantage of a protein's unique structure and chemistry in order to separate it from other molecules.
  - Increasing the salt concentration causes selective "salting out" (precipitation) of proteins with different solubilities
  - A protein's ionic charge, polarity, size, and ligand-binding ability influence its chromatographic behavior.
  - Gel electrophoresis and its variations can separate proteins according to charge, size, and isoelectric point.
  - The overall size and shape of macromolecules and larger assemblies can be assessed through ultracentrifugation

Protein Purification

- Often involve increasing a protein with <0.1% dry weight to >98% purity for analysis and functional studies
- Modern molecular biology usually involve cloning and over-expression (up to ~40% of total protein);
- Target protein is often tagged (his tag or small protein domains) for ease of purification
- Need to consider how many environmental factors could destabilize and irreversibly damage the target protein
  - pH, temperature, degradative enzymes, surface absorption, aggregation etc
- Assays needed to quantify the target protein
  - Sensitivity and specificity
  - Activity (e.g. for enzymes), immuno-assays (antibodies)
- Usually require multiple stages: minimize loss!
- Very protein specific: trial-and-error, "an art"

Many Physical Properties Can Be Explored

TABLE 5-2 Protein Purification Procedures

Protein Characteristic	Purification Procedure
Solubility	**Salting out**
Ionic Charge	**Ion exchange chromatography** **Electrophoresis** **Isoelectric focusing**
Polarity	**Hydrophobic interaction chromatography**
Size	**Gel filtration chromatography** **SDS-PAGE**
Binding Specificity	**Affinity chromatography**

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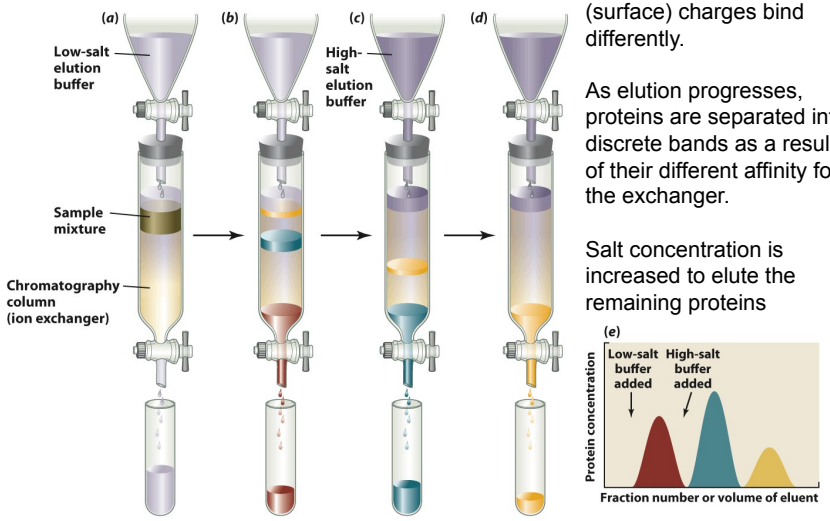
Chromatography

- The most commonly used approach for protein purification
- A mixture of substances in the solution ("mobile" phase) are percolated through a column containing a porous solid matrix ("stationary" phase)
- The substances interact differently with the stationary phase, leading to different retarding forces and thus different migration rates and separation!
- HPLC (high performance liquid chromatography): controlled flow rates at high pressure and specific matrix; thus improved speed, resolution and reproducibility
- Several major types in common use and exploit different properties
  - Ion exchange chromatography: separate ions and anions
  - Hydrophobic interaction chromatography: nonpolar solutes
  - Gel filtration chromatography: size separation
  - Affinity chromatography: specific binding (immunoaffinity, metal chelate)

Ion Exchange Chromatography



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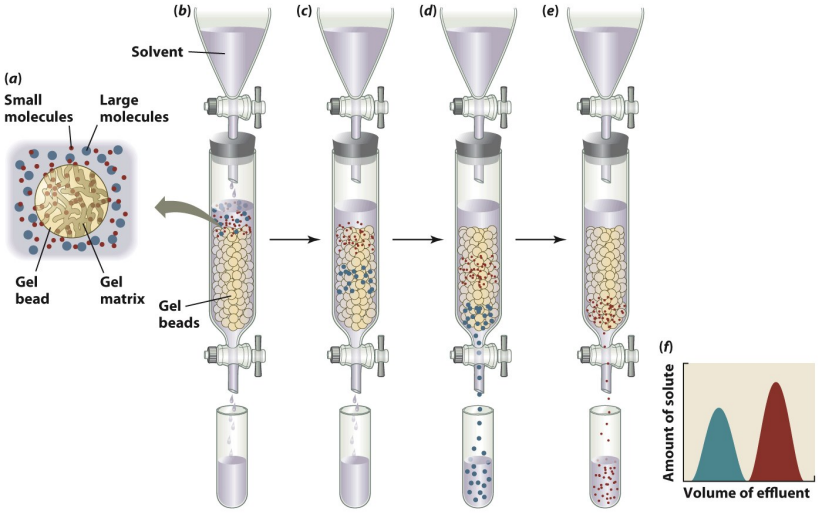
Proteins with different (surface) charges bind differently.

As elution progresses, proteins are separated into discrete bands as a results of their different affinity for the exchanger.

Salt concentration is increased to elute the remaining proteins

Gel Filtration Chromatography



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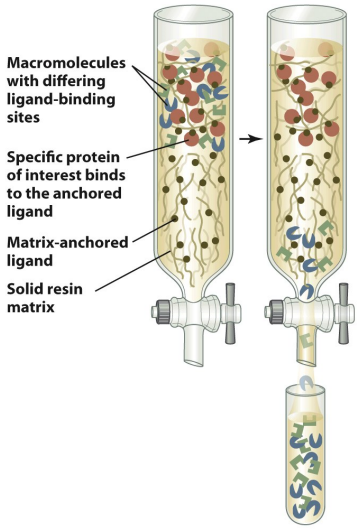
Affinity Chromatography



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Target recombinant protein can be expressed with specifically designed tags, allowing them to be reliably separated by corresponding matrix materials.

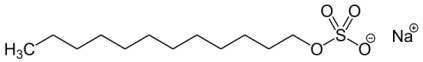
Can be highly specific (instead of relying on small differences in general physical properties)

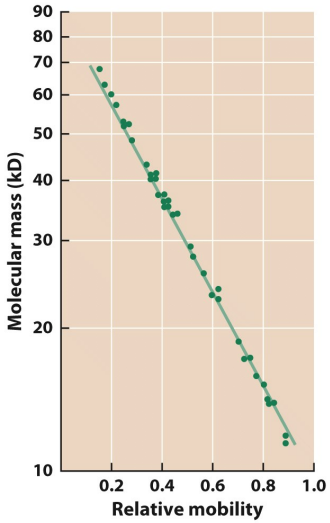
Much much more powerful!

His tag <-> Zn2+/Ni2+
Small proteins <-> antibodies

Electrophoresis: Separation by Charge & Size

- Polyacrylamide gel electrophoresis (PAGE): smaller proteins with larger net charges move faster
  - High pH (~9): almost all proteins are charged
  - Visualized by staining, radioactive labeling, immunoblotting (western blotting)
- SDS-PAGE:
  - SDS (sodium dodecyl sulfate) is a detergent that denatures virtually all proteins;
  - it also coats the unfolded proteins, effectively removing charge and shape dependence.
  - Proteins separate almost purely by molecular weight via gel filtration effects!





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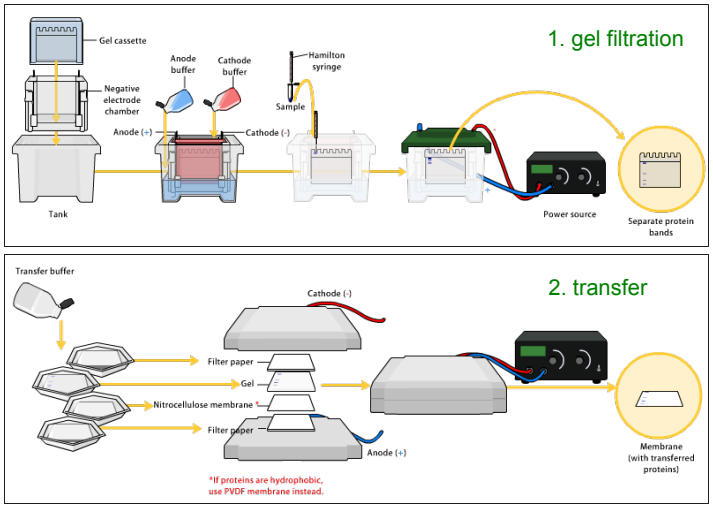
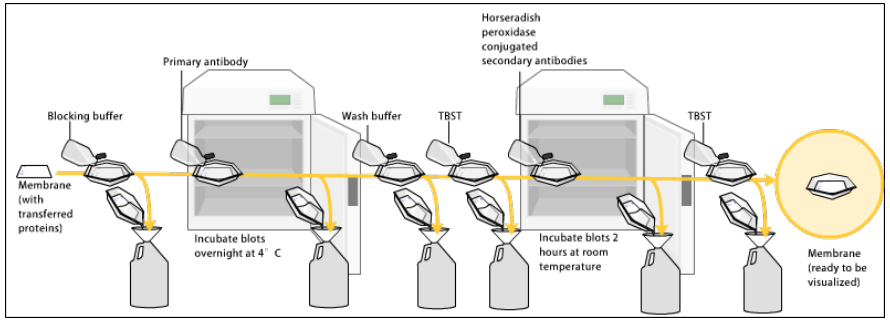
Western Blot w/ SDS-PAGE



https://en.wikipedia.org/wiki/Western_blot

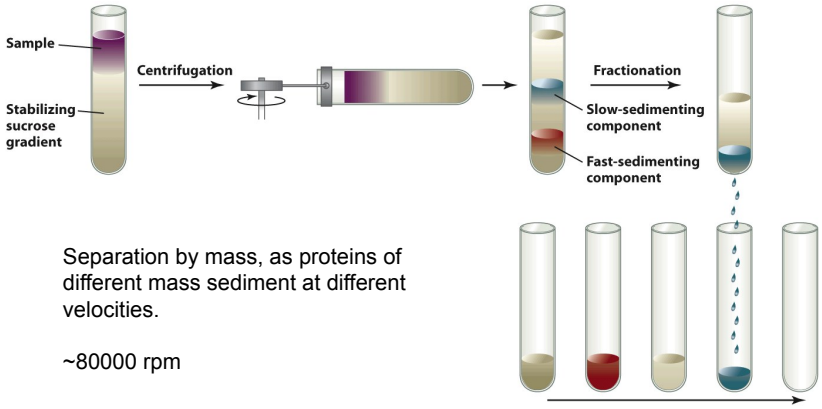
Western Blot w/ SDS-PAGE

3. Blocking and incubation



https://en.wikipedia.org/wiki/Western_blot

Ultracentrifugation



Separation by mass, as proteins of different mass sediment at different velocities.

~80000 rpm

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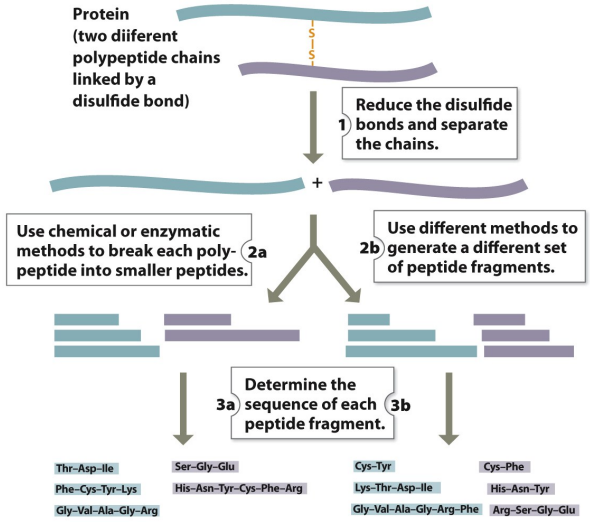
5.3 Protein Sequencing

- **Key Concepts 5.3**
  - To be sequenced, a protein must be separated into individual polypeptides that can be cleaved into sets of overlapping fragments.
    - Protease digestion: trypsin, chymotrypsin etc
  - The amino acid sequence can be determined by Edman degradation, a procedure for removing N-terminal residues one at a time.
  - Mass spectrometry can identify amino acid sequences from the mass-to-charge ratio of gas-phase protein fragments.
    - ESI: Electrospray Ionization
  - Protein sequence data are deposited in online databases.

Process Diagram: Protein Sequencing



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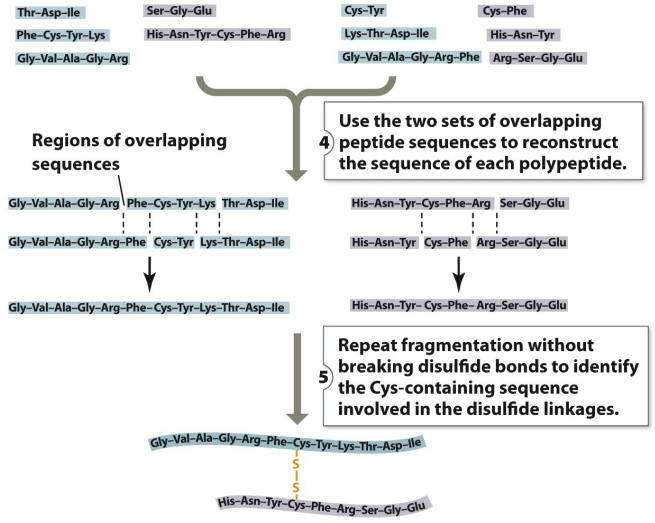
Process Diagram: Protein Sequencing



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Electrospray Ionization (ESI) Mass Spectrometry

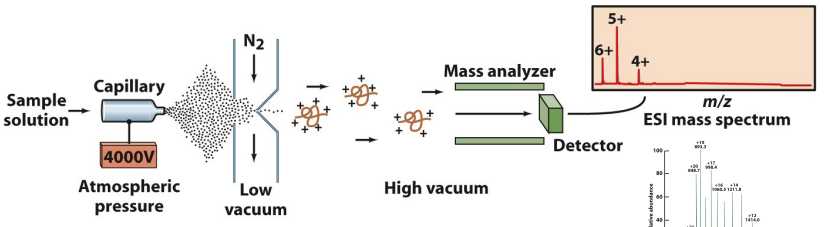

Figure 5-17a
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- Tandem mass spec: first one select the peptide ion to be passed through a collision cell; resulting fragments detected by a second mass spec
  - Precision of m/z ratio allows small fragments to be sequenced directly

Protein Sequence Databases

TABLE 5-5 Internet Addresses for the Major Protein and DNA Sequence Data Banks

Data Banks Containing Protein Sequences
ExPASy Proteomics Server: http://expasy.org/
Protein Information Resource (PIR): http://pir.georgetown.edu/
UniProt: http://www.uniprot.org/
Data Banks Containing Gene Sequences
GenBank: http://www.ncbi.nlm.nih.gov/genbank/
European Bioinformatics Institute (EBI): http://www.ebi.ac.uk
GenomeNet: http://www.genome.jp/

Table 5-5
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