Protein Analysis and Purification

First, to acquire a protein, need to **lyse** cells by one of many methods:

- grinding
- freeze/thawing
- detergents
- sonicating
- rapid pressure changes

Then you can **centrifuge** to roughly sort proteins.

Extract @ low speed (5000G) for 10 mins
  Pellet = nuclei
Supernatant @ medium speed (10,000G) for 20 mins
  Pellet = mitochondria
Supernatant @ high speed (100,000G) for 2 hours
  Pellet = microsomes, membranes and ribosomes
Supernatant = soluble proteins

Protein Analysis - determining what proteins are present

1. **SDS-PAGE:** Separation by molecular weight
   - negative charge provided by SDS: one SDS / 2 a.a.
   - disulfide bonds reduced by β-mercaptoethanol
   - long polyacrylamide chains crosslinked with bisacrylamide create a sieve through which short proteins move quickly and long proteins move slowly
   - Temed is the stinky substance that initiates bis-crosslinking
   - "Run to red": the negatively charged proteins migrate towards the positive electrode (which is always red)
   - detect with Coomassie blue or silver stain
2. **Western blot**
   - uses antibody to protein (so Ab must be commercially available or made)
   - primary antibody binds to protein; a secondary antibody with some sort of tag (fluorescence, radiation, etc) binds to the primary antibody
   - detection of secondary antibody shows where (if present) the protein of interest is in a sample
3. **Isoelectric Focusing (IEF):** Separation by pI.
   - pI = the pH at which the charge on the whole protein is neutral
   - pH gradient set up by pre-running the gel with ampholytes
   - below pI, the protein is positively charged
   - above pI, the protein is negatively charged (use Zwitterion example)
4. **2D gel**
   - first perform IEF, then do SDS-PAGE
   - the IEF has NO SDS
   - very helpful if you have multiple samples that are similar in pI and weight.

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Protein Purification - purifying protein for further studies

1. Salting Out

- makes use of different solubilities
- as ionic strength increases, solubility decreases; thus, protein "falls out" of solution
- different salt concentrations needed for different proteins; that is, while one protein will salt out at salt concentration X molar, a different protein will salt out at salt concentration (X+N) molar.
- $(NH_4)_2SO_4$ mentioned in class

2. Dialysis

- separates based on size
- small molecules, like salt contaminates, go through the bag, while large proteins remain in the bag. At equilibrium, there will be an equal concentration of salt contaminates both inside and outside of the bag. Thus, you want to use a very large volume of liquid outside of the bag to get rid of as many contaminates from inside the bag as possible
- ideal for separating contaminates from protein
- not good for separating a "small" protein from a "larger" protein - usually, neither can pass though the bag

3. Gel Filtration Chromatography

- better way of separating by size
- fill column with relatively large (0.1mm) beads made from carbohydrates. The beads have small pores throughout them.
- small proteins run both outside and inside the beads - thus, they travel slowly
- large proteins cannot get inside the beads - thus, they travel through the column quickly
- large proteins elute first, small proteins elute last
- agarose, polyacrylamide are both types of gel beads

4. Ion Exchange Chromatography

- separation by charge
- anion = negative
- cation = positive
- to select for negative proteins, use DEAE column (anion-exchange)
- to select for positive proteins, use CM column (cation-exchange)
- pass the solution with your protein of interest over the column
- use a higher concentration of ion (positive ion for positive protein, negative ion for negative protein) to elute out your protein of interest

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5. Affinity Chromatography

- separation by affinity for a specific chemical group.
- very specific: can get > 10^4 purification in one step
- requires that you know a ligand for your protein and that you have a column with ligand attached (ex: Histidine binds Nickle)
- elute with substance that competes with the binding of the beads to the protein.
- examples:
    * Elute $(His)_6$-tagged protein with imidazole, functional group of Histidine
    * Elute Maltose Binding Protein with maltose

You will want to test your specific activity of your protein:

Simply the total activity ÷ total protein

if you radio labeled something then it would be:

$$\frac{\text{nmoles *label}}{\text{(ug template)*(minutes)*(mg extract)}}$$