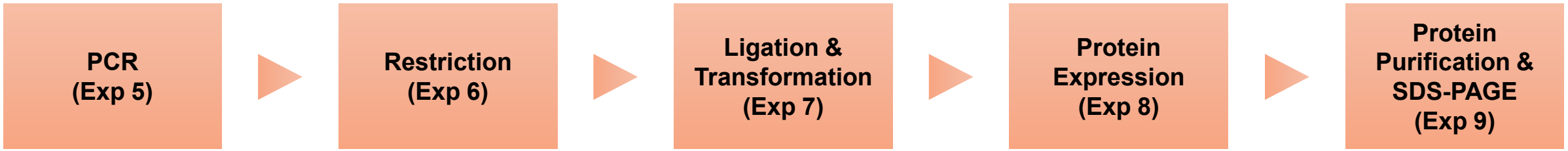
Experiment 9. Protein Purification and SDS-PAGE II

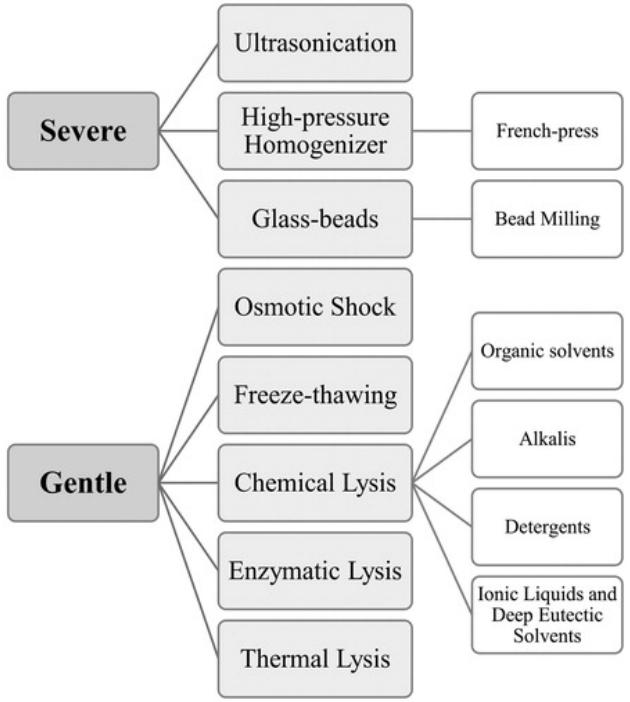
CH353 Chemistry Major Laboratory III

Experimental Procedure



Cell lysis

Cell membrane and walls must be disrupted to extract target proteins. In physical disruption methods, the cell membrane is physically broken down by shear or external forces to release cellular components. Depending on the stability of the target proteins, one of the following methods can be used: sonication, manual grinding, homogenization and freeze-thaw. Lysing a wide range of cells with high efficiency is important, but protein denaturation and aggregation can occur due to localized heating. In solution-based cell lysis, detergents break the lipid barrier surrounding cells by solubilizing proteins and disrupting lipid-lipid, protein-protein, and protein-lipid interactions.



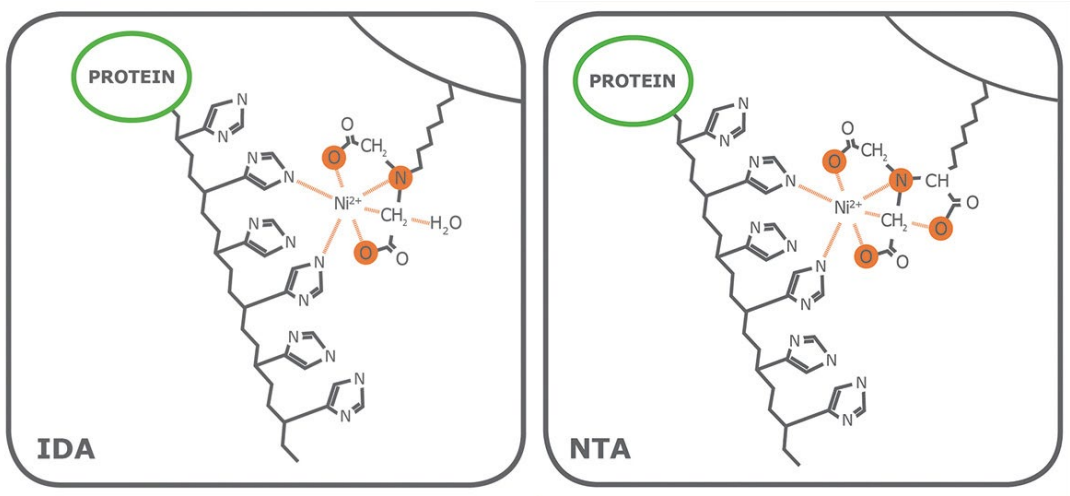
Ultrasonicator

Protein Purification

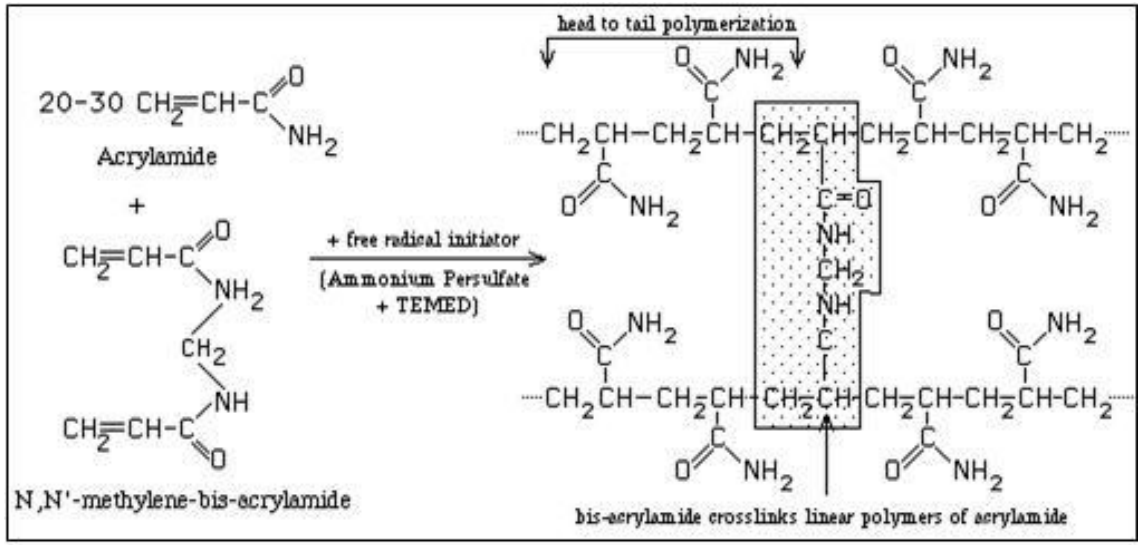
Proteins can be purified based on different properties as follows:

- Separation based on size: Size exclusion chromatography
- Separation based on charge or hydrophobicity: Ion exchange chromatography
- Separation by affinity difference: Affinity chromatography

Ligand (stationary phase)	Target protein
Substrate analogue	Enzymes
Antibody	Antigen tagged proteins (FLAG, Myc, HA, NE)
Lectin	Glycoproteins
Hormone	Receptors
Avidin	Biotinylated proteins
Glutathione	GST fusion proteins
Amylose	MBP fusion proteins
Protein A & G	Immunoglobulins
Metal ions	His-tagged proteins



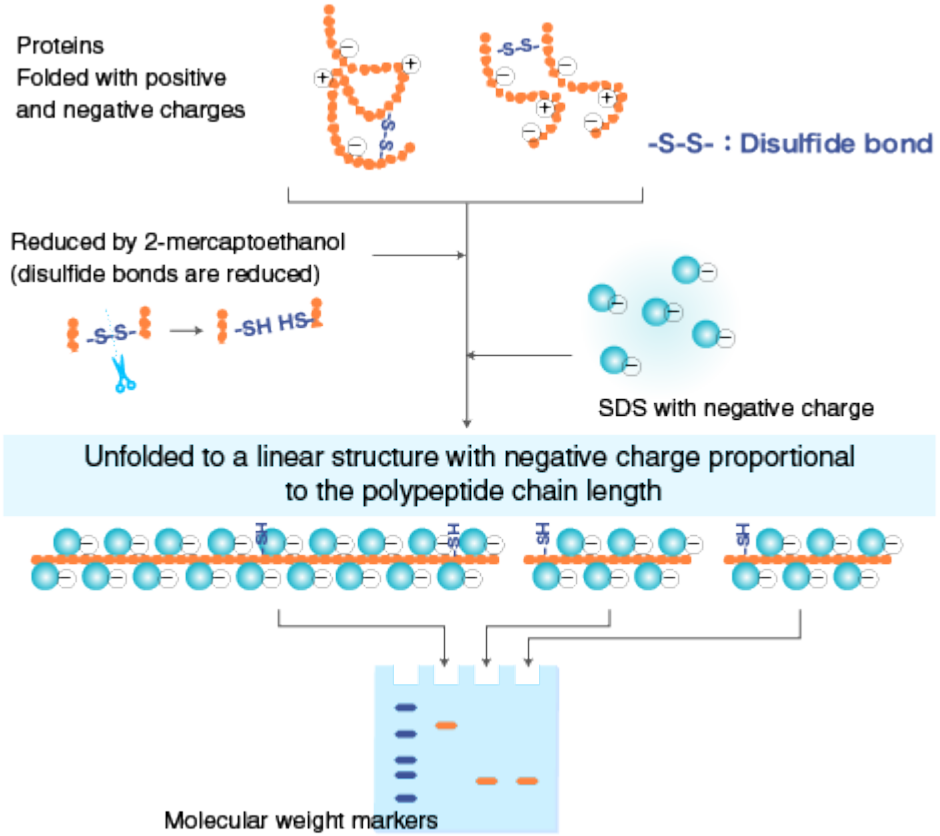
Polyacrylamide gel

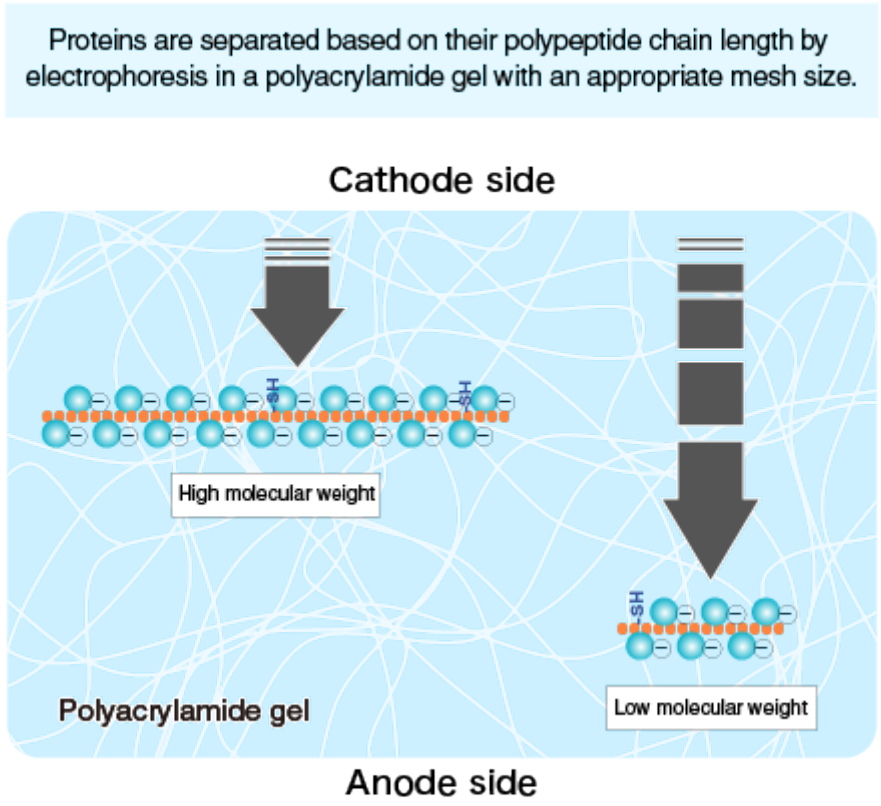


Acrylamide cross-linked by N,N’-methylene bisacrylamide.
The polymerization is initiated by APS(ammonium persulfate) free radical, which is generated by TEMED.
We can control gel pore size by changing acrylamide concentration.

What is SDS-PAGE?

Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis



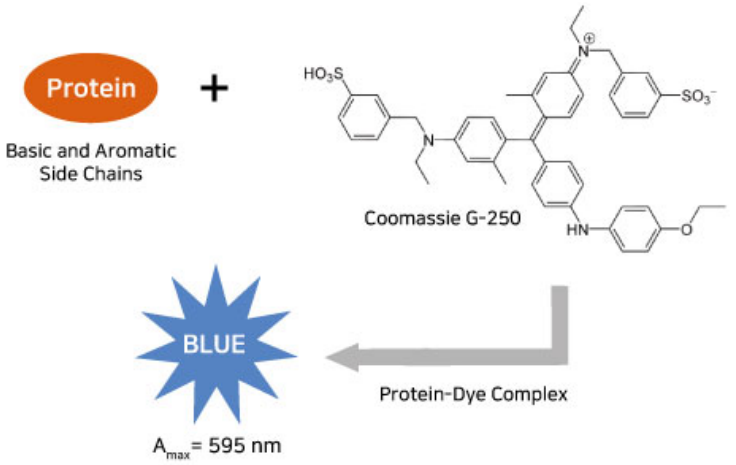


Native PAGE

Native PAGE can distinguish proteins with different charge & shape/size.
In Native PAGE, you don't denature the proteins, so the complexes (if they're strong enough) stay together

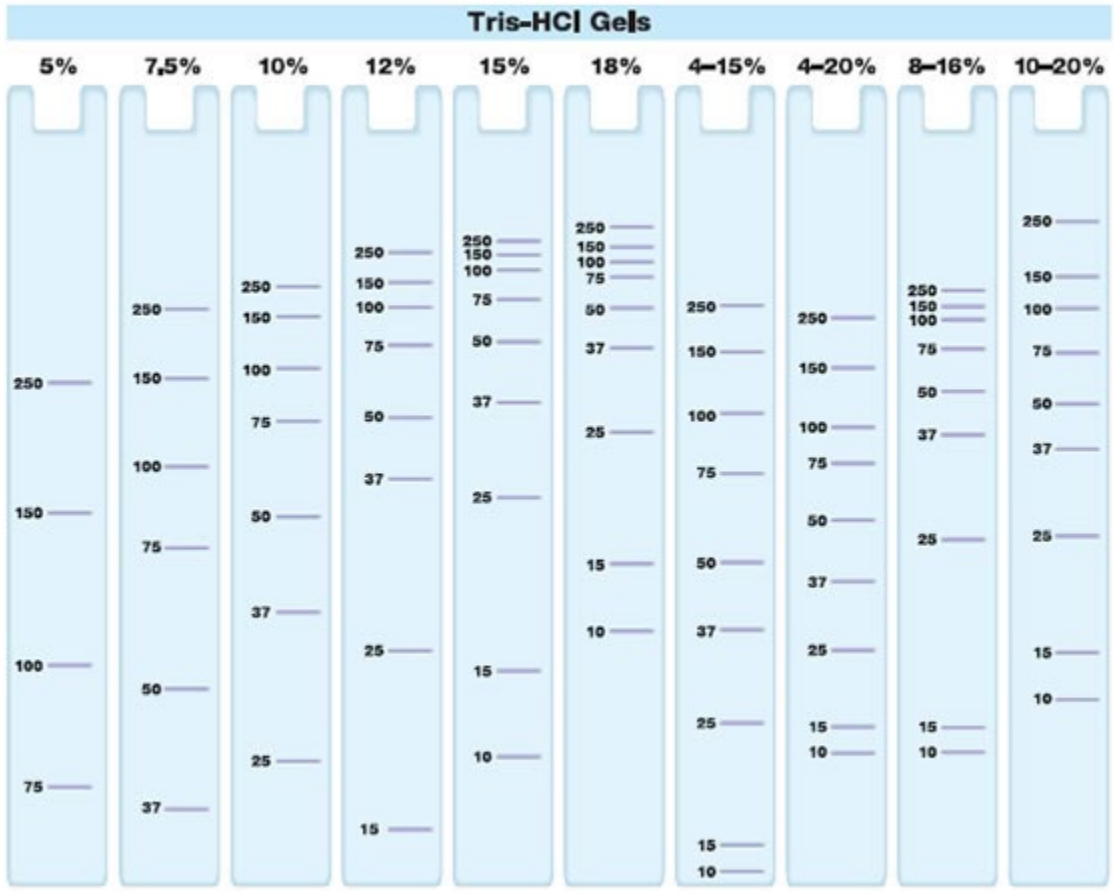
Staining solution

40 % Methanol
10 % Acetic acid
0.1 % Dye (CoomassieBrilliant Blue R250)



Acrylamide concentration

15% gel is appropriate for GFP (GFP : 26.9 kDa)



Procedure – Protein purification

Before the class (What TA did)

1. Cell pellet is resuspended with lysis(equilibration) buffer. (50 mM Tris-HCl pH8.0, 500 mM NaCl)
2. Sonication to break the cell membrane.
3. Centrifuge lysate at 12000 rpm for 15 min.

Step 1. Load Ni-IDA resin(final 1mL, load resin mixture about 2mL) to the column.

Step 2. Equilibrate Ni-IDA column with 5X volume of equilibration buffer. (50 mM Tris-HCl pH8.0, 500 mM NaCl)

- Please, load buffer along the wall to avoid digging the resin.

Step3. Load lysate(supernatant) containing GFP onto the pre-equilibrated Ni-IDA column.

- Please, collect flow-through solution for PAGE analysis.

Procedure – Protein purification

Step 4. Wash the Ni IDA column with 5X volume of wash buffer (50 mM Tris-HCl pH8.0, 500 mM NaCl, 50 mM imidazole)

- Please, collect washing solution for PAGE analysis.

Step 5. Elute the protein with elution buffer (50 mM Tris-HCl pH8.0, 500 mM NaCl, 500 mM imidazole)

- Please, collect eluate for PAGE analysis.

Procedure – SDS PAGE

Step 1. Mix your samples with 5X SDS-PAGE loading buffer

- Sample 20ul + Buffer 5ul
- Label each sample clearly not to be confused

Step 2. Boil the samples in a heat block at 95 ºC for 5 min

Step 3. Set up the electrophoresis equipment

1) Put up the casting cradle on the bottom chamber
2) Fix your gel to the casting cradle
3) Pour the 1X SDS-running buffer to fill the chamber

Procedure – SDS PAGE

Step 4. Loading sample

1) Carefully load your sample to the well (5 μL for each sample)

2) Load the protein size-marker – 5 μL

Step 5. Running electrophoresis (150V, 60min)

- Check the electrode(black-black / red-red) before closing the upper chamber

Step 6. Pour the staining solution then shake for 30min.

Step 7. Remove staining solution and pour de-staining solution.

Analysis & Discussion

1. What does the color of Ni-IDA beads indicate in each step of the experiment. Analyze the reasons for the process of color change.
   – before equilibration, after equilibration, flow-through, wash, elution.

2. In the SDS-PAGE gel, which is the band of the protein of interest (GFP)?

3. From the SDS-PAGE gel image, can you say that you have recovered most of the over-expressed GFP with the resin? How can you know that?

4. From the SDS-PAGE gel, can you see if the purification is well-performed? Explain your answer regarding your gel image.