Texas A&M University-Corpus Christi
CHEM4402 Biochemistry II Laboratory
Laboratory 13: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

In this final laboratory, we will use the technique of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to examine the timed GFP expression samples from our induction experiments, examine the ability of gel permeation chromatography to clean up a crude protein extract, and estimate the molecular weight of GFP by comparing its migration to those of molecular weight markers of known size. SDS-PAGE is a common analytical technique for proteins (figure 1). The principle of separation is the same as for the agarose gel electrophoresis used in the analysis of our PCR products and restriction digests. Molecules are separated on the basis of their mass/charge ratio. The gel, made from a polymer of acrylamide, serves as a molecular sieve. It retards the movement of larger molecules more than smaller ones. As with agarose gel electrophoresis, the buffer (Tris-glycine-SDS in this case) is a source of electrolytes (ions) that enables current to flow through the gel. Unlike agarose gel electrophoresis, the SDS-PAGE apparatus holds the gel in a vertical position. Polyacrylamide gels are typically very thin (2-3 mm), and require the rigidity of glass or plastic plates for support. Agarose gels are usually much thicker, and can support themselves as long as they have a base.

Other differences also distinguish SDS-PAGE from agarose gel electrophoresis, making it a common analytical technique for protein analysis. For instance, unlike DNA, proteins do not all carry a uniform charge. Different proteins will have characteristic *isoelectric point* (pI) values, which are a function of their amino acid sequence. The net charge on a protein, therefore, will vary with the pH. Proteins with a pI value less than the pH used for separation will have a net negative charge, while those with a higher value will have a net positive charge. Thus, it is conceivable that a mixture of proteins from a crude extract could have some that migrate towards the positive electrode, and others that migrate towards the negative electrode. Furthermore, proteins come in a variety of shapes and sizes: rod-like structures, spheres, fibrous structures, ellipses, etc. which can affect their ability to move through a polyacrylamide gel.

To overcome these difficulties proteins are denatured (2º, 3º, and 4º eliminated) with the detergent sodium dodecyl sulfate and the reducing agent β-mercaptoethanol prior to loading on a polyacrylamide gel. Both of these reagents were contained in the *SDS loading dyes* added to your samples in previous labs. Sodium dodecyl sulfate unfolds the protein and coats it with molecules that posses a uniform negative charge. β-mercaptoethanol reduces (breaks) disulfide (-S-S-) bonds in the protein's primary structure. Recall that disulfide bonds are covalent, which are much stronger than the H-bonds, ionic interactions and van der Waals forces that dominate protein structure. The combination of these two substances in the presence of heat (boiling water bath) completely denatures the proteins so they resemble strands of negatively charged spaghetti. The separation then resembles electrophoresis of DNA molecules on an agarose gel, i.e. it based solely on the charge:mass ratio. Larger proteins will move more slowly, and remain towards the top of the gel, while smaller proteins will move more quickly and further down the gel.

Today we will analyze the timed samples from our GFP induction lab (0, 45 and 90 minute time points), the cell-free extract, and the fractions from our size exclusion chromatography

purification ($A_{476\text{-}1}$, $A_{476\ peak}$, A_{476+1}) on a polyacrylamide gel. After the gels finish running, we will stain them with a Coomassie Blue dye to visualize the proteins. Unfortunately, the de-staining process takes more time than class will allow. Therefore, we will analyze the gels next week. As with the agarose gels, we will be taking pictures of our results.

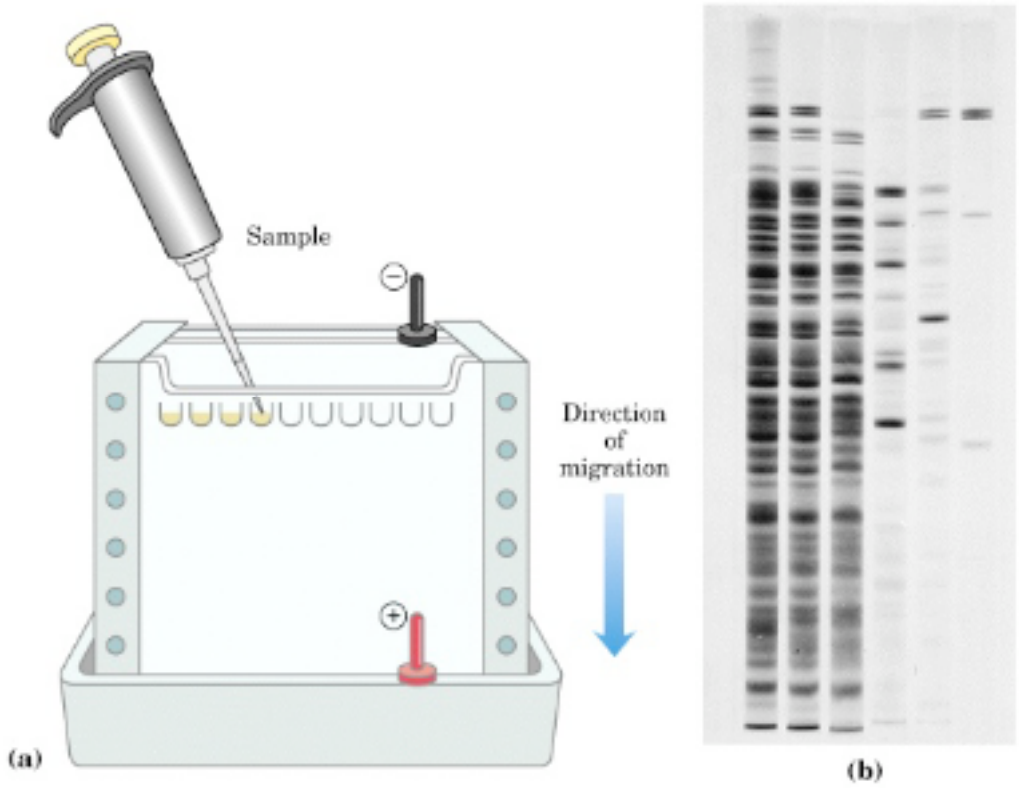

Figure 1. Polyacrylamide gel electrophoresis. (a) gel apparatus and example of sample loading procedure (b) electrophoresis results after staining. Left three lanes represent crude protein extracts. Right three lanes represent samples subjected to various purification techniques.

Materials & Equipment

400 ml SDS running buffer	Molecular weight markers (MWM)
Bio-Rad criterion gel	induction samples (0, 45, 90 minutes)
Power supply	Chromatography samples ($A_{476\text{-}1}$, $A_{476\ peak}$, A_{476+1})
Gel tank	Cell-free extract
Bio-Safe Coomassie Stain	gel staining box
12-well gel loading guide	

Procedure

For today's exercise, you will be using 12.5% acrylamide, precast (pre-polymerized) gels. These offer several advantages over pouring your own gel. Aside from simplifying the setup procedure and saving time, precast gels also reduce hazardous waste materials in the lab. While polymerized acrylamide is safe, it is a neurotoxin prior to polymerization. Nevertheless, always **wear gloves and eye protection** as a precaution.

A. Gel Setup

1. Start a boiling water bath using a 600 ml beaker with 250 ml of water.
2. Remove the gel cassette from its storage container.
3. Gently remove the combs and rinse the wells thoroughly with distilled water.
4. Remove the tape from the bottom of the cassette.
5. Insert the gel into one of the slots of the gel tank, as demonstrated by your instructor. Make sure the upper buffer chamber of the gel is facing toward the center of the tank.
6. Fill the upper buffer chamber with 60 ml SDS running buffer.
7. Insert the 12-well sample loading guide into the upper buffer chamber.
8. Gently flush the individual wells with 200 ul of buffer from the reservoir using a P-200 pipet.
9. Puncture all sample (0,45,90 min. inductions samples, cell free extract sample, $A_{476\text{-}1}$, $A_{476\ peak}$, $A_{476+1)}$ and molecular weight marker tube lids with a 16 gauge needle.
10. Place sample and molecular weight marker tubes in a boiling water bath for 2 minutes.
11. Allow samples and markers to cool to room temperature (3-4 minutes).
12. Load your samples and markers, using the gel well loading guide, in order from left to right according to the table below (20 ul of markers, 40 ul of samples) :

Lane	Sample
1	Molecular Weight Markers
2	0 time point (induction)
3	45 min. time point
4	90 min time point
5	Cell Free extract
6	no sample
7	A476 (-1) fraction
8	A476 peak fraction
9	A476 (+1) fraction
10	no sample
11	no sample
12	no sample

13. Fill the bottom tank reservoir to fill line with SDS running buffer (~ 300 ml).
14. Remove the gel well guide. Place the lid on the gel tank. Make sure to align the color-coded plugs.
15. Insert electrical leads into a power supply. Turn on power supply and run gel at a constant 200 V for approximately 45 minutes.

B. Gel Removal and Staining

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the lid and carefully lift out the gel cassette. Discard the running buffer down the sink.
3. Use the cassette opening tool built into the lid of the gel tank to break the weld-joint on the gel cassette, as demonstrated by your instructor. In brief, place the gel cassette's upper buffer chamber over the built-in opening wedge of the lid. Push the cassette straight down until the upper edge of the upper buffer chamber contacts the top of the lid and the weld-joint at the top of the cassette is broken. Pull the two cassette halves apart.
4. Wet a gloved finger and work underneath gel to loosen from plate. Carefully transfer to a staining box containing 200 ml of distilled water. Place box on a rotary shaker for 5 min. Remove the water (down the sink is OK) and replace with 200 ml of fresh water. Shake for 5 min. and remove water again. Repeat once more.
5. Add 50 ml of Bio-Safe Coomassie Blue Stain. Shake gently for 30 minutes.
6. Drain coomassie blue stain from container to the "used" stain container. Rinse gel with 200 ml of distilled water and gentle shaking for 20 minutes.
7. Replace water with 200 ml of fresh, distilled water. Place a crumpled Chem-wipe in your gel box to help absorb the stain. Place on the shaker for overnight stain removal.
8. The next day, one lab partner needs to return to the lab to replace the water, remove the chem wipe and store the gel in the refrigerator. Be sure that your gel box is labeled with a piece of tape with your name and lab section. We will take photos of your gel next week.

C. Gel Analysis (next week)

1. Retrieve your gel from the refrigerator. Go with your instructor to take a photograph of your gel.
2. Use Microsoft Excel to prepare a standard curve by plotting the logarithmic value of the molecular mass (M_r) of each molecular weight marker (y axis) against its migration distance (in mm, x-axis) from the well (see figure 2). Include the equation for the line of best fit for your data. Use this equation to estimate the M_r for GFP. Prepare a figure legend (figure no. and one sentence description) that also includes your estimate.
3. Prepare a second figure with your gel photo as done previously with DNA agarose gel electrophoresis images. Prepare a numbered, descriptive figure legend that also identifies each lane (including molecular weight markers) and includes pertinent electrophoresis information (e.g. % acrylamide, running buffer, voltage used for electrophoresis, etc). Both your photo and standard curve figures will not be graded separately, but are to be included as part of your final lab report.

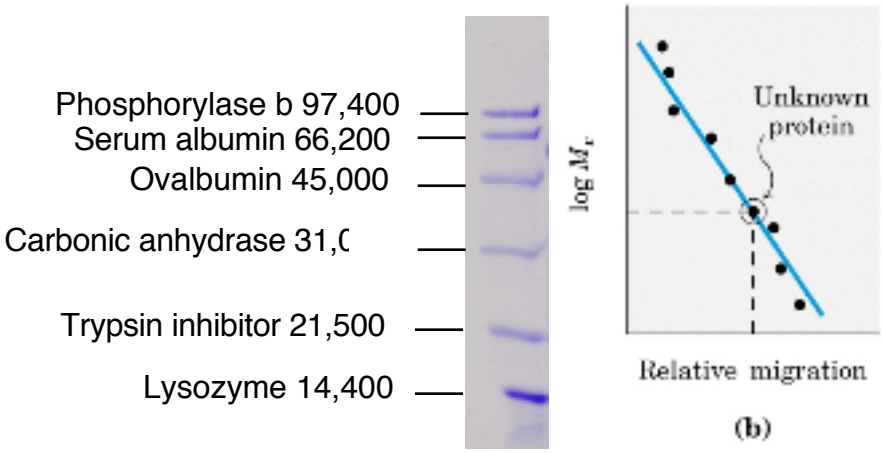

Figure 2. Sizes of protein molecular weight markers (M_r – molecular mass) and example of molecular mass determination (b).