Analysis of Mouse Proteins by SDS Polyacrylamide Electrophoresis

Prior to lab you should:

- Make sure you:
  - understand how electrophoresis works (how are molecules separated?)
  - know what factor determine the rate of movement of polypeptides during SDS-PAGE
  - know the role of each of the following in the procedure:
    - SDS (ionic detergent)
    - Polyacrylamide
    - electric field
  - understand how to prepare a standard curve
  - understand the concept of a tissue "protein profile"
  - can distinguish between luxury and housekeeping proteins.

I. Objective:

- **To use gel electrophoresis to generate the protein profiles of the cells of different mouse tissues**
- **Identify several housekeeping and luxury proteins in mouse tissues.**
- **Determine the molecular weights of these proteins**

II. Background:

Protein Profiles

Every cell in every tissue of an organism contains a large array of proteins. The number of proteins varies from tissue to tissue, but averages in the neighborhood of 10,000 different proteins in every cell type. Some of these proteins function as enzymes, others regulate gene activity, still others are part of the cytoskeleton or the membrane. These proteins can range in molecular weight from 5000 daltons (~45 amino acids) to over 600,000 daltons (~5500 amino acids).

Some of the proteins in cells perform "housekeeping" tasks or tasks that are common (and probably necessary) to all cells. Examples of these proteins would include the enzymes of glycolysis, the proteins that package DNA in the nucleus or cytoskeletal proteins. These proteins are found in all the cell types because they perform some cellular function required in all cells. Commonly, these proteins are called "**housekeeping proteins**". A good working definition of a housekeeping protein is any protein that is expressed in all the cell types of an organism.

Other proteins may be associated with a special physiological role of a cell. For example the proteins that makeup striated muscle fibers are found only in muscle, the enzymes necessary for cholesterol metabolism are found in liver and the membrane receptors for

neurotransmitters are found in brain cells. Each of these proteins is only expressed in those cells that require the proteins for their physiologic roles. Proteins that are not expressed in all cell types but only in one or a few cell types are called "**luxury proteins.**" In a sense, what makes one tissue different from another is which set of luxury proteins are found in that tissue.

A protein profile of a cell is a description of all the proteins expressed in that cell. Protein profiles are useful for identifying housekeeping proteins and luxury proteins. For example, to identify housekeeping proteins you could compare the protein profiles of several different tissues. Housekeeping proteins would be proteins found in all the tissues. Luxury proteins, on the other hand, would be proteins that are found in one (or just a few) of the tissues and not in other tissues.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis is the technique of separating charged particles in an electrical field. An electrical field is established by passing an electrical current through some medium. The charged particles in the medium then move in response to the electrical field. Positively charged particles move to the negative electrode (cathode) and negatively charged particles move to the positive electrode (anode). In the most general electrophoresis, three factors control the relative rate of movement of a particle: mass, shape and charge. For particles that differ in molecular mass, smaller particles move faster than larger particles. For particles that differ in shape, particles with less surface area move faster than particles with more surface area. And for particles that differ in charge, the greater the total charge of a particle, the faster it will move towards the electrode.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a special type of electrophoresis used to separate polypeptides. It differs from other forms of electrophoresis because it separates polypeptides based only on their molecular weight. The "polyacrylamide" of SDS-PAGE refers to the gel-like medium through which the electric field is passed. It is a molecular mesh of acrylamide polymers through which polypeptides can move. This mesh acts as a molecular sieve, helping to separate the larger proteins from the smaller proteins. The SDS refers to an ionic detergent in the polyacrylamide gel called sodium dodecyl sulfate (Fig 1). SDS plays two roles in SDS-PAGE. First, it denatures the protein resulting in all the proteins have a similar shape. Therefore, differences in protein shape are not important factors during SDS-PAGE. Second, when SDS denatures the proteins, hundreds of SDS molecules coat the open polypeptide (Fig 2). Each of these SDS molecules has a negative charge. When SDS coats polypeptides, it gives all the polypeptides a uniform negative charge. Therefore, differences in the charge of the polypeptide are not important factors during SDS-PAGE. **The only factor that affects the rate of migration of polypeptides during SDS-PAGE are their molecular weight. If one polypeptide migrates faster than another polypeptide, that is evidence that the faster polypeptide has a lower molecular weight.**

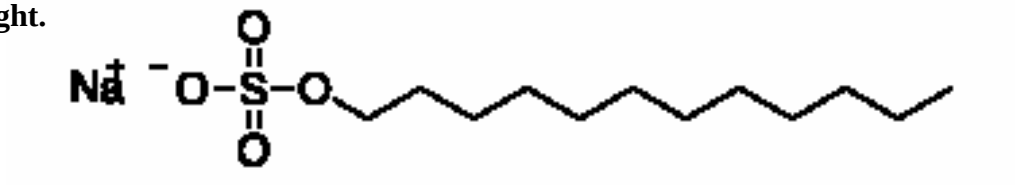


Figure 1. Sodium Dodecyl Sulfate (SDS)

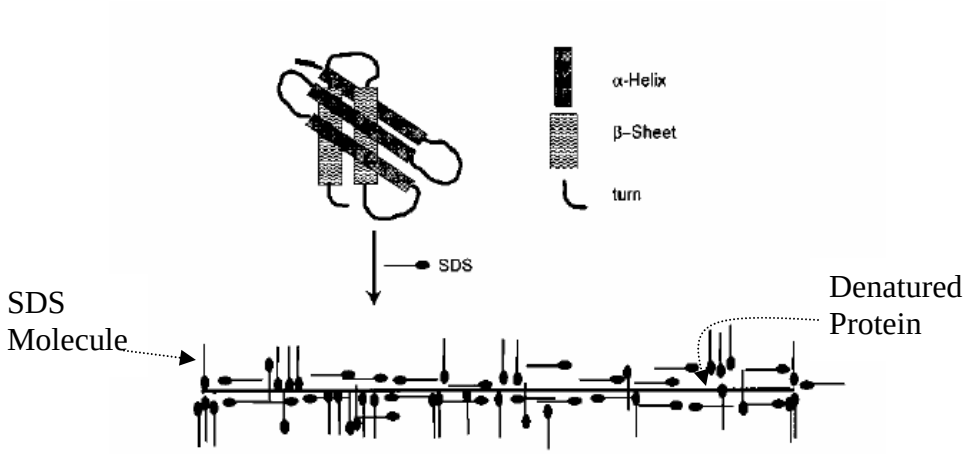


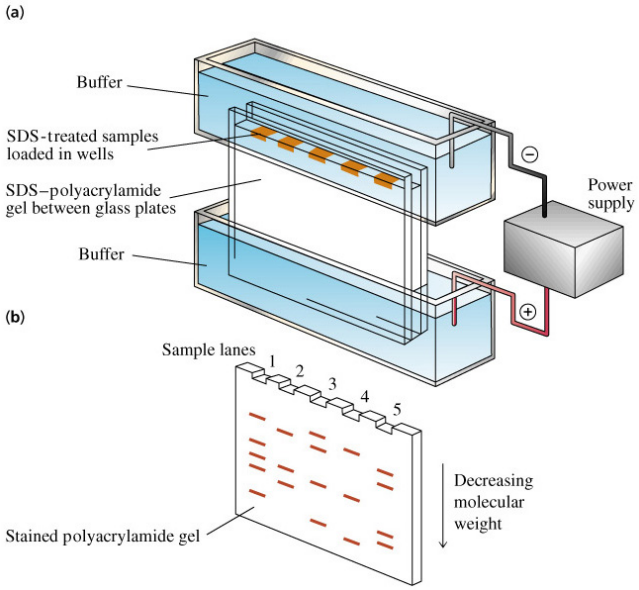
Figure 2. SDS Complexes with unfolded proteins and coats them so that they bear an overall negative charge from the SO_4^- groups . Note that the protein must be unfolded to completely complex with SDS.

SDS-PAGE and Protein Profiles

SDS-PAGE is a useful tool to determine the protein profiles of tissues. Basically, the proteins of any tissue can be extracted into a buffer. The proteins are denatured by adding an SDS reagent and boiling 2 minutes. The samples are then applied to a polyacrylamide gel and a high voltage is applied across the gel. All the proteins migrate towards the anode, with smaller proteins migrate faster than larger proteins. When the electric voltage is shut-off and the gel is stained for proteins, a series of bands is observed in the gel. Each band corresponds to a protein. Bands near the top of the gel correspond to larger proteins and bands near the bottom of the gel correspond to smaller proteins.

SDS Gel Electrophoresis Set-Up

The general arrangement of an SDS gel electrophoresis setup is diagramed below. The gel itself is made of two parts an upper "stacking gel and a lower "running gel."

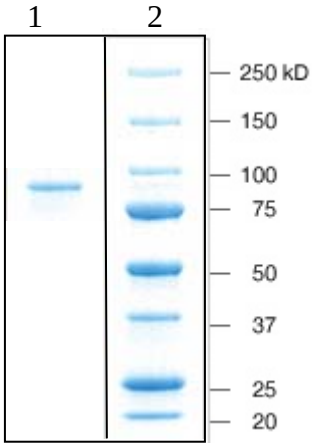


Molecular Weight Determination

Since the binding of the SDS during SDS-PAGE causes all proteins to denature and have a similar charge and surface area, the only factor affecting the rate of migration of polypeptides is molecular weight. The distance migrated by a polypeptide is inversely proportional to the log of the polypeptide's MW. Because of this relationship it is possible to determine molecular weight of unknown proteins by comparing their migration to protein standards (polypeptides with a known molecular weight.)

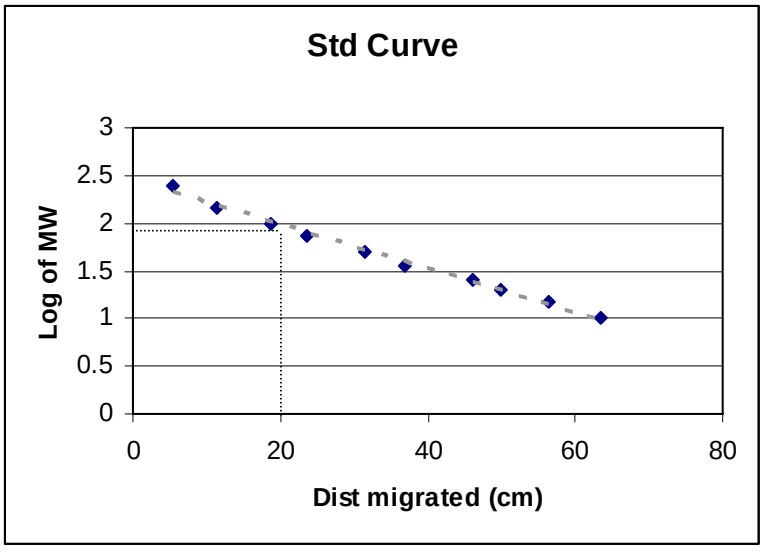
To estimate the molecular weight of polypeptides, a standard curve must first be generated using protein standards. Protein standards are a set of polypeptides with known molecular weights. These proteins are run on the same gel as the unknown proteins that you are analyzing.

Consider the example below. On the left is a photo of an SDS-PAGE gel. Lane 1 contains a single unknown protein. Lane 2 contains a series of protein standards – their molecular weights are noted on the right. Note that the larger molecular weight polypeptides migrate slower than the smaller polypeptides. To generate the standard curve the distance that each standard protein has migrated from the top of the gel is measured. Then this distances is plotted against the log of the size of each protein



MW of Stds (Kd)	Log of MW	Distance Migrated (mm)
250	2.39	5.5
150	2.17	115
100	2.00	18.7
75	1.87	23.5
50	1.70	31.5
37	1.56	37
25	1.40	46
20	1.30	50

The unknown protein (lane 2) migrated 20mm. Using the std curve, this corresponded to a log MW of 1.90. The inverse log gave a molecular weight of 89 Kd.



III. Materials:

Each Station should have:
gel apparatus with gel
P1000/p200/p20 pipettes &tips
microcentrifuge tubes
Gel loading tips
Ice
Weight boats
Dissection tools
Sand
Razor blades
Mortar and Pestle
Plastic Spatula
Waste Beaker
Sharpie

Common Solutions:
SDS Sample buffer 5X
Homogenizing buffer (TE)
Molecular weight standards
Tank Buffer

IV. Procedure:

A. Preparation of Protein Samples from Mouse Tissues:

1. Each group will excise a mouse tissue sample with the assistance of the instructor. The tissues (liver, lung, muscle, kidney, or heart) will be placed on a weight boat and chopped into several pieces with razor blades.

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2. Place the tissue in a clean mortar. Add 500µl of homogenization buffer and a pinch of sand. Grind the tissue to a fine slurry using the pestle.

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3. Use a P1000 to transfer most of the slurry to a microcentrifuge tube. Centrifuge the sample 2 minutes at high speed. A liquid supernatant containing soluble proteins will be visible above a pellet of cell debris.

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4. Into a clean microfuge tube pipette
   45µl of TE (40µl for heart and lung samples)
   5 µl of tissue supernatant liquid (10µl of heart or lung)
   12.5 µl sample buffer.
   - boil for 3 minutes to denature the proteins
   - centrifuge at top speed for 2 minute. in the microcentrifuge

B. Loading Samples on the Gels

5. Each group should load their sample into two lanes of an SDS-PAGE gel. You will use a P20 micropipetter and a special gel loading tip. Make sure you load you samples into the correct lane as described below.

Lane 1: **10 µl** standards
Lane 2: **3 µl** kidney
Lane 3: **10 µl** kidney
Lane 4: **3 µl** lung
Lane 5: **10 µl** lung
Lane 6: **3 µl** liver
Lane 7: **10 µl** liver
Lane 8: **3 µl** heart
Lane 9: **10 µl** heart
Lane 10: **3 µl** muscle
Lane 11: **10 µl** muscle
Lane 12: **10 µl** standard

6. Your instructor will help you set up your gel in the running apparatus so that it can be run at **200 volts** for **45 minutes**. You will be able to follow electrophoresis by watching the colored protein standards separate

C. Staining and Destaining

7. Your instructor will remove the gel from the running apparatus and stain it with the protein stain Coomassie blue for 1 hour. The gel will be treated with a destaining solution to remove un-bound stain and the gel will be photographed with a digital camera. The photos will be posted on the Resource section of the BIO211 web site.

V. Short Report **Name: _____**

Due at next laboratory session

1. Print the photo of your group's gel from the BIO211 web page and attach the photo to the back of this report. Identify one house keeping protein in your tissue sample and draw a circle around it and label it. Identify one luxury protein your sample and draw a box around it and label it.

2. Measure the distances that all the standard proteins migrated on your gel. Prepare a standard curve. You can draw it using Microsoft excel or by hand. (It should look something like the standard curve above.) Either attach the standard curve to the back of your report, or paste it in the space below.

3. Using your standard curve, estimate the molecular weight of your luxury protein and the housekeeping protein you identified in question 1.

4. Every tissue should contain 10,000 different proteins.
    a. How many proteins can you identify in the your tissue sample using gel electrophoresis? (Hint: count the number of bands)
    b. Speculate on why gel electrophoresis cannot detect all 10,000 different proteins.

5. Explain why it is important for cells in different tissues to have different proteins.