

Project 1: Myoglobin—

Isolation from ground beef; UV-Vis spectroscopic characterization of oxidized and reduced heme and characterization of purity by SDS-PAGE

PRE-LAB ASSIGNMENT:

Read the entire laboratory project described in the following pages, as well as the *J. Chem. Educ.* source paper.¹

- Prepare, on a typed sheet of paper, the Project Objectives of this lab (include the major experimental techniques you will use in each part); on the same sheet, complete the assignment below:
 - 1) Summarize the chemical and spectroscopic differences between oxy-myoglobin and met-myoglobin.
 - 2) If 50.0 μL of met-Mb stock solution is diluted by adding 950.0 μL of buffer, and the measured A_{409} is 1.402, what is [Mb] in the original stock solution?
 - 3) Give balanced net ionic equations for the oxidation of oxy-Mb (Mb-heme- Fe^{2+}) by ferricyanide, and the reduction of met-Mb (Mb-heme- Fe^{3+}) by dithionite.²
 - 4) What type of chromatography is carried out with Sephadex G-25 resin? The Mb oxidation solution contains brown Mb and yellow ferricyanide. Which elutes first? Why?

Introduction: Myoglobin Extraction, Characterization, Oxidation, and Chromatographic Separation:

¹ Bylka, S.A.; Andersson, L.A. "Microburger biochemistry: extraction and spectral characterization of myoglobin from hamburger" *J. Chem. Educ.* 1997, 74, 426-430.

² The formula for the oxidizing agent ferricyanide $\text{Fe}(\text{CN})_6^{3-}$ is actually $\text{K}_3\text{Fe}(\text{CN})_6$; it is reduced to ferrocyanide, $\text{Fe}(\text{CN})_6^{4-}$. The reducing agent dithionite, $\text{S}_2\text{O}_4^{2-}$ is oxidized to sulfite, SO_3^{2-} , with the following half-reaction: $\text{S}_2\text{O}_4^{2-} + 2 \text{H}_2\text{O} \rightarrow 2 \text{SO}_3^{2-} + 2\text{e}^- + 4 \text{H}^+$

Proteins often contain bound cofactors that allow them to function. For example, hemoglobin (Hb), myoglobin (Mb), cytochromes, and chlorophylls all use heme cofactors. In Hb and Mb, the heme complexes with an Fe^{2+} ion that reversibly binds O_2 . The heme- Fe^{2+} complex is unusual in several respects: (a) It can be oxidized (or reduced); (b) it can bind several other small ligands besides O_2 (e.g., CO, N_3^- , CN^- , all of which are potent poisons); (c) it absorbs strongly in the Vis and UV regions and (d) its absorbance spectrum changes dramatically depending on the ligand bound and the oxidation state of the heme. In this lab project, student pairs will extract Mb (MW 17.3 kDa) from cow muscle (i.e., hamburger), oxidize and reduce it, and then spectrophotometrically characterize myoglobin. Please review Beer's Law ($A_\lambda = \epsilon_\lambda c \ell$), and centrifugation.

SDS-PAGE of ground beef supernatant:

Living tissues contain thousands of proteins, so purifying a protein of interest is crucial, as is determining important physical characteristics of the protein (e.g., molecular weight). SDS-PAGE is a powerful technique that allows biochemists to determine the level of purity of tissue extracts. At the same time, it allows for the determination of the approximate molecular weight of each protein in a discernible band. Please read the section of your biochemistry text on SDS-PAGE.

Day 1 Procedure:

The first week of this lab student pairs will isolate Mb, oxidize and reduce it, and then purify the oxidized (met)Mb using size-exclusion chromatography on a Sephadex G-25 gel filtration column. You will find specific instructions for the experiment in the Bylka/Andersson paper, as pasted below. **Please note that modifications of the Bylka/Andersson procedure to suit our laboratory and instruments are listed immediately following the excerpt.**

A. Microburger Biochemistry: Extraction and Spectral Characterization of Myoglobin from Hamburger

Sheri A. Bylkas and Laura A. Andersson*

Department of Biochemistry, 103 Willard Hall, Kansas State University, Manhattan, KS 66506

Journal of Chemical Education • Vol. 74 No. 4 April 1997; pp. 426-430.

p. 427, left column, bottom:

Myoglobin Extraction and Partial Purification

Each student or pair of students should have a 10-g “microburger”, prepared before class from very lean ground steak.³ This sample is placed in a disposable centrifuge tube and 20 mL (two volumes) of buffer is added. We used 20 mM potassium phosphate, pH 5.6. (Other buffers that can be used are 100 mM sodium phosphate or 100 mM potassium phosphate, both at pH 7.) Now the student mixes the sample with a glass rod for 1 min, to break open the cells and release the Mb, as shown in Figure 2. The student should be careful: rough or extensive mixing can result in release of fats and nucleic acids contained in the meat sample.

Next, to pack the pellet, the sample is centrifuged for ca. 15 min at 10,000 rpm or for 60 min at 5000 rpm using a Beckman JA-20 rotor. (The 1-hour time was originally planned to permit the students to pour and/or equilibrate their gel filtration columns.) Students should be advised to balance their tube against another tube, as demonstrated by the instructor.

After centrifugation, the student will usually observe a whitish-gray pellet and a reddish supernatant containing the Mb. This is clear visual evidence that the majority of the color in the meat comes from the soluble heme protein. There might also be a top layer of fat, which should be avoided in pipetting and discarded. The Mb solution (supernatant) is now carefully removed with a Pasteur pipet. To prepare the Mb sample for electronic absorption spectroscopy, the students dilute 1.0 mL of supernatant with 3.0 mL of the initial buffer.

Please note these important modifications of the above Bylka/Andersson protocol:

1. *Myoglobin Extraction...* second paragraph. You will use an SS-34 rotor for centrifuging your myoglobin extracts. Centrifuge for 15 min. at 10,000 rpm, as you will prepare your gel filtration column on lab day 2.
2. *Myoglobin Extraction...*, last sentence (“To prepare the Mb sample...”): Take a Vis scan (330-700 nm) of a 250 μ L aliquot of the Mb-containing supernatant, diluted to 1 mL. Dilute further, if necessary. Student PAIRS then use the sample with highest [Mb].

B. Oxidation, Reduction, Separation, and Spectroscopic Characterization (Bylka and Andersson, p. 427, right column, middle:)

Oxidation and Reduction

The remaining Mb supernatant is divided in half. One half is to be oxidized by thorough mixing with approximately 15 crystals of potassium ferricyanide ($K_3[Fe(CN)_6]$). This chemical converts Fe^{2+} (ferrous) heme proteins to the Fe^{3+} (ferric) form. Here it will produce Met-Mb (sample A), which is yellow to yellowish brown because of the ferricyanide. With the other half of the Mb supernatant, the student will mix approximately 20 crystals of sodium dithionite ($Na_2S_2O_4$). This is a reducing agent that will convert Fe^{3+} heme protein to the Fe^{2+} form, producing bright red Oxy-Mb (sample B). Both samples should sit for 5 min before absorbance is read.

3. *Oxidation and Reduction*, 1st paragraph (“The remaining Mb...”): The formula for the oxidizing agent ferricyanide $Fe(CN)_6^{3-}$ is actually $K_3Fe(CN)_6$; it is reduced to ferricyanide, $Fe(CN)_6^{4-}$. The reducing agent dithionite, $S_2O_4^{2-}$ is oxidized to sulfite, SO_3^{2-} , with the following half-reaction: $S_2O_4^{2-} + 2 H_2O \rightarrow 2 SO_3^{2-} + 2e^- + 4 H^+$

EACH student uses a centrifuge to extract Mb from ground beef. Afterwards, use the sample with the highest [Mb]; split the Mb-containing supernatant into three (not two) equal portions. One portion you will save as an unreacted control; a second portion you will reduce to Mb using dithionite ($Na_2S_2O_4$) reducing agent; the last portion you will oxidize to metMb using ferricyanide ($K_3Fe(CN)_6$) oxidizing agent. Meanwhile, the second student in each pair will oxidize about 4 mLs of the stockrooms’ 0.40 mM Mb stock solution in the same manner, using ferricyanide. You now have two metMb samples to work with: one from hamburger and one from pure Mb (stockroom solution).

p. 428, "Advanced Laboratory" section

Electronic Absorption Spectroscopy

The buffer used to extract Mb from the meat is placed in a clean 1-cm cuvette. Following directions from the lab instructor for the particular instrument (scanning spectrophotometer), a baseline absorbance curve is to be obtained from 700 to 300 nm.⁴ This baseline will be subtracted automatically from the spectrum of the sample when it is scanned. Next, the supernatant sample is placed in the cuvette, and the sample is scanned from 700 to 300 nm. The sample will contain both Mb-O₂ and Mb-H₂O and the spectrum will show all the peaks listed in Table 1. Students should label all peaks observed; their spectra can be compared with those in Figure 3.

This experiment is usually completed in a 2nd lab session. The samples should be labeled, covered with Parafilm, and refrigerated until the next class. At that time, the samples are removed from the refrigerator. The sample may have become slightly cloudy owing to the presence of a small amount of denatured protein. An approximately 10-min centrifugation step using a bench-top centrifuge will enable students to separate their meat supernatant (Mb extract) from the denatured pellet before beginning the next experiments.

Day 2 Procedure:*Oxidation and Reduction Reactions and Gel Filtration Chromatography*

Column and Sample Preparation

This lab can use columns prepared in advance or purchased, or the students can pour and equilibrate their own columns. Two chromatography columns, each containing 10 mL of Sephadex G-25 equilibrated in the working buffer, are required.⁵ Next the students will need to equilibrate each column with 3–5 volumes (30–50 mL) of buffer, if this step was not already done. (Usually, the students equilibrate their columns for lab period two while the samples are in the centrifuge.)

Now the students will divide the Mb supernatant in half and follow the procedures described for the basic experiment for oxidation and reduction of the two parts of the sample. The samples should be mixed thoroughly and allowed to incubate for about 5 min. Students should be able to observe color changes for both samples. The sample to which the oxidizing agent was added, producing Met-Mb, will be a yellow-brownish color and the sample to which the reducing agent was added, producing Oxy-Mb, should be bright red.

Desalting Experiment

A gel filtration chromatography⁵ column with a 10.0-mL bed volume ($V = \pi r^2 h$, where V is column volume and h is column bed height) can efficiently separate 10–15% in a “desalting experiment”. So an appropriate sample volume to be loaded is on the order of 1.0–1.5 mL. Each protein sample (Met-Mb or Oxy-Mb) is gently loaded onto a separate column, being careful not to disturb the gel bed. In the case of the Met-Mb sample, there will initially be a yellowish-brown band (protein + ferricyanide) that will move down the column, separating into a lower brown band of MetMb (ca. 17,000 Da) and an upper yellow band of the oxidizing agent, which will be slowed down by the beads in the column. This separation is shown in Figure 4. The brown band will be collected. To ensure the collection of all the protein,

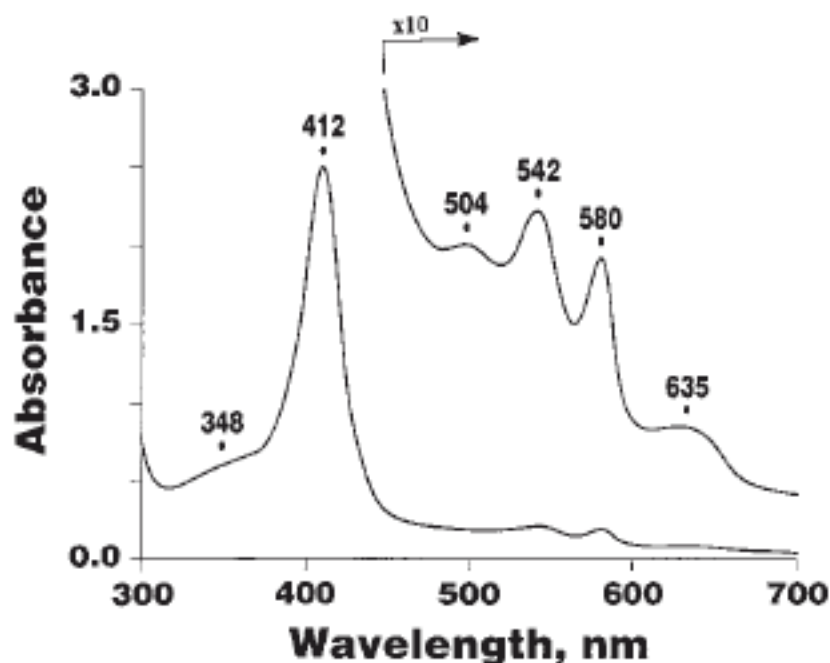


Figure 3. Electronic absorption (UV-vis) spectrum of the meat supernatant. Comparison of this figure with Table 1 reveals that the supernatant is a mixture of Oxy-Mb and Met-Mb.

students can put a white piece of paper behind the column to better observe the color of the drops as they elute.

In the case of the Oxy-Mb sample, the bright red protein moves quickly down the column, whereas the colorless reducing agent is also slowed down by the beads in the column. The bright red band is to be collected and the students can use the white-paper method again. An important point is to collect only the colored drops. This is because although the sodium dithionite is not colored, if collected with the sample it will interfere with the spectrum.

Electronic Absorption Spectra

The buffer that was used for the original extraction is placed into a clean 1-cm cuvette and the instrument's baseline absorbance curve is obtained from 700 nm to 300 nm, as described previously.⁴ The samples eluted from the

two columns will most likely be too concentrated to be on scale in the Soret region. Students can check this by examining the column eluates at the Soret peaks listed in Table 1. We usually prepare the sample by adding 2.5 mL buffer to 0.5 mL of the column eluate. The “homogeneous” (post-chromatography) Oxy-Mb and Met-Mb samples are scanned from 700 to 300 nm. For Met-Mb, the sample should show all the peaks in Figure 5A; for Oxy-Mb, the sample should look very similar to the spectrum shown in Figure 5B.

Here are some more important modifications of the above Bylka/Andersson protocol:

1. *Column and Sample Preparation*, first paragraph: You will use only one column, separating only the oxidized Mb (not reduced). Pour a Sephadex G-25 gel column:
 - a. Fill the column half way with buffer.
 - b. swirl the Sephadex stock suspension, and take \approx 15-20 mLs for your column
 - c. using a Pasteur pipet add swirled Sephadex to your column, while it slowly drains;
 - d. stop when it looks like the gel bed will reach 2/3 the height of the column
 - e. slowly drain the buffer as the gel packs; never drain buffer below the top of the gel bed
 - f. CAREFULLY rinse the gel with about 20-30 mL of buffer. Try to keep the top of the gel bed flat, smooth, horizontal, and undisturbed.

2. *Column and Sample Preparation*, last paragraph: Student #1 will run at least two 1.5 mL samples of the oxidized metMb (brown) solution down the column, according to the following instructions:
 - a. Drain the buffer down to the top of the gel bed.
 - b. Add 1.5 mL of protein solution CAREFULLY, SLOWLY to the top of the gel bed. Try not to ruffle or disturb the top of the gel bed.
 - c. Drain the protein sample into the gel bed.
 - d. Add buffer CAREFULLY, SLOWLY to the top of the gel bed, while draining.
 - e. Collect fractions:
 - i. initial “void” volume (clear)
 - ii. Mb (brown)
 - iii. Mb? light brown, but NOT yet yellow
 - iv. FeCN (yellow)
 - v. clear- no more FeCN left on column
 - f. Once the FeCN is washed from the column, run your next protein sample.
 - g. Combine and keep only the early brown metMb fractions; discard all later yellow ferricyanide fractions.

3. The second student now purifies at least two 1.5 mL samples of the metMb derived from the stockroom solution, following all of the steps in #2 above.

4. Take Vis scans of the reduced Mb, the oxidized metMb, and the Sephadex-purified metMb.

DAY 3: SDS-PAGE on ground beef supernatant**PROCEDURE:**

A. Summary: You will be running four different myoglobin samples (a-d), plus two types of protein MW standards (e, f) on SDS-PAGE:

- a. ground beef supernatant, unpurified: Bspnt
- b. ground beef supernatant, reduced, unpurified: Bred
- c. Stockroom's pure Mb solution, oxidized (metMb), Sephadex-purified: Gmet
- d. ground beef supernatant, oxidized (metMb), Sephadex-purified: Bmet
- e. Kaleidoscope pre-stained (colored) protein MW standards: Kal
- f. Precision Plus unstained protein MW standards: PP+

B. Sample Preparation Protocol:

1. Obtain six 0.6 mL Eppendorf tubes and label appropriately for each Mb sample, *e.g.* Bspnt, Bred, Bmet, Gmet, Kal, PP+.
2. Pick up a 250-mL beaker with glass beads and covered with a sheet of aluminum foil.
3. Punch 4 small holes near the center of the aluminum foil so that the small Eppendorf tubes can be suspended from the foil and not fall into the beaker.
4. Add water to a height that will cover the bottom of the Eppendorf tube when it is inserted into the hole in the aluminum foil.
5. Place the 250-mL beaker with the glass beads, the water, and the foil cover on the hot plate and set the heat setting on about three-quarters of the maximum setting.
6. The four myoglobin samples (a-d above) must be converted to an SDS-protein complex. (But not the MW standards, e and f; they are already in this form.) Add 10 μL of the appropriate myoglobin sample solution (a-c) and 10 μL of gel sample buffer into each of the four tubes (a-c). For the Bmet sample (d), add 30 μL to 30 μL of gel sample buffer. Be sure that the microcentrifuge tubes are shut tightly. Vortex all four tubes. Place your four tubes in the microfuge in larger Eppendorf tubes already in the rotor; arrange each tube diametrically across from another tube, close the lid and centrifuge in microfuge for 10s. Remove the tubes and make certain that the lids are securely and completely closed.
7. After the water boils, heat samples in the boiling water bath for 5 minutes. Remove the Eppendorf tubes from the bath (you may want to use a forceps to avoid burning your fingers) and place them on ice to cool for about a minute.
8. When the samples have cooled, transfer them back to the microcentrifuge. Balance the tubes and centrifuge as in step 6 above for 10 s. (This brings any water droplets that have condensed near the top of the tube back down to the bottom of the tube.)
9. Ascertain the location of the two types of protein MW Standards; you'll load, per lane, 7 μL of Kaleidoscope pre-stained standards, and 10 μL of PP+ MW standards.

C. Preparation for Electrophoresis.

GEL PREPARATION:

1. Remove the gel from the plastic storage pouch.
2. Cut along the dotted line at the bottom of the gel cassette with a razor blade; pull the clear tape at the bottom of the cassette to expose the bottom edge of the gel.
3. Gently remove the comb and rinse the wells thoroughly with distilled water. Dry wells as much as possible by holding the gel upside down and using a Kimwipe.

Apparatus Assembly

1. Rinse thoroughly the electrophoresis apparatus:
 - a. One clear plastic large outer tank
 - b. One green plastic lid with red and black lead wires
 - c. One electrode frame/clamping assembly, and
 - d. Two plastic gel cassettes with a thick clear plastic window and 4 corner screws
2. Hold the gel cassette with the four corner-screws (knurled knobs) facing you and the longer two vertical tabs pointing up. Loosen the 4 corner screws. Place your gel plates in the cassette, on the side furthest from the screws, with the short plate facing away from you (furthest from the screws). Tighten the screws, but not super-tight.
3. Make sure that there is a small amount of Vaseline on the U-shaped gray rubber liner in the electrode frame. This should prevent leaking.
4. Hold your gel cassette (with clamped gel) so that the two longer corner tabs point up and the two shorter corner tabs point down. Slide the longer corner tabs of your cassette into the slots at the top of electrode clamping frame; then use pressure at the bottom to snap the shorter tabs into the bottom of the frame.
5. Hold the frame so that the red electrode is closest to you; record in your notebook which side of the frame (left or right) holds your gel cassette.
6. Two gel cassettes will be placed in each electrode/clamping frame, 1 on each side. Be sure the short plate of the cassette faces **inward** toward the gel on the opposite side.
7. Prepare the electrode buffer solution: to 50 mL of the 10× stock solution, add 450 mL of distilled water. When mixing be sure to pour the buffer and the water down the side of the graduated cylinder to minimize bubble formation. Mix the solution well.
8. Add running buffer to the *upper* electrophoresis chamber, between the two electrodes of the gel-loaded clamping frame. Be sure to do this slowly, along the side of the apparatus (a funnel may help). Allow the clamping frame to sit on paper towels for a few minutes in order to check for leaks. The paper towel should remain dry. (The inner chamber should be filled with enough buffer to reach halfway between the tops of the taller and shorter plates of the cassettes.)
9. Lower this assembly into the outer chamber.
10. Slowly add 200 mL of buffer to the *lower* tank; try not to make too many bubbles.
11. If you see bubbles along the bottom of the gel plate, tip the electrophoresis module sideways to allow any bubbles to rise to the surface, and/or use a plastic pipet to remove the bubbles. (The presence of large bubbles can insulate the gel from electrical current flow, leading to errors in the sample run.)
12. Slowly add additional buffer to the lower chamber. Buffer contacting the gel plate will help cool the plate and prevent overheating during the run.

D. Gel loading:

1	2	3	4	5	6	7	8	9	10
Kal-7	PP+	Gmet	Bspnt	Bred	Bmet-10	Bmet-20	Kal.	PP+	Bmet-15

1. In each gel lane you'll load one standard or one sample. Load lanes asymmetrically across the gel so you'll always know which lane is #1. One example layout is shown above: top row = lane #; Bmet-15 = 15 μ L of Bmet loaded in the lane.
2. Be sure to load duplicates of the protein MW standard markers (in two separate lanes). Whatever layout you decide to use, record it as a table in your lab notebook.
3. Obtain a 2-20 μ L micropipettor with a special gel loading tip. You will load 10 μ L of each sample (unless otherwise specified) in a separate well in the gel. For the Kaleidoscope standard markers, load only 7 μ L.
4. When drawing the sample into the micropipet, make sure there are no air bubbles in the sample and that there is no air between the end of the tip and the solution.
5. When expelling the sample from the pipet, make sure that the tip is actually in the well so that the sample does not end up in the electrode buffer. When dispensing, push the pipet plunger to the first stop and remove the tip from the well before releasing the button. This will prevent air bubbles from forming in the sample.
6. Load the samples in the wells carefully, but quickly (within 5-10 min.) so that the samples do not start diffusing out of the wells.
7. After all samples are loaded in both gels, place the green plastic lid over the apparatus making sure to align the color-coded banana plugs and jacks. *Note which side of the electrophoresis chamber contains your gel.*
8. Turn on the power supply: black switch on lower left. Run/ Modify/ Set to: 0200 V, 060 mA (**per gel**), 300W/ Exit/ Run. Choose an appropriate current based on the number of gels that will be run. For example, running four gels requires a current of 240 mA. The voltage should be constant at 200 V; the current should start at approximately 60 mA/gel, and drop to approximately 30 mA/gel by the end of the run.
9. Once the power supply is on and electrophoresis has begun, check that the electrical circuit is closed by watching for the evolution of gas bubbles from the wires inside the chamber. Check every once in a while that the gel is not getting hot (warm is OK).
10. Allow the current to run for at least 35 minutes or until the tracking dye is at the bottom of the gel. Record the volts and the milli-amps at the beginning and end of the run. The current will change during the run.
11. Turn power supply off and remove lid from apparatus. Place outer chamber with electrode in the sink. Lift the electrode and clamping frame out of the outer chamber and discard the buffer solution in the outer chamber.
12. At the bottom of the clamping frame, pull down on the frame and also push up on the gel cassette to free each cassette. Be careful not to drop the cassettes. Take each gel and use a spatula to pry off the smaller plate of each cassette. Be sure that the smaller plate is pointed upward and that the spatula does not tear the gel.
13. Add water to a depth of about 1 cm to a small plastic container (can use a pipet box lid). Place the gel in the water for rinsing. This is best accomplished by turning cassette plate with gel over so that the gel is facing the water, then use a spatula to carefully dislodge the gel from the cassette plate.

14. Swirl the gel in water for 30 sec, and leave it to rinse for 5 minutes. Drain off the water and replace with fresh deionized water. Repeat 2× more for a total of 3 rinses over a period of 15 minutes. Be careful not to tear gel when changing water. During the final rinsing, record the qualitative positions and the colors seen in the Kaleidoscope pre-stained standard lanes; draw a picture in your lab notebook.
15. After the last rinse is poured off, add enough Bio-Safe Coomassie Blue Staining Reagent to completely cover the gel. Swirl for 30 sec and allow gel to stain for approximately 1 hour.
16. When one hour has passed, drain the staining reagent into the waste container and cover the gel with water. As in step 14 above, rinse three times changing water every 5 minutes, for a total of 15 minutes.
17. Carefully wrap your stained/rinsed gel in Saran Wrap; try to smooth out all wrinkles. Now, take pictures of the gel using the Gel Doc system downstairs in the Molecular Biology lab (see instructions below). Store gel, wrapped in damp paper towel, in the fridge.

E. Gel Doc Instructions:

*Take a picture of your stained gel, then Analyze stained bands in your pictured gel*This is a new system. You will be trained on site by the instructor.

Results and Analysis: Data analysis is described separately for each of the four parts of this lab project. Here are a few final notes concerning the SDS-PAGE MW determination of Mb in Part D:

1. Make a table that includes the colors you observe for your Kaleidoscope pre-stained standard markers, their size (in kDa) and the distance migrated from the bottom of the well. Kal. Standard MWs are, in kDa: 216.0 (light blue), 132.0 (light pink), 78.0 (olive green), 45.7 (pink), 32.5 (orange), 18.4 (purple), 7.6 (blue). PP+ standard MWs are, in kDa: 250.000, 150.000, 100.000, 75.000, 50.000, 37.000, 25.000, 20.000, 15.000, and 10.000.
2. If the migration distance of standard proteins in duplicate lanes seems to be about the same, average the values from the two different lanes and use the average to make a standard curve. If the duplicate lanes are very different, make 2 standard curves and use the curve for the standard lane closest to your other proteins.
3. Make a standard curve in Microsoft Excel (or by hand) by plotting the **log** of the molecular weight of the standard marker proteins (y-axis) vs. the distance migrated (x-axis). If the data points fit a straight line, add a linear trendline in Excel and display the R²-value and the equation on the graph. If data points do not fit a straight line, draw the best-fit curve through the points.
4. Make another table with the results from the other lanes of your gel. Note the # of bands seen, the distances migrated, and identify which bands are darkly stained (high concentration of protein) and which are lightly stained (low conc.). Identify the myoglobin band.
5. Read from your graph (if you drew the curve fit by hand) or use the straight-line equation to determine the approximate molecular weight of the protein subunits for each band observed in your clearest "Bmet" lane. Using the distance migrated for each band as an x-value, use your calibration curve equation to calculate a y-value (log MW), and take the antilog of this value (10^y) to calculate the MW.
6. Carry out statistical analysis on the linear regression (as you did for the aspartame calibration curve in Project 1) of the semi-log calibration curve (logMW vs. migration distance). This will give you uncertainties in the slope and intercept, which will in turn allow you to estimate the uncertainty in your derived value of MW(Mb).
7. Compare your experimental MW for myoglobin to its literature value (Protein Data Bank, UniProt, or ExPASy: myoglobin from horse skeletal muscle). Calculate the percent error in your estimation.

Post-Lab

A. Informal Written Report: Be sure to include.

1. Exemplary absorbance scans, labeled.
2. Observations, and raw data on solutions made, with sample calculations.
3. Table of data used to create protein MW Standard Curve.
4. Table of experimentally-determined MW for all protein bands in Bmet.
5. Pictures of Gels. Label lanes appropriately. Also, label each band of one of the standard lanes with the molecular weights (kDa).
6. Standard Curve (made from your gel)

B. Post-Lab Questions:

1. How do oxy-Mb and metMb differ? Explain the color difference between fresh and aged meat.
2. Use molar absorptivity values from the literature to calculate [oxyMb] in your reduced sample, and [metMb] in your oxidized sample.
3. Estimate the % of total protein in the Bmet sample that is myoglobin.
4. Since the Bmet sample is not pure myoglobin, did the presence of other proteins alter the SDS-PAGE mobility of myoglobin? Is this surprising?