“Meat Mimicry: Burgers without Blood” Workshop Protocol

Protocol by Lauren Blake, adapted from Eric Johnson’s myoglobin extraction protocol
Email: lblake8@jhu.edu

For this lab you can choose to characterize either Impossible or Beyond ground “beef.” You will notice they are both colored red. Impossible beef is colored using soy leghemoglobin, which was produced and purified from a genetically modified yeast organism. Beyond Meat uses beet root extract to color its meat red. In groups of two, we will extract the colorants used to produce red pigment in each of these meat substitutes, and then perform a spectroscopic characterization with various perturbations of the colorants.

NOTE: Do NOT consume the beef in this lab! We will be grilling out afterwards so you do not be tempted to try the meat that is used in the lab. Please use gloves to handle the meat.

Colorant Extraction

1. Using a weight boat from the lab, each student will weigh out approximately 25 grams of the plant-based beef. Each group should have at least one person weighing out Impossible and Beyond beef.
2. Return to your lab bench with your weighed beef and empty the contents into a Ziploc bag. Using a sharpie, label the bag with Impossible or Beyond beef and your name.
3. Once you have placed your beef into the bag, ensure the bag is tightly sealed and try to remove as much air as possible. Any openings to the bag will result in leakage of water into the bag and dilute your colorant.
4. Once the bag has been properly sealed, bring the bag to the sous vide station and place the bag into the water bath. The sous vide water bath should be at a temperature of 57 degrees.
5. Let the bag sit in the water bath for 30-60 minutes while we continue the lecture.

Crude Purification

The sous vide will hold the meat at a constant temperature of 57 C for the entire time it is in the water bath. At this temperature the meat analogue will condense and this will force the liquid contents of the plant-based beef out of the meat. 57 C is not hot enough to “cook” or denature the protein, so the leghemoglobin and betalain will be removed from the beef, but should still be biochemically active. For the Impossible beef, leghemoglobin will form the majority of the protein content of this liquid. For the Beyond beef, there will be a lower concentration of the betalain colorant from beet root extract that makes the Beyond Beef a light pink.

1. Remove the plastic bag from the sous vide water bath and return to your lab bench. You should see some liquid collecting at the bottom of the bag. Squeeze the beef so that more liquid is extracted, use the P1000 to pipette 1 mL of liquid and place in a 1.5 mL Eppendorf tube. Label your tube with your name and beef brand, as well as “Centrifuge.”
2. Place your tubes into the centrifuge. One of the volunteers will operate the centrifuge. The centrifuge will be run at 5000 rpm for 5 minutes.
3. While the centrifuge is running, take a new 1.5 mL Eppendorf tube tube and label it with your name and beef brand, and the words “Crude Extract”.
4. After the centrifuge has run, analyze the different layers that have formed in the tube. Fat has a low density so it will rise to the top during the centrifugation process. The middle portion is where your soluble protein and colorant (leghemoglobin or betalin) should be, and the bottom portion is where the heavier aggregated proteins are. Use a P1000 to carefully pipette 700 uL from the middle region of the “Centrifuge” tube and put into your “Crude Extract” tube, careful not to disturb the fat or the aggregated regions too much.

NanoDrop Setup

1. If using the Nanodrop for the first time that day, first clean the instrument using DI water and dry with Kimwipe.
2. When opening the Nanodrop software, select UV-Vis mode for acquisition.
3. Activate the Nanodrop by pipetting 2 uL of DI water onto the stand. Make sure the Nanodrop arm is down before activating the instrument. Wipe off the water afterwards both on the arm of the nanodrop and on the pedestal.
4. Add 2 uL of DI water to the Nanodrop again. This water will serve as our “Blank.”
5. Press the “Blank” button on the NanoDrop touch pad to blank the instrument.
6. You are now ready to add your samples to the Nanodrop.
7. NOTE: After each use. clean the pedestal with DI water and dry with Kimwipe.

Initial Leghemoglobin Absorbance Spectrum

1. Take an initial spectrum of the un-perturbed colorants. Carefully pipette 2 uL of your sample and dispense it onto the pedestal of the Nanodrop
2. Bring down the Nanodrop arm and click the “Measure” button.
3. Record the wavelength peaks and absorption readings:

Peak wavelength(s): _____

Corresponding absorption reading:_____

4. If you are collecting the spectrum from the Beyond beef, the signal from the betalain may be too dilute to show up on the spectrum. If this is the case, crush up some raw beets, collect as much juice as possible (you may dilute into water, if you like) and re-try.

Reduced Leghemoglobin Spectrum

1. On the lab bench will be a small tube labeled “DT”. This is a reductant, it will do two things when you add it to the solution. First it will destroy all molecular oxygen in the solution, creating an anaerobic environment. Secondly it will reduce the iron within the heme molecule of the leghemoglobin from Fe2+ to Fe (remember- reduction is the GAIN of electrons, so a less positive charge will result). This reduced state of iron is unable to bind oxygen and should turn the solution darker red.
2. Take a new Eppendorf tube labeled “PERTURBED” and using the P200, add 100 µl of “CRUDE EXTRACT” to the “DT” tube. Take a P20 and transfer 10 µl of solution from the “DT” tube to the PERTURBED solution.
3. Close the lid of the PERTURBED solution and invert slowly.
4. Add 2 uL of the PERTURBED solution onto the NanoDrop pedestal.
5. Press the “Measure” button on the NanoDrop.
6. Record the wavelength peaks and absorption readings:

Peak wavelength(s): _____

Corresponding absorption readings:_____

Oxy-leghemoglobin Spectrum

1. Open the PERTURBED eppendorf tube to allow oxygen back into the sample, and *slowly* invert the tube for approximately 2 minutes. What you are doing is mixing air into your sample the air will eventually over power the DT reductant and allow the heme in the leghemoglobin to bind oxygen again. This should result in a brighter red sample.
2. Add 2uL of this re-oxygenated leghemoglobin onto the NanoDrop pedestal.
3. Press the “Measure” button.
4. Record the wavelength peaks and absorption readings:

Peak wavelength(s): _____

Corresponding absorption readings:_____

Peroxide Damage to Leghemoglobin

1. On the lab bench will be a small tube labeled “Hydrogen Peroxide.” This should
2. Using a P20, pipette 20 µl of “Peroxide” solution into your perturbed sample.
3. Close the lid to the Eppendorf tube and invert two time. Wait 2 minutes.
4. Add 2uL of this re-oxygenated leghemoglobin onto the NanoDrop pedestal.
5. Press the “Measure” button on the NanoDrop
6. Record the wavelength peaks and absorption readings:

   Peak wavelength(s): _____

   Corresponding absorption readings:_____

7. If changes are not seen in the spectrum or in the color of the hemoglobin, try adding 10 uL of bleach (a stronger and more concentrated oxidizing agent) until you see a change in color.

Other perturbations you can try for fun if there is time at the end:

1. Heat the CRUDE EXTRACT solution to 80 degrees Celsius on a heating block for just a few minutes and observe the changes in the solution
   Observations:

2. Add a “chelating agent” such as 20 uL of 0.5 M EDTA or EGTA to the CRUDE EXTRACT solution. Chelating agents bind metals very well and can remove them from other bound molecules, like heme.
   Observations:

3. Check the absorption spectra of a yellow solution such as turmeric. In turmeric, the primary coloring agent is called curcumin, and it usually has an absorption peak around 420 nm.

   Draw the peak you see below, label the x and y axis. Where is the absorption peak?