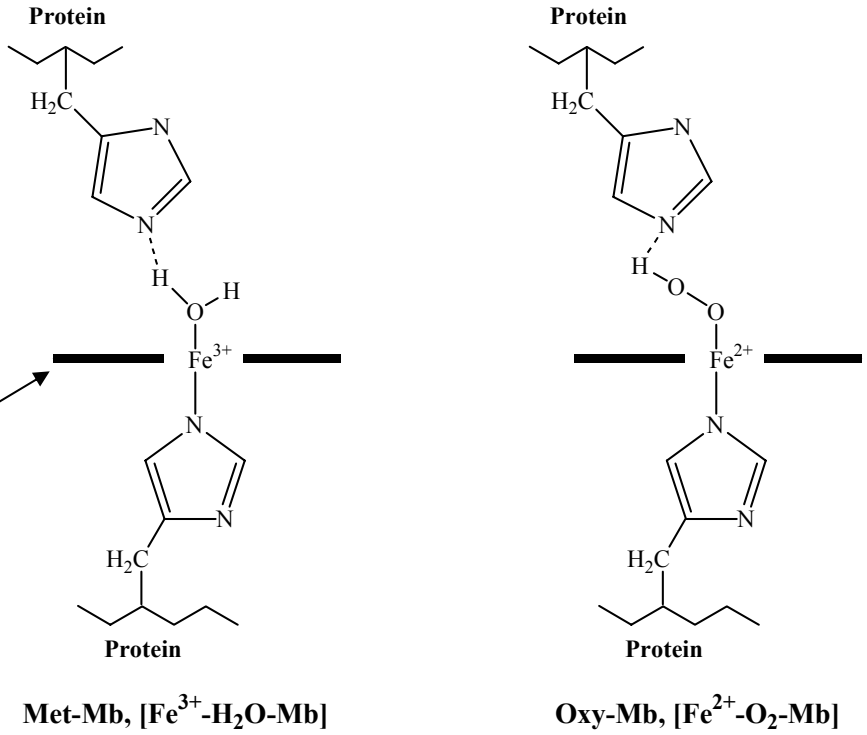
7. Iron in Myoglobin[1]

PART 1: Background

Mammalian myoglobin (Mb) is a monomeric oxygen-binding protein found in cardiac and skeletal muscle and is one of the most studied proteins in biochemistry. It is known to be a monomeric (one peptide chain) protein of 153 amino acids in length and 17.2 kDaltons. It contains the prosthetic group heme, a porphyrin ring system that includes an iron ion. Its primary function is to store oxygen until the tissue requires it. The protein has a single heme group that is ligated to the protein by histidine residue His-93. The iron in the heme can be found in two oxidation states: Fe^{2+} or Fe^{3+}. Oxygen binds to heme when iron is in the 2+ state (Oxy-Mb) but cannot bind when the iron is in the 3+ state (Met-Mb) shown in **Figure** 1. When oxygen binds to Mb, a significant change in the conformation of the heme occurs- the heme becomes planar. This slight movement is crucial to the physiological properties of both Mb and hemoglobin. In vivo, Oxy-Mb and deoxy-Mb (the unligated ferrous state) are the two most common forms. However, in a nonliving system, Oxy-Mb is slowly converted to Met-Mb as the heme-bound O_2 molecule is released and an active-site H_2O molecule is bound.

Figure 1

Edge view of porphyrin



Met-Mb, [Fe^{3+}-H_2O-Mb] **Oxy-Mb, [Fe^{2+}-O_2-Mb]**

Myoglobin is easily extracted from a lean ground steak patty. The meat contains very high levels of both Oxy-Mb and Met-Mb, which have unique spectral properties (see **Table 1**) that can be used to accurately identify and characterize the sample identity with respect to both oxidation state and functional state. One can use chemical agents to duplicate the oxidizing and reducing reactions of living systems. Using redox chemistry, the functionally important state, Oxy-Mb, can be converted to inactive Met-Mb.

Today we will obtain an extraction of Mb and experimentally show the redox changes in the protein. The extraction is relatively simple because Mb is a water-soluble protein. We will then use spectroscopy to follow the redox reactions of the heme prosthetic group.

[1] Adapted from Bylkas, S. A., Andersson, L.A., *J. Chem. Ed.* **1997**, *74*, 426.

Table 1: Absorption Peaks for Myoglobin Complexes

Sample	Visible Region[a]				Soret Region[a,b]	
Met-Mb	635			504	409 (179)	
Oxy-Mb		580	542		417 (128)	348

[a] Wavelengths given in units of nm; extinction coefficient is in parentheses, in units of $mM^{-1}cm^{-1}$.
[b] The Soret region is the part of the spectrum, which absorbs porphyrin rings strongly.

Part 2: Methods

I. Myoglobin Extraction
   A. Place 10g lean ground steak and 20 mL of buffer (20 mM potassium phosphate, pH 5.6) to a centrifuge tube.
      1. Mix sample with a glass rod for 1 minute to break open the cells. Take care not to be too rough lest you also release fats and nucleic acids.
      2. Centrifuge sample for 15 minutes at 10,000 rpm.
   B. Remove aqueous layer that contains the Mb.
      1. Be sure to note the color of the supernatant and pellet
      2. Be careful not to remove any fat layer that may be on the very top.

II. Obtain absorption spectra
   A. Divide your Mb extract into three aliquots of approximately 5, 5, and 5 mL labeling them Extract, Sample A, and Sample B, respectively.
   B. Redox reactions
      1. To Sample A (a 5 mL aliquot), add approximately 8 crystals of potassium ferricyanide ($K_3[Fe(CN)_6]$). Mix and note the color of this solution.
      2. To Sample B (a 5 mL aliquot), add approximately 10 crystals of sodium hydrosulfite ($Na_2S_2O_4$). Mix and note the color of this solution.
   C. Absorption spectra
      1. First test the Mb extract.
         a. Make a 1:4 dilution of your sample using buffer.
         b. Run a spectrum from 300 nm to 700 nm. You may need to use two different y-axes to clearly see all of the peaks. In other words, you may have to blow up the spectrum in the region of 450-700 nm.
         c. Note where all of the peaks occur and their absorption. Be sure to specifically note the absorbance at the wavelengths in table 1.
      2. Test Sample A and B
         a. Make dilutions of each sample as in the extract solution.
         b. Run the spectra and record the data exactly as you did for the extract solution.

Part 3: Analysis & Discussion

1. What types of molecules where in the supernatant? What was in the pellet? Explain the color difference between the supernatant and pellet. Why would you have to stir more vigorously to release nucleotides and fats than to release Mb?
2. Write out the redox reactions that occur in your experiment. Be sure to explain the colors that you noted in your experiment by the oxidation state of each solution.
3. Compare and contrast the differences in spectra you collected. What is the concentration of Oxy-Mb and Met-Mb in your extract? In Sample A? In Sample B? Hint: Use the extinction coefficient in Table 1 and Beer's Law: $A = \varepsilon cb$. If you are unfamiliar with this, see the instructor.
4. Why is fresh meat red whereas aged meat begins to turn brown? The body more readily absorbs Fe^{2+} than Fe^{3+}. What does this suggest about the dietary availability of iron in aged meat?
5. Consider the difference between white (breast) and dark (thigh) meat of chicken. Propose a reason for the observed difference in color? Be sure to discuss the physiological functioning of the muscles for white and dark meat. Eating which type of meat would give you more iron in your diet.