Modeling Pre-lab Homework Assignment
Oxygen Binding to Myoglobin and Hemoglobin

Make sure to watch the video on Canvas!!!!!!

<u>Homework</u>: This assignment is to be done individually and uploaded **BEFORE** the start of the lab. A video is posted on Canvas that covers the coding syntax you need in order to complete this assignment. It is required to watch the video before you start the assignment.

Organize your code and answers clearly in one .Rmd file. Enter all answers to boldface questions as comments in the code.

This is an individual assignment, but you are allowed to work together in groups and discuss coding and answers. That said, you are responsible for all of the material in this homework assignment. **DO NOT COPY** *from anyone that you work with. You are NOT allowed to share code. You need to write the code and answer the questions yourself. Try the coding yourself first before seeking help.*

Be sure to include your name in the file name as follows: lastname_firstname_labday.Rmd. Also type your full name as the first comment in your .Rmd file. <u>**Upload 1) the .Rmd file and 2) the knitted html file to Canvas before the start of your lab.**</u>

Due: BEFORE the start of your lab period in week 7
Total Points: 10 points

If you have any questions, please do not hesitate to ask. Best of luck!

PART I: Myoglobin

We will begin by describing how the fractal O_2 saturation of myoglobin depends on the partial pressure of oxygen (pO_2). Since myoglobin only binds a single O_2 molecule, we will consider the simple binding kinetics.

$$Mb + O_2 \leftrightarrow MbO_2 \qquad \text{Eq. 1}$$

If this reaction is allowed to equilibrate, we can define an equilibrium constant *Keq* in terms of those equilibrium concentrations:

$$Keq = \frac{(MbO_2)}{(Mb)(O_2)} \qquad \text{Eq. 2}$$

or we can express the O_2 concentration in terms of partial pressure (pO_2) instead,

$$Keq = \frac{(MbO_2)}{(Mb) \cdot pO_2} \qquad \text{Eq. 3}$$

We are more interested in what **fraction of total myoglobin is oxygenated**, rather than the absolute concentration of oxygenated myoglobin. Therefore, we define the fractional O_2 saturation of myoglobin (*Y*) as:

$$Y = \frac{MbO_2}{Mb_{tot}} \qquad\qquad \text{Eq. 4}$$

$$Y = \frac{MbO_2}{MbO_2 + Mb} \qquad\qquad \text{Eq. 5}$$

Making the substitution with the equilibrium expression ($MbO_2 = Keq \cdot Mb \cdot pO_2$), we get:

$$Y(pO_2) = \frac{pO_2}{pO_2 + p50} \qquad\qquad \text{Eq. 6}$$

where p50 = 1/Keq.

Modeling Myoglobin Oxygen Dissociation Curves

Program the equation above for the fraction of total myoglobin that is oxygenated (Eq. 6). Produce a range of pO_2 values and start with a p50=35.6 mmHg (cat blood).

1. Graph your results (include axes labels with units, and title). What is the shape of the curve in your plot? Do you get a sigmoidal or hyperbolic curve?

2. Choose 5 different p50 values – plot all of the curves on one graph (be sure to include the "control" from above). Graph a horizontal line at the ½ way point in order to discuss the effects of changing P50. Include axes labels with units, title, and a legend. Comment on your findings. What is the relationship between p50 and myoglobin saturation curves?

PART II: Hemoglobin

Let's plot some "real" hemoglobin saturation data. Then we will explore assumptions made about *Keq* values and how it affects our ability to fit our model to the "real" data.

Handling Experimental Data
Import the data file you downloaded from Canvas into the working environment in R (Experimental_Data.csv) – **SAVE** the file on your computer and set the **working directory through R**.
- In RStudio → session → set working directory → choose directory (choose directory where you saved the experimental file – the DESKTOP (or wherever you saved the experimental file)).
- In the console window type: **getwd()** – this will give you the exact pathway to your file. Copy and paste this pathway when you read in your .csv file. For example
  o Variablename=read.csv("/Users/esmaelhaddadian/Desktop/ Experimental_Data.csv") # data frame
  o Variablename=read.csv("/Users/esmaelhaddadian/Desktop/Temp_data_ExpF22.csv") # data frame

- This tells the software exactly where the file lives to be read in.
- Read in the files by assigning the whole .csv file to a variable name – which will be a matrix with dimensions of 554x2 (554 rows and 2 columns) for the experimental data file and 4474x14 (4474 rows and 14 columns) for the temperature sensitive data.

  Once the data is read into the environment as 2 matrices with dimensions of 554×2 and 4474×14 - we will manipulate the data frames using 3 different techniques. Each of the 3 techniques will be applied to each of the 2 data sets.

I. **Assign each column to a specific variable**
   Variable_column=Variablename$columnName → where columnName is the name of the column explicitly written in the .csv file. You need to look at the .csv files to see the tag for each column.

II. **Create mini-matrices that contain the data that corresponds to each data type.** For experimental data you will create 1 mini-matrix containing percent bound versus partial pressure of O_2. For the temperature sensitive data, you will create 7 mini-matrices, each containing the percent bound as a function of the partial pressure of O_2 dependent on the temperature (there are seven different temperatures).

Choose either II.1 or II.2 to create the mini-matrices

II.1 Example syntax using the matrix you read in originally (554x2 and 4474x14).
a. Exp_data <- data.frame(x=experimental[,1], y=experimental[,2])
b. Temp_32<-data.frame(x=temp[,1], y=temp[,2])

II.2 Example syntax using the array you created from #I
c. data <- data.frame(x= data_PO2, y= data_Y)
d. T32 <- data.frame(x= data_pO2_32, y= data_Y_32)

III. **Work with the original matrix you read in** – remembering the order of the columns and what each column corresponds to. In order to access positions in a matrix, the syntax is: matrix[row,column]. You will use the original matrix to plot. Please see below #3.

Now that you have read in the data and have it in a nice form in your .Rmd file, let's plot the data, while understanding what it is that we are plotting.

3. Plot the data! You will create 6 plots using the 3 techniques from #I, #II and #III, for the experimental and temperature sensitive data. Use points (include axes labels with units, title, and add a legend). Remember to assign a color to each of the curves – then you can add a legend. Please add limits to the x- and y- axes using ylim=c(min, max) and xlim=c(min, max) within the plot function. Graph a horizontal line at the ½ way point (0.5) using abline(h=50,lty=2)).

4. Describe what you see. Use comments in the .Rmd file. in order to discuss the qualitative effects of temperature change on P50. What is the relationship between temperature and hemoglobin oxygen saturation curves (use the ½ waypoint as a reference)?

During the lab, we will use the experimental data to fit the data to different mechanistic binding schemes.

If you have further questions, please email Dr. Haddadian: haddadian@uchicago.edu