Myoglobin Stability

Read:
Sykes, P. A.; Shiue, H.; Walker, J. R.; and Bateman, R. C., Jr. (1999) Determination of Myoglobin Stability by Visible Spectroscopy. **J. Chem. Ed. 76:** 9; pp. 1283-1284.

With you lab partner, design an experiment to test the effect of temperature on the stability of myoglobin (K_{eq}). You will need to collect a denaturation profile in solutions at three temperatures.

Your group will be provided with the following:

- 1 mL cuvette
- Small glass test tubes
- Parafilm
- Visible Spectrophotometers
- Adjustable pipettes
- Ice
- Water bath at 28 C
- 7.5 mL of 2 mg/mL equine myoglobin in 50 mM phosphate buffer (pH 7)
- 25 mL of 5.00 M GuHCl in 50 mM phosphate buffer (pH 7)
- 50 mL of 50 mM phosphate buffer (pH 7)

Suggestions:

- Mix all solution components thoroughly before adding the protein.
- Use 0.2 M increments GuHCl concentration.

Data Analysis:

1. Plot Absorbance vs. [GuHCl] at each temperature value seperately.
2. Use non-linear fitting in Excel to determine the A_{folded}; $A_{unfolded}$; K°_{eq} ; and m at each temperature value. Determine the standard error of each parameter.

$$Mb_{folded} \overset{K_{eq}}{\Leftrightarrow} Mb_{unfolded}$$

$$K_{eq} = \frac{[Mb_{unfolded}]}{[Mb_{folded}]}$$

$$K_{eq} = \frac{\left(A_{folded} - A\right)}{\left(A - A_{unfolded}\right)}$$

$$A = \frac{\left(A_{folded} + K_{eq}(A_{unfolded})\right)}{\left(K_{eq} + 1\right)}$$

$$\frac{\partial \ln K_{eq}}{\partial [GuHCl]} = m$$

$$K_{eq} = K_{eq}^{\circ} e^{m[GuHCl]}$$

$$A = \frac{\left(A_{folded} + \left(K_{eq}^{\circ} e^{m[GuHCl]}\right)\left(A_{unfolded}\right)\right)}{\left(\left(K_{eq}^{\circ} e^{m[GuHCl]}\right) + 1\right)}$$

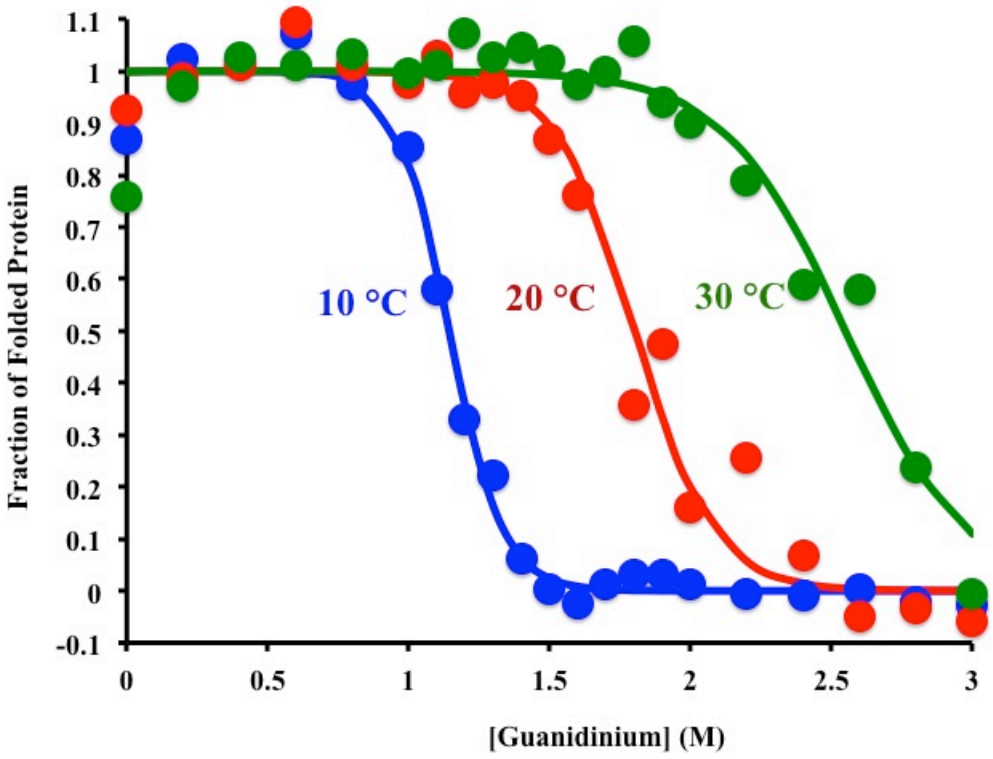
3. Create a column graph that presents the effect of temperature on K_{eq}°, being sure to include standard errors. Write a figure caption.

4. Create a column graph the presents the effect of temperature on m, being sure to include standard errors. Write a figure caption.

5. For each temperature set, determine the faction of folded protein by subtracting the best-fit $A_{unfolded}$ from each data point and dividing the difference by the best-fit A_{folded}.

6. Create a single graph that presents the effect of temperature on the fraction of folded protein data with fitting lines. Write a figure caption. Use the image below as a guide, though your plot will have different temperatures and may have very different looking data. Your plot should be formatted exactly as the example when you are done.

7. Summarize the effects of temperature on the stability of myoglobin.


Fraction of Folded Protein
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0
0.5
1
1.5
2
2.5
3
[Guanidinium] (M)
10 °C
20 °C
30 °C