## NONCOOPERATIVE LIGAND BINDING: HOW STRONGLY DOES AZIDE BIND TO MYOGLOBIN?

**Caution:** Since azide binds to myoglobin and hemoglobin, it is a poison to all animals that utilize heme-containing proteins to transport $O_2$ (this includes you!). Wear goggles and gloves while in the laboratory and dispose of all solutions in the proper waste container.

**Stock solutions:**

2 mM metmyoglobin (Mb)
20 mM Sodium Phosphate buffer pH 7.4
0.500 mM Sodium azide
0.500 mM Sodium Chloride

**Table 1: Myoglobin Binding Set**

| Tube | [Mb] (mM) | [azide] (mM) | [NaCl] (mM) | Total Vol. (mL) |
|---|---|---|---|---|
| 1 | 0.050 | 0.00000 | 0.16667 | 3.00 |
| 2 | 0.050 | 0.00083 | 0.16583 | 3.00 |
| 3 | 0.050 | 0.00250 | 0.16417 | 3.00 |
| 4 | 0.050 | 0.00500 | 0.16167 | 3.00 |
| 5 | 0.050 | 0.01000 | 0.15667 | 3.00 |
| 6 | 0.050 | 0.01500 | 0.15167 | 3.00 |
| 7 | 0.050 | 0.02000 | 0.14667 | 3.00 |
| 8 | 0.050 | 0.04000 | 0.12667 | 3.00 |
| 9 | 0.050 | 0.06000 | 0.10667 | 3.00 |
| 10 | 0.050 | 0.10000 | 0.06667 | 3.00 |
| 11 | 0.050 | 0.16667 | 0.00000 | 3.00 |

### Lab Activity

Mix each of the solutions described in Table 1 in glass test tubes. Be sure to add myoglobin last to each solution. Allow each solution to reach equilibrium (~30 minutes at room temp). Blank the spectrophotometer with Tube 1. Record the absorbance at 425 nm of each solution in Table 1.

### Data Analysis

Your data should fit to the following equation where $A_{observed}$ is the observed absorbance at 425 nm, $A_{max}$ is the absorbance of metmyoglobin bound to azide, and $\theta$ is the fractional saturation as defined in the classroom/pre-lab activity:

$$A_{observed} = A_{\max}\theta$$

1. Use non-linear regression with Microsoft Excel to solve for the $A_{max}$ and $K_D$ parameters. Determine the standard deviations associated with each fit parameter.

2. In utilizing the above derived equation, it is assumed that the $[N_3^-]_{free}$ is approximated by the $[N_3^-]_T$ .
   a. How does your best-fit value for the $K_D$ of azide to metmyoglobin compare to $[Mb]_T$?
   b. Is $[N_3^-]_{free}$ well approximated by $[N_3^-]_T$ for your experiment?

3. The expression for θ which includes $[N_3^-]_T$ rather than $[N_3^-]_{free}$ takes on a quadratic form:

$$\theta = \frac{\left([Mb]_T + [N_3^-]_T + K_D\right) - \sqrt{\left([Mb]_T + [N_3^-]_T + K_D\right)^2 - \left(4(Mb]_T[N_3^-]_T\right)}}{2[Mb]_T}$$

Use non-linear regression with Microsoft Excel to solve for the $A_{max}$ and $K_D$ parameters using this expression for θ that does not make assumptions about $[N_3^-]_{free}$. Determine the standard deviations associated with each fit parameter.

4. Are the best-fit $K_D$ values for the two models significantly different?

5. Using the best-fit value of $A_{max}$, covert each $A_{observed}$ into a theta value:

$$\theta = \frac{A_{observed}}{A_{\max}}$$

6. Make a plot of $\theta$ versus $\left[N_3^-\right]_T$. This should be hyperbolic.

7. Make a plot of $\log\left(\frac{\theta}{1-\theta}\right)$ versus $\log\left[N_3^-\right]_T$. This is called a Hill plot. Add a linear trendline to your data points.

8. Use algebra to define an expression for $\log\left(\frac{\theta}{1-\theta}\right)$ from your expression for $\theta$ from your pre-lab.

   a. What does the slope of the Hill plot indicate?
   b. What does the y-intercept on a Hill plot indicate?
   c. How does the best-fit value for the $K_D$ from the Hill plot compare to the best-fit values from the non-linear models?