COOPERATIVE LIGAND BINDING: OXYGEN BINDING TO HEMOGLOBIN

For hemoglobin (Hb), four monomers interact to form a tetrameric quaternary structure. When one subunit of hemoglobin binds an oxygen molecule, it may undergo a conformational change, which transmits a signal to the adjacent subunits and positively influences their affinity to bind an oxygen molecule. This is called positive cooperativity. Imagine a situation where hemoglobin has all four sites either bound by oxygen or all four sites are unoccupied. In this scenario, there are no intermediate binding states (i.e., Hb with three oxygen bound instead of four). This situation is called the “completely cooperative” limit.

$$Hb:\left(O_2\right)_4 \overset{K_D}{\Leftrightarrow} Hb_{free} + 4O_2$$

1. Derive an expression for θ for this completely cooperative model.

2. Assume that the K_D for the completely cooperative hemoglobin model is 2 x 10^6 mm Hg. Oxygen is going to be monitored as a partial pressure in mm Hg (pO_2). Calculate θ values for the pO_2 range from 1 to 150 mm Hg using a 10 mm Hg interval.

3. Make a plot of θ versus pO_2. Notice that mathematics predicts a fundamentally different experimental result for the cooperative model as compared to the noncooperative one found for myoglobin.

4. Make a plot of $\log\left(\frac{\theta}{1-\theta}\right)$ versus $\log\left(pO_2\right)$ for the completely cooperative model of hemoglobin. This is called a Hill plot. Add a linear trendline to the data points.

5. Use algebra to define an expression for $\log\left(\frac{\theta}{1-\theta}\right)$ from your expression for θ in Question #3.
   a. What does the slope of the Hill plot indicate?
   b. What does the y-intercept indicate?

While the completely cooperative model is a useful thought problem, experimental data for oxygen binding to hemoglobin does not present a linear Hill plots with a slope equal to four. Archibald Vivian Hill [The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. 1910. *Journal of Physiology* **40:**iv-vii] first described the non-integer value for the slope (h) on the Hill plot:

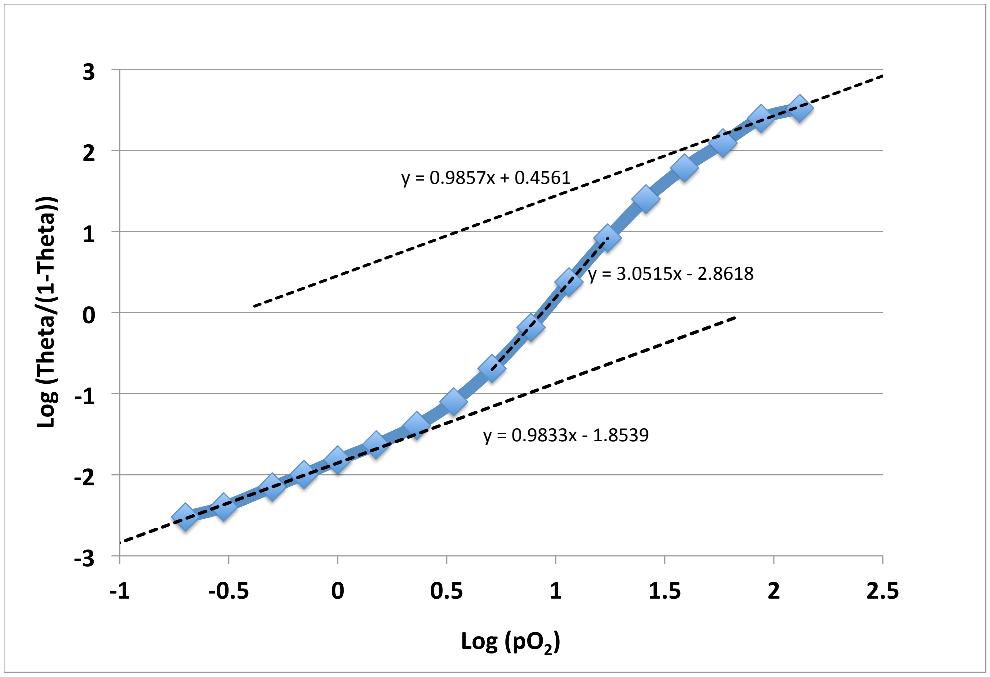
$$\theta = \frac{\left(pO_2\right)^h}{\left(P_{50}\right)^h + \left(pO_2\right)^h} \qquad \log\left(\frac{\theta}{1-\theta}\right) = h\left[\log\left(pO_2\right)\right] - h\left[\log\left(P_{50}\right)\right]$$

The P_{50} is the pO_2 at which half of the binding sites on Hb are bound to oxygen and θ = 0.5.

$$K_D = \left(P_{50}\right)^h$$

If h = 1, then the system behaves non-cooperatively.
If h > 1, then the system behaves with positive cooperativity.
If h < 1, then the system behaves with negative cooperativity.

Below is an actual Hill plot for oxygen binding to purified Hb:



6. How does the actual Hb data compare to the completely cooperative model for 4 binding sites? How does it compare to the non-cooperative binding model for Mb?

The MWC model proposes that Hb exists in two conformations, one that binds O_2 weakly (T-state) and one that binds O_2 tightly (R-state). The four, individual oxygen-binding sites act independently (non-cooperatively) when Hb is in the T-state at low oxygen concentrations.

7. At low oxygen pressure, what observation in the actual Hill graph supports that Hb acts non-cooperatively in the T-state?

8. Determine the association binding constant for the T-state (called K_{site}^{T}). Use your knowledge of non-cooperative systems and the fit-line to the low oxygen concentration data.

Since there are multiple sites and ways for each oxygen molecule to bind, the T-state binding constant receives a statistical factor for the process of binding at all four sites.

9. Determine the value of the equilibrium constant at each point during the binding scheme below:

$$T_0 + 4O_2 \overset{4K_{site}^T}{\Leftrightarrow} T_1 + 3O_2 \overset{\frac{3}{2}K_{site}^T}{\Leftrightarrow} T_2 + 2O_2 \overset{\frac{2}{3}K_{site}^T}{\Leftrightarrow} T_3 + O_2 \overset{\frac{1}{4}K_{site}^T}{\Leftrightarrow} T_4$$

The four, individual oxygen-binding sites act independently (non-cooperatively) when Hb is in the R-state at high oxygen concentrations.

10. At high oxygen pressure, what observation in the actual Hill graph supports that Hb acts non-cooperatively in the R-state?

11. Determine the association binding constant for the R-state (called K_{site}^{R}).

12. Similarly, determine the value of the equilibrium constant at each point during the binding scheme below:

$$R_0 + 4O_2 \overset{4K_{site}^{R}}{\Leftrightarrow} R_1 + 3O_2 \overset{\frac{3}{2}K_{site}^{R}}{\Leftrightarrow} R_2 + 2O_2 \overset{\frac{2}{3}K_{site}^{R}}{\Leftrightarrow} R_3 + O_2 \overset{\frac{1}{4}K_{site}^{R}}{\Leftrightarrow} R_4$$

Hb without oxygen bound is overwhelmingly in the T-state:

$$T_0 \overset{K_0^{T\to R}}{\Leftrightarrow} R_0 \qquad \text{where } K_{T_0 \to R_0} = \frac{[R_0]}{[T_0]} = 1.75x10^{-6}$$

13. Determine the value of the equilibrium constants describing the conversion of the T-state to R-state at each point along the binding pathway:

$$T_1 \overset{K_1^{T\to R}}{\Leftrightarrow} R_1 \quad K_1^{T\to R} = ?$$

$$T_2 \overset{K_2^{T\to R}}{\Leftrightarrow} R_2 \quad K_2^{T\to R} = ?$$

$$T_3 \overset{K_3^{T\to R}}{\Leftrightarrow} R_3 \quad K_3^{T\to R} = ?$$

$$T_4 \overset{K_4^{T\to R}}{\Leftrightarrow} R_4 \quad K_4^{T\to R} = ?$$

14. Use the handy equation $\Delta G^o = -RT \ln K_{obs}$ to determine the standard free energy change for converting from the T-state to the R-state at each point along the binging pathway.

15. How many oxygen molecules must bind for the R-state to become energetically favored over the T-state (i.e., the standard free energy change for converting from the T to the R state becomes negative)?