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**Cooperativity of hemoglobin**

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**1. MWC model:** Consider the Monod, Wyman, Changeux (MWC) model of cooperativity in hemoglobin:  $n = 4$  sites that can be in a relaxed (R) or tense (T) state, the latter favored by a factor  $L$ , with normalized oxygen fugacities of  $\alpha$  and  $c\alpha$  per site in the two states, such that the overall fractional occupancy of oxygen is

$$Y = \alpha \frac{(1 + \alpha)^{n-1} + Lc(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + L(1 + c\alpha)^n}.$$

(a) Estimate the effective Hill coefficient  $n_h$  for hemoglobin, either from the maximal slope of  $\log[Y/(1 - Y)]$  vs  $\log \alpha$  in a Hill plot, or from the slope at half-saturation. Comment on the relative importance of parameters  $c \ll 1$  and  $L \gg 1$  in determining  $n_h$ , by considering the limits of  $c \rightarrow 0$  and  $L \rightarrow \infty$ .

(b) How does the probability of being in the  $T$  state change as more oxygen molecules are bound? Calculate the equilibrium probability  $P_T$  of being in the  $T$  state, and plot it as a function of the normalized oxygen pressure  $\alpha$ . Next calculate the conditional probability  $P_T(i)$  of being in the  $T$  state, given that  $i$  molecules of oxygen are bound. Plot  $P_T(i)$  as a function of  $i$ . What is the effect of oxygen on hemoglobin conformation?

(c) Different species have different mechanisms of adaptation to hypoxia at high altitude. In humans, adaptation to high altitude involves, among other factors, rapid elevation of the level of 2,3 - DPG (aka 2,3 - BPG) molecule, which is synthesized in red-blood cells and binds preferentially to the  $T$  state of hemoglobin. Some birds that fly at high altitude are adapted by having the hemoglobin with a mutation at the interface between its  $\alpha$  and  $\beta$  domains, making the transition between the two conformational states of hemoglobin easier, i.e. reducing the free energy difference between the  $R$  and  $T$  states. How do these two mechanisms of adaptation affect the saturation curve of hemoglobin? Do you expect them to have similar or different effects on oxygen uptake in the lungs, and release in the tissues?

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**2. Binding to non-identical sites:** Consider a variant of the MWC model, with the sites that have different affinities,  $K_1, K_2, \dots, K_n$ , for oxygen.

(a) Show that at both very low, and very high pressures, the model can be approximated by the classical MWC model with identical sites, but with an effective binding constant  $K_{eff}$ . In either regime, which sites contribute most to  $K_{eff}$ , the strongest or the weakest?

(b) Can the above model, in the simplified limit of only one state – say for  $L = 0$ , lead to a sigmoidal Hill plot with  $n_h < n$ ?

(c) Consider another variant of the model, with different affinities but with extreme cooperativity as considered by Hill, i.e. with either no site occupied or all sites occupied. Can such a model provide a sigmoidal Hill plot with  $n_h < n$ ?

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