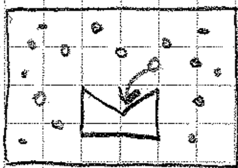
① 4/23/09

Heamoglobin and Myoglobin

Outline

1. Binding reaction, Scatchard plot
2. Binding to n independent sites
3. Hill equation, Hill cofficient
4. MWC model

1. Simple binding reaction



$P + L \rightleftarrows P.L$

$Y = \dfrac{[PL]}{[PL]+[P]}$ ← site occupancy

E_b - binding energy

——— free F_{free} $\qquad F_{free} = 0 - TS$

——— bound F_{bound} $\qquad F_{bound} = -E_b \quad (E_b > 0)$

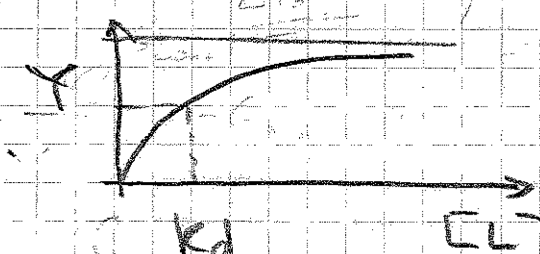
Estimate entropy in the free state: $S = \log \omega$

ω - # of states per molecule $= \dfrac{V}{N_L} = \dfrac{1}{[L]}$, where

$[L] = \dfrac{N_L}{V}$ - concentration $\Rightarrow \boxed{S = -\log [L]}$

$$Y = \frac{e^{-F_{bound}/T}}{e^{-\frac{F_{bound}}{T}} + e^{-\frac{F_{free}}{T}}} = \frac{e^{E_b/T}}{e^{E_b/T} + \underbrace{e^{S}}_{=\frac{1}{[L]}}} = \frac{[L]}{K_d + [L]}$$

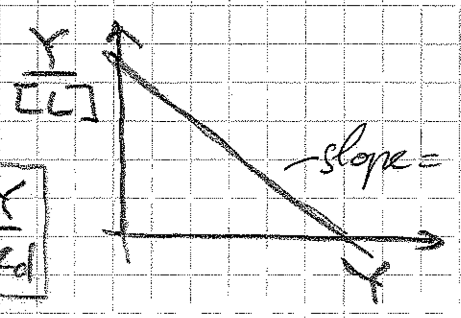
where $\boxed{K_d \equiv e^{-E_b/T}}$



* Scatchard Plot

$Y(K_d + [L]) = [L]$

$\dfrac{Y}{[L]} K_d + Y = 1 \Rightarrow \boxed{\dfrac{Y}{[L]} = \dfrac{1}{K_d} - \dfrac{Y}{K_d}}$



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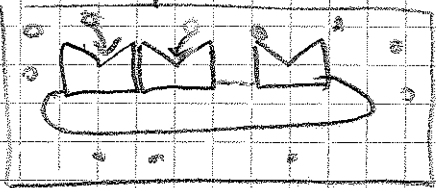
2. Independent sites

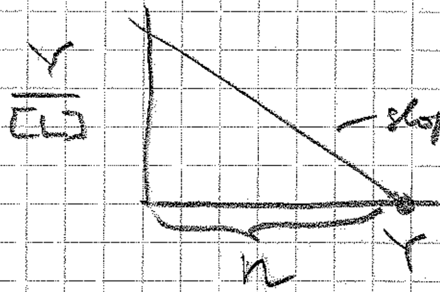


occupancy of a site

$$Y = \frac{[L]}{K_d + [L]}$$

occupancy of the protein

$$Y_p = \frac{n[L]}{K_d + [L]}$$



Scatchard plot

$$\frac{Y_p}{n[L]} = \frac{1}{K_d} - \frac{Y}{nK_d} \Rightarrow \boxed{\frac{Y_p}{[L]} = \frac{n}{K_d} - \frac{Y}{K_d}}$$

3. Cooperative binding to n sites

Hill model of hemoglobin (1910)

n sites, either all occupied or all vacant

$$M + nL \rightleftarrows ML_n \qquad K_d^{(n)} = \frac{[M][L]^n}{[ML_n]}$$

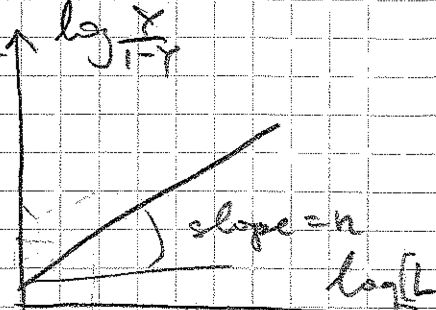
$$Y = \frac{e^{nE_b/T}}{e^{nE_b/T} + \underbrace{e^{-n\log[L]}}_{\text{entropy loss upon binding of n ligands}}} =$$

$$\boxed{Y = \frac{[L]^n}{\underset{\substack{\| \\ K_d^n}}{K_d^{(n)}} + [L]^n}}$$

Hill equation

Scatchard is non-linear

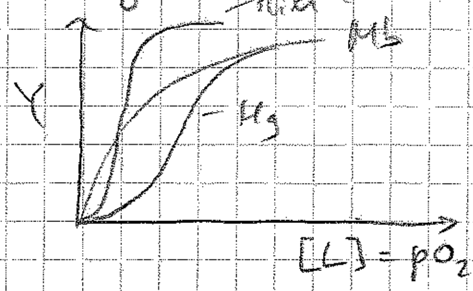
Hill plot



$$\frac{Y}{1-Y} = \frac{[L]^n}{K_d^{(n)}}$$

$$\log\frac{Y}{1-Y} = n\log[L] - \log K_d^{(n)}$$

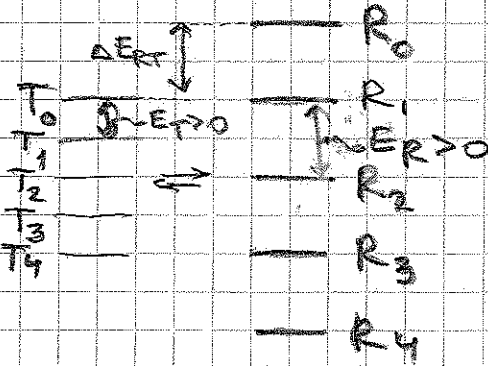
③ Hemoglobin

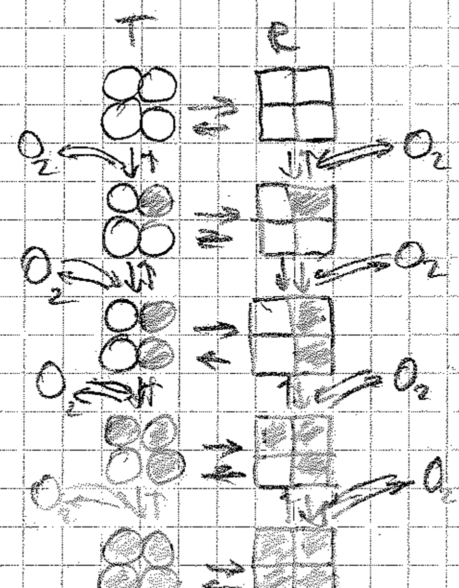


$\Rightarrow$ need a better model

* Monod - Wyman - Changeux model (1965) (MWC)

1) Hemoglobin consists of $n=4$ domains
2) All domains can be either in R or in T states.
3) O_2 binds to the R state with higher affinity
4) In the unbound form, T state has a lower energy





Parameters of the model

$$L = \frac{[T_0]}{[R_0]} = e^{-\Delta E_{RT}} \gg 1$$

$$\alpha \equiv \alpha_R = \frac{[L]}{K_d^R} = [L]\, e^{E_R/T}$$

$$c = \frac{K_R}{K_T} = \frac{\alpha_T}{\alpha_R} = e^{(E_T - E_R)/T} \ll 1$$

$$Z = \sum_{i=0}^{n} \left[w(R_i) + w(T_i) \right], \text{ where } w(R_i) = \alpha^i C_n^i$$

$$w(T_i) = L \cdot (\alpha c)^i C_n^i$$

$$= \sum_{i=0}^{n} C_n^i \alpha^i + L \sum_{i=0}^{n} C_n^i (\alpha c)^i =$$

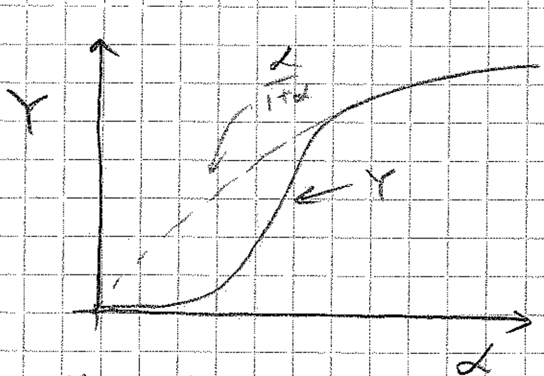
$$= (1+\alpha)^n + L(1+\alpha c)^n$$

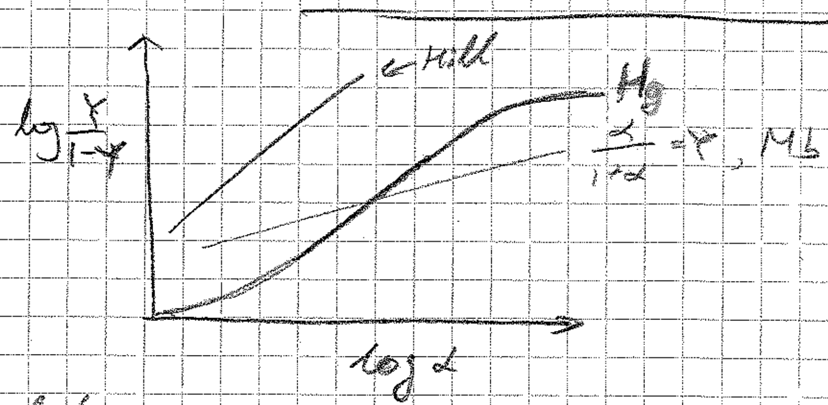
(4) $$Y = \frac{\frac{1}{n}\sum_{i=0}^{n} i\,[w(R_i) + w(T_i)]}{Z}$$

$$\sum_{i=0}^{n} i\,w(R_i) = \sum_{i=0}^{n} i\,C_n^i \alpha^i = \alpha \sum_{i=1}^{n} i\,C_n^i \alpha^{i-1} = \alpha \frac{\partial}{\partial \alpha} \sum C_n^i \alpha^i = \alpha \frac{\partial}{\partial \alpha} (1+\alpha)^n$$

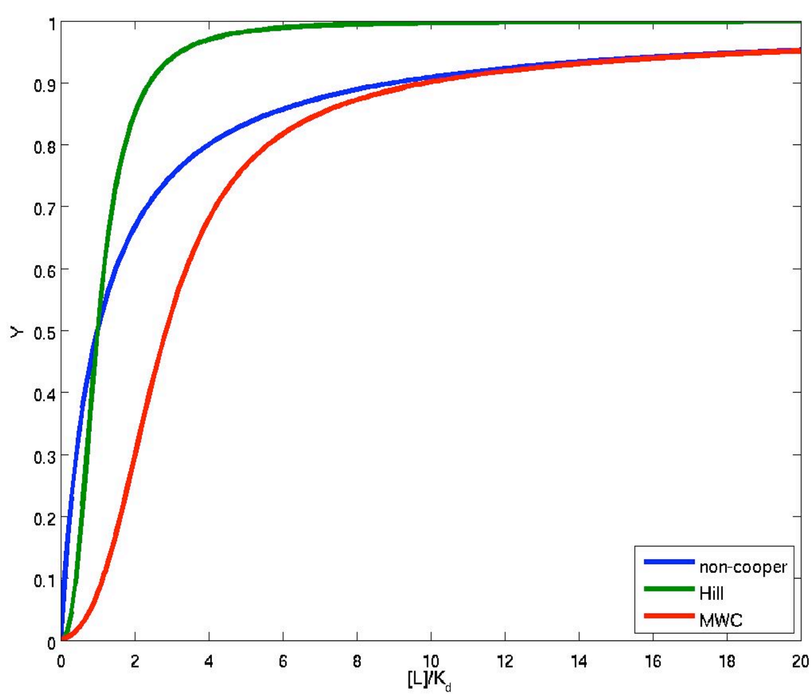
$$= \alpha n (1+\alpha)^{n-1}$$

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

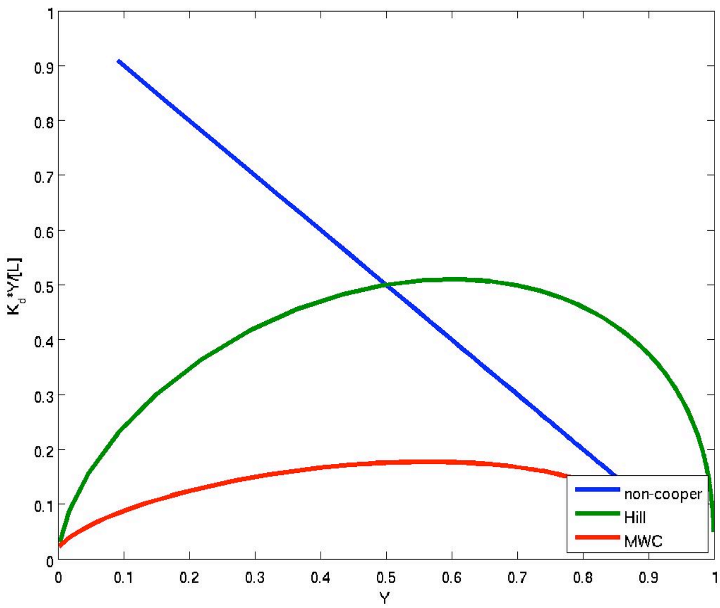




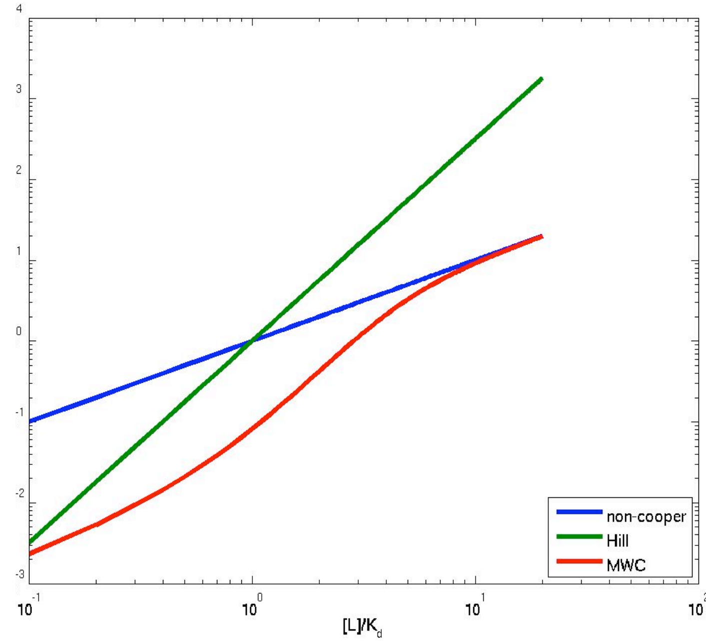
- effect of parameters c and L
- Bohr effect
- Heterotropic regulation



SCATCHARD PLOT



HILL PLOT



SENSETIVITY OF MWC TO PARAMETERS