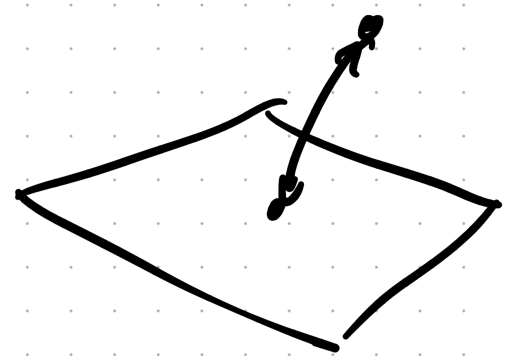
Ligand Binding



affinity for a ligand - titration experiment

Chemical Eq

$$M + L \rightleftharpoons ML$$

$$K_b = \frac{[ML]_{eq}}{[M]_{eq}[L]_{eq}},$$ units are $1/M$

b → bind

$$ML \rightleftharpoons M + L$$

$$K_d = K_b^{-1} = \frac{[M][L]}{[ML]}$$ units of "M"

low K_d is high affinity!

very high affinity

pM ~ 10^{-12} M

most protein-protein interactions

μM → mM

10^{-6} 10^{-3}

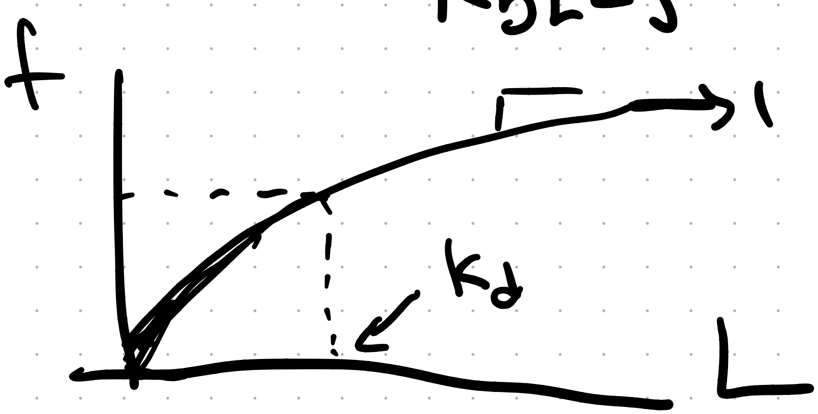
what fraction is bound

$$\langle f \rangle = \frac{[ML]}{[M]+[ML]} = \frac{k_b[M][L]}{[M]+k_b[M][L]} = \frac{k_b[L]}{1+k_b[L]}$$

$$= \frac{\# ML}{\# M\ \text{total}}$$

$$K_b = \frac{[ML]}{[M][L]}$$

$$= \frac{1}{\frac{1}{k_b[L]}+1} = \frac{1}{1+\left(K_d/[L]\right)}$$



K_d is [L] where $f = 1/2$

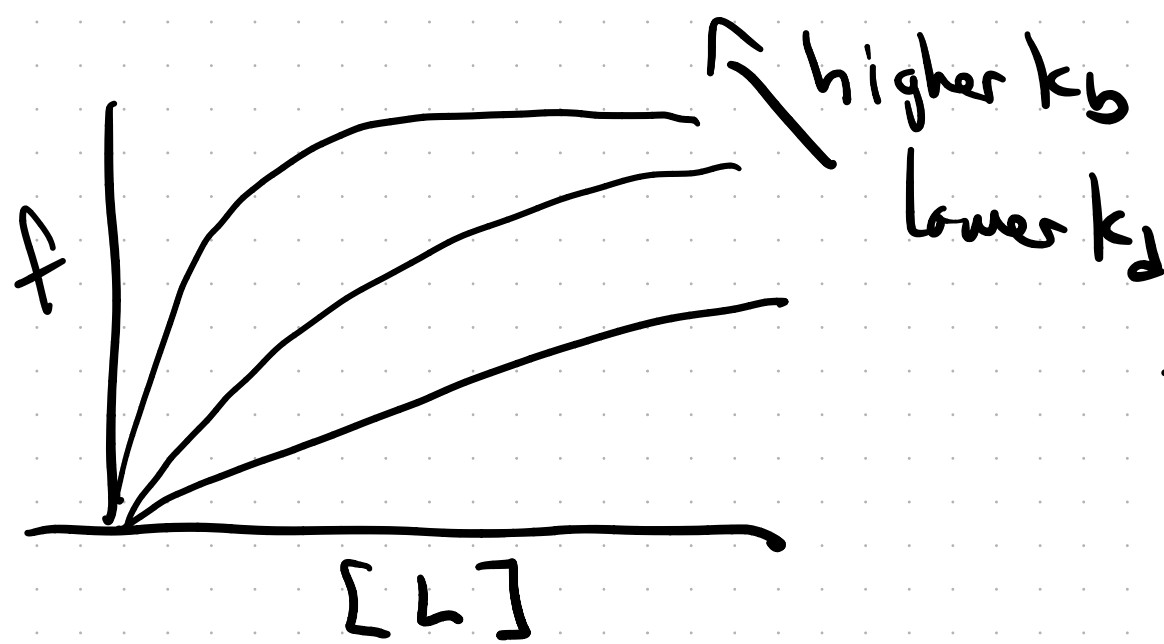
$$\langle f \rangle = \frac{K_b[L]}{1 + K_b[L]}$$

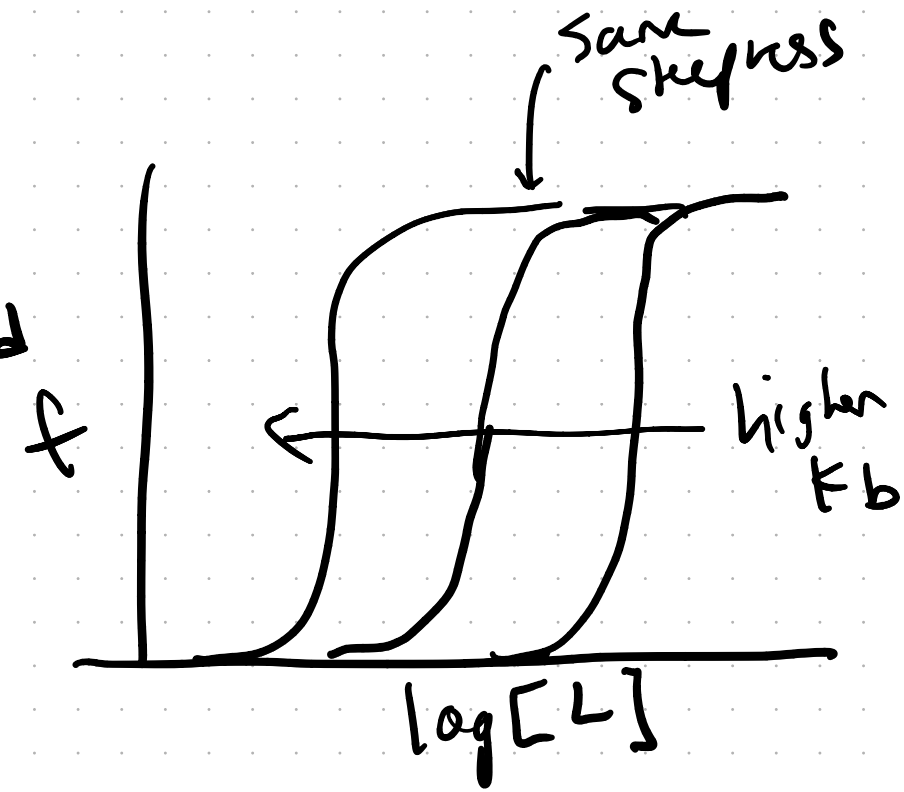
$L + M \rightleftharpoons ML$

Unbound $\rightleftharpoons$ bound

$$\langle f \rangle_{2S} = \frac{K_{eq}}{1 + K_{eq}} = \frac{e^{-\beta\Delta\epsilon}}{1 + e^{-\beta\Delta\epsilon}}$$

$$K = \frac{[\text{bound}]}{[\text{unbound}]}$$





Binding Capacity $\longleftrightarrow$ Heat Capacity

amount bound complex changes for increasing ligand

$$C_L = \frac{df}{d\log 10[L]} = 2.303\,\frac{df}{d\ln[L]}$$

$$\log_{10} x = \frac{\ln x}{\ln 10}$$

$$C_L = 2.303 \frac{df}{d\ln[L]}$$

$$\frac{d\ln[x]}{dx} = \frac{1}{[x]}$$

$$d\ln[x] = \frac{d[x]}{[x]}$$

$$= 2.303 \cdot [L] \frac{df}{d[L]}$$

$$f = \frac{K_b[L]}{1 + K_b[L]}$$

$$= 2.303[L] \cdot \left[\frac{(1+K_b[L])K_b - K_b[L]K_b}{(1+K_b[L])^2} \right]$$

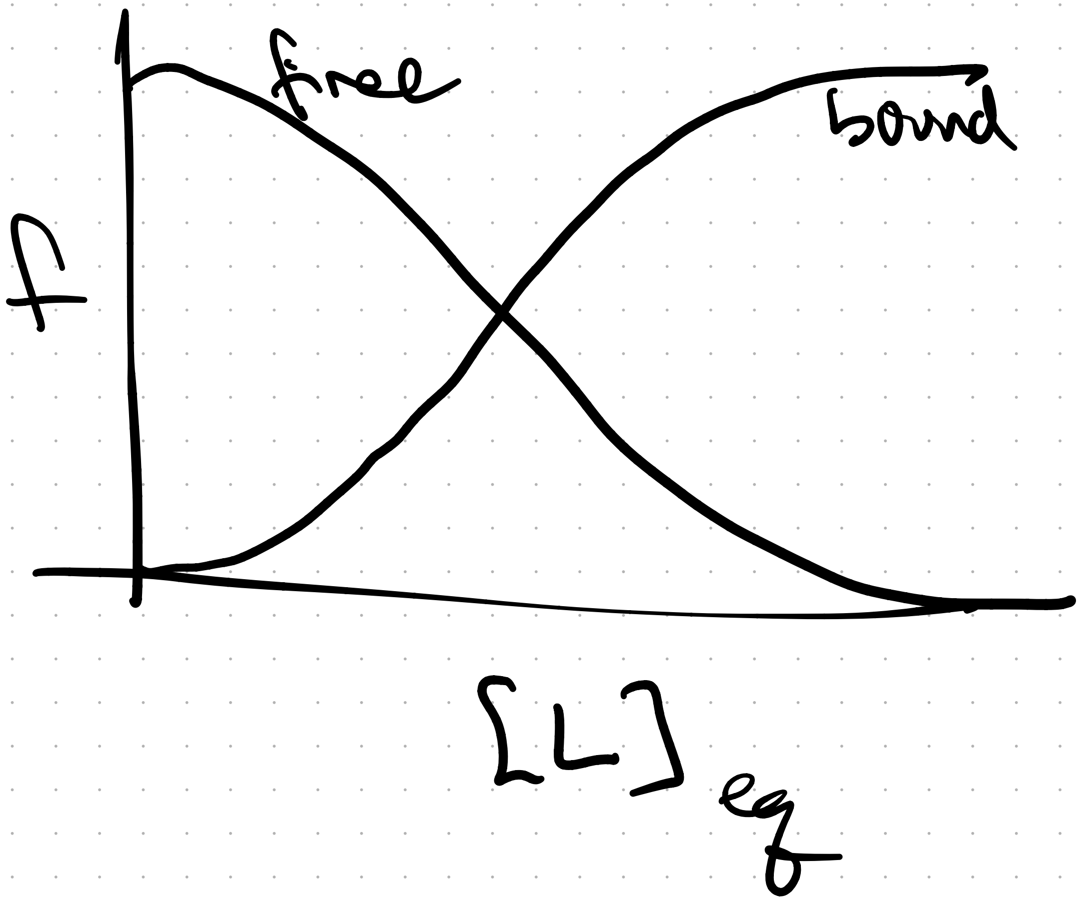
$$= 2.303[L] \cdot \left[\frac{(1+K_b[L])K_b - K_b[L]K_b}{(1+K_b[L])^2} \right]$$

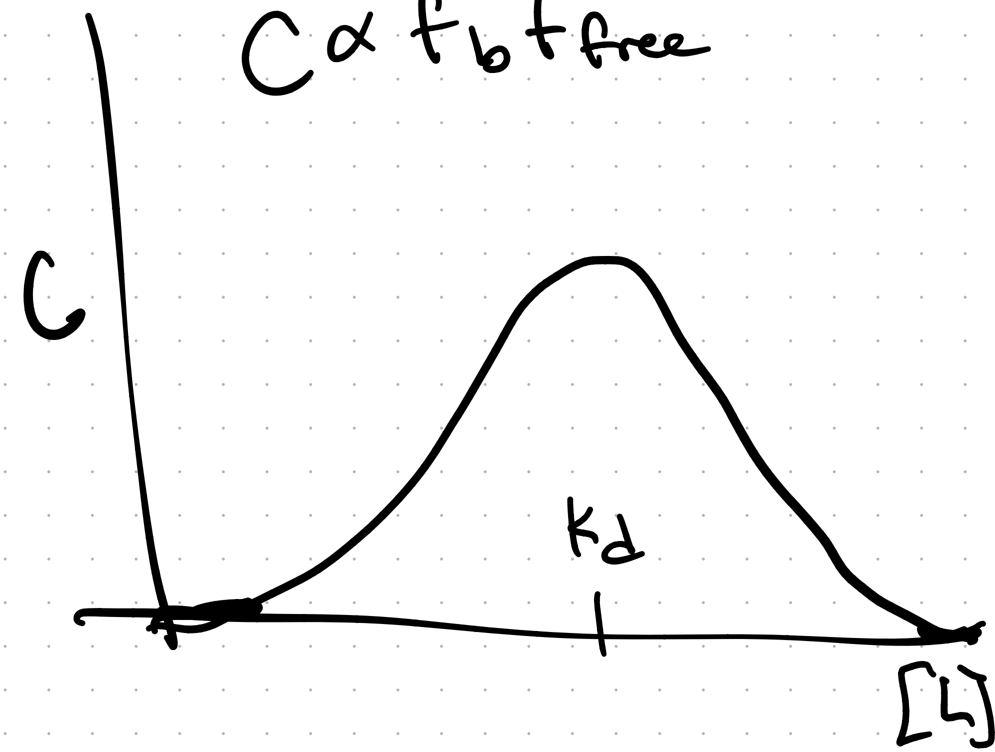
$$= 2.303 \frac{[L]K_b}{(1+K_b[L])^2}$$

$$f_b = \frac{[L]K_b}{1+K_b[L]}$$

$$f_u = \frac{1}{1+K_b[L]}$$

$$= 2.303 f_b f_{free}$$





Practical Issues: 409–411

$$K_b = \frac{[ML]}{[M][L]}$$

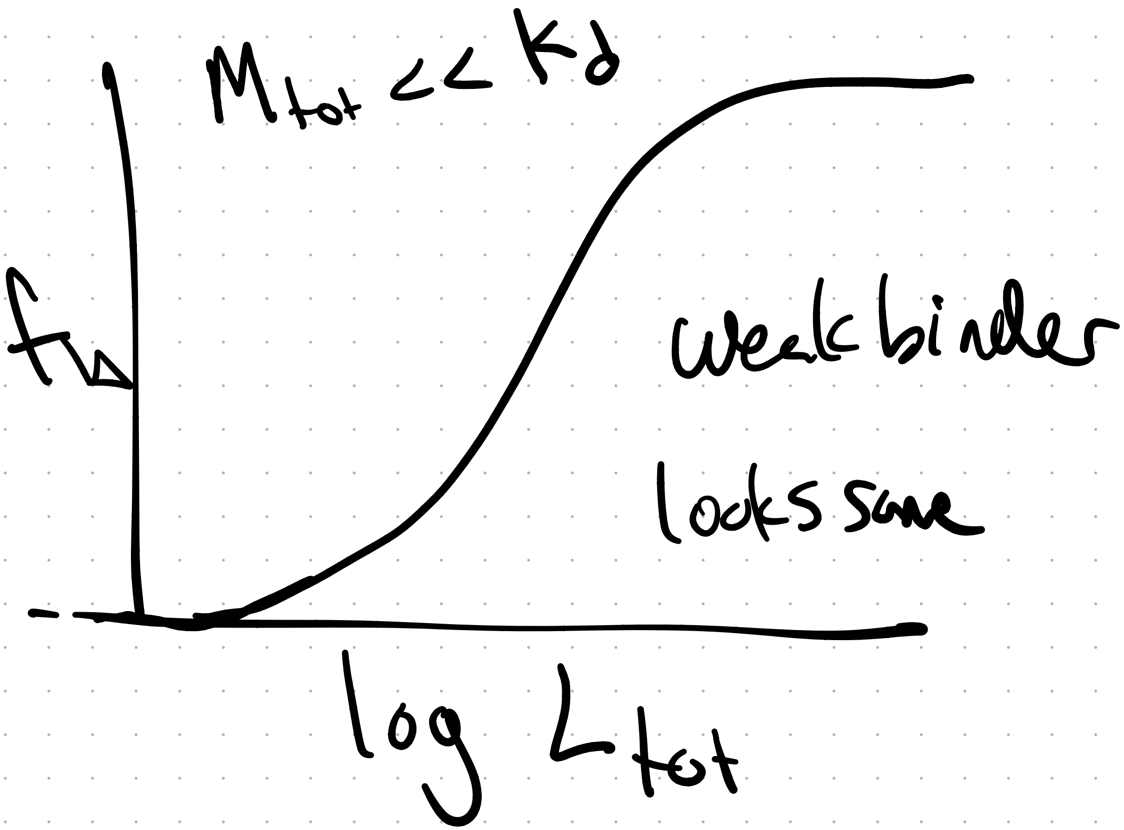
$$[M_{total}] = [M] + [ML]$$

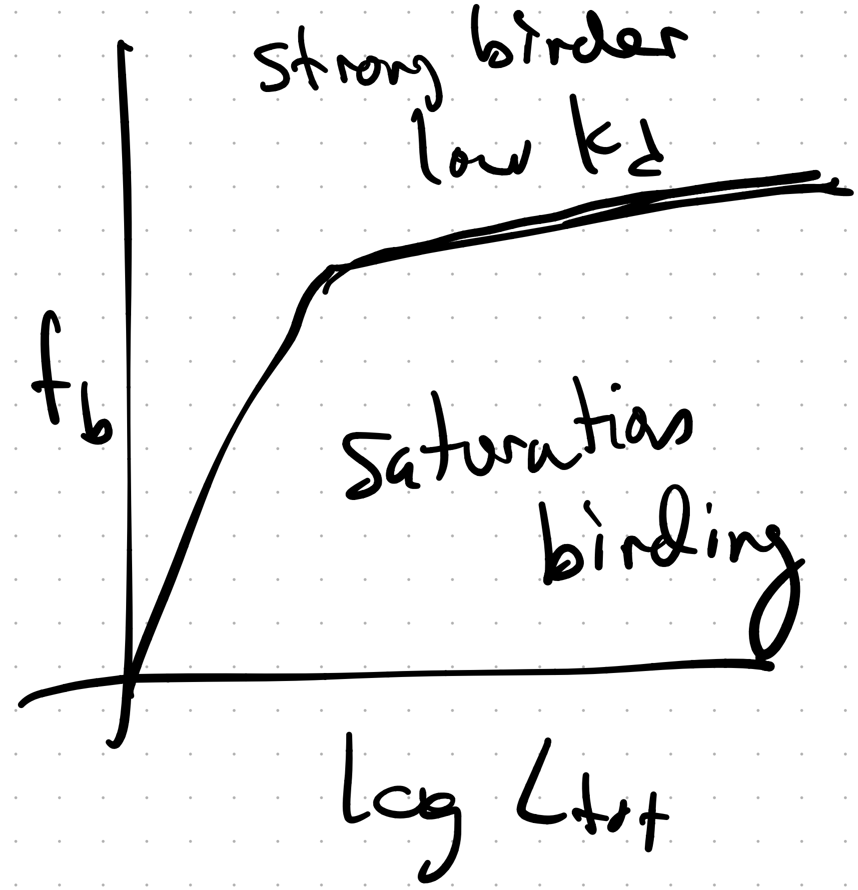
$$[L_{total}] = [L] + [ML]$$

$$K_b = \frac{[ML]}{([L_{tot}] - [ML])([M_{tot}] - [ML])}$$

Solve this for [ML]

$$f_b = \frac{[ML]}{[M_{tot}]}$$


$M_{tot} << K_d$
f_b
weak binder
looks same
log L_{tot}


strong binder
low K_d
f_b
saturation binding
log L_{tot}

Bind multiple ligands

Hemoglobin – binds $4 O_2$
cooperatively

$$M + L \underset{K_1}{\rightleftharpoons} ML + L \underset{K_2}{\rightleftharpoons} ML_2 + L \underset{K_3}{\rightleftharpoons}$$

$$K_1 = \frac{[ML]}{[M][L]} \quad K_2 = \frac{[ML_2]}{[ML][2]} \quad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$M + nL \rightleftarrows ML_n$$

$$\beta_n = \frac{[ML_n]}{[M][L]^n}$$

$$K_1 = \frac{[ML]}{[M][L]} \quad K_2 = \frac{[ML_2]}{[ML][L]} \quad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$\beta_n = K_1 \cdot K_2 \cdot K_3 \cdot \cdots K_n$$

$$\Delta G_n = -k_B T \ln \beta_n$$

K units $[M]^{-1}$

β_n units of $[M]^{-n}$

Cooperativity

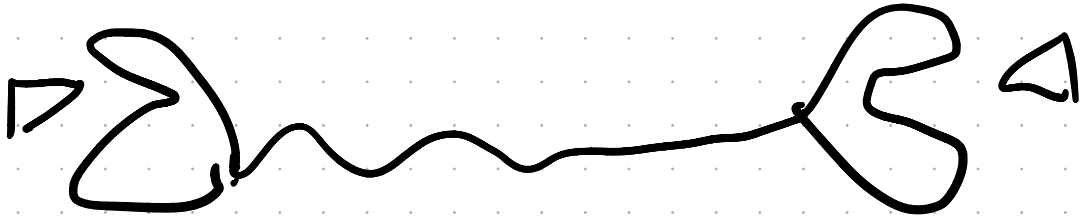
K increases, step positive cooperativity

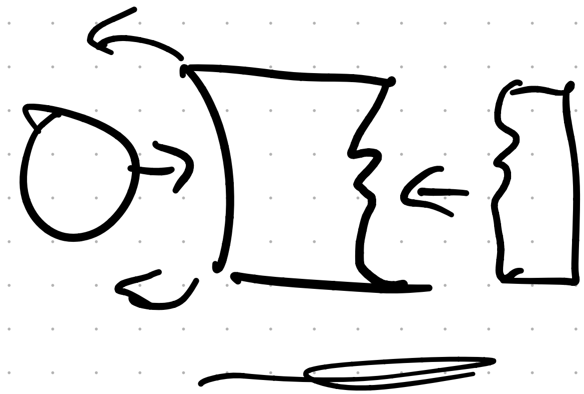
K_1 vs K_2

K decreases, step has negative cooperativity

K_1 vs K_2

no coop





What fraction of binding sites are occupied, s sites

$$f_b = \sum_{i=0}^{s} \left(\frac{i}{s}\right) P_i = \frac{1}{s} \sum_{i=0}^{s} i \, \frac{[ML_i]}{\sum_{i=0}^{s} [ML_i]}$$

$$f_b = \frac{1}{S} \cdot \frac{1 \cdot [ML] + 2[ML_2] + 3[ML_3] + \cdots}{1 + [ML] + [ML_2] + [ML_3] + \cdots}$$

$$f_b = \frac{1}{S} \frac{\sum_{i=0}^{S} i [ML_i]}{\sum_{i=0}^{S} [ML_i]}$$

$$\beta_i = \frac{[ML_i]}{[M][L]^i}$$

$$[ML_i] = [M][L]^i$$

$$= \frac{1}{S} \frac{\sum i \beta_i [L]^i}{\sum \beta_i [L]^i} \leftarrow P$$

$$\mu = \mu_0 + k_B T \ln[L]$$

$$P = \sum_{i=0}^{S} \beta_i [L]^i \qquad \beta_0 = 1 \qquad \beta_i = e^{-\Delta G^\circ / RT}$$

$$P = \sum_{i=0}^{s} \beta_i [L]^i$$

$$f_b = \frac{1}{sP} \cdot \sum_{i=1}^{s} i \, \beta_i [L]^i$$

$$\frac{\partial P}{\partial [L]} = \left(\sum_{i=0}^{s} i \, \beta_i [L]^{i-1} \right) \frac{[L]}{[L]}$$

$$\frac{\partial P}{\partial [L]} = \frac{1}{[L]} \sum_{i=0}^{s} i \, \beta_i [L]^i$$

$$\frac{\partial P}{\partial [L]} = \frac{1}{[L]} \sum_{i=0}^{S} i \beta_i [L]^i$$

$$\frac{[L] \frac{\partial P}{\partial [L]}}{SP} = \frac{\sum_{i=0}^{S} i \beta_i [L]^i}{SP} = f_b$$

$$f_b = \frac{[L]}{SP} \frac{dP}{d[L]} = \frac{[L]}{S} \frac{d \ln(P)}{d[L]} = \boxed{\frac{1}{S} \frac{d \ln(P)}{d \ln[L]}}$$

$$\text{Population of } ML_i = \frac{\beta_i [L]^i}{P}$$

Binding Capacity

$$C_L = 2.303 \frac{\partial f}{\partial \ln[L]} = \frac{2.303}{SP}\left[\frac{P''}{} - \frac{P'^2}{P}\right]$$

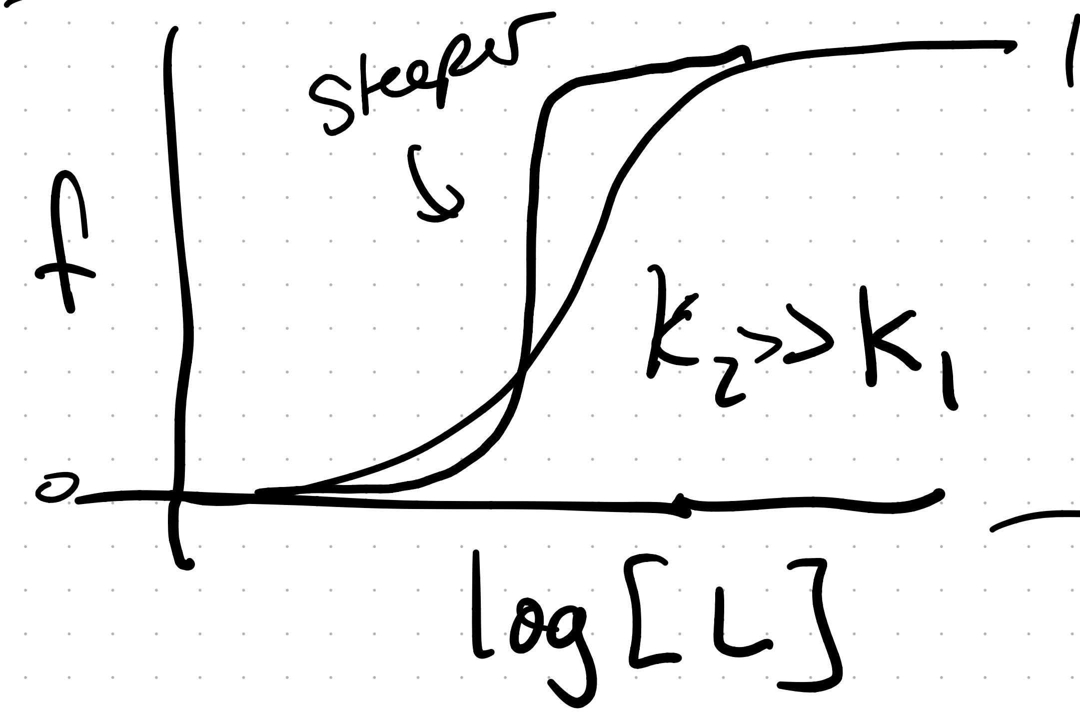
2 sites

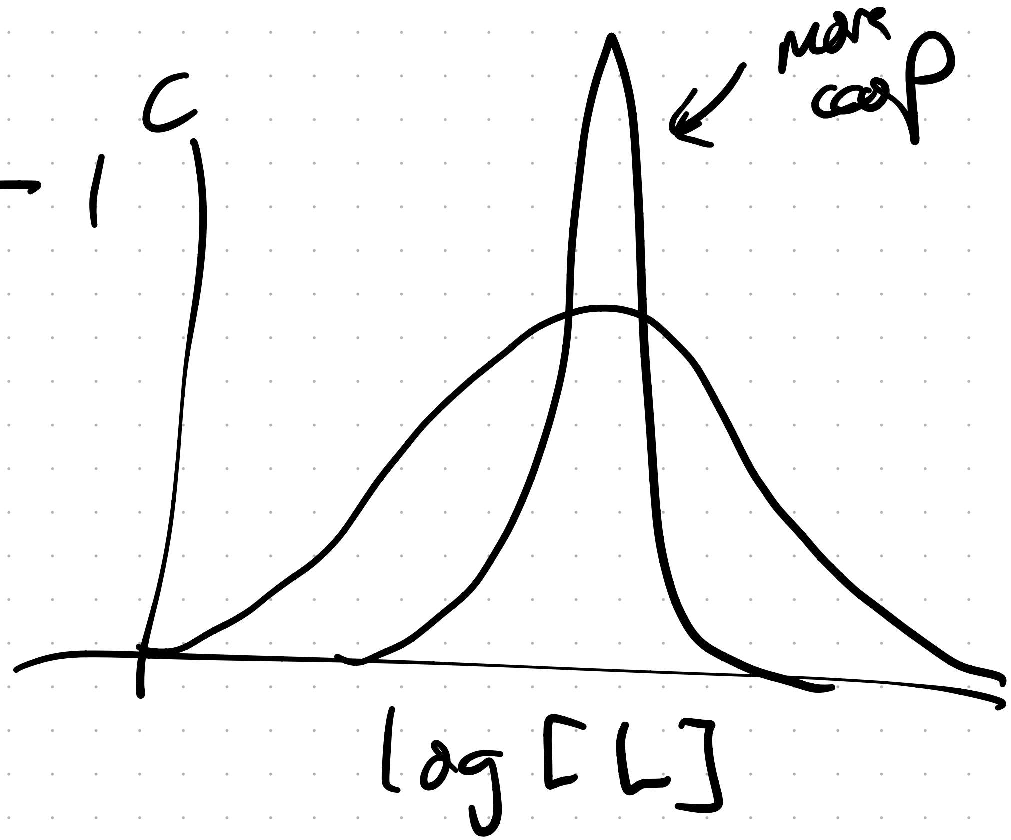
$$\frac{\partial}{\partial \ln[L]}$$

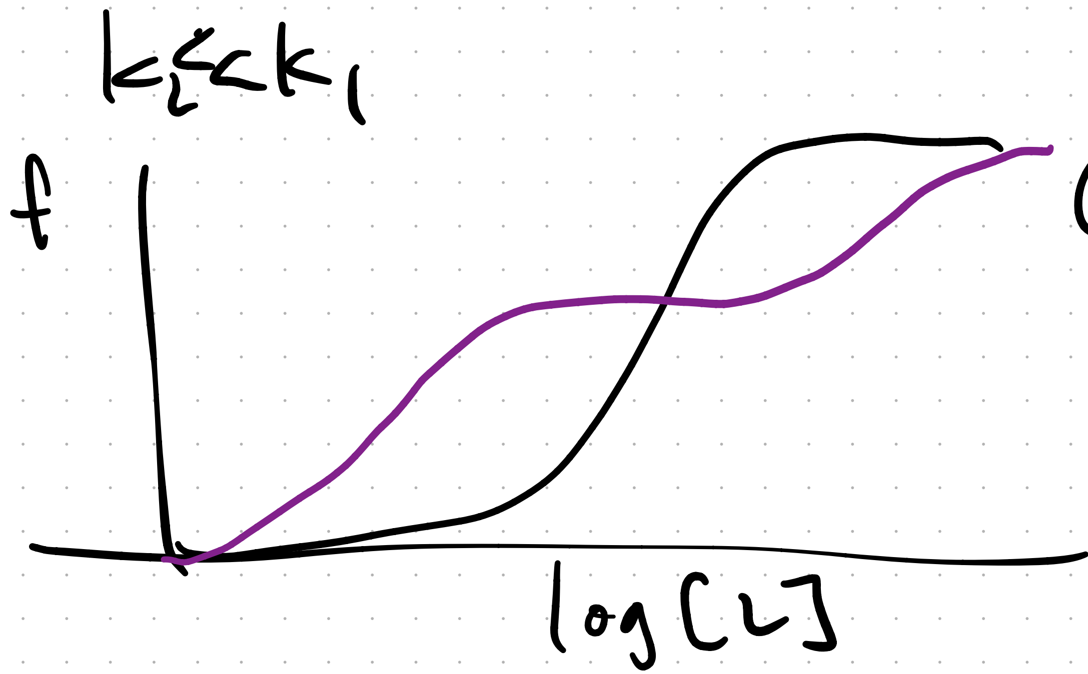
$$P = \sum_{i=0}^{S} \beta_i [L]^i$$

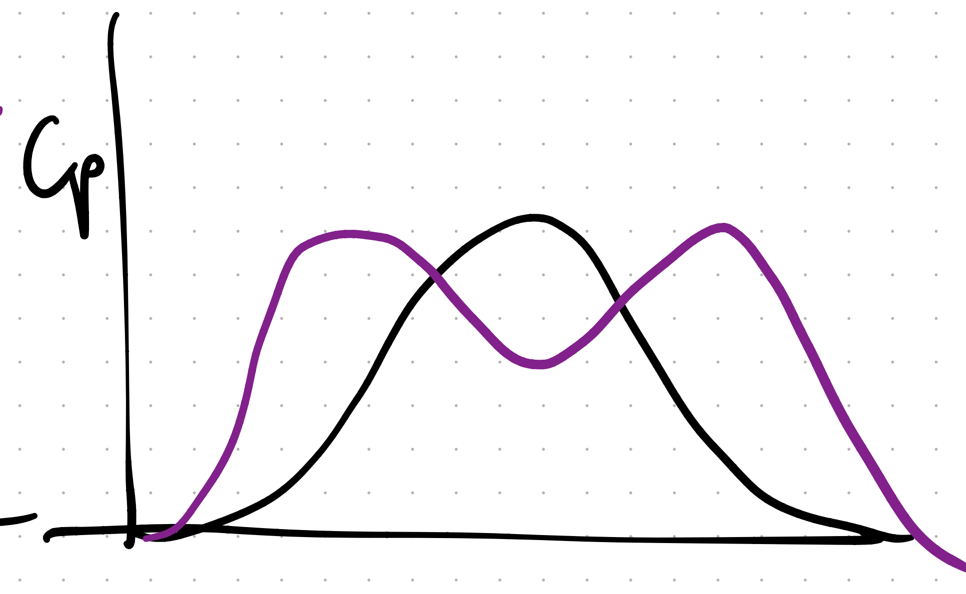
$$= 1 + K_1[L] + K_1 K_2 [L]^2$$

Eg 2 ligand coop



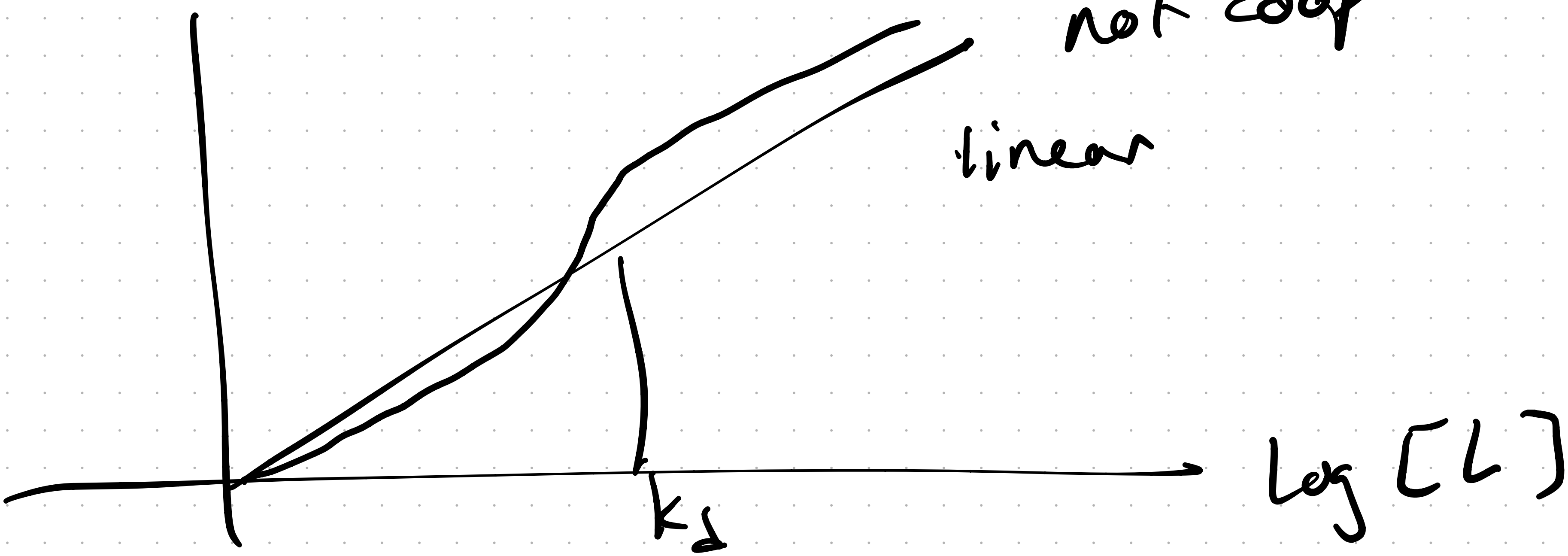






Hill plot / coeff

$\log(f_b/f_u)$ vs $\log[L]$



coop, pos slope, value of $n>1$

neg coop , , value of $n<1$

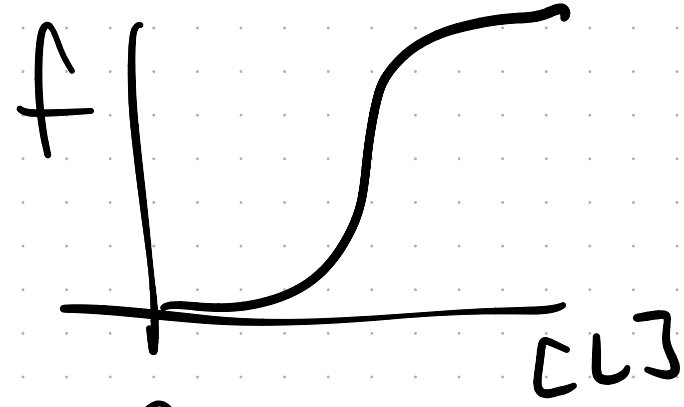
$$M + n_H L \rightleftharpoons ML_{n_H}$$

$$f_b = \frac{\beta_{n_H}[L]^{n_H}}{1 + \beta[L]^{n_H}} \qquad f_u = \frac{1}{1+\beta[L]^{n_H}}$$

$$\ln\left(f_b / f_u\right) = \ln\left(\beta_n [L]^{n_H}\right)$$

$$= \underline{n_H} \ln[L] + \ln \beta_N$$

$$f_b = \frac{\beta_{n_H}[L]^{n_H}}{1 + \beta[L]^{n_1}}$$



$$= \frac{1}{1 + \frac{1}{\beta[L]^{n_H}}} = \frac{1}{1 + \left(\frac{K_d}{[L]}\right)^{n_H}}$$

$$K^n = \beta_n$$