Ligand Binding

Ligand binding is very important (think drug design) and these are some of the most common kinds of expts we do – titrate in a ligand & see how much bound

Analogous to adsorbing on surface

see exam

Have enough time to cover key parts

Chemical Eq:

$$M + L \rightleftharpoons ML$$

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

bind

very common to see K_d (dissociation)

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

low K_d is high affinity!

pM → mM

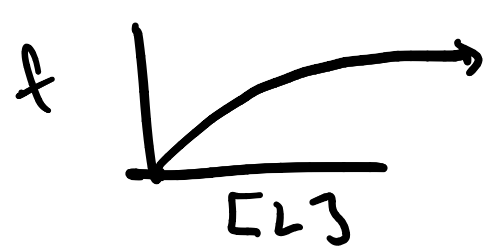
~ mM protein protein interactions

what fraction is bound? @ Eq

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

$$= \frac{K_B[L]}{1 + K_B[L]} = \frac{1}{1 + K_d/[L]}$$

so K_d is $[L]$ at which half bound! ☆

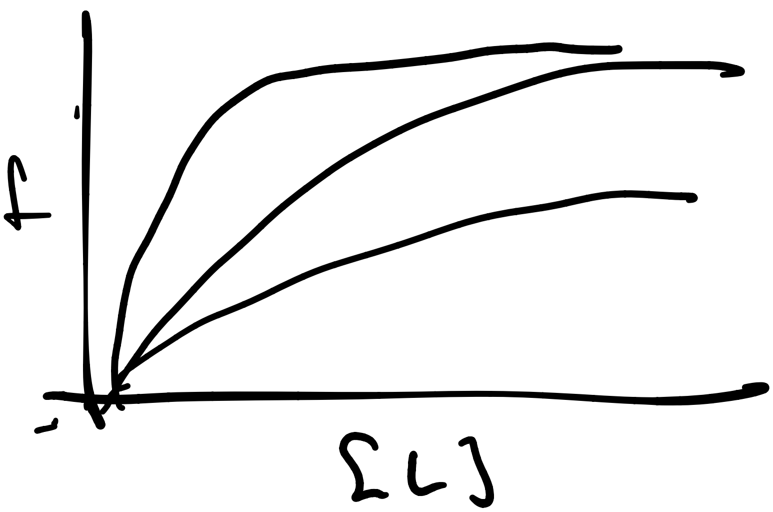
 for simple case

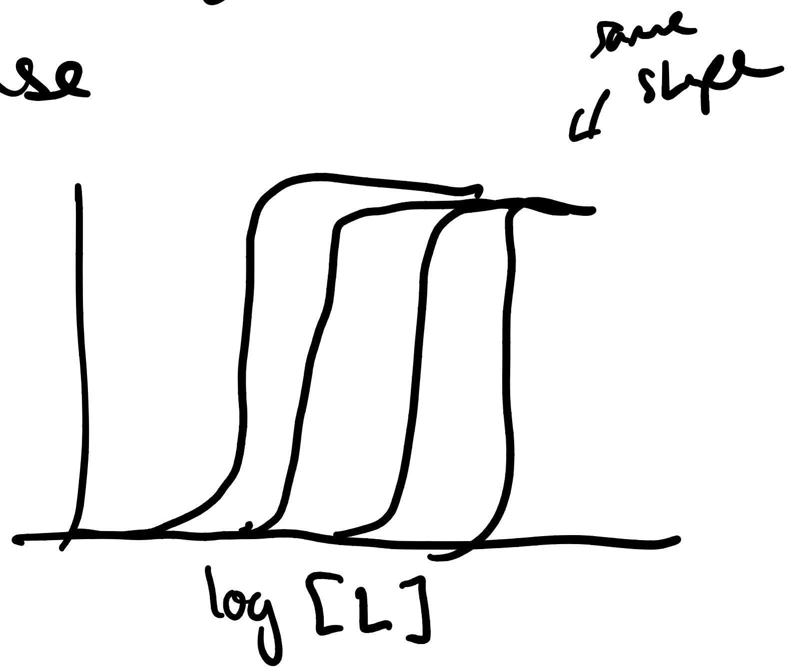
Like 2 state model

Bound / unbound $f = \frac{K}{1+K}$

here "K" = $K_b[L]$

lower K_d / higher K_b has sharper response





Binding capacity is like heat capacity - how much does frac bound change with concentration

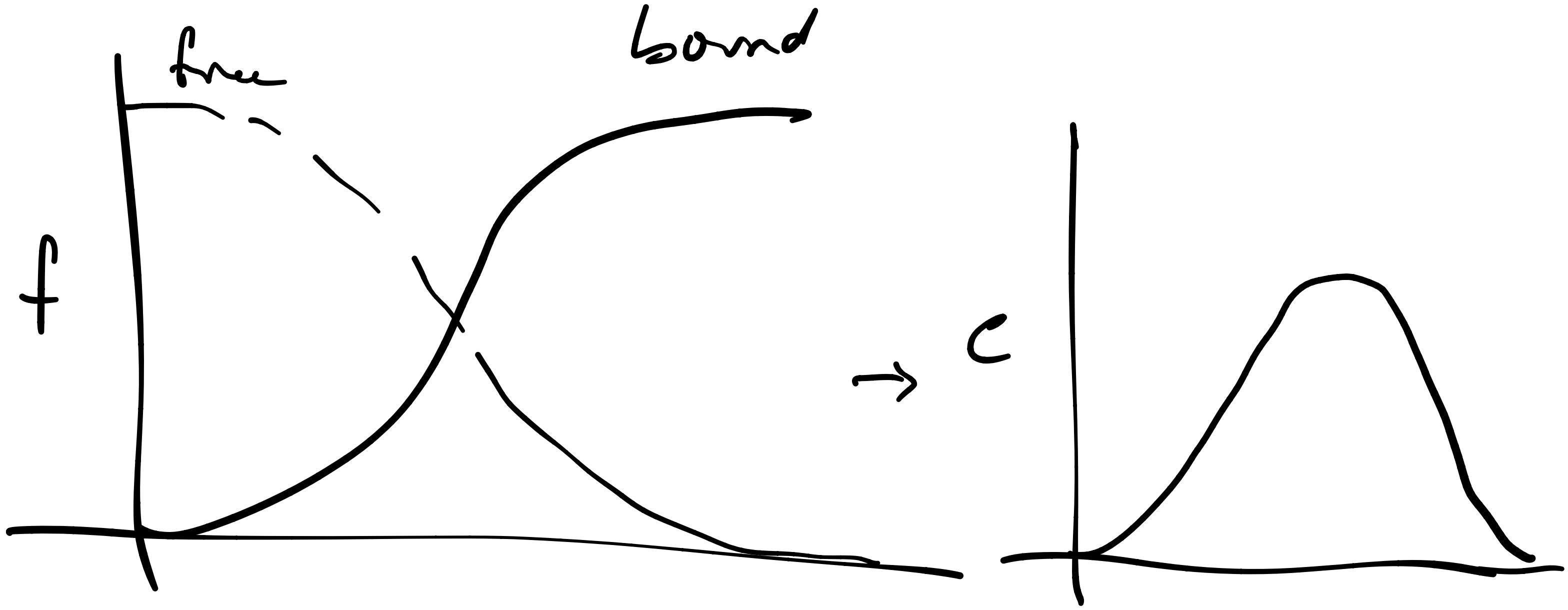
$$C_L \equiv \frac{df_{bound}}{d\log_{10}(L)} = 2.303 \frac{df}{d\ln[L]}$$

$$= 2.303\,[L] \cdot \frac{df}{d[L]} \quad , \quad f = \frac{K_b[L]}{1+K_b[L]}$$

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

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

$$= 2.303 \cdot f_b \cdot f_{free} = \frac{1}{1+k[L]}$$



like max in heat capacity curve for melting transition!

Several practical issues w/ measuring on 409-411

① One to discuss [L] is free ligand conc at eq but actually control

$$[L]_{tot} = [L] + [ML]$$

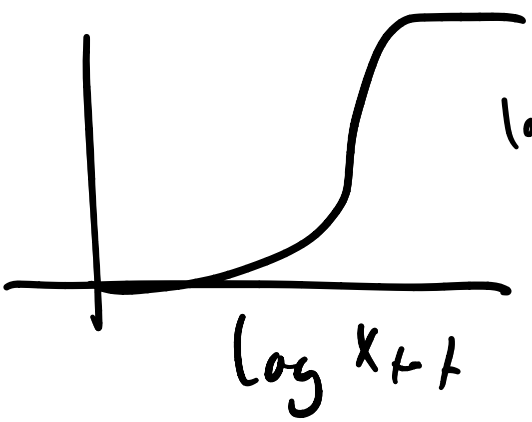
$$[M]_{tot} = [M] + [ML]$$

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

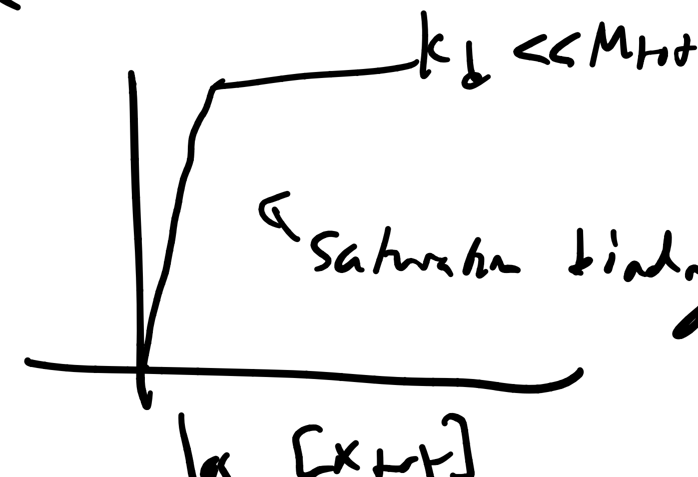
Can solve for $[ML]$ in terms of known concentrations

$$f = \frac{[ML]}{[M_{tot}]}$$ and fit K_b

When $M_{tot} \ll K_d$ (weak binder, very low conc)



strong binder



Binding multiple ligands

eg Hemoglobin - binds $4\ O_2$

cooperatively

$$M + L \rightleftarrows ML + L \rightleftarrows ML_2 + L \cdots$$

$$K_1 = \frac{[ML]}{[M][L]}$$

Overall reaction to that point

$$\beta_1 = K_1$$

$$K_2 = \frac{[ML_2]}{[ML][L]}$$

$$\beta_2 = K_1 K_2 = \frac{[ML_2]}{[M][L]^2}$$

$\vdots$

etc

doesn't have to be stepwise, if at eq then $\beta_N = \frac{[ML_N]}{[M][L]^n}$ has

$$\Delta G_N = -k_B T \ln \beta_N$$

- If cooperative / stepwise, not all k's are the same

pos cooperative – k's inverse
neg cooperativity k's decrease

- k's casier to work with, all $[M]^{-1}$, same magnitude. β's get small b/c of units, some math easier

What is frac ligand sites occupied?

s binding sites

$$f_b = \sum f_i P_i$$

$$= \sum_{i=0}^{s} \frac{i}{s} P_i = \frac{1}{s} \sum_{i=0}^{s} i \frac{[ML_i]}{\sum_{i=0}^{n} [ML_i]}$$

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

$$f_b = \frac{1}{s} \frac{\sum i [ML_i]}{\sum [ML_i]} = \frac{1}{s} \frac{\sum i \beta_i [L]^i}{\sum \beta_i [L]^i}$$

but $\beta_i = \frac{[ML_i]}{[M_0][L]^i}$

Denom is "binding polynomial"

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

where $\beta_0 = 1$

like partition function, actually

* grand canonical b/c

$$\mu_i = \mu_i^\circ + k_B T \ln [i]$$

and $\beta_i = e^{-\Delta G^\circ / RT}$

$\oplus$ final surface binding

Can get properties like $\langle f_b \rangle$ from P

$$f_b = \frac{1}{SP} \cdot \sum_{i=1}^{S} i\, \beta_i [x]^i$$

$$= \frac{1}{SP} [x] \frac{\partial}{\partial [x]} P$$

useful

$$= \frac{1}{S} \frac{[x]}{P} \frac{\partial P}{\partial [x]} = \frac{[x]}{S} \frac{\partial \ln P}{\partial [x]}$$

$$= \frac{1}{S} \frac{\partial \ln P}{\partial \ln [x]}$$

← easy to remember

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

Binding capacity

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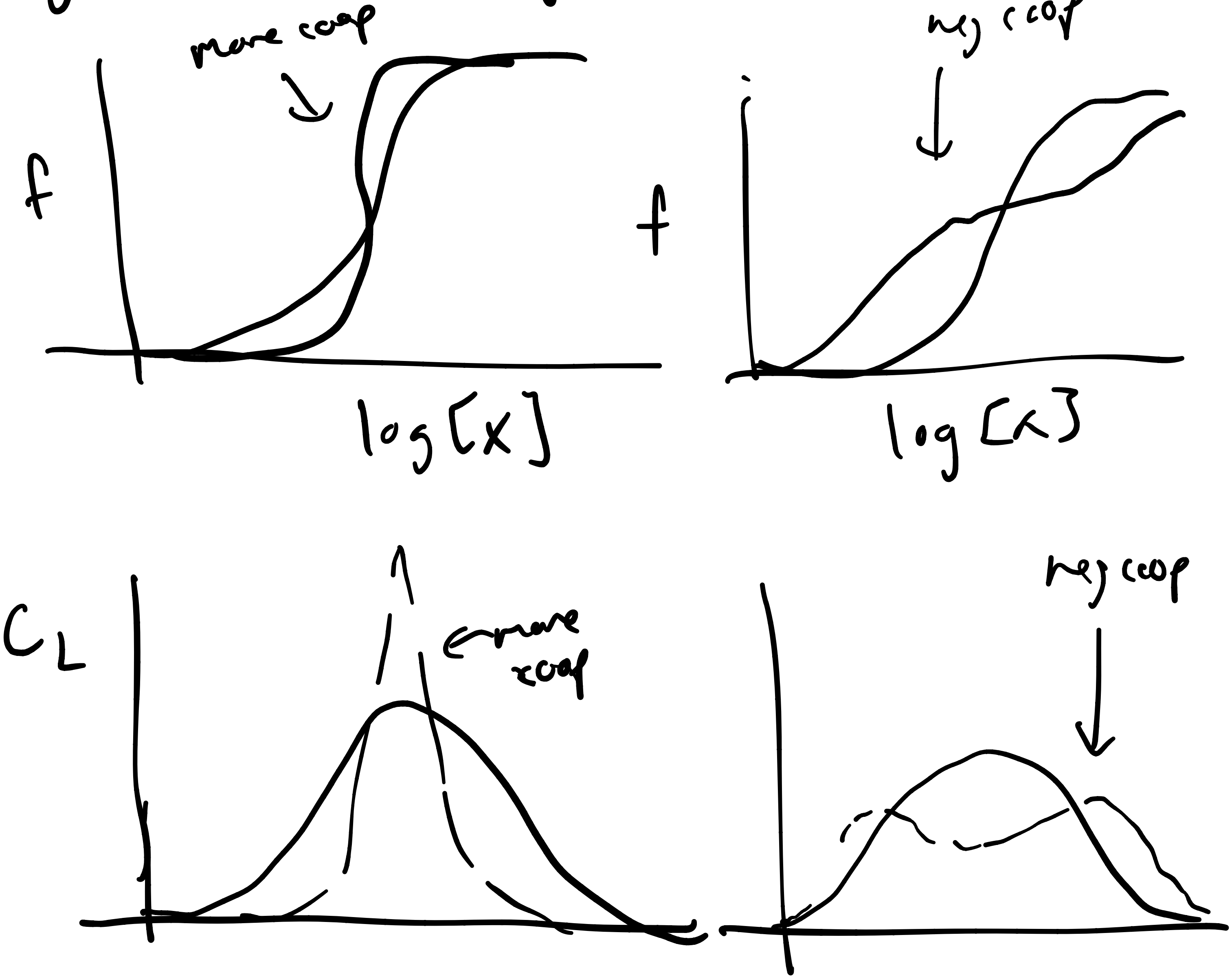
$\frac{\partial}{\partial \ln[x]}$

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

Eg 2 ligands Coop



Hill plot / ~~coeff~~

$\log \frac{f_b}{f_u}$ vs $\log [L]$

~~eg~~ no coop $\quad f_b = \frac{[L] K}{1 + [L] K} \quad f_u = \frac{1}{1 + [L]/K}$

$\log \frac{f_b}{f_u} = \log [L] K = \log K + \log [L]$

linear!

if pos coop, slope up

If neg coop, slope down

at 50%

$n_H = \frac{\partial \log f_b / f_u}{\partial [\log [X]]}$ > 1 if pos

< 1 if neg

effect num coop bind

Can show

$$C_L = 2.303 \langle f_b \rangle \langle f_u \rangle n_H$$

Steepness of transition

Last: simple model, 1 step coop

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

$$K = \beta_N = \frac{[ML_N]}{[M][L]^N}$$

$$f_b = \frac{\beta [L]^n}{1 + \beta [L]^n}$$

$$f = \frac{[ML_N]}{[M] + [ML_N]}$$

$$f_u = \frac{1}{1 + \beta [L]^n}$$

$$\ln f_b/f_u = \ln \beta [L]^n$$

$$\frac{\partial \ln f_b/f_u}{\partial \log [L]} = n$$

fit:

$$f = \frac{1}{1 + \left(\frac{K_A}{L}\right)^{n_H}}$$

$\beta = K^{n_H}$

common

final - Hill langmuir