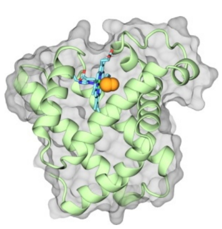
Selected Applications of Chemical Equilibrium to Biochemistry: Ligand Binding, Bioenergetics

Chang, Sections 6.4-6.5

Biological Standard State

Discussed on whiteboard

Binding of ligands and metal ions to macromolecules: A. One binding site per macromolecule



Myoglobin: 1 binding site for O_2

Binding equilibrium

Association

$$\mathrm{P + L \rightarrow PL} \qquad K_a = \frac{a_{\mathrm{PL}}}{a_{\mathrm{P}} a_{\mathrm{L}}} \cong \frac{[\mathrm{PL}]}{[\mathrm{P}][\mathrm{L}]}$$

Dissociation

$$\mathrm{PL \rightarrow P + L} \qquad K_d \cong \frac{[\mathrm{P}][\mathrm{L}]}{[\mathrm{PL}]}$$

↑ Dissociation Constant

Determining K_d

Numerator: concentration of L bound to P

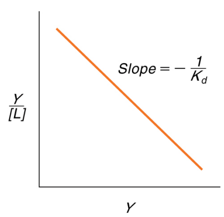
measurable

Denominator: concentration of all forms of P

Define: $Y \equiv \frac{[\mathrm{PL}]}{[\mathrm{P}] + [\mathrm{PL}]}$

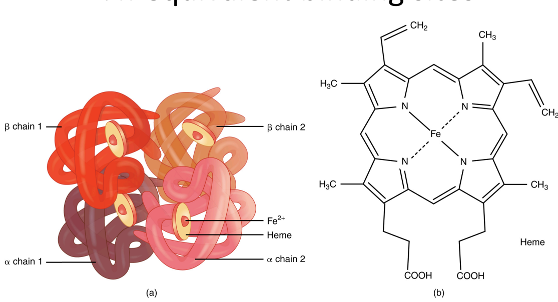
↓ algebra

$$\frac{Y}{[\mathrm{L}]} = \frac{1}{K_d} - \frac{Y}{K_d}$$



Obtain K_d by plotting Y/[L] vs Y: slope = -1/K_d

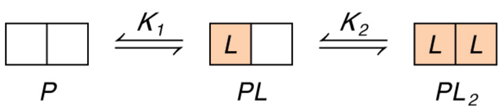
B. *n* equivalent binding sites



Hemoglobin: 4 binding sites for O_2

Caveat: Hemoglobin's bind sites not really equivalent

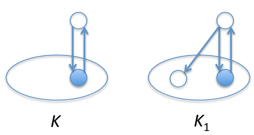
2 equivalent sites



$$\mathrm{P} + \mathrm{L} \rightarrow \mathrm{PL} \qquad K_1 = \frac{[\mathrm{P}][\mathrm{L}]}{[\mathrm{PL}]}$$

$$\mathrm{PL} + \mathrm{L} \rightarrow \mathrm{PL}_2 \qquad K_2 = \frac{[\mathrm{PL}][\mathrm{L}]}{[\mathrm{PL}_2]}$$

Express K_1 in terms of K



K = dissociation constant from any one site
$K = 2K_1$

Y in terms of [L] and K

$$Y \equiv \frac{[\mathrm{PL}] + 2[\mathrm{PL}_2]}{[\mathrm{P}] + [\mathrm{PL}] + [\mathrm{PL}_2]} = \ldots = \frac{2[\mathrm{L}]}{[\mathrm{L}] + K}$$

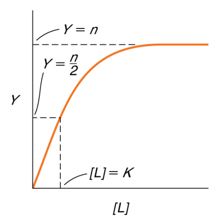
Generalize to *n* equivalent sites

$$K_i = \left(\frac{i}{n-i+1}\right)K$$

$$Y = \frac{n[\mathrm{L}]}{[\mathrm{L}]+K}$$

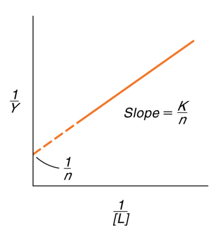
Determining *n* and *K*: Plotting method 1 (Direct plot)

$$Y = \frac{n[\mathrm{L}]}{[\mathrm{L}]+K}$$



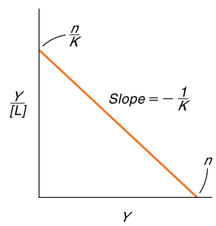
Plotting method 2 (double reciprocal plot)

$$\frac{1}{Y} = \frac{1}{n} + \frac{K}{n[\mathrm{L}]}$$



Plotting method 3 (Scatchard plot)

$$\frac{Y}{[L]} = \frac{n}{K} - \frac{Y}{K}$$

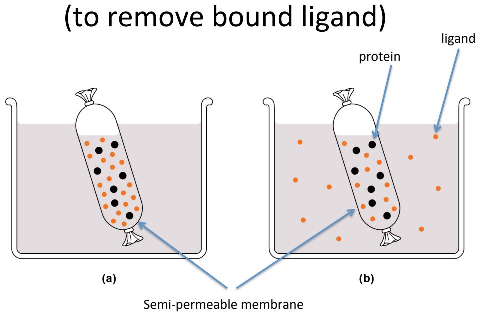


n non-equivalent sites

Possible but harder → for the expert

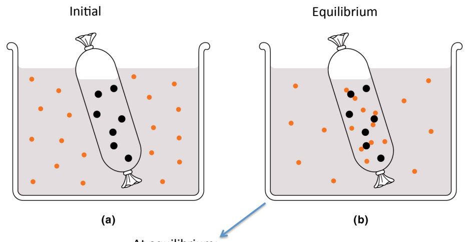
We won't cover

Equilibrium dialysis (to remove bound ligand)



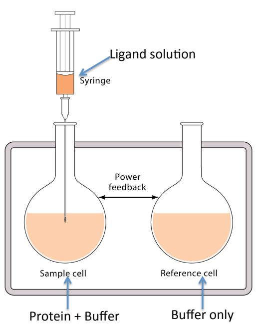
Can remove all ligand with repeated 'rinsings'

Equilibrium dialysis (to determine *n* and *K*)



At equilibrium:
Interior [L] = Exterior [L] (known or measurable)
Total P concentration (all forms) known
→Can determine *Y* → *n*, *K* as before

Isothermal titration calorimetry (ITC)

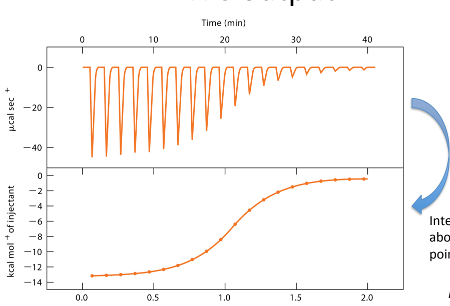


Make repeated injections:
Measure power needed to keep sample at same temperature as buffer

$q = V\Delta_r H^o [L]_{bound}$

$P + nL \rightarrow PL_n$

ITC output



Integrate peak above to get point below

↓ *Curve fitting*

K_a, n, $\Delta_r H^o$

↓ *Get* $\Delta_r G^o$

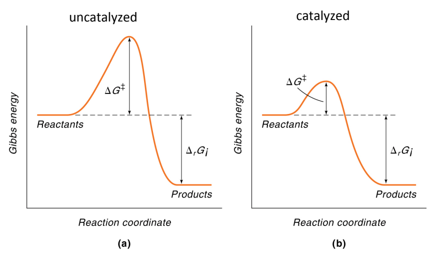
$\Delta_r G^o = -RT K_a$ → *Get* $\Delta_r S^o$ → $\Delta_r G^o = \Delta_r H^o - T\Delta_r S^o$

Bioenergetics

OVERVIEW

- Reactions must be exergonic ($\Delta_r G < 0$) to be spontaneous.
- Building a protein (and many other biomolecules) is highly endergonic.
- Must be coupled with other reactions that are highly exergonic, to give a net reaction that is also exergonic.
- Chemical reactions in biological systems are made efficient (rates are increased) by catalysis.

Catalysis



- Reaction barrier ($\Delta G^{\ddagger}$)reduced → Rate increased
- Gibbs energy of reaction ($\Delta_r G$) not affected → Equilibrium concentrations unchanged

Coupled reactions

1. Outline effect of stoichiometry and summer reactions on board.

2. Question: How make the following reaction – extraction of copper from ore - "go"?

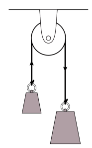
$Cu_2S(s) \rightarrow 2Cu(s) + S(s) \quad \Delta_r G^o = 86.2$ kJ/mol

Couple reactions (cont'd)

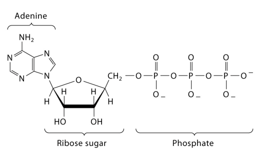
Answer: Couple to an exergonic reaction.

$Cu_2S(s) \rightarrow 2Cu(s) + S(s)$ $\Delta_r G^o$ = 86.2 kJ/mol
$S(s) + O_2(g) \rightarrow SO_2(g)$ $\Delta_r G^o$ = -300.1 ..

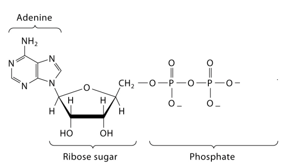
$Cu_2S(s) + O_2(g) \rightarrow SO_2(g)$ $\Delta_r G^o$ = -213.9 ..



ATP, ADP (adenosine triphosphate)



ATP



ADP

ATP → ADP

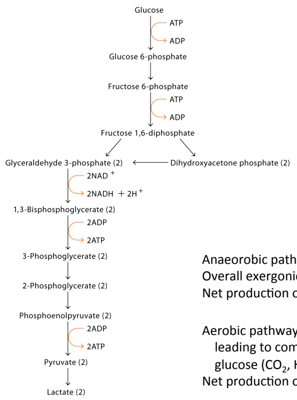
$ATP^{4-} + H_2O \rightarrow ADP^{3-} + H^+ + HPO_4^{2-}$

or (biochemist's shorthand)

$ATP + H_2O \rightarrow ADP + P_i$

$\Delta_r G^{of}$ = -30.5 kJ/mol (pH=7, T=310 K)

Exergonic → Can be coupled with endergonic reactions to drive them



Glycolytic pathway

Anaerobic pathway
Overall exergonic
Net production of 2 moles ATP

Aerobic pathway adds more steps leading to complete combustion products of glucose (CO_2, H_2O)
Net production of 38 moles ATP

Efficiency of glycolytic pathway (aerobic)

Combustion of glucose:

$C_6H_{12}O_6 + 6O_2(g) \rightarrow 6CO_2(g) + 6\ H_2O(l)$ $\quad \Delta_r G^o = -2879$ kJ/mol

$ADP^{3-} + H^+\ HPO_4^{2-} \rightarrow ATP^{4-} + H_2O$ $\quad \Delta_r G^{of} = 30.5$ kJ/mol

Efficiency = (38 x 30.5 / 2879) = 40 %

Energetics of building proteins