Lecture 3 Hemoglobin

1. Law of Mass Action

Can derive formally from the condition for equilibrium $\Delta G = 0$ (p268.)

Include the concentration dependence of the chemical potentials $\mu = \mu_0 + k_B T \ln c/c_0$.

But the result can be seen directly from rate equations:

$$A + B \underset{k-}{\overset{k+}{\rightleftharpoons}} AB$$

Forward rate $= [A][B]k+$ } equal in eqm.
Reverse rate $= [AB]k-$

$$[A][B]k+ = [AB]k-$$

$$\Rightarrow \frac{[AB]}{[A]\cdot[B]} = \frac{k+}{k-} = K_{eq}. \quad \text{equilibrium constant.}$$

All chemical reactions can be characterised by an equilibrium constant. Units depend on the "order" of the reaction, eg valencies of the reacting species. Here $[K_{eq}] = \text{conc}^{-1}$.

2. Ligand - Receptor binding.

We already saw the "Langmuir isotherm" for binding probability of a ligand to receptor concentrations: $[L]$ $[R]$

$$L + R \rightleftharpoons LR.$$

$$\frac{[L][R]}{[LR]} = K_d \quad \text{equilib. const. (units = conc)}$$

$$P_{bound} = \frac{[LR]}{[LR]+[R]} = \frac{[LR]/[R]}{1+[LR]/[R]} = \frac{[L]/K_d}{1+[L]/K_d}.$$

This is the same as the result before:

$$P_{bound} = \frac{(c/c_0)\, e^{-\beta\Delta\varepsilon}}{1 + (c/c_0)\, e^{-\beta\Delta\varepsilon}} \quad \text{where } c = [L]$$

Comparing: $K_d = c_0\, e^{+\beta\Delta\varepsilon}$.

Where c_0 = concentration of lattice sites in solution

$\Delta\varepsilon$ = binding energy $= \varepsilon_b - \varepsilon_{sol.}$ [2.10]

Two results allow conversion back/forth from microscopic state params to macro concentrations.

3. Entropy of binding.

Intuitively, there is no entropy associated with $P_{bound} = 0$ or $P_{bound} = 1$, fully specified.

Use Shannon Entropy to look in between:

$$S = -\sum_i p_i \ln p_i = \text{Missing Information}$$

Here there are 2 states bound & unbound.

$$S = -P_{bound} \ln P_{bound} - P_{unbound} \ln P_{unbound}.$$

with $P_{bound} + P_{unbound} = 1$

$$P_{bound} = \frac{[L]/K_d}{1 + [L]/K_d} = \frac{\alpha}{1+\alpha} \qquad P_{unbound} = \frac{1}{1+\alpha}.$$

$$S = -\frac{\alpha}{1+\alpha} \ln \frac{\alpha}{1+\alpha} - \frac{1}{1+\alpha} \ln \frac{1}{1+\alpha}$$

$$= \frac{-1}{1+\alpha}\left[\alpha \ln \alpha - \alpha \ln(1+\alpha) + \cancel{\ln 1} - \ln(1+\alpha)\right]$$

$$= \frac{+1}{1+\alpha}\left[(1+\alpha)\ln(1+\alpha) - \alpha \ln \alpha\right]$$

$$= \ln(1+\alpha) - \frac{\alpha}{1+\alpha} \ln \alpha.$$

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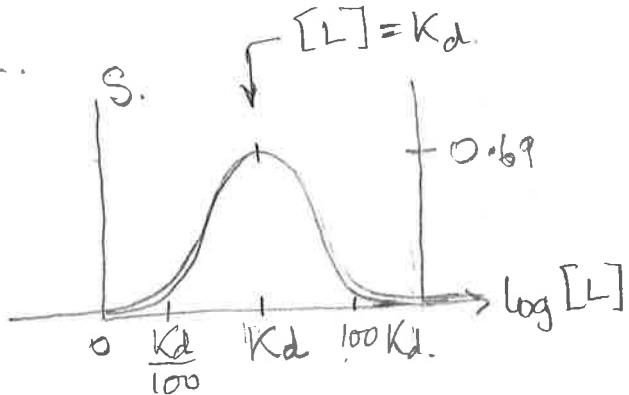
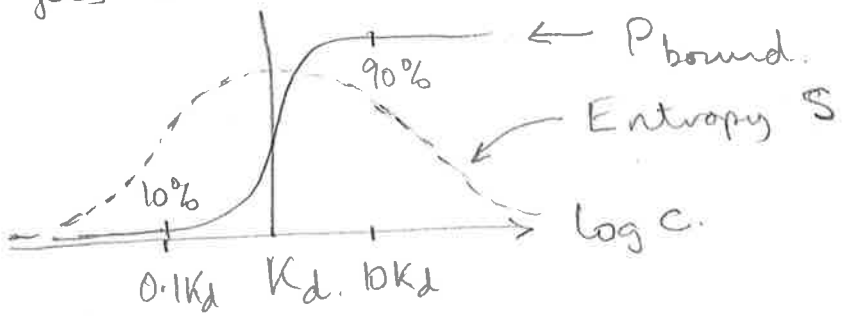
$\alpha \ll 1 \quad S \to \alpha - \alpha \ln \alpha \to 0$

$\alpha \gg 1 \quad S \to \ln \alpha - \ln \alpha \to 0$

$\alpha = 1 \quad S = \ln 2 - 0 \neq 0$



This goes with the "titration curve" we saw before



log c is the same scale as energy or chem potential

$$\mu = \mu_0 + k_B T \ln c/c_0.$$ or pH for $[H^+]$

4. Hill Function

Treat higher order reactions the same way.

When two ligands bind the receptor at the same time:

$$L + L + R \underset{k-}{\overset{k+}{\rightleftharpoons}} L_2R.$$

$$\left.\begin{aligned} \text{Forward rate} &= [L]^2[R]\,k_+ \\ \text{Backward} &= [L_2R]\,k_- \end{aligned}\right\} K_d^2 = \frac{[L]^2[R]}{[L_2R]}.$$

New K_d is squared to keep units of conc.

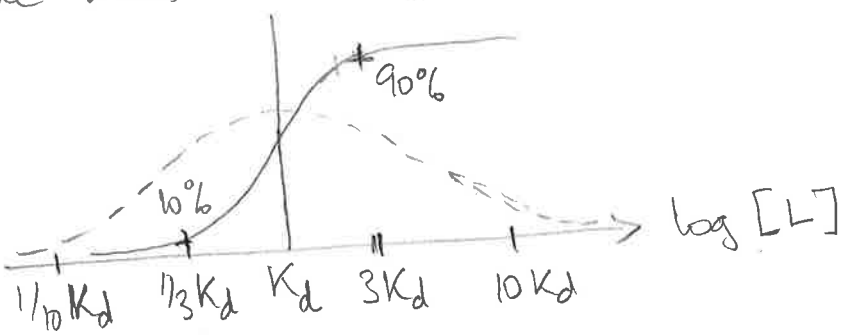
$$P_{bound} = \frac{[L_2R]}{[L_2R]+[R]} = \frac{([L]/K_d)^2}{1+([L]/K_d)^2}$$

Generalise to n+1 th-order reaction [n is a measure of cooperativity]

$$P_{bound} = \frac{([L]/K_d)^n}{1+([L]/K_d)^n}$$ Hill function

Shannon entropy, as before, with $\alpha = ([L]/K_d)^n$.

Both curves get narrower, as seen on log scale most easily:



5. Reaction Free Energy (out of equilib).

$$A + B \rightleftharpoons AB \qquad K_{eq} = \frac{[A][B]}{[AB]}$$

Definition $\mu = \frac{\partial G}{\partial N}$ chemical potential.

$$\Delta G = \mu_A \Delta N_A + \mu_B \Delta N_B + \mu_{AB} \Delta N_{AB}$$

$\Delta N_A = +1$, $\Delta N_B = +1$, $\Delta N_{AB} = -1$ ← one molecule reacts

Recalling that $\mu_A = \mu_{A0} + k_B T \ln [A]/[\text{ref}A]$

$$\Delta G = \mu_{A0} + k_B T \ln \frac{[A]}{[\text{ref}A]} + \mu_{B0} + k_B T \frac{[B]}{[\text{ref}B]} - \mu_{AB0} - k_B T \frac{[AB]}{[\text{ref}AB]}$$

$$= \mu_{A0} + \mu_{B0} - \mu_{AB0} + k_B T \frac{[A][B]}{[AB]} - k_B T \frac{[A_{ref}][B_{ref}]}{[AB_{ref}]}$$

$$= \Delta G_0 + k_B T \frac{[A][B]}{[AB]}$$

where ΔG_0 includes the equilibrium concs of the species: $\Delta G_0 = -k_B T \ln K_{eq}$.

Note: if reaction is in equilibrium, the concentrations obey K_{eq} def and $\Delta G = 0$.

But out of equilibrium reaction generates a driving force

6. Examples

i) ATP drives many energetic processes in cells

$$ADP + P_i \rightleftharpoons ATP.$$

Standard state free energy $\Delta G_0 = -12.5\ k_B T$ /mol.

Typical E. coli numbers:

$[ATP] = 5$ mM

$[ADP] = 0.5$ mM

$= [P_i] = 10$ mM (same as Coca cola)

$$\frac{[ADP][P_i]}{[ATP]} = \frac{0.5 \times 10}{5} \times 10^{-3} = 10^{-3}$$

$$\ln 10^{-3} = -6.9$$

$$\Delta G = (-12.5 - 6.9)\ k_B T = -19.4\ k_B T \text{ in E. coli}$$

ii) Expts to determine K_{eq}.

Measure some physical property such as light absorption spectrum:

A Myoglobin changes colour when O_2 binds.

B. HIV protein to CD4 receptor in solution.

C. Transcription factor NtrC to DNA.

Methods:

slide A. Affinity chromatography.

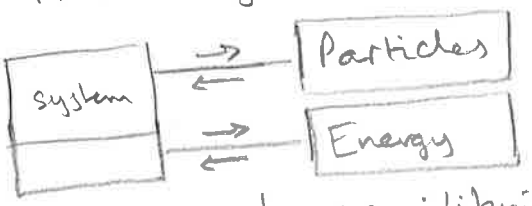
B. Dialysis to equilibrium (open bag and assay)

C. Surface plasmon resonance (for immob. receptors)
change of mass detected by reflectivity

D. Calorimetry

7. The Gibbs Distribution (p289, ch7)

New concept of particle reservoir.
as well as temperature reservoir.
New thermodynamic variable = N = particle number.



System comes to equilibrium with reservoir.

$N_{tot} = N_S + N_r$ total particles
$E_{tot} = E_S + E_r$ total energy

S = system
r = reservoir

We want the probability:

$p(\text{state}) \propto$ no. of states available to reservoir for system in that state.

$\propto e^{S/k_B}$ since $S = k_B \ln W_{res}$.

$\boxed{S = k_B \ln W}$

Comparing two states

$$\frac{p(E_S^{(1)}, N_S^{(1)})}{p(E_S^{(2)}, N_S^{(2)})} = \frac{W_{res}^{(1)}}{W_{res}^{(2)}} = \frac{e^{S_r(E_{tot}-E_S^{(1)}, N_{tot}-N_S^{(1)})/k_B}}{e^{S_r(E_{tot}-E_S^{(2)}, N_{tot}-N_S^{(2)})/k_B}}$$

linear expansion of entropy (Taylor series)

$$S_{res}(E_{tot}-E_S, N_{tot}-N_S) = S_{res}(E_{tot}, N_{tot}) - \frac{\partial S_r}{\partial E}E_S - \frac{\partial S_r}{\partial N}N_S$$

thermo definitions: $1/T$, $-\mu/T$

$$\Rightarrow \frac{p(E_S^{(1)}, N_S^{(1)})}{p(E_S^{(2)}, N_S^{(2)})} = \frac{e^{-(E_S^{(1)} - \mu N_S^{(1)})/k_BT}}{e^{-(E_S^{(2)} - \mu N_S^{(2)})/k_BT}}$$

Analogous to Boltzmann, all possible microstates, i:

$$p(E_S^{(i)}, N_S^{(i)}) = \frac{1}{Z} e^{-(E_S^{(i)} - \mu N_S^{(i)})/k_BT}$$

Gibbs Distribution

$$Z = \sum_i e^{-(E_S^{(i)} - \mu N_S^{(i)})/k_BT}$$

Grand Partition Function

The Grand Partition Function allows us to obtain the average particle number:

$$\langle N\rangle = \frac{1}{\beta}\frac{\partial}{\partial\mu}\ln\mathcal{Z} = \frac{1}{\beta}\frac{\partial}{\partial\mu}\ln\sum_i e^{-\beta(E^{(i)}-\mu N^{(i)})}$$

$$= \frac{1}{\mathcal{Z}}\frac{1}{\beta}\sum_i \beta N^{(i)} e^{-\beta(E^{(i)}-\mu N^{(i)})}$$

$$= \frac{1}{\mathcal{Z}}\sum_i N^{(i)} e^{-\beta(E^{(i)}-\mu N^{(i)})}$$

$$= \sum_i N^{(i)} p(E^{(i)}, N^{(i)}) \quad \text{usual form.}$$

This is in addition to $\langle E\rangle = -\frac{\partial}{\partial\beta}\ln\mathcal{Z} = \frac{-1}{\mathcal{Z}}\frac{\partial}{\partial\beta}\mathcal{Z}$.

8. Ligand-Receptor (take 3).

The average $\langle N\rangle$ is the same thing as p_{bound} for a ligand-receptor system. Think of solution as the reservoir.

Each receptor can be in one of 2 states:

$\sigma = 0$ unbound
$\sigma = 1$ bound. $\Big\}$ $E_{state} = \varepsilon_b\sigma$ $\quad N_{state} = \sigma$

$$\mathcal{Z} = \sum_{\sigma=0}^{1} e^{-\beta(\varepsilon_b\sigma - \mu\sigma)} = 1 + e^{-\beta(\varepsilon_b-\mu)}$$

$$p_{bound} = \langle N\rangle = \frac{e^{-\beta(\varepsilon_b-\mu)}}{1+e^{-\beta(\varepsilon_b-\mu)}} \quad \text{as before.}$$

Recall connection between μ and concentration:

$$\mu = \mu_0 + k_bT\ln c/c_0$$

$$\Rightarrow \langle N\rangle = \frac{c/c_0\, e^{-\beta(\varepsilon_b-\mu_0)}}{1 + c/c_0\, e^{-\beta(\varepsilon_b-\mu_0)}} \quad \text{as seen before}$$

9. Phosphorylation p 293

This is used in energy activation to drive processes that are otherwise unfavorable.

Also used as a switch:

Regulation of protein (enzyme) activity.

- much faster than synthesising / denaturing.
  + more efficient for cell.

2-state system:

"Covalent post-translational modification"

kinase / phosphatase on/off or off/on.

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OH group of serine (S)
threonine (T) extra CH_3.
tyrosine (Y) extra  -OH

in prokaryotes, also histidine (H)
aspartate (D).

10. Allosteric enzyme (eg Phosphofructokinase).

Model of a 2-state protein system.

Catalytic site +
Binding site for control molecule (eg P).

Mechanism of control is to tip the energy landscape: Rapid response.

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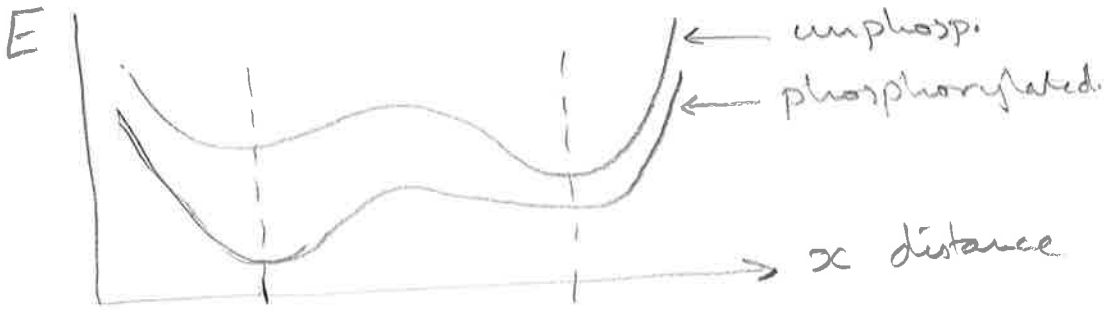

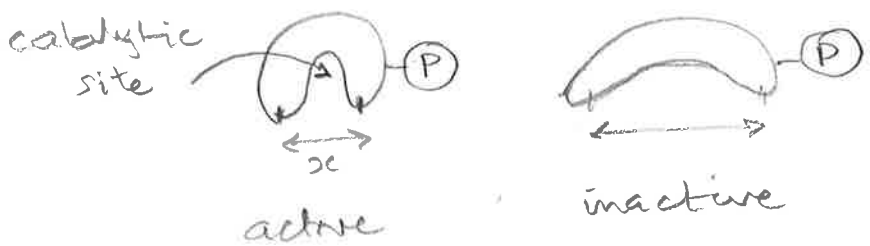

might be two folded domains connected by "hinge"

11. Signal transduction p296.
(mostly prokaryotes)
eg lac repressor, switches enzymatic pathway for new food source, lactose.
But food is outside cell,
enzymes are inside, control of DNA.
Signal has to cross cell wall / membrane.
slide "Sensor histidine kinase" = relay.
i) binds environmental signal.
ii) enables ATP → Phosphorylation inside
iii) transfers P to "response regulator" aspartate
iv) = "transcription factor" 1000 x binds DNA.
slide Large variety of responses | eg lac repressor
eg temperature
osmolarity
metal ions
slide High specificity demonstrated by
Comparing resp. regulators on gel. (^{32}P)
^{31}P is only stable isotope 14-day half life

12. Hemoglobin.

Cooperativity = binding energy depends number of ligands already bound.

Variant of allostery – mediated by conformation change of protein.

Specifically, O_2 binds to heme which changes shape:

oxy : planar heme config.

deoxy : Fe moves out of plane
proximal histidine follows.
conformation change.

slides
movies

Electronic state of Fe still not understood.

Fe^{3+} ion smaller than Fe^{2+}
oxy deoxy

[but Fe in Hb is always diamagnetic
so no unpaired electrons.]

Studied in 1965 by Monod-Wyman-Changeux (MWC).

Tense (T) $\rightleftharpoons$ Relaxed (R)

no oxygen

lower ... higher affinity for O_2

Binding energy $\varepsilon_T > \varepsilon_R$

Coupling between the two binding energies and the conformation leads to cooperativity.

13 "Dimoglobin"

Simplify to two site MWC model.

Three state variables

$\sigma_1 = 0, 1$ } occupation of hemes by ligand

$\sigma_2 = 0, 1$ }

$\sigma_m = 0, 1$ T or R state of receptor.

Eight terms in partition function:

$$E = (1-\sigma_m)(\sigma_1+\sigma_2)\varepsilon_T + \sigma_m\left[\varepsilon + (\sigma_1+\sigma_2)\varepsilon_R\right]$$

Introduce concentration variables:

$x = e^{-\beta(\varepsilon_T - \mu)} = c/c_0\, e^{-\beta(\varepsilon_T - \mu_0)}$ as before

$y = \cdots \varepsilon_R = \cdots \varepsilon_R \cdots$

$$Z = 1 + 2x + x^2 + e^{-\beta\varepsilon}(1 + 2y + y^2)$$
$$= (1+x)^2 + e^{-\beta\varepsilon}(1+y)^2$$

$$\langle N \rangle = \frac{1}{\beta}\frac{\partial}{\partial \mu}\ln Z$$
$$= \frac{1}{\beta}\frac{1}{Z}\left(2(1+x)\beta x + e^{-\beta\varepsilon}\, 2(1+y)\beta y\right)$$
$$= \frac{2}{Z}\left(x + x^2 + e^{-\beta\varepsilon}(y + y^2)\right).$$

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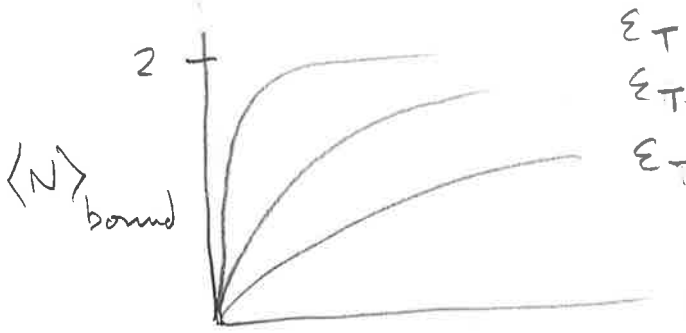

$\varepsilon_T - \varepsilon_R = 4kT$

$\varepsilon_T - \varepsilon_R = 2kT$

$\varepsilon_T - \varepsilon_R = 0$

$\varepsilon = 2kT$

This illustrates the sharper cooperative binding of the MWC model.

14 Ising model.

This is the basis of the Adair and Pauling models used to explain hemoglobin data in 1925.

Interaction between sites is included by a cross-term with its own energy J.

Dimoglobin again for simplicity

$$\left.\begin{matrix}\sigma_1 = 0,1 \\ \sigma_2 = 0,1\end{matrix}\right\} \quad E = \varepsilon(\sigma_1 + \sigma_2) + J\sigma_1\sigma_2$$

(nonlinear now)

Single concentration variable:

$$x = e^{-\beta(\varepsilon-\mu)} = c/c_0\, e^{-\beta(\varepsilon-\mu_0)}$$

$$Z = 1 + 2x + x^2 e^{-\beta J}$$

$$\langle N\rangle = \frac{1}{\beta}\frac{\partial}{\partial\mu}\ln Z$$

$$= \frac{1}{\beta}\frac{1}{Z}\left(2\beta x + 2x\beta x e^{-\beta J}\right)$$

$$= \frac{2}{Z}\left(x + x^2 e^{-\beta J}\right)$$

Verify correct behaviour when $J \to 0$

$$\langle N\rangle = \frac{2(x+x^2)}{(1+x)^2} = 2\,\frac{x}{1+x}$$ = twice standard L-R result.

When J is large and negative, the x^2 term dominates:

$$\langle N\rangle \to \frac{2x^2 e^{-\beta J}}{1 + x^2 e^{-\beta J}}$$ = n=2 Hill function

Binding curve sharpens.

slide

Full model of 4 sites worked in text.

Good agreement with real data of Imai.

Pauling model = single J

Adair model = higher order site interactions.