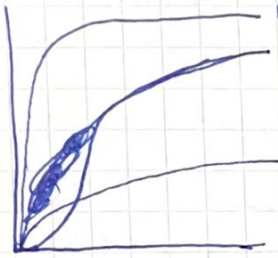
NON-LINEAR LEAST SQUARES & HEMOGLOBIN

Allostery and Cooperativity in Binding

- Allostery is defined as the binding of a ligand to one binding site of a protein.
- Often this affects binding of a ligand at a different, and often distant binding site of a protein.
- Cooperativity is defined as the binding of a ligand at one binding site. This affects the binding of the same type of ligand at a different site.

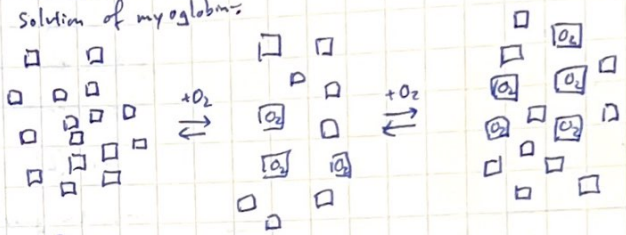
Example: Hemoglobin - binds up to 4 O_2 cooperatively



- A sigmoidal binding curve indicates that this protein binds cooperatively with the ligand.

Non cooperative binding: → Single site

Solution of myoglobin:

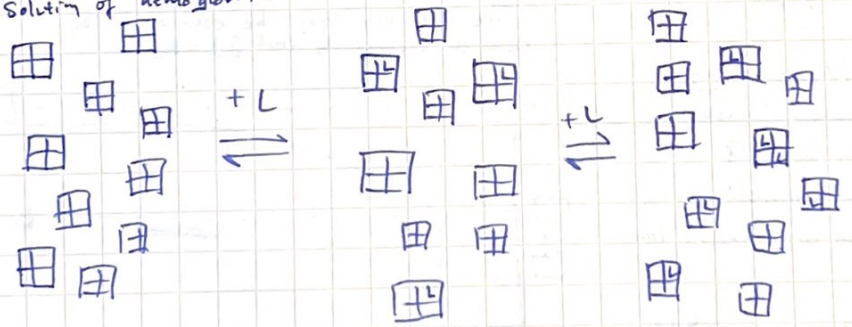


Summary of single-site, noncooperative binding:

- All binding sites have the same binding affinity
- At low [L], solution contains [P] & small amount of [P·L]

Noncooperative binding → Tetrameric protein

Solution of hemoglobin:



All subunits have the same probability of binding L

All empty subunits still have the same probability of binding L.

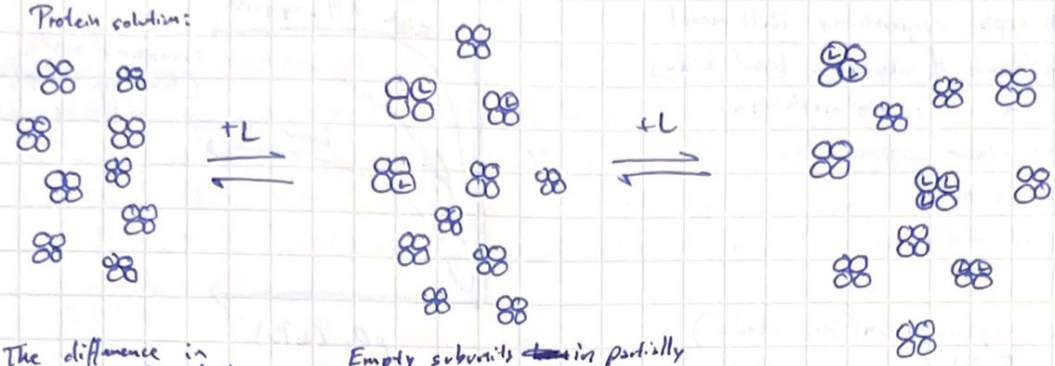
Multi-site non cooperative:

- All binding sites have the same affinity, always.
- At very low [L], the solution contains [P] and a small amount of $[P\cdot(L)_1]$
- Higher [L] - solution contains less [P], more $[P\cdot(L)_1]$ and small amount of $[P\cdot(L)_2]$

Cooperative ligand binding

- A cooperative tetrameric protein, , assume that all circles (subunits) in the empty tetramers have the same (low) affinity for L.

Protein solution:



The difference in cooperative ligand binding:

Empty subunits in partially filled tetramers have a higher affinity for ligands than in the empty tetramers.

Results in many almost filled proteins and a lot of unfilled subunits.

Positive affinity is defined as an increase in ligand affinity with the binding of one protein subunit. This form of affinity is displayed in the above diagram.

Summary of binding types

1) Single site, non-cooperative
   - All binding sites have the same affinity
   - At low [L], solution contains [P] and small amounts of [P·L]
   - At high [L], solution contains less [P] and more [P·L]
2) Multi-site, non-cooperative
   - All binding sites have the same affinity, always
   - At very low [L], solution contains [P] and small amounts of [P·L]
   - At higher [L], solution contains less [P], more $[P\cdot(L)_1]$ and small amounts of $[P\cdot(L)_2]$
3) Multi-site, cooperative:
   - All binding sites have the same binding affinity, in empty protein.
   - Binding affinity increases, as ligand is bound (positive affinity / cooperativity)
   - At very low [L] - solution contains [P] and a small amount of $[P\cdot(L)_1]$
   - At high [L] - solution contains less [P] and more $[P\cdot(L)_2]$ and $[P\cdot(L)_3]$ etc...

The Infinite Cooperativity Mechanism: Equation for $Y = f(L)$:

$P + nL \rightleftharpoons P\cdot(L)_n$ If $n = 1$, non cooperative. If $n = 2, 3, \ldots$, it is cooperative.

$$K_d = \frac{[P][L]^n}{[P\cdot(L)_n]} \quad \therefore \quad Y = \frac{[L]^n}{[L]^n + K_d} = \frac{[L]^n}{[L]^n + (C_{0.5})^n}$$

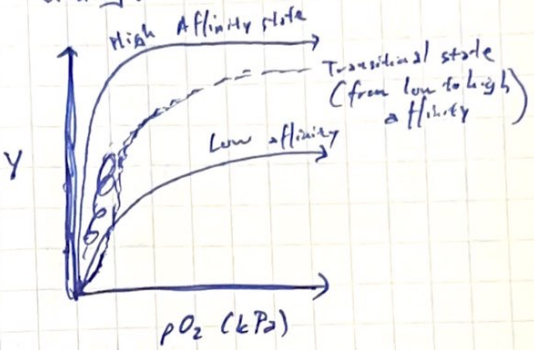
$C_{0.9} = [L]$ at which $Y = 0.5$

The infinite cooperativity mechanism assumes that as soon as a ligand is bound to the subunit of a tetrameric protein, all other subunits of that protein become infinitely more likely to bind to another ligand.

HEMOGLOBIN TOPICS

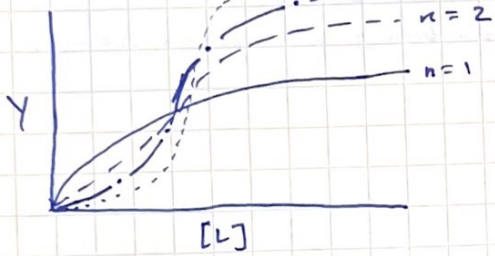
→ What are cooperativity and allostery?
→ Cooperative binding of O_2 by hemoglobin
→ Simple models to explain cooperativity – Hill model
→ Biological significance of cooperative ligand binding
→ Hemoglobin structure — Perutz mechanism
→ Better models to explain cooperativity:
- Sequential model
- Concerted model

O_2 binding to hemoglobin exhibits a sigmoidal binding curve, indicating cooperative binding.



The infinite cooperativity mechanism (Hill-model)

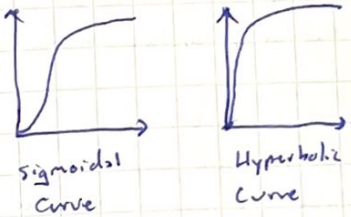
$$Y = \frac{[L]^n}{[L]^n + K_d} = \frac{[L]^n}{[L]^n + (C_{0.5})^n}$$



Note how when $n=1$, the function is hyperbolic.

When $n>1$, the function becomes more sigmoidal

Note the difference between sigmoidal and hyperbolic curves:



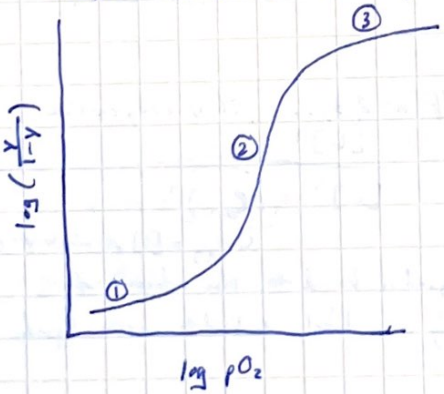
The Hill Plot:

$$Y = \frac{[L]^n}{[L]^n + K_d} \xrightarrow{\text{(algebra)}} \log\left(\frac{Y}{1-Y}\right) = n(\log[L]) - \log(K_d)$$

Plot: $\log\left(\frac{Y}{1-Y}\right)$ vs. $\log[L]$ would result in a straight line in which the slope would be equal to n. This value, also denoted as n_H, is known as the "Hill Coefficient."

When graphed however, one would expect $n = n_H$. However this is not the case, indicating that Hill's model is not 100% accurate. Additionally, the graph is not wholly linear either.

Therefore it is best to model cooperative binding in parts:



① At this low affinity state, $n_H = 1$. Hemoglobin exhibits the following binding pattern:

$$Hb + O_2 \rightleftharpoons Hb(O_2)$$

② At moderate affinity, $n_H = 3$. Hemoglobin binds as:

$$Hb + 3O_2 \rightleftharpoons Hb(O_2)_3$$

More proteins are bound to ligands all at once!

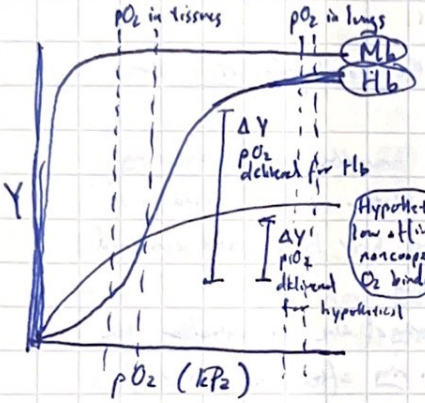
③ At high affinity, $n_H = 1$. Hemoglobin binds as such:

$$Hb(O_2)_3 + O_2 \rightleftharpoons Hb(O_2)_4$$

Significance of cooperative ligand binding

→ For a cooperative system, a small change in concentration (X-axis) may result in a large change in response (Y-axis)

→ Myoglobin's function is to bind and store O_2 in cells. It binds to O_2 non-cooperatively, with high affinity, and reversibly.
→ Hemoglobin's function is to deliver O_2 to cells. Therefore Hb has high binding affinity in areas with high $[O_2]$, such as the lungs, and lower binding affinity in areas with low $[O_2]$, such as peripheral tissues.
- Therefore Hb is better for O_2 distribution while myoglobin is better for transport
- The sigmoidal O_2 binding curve of hemoglobin due to cooperativity makes this ideal for delivering O_2 to tissues.



Note in the diagram to the left that in areas with both high and low pO_2, Myoglobin (Mb) is unlikely to release any O_2 due to its high affinity for O_2 at that pO_2.
→ On the other hand, there is a noticeable difference in affinity between the pO_2 in lungs for Hb and the pO_2 in tissues.

Also notice that the ΔY for the cooperative binding Hb is much greater than the ΔY for the hypothetical low affinity non-cooperative O_2 binder. This is because the slope of sigmoidal curves are much steeper than hyperbolic curves at a moderate pO_2 level.

For non-cooperative proteins: How do fractional saturations (Y) change as $[L]_t$ change?

Recall that $P + L \rightleftharpoons P{\cdot}L$ $\quad Y = \dfrac{[L]}{K_d + [L]}$ and $n = 1$ for non-cooperatively bound proteins.

Suppose that $[L]_t$ is at a value such that $Y = 0.25$. By what factor must $[L]_t$ be increased so that $Y = 0.75$?

$Y = 0.25$ when $[L] = K_d/3$
$Y = 0.5$ when $[L] = K_d$
$Y = 0.75$ when $[L] = 3K_d$

→ 9 fold increase.

$Y_4 = \dfrac{[L]}{K_d + L}$ $\quad K_d + [L] = 4[L]$ $\quad [L] = K_d/3$

$\dfrac{3}{4} = \dfrac{[L]}{K_d + L}$ $\quad 3(K_d + [L]) = 4[L]$ $\quad [L] = 3K_d$

Likewise, to go from $Y = 0.1$ to $Y = 0.9$, it is an 81 fold increase.

Suppose that $n > 1$ (→ cooperative binding). What is the factor of change between $0.25 = Y$ and $0.75 = Y$, as well as $0.9 = Y$ and $0.1 < Y$?

$\dfrac{[L]_{0.75}}{[L]_{0.25}} < 9$ $\qquad \dfrac{[L]_{0.9}}{[L]_{0.1}} < 81$

Why is it less for cooperative binding? – A large change in saturation (Y) results from a small change in ligand concentration $[L]$.

Perutz Mechanism

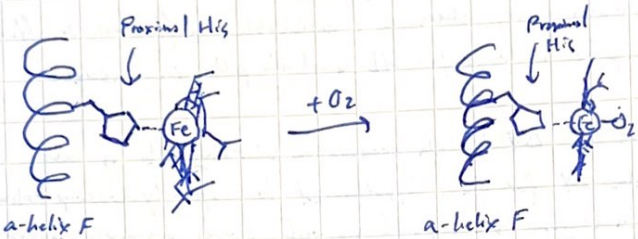
→ The discovery of the structure of hemoglobin allowed for researchers to more accurately model O_2 binding in hemoglobin. Max Perutz was the one to determine the structure of Hb via x-ray crystallography. He found deoxyhemoglobin (no O_2 bound) and standard hemoglobin (oxyhemoglobin)

In the structure of deoxyhemoglobin, Perutz noticed that there were ionic bonds, namely the Asp FG1 $^{(-)}$ to His HC3 $^{(+)}$ and Lys C5 $^{(+)}$ to C-terminus $^{(-)}$ of the β-subunit which helped to stabilize deoxyhemoglobin. Note that Lys C5 is on the α-subunit, so the Lys C5 - C-terminus bond is formed between subunits. Particularly, the ionic bonds form between αβ-dimers.

→ These ionic bonds are not found in oxyhemoglobin.

→ The individual αβ dimers are held together by hydrophobic bonds.

The Perutz Mechanism found that a change in structure occurs within the porphyrin ring due to the Fe in the center binding to oxygen.



α-helix F

deoxy-Hb
(T-state)

α-helix F

oxy-Hb
(R-state)

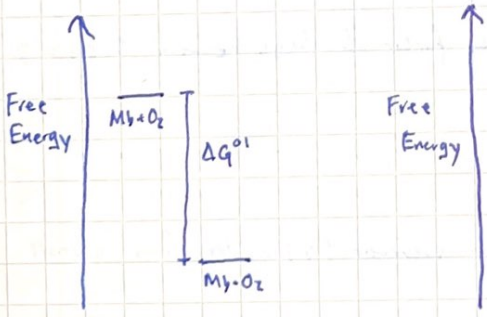
- Notice how the porphyrin ring becomes flatter with the binding of O_2, and how Fe is more centered in the ring.
- The moving of the iron into the porphyrin ring after the binding of O_2 pulls on the proximal histidine, which pulls on the alpha helix as a whole.
- The pulling of the alpha helix then dissociates bonds of the neighboring subunits in oxy-hemoglobin

- There is also a distal histidine on the other side of the porphyrin ring responsible for hydrogen bonding the oxygen bound to iron.

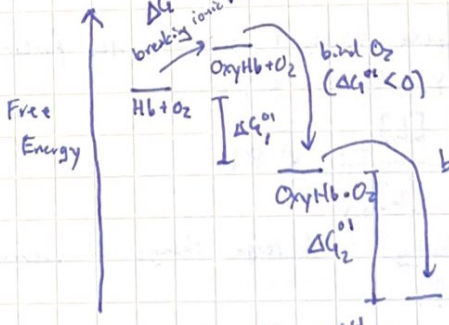
Q: Why are the ionic bonds in deoxy Hb relevant for binding affinity and cooperativity?

A: Defining "T-state" - Hb is in the deoxy conformation, and is stabilized by ionic bonds.
Defining "R-state" - Hb is in the oxy conformation (even if no O_2 is bound) - Key thing is that the ionic bonds are broken.

Thermodynamics - compare ΔGs:



Mb - binds O_2, high affinity
(large negative $\Delta G^{o\prime}$)



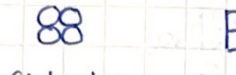
Hb - binds weakly to 1st O_2

Essentially, the ionic bonds stabilizing the deoxy Hb "resist" binding of O_2. Therefore after this first barrier is broken, O_2 may bind much more easily to the Hb.

Two other models used to explain cooperativity

- Sequential Model
- Concerted model

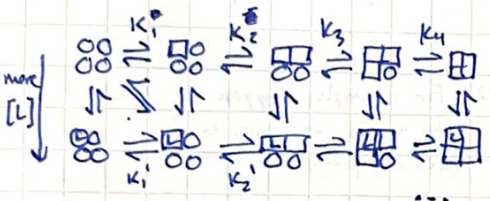
Sequential model (KNF Model)



Circles - low affinity subunits

Squares - high affinity subunits

~~Bound ligands are~~



Over time, the ligands allow for the equilibria to shift toward more active (more affinity) in the protein.

→ The sequential model stated that subunits in ~~the~~ the multimer can be in either conformation.

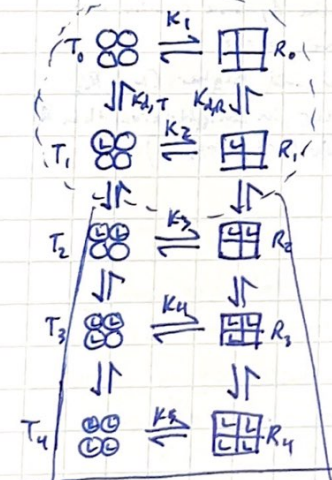
( , , , , , etc...)

→ A protein in solution is in equilibrium with all species; and therefore has a K value for each:

- If ligand is not present, the equilibrium favors low affinity species. Which means $K_1 < 1$ and $K_2 \ll 1$, $K_3 \ll\ll 1$, etc...
- When ligands are present, many more equilibria appear (left) and the binding of one ligand to a subunit increases the probability that neighboring subunit(s) switch to higher affinity conformations

$K_1' > K_1$, and $K_2' > K_2$.

Concerted model (MWC Model)



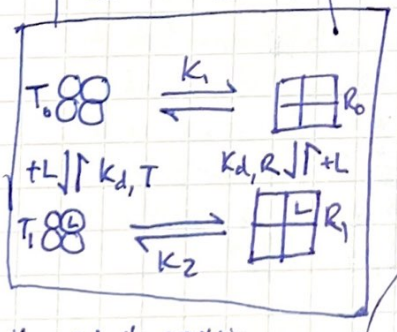
→ Similarly to the ~~new~~ sequential model, ○ represents a subunit in the low affinity state, whereas □ represents a subunit in the high affinity state.

We can mathematically prove that $K_1 < K_2 < K_3 < K_4 < K_5$.

Rules:

1) Subunits can either be low or high affinity, in which ligand binding is preferential to R state
2) All subunits in each tetramer are either circles ("T-state") or squares ("R" state)
3) In the absence of L, equilibrium favors low affinity. That is, $K_1 < 1$.

If these rules are followed, it can be proven that equilibria shift to favor R state, as ligand concentration increases.



$K_1 = \frac{[R_0]}{[T_0]}$ Since $[T_0] > [R_0]$, $K < 1$.

$K_{d,T} = \frac{[L][T_0]}{[T_1]}$ $K_{d,R} = \frac{[L][R_0]}{[R_1]}$ $K_{d,T} > K_{d,R}$ (Since T state has low affinity)

$K_2 = \frac{[R_1]}{[T_1]}$ What is the ratio of $K_2/K_{d,T}$?

What is the ratio of $K_1/K_{d,R}$?

$\frac{K_2}{K_{d,T}} = \frac{[R_1]}{[T_1]}\left(\frac{[T_1]}{[L][T_0]}\right) = \frac{[R_1]}{[L][T_0]}$

$\frac{K_1}{K_{d,R}} = \frac{[R_0]}{[T_0]}\left(\frac{[R_1]}{[L][R_0]}\right) = \frac{[R_1]}{[T_0][L]}$

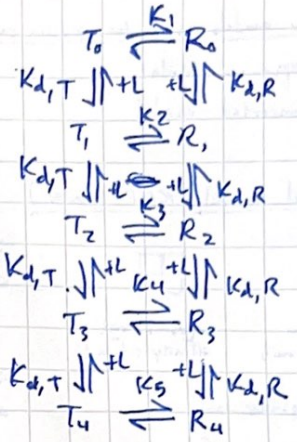
These are equal!

If we set the expression

$\frac{K_2}{K_{d,T}} = \frac{K_1}{K_{d,R}}$ $K_2 = K_1\left(\frac{K_{d,T}}{K_{d,R}}\right)$ $K_2 > K_1$

Concerted Model (cont'd)

Note that in the concerted model, $K_{d,T} = K_{d,R}$ every step of the way:



This may seem counterintuitive in that there is no change to the binding affinity of the subunit, whereas in fact this means that there will be more and more high-affinity binding than low-affinity binding proteins present, since $K_1 < K_2 < K_3 < K_4 < K_5$.

To summarize:

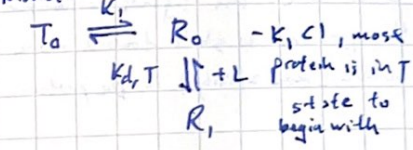
→ In the absence of L, most subunits have low affinity (in T-state), as $K_1 < 1$.

→ Increasing [L] results in more and more binding sites which have higher affinity (more R states)

→ The preferential binding of ligands to one protein form pulls the equilibrium to that protein form.

↳ For example, suppose L binds only to R_0 and not to T_0 (extreme preferential binding)

Model:



- $K_1 < 1$, most protein is in T state to begin with
- With [L] increasing, R_0 turns into R_1, shifting the dynamic equilibrium of K_1 towards more R_0
- With higher and higher [L], R_1 is much greater in concentration than B To (Le Châtelier's principle)

Can we derive an equation for $Y = f([L])$ for the concerted model?

$$Y = \frac{a(1+a)^{n-1} + \frac{ca}{K_1}(1+ca)^{n-1}}{(1+a)^n + (1+ca)^n / K_1}$$

Parameters & Variables:

- n = # of binding sites on protein
- $K_1 = \frac{[R_0]}{[T_0]}$
- $c = \frac{K_{d,R}}{K_{d,T}}$
- $a = \frac{[L]}{K_{d,R}}$

↓ If this complicated function is graphed, it results in a sigmoidal curve which fits for the data given (can use NLLS).