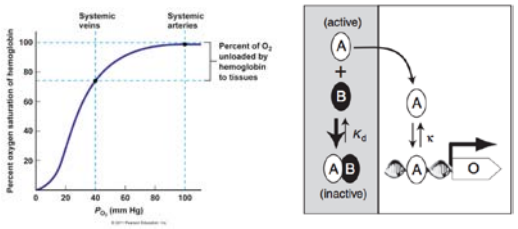


MCB 135 Lecture 7
Inhibition and Cooperativity

Today's Outline

- Cooperativity
  - Review of simple binding
  - Effect of multiple independent binding sites
  - Hill curves
  - Implications for gene regulation
  - Monod-Wyman-Changeux model
- Allosteric regulation
  - Competitive and non-competitive inhibition
  - Generation of switch-like behaviors

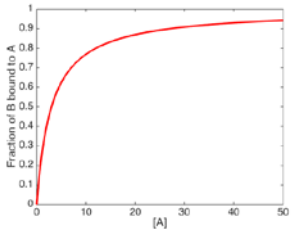
Recap: Simple binding curves are hyperbolic

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AB$$

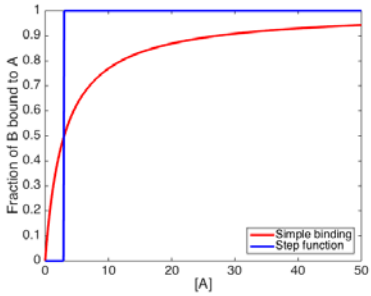
Fraction of B bound to A:

$$\frac{[AB]}{[B]+[AB]} = \frac{[A]}{K+[A]}$$

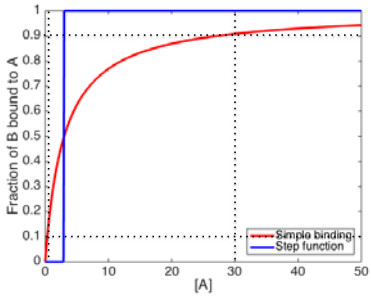
$$K = \frac{k_{-1}}{k_1}$$



Step functions do not approximate simple binding curves very well



How hard is it to switch from "mostly not bound" to "mostly bound"?



How hard is it to switch from "mostly not bound" to "mostly bound"?

What is [A] when 10% of B is bound to A?

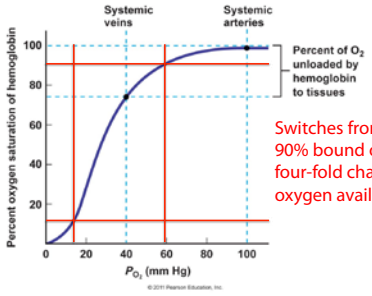
$$0.1 = \frac{[A]}{K+[A]} \Longrightarrow [A] = \frac{K}{9}$$

What is [A] when 90% of B is bound to A?

$$0.9 = \frac{[A]}{K+[A]} \Longrightarrow [A] = 9K$$

…we must increase [A] by a factor eighty-one!

Hemoglobin, an oxygen-binding protein, has a sigmoidal curve



Switches from 10% to 90% bound over an ≈ four-fold change in oxygen availability

Hemoglobin has four oxygen binding sites:

Is this responsible for its sigmoidal curve?

Suppose all four sites are identical and independent (i.e., each site is unaffected by the binding of O_2 elsewhere) with binding reaction rate k_{on} and dissociation reaction rate k_{off}

$$P + X \rightleftharpoons PX$$

$$PX + X \rightleftharpoons PX_2$$

$$PX_2 + X \rightleftharpoons PX_3$$

$$PX_3 + X \rightleftharpoons PX_4$$

P: Protein (hemoglobin)
X: Ligand (O_2)

Finding the binding curve when sites are independent

What is the total concentration of binding sites?

$$4[P_{tot}] = 4([P] + [PX] + [PX_2] + [PX_3] + [PX_4])$$

What is the total concentration of bound sites?

$$[PX] + 2[PX_2] + 3[PX_3] + 4[PX_4]$$

What is the fraction of sites bound?

$$\bar{v} = \frac{[PX] + 2[PX_2] + 3[PX_3] + 4[PX_4]}{4([P] + [PX] + [PX_2] + [PX_3] + [PX_4])}$$

Finding the binding curve when sites are independent

Assume that equilibrium has been reached:

$$P + X \underset{k_{off}}{\overset{4k_{on}}{\rightleftharpoons}} PX$$

$$4k_{on}[P][X] = k_{off}[PX]$$

$$\Longrightarrow [PX] = \frac{4k_{on}[X]}{k_{off}}[P] = 4K[X][P]$$

Finding the binding curve when sites are independent

Assume that equilibrium has been reached:

$$PX + X \underset{2k_{off}}{\overset{3k_{on}}{\rightleftharpoons}} PX_2$$

$$3k_{on}[PX][X] = 2k_{off}[PX_2]$$

$$[PX_2] = \frac{3k_{on}[X]}{2k_{off}}[PX] = 6K^2[X]^2[P]$$

Finding the binding curve when sites are independent

Find expressions for the concentration of each complex by this method:

$$[PX] = 4K[X][P] \qquad [PX_3] = 4K^3[X]^3[P]$$
$$[PX_2] = 6K^2[X]^2[P] \qquad [PX_4] = K^4[X]^4[P]$$

Then plug them into our expression for the fraction of sites bound:

$$\bar{\nu} = \frac{[PX] + 2[PX_2] + 3[PX_3] + 4[PX_4]}{4([P] + [PX] + [PX_2] + [PX_3] + [PX_4])}$$

Finding the binding curve when sites are independent

$$\bar{\nu} = \frac{4K[X][P]\left(1 + 3K[X] + 3K^2[X]^2 + K^3[X]^3\right)}{4[P]\left(1 + 4K[X] + 6K^2[X]^2 + 4K^3[X]^3 + K^4[X]^4\right)}$$
$$= \frac{K[X](1 + K[X])^3}{(1 + K[X])^4} = \frac{K[X]}{1 + K[X]} = \frac{[X]}{K' + [X]}$$

Four independent binding sites cannot explain hemoglobin's sigmoidal binding curve!

If the sites are not independent, then binding affinity at each site depends on the occupancy of the other sites.

Perhaps the sites are also not identical.

How could this be modeled?

Modeling with non-identical, non-independent binding sites

How many distinct states of oxygen binding are there?

$2^4 = 16$

How many transitions are there out of any given state (that involve gaining or losing a single O_2 molecule)?

4

How many "on" and "off" reaction rates are there to fit?

16 x 4 = 64

"With four parameters I can make an elephant, and with five I can make him wiggle his trunk."

-- John von Neumann

Modeling with identical, non-independent binding sites

How many distinct states of oxygen binding are there?

5

How many transitions are there out of a given state (that involve gaining or losing a single O_2 molecule)?

1 (P and PX_4) or 2 (PX, PX_2, and PX_3)

How many "on" and "off" reaction rates are there to fit?

8

Modeling with identical, non-independent binding sites

$$P + X \underset{k_{-1}}{\overset{4k_1}{\rightleftharpoons}} PX$$

$$PX + X \underset{2k_{-2}}{\overset{3k_2}{\rightleftharpoons}} PX_2$$

$$PX_2 + X \underset{3k_{-3}}{\overset{2k_3}{\rightleftharpoons}} PX_3$$

$$PX_3 + X \underset{4k_{-4}}{\overset{k_4}{\rightleftharpoons}} PX_4$$

Define four association constants, K_i:

$$K_i = \frac{k_i}{k_{-1}}$$

Finding an expression for the fraction of sites bound

Find expressions for the concentration of each complex by this method

$$[PX] = 4K_1[P][X] \qquad [PX_3] = 4K_1K_2K_3[P][X]^3$$
$$[PX_2] = 6K_1K_2[P][X]^2 \qquad [PX_4] = K_1K_2K_3K_4[P][X]^4$$

Then plug them into our expression for the fraction of sites bound:

$$\bar{\nu} = \frac{[PX] + 2[PX_2] + 3[PX_3] + 4[PX_4]}{4([P] + [PX] + [PX_2] + [PX_3] + [PX_4])}$$

Finding an expression for the fraction of sites bound

$$\bar{\nu} = \frac{K_1[X] + 3K_1K_2[X]^2 + 3K_1K_2K_3[X]^3 + K_1K_2K_3K_4[X]^4}{1 + 4K_1[X] + 6K_1K_2[X]^2 + 4K_1K_2K_3[X]^3 + K_1K_2K_3K_4[X]^4}$$

This general expression is called the *Adair equation*.

Finding an expression for the fraction of sites bound

$$\bar{\nu} = \frac{K_1[X] + 3K_1K_2[X]^2 + 3K_1K_2K_3[X]^3 + K_1K_2K_3K_4[X]^4}{1 + 4K_1[X] + 6K_1K_2[X]^2 + 4K_1K_2K_3[X]^3 + K_1K_2K_3K_4[X]^4}$$

A special case: $K_4 >> K_1, K_2, K_3$

This is an example of *cooperativity* because the binding of earlier molecules "helps" the last one bind.

$$\bar{\nu} \approx \frac{K_1K_2K_3K_4[X]^4}{1 + K_1K_2K_3K_4[X]^4} = \frac{[X]^4}{K + [X]^4}$$

Cooperativity and Hill curves

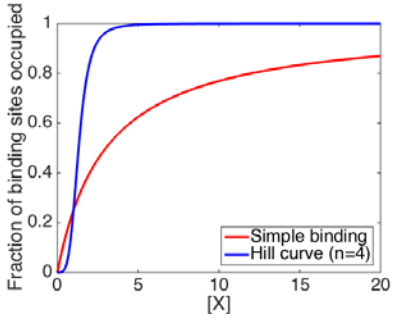
$$\bar{\nu} \approx \frac{K_1K_2K_3K_4[X]^4}{1 + K_1K_2K_3K_4[X]^4} = \frac{[X]^4}{K + [X]^4}$$

… is just a specific example of a *Hill equation.*

The Hill equation: $\bar{\nu} = \frac{[X]^n}{K + [X]^n}$

Hill coefficient (always ≤ # of binding sites)

Comparing simple and cooperative binding



How hard is it to switch from "mostly not bound" to "mostly bound"?

What is [X] when 10% of sites are bound?

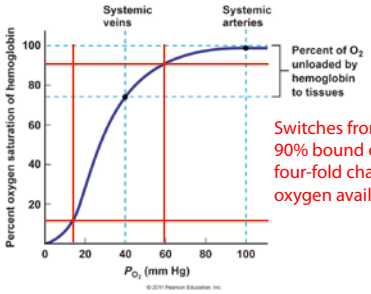
$$0.1 = \frac{[A]^4}{K + [A]^4} \Longrightarrow [A] = \sqrt[4]{\frac{K}{9}}$$

What is [X] when 90% of sites are bound?

$$0.9 = \frac{[A]^4}{K + [A]^4} \Longrightarrow [A] = \sqrt[4]{9K}$$

…we must increase [A] 3-fold (not 81-fold)!

Hey, not bad!



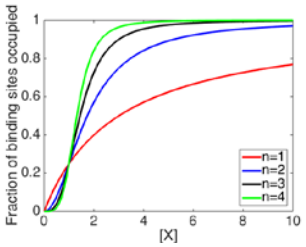
Switches from 10% to 90% bound over an ≈ four-fold change in oxygen availability

The maximum slope of the Hill curve is proportional to *n*

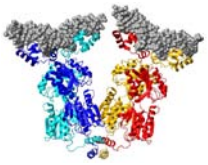
$$\bar{v} = \frac{[X]^n}{K^n + [X]^n}$$

$$\frac{d\bar{v}}{d[X]} = \frac{nK^n[X]^{n-1}}{\left([X]^n + K^n\right)^2}$$

$$\left.\frac{d\bar{v}}{d[X]}\right|_{[X]=K} = \frac{n}{4K}$$



Cooperativity in transcription factor binding



The Lac repressor is a dimer-of-dimers.

Its three binding sites are within 500 bp of each other in a 5 Mbp genome.

Cooperativity in transcription factor binding

Finding the first site is slow.

Once bound, the other end of the protein more quickly finds a second, nearby, binding site.

Cooperativity in transcription factor binding

Finding the first site is slow.

Once bound, the other end of the protein more quickly finds a second, nearby, binding site.

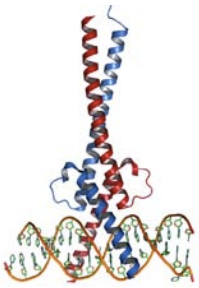
If the repressor falls off of one site, it can quickly reattach.

Many transcription factors are dimers that bind palindromic sequences

Example:
Basic helix-loop-helix/leucine zipper transcription factors

5′-TTACGTAA-3′
3′-AATGCATT-5′

Notice that in this case the two binding sites are right next to one another.



A Hill curve can describe the rate of target gene transcription

For a gene regulated by a transcriptional activator (with maximum expression rate c):

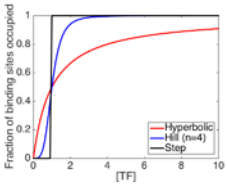
$$\text{Expression rate} = c \cdot \text{Fraction of time a transcriptional activator is bound at its site} = \frac{c[\text{TF}]^n}{K + [\text{TF}]^n}$$

For a gene regulated by a transcriptional repressor:

$$\text{Expression rate} = c \cdot \text{Fraction of time a transcriptional repressor is } \textit{not} \text{ bound at its site} = c\left(1 - \frac{[\text{TF}]^n}{K + [\text{TF}]^n}\right) = \frac{cK}{K + [\text{TF}]^n}$$

(Assumes that the "average fraction of sites occupied" is a good estimate of the "fraction of time a given site is occupied.")

In the Alon textbook, Hill curves are approximated as logic functions



$$\theta(t) = \begin{cases} 1 & : t > 0 \\ 0 & : t \le 0 \end{cases}$$

The variable t can be a Boolean:
- True: 1
- False: 0

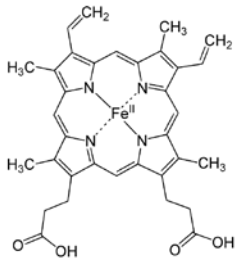
$$\frac{dP}{dt} = \alpha\theta\left([\text{Activating TF}] > K\right) - \beta P$$

Is there a biological reason to choose this eight-parameter model for hemoglobin?

$$P + X \underset{k_{-1}}{\overset{4k_1}{\rightleftharpoons}} PX$$
$$PX + X \underset{2k_{-2}}{\overset{3k_2}{\rightleftharpoons}} PX_2$$
$$PX_2 + X \underset{3k_{-3}}{\overset{2k_3}{\rightleftharpoons}} PX_3$$
$$PX_3 + X \underset{4k_{-4}}{\overset{k_4}{\rightleftharpoons}} PX_4$$

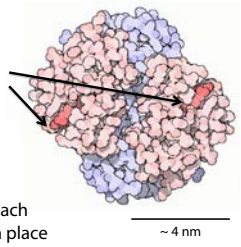
or is there a simpler, more biologically-grounded explanation for cooperativity?

An iron atom in a small molecule called heme binds oxygen



Heme is present at each of hemoglobin's four O_2 binding sites

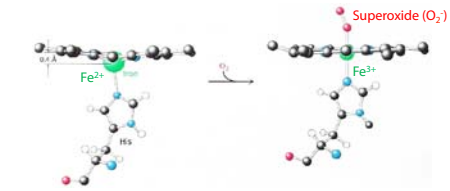
Two of four heme groups



Amino acid side chains in hemoglobin interact with each heme molecule to hold it in place

PDB May 2003 Molecule of the Month

Oxygen binding pulls upward on an amino acid bound to the iron atom

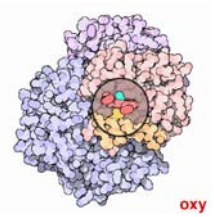


Pulling this amino acid causes others, attached through the protein's backbone, to move as well

Those local movements have global effects on hemoglobin's shape

Hemoglobin's structure has been determined when:
a) No O_2 is bound (1960)
b) All sites have O_2 bound (1970)

This animation shows both of those structures and simulates a transition between them.

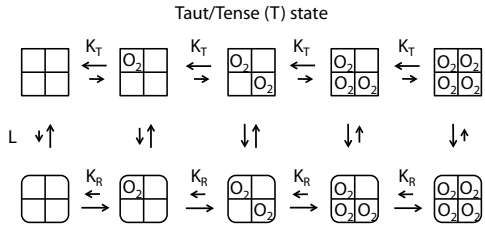


Monod-Wyman-Changeux (1965)

- Hemoglobin exists in two folding states, tensed/taut (T) and relaxed (R)
  - Essentially the "oxy" and "deoxy" crystal structures just shown, but with variable numbers of O_2 molecules bound
- All four binding sites behave identically
- Binding sites in R-state hemoglobin have a higher affinity for oxygen

Monod-Wyman-Changeux (1965)

Taut/Tense (T) state

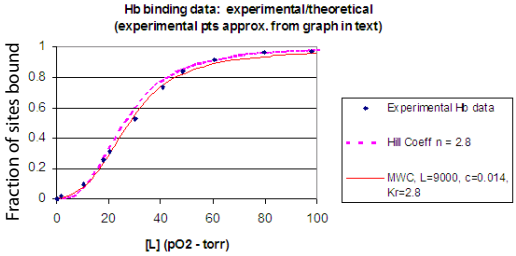


Relaxed (R) state

…only three parameters! (L, K_R, K_T)

On problem set 2, you'll find the expression for fraction of sites bound under the MWC model (with just two sites, for simplicity)

The MWC model fits hemoglobin experimental data well



Data/plot by Henry Jakubowski

Cooperativity is one way to get switch-like behavior from binding curves.

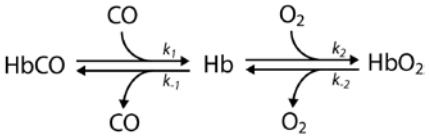
But can we regulate binding without changing ligand concentration?

Carbon monoxide and oxygen compete for the same binding sites on hemoglobin:

Oxygen gas: :O═O:

Carbon monoxide: •C≡O: ← >200x higher affinity!

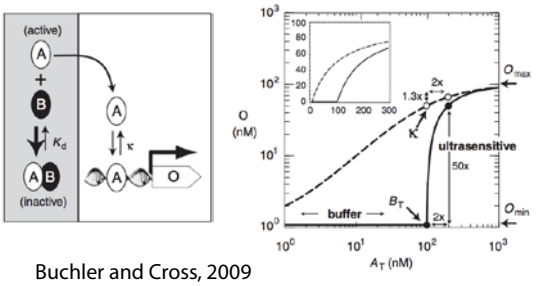
Competitive inhibition of hemoglobin



$$[HbO_2] = \frac{K_1[O_2][Hb_{tot}]}{1 + K_1[O_2] + K_2[CO]}$$

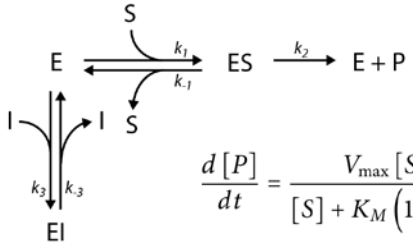
Suggests a cure: increasing ppO_2!

Competitive inhibition can generate switch-like behaviors



Buchler and Cross, 2009

Enzymes also experience competitive inhibition



$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{[S] + K_M\left(1 + \frac{[I]}{K_I}\right)}$$

We can still always reach V_{max} is [S] >>[I]

Hemoglobin's oxygen affinity changes with environmental cues

Cellular respiration uses up O_2 & produces CO_2

- Tissues that need O_2 the most tend to have high [CO_2]

Carbon dioxide is carried through blood in several ways:

- Bound by hemoglobin (~10%)
- Converted to carbonic acid (~80%)

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

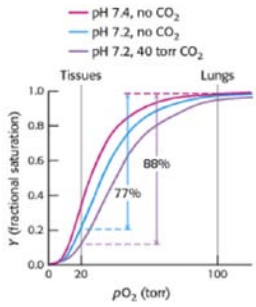
Hemoglobin's oxygen affinity changes with environmental cues

Cellular respiration uses up O_2 & produces CO_2
- Tissues that need O_2 the most tend to have high [CO_2]

Carbon dioxide is carried through blood in several ways:
- **Bound by hemoglobin (~10%)**
- **Converted to carbonic acid (~80%)**

Both pH sensitivity and CO_2 binding regulate hemoglobin's affinity for oxygen

The Bohr Effect

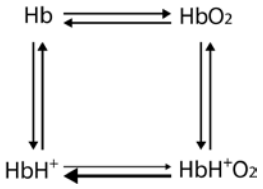


Stryer Fig. 7.21

Unlike carbon monoxide, CO_2 and H^+ don't get anywhere near the O_2 binding sites.

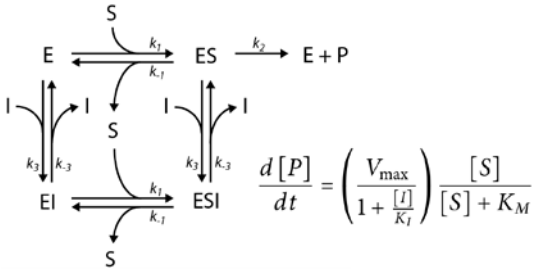
We say that they influence O_2 affinity *allosterically*.

Allosteric inhibition in hemoglobin



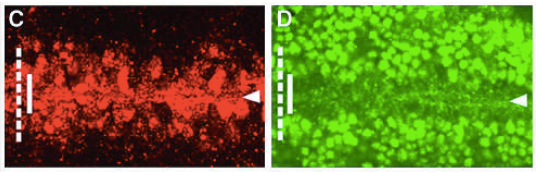
Unlike carbon monoxide poisoning, this oxygen release is adaptive

Non-competitive inhibition of enzymes



$$\frac{d[P]}{dt} = \left(\frac{V_{max}}{1+\frac{[I]}{K_I}}\right)\frac{[S]}{[S]+K_M}$$

Can't reach the original V_{max} just by adding more S



Next time:
Zero-order ultrasensitivity