TOPICS

- Contexts for biomolecular binding
- Specificity
- Binding Kinetics and Thermodynamics
- Enzyme Function and Kinetics

Contexts for Biomolecular Binding

The key to the way biology manages complexity is the great specificity of binding displayed by biomolecules. You are familiar with DNA base-pairing as one example. Many proteins are designed to recognize and bind to specific target molecules. Example include:

Antibodies – bind to invading molecules
Cell membrane receptors – bind to target ligands that are needed for cell function
Enzymes – bind to substrates and catalyze their reactions
Lectins – bind to saccharides

Examples

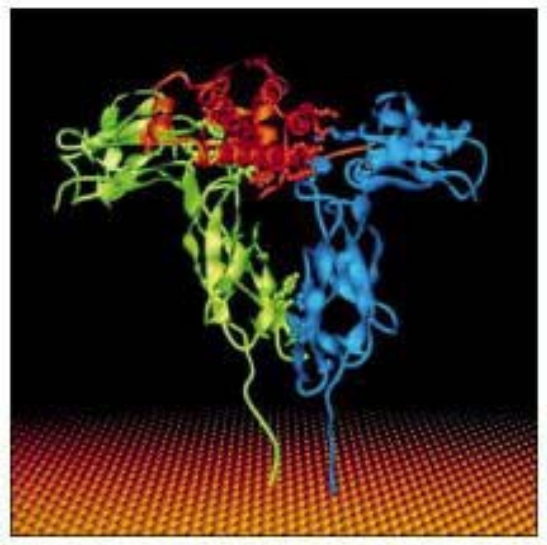


Figure 15-62. The three-dimensional structure of human growth hormone bound to its receptor. The hormone *(red)* has cross-linked two identical receptors (one shown in *green* and the other in *blue)*. Hormone binding activates cytoplasmic tyrosine kinases that are tightly bound to the cytosolic tails of the receptors (not shown). The structures shown were determined by x-ray crystallographic studies of complexes formed between the hormone and extracellular receptor domains produced by recombinant DNA technology. It was entirely unexpected that a monomeric ligand such as growth hormone would cross-link its receptors, as it requires that the two identical receptors recognize different parts of the hormone. As mentioned earlier, EGF does the same thing. (From A.M. deVos, M. Ultsch, and A.A. Kossiakoff, *Science* 255:306–312, 1992. © AAAS.)
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookH

L&term=receptor+ligand+x-ray+AND+mboc4%5Bbook%5D+AND+373896%5Buid%5D&rid=mboc4.figgrp.2868

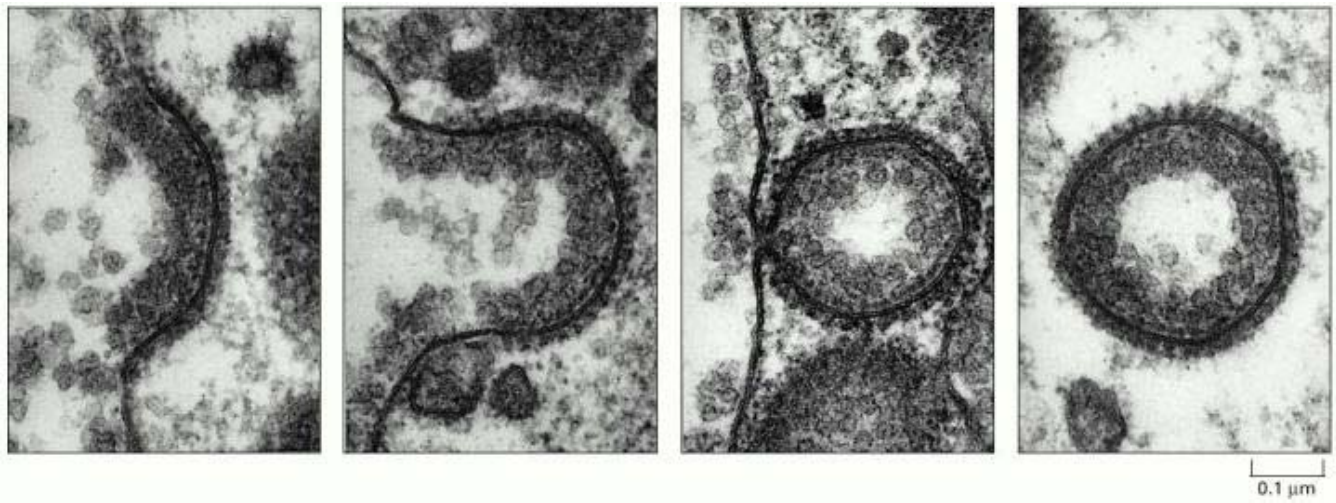


Figure 13-41. The formation of clathrin-coated vesicles from the plasma membrane. These electron micrographs illustrate the probable sequence of events in the formation of a clathrin-coated vesicle from a clathrin-coated pit. The clathrin-coated pits and vesicles shown are larger than those seen in normal-sized cells. They are involved in taking up lipoprotein particles into a very large hen oocyte to form yolk. The lipoprotein particles bound to their membrane-bound receptors can be seen as a dense, fuzzy layer on the extracellular surface of the plasma membrane—which is the inside surface of the vesicle. (Courtesy of M.M. Perry and A.B. Gilbert, *J. Cell Sci.* 39:257–272, 1979. © The Company of Biologists.)
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.2389

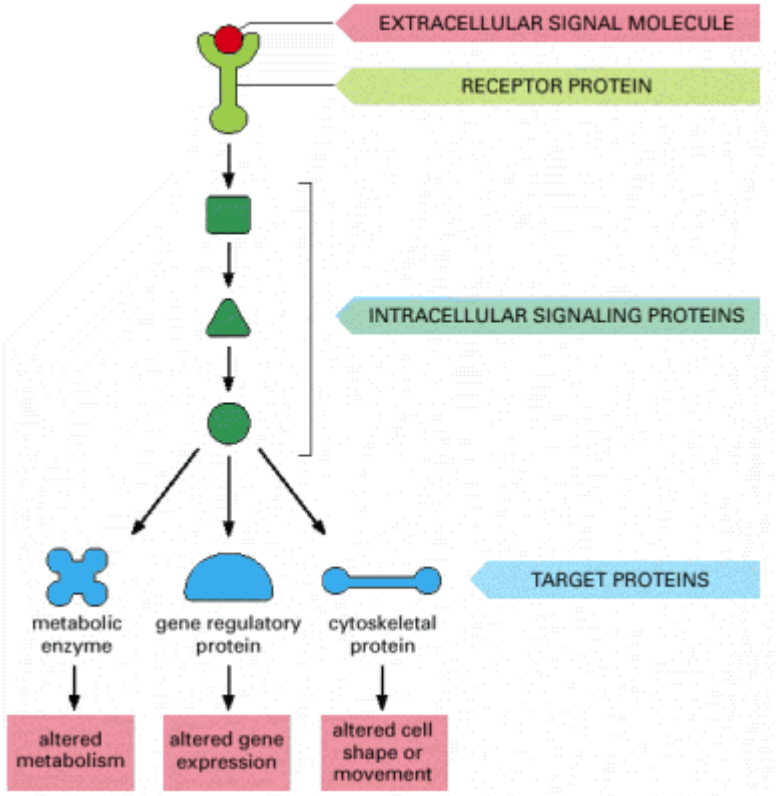


Figure 15-1. A simple intracellular signaling pathway activated by an extracellular signal molecule. The signal molecule binds to a receptor protein (which is usually embedded in the plasma membrane), thereby activating an intracellular signaling pathway that is mediated by a series of signaling proteins. Finally, one or more of these intracellular signaling proteins interacts with a target protein, altering the target protein so that it helps to change the behavior of the cell. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.2741

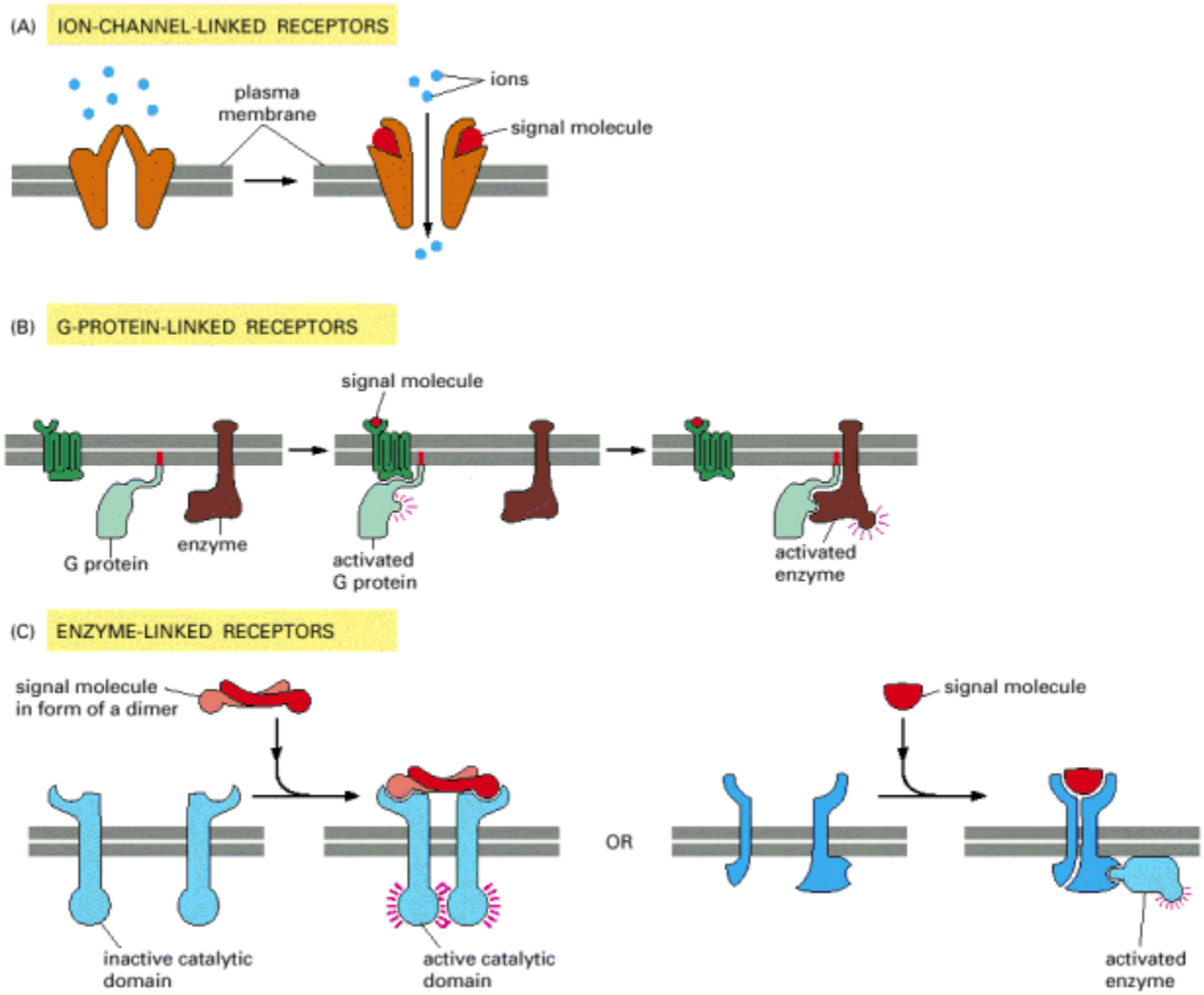
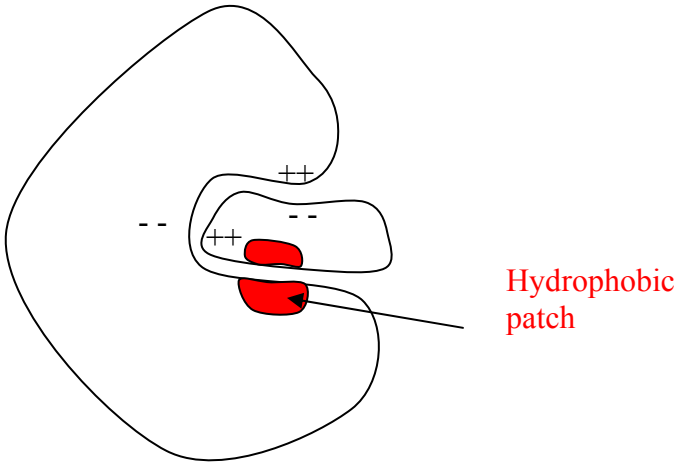


Figure 15-15. Three classes of cell-surface receptors. (A) Ion-channel-linked receptors, (B) G-protein-linked receptors, and (C) enzyme-linked receptors. Although many enzyme-linked receptors have intrinsic enzyme activity, as shown on the left, many others rely on associated enzymes, as shown on the right.
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4.figgrp.2767

The transport proteins we discussed in Lecture 14 are also examples of highly specific molecular recognition systems. The origin of molecular recognition specifity lies in the same set of intermolecular forces that drive protein folding and lipid self-assembly.



We will discuss the key characteristics of molecular recognition in the context of enzyme-catalyzed reaction kinetics.

Binding Kinetics and Thermodynamics

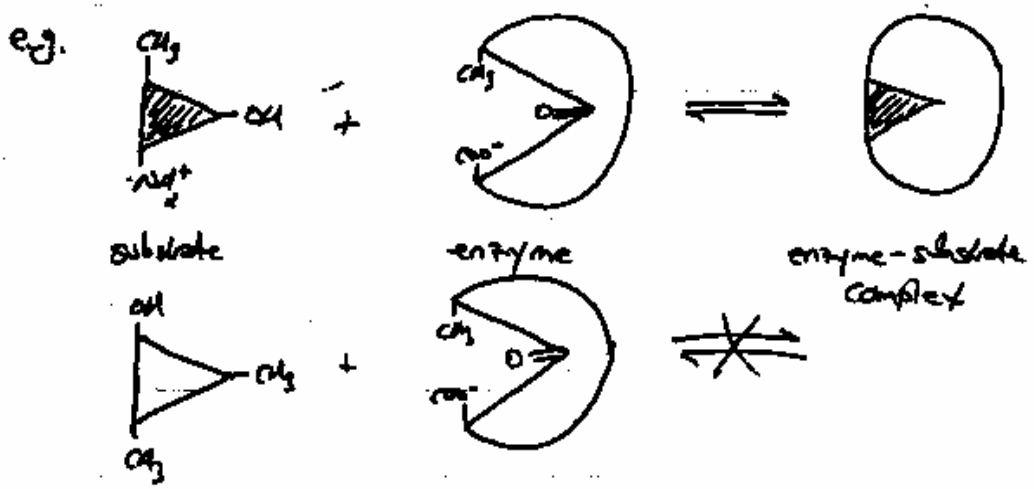
Molecular Recognition = Binding
binding kinetics and thermodynamics
contexts: recognition, regulation, catalysis

Binding - the noncovalent and specific association of two molecules

e.g. - two complementary strands of DNA
- ribozyme and Shine-Dalgarno box
- mRNA codon + tRNA anticodon
- antibody and antigen
- enzyme and substrate
- HIV and CD4 on T cells

noncovalent binding involves H-bonds, electrostatic interactions (+ve and -ve charge groups interacting) and hydrophobic interactions (hydrophobic groups clustering to avoid water)

specificity is conferred by shape (spatial) complementarity between the molecules and their interacting groups



Note that because noncovalent interactions are used, binding is reversible

Specificity is important because cells, organs contain 1000's of different species and are constantly 1000's of different events (reactions, protein expression, replication, motion etc) simultaneously

Kinetics and thermodynamics

Consider specific interaction between a protein and a small molecule (ligand): can write a binding reaction

$$\text{protein} + \text{ligand} \rightleftharpoons \text{protein-ligand complex}$$

$$P + L \rightleftharpoons PL$$

Mass action kinetics $\Rightarrow$ (rate of rxn) $\propto \prod_{\substack{i=1 \\ \text{reactants}}}^{n} [\text{reactant } i]^{(\text{stoichiometric coefficient})}$

forward rxn: binding $P + L \longrightarrow PL$

proportionality constant

$$r_{fwd} = k_{fwd}[P]^1[L]^1$$

reverse rxn: release $PL \longrightarrow P + L$

proportionality constant

$$r_{rev} = k_{rev}[PL]^1$$

net rate of rxn in forward direction:

$$r_{net} = k_{fwd}[P][L] - k_{rev}[PL]$$

recall from stoichiometry, rate of changes of all species taking part in a reaction are linked by stoichiometry

$$\frac{(\text{rate of change species 1})}{\nu_1} = \frac{(\text{rate of disappearance in rxn species 2})}{\nu_2} = \text{etc.}$$

So, can write:

$$r_{net} = \frac{d[P]/dt}{-1} = \frac{d[L]/dt}{-1} = \frac{d[PL]/dt}{1} = \underbrace{k_{fwd}[P][L] - k_{rev}[PL]}$$

this specifies what $R_i(t)$ looks like in M-balls with rxns...

Let's consider the reaction at equilibrium ($r_{fwd} = r_{rev}$) $\Rightarrow r_{net} = 0$

$$0 = k_{fwd}[P][L] - k_{rev}[PL]$$

$$k_{fwd}[P][L] = k_{rev}[PL]$$

$$\underbrace{K}_{\text{equilibrium (association) constant}} \equiv \frac{k_{fwd}}{k_{rev}} = \frac{[PL]}{[P][L]} \equiv \prod_{i=1}^{n \text{ species}} [\text{species } i]^{\nu_i}$$

rxn.

Often, biological binding interactions are specified in terms of an equilibrium dissociation constant,

$$K_d \equiv \frac{k_{rev}}{k_{fwd}} = \frac{[P][L]}{[PL]}$$

K_d represents the ligand conc at which the binding constant on the protein is half saturated ($[P] = [PL]$)

The smaller K_d, the tighter the binding

eg. $K_d = 1\ mM \Rightarrow$ if $[L] > 1\ mM \Rightarrow$ } more than half of protein is in complex form

$= 1\ nM \Rightarrow$ if $[L] > 1\ nM \Rightarrow$ }

Approximate treatment of fraction of bound species, $f_{PL,}$

Can combine equilibrium expressions with species balances within streams

Total protein at equilibrium, P_T

$$P_T = \underset{\text{unbound protein}}{[P]} + \underset{\text{bound protein}}{[PL]} = [P] + \frac{[P][L]}{K_d} = [P]\left(1 + \frac{[L]}{K_d}\right)$$

$$L_T = \underset{\text{'free' ligand}}{[L]} + \underset{\text{bound ligand}}{[PL]} = [L] + \frac{[P][L]}{K_d} = [L]\left(1 + \frac{[P]}{K_d}\right)$$

Now, $[P]$ and $[L]$ can be hard to measure, but we will often know P_T and L_T based on how the system or stream was prepared

Can solve these two equations simultaneously to eliminate $[L]$ and solve for $[P]$ in terms of P_T, L_T and K_d...

$$K_d\left(\frac{P_T}{[P]} - 1\right) = [L] = \frac{L_T}{(1 + [P]/K_d)}$$

$$\Rightarrow [P]^2 + (K_d + L_T - P_T)[P] - P_T K_d = 0$$

solve for $[P]$ using quadratic formula

$$\Rightarrow [P] = \frac{-(K_d + L_T - P_T) \pm \sqrt{(K_d + L_T - P_T)^2 + 4 P_T K_d}}{2}$$

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Which not?

expect $\lim_{L_T \to \infty} [PL] = \phi \overset{\checkmark}{=} \frac{-L_T \oplus \sqrt{L_T^2}}{2} \Rightarrow +ve$

huge conc of ligand
bind to leave no unbound protein

check $\lim_{P_T \to \infty} [PL] = P_T = \frac{-(-P_T) + \sqrt{-P_T^2 + 4P_T K_D}}{2}$?

can't saturate, huge conc of total protein $\overset{\checkmark}{=} \frac{P_T + \sqrt{P_T^2}}{2}$

Now, we can ask questions about fraction of protein bound, fraction of ligand bound etc...

fraction protein bound, $f_{PL} = \frac{P_T - [P]}{P_T} = 1 - \frac{-(K_D + L_T - P_T) + \sqrt{(K_D + L_T - P_T)^2 + 4P_T K_D}}{2P_T}$

now check limits

expect $\lim_{K_D \to 0} f_{PL} = 1 \overset{\checkmark}{=} 1 + \frac{(L_T - P_T) - \sqrt{(L_T - P_T)^2}}{2P_T}$

infinitely small dissociation constants suggest everything is bound... if there is enough ligand

expect $\lim_{P_T, K_D \to \infty} f_{PL} = \phi = 1 + \frac{(K_D - P_T) - \sqrt{(K_D - P_T)^2 + 4P_T K_D}}{2P_T}$

this limit implies that there is absolutely no binding $\overset{\checkmark}{=} 1 + \frac{(K_D - P_T) - \sqrt{(K_D + P_T)^2}}{2P_T}$

How Enzymes Work

Impact on activation energy….

How do enzymes accelerate reactions?

Stabilize transition states – active site binds transition state complex most tightly

proximity effect – they bring reactants (substrates) close together by binding them

orientation effect – bound reactants (substrates) are held in a position relative to one another that facilitates reaction

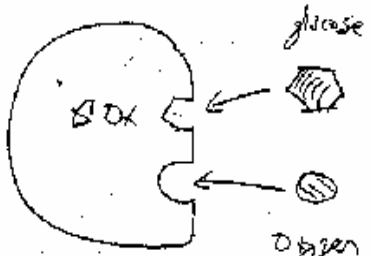
all catalysts – biological (e.g. enzymes, ribozymes and abzymes) and chemical (e.g. Grubbs used for alkene) work this way

2- enzymes are specific – only glucose, out of all the substances in blood, binds and reacts via GOX

specificity or specificity means that only the particular reactants (substrates) that the enzyme uses in reactions can be bound – lock and key model

GOX = glucose oxidase, an enzyme that catalyzes the oxidation of glucose with O_2 to form gluconic acid

e.g.



other species won't have the right shape, size or chemical composition to bind

the binding forces are the "active sites"
this is where the rxn takes place

Enzyme Kinetics

Consider an enzyme E and a substrate S. Enzyme catalysis happens by binding of the substrate to the enzyme, whereupon a reaction occurs. Then the product dissociates from the enzyme, freeing up the enzyme to bind to another substrate molecule. An enzyme usually has one *active site* where the binding occurs. Sometimes there can be more than one, but we will focus on the case of 1 site. Since there is one site only, when it is bound to a substrate, that site cannot bind another one until the substrate leaves.

This is represented by the following sequence of events:

$$E + S \underset{k_r}{\overset{k_f}{\rightleftarrows}} ES \xrightarrow{k_{cat}} E + products \qquad [1]$$

where the constants k_f, k_r, and k_{cat} are the rate constants for the forward E+S → ES reaction, for the reverse ES → E+S reaction, and for the ES → E+products reaction. Note that the last reaction is assumed to be irreversible, where the E+S binding is reversible. S can dissociate from E without reacting to form products.

Now, the rate of the reaction, *v*, is equal to the rate at which product P appears.

$$v = \frac{d[P]}{dt} = k_{cat}[ES] \qquad [1]$$

But – how do we know the concentration of ES complex? All we know about the enzyme is the total concentration of enzyme [E_o] in our system. Here we apply a material balance, recognizing that the total amount of enzyme in any form (bound or unbound) does not change:

$$[E_o] = [E] + [ES] \qquad [2]$$

We also know that the concentration of ES complex, [ES] must follow

$$\frac{d[ES]}{dt} = k_f[E][S] - \left(k_r + k_{cat}\right)[ES] \qquad [3]$$

To a very good approximation, we can make the *quasi-steady state approximation*, namely that

$$\frac{d[ES]}{dt} = 0 \qquad [4]$$

In that case:

$$[ES] = \frac{k_f [E][S]}{k_r + k_{cat}} \qquad [5]$$

$$v = k_{cat}[ES] = \frac{k_{cat} k_f [E][S]}{k_r + k_{cat}} \qquad [6]$$

The problem is that we do not know the concentration of free enzyme E, but we can relate it to [S] via eqn. [4]. Substitute $[ES] = [E_o] - [E]$ into eqn. [5]. This gives

$$[E_o] - [E] = \frac{k_f [E][S]}{k_r + k_{cat}}$$

Rearrange:

$$[E] = \frac{[E_o]}{1 + \frac{k_f [S]}{k_r + k_{cat}}}$$

Rearrange some more to get

$$[E] = \frac{[E_o](k_r + k_{cat})}{k_r + k_{cat} + k_f [S]} \qquad [7]$$

Plug eqn [7] into eqn [6]

$$v = \frac{k_{cat} k_f [S]}{k_r + k_{cat}} \frac{[E_o](k_r + k_{cat})}{k_r + k_{cat} + k_f [S]}$$

which is rearranged to

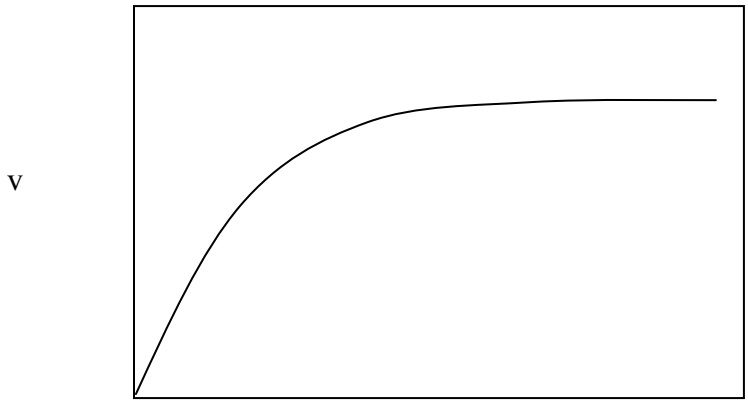
$$v = \frac{k_{cat}[S][E_o]}{\frac{k_r + k_{cat}}{k_f} + [S]}$$

Lumping the collection of rate constants in the denominator, we write this as

$$v = \frac{k_{cat}[S][E_o]}{K_m + [S]} \qquad [8]$$

where K_m is sometimes called the Michaelis-Menten constant.

Equation [8] is called the Michaelis-Menten rate law for enzyme kinetics. It follows the same type of saturation kinetics that we saw for the Monod cell proliferation model.



turnover number
specificity constant

Consider $v = \frac{V_{max}[S]}{K_m + [S]} = \frac{k_2[E_0][S]}{K_m + [S]}$

k_2 (A.K.A. k_{cat}) ≡ the $\frac{\text{turnover number}}{\text{catalytic activity}}$

the maximum amount of substrate that can be converted into product per unit time per unit enzyme (assuming 1 active site per enzyme)

$\frac{k_2}{K_m}$ ≡ the $\frac{\text{specificity constant}}{\text{catalytic efficiency}}$

an effective 2° rate constant for collisions between enzyme and substrate that result in formation of product

$\frac{k_{cat}}{K_m} < 10^8$ to 10^9 $\frac{1}{\text{sec} \cdot M}$ set by diffusion limitations

$K_m \sim$ on the order physiological concentration

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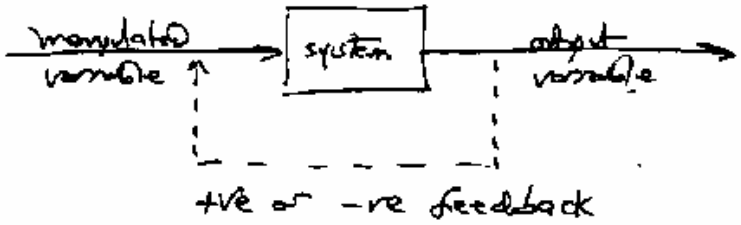
Contexts for Binding

② Control

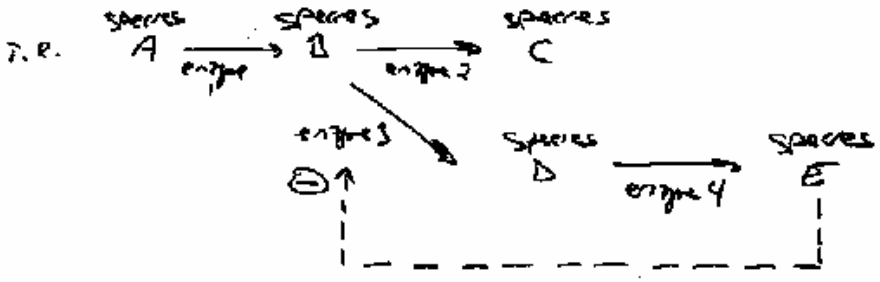
Control and coordination of cellular processes is achieved through binding interactions

Cells continuously exchange flows of C, N and other species through their metabolic networks so that they can survive in diverse environments – don't want an over-abundance or under-abundance of any material

Types of feedback control

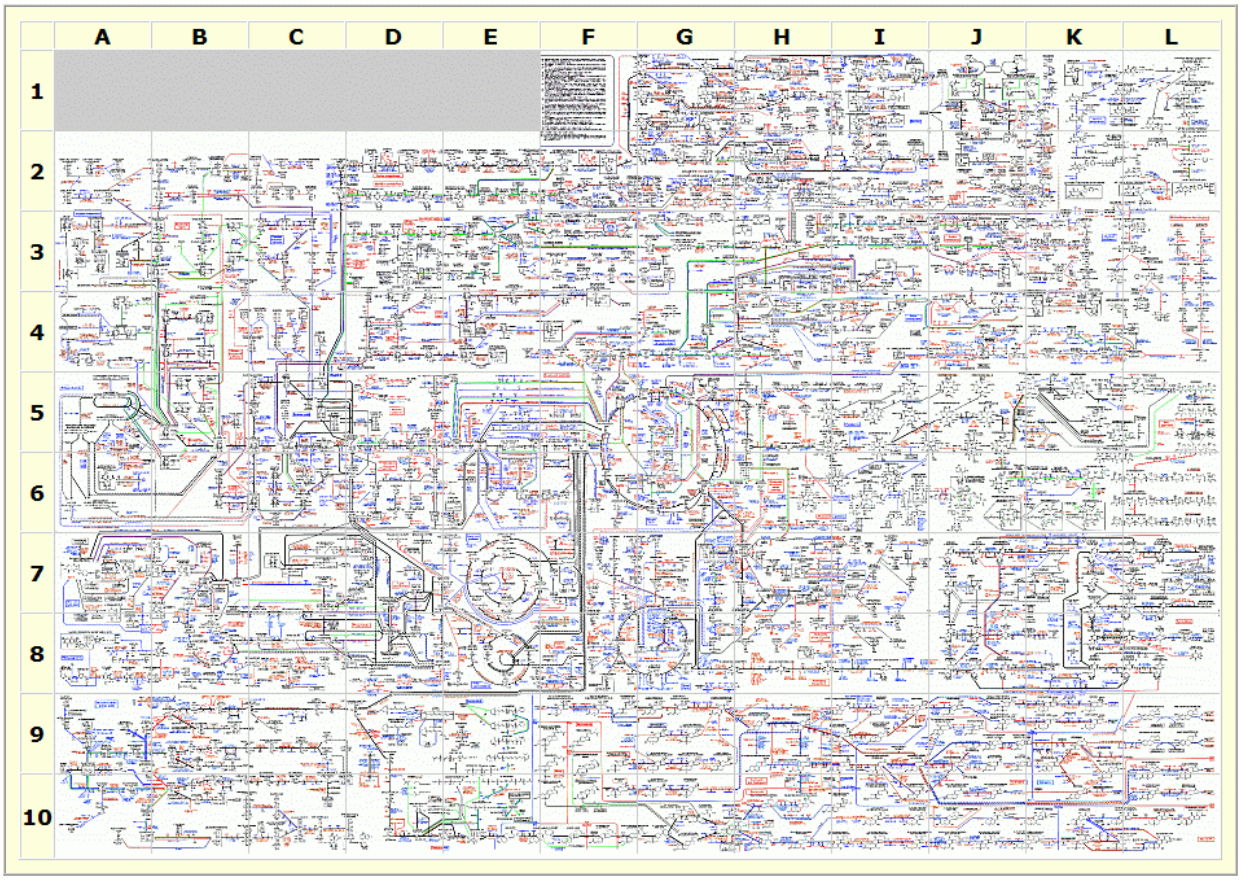


A. Can inhibit an enzyme – manipulate flows through a metabolic pathway: an end product (intermediate) may actually work to turn an enzyme off, or at least reduce its activity



here, an accumulation of species E may be used to turn off or reduce activity of enzyme 3, diverting flow of C, or N to species C instead

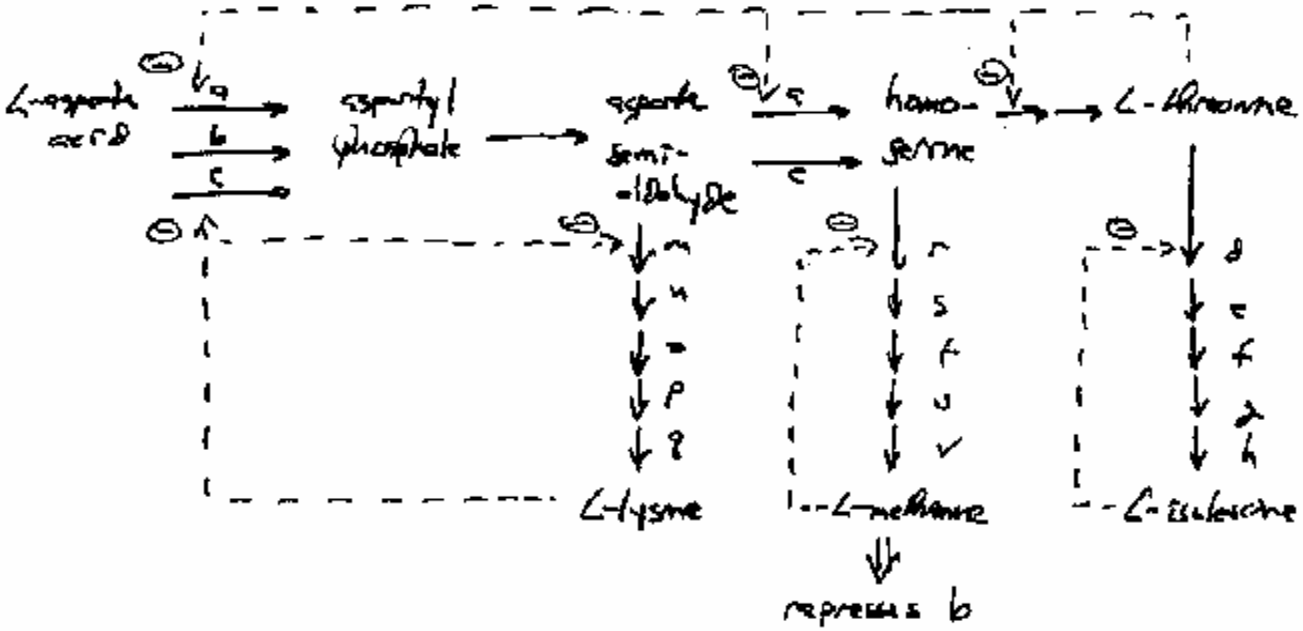
A brief overview of cellular metabolism…



http://www.expasy.org/cgi-bin/show_thumbnails.pl

http://www.genome.jp/kegg/pathway.html

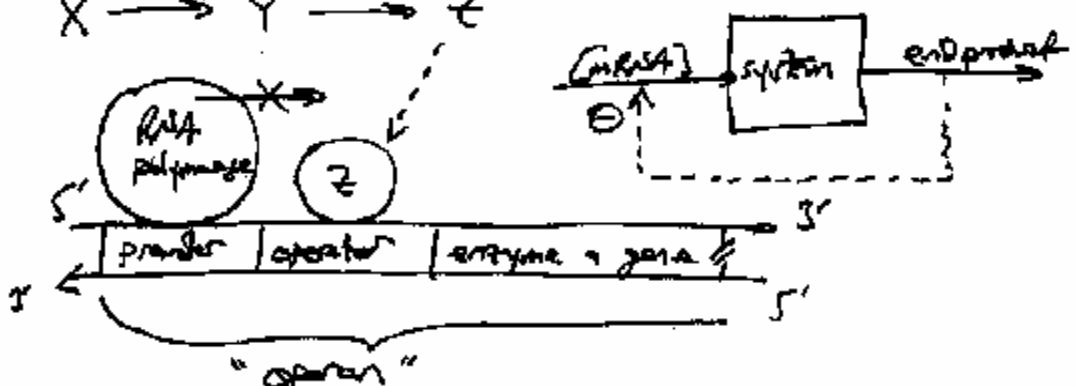
Consider the aspartate pathway in *E. coli*:



control flows of C, N through pathways so that correct levels of L-lysine, L-methionine, L-isoleucine, L-threonine → L-aspartic acid are maintained...

B. Can <u>repress</u> an enzyme: prevent enzyme from being made by binding an intermediate or an endproduct to the DNA that codes for the enzyme (gene), blocking transcription

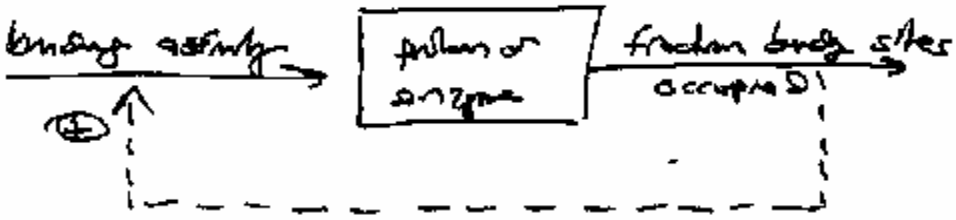
I.P. $X \xrightarrow{a} Y \xrightarrow{b} Z$



Z binds, prevents transcription of enzyme a gene ⇒ no more enzyme a made ⇒ [Z] decays ⇒ Z dissociates ⇒ transcription occurs

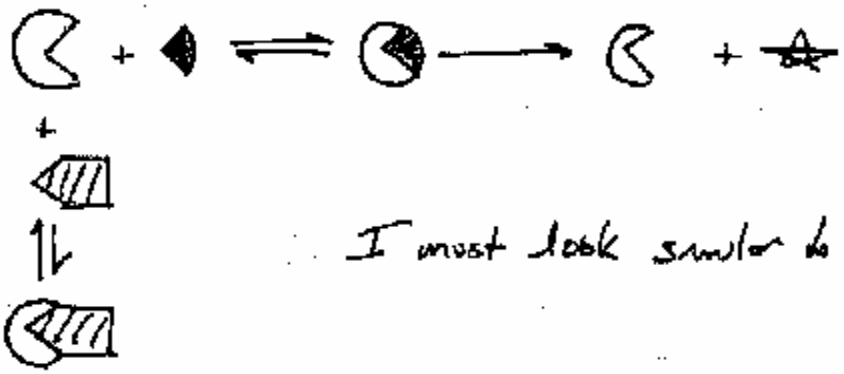
C. Allostery : positive feedback control
some proteins, enzymes have more than one binding site for a ligand or substrate : binding of 1st ligand or substrate increases binding affinity or activity of subsequent ligand or substrate binding

e.g. hemoglobin : binding of 1st molecule of O_2 improves binding strength of 2nd, 3rd, 4th molecule ...



Enzyme Inhibition : common modes of inhibition

1. Competitive inhibition
   inhibitor competes w/ substrate for the active site of enzyme



I must look similar to S...

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

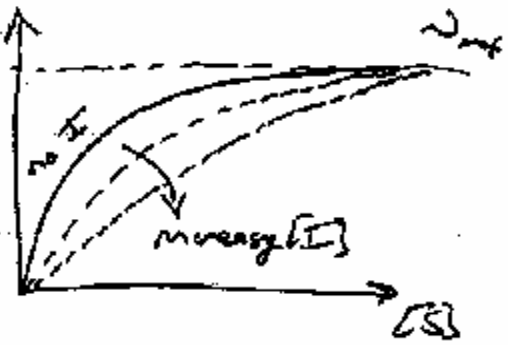
$$+ \; I \qquad k_{I_1} \uparrow\downarrow k_{-I_1} \qquad EI$$

$$K_m \equiv \frac{k_{-1}}{k_1}, \quad \text{substrate dissociation constant} = \frac{[E][S]}{[ES]}$$

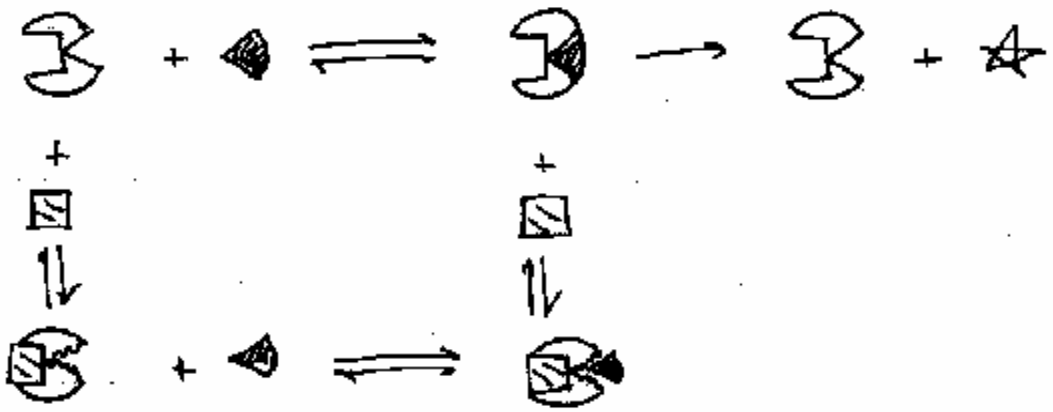
$$K_I \equiv \frac{k_{-I_1}}{k_{I_1}}, \quad \text{inhibitor dissociation constant} = \frac{[E][I]}{[EI]}$$

$$v_{comp} = \frac{v_{max}[S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

effectively increases K_m ⇒ or the [S] required for binding



2. Non competitive inhibition.
Inhibitor binds somewhere other than active site (S binding site) and reduces enzymes ability to bind substrate



$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$+ I \quad \Vert K_I \qquad + I \quad \Vert K_I$$

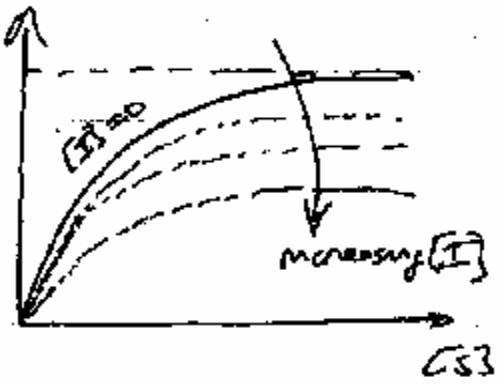
$$EI + S \underset{}{\overset{K_m}{\rightleftharpoons}} ESI$$

$$K_m = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[ESI]}$$

$$K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]}$$

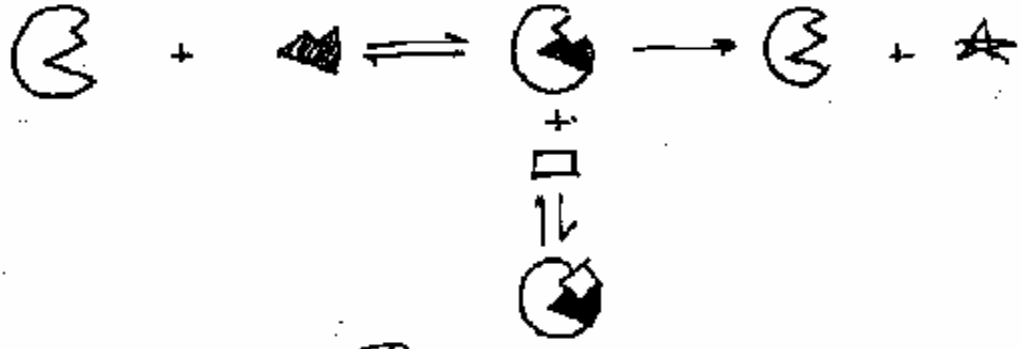
$$v_{non\,comp} = \frac{V_{max}[S]}{(K_m + [S])\left(1 + \frac{[I]}{K_I}\right)}$$

effectively reduces V_{max}



3. Uncompetitive inhibition

uncompetitive inhibitors bind only to the ES complex, not to the enzyme itself



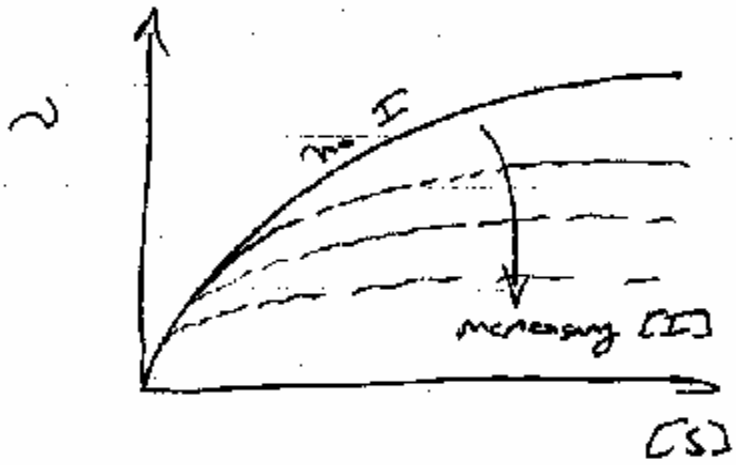
$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$\downarrow\uparrow K_I$$

$$ESI$$

$$v_{uncomp} = \frac{\dfrac{v_{max}}{(1+[I]/K_I)}[S]}{\dfrac{K_m}{(1+[I]/K_I)} + [S]}$$

both v_{max} and K_m reduced



4. Substrate inhibition

special case of uncompetitive inhibition where high concentrations of substrate will lead to binding of another substrate molecule to the ES complex

$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

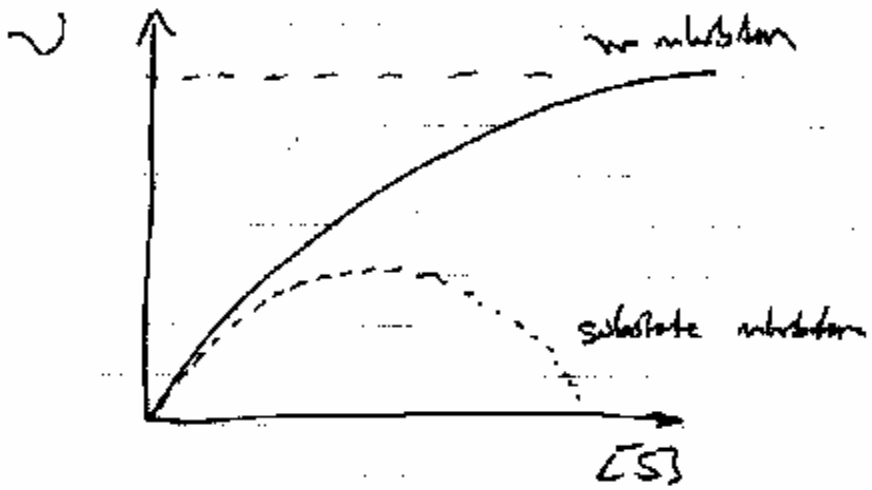
$$+ \; S$$

$$k_{s_2} \; \| \; k_{s_1}$$

$$[ES_2]$$

$$K_s \equiv \frac{k_{s-1}}{k_{s_1}} = \frac{[ES][S]}{[ES_2]}$$

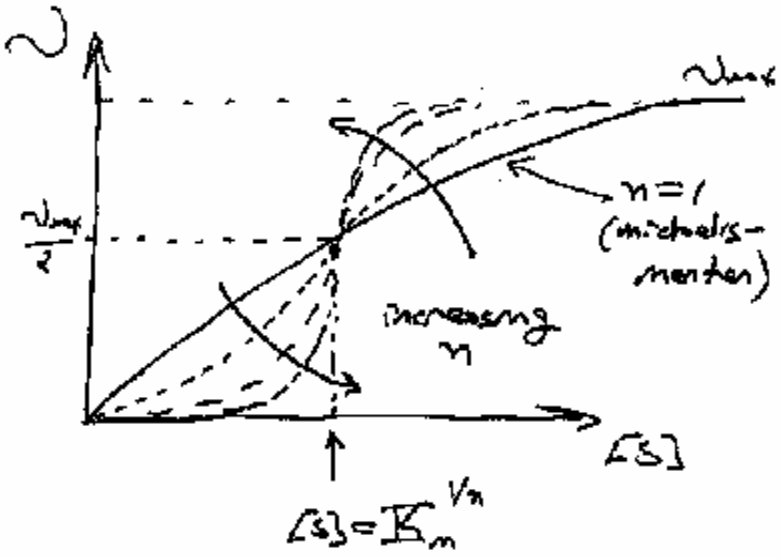
$$v_{sub\ inhib.} = \frac{\frac{v_{max}}{(1+[S]/K_s)}[S]}{\frac{K_m}{(1+[S]/K_s)} + [S]} = \frac{v_{max}[S]}{K_m + [S] + \frac{[S]^2}{K_s}}$$



these inhibitory mechanisms are contrasts control mechanisms

Allostery

$$v = \frac{V_{max}[S]^n}{K_m + [S]^n}$$



$n \equiv$ cooperativity coefficient, corresponds roughly to the number of substrate binding sites

→ as n increases, control becomes on/off or discrete

→ a good way for cell to maintain [S] ~ constant, near K_m