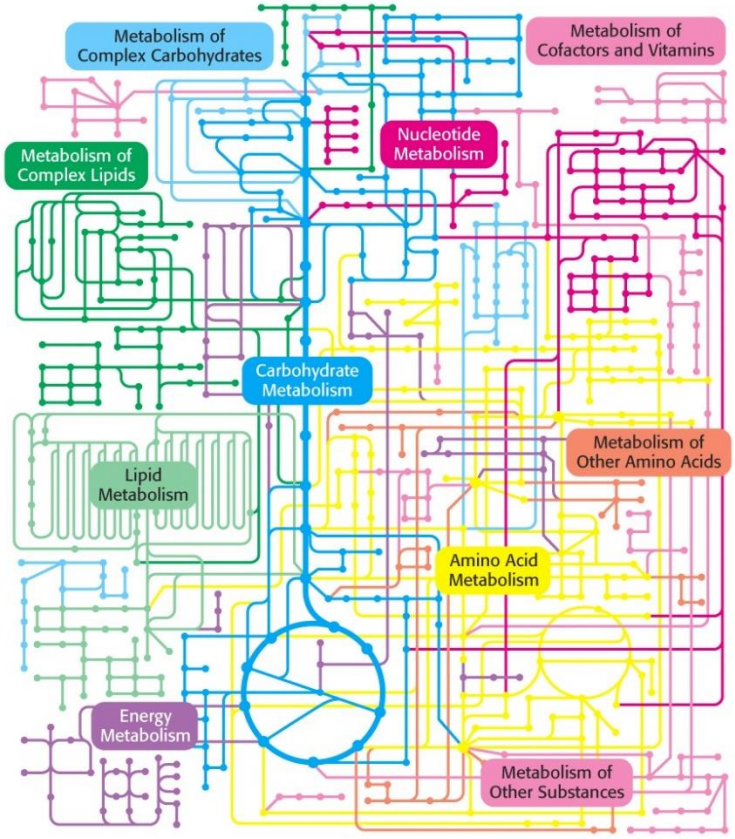
Enzyme kinetics

vladimir.damjanovic@mef.hr

Enzymes - biological catalysts

- enzymes serve as biocatalysts of chemical reactions in living systems (enzymes in every single cell ensure proper order and control of metabolic processes)



- for many chemical reactions in a living cell there is an energy barrier which prevents them from being carried out fast; that energy barrier is overcome by the action of an enzyme

- the role of enzymes in living cells is to speed up energetically favorable reactions to the rate that will enable normal growth and development

Enzymes - biological catalysts

- although enzymes are almost exclusively proteins, some RNA molecules (ribozymes) can also assume the role of biological catalysts

- the most distinguishing enzyme properties are their catalytic power and specificity

Catalytic power: a measure of the ability of an enzyme to accelerate a chemical reaction (compared to non-catalyzed reaction)

- many reactions in biological systems would proceed with negligible rate without the presence of an enzyme

- enzymes accelerate chemical reactions in an organism by factors of more than a million

Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Enzymes - biological catalysts

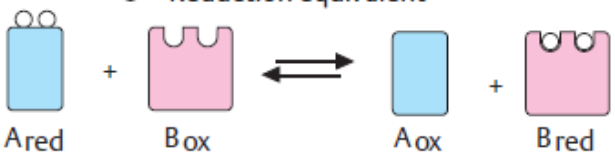
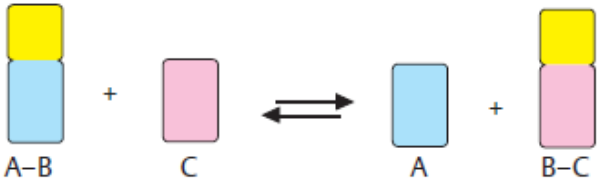
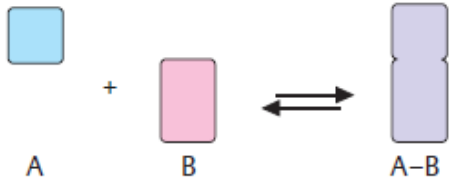
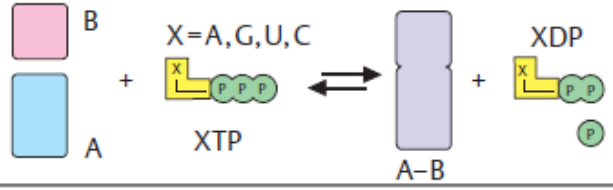
Specificity: enzymes are highly specific in regards to

I. the type of chemical reaction they catalyze

II. choice of reactant (substrate)
    i. absolute specificity
    ii. stereochemical specificity
    iii. group specificity

- specificity of an enzyme is the consequence of a precise interaction between the enzyme and its substrate (as a result of characteristic three-dimensional structure of the enzyme)

International enzyme classification

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent  	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	 	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	 	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	 	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	 	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	 	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

oxidoreductases
oxidation-reduction;
transfer of electrons (hydride ion or H atom)

transferases
group transfer

hydrolases
hydrolysis;
transfer of functional groups to water

lyases
addition to double bonds or elimination of a chemical group with a new double bond formation

isomerases
isomerization;
intramolecular group transfer with isomer formation

ligases
joining of two substrates with ATP consumption;
formation of *C–C*, *C–S*, *C–N* and *C–O* bonds by condensation reaction coupled with ATP hydrolysis

Enzyme structure

- enzymes are active only in their native protein conformation (primary, secondary, tertiary and quaternary structure essential for catalytic activity)

- protein component is enough for catalytic activity of „simple enzymes"

- however, catalytic activity of many „complex enzymes" depends on the presence of small molecules or ions termed cofactors (capable of catalyzing chemical reactions which cannot proceed in the presence of only the standard 20 amino acids in enzyme structure)

- complete catalytically active enzyme (HOLOENZYME): protein part (APOENZYME or APOPROTEIN) and non-protein part (COFACTOR)

- cofactors:

1) metals and metal ions

2) coenzymes (small organic molecules, most often vitamin derivatives)

- depending on the bond they form with the protein portion of the enzyme they can be categorized into:

  a) prostethic groups: coenzymes are tightly bound to the enzyme (with strong noncovalent interactions or, more often, with a covalent bond)

  b) cosubstrates: weakly bound with noncovalent interactions

Cofactors

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

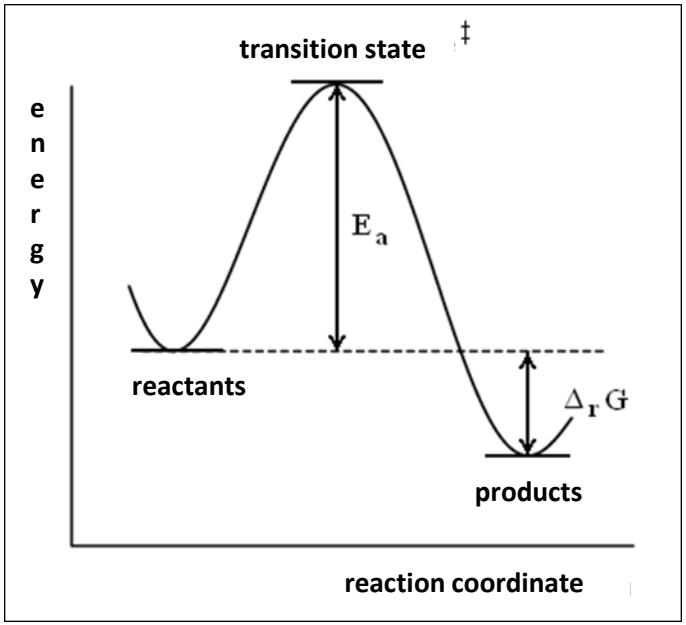
Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Enzymes affect reaction kinetics only, NOT thermodynamics

- even though a reaction may be thermodynamically spontaneous, i.e. exergonic ($\Delta G' < 0$), that doesn't mean that it will occur at an noticeable rate (depending on the activation energy, E_a or $\Delta G^{\ddagger}$)

$$\Delta G' = \Delta G^{\Theta'} + RT \ln Q$$

THERMODYNAMICS
tells us about the direction of a reaction



$$k = Ae^{-\frac{E_a}{RT}}$$

KINETICS
tells us how fast a reaction occurs (reaction rate)

in equilibrium:

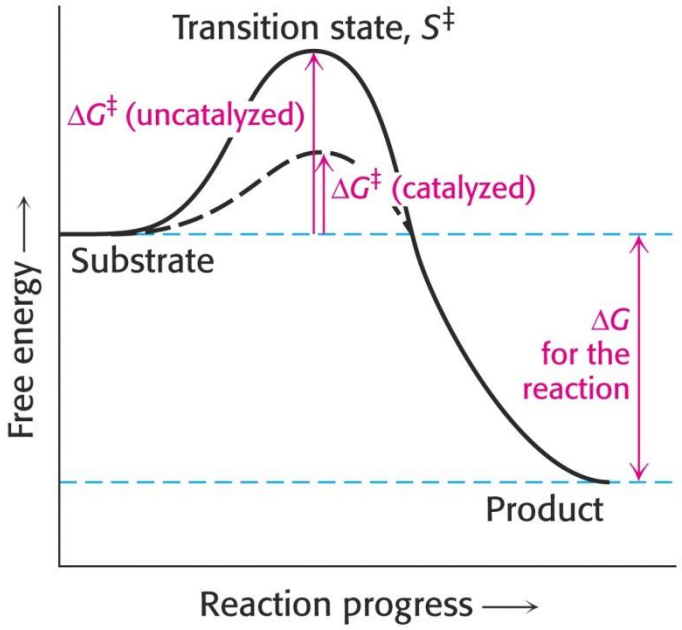
$$\Delta G' = 0$$
$$\Delta G^{\Theta'} = -RT \ln K^{\Theta}$$

- enzymes cannot change thermodynamics laws, hence <u>enzymes cannot change reaction equilibria</u> (which solely depends on the difference of free energy between reactants and products)

- enzymes <u>accelerate the achievement of equilibrium but do not shift the position of equilibrium</u>

Enzymes affect reaction kinetics only, NOT thermodynamics

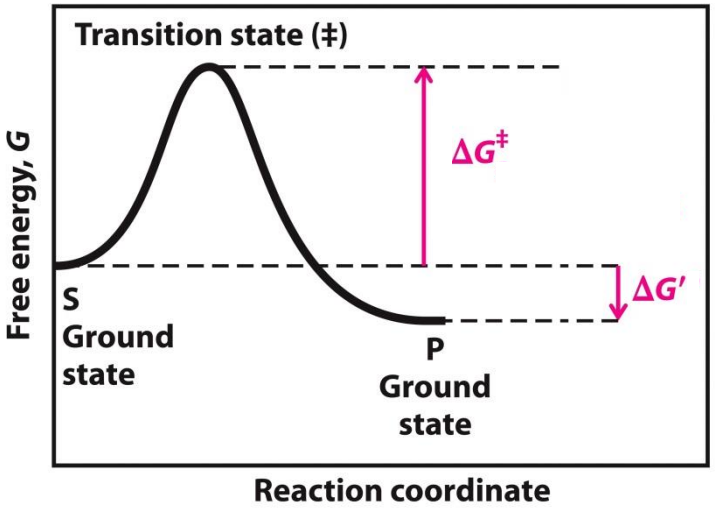
- enzymes accelerate chemical reactions by changing the reaction mechanism (the reaction occurs through different transition states than non-catalyzed reaction)
- reaction occurs through a reaction path in which transition state(s) energy is lower when compared to the transition state(s) energy of an non-catalyzed reaction
- hence, activation energy of a catalyzed reaction is lower than that of an non-catalyzed reaction

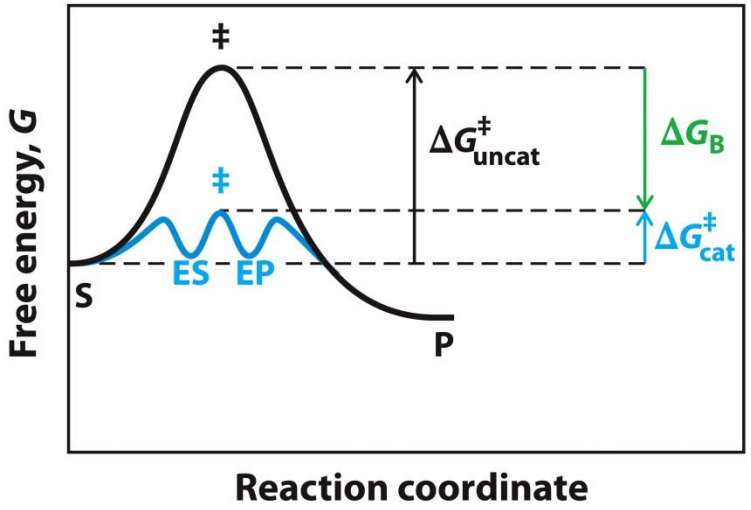


Enzymes affect reaction kinetics only, NOT thermodynamics

- enzymes function to lower the activation energy, or, in other words, enzymes facilitate the formation of the transition state

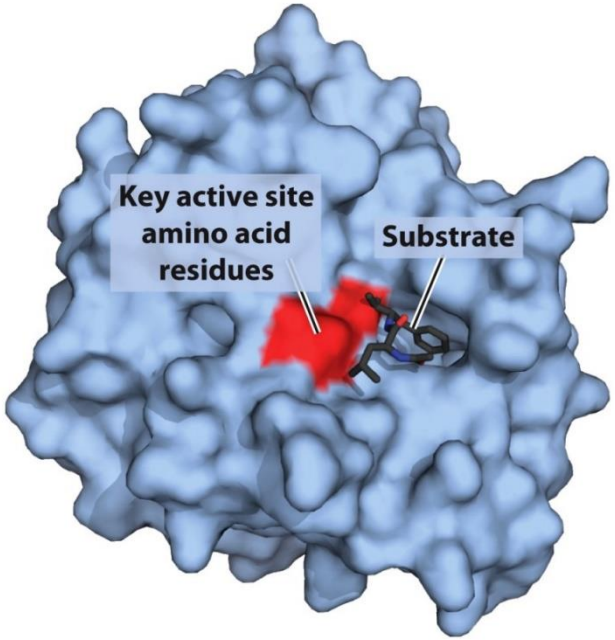
- by stabilizing the transition state (by formation of weak noncovalent interactions between the substrate and the enzyme in the transition state), binding energy, ΔG_B, is released; as a consequence activation energy of the reaction is lower





Enzyme structure: active site as a catalytic center of the enzyme

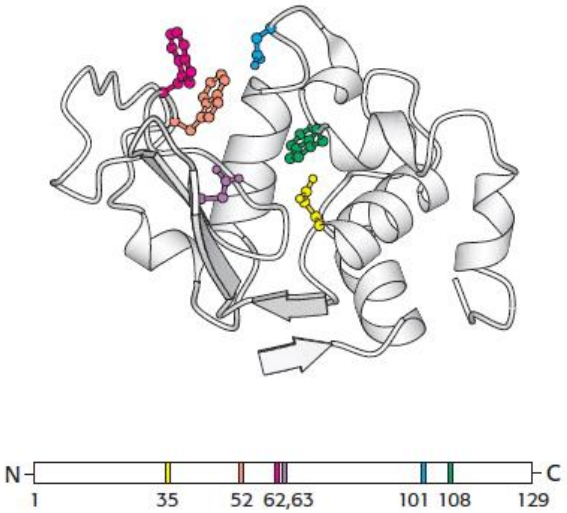
- catalytic power of an enzyme originates form its ability to orientate the substrate in a way that makes the formation of transition state easier
- the enzyme and the substrate form a transient complex termed enzyme-substrate (ES) complex
- substrates (and cofactors, if there are any) bind to a specific part of the enzyme termed active site, which represents the catalytic center
- the surface of the active site is lined with amino acid residues with substituent groups, i.e catalytic groups that bind the substrate and catalyze its chemical transformation; the interaction between E and S in the active site leads to the formation of transition state



Enzyme structure: active site as a catalytic center of the enzyme

- common properties of active sites:

- three dimensional groove or pocket formed by amino acid residues from different parts of primary structure (residues that can be very far apart in the amino acid sequence can interact in the active site)



- they take up a relatively small portion of the whole enzyme macromolecule („the extra" amino acids serve as scaffolding on which a 3D active site is formed, they form regulatory sites and tunnels through which the substrates are transported to the active site)

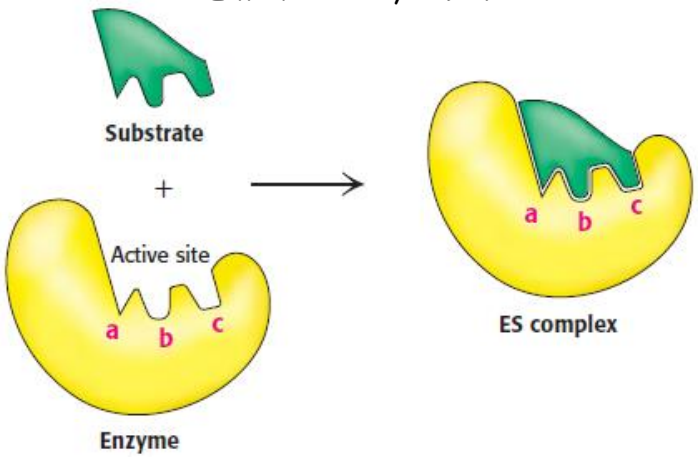
- substrates bind enzymes through many weak noncovalent interactions (reversible interactions such as electrostatic interactions, H bonds, van der Waals forces and hydrophobic interactions) → all of these interactions are significant and strong enough only when multiple S atoms come into the vicinity of multiple E atoms

Enzyme structure: active site as a catalytic center of the enzyme

- common properties of active sites:

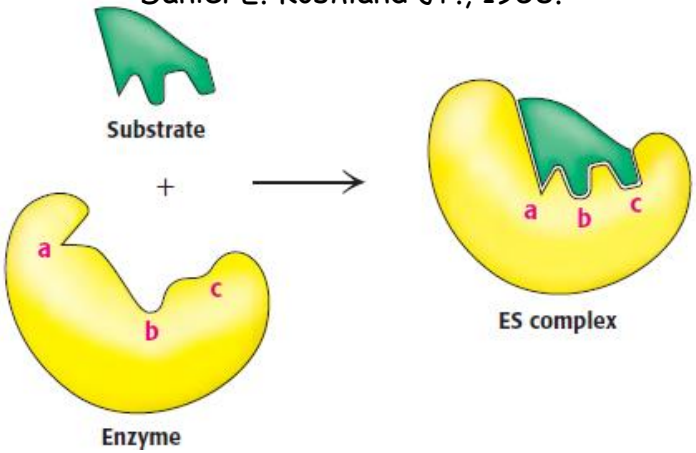
- the specific binding depends on a strictly defined atom layout in the active site: E and S interact with short-range forces that require close contact: <u>that indicates SHAPE COMPLEMENTARITY of the substrate and active site; S needs to have a correct shape in order to fit in the active site</u>

Key and lock model
Emil Fischer, 1890.



- rigid model

Induced fit model
Daniel E. Koshland Jr., 1958.



- enzymes are flexible and the shape of active sites can significantly change when substrates are bound; active sites of some enzymes adopt a structure complementary to the substrate only AFTER the substrate is bound ("hand in the glove")

- active site with its shape (geometric complementarity) and amino acid residue layout (electron complementarity) specifically recognizes the reaction substrates and binds them in the vicinity and in orientation that is suitable for that chemical reaction

- *the full set of interactions is achieved when the substrate is in the transition state*

Factors that affect enzyme activity

- enzyme activity actually represents the rate of an enzyme-catalyzed reaction
- the following factors can affect the rate of an enzyme-catalyzed reaction:

I. substrate concentration, [S]

II. enzyme concentration, [E]

III. the pH of the reaction environment

IV. temperature, T

V. the presence of certain effectors: activators and inhibitors

Michaelis-Menten model

- at the beginning of the 20th century the foundations of enzyme kinetics were laid by M. Menten i L. Michaelis

- assumption: substrate S can be transformed to product P only if it comes into functional contact with the enzyme E and forms enzyme-substrate (ES) "complex"

- Michaelis-Menten model is valid for mono-substrate reactions where ES represents all of the forms of the complex (starting from the unchanged substrate, through all of the intermediate states, to the EP complex)

k_1 – formation (of ES from E and S) rate constant
k_{-1} – dissociation (of ES to E and S) rate constant
k_2 - formation (of P from ES) rate constant
k_{-2} - formation (of ES from E and P) rate constant

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Maud Menten
1879-1960

- the model assumes that initial rate (v_0) is measured; under that conditions the reverse reaction, E + P → ES, can be disregarded since [P] ≈ 0 or/and $k_2 \gg k_{-2}$

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

Leonor Michaelis
1875-1949

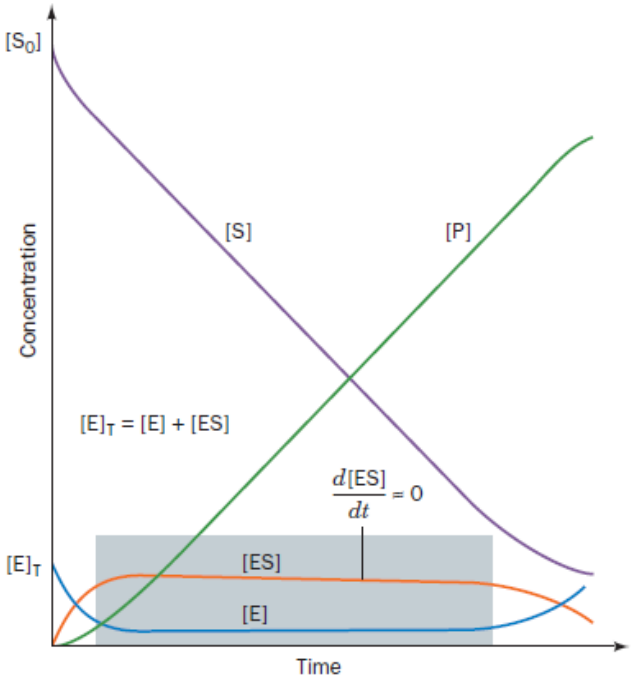
The progress of enzyme-catalyzed reaction

- reaction progresses through three stages until equilibrium is achieved:

1) pre-steady state: ES concentration increases

2) steady state: ES concentration is constant

3) post-steady state: ES concentration decreases



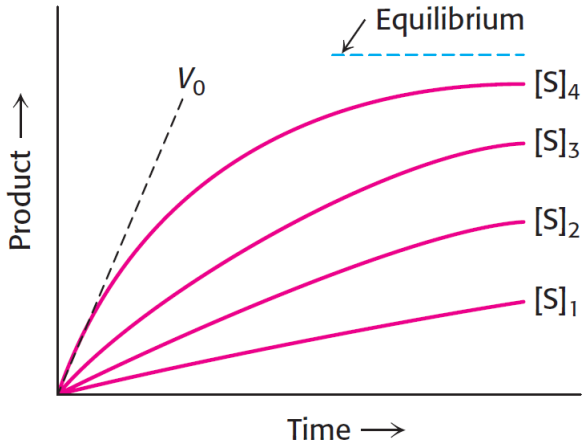
- when E is mixed with a significant excess of S, there is a short period of pre-steady state (too short to be noticeable by conventional techniques, milliseconds) in which [ES] increases; the reaction soon reaches steady state where [ES] is almost constant → the reaction initial rate mostly reflects steady state and the analysis of initial rates is referred to as steady state kinetics

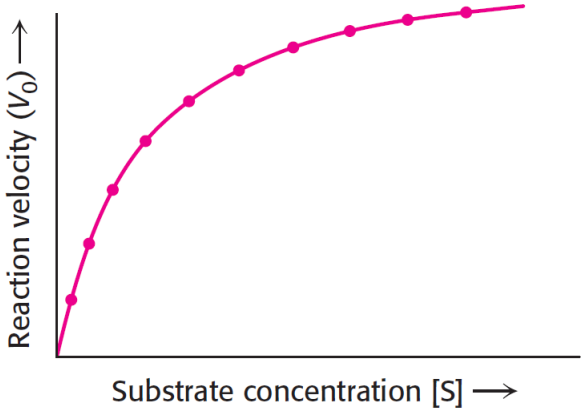
Michaelis-Menten model
Effect of substrate concentration on the rate of an enzyme-catalyzed reaction (with constant enzyme concentration)

- constant enzyme concentration, i.e. [E] = const., defined temperature, pH and ionic strength

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

- determination of initial rates, v_0, for different substrate concentrations, [S], by measuring the rate of product formation at the beginning of the reaction, prior to product accumulation





- the goal is to obtain an expression which shows the dependence of rate of enzyme-catalyzed reaction on substrate and enzyme concentration, as well as the rate of individual steps

Michaelis-Menten model

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

- under the conditions when the reverse reaction can be ignored, initial rate of product formation, v_0, equals:

$$v_0 = k_2\,[ES]$$

- the assumption of steady state for intermediar ES complex ($d[ES]/dt \approx 0$ and $v_{\text{formation ES}} \approx v_{\text{breakdown ES}}$)

$$v_{\text{formation ES}} = k_1\,[E][S]$$
$$v_{\text{breakdown ES}} = k_{-1}\,[ES] + k_2\,[ES]$$

- further on: $[E]_0 = [E] + [ES]$ and $[S]_0 = [S] + [ES] + [P]$

- however, since under these conditions $[S]_0 \gg [E]_0$ and under initial rate of the reaction ($t \approx 0$) when $[P] \approx 0$ then $[S]_0 \approx [S]$

$$v_{\text{formation ES}} = k_1\,([E]_0 - [ES])[S]$$
$$v_{\text{breakdown ES}} = (k_{-1} + k_2)\,[ES]$$

Michaelis-Menten model

$$v_{\text{formation ES}} = v_{\text{breakdown ES}}$$

$$k_1([\mathrm{E}]_0 - [\mathrm{ES}])[\mathrm{S}] = (k_{-1} + k_2)[\mathrm{ES}]$$

$$k_1[\mathrm{E}]_0[\mathrm{S}] - k_1[\mathrm{ES}][\mathrm{S}] = (k_{-1} + k_2)[\mathrm{ES}]$$

$$[\mathrm{ES}](k_{-1} + k_2) + [\mathrm{ES}]k_1[\mathrm{S}] = k_1[\mathrm{E}]_0[\mathrm{S}]$$

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E}]_0[\mathrm{S}]}{(k_{-1} + k_2) + k_1[\mathrm{S}]} \Big/ \frac{1}{k_1}$$

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{\left(\frac{k_{-1} + k_2}{k_1}\right) + [\mathrm{S}]}$$

- the above expression can be simplified by introducing a new constant which encompasses these three constants: Michaelis constant K_M

$$K_{\mathrm{M}} = \frac{k_{-1} + k_2}{k_1}$$

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_{\mathrm{M}} + [\mathrm{S}]}$$

Michaelis-Menten model

- by taking the expression for [ES] and substituting it in the expression for initial rate we get the following expression:

$$v_0 = k_2\,[\mathrm{ES}] = k_2 \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}$$

- the highest possible reaction rate will be reached in the conditions when all of the enzymes catalytic sites are saturated with the substrate, i.e. when all enzyme molecules are in the form of ES complex

- in that conditions $[ES] = [E]_0$, and maximum rate of the reaction, V_{max}, equals:

$$V_\mathrm{max} = k_2\,[\mathrm{E}]_0$$

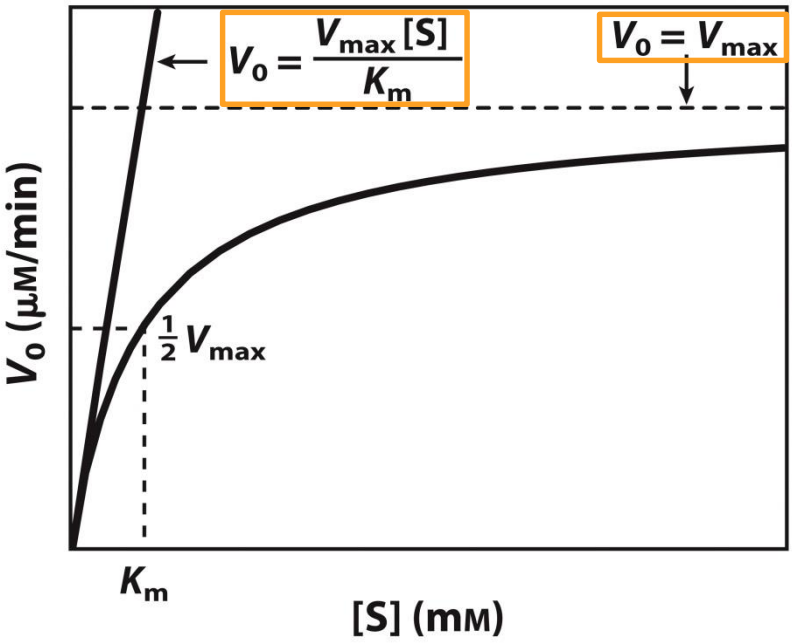
- by substituting V_{max} in the expression for v_0 we get the Michaelis-Menten equation:

$$v_0 = V_\mathrm{max} \frac{[\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}$$

Michaelis-Menten equation

$$v_0 = V_{\max} \frac{[S]}{K_M + [S]}$$

- Michaelis-Menten equation explains the kinetic data of v_0 dependency on [S]



at very low substrate concentrations:

$[S] \ll K_M$

$$v_0 = \frac{V_{\max}}{K_M}[S] = const.\,[S]$$

it is a **first order reaction regarding the substrate** and the rate is proportional to substrate concentration

at very high substrate concentrations

$[S] \gg K_M$

$$v_0 \approx V_{\max}$$

it is a **zero order reaction regarding the substrate**, the rate is maximal, independent of substrate concentration; the reaction proceeds with constant rate

saturation effect (saturation kinetics): substrate is bound to all active sites on enzyme molecules

substrate concentration equal to Michaelis constant

$[S] = K_M$

$$v_0 = \frac{V_{\max}}{2}$$

K_M and V_{max} are important enzyme characteristics; interpreting K_M and V_{max} kinetic parameters

The meaning of Michaelis constant K_M [mol L^{-1}]

- it is specific for a certain enzyme-substrate pair (K_M for a certain enzyme depends on the substrate, but on the reaction conditions also: pH, temperature and ionic strength)

- dual meaning of Michaelis constant:

1.

- K_M provides a measure of the substrate concentration required for significant catalysis to occur, i.e. at which the catalytic effect of an enzyme is significant

- K_M equals substrate concentration at which the reaction rate is half its maximal value, i.e. the concentration of substrate at which half the active sites are filled

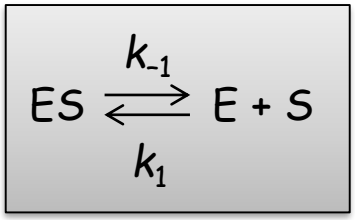
$$K_M = [S] \text{ then } v_0 = V_{max}/2$$

K_M and V_{max} are important enzyme characteristics; interpreting K_M and V_{max} kinetic parameters

The meaning of Michaelis constant K_M [mol L^{-1}]

2.

- K_M equals ES complex dissociation constant (**only when $k_{-1} \gg k_2$**, i.e. when ES complex dissociates to E and S much faster than P is formed)



$$K_M = \frac{k_{-1} + k_2}{k_1} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} = K_d$$

- under these conditions, K_M is a measure of the strength of the ES complex: high K_M value indicates weak binding (ES prone to dissociation), low K_M value indicates strong binding (more stable ES)

- therefore, K_M reflects enzyme affinity towards a certain substrate and tells us how "good of a pair" enzyme and substrate are (the lower the K_M value, the affinity of E towards a specific S is higher and E and S are „a better pair")

K_M and V_{max} are important enzyme characteristics; interpreting K_M and V_{max} kinetic parameters

The meaning of maximal rate V_{max} [mol L^{-1} s^{-1}]

- maximal rate of a reaction reveals the turnover number of an enzyme which is equal to the kinetic constant k_2 (which is also called k_{cat}) when the total concentration of active sites, i.e. total enzyme concentration $[E]_0$ is known

$$ES \xrightarrow{k_2} E + P$$

$$V_{\max} = k_2[\mathrm{E}]_0 \rightarrow k_2 = k_{\mathrm{cat}} = \frac{V_{\max}}{[\mathrm{E}]_0}$$

- turnover number of an enzyme: the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate

Parameters K_M, V_{max} and k_{cat}

- K_M , V_{max} and k_{cat} are main parameters of the efficiency of an enzyme-catalyzed reaction (they are determined experimentally)

k_{cat}/K_M ratio: measure of catalytic efficiency

Enzymes for which k_{cat}/K_M is close to the diffusion-controlled rate of encounter

Enzyme	k_{cat}/K_M ($s^{-1}M^{-1}$)
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β-Lactamase	1×10^8
Superoxide dismutase	7×10^9

Maximum turnover numbers of some enzymes

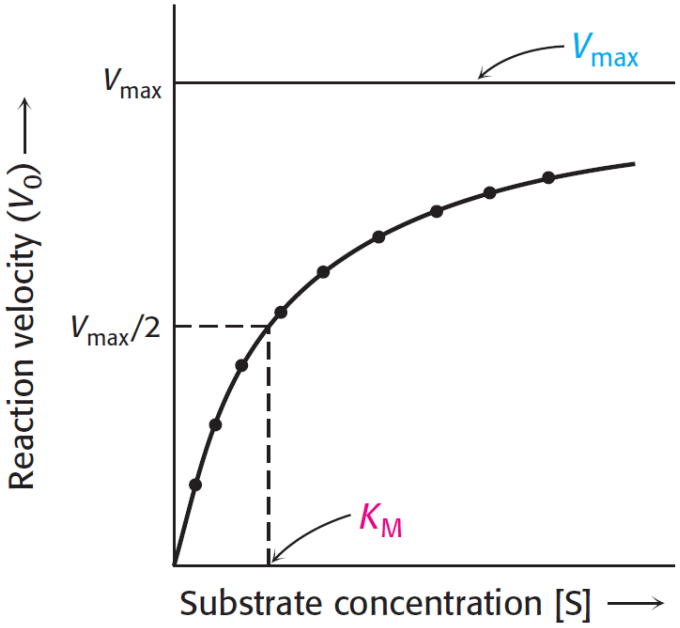
Enzyme	Turnover number (per second)
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

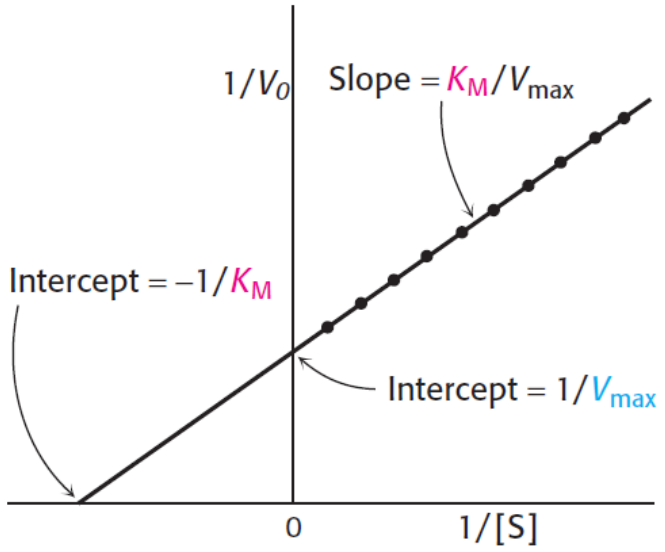
K_M values of some enzymes

Enzyme	Substrate	K_M(μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

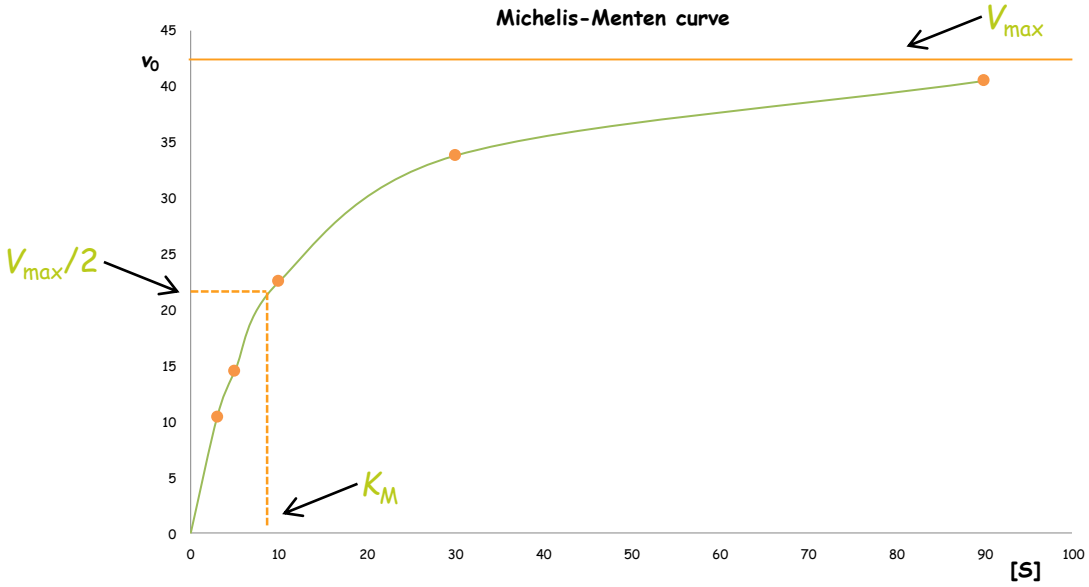
Graphical determination of K_M and V_{max}

- Michaelis-Menten equation describes a rectangular hyperbola with asymptotes in [S] = $-K_M$ i $v_0 = V_{max}$
- it is not possible to precisely determine V_{max} or K_M values
- Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting experimental data and determining K_M and V_{max} parameters



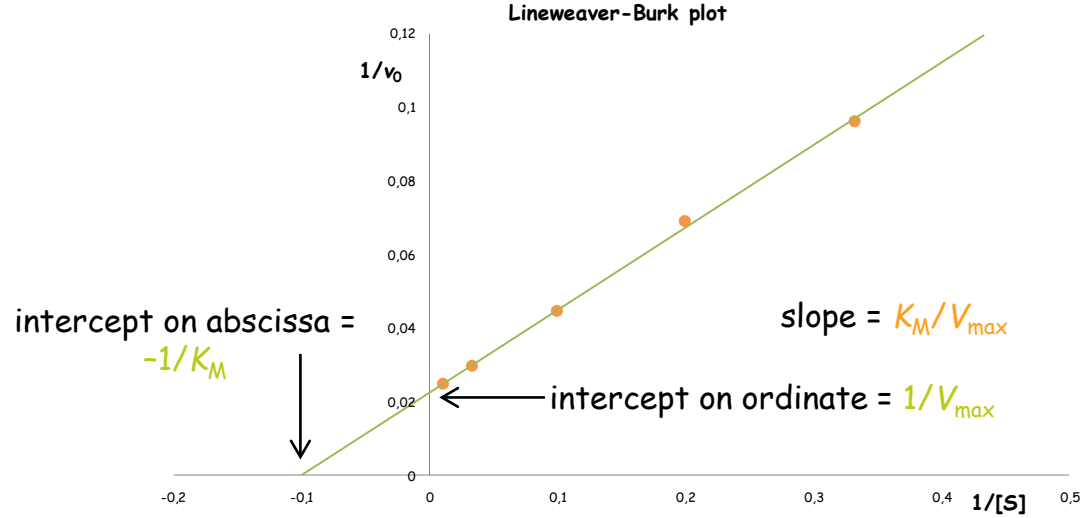


Linearization of Michaelis-Menten equation: Lineweaver-Burk plot



Michaelis-Menten equation

$$v_0 = V_{\max} \frac{[S]}{K_M + [S]}$$



Lineweaver-Burk equation

reciprocal form of Michaelis-Menten equation

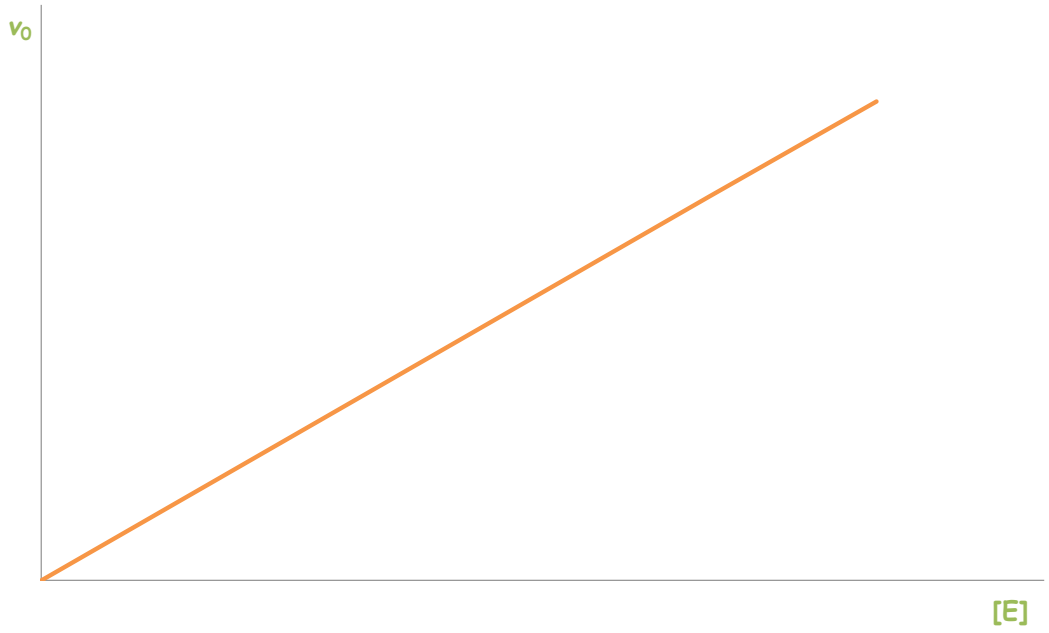
Lineweaver-Burk plot

a plot of $1/v_0$ *versus* $1/[S]$

$$\frac{1}{v_0} = \frac{K_M}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}}$$

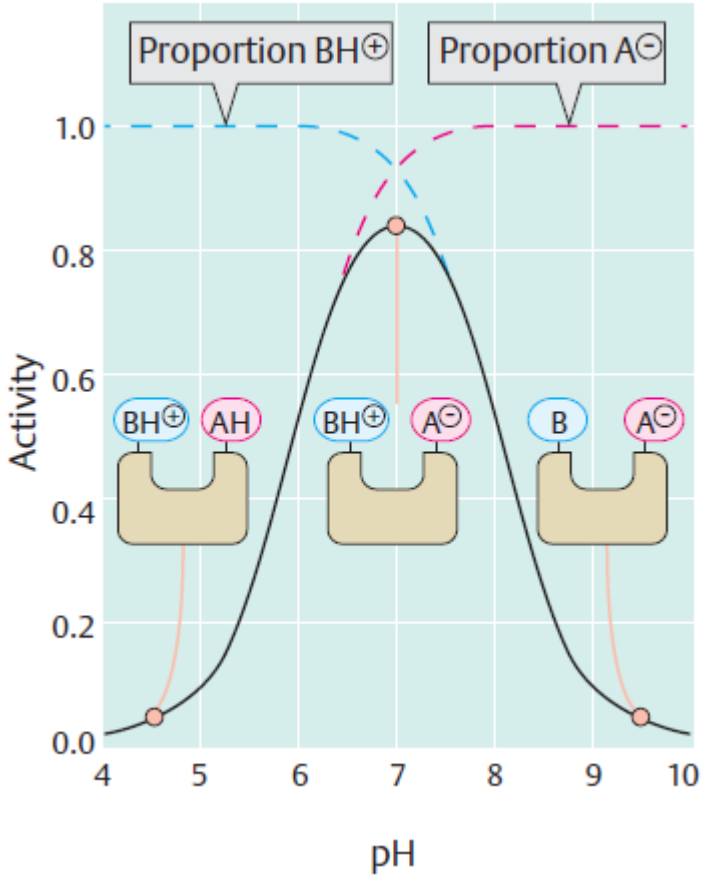
The effect of enzyme concentration on the rate of an enzyme-catalyzed reaction

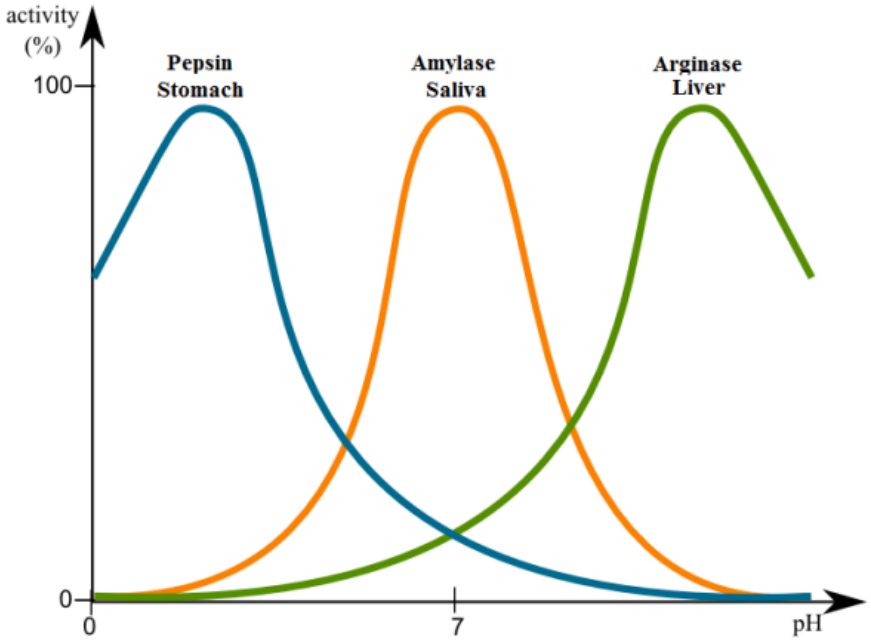
- under conditions when the enzyme is fully saturated with the substrate (zero order reaction regarding the substrate); $c(S) \gg c(E)$
- enzyme activity is proportional to its concentration (first order reaction regarding enzyme concentration: the rate is linearly dependent on [E])



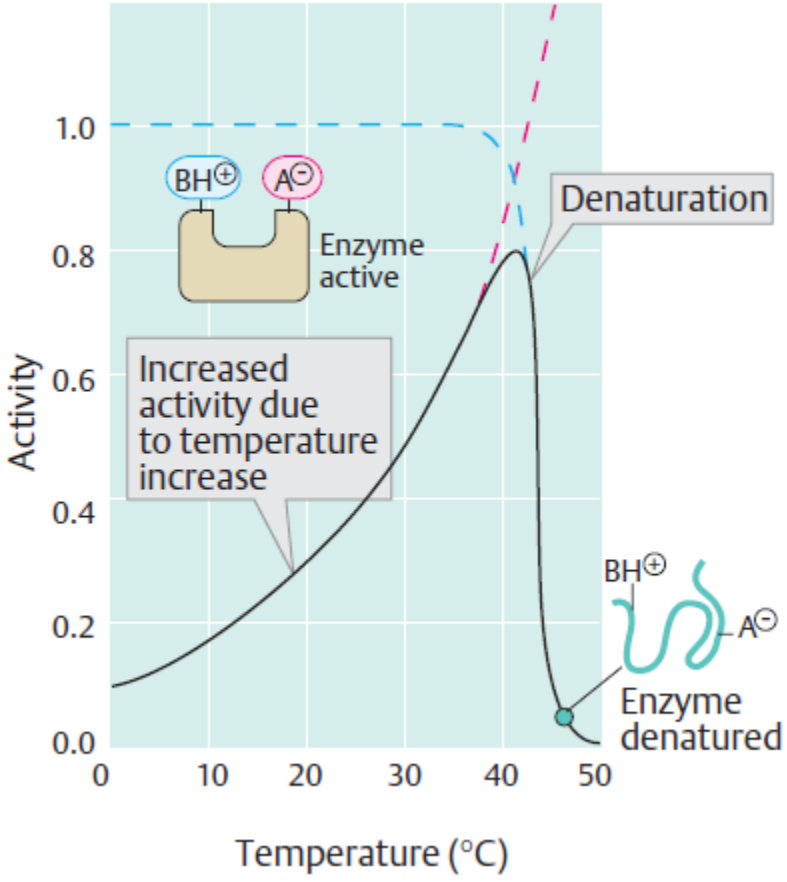
The effect of pH on the velocity of an enzyme-catalyzed reaction

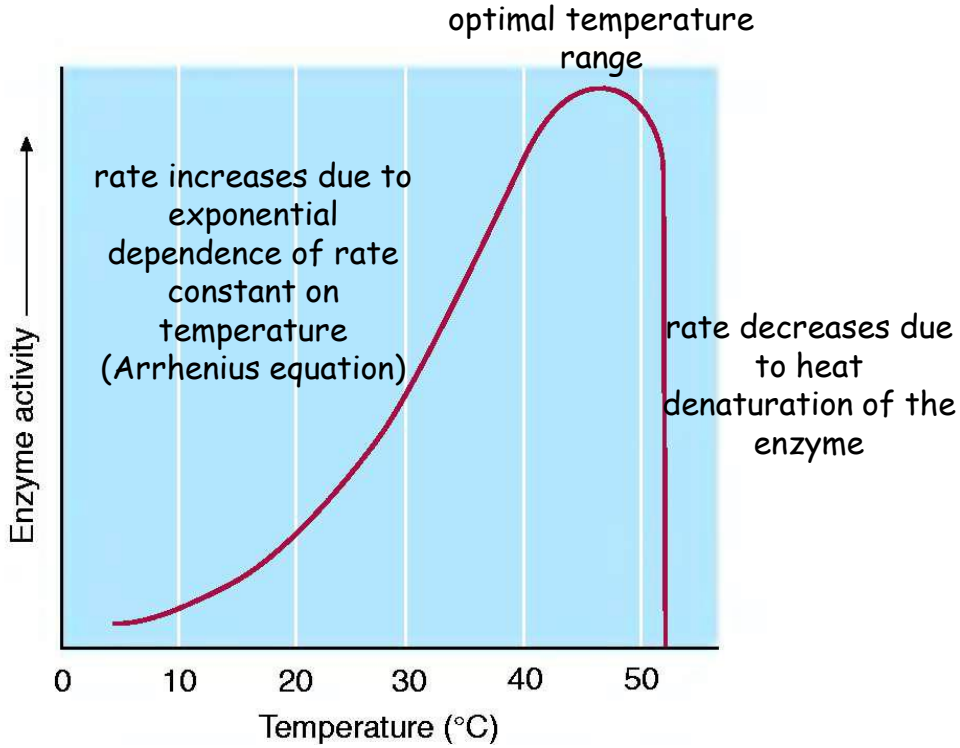
- ionizable amino acid side chains in the enzyme active site
- ionizable substrate functional groups





The effect of temperature on the rate of an enzyme-catalyzed reaction





Inhibition of enzyme activity

- activity of many enzymes can be inhibited by binding specific small molecules or ions
- this means of inhibiting enzyme activity serves as a major control mechanism in biological systems; in addition, many drugs and toxic agents act by inhibiting enzymes
- there are two broad classes of enzyme inhibitors: reversible and irreversible

1) irreversible inhibition

- irreversible inhibitor dissociates very slowly from its target enzyme (dissociation of enzyme-inhibitor complex, EI, is very slow) because it has become tightly bound to the enzyme, either covalently or noncovalently

2) reversible inhibition

- reversible inhibitors are characterized by a rapid dissociation of the enzyme-inhibitor complex, EI, since the "bond" to the target enzyme is much weaker
- according to the specificity of both binding site and enzyme form that is bound by the inhibitor, we can distinguish between three types of reversible inhibition:
  a) competitive inhibition
  b) noncompetitive inhibition
  c) uncompetitive inhibition

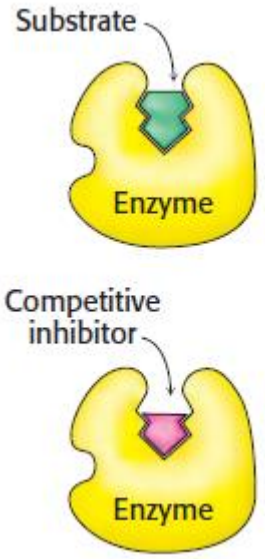
Reversible inhibition
Competitive inhibitor

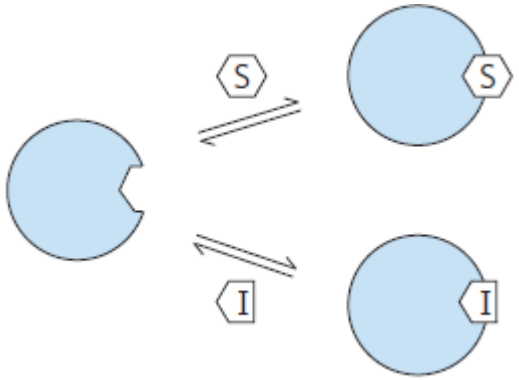
a) competitive inhibitor

- structurally similar to the substrate, so it competes with the substrate for the active site of an enzyme; while the inhibitor (I) occupies the active site, it prevents binding of the substrate to the enzyme

- <u>the enzyme can bind either the substrate (and form ES complex) or the inhibitor (and form EI complex), but never both (ESI complex cannot exist)</u>

- at any inhibitor concentration, the competition can be biased to favor the substrate simply by adding more substrate (S successfully competes with I for binding the active site)

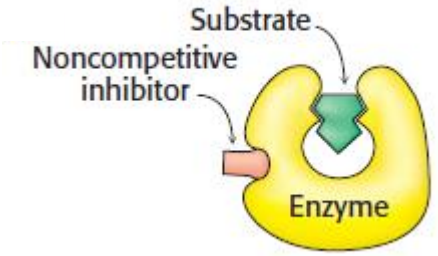


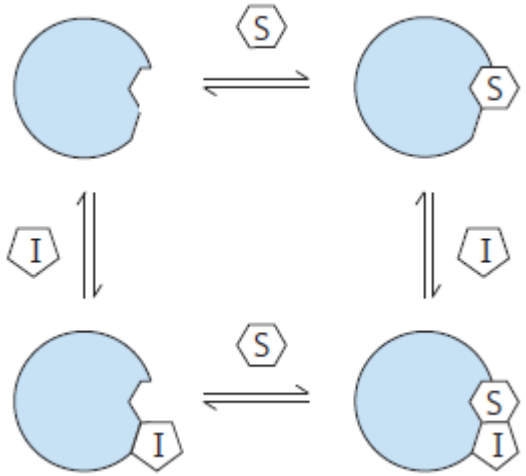


Reversible inhibition
Noncompetitive inhibitor

b) noncompetitive inhibitor

- binds at a site important for enzyme activity, but distinct from the active site
- inhibitor and substrate can bind the enzyme at the same time at different binding sites
- <u>inhibitor can bind the enzyme, but also enzyme-substrate complex; EI as well as ESI complex can be formed</u>
- noncompetitive inhibition cannot be overcome by increasing the substrate concentration

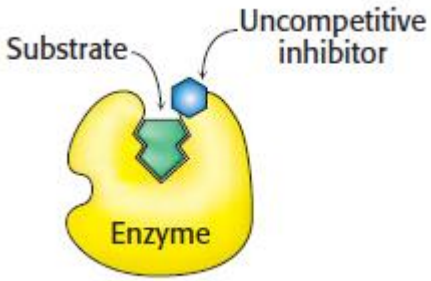


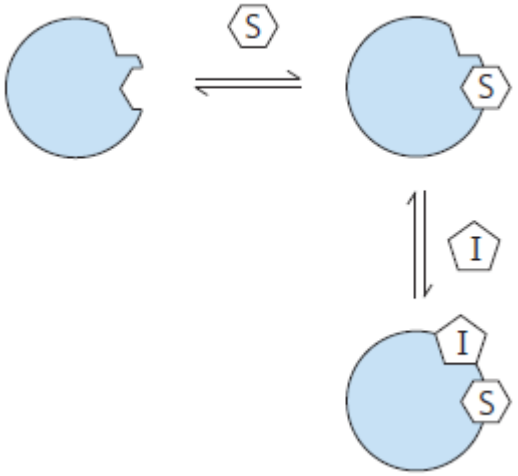


Reversible inhibition
Uncompetitive inhibitor

c) uncompetitive inhibitor

- binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex
- <u>binding site for the inhibitor is formed only after the substrate interacted with the enzyme, only ESI complex is possible</u>
- unncompetitive inhibition cannot be overcome by increasing the substrate concentration

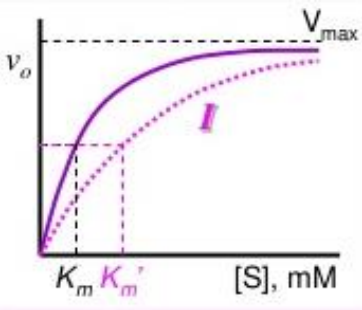




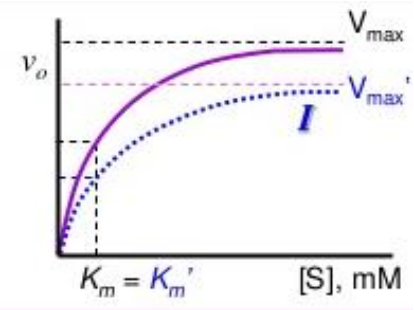
Reversible inhibition

- competitive, noncompetitive and uncompetitive reversible inhibition can be distinguished kinetically
- inhibition type is determined by measuring initial rates at different substrate concentrations without the inhibitor, and in the presence of certain inhibitor concentration

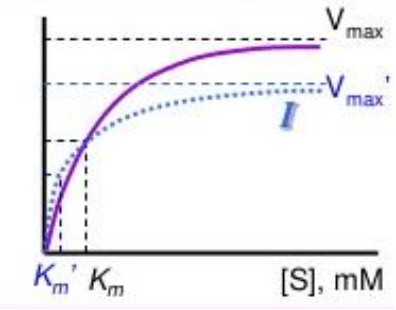
competitive inhibitor



noncompetitive inhibitor

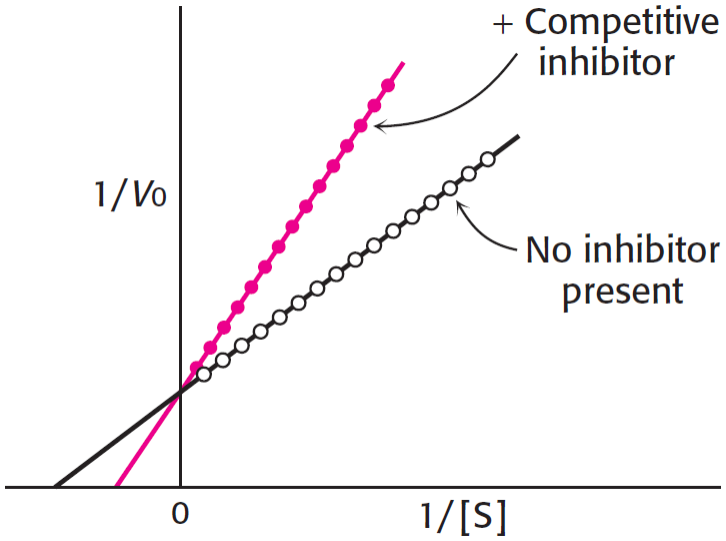


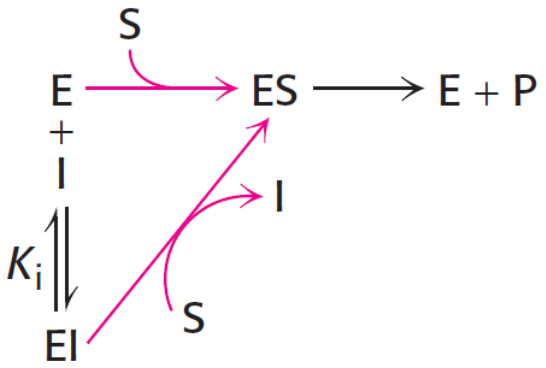
uncompetitive inhibitor



Competitive inhibitor

- increases the apparent value of K_M, does not change V_{max}





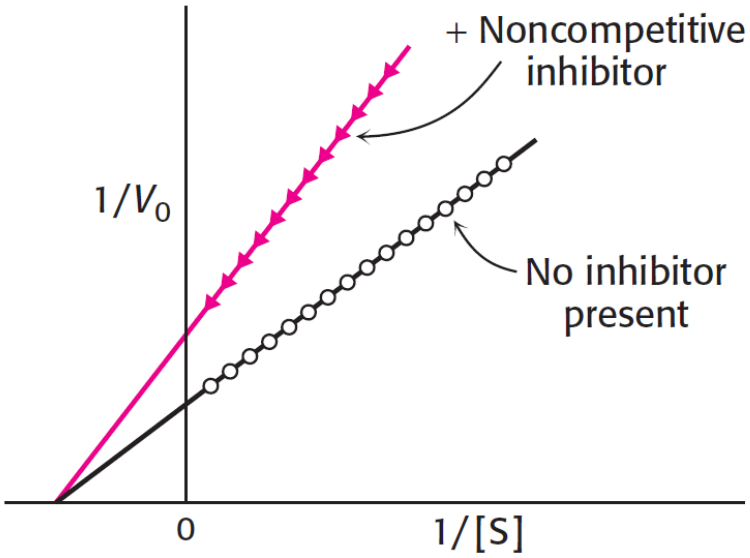
$$K_{M\,i} = K_M\left(1 + \frac{[I]}{K_i}\right)$$
$$V_{max\,i} = V_{max}$$

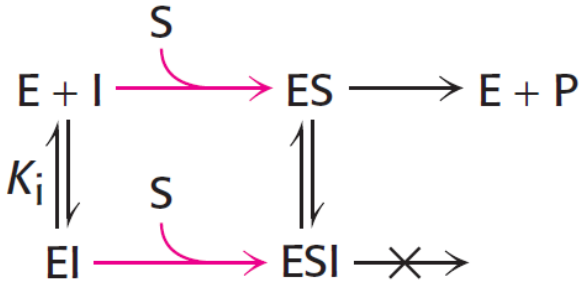
- methanol or ethylene glycol (antifreeze) poisoning is treated with an ethanol solution: competitive inhibition of alcohol dehydrogenase

K_i –inhibitor dissociation constant; the lower the value of the constant, the stronger the inhibitor

Noncompetitive inhibitor

- decreases the apparent value of V_{max}, does not change K_M



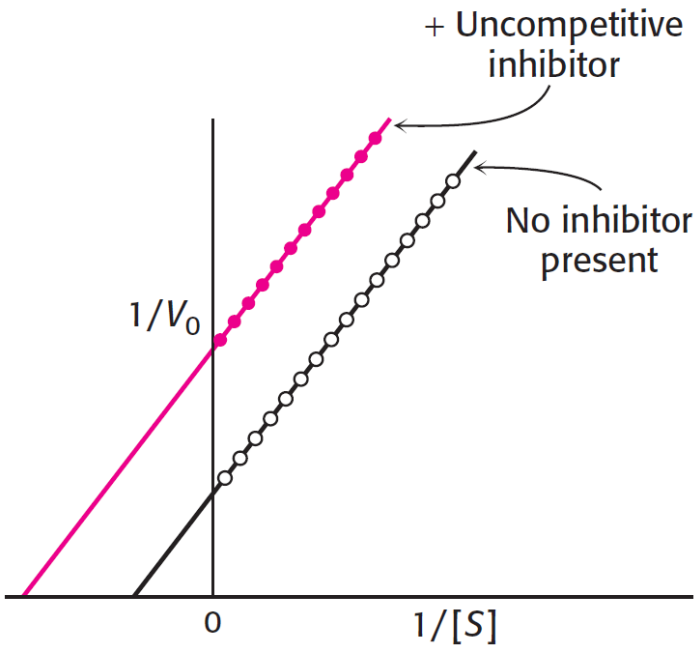


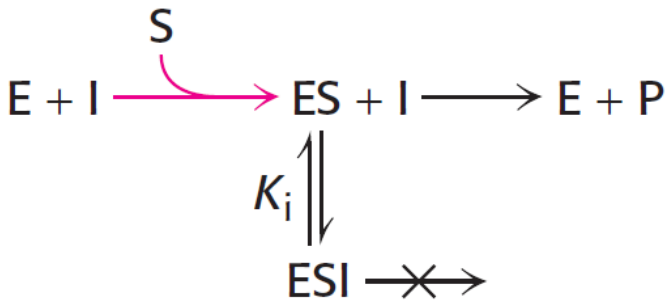
$$K_{M\,i} = K_M$$

$$V_{max\,i} = \frac{V_{max}}{\left(1 + \frac{[I]}{K_i}\right)}$$

Uncompetitive inhibitor

- decreases the apparent values of K_M and V_{max}



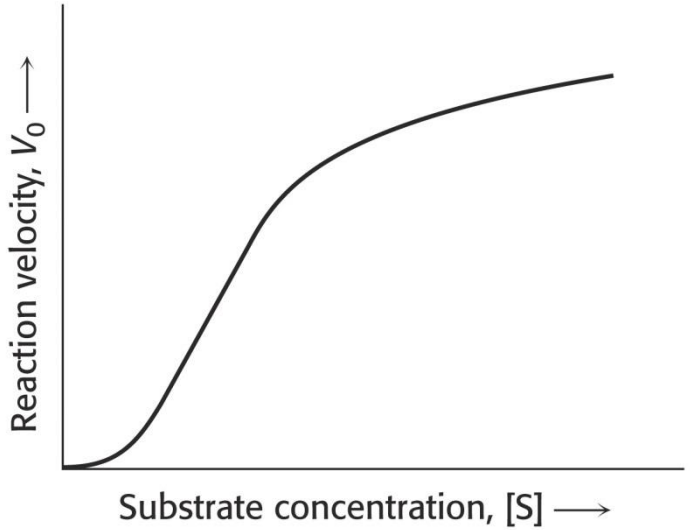


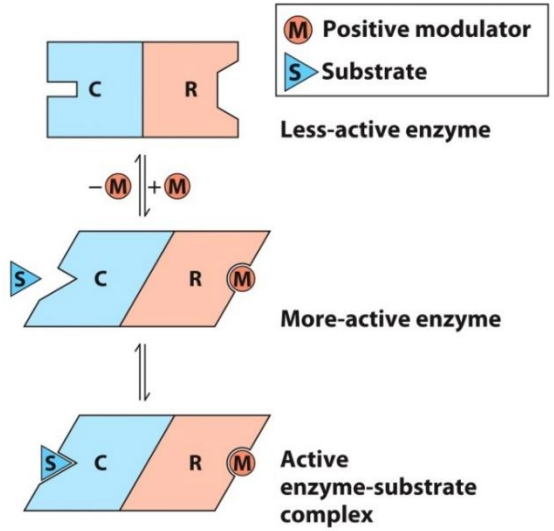
$$K_{M\,i} = \frac{K_M}{\left(1 + \frac{[I]}{K_i}\right)}$$

$$V_{max\,i} = \frac{V_{max}}{\left(1 + \frac{[I]}{K_i}\right)}$$

Allosteric enzymes do NOT obey Michaelis-Menten kinetics

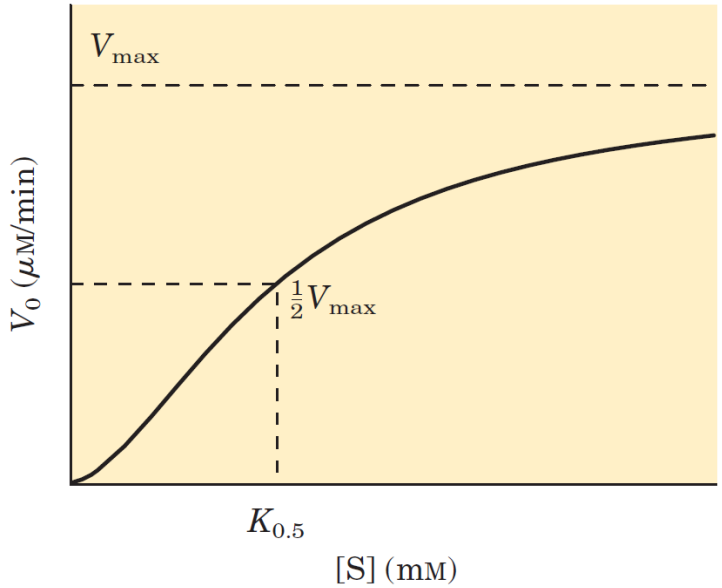
- an important group of enzymes that do not obey Michaelis-Menten kinetics are allosteric enzymes
- allosteric enzymes consist of multiple subunits (quaternary structure) and multiple active sites in one enzyme macromolecule
- allosteric enzymes often display sigmoidal plots of the reaction rate (v_0) versus substrate concentration [S]
- in addition to active sites, allosteric enzymes also contain specific allosteric (regulatory) binding sites which can reversibly bind allosteric regulatory molecules (allosteric activators or allosteric inhibitors) which change the activity i.e. catalytic properties of allosteric enzymes
- the catalytic properties of allosteric enzymes can thus be adjusted to meet the immediate needs of a cell and for this reason, allosteric enzymes are key regulators of metabolic pathways in the cell





Allosteric enzymes do NOT obey Michaelis-Menten kinetics

- sigmoidal plot is characteristic for multimeric enzymes i.e. enzymes with quaternary structure
- sigmoidal plot is a consequence of cooperative substrate binding effect of allosteric enzymes
- cooperative effect: the binding of substrate to one active site can affect the properties of other active sites in the same enzyme molecule; the binding of substrate to one active site of the enzyme facilitates substrate binding to the other active sites



- $[S]_{0,5}$ or $K_{0,5}$ – substrate concentration necessary to achieve half the maximal rate

$[S] = K_{0,5} = [S]_{0,5}$ follows $v_0 = V_{max}/2$

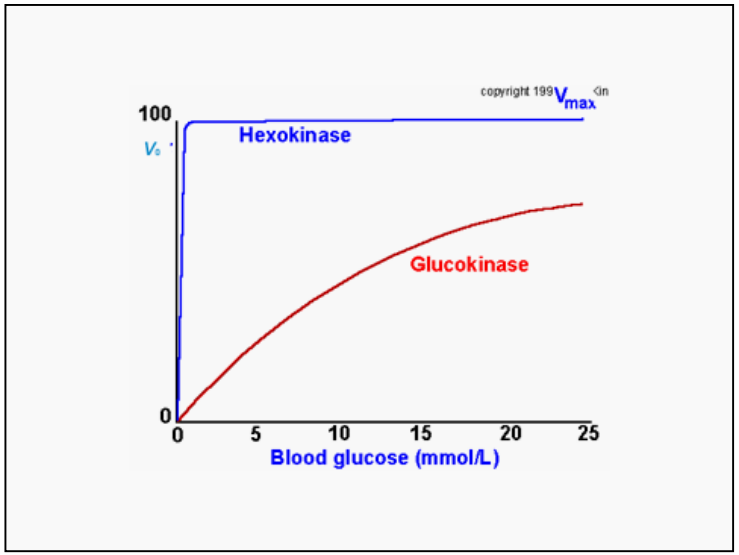
Multiple forms of enzymes (isoenzymes; isozymes)

- isoenzymes: homologous enzymes within a single organism that catalyze the same reaction but differ slightly in structure (amino acid sequence) and more obviously in kinetic parameters, as well as regulatory properties
- they are often expressed in a distinct tissue or organelle or at a distinct stage of development
- isoenzymes provide an avenue for varying regulation of the same reaction at distinct locations or time and in that way enable fine regulation of metabolic processes in order to meet the metabolic needs of a specific tissue or organ

Example: Significance of kinetic parameters of glucokinase (liver) and hexokinase in glucose homeostasis

K_{50} hexokinase II (sceletal muscles) = 0.05 mmol/L

K_{50} glucokinase (liver) = 10 mmol/L



Units used to express enzyme activity

- to express specific enzyme activity at defined temperature, pH, ionic strength and substrate concentration conditions (the enzyme is usually saturated with the substrate) we use:

I. international unit of enzyme activity (U or IU)

- enzyme activity of one U represents the amount of enzyme that catalyzes the formation of 1 μmol of product per minute

$$U = \mu mol\ min^{-1}$$

II. katal (kat)

- enzyme activity of one katal represents the amount of enzyme that catalyzes the formation of 1 mol of product per second

$$kat = mol\ s^{-1}$$

$1\ U = 1{,}6 \cdot 10^{-8}\ kat$
$1\ kat = 6 \cdot 10^{7}\ U$