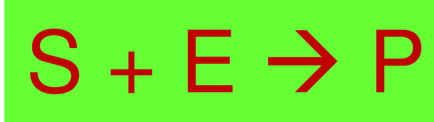
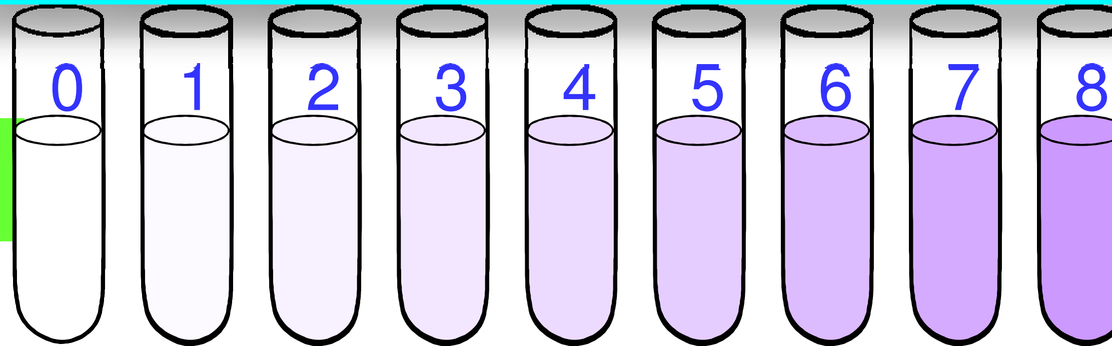
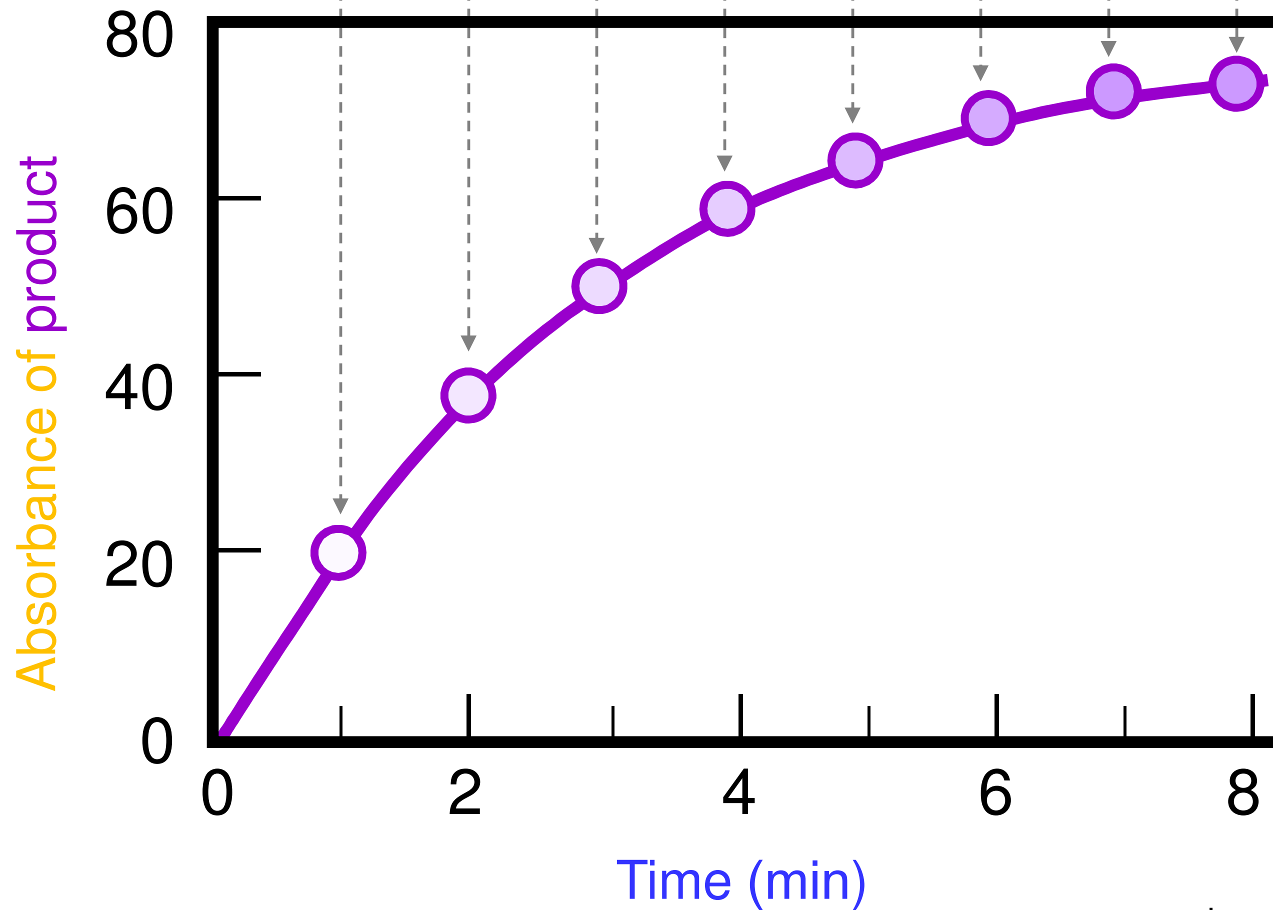
Enzyme Kinetics

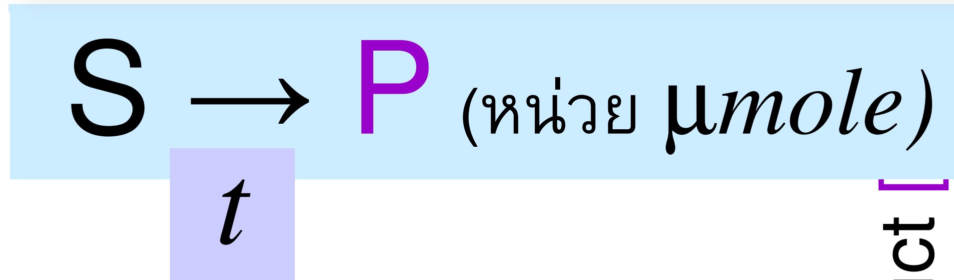






Juang RH (2004) BCbasics

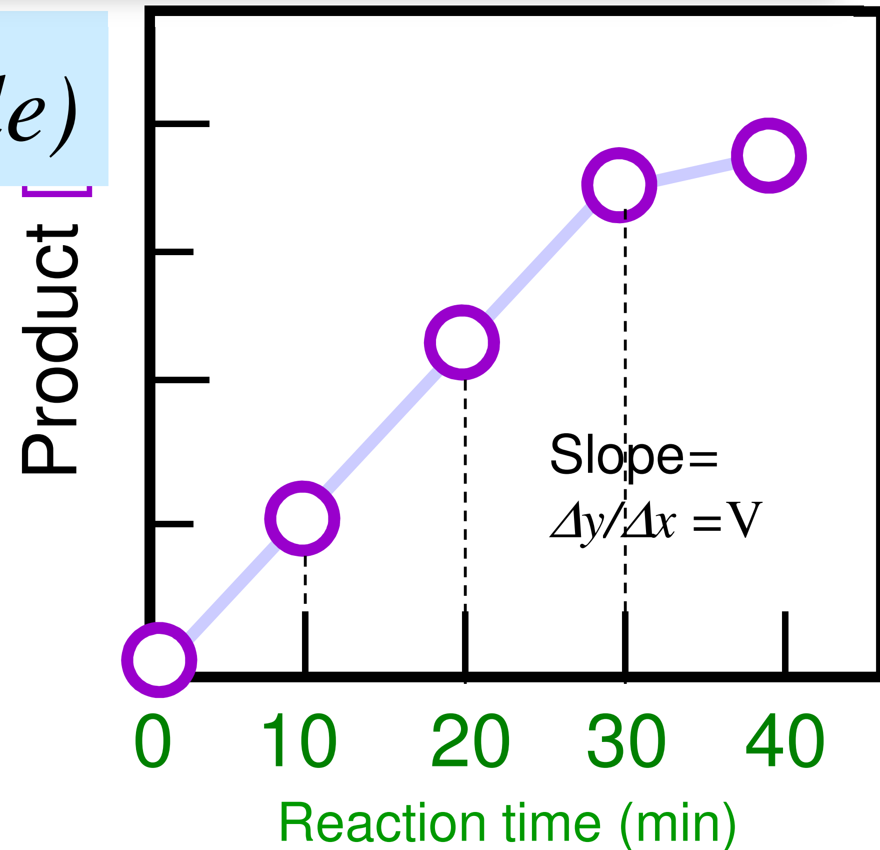
Rate of reaction (V) คือ อัตราการเกิดผลิตภัณฑ์/หน่วยเวลา



$V = [P] / min$

ความเร็วของปฏิกิริยา

อัตราการเกิดผลิตภัณฑ์ ต่อนาที



Enzyme activity = μmole /min

หน่วยของ กิจกรรมของเอนไซม์

ปริมาณสารตั้งต้น ที่ลดลง หรือ
ปริมาณผลิตภัณฑ์ที่เกิดขึ้น ต่อนาที

Enzyme activity คือ อัตราการเกิดผลิตภัณฑ์ หน่วยไมโครโมล/หน่วยเวลาเป็นนาที

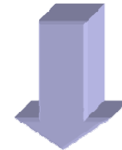
Juang RH (2004) BCbasics

Specific activity = enzyme activity หน่วยเป็น unit/ มิลลิกรัมของโปรตีน

$$V = [P] / min$$

ความเร็วของปฏิกิริยา

อัตราการเกิดผลิตภัณฑ์ ต่อนาที



Enz activity = $\mu mole$ /min

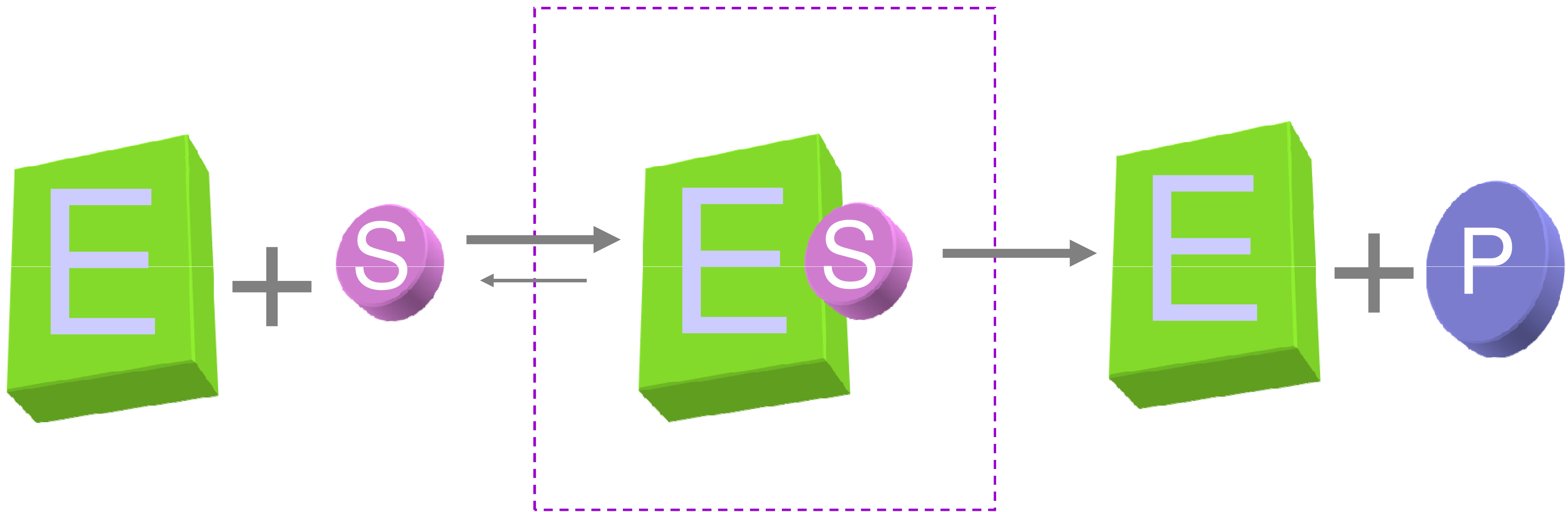
หน่วยของ กิจกรรมของเอนไซม์

ปริมาณสารตั้งต้น ที่ลดลง หรือ
ปริมาณผลิตภัณฑ์ที่เกิดขึ้น ต่อนาที

$$\text{Specific Activity} = \frac{\text{Enz Activity Units}}{\text{Protein (mg)}}$$

Juang RH (2004) BCbasics

Steady state

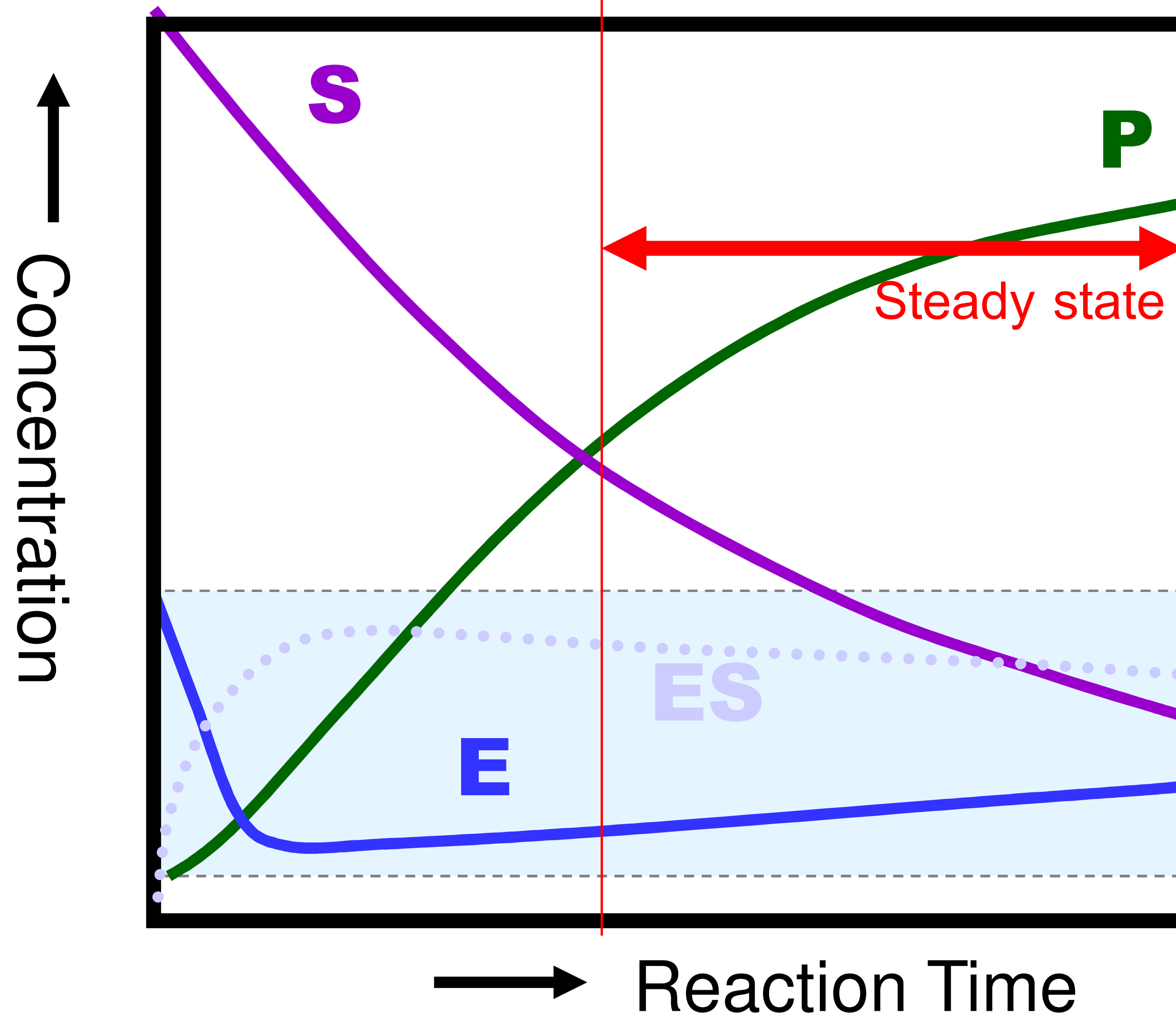


rate of production [ES] = rate of consumption [ES]

So the concentration of [ES] constant.

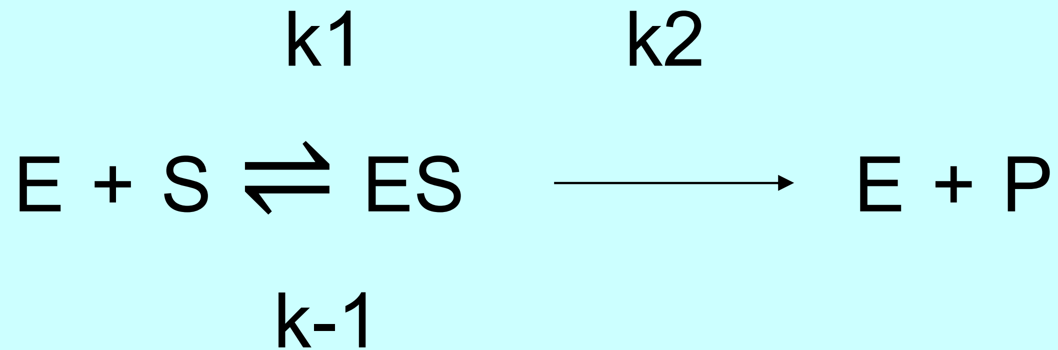
Michaelis-Menten Hypothesis:at Steady state

1) No free Enz (all enzymes are active)
2) [ES] const (according to steady state theory 3) [P] V_{max}



Juang RH (2004) BCbasics

How to derive equation for Km and Vmax



Rate of rx. $V_i = \frac{d[ES]}{dt}$ Vi = initial velocity

$= k_1 [E][S] - k_{-1}[ES] - k_2 [ES]$

$= k_1 \{[E_t] - [ES]\}[S] - k_{-1}[ES] - k_2[ES]$

$= k_1 [E_t][S] - k_1[ES][S] - k_{-1}[ES] - k_2[ES]$

How to derive equation for Km and Vmax (cont.)

Hypothesis → [ES] constant

Thus

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

$$k_1 [E_t] [S] - k_1[ES] [S] - k_{-1}[ES] - k_2[ES] = 0$$

$$k_1 [E_t] [S] = k_1[ES] [S] + k_{-1}[ES] + k_2[ES]$$

$$k_1 [E_t] [S] = [ES] \{k_1 [S] + k_{-1} + k_2 \}$$

$$k_1 [E_t] [S] = [ES] \{k_1 [S] + k_{-1} + k_2\}$$

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

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

Km

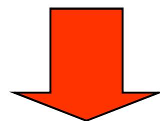
$$k2\,[ES] = Vi$$

$$k2\,[Et] = Vmax$$

$$k2\,[ES] = \frac{k2\,[Et]\,[S]}{[S] + Km}$$

Vmax and Km have meaning
→ application

Km = Michaelis-Menten const.



$$Vi = \frac{Vmax\,[S]}{[S] + Km}$$

Michaelis – Menten equation

Relationship between initial velocity (V) and substrate concentration [S]

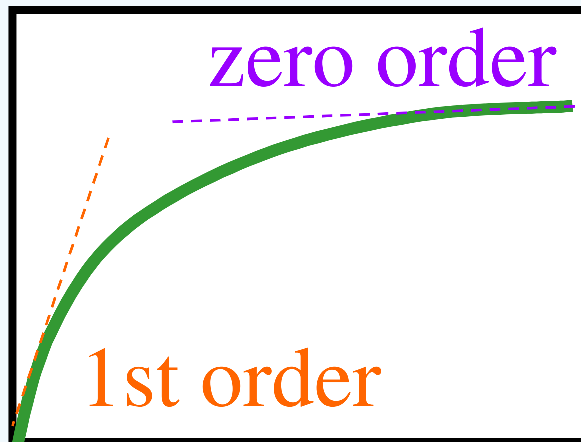
Vmax: Enzyme activity → 1 Enz unit = …μmol/min

Vmax บอกทางอ้อมถึงปริมาณเอนไซม์ ถ้า Vmax มาก คาดว่าปริมาณเอนไซม์มาก

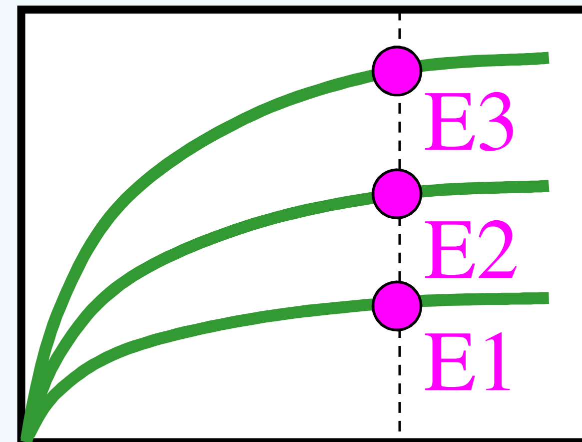
Vmax : enzyme activity

Amount of enzyme = …..μmol/min = 1 Enzyme unit

$$V_i = \frac{V_{max}[S]}{K_m + [S]}$$



[S] = Low → High



[S] = Fixed concentration

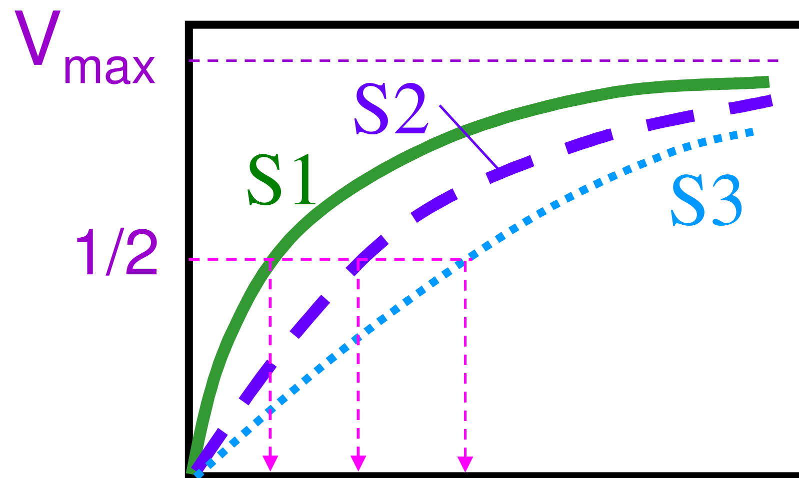
Juang RH (2004) BCbasics

K_m: Affinity with substrate → [S] about 2-5 Km

Km บอกถึงความชอบพอของเอนไซม์กับซับสเตรท Km กับซับสเตรทใดน้อย คาดว่าเอนไซม์ชอบซับสเตรทนั้นมาก

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

When using different substrate



S1 S2 S3

K_m Affinity changes

- Km is a constant
- Small Km means tight binding
- High Km means weak binding
- Useful to compare Km for different substrates for one enzyme

Hexokinase :

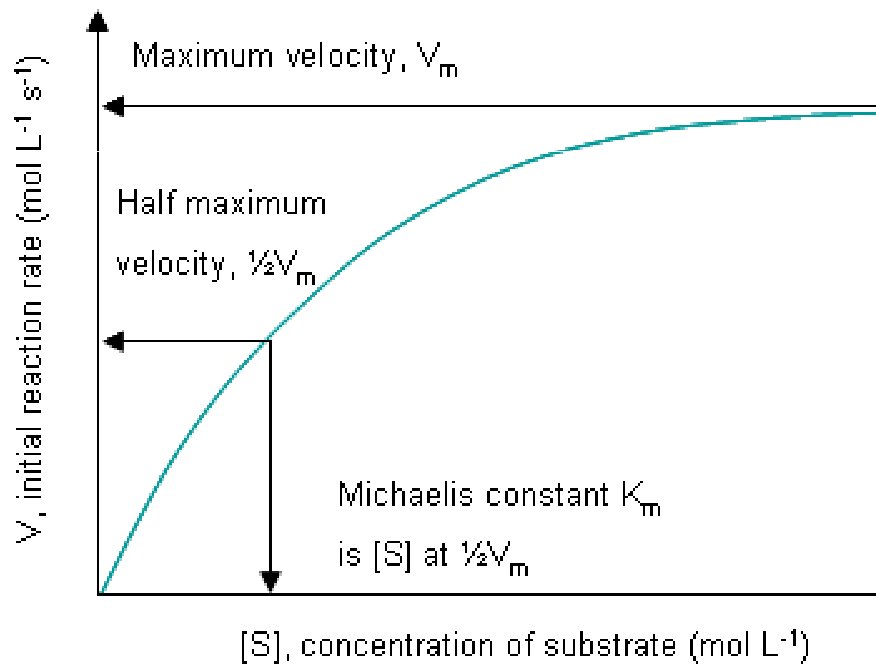
D-fructose – 1.5 mM

D-glucose – 0.15 mM

Juang RH (2004) BCbasics

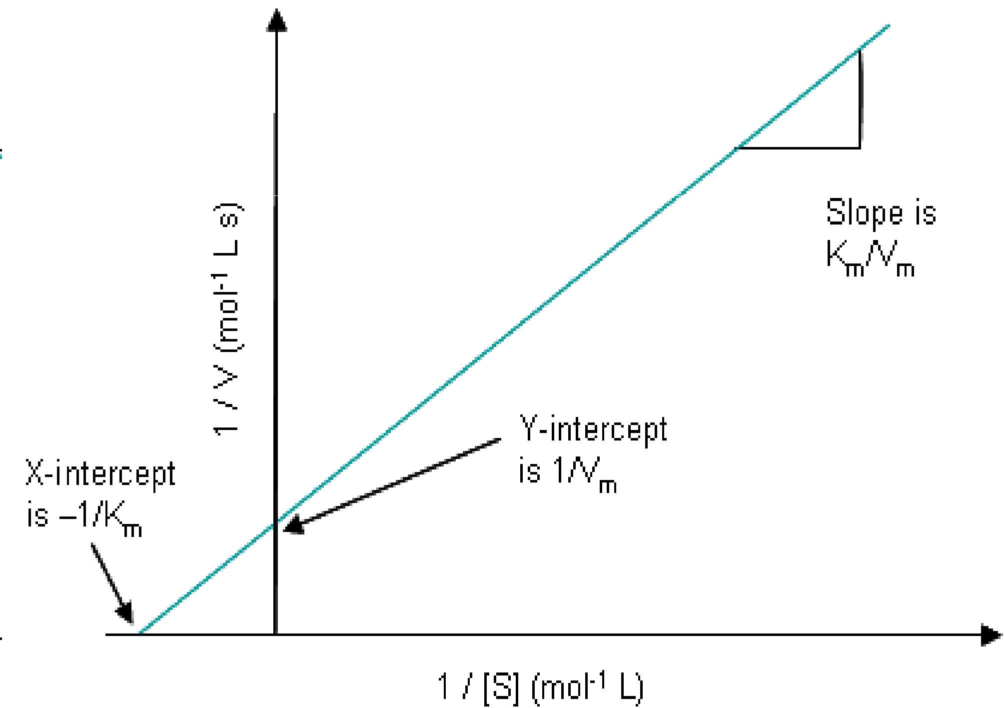
Direct plot & Lineweaver-Burk plot

Direct plot



www.steve.gb.com/science/enzymes.html

Lineweaver-Burk plot (Double reciprocal plot)



www.steve.gb.com/science/enzymes.html

Turn Over Numbers of Enzymes

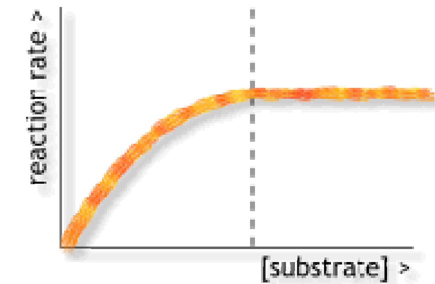
Enzymes	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	140,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (ATPase)	ATP	0.4

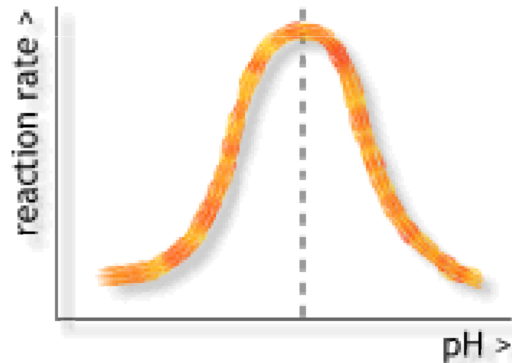
The number of product transformed from substrate by one enzyme molecule in one second

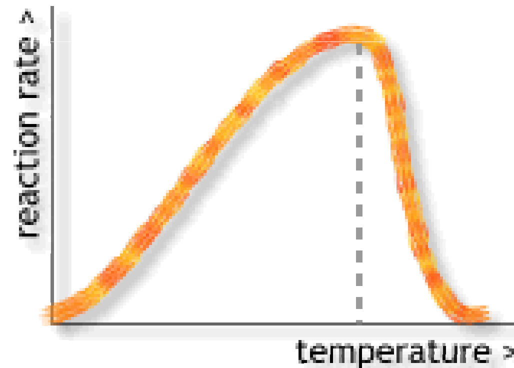
Adapted from Nelson & Cox (2000) Lehninger Principles of Biochemistry (3e) p.263

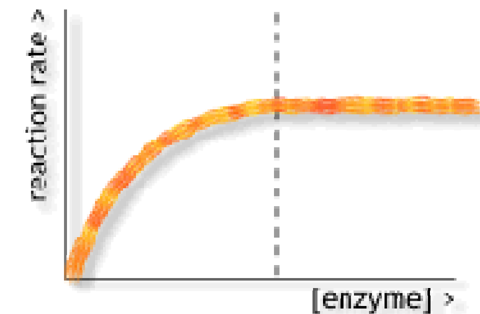
Factors affecting enzyme action

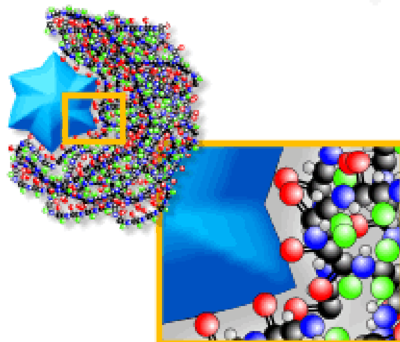
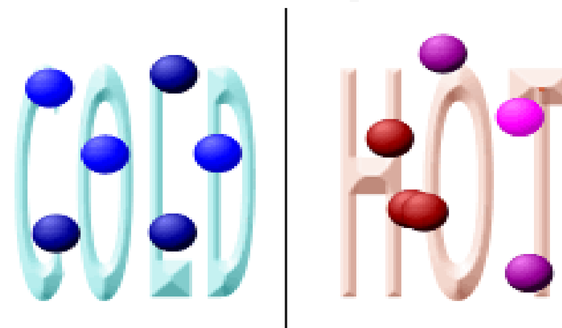
- pH
- Temperature
- Substrate concentration
- Enzyme concentration









www.blobs.org/science/enzyme/index.shtml

Enzyme inhibition

Irreversible inhibition

Involve with formation of breaking covalent bond

Reversible inhibition

1. Competitive
2. Noncompetitive
3. Uncompetitive

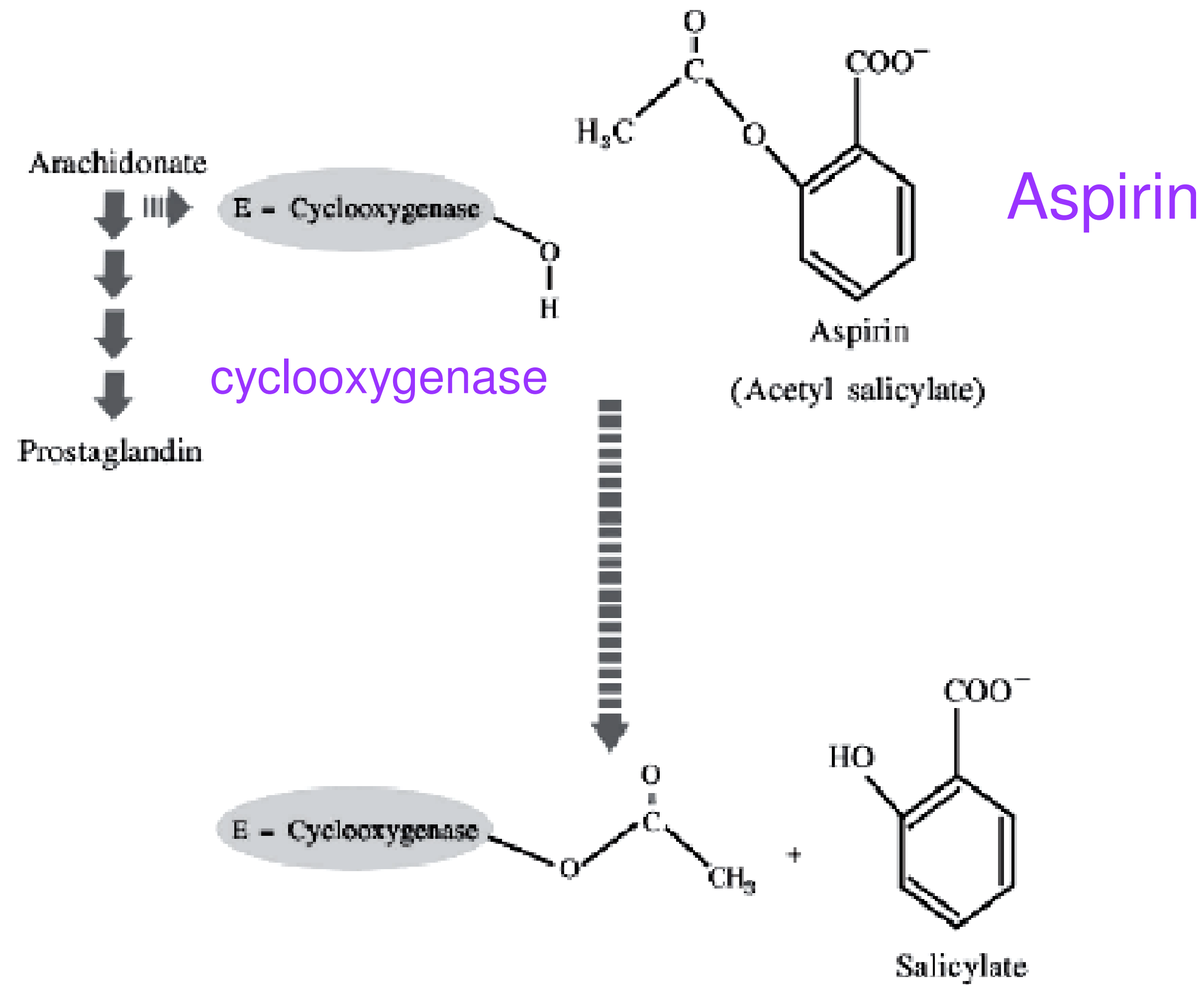
Involve with formation of non-covalent bond

Enzyme inhibitors are important because they can be used to…….

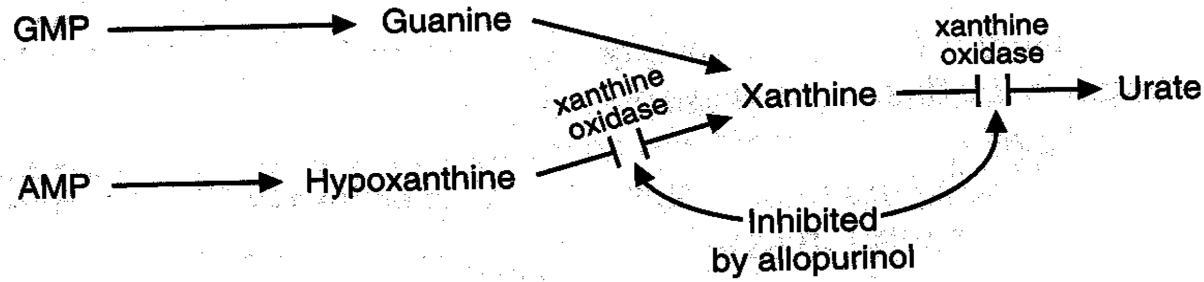
1) gain information about
- the shape on the enzyme active site and the amino acid residues in the active site
- the chemical mechanism
- the regulation or control of a metabolic pathway

2) design new drug

Example of irreversible inhibition - aspirin



Example of irreversible inhibition - allopurinol



Mo = S xanthine oxidase

$H_2O + H^+$ $3H^+, 2e^-$

Hypoxanthine

Xanthine

enz Mo^{VI}

$H_2O + H^+$ $3H^+, 2e^-$

Xanthine–enzyme complex

Urate

xanthine oxidase

Allopurinol

Alloxanthine (oxypurinol)

Reversible inhibition mechanism

	Competitive	Non-competitive	Uncompetitive
Cartoon Guide	Substrate S E I Inhibitor Compete for active site	S E S I I Different site	E S I S I
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+ \qquad\qquad +$ $I \qquad\qquad I$ $\downarrow\uparrow \qquad\qquad \downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EIS
	[*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	[*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	[*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].

Juang RH (2004) BCbasics

Enzyme inhibition plots

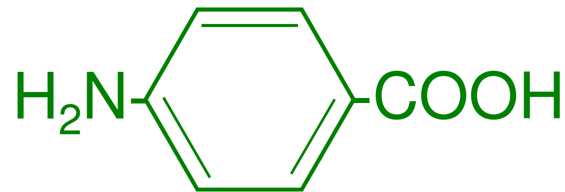
	Competitive	Non-competitive	Uncompetitive
Direct Plots	V; V_{max}; I; K_m K_m'; [S], mM	V; V_{max}; V_{max}'; I; $K_m = K_m'$; [S], mM	V_{max}; V_{max}'; I; K_m' K_m; [S], mM
	V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged	Both V_{max} & K_m decreased
Double Reciprocal	$1/V$; I; Intersect at Y axis; $1/V_{max}$; $1/K_m$; 1/[S]	$1/V$; I; Intersect at X axis; $1/V_{max}$; $1/K_m$; 1/[S]	$1/V$; I; Two parallel lines; $1/V_{max}$; $1/K_m$; 1/[S]

Juang RH (2004) BCbasics

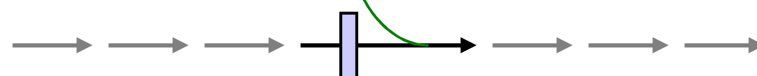
Example of reversible inhibition – sulfa drug

Domagk (1939)

Para-aminobenzoic acid (PABA)



Bacteria needs PABA for the biosynthesis of folic acid



Folic acid → Tetrahydro-folic acid

Sulfa drugs has similar structure with PABA, and inhibit bacteria growth.

Sulfanilamide
Sulfa drug (anti-inflammation)

Adapted from Bohinski (1987) Modern Concepts in Biochemistry (5e) p.197

Example of reversible inhibition

Enz = ACE (angiotensin converting enzyme)

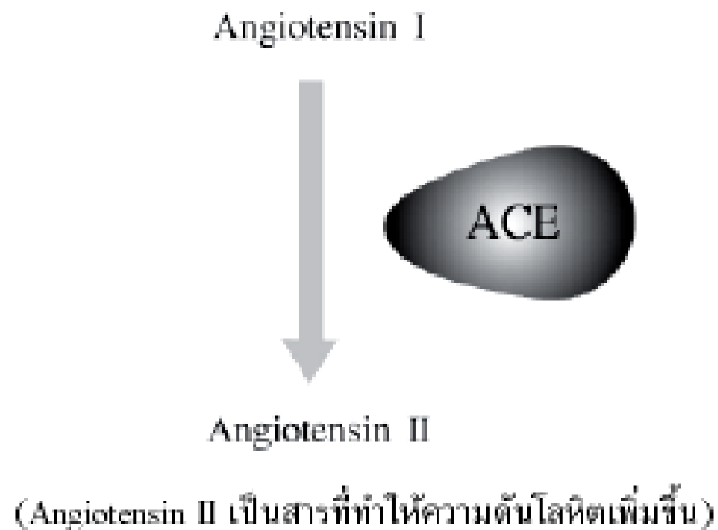
Substrate = angiotensin I

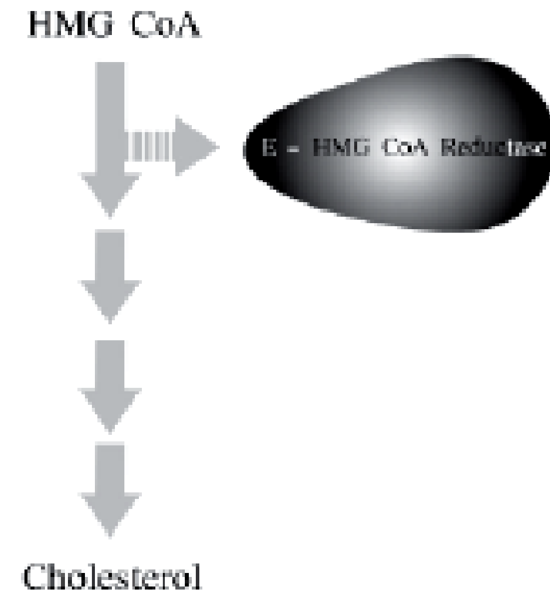
Competitive inhibitor = Captopril and enalapril (blood pressure ↓)

Enz = HMG CoA reductase

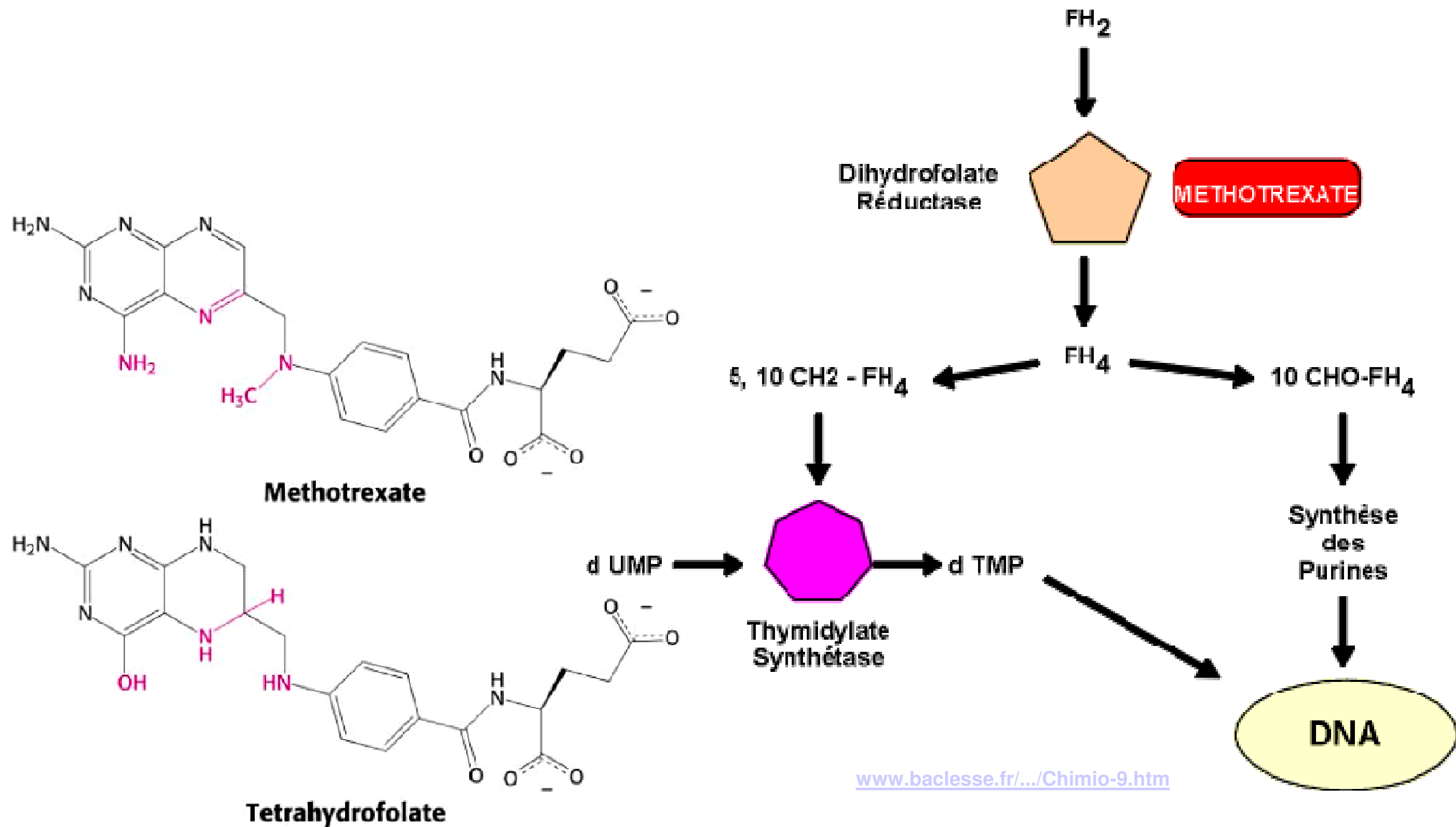
Substrate = HMG CoA

Competitive inhibitor = lavastatin and mevilonin





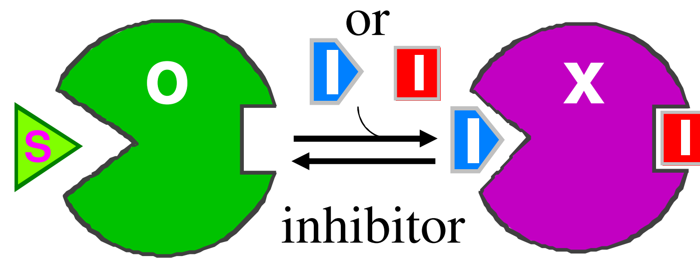
Example of reversible inhibition – cancer treatment



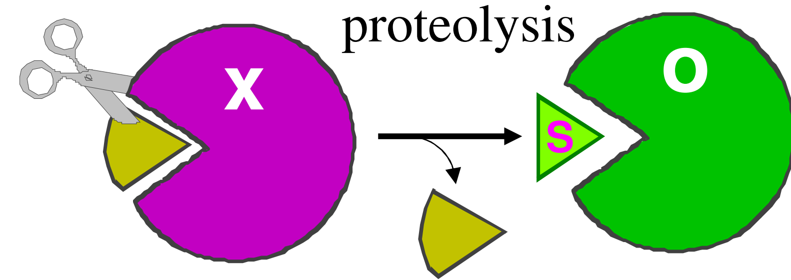
oregonstate.edu/.../enzymesoutline.html

Enzyme Regulation

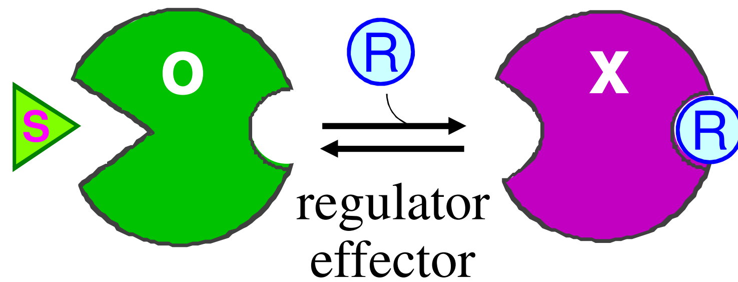
Inhibitor



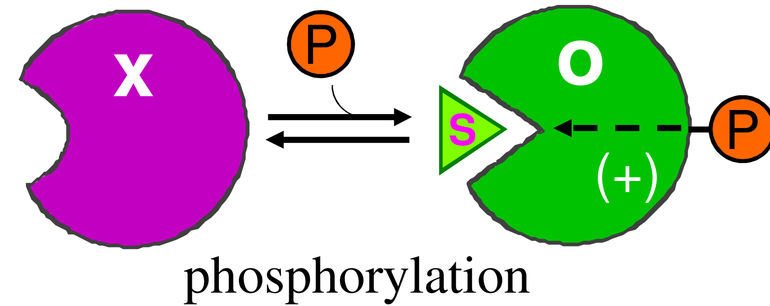
Proteolysis



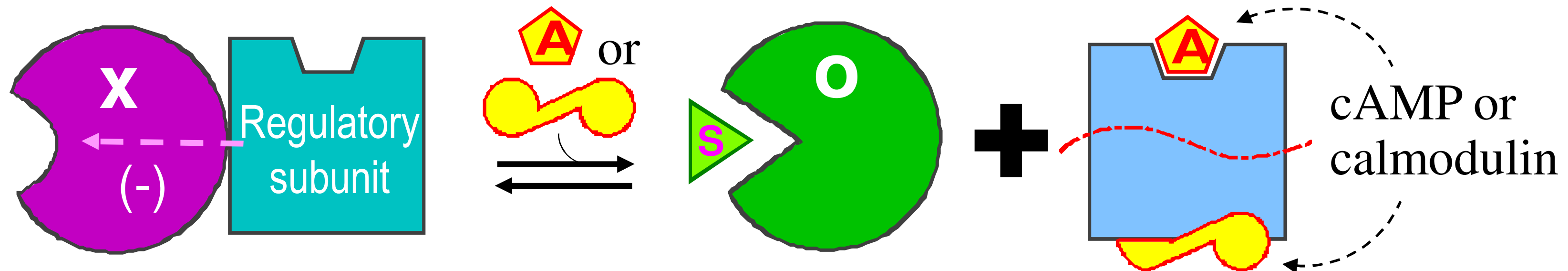
Feedback regulation



Covalent modification(Phosophorylation)



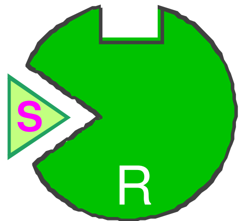
Signal transduction

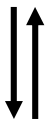


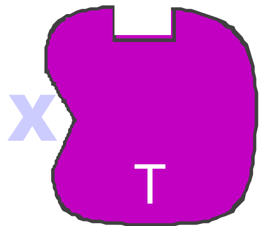
Juang RH (2004) BCbasics

Allosteric enzyme

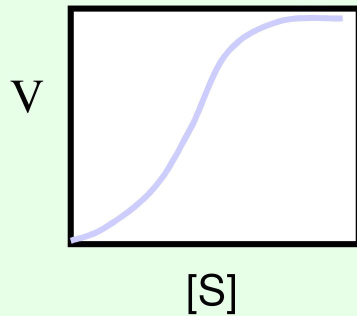
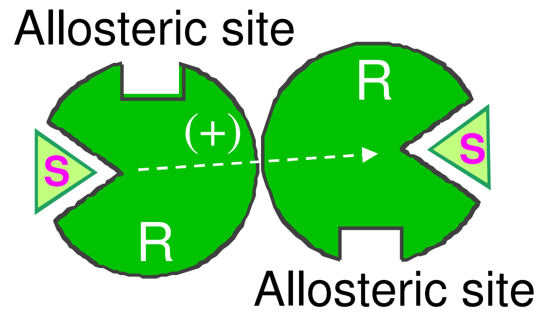
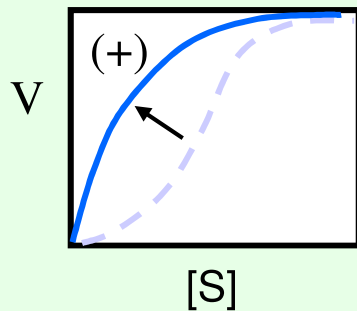
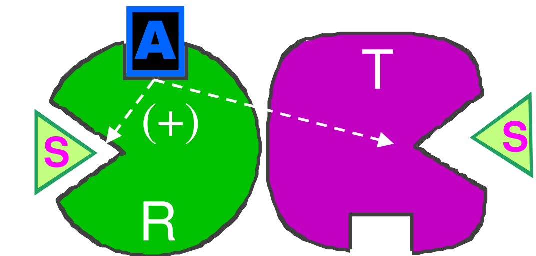
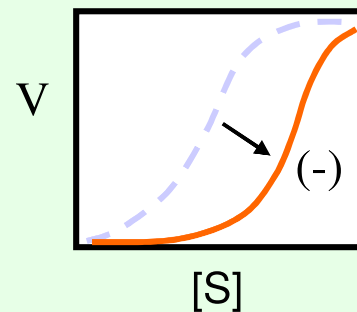
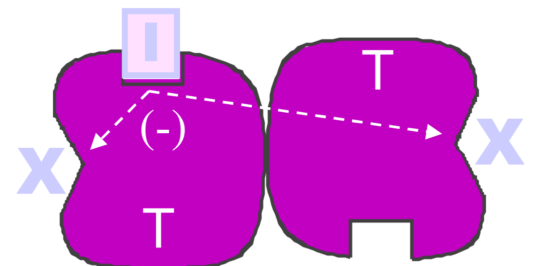
R = Relax
(active)







T = Tense
(inactive)

Kinetics	Models	Cooperation
		Homotropic (+) Concerted
		Heterotropic (+) Sequential
		Heterotropic (-) Concerted

Juang RH (2004) BCbasics

Summary

Enzyme is a biological catalyst. It decreases Ea but not ΔG.

Enzyme can be classified into 6 classes. Isoenzymes are different but catalyze the same rx.

Km → affinity for substrate, Vmax→ enzyme activity

Enzyme inhibition consists of irreversible and reversible inhibition. Competitive inhibition is advantage to drug design.

There are many ways to regulate enzymes. Allosteric enzyme is one type of enzyme regulation.