

Gli enzimi

Gli enzimi sono le proteine¹ che catalizzano² le reazioni chimiche che avvengono nei sistemi biologici

¹con l'eccezione dei ribozimi (RNA catalitici)

²Un catalizzare è una sostanza che promuove una reazione chimica senza subire alcuna modificazione permanente

Proprietà generali delle reazioni catalizzate dagli enzimi

- Elevate velocità di reazione (10^6 - 10^{12} volte maggiori rispetto a quella delle reazioni non catalizzate)
- Avvengono in condizioni più moderate
- Maggiore specificità di reazione
- Sono spesso soggette a regolazione

Specificità di substrato

- Gli enzimi sono stereospecifici
- Sono selettivi riguardo l'identità chimica dei loro substrati (specificità geometrica)

Il grado di specificità geometrica può tuttavia variare considerevolmente

Enzimi in biotecnologie

Table 10.1 Selected attributes of industrial enzymes, as compared to those of proteins used for healthcare purposes

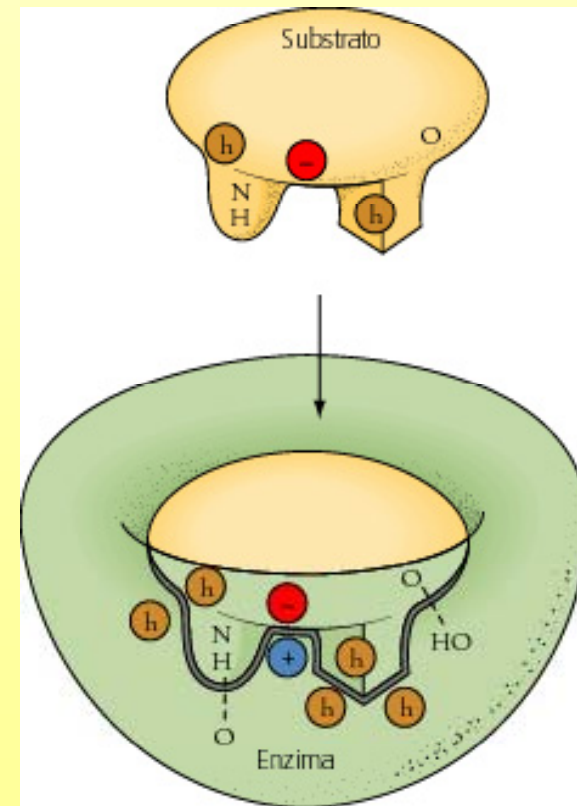
Industrial enzymes	Medical/diagnostic proteins
Produced in large quantities	Produced in small quantities
Partially purified at best	Extensively purified
Economic considerations critical	Economic considerations are of secondary importance to functional excellence of product
Function: catalytic	Function: various (hormones, growth factors, cytokines, other regulatory factors, blood factors, vaccines, antibodies, enzymes)
Source: mainly microbial and recombinant	Source: mainly human or animal and recombinant products
Mainly secreted into the extracellular medium by producer strains	May be intracellular or extracellular

Specificità di substrato

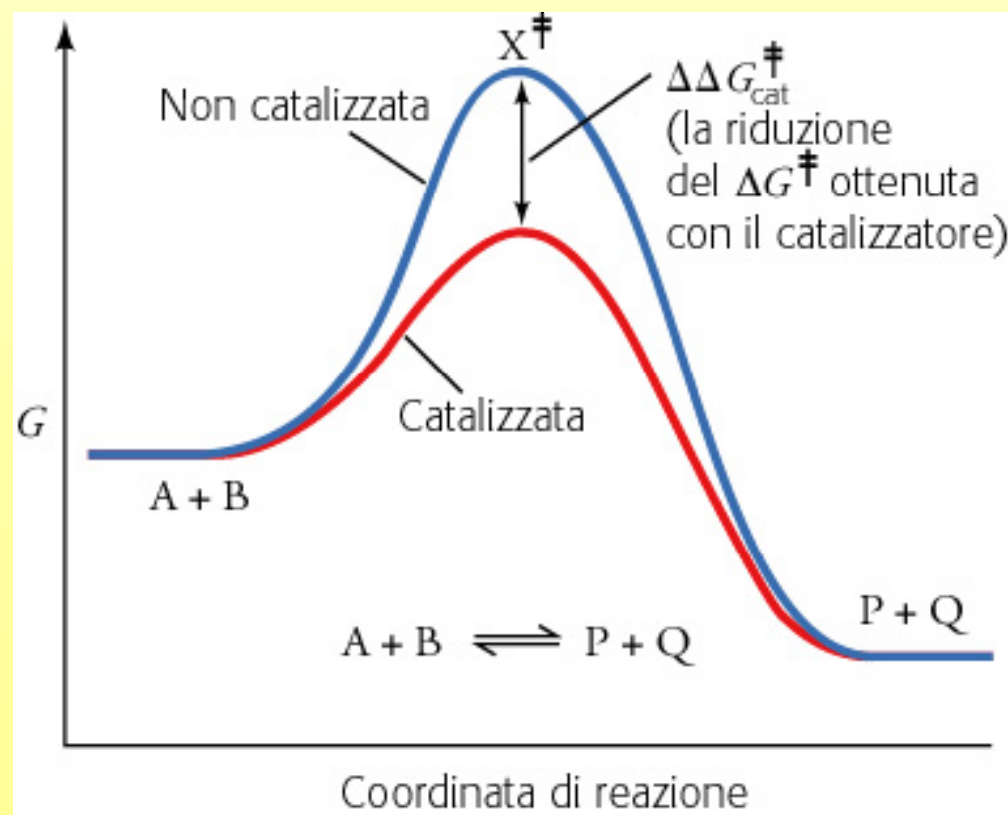
Complementarità tra sito attivo e substrato

Modello Chiave-serratura
(Fisher 1894)

Adattamento indotto
(Koshland 1958)

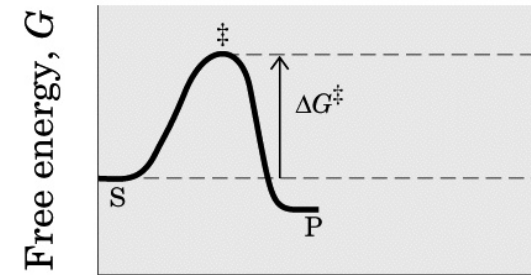
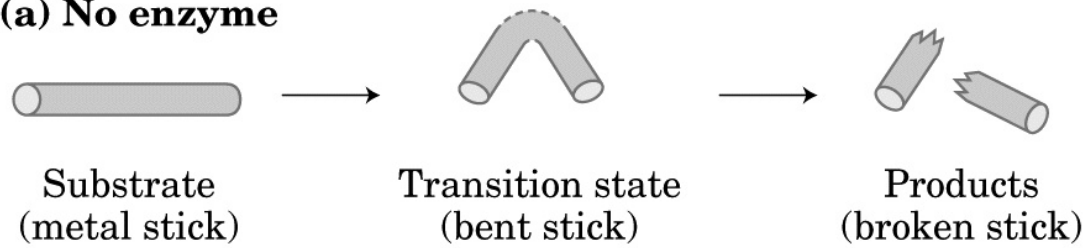


I catalizzatori aumentano la velocità della reazione abbassando l'energia di attivazione

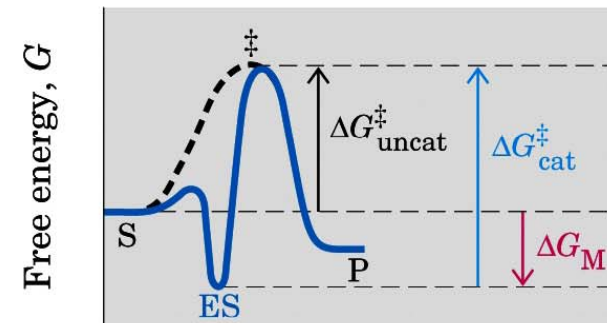
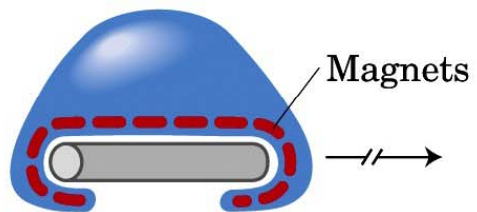


**I catalizzatori non
modificano gli equilibri
chimici delle reazioni**

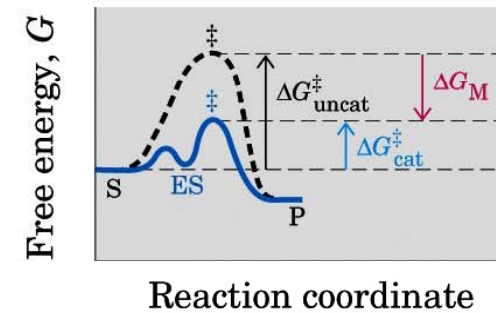
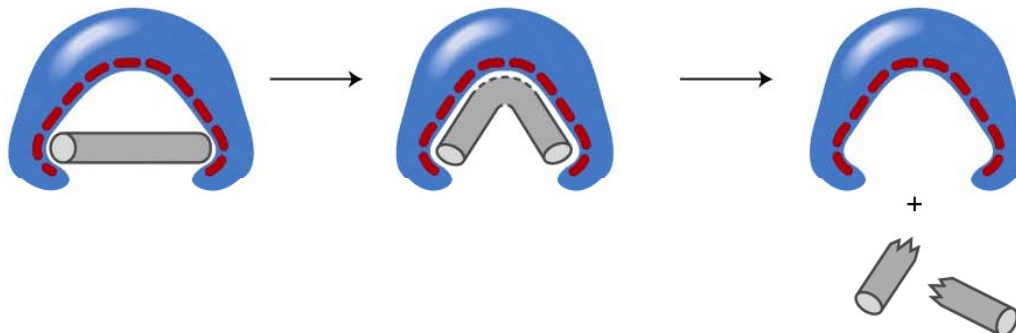
(a) No enzyme

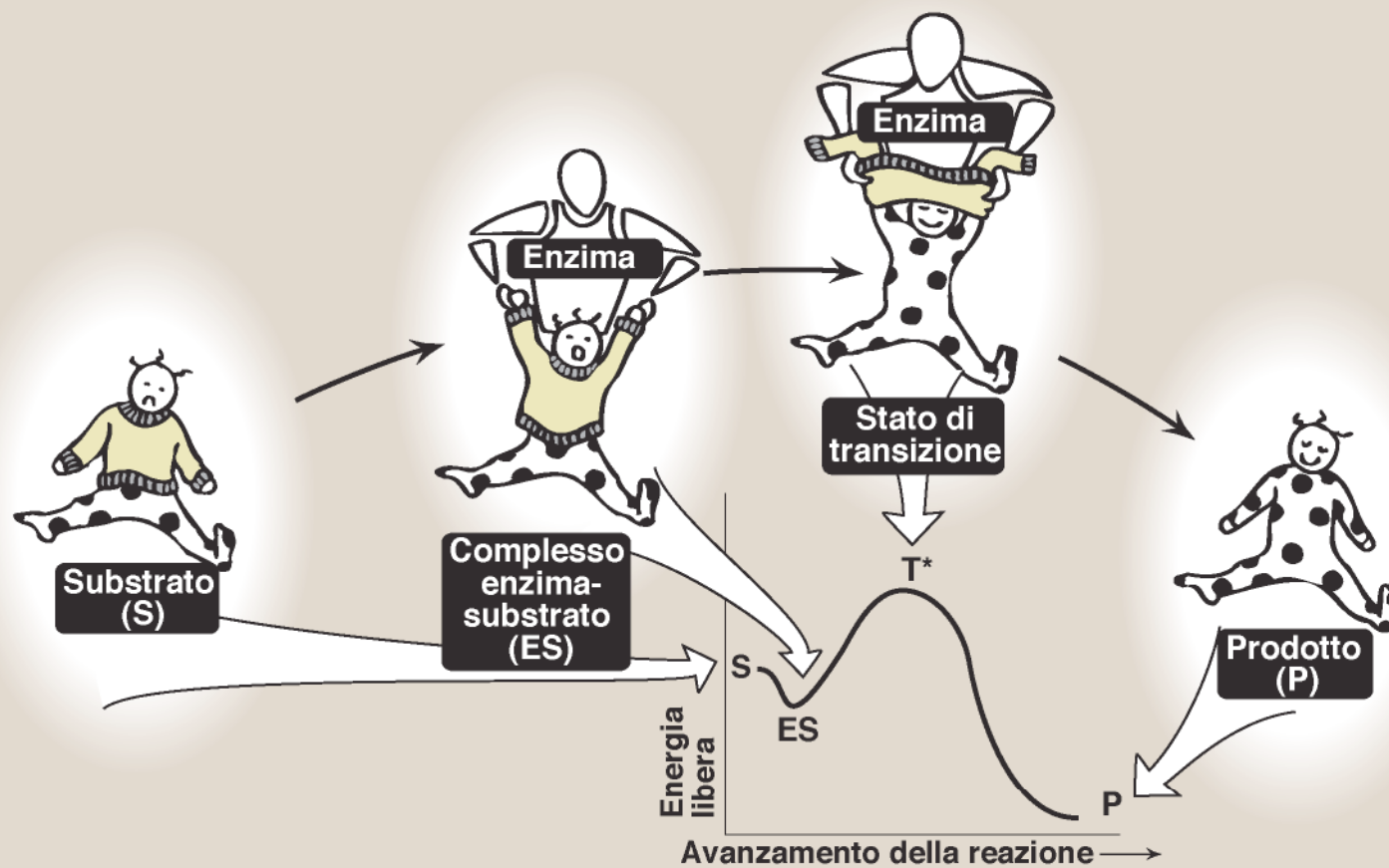


(b) Enzyme complementary to substrate



(c) Enzyme complementary to transition state





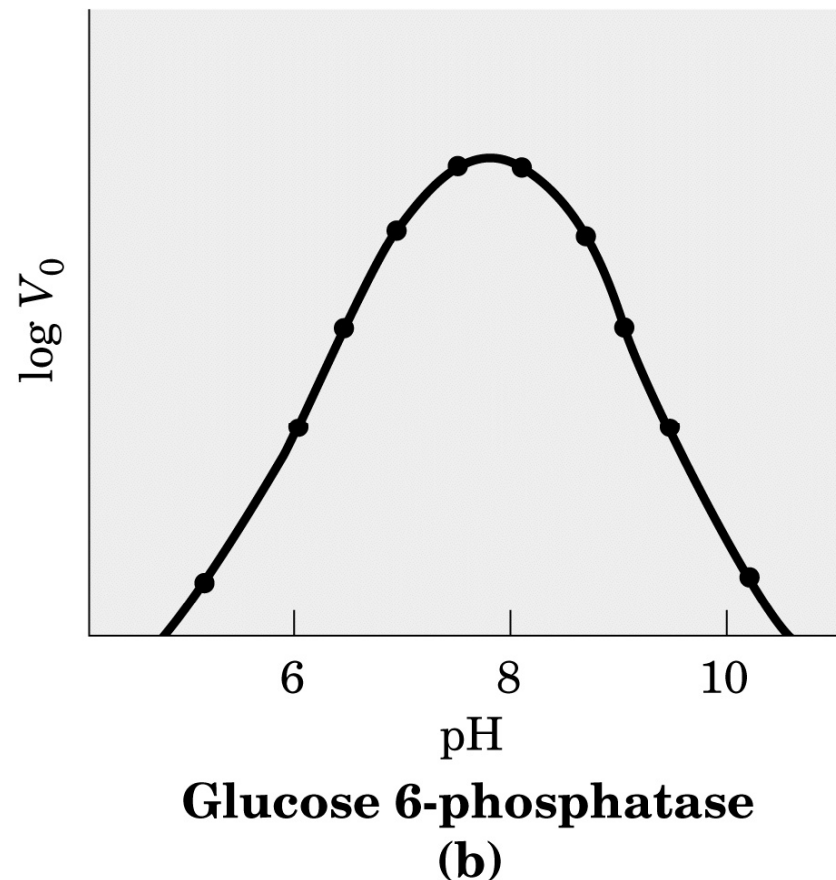
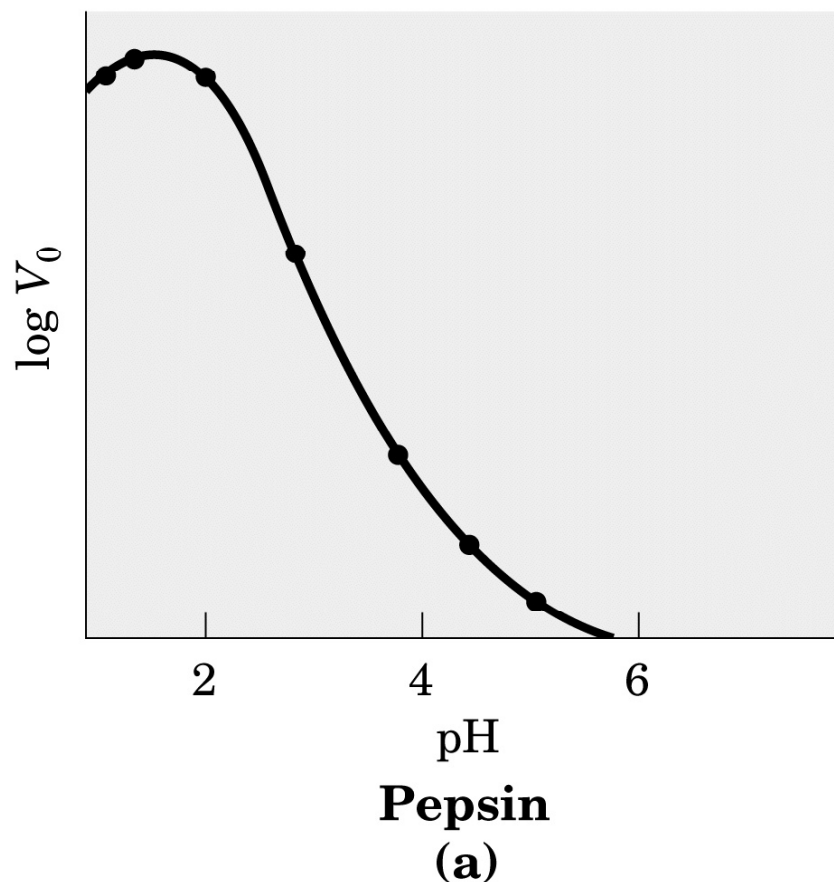
Meccanismi di catalisi:

1) Catalisi acido-basica

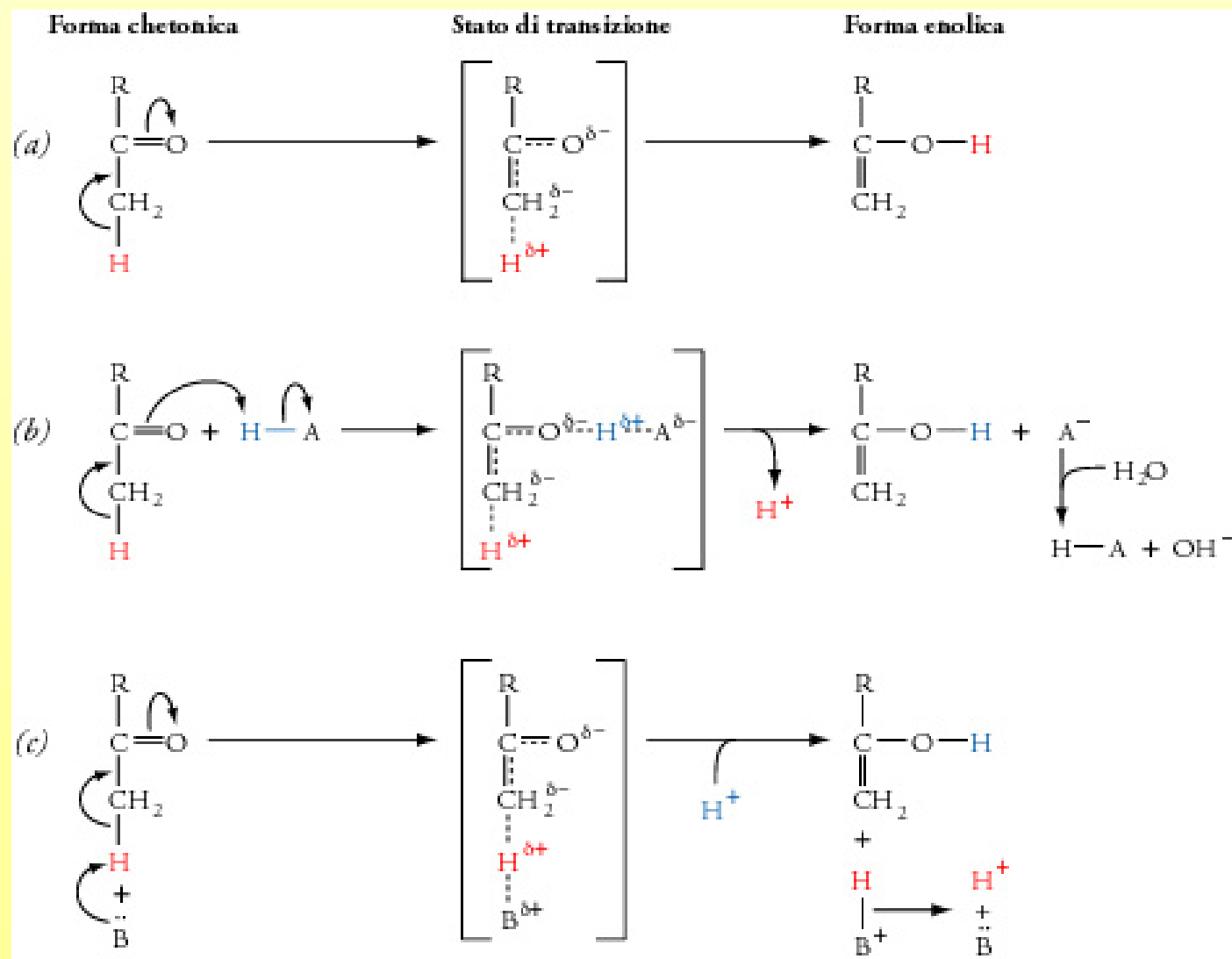
- La catalisi acida generale è un processo in cui il trasferimento temporaneo di un protone da un acido abbassa l'energia libera dello stato di transizione di una reazione
- Si parla di catalisi basica generale se la velocità di reazione viene accelerata dalla sottrazione temporanea di un protone da parte di una base

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	$\text{R}-\text{COOH}$	$\text{R}-\text{COO}^-$
Lys, Arg	$\text{R}-\overset{\text{H}}{\underset{\text{H}}{\text{N}^+}}$	$\text{R}-\ddot{\text{N}}\text{H}_2$
Cys	$\text{R}-\text{SH}$	$\text{R}-\text{S}^-$
His	$ \begin{array}{c} \text{R}-\text{C}=\text{CH} \\ \diagup \quad \diagdown \\ \text{HN} \quad \text{N}^+ \\ \diagdown \quad \diagup \\ \text{C} \\ \text{H} \end{array} $	$ \begin{array}{c} \text{R}-\text{C}=\text{CH} \\ \diagup \quad \diagdown \\ \text{HN} \quad \text{N}: \\ \diagdown \quad \diagup \\ \text{C} \\ \text{H} \end{array} $
Ser	$\text{R}-\text{OH}$	$\text{R}-\text{O}^-$
Tyr	$ \text{R}-\text{C}_6\text{H}_4-\text{OH} $	$ \text{R}-\text{C}_6\text{H}_4-\text{O}^- $

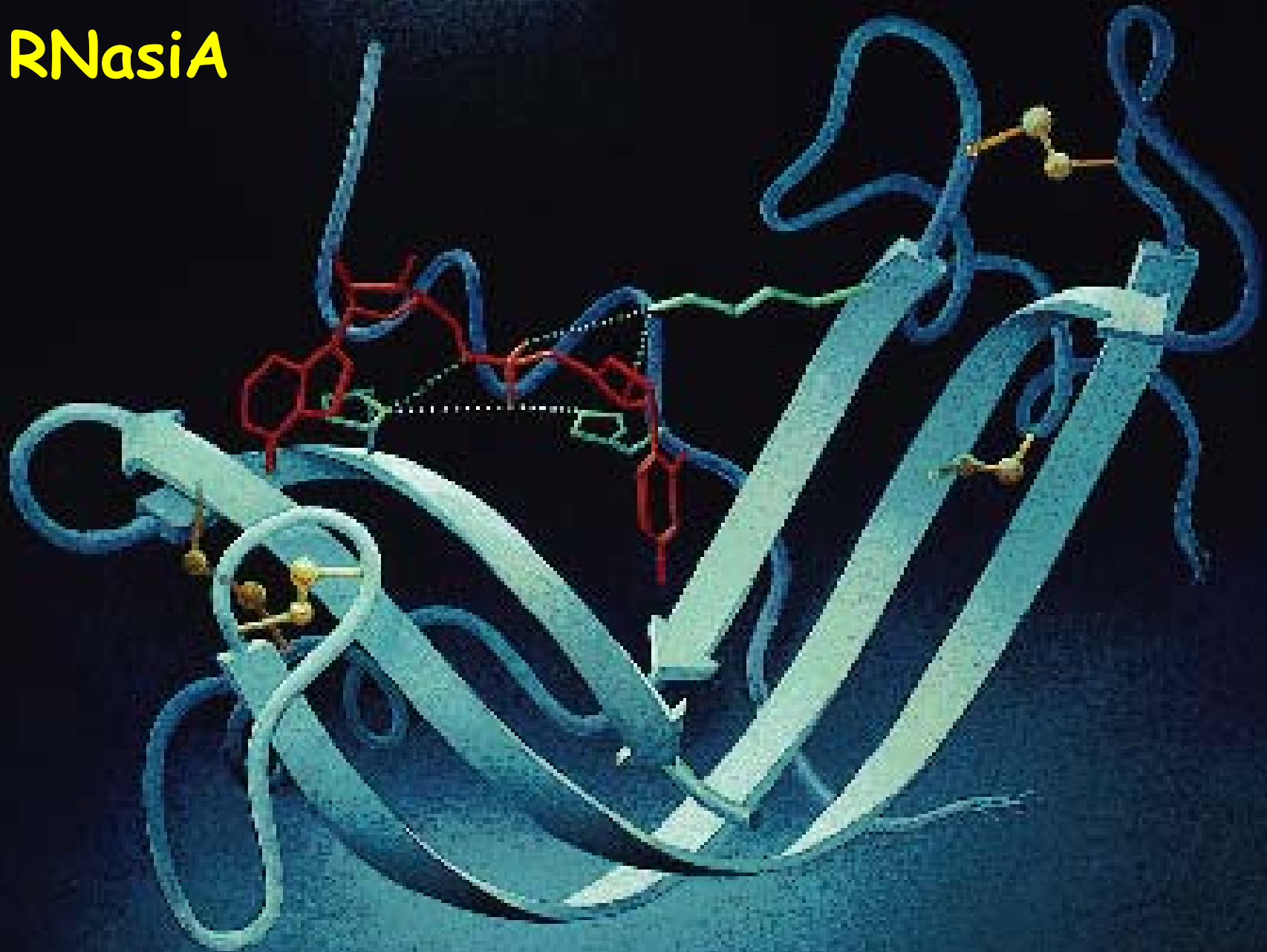
L'attività di questi enzimi è fortemente influenzata dal pH



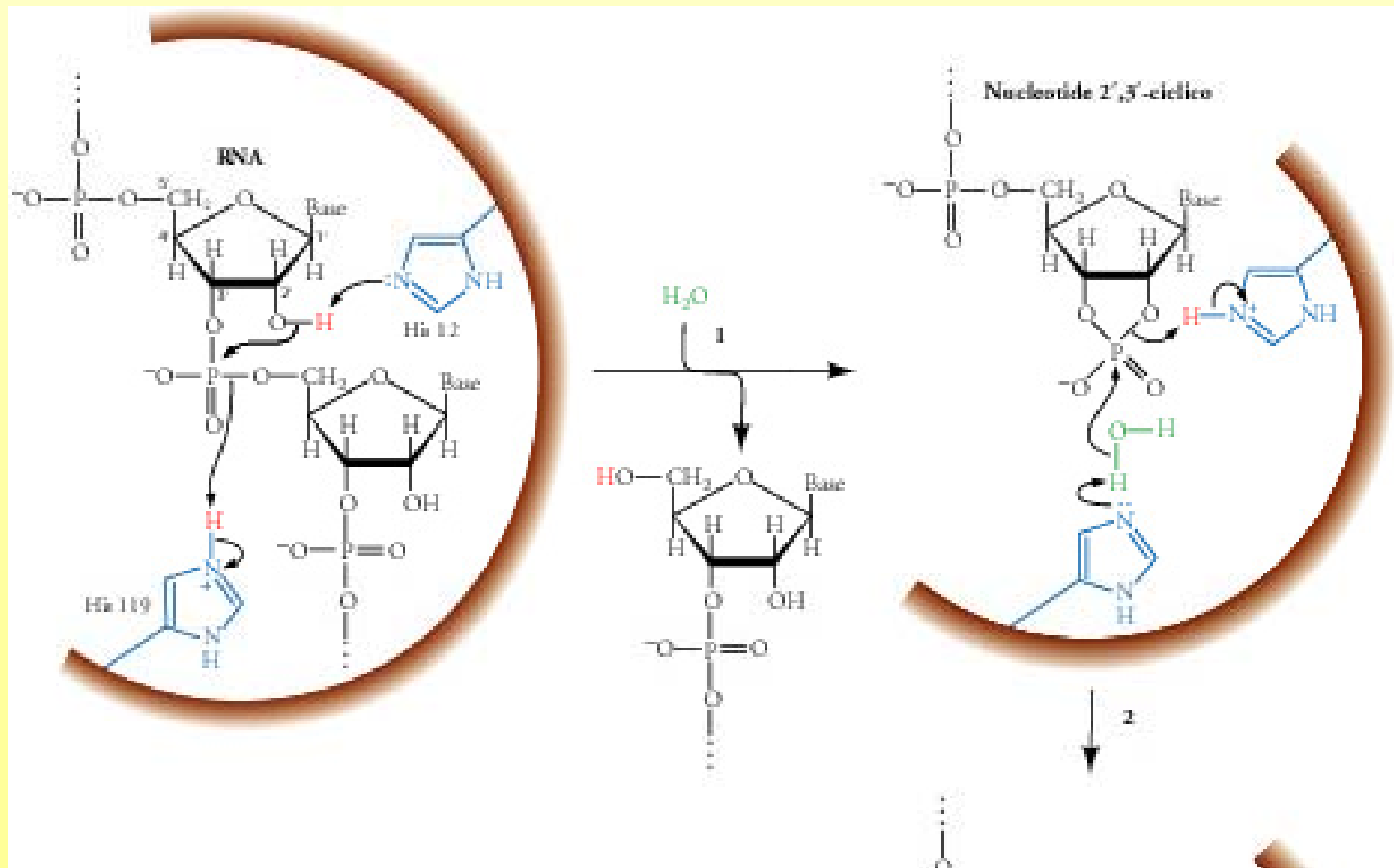
Catalisi acido-basica

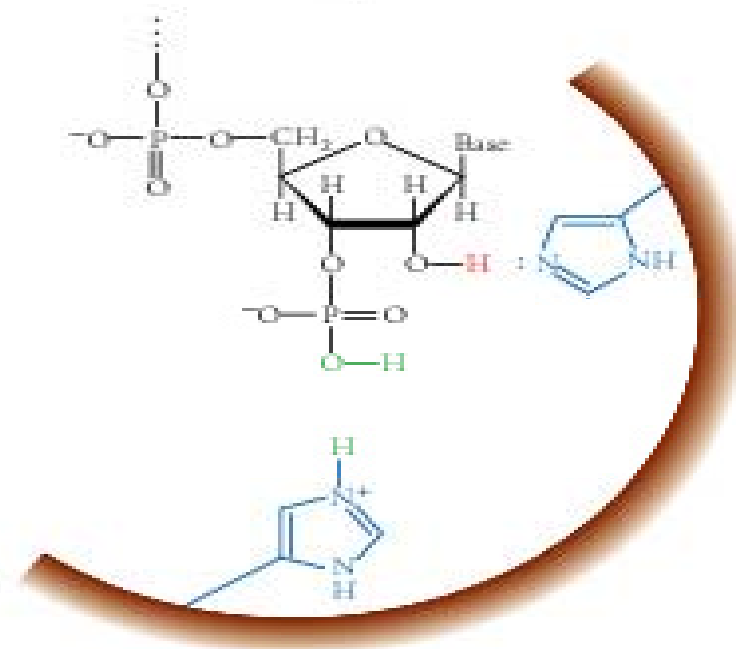
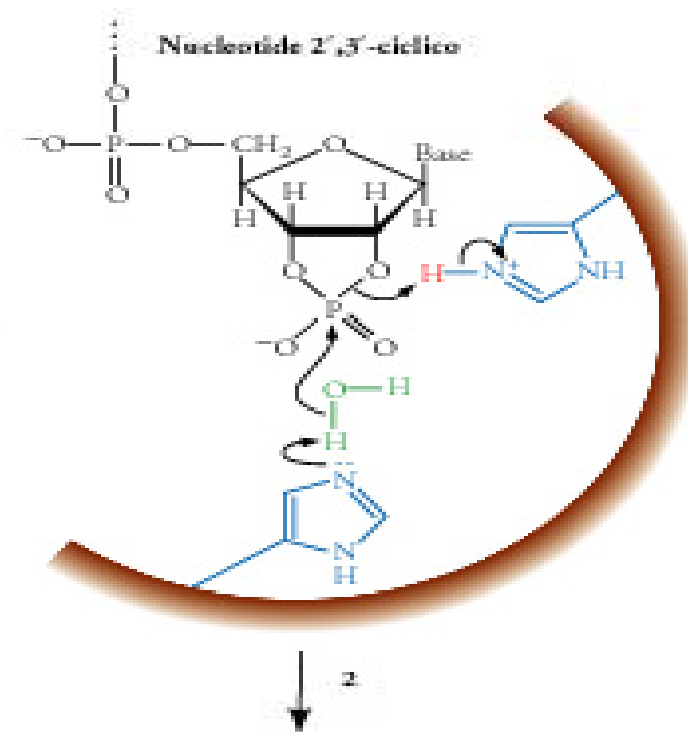
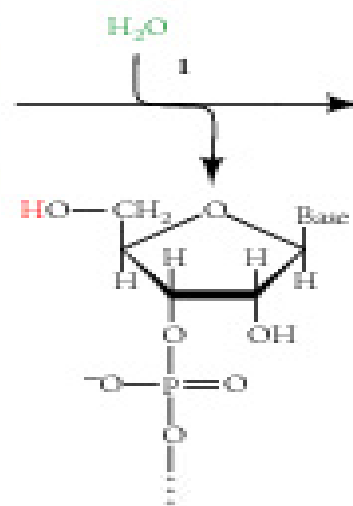
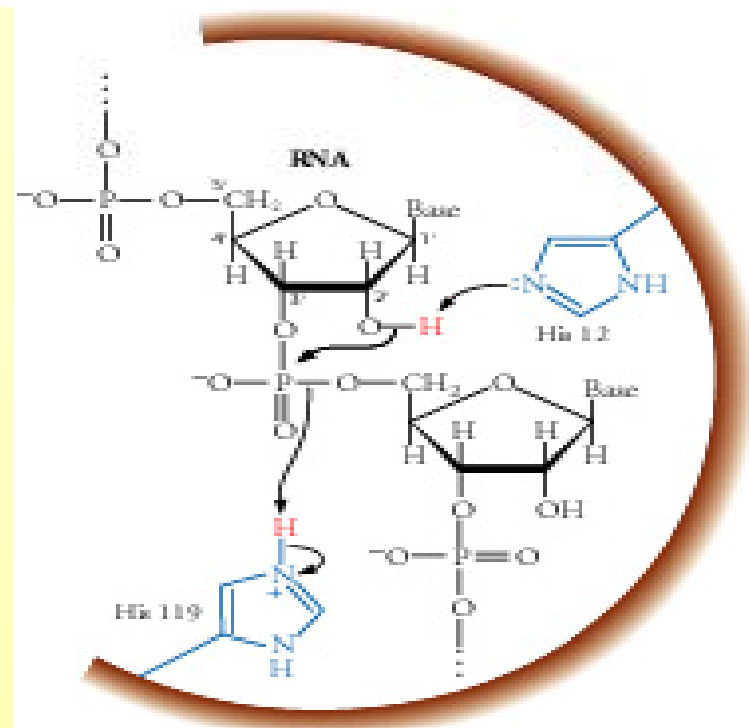


RNasiA



Meccanismo catalitico dell'RNasi A

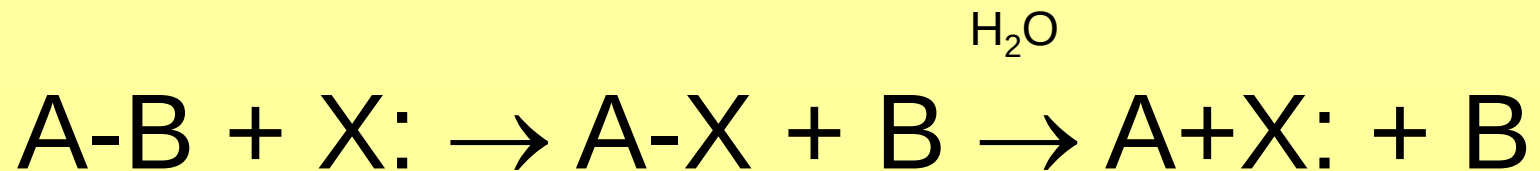
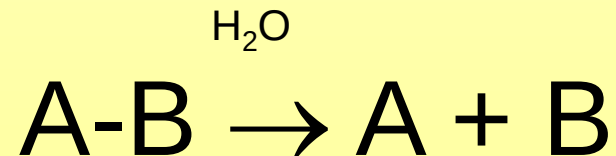




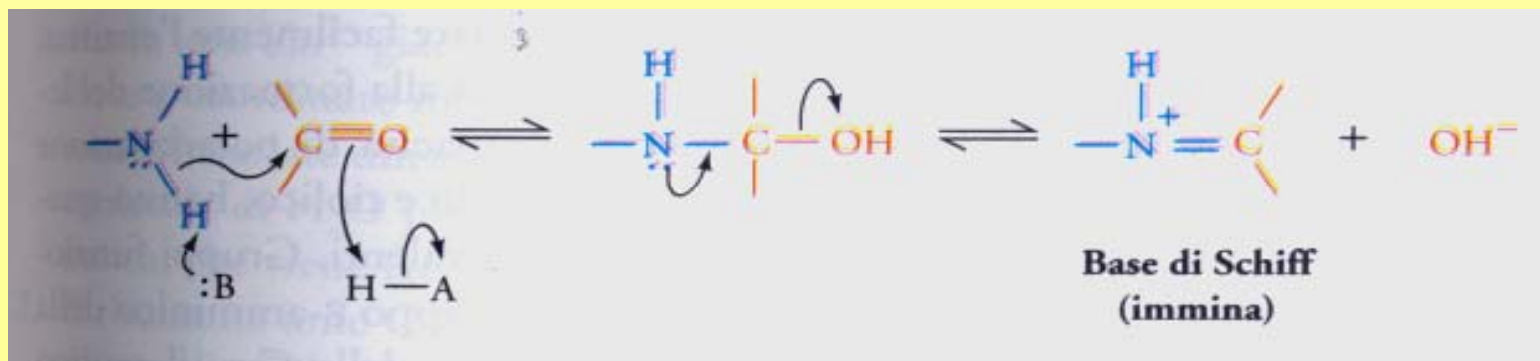
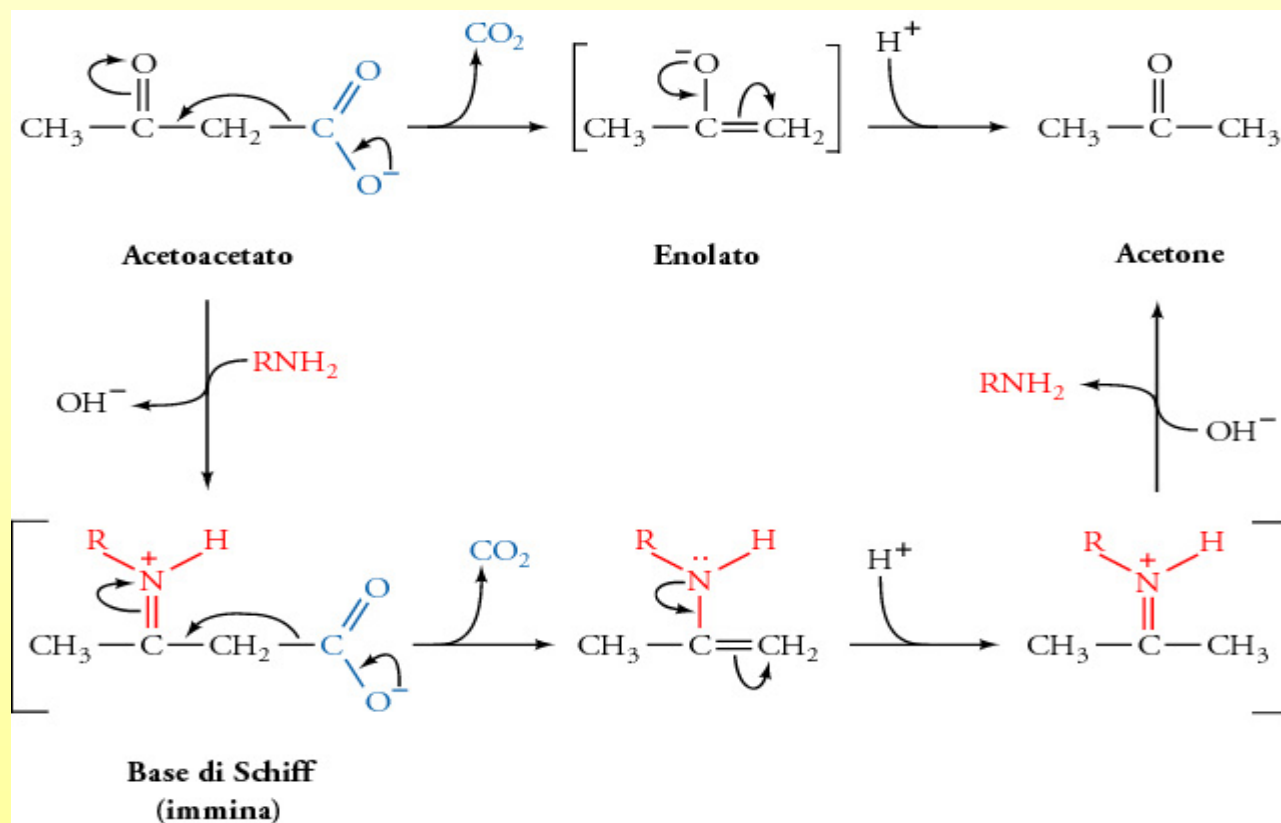
Meccanismi di catalisi:

2) Catalisi covalente (nucleofilica)

La catalisi covalente coinvolge la formazione di un legame covalente transitorio tra l'enzima e il substrato

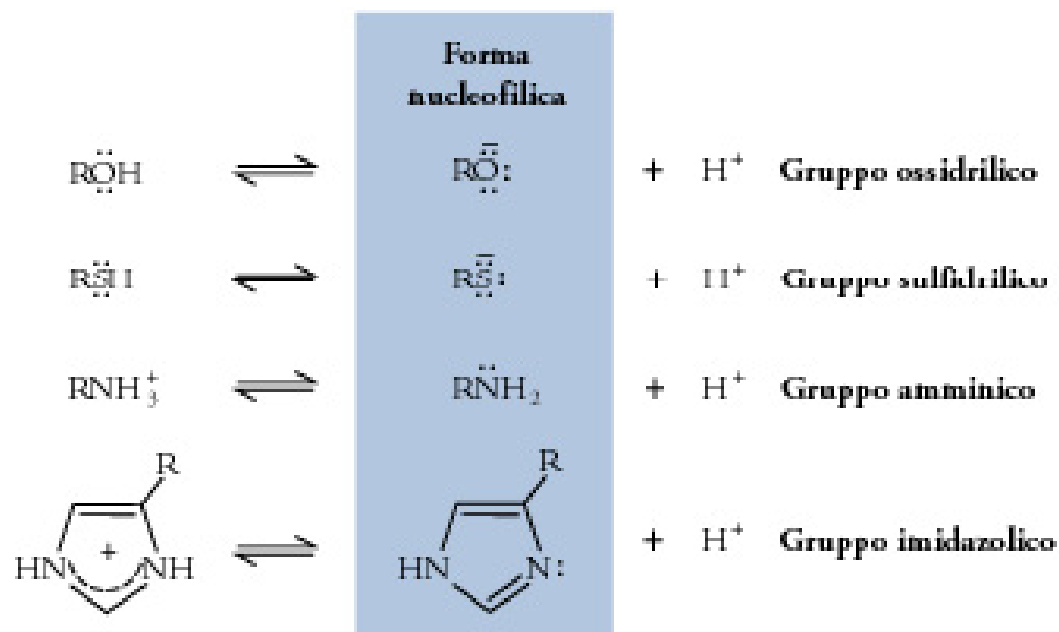


Catalisi covalente (o nucleofilica)

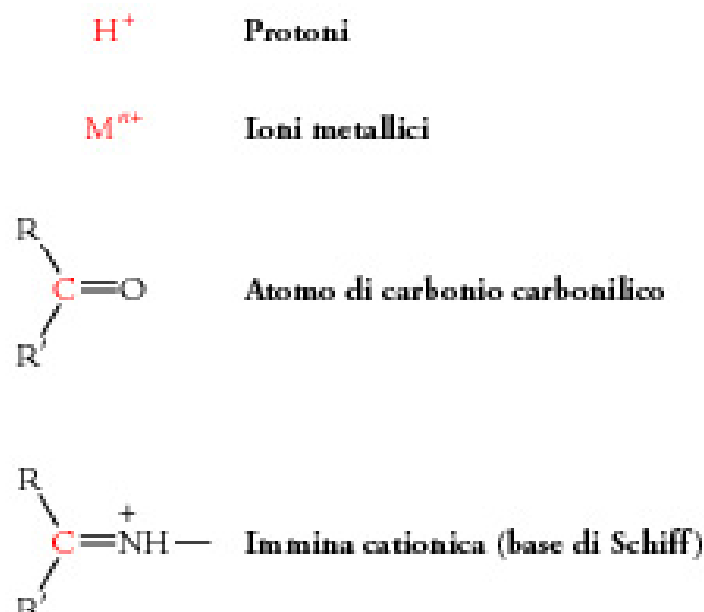


Gruppi nucleofilici ed elettrofilici

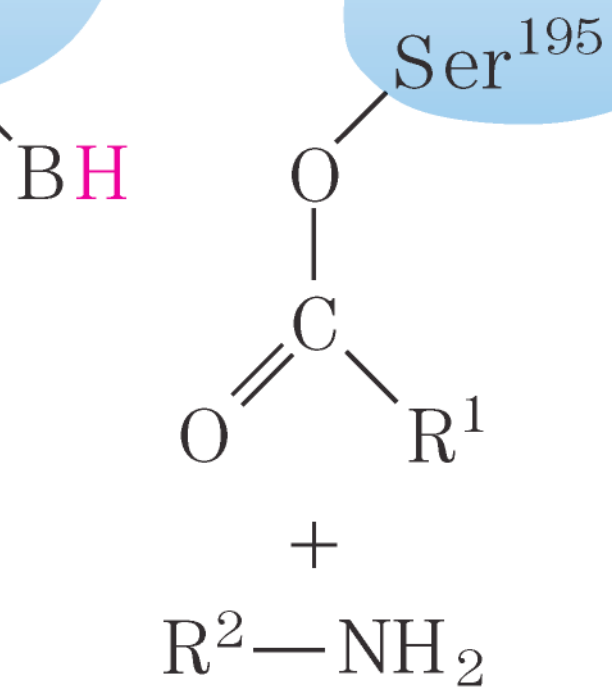
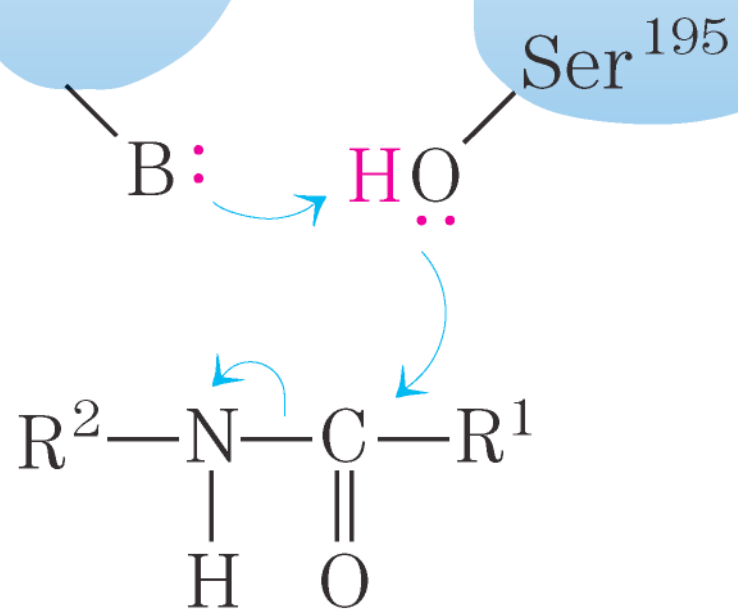
(a) Gruppi nucleofilici



(b) Gruppi elettrofilici

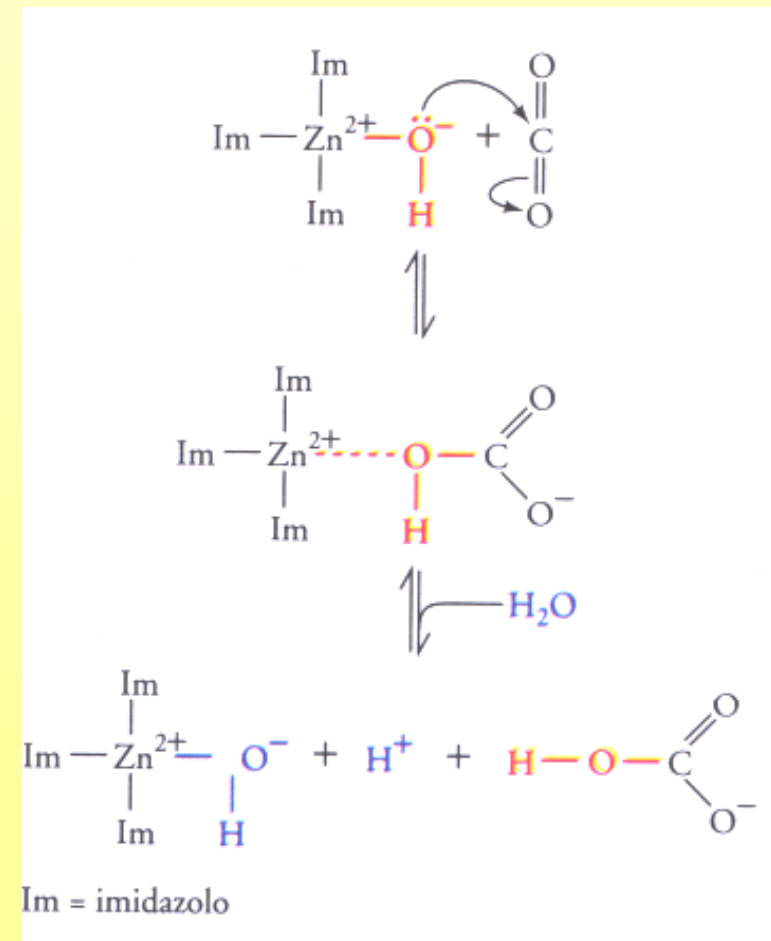
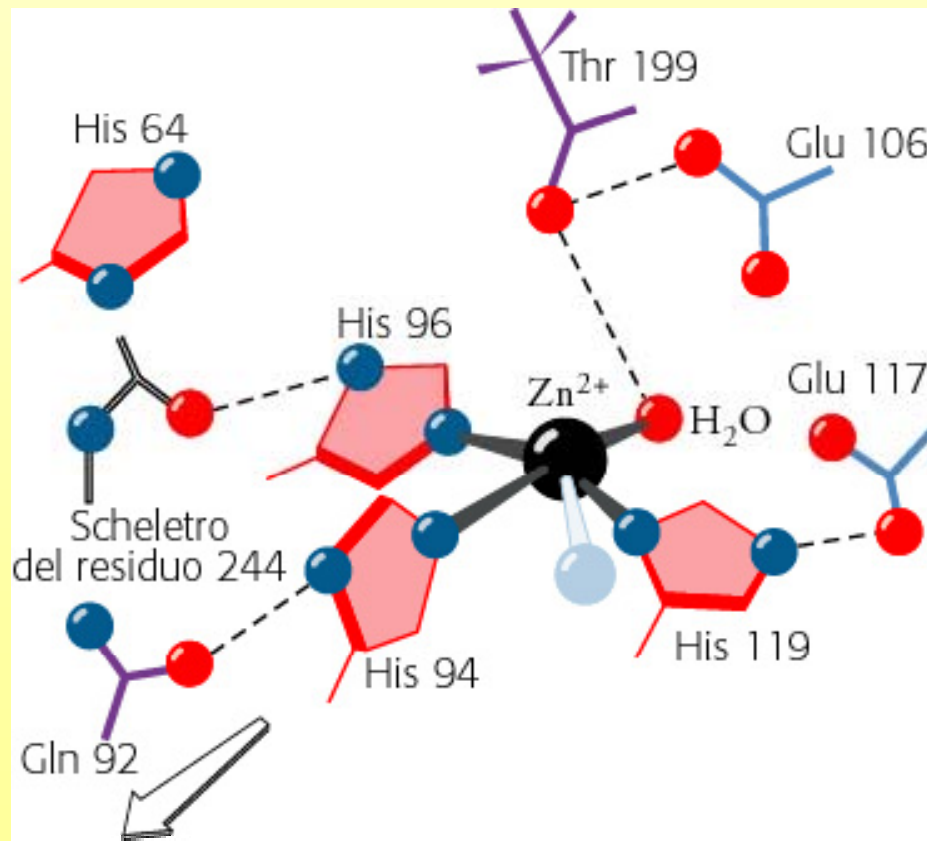


Chimotripsina



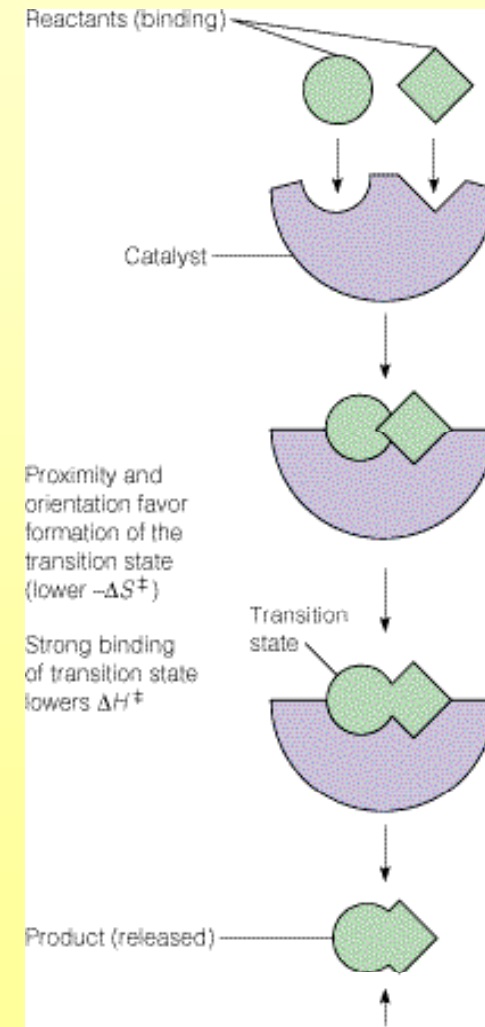
Meccanismi di catalisi:

3) Catalisi favorita da ioni metallici

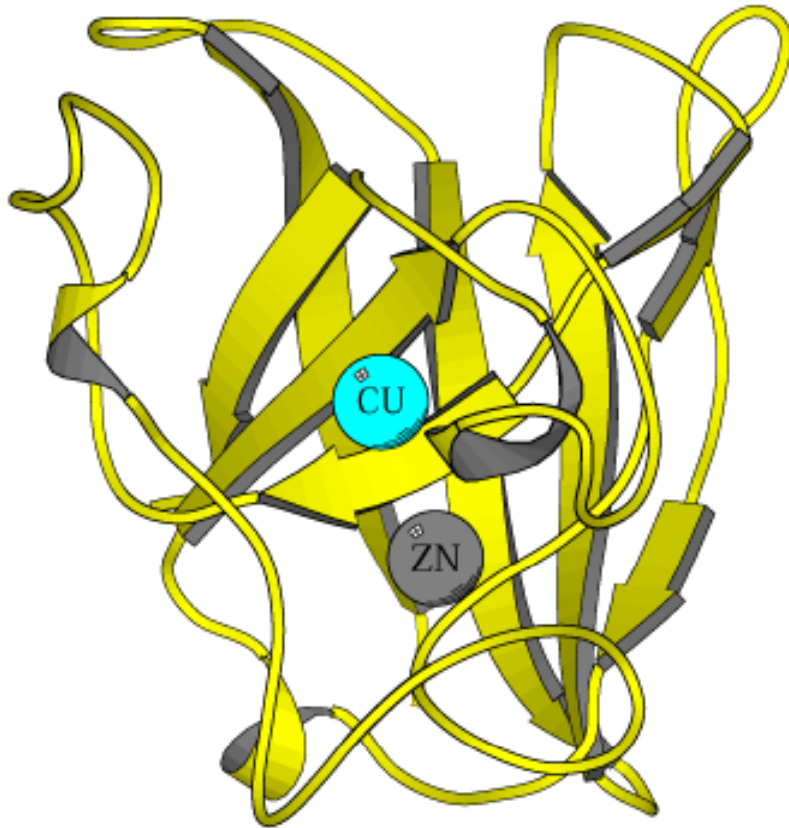
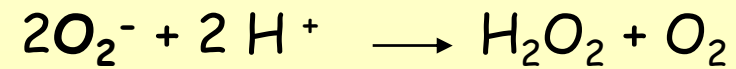


Effetti di prossimità e orientamento

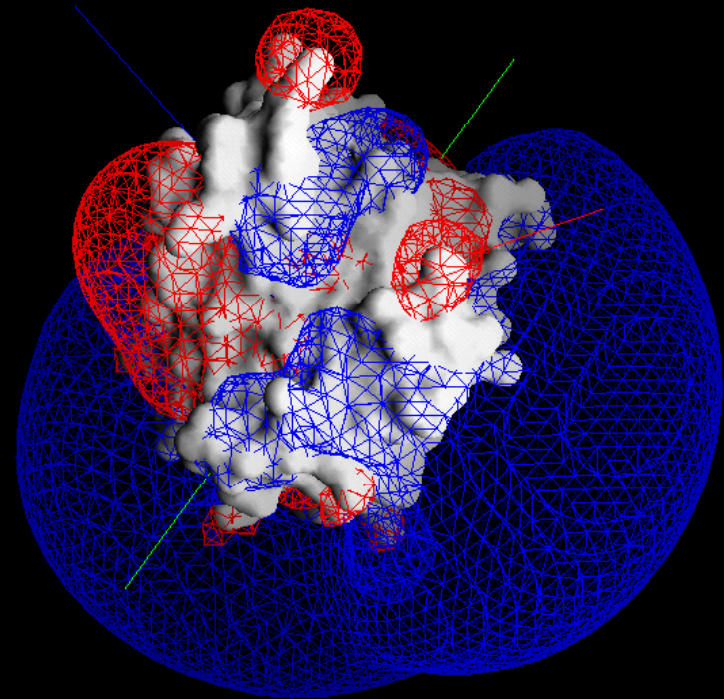
- Gli enzimi che catalizzano reazioni a più substrati agiscono mettendo i substrati in contatto tra loro
- Gli enzimi legano i loro substrati secondo un orientamento appropriato in modo da favorire la reazione
- Gli enzimi stabilizzano i movimenti relativi traslazionali e rotazionali dei loro substrati e dei loro gruppi catalitici



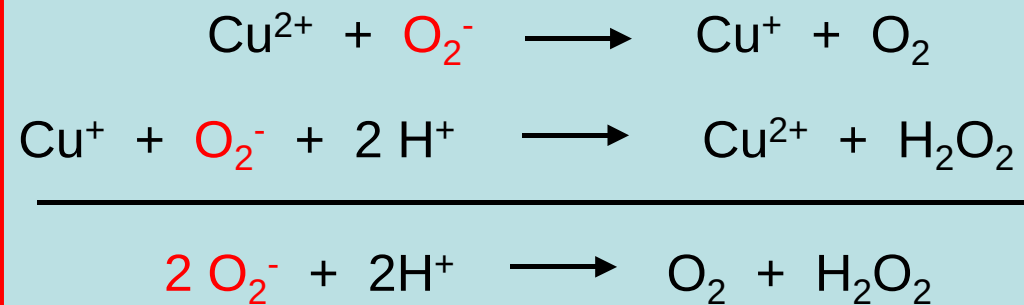
Orientamento del substrato



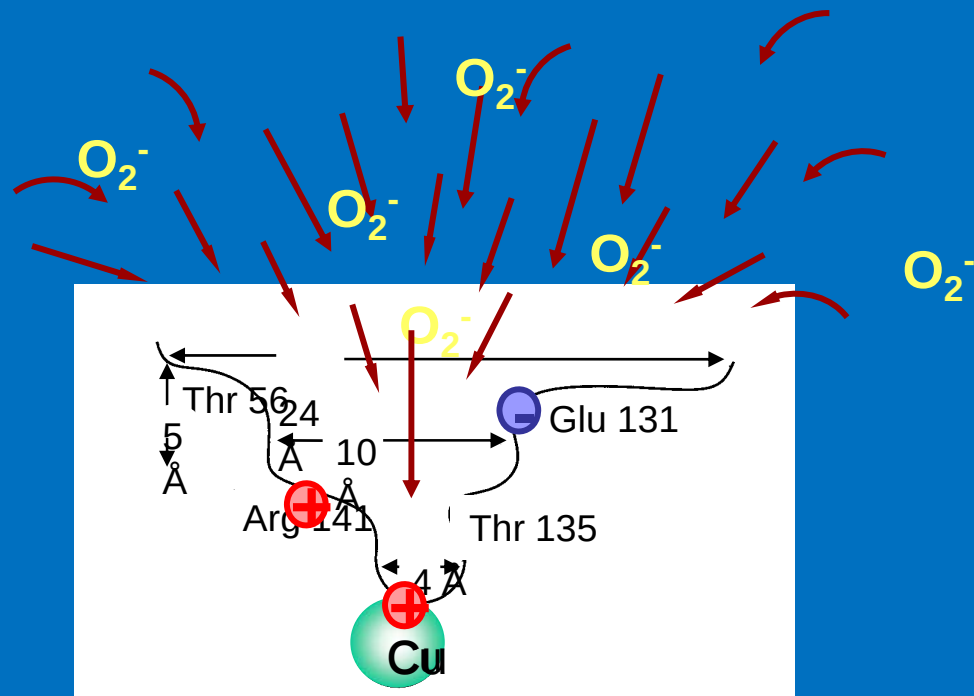
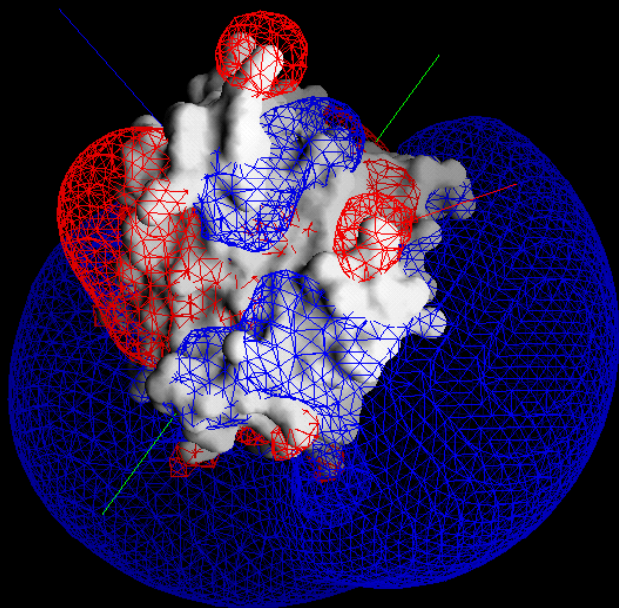
E. coli wt sito attivo visto di fronte



Cu,Zn superoxide dismutases



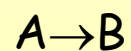
E. coli wt sito attivo visto di fronte



Cinetica enzimatica

Velocità delle reazioni chimiche:

1) Reazioni di primo ordine



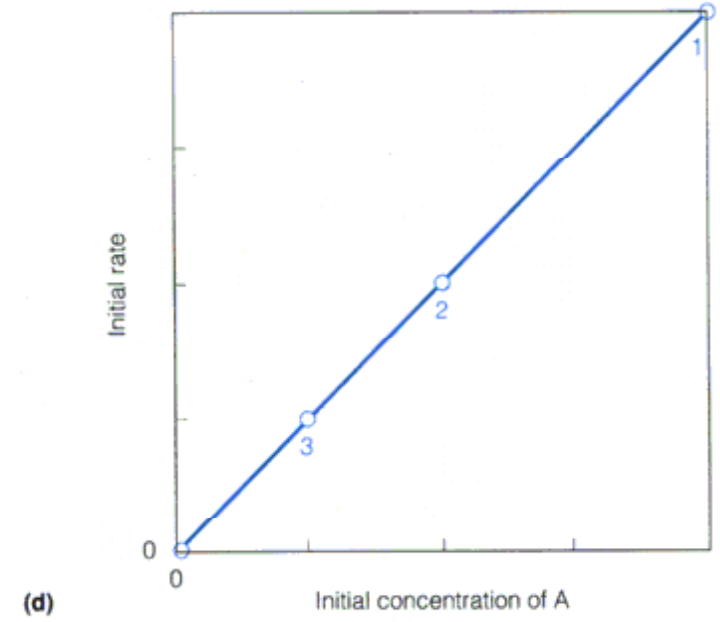
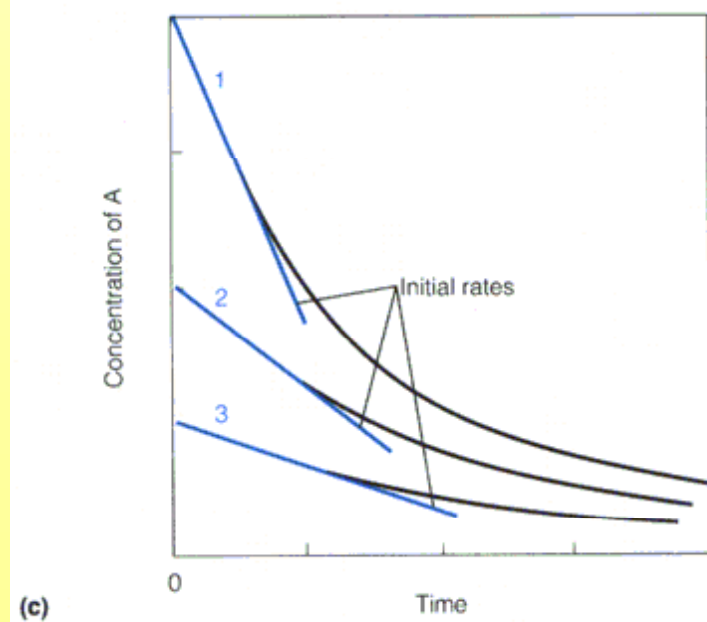
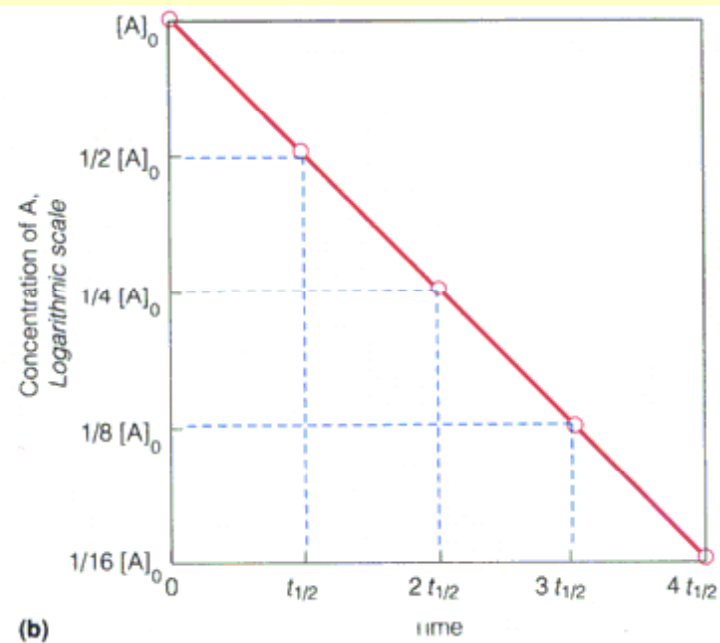
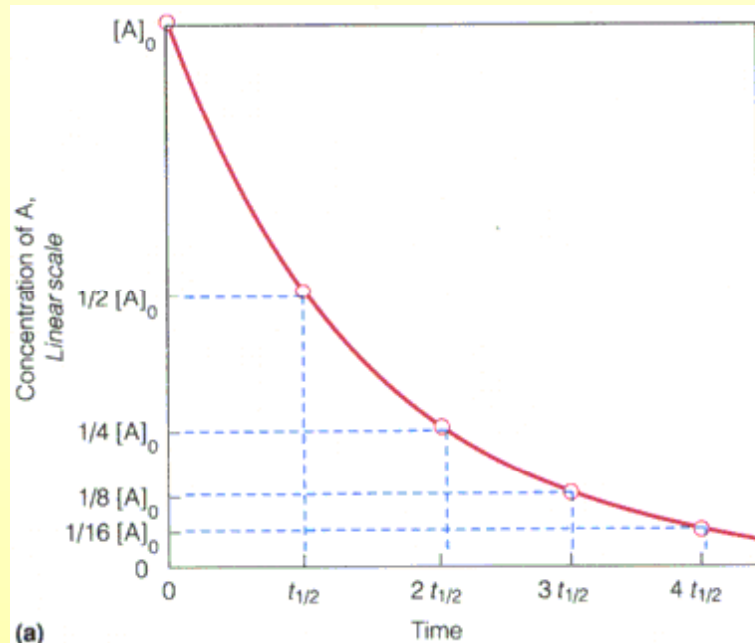
$$V = d[B]/dt \quad \text{oppure} \quad V = -d[A]/dt$$

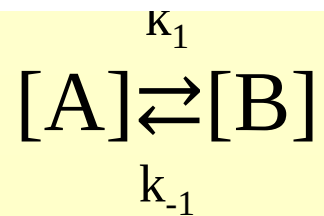
$$V = d[B]/dt = -d[A]/dt = k_1[A]$$

k = Costante di velocità s^{-1}

$$\text{Integrando} \quad \ln[A]_0/[A] = k_1 t$$

$$\text{oppure} [A]/[A]_0 = e^{-k_1 t}$$





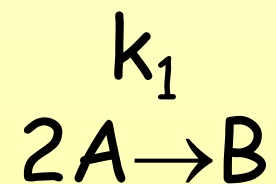
$$-V = d[A]/dt = -k_1[A] + k_{-1}[B]$$

$$0 = -k_1[A]_{eq} + k_{-1}[B]_{eq}$$

$$[B]_{eq}/[A]_{eq} = k_1/k_{-1} = K$$

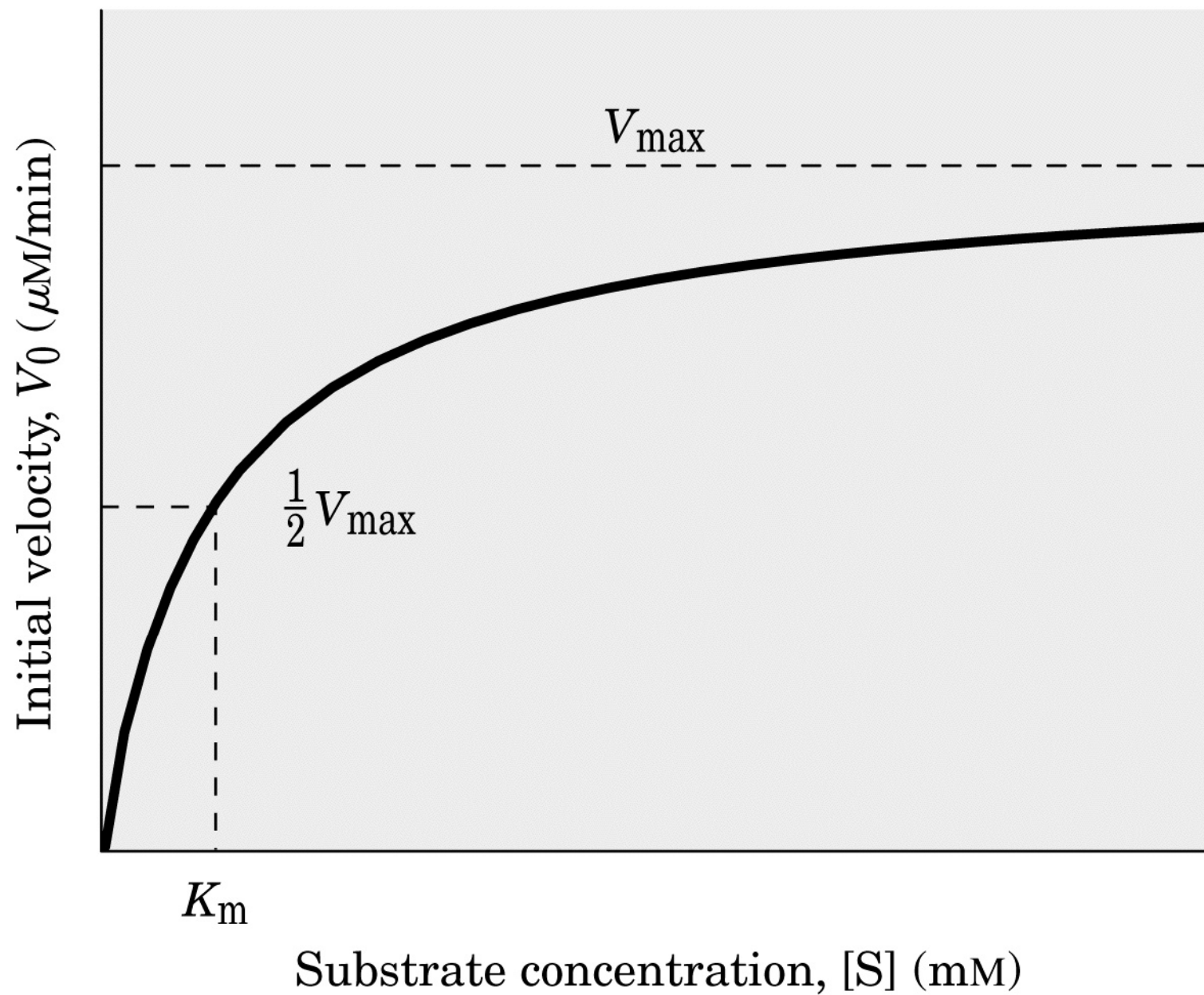
(costante di equilibrio)

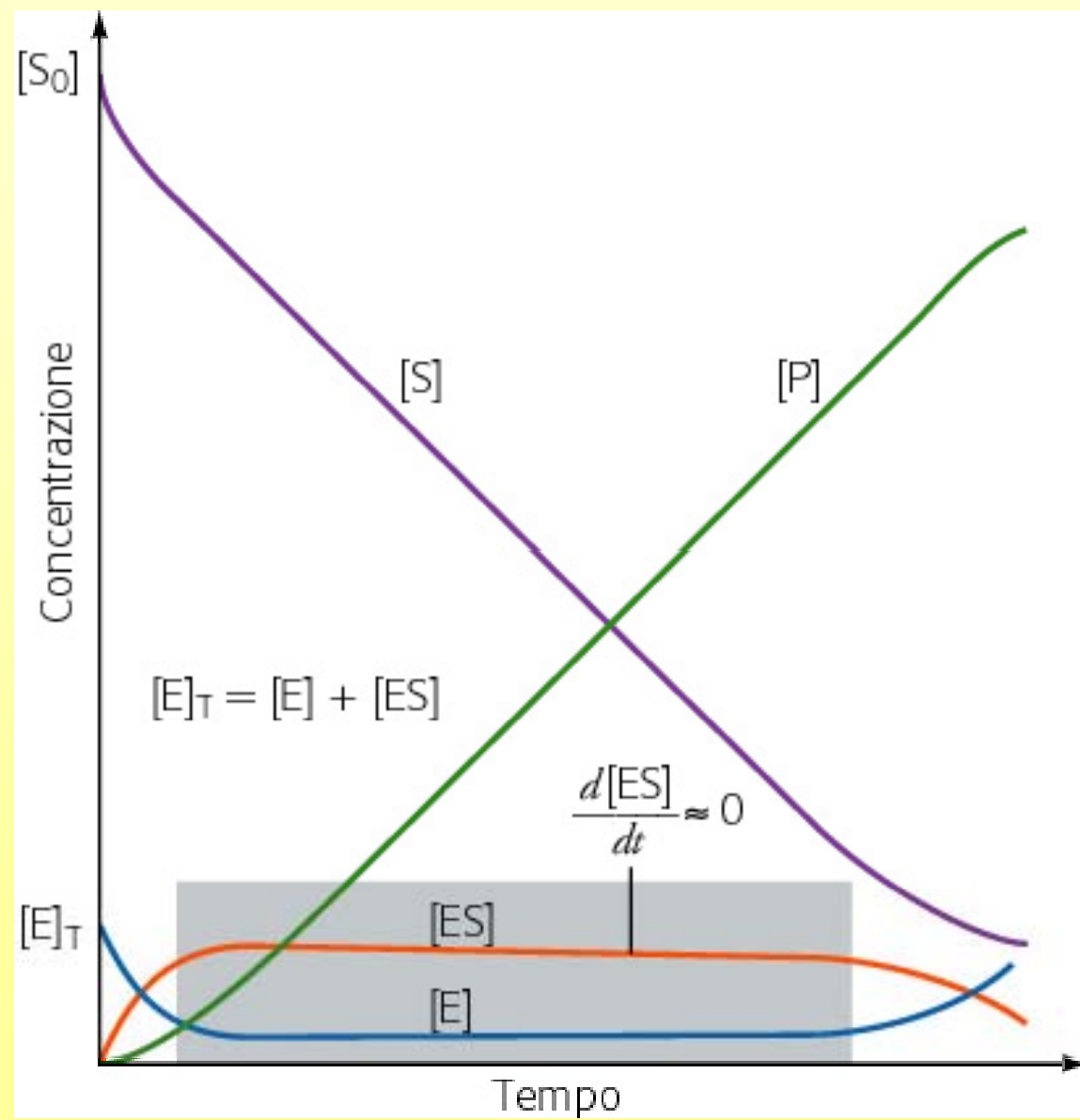
Reazioni di secondo ordine



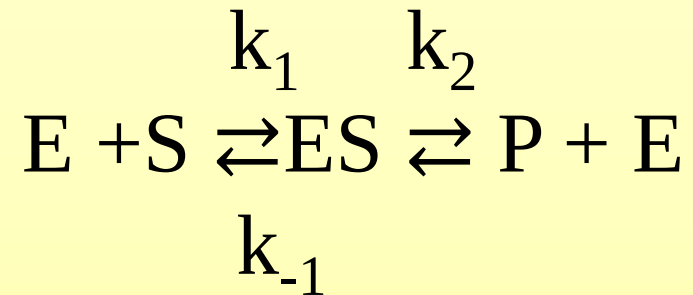
$$V = d[B]/dt = k_2[A]^2$$

$$(\text{mol/L})\text{s}^{-1}$$





Cinetica della catalisi enzimatica

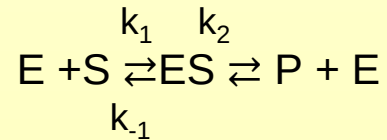


$$v = d[\text{P}]/dt = k_2[\text{ES}]$$

$$d[\text{ES}]/dt = k_1[\text{E}][\text{S}] - k_{-1}[\text{ES}] - k_2[\text{ES}]$$

Cinetica della catalisi enzimatica

L'equazione di Michaelis-Menten



$$v = d[P]/dt = k_2[ES]$$

Se $[S] \gg [E]$ allora $d[ES]/dt = 0$ $v_0 = k_2[ES]$
 $[E]_T = [E] + [ES]$ oppure $[E] = [E]_T - [ES]$

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

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

$$([E]_T - [ES])[S]/[ES] = (k_{-1} + k_2)/k_1 = K_M$$

$$K_M[ES] = ([E]_T - [ES])[S]$$

$$[ES] = [E]_T[S] / (K_M + [S])$$

$$v_0 = k_2[E]_T[S] / (K_M + [S])$$

$$V_{max} = k_2[E]_T$$

$$v_0 = V_{max}[S] / (K_M + [S]) \quad \text{Equazione di Michaelis-Menten}$$

$$\text{Se } v_0 = 1/2 V_{max} \quad \text{allora} \quad K_M = [S]$$

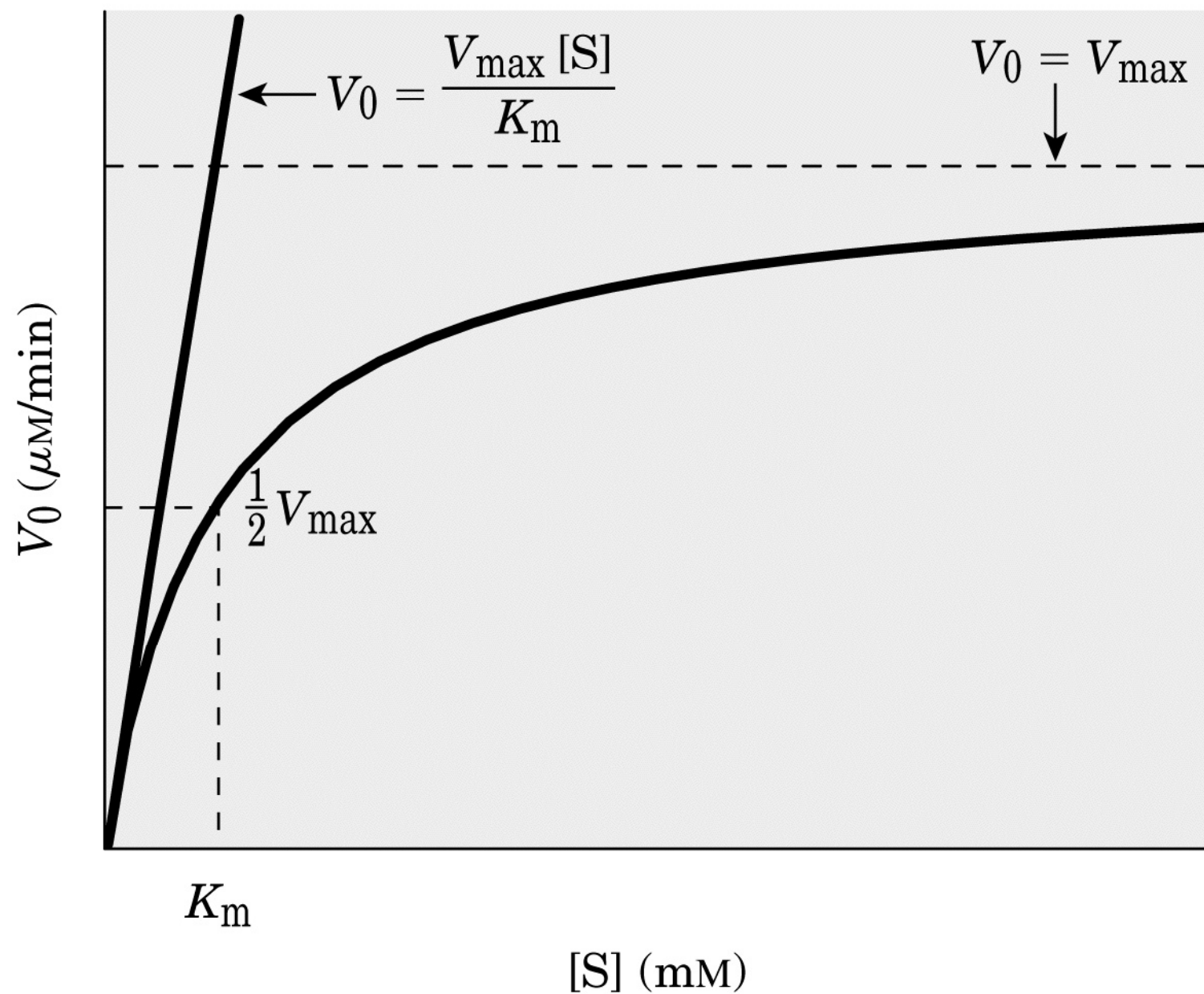


table 8–6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β -Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

E' possibile definire la costante catalitica di un enzima, k_{cat} , come:

$$K_{cat} = V_{max} / [E]_T$$

Questa costante misura la velocità del processo catalitico (il numero delle molecole di substrato trasformate in un secondo) ed è detta anche numero di turnover

table 8–7

Turnover Numbers (k_{cat}) of Some Enzymes

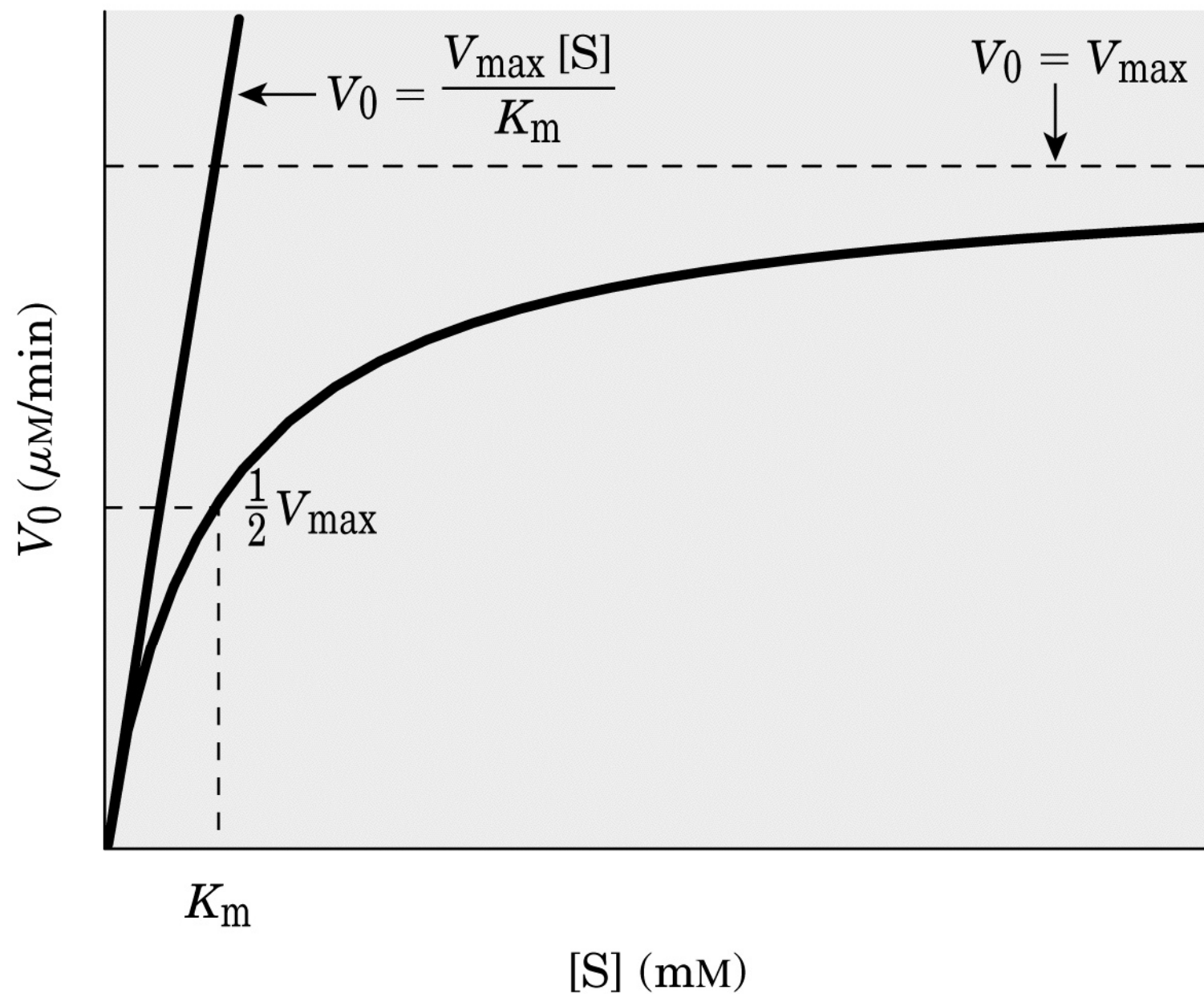
Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	140,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

table 8–8

Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$)

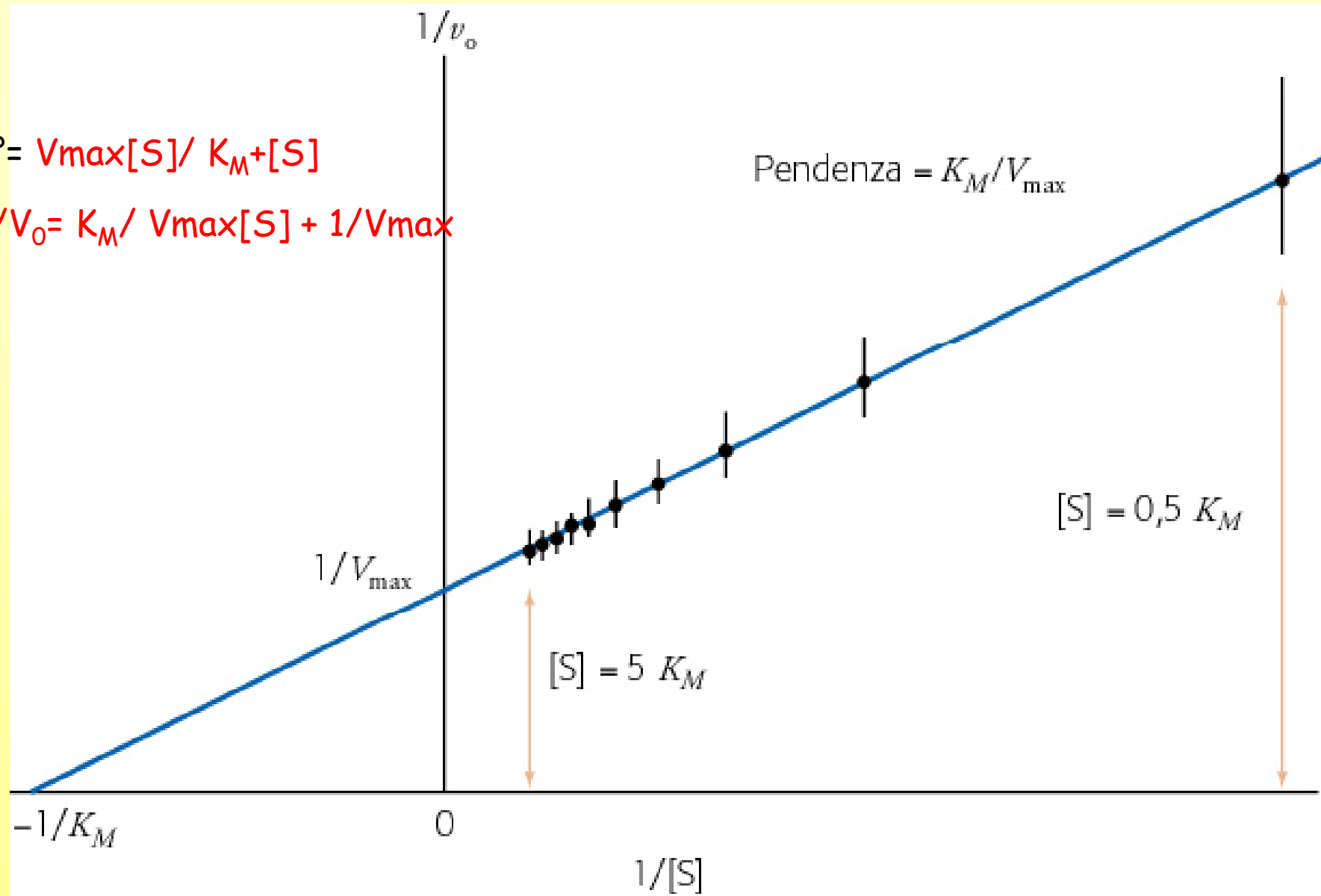
Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($\text{M}^{-1}\text{s}^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β -Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8
Triose phosphate isomerase	Glyceraldehyde 3-phosphate	4.3×10^3	4.7×10^{-4}	2.4×10^8

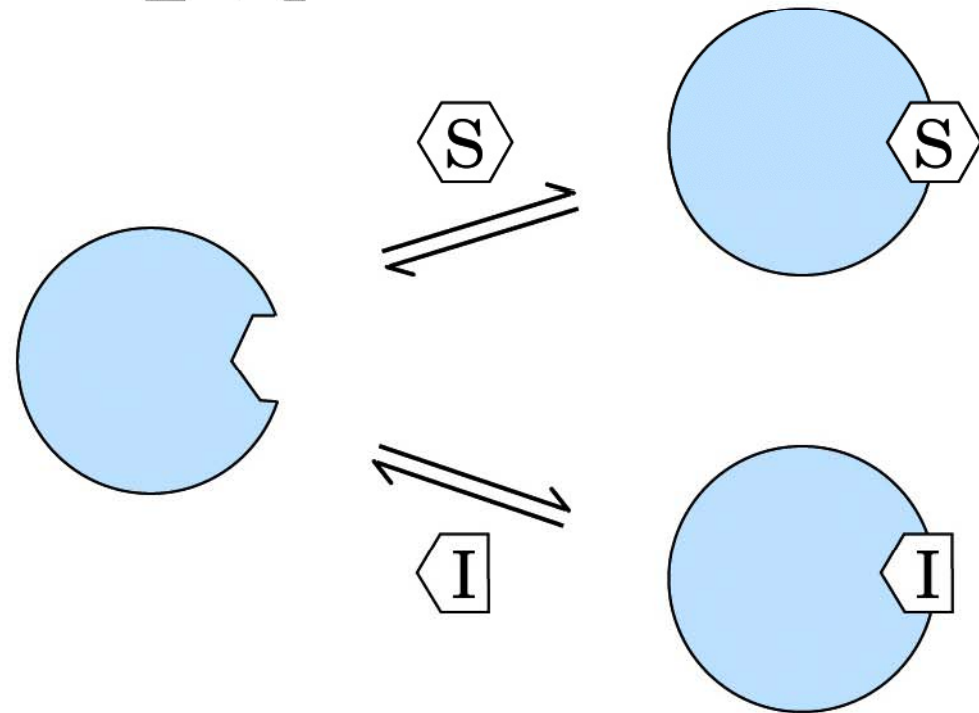
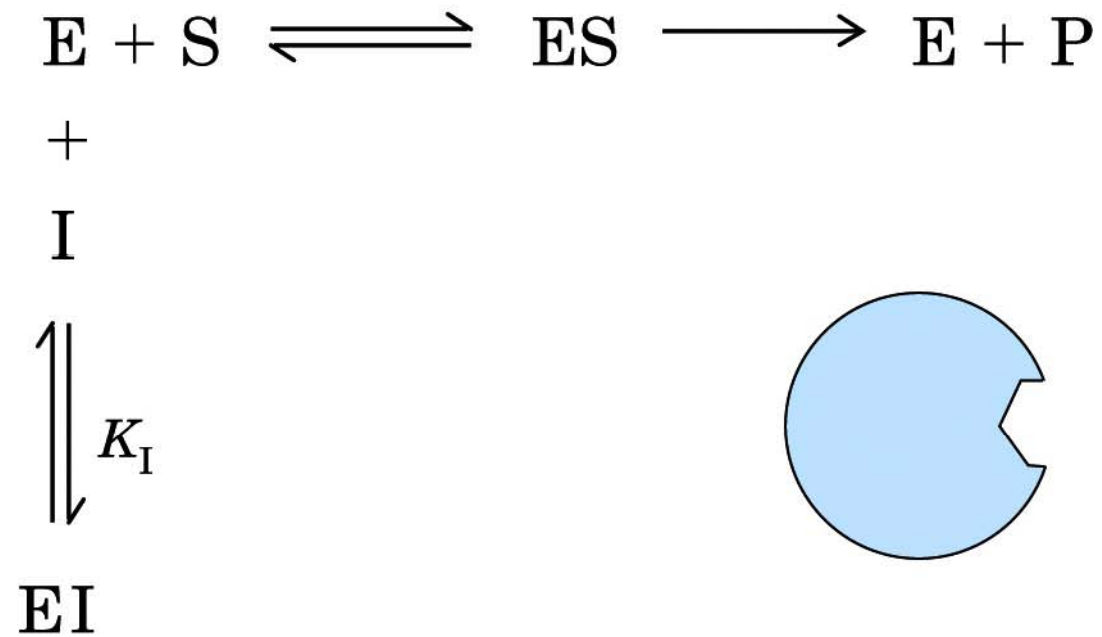
Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W.H. Freeman and Company, New York.



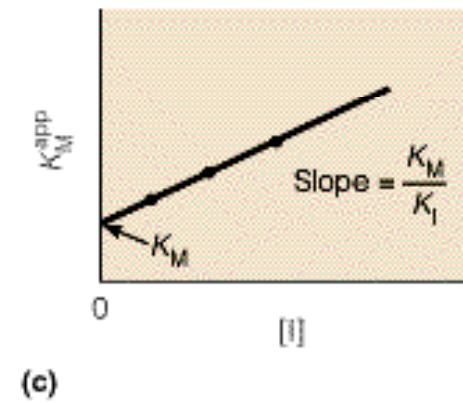
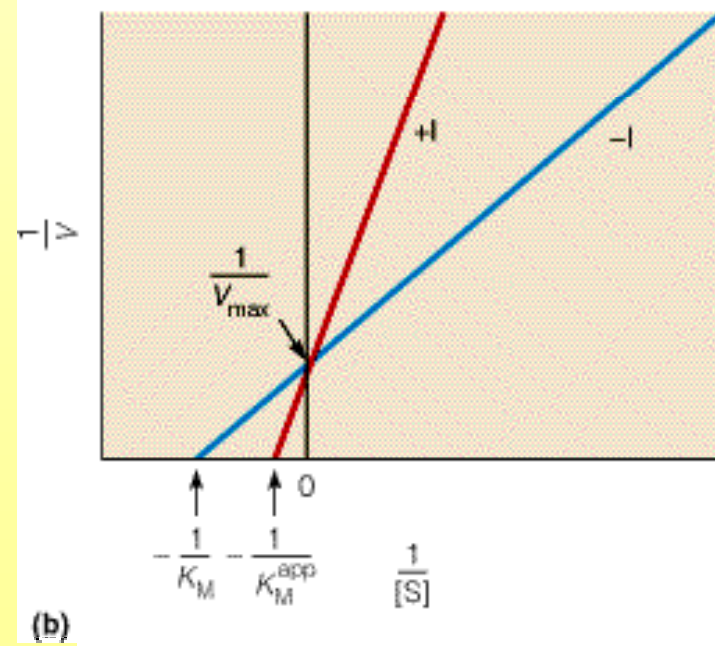
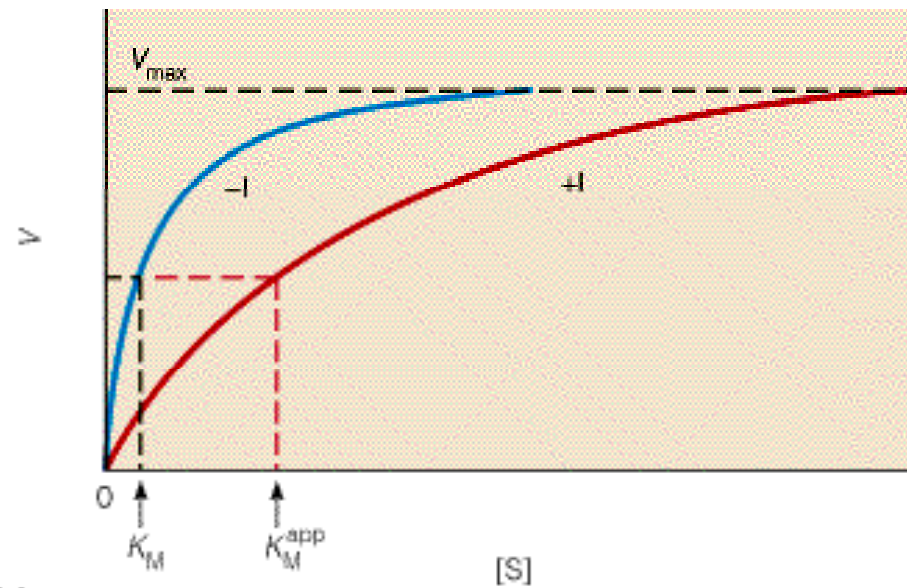
$$v^o = V_{\max}[S] / K_M + [S]$$

$$1/V_0 = K_M / V_{\max}[S] + 1/V_{\max}$$

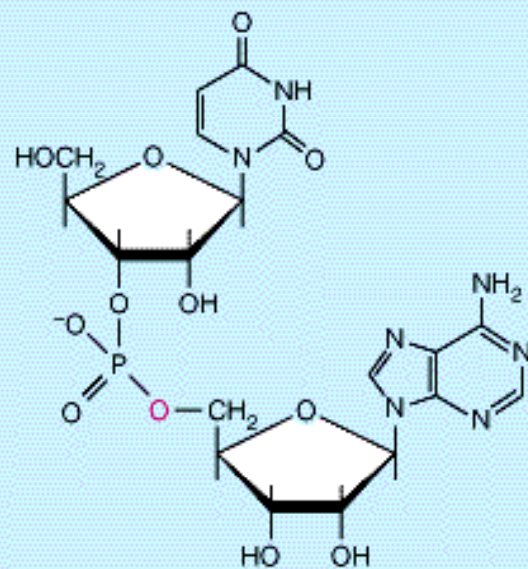




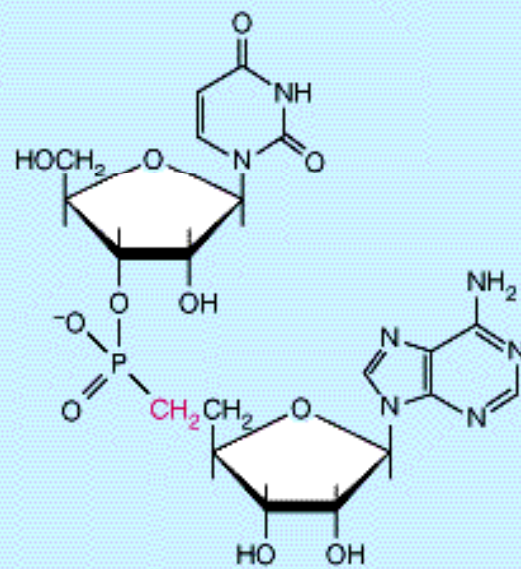
(a) Competitive inhibition



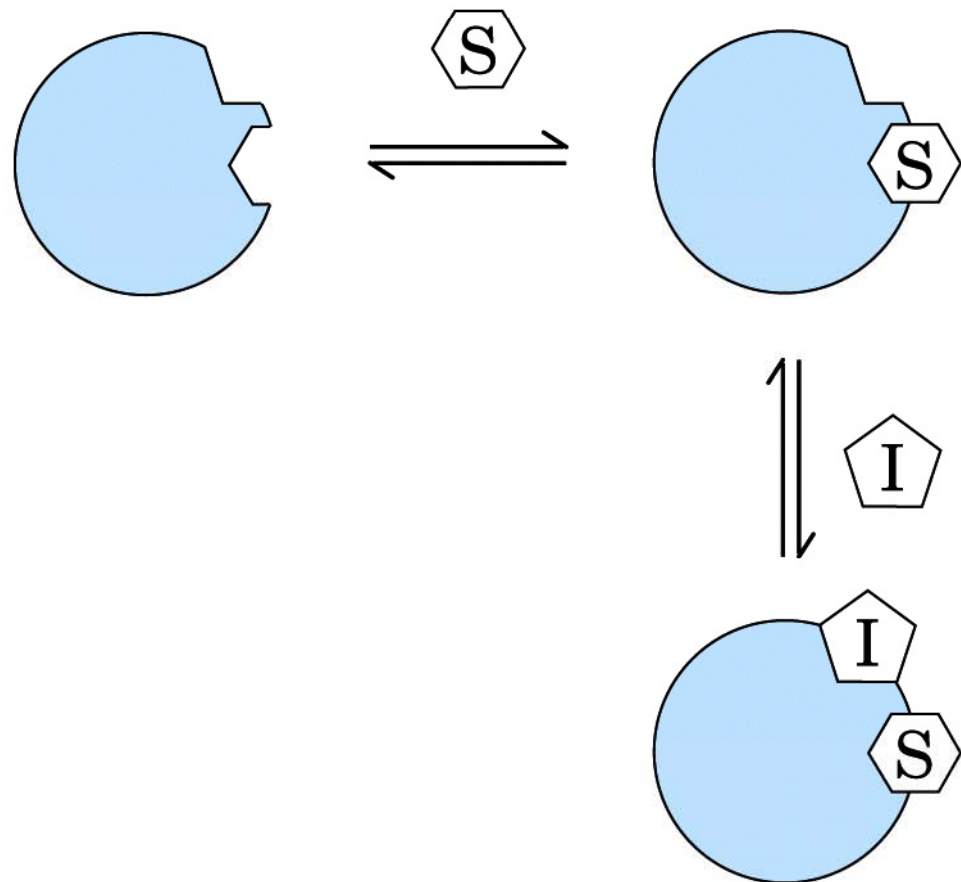
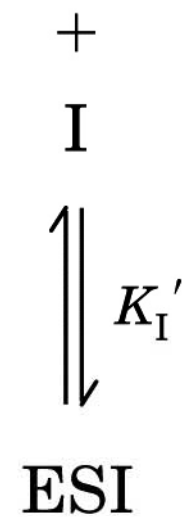
$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{1}{V_{\max}}$$



UpA: ribonuclease substrate

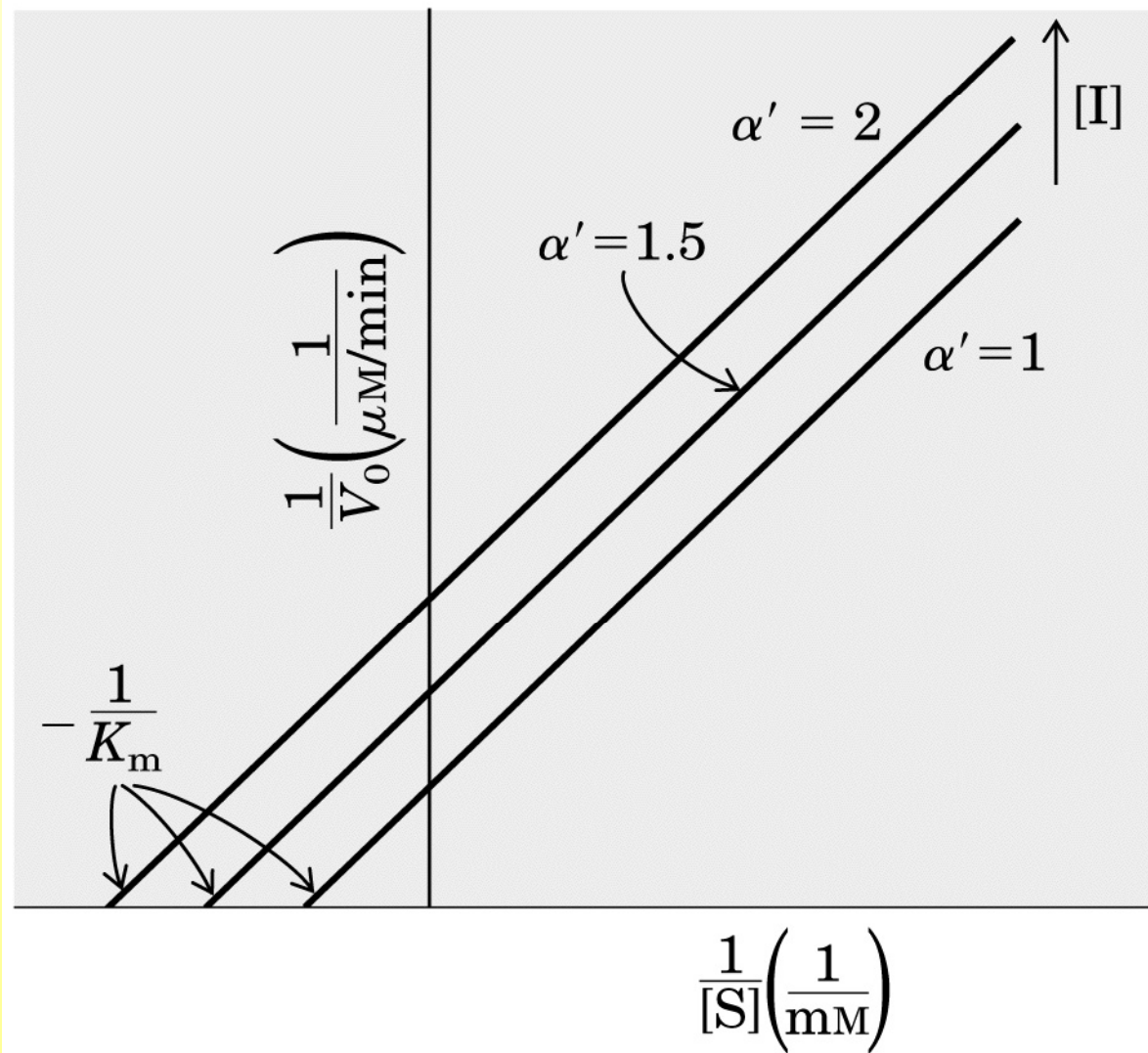


UpcA: competitive inhibitor of ribonuclease



(b) Uncompetitive inhibition

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$



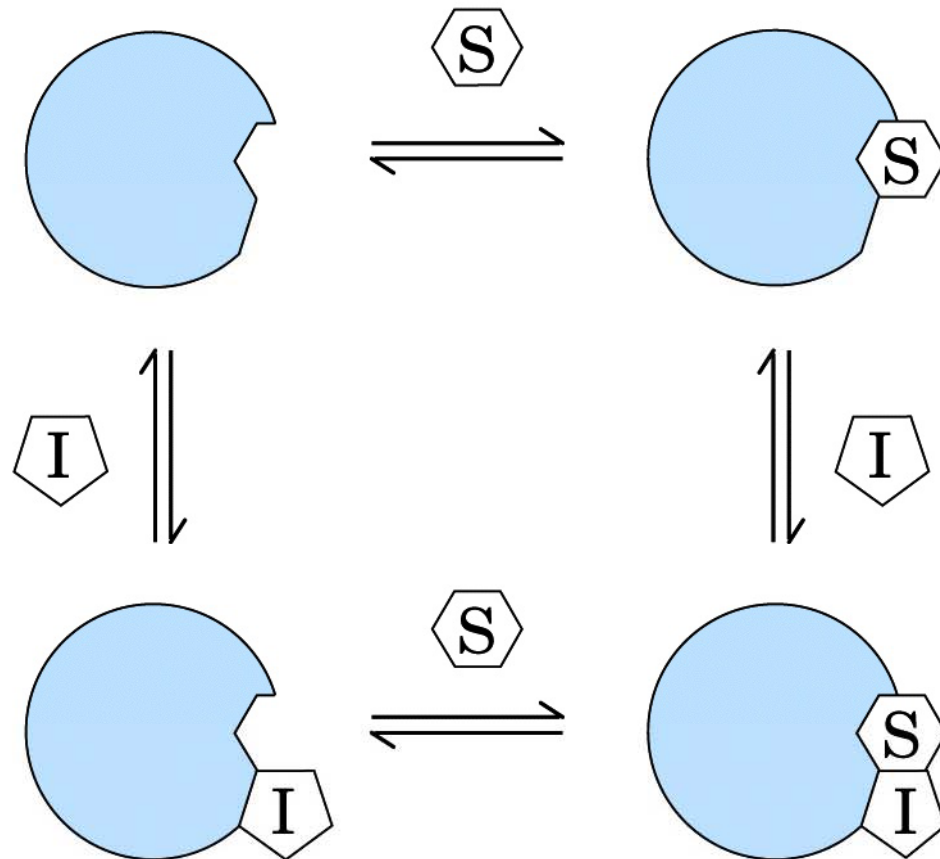
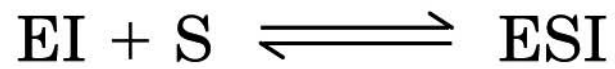
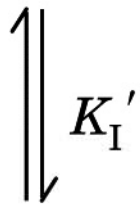
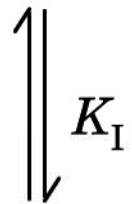


+

+

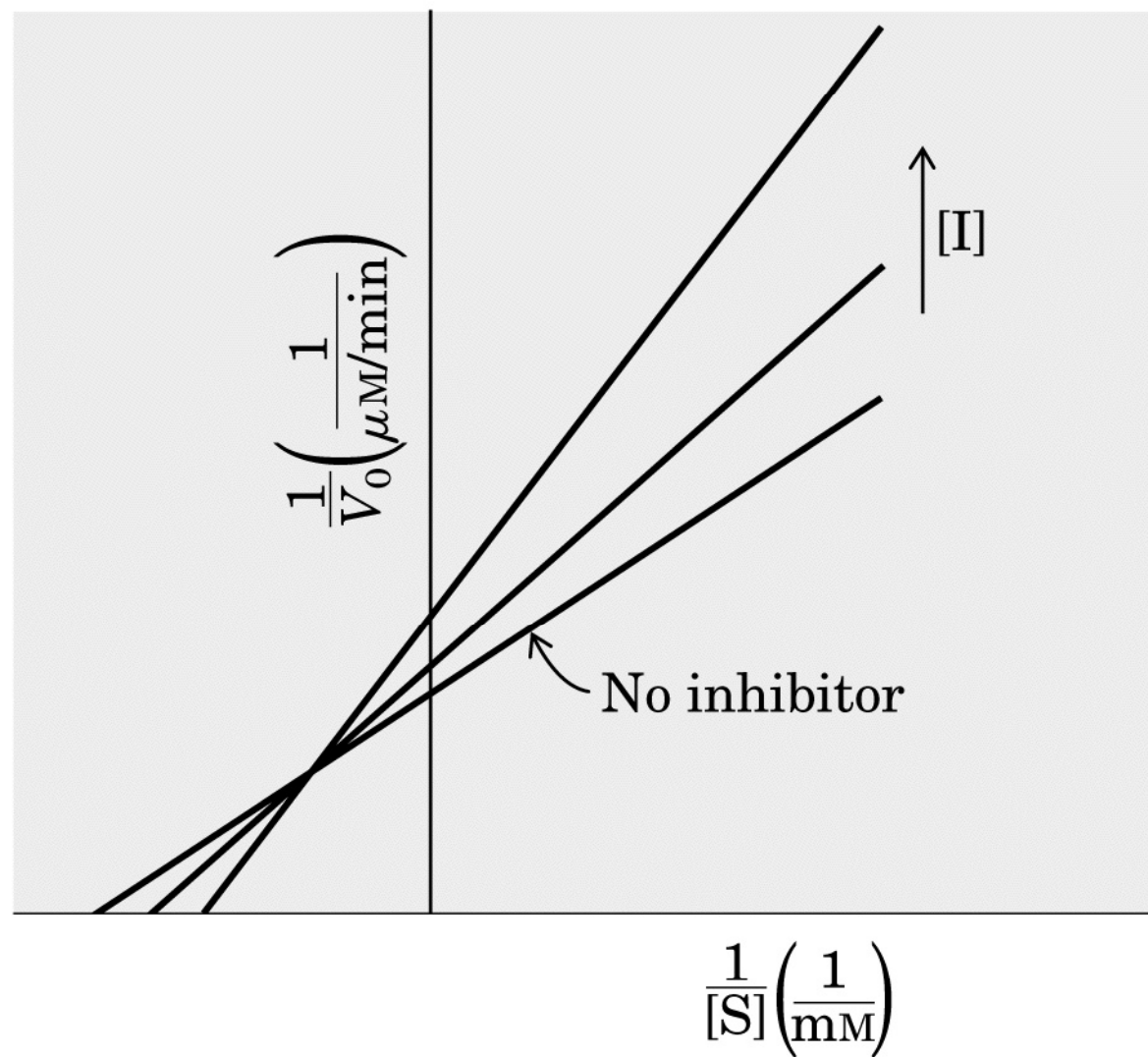
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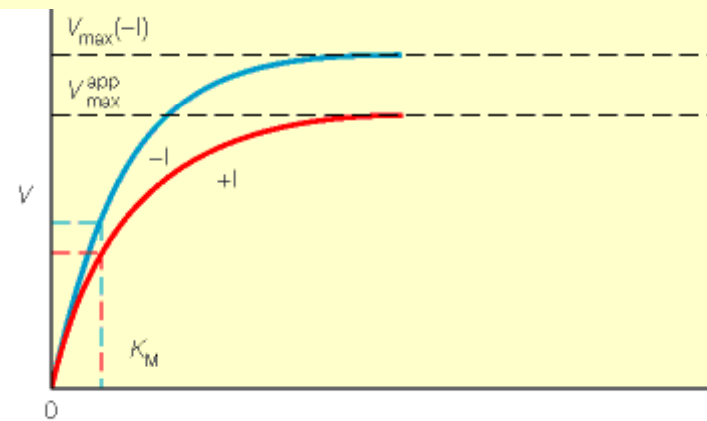
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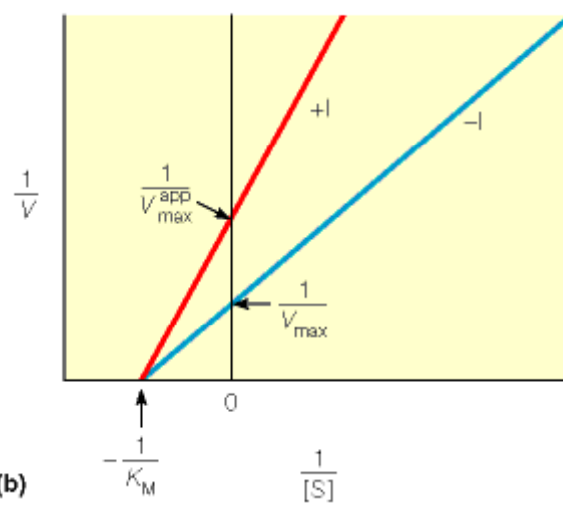
(c) Mixed inhibition

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$

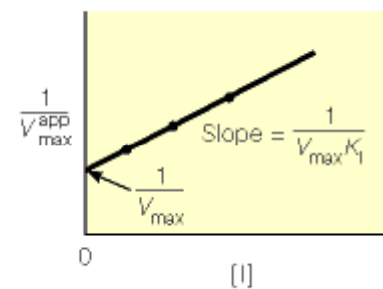




(a) [S]

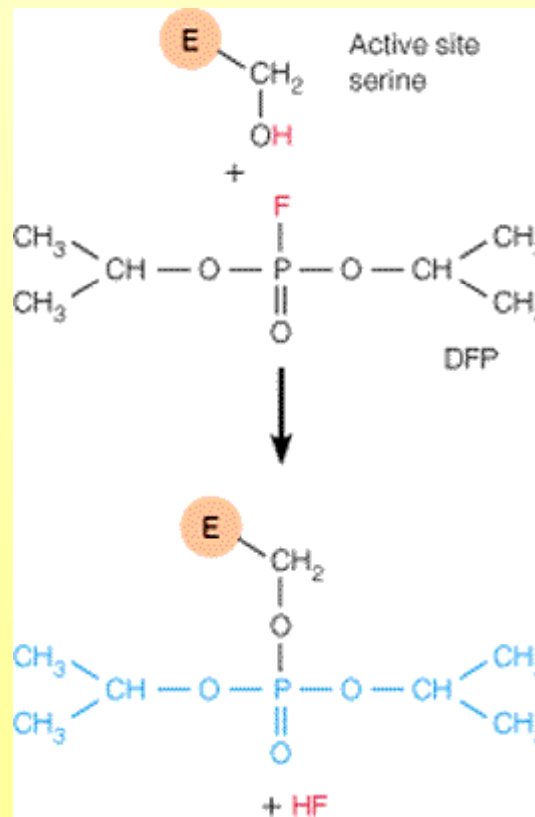


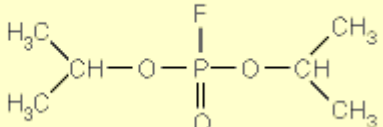
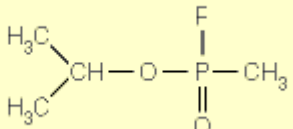
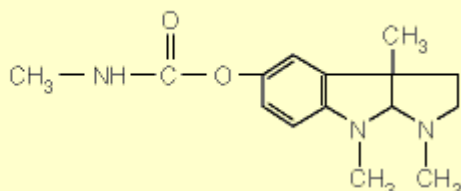
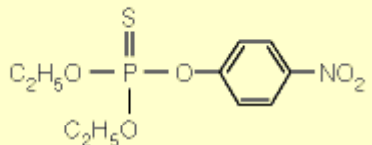
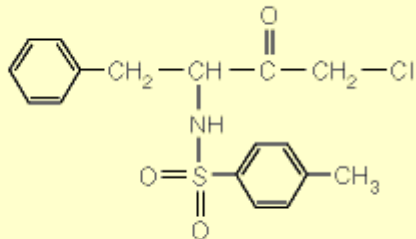
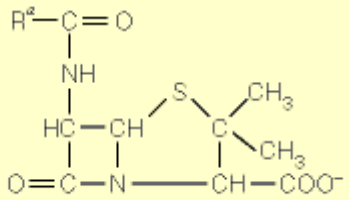
(b)



(c)

Inibizione irreversibile



Name	Formula ^a	Source	Mode of Action
Cyanide	CN ⁻	Bitter almonds	Reacts with enzyme metal ions (i.e., Fe, Zn, Cu); respiratory chain enzymes are primary targets (see Chapter 15)
Diisopropyl fluorophosphate (DFP)		Synthetic	Inhibits enzymes with active site serine, including acetylcholinesterase
Sarin		Synthetic (nerve gas)	Like DFP
Physostigmine		Calabar beans	Like DFP
Parathion		Synthetic (insecticide)	Like DFP, but especially inhibitory to insect acetylcholinesterase
N-Tosyl-L-phenylalaninechloromethyl ketone (TPCK)		Synthetic	Reacts with His 57 of chymotrypsin
Penicillin		From <i>Penicillium</i> fungus	Inhibits enzymes in bacterial cell wall synthesis (see Chapter 16)