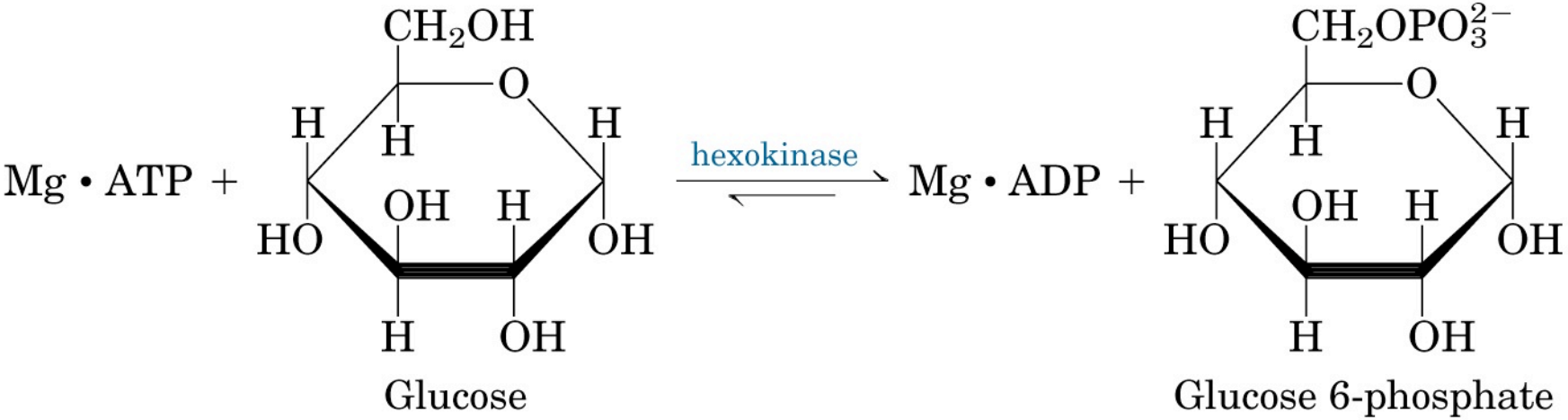
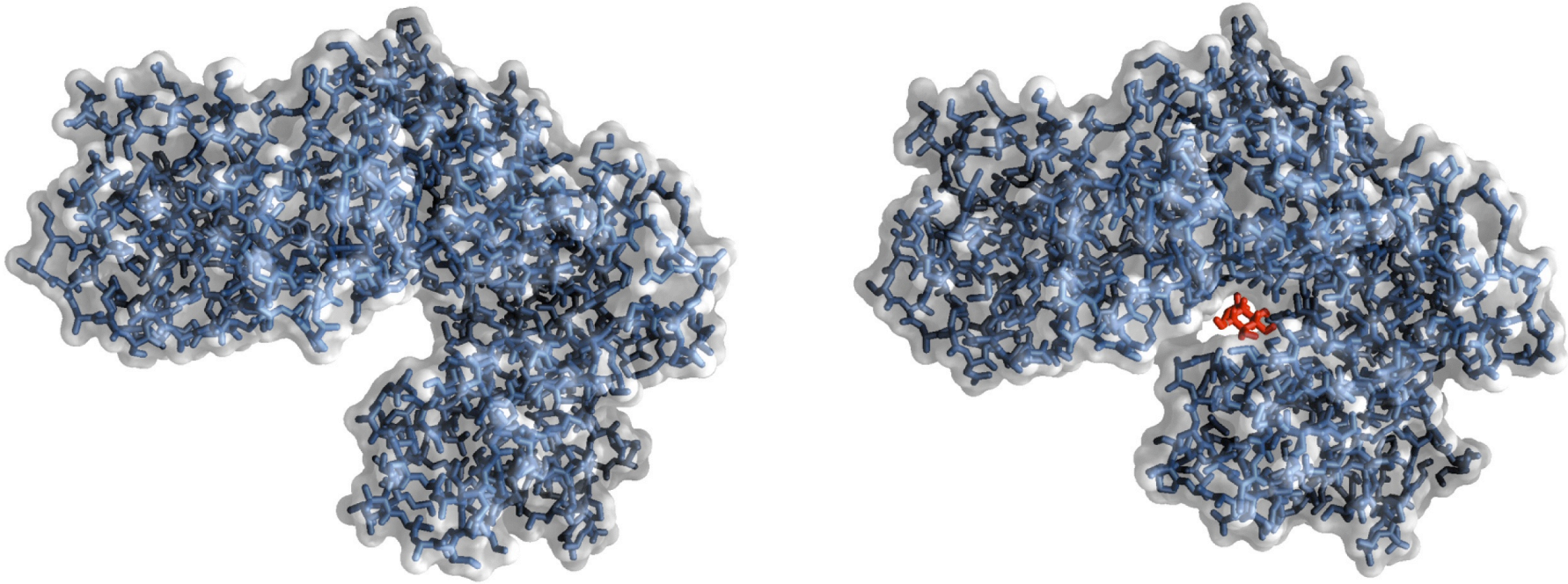
MECCANISMI DI CATALISI

Fattori che influenzano $\Delta G^{\ddagger}$:

- Minore libertà di movimento delle molecole
- Desolvatazione
- Distorsione del substrato
- Allineamento corretto gruppi funzionale dell'enzima

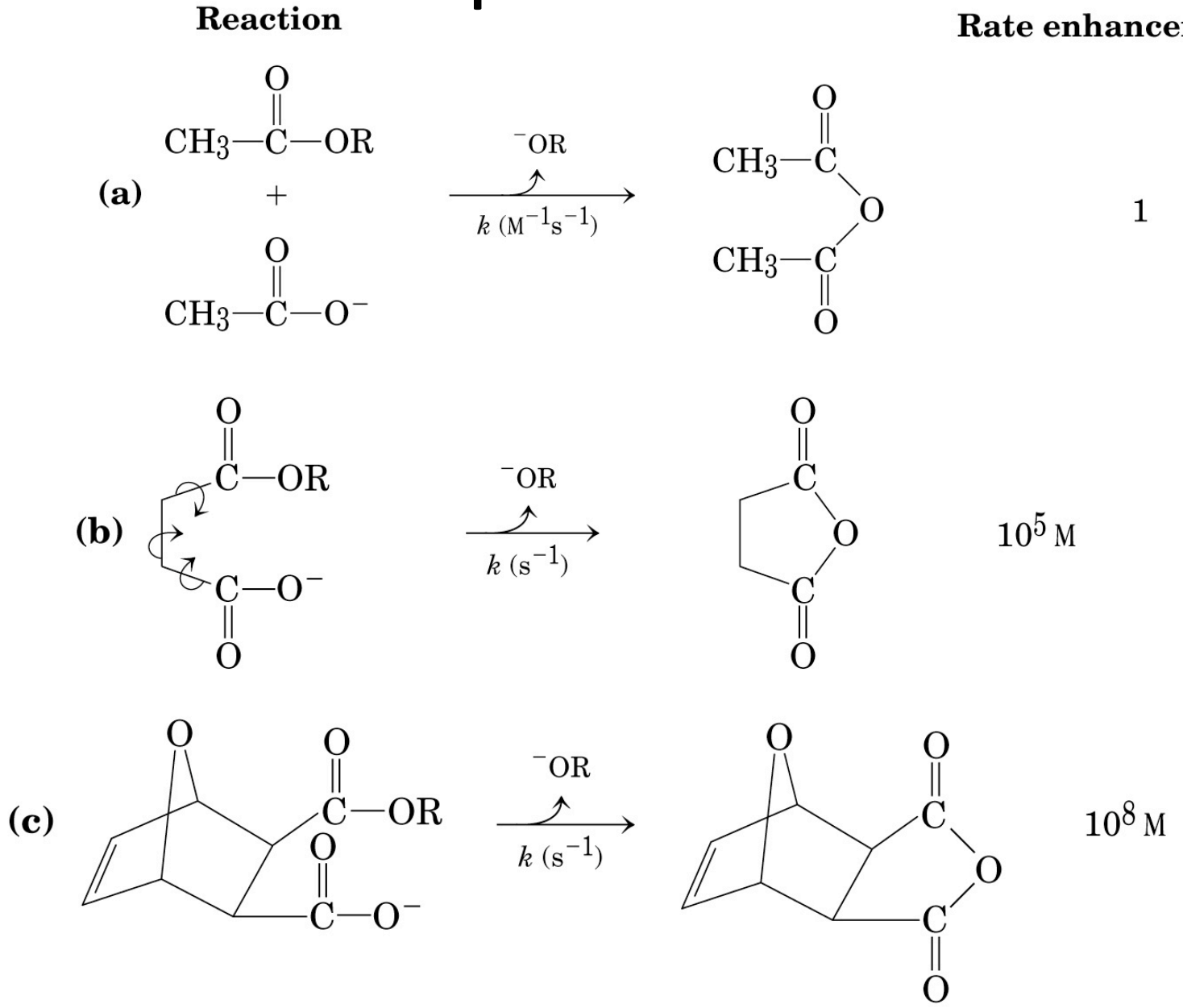
ADATTAMENTO INDOTTO



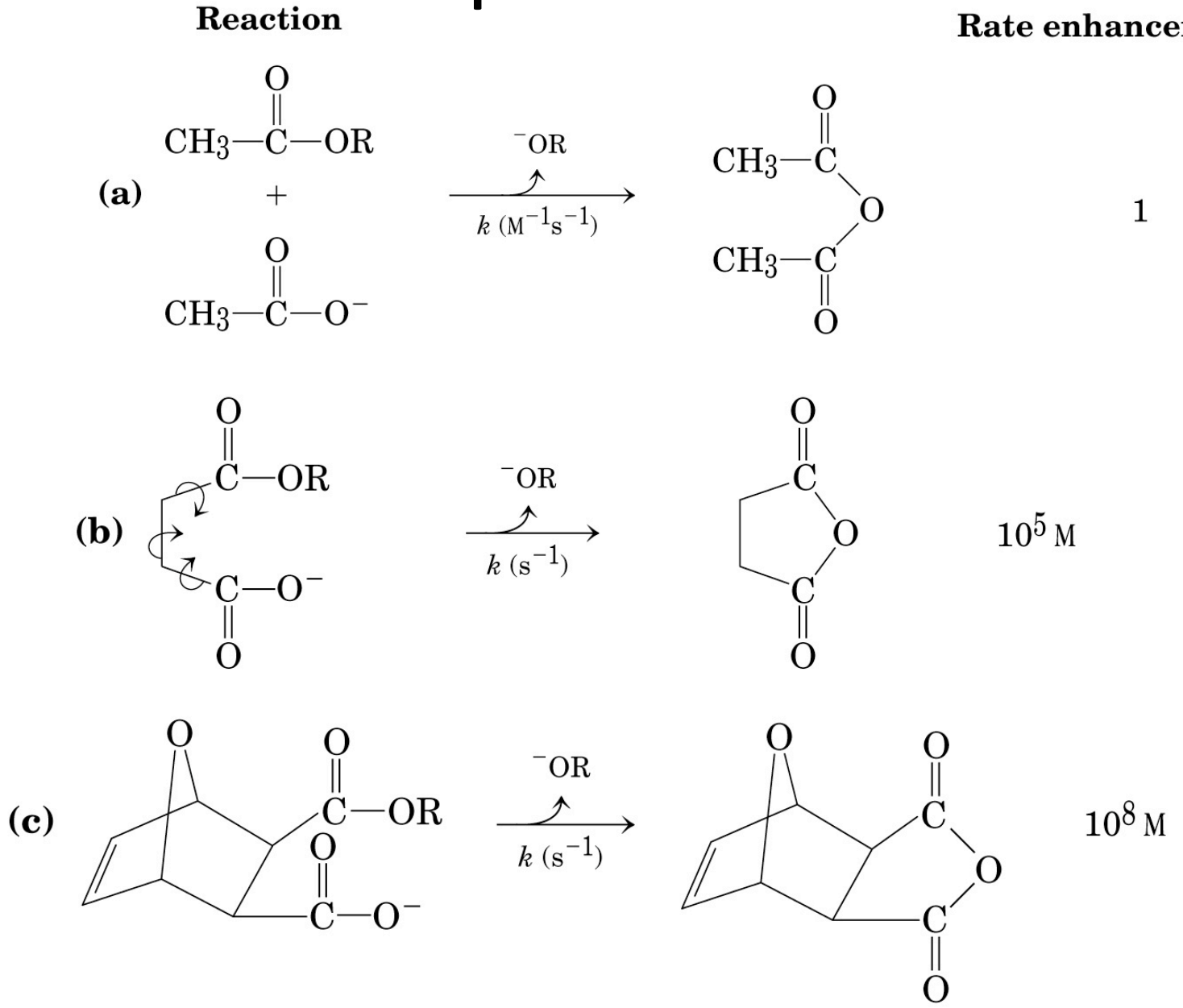


(a) (b)

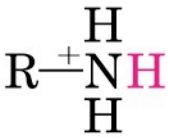
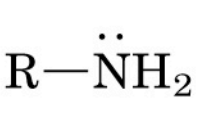
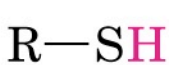
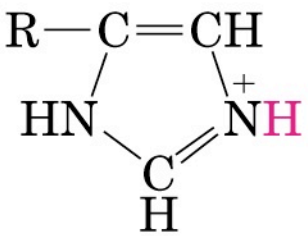
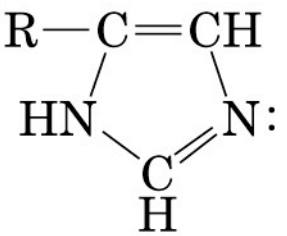
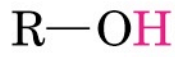
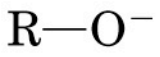
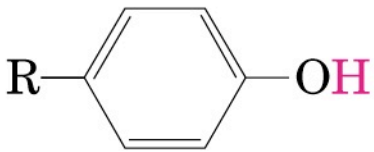
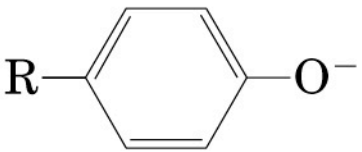
Catalisi per riduzione entropica



Catalisi per riduzione entropica



Catalisi acido base generale

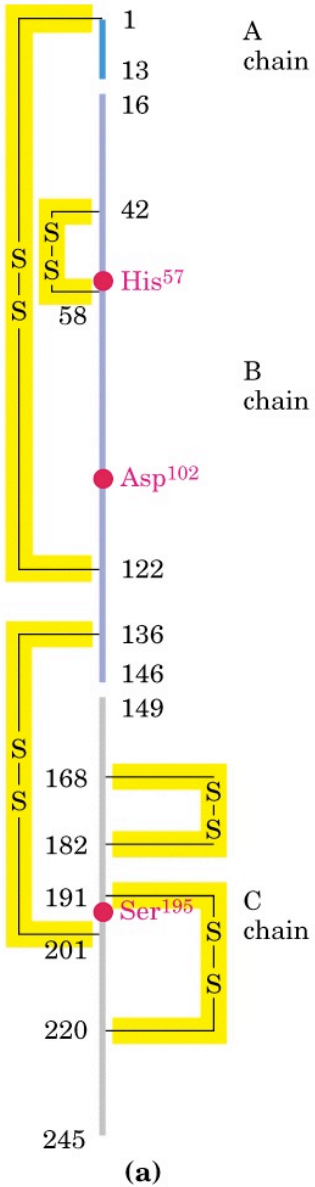
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

CATALISI COVALENTE

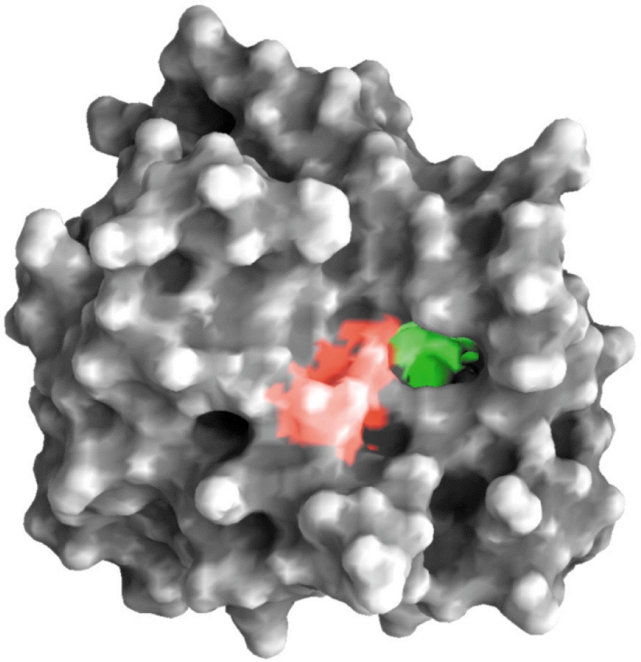
Si attua attraverso la formazione
di un legame covalente transitorio
tra una catena laterale amminoacidica
dell' enzima o tra un cofattore enzimatico
ed il substrato.

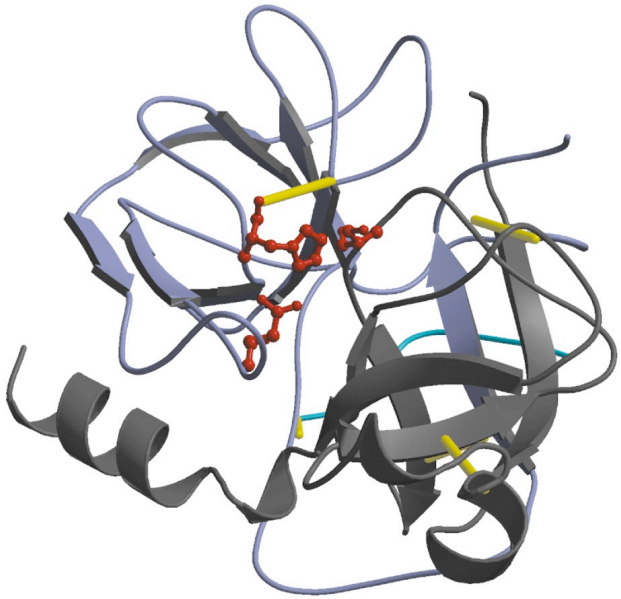
CHIMOTRIPSINA

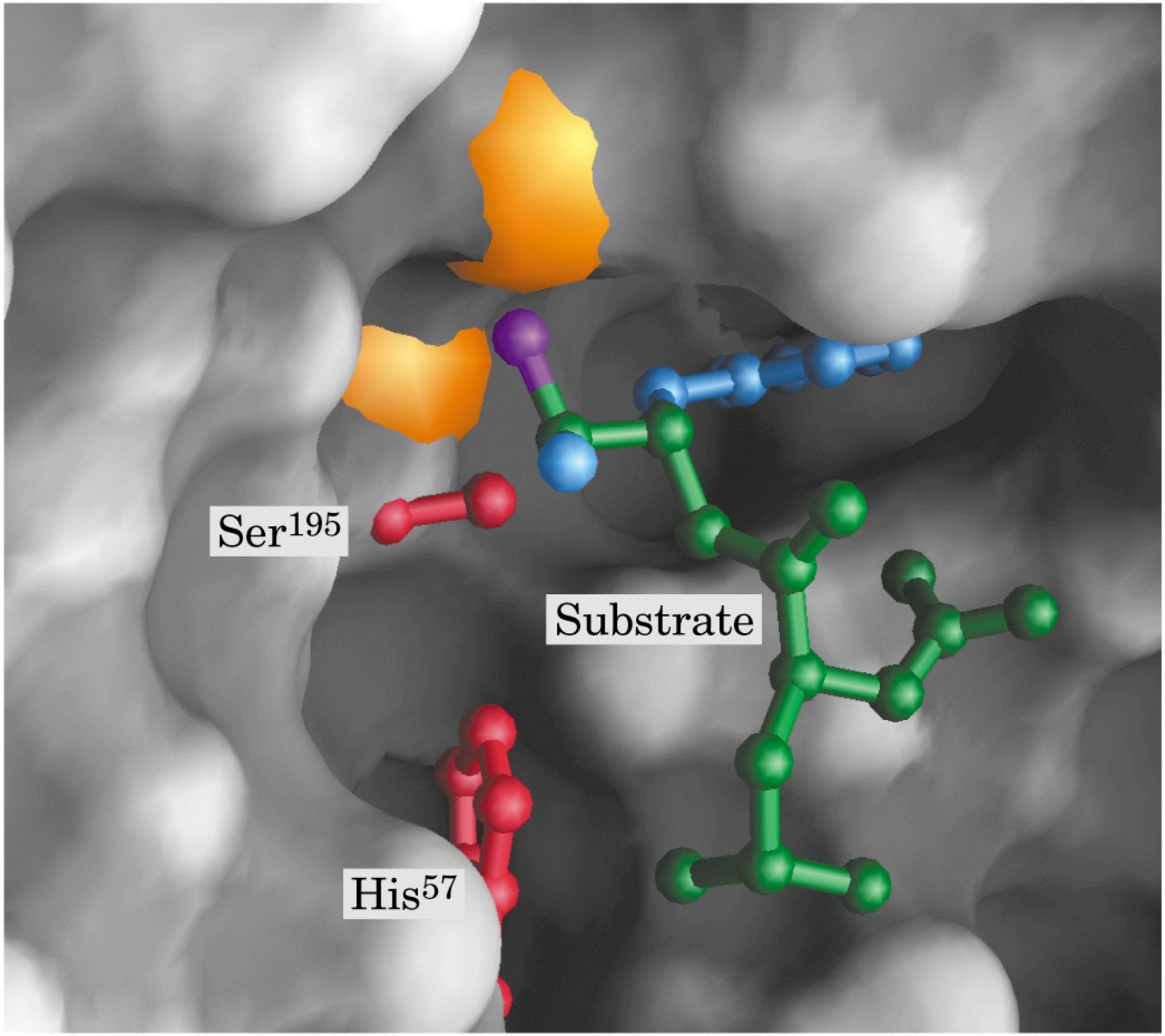
P M 25.000 Da
3 CATENE
2 -S-S- INTER
3 -S-S- INTRA
CATALISI:
Covalente
basica generale
AA CATALITICI
Ser 195
Asp 102
HIS 57
FATTORE DI ACCELERAZIONE:
10^9



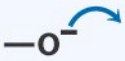
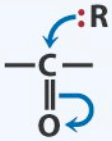
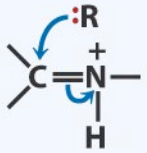
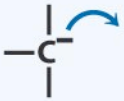
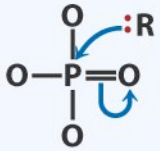
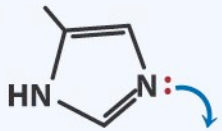
(a)

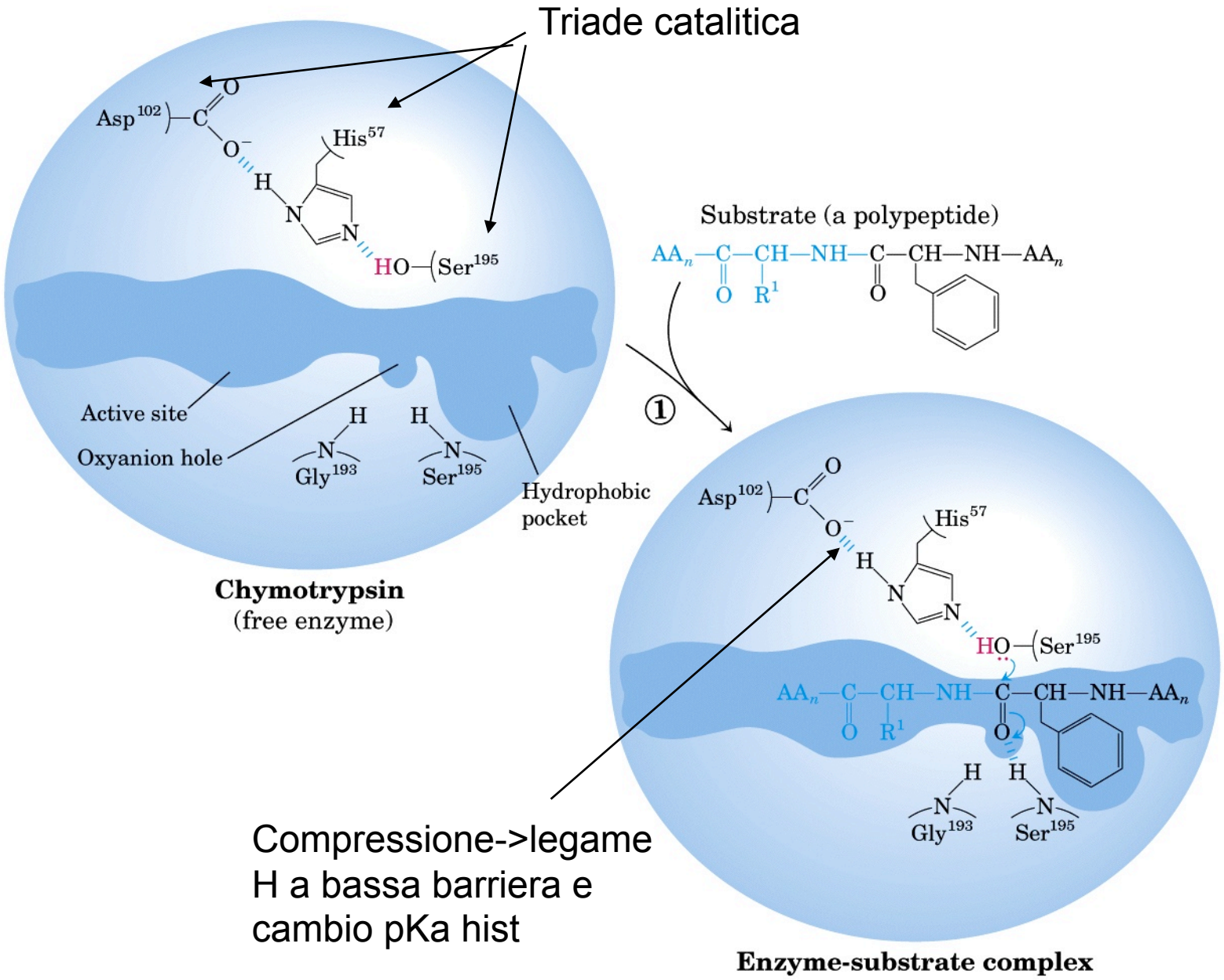

(b)


(c)



(d)

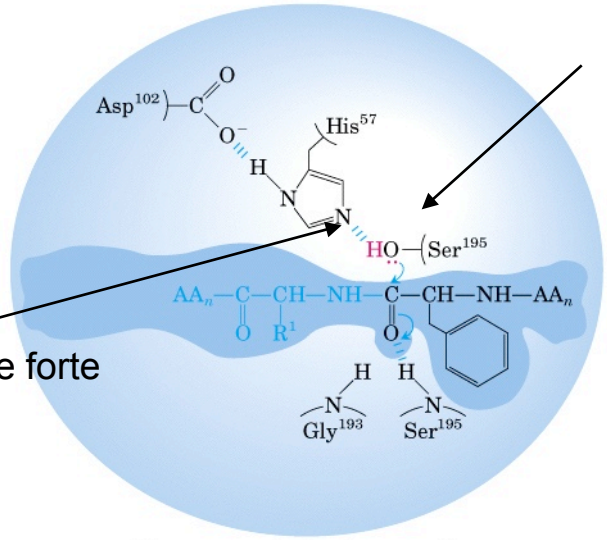
Nucleophiles	Electrophiles
 Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)	 Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)
 Negatively charged sulfhydryl	 Pronated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)
 Carbanion	 Phosphorus of a phosphate group
 Uncharged amine group	 Proton
 Imidazole	
 Hydroxide ion	



Compressione->legame H a bassa barriera e cambio pKa hist

Attacco dello ione alcossido al carbonile

Deprotonazione da base forte

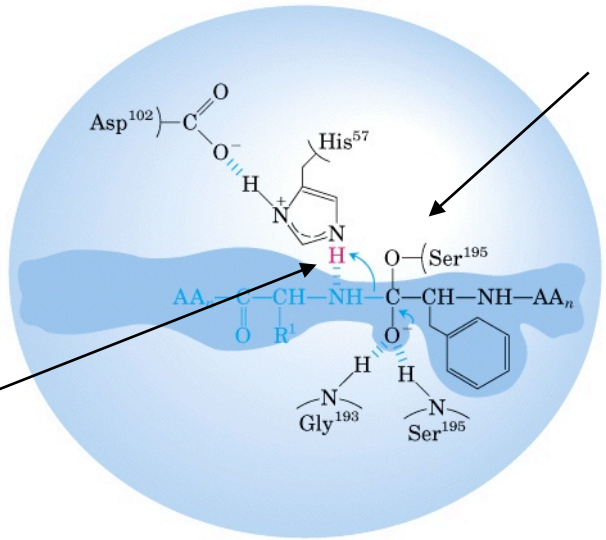


Enzyme-substrate complex

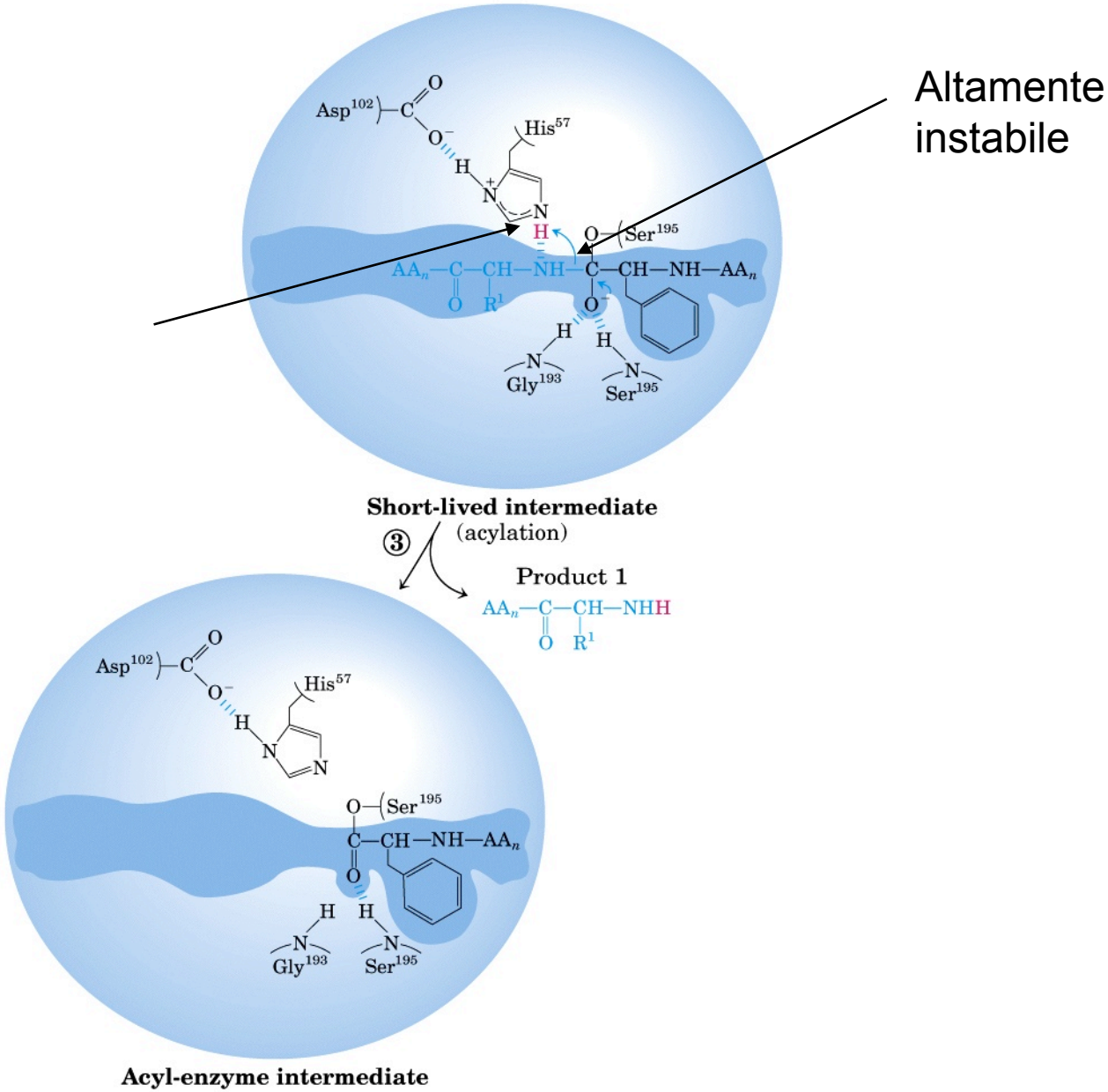
②

Formazione del l'intermedio tetraedrico con carica negativa stabilizzata da ponti H (i.e.energia legame per catalisi)

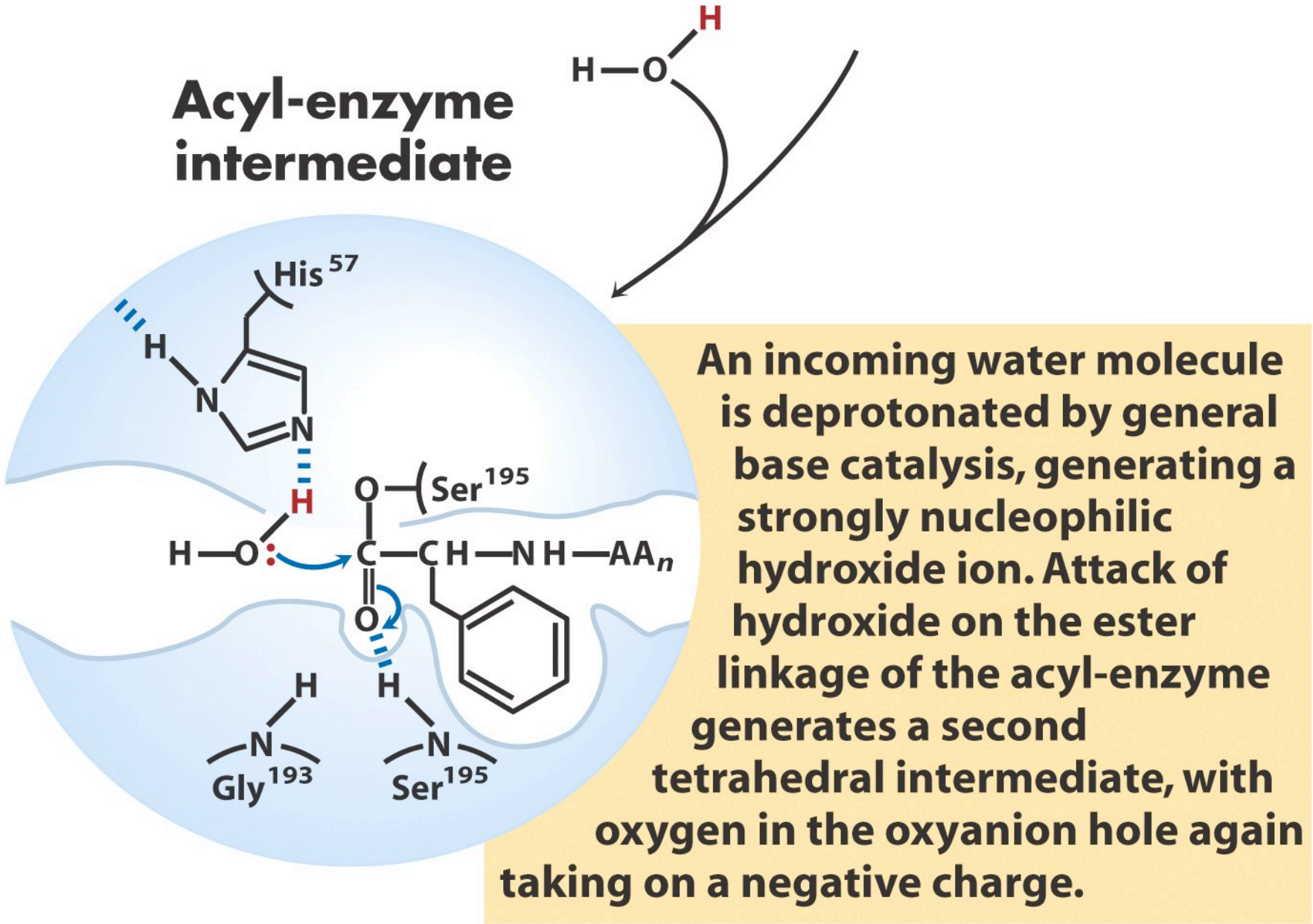
Protonazione del gruppo uscente favorendo la rottura



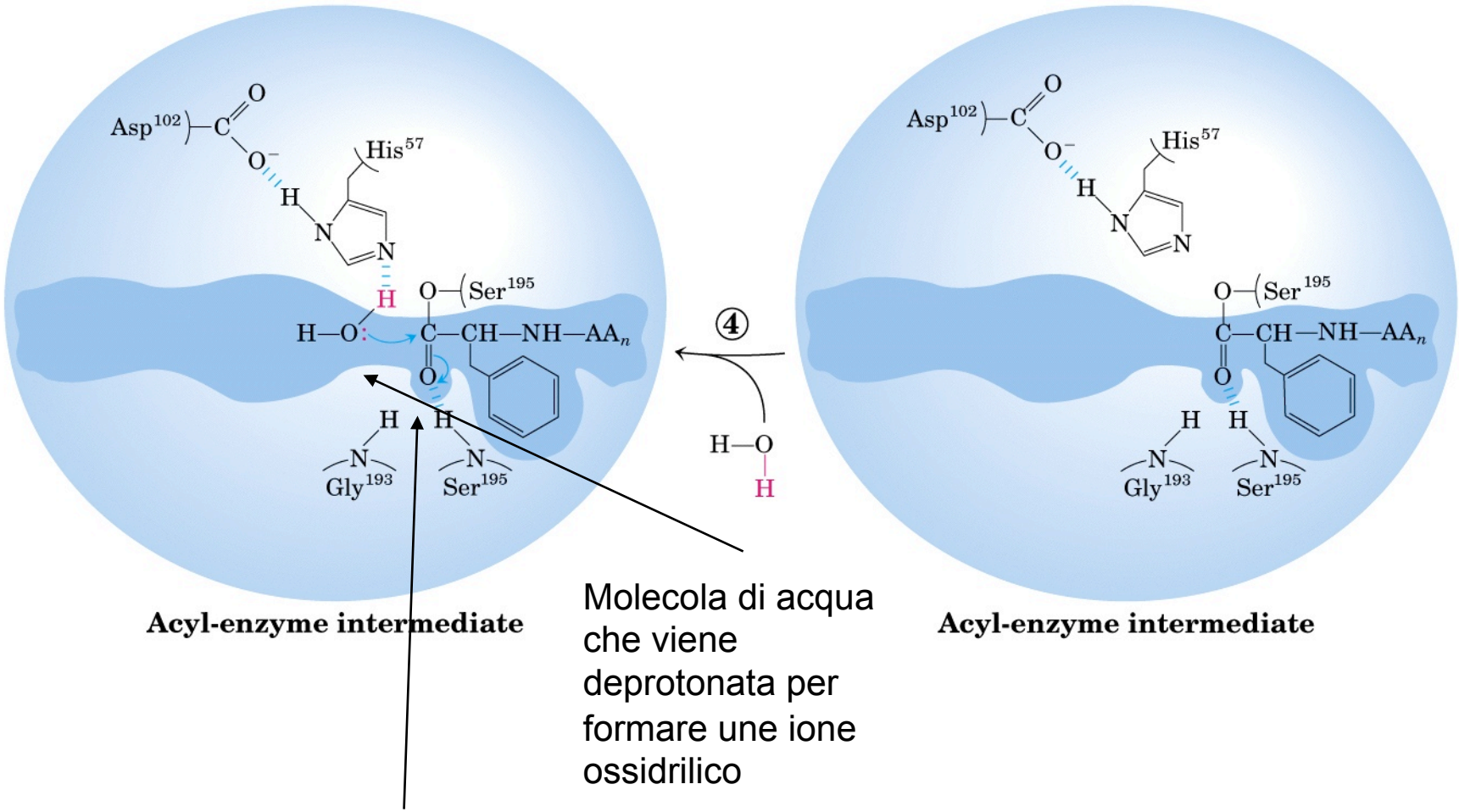
Short-lived intermediate
(acylation)



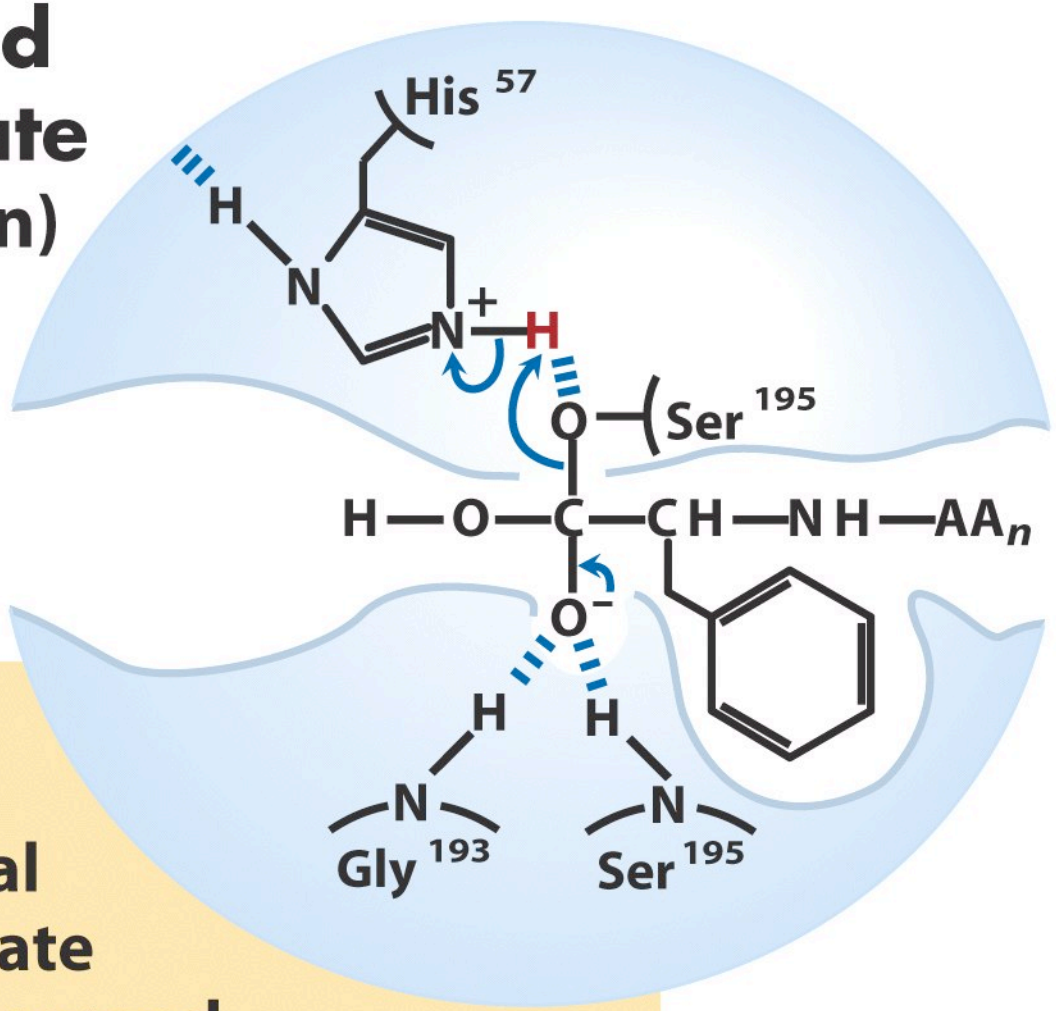
Altamente instabile



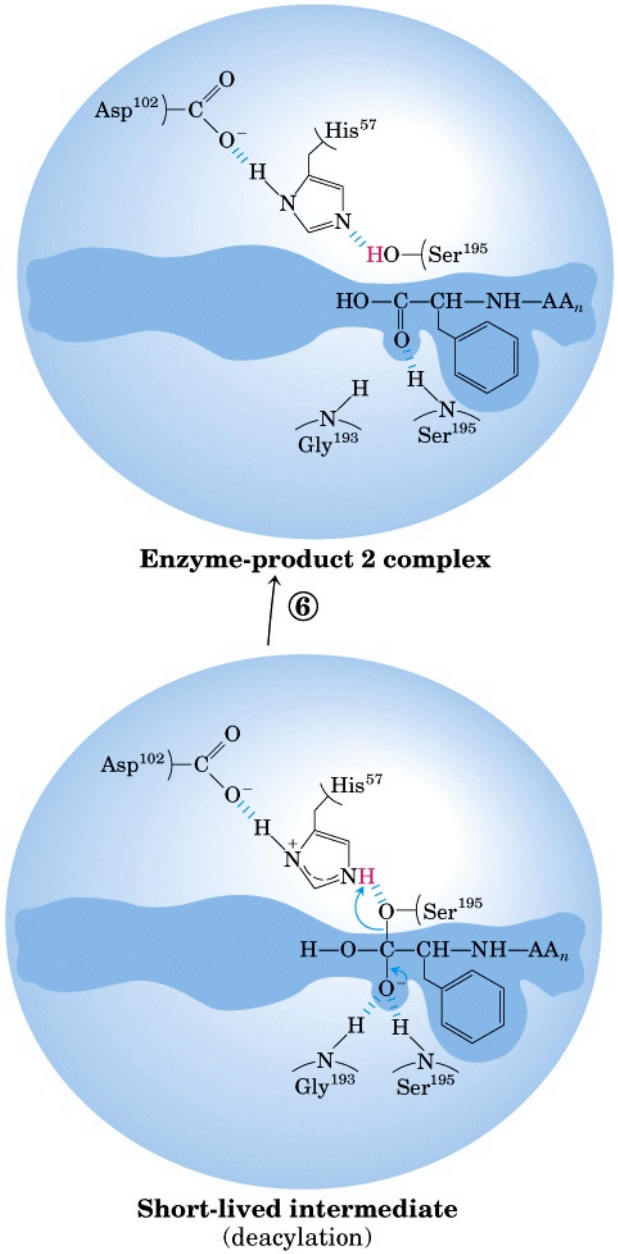
An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.


Asp102
His57
Ser195
Gly193
Ser195
④
H—O
H
Acyl-enzyme intermediate
Acyl-enzyme intermediate
Molecola di acqua che viene deprotonata per formare une ione ossidrilico
Presenza forte nucleofilo porta nuovamente a carica negativa

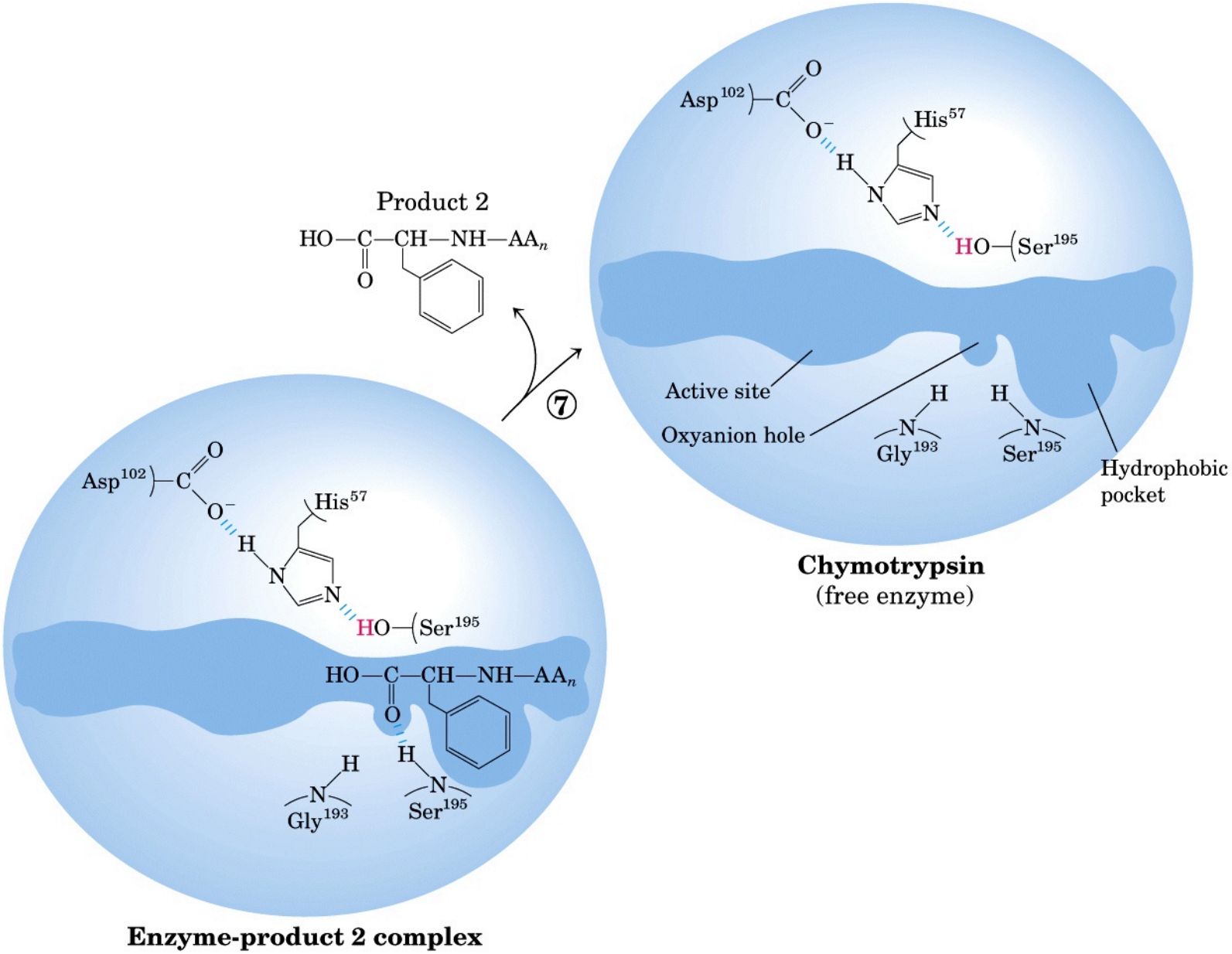
Short-lived intermediate (deacylation)

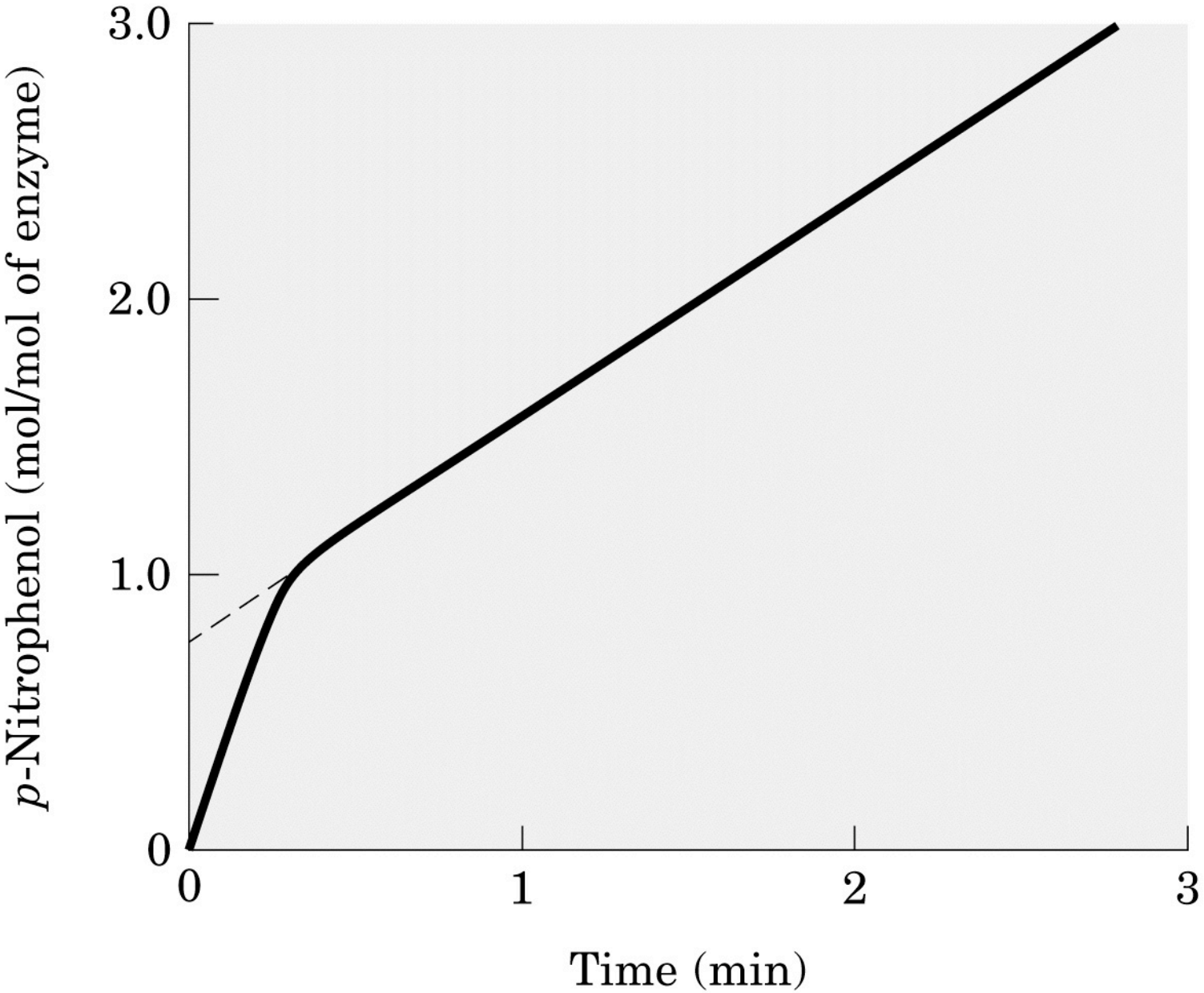


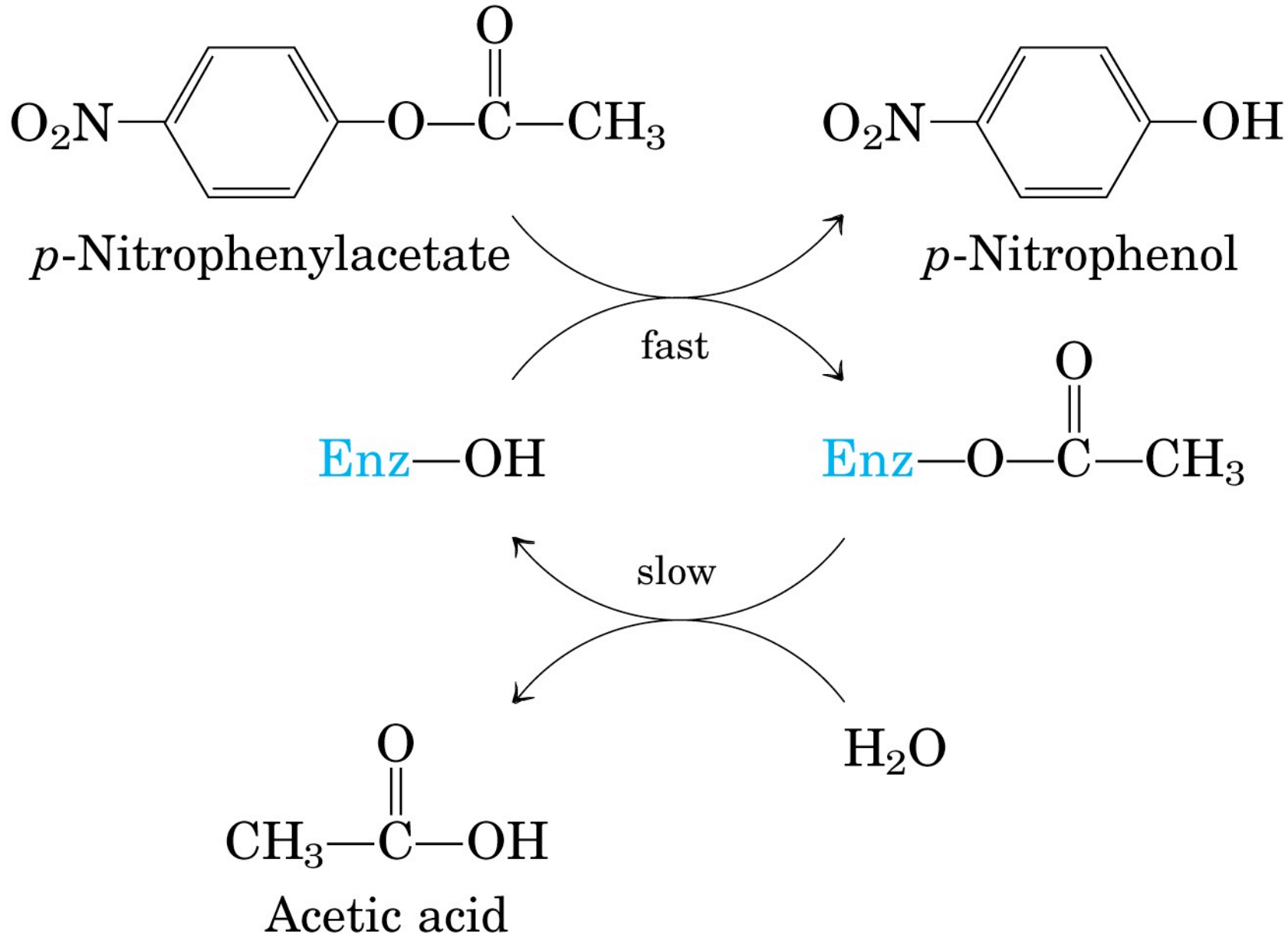
Collapse of the tetrahedral intermediate forms the second product, a carbohydrate anion, and displaces Ser^{195}.

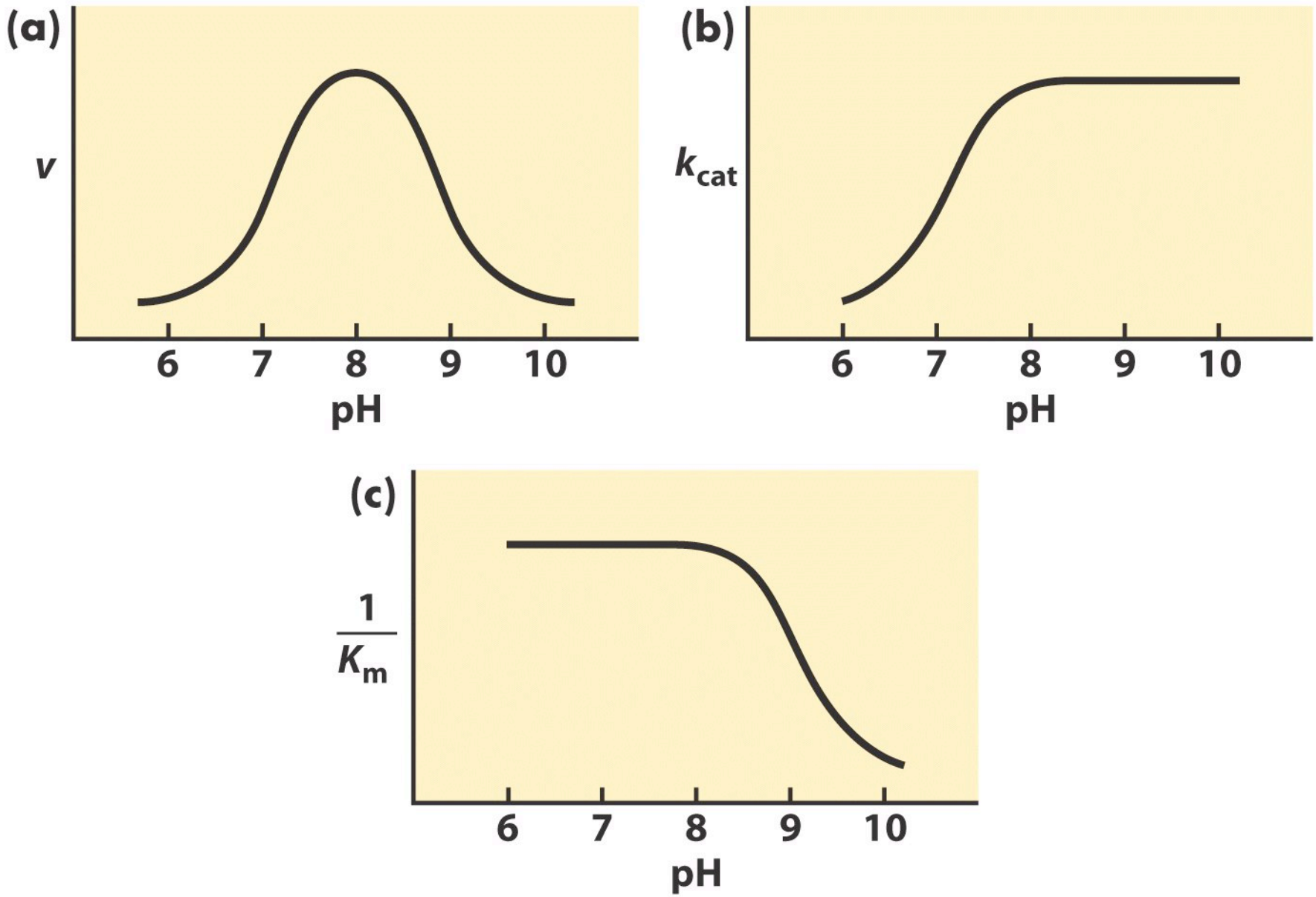


Ripetizione del primo ciclo di deprotonazione


Product 2
HO—C—CH—NH—AA$_n$
O
⑦
Asp^{102}
His^{57}
Ser^{195}
Active site
Oxyanion hole
Gly^{193}
Hydrophobic pocket
Chymotrypsin
(free enzyme)
Enzyme-product 2 complex

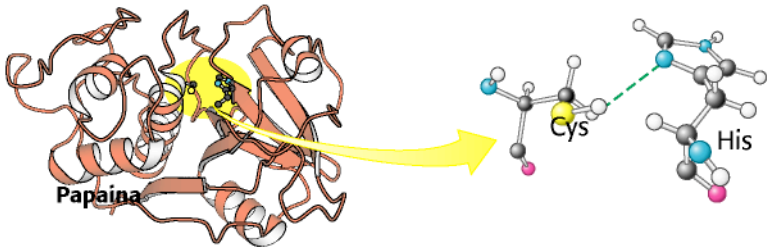

3.0
2.0
1.0
0
0
1
2
3
p-Nitrophenol (mol/mol of enzyme)
Time (min)


O
O2N
O—C—CH3
p-Nitrophenylacetate
O2N
OH
p-Nitrophenol
fast
Enz—OH
O
Enz—O—C—CH3
slow
H2O
O
CH3—C—OH
Acetic acid


(a)
v
6
7
8
9
10
pH
(b)
k_{cat}
6
7
8
9
10
pH
(c)
$\frac{1}{K_m}$
6
7
8
9
10
pH

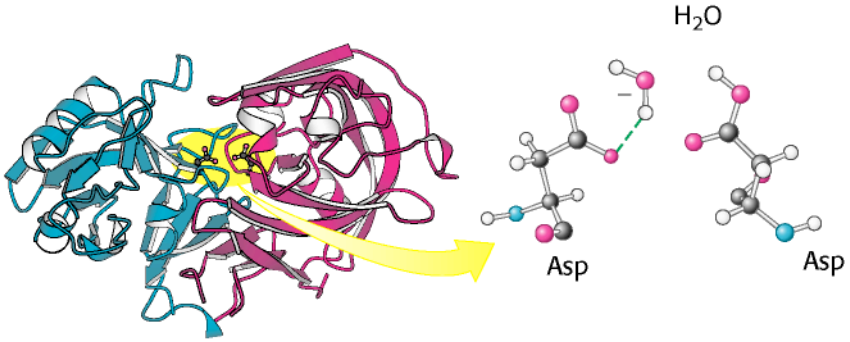
- Cistein-proteasi
- Aspartil-proteasi
- Metalloproteasi

CISTEINA PROTEASI

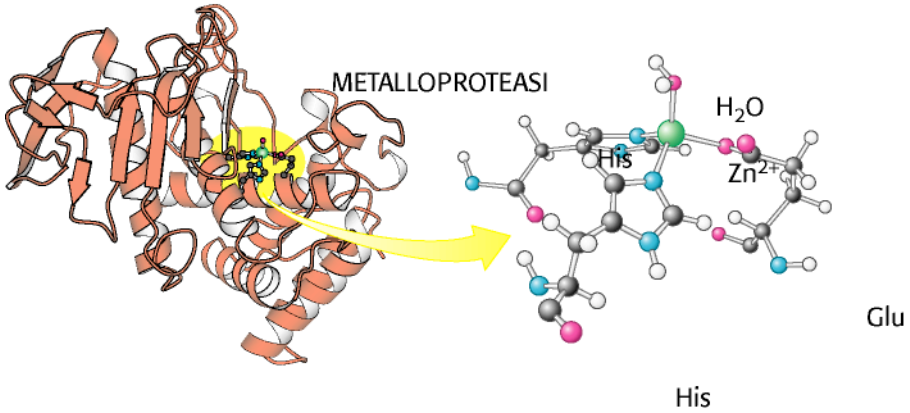


Papaina

ASPARTIL PROTEASI

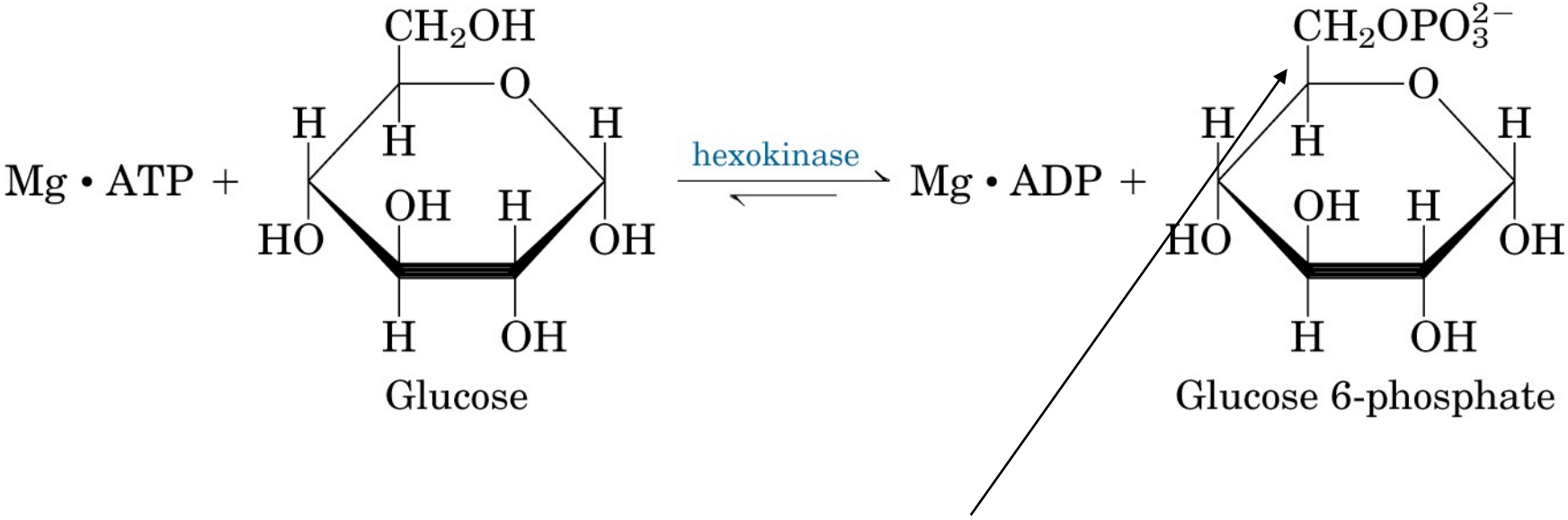


Renina



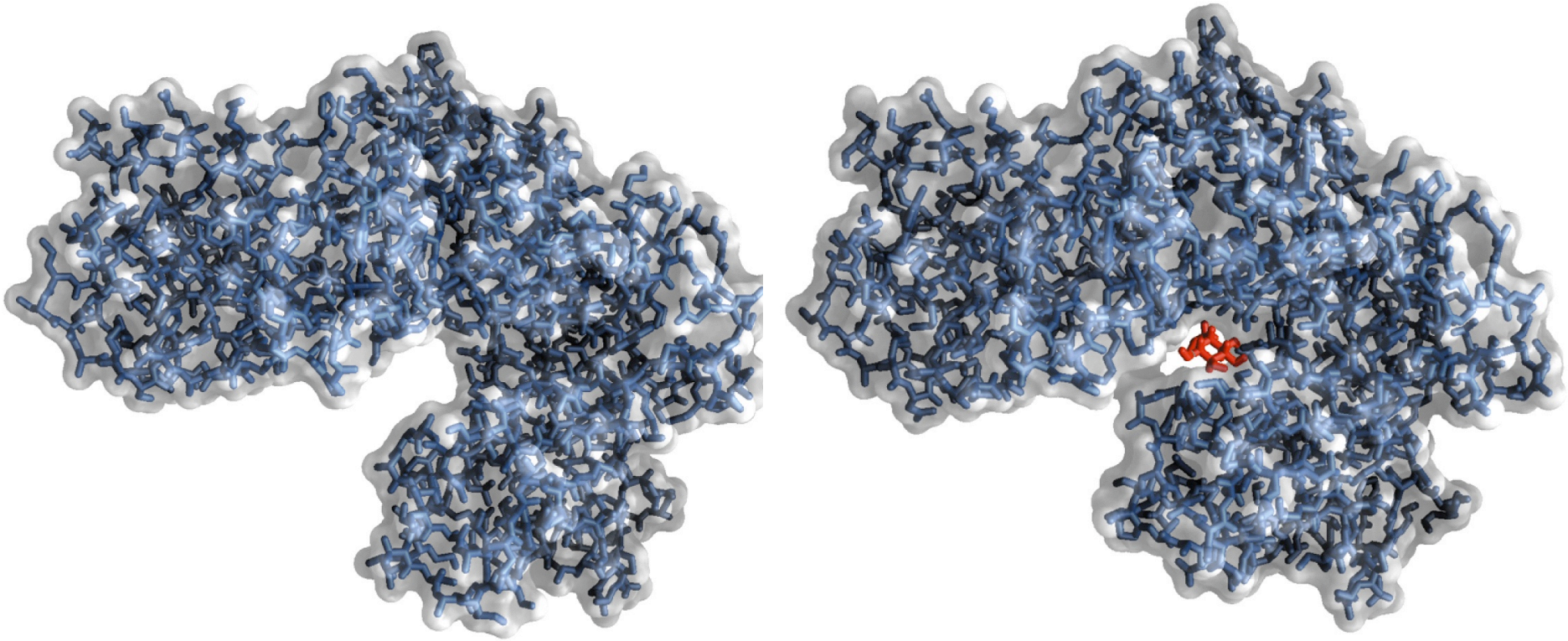
Termolisina

ESOCHINASI

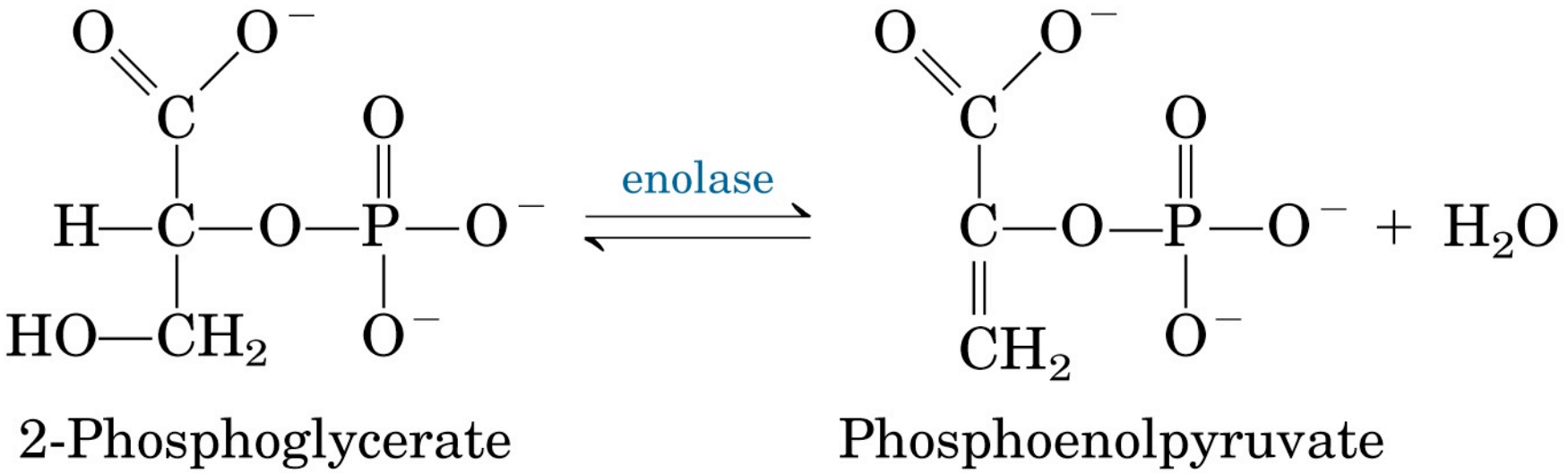


Gruppo ossidrilico sul C6 ha reattività simile all'acqua, ma c'e specificità di 10E6

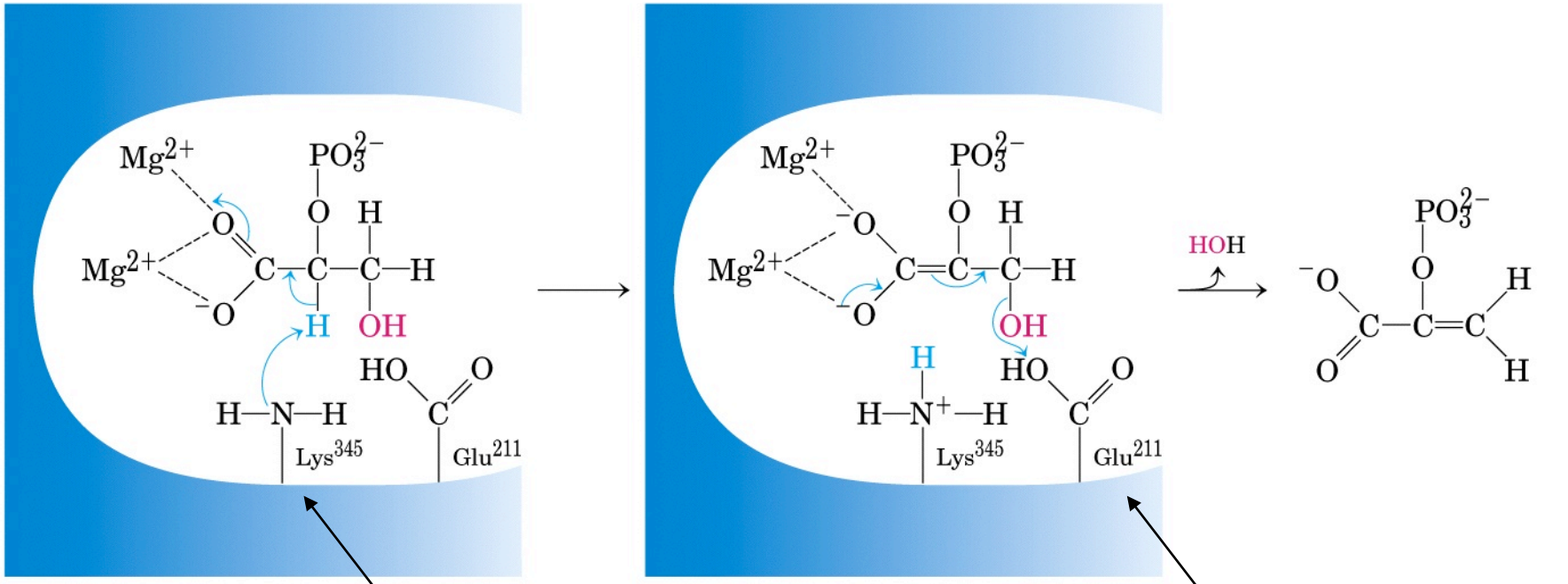
ADATTAMENTO INDOTTO



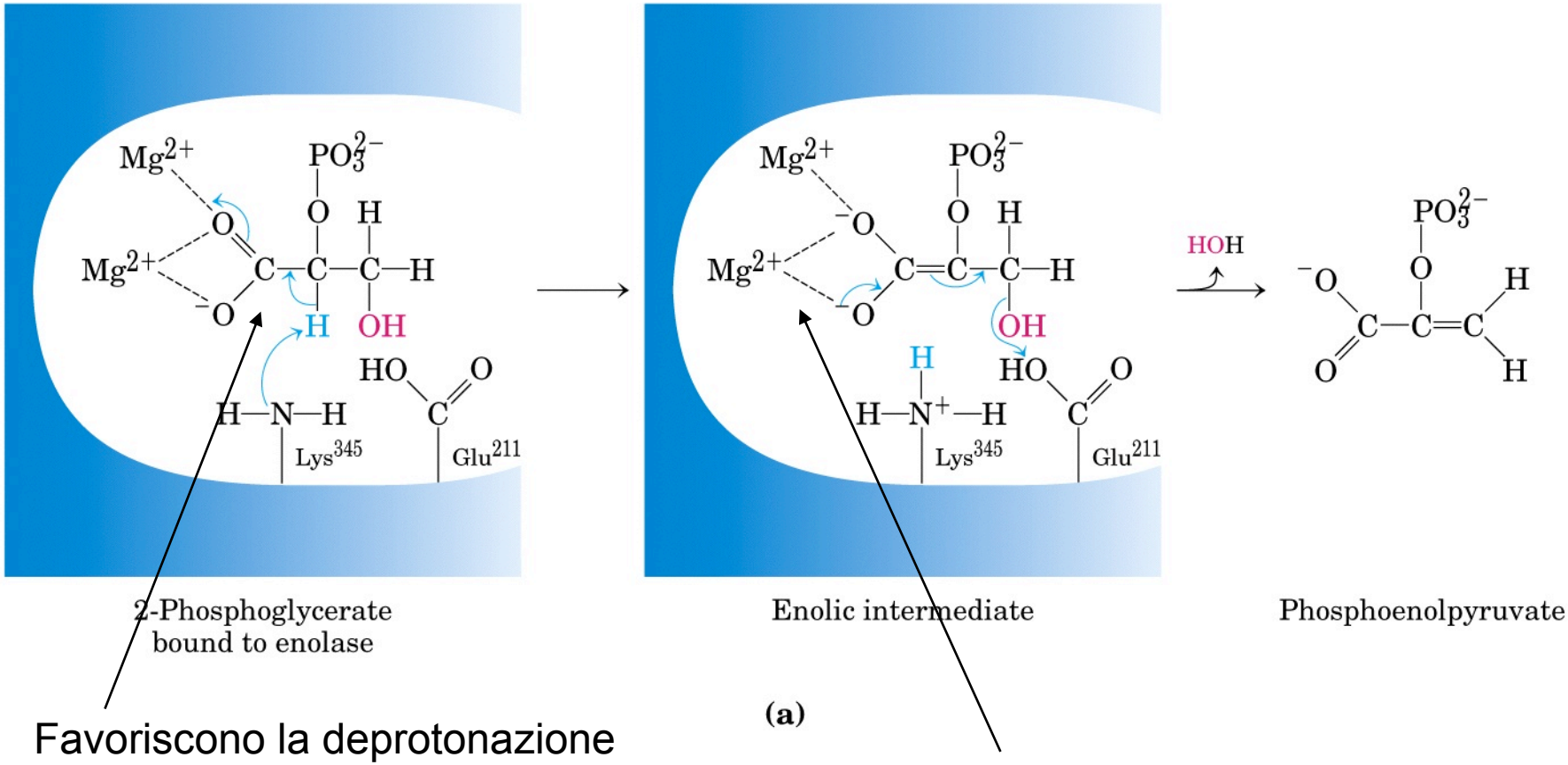
ENOLASI



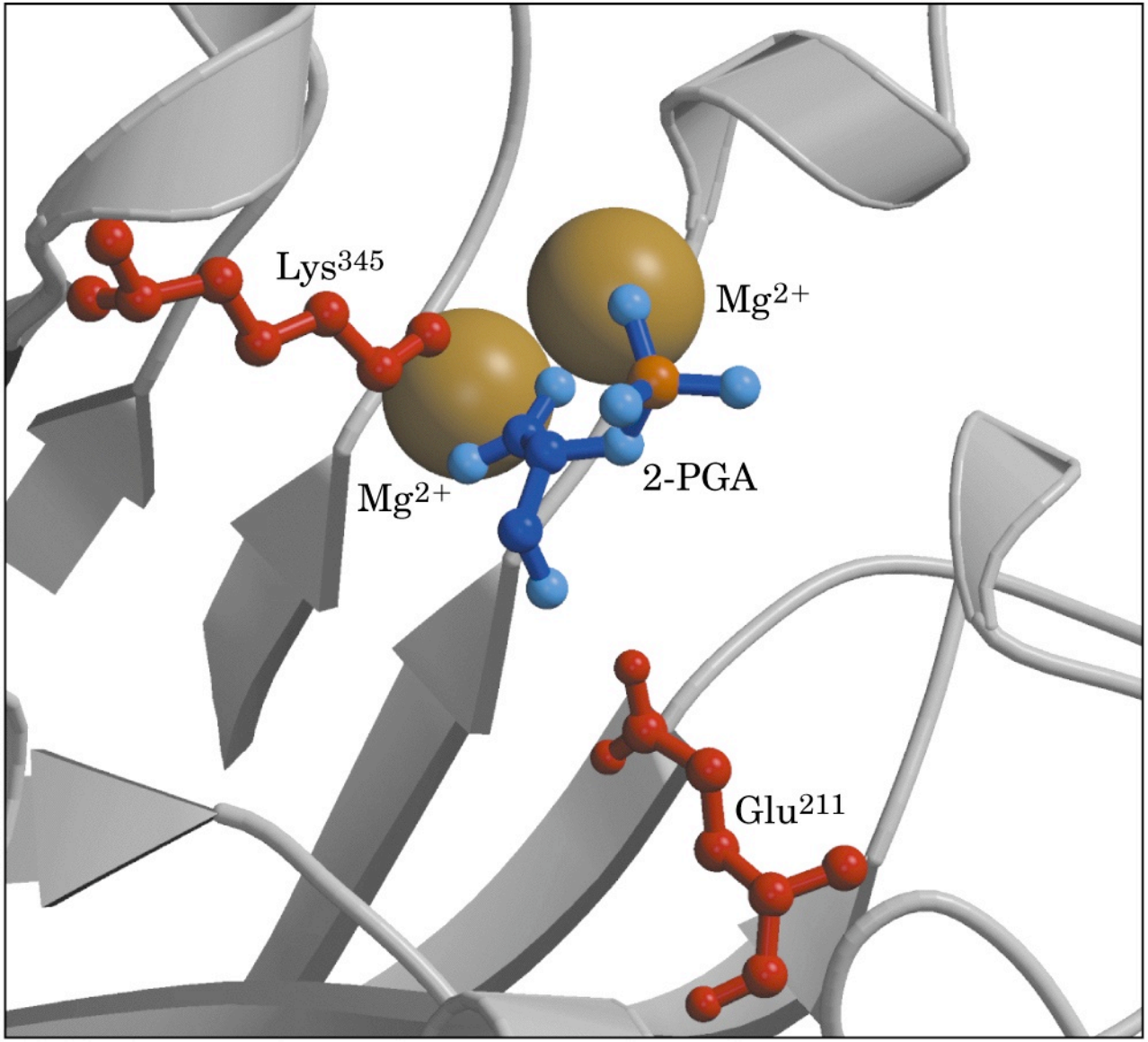
Catalisi da ioni metallici
Acido-base generale
Stabilizzazione stato transizione


2-Phosphoglycerate bound to enolase
Enolic intermediate
Phosphoenolpyruvate
B generale
A generale
(a)

CATALISI DA IONI METALLICI

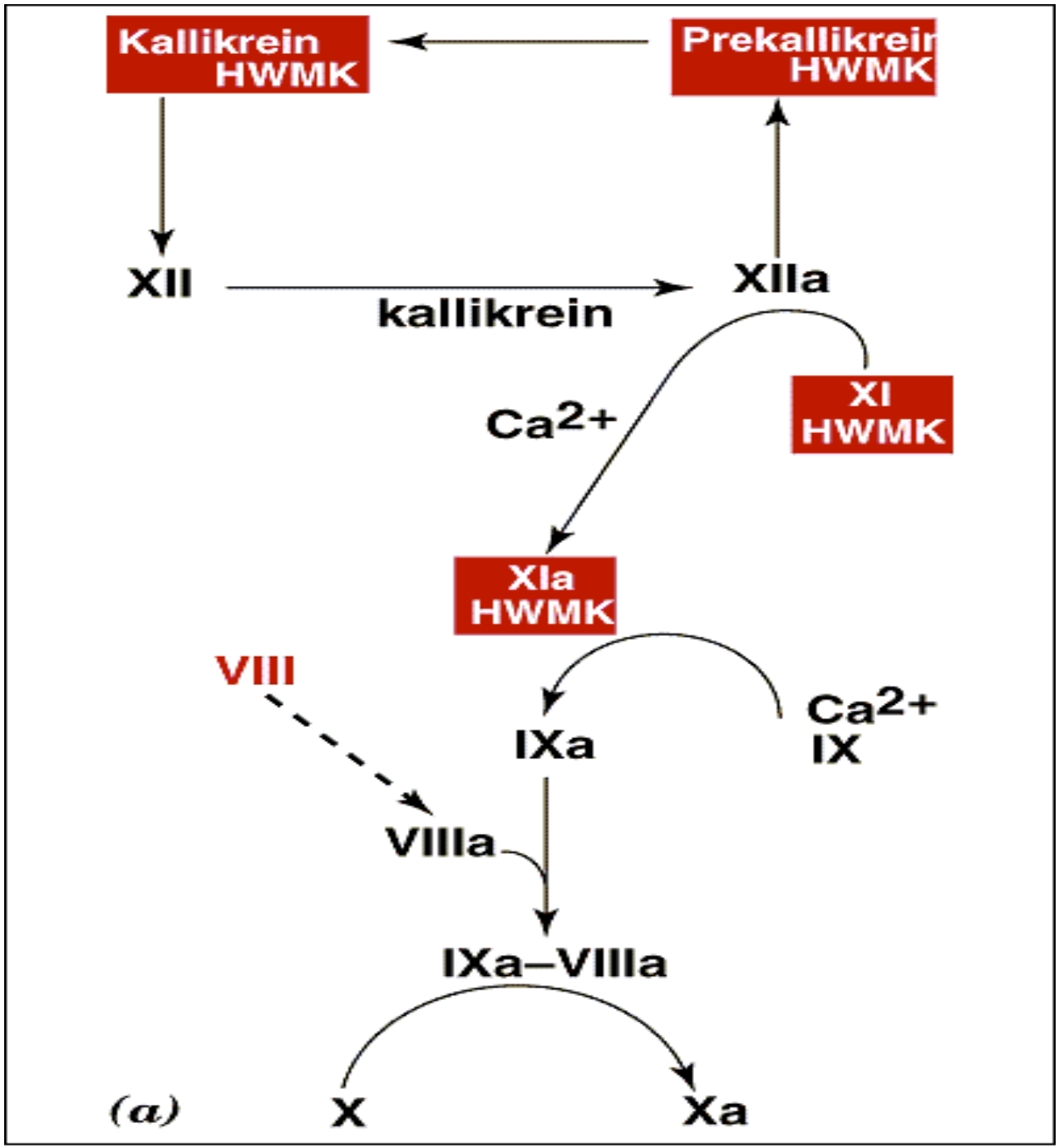


CATALISI BASICA GENERALE

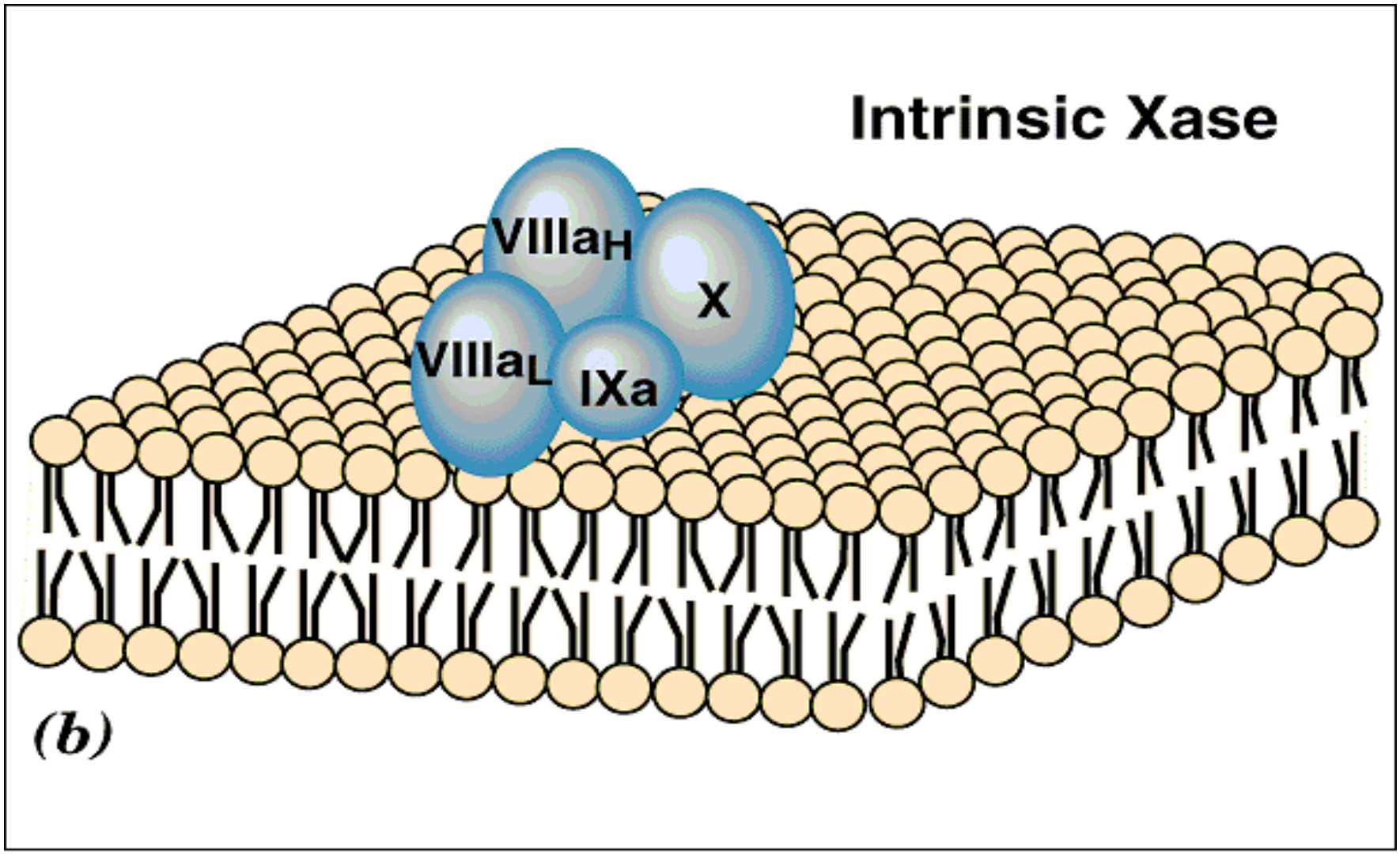


(b)

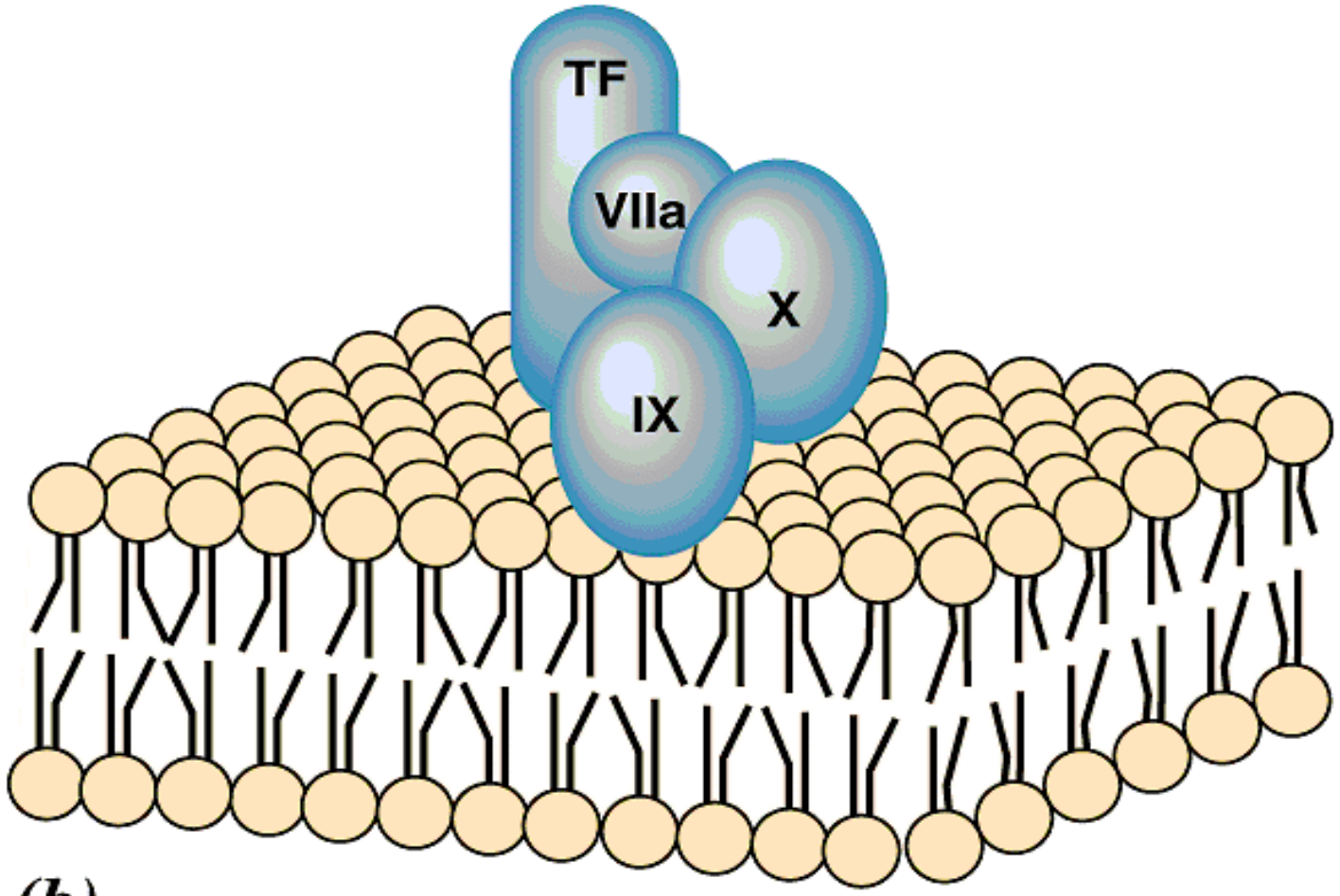
VIA INTRINSECA DELLA COAGULAZIONE



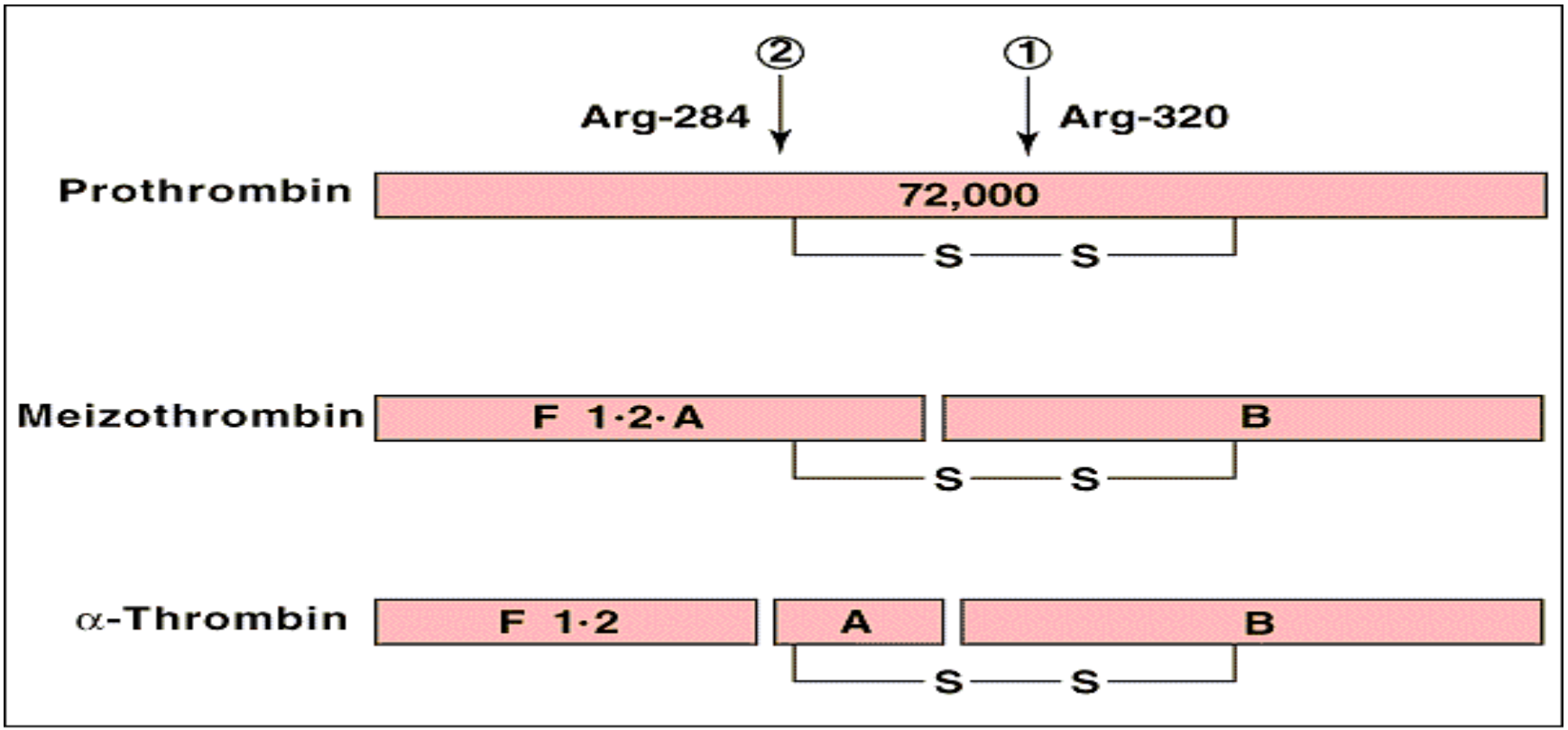
ATTIVAZIONE del FATTOR X

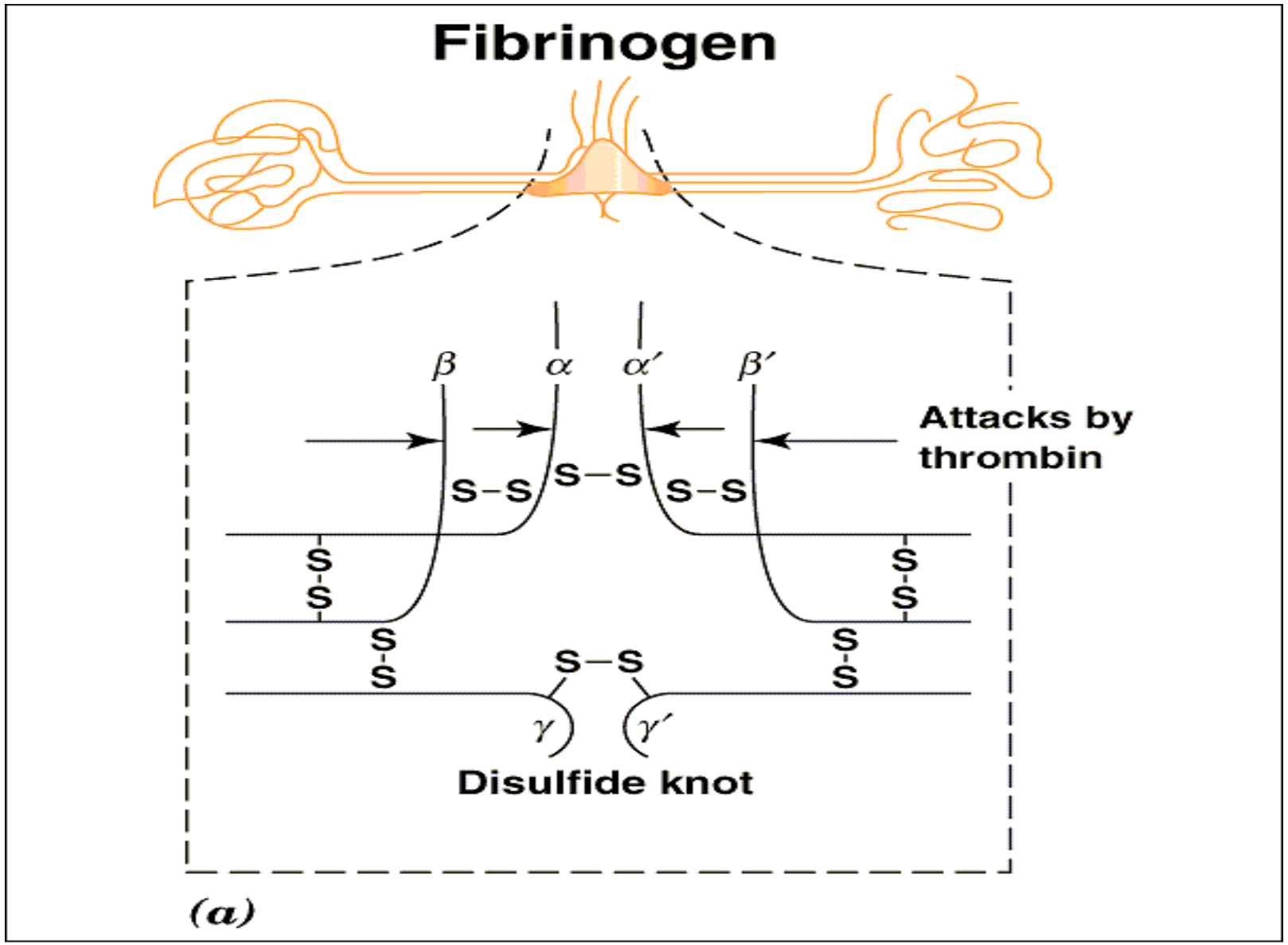


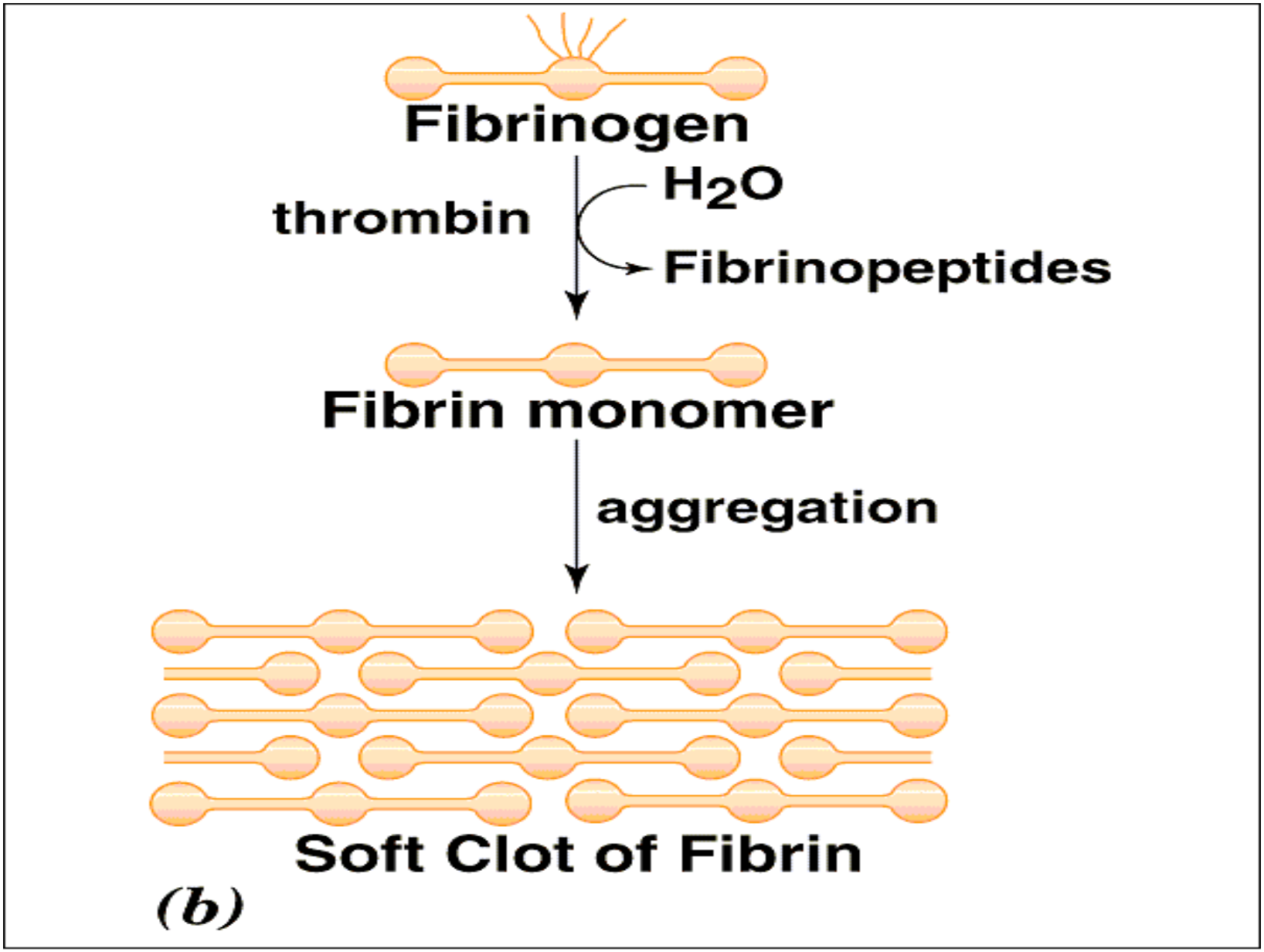
Extrinsic Xase

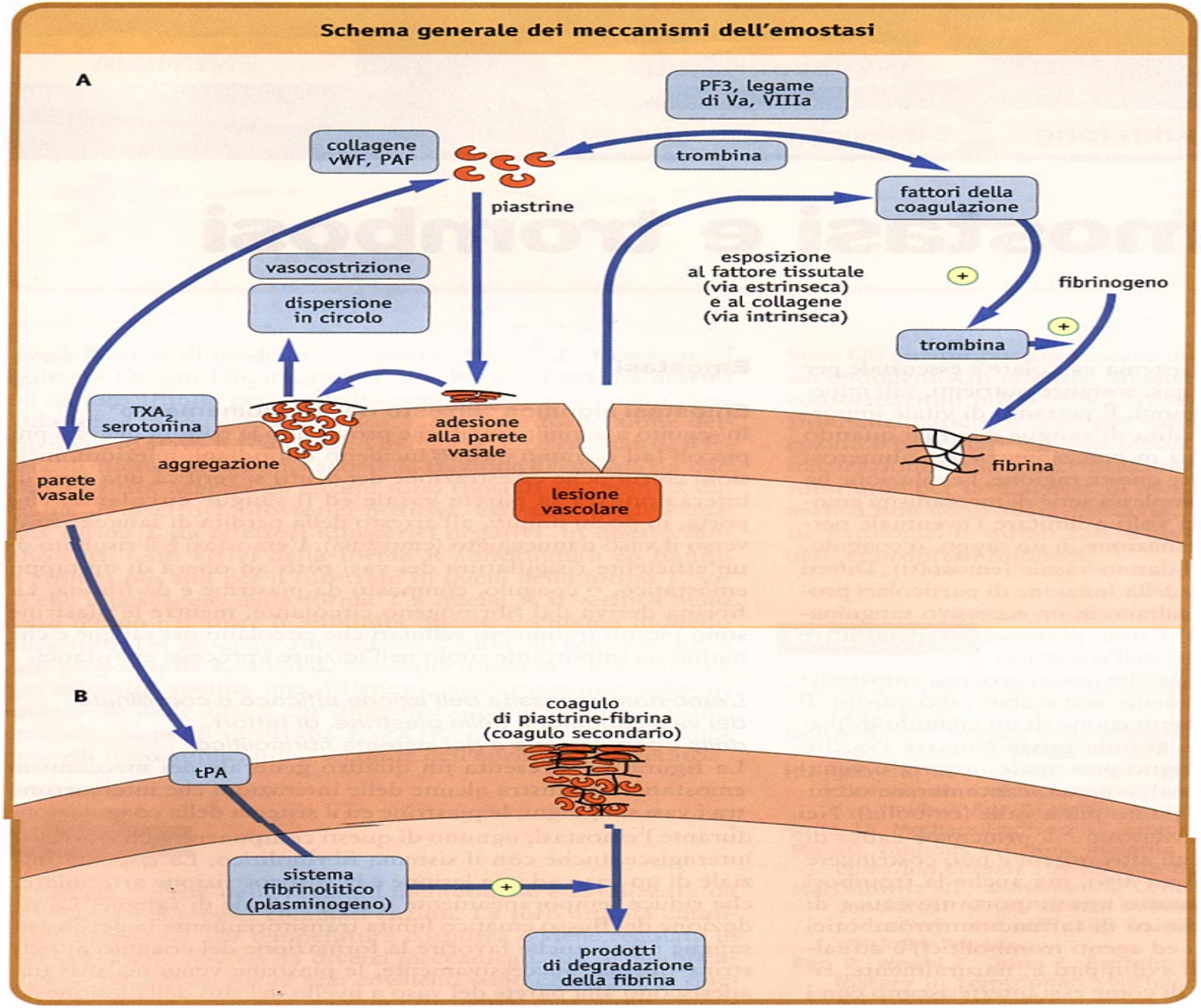


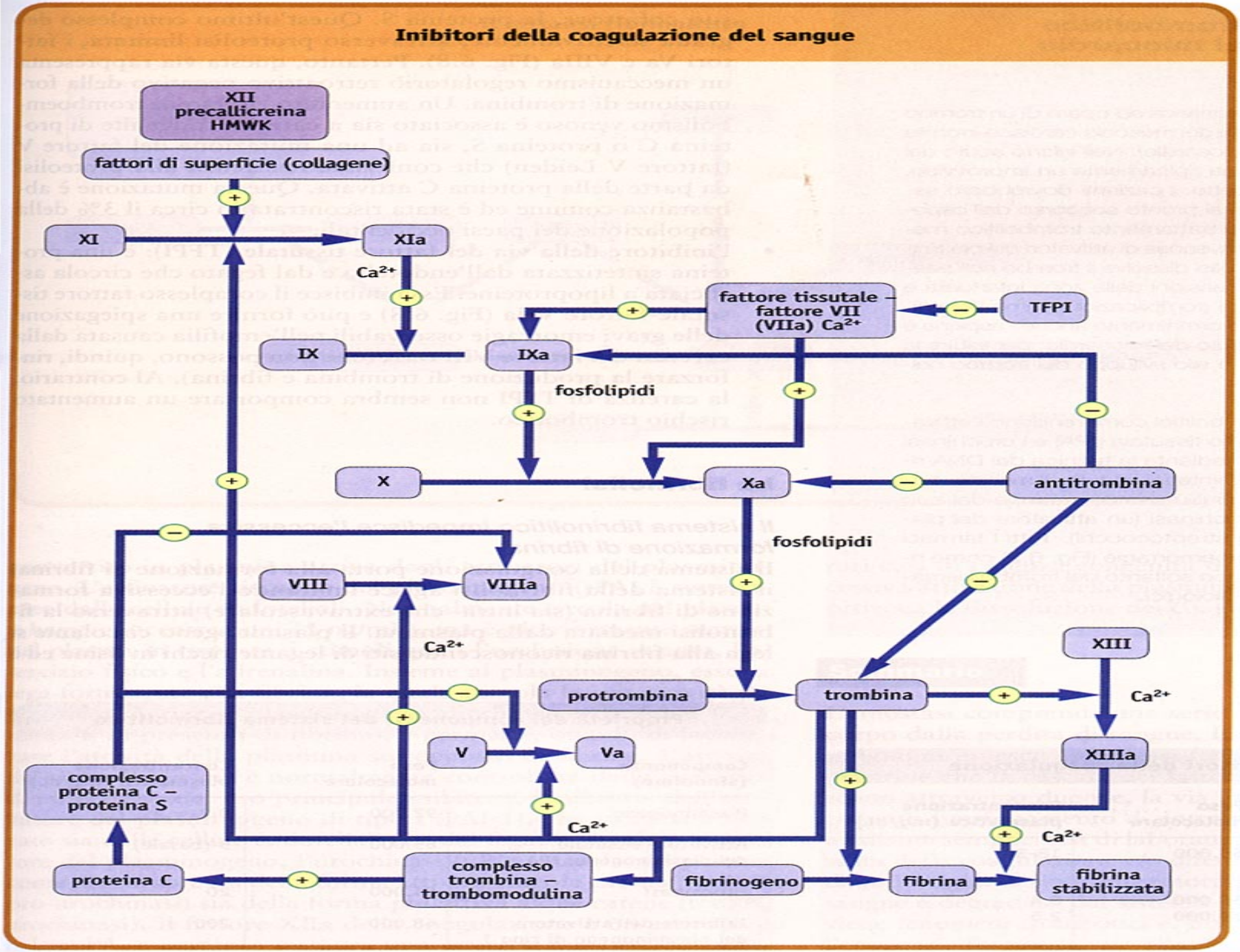
ATTIVAZIONE PROTEOLITICA DI TROMBINA



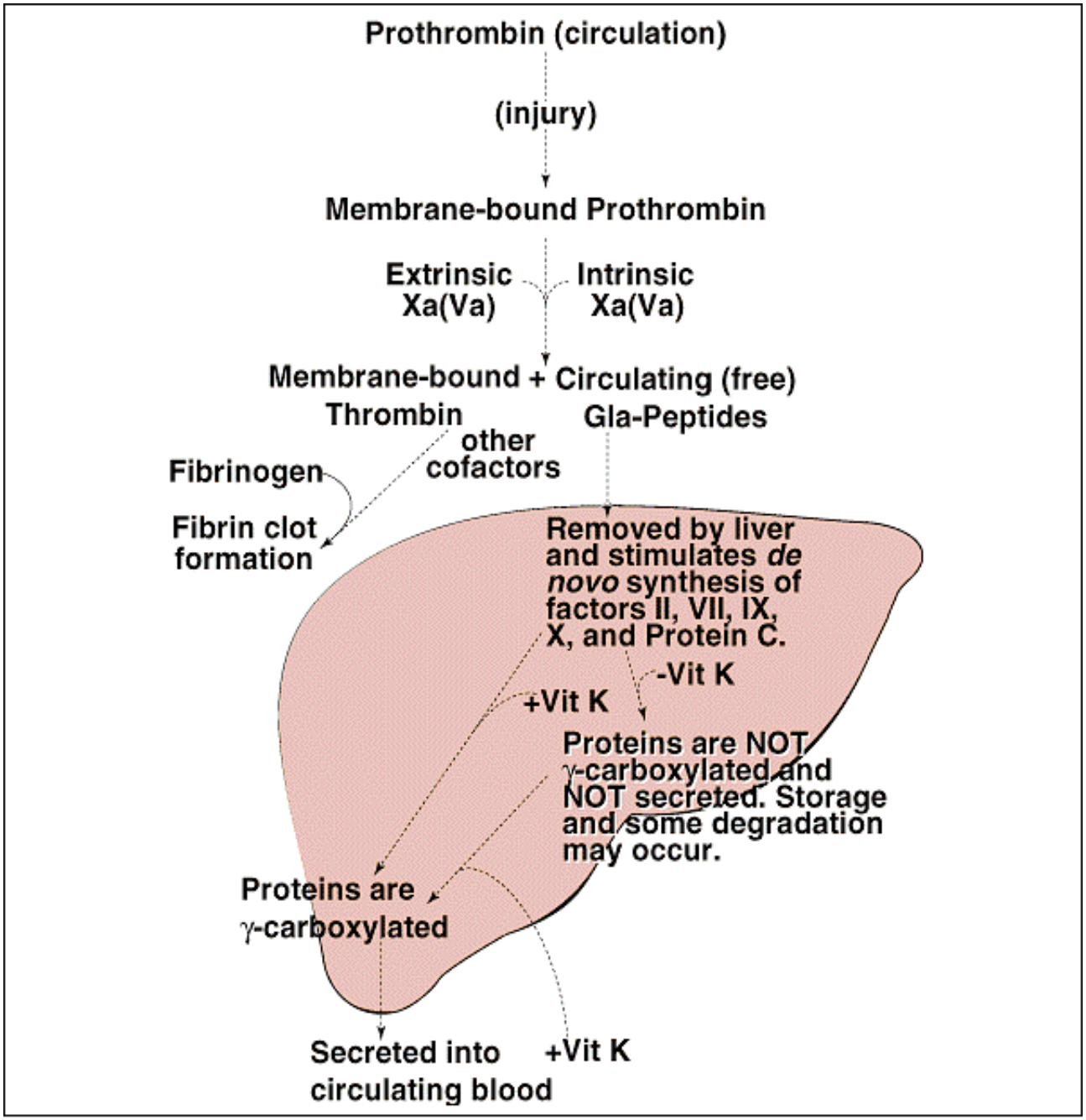


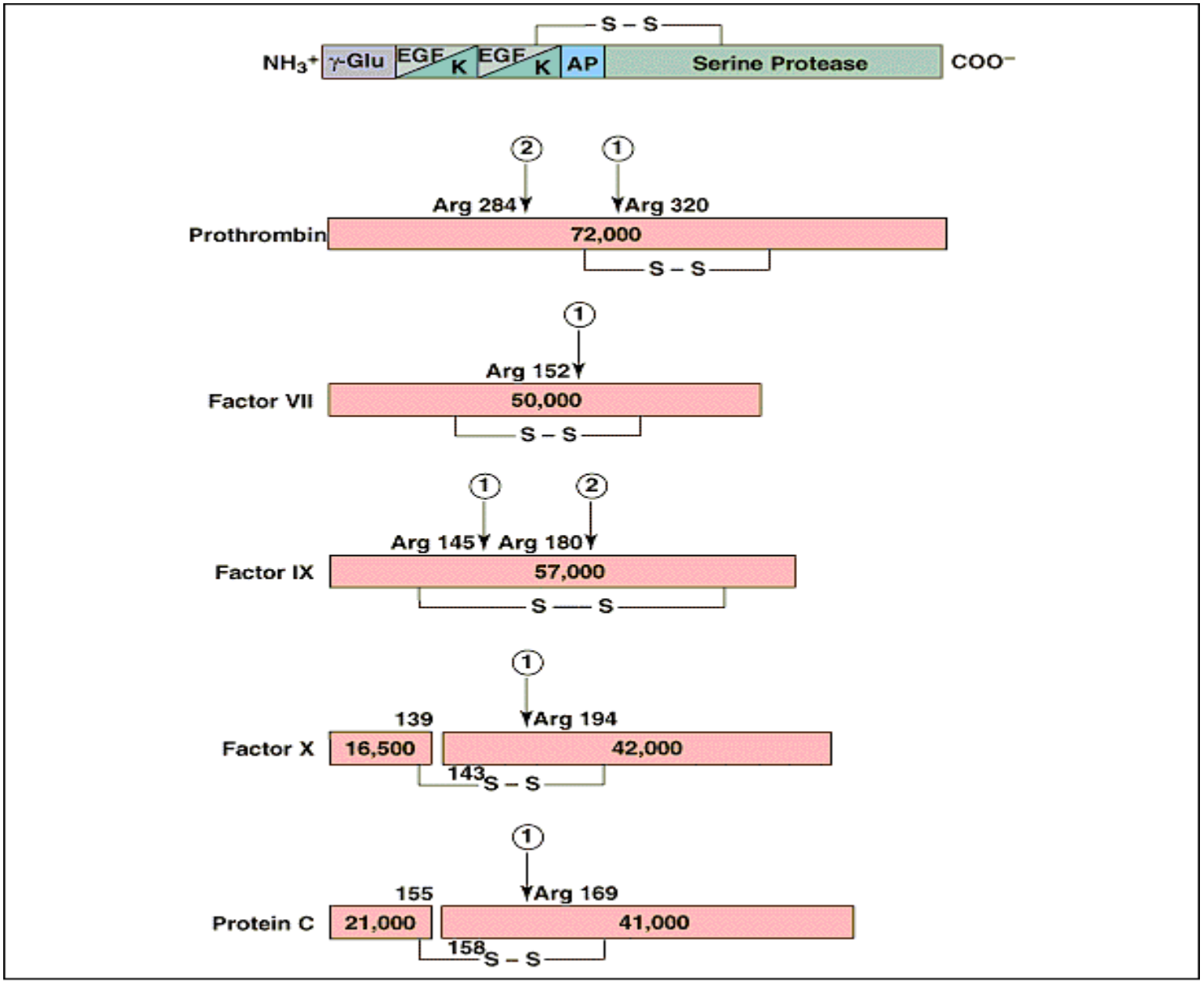



Schema generale dei meccanismi dell'emostasi
A
PF3, legame di Va, VIIIa
collagene vWF, PAF
trombina
piastrine
fattori della coagulazione
esposizione al fattore tissutale (via estrinseca) e al collagene (via intrinseca)
vasocostrizione
dispersione in circolo
fibrinogeno
trombina
TXA2 serotonina
adesione alla parete vasale
aggregazione
parete vasale
lesione vascolare
fibrina
B
coagulo di piastrine-fibrina (coagulo secondario)
tPA
sistema fibrinolitico (plasminogeno)
prodotti di degradazione della fibrina

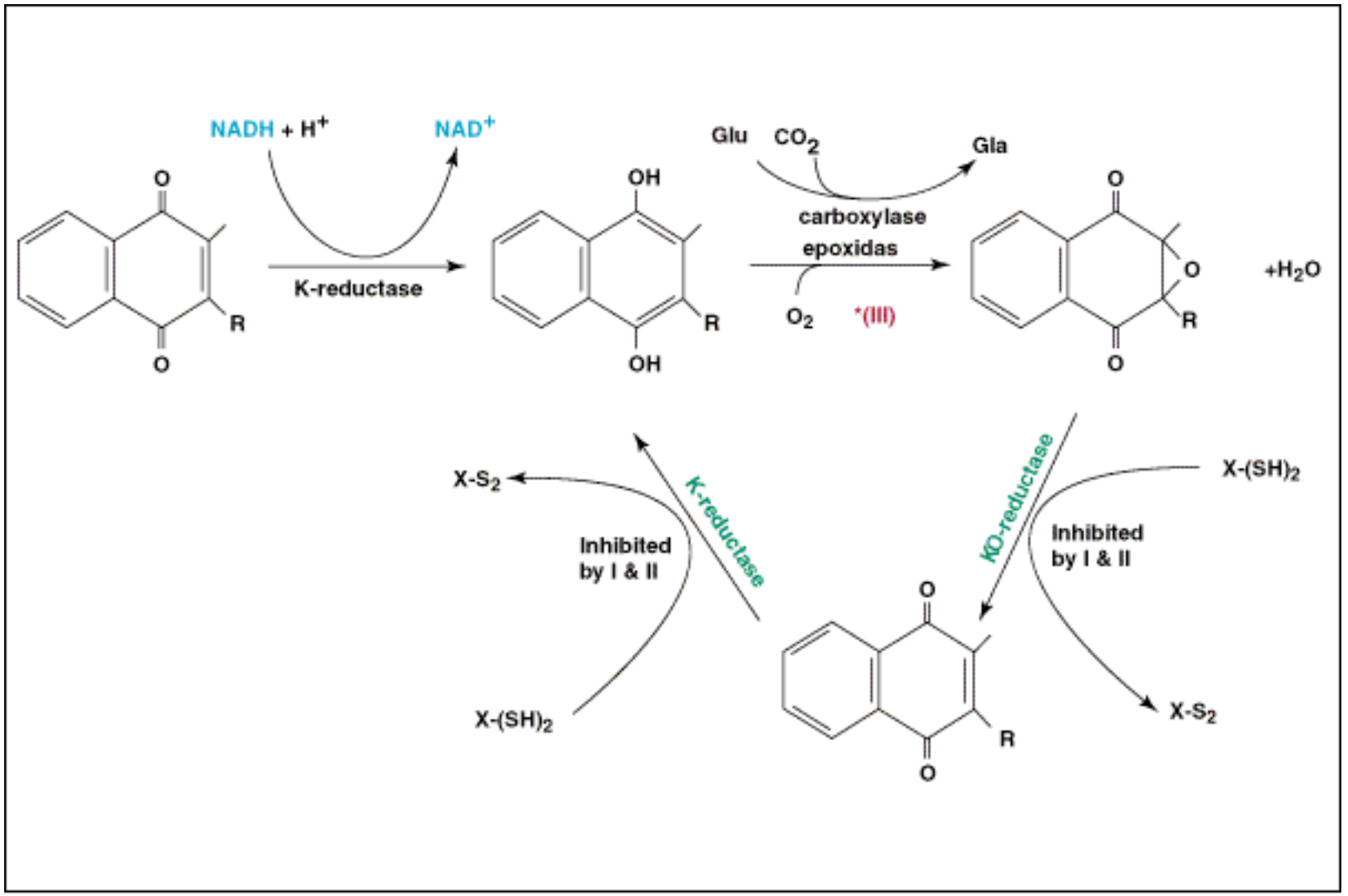

Inibitori della coagulazione del sangue
XII precallicreina HMWK
fattori di superficie (collagene)
XI
XIa
Ca2+
IX
IXa
fattore tissutale – fattore VII (VIIa) Ca2+
TFPI
fosfolipidi
X
Xa
antitrombina
fosfolipidi
VIII
VIIIa
Ca2+
protrombina
trombina
XIII
Ca2+
XIIIa
V
Va
complesso proteina C – proteina S
Ca2+
Ca2+
proteina C
complesso trombina – trombomodulina
fibrinogeno
fibrina
fibrina stabilizzata

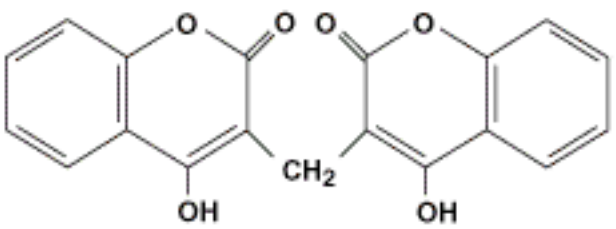
Ruolo dei residui di ac. γ carbossiglutammico nella regolazione della sintesi *de novo* dei fattori della coagulazione



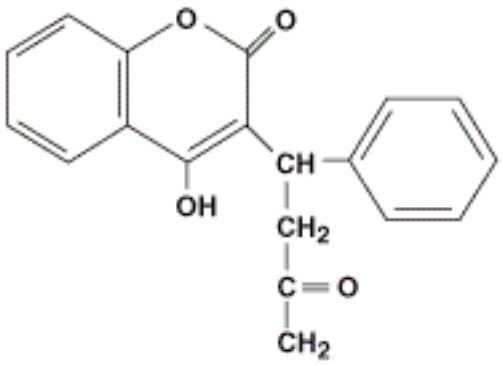


CICLO DELLA VITAMINA K

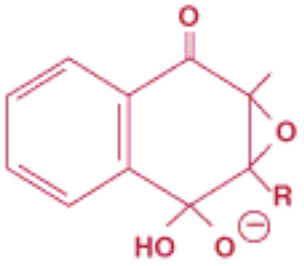




(I)



(II)



(III)*

pKa-20

Ruolo dei residui di ac. γ carbossiglutammico nella regolazione della sintesi *de novo* dei fattori della coagulazione