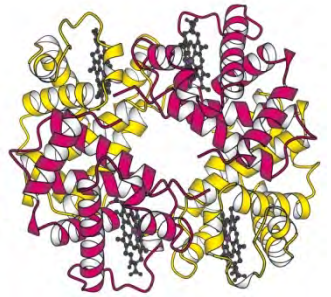
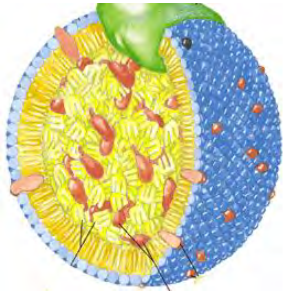


BIOCHEMISTRY REVIEW

Overview of Biomolecules

Chapter 6

Enzymes



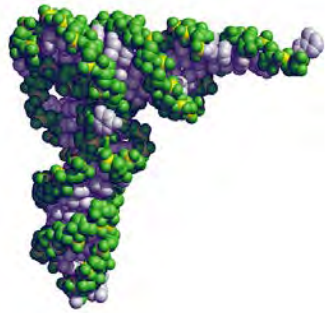


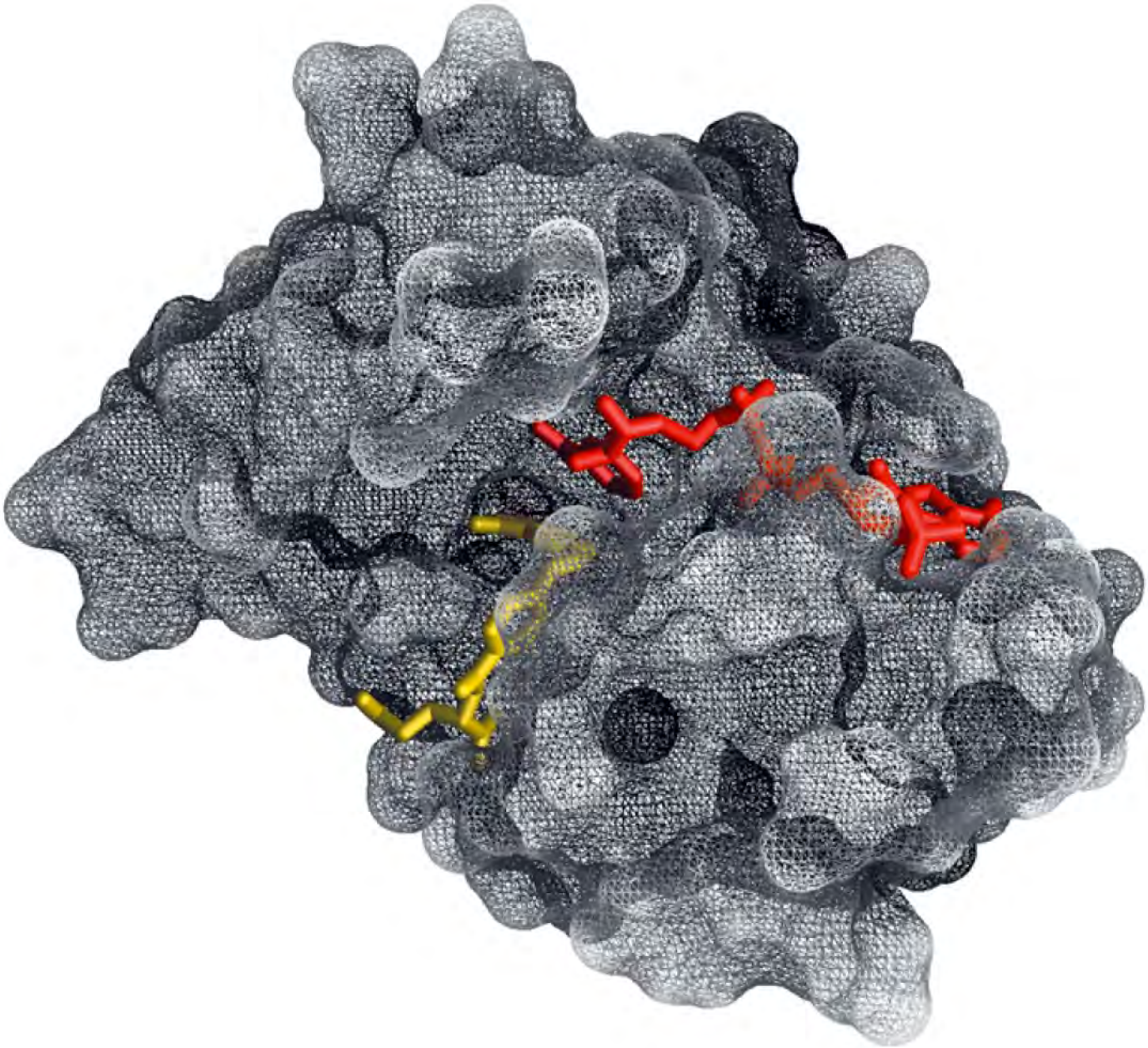
TABLE 8.1 **Rate enhancement by selected enzymes**

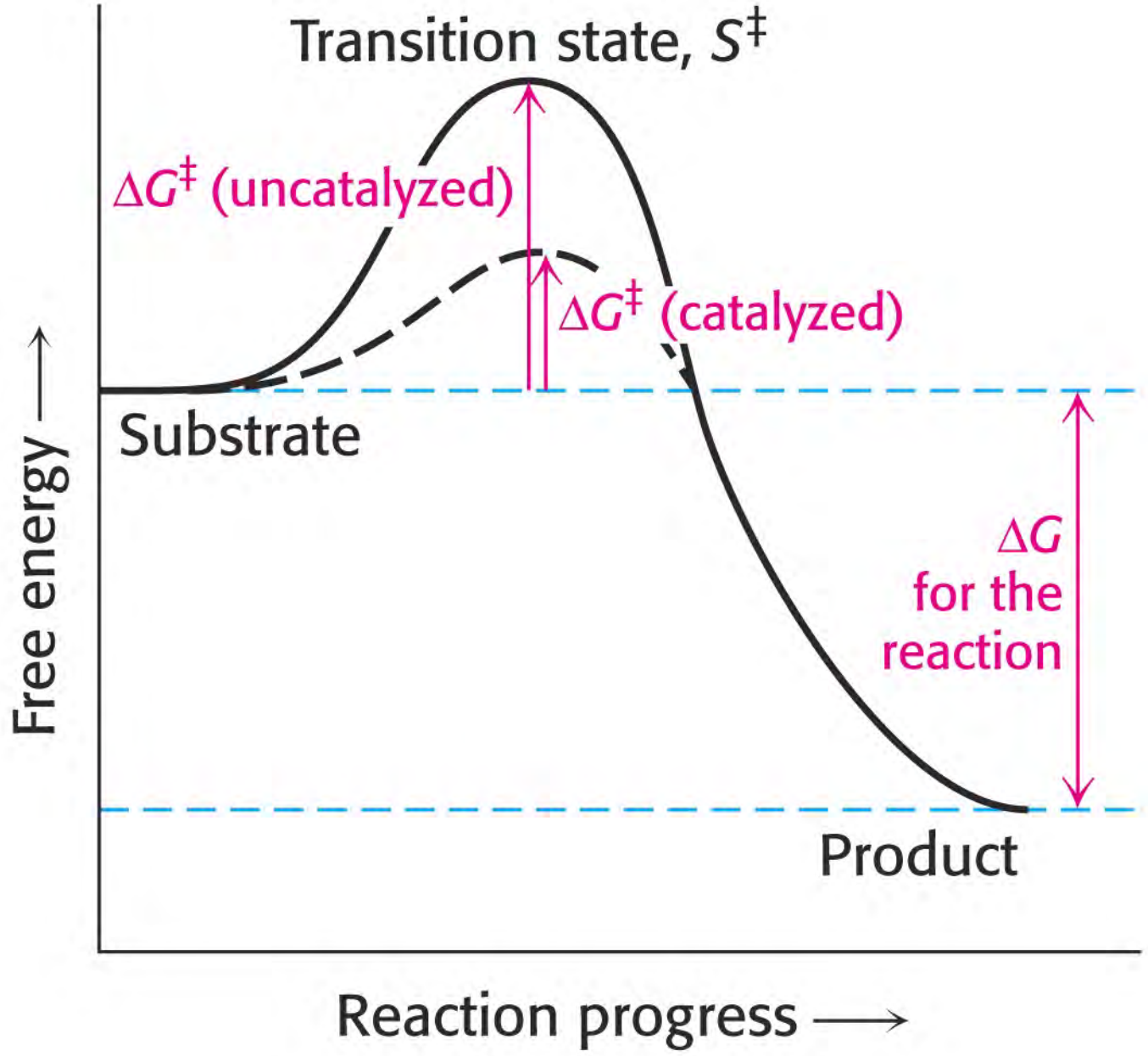
Enzyme	Nonenzymatic half-life	Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat}/k_{un})
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	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}

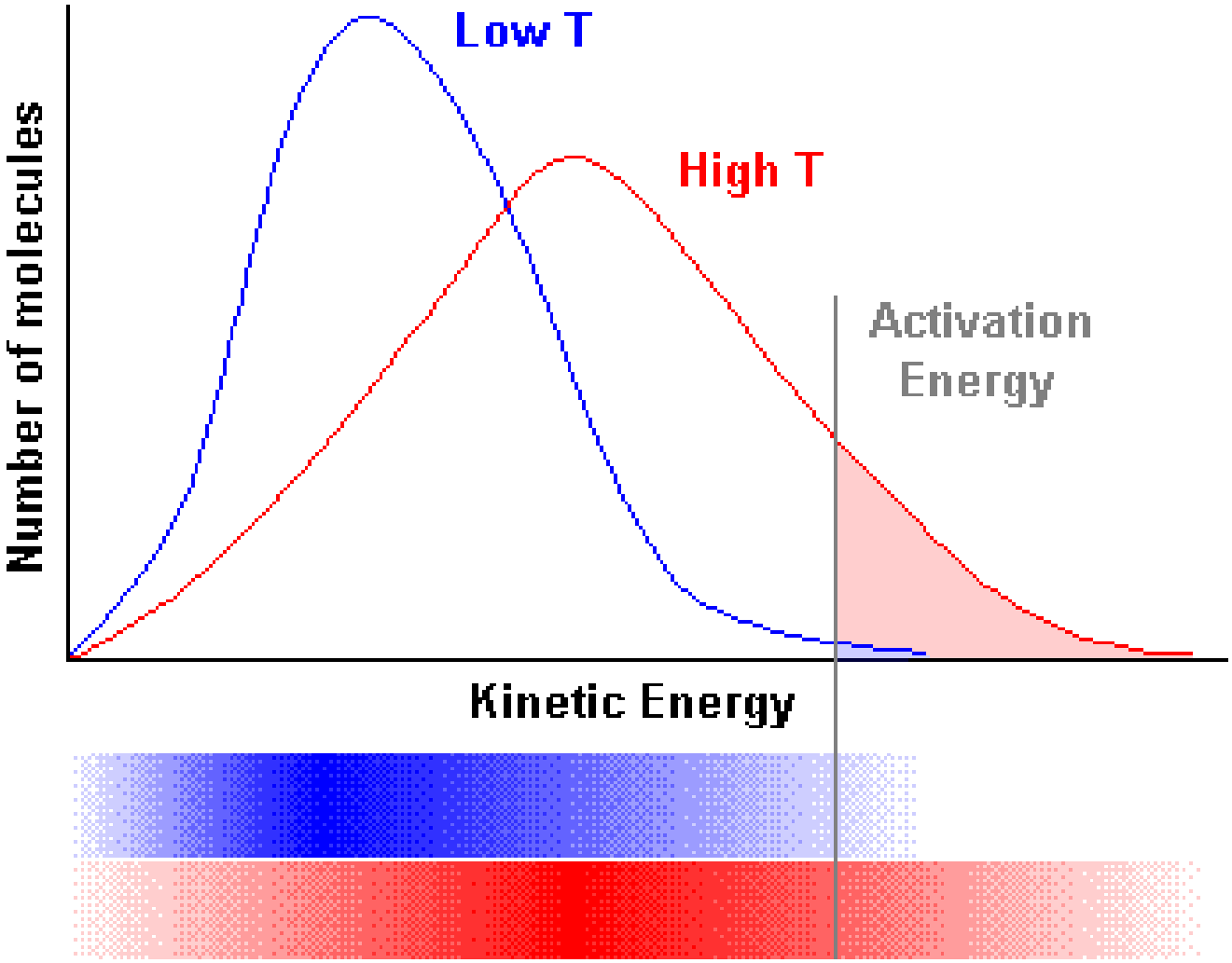
Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wofenden. *Science* 267 (1995):90–93.

TABLE 8.2 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase




Transition state, $S^{\ddagger}$
$\Delta G^{\ddagger}$ (uncatalyzed)
$\Delta G^{\ddagger}$ (catalyzed)
Substrate
ΔG for the reaction
Product
Free energy ⟶
Reaction progress ⟶


Low T
High T
Activation Energy
Number of molecules
Kinetic Energy

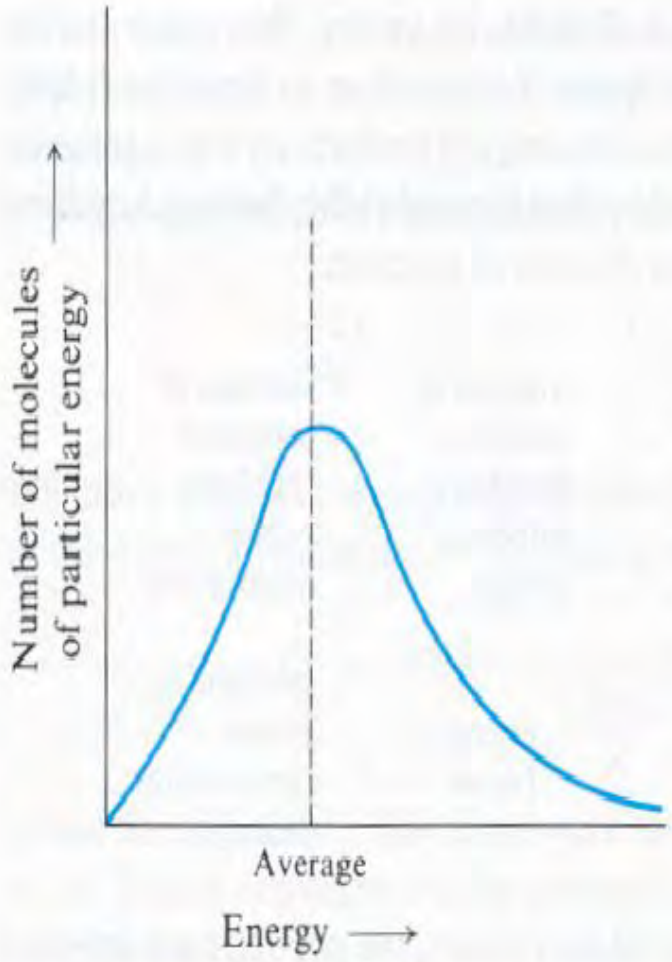


Figure 2.6 Distribution of kinetic energy among molecules.

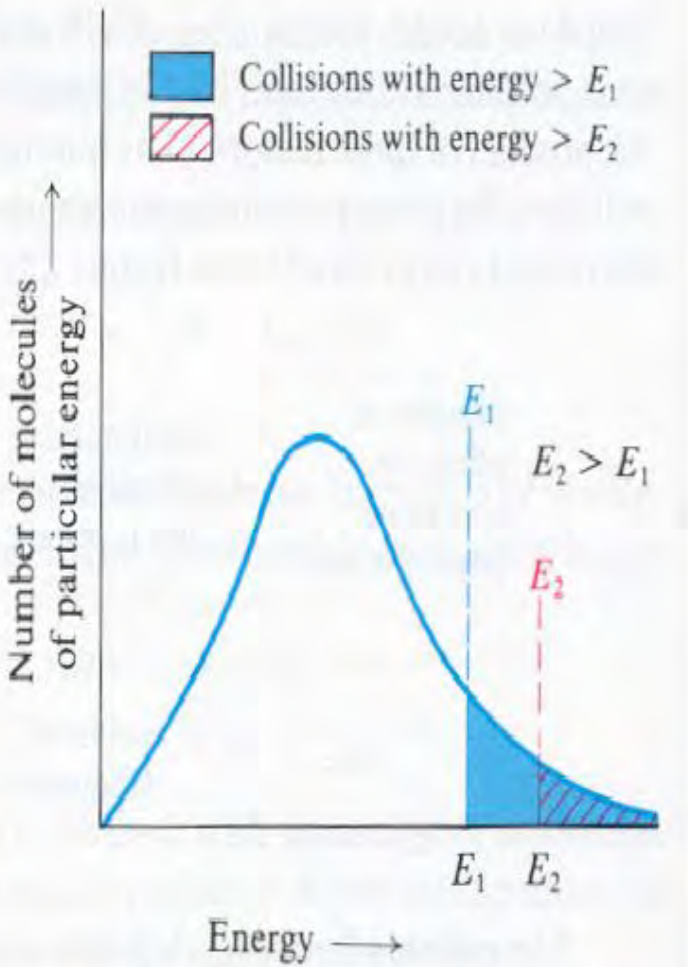


Figure 2.7 Distribution of kinetic energy among collisions.

Are You Getting It??

Which of the following things will an enzyme do?
(multiple answers)

a) An enzyme will increase the number of random collisions with the substrate.

b) An enzyme will bind the substrate and increase the rate of the reaction.

c) An enzyme will increase the kinetic energy of the substrate molecules.

d) An enzyme will lower the activation energy of the reaction.

Are You Getting It??

Answer

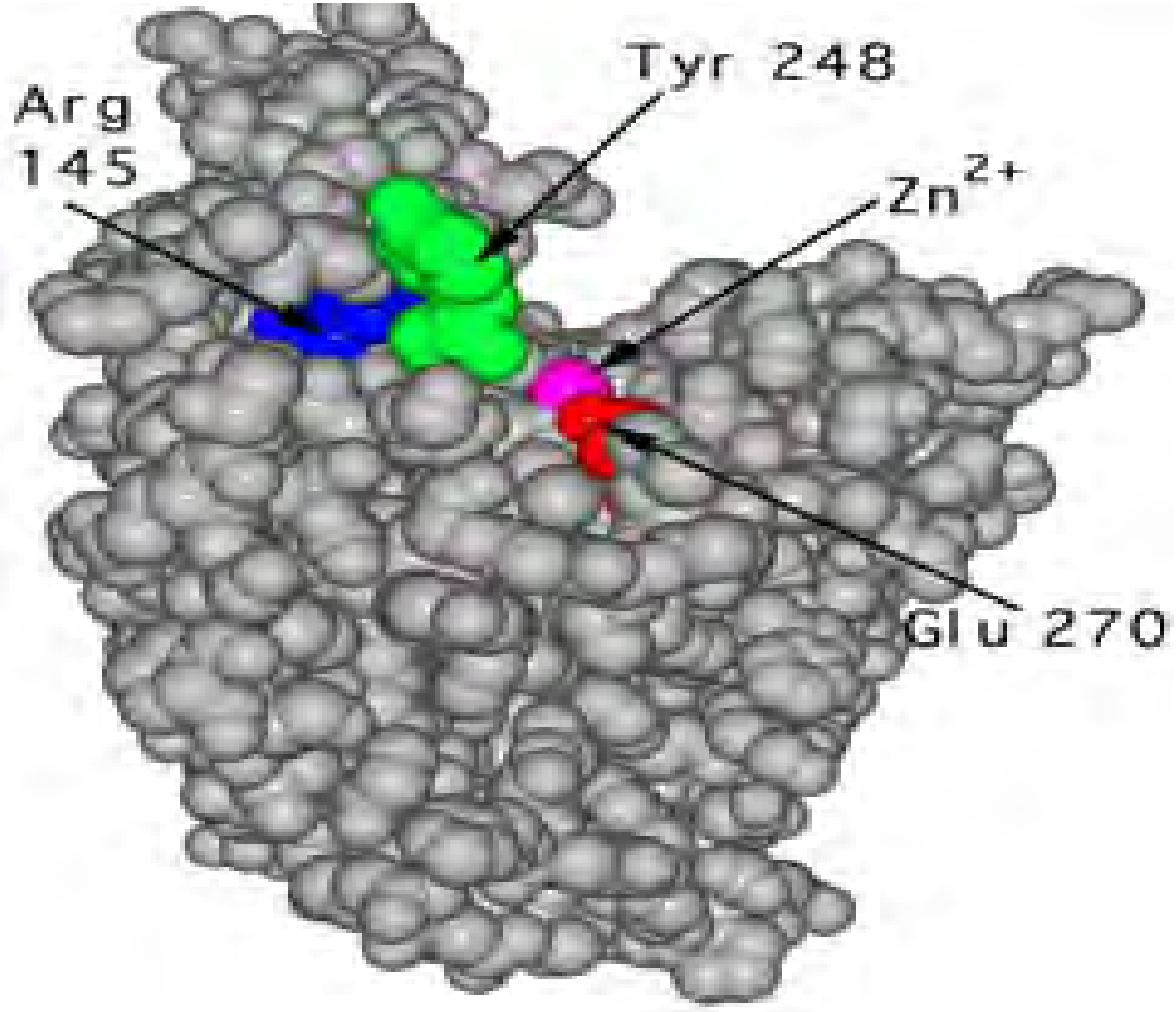
Which of the following things will an enzyme do?

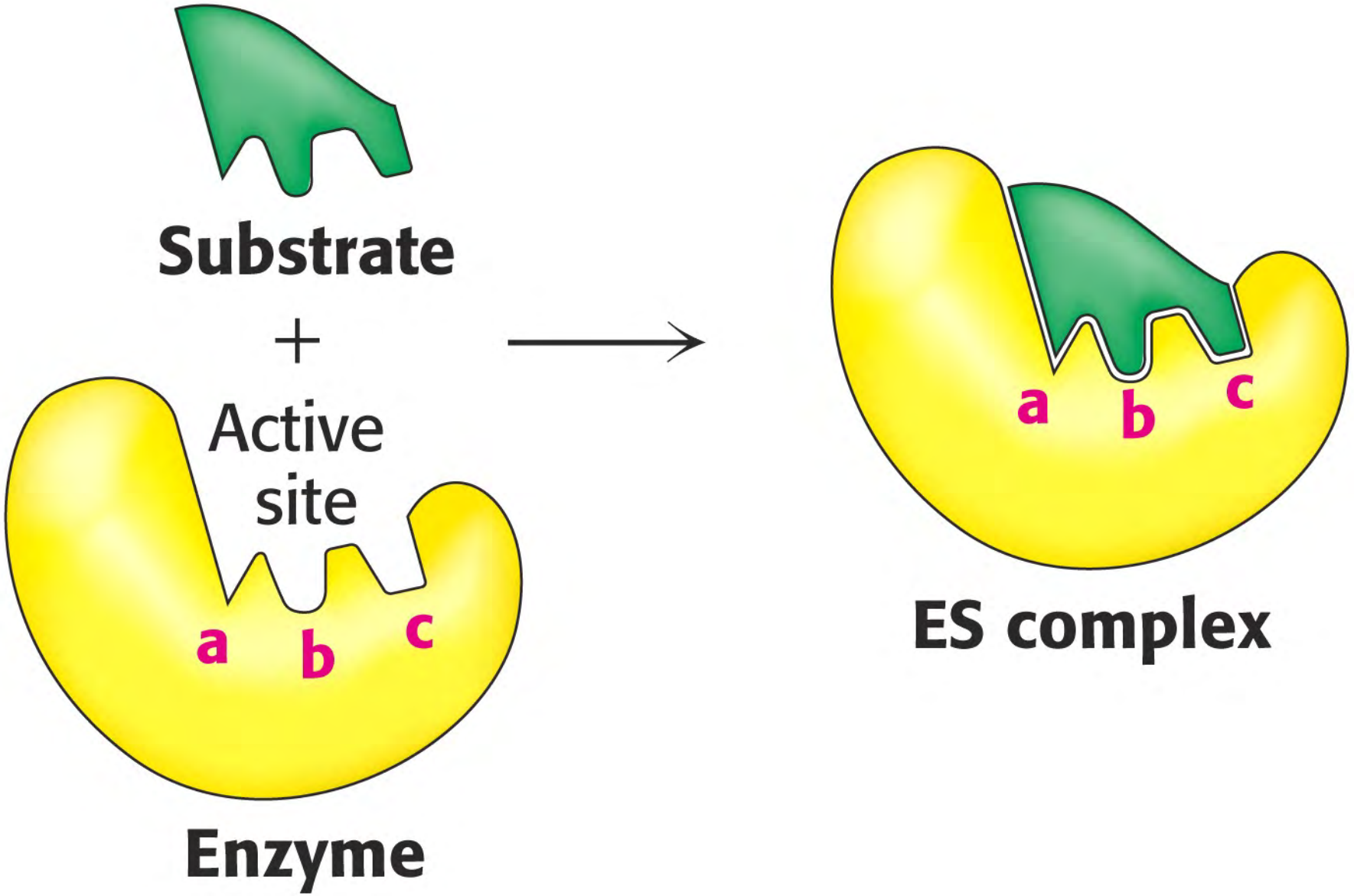
a) An enzyme will increase the number of random collisions with the substrate.

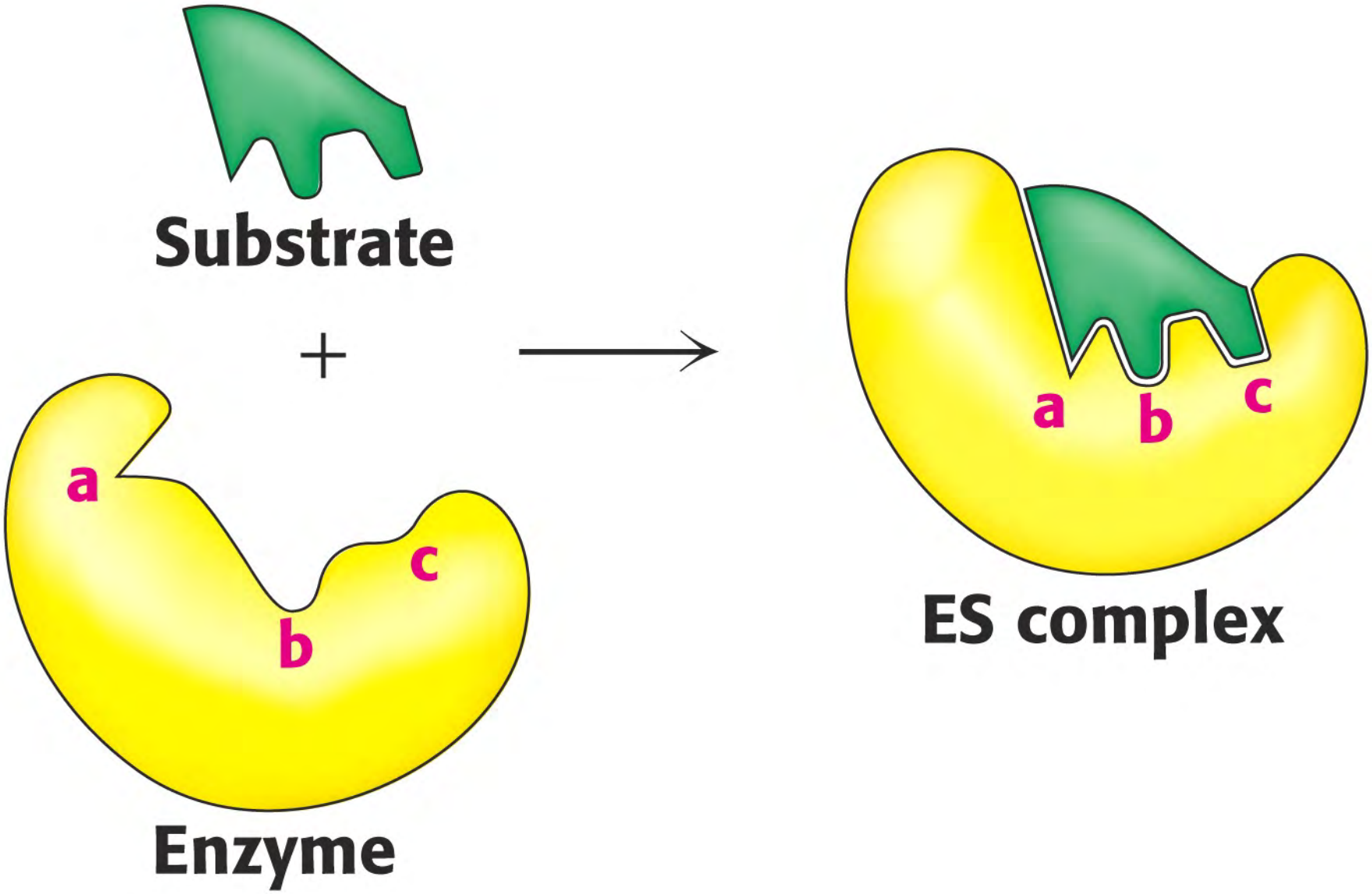
b) An enzyme will bind the substrate and increase the rate of the reaction.

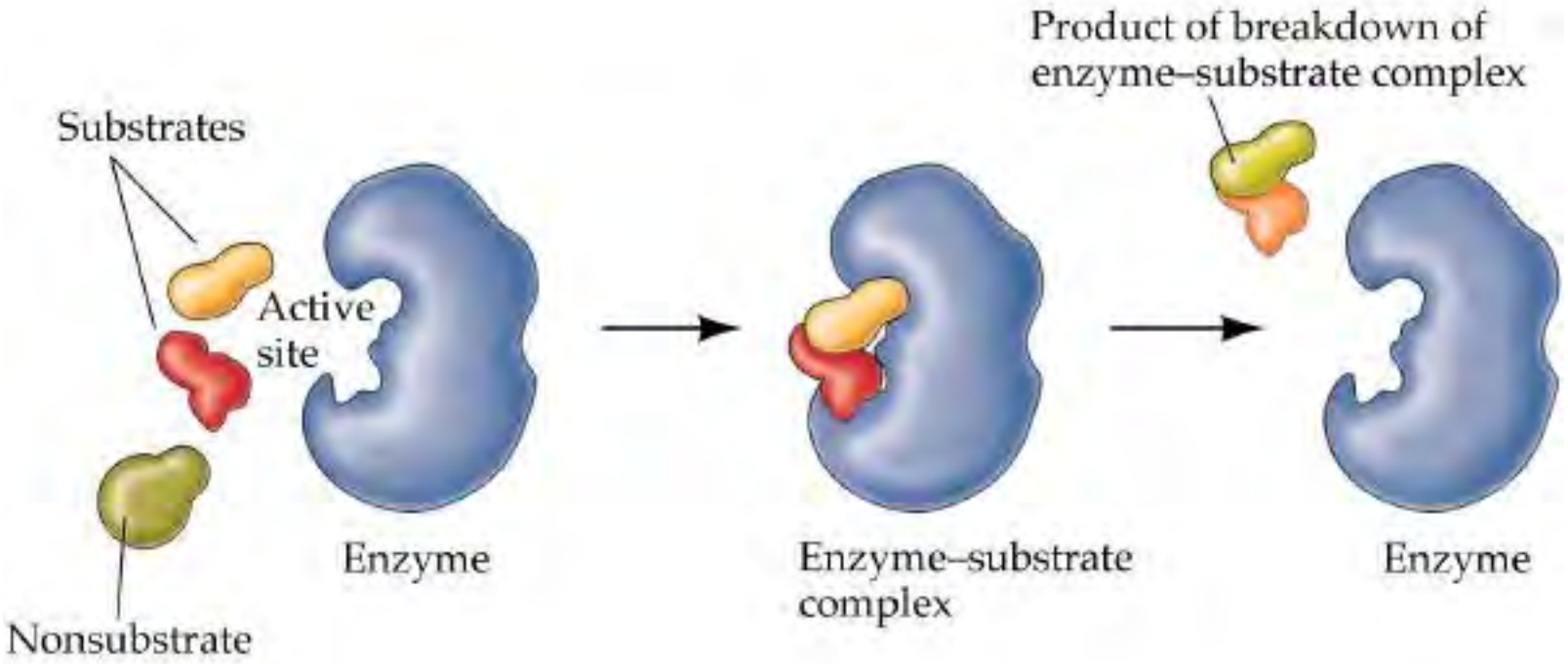
c) An enzyme will increase the kinetic energy of the substrate molecules.

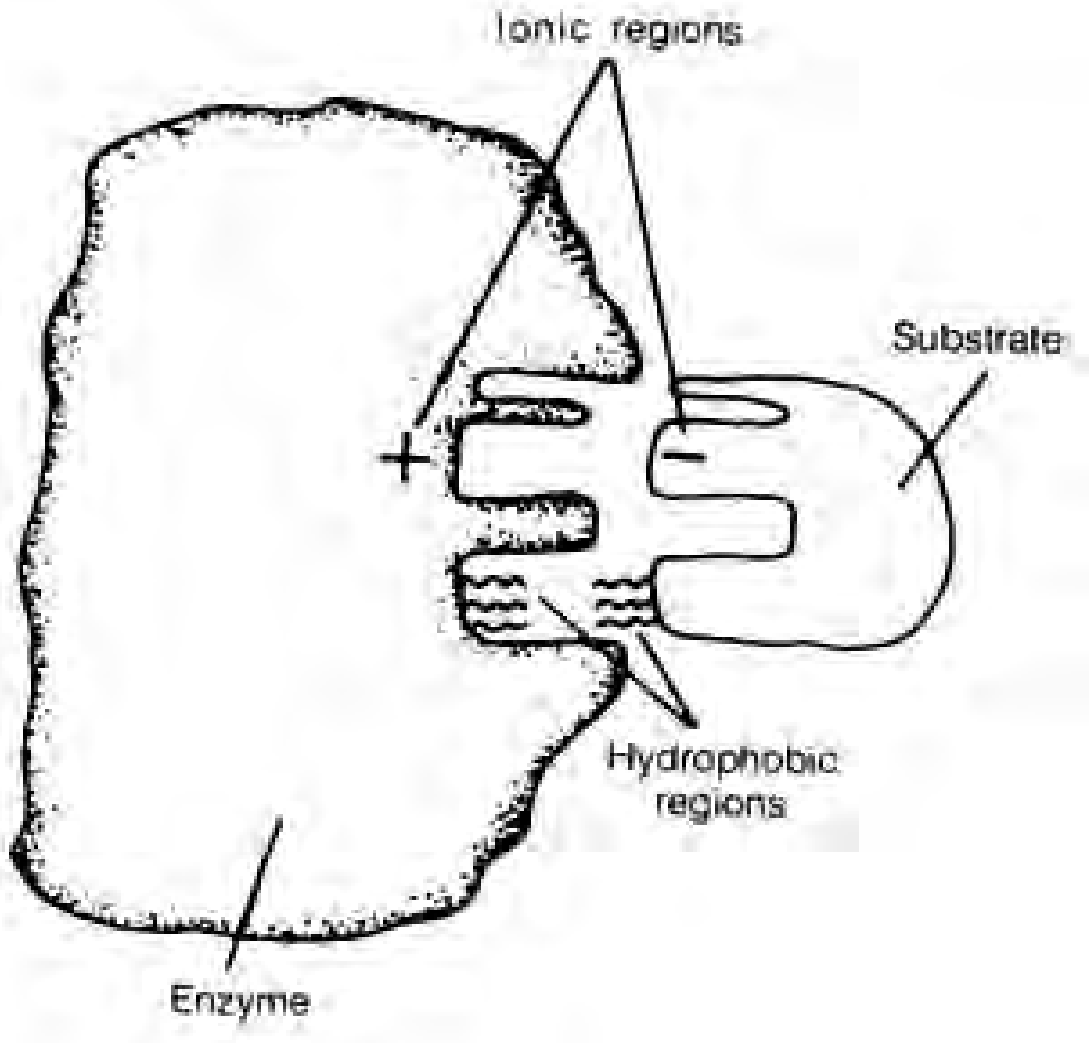
d) An enzyme will lower the activation energy of the reaction.


Tyr 248
Arg
145
Zn^{2+}
Glu 270


Substrate
+
Active site
a
b
c
Enzyme
ES complex


Substrate
+
a
b
c
Enzyme
a
b
c
ES complex




Ionic regions
Substrate
+
−
Hydrophobic regions
Enzyme

Are You Getting It??

Which characteristics are generally part of enzyme-substrate binding? ***(multiple answers)***

a) One enzyme can bind many different substrates.
b) The substrate binds to many amino acids in the enzyme.
c) The substrate forms non-covalent bonds with the enzyme.
d) The substrate and active site have complementary shapes.
e) The active site can denature as the substrate binds.

Are You Getting It??

Answer

Which characteristics are generally part of enzyme-substrate binding?

a) One enzyme can bind many different substrates.

b) The substrate binds to many amino acids in the enzyme.

c) The substrate forms non-covalent bonds with the enzyme.

d) The substrate and active site have complementary shapes.

e) The active site can denature as the substrate binds.

Are You Getting It??

An enzyme has several Asp residues in its active site. What kind of substrate could bind to this enzyme? ***(multiple answers)***

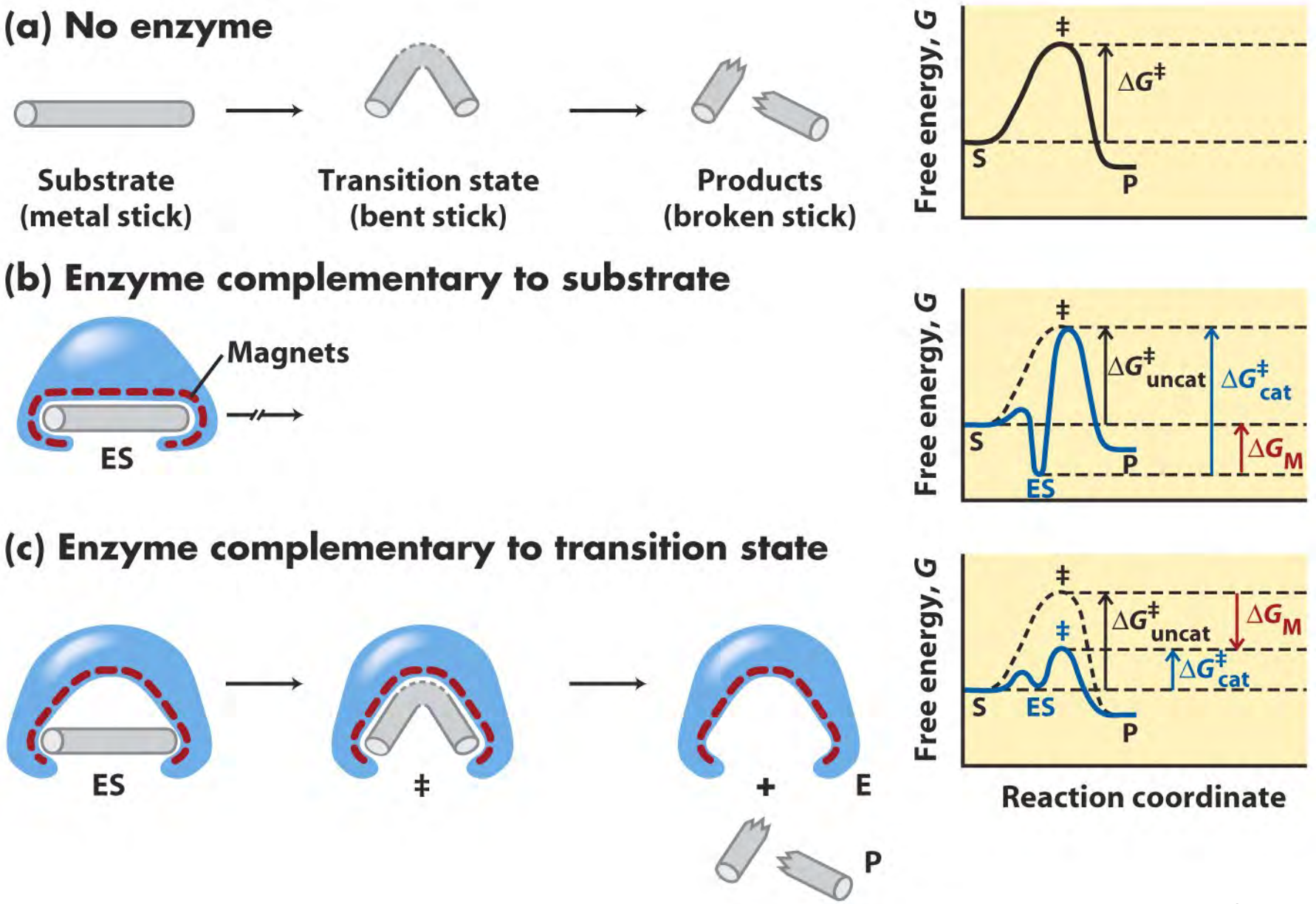
a) a hydrocarbon
b) a carboxylic acid
c) an amine
d) an alcohol
e) a thiol

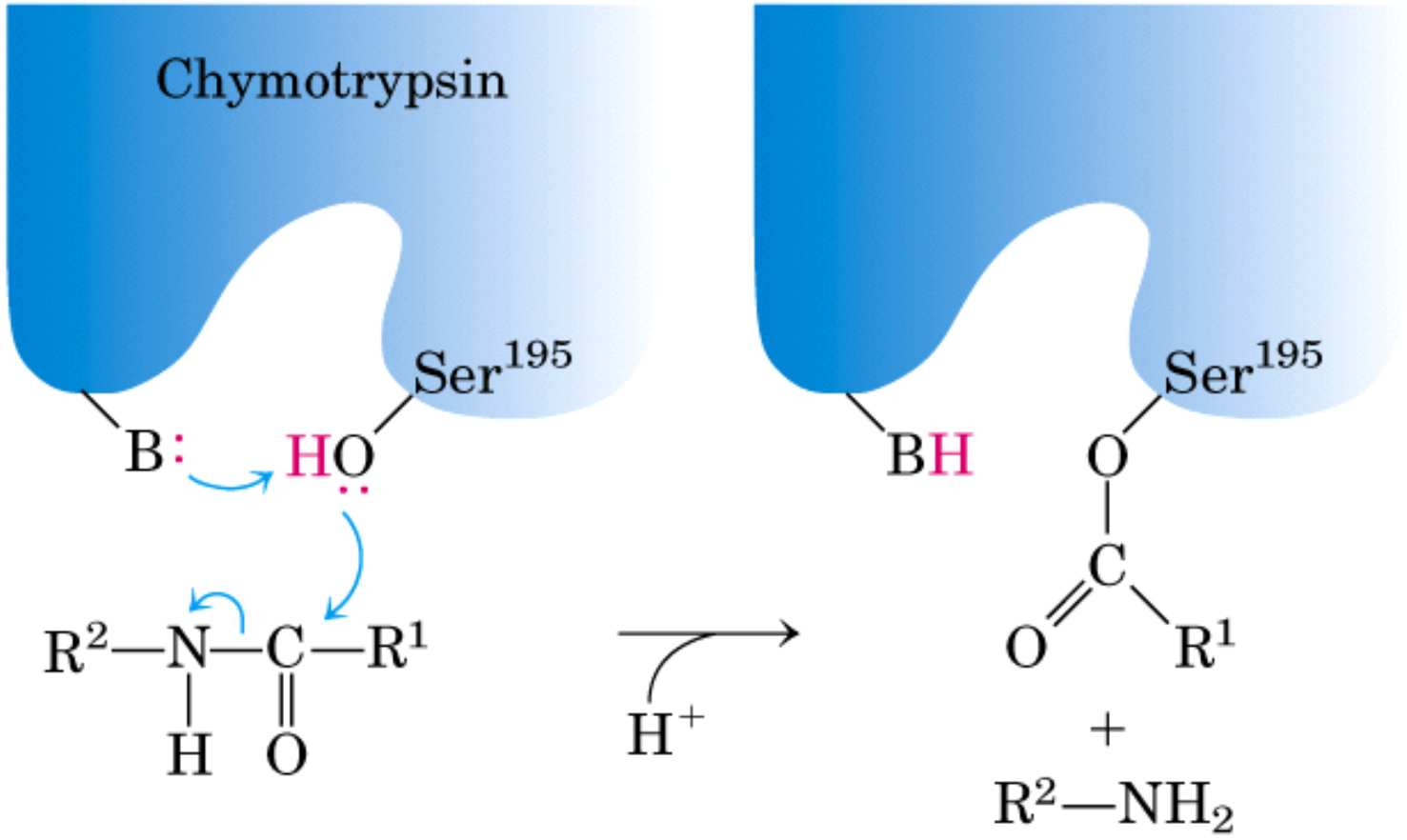
Are You Getting It??

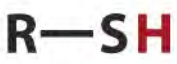
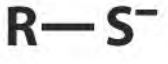
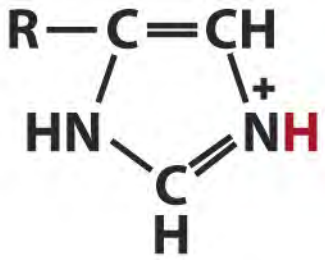
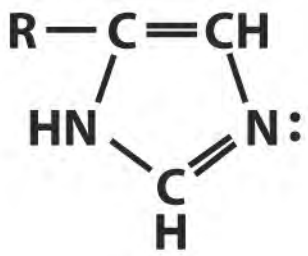
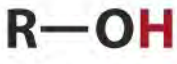
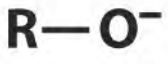
Answer

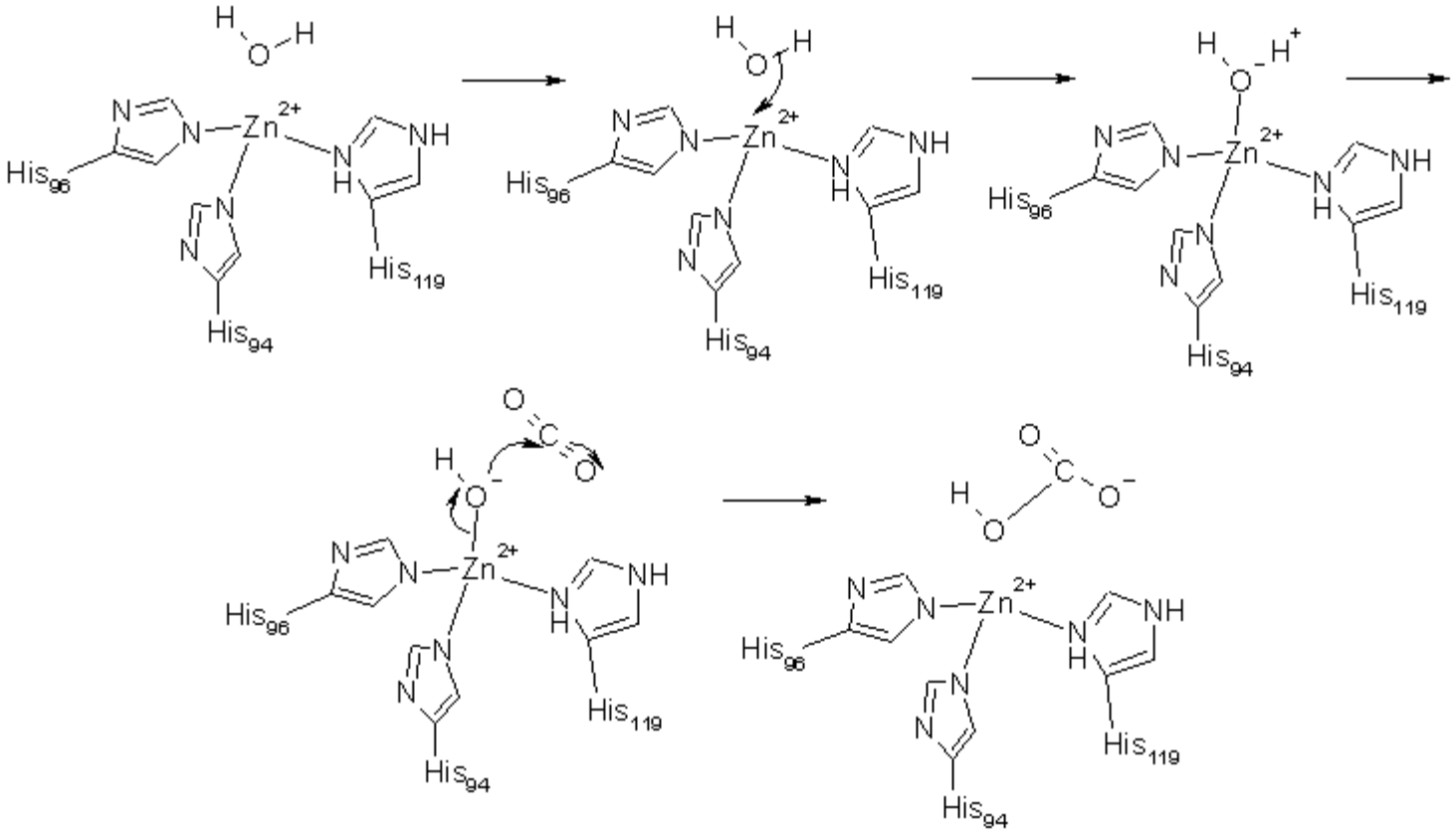
An enzyme has several Asp residues in its active site. What kind of substrate could bind to this enzyme?

a) a hydrocarbon
b) a carboxylic acid
c) an amine
d) an alcohol
e) a thiol


(a) No enzyme
Substrate
(metal stick)
Transition state
(bent stick)
Products
(broken stick)
Free energy, G
‡
ΔG‡
S
P
(b) Enzyme complementary to substrate
Magnets
ES
Free energy, G
‡
ΔG‡uncat
ΔG‡cat
ΔGM
S
P
ES
(c) Enzyme complementary to transition state
ES
‡
+
E
P
Free energy, G
‡
ΔG‡uncat
ΔGM
‡
ΔG‡cat
S
ES
P
Reaction coordinate


Chymotrypsin
Ser195
B
HO
R2—N—C—R1
H O
H+
BH
O
C
O
R1
+
R2—NH2

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


His96
His119
His94
Zn2+

Are You Getting It??

Which of the following mechanisms could be used by an enzyme to catalyze a reaction? *(multiple answers)*

a) The substrate is exactly complementary to the active site.
b) A histidine residue donates a proton to the substrate.
c) A ferric ion prosthetic group stabilizes a negatively charged transition state.
d) A stable ester bond forms between the enzyme and the transition state.

Are You Getting It??

Answer

Which of the following mechanisms could be used by an enzyme to catalyze a reaction?

a) The substrate is exactly complementary to the active site.

b) A histidine residue donates a proton to the substrate.

c) A ferric ion prosthetic group stabilizes a negatively charged transition state.

d) A stable ester bond forms between the enzyme and the transition state.

Are You Getting It??

An enzyme is active at pH=7.0 but is inactive at pH=10.0. Which amino acid would probably be essential for this enzyme to catalyze its reaction?

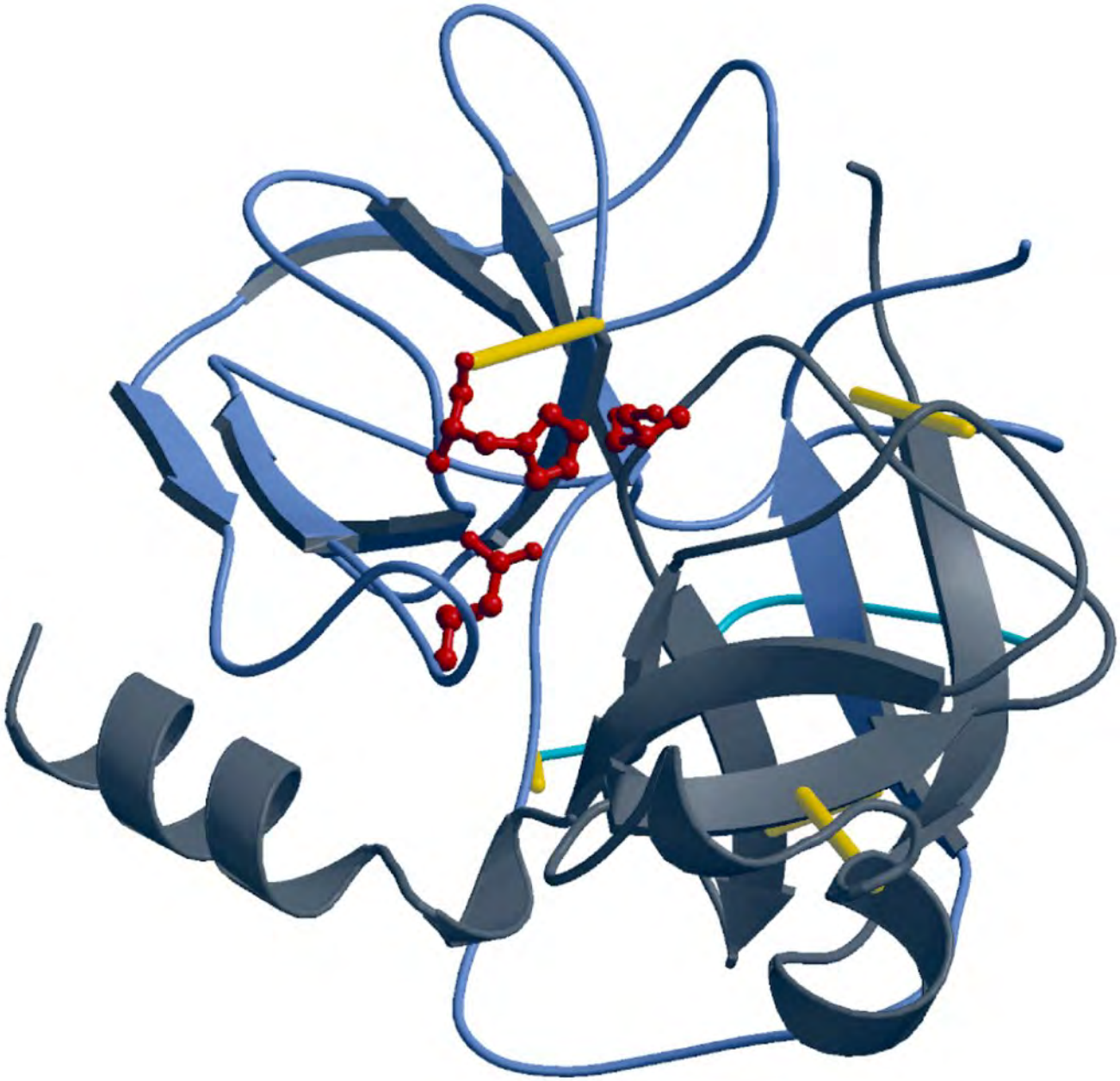
a) glutamate
b) lysine
c) tryptophan
d) methionine
e) serine

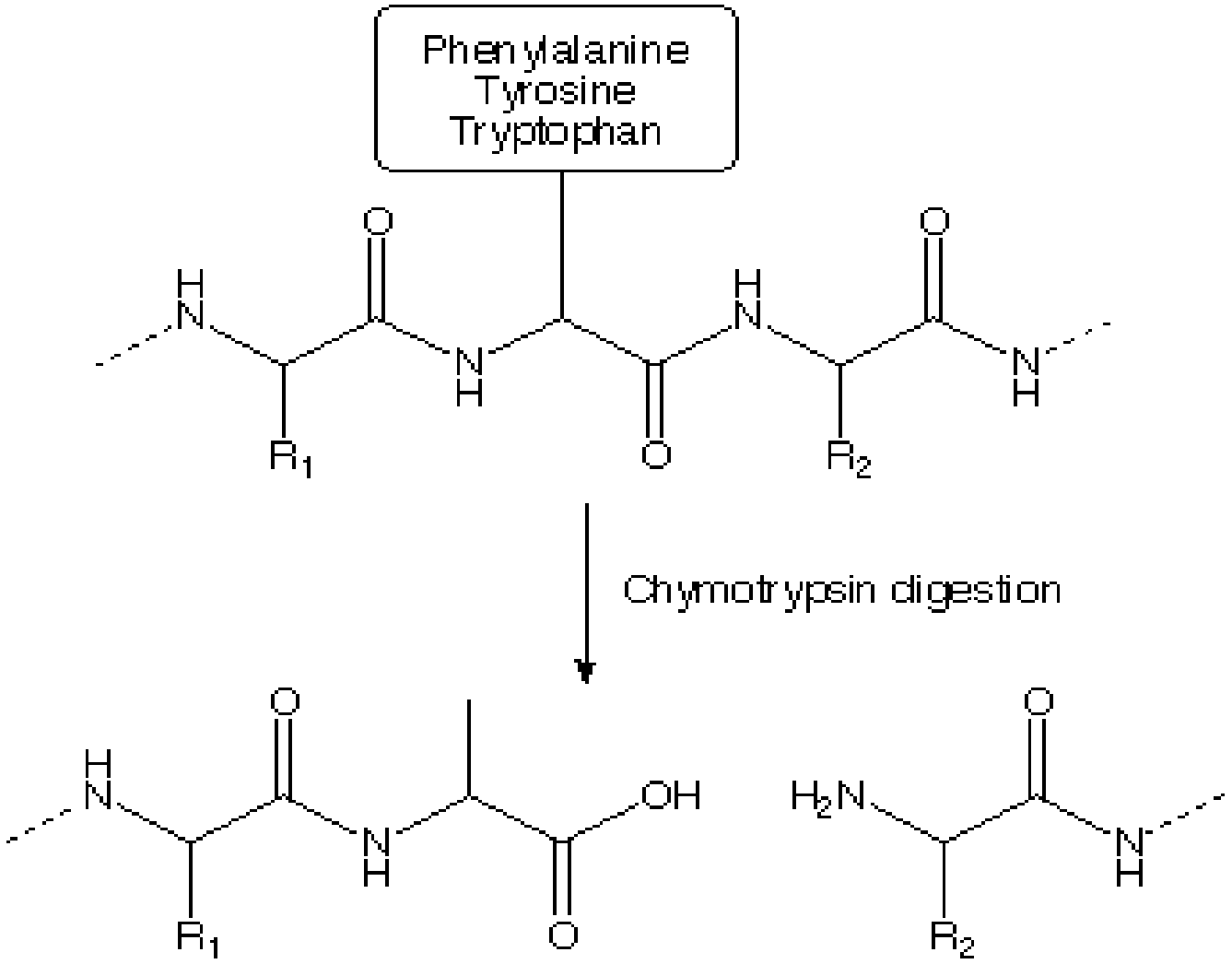
Are You Getting It??

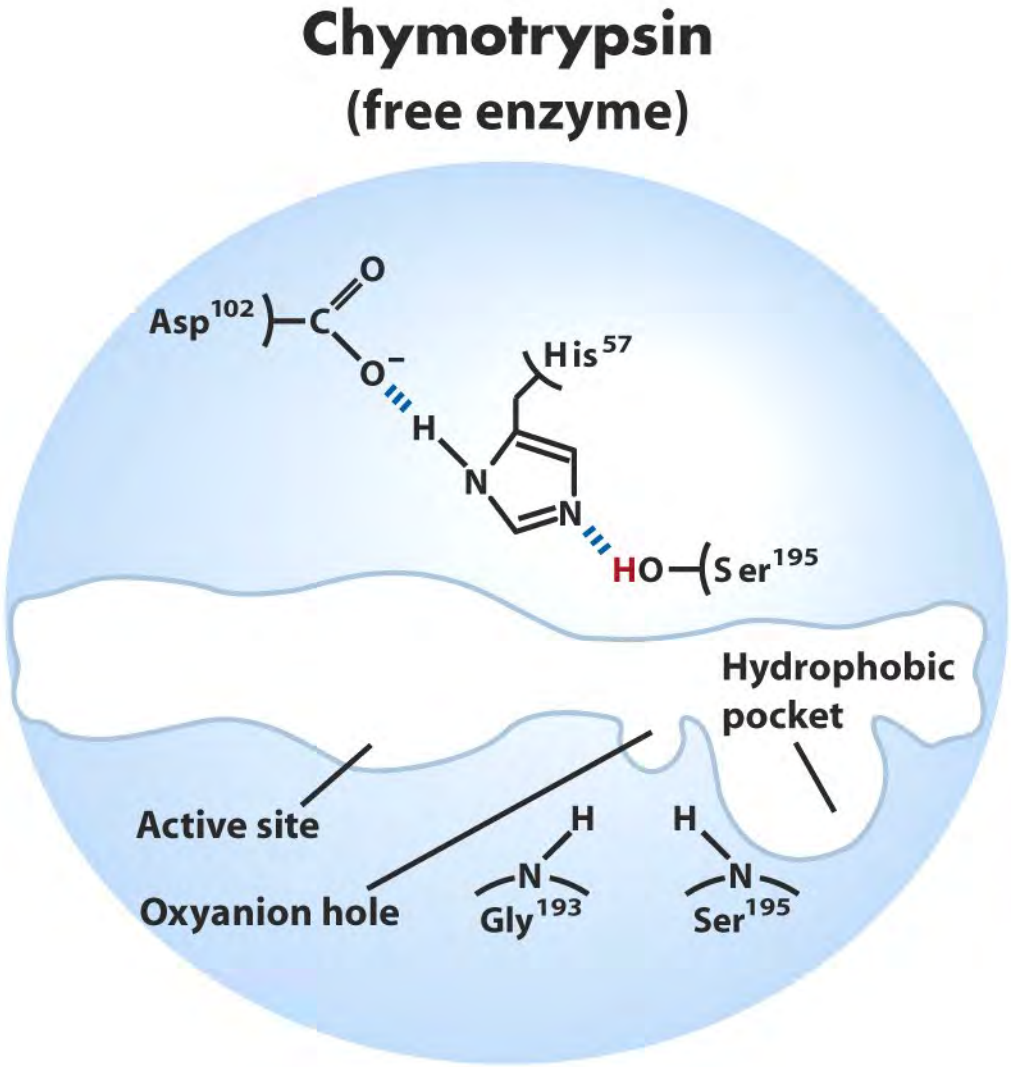
Answer

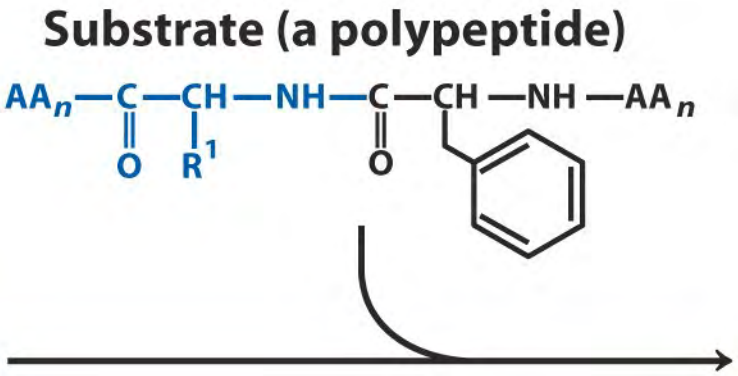
An enzyme is active at pH=7.0 but is inactive at pH=10.0. Which amino acid would probably be essential for this enzyme to catalyze its reaction?

a) glutamate
b) lysine
c) tryptophan
d) methionine
e) serine



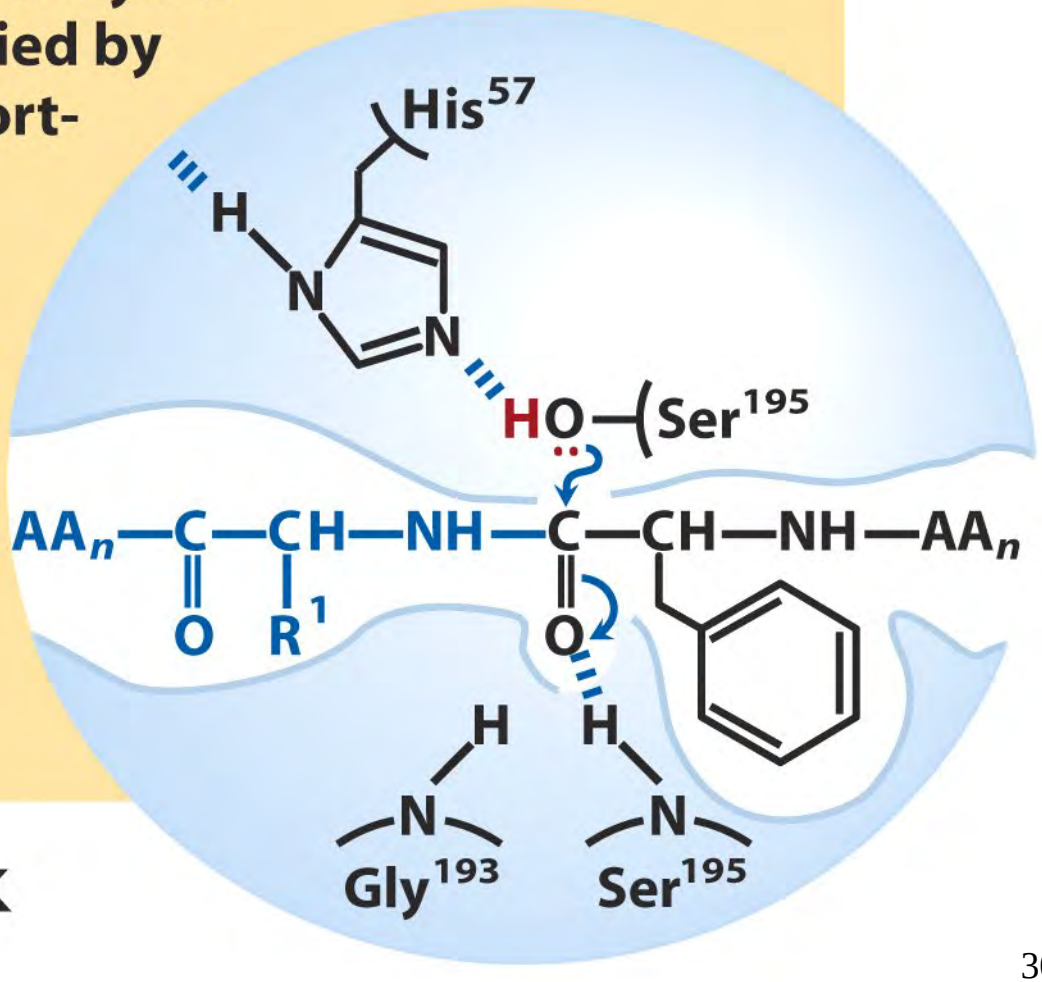

Phenylalanine
Tyrosine
Tryptophan
Chymotrypsin digestion





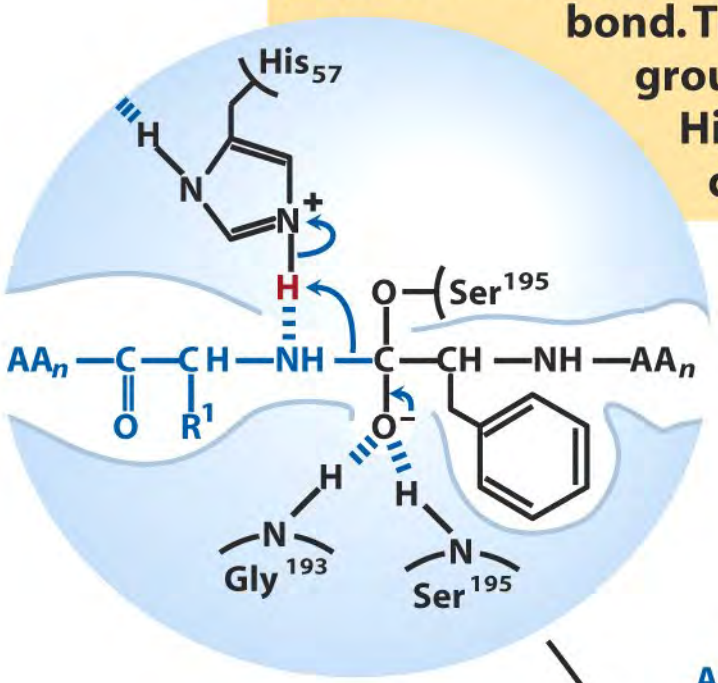
When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.

Interaction of Ser^{195} and His^{57} generates a strongly nucleophilic alkoxide ion on Ser^{195}; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accom-panied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bond-ing in the oxyanion hole.



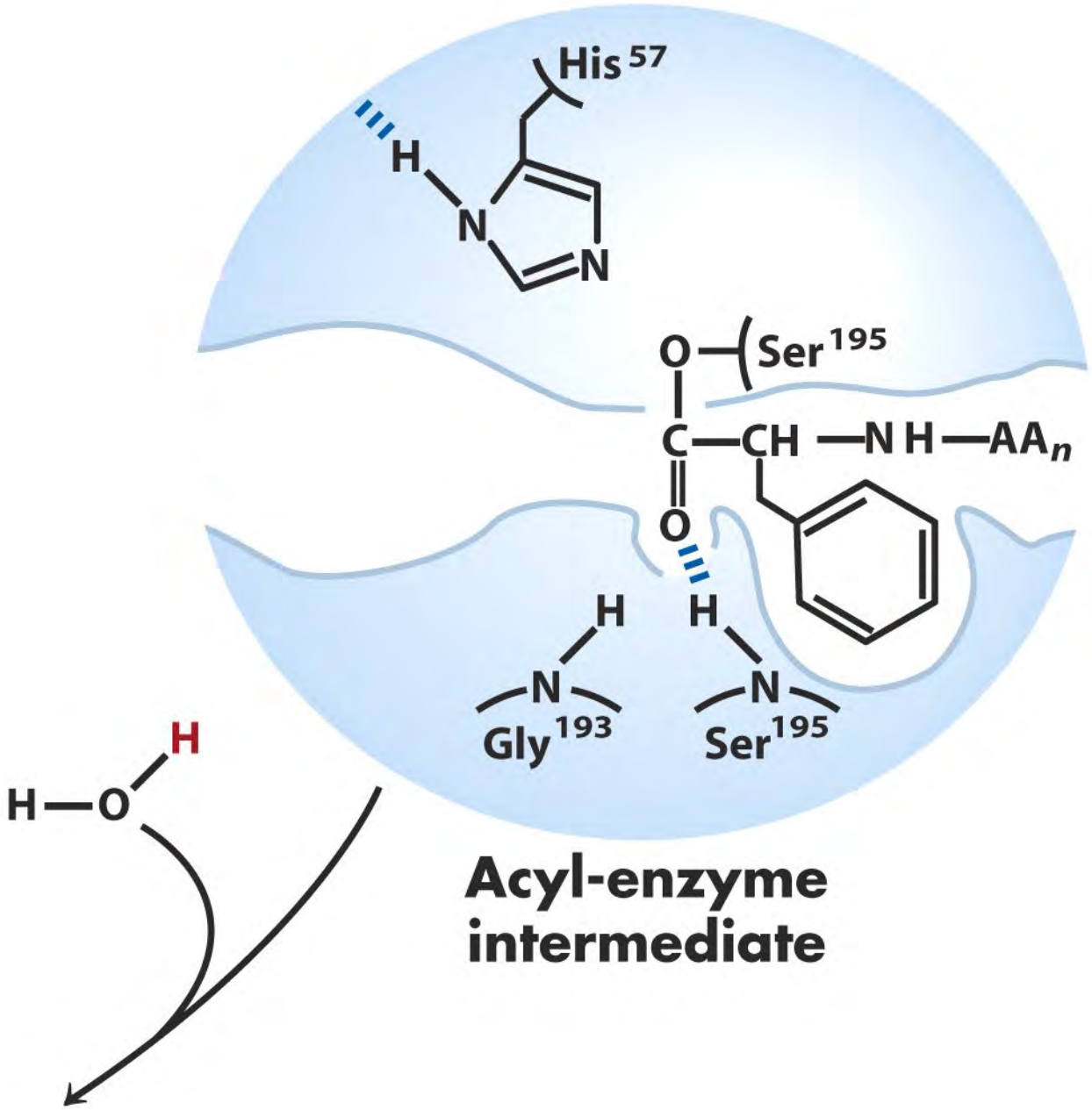
ES complex

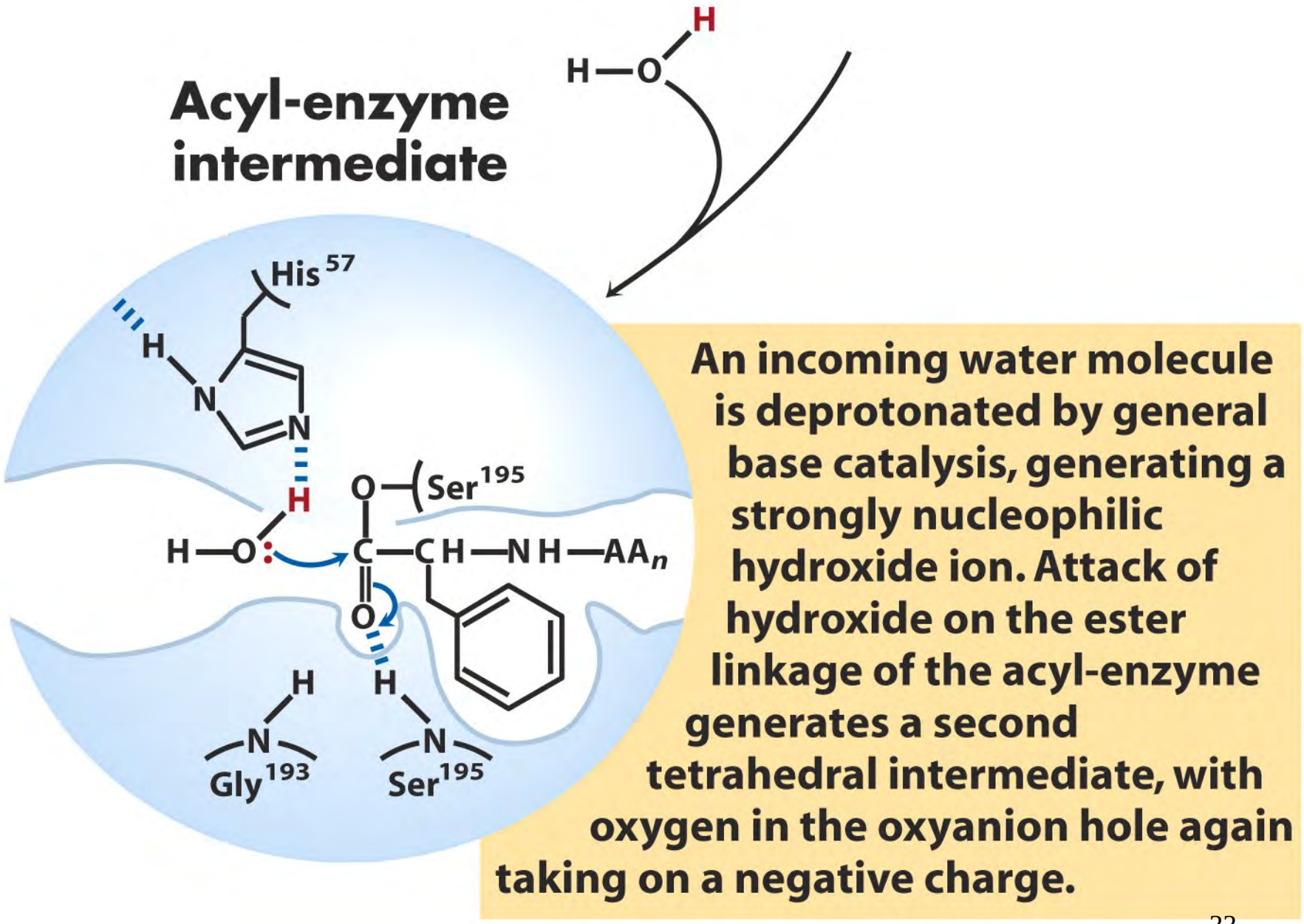
Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral inter-mediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His^{57}, facilitating its displacement.



Short-lived intermediate (acylation)

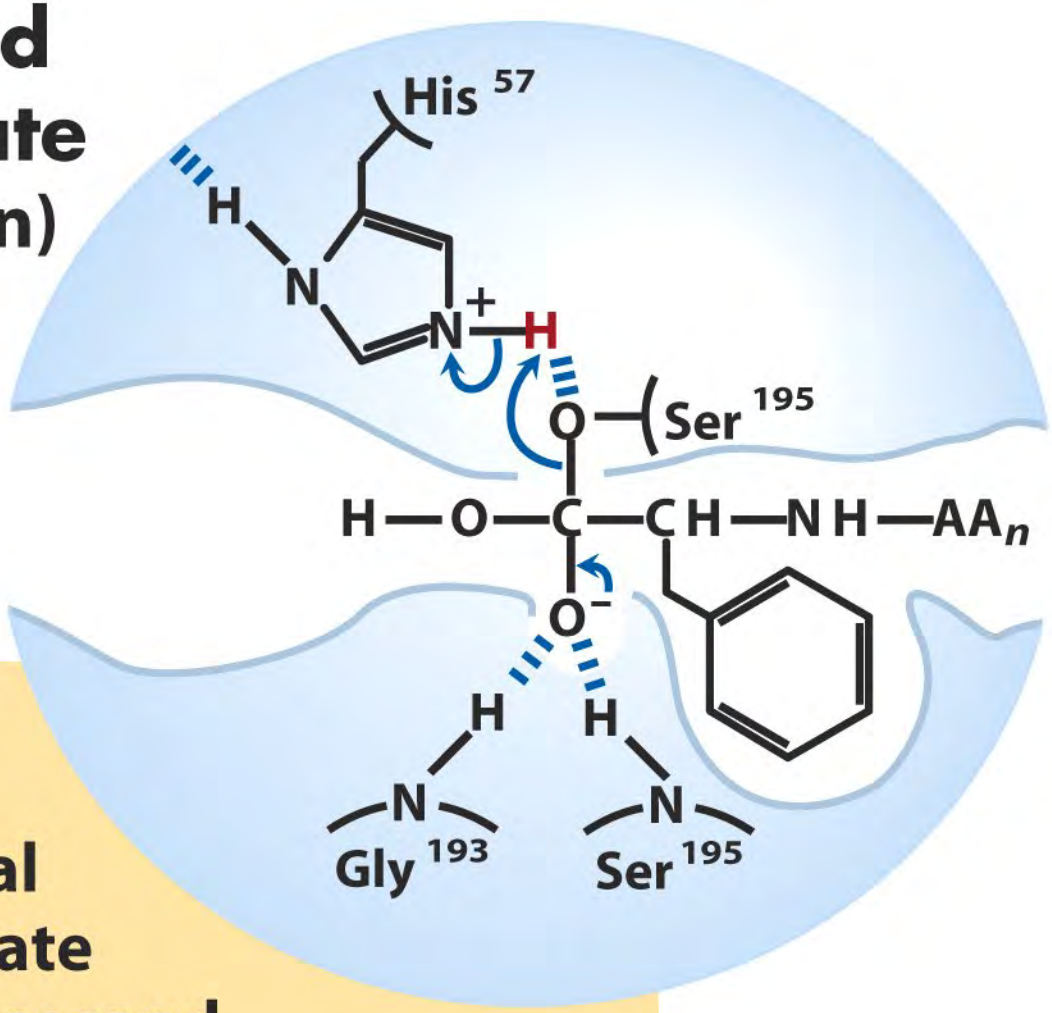
Product 1


His 57
H
N
N
O
Ser 195
C
CH
NH
AA$_n$
O
H
N
Gly 193
H
N
Ser 195
H
H
O
Acyl-enzyme
intermediate

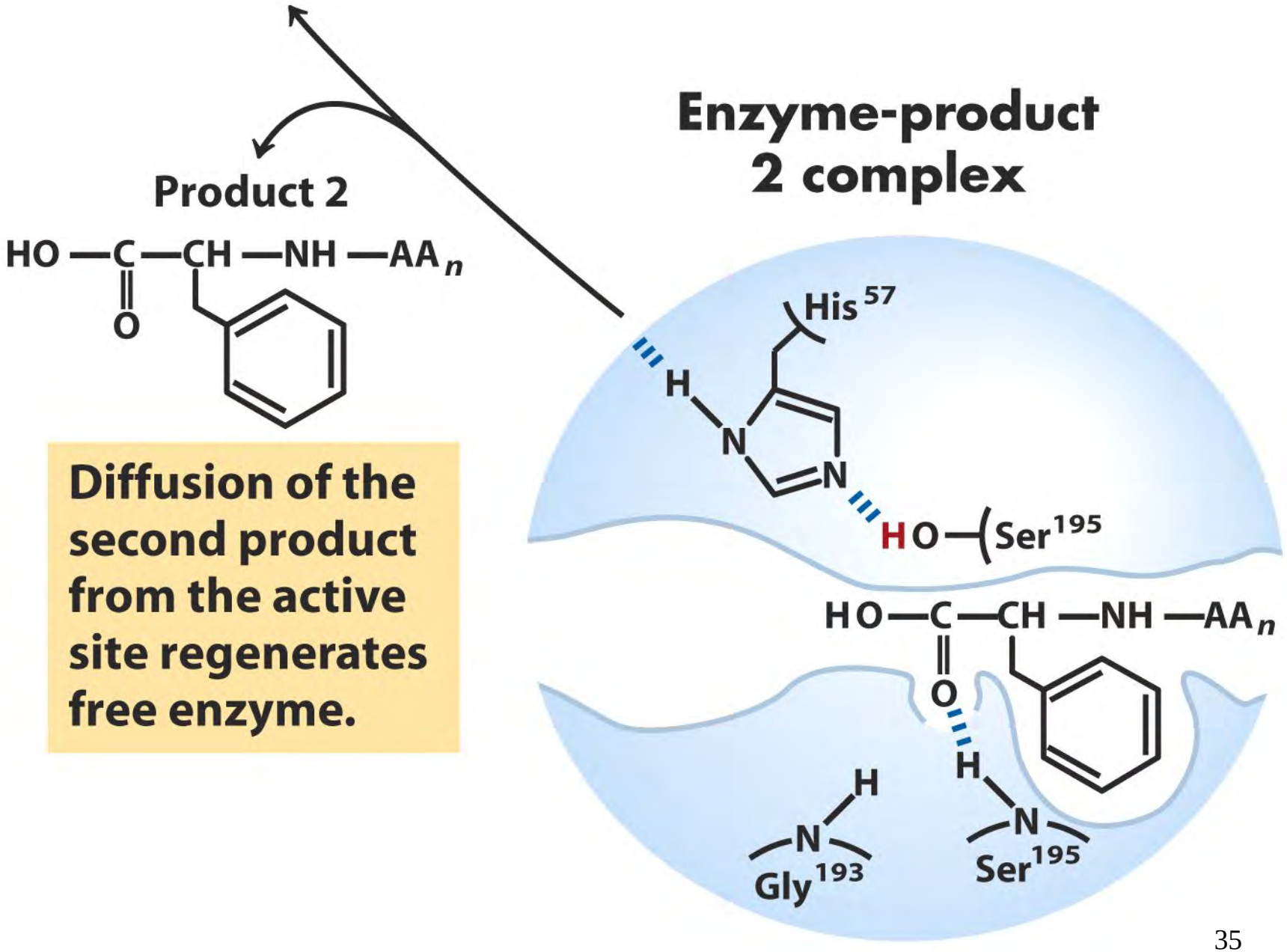


An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

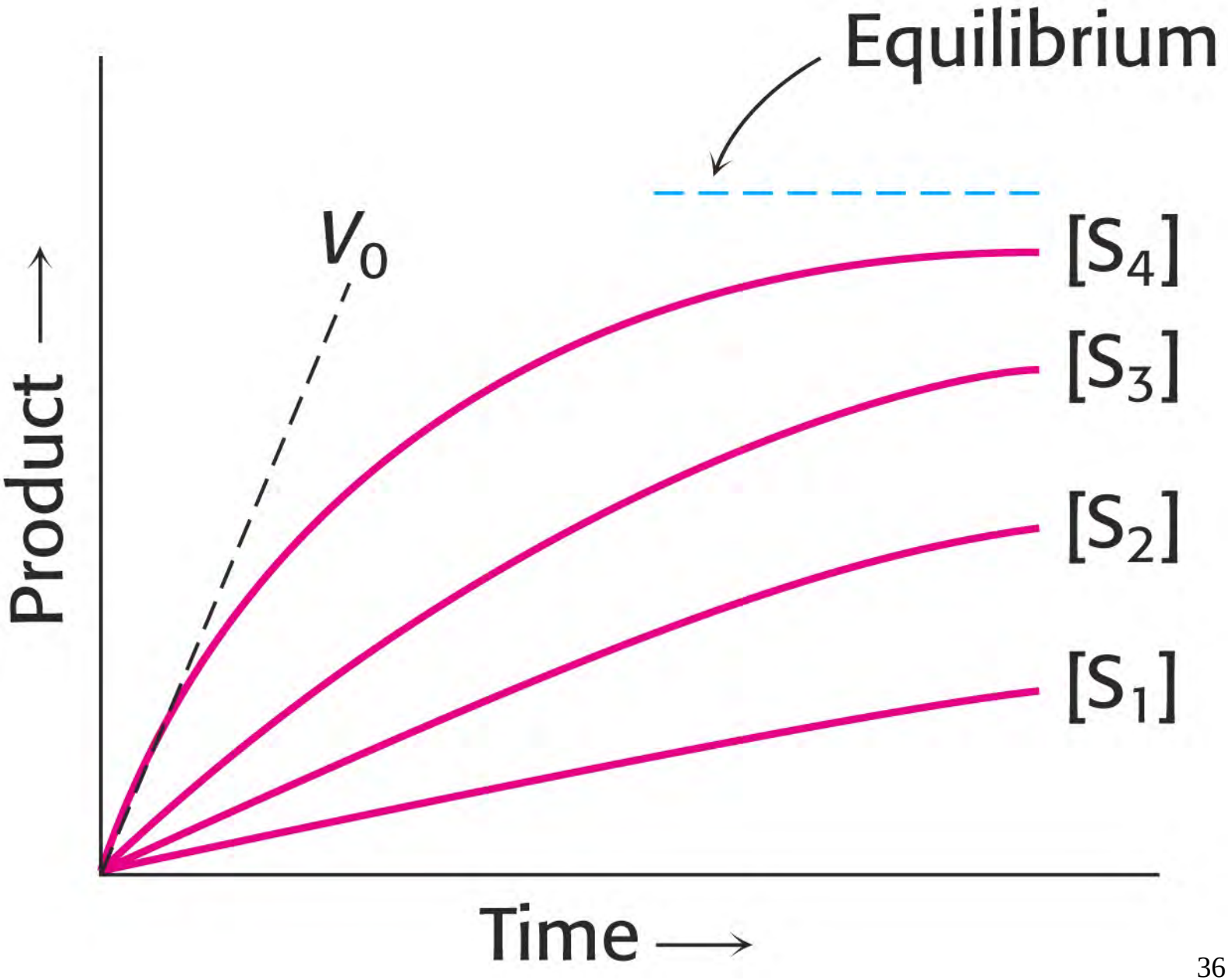
Short-lived intermediate (deacylation)

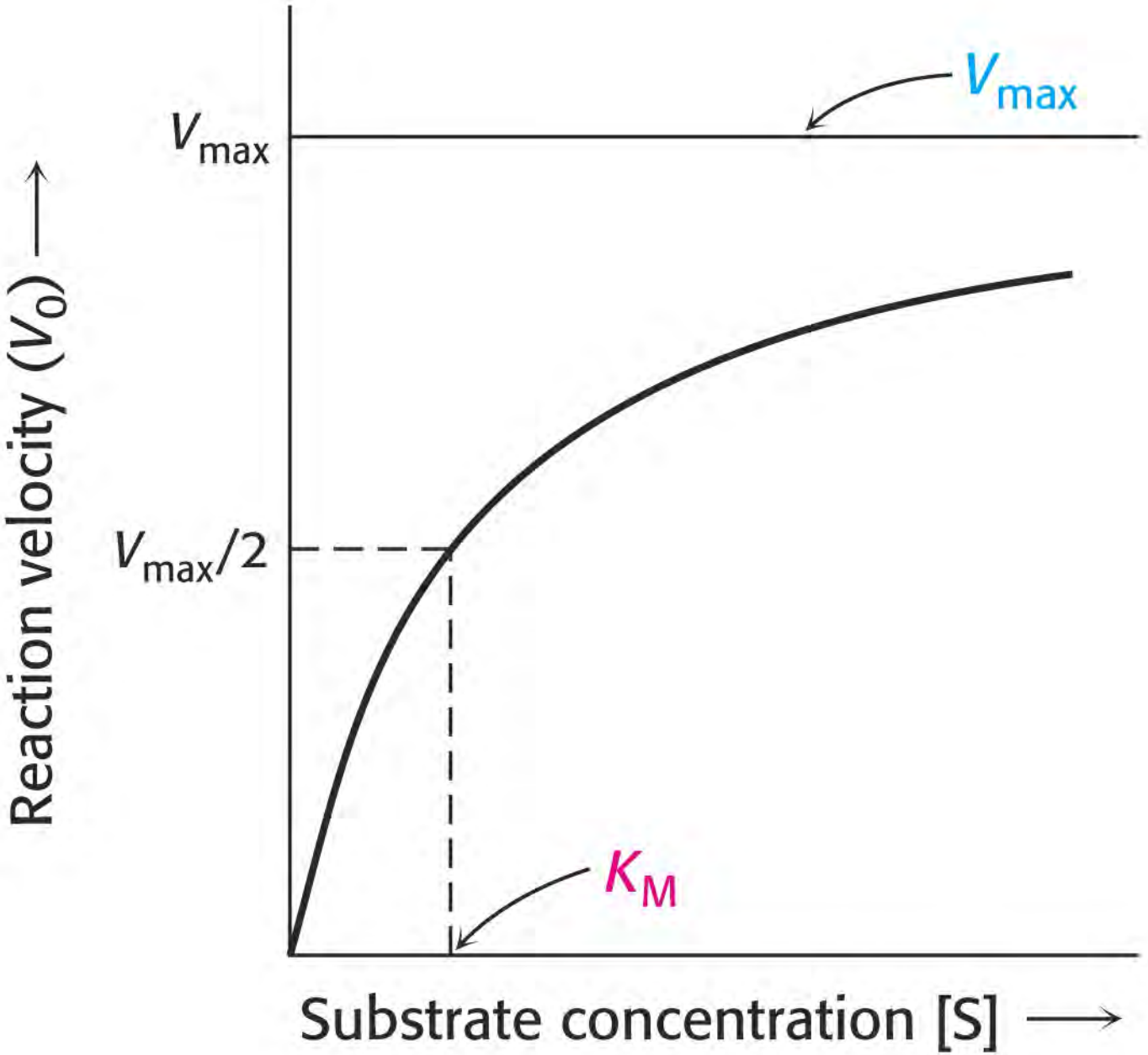


Collapse of the tetrahedral intermediate forms the second product, a carbohydrate anion, and displaces Ser^{195}.



Diffusion of the second product from the active site regenerates free enzyme.


Equilibrium
V_0
$[S_4]$
$[S_3]$
$[S_2]$
$[S_1]$
Product ⟶
Time ⟶


V_{max}
V_{max}
$V_{max}/2$
K_M
Reaction velocity (V_0) ⟶
Substrate concentration [S] ⟶

$$V_0 = \frac{[S] \bullet V_{MAX}}{([S] + K_M)}$$

Other models which give the Michaelis-Menten Equation:

#4. $E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \xrightarrow{k2} E + P$

with $\Delta S = 0$ and full integration, Michaelis-Menten after a few milliseconds.

$V_{max} = k_2 E_o$

$K_m = (k_1 + k_2)/k_1$

#5. $E + S \underset{k-1}{\overset{k1}{\rightleftarrows}} ES \underset{k-2}{\overset{k2}{\rightleftarrows}} E + P$

Steady-state derivation, with $\Delta S = 0$ and $P_o = 0$

$V_{max} = k_2 E_o$

$K_m = (k_1 + k_2)/k_1$

REVERSIBLE!

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	*Substrate*	K_m (mM)
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

Are You Getting It??

An enzyme with a small Km must have which characteristic?

a) It would have a large Vmax.

b) It would have a small Vmax.

c) Its reaction would be reversible.

d) Its reaction would be irreversible.

e) It would work well even when small amounts of substrate are present.

f) It would work well only if large amounts of substrate are present.

Are You Getting It??

Answer

An enzyme with a small Km must have which characteristic?

a) It would have a large Vmax.

b) It would have a small Vmax.

c) Its reaction would be reversible.

d) Its reaction would be irreversible.

e) It would work well even when small amounts of substrate are present.

f) It would work well only if large amounts of substrate are present.

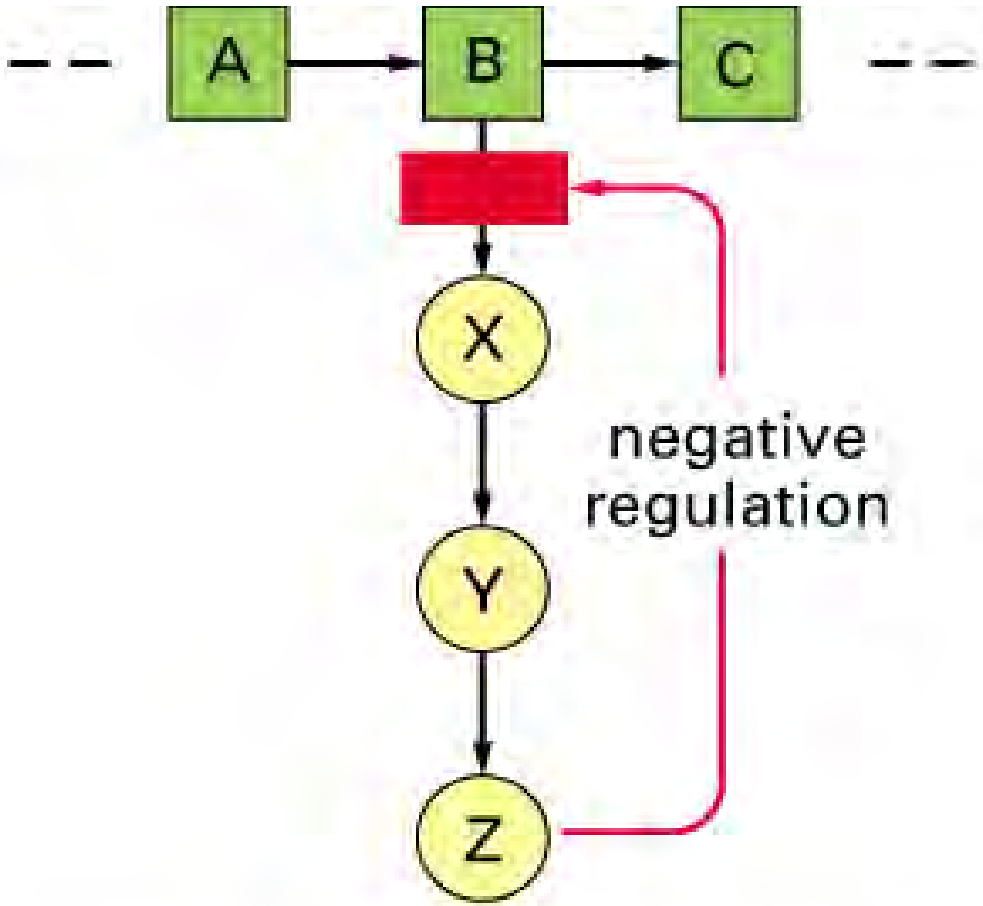
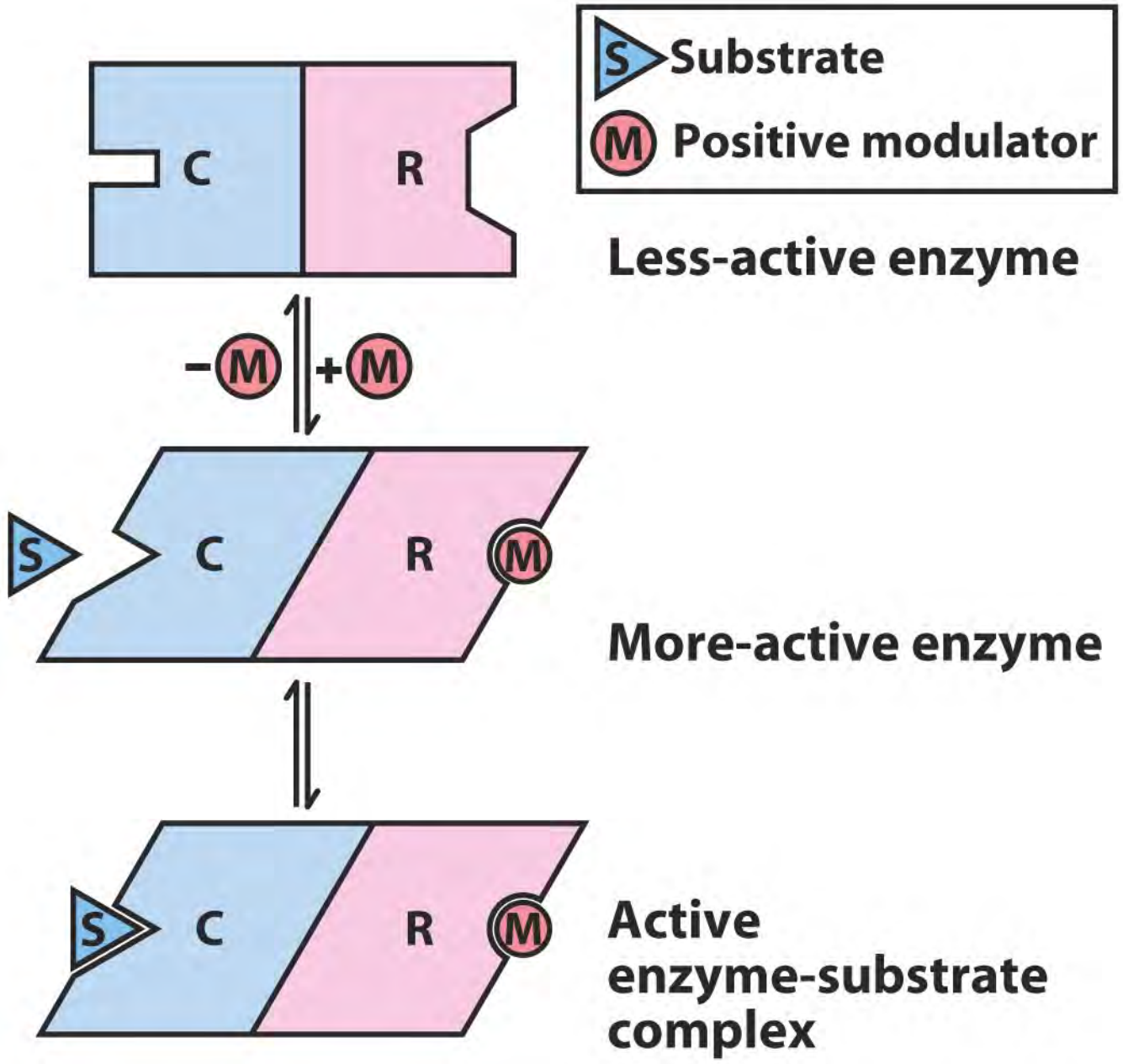
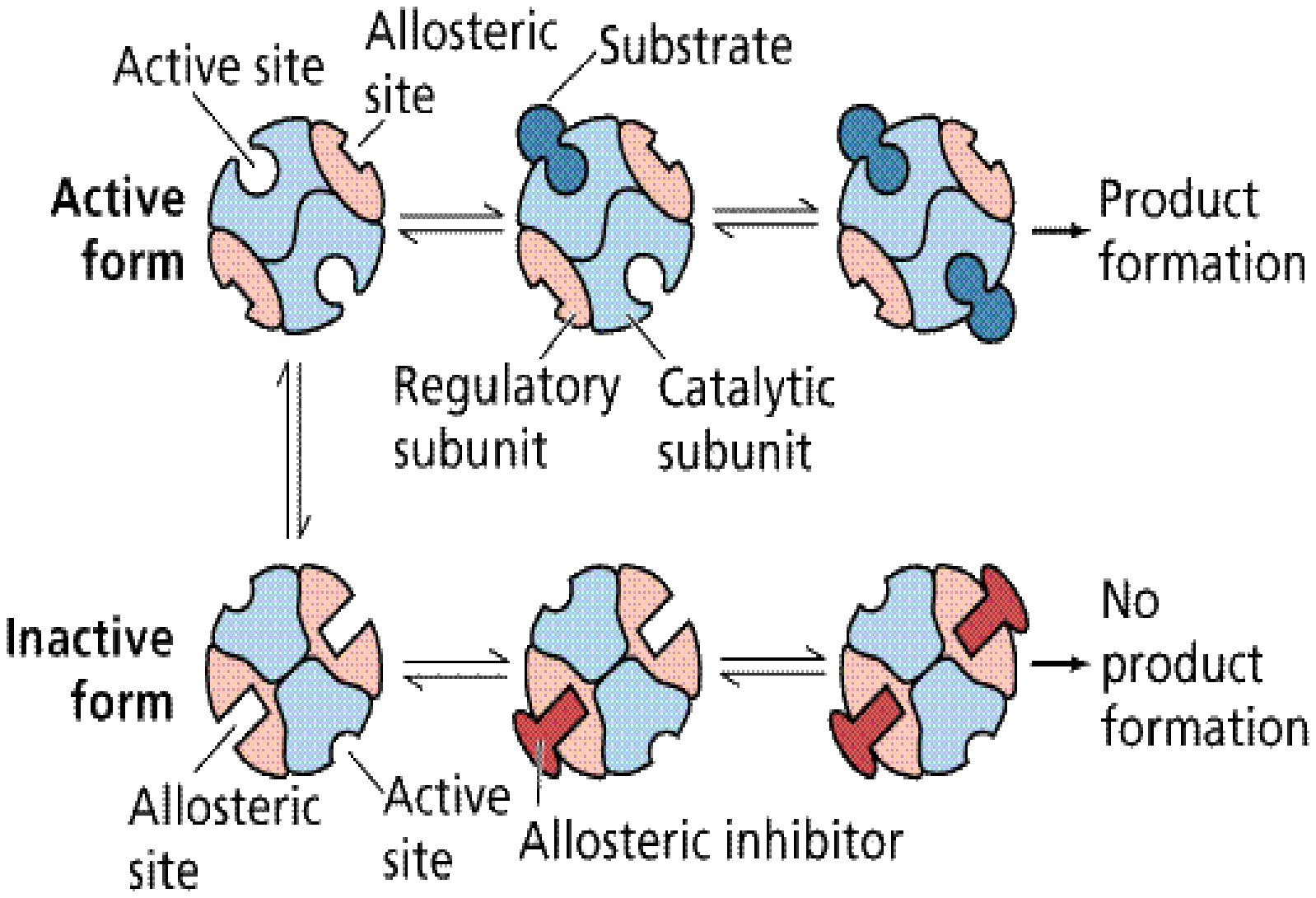


Figure 3–55. Molecular Biology of the Cell, 4th Edition.


S Substrate
M Positive modulator
C
R
Less-active enzyme
– M
+ M
S
C
R
M
More-active enzyme
S
C
R
M
Active enzyme-substrate complex


Active site
Allosteric site
Substrate
Active form
Product formation
Regulatory subunit
Catalytic subunit
Inactive form
No product formation
Allosteric site
Active site
Allosteric inhibitor

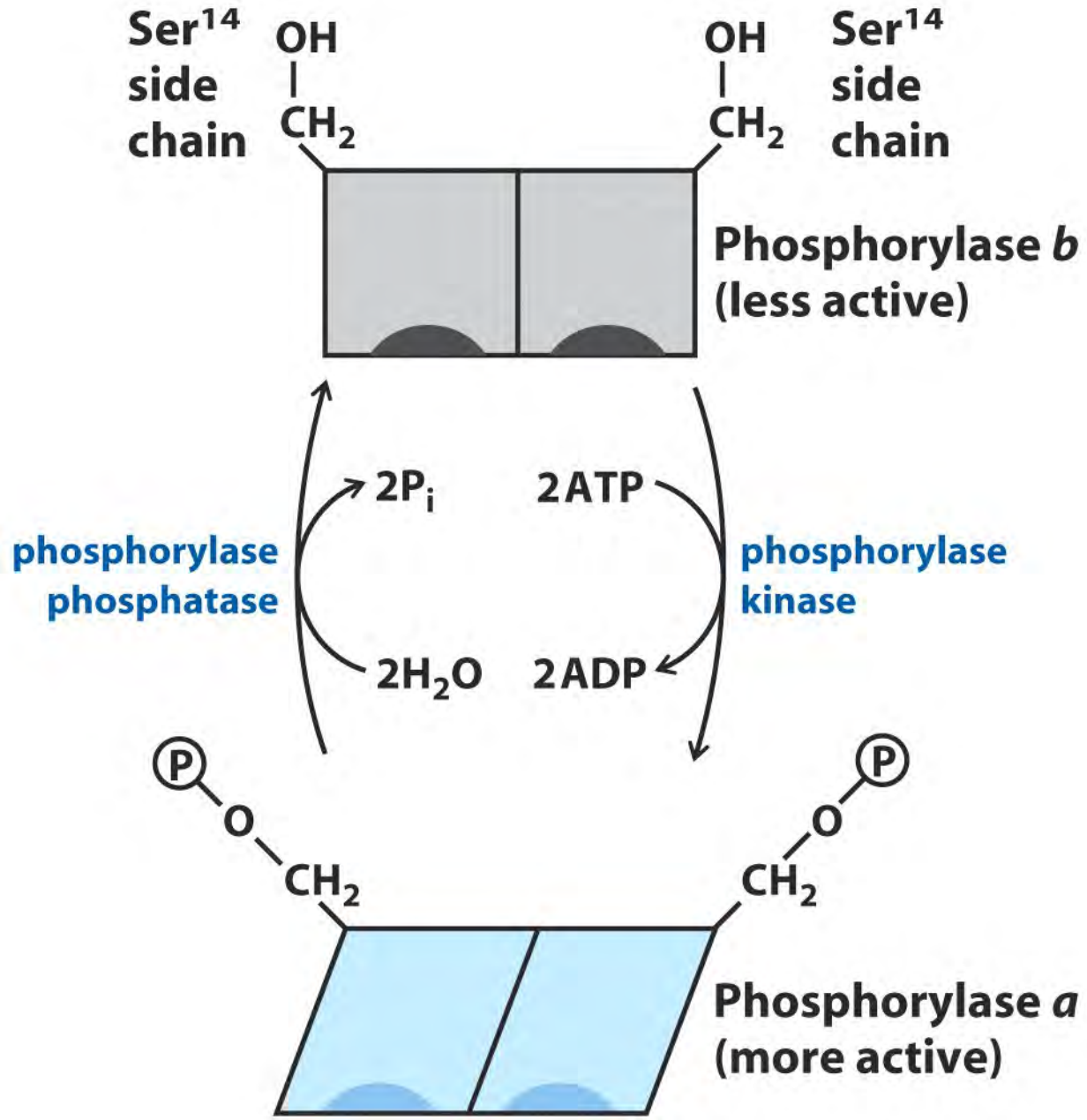
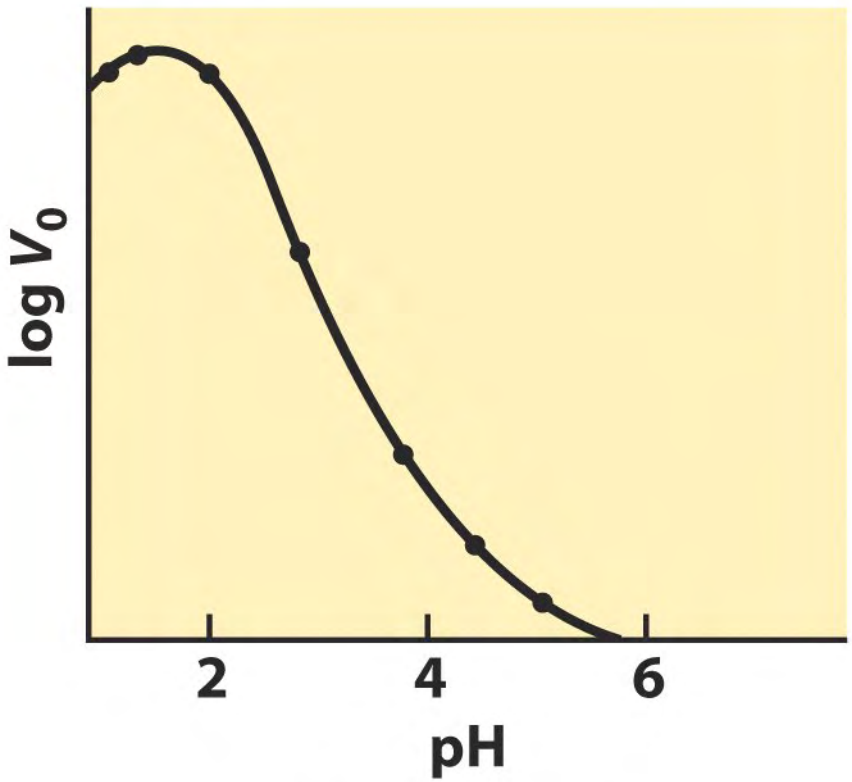
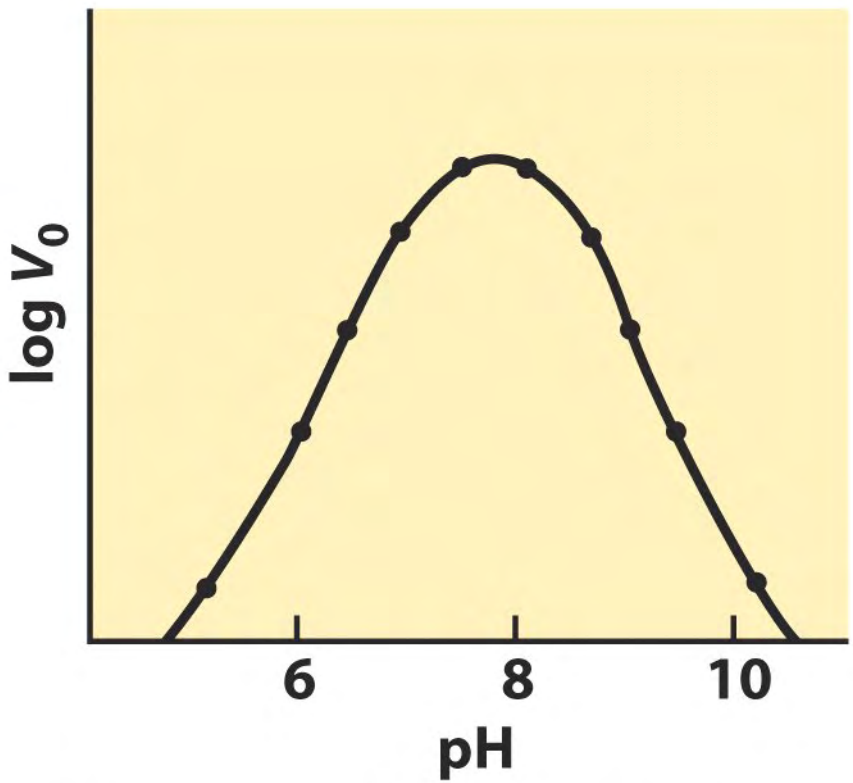

Ser14 side chain
OH
CH2
OH
CH2
Ser14 side chain
Phosphorylase b (less active)
2Pi
2ATP
phosphorylase phosphatase
phosphorylase kinase
2H2O
2ADP
P
O
CH2
P
O
CH2
Phosphorylase a (more active)

TABLE 10.1 Common covalent modifications of protein activity

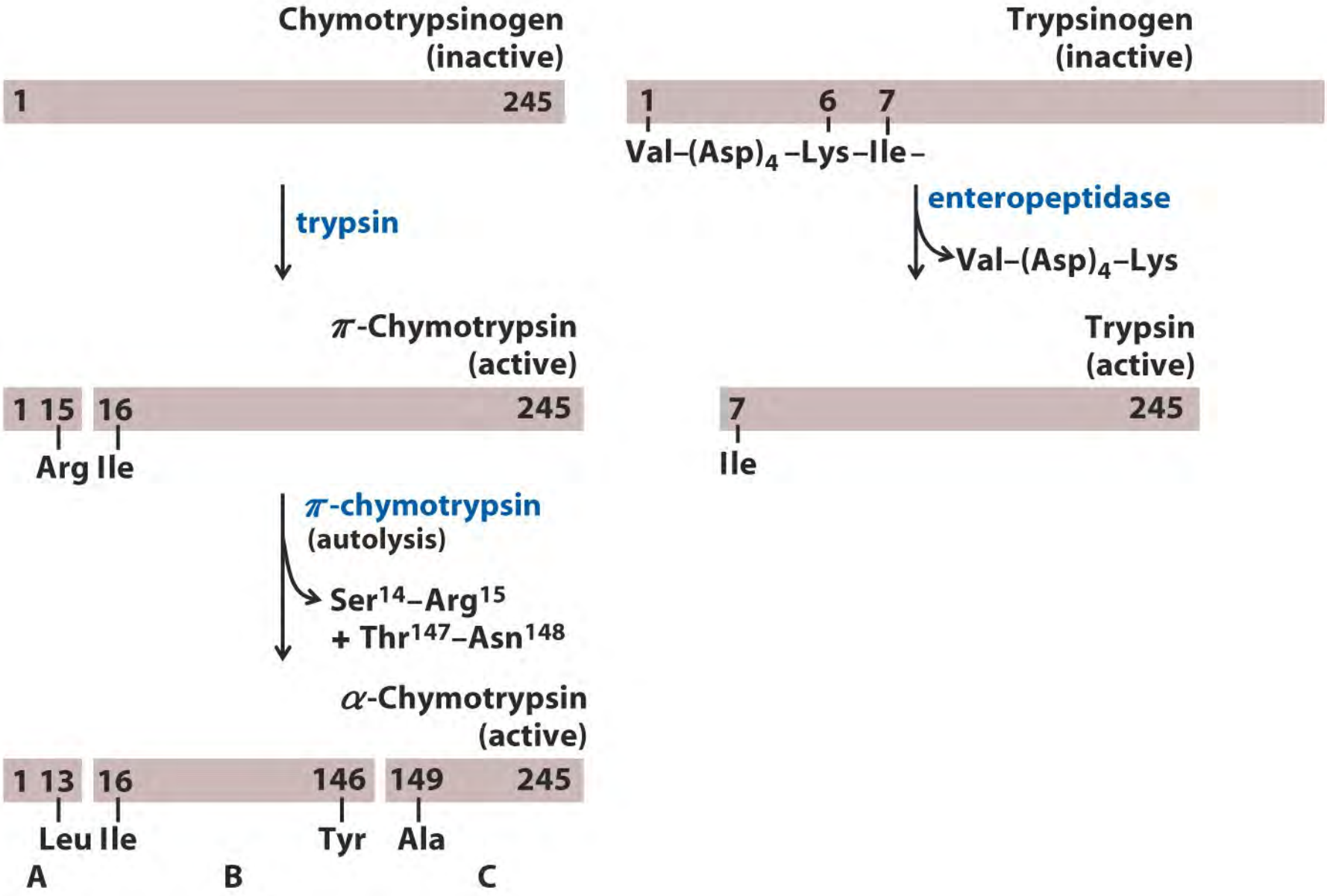
Modification	Donor molecule	Example of modified protein	Protein function
Phosphorylation	ATP	Glycogen phosphorylase	Glucose homeostasis; energy transduction
Acetylation	Acetyl CoA	Histones	DNA packing; transcription
Myristoylation	Myristoyl CoA	Src	Signal transduction
ADP-ribosylation	NAD	RNA polymerase	Transcription
Farnesylation	Farnesyl pyrophosphate	Ras	Signal transduction
γ-Carboxylation	HCO_3^-	Thrombin	Blood clotting
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate	Fibrinogen	Blood-clot formation
Ubiquitination	Ubiquitin	Cyclin	Control of cell cycle



(a) Pepsin



(b) Glucose 6-phosphatase


Chymotrypsinogen
(inactive)
1
245
trypsin
π-Chymotrypsin
(active)
1 15
16
245
Arg Ile
π-chymotrypsin
(autolysis)
Ser14–Arg15
+ Thr147–Asn148
α-Chymotrypsin
(active)
1 13
16
146
149
245
Leu Ile
Tyr
Ala
A
B
C
Trypsinogen
(inactive)
1
6
7
Val–(Asp)4–Lys–Ile–
enteropeptidase
Val–(Asp)4–Lys
Trypsin
(active)
7
245
Ile

Are You Getting It??

When an enzyme is regulated,
(multiple answers)

a) it can be inhibited by an effector.
b) it can be activated by a covalent modifier.
c) it can undergo a conformational change.
d) it can contain a regulatory subunit.
e) it can be more active at acidic pH.

Are You Getting It??

Answer

When an enzyme is regulated,

a) it can be inhibited by an effector.
b) it can be activated by a covalent modifier.
c) it can undergo a conformational change.
d) it can contain a regulatory subunit.
e) it can be more active at acidic pH.