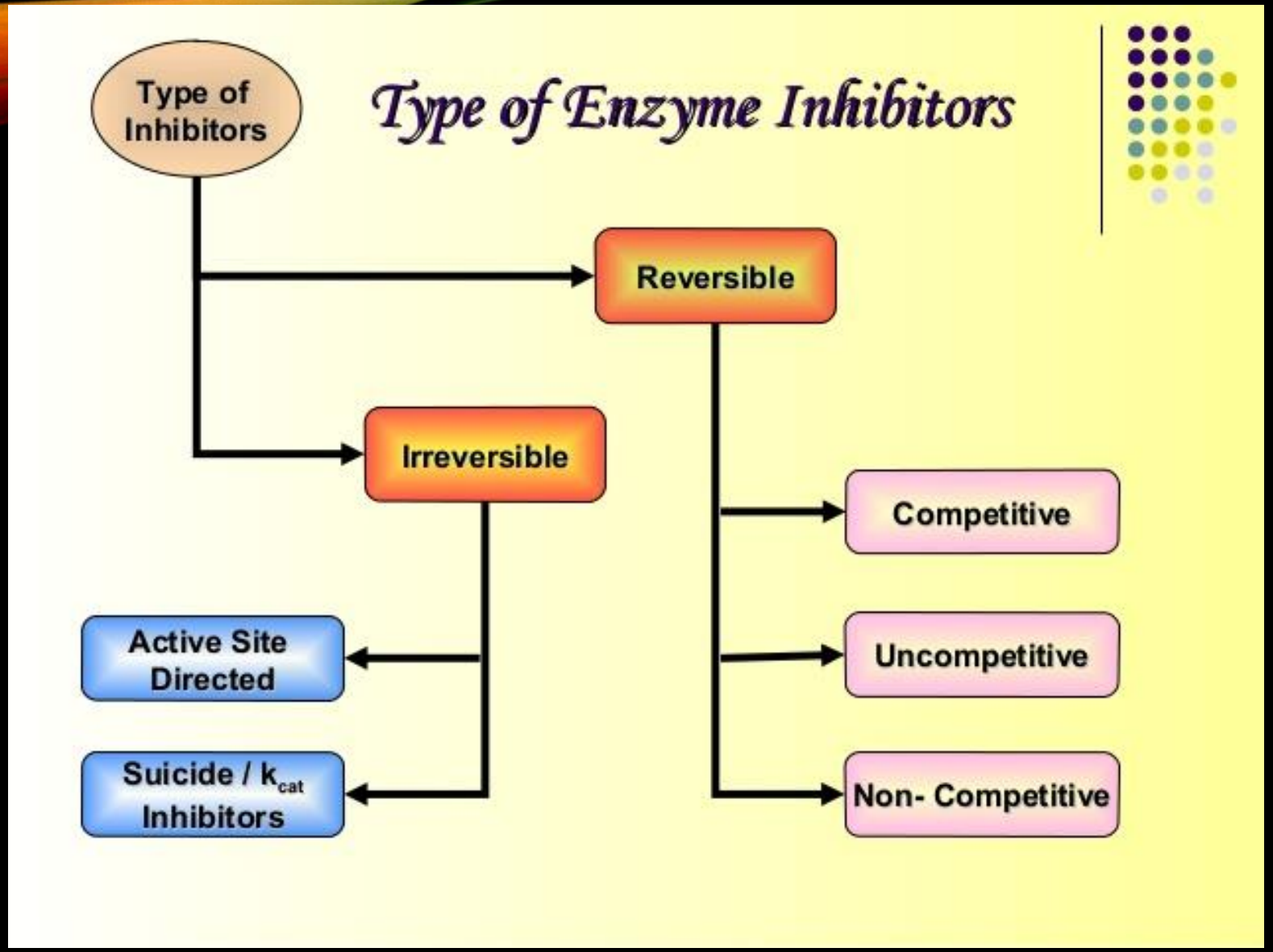
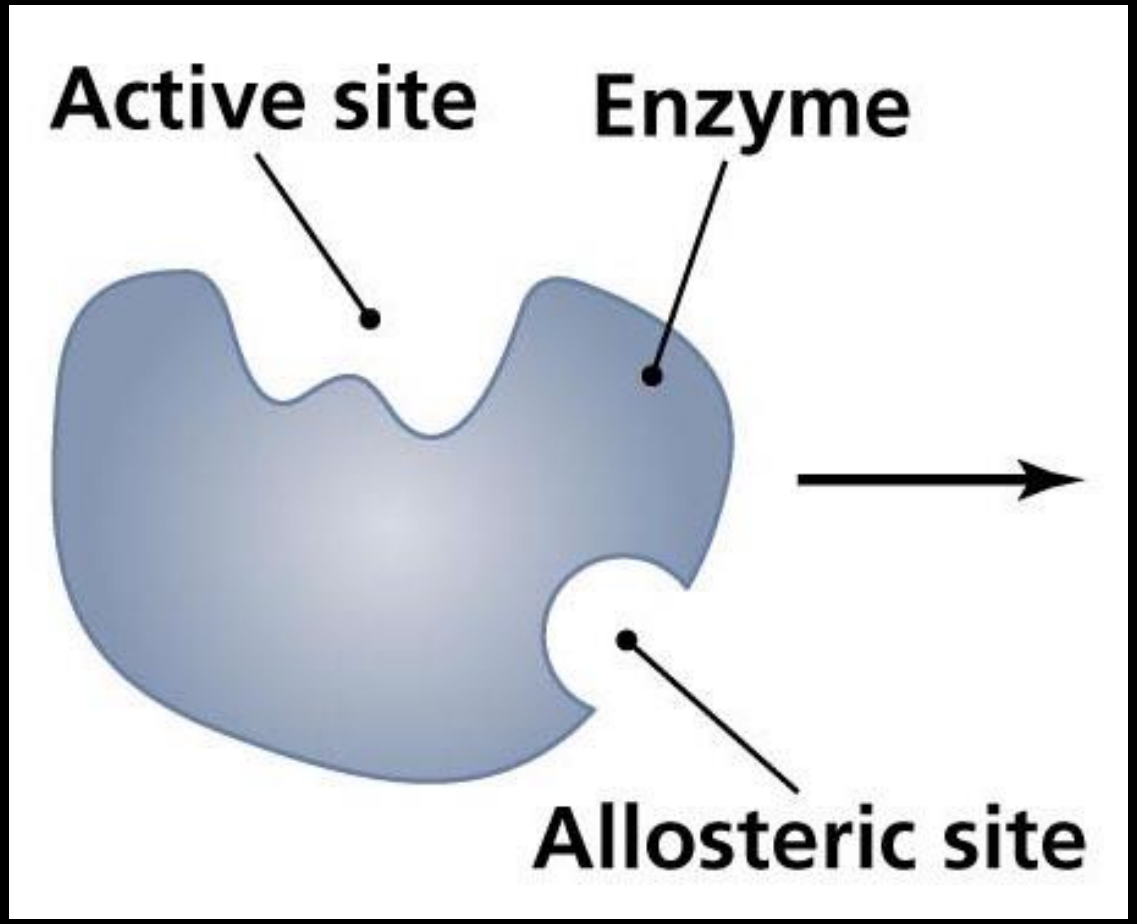
REGULATION OF ENZYME ACTIVITY

Dr Bela Goyal

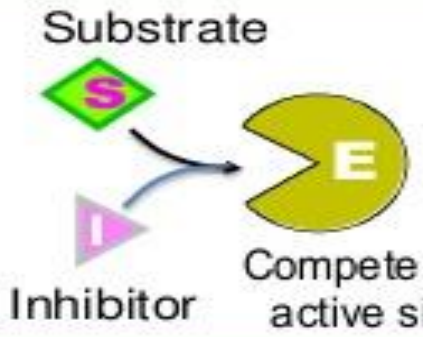
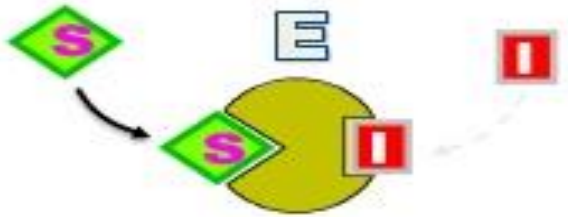
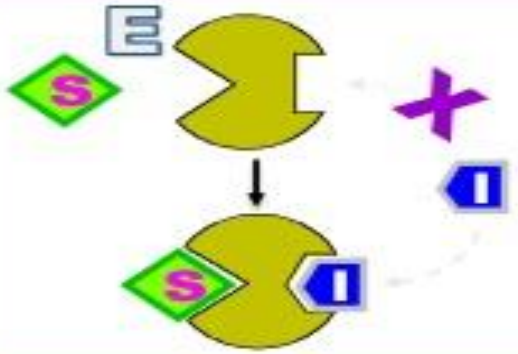


Depending on the types of bond between inhibitor and enzyme

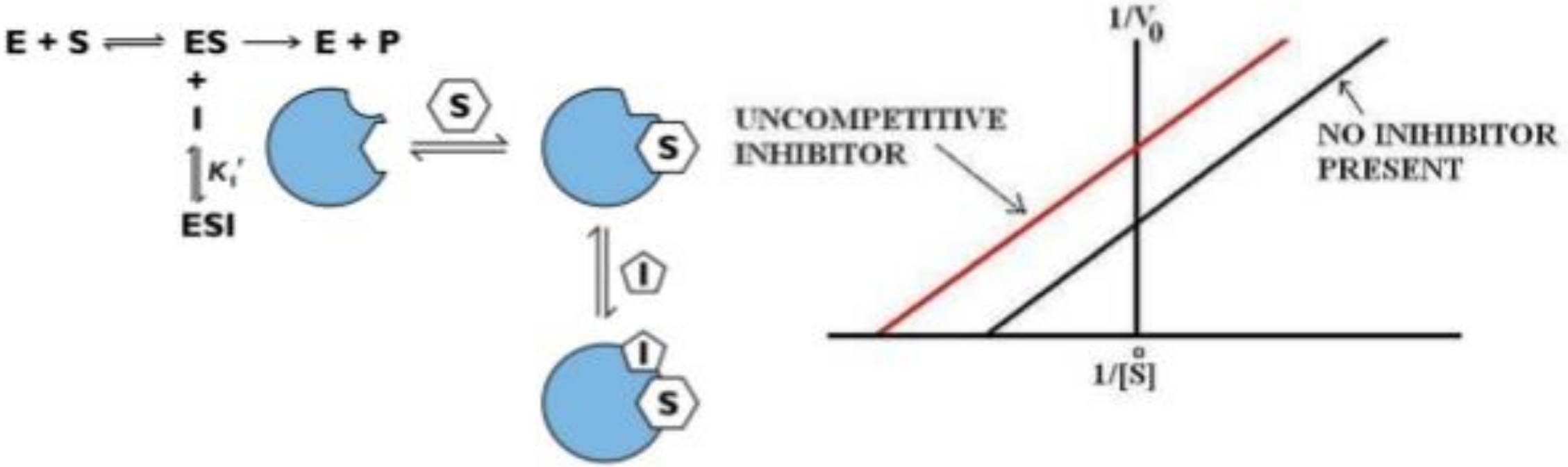
Active site vs allosteric site



Enzyme Inhibition (Mechanism)

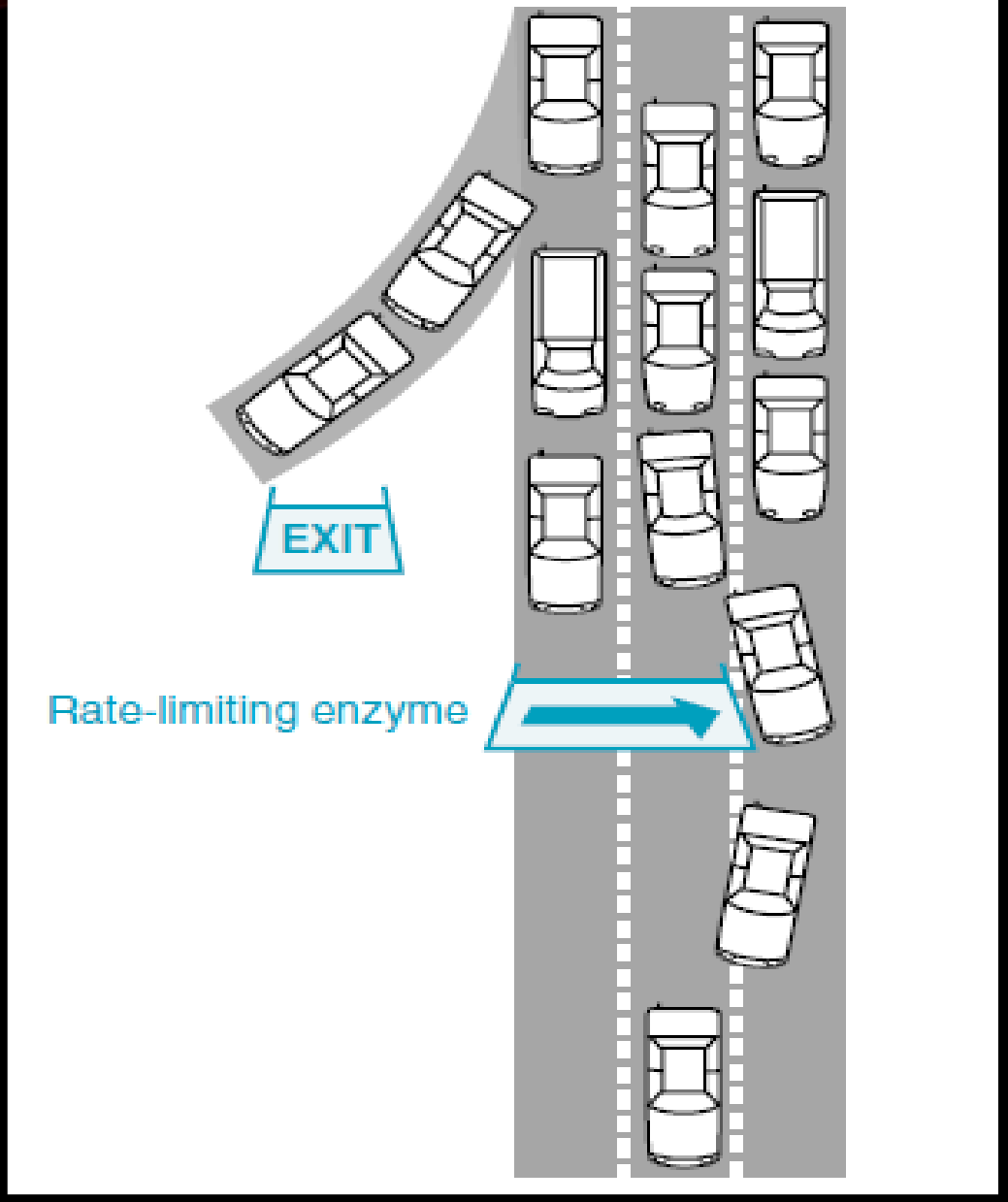
	Competitive	Non-competitive	Uncompetitive
Cartoon Guide			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Updownarrow$ EI	$E + S \rightleftarrows ES \rightarrow E + P$ $+ \quad +$ $I \quad I$ $\Updownarrow \quad \downarrow\uparrow$ $EI + S \rightarrow EIS$	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\Updownarrow$ 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*].

Uncompetitive inhibition of enzymes



Uncompetitive inhibition- inhibitors binds to ES Complex

Vmax & Km decreased eg Alkaline Phosphatase by phenylalanine


EXIT
Rate-limiting enzyme

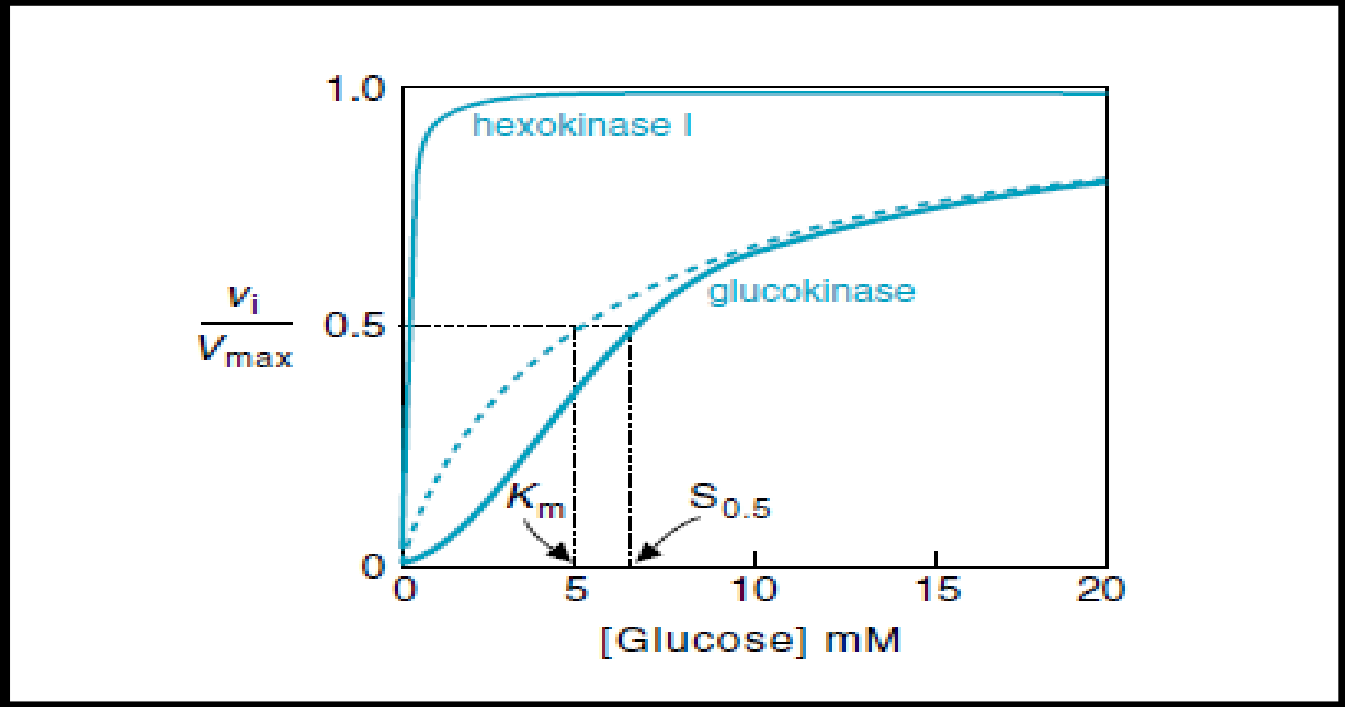
- REGULATION BY SUBSTRATE AND PRODUCT CONCENTRATION
- REGULATION THROUGH CONFORMATIONAL CHANGES
- REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME
- REGULATION OF METABOLIC PATHWAYS

REGULATION BY SUBSTRATE AND PRODUCT CONCENTRATION

- Velocity and Substrate Concentration: Michael menton equation
- Reversible Inhibition within the Active Site

SUBSTRATE CONCENTRATION

- HEXOKINASE ISOZYMES HAVE DIFFERENT Km VALUES FOR GLUCOSE



REVERSIBLE INHIBITION

- **Reversible Inhibition within the Active Site**
- COMPETITIVE INHIBITION

- NONCOMPETITIVE AND UNCOMPETITIVE INHIBITION

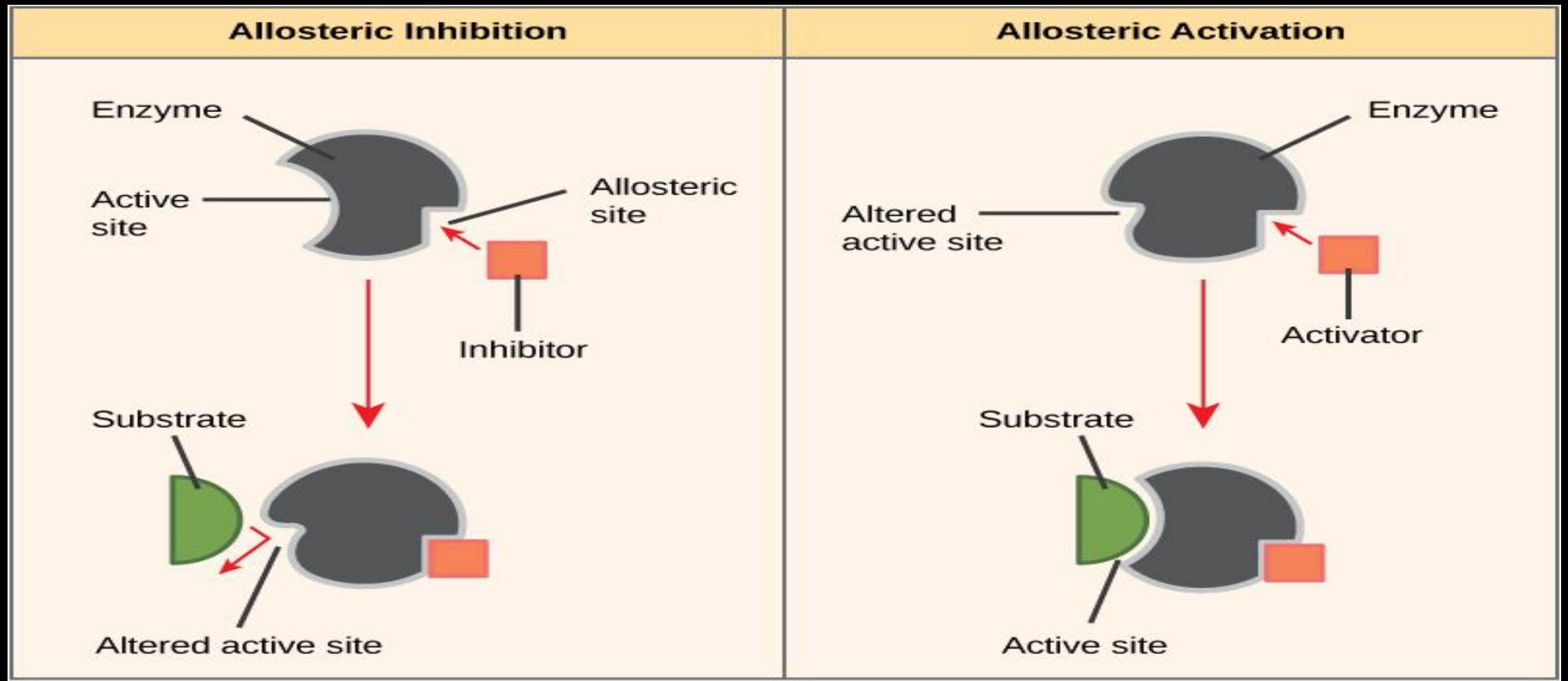
- SIMPLE PRODUCT INHIBITION IN METABOLIC PATHWAYS

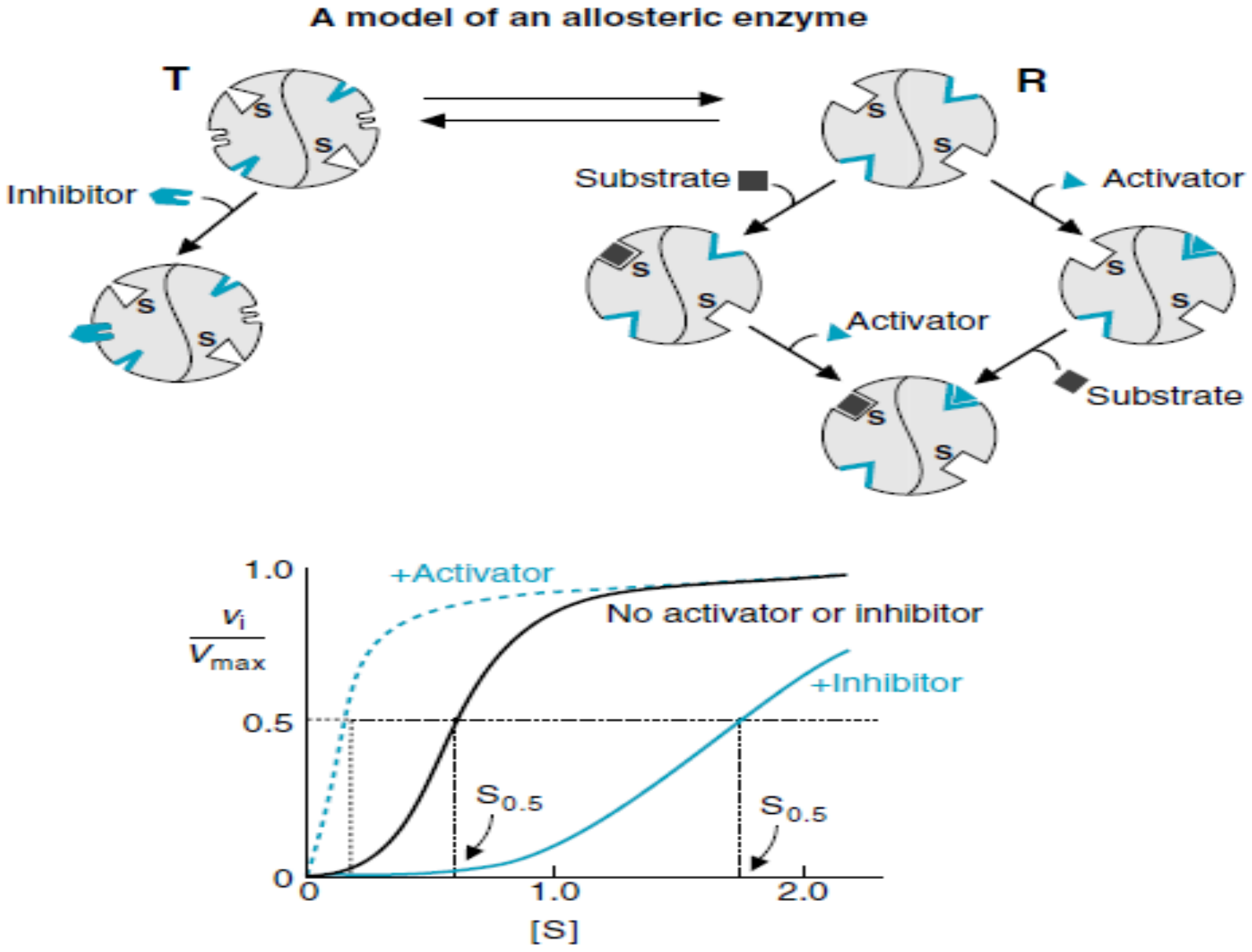
- Product inhibition of hexokinase by glucose 6-phosphate

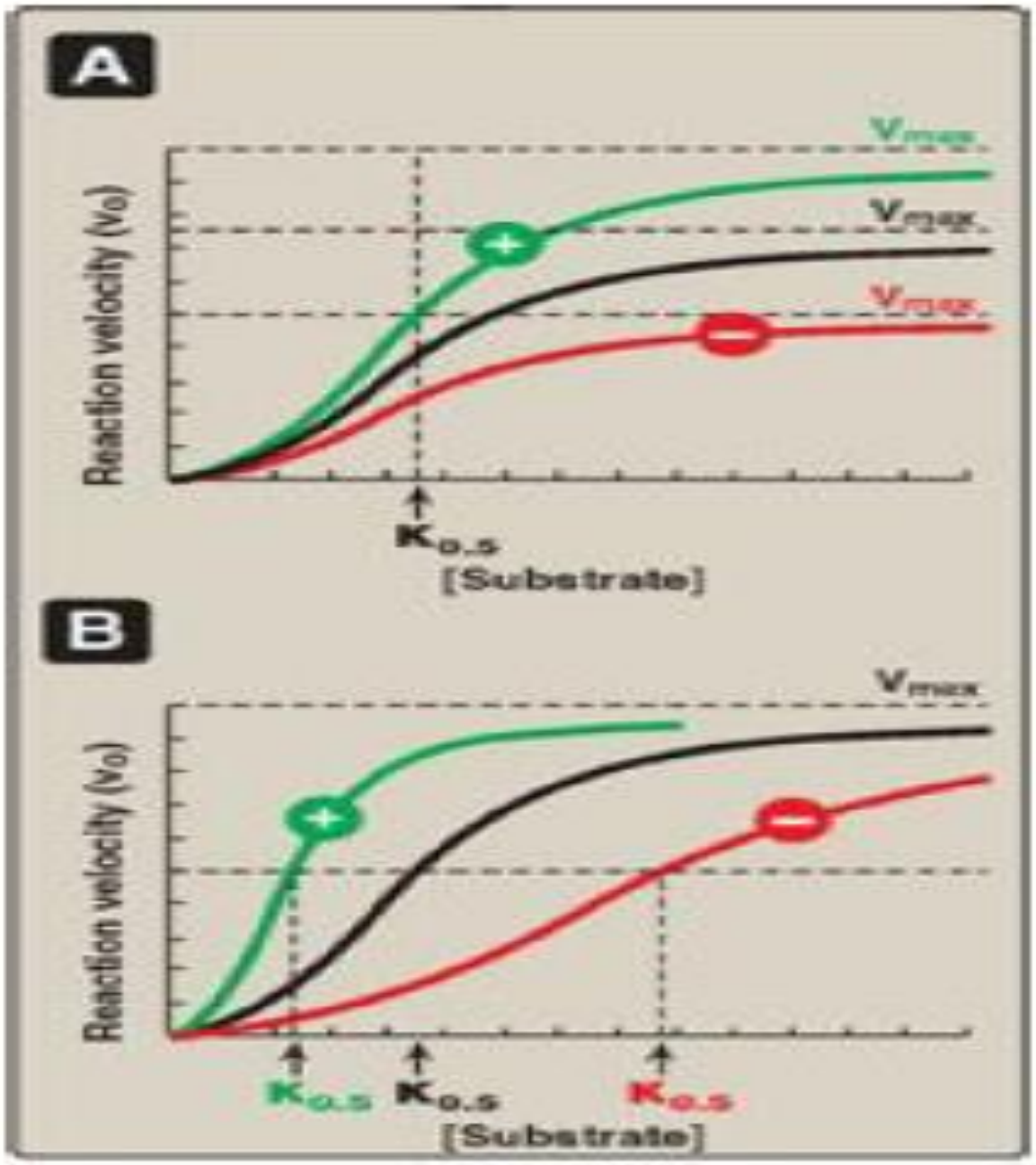
REGULATION THROUGH CONFORMATIONAL CHANGES

- A. Conformational Changes in Allosteric Enzymes
- B. Conformational Changes from Covalent Modification
- C. Proteolytic Cleavage

Allosteric enzymes




A model of an allosteric enzyme
T
R
S
Inhibitor
Substrate
Activator
Activator
Substrate
1.0
$\frac{v_i}{V_{max}}$
0.5
0
+Activator
No activator or inhibitor
+Inhibitor
$S_{0.5}$
$S_{0.5}$
0
1.0
2.0
[S]


A
Reaction velocity (v0)
Vmax
Vmax
Vmax
K0.5
[Substrate]
B
Reaction velocity (v0)
Vmax
K0.5
K0.5
K0.5
[Substrate]

CONFORMATIONAL CHANGES IN ALLOSTERIC ENZYMES

- COOPERATIVITY IN SUBSTRATE BINDING TO ALLOSTERIC ENZYMES

- ALLOSTERIC ACTIVATORS AND INHIBITORS
  - **Homotropic effectors**

  - **Heterotropic effectors**

Table 6.6: Allosteric enzymes and its modulators

Pathway	*Enzyme*	*Inhibitor*	*Activator*
Glycolysis	Phosphofructokinase-I	ATP	AMP
Pyruvate to acetyl-CoA	Pyruvate dehydrogenase	ATP	-
TCA cycle	Isocitrate dehydrogenase	ATP	ADP
Gluconeogenesis	Pyruvate carboxylase	-	Acetyl-CoA
Fatty acid synthesis	Acetyl-CoA carboxylase	-	Citrate

ALLOSTERIC ENZYMES IN METABOLIC PATHWAYS

- much stronger effect on enzyme velocity
- may function as activators
- need not bear any resemblance to substrate or product of the enzyme
- rapid

- Allosteric enzymes are regulated by molecules called effectors that bind noncovalently at a site other than the active site

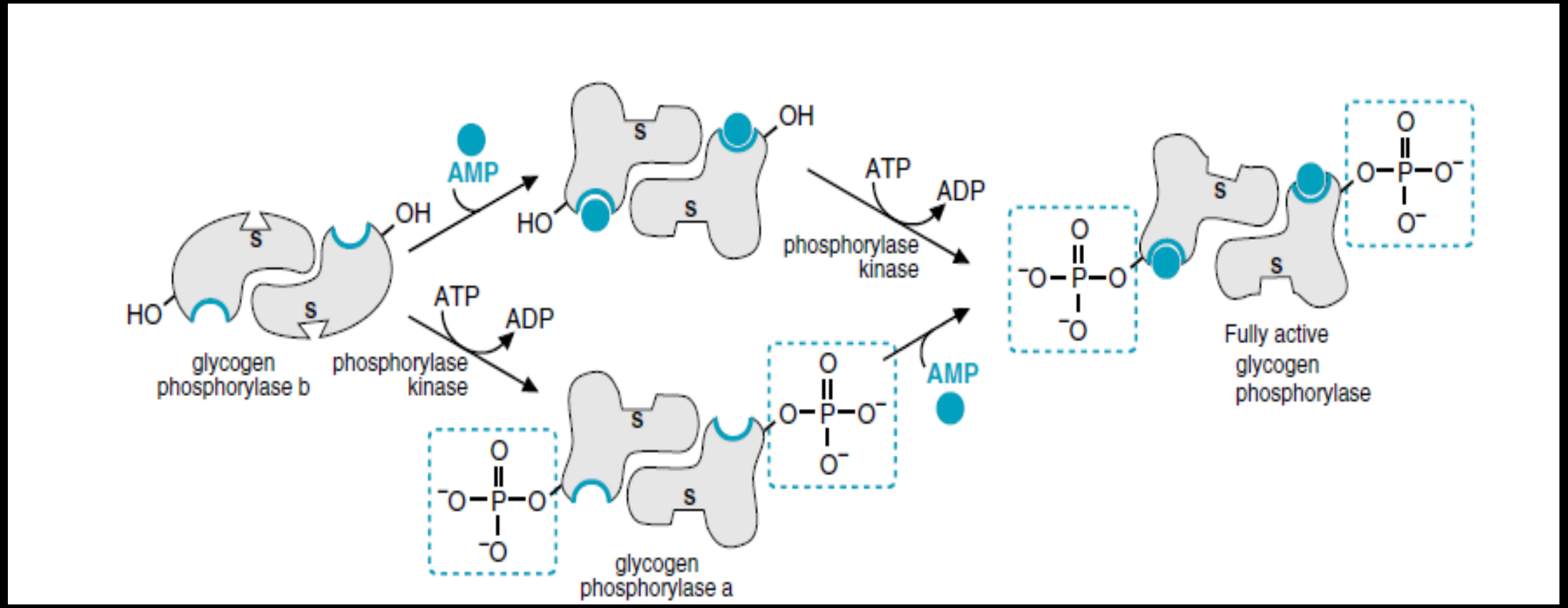
- Positive and negative effectors can affect the affinity of the enzyme for its substrate (K0.5), modify the maximal catalytic activity of the enzyme (Vmax), or both

- **Which of the following describes a characteristic of most allosteric enzymes?**

- (A) They are composed of single subunits.
- (B) In the absence of effectors, they generally follow Michaelis-Menten kinetics.
- (C) They show cooperativity in substrate binding.
- (D) They have allosteric activators that bind in the catalytic site.
- (E) They have irreversible allosteric inhibitors that bind at allosteric sites.

COVALENT MODIFICATION

- PHOSPHORYLATION



ZYMOGEN CLEAVAGE

- Proteases
- Blood clotting factors

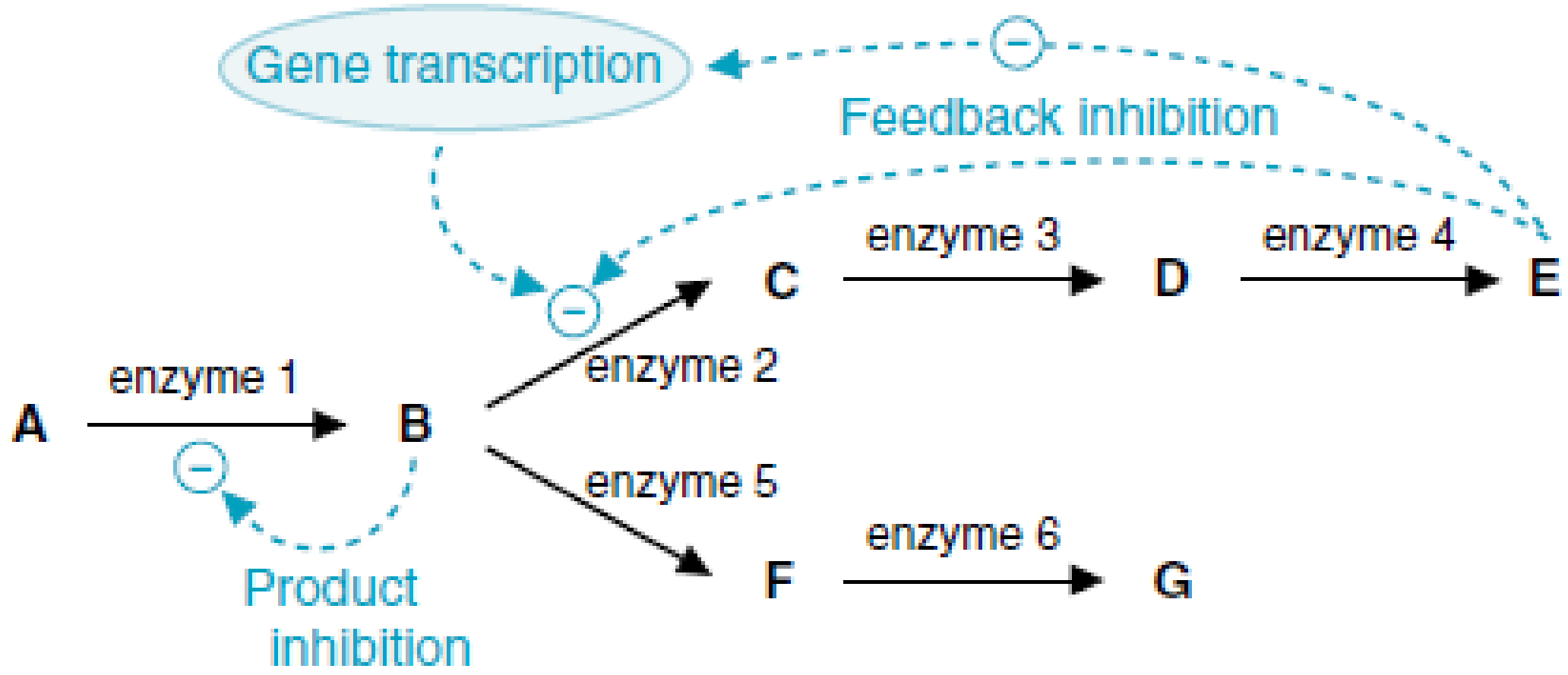
REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

- Regulated Enzyme Synthesis
- Regulated Protein Degradation

REGULATION OF METABOLIC PATHWAYS

- REGULATION OCCURS AT THE RATE-LIMITING STEP
- FEEDBACK REGULATION
- FEED-FORWARD REGULATION
- TISSUE ISOZYMES OF REGULATORY PROTEINS
- COUNTER-REGULATION OF OPPOSING PATHWAYS
- SUBSTRATE CHANNELING THROUGH COMPARTMENTATION

FEED BACK REGULATION



- A rate-limiting enzyme catalyzes the first step in the conversion of a toxic metabolite to a urinary excretion product. Which of the following mechanisms for regulating this enzyme would provide the most protection to the body?

(A) The product of the pathway should be an allosteric inhibitor of the rate-limiting enzyme.

(B) The product of the pathway should act through gene transcription to decrease synthesis of the enzyme.

(C) The toxin should act through gene transcription to increase synthesis of the enzyme.

(D) The product of the first enzyme should allosterically activate the subsequent enzyme in the pathway

REGULATOR EVENT	TYPICAL EFFECTOR	RESULTS	TIME REQUIRED FOR CHANGE
Substrate availability	Substrate	Change in velocity (v_0)	Immediate
Product inhibition	Reaction product	Change in V_{max} and/or K_m	Immediate
Allosteric control	Pathway end product	Change in V_{max} and/or $K_{0.5}$	Immediate
Covalent modification	Another enzyme	Change in V_{max} and/or K_m	Immediate to minutes
Synthesis or degradation of enzyme	Hormone or metabolite	Change in the amount of enzyme	Hours to days