Reaction Kinetics

- Simple rate equations describe the progress of first-order and second-order reactions.
- The Michaelis–Menten equation relates the initial velocity of a reaction to the maximal reaction velocity and the Michaelis constant for a particular enzyme and substrate.
- An enzyme's overall catalytic efficiency is expressed as k_{cat}/K_M.
- A Lineweaver–Burk plot can be used to present kinetic data and to calculate values for K_M and V_{max}.
- Bisubstrate reactions can occur by an Ordered or Random sequential mechanism or by a Ping Pong mechanism.

Reaction Kinetics

- Enzyme kinetics: Study of the rates of enzyme- catalyzed reaction
- What are the uses of studying kinetics?

What we will cover:

1. Kinetics
2. Inhibition
3. Regulation

Plot of First-Order Rate Equation

$$A \longrightarrow P$$

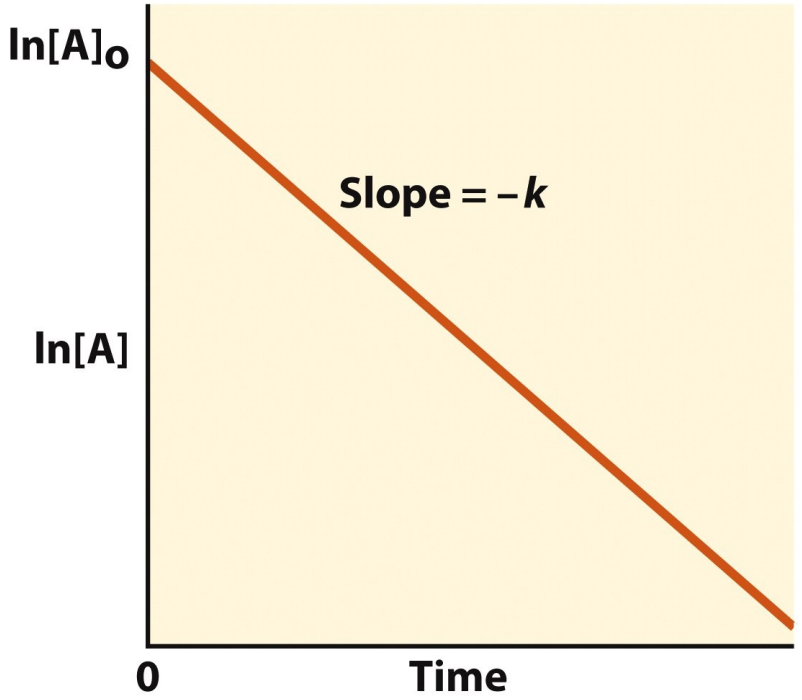
The rate V is
1. quantities of A to disappear in a specified unit of time or
2. quantities of P to appear in a specified unit of time

$$V = -\Delta A/\Delta T = \Delta P/\Delta T$$

The rate of the reaction is directly related to the concentration of A by a proportionality constant, k.
k = rate constant

$$V = k[A]$$

Reactions that are directly proportional to the reactant concentration are ***first-order reactions***.



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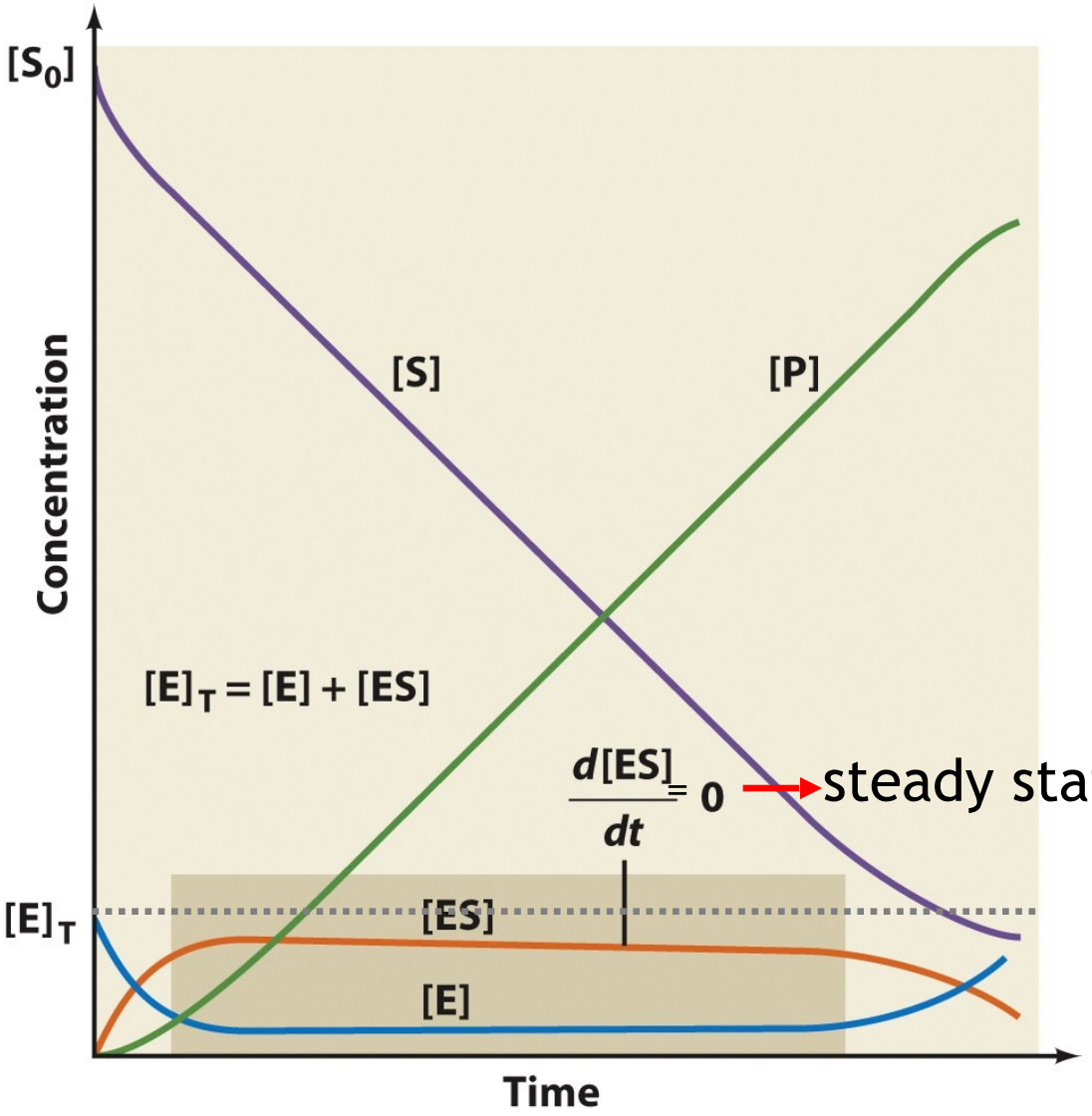
When 2A ⟶ P or A + B ⟶ P : bimolecular reaction.

- pseudo 1st order reaction
- zero order reaction

Progress Curve: Simple Enzyme-Catalyzed Reaction



[S]: concentration of substrate
[P]: concentration of product
[E]: concentration of enzyme
[ES]: enzyme bound to substrate
$[E]_T$: concentration of total enzyme

→ steady state assumption

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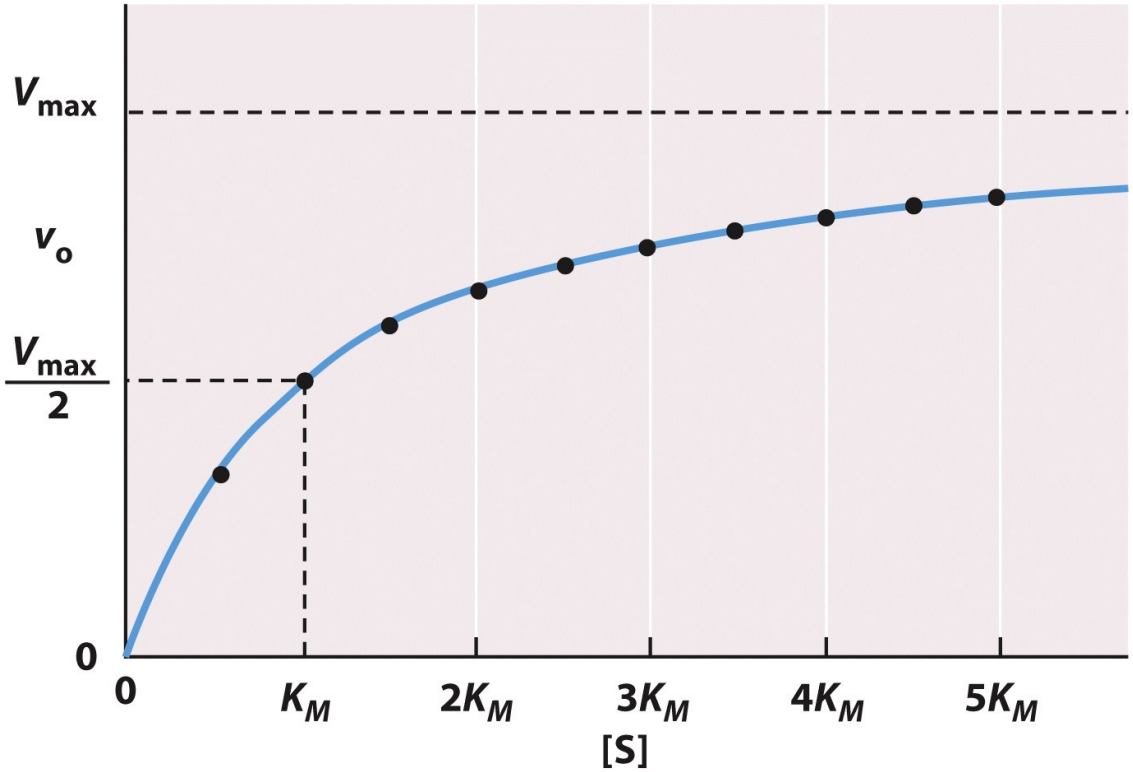
Michaelis-Menten Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Three assumptions

1. ES is a necessary intermediate step
2. k_{-2} is negligible due to small [P]
3. Steady state: [ES] is a constant independent on [S] or [P]



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M-M equation

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

2. Introduce $[E]_T$
   $[E] = [E]_T - [ES]$

3. Introduce $V_0 = k_2[ES]$

4. Find V_{max} when $[ES] = [E]_T$

Meaning of K_M

The physiological consequence of K_M in alcohol sensitivity

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

acetaldehyde

$$CH_3CHO + NAD^+ + H_2O \xrightleftharpoons{\text{aldehyde dehydrogenase}} CH_3COO^- + NADH + 2H^+$$

acetaldehyde

There are two forms of the AD: a low K_M mitochondrial form and a high K_M cytoplasmic form. What happen to the susceptible people?

Double-Reciprocal (Lineweaver-Burk) Plot

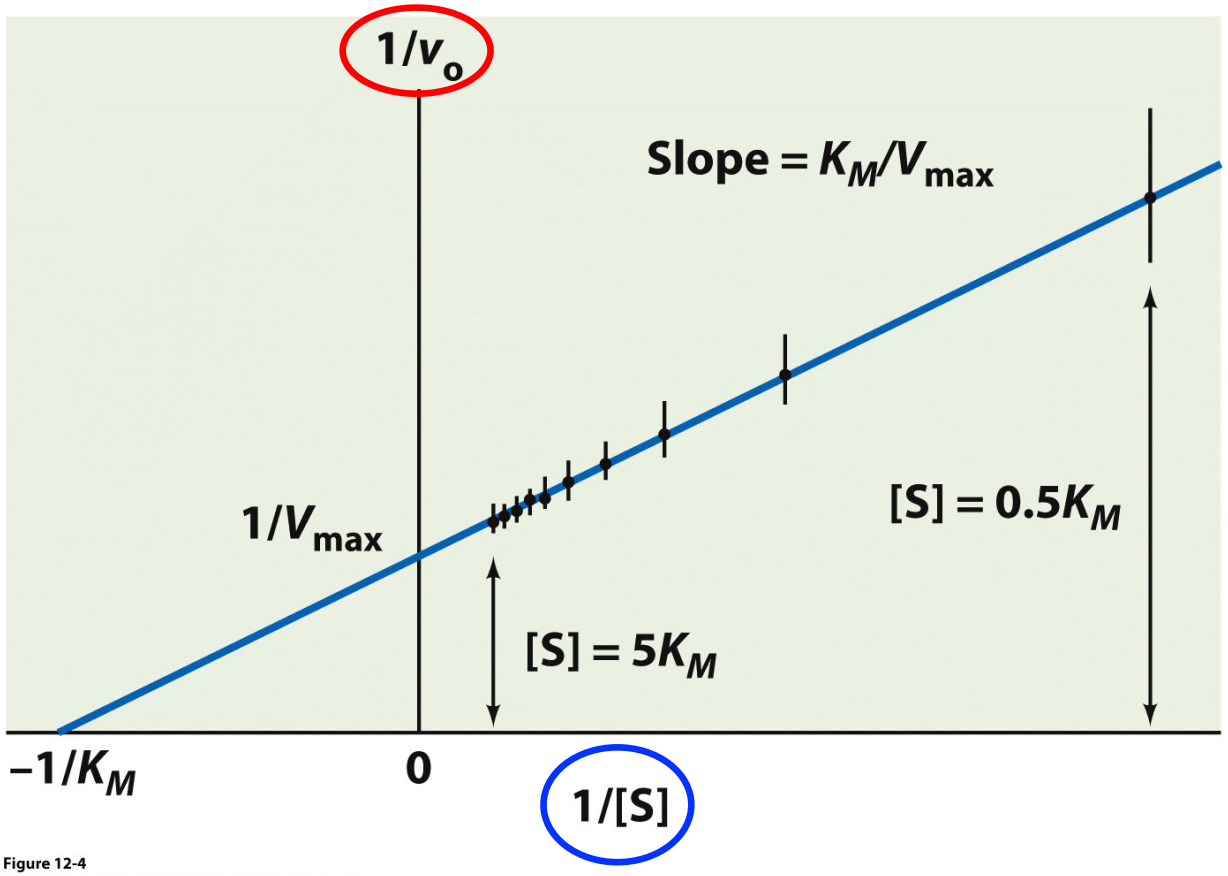

Figure 12-4

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double-reciprocal of MM equation

$V_0 = V_{max}\,[S]/([S] + K_M)$

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

Enzyme Kinetic Parameters

a measure of catalytic efficiency

TABLE 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

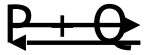
Donald Voet&Judith G. Voet&Charlotte W. Pratt (2016). Fundamentals of biochemistry, binder ready version life at the molecular level Wiley pp.368 TABLE12-1 https://www.amazon.co.jp/Fundamentals-Biochemistry-Binder-Ready-Version/dp/1118918436/ref=dp_ob_title_bk 2019/6/10

Vmax: The maximal rate reveals the **turnover number** of an enzyme which is the number of substrate molecules converted into product by **an** enzyme molecule in a unit time when the enzyme is fully saturated with substrate = k_{cat} (= k_2 when the V is maximum)

$$V_{max} = k_{cat}[E]_T$$

Q: a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when the enzyme is fully saturated with substrate. What is the k_{cat}?

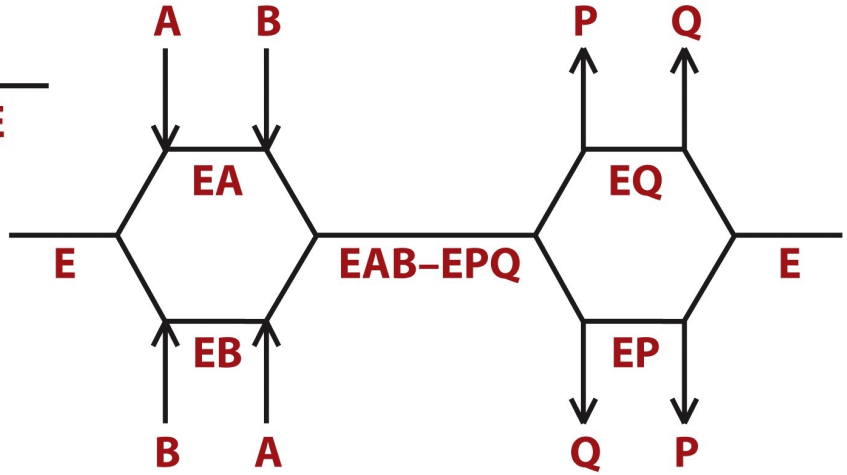
Bisubstrate Reactions

Most biological reactions: A + B  P + Q
(1) sequential (ordered and random) and (2) ping-pong reaction

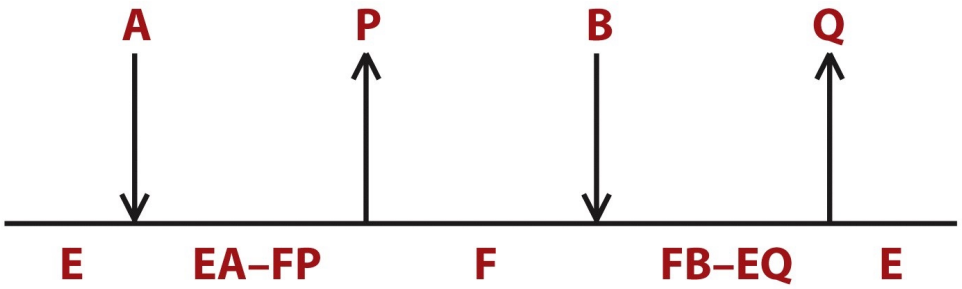
Many enzymes use NAD^+ or NADH
ex) lactate dehydrogenase

random

ex) creatine kinase



ping pong



: Group-transfer reactions in which one or more products are released before all substrates have been added.
F: enzyme temporarily modified with a functional group
ex: aspartate aminotrasferase

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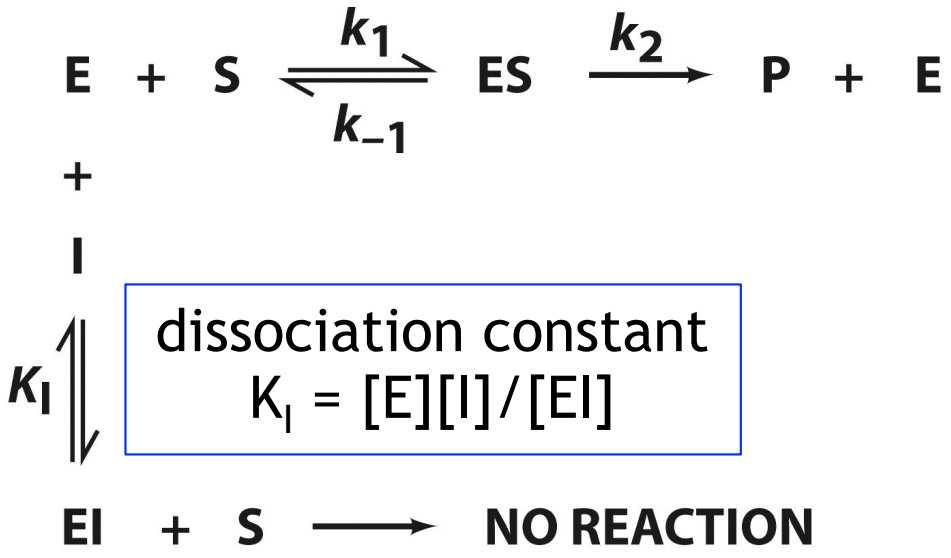
Enzyme Inhibition

- Enzyme inhibition is a major control mechanism in biological systems.
- Many drugs and toxic agents act as inhibitors (transition state inhibitors!).
- Enzyme inhibitors interact reversibly or irreversibly (ex: Penicillin and Aspirin) with an enzyme to alter its K_M and/or V_{max} values. Among reversible inhibitors belong competitive and uncompetitive inhibitors.
- **A competitive inhibitor** binds to the enzyme's active site and increases the apparent K_M for the reaction.
- **An uncompetitive inhibitor** binds to ES complex and affects catalytic activity such that both the apparent K_M and the apparent V_{max} decrease.
- **A noncompetitive inhibitor or mixed inhibitor** can bind to free enzymes and reduce the number of available (functional) enzyme: decrease the turnover number. They alters both catalytic activity and substrate binding such that the apparent V_{max} decreases and

Competitive Enzyme Inhibition

- Inhibitors mimic the substrate: compete for the same site.
- The inhibition can be overcome by adding more substrate.
- Often act as drugs: e.g. ibuprofen, Statins



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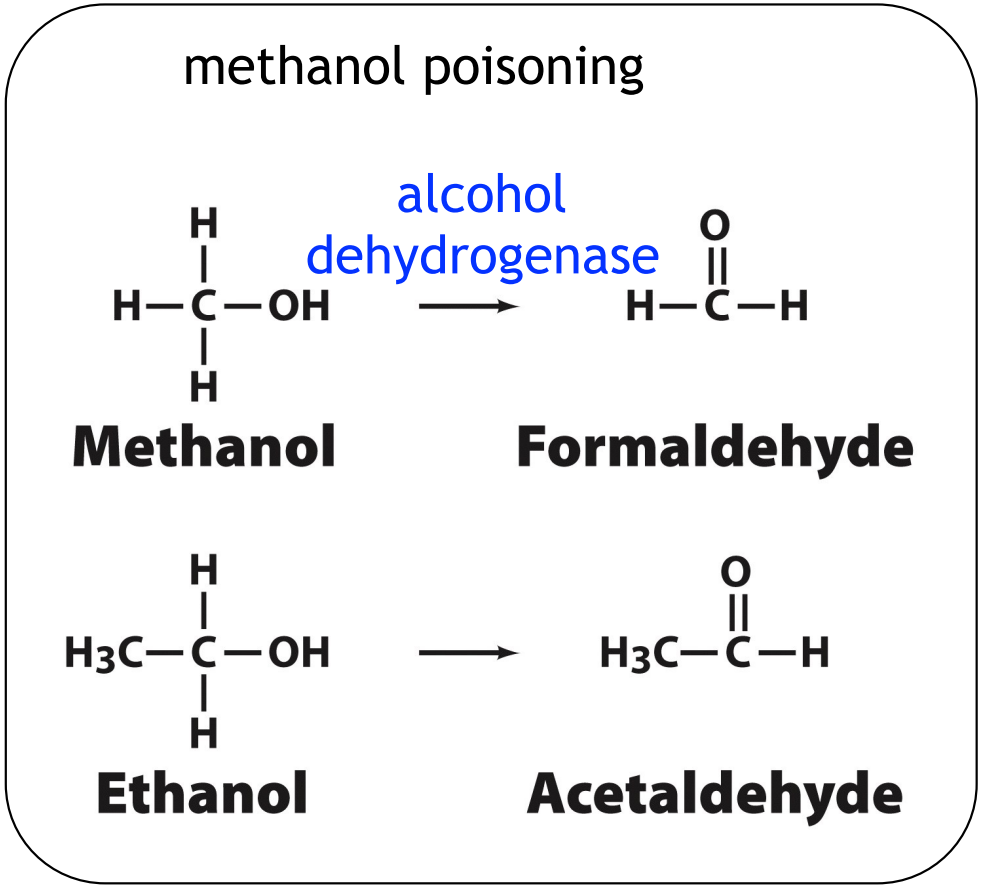
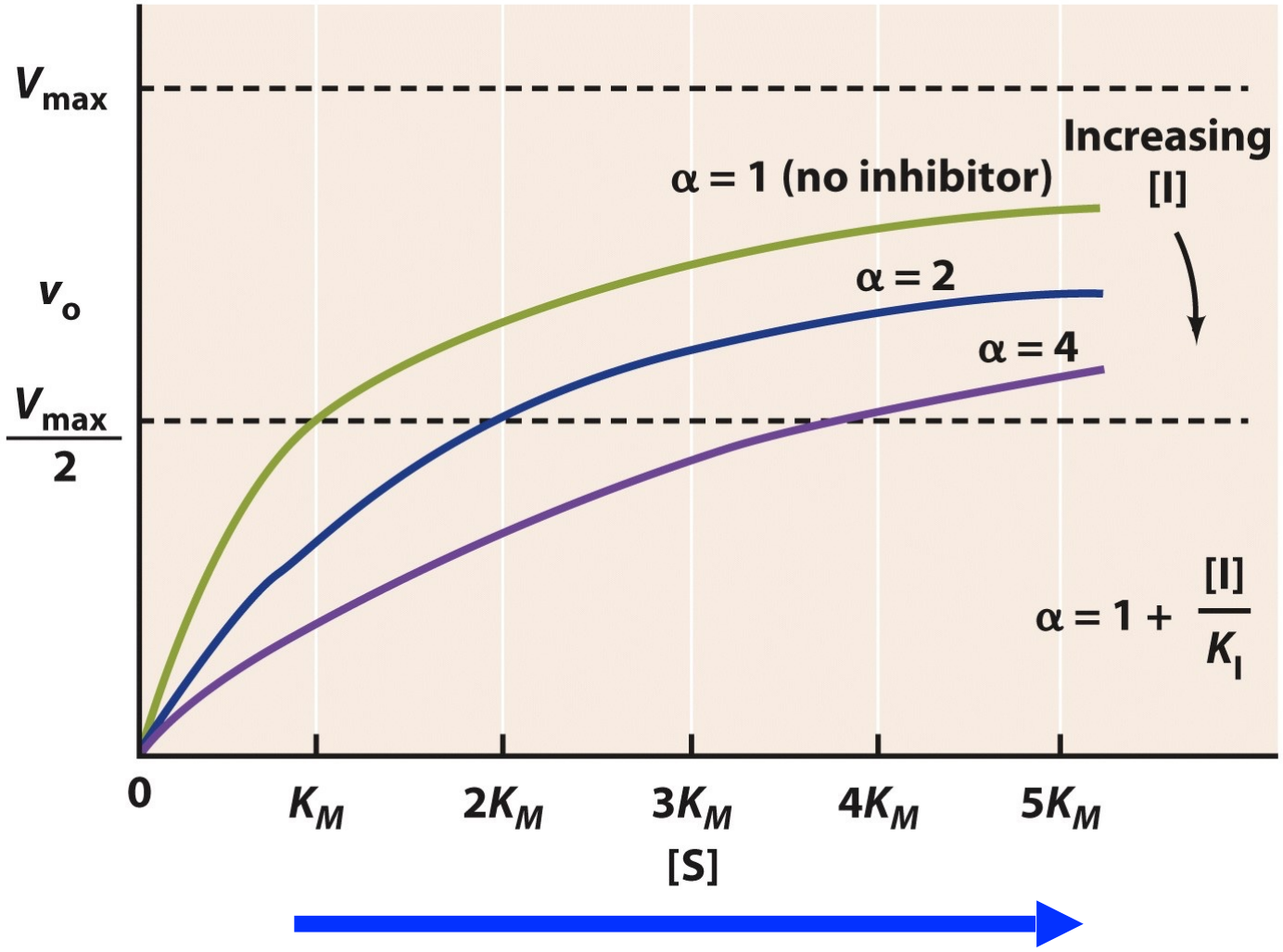


Competitive Enzyme Inhibition



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more inhibitors → increases K_M

More substrate is needed to obtain the same reaction rate.

The apparent $K_M = K_M(1+[I]/K_I)$

Competitive Enzyme Inhibition

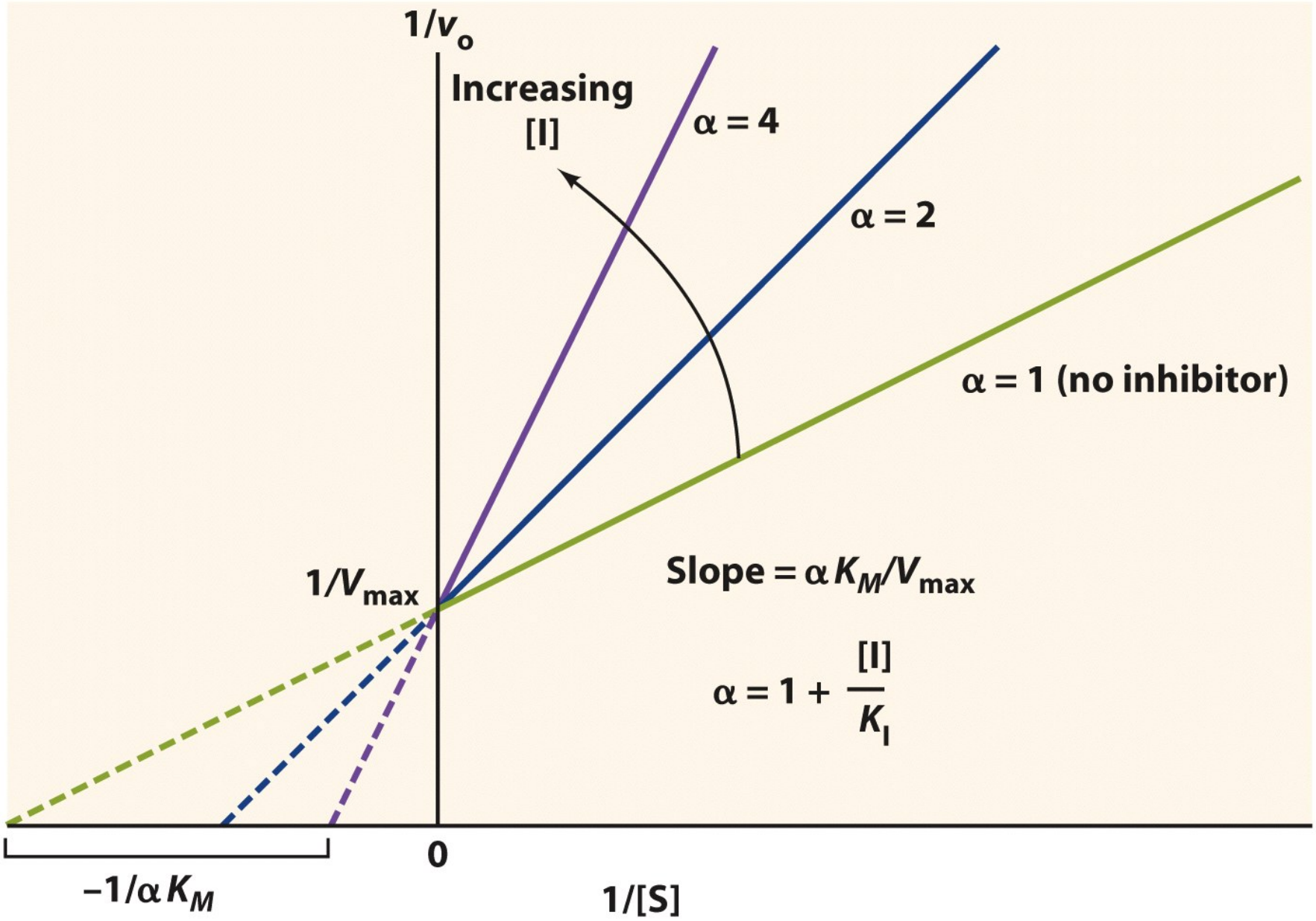

Figure 12-8

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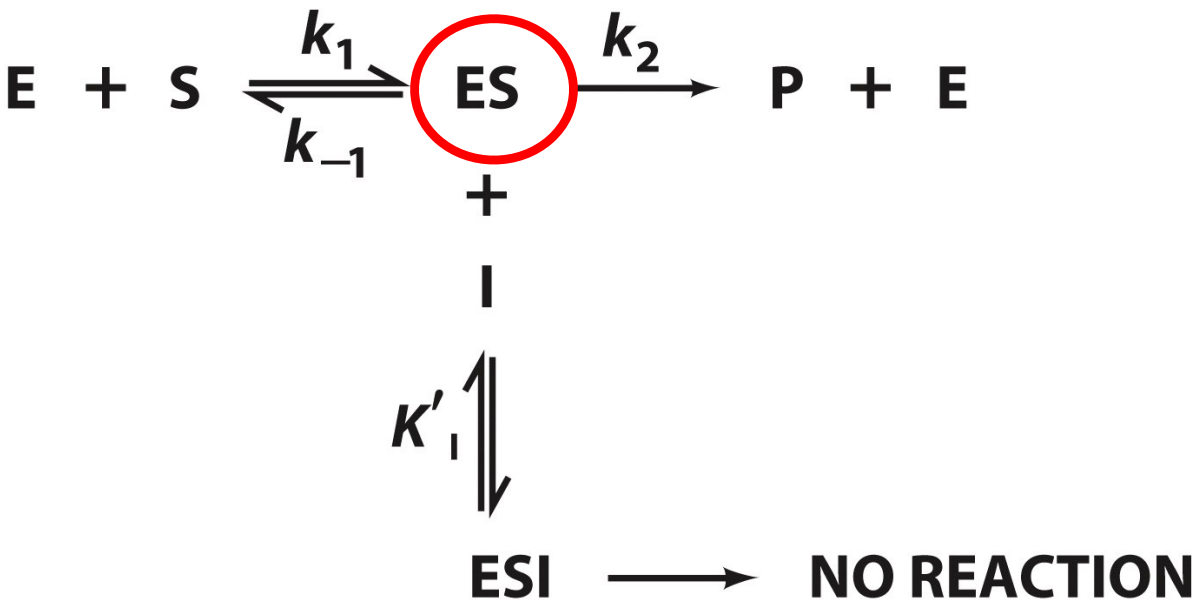
Uncompetitive Enzyme Inhibition



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ESI: Enzyme-Substrate-Inhibitor complex: a certain portion of ESI always exists, thus decreases V_{max} – as if some enzymes are kidnapped in ES form! What would happen to K_M?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$K_M = (k_{-1} + k_2)/k_1$$

Uncompetitive Enzyme Inhibition

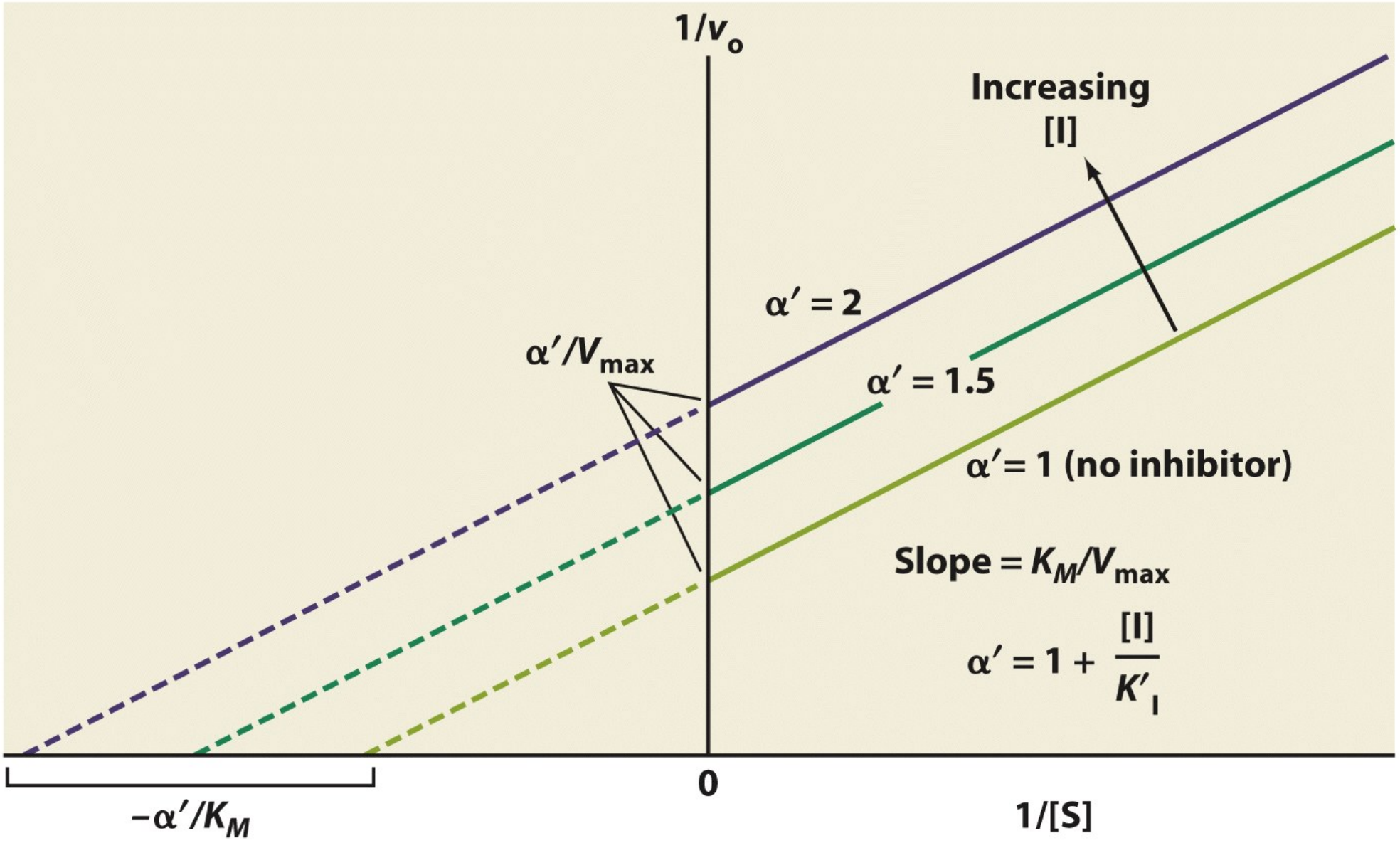

Figure 12-9

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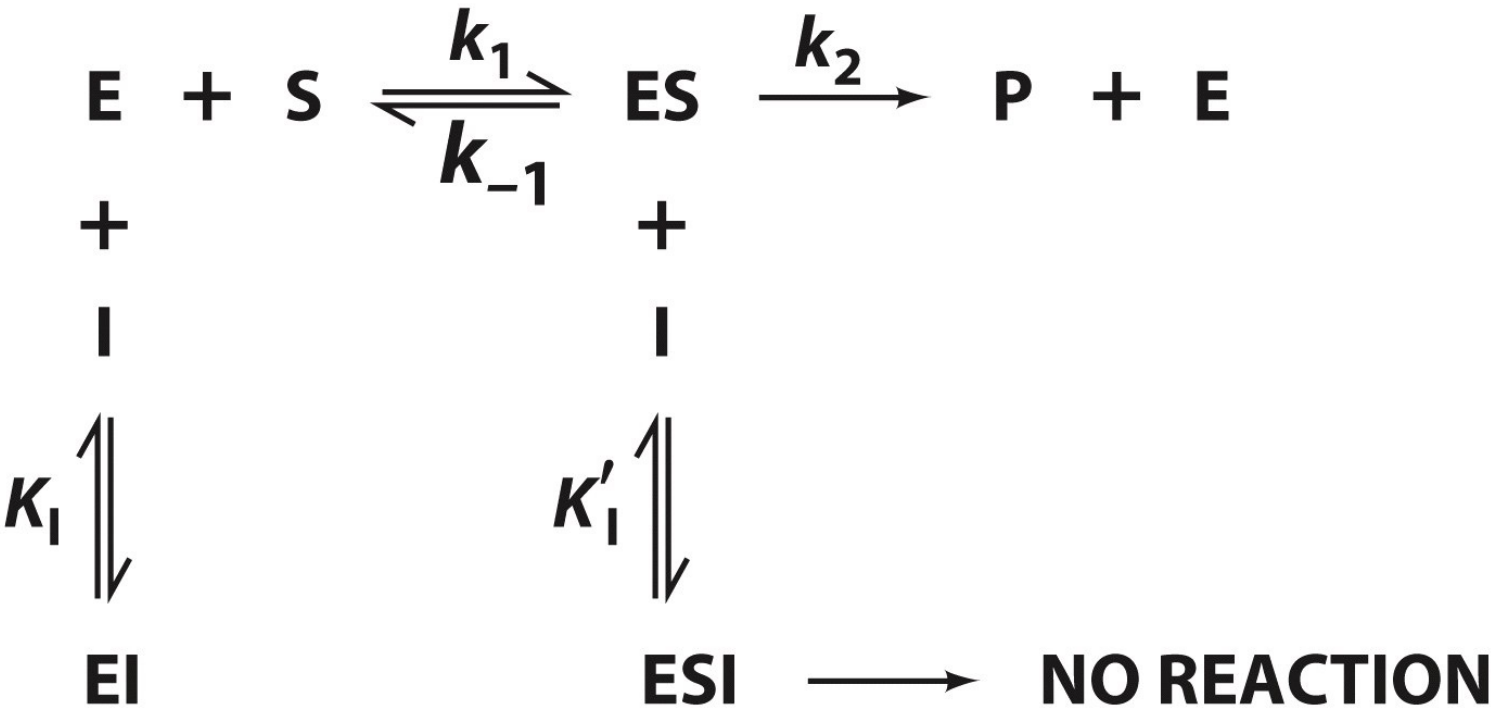
Mixed and Noncompetitive Enzyme Inhibition



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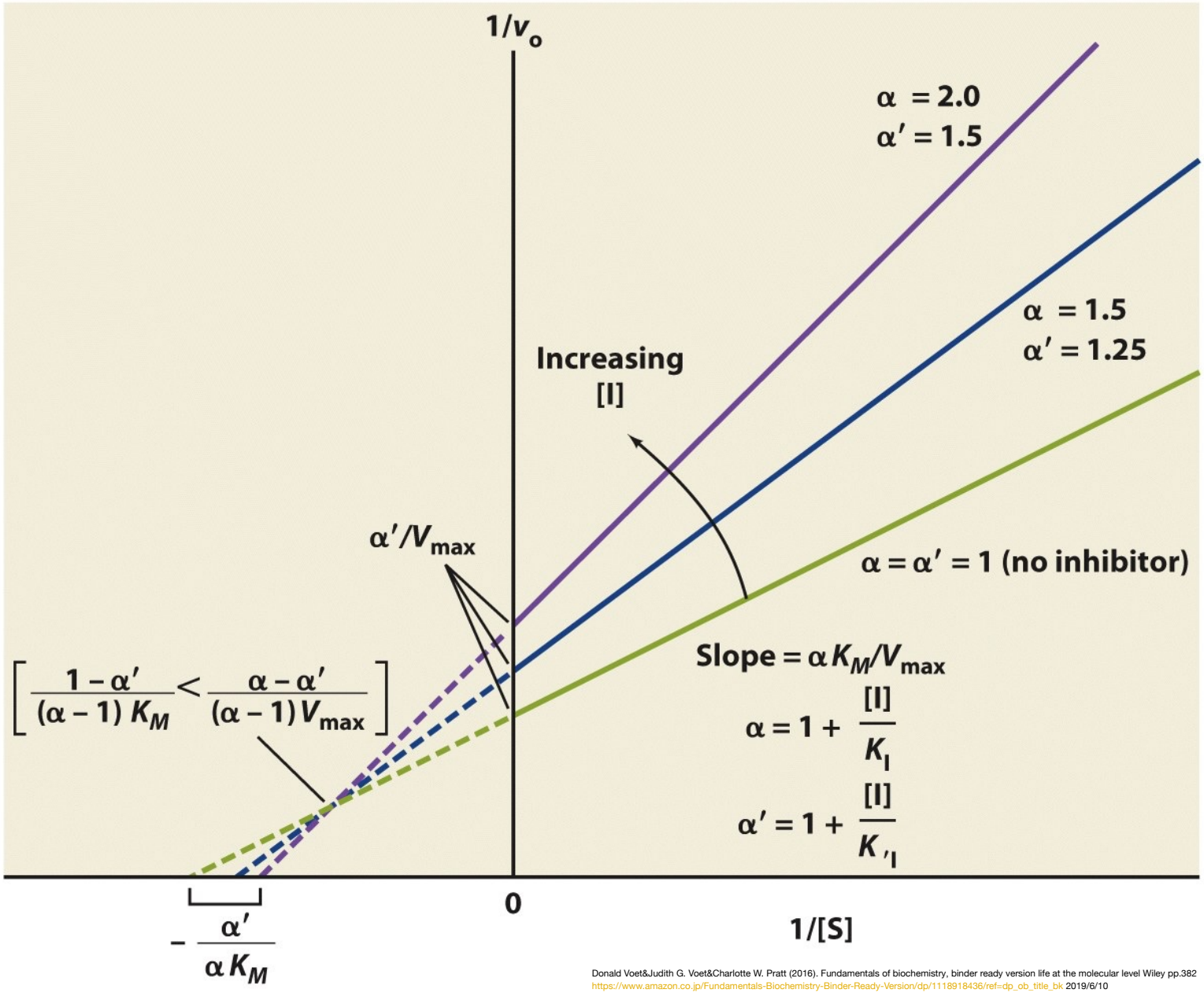
Mixed and Noncompetitive Enzyme Inhibition



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Enzyme Inhibitor Effects

TABLE **12-2** Effects of Inhibitors on Michaelis–Menten Reactions[a]

Type of Inhibition	Michaelis–Menten Equation	Lineweaver–Burk Equation	Effect of Inhibitor
None	$v_o = \frac{V_{max}[S]}{K_M + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	None
Competitive	$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	Increases K_M^{app}
Uncompetitive	$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{K_M/\alpha' + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases V_{max}^{app}; may increase or decrease K_M^{app}

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K'_I}$.

Donald Voet&Judith G. Voet&Charlotte W. Pratt (2016). Fundamentals of biochemistry, binder ready version life at the molecular level Wiley pp.380 TABLE 12-2
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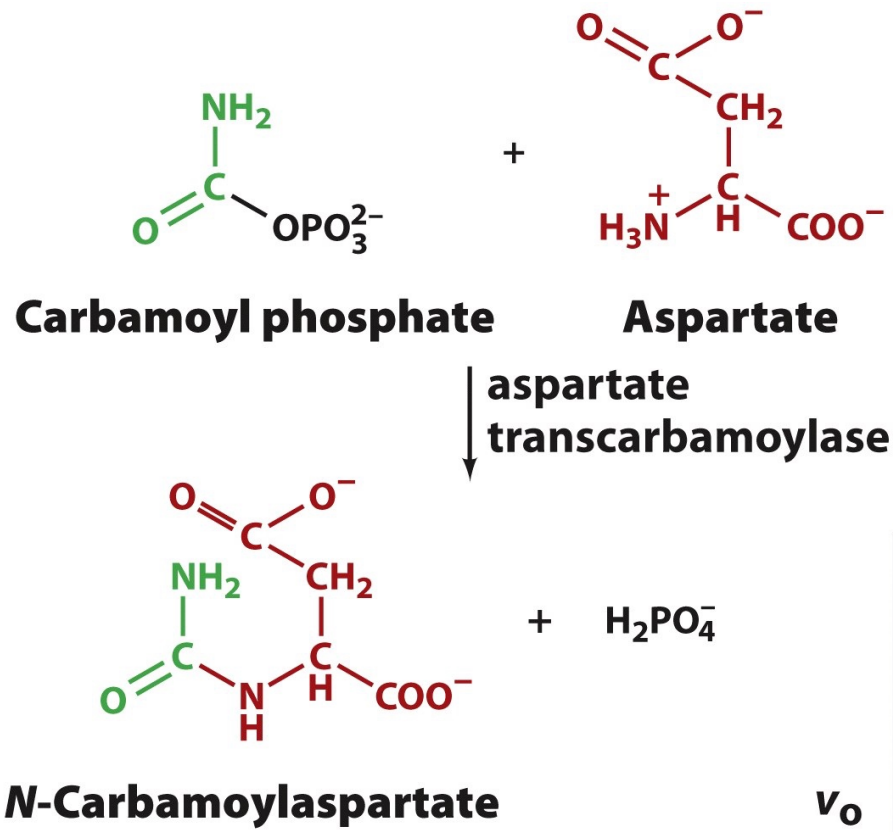
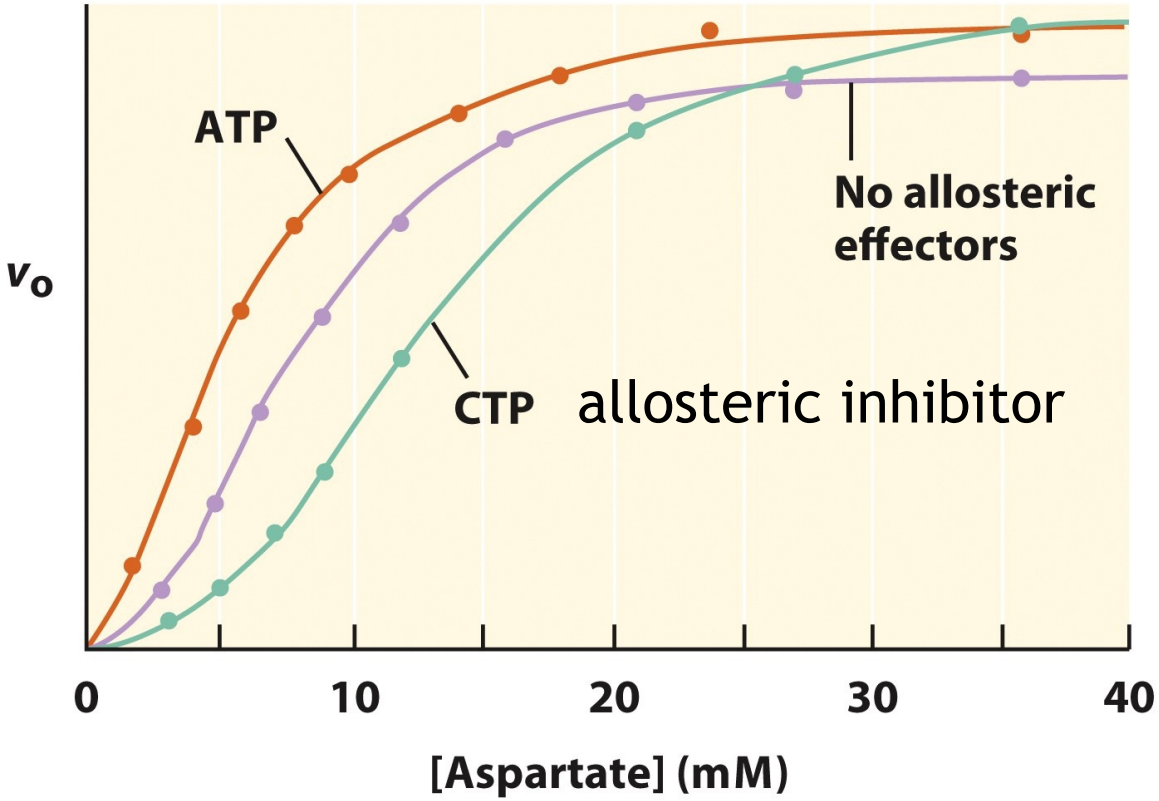
Control of Enzyme Activity

- Allosteric effectors bind to multisubunit enzymes such as aspartate transcarbamoylase, thereby inducing cooperative conformational changes that alter the enzyme's catalytic activity. (They don't follow MM)
- Phosphorylation and dephosphorylation of an enzyme such as glycogen phosphorylase can control its activity by shifting the equilibrium between more active and less active conformations.

Aspartate Transcarbamoylase Reaction



allosteric control:
- distinct regulatory site
- multisubunit
- cooperativity
- feedback inhibition



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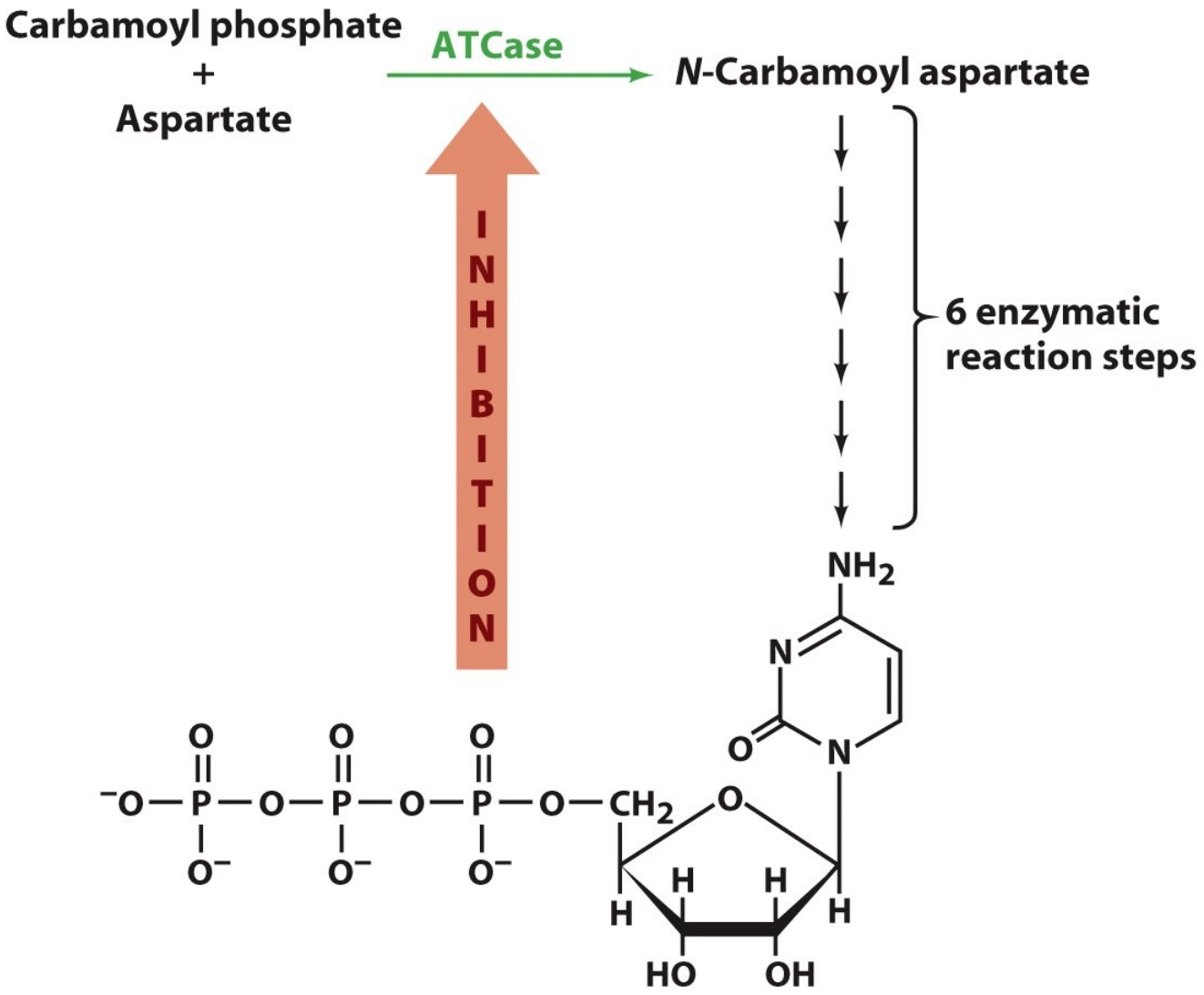
Pyrimidine Biosynthesis: ATCase Feedback Inhibition



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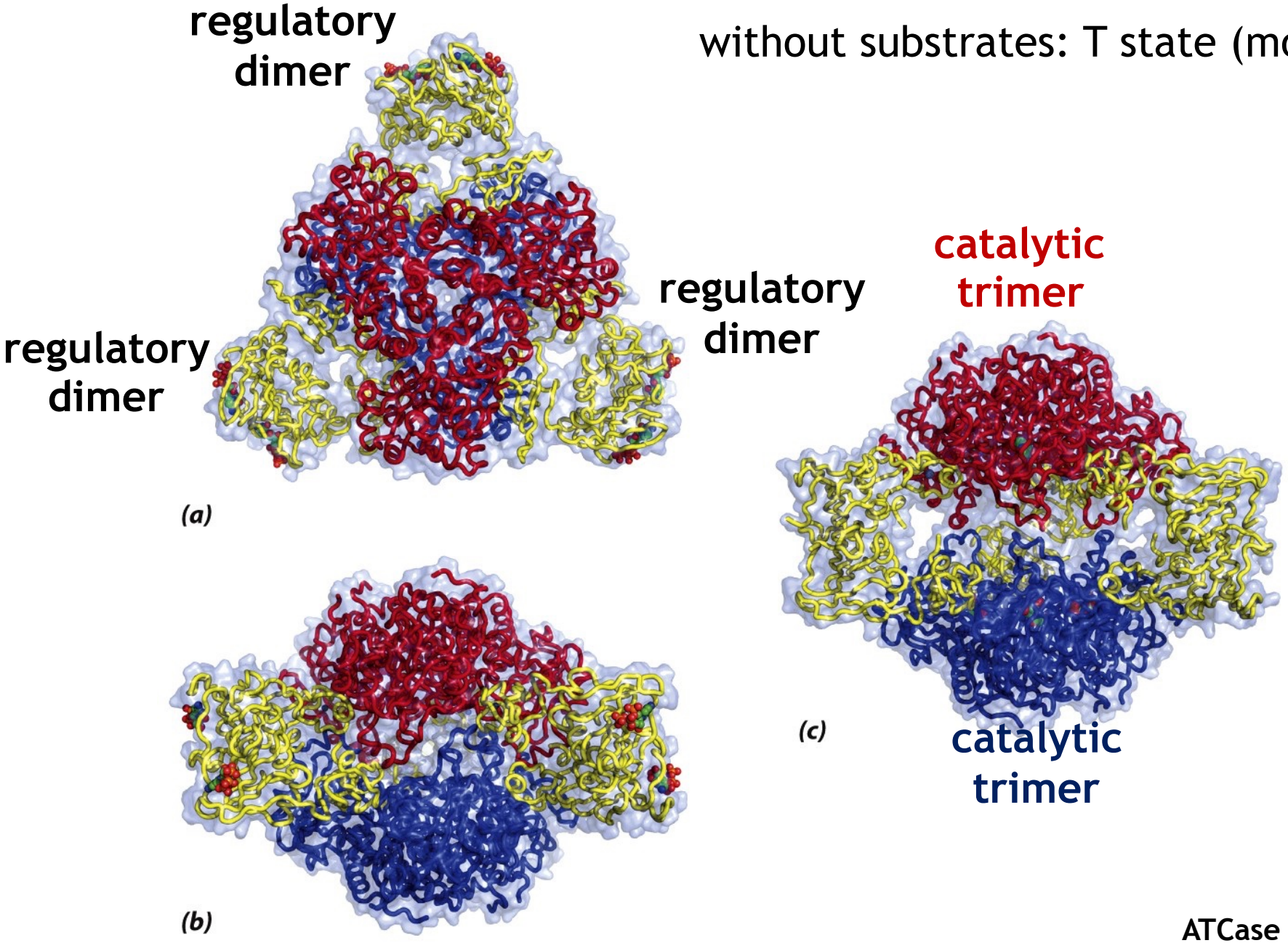
ATCase: T-State vs. R-State

without substrates: T state (more stable)



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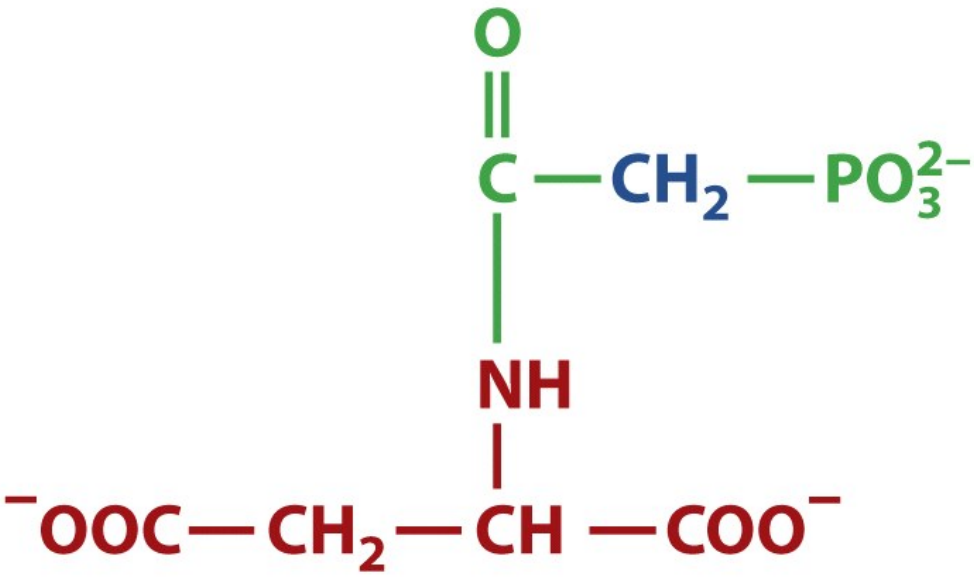
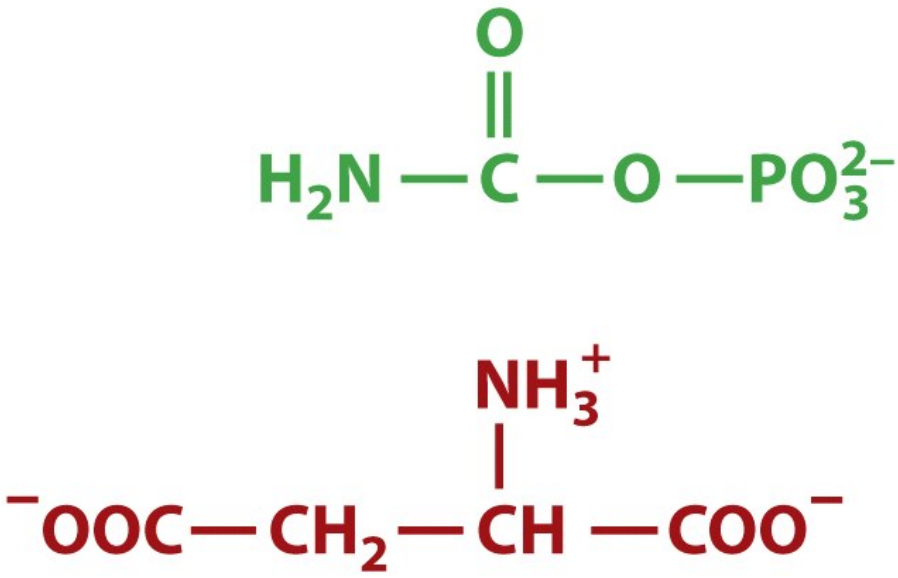
ATCase from *E. coli*
PDBids 5AT1 and 8ATC

Unreactive Bisubstrate Analog



***N*-(Phosphonacetyl)-L-aspartate (PALA)**



Carbamoyl phosphate

+

Aspartate

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ATCase: Conformational Changes

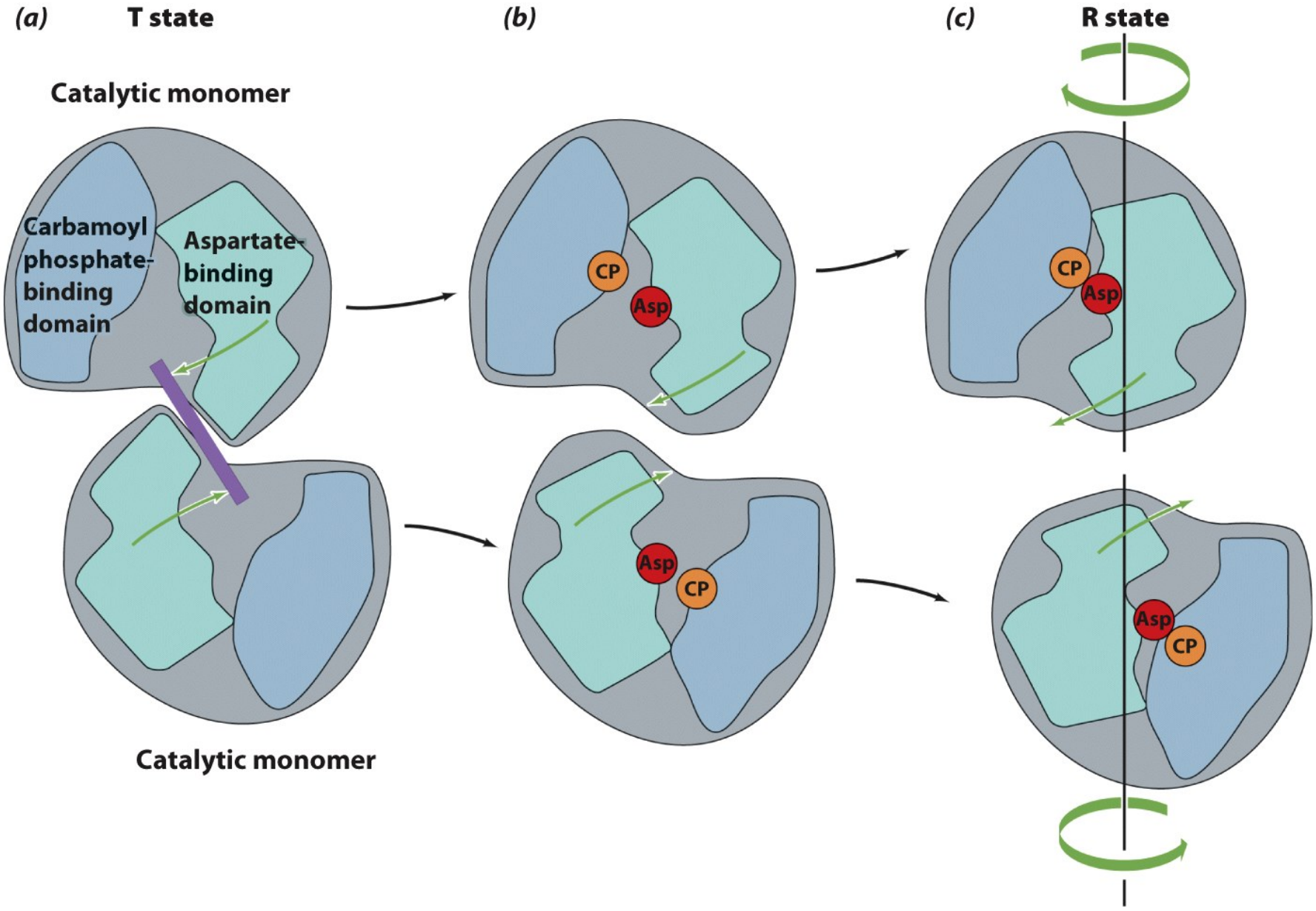

Figure 12-14

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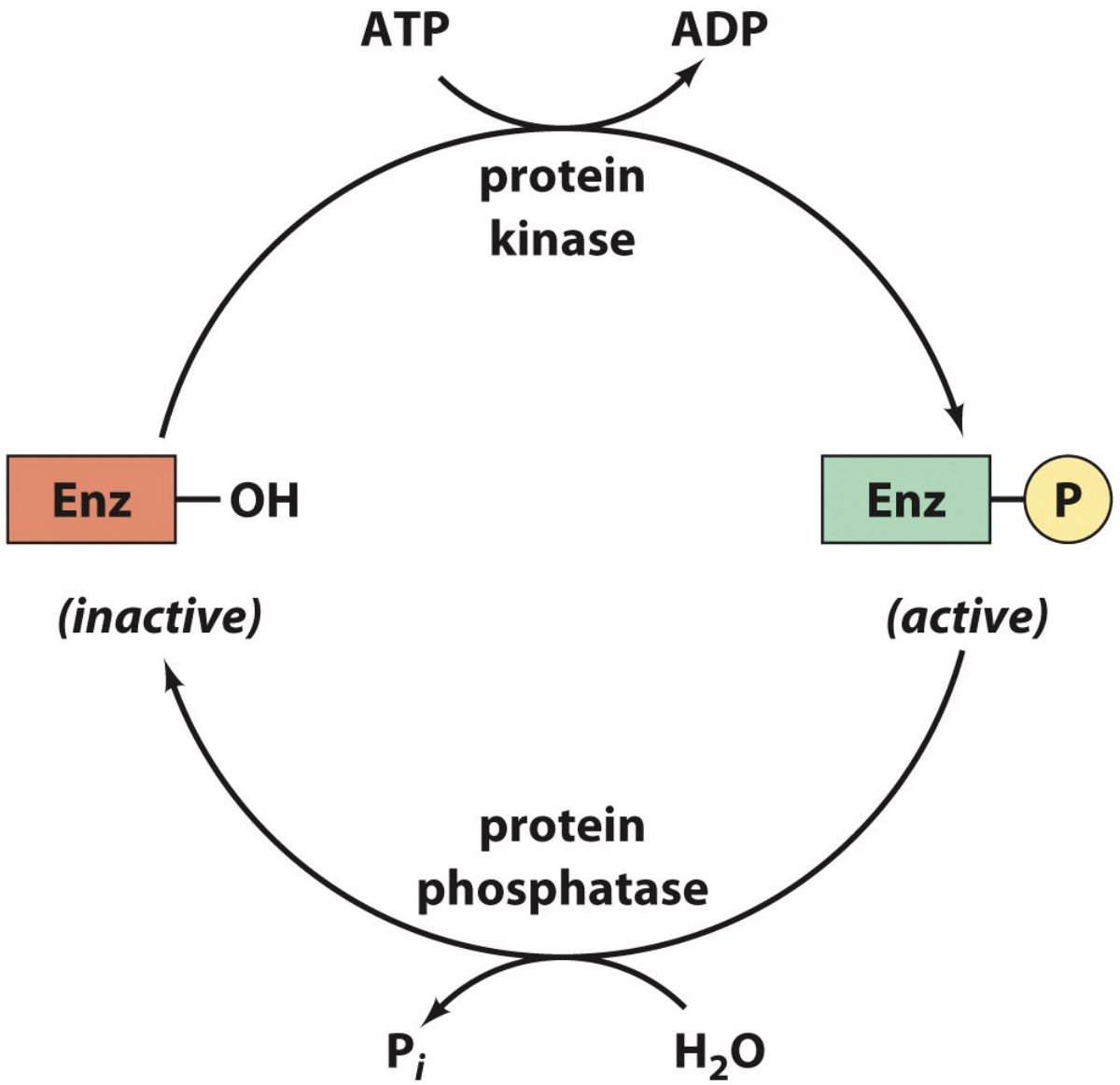
Control by Covalent Modification: Phosphorylation



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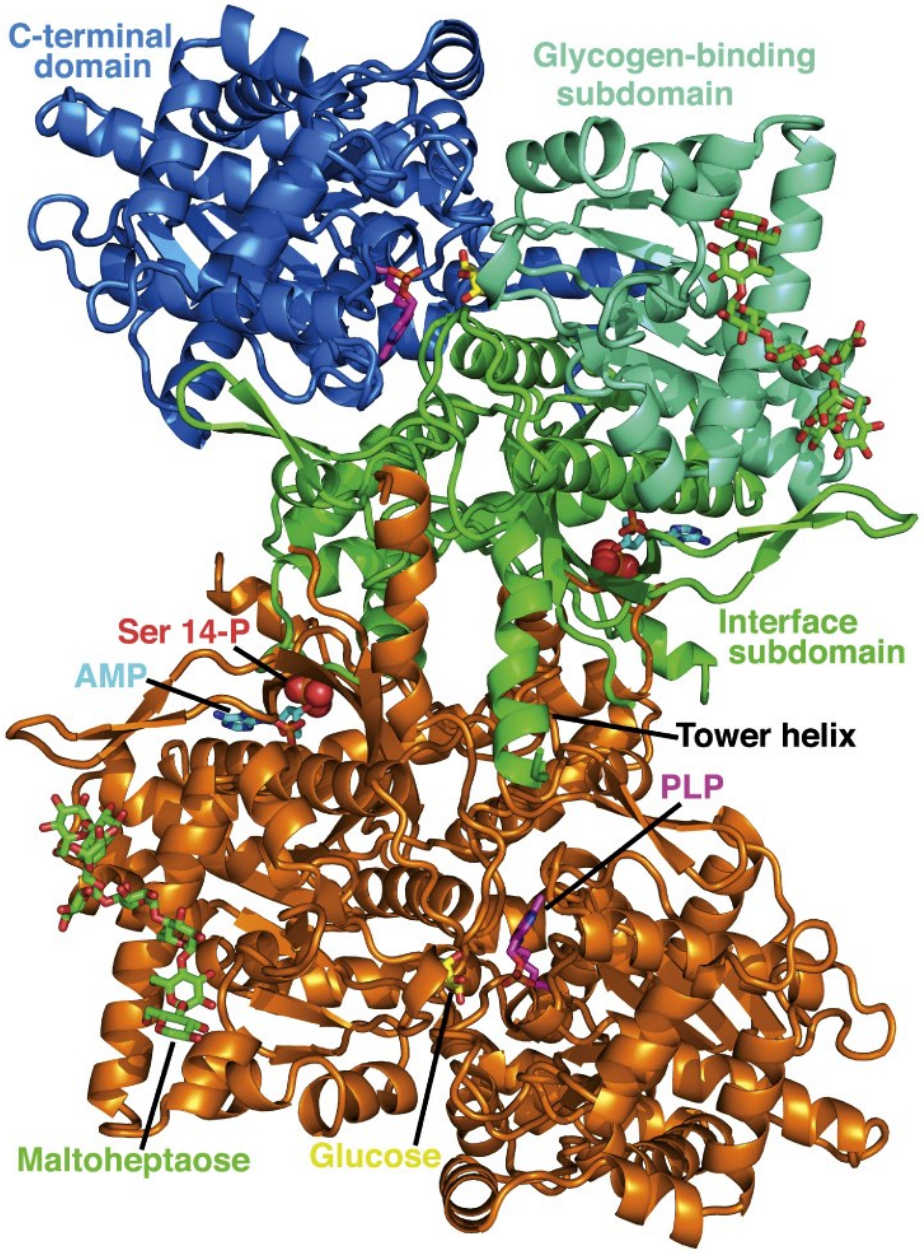
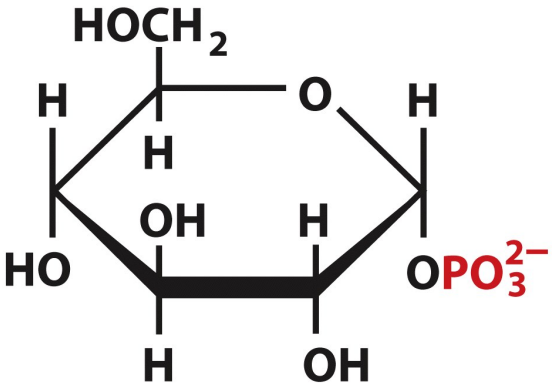
Rabbit Muscle Glycogen Phosphorylase





Glucose-1-phosphate (G1P)

Figure 12-15
X-Ray structure coordinates courtesy of Stephen Sprang, University of Texas Southwest Medical Center

Conformational Changes: Glycogen Phosphorylase

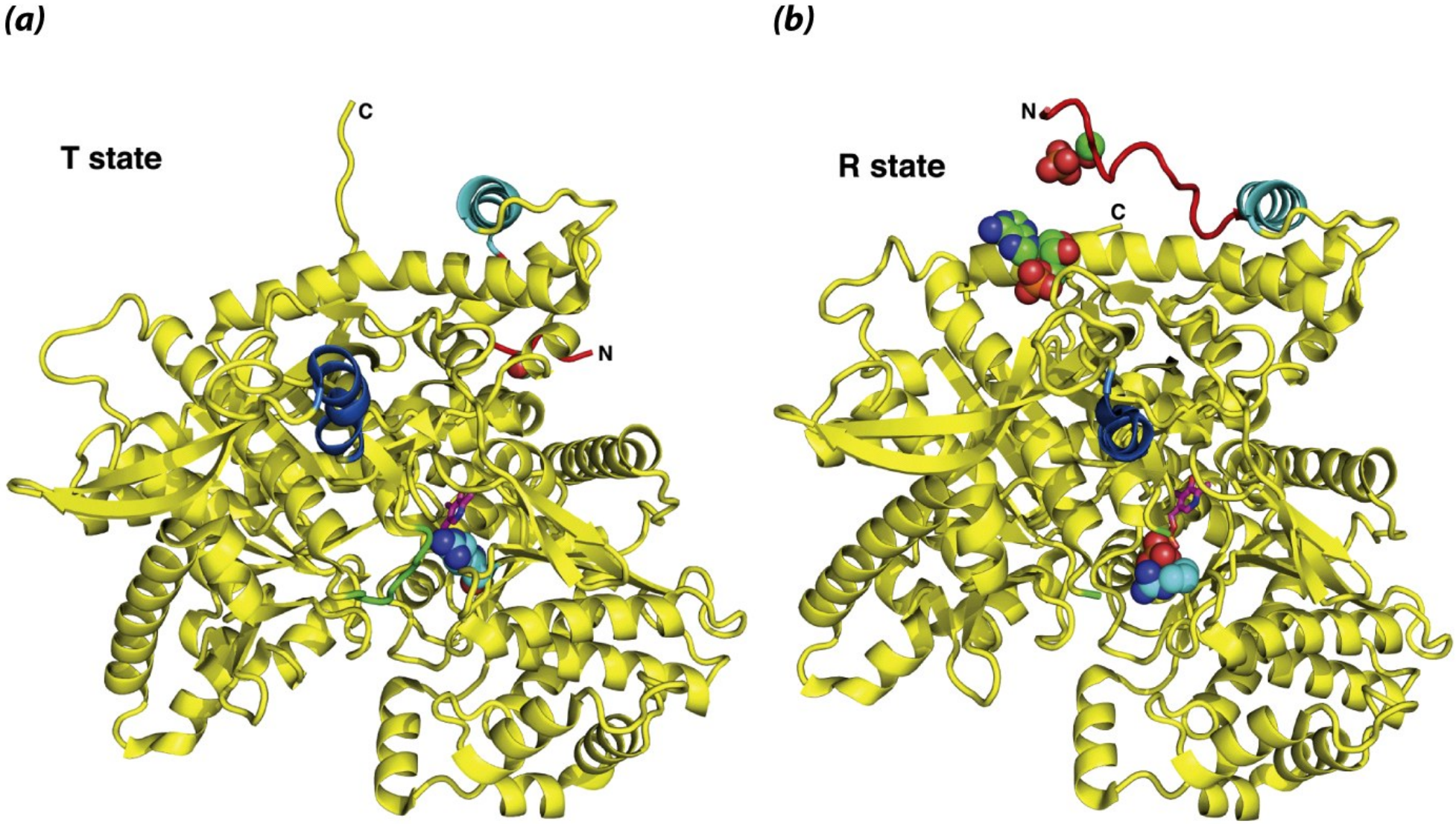


Figure 12-16

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Glycogen phosphorylase
PDBids 8GPB and 7GPB

Glycogen Phosphorylase: Control by Phosphorylation

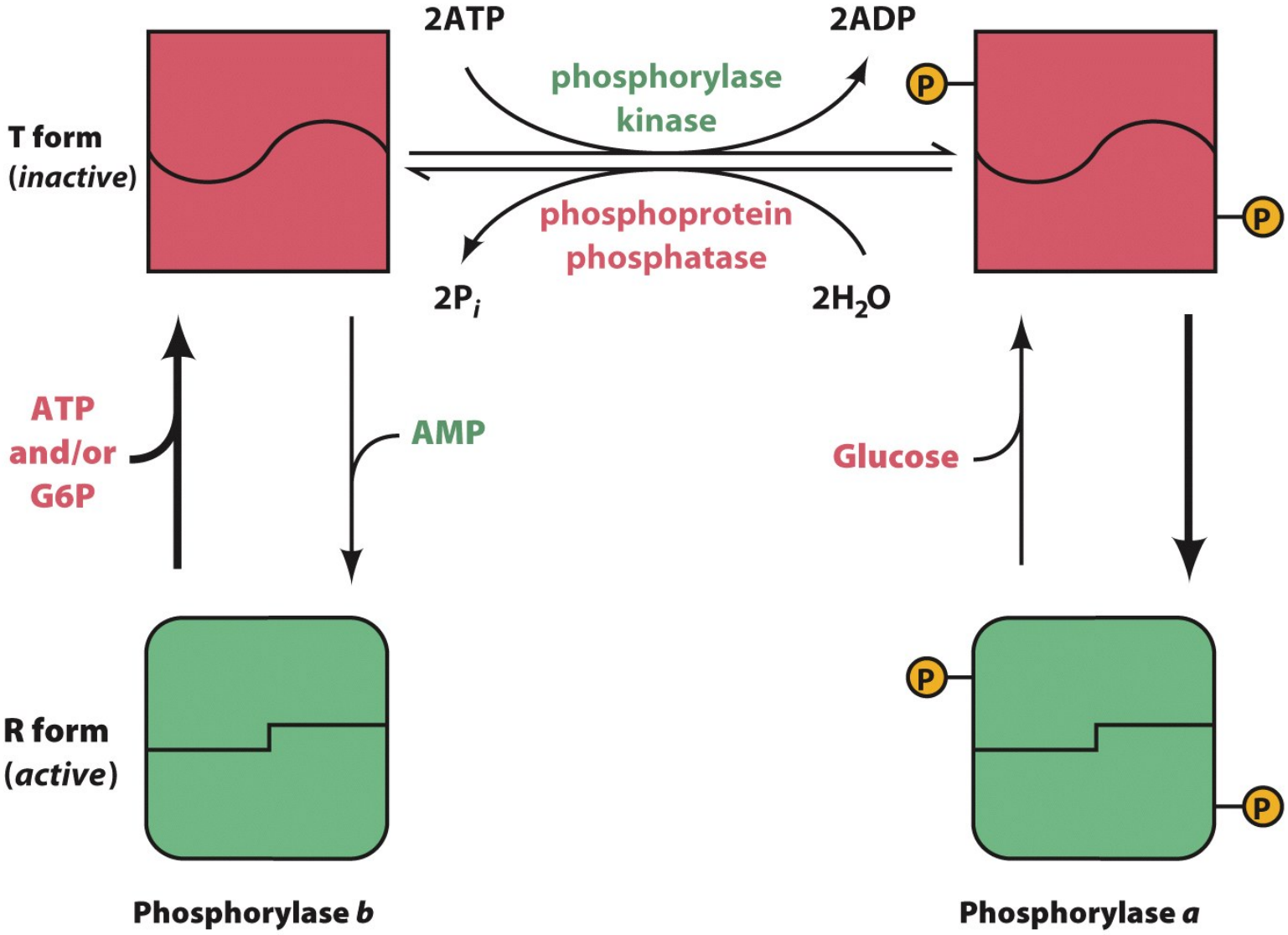

Figure 12-17

Donald Voet&Judith G. Voet&Charlotte W. Pratt (2016). Fundamentals of biochemistry, binder ready version life at the molecular level Wiley pp.390
https://www.amazon.co.jp/Fundamentals-Biochemistry-Binder-Ready-Version/dp/1118918436/ref=dp_ob_title_bk 2019/6/10