Welcome to BCII lesson 2

Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

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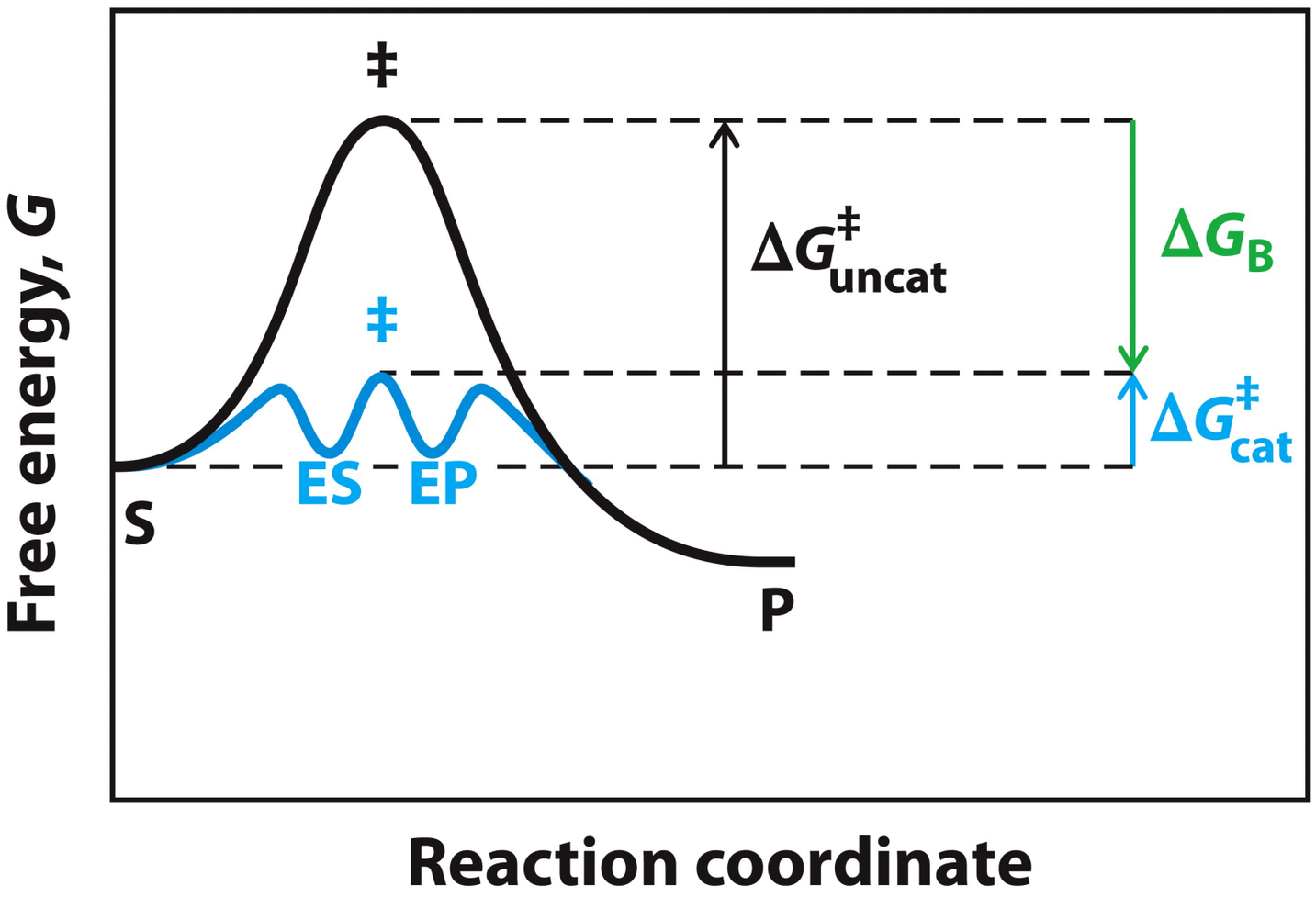
Enzymes Affect Rxn Rates

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$



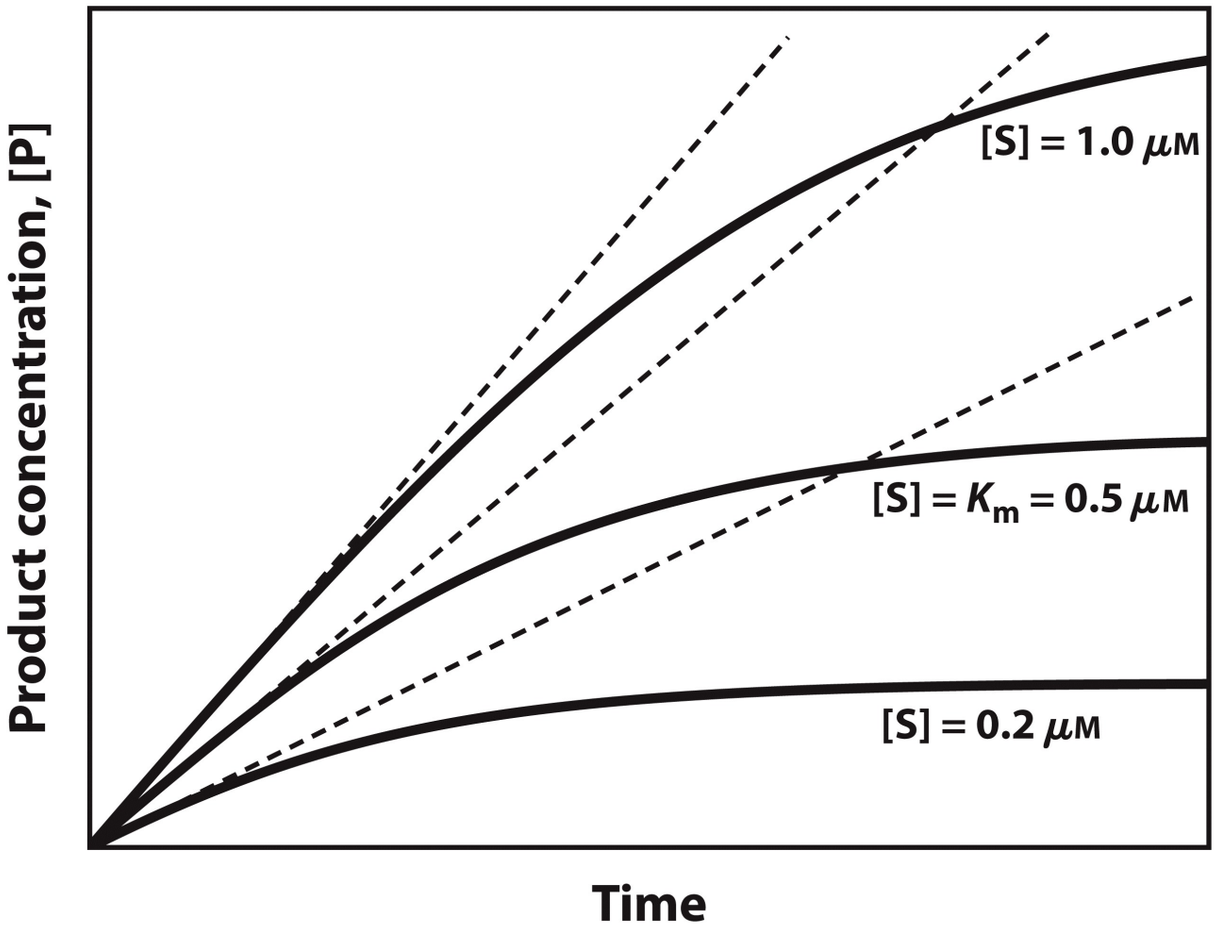
$$\boldsymbol{k} = (\mathrm{k}T/h) * e^{-\Delta G^{\ddagger}/RT}$$

$$V = \boldsymbol{k}[S]$$

What can we learn on Enzymes by looking at Rxn Rates?

Intro to Enzyme Kinetics

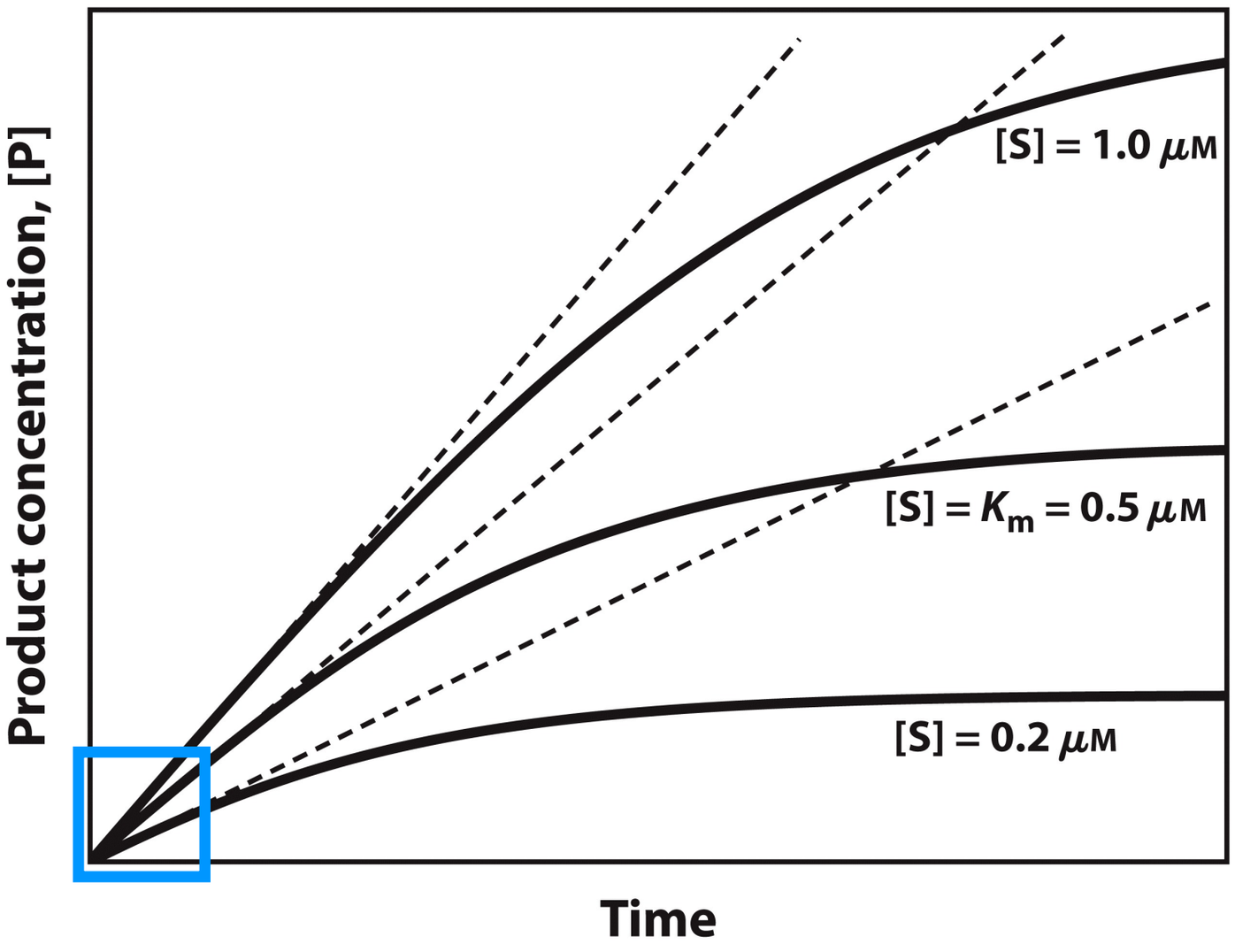
The oldest approach to understanding **enzyme mechanisms**, and the one that remains the most important, is to determine the rate of a reaction and **how it changes in response to changes in experimental parameters.** This is the discipline known as **enzyme kinetics**. A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S].



Studying the effects of substrate concentration is complicated by the fact that [S] changes during the course of an in vitro reaction as substrate is converted to product. One simplifying approach in kinetic experiments is to measure the initial rate (initial velocity), designated V_0

V_0

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$



$$V = [P]/t = \boldsymbol{k}\,[S]$$

$$V_t = \boldsymbol{k}\,[S]_t$$

$$[S]_t = [S]_0 - [P]_t$$

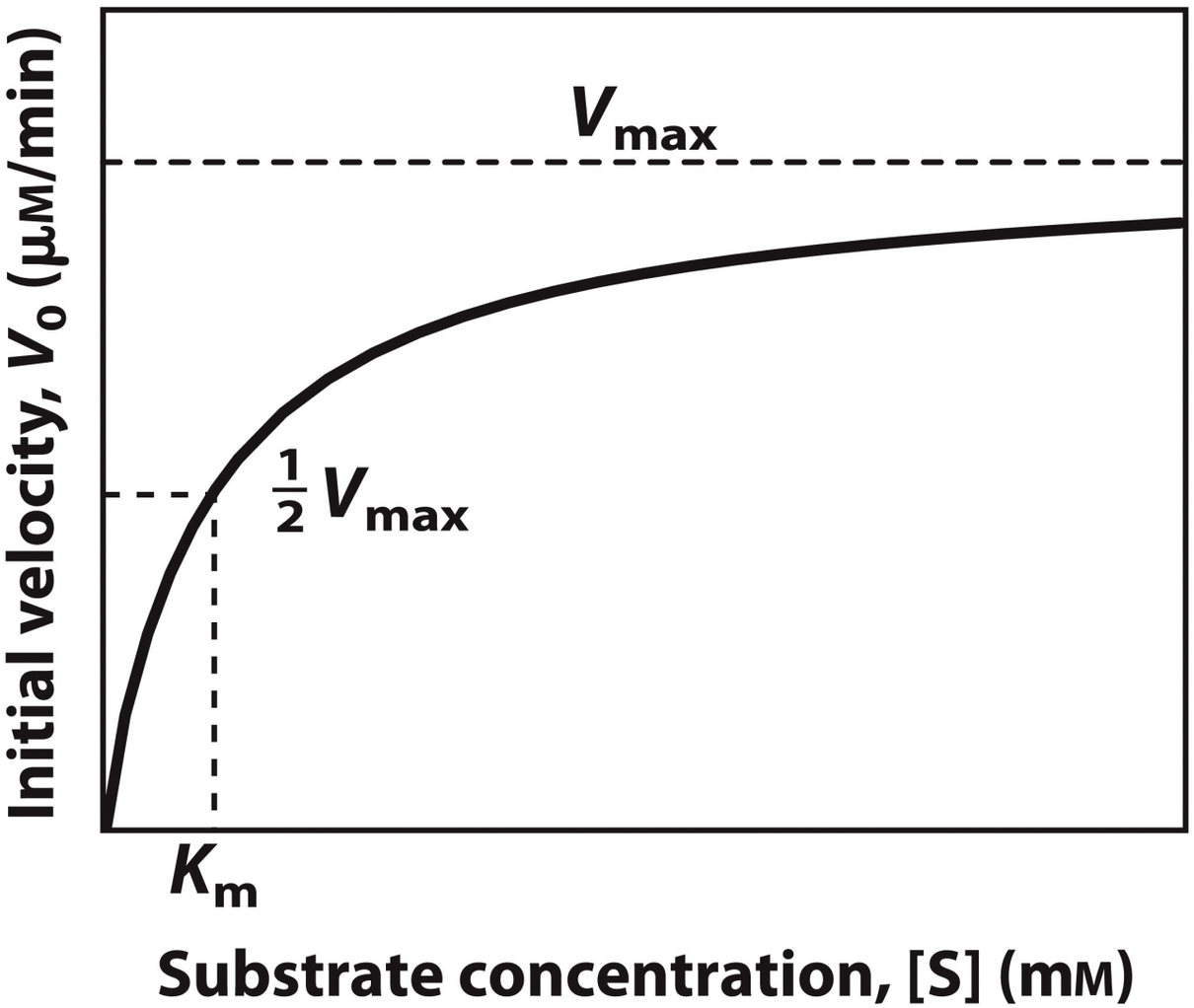
$$V_t = \boldsymbol{k}\,([S]_0 - [P]_t)$$

$$V_0 = \boldsymbol{k}\,([S]_0 - 0)$$

$$\boxed{V_0 = \boldsymbol{k}\,[S]_0}$$

Effect of [S] on V_0

The effect on V_0 of varying [S] when the enzyme concentration is held constant is shown. This is the appearance of a V_0 vs [S] kinetic plot for a typical enzyme.



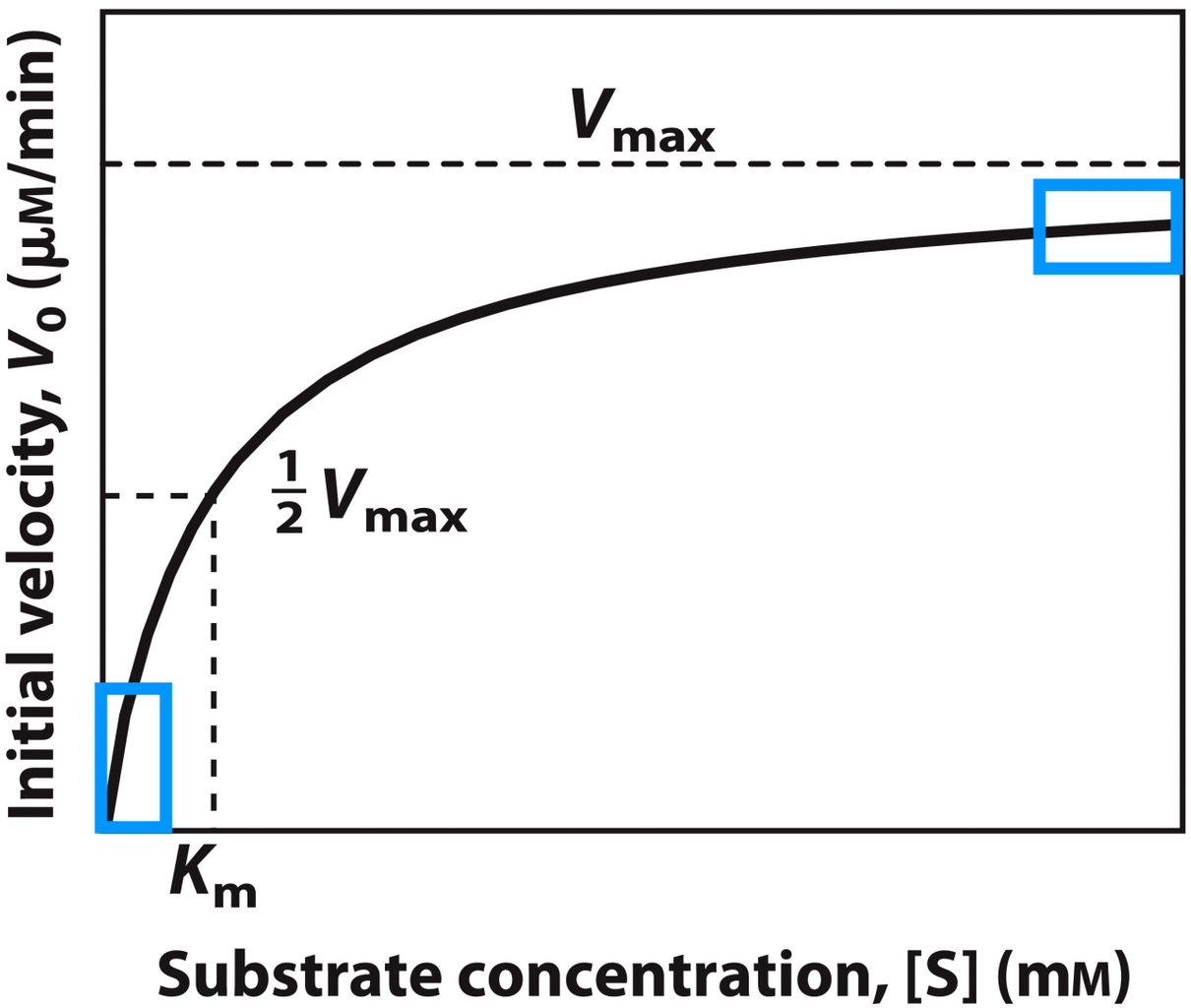
At relatively low concentrations of substrate, V_0 increases almost linearly with an increase in [S]. At higher substrate concentrations, V_0 increases by smaller and smaller amounts in response to increases in [S]. Finally, a point is reached beyond which increases in V_0 are vanishingly small as [S] increases. This plateau-like V_0 region is close to the maximum velocity, V_{max}.

Effect of [S] on V_0

How can we 'intuitively' explain this?

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$

$$E + S \rightleftarrows ES \rightarrow E + P$$



When [E] > [S] Then $V_0 = k$ [S]

When [S] >> [E] then all E will be engaged with S in a ES complex. E is **saturated**

At this point adding more S will not make the Reaction Faster

Effect of [S] on V_0

How can we formally describe this?

The ES complex is the key to understanding the kinetic behavior of an enzyme. The enzyme first combines with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES$$

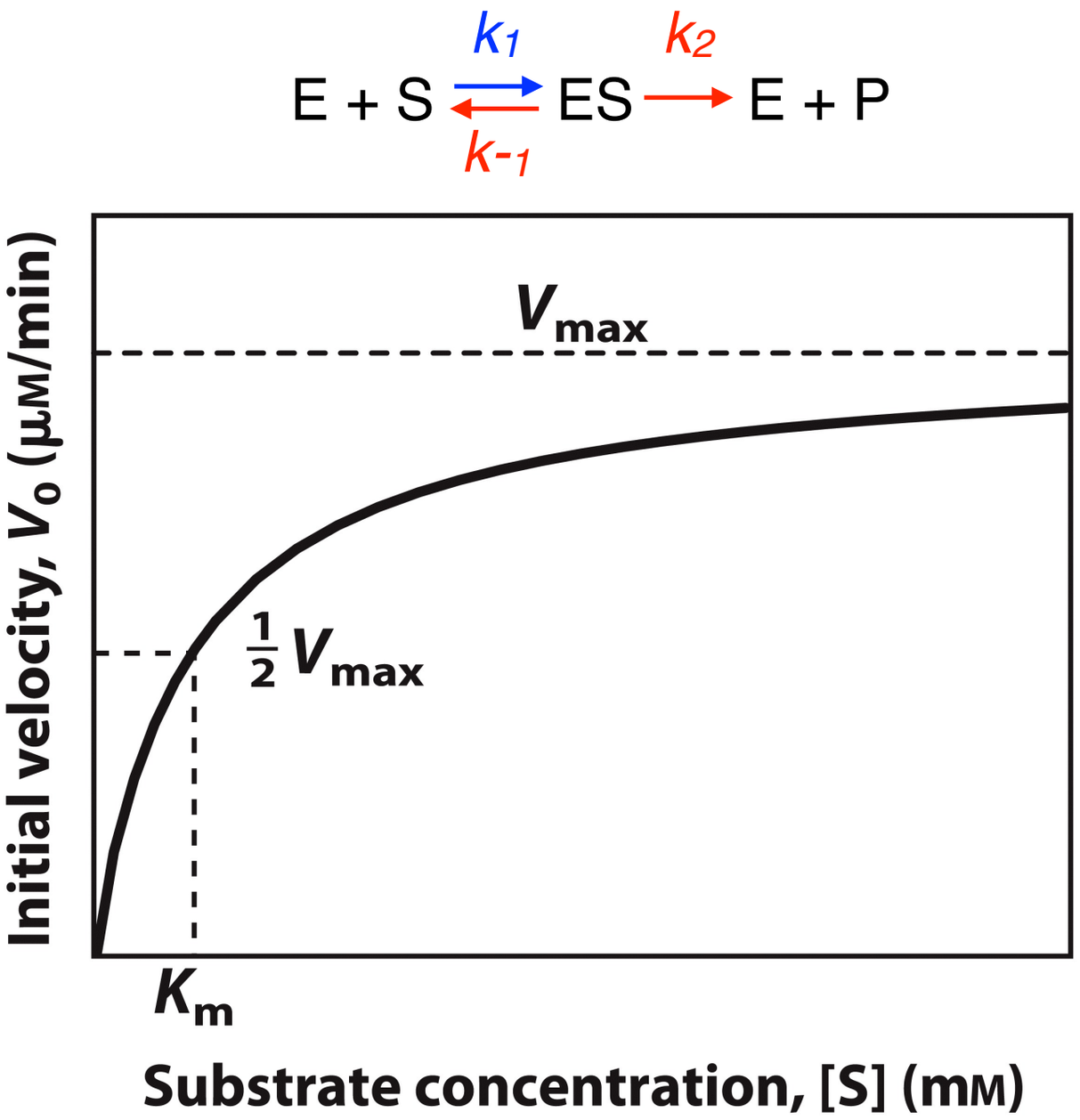
The ES complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

$$ES \xrightarrow{k_2} E + P$$

If the slower second reaction limits the rate of the overall reaction, the overall rate must be proportional to the concentration of the species that reacts in the second step, i.e., ES.

$$V_0 = k_2 [ES]$$

The Role of the ES Complex



Eq1: rate of [ES] production

$= k_1[E]_{free}[S]$

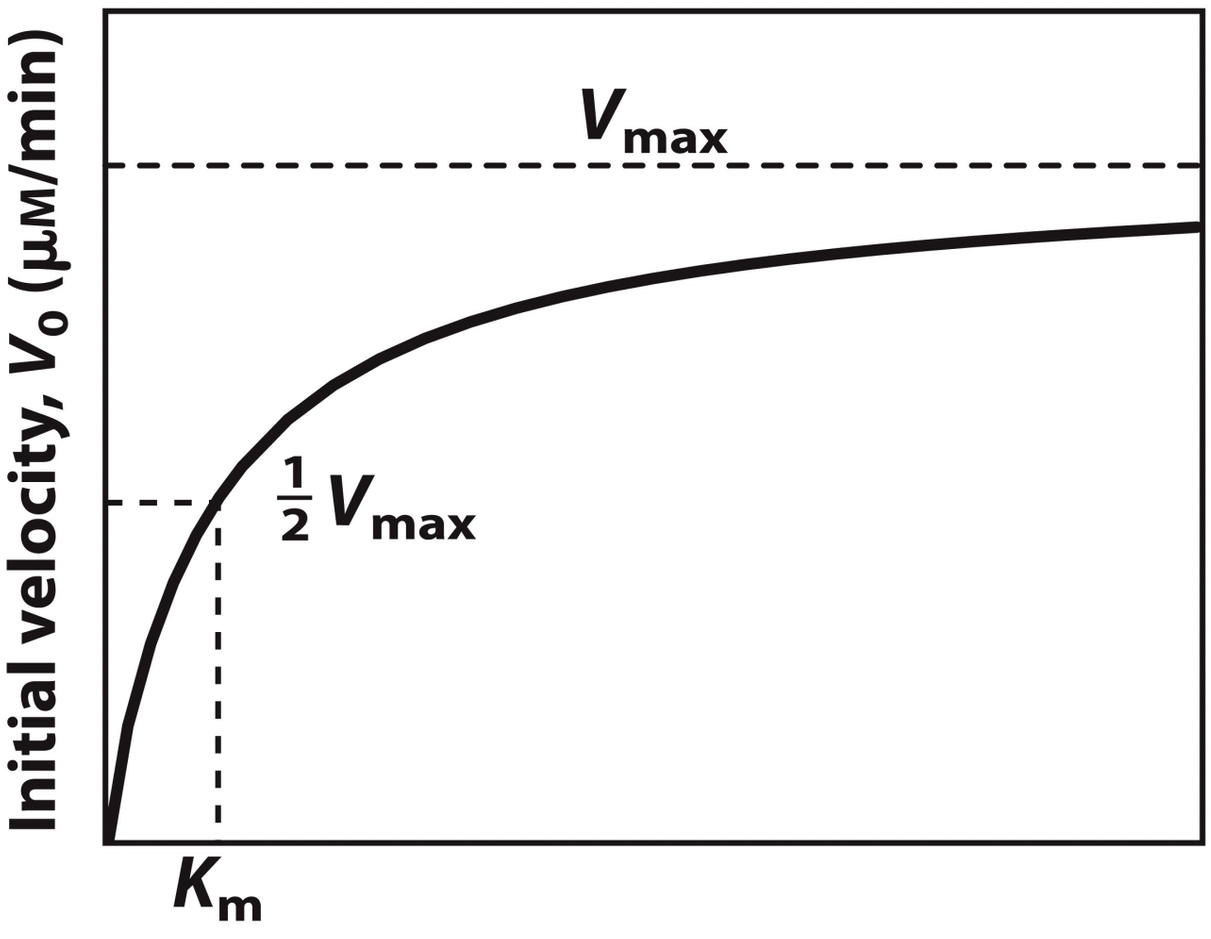
$= k_1([E]_{tot} - [ES])[S]$

Eq2: rate of [ES] breakdown

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

The Role of the ES Complex

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



Steady state assumption

Here we assume that V_o reflects a condition where [ES] is constant

that is

[ES] production = [ES] breakdown

Eq3

$$k_1([E]_{tot} - [ES])[S] = k_{-1}[ES] + k_2[ES]$$

Derivation of the Michaelis-Menten Equation

Eq3 $k_1([E]_{tot} - [ES])[S] = k_{-1}[ES] + k_2[ES]$

Eq4 $k_1[E]_{tot}[S] - k_1[ES][S] = (k_{-1} + k_2)[ES]$

Eq5 $k_1[E]_{tot}[S] = (k_1[S] + k_{-1} + k_2)[ES]$ Here we solve for [ES]

Eq6 $$[ES] = \frac{k_1[E]_{tot}[S]}{k_1[S] + k_{-1} + k_2}$$

Here we simplify combining the rate constants

Eq7 $$[ES] = \frac{[E]_{tot}[S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

Eq8 $$\frac{k_{-1} + k_2}{k_1} = K_m$$

Michaelis constant

Derivation of the Michaelis-Menten Equation

Eq9 $$[ES] = \frac{[E]_{tot}[S]}{[S] + K_m} \longrightarrow V_0 = k_2 [ES]$$

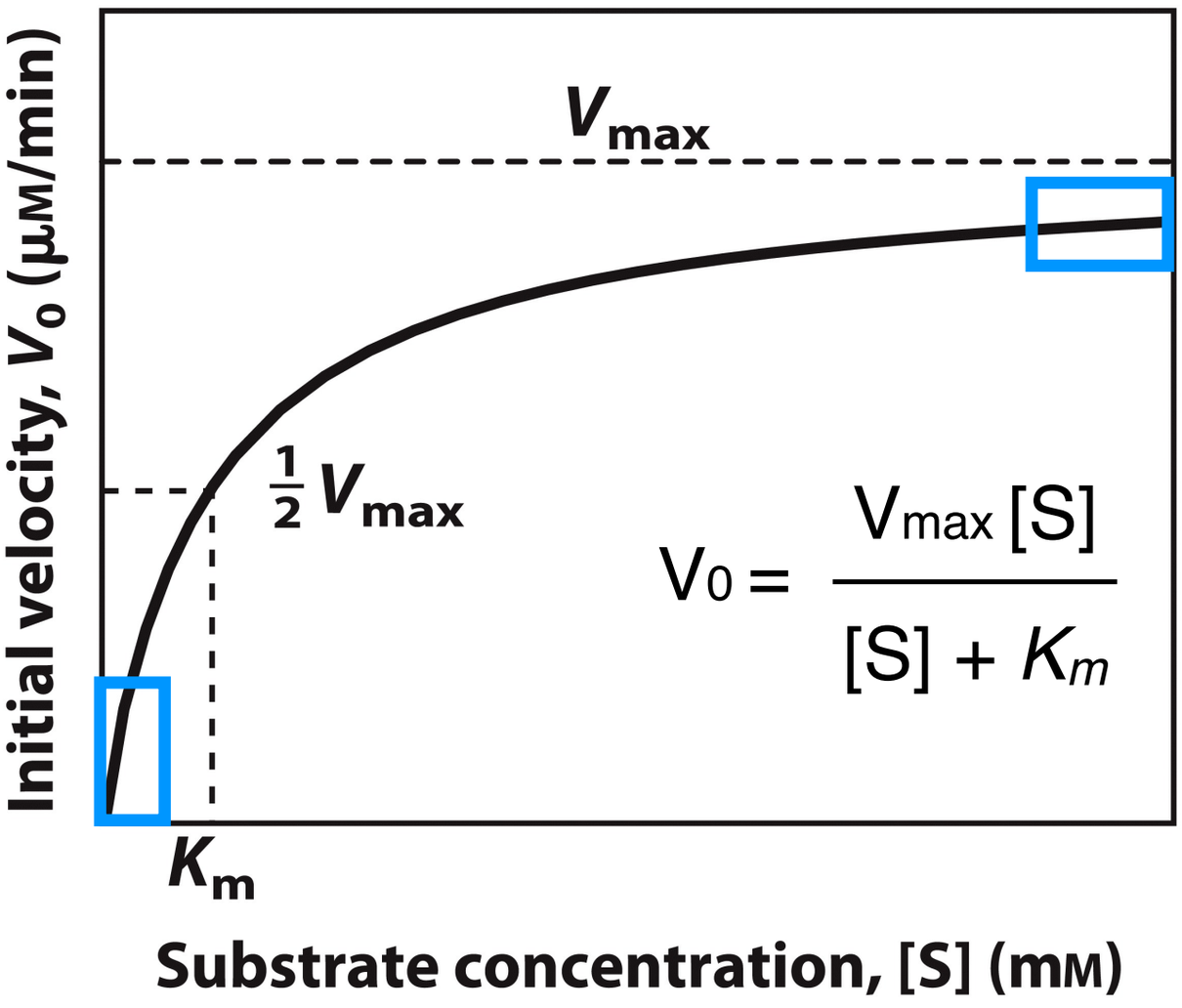
Eq10 $$V_0 = \frac{k_2 [E]_{tot}[S]}{[S] + K_m} \qquad V_{max} = k_2 [E]_{tot}$$

Eq11 $$\boxed{V_0 = \frac{V_{max}[S]}{[S] + K_m}}$$ Michaelis-Menten Equation

The MM equation describes the kinetic behavior of many enzymes, and all enzymes that exhibit a hyperbolic dependence of V_0 on [S] are said to follow Michaelis-Menten kinetics. Even though the MM equation holds true for many enzymes, both the magnitude and the real meaning of V_{max} and K_m can differ from one enzyme to another. This is an important limitation of the steady-state approach to enzyme kinetics.

Validation of the Michaelis-Menten Equation

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$



When [S] is low i.e., $[S] << K_m$

then

$$V_0 = \frac{V_{max}[S]}{K_m} \qquad V_0 = k[S]$$

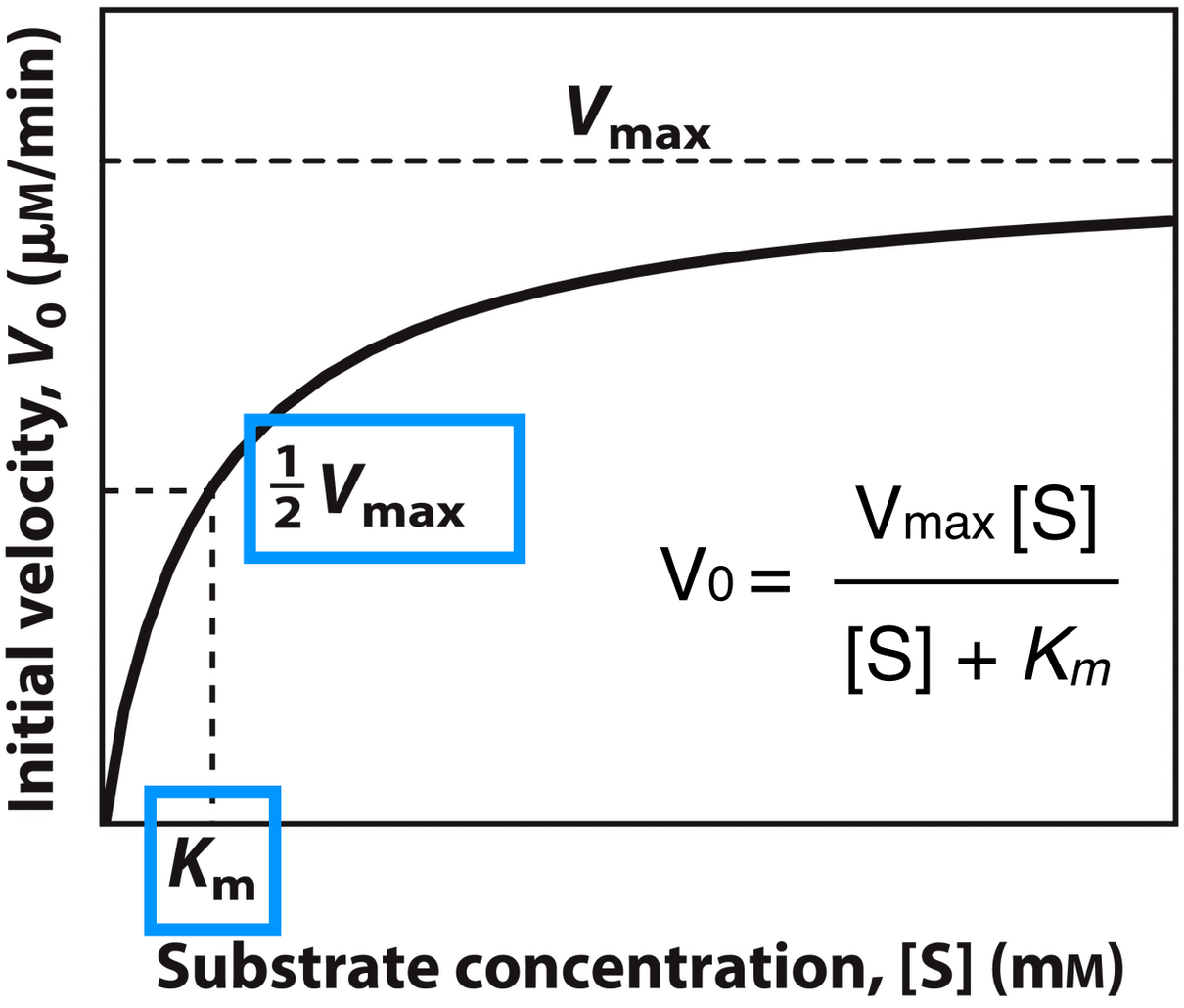
When [S] is high i.e., $[S] >> K_m$

then

$$V_0 = \frac{V_{max}[S]}{[S]} \qquad V_0 = V_{max}$$

Validation of the Michaelis-Menten Equation

$$E + S \rightleftarrows ES \rightleftarrows EP \rightleftarrows E + P$$



When $V_0 = \frac{V_{max}}{2}$

then

$$\frac{[S]}{[S] + K_m} = \frac{1}{2} \qquad K_m = [S]$$

K_m is equivalent to the substrate concentration at which V_0 is one-half V_{max}.

Double-reciprocal Plot

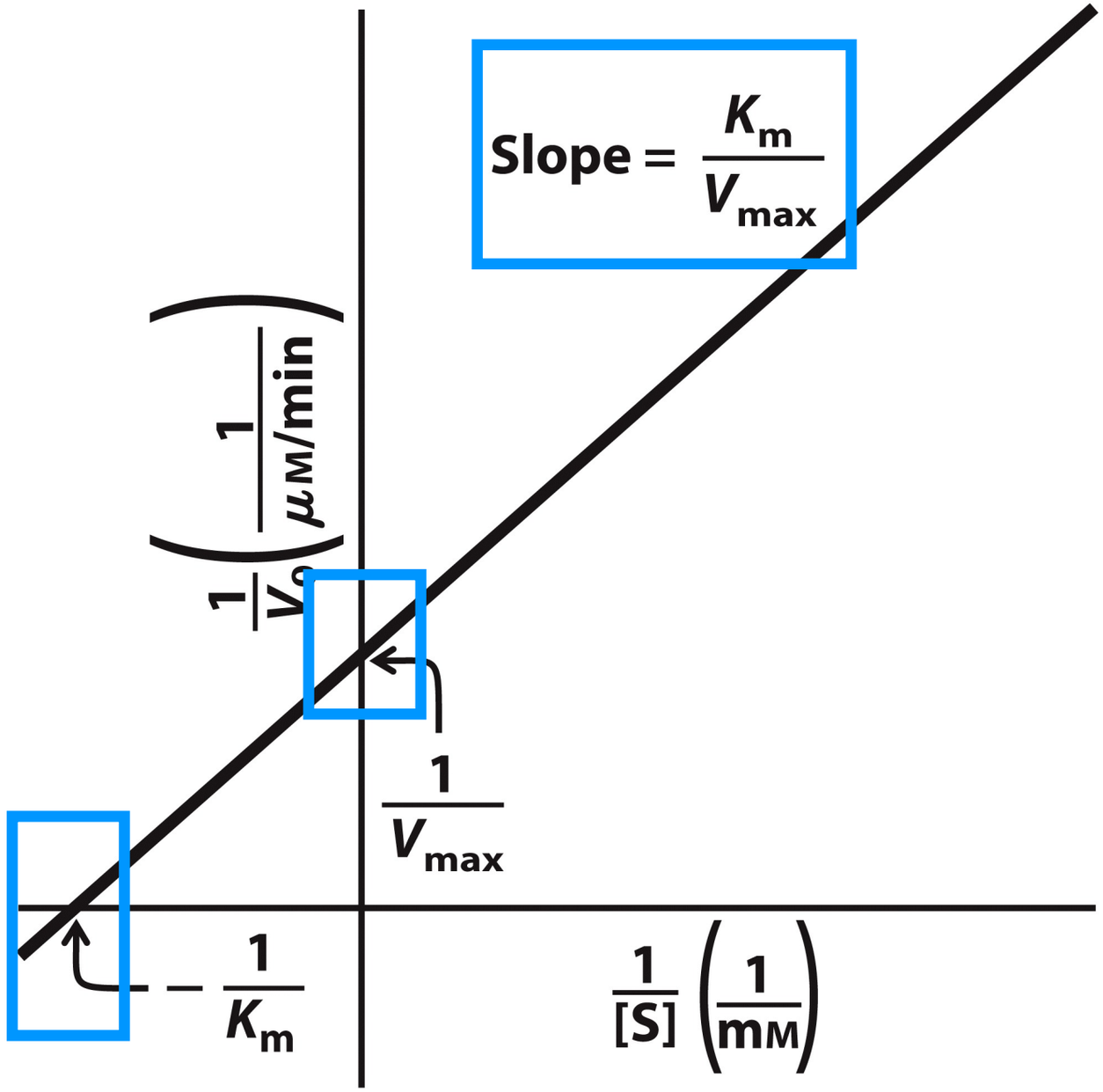
Because the plot of V_0 vs [S] for an enzyme-catalyzed reaction asymptotically approaches the value of V_{max} at high [S], it is difficult to accurately determine V_{max} (and thereby, K_m) from such graph. The problem is readily solved by converting the Michaelis-Menten kinetic equation to the so-called double-reciprocal equation (Lineweaver-Burk equation) which describes a linear plot from which V_{max} and K_m can be easily obtained

$$V_0 = \frac{V_{max}[S]}{[S] + K_m} \longrightarrow \frac{1}{V_0} = \frac{[S] + K_m}{V_{max}[S]} \longrightarrow \frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

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

$$y = mx + b$$

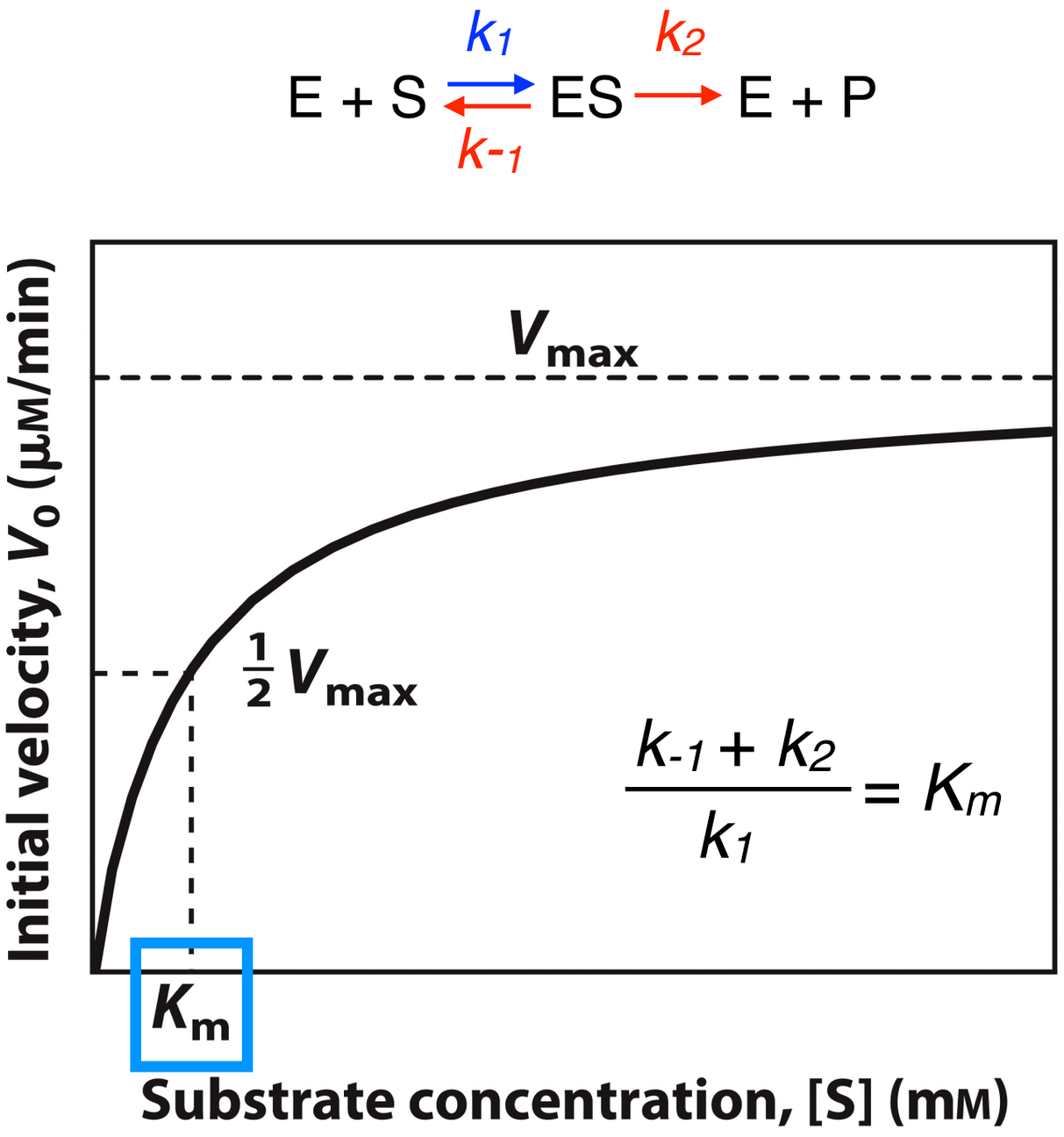
Double-reciprocal Plot



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

The plot of $1/V_0$ vs $1/[S]$ gives a straight line, the y-intercept of which is $1/V_{max}$, the x-intercept of which is $-1/K_m$ and the slope of which is K_m/V_{max}

The Meaning of the K_m



The K_m is sometimes used as an indicator of the affinity of an enzyme for its substrate. The actual meaning of the K_m depends on the reaction mechanism such as the number and relative rates of the individual steps. For example, for a reaction with two steps, $K_m = (k_2 + k_{-1})/k_1$. If k_2 is rate-limiting, then $k_2 << k_{-1}$, and K_m reduces to k_{-1}/k_1, which is the dissociation constant, K_d of the ES complex. Where these conditions hold, K_m represents a measure of the affinity of the enzyme for its substrate. However this scenario often doesn't apply and K_m cannot always be considered a simple measure of the affinity of an enzyme for its substrate.

The Meaning of the K_m

The K_m can vary greatly from enzyme to enzyme, and even for different substrates of the same enzyme

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

V_{max}

The meaning of the quantity V_{max} also varies greatly from one enzyme to the next. If an enzyme reacts via the two-step MM mechanism, then $V_{max} = k_2[E_t]$. However, the number of reaction steps and the identity of the rate-limiting step can vary from enzyme to enzyme. Therefore, it is useful to define a more general rate constant, $\boldsymbol{k_{cat}}$, to describe the rate constant of the rate limiting step(s) of any enzyme-catalyzed reaction at saturation. With the modification that $V_{max} = k_{cat}[E_t]$, the MM equation becomes

$$V_0 = \frac{k_{cat}[E]_{tot}[S]}{[S] + K_m}$$

The constant k_{cat} is a first-order rate constant and hence has the units of reciprocal time (s^{-1}). It is also called the turnover number for the enzyme-catalyzed reaction. It is equivalent to the **number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate**.

k_{cat}

TABLE 6–7 Turnover Number, 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	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

The Specificity Constant (k_{cat} / K_m)

$$V_0 = \frac{k_{cat}[E]_{tot}[S]}{[S] + K_m} \longrightarrow \text{When } [S] \ll K_m \longrightarrow V_0 = \frac{k_{cat}}{K_m}[E]_{tot}[S]$$

Together, the parameters k_{cat} and K_m can be used to evaluate the catalytic efficiency of an enzyme. The best way to determine the catalytic efficiency of an enzyme is to determine the ratio of k_{cat}/K_m for its reaction. This parameter, sometimes called the specificity constant, is the rate constant for the conversion of E + S to E + P.

V_0 in this case depends on the concentration of two reactants, $[E_t]$ and [S]. Therefore, this is a second-order rate equation and the constant k_{cat}/K_m is a second-order rate constant with the units $M^{-1}s^{-1}$. There is an upper limit to k_{cat}/K_m, imposed by the rate at which E and S can diffuse together in an aqueous solution. This diffusion-controlled limit is 10^8 to 10^9 $M^{-1}s^{-1}$, and many enzymes have a k_{cat}/K_m near this range. Such enzymes are said to have achieved catalytic perfection.

The Specificity Constant (k_{cat} / K_m)

TABLE 6–8 **Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)**

Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($M^{-1}s^{-1}$)
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	1.1×10^0	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

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

Introduction to Enzyme Inhibition

Enzyme inhibitors are molecules that **interfere with catalysis, slowing or halting enzymatic reactions**. Enzyme inhibitors are among the most important pharmaceutical agents known. For example, aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that cause pain. The study of enzyme inhibitors also has provided valuable information about enzyme mechanisms and has helped define metabolic pathways. There are two broad classes of enzyme inhibitors: **reversible** and **irreversible** inhibitors.

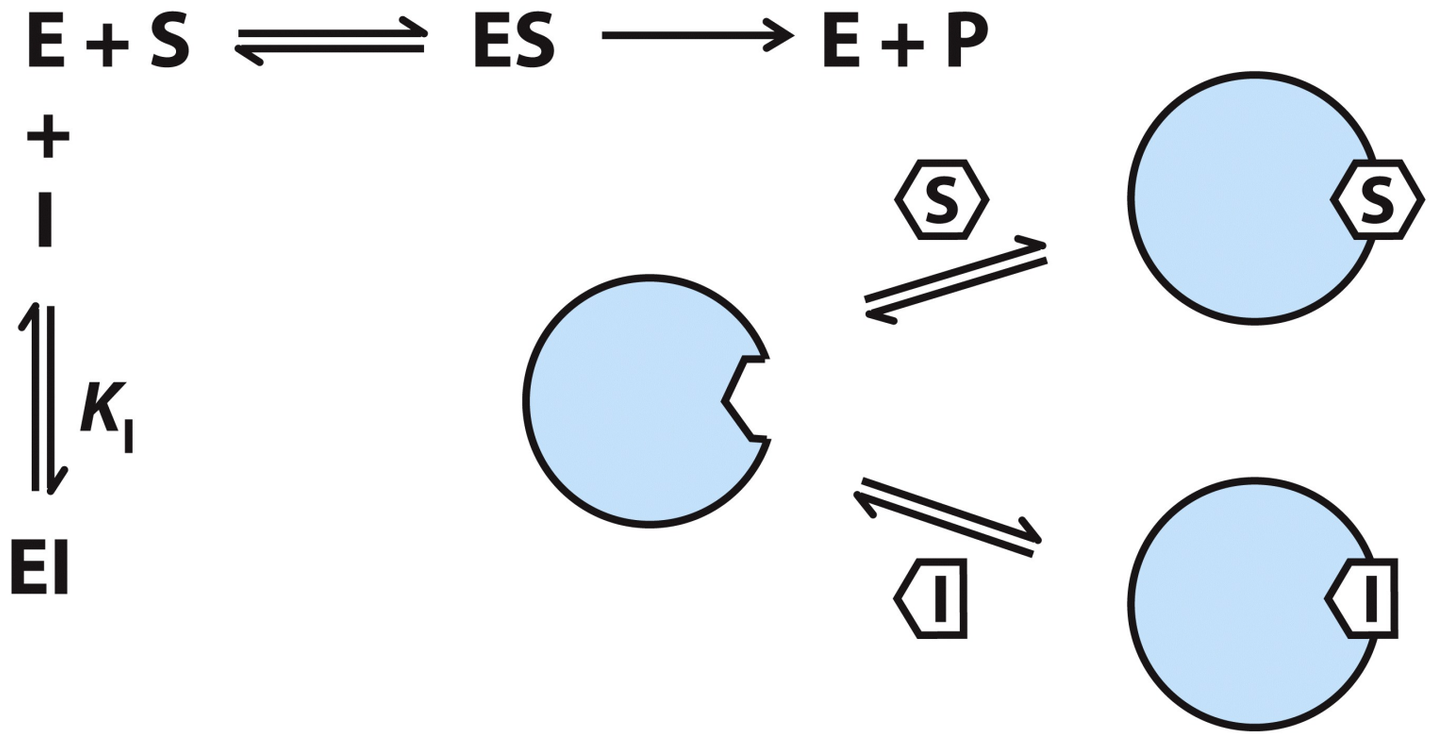
reversible inhibitors can be Competitive, non-Competitive, or Uncompetitive

irreversible inhibitors can also be suicide inactivators or transition state analogs

Competitive Inhibition

A competitive inhibitor (I) competes with the substrate for binding to the active site of an enzyme. While the inhibitor occupies the active site, it prevents the binding of the substrate to the enzyme. Many competitive inhibitors are structurally similar to the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis. Competitive inhibition can be analyzed quantitatively by steady-state kinetics.

Competitive inhibition



Competitive Inhibition

In the presence of a competitive inhibitor, the MM equation becomes

$$V_0 = \frac{V_{max}[S]}{[S] + K_m} \longrightarrow V_0 = \frac{V_{max}[S]}{[S] + \alpha K_m}$$

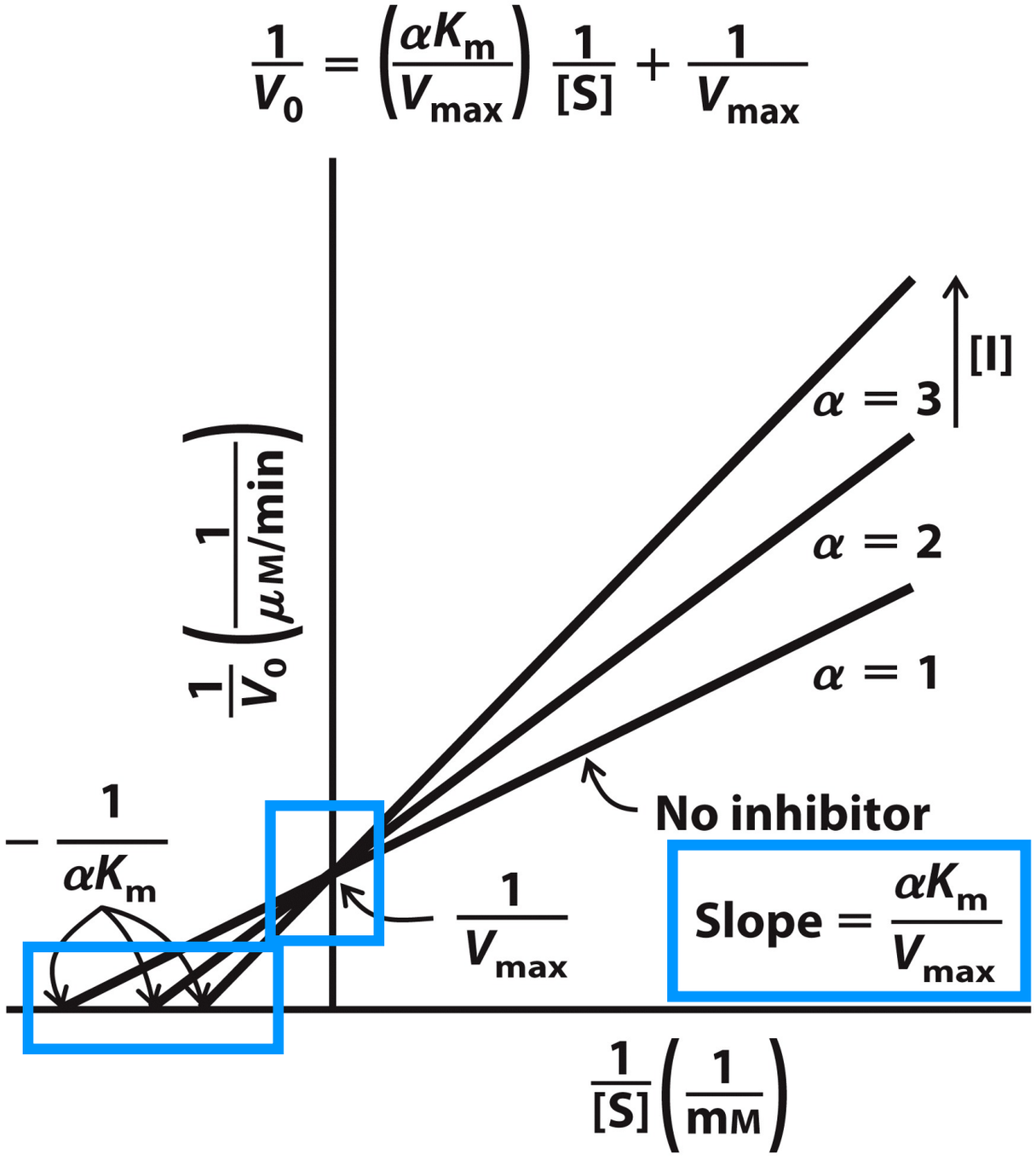
where

$$\alpha = 1 + \frac{[I]}{k_I} \quad \text{and} \quad k_I = \frac{[E][I]}{[EI]}$$

The experimentally determined variable aK_m, the K_m observed in the presence of the competitive inhibitor, is often called the “apparent” K_m.

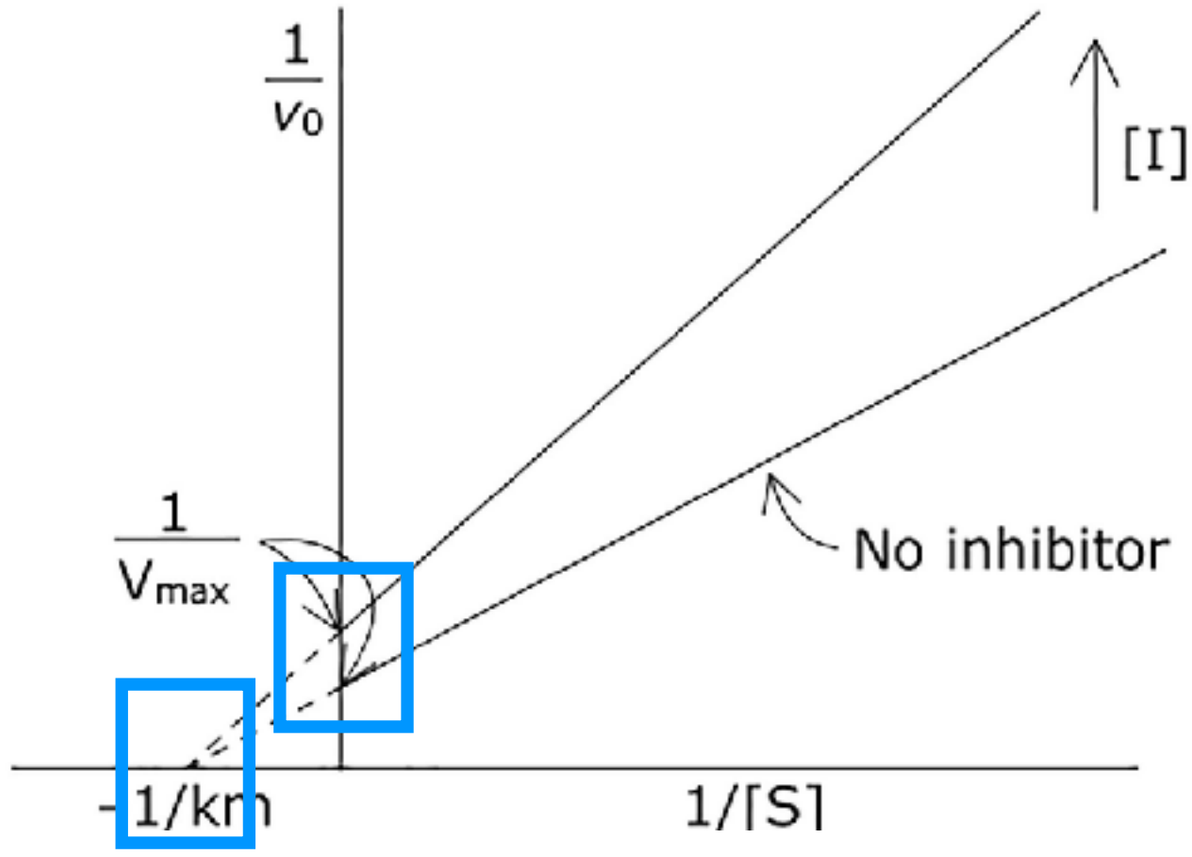
Competitive Inhibition

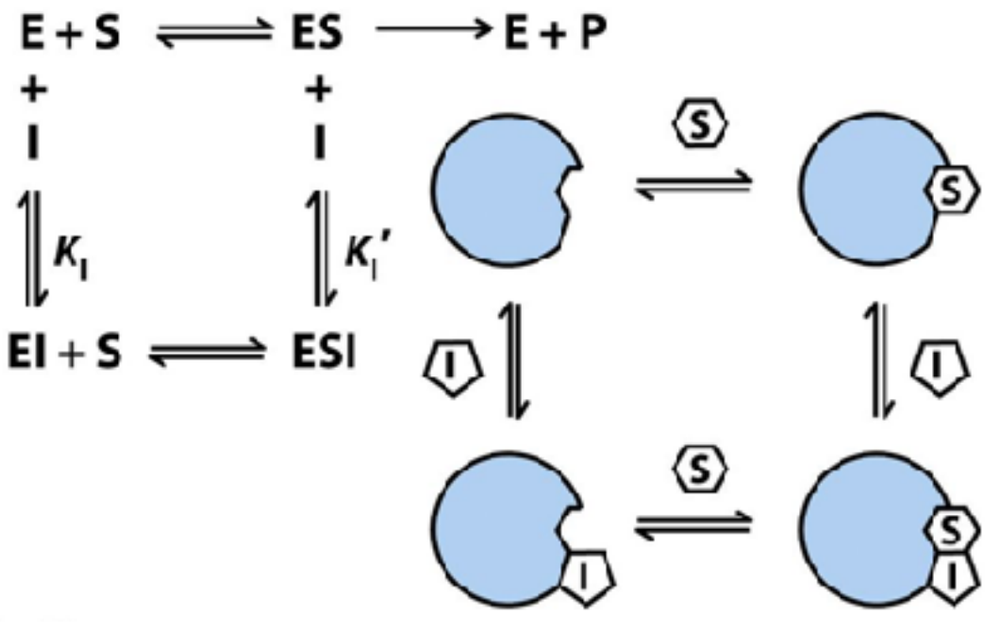
When [S] far exceeds [I], the probability that an inhibitor will bind to the enzyme is minimized and the reaction exhibits a normal V_{max}. However, the [S] at which V_0 = 1/2 V_{max}, the apparent K_m, increases in the presence of inhibitor by the factor a. This affect on apparent K_m, combined with the absence of an effect on V_{max}, is diagnostic of competitive inhibition and is readily revealed in a double-reciprocal kinetic plot. The equilibrium constant for inhibitor binding, K_I, can also be obtained from these plots.



non-Competitive Inhibition

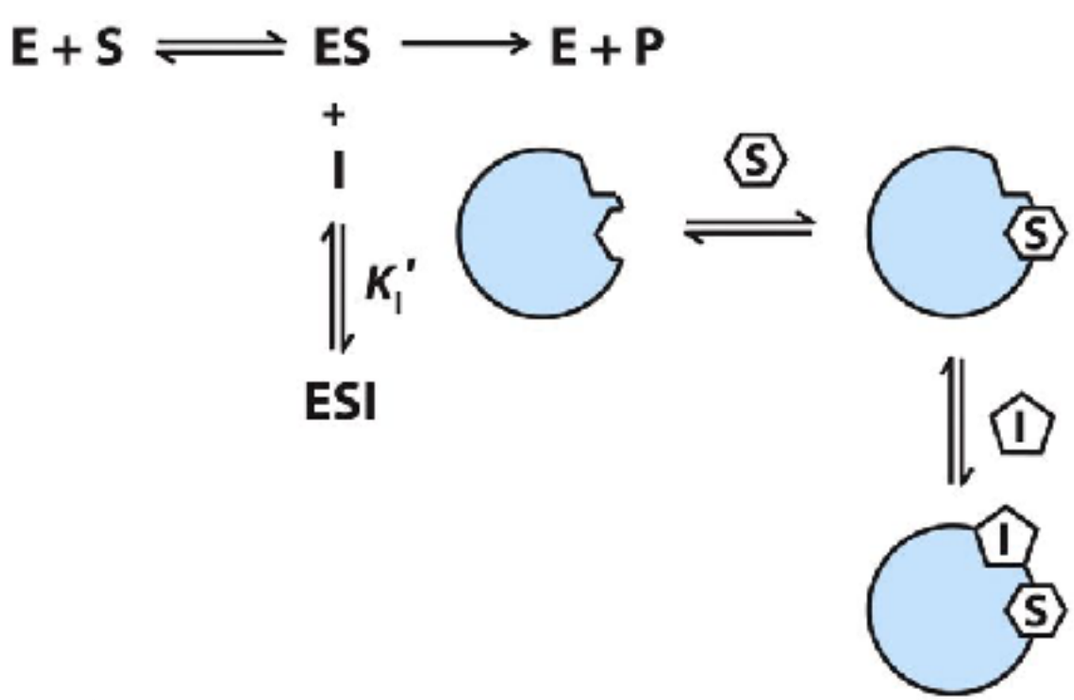
A non-competitive inhibitor (I) binds the enzyme in a different site and independently from S. It does not interfere with the binding of S to E but reduces the [ ] of active ES. And thus the V_{max} competes with no effect on the K_m





Uncompetitive Inhibition

An uncompetitive inhibitor (I) binds the enzyme in a different site but it does bind only the ES complex. It does not interfere with the binding of S to E but reduces the [ ] of active ES

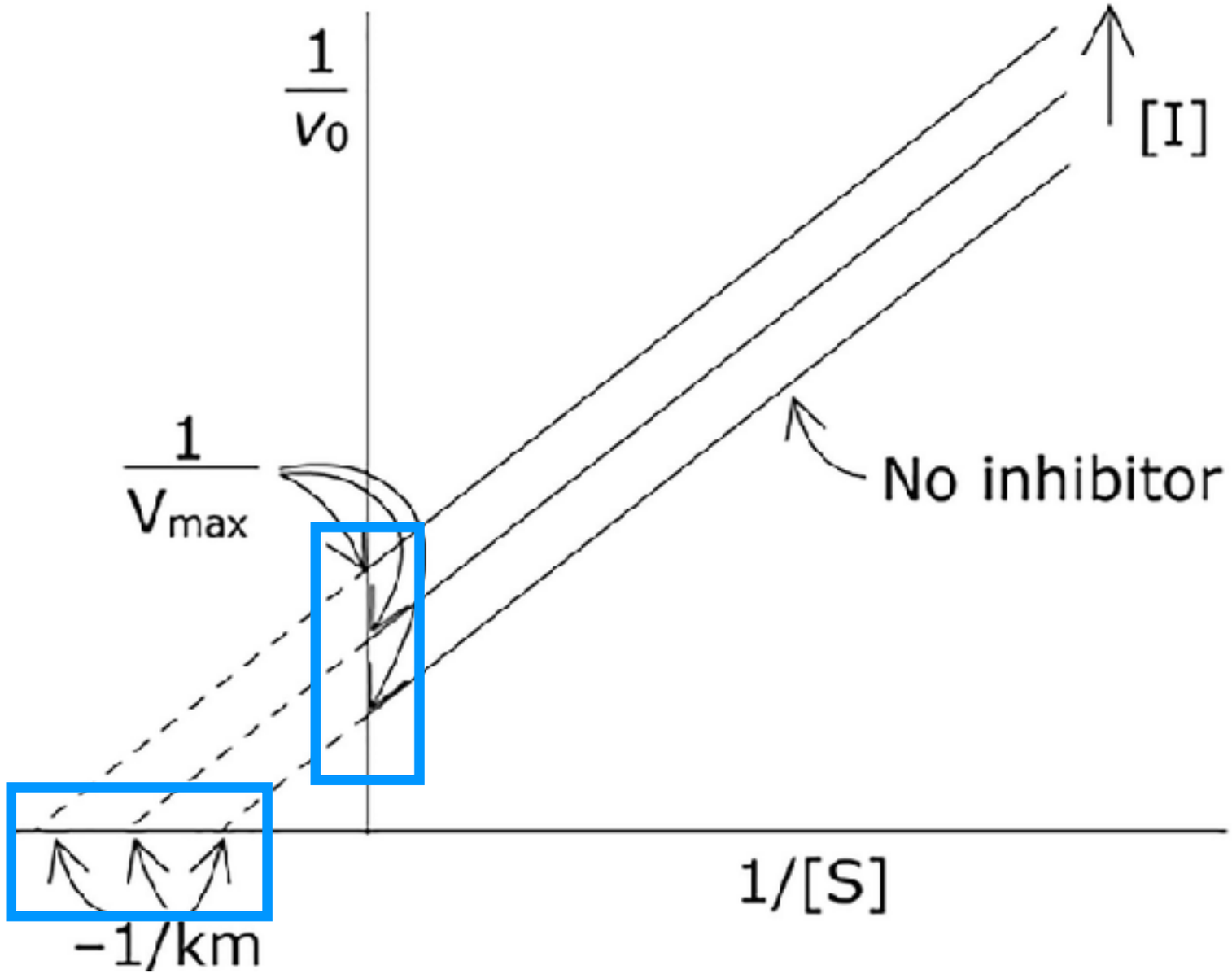


$$V_0 = \frac{V_{max}[S]}{\alpha[S] + K_m}$$

$$\alpha = 1 + \frac{[I]}{k_I}$$

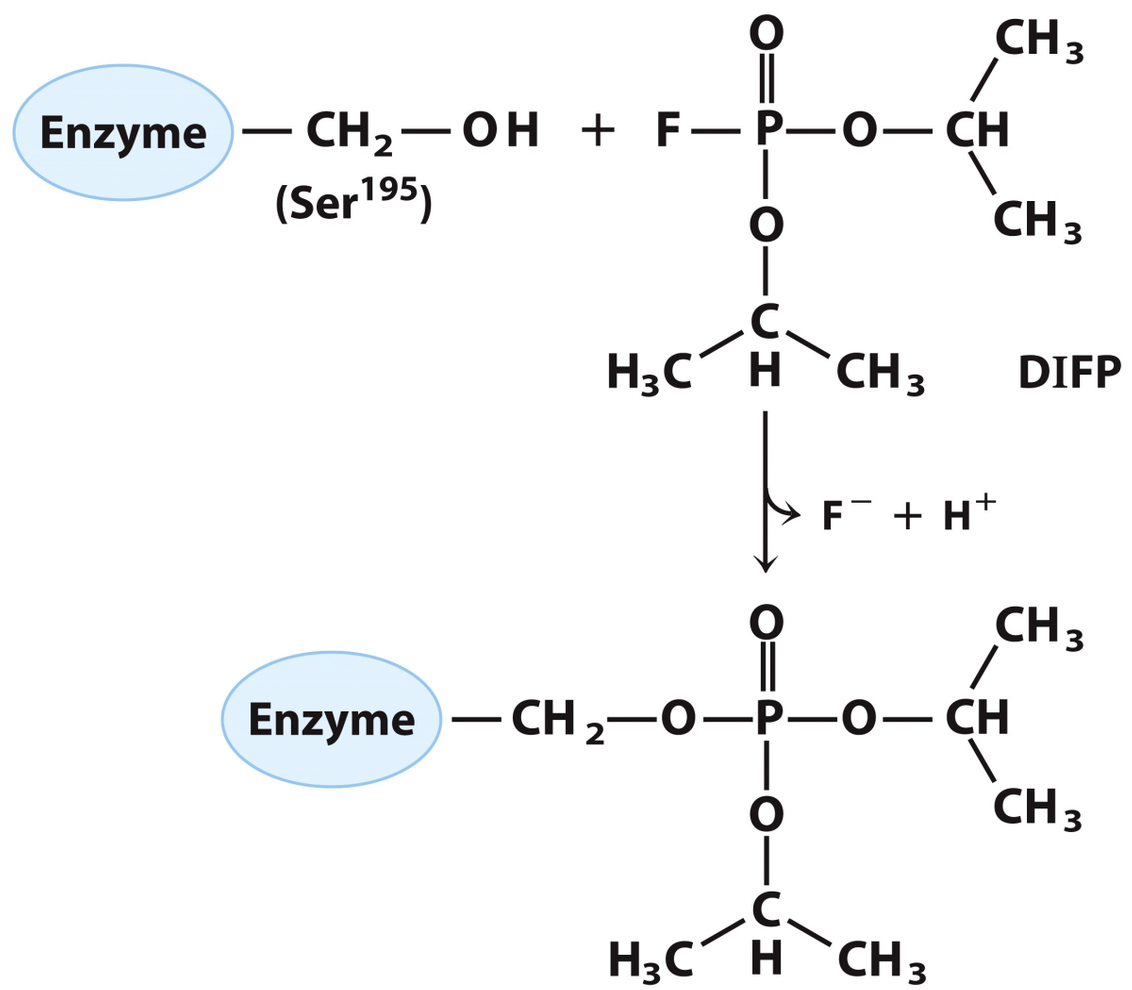
$$k_I = \frac{[ES]\,[I]}{[ESI]}$$

Uncompetitive Inhibition



Irreversible Inhibition

Irreversible inhibitors bind covalently to or destroy a functional group on an enzyme that is essential for the enzyme's activity. They also can inhibit an enzyme by forming a particularly stable noncovalent association with the enzyme. An example of a irreversible covalent inhibitor of the protease, chymotrypsin. Chymotrypsin contains a reactive serine residue in its active site that is intimately involved in catalysis of peptide bond cleavage. This serine will react with the inhibitor diisopropylfluorophosphate (DIFP) which modifies the serine residue irreversibly, and thereby inhibits the proteolytic activity of the enzyme.

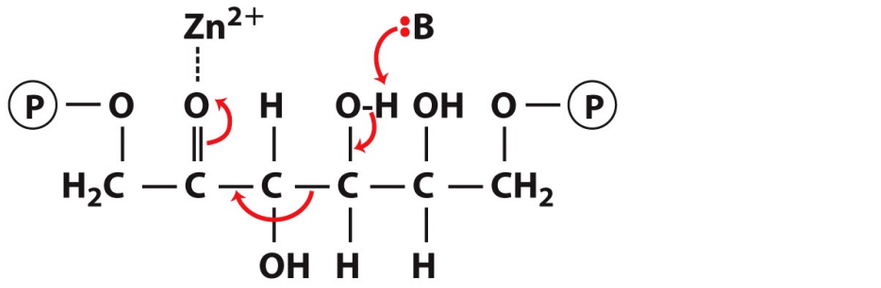


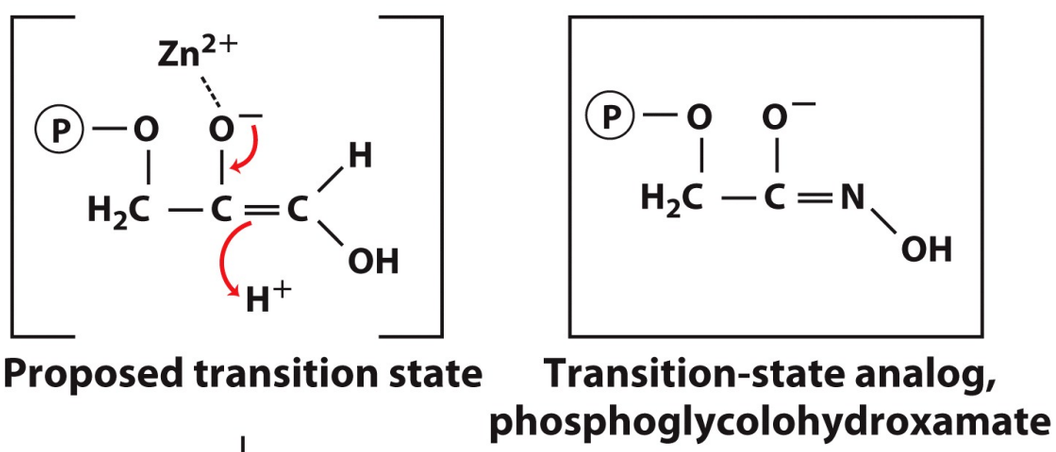
Suicide Inhibition

A special class of irreversible inhibitors are the mechanism-based (suicide) inactivators. These compounds are relatively unreactive until they bind to the active site of a specific enzyme. A suicide inactivator undergoes the first few chemical steps of the normal enzymatic reaction, but instead of being transformed into the normal product, the inactivator is converted into a very reactive compound that combines irreversibly with the enzyme. These inhibitors earn their name because they hijack the normal enzyme reaction mechanism to inactivate the enzyme. Because drugs that serve as mechanism-based inactivators are highly specific for their target enzymes, they often have the advantage of few side effects.

Transition-state Analogs

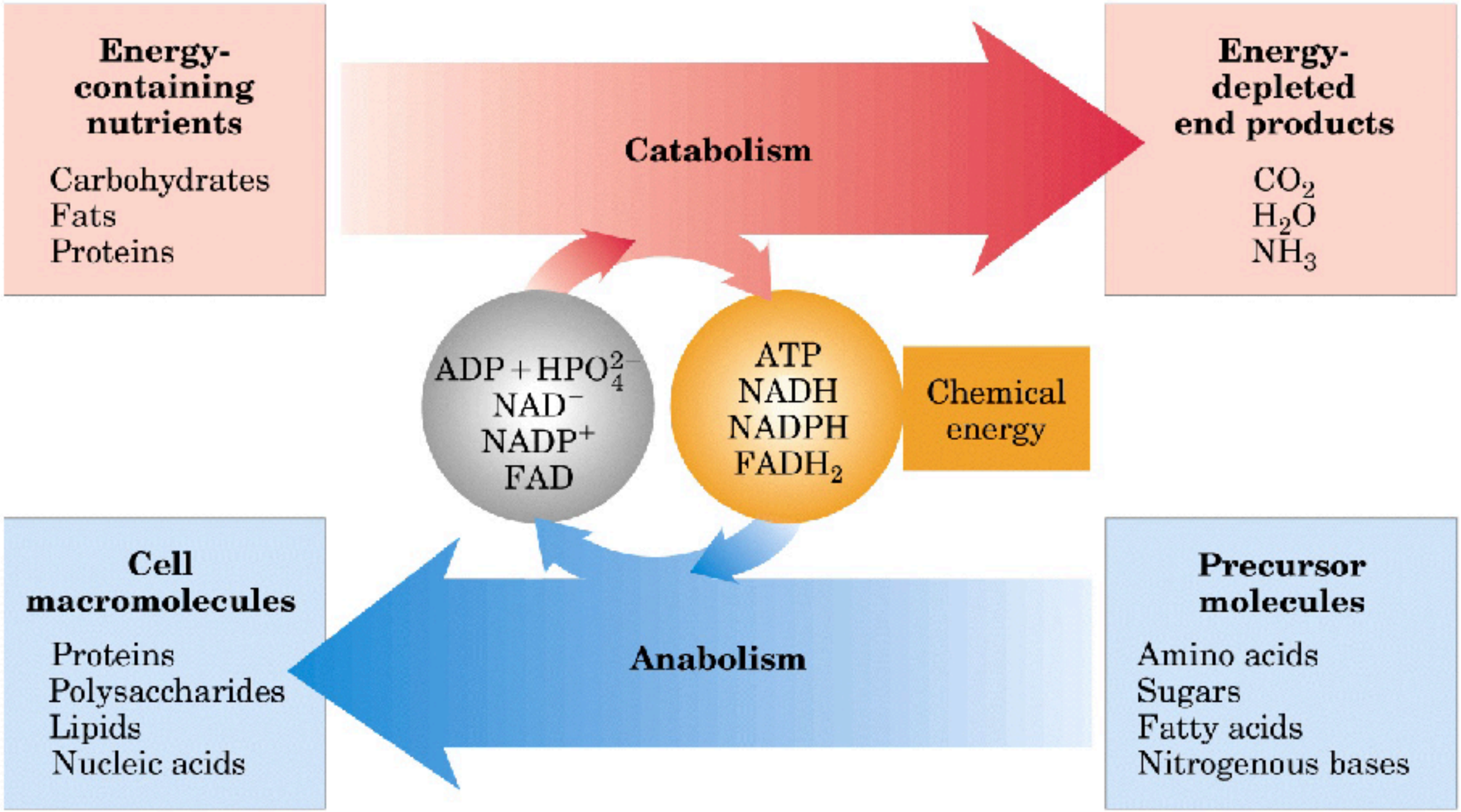
These inhibitors commonly resemble the predicted transition state structure of the reaction and are called transition-state analogs. These compounds bind more tightly to an enzyme than the substrate because they fit into the active site better. For example, transition state analogs designed to inhibit the glycolytic enzyme aldolase bind to that enzyme more than four order of magnitude more tightly than its actual substrates. Observations that such molecules are effectively irreversible inhibitors of enzymes, support the concept that enzyme active sites are most complementary to that of the transition state of the reaction.



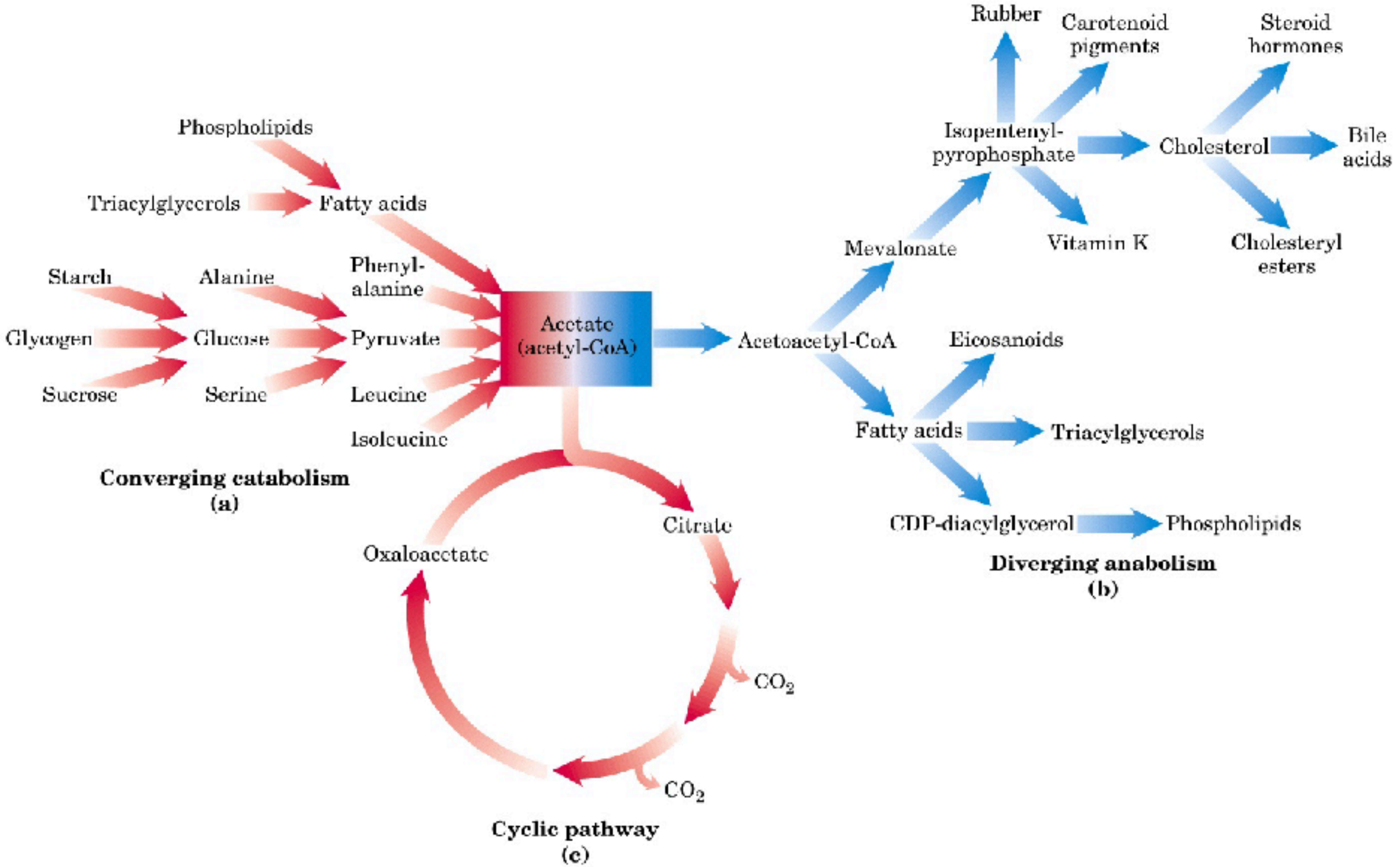


10’ Break

Catabolism <=> Anabolism



Convergence <-> Divergence



The second law

The second law of thermodynamics states that the total entropy of an isolated system can never decrease over time. Isolated systems spontaneously evolve towards **thermodynamic** equilibrium, the state with **maximum entropy**.

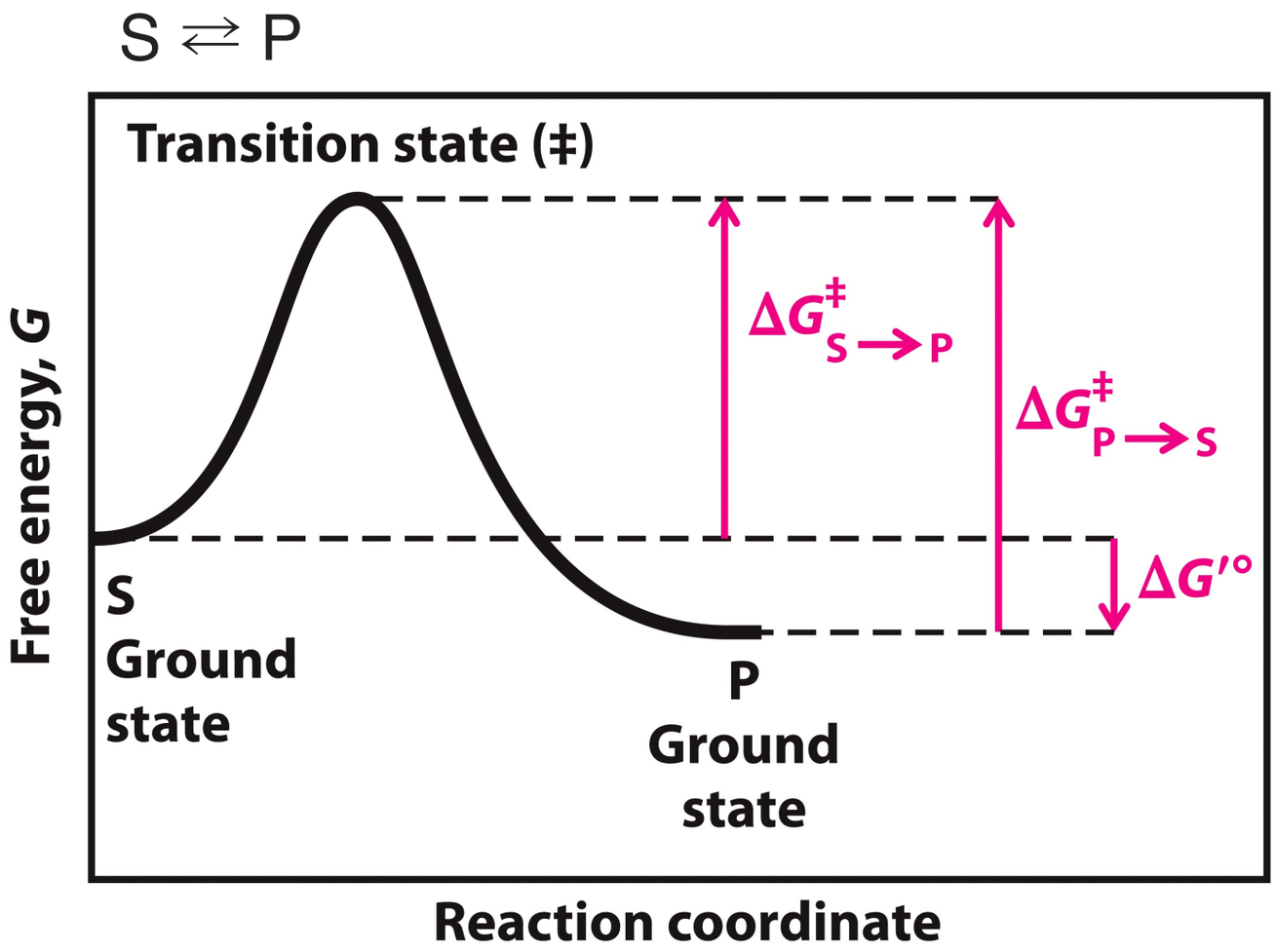
In a chemical reaction entropy increases when the products of the reaction are less complex and more disordered than its substrates

Many Biochemical reactions **'produce' order** (*i.e.,* in biosynthetic pathways) which seems to contradict the second law.

The order produced by cells in their growth and division is compensated by taking **free energy** from the environment in the form of nutrients or solar light and exchanging it for heat and entropy.

How do cells exploit free energy?

Variations in ΔG are additive



In case of sequential reactions,

$A \rightleftarrows B \quad B \rightleftarrows C$

each of the two reactions will have its characteristic $\Delta G'^0$ ($\Delta G_1'^0$ and $\Delta G_2'^0$)

As the reactions are sequential we can write them in the form

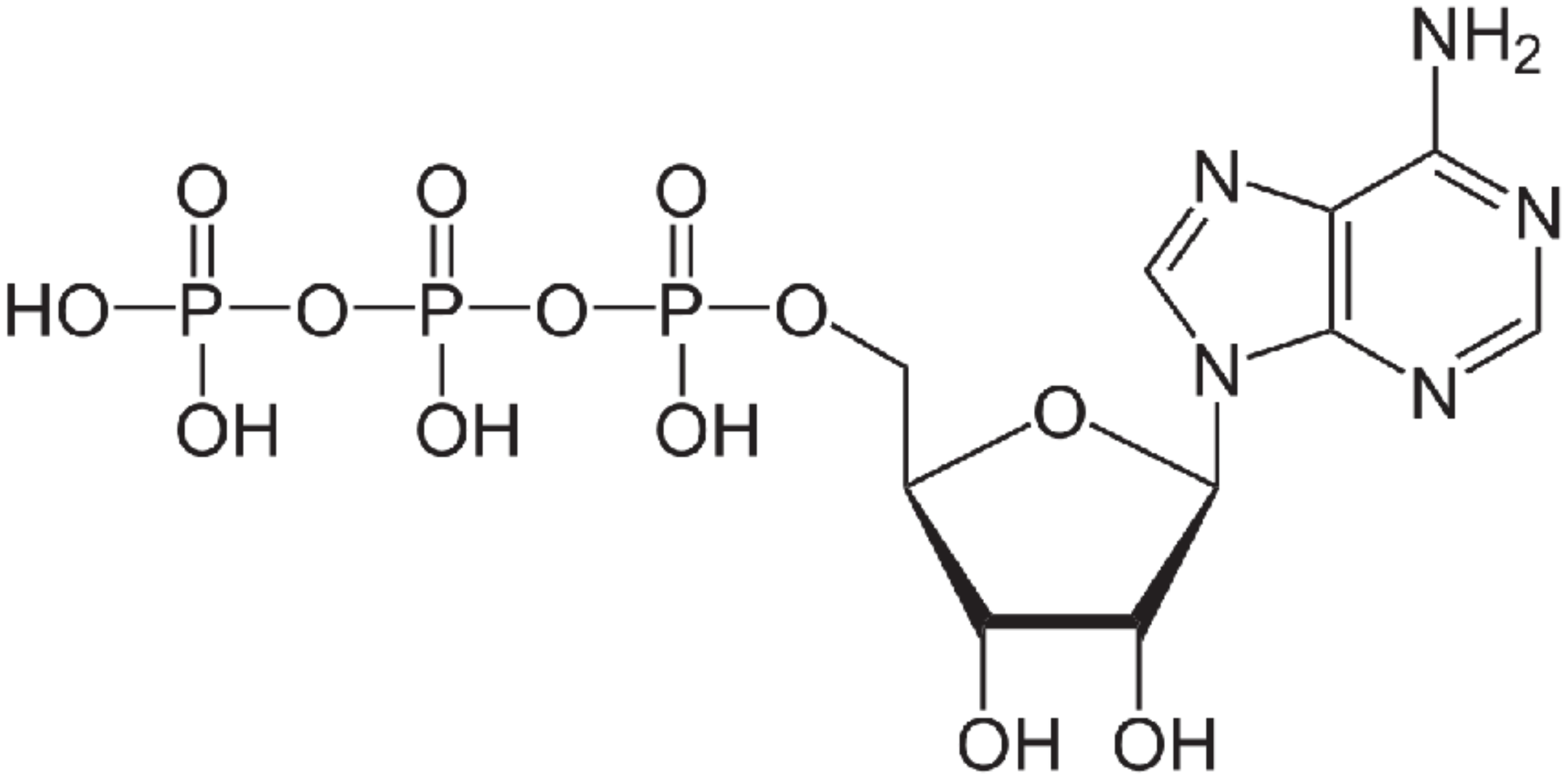
$A \rightleftarrows C$

Here the overall ΔG variation ($\Delta G_{tot}'^0$) is the sum of the $\Delta G'^0$ associated of the individual reactions:

$$\Delta G_{tot}'^0 = \Delta G_1'^0 + \Delta G_2'^0$$

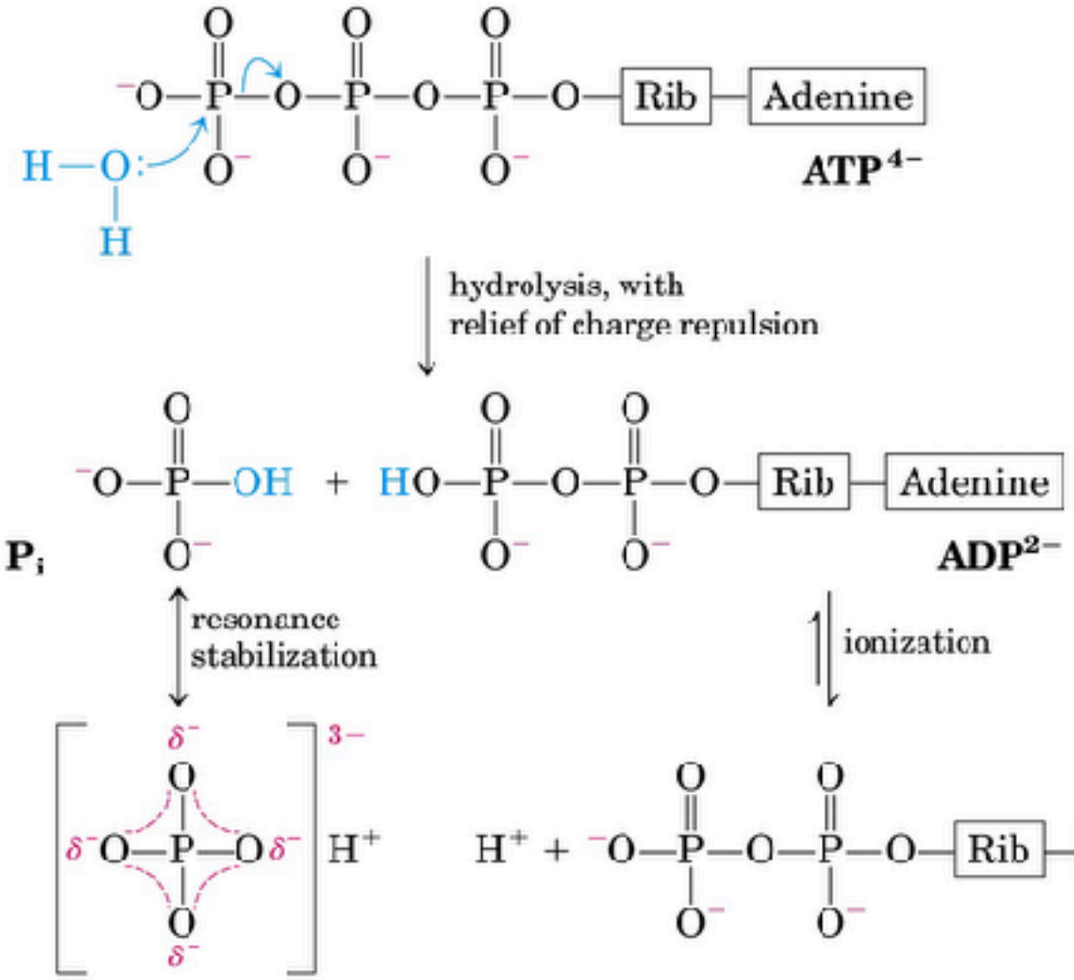
One can make an unfavourable reaction favourable by coupling it with a highly favourable reaction

the ATP



ATP: Adenosine triphosphate is the **energy currency** of the cell.

the ATP

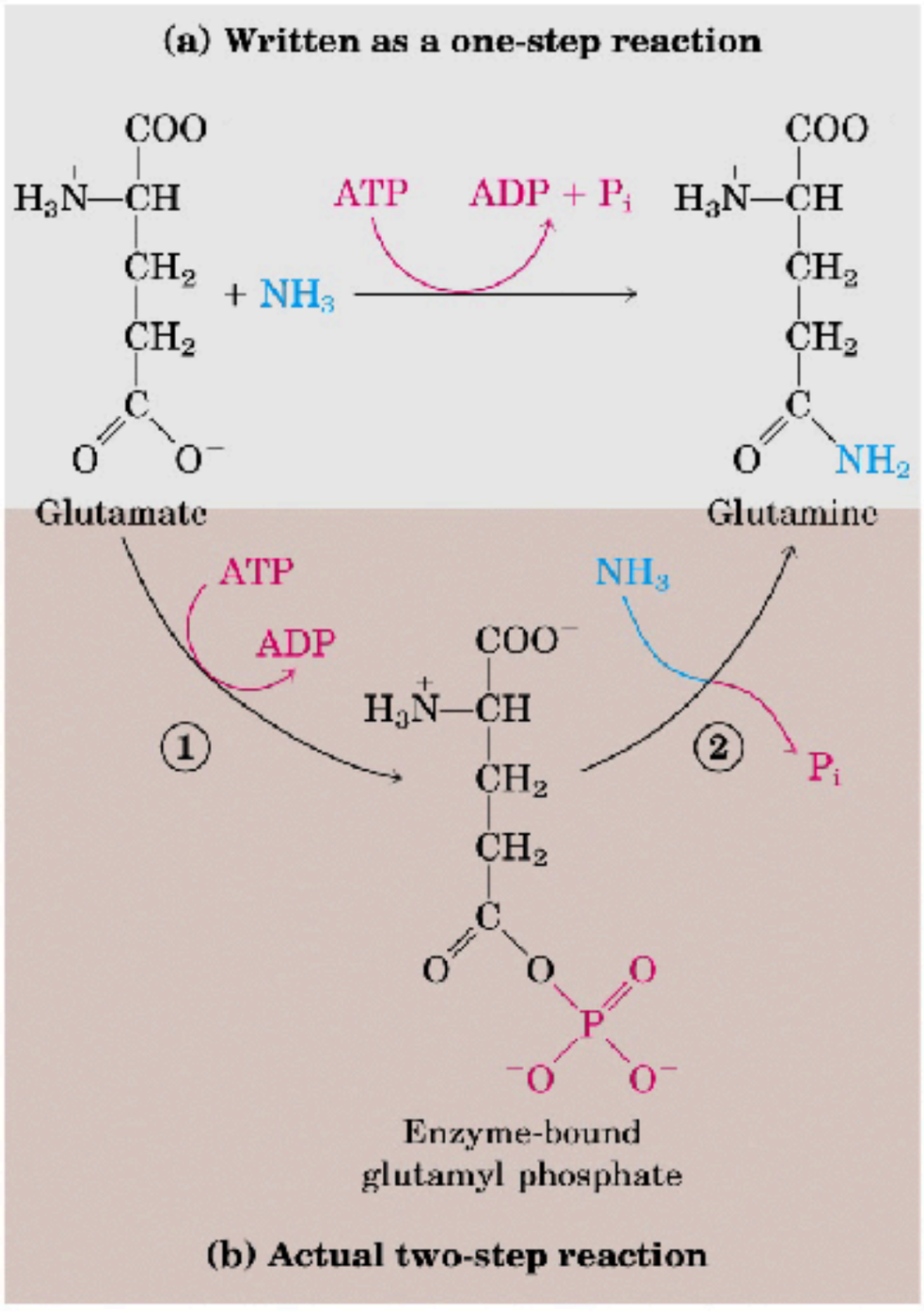


$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^\circ = -30.5\ \text{kJ/mol}$$

Why is the hydrolysis of ATP highly exergonic?

- Relieves electrostatic repulsion between the negatively charges phosphates.
- Inorganic phosphate can be stabilized by resonance hybrid.
- ADP2- can ionize.
- The products are better solvated than the reactants.

the ATP

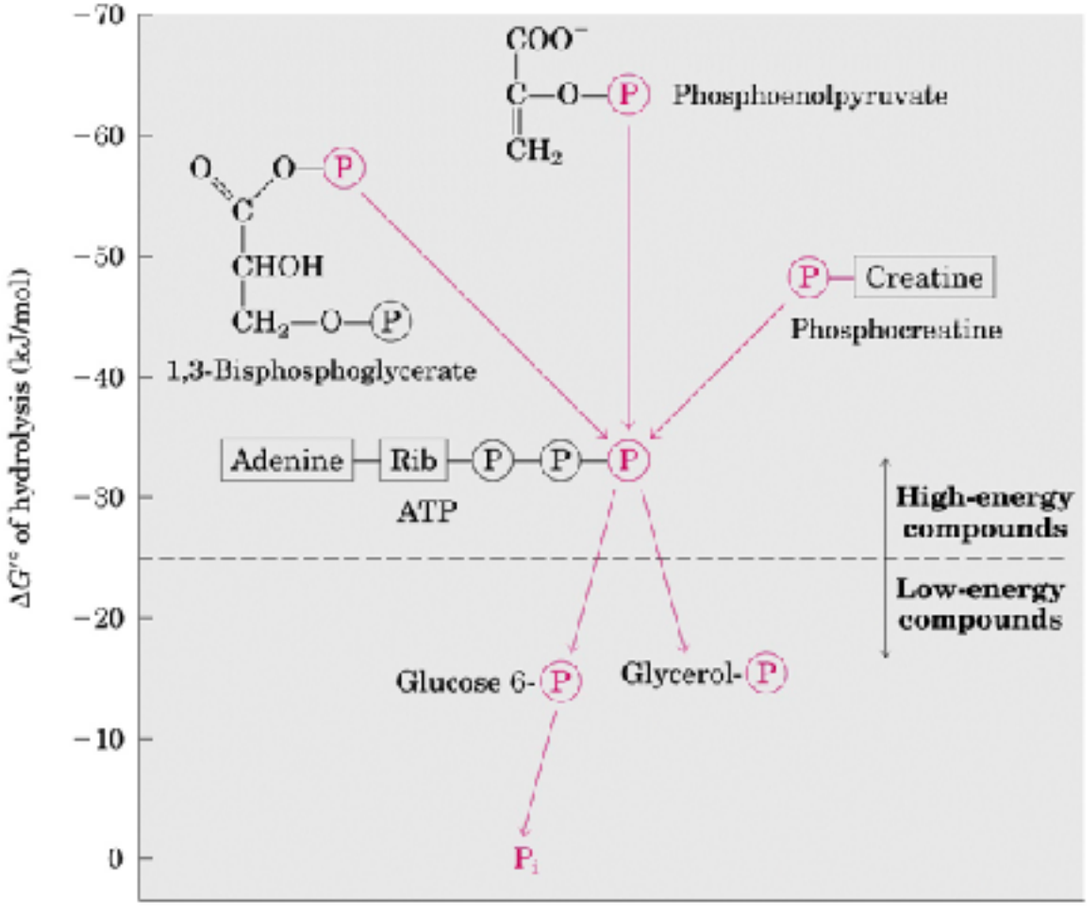


The ATP provides energy by transferring its phosphate group and not by mere hydrolysis.

Often we use to say that a given reaction is coupled to ATP hydrolysis which provides the energy required for the reaction to happen.

ATP hydrolysis per se only produces heat. In many reactions ATP is used as a phosphate donor to a substrate that, once phosphorylated, acquires an higher free energy.

High energy phosphorylated compounds



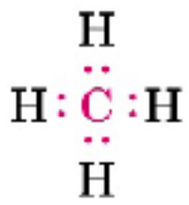
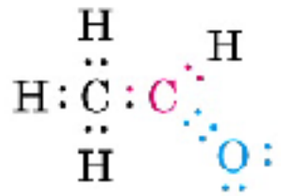
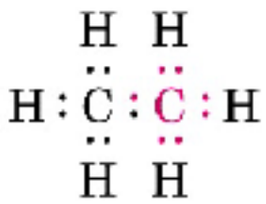
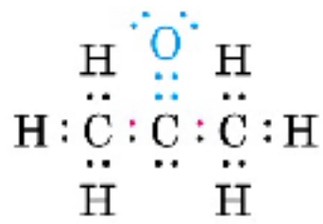
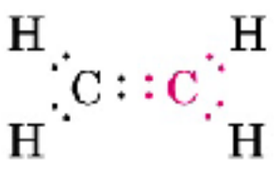
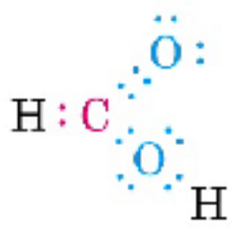
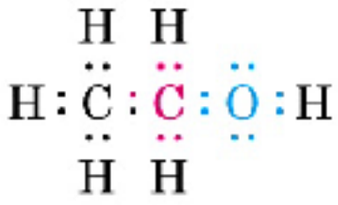
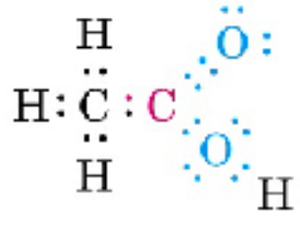
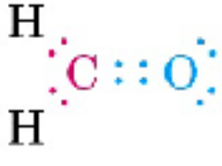
Phosphoenolpyruvate	−61.9
1,3-bisphosphoglycerate (⟶ 3-phosphoglycerate + P_i)	−49.3
Phosphocreatine	−43.0
ADP (⟶ AMP + P_i)	−32.8
ATP (⟶ ADP + P_i)	−30.5
ATP (⟶ AMP + PP_i)	−45.6
AMP (⟶ adenosine + P_i)	−14.2
PP_i (⟶ $2P_i$)	−19
Glucose 1-phosphate	−20.9
Fructose 6-phosphate	−15.9
Glucose 6-phosphate	−13.8
Glycerol 1-phosphate	−9.2
Acetyl-CoA	−31.4

How to produce Hi-NRG compounds

Biological Oxidation-Reduction

- The flow of electrons can do it.
- Electrons flow from a reducing agent to an oxidizing agent due to their different electron affinities.
- This difference in affinities is called the electromotive force (emf).
- The reducing agent undergoes oxidation and the oxidized undergoes reduction.

Electronegativity series: O > N > S > C > H

Methane		8	Acetaldehyde (aldehyde)		3
Ethane (alkane)		7	Acetone (ketone)		2
Ethene (alkene)		6	Formic acid (carboxylic acid)		2
Ethanol (alcohol)		5	Carbon monoxide		2
Acetylene (alkyne)		5	Acetic acid (carboxylic acid)		1
Formaldehyde		4	Carbon dioxide		0

Dehydrogenation = Oxidation

- Carbon is less electronegative than all atoms it is bound to, except hydrogen.

- Thus all atoms that bind to carbon oxidize it except hydrogen.

- Thus removing a hydrogen and replacing that bond with any other atom (including carbon) is synonymous with oxidation.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$\Delta G'^0 = -2{,}840 \text{ kJ/mol}$$

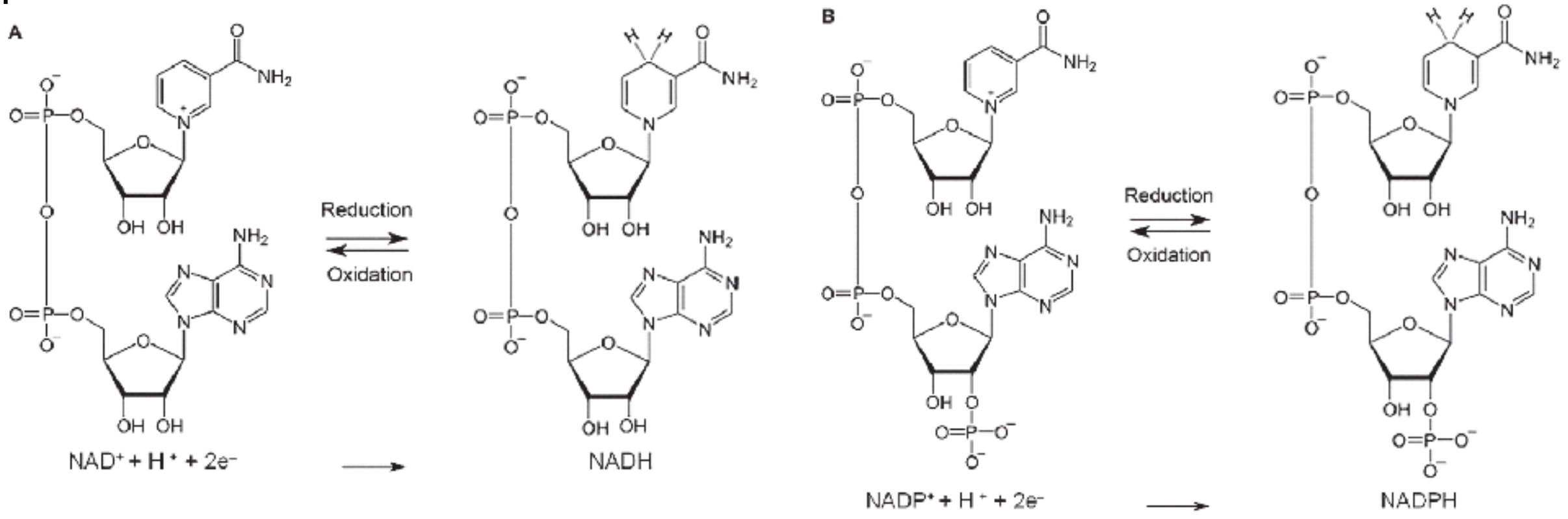
How to store/use this free NRG

Electron Carriers

NAD^+, $NADP^+$, FAD & FMN

Electron carriers can undergo reversible oxidation-reduction cycles. Their reduction allows to store the free energy deriving from the oxidation of substrates.

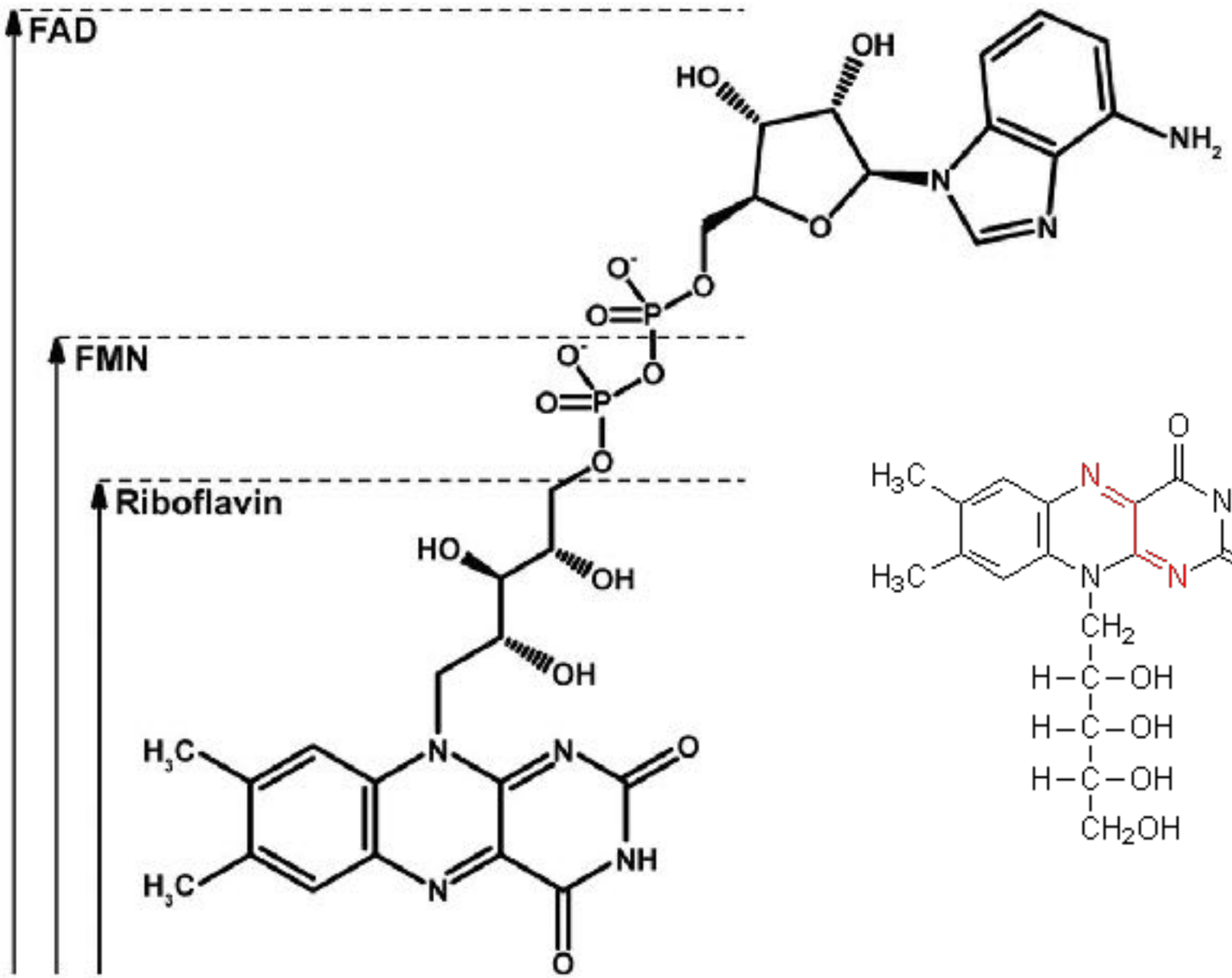
Electron carriers include specific nucleotide-based molecules, quinones, and proteins.



Nicotinamide adenine dinucleotide (NAD) NAD-phosphate (NADP)

Electron Carriers

NAD^+, $NADP^+$, FAD & FMN



Flavin adenine dinucleotide (FAD) Flavin mononucleotide (FMN)

Enzymes II - Take Home Messages

- Enzymes Kinetics describe how reactions change in response to changes in experimental parameters
- Concepts of Vo, Vmax KM, kcat and specificity constant
- The MM equation and its derivation
- the principle of reversible and irreversible Enzyme Inhibition

- Cell metabolism can be divided in Catabolic and Anabolic phases that involve converging/ cyclic and diverging pathways respectively
- ATP is the energy currency of the cell
- ATP can be produced by oxidation of nutrients
- Oxidative reactions require electron carriers such as NAD+, NADP+, FMN, and FAD

Questions?