

# Seminar 9: Enzyme kinetics

# Enzyme kinetics is similar to the kinetics you already know

In both, kinetics and enzyme kinetics we will study the speed of  
chemical reactions



$$V = -\frac{dA}{dt} = \frac{dB}{dt}$$

# Enzyme kinetics is similar to the kinetics you already know

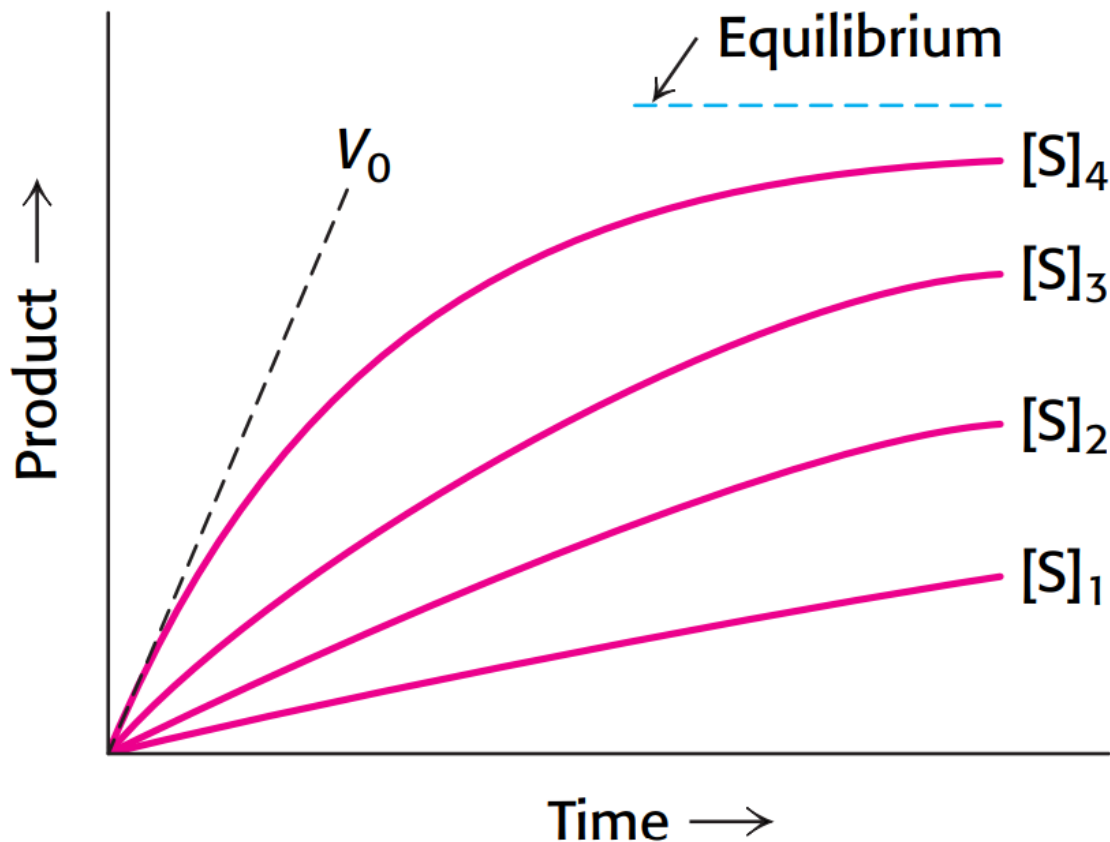
In both, kinetics and enzyme kinetics we will study the speed of  
chemical reactions

In enzyme kinetics **substrates (S)** are transformed into **products (P)**



$$V = -\frac{dS}{dt} = \frac{dP}{dt}$$

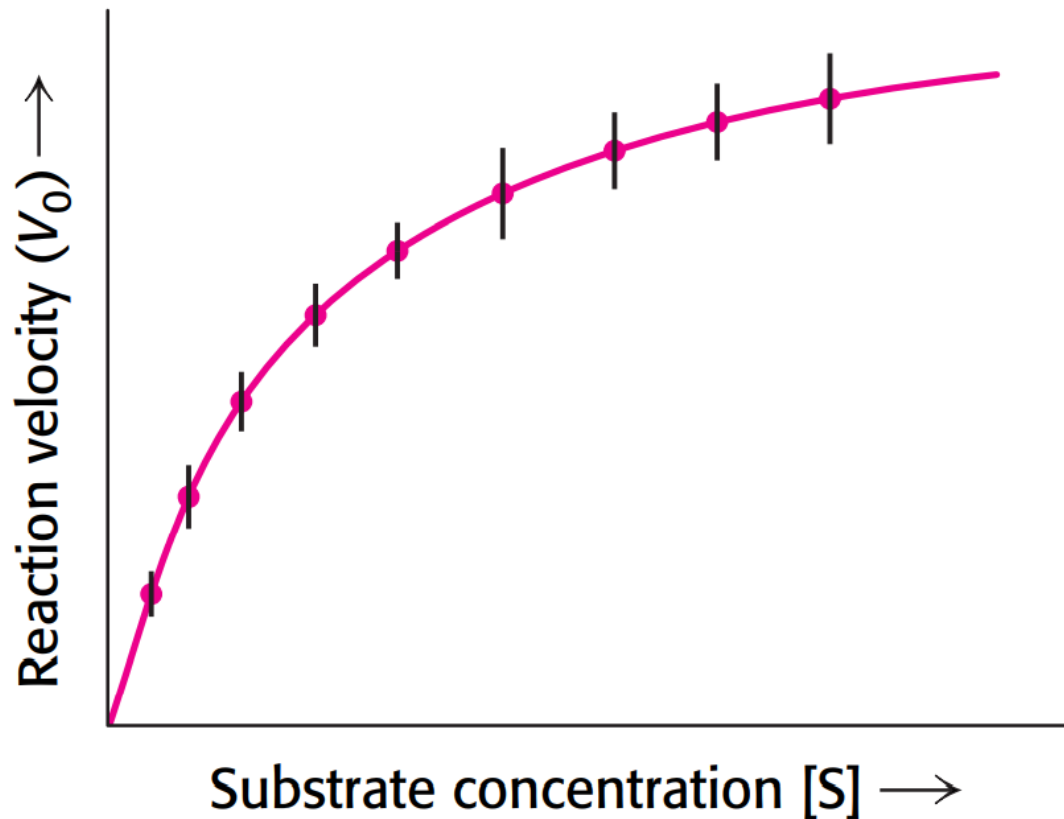
Given a constant amount of enzyme, we see how the initial speed of a reaction depends on the concentration of substrate



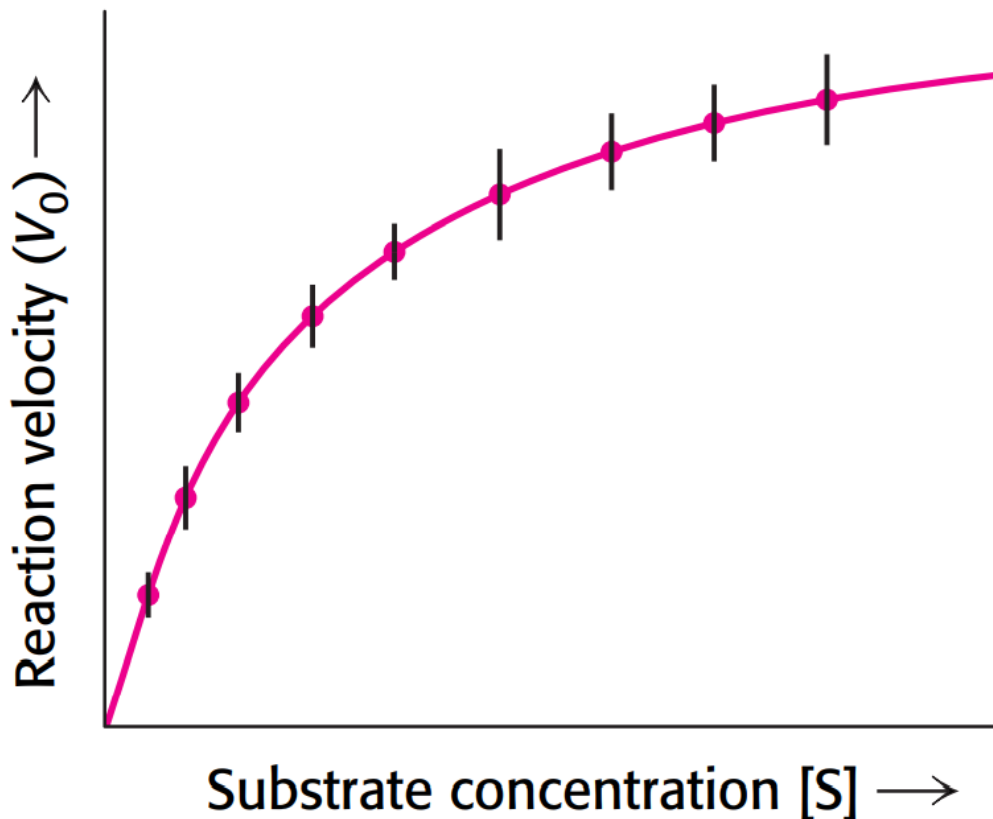
The slope of these curves is the speed of the reaction, because:

$$V = \frac{dP}{dt}$$

If we increase the amount of substrate, we see how the speed of the reaction goes up, until we reach maximum speed



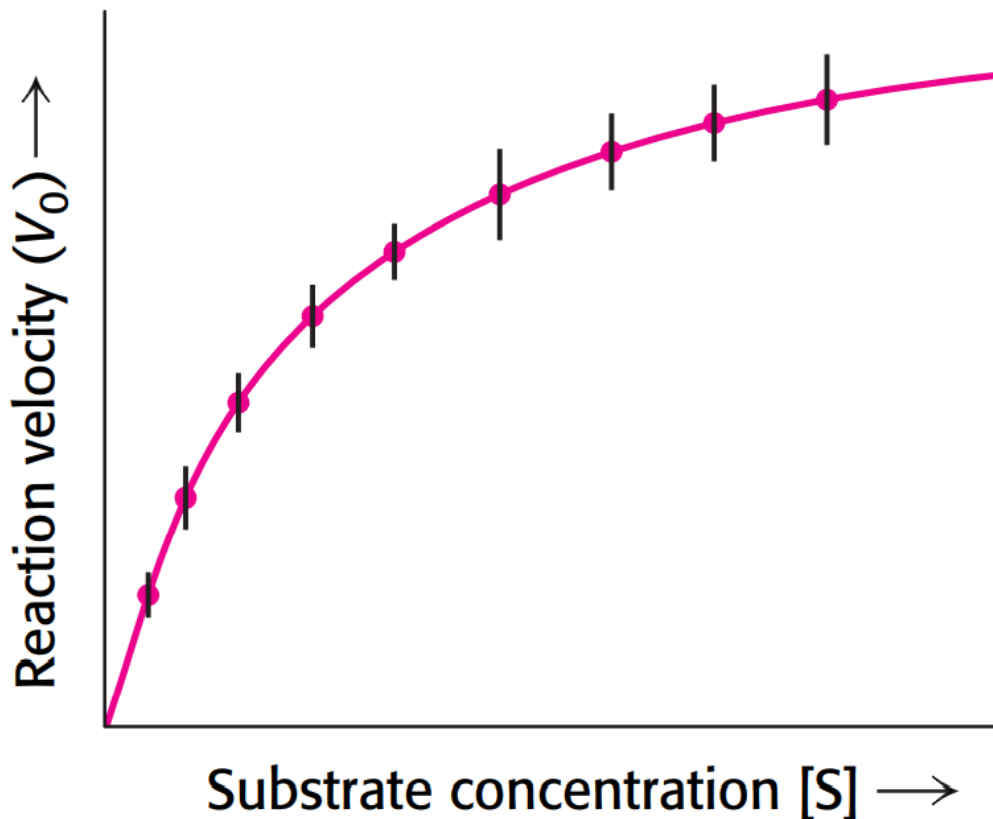
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**Why the speed of the reaction stops increasing while we increase substrate concentration?**

**How would you improve maximum speed?**

**If we increase the amount of substrate, we see how the speed of the reaction goes up, until we reach maximum speed**



At maximum speed all enzyme molecules are occupied by substrate.

The reaction cannot go any faster unless you add more enzyme molecules to the system.

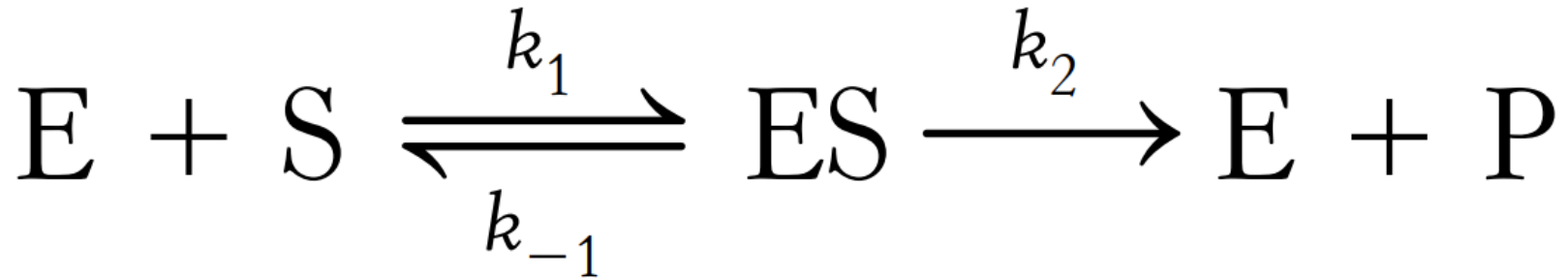
If we increase the amount of substrate, we see that the reaction rate increases.

**To explain this behaviour,  
Michaelis and Menten  
developed their model for  
enzyme kinetics**

substrate concentration  $[S] \rightarrow$



# Michaelis Menten model for enzyme kinetics

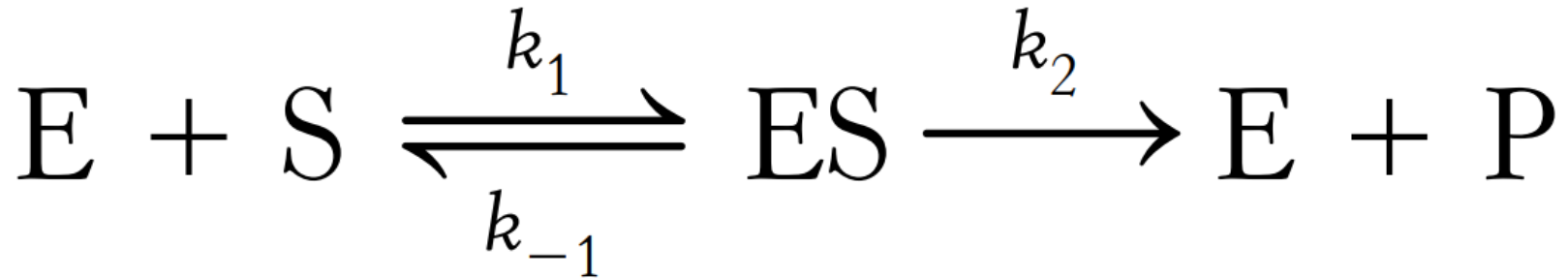


In chemical kinetics we saw that we can express the speed of a reaction as the product of the reactants and a constant:



$$V = k \cdot [\text{A}]^x \cdot [\text{B}]^y$$

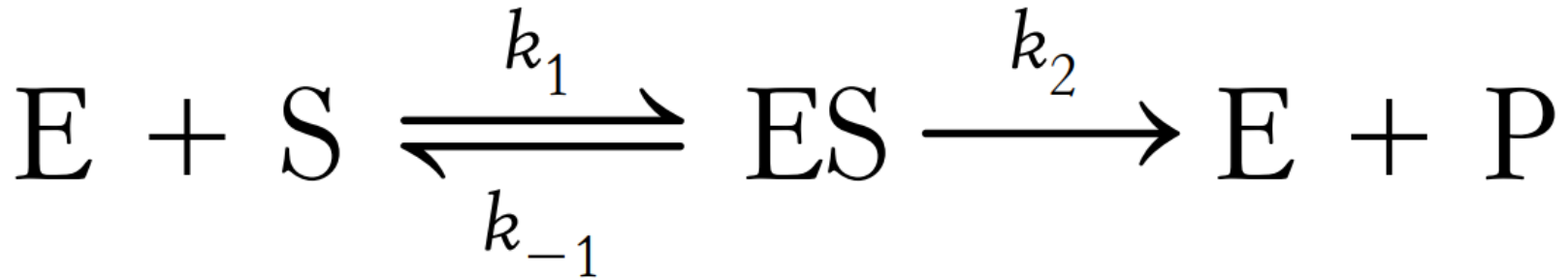
# Michaelis Menten model for enzyme kinetics



If we adapt the formula of classical kinetics to the Michaelis Menten system, we get the following expression:

$$V = k_2 \cdot [\text{ES}]$$

# Michaelis Menten model for enzyme kinetics

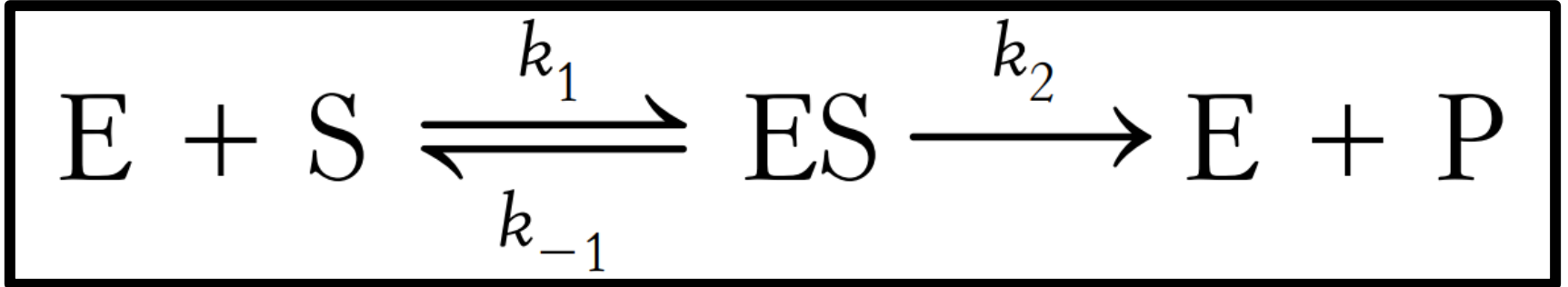


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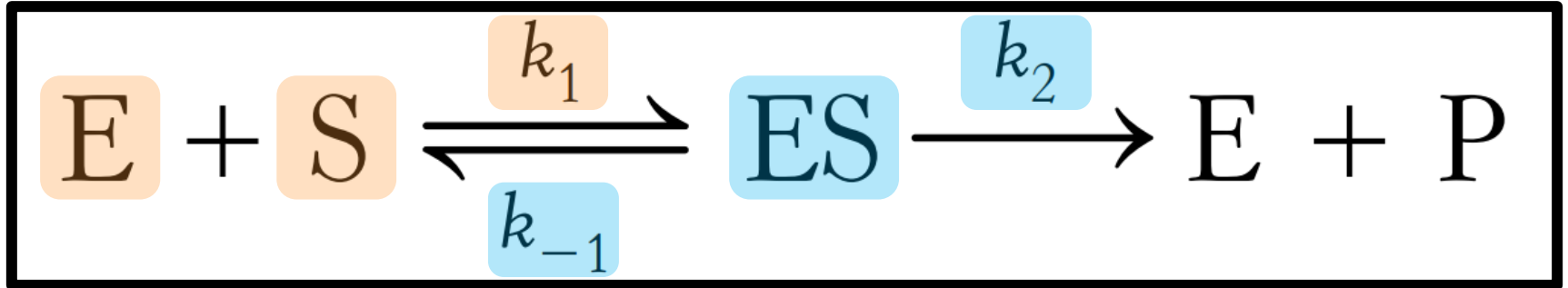
[ES] is very hard to know, so we will try to express this value as a combination of other values that we can know

# Michaelis Menten model for enzyme kinetics



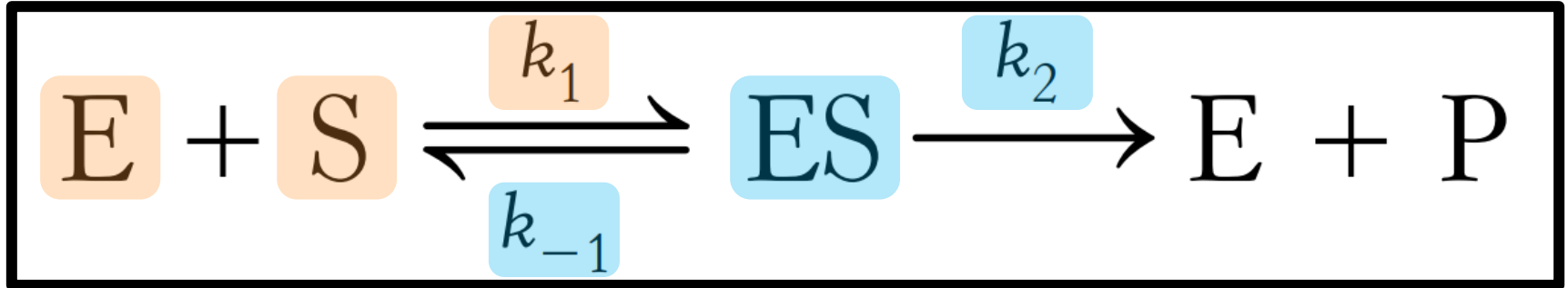
To quantify [ES] we can consider what elements in the system contribute to the **formation** of ES and the **decomposition** of ES

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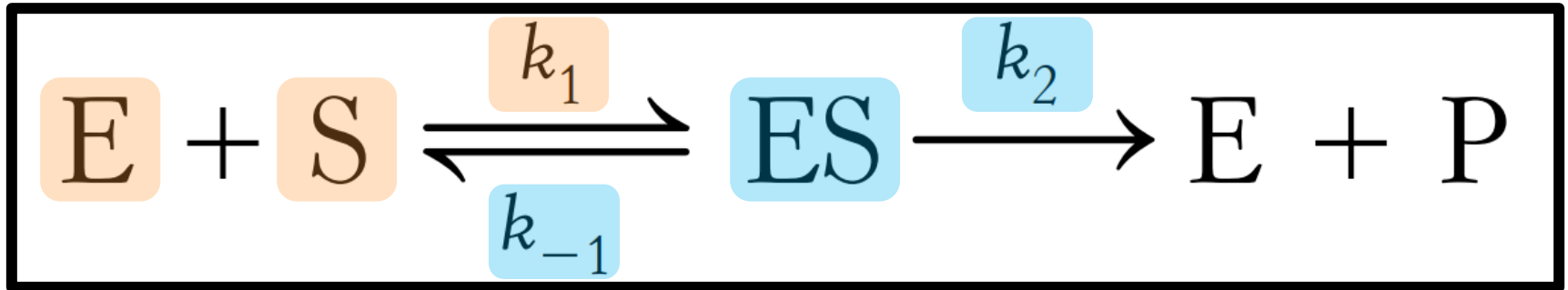
**Formation of ES:**

$$k_1 \cdot [\text{E}] \cdot [\text{S}]$$

**Decomposition of ES:**

$$k_{-1} \cdot [\text{ES}] + k_2 \cdot [\text{ES}]$$

# Michaelis Menten model for enzyme kinetics



To quantify [ES] we can consider what elements in the system contribute to the **formation** of ES and the **decomposition** of ES

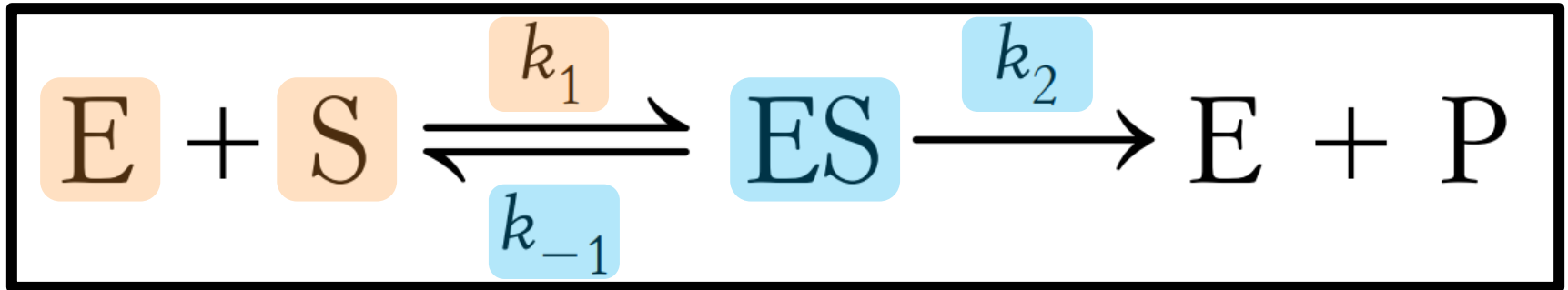
**Formation of ES:**  
 $k_1 \cdot [\text{E}] \cdot [\text{S}]$

**Decomposition of ES:**  
 $k_{-1} \cdot [\text{ES}] + k_2 \cdot [\text{ES}]$

We are going to combine these two equations by assuming that the chemical system is in a moment where formation and decomposition of

ES is in equilibrium. Therefore,  $\frac{d[\text{ES}]}{dt} = 0$

# Michaelis Menten model for enzyme kinetics



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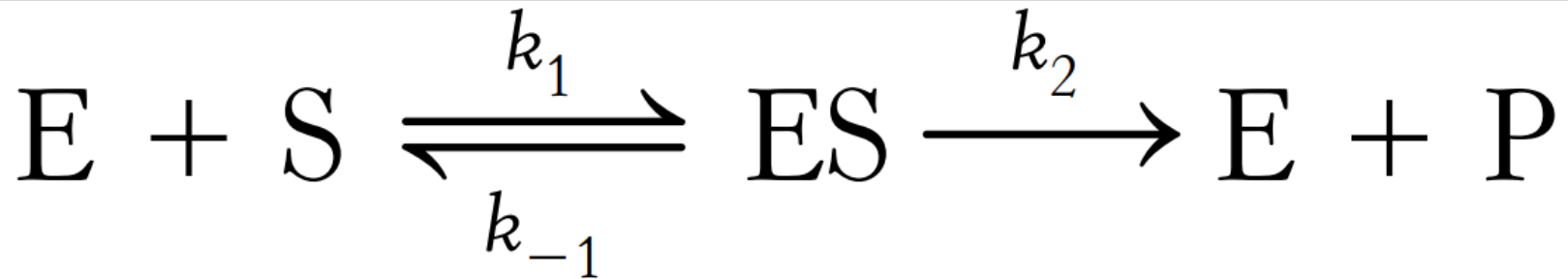
**Formation of ES:**  
 $k_1 \cdot [\text{E}] \cdot [\text{S}]$

**Decomposition of ES:**  
 $k_{-1} \cdot [\text{ES}] + k_2 \cdot [\text{ES}]$

$$\frac{d[\text{ES}]}{dt} = 0 = k_1 \cdot [\text{E}] \cdot [\text{S}] - K_{-1} \cdot [\text{ES}] - K_2 \cdot [\text{ES}]$$



# Michaelis Menten model for enzyme kinetics



$$\frac{d[ES]}{dt} = 0 = k_1 \cdot [E] \cdot [S] - K_{-1} \cdot [ES] - K_2 \cdot [ES]$$

$$0 = k_1 \cdot [E] \cdot [S] - [ES] \cdot (K_{-1} + K_2)$$

$$\frac{[E] \cdot [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = KM$$

# The Michaelis constant ( $K_M$ )

$K_M$  is a ratio of concentrations, but it is also the concentration of substrate at which the reaction takes place at 50% of maximum speed

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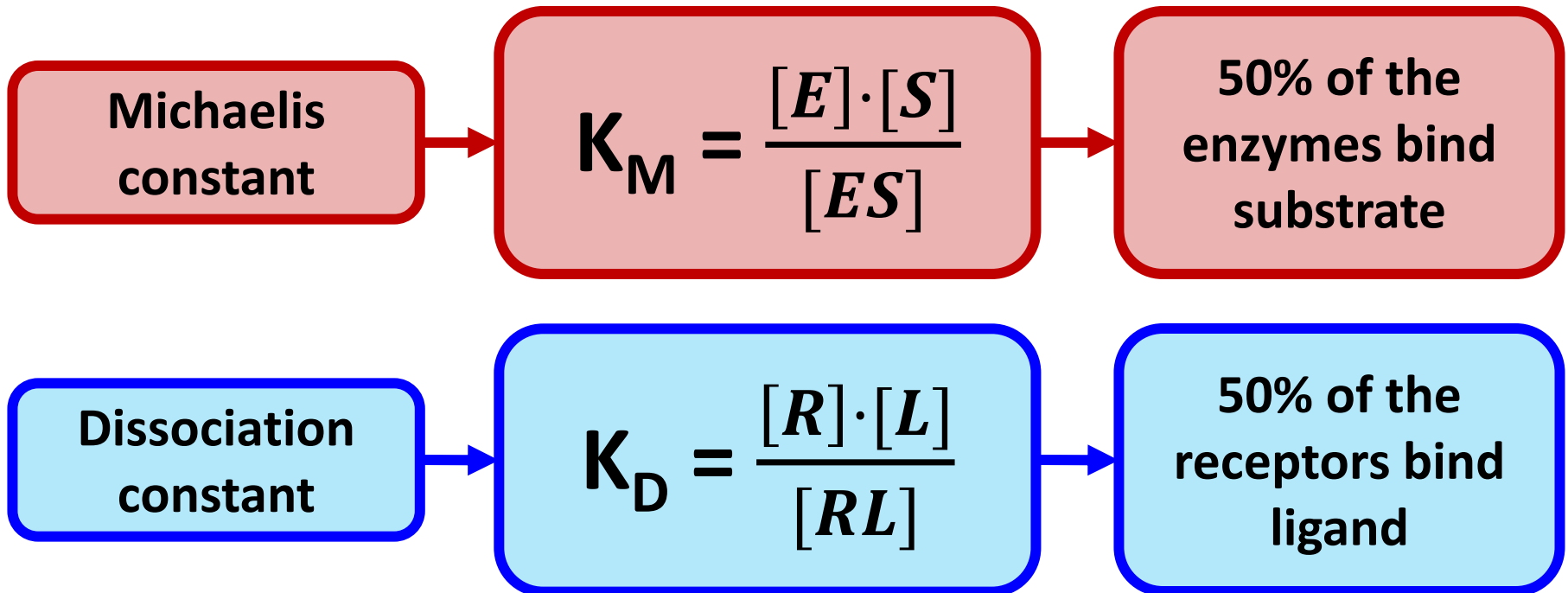
Dissociation  
constant

$$K_D = \frac{[R] \cdot [L]}{[RL]}$$

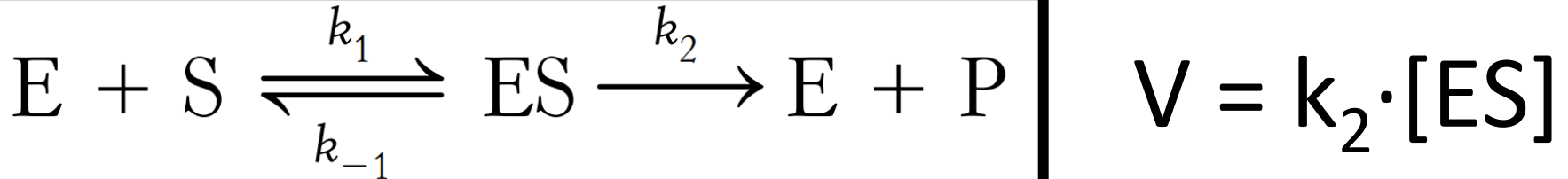
# The Michaelis constant ( $K_M$ )

$K_M$  is a ratio of concentrations, but it is also the concentration of substrate at which the reaction takes place at 50% of maximum speed

As it happened with the dissociation constant, the Michaelis constant is a measure of the affinity between the enzyme and the substrate



# Michaelis Menten model for enzyme kinetics



Remember that we are still trying to express [ES] as something we can measure easily, we can apply the following operation:

$$K_M = \frac{[E] \cdot [S]}{[ES]} \longrightarrow [ES] = \frac{[E] \cdot [S]}{K_M}$$

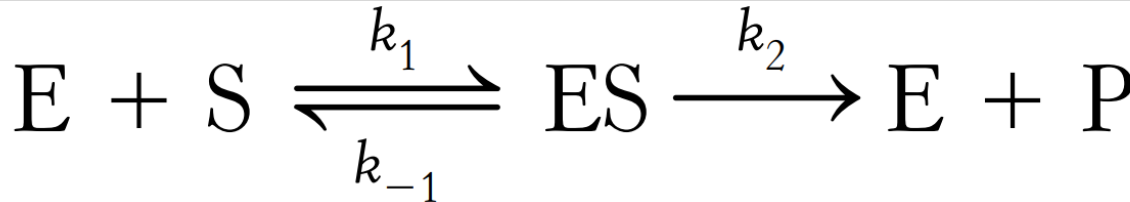
**We can estimate [S] because:**

$$[S] \gg [ES] \text{ and } [S]_{\text{total}} \approx [S]$$

**We cannot estimate [E] because:**

$$[E] \text{ similar to } [ES] \text{ and } [E]_{\text{total}} \neq [E]$$

# Michaelis Menten model for enzyme kinetics



$$V = k_2 \cdot [ES]$$

$$[ES] = \frac{[E] \cdot [S]}{K_M}$$

Then, we have to express  $[E]$  as a combination of parameters that can be easily measured:

$$[E]_{\text{total}} = [E] + [ES] \longrightarrow [E] = [E]_{\text{total}} - [ES]$$

$[E]_{\text{total}}$  is a value that we can know easily, is the concentration of total enzyme I have in my chemical system

# Michaelis Menten model for enzyme kinetics

Now we have 3 different equations that we can integrate together to come up with one expression that explains the speed of a reaction

$$V = k_2 \cdot [ES]$$

$$[ES] = \frac{[E] \cdot [S]}{K_M}$$

$$[E] = [E]_{\text{total}} - [ES]$$

$$V = k_2 \cdot \frac{([E]_{\text{total}} - [ES]) \cdot [S]}{K_M}$$

$$V = k_2 \cdot \frac{[E]_{\text{total}} \cdot [S] / K_M}{1 + [S] / K_M}$$

$$V = k_2 \cdot [E]_{\text{total}} \cdot \frac{[S]}{[S] + K_M}$$

# Michaelis Menten model for enzyme kinetics

This final formula enables us to express the speed of a reaction using concentrations and constants that can be easily measured

$$V = k_2 \cdot [E]_{total} \cdot \frac{[S]}{[S] + KM}$$



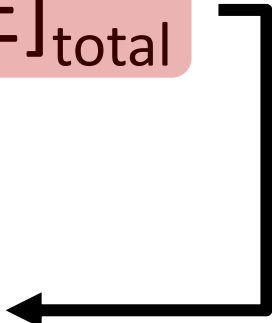
# Michaelis Menten model for enzyme kinetics

The maximum speed of the reaction will involve that  $[E]_{\text{total}}$  is involved in the reaction

$$V = k_2 \cdot [ES] \longrightarrow V_{\text{max}} = k_2 \cdot [E]_{\text{total}}$$

# Michaelis Menten model for enzyme kinetics

We can relate that to the formula of the speed of the reaction

$$V = k_2 \cdot [ES] \longrightarrow V_{\max} = k_2 \cdot [E]_{\text{total}}$$
$$V = k_2 \cdot [E]_{\text{total}} \cdot \frac{[S]}{[S] + KM}$$


# Michaelis Menten model for enzyme kinetics

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$$V = k_2 \cdot [ES] \longrightarrow V_{\max} = k_2 \cdot [E]_{\text{total}}$$

$$V = k_2 \cdot [E]_{\text{total}} \cdot \frac{[S]}{[S] + KM}$$

$$V = V_{\max} \cdot \frac{[S]}{[S] + KM}$$

# Michaelis Menten model for enzyme kinetics

See that when  $[S] = K_M$ ;  $V = V_{\max} \cdot 1/2$ .

$$V = V_{\max} \cdot \frac{[S]}{[S] + K_M}$$

$$V = V_{\max} \cdot \frac{K_M}{K_M + K_M} \longrightarrow V = V_{\max} \cdot \frac{1}{2}$$