

# Kinetic chemistry

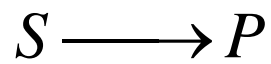
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- Definitions
  - Substrate
  - Product
  - Enzyme
- Law of mass action
  - reaction rate
  - reversible reactions
  - steady state
- Enzyme-catalyzed reactions
  - Michaelis – Menten model
  - Michaelis – Menten kinetics
  - $K_M$  significance
  - $V_{max}$  &  $k_2$  ( $k_{cat}$ ) significance
- Hill equation : allosterism
  - comparison
  - Hill coefficient
  - $K_M$  constant
- Hill eq in gene modelling

# [ Definitions ]

## ■ Substrate

- a molecule upon which an enzyme act
- is converted to a product

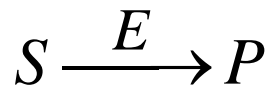


## ■ Product

- a molecule that is the result of a chemical reaction
- comes from a substrate

## ■ Enzyme

- a catalyst of a reaction
- accelerates the rate of a reaction

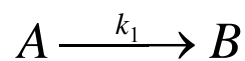


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[ ]  
Law of mass action

## [ Law of mass action ]

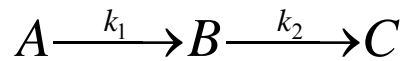
- reaction rate



$$r = \frac{-d[A]}{dt} = \frac{d[B]}{dt}$$

$$r = k_1[A]$$

rate constant

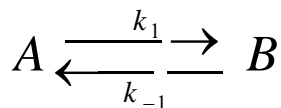


if  $k_2 \ll k_1$ , then  $B \rightarrow C$  is the **rate-determining step** of the reaction, and the reaction rate depends mainly on  $k_2$

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## [ Law of mass action ]

- reversible reactions



$$r = \frac{-d[A]}{dt} = \frac{d[B]}{dt}$$

$$r = k_1[A] - k_{-1}[B]$$

- steady state

- if  $k_1$  and  $k_{-1}$  are equal, A and B do not change in time

- then  $r = 0$

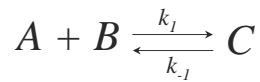
$$r = k_1[A] = k_{-1}[B]$$

$$\frac{k_{-1}}{k_1} = \frac{[A]}{[B]} = K_{eq}$$

equilibrium constant, tells us the extent of the reaction, NOT its speed.

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## [ Law of mass action ]



- we assume that the rate of forward reaction is linearly proportional to the concentrations of A and B, and the back reaction is linearly proportional to the concentration of C

$$r = \frac{-d[A]}{dt} = \frac{-d[B]}{dt} = \frac{d[C]}{dt}$$

$$r = k_1[A][B] - k_{-1}[C]$$

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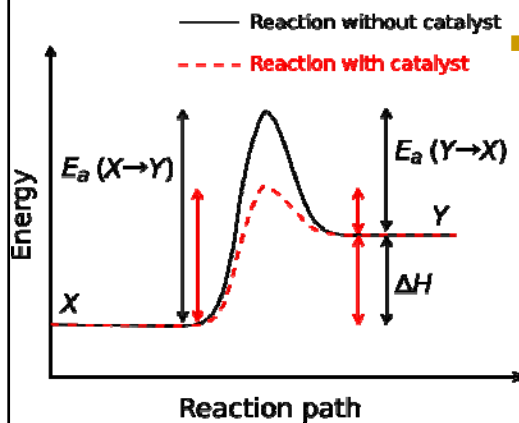
- steady state

$$k_{-1}[C] - k_1[A][B] = 0$$

$$K_{eq}[C] = [A][B], \quad K_{eq} = \frac{k_{-1}}{k_1}$$

Enzyme-catalyzed reactions :  
Michaelis – Menten model

## [ Enzyme-catalyzed reactions ]



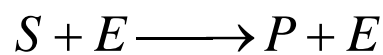
### ■ Enzymes are catalysts

- speed up the rate of a reaction
- without changing the extent of the reaction
- highly specific
- highly regulated

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## [ Enzyme-catalyzed reactions ]

- Suppose an enzyme were to react with a substrate, giving a product

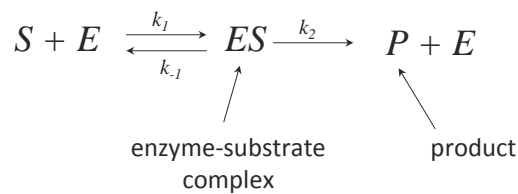


- Applying the law of mass action to this reaction, the rate of reaction would be a linearly increasing function of  $[S]$  : as  $[S]$  gets very big, so would the reaction rate

but, in reality, the reaction rate saturates...

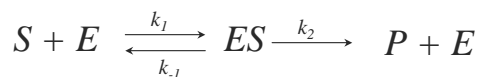
## [ Enzyme-catalyzed reactions ]

- Leonor Michaelis & Maud Menten (1913) proposed a mechanism for a saturating reaction rate
  - A specific enzyme-substrate complex is a necessary intermediate in catalysis
  - The product does not revert to the original substrates



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## [ Michaelis – Menten model ]



- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>■ affinity phase           <ul style="list-style-type: none"> <li>○ S joins active centre of E and forms ES complex</li> </ul> </li> </ul> | <ul style="list-style-type: none"> <li>■ catalysis phase           <ul style="list-style-type: none"> <li>○ transformation of S to P and recovering of E</li> <li>○ is the step that limits the reaction</li> </ul> </li> </ul> |
|---|---|

$$K_S = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$

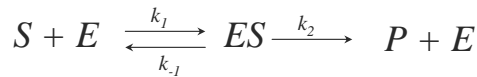
ES complex dissociation constant

$$\frac{d[P]}{dt} = k_2[ES]$$

catalytic constant ( $k_{cat}$ )

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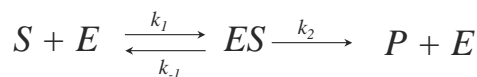
## [ Michaelis – Menten model ]



- Relates catalysis rate with substrate concentration
- Assumptions :
  1. **P** is not converted in **S**
    - true when **[P]** is very low (at the beginning of the reaction). We consider initial rates ( $V_0$ )
  2.  $k_2 < k_1, k_{-1}$ 
    - **steady state** is reached : **ES** formation rate is equal to **ES** decomposition rate
    - **[ES]** is considered constant
  3. **[E] << [S]**
    - **[S] ≈ [S]<sub>initial</sub>**

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## [ Michaelis – Menten kinetics ]



$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

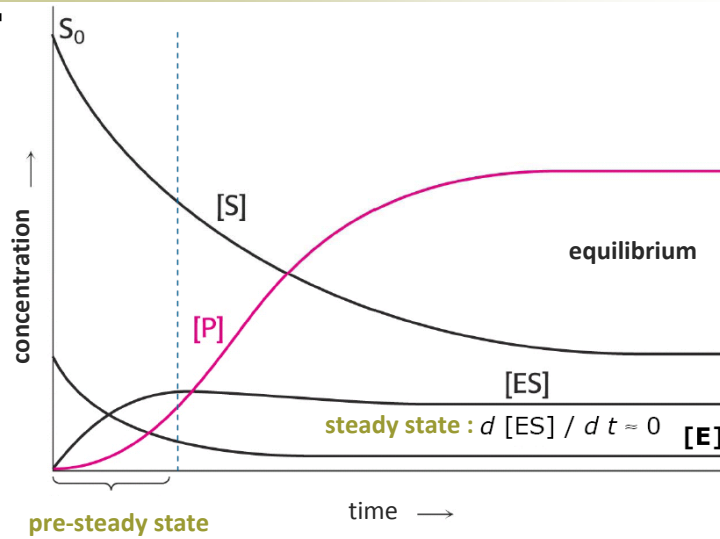
$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

↑  
catalytic  
constant ( $k_{cat}$ )

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# Michaelis – Menten kinetics



# Michaelis – Menten kinetics

$$V_0 = k_2[ES]$$

steady state :  $k_1[E][S] = k_{-1}[ES] + k_2[ES]$

$$[ES] = [E][S] \frac{k_1}{k_{-1} + k_2} \quad \left\{ \begin{array}{l} [E]_t = [E] + [ES] \\ K_M = \frac{k_{-1} + k_2}{k_1} \leftarrow K_M: \text{Michaelis constant} \end{array} \right.$$

$$[ES] = [E]_t \frac{[S]}{K_M + [S]} \quad \left\{ \begin{array}{l} k_2[E]_t = V_{\max} \leftarrow \text{maximum rate when } [E] = [E]_t \end{array} \right.$$

$$V_0 = k_2[ES] = k_2[E]_t \frac{[S]}{K_M + [S]}$$

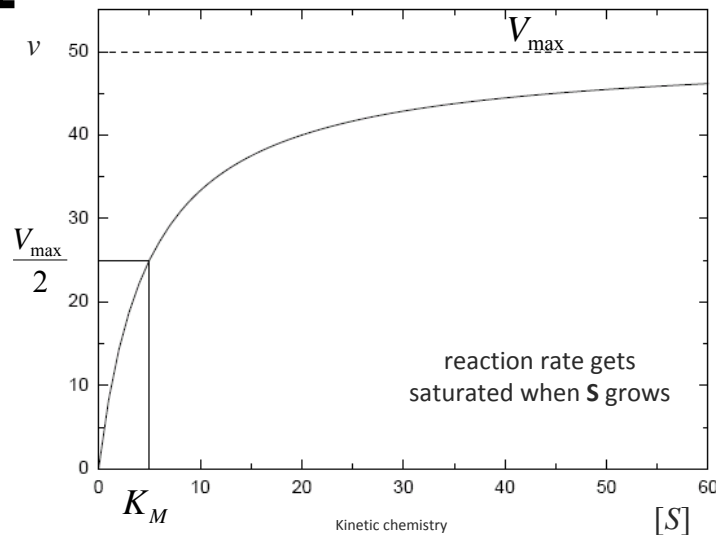
Michaelis – Menten equation

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

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## Michaelis – Menten kinetics



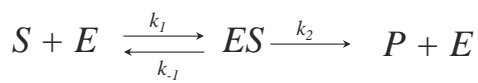
## $K_M$ significance

- two meanings :
- $K_M$  is  $[S]$  for which  $v_0 = v_{max}/2$
- when  $k_2 \ll k_{-1}$ ,  $K_M \approx K_S$  (ES complex dissociation constant)
 
$$K_M = \frac{k_{-1} + k_2}{k_1} \approx K_S = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$
  - Represents the inverse of the enzyme's affinity for the substrate
  - $K_M$  has concentration units (M)
- for a given enzyme
  - $K_M$  changes for substrate and conditions (pH, temperature, ionic force, ...)

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# [ $V_{max}$ & $k_2$ ( $k_{cat}$ ) significance ]

- $V_{max}$  represents the exchange number of the enzyme
  - Exchange number =  $k_{cat}$ 
    - number of substrate molecules converted in product per unit of time and for each molecule of enzyme, on saturating conditions



$$V_{max} = k_{cat} [E]_t \quad k_{cat} = \frac{V_{max}}{[E]_t}$$

$\text{M s}^{-1}$        $\text{M}$        $\text{s}^{-1}$

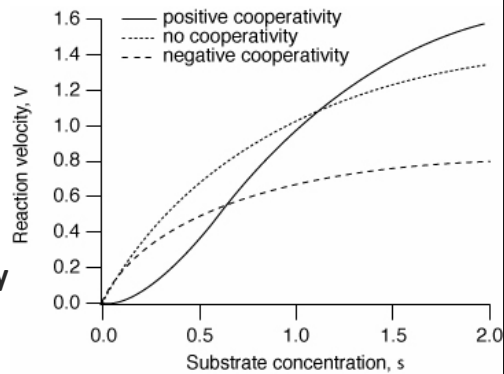
$1/k_{cat}$  is the time needed to convert one molecule of substrate in product

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allosterism & enzymes :  
Hill equation

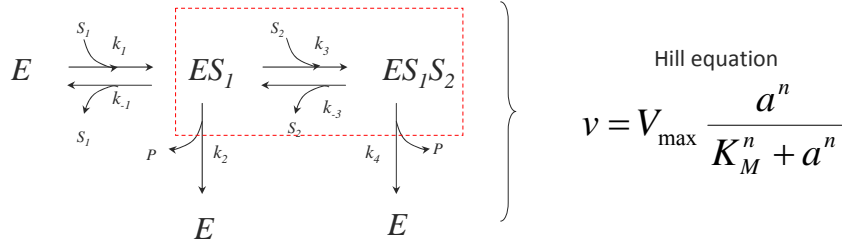
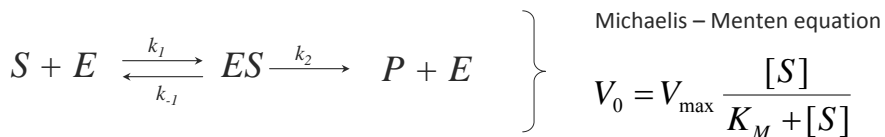
## Hill equation : allosterism

- a reaction can bind more than one molecule from a given substrate
- usually, the binding of the first **S** changes the rate at which the second **S** binds
- If the binding rate of the second **S** is **increased**, it's called **positive cooperativity**
- If the binding rate of the second **S** is **decreased**, it's called **negative cooperativity**



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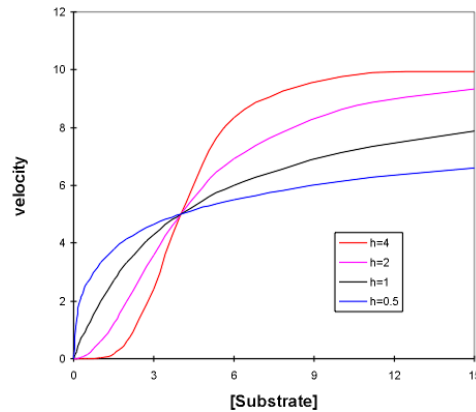
## Hill equation : comparison



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## Hill equation : Hill coefficient

- indicates the degree of cooperation
- a Hill coefficient of 1 indicates completely independent binding
  - independent of whether or not additional ligands are already bound
- a coefficient > 1 indicates cooperative binding
  - oxygen binding to hemoglobin :
    - Hill coefficient of 2.8 – 3.0

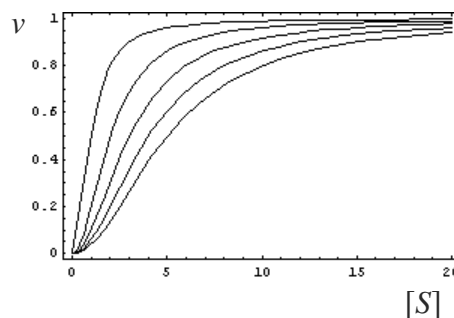


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## Hill equation : $K_M$ constant

- same significance than with M-M model
  - [S] for which  $v_0 = v_{max}/2$
  - represents the inverse of the enzyme's affinity for the substrate
- $\uparrow K_M \rightarrow \downarrow$  affinity

$$v = V_{\max} \frac{a^n}{K_M^n + a^n}$$



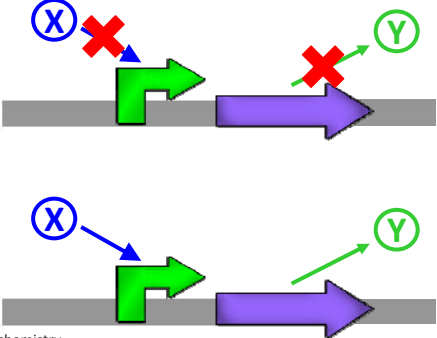
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# Hill equation in gene modelling

## Hill equation in gene modelling

$$v = V_{\max} \frac{a^n}{K_M^n + a^n} \longrightarrow y = \frac{\left(\frac{x}{k}\right)^n}{1 + \left(\frac{x}{k}\right)^n}$$

- assumptions :
  - $V_{\max} = 1$
  - divide by  $K_M$
  - not interested for **substrate** and **enzyme**, but **transcription factor** and **gene activation**

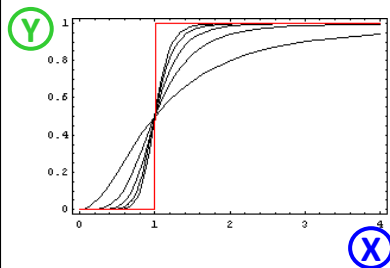


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## Hill equation in gene modelling

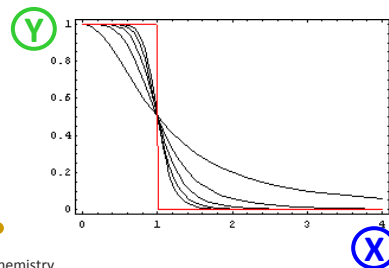
○ activation

$$y = \frac{\left(\frac{x}{k}\right)^n}{1 + \left(\frac{x}{k}\right)^n}$$



○ repression

$$y = \frac{1}{1 + \left(\frac{x}{k}\right)^n}$$



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## sources

- J. Salgado course on *Biochemistry* at University of Valencia
- P.S. Thiagarajan lecture on *Reaction kinetics* at National University of Singapore
- J. Keener, J. Sneyd, *Mathematical Physiology*, Springer, 1998