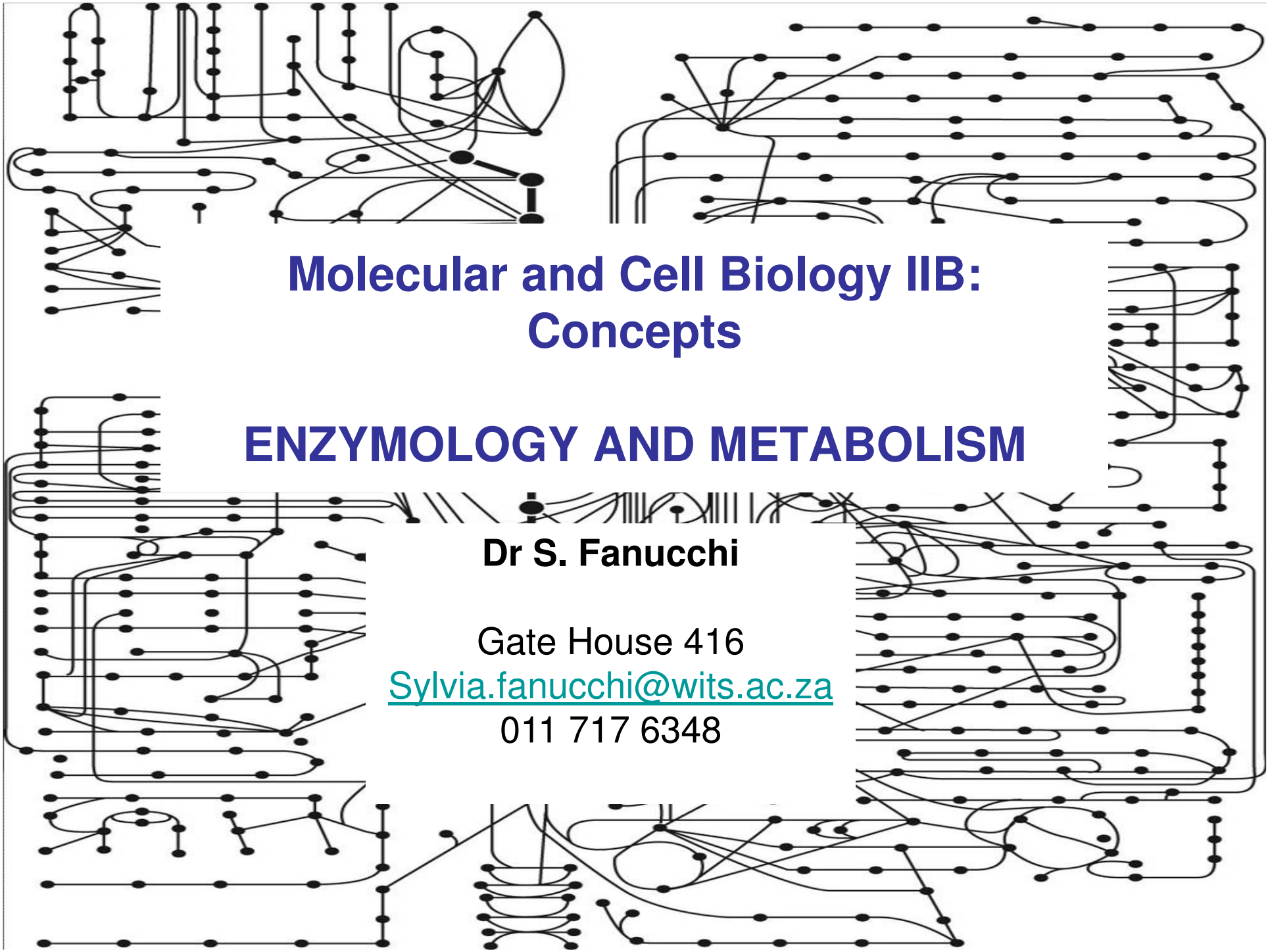

# Molecular and Cell Biology IIB: Concepts

# ENZYMOLOGY AND METABOLISM

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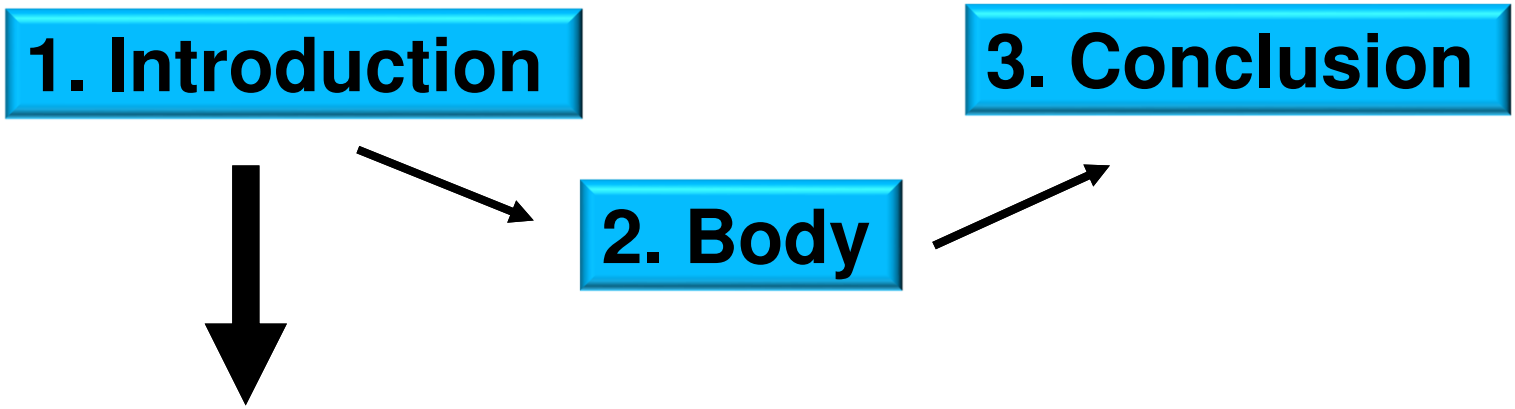
# The Argumentative Essay

- Divided into three sections



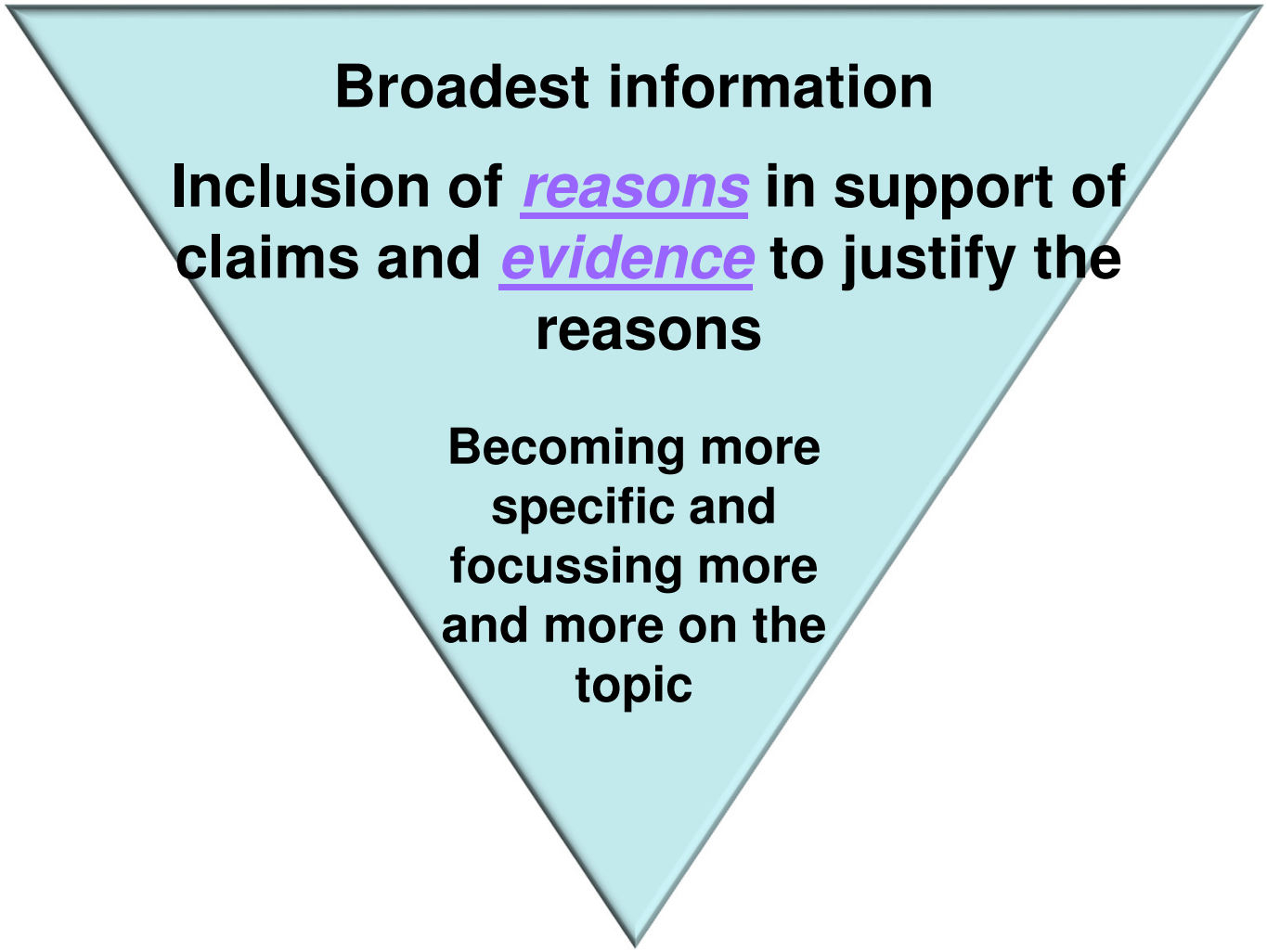

- What is the context?
- Why is it important?
- What is the outline of the essay?
- **What is your main claim?**

# The Main Claim

i.e. what is the position that you support?

Make your main claim:

# The Body of the Essay



# A good paragraph contains

**TTEW**

- A **Transition** sentence
- A **Topic** sentence
- **Evidence** from the literature
- A **Wrap-up** sentence

# The Conclusion

- Wraps up argument by moving back from specific to more general information regarding the topic

The conclusion should do the following:

- Restate the **topic/main claim** and its importance
- **Summarise** why the reader should align with your position
- State possible **future research possibilities**

# Enzymes – Kinetics and Specificity GG
# Chapter 13

- Enzymes **accelerate** and **control** biochemical reaction **rates**

- Exert **kinetic control** over thermodynamic potentiality

# The four distinctive features of enzymes

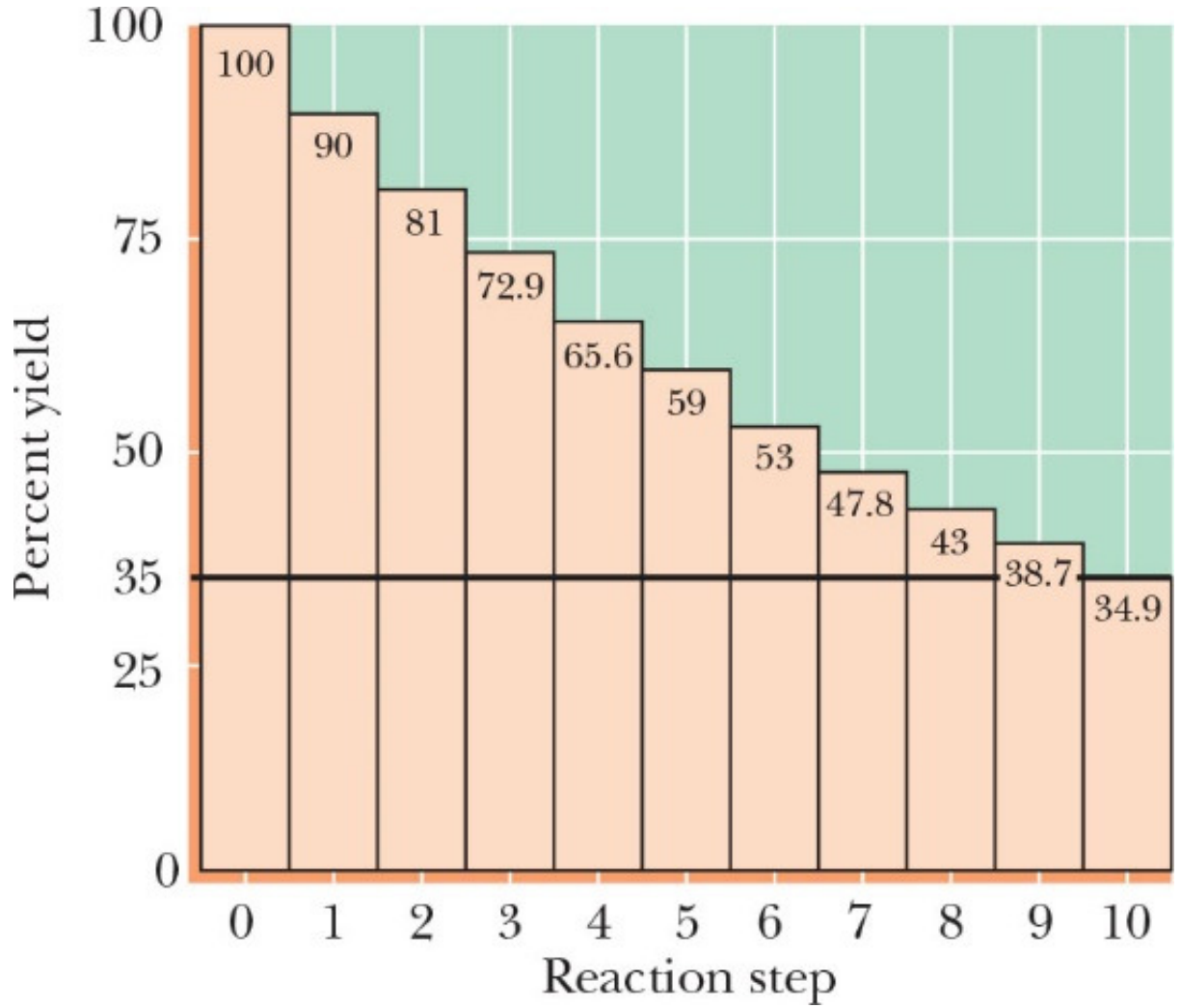
1. Catalytic power:

2. Specificity

3. Regulation

4. Mild conditions

# Enzymes have high specificity and produce more than 90% yield



# Enzyme nomenclature

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

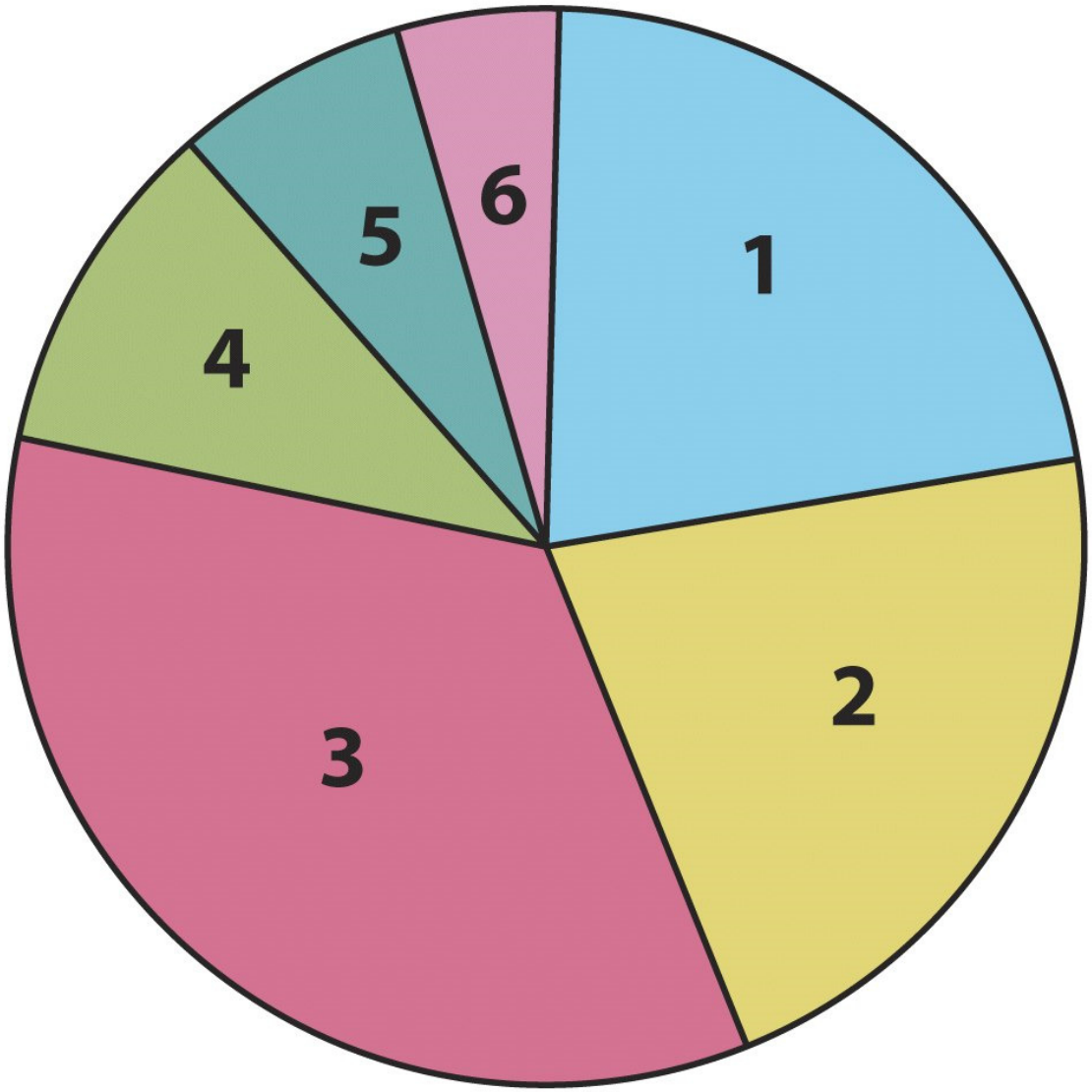
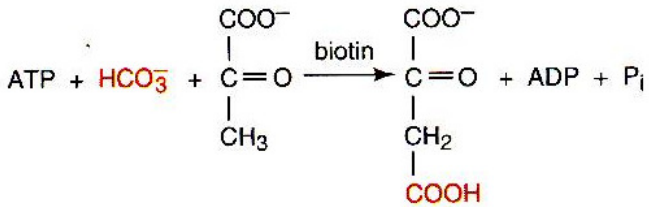
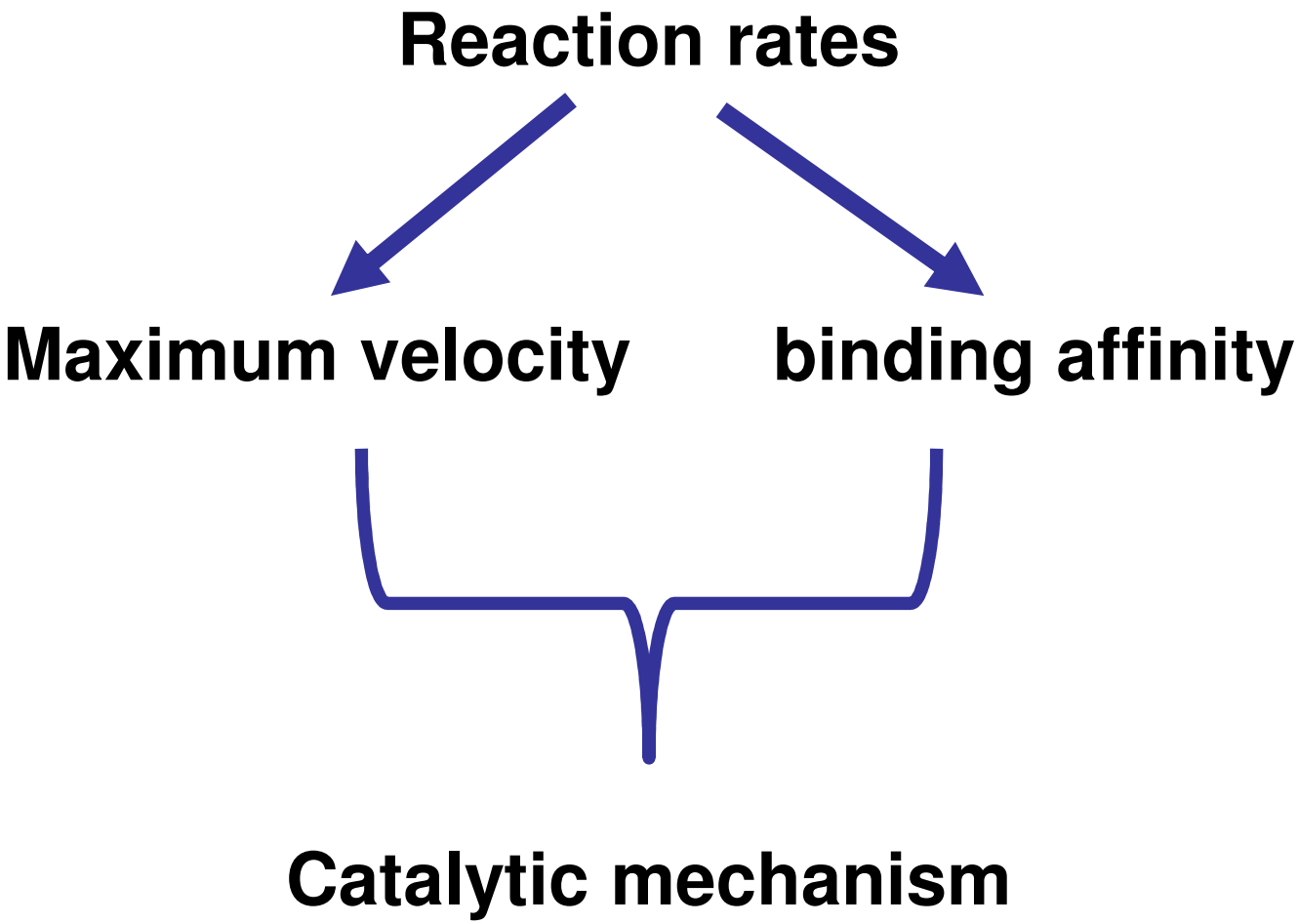


**TABLE 13.1 Systematic Classification of Enzymes According to the Enzyme Commission**

| E.C. Number | Systematic Name and Subclasses |
|---|---|
| 1 | *Oxidoreductases* (oxidation–reduction reactions) |
| 1.1 | Acting on CH—OH group of donors |
| 1.1.1 | With NAD or NADP as acceptor |
| 1.1.3 | With $O_2$ as acceptor |
| 1.2 | Acting on the $\rangle C{=}O$ group of donors |
| 1.2.3 | With $O_2$ as acceptor |
| 1.3 | Acting on the CH—CH group of donors |
| 1.3.1 | With NAD or NADP as acceptor |
| 2 | *Transferases* (transfer of functional groups) |
| 2.1 | Transferring C-1 groups |
| 2.1.1 | Methyltransferases |
| 2.1.2 | Hydroxymethyltransferases and formyltransferases |
| 2.1.3 | Carboxyltransferases and carbamoyltransferases |
| 2.2 | Transferring aldehydic or ketonic residues |
| 2.3 | Acyltransferases |
| 2.4 | Glycosyltransferases |
| 2.6 | Transferring N-containing groups |
| 2.6.1 | Aminotransferases |
| 2.7 | Transferring P-containing groups |
| 2.7.1 | With an alcohol group as acceptor |
| 3 | *Hydrolases* (hydrolysis reactions) |
| 3.1 | Cleaving ester linkage |
| 3.1.1 | Carboxylic ester hydrolases |
| 3.1.3 | Phosphoric monoester hydrolases |
| 3.1.4 | Phosphoric diester hydrolases |
| 4 | *Lyases* (bond cleavage by means other than hydrolysis or oxidation) |
| 4.1 | C—C lyases |
| 4.1.1 | Carboxy lyases |
| 4.1.2 | Aldehyde lyases |
| 4.2 | C—O lyases |
| 4.2.1 | Hydrolases |
| 4.3 | C—N lyases |
| 4.3.1 | Ammonia lyases |
| 5 | *Isomerases* (isomerization reactions) |
| 5.1 | Racemases and epimerases |
| 5.1.3 | Acting on carbohydrates |
| 5.2 | *Cis-trans* isomerases |
| 6 | *Ligases* (formation of bonds with ATP cleavage) |
| 6.1 | Forming C—O bonds |
| 6.1.1 | Amino acid–RNA ligases |
| 6.2 | Forming C—S bonds |
| 6.3 | Forming C—N bonds |
| 6.4 | Forming C—C bonds |
| 6.4.1 | Carboxylases |



# Enzyme Kinetics



# Basic principles of chemical kinetics

**A → P**

- The **velocity (or rate):**

- i.e. $v = \frac{d[P]}{dt}$ or $v = -\frac{d[A]}{dt}$

Rate law: $\boldsymbol{v = -\frac{d[A]}{dt} = k[A]}$

Therefore **rate is proportional to the concentration of A** and ***k* is the proportionality constant or rate constant.**

# Terminology

- For an elementary reaction, the **order** for any reactant is given by its exponent in the rate equation.
- *v* is first order w.r.t. A
- The number of molecules that must simultaneously interact is defined as the **molecularity** of the reaction
- A → P is a

**A → P**

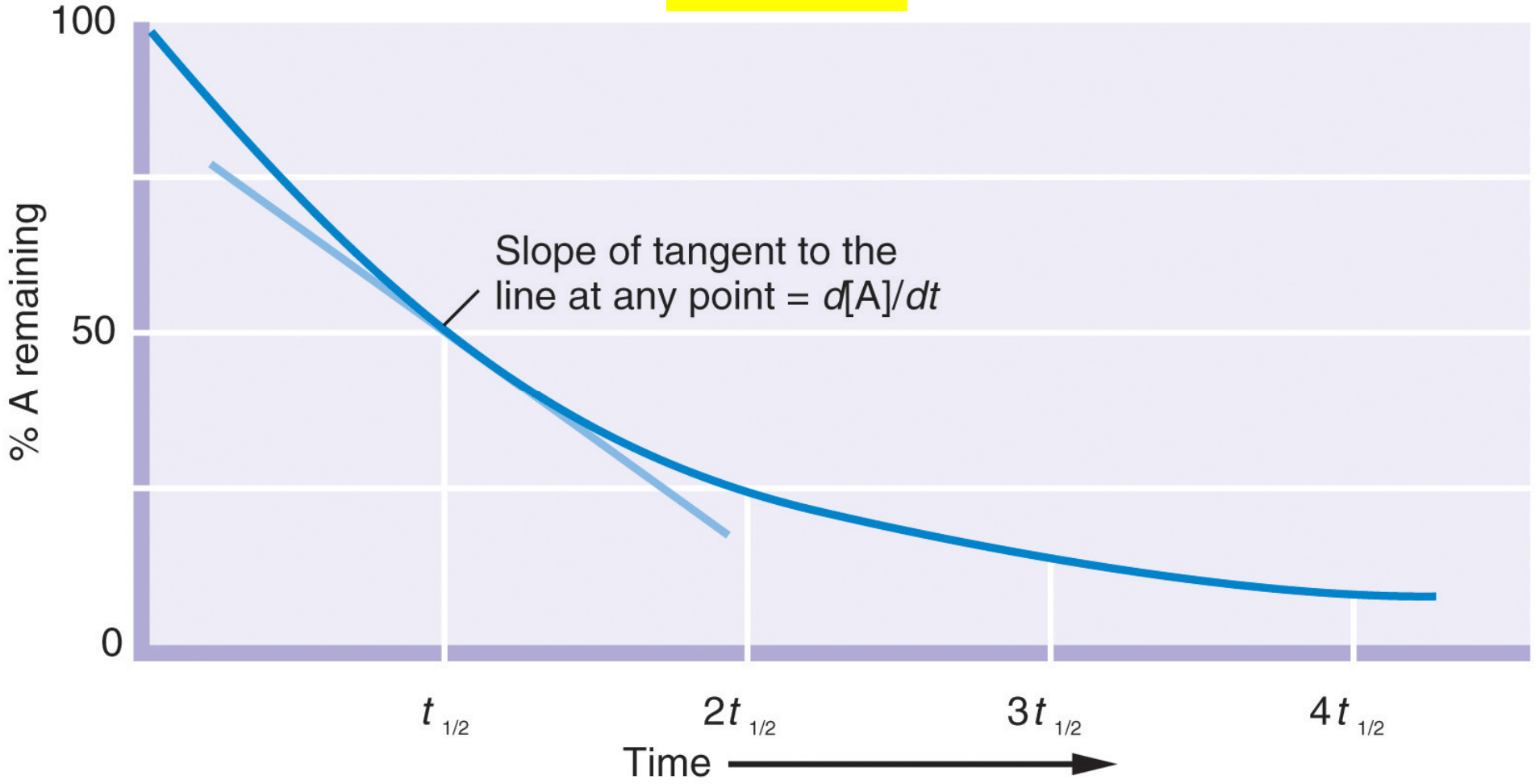



Fig. 13-4, p. 387

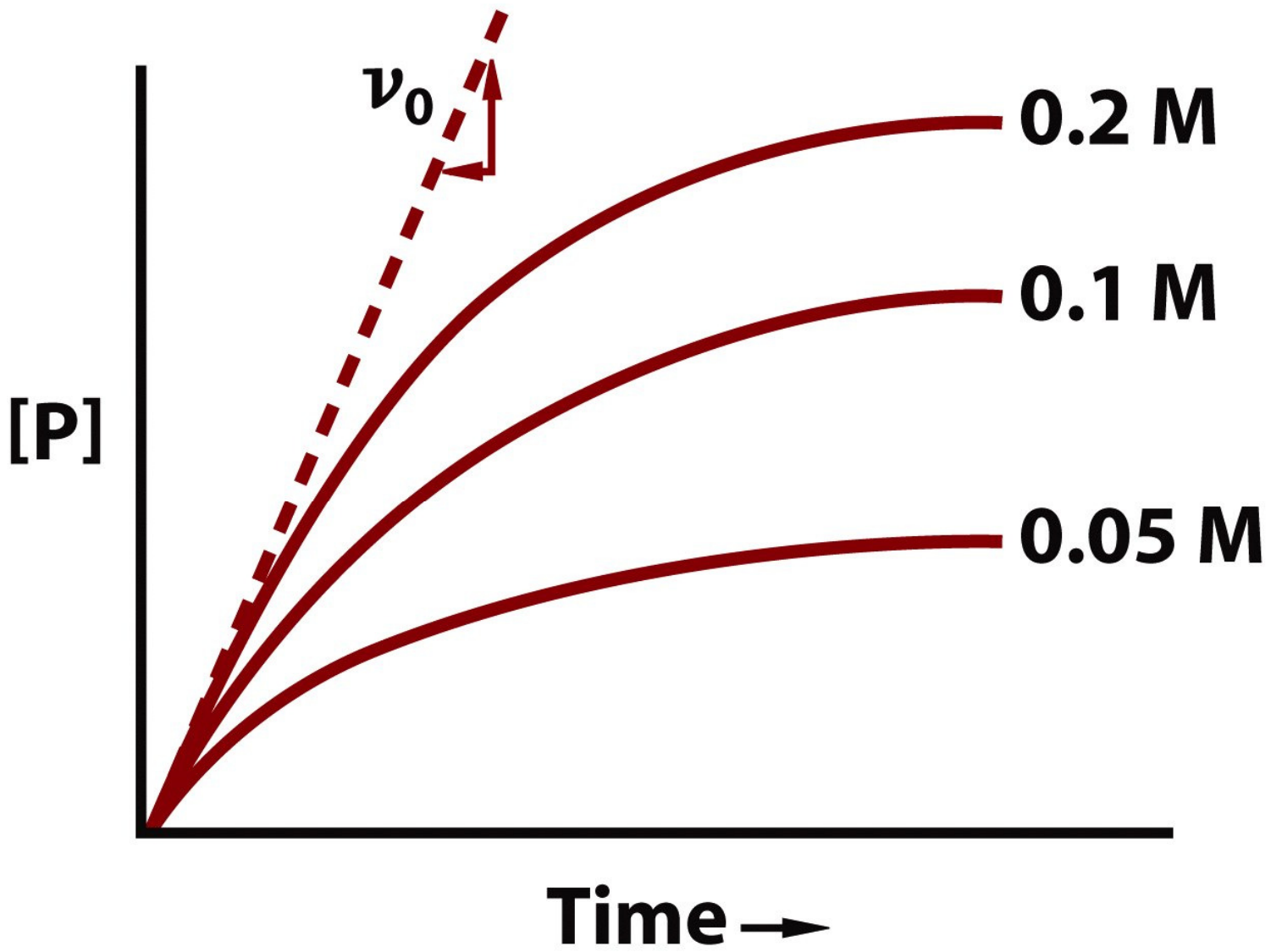



Figure 5-1a Principles of Biochemistry, 4/e

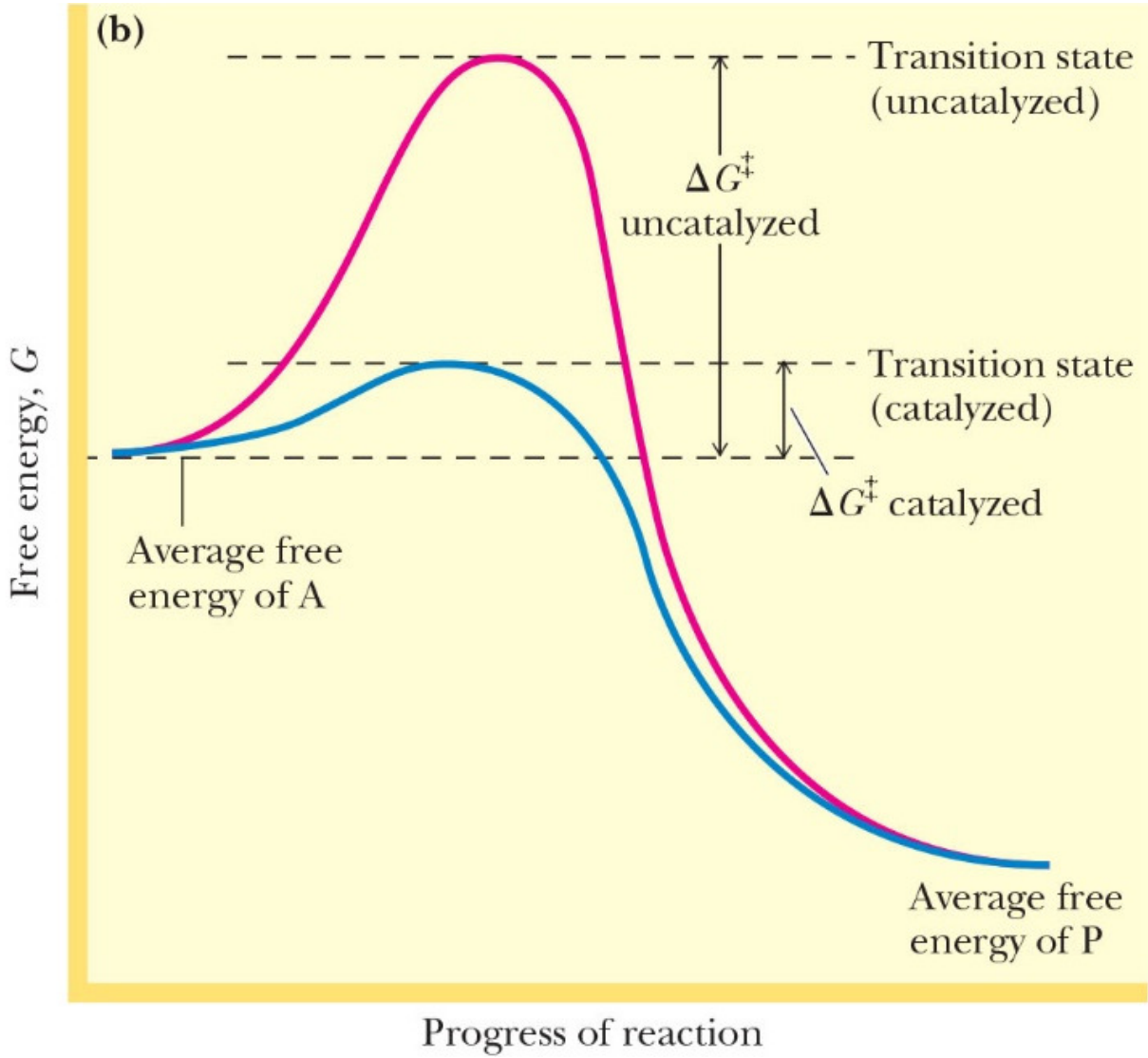
# $A + B \rightarrow P + Q$

- What is the molecularity of this reaction?
- What is the velocity defined as?
- What is the rate law?
- What is the order of the reaction?

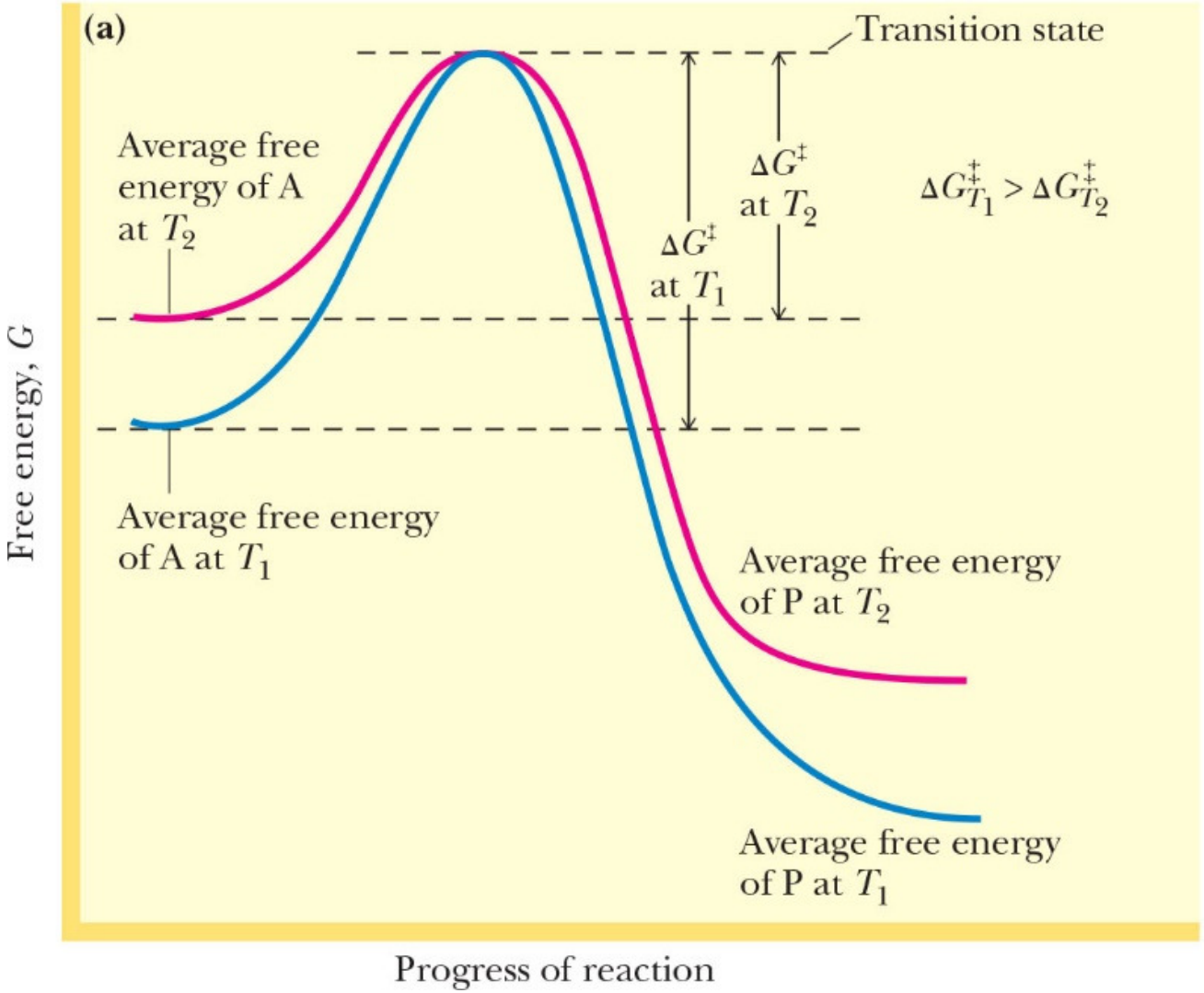
**What would be an example of a reaction that is second order overall and also second order w.r.t. A?**

# How do catalysts work?

**A → P**



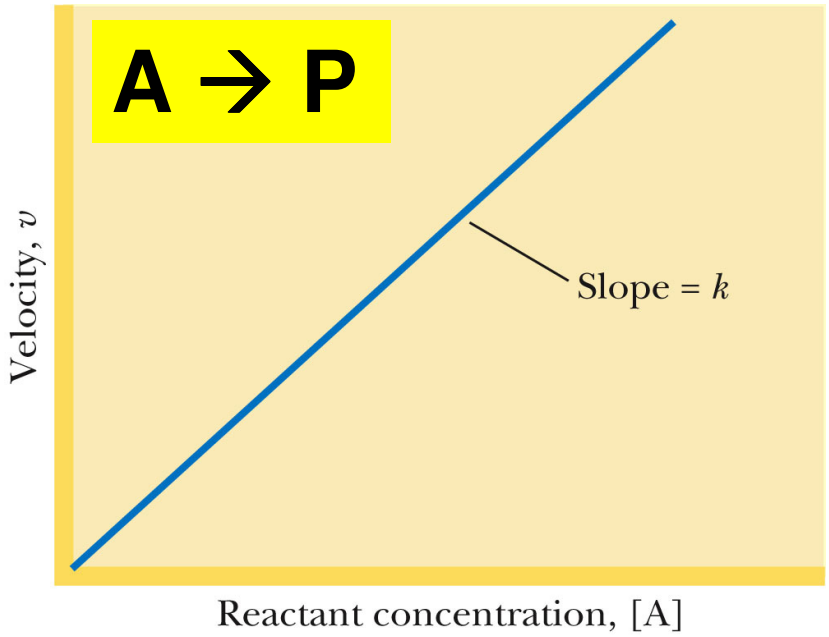
# Discuss the difference between temperature and a catalyst on reaction rate

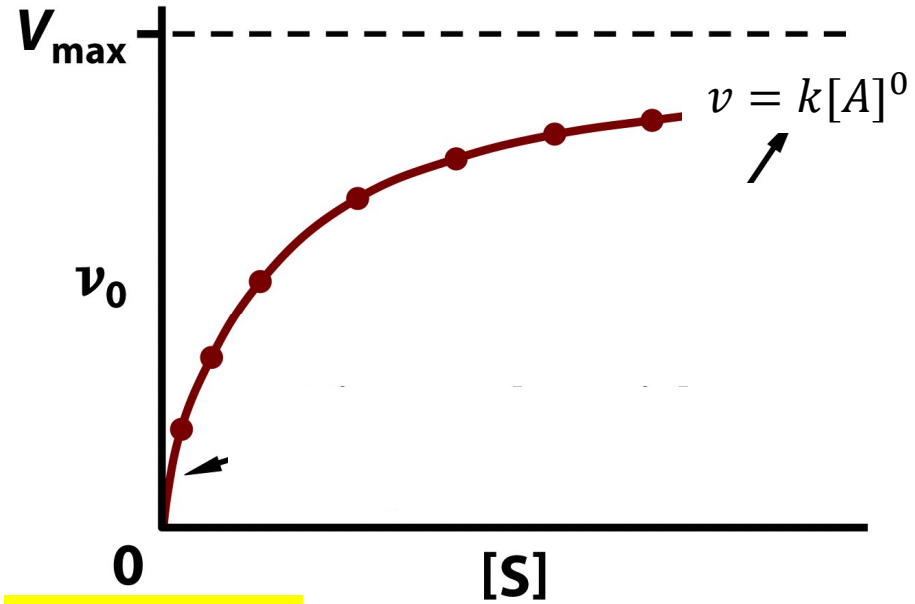


# Three important characteristics of a catalyst

1. They are **regenerated** after the reaction cycle.
2. They have no effect on the **overall free energy change** in the reaction.
3. They can only speed up the reaction of **favourable reactions**. They have no effect on a non spontaneous reaction i.e. they cannot change the equilibrium of the reaction

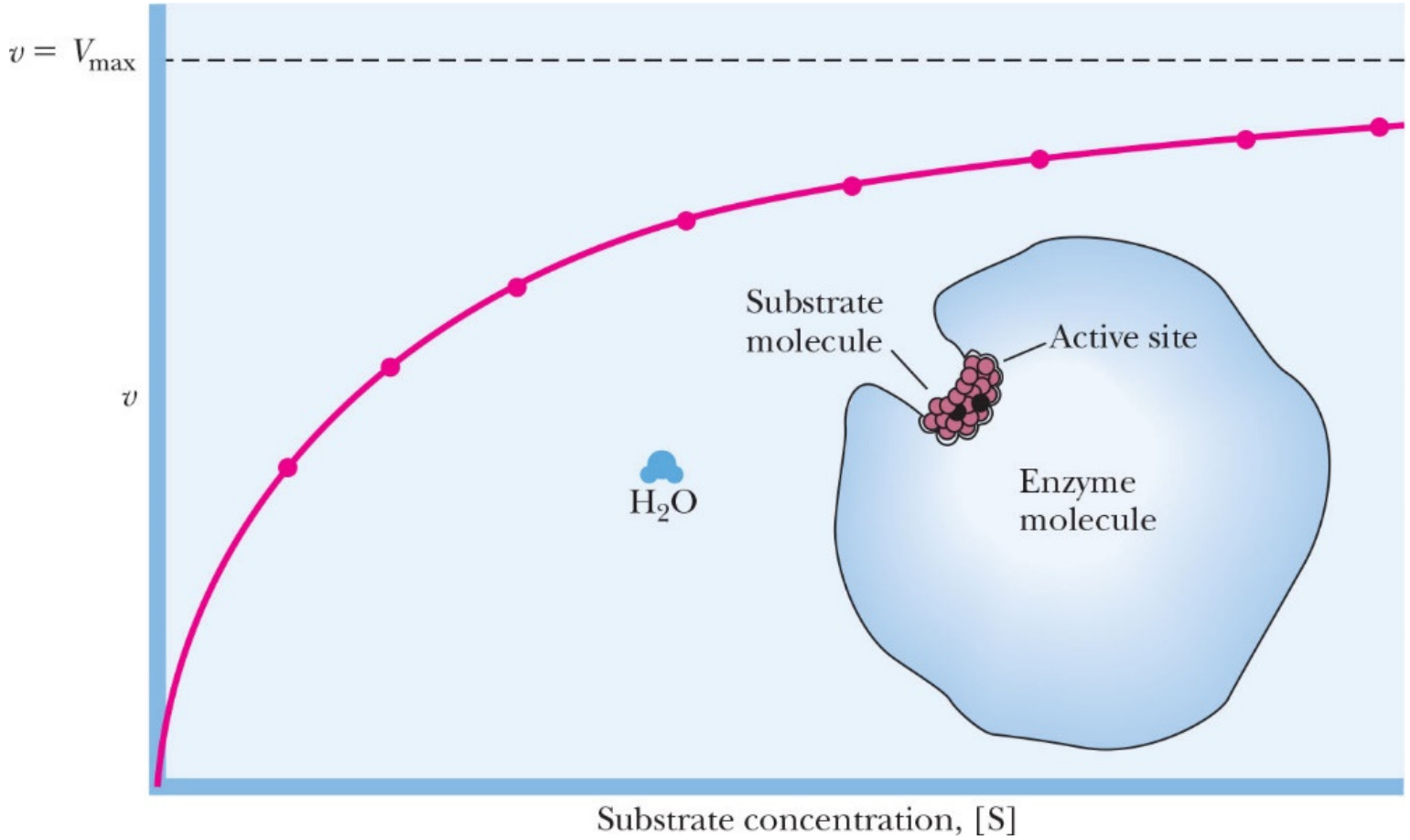
# How do we study enzyme kinetics?



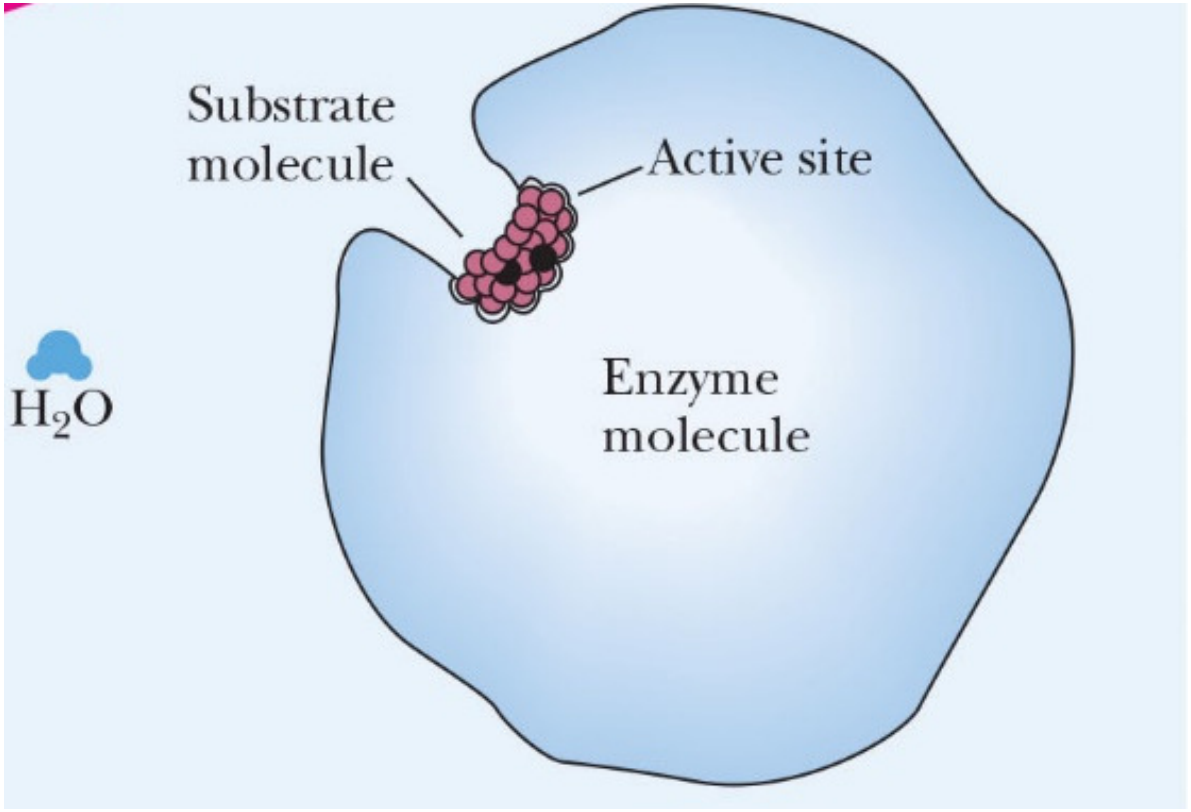




A → P
E

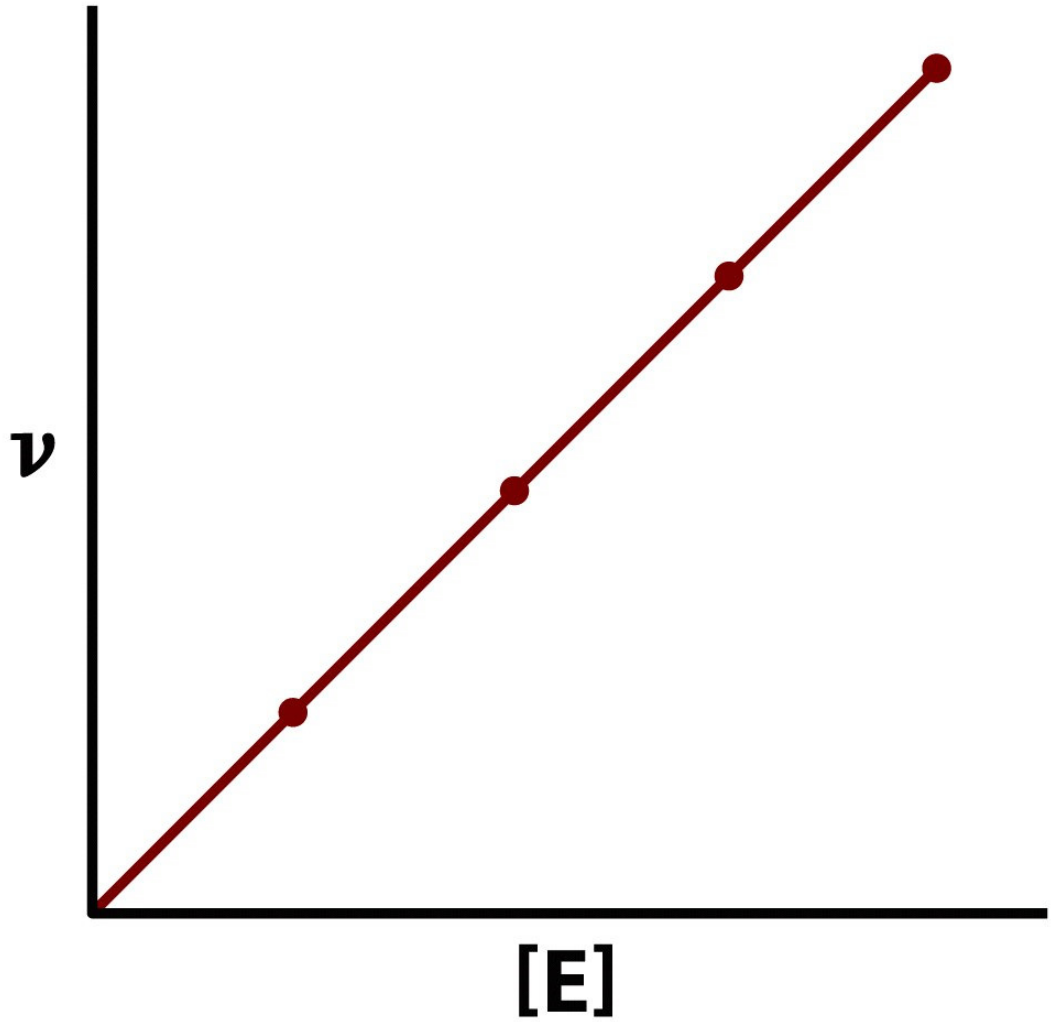
The system becomes
**SATURATED** with the substrate



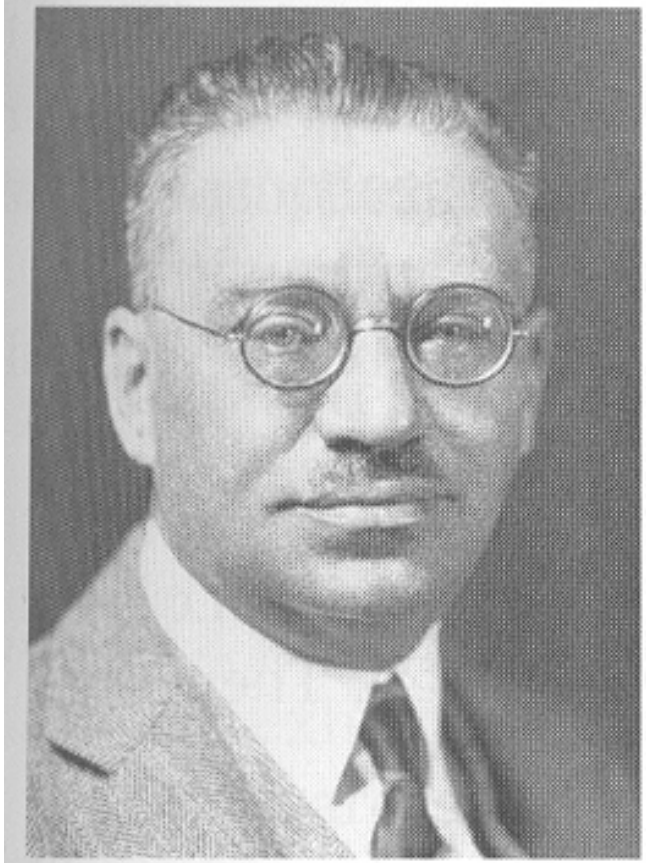
# How does an enzyme interact with its substrate?



# Effect of enzyme concentration on $v_0$



# Michaelis-Menten Enzyme Kinetics



Leonor Michaelis;
1875–1949

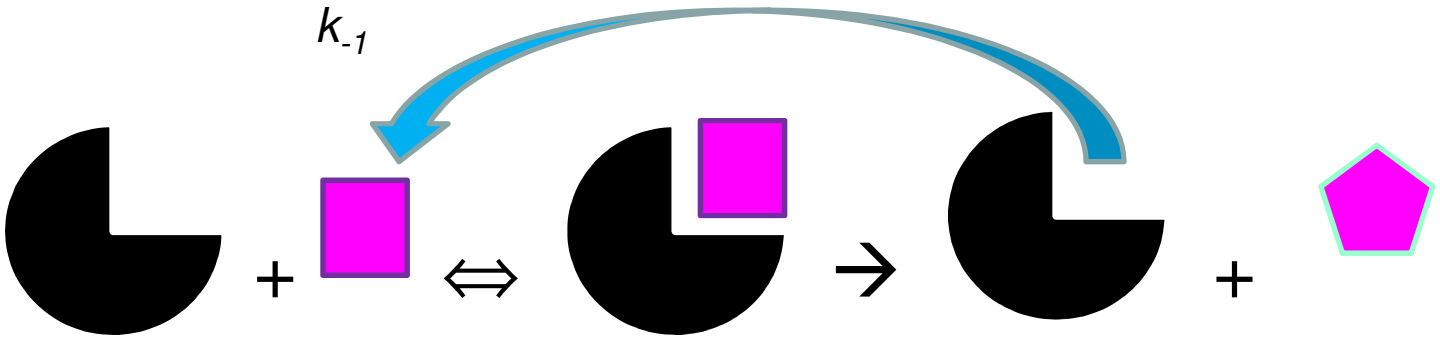
Maud Menten,
1879–1960

# E and S associate **reversibly** to form an enzyme-substrate complex (ES)

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

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

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



**Show mathematically why the assumption that the back reaction (E+P → ES) is negligible when measuring initial velocity holds**

# Briggs Huldane assumption

The **rate of formation** of the ES complex from enzyme and substrate is equal to the **rate of its breakdown** into enzyme and product

What does this tell you about the concentration of the ES complex?

**Draw a plot of concentration vs time for an enzyme catalysed reaction and include the following species:**

Substrate
Product
Enzyme
ES complex

# Derivation of the Michaelis-Menten equation

- 2 simplification assumptions:

- Knowing this, and knowing $E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P$, we are

  equipped to describe the initial velocity ($v_0$) of the reaction as a function of [S]

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

Total enzyme $[E_T] = [E] + [ES]$ $\therefore [E] = [E_T] - [ES]$

Rate of ES formation? $\boldsymbol{v_f = k_1[E][S] = k_1([E_T] - [ES])[S]}$

Rate of ES disappearance? $\boldsymbol{v_d = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES]}$

At steady state $\frac{d[ES]}{dt} = 0$ and therefore $v_f = v_d$

$$\therefore k_1([E_T] - [ES])[S] = (k_{-1} + k_2)[ES]$$

Rearranging to solve for all rate constants gives

$$\frac{([E_T] - [ES])[S]}{[ES]} = \frac{(k_{-1} + k_2)}{k_1} = K_M$$

# What are the units of $K_M$?

Given: $E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P$

and $K_M = \frac{(k_{-1}+k_2)}{k_1}$

$$\frac{([E_T] - [ES])[S]}{[ES]} = K_M$$

Solving for [ES] gives

$$\frac{[E_T][S]}{[ES]} - \frac{[ES][S]}{[ES]} = K_M$$

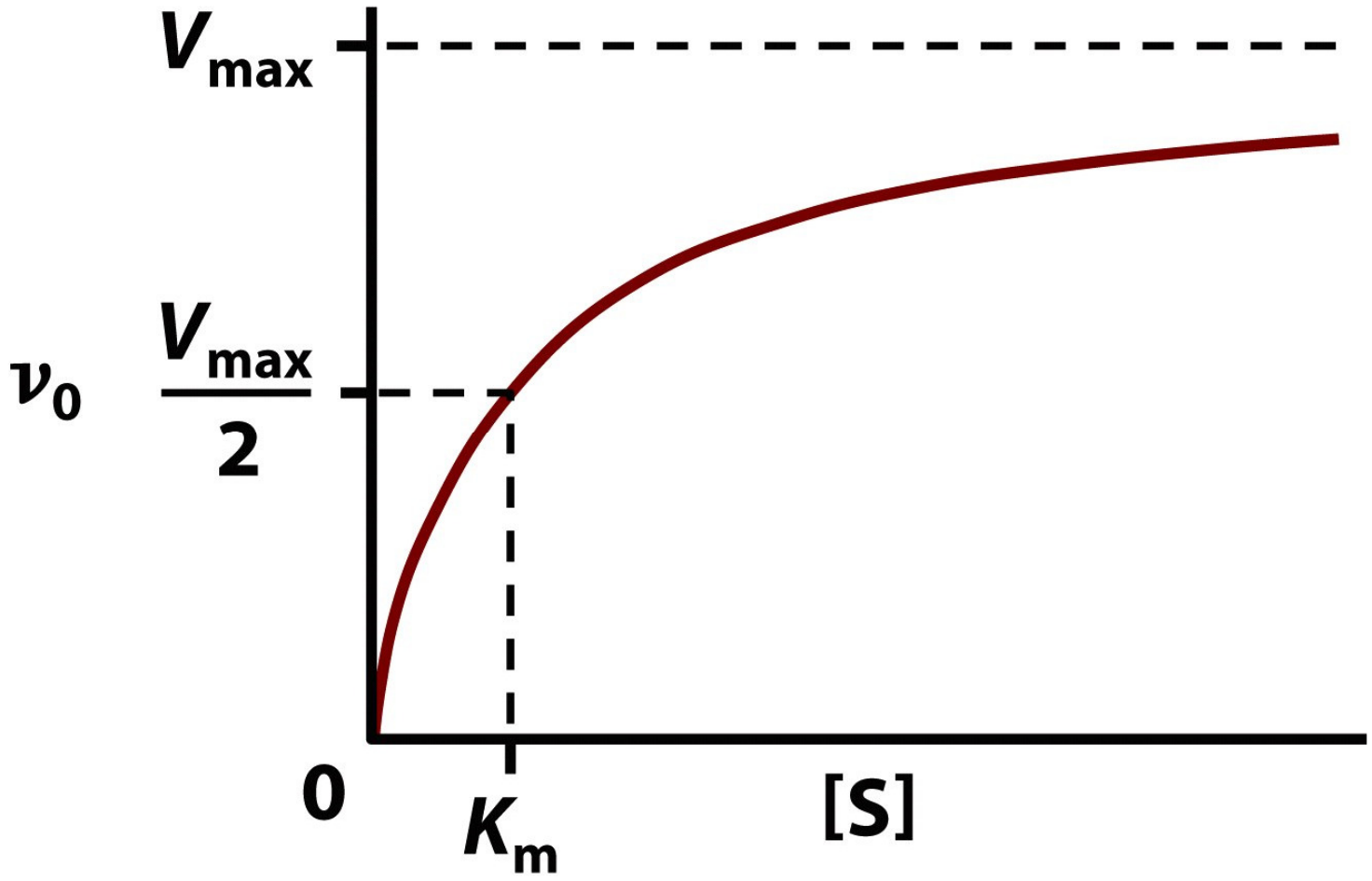
$$\therefore K_M + [S] = \frac{[E_T][S]}{[ES]} \text{ and } [ES] = \frac{[E_T][S]}{K_M + [S]}$$

The rate of product formation is given by $v = \frac{d[P]}{dt} = k_2[ES]$

Substituting for [ES] above: $\mathrm{v} = \frac{k_2[E_T][S]}{K_M + [S]}$

When [S]>>>[$E_T$], [$E_T$] = [ES] and $v = k_2[E_T] = v_{max}$

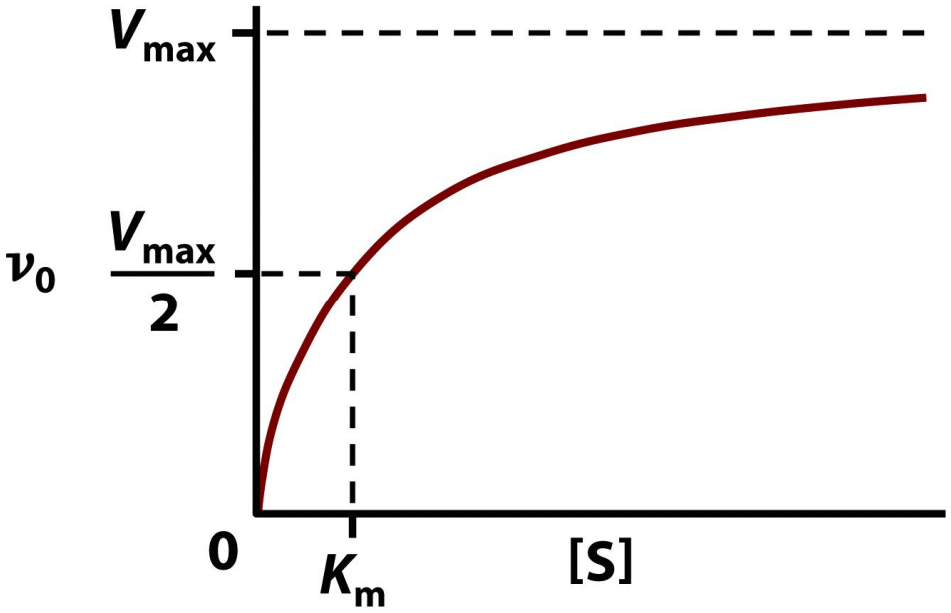
$$\mathrm{v} = \frac{v_{max}\,[S]}{K_M + [S]}$$


$V_{max}$
$\nu_0$
$\frac{V_{max}}{2}$
0
$K_m$
[S]

# $K_m$ and $V_{max}$ once known explicitly, define the rate of the enzyme-catalyzed reaction provided:

- The reaction involves only one substrate, or if the reaction is multi-substrate the concentration of the other substrates is held constant
- [ES] is in a steady state
- The reaction ES $\rightarrow$ P is irreversible, or the experiment is limited to observing only initial velocities where [P] = 0
- $[S]_0 > [E_T]$ and $[E_T]$ is held constant
- All other variables that might influence the rate of the reaction are held constant

# What is the significance of the $K_M$ value?



# Question:

If an enzyme could catalyse 2 substrates how would you determine which substrate would be favoured?

# Turnover number/kinetic efficiency

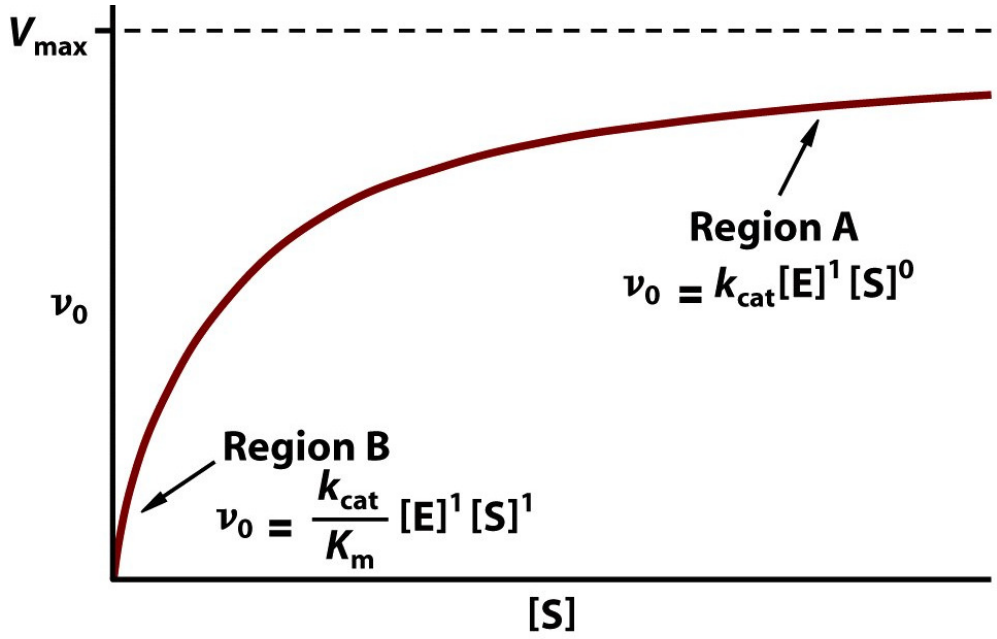
- Turnover number (molecular activity) = $k_{cat}$
- $k_{cat}$ measures **maximal catalytic activity**

- For $E + S \underset{}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P$, under conditions of initial velocity, $\boldsymbol{k_{cat} = k_2}$
- At saturating [S], $\boldsymbol{v = v_{max} = k_2[E_T]}$
- $\therefore \boldsymbol{k_2 = \frac{v_{max}}{[E_T]} = k_{cat}}$

# Catalytic efficiency

- $k_{cat}$ is only informative at very high substrate concentration which isn't physiological
- But we know that $v_{max} = kca_t[ET]$
- So, from the Michaelis Menten equation: $\mathrm{v} = \frac{v_{max}\,[S]}{K_M + [S]}$, we can say $\mathrm{v} = \frac{k_{cat}[E_T]\,[S]}{K_M + [S]}$
- And at conditions where [S]<<<<<$K_M$
- $\mathrm{v} = \left(\frac{k_{cat}}{K_M}\right)[E][S]$
- $\left(\frac{k_{cat}}{K_M}\right)$
- Enzymes that reach **catalytic perfection**

# Meanings of $k_{cat}$ and $k_{cat}/K_M$



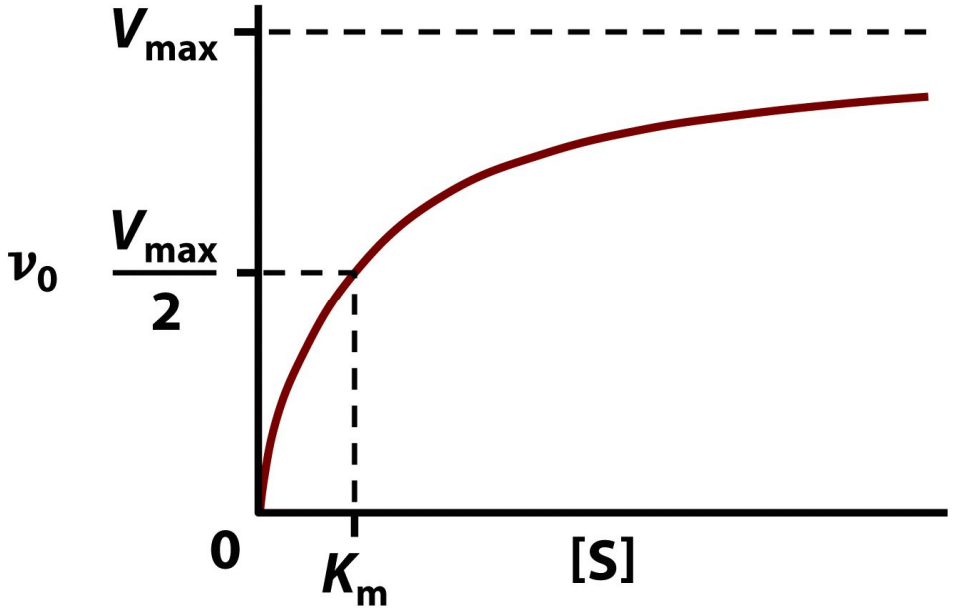

Region A: $ES \xrightarrow{k_{cat}} E + P$

Region B: $E + S \xrightarrow{\frac{k_{cat}}{K_m}} E + P$

$(E + S \longrightarrow ES \longrightarrow E + P)$

# Linear plots derived from the Michaelis Menten equation

- How is $K_M$ and $v_{max}$ determined from the Michaelis Menten equation?
- Rearrange the MM equation to yield a straight line



Take the **double reciprocal** of the Michaelis- Menten equation (ie the reciprocal of both sides)

$$\mathrm{v} = \frac{v_{max}\,[S]}{K_M + [S]}$$

# Lineweaver-Burk plot

$$\frac{1}{v} = \frac{K_M}{v_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{v_{\max}}$$