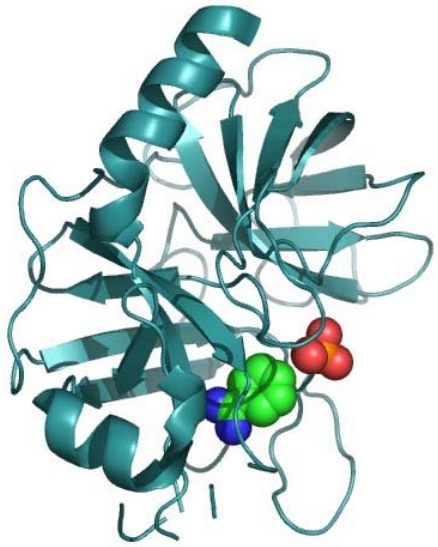
# Enzymes

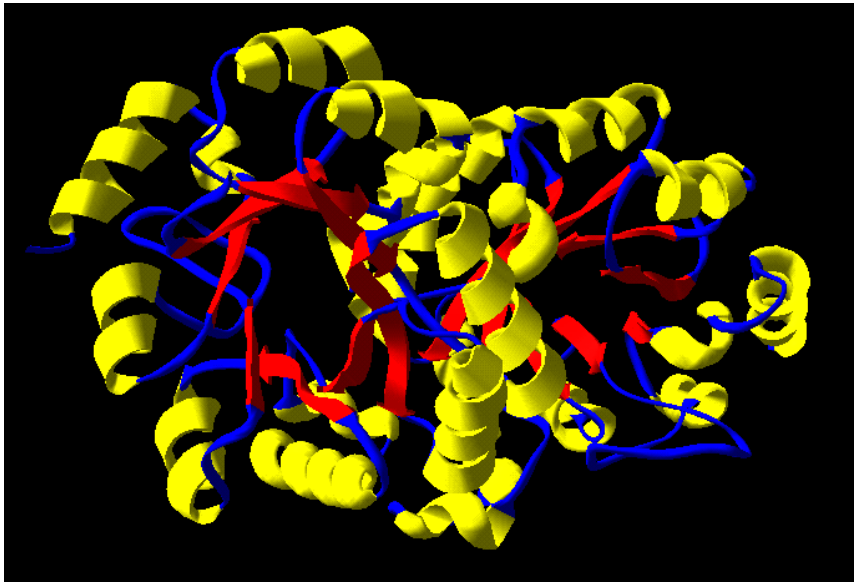
Substrate $\xrightarrow{\text{Enzyme}}$ Product



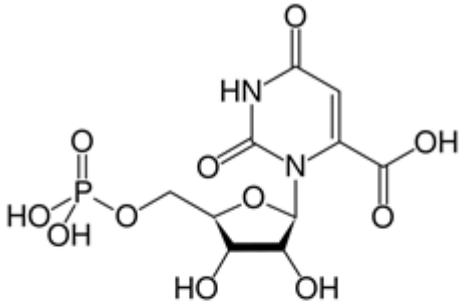
**Aim**: understanding the basic concepts of enzyme catalysis and enzyme kinetics

# Enzymes are **efficient**

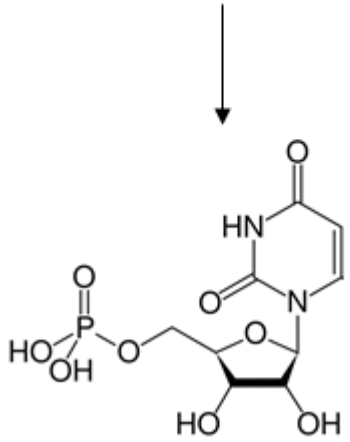
| **Enzyme** | **Reaction** | **Uncatalysed ($k_{uncat}$ $s^{-1}$)** | **Catalysed ($k_{cat}$ $s^{-1}$)** | **Rate enhancement ($k_{cat}/k_{uncatat}$)** |
|---|---|---|---|---|
| OMP-decarboxylase | Decarboxylation | $2.8 \times 10^{-16}$ | 39 | $1,4 \times \mathbf{10^{17}}$ |
| Carboxypeptidase A | Peptide hydrolysis | $3 \times 10^{-9}$ | 578 | $1,9 \times 10^{11}$ |
| Carboanhydrase | $CO_2$-hydratisation | $1,3 \times 10^{-1}$ | $1 \times \mathbf{10^{6}}$ | $7,7 \times 10^{6}$ |



Orotidine monophosphate decarboxylase

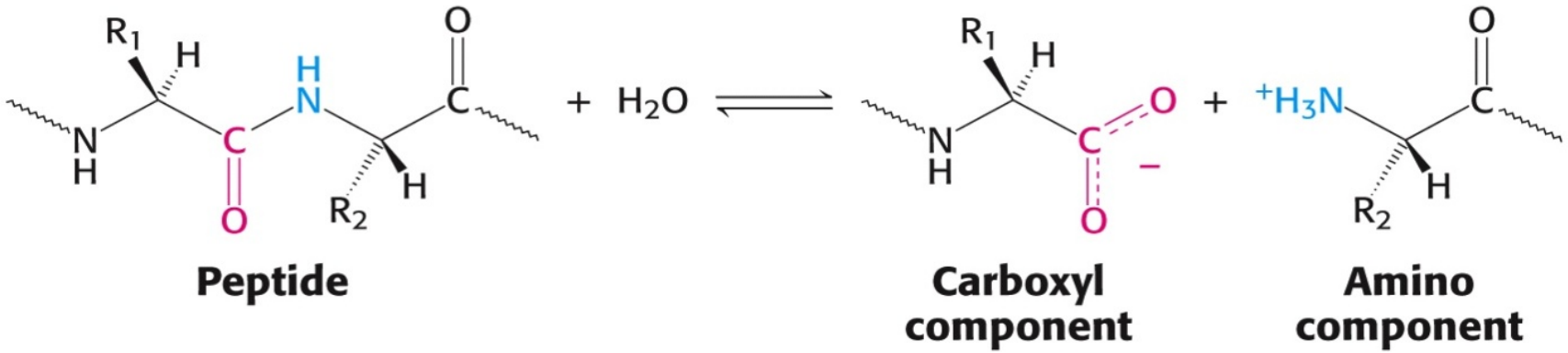



Orotidine monophosphate




Uridine monophosphate

# Enzymes are **specific**




Substrate specificity varies:

Trypsin: $R_1$ = Lys/Arg
Thrombin: $R_1$ = Arg, $R_2$ = Gly

The chance for DNA-polymerase I to add an incorrect nucleotide is < 0,00001 %

# Cofactor - Coenzyme - Cosubstrate – Prosthetic group

Many enzymes use metal ions or small molecules to achieve catalysis: **COFACTOR**

*Apoenzyme + Cofactor = Holoenzyme*

2 kinds of cofactors

- **Metal ions** e.g. $Zn^{2+}$
- **coenzymes** small org. molecules, e.g. $NAD^+$

The **cofactor** can be tightly bound to the enzyme – **prothetic group**

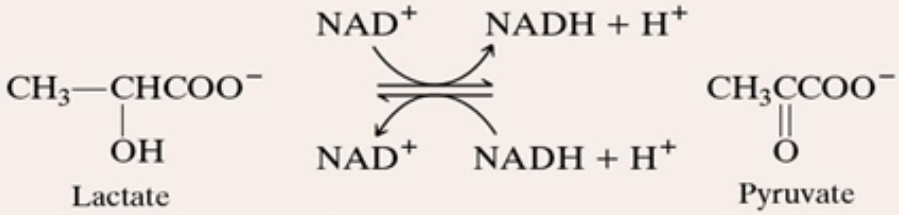
The **coenzyme** can be free like a substrate and is then also called **cosubstrate**, e.g. $NAD^+$

# Six enzyme classes

| Class | Type of reaction | Example |
|---|---|---|
| 1. Oxidoreductases | Oxidation-reduction | Lactate dehydrogenase |
| 2. Transferases | Group transfer | Nucleoside monophosphate kinase (NMP kinase) |
| 3. Hydrolases | Hydrolysis reactions (transfer of functional groups to water) | Chymotrypsin |
| 4. Lyases | Addition or removal of groups to form double bonds | Fumarase |
| 5. Isomerases | Isomerization (intramolecular group transfer) | Triose phosphate isomerase |
| 6. Ligases | Ligation of two substrates at the expense of ATP hydrolysis | Aminoacyl-tRNA synthetase |

EC-number (Enzyme Commission)
Example:  Chymotrypsin = EC 3.4.21.1

1. Oxidoreductases




Lactate Pyruvate

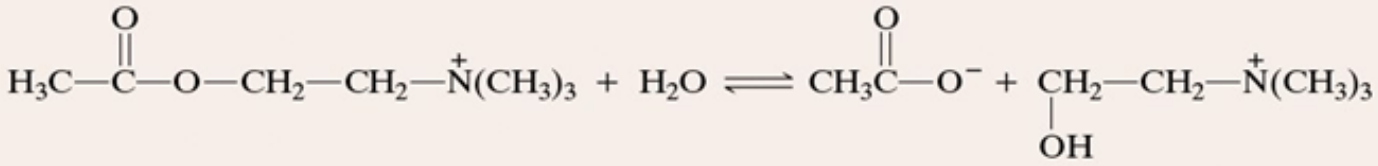
Common name: Lactate dehydrogenase
Official name: L-Lactate:$NAD^+$ oxidoreductase
Official number: 1.1.2.3

2. Transferases

$$(d\text{NMP})_n + d\text{NTP} \rightleftharpoons (d\text{NMP})_{n+1} + \text{PP}_\text{i}$$

$(d\text{NMP})_n$ = DNA with $n$ nucleotides
$d$NTP = deoxynucleoside triphosphate
$(d\text{NMP})_{n+1}$ = DNA with $n + 1$ nucleotides
$\text{PP}_\text{i}$ = Pyrophosphate
Common name: DNA polymerase
Official name: Deoxynucleoside triphosphate:DNA deoxynucleotidyltransferase (DNA-directed)
Official number: 2.7.7.7

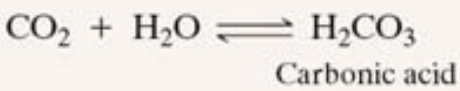
3. Hydrolases




Acetylcholine Acetate Choline

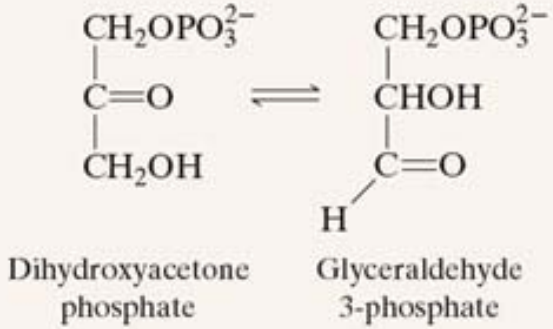
Common name: Acetylcholinesterase
Official name: Acetylcholine acetylhydrolase
Official number: 3.1.1.7

4. Lyases



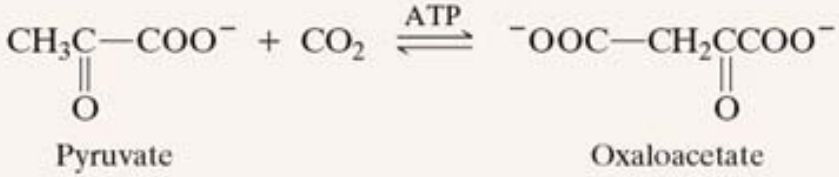

Common name: Carbonic anhydrase
Official name: Carbonate hydrolyase
Official number: 4.2.1.1

5. Isomerases



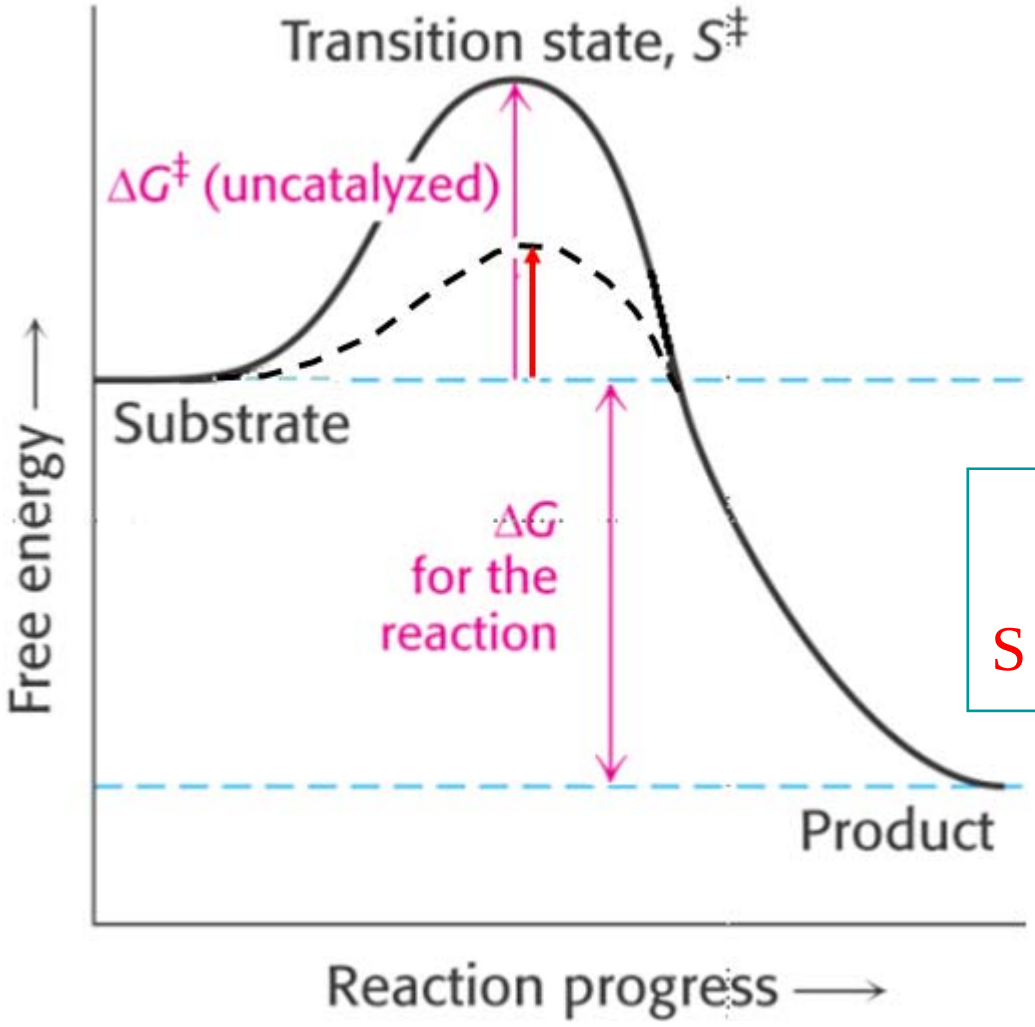

Common name: Triose phosphate isomerase
Official name: D-Glyceraldehyde-3-phosphate ketoisomerase
Official number: 5.3.1.1

6. Ligases




Common name: Pyruvate carboxylase
Official name: Pyruvate $CO_2$ ligase (ADP-forming)
Official number: 6.4.1.1

# Stabilization of transition state




**S**ubstrate → **P**roduct

Enzyme catalysed

$S + E \rightarrow ES \rightleftharpoons ES^{*} \rightarrow P + E$

# Active site

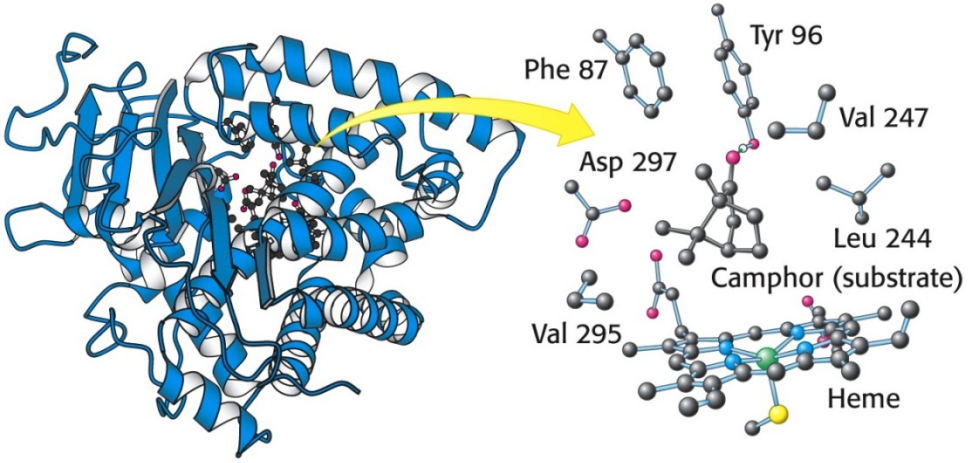
- Catalysis always includes the formation of Enzyme-Substrate complex

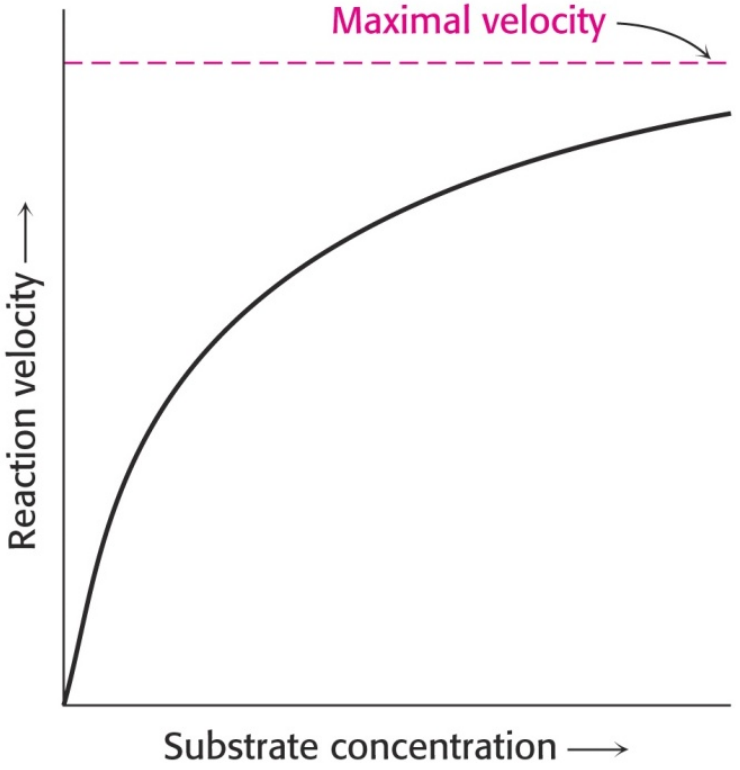
- The substrate binds to an ACTIVE SITE

X-Ray

- Evidence: Fluorescence

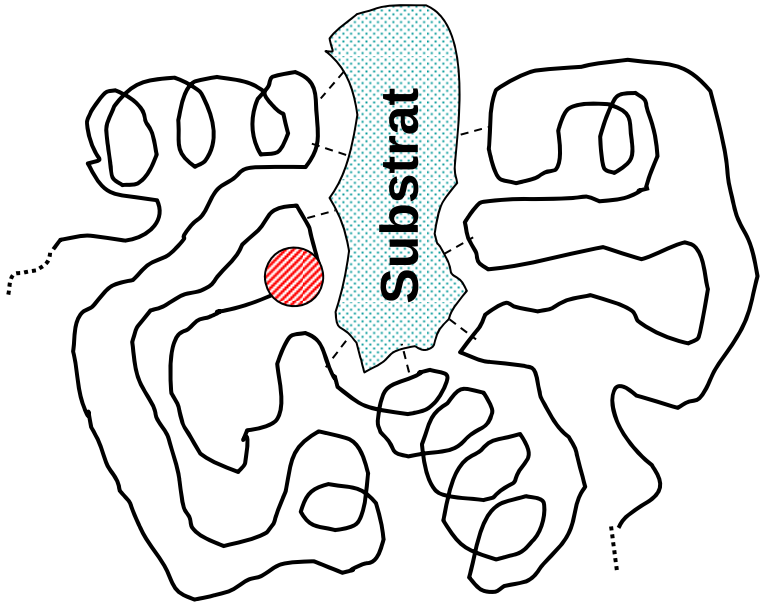
Saturation kinetics



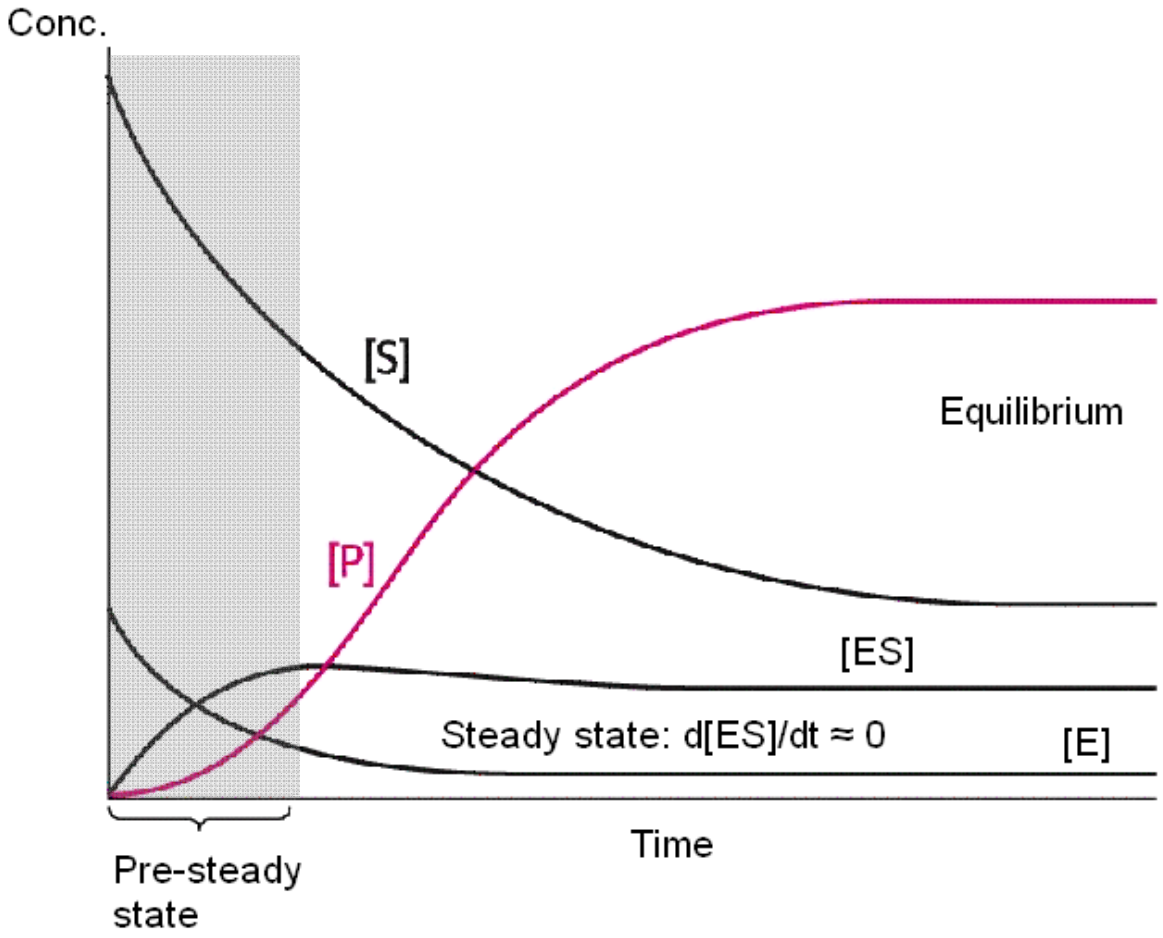
# General properties of active site

- Active site is a cavity
- Substrate is bound through weak interactions
- Specificity is based on how well a substrate fit
  (lock and key or induced fit)
- Active site can provide a water-free
  environment (solvent effect)
- Catalytic groups in the active site:
  - from side chain of amino acid
  - from cofactor



# Enzyme kinetics

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

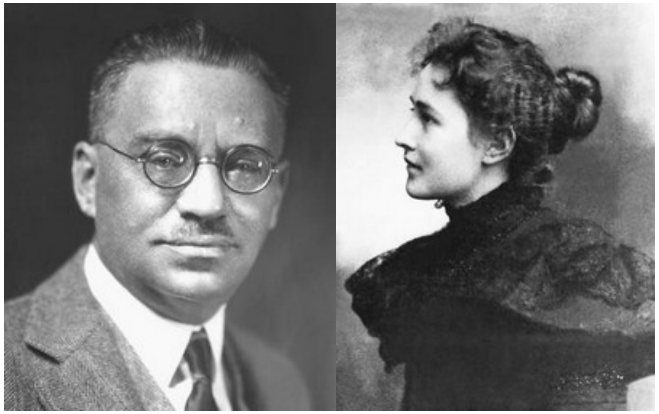


# Michaelis-Menten kinetics

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

You can formulate the Michelis-Menten equation assuming STEADY-STATE kinetics

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



$V$ = the initial reaction rate

$V_{max}$ = the maximal rate = $k_2\,[E]_{tot}$

$K_M$ = Michaelis-Menten constant

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

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

$$V = \frac{d[P]}{dt} = k_2[ES] \qquad \frac{d[ES]}{dt} = k_1[E][S] - [ES](k_{-1} + k_2)$$

At steady state: $\frac{d[ES]}{dt} = 0$ $\qquad k_1[E][S] - [ES](k_{-1} + k_2) = 0$

$$[E] = [E]_0 - [ES]$$

$$k_1([E]_0 - [ES])[S] - [ES](k_{-1} + k_2) = 0$$

$$k_1[E]_0[S] - [ES](k_1[S] + k_{-1} + k_2) = 0$$

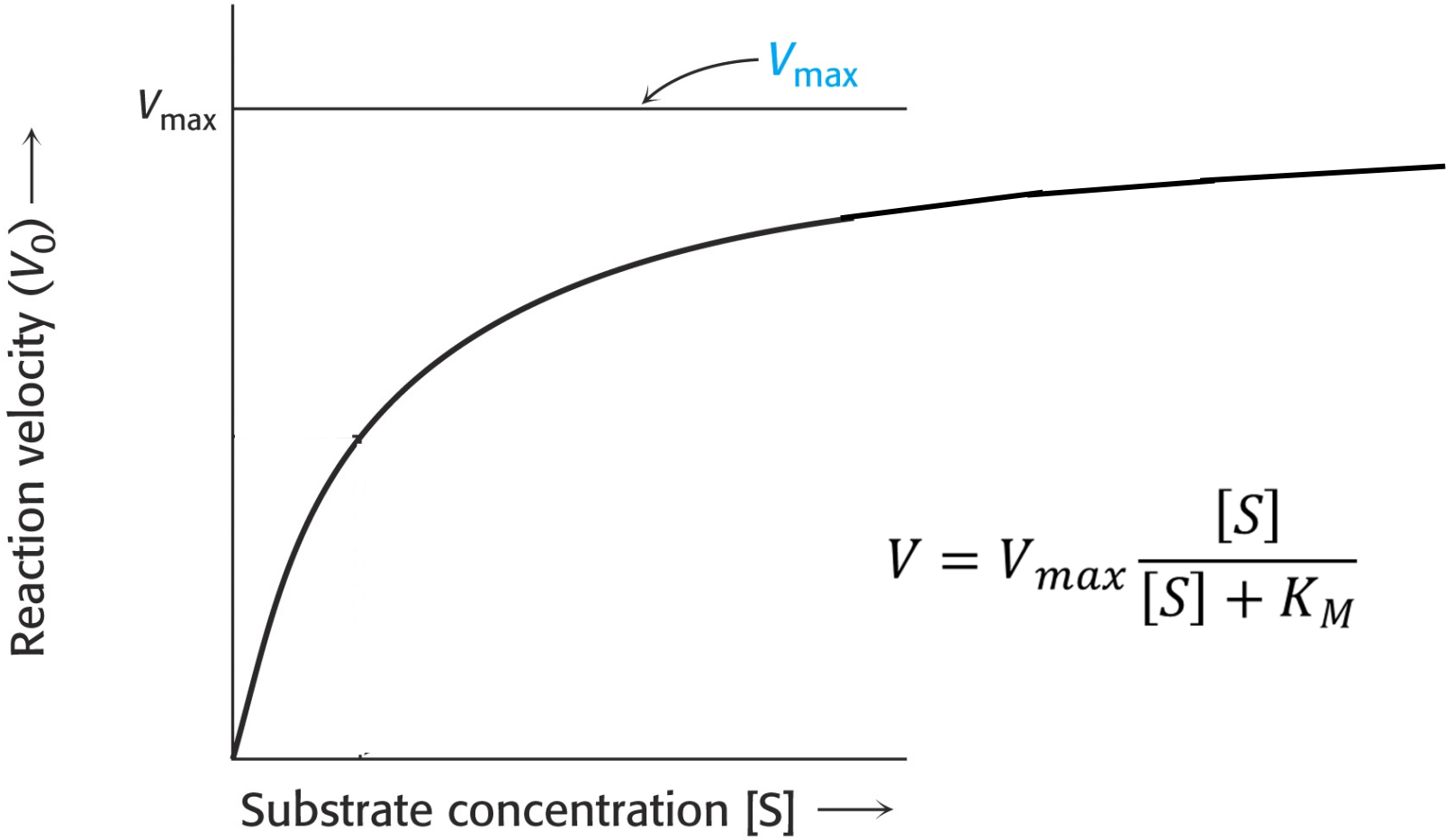
$$[ES] = \frac{k_1[E]_0[S]}{k_1[S] + k_{-1} + k_2} = \frac{[E]_0[S]}{[S] + \frac{k_{-1} + k_2}{k_1}}$$

Let $\qquad K_M = \frac{k_{-1} + k_2}{k_1}$ $\qquad$ then $\qquad [ES] = \frac{[E]_0[S]}{[S] + K_M}$

$$V = k_2[ES] = \frac{k_2[E]_0[S]}{[S] + K_M} = V_{max}\frac{[S]}{[S] + K_M}$$

# Michaelis-Menten kinetics

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




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

# What do $V_{max}$ and $k_{cat}$ mean?


$$V_{max} = k_2[E]_0$$

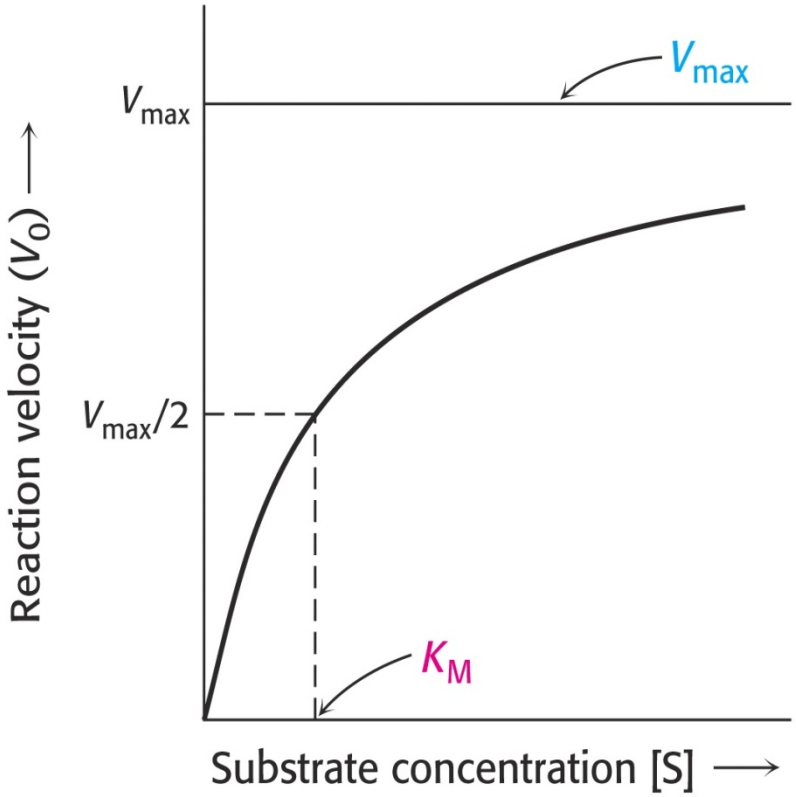
$$V_{max} = k_{cat}[E]_0$$

$k_2$ or $k_{cat}$ is called **turnover number**, it tells what an enzyme can do **at best**

| **Enzyme** | **Function** | $k_{cat}$ |
|---|---|---|
| Carboanhydrase | Hydratises $CO_2$ | 600 000 $s^{-1}$ |
| Lactate dehydrogenase | Oxidises lactate | 1 000 $s^{-1}$ |
| Lysozyme | Open up cell walls | 0,5 $s^{-1}$ |

# What is the meaning of $K_M$?

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




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

$K_M$ : substrate concentration at

$V = V_{max} / 2$

Low $K_M$ indicates that enzyme has **high affinity** for substrate

# What is the meaning of $k_{cat}/K_M$?

$$V = \frac{k_{cat}[E]_0[S]}{[S] + K_M}$$

The cell often works at substrate concentrations much lower than $K_M$

When $[S] \ll K_M$ $$V = \frac{k_{cat}}{K_M}[E]_0[S]$$

$K_{cat}/K_M$ is a good indicator of efficiency in the cell

Some enzymes have a $k_{cat}/K_M$ value close to $10^8$ $M^{-1}s^{-1}$

They have reached KINETIC PERFECTION!

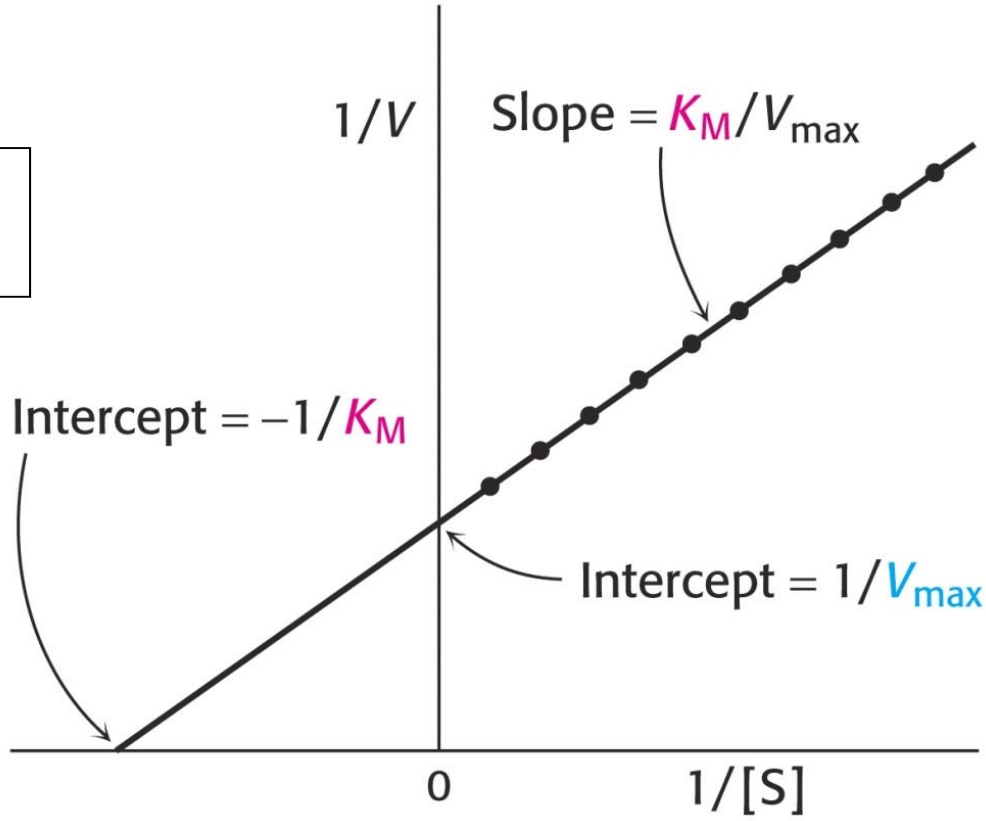
Example: Acetylcholinesterase

# How to determine $K_M$ och $V_{max}$?

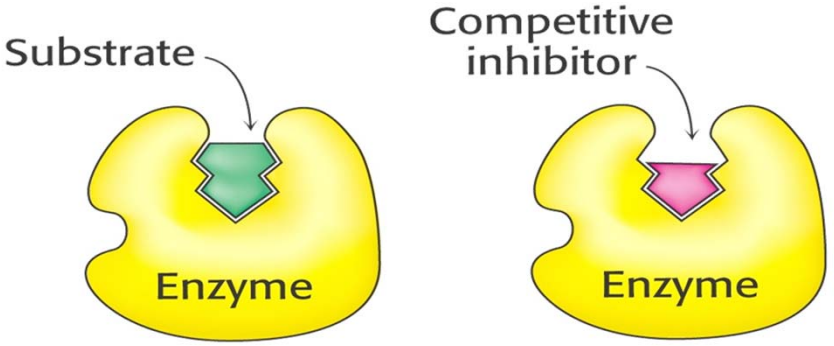
$$V = V_{max}\frac{[S]}{[S] + K_M} \quad \Rightarrow \quad \frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\frac{1}{[S]}$$

1) Determine V for a number of [*S*]
2) Plot 1/*V* vs. 1/[*S*]

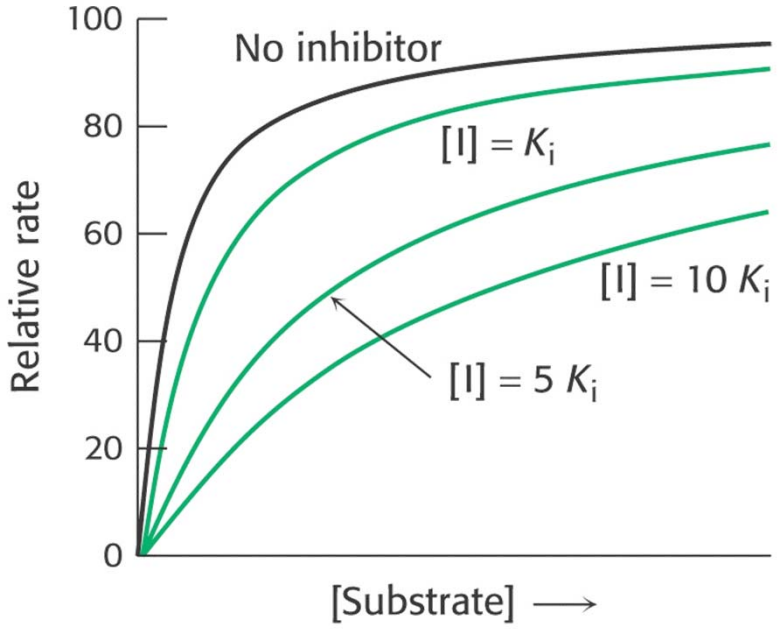
Lineweaver-Burk plot



# Enzyme inhibition
# A. Competitive inhibition




EI-complex

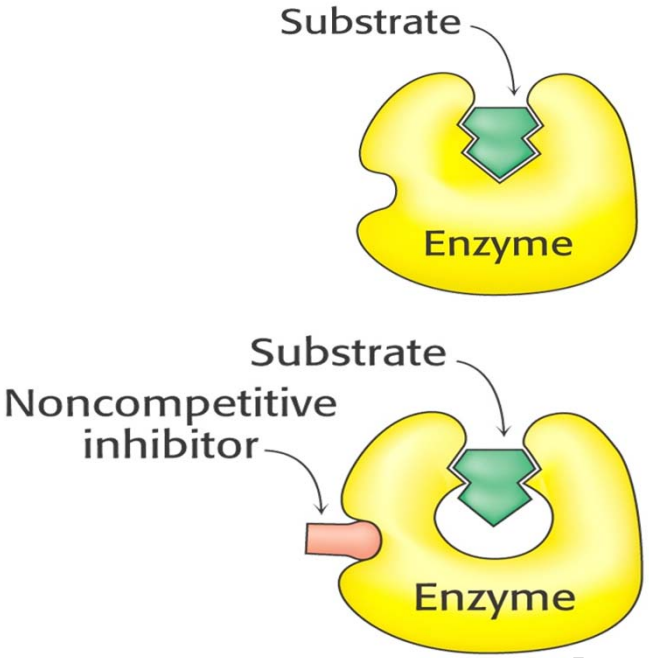



Competitive inhibition can be counteracted by adding more substrate

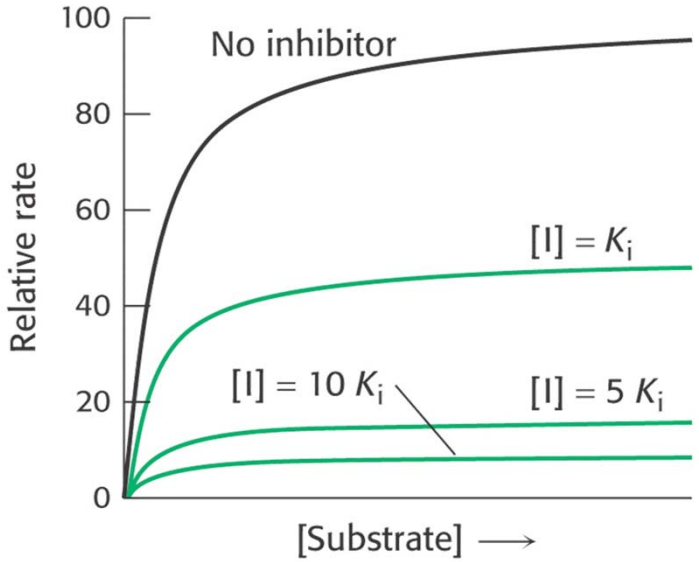
$$V = V_{\max} \frac{[S]}{[S] + K_M \cdot (1 + \frac{[I]}{K_i})}$$

$K_i = K_{diss}$ for the EI complex

# Enzyme inhibition
# B. Non-competitive inhibition




EI-complex




$$V = \frac{V\,\max}{(1+\frac{[I]}{K_i})} \cdot \frac{[S]}{K_M + [S]}$$

Non-competitive inhibition can´t be diminished by more substrate

$K_i = K_{diss}$ for the EI-complex

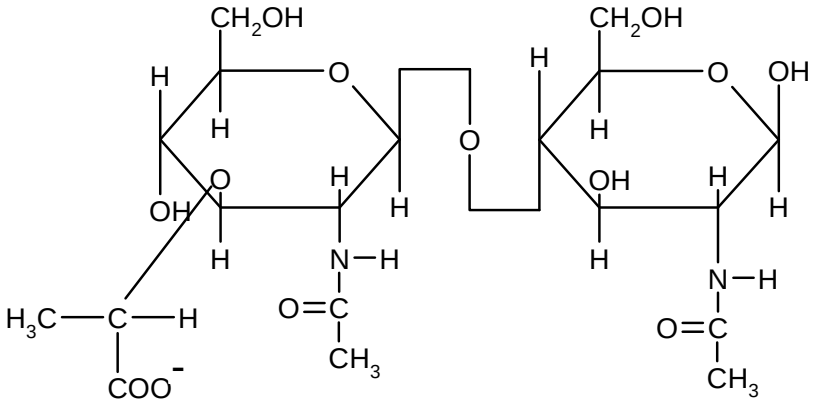
# Lysozyme hydrolyses bacterial cell walls

**- -O–NAG–O–NAM–O–NAG–O–NAM - -**

**$H_2O$**

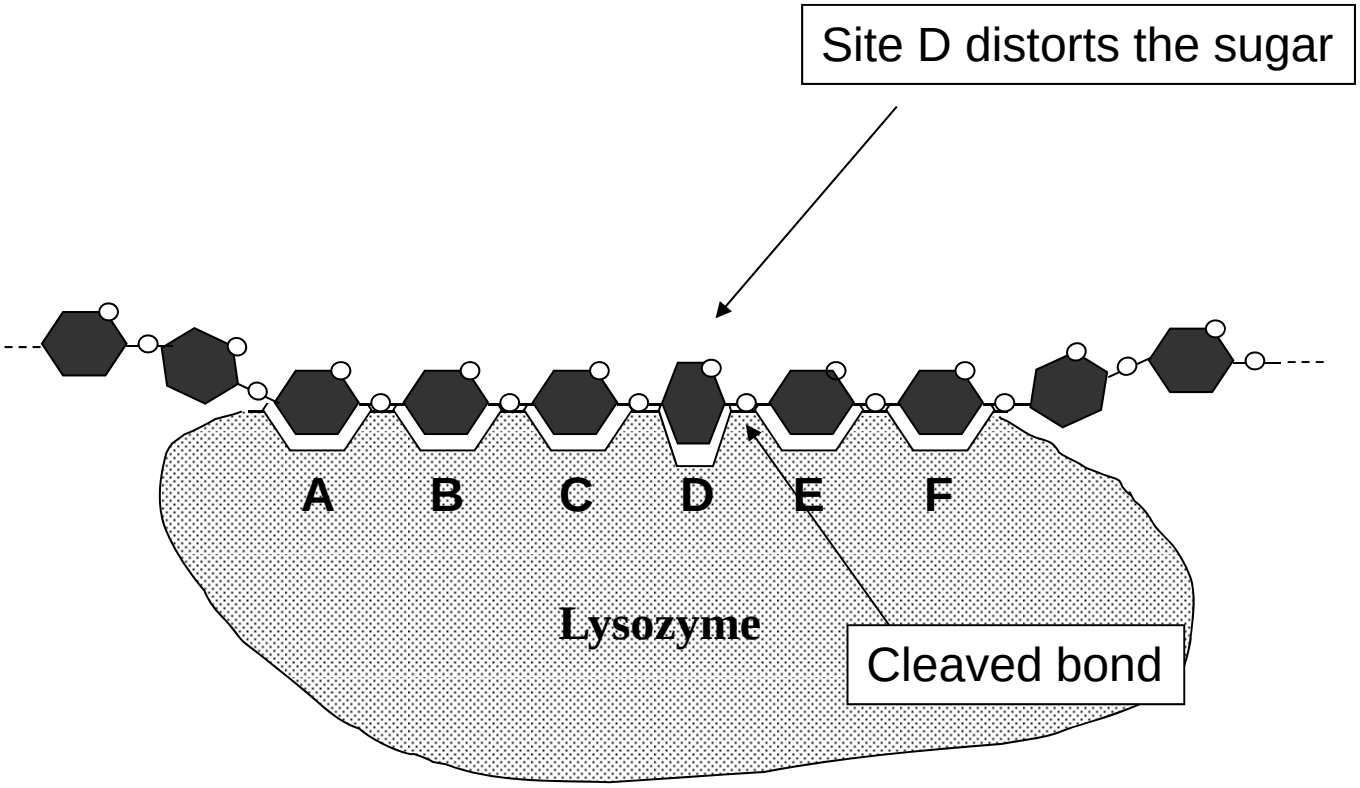
**- -O–NAG–O–NAM–OH + HO–NAG–O–NAM- -**

NAM: N-acetylmuramic acid

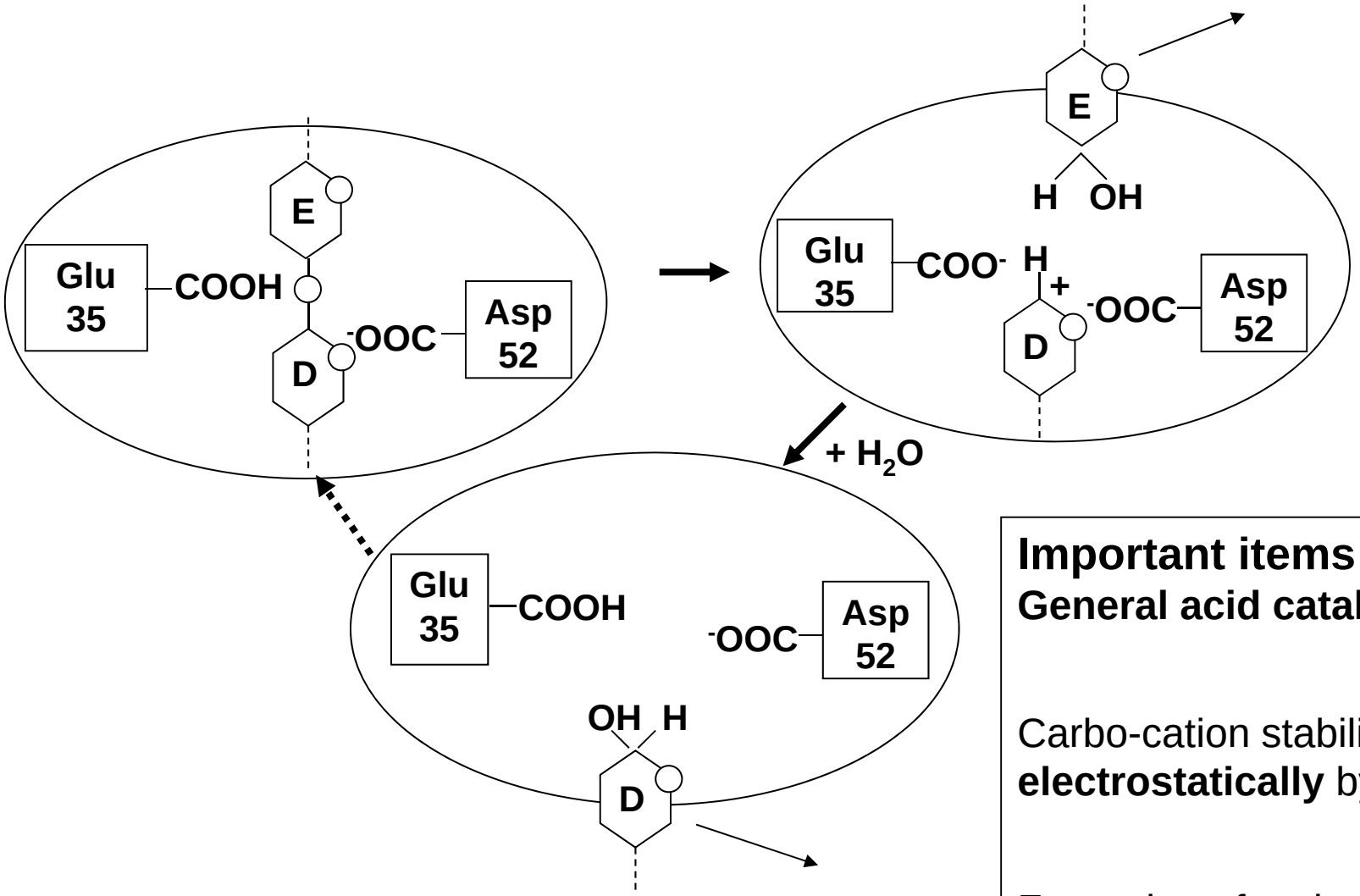



NAG: N-acetylglucosamine

# The active site of Lysozyme



# The catalytic mechanism of Lysozyme

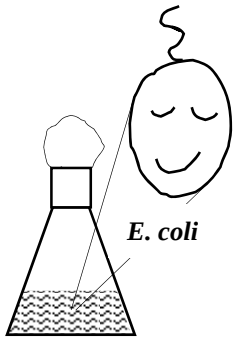



**Important items:**
**General acid catalysis**

Carbo-cation stabilised **electrostatically** by Asp 52

Formation of carbo-cation favoured by the **steric** effect in site D

# Enzyme activity is controlled




Very important for the cell to be able to control the enzyme activity!

# Different ways to control enzyme activity

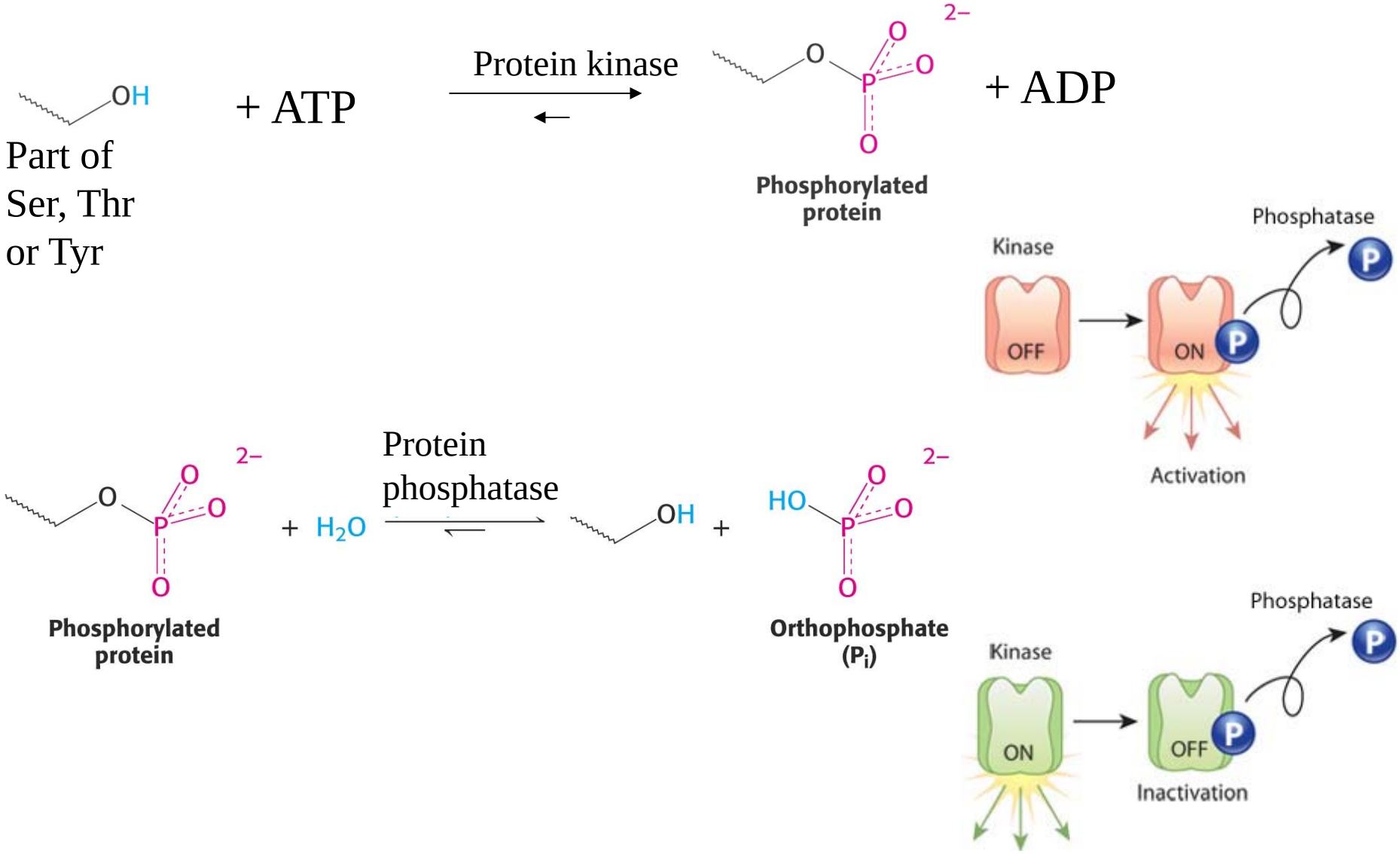
**A.** Make new enzyme molecules – degrade (slow control)

1. **synthesis** (transcription/translation)
2. synthesis of particular **isoenzyme**
3. **degrade** the enzyme

**B.** Control of the activity of existing enzymes (fast control)

1. **Allosteric control**
   - **-** often feed-back inhibition
2. **Reversible covalent modification**
   - **-** e.g. phosphorylation/dephosphorylation of enzyme
3. **Proteolytic activation**
   - removing part of the polypeptide

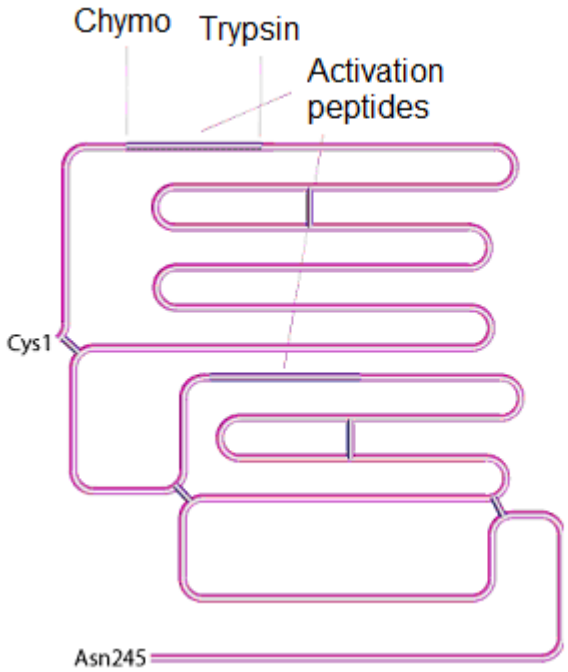
# Phosphorylation is made by **kinases**
# Dephosphorylation by **phosphatases**



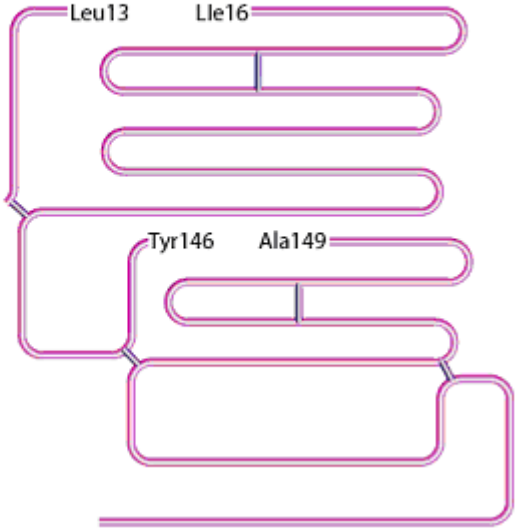
# Proteolytic cleavage activates proteins

Some enzymes are synthesised as **inactive** pro-enzymes = **zymogens**. They are cleaved and refolded to active proteins when needed

Chymotrypsinogen




α-Chymotrypsin



# Enzymes in washing powder

**Why?**

Dirty spots will disappear more easily

- lower temperature

- energy is saved

**Which enzymes?**

- Proteases degrade proteins

- Lipases degrade fats

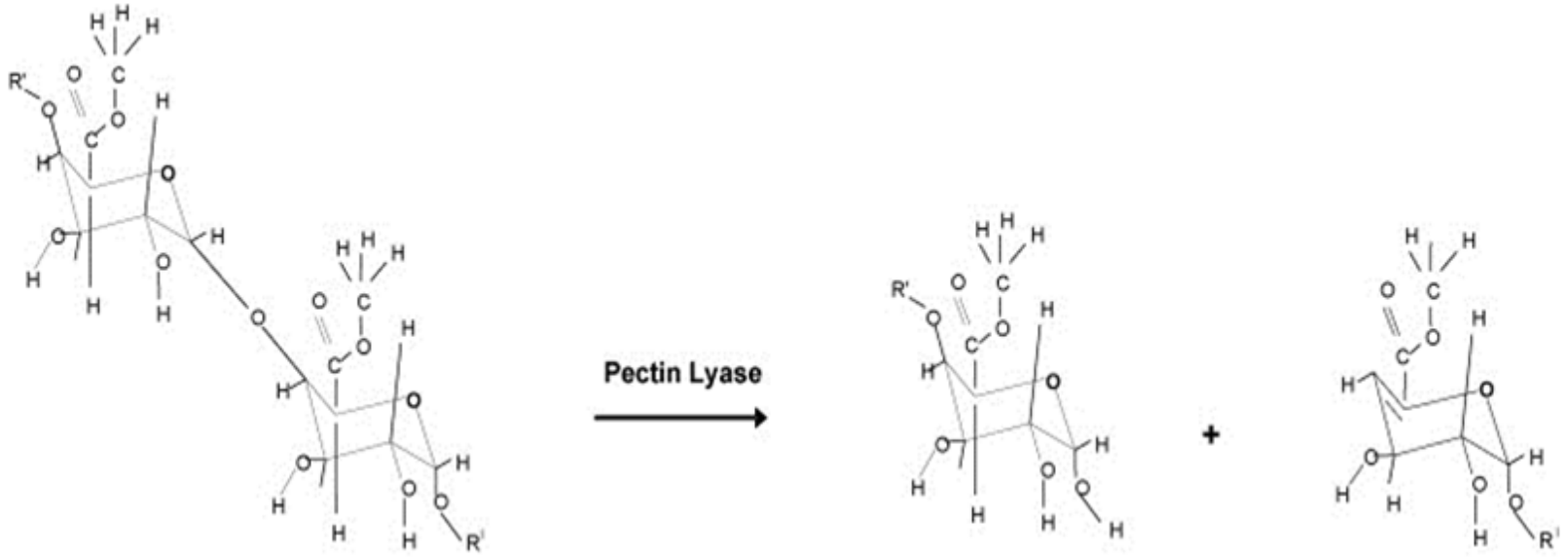
- Amylases degrade carbohydrates

- Cellulases degrade cellulose

# Treatment of cotton fibres to facilitate dyeing

In order to dye cotton fibres, non-cellulose material (pectin) must be removed. This can be done in alkaline solutions.

The enzyme pectin lyase can be used instead, requiring lower temperature and resulting in fewer waste.



# Summary

- Enzymes are efficient and specific
- Cofactor - Coenzyme - Cosubstrate - Prosthetic group
- 6 enzyme classes: EC-number
- Enzymes perform catalysis by stabilizing transition state
- Active site of enzyme
- Michaelis-Menten kinetics
- $K_M$, $V_{max}$, $k_{cat}$ and $k_{cat}/K_M$
- Enzyme inhibition
- Control of enzymatic activity
- Technical use of enzymes