2017-2018
Licence Internationale Part 1
Jean Chaudière

Enzymes

Protein catalysts

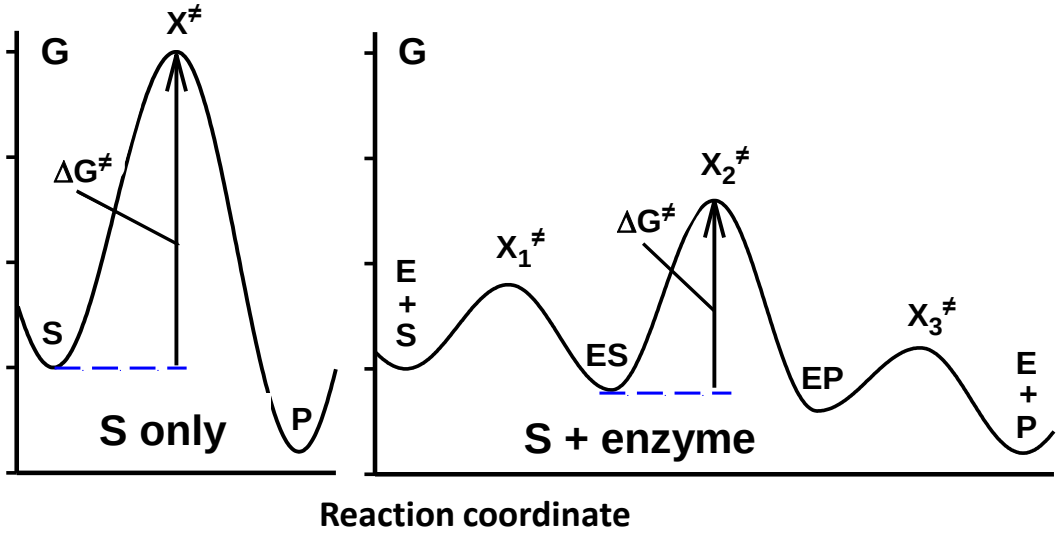
- **Multiply uncatalyzed reaction rates by a very high factor**
- **High specificity for recognition and transformation of their substrates**
- **Work optimally at temperature, pressure and ionic strength of the biological compartment in which they are found**
- **Often regulated by inhibitors or activators**
  including auxiliary enzymes

Why enzymes are very efficient catalysts ?

Protein evolution has led to optimal complexation of transition states along a reactional sequence

affinity for transition states >> affinity for substrates

Comparison between an elementary reaction and the same reaction catalyzed by an enzyme



Human Phospholipase A_2

complexed with a phospholipid substrate analog (whose binding invoves one of the two calcium atoms)

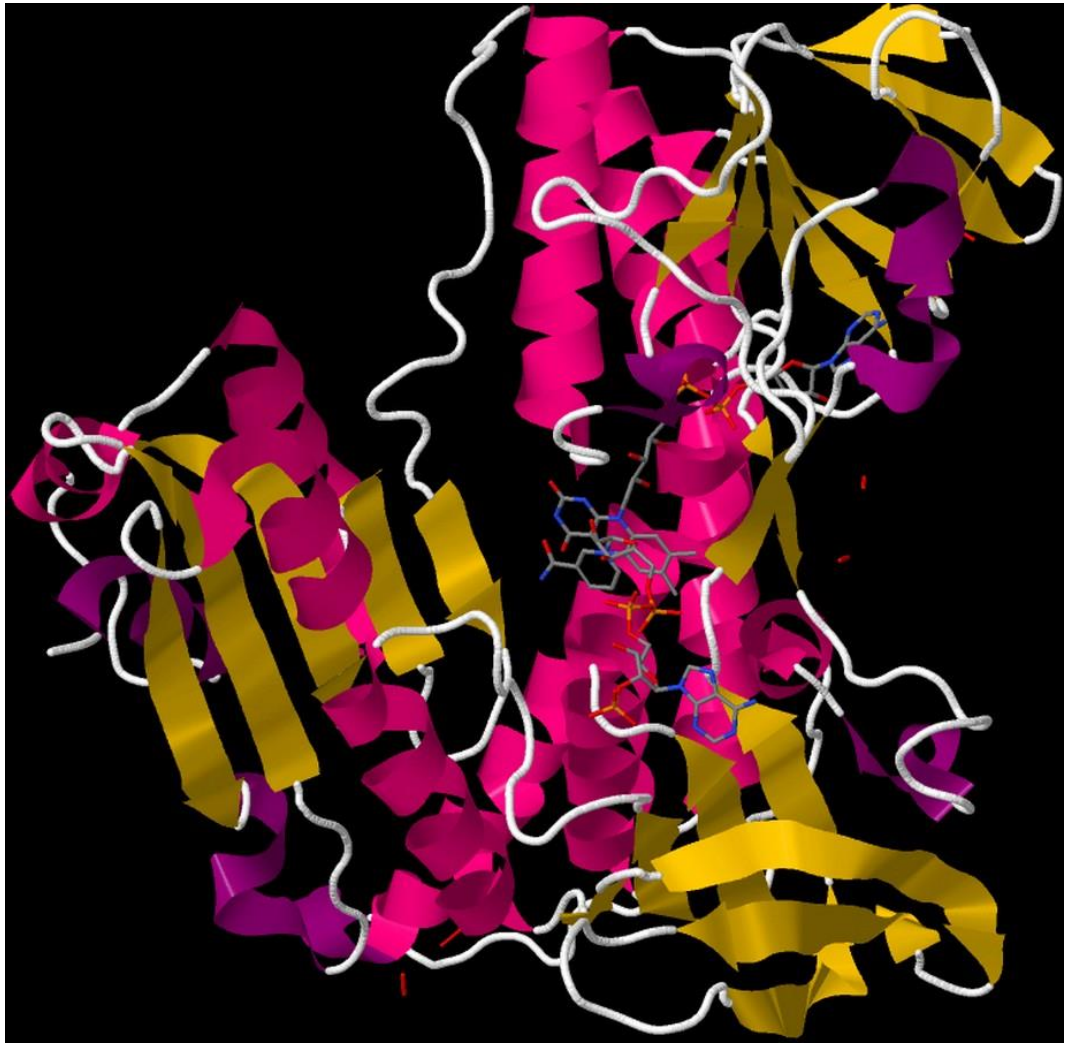
(1KQU)

$$\text{Phospholipid} + H_2O \xrightarrow{PLA_2} \text{FFA} + \textit{products}$$

Human glutathione peroxidase (1GP1)

Homotetramer
(4 active sites containing selenium)

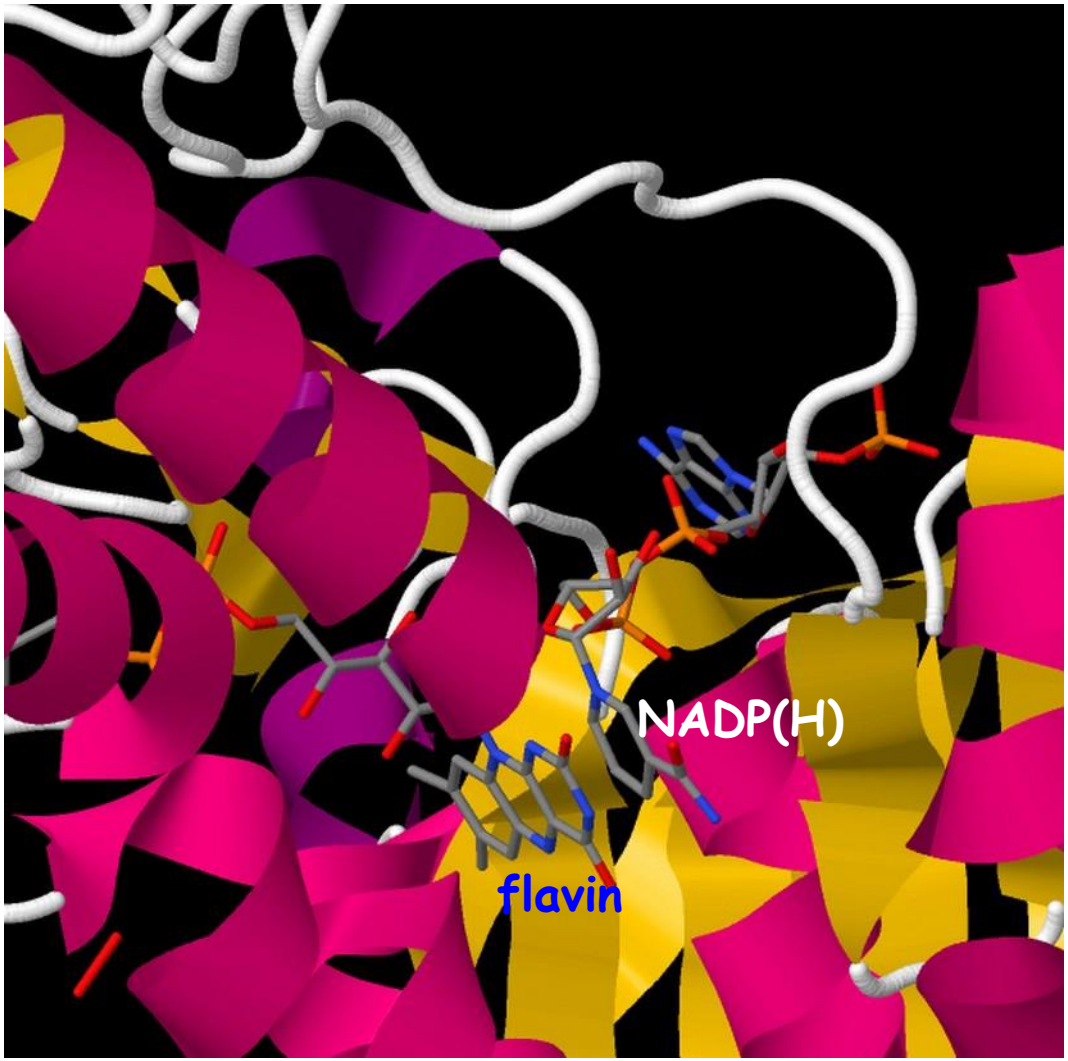
$$2\ GSH + H_2O_2 \xrightarrow{\text{Se-GPx}} GSSG + 2\ H_2O$$



Human GSSG reductase (3DJG)

Homodimer

$$GSSG + NADPH \xrightarrow{GR} 2\ GSH + NADP^{+}$$



Human GSSG reductase (3DJG)

Zooming on the active site

Cyclooxygenase COX2

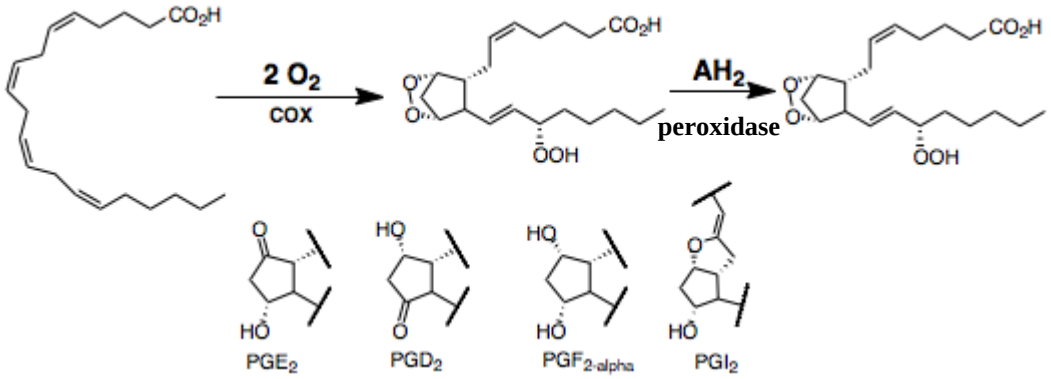
(3HS5)

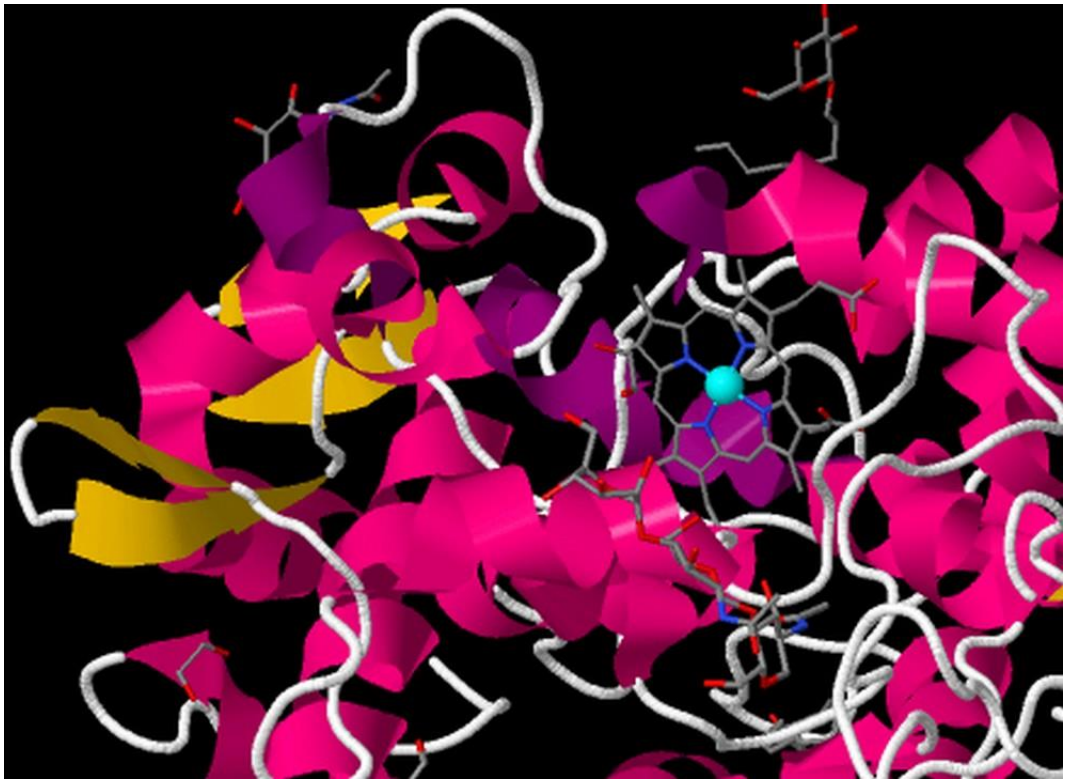
Homodimer
complexed with a substrate analog, polyunsaturated fatty acid C20:5 ω3

$$C20{:}4\omega6 + 2\ O_2 \xrightarrow{COX2} PGG_2 \xrightarrow{COX2} PGH_2$$

PGH_2 is the precursor of several prostanoids (hormones that include prostaglandins and thromboxanes)

COX2 is the target of some non-steroidal anti-inflammatory compounds which include diclofenac





Cyclooxygenase COX2

(3HS5)

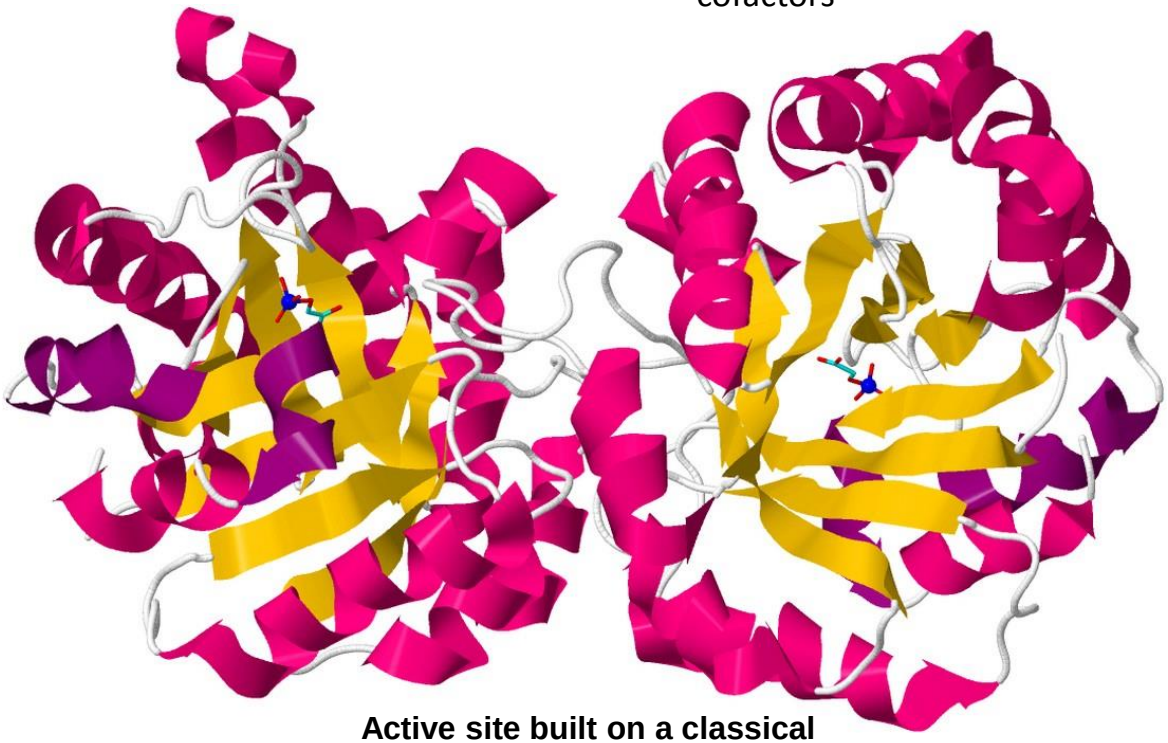
Zooming on the porphyrin active site
(iron is colored in *)**

Active site = binding site(s) + catalytic site

Pocket, crevice

Residues that are essential for binding of substrates and cofactors

Residues that are essential for catalytic transformation ± prosthetic group

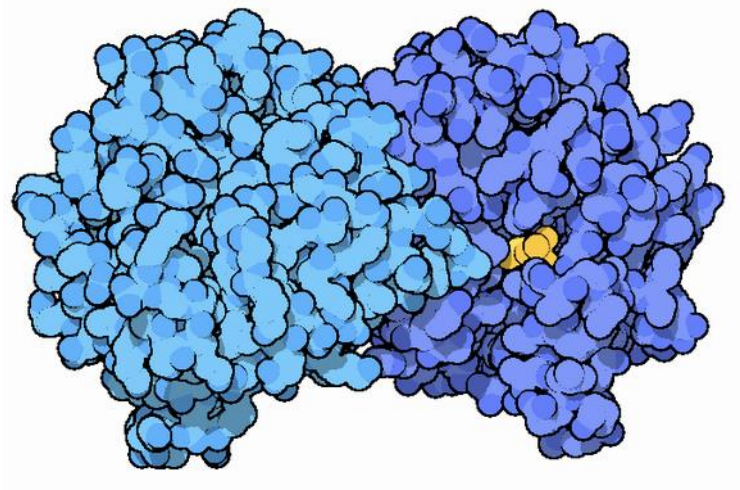


Active site built on a classical supersecondary structure
(β barrel)

example of
triose phosphate isomerase
(3YPI)

Homodimer

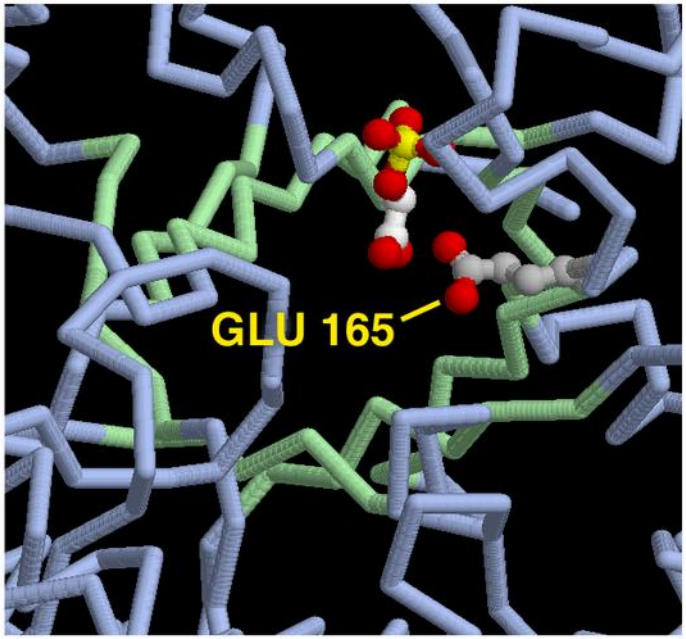
complexed with
3-Phosphoglycolate
(substrate analog)



Triose phosphate isomerase

(3YPI)

Note that if all atoms are represented as here, the 3-dimensional structure of the crystallized enzyme (obtained from X-Ray diffraction) is hardly useful.
The most interesting features are buried under the surface.
At least we see here that the enzyme has largely entrapped its substrate (in yellow).

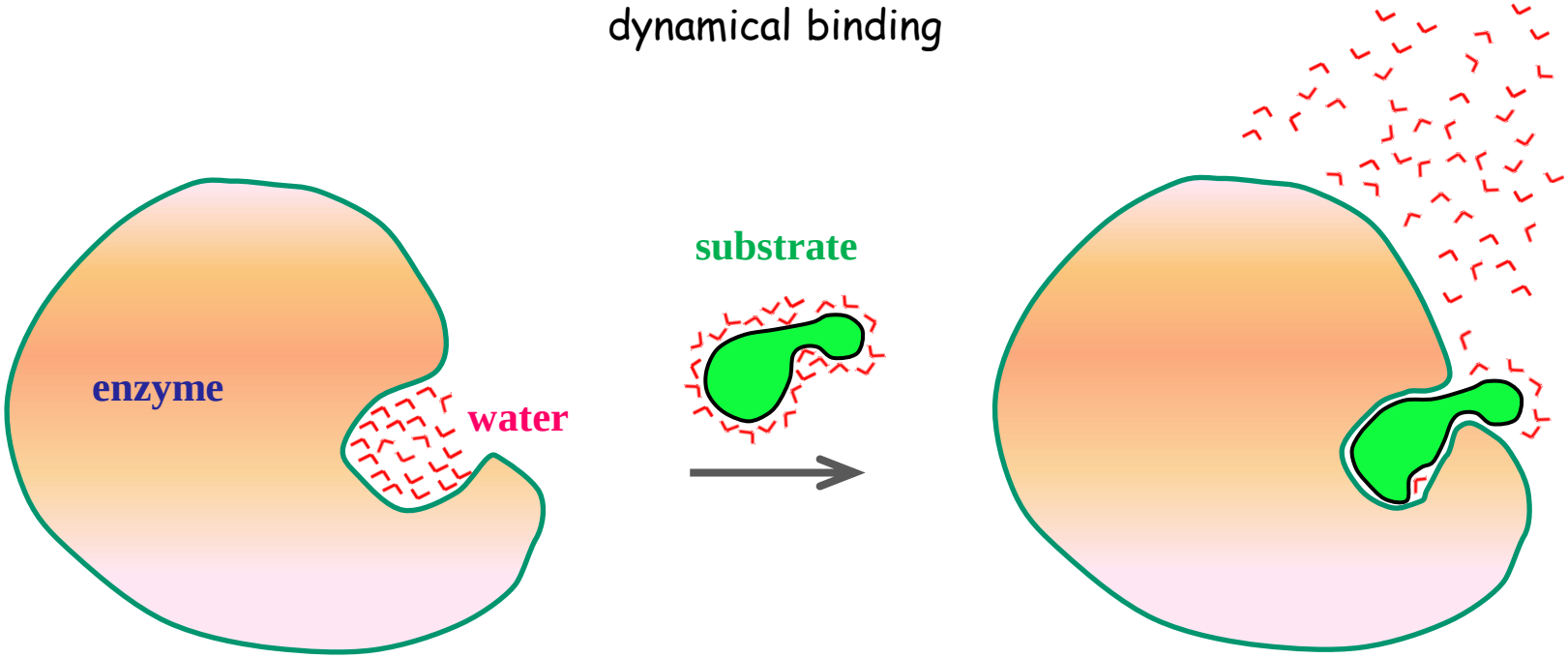


Zooming on the active site

Here, a glutamate sidechain is essential. Enzyme activity is lost if this aminoacid residue (number 165 in the AA sequence) is mutated for another one.

Induced fit

dynamical binding



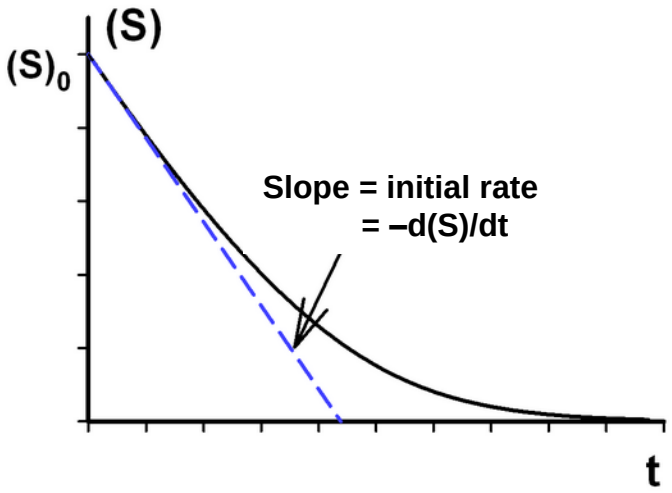
Van der Waals bonds
Hydrogen bonds
Electrostatic Interactions
Hydrophobic Interactions

Many water-soluble enzymes are able to catalyze reactions in a microenvironment from which water has been excluded.

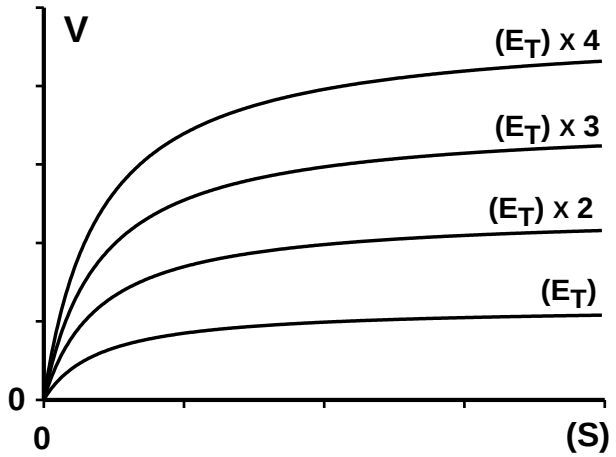
This environment looks more like organic solvent than water.

Enzyme kinetics

Consumption Curve



Rate curve



One substrate model with a single intermediate complex :

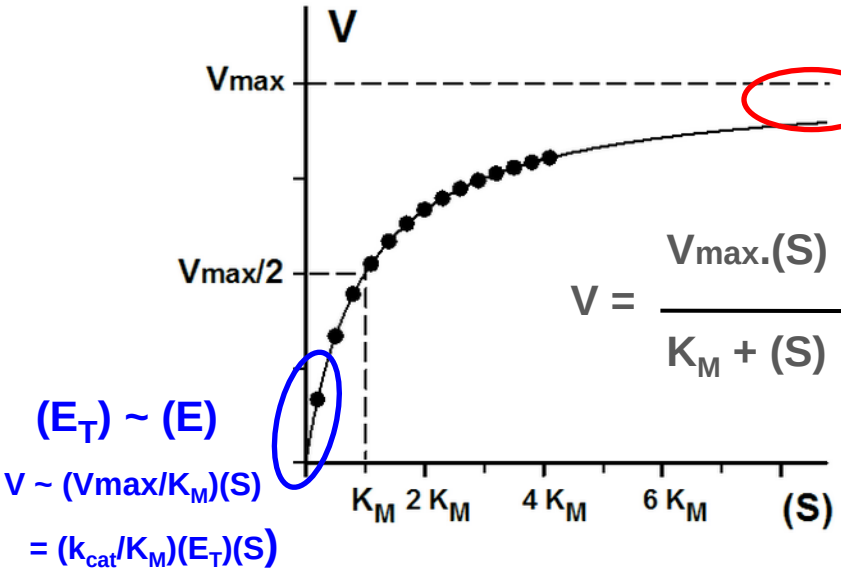
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Henri; Michaelis & Menten;
Briggs & Haldane

$$V = \frac{V_{max}.(S)}{K_M + (S)}$$

"Michaelis-Menten" equation

Hyperbolic rate curve



$(E_T) \sim (ES)$

$V \sim Vmax = k_{cat}.(E_T)$

$$V = \frac{Vmax.(S)}{K_M + (S)}$$

"Michaelis-Menten" equation

$(E_T) \sim (E)$

$V \sim (Vmax/K_M)(S)$

$= (k_{cat}/K_M)(E_T)(S)$

K_M = concentration (S) for which V = Vmax/2

$k_{cat} = Vmax/(E_T)$

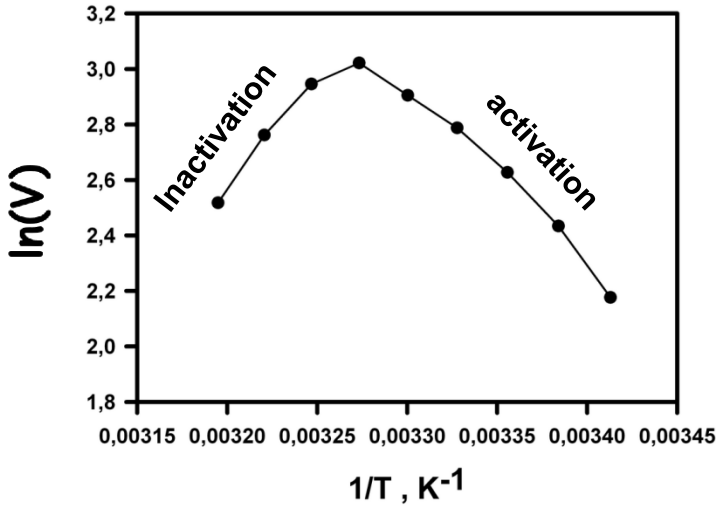
Catalytic constant (apparent 1st order rate constant)
Turnover number = number of catalytic cycles realized by one enzyme molecule per unit of time in saturating conditions

Some examples of one-substrate reactions

Transformation	Substrate	Product(s)	Example of enzyme
Isomerization	aldose	ketose	phosphoglucose isomerase
	anomer α (or β)	anomer β (or α)	galactose mutarotase
	ose	epimer	UDP-galactose 4-epimerase
	chiral substrate	enantiomer	proline racemase
Intramolecular	anthrone	naphtacenone	tetracenomycin cyclase
Condensation	ATP	Cyclic AMP	adenylate cyclase
Hydration/	CO_2	HCO_3^-	carbonic anhydrase
Dehydration	alkene	alcohol	fumarase
Hydrolysis	ester	acide + alcohol	acetylcholinesterase
	lactone	acide-alcohol	gluconolactonase
	amide	acide + amine	fatty acid amide hydrolase
	anhydride	acide + acide	pyrophosphatase
	polypeptide	(oligo)peptides	α-chymotrypsin
	polysaccharide	(olig)osides	lysozyme
	guanidine	urea + amine	arginase

Typical effect of temperature

Arrhenius graph



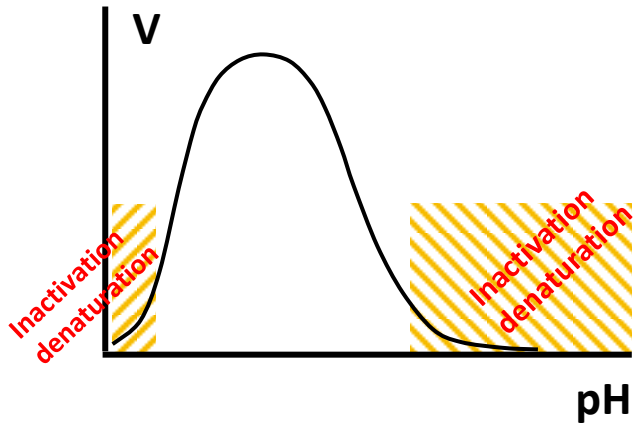
When T° increases, the enzymatic reaction rate Increases but above a threshold temperature the protein is denatured (it loses its 3D structure) and the activity is irreversibly lost

ex of non linear Arrhenius graph obtained for an arbitrary (S) value

(dihydroflavonol reductase)

Typical effect of pH

Bell curve



Enzymes are usually inactivated (irreversible inhibition) at very low pH (very acidic) as well as at very high pH (very basic).
But the enzyme rate may be markedly reduced by reversible inhibition on more extended acidic and/or basic regions.

Each enzyme has its own pH profile :
The maximal rate may be observed on a narrow pH interval or on a more extended plateau

If one wants to use an enzyme in biotechnology, it is necessary to maintain (with an appropriate buffer) a pH that is not far from the pH region for which the rate is maximal.

The toolbox of coenzymes

Nicotinamide Adenine Dinucleotide — **NAD^+** *Oxidation of –CHOH- to >C=O*; **NADPH** *Reduction of >C=O to –CHOH- and of >C=N- to –CH-NH-*

Flavin Adenine Dinucleotide — **FAD** *Oxidation alkane > alkene and thiol > disulfide*; **$FADH_2$** *Reduction disulfide > thiol; Monooxygenation of nucleophiles and electrophiles (with O_2)*

ATP — **ATP** *Phosphorylation of alcohols RCHOH- to esters RCH-OPO_3^{2-} and of carboxylates $RCOO^-$ to electrophilic mixed anhydride RCO-OPO_3^{2-}*

Coenzyme A — **coASH** *Activation of Carboxylates $RCOO^-$ to electrophilic thioesters RCOScoA*

Thiamin pyrophosphate — **TPP** *Decarboxylation of α-ketoacids $RCOCOO^-$ > $RCOO^-$ + CO_2 ; Transketolisation*

Lipoic Acid — *Decarboxylation of α-ketoacids (with TPP)*

Biotin — *Carboxylation of keto-enolates $RCOCH_3$ + CO_2 > $RCOCH_2$-COO^-*

Pyridoxal Phosphate — **PLP** *Transformations of α-Aminoacids (racemisation, α-decarboxylation, transaminations, β-eliminations etc)*

Glutathione — **GSH** *Reduction of Hydroperoxides ROOH + 2 GSH > ROH + GSSG*

S-AdenosylMethionine — **SAM** *Methylation; Propylamination*

and others (cytochrome P450, Tetrahydrofolate, PQQ, TPQ etc.)

Green Chemistry

Several thousands classified enzymes

EC 1. Oxydo-réductases
EC 2. Transférases
EC 3. Hydrolases
EC 4. Lyases
EC 5. Isomérases
EC 6. Ligases

Clean synthetic processes

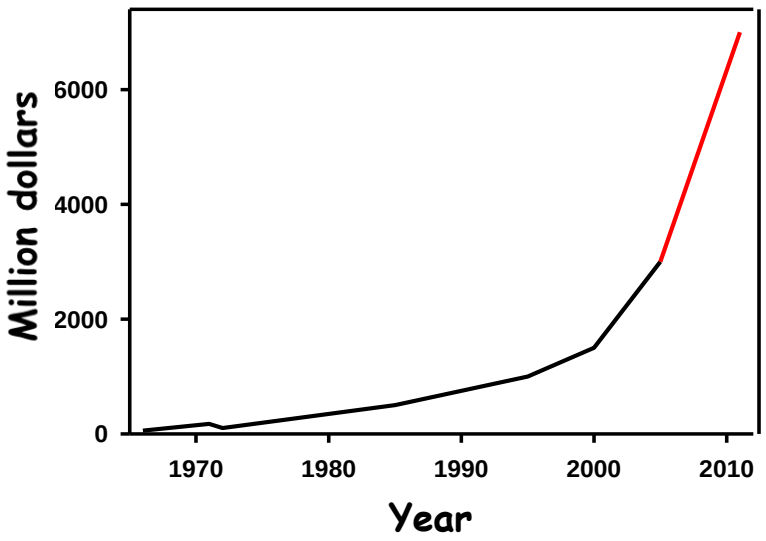
- To reduce toxic waste and reduce green house gas effects
- To limit energy expenditure
- To minimize production costs

Paul Anastas et John Warner (1998)
Green Chemistry: Theory and Practice

- *the twelve principles of green chemistry*

1. Prevent waste
2. Atom Economy
3. Less Hazardous Synthesis
4. Design Benign Chemicals
5. Benign Solvents & Auxiliaries
6. Design for Energy Efficiency
7. Use of Renewable Feedstocks
8. Reduce Derivatives
9. Catalysis (vs. Stoichiometric)
10. Design for Degradation
11. Real-Time Analysis for Pollution Prevention
12. Inherently Benign Chemistry for Accident Prevention

Enzyme market evolution



Main producers of enzymes

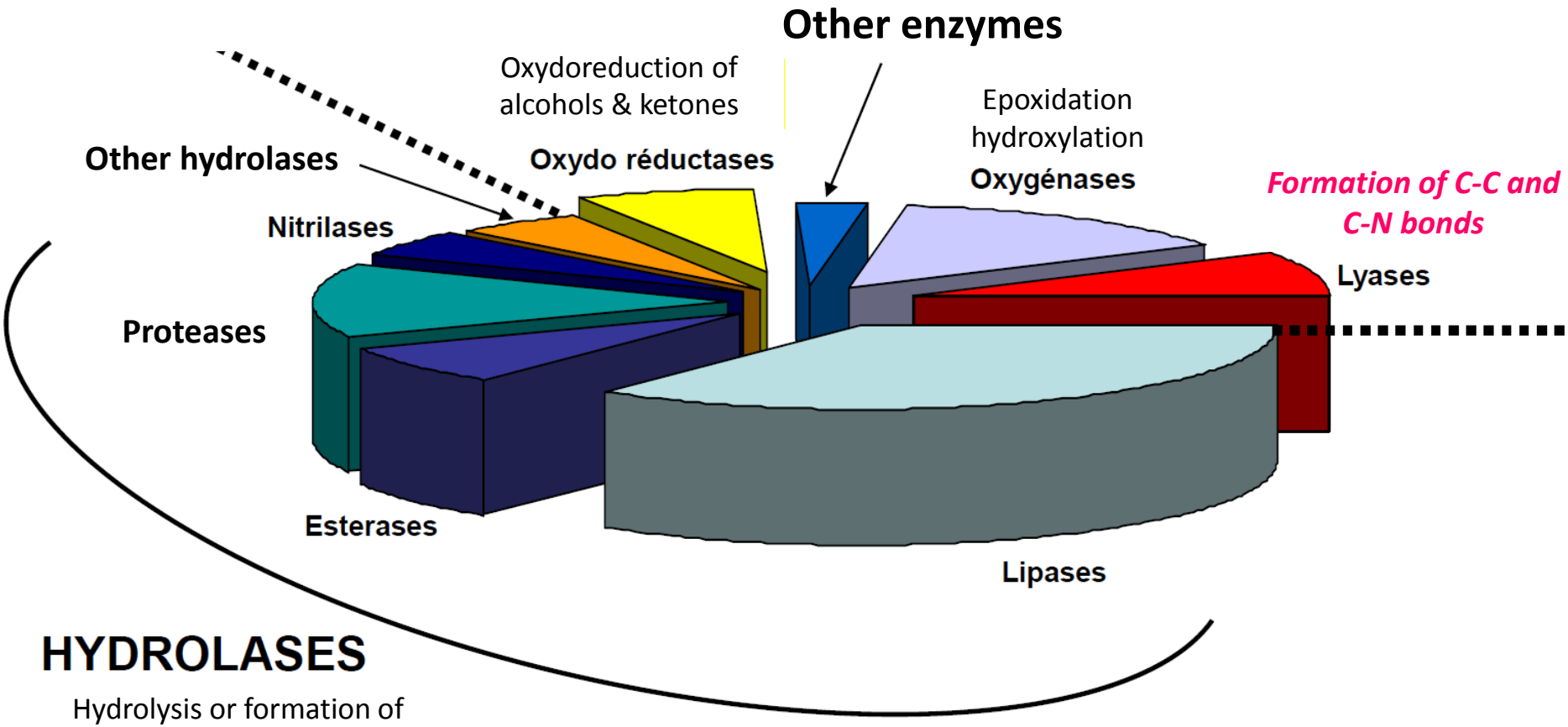
Novozyme (Denmark)	**50%**
DSM (Holland)	**20%**
Genencor International (USA)	**15%**
Solvay-Miles (USA)	**5%**
Amano, Nagase (Japon)	**< 1%**

Production of enzymes by Novozyme at Kalundborg

Industrial fermenter

Enzyme reactors of Novozyme in North America

Main classes of enzymes used in industrial processes



Enzyme Catalysis, Ed. K. Drauz and H. Waldmann, Wiley-VCH verlag GmbH, Weinheim 2002

Erroneous dogmas in enzymology

1. Enzymes only act on their natural substrates.

2. Enzymes only work properly in aqueous medium

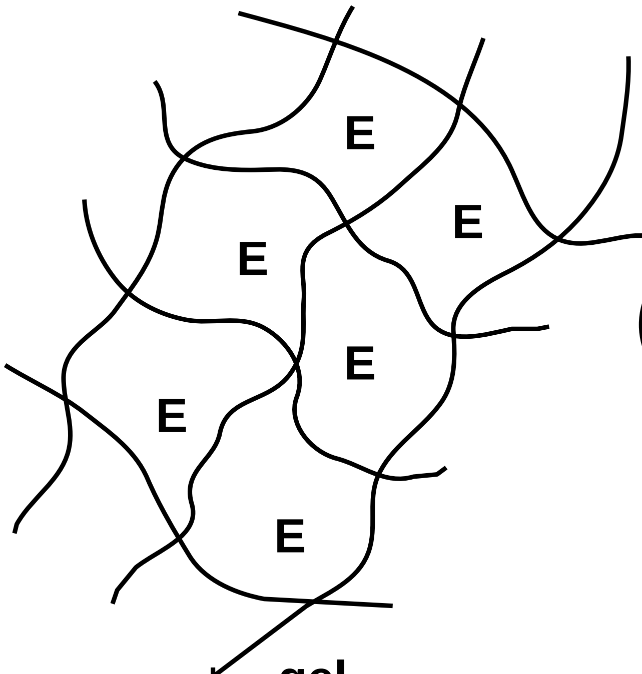
3. Enzymes do not tolerate high substrate concentrations

4. Enzymes are too unstable as production tools.

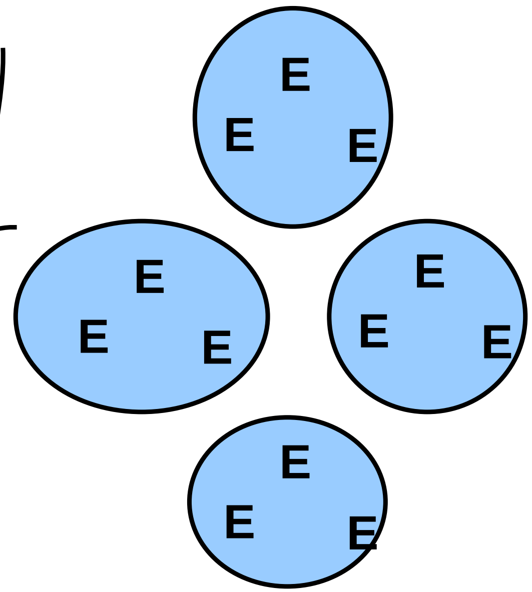
5. Enzymes are too expensive for scaling-up in fine chemistry

Inclusion

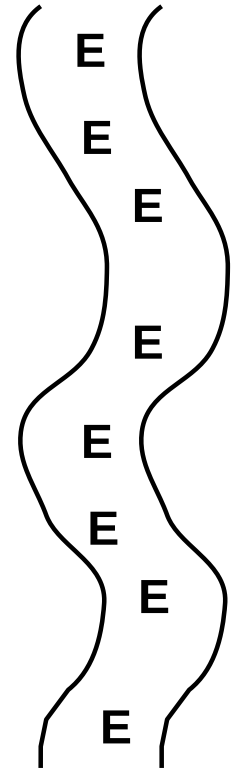
Enzymes sequestered in a porous insoluble matrix



gel

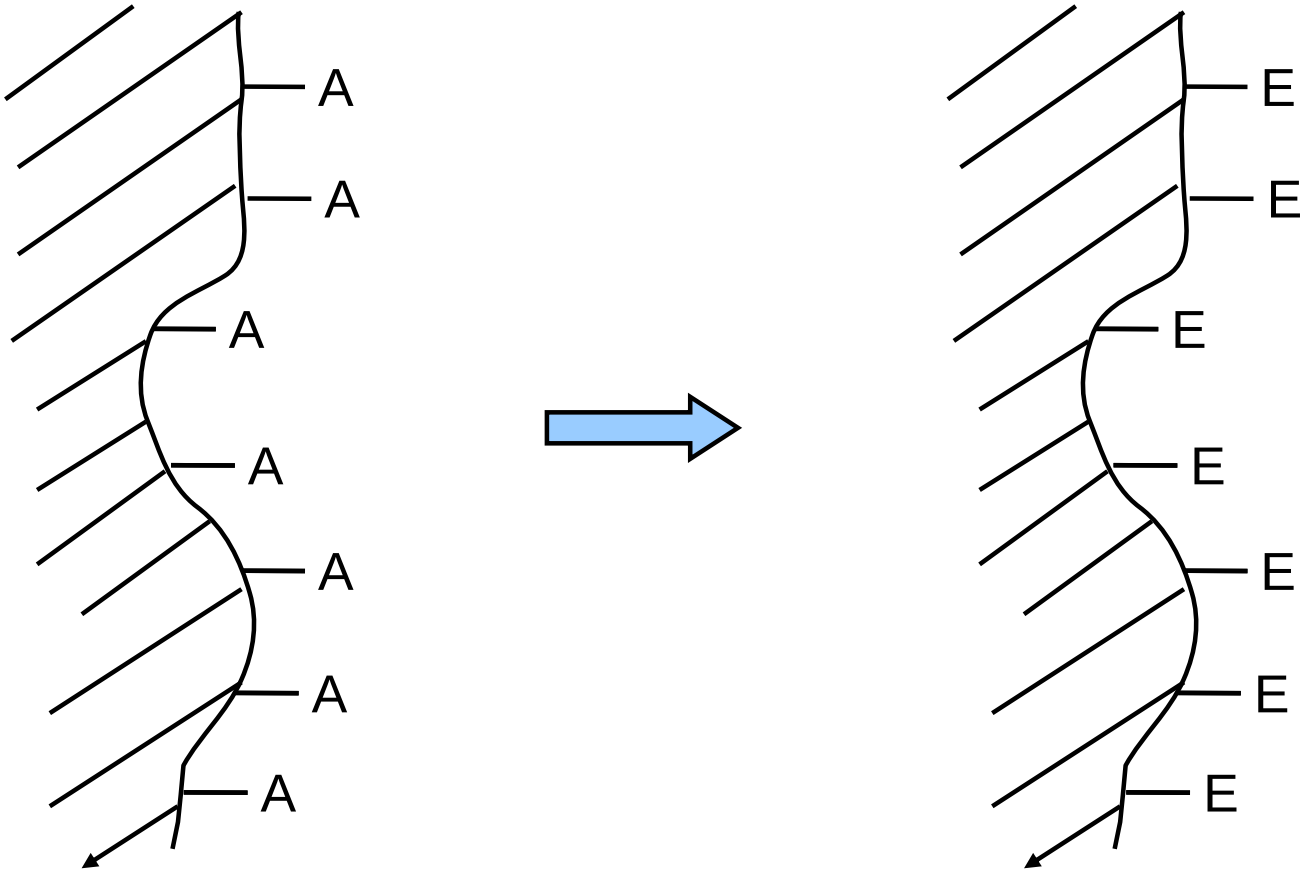


microcapsules



Hollow fibers

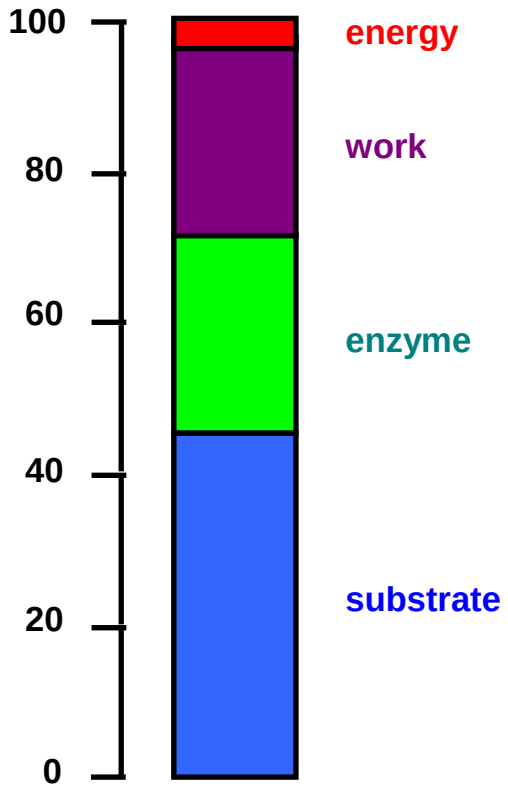
Covalent Fixation



Activation of the support

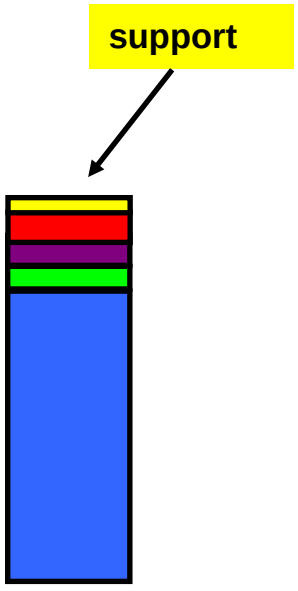
enzyme bound with a spacer

Decreased cost of continuous processes with immobilized enzymes



Discontinuous process
(batch)

Resolution of the racemic mixture of D,L-methionine by acylase (Tanabe-Seiyaku)



Continuous process
(immobilized enzyme)

Tanabe Seiyaku (Japan) has been using enzymes from microorganisms to produce aminoacids since 1970. The immobilization of enzymes on a solid support to use them repeatedly has induced 40% cost savings. By genetic modification of the microorganisms to optimize the enzymes, the company has multiplied its productivity by 15 and strongly reduced its organic waste.

Enzymes are also drug targets in drug design …

Examples of drugs which are enzyme inhibitors

Antibiotics :

Penicillins = irreversible inhibitors of bacterial transpeptidases

Anti-viral :

Protease inhibitors (HIV)
Reverse transcriptase inhibitors (HIV) : nucleosides; others.
Neuraminidase inhibitors (influenza virus, types A and B).

Anti-inflammatory :

Aspirin = inhibitor of type 1 cycloxygenase.
Diclofenac = irreversible inhibitor of type 2 cycloxygenase.

Anti-cancer :

Inhibitors of dihydrofolate reductase; ex: methotrexate, structural analog of FH_4.
Inhibitors of tyrosine kinases (chronic myeloid leukemia; gastro-intestinal tumors etc..).

Cardio-vascular :

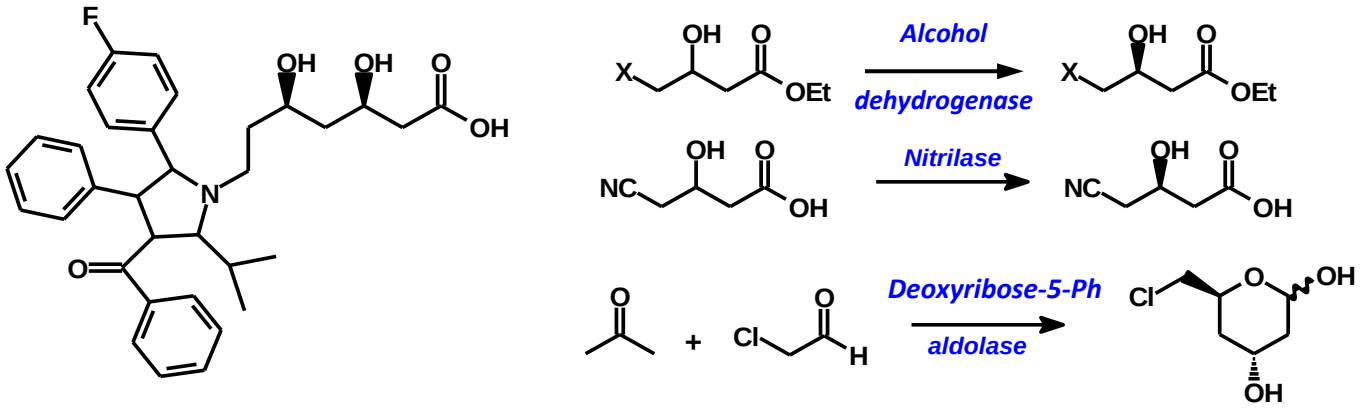
Inhibitors of the angiotensin conversion enzyme (hypertension, cardiac insufficiency)
Inhibitors of type 3 phosphodiesterase (cardiac flux)
Inhibitors of type 5 phosphodiesterase (c-GMP specific) : Viagra.

Central Nervous System :

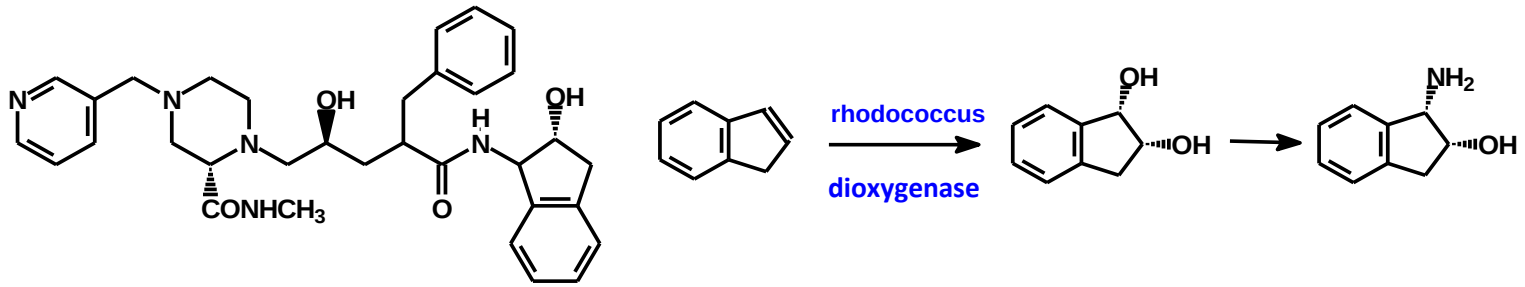
Inhibitors of dopa decarboxylase (Parkinson).

Examples of enzymatic steps in synthesis of complex drugs

1) Atorvastatin; inhibitor of HMG-CoA reductase: 3 enzymatic steps



2) Indinavirsulfate; inhibitor of HIV protease: 1 enzymatic step



Partially enzymatic production of a drug: Step sequence in process development