facultad de ciencias bioquímicas y farmacéuticas

TÓPICOS DE ENZIMOLOGÍA

2025

Enzymes are biological catalysts (also known as biocatalysts) that speed up biochemical reactions in living organisms.

The word 'enzyme' was first used by the German physiologist Wilhelm **Kühne in 1878**, when he was describing the ability of yeast to produce alcohol from sugars, and it is derived from the **Greek words en (me** **'within') and zume (meaning 'yeast')**

They can also be extracted from cells and then used to catalyze a wide range of commercially important processe

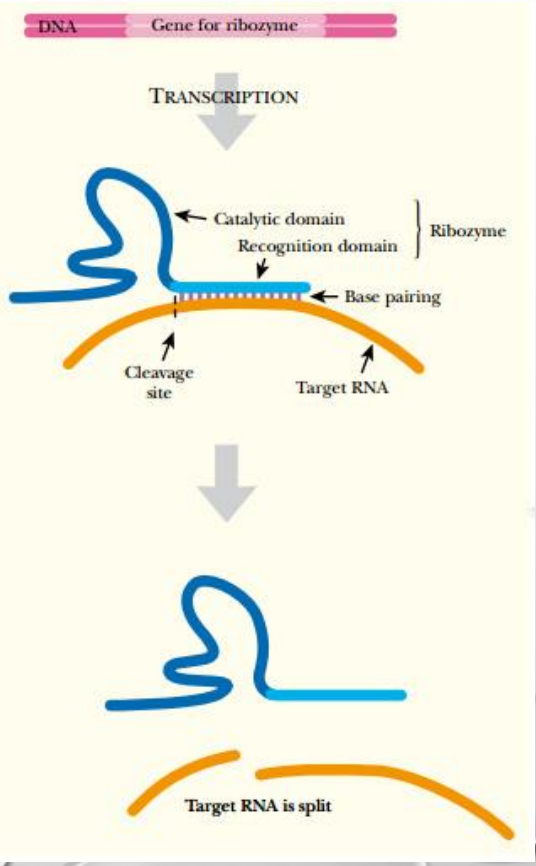
- the production of sweetening agents
- the modification of antibiotics/drugs
- used in washing powders and various cleaning products
- key role in analytical devices and assays that have clinical, forensic and environmental applications.

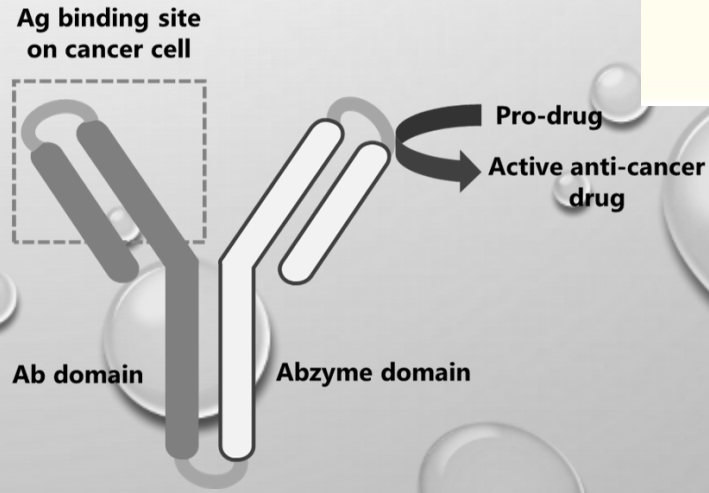
In the late XIX century and early XX century, significant advances were made in the extraction, characterization and commercial exploitation of many enzymes, but it was not until the **1920s that enzymes were crystallized, revealing that catalytic activity is associated with protein molecules**

For the next 60 years or so it was believed that all enzymes were proteins, but …..

1980s it was found that some ribonucleic acid (RNA) molecules are also able to exert catalytic effects.

1980s, biochemists also developed the technology to generate antibodies that possess catalytic properties. These so-called 'abzymes' have significant potential both as novel industrial catalysts and in therapeutics



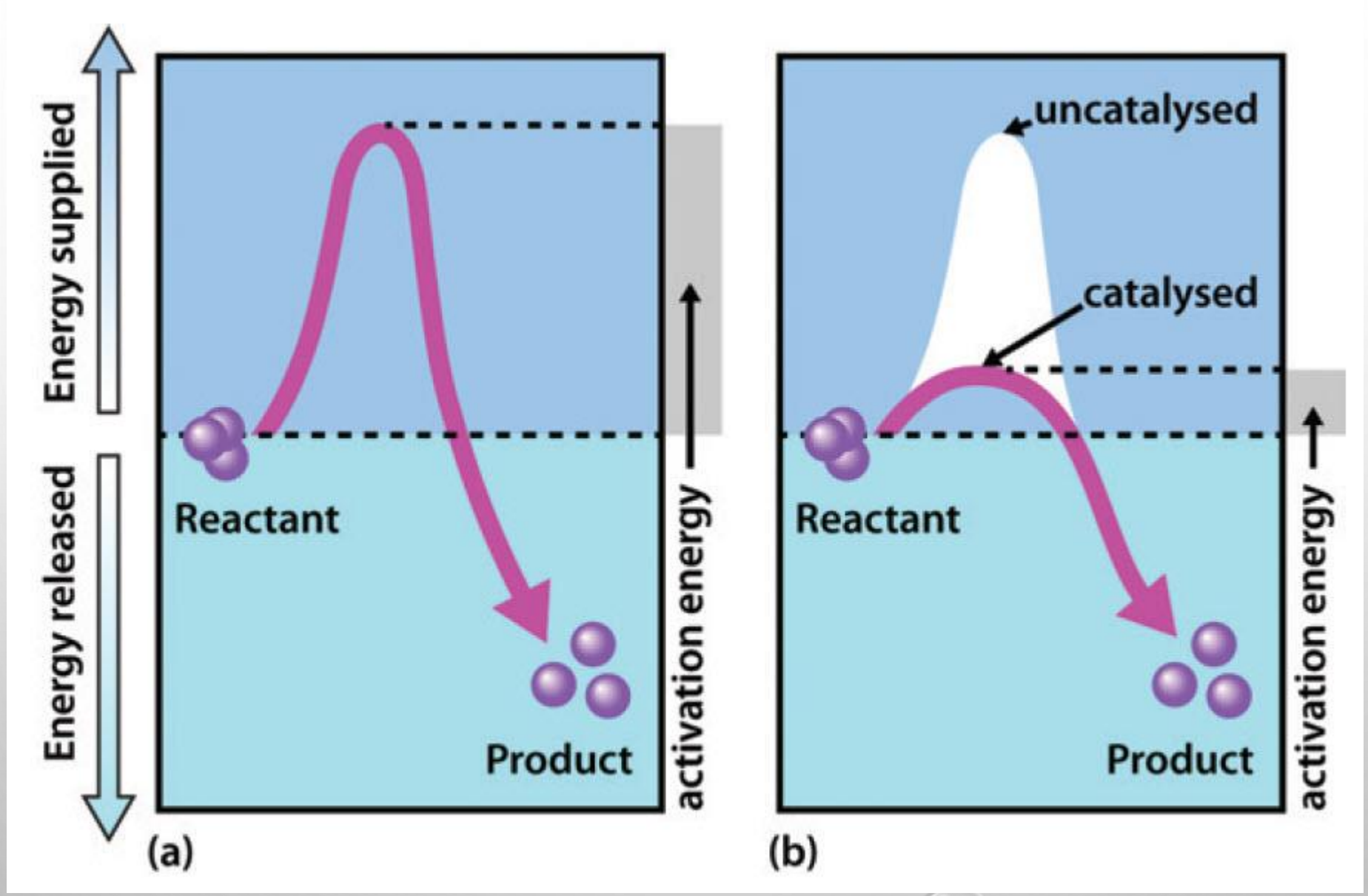


As catalysts,

- enzymes are only required in very low concentrations,
- speed up reactions without themselves being consumed during the reaction.

- POTENT!!! enormous catalytic activity of enzymes best be expressed by a constant, k_{cat}
- k_{cat} : ***turnover rate, turnover frequency*** or ***turnover number.*** This constant represents the number of substrate molecules that can be converted to product by a single enzyme molecule per unit time (usually per minute or per second)

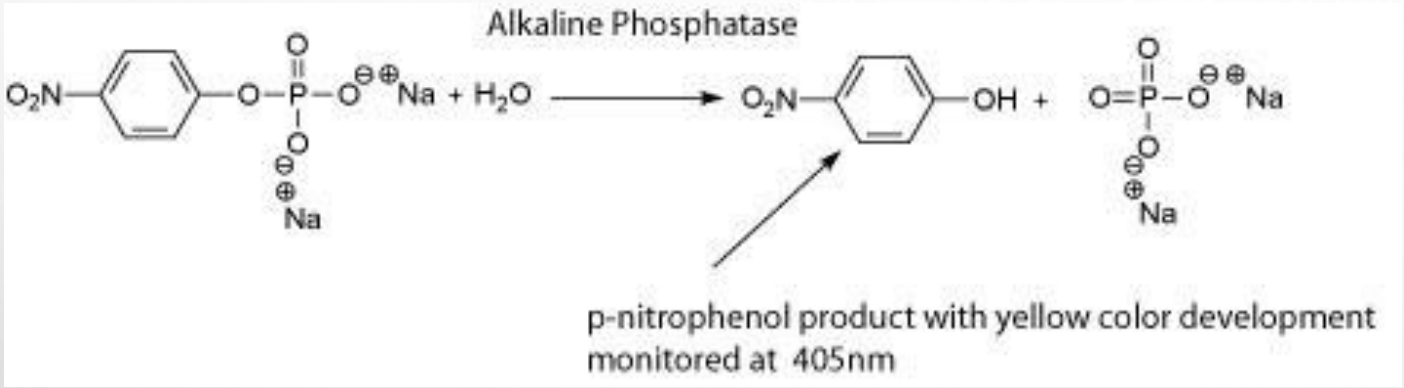
Enzyme	Turnover rate (mole product s^{-1} mole enzyme^{-1})
Carbonic anhydrase	600 000
Catalase	93 000
β–galactosidase	200
Chymotrypsin	100
Tyrosinase	1


Energy supplied
Energy released
Reactant
Product
activation energy
(a)
uncatalysed
catalysed
Reactant
Product
activation energy
(b)

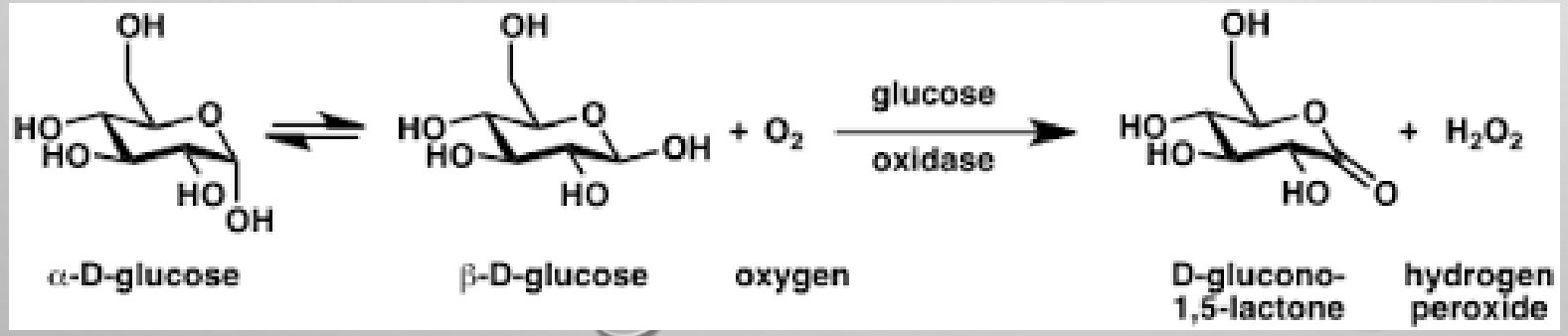
As catalysts,

- SPECIFIC!!!

❑ enzymes demonstrate **group specificity.** For example, alkaline phosphatase can remove a phosphate group from a variety of substrates.



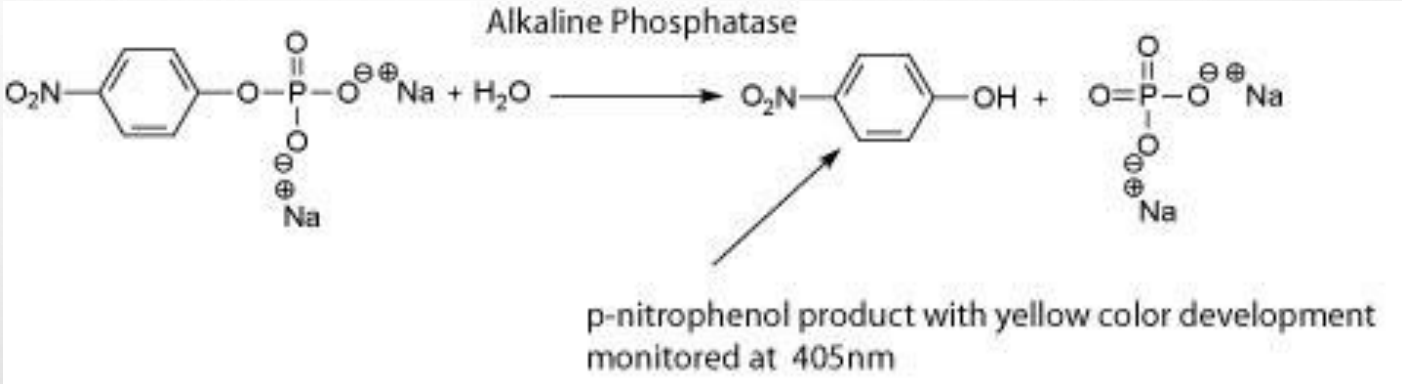
❑ Other enzymes demonstrate much higher specificity, **absolute specificity.** Eg. glucose oxidase total specificity for its substrate, β-D-glucose, and virtually no activity with any other monosaccharides.

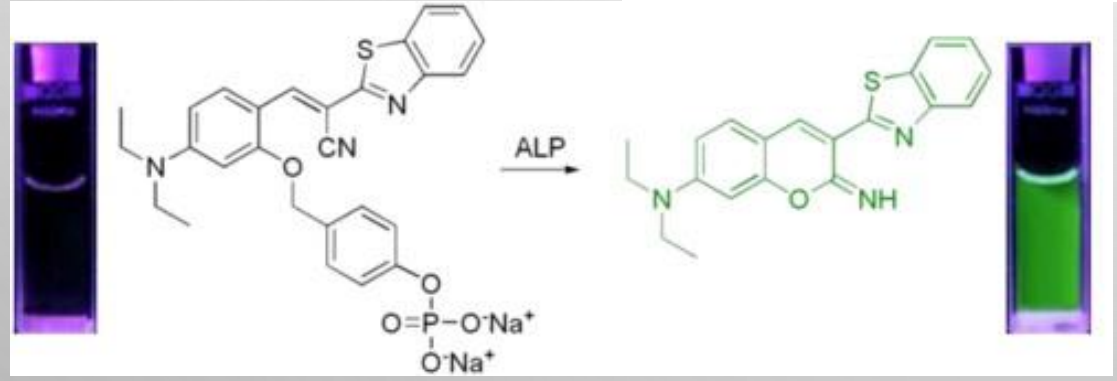


SPECIFICITY…..

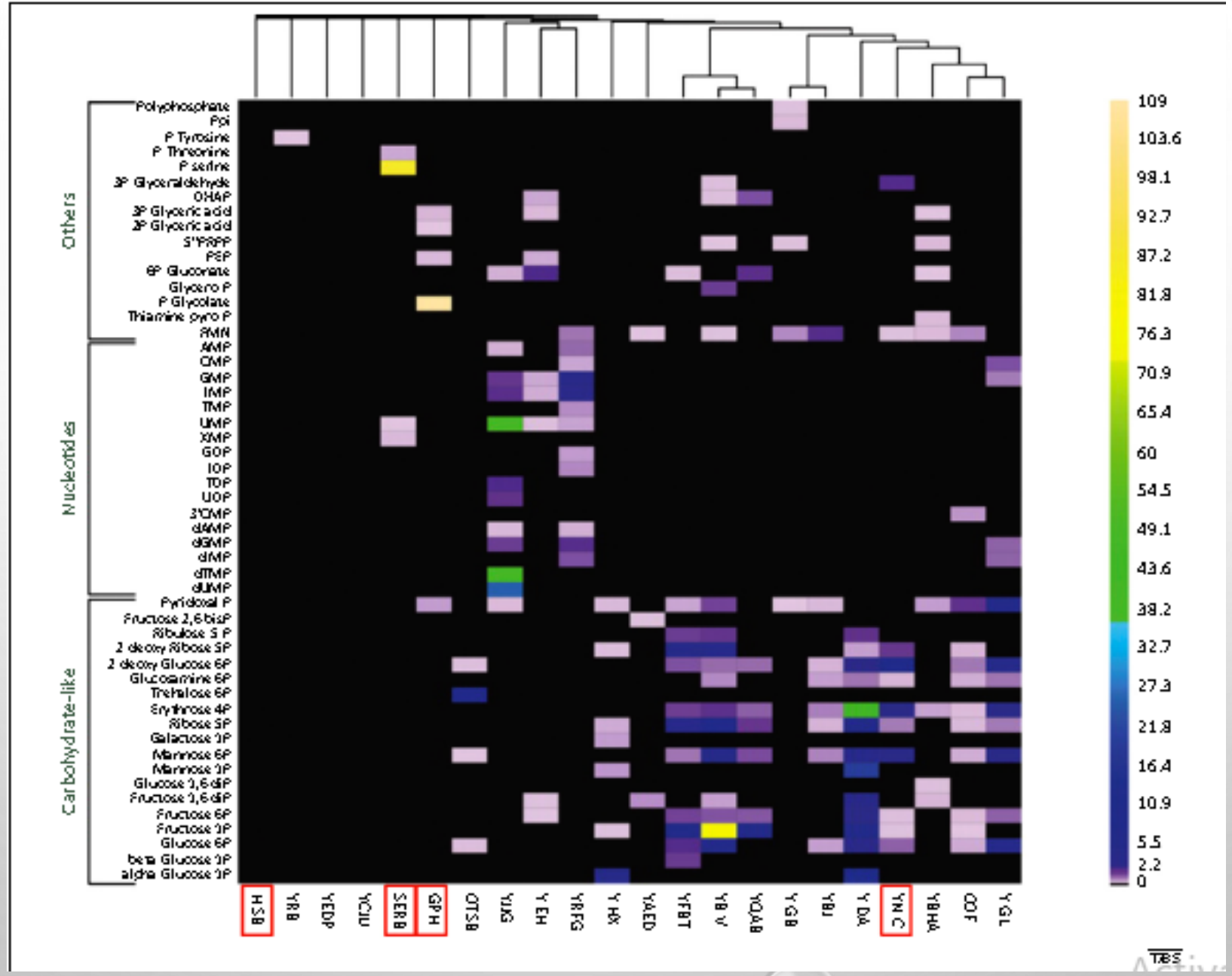
Accidental binding of non-canonical substrates in a reactive active site environment sometimes results in a chemical reaction; such reactions are referred to as '**promiscuous**'.

'**Substrate promiscuity**' occurs when enzymes carry out their typical catalytic functions using non-canonical substrates.

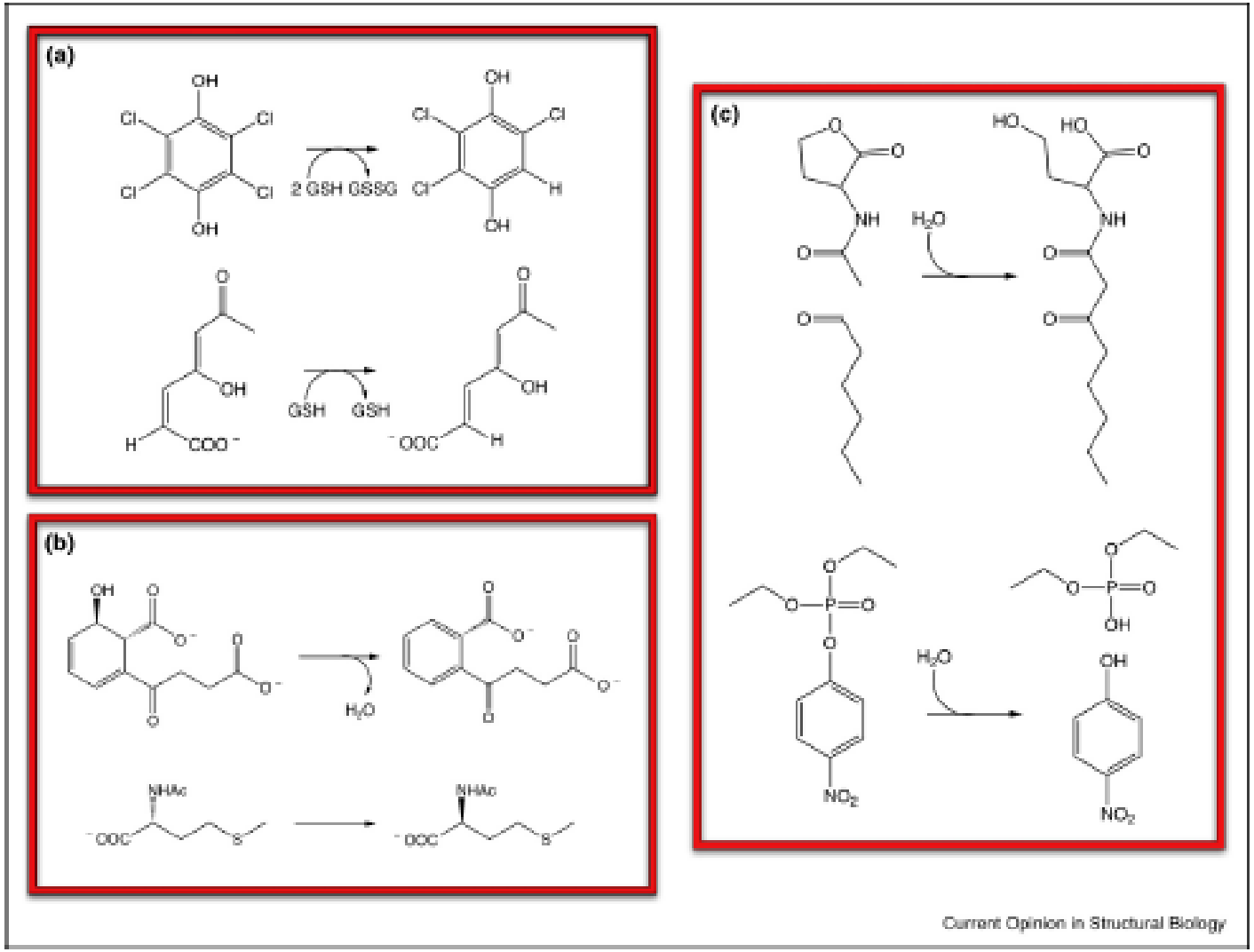




23 haloacid dehalogenase-like phosphatases from E. coli with 80 physiological substrates
Activities are given in units
of mmol/min/mg of protein.

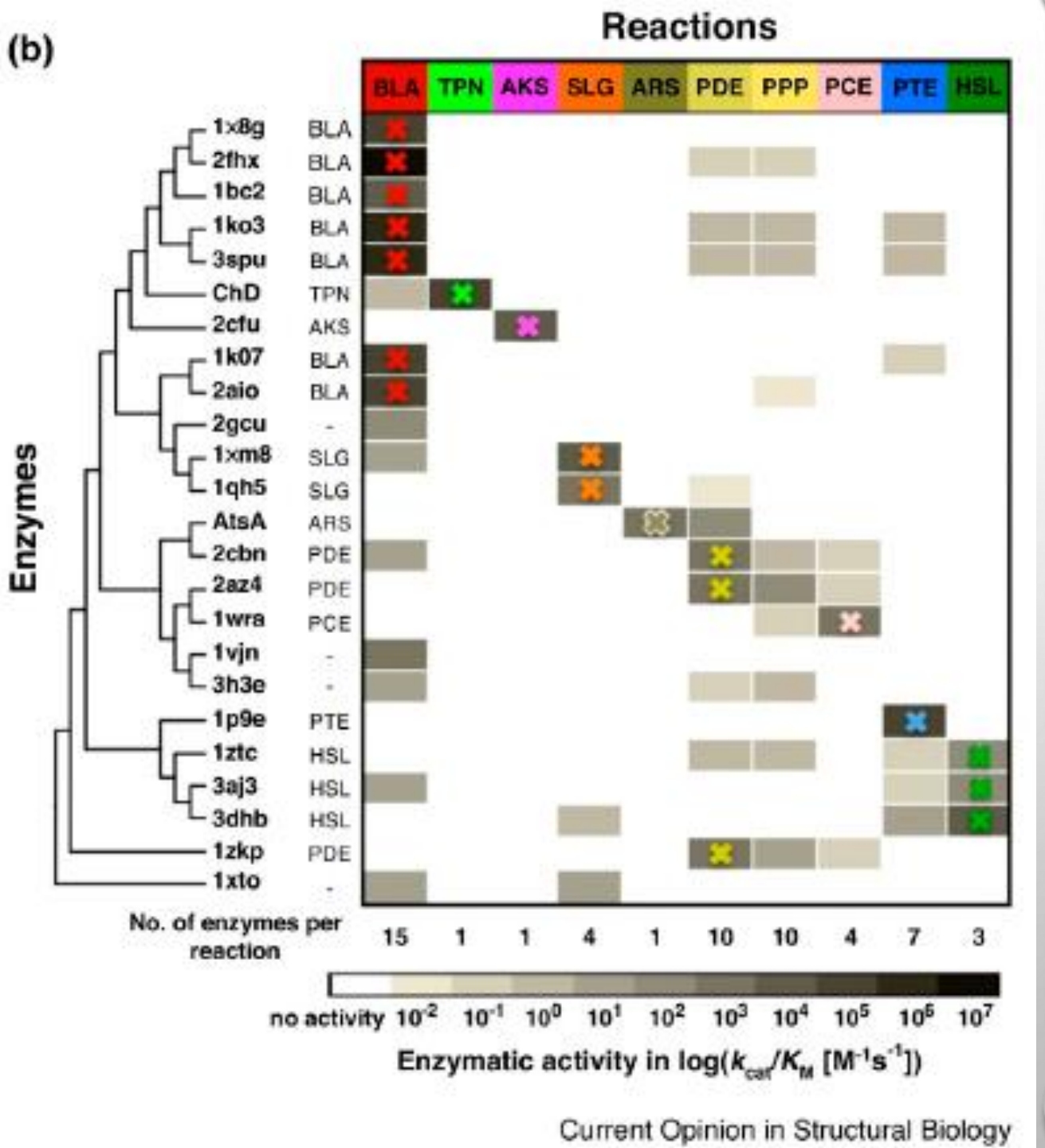


'Catalytic promiscuity' occurs when the catalytic abilities of the active site are used to catalyze a distinctly different type of reaction



Examples of catalytic promiscuity. **(a)** Tetrachlorohydroquinone dehalogenase catalyzes isomerization of maleylacetate [55]. (GSH, glutathione; GSSG, glutathione disulfide). **(b)** *o*-Succinylbenzoate synthase catalyzes racemization of *N*-acyl amino acids [56]. **(c)** PON1 (a homoserine lactone hydrolase) catalyzes hydrolysis of paraoxon [43].

Enzymes from 15 families within the metallo- b-lactamase superfamily often display catalytic promiscuity with substrates for enzymes in a different family.
Abbreviations:
BLA, beta-lactamase;
TPN, chlorothalonil dehalogenase;
AKS, alkylsulfatase;
SLG, glyoxalase II;
ARS, arylsulfatase;
PDE, phosphodiesterase;
PPP, phosphonatase;
PCE, phosphorylcholine esterase;
PTE, phosphotriesterase;
HSL, homoserine lactonase.



But first: when is a reaction promiscuous?

- enzymes with broad substrate specificity as promiscuous…. can be defined as 'indiscriminate'.

Broad-specificity enzymes often have large active sites that can accommodate substrates of varying sizes and shapes. Hydrophobic active sites that foster binding by hydrophobic effects rather than specific electrostatic and hydrogen bonding interactions also likely enable binding of physiologically irrelevant substrates.

- restrict the term to secondary reactions that are physiologically irrelevant.

In some cases, such as the glutathione S-transferases that detoxify electrophilic compounds in the mammalian liver, broad specificity is clearly an evolutionarily beneficial trait.
This is clearly a different situation from the existence of many very inefficient secondary activities in an enzyme, none of which makes a contribution to the fitness of the organism.

Promiscuous activities are important because

- **they provide the starting point for evolution of new enzymes**. Even though they are usually less efficient than primary activities, promiscuous activities can accelerate reactions by factors up to 10^{26}.
- When **an environmental change makes a promiscuous activity important for fitness, gene duplication followed by divergence** can allow evolution of a new enzyme while the original activity is maintained.
- **provide useful starting points for novel enzymes** in engineered pathways for synthesis of valuable chemicals and pharmaceuticals
- **Multiple promiscuous activities can be patched together**, either in nature or in the laboratory, **to generate new metabolic pathways** (e.g. for degradation of anthropogenic pollutants)

Enzyme names and classification

Enzymes typically have common names (often called 'trivial names')

- related to the reaction they catalyse, with the suffix *-ase* (e.g. oxidase, dehydrogenase, carboxylase),
- Proteolytic enzymes generally have the suffix *-in* (e.g. trypsin, chymotrypsin, papain).
- Often trivial name indicates the substrate on which the enzyme acts (e.g. glucose oxidase, alcohol dehydrogenase, pyruvate decarboxylase).
- Some trivial names provide little info …catalase, diastase, invertase

Due to the growing complexity of and inconsistency in the naming of enzymes, the International Union of Biochemistry set up the **Enzyme Commission**…

The first Enzyme Commission Report was published in 1961, and provided a systematic approach to the naming of enzymes.
The sixth edition, published in 1992…..nearly 3 200 different enzymes, and supplements published annually have now **extended this number to over 5 000**

Within this system, all enzymes are described by a four-part Enzyme Commission (EC) Number

lactate dehydrogenase EC number 1.1.1.27, and correctly called l-lactate: NAD+ oxidoreductase

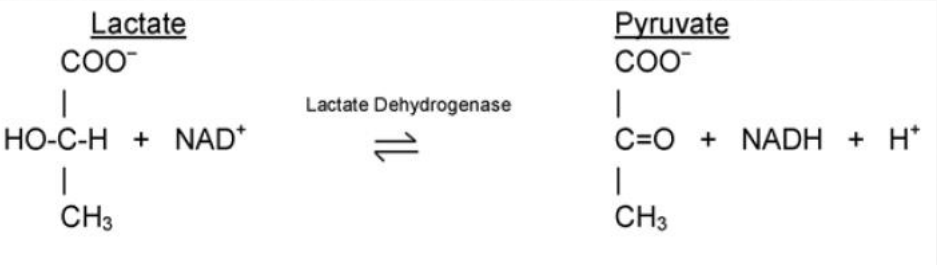
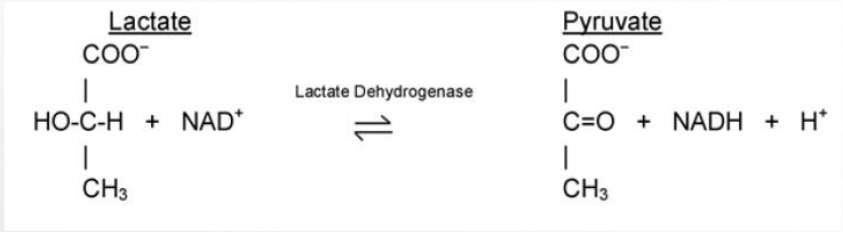


Table 2. Enzyme Classification: Main classes of enzymes in EC system.

First EC digit	Enzyme class	Reaction type
1.	Oxidoreductases	Oxidation/reduction
2.	Transferases	Atom/group transfer (excluding other classes)
3.	Hydrolases	Hydrolysis
4.	Lyases	Group removal (excluding 3.)
5.	Isomerases	Isomerization
6.	Ligases	Joining of molecules linked to the breakage of a pyrophosphate bond

lactate dehydrogenase EC number 1.1.1.27, l-lactate: NAD+ oxidoreductase



Enzyme Nomenclature Database (available at http://enzyme.expasy.org)

Table 3. Enzyme Classification: Secondary classes of oxidoreductase enzymes in EC system.

Oxidoreductases: second EC digit	Hydrogen or electron donor
1.	Alcohol (CHOH)
2.	Aldehyde or ketone (C=O)
3.	—CH—CH—
4.	Primary amine ($CHNH_2$ or $CHNH_3^+$)
5.	Secondary amine (CHNH)
6.	NADH or NADPH (when another redox catalyst is the acceptor)

Table 4. Enzyme Classification: Tertiary classes of oxidoreductase enzymes in EC system.

Oxidoreductases: third EC digit	Hydrogen or electron acceptor
1.	NAD^+ or $NADP^+$
2.	Fe^{3+} (e.g. cytochromes)
3.	O_2
4.	Other

Enzyme structure and substrate binding

Amino acid-based enzymes are globular proteins that range in size from less than 100 to more than 2 000 amino acid residues.

a specific three-dimensional structure, incorporating a small area known as the active site…..may well involve only a small number (less than 10) of the constituent amino acids. SHAPE AND CHARGE!

FIRST hypothesis that enzyme specificity results from the complementary nature of the substrate German chemist Emil Fischer in 1894, 'lock and key hypothesis'

But enzymes are not rigid structures……

1958 Daniel Koshland extended Fischer's ideas and presented the 'induced-fit model' of substrate and enzyme binding

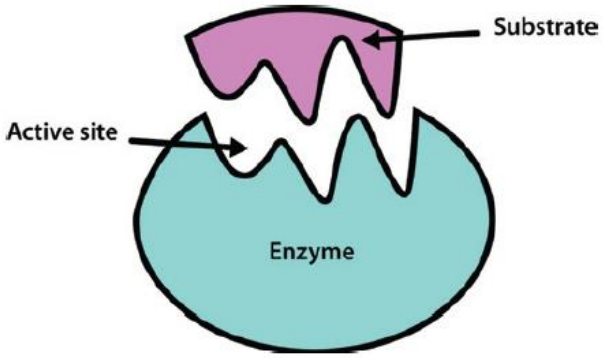
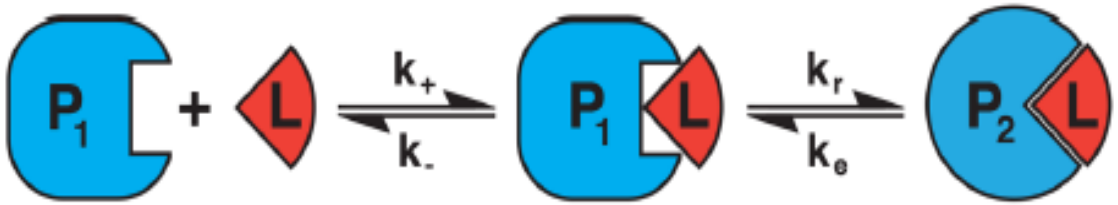


Figure 1. Representation of substrate binding to the active site of an enzyme molecule.

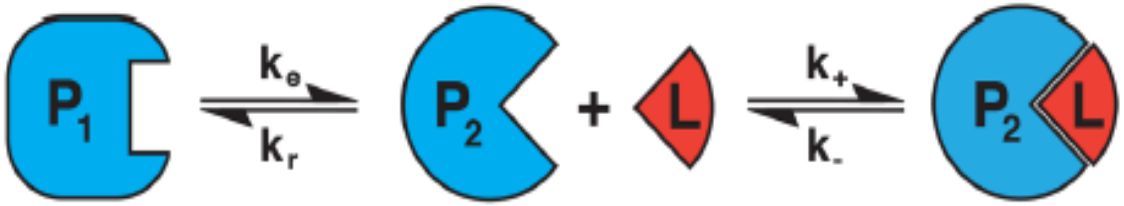
enzyme molecule changes its shape slightly to accommodate the binding of the substrate
HAND-IN-GLOVE MODEL

But models continue evolving……

(a) induced-fit binding mechanism



(b) conformational-selection binding mechanism



Topics during the course

Enzyme kinetics is the study of factors that determine the speed of enzyme-catalyzed reactions.

Enzyme Km y Vmax--- models
Interpretation

Inhibitors…. Apps

pH, Temp

Enzymes in unconventional media

Immobilization of enzymes & kinetics

Assays & Analytical apps.

Enzyme evolution

Bio-catalysis

In sillico tools

¤	semana¤	FECHA¶ Y¶ HORA¤	CLASE·¤	DOCENTE·¤	Observaciones/·aula¤
1¤	11-de-agosto¤	Jue·14/8¶ 9:00¤	Inaugural·-Organización¶ ¤	Gabriela·Coux¤	virtual¤
¤	18-de-agosto¤	LIBRE¤			
2¤	25-de-agosto¶ SAMIGE¤	28/8¶ 11:00¤	Repaso·y·conceptos·básicos¤	Anabella¶ Lodeyro¤	¤
3¤	1-de-septiembre¤	04/08¶ 9:00¤	Inhibidores·y·*High-throughput-Screening*.·¤	Ana¶ Bortolotti¤	¤
4¤		05/09¶ 11:00¤	Inmovilización·de·enzimas.· Cinética·de·enzimas· inmovilizadas¤	Anabella¤	¤
5¤	8-de-septiembre¤	11/09¤	Herramientas·*in·sillico*·para·el·estudio·de·enzimas.·BRENDA¤	Ana·Bortolotti¤	¤
6¤		12/09¶ 11:00¤	Efecto·del·pH·y·temperatura¤	Gabi¤	¤
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7¤	22-de-septiembre¤	26/09¶ 11:00¤	Aplicaciones·analíticas·de·enzimas.·¤	Julia¤	¤
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12¤	¤	¿??¤	¤	¤	¤

Nota·promoción:¶

Actividades·in-sillico/·Q&A¤	Informe·(Práctico·+¶ Brenda)¤	Seminario· bibliográfico¤	Proposal¤	=1¤	¤
0.1¤	0.3¶ ¤	0.4¤	0.2¤	=1¤	¤