# 3rd lecture: ENZYMES



## Comparison of chemical and enzymatic catalysis

| Reaction | Catalyst | Activation energy kJ/mol | $k_{rel}$ 25 °C |
|---|---|---|---|
| $H_2O_2 \rightarrow H_2O + 1/2O_2$ | -<br>$I^{-1}$<br>catalase | 75<br>56,5<br>26,8 | 1<br>$2{,}1.10^3$<br>$3{,}5.10^8$ |
| Casein + $nH_2O$ →(n+1) peptide | $H^+$<br>trypsin | 86<br>50 | 1<br>$2{,}1.10^6$ |
| Sucrose + $H_2O$ → glucose+fructose | $H^+$<br>invertase | 107<br>46 | 1<br>$5{,}6.10^{10}$ |
| Linoleic acid + $O_2$ → linolene peroxide | -<br>$Cu^{2+}$<br>lipoxygenase | 150-270<br>30-50<br>16,7 | 1<br>$\sim 10^2$<br>$\sim 10^7$ |



## ENZYMES

A many proteins are known with different biological functions:

Regulator proteins
Transport proteins
Protecting proteins
Toxins
Reserve proteins
Contractile proteins
Structural proteins

**ENZYMES - catalysts of reactions**

ε ν ζ υ μ η = "in yeast" (greek) 1878 Kühne



## Catalysis

General cases of the enzymatic catalysis (taken from general chemistry):

1. acid-base catalysis
2. covalent catalysis
3. metal ion catalysis

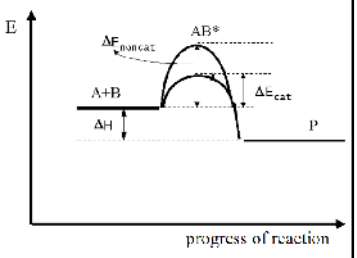


## THERMODYNAMICS OF CATALYSIS

1930- years: Eyring:

During the reaction a higher energy transition complex is formed - activation energy (ΔE*) is neded:

$$k_r = \frac{kT}{h} e^{\frac{\Delta S^*}{R}} \cdot e^{-\frac{\Delta H^*}{RT}} \approx const \cdot e^{-\frac{\Delta E^*}{RT}}$$



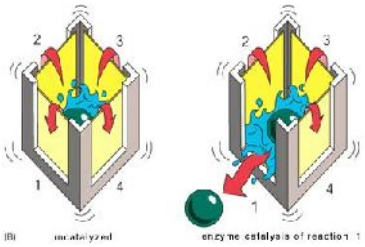

$k_r$ – reaction rate constant
T - absolute temperature (Kelvin)
k - Boltzmann constant (1,37.10-23 J/°K)
h - Planck constant (6,62.10-34 Js)

This energy is reduced by catalysts – the reaction rate is higher but the chemical equilibrium is not affected.



## ENZYMES

In a cell the organic compounds may react on many different way – but these reactions are very slow because of the activation energy barrier. The enzymes open a certain reaction route.



## Enzyme-substrate complex

A higher energy transition complex is formed:

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

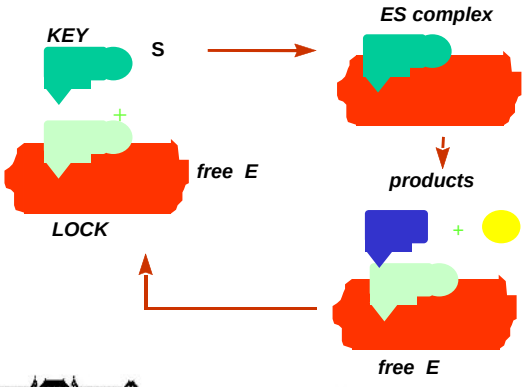
The substrate attached to the substrate binding site, that is only a small portion of the surface of the enzyme molecule (sack/pocket).

Other domains on the surface:

- Catalytic domain = **ACTIVE CENTER** – the site for chemical reaction
- Sites for modulators (inhibitors, activators, S, P, metal ions)
- Sites for covalent modification of enzyme (phosphorylation, glycosylation, proteolysis)



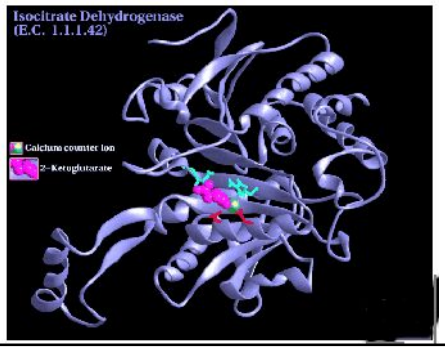
## Lock and key model






## Substrate binding site

The substrate binding site is only a small spot/pocket on the surface of enzyme molecule

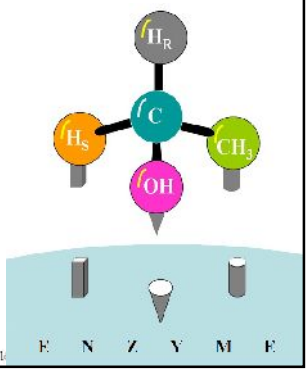





## Orientation effect

„Three-point attachment": at least three functional groups of the substrate molecule bind to the enzyme - precise positioning, no rotation.

Only the proper optical isomer can attach – this is the base of **stereospecificity.**






## Enzyme-substrate interactions

… between the molecular surfaces:

Secondary (noncovalent) interactions:

- electrostatic
- Van der Waals and
- hydrophobic interactions

Effects in enzyme-catalysis:

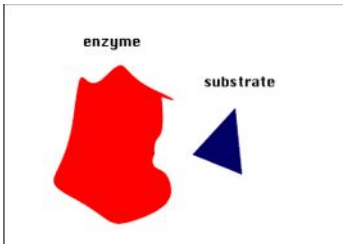
- lock and key model
- proximity effect
- orientation effect
- induced fit (Koshland-conformation change)



## Induced fit

In close approach (proximity) the form of the protein changes in interaction (Koshland, 1958), tends to complementarity and catches the substrate.

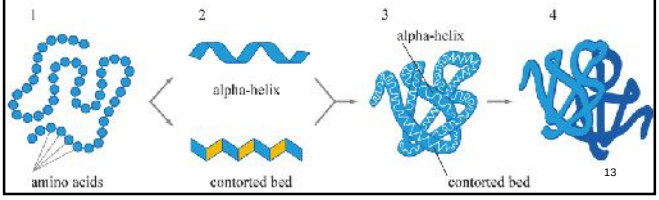
http://www.chem.ucsb.edu/~molvisual/ABLE/induced_fit/index.html



## How is the proper surface formed?

The folded peptide chains form the three dimensional structure of protein (tertiary, quaternary structure). The side chains of amino acids can be:

- apolar (alkyl groups)
- polar (-OH, -SH groups)
- ionic (-$NH_2$, -COOH groups)

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## Reactive side chains

Acidic: –COOH: Asp, Glu Basic: -$NH_2$: Lys, Arg
terminal –COOH and -$NH_2$
Amide: –CO-$NH_2$: Asn, Gln

Polar: -OH: Ser, Thr -SH: Cys, -S-$CH_3$: Met

Imidazole: His Guanidine: Arg

H-bonds: C=O …… H-O- C=O …… H-NH-

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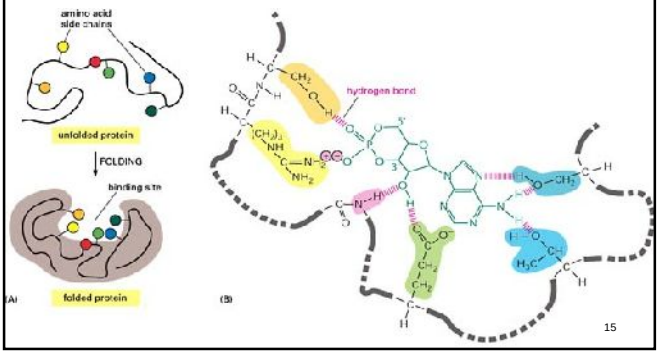
## Conformation of active center

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## Enzyme catalysed reactions

Only thermodynamically possible reactions can be catalysed
$\Delta G < 0$

All enzyme catalysed reactions are reversible, tends to an equilibrium. but: the equilibrium can be shifted, e.g.. with product removal.

Proteins are denaturable: t, pH, ionic strength (salting out), organic solvents

Specify: substrate-specifity
group-specifity
stereo-specifity
region-specifity

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## Pros for enzyme catalysed reactions

Higher reaction rate: even $10^6$-$10^{12}$ x faster

Mild reaction condition (temperature, pressure, pH)

Sophisticated selectivity, better than in organic chemistry

Easy control

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## Necessary reaction partners

**HOLOENZYME**

**APOENZYME** + **COFACTOR**

**METAL ION**
Mg, Ca, Zn,
Fe, Cu, Mo

**COENZYME**

**Prostetic group**
stable covalent bond
FAD($H_2$), Pyridoxal-P($B_6$)

**Cosubstrate**
Sztoichiometric use,
must be regenerated
NAD(H), ATP

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## Nomenclature of enzymes

1. To substrate: urea + water ⇌ $CO_2$ + $2NH_3$

urease

**S-name + ase**

2. To substrate and reaction: EtOH → AcO → AcOH

**alcohol-dehydrogenase**

**S-name + reaction name + ase**

3.Trivial names:

pepsin, trypsin, rennin – all peptidases **+ -in**

4. IUB, IUPAC, IUBMB 1964,1972,1978 Enzyme Commission: systematical nomenclature

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## Nomenclature of enzymes

catalogue number

cosubstrate

**E.C.1.1.1.49. D-glucose-6P: NADP 1-oxydoreductase**

the reaction

substrate

target on the 1st C-atom

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| Group | Reaction catalyzed | Typical reaction | Enzyme example(s) with trivial name |
|---|---|---|---|
| EC 1 Oxidoreductases | To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another | AH + B → A + BH (reduced) A + O → AO (oxidized) | Dehydrogenase, oxidase |
| EC 2 Transferases | Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group | AB + C → A + BC | Transaminase, kinase |
| EC 3 Hydrolases | Formation of two products from a substrate by hydrolysis | AB + $H_2O$ → AOH + BH | Lipase, amylase, peptidase |
| EC 4 Lyases | Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved | RCOCOOH → RCOH + $CO_2$ or [X-A-B-Y] → [A=B + X-Y] | Decarboxylase |
| EC 5 Isomerases | Intramolecule rearrangement, i.e. isomerization changes within a single molecule | AB → BA | Isomerase, mutase |
| EC 6 Ligases | Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP | X + Y+ ATP → XY + ADP + Pi | Synthetase |

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