CHEM-E4109

MODERN METHODS IN BIOCATALYSIS

chapter #1: basic concepts of enzyme catalysis

Jan Deska
Bioorganic
Chemistry

1.3.2022

Aim of the Course

FOSTERING THE **UNDERSTANDING** OF…

- the basic biosynthetic principles
- ideas &benefits of catalysis regarding efficiency & selectivity
- the molecular mechanistic basis of enzymatic activation
- the relevance of biocatalysis in organic chemistry

material taken from:

- original literature (indicated on the bottom of the slides)
- K. Faber, *Biotransformations in Organic Chemistry*, 6th ed, 2011, Springer

Grading of the Course

- pass/fail course

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Requirements to pass

- Submission of learning diaries
  - ✓ Weekly inbox on MyCourses
  - ✓ brief summary of the sessions
  - ✓ What was of particular relevance/interest
  - ✓ Thoughts/comments/concerns...

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- Seminar presentation
    - ✓ 15 min (12+3, talk + discussion)
    - ✓ Topic: current literature

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Preliminary Schedule

1.3. Introduction & basic principles

4.3. Oxidoreductases I (reduction catalysis)

8.3. Oxidoreductases II (oxygenation catalysis)

11.3. Transferases (transaminases)

15.3. Lyases I (decarboxylases and transketolases)

18.3. Lyases II (aldolases)

22.3. Hydrolases (lipases, esterases, epoxide hydrolases)

25.3. Directed evolution

29.3. Muta- and semisynthesis

1.4. Enzymes in non-natural reactions

12./13.4. Seminar presentations

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Seminar

- ✓ you choose one day (12th or 13th)
- ✓ you choose a topic (from my list or your own suggestion)
- ✓ you prepare & present your talk (on that one day, no need to attend both days)
- ✓ you listen to the other talks & ask good questions

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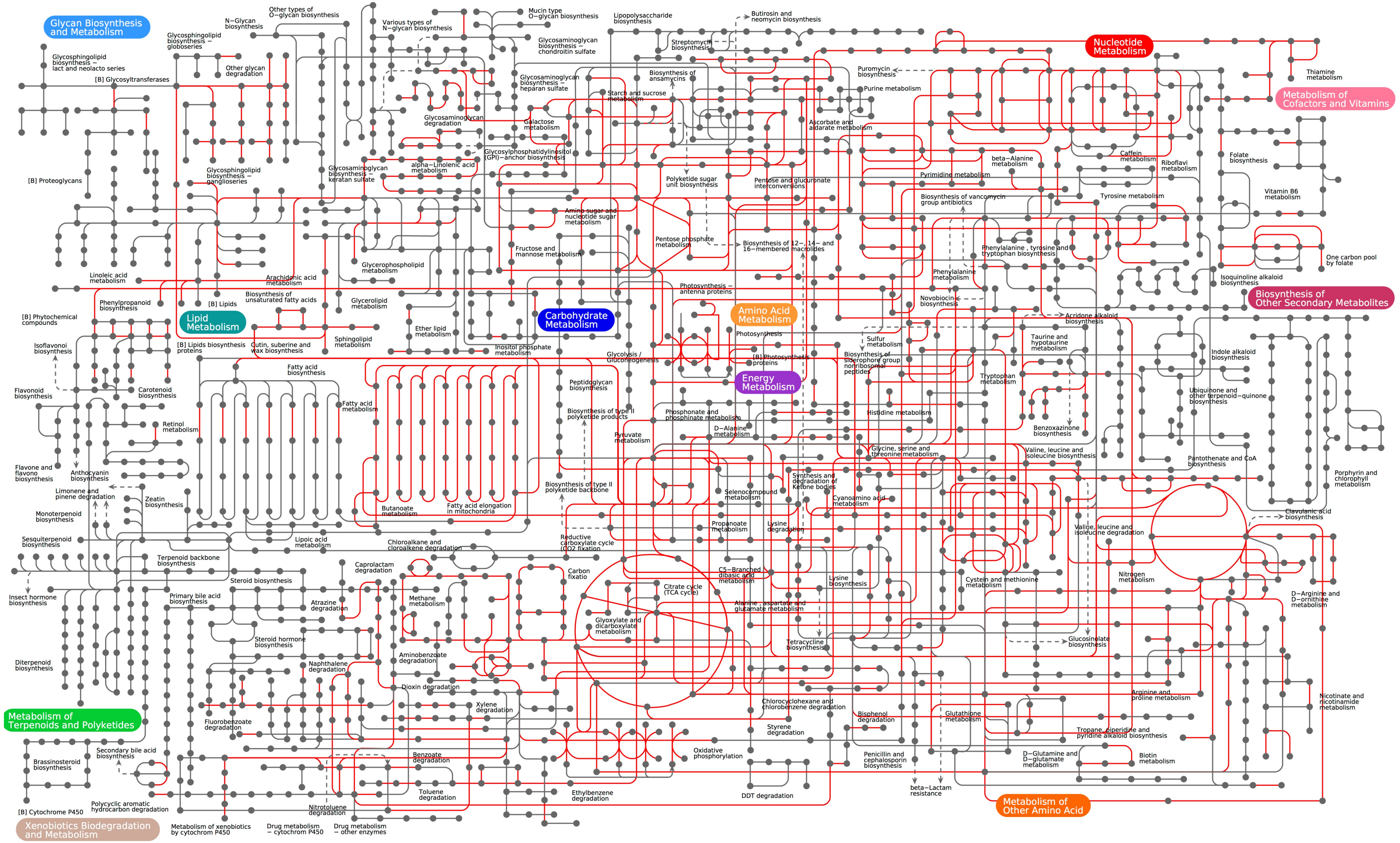
Today's Menu

After today's session you will be able to...

- recognize basic protein structures
- explain catalysis on a general level
- ...and how different kinds of selectivity can be achieved
- name typical vitamins cofactors and their general role in biocatalytic transformations

Biochemistry from a Chemist's perspective



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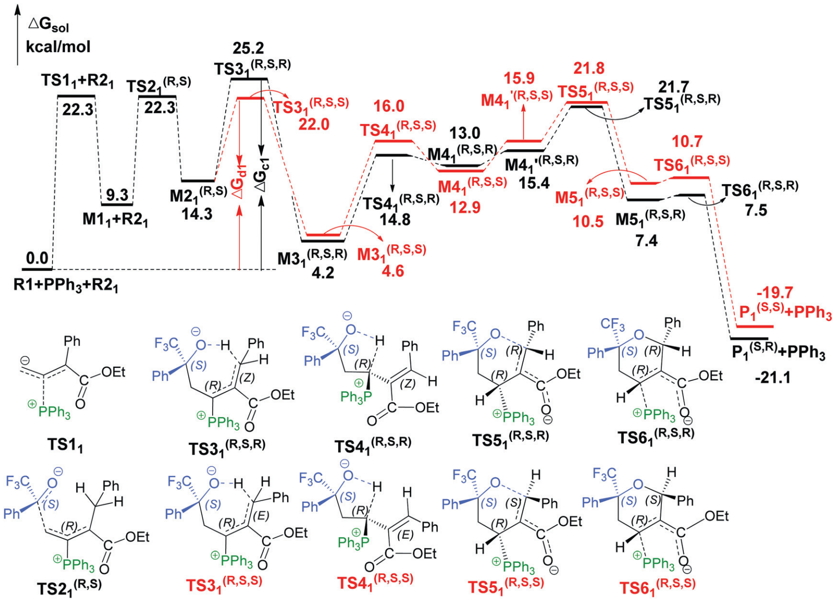
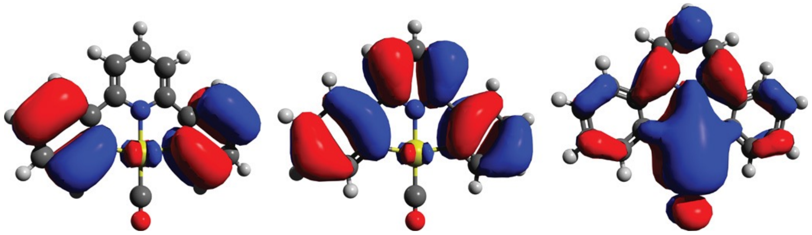
Chemical catalysis from a Biologist's perspective





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What is Biocatalysis?

catalysis in general

- acceleration of a certain reaction by lowering its activation energy
- more accurate: in many cases catalysis makes reactions happen that are not happening at all under uncatalyzed conditions
- probably the crucial element for any industrial (chemical) development

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biocatalysis

- proteins – evolved by Nature to conduct a certain transformation – are used to facilitate chemical processes
- these **enzymes** can be obtained from various natural sources, mainly of bacterial, & fungal origin
- size of the enzymes can range from a few to very very many amino acids

  4-oxalocrotonate tautomerase **62 amino acids** vs fatty acid synthases over **2500 aa**

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major advantages over chemical catalysis

- very high selectivities (chemo-, regio, and stereoselectivity)
- particularly mild conditions (important pillar of the *Green Chemistry* idea)

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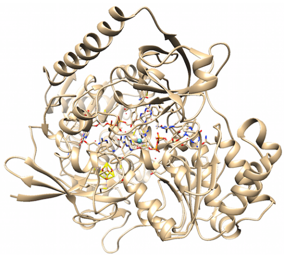
Reaction Systems

isolated enzymes



pros

- highly controlled conditions
- simple reaction equipments
- high catalyst concentrations
- nonaqueous environment possible

cons

- protein stability
- issues related to cofactors/cosubstrates

whole cell biocatalysis

pros

- optimal conditions
- low enzyme degradation
- cofactor recyclic obsolete
- metabolic engineering possible

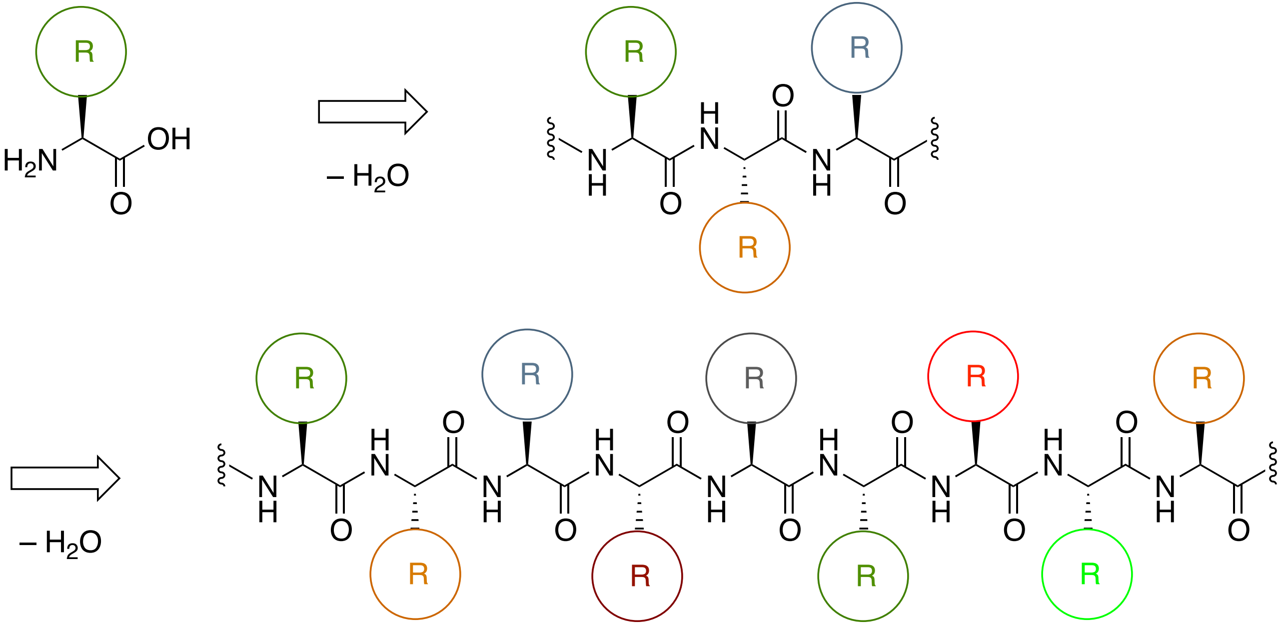
cons

- massiv amount of organic biomass as waste
- degradative side reactions

Structure & Catalysis

Structure of Proteins

- primary, secondary and tertiary structures



- primary structure depicts a virtual linear alignment
- various R's can provide various chemical functions (acidic, basic, aromatic,...)

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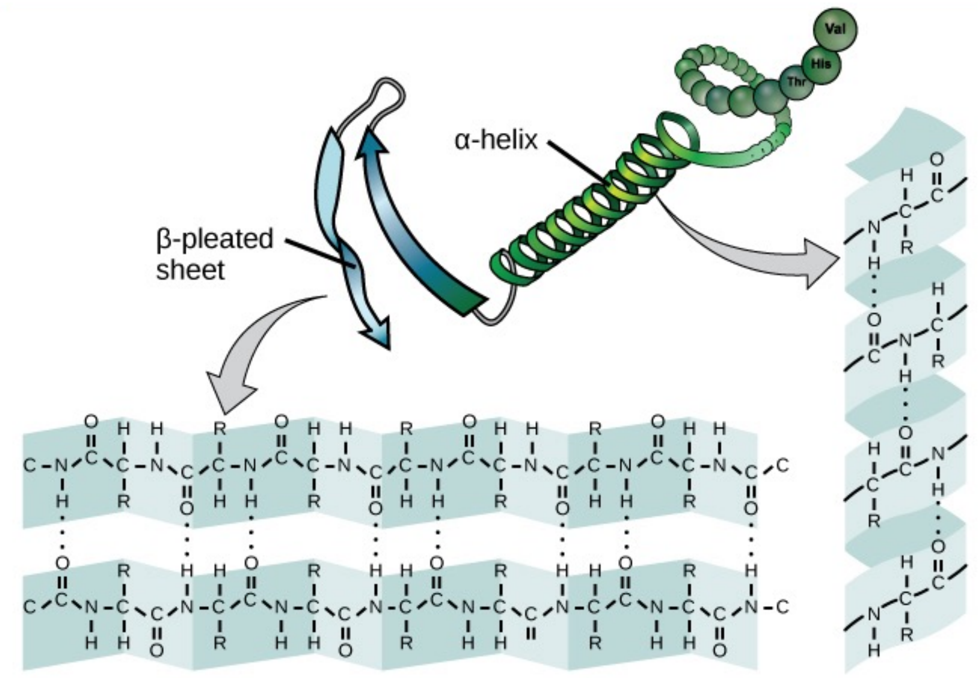
Structure of Proteins

- primary, secondary and tertiary structures



- secondary structure describes 3D-objects formed through H-bonding interactions

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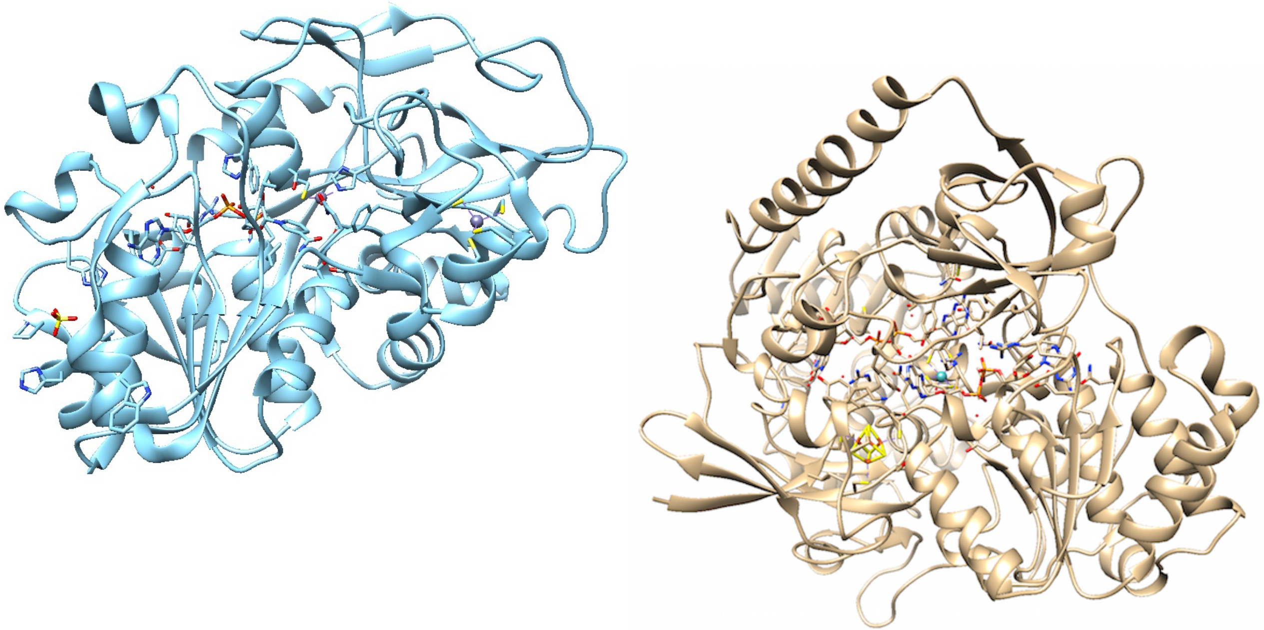
Structure of Proteins

- primary, secondary and tertiary structures



- tertiary structure shows arrangement of subunits to form a complex bulk structure
- well-defined 3D structure provides **active site** to bind and transform the substrate

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Functional Principle

$$E + S \underset{}{\overset{\text{binding}}{\rightleftharpoons}} ES \xrightarrow{\text{catalysis}} E + P$$

affinity of the enzyme to bind a particular substrate

(related to K_M)

Michaelis constant

turnover of the substrate by the enzyme

(often expressed as k_{cat})

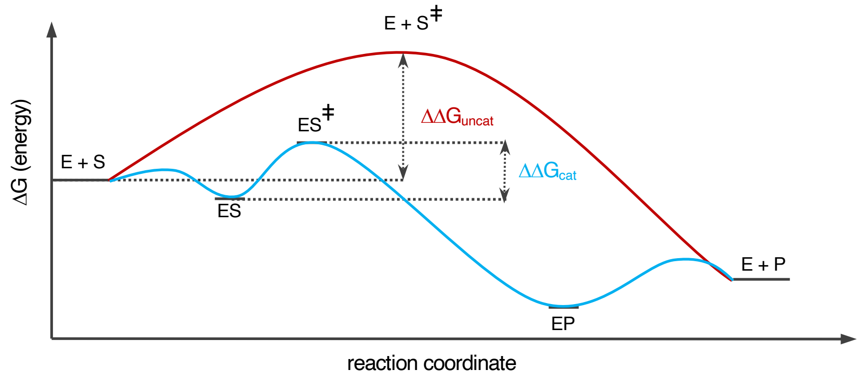
catalytic constant or turnover number

E = enzyme

S = substrate

P = product

ES = enzyme-substrate complex



uncatalyzed reaction

catalyzed reaction

$\Delta\Delta G_{cat} < \Delta\Delta G_{uncat}$

= acceleration

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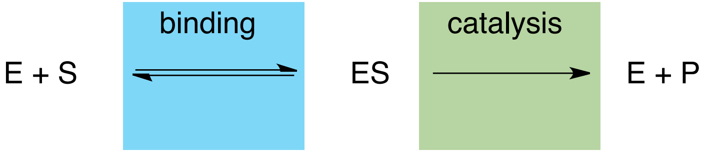
Functional Principle



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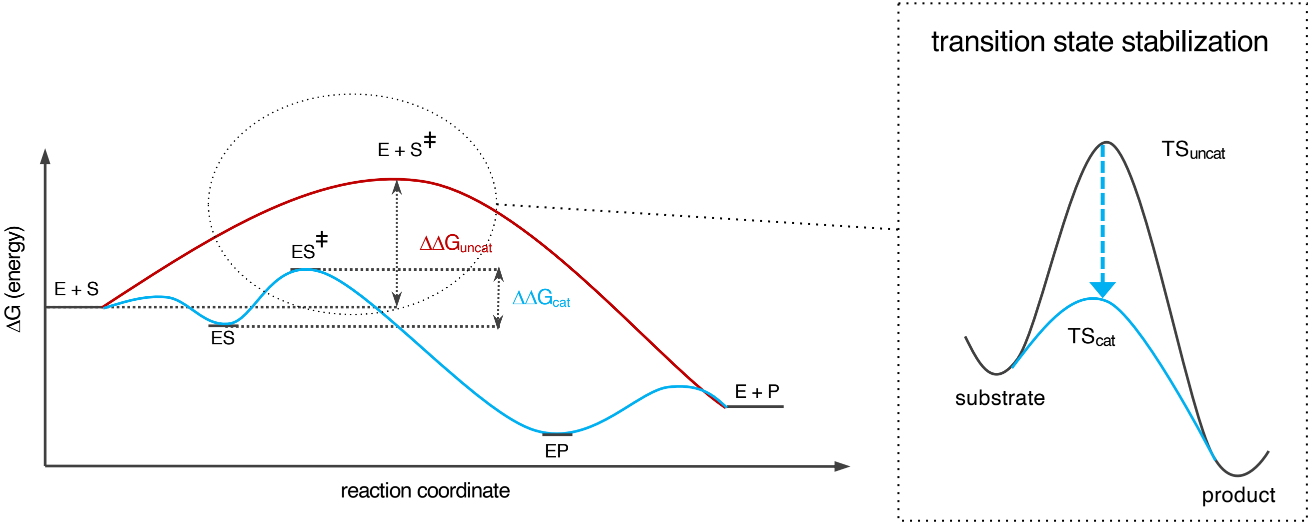

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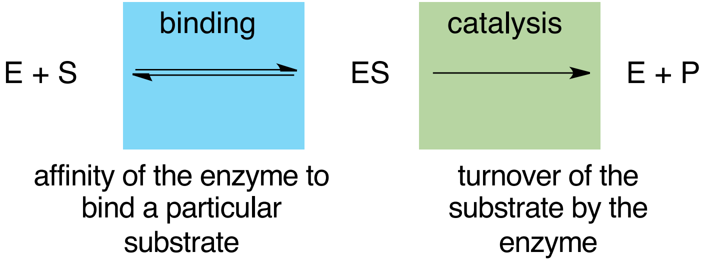
Functional Principle



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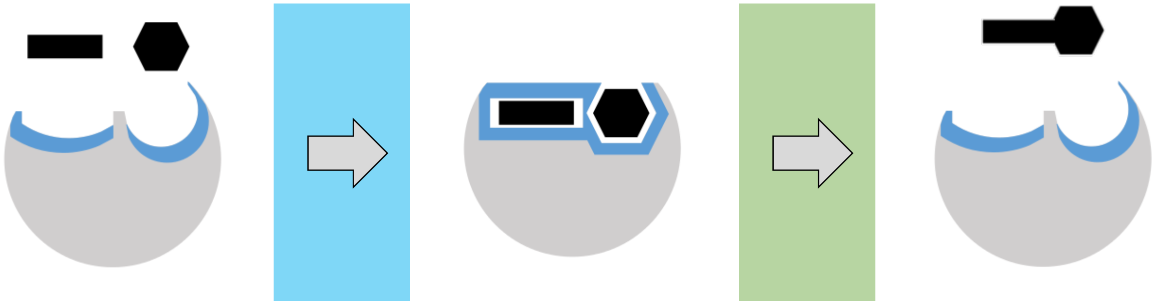
lock-key model (H. E. Fischer, 1894)

- comprehensible model, explains selectivity of enzymes but fails to explain transition state stabilization



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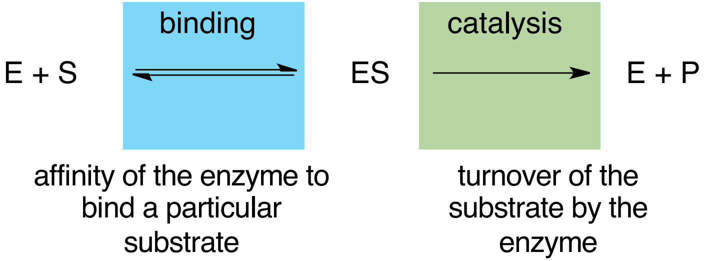
Functional Principle



E = enzyme

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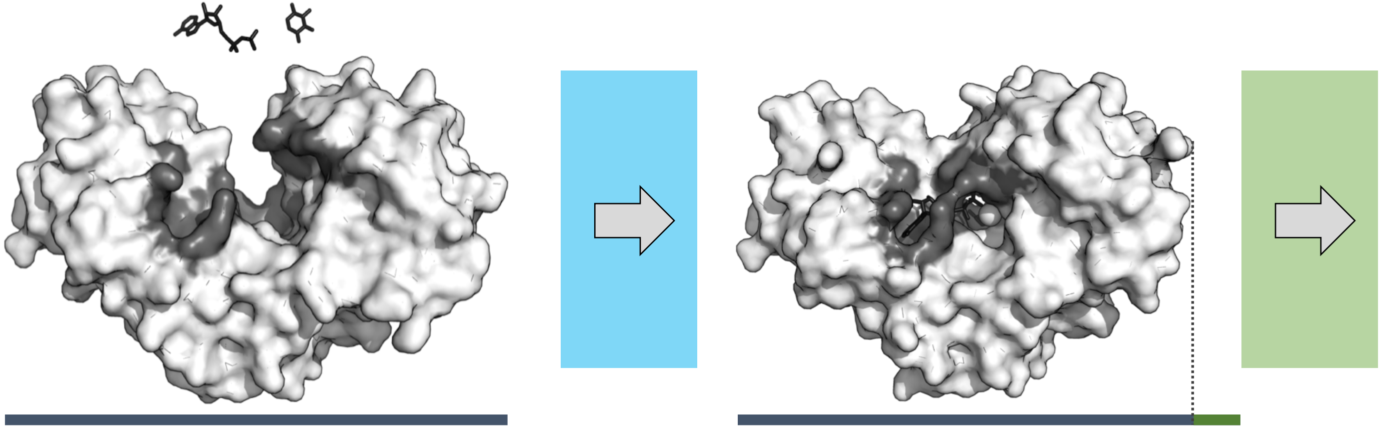
induced fit model (D. E. Koshland, 1958)

- flexibility of the protein allows to adapt and reshape during protein-substrate binding (like a hand fits into a glove)



hexokinase + ATP + xylose

squeezed by 9% upon substrate binding

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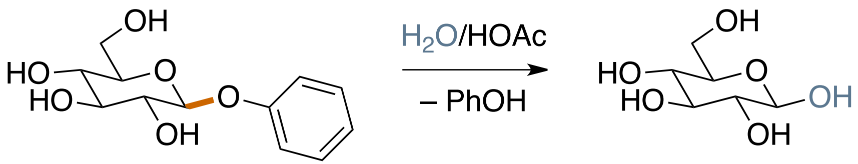
Enzyme-substrate complexes: pseudo-intramolecularity

intramolecular reactions faster than their intermolecular counterparts



three components required
(water, acid, glycoside)

k_{rel} = 1

Rye, Withers, *Curr. Opin. Chem. Biol.* **2000**, *4*, 573-580.

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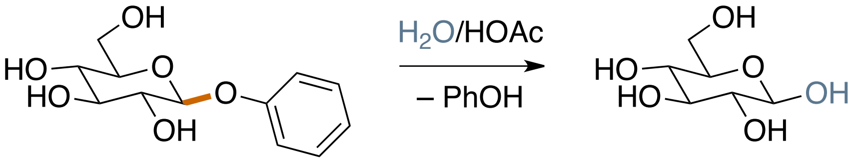

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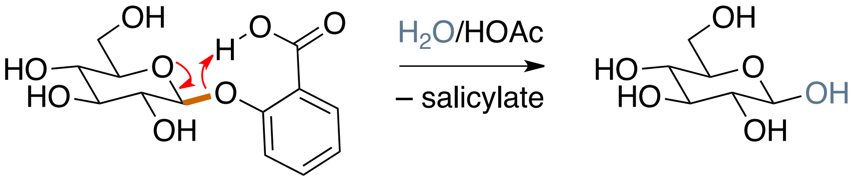

bimolecular reaction
(water & activated glycoside)

$k_{rel} = 10^4$

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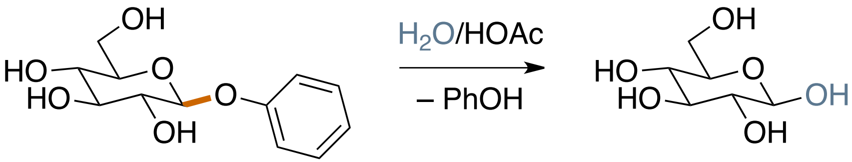

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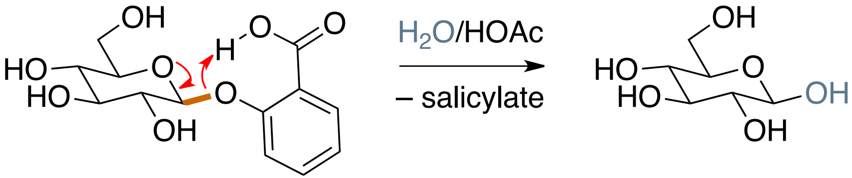

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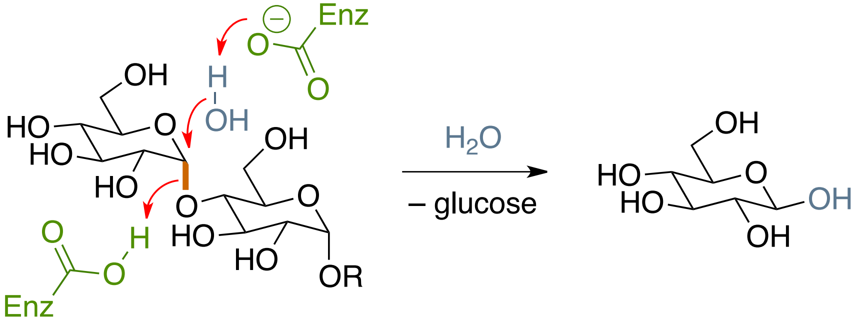


pseudo-monomolecular
reaction

$k_{rel} = 10^{17}$

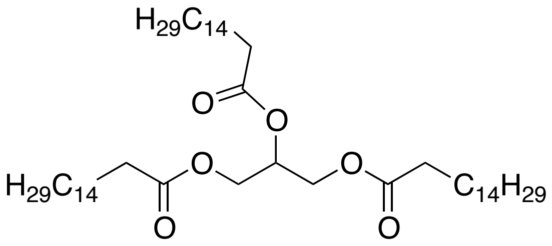
Rye, Withers, *Curr. Opin. Chem. Biol.* **2000**, *4*, 573-580.

Conclusions from a hand-glove analogy

in addition to rate enhancement, flexible binding of a three-dimensional amino acid based reaction vessel results in **selectivity**

i.e. in fat degradation

Regioselectivity



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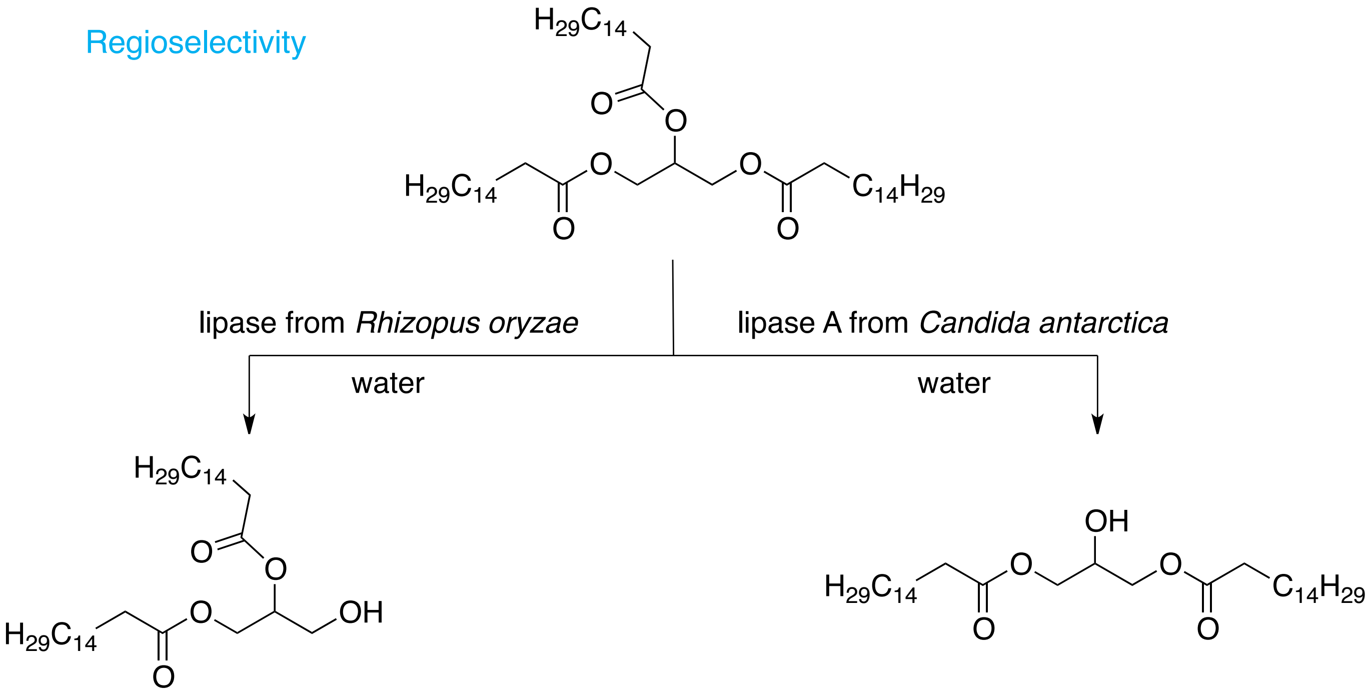

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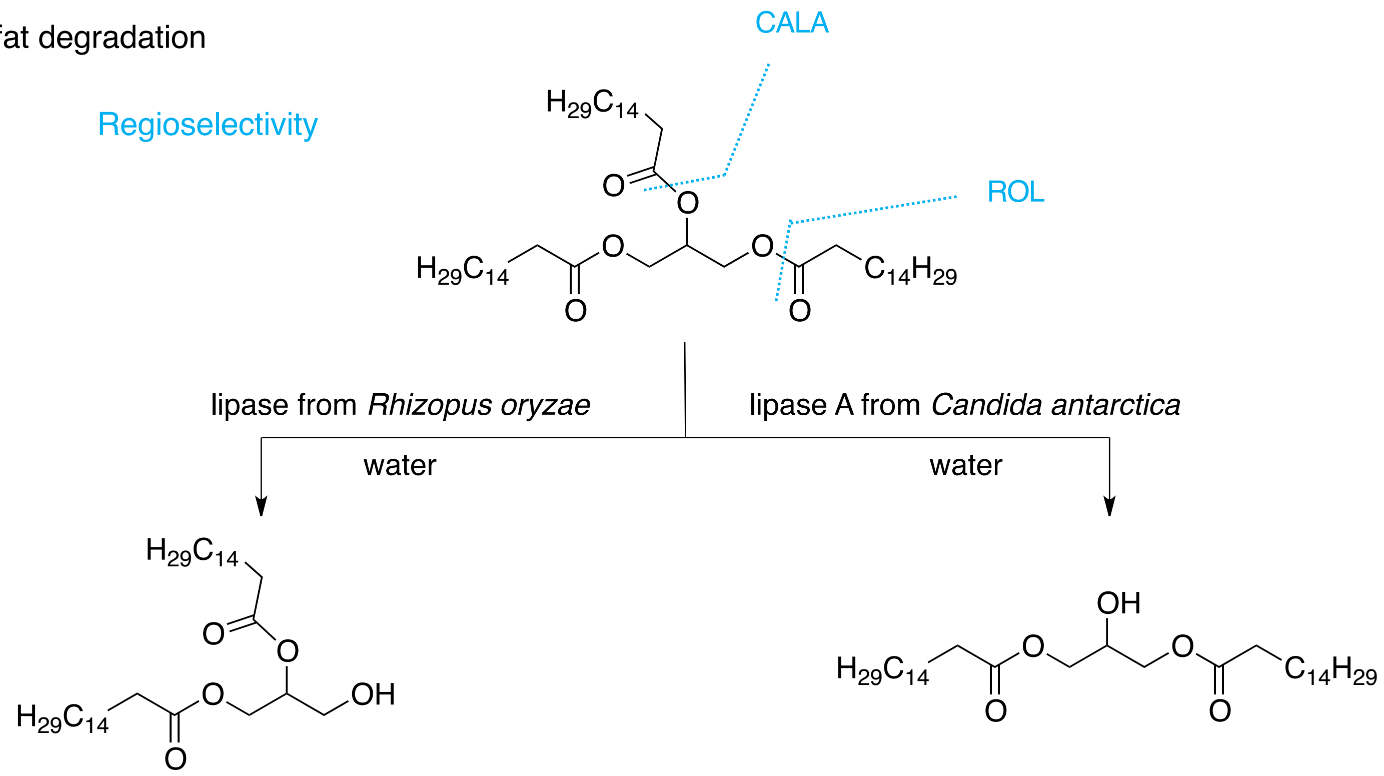

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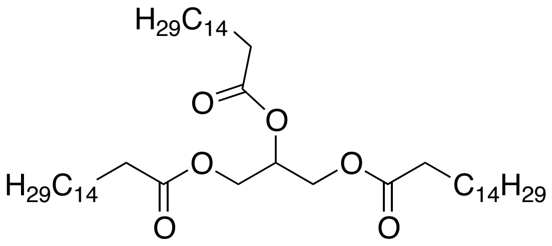
Stereoselectivity



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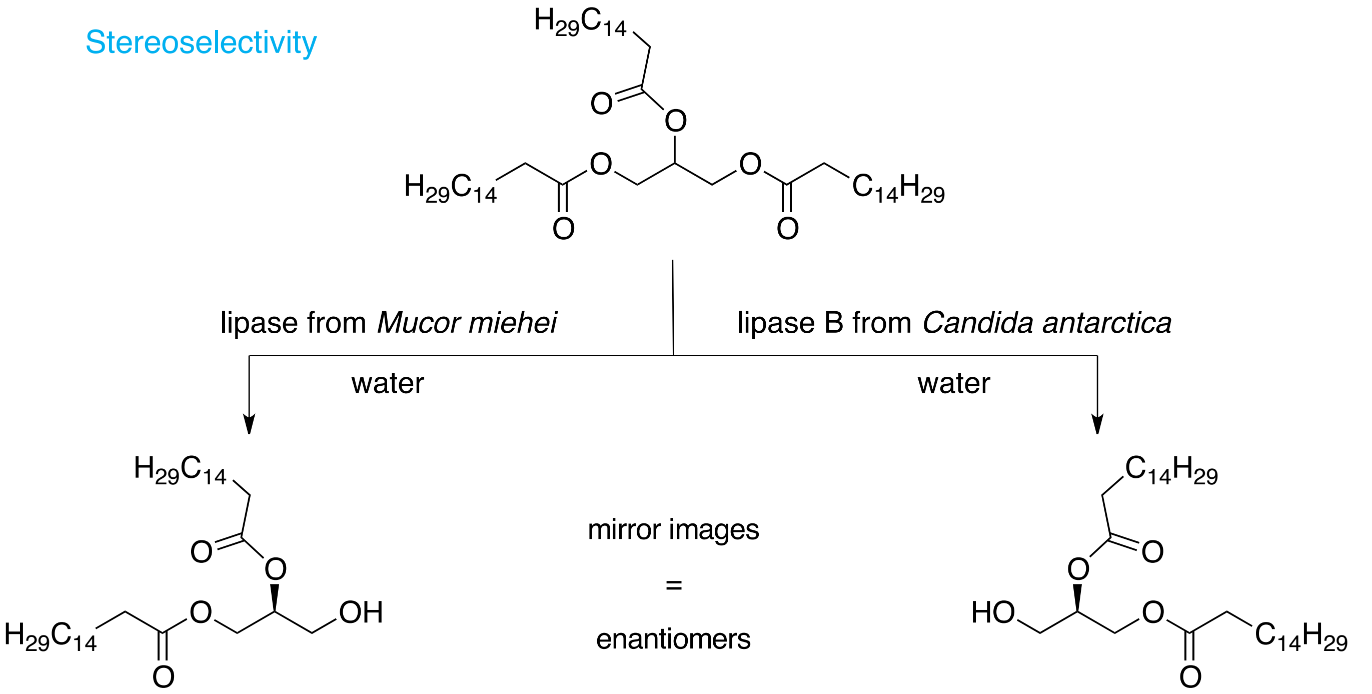
Stereoselectivity



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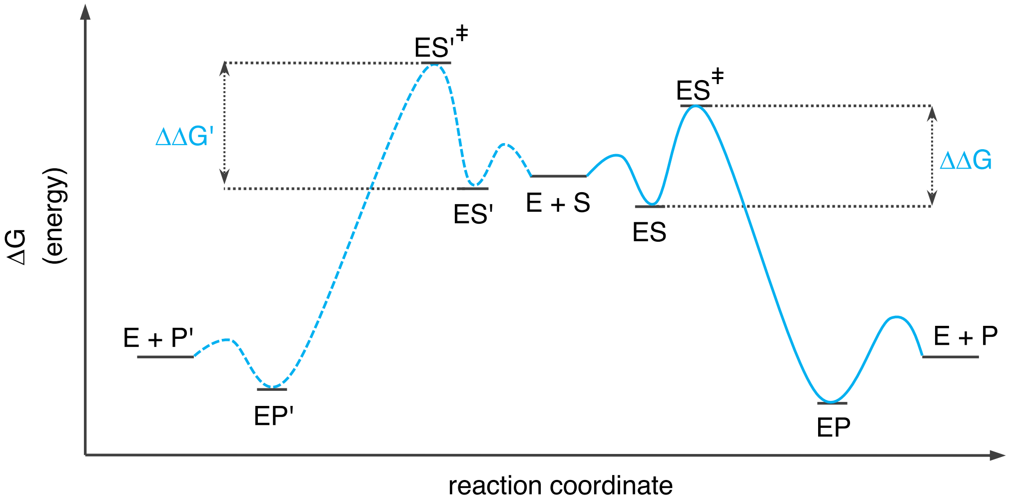
Conclusions from a hand-glove analogy

in addition to rate enhancement, flexible binding of a three-dimensional amino acid-based reaction vessel results in **selectivity**

$\Delta\Delta G < \Delta\Delta G'$ =
product selectivity

energy difference	product ratio P : P'
0.5 kcal/mol	2.3 : 1
1.0 kcal/mol	5.4 : 1
2.0 kcal/mol	30 : 1
3.0 kcal/mol	200 : 1

at 25 °C



$\Delta G = -RT \ln K$

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Vitamins & Cofactors

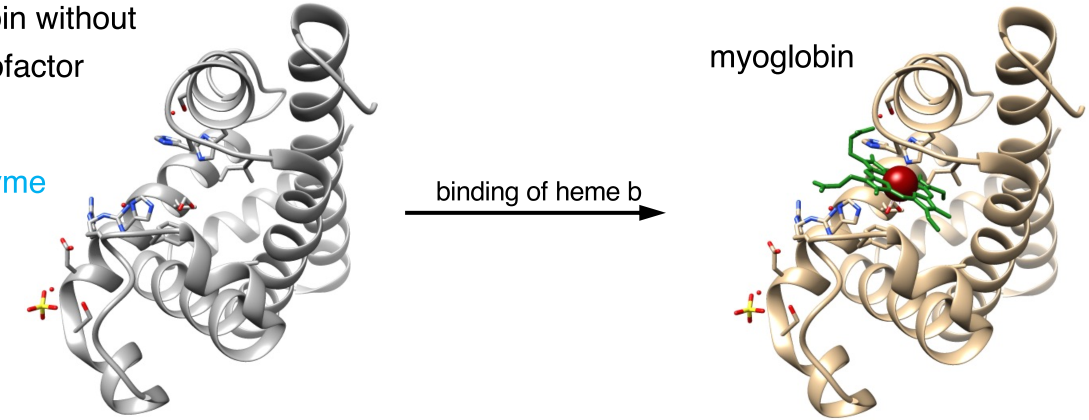
Vitamins & Cofactors

transition state stabilization can be achieved by many factors such as

- lipophilic attractive forces
- acid/base chemistry
- electrostatic interactions
- covalent bonding

multiple weak interactions sum up to a strong stabilizing effect

often, catalytically active enzymes and transport proteins contain – in addition to the poly-amino acid bulk structure – small organic molecules or metal ions to act as **cofactor**



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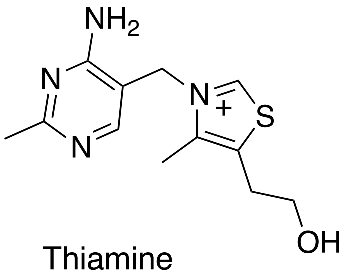
Vitamins & Cofactors



Thiamine

Vitamin B_1

function

- source of nucleophilic *N*-heterocyclic carbenes

mode of action

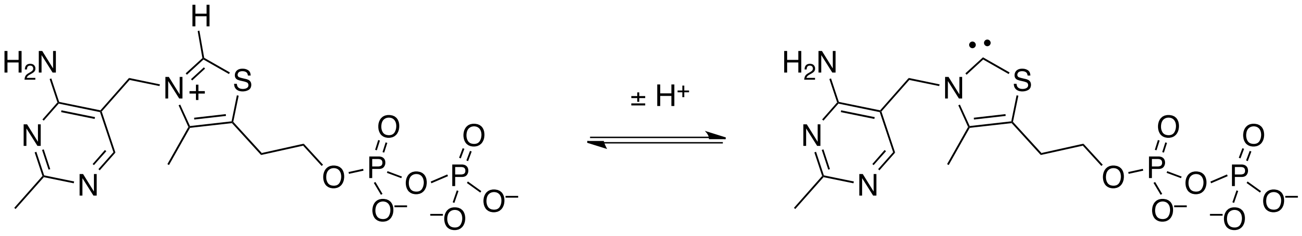
- carbene catalysis

found in

- decarboxylases, aldehyde lyases

Thiamine pyrophosphate

TPP



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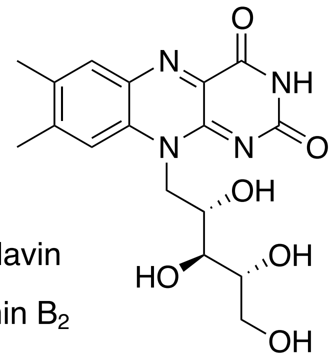
Vitamins & Cofactors



Riboflavin

Vitamin B_2

function

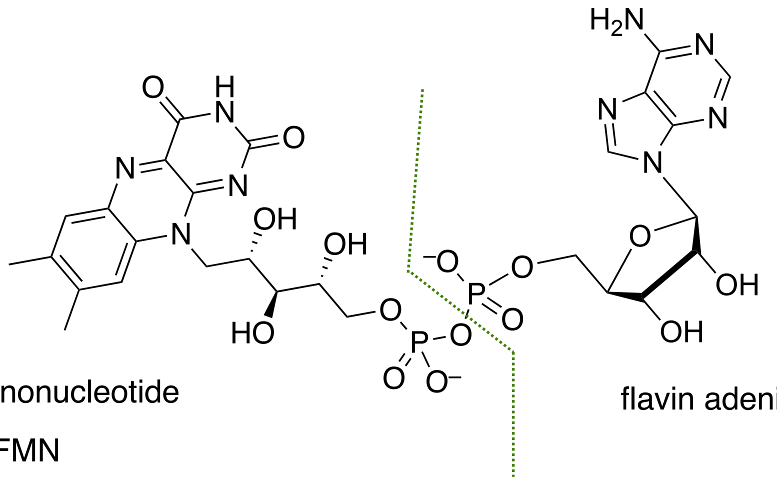
- redox cofactor

mode of action

- hydride transfer **or** hydroperoxide formation

found in

- reductases, monooxygenases, oxidases,...



flavin mononucleotide

FMN

flavin adenine dinucleotide

FAD

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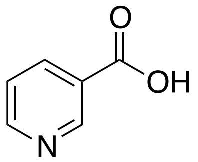
Vitamins & Cofactors

nicotinic acid, niacin

Vitamin B_3



function

- redox cofactor

mode of action

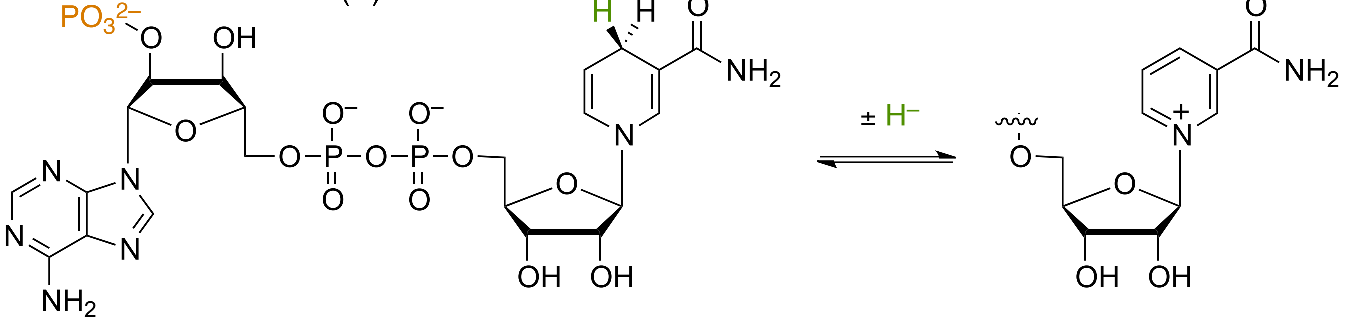
- hydride transfer

found in

- reductases, monooxygenases,...

nicotinamide adenine dinucleotide (phosphate)

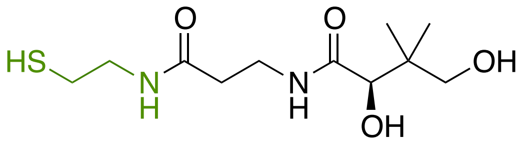
NAD(P)H



Vitamins & Cofactors

pantothenic acid

Vitamin B_5



pantetheine (part of coenzyme A)

function

- conjugation and activation of carboxylates

mode of action

- thioester formation

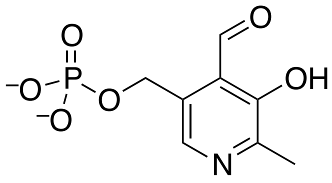
found in

- fatty acid synthases, polyketide synthases

pyridoxal 5'-phosphate

Vitamin B_6

PLP or P5P



function

- "ammonia" shuttle, CH-acidification

mode of action

- imine formation

found in

- decarboxylases, transaminases

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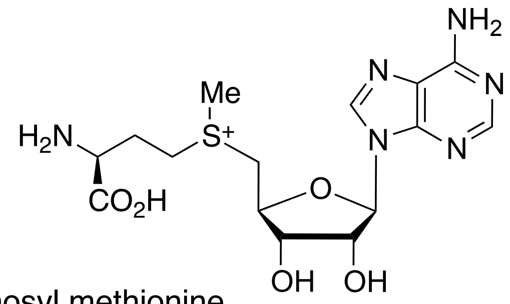
Vitamins & Cofactors



S-adenosyl methionine

SAM

function

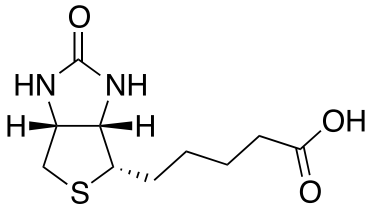
- methyl transfer

mode of action

- electrophilic or radical methylation

found in

- methyltransferases



biotin

Vitamin H

function

- CO_2 activation

mode of action

- carboxamide formation

found in

- carboxylases

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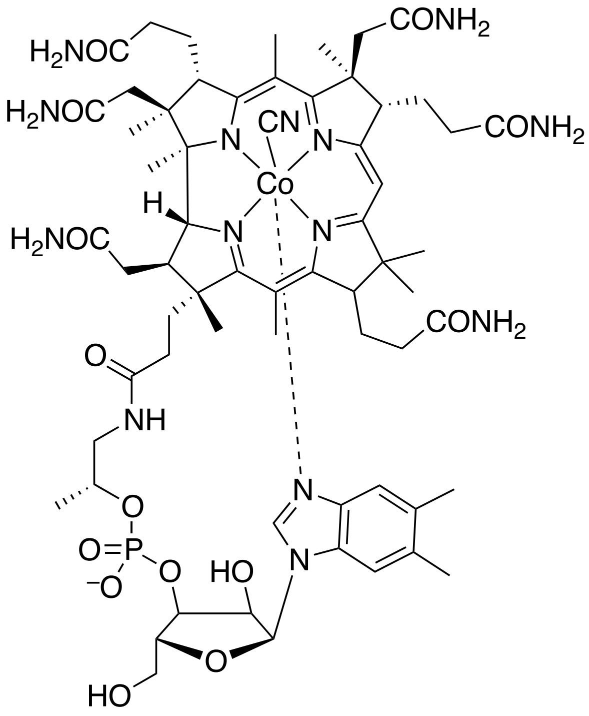
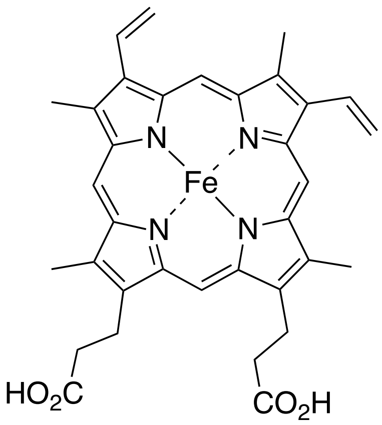
Vitamins & Cofactors



cyanocobalamin

Vitamin B_{12}



heme B

function

- oxygenations, radical initiation

mode of action

- e.g. Fe=O formation

found in

- monooxygenases, peroxidases

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Relevance

Classifications

all enzymes are systematically arranged in **6 classes** and classified according to their **enzyme commission number** (EC number)

EC 1.a.b.c	Oxidoreductases	oxidation & reduction reactions e.g. dehydrogenases, oxidases, oxygenases
EC 2.a.b.c	Transferases	transfer of functional groups from one substrate to another (methyl-, acyl-, amino-,...) e.g. kinases, transaminases, glycosyl transferases
EC 3.a.b.c	Hydrolases	hydrolytic cleavage (but also reverse reaction) e.g. lipases, proteases, amylases
EC 4.a.b.c	Lyases	non-hydrolytic cleavage or bond formation (C-C or C-X) e.g. decarboxylases, aldolases
EC 5.a.b.c	Isomerases	intramolecular rearrangement reactions (isomerizations, racemizations, epimerizations) e.g. isomerases, mutases
EC 6.a.b.c	Ligases	synthesis of C-C or C-X bonds with simultaneous breakdown of ATP e.g. synthetases, DNA-ligases

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EC 3.a.b.c	Hydrolases	hydrolytic cleavage (but also reverse reaction) e.g. lipases, proteases, amylases
EC 4.a.b.c	Lyases	non-hydrolytic cleavage or bond formation (C-C or C-X) e.g. decarboxylases, aldolases
EC 5.a.b.c	Isomerases	intramolecular rearrangement reactions (isomerizations, racemizations, epimerizations) e.g. isomerases, mutases
EC 6.a.b.c	Ligases	synthesis of C-C or C-X bonds with simultaneous breakdown of ATP e.g. synthetases, DNA-ligases

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Classifications

all enzymes are systematically arranged in **6 classes** and classified according to their **enzyme commission number** (EC number)

EC 1.a.b.c	Oxidoreductases	oxidation & reduction reactions e.g. dehydrogenases, oxidases, oxygenases
EC 2.a.b.c	Transferases	transfer of functional groups from one substrate to another (methyl-, acyl-, amino-,...) e.g. kinases, transaminases, glycosyl transferases
EC 3.a.b.c	Hydrolases	hydrolytic cleavage (but also reverse reaction) e.g. lipases, proteases, amylases
EC 4.a.b.c	Lyases	non-hydrolytic cleavage or bond formation (C-C or C-X) e.g. decarboxylases, aldolases
EC 5.a.b.c	Isomerases	intramolecular rearrangement reactions (isomerizations, racemizations, epimerizations) e.g. isomerases, mutases
EC 6.a.b.c	Ligases	synthesis of C-C or C-X bonds with simultaneous breakdown of ATP e.g. synthetases, DNA-ligases

recently added:
EC 7.a.b.c
Translocases

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Biocatalysis on an Industrial Scale

t/a			
	>1.000.000	high-fructose corn sirup	glucose isomerase
	> 100.000	lactose-free dairy products	lactase
	> 10.000	acrylamide	nitrilase
	> 1.000	nicotinamide	nitrilase
		D-pantothenic acid	aldonolactonase
		6-aminopenicillanic acid	penicillin amidase
		aspartame	thermolysin
		L-aspartate	aspartase
		D-phenylglycine	hydantoinase
	> 100	ampicillin	penicillin amidase
		L-methionine, L-valine	aminoacylase
		L-DOPA	beta-tyrosinase
		S-methoxyisopropylamine	lipase

additionally widespread in brewing, laundry additives, paper & pulp, biofuels,...

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Nature as Inspiration

Ronald Breslow

"In Biomimetic Chemistry, we take what we have observed in Nature and apply its principles to the invention of novel synthetic compounds that can achieve the same goals... As an analogy, we did not simply make larger versions of birds when we invented airplanes, but we did take the idea of the wing from Nature, and then used the aerodynamic principles in our own way to build a jumbo jet."

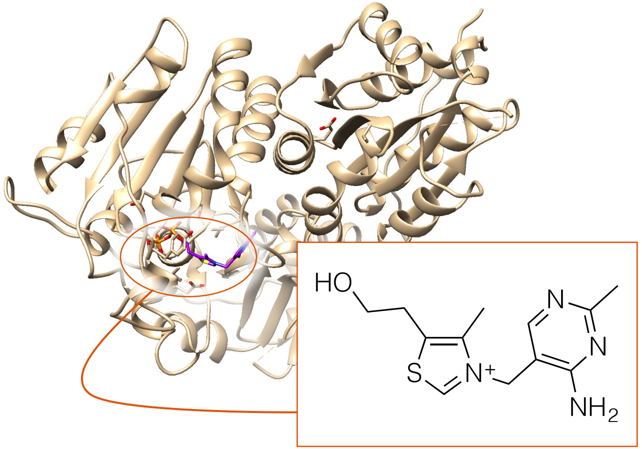
Synthetic compounds that mimic biological materials' functions or properties:

- bioactivity (medicinal chemistry)
- light-activated (photovoltaics & photocatalysis)
- natural binding modes (organocatalysis)

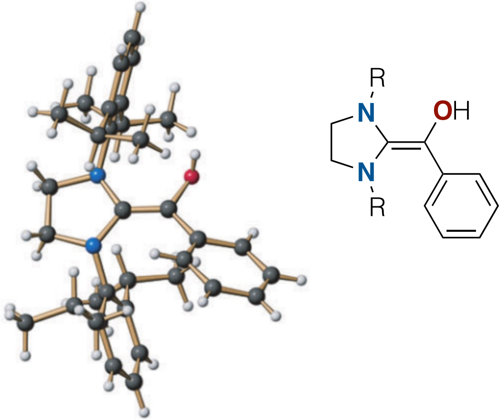
Nature as Inspiration

- **biomimetic catalysis**

= imitation of the catalytic principles of enzyme catalysts



thiamine-dependent decarboxylases/transketolases



Breslow intermediate as motif in
a^1-d^1 umpolung reactions

modern carbene catalysis

a) Breslow, *J. Am. Chem. Soc.* **1957**, *79*, 1762-1763; b) Breslow, *J. Am. Chem. Soc.* **1958**, *80*, 3719-3726;
c) Berkessel, Yatham, Elfert, Neudörfl, *Angew. Chem. Int. Ed.* **2013**, *52*, 11158-11162.

Wishes?
Suggestions?
Questions?