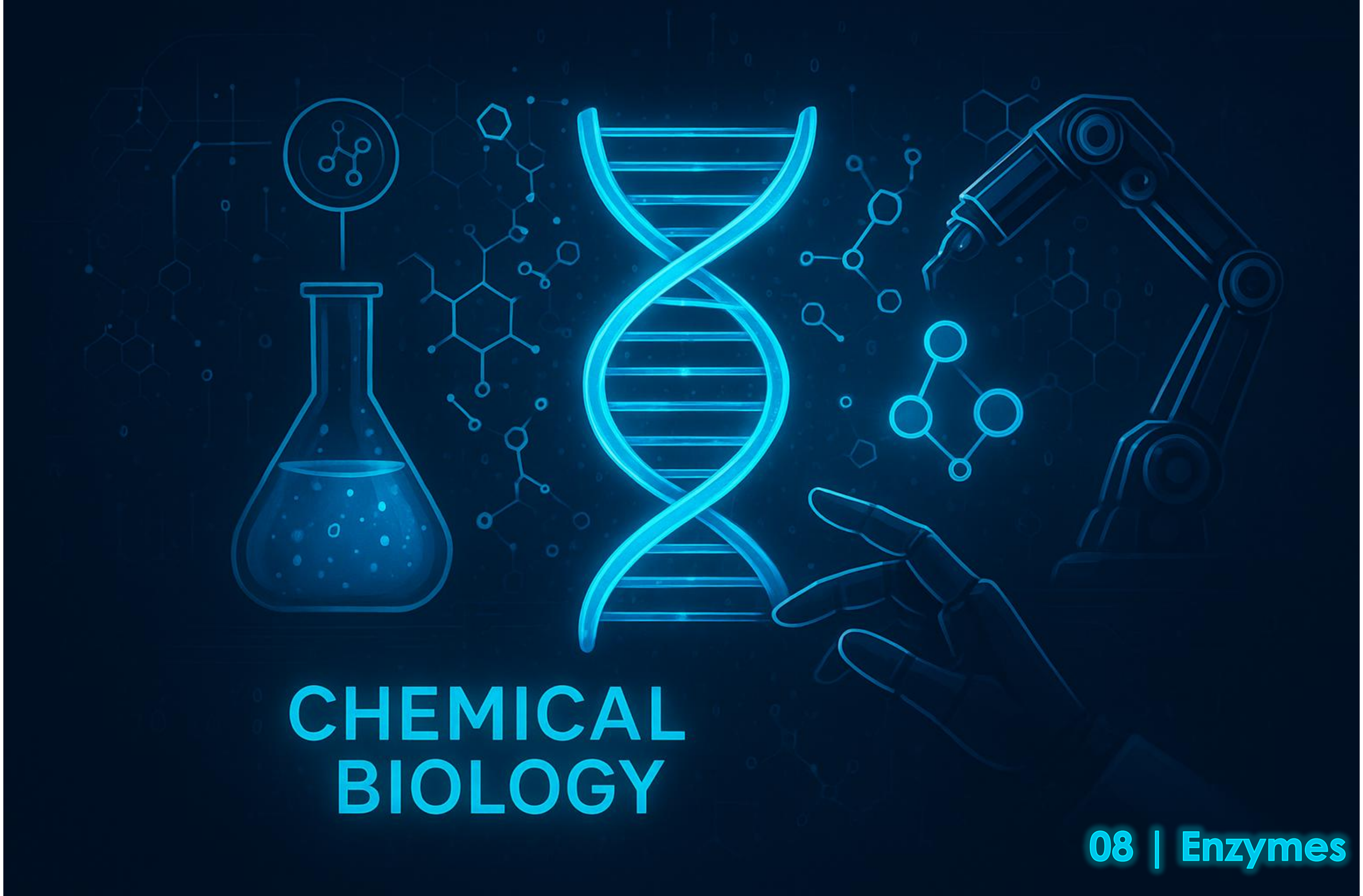
EPFL



CHEMICAL BIOLOGY

08 | Enzymes

Prof. Dr. Markus Jeschek, LSAM

CH-313

General Course Information

- Moodle: https://go.epfl.ch/CH-313
  - Lecture slides (evening before the lecture)
  - Distributed presentation topics (assignments)
  - Forum (for questions and announcements)

- Examination (written, graded, detailed information will follow)
- Contact:
  - Moodle forum (for questions)
  - markus.jeschek@epfl.ch

- **"Concepts over details!"**
- **Interact! Ask! Discuss! Anytime!**

Group Presentations

- Critical discussion of primary literature
- Illustrative examples for topics from the lecture

- Why?
  - Repetition of core concepts, techniques etc.
  - Presentation skills and critical discussion of research
  - Insight into current research topics

- How?
  - Two students per group
  - Assignments distributed one week before delivery of presentation (via Moodle)
  - **Send slides: markus.jeschek@epfl.ch (Mon evening before presentation)**
  - **15 min presentation (both group members should present!)** + Q&A

Tipps for Group Presentations

- Rough structure
  - Short intro on general topic
  - Main presentation according to assignment
  - Brief outlook incl. points of criticism/open questions/personal opinion as kick-starter for the discussion

- Everybody should participate in the discussion, incl. constructive(!) feedback on presentation style

- Questionnaires with different points, feedback by peers

- Typical assignment:
  - You will receive a certain topic including a related publication
  - Introduce the topic using the publication
  - present the motivation behind the research, methodology, key results (not every graph!)
  - Additional questions will be provided hinting towards central points
  - Be encouraged to look/present beyond the questions and the provided paper

Group Presentations – Schedule

#	Name1	Name2	Presentation on…	Assignment on…
1	Wininger Quentin	Jeremy	**Sep 23, 2025**	Sep 16, 2025
2	Ema	Ariane	**Sep 30, 2025**	Sep 23, 2025
3	Benjamin	Matthieu	**Oct 7, 2025**	Sep 30, 2025
4	Ivana	Ipek	**Oct 14, 2025**	Oct 7, 2025
5	Mridhula	Elodie	**Oct 28, 2025**	Oct 21, 2025
6	Abigail	Robin	**Nov 4, 2025**	Oct 28, 2025
7	Eva	Florian	**Nov 11, 2025**	Nov 4, 2025
8	Bastien	Axel	**Nov 18, 2025**	Nov 11, 2025
9	Melodie	Siolène	**Nov 25, 2025**	Nov 18, 2025
10	Nicole	Maria	**Dec 2, 2025**	Nov 25, 2025

Course Topics – Overview

- Week 1 | Introduction + DNA
- Week 2 | DNA
- Week 3 | DNA
- Week 4 | DNA
- Week 5 | DNA/RNA
- Week 6 | RNA/Translation
- Week 7 | Translation
- **Week 8 | Enzymes (Zoom)**
- Week 9 | Enzymes (Zoom)
- Week 10 | Enzymes/Metabolism (Zoom)
- Week 11 | Metabolism (Zoom)
- Week 12 | Engineering
- Week 13 | Engineering
- Week 14 | LSAM Intro + Exam Preparation

!Due to paternity leave the next lectures will be delivered via Zoom!

[tentative schedule]

Zoom Info

- Week 1 | Introduction + DNA
- Week 2 | DNA
- Week 3 | DNA
- Week 4 | DNA
- Week 5 | DNA/RNA
- Week 6 | RNA/Translation
- **Week 7 | Translation**
- **Week 8 | Enzymes (Zoom)**
- **Week 9 | Enzymes (Zoom)**
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!Due to paternity leave the next lectures will be delivered via Zoom!

https://epfl.zoom.us/j/68900732223

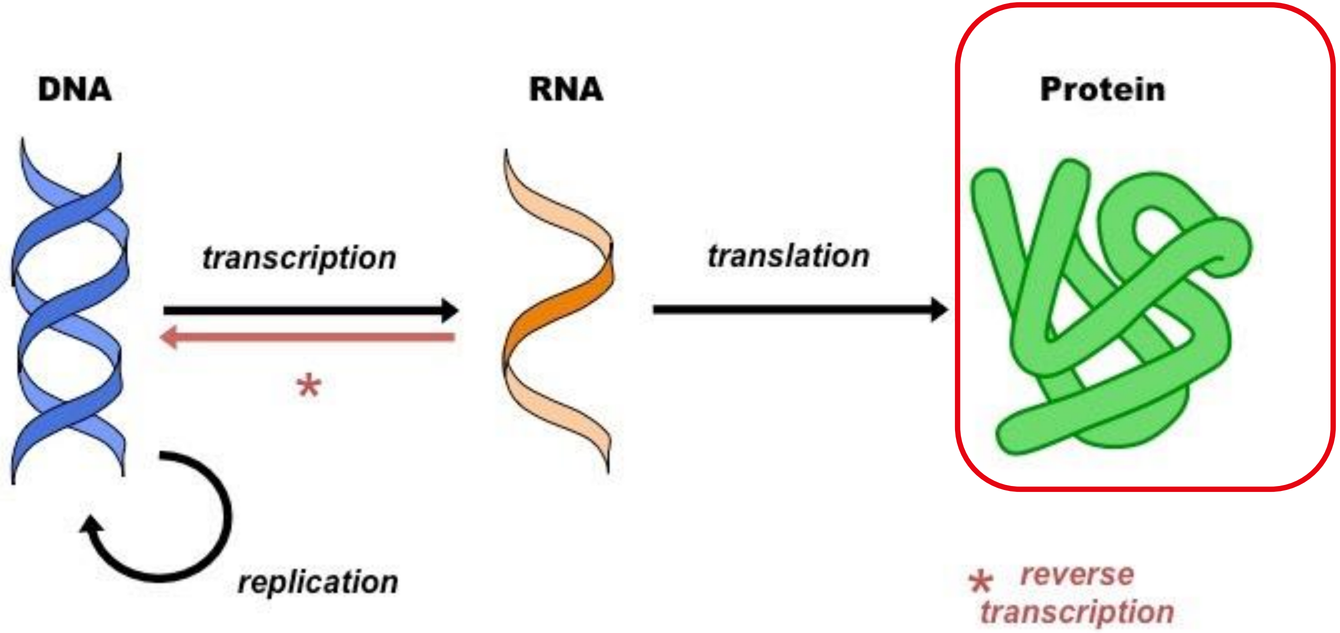
Meeting ID: 689 0073 2223

[tentative schedule]

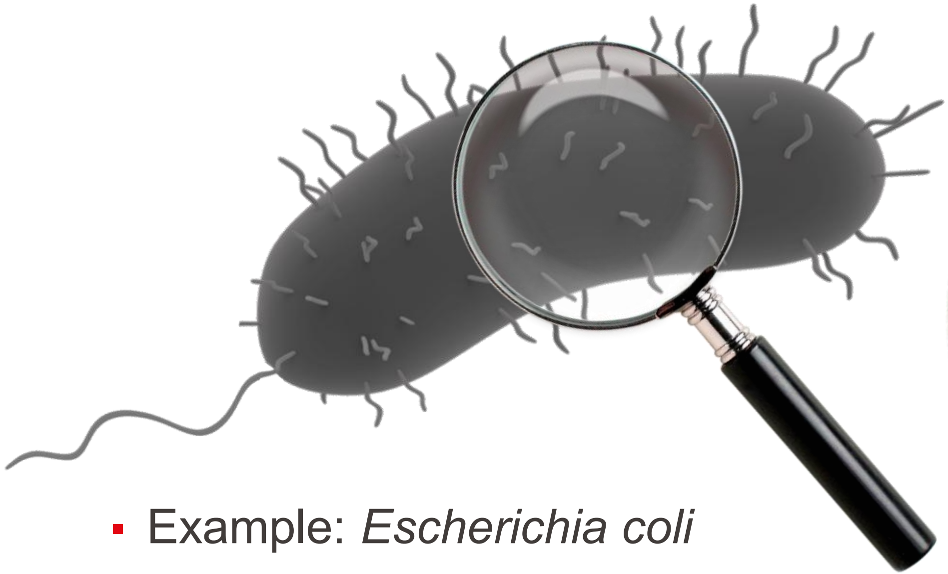
Protein – Enzymes

The Central Dogma

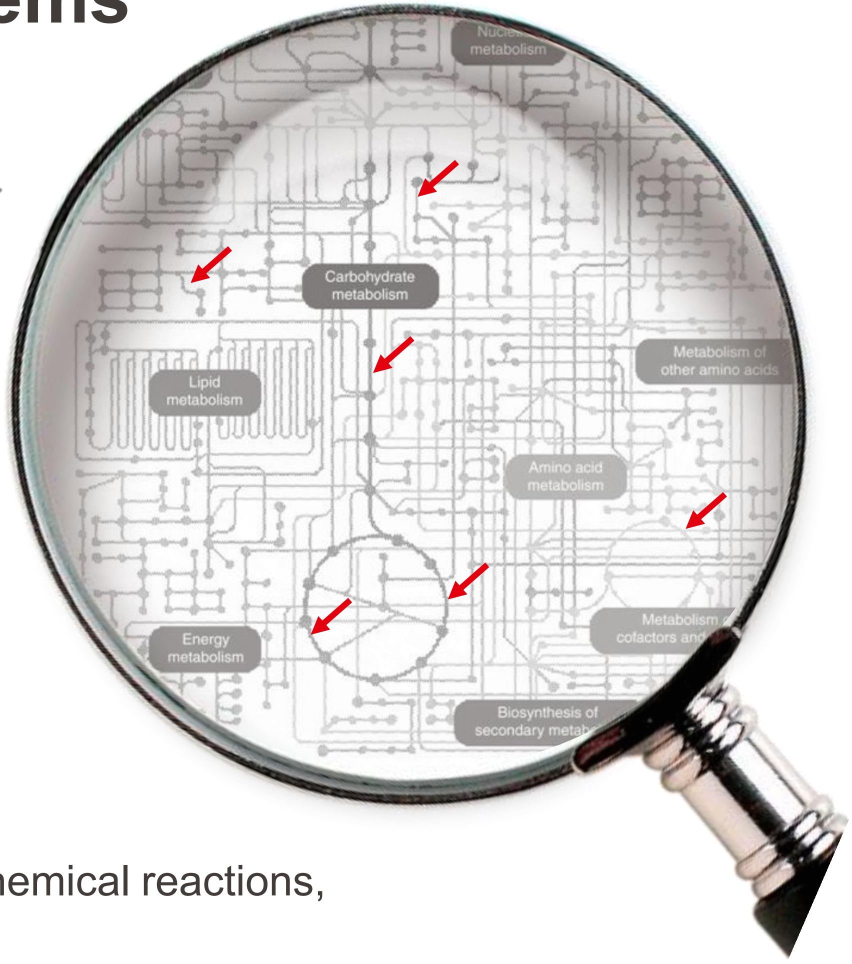
- …of Molecular Biology



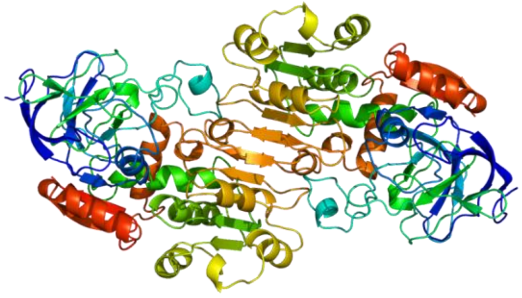
Complexity of Biosystems



- Example: *Escherichia coli*
  - approx. 4000 genes
  - **600-800 enzymes**
  - approx. 1000 metabolites
  - Complex network of molecules, biochemical reactions, regulation and interaction



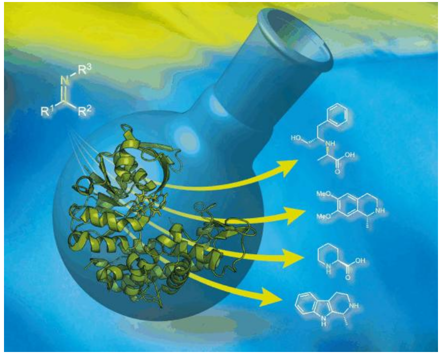
Enzymes – Intro



- catalysts built up of biological macromolecules
- “biocatalysts” (the catalysts of life)
- mostly proteins! (rarely nucleic acids, ribozymes etc.)

- “enzyme” from Greek “en zýmē” (“in yeast”/”in leaven”)
- Initially defined as sth. “dead”/ “outside of living cells”

- field of biocatalysis



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Enzymes – Structure

primary structure

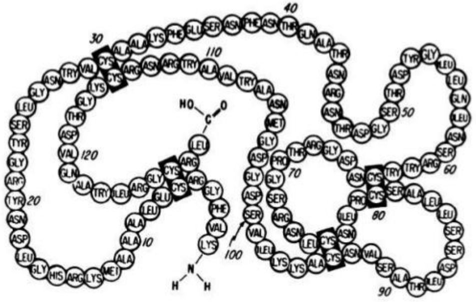
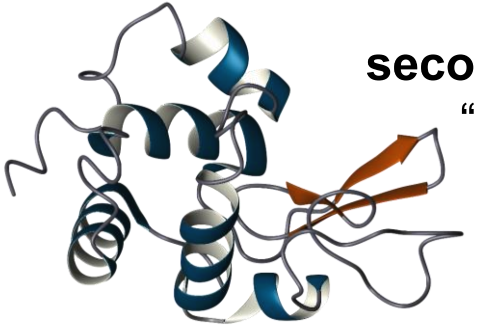
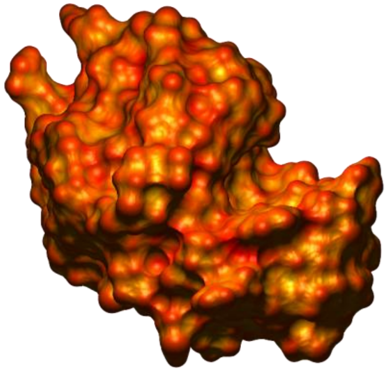


FIG. 1.—Amino acid sequence of hen egg-white lysozyme reproduced from Canfield and Liu, 1965.

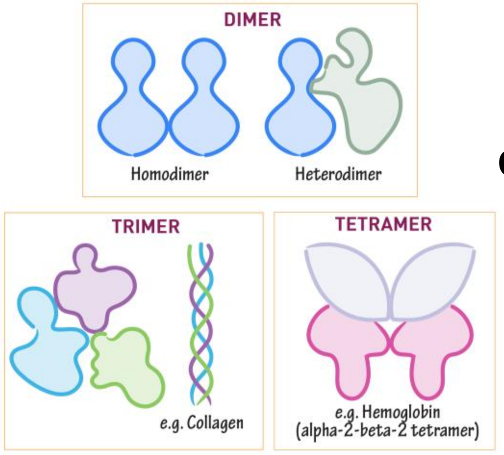
secondary structure
"ribbon" view



tertiary structure
"surface" view



quartenary structure

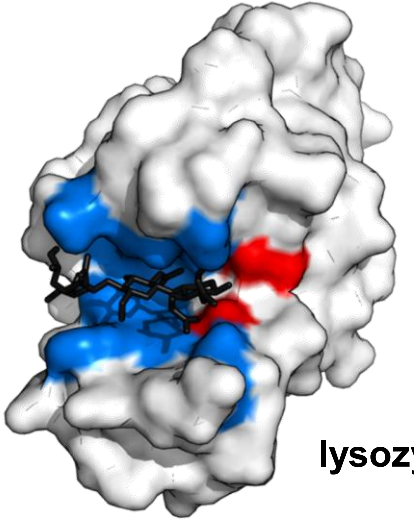


Q: What does lysozyme do? Where do you find it?

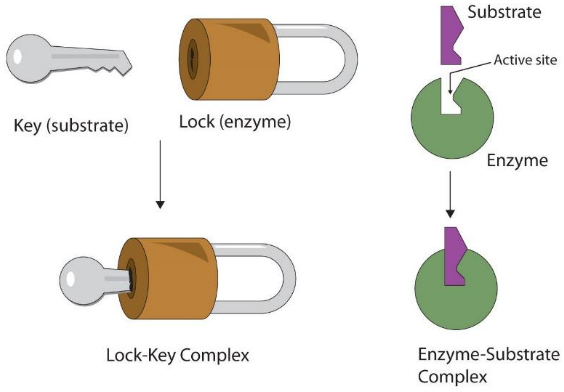
RECAP!

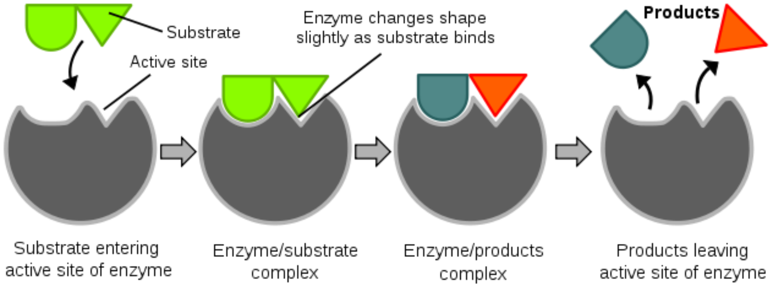
Enzymes – Active Site

- (Main) functional site in the protein
- Few key amino acid residues directly responsible for catalysis
- Substrate binding and conversion to product
- “Lock and key” vs. “induced fit” mechanism



lysozyme active site





RECAP!

Enzymes – Brief History

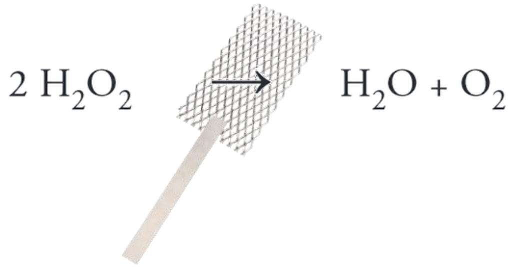
- 1783 Spallanzani digests meat with gastric juice
- 1836 Schwann calls the corresponding active substance „pepsin“
- 1876 Kühne coins the term „enzyme“ (=„in yeast“), in recognition of the role of yeast in alcoholic fermentation
- 1897 Buchner carries out alcoholic fermentation of with a cell-free yeast extract → end of ”vis vitalis“
- 1908 Rosenthaler uses almond extracts to achieve chemical transformations
- 1926 Sumner crystallizes urease from jack bean: proteins!
- 1930 Northrop establishes firmly the protein character with a series of crystallization projects, starting with pepsin
- 1951 Sanger & Tuppy determine amino acid sequence of insulin beta-chain – start of modern era of molecular biology
- 1978 Noble prize for restriction enzymes (Arber, Smith)
- 1980’s Directed evolution (enzyme engineering)
- 2008 First computer-designed enzyme
- 2018 Nobel Prize in Chemistry for Directed Evolution of Enzymes (F. H. Arnold)

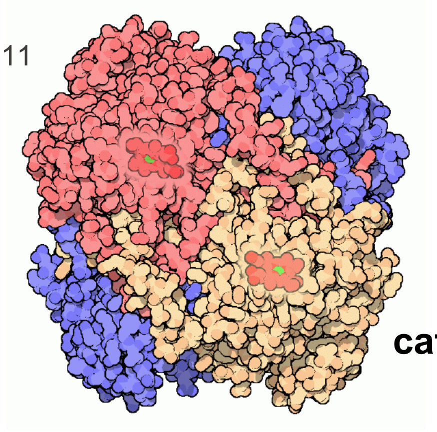
Enzymes are Highly Efficient

- Example: decomposition of hydrogen peroxide

$$2\,H_2O_2 \longrightarrow H_2O + O_2$$

  - without catalyst: relative reaction speed := 1
  - with platinum: relative reaction speed = ~ 2 x 10^4
  - with catalase: relative reaction speed = ~ 2 x 10^{11}

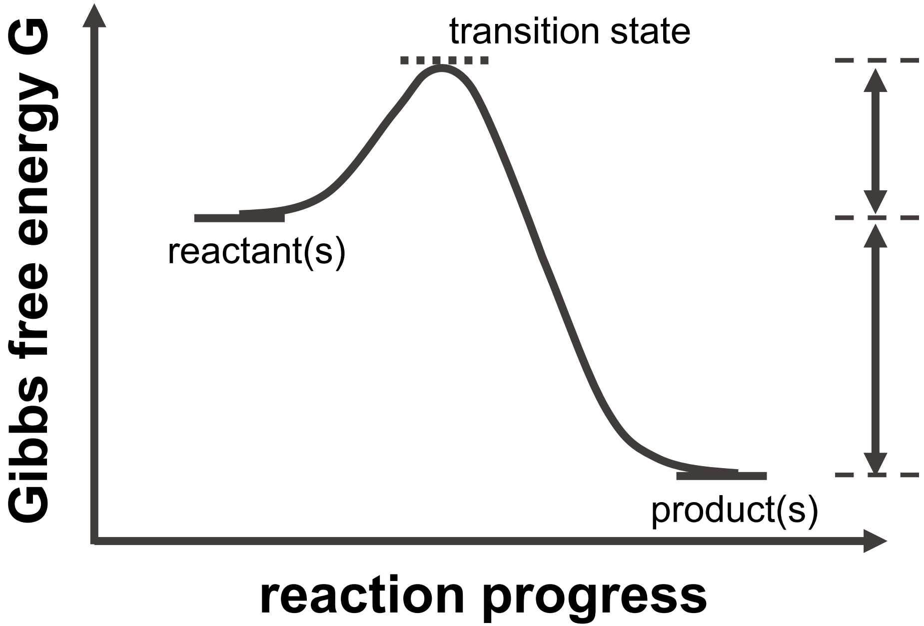




catalase

- How do enzymes achieve that?

Energy Diagram of a Chemical Reaction



activation energy ($\Delta G^{\ddagger} > 0$)

reaction enthalpy ($\Delta G < 0$ for exergonic reactions)

Transition state: transient, short-lived high energy state

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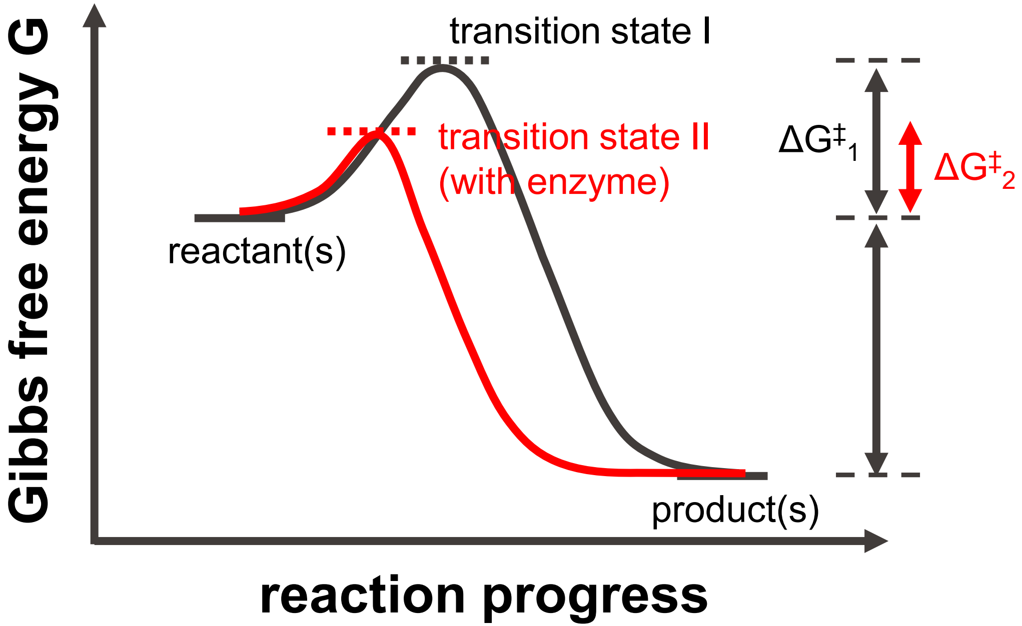
How do Enzymes Work?



Arrhenius equation:

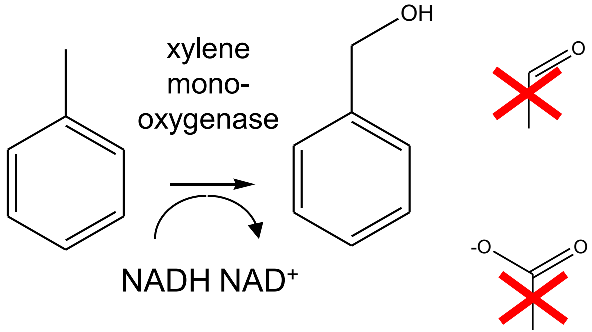
$$k = A \cdot e^{-\frac{\Delta G^{\ddagger}}{R \cdot T}}$$

A: frequency factor [1/s]
$\Delta G^{\ddagger}$: activation energy [J/mol]
R: gas constant, 8.314 [J/(mol*K)]
T: absolute temperature [K]

- Enzymes lower activation energies by stabilizing transition state structures (= different transition state)
- Faster reaction!
- Enzymes do not change the thermodynamic equilibrium!
- Dependence of reaction velocity (rate constant k) on $\Delta G^{\ddagger}$ → Arrhenius equation

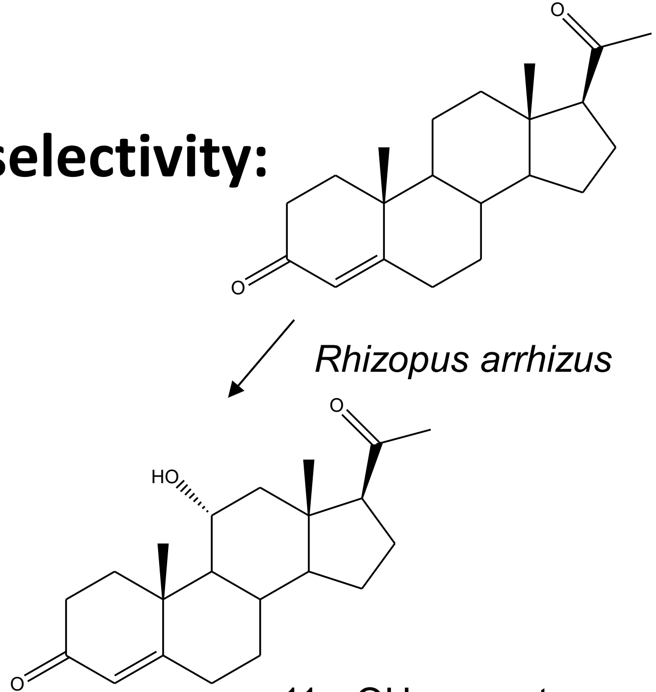
Enzymes are Highly Selective

chemoselectivity:



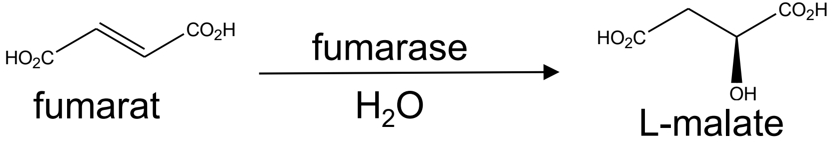
regioselectivity:

progesterone



11α-OH-progesterone

enantioselectivity:



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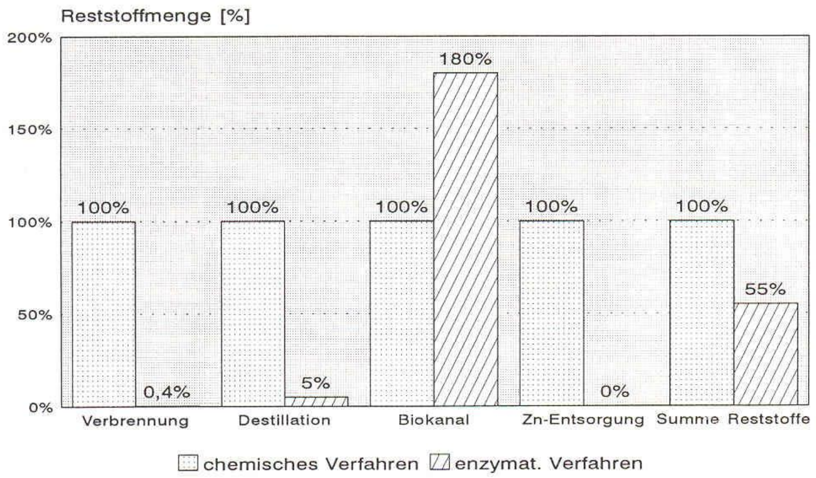
Enzymes are “Green”

E-factor = total waste / product

Table 1. E-Factors across the chemical industry

Industry sector	Annual production (t)	E-factor	Waste produced (t)
Oil refining	10^6-10^8	Ca. 0.1	10^5-10^7
Bulk chemicals	10^4-10^6	<1–5	10^4-$5{\times}10^6$
Fine chemicals	10^2–10^4	5–50	5×10^2–5×10^5
Pharmaceuticals	10–10^3	25–100	2.5×10^2–10^5



Example: waste streams β-lactame production

Q: What are beta-lactams used for? How are they counteracted by e.g. bacteria?

Pros and Cons of Enzymes

- Enzymes are excellent catalysts…
  - fast, efficient
  - selective (chemo-, regio-, stereo-)
  - work under “mild” reaction conditions (water, pH ~7, 10-40 °C, low salt etc.)
  - environmentally friendly (mostly)
  - can be easily combined! (cascade reactions)
- …but…
  - can be expensive
  - cofactor dependence
  - reduced activity in organic solvents
  - inhibition
  - stability issues

Enzyme Commission Number (EC)

- Enzyme commission (founded 1956, part of the International Union of Biochemistry and Molecular Biology, IUBMB)
- Functional classification (catalyzed reaction)

subclass (substrate class)

serial number in sub-subclass

EC X . X . X . X

main reaction type (7 classes)

sub-subclass (substrate)

Example: EC 2.3.2.13 "transglutaminase"

2: transferases
2.3: acyltransferases
2.3.2: aminoacyltransferases
2.3.2.13: protein-glutamine γ-glutamyltransferase

"meat glue"

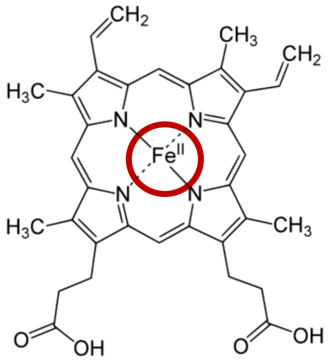
Enzyme Classes

- **EC 1: Oxidoreductases** → redox reactions
- **EC 2: Transferases** → group transfer
- **EC 3: Hydrolases** → hydrolysis with H_2O
- **EC 4: Lyases** → cleavage (or joining) of molecules (non-hydrolytic!) forming double bonds or ring structures
- **EC 5: Isomerases** → isomerization (racemization, epimerization, rearrangement); mixtures (equilibrium!)
- **EC 6: Ligases** → joining of molecules at expense of ATP hydrolysis
- **EC 7: Translocases** → translocating molecules or ions across membranes (new class, since 2018)

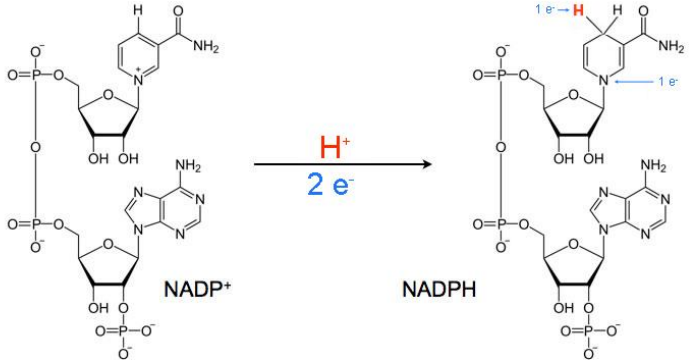
Cofactors

- Small, non-protein molecules essential for enzymatic activity
- Holoenzyme = apoenzyme (protein part) + cofactor
- Types:
  - **coenzymes** ("cosubstrate"): organic molecules, non-covalent, dissociate after catalysis, donor/acceptors of groups/e^-/H^+ etc. (e.g. NAD(P)H, ATP, coenzyme A etc.)
  - **prosthetic groups**: organic molecules, covalently or non-covalently bound to protein, no dissociation (e.g. flavins, heme porphyrin, biotin etc.)
  - **metal ions**: in metalloenzymes (e.g. Fe → oxygenases, Ni → urease etc.); ~30% of enzymes (Mg, Zn, Fe, Cu, Co, Ni, Mn, Mo, Se, V etc.)

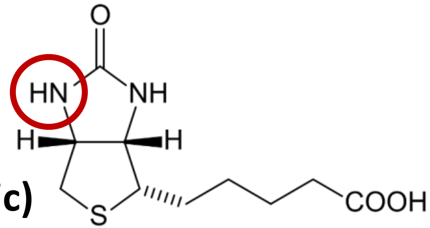
Cofactors – Examples



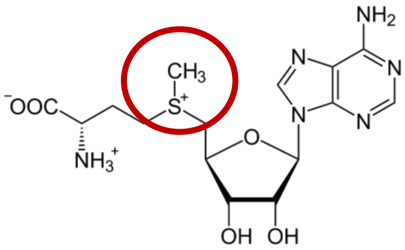
heme / iron porphyrins (prosthetic)
→ redox reactions, oxygen transfer



NAD(P)$^+$ / NAD(P)H (coenzyme)
→ redox reactions, transfer of "H$^-$"



D-biotin (prosthetic)
→ carboxylations



***S*-adenosyl methionine (SAM, coenzyme)**
→ methyl (C_1) group transfer

Q: In which enzyme classes do you expect these cofactors?

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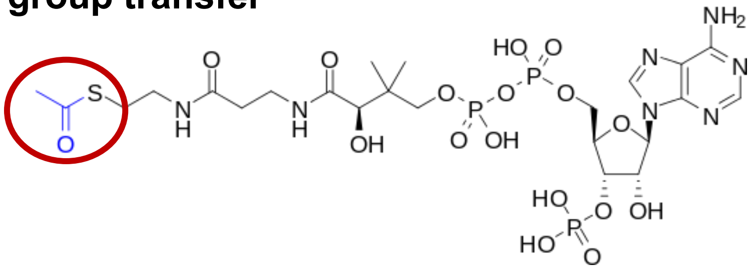
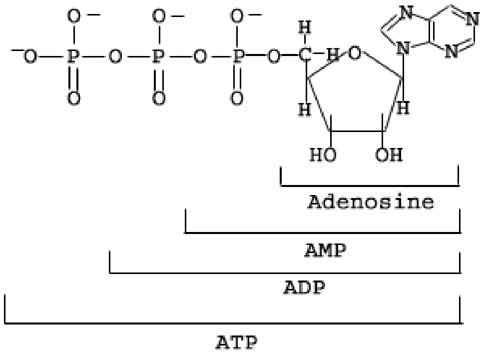
Cofactors – Examples

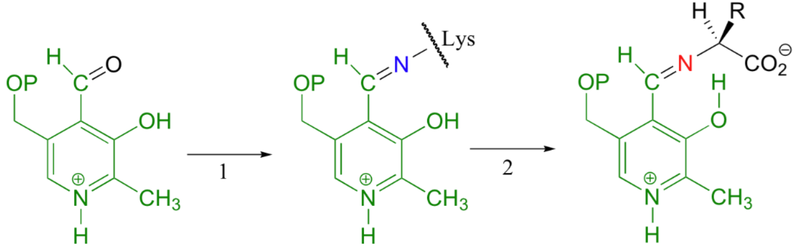
coenzyme A (CoA-SH) / acetyl-CoA
→ acyl group transfer



ATP / ADP / AMP (coenzyme)
→ phosphate transfer, "energy"

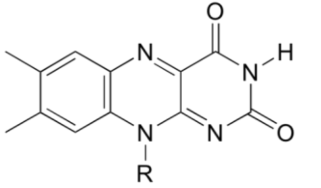


pyridoxal phosphate (PLP, coenzyme)
→ transamination, deamination etc.

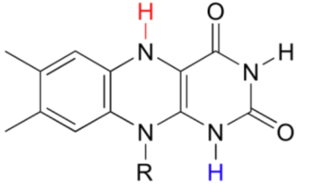


free PLP enzyme-PLP amino acid-PLP

riboflavins (prosthetic)
→ redox reactions, single electron transfer



FAD or FMN (oxidized flavin)

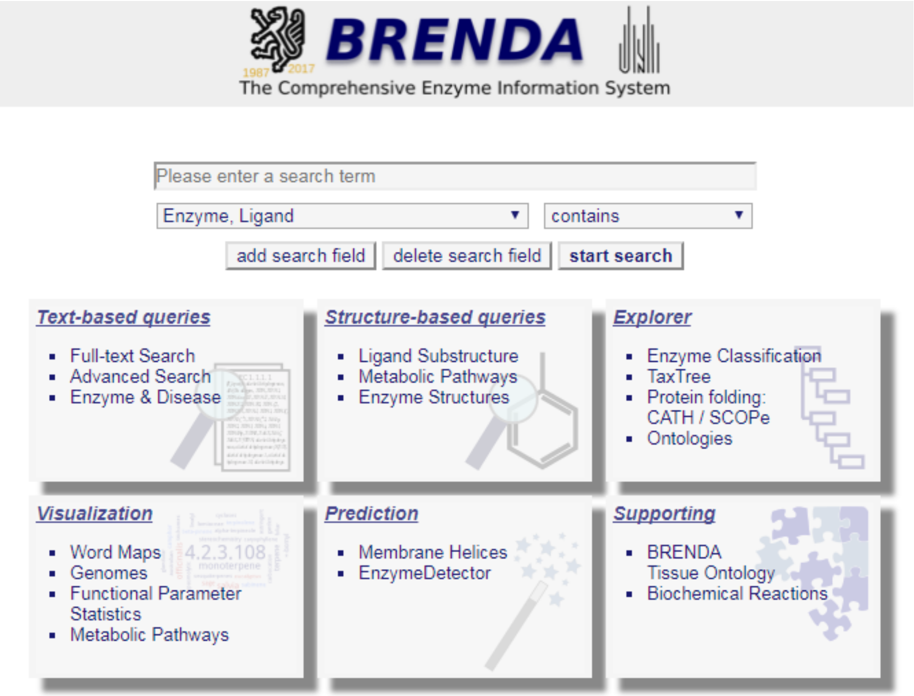


$FADH_2$ or $FMNH_2$ (reduced flavin)

Q: In which enzyme classes do you expect these cofactors?

BRENDA

- https://www.brenda-enzymes.org/
- Enzyme database
- Useful search tool:
  - substrates, products
  - main and side reactions
  - organisms
  - literature references
  - etc.



Enzymes in Industry

- as catalysts for synthetic chemical reactions (= biocatalysis)
- in detergents
- for bleaching
- food/feed processing or as additives
- pharmaceuticals
- etc.

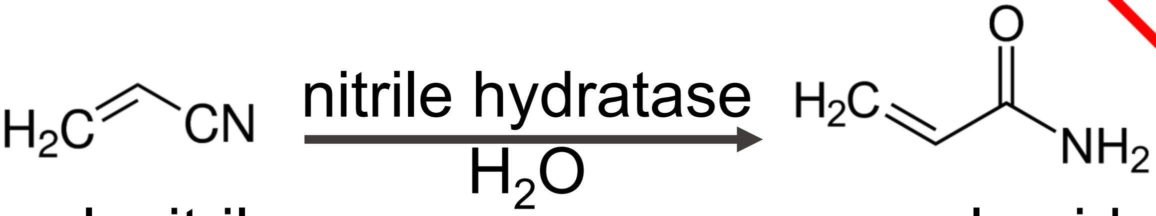
→ enzymes appear both in industrial processes and in/as products!

Q: Which enzyme class is the most important one in industry?

Enzymes in Industry - Examples

- β-lactame antibiotics (e.g. **penicillin acyclase**)
- acryl amide production with **nitrile hydratase**
- high-fructose corn syrup (**amylases, xylose isomerase**)
- **cellulases, laccases** for denim treatment, bleaching
- enzymes in detergents (**lipases, proteases, amylases, cellulases etc.**)
- **phytase** as feed additive
- enzymes as pharmaceuticals **(thrombolytic enzymes, uricas**e etc.)
- etc. etc.

Nitrile Hydratase

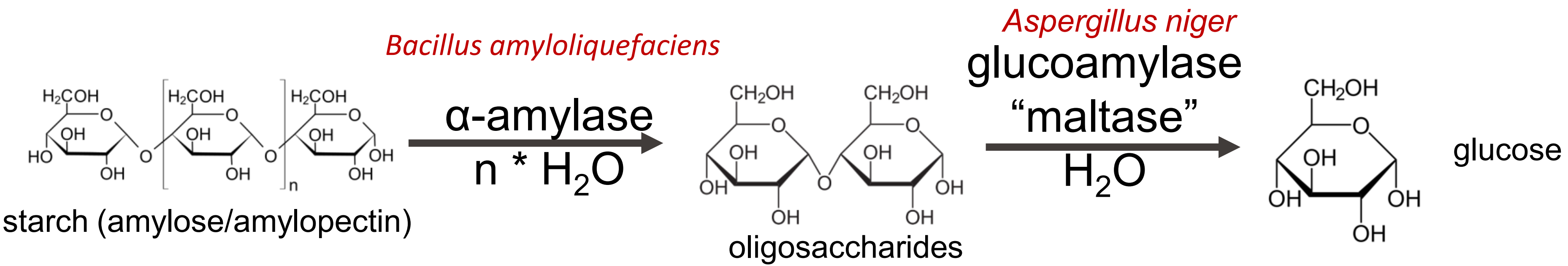


acrylonitrile → acrylamide

- polymer synthesis, flocculation agent (water treatment), dyes, glue, solvent etc.
- immobilized cells (Rhodococcus sp.), T = 5 °C, 100% yield, > 30,000 t annually
- Nitto Chemical Industry (Japan)
- Chemical process: copper catalyst, side products (acrylic acid etc.), polymerization

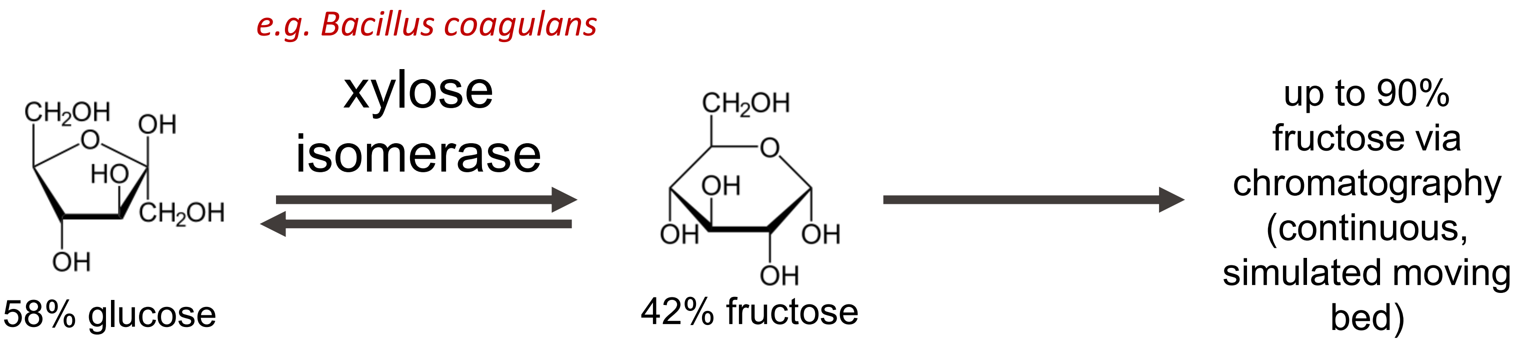
Q: Which enzyme class does nitrile hydratase belong to?

High-fructose corn syrup (HFCS)



novo nordisk®

DSM



70% sweetness of sucrose

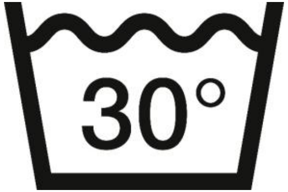
120% sweetness of sucrose

Q: Which enzyme classes are involved here?

Enzymes in Detergents

novozymes

- laundry, dish washing
- hydrolytic enzyme cocktail:
  - lipases: oil and fat stains
  - amylases: starch remainders
  - proteases: e.g. egg, blood, grass
  - cellulases: smoothening of surface, stain removal
  - etc.



- washing at lower temperatures possible
- enzymes must be stable against heat, surfactants, bleach etc.!

  → thermophilic enzymes, enzyme engineering