EPFL

CHEMICAL BIOLOGY

09 | Enzymes

CH-313

Prof. Dr. Markus Jeschek, LSAM

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 (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)**
- **Week 10 | Enzymes (Zoom)**
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- Week 14 | LSAM Intro + Exam Preparation

!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]

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Protein – Enzymes

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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”

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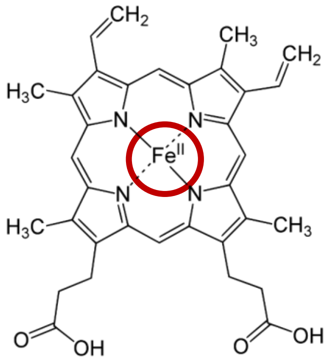
Enzyme Classes

- **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)

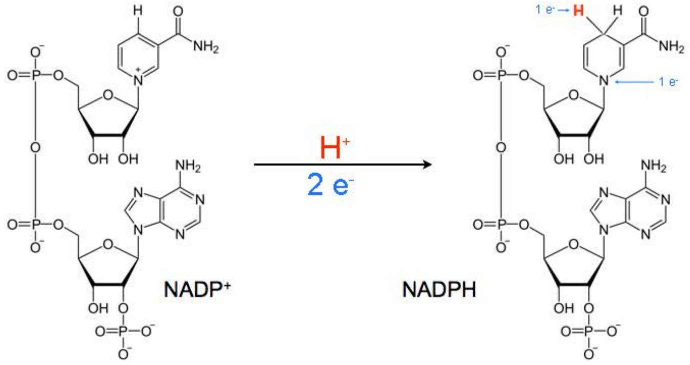
Cofactors

- 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.)

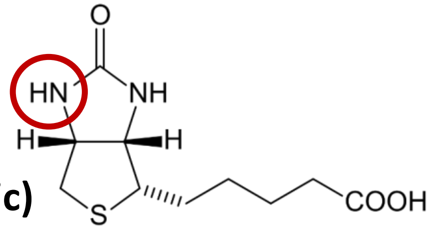
Cofactors – Examples



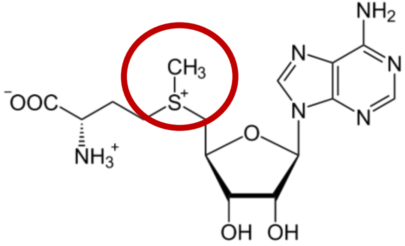
heme / iron porphyrins (prosthetic)
→ redox reactions, oxygen transfer



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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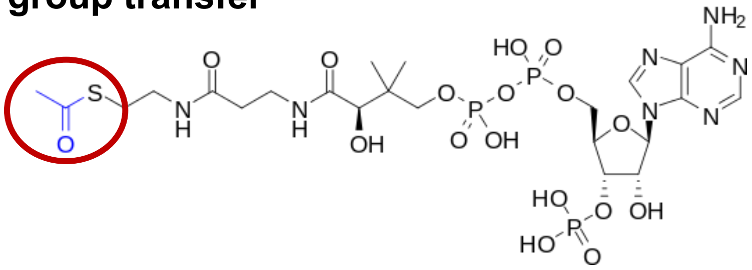
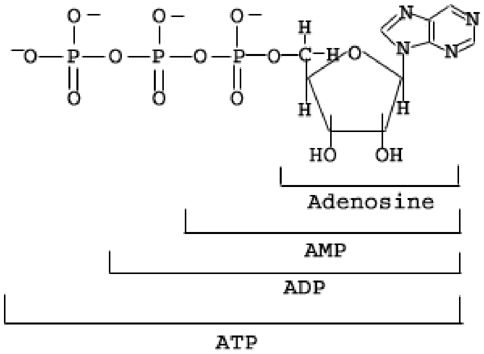
Cofactors – Examples

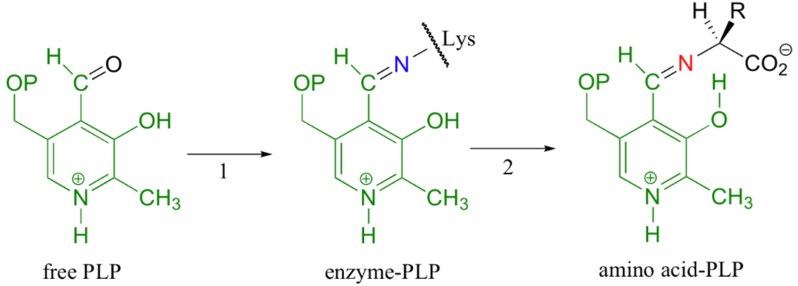
coenzyme A (CoA-SH) / acetyl-CoA
→ acyl group transfer



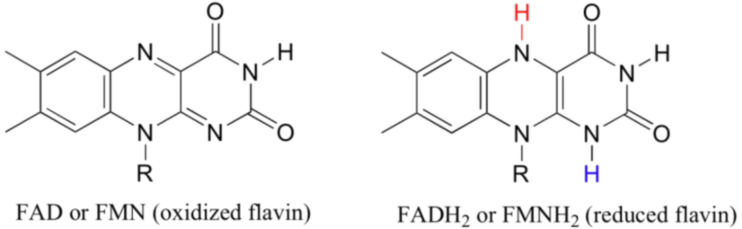
ATP / ADP / AMP (coenzyme)
→ phosphate transfer, "energy"



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



riboflavins (prosthetic)
→ redox reactions, single electron transfer



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.

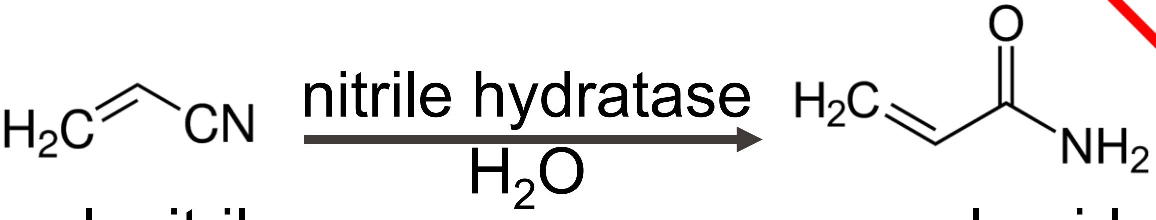
→ enzymes appear both in industrial processes and in/as products!

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

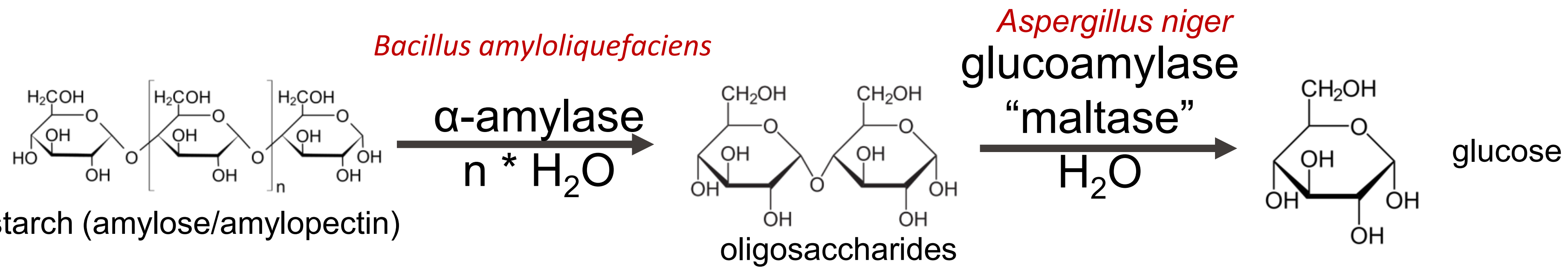


acrylonitrile → acrylamide

- 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

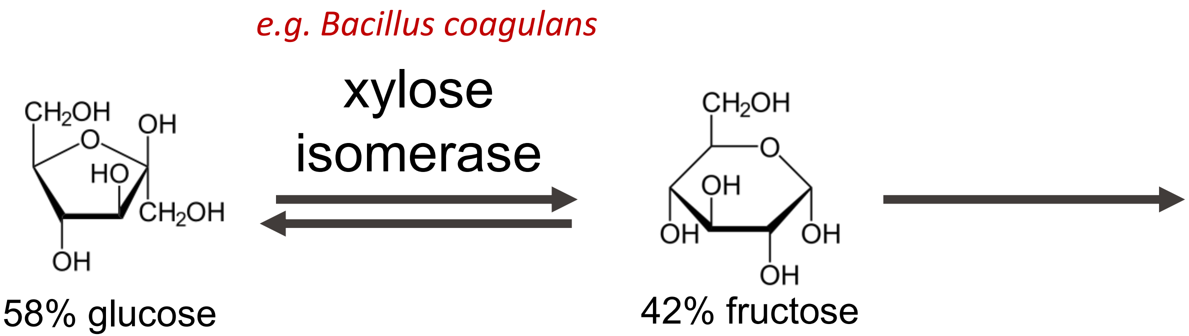
Q: Which enzyme class does nitrile hydratase belong to?

High-fructose corn syrup (HFCS)



novo nordisk®

DSM



up to 90% fructose via chromatography (continuous, simulated moving bed)

70% sweetness of sucrose

120% sweetness of sucrose

Q: Which enzyme classes are involved here?

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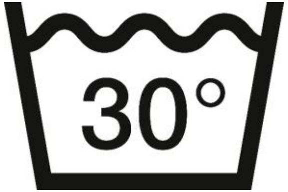
Enzymes in Detergents

- 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

novozymes



Protein – Enzymes
(Kinetics)

Enzyme Kinetics

S: substrate
P: product
v: reaction rate

- Mathematical description of substrate/product concentrations of enzymatic reactions as a function of time → reaction rate ***v***
- Important for…
  - enzyme characterization
  - enzyme optimization/engineering
  - identification of catalytic mechanism and regulation (e.g. inhibition)
  - bioprocess planning
- Basic reaction model: **Michaelis-Menten**

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$$

Michaelis-Menten Model

"binding" / "catalysis"

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

S: substrate
P: product
v: reaction rate
E: enzyme
(ES): enzyme-substrate complex
k_1: rate constant (ES) formation
k_{-1}: rate constant (ES) disintegration
k_2: rate constant P formation

- equivalent to a **one-substrate (1st order) reaction at its start** (no P around yet)
- simplifying assumptions:
  - (ES) → E + P is the rate-limiting step: $v = k_2\,[(ES)]$
  - E+S and (ES) in steady-state (equilibrium): $k_{1/-1} \gg k_2 \rightarrow \frac{d[(ES)]}{dt} = 0$
  - S is present in excess (enzyme is saturated): $[S] \gg [ES] \rightarrow [S]=[S]_0$

Q: Why is there no k_{-2} considered?

Michaelis-Menten (MM) Model

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

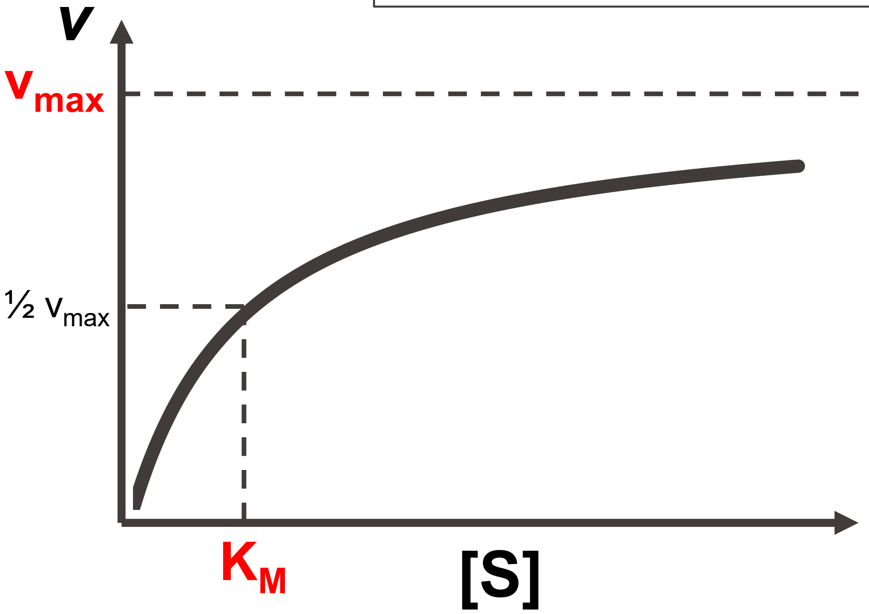
"binding" (k_1), "catalysis" (k_2)

S: substrate
P: product
v: reaction rate
E: enzyme
(ES): enzyme-substrate complex
k_1: rate constant (ES) formation
k_{-1}: rate constant (ES) disintegration
k_2: rate constant P formation

Michaelis-Menten equation:

$$-\frac{d[S]}{dt} = v = \frac{v_{max} * [S]}{K_M + [S]}$$

K_M: [S] at which v = 0.5 v_{max}



Q: How do you get to the Michaelis-Menten plot experimentally?

Enzyme Kinetics – Key Parameters

$$\frac{k_{cat}}{K_M} := \text{"catalytic efficiency"} \qquad v_{max} = k_{cat} \cdot E_0$$

- Catalytic efficiency
  - Comparison of enzyme performance
  - derived from v_{max} and K_M
  - limited by diffusion (practical maximum ~ 10^9 M^{-1} s^{-1})

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Table 12-1

- Unit (U)
  - "enzyme amount to convert one µmol substrate in one minute" [µmol/min]
  - in enzyme literature, commercial enzymes (U/mL, U/mol, U/mg etc.)
- Katal (kat)
  - "enzyme amount to convert one mol substrate in one second" [mol/s] (= 60×10^6 U)
  - SI unit, rarely use

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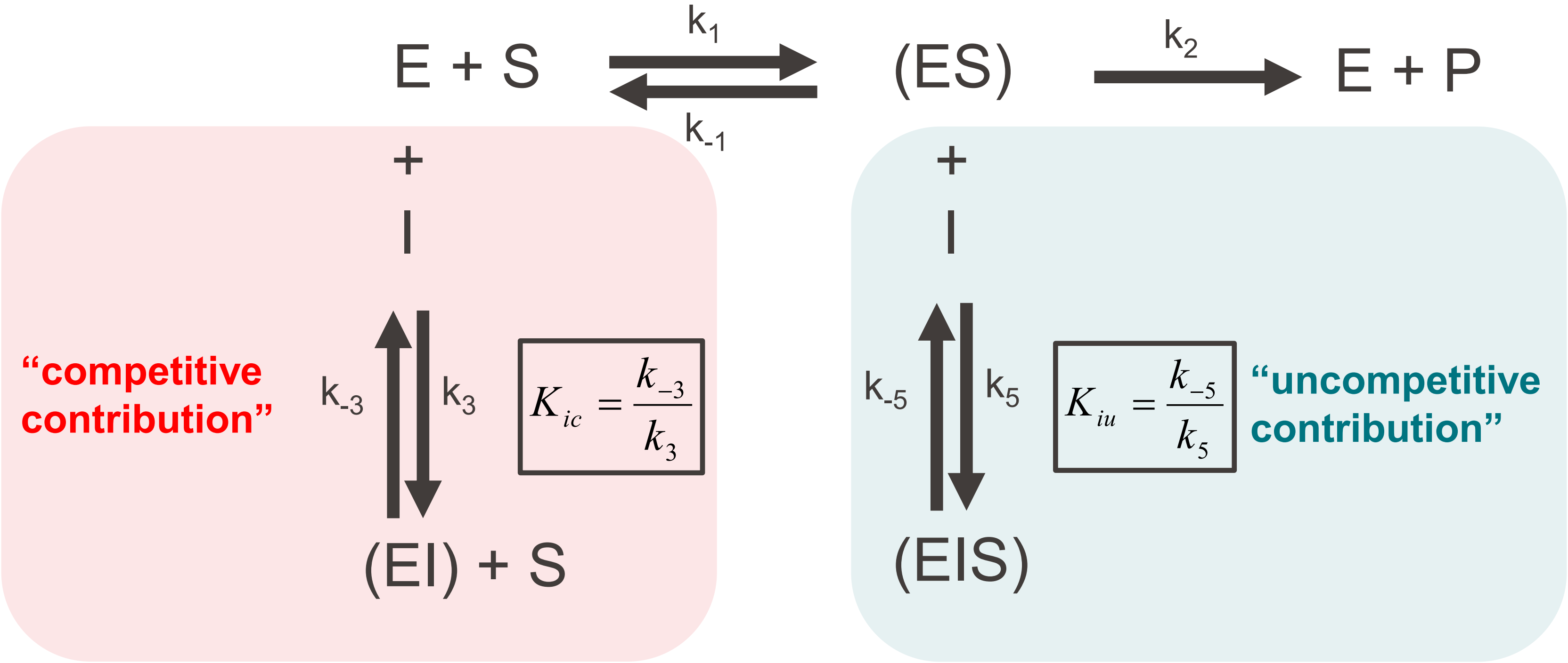
Enzyme Inhibition

- decrease in enzyme activity by inhibitor
- reversible and irreversible
  - reversible: can be recovered by exchange of medium
    - competitive
    - uncompetitive
    - Mixed
  - irreversible (“inactivation”): mainly covalent modification/denaturation of the enzyme; cannot be recovered
- inhibitors can bind at the active site or at other sites in the enzyme (allosteric sites)

Reversible Inhibition (Expanded MM)



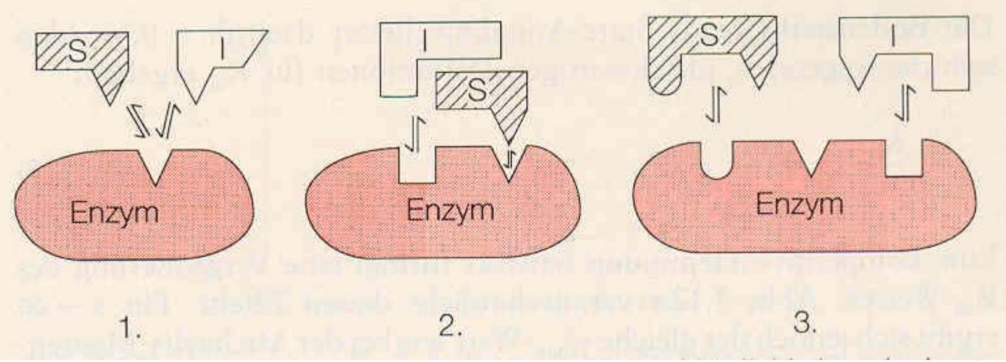
Competitive Inhibition

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

$$+$$
$$I$$
$$k_{-3} \updownarrow k_3$$
$$(EI) + S$$

"competitive contribution"

$$K_{ic} = \frac{k_{-3}}{k_3}$$



$$v = \frac{v_{max}[S]}{\alpha K_M + [S]}$$

$$\alpha = 1 + \frac{[I]}{K_I}$$

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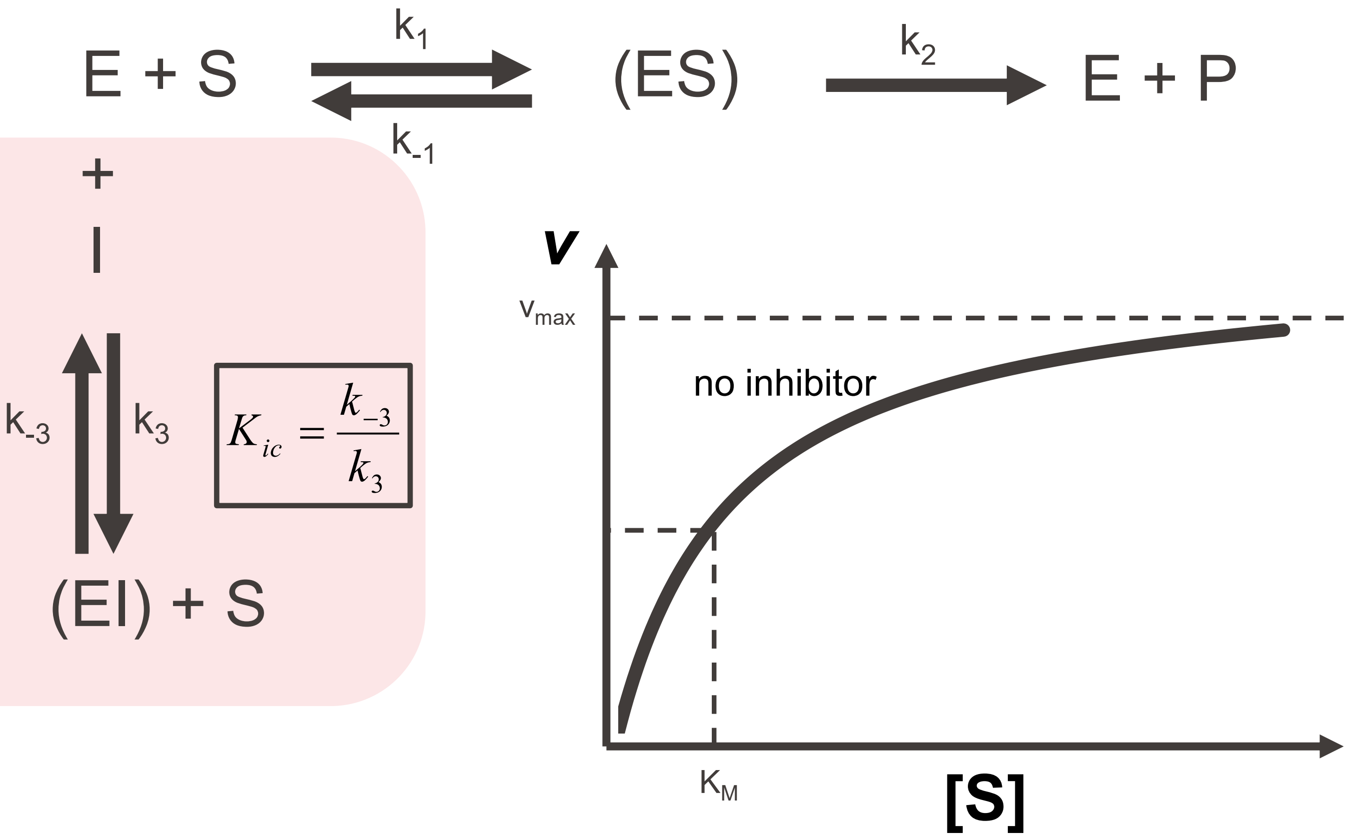
Competitive Inhibition



$$K_{ic} = \frac{k_{-3}}{k_3}$$

“competitive contribution”

Q: How does the curve for a competitive inhibitor look like? What happens to v_{max} and K_M?

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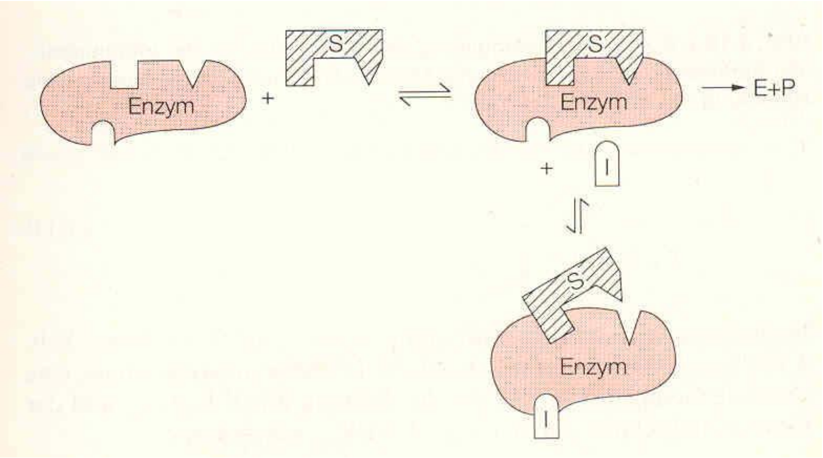
Uncompetitive Inhibition

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

$$(ES) + I \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} (EIS)$$



$$K_{iu} = \frac{k_{-5}}{k_5}$$

"uncompetitive contribution"

$$v = \frac{v_{\max}{}^{app} * [S]}{K_m{}^{app} + [S]}$$

$$v_{\max}{}^{app} = \frac{v_{\max}}{1 + \frac{[I]}{K_{iu}}} \qquad K_m{}^{app} = \frac{Km}{1 + \frac{[I]}{K_{iu}}}$$

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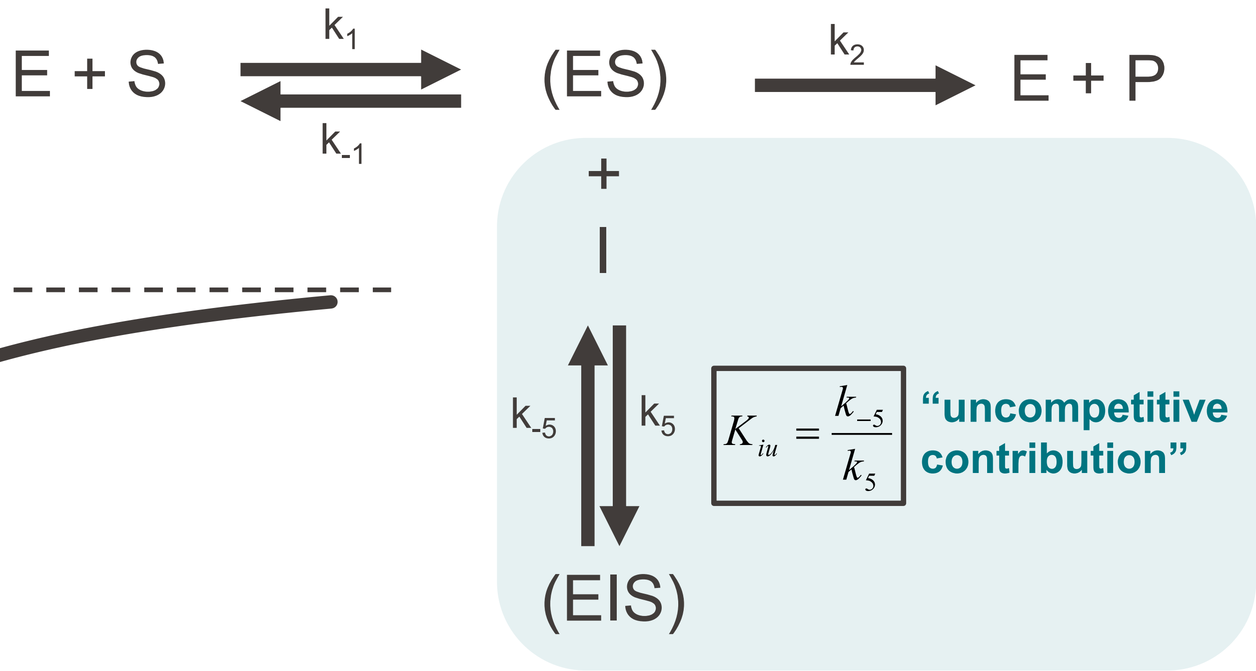
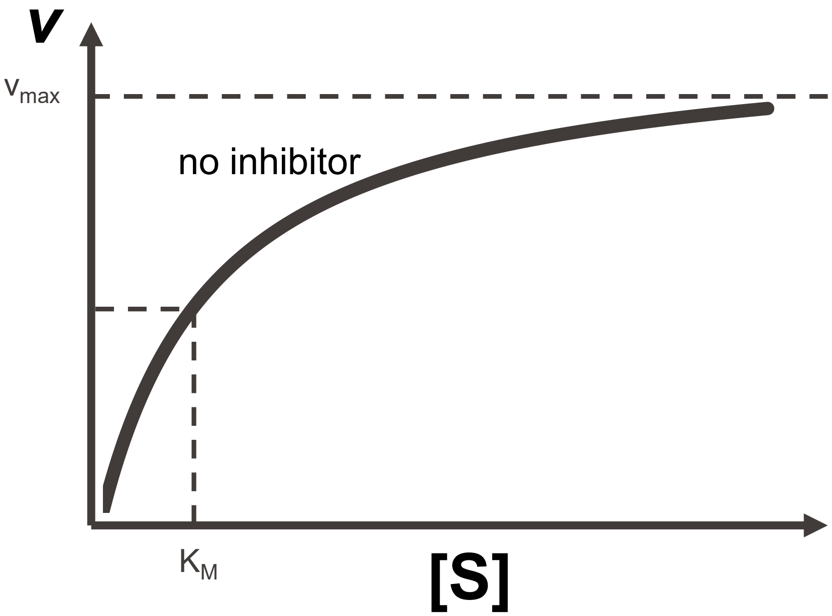
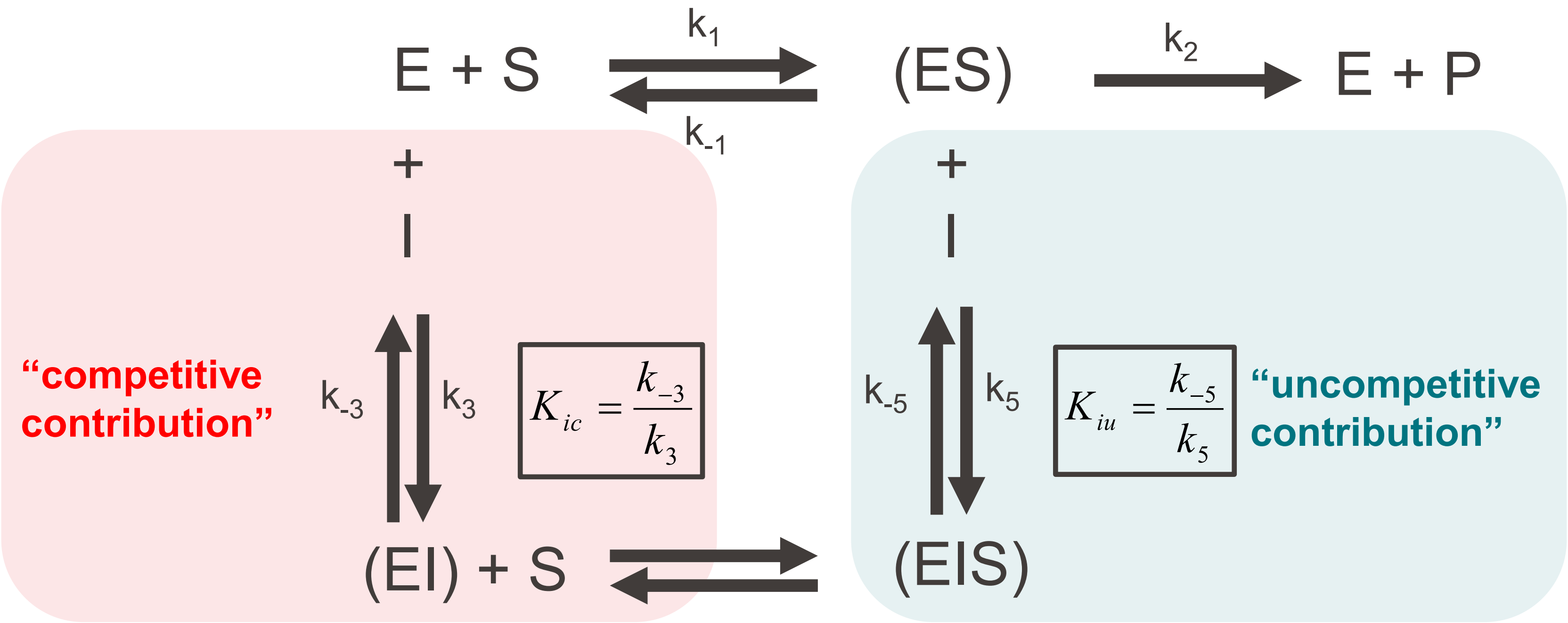
Uncompetitive Inhibition





Q: How does the curve for an uncompetitive inhibitor look like? What happens to v_{max} and K_M?

Mixed Inhibition (= comp. + uncomp.)



$$v = \frac{v_{\max}{}^{app} * [S]}{K_m{}^{app} + [S]}$$

$$v_{\max}{}^{app} = \frac{v_{\max}}{1 + \frac{[I]}{K_{iu}}}$$

$$K_m{}^{app} = \frac{K_m * \left(1 + \frac{[I]}{K_{ic}}\right)}{1 + \frac{[I]}{K_{iu}}}$$

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Inhibition – Special Cases

- **non-competitive inhibition**: special case of mixed inhibition, in which the inhibitor binds equally well to E and to (ES)

  → $K_{ic} = K_{iu}$ → $K_m^{app} = K_{M,\ (uninhibited)}$

- **substrate inhibition:** substrate acts as uncompetitive inhibitor binding at an allosteric site at high concentrations

- **product inhibition:** product remains in the active site and blocks access for the substrate → special case of competitive inhibition

Q: What could be the biological role of substrate/product inhibition?