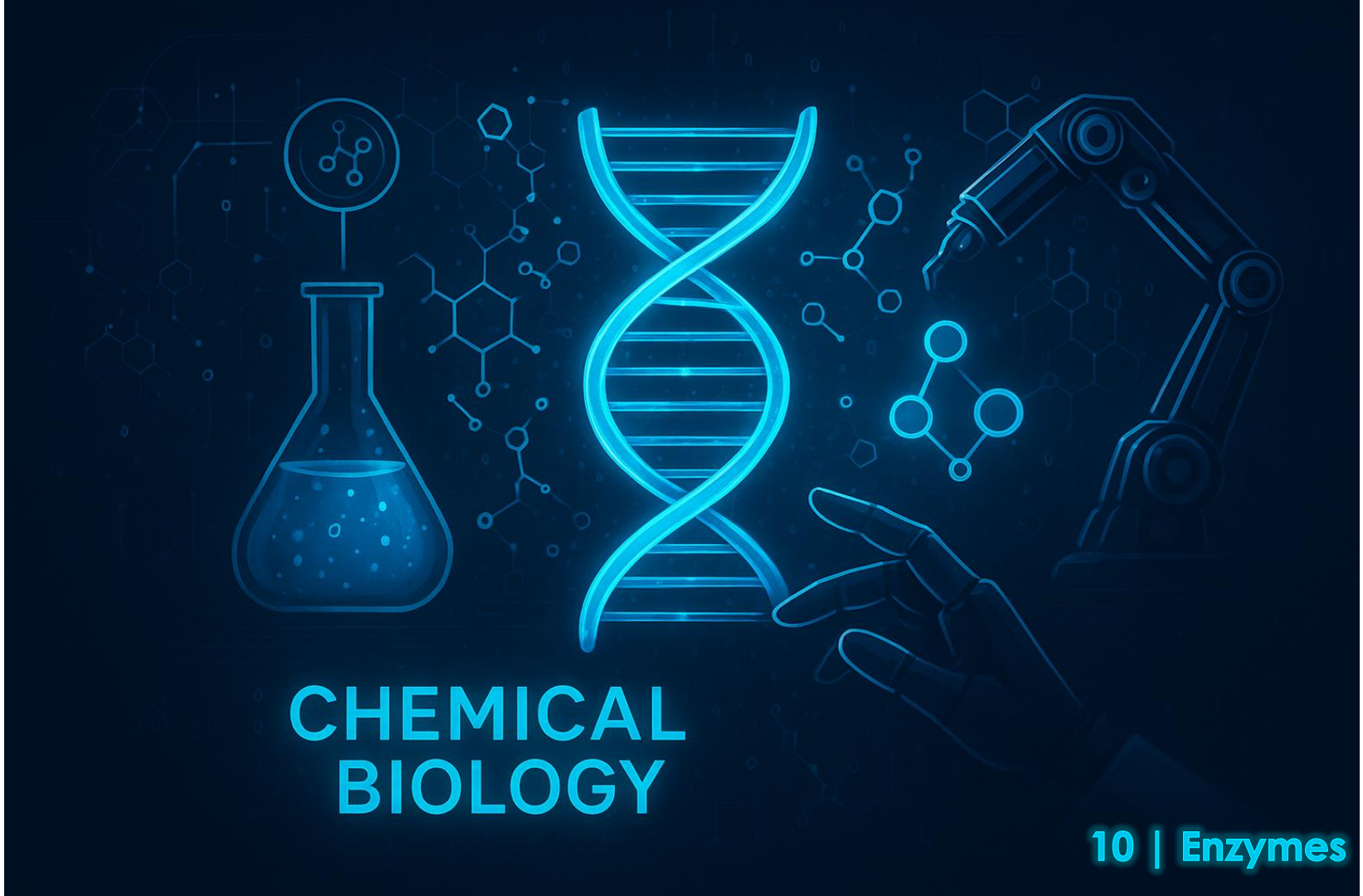

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General Course Information

- 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!**

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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]

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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)**
- **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!

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

Meeting ID: 689 0073 2223

[tentative schedule]

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Protein – Enzymes
(Kinetics)

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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**

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

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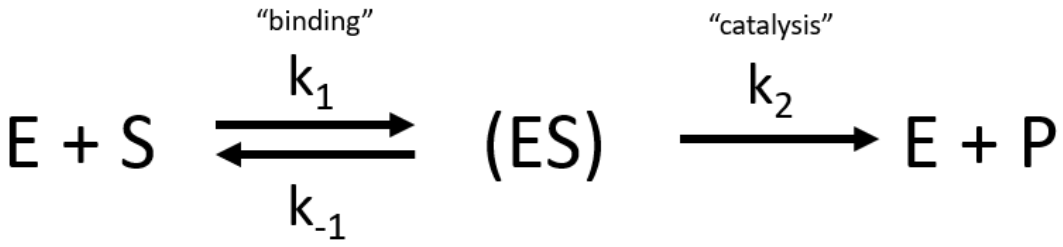
Michaelis-Menten Model



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?

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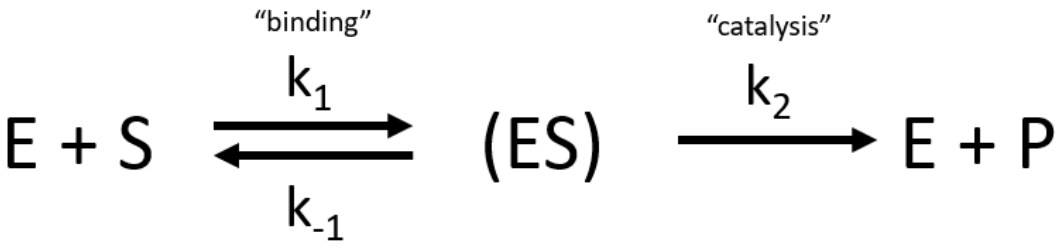
Michaelis-Menten (MM) Model

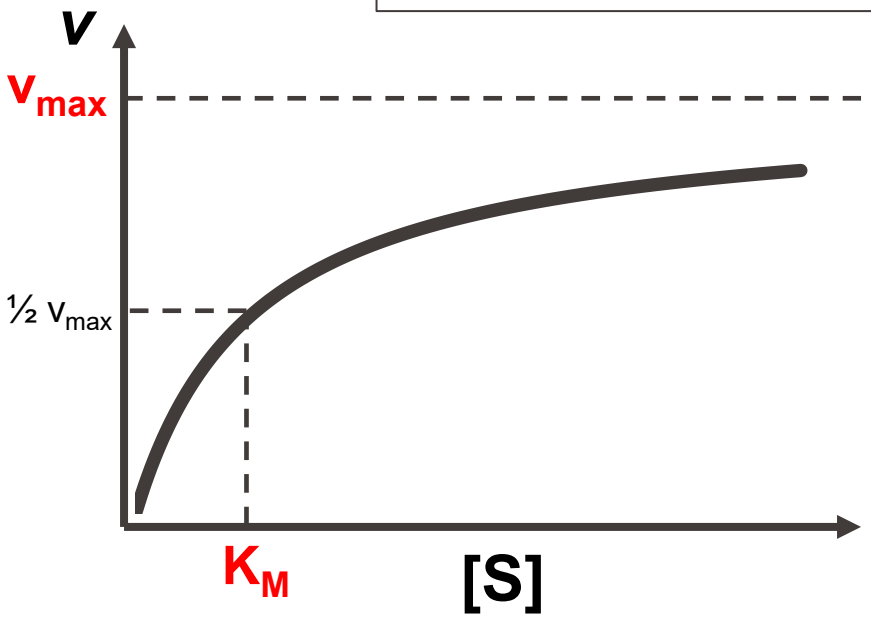


S: substrate
P: product
v: reaction rate
E: enzyme
(ES): enzyme-substrate complex
k_1: rate constant (ES) formation
k_{-1}: rate constant (ES) disintegration
k_2: rate constant P formation

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?

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Enzyme Kinetics – Key Parameters

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

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

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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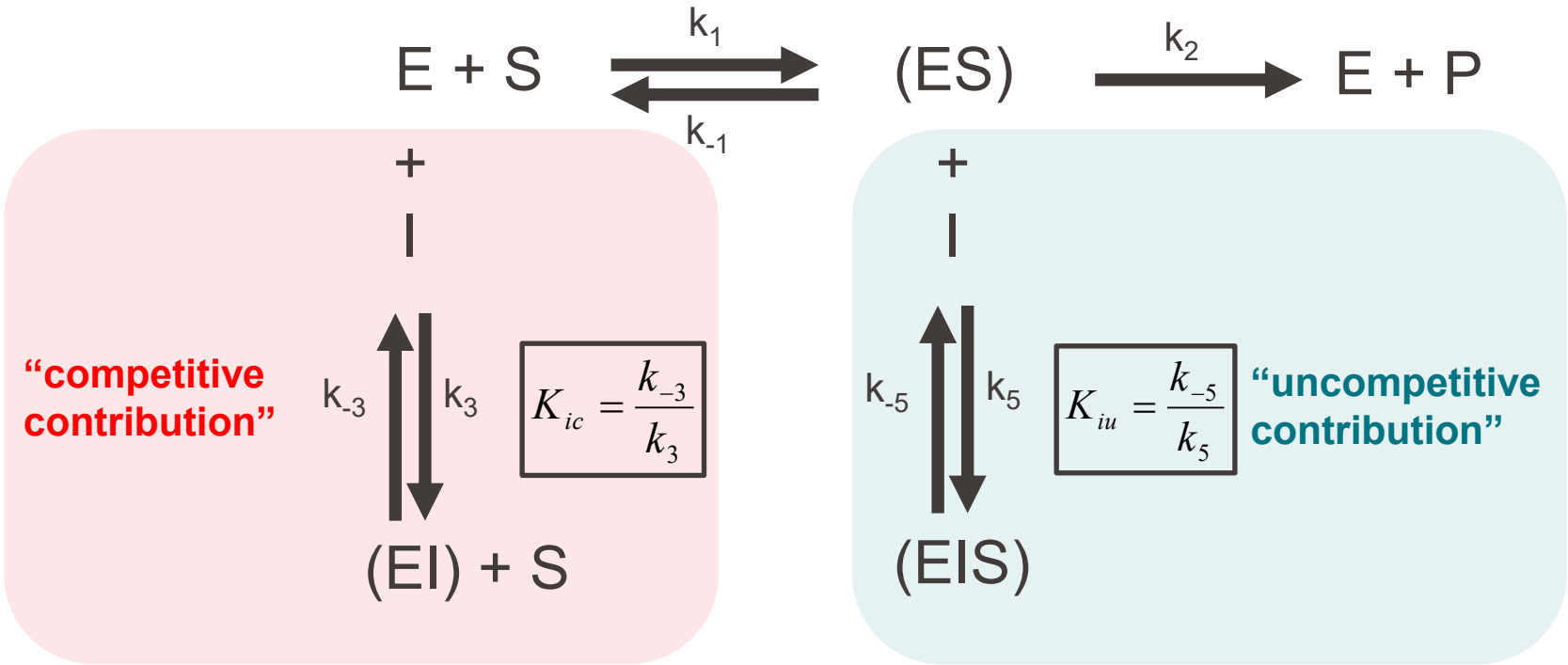
Enzyme Inhibition

- decrease in enzyme activity by inhibitor
- reversible and irreversible
  - reversible: can be recovered by exchange of medium
    - competitive
    - uncompetitive
    - mixed
  - irreversible (better “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)



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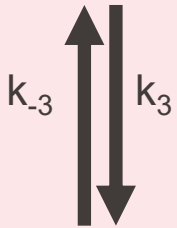
Competitive Inhibition

+

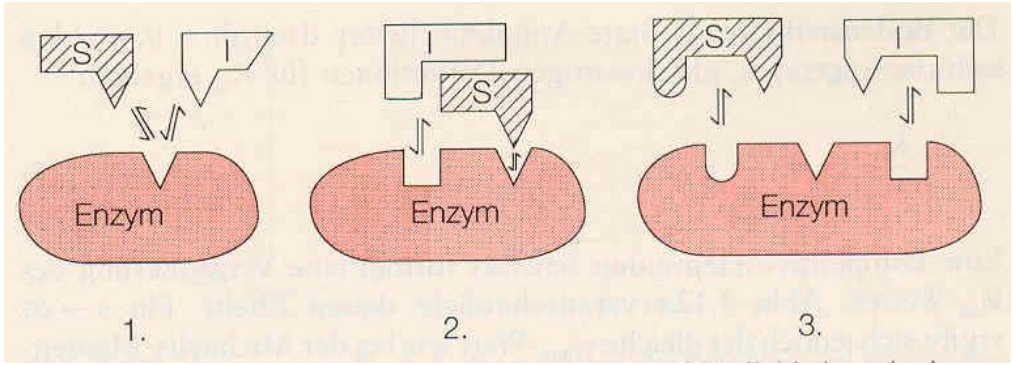
I

"competitive contribution"



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

(EI) + S



$$v = \frac{v_{max}[S]}{\alpha K_M + [S]} \qquad \alpha = 1 + \frac{[I]}{K_I}$$

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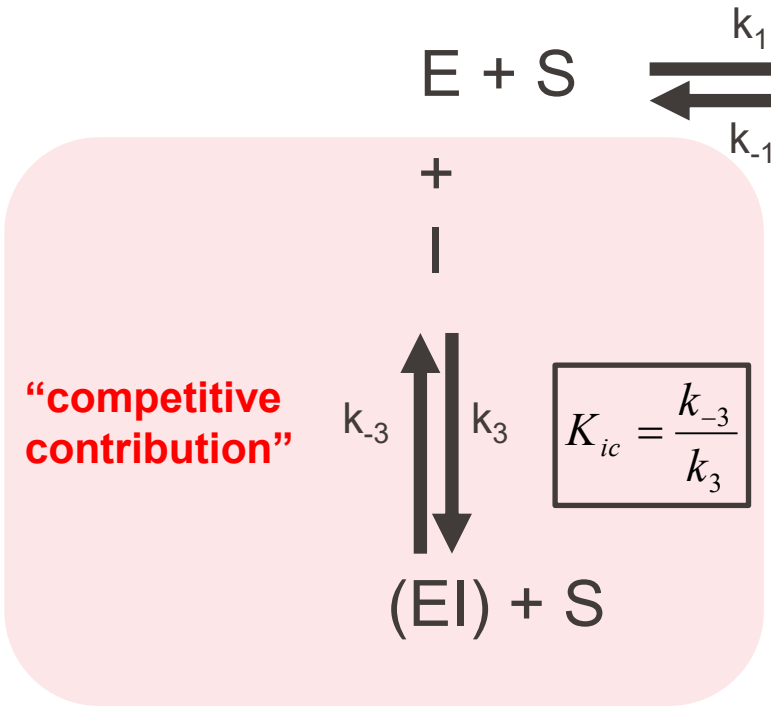
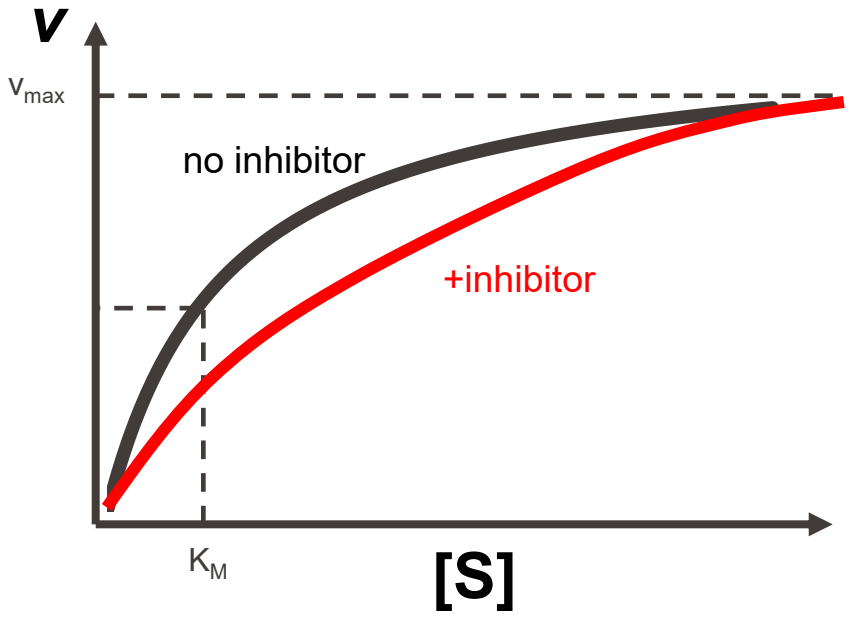
Competitive Inhibition





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

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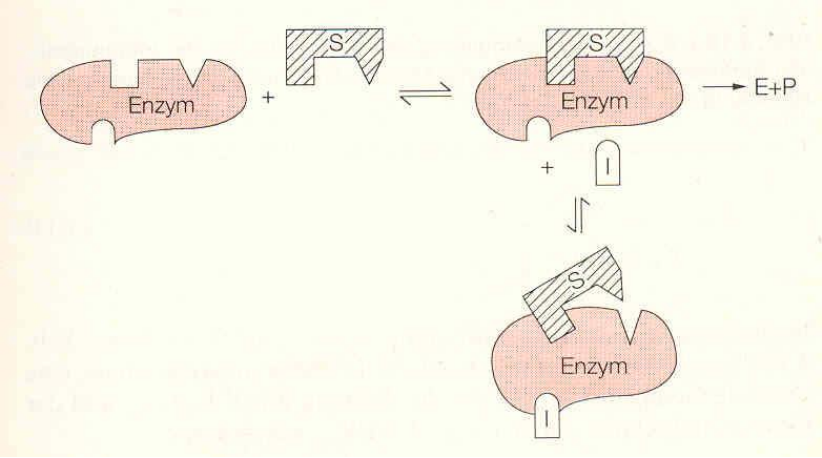
Uncompetitive Inhibition

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



$$+ I$$

$$k_{-5} \quad k_5$$

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

“uncompetitive contribution”

$$(EIS)$$

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

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

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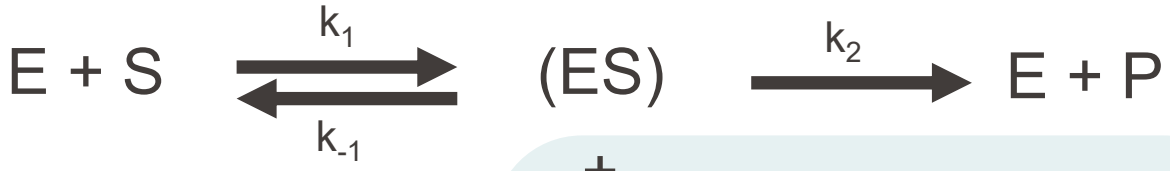
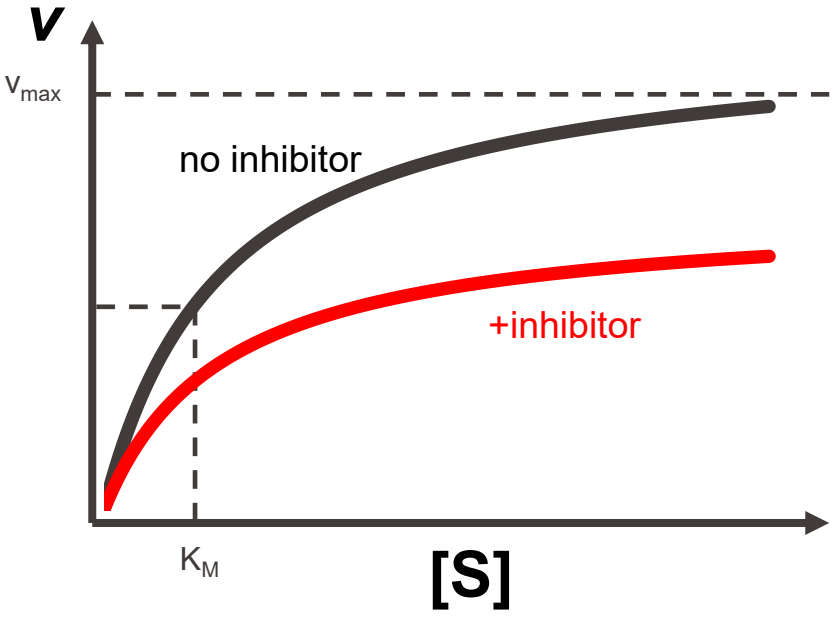
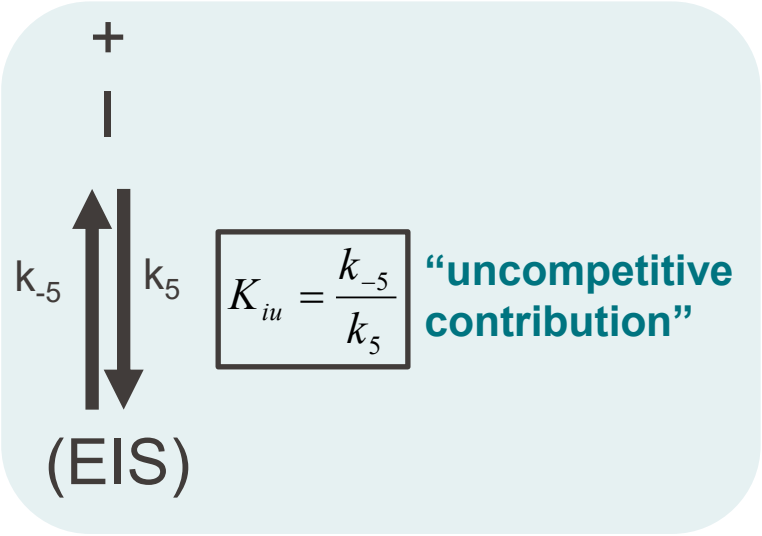
Uncompetitive Inhibition







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

“uncompetitive contribution”

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

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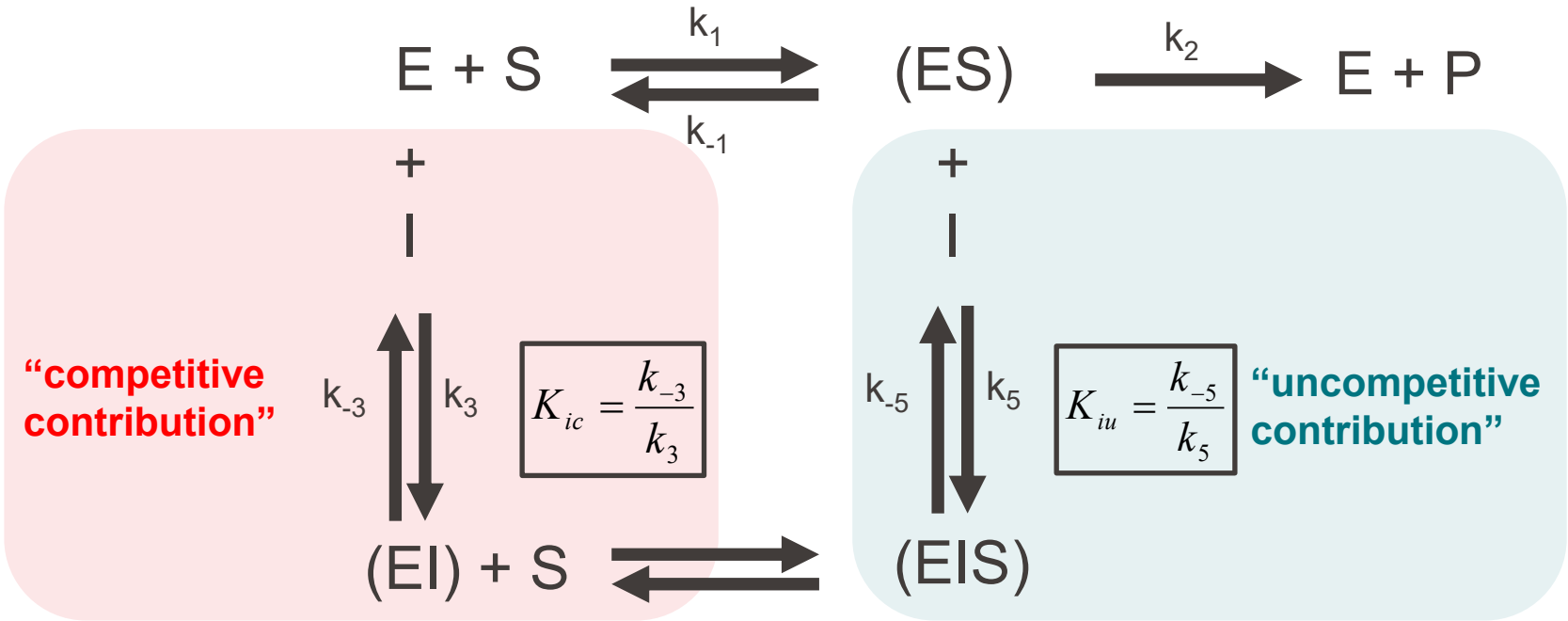
Mixed Inhibition (= comp. + uncomp.)



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

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

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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?

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Temperature Dependence

- Temperature influences both k_{cat} and K_M!
- assumption: influence on K_M is negligible (why?)
- effect on k_{cat} described by Arrhenius equation

$$v = \frac{k_{cat}(T)[E_0][S]}{K_M(T) + [S]}$$

Arrhenius equation:

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

- But: enzyme inactivation at high T!

$$E_{act} \xrightarrow{k_d} E_{inact}$$

$$\ln k_{cat} = \ln A - \frac{\Delta G^{\ddagger}}{R} \cdot \frac{1}{T}$$

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

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Kinetics of Enzyme Inactivation

- assumption: 1st order kinetics

$$E_{act} \xrightarrow{k_d} E_{inact}$$

$$\frac{d[E_{act}]}{dt} = k_d \cdot [E_{act}]$$

$$[E_{act}] = [E_{act}]_0 \cdot e^{-k_d \cdot t}$$

Arrhenius:

$$\ln k_d = \ln A_d - \frac{\Delta G^{\ddagger}{}_d}{R} \cdot \frac{1}{T}$$

$$t_{1/2} =$$

Q: Derive the formula for the enzyme half life $t_{1/2}$!

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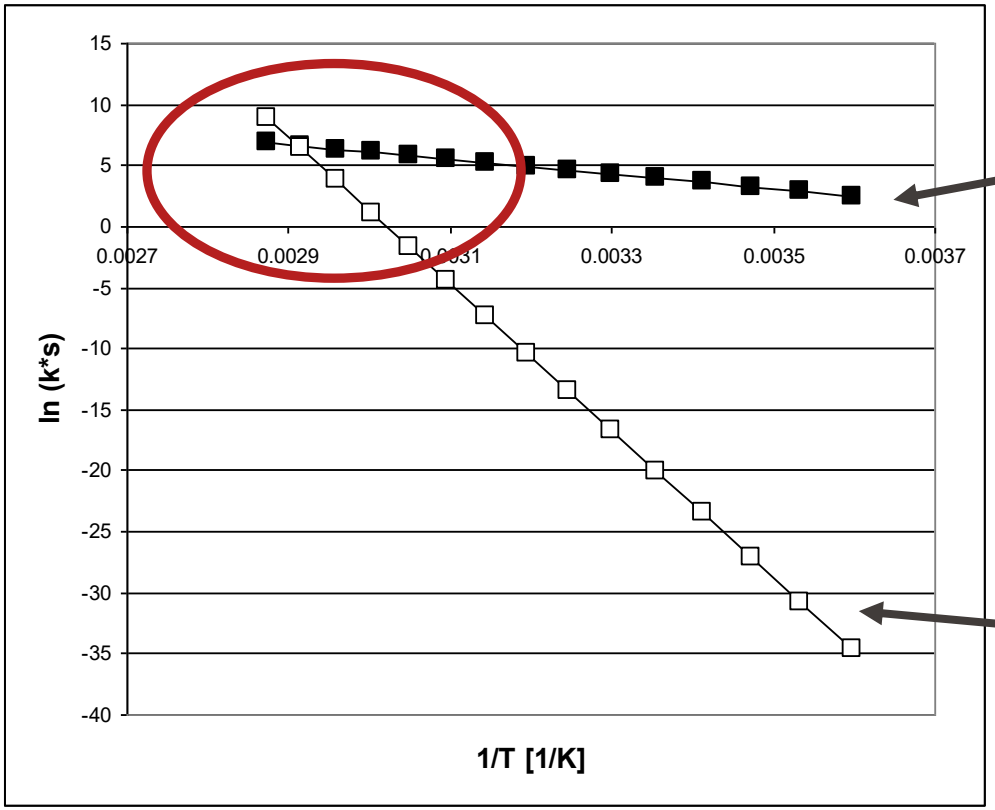
Temperature Dependence



Typical $\Delta G^{\ddagger}$:

- **enzymatic reaction: ~10-100 kJ/mol**
- **thermal enzyme inactivation: ~200-500 kJ/mol**

"reaction":

$$\ln k_{cat} = \ln A - \frac{\Delta G^{\ddagger}}{R} \cdot \frac{1}{T}$$

→ There has to be a T_{opt}!

"inactivation":

$$\ln k_d = \ln A_d - \frac{\Delta G^{\ddagger}{}_d}{R} \cdot \frac{1}{T}$$

Q: Name reasons other than T for enzyme inactivation!

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