

# *E2 - Proteine*



4. Tag: Enzymkinetik - Bestimmung und Datenbanken

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# Reaktionsordnung



Reaktion 0. Ordnung: z.B. konst. Zerfall



Reaktion 1. Ordnung: z.B. radioaktiver Zerfall



Reaktion 2. Ordnung: z.B. zwei Reaktanten reagieren miteinander



# Einfache Reaktionskinetik I

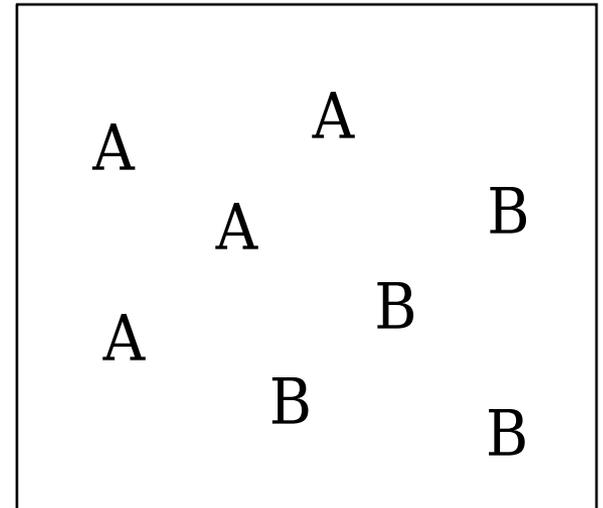
Einfachster Fall:



Reaktionsgeschwindigkeit:

$$d[A]/dt = dA/dt = A' = -k^*[A] = -k^*A$$

$$d[B]/dt = dB/dt = B' = k^*[A] = k^*A$$



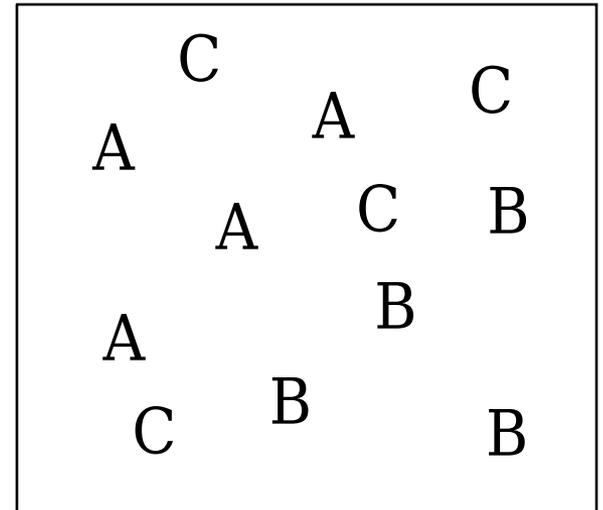
# Einfache Reaktionskinetik II

Zwei Reaktanten:



Reaktionsgeschwindigkeit:

$$d[A]/dt = dA/dt = A' = -k*[A]*[B] = -k*A*B$$
$$d[B]/dt = dB/dt = B' = -k*[A]*[B] = -k*A*B$$



# Einfache Reaktionskinetik III

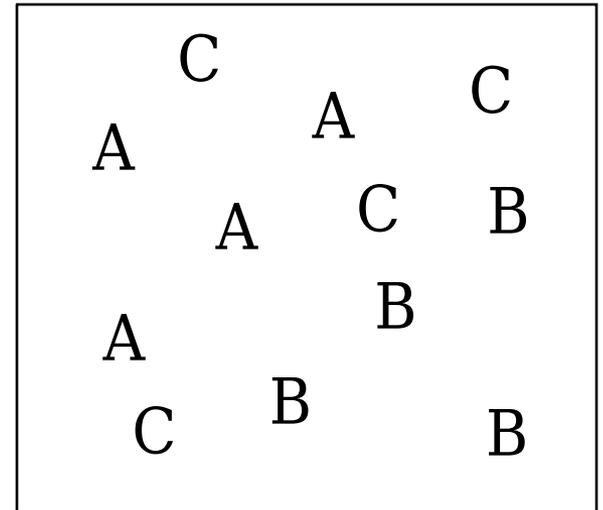
Drei Reaktanten:



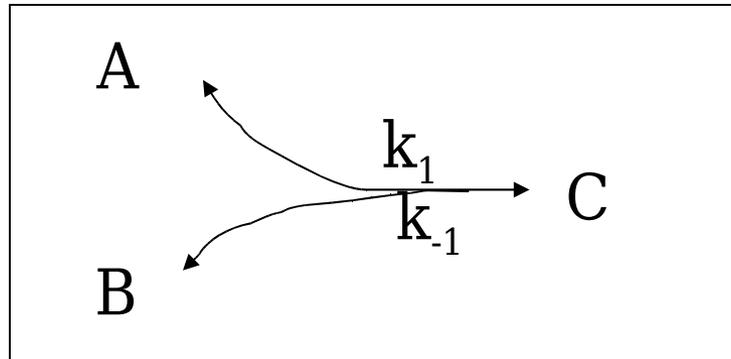
Reaktionsgeschwindigkeit:

$$d[A]/dt = dA/dt = A' = -k*[A]*[B]^2 = -k*A*B^2$$

$$d[B]/dt = dB/dt = B' = -k*[A]*[B]^2 = -k*A*B^2$$



# Reaktionskinetik - Reversibilität



Reversible Reaktion

$$K = \frac{[C]}{[A] * [B]}$$

Gleichgewichtskonstante

$$\frac{d[A]}{dt} = -k_1 * [A] * [B] + k_{-1} * [C]$$

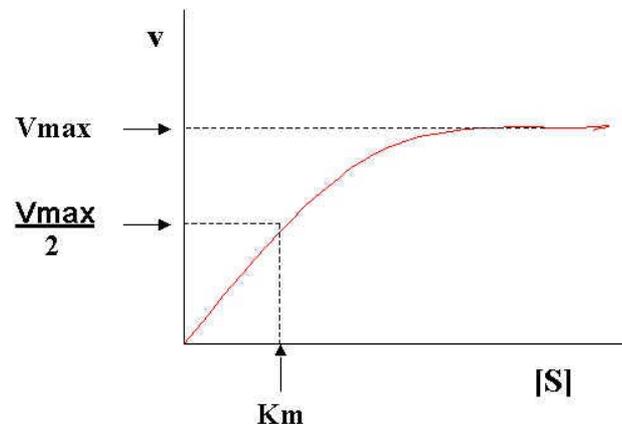
$$\frac{d[B]}{dt} = -k_1 * [A] * [B] + k_{-1} * [C]$$

Systemgleichungen

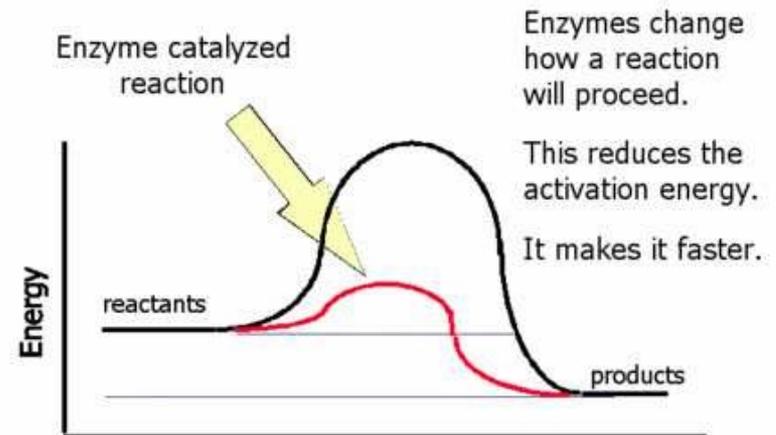
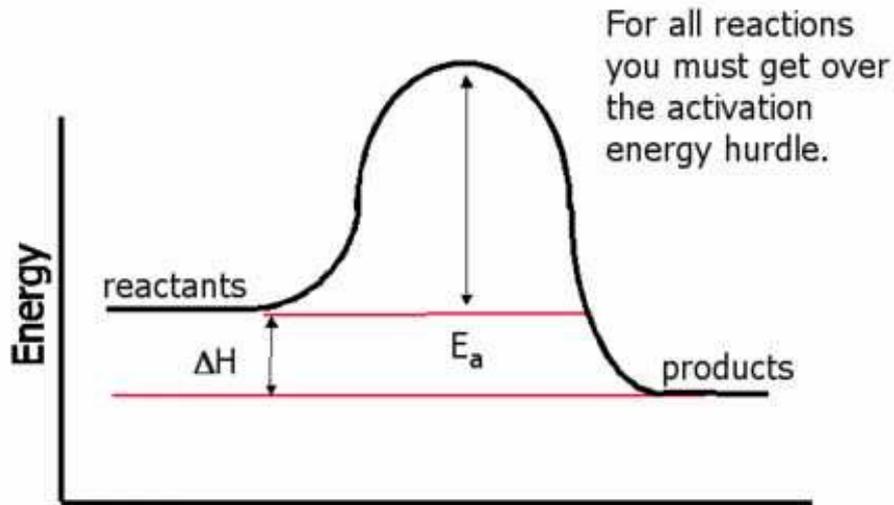
$$\frac{d[C]}{dt} = k_1 * [A] * [B] - k_{-1} * [C]$$

# Enzyme, die Biokatalysatoren

- Die Geschwindigkeit biochemischer Reaktionen folgt meist einer Sättigungskurve.
- Wenn alle Enzyme belegt sind, kann die Geschwindigkeit nicht mehr zunehmen, auch wenn mehr Substrat dazukommt.
- Die Substratkonzentration bei halbmaximaler Geschwindigkeit entspricht  $K_m$ .



# Effekt von Enzymen auf Aktivierungsenergie



# Michaelis-Menten Equation



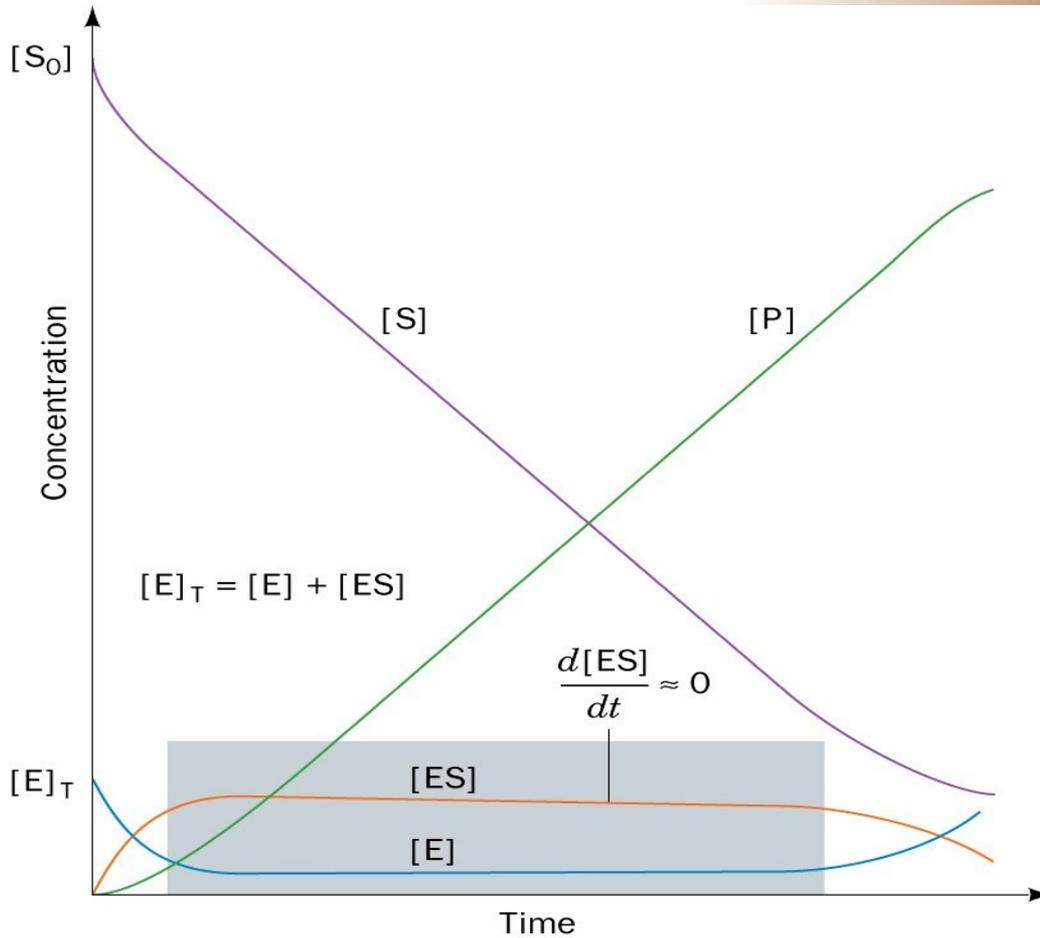
Reaktionsgeschwindigkeit

$$v = \frac{d[\text{P}]}{dt} = k_2[\text{ES}]$$

Gleichung für ES

$$\frac{d[\text{ES}]}{dt} = k_1[\text{E}][\text{S}] - k_{-1}[\text{ES}] - k_2[\text{ES}]$$

# Komponenten in einer simplen Michaelis-Menten-Reaktion



# Michaelis-Menten

- Annahme des schnellen GG

$$K_S = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

- Annahme des Steady States

$$\frac{d[ES]}{dt} = 0$$


$$[E]_T = [E] + [ES]$$

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$\frac{([E]_T - [ES])[S]}{[ES]} = \frac{k_{-1} + k_2}{k_1}$$

→

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

A decorative graphic consisting of a horizontal arrow pointing to the right. The arrow has a black shaft on the left and a multi-colored, gradient tip on the right, transitioning from dark brown to light tan.
$$K_M[\text{ES}] = ([\text{E}]_T - [\text{ES}])[S]$$

$$[\text{ES}] = \frac{[\text{E}]_T[S]}{K_M + [S]}$$

# Michaelis-Menten

- Maximalgeschwindigkeit
- Michaelis-Menten

$$V_{\max} = k_2[E]_T$$

$$v_o = \frac{V_{\max}[S]}{K_M + [S]}$$

$K_M$

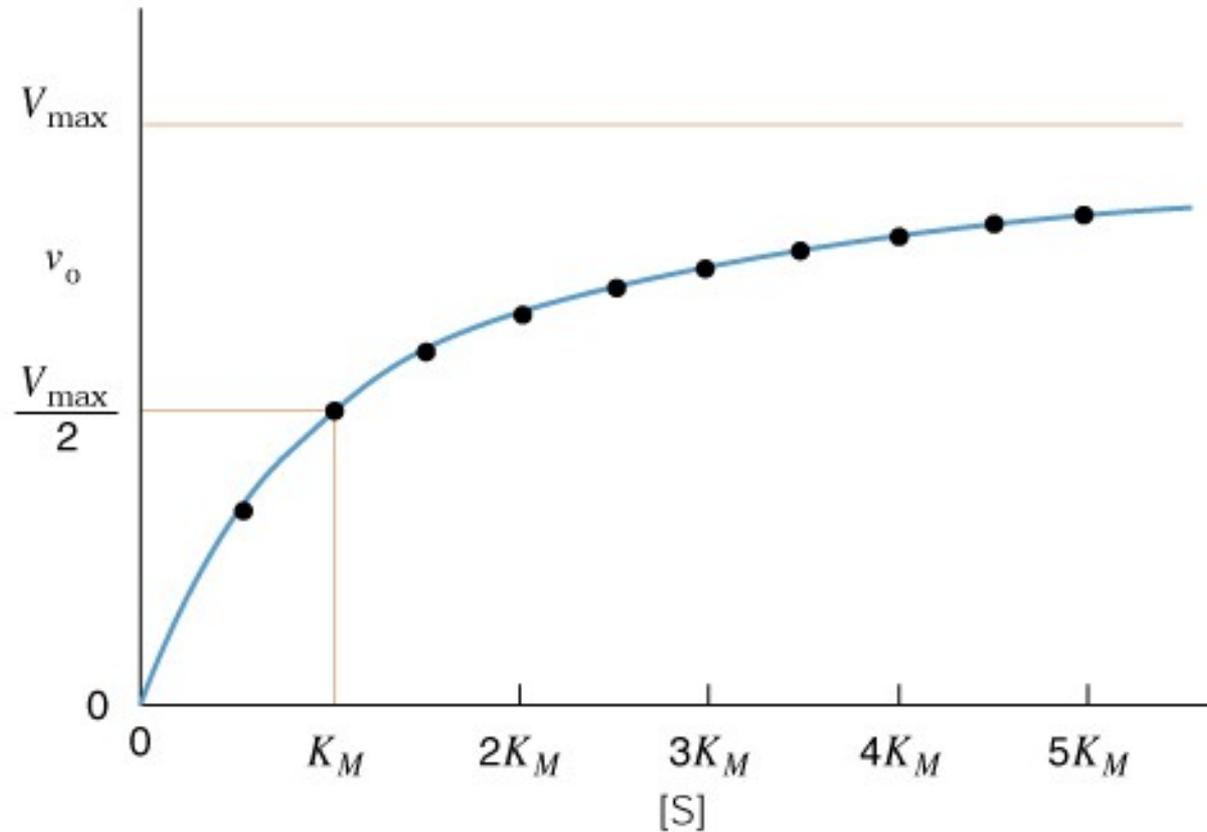


Figure 12-3. Key to Function. A plot of the initial velocity  $v_0$  of a simple enzymatic reaction versus the substrate concentration  $[S]$ .

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$k_{cat}/K_M$  als Maß für die Effizienz (turnover number)



$$k_{cat} = \frac{V_{max}}{[E]_T}$$

# Beispiele

Enzyme	Substrate	$K_M (M)$	$k_{cat} (s^{-1})$	$k_{cat}/K_M (M^{-1} \cdot s^{-1})$
Acetylcholinesterase	Acetylcholine	$9.5 \times 10^{-5}$	$1.4 \times 10^4$	$1.5 \times 10^8$
Carbonic anhydrase	CO <sub>2</sub>	$1.2 \times 10^{-2}$	$1.0 \times 10^6$	$8.3 \times 10^7$
	HCO <sub>3</sub>	$2.6 \times 10^{-2}$	$4.0 \times 10^5$	$1.5 \times 10^7$
Catalase	H <sub>2</sub> O <sub>2</sub>	$2.5 \times 10^{-2}$	$1.0 \times 10^7$	$4.0 \times 10^8$
Chymotrypsin	<i>N</i> -Acetylglycine ethyl ester	$4.4 \times 10^{-1}$	$5.1 \times 10^{-2}$	$1.2 \times 10^{-1}$
	<i>N</i> -Acetylvaline ethyl ester	$8.8 \times 10^{-2}$	$1.7 \times 10^{-1}$	1.9
	<i>N</i> -Acetyltyrosine ethyl ester	$6.6 \times 10^{-4}$	$1.9 \times 10^2$	$2.9 \times 10^5$
Fumarase	Fumarate	$5.0 \times 10^{-6}$	$8.0 \times 10^2$	$1.6 \times 10^8$
	Malate	$2.5 \times 10^{-5}$	$9.0 \times 10^2$	$3.6 \times 10^7$
Superoxide dismutase	Superoxide ion (O <sub>2</sub> <sup>-</sup> )	$3.6 \times 10^{-4}$	$1.0 \times 10^6$	$2.8 \times 10^9$
Urease	Urea	$2.5 \times 10^{-2}$	$1.0 \times 10^4$	$4.0 \times 10^5$

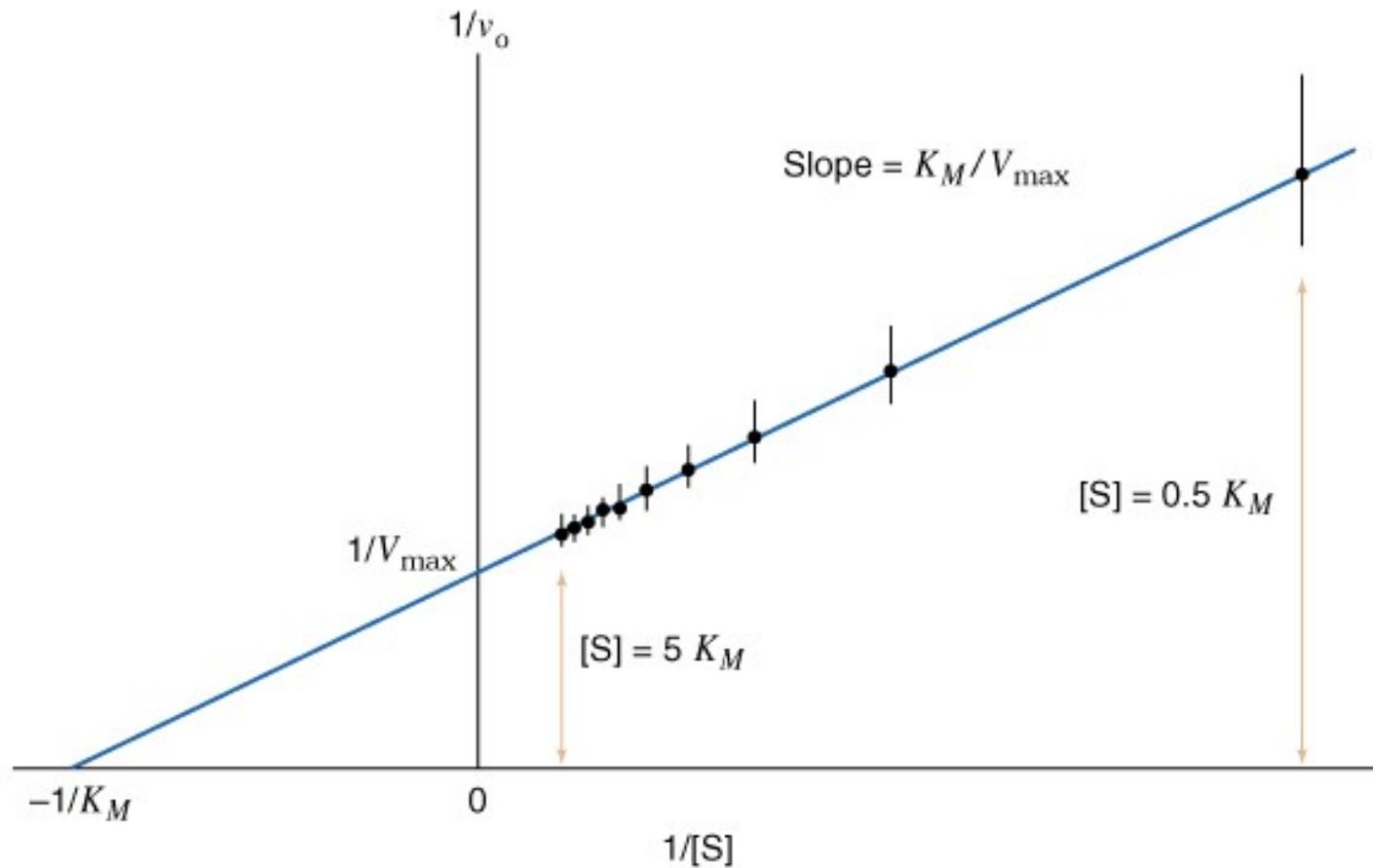
# Lineweaver-Burk

$$V = V_{max} \frac{[S]}{K_m + [S]}$$

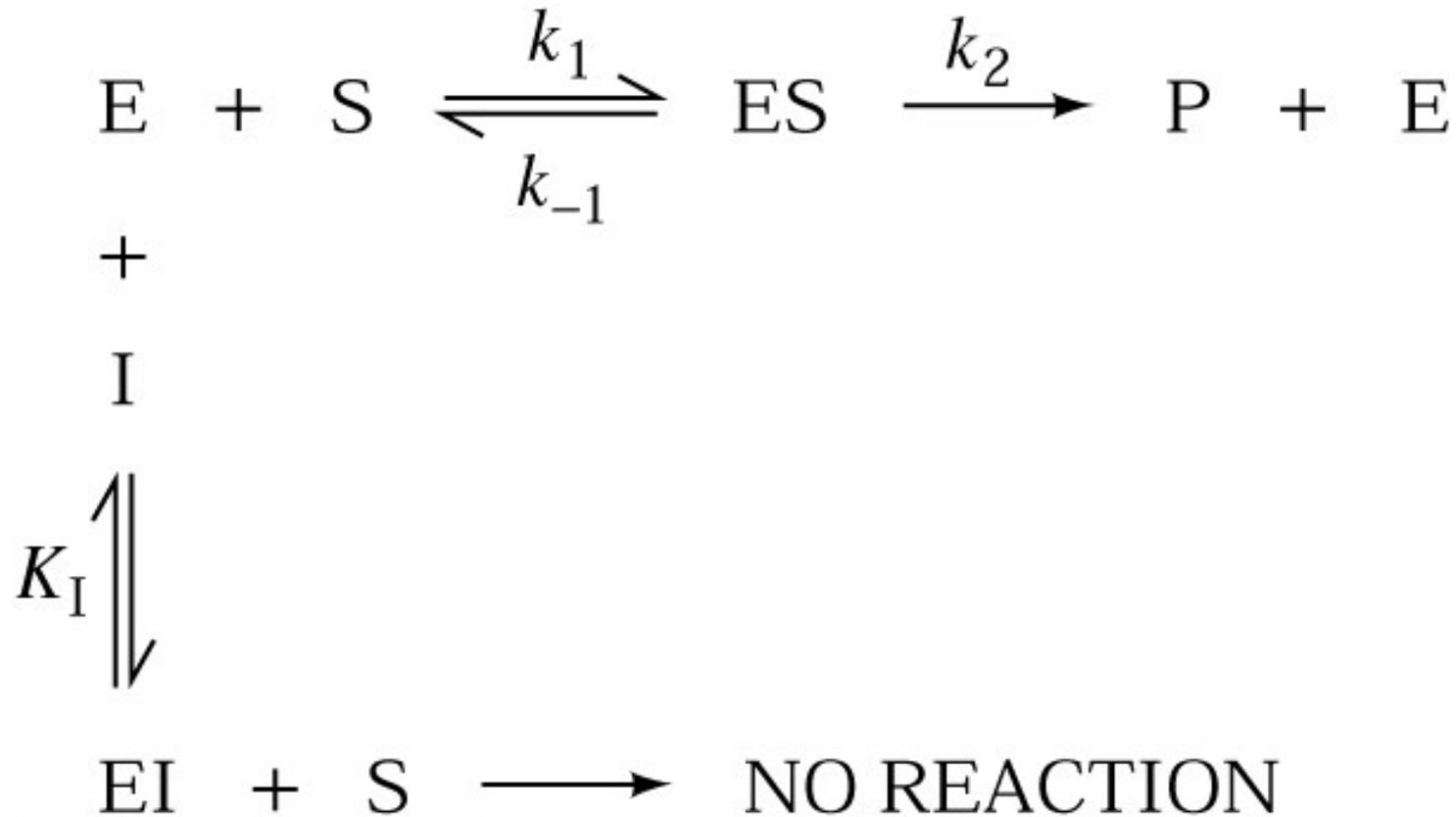
-> reziprok:

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max} [S]} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

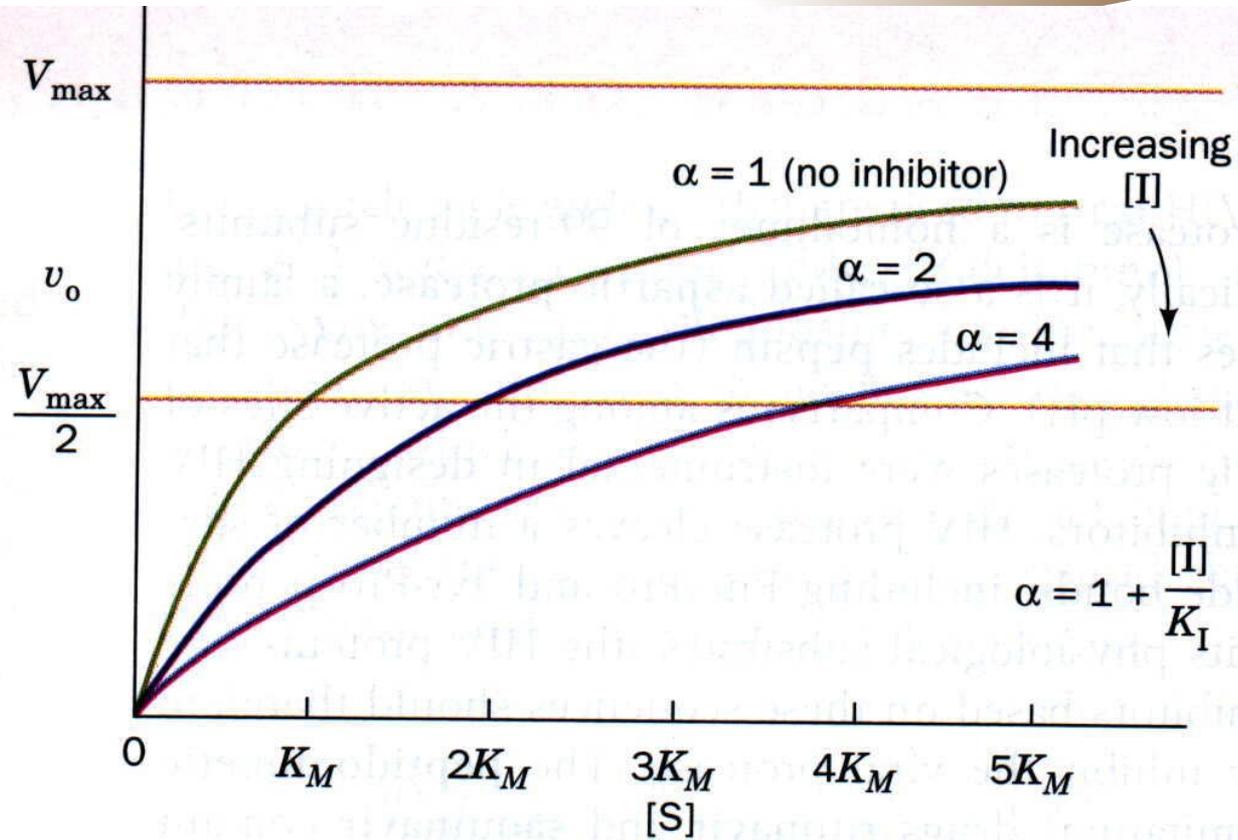
# Lineweaver-Burk



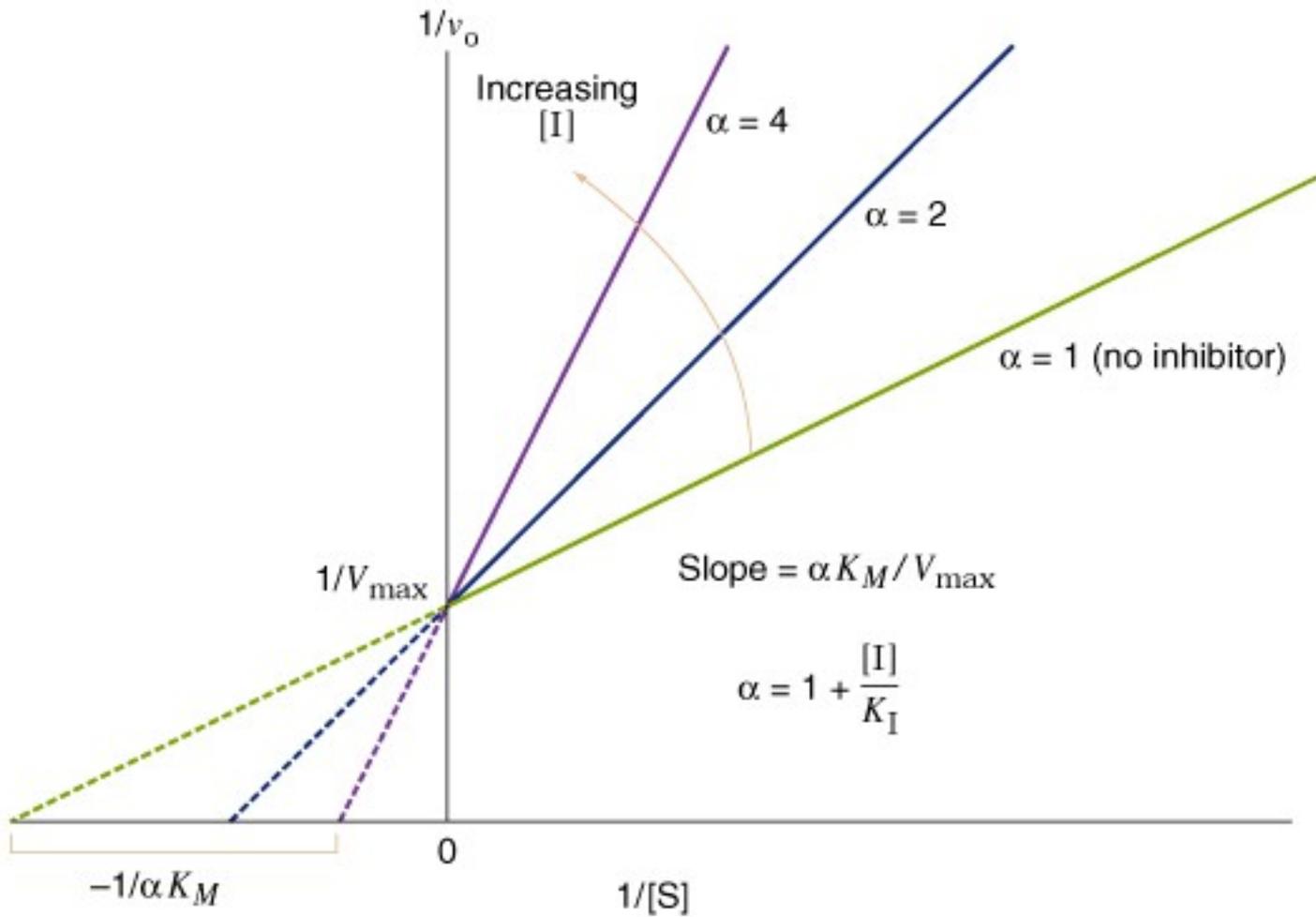
# Kompetitive Hemmung



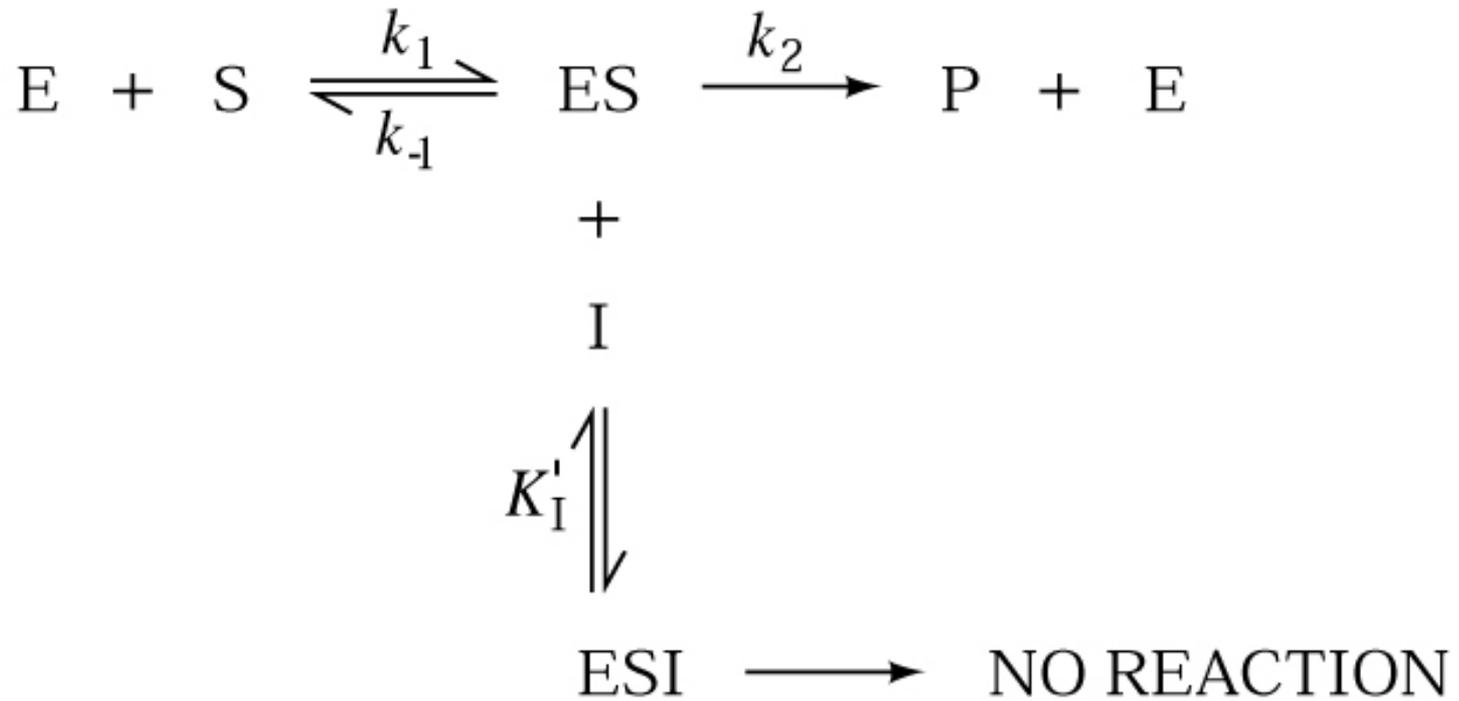
# Kompetitive Hemmung

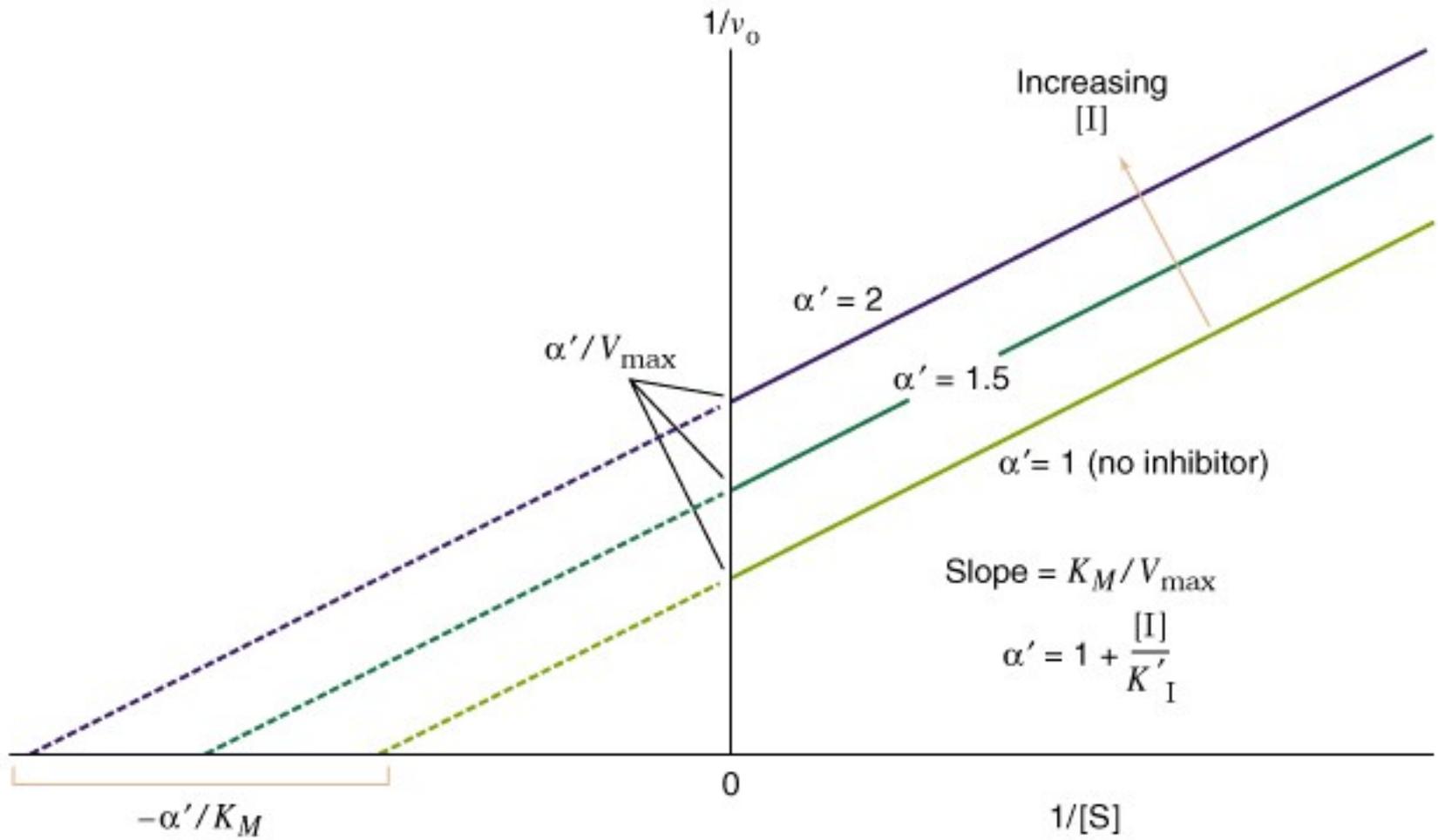


**Figure 12-6.** A plot of  $v_0$  versus  $[S]$  for a Michaelis–Menten reaction in the presence of different concentrations of a competitive inhibitor.

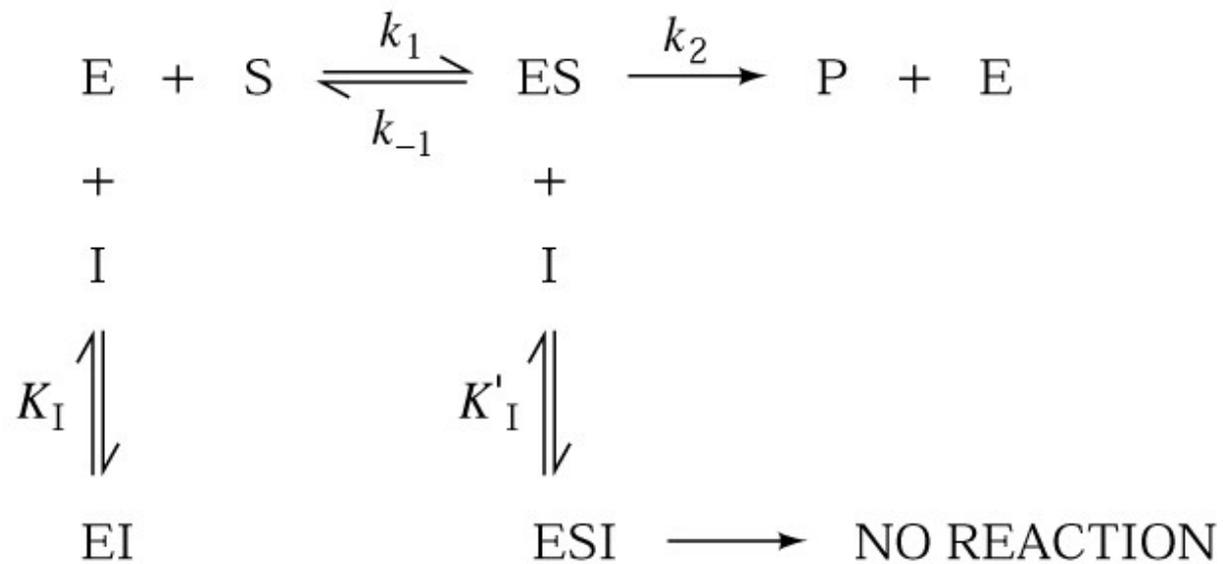


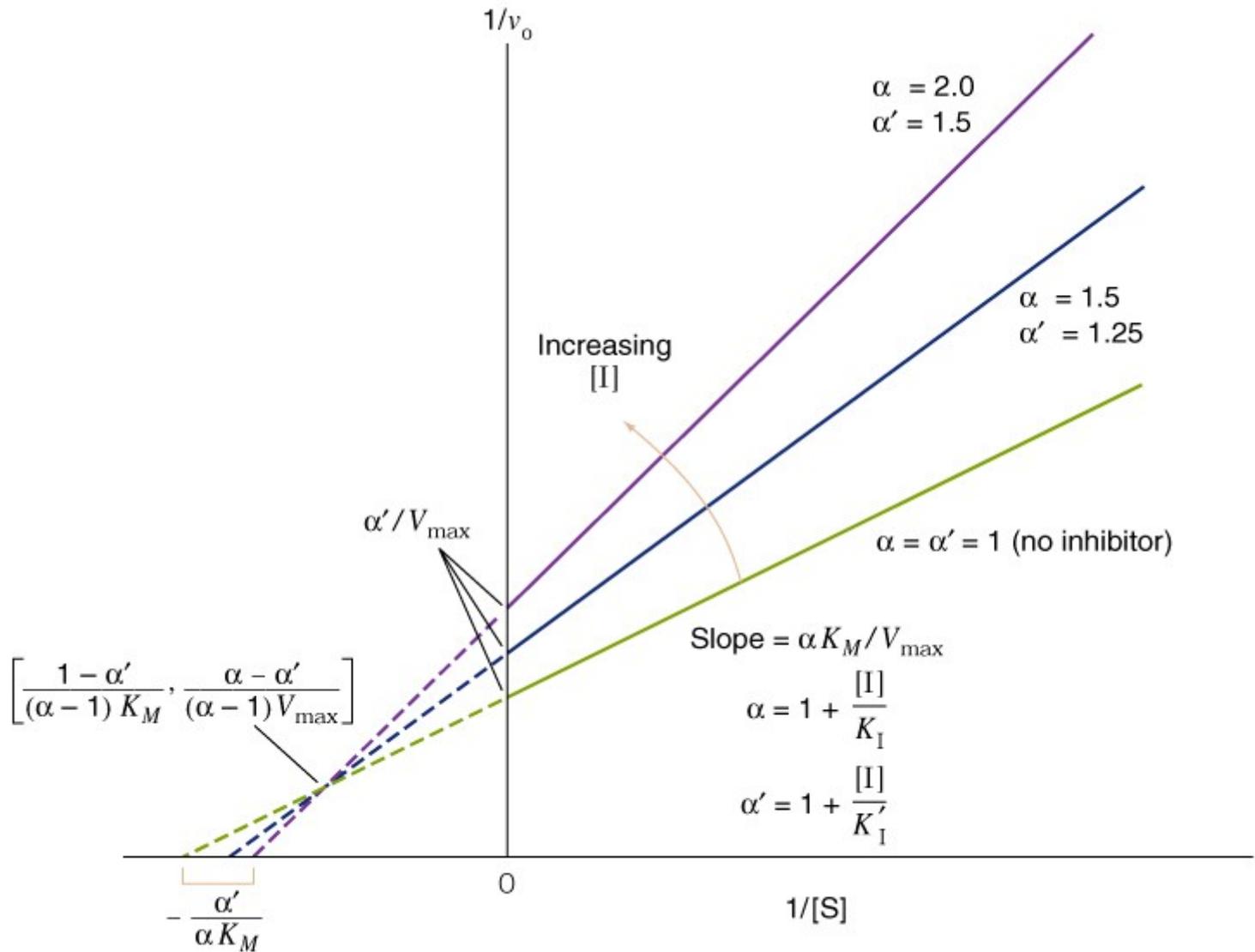
# Unkompetitive Hemmung





# Mixed



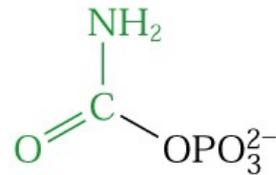


# Effekte von Inhibitoren auf Parameter der Michaelis–Menten-Gleichung<sup>a</sup>

Type of Inhibition	$V_{\max}^{\text{app}}$	$K_M^{\text{app}}$
None	$V_{\max}$	$K_M$
Competitive	$V_{\max}$	$\alpha K_M$
Uncompetitive	$V_{\max}/\alpha'$	$K_M/\alpha'$
Mixed	$V_{\max}/\alpha'$	$\alpha K_M/\alpha'$

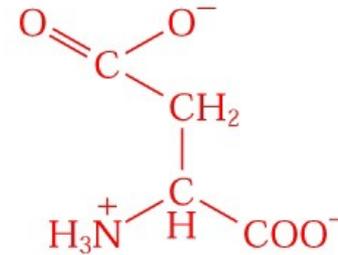
$$^a\alpha = 1 + \frac{[I]}{K_I} \text{ and } \alpha' = 1 + \frac{[I]}{K_I'}$$

# Beispiel Regulation: Aspartate transcarbamylase



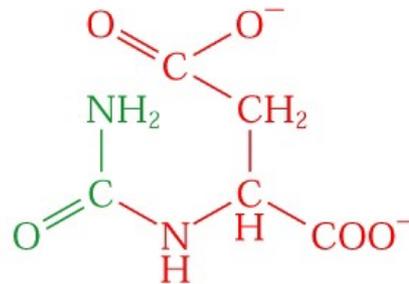
**Carbamoyl phosphate**

+



**Aspartate**

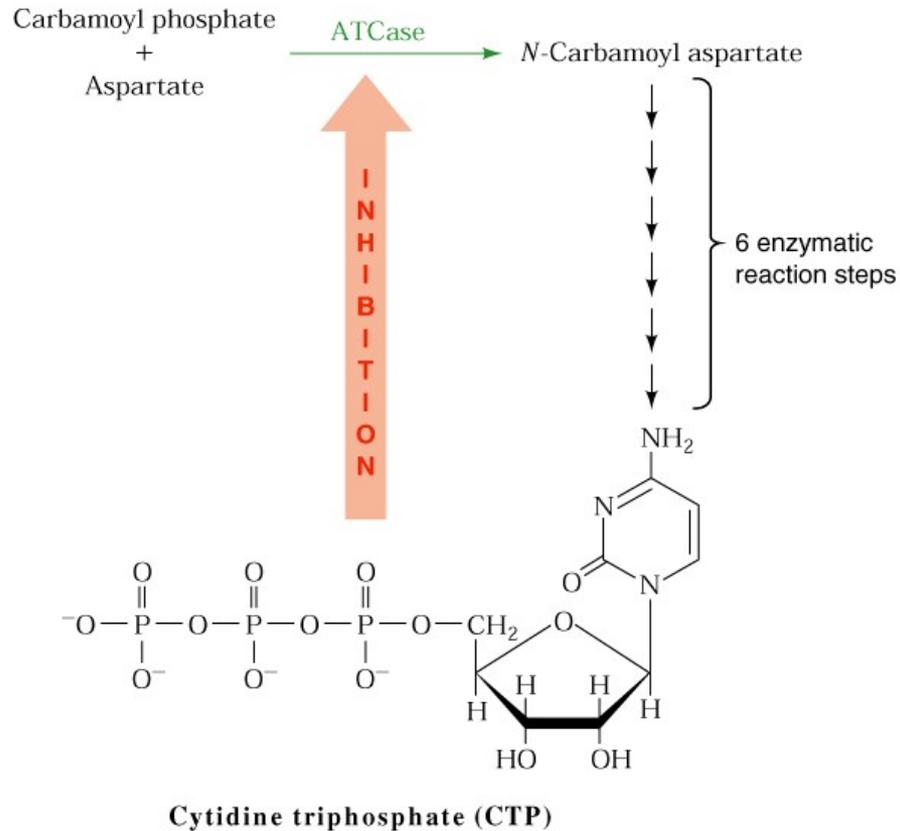
↓ aspartate  
transcarbamoylase



**N-Carbamoyl aspartate**

+ H<sub>2</sub>PO<sub>4</sub><sup>-</sup>

# Feedback Inhibition



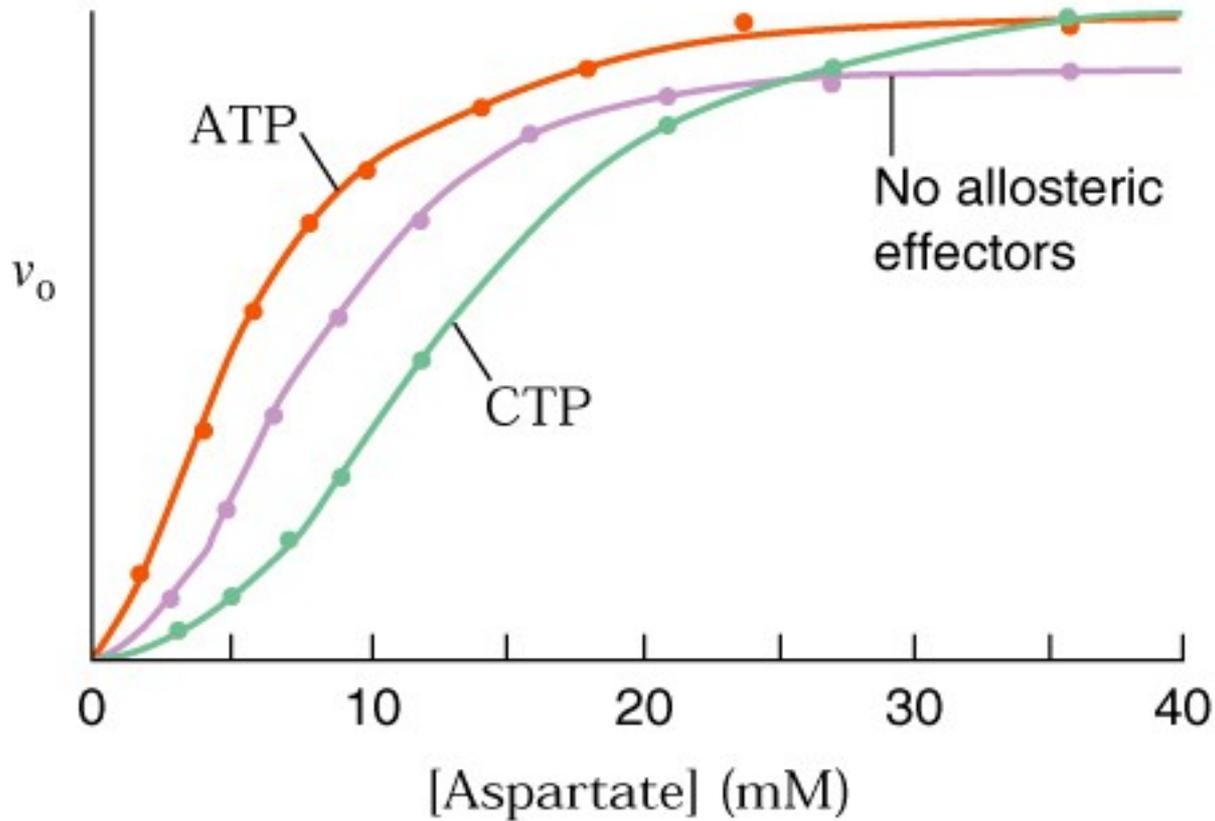
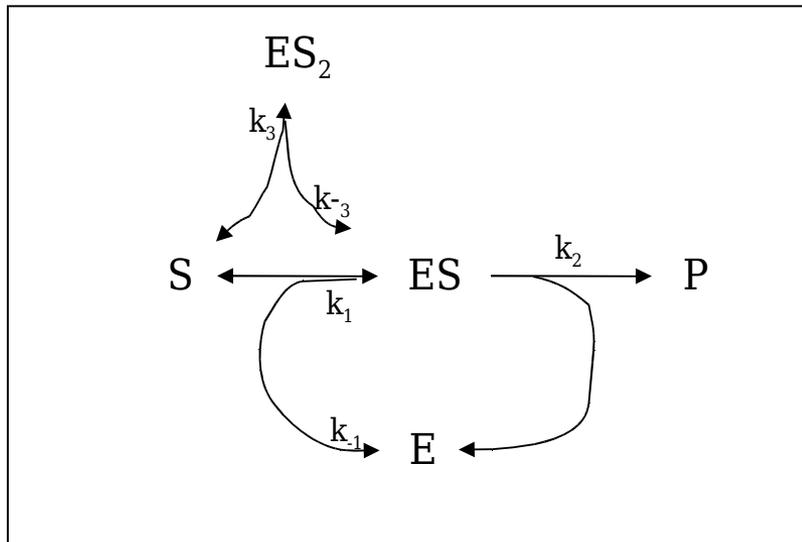


Figure 12-10. Plot of  $v_0$  versus [Aspartate] for the ATCase reaction. [After Kantrowitz, E.R., Pastra-Landis, S.C., and Lipscomb, W.N., *Trends Biochem. Sci.* 5, 125 (1980).]

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# Substratinhibition

Katalyse mit Substratinhibition:



Analoge Vorgehensweise liefert:

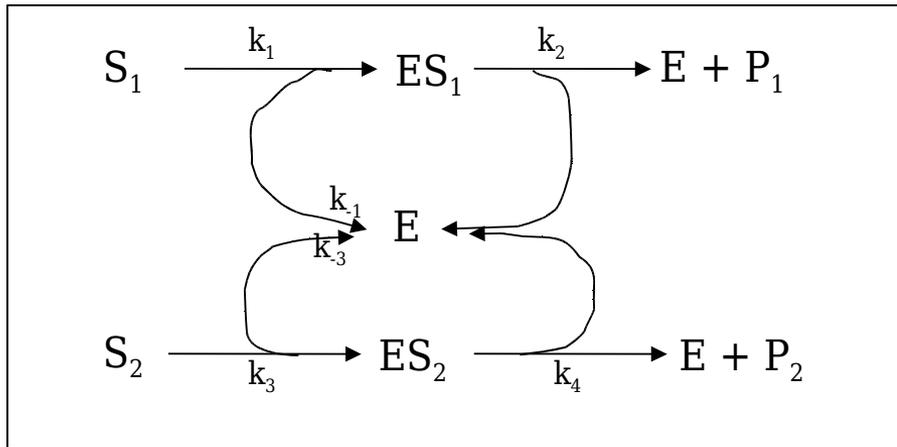
$$\frac{dS}{dt} = -k_2 * E_0 * \frac{S}{(K_m + S + K_i * S^2)}$$

mit

$$K_i = \frac{(k_{-1} + k_2) * k_3}{k_1 * k_{-3}}$$

# Kompetitive Reaktionen

Zwei konkurrierende Substrate:



Partieller steady state:

$$d ES_1/dt = k_1 * E * S_1 - (k_{-1} + k_2) * ES_1 = 0$$

$$d ES_2/dt = k_3 * E * S_2 - (k_{-3} + k_4) * ES_2 = 0$$

$$\text{Gesamtgeschwindigkeit } v = k_2 * ES_1 + k_4 * ES_2$$

$$E_0 = E + ES_1 + ES_2$$

Bsp: Teilgeschwindigkeit:

$$\frac{dP_1}{dt} = k_2 * E_0 * \frac{S_1 * K_{m2}}{(S_1 * K_{m2} + S_2 * K_{m1} + K_{m1} * K_{m2})}$$

Das erste Substrat A bindet an das Enzym, um den Enzymsubstratkomplex EA zu bilden. Dann bindet das zweite Substrat B. Die Reaktion produziert EPQ und Produkt P wird vor Produkt Q freigesetzt.



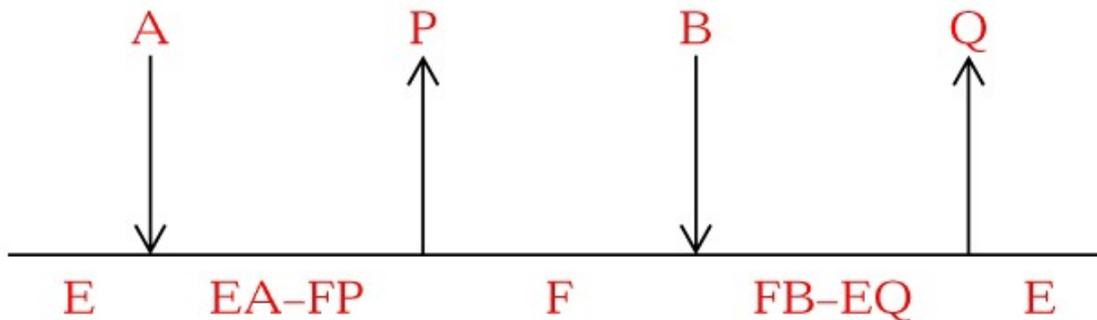
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Davon abgeleitet ergibt sich:

$$V = V_1 * \frac{S_1 * S_2}{(K_i S_1 * K_m S_2 + K_m S_2 * S_1 + K_m S_1 * S_2 + S_1 * S_2)}$$

# Ping-Pong

Das erste Substrat A vereinigt sich wiederum mit E zu EA. Dann wird jedoch schon das erste Produkt P freigesetzt. Das zweite Substrat B bindet nun und Produkt Q wird freigesetzt.



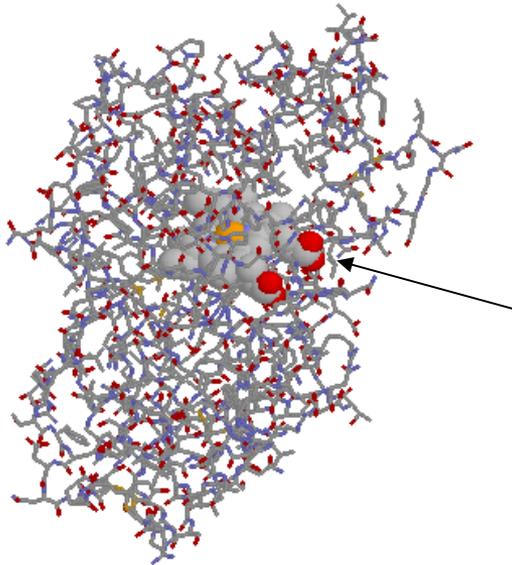
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Hier ergibt sich folgende Geschwindigkeit:

$$V = V_1 * \frac{S_1 * S_2}{(K_m S_2 * S_1 + K_m S_1 * S_2 + S_1 * S_2)}$$

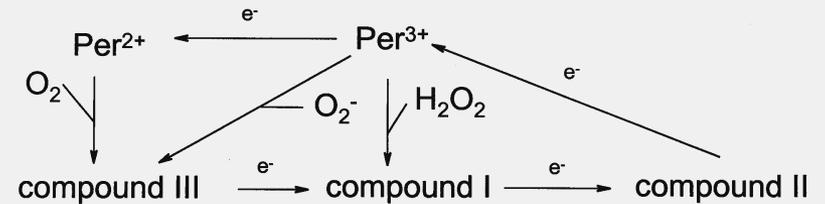
# Fallbeispiel 1 - Peroxidase

## Struktur



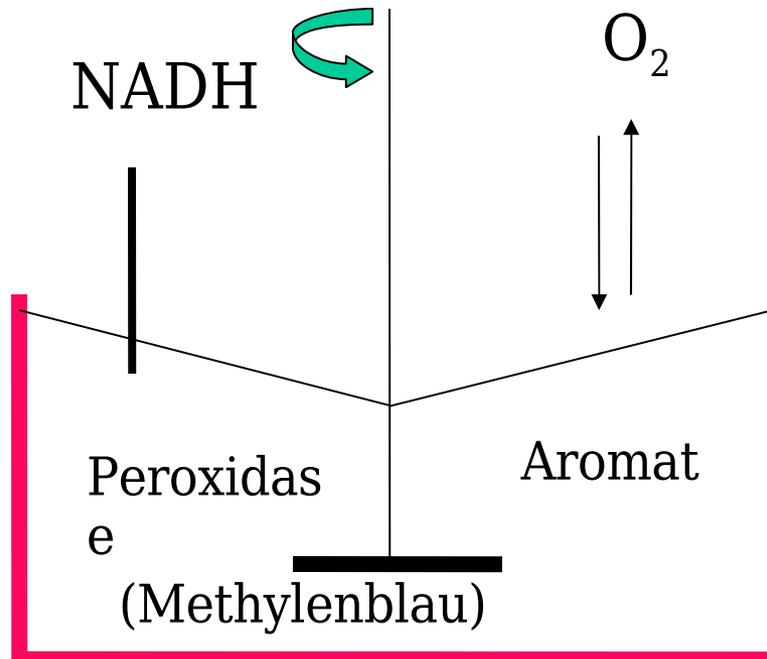
Häm-  
grupp  
e

## Enzymintermediate



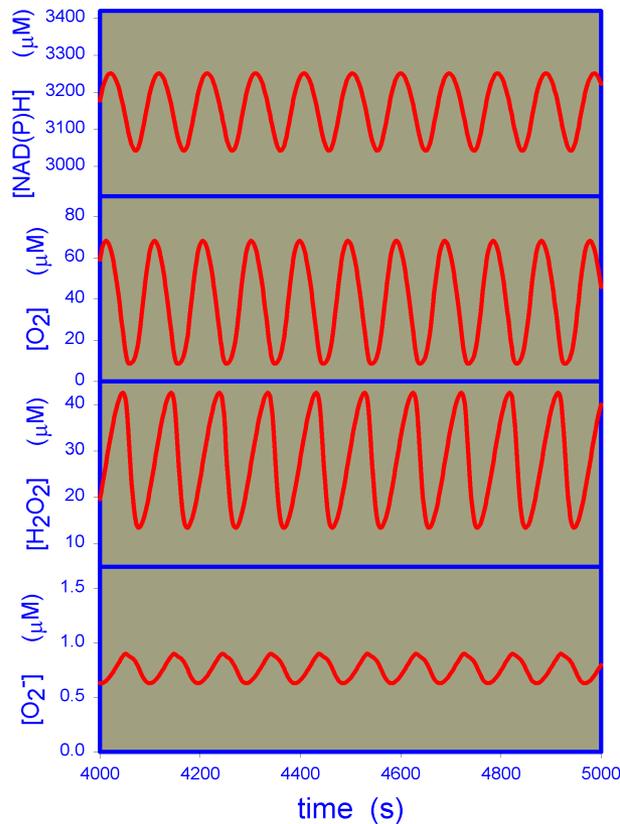
# Fallbeispiel 1 - Peroxidase

in vitro



# Fallbeispiel 1 - Peroxidase

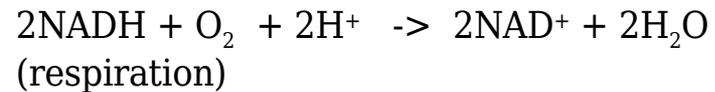
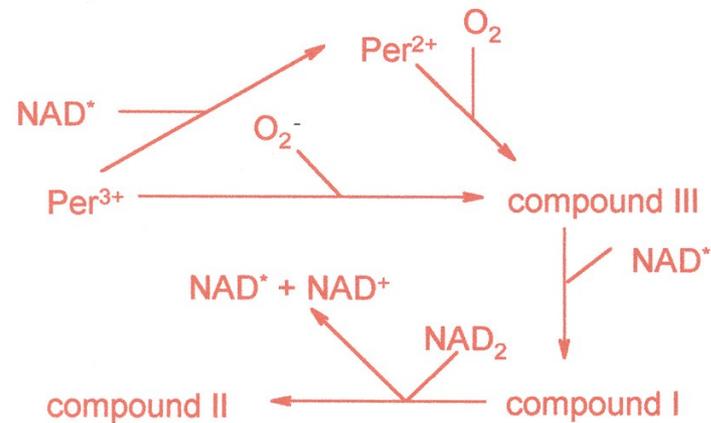
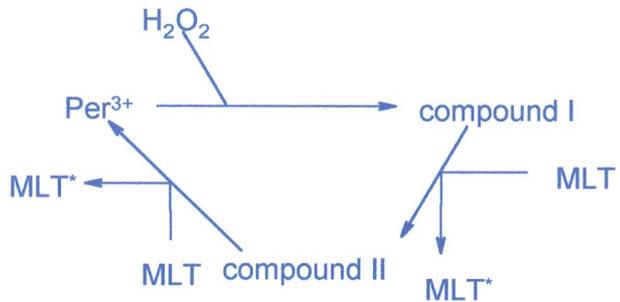
## *Kinetik*



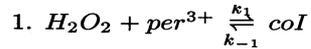
- Die Reaktion oszilliert unter verschiedenen Bedingungen
- Die Oszillationen können viele Stunden andauern

# Fallbeispiel 1 - Peroxidase

## Elementarreaktionen

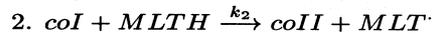


# Fallbeispiel 2 - Peroxidase



$$k_1[H_2O_2]_p[Per^{3+}]_p - k_{-1}[coI]_p$$

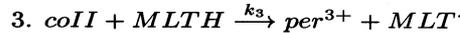
$$k_1 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



$$k_2[coI]_p[MLTH]_p$$

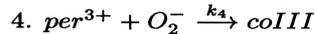
$$k_{-1} = 58 \text{ s}^{-1}$$

$$k_2 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



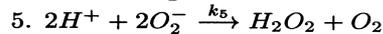
$$k_3[coII]_p[MLTH]_p$$

$$k_3 = 4.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$$



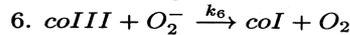
$$k_4[per^{3+}]_p[O_2^-]_p$$

$$k_4 = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



$$k_5[O_2^-]_p^2$$

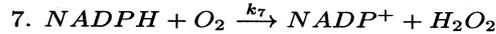
$$k_5 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



$$k_6[coIII]_p[O_2^-]_p$$

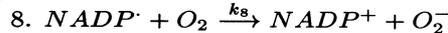
$$k_6 = 1.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$$

Reactions occurring in cytoplasm:



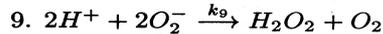
$$k_7[NADPH]_c[O_2]_c$$

$$k_7 = 1 \text{ M}^{-1} \text{ s}^{-1}$$



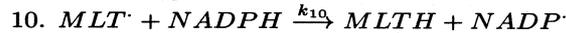
$$k_8[NADP^+]_c[O_2]_c$$

$$k_8 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



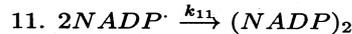
$$k_9[O_2^-]_c^2$$

$$k_9 = 5.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$$



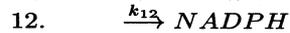
$$k_{10}[MLT']_c[NADPH]_c$$

$$k_{10} = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



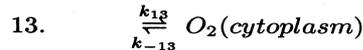
$$k_{11}[NADP^+]_c^2$$

$$k_{11} = 6.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$$



$$k_{12}$$

$$k_{12} = 22-35 \mu\text{M s}^{-1}$$

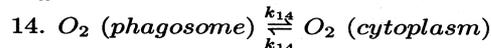


$$k_{13} - k_{-13}[O_2]_c$$

$$k_{13} = 12.5 \mu\text{M s}^{-1}$$

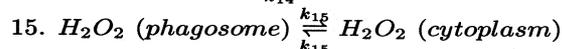
$$k_{-13} = 4.5 \times 10^{-2} \text{ s}^{-1}$$

Diffusion terms:



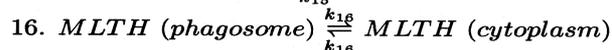
$$k_{14}([O_2]_p - [O_2]_c)$$

$$k_{14} = 30 \text{ s}^{-1}$$



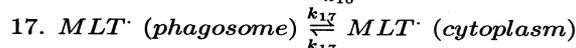
$$k_{15}([H_2O_2]_p - [H_2O_2]_c)$$

$$k_{15} = 30 \text{ s}^{-1}$$



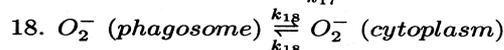
$$k_{16}([MLTH]_p - [MLTH]_c)$$

$$k_{16} = 10 \text{ s}^{-1}$$



$$k_{17}([MLT']_p - [MLT']_c)$$

$$k_{17} = 10 \text{ s}^{-1}$$



$$k_{18}([O_2^-]_p - [O_2^-]_c)$$

$$k_{18} = < 0.01 \text{ s}^{-1}$$

NADPH oxidase:

Beispiel für Systemgleichung:

$$coII' = k_2 * coI * MLTH - k_3 * coII * MLTH$$

# Einschub: Einheiten

Units:

- **UNIT OF ACTIVITY** - the amount of enzyme required to catalyze a reaction rate of transformation of 1 micromole of substrate/minute under standard conditions\*.

***Example: if 0.1 milligram/ml of enzyme catalyzes the conversion of 10 micromoles of substrate per minute, then that milliliter contains 10 units of activity.***

\*STANDARD CONDITIONS - conditions appropriate to a given enzyme (i.e. VARIABLE STANDARD!). However, when possible:

- Temperature = 30°C
- pH at Optimum
- Substrate concentration at 10X its half saturation concentration (10 Km)

## Einschub: Einheiten

- **SPECIFIC ACTIVITY** - amount of activity per amount of protein, Typically expressed as Units/mg of protein.
- **MOLECULAR ACTIVITY** - (previously called "turnover number") the number of molecules of substrate transformed per minute per molecule of enzyme.
- **CATALYTIC CENTER ACTIVITY** - molecular activity on a per site basis.

**NOTE:** definition of units is a progressive and iterative process dependent on purity of the enzyme & its substrates, measurement of its molecular weight, improved measures of  $V_{max}$  &  $K_m$ , discovery of the number of sites and the evolving "STANDARD" conditions.

# Datenbanken im Netz

<http://www.brenda.uni-koeln.de/>

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The Comprehensive Enzyme Information System Release 2

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Nomenclature	Reaction & Specificity	Functional Parameters
Enzyme Names EC Number Common/ Recommended Name Systematic Name Synonyms CAS Registry Number	Pathway Catalysed Reaction Reaction Type Natural Substrates and Products Substrates and Products Substrates Natural Substrate Products Natural Product	Km Value Ki Value pl Value Turnover Number Specific Activity pH Optimum pH Range Temperature Optimum Temperature Range
Isolation & Preparation	Inhibitors Cofactors Metals/Ions Activating Compounds Ligands	Organism-related information
Purification Cloned Renatured Crystallization	Enzyme Structure	Organism Source Tissue Localization
Stability	Sequence/ SwissProt link 3D-Structure/ PDB link Molecular Weight Subunits Posttranslational Modification	Disease & References
pH Stability Temperature Stability General Stability Organic Solvent Stability Oxidation Stability Storage Stability		Disease References
		Application & Engineering
		Engineering Application

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Reaction Search

Return only reactions having kinetic data matching all criteria (blue and grey)

Search criteria in blue are used to define the search conditions for reactions, independently if there is or not kinetic data for these reactions.

Search Reaction



Specify Search Criteria:

Submit Search

Reset Form

SBML Model Setup



with **Reactant(s)**

[ + ] [ - ]



in **Pathway(s)**

[ + ] [ - ]



having **Enzyme(s)**

[ + ] [ - ]

in **Publication**

[ + ] [ - ]

in **Organism(s)**

[ + ] [ - ]

in **Tissue(s)/Cell Type(s)**

[ + ] [ - ]

in **(Intra/Extra)Cellular Location(s)**

[ + ] [ - ]

Having **Kinetic Data** Determined for Specific Experimental Conditions

[ + ] [ - ]

having **Kinetic data**

[ + ] [ - ]



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