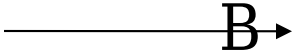
E2 - Proteine

4. Tag: Enzymkinetik - Bestimmung und Datenbanken

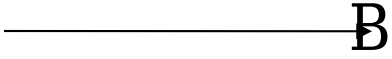
Ursula Kummer, Sven Sahle
Femke Mensonides, Irina Surovtsova, Jürgen Zobeley

Reaktionsordnung

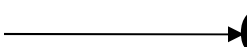
Reaktion 0. Ordnung: z.B. konst. Zerfall

A (unendlicher Pool)  B

Reaktion 1. Ordnung: z.B. radioaktiver Zerfall

A  B

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

A + B  C

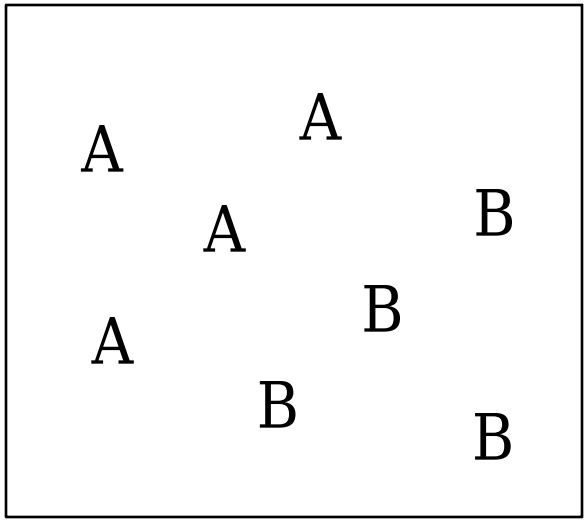
Einfache Reaktionskinetik I

Einfachster Fall:

A -------> B

Reaktionsgeschwindigkeit:

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



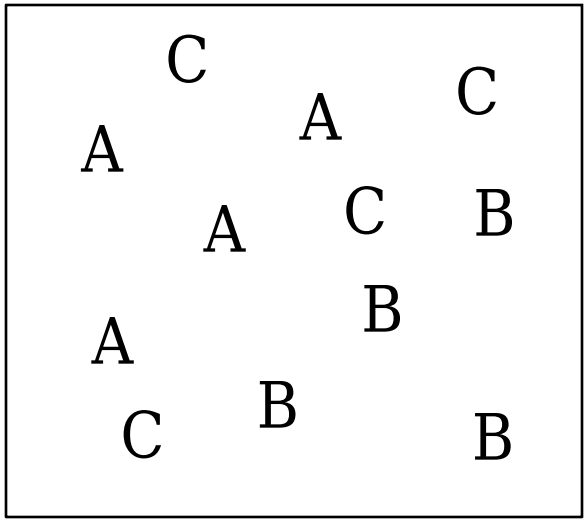
Einfache Reaktionskinetik II

Zwei Reaktanten:

A + B -------> C

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



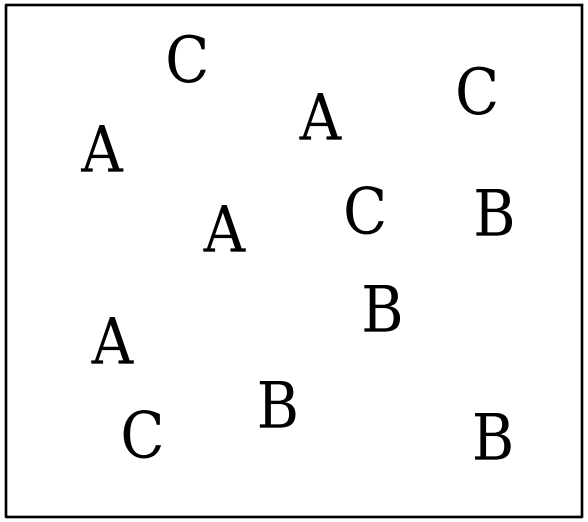
Einfache Reaktionskinetik III

Drei Reaktanten:

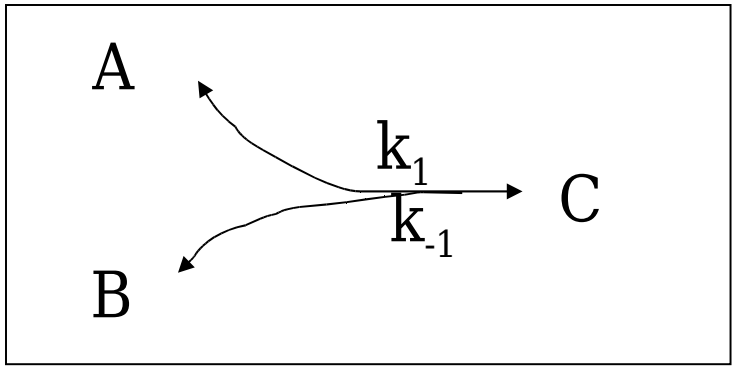
A + 2B -------> C

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

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

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

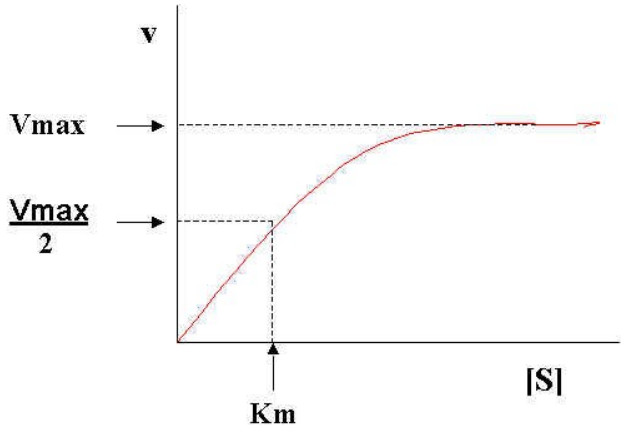
Systemgleichungen

Enzyme, die Biokatalysatoren

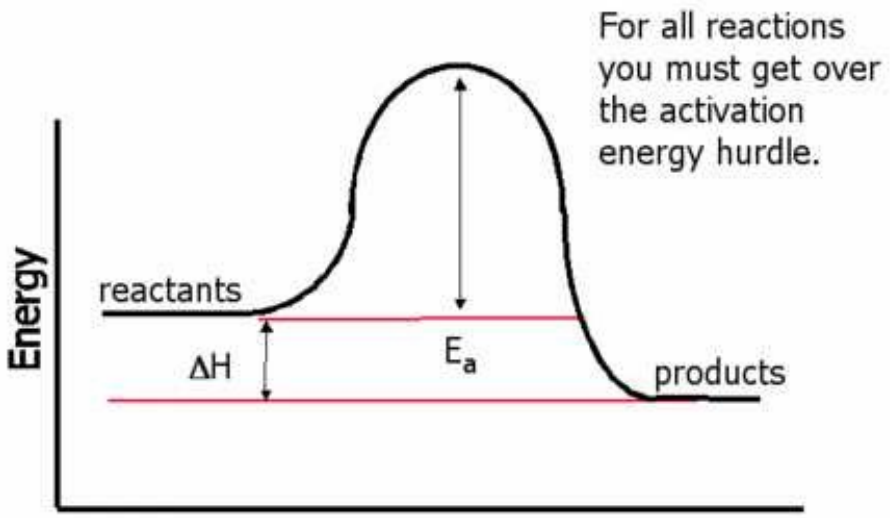
- Die Geschwindigkeit biochemischer Reaktionen folgt meist einer Sättigungskurve.

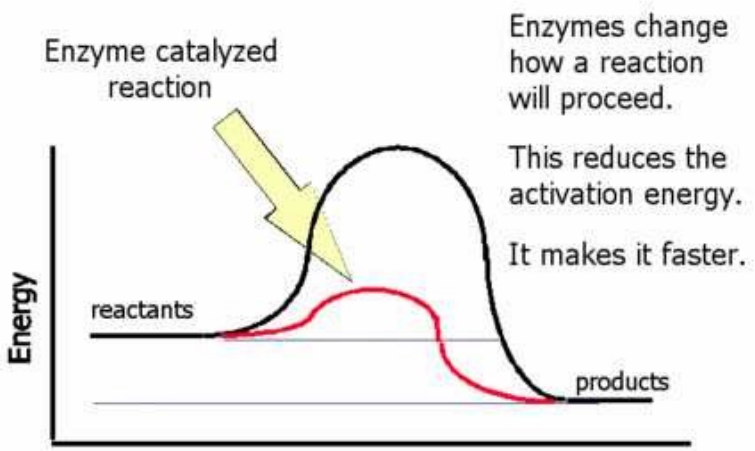
- Wenn alle Enzyme belegt sind, kann die Geschwindigkeit nicht mehr zunehmen, auch wenn mehr Substrat dazukommt.

- Die Substratkonzentration bei halbmaximaler Geschwindigkeit entspricht Km.



Effekt von Enzymen auf Aktivierungsenergie





Michaelis-Menten Equation

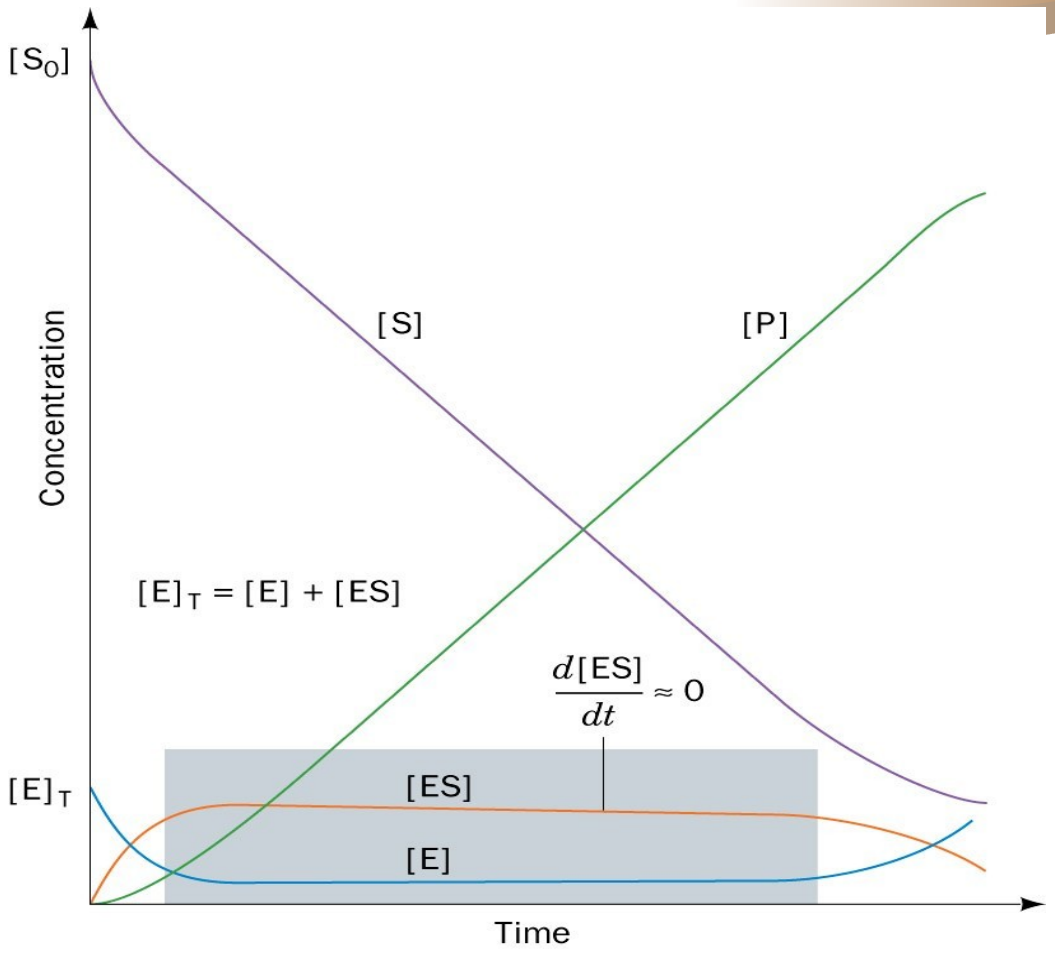
Reaktionsgeschwindikeit

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

Gleichung für ES

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

Komponenten in einer simplen Michaelis–Menten-Reaktion



Michaelis-Menten

- Annahme des schnellen GG

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

- Annahme des Steady States

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

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

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

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

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

$$K_M[\mathrm{ES}] = ([\mathrm{E}]_\mathrm{T} - [\mathrm{ES}])[\mathrm{S}]$$

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_M + [\mathrm{S}]}$$

Michaelis-Menten

- Maximalgeschwindigkeit

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

- Michaelis-Menten

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

K_M

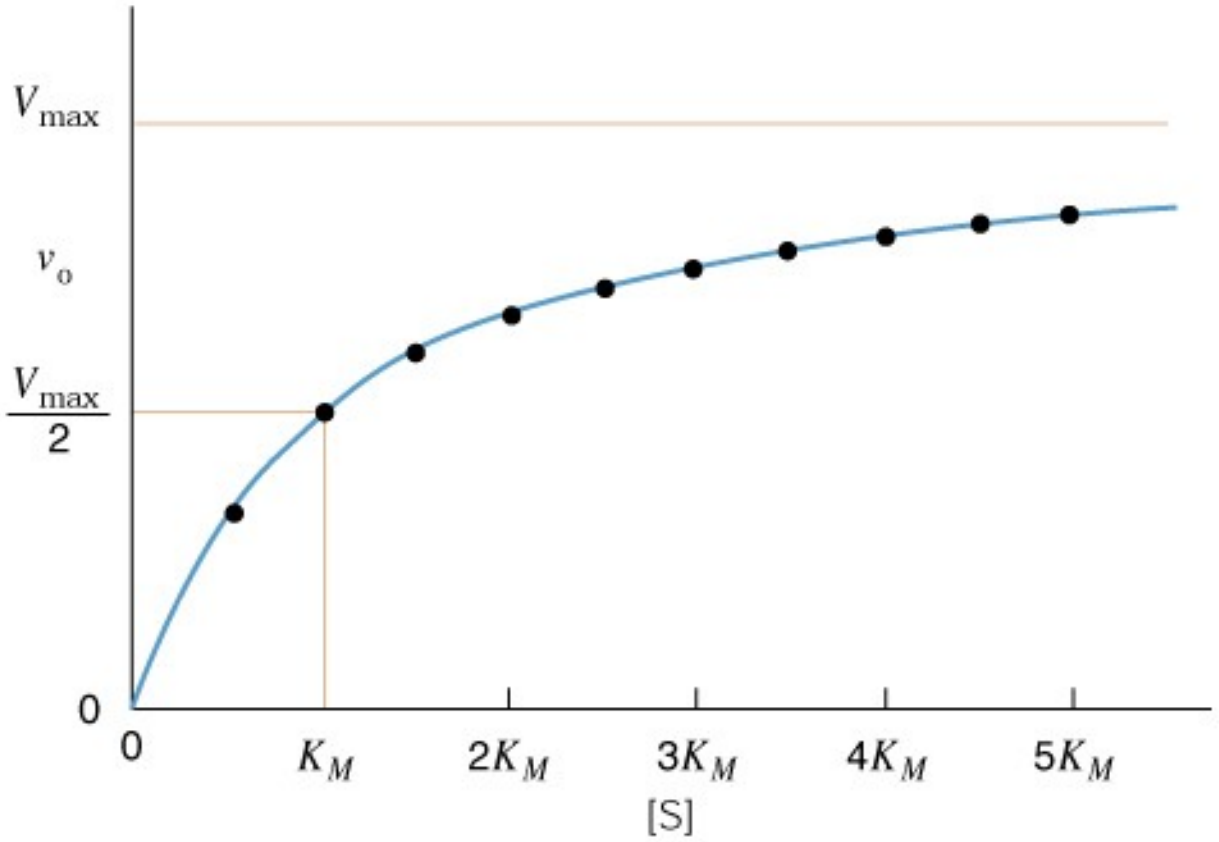
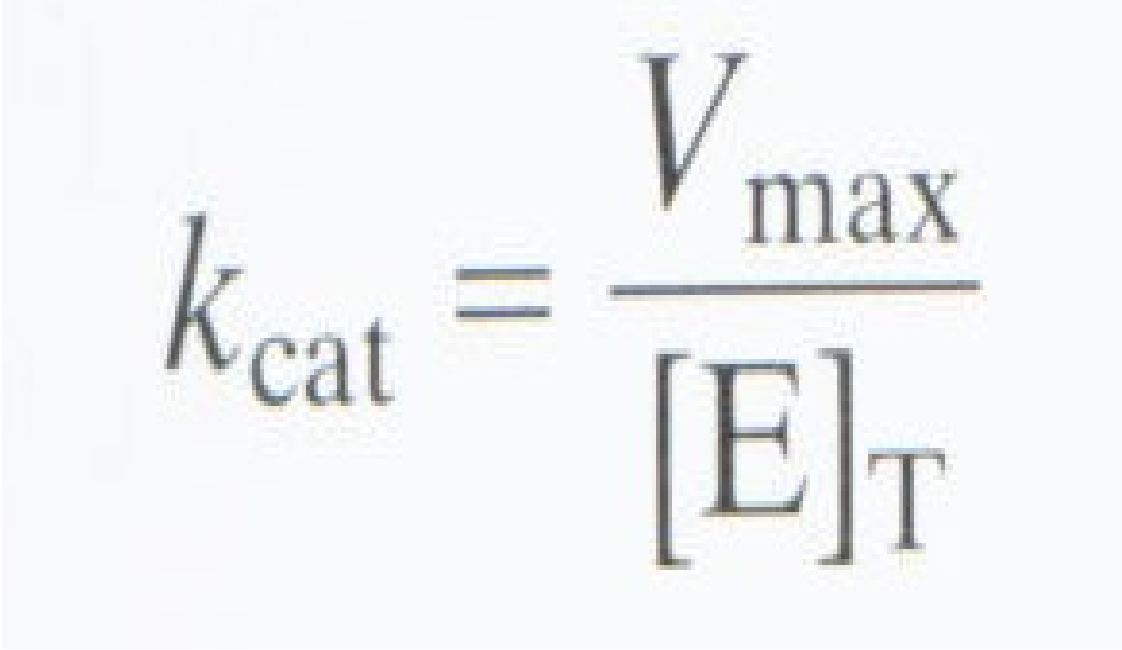


Figure 12-3. Key to Function. A plot of the initial velocity v_o of a simple enzymatic reaction versus the substrate concentration [S].

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	9.5×10^{-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}
Superoxide dismutase	Superoxide ion ($O_2^{\cdot -}$)	3.6×10^{-4}	1.0×10^{6}	2.8×10^{9}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

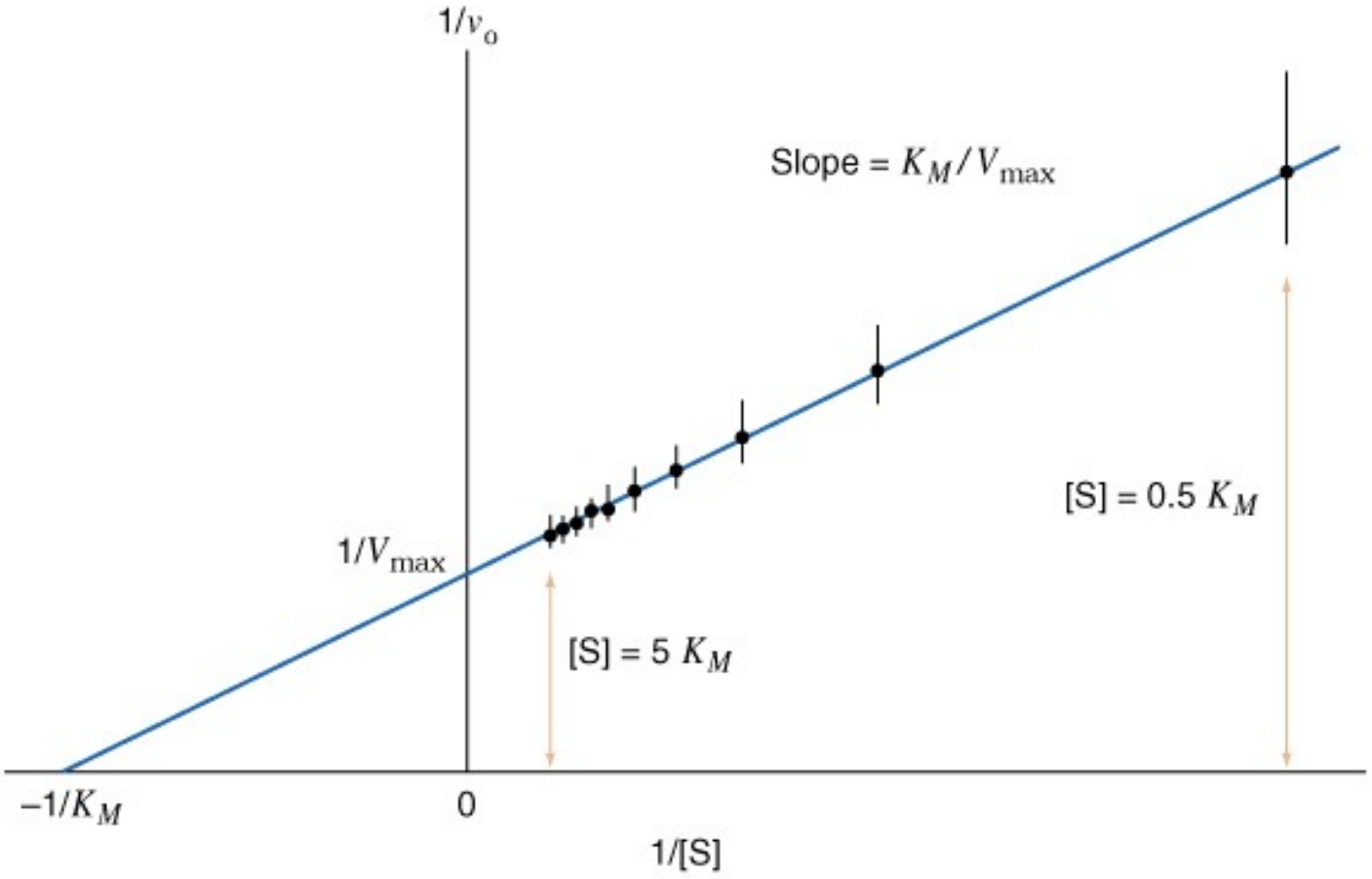
Lineweaver-Burk

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

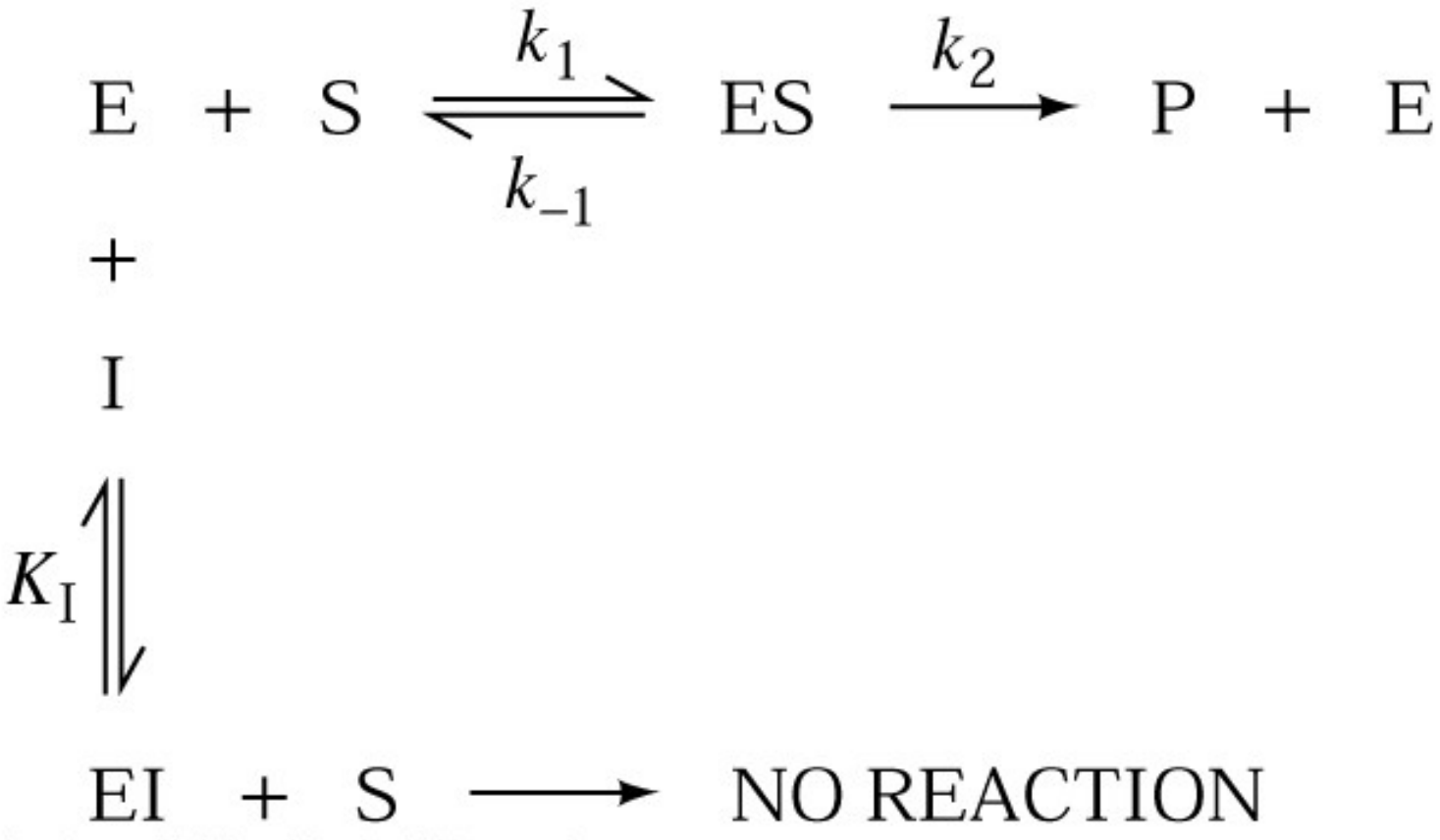
-> reziprok:

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

Lineweaver-Burk



Kompetitive Hemmung



Kompetitive Hemmung

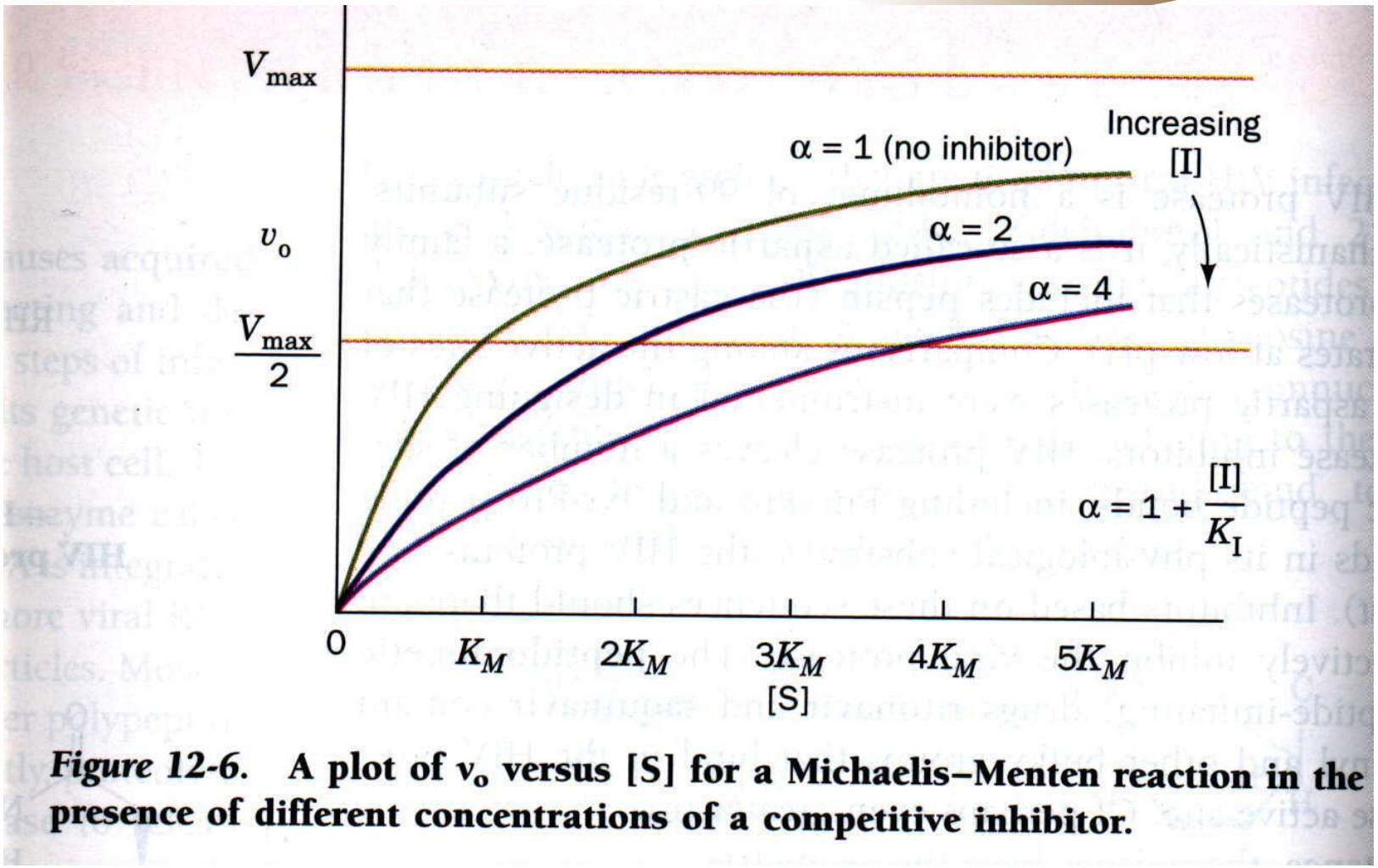
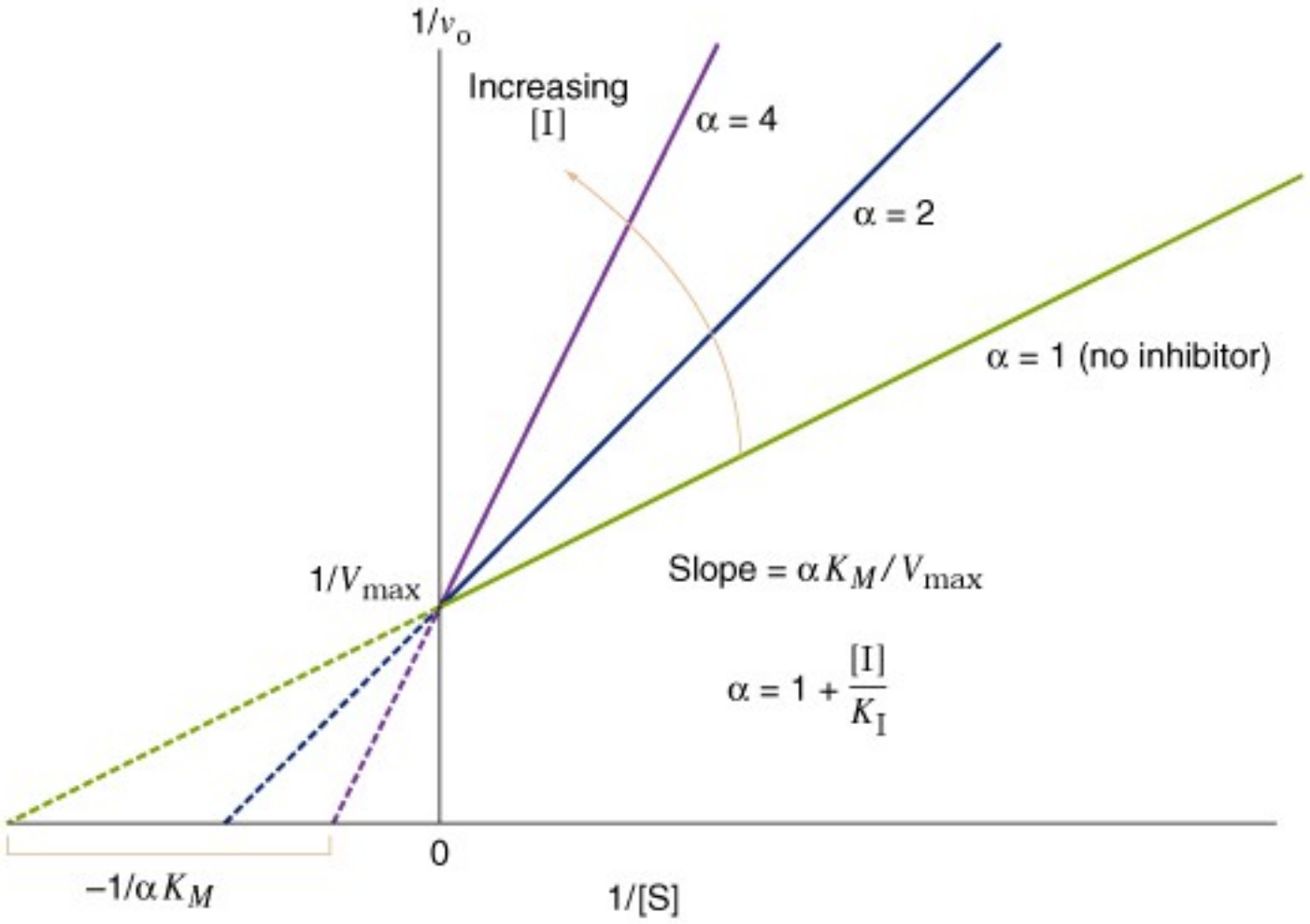
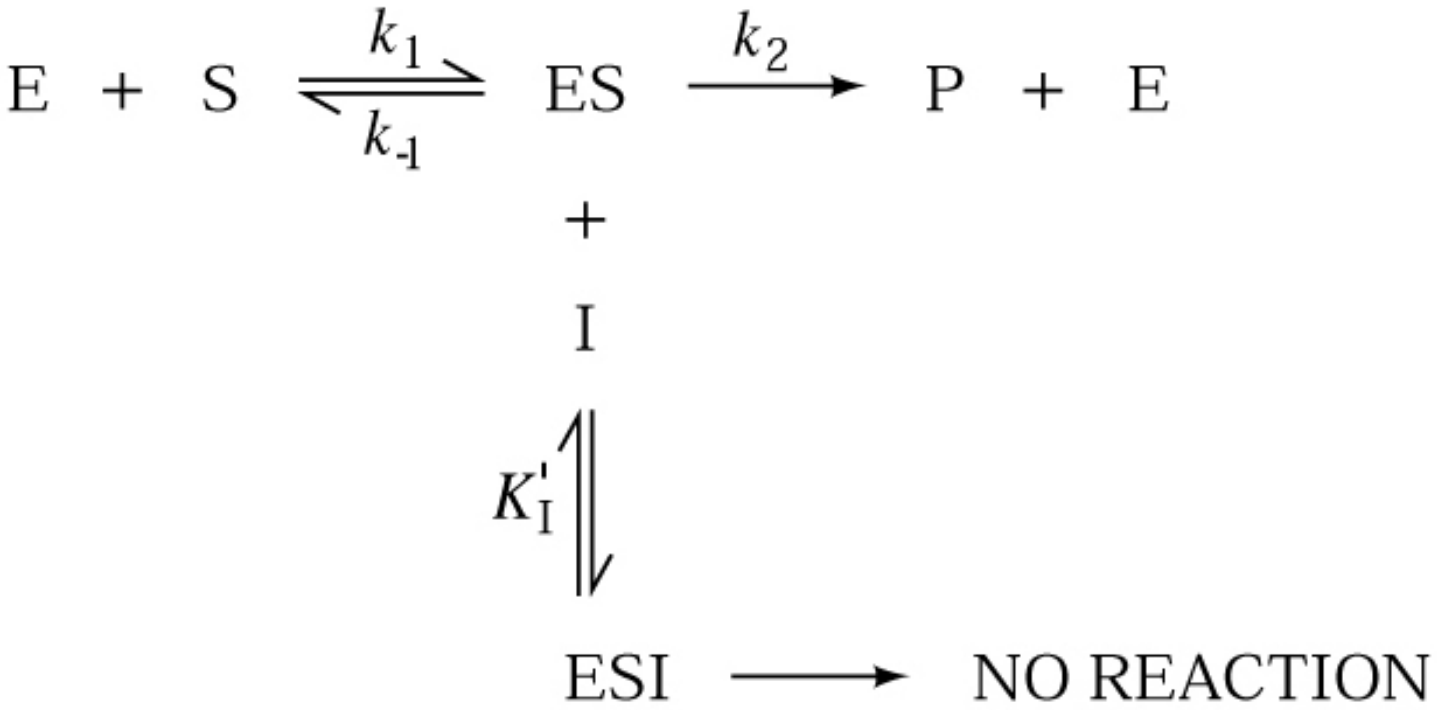
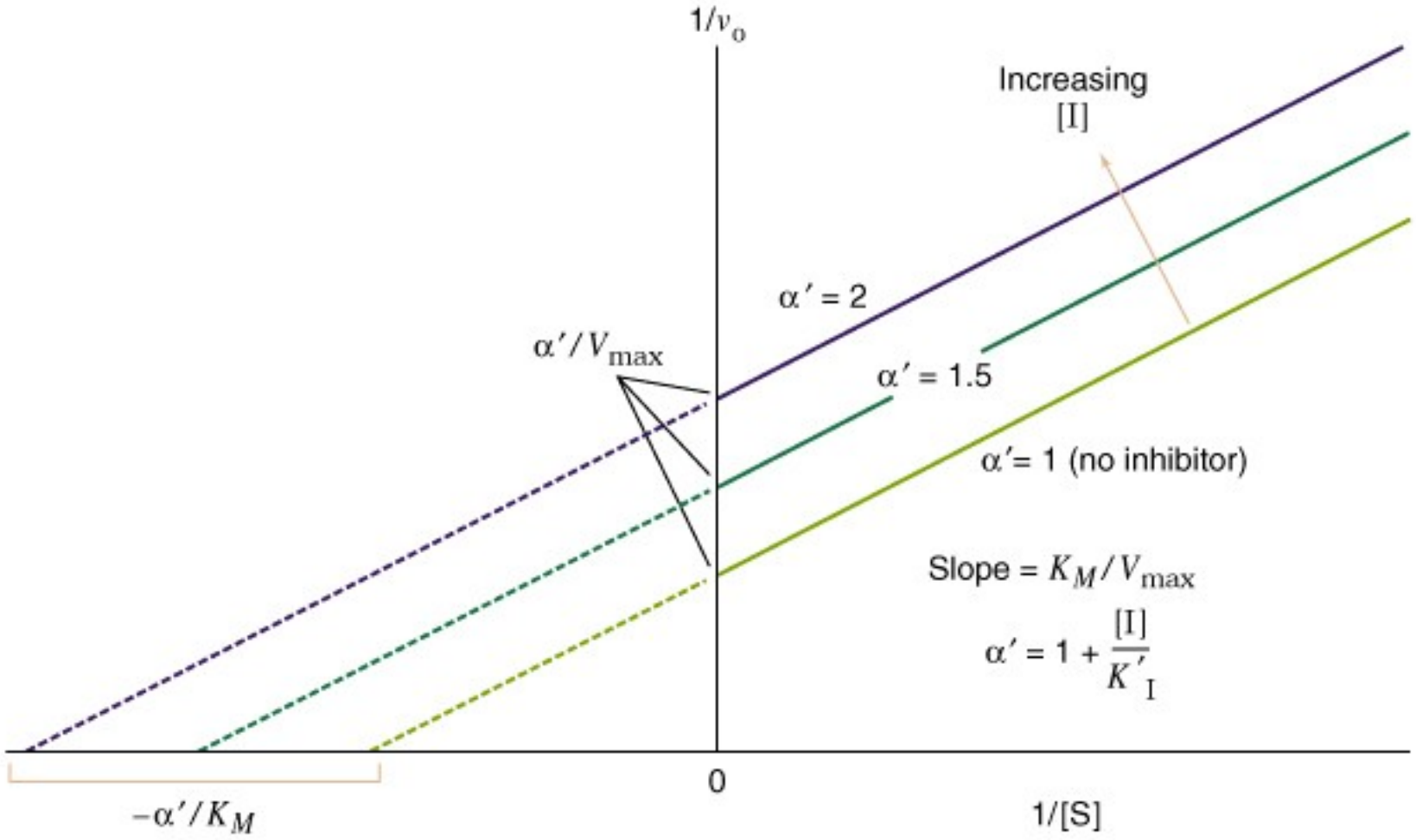


Figure 12-6. **A plot of v_o versus [S] for a Michaelis–Menten reaction in the presence of different concentrations of a competitive inhibitor.**

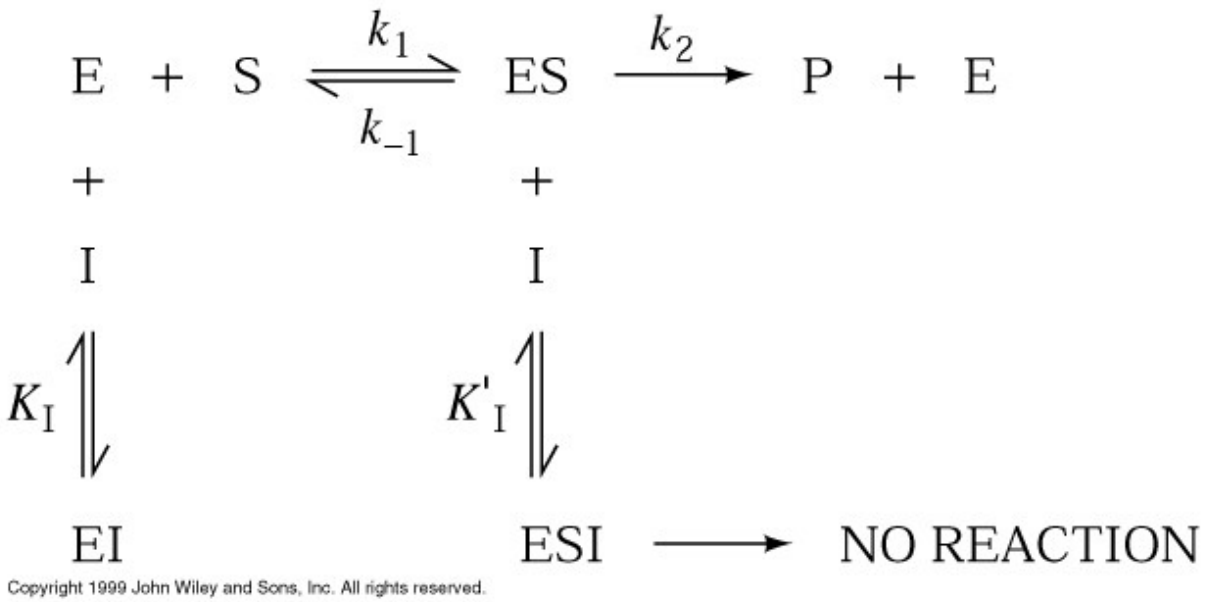


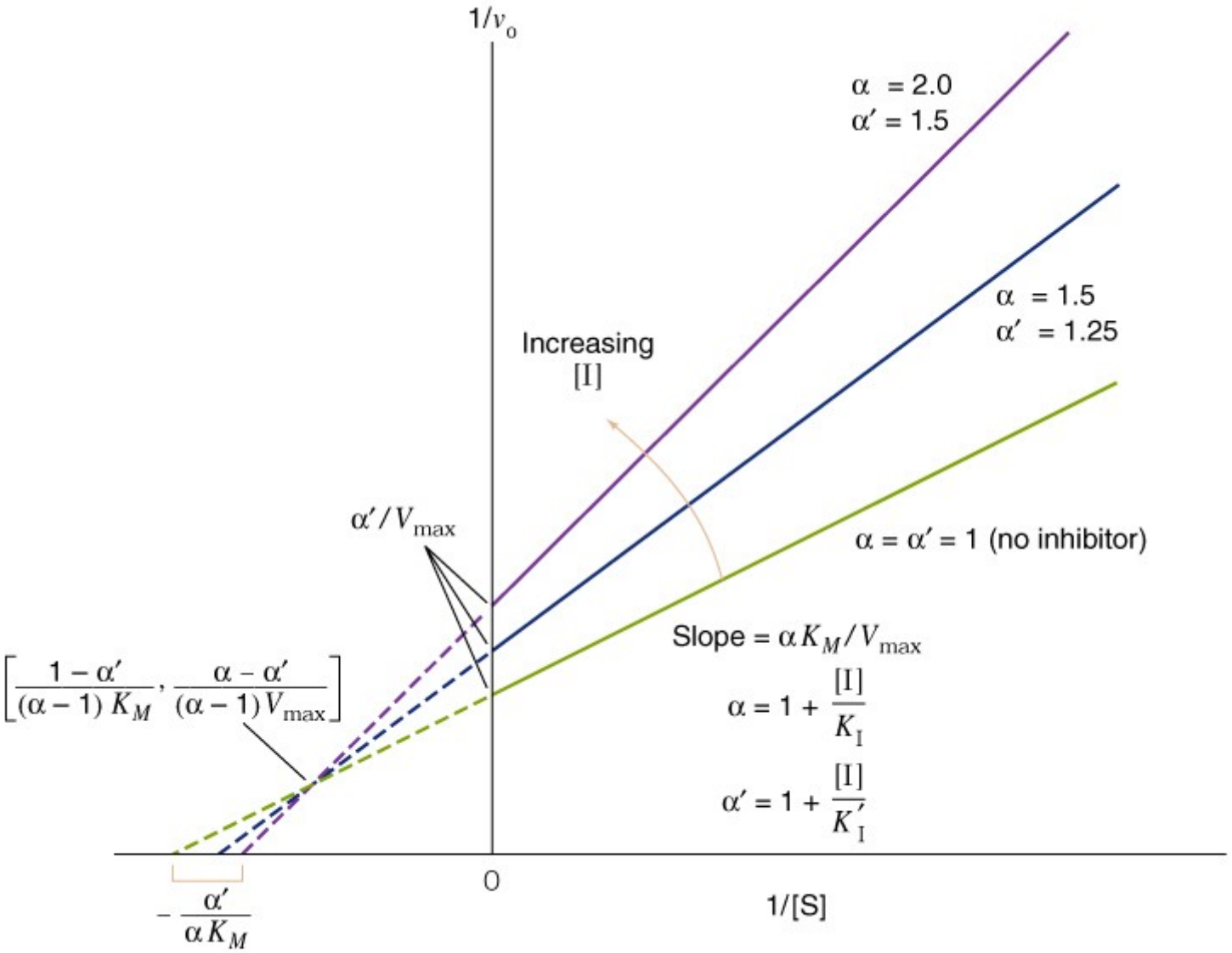
Unkompetitive Hemmung





Mixed



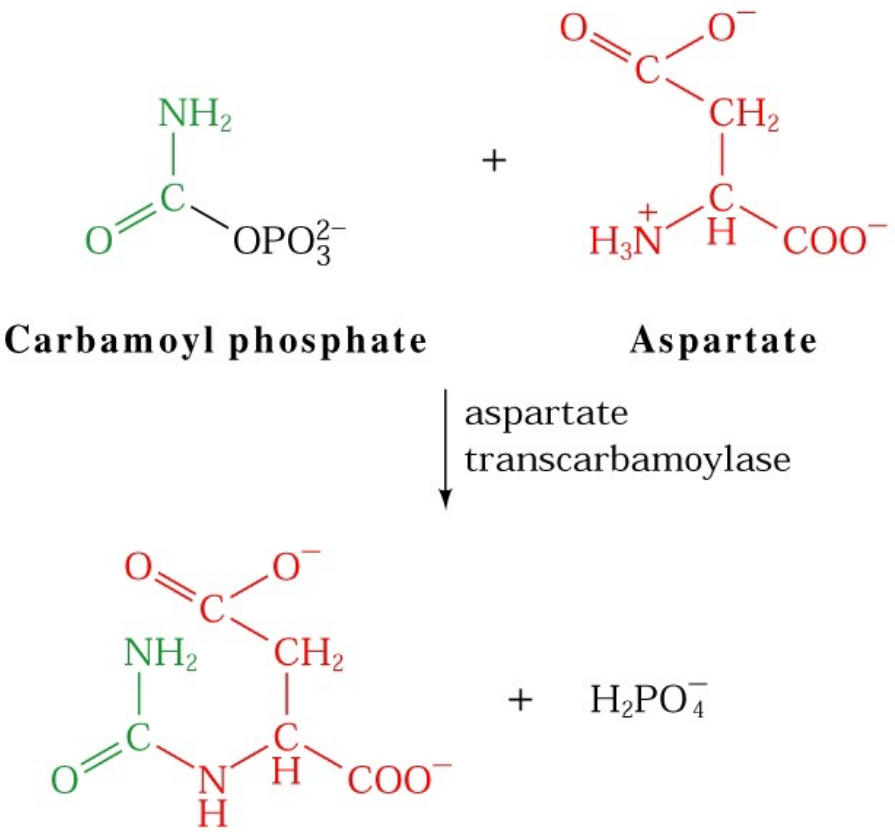


Effekte von Inhibitoren auf Parameter der Michaelis–Menten-Gleichung[a]

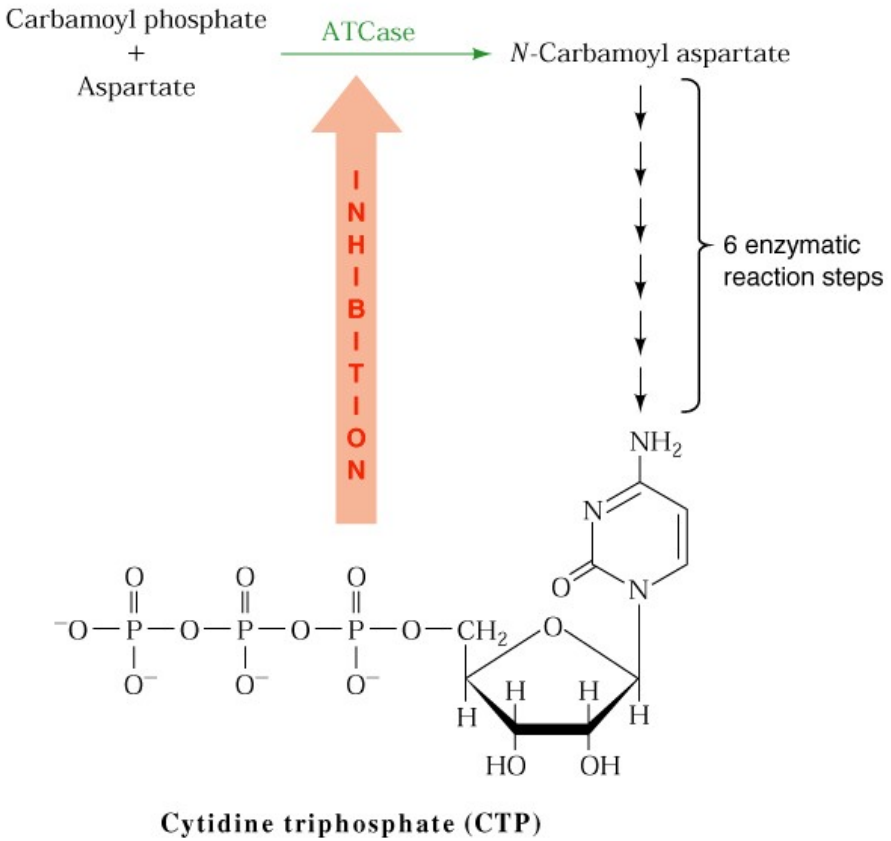
Type of Inhibition	V_{max}^{app}	K_M^{app}
None	V_{max}	K_M
Competitive	V_{max}	αK_M
Uncompetitive	V_{max}/α'	K_M/α'
Mixed	V_{max}/α'	$\alpha K_M/\alpha'$

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

Beispiel Regulation: Aspartate transcarbamylase



Feedback Inhibition



Cytidine triphosphate (CTP)

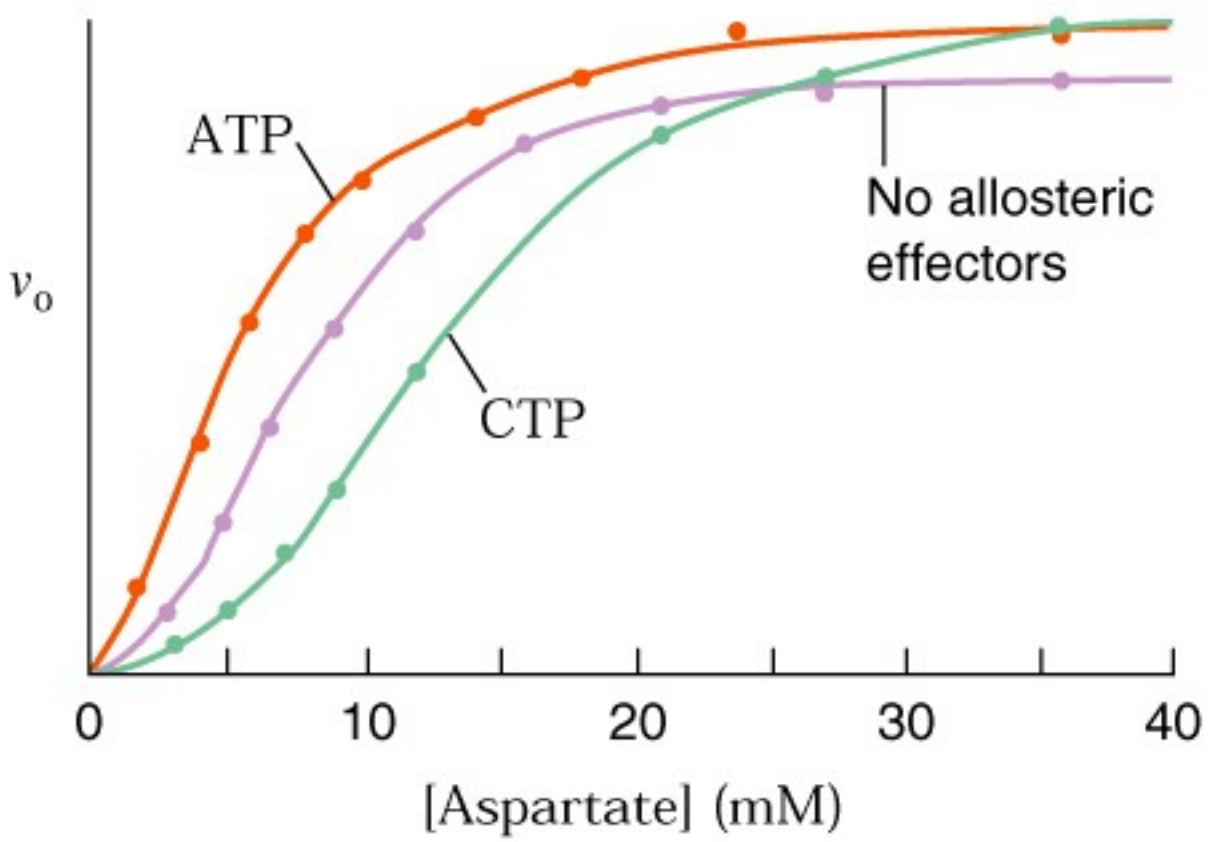
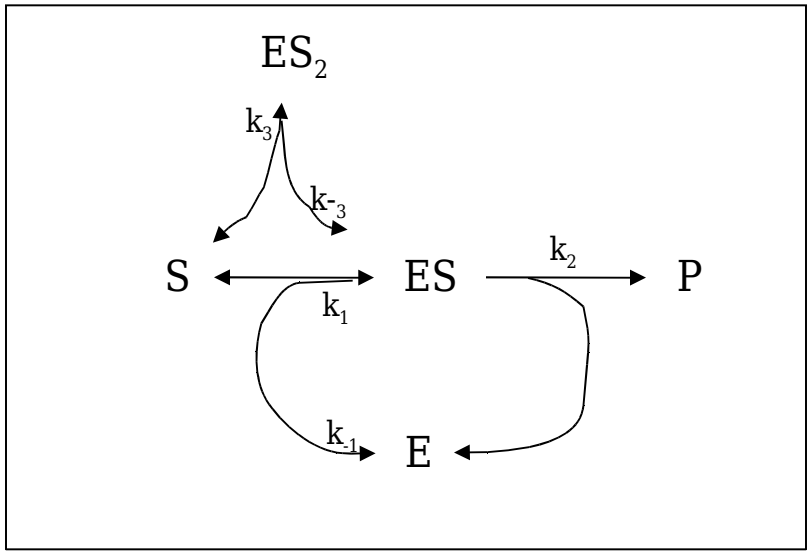


Figure 12-10. Plot of v_o versus [Aspartate] for the ATCase reaction. [After Kantrowitz, E.R., Pastra-Landis, S.C., and Lipscomb, W.N., *Trends Biochem. Sci.* 5, 125 (1980).]

Substratinhibition

Katalyse mit Substratinhibition:



Analoge Vorgehensweise liefert:

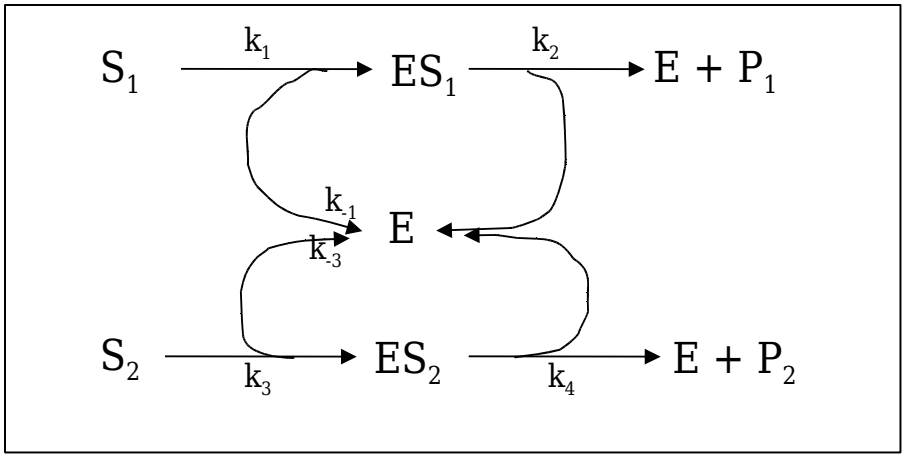
$$\frac{dS}{dt} = -k2 * E0 * \frac{S}{(Km + S + Ki * S^2)}$$

mit

$$Ki = \frac{(k-1 + k2) * k3}{k1 * k-3}$$

Kompetitive Reaktionen

Zwei konkurrierende Substrate:



Partieller steady state:

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

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

Gesamtgeschwindigkeit $v = k_2*ES_1 + k_4*ES_2$

$E_0 = E + ES_1 + ES_2$

Bsp: Teilgeschwindigkeit:

$$\frac{dP1}{dt} = k2*E0*\frac{S1*Km2}{(S1*Km2+S2*Km1+Km1*Km2)}$$

BiBi

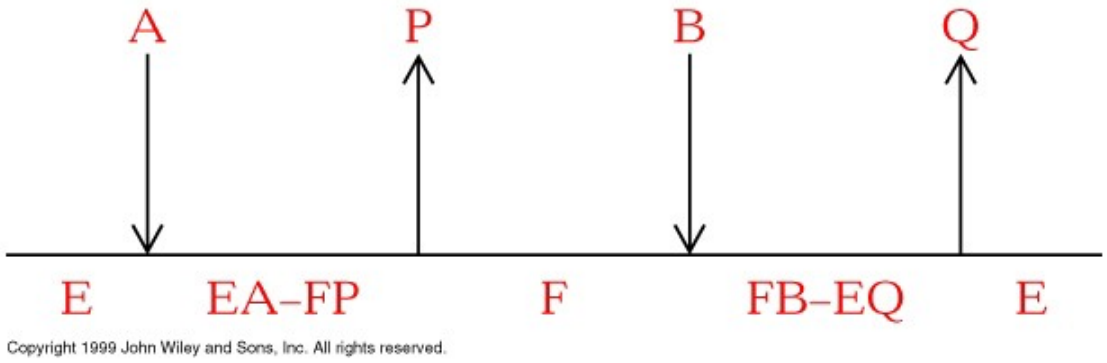
Das erste Substrat A bindet an das Enzym, um den Enzymsubstrat
komplex EA zu bilden. Dann bindet das zweite Substrat B.
Die Reaktion produziert EPQ und Produkt P wird vor Produkt
Q freigesetzt.

Davon abgeleitet ergibt sich:

$$V = V1 * \frac{S1 * S2}{(KiS1 * KmS2 + KmS2 * S1 + KmS1 * S2 + S1 * S2)}$$

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.

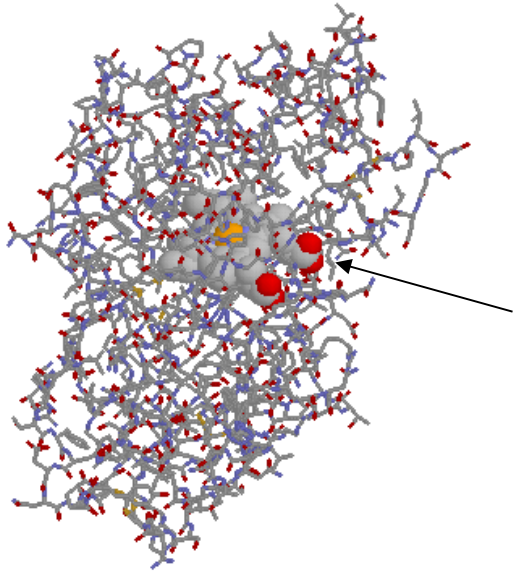


Hier ergibt sich folgende Geschwindigkeit:

$$V = V1 * \frac{S1 * S2}{(KmS2 * S1 + KmS1 * S2 + S1 * S2)}$$

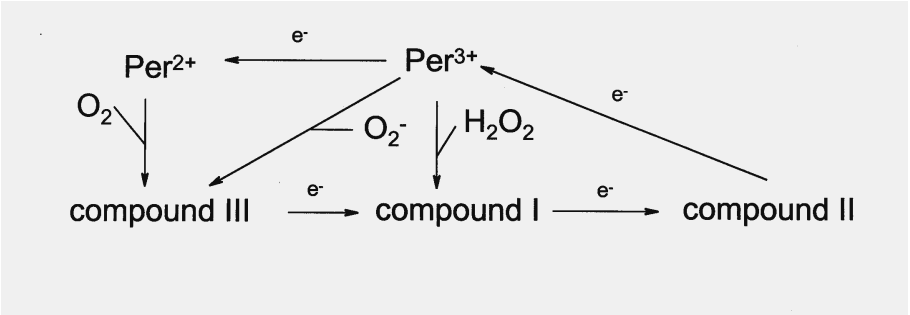
Fallbeispiel 1 - Peroxidase

Struktur



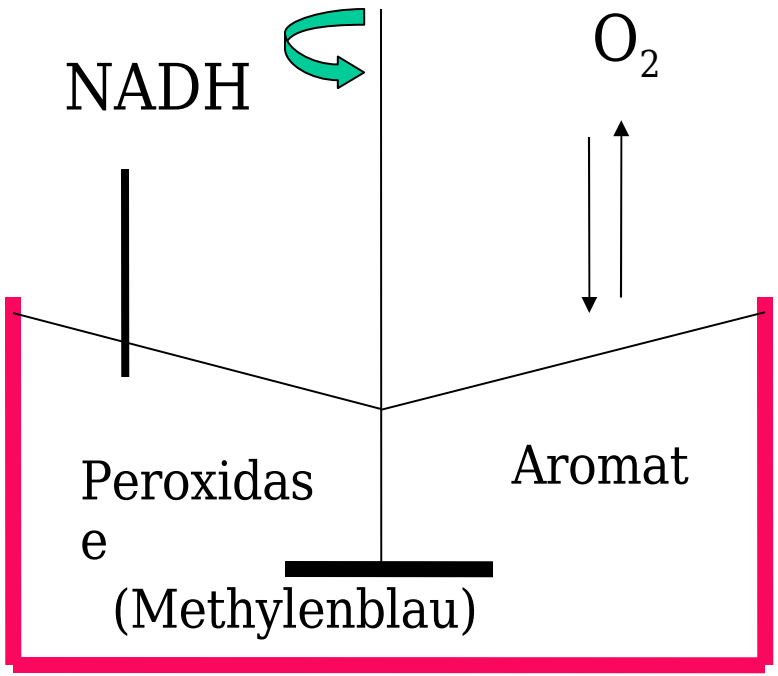
Häm-
gruppe

Enzymintermediate



Fallbeispiel 1 - Peroxidase

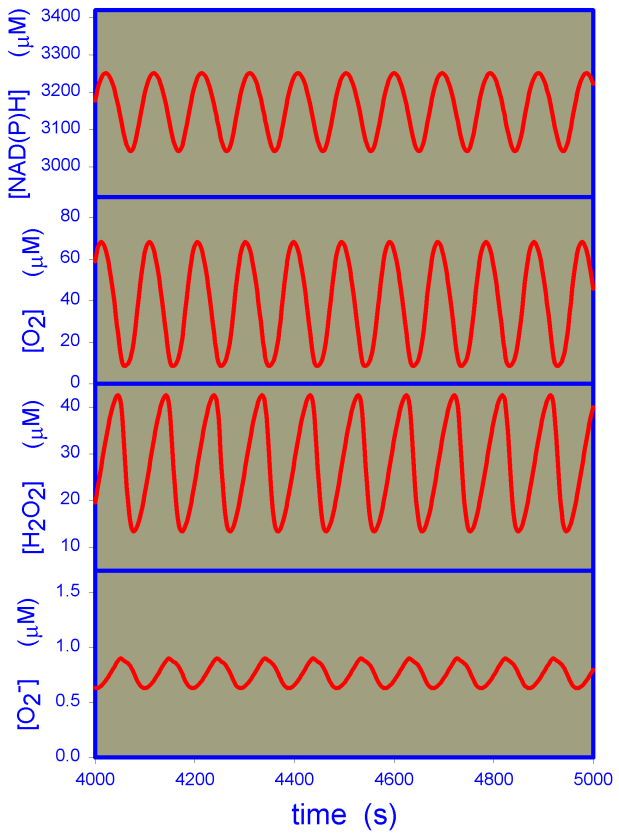
in vitro



$$2NADH + O_2 \longrightarrow 2NAD^+ + 2H_2O$$

Fallbeispiel 1 - Peroxidase

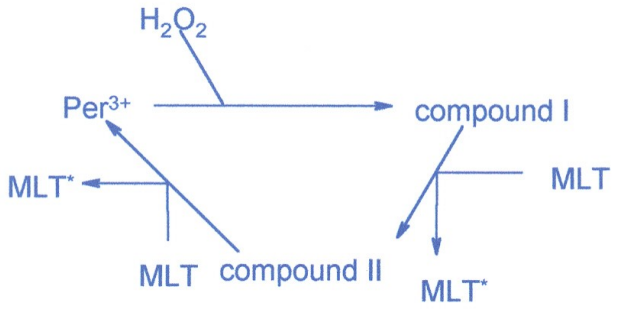
Kinetik

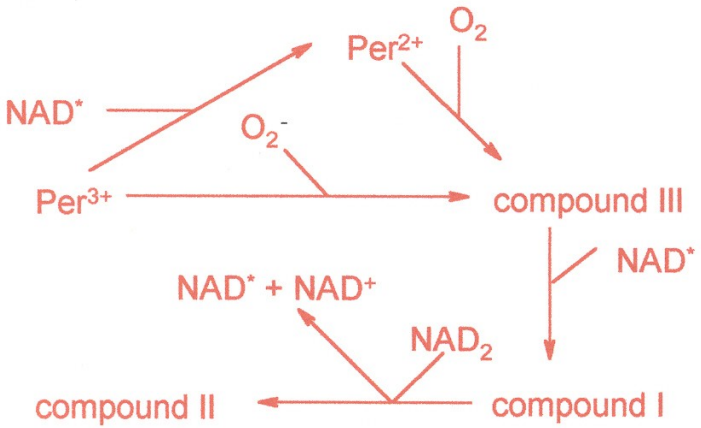


- Die Reaktion oszilliert unter verschiedenen Bedingungen
- Die Oszillationen können viele Stunden andauern

Fallbeispiel 1 - Peroxidase

Elementarreaktionen





$NADH + MLT^* \longrightarrow NAD^* + MLT + H^+$

$NAD^* + O_2 \longrightarrow NAD^+ + O_2^-$

$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$

$2NAD^* \longrightarrow NAD_2$

$NADH + O_2 + H^+ \longrightarrow NAD^+ + H_2O_2$

$NAD^+ \longrightarrow NADH$ (enzymatic)

$\rightleftharpoons O_2$

$2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O$
(respiration)

Fallbeispiel 2 - Peroxidase

1. $H_2O_2 + per^{3+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} coI$	$k_1[H_2O_2]_p[Per^{3+}]_p - k_{-1}[coI]_p$	$k_1 = 5.0 \times 10^7\ M^{-1}\ s^{-1}$ $k_{-1} = 58\ s^{-1}$
2. $coI + MLTH \xrightarrow{k_2} coII + MLT^{\cdot}$	$k_2[coI]_p[MLTH]_p$	$k_2 = 1.0 \times 10^7\ M^{-1}\ s^{-1}$
3. $coII + MLTH \xrightarrow{k_3} per^{3+} + MLT^{\cdot}$	$k_3[coII]_p[MLTH]_p$	$k_3 = 4.0 \times 10^3\ M^{-1}\ s^{-1}$
4. $per^{3+} + O_2^- \xrightarrow{k_4} coIII$	$k_4[per^{3+}]_p[O_2^-]_p$	$k_4 = 1.1 \times 10^7\ M^{-1}\ s^{-1}$
5. $2H^+ + 2O_2^- \xrightarrow{k_5} H_2O_2 + O_2$	$k_5[O_2^-]_p^2$	$k_5 = 1.0 \times 10^7\ M^{-1}\ s^{-1}$
6. $coIII + O_2^- \xrightarrow{k_6} coI + O_2$	$k_6[coIII]_p[O_2^-]_p$	$k_6 = 1.0 \times 10^5\ M^{-1}\ s^{-1}$
Reactions occurring in cytoplasm:		
7. $NADPH + O_2 \xrightarrow{k_7} NADP^+ + H_2O_2$	$k_7[NADPH]_c[O_2]_c$	$k_7 = 1\ M^{-1}\ s^{-1}$
8. $NADP^{\cdot} + O_2 \xrightarrow{k_8} NADP^+ + O_2^-$	$k_8[NADP^{\cdot}]_c[O_2]_c$	$k_8 = 5.0 \times 10^7\ M^{-1}\ s^{-1}$
9. $2H^+ + 2O_2^- \xrightarrow{k_9} H_2O_2 + O_2$	$k_9[O_2^-]_c^2$	$k_9 = 5.0 \times 10^8\ M^{-1}\ s^{-1}$
10. $MLT^{\cdot} + NADPH \xrightarrow{k_{10}} MLTH + NADP^{\cdot}$	$k_{10}[MLT^{\cdot}]_c[NADPH]_c$	$k_{10} = 1.0 \times 10^7\ M^{-1}\ s^{-1}$
11. $2NADP^{\cdot} \xrightarrow{k_{11}} (NADP)_2$	$k_{11}[NADP^{\cdot}]_c^2$	$k_{11} = 6.0 \times 10^7\ M^{-1}\ s^{-1}$
12. $\xrightarrow{k_{12}} NADPH$	k_{12}	$k_{12} = 22\text{-}35\ \mu M\ s^{-1}$
13. $\underset{k_{-13}}{\overset{k_{13}}{\rightleftharpoons}} O_2(cytoplasm)$	$k_{13} - k_{-13}[O_2]_c$	$k_{13} = 12.5\ \mu M\ s^{-1}$ $k_{-13} = 4.5 \times 10^{-2}\ s^{-1}$
Diffusion terms:		
14. $O_2\ (phagosome) \underset{k_{14}}{\overset{k_{14}}{\rightleftharpoons}} O_2\ (cytoplasm)$	$k_{14}([O_2]_p - [O_2]_c)$	$k_{14} = 30\ s^{-1}$
15. $H_2O_2\ (phagosome) \underset{k_{15}}{\overset{k_{15}}{\rightleftharpoons}} H_2O_2\ (cytoplasm)$	$k_{15}([H_2O_2]_p - [H_2O_2]_c)$	$k_{15} = 30\ s^{-1}$
16. $MLTH\ (phagosome) \underset{k_{16}}{\overset{k_{16}}{\rightleftharpoons}} MLTH\ (cytoplasm)$	$k_{16}([MLTH]_p - [MLTH]_c)$	$k_{16} = 10\ s^{-1}$
17. $MLT^{\cdot}\ (phagosome) \underset{k_{17}}{\overset{k_{17}}{\rightleftharpoons}} MLT^{\cdot}\ (cytoplasm)$	$k_{17}([MLT^{\cdot}]_p - [MLT^{\cdot}]_c)$	$k_{17} = 10\ s^{-1}$
18. $O_2^-\ (phagosome) \underset{k_{18}}{\overset{k_{18}}{\rightleftharpoons}} O_2^-\ (cytoplasm)$	$k_{18}([O_2^-]_p - [O_2^-]_c)$	$k_{18} = <\ 0.01\ s^{-1}$
NADPH oxidase:		

Beispiel für Systemgleichung:
coII’ = k2*coI*MLTH - k3*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)**

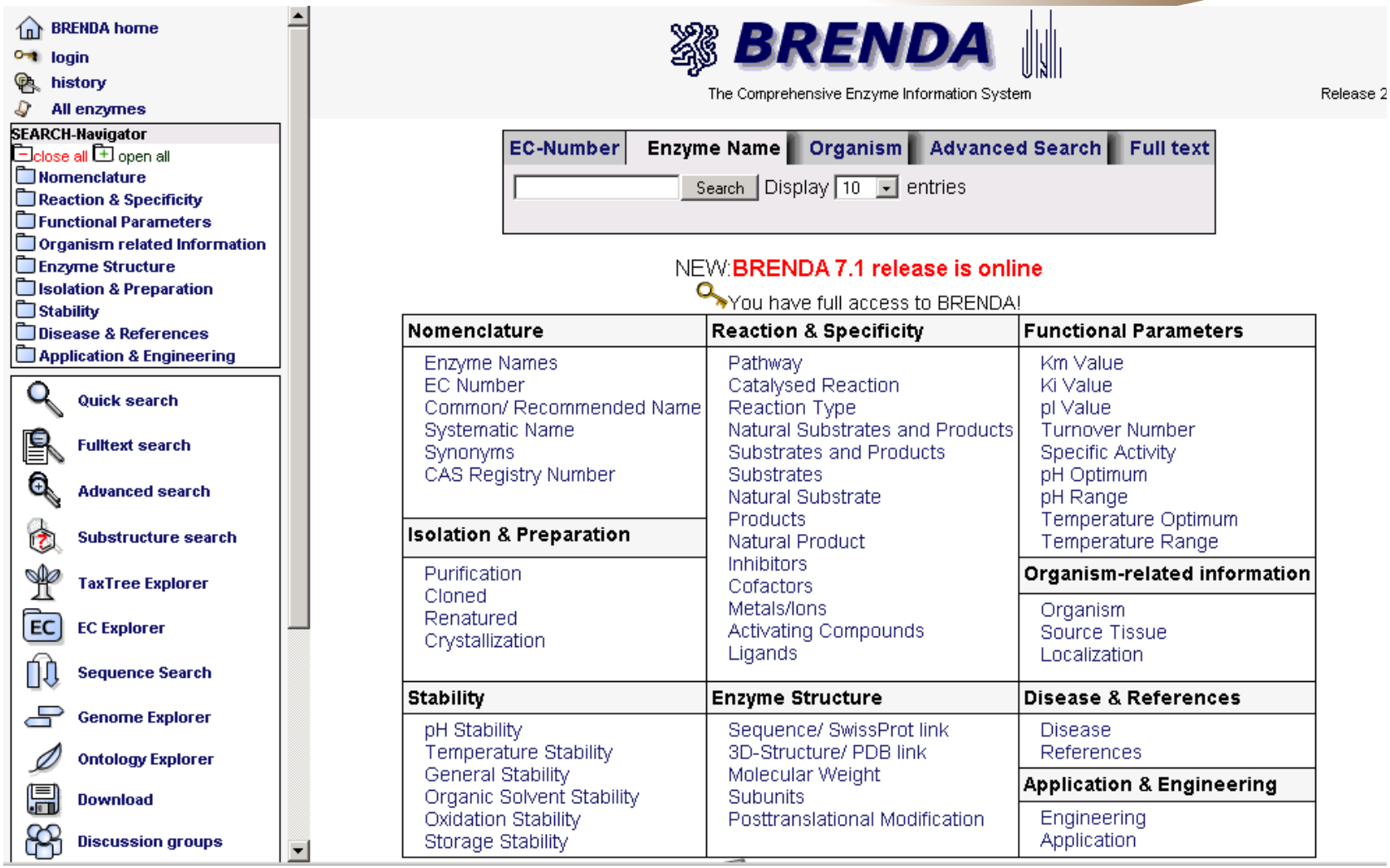
Einschub: Einheiten

- **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 Vmax & Km, discovery of the number of sites and the evolving "STANDARD" conditions.

Datenbanken im Netz

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



Datenbanken im Netz

http://sabio.villa-bosch.de/SABIORK/