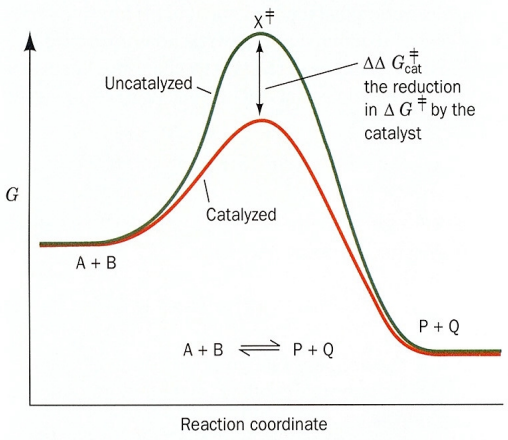
Enzyme



Pasteur (1850)	**force vitale**
Liebig (1850)	**Fermente = chemische Substanzen**
Kühne (1878)	**„En - zym“ = „in Hefe/Sauerteig“; Trypsin**
Buchner (1897)	**zellfreier Hefeextrakt produziert Alkohol**
Fischer (1894)	**Schlüssel-Schloss-Prinzip**
Sumner (1926)	**Enzyme sind Proteine (Urease)**
Northrop & Kunitz (1930)	**Kristallisation von Trypsin, Chymotrypsin, Elastase**

Substratspezifität
Wirkungsspezifität
Stereospezifität
geometrische Spezifität

im Vergleich zu chemischen Katalysatoren:

- **höhere Geschwindigkeit**
- **mildere Bedingungen**
- **höhere Spezifität**
- **regulierbar**

Enzymklassifikation

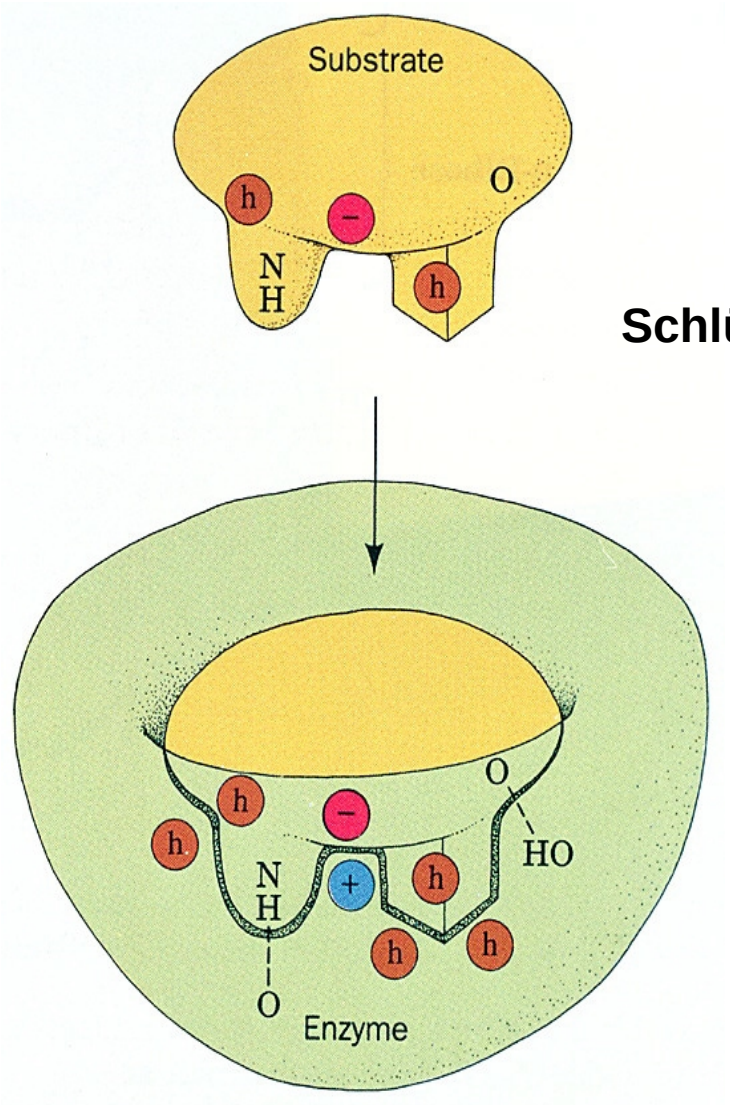
ENZYME CLASSIFICATION ACCORDING TO REACTION TYPE

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

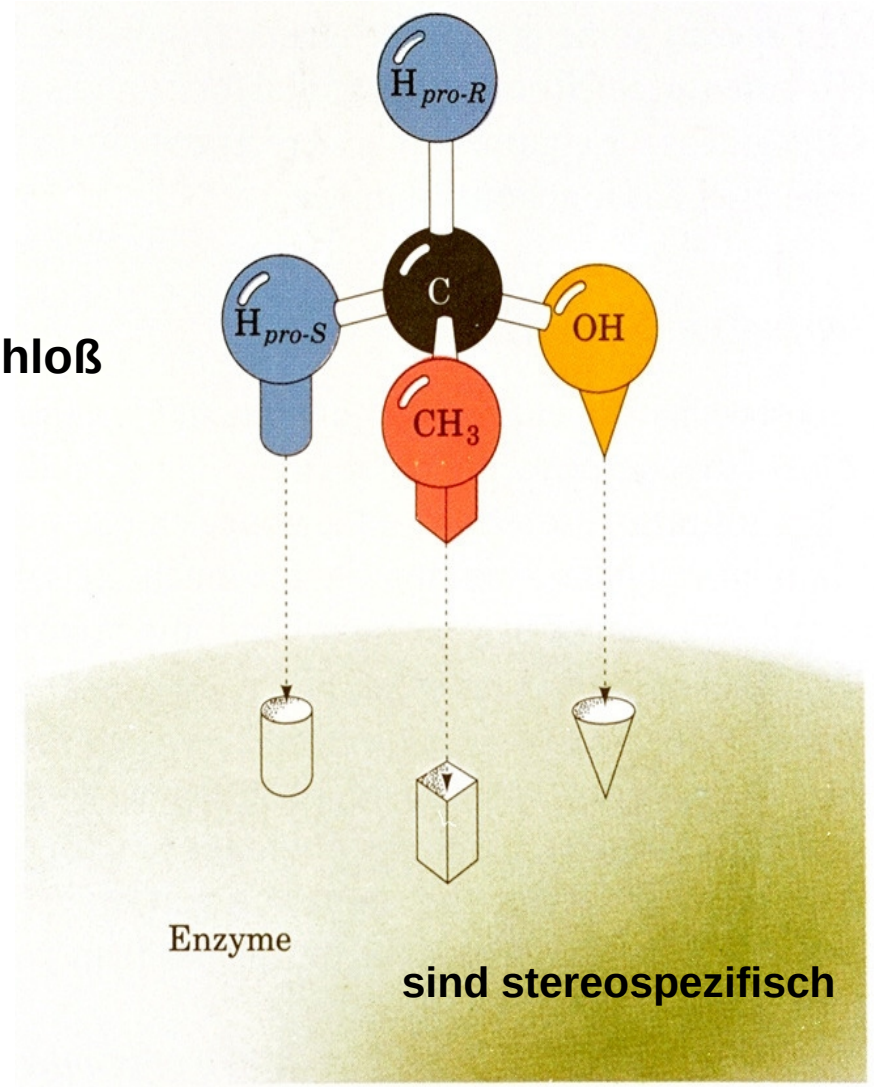
Dehydrogenasen
Kinasen
Amylase
Carboanhydrase
Prolin cis/trans Isomerase
Aminoacylsynthetase

EC Nummer:
Beispiel Carboxypeptidase, eine Peptidyl-L-aminosäurehydrolase, EC 3.4.17.1;
EC = Enzyme Commission, 3 = Enzymklasse (Hydrolasen),
4 = Unterklasse (Peptidasen), 17 = Unterunterklasse(Metallocarboxypeptidasen),
1 = Nummer des Enzyms in dieser Unterunterklasse

Enzym-Substratkomplex

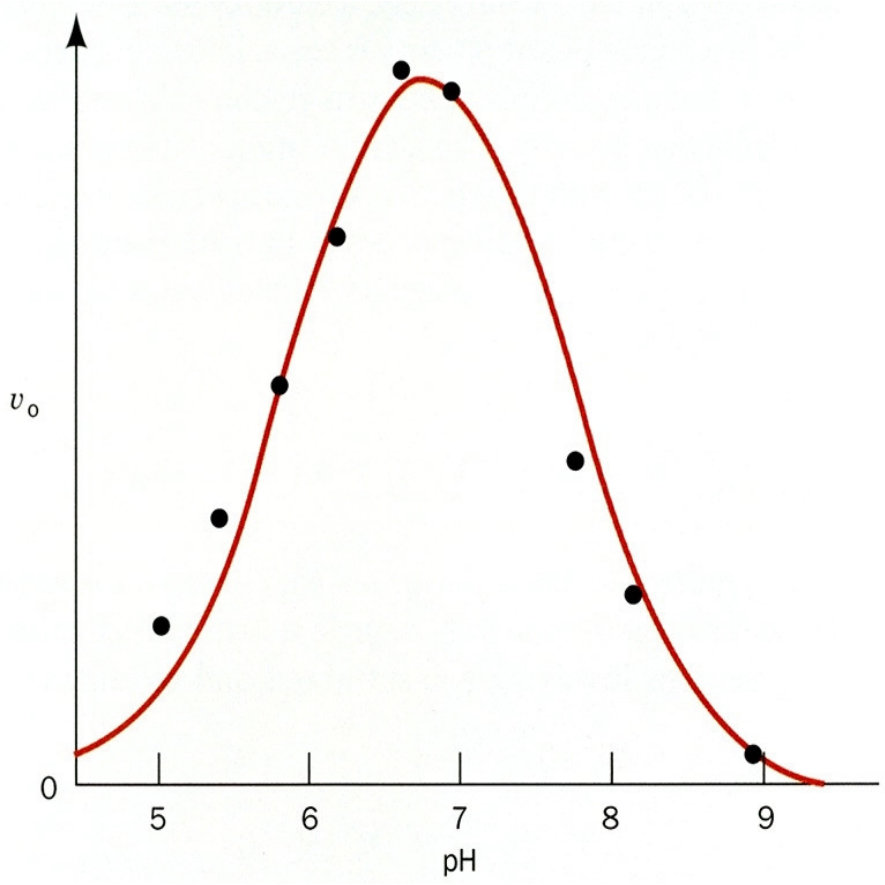


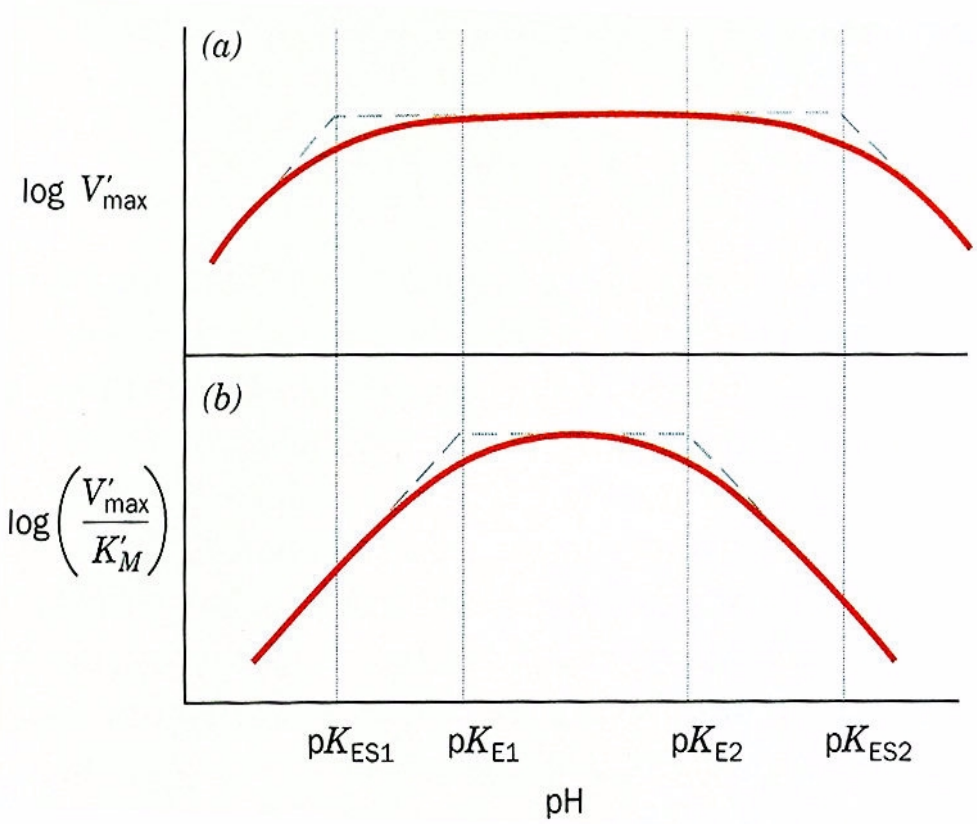
Schlüssel - Schloß Prinzip



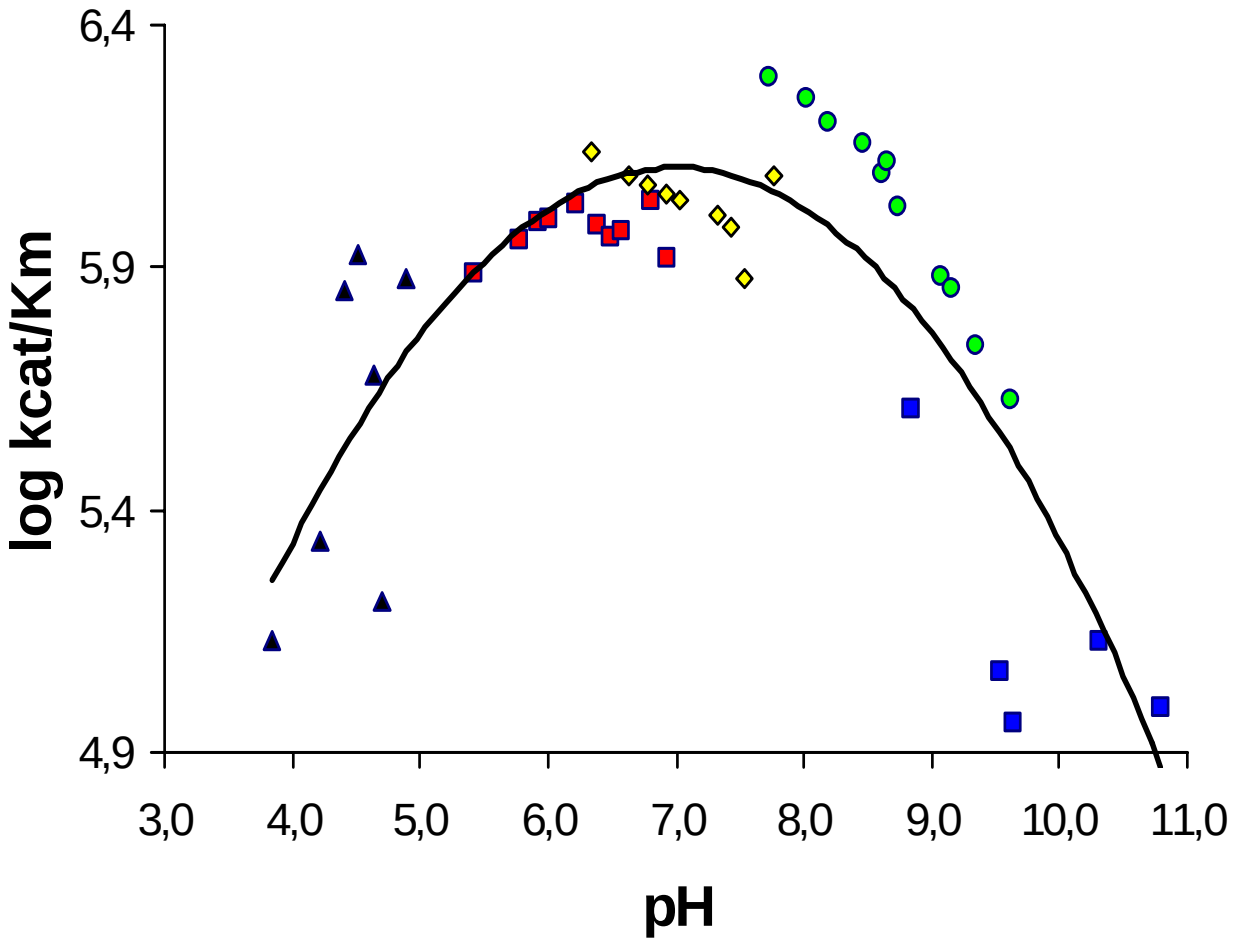
sind stereospezifisch

pH-Abhängigkeit von Enzymen



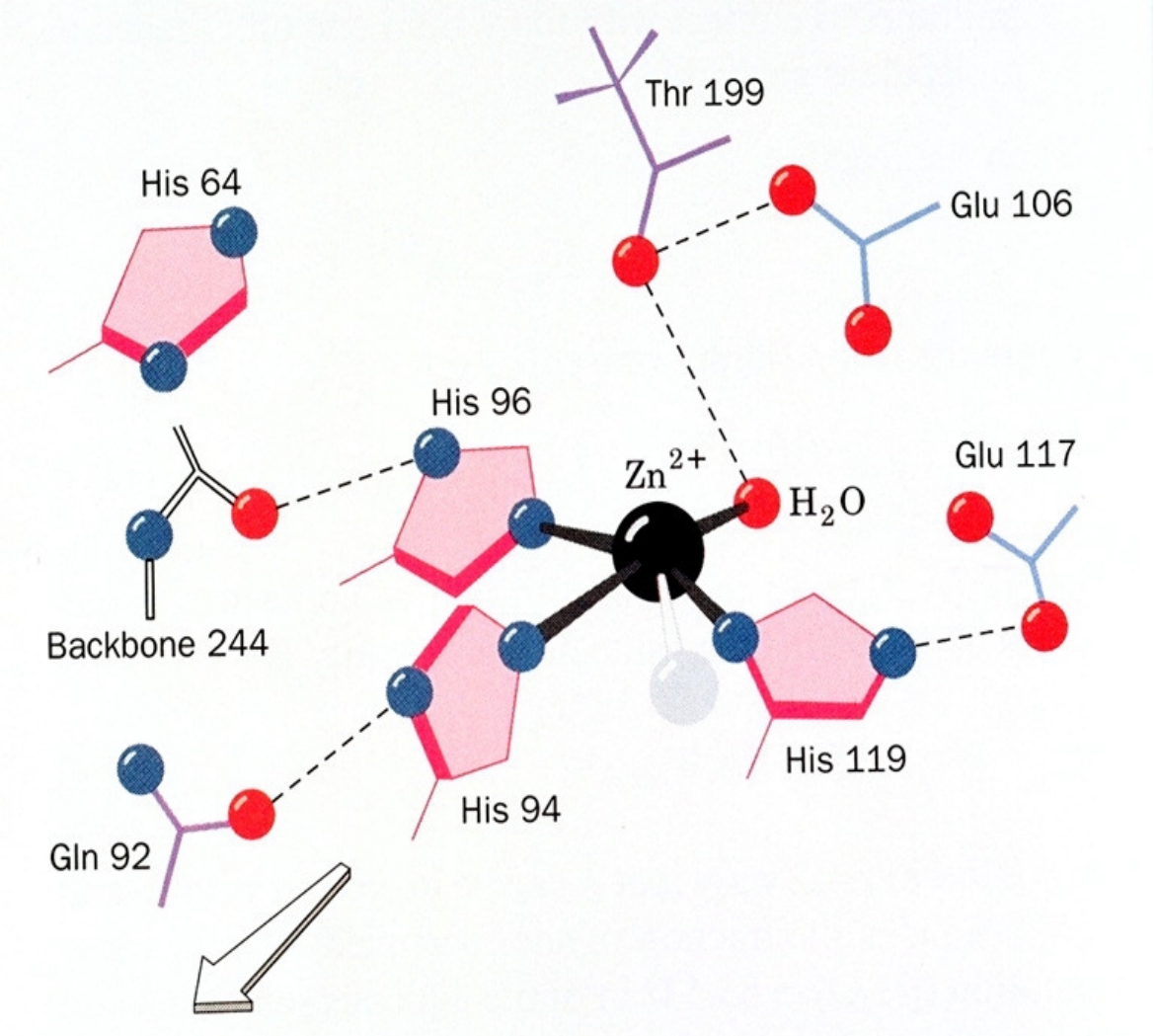


pH-activity profile of astacin



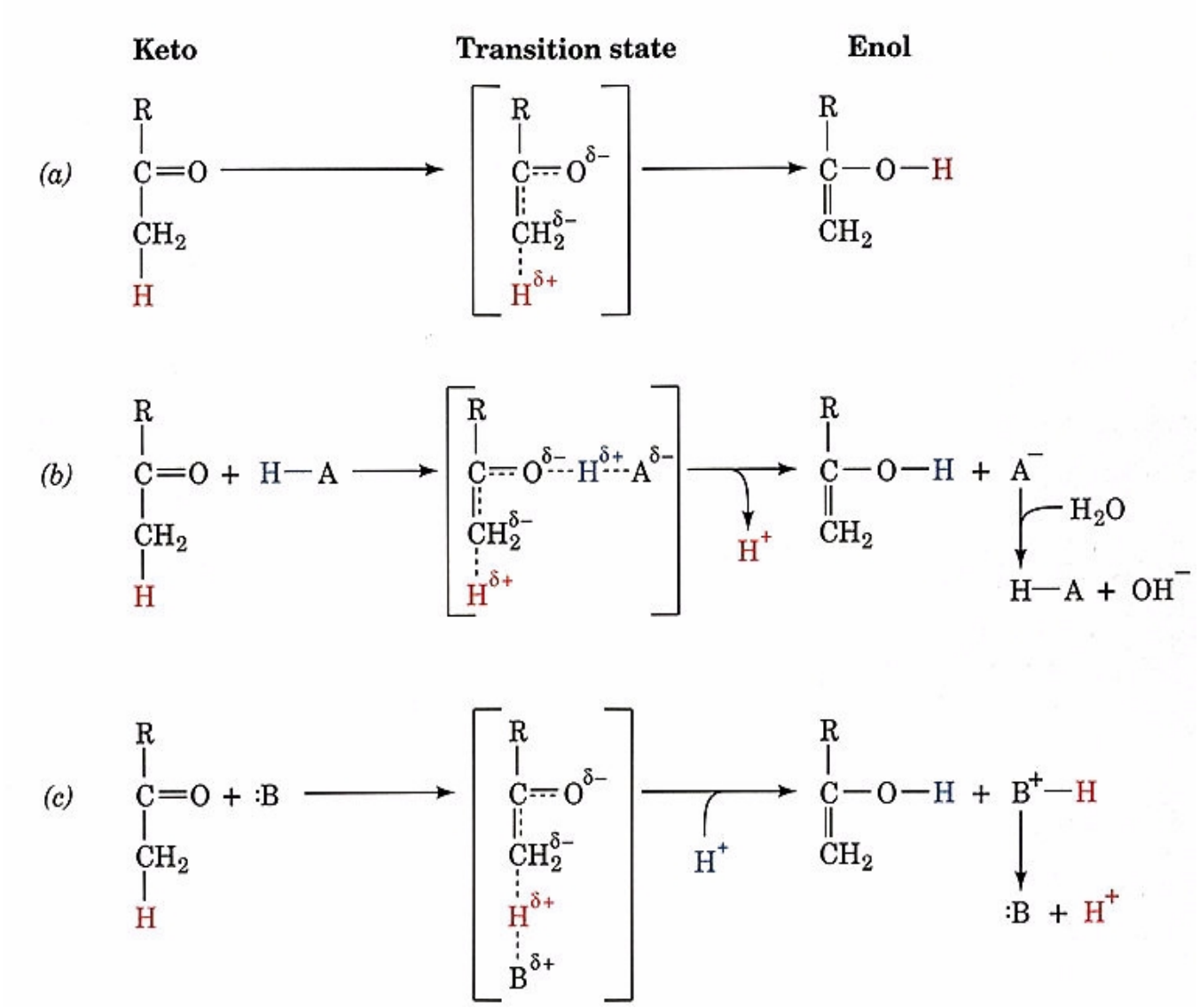
Metallionenkatalyse

Carboanhydrase

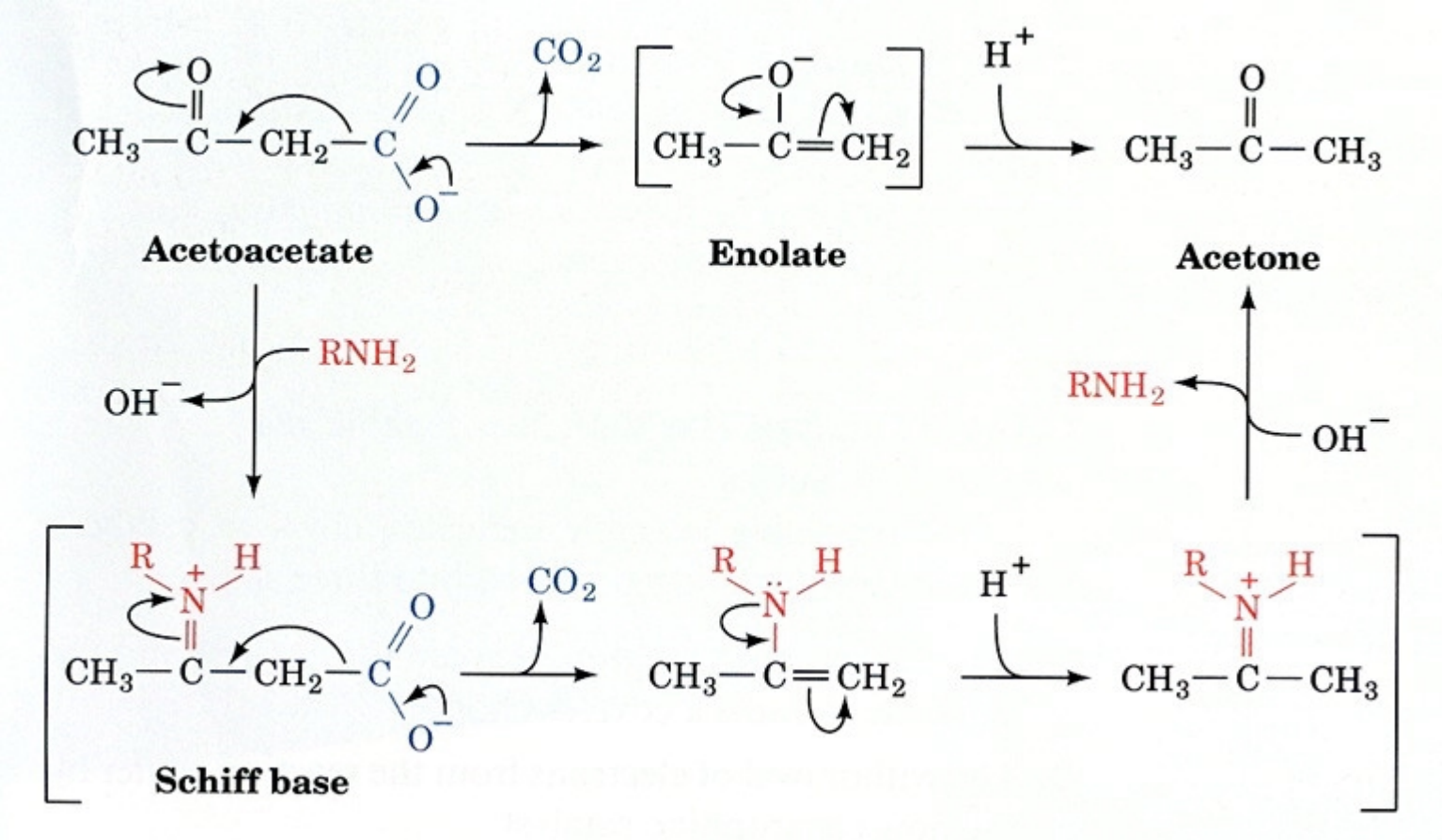


Säure-Basekatalyse

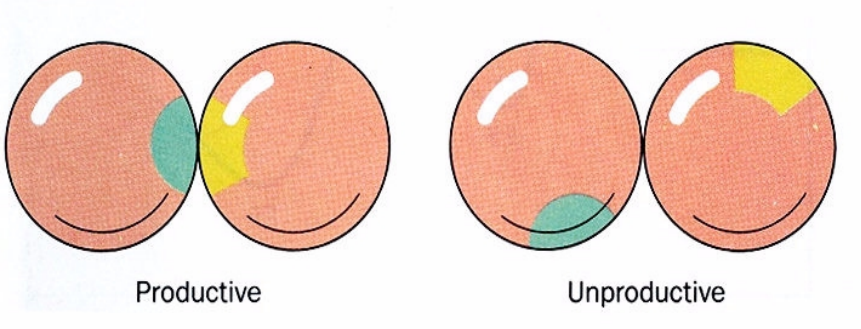
Mechanismus der Keto-Enol Tautomerisierung

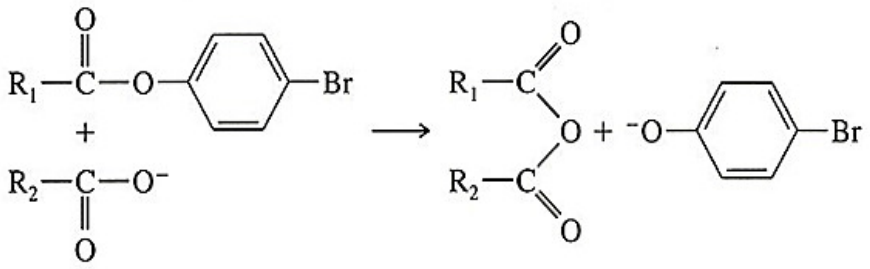


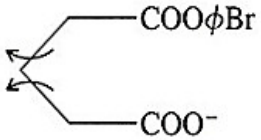
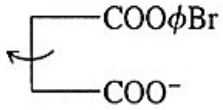
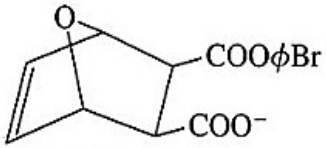
Decarboxylierung von Acetoacetat über ein kovalentes Zwischenprodukt



katalytische Wirkung durch Nachbargruppeneffekte

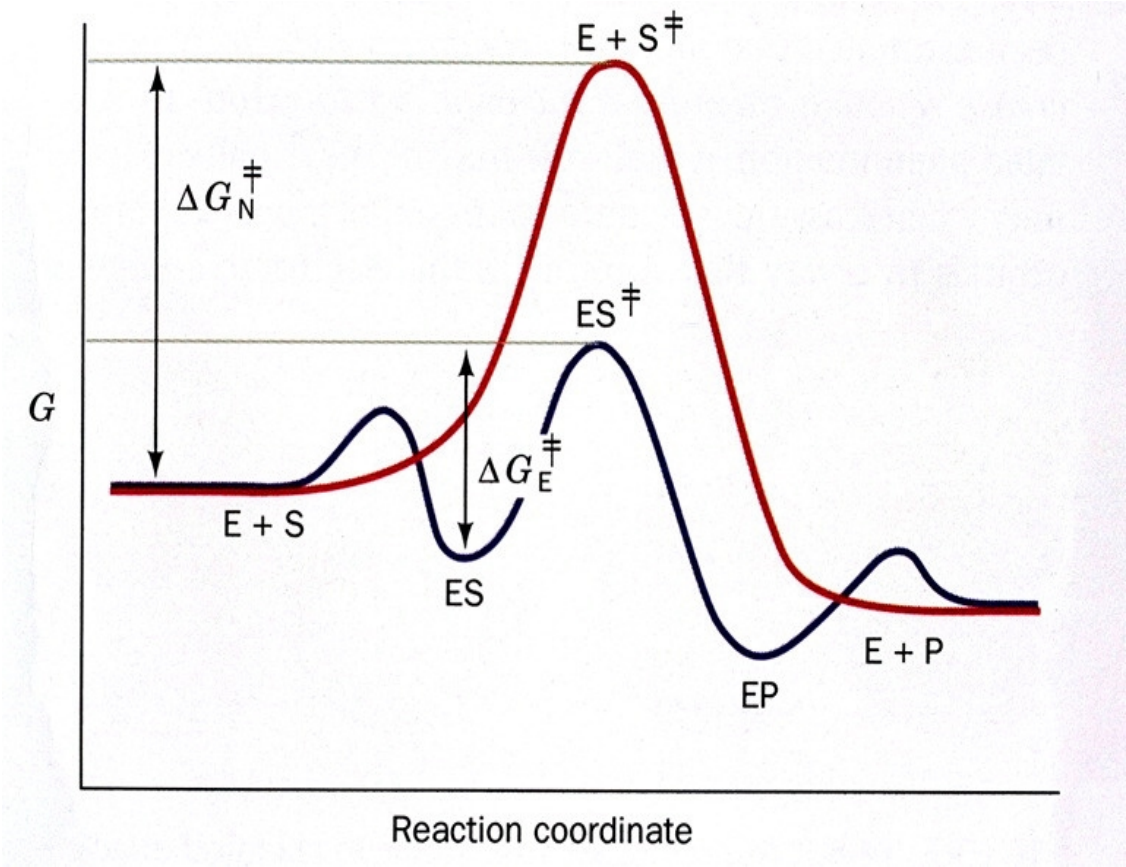




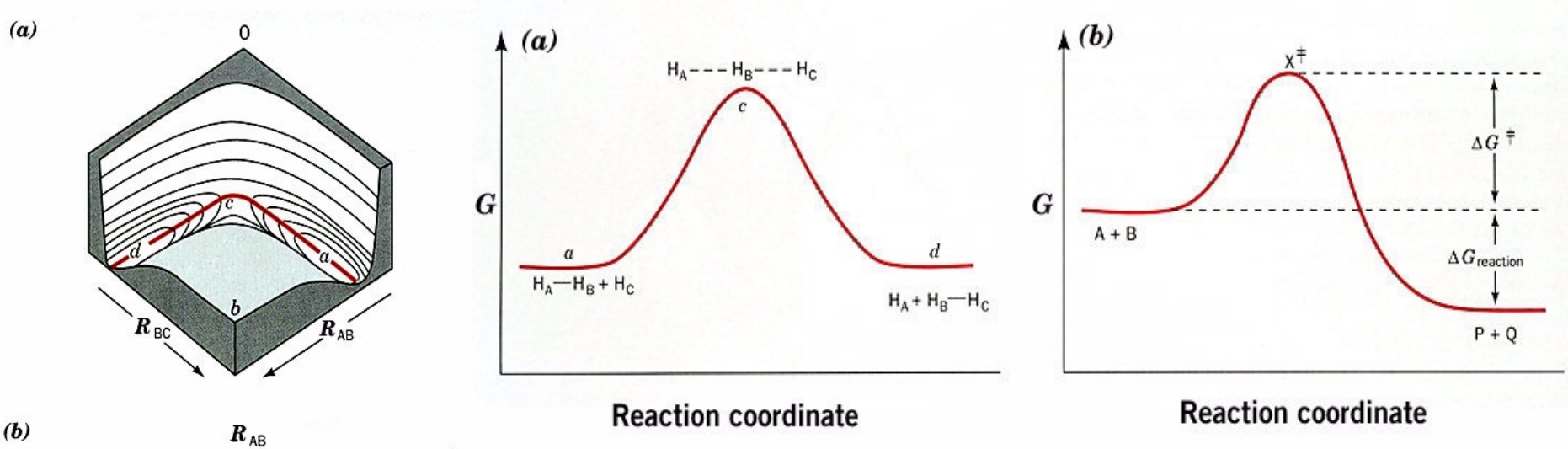
Reactants[a]	Relative Rate Constant
$CH_3COO\phi Br$ + CH_3COO^-	1.0
	$\sim 1 \times 10^3$
	$\sim 2.2 \times 10^5$
	$\sim 5 \times 10^7$

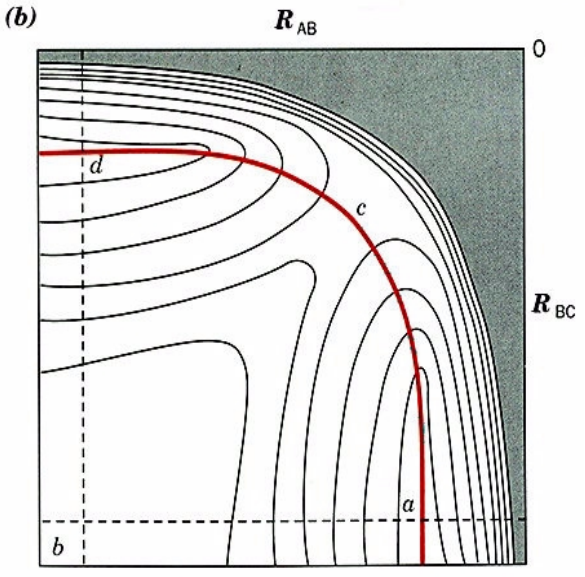
[a] Curved arrows indicate rotational degrees of freedom.
Source: Bruice, T.C., *Annu. Rev. Biochem.* **45,** 353 (1976).

Katalyse durch Stabilisierung des Übergangszustandes



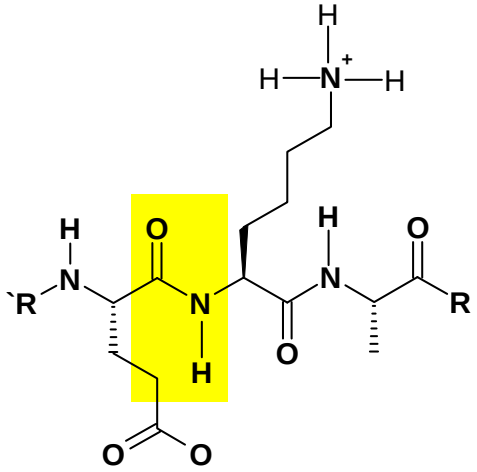
Theorie des Übergangszustandes





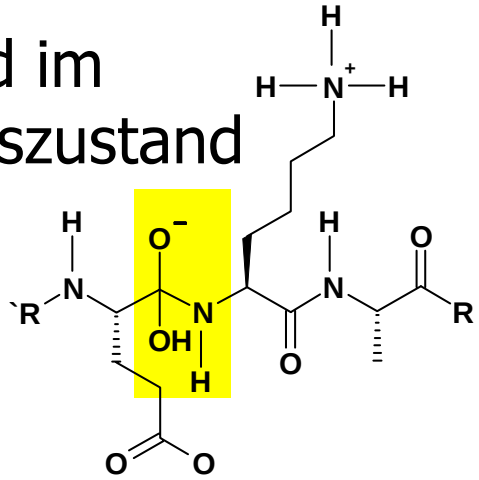
spontane Reaktion

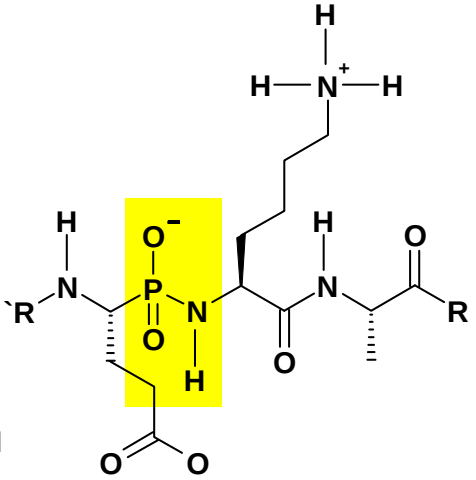
Enzyme binden nicht den Grundzustand besonders gut, sondern den Übergangszustand (ÜZ).



Peptid im Grundzustand

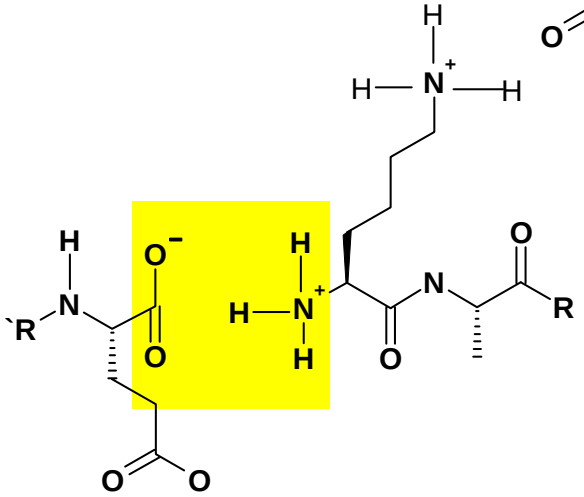
Peptid im Übergangszustand





Übergangszustand -Analogon

Hydrolysiertes Peptid



Katalytische Antikörper

William Jencks (1969)

Prinzip:
Antikörper binden mit hoher Affinität an Peptide, Kohlenhydrate, Nucleinsäuren, Lipide, usw. Sie binden den „Grundzustand“.

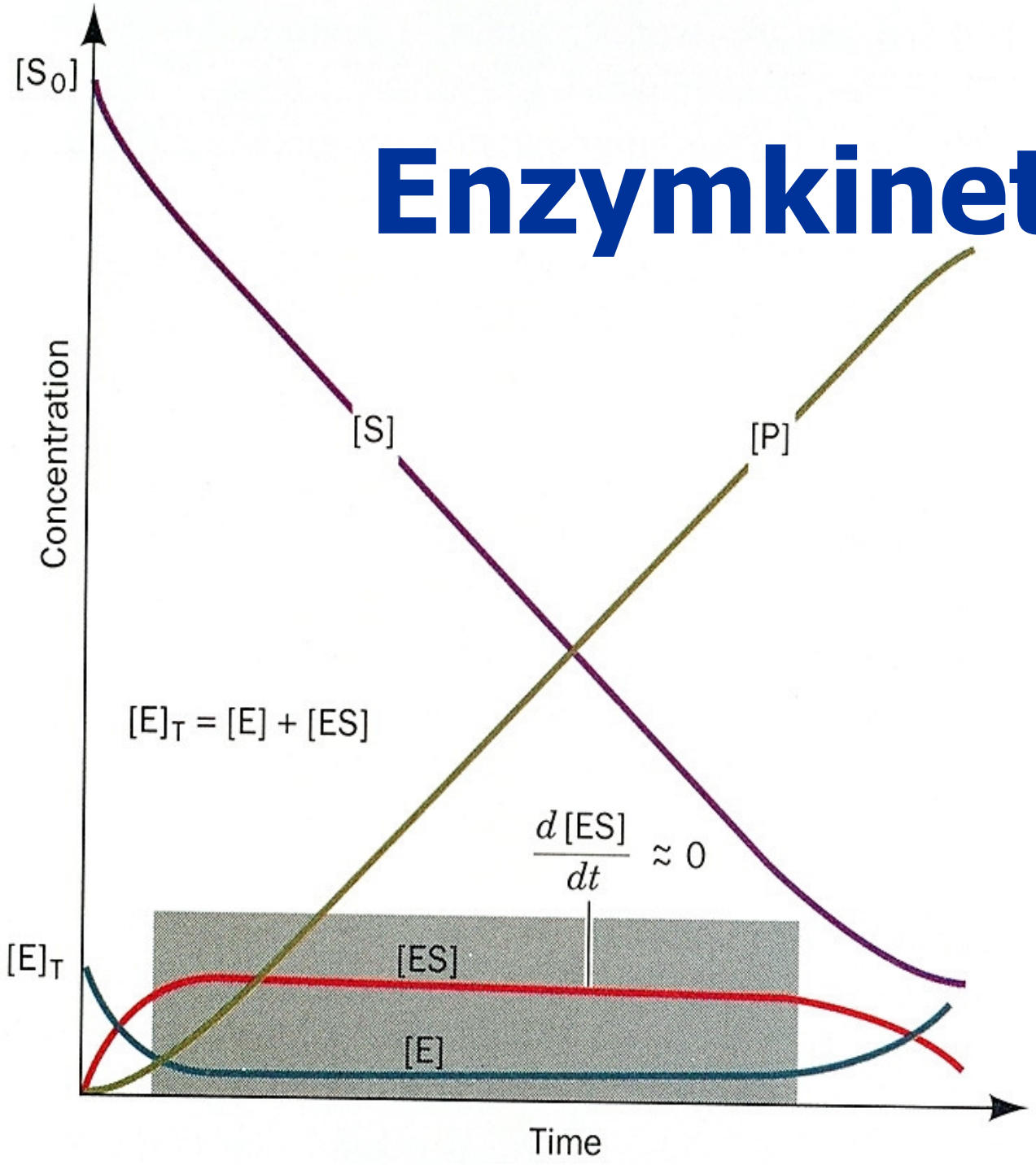
Enzyme binden nicht den Grundzustand einer Struktur sondern den „Übergangszustand“.
Dadurch erhöhen sie die Existenzdauer dieses Übergangszustandes und ermöglichen dadurch, dass eine Reaktion leichter ablaufen kann (Katalysatorfunktion).

Antikörper gegen den ÜZ

-Immunisiert man ein Wirbeltier mit einem Analogon des ÜZ einer chemischen Reaktion, so erhält man Antikörper, die den ÜZ dieser Reaktion stabilisieren.

- Dadurch können diese Antikörper als Katalysatoren wirken, weil sie die Wahrscheinlichkeit der Existenz des ÜZ erhöhen.

Enzymkinetik



Die Kinetik der Invertinwirkung.

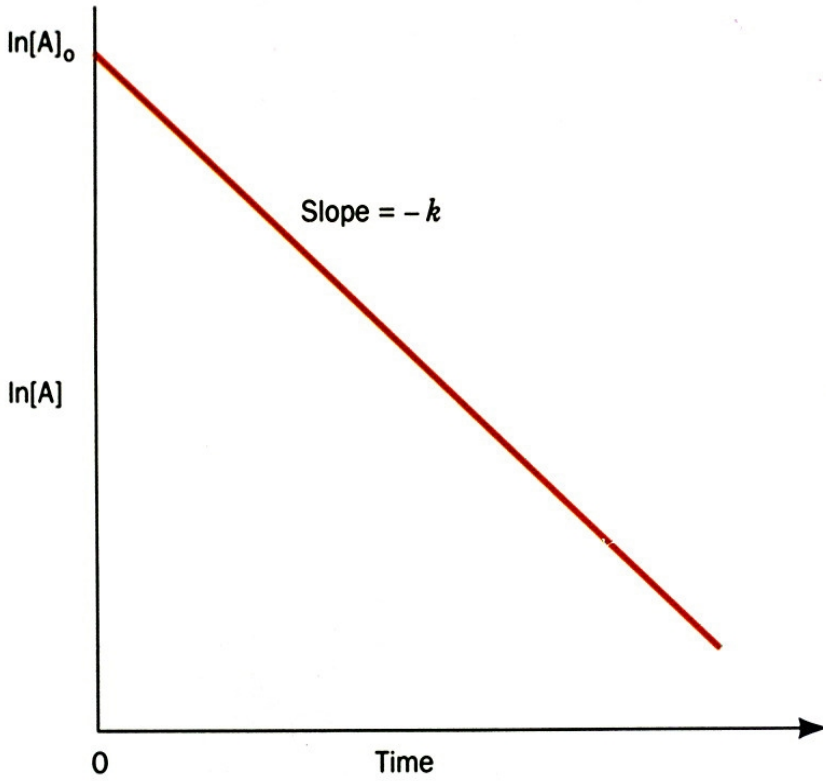
Von

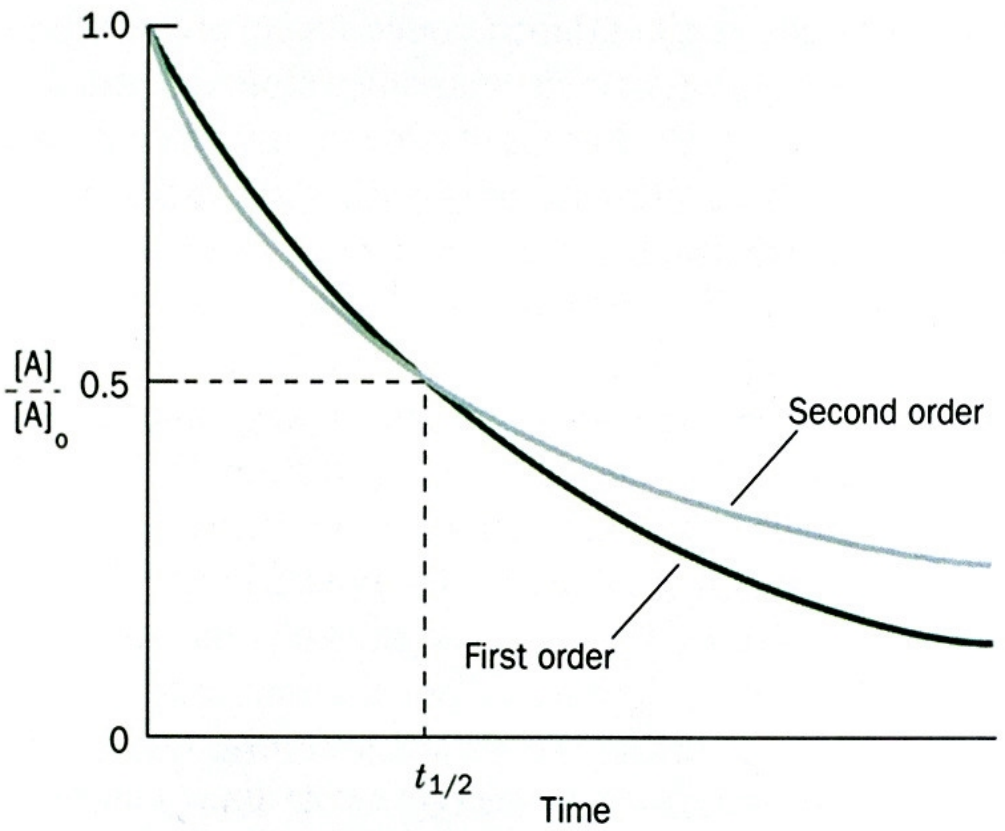
L. Michaelis und Miß Maud L. Menten.

(Eingegangen am 4. Februar 1913.)

Mit 19 Figuren im Text.

Enzymkinetik





Enzymkinetik

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

$$v = \frac{d[P]}{dt} = k_2[ES] \quad (1)$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] \quad (2)$$

1. Annahme: Gleichgewicht
(Henri, Michaelis, Menten) $k_{-1} >> k_2$

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

2. Annahme: Fließgleichgewicht (steady state)
(Briggs, Haldane) [ES] = const.

$$\frac{d[ES]}{dt} = 0 \quad (3)$$

$$[E]_t = [E] + [ES] \quad (4)$$

aus (2,3,4)

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

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

$$[ES] = \frac{[E]_t[S]}{K_m + [S]} \quad (5) \text{ wobei } K_m = \frac{k_{-1} + k_2}{k_1} \quad (6)$$

(5) in (1)

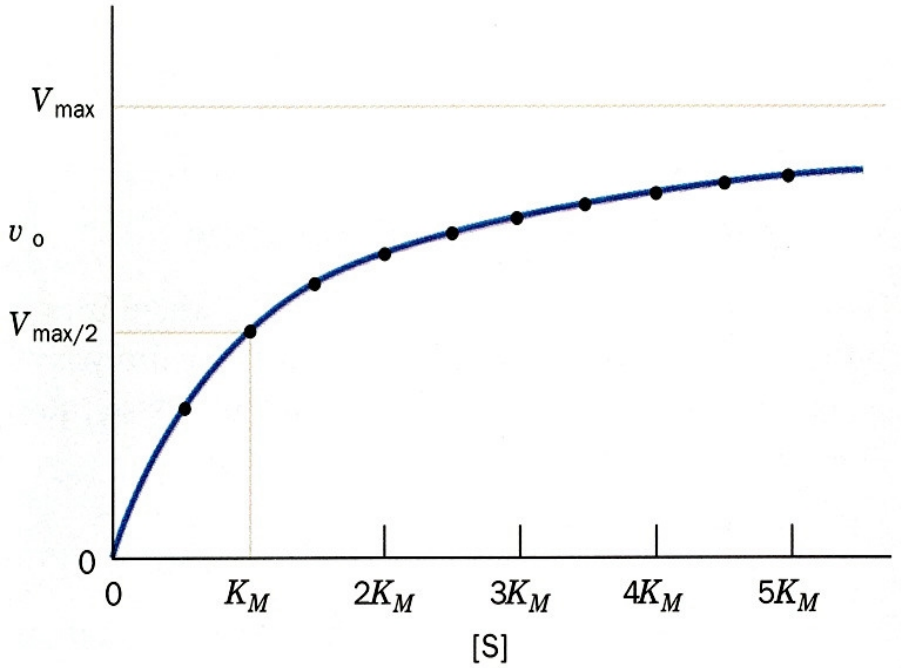
$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E]_t[S]}{K_m + [S]}$$

$$V_{max} = k_2[E]_t \qquad v = \frac{V_{max}[S]}{K_m + [S]}$$

Michaelis-Menten-Kinetik

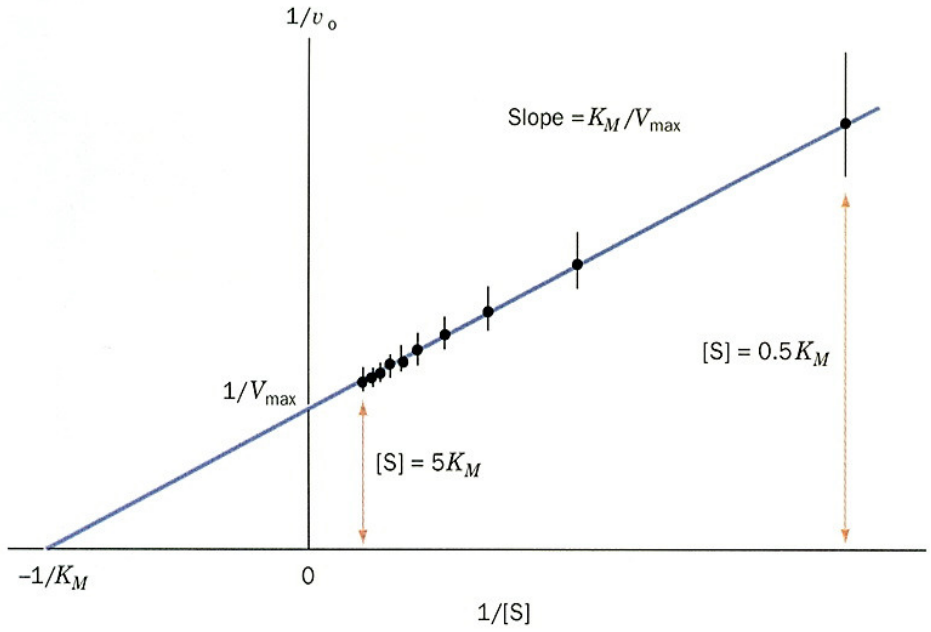
Michaelis-Menten Diagramm

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



Lineweaver-Burk Diagramm

$$1/v = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$



kinetische Parameter von Enzymen

Umsatzzahl:

$$k_2 = k_{cat} = \frac{V_{max}}{[E]_t}$$

;

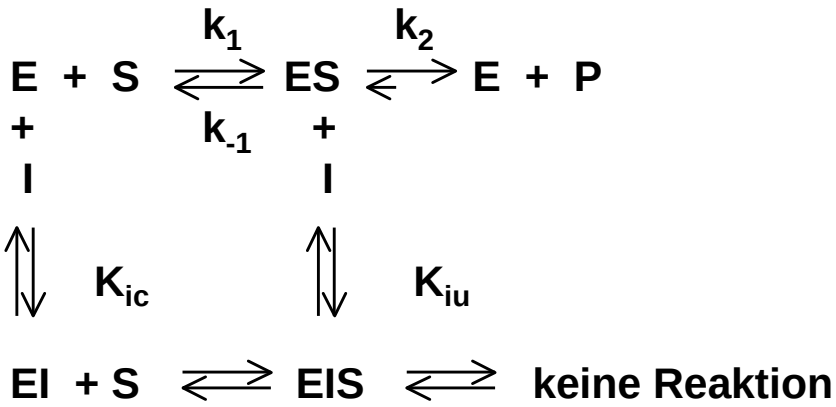
Spezifitätskonstante (Geschwindigkeitskonstante 2. Ord.)

für $[S] << K_m$ folgt: $v = \frac{k_{cat}}{K_m} [S] [E]_t$

$k_{cat}/K_m = k_2/K_m = k_1 \times k_2 / k_{-1} + k_2$; max., wenn $k_2 >> k_{-1}$; also wenn Produktbildung favorisiert ist vorDekomp. des [ES]. Aber k_1 ist maximal so groß wie die Frequenz der Molekülzusammenstöße.

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}\ s^{-1}$)
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}

Enzymhemmung

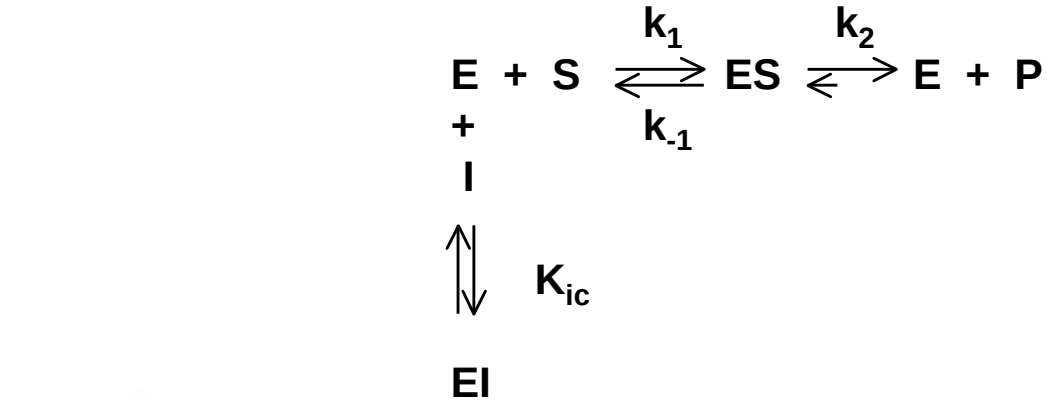


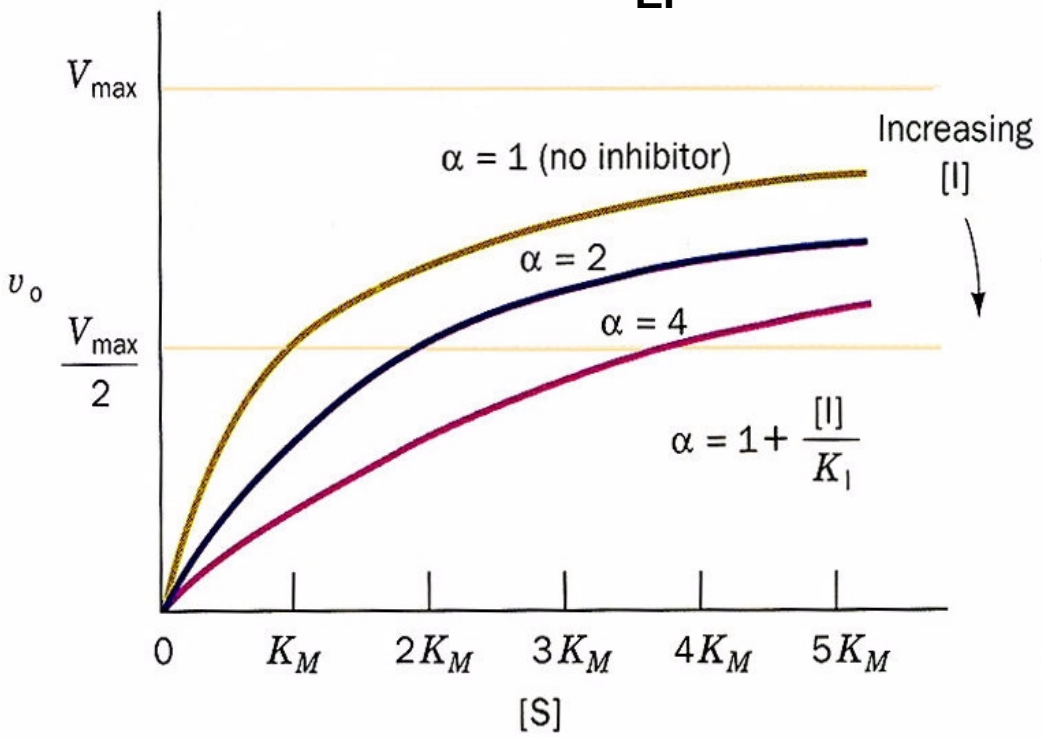
Inhibitorkinetik

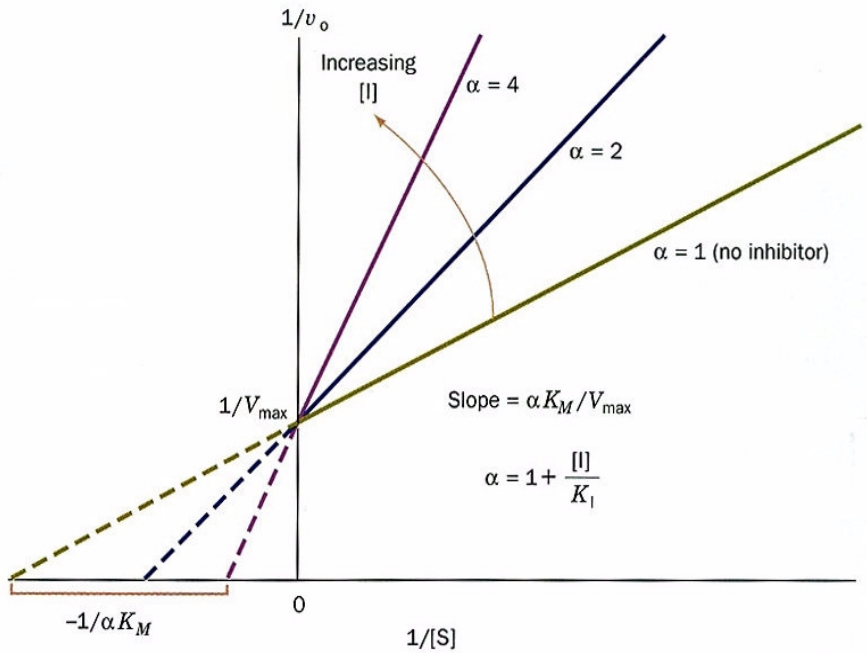
$$v = \frac{V_{max}\ [S]}{K_m + [S]}; \qquad 1/v = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$v = \frac{V_{max}\ [S]}{K_m\,(1+[I]/Ki) + [S]}; \quad 1/v = \frac{K_m\,(1+[I]/Ki)}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

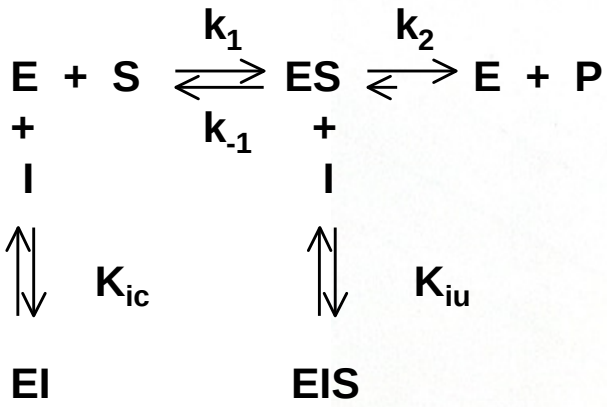
kompetitive Hemmung



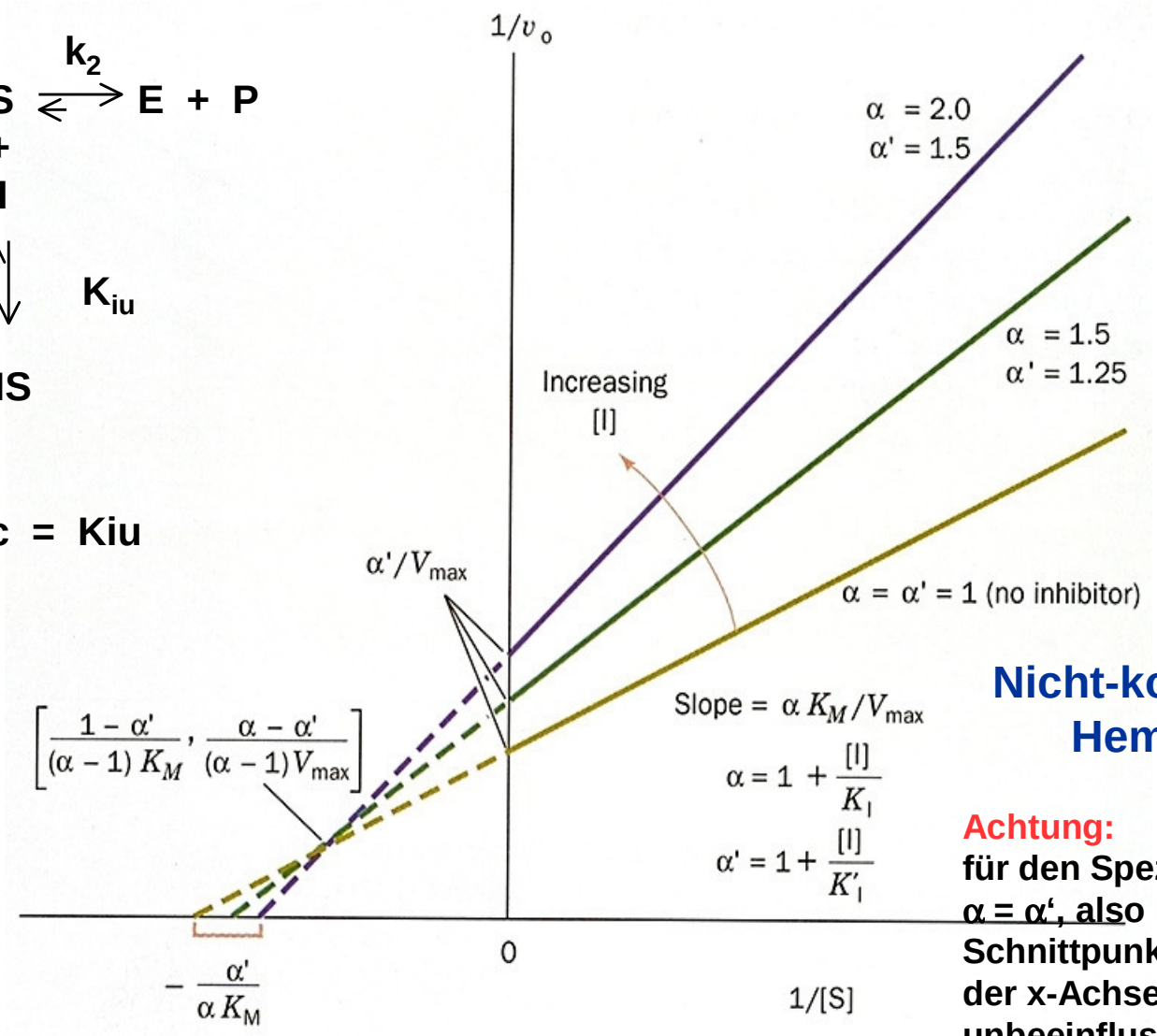




Gemischte Hemmung



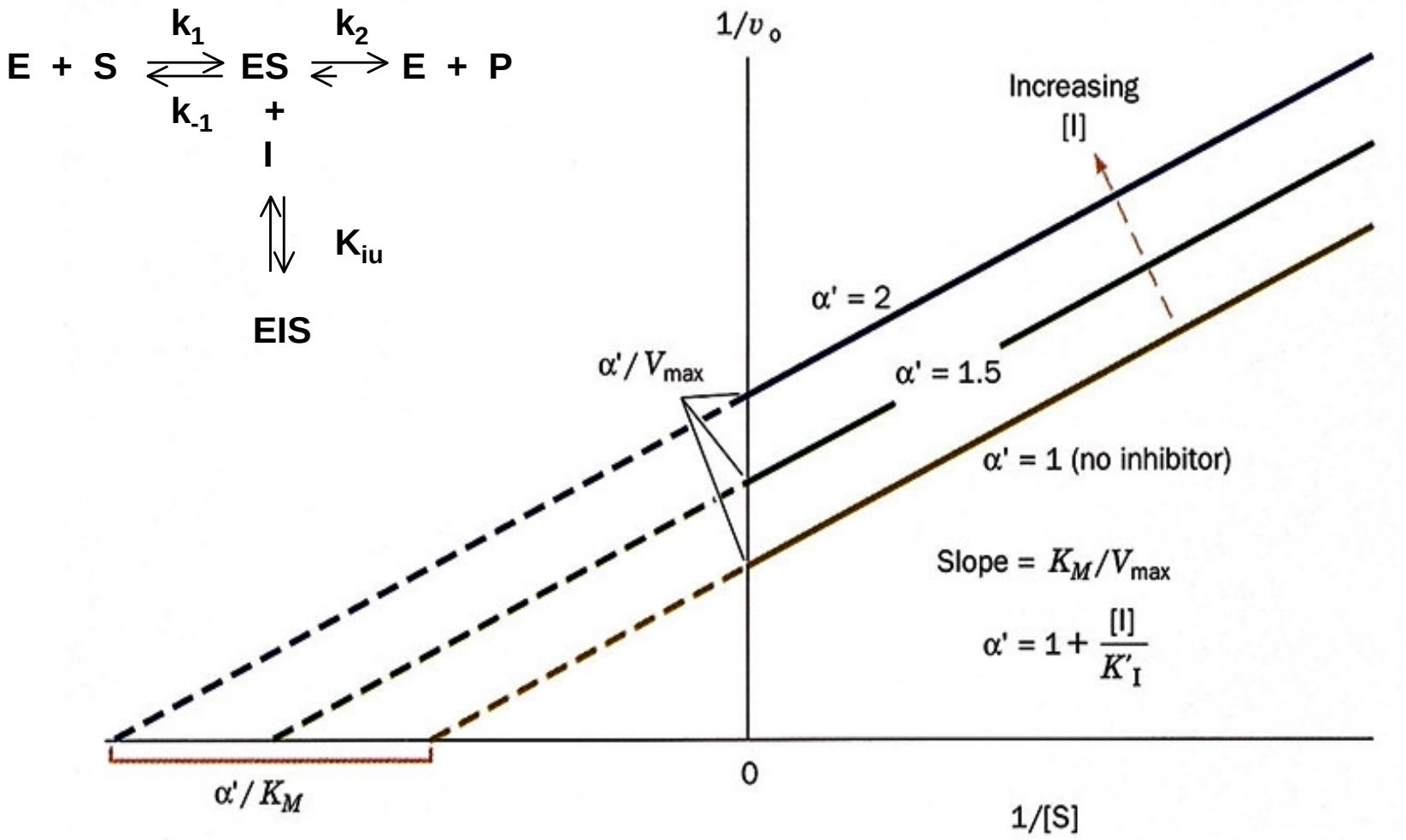
Spezialfall: Kic = Kiu



Nicht-kompetitive Hemmung

Achtung:
für den Spezialfall
α = α', also $K_{ic} = K_{iu}$, liegt der Schnittpunkt der Geraden auf der x-Achse, d.h. K_m bleibt unbeeinflusst.

Unkompetitive Hemmung

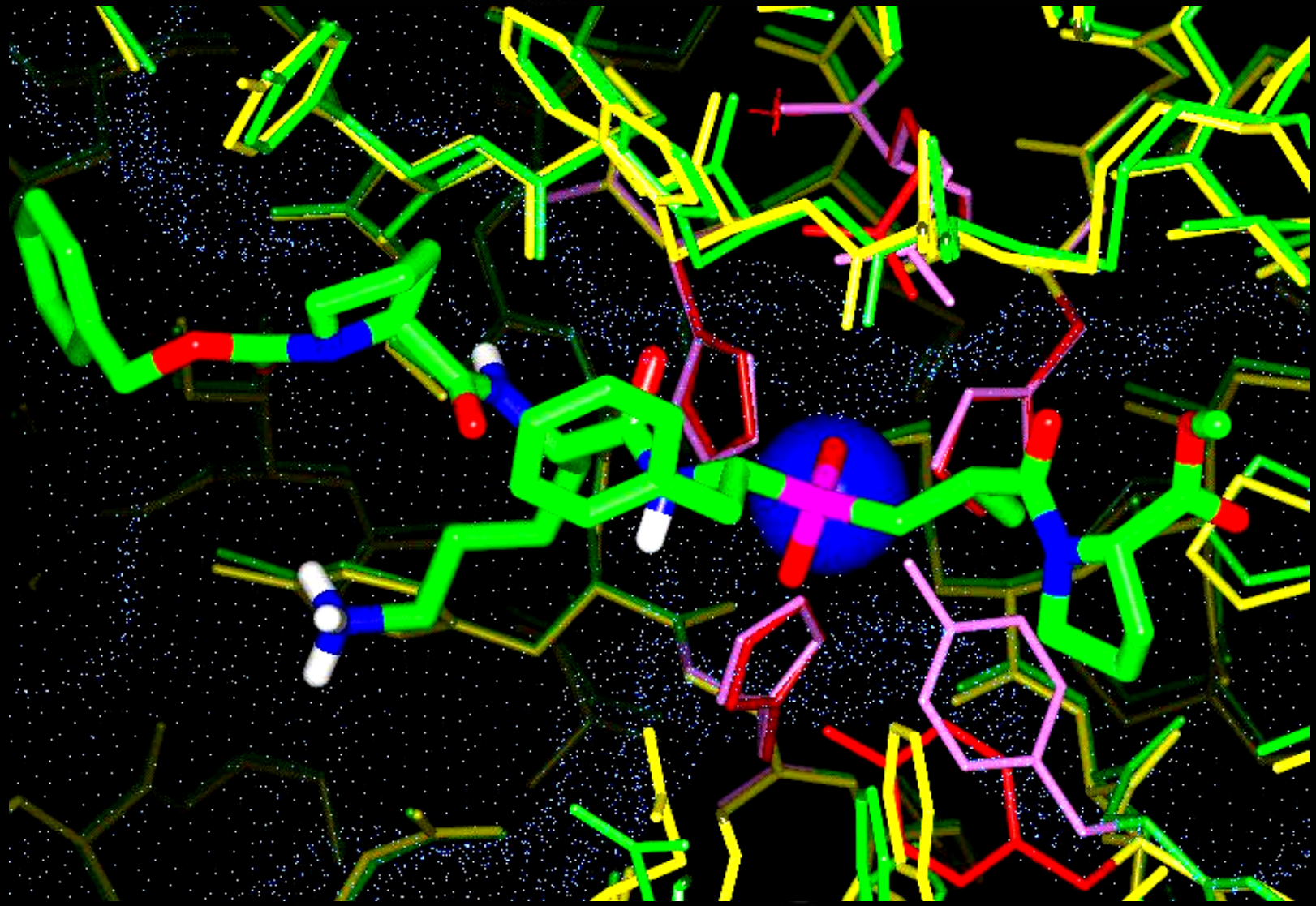


Reversible Enzymhemmung

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'	$\alpha K_M/\alpha'$

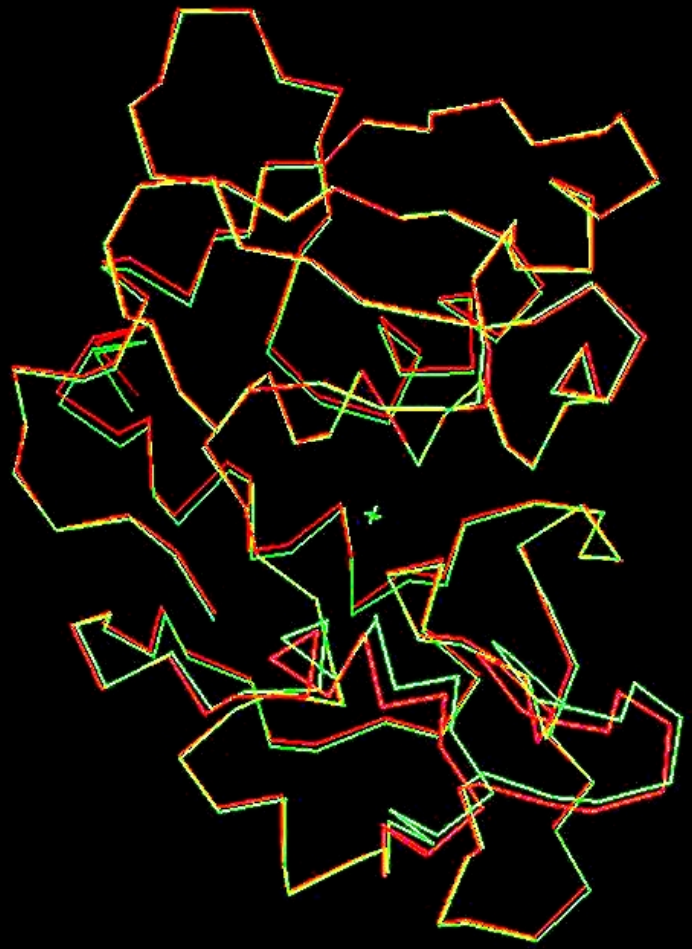
[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K_I'}$

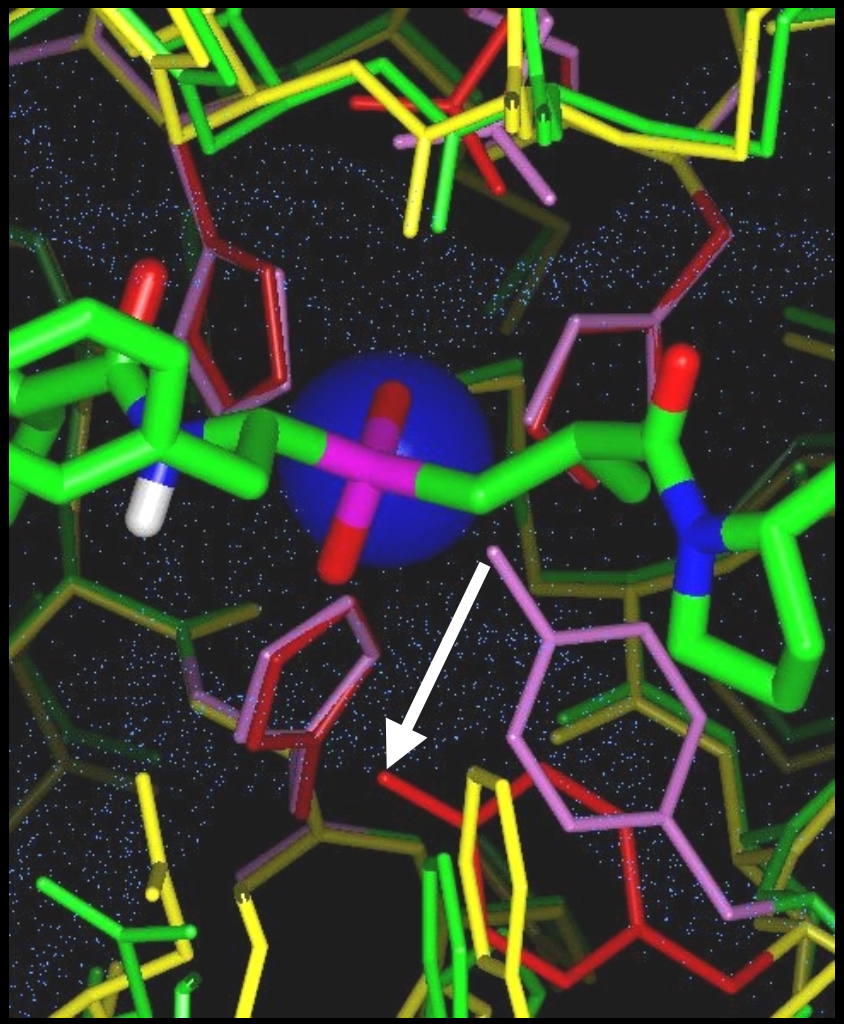
Zink-Hydrolase-Inhibitor Komplex



Grams, F., Dive, V., Yiotakis, A., Yiallouros, I., Vassiliou, S., Zwilling, R., Bode, W. & Stöcker, W. Nature Struct. Biol. 3, 671-675 (1996).

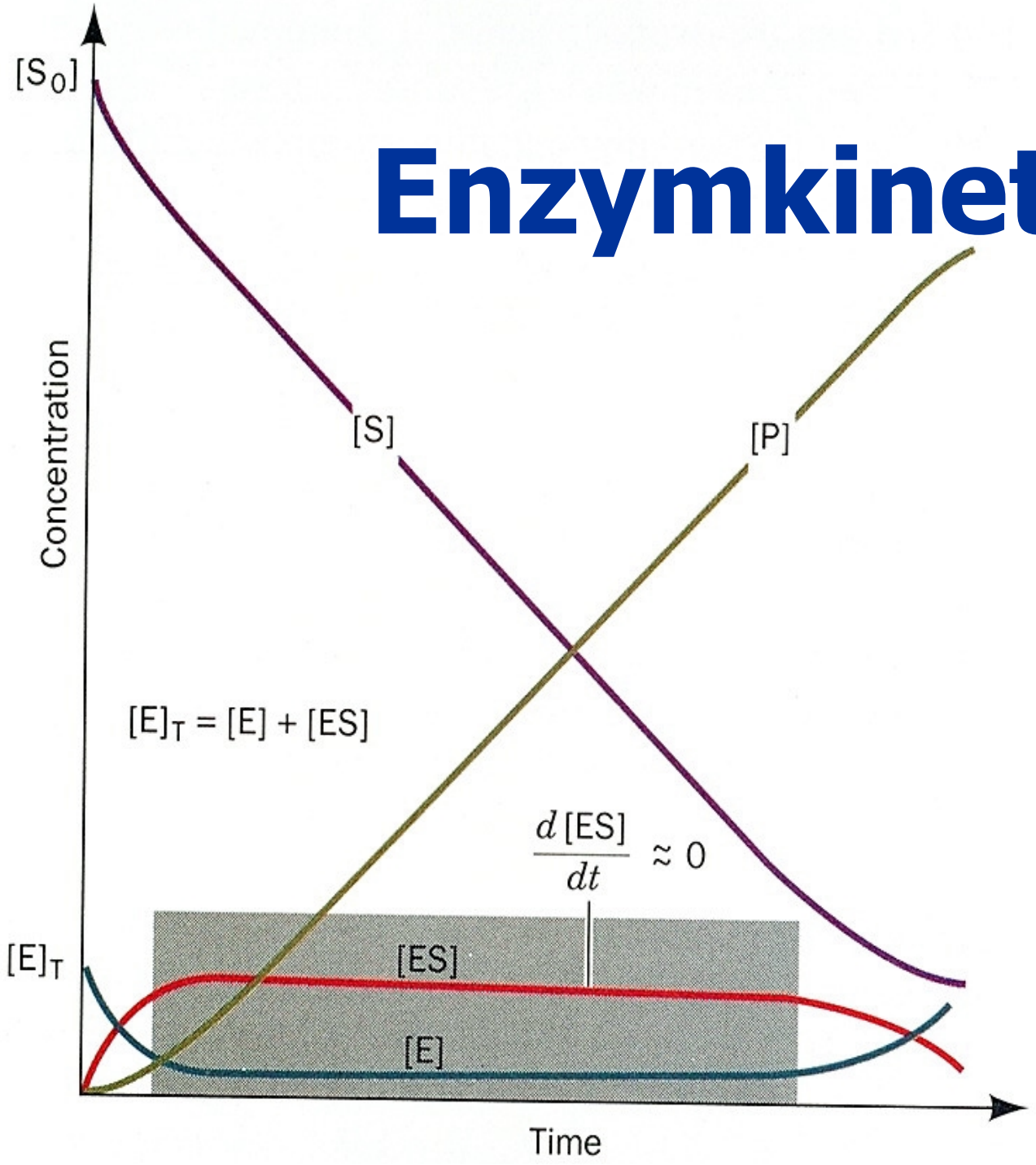
Konformationsänderung (Induced Fit) bei der Katalyse



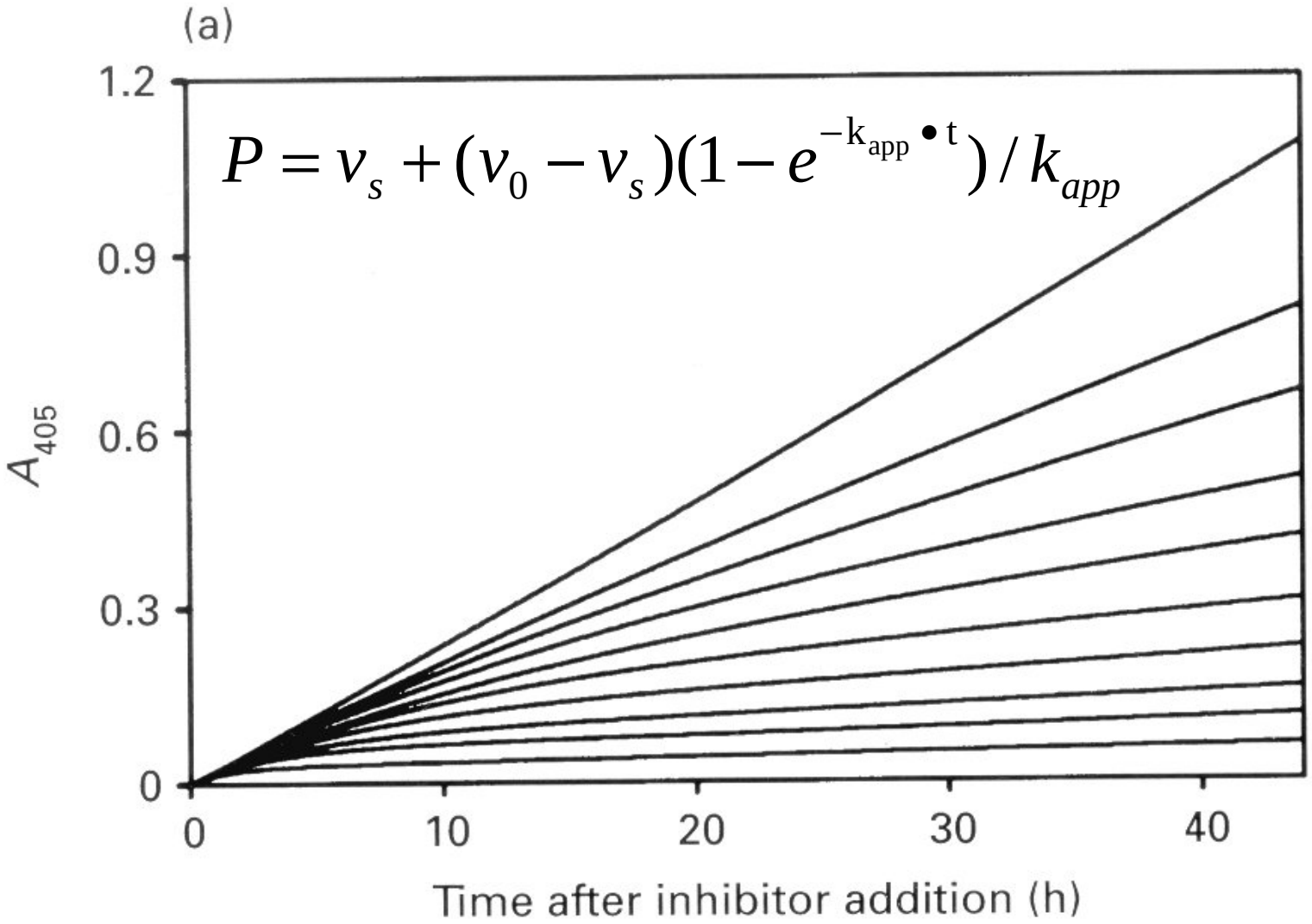


Grams, F., Dive, V., Yiotakis, A., Yiallouros, I., Vassiliou, S., Zwilling, R., Bode, W. & Stöcker, W. Nature Struct. Biol. 3, 671-675 (1996).

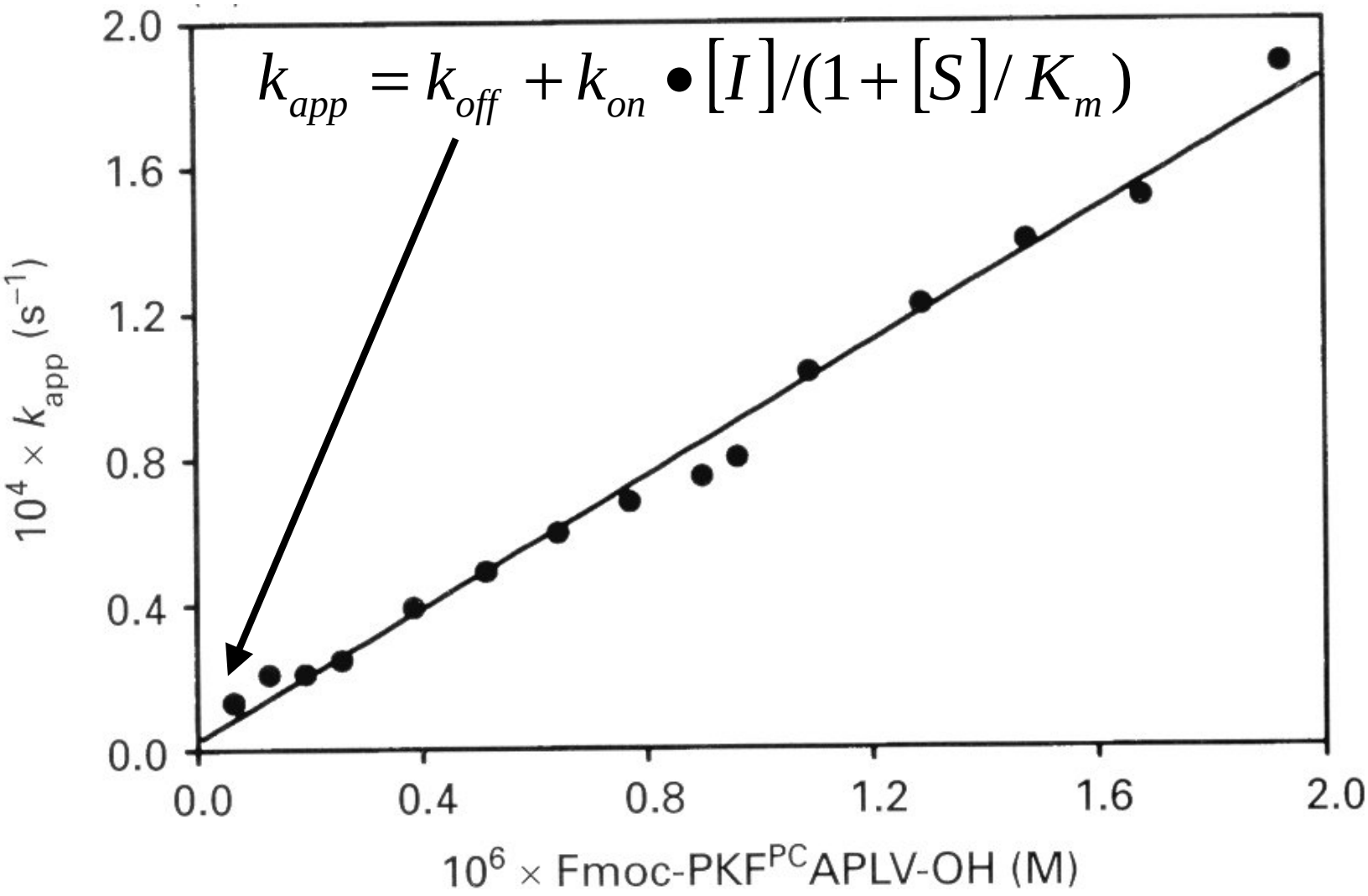
Enzymkinetik



Slow Binding Inhibition



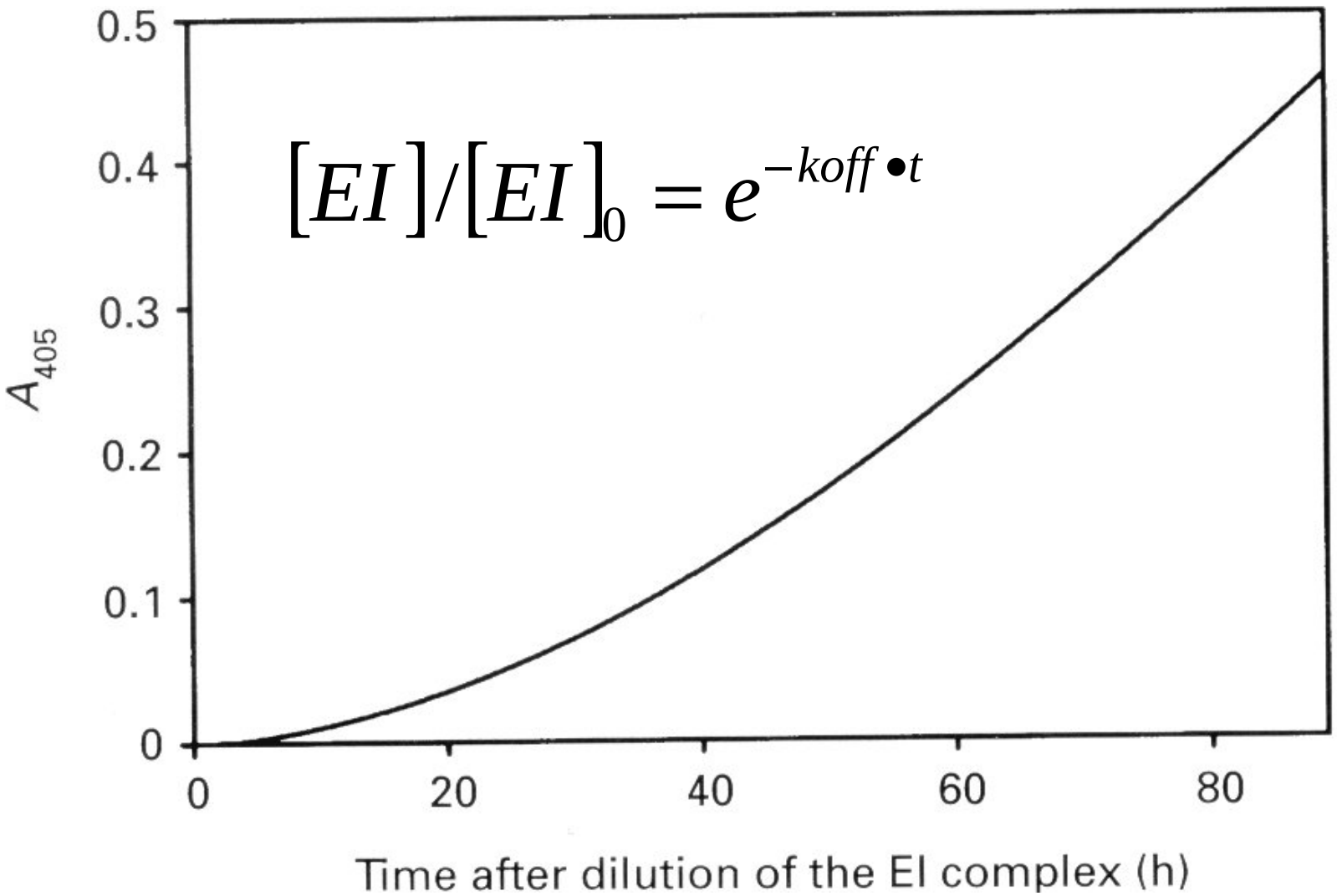
k_{off}


$k_{app} = k_{off} + k_{on} \bullet [I]/(1 + [S]/K_m)$
2.0
1.6
1.2
0.8
0.4
0.0
$10^4 \times k_{app}$ (s^{-1})
0.0
0.4
0.8
1.2
1.6
2.0
$10^6 \times$ Fmoc-PKF^{PC}APLV-OH (M)

$$K_i = \frac{k_{off}}{k_{on}}$$

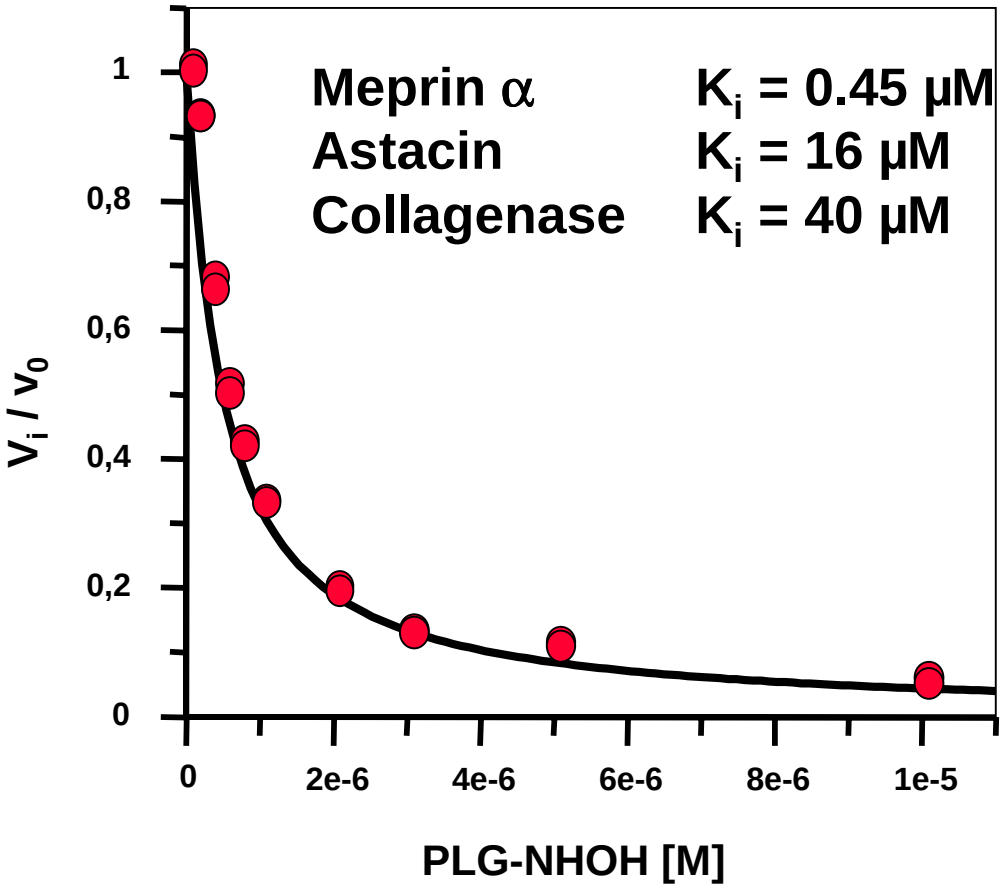
$$\frac{1}{t_{1/2}} = k_{off} + k_{on}[I]_o$$

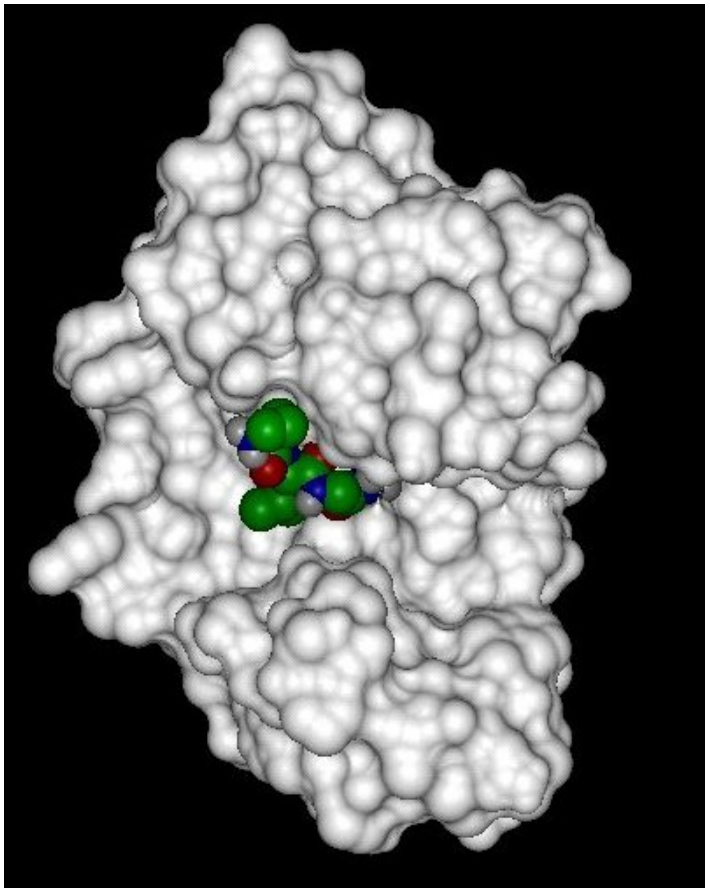
k_{off}


$[EI]/[EI]_0 = e^{-koff \bullet t}$
A_{405}
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
Time after dilution of the EI complex (h)

Inhibition of Human Meprin α by PLG-NHOH

$$\frac{v_i}{v_o} = \sqrt{1 - \left[(I_o + E_o + K_i) - (I_o + E_o + K_i)^2 - 4 \bullet E_o \bullet I_o \right]} / 2 \bullet E_o$$

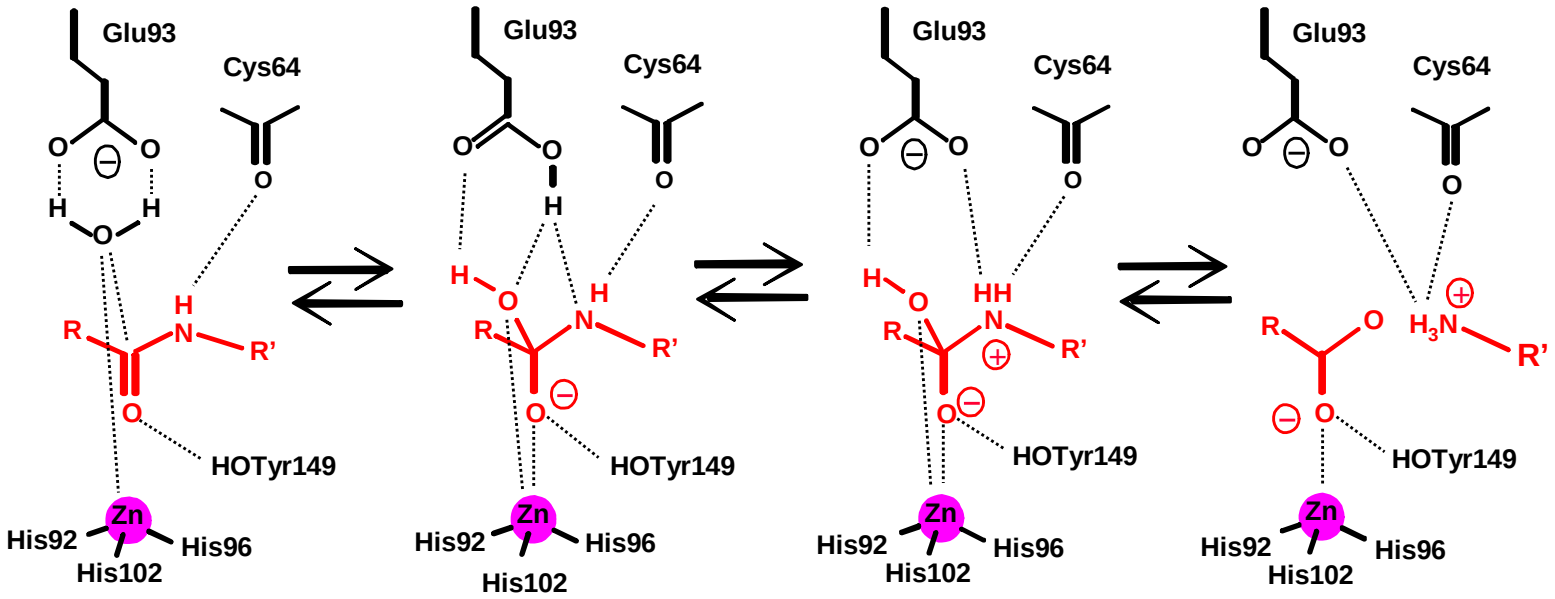




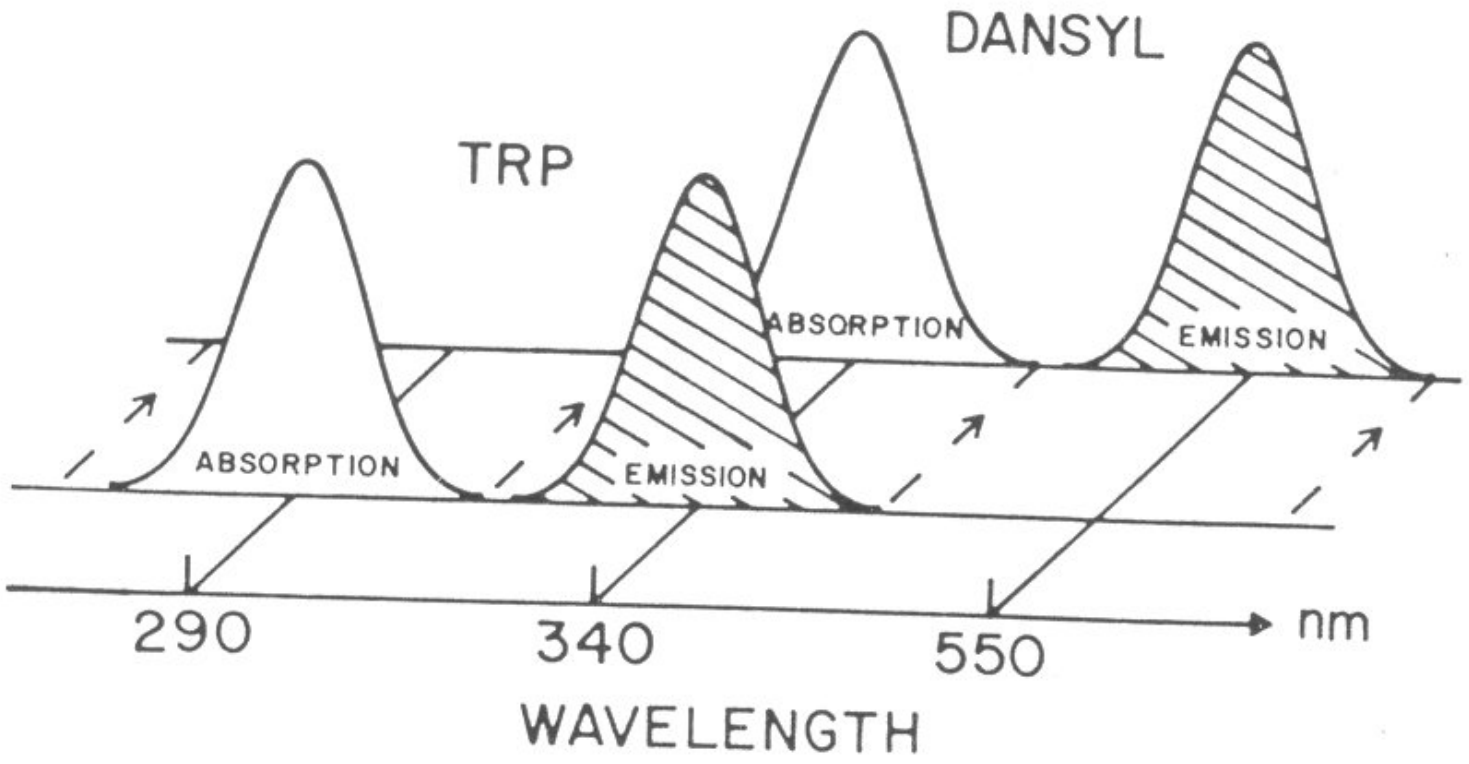
Enzym-Hemmung durch Übergangszustand-Analoga

P_4 P_3 P_2 P_1	P_1' P_2' P_3' P_4'	k_{on} $[M^{-1}s^{-1}]$	k_{off} $[10^{-4}s^{-1}]$	K_i [µM]	$t_{1/2}$ (@[I]=K_i)
CBZ-Pro-Lys-Phe$\Psi(PO_2CH_2)$	Ala-Pro	14	2	14	42 min
CBZ-Pro-Lys-Phe$\Psi(PO_2CH_2)$	Gly-Pro			580	
CBZ-Pro-Lys-Phe$\Psi(PO_2CH_2)$	Ala-Pro-Leu	52	0.1	0.2	11 h
CBZ-Pro-Lys-Phe$\Psi(PO_2CH_2)$	Gly-Pro-Leu			11	
CBZ-Pro-Lys-Lys$\Psi(PO_2CH_2)$	Ala-Pro-Leu-Val	344	0.4	0.1	4 h
FMOC-Pro-Lys-Phe$\Psi(PO_2CH_2)$	Ala-Pro-Leu-Val	97	0.04	0.04	34 h
FMOC-Lys-Phe$\Psi(PO_2CH_2)$	Ala-Pro-Leu-Val	168	3	1.8	27 min
FMOC-Phe$\Psi(PO_2CH_2)$	Ala-Pro-Leu-Val	163	2	1.3	38 min

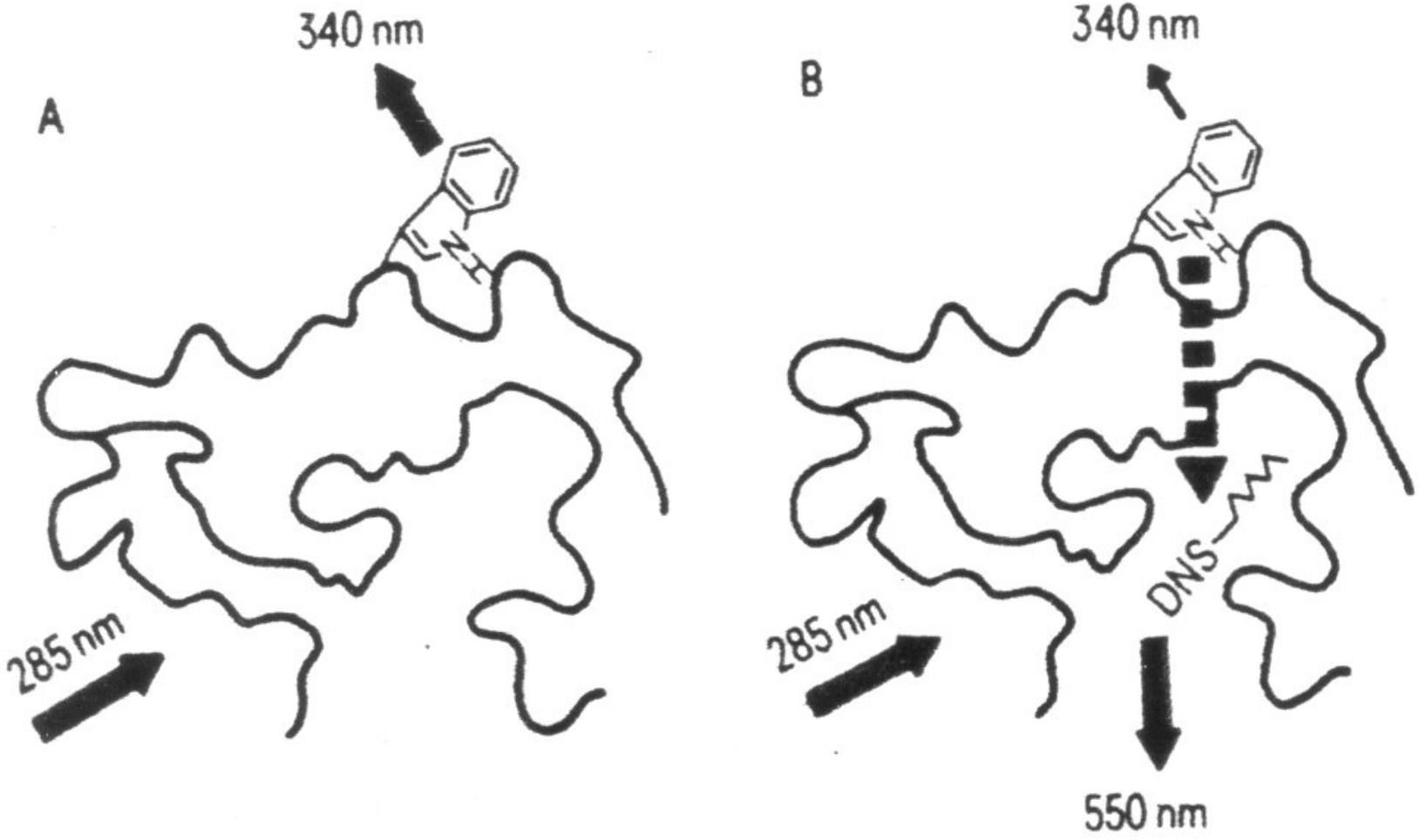
Katalyse durch Zink-Hydrolasen



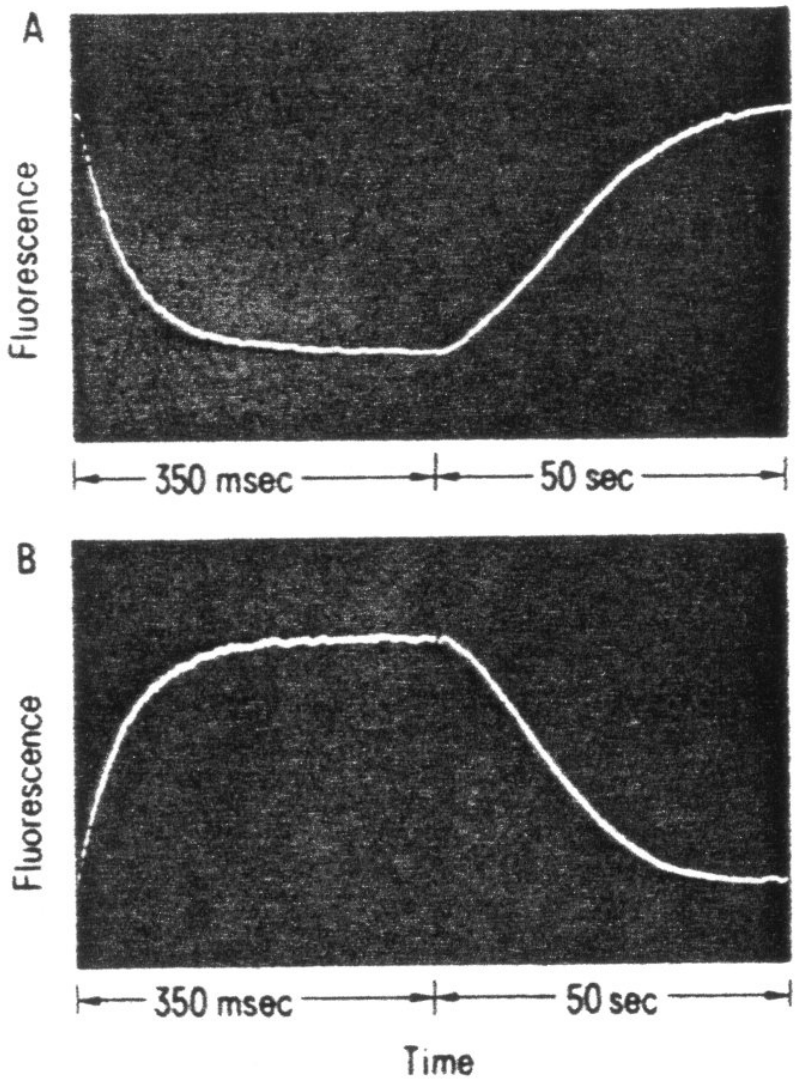
Radiationless Energy Transfer RET (FRET)



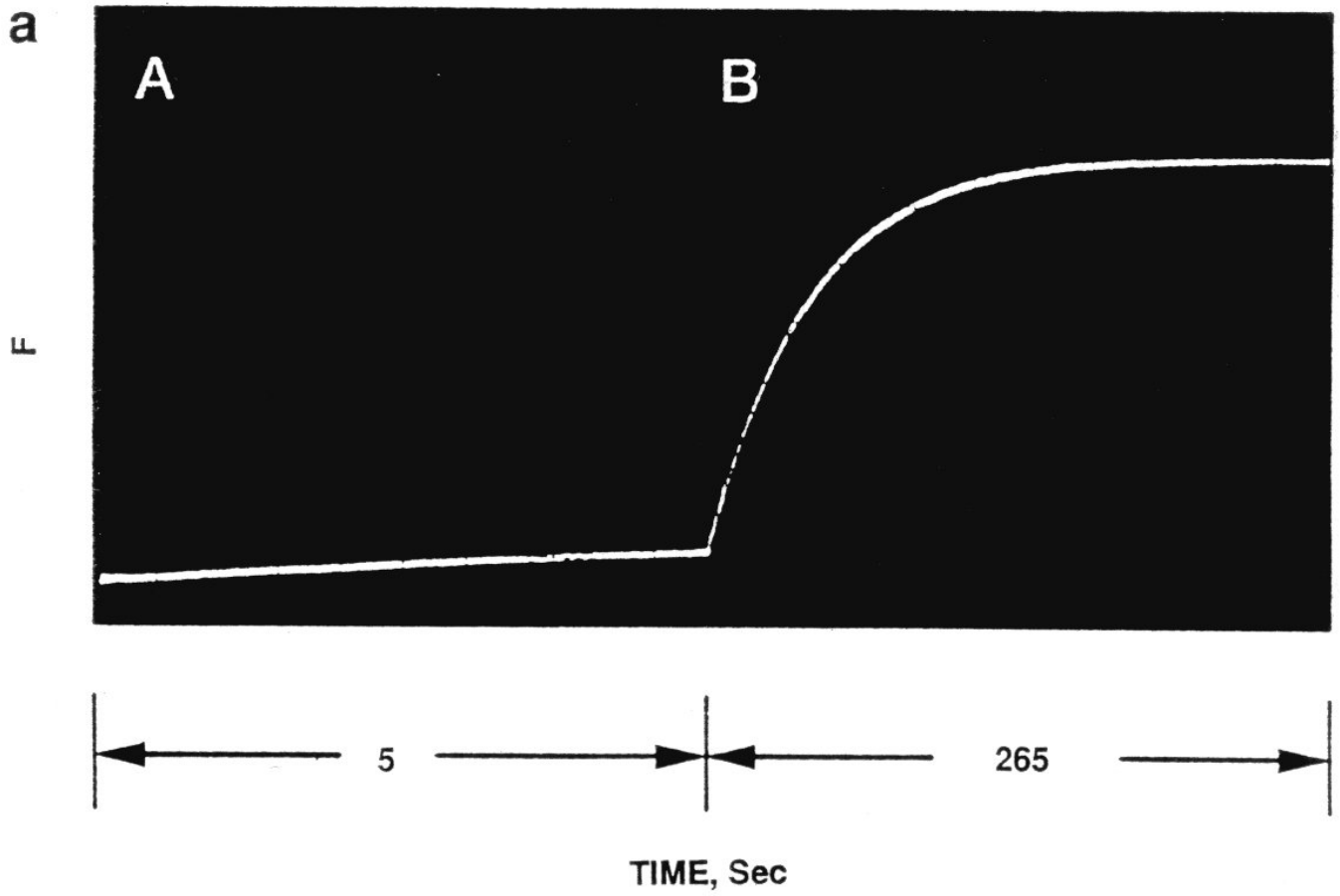
ES-Komplex



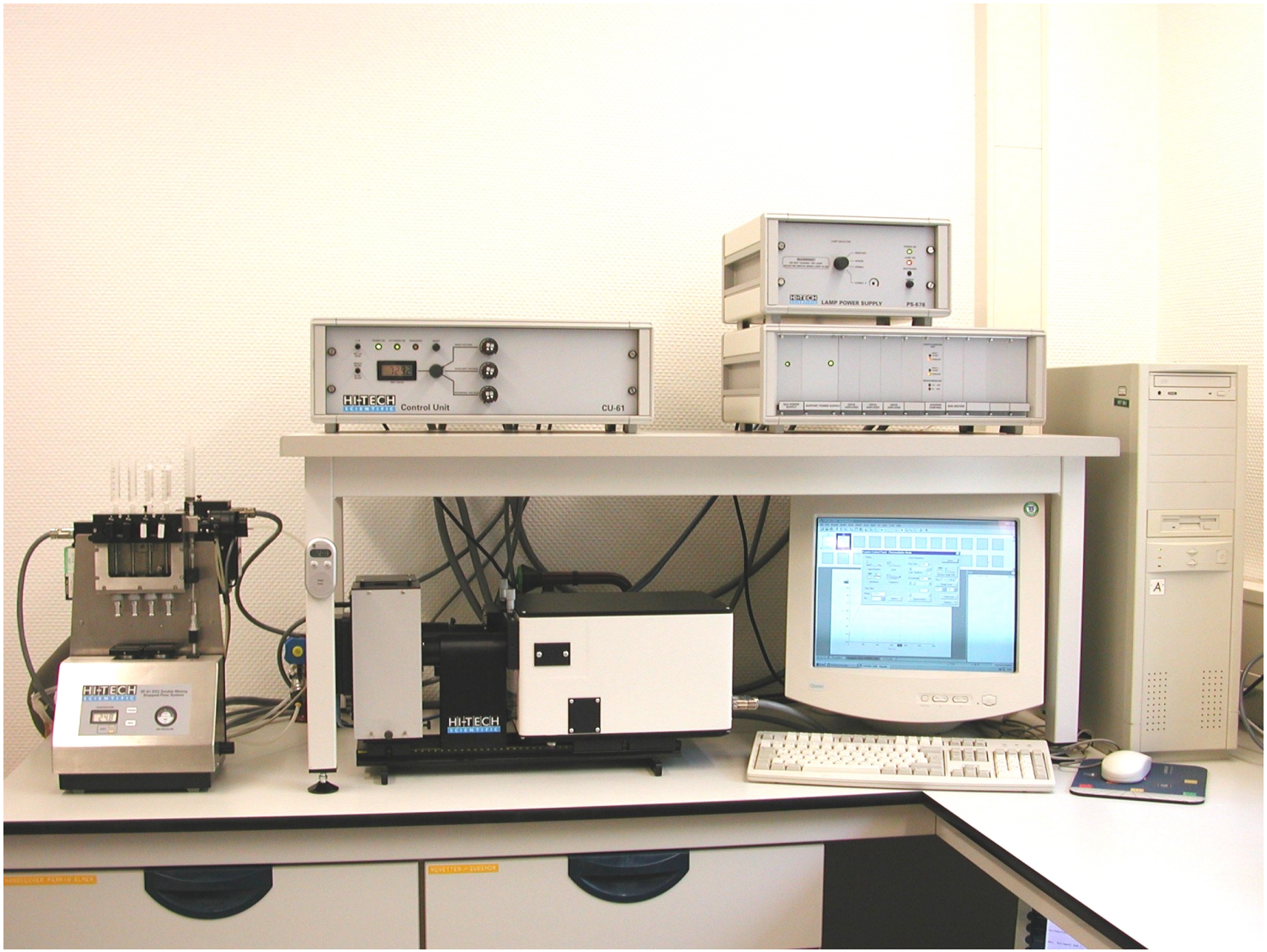
Tryptophan oder Dansyl Fluoreszenz



Quenched Fluorescent Substrate



Stopped Flow Kinetik


HI-TECH SCIENTIFIC
Control Unit
CU-61
HI-TECH SCIENTIFIC
LAMP POWER SUPPLY
PS-678
HI-TECH SCIENTIFIC
HI-TECH SCIENTIFIC

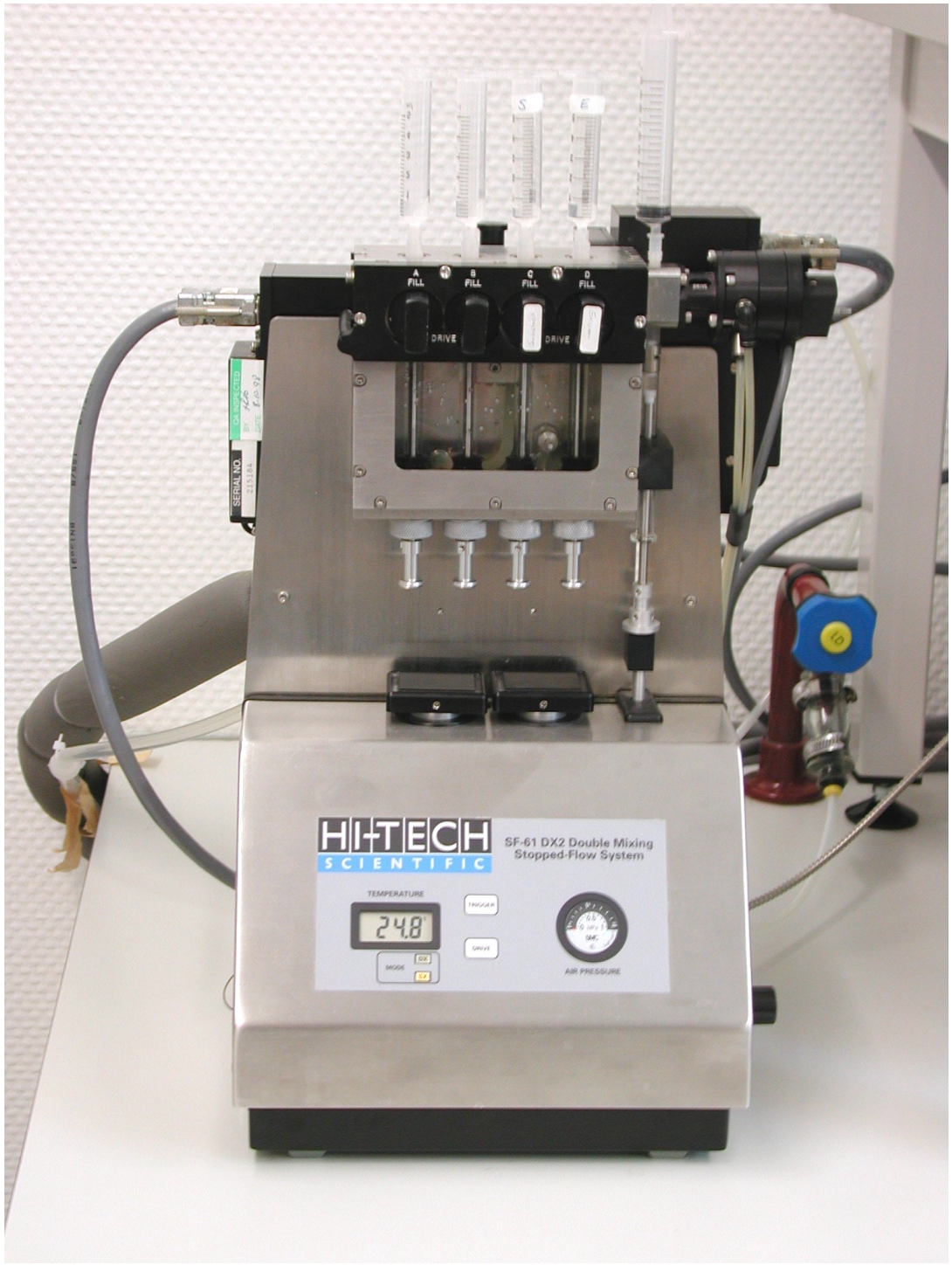

A FILL
B FILL
C FILL
D FILL
DRIVE
DRIVE
SERIAL NO.
HI-TECH
SCIENTIFIC
SF-61 DX2 Double Mixing
Stopped-Flow System
TEMPERATURE
24.8
TRIGGER
DRIVE
MODE
AIR PRESSURE

Fig.1.2.3a: Single Mixing Mode: START

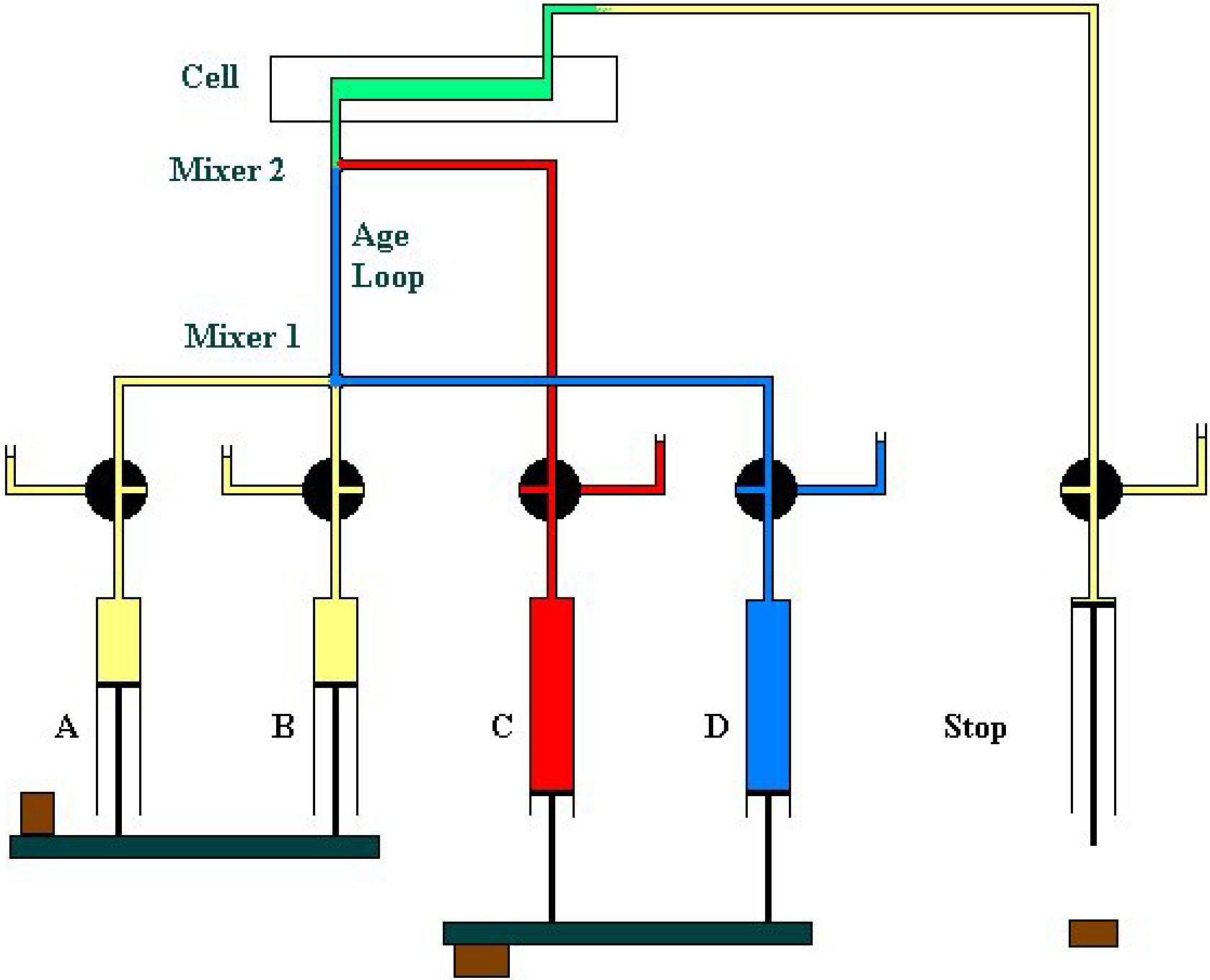


Fig.1.2.3b: Single Mixing Mode: FINISH

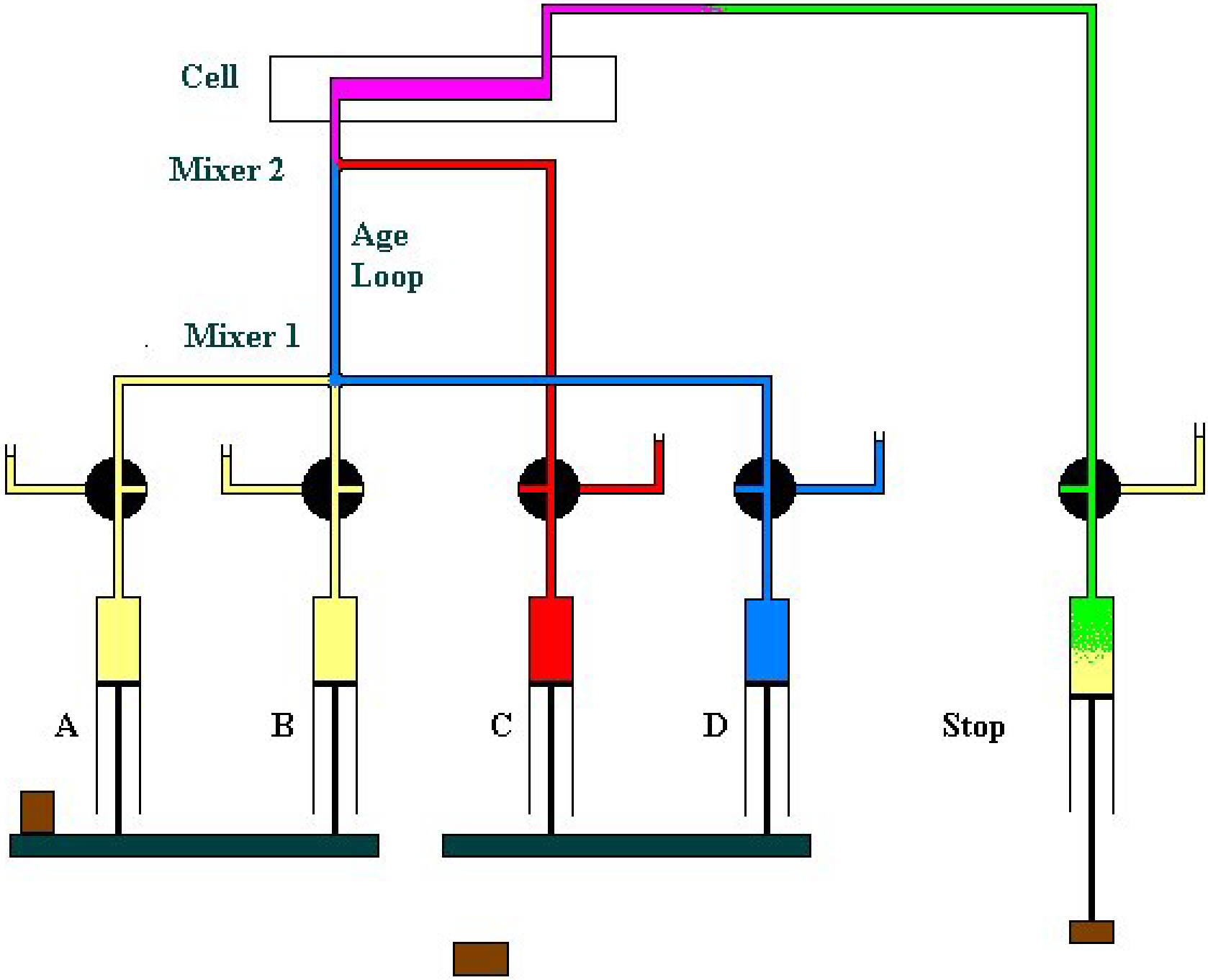


Fig.1.2.2a: Double Mixing Mode: START

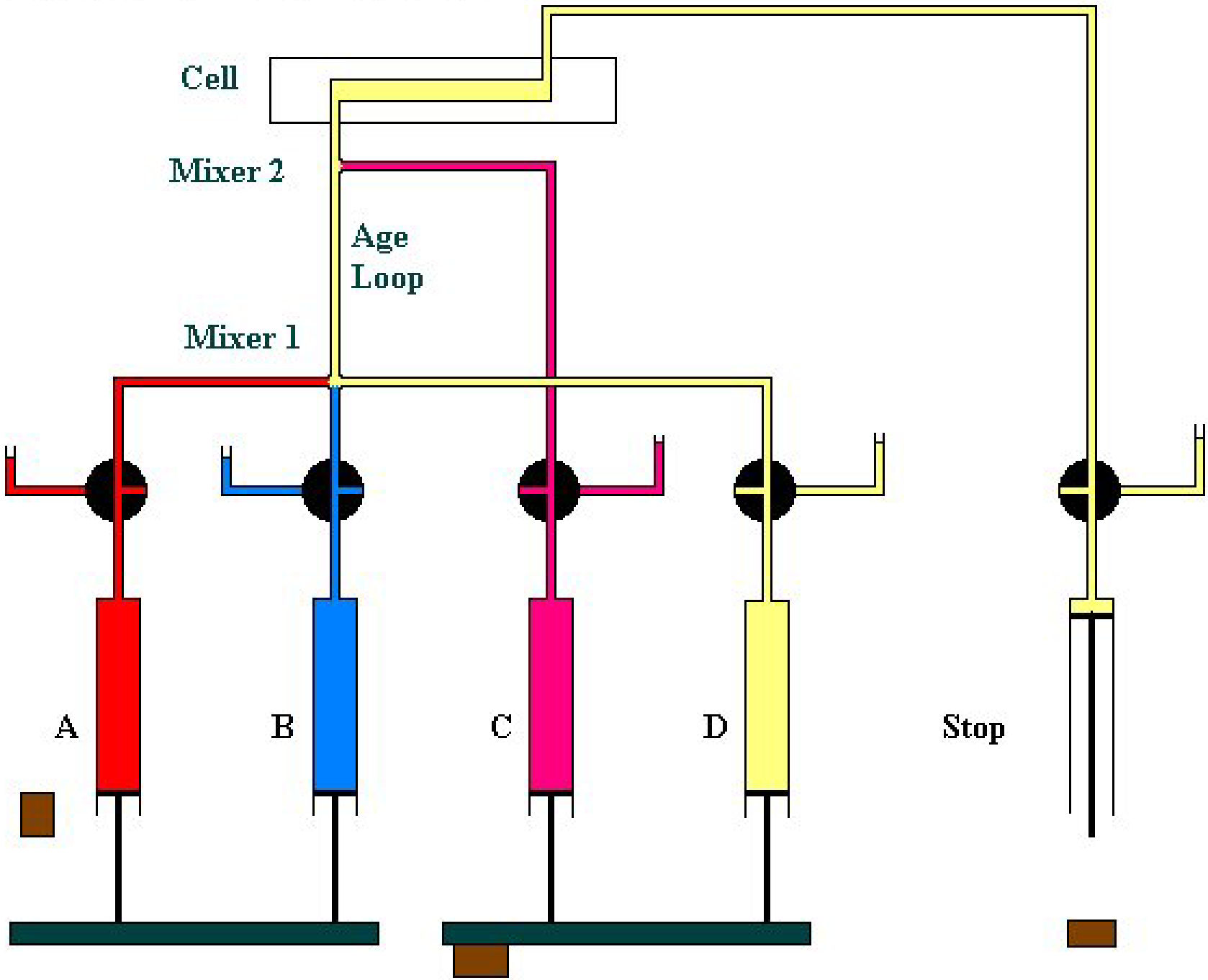


Fig.1.2.2b: Double Mixing Mode: FIRST STOP

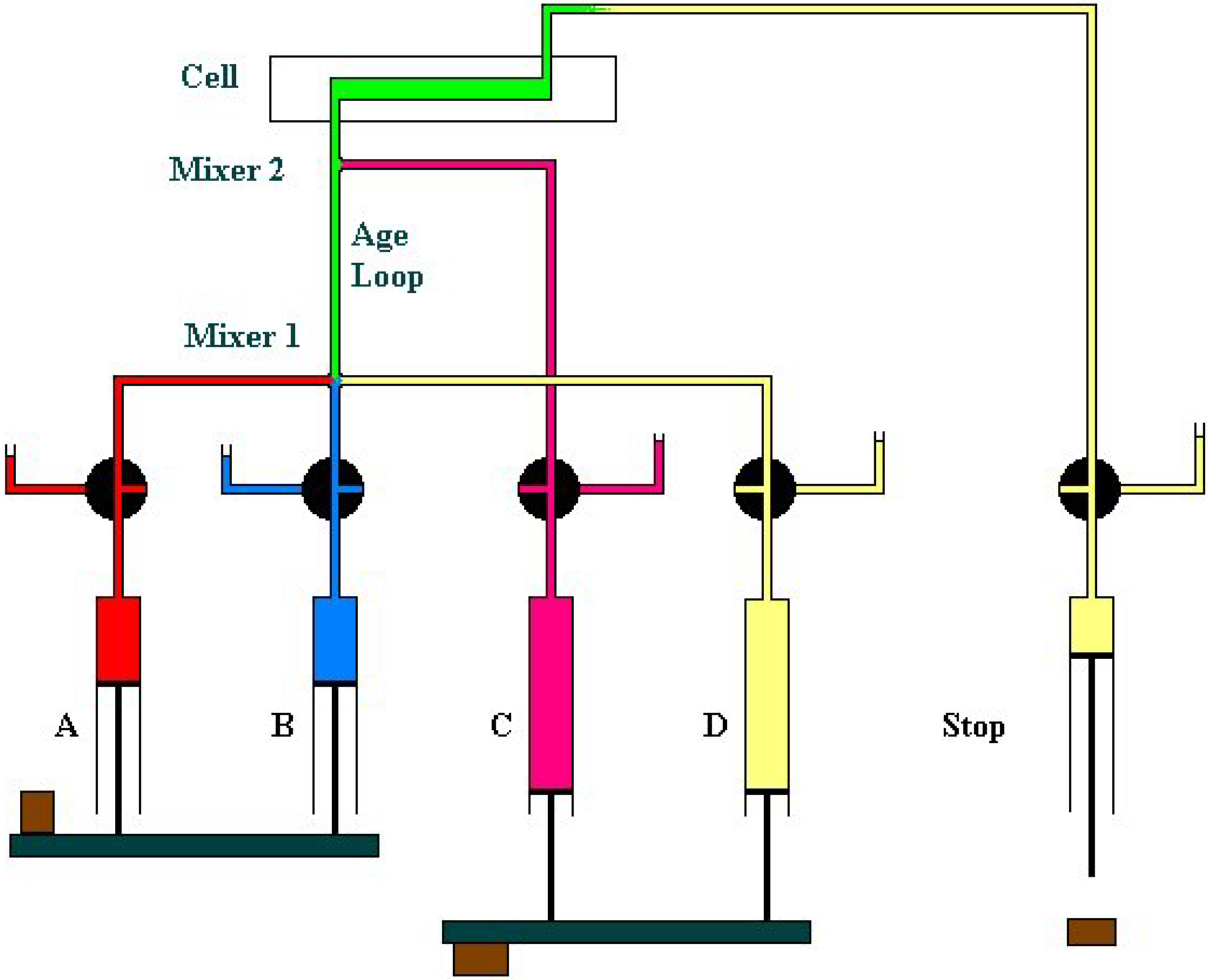


Fig.1.2.2c: Double Mixing Mode: FINISH