4. ENZYME KINETICS

Enzyme kinetics

Investigation of enzymatic reaction rate, identification of parameters.

$$E + S \leftrightarrow E + P$$

For stoichiometric calculations all components should be given in moles or grams. But: enzymes are not pure proteins!

→ amount of enzymes is measured through their catalytic effect → *ACTIVITY*

Enzyme kinetics

One **UNIT** is the amount of the enzyme which consumes 1 μmol substrate or forms 1 μmol product during 1 minute *at given reaction circumstances*.

SI: 1 Katal: 1 mol substrate (product) during 1 s.

(too huge!!) → nKat = 10^{-9} Kat (nanoKatal)

1 Kat = $6*10^{7}$ U, 1 U =$1.666*10^{-8}$ Kat,

1 U= 1/60 μKat, 1 U = 16.67 nKat

Specific activity: U/mass or U/volume → U/mg, U/ml

Michaelis-Menten kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Conditions:

- $k_{-2} = 0$ (the second step is irreversible)
- the first step reaches the equilibrium quickly = **RAPID EQUILIBRIUM:** $k_1 SE = k_{-1} (ES)$

  Dissociation constant of (ES): $K_s = \frac{k_{-1}}{k_1} = \frac{S.E}{(ES)}$

- stable ES complex, EP complex negligible

Michaelis-Menten kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- one active centre, one substrate
- concentration can be applied (instead of activity)
- (S) **>>** (E_0) i.e. $E_0 / S \ll 1$

Reaction rate: $$V = \frac{dP}{dt} = k_2 (ES)$$

Mass balance for E: $$E + (ES) = E_o$$

Divide these equations!

Michaelis-Menten kinetics

Divide the two equations: $\frac{V}{E_o} = \frac{k_2(ES)}{E+(ES)}$

substitute: $K_s = \frac{k_{-1}}{k_1} = \frac{S.E}{(ES)}$

$$\frac{V}{E_o} = \frac{k_2 \frac{S}{K_s} E}{E + \frac{S}{K_s} E}$$

Rearrange: $\frac{V}{k_2 E_o} = \frac{\frac{S}{K_s}}{1+\frac{S}{K_s}} = \frac{S}{K_s + S}$

$V_{max} = k_2 E_o$ because $V = \frac{dP}{dt} = k_2(ES)$

Michaelis-Menten kinetics

The rate equation:

$$V = V_{max} \frac{S}{K_s + S} \quad \text{or} \quad \frac{V}{V_{max}} = \frac{\frac{S}{K_s}}{1 + \frac{S}{K_s}}$$

M és M

Maud Menten
1879-1960

Leonor Michaelis
1875-1949

Michaelis, L., Menten, M. (1913) Die kinetik der invertinwirkung, *Biochemische Zeitung 49*, 333-369

Briggs-Haldane kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

The same differential eqution but the condition:

$$\frac{dS}{dt} = -k_1 ES + k_{-1}(ES)$$

(quasi) steady state:

d(ES)/dt =0

$$\frac{d(ES)}{dt} = k_1 ES - k_{-1}(ES) - k_2(ES)$$

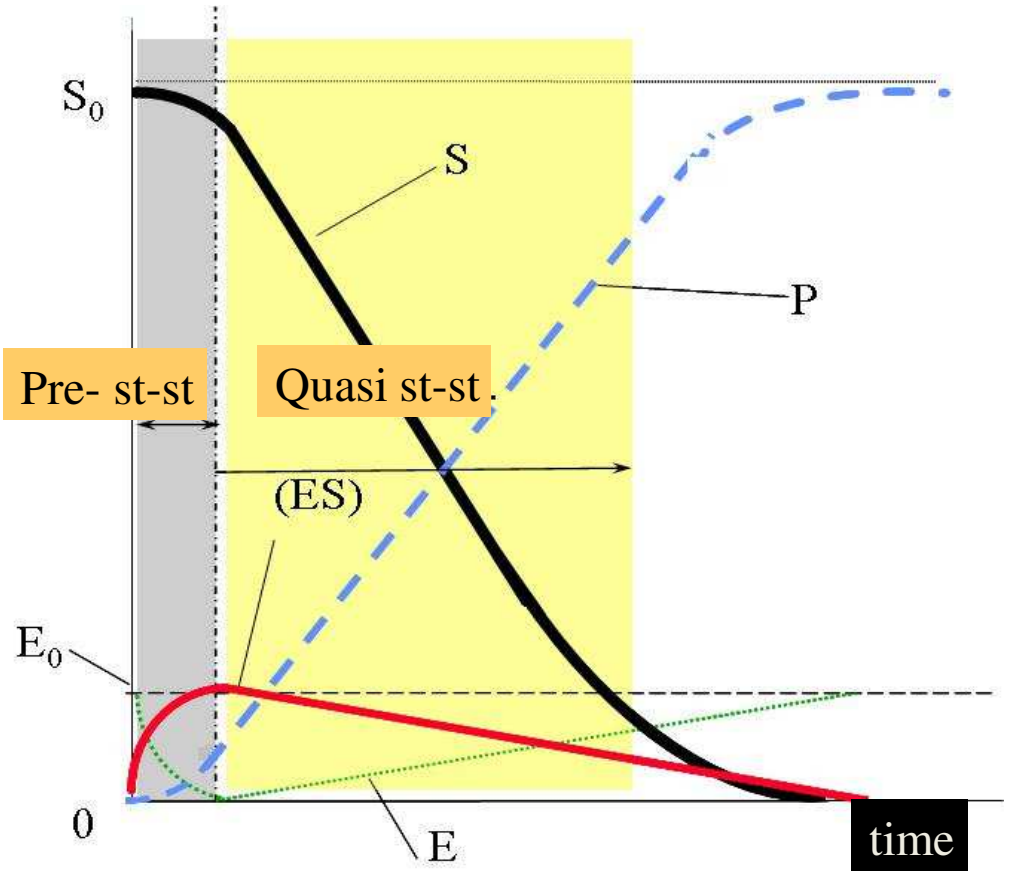
$$\frac{dP}{dt} = k_2(ES)$$

(S) >> (E$_0$) i.e. E$_0$/S << 1

$k_1ES > k_{-1}(ES)$ ill. $k_1ES > k_2(ES)$

Briggs-Haldane kinetics

After a short transition period (pre-steady state) the rate is almost constant (quasi-steady state).



Briggs, G. E., and Haldane, J. B. (1925) A Note on the Kinetics of Enzyme Action, *Bio-chem J 19*, 338-339.

Briggs-Haldane kinetics

$$\frac{d(ES)}{dt} = k_1 \cdot E \cdot S - k_{-1}(ES) - k_2(ES) = 0$$

$$k_1 \cdot E \cdot S = (k_{-1} + k_2)(ES)$$

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

$$E + (ES) = E_o$$

$$V = \frac{k_2 E_o S}{K_m + S} = V_{max} \frac{S}{K_m + S}$$

$K_m = (k_{-1} + k_2) / k_1$

Michaelis constant

Discussion

Michaelis-Menten

$$V = V_{max} \frac{S}{K_s + S}$$

$$K_s = \frac{k_{-1}}{k_1}$$

Briggs-Haldane

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

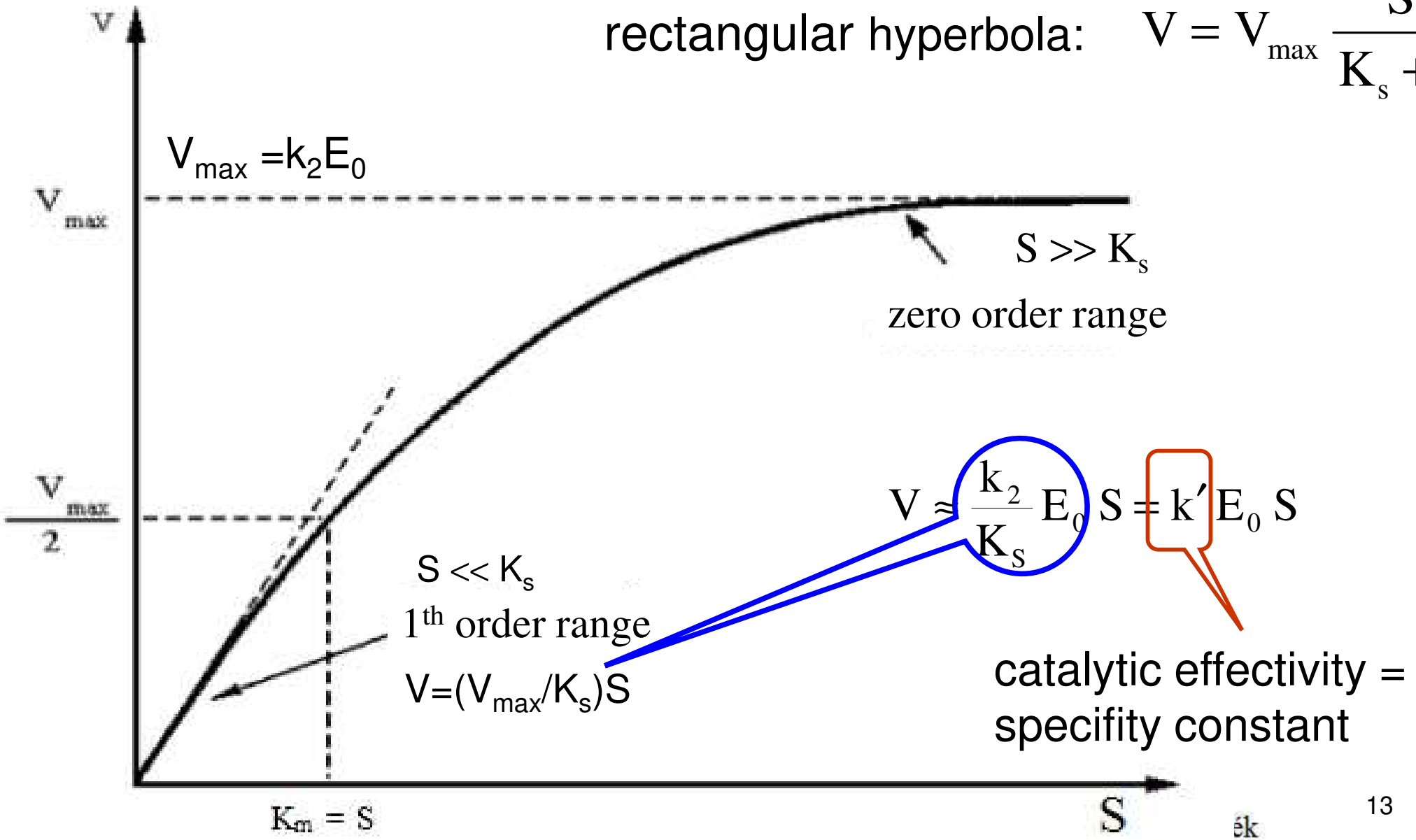
$$K_m = \frac{k_{-1} + k_2}{k_1}$$

$$K_m = K_s + \frac{k_2}{k_1}$$

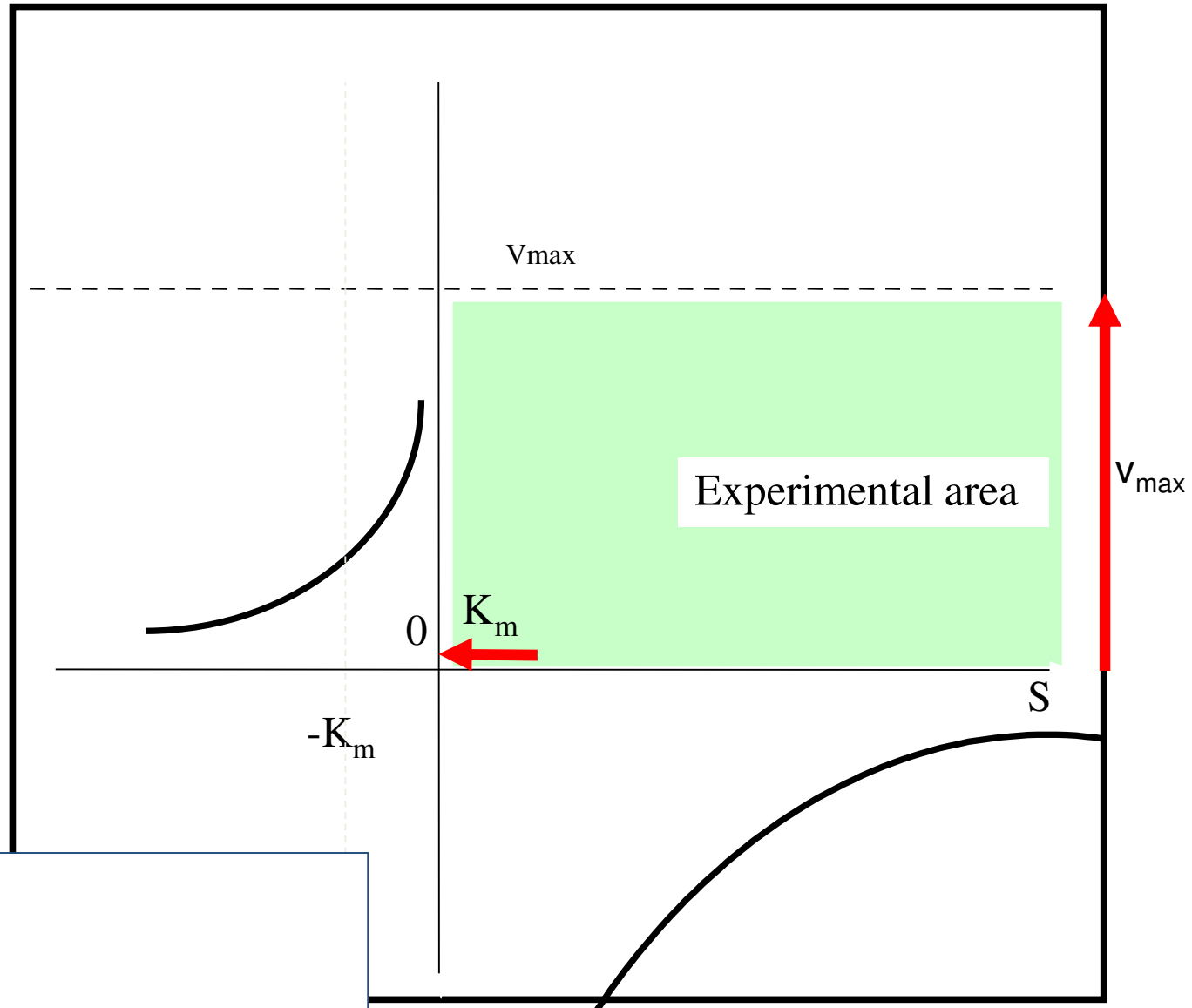
if (k_1) >> (k_2) the two constants are equal!

Discussion

rectangular hyperbola: $V = V_{max} \frac{S}{K_s + S}$



Hyperbola

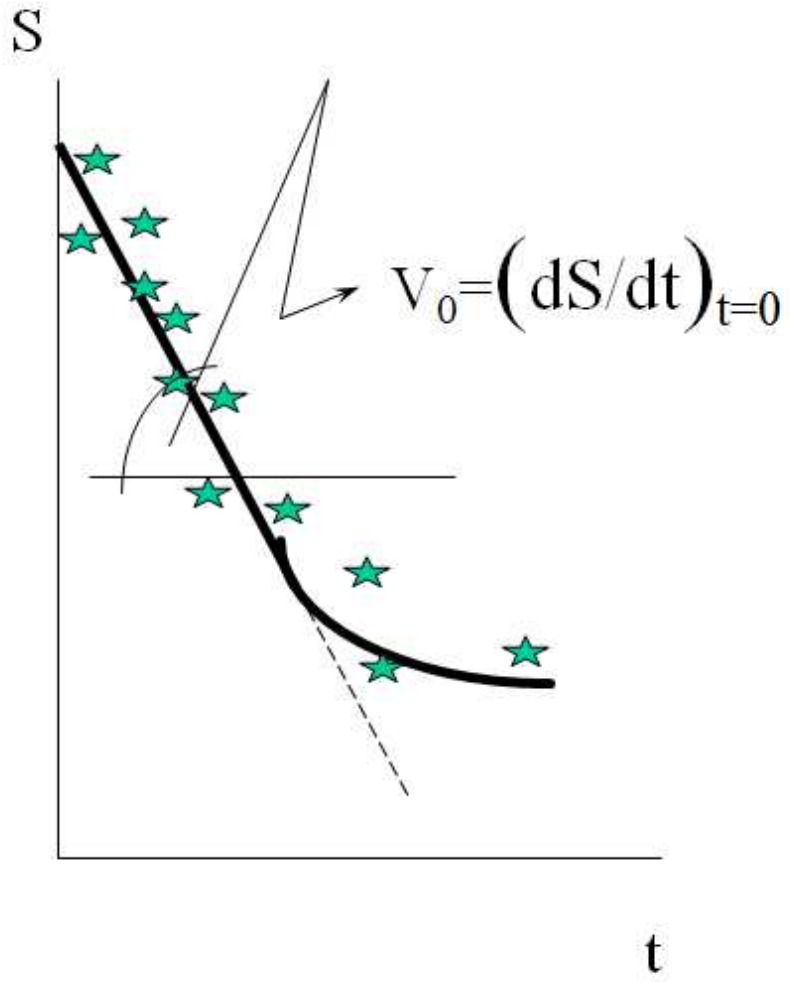


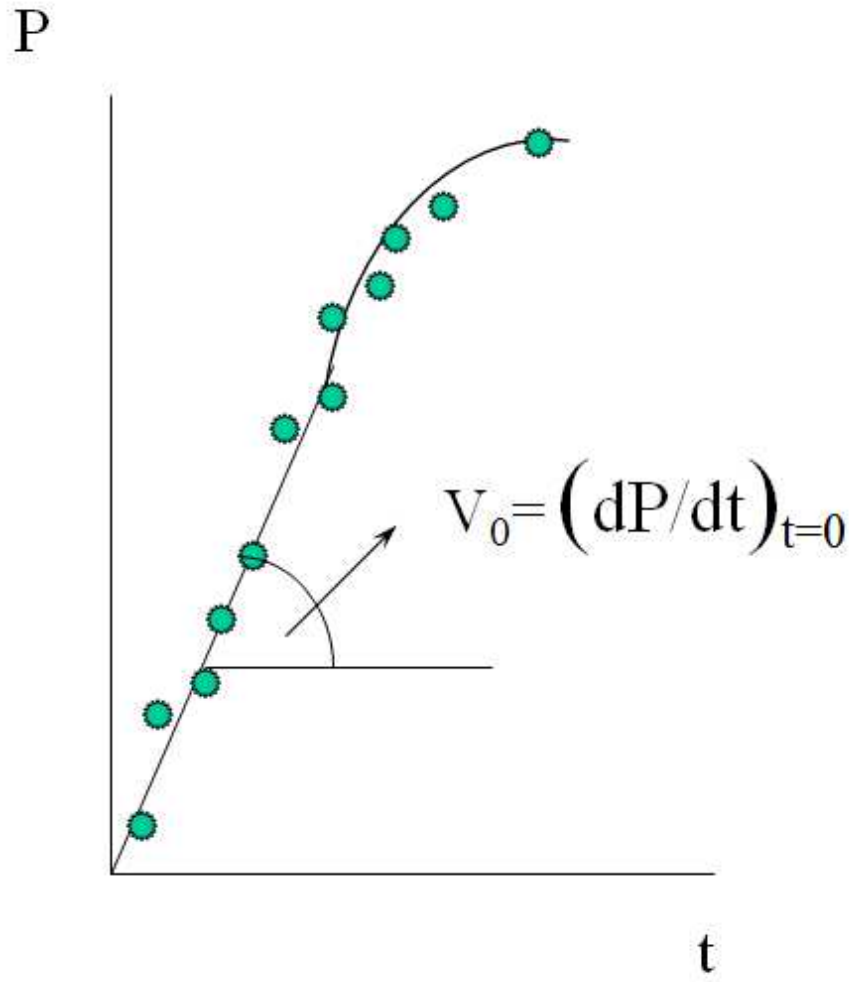
$$y = \frac{a}{x}$$

$$V = \frac{V_m S + K_s V_m - K_s V_m}{S + K_m} = -\frac{K_s V_m}{S + K_m} + V_m$$

How to measure reaction rate?

In M-M and B-H equations V means initial reaction rate ($V_0 \rightarrow$ extrapolated to t=0).





Parameter estimation

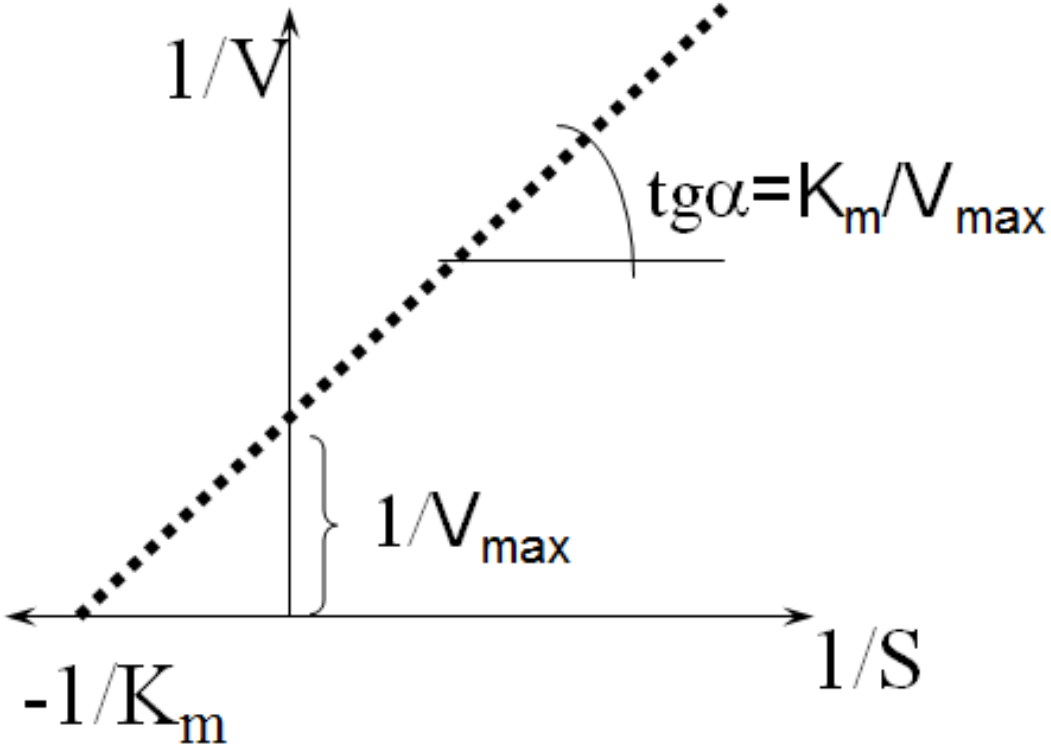
Linearised diagrams are used:

- Calculation of nonlinear regression was complicated without computers
- It provides additional info about enzyme inhibition

1. Lineweaver-Burk plot

1/v – 1/S

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

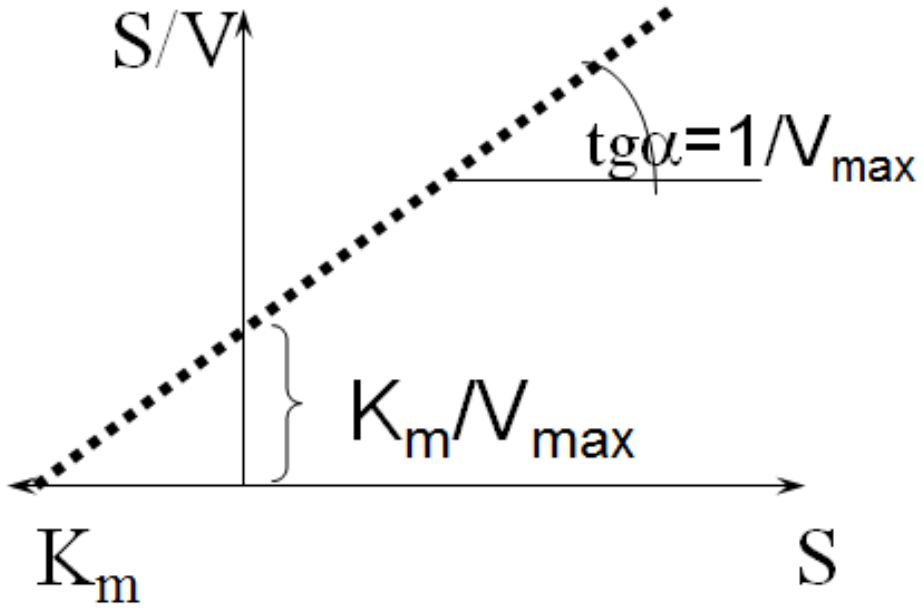


Linearised forms

2. Hanes-Langmuir plot

S/v – S

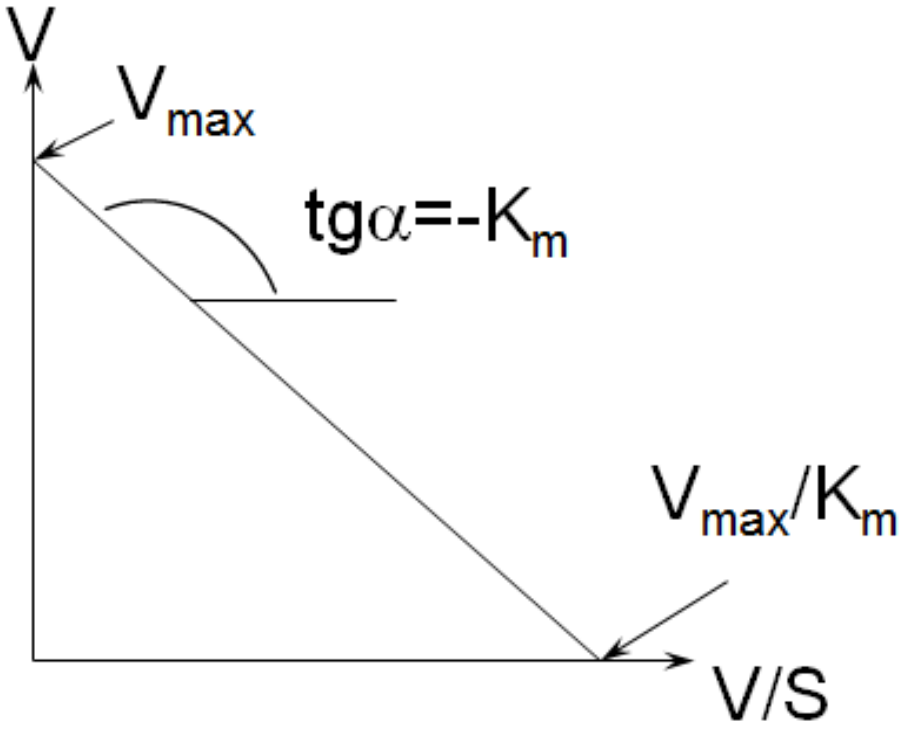
$$\frac{S}{V} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} \cdot S$$



3. Eady-Hofstee plot

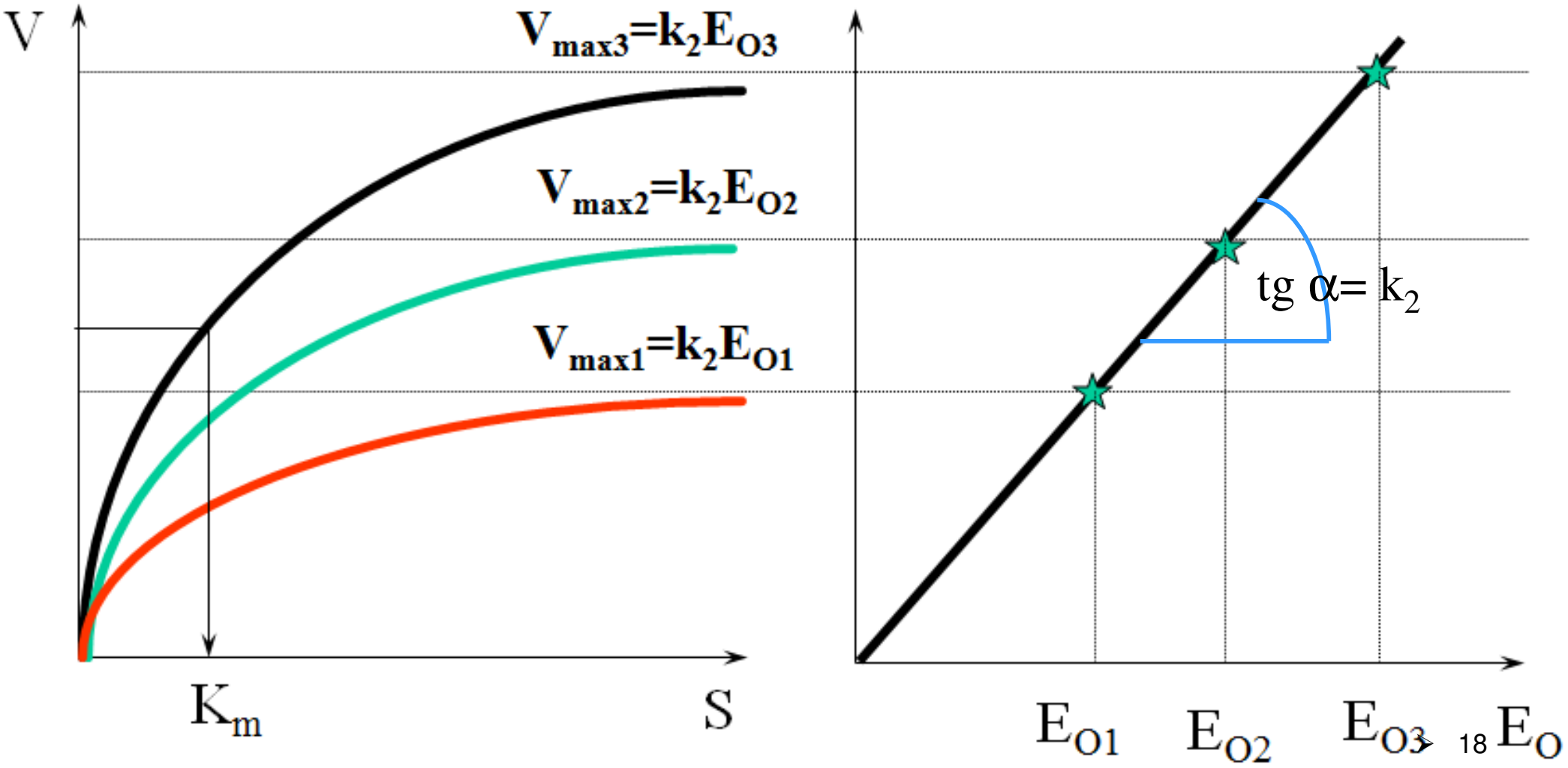
v/S – v

$$V = V_{max} - K_m \frac{V}{S}$$



Effect of enzyme concentration

If $v_{max} = k_2.E_0$, then:



Interpretation of kinetic parameters

V_{max} : its not a climax, but limit → border of rate

It's not an enzyme feature, it depends on E_0:

$V_{max} = k_2 \cdot E_0$ → = **ACTIVITY**

k_2 is the real enzyme feature = turnover number [s^{-1}] →

transformation frequency

Extending to every enzymes and every kinetics:

$V_{max} = k_{cat} \cdot E_0$

k_{cat} [s^{-1}]: Turnover frequency of one enzyme molecule (at S-saturation): how many substrate molecules are transformed in one second by one enzyme molecule.

Kinetic parameters: K_s, K_m

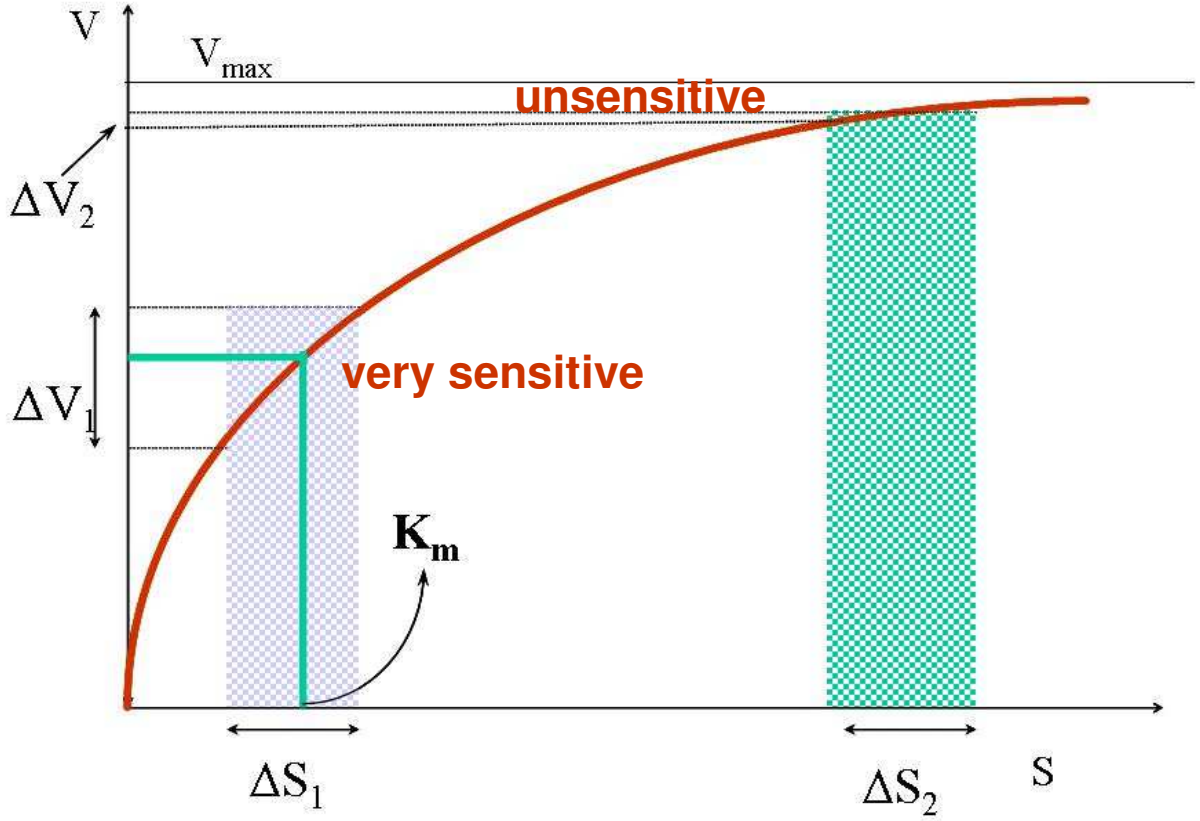
- Affinity of enzyme to substrate
- Usually the S concentration in a living cell – easy adaption to changes
- K_S has changed → Inhibitor? Activator?
- Enzyme analytics:

- activity measurement:

  $S>>K_S$ $v=v_{max}$

- substrate measurement:

  $S<<K_S$ linear range



Interpretation of kinetic parameters

k_1 10^7-10^{10} $dm^3mol^{-1}min^{-1}$ [max. value (~10^{11}) limited by diffusivity of small molecules]

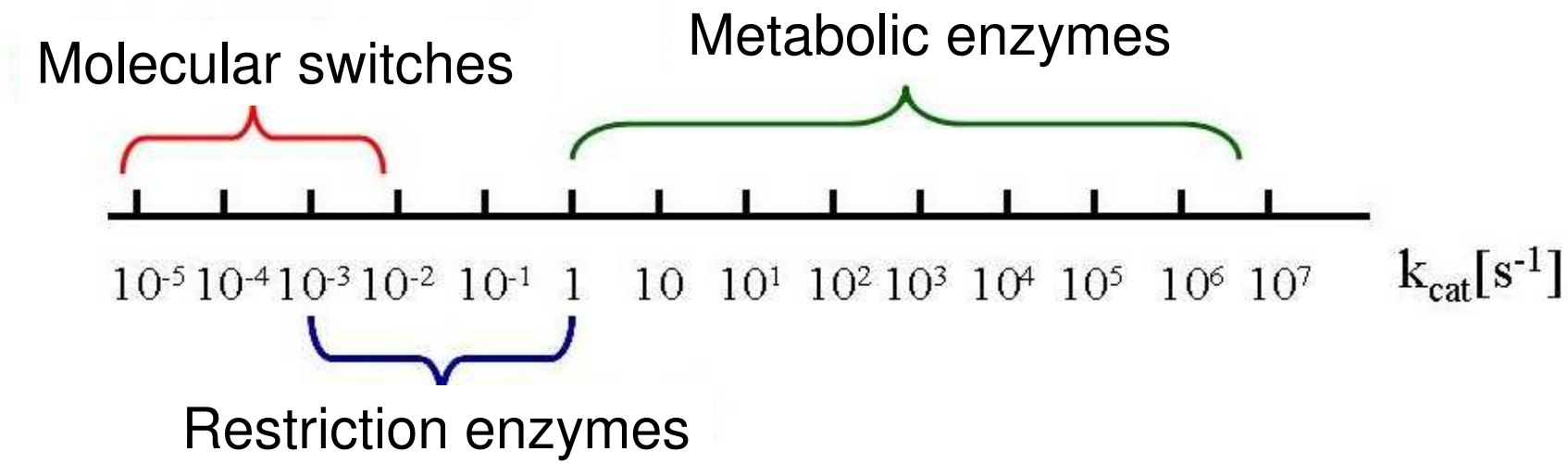
k_{-1} 10^2-10^6 min^{-1}

k_2 50-10^7 min^{-1}

K_m 10^{-6} - 10^{-2} mol/dm^3

TABLE 13-1. THE VALUES OF K_M, k_{CAT}, AND k_{CAT}/K_M FOR SOME ENZYMES AND SUBSTRATES

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}


Molecular switches
Metabolic enzymes
10-5 10-4 10-3 10-2 10-1 1 10 101 102 103 104 105 106 107
kcat[s-1]
Restriction enzymes

Reversible reactions

Many enzyme catalysed reactions - mainly biopolymer hydrolysis - are highly shifted to the right hand side, practically k_{-2} may really be neglected.

But conversions like

glucose $\rightleftharpoons$ **fructose**

(glucose isomerase)

~50 : 50 %

are of reversible character.

Reversible reactions

While $k_{-2} = 0$ in both kinetic models reactions seems to be irreversible. Models for reversible (equilibrium) reactions are built up from models of two countercurrent irreversible reaction.

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

$$K_{mp} = \frac{k_2 + k_{-1}}{k_{-2}}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$V_{maxs} = k_2 E_o$$

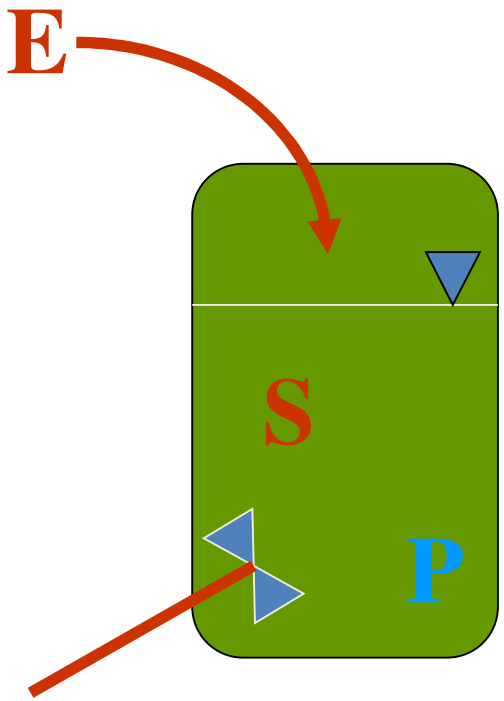
$$V_{maxp} = k_{-1} E_o$$

$$K_1 = \frac{k_1}{k_{-1}} \qquad K_2 = \frac{k_2}{k_{-2}}$$

$$K_{eq(uilibrium)} = K_1 K_2 = \frac{k_1 k_2}{k_{-1} k_{-2}}$$

$1/K_S$ K_P

Reversible reactions



WHAT WILL HAPPEN?

S → P or P → S

?

What does it depend on?

K_{eq} , S , P value!

Presume the presence of EP complex:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \rightleftharpoons E + P$$

Reversible reactions

The netto rate is the difference of the two processes:

$$V_{netto} = V_{fore} - V_{back} = k_2(ES) - k_{-2}(EP)$$

Repeat the previous deduction, divide the equation with:

$$E_o = E + (ES) + (EP)$$

$$\frac{v_{fore}}{E_o} = \frac{k_2(ES)}{E + (ES) + (EP)} \qquad \frac{v_{back}}{E_o} = \frac{k_{-2}(EP)}{E + (ES) + (EP)}$$

From these:

$$\Delta v = \frac{E_0 k_2(ES) - E_0 k_{-2}(EP)}{E + (ES) + (EP)}$$

Reversible reactions

Substitute v_{max}:

$$\Delta v = \frac{v_{max\,S}(ES) - v_{max\,P}(EP)}{E + (ES) + (EP)}$$

Substitute complex concentrations:

$$(ES) = E\frac{S}{K_s} \qquad (EP) = E\frac{P}{K_P}$$

$$\Delta v = \frac{v_{max\,S}\frac{S}{K_s}E - v_{max\,P}\frac{P}{K_p}E}{E + \frac{S}{K_s}E + \frac{P}{K_p}E}$$

equals

$$\Delta V = \frac{V_{maxs}\left(S - \frac{P}{K_{eq}}\right)}{K_{ms}\left(1 + \frac{P}{K_{mp}}\right) + S}$$

$= S_{equilibrium}$

Reversible M-M equation