STEADY-STATE

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STEADY-STATE

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

Briggs and Haldane:

- k_{cat} not necessarily << k_1 and k_{-1}
- when reaction velocity is constant, [ES] is constant or the variation of [ES] is << [S]

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

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{cat}[ES] = k_1[S]([E_t] - [ES]) - k_{-1}[ES] - k_{cat}[ES] =$$

$$= k_1[S][E_t] - [ES](k_1[S] + k_{-1} + k_{cat})$$

$$\frac{d[ES]}{dt} = 0 \quad \Rightarrow \quad k_1[S][E_t] = [ES](k_1[S] + k_{-1} + k_{cat}) \qquad \frac{[E_t]}{[ES]} = 1 + \frac{k_{-1} + k_{cat}}{k_1[S]}$$

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$$\frac{[E_t]}{[ES]} = 1 + \frac{k_{-1} + k_{cat}}{k_1[S]} \qquad [ES] = \frac{[E_t]k_1[S]}{k_1[S] + k_{-1} + k_{cat}} = \frac{[E_t][S]}{[S] + \frac{k_{-1} + k_{cat}}{k_1}}$$

$$[ES] = \frac{[E_t][S]}{\frac{k_{-1} + k_{cat}}{k_1} + [S]} \qquad v = \frac{k_{cat}[E_t][S]}{\frac{k_{-1} + k_{cat}}{k_1} + [S]} = \frac{k_{cat}[E_t][S]}{K_m + [S]}$$

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

When $k_{cat} \ll k_1$ and k_{-1} $K_m = K_s$

Carbonic anhydrase features Briggs-Haldane kinetics

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John Burdon Sanderson Haldane: Enzymes, Longmans Green & Co. London, 1930
Reversible Reactions: pp.80-83

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrow}} ES \underset{k_4}{\overset{k3}{\leftrightarrow}} EP \underset{k_6}{\overset{k_5}{\leftrightarrow}} P + E$$

$$\frac{d[ES]}{dt} = k_1[S]\left([Et] - [ES] - [EP]\right) + k_4[EP] - (k_2 + k_3)[ES]$$

$$\frac{d[EP]}{dt} = k_6[P]\left([Et] - [ES] - [EP]\right) + k_3[ES] - (k_4 + k_5)[EP]$$

Under steady-state conditions: both d[ES]/dt and d[EP]/dt = 0

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Under steady-state conditions:

$$k_1[S]([Et]-[ES]-[EP])+k_4[EP]-(k_2+k_3)[ES]=0$$

$$k_6[P]([E_t]-[ES]-[EP])+k_3[ES]-(k_4+k_5)[EP]=0$$

Let us consider [ES] and [EP]:

$$[ES]=\frac{k_1[S][E_t]+[EP](k_4-k_1[S])}{k_1[S]+k_2+k_3} \qquad [EP]=\frac{k_6[P][E_t]+[ES](k_3-k_6[P])}{k_6[P]+k_4+k_5}$$

$$\frac{[ES]}{[E_t]}=\frac{k_1[S]+[EP]/[E_t](k_4-k_1[S])}{k_1[S]+k_2+k_3} \qquad \frac{[EP]}{[E_t]}=\frac{k_6[P]+[ES]/[E_t](k_3-k_6[P])}{k_6[P]+k_4+k_5}$$

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$$\frac{[ES]}{[E_t]} = \frac{k_1[S] + [EP]/[E_t](k_4 - k_1[S])}{k_1[S] + k_2 + k_3} \qquad \frac{[EP]}{[E_t]} = \frac{k_6[P] + [ES]/[E_t](k_3 - k_6[P])}{k_6[P] + k_4 + k_5}$$

$$\frac{[ES]}{[E_t]} = \frac{k_1[S] + (k_4 - k_1[S])\left(\frac{k_6[P] + [ES]/[E_t](k_3 - k_6[P])}{k_6[P] + k_4 + k_5}\right)}{k_1[S] + k_2 + k_3} =$$

$$\frac{k_1[S](k_4 + k_5 + k_6[P]) + k4k6[P] - k_1k_6[S][P] + [ES]/[E_t](k_4k_3 + k_1k_6[S][P] - k_4k_6[P] - k_1k_3[S])}{(k_4 + k_5 + k_6[P])(k_1[S] + k_2 + k_3)}$$

$$\frac{[ES]}{[E_t]}\left[(k_4 + k_5 + k_6[P])(k_1[S] + k_2 + k_3) - k_4k_3 - k_1k_6[S][P] + k_4k_6[P] + k_1k_3[S]\right] =$$

$$k_1k_4[S] + k_1k_5[S] + k_1k_6[S][P] + k_4k_6[P] - k_1k_6[S][P]$$

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$$\frac{[ES]}{[E_t]}\left[(k_4+k_5+k_6[P])(k_1[S]+k_2+k_3)-k_4k_3-k_1k_6[S][P]+k_4k_6[P]+k_1k_3[S]\right]=$$

$$k_1k_4[S]+k_1k_5[S]+k_1k_6[S][P]+k_4k_6[P]-k_1k_6[S][P]$$

$$\frac{[ES]}{[E_t]}\left[k_1[S](k_3+k_4+k_5)+k_6[P](k_2+k_3+k_4)+k_2k_5+k_3k_5+k_2k_4\right]=k_1[S](k_4+k_5)+k_4k_6[P]$$

$$\frac{[ES]}{[E_t]}=\frac{k_1[S](k_4+k_5)+k_4k_6[P]}{k_1[S](k_3+k_4+k_5)+k_6[P](k_2+k_3+k_4)+k_2k_5+k_3k_5+k_2k_4}$$

And :

$$\frac{[EP]}{[E_t]}=\frac{k_6[P](k_2+k_3)+k_1k_3[S]}{k_1[S](k_3+k_4+k_5)+k_6[P](k_2+k_3+k_4)+k_2k_5+k_3k_5+k_2k_4}$$

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$$\frac{[ES]}{[E_t]} = \frac{k_1[S](k_4 + k_5) + k_4k_6[P]}{k_1[S](k_3 + k_4 + k_5) + k_6[P](k_2 + k_3 + k_4) + k_2k_5 + k_3k_5 + k_2k_4}$$

$$\frac{[EP]}{[E_t]} = \frac{k_6[P](k_2 + k_3) + k_1k_3[S]}{k_1[S](k_3 + k_4 + k_5) + k_6[P](k_2 + k_3 + k_4) + k_2k_5 + k_3k_5 + k_2k_4}$$

$$v = k_3[ES] - k_4[EP] = \frac{(k_1k_3k_5[S] - k_2k_4k_6[P])[E_t]}{k_1[S](k_3 + k_4 + k_5) + k_6[P](k_2 + k_3 + k_4) + k_2k_5 + k_3k_5 + k_2k_4}$$

$$v = \frac{(k_1k_3k_5[S] - k_2k_4k_6[P])[E_t]}{k_1[S](k_3 + k_4 + k_5) + k_6[P](k_2 + k_3 + k_4) + k_2k_5 + k_3k_5 + k_2k_4} = \frac{VK'[S] - V'K[P]}{KK' + K'[S] + K[P]}$$

With :

$$V = \frac{k_3k_5[E_t]}{k_3 + k_4 + k_5} \qquad V' = \frac{k_2k_4[E_t]}{k_2 + k_3 + k_4} \qquad K = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_1(k_3 + k_4 + k_5)} \qquad K' = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_6(k_2 + k_3 + k_4)}$$

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$$v = \frac{VK'[S] - V'K[P]}{KK' + K'[S] + K[P]}$$

$$V = \frac{k_3 k_5 [E_t]}{k_3 + k_4 + k_5} \qquad V' = \frac{k_2 k_4 [E_t]}{k_2 + k_3 + k_4} \qquad K = \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_1 (k_3 + k_4 + k_5)} \qquad K' = \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_6 (k_2 + k_3 + k_4)}$$

If $[P] = 0$:

$$v = \frac{VK'[S]}{KK' + K'[S]} = \frac{V[S]}{K + [S]} \qquad \text{with } V = V_{max} \quad \text{and } K = K_m$$

At equilibrium:

$$v = \frac{VK'[S] - V'K[P]}{KK' + K'[S] + K[P]} = 0 \qquad VK'[S] = V'K[P] \qquad \frac{[S]}{[P]} = \frac{V'K}{VK'} = \frac{k_2 k_4 k_6}{k_1 k_3 k_5}$$

Haldane relationship

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$$v = \frac{k_{cat}[E_t][S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver and Burk:
double-reciprocal plot

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]} = \frac{1}{V_{max}}\left(\frac{K_m}{[S]} + 1\right) \qquad \frac{1}{v} = y \qquad \frac{1}{[S]} = x$$

$$y = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \bullet x \qquad y = a + b \bullet x$$

Linear plot, easier to handle!

Slope: K_m/V_{max} Intercepts → x: $-1/K_m$; y: $1/V_{max}$

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The reciprocal plot features crouded points

[S] (µM)	v (µM/s)	1/[S] (μM^{-1})	1/v (s/µM)	v/[S] (s^{-1})
25	0.708	0.0400	1.4124	0.0283
50	1.195	0.0200	0.8368	0.0239
75	1.551	0.0133	0.6447	0.0207
100	1.821	0.0100	0.5491	0.0182
200	2.468	0.0050	0.4052	0.0123
300	2.799	0.0033	0.3573	0.0093
500	3.135	0.0020	0.3190	0.0063
750	3.336	0.0013	0.2998	0.0044
1000	3.446	0.0010	0.2902	0.0034

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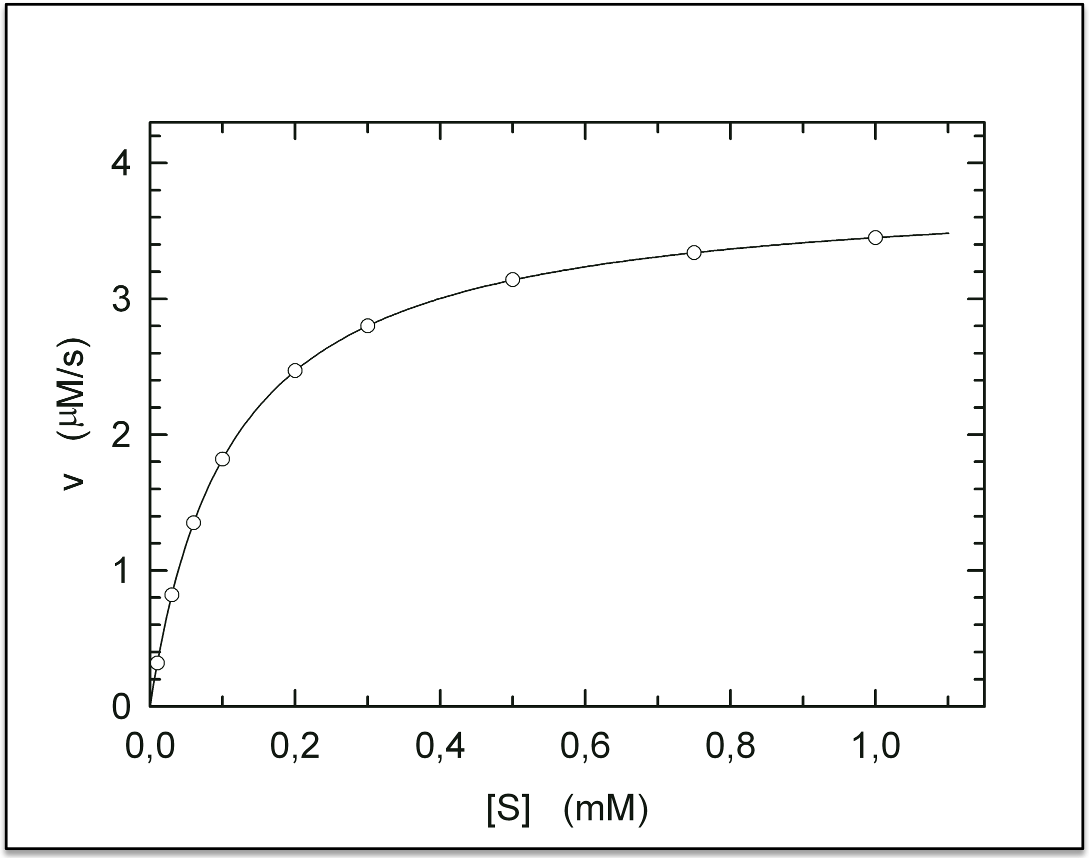
v vs. [S] plot



y = (a•x)/(b+x)

a = V_{max}

b = K_m

- Points well separated
- Fitting by computer

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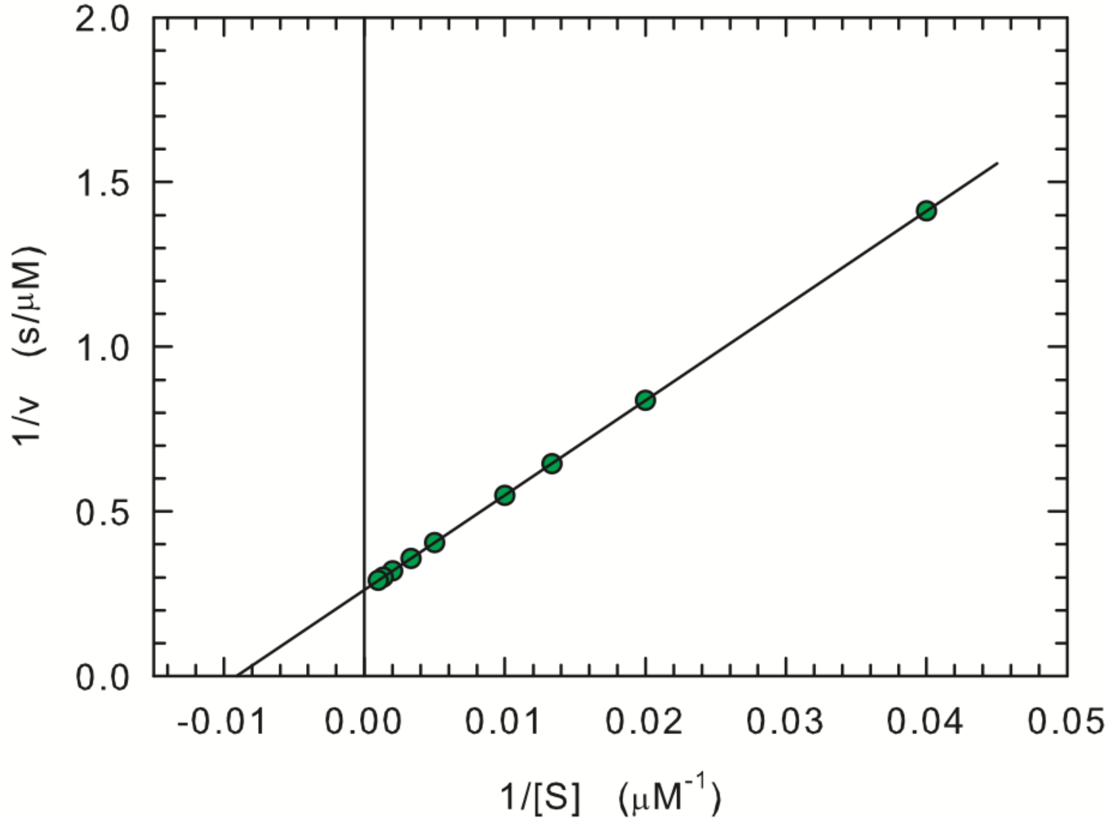
Lineweaver-Burk plot



$y = a + b \bullet x$

$a = 1/V_{max}$

$b = -1/K_m$

- Points at high [S] crouded
- Easy fitting

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$$v = \frac{k_{cat}[E_t][S]}{K_m + [S]} \qquad v = \frac{V_{max}[S]}{K_m + [S]}$$

Eadie-Hofstee:

$v(K_m + [S]) = V_{max}[S]$ $\qquad$ $v = V_{max} – v \bullet K_m/[S]$

$y = v$ $\qquad$ $x = v/[S]$ $\qquad$ $y = V_{max} - K_m \bullet x$

Slope: $-K_m$ $\quad$ Intercepts

→ x: V_{max}/K_m

→ y: V_{max}

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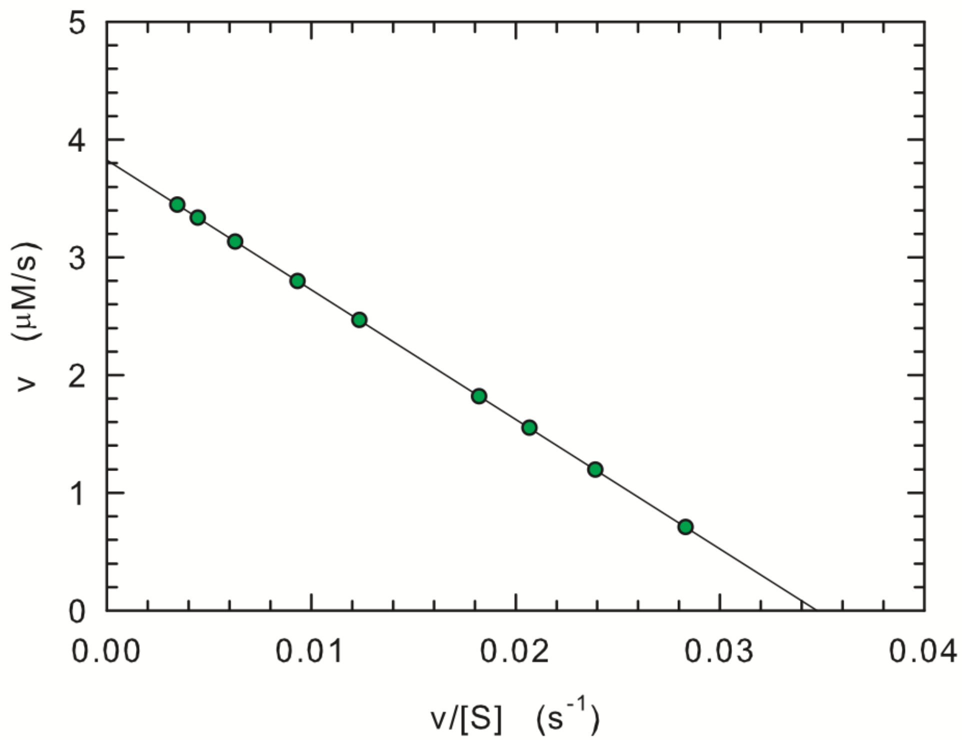
Eadie-Hofstee plot



$y = a - b \cdot x$

$a = V_{max}$

$b = K_m \cdot x$

- Points well separated
- Easy fitting

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