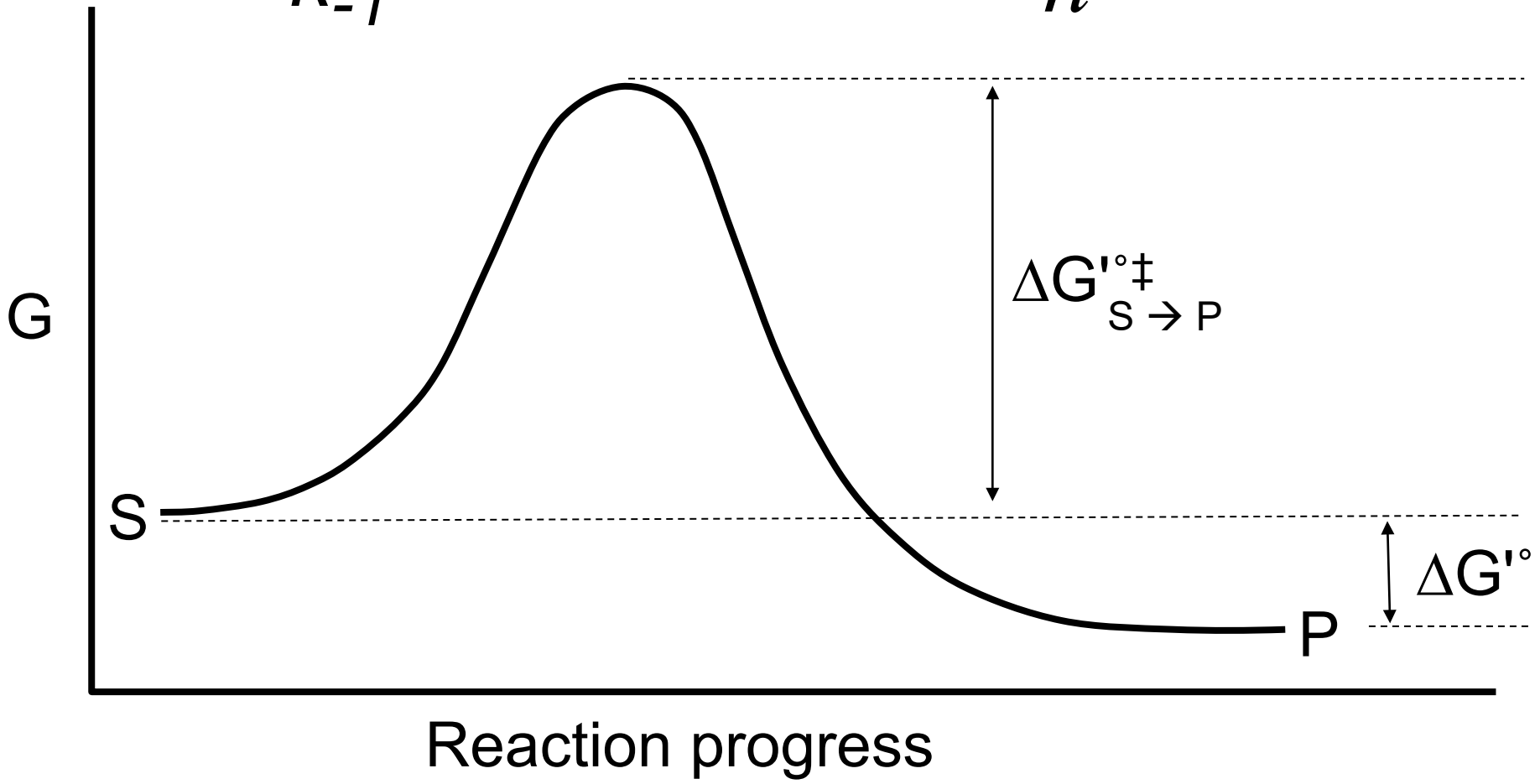
Enzyme Kinetics: The single substrate case

Reaction coordinate for S → P

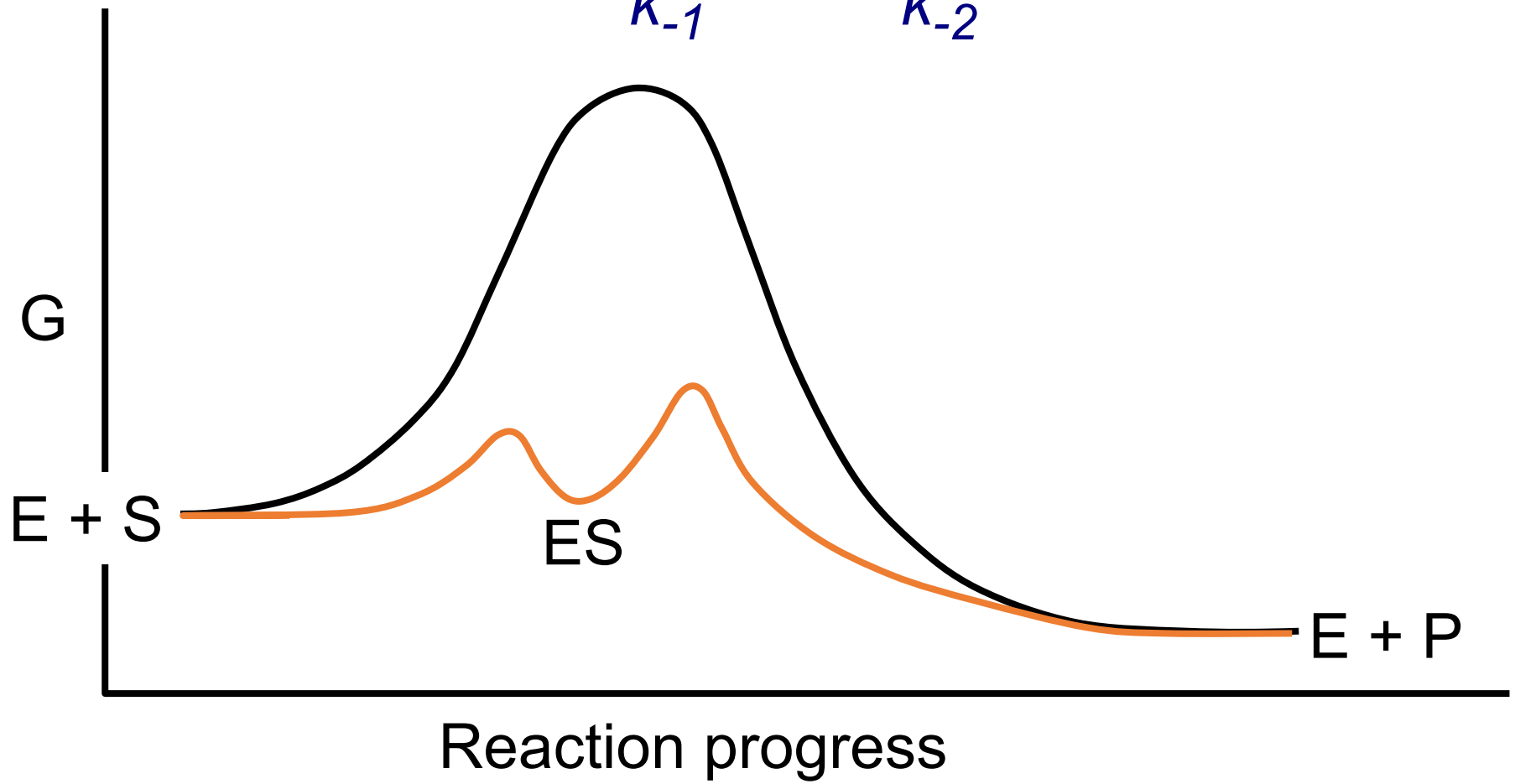
$$S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$$

$$k_1 = \frac{KT}{h} e^{-\Delta G^{\ddagger} / RT}$$

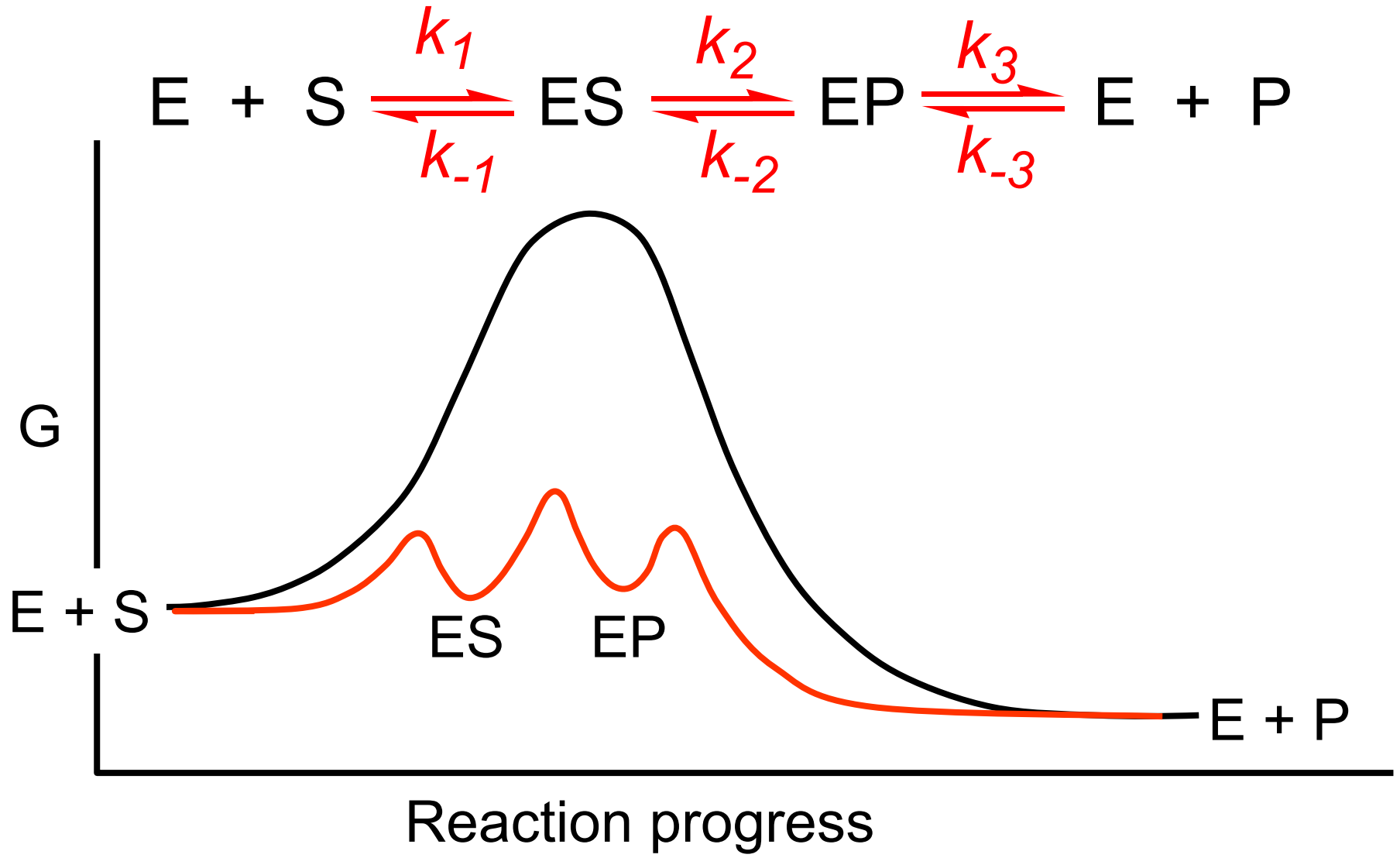


Reaction coordinate for S → P following the kinetic mechanism:

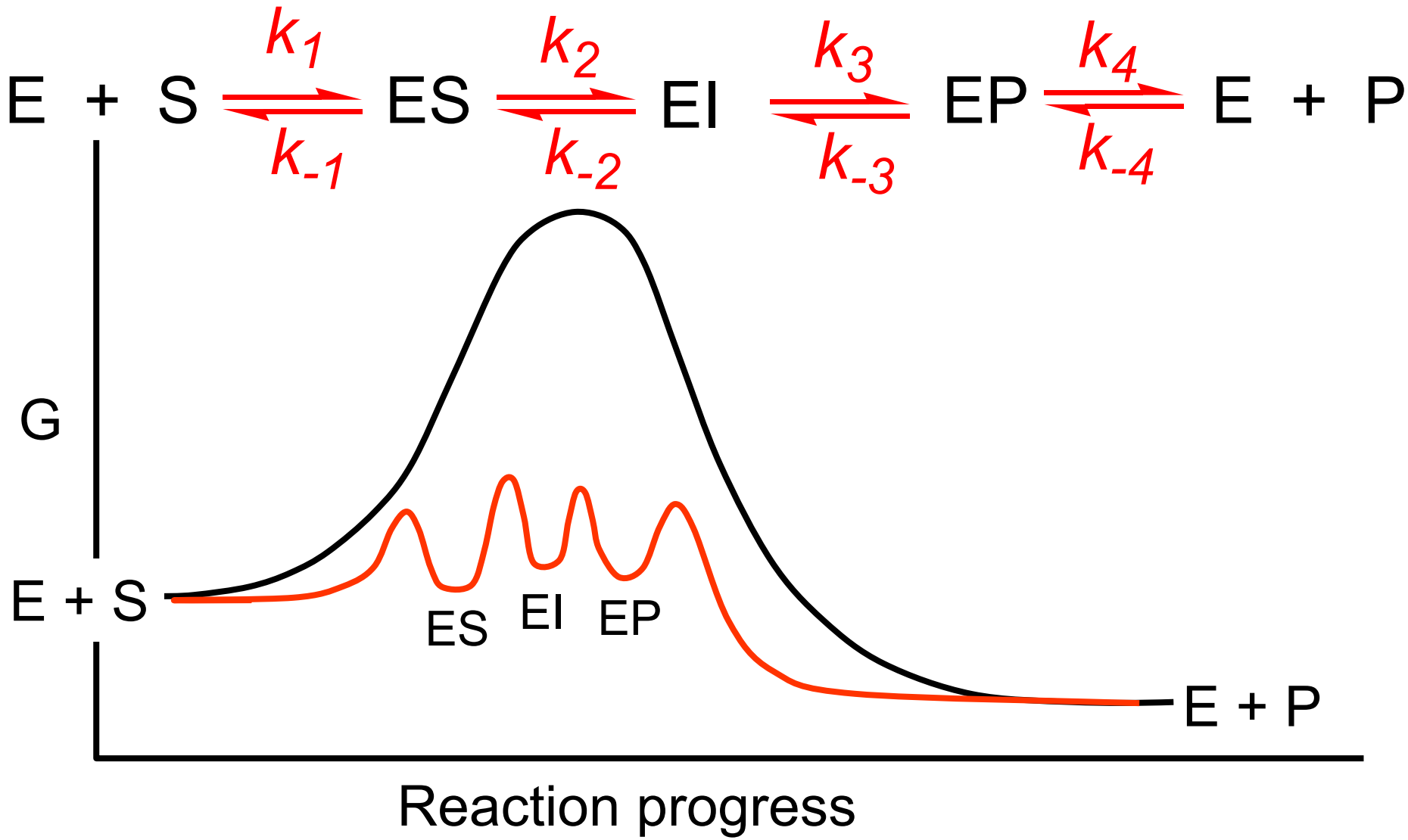
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$



Reaction coordinate for S → P following the kinetic mechanism:



Reaction coordinate for S → P following the kinetic mechanism:



Single substrate model: Steady state assumption

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

- E & P do not react back to form ES to any appreciable degree (design enzyme assay to assure this)

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

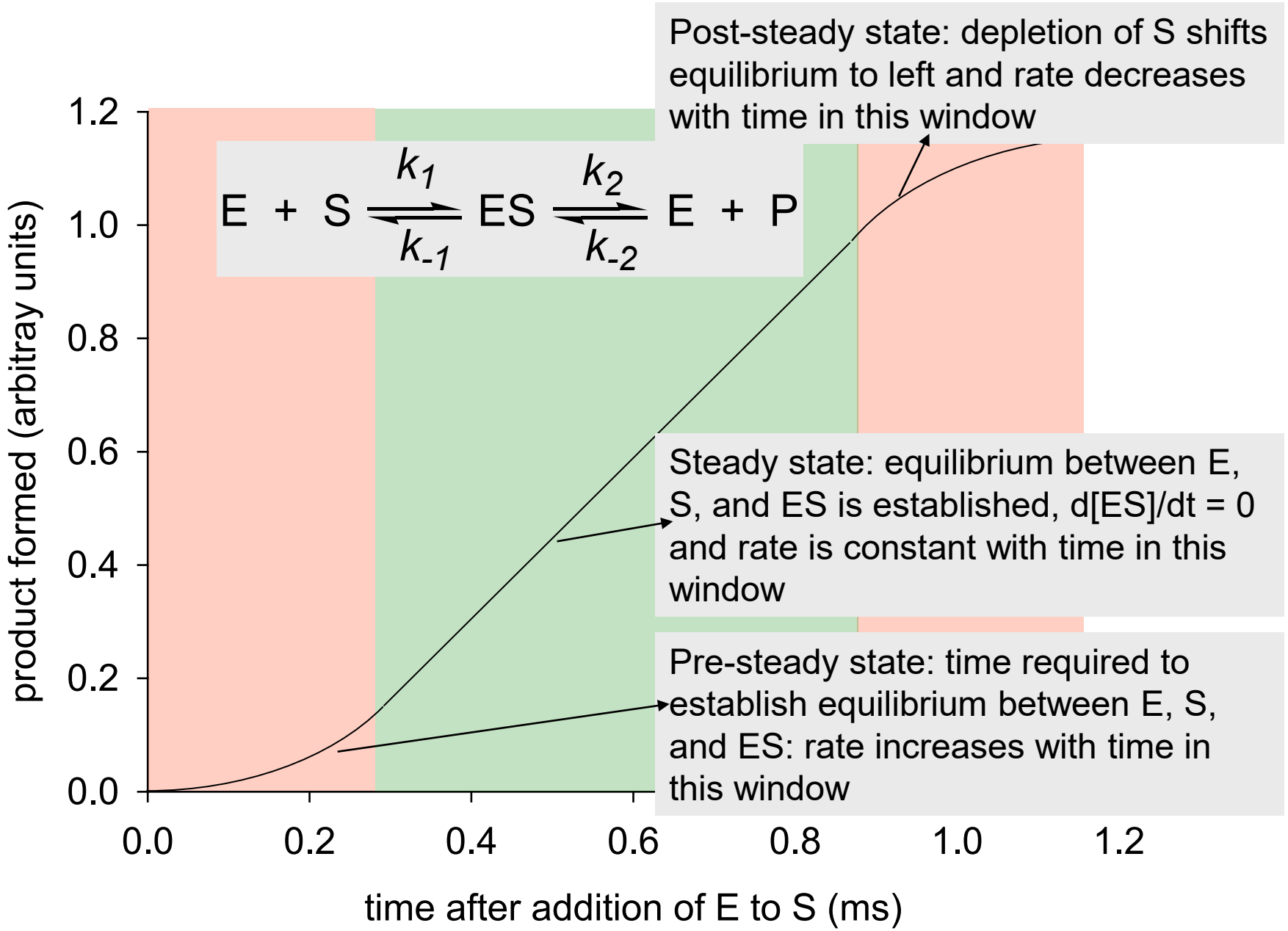
- Equilibrium of first step is rapidly established

$k_{-1} >> k_2$ $\qquad$ $K_a = \frac{k_1}{k_{-1}} = \frac{[\mathrm{ES}]}{[\mathrm{E}][\mathrm{S}]}$

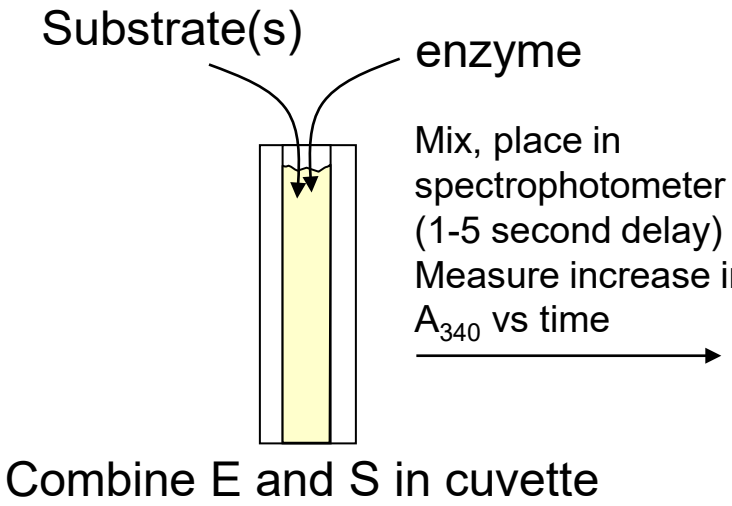
- [ES] remains constant during period in which enzyme activity is being measured

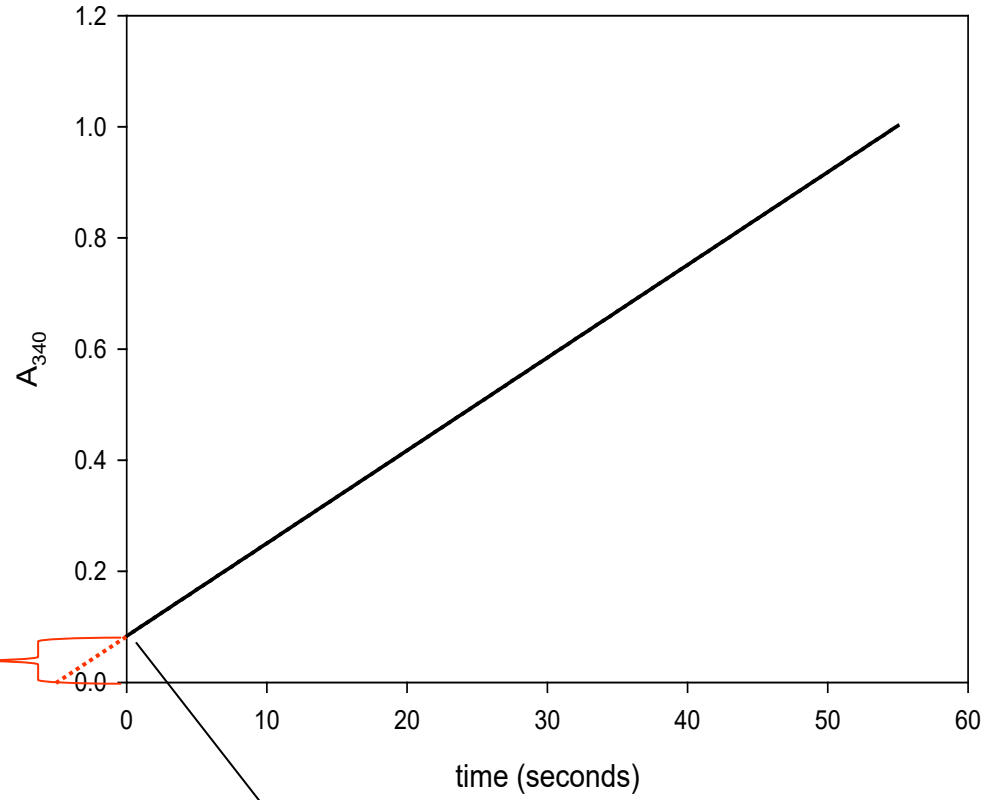
$$\frac{d[\mathrm{ES}]}{dt} = 0$$

Steady vs. pre-steady state



- The pre-steady state is usually only observed in the ms time frame (not seen under conditions most enzyme assays are conducted under)
- Example: lactate dehydrogenase: lactate + NAD^+ → pyruvate + NADH

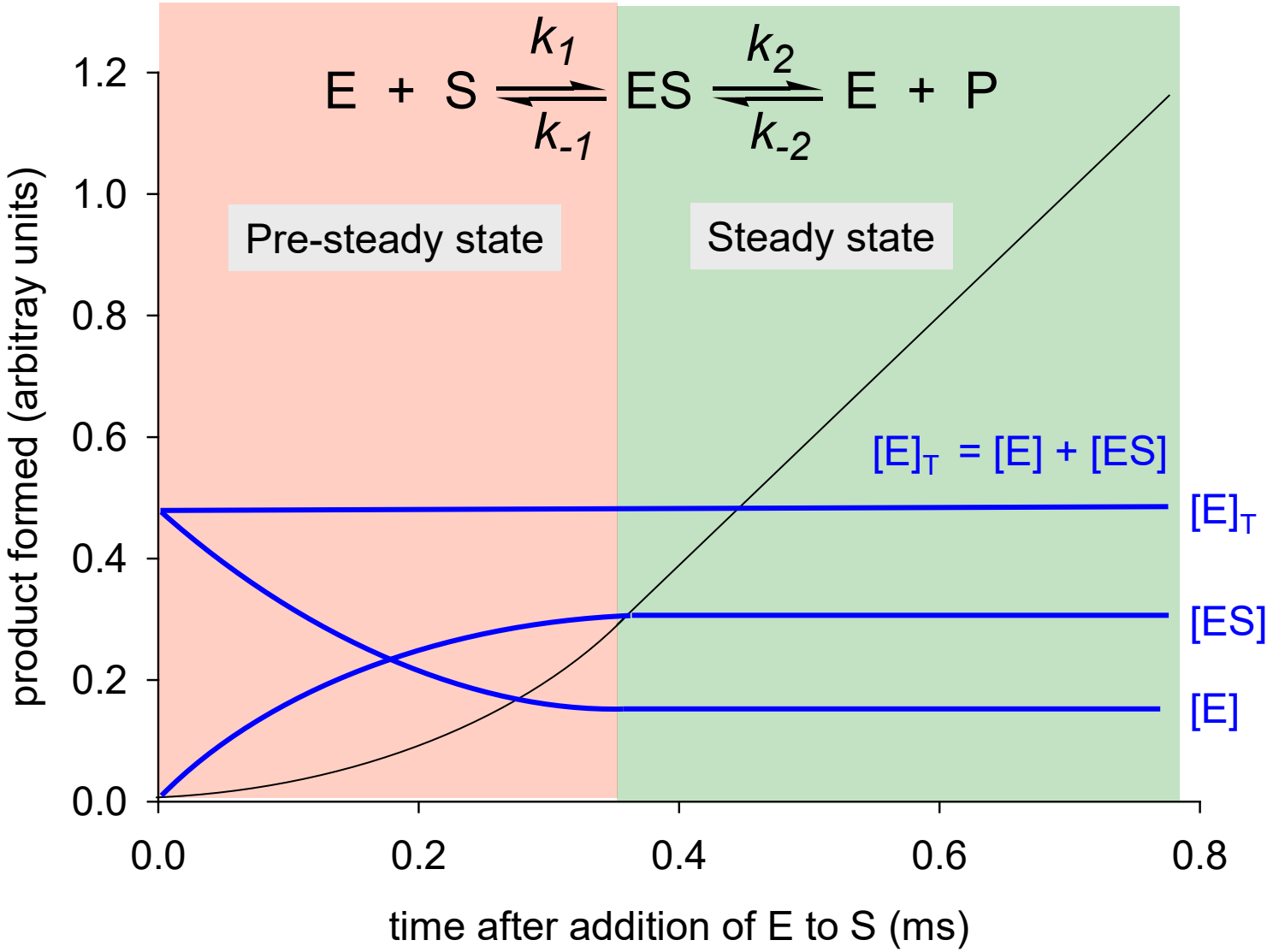




product is formed during delay time in starting measurement (time to mix, put in spec, press "go")

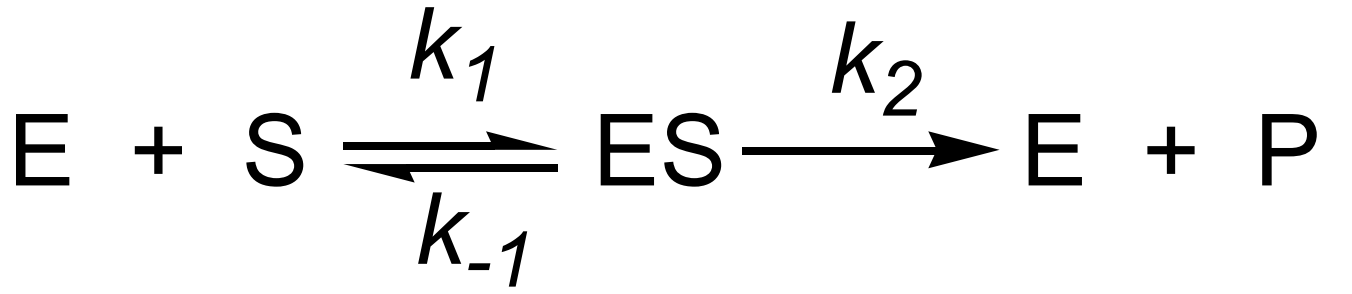
Initial A_{340} reading at time at which data recording is started is not 0.

[E], [ES], and $[E]_T$ during establishment of the steady state



The relative concentrations of E, S and ES in the steady state are determined by how much E_T and S are present in the assay

Derivation of a rate law for the steady-state



Equation 1

$$\frac{d[\mathrm{ES}]}{dt} = 0 = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}]$$

Equation 2

$$[\mathrm{E}]_T = [\mathrm{E}] + [\mathrm{ES}]$$

Equation 3

$$\frac{d[\mathrm{P}]}{dt} = v_0 = k_2[\mathrm{ES}]$$

Algebraic manipulation of these three expressions allows the rate law to be determined

Derivation of a rate law for the steady-state

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

Equation 1

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

Solve for $k_1[E][S]$

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

Combine like terms

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

Equation 2

(4) $$[E]_T = [E] + [ES]$$

Solve for [E]

(5) $$[E] = [E]_T - [ES]$$

Substitute (5) into (3)

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

Expand left side

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

Solve for $k_1[E]_T[S]$

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

Combine like terms

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

Solve for [ES]

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

Multiply N and D by $1/k_1$

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

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

Define constant K_m

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

Substitute 13 into 12

(14) $$[ES] = \frac{[E]_T[S]}{[S] + K_m}$$

Equation 3

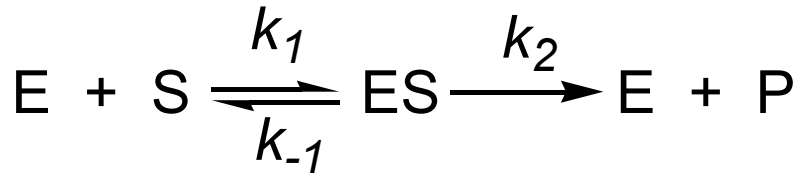
(15) $$\frac{d[P]}{dt} = v_0 = k_2[ES]$$

Solve for [ES]

(16) $$[ES] = \frac{v_0}{k_2}$$

Continued next page.

Derivation of a rate law for the steady-state



Substitute (16) into (14)

$$(14)\quad [ES] = \frac{[E]_T[S]}{[S]+K_m} \qquad (16)\quad [ES] = \frac{v_0}{k_2}$$

$$(17)\quad \frac{v_o}{k_2} = \frac{[E]_T[S]}{[S]+K_m}$$

Solve for v_o

$$(18)\quad v_o = \frac{k_2[E]_T[S]}{[S]+K_m}$$

The maximal velocity for the enzyme (V_{max}) will occur when the concentration of S is infinitely high, such that essentially all of $[E]_T$ is in the form of [ES]

$$v_0 = k_2[ES]$$

$$(19)\quad V_{max} = k_2[E]_T$$

Substitute (19) into (18)

$$(20)\quad v_o = \frac{V_{max}[S]}{[S]+K_m}$$

Michaelis-Menten equation

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

Equation for fractional saturation of oxygen binding to myoglobin

$$\theta = \frac{[O_2]}{[O_2]+K_d}$$

Equation giving a rectangular hyperbola

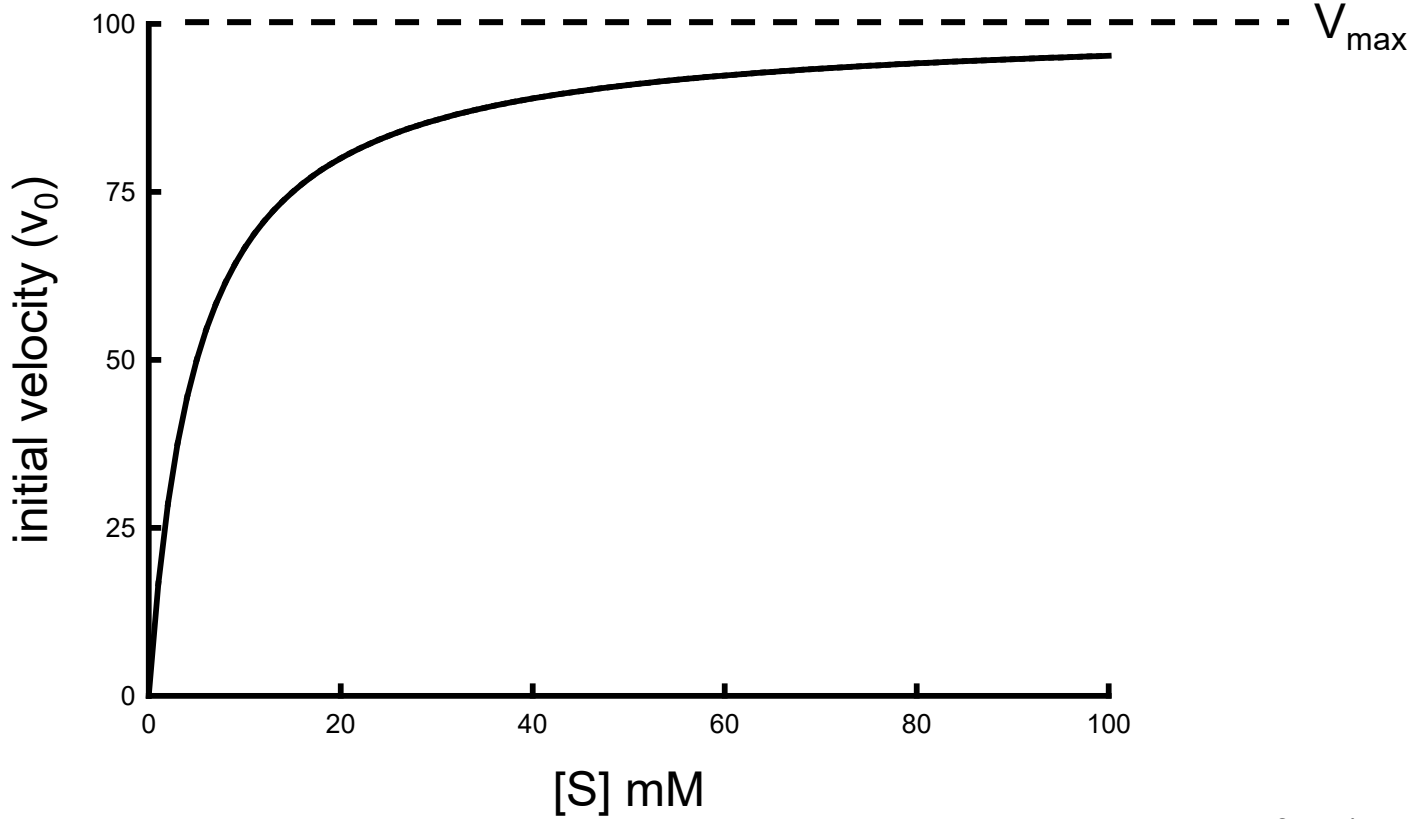
$$y = \frac{x}{x+C}$$

Michaelis-Menten Equation for the single substrate case

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

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

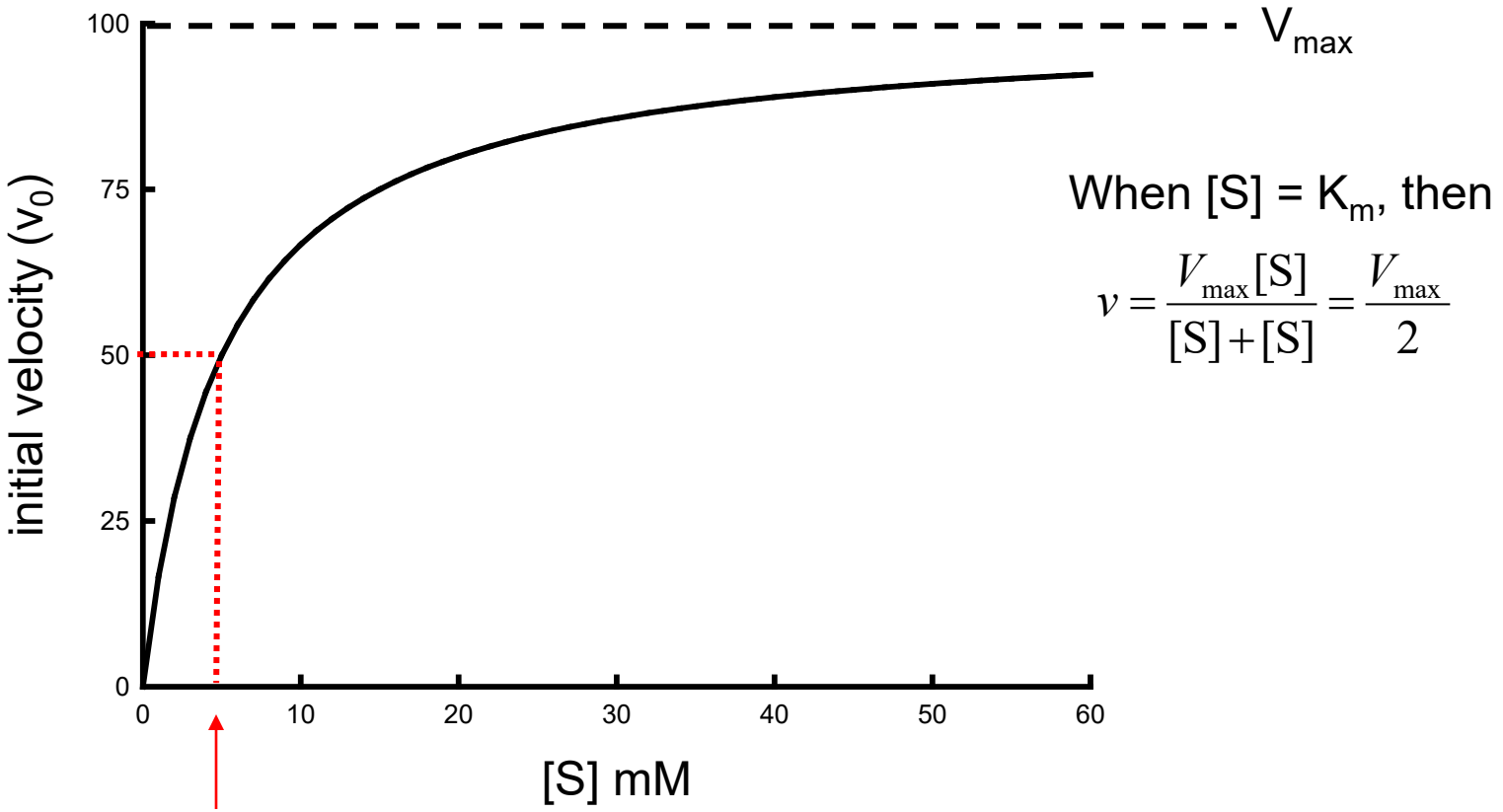
$$K_m = \frac{k_{-1} + k_2}{k_1}$$



S. Ensign, enzyme kinetics

Michaelis-Menten Equation for the single substrate case

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad v = \frac{V_{max}[S]}{K_m + [S]} \qquad K_m = \frac{k_{-1} + k_2}{k_1}$$

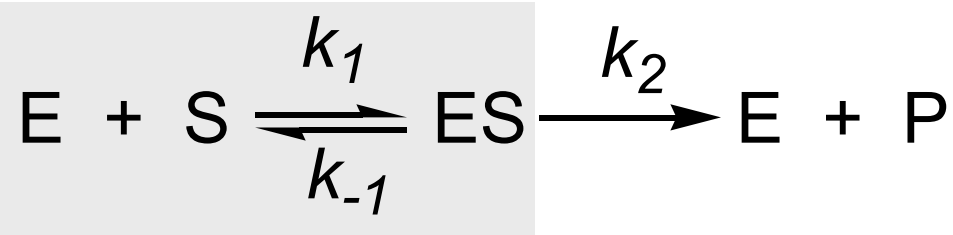


When [S] = K_m, then

$$v = \frac{V_{max}[S]}{[S] + [S]} = \frac{V_{max}}{2}$$

K_m = concentration of substrate that results in a rate equal to ½ V_{max}

K_D vs. K_m (single substrate case)



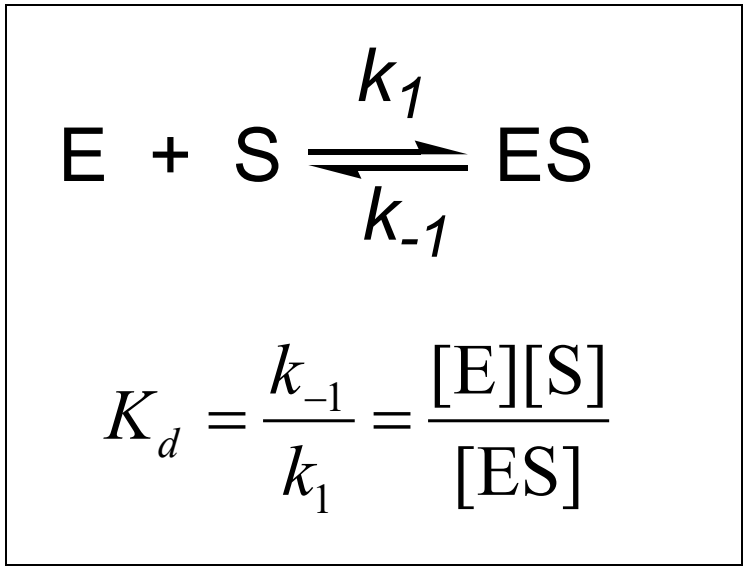
$$K_m = \frac{k_{-1} + k_2}{k_1}$$

Consider the equilibrium for the formation of ES from E and S (disregard 2nd step)

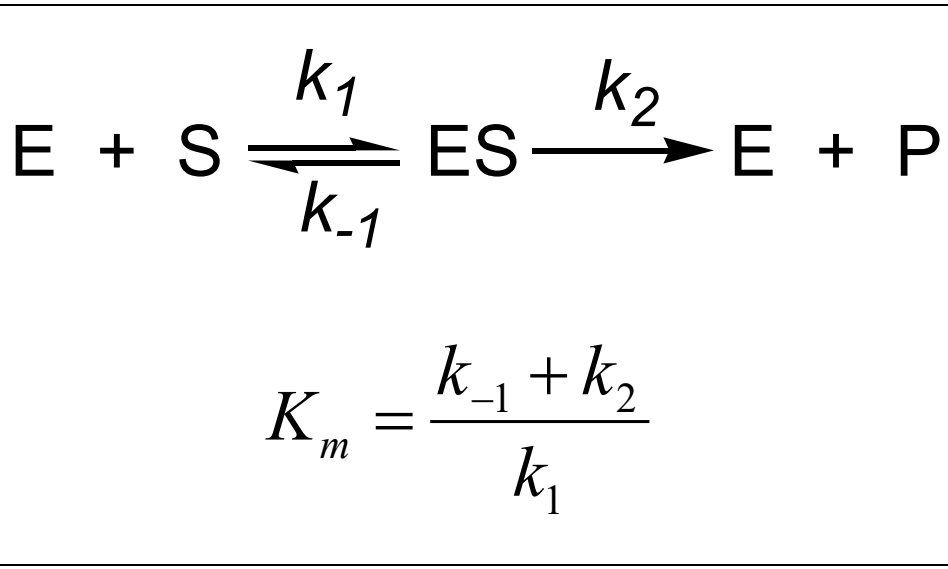
Association equilibrium constant: $$K_a = \frac{k_1}{k_{-1}} = \frac{[\mathrm{ES}]}{[\mathrm{E}][\mathrm{S}]}$$

Dissociation equilibrium constant: $$K_d = \frac{1}{K_a} = \frac{k_{-1}}{k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

K_D vs. K_m (single substrate case)



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

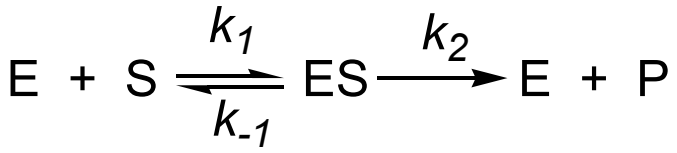


$$K_m = \frac{k_{-1} + k_2}{k_1}$$

When $k_{-1} >> k_2$, then $K_m \approx \frac{k_{-1}}{k_1} = K_d$

K_m approximates the affinity of an enzyme for it's substrate only when $k_{-1} >> k_2$

The numerical values of K_m and V_{max} vary greatly for different enzymes



$$K_m = \frac{k_{-1} + k_2}{k_1} \qquad v = \frac{V_{\max}[S]}{K_m + [S]}$$

- K_m: high nM/low μM → high mM
- Physiological significance?
  - Available [S]
  - Need to regulate substrate conversion rate?
- V_{max} units: μmol/min; tells nothing about $[E]_T$ in assay (can report as μmol/min/mg)
-
- $V_{max} / [E]_T = k_{cat}$ $v_0 = k_2[ES]$ $V_{\max} = k_2[E]_T$

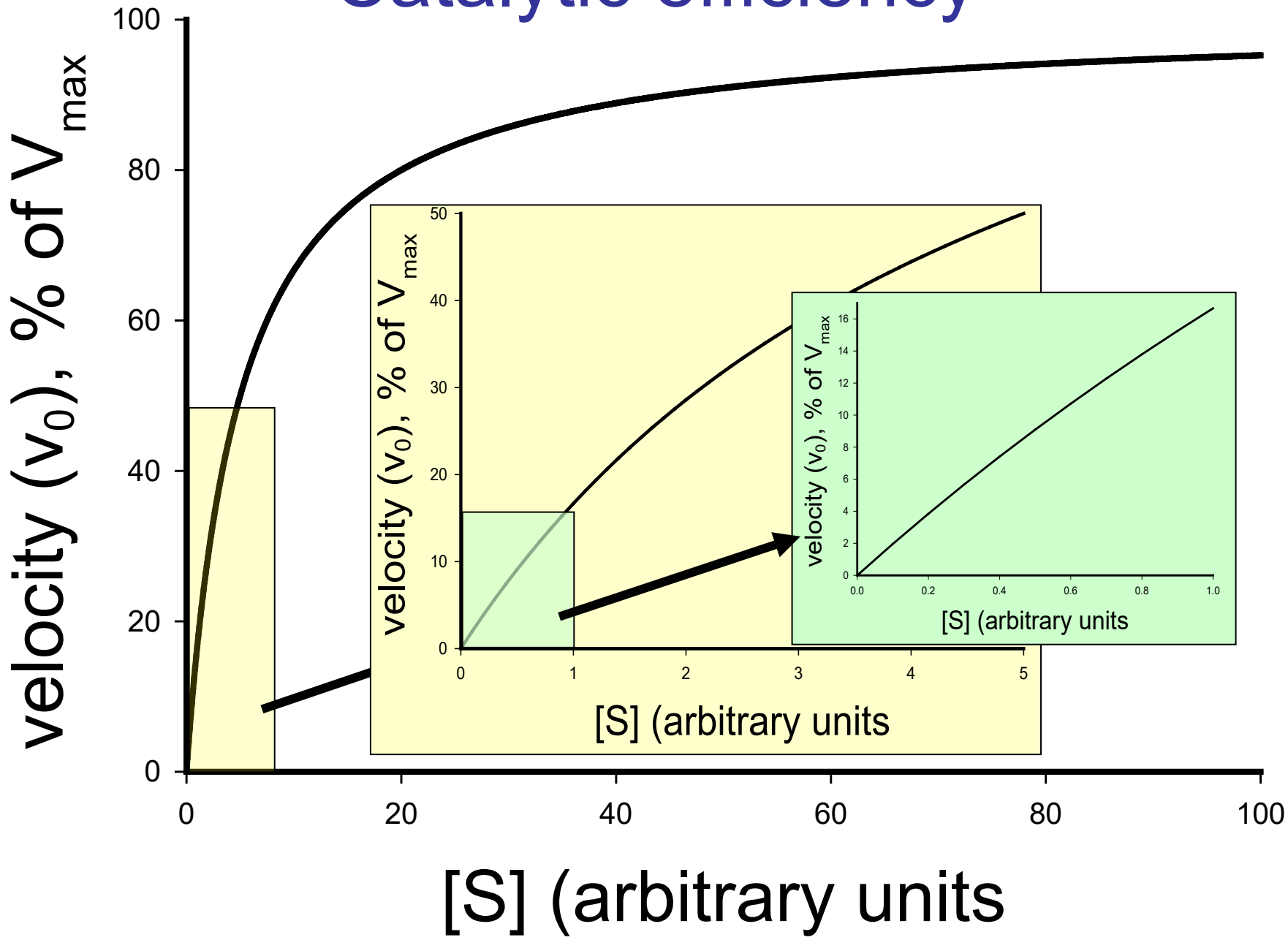
k_{cat}: turnover number

- The number of reaction processes (turnovers) that each active site catalyzes per unit time (per s, per min)
- A first order rate constant (s^{-1} or min^{-1})
- Calculate k_{cat} for a homodimeric (α_2) enzyme of Mr = 126,000 exhibiting V_{max}=10,000 U/mg
- For the simple kinetic mechanism,

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

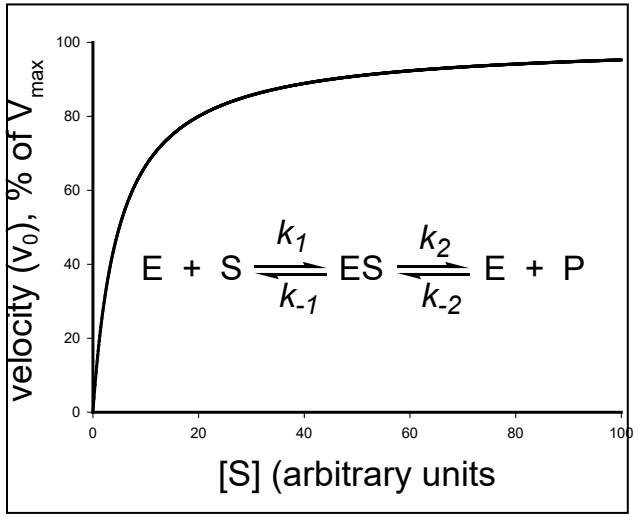
$k_{cat} = k_2$ when step 2 is the RDS

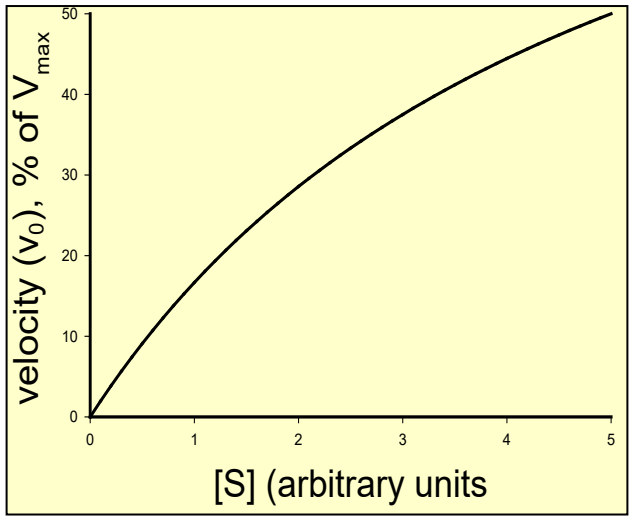
Catalytic efficiency

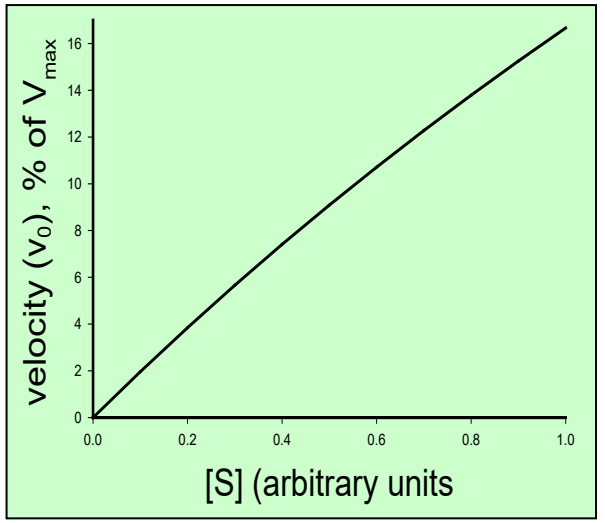


S. Ensign, enzyme kinetics

An enzyme is most "efficient" at converting substrate to product at low concentrations of substrate







$$v = \frac{V_{\max}[\mathrm{S}]}{K_m + [\mathrm{S}]}$$; when [S] << K_m, $$v \approx \frac{V_{\max}[\mathrm{S}]}{K_m}$$; $$V_{\max} = [\mathrm{E}]_T \cdot k_{cat}$$; $$v \approx \frac{k_{cat}}{K_m}[\mathrm{E}]_T[\mathrm{S}]$$

$$[\mathrm{E}]_T = [\mathrm{E}] + [\mathrm{ES}]$$; when [S] << K_m, $[\mathrm{E}] >> [\mathrm{ES}]$ and $[\mathrm{E}]_T \approx [\mathrm{E}]$

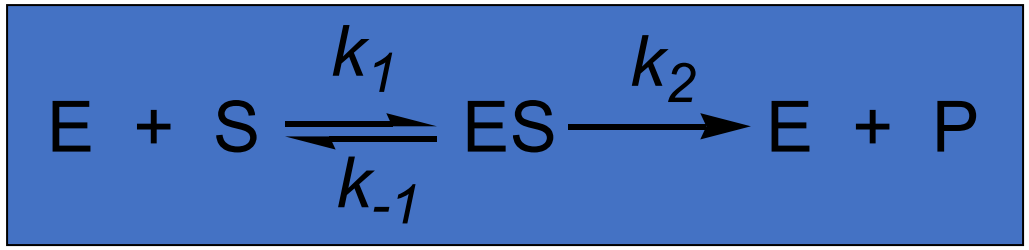
thus $$v \approx \frac{k_{cat}}{K_m}[\mathrm{E}][\mathrm{S}]$$

A 2nd order rate equation of form $rate = k[\mathrm{A}][\mathrm{B}]$

Catalytic efficiency

- The ratio $\frac{k_{cat}}{K_m}$
- A second order rate constant (units of $M^{-1} \cdot s^{-1}$)
- A high k_{cat} and low K_m contribute to high catalytic efficiency
- The magnitude of k_{cat}/K_m differs dramatically for different enzymes

Is there an upper limit to the catalytic efficiency of an enzyme?



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

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

For the single intermediate model shown above, $k_{cat} = k_2$

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

$\frac{k_{cat}}{K_m}$ is greatest when $k_2 >> k_{-1}$, reducing above expression to

$\frac{k_{cat}}{K_m} \approx k_1$ and thus the rate of reaction is dictated by the magnitude of k_1

Is there an upper limit to the catalytic efficiency of an enzyme?

$$E + S \xrightarrow{k_1} ES \xrightarrow{fast} E + P \qquad v = k_1[E][S]$$

- The diffusion controlled upper limit to a bimolecular second order reaction is 10^8 to 10^9 $M^{-1}s^{-1}$
- Enzymes with k_{cat}/K_m values in this range are "perfect enzymes": they "instantaneously" catalyze a reaction every time E and S collide!
- It is impossible to exceed this diffusion controlled limit for a catalyzed (thus bimolecular) reaction

How do you improve a perfect enzyme?

Reversible reactions (single S and P case)

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

$$v = \frac{\frac{V^f_{\max}[S]}{K^S_m} - \frac{V^r_{\max}[P]}{K^P_m}}{1 + \frac{[S]}{K^S_m} + \frac{[P]}{K^P_m}}$$

$$K^S_m = \frac{k_{-1} + k_2}{k_1} \qquad K^P_m = \frac{k_{-1} + k_2}{k_{-2}}$$

$$V^f_{\max} = [E]_T \cdot k_2 \qquad V^r_{\max} = [E]_T \cdot k_{-1}$$

At equilibrium, rate of S → P = rate of P → S and v = 0. Substitute 0 into the equation for v and solve for [P]/[S] (i.e., K_{eq})

The Haldane Relationship

$$K_{eq} = \frac{[\mathrm{P}]}{[\mathrm{S}]} = \frac{V_{\max}^{f} \; K_{m}^{P}}{V_{\max}^{r} \; K_{m}^{S}}$$

- The kinetic parameters of a reversible enzymatic reaction are related by the equilibrium constant for the overall reaction

Models with two or more intermediates

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

Two intermediate case: $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$

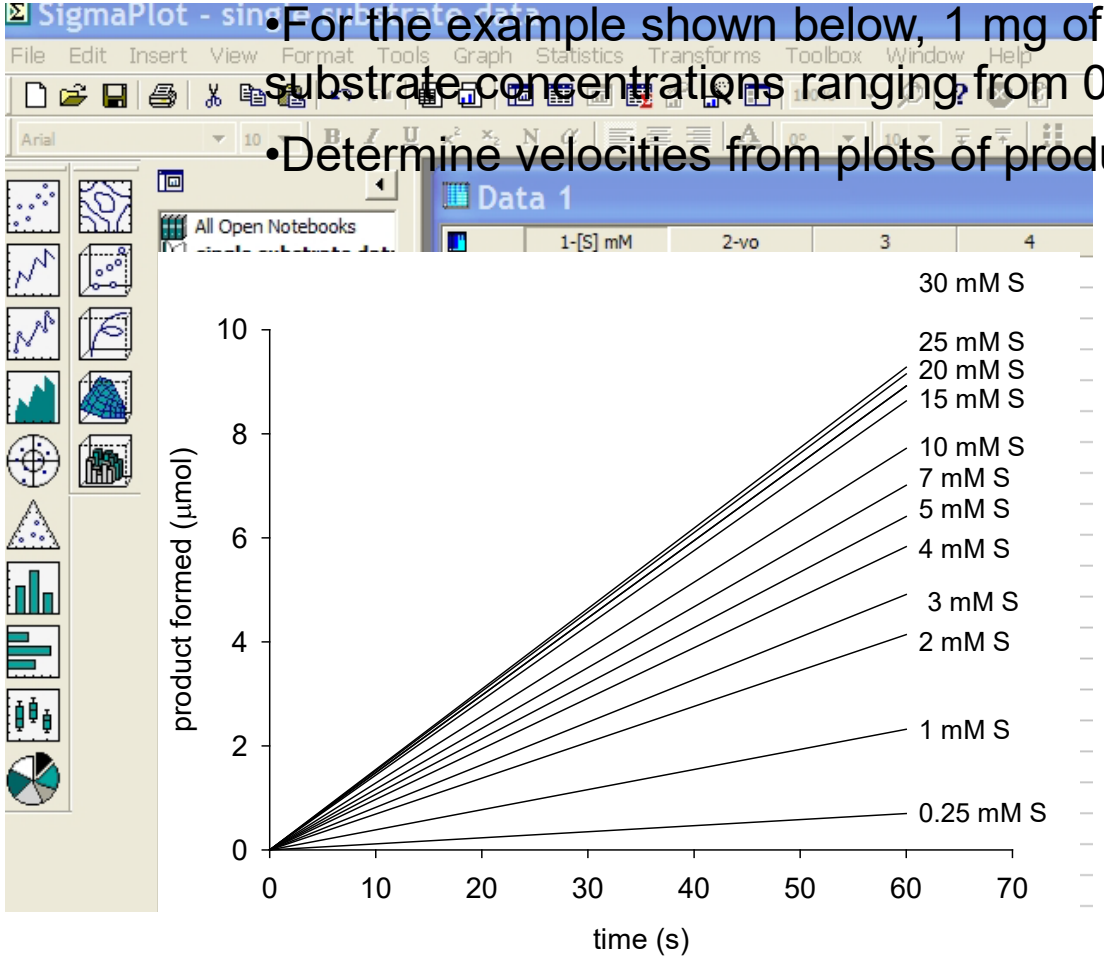
Three intermediate case: $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EI \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P$

- The overall Michaelis-Menten equations are the same as for the one intermediate model
- Kinetic parameters ($V_{\max}^{f}, K_m^{S}, V_{\max}^{r}, K_m^{P}$) are defined in terms of 6 or 8 kinetic constants for the examples shown
- The RDS for product determination defines k_{cat}
- Steady state kinetics cannot distinguish the number or nature of intermediates in an enzymatic reaction.

Black box model for steady state enzyme kinetics

Analysis of kinetic data: single substrate case

- Conduct enzyme assays with constant [E] and varying [S]
- Choose a wide range of substrate concentrations both below, around, and above estimated K_m
- For the example shown below, 1 mg of enzyme was assayed with substrate concentrations ranging from 0.25 mM to 30 mM
- Determine velocities from plots of product formed vs. time for each [S]

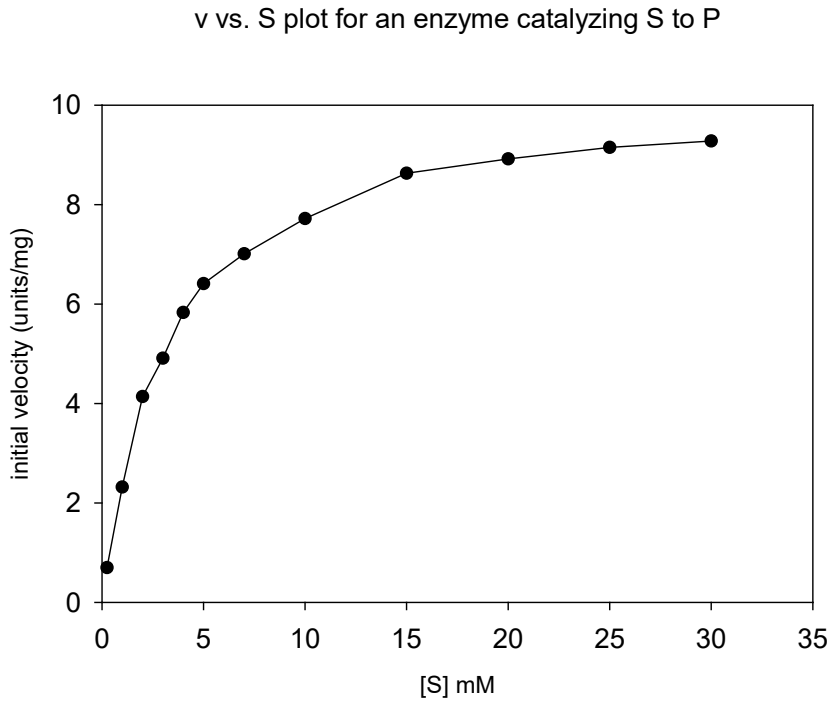


Analysis of kinetic data: single substrate case

- Create a plot of initial velocity vs. [S]

single S data

S	v
0.25	0.7
1	2.32
2	4.14
3	4.91
4	5.83
5	6.41
7	7.01
10	7.72
15	8.63
20	8.92
25	9.15
30	9.28



Estimate V_{max} and K_m from looking at this graph?

Methods used to determine K_m and V_{max}

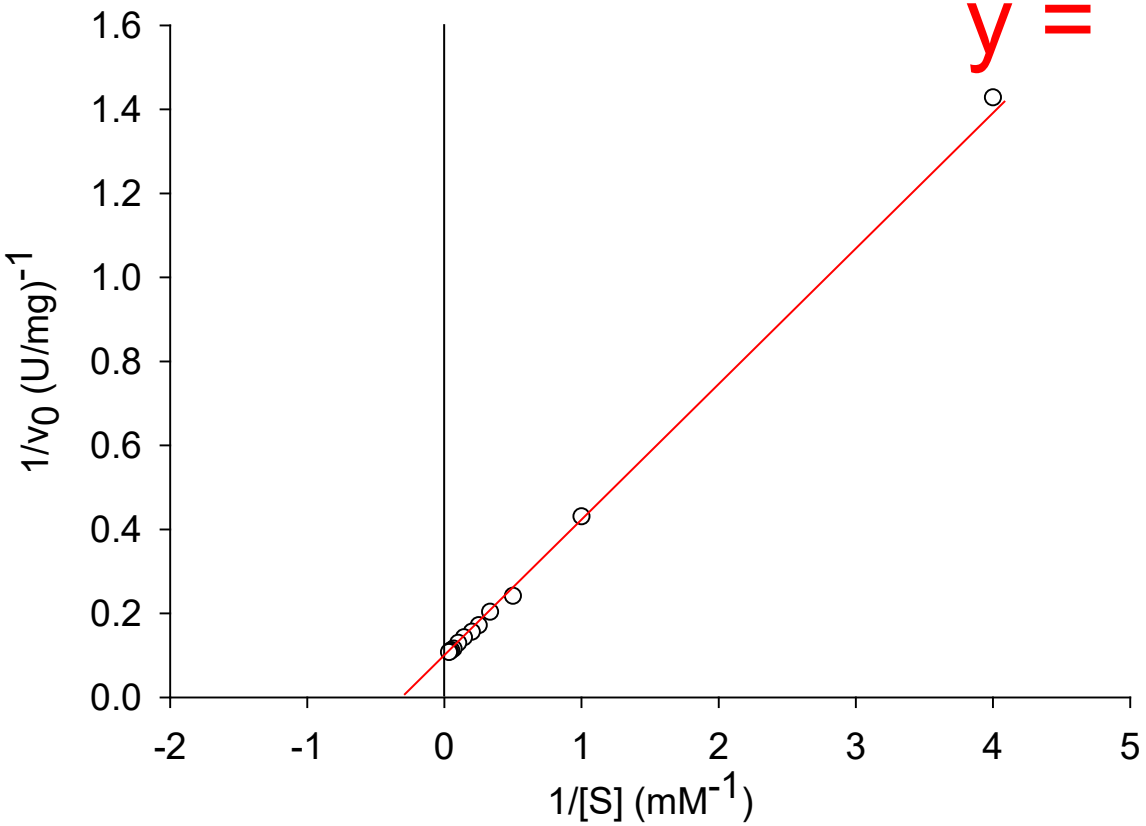
- Estimate from v vs. S plot (qualitative at best)
- Algebraic rearrangement of Michaelis-Menten equation; replot data and estimate parameters from replots (useful for visual purposes)
- Nonlinear regression of v vs. S data- the only acceptable way for determining kinetic constants for publication

Algebraic rearrangement of the Michaelis-Menten Equation

single S data	
S	v
0.25	0.7
1	2.32
2	4.14
3	4.91
4	5.83
5	6.41
7	7.01
10	7.72
15	8.63
20	8.92
25	9.15
30	9.28

$$v = \frac{V_{max}[S]}{K_m + [S]} \Longrightarrow \frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = \underbrace{m}\ x + b$$



1/S	1/v
4	1.428571
1	0.431034
0.5	0.241546
0.333333	0.203666
0.25	0.171527
0.2	0.156006
0.142857	0.142653
0.1	0.129534
0.066667	0.115875
0.05	0.112108
0.04	0.10929
0.033333	0.107759

- Lineweaver-Burke (double reciprocal) plot
- y-int = $1/V_{max}$
- x-int = $-1/K_m$
- slope = K_m/V_{max}
- DON'T fit the double reciprocal data using linear regression or you will introduce errors (trumpet effect)

Fitting Kinetic data to the Michaelis-Menten Equation (rectangular hyperbola) using a free on line tool: http://statpages.info/nonlin.html

- Statpages credit: John Pezullo
- Page is no longer maintained or updated, so I have created a modified mirror page in case the above site gets taken down:
  - http://ensignchemistry.com/enzymology/curve_fitting.html
- See file: “kinetic curve fitting codes”

Fitting Kinetic data to the Michaelis-Menten Equation (rectangular hyperbola) using nonlinear regression

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

Instructions:

1. Enter the number of data points: 12
2. Enter the number of independent variables: 1 (1 for single substrate M-M equation; 2 for bisubstrate, 2 for C, UC, and mixed inhibition)
3. Enter the number of parameters: 2 (deduce from form of M-M equation used; will be 2 for single substrate M-M)
4. Enter the formula for the function to be fitted:
   $Y =$ a*x1/(b+x1)
   Use "" for multiplication and "/" for division when entering formulas. Define parameters (a, b, c) and variables (x1, x2, x3) as described above. Be sure to use parentheses approriately to get the correct hierarchy*
5. Type (or paste) the [x,y] data:

```
0.25    0.7
1       2.32
2       4.14
3       4.91
4       5.83
5       6.41
7       7.01
10      7.72
```

   Data can be typed in directly, or pasted from a text file or spreadsheet. The first column(s) contain the independent variable(s) (x1, x2..) while the final column contains the dependent variable y (velocity for enzyme data)
6. Enter initial guesses for the parameters from evaluation of the data (e.g. Lineweaver-Burke plots and appropriate slope and intercept replots). If the data fails to converge, provide better initial guesses. :

a (or p_1)=	10	±		, p=	
b (or p_2)=	3	±		, p=	
c (or p_3)=	0	±		, p=	
d (or p_4)=	0	±		, p=	
e (or p_5)=	0	±		, p=	
f (or p_6)=	0	±		, p=	
g (or p_7)=	0	±		, p=	
h (or p_8)=	0	±		, p=	

   Place the value "0" (zero) in all parameter boxes for which a value is not defined in the equation to be fit. Leave the fields to the right of the parameters blank; these will be populated with the standard errors upon iteration.
7. Click the Iterate button once for each iteration cycle. Watch how the initial guesses change in response to the iteration. Continue to click the "iterate" button until the values converge to unchanging values. If the parameters do not converge on reasonable numbers, provide better initial guesses and try again.

Fitting Kinetic data to the Michaelis-Menten Equation (rectangular hyperbola) using nonlinear regression

6. Enter initial guesses for the parameters from evaluation of the data (e.g. L

a (or p_1)=	10.31581092113	±	0.084894623156	p=	5e-7
b (or p_2)=	3.175900437348	±	0.093749889829	p=	5e-7
c (or p_3)=	0	±	1	p=	1.000000
d (or p_4)=	0	±	1	p=	1.000000
e (or p_5)=	0	±	1	p=	1.000000
f (or p_6)=	0	±	1	p=	1.000000
g (or p_7)=	0	±	1	p=	1.000000
h (or p_8)=	0	±	1	p=	1.000000

Place the value "0" (zero) in all parameter boxes for which a value is not defined in the equ

7. Click the Iterate button once for each iteration cycle. Watch how the init
the parameters do not converge on reasonable numbers, provide better init
If you encounter problems getting the parameters to converge, you can spe
making the convergence slower but more stable. Change this value back to 1.0 once the i

RMS Err = 0.105620199095 Weighted Standard Error of Estimate: the average scat
points to within their intrinsic errors.

```
Y = a*x1/(b+x1)
 x1   Y yc Y-yc SEest YcLo YcHi
 0.2500 0.7000 0.7528 -0.0528 0.0158 0.7177 0.7879
 1.0000 2.3200 2.4703 -0.1503 0.0399 2.3813 2.5593
 2.0000 4.1400 3.9861 0.1539 0.0481 3.8789 4.0933
 3.0000 4.9100 5.0110 -0.1010 0.0471 4.9059 5.1161
 4.0000 5.8300 5.7503 0.0797 0.0438 5.6526 5.8479
 5.0000 6.4100 6.3087 0.1013 0.0404 6.2188 6.3986
 7.0000 7.0100 7.0962 -0.0862 0.0358 7.0165 7.1760
 10.0000 7.7200 7.8293 -0.1093 0.0352 7.7510 7.9076
```

V_{max} ± std error

K_m ± std error

Theoretical v values using parameters returned from the curve fit

points to within their intrinsic errors.

```
Y = a*x1/(b+x1)
x1      Y       yc      Y-yc    SEest   YcLo    YcHi
0.25    0.7     0.7527809921646346      -0.05278099216463461
0.015752330790615998    0.7176826119207237      0.7878793724085454
1       2.32    2.470320132365934       -0.150320132365934
0.03993819349781473     2.381332291755308       2.5593079729765598
2       4.14    3.98609325894304        0.1539067410569599
0.04809482898551111     3.8789313019007876      4.093255215985292
3       4.91    5.010999299188705       -0.10099929918870476
0.047149592825910566    4.905943459557961       5.116055138819449
```

Optional) click here for ☑ Tab-delimited output (more convenient for pasting the results into spreadshee

Tab-delimited data → textedit (pc) → open in excel:

	A	B	C	D	E	F	G
1	x1	Y	yc	Y-yc	SEest	YcLo	YcHi
2	0.25	0.7	0.752781	-0.05278	0.015752	0.717683	0.787879
3	1	2.32	2.47032	-0.15032	0.039938	2.381332	2.559308
4	2	4.14	3.986093	0.153907	0.048095	3.878931	4.093255
5	3	4.91	5.010999	-0.101	0.04715	4.905943	5.116055
6	4	5.83	5.750253	0.079747	0.043807	5.652646	5.84786
7	5	6.41	6.30867	0.10133	0.040356	6.21875	6.398589
8	7	7.01	7.096244	-0.08624	0.035796	7.016486	7.176003
9	10	7.72	7.829302	-0.1093	0.03515	7.750983	7.907622
10	15	8.63	8.513315	0.116685	0.040961	8.422047	8.604582
11	20	8.92	8.902188	0.017812	0.047329	8.796731	9.007644
12	25	9.15	9.153045	-0.00304	0.052485	9.036101	9.269988
13	30	9.28	9.328287	-0.04829	0.056523	9.202347	9.454227
14							
15	Corr. Coef	0.99935	r*r =	0.9987			
16	RMS Error	0.10562	d.f =	10	SSq =	0.111556	
17	AIC =	-50.1376	AIC(corre	-47.1376			
18							
19	Parameter Estimates...						
20	p1=	10.31581		0.084895	p=	3.33E-16	
21	p2=	3.1759		0.09375	p=	1.19E-11	
22							
23	Covariance Matrix Terms and Error-Correlations...						
24	B(1,1)=	0.007207	r=	1			
25	B(1,2)=B(2	0.006673	r=	0.838484			
26	B(2,2)=	0.008789	r=	1			

Fitting Kinetic data to the Michaelis-Menten Equation (rectangular hyperbola) using nonlinear regression

Try putting in some bad initial guesses and see what happens…

Instructions:

1. Enter the number of data points: 12
2. Enter the number of independent variables: 1 (1 for single substrate M-M equation; 2 for bisubstrate, 2 for C, UC, and mixed inhibition)
3. Enter the number of parameters: 2 (deduce from form of M-M equation used; will be 2 for single substrate M-M)
4. Enter the formula for the function to be fitted:
   $Y =$ a*x1/(b+x1)
   Use "" for multiplication and "/" for division when entering formulas. Define parameters (a, b, c) and variables (x1, x2, x3) as described above. Be sure to use parentheses approriately to get the correct hierarchy*
5. Type (or paste) the [x,y] data:

```
0.25    0.7
1       2.32
2       4.14
3       4.91
4       5.83
5       6.41
7       7.01
10      7.72
```

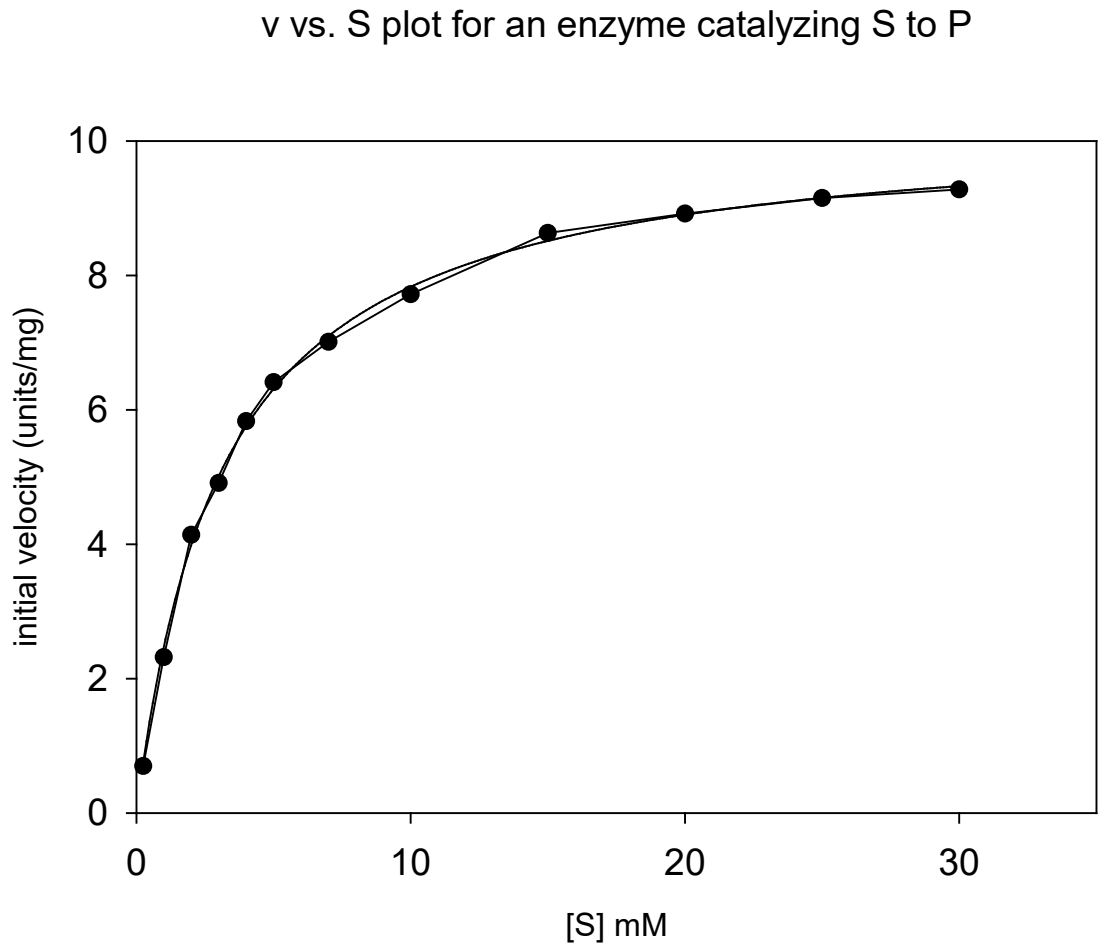
   Data can be typed in directly, or pasted from a text file or spreadsheet. The first column(s) contain the independent variable(s) (x1, x2..) while the final column contains the dependent variable y (velocity for enzyme data)
6. Enter initial guesses for the parameters from evaluation of the data (e.g. Lineweaver-Burke plots and appropriate slope and intercept replots). If the data fails to converge, provide better initial guesses. :

a (or p_1) =	10	±	, p=	
b (or p_2) =	3	±	, p=	
c (or p_3) =	0	±	, p=	
d (or p_4) =	0	±	, p=	
e (or p_5) =	0	±	, p=	
f (or p_6) =	0	±	, p=	
g (or p_7) =	0	±	, p=	
h (or p_8) =	0	±	, p=	

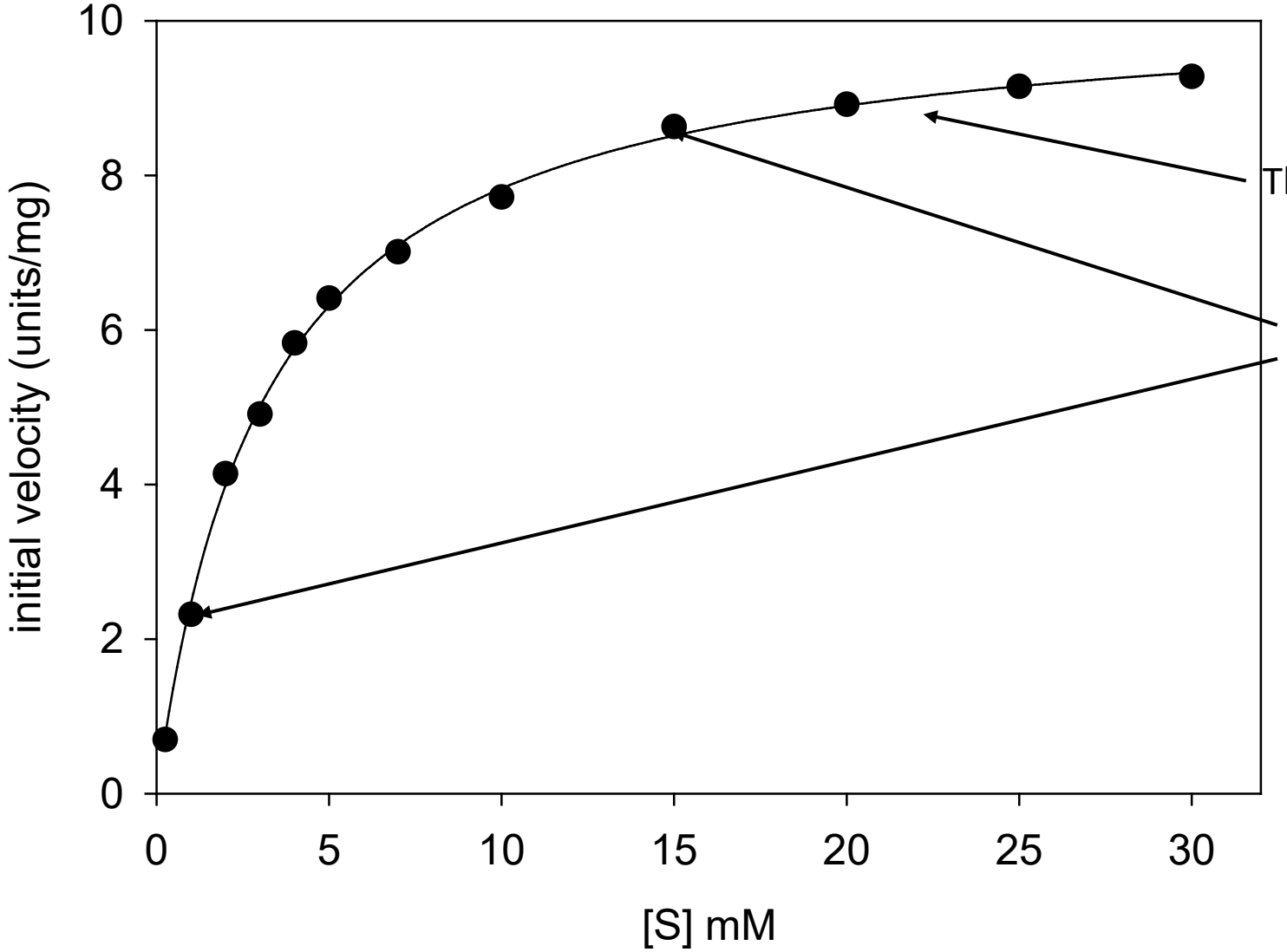
   Place the value "0" (zero) in all parameter boxes for which a value is not defined in the equation to be fit. Leave the fields to the right of the parameters blank; these will be populated with the standard errors upon iteration.
7. Click the Iterate button once for each iteration cycle. Watch how the initial guesses change in response to the iteration. Continue to click the "iterate" button until the values converge to unchanging values. If the parameters do not converge on reasonable numbers, provide better initial guesses and try again.

Making a publication quality graph showing the nonlinear regression fit of the experimental data

- Make a v vs s plot with both the experimental and theoretical velocity data plotted on the x axis
- Remove the line connecting data points for the experimental data
- Add a smoothed line (spline) going through the theoretical data points
- Hide the theoretical data points
- You will now have a graph showing the experimental data points, and with a line showing the shape of the hyperbola based on the nonlinear regression (see next slide)



Making a publication quality graph showing the nonlinear regression fit of the experimental data



The line is a spline curve for the theoretical data

The data points are the experimental data points. DO NOT show the theoretical data points on the graph

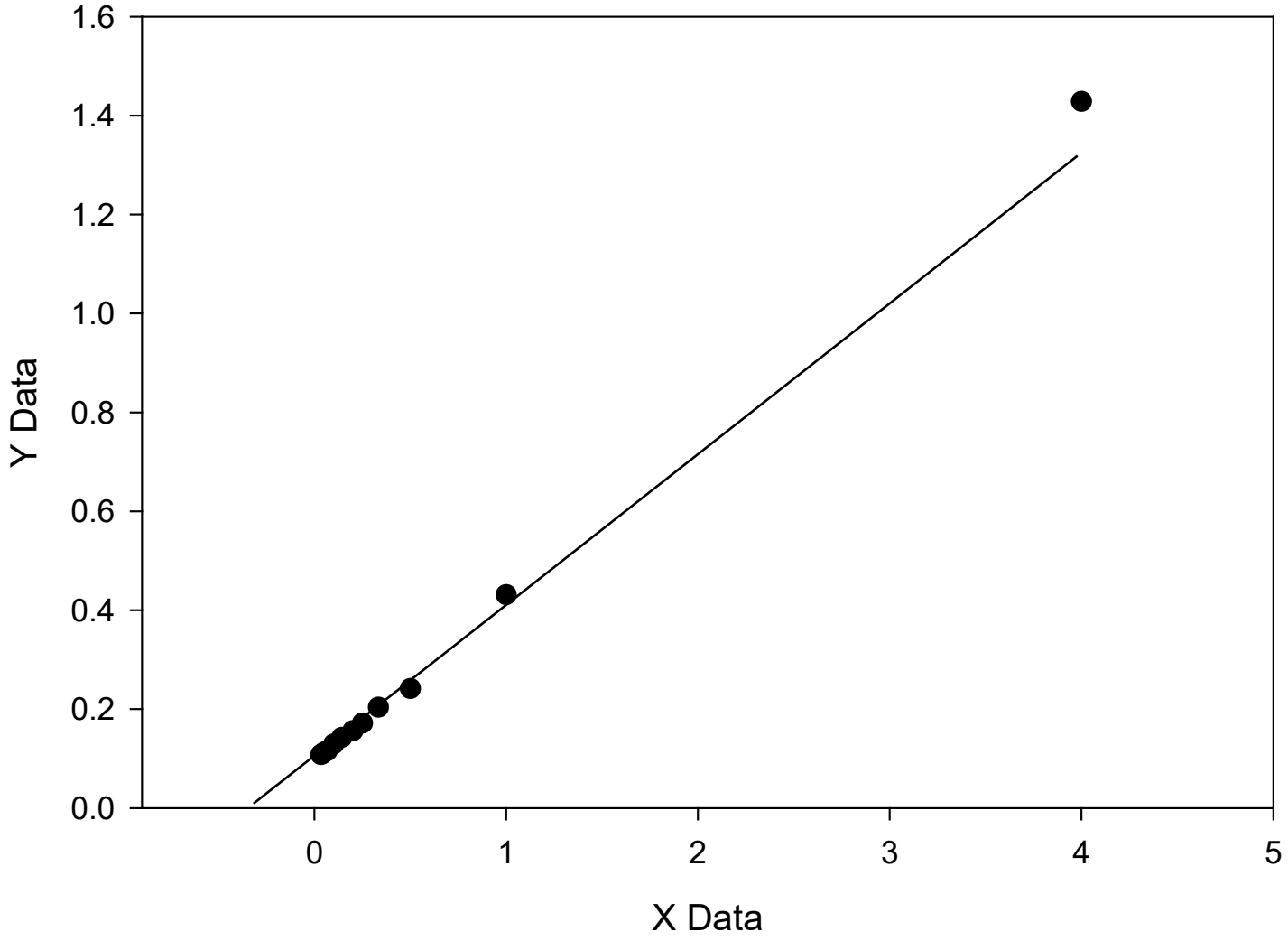
Adding a Lineweaver-Burke Plot to your v vs. S plot

- Transform your s, v_{expt} and $v_{theoretical}$ data to 1/s, $1/v_{expt}$, and $1/v_{theor}$

single S data						
S	v	v(theor)		1/S	1/v	1/v(theor)
0.25	0.7	0.752781		4	1.428571	1.328408
1	2.32	2.47032		1	0.431034	0.404806
2	4.14	3.986093		0.5	0.241546	0.250872
3	4.91	5.010999		0.333333	0.203666	0.199561
4	5.83	5.750253		0.25	0.171527	0.173905
5	6.41	6.30867		0.2	0.156006	0.158512
7	7.01	7.096244		0.142857	0.142653	0.14092
10	7.72	7.829302		0.1	0.129534	0.127725
15	8.63	8.513315		0.066667	0.115875	0.117463
20	8.92	8.902188		0.05	0.112108	0.112332
25	9.15	9.153045		0.04	0.10929	0.109253
30	9.28	9.328287		0.033333	0.107759	0.107201

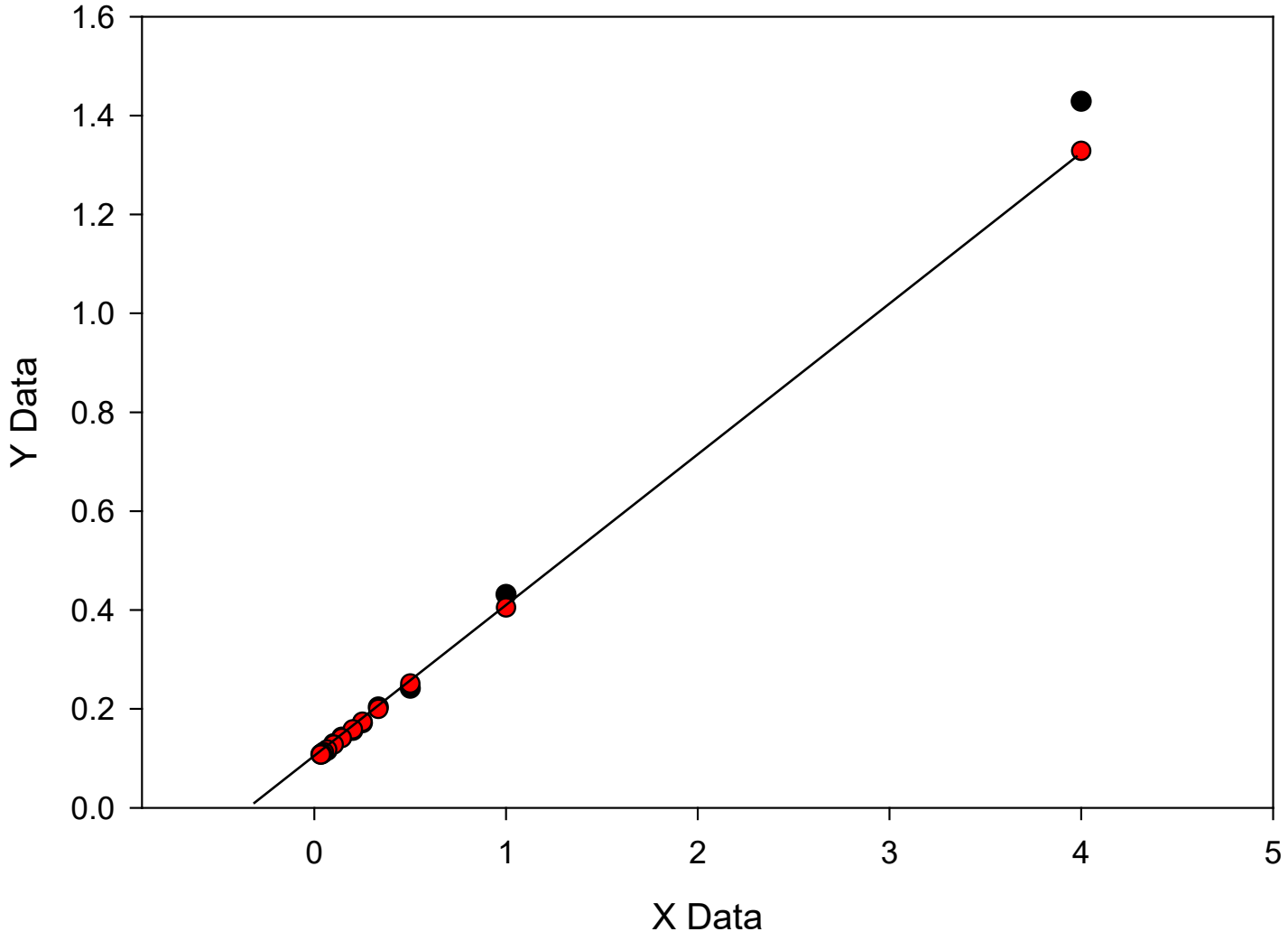
Adding a Lineweaver-Burke Plot to your v vs. S plot II

- Plot both $1/v_{expt}$ AND $1/v_{theor}$ vs 1/s and make a scatter plot that looks like this:
- The black circles are the experimental data
- The red circles are the theoretical data
- Add a linear regression line to the theoretical data, NOT the experimental data
- Extend the line to intersect the x axis
- Hide the theoretical data points, but keep the regression line



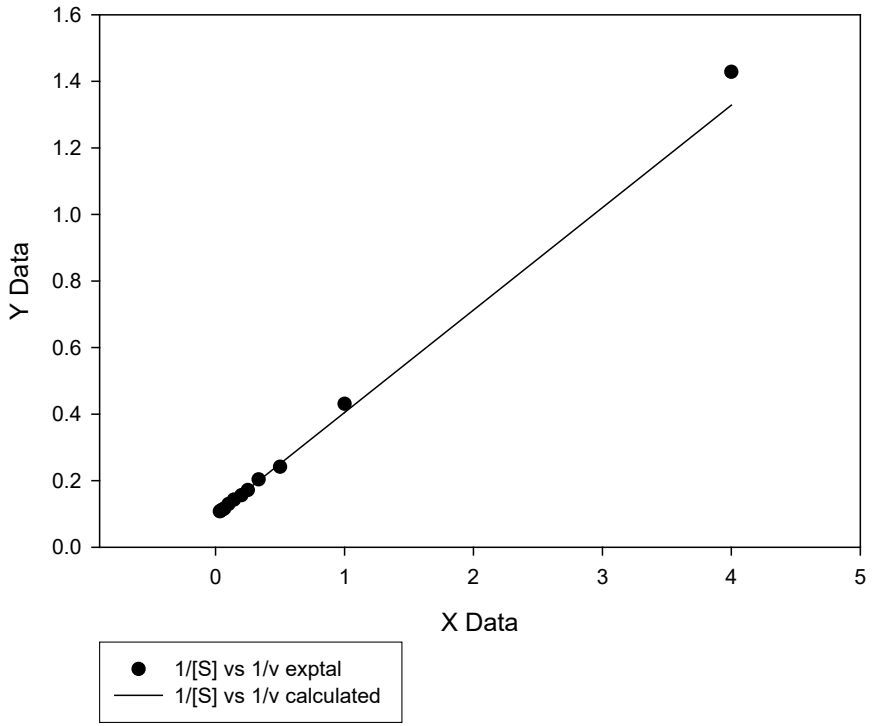
Adding a Lineweaver-Burke Plot to your v vs. S plot II

- Plot both $1/v_{expt}$ AND $1/v_{theor}$ vs 1/s and make a scatter plot that looks like this:
- The black circles are the experimental data
- The red circles are the theoretical data
- Add a linear regression line to the theoretical data, NOT the experimental data
- Extend the line to intersect the x axis
- Hide the theoretical data points, but keep the regression line



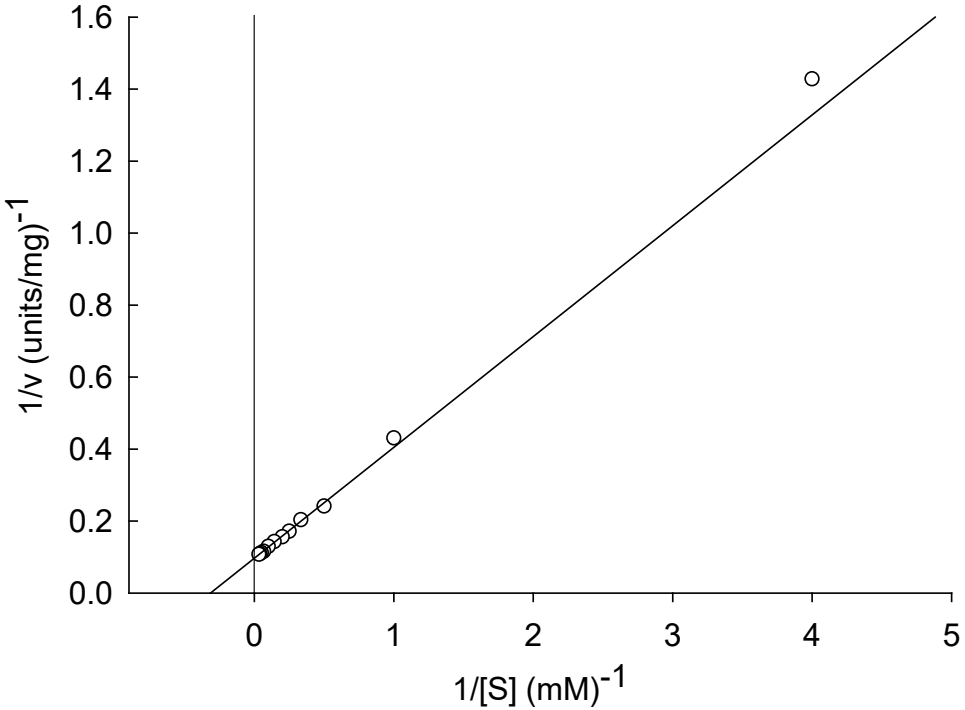
Adding a Lineweaver-Burke Plot to your v vs. S plot III

•Note: the line you have generated for your plot is NOT the same line you would have obtained by performing a linear regression of the experimental data points. The line shown at right is a correct rendering, because it is fitting the data points using the rectangular hyperbola, NOT linear regression of the double reciprocal data. If you had fit the experimental double reciprocal data using linear regression, it would have treated all points equally and generated an incorrectly fit line.

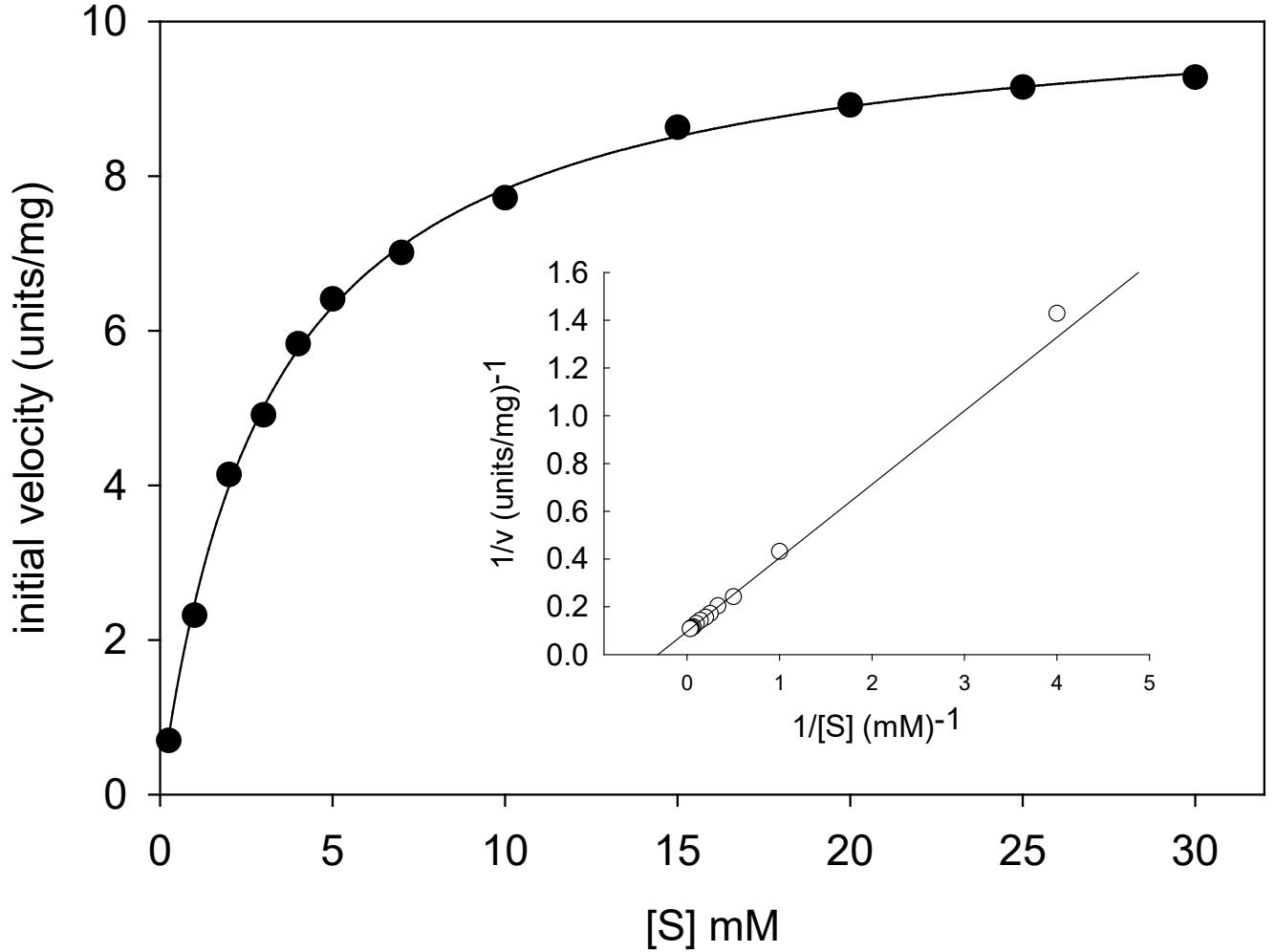


Adding a Lineweaver-Burke Plot to your v vs. S plot IV

•When formatted, you will have a publication quality, properly fit line for your Lineweaver-Burke plot that you can paste into your hyperbola plot as an inset.



Publication-ready Kinetic Plot



Expanding beyond S →P

- One substrate, one product $S \rightarrow P$
- One substrate, two products $S \rightarrow P_1 + P_2$
- Two substrates, one product $A + B \rightarrow C$
- Two substrates, two products $A + B \rightarrow C + D$
- Three substrates, one product $A + B + C \rightarrow D$

Most biochemical reactions involve two substrates

Two substrate reactions

- In what order do substrates bind?
- In what order are products released?
- How are different kinetic mechanisms distinguished?
- How are kinetic constants determined for two substrate reactions?

Two substrate reactions

- Sequential: both substrates must combine with enzyme before a reaction occurs and product(s) are released
  - “Ordered substrate binding”
  - “Random substrate binding”
- Ping-pong (double displacement): One or more products are released between binding of first and second substrates

Kinetic mechanisms of two substrate reactions

- Sequential
  - Ordered or random substrate binding?
  - Ordered or random product release?
- Ping-pong
  - Always ordered, by nature of the reaction

Designate substrates as S_1 and S_2, or A and B

Designate product(s) as P_1 and P_2, or P and Q

$$E + S_1 + S_2 \rightleftharpoons E + P_1 + P_2$$

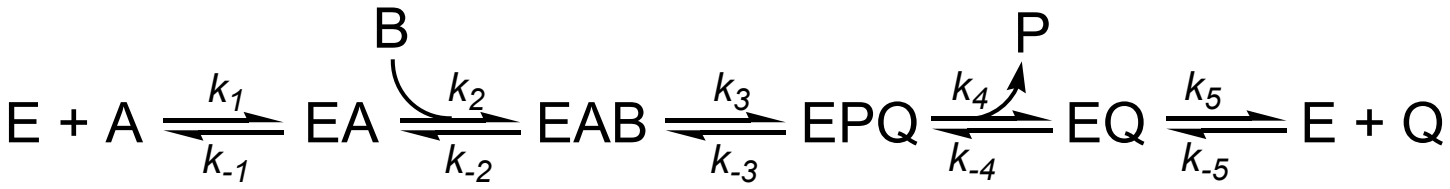
$$E + A + B \rightleftharpoons E + P + Q$$

Writing kinetic mechanisms for two substrate reactions

1. Use equilibrium expressions (as for single substrate reactions)
2. Use horizontal lines to denote the enzyme surface, vertical arrows to denote incoming substrates and outgoing products

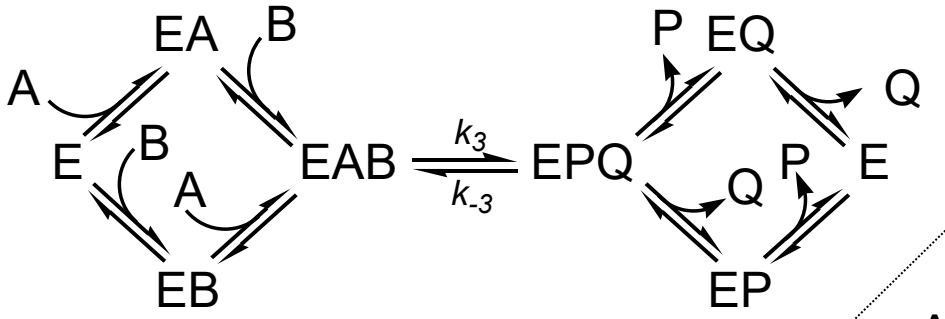
Example kinetic mechanisms for sequential reactions involving 2 substrates and 2 products

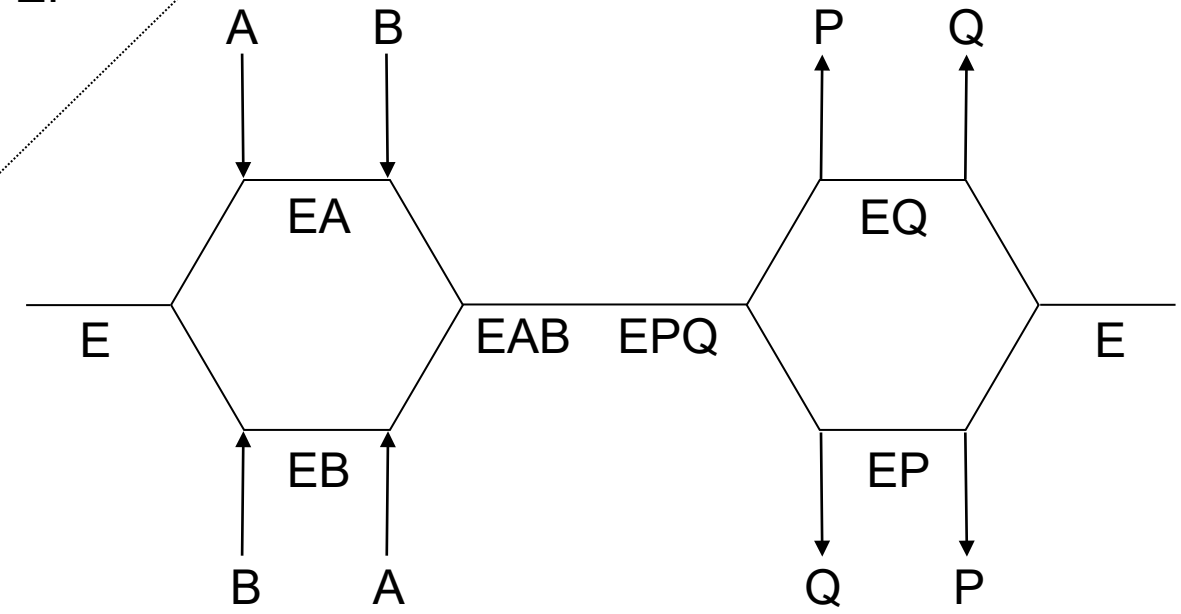
- Ordered with respect to substrate addition and product release:



Example kinetic mechanisms for sequential reactions involving 2 substrates and 2 products

- random with respect to substrate addition and product release:



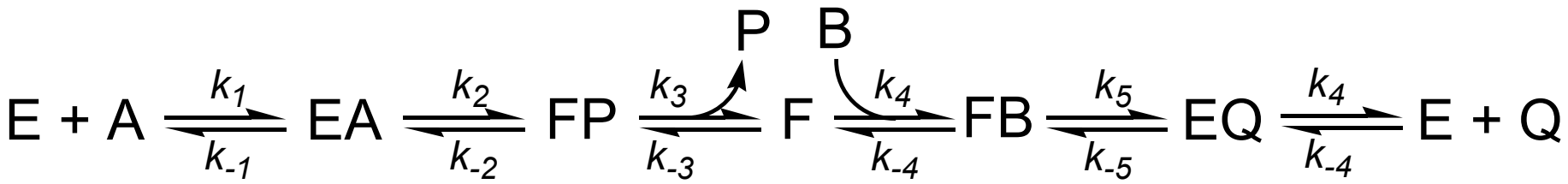


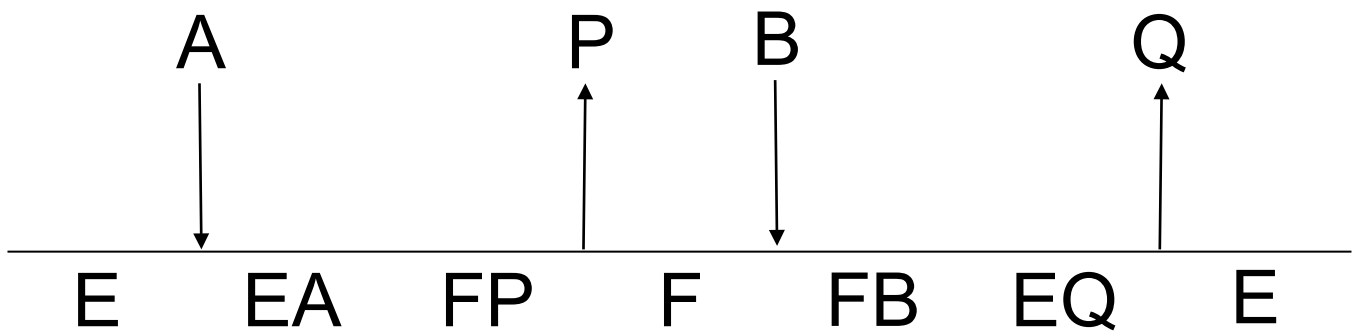
Other possible kinetic mechanisms for sequential reactions involving 2 substrates and 2 products

- Ordered with respect to substrate binding, random with respect to product release
- Random with respect to substrate binding, ordered with respect to product release

Kinetic mechanism for a ping-pong (double displacement) reaction involving 2 substrates and 2 products

- "F" designates a modified form of the enzyme (e.g., enzyme with a covalent bond to a fragment of the first substrate)





Two substrate reactions

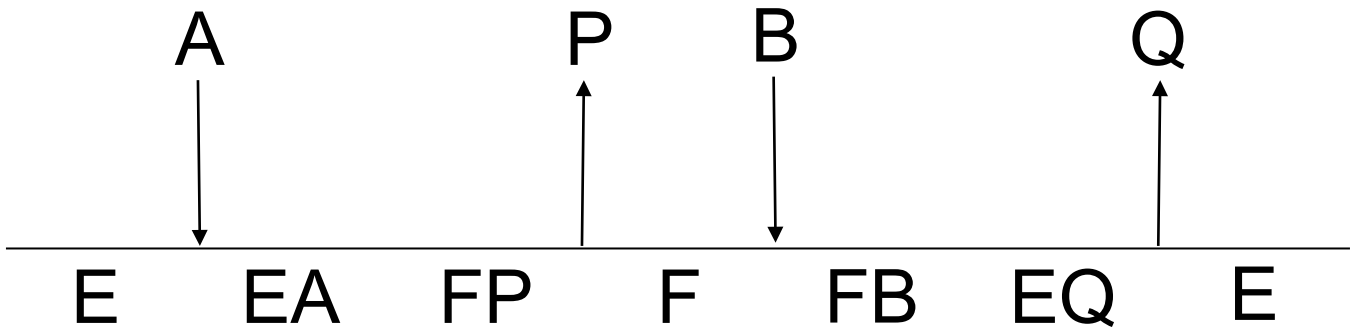
- In what order do substrates bind?
- In what order are products released?
- How are different kinetic mechanisms distinguished?
- How are kinetic constants determined for two substrate reactions?

- Collect kinetic data
- Analyze kinetic data using Michaelis-Menten equations derived for ping-pong, ordered sequential, and random sequential mechanisms
- Analyze kinetic data and curve fits statistically and graphically
- Deduce kinetic mechanism from best fits

Collection and analysis of kinetic data for two substrate reactions

- Conduct enzyme assays with constant [E] and varying [A] with a fixed, subsaturating [B] present in each assay
- Select a second fixed [B] (e.g., twice that used for the first set of assays), and repeat the kinetic measurements for each varied [A]
- Repeat this procedure for as many different fixed [B] desired
- Construct a graph containing each of the v vs. [A] plots obtained for different [B]
- Replot each v vs. [A] plot in double reciprocal form
- The pattern of double reciprocal lines (parallel or intersecting) reveals whether the kinetic mechanism is ping-pong or ordered

Michaelis-Menten Equation for a Bimolecular Ping-Pong reaction

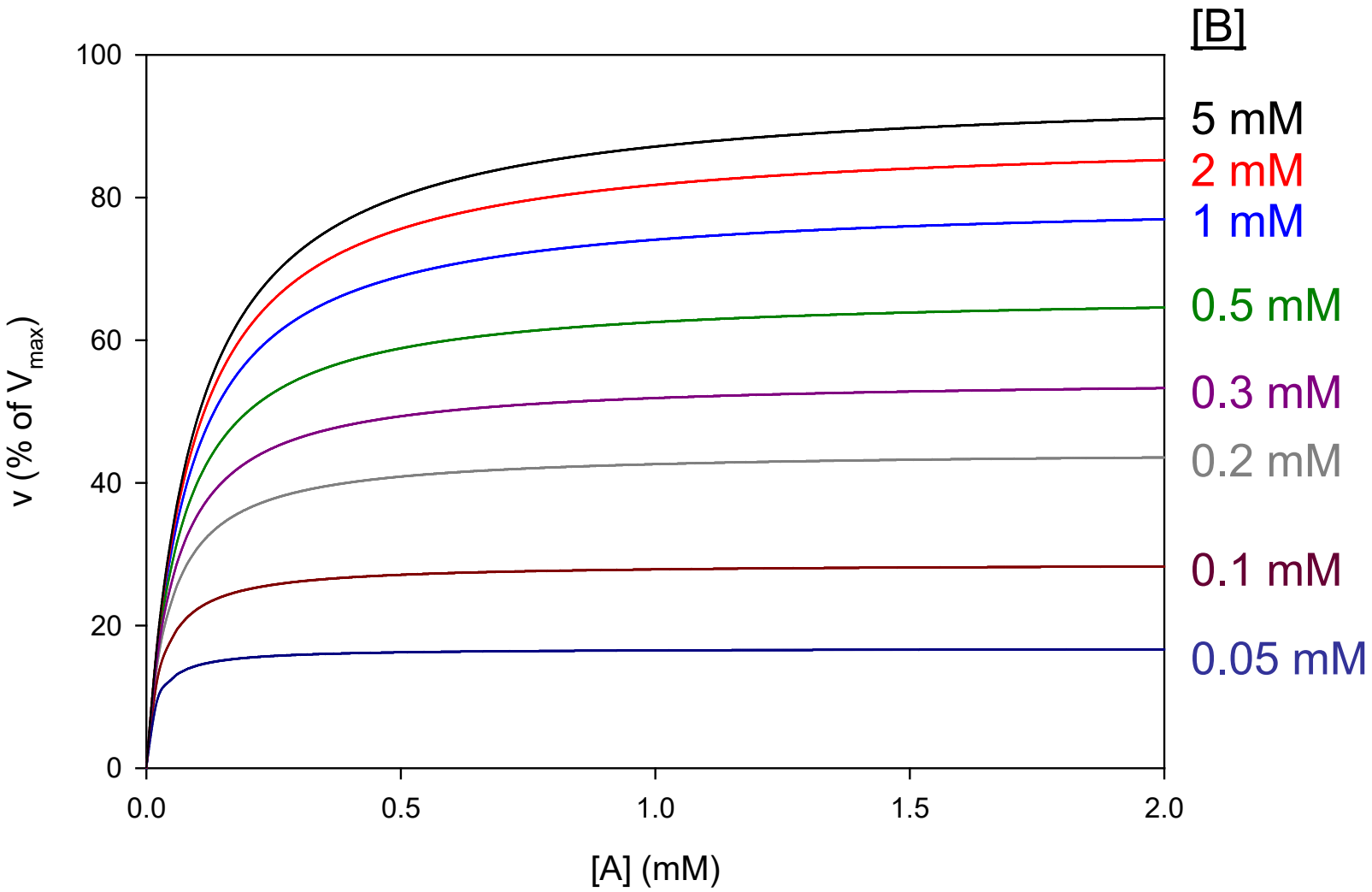


$$v = \frac{V_{\max}[A]\cdot[B]}{K_{m,a}\cdot[B] + K_{m,b}\cdot[A] + [A]\cdot[B]}$$

$K_{m,a} = K_m$ for substrate A
$K_{m,b} = K_m$ for substrate B

V vs. S plots for a 2 substrate ping-pong kinetic mechanism

For the hypothetical enzyme example shown below, $K_{m,a}$ =0.1 mM , $K_{m,b}$ = 0.25 mM, and V_{max} = 100 U/mg.



Algebraic rearrangement of the Michaelis-Menten Equation for a Bimolecular Ping-Pong reaction in the form of y = mx + b

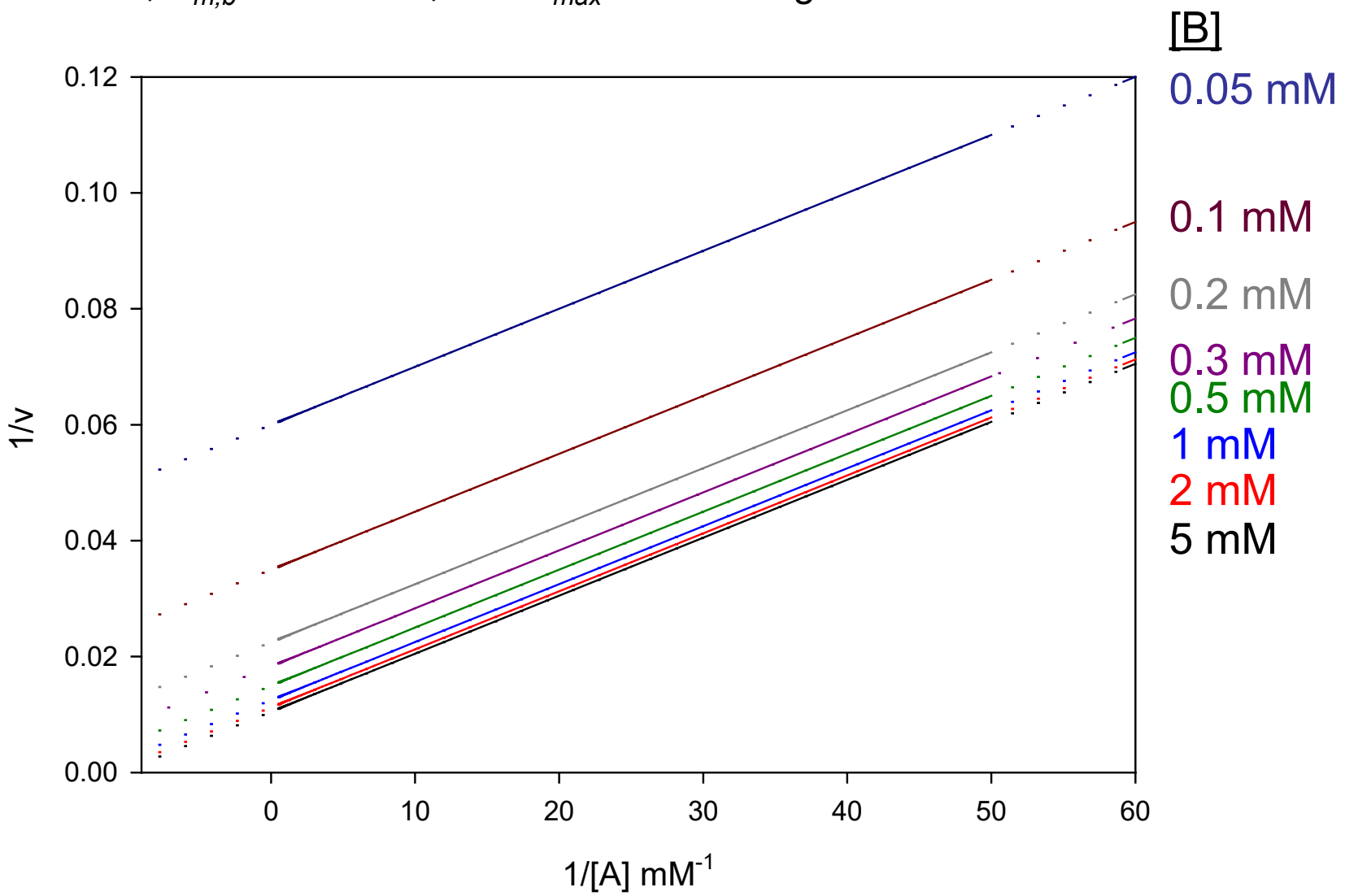
$$\frac{1}{v} = \frac{K_{m,a}}{V_{max}} \cdot \frac{1}{[A]} + \frac{1}{V_{max}} \cdot \left(1 + \frac{K_{m,b}}{[B]}\right)$$

y = m x + b

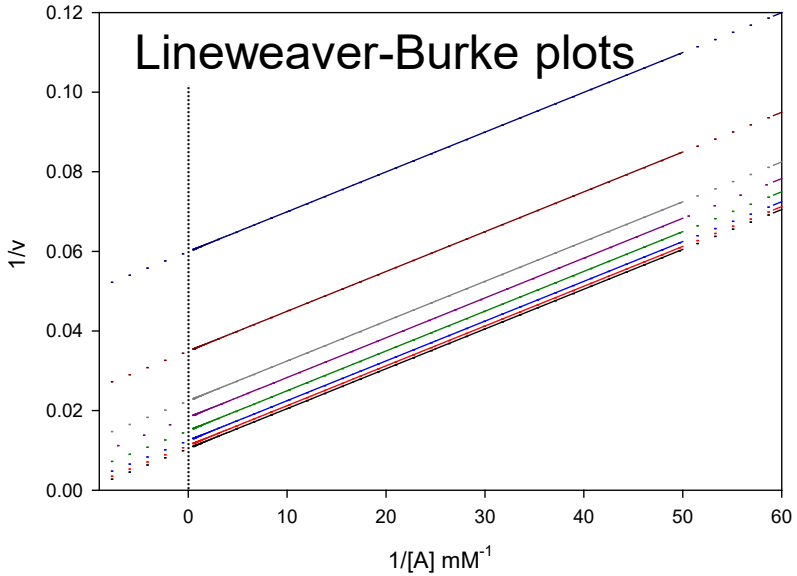
$K_{m,a} = K_m$ for substrate A
$K_{m,b} = K_m$ for substrate B

Reciprocal plots for a 2 substrate ping-pong kinetic mechanism

For the hypothetical enzyme example shown below, $K_{m,a}$ =0.1 mM , $K_{m,b}$ = 0.25 mM, and V_{max} = 100 U/mg.



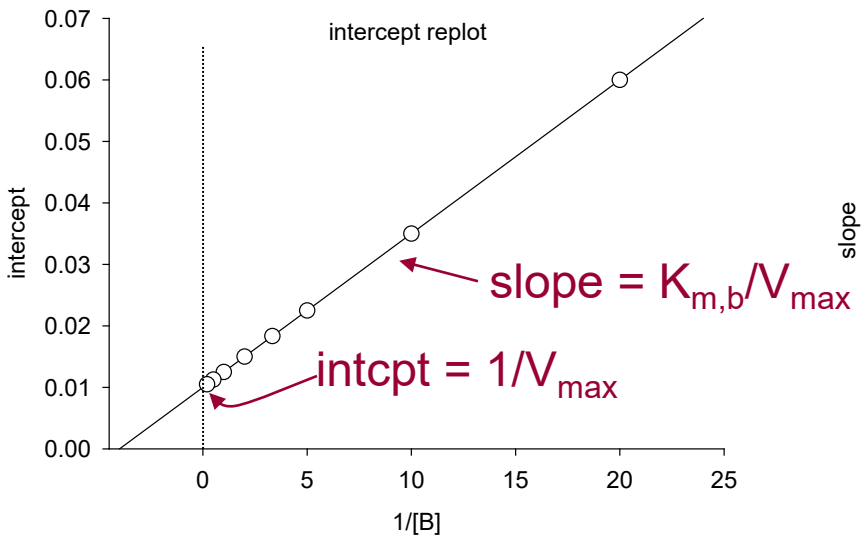
Graphical estimation of kinetic parameters for a 2 substrate ping pong reaction from Lineweaver-Burke plots

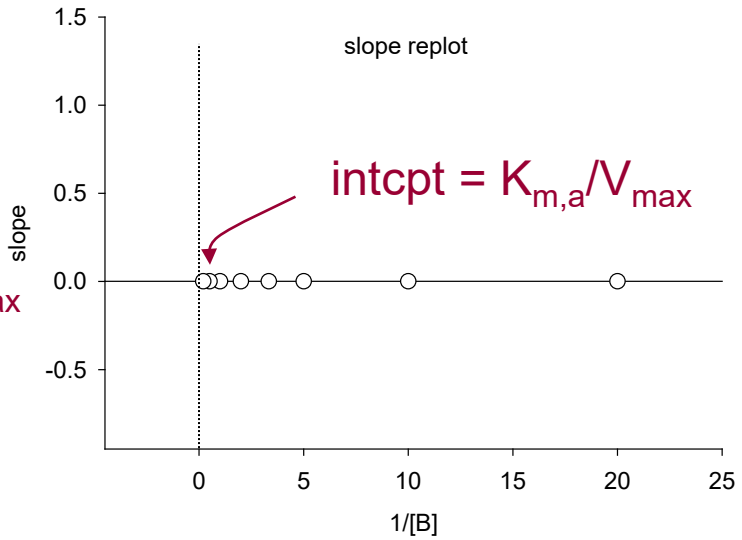


$$\frac{1}{v} = \frac{K_{m,a}}{V_{max}} \cdot \frac{1}{[A]} + \frac{1}{V_{max}} \cdot \left(1 + \frac{K_{m,b}}{[B]}\right)$$

replots data

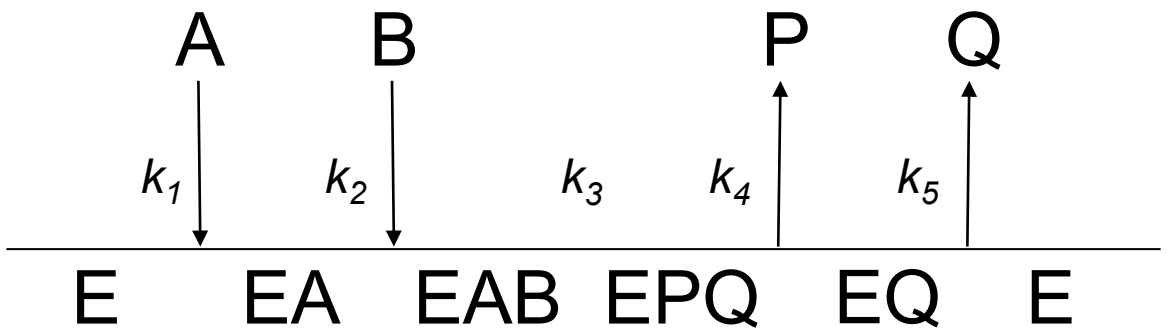
	1-[B]	2-slope	3-intercept	4-1/[B]
1	0.0500	1.0000e-3	0.0600	20.0000
2	0.1000	1.0000e-3	0.0350	10.0000
3	0.2000	1.0000e-3	0.0225	5.0000
4	0.3000	1.0000e-3	0.0183	3.3333
5	0.5000	1.0000e-3	0.0150	2.0000
6	1.0000	1.0000e-3	0.0125	1.0000
7	2.0000	1.0000e-3	0.0113	0.5000
8	5.0000	1.0000e-3	0.0105	0.2000





S. Ensign, enzyme kinetics

Michaelis-Menten Equation for an ordered sequential bimolecular reaction



$$v = \frac{V_{\max}[A]\cdot[B]}{K_{ia}\cdot K_{m,b} + K_{m,a}\cdot[B] + K_{m,b}\cdot[A] + [A]\cdot[B]}$$

$K_{m,a}$ = K_m for substrate A
$K_{m,b}$ = K_m for substrate B
K_{ia} = the dissociation constant for the first substrate for an ordered sequential mechanism.

Algebraic rearrangement of the Michaelis-Menten Equation for an ordered sequential bimolecular reaction in the form of y = mx + b

Equation where A is varied at fixed concentrations of B:

$$\frac{1}{v} = \underbrace{\frac{K_{m,a}}{V_{\max}} \cdot \left(1 + \frac{K_{ia} \cdot K_{m,b}}{K_{m,a} \cdot [B]}\right)}_{m} \cdot \frac{1}{[A]} + \underbrace{\frac{1}{V_{\max}} \cdot \left(1 + \frac{K_{m,b}}{[B]}\right)}_{b}$$

y = m x + b

$K_{m,a} = K_m$ for substrate A

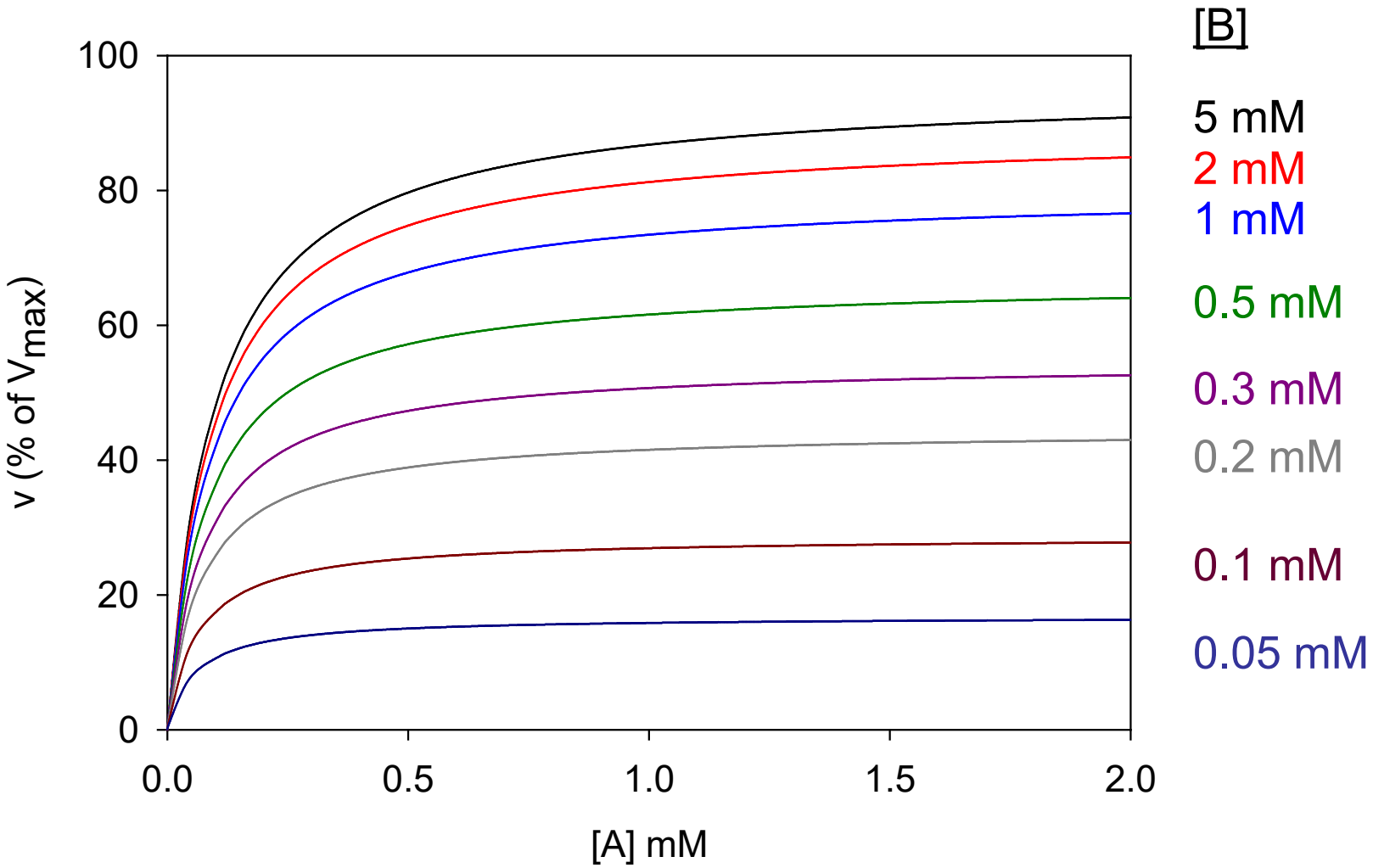
$K_{m,b} = K_m$ for substrate B

K_{ia} = the dissociation constant for the first substrate for an ordered sequential mechanism (assumes A is substrate that binds first).

If the order of binding is random, then $K_{ia} \cdot K_{m,b} = K_{ib} \cdot K_{m,a}$

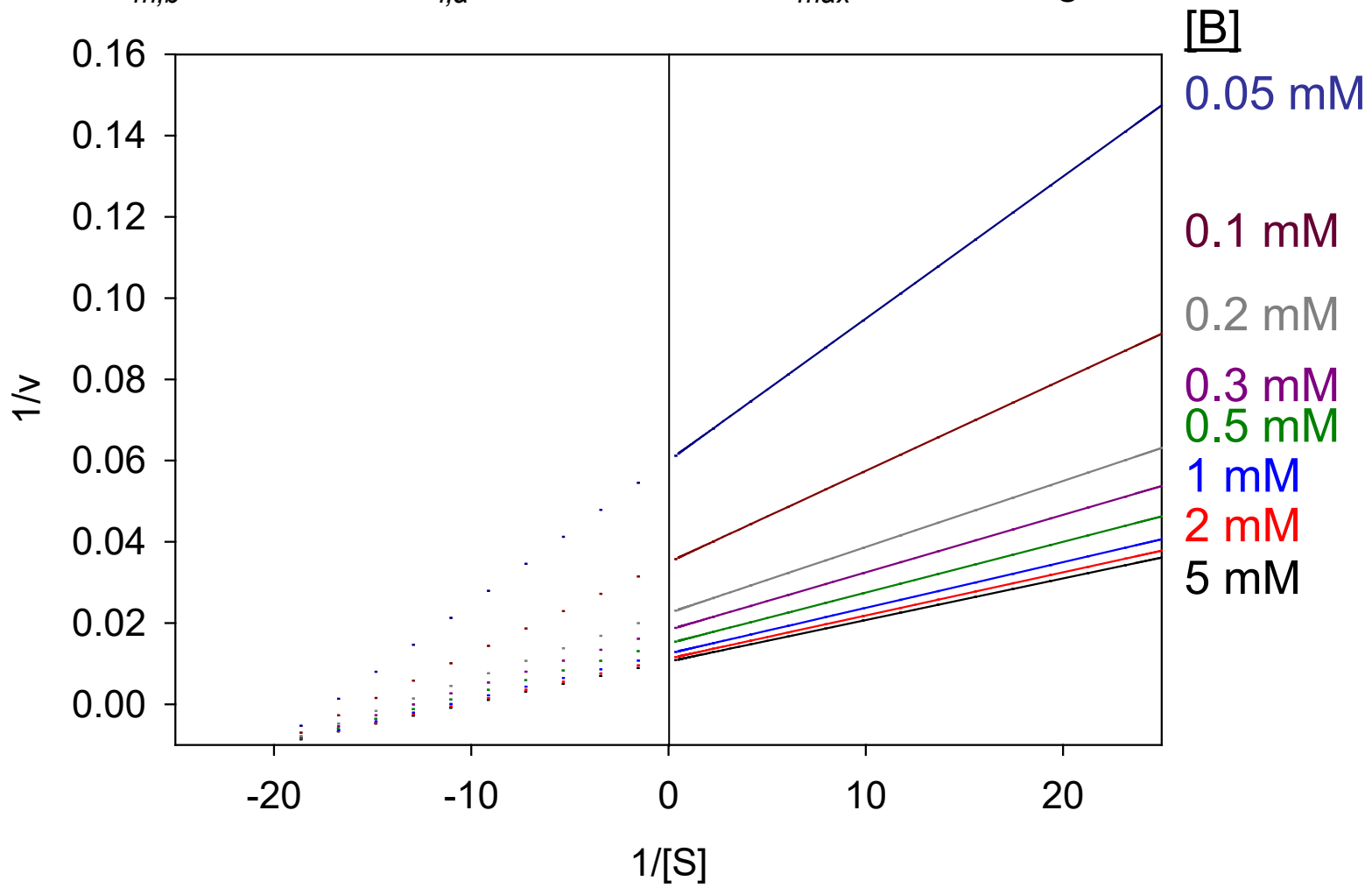
V vs. S plots for a 2 substrate ordered sequential kinetic mechanism

For the hypothetical enzyme example shown below, $K_{m,a}$ =0.1 mM , $K_{m,b}$ = 0.25 mM, $K_{i,a}$ = 0.05 mM and V_{max} = 100 U/mg.

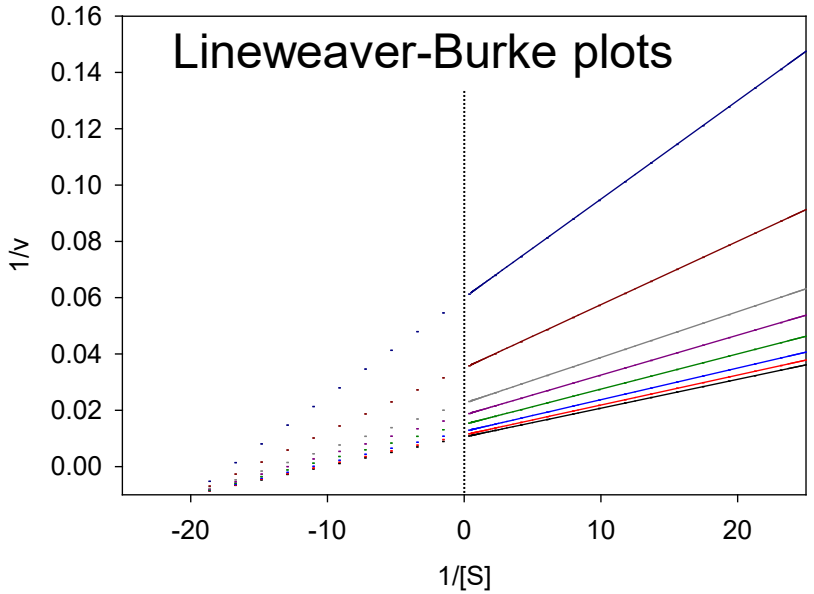


Reciprocal plots for a 2 substrate ordered sequential kinetic mechanism

For the hypothetical enzyme example shown below, $K_{m,a}$ =0.1 mM , $K_{m,b}$ = 0.25 mM, $K_{i,a}$ = 0.05 mM and V_{max} = 100 U/mg.

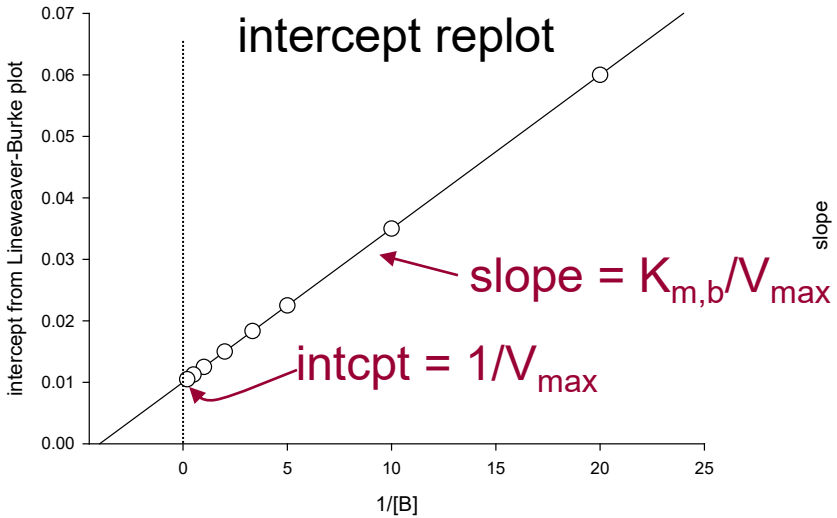


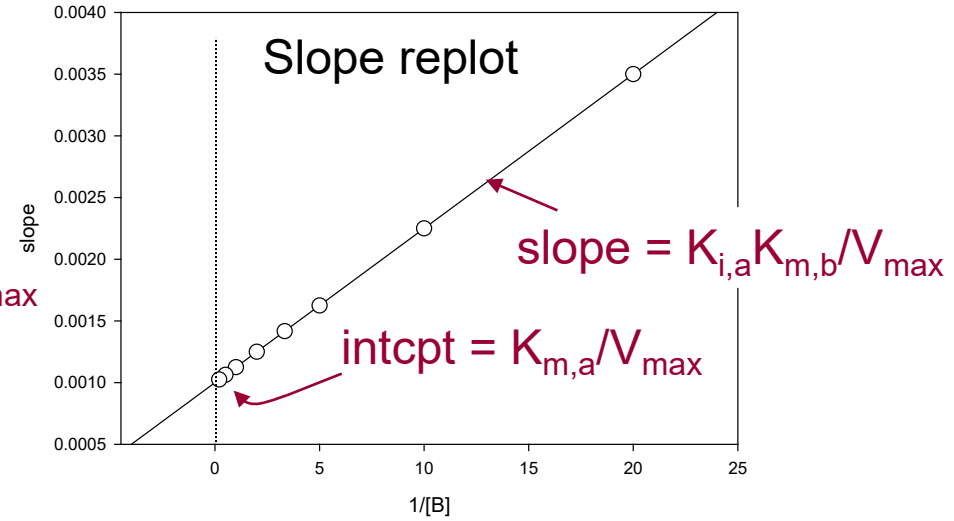
Graphical estimation of kinetic parameters for a 2 substrate ordered sequential reaction from Lineweaver-Burke plots



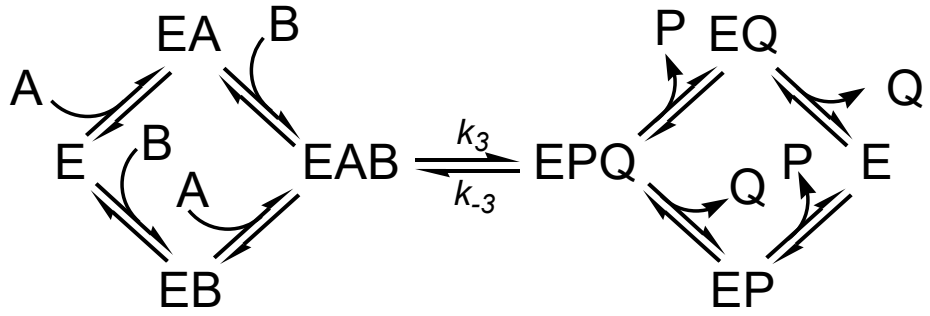
$$\frac{1}{v} = \frac{K_{m,a}}{V_{\max}} \cdot \left(1 + \frac{K_{ia} \cdot K_{m,b}}{K_{m,a} \cdot [B]}\right) \cdot \frac{1}{[A]} + \frac{1}{V_{\max}} \cdot \left(1 + \frac{K_{m,b}}{[B]}\right)$$

1-[B]	2-slope	3-intercept	4-1/[B]
0.0500	3.5000e-3	0.0600	20.0000
0.1000	2.2500e-3	0.0350	10.0000
0.2000	1.6250e-3	0.0225	5.0000
0.3000	1.4167e-3	0.0183	3.3333
0.5000	1.2500e-3	0.0150	2.0000
1.0000	1.1250e-3	0.0125	1.0000
2.0000	1.0625e-3	0.0113	0.5000
5.0000	1.0250e-3	0.0105	0.2000





Michaelis-Menten Equation for a random sequential bimolecular reaction



$$v = \frac{V_{max}[A]\cdot[B]}{\alpha K_a \cdot K_b + \alpha K_a \cdot [B] + \alpha K_b \cdot [A] + [A]\cdot[B]}$$

K_a = dissociation constant for substrate A

K_b = dissociation constant for substrate B

α = binding interaction factor

if α = 1, substrate binding is independent

if α > 1, binding of one substrate decreases affinity of other

if α < 1, binding of one substrate increases affinity of other

Algebraic rearrangement of the Michaelis-Menten Equation for a random sequential bimolecular reaction in the form of y = mx + b

Equation where A is varied at fixed concentrations of B:

$$\frac{1}{v} = \frac{\alpha K_a}{V_{max}} \cdot \left(1 + \frac{K_b}{[B]}\right) \cdot \frac{1}{[A]} + \frac{1}{V_{max}} \cdot \left(1 + \frac{\alpha K_b}{[B]}\right) \quad (31)$$

y = m x + b

K_a = dissociation constant for substrate A

K_b = dissociation constant for substrate B

α = binding interaction factor

if α = 1, substrate binding is independent

if α > 1, binding of one substrate decreases affinity of other

if α < 1, binding of one substrate increases affinity of other

How do you discriminate an ordered from a random sequential mechanism?

- Examine product inhibition profiles (we can discuss this more after discussing the various types of reversible enzyme inhibitors-C, NC, UC)
- Lactate dehydrogenase provides a good example to illustrate this

Analysis of two substrate kinetic data

- Conduct enzyme assays with constant [E] and varying [A] with a fixed, subsaturating [B] present in each assay
- Select a second fixed [B] (e.g., twice that used for the first set of assays), and repeat the kinetic measurements for each varied [A]
- Repeat this procedure for as many different fixed [B] desired
- Construct a graph containing each of the v vs. [A] plots obtained for different [B]
- Replot each v vs. [A] plot in double reciprocal form
- The pattern of double reciprocal lines (parallel or intersecting) reveals whether the kinetic mechanism is ping-pong or ordered
- Slope and intercept replots are useful for visual purposes and estimating kinetic parameters
- Fit your data to the appropriate M-M equation to get your kinetic parameters

An actual example to illustrate how to analyze two substrate data

Biochemistry **2002,** *41,* 2727−2740 2727

Characterization of the 2-[(*R*)-2-Hydroxypropylthio]ethanesulfonate Dehydrogenase from *Xanthobacter* Strain Py2: Product Inhibition, pH Dependence of Kinetic Parameters, Site-Directed Mutagenesis, Rapid Equilibrium Inhibition, and Chemical Modification†

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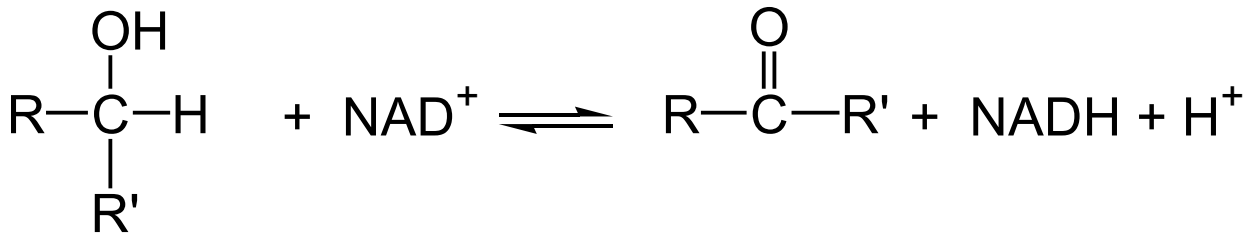
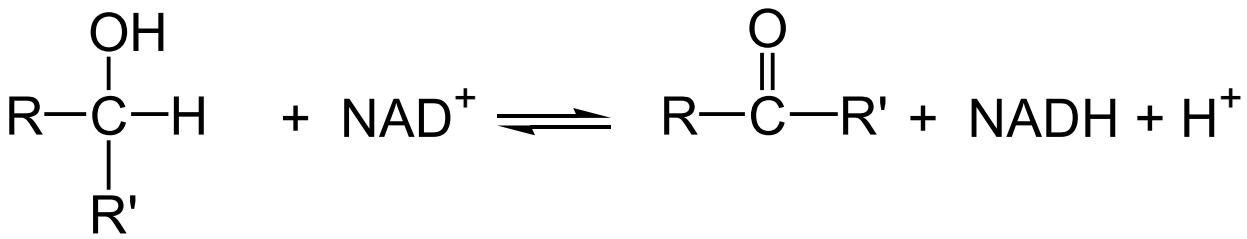
ABSTRACT: Although the short-chain dehydrogenase/reductase (SDR) superfamily contains a very large number of members defined in annotated databases and by biochemical and structural studies, very few SDR enzymes have been identified that have a homologous partner catalyzing the same reaction but with an opposite stereospecificity. In the present study we have cloned and expressed one of these enzymes, the 2-[(*R*)-2-hydroxypropylthio]ethanesulfonate (R-HPC) dehydrogenase, that is part of the coenzyme M-dependent pathway of alkene and epoxide metabolism in *Xanthobacter* strain Py2. Investigation of the kinetic mechanism using product inhibition suggested that a compulsory-ordered ternary complex mechanism was followed. The pH dependence of k_{cat}/K_m indicated the presence of a single ionizable residue of catalytic importance ($pK_a = 6.9$) that was proposed to be Y155 of the catalytic triad. Amino acid substitutions of the putative catalytic triad residues produced inactive enzymes (S142C, Y155F, Y155E, and K159A) or enzyme with a greatly decreased activity (S142A). Inhibitors were investigated as probes of the molecular features of R-HPC that contribute to substrate binding. 2-[(*S*)-2-Hydroxypropylthio]-ethanesulfonate (S-HPC) and 2-(2-methyl-2-hydroxypropylthio)ethanesulfonate were found to be competitive inhibitors of R-HPC with K_{ic} values close to the K_m for R-HPC. The arginine-specific modifiers 2,3-butanedione and phenylglyoxal were found to be inactivators, and inactivation could be protected against by the addition of R-HPC. 2,3-Butanedione was found to reduce enzyme activity with R-HPC as a substrate much more dramatically than with substrates that lacked a sulfonate moiety [e.g., 2-propanol, (*R*)-2-pentanol, and (*R*)-2-heptanol]. Amino acid analyses of enzyme modified by 2,3-butanedione in the presence and absence of S-HPC suggested protection of a single arginine residue. On the basis of these results, we propose that one or more active site arginines play a key role in substrate binding via an ionic interaction with the sulfonate moiety of R-HPC.



An actual example to illustrate how to analyze two substrate data

- NAD^+ = substrate A
- R-HPC = substrate B (the alcohol)
- Graphical analysis of the data indicates sequential (not ping pong)
- Product inhibition profiles suggest ordered sequential, with NAD^+ binding before R-HPC

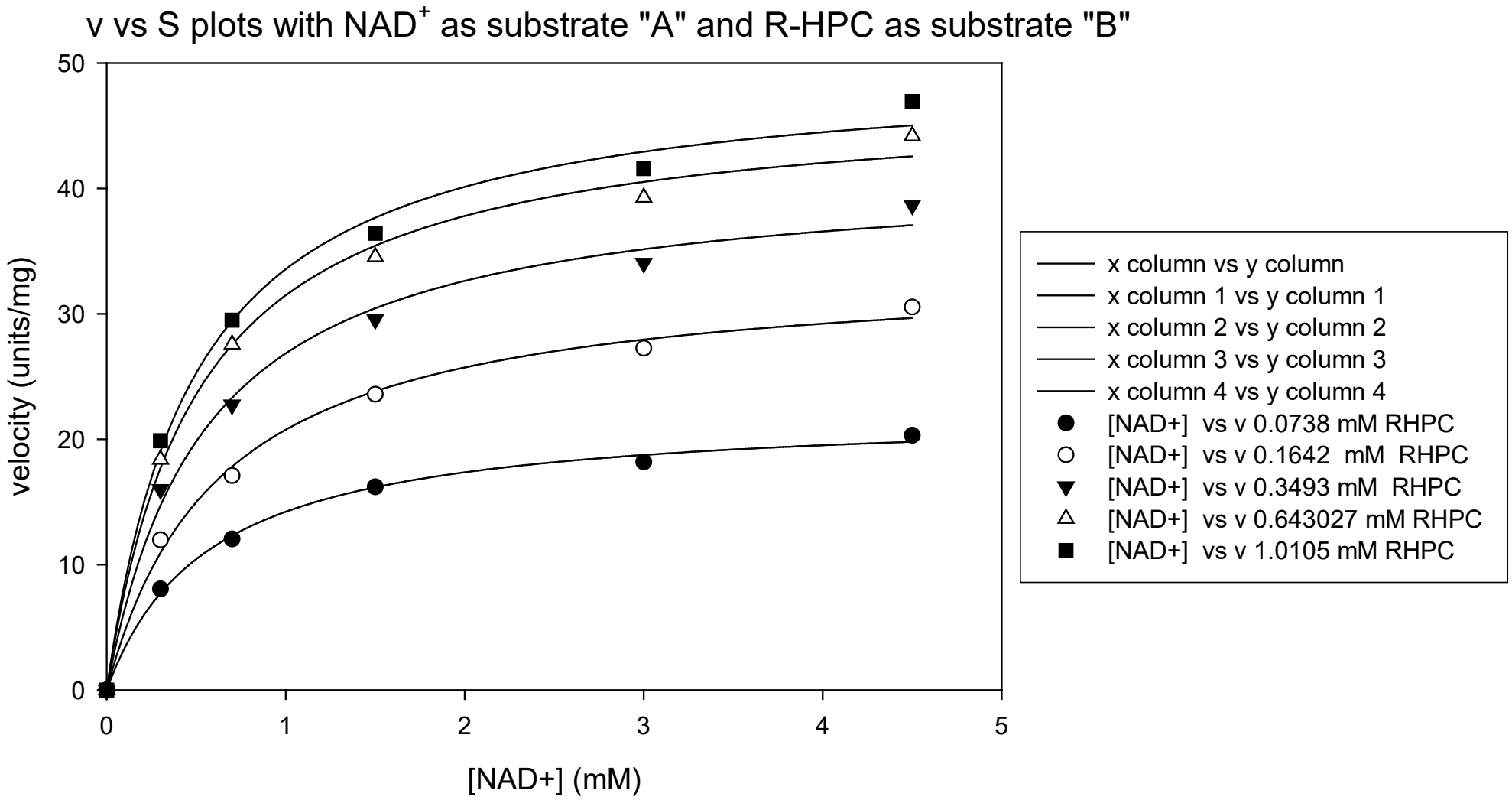


Graph the data

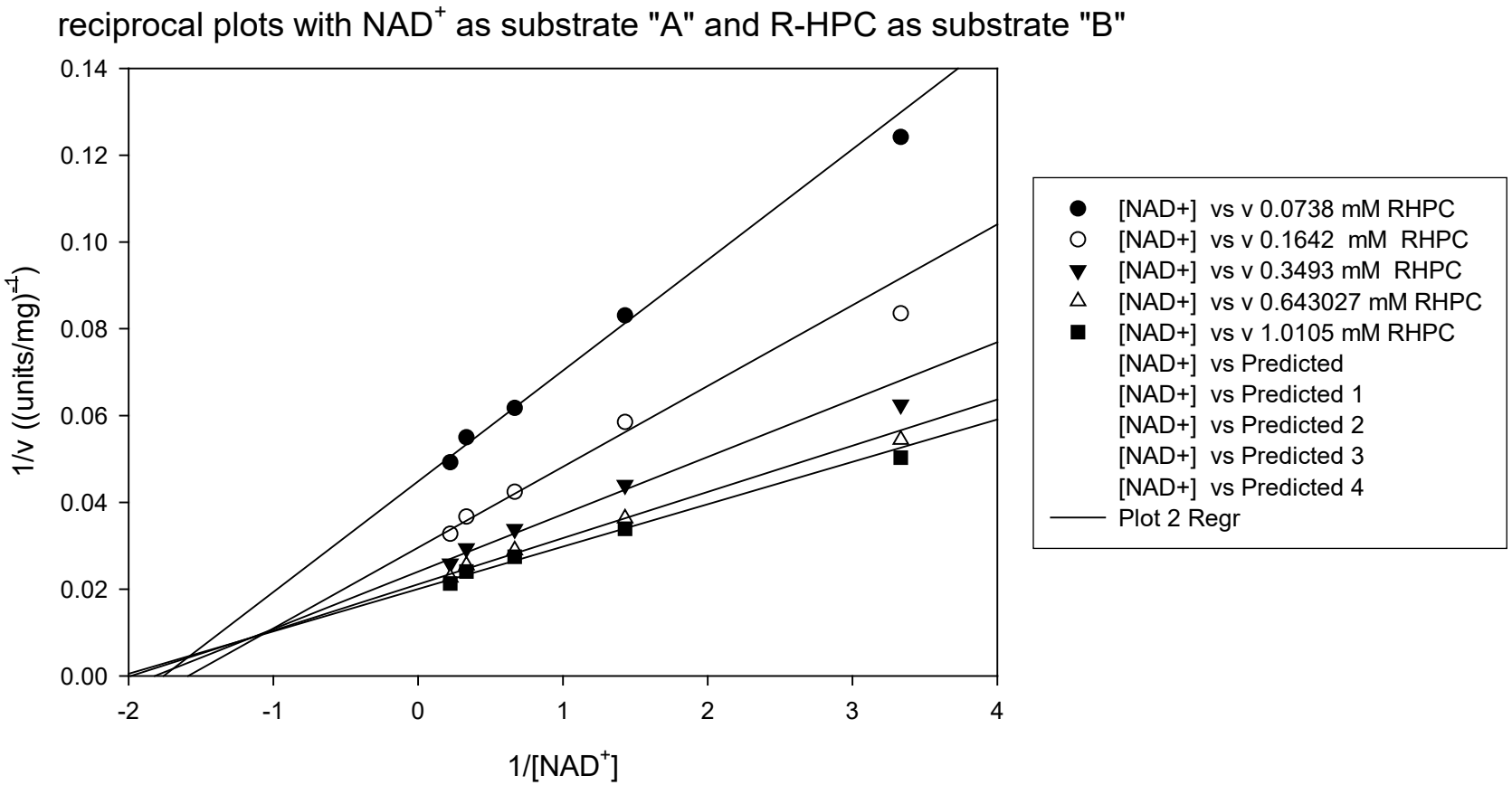
- Make scatter plots of each v vs. S curve on a single page
- Fit each individual curve to a rectangular hyperbola
- Add the lines for the curve fits
- Make double reciprocal plots (1/v vs. 1/S) on a single page
- Add the linear regressions for the theoretical data to complete the Lineweaver-Burke plots
- Determine the slopes and intercepts of each 1/v vs. 1/S plot, and replot the slopes and intercepts vs. 1/[B] for visual estimation of kinetic parameters

1-[NAD+]	2-v 0.0738 mM RHPC	3-v 0.1642 mM RHPC	4-v 0.3493 mM RHPC	-v 0.643027 mM RHP	6-v 1.0105 mM RHPC
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.3000	8.0520	11.9680	15.9980	18.3740	19.8820
0.7000	12.0390	17.0890	22.7320	27.5490	29.4900
1.5000	16.1990	23.5827	29.5530	34.5370	36.4190
3.0000	18.1830	27.2560	34.0350	39.2700	41.5810
4.5000	20.3120	30.5370	38.6740	44.1600	46.9240

v vs. S curves for R-HPCDH data



Reciprocal plots of R-HPCDH data

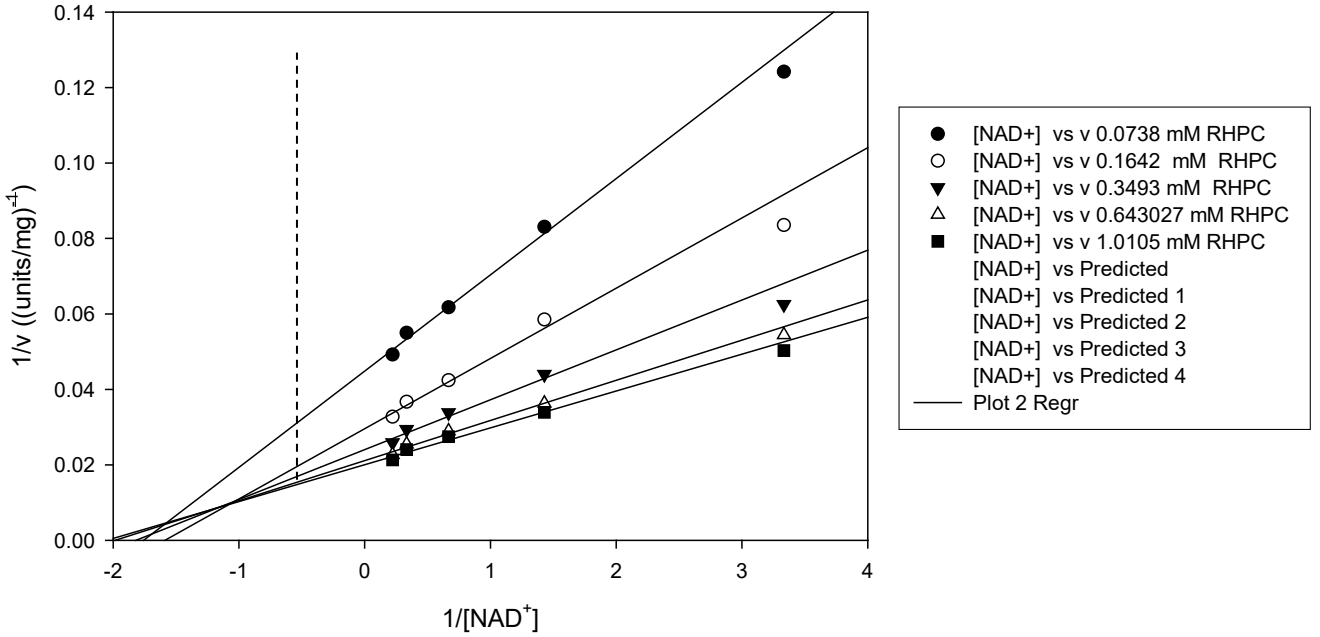


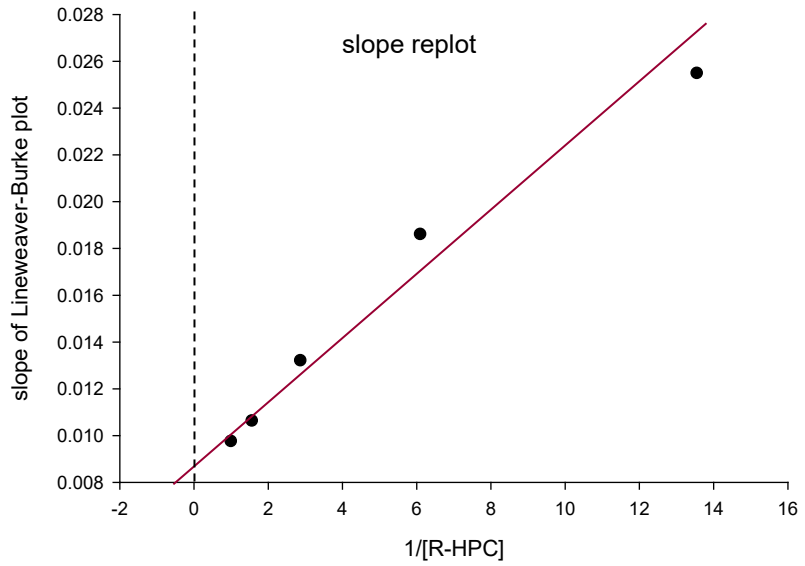
Note how the lines intersect pretty well at a common point, indicative of a sequential reaction. Is it a concern that the top line does not intersect with the others as well?

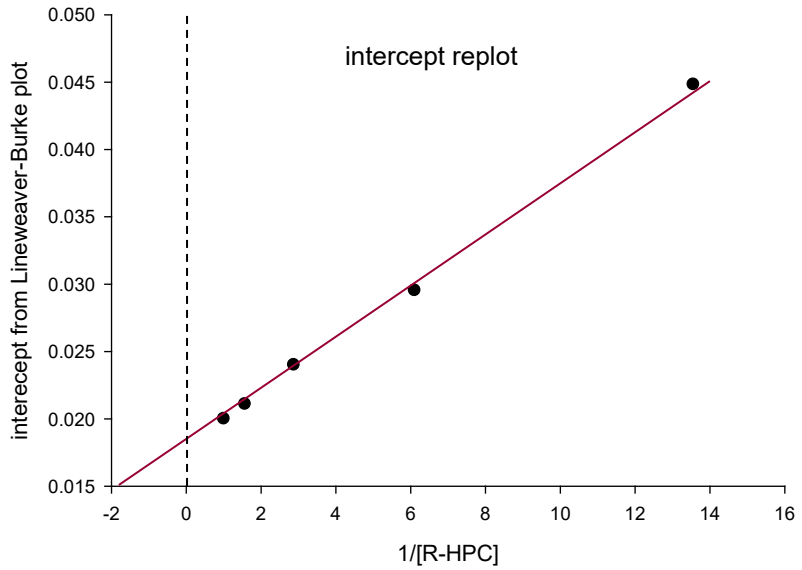
Slope and intercept replots of R-HPCDH data

- Use the slopes and intercepts determined from linear regression of the predicted data points

1-[R-HPC]	2-slope	3-intecept	4-1/[R-HPC]
0.0738	0.0255	0.0449	13.5413
0.1642	0.0186	0.0296	6.0897
0.3493	0.0132	0.0240	2.8628
0.6430	0.0106	0.0211	1.5551
1.0105	9.7675e-3	0.0200	0.9896







S. Ensign, enzyme kinetics

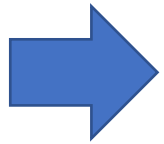
Analysis of two substrate kinetic data

- Conduct enzyme assays with constant [E] and varying [A] with a fixed, subsaturating [B] present in each assay
- Select a second fixed [B] (e.g., twice that used for the first set of assays), and repeat the kinetic measurements for each varied [A]
- Repeat this procedure for as many different fixed [B] desired
- Construct a graph containing each of the v vs. [A] plots obtained for different [B]
- Replot each v vs. [A] plot in double reciprocal form
- The pattern of double reciprocal lines (parallel or intersecting) reveals whether the kinetic mechanism is ping-pong or ordered
- Slope and intercept replots are useful for visual purposes and estimating kinetic parameters
- Fit your data to the appropriate form of the M-M equation to get your kinetic parameters

Analysis of two substrate kinetic data using nonlinear regression

- Arrange all data into 3 columns:

1-[NAD+]	2-v 0.0738 mM RHPC	3-v 0.1642 mM RHPC	4-v 0.3493 mM RHPC	-v 0.643027 mM RHP	6-v 1.0105 mM RHPC
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.3000	8.0520	11.9680	15.9980	18.3740	19.8820
0.7000	12.0390	17.0890	22.7320	27.5490	29.4900
1.5000	16.1990	23.5827	29.5530	34.5370	36.4190
3.0000	18.1830	27.2560	34.0350	39.2700	41.5810
4.5000	20.3120	30.5370	38.6740	44.1600	46.9240



1-[NAD+]	2-[R-HPC]	3-v
0.3000	0.0738	8.0520
0.3000	0.1642	11.9680
0.3000	0.3493	15.9980
0.3000	0.6430	18.3740
0.3000	1.0105	19.8820
0.7000	0.0738	12.0390
0.7000	0.1642	17.0890
0.7000	0.3493	22.7320
0.7000	0.6430	27.5490
0.7000	1.0105	29.4900
1.5000	0.0738	16.1990
1.5000	0.1642	23.5827
1.5000	0.3493	29.5530
1.5000	0.6430	34.5370
1.5000	1.0105	36.4190
3.0000	0.0738	18.1830
3.0000	0.1642	27.2560
3.0000	0.3493	34.0350
3.0000	0.6430	39.2700
3.0000	1.0105	41.5810
4.5000	0.0738	20.3120
4.5000	0.1642	30.5370
4.5000	0.3493	38.6740
4.5000	0.6430	44.1600
4.5000	1.0105	46.9240

Analysis of two substrate kinetic data using nonlinear regression

$$v = \frac{V_{max}[A]\cdot[B]}{K_{ia}\cdot K_{m,b} + K_{m,a}\cdot[B] + K_{m,b}\cdot[A] + [A]\cdot[B]}$$

- Formula: Y = a*x1*x2/(d*c+c*x1+b*x2+x1*x2)
- Number of data points = (varies for data) *(25 here)*
- number of independent variables = 2 (x1, x2)
  - x1 = [A] *(NAD+)*
  - x2 = [B] *(R-HPC)*
- number of parameters = 4 (a, b, c, d)
  - a = Vmax
  - b = Kma
  - c = Kmb
  - d = Kia
- Y = velocity

x1	x2	Y
0.3000	0.0738	8.0520
0.3000	0.1642	11.9680
0.3000	0.3493	15.9980
0.3000	0.6430	18.3740
0.3000	1.0105	19.8820
0.7000	0.0738	12.0390
0.7000	0.1642	17.0890
0.7000	0.3493	22.7320
0.7000	0.6430	27.5490
0.7000	1.0105	29.4900
1.5000	0.0738	16.1990
1.5000	0.1642	23.5827
1.5000	0.3493	29.5530
1.5000	0.6430	34.5370
1.5000	1.0105	36.4190
3.0000	0.0738	18.1830
3.0000	0.1642	27.2560
3.0000	0.3493	34.0350
3.0000	0.6430	39.2700
3.0000	1.0105	41.5810
4.5000	0.0738	20.3120
4.5000	0.1642	30.5370
4.5000	0.3493	38.6740
4.5000	0.6430	44.1600
4.5000	1.0105	46.9240

Analysis of two substrate kinetic data using nonlinear regression

- You must put in good initial guesses using statpages.info fitting. Get these from the slope and intercept replots

1. Enter the number of data points: 25
2. Enter the number of independent variables: 2 (1 for single substrate M-M equation; 2 for bisubstrate, 2 for C, UC, and mixed inhibition)
3. Enter the number of parameters: 4 (deduce from form of M-M equation used; will be 2 for single substrate M-M)
4. Enter the formula for the function to be fitted:
   $Y =$ a*x1*x2/(d*c+c*x1+b*x2+x1*x2)
   Use "" for multiplication and "/" for division when entering formulas. Define parameters (a, b, c) and variables (x1, x2, x3) as described above. Be sure to use parentheses approriately to get the correct hierarchy*
5. Type (or paste) the [x,y] data:

```
3.0000  0.6430  39.2700
3.0000  1.0105  41.5810
4.5000  0.0738  20.3120
4.5000  0.1642  30.5370
4.5000  0.3493  38.6740
4.5000  0.6430  44.1600
4.5000  1.0105  46.9240
```

Data can be typed in directly, or pasted from a text file or spreadsheet. The first column(s) contain the independent variable(s) (x1, x2..) while the final column contains the dependent variable y (velocity for enzyme data)

6. Enter initial guesses for the parameters from evaluation of the data (e.g. Lineweaver-Burke plots and appropriate slope and intercept replots). If the data fails to converge, provide better initial guesses. :

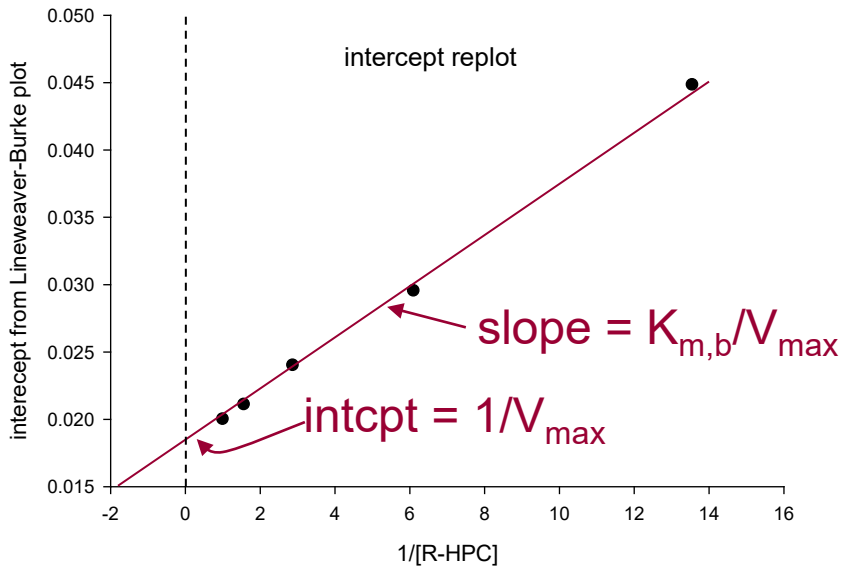
a (or p_1)=		±		, p=	
b (or p_2)=		±		, p=	
c (or p_3)=		±		, p=	
d (or p_4)=		±		, p=	
e (or p_5)=	0	±		, p=	
f (or p_6)=	0	±		, p=	
g (or p_7)=	0	±		, p=	
h (or p_8)=	0	±		, p=	

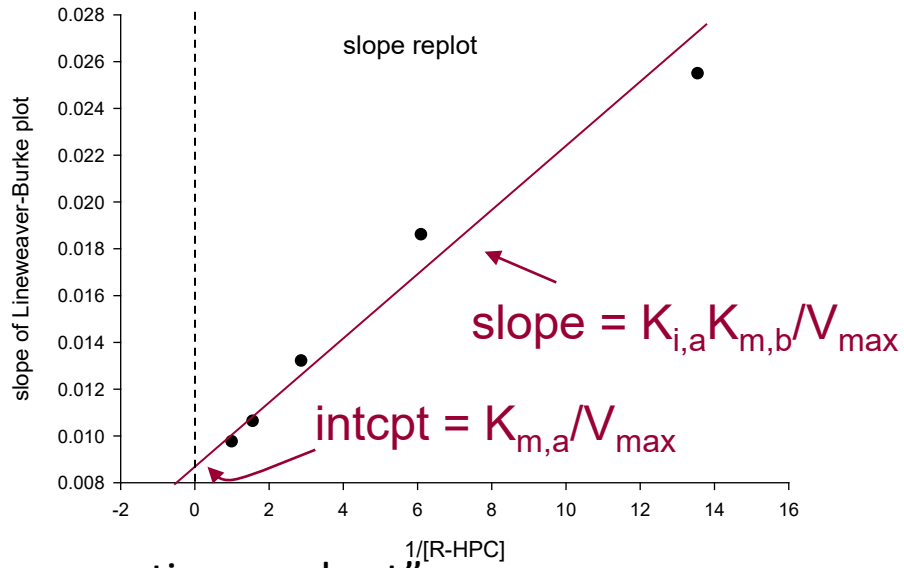
Place the value "0" (zero) in all parameter boxes for which a value is not defined in the equation to be fit. Leave the fields to the right of the parameters blank; these will be populated with the standard errors upon iteration.

7. Click the Iterate button once for each iteration cycle. Watch how the initial guesses change in response to the iteration. Continue to click the "iterate" button until the values converge to unchanging val

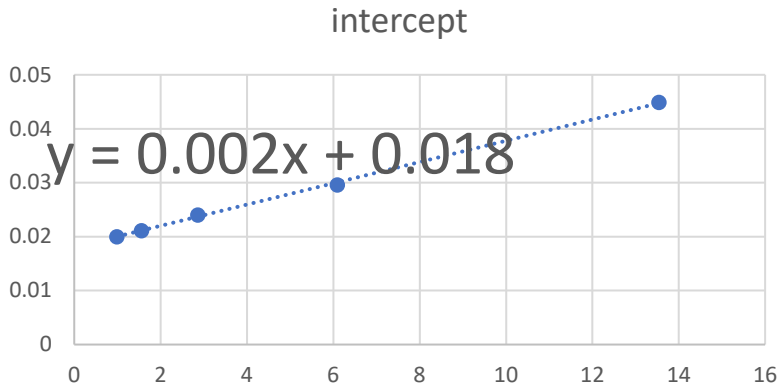
Slope and intercept replots of R-HPCDH data

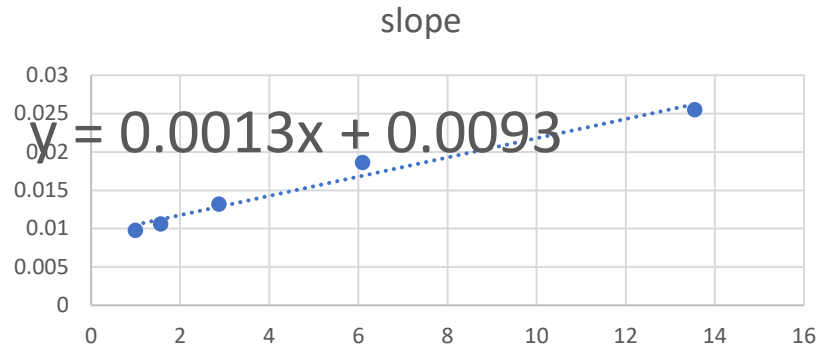
1/RHPC	intercept		1/RHPC	slope
13.5413	0.0449		13.5413	0.0255
6.0897	0.0296		6.0897	0.0186
2.8628	0.024		2.8628	0.0132
1.5551	0.0211		1.5551	0.0106
0.9896	0.02		0.9896	9.77E-03





Linear regression in excel: right click data points, select “add trend line”, “display equation on chart”





intcpt = $1/V_{max}$; Vmax = 1/intcpt = 1/0.018 = 55.6

slope = $K_{m,b}/V_{max}$; $K_{m,b}$ =V_{max}*slope = 55.6*0.002 = 0.112

intcpt = $K_{m,a}/V_{max}$; $K_{m,a}$ = intcpt*V_{max} = 0.0093*55.6 = 0.517

slope = $K_{i,a}K_{m,b}/V_{max}$; $K_{i,a}$ = slope*$V_{max}/K_{m,b}$ = 0.0013*55.6/0.112= 0.645

Analysis of two substrate kinetic data using nonlinear regression

$$v = \frac{V_{\max}[A]\cdot[B]}{K_{ia}\cdot K_{m,b} + K_{m,a}\cdot[B] + K_{m,b}\cdot[A] + [A]\cdot[B]}$$

- Formula: Y = a*x1*x2/(d*c+c*x1+b*x2+x1*x2)
- Number of data points = (varies for data) *(25 here)*
- number of independent variables = 2 (x1, x2)
  - x1 = [A] *(NAD+)*
  - x2 = [B] *(R-HPC)*
- number of parameters = 4 (a, b, c, d)
  - a = Vmax ~55.6
  - b = Kma ~0.517
  - c = Kmb ~0.112
  - d = Kia ~0.645
- Y = velocity

6. Enter initial guesses for the parameters from evaluation of the data (e.g.

a (or p_1)=	55.6	±		, p=	
b (or p_2)=	0.517	±		, p=	
c (or p_3)=	0.112	±		, p=	
d (or p_4)=	0.645	±		, p=	
e (or p_5)=	0	±		, p=	
f (or p_6)=	0	±		, p=	
g (or p_7)=	0	±		, p=	
h (or p_8)=	0	±		, p=	

x1	x2	Y
0.3000	0.0738	8.0520
0.3000	0.1642	11.9680
0.3000	0.3493	15.9980
0.3000	0.6430	18.3740
0.3000	1.0105	19.8820
0.7000	0.0738	12.0390
0.7000	0.1642	17.0890
0.7000	0.3493	22.7320
0.7000	0.6430	27.5490
0.7000	1.0105	29.4900
1.5000	0.0738	16.1990
1.5000	0.1642	23.5827
1.5000	0.3493	29.5530
1.5000	0.6430	34.5370
1.5000	1.0105	36.4190
3.0000	0.0738	18.1830
3.0000	0.1642	27.2560
3.0000	0.3493	34.0350
3.0000	0.6430	39.2700
3.0000	1.0105	41.5810
4.5000	0.0738	20.3120
4.5000	0.1642	30.5370
4.5000	0.3493	38.6740
4.5000	0.6430	44.1600
4.5000	1.0105	46.9240

Analysis of two substrate kinetic data using nonlinear regression

Be sure to iterate until the values no longer change. Here is what you end up with. Compare these numbers to your initial guesses from the intercept and slope replots. The numbers below, with their standard errors, are the numbers you report when you publish your data.

6. Enter initial guesses for the parameters from evaluation of the data (e.g. Line

a (or p_1)=	55.00977463651	±	1.452688132807	, p=	5e-7
b (or p_2)=	0.463554321482	±	0.051612969840	, p=	5e-7
c (or p_3)=	0.106694812940	±	0.011758483401	, p=	5e-7
d (or p_4)=	0.776863494422	±	0.206706457062	, p=	0.001156
e (or p_5)=	0	±	1	, p=	1.000000
f (or p_6)=	0	±	1	, p=	1.000000
g (or p_7)=	0	±	1	, p=	1.000000
h (or p_8)=	0	±	1	, p=	1.000000

Place the value "0" (zero) in all parameter boxes for which a value is not defined in the equati

- Estimates:
  - a = Vmax ~55.6
  - b = Kma ~0.517
  - c = Kmb ~0.112
  - d = Kia ~0.645

Michaelis-Menten equation for a bisubstrate ping-pong reaction

$$v = \frac{V_{\max}[A]\cdot[B]}{K_{m,a}\cdot[B] + K_{m,b}\cdot[A] + [A]\cdot[B]}$$

- Formula: $Y = a*x1*x2/(b*x2+c*x1+x1*x2)$
- Number of data points = (varies for data)
- number of independent variables = 2 (x1, x2)
  - x1 = [A]
  - x2 = [B]
- number of parameters = 3 (a, b, c)
  - a = Vmax
  - b = Kma
  - c = Kmb
- Y = velocity

Sample data:

0.2	0.025	8.6
0.25	0.025	9.2
0.333	0.025	9.9
0.5	0.025	10.6
1	0.025	11.7
2	0.025	12.2
2.5	0.025	12.3
3.33	0.025	12.42
4	0.025	12.44
5	0.025	12.5
0.2	0.05	12.2
0.25	0.05	13.5
0.333	0.05	15.1
0.5	0.05	17.1
1	0.05	19.75
2	0.05	21.3
2.5	0.05	21.75
3.33	0.05	22
4	0.05	22.2
5	0.05	22.5
0.2	0.1	15.6
0.25	0.1	17.8
0.333	0.1	20.6
0.5	0.1	25.55
1	0.1	30.3
2	0.1	34.2
2.5	0.1	35.1
3.33	0.1	36.1
4	0.1	36.6
5	0.1	37.2
0.2	0.25	18.8
0.25	0.25	22
0.333	0.25	26.4
0.5	0.25	33.2
1	0.25	44.5
2	0.25	53.75
2.5	0.25	56
3.33	0.25	58.6
4	0.25	59.9
5	0.25	61.3
0.2	0.5	20.1
0.25	0.5	23.8
0.333	0.5	29.1
0.5	0.5	37.5
1	0.5	52.8
2	0.5	66.3
2.5	0.5	69.8
3.33	0.5	73.9
4	0.5	76
5	0.5	78.3

S. Ensign, enzyme kinetics

“Apparent” vs. “true” K_m and V_{max} values for bisubstrate reactions

- “True” K_m and V_{max} values are derived from a complete three variable analysis of kinetic data, as described above
- If K_m and V_{max} values are determined for only one substrate at a high, nominally “saturating” concentration of the second substrate, then the constants are “apparent” K_m and V_{max}

Allosteric enzymes

- Multisubunit enzymes which exhibit cooperativity for substrate binding
- Sigmoidal curves for substrate binding instead of hyperbolic
- Michaelis-Menten kinetics does not apply

Enzyme Inhibition

Inhibitors: Compounds that reduce the activity of an enzyme by combining with in a manner that influences the binding of substrate and/or its k_{cat} .

Uses:
- Probes of catalytic mechanism
- Elucidate metabolic pathways
- Pharmaceuticals-chemotherapeutic action

Two broad classes of “reversible” inhibitors:

- Rapid equilibrium
  - Competitive
  - Noncompetitive
  - Uncompetitive
- Slow-(tight) binding

Irreversible inhibitors: inactivators (do not dissociate), include “mechanism-based inactivators (suicide substrates)

Distinguishing rapid-equilibrium and slow-binding inhibitors

- Rapid-equilibrium: equilibrium between E, I, and EI is rapidly established (i.e. within mixing time prior to initiating assay).

$$E + I \overset{\text{fast}}{\rightleftharpoons} EI$$

- Slow- (tight) binding:
  - Scenario 1: equilibrium between E, I and EI is not established rapidly (i.e. within time frame of assay)

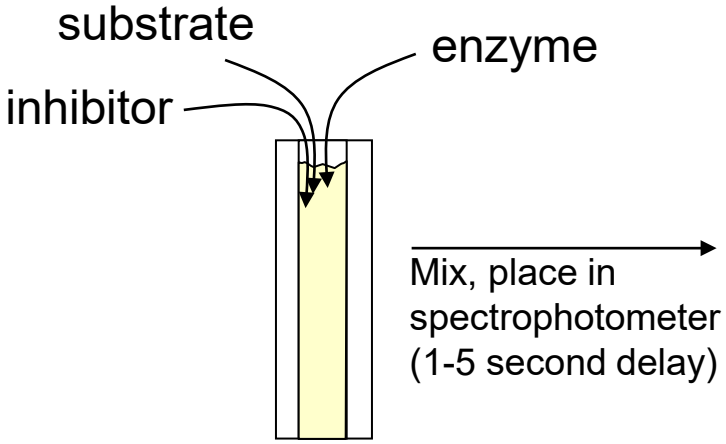
$$E + I \overset{\text{slow}}{\rightleftharpoons} EI$$

  - Scenario 2: equilibrium between E, I, and EI is rapidly established, but EI complex slowly reacts further to form a second inhibited form EI*.

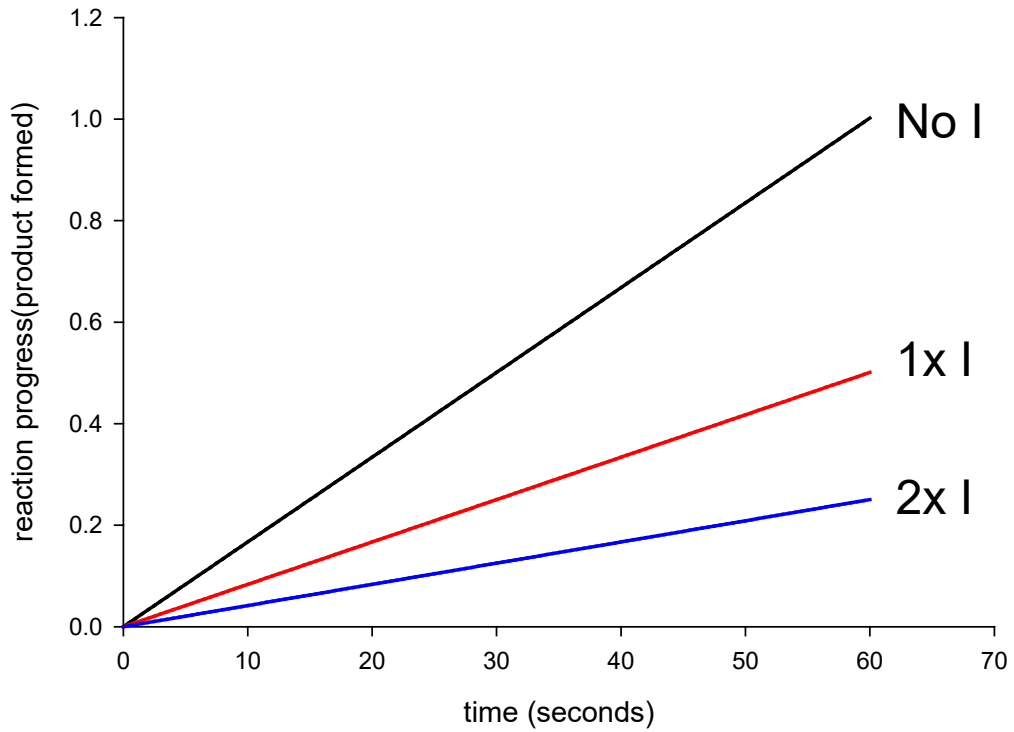
$$E + I \overset{\text{fast}}{\rightleftharpoons} EI \overset{\text{slow}}{\rightleftharpoons} EI^*$$

Distinguishing rapid-equilibrium and slow-binding inhibitors

- Rapid-equilibrium: equilibrium between E, I, and EI is rapidly established (i.e. within mixing time prior to initiating assay).

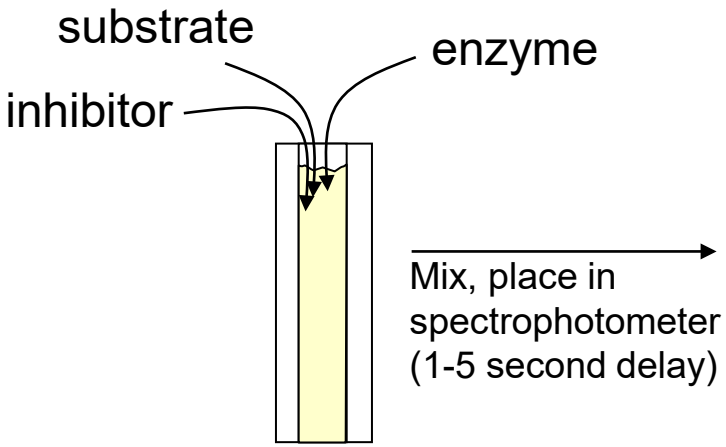


Combine E, S, and I simultaneously



Distinguishing rapid-equilibrium and slow-binding inhibitors

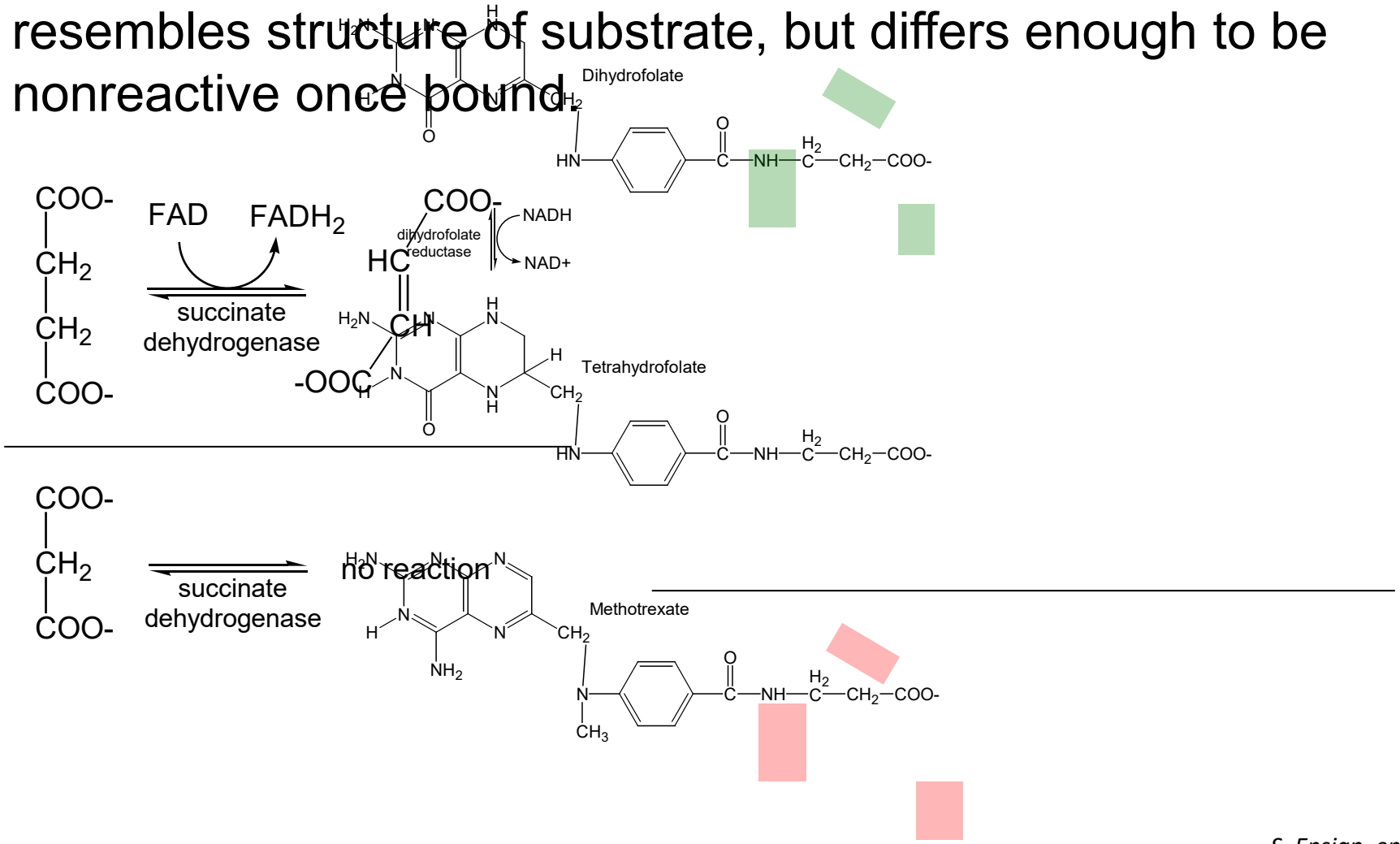
- Slow-binding: equilibrium between E, I, and EI (or EI*) is not rapidly established (i.e. within mixing time prior to initiating assay). The time required for establishment of the equilibrium is observed in the “steady state”



Combine E, S, and I simultaneously

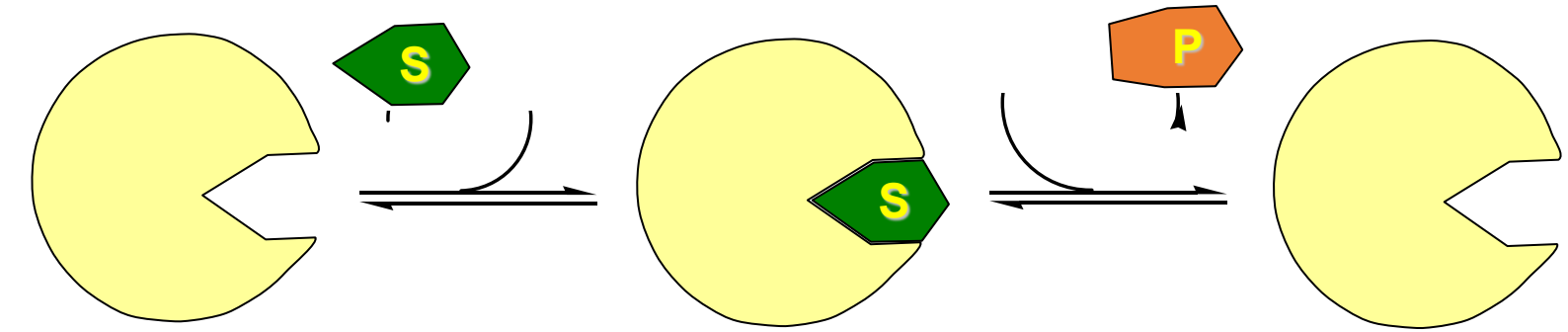
Competitive Inhibition

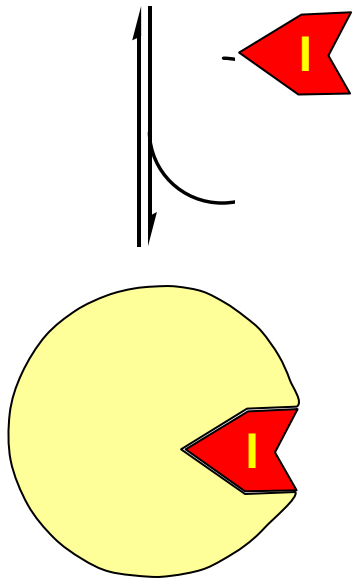
Competitive inhibitor: a compound that competes with substrate for binding to enzyme active site. Usually resembles structure of substrate, but differs enough to be nonreactive once bound.



Competitive Inhibition

Competitive inhibitor: a compound that competes with substrate for binding to enzyme active site. Usually resembles structure of substrate, but differs enough to be nonreactive once bound.



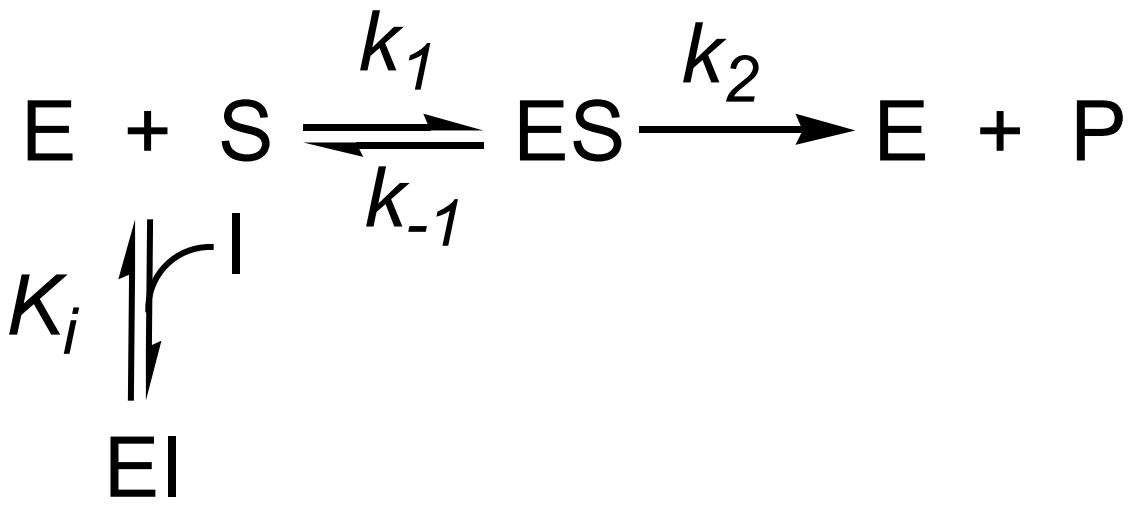


$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$K_i \quad I$$

$$EI$$

Inhibition constant K_I



The inhibition constant K_I is the thermodynamic dissociation constant for the nonproductive EI complex

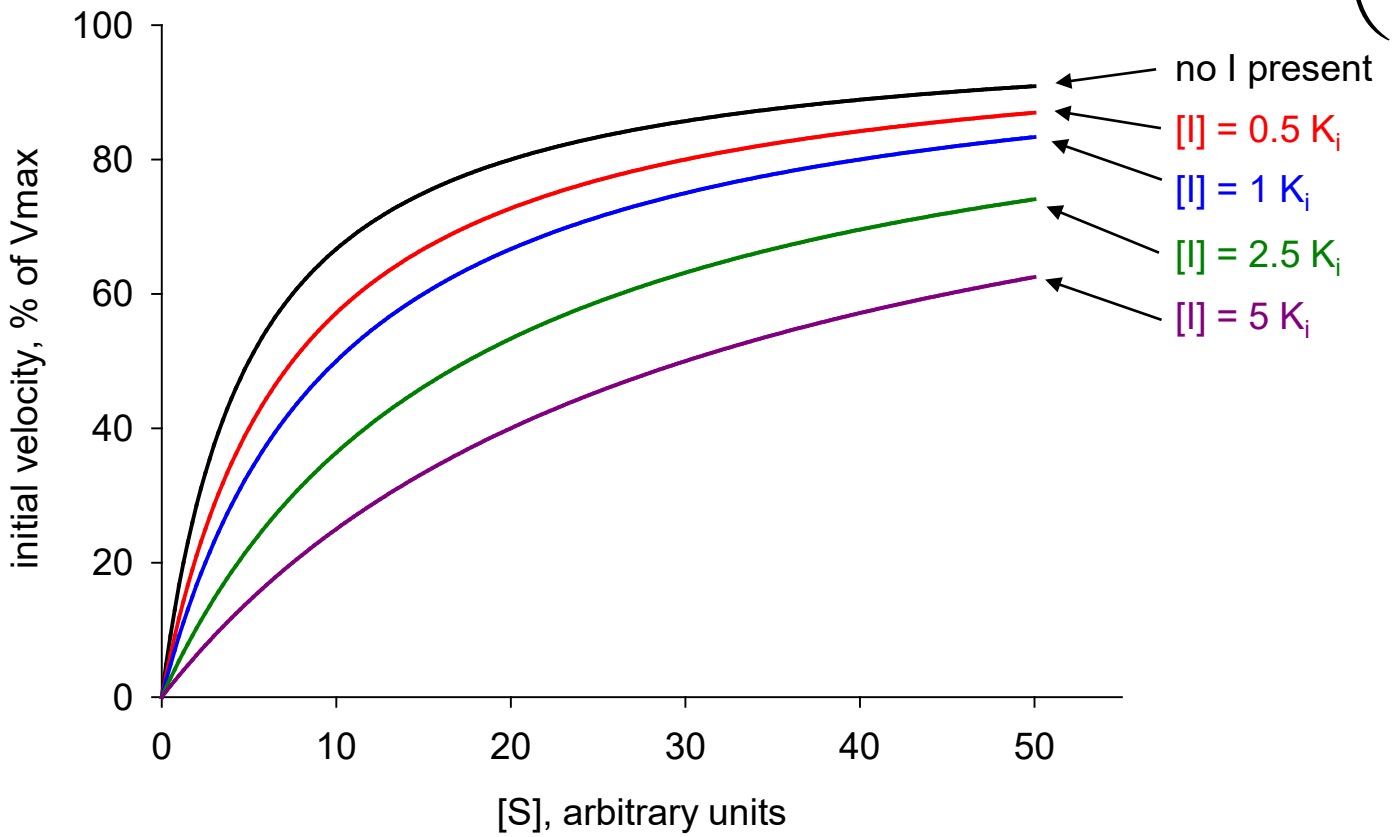
$$EI \rightleftharpoons E + I \qquad K_i = \frac{[E][I]}{[EI]}$$

The smaller the value of K_i, the more potent the inhibitor

Michaelis-Menten equation in the presence of a competitive inhibitor (single substrate case)

Without inhibitor: $$v = \frac{V_{max}[S]}{K_m + [S]}$$

With inhibitor: $$v = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]}$$



Algebraic rearrangement of the Michaelis-Menten equation in the presence of a competitive inhibitor

Without inhibitor:

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

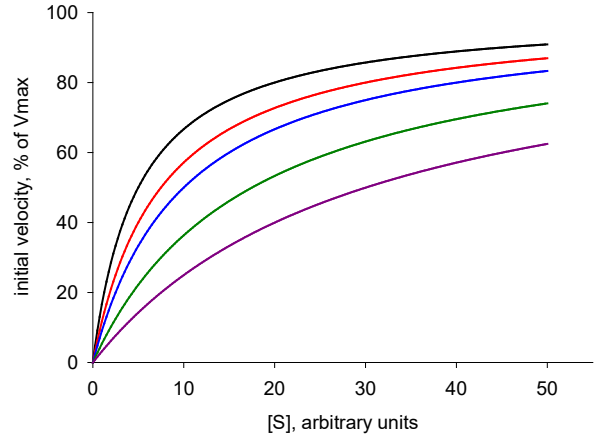
y = m x + b

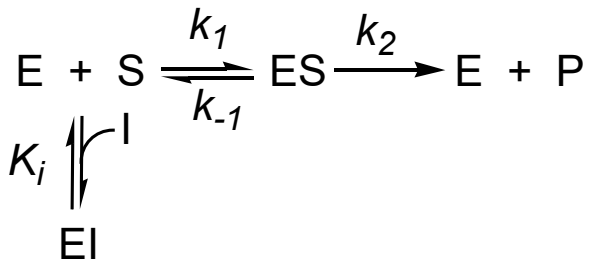
With inhibitor:

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right) \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

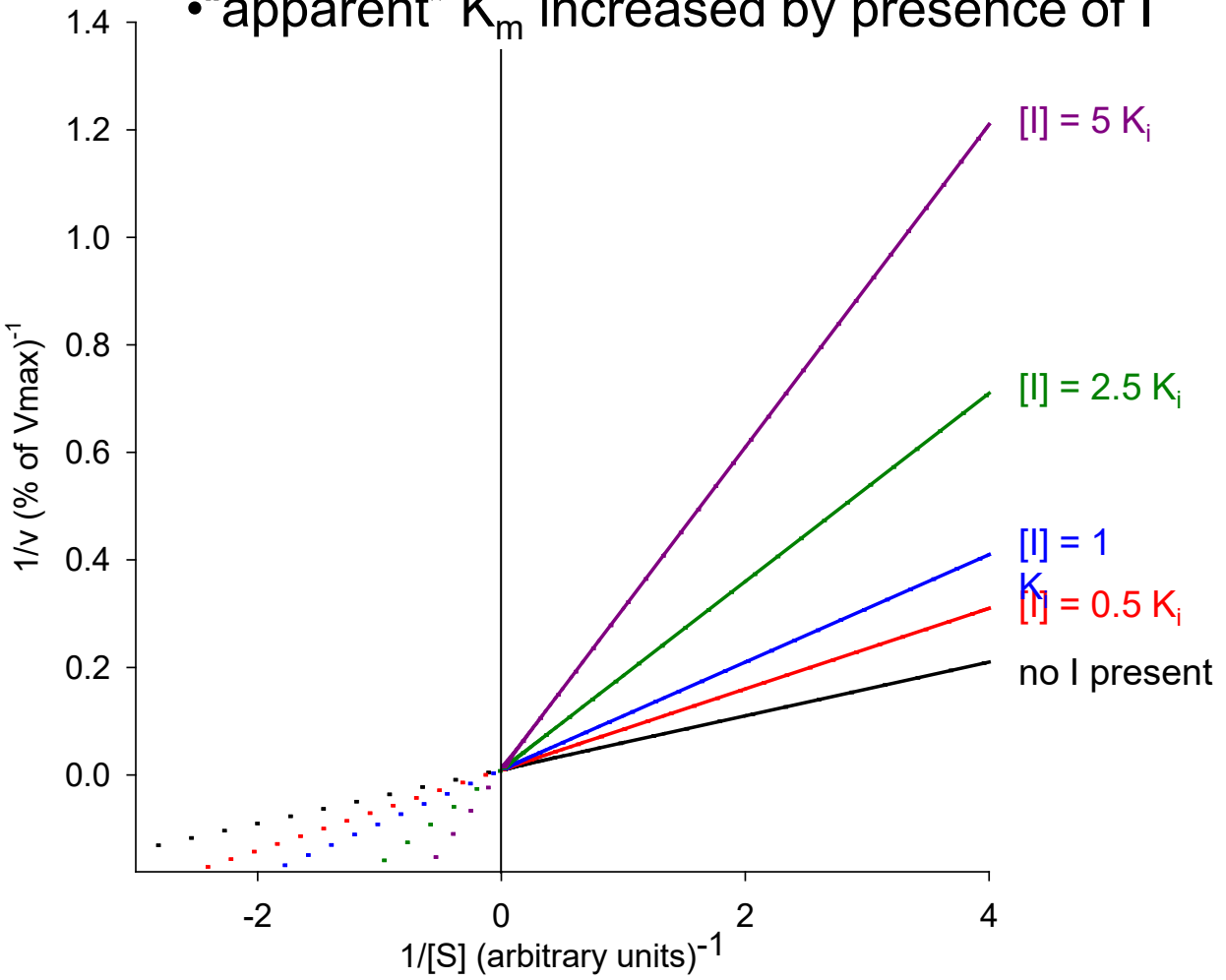
y = m x + b

Lineweaver-Burke plots in the presence of a competitive inhibitor



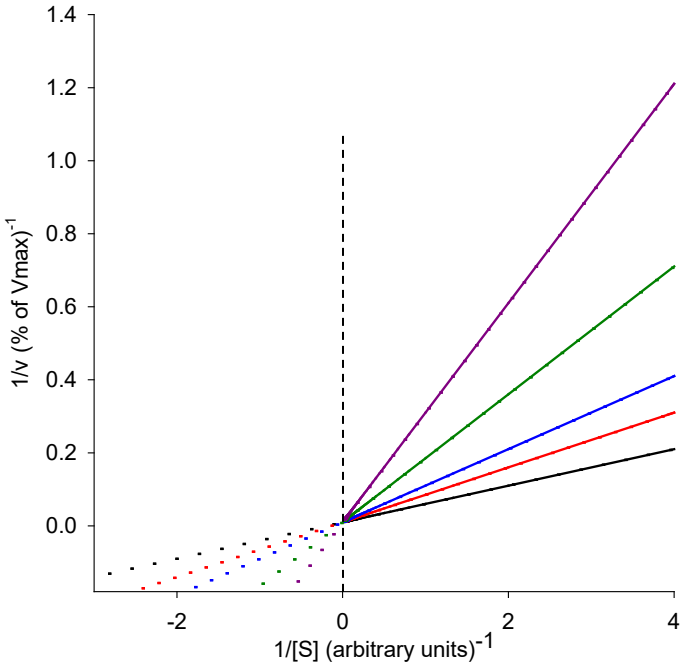


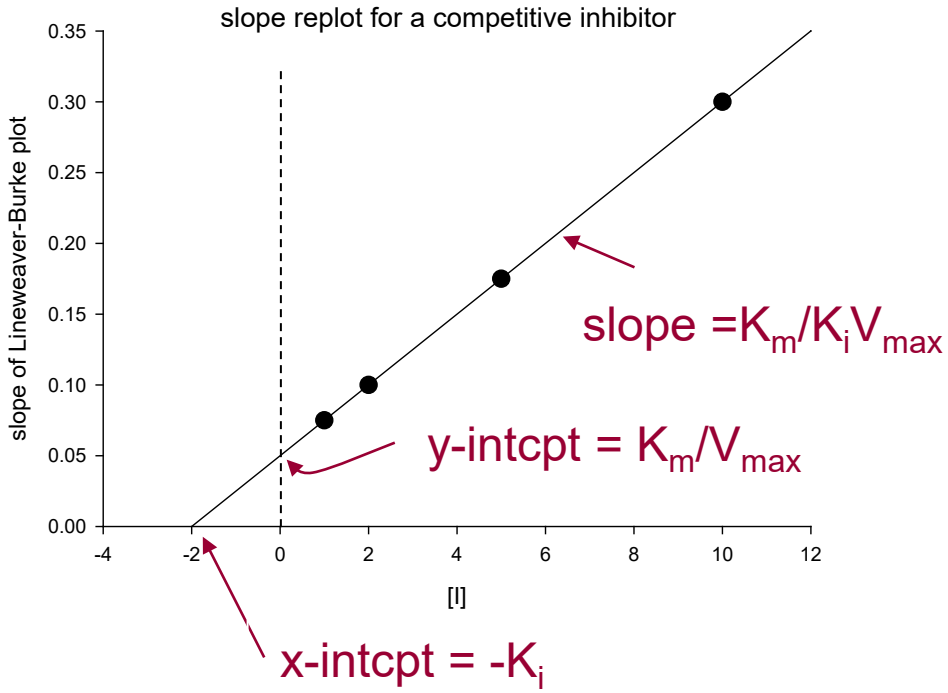
- V_{max} unaffected by presence of I
- "apparent" K_m increased by presence of I



S. Ensign, enzyme kinetics

Slope replot for estimation of kinetic parameters with a competitive inhibitor

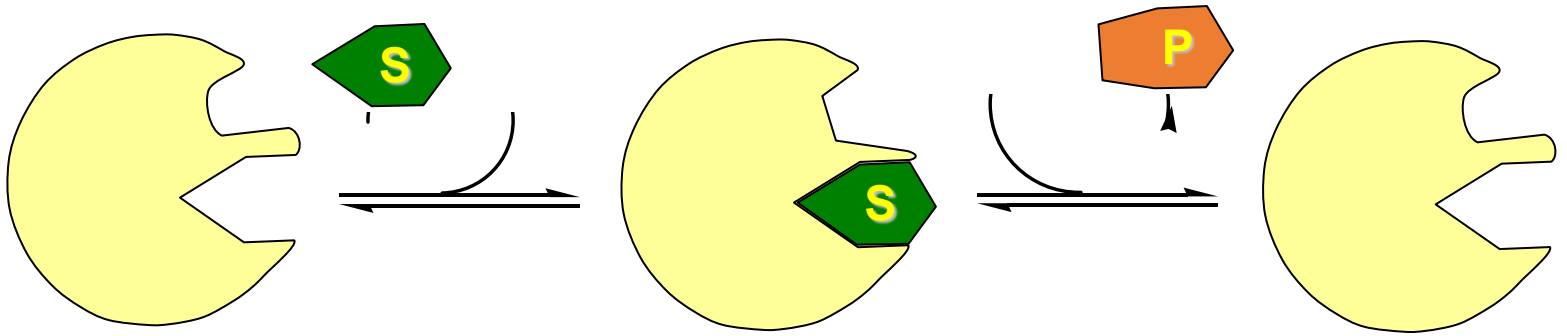


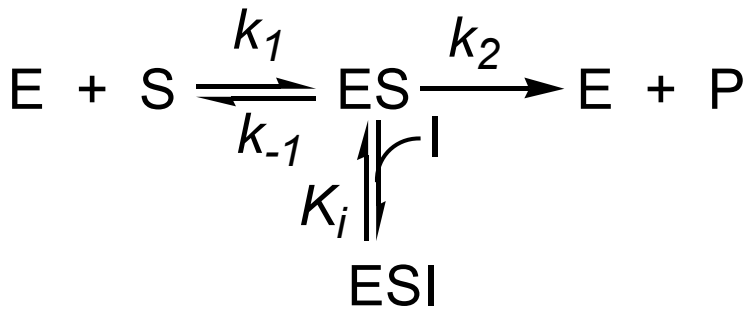


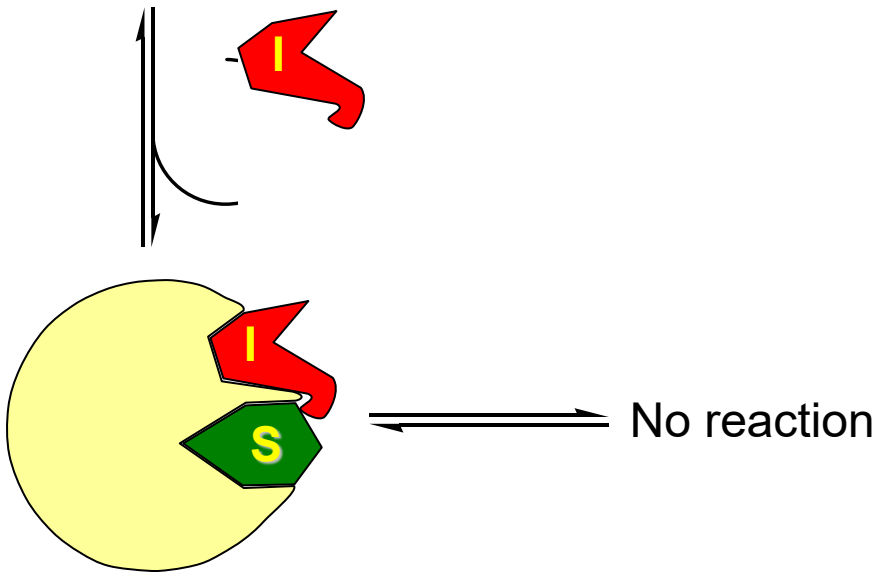
For this example, V_{max} = 100, K_m = 5, and K_i = 2

Uncompetitive Inhibition

Uncompetitive inhibitor: a compound that inhibits activity by binding the ES complex but not the free enzyme E.

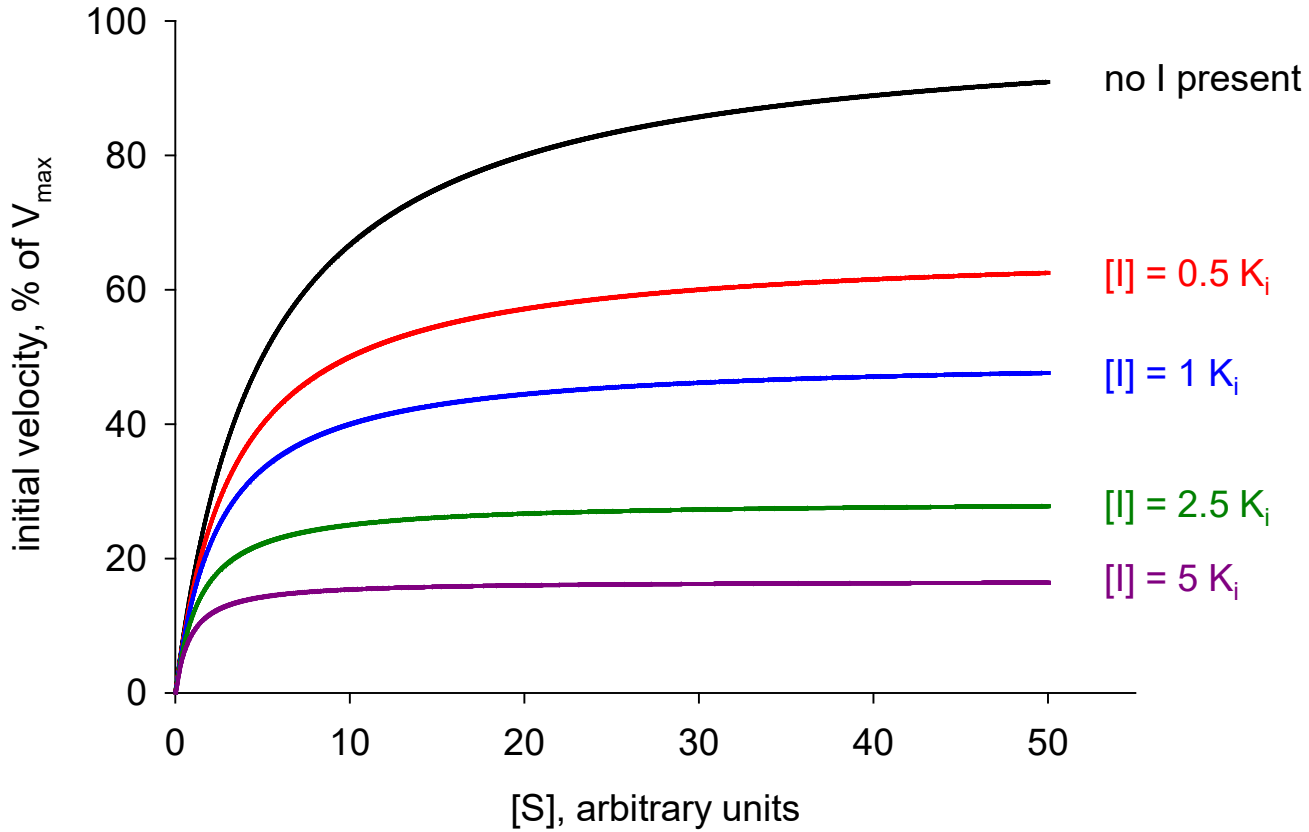






Michaelis-Menten equation in the presence of an uncompetitive inhibitor (single substrate case)

$$v = \frac{V_{\max}[\mathrm{S}]}{K_m + [\mathrm{S}]\left(1 + \frac{[\mathrm{I}]}{K_i}\right)}$$



S. Ensign, enzyme kinetics

Algebraic rearrangement of the Michaelis-Menten equation in the presence of an uncompetitive inhibitor

Without inhibitor:

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

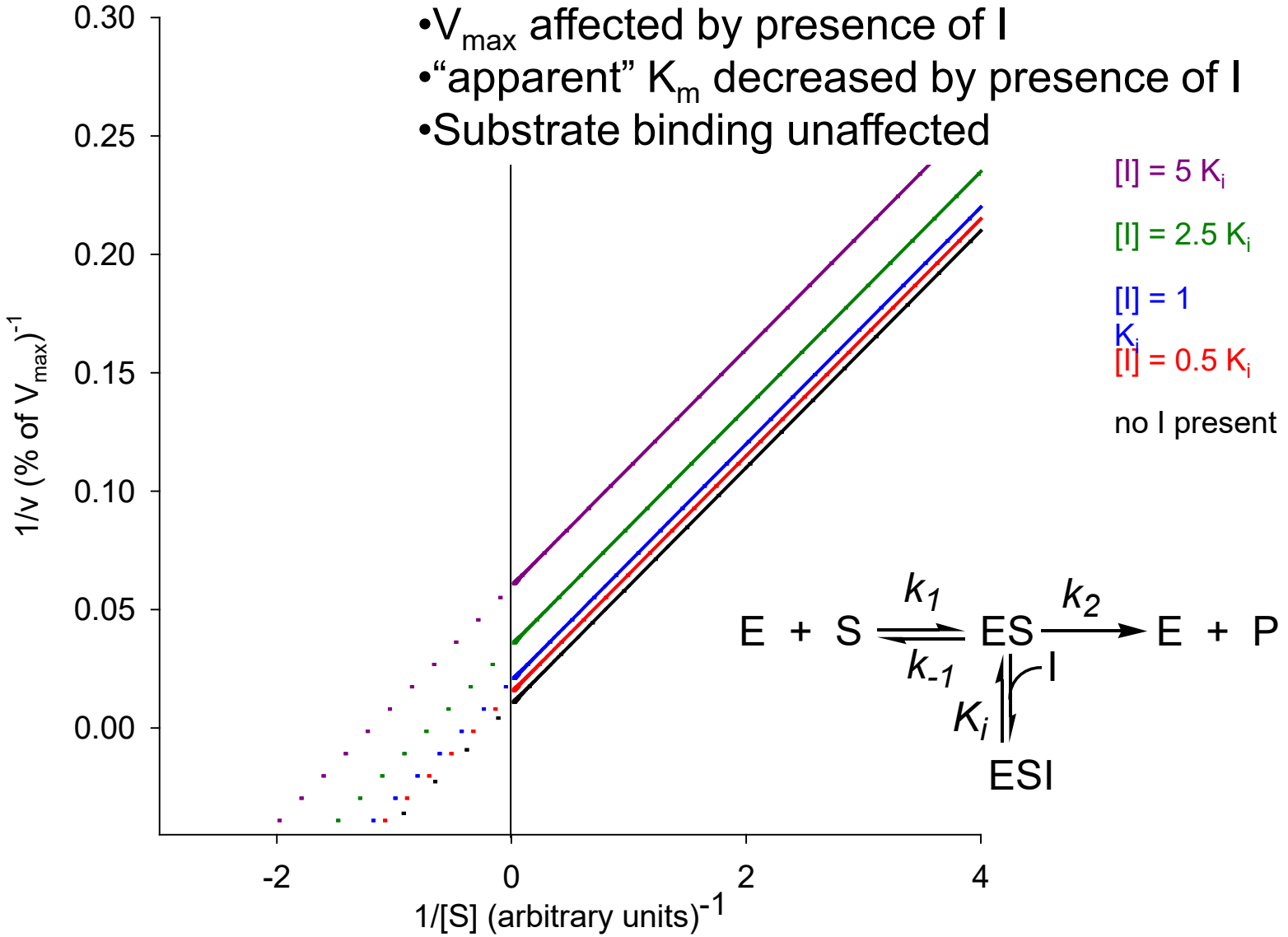
$$y = \underbrace{m}\ x + b$$

With inhibitor:

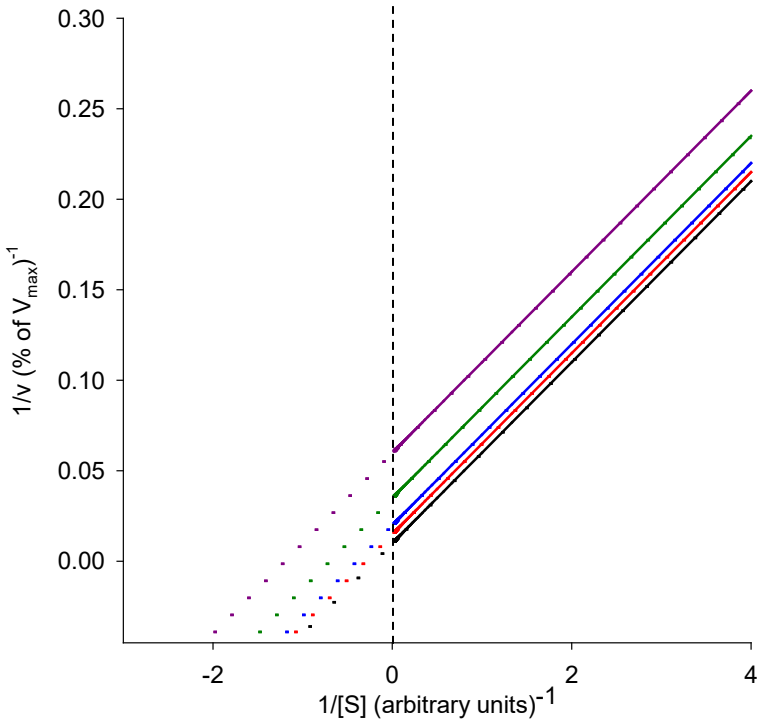
$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$$

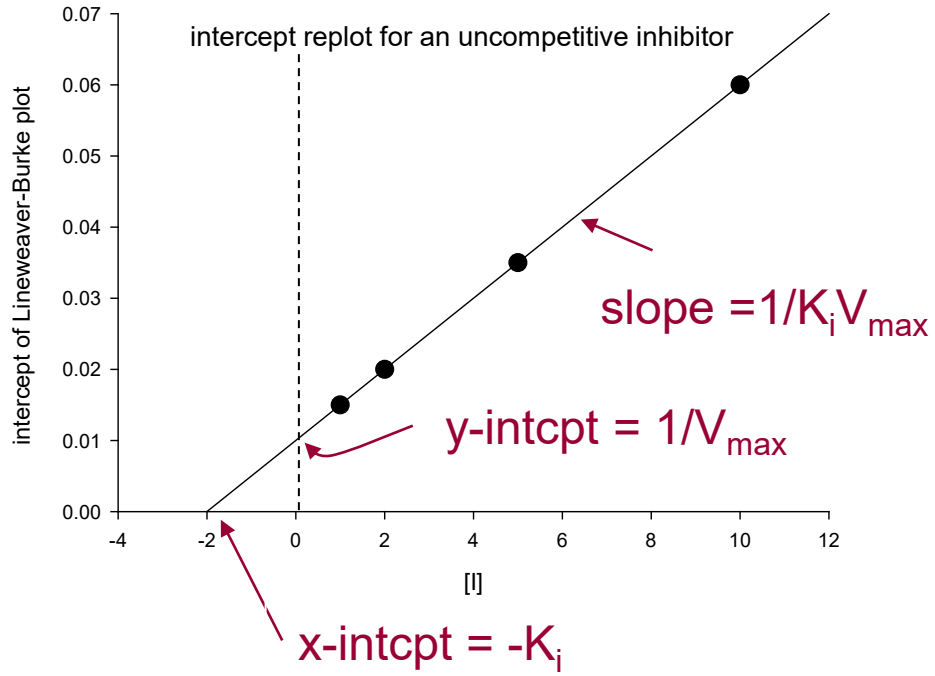
$$y = \underbrace{m}\ x + b$$

Lineweaver-Burke plots in the presence of an uncompetitive inhibitor



Intercept replot for estimation of kinetic parameters with an uncompetitive inhibitor

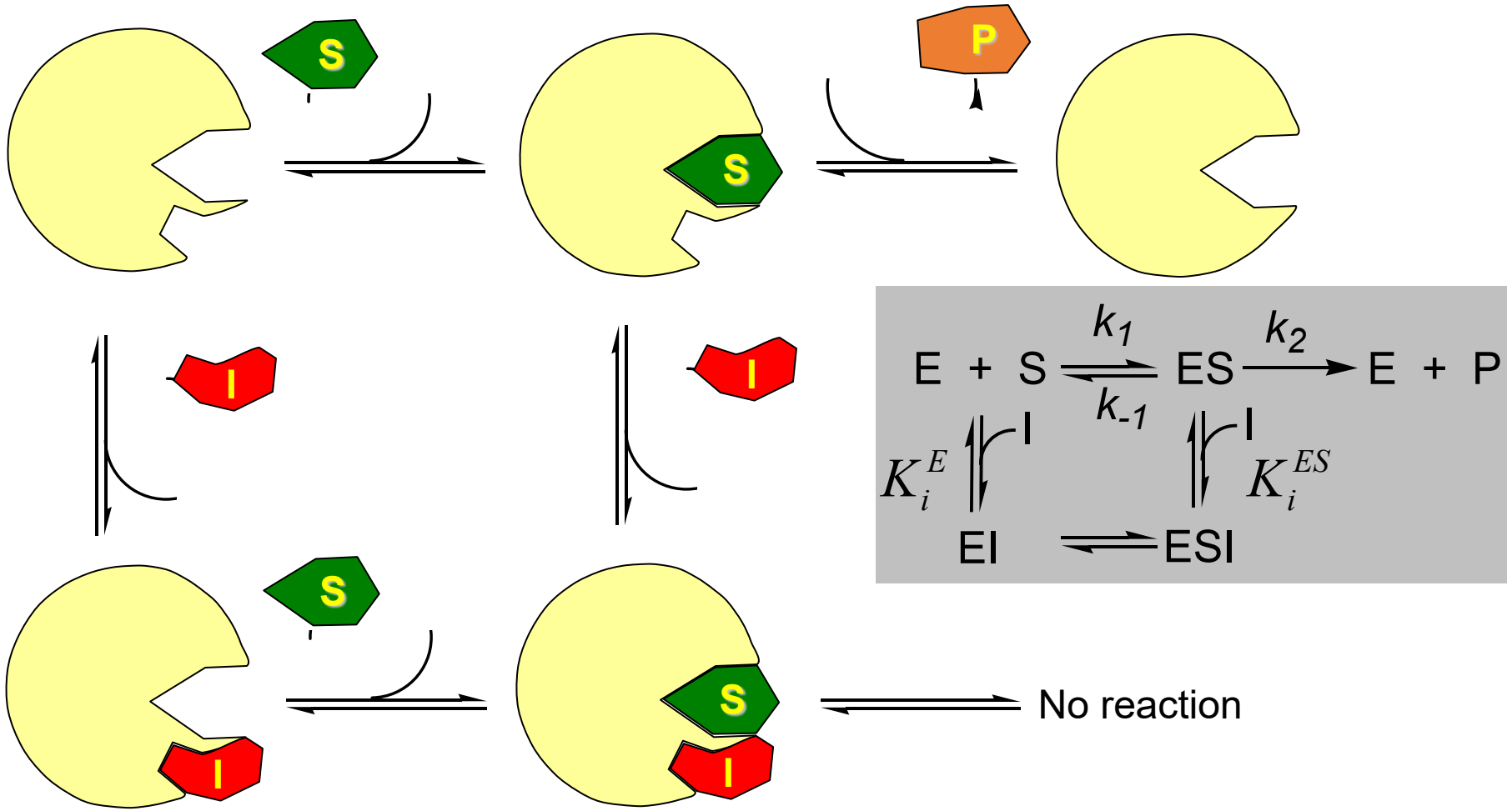




For this example, V_{max} = 100, K_m = 5, and K_i = 2

Mixed (noncompetitive) Inhibition

Mixed inhibitor: a compound that inhibits activity upon binding either the free enzyme or the ES complex



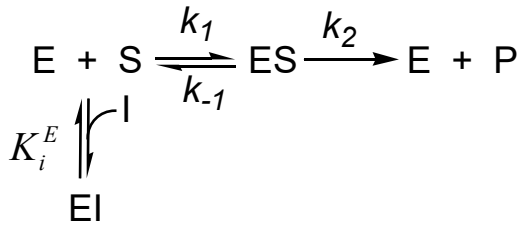
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$K_i^E: \quad E + I \rightleftharpoons EI$$

$$K_i^{ES}: \quad ES + I \rightleftharpoons ESI$$

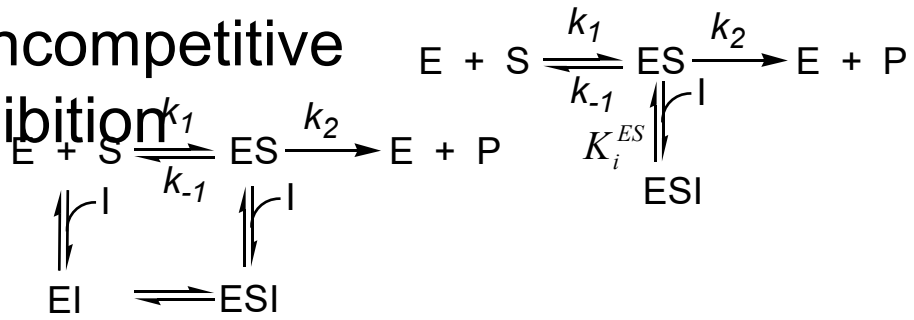
Inhibition constant nomenclature

- Competitive inhibition



$$K_i^E = K_{is} = \frac{[E][I]}{[EI]}$$

- Uncompetitive inhibition



$$K_i^{ES} = K_{ii} = \frac{[ES][I]}{[ESI]}$$

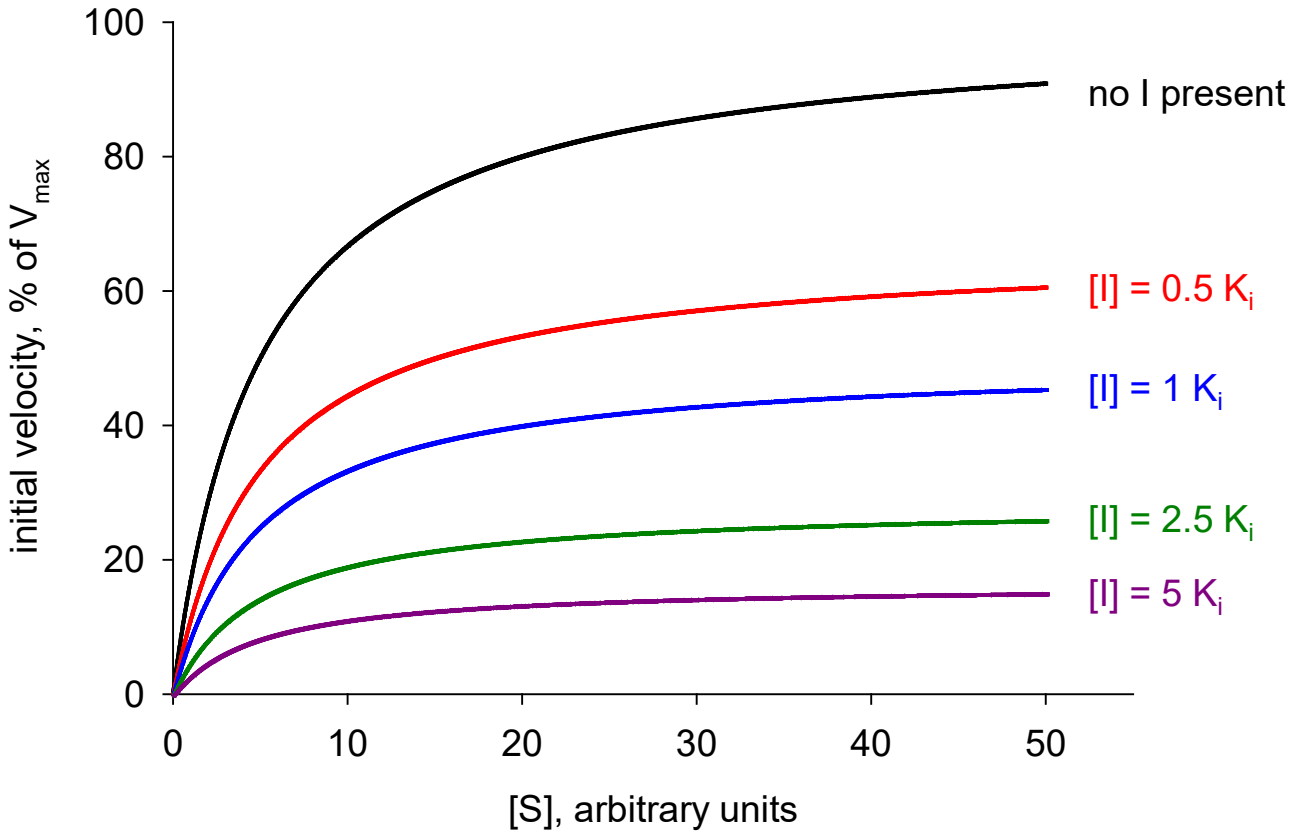
- Mixed inhibition

K_i^E K_i^{ES}

$$K_i^E = \frac{[E][I]}{[EI]} \qquad K_i^{ES} = \frac{[ES][I]}{[ESI]}$$

Michaelis-Menten equation in the presence of a mixed inhibitor (single substrate case)

$$v = \frac{V_{\max}[\mathrm{S}]}{K_m\left(1+\frac{[\mathrm{I}]}{K_i^E}\right)+[\mathrm{S}]\left(1+\frac{[\mathrm{I}]}{K_i^{ES}}\right)}$$



Algebraic rearrangement of the Michaelis-Menten equation in the presence of a mixed inhibitor

Without inhibitor:

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

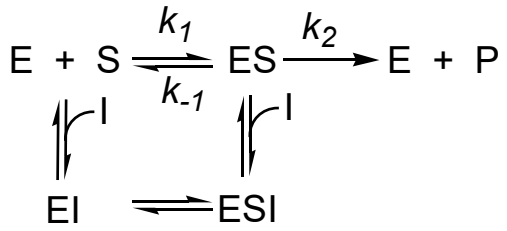
$$y = \underbrace{\quad}_{m} \quad x + \quad b$$

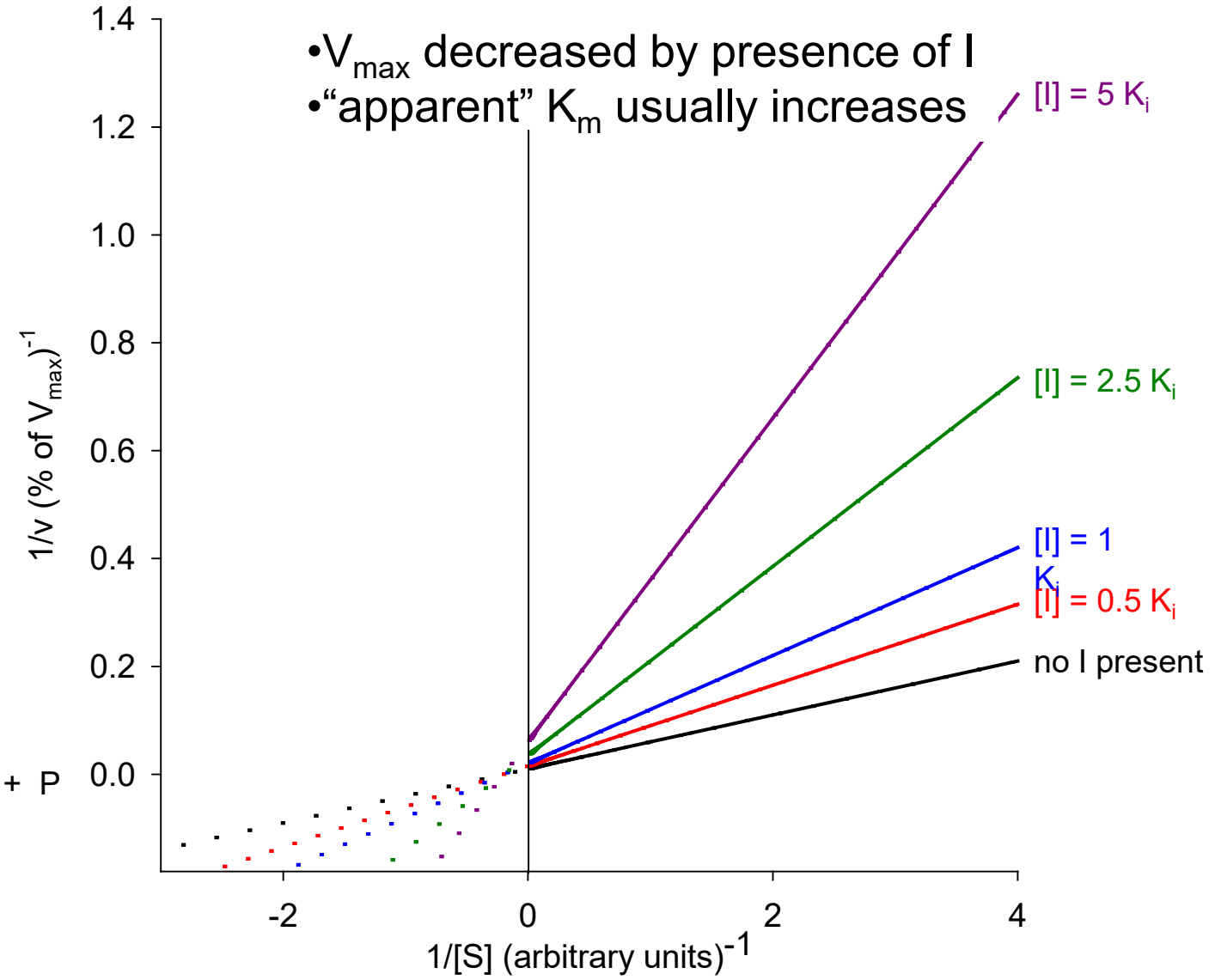
With inhibitor:

$$\frac{1}{v} = \underbrace{\frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i^E}\right)}_{m} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i^{ES}}\right)$$

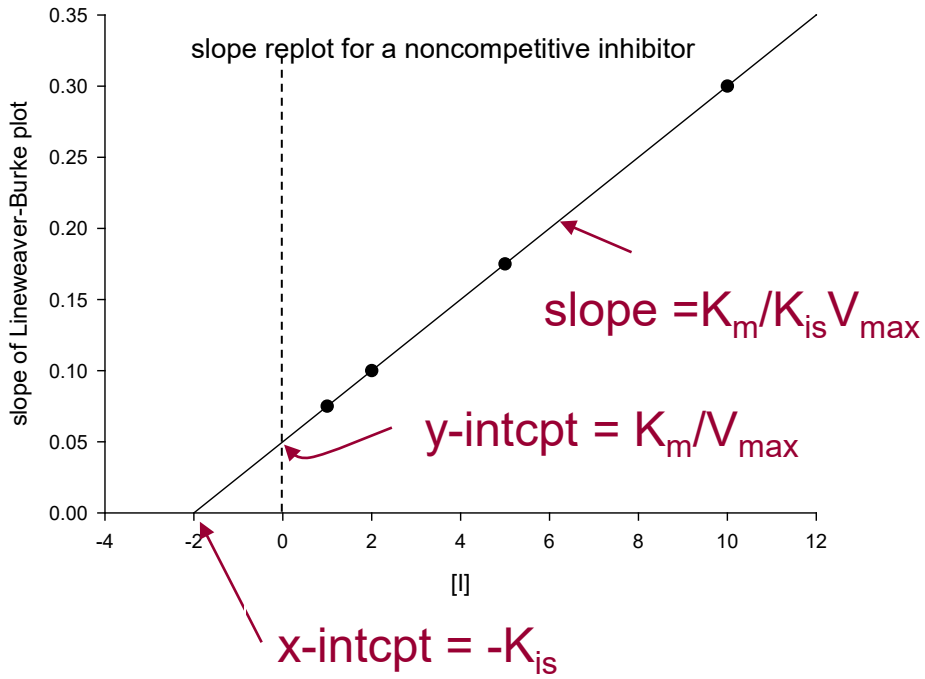
$$y = \qquad\qquad m \qquad\qquad x \; + \qquad b$$

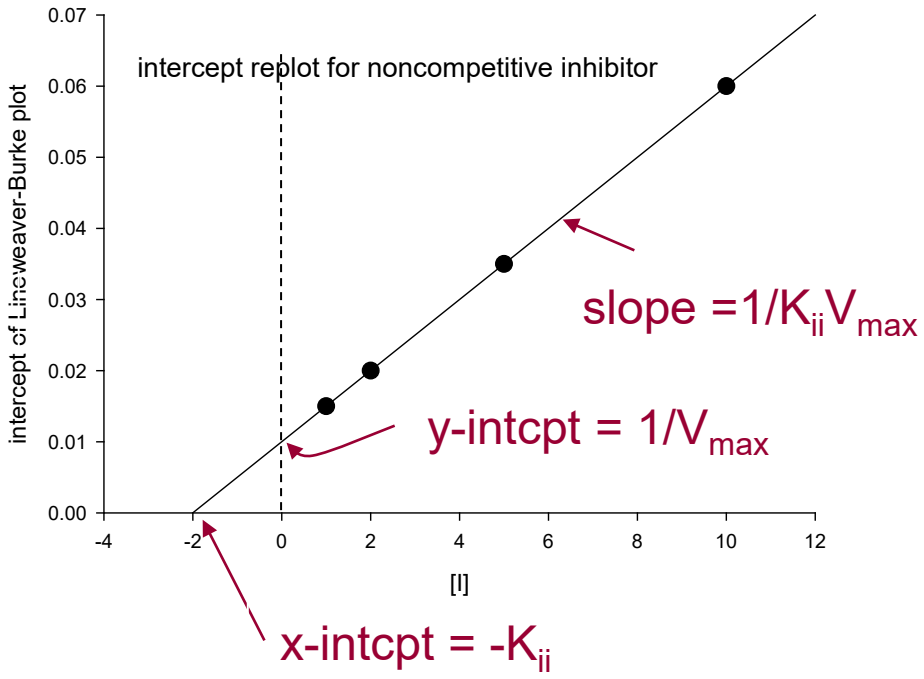
Lineweaver-Burke plots in the presence of a mixed inhibitor





Slope and intercept replots with a mixed inhibitor





For this example, V_{max} = 100, K_m = 5, K_{is} = 2, and K_{ii} = 2

Analysis of kinetic data for a single substrate enzyme with a rapid-equilibrium inhibitor

- Conduct enzyme assays with constant [E] and varying [S] and with no I present
- Conduct enzyme assays with the same constant [E] and varying [S] as above, but with a fixed, subsaturating [I] present in each assay
- Select a second fixed [I] (e.g., twice that used for the first set of assays), and repeat the kinetic measurements for each varied [S]
- Repeat this procedure for as many different fixed [I] desired
- Construct a graph containing each of the v vs. [S] plots obtained for different [I]
- Replot each v vs. [S] plot in double reciprocal form
- The pattern of double reciprocal lines (intersecting on y-axis, parallel or intersecting in 3rd or 4th quadrant) reveals whether the inhibitor is C, UC, or NC
- Slope and intercept replots are useful for visual purposes and estimating kinetic parameters
- Fit your data to the appropriate M-M equation to get your kinetic parameters

An actual example to illustrate how to analyze inhibitor data

5372 *Biochemistry* **1995,** *34,* 5372−5381

Reactivity of Carbon Monoxide Dehydrogenase from *Rhodospirillum rubrum* with Carbon Dioxide, Carbonyl Sulfide, and Carbon Disulfide

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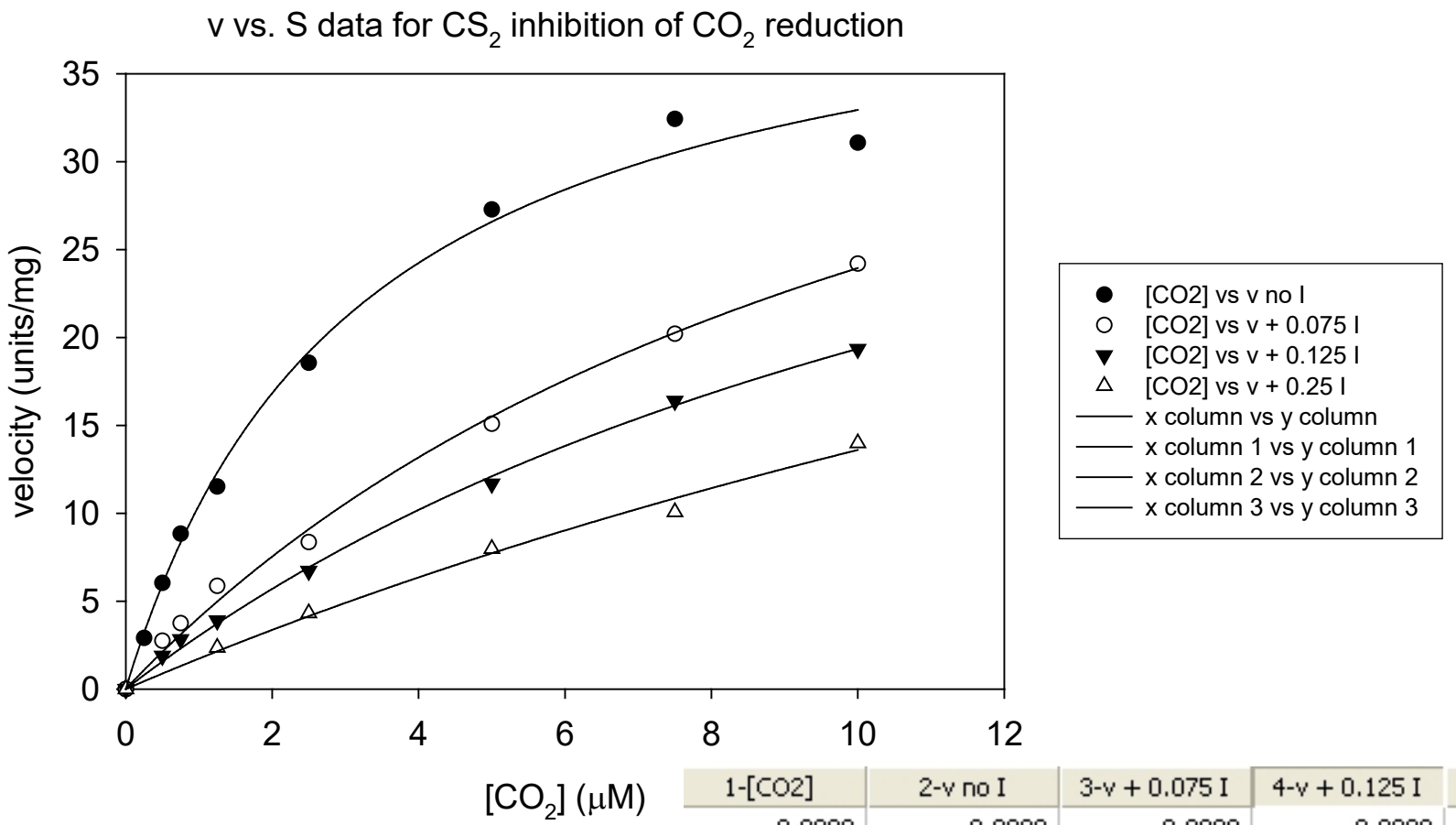
Received November 23, 1994; Revised Manuscript Received February 8, 1995[⊗]

ABSTRACT: The reactivities of CO_2 and the related compounds COS and CS_2 with the nickel- and iron−sulfur-containing carbon monoxide dehydrogenase (CODH) from *Rhodospirillum rubrum* have been investigated. Both CO_2 and COS were substrates for CODH in a reductant-dependent reaction resulting in the formation of CO. CO_2 was reduced to CO and H_2O, while COS was reduced to CO and H_2S. CO was a potent inhibitor of CO_2 reduction at dissolved concentrations as low as 1 μM, but this inhibition could be prevented by quantitatively trapping CO as it was formed by including reduced hemoglobin in the assays. The addition of hemoglobin to the assays also allowed the formation of CO to be monitored in real time by following the decrease in absorbance at 433 nm resulting from carboxyhemoglobin formation. A variety of low-potential reductants, including dithionite, titanium(III) citrate, and dithionite-reduced viologens (methyl and benzyl), were suitable electron donors for the reduction of CO_2 and COS. Dithionite-reduced methyl viologen supported the highest rates of CO_2 and COS reduction, and the stimulation of CO_2 reduction (170-fold increased rate over dithionite alone) was much more dramatic than the stimulation of COS reduction (2.6-fold increased rate over dithionite alone). CO_2 was reduced to CO with a K_m for CO_2 of 190 μM and a V_{max} of 44 μmol of CO formed min^{-1} (mg of protein)$^{-1}$, while COS was reduced with a K_m for COS of 2.2 μM and a V_{max} of 0.51 μmol of CO formed min^{-1} (mg of protein)$^{-1}$. The CO_2/COS analog CS_2 interacted with CODH as a reversible, rapid-equilibrium inhibitor of CO_2 reduction, and the pattern of inhibition observed for CS_2 was competitive vs CO_2 (K_i = 43 μM).

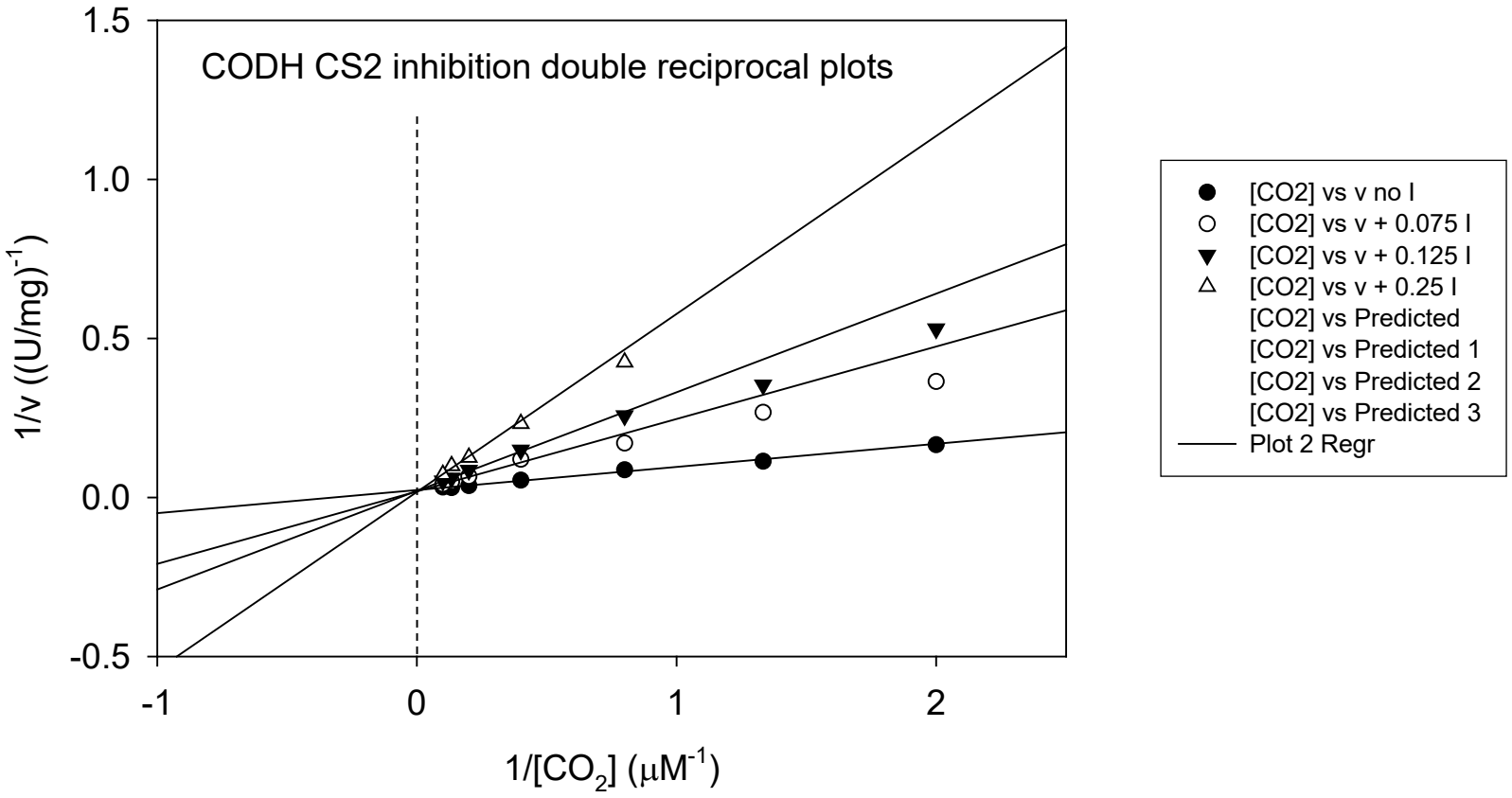
$$CO + H_2O + 2\,MV_{ox} \rightleftharpoons CO_2 + 2H^+ + 2MV_{red}$$

$$CO_2 + 2H^+ + 2MV_{red} \rightleftharpoons CO + H_2O + 2\,MV_{ox}$$



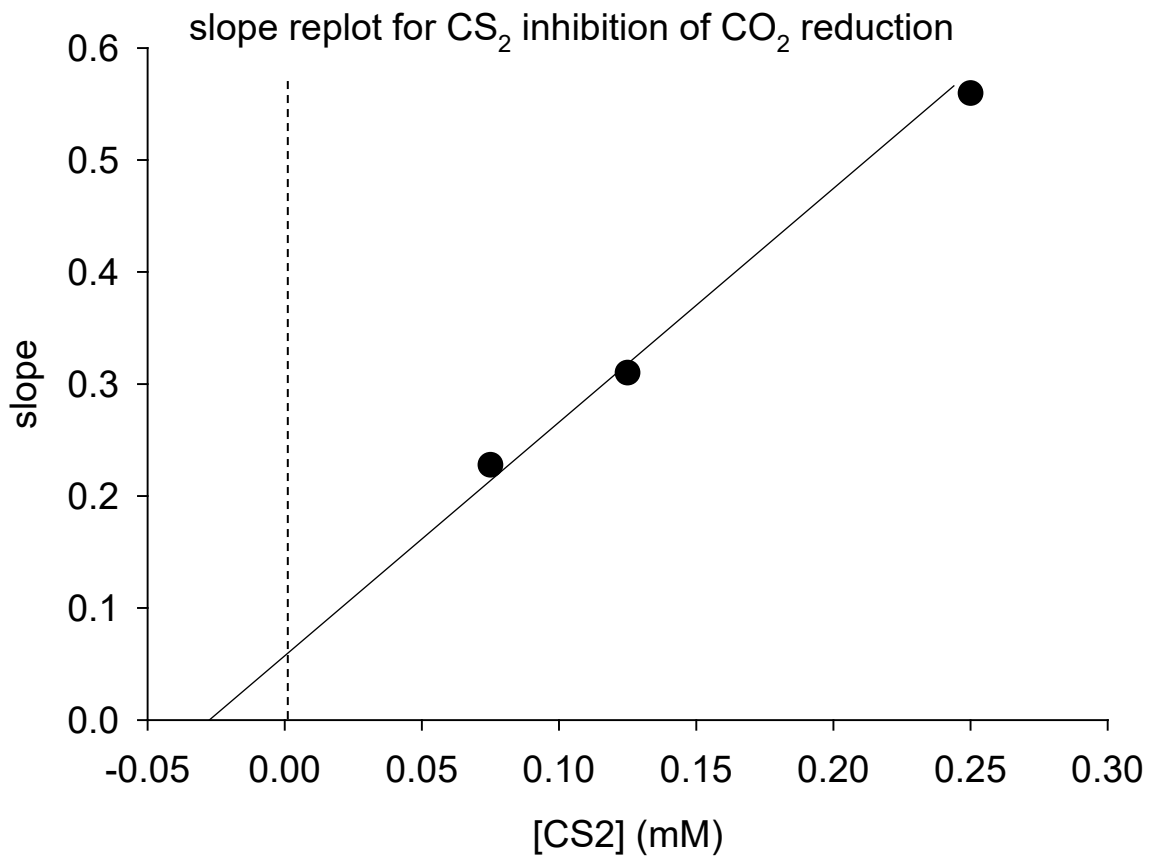
1-[CO2]	2-v no I	3-v + 0.075 I	4-v + 0.125 I	5-v + 0.25 I
0.0000	0.0000	0.0000	0.0000	0.0000
0.2500	2.9066			
0.5000	6.0368	2.7432	1.8863	
0.7500	8.8317	3.7408	2.8294	
1.2500	11.5147	5.8605	3.8904	2.3531
2.5000	18.5577	8.3544	6.7198	4.3140
5.0000	27.2775	15.0878	11.6712	7.9743
7.5000	32.4200	20.2002	16.3868	10.0659
10.0000	31.0785	24.1903	19.3341	13.9877

Reciprocal plots of CODH inhibition data



The straight lines are the linear regressions of the predicted data points obtained from the hyperbola regression fits of the original data. Note how data points nearer the origins (i.e. the larger numbers) are weighted greater than the points further from the origin (the smaller numbers)

Slope replot of CODH inhibition data



Analysis of inhibition data using nonlinear regression

$$v = \frac{V_{max}[S]}{K_m\left(1+\frac{[I]}{K_i}\right)+[S]}$$

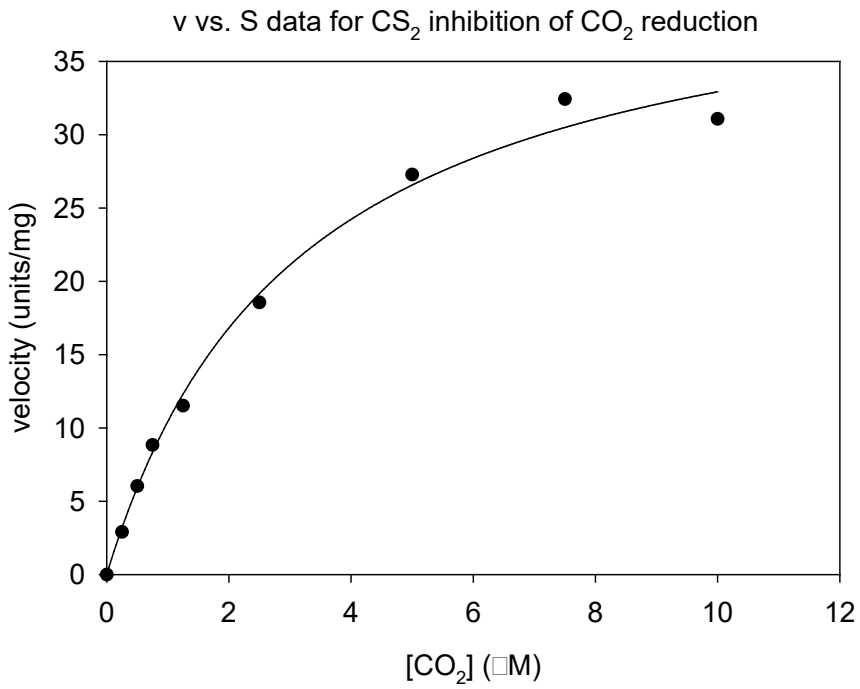
- Arrange data in 3 columns (S, I, and v)
- Formula: Y = a*x1/(b*(1+x2/c)+x1)
- Number of data points = (varies for data)
- number of independent variables = 2 (x1, x2)
  - x1 = [S]
  - x2 = [I]
- number of parameters = 3 (a, b, c)
  - a = Vmax
  - b = Km
  - c = Kis
- Y = velocity

x1	x2	Y
0	0	0
0.25	0	2.9066
0.5	0	6.0368
0.75	0	8.8317
1.25	0	11.5147
2.5	0	18.5577
5	0	27.2775
7.5	0	32.42
10	0	31.0785
0.5	0.075	2.7432
0.75	0.075	3.7408
1.25	0.075	5.8605
2.5	0.075	8.3544
5	0.075	15.0878
7.5	0.075	20.2002
10	0.075	24.1903
0.5	0.125	1.8863
0.75	0.125	2.8294
1.25	0.125	3.8904
2.5	0.125	6.7198
5	0.125	11.6712
7.5	0.125	16.3868
10	0.125	19.3341
1.25	0.25	2.3531
2.5	0.25	4.314
5	0.25	7.9743
7.5	0.25	10.0659
10	0.25	13.9877

Analysis of inhibition data using nonlinear regression

- You must put in good initial guesses using statpages.info fitting.
- Get the values of V_{max} (parameter a) and Km (parameter b) from the v vs S data where no I was present

[S]	v (no I)
0	0
0.25	2.9066
0.5	6.0368
0.75	8.8317
1.25	11.5147
2.5	18.5577
5	27.2775
7.5	32.42
10	31.0785



1. Enter the number of data points: 9
2. Enter the number of independent variables: 1 (1 for single substrate M-
3. Enter the number of parameters: 2 (deduce from form of M-M equation u
4. Enter the formula for the function to be fitted:
   $Y =$ a*x1/(b+x1)
   Use "" for multiplication and "/" for division when entering formulas. Define parameter.*
5. Type (or paste) the [x,y] data:

```
0       0
0.25    2.9066
0.5     6.0368
0.75    8.8317
1.25    11.5147
2.5     18.5577
5       27.2775
7.5     32.42
```

Data can be typed in directly, or pasted from a text file or spreadsheet. The first column(s

6. Enter initial guesses for the parameters from evaluation of the data (e.g. I

a (or p_1)=	35	±	, p=	
b (or p_2)=	2	±	, p=	
c (or p_3)=	0	±	, p=	

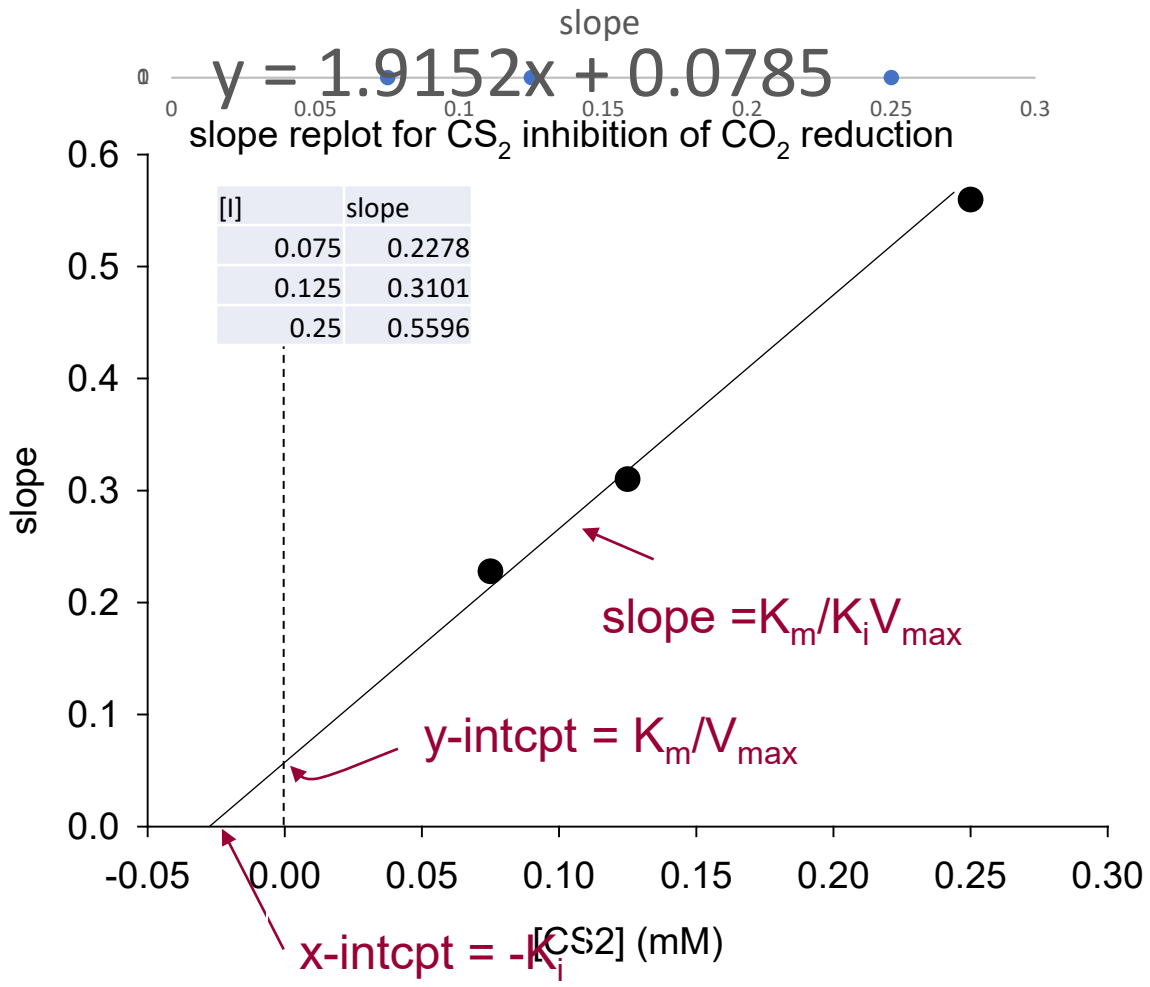
Iterate to convergence:

6. Enter initial guesses for the parameters from evaluation of the data (e

a (or p_1)=	43.28633202796	± 2.164841440176	, p=	5e-7
b (or p_2)=	3.148406172936	± 0.399801101918	, p=	0.000101
c (or p_3)=	0	± 1	, p=	1.000000

Analysis of inhibition data using nonlinear regression

- You must put in good initial guesses using statpages.info fitting.
- Get the values of V_{max} (parameter a) and Km (parameter b) from the v vs S data where no I was present (43.3 and 3.15 for this example)
- Get the value of K_{is} from the slope replot:



slope $= K_m/K_iV_{max}$;

$K_i = K_m/V_{max}$/slope = 3.15/43.3/1.9152 = 0.038

1. Enter the number of data points: 28
2. Enter the number of independent variables: 2 (1 for single substrate M-
3. Enter the number of parameters: 3 (deduce from form of M-M equation u
4. Enter the formula for the function to be fitted:
   $Y =$ a*x1/(b*(1+x2/c)+x1)
   Use "" for multiplication and "/" for division when entering formulas. Define parameters*
5. Type (or paste) the [x,y] data:

```
0       0       0
0.25    0       2.9066
0.5     0       6.0368
0.75    0       8.8317
1.25    0       11.5147
2.5     0       18.5577
5       0       27.2775
7.5     0       32.42
```

   Data can be typed in directly, or pasted from a text file or spreadsheet. The first column(s)
6. Enter initial guesses for the parameters from evaluation of the data (e.g. I
   a (or p_1)= 43.3 ± , p=
   b (or p_2)= 3.15 ± , p=
   c (or p_3)= 0.038 ± , p=

Analysis of two substrate kinetic data using nonlinear regression

Be sure to iterate until the values no longer change. Here is what you end up with. Compare these numbers to your initial guesses. The Ki value below is the one you report when you publish. Use the v vs S data without I to get the values of K_m and V_{max}.

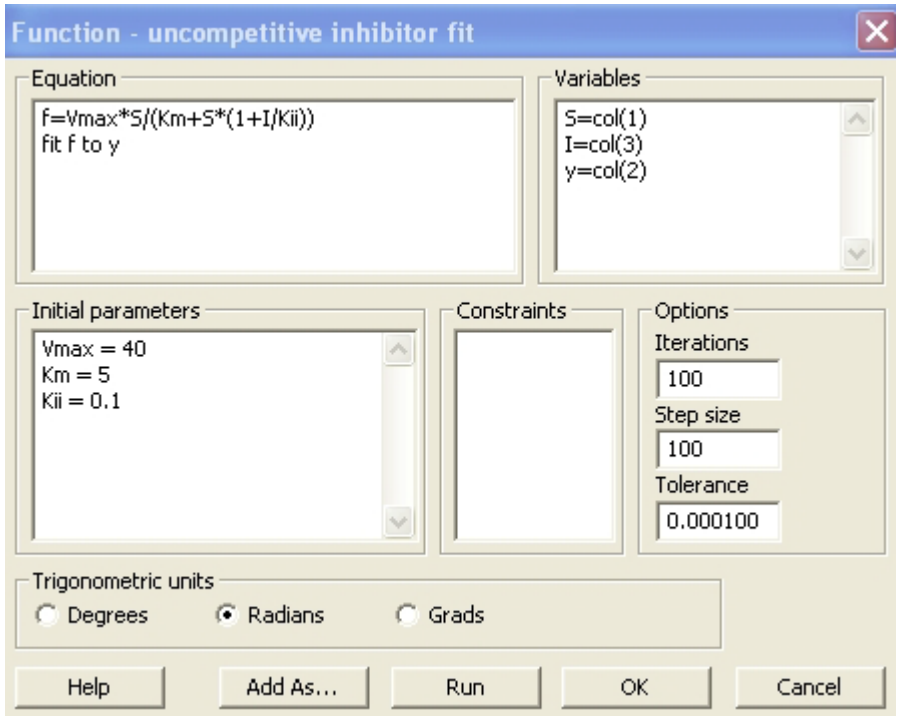
Enter initial guesses for the parameters from evaluation of the data (e.g

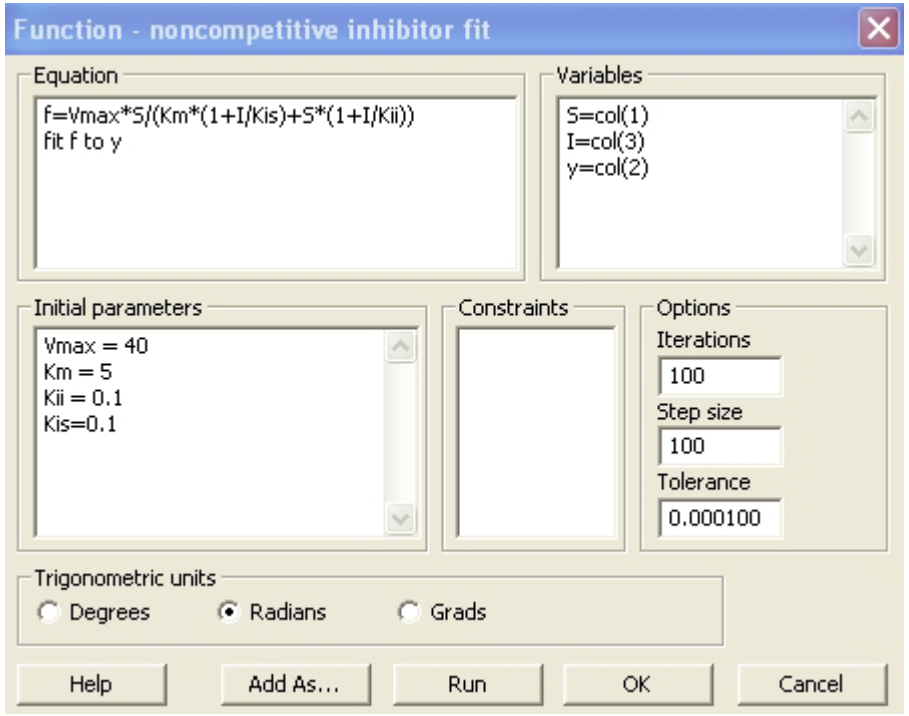
a (or p_1)=	44.08685834303	±	1.42017639482 6	, p=	5e-7
b (or p_2)=	3.277455055066	±	0.267717573812	, p=	5e-7
c (or p_3)=	0.042211321190	±	0.002739530755	, p=	5e-7
d (or p_4)=	0	±	1	p=	1.000000

- Inputs:
  - a = Vmax 43.3 ±
  - b = Km 3.15 ±
  - c = Kis ~0.038

- Numbers to report
  - a = Vmax 43.3 ± 2.16
  - b = Km 3.15 ± 0.40
  - c = Kis 0.0422 ± 0.0027

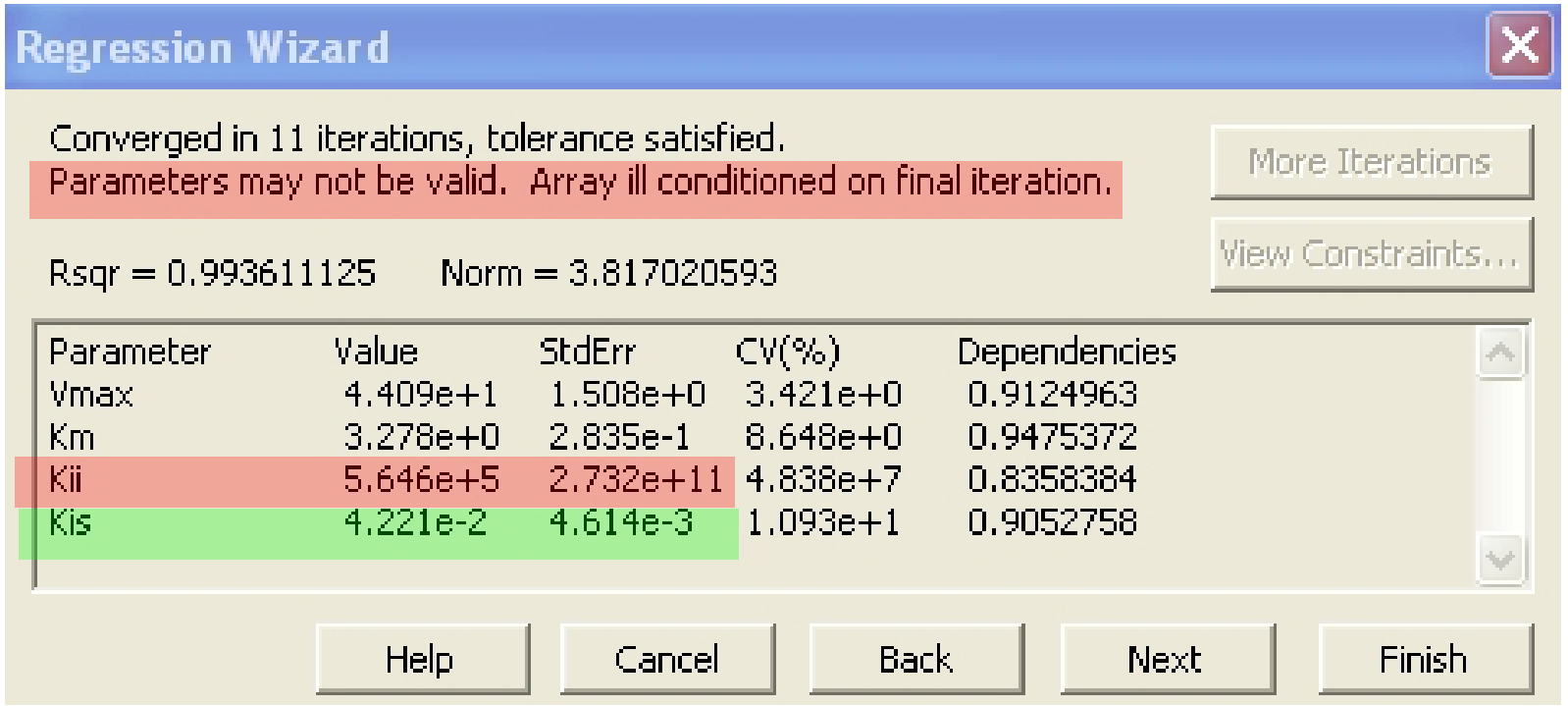
Analysis of inhibition data using Sigmaplot 9.0: equations for noncomp. and uncomp. inhibitors





Note: if you aren't sure what type of inhibition you have up front, use the noncompetitive inhibition fit, and examine the magnitudes of K_{is} and K_{ii} to give an indication of what type of inhibitor you are dealing with

Results of analyzing the "CS_2 inhibition of CODH" data using the noncompetitive inhibitor fit



The magnitudes and standard errors of the inhibition constants tell you whether they contribute to the type of inhibition

Product inhibition patterns for ordered and random sequential bisubstrate reactions

Mechanism	Substrate		Inhibitor	Pattern?
	Variable	Fixed		
Ordered	A	B (NS or S)	Q	C
Ordered	B	A (NS)	Q	NC
Ordered	B	A (S)	Q	None
Ordered	A	B (NS)	P	NC
Ordered	A	B (S)	P	UC
Ordered	B	A (NS or S)	P	NC
Random	A	B (NS)	Q	C
Random	A	B (S)	Q	None
Random	A	B (NS)	P	C
Random	A	B (S)	P	None

Ordered applies to both substrate binding and product release in this example.
A= first substrate to bind for the ordered mechanism
B=second substrate to bind for the ordered mechanism
P= first product released for the ordered mechanism
Q=second product released for the ordered mechanism
NOTE: these assignments of A, B, P, and Q apply only to the ordered mechanism.
NS = nonsaturating concentration of substrate
S = saturating concentration of substrate of interest