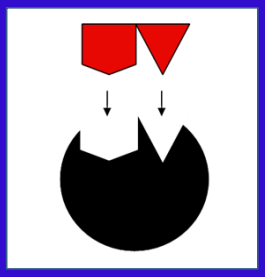
SCIENCE

Enzyme kinetics



Prof Peter O'Donoghue

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Chemical reactions

- molecular reactants collide
- bonds break, new bonds form
- catabolic (breakdown) vs anabolic (building)

$$A + B \leftrightarrows AB$$

Kinetics (rate of reaction) depends on:
- chemical nature of reactants
- physical nature of reactants
- concentration of reactants
- temperature
- presence of catalysts

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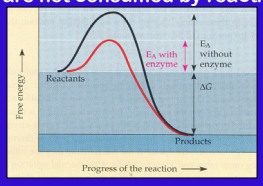
Catalysts

substances that change reaction rate
(by many orders of magnitude),
but are not consumed by reaction



catalysts in living systems = enzymes (proteins)
lower activation energy (so more collisions proceed to reactions)

activation energy for sucrose to glucose = 107 kJ/mol
saccharase lowers it to 36 kJ/mol, accelerates rate by ~10^{12}

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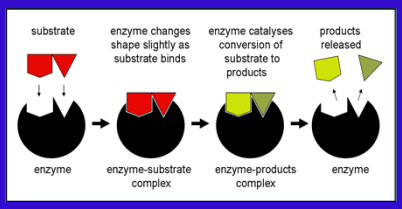
Enzymes

lower activation energy (so more collisions proceed to reactions)



active sites on folded proteins
'lock-and-key' model
'induced-fit' model

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Reaction kinetics

For the reaction: R → P

(reactant) (product)

reaction rate: $d[P]/dt = k[R]^n$

where constant k = proportionality (rate) constant
and exponent n = order of reaction
(must be determined experimentally)

NB: rate proportional to amount times constant

when n = 0 (zero-order); reaction rate = k
when n = 1 (first order); reaction rate = k[R]
when n = 2 (second order); reaction rate = $k[R]^2$

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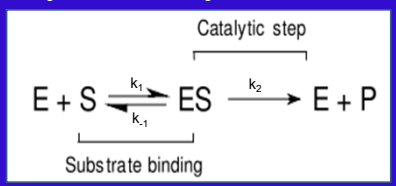
Enzyme kinetics

action of enzyme described by Michaelis-Menten equation



we want an equation for the rate of formation of P

$d[P]/dt = k_2[ES]$

but in terms of [E] and/or [S]
involves 2 steps to replace [ES]

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Enzyme kinetics

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

first step:

determine $d[ES]/dt$ = formation minus decay

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

which at steady state will = 0

so rearranges to

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

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Enzyme kinetics

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

second step:

determine $[E]_0$ = total concentration of enzyme (which is constant as enzyme not consumed)

$[E]_0 = [E] + [ES]$ rearranges to $[E] = [E]_0 - [ES]$

which is used to substitute [E] in eq. 1 to give

$$[ES] = \frac{k_1[E]_0[S]}{k_2 + k_{-1} + k_1[S]} \quad \text{.....2}$$

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Algebraic check

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

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

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

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

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

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

$$[ES] = \frac{k_1[E]_0[S]}{k_2 + k_{-1} + k_1[S]} \quad \text{.....2}$$

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Enzyme kinetics

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

remember $d[P]/dt = k_2[ES]$

substitute derived equation for [ES] from eq. 2 to give:

$$\frac{d[P]}{dt} = \frac{k_2k_1[E]_0[S]}{k_2 + k_{-1} + k_1[S]}$$

let $K_M = (k_2 + k_{-1})/k_1$ M-M constant (decay/formation)

gives $\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_M + [S]}$ M-M equation

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Enzyme kinetics

$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_M + [S]}$ M-M equation

says rate of product formation is dependent on:

- total enzyme concentration $[E]_0$
- substrate concentration [S]
- rate constants

values of rate constants k (thus K_M) need to be determined for each specific enzyme-substrate reaction

typically done by plotting reaction rates against [S]

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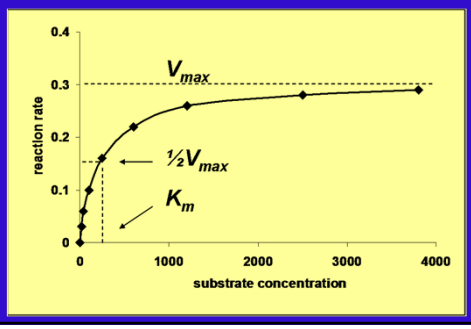
Enzyme kinetics

plots yield characteristic rectangular hyperbola shape



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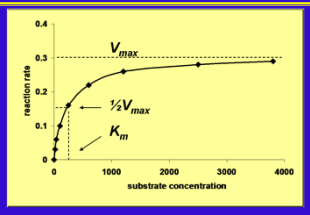
$$\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_M + [S]}$$ M-M equation



when [S] >> K_M, $d[P]/dt = (rate_{max}[S])/(K_M + [S])$
enzyme is saturated and rate is constant

when [S] << K_M, $d[P]/dt = (k_2[E]_0[S])/K_M$
rate depends on concentrations of both

when [S] = K_M, $d[P]/dt = (rate_{max})/2$
half maximum rate (= operational definition)

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Enzyme kinetics

Enzyme reactivity (catalytic power)
quantitated (and visualized) by three-step process:

1. measuring enzyme reactions against different substrate concentrations [*S*]
   (plot OD against time for each [*S*])
2. calculating average rates of reaction (= enzyme velocity, V_i)
   (plot V_i against [*S*])
3. determining Michaelis-Menten constant (K_m) which is substrate concentration required for enzyme to reach ½ maximum velocity (½ V_{max})
   (plot $1/V_i$ against $1/[S]$)

[= Lineweaver-Burk double reciprocal plot]

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Do an example!

Metabolism of alcohol by liver alcohol dehydrogenase
[classical ADH = EC 1.1.1.1]

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

ADH EtOH ADH acetaldehyde

reaction proceeds by reduction of cofactor NAD^+
(nicotinamide adenine dinucleotide) to NADH

Measure NADH by spectroscopy
UV absorbance (OD_{340nm})

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Step 1.

Set up experiment

Use single enzyme concentration (1/30 stock)

Add to five ethanol concentrations [5-80 mM]

Record $OD_{340\ nm}$ at 0, 15, 45, 75 secs

TUBE	1	2	3	4	5
[*S*]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)					
OD (t = 15 s)					
OD (t = 45 s)					
OD (t = 75 s)					

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Step 1.

Set up experiment

Use single enzyme concentration (1/30 stock)

Add to five ethanol concentrations [5-80 mM]

Record $OD_{340\ nm}$ at 0, 15, 45, 75 secs

TUBE	1	2	3	4	5
[*S*]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)	0	0	0	0	0
OD (t = 15 s)	0.10	0.12	0.15	0.18	0.20
OD (t = 45 s)	0.30	0.41	0.52	0.61	0.69
OD (t = 75 s)	0.50	0.69	0.88	1.03	1.13

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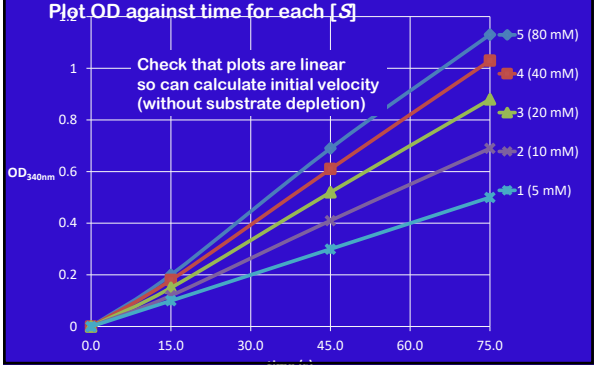
Plot 1.

Plot OD against time for each [*S*]



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Step 2.

Calculate average rate of reaction (initial velocity, V_i) expressed as change in OD per minute ($\Delta OD / \Delta t$)
= [OD at 75 sec - OD at 15 sec] / [1.25 – 0.25 min]

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)	0	0	0	0	0
OD (t = 15 s)	0.10	0.12	0.15	0.18	0.20
OD (t = 45 s)	0.30	0.41	0.52	0.66	0.78
OD (t = 75 s)	0.50	0.69	0.88	1.03	1.13
OD 75s – OD 15s					
V_i = ΔOD/min					

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Step 2.

Calculate average rate of reaction (initial velocity, V_i) expressed as change in OD per minute ($\Delta OD / \Delta t$)
= [OD at 75 sec - OD at 15 sec] / [1.25 – 0.25 min]

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)	0	0	0	0	0
OD (t = 15 s)	0.10	0.12	0.15	0.18	0.20
OD (t = 45 s)	0.30	0.41	0.52	0.66	0.78
OD (t = 75 s)	0.50	0.69	0.88	1.03	1.13
OD 75s – OD 15s	0.50-0.10	0.69-0.12	0.88-0.15	1.03-0.18	1.13-0.20
V_i = ΔOD/min	0.40	0.57	0.73	0.85	0.93

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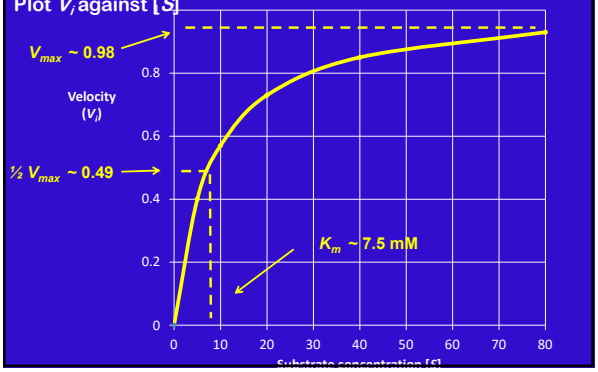
Plot 2.

Plot V_i against [S]



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Step 3.

Calculate reciprocals

Reciprocals of [S] = 1/[S]
Reciprocal of V_i = 1/ V_i

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
1/[S]					
V_i	0.40	0.57	0.73	0.85	0.93
$1/V_i$					

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Step 3.

Calculate reciprocals

Reciprocals of [S] = 1/[S]
Reciprocal of V_i = 1/ V_i

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
1/[S]	0.20	0.10	0.05	0.025	0.0125
V_i	0.40	0.57	0.73	0.85	0.93
$1/V_i$	2.50	1.75	1.37	1.18	1.08

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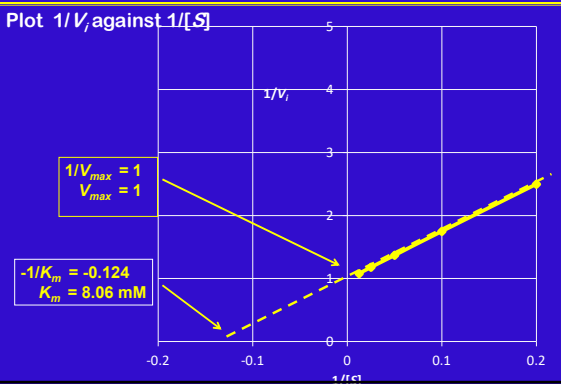
Plot 3.

Plot $1/V_i$ against 1/[S]



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Check

Rough plot: V_{max} = 0.98 ΔOD/min

K_m = 7.50 mM

Lineweaver-Burk double reciprocal plot:

V_{max} = 1.00 ΔOD/min

K_m = 8.06 mM

Good congruence!

Results applicable only to:

• this enzyme preparation/concentration/temperature/etc.

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So what?

It takes a substrate concentration of 8 mM to achieve one-half the maximum enzyme velocity

8 mM ethanol = 0.037% [legal limit = 0.05%]

this means that our alcohol detoxification system (enzyme catalyzed metabolic breakdown) is easily overwhelmed by one drink!

To get to legal limit of 0.05% (= 0.5 g/L),
0.7 of a 70 kg man = 50 L which holds 25 g alcohol
0.6 of a 50 kg woman = 30 L which holds 15 g alcohol
[applicable only to first hour!]

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So what?

Catalytic power substantially influenced by other reagents

Let's examine the effect of 2,2,2-trifluoroethanol (a solvent used in organic chemistry, fabric production and the pharmaceutical industry; toxic to humans)

Does it
• up-regulate (enhance) or
• down-regulate (inhibit) alcohol metabolism, and how?

Repeat the previous experiment, but add 2 mM TFE to each tube

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Step 1.

Use single enzyme concentration (1/30 stock)

Add to 5 ethanol concentrations [5-80 mM] + TFE [2 mM]

Record $OD_{340\ nm}$ at 0, 15, 45, 75 secs

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)	0	0	0	0	0
OD (t = 15 s)	0.03	0.06	0.09	0.11	0.15
OD (t = 45 s)	0.09	0.18	0.30	0.40	0.55
OD (t = 75 s)	0.14	0.30	0.48	0.70	0.95

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Plot 1.

Plot OD against time for each [S]

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Step 2.

Calculate average rate of reaction (initial velocity, V_i)

expressed as change in OD per minute (ΔOD / Δt)

= [OD at 75 sec - OD at 15 sec] / [1.25 – 0.25 min]

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
OD (t = 0 s)	0	0	0	0	0
OD (t = 15 s)	0.03	0.06	0.09	0.11	0.15
OD (t = 45 s)	0.09	0.18	0.30	0.40	0.51
OD (t = 75 s)	0.14	0.30	0.48	0.70	0.95
OD 75s – OD 15s	0.14-0.03	0.30-0.06	0.48-0.09	0.70-0.11	0.95-0.15
V_i = ΔOD/min	0.11	0.24	0.39	0.59	0.80

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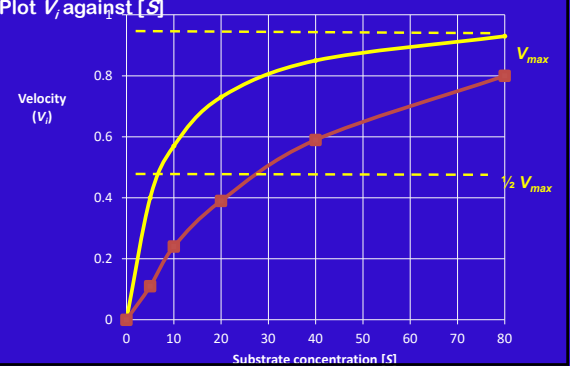
Plot 2.

Plot V_i against $[S]$



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Step 3.

Calculate reciprocals

Reciprocal of $[S] = 1/[S]$

Reciprocal of $V_i = 1/V_i$

TUBE	1	2	3	4	5
$[S]$	5 mM	10 mM	20 mM	40 mM	80 mM
$1/[S]$	0.20	0.10	0.05	0.025	0.0125
V_i	0.11	0.24	0.39	0.59	0.74
$1/V_i$	9.1	4.16	2.56	1.69	1.35

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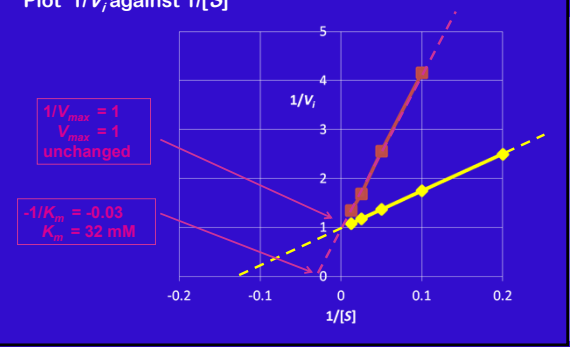
Plot 3.

Plot $1/V_i$ against $1/[S]$



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So what?

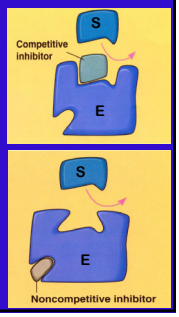
In the presence of TFE, it now takes a substrate concentration of 32 mM to achieve one-half the maximum enzyme velocity

(compared to 8 mM in absence of TFE)

TFE is therefore a potent inhibitor, but is it competitive or noncompetitive?

- competitive inhibitor involves direct binding to active site
- noncompetitive inhibitor involves binding elsewhere but changes active site

Lineweaver-Burk plots can differentiate these!



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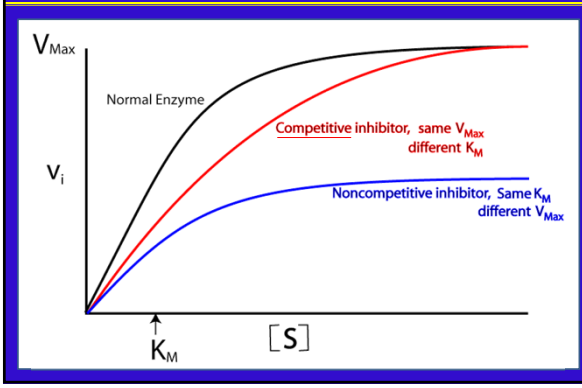
Competitive vs noncompetitive inhibition



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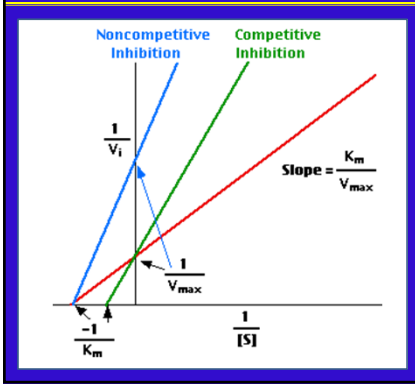
Competitive vs noncompetitive inhibition



Competitive inhibition

V_{max} unchanged

K_m changes

Noncompetitive inhibition

V_{max} changes

K_m unchanged

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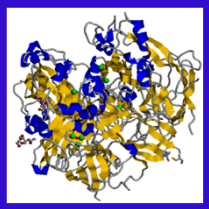
Enzyme kinetics

Enzymes are very potent catalysts

Need to understand their action



quantitative understanding of rates of reactions under wide variety of conditions

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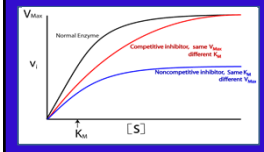
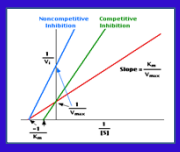
Questions?

Why would a double reciprocal plot turn a rectangular hyperbola into a straight line?





M-M equation $\frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{K_M + [S]}$

when enzyme is saturated {i.e. [S] >> K_M}, rate becomes constant,

$V = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_M + [S]}$

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Data transformation

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

take reciprocal of both sides

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

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

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

= equation for a straight line y = mx + c

where gradient (m) = $\frac{K_M}{V_{max}}$ and y intercept = $\frac{1}{V_{max}}$

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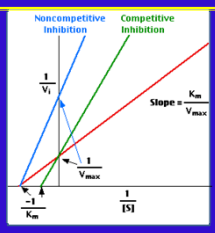
Intercept

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

gradient = $\frac{K_M}{V_{max}}$ y intercept = $\frac{1}{V_{max}}$



check y intercept, when x = 0; that is, when 1/[S] = 0

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

$$\frac{1}{V} = 0 + \frac{1}{V_{max}}$$

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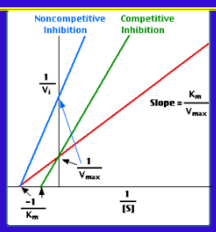
Intercept

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

gradient = $\frac{K_M}{V_{max}}$ y intercept = $\frac{1}{V_{max}}$



check x intercept, when y = 0; that is, when 1/V = 0

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

$$0 = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

$$\frac{K_M}{V_{max}[S]} = \frac{-1}{V_{max}}$$

$$\frac{1}{[S]} = \frac{-1}{K_M}$$

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Ain't maths great!!!

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Enzyme kinetics

blank worksheets

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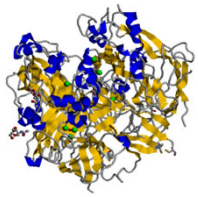
Enzyme kinetics

Enzymes are very potent catalysts

Need to understand their action



quantitative understanding of rates of reactions under wide variety of conditions

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Exemplar

Metabolism of alcohol by liver alcohol dehydrogenase [classical ADH = EC 1.1.1.1]

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

ADH EtOH ADH acetaldehyde

reaction proceeds by reduction of cofactor nicotinamide adenine dinucleotide NAD^+ to NADH (measured by OD_{340nm})

react ADH against doubling dilutions of EtOH in 5 tubes

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Table 1.

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
$OD_{(t = 0 s)}$	0	0	0	0	0
$OD_{(t = 15 s)}$	0.10	0.12	0.15	0.18	0.20
$OD_{(t = 45 s)}$	0.30	0.41	0.52	0.66	0.78
$OD_{(t = 75 s)}$	0.50	0.69	0.88	1.03	1.13
$OD_{(75s)} - OD_{(15s)}$					
V_i					
$1/V_i$					
1/[S]					

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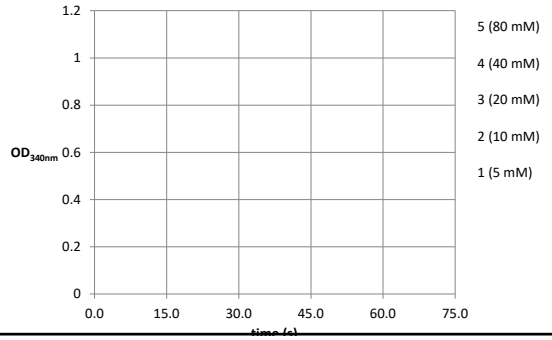
Plot 1A.

Plot OD against time for each [*S*]



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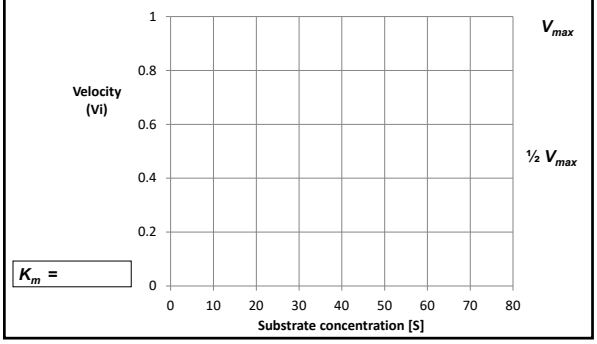
Plot 1B.

Plot V_i against [*S*]



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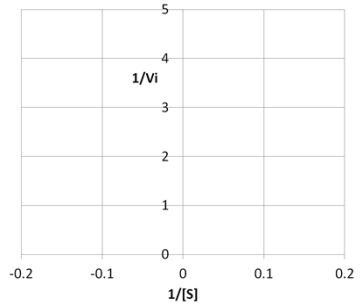
Plot 1C.

Plot $1/V_i$ against $1/[S]$

$1/V_{max}$ =
V_{max} =

$-1/K_m$ =
K_m =



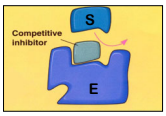
49

Inhibitors (competitive vs noncompetitive)

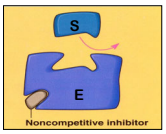
Catalytic power substantially influenced by other reagents

Examine effect of solvent TFE (2,2,2-trifluoroethanol) on ADH metabolic breakdown of EtOH

Does it:
- compete for active sites on ADH?
- change ADH active sites?



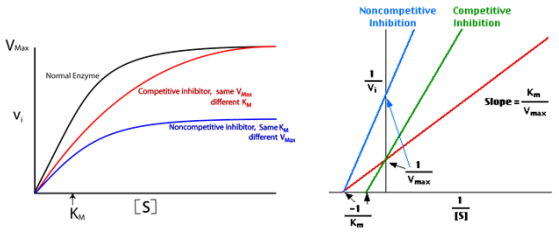
Repeat the previous experiment, but add 2 mM TFE to each tube



50

Inhibitors (competitive vs noncompetitive)

Lineweaver-Burk plots can differentiate between competitive and noncompetitive inhibition



51

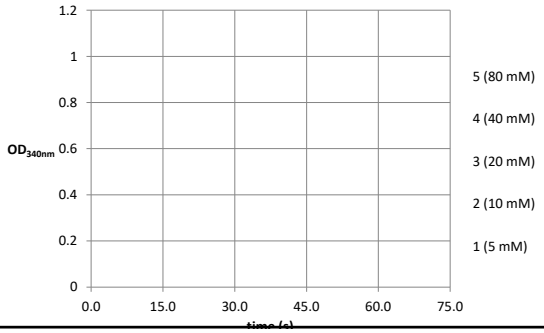
Table 2.

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
$OD_{(t = 0 s)}$	0	0	0	0	0
$OD_{(t = 15 s)}$	0.03	0.06	0.09	0.11	0.15
$OD_{(t = 45 s)}$	0.09	0.18	0.30	0.40	0.51
$OD_{(t = 75 s)}$	0.14	0.30	0.48	0.70	0.95
$OD_{(75s)} - OD_{(15s)}$					
V_i					
$1/V_i$					
1/[S]					

52

Plot 2A.

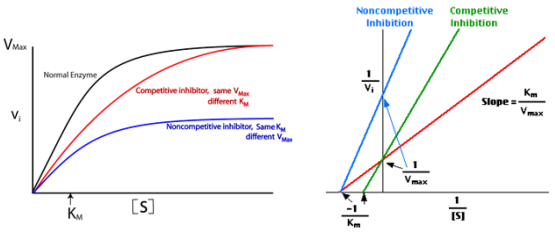
Plot OD against time for each [S]



53

Inhibitors (competitive vs noncompetitive)

Lineweaver-Burk plots can differentiate between competitive and noncompetitive inhibition



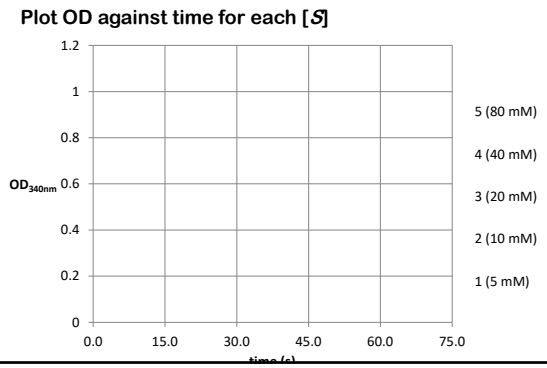
54

Table 2.

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
$OD_{(t = 0 s)}$	0	0	0	0	0
$OD_{(t = 15 s)}$	0.03	0.06	0.09	0.11	0.15
$OD_{(t = 45 s)}$	0.09	0.18	0.30	0.40	0.51
$OD_{(t = 75 s)}$	0.14	0.30	0.48	0.70	0.95
$OD_{(75s)} - OD_{(15s)}$					
V_i					
$1/V_i$					
1/[S]					

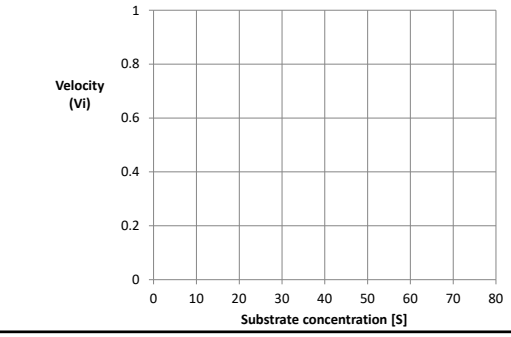
55

Plot 2A.



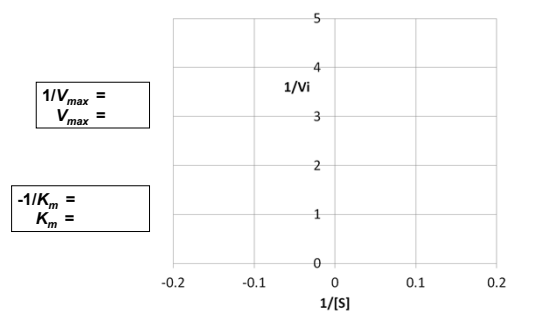
56

Plot 2B.



57

Plot 2C.



58

59

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Enzyme kinetics

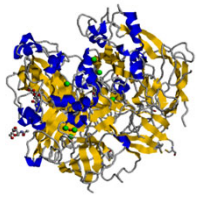
completed worksheet

61

Enzyme kinetics

Enzymes are very potent catalysts

Need to understand their action



quantitative understanding of rates of reactions under wide variety of conditions

62

Exemplar

Metabolism of alcohol by liver alcohol dehydrogenase [classical ADH = EC 1.1.1.1]

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

ADH EtOH ADH acetaldehyde

reaction proceeds by reduction of cofactor nicotinamide adenine dinucleotide NAD^+ to NADH (measured by OD_{340nm})

react ADH against doubling dilutions of EtOH in 5 tubes

63

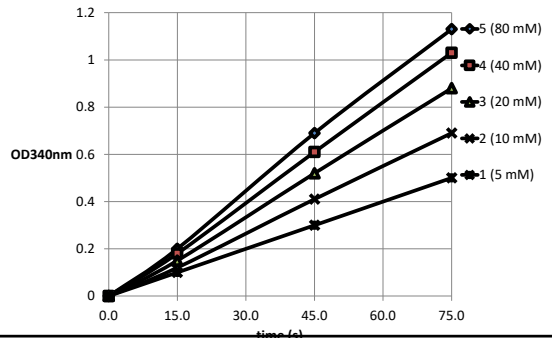
Table 1.

TUBE	1	2	3	4	5
[S]	5 mM	10 mM	20 mM	40 mM	80 mM
$OD_{(t = 0 s)}$	0	0	0	0	0
$OD_{(t = 15 s)}$	0.10	0.12	0.15	0.18	0.20
$OD_{(t = 45 s)}$	0.30	0.41	0.52	0.66	0.78
$OD_{(t = 75 s)}$	0.50	0.69	0.88	1.03	1.13
$OD_{(75s)} - OD_{(15s)}$	0.50-0.10	0.69-0.12	0.88-0.15	1.03-0.18	1.13-0.20
V_i	0.40	0.57	0.73	0.85	0.93
$1/V_i$	2.50	1.75	1.37	1.18	1.08
1/[S]	0.20	0.10	0.05	0.025	0.0125

64

Plot 1A.

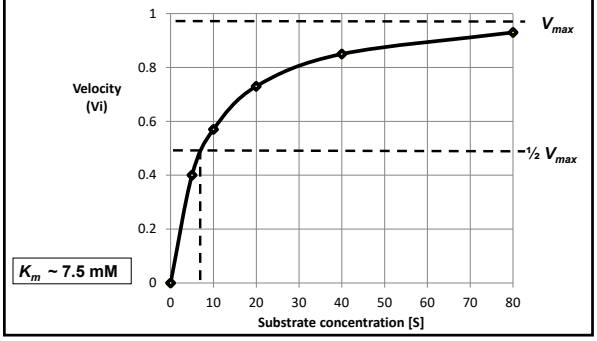
Plot OD against time for each [*S*]



65

Plot 1B.

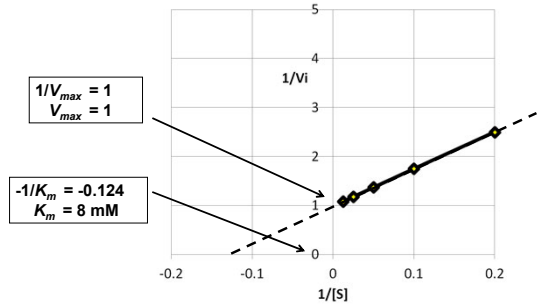
Plot V_i against [*S*]



66

Plot 1C.

Plot $1/V_i$ against $1/[S]$



67

Inhibitors (competitive vs noncompetitive)

Catalytic power substantially influenced by other reagents

Examine effect of solvent TFE (2,2,2-trifluoroethanol) on ADH metabolic breakdown of EtOH

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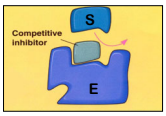
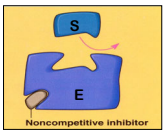
- change ADH active sites?



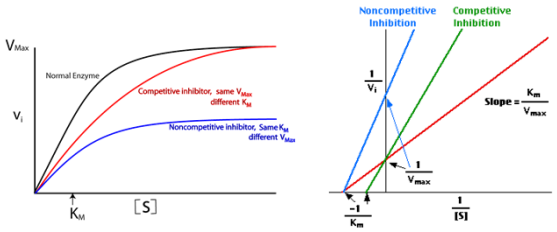
Repeat the previous experiment, but add 2 mM TFE to each tube



68

Inhibitors (competitive vs noncompetitive)

Lineweaver-Burk plots can differentiate between competitive and noncompetitive inhibition



69

Table 2.

TUBE	1	2	3	4	5
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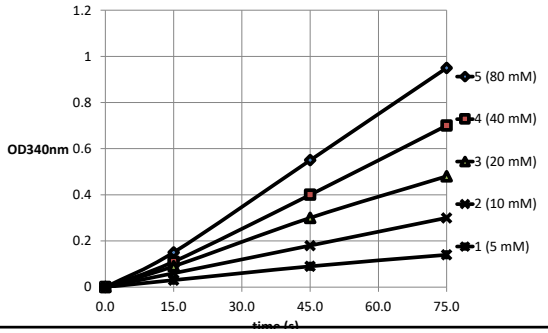
$OD_{(t = 0 s)}$	0	0	0	0	0
$OD_{(t = 15 s)}$	0.03	0.06	0.09	0.11	0.15
$OD_{(t = 45 s)}$	0.09	0.18	0.30	0.40	0.51
$OD_{(t = 75 s)}$	0.14	0.30	0.48	0.70	0.95
$OD_{(75s)} - OD_{(15s)}$	0.14-0.03	0.30-0.06	0.48-0.09	0.70-0.11	0.95-0.15
V_i	0.11	0.24	0.39	0.59	0.80
$1/V_i$	9.1	4.16	2.56	1.69	1.35
1/[S]	0.20	0.10	0.05	0.025	0.0125

70

Plot 2A.

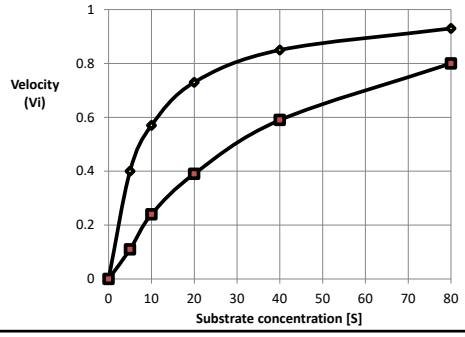
Plot OD against time for each $[S]$



71

Plot 2B.

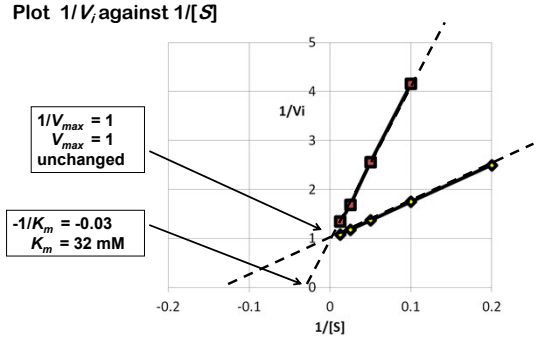
Plot V_i against $[S]$



72

Plot 2C.

Plot $1/V_i$ against $1/[S]$



$1/V_{max}$ = 1
V_{max} = 1
unchanged

$-1/K_m$ = -0.03
K_m = 32 mM

73