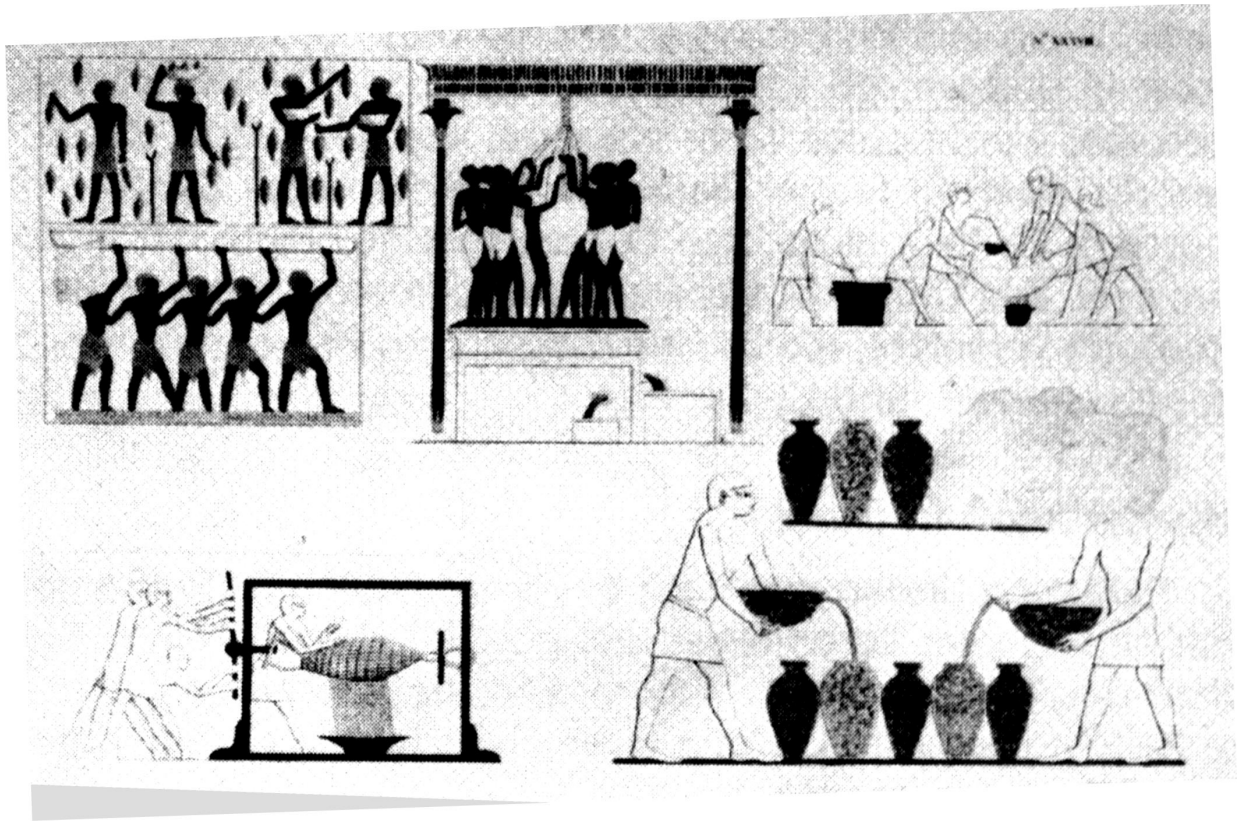
Enzyme Reaction Kinetics

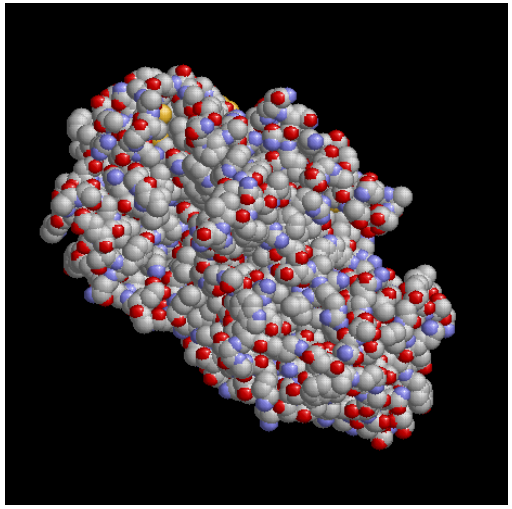
Egyptian Wine-Making

- Crushing grapes, filtration, fermentation
- Mysterious bubbling and gas evolution
- ‘Magical’ transformation of sweetness to alcohol
- ‘Essential’ characteristic of living materials



Binding and Reaction

- Enzymes catalyze reactions by increasing rate approx. 10^3-10^{10}
- Decrease activation energy barrier by restricting possible conformational structures of substrate (binding)
- Place reactive groups in proximity to substrate to enhance reaction
- Enhance release of product/binding of new substrate



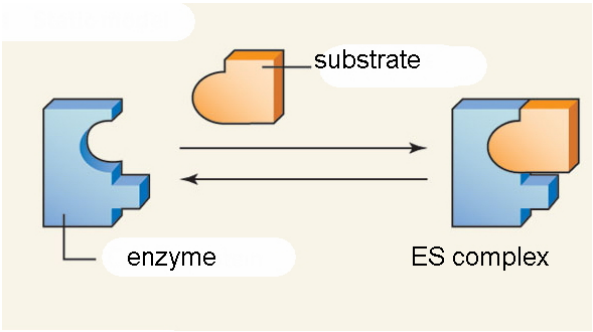
3D computer model of enzyme

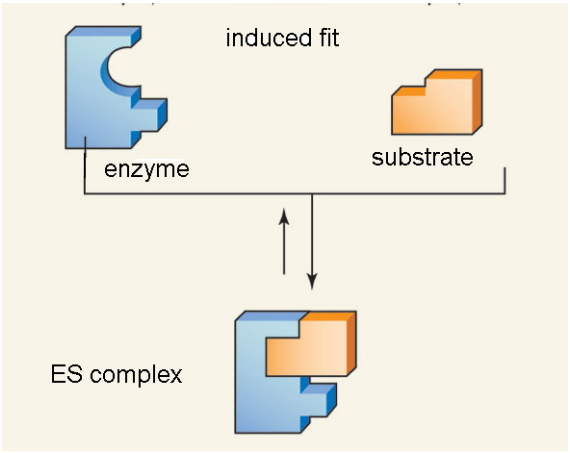
Binding concepts

1920's Emil Fischer - key-lock analogy (binding site)

1960's Daniel Koshland - glove-hand analogy (induced fit)

Binding is complex, 3D fit of substrate to enzyme, due to a combination of hydrophobic, ionic and hydrogen bonding mechanisms





Binding concepts

Conformational changes are major issues in enzymatic performance, both in substrate binding and product release

Effectors of conformational changes may be substrate/product or other molecules (inhibitors/activators)

Due to multiple binding sites on enzyme/ protein structure (allosteric activity)

Development of the Kinetic Reaction model for Enzymes

- 1850 Ludwig Wilhelmy noted 1st order reaction kinetics for acid hydrolysis of sucrose
- S->P

1st order kinetics

rate of reaction

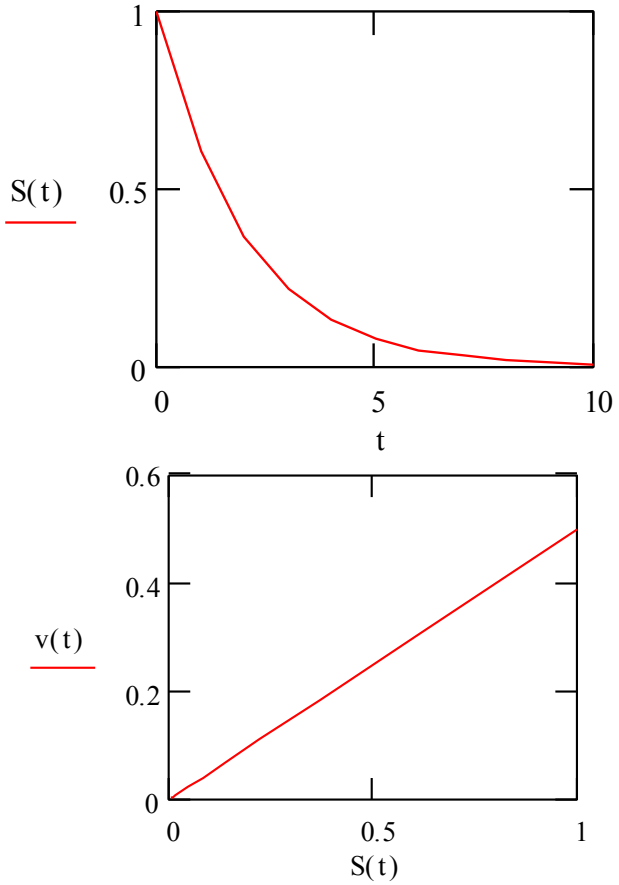
v= -dS/dt = k*S

S(t)=exp(-kt)

k=reaction constant

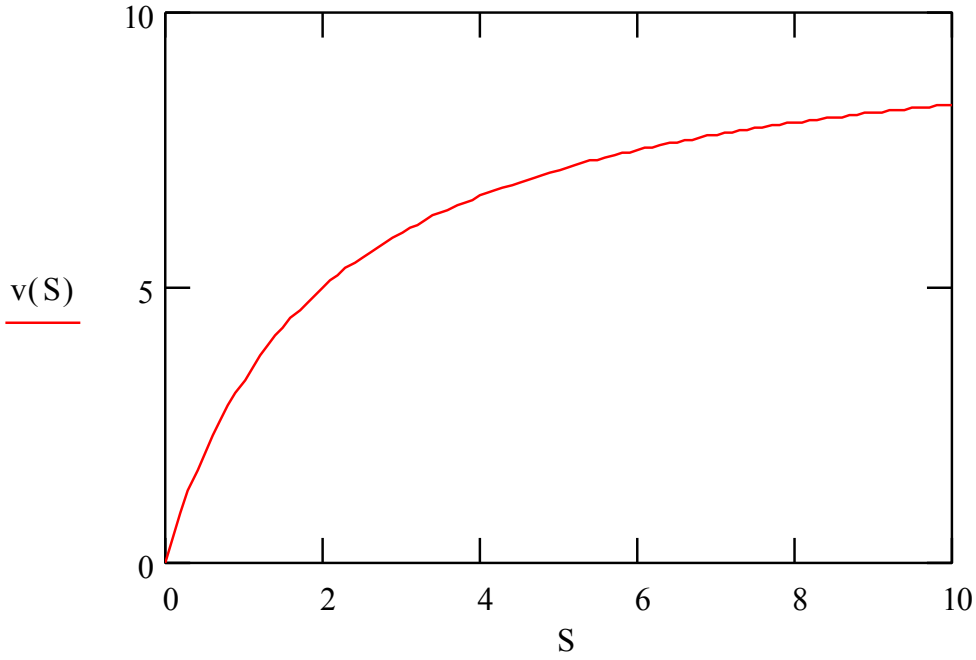
S = substrate

t = time



Development of the Kinetic Reaction model for Enzymes

- Victor Henri and Adrian Brown (1902) examined kinetics of invertase on sucrose
- Found at low [S] the reaction was 1st order
- But at high [S], the reaction was zero order
- E+S->ES->EP->E+P



Michealis and Menten

- Leonor Michaelis and Maud Menten derived the mathematical relationship
- d[S]/dt = A*[S]/(B+[S])
- Michaelis-Menten equation

Michealis and Menten equation derivation

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

$k_1[E][S] = k_{-1}[ES]$ (equilibrium assumption)

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

Michealis and Menten equation derivation

$[E][S]/[ES] = k_{-1}/k_1 = K_s$

Mass balance on enzyme

$[E]=[E_0]-[ES]$

Plugging into equilibrium assumption

$([E_0]-[ES])[S]/[ES] = K_s$

Re-arranging,

$[ES]=[E_0][S]/(K_s+[S])$

Michealis and Menten equation derivation

$[ES]=[E_0][S]/(K_s+[S])$

From the 2nd reaction,

$v=k_2[ES]$

$= ks[E_0][S]/(K_s+[S])$

$= V_m[S]/(K_m+[S])$ $\quad Vm=k_2[E_0]$

$K_m=K_S$

$v=-d[S]/dt=d[P]/dt$

Problems

- M-M equation fit the observed data
- Problem with equilibrium assumption
  - k_1/k_{-1} reaction cannot be at equilibrium since k_2 reaction occurs or k_2 reaction must be very slow by comparison
  - ES not detectable

Briggs-Haldane analysis

Instead of equilibrium, assume steady state for ES

$d[ES]/dt = 0$

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

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

Re-arranging,

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

Briggs-Haldane analysis

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

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

Mass balance on enzyme

$[E] = [E_0] - [ES]$

$[ES] = k_1([E_0] - [ES])[S]/(k_{-1} + k_2)$

Re-arranging for [ES],

$[ES] = [E_0][S]/\{(k_{-1} + k_2)/k_1 + [S]\}$

Briggs-Haldane analysis

$[ES] = [E_0][S]/\{(k_{-1} + k_2)/k_1 + [S]\}$

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

$= [E_0][S]/\{K_M + [S]\}$

Recall the $v = k_2[ES]$, so

$v = k_2 [E_0][S]/\{K_M + [S]\}$ define $V_M = k_2 [E_0]$

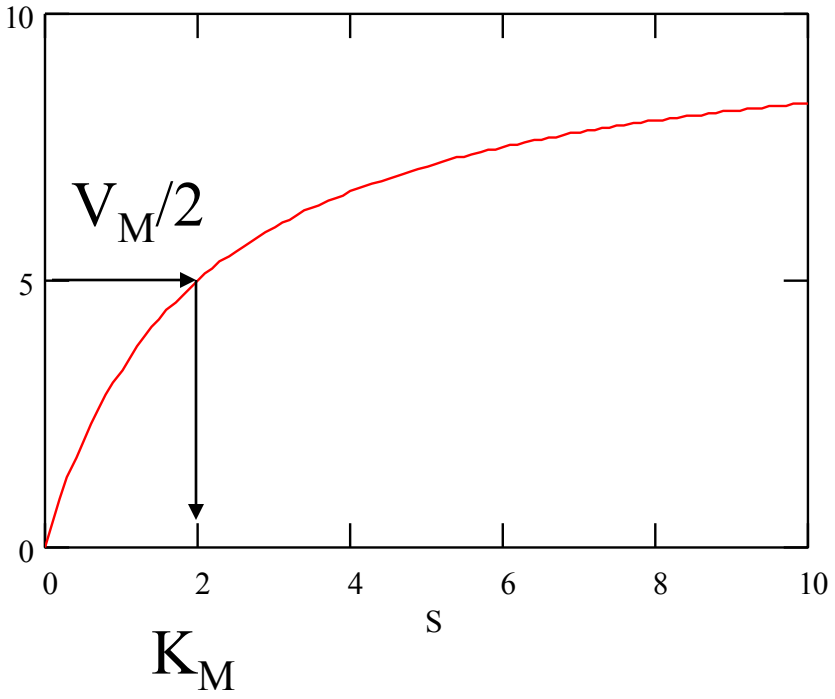
$v = V_M[S]/(K_M + [S])$

$v=V_M[S]/(K_M+[S])$

$V_M = k_2[E_0]$, maximum rate of reaction, when all enzyme molecules are making product

K_M = Michaelis constant, measurement of binding affinity (low K_M=strong binding affinity)

also corresponds to value of [S] where $v=V_M/2$



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

$K_M = (k_{-1} + k_2)/k_1$ (Why does this measure binding?)

Note that low K_M means $k_1 > (k_{-1} + k_2)$
or that the enzyme strongly binds the substrate,
whereas high K_M means $(k_{-1} + k_2) > k_1$
or the enzyme prefers to release/react the substrate
K_M values usually in range of 10^{-2} to 10^{-6} mol/L

$v=V_M[S]/(K_M+[S])$

When $K_M >> [S]$
(low substrate concentration),
$v=V_M/K_M [S] = k[S]$
e.g. a 1st order reaction
When $[S]>>K_M$
(high substrate concentration),
$v=V_M[S]/[S] = V_M$
eg. a zero order reaction