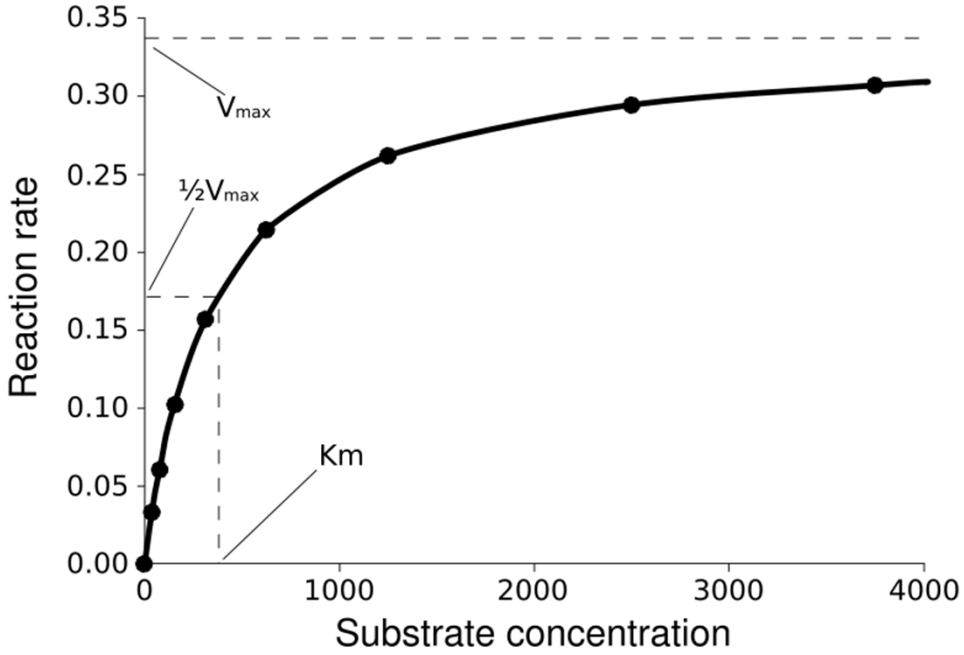
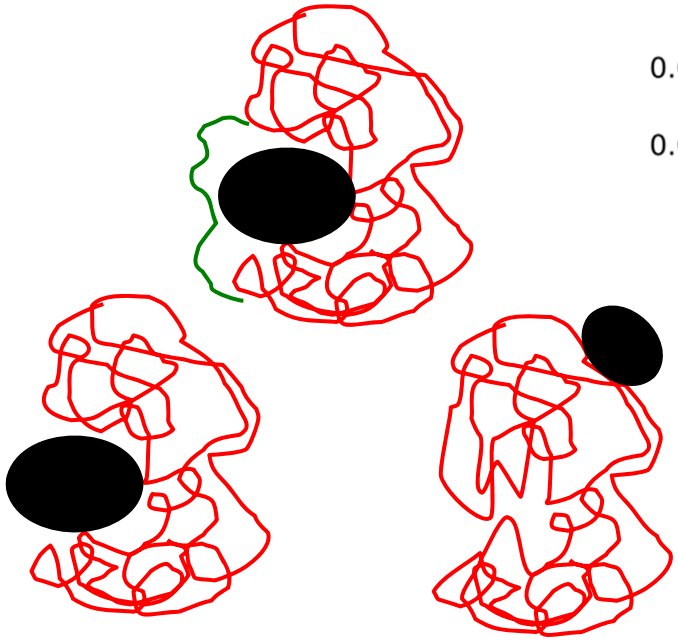
Enzyme Regulation and Mechanisms

Leonor Michaelis
1875–1949

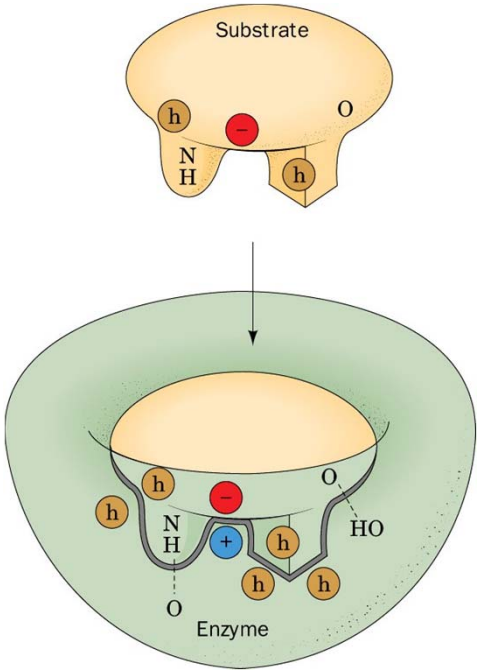
Maud Menten
1879–1960





$$\frac{d[P]}{dt} = k_2[E_0]\frac{[S]}{K_m + [S]} = V_{\max}\frac{[S]}{K_m + [S]}$$

Last Week...


Substrate
h
O
N
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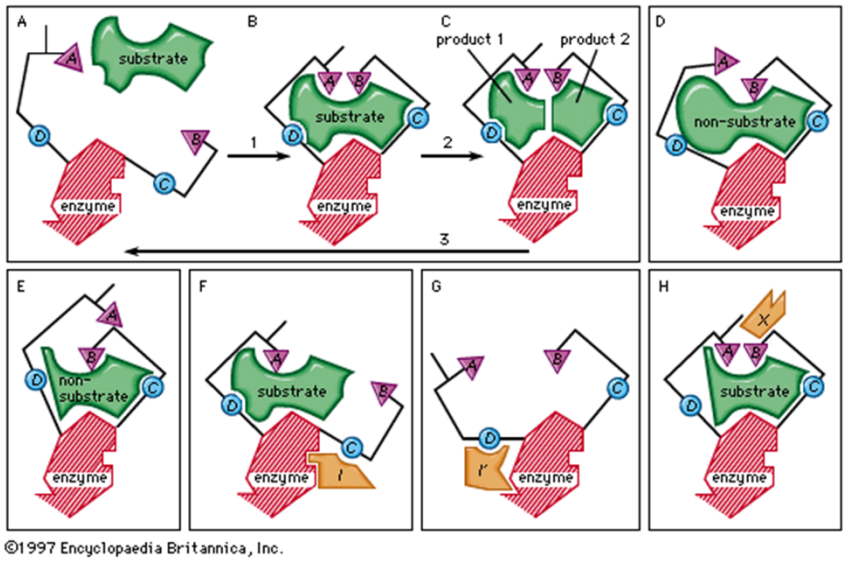
h
Enzyme
HO


A
substrate
B
C
product 1
product 2
D
non-substrate
enzyme
1
2
3
E
non-substrate
F
substrate
G
I'
H
X
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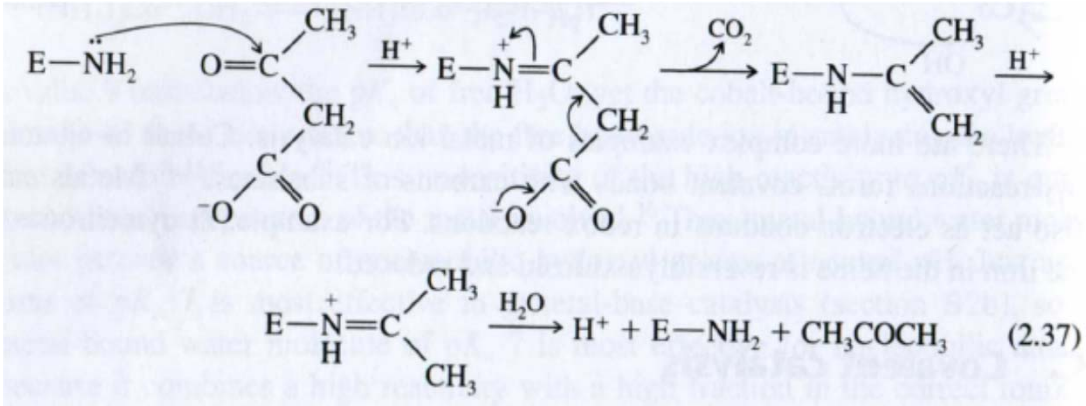
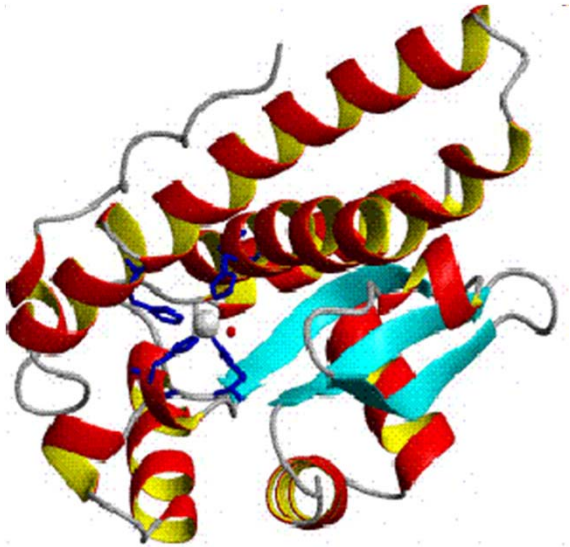

E—NH2
O=C
CH3
CH2
H+
CO2
E—N—C
H
CH2
⁻O
O
H2O
H+ + E—NH2 + CH3COCH3
(2.37)



Enzymes *in vitro*

- Ok, so we know a few tricks that enzymes use to catalyze reactions by stabilizing the transition state.

- But how do we uncover these mechanisms?

- How do we figure out their fundamental properties as enzymes?

We'd better purify the enzyme, get it into a test tube and study it in vitro!

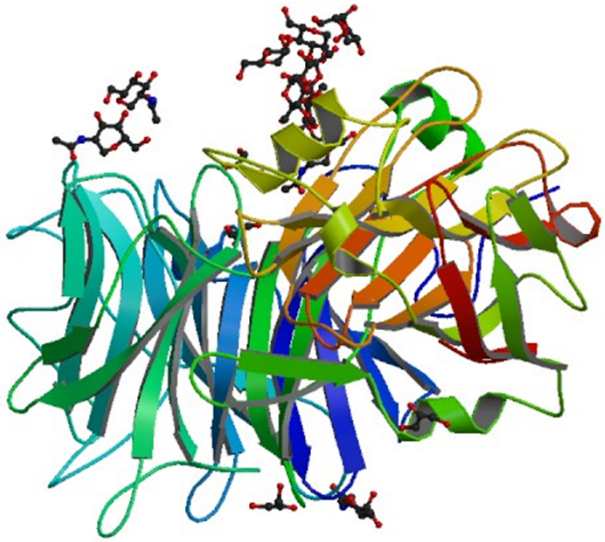
- Enter these two characters:

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

Biochemische Zeitschrift **49**, 333 (1913)

Michaelis-Menten Kinetics



Invertase: Hydrolyzes β-D-Fructose

Now in any moment according to the law of mass action

(1) $S(\Phi - \varphi - u_1 - u_2) = k\varphi$

(2) $F(\Phi - \varphi - u_1 - u_2) = k_1 u_1$

(3) $G(\Phi - \varphi - u_1 - u_2) = k_2 u_2$

(4) $\varphi = S(\Phi - u_1 - u_2)/(S + k)$

From (1) it follows

(4) $\varphi = S(\Phi - u1 - u2)/(S + k)$

We can eliminate u_1 and u_2 if we first find by division of (2) and (3):

$$u_2 = (k_1/k_2)\cdot u_1$$

and further by division of (1) and (3)

$$u_1 = (k/k_1)\cdot\varphi\cdot(F/S)$$

so that

$$u_1 + y_2 = k\cdot\varphi\cdot(F/S)(1/k_1 + 1/k_2)$$

Let us refer next to the abbreviation

$$1/k_1 + 1/k_2 = q$$

so that

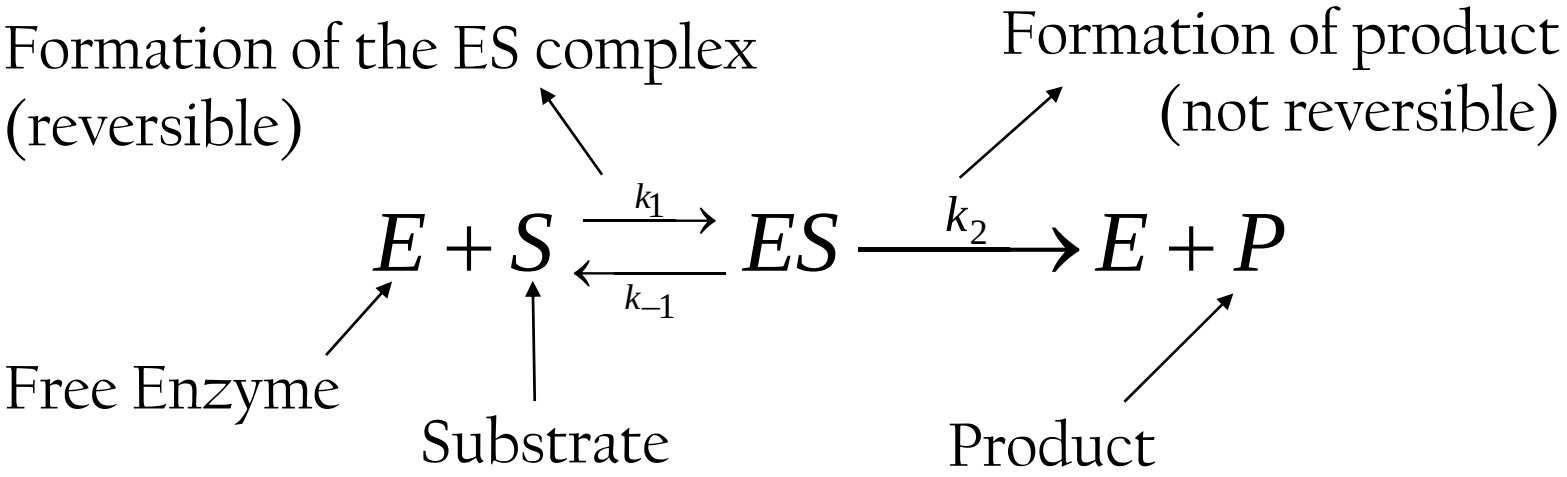
$$u_1 + y_2 = k\cdot q\cdot\varphi F/S$$

This gives, substituted in (4) and solved for φ,

(4) $\varphi = \Phi.S/[S + k(1+qF)]$

The Michaelis-Menten Equation Explained

- Assumption 1: All enzyme reactions occur via the following steps:



This system is described by the following set of differential equations:

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

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

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

The Michaelis-Menten Equation Explained

Assumption 2: There is always a huge amount of S compared to E, *i.e.* [S]››[E]

Assumption 3: The E+S / ES equilibrium is established before we start watching the reaction

- Together, these assumptions imply that the enzyme/substrate complex concentration, [ES], is constant!

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

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

- Rearrange in terms of [ES]:

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

- This is the Steady State Assumption (Briggs-Haldane)

The Michaelis-Menten Equation Explained

- Our steady state equation contains a term that we don't like: We don't know the concentration of free enzyme, [E].

- We get rid of [E] by describing it in terms of the amount of enzyme we started with: $[E]=[E_0]-[ES]$

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

- Our rate terms are annoying. Lets combine them into a prettier, single term:

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

$$[ES]=\frac{([E_0]-[ES])[S]}{K_M}$$

The Michaelis-Menten Equation Explained

- Looking better, but there's still an ugly spot: We have two [ES] terms. We want just one. So we have to factor it out!

$$[ES] = \frac{([E_0] - [ES])[S]}{K_M} \longrightarrow [ES] = [E]_0 \frac{[S]}{K_M + [S]}$$

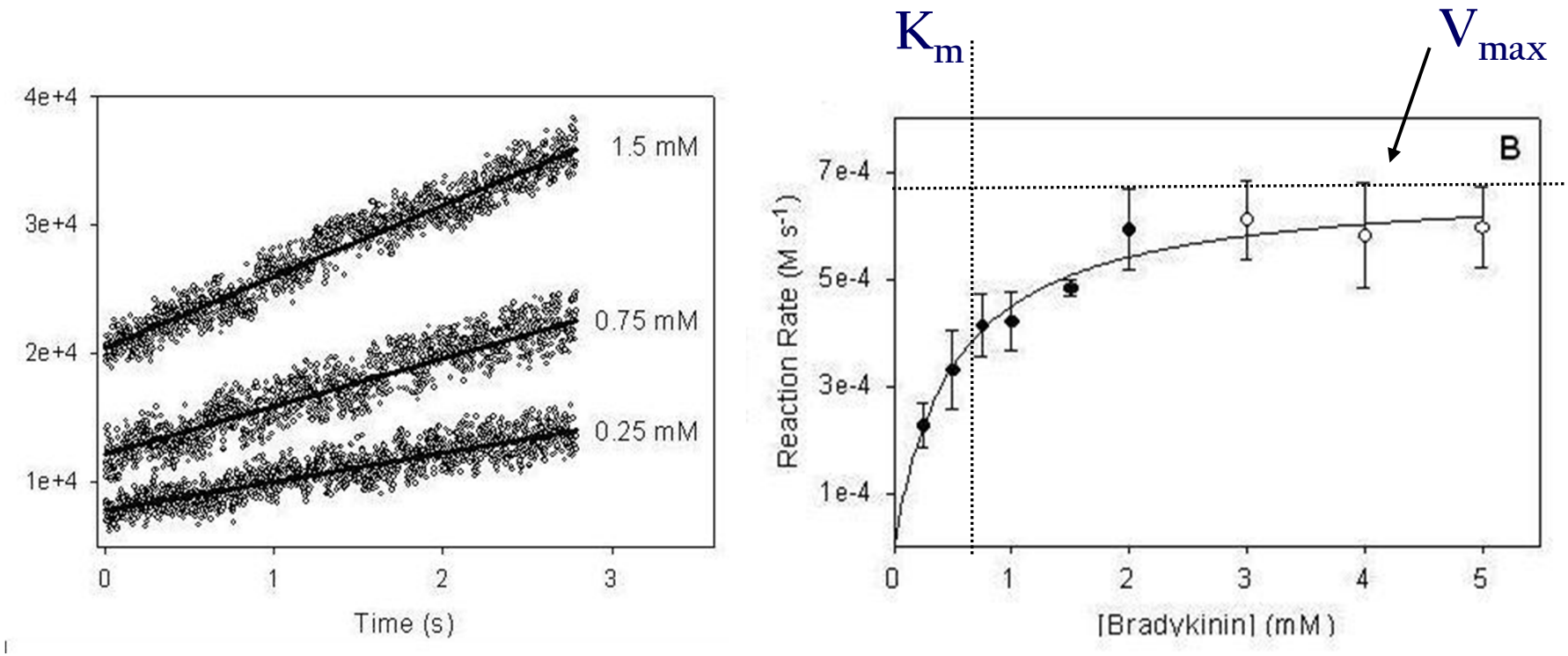
- So we can now describe [ES] in terms of things that we know ($[E]_0$ and [S]). But we're not going to be watching [ES]!! We'll be watching [P]!

- Fortunately: $\frac{d[P]}{dt} = V = k_2[ES]$

-Subbing in: $V = k_2[E]_0 \frac{[S]}{K_M + [S]} = \frac{V_{max}[S]}{K_M + [S]}$

Michaelis-Menten Kinetics

- So what happens if you monitor d[P]/dt at different [S]…



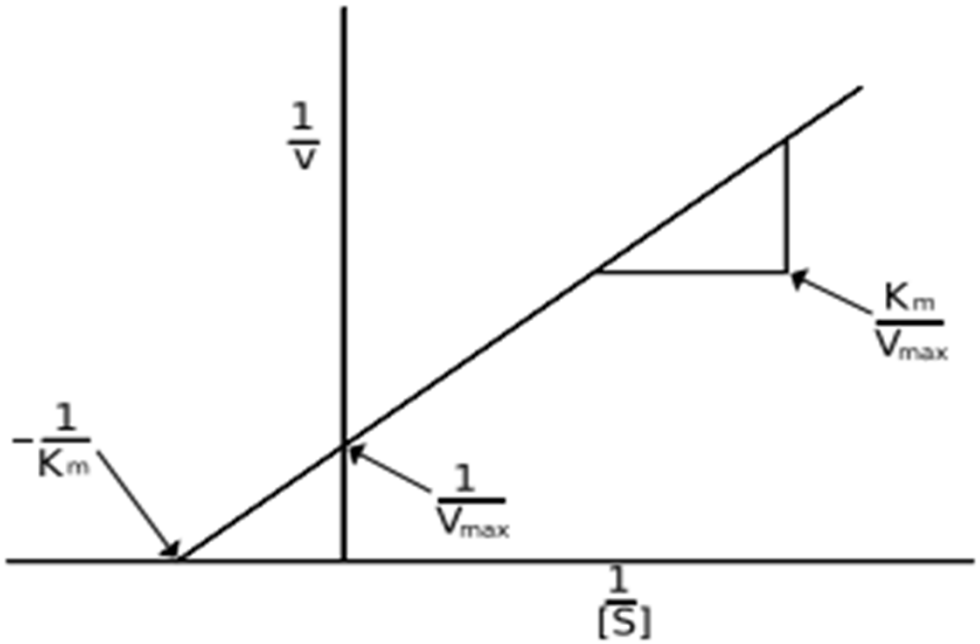
- K_m = the [S] at $V_{max}/2$. It also =[E]+[S]/Σ[ES].
- k_2 = maximum number of turnovers/sec. It cannot be greater than any forward microscopic rate.

Linearized Michaelis-Menten Kinetics

- For some strange reason, people today still want to linearize Michaelis-Menten kinetic data! They are crazy.

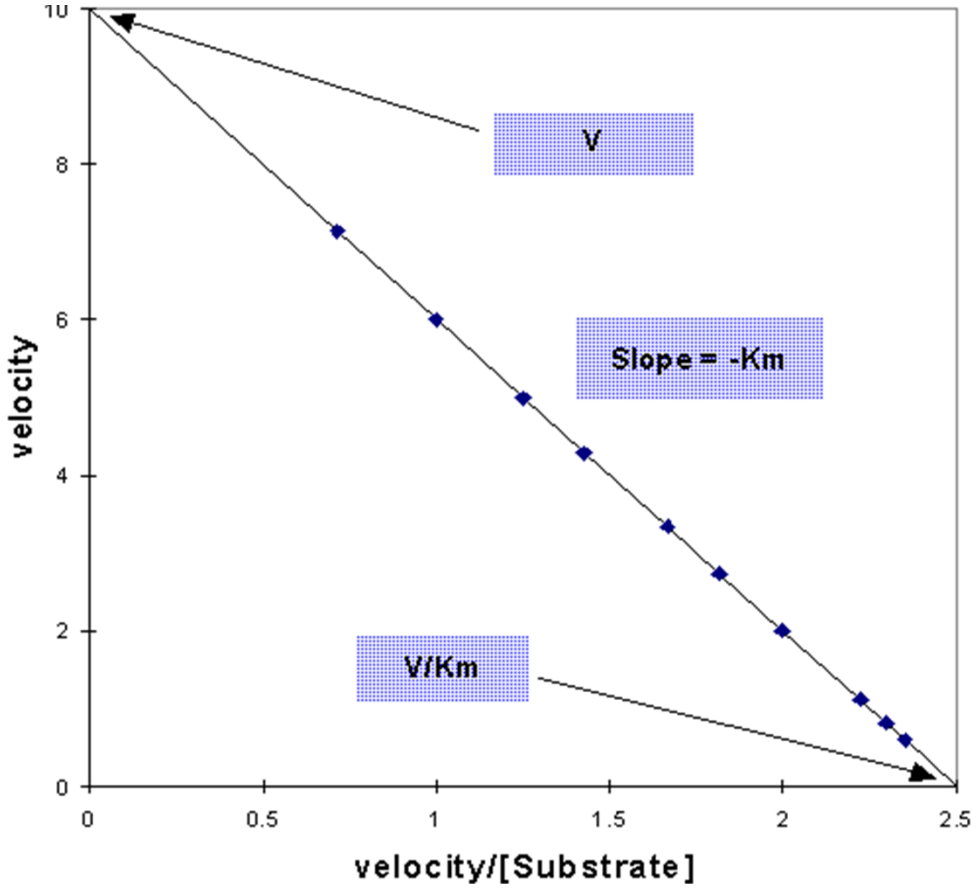
$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}$$

- This is called a 'Lineweaver-Burke' or 'Double Reciprocal' plot



The Eadie-Hofstee Plot

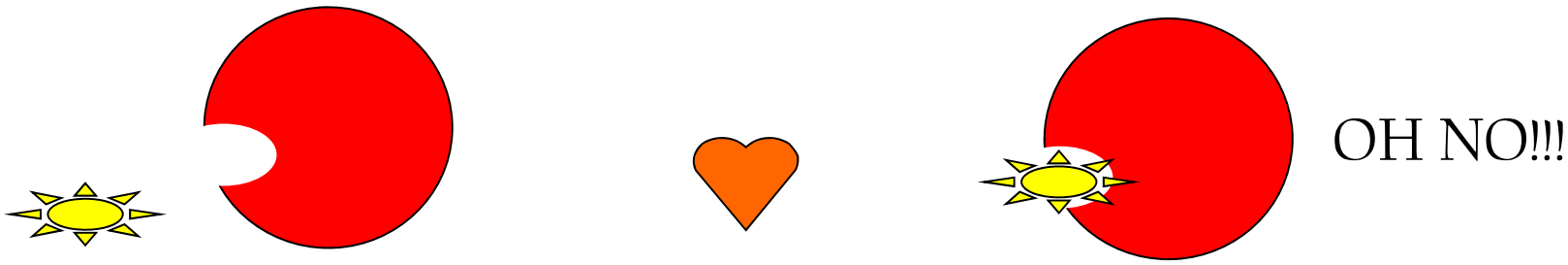
$$\frac{V_{max}}{v} = \frac{V_{max}(K_m + [S])}{V_{max}[S]} = \frac{K_m + [S]}{[S]} \qquad v = -K_m \frac{v}{[S]} + V_{max}$$



Competitive Inhibition

- Understanding enzyme inhibition is important for us because it is '**the**' basis of metabolic control:

- Competitive inhibition: Inhibitor binds to enzyme at active site



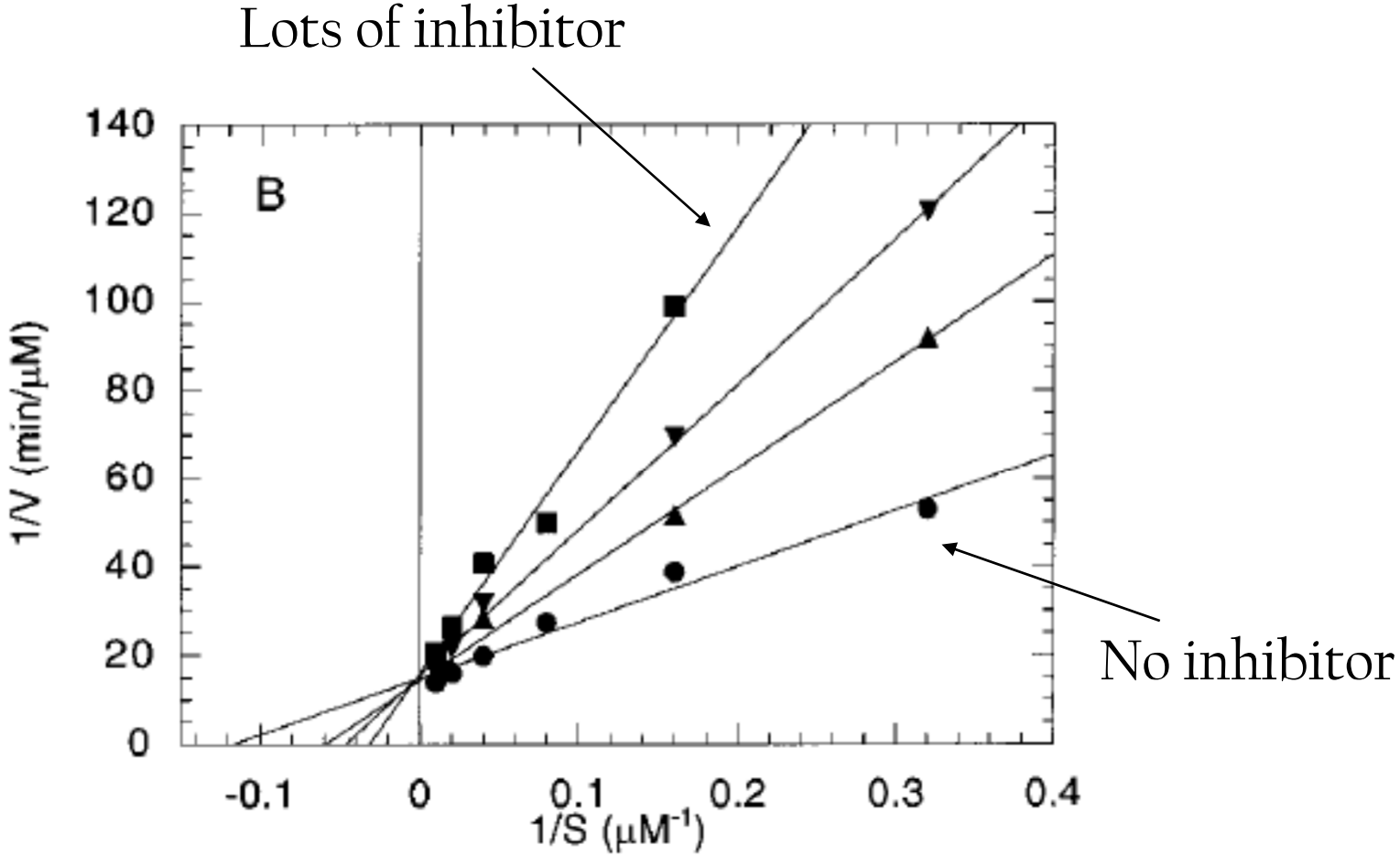
- Effect: Takes a percentage of enzymes 'out of action'

- Effect in terms of Michaelis-Menten: Lowers [ES], ***increases*** K_M but has ***no effect*** on V_{max}

- Metabolic Example use: Feedback inhibtion!

Competitive inhibition

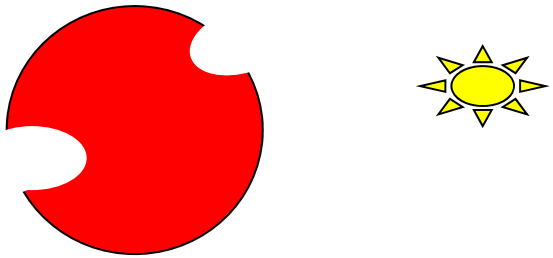
- Lineweaver-Burke plots of product inhibition in HepC protease:



Biochemistry, Vol. 37, No. 25, 1998

Non-competitive Inhibition

- Non-competitive: Inhibitor binds somewhere other than the active site



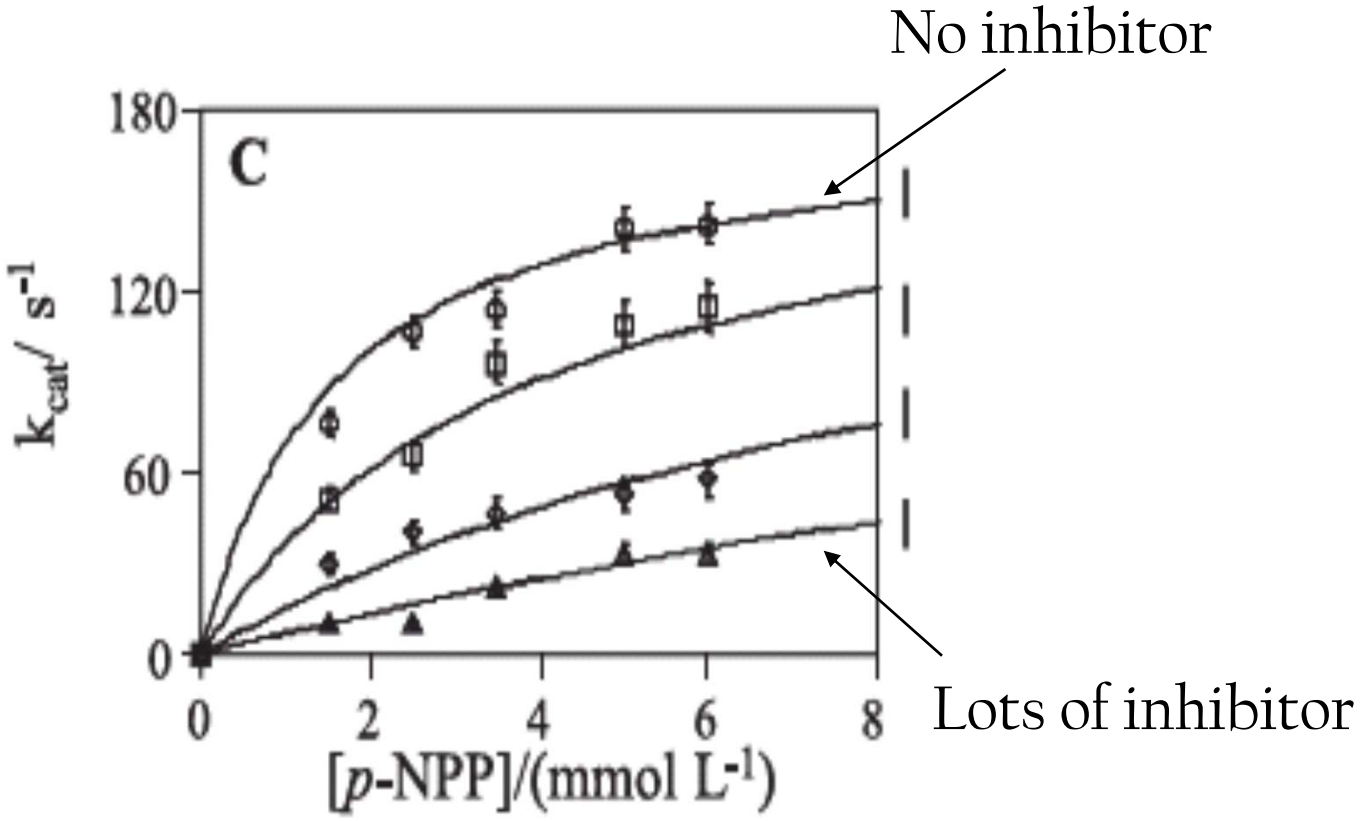
- Effect: Reduces the catalytic efficiency of the enzyme, but not it's ability to bind substrate

- Effect in terms of Michaelis-Menten: Lowers k_2, changes V_{max} but ***not*** K_M

- Metabolic Example use: General inhibition!

Non-competitive Inhibition

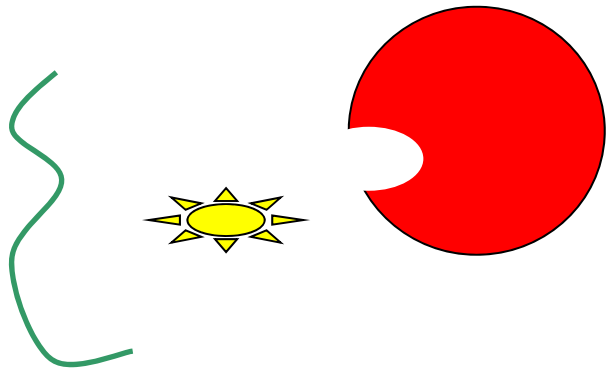
- See! Decreases V_{max}!



J. Braz. Chem. Soc., Vol. 17, No. 8, 1558-1565, 2006

Un-competitive Inhibition

- Un-competitive: Inhibitor binds the ES complex



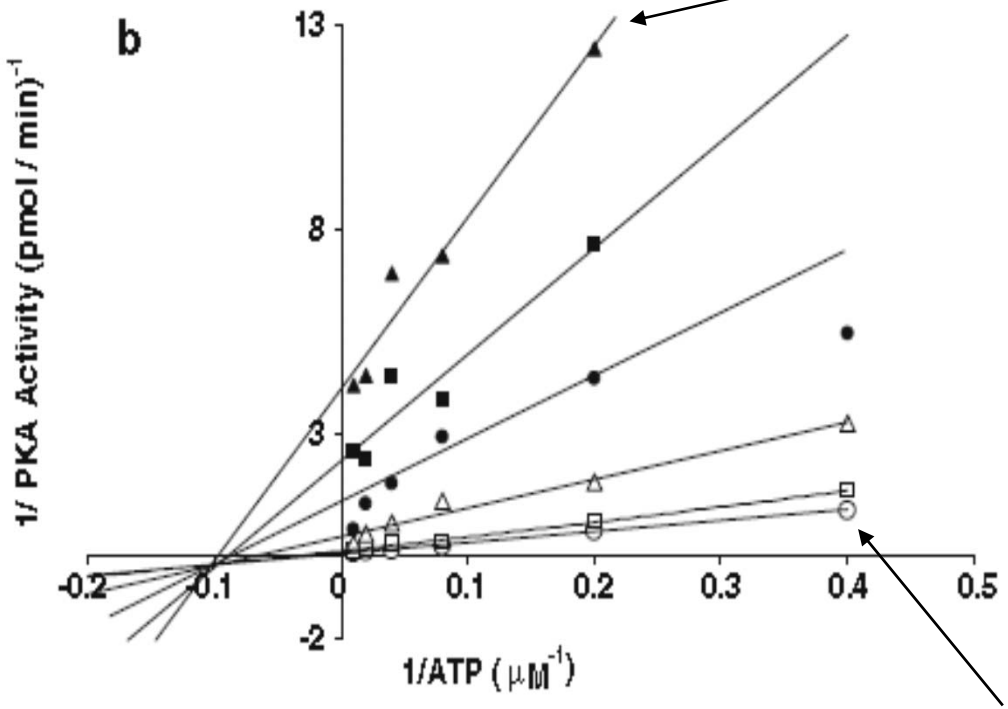
- Effect: Takes some enzyme out of action ***and*** reduces catalytic efficiency

- Effect in terms of Michaelis-Menten: Lowers k_2, changes V_{max} and K_M

- Metabolic use: General inhibition

Mixed Inhibition Example

- Looks like both competive and non-competitive!



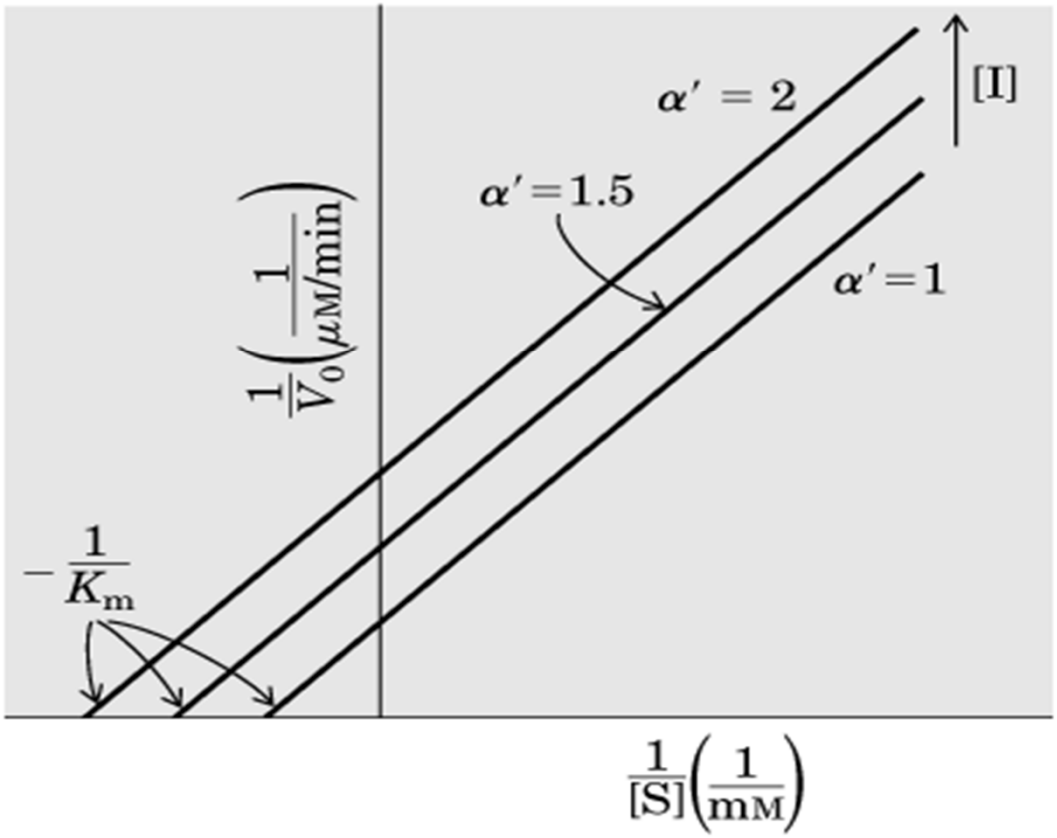
Lots of inhibitor

No inhibitor

J Biol Inorg Chem (2006) 11: 379–388

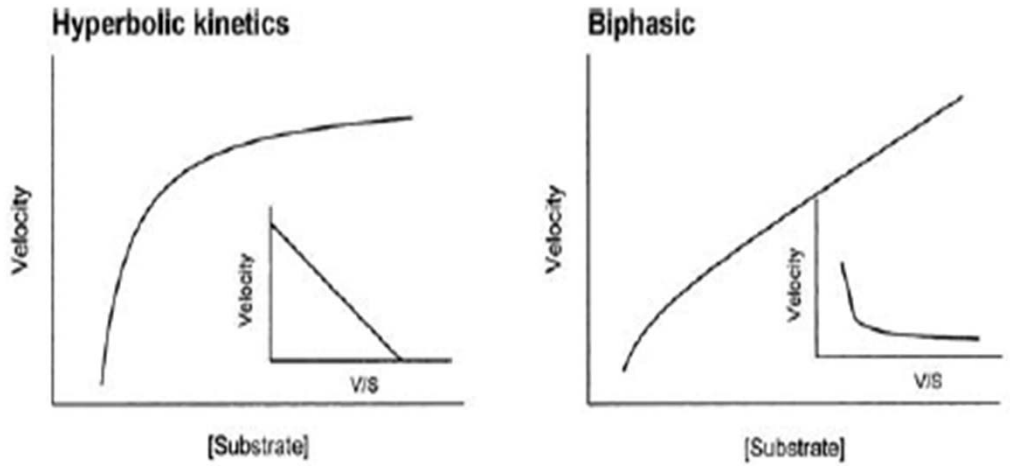
Uncompetitive Inhibition

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



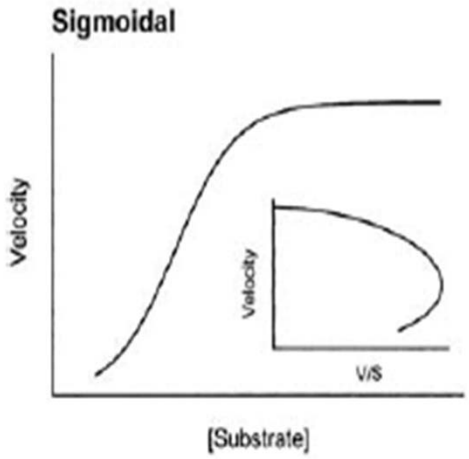
K_M/V_{max} = unchanged!!

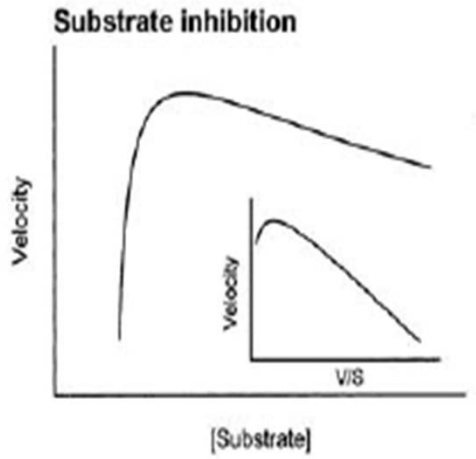
Non-Michaelis Menten Kinetics



Second active site with different activity

Homotropic activation





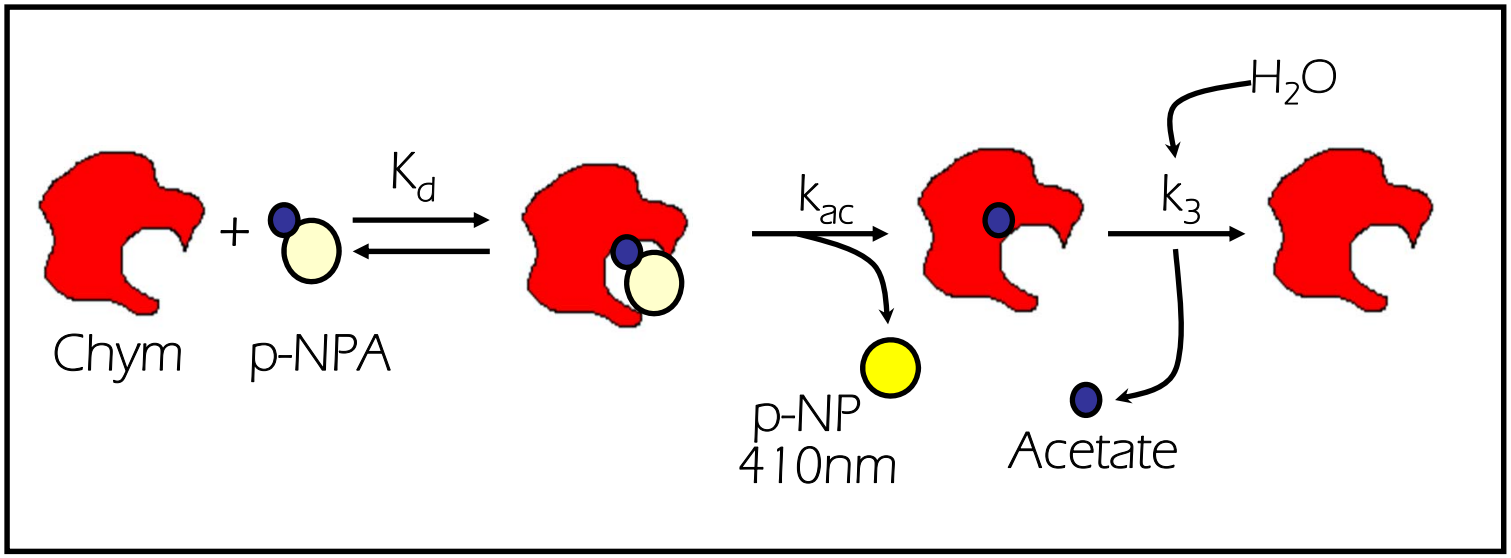
Substrate Inhibition

Annu. Rev. Pharmacol. Toxicol. 2005. 45:291–310

Pre-steady State Enzyme Kinetics

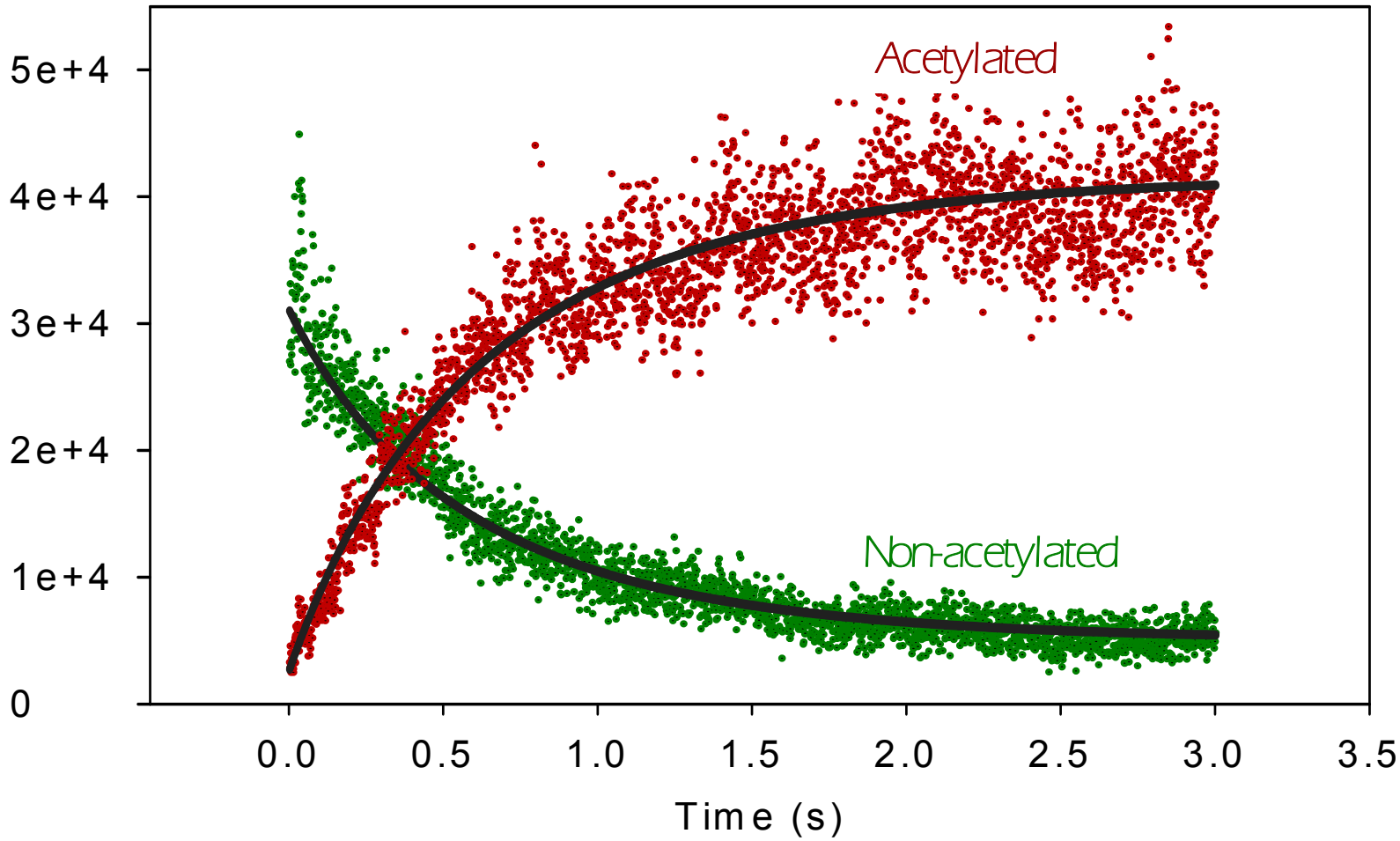
- Michaelis-Menten kinetics is a 'steady state' analysis. It can give us macroscopic parameters for the reaction, but not microscopic parameters.

- To get at the microscopic rates, we have to study the reaction prior to the establishment of one or more internal equilibria

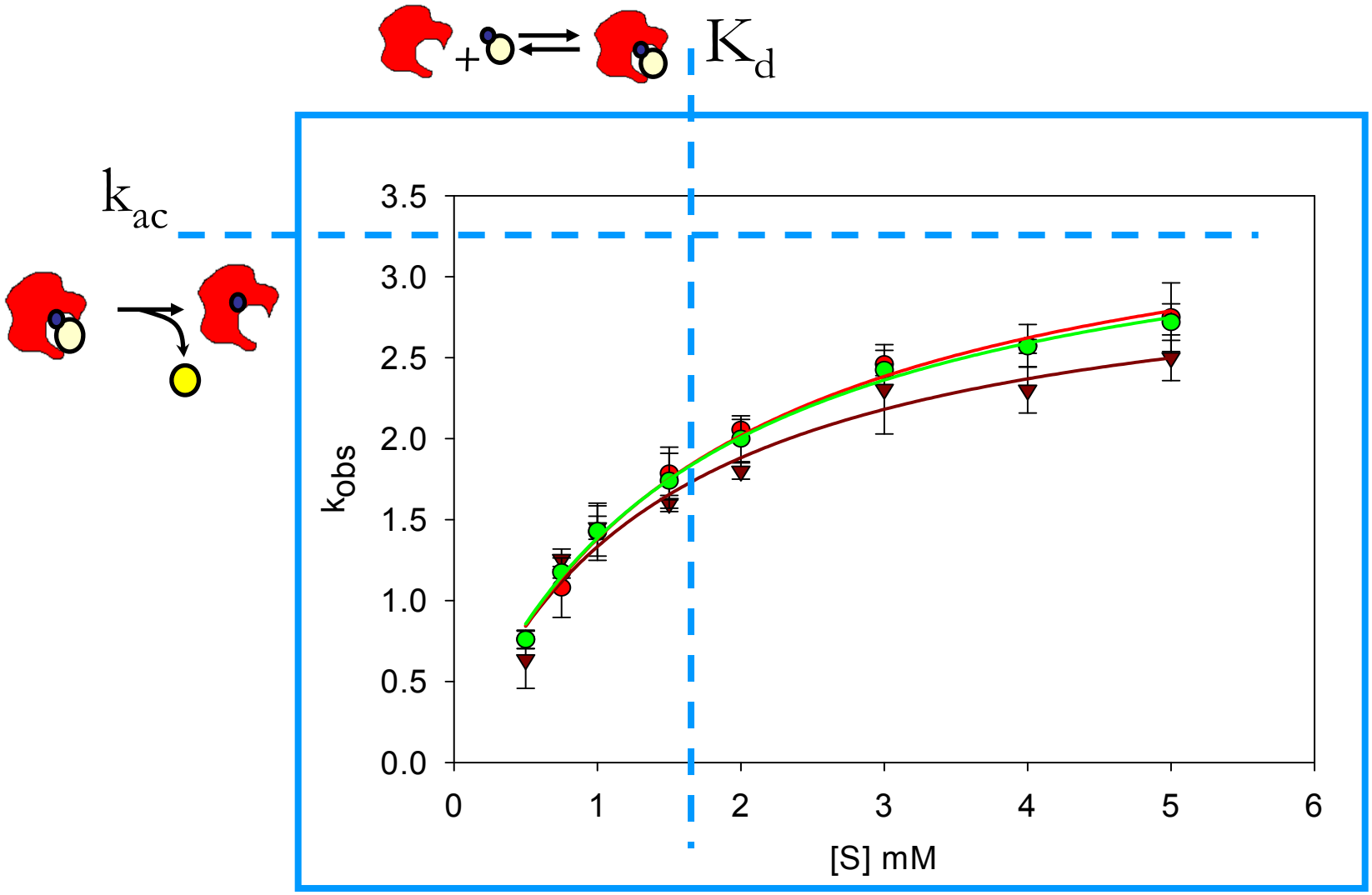


- In a 'steady state' analysis, k_{ac} and k_3 cannot be isolated from each other

Pre-steady State Enzyme Kinetics



Pre-steady state saturation plot



Linearized Michaelis-Menten Kinetics

Linearized Michaelis-Menten Kinetics