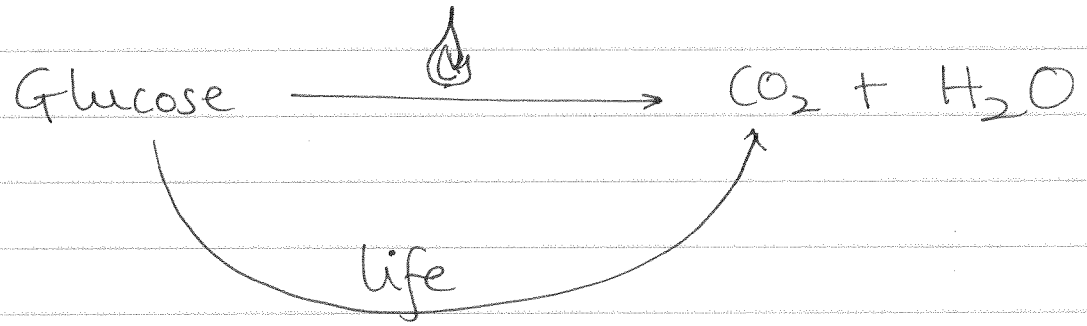
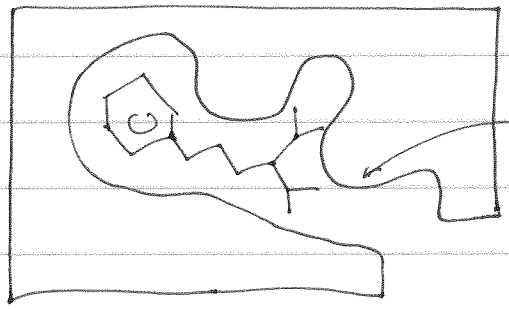
ENZYME CATALYSIS

* Traditional chemical engineers view enzymes & biology with suspicion.

* Think of biology as <u>slow combustion.</u>



Why is biocatalysis so powerful?



moving parts

⇓

these are like fingers that bring reacting parts of the molecules close to one another

@ Why is the study of enzymes important?

Key industries that use biocatalysis as the foundation technology:

→ biofuels

→ biochemicals

→ biopolymers

→ chemical manufacturing

→ pharmaceuticals

→ food

→ environmental remediation

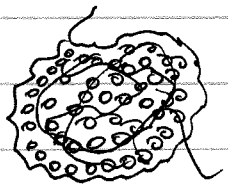
* The biocatalysis-based industries are an $\underline{\$80\text{ bn.}}$ enterprise.

* Sales of pure enzymes ≡ $\underline{\$5\text{ bn./yr.}}$

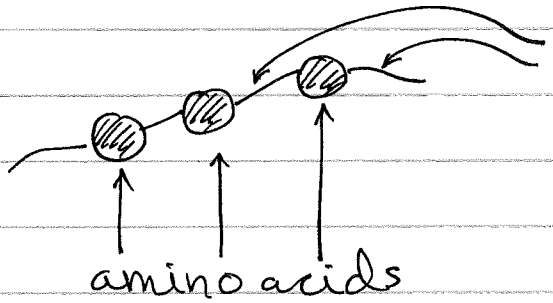
* Total biotech market ≡ $215bn.

What are enzymes? [They are also called proteins]

* Think of enzymes are beads on strings that have been rolled & twisted into a solid object.



⟹ Let us unwind the beaded string

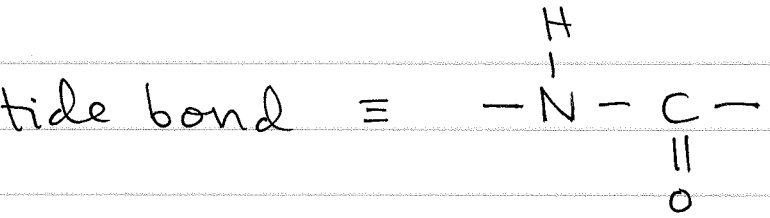


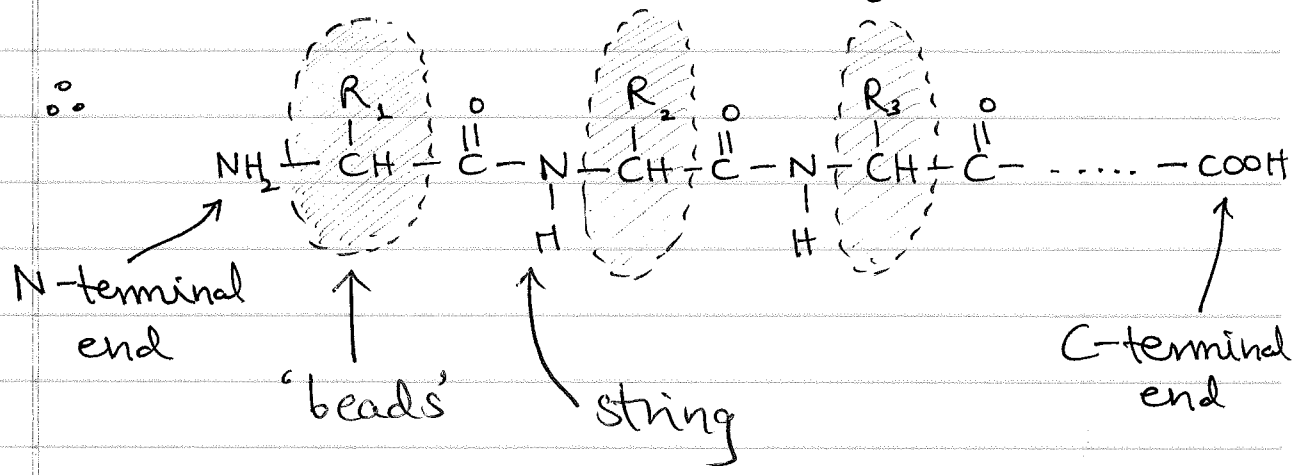
In general,

$$H_2N-\underset{\displaystyle R}{\underset{|}{C}}H-COOH$$

amino acid

The R-group could be acidic, basic, hydrophobic, aromatic etc.

Peptide bond ≡ 



Now enzymes exhibit 4 levels of structures:

① <u>Primary structure</u> (1° structure)
↖ notation

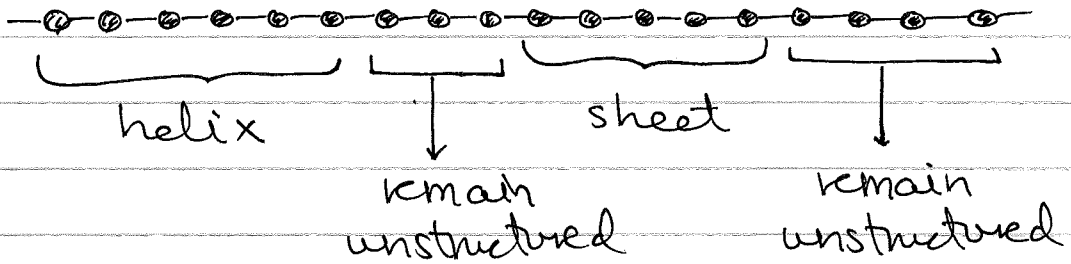
'stretched out string'

1° structure ≡ amino acid sequence

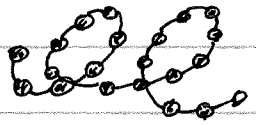
② <u>Secondary (2°) structure</u>:

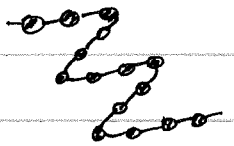
If you let go the stretched string, it will collapse into an <u>ordered</u> structure.

some amino acidsequences fold into helices, some fold into sheets. Some don't fold at all!

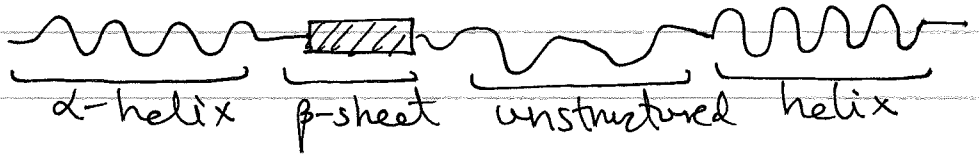


Folding into a structure is an energy minimization process. If stretches are unstructured, it is likely they are already at an energy minimum.

 α-helix ≡ spring-like

 β-sheet ≡ zig-zag & look like a sheet of paper

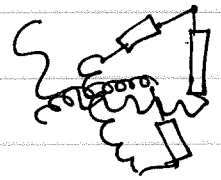
(3) Tertiary structure (3°):



Collapsing continues & the 2° structures

collapse onto themselves to resemble a 3-D blob that is made of helices, sheets & strands. Sometimes, helices & sheets might covalently bond.

This is known as the 'protein folding problem'



} 3° structure

(4) Quaternary structure (4°):

Blobs might dimerise or associate with other proteins to form complex structures.

* Supplying energy or destabilising the folded amino acid sequences leads to 'denaturation' of the protein/enzyme.

e.g. scambled / boiled eggs

soy protein / tofu

* Sometimes, proteins/enzymes require cofactors (or partners) to function properly.

* We will look only at simple cases in this course

Incidentally, there are only 6 types of enzymes.

(1) oxidoreductases :

$$AH_2 + B \longrightarrow A + BH_2$$

oxidation ≡ loss of e^- ≡ loses H
reduction ≡ gain of e^- ≡ adds H

eg. drug metabolism

(2) transferases :

$$AB + C \longrightarrow A + BC$$

eg. methyl transferases
acyltransferases

(3) Hydrolases :

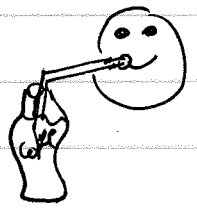
$$AB + H_2O \longrightarrow AH + BOH$$

The reverse rxn. is more popular

↳ anhydrases (or dehydroses)

where? COca-cola & carbonic anhydrase

does not lose fizz when opened (takes a while!)



fizzes as soon as you drink it (that's carbonic anhydrase acting)

$$H_2CO_3 \xrightarrow{CA} CO_2 + H_2O$$

(4) Isomerases:

$$A \longrightarrow A'$$

eg.: Glucose-6-phosphate to fructose-6-phosphate

(5) Lyases: break apart molecules

$$AB \longrightarrow A + B$$

enzyme + cofactor ≡ working enzyme

enzyme: correctly folded amino acid chain

cofactor: often metal ions or vitamins

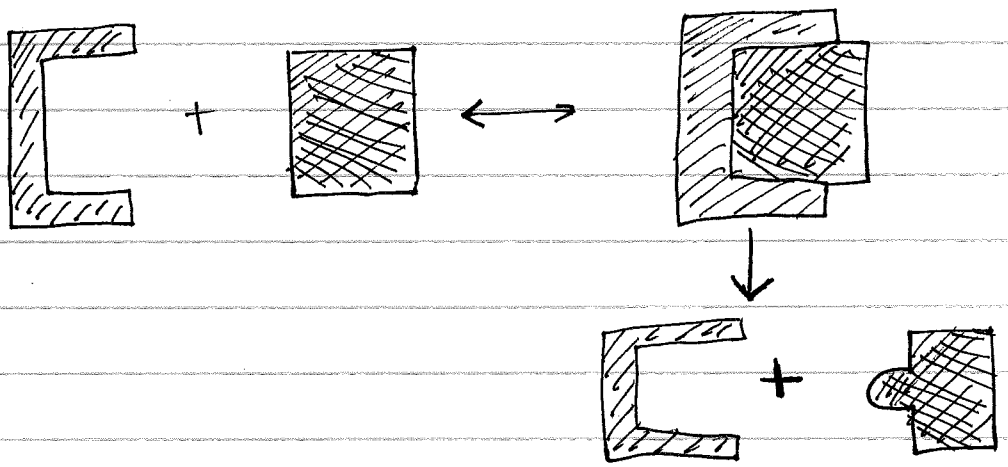
Nomenclature: enzyme alone = APOENZYME

cofactor = PROSTHETIC GROUP

working enzyme = HOLOENZYME (ˈwhole enzyme)

(e) How do enzymes work?

* Simplest model: LOCK & KEY model



∴ 

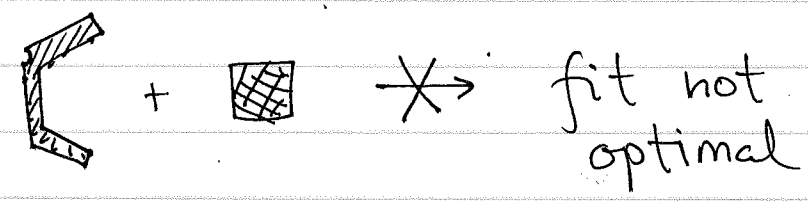
$E + S \longleftrightarrow ES \longrightarrow E + P$

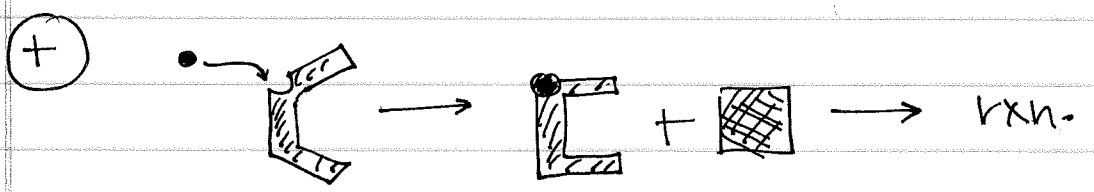
→ Michaelis-Menten Mechanism

- Leonor Michaelis
- Maud Menten (Canadian)
- 1913

There could be other complexities involved.

eg.





⑥ Ligases: glue together molecules

$$A + B \longrightarrow AB$$

Lyases & ligases ≡ recombinant DNA technology

very important innovation in the last 50 years

① Analysis of Michaelis-Menten kinetics:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Conc. $\dfrac{d[P]}{dt}$ = rate of reaction = $k_3[ES]$

also called reaction 'velocity'

elementary kinetics

* Can we measure [ES]? What about [E]?

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES]$$

We make the pseudo-steady state assumption for [ES].

$$\therefore \frac{d[ES]}{dt} = 0 \Rightarrow [ES] = \left(\frac{k_1}{k_2+k_3}\right)[E][S]$$

Typically, one can measure [S] & $[E]_0$

* But [E] is harder to measure.

∴ Re-write [E] in terms of measurable quantities.

$$[E]_0 = [E] + [ES]$$

↑ The enzyme can exist in 1 of 2 forms

$$\therefore [E] = [E]_0 - [ES]$$

$$\& [ES] = \left(\frac{k_1}{k_2+k_3}\right)[E][S]$$

↖ Subst.

$$\therefore [E]\left\{1 + \frac{k_1}{k_2+k_3}[S]\right\} = [E]_0$$

$$\therefore [E] = \frac{(k_2+k_3)[E]_o}{k_2+k_3+k_1[S]}$$

Now, $\frac{d[P]}{dt} = k_3[ES] = \left(\frac{k_1 k_3}{k_2+k_3}\right)[E][S]$

$$\therefore \frac{d[P]}{dt} = \left(\frac{k_1 k_3}{k_2+k_3}\right)\frac{(k_2+k_3)[E_o][S]}{\{k_2+k_3+k_1[S]\}}$$

$$\therefore \frac{d[P]}{dt} = \frac{k_1 k_3 [E]_o [S]}{k_2+k_3+k_1[S]}$$

$$\therefore \frac{d[P]}{dt} = \frac{k_3[E]_o[S]}{\left(\frac{k_2+k_3}{k_1}\right)+[S]}$$

Let $\frac{d[P]}{dt} = V_E$ .... velocity

$$\therefore V_E = \frac{k_3[E]_o[S]}{\left(\frac{k_2+k_3}{k_1}\right)+[S]}$$

Let $\frac{k_2+k_3}{k_1} = K_M$ .... Michaelis-Menten constant

$$\therefore \quad v_E = \frac{k_3[E]_o[S]}{K_M + [S]}$$

What is $k_3[E]_o$?

If $[E] = 0$, then $[E]_o = [ES]$

$\therefore$ If $[E] = 0$, $k_3[E]_o = \underbrace{k_3[ES]}_{\left.\frac{d[P]}{dt}\right|_{[E]=0}}$

This is the fastest rate @ which the reaction can occur

$\therefore \quad k_3[E]_o \equiv$ maximum velocity

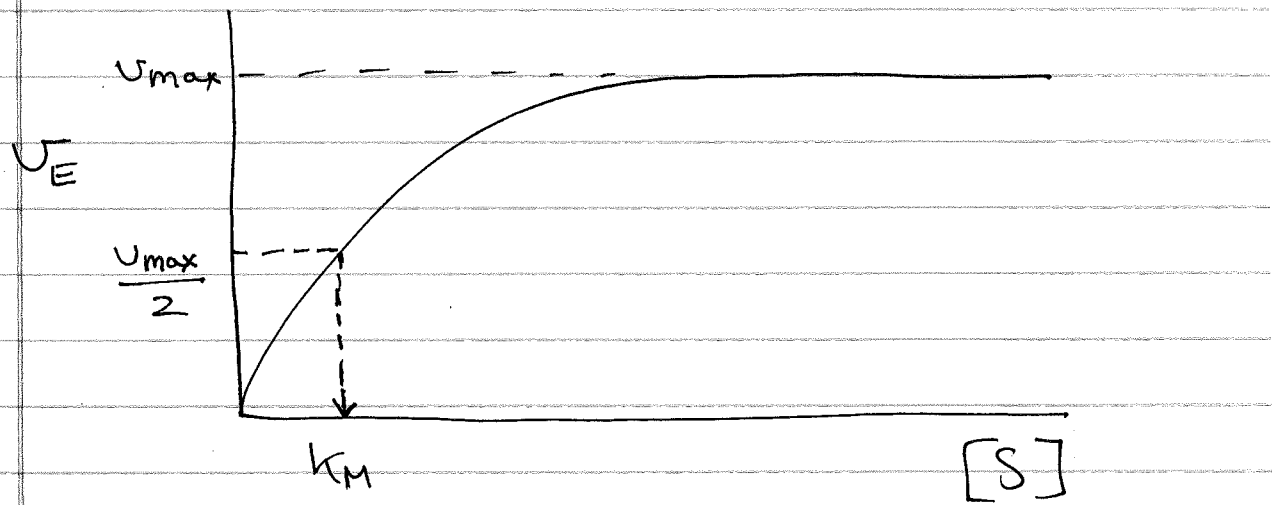
$$\therefore \quad v_E = \frac{v_{max}[S]}{K_M + [S]} \longrightarrow \text{Michaelis Menten Rate}$$

* What does K_M signify?

If $K_M = [S]$, $v_E = \frac{v_{max}[S]}{[S] + [S]}$

$\therefore \quad v_E = \frac{v_{max}}{2}$ when $K_M = [S]$

$\therefore$ K_M is the substrate concentration for which rate = $\frac{v_{max}}{2}$.



What happens when [S] is very large?

i.e. $K_M \ll [S]$

$\therefore \quad v_E \simeq \frac{v_{max}[S]}{[S]}$

$\therefore \quad v_E \simeq v_{max}$ → [S] very large

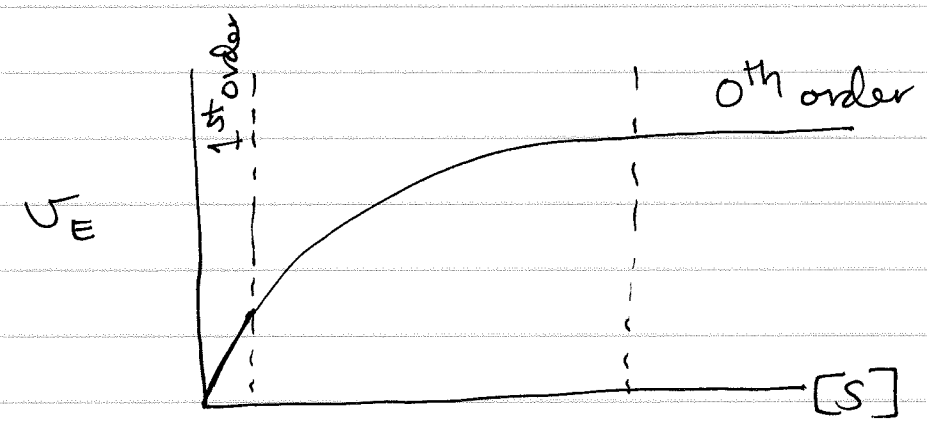
What happens if [S] is very low?

i.e. $K_M \gg [S]$

$$\therefore \quad v_E \cong \frac{v_{max}[S]}{K_M}$$

$$\therefore \quad v_E \approx \left(\frac{v_{max}}{K_M}\right)[S]$$

$$\boxed{\text{i.e. } v_E \approx k[S]} \longrightarrow 1^{st} \text{ order}$$



<u>Data-fitting</u> : $v_E = \frac{v_{max}[S]}{K_M + [S]}$

similar to $-\frac{dC_A}{dt}$

non-linear form

Note, $v_E = -r_S$

$$v_E = \frac{v_{max}[S]}{K_M + [S]}$$

$$\therefore \frac{K_M + [S]}{v_{max}[S]} = \frac{1}{v_E}$$

$$\therefore \frac{1}{v_E} = \frac{1}{v_{max}} + \frac{K_M}{v_{max}[S]}$$

y-axis y-intercept slope x-axis

$\therefore$ Plot $\left(\frac{1}{v_E}\right)$ versus $\frac{1}{[S]}$

i.e. $\frac{1}{\left(\frac{-dC_S}{dt}\right)}$ versus $\frac{1}{C_S}$ } Lineweaver Burke plot

There are other ways to linearise the Michaelis-Menten expression.

$$v_E = \frac{v_{max}[S]}{K_M + [S]}$$

$$\therefore v_{max}[S] = v_E(K_M + [S]) \longrightarrow (a)$$

$$\therefore v_{max} = \frac{v_E K_M}{[S]} + v_E$$

$$\therefore v_E = v_{max} - \left(\frac{v_E}{[S]}\right) K_M$$

v_E: y-axis; $\frac{v_E}{[S]}$: x-axis; K_M: negative slope

v_E versus $\frac{v_E}{[S]}$ $\equiv$ Eadie-Hofstee plot

Also, rewriting (a),

$$v_{max}[S] = v_E K_M + v_E[S]$$

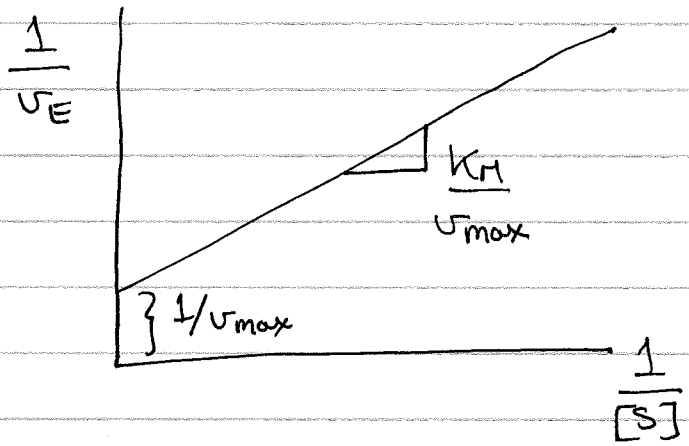
$\therefore$ $v_{max} v_E \Rightarrow$ divide across,

$$\frac{[S]}{v_E} = \frac{K_M}{v_{max}} + \frac{[S]}{v_{max}}$$

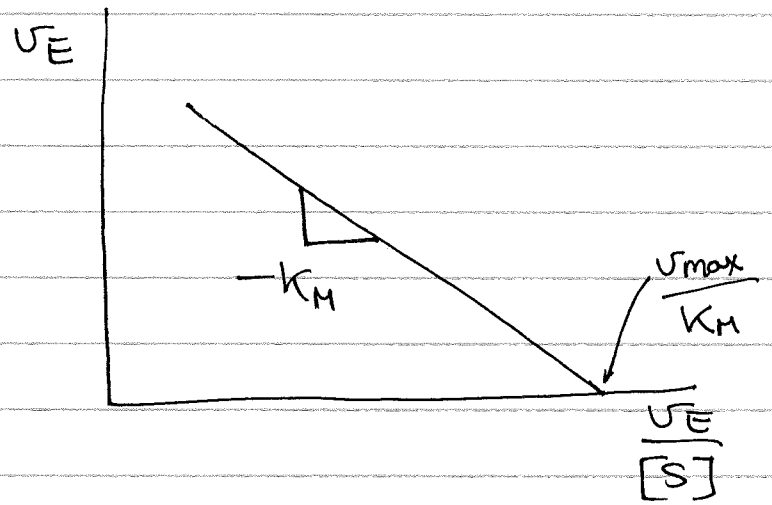
$$\therefore \quad \frac{[S]}{v_E} = \left(\frac{1}{v_{max}}\right)[S] + \frac{K_M}{v_{max}}$$

$\frac{[S]}{v_E}$ versus $[S]$ $\rightarrow$ Hanes-Woolf plot

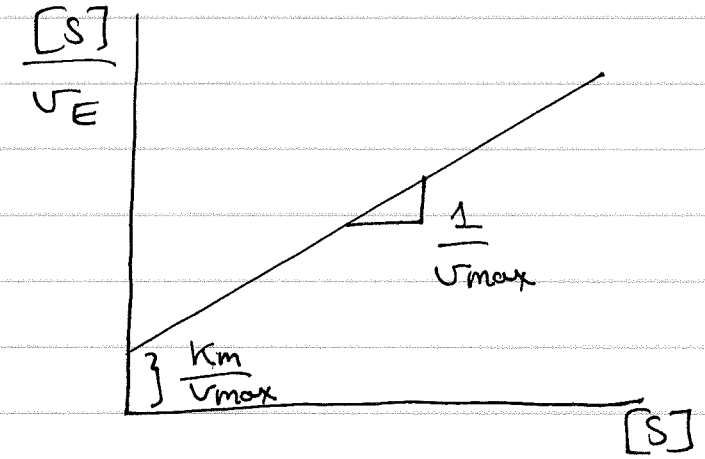
$$\therefore \quad v_E = \frac{v_{max}[S]}{K_M + [S]} \qquad \{v_E = -r_S\}$$



Lineweaver-Burke Plot



Eadie-Hofstee Plot



Hanes–Woolf plot

Which to use?

* Lineweaver-Burke most common.
  → easiest too!

(e) What happens if?

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

↓

Briggs–Haldane Equation

→ The rate expression simplifies down to the MM equation (constants different, of course!)