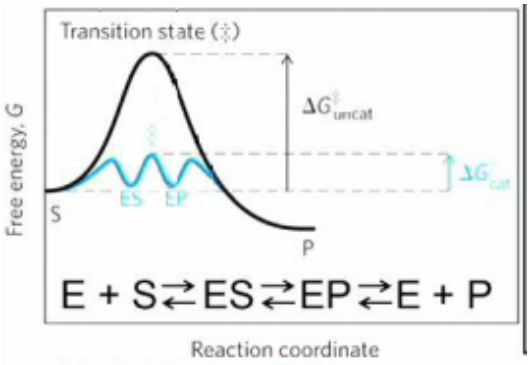
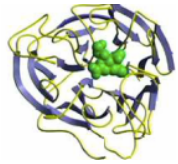
LECTURE 10 NOTES

ENZYMES

Are biomolecules that have a distinctive structure and act as catalysts using weak interactions, acid-base, covalent and metal ion catylsis. i.e. they provide an active site for a reaction to proceed which lowers the activitation energy so that the reaction is sped up (affects the rates, not the position of the equilibria). The **active sites** are lined with functional groups to bind the substrate.



Enzymes are highly specific and are characterised by how they stabilise the transition state such that you see the formation of a **complex** (intermediate) between the formation of the product and the reactants. See diagram.



IMPORTANT ENZYMES

Some diseases are caused by excessive or deficient enzymatic activity e.g. phenylketonuria (PKU). As such, you also find that many drugs target enzymes by either inhibiting or activating an enzyme.

FUNCTION OF ENZYMES

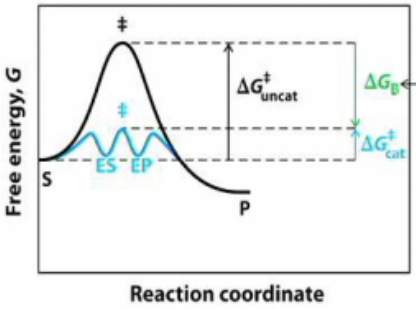
Often can't work alone but require **co-factors** (small inorganic molecules like Mg^{2+}) or **co-enzymes** which are more complex molecules that transiently carry functional groups during the catalysis e.g. biocytin, NADH. Another key thing about enzymes is that they can be so favourable that they can be coupled with unfavourable reactions to help them 'go'

- Important example: The unfavourable production of GTP in the Krebs cycle is coupled to hydrolysis of a thioester bond in succinyl-CoA, which is favourable

HOW ENZYMES WORK

The higher the activation energy, the more (usually thermal) energy required for the reactants to convert to products. Remember that at the transition state, it could form the reactant again, or product. Enzymes reduces the activation energy at the transition state but do not influence the overall free energy change or the equilibria, only the rate of reaction. You will have a multichoice question on this.

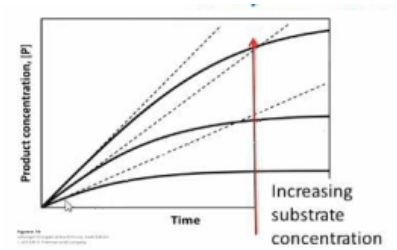
Most reactions occur in multiple steps. Enzymes can enable a reaction to have stable **reaction intermediates** which dramatically lower the free energy of the total transition state. Reaction intermediates lower the free energy because the enzymes stabilise the intermediates by binding them, such that getting from one intermediate to another only requires overcoming a much smaller transition states.



Binding free energy is the difference between the activation energies of the uncatalysed and catalysed reactions. *Handy note, the energy produced by the bond formation of the intermediates, get's the molecules part way up the hill to the next transition state

ENZYMES WORK BY;

Binding substrates in the correct orientation relative to the active groups, providing catalytically active groups and by stabilising the transition state. They wouldn't be able to do all of this without thte **weak binding interactions** between the enzyme and substrate in the first place.



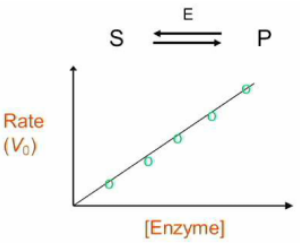
ENZYME KINETICS

Rate decreases with time because the [R] run out, the reverse reactions starts working and enzymes are usually unstable under reaction conditions in general anyway!

INVESTIGATING CHANGING RATES

(1) **Changing enzyme concentration** - the rate of a catalysed reaction is **directly proportional** to the amount of enzyme in solution i.e. doubling the enzyme will double the rate

(2) **Substrate concentration** – increasing the concentration of substrate available will increase the speed of the reaction in a hyperbolic like pattern until you reach a value of V which is the maximum ***Note** – the value of ⌈S⌉ at $1/2V_{max}$ is the Michaelis constant, Km. Note the similarity between Km and Kd

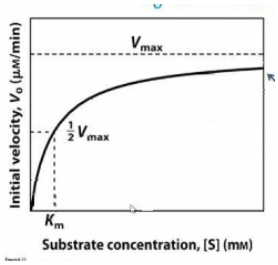


MICHAELIS MENTEN THEORY

Involves a multistep reaction with respect to the intermediate (therefore, 3 rate constants to think about)

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

Rate of ES breakdown to form product (V_0) = k_2[ES]
Rate of ES formation = k_1[E].[S] = k_1([E_t] – [ES]) [S]
Rate of ES breakdown = k_{-1}[ES] + k_2[ES]



There are four assumption which make the Michaelis Menten Theory plausible; (1) the ES conversion to E+P is **irreversible** (2) the ⌈ES⌉ remains constant (remains at steady state). (3) we assume that ⌈S⌉ is much greater than the concentration of enzyme⌈E_t⌉ (4) initially, the ⌈S⌉ is much greater than ⌈P⌉ (to keep the rate of the back reaction low)

- $[E][S]/[ES] = (k_2 + k_{-1})/k_1$, defined as K_m, the Michaelis constant

$$[ES] = \frac{[E_t][S]}{[S]+K_m}$$

$$V_0 = \frac{k_2[E_t][S]}{(K_m+[S])} \text{ (Substituting } [ES] \text{ in } V_o = k_2[ES])$$

$$V_0 = \frac{V_{max}[S]}{(K_m+[S])} \text{ (Substituting } V_{max} \text{ for } k_2[E_t])$$

MICHAELIS MENTEN THEORY

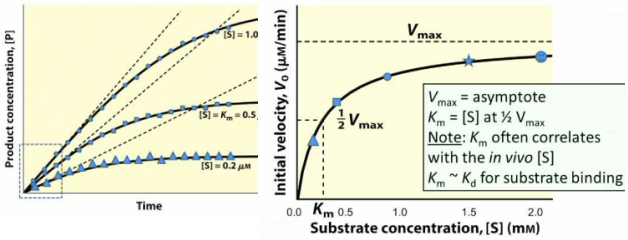
Note its similarity to the ligand binding equation. The crucial step to remember when deriving this equation is that dP/dt (i.e. LHS of the equation) is k2{E_t} (E total). However, E total is actually ⌈ES⌉ + ⌈E⌉. Therefore, confusingly enough, we actually have to define ⌈E⌉ as ⌈Et⌉ – ⌈ES⌉ in order to get the proper equation. *Note, we get the equation in terms of speed by doing a few substitions…

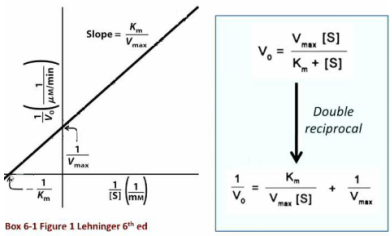
$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

MEASURING MM EXPERIMENTALLY

(1) Plot ⌈P⌉ at varying ⌈S⌉ across time to determine the V(initial)

(2) Plot the initial velocity as a function of ⌈S⌉ and use the asymptote to determine your Vmax, and therefore your Michaelis Menten constant





Box 6-1 Figure 1 Lehninger 6th ed

LINEWEAVER-BURK ANALYSIS

Just takes the inverse of the MM equation so that you get a linear graph instead of a hypbola for the step 2 describe above

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

LECTURE 11 NOTES

COMPARING KM AND KD

Note, at steady state of ⌈ES⌉, k2 is apparently a lot smaller than k_{-1} which means that we can basically assume the k_{-1} is doing the majority of the reversing of the ⌈ES⌉ complex. Once you understand this assumption, Km is roughly equal so Kd! Note that steady state doesn't mean equilbrium! It is a different concept.

MM CONSTANTS

In general, a low Km corresponds to a high affinity of the enzyme for its substrate, much like the rule that we learnt for the differing Kd values.

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

TURNOER NUMBER

Also known as k_2 or k_{cat}. It is usually the rate determining step and is defined as the number of molecules of substrate converted to product per unit time per enzyme molecule **at Vmax**. Recall that k values are always measured in the units s^{-1}.

CONVERTING TO VMAX

The units for Vmax are micro-concentration product per time. Therefore, it can be linked to k_{cat} through the very simple equation:

$$k_{cat} = {V_{max}}/{[E_t]}$$

NOTE: $V_{max} = k_{cat}$ **if** V_{max} **is expressed in units of (μ)mole product per time/(μ)mole of enzyme**

THE SPECIFICITY CONSTANT

k_{cat}/K_m

It is defined as the rate constant for the conversion of E+S to E+P. It is a simple way of us being able to summarise all of the millions of K constants we now have, into one value. Because it is a rate constant, high values of the specificity constant indicates efficient use i.e.

- You want **kcat** to be large – indicates that lots of reactant is being turned over to product
- You want **Km** to be small – indicates the the prodcuts are being bound tightly

DERIVING FROM THE SPECIFICITY CONSTANT

$$V_0 = \frac{V_{max}[S]}{(K_m + [S])}$$

Remembering this equation for V0, we can now say that, if [S] is insignificant in comparison to Km, the equation becomes that middle one.

$$V_0 = \frac{V_{max}[S]}{K_m}$$

And *then*, once you use the equation in the top right corner, we can figure out we can substitute kcat x [Et] into this equation such that we finally get;

$$V_0 = \frac{k_{cat}[E_t][S]}{K_m}$$

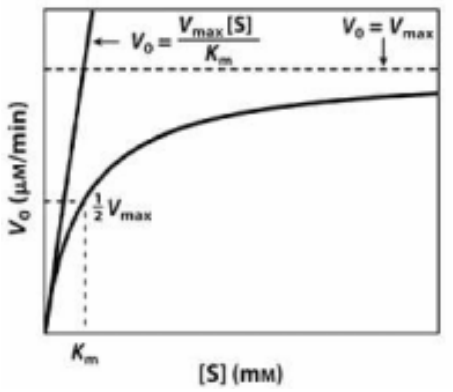


TABLE 6–9	Effects of Reversible Inhibitors on Apparent V_{max} and Apparent K_m	
Inhibitor type	**Apparent V_{max}**	**Apparent K_m**
None	V_{max}	K_m
Competitive	V_{max}	αK_m
Uncompetitive	V_{max}/α'	K_m/α'
Mixed	V_{max}/α'	$\alpha K_m/\alpha'$

INHIBITORS OF ENZYMES

Basically refers to drugs, poisons or intermediates which stop or prevent the action of an enzyme. There are three types of reversible inhibition. Note the irreversible enzymes tend to be the poisons.

Double note: Note, when the inhibitor is bound, the enzyme has *no* activity, this gives it the definition of 'inhibitor'

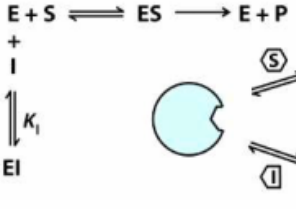
(1) **Competitive** – the inhibitor competed with the substrate for binding at the active site of the enzyme
   a. Increases the Km i.e. is affecting the strength of binding
   b. No change to the Vmax
(2) **Uncompetitive** – the inhibitor binds only to the ES complex at a site which is not the active site
   a. Decreases the Km i.e. kinetics have changed which drops the Km (hasn't bound it tighter!)
   b. Decreases the Vmax
(3) **Mixed** – like uncompetitive, but it can bind to either the ES or the E complex
   a. Either increases or decreases the Km
   b. Decreases the Vmax

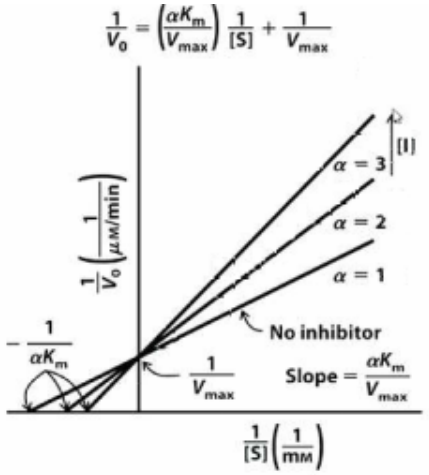
COMPETITIVE INHIBITION

The more inhibitor we put in, the lower the binding value (Km) of the reaction becomes. Competitive inhibitor sees the introduction of the complex [EI]. When you see the double reciprocal plot representing competitive inhibition, you can tell almost immediately that you have a competitive inhibitor.

I.e. The gradient changes, but the y-intercept stays the same. This is because 1/Km has becaome 1/αKm, then the reciprocal is /αKm and the gradient is α.

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$



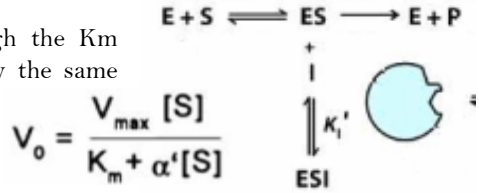


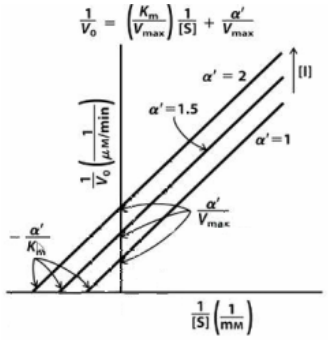
UNCOMPETITIVE INHIBITION

Forms a new complex [ESI]. They are easy to detect on the plots because the Vmax (y-intercept of the Lineweaver-Burk analysis) is changing, not the slope of the lines.

It doesn't affect the slope, even though the Km decreases, because the Km decreases by the same amount for each line (see the equation).

$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

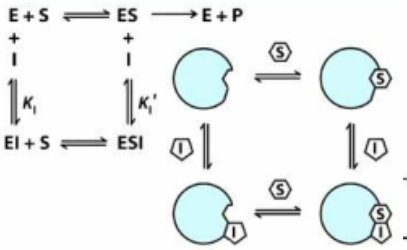


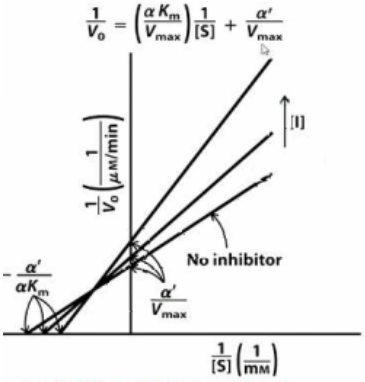


MIXED INHIBITION

Involve the simultaneous binding of [EI] and [ESI] which means that we deal with alpha and alpha prime in the equation, and see a slope difference and a y-intercept different, on the graphs

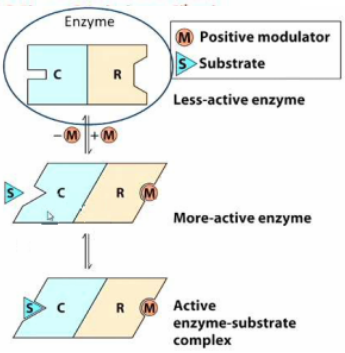
$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$





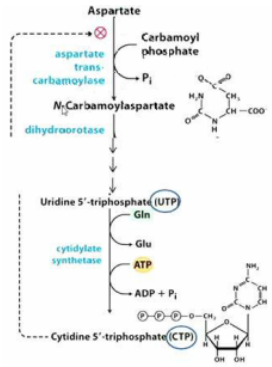
ALLOSTERIC ENZYMES

Often regulate metabolic pathways by changing *activity* in response to changes in the concentration of the molecules around them. They are reversible, and they are non-covalent, but they don't bind at the same sites as the competitive and uncompetitive inhibitors. ***Allosteric modulators / effectors** effect allosteric enzymes. Positive modulators activate and negative modulators inhibit allosteric enzymes



ALLOSTERIC ENZYME FUNCTION

Allosteric enzymes are made up of two subunits, the catalytic subunit (C) and the regulatory subunit (R). The modulator binds to the regulatory site and causes a conformation change to the active site which allows the substrate to then come in and bind with a high affinity.



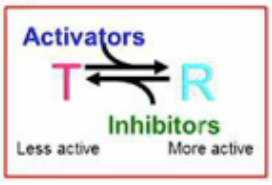
EXAMPLE ALLOSTERIC ENZYME: ATCase

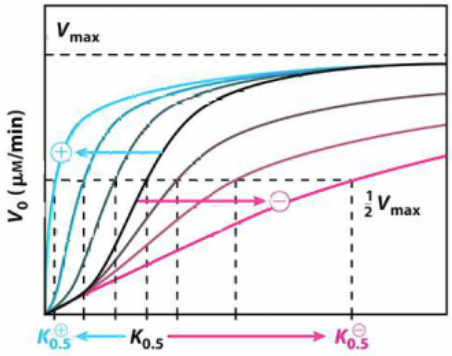
ATCase catalyses the first step in the *E. coli* pathway to produce UTP and finally CTP (nucleotides). The system works as a feedback system, because the final product, CTP, inhibits ATCase when present at high levels (it is a negative modulator). On the other hand, ATP is an intermediate in the step and acts like a positive modulator to keeep the pathway going. The structure of ATCase involves;

- 12 subunits; 6 catalytic subunits as 2 x trimeric complexes and 6 regulatory subunits arranged as 3 x dimeric complexes
- ATP is the activator of the active R state
- CTP is the inhibitor, which binds and stabilises the inactive T state

KINETIC OF ALLOSTERIC ENZYMES

Allowsteric enzymes do not show M-M kinetics. Instead, their graph showing kinetics (V0 versus [S]) looks very much like the graph representing the T state and R state complex of Hb. Note, the black line represents the average of both curves.

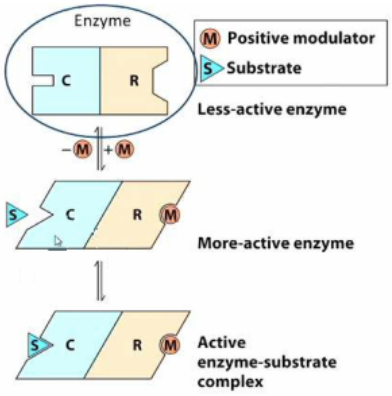




- Remember that [S] has a significant effect on equilibirum
- Note, you can't technically call that 1/2Vmax value the M-M coefficient because you're not dealing with a MM plot
- Explain what is going on in that graph

ENZYMES WITHOUT REGULATORY SUBUNITS (LIGHT BULB)

- Forms 2 trimers
- Are catalytically active (active site is still there)
- Displays Michaelis-Menten kinetics
- Are not modulated, helped, or inhibited by ATP or CTP



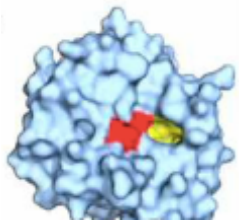
ENZYMES WITHOUT CATALYTIC SUBUNITS (SWITCH)

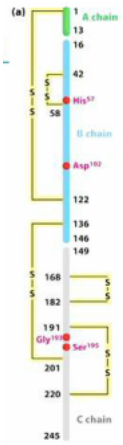
- Forms 3 dimers
- Are catalytically inactive because the active site has been taken away
- Bind both ATP and CTP, though for no helpful reason

ALPHA-CHYMOTRYPSIN STRUCTURE

A pancratic protease involved in digestion i.e. it breaks down protein. It does this by hydrolysing the peptide bonds on the carboxyl side of Tyr, Trp and Phe.

(1) 5 disulfide bonds within the A, B and C chains
(2) Hydrophobic pockets which bind Phe, Tyr or Trp in the substrate position for efficient creation of the ES complex.
(3) The **oxyanion hole** is the active site and is described as a **catalytic triad** of Asp102, His57 and Ser195.

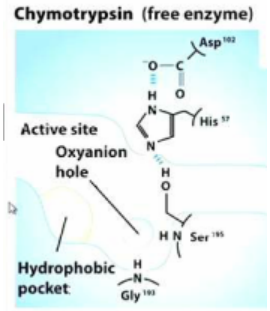


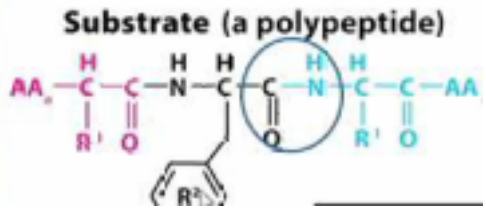


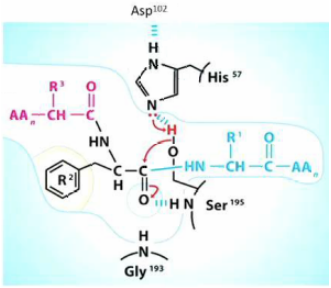
ALPHA-CHYMOTRYPSIN FUNCTION

Its reaction mechanism uses acid-base catalysis and the formation/breaking of covalent bonds;

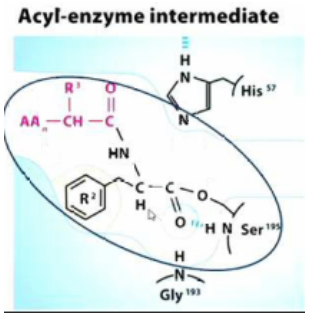
(1) **Pre-acylation –** substrate (a protein) comes in and binds to the aromatic residue adjacent to the peptide bond before nestling into a hydrophobic pocket of the chymotrypsin. This gives you the **ES complex.**







(2) **Acylation** – cleavage of substrate pepside bond - addition of an acyl chain. It acylates a Serine residue (His is deprotonated to accept the proton from Ser) to form an acyl enzyme intermediate.
   a. **Asp forms a hydrogen bond with His** – this deprotonates Ser (catalytic triad)
   b. **Ser side chain binds to the sliced carbonyl carbon of the protein main chain** – note that, here, serine is acting as a nucleophile, and the carbonyl half of the protein bond which can be slightly electrophilic because of its double bond quality
   c. **Carbonyl group is stabilised by hydrogen –** the hydrogens come from the serine and the glycine functional groups. i.e. the carbonyl is starting to be quite happy without the peptide bond
   d. **Peptide bond is cleaved** – now you have an acyl linkage with the carbonyl part of the polypeptide and the serine. (The N terminus part iso just released from the substrate)



(3) **Deacylation** – returns the enzyme to its original form whilst hydrolysing its bonds. A water molecule activated by the basic histidine acts as a nucleophile. The oxygen of water attacks the carbonyl carbon of the serine-bound acyl group and the serine -OH group is regenerated. The remaining bound protein fragment of the substrate is released to regenerate free enzyme