Enzymes do not affect K_{eq}

- Enzymes emerge unchanged at the completion of a reaction.
- The enzyme has **no effect on ΔG^0**, which is a function of the initial and final states of the reactants

$$\Delta G^0 = - RT \ln K_{eq}$$

Calculation of K_{eq} including enzyme

$$A + B + Enz \rightleftharpoons P + Q + Enz$$

$$K_{eq} = \frac{[P][Q][Enzyme]}{[A][B][Enzyme]}$$

reduces to one identical to that for the reaction without enzyme

$$K_{eq} = \frac{[P][Q]}{[A][B]}$$

- Enzyme concentration has therefore **no effect on K_{eq}**
- Because enzymes have no effect on **K_{eq}** they **do not affect ΔG^0** as the factors R and T are constant (from the equation $\Delta G^0 = - RT \ln K_{eq}$)
- However equilibrium is reached much faster when enzyme is present because of increased rate of reaction

-As substrate concentration is increased , the v_i increases until it reaches a maximum value V_{max}.

-When further increases in substrate concentration do not further increase v_i the enzyme is 'saturated' with substrate

-Curve is hyperbolic

-At points A or B increasing or decreasing substrate concentration will increase or decrease the ES complexes with a corresponding change in v_i

-At point C, essentially all the enzyme is present as the ES complex

-no free enzyme remains

-further increase in [S] can not increase the rate of the reaction

-Case B is where half the enzyme molecules are 'saturated with' substrate and the velocity is accordingly half the maximal velocity ($V_{max}/2$) at a particular enzyme concentration

- The [ES] is key to understanding the kinetic behaviour
  - The enzyme binds substrate in a relatively fast manner
  - The ES complex then breaks down in a slower second step to form product. Because it is slow it must be rate limiting

$$E + S \rightleftharpoons ES$$

- At low [S] most of the enzyme is in the uncombined E form. The rate is proportional to [S] because the equilibrium of the above is shifted towards formation of ES as [S] increase
- V_{max} is observed when virtually all enzyme is present in the ES complex and [E] is small
- V_{max} reveals the turn-over number of the enzyme ie the number of substrate molecules converted to product by an enzyme molecule per unit time when the enzyme is fully saturated
- The turn-over number is equal to the rate constant k_2, (k_{cat})
- V_{max} reveals the turn-over number of an enzyme if the concentration of the active sites [E_t] is known

$$V_{max} = k_2[E_t] \qquad k_2 = V_{max} / [E_t]$$

- In the Michaelis-Menten expression, the [S] that gives $v_i = \frac{V_{max}}{2}$ is

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

But when $k_{-1} >> k_2$, then $k_2 + k_{-1} \approx k_{-1}$ and $[S] = \frac{k_{-1}}{k_1} = Kd$

Under these conditions, $1/K_m = 1/K_d$ = affinity.

If $k_2 + k_{-1} \neq k_{-1}$, then $1/K_m$ underestimates the affinity $1/K_d$

- Km equals to the dissociation constant of the ES complex if K_2 is much smaller than K_{-1}
- When this condition is met , K_m is a measure of the strength of the ES complex: a high K_m indicates weak binding ; a low Km indicates strong binding

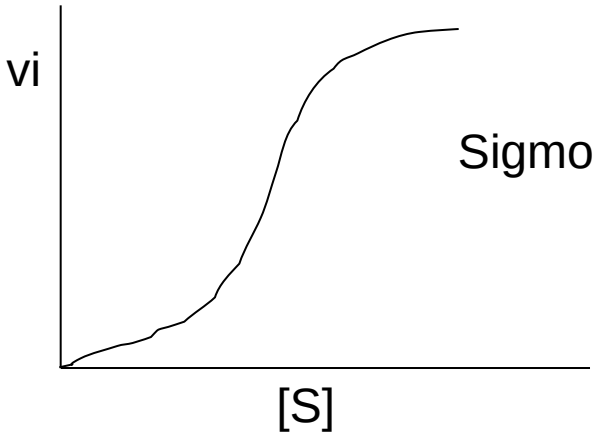
The Hill equation describes the behaviour of enzymes that exhibit cooperative binding of substrate

-Some enzymes bind their substrates in a cooperative fashion analogous to the binding of oxygen by hemoglobin

-Cooperative binding is encountered in multimeric enzymes that bind substrates at multiple sites

-In enzymes that display positive cooperativity in binding to substrate, the shape of the curve that relate vi to changes in [S] is sigmoidal

-Neither the Michaelis-Menten equation nor the Lineweaver-Burk plot can be used to evaluate cooperative saturation kinetics



Sigmoid saturation kinetics