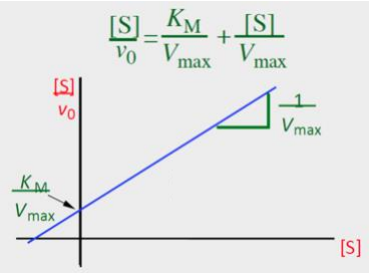
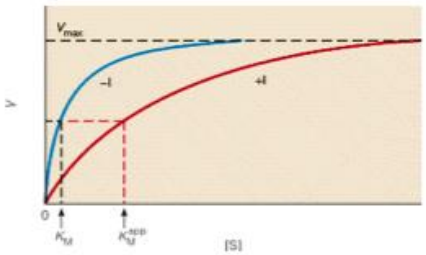
- **Hanes-Woolf plot** – slope is $1/V_{max}$, y-axis intercept is K_M/V_{max}, even weighting of the data.



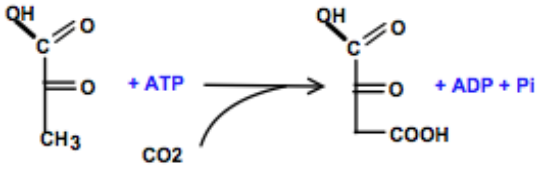
Enzyme Regulation

- **pH**
  - Usually only active in a **narrow pH range** (usually 5-9) due to pH sensitivity of substrate binding, reduced catalytic efficiency of the enzyme, ionisation of substrate, protein structural changes (usually at pH extremes).
  - Kinetic analysis as a function of pH provides information about the nature of functionally (catalytically) important residues.
  - Tend to get a bell-shaped curve when looking at pH and reaction rate, different enzymes have **different optimum pH** suited to environments where they work.
  - **Denaturing** is normally only at extreme changes in pH.
  - pH affects the state of the groups in the active site by protonation, as well as the substrate.
- **Temperature**
  - Increase in temperature gradually increases the rate to a maximum, increasing enzyme activity by making more flexible, then begins to affect active site (reversible), before fairly rapid decrease in rate until enzyme becomes too flexible and eventually denatures.
  - Relatively small impact from increased number of collisions as temperature increases.
- **Types of regulation**
  - Enzyme flexibility provides opportunity for regulation.
  - Can be positive (activation) or negative (inhibition).
  - Can involve covalent binding or non-covalent (ionic, hydrophobic, van der Waals forces) interactions with the regulatory molecule.
  - Can be reversible (covalent and non-covalent) or irreversible (post translational modifications, protein cleavage, irreversible regulatory molecule binding), from point of view of cell economy it doesn't make sense to have many reactions completely irreversible.
  - **Non-covalent regulation** – highly specific so only the target enzyme is affected, two main types are allosteric regulation and simple inhibitors, can bind to E (competitive), E and ES (non-competitive), and ES (uncompetitive) which is more complicated because this is usually in multisubstrate reactions.
- **Competitive Inhibition** – structure similar to substrate, occupies active site, inhibitor (I) and substrate (S) compete, binding of I and S is mutually exclusive, effect is reversed by increasing substrate concentration.
  - **Kinetics of competitive inhibition** – if [S] increases to infinity then all I is displaced so V_{max} remains unchanged, at all [S] I will move equilibrium from E to ES so [E][S]/[ES] will increase so K_M will appear to increase to K_{app} and apparent affinity for substrate is decreased.

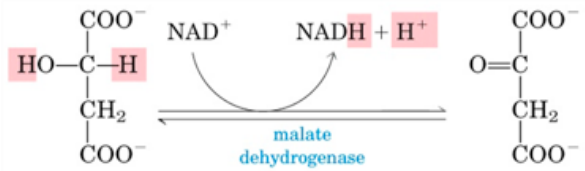


It is assumed that I binds reversibly to the enzyme and is in rapid equilibrium with it so that K_I = [E][I]/[EI], then $\boldsymbol{v_o = V_{max}[S] / K_M(1 + [I]/K_I) + [S]}$, and $V_{max} = k_2[E]_T$.

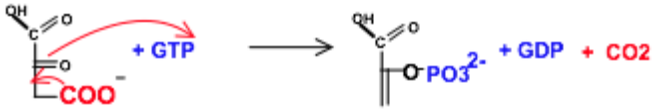
- **Pyruvate carboxylase** – converts pyruvate to oxaloacetate in the mitochondria by carboxylating it (biotin is carboxylated in the biotin carboxylase domain, using ATP, then biotin carrier domain swings CO_2 to the carboxyl transferase domain where pyruvate is carboxylated), Mg^{2+} coordinates.



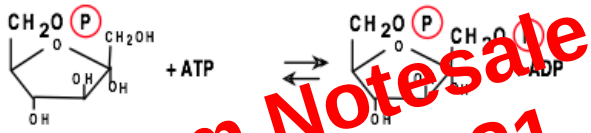
- **Malase** turns oxaloacetate to **malate** to get it out of the mitochondria via the malate shuttle using **malate dehydrogenase** with NAD then it is converted back with NADH and H^+.



- **PEP carboxykinase** – converts oxaloacetate to PEP, requires GTP (made from ATP), requires Mg^{2+} for phosphoryl transfer, occurs outside the mitochondria.



- **Fructose-1,6-bisphosphatase** – converts fructose-1,6-bisphosphate to fructose-6-phosphate (the reverse of the rate determining step in glycolysis), requites ATP, very highly regulated (reciprocal regulation).



- Regulatory steps have become important targets for treating diabetes and obesity – **glucagon** (increases blood glucose) and **insulin** (lowers blood glucose) are important regulators, both act on **phosphofructokinase 2**, insulin dephosphorylates and pushes to pyruvate, glucagon phosphorylates pushes towards glucose.