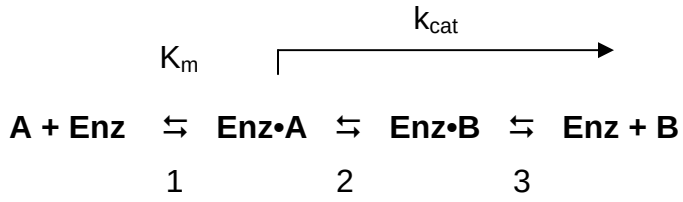
Basic Enzyme Theory

In a metabolic pathway, the interconversion of two intermediates must be catalyzed by a specific enzyme. If the mass-action-ratio lies far away from equilibrium (large -ΔG) then a change in enzymatic activity will directly result in a change in the rate of conversion of the reactant to product. If this reaction step is rate limiting in a metabolic pathway, then this enzyme's activity will directly control the rate of metabolic flux through the entire pathway.

The enzymatic conversion of substrate to product comprises three basic steps.

For an enzyme to carry out its catalytic function, it must be capable of three things: 1) it must bind the substrate, 2) it must convert the substrate into product, and 3) it must release the product for capture by the next enzyme in the metabolic sequence. This is shown in the following model for an enzyme (Enz) catalyzing the net conversion of A to B in the reaction A ⇆ B:



Substrate binding is described by K_m

The overall velocity of the forward reaction A→B is dictated by the affinity of substrate (A) binding to the Enz active site (Step 1), followed by how quickly the bound substrate can be converted into product (Step 2), and how quickly the product (B) can then be released (Step 3). The binding affinity corresponds simply to the K_{eq} for this reaction (Step 1). The greater the value of K_{eq}, the more favorable this step is. However, in enzymology, it is the inverse of K_{eq} that is used (…just to make things confusing for students!!). This term is called K_m, where $K_m = 1/K_{eq}$[1]. Thus, the lower the value of K_m, the more favorable the binding step is, and in most cases the higher the rate of product formation. K_m has the dimensions of concentration; the units for K_m are uM or mM.

Substrate processing and product release are described by k_{cat}

Steps 2 and 3 are combined into a single term called k_{cat}, which represents the combined efficiencies of both the substrate processing step and the ability to release the product. k_{cat} represents the overall "speed" at which a *single enzyme molecule* can carry out these steps (2 and 3). The units for k_{cat} are sec^{-1}. An enzyme with $k_{cat} = 10\ sec^{-1}$, means that one enzyme molecule will convert ten molecules of A to B every second. The actual rate (in uM/sec) at which B is produced in the bulk solution will therefore depend on both k_{cat} as well

[1] This is a very simplified explanation. A more advanced treatment reveals that several qualifications of this explanation are necessary.

as the number of enzyme molecules. BUT - it is the number of enzyme molecules that are *bound with substrate* that is relevant; hence the reason why K_m is also critical. The overall velocity by which an enzyme converts A to B therefore depends on at least three parameters: K_m, k_{cat}, and the enzyme concentration.

Reaction rate increases *hyperbolically* with substrate concentration

In addition to K_m, k_{cat}, and [E], the rate of product formation will also depend on the concentration of substrate. For example, as the concentration of A increases, more enzyme will become bound and the rate of formation of B (the reaction rate) will increase. However, the reaction rate does not increase linearly with the substrate concentration - it increases *hyperbolically* (see Fig. 6.11 Lehninger). This means that it requires more and more substrate to bind the remaining free enzyme molecules as the total enzyme becomes more and more "saturated" with bound substrate. In theory, true enzyme saturation and therefore maximal velocity (V_{max}) occurs only at infinite substrate concentration. In such a hypothetical case, the maximum rate is dictated by only by k_{cat} and the enzyme concentration: $\mathbf{V_{max} = k_{cat}[Enz]}$. If k_{cat} = 10 sec^{-1} and [Enz] = 10 uM, then V_{max} = 100 uM/sec.

If less than 100% of all enzyme molecules are bound (ie. sub-saturating conditions) then the overall rate of product formation will be less than maximum. It will be less by some factor that describes substrate binding. One may assume that this factor must be some function of the substrate concentration [s] and K_m, because these are the only two parameters that influence the extent of substrate binding to enzyme. The mathematical form of this factor is given by: $([s]/([s]+K_m)$, which describes the substrate concentration *relative to the K_m*. The reaction rate (v) at any sub-saturating concentration of substrate is therefore given by:

$$\mathbf{v = V_{max} \quad x \ [s]/([s]+K_m) \quad or}$$

$$\mathbf{v = k_{cat}[Enz] \ x \ [s]/([s]+K_m)}$$

This is the well-known Michaelis-Menten equation. It reveals that K_m corresponds to the substrate concentration when the velocity is one-half the maximum. This is true because when $[s]=K_m$, $[s]/([s]+K_m)= ½$, and therefore $v=V_{max}/2$. It also demonstrates that V_{max} occurs only as [s] approaches infinity, because only then does $[s]/([s]+K_m)$ approach 1, allowing v to approach V_{max}.

Implications for Regulation of Metabolism

Finally, the Michaelis-Menten equation demonstrates how flux (flow or velocity) through a metabolic pathway can be regulated by 1) changing the k_{cat} or K_m values of key regulatory enzymes by, for example, allosteric or covalent modification. 2) Metabolic flux can also be changed by altering enzyme levels by gene transcription/translation. Finally, 3) the availability of substrate can be regulated by compartmentation or other mechanisms.