Michaelis-Menten (steady-state) Kinetics

The Michaelis-Menten model for enzyme kinetics presumes a simple 2-step reaction:

Step 1: Binding – the substrate binds to the enzyme

Step 2: Catalysis – the substrate is converted to product and released

(Note that enzymes not matching this reaction scheme may still show similar kinetics.)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

The Michaelis-Menten equation shows how the initial rate of this reaction, V_o, depends on the substrate concentration, [S]: $V_o = \frac{V_{max}[S]}{K_m + [S]}$

Several simplifying assumptions allow for the derivation of the Michaelis-Menten equation:

(1) The binding step ($E + S \rightleftharpoons ES$) is fast, allowing the reaction to quickly reach equilibrium ratios of [E], [S], and [ES]. The catalytic step ($ES \rightleftharpoons E + P$) is slower, and thus rate-limiting.

(2) At early time points, where initial velocity (V_o) is measured, [P] ≈ 0.

(3) ES immediately comes to steady state, so [ES] is constant (throughout the measured portion of the reaction).

(4) [S] >> [E_T], so the fraction of S that binds to E (to form ES) is negligible, and [S] is constant at early time points.

(5) The enzyme exists in only two forms: free (E), and substrate-bound (ES). Thus, the total enzyme concentration (E_T) is the sum of the free and substrate-bound concentrations: [E_T] = [E] + [ES]

A derivation of the Michaelis-Menten equation shows how to use the above assumptions to describe the rate of the enzyme-catalyzed reaction in terms of measurable quantities:

From (1), we know the overall rate of the reaction is determined by the rate of the catalytic step:	$V_o = k_2[\mathrm{ES}] - k_{-2}[\mathrm{E}][\mathrm{P}]$
From (2), the second term equals zero, so we are left with:	$V_o = k_2[\mathrm{ES}]$
We want to describe V_o in measurable quantities, but [ES] is not easy to measure. However [S] is known, from (4). To express [ES] in terms of [S], we can start from (3):	Rate of formation of ES = Rate of breakdown of ES $k_1[\mathrm{E}][\mathrm{S}] + k_{-2}[\mathrm{E}][\mathrm{P}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$
From (2), this simplifies to:	$k_1[\mathrm{E}][\mathrm{S}] = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}]$
We can factor out [ES] and group the rate constants:	$k_1[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]\{k_{-1} + k_2\}$ $[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]\left\{\frac{k_{-1} + k_2}{k_1}\right\}$
This ratio of rate constants is defined as **the Michaelis Constant, K_m**:	$\boldsymbol{K_m = \frac{k_{-1} + k_2}{k_1}}$
Substituting in K_m for the rate-constant ratio gives:	$[\mathrm{E}][\mathrm{S}] = [\mathrm{ES}]K_m$
Just as [ES] is not easy to measure, [E] is also not easy to measure. However, [E_T] is known. Rearranging (5) for [E] and substituting, we get:	$\{[\mathrm{E_T}] - [\mathrm{ES}]\}[\mathrm{S}] = [\mathrm{ES}]K_m$
We are still trying to get an expression for [ES] in terms of measurable quantities. Here we can multiply, rearrange, factor, and divide, to get [ES] in terms of [E_T], [S], and K_m:	$[\mathrm{E_T}][\mathrm{S}] - [\mathrm{ES}][\mathrm{S}] = [\mathrm{ES}]K_m$ $[\mathrm{E_T}][\mathrm{S}] = [\mathrm{ES}]K_m + [\mathrm{ES}][\mathrm{S}]$ $[\mathrm{E_T}][\mathrm{S}] = [\mathrm{ES}]\{K_m + [\mathrm{S}]\}$ $\frac{[\mathrm{E_T}][\mathrm{S}]}{K_m + [\mathrm{S}]} = [\mathrm{ES}]$
Now we can substitute our expression for [ES] into the rate equation:	$V_o = k_2[\mathrm{ES}] = \frac{k_2[\mathrm{E_T}][\mathrm{S}]}{K_m + [\mathrm{S}]}$
At high [S] (when [S] >>> K_m), nearly all enzyme will have substrate bound, and [ES] approaches [E_T]. This is when V_o approaches V_{max}. Since $V_o = k_2$[ES], (Or, mathematically, when [S] >>> K_m, K_m is negligible, and the equation simplifies to:)	$V_{max} = k_2[E_T]$ $\left(V_{max} = \frac{k_2[\mathrm{E_T}][\mathrm{S}]}{[\mathrm{S}]} = k_2[\mathrm{E_T}]\right)$
Substituting V_{max} in to the rate equation gives **the Michaelis-Menten equation**:	$\boldsymbol{V_o = \frac{V_{max}[\mathrm{S}]}{K_m + [\mathrm{S}]}}$