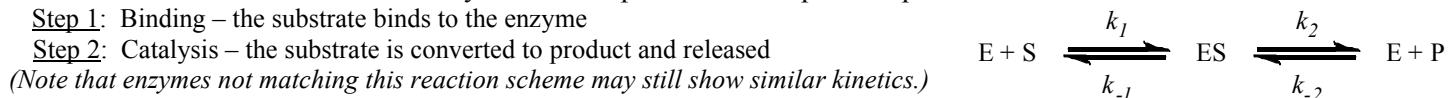


Michaelis-Menten (steady-state) Kinetics

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



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 ($\text{E} + \text{S} \rightleftharpoons \text{ES}$) is fast, allowing the reaction to quickly reach equilibrium ratios of [E], [S], and [ES]. The catalytic step ($\text{ES} \rightleftharpoons \text{E} + \text{P}$) is slower, and thus rate-limiting.
- (2) At early time points, where initial velocity (V_o) is measured, $[\text{P}] \approx 0$.
- (3) ES immediately comes to steady state, so $[\text{ES}]$ is constant (throughout the measured portion of the reaction).
- (4) $[\text{S}] \ggg [\text{E}_T]$, so the fraction of S that binds to E (to form ES) is negligible, and $[\text{S}]$ 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: $[\text{E}_T] = [\text{E}] + [\text{ES}]$

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:	$V_o = k_2[\text{ES}] - k_{-2}[\text{E}][\text{P}]$
From (2), the second term equals zero, so we are left with:	$V_o = k_2[\text{ES}]$
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 $k_1[\text{E}][\text{S}] + k_{-2}[\text{E}][\text{P}] = k_{-1}[\text{ES}] + k_2[\text{ES}]$
From (2), this simplifies to:	$k_1[\text{E}][\text{S}] = k_{-1}[\text{ES}] + k_2[\text{ES}]$
We can factor out [ES] and group the rate constants:	$k_1[\text{E}][\text{S}] = [\text{ES}]\{k_{-1} + k_2\}$ $[\text{E}][\text{S}] = [\text{ES}] \left\{ \frac{k_{-1} + k_2}{k_1} \right\}$
This ratio of rate constants is defined as the Michaelis Constant, K_m :	$K_m = \frac{k_{-1} + k_2}{k_1}$
Substituting in K_m for the rate-constant ratio gives:	$[\text{E}][\text{S}] = [\text{ES}]K_m$
Just as [ES] is not easy to measure, [E] is also not easy to measure. However, $[\text{E}_T]$ is known. Rearranging (5) for [E] and substituting, we get:	$\{[\text{E}_T] - [\text{ES}]\}[S] = [\text{ES}]K_m$
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 $[\text{E}_T]$, [S], and K_m :	$[\text{E}_T][\text{S}] - [\text{ES}][\text{S}] = [\text{ES}]K_m$ $[\text{E}_T][\text{S}] = [\text{ES}]K_m + [\text{ES}][\text{S}]$ $[\text{E}_T][\text{S}] = [\text{ES}]\{K_m + [\text{S}]\}$ $\frac{[\text{E}_T][\text{S}]}{K_m + [\text{S}]} = [\text{ES}]$
Now we can substitute our expression for [ES] into the rate equation:	$V_o = k_2[\text{ES}] = \frac{k_2[\text{E}_T][\text{S}]}{K_m + [\text{S}]}$
At high [S] (when $[\text{S}] \ggg K_m$), nearly all enzyme will have substrate bound, and [ES] approaches $[\text{E}_T]$. This is when V_o approaches V_{max} . Since $V_o = k_2[\text{ES}]$,	$V_{max} = k_2[\text{E}_T]$ $\left(V_{max} = \frac{k_2[\text{E}_T][\text{S}]}{[\text{S}]} = k_2[\text{E}_T] \right)$
(Or, mathematically, when $[\text{S}] \ggg K_m$, K_m is negligible, and the equation simplifies to:)	$V_o = \frac{V_{max}[\text{S}]}{K_m + [\text{S}]}$
Substituting V_{max} in to the rate equation gives the Michaelis-Menten equation :	