

Introduction to Enzyme Kinetics

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Key concepts, definitions, and equations for understanding how enzymes catalyze reactions and how their rates are measured.

1. Basic Terminology

Term	Definition
Enzyme	Biological catalyst that speeds up a chemical reaction without being consumed.
Substrate (S)	Molecule upon which an enzyme acts.
Product (P)	Molecule(s) generated by the enzymatic reaction.
Active site	Region of the enzyme where substrate binding and catalysis occur.
Catalytic constant (k _{cat})	Turnover number; maximum number of substrate molecules converted to product per enzyme molecule per unit time (s ⁻¹).
Michaelis constant (K _M)	Substrate concentration at which the reaction rate is half of V _{max} ; reflects affinity of enzyme for substrate.
V _{max}	Maximum reaction velocity when the enzyme is saturated with substrate.
Rate (v)	Change in product concentration per unit time (e.g., μM·s ⁻¹).
Specific activity	Enzyme activity per milligram of protein (U·mg ⁻¹).

2. The Michaelis-Menten Model

2.1 Reaction scheme



- E – free enzyme
- ES – enzyme–substrate complex
- k₁, k₋₁ – association/dissociation rate constants
- k₂ – catalytic rate constant (often called k_{cat})

2.2 Derivation assumptions

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1. **Steady-state approximation** – concentration of ES remains constant after an initial transient.
2. **Initial-rate conditions** – product concentration is negligible, so reverse reaction ($P \rightarrow S$) can be ignored.
3. **Single substrate** – only one substrate binds to the enzyme.

2.3 Michaelis-Menten equation

$$[v = \frac{V_{\max}[S]}{K_M + [S]}]$$

- When ($[S] \ll K_M$): ($v \approx \frac{V_{\max}}{K_M}[S]$) (first-order with respect to S).
- When ($[S] \gg K_M$): ($v \approx V_{\max}$) (zero-order; enzyme saturated).

2.4 Relationship to kinetic constants

$$[V_{\max} = k_{\text{cat}}[E]_{\text{T}}]$$

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

- **Low (K_M)** → high affinity (enzyme reaches half- $V_{\max}$ at low $[S]$).
 - **High (k_{cat})** → fast turnover.
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3. Experimental Determination of Kinetic Parameters

3.1 Initial-rate method

1. **Prepare a series of substrate concentrations** (typically 5–10 points spanning $<0.1K_M$ to $>10K_M$).
2. **Measure initial reaction rates** (v_0) under identical enzyme concentration, pH, temperature, and ionic strength.
3. **Plot data:**
 - **Michaelis-Menten plot** (v vs. $[S]$) – visual fit.

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- **Linear transformations** for parameter extraction:

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