Kinetics of enzymatic reactions
Biochemical Kinetics: the science that studies rates of chemical reactions

An example is the reaction \( A \rightarrow P \). The velocity, \( v \), or rate, of the reaction \( A \rightarrow P \) is the amount of \( P \) formed or the amount of \( A \) consumed per unit time, \( t \). That is,

\[
\nu = \frac{d[P]}{dt}
\]

or

\[
\nu = \frac{-d[A]}{dt}
\]
Reaction Rate Law

- The rate is a term of change over time
- The rate will be proportional to the conc. of the reactants
- It is the mathematical relationship between reaction rate and concentration of reactant(s)
- For the reaction \((A + B \rightarrow P)\), the rate law is

\[
\text{Rate} = \frac{-\Delta[A]}{\Delta t} = \frac{-\Delta[B]}{\Delta t} = \frac{\Delta[P]}{\Delta t} = -\frac{d[A]}{dt} = k[A]
\]

- From this expression, the rate is proportional to the concentration of A, and \(k\) is the rate constant
Enzymatic reactions may either have a simple behavior or complex (allosteric) behavior.

Simple behavior of enzymes: as the concentration of the substrate rises, the velocity rises until it reaches a limit.

Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots.

Enzyme is saturated: far more substrate than it can deal with.
The maximal rate, $V_{\text{max}}$, is achieved when the catalytic sites on the enzyme are saturated with substrate.

$V_{\text{max}}$ reveals the turnover number of an enzyme.

The number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated with substrate.

At $V_{\text{max}}$, the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction.

Enzyme kinetics
The Michaelis constant (K_m)

- For a reaction:

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P
\]

- \( K_m \), called the Michaelis constant is

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

- In other words, \( K_m \) is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex.

- \( K_m \) describes the affinity of enzyme for the substrate.
Expression of enzyme kinetic reactions
Michaelis-Menten equation

- A quantitative description of the relationship between the rate of an enzyme catalyzed reaction ($V_0$) & substrate concentration [S]

- The rate constant ($K_m$) and maximal velocity ($V_{max}$)

$V_0 = V_{max} \frac{[S]}{[S] + K_M}$

The substrate concentration at which $V_0$ is half maximal is $K_m$

When [S] << $K_m$
Linear relation $V = X[S]$

When [S] >> $K_m$
Independent relation

$V_0 = \frac{V_{max} [S]}{K_m}$

$\frac{1}{2} V_{max} = \frac{V_{max} [S]}{K_m + [S]}$

$K_m = [S]$, when $V_0 = \frac{1}{2} V_{max}$

[S] (mM)
The Michaelis constant (Km)

- The lower the $K_m$ of an enzyme towards its substrate, the higher the affinity.

- When more than one substrate is involved? Each will have a unique $K_m$ & $V_{max}$.

- $K_m$ values have a wide range. Mostly between (10^-1 & 10^-7 M).
**KM & KD**

**[E], KM & Vmax**

- $K_D$: dissociation constant, The actual measure of the affinity
- $K_D = \frac{k_{-1}}{k_1}$

When you increase the enzyme concentration, what will happen to $V_{max}$ & $K_m$?
Vmax & kcat

- For the enzymatic reaction
  \[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

- The maximal rate, $V_{\text{max}}$, is equal to the product of $k_2$, also known as $k_{\text{cat}}$, and the total concentration of enzyme
  \[ V_{\text{max}} = k_2 [E]_T \]

- $k_{\text{cat}}$, the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated
  \[ k_{\text{cat}} = \frac{V_{\text{max}}}{[E]_T} \]

- In other words, the maximal rate, $V_{\text{max}}$, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known
Example

- A $10^{-6}$ M solution of carbonic anhydrase catalyzes the formation of $0.6$ M $\text{H}_2\text{CO}_3$ per second when it is fully saturated with substrate.
  - Hence, $k_{\text{cat}}$ is $6 \times 10^5$ s$^{-1}$
  - $3.6 \times 10^7$ min$^{-1}$

- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is $1.7$ μs for carbonic anhydrase.

- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to $10^4$ per second.
Reaction rate (v); Enzyme activity; Specific activity; Turnover number

- Reaction rate; measures the **concentration** of substrate consumed (or product produced) **per unit time** (mol/[L.s] or M/s)
- Enzyme activity; measures the **number of moles** of substrate consumed (or product produced) **per unit time** (mol/s)
  - Enzyme activity = rate of reaction × reaction volume
- Specific activity; measures **moles of substrate converted per unit time per unit mass of enzyme** (mol/[s.g])
  - Specific activity = enzyme activity / actual mass of enzyme
  - This is useful in determining enzyme purity after purification
- Turnover number; measures **moles of substrate converted per unit time per moles of enzyme** (min⁻¹ or s⁻¹)
  - Turnover number = specific activity × molecular weight of enzyme
A solution contains initially $25 \times 10^{-4}$ mol L$^{-1}$ of peptide substrate and 1.5 μg chymotrypsin in 2.5 ml volume. After 10 minutes, $18.6 \times 10^{-4}$ mol L$^{-1}$ of peptide substrate remain. Molar mass of chymotrypsin is 25,000 g mol$^{-1}$.

- How much is the rate of the reaction?
  - (conc./time)
- How much is the enzyme activity?
  - (mol./time)
- How much is the specific activity?
  - (enz. Act. / enz. Mass)
- How much is the turn over number?
  - (sp. Act. X enz. molar mass)
Determining the $K_m$ from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach $V_{max}$.

This prevents the calculation of both $V_{max}$ & $K_m$.

Lineweaver-Burk plot: A plot of $1/v_0$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an $y$-intercept of $1/V_{max}$ and a slope of $K_m/V_{max}$.

The intercept on the $x$-axis is $-1/K_m$. 

\[
\frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}
\]
A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, $V_{\text{max}}$ of this enzyme is ... & $K_m$ is ...?

<table>
<thead>
<tr>
<th>Substrate Concentration (μM)</th>
<th>Initial velocity (μmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
</tr>
<tr>
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<tr>
<td>1000</td>
<td>698</td>
</tr>
<tr>
<td>5000</td>
<td>699</td>
</tr>
</tbody>
</table>

You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme converts 9.6 μmol per min at 25°C. the turn-over number ($k_{\text{cat}}$) for the enzyme is:

A. 9.6 s$^{-1}$            B. 48 s$^{-1}$            C. 800 s$^{-1}$
D. 960 s$^{-1}$            E. 1920 s$^{-1}$