

# Enzyme kinetics

BIOS 10016

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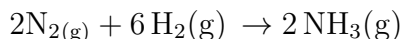
## Objectives

- Describe the principles behind enzyme kinetics.
- Describe the criteria and assumptions required to analyze a system using Michaelis-Menten kinetics, and the parameters that are yielded.
- Prepare Michaelis-Menten and Lineweaver-Burk plots from raw data.
- Calculate elements of the Michaelis-Menten equation when provided sufficient information.
- Calculate the turnover number and specificity constant.
- Extract kinetic data from the Lineweaver-Burk plot.
- Describe the limits at which the turnover number and the specificity constant drive the reaction.

# Enzymes

## General properties of enzymes

- Have high reaction rates ( $10^6$  to  $10^{12}$  times faster than uncatalyzed reactions)
- Mild reaction conditions



- Regulated activity
- Reaction specificity
  - Absolute specificity: only one substrate (urease  $\rightarrow$  urea)
  - Relative specificity: few related substrates (e.g. hexokinase  $\rightarrow$  glucose and fructose)
  - Stereospecific specificity: only one isomer (D or L form)

## Active sites

Substrates bind using noncovalent interactions to **active sites**. Active sites are a unique microenvironment that binds the substrate and catalyzes the reaction.

**Induced fit** = changing the shape of the enzyme to fit the substrate.

Initial binding of the substrate to the active site depends on IMFs.

## Structural complementarity

**Geometric**: substrate and enzyme structures match structurally

**Electronic**: substrate and enzyme structures match electronically (opposite charges)

**Stereospecificity**: chiralities match

## Binding energy and transition states

or  $\Delta G_B$

The energy associated with substrate binding to the active site that pays for the entropy associated with binding.

Energy comes from:

1. Enthalpy from IMFs
2. Entropy of  $\text{H}_2\text{O}$
3. Entropy of the substrate

Transition states may block spontaneous processes. The transition state is short-lived, high-energy, and determines reaction rate.

To speed up: lower activation energy or increase  $\Delta G$  of S.

**Transition state analogs** are excellent enzyme inhibitors. The molecules mimic a transition state and bind to the active site, preventing substrate binding.

**Dissociation constant** - how tightly a substrate binds to a protein or enzyme. A smaller  $K_D$  means stronger binding. ( $K_i$  is  $K_D$  for an inhibitor.)

## Arrhenius equation

$$k = Ae^{-\frac{E_a}{RT}} \quad (1)$$

where  $k$  is the rate constant,  $A$  is the pre-exponential factor,  $E_a$  is the activation energy,  $R$  is the gas constant, and  $T$  is the temperature in Kelvin.

# Kinetics

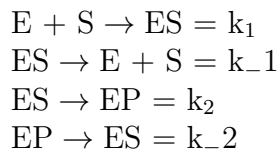
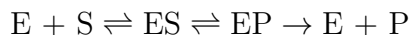
## Enzyme kinetics

Consider a first-order reaction: an enzyme E converts one substrate S into one product P.



$$\text{rate} = \frac{d[P]}{dt} = \frac{-d[S]}{dt}$$

An enzyme is like a factory assembly line - rate of product formation depends on the time it takes to make a product ( $V_{max}$ ,  $k_{cat}$ ) and the time it takes for materials to arrive



## Dependence of reaction rate on [S]

The more substrate, the greater the chance for collisions

$$\text{rate} = \text{velocity} = V \propto [S]$$

To study enzyme kinetics, study it far away from equilibrium

$v_0$  = initial rate of reaction (initial velocities)

## Reaction order

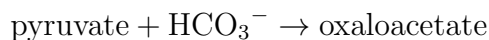
The number of molecules that react in the rate determining step (slowest step)

**first-order reaction** rate =  $k[S]$ , rate  $\propto [S]$

**zero-order reaction** reaction rate is not proportional to  $[S]$ , observed when  $[S] \gg [E]$   
on the flat line

$$\text{rate} = k_{cat}$$

**second-order reaction** reaction rate  $\propto [S]_1$  and  $[S]_2$



$$\text{rate} = k[S]_1[S]_2$$

**pseudo first-order reaction** reaction rate  $\propto [S]_1$  only (the independent substrate is present in excess amounts)



$$\text{rate} = k[S]_1$$

## Michaelis-Menten kinetics

- Simplest, but very useful model
- Requires collection of initial velocity data
- Can be applied to study enzyme kinetics providing..
  - 1st or pseudo 1st order
  - hyperbolic
- Based upon the reaction  $E + S \rightleftharpoons ES \rightarrow E + P$  or binding  $\rightleftharpoons$  processing
- **Michaelis-Menten complex** =  $K_m$  = **enzyme-substrate complex** (ES)
- Looking only at initial velocities (no  $k_{-2}$ )
- Assumptions:
  - Minimal amount of enzyme  $[E] \ll [S]$
  - No product ( $<10\%$ ) and the forward reaction predominates,  $V = V_0$
  - $k_{-1} \gg k_2$  ( $ES \rightarrow E + S$  is much faster than  $ES \rightarrow E + P$ )
  - Steady state condition ( $[ES]$  will not change)

## The Michaelis-Menten equation

$$v_0 = \frac{V_{max}[S]}{[S] + K_m} \quad (2)$$

where:

- $v_0$  = initial velocity
- $V_{max}$  = maximum velocity, line approaches this value asymptotically
- $[S]$  = substrate concentration
- $K_m$  = Michaelis constant (the substrate concentration at which the reaction rate is half of  $V_{max}$ )

## Extracting $K_m$ and $V_{max}$ values

Linear plots:

- **The Lineweaver-Burke Double Reciprocal Plot**
- Woolf-Hofstee Plot
- Hanes-Woolf Plot
- Eadie-Scatchrd Plot

Deriving the Lineweaver-Burk relationship:

$$v_0 = \frac{V_{max}[S]}{[S] + K_m}$$
$$\frac{1}{v_0} = \frac{[S] + K_m}{V_{max}[S]}$$
$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \left( \frac{1}{[S]} \right) + \frac{1}{V_{max}}$$

Slope:  $K_m/V_{max}$

x-int:  $\frac{-1}{K_m}$

## The Michaelis constant, $K_m$

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

$k_2$  is negligible. Cancel and rearrange to get:

$$K_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

Units: M

Small value = tighter binding (high affinity)

Large value = weaker binding (low affinity)

The constant value depends on

- enzyme type
- substrate
- reaction conditions (pH, temperature, ionic strength)

$K_m$  is an approximation of substrate affinity, provided  $k_2 \ll k_{-1}$ .

$V_{max}$

A value that depends on enzyme properties

Has units: amount/time

**Turnover number,  $k_{cat}$ ,  $k_2$**

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

units:  $s^{-1}$

**The specificity constant**

A useful term to describe enzyme-substrate systems

Quantitative term to assess **catalytic efficiency** (how efficiently an enzyme converts substrate to product)

Specificity constant =  $\frac{k_{cat}}{K_m}$  (rate of processing/binding of S)

Units:  $M^{-1}s^{-1}$

$$\frac{k_{cat}}{K_m} = \frac{\text{higher rate}}{\text{higher affinity}} = \frac{k_{cat} \text{ is bigger}}{K_m \text{ is smaller}}$$

**Dual nature of the Michaelis-Menten equation**

Combination of 0-order and 1st-order kinetics

The Michaelis Menten equation describes a hyperbolic dependence on [S]

Low-substrate concentration:  $v = \frac{k_{cat}}{K_m}$

High-substrate concentration:  $v = k_{cat}$