Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 11

Enzyme Kinetics, Continued

15 February 2022

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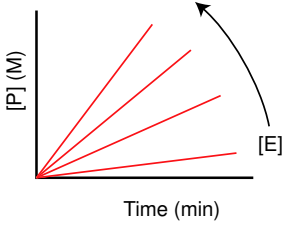
COVID-19 Exposure in BIOL 3515/CHEM 3515

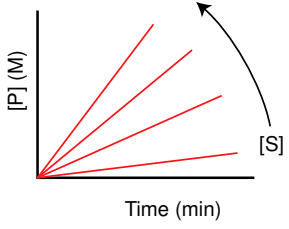
- Exposures:
  - Wednesday lab section: 9 February
  - Thursday lecture and quiz: 10 February
- If you are vaccinated and boosted:
  - Continue coming to campus unless you have COVID symptoms or a positive COVID test.
  - Wear a face mask for 5 days, test after 5 days and continue wearing a mask another 5 days.
- If you are not vaccinated and boosted:
  - Quarantine at home for 5 days.
  - 5 days after exposure get a PCR test. If the test is negative, you may return to campus but must wear a mask for another 5 days.
- 10 day period ends, Sunday, 20 February.

Kinetic Analysis of Enzyme-Catalyzed Reactions

- $E + S \rightarrow E + P$
  E: Enzyme, S: Substrate (reactant), P: Product
- The basic experiment:

  Measure reaction rate (“velocity”) as a function of both enzyme concentration ([E]) and substrate concentration ([S]).





- Rate = $\frac{d[P]}{dt}$ (slope of [P] vs. time)

Mechanistic Framework for Interpreting Enzyme Kinetics

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P}$$

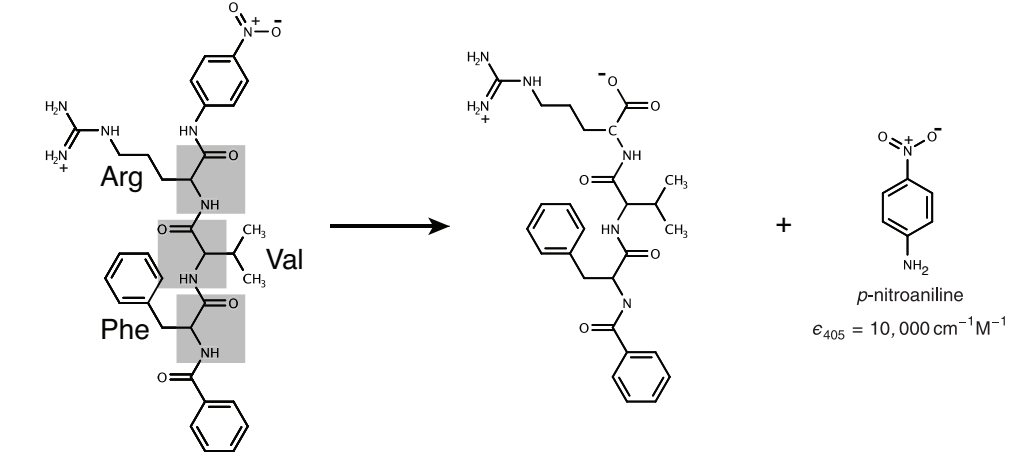
$$V = \frac{[\mathrm{S}]V_{\mathrm{max}}}{K_{\mathrm{m}} + [\mathrm{S}]}$$

$$K_{\mathrm{m}} = \frac{k_{-1} + k_{\mathrm{cat}}}{k_1}$$

$$V_{\mathrm{max}} = k_{\mathrm{cat}} \times \text{Enzyme Concentration}$$

A Chromogenic Substrate for Studying Trypsin

Benzoyl-Phe-Val-Arg-*p*-nitroanilide



- Chromogenic: generates color.
- Nitroanilide (substrate) is colorless.
- Nitroaniline (product) absorbs violet light. Solutions look yellowish.

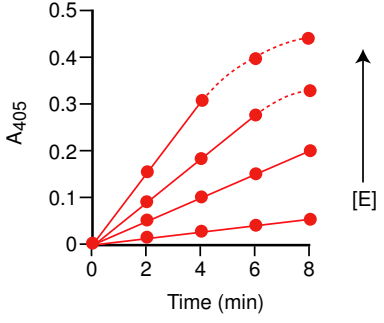
Outline of Enzyme-kinetics Experiment

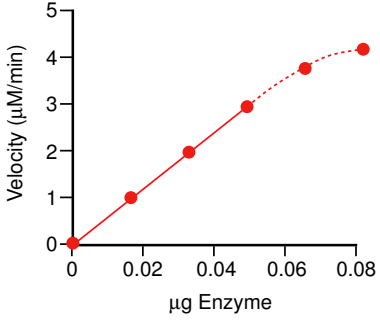
- This week:
  1. Measure velocity as a function of enzyme concentration.
  2. Determine enzyme concentration by titration with an inhibitor.
- Next week:
  1. Determine enzyme concentration by reaction with a “burst substrate”.
  2. Measure reaction velocity as a function of substrate concentration.
- Data analysis. Calculate:
  - K_{m}
  - V_{max}
  - k_{cat}

Experiment 3, Part A:
Velocity as a Function of Enzyme Concentration

- The Michaelis-Menten equation predicts that V is proportional to $[E]_T$:

$$V = \frac{V_{max}[S]}{K_m + [S]} = \frac{k_{cat}[E]_T[S]}{K_m + [S]}$$





- What might cause deviations from linearity in these plots?

Experiment 3, Part B: Measurement of Trypsin Concentration

- To determine k_{cat}, we need to know both V_{max} and the total enzyme concentration:

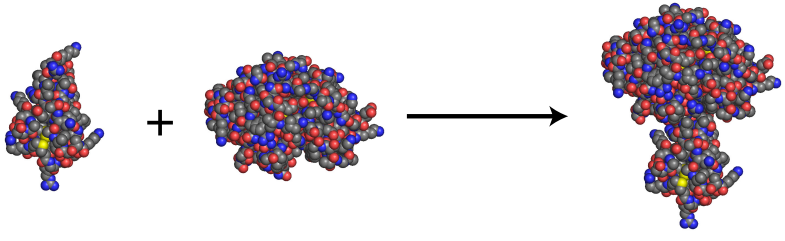
$$V_{max} = [E]_T k_{cat}$$

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

(k_{cat} is more interesting than V_{max}!)

- Why not just measure enzyme concentration by UV absorbance or Bradford assay?
  - Both measure protein concentration.
    Not all of the protein may be active enzyme.
  - This is a particular problem with proteases, which tend to lose activity due to proteolysis.

Experiment 3, Part B:
Measurement of Trypsin Concentration Using BPTI



BPTI (I) Trypsin (E) Complex (E·I)

- Strength of binding is commonly expressed as a dissociation equilibrium constant:

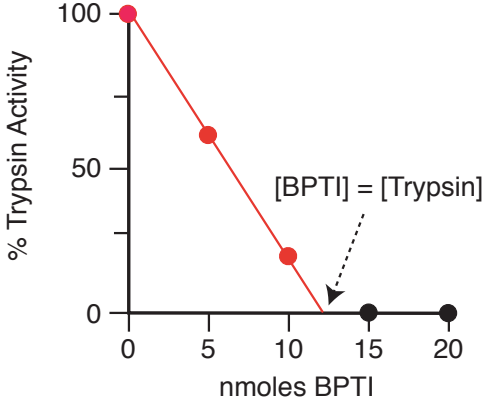
$$K_d = \frac{[E][I]}{[E \cdot I]}$$

Related to, but not identical to, K_m.
Has units of concentration, and a low value indicates tight binding.

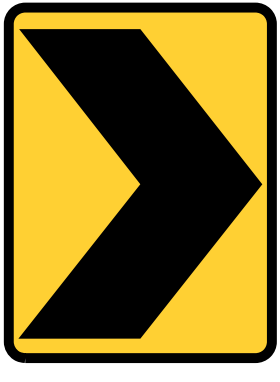
- For BPTI and bovine β-trypsin, $K_d \approx 10^{-12}$ M.
One of the tightest biological complexes known.

Experiment 3, Part B:
Titration of Trypsin with Bovine Pancreatic Trypsin Inhibitor

- Determine [BPTI] from A_{280}
- Mix fixed [trypsin] with increasing [BPTI]
- Measure residual trypsin activity
- Calculate trypsin concentration from equivalence point.



Warning!



Direction Change

A Closer Look at the Michaelis-Menten Mechanism

The Michaelis-Menten Mechanism and Equation

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P}$$

$$V = \frac{[\mathrm{S}]V_{\mathrm{max}}}{K_{\mathrm{m}} + [\mathrm{S}]}$$

The Individual Steps and Rate Constants

- $E + S \xrightarrow{k_1} ES$
  - A second-order reaction
  - In the absence of other reactions:

$$\frac{d[ES]}{dt} = k_1[E][S]$$

  - The second-order rate constant, k_1, has units of $M^{-1}s^{-1}$ or $M^{-1}min^{-1}$

$$\frac{d[ES]}{dt} \quad \text{Units:} \quad M \cdot min^{-1}$$

$$k_1[E][S] \quad \text{Units:} \quad M^{-1}min^{-1} \cdot M \cdot M = M \cdot min^{-1}$$

The Individual Steps and Rate Constants

- $\mathrm{E} + \mathrm{S} \xleftarrow{k_{-1}} \mathrm{ES}$
  - A first-order reaction.
  - In the absence of other reactions:

$$\frac{d[\mathrm{ES}]}{dt} = -k_{-1}[\mathrm{ES}]$$

  - The first-order rate constant, k_{-1}, has units of s^{-1} or min^{-1}

$$\frac{d[\mathrm{ES}]}{dt} \quad \text{Units:} \quad \mathrm{M} \cdot \mathrm{min}^{-1}$$

$$k_{-1}[\mathrm{ES}] \quad \text{Units:} \quad \mathrm{min}^{-1} \cdot \mathrm{M} = \mathrm{M} \cdot \mathrm{min}^{-1}$$

The Individual Steps and Rate Constants

- $\mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P}$
  - A first-order reaction
  - In the absence of other reactions:

$$\frac{d[\mathrm{ES}]}{dt} = -k_{\mathrm{cat}}[\mathrm{ES}]$$

  - The first-order rate constant, k_{cat} has units of s^{-1} or min^{-1}

$$\frac{d[\mathrm{ES}]}{dt} \quad \text{Units:} \quad \mathrm{M} \cdot \mathrm{min}^{-1}$$

$$k_{\mathrm{cat}}[\mathrm{ES}] \quad \text{Units:} \quad \mathrm{min}^{-1} \cdot \mathrm{M} = \mathrm{M} \cdot \mathrm{min}^{-1}$$

  - k_{cat} is also called the *turnover number*; the maximum number of times that an enzyme molecule can convert substrate to product per unit time.

An Important Point

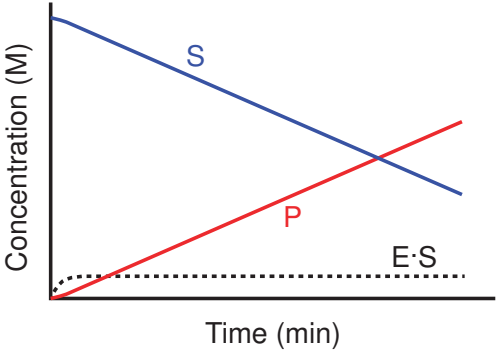
- The rate constants, k_1, k_{-1} and k_{cat} are konstant!

  They do not change with concentration.

- The fine print:

  This is an assumption that may break down at extreme concentrations. The values of the rate constants generally depend on other factors, such as temperature or other solution conditions.

Approaching Steady State after Mixing Substrate and Enzyme



$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P}$$

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_{\mathrm{cat}}[\mathrm{ES}]$$

At steady state:

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_{\mathrm{cat}}[\mathrm{ES}] = 0$$

Solving this equation gives the Michaelis-Menten equation.

The Michaelis-Menten Mechanism and Equation

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_{\mathrm{cat}}} \mathrm{E} + \mathrm{P}$$

$$V = \frac{[\mathrm{S}]V_{\mathrm{max}}}{K_{\mathrm{m}} + [\mathrm{S}]}$$

where:

$$V_{\mathrm{max}} = k_{\mathrm{cat}}\left([\mathrm{E}] + [\mathrm{E}\cdot\mathrm{S}]\right) = k_{\mathrm{cat}}[\mathrm{E}]_{\mathrm{T}} \qquad \text{Units}: \ \mathrm{M}\cdot\mathrm{min}^{-1} = \mathrm{M/min}$$

and:

$$K_{\mathrm{m}} = \frac{k_{-1} + k_{\mathrm{cat}}}{k_1} \qquad \text{Units}: \ \frac{\mathrm{min}^{-1} + \mathrm{min}^{-1}}{\mathrm{min}^{-1}\mathrm{M}^{-1}} = \mathrm{M}$$

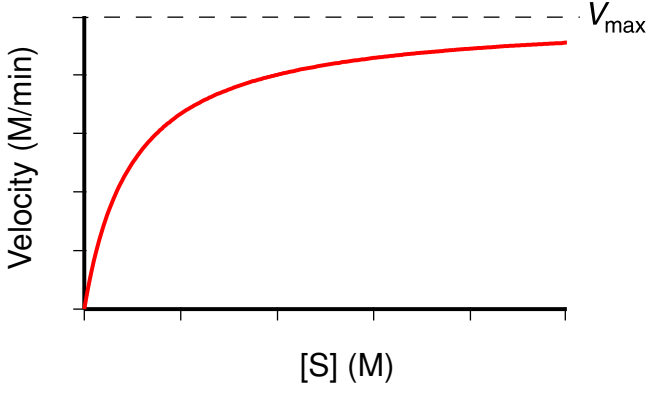
The fraction, V/V_{max}, represents the fraction of enzyme with substrate bound.

When $[S] \gg K_m$:

$[S] \gg K_m$

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

$$V \approx \frac{[S]V_{max}}{[S]} = V_{max}$$



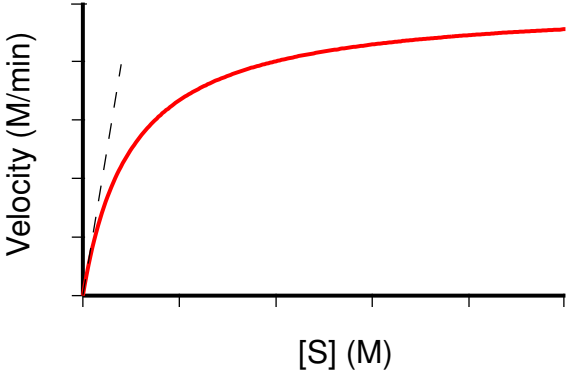
When V/V_{max} approaches 1, nearly all of the enzyme molecules have substrate bound.

When $[S] \ll K_m$:

$$[S] \ll K_m$$

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

$$V \approx \frac{[S]V_{max}}{K_m}$$



In this regime, the fraction of enzyme with substrate bound is:

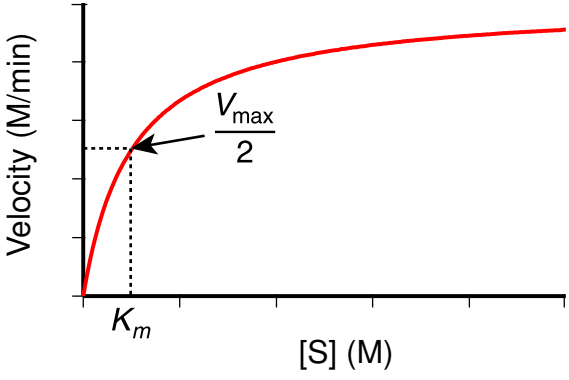
$$\frac{V}{V_{max}} \approx \frac{[S]}{K_m}$$

When [S] equals K_m:

$$[S] = K_m$$

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

$$V = \frac{[S]V_{max}}{2 \times [S]} = \frac{V_{max}}{2}$$



When $V/V_{max} = 1/2$, one half of the enzyme molecules have substrate bound.

K_m is not equal to 1/2 V_{max}!

- The equation:

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

- The units:
  - K_m has units of concentration, like [S].
  - V_{max} has units of reaction velocity, *e.g.,* M/min, like V.

- K_m equals the substrate concentration at which $V = V_{max}/2$.

- Writing $K_m = V_{max}/2$ in a lab report or quiz will cost lots of points!

Clicker Question #3

- For a particular enzyme:

  $K_m = 10\,\mu M$

  $k_{cat} = 15\,s^{-1}$

- If the concentrations are:

  $[S] = 25\,\mu M$

  $[E]_T = 2 \times 10^{-8}\,M$

- Which of these approximations is reasonable?

  A) $V \approx V_{max}$

  B) $V \approx [S]V_{max}/K_m$

  C) Neither

Clicker Question #4

- Given:

$$K_m = 10\,\mu\text{M}$$
$$k_{cat} = 15\,\text{s}^{-1}$$
$$[\text{S}] = 25\,\mu\text{M}$$
$$[\text{E}]_T = 2 \times 10^{-8}\,\text{M}$$

- What is the reaction velocity?

A) $V \approx 2 \times 10^{-8}\,\text{M/s}$

B) $V \approx 2 \times 10^{-6}\,\text{M/s}$

C) $V \approx 0.2\,\mu\text{M/s}$

D) $V \approx 120\,\mu\text{M/min}$

Using the Michaelis-Menten Equation

- $V = \dfrac{[\mathrm{S}]V_{\max}}{K_\mathrm{m} + [\mathrm{S}]}$

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

- $K_\mathrm{m} = 10\,\mu\mathrm{M}$
  $k_{\mathrm{cat}} = 15\,\mathrm{s}^{-1}$
  $[\mathrm{S}] = 25\,\mu\mathrm{M}$
  $[\mathrm{E}]_\mathrm{T} = 2 \times 10^{-8}\,\mathrm{M}$

- $$\begin{aligned} V_{\max} &= k_{\mathrm{cat}}[\mathrm{E}]_\mathrm{T} \\ &= 15\,\mathrm{s}^{-1} \times 2 \times 10^{-8}\,\mathrm{M} \\ &= 3 \times 10^{-7}\,\mathrm{Ms}^{-1} \\ &= 0.3\,\mu\mathrm{Ms}^{-1} \end{aligned}$$

- $$\begin{aligned} V &= \frac{[\mathrm{S}]V_{\max}}{K_\mathrm{m} + [\mathrm{S}]} \\ &= 0.3\,\mu\mathrm{Ms}^{-1} \times \frac{25\,\mu\mathrm{M}}{25\,\mu\mathrm{M} + 10\,\mu\mathrm{M}} \\ &= 0.3\,\mu\mathrm{Ms}^{-1} \times 0.71 \\ &\approx 2 \times 10^{-7}\,\mathrm{Ms}^{-1} \end{aligned}$$