Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 10

A Brief Introduction to Enzyme Kinetics

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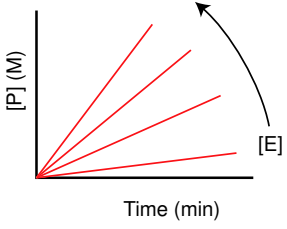
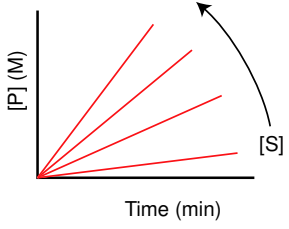
Kinetic Analysis of Enzyme-Catalyzed Reactions

- $E + S \rightarrow E + P$
  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]).

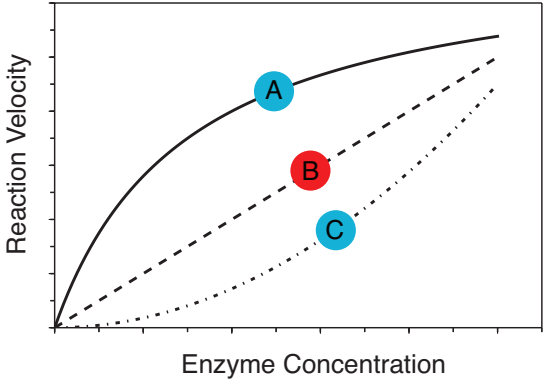




- Rate = $\frac{d[P]}{dt}$ (slope of [P] vs. time)

Clicker Question #3

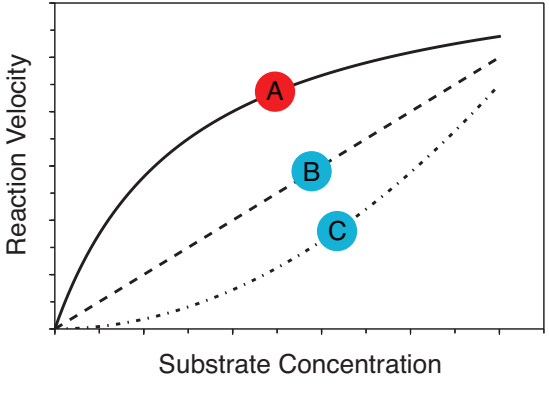
How does velocity change with enzyme concentration?



All answers count (for now).

Clicker Question #4

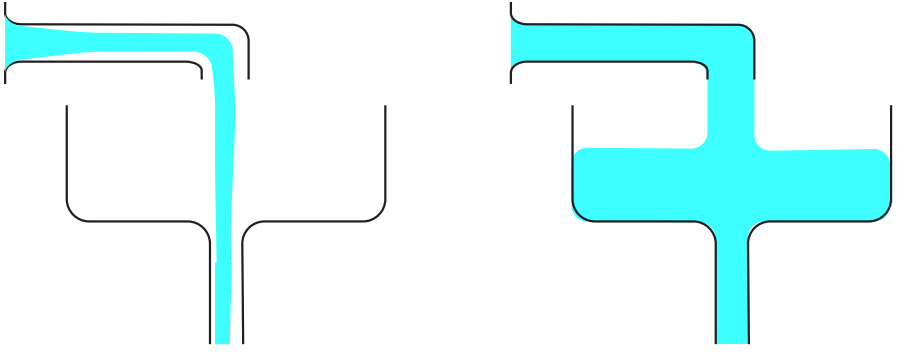
How does velocity change with substrate concentration?



All answers count (for now).

Why Doesn't Velocity Increase Linearly with Substrate Concentration?

A plumbing analogy:



Our Heroes

- Leonor Michaelis (1875–1949)
- Maude Leonora Menten (1879–1960)

Michaelis, L. & Menten, M. L. (1913). Die kinetik der invertinwirkung. *Biochem. Z.*, 49, 333–369.

Johnson, K. A. & Goody, R. S. (2011). The original Michaelis constant: Translation of the 1913 Michaelis-Menten paper. *Biochemistry*, 50, 8264–8269. http://dx.doi.org/10.1021/bi201284u

A Simple Mechanism Accounts for Hyperbolic Kinetics

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

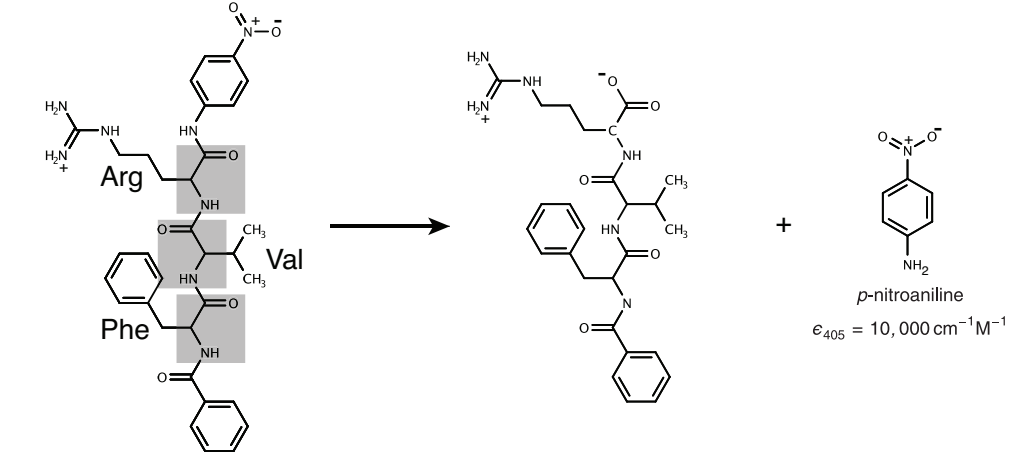
$$V = \frac{[\mathrm{S}]V_{\mathrm{max}}}{K_{\mathrm{m}} + [\mathrm{S}]}$$

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

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

A Chromogenic Substrate for Studying Trypsin

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

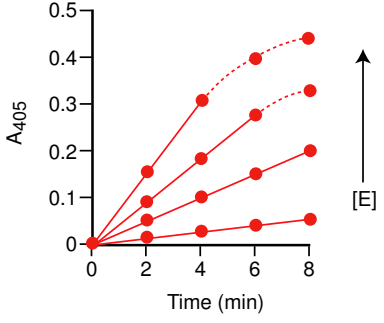


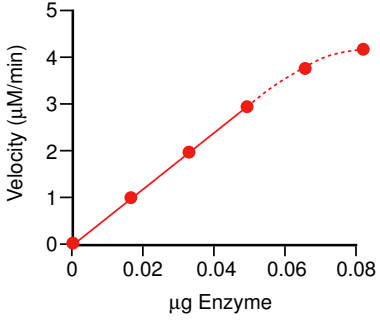
- Chromogenic: generates color.
- Nitroanilide (substrate) is colorless.
- Nitroaniline (product) absorbs violet light. Solutions look yellowish.

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?