

Enzyme Kinetics

BTEC 2020

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Enzymes

- proteins that catalyze (increase the rates of) chemical reactions.



E = enzyme

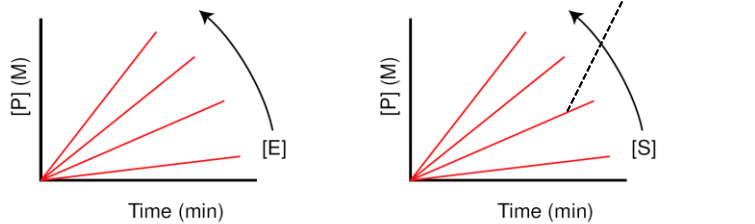
S = substrate

ES = enzyme-substrate complex

P = product

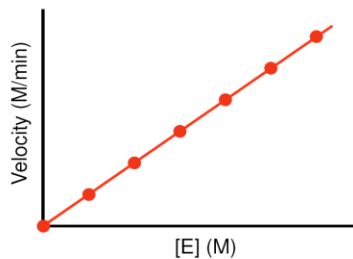
Enzyme kinetics

- study of how enzymes catalyze reactions; involves measuring rates of reactions.
- Basic experiment: measure production of product over time as a function of
 - enzyme concentration
 - substrate concentration



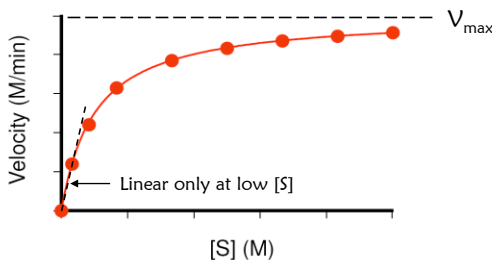
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Velocity of an Enzyme-Catalyzed Reaction is Generally Proportional to Enzyme Concentration



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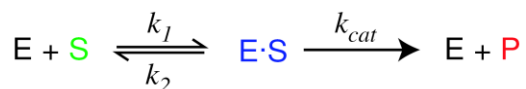
The Velocity Does Not Increase Linearly with Substrate Concentration!



V_{\max} = maximal velocity,
reached when enzyme is “saturated” with substrate

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V_{\max} is dependent on k_{cat} and [enzyme]

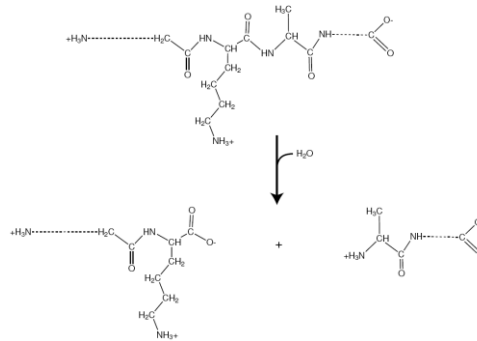


- k_{cat} = *turnover number* of the enzyme
 - the maximum number of substrate molecules converted to products per active site per unit time
 - the number of times the enzyme “turns over” per unit time
- $k_{\text{cat}} \cdot [\text{E}]_{\text{total}} = V_{\max}$
- V_{\max} is determined experimentally (for a particular [E])
- k_{cat} is calculated from [E] and V_{\max}
 - a property of the enzyme and reaction conditions (temperature, pH)

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Trypsin

- Protease
- Hydrolyzes peptide bond C-terminal to lysine or arginine



- Can we easily monitor the hydrolysis of peptide bonds in an ordinary protein?

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BAPNA: a chromogenic substrate for trypsin

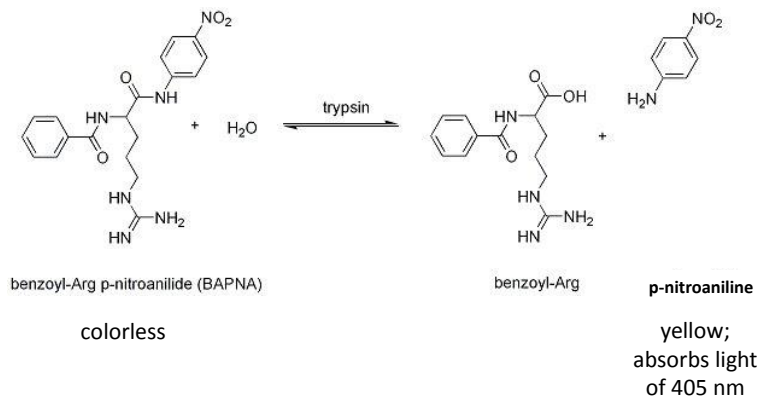


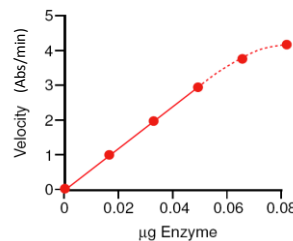
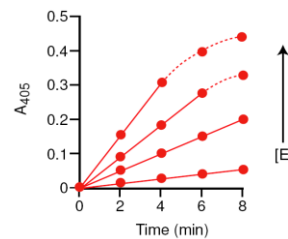
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Trypsin kinetics experiments

- Day 1: Measure reaction velocity as a function of enzyme concentration to establish that
 - velocity is constant with respect to time
 - velocity is proportional to enzyme concentration

Velocity as a function of enzyme concentration

- Measure absorbance over time with varied amounts of enzyme
- Determine slope of each line (Abs / min)
- Slope = velocity
- Plot velocity vs. amount of enzyme



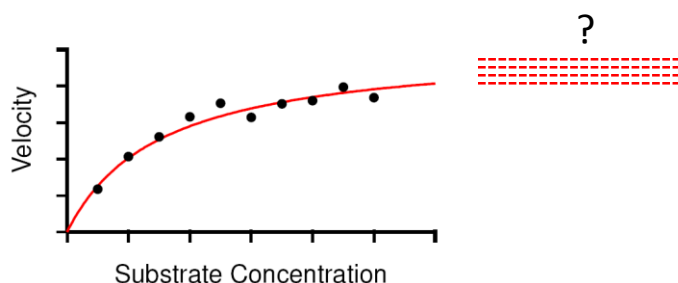
What might cause deviations from linearity in these plots?

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Trypsin kinetics experiments

- Day 1: Measure reaction velocity as a function of enzyme concentration to establish that
 - velocity is constant with respect to time
 - velocity is proportional to enzyme concentration
- Day 2:
 - Measure reaction velocity as a function of substrate concentration
 - determine V_{\max} and turnover number
 - Measure reaction velocity as a function of substrate concentration in the presence of soybean trypsin inhibitor
 - determine V_{\max} and turnover number

Velocity as a function of substrate concentration



- Michaelis-Menten plot
- curve is hyperbolic
- Excel will not fit hyperbolic equations
 - so M-M on Excel won't give us a good estimate of V_{\max}

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the **Lineweaver-Burk** plot: $1/V$ vs. $1/[S]$

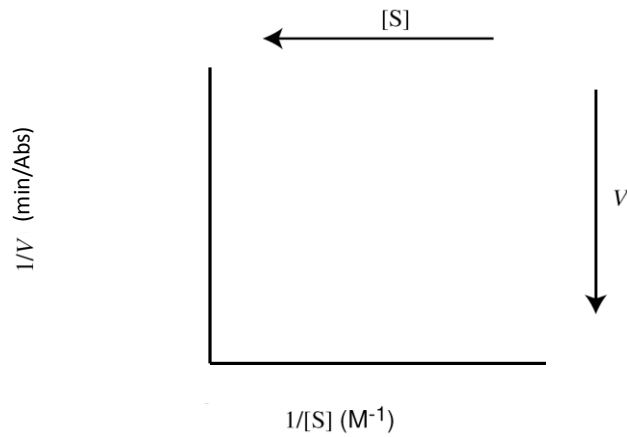


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the **Lineweaver-Burk** plot: $1/V$ vs. $1/[S]$

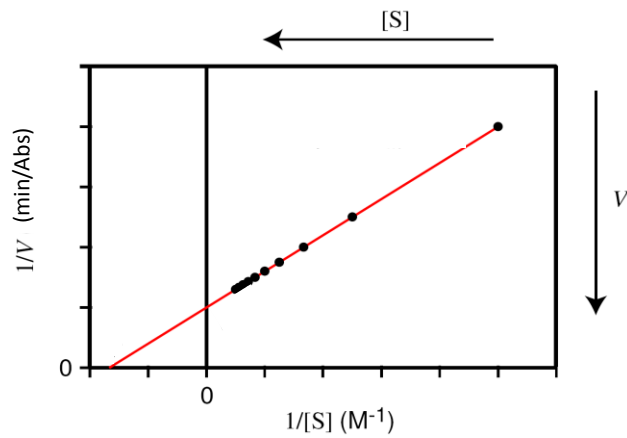


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the **Lineweaver-Burk** plot: $1/V$ vs. $1/[S]$

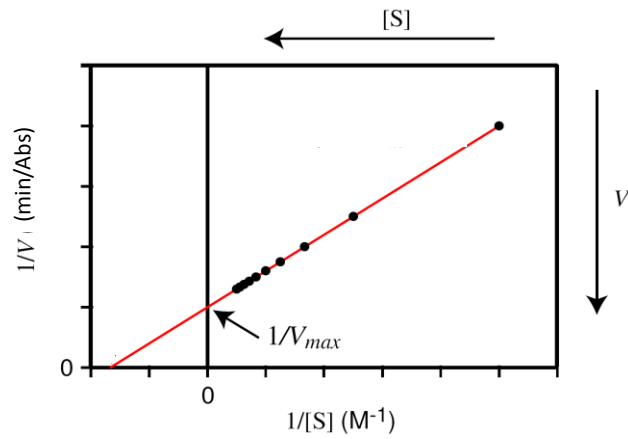


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