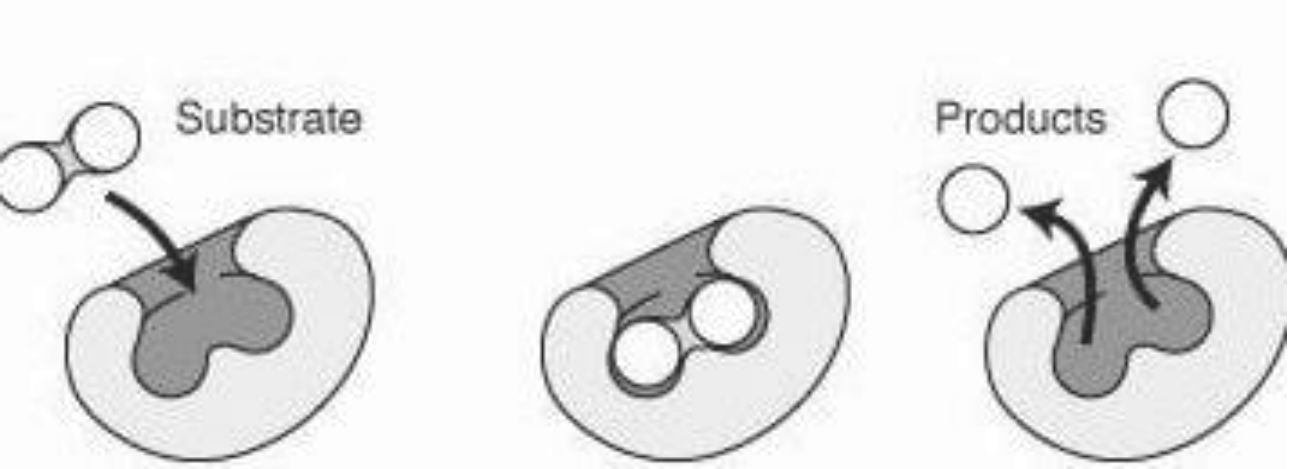
ACTIVE SITE INVESTIGATIONS
(KINETIC STUDIES)



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ACTIVE SITE INVESTIGATION:

1. The active site is a region on the surface of an enzyme where the substrate binds and undergoes a chemical reaction
2. **Active site Investigations** aims to understand the structure and function of the active site of a protein or enzyme.

KINETIC STUDIES:

1. **Kinetic studies are a powerful tool for investigating active sites because they can provide information about:**
2. The rates of reaction
3. The binding affinities of substrates and inhibitors
4. the mechanisms of catalysis.

PURPOSE OF KINETIC STUDIES:

1. Understanding Enzyme Dynamics
2. Evaluating the availability of active sites in macromolecular catalysts.
3. Explain how these rates depend upon various factors such as temperature, pressure, and the presence of catalysts
4. Determining Reaction Rate Models
5. Provides a Quantitative Measurement of Rates of Reactions.

TECHNIQUES IN KINETIC STUDIES

Initial Rate Studies

- It measure the rate of reaction at different substrate concentrations.

Progress Curves

- It measure the concentration of the product or substrate over time.

Steady-State Kinetics

- It measure the rate of reaction at a constant concentration of substrate.

TECHNIQUES IN KINETIC STUDIES:

Transient Kinetics

It measure the rate of reaction very quickly, often using stopped-flow or pulse-chase methods.

Single Molecule Kinetics

Observe individual enzyme-substrate interactions in real-time.

Michaelis-Menten Kinetics

This model helps to determine how quickly an enzyme converts substrate into product.

TECHNIQUES IN KINETIC STUDIES:

Lineweaver Burk Plot

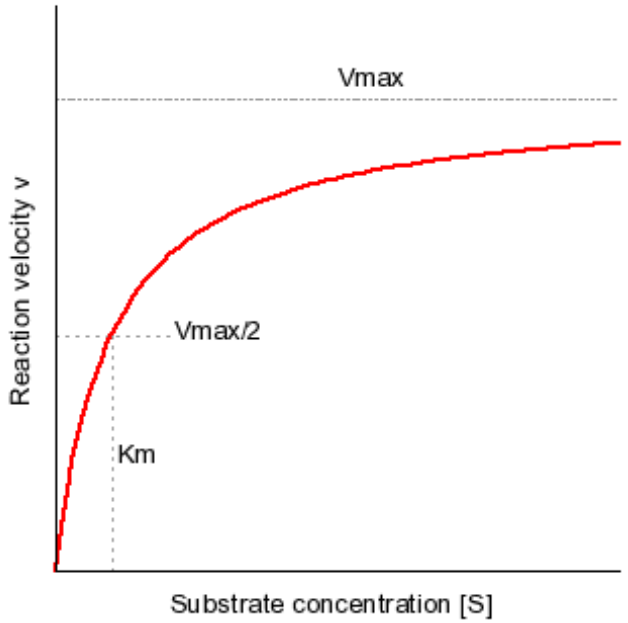
- This double reciprocal plot simplifies the determination of kinetic parameters, such as Km and Vmax and allows for easy visualization of enzyme inhibition.

Molecular Dynamics Simulations

- Simulating the movements of atoms and molecules to understand enzyme dynamics.

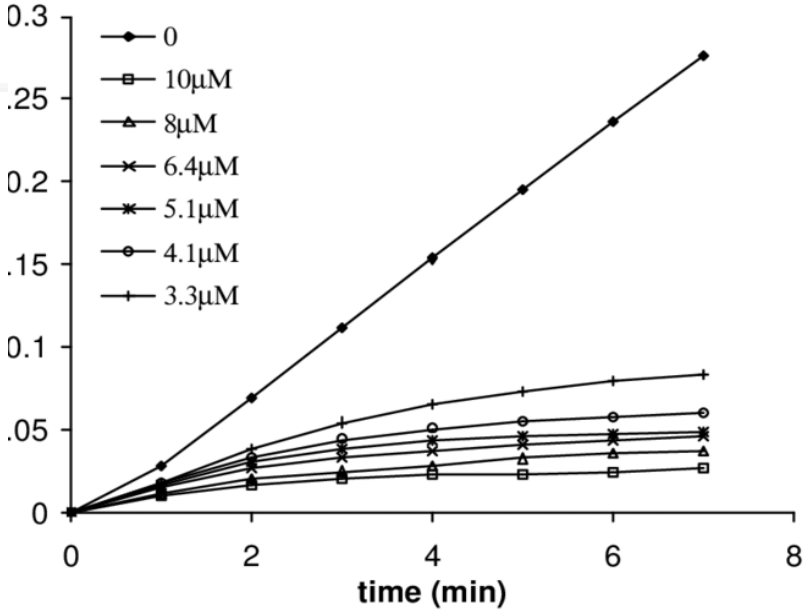
1. INITIAL RATE STUDIES:

- They are performed by measuring the rate of reaction at a series of different substrate concentrations.
- The data can be plotted in a **Michaelis-Menten plot**, which is a graph of the rate of reaction versus the substrate concentration.
- The Michaelis-Menten equation can be used to fit the data to the plot, and the Km can be determined from the equation



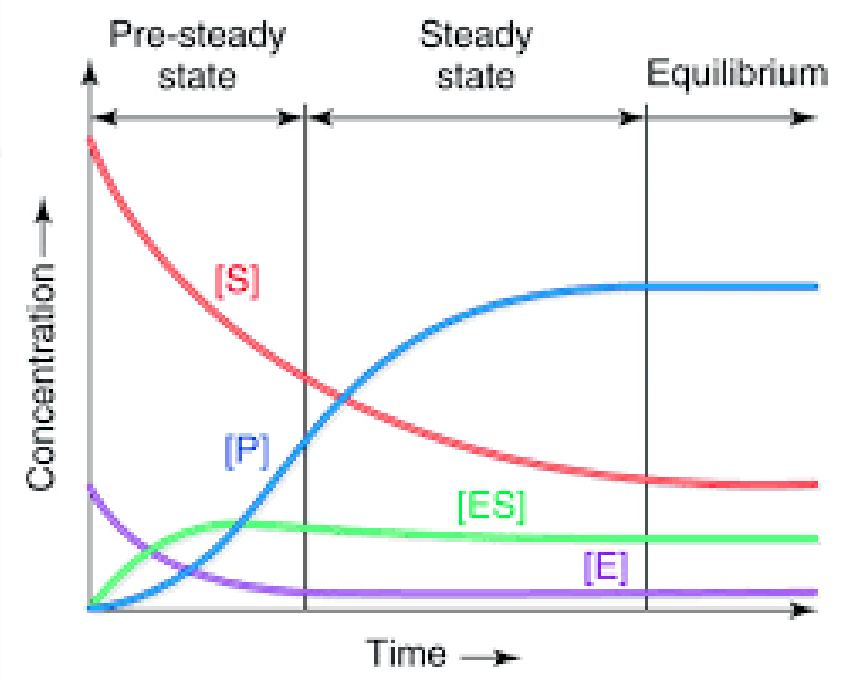
2. PROGRESS CURVES:

- They are typically generated by measuring the concentration of the product or substrate over time.
- These studies can be used to determine the rate of the reaction and the mechanism of catalysis.
- The progress curve will be **Hyperbolic**.



3. STEADY STATE KINETICS

- They are typically performed by measuring the rate of reaction at a **constant concentration** of enzyme and substrate complex.
- The data from these studies → used to determine the Vmax of the reaction and the Km.
- **Vmax** is the **maximum** rate of reaction that can be achieved under the conditions of the experiment.
- **Km** is the **substrate concentration** at which the rate of reaction is half of the Vmax



4. TRANSIENT KINETICS:

- Transient kinetics are typically performed using **Stopped-flow or Pulse-chase methods.**
- **Stopped-flow methods** involve **mixing the enzyme and substrate** very quickly and then measuring the reaction.
- **Pulse-chase methods** involve **adding a pulse of substrate** to the enzyme and then measuring the reaction over time.

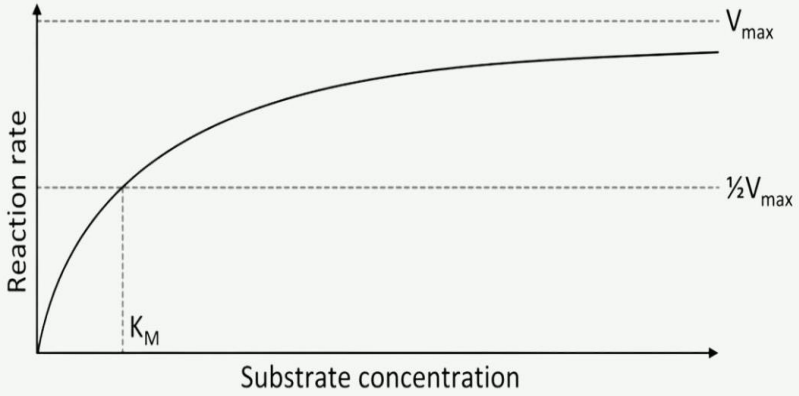
5. SINGLE MOLECULE KINETICS

- Single Molecule Kinetics involves monitoring the reactions of individual molecules (**enzymes and substrates**) with **high spatial and temporal resolution.**

- Single-molecule kinetics allows the direct observation of individual enzyme-substrate binding events and reaction steps.

6. MICHAELIS MENTION EQUATION:

- This model is used to describe enzyme-catalyzed reactions involving one substrate and one product.
- The model assumes that the **reaction proceeds through an enzyme-substrate complex,** which then breaks down to form the product and the enzyme.
- The rate of the reaction is determined by the **rate of formation of the enzyme-substrate complex and the rate of breakdown of the complex**



7. LINEWEAVER BURK PLOT:

- Taking the reciprocal of both sides of the Michaelis-Menten equation yields the Lineweaver-Burk equation.

$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}$$

- The purpose of taking the reciprocal is to **linearize the relationship between 1/v and 1/[S],** making it easier to determine kinetic parameters graphically.

- The Lineweaver-Burk Plot is particularly useful for studying enzyme inhibition.

8. MOLECULAR DYNAMICS SIMULATIONS:

- Molecular dynamics simulations play a key role in enzyme kinetics studies.
- They provide insights into the **structure and dynamics of enzyme-catalyzed reactions.**
- The simulations are based on classical mechanics and use force fields to describe the interactions between atoms.