Rational Design of Enzyme Inhibitors

Introduction to Enzymes as Drug Targets

- **Enzymes** are biological catalysts that increase the rate of a reaction by lowering its **activation-energy barrier**.
- They do not change the overall equilibrium of the reaction; an inhibitor changes how rapidly the enzyme carries out its catalytic function.
- An **enzyme inhibitor** is a substance that reduces an enzyme-catalysed reaction rate by interacting with the enzyme or a defined enzyme state.
    - An inhibitor may compete with a substrate, bind to a different enzyme state or form a reversible or irreversible covalent interaction.
- The observed inhibition therefore depends on the mechanism and experimental conditions; one low IC50 value does not establish selectivity or therapeutic usefulness.

Why Enzymes Are Useful Drug Targets

1. Defined Biochemical Function

- The enzyme links a substrate to a product, so its activity can often be measured directly or through a coupled readout.
    - Inhibiting a disease-relevant reaction can reduce an unwanted product or allow a useful substrate to accumulate.

2. Recognisable Binding Sites

- An active site recognises a substrate, cofactor or transition-state-like arrangement.
- An allosteric site provides a different opportunity to change catalytic activity.

Examples of Therapeutic Enzyme Targets

- **Statins** inhibit HMG-CoA reductase in cholesterol biosynthesis.
- **ACE inhibitors** reduce angiotensin-converting-enzyme activity.
- **Acetylcholinesterase inhibitors** slow the breakdown of acetylcholine.

Requirements for a Useful Inhibitor

- The compound must reach the enzyme, bind in a productive way and remain present for a useful period.
- Selectivity is important when related enzymes perform essential functions in healthy tissues.
- A low concentration that inhibits a purified enzyme is an initial measurement rather than a complete prediction of therapeutic benefit.

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Enzyme Kinetics

- **Enzyme kinetics** studies how reaction rate changes with substrate concentration, enzyme concentration and experimental conditions.
- For inhibitor design, these measurements connect a chemical binding event with loss of catalytic activity and help distinguish different mechanisms of inhibition.

The Michaelis–Menten Model

- A simple single-substrate model is:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

  - The enzyme binds substrate to form a complex, and the complex produces product while releasing enzyme.

Initial-Rate Conditions

- Initial rates are measured before substantial substrate depletion or product accumulation changes the reaction.
  - For the simple model, substrate is normally present in excess over active enzyme sites.
  - The steady-state approximation treats the concentration of the enzyme–substrate complex as approximately constant after the initial transient.

Michaelis–Menten Equation

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

- The initial rate is v, substrate concentration is [S], and the maximum rate at substrate saturation is V_{max}.
- K_m is the substrate concentration at which the rate is half of V_{max} in this simple model.

Meaning of the Parameters

- Read each parameter as an answer to a different question: how much product the enzyme system can form, how much substrate is needed, how rapidly one active site turns over, and how efficiently dilute substrate is used.

A. Maximum Rate

  - **Vmax** is the limiting initial rate approached at saturating substrate concentration for a fixed amount of active enzyme.
  - Its units are amount or concentration of product per unit time, such as µmol/min or µmol/L/min; doubling the active-enzyme amount doubles Vmax under otherwise identical conditions.

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

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B. Michaelis Constant

- **Km** is the substrate concentration at which the initial rate is half Vmax in a Michaelis–Menten system.
- It has concentration units and is a property of the enzyme–substrate system under stated conditions; it is not generally a direct measure of binding affinity.

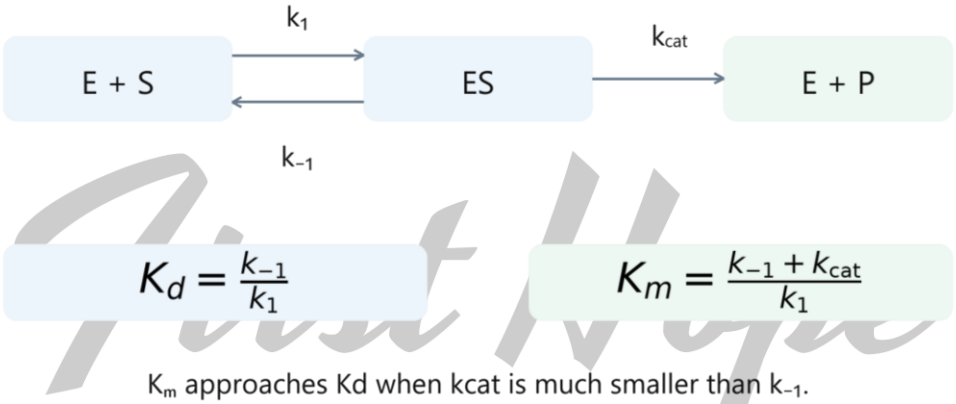
$$K_m = \frac{k_{-1} + k_{cat}}{k_1}$$

- It is not generally identical to the substrate dissociation constant.

$$K_d = \frac{k_{-1}}{k_1}$$

- The two values approach one another when catalytic conversion is much slower than substrate dissociation under the model's assumptions.

Why the Michaelis constant is not always a binding constant



For the simple steady-state mechanism, K_m includes substrate dissociation and catalytic turnover, whereas K_d describes only the binding equilibrium

C. Turnover Number

- The turnover number describes the maximal number of catalytic events per active site per unit time.

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

- The units of enzyme and rate must be consistent when this ratio is calculated.
- If V_{max} is 20 micromolar per minute and the active-site concentration is 0.10 micromolar, k_{cat} is 200 per minute.
- An inactive fraction of the protein preparation can distort this calculation if total protein is mistaken for active enzyme.

D. Catalytic Efficiency

- At low substrate concentration, the ratio of turnover number to K_m describes how effectively an enzyme uses dilute substrate in the simple model.

$$\text{Catalytic efficiency} = \frac{k_{cat}}{K_m}$$

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Behaviour at Different Substrate Concentrations

1. Low Substrate Concentration

- When [S] is much smaller than K_m, the rate increases approximately in direct proportion to substrate concentration.

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

2. Substrate Concentration Equal to Km

$$v = \frac{V_{max}}{2}$$

- At this substrate concentration, the observed rate is halfway to the limiting rate; this is the operational meaning of Km.

3. High Substrate Concentration

- When [S] is much larger than K_m, most active sites are occupied and the rate approaches V_{max}.
- Substrate binding is no longer the main limitation at saturation, so a further increase in substrate produces only a small increase in rate.

Worked Calculation

- Consider an enzyme with V_{max} of 100 micromoles per minute and K_m of 2 mM.
- At a substrate concentration of 2 mM:

$$v = \frac{100 \times 2}{2 + 2} = 50 \text{ micromoles per minute}$$

- At a substrate concentration of 6 mM:

$$v = \frac{100 \times 6}{2 + 6} = 75 \text{ micromoles per minute}$$

- Tripling substrate from 2 to 6 mM does not triple the rate because the enzyme is moving toward saturation.

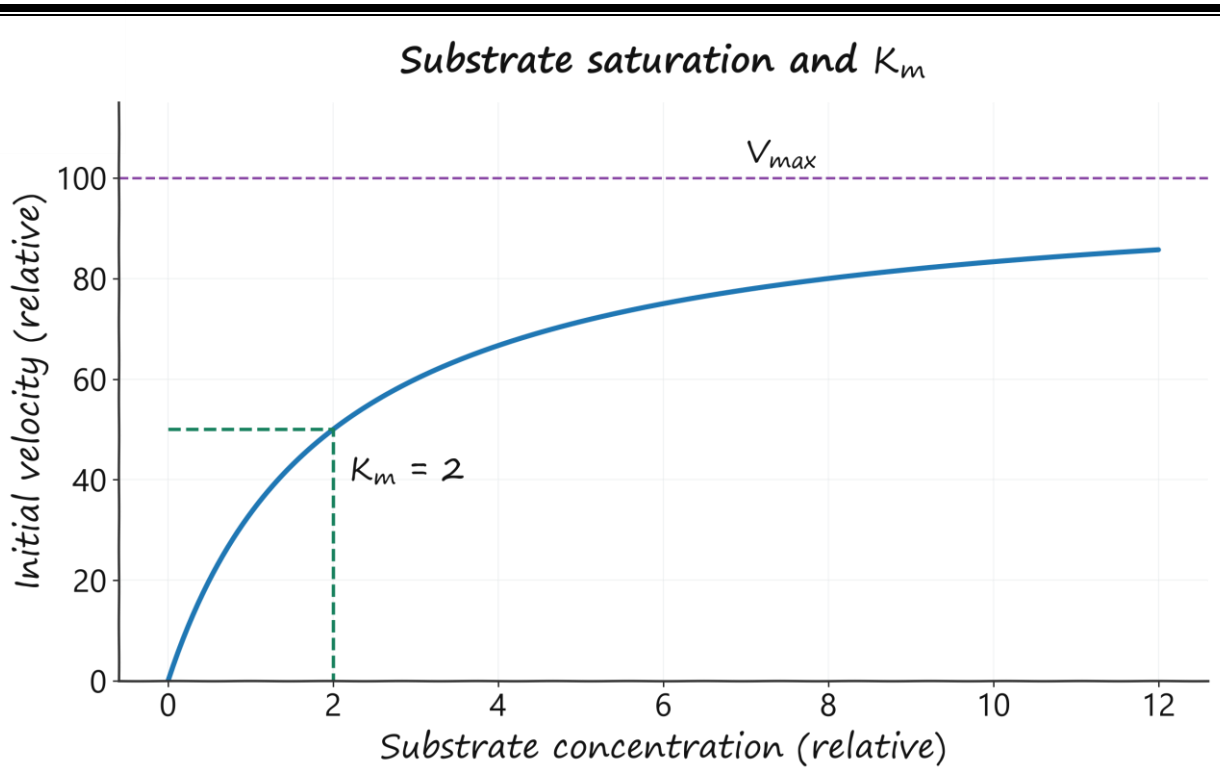
Graphical Representation

1. Direct Rate–Substrate Plot

- A Michaelis–Menten plot is hyperbolic for the simple model.
- Fitting the direct data by a suitable nonlinear method is generally preferable to estimating parameters from a hand-drawn reciprocal plot.

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In the simple Michaelis–Menten model, the velocity is half of V_{max} when substrate concentration equals K_m

2. Lineweaver–Burk Form

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

- The reciprocal form has a slope of K_m/V_{max} and a vertical intercept of $1/V_{max}$.
- The x-intercept is negative reciprocal Km; the intercepts are extrapolations, and the negative horizontal region does not represent a physically negative substrate concentration.
- It is useful for teaching patterns but magnifies errors in low-substrate measurements.

Factors Affecting Measured Activity

- Temperature, pH, cofactors, ionic conditions and enzyme stability can change the observed rate.
- Substrate inhibition, cooperativity or multiple substrates can require a model more complex than the simple Michaelis–Menten expression.

When the Simple Model Is Inadequate

- Cooperative enzymes can give sigmoidal rather than ordinary hyperbolic response curves.
- Multiple substrates, slow conformational changes and substrate inhibition can require other models.
- A good fit should be supported by the experimental design and plausible mechanism, not chosen only because the equation is familiar.

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