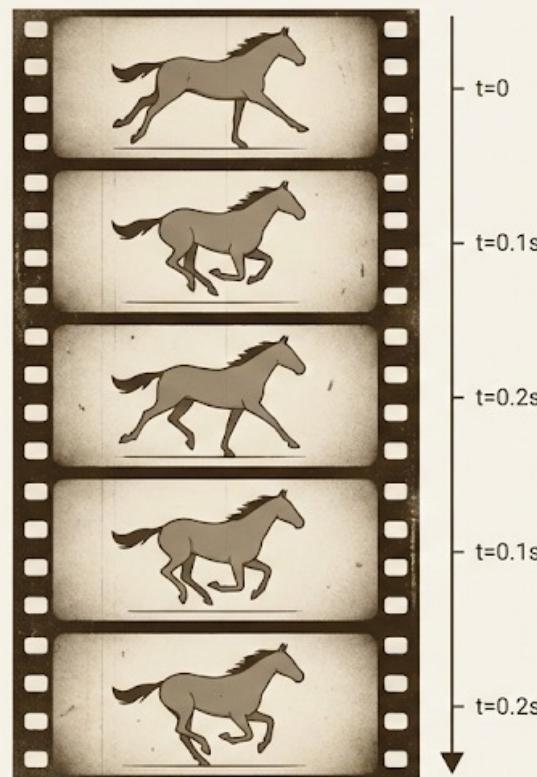


CHAPTER 8

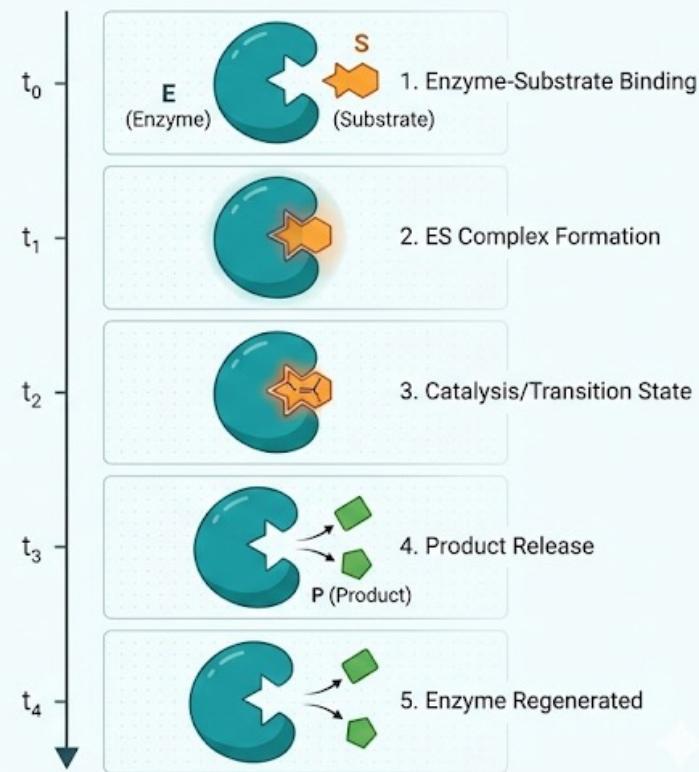
Kinetics and Regulation

- 7.1 Kinetics Is the Study of Reaction Rates
- 7.2 The Michaelis–Menten Model Describes the Kinetics of Many Enzymes
- 7.3 Allosteric Enzymes Are Catalysts and Information Sensors
- 7.4 Enzymes Can Be Studied One Molecule at a Time

Muybridge Horse Gallop (1878)



Enzyme-Substrate Reaction (Modern View)



THERMODYNAMICS (ΔG)



Spontaneity (Will it go?)



Spontaneous
($\Delta G < 0$)

Non-spontaneous
($\Delta G > 0$)



Equilibrium (How far?)

Equilibrium ($\Delta G = 0$)



Direction (Which way?)

Forward or Reverse



Energy State (Stable?)

Final State Energy

KINETICS (V)



Rate (How fast?)



Mechanism (How?)



Activation Energy (How hard?)

Activation Energy Barrier



Time (How long?)

Reaction Duration



SAME REACTION, DIFFERENT QUESTIONS.

[S] low



Hungry enzyme

Learning objective 3: Explain what reaction velocity is.

MEASURING THE RATE OF REACTION (VELOCITY)

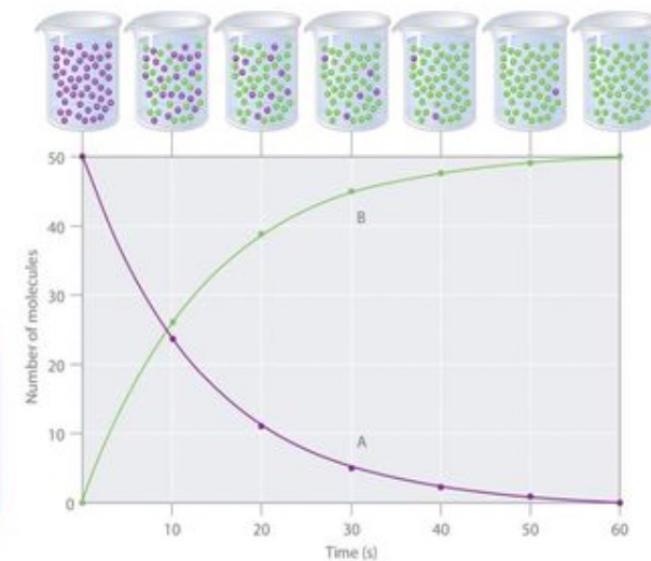
The progress of an enzyme catalyzed reaction may be followed by measuring either the

- amount of substrate consumed, or
- Amount of product formed

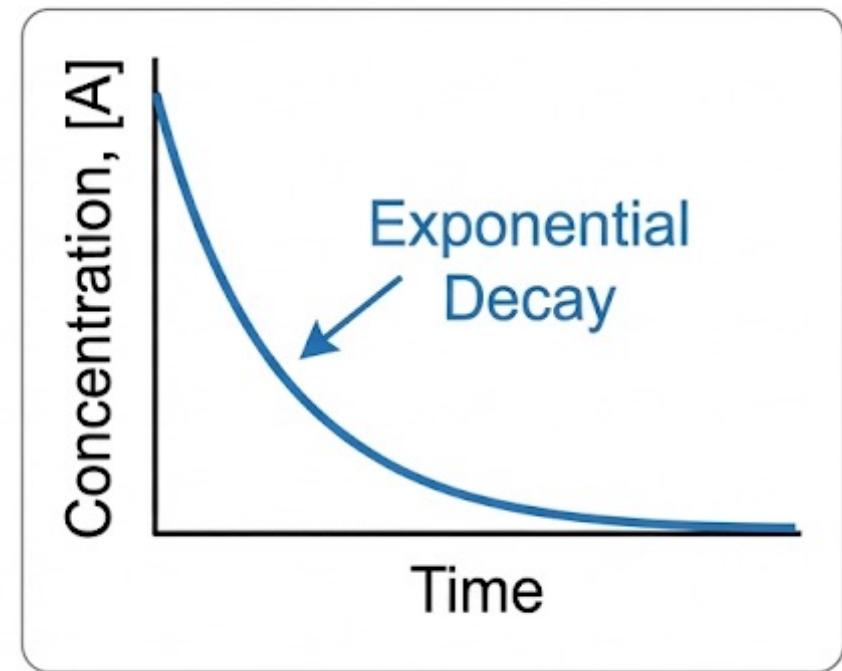
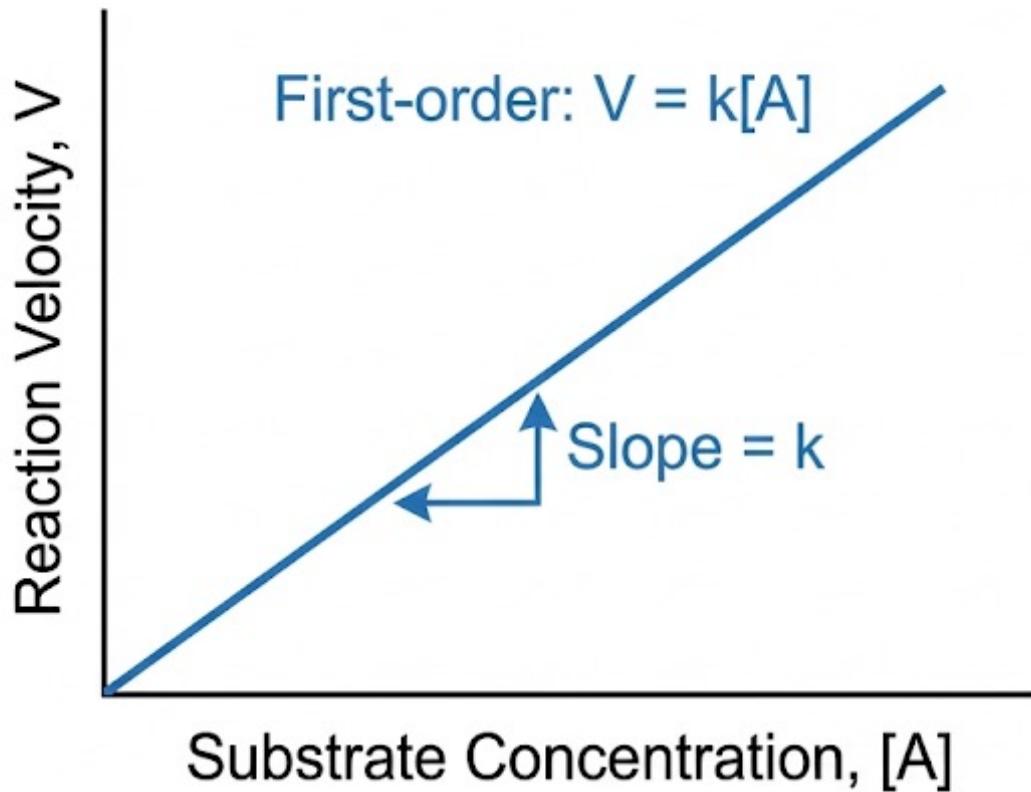
} per unit time.

Velocity (V) = rate of reaction = change in [P] or [S] per unit time

Unit : **$\mu\text{moles}/\text{minute}$**



Section 7.1 First Order Reaction Rates



At low $[S]$, enzyme reactions behave first-order.

Section 7.1 Second Order Reaction Rates

- Many important biochemical reactions are bimolecular or second-order reactions.
$$2A \rightarrow P \quad \text{or} \quad A + B \rightarrow P$$
- The rate equations for these reactions are

$$V = k[A]^2 \quad \text{and} \quad V = k[A][B]$$

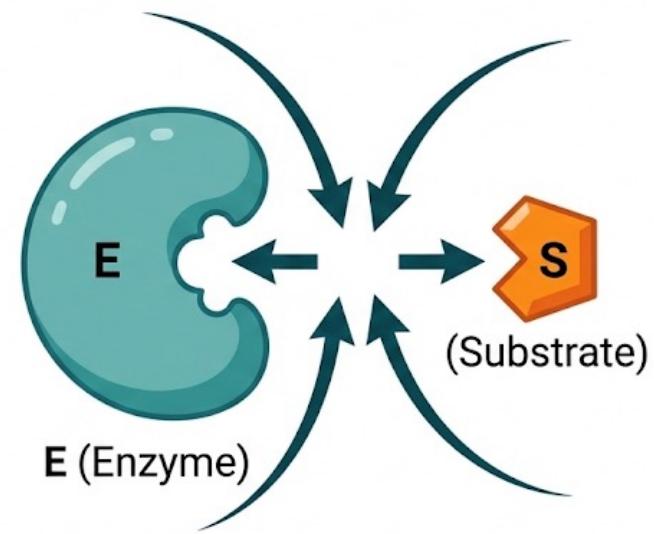
- The proportionality constant for second-order reactions has the units $M^{-1}s^{-1}$.

Reactions involving enzymes are typically a series of second order reactions because it takes two to Tango ;)

Second-order: $V = k[E][S]$



Molecular Collision



Rate depends on concentration of BOTH partners.

Graph Model of Reaction Velocity Versus Substrate Concentration in an Enzyme-catalyzed Reaction



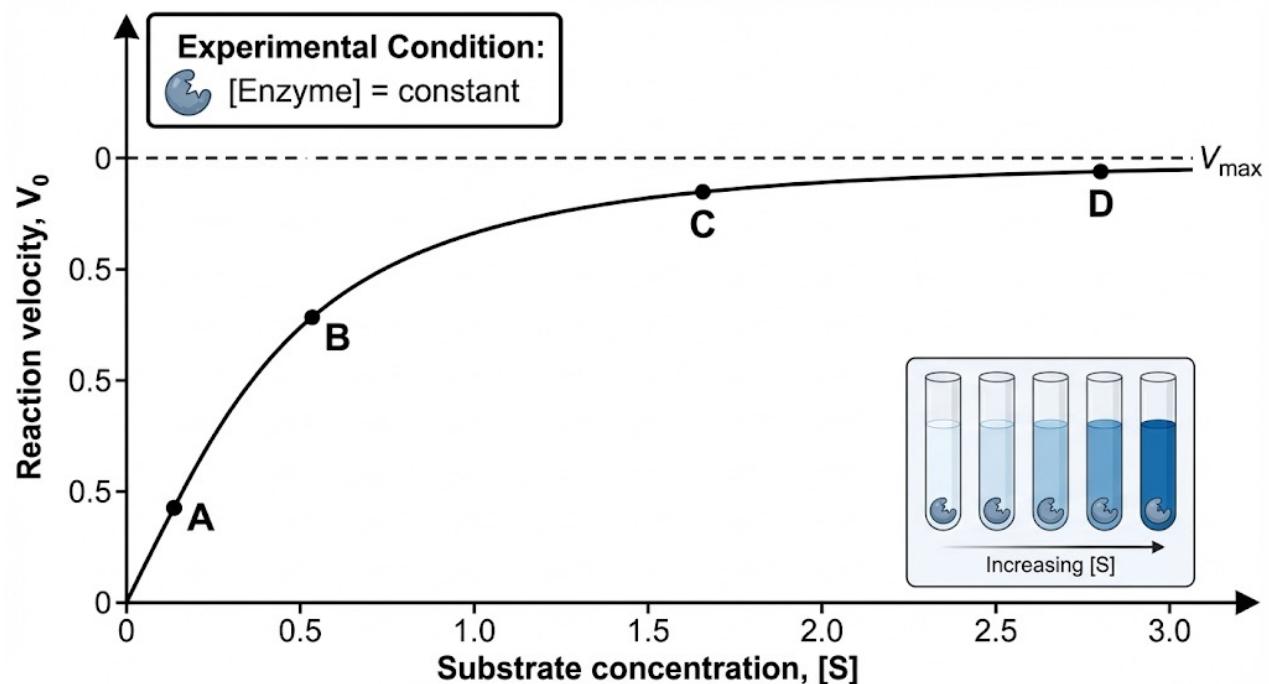
Leonor Michaelis



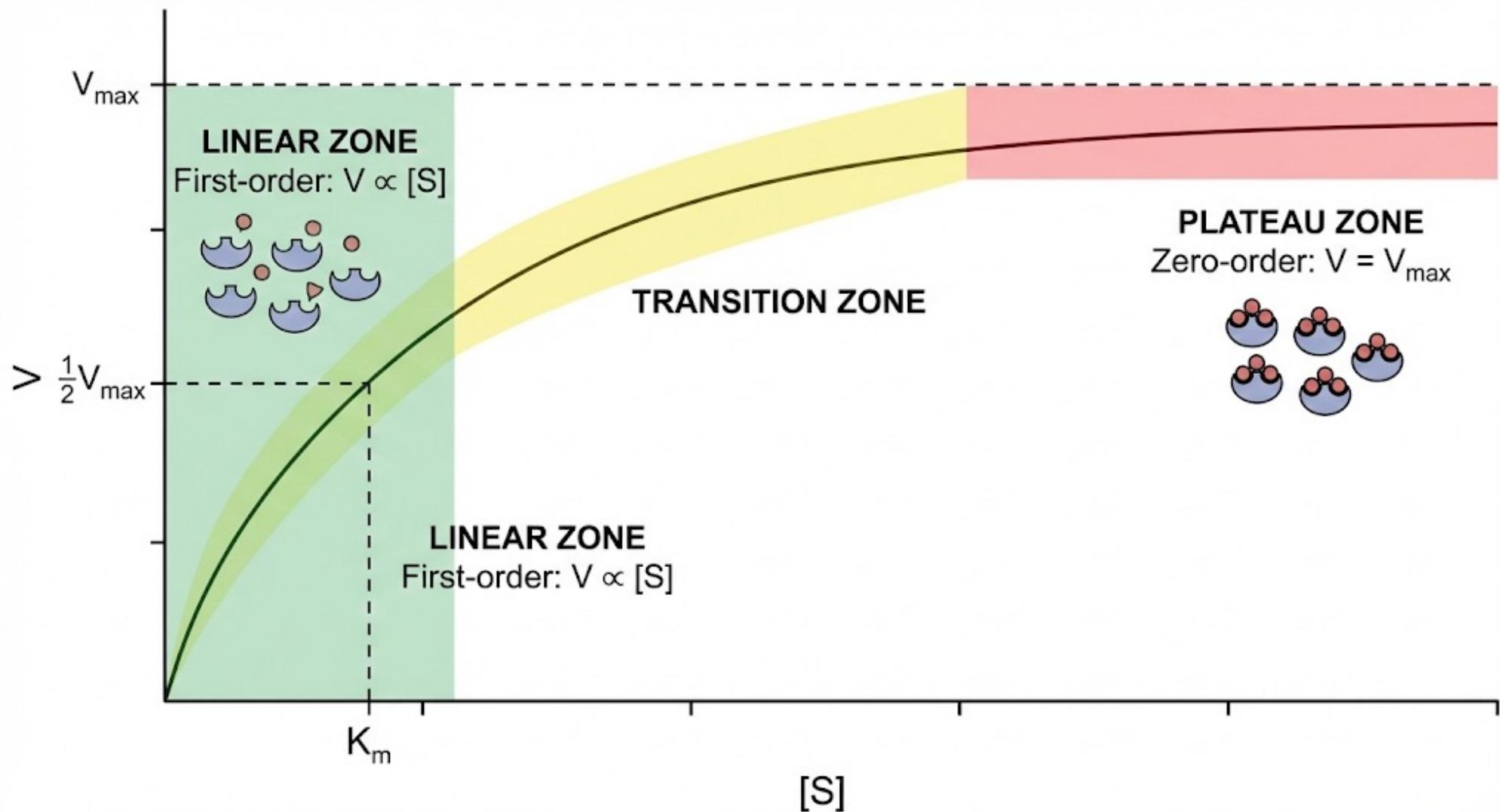
Maude Menton



- A. As $[S]$ is first increased, the **initial rate or velocity (V_0)** increases with increasing substrate concentration
 - i. **V is proportional to $[S]$**
- B. As $[S]$ increases, V increases less and less
 - i. **V is NOT proportional to $[S]$ in this range**
- C. Finally, V doesn't increase anymore and velocity reaches its maximum (V_{max})
 - i. **Enzyme is working as fast as it can**
- D. Velocity won't change no matter how much substrate is present. At this point, the enzyme is **saturated** with substrate, S .

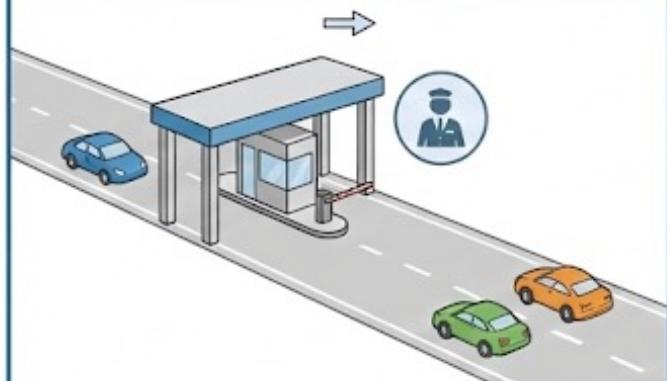


Anatomy of a Michaelis-Menton Graph



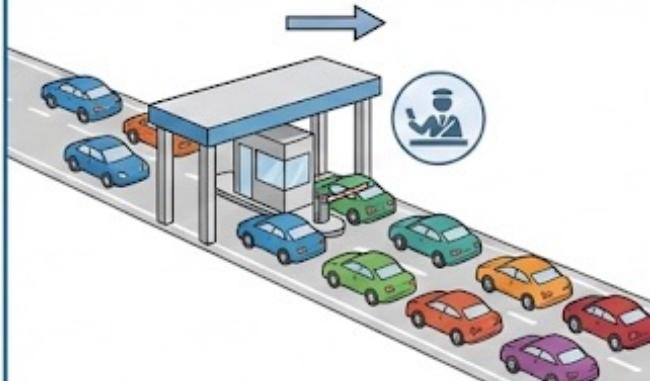
Toll Booth Analogy

Low [S]: $V \propto [S]$



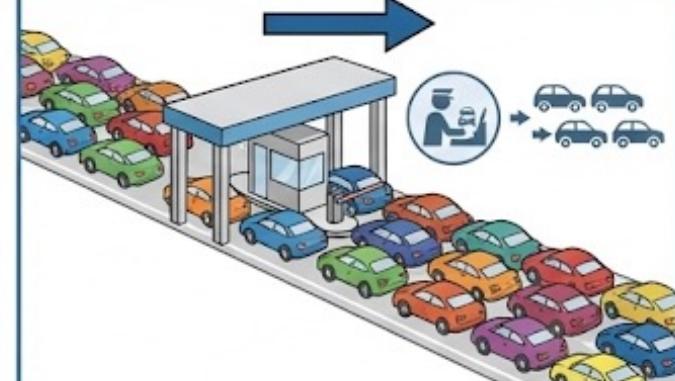
Low substrate concentration:
Velocity is proportional to substrate.

$[S] = K_m: V = \frac{1}{2}V_{max}$

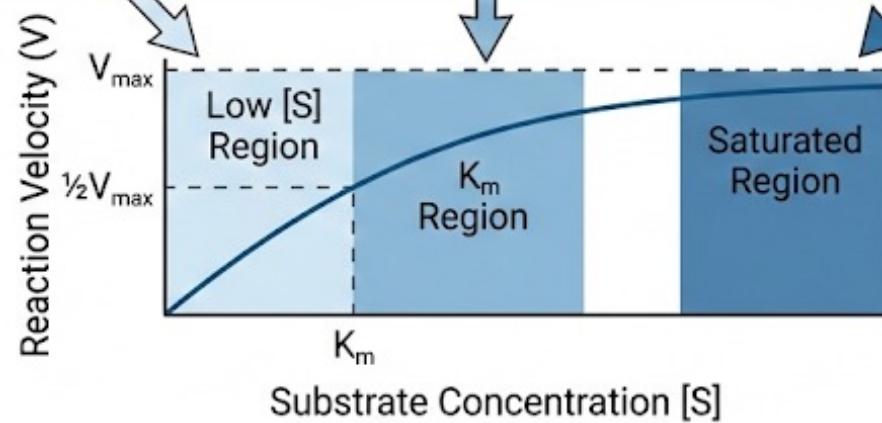


Substrate concentration = K_m :
Velocity is half of maximum.

High [S]: $V = V_{max}$ (saturated)

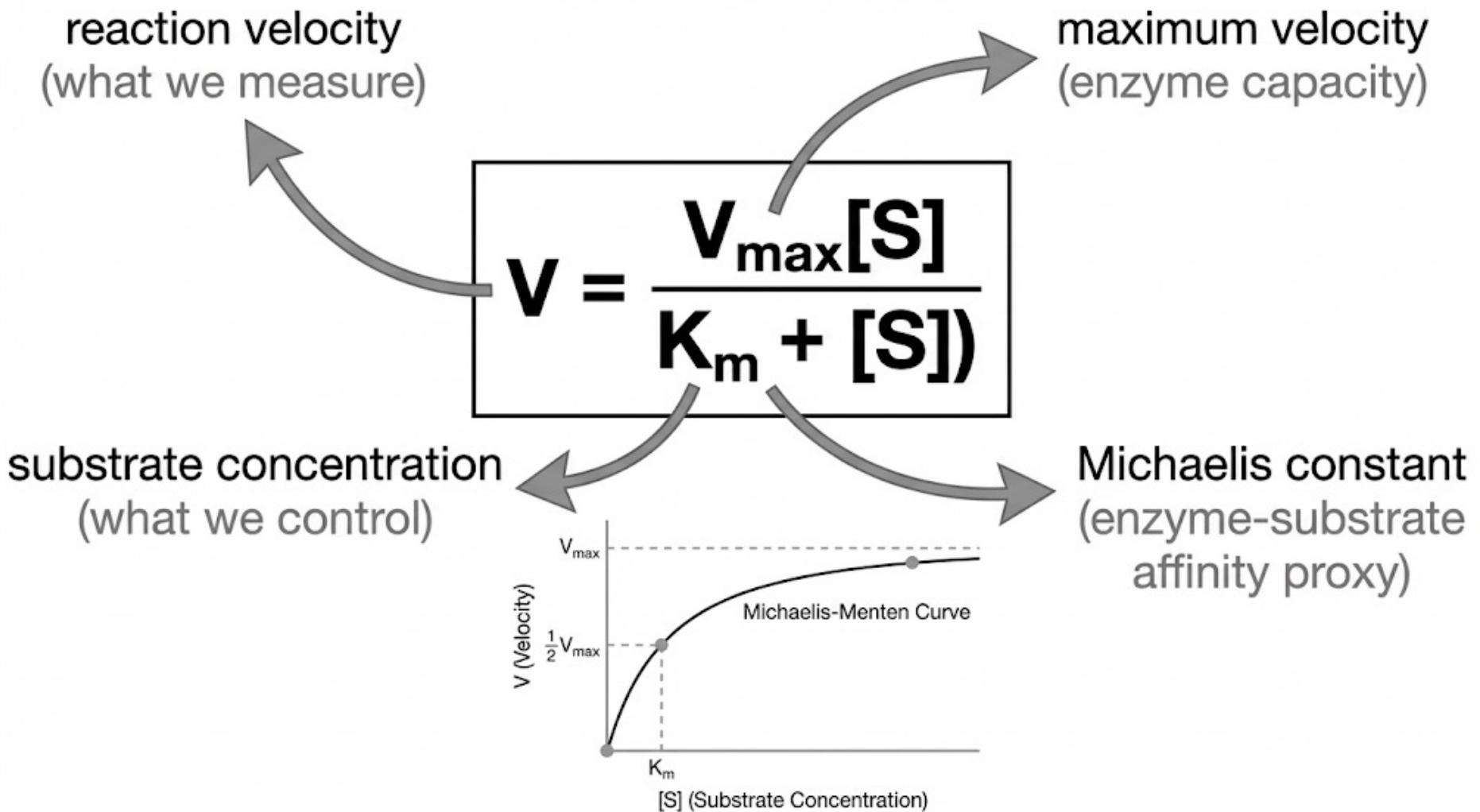


High substrate concentration:
Velocity is at maximum (saturated).



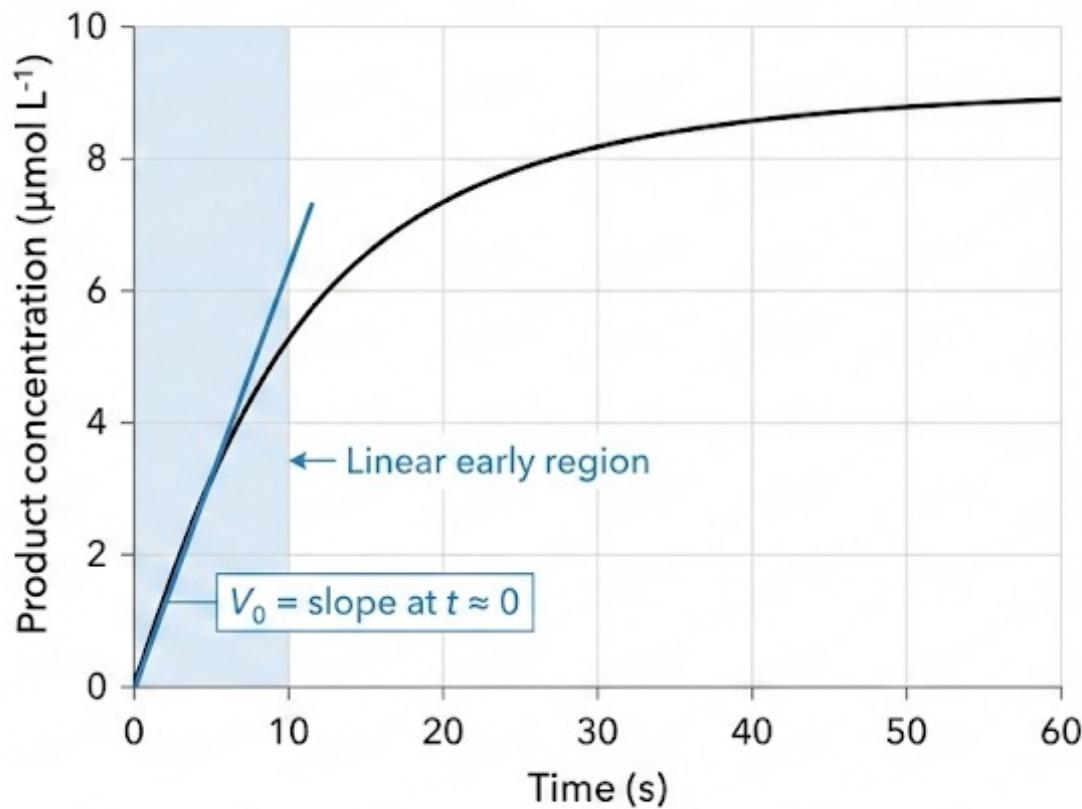
The enzyme (toll booth) has maximum capacity.

Quantitative Expression of Enzyme Behavior



Learning objective 4: Explain how reaction velocity is determined and how reaction velocities are used to characterize enzyme activity.

importance of initial velocity (V_0)

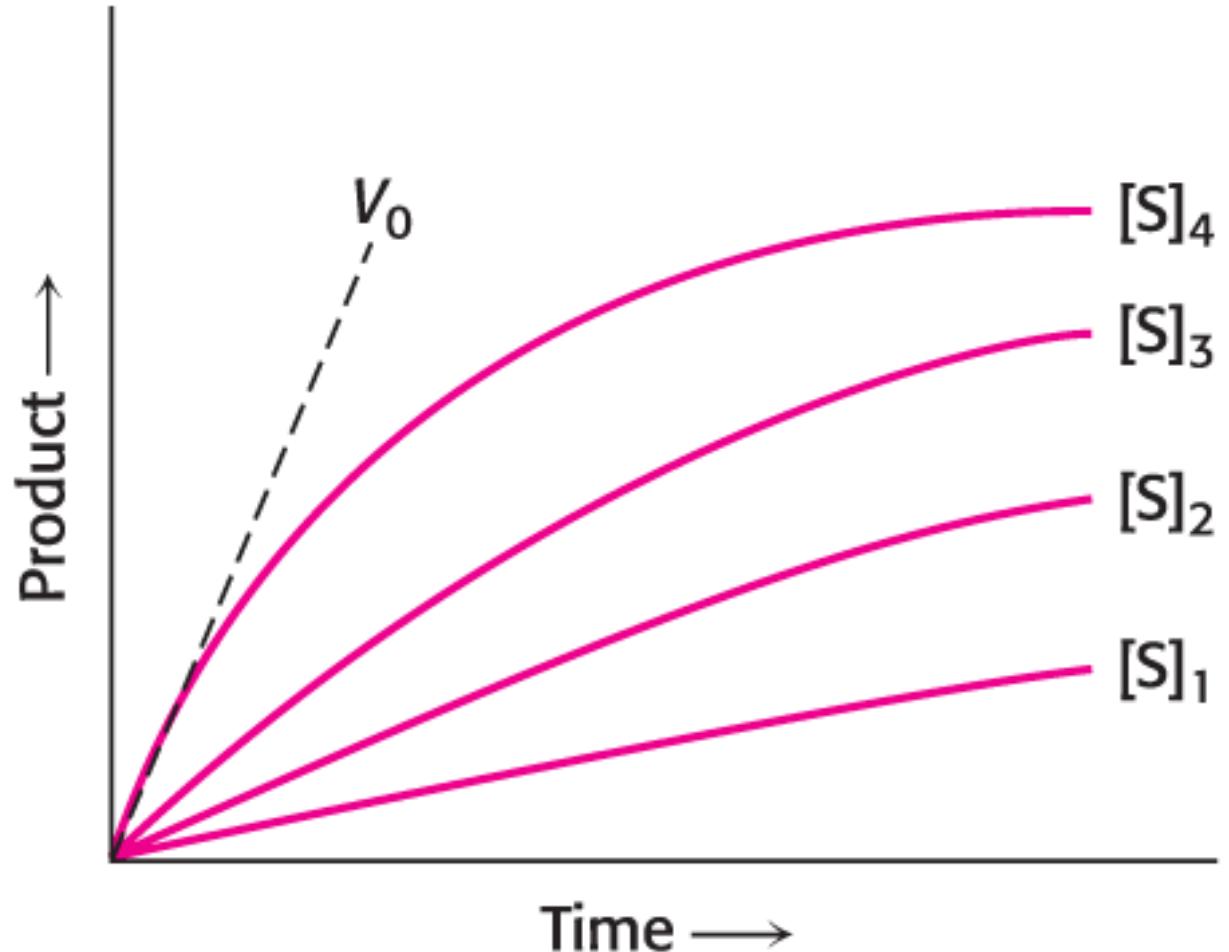


Why measure V_0 ?

- At the start, $[S]$ is nearly constant, so the rate reflects enzyme activity.
- Early data are linear, so V_0 is easy to measure and compare across conditions.

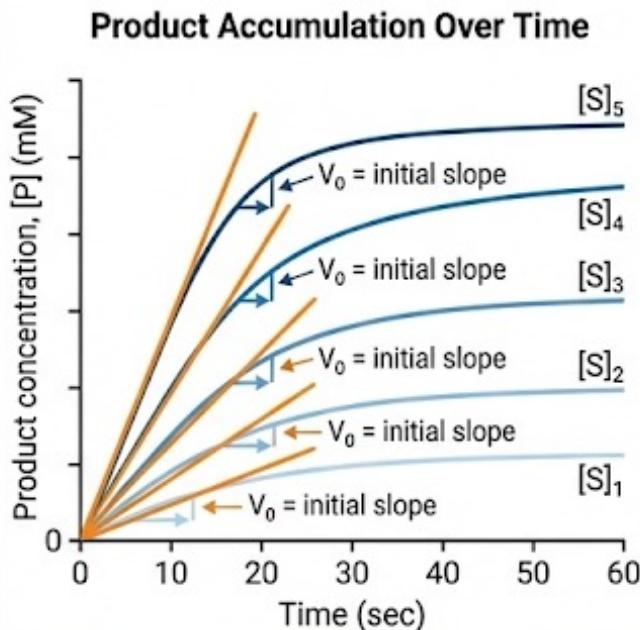
Calculating Initial Velocity

- The initial velocity is determined by measuring product formation as a function of time and then determining the velocity soon after the reaction has started.



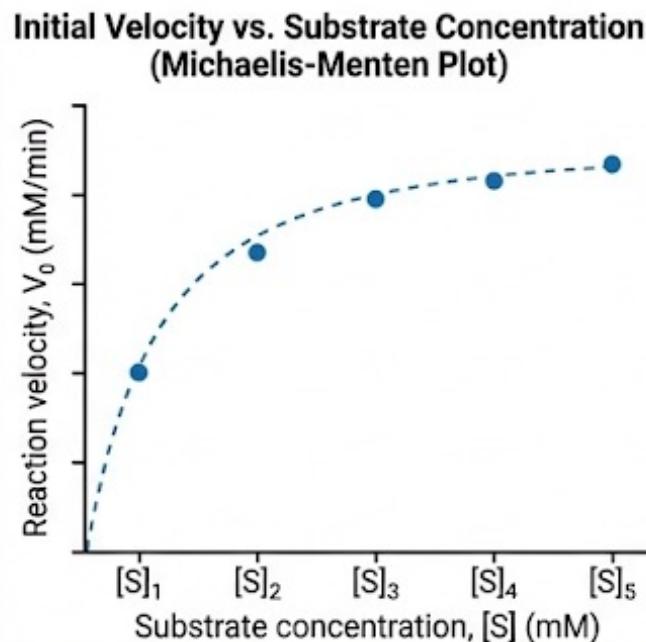
Experimental Workflow for Determining Enzyme Kinetic Parameters

Step 1: Measure Progress Curves



Measure product formation at multiple substrate concentrations. Calculate V_0 from early linear region (typically first 5-10% of reaction).

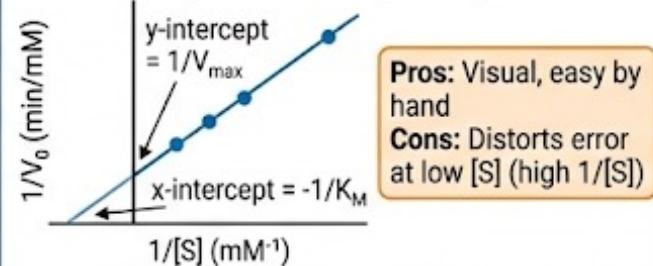
Step 2: Plot V_0 vs $[S]$



Plot initial velocities against substrate concentrations. Data should follow hyperbolic pattern.

Step 3: Determine K_M and V_{\max}

Historical: Lineweaver-Burk Plot (Double-Reciprocal)



Pros: Visual, easy by hand
Cons: Distorts error at low $[S]$ (high $1/[S]$)

Modern: Non-linear Regression



Michaelis-Menten

$$V_0 = \frac{V_{\max}[S]}{(K_M + [S])}$$

Output: $K_M = 2.3 \pm 0.2 \text{ mM}$, $V_{\max} = 0.65 \pm 0.03 \text{ mM/min}$

Pros: More accurate, proper error estimation.
Cons: Requires specialized software.

Key experimental considerations:

(1) Use $[E] \ll [S]$ so substrate depletion is negligible (steady-state assumption).



(2) Measure true initial velocities before $[S]$ changes significantly (first 5-10% conversion).



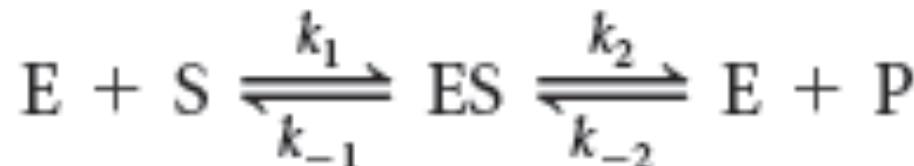
(3) Include $[S]$ values both below and above K_M for accurate determination.



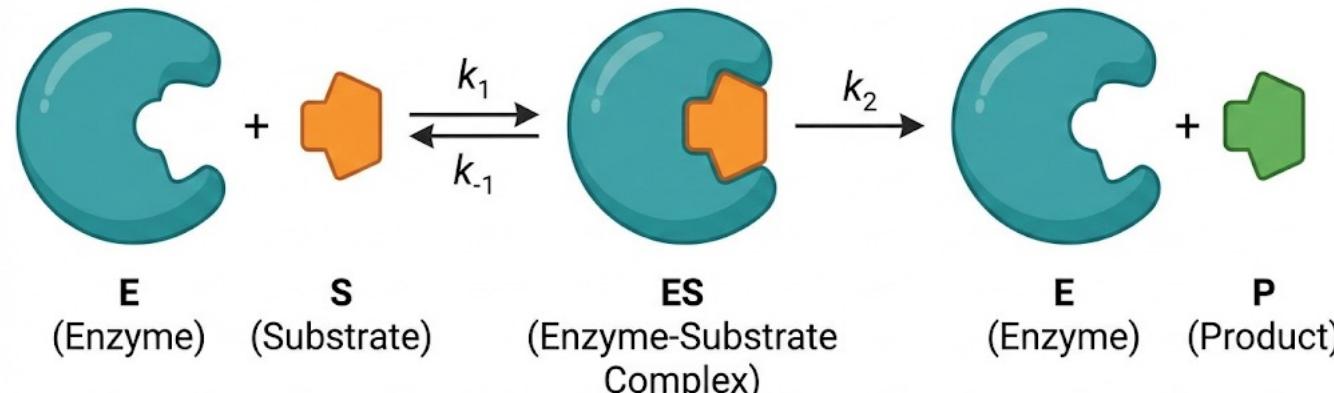
Section 7.2 The Michaelis–Menten Model Describes the Kinetics of Many Enzymes

A common means of investigating enzyme kinetics is to measure velocity as a function of substrate concentration with a fixed amount of enzyme.

Consider a simple reaction in which the enzyme E catalyzes the conversion of S → P.

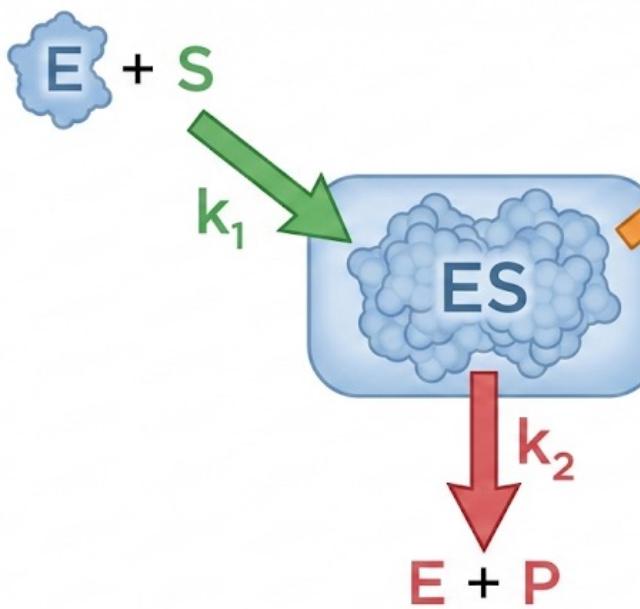


with k_1 , k_2 being the rate constant for the indicated reaction steps. To ignore the reverse reaction of P → S, we measure activity when $[P] \approx 0$.



Note: k_2 is often called k_{cat} .

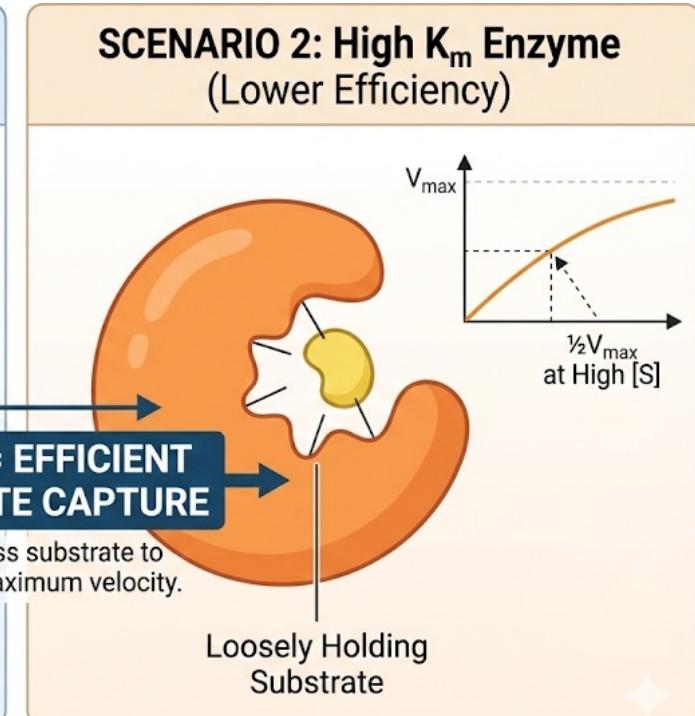
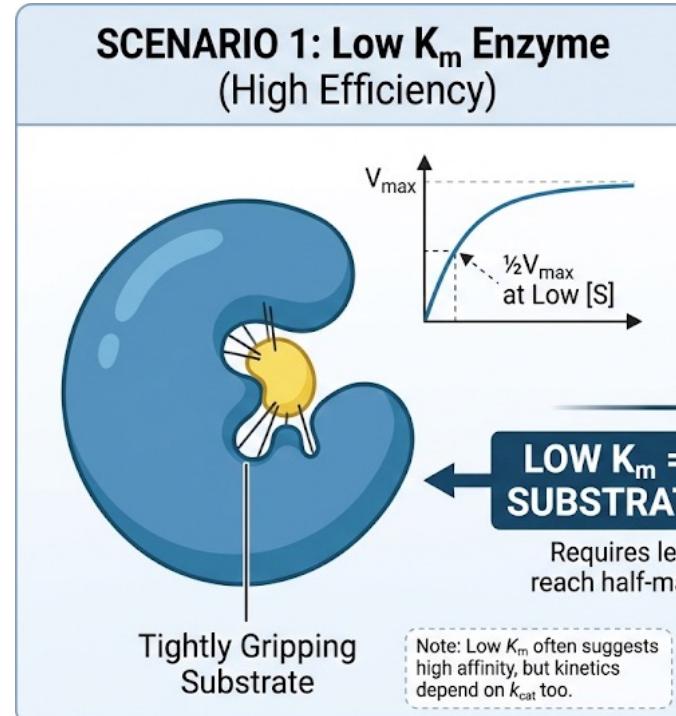
Under these conditions, the velocity is called the initial velocity or V_0 .



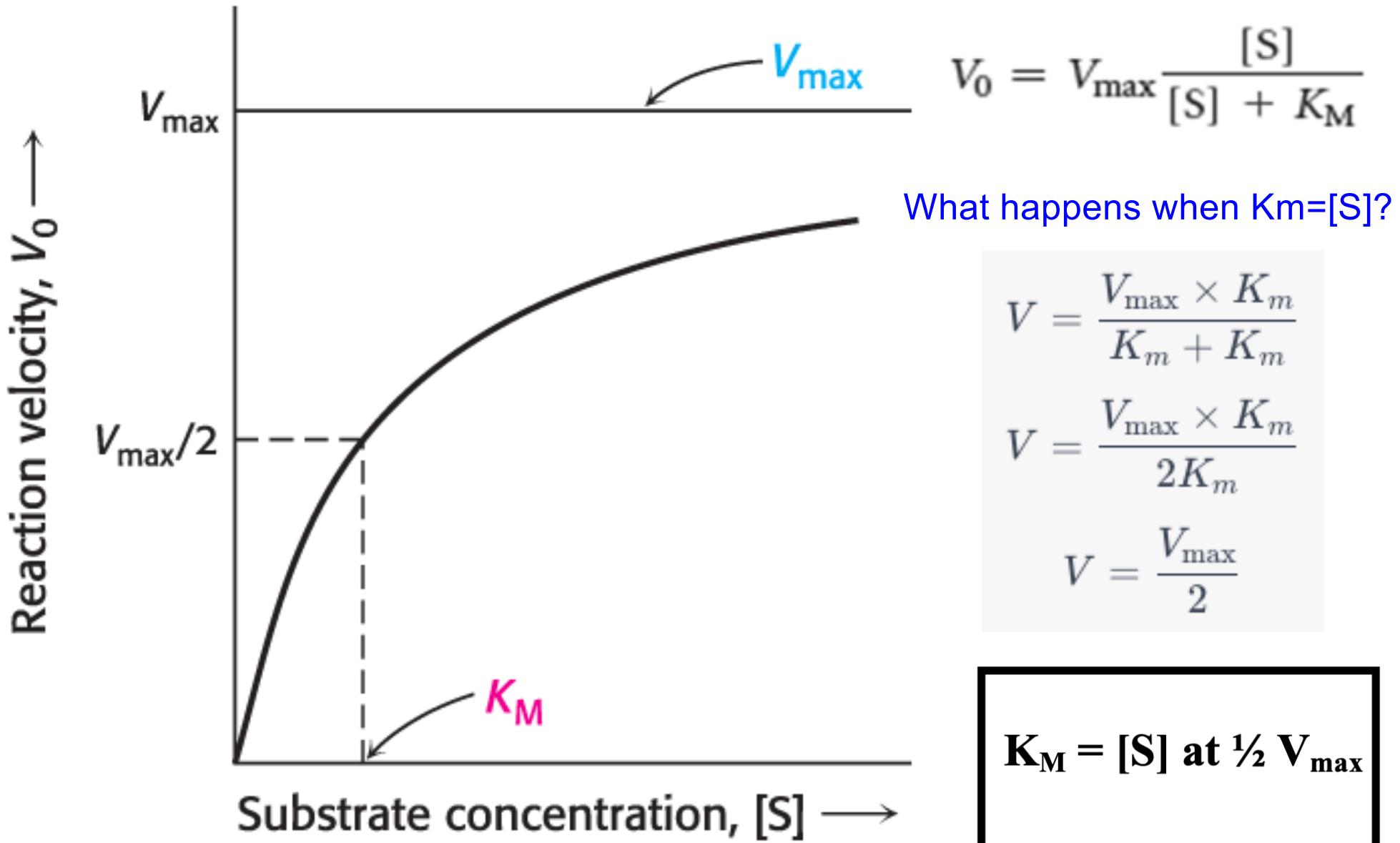
$K_M = \text{substrate concentration at } \frac{1}{2} V_{\max}$

$$K_M = (k_{-1} + k_2)/k_1$$

Understanding K_m – the Michaelis Constant

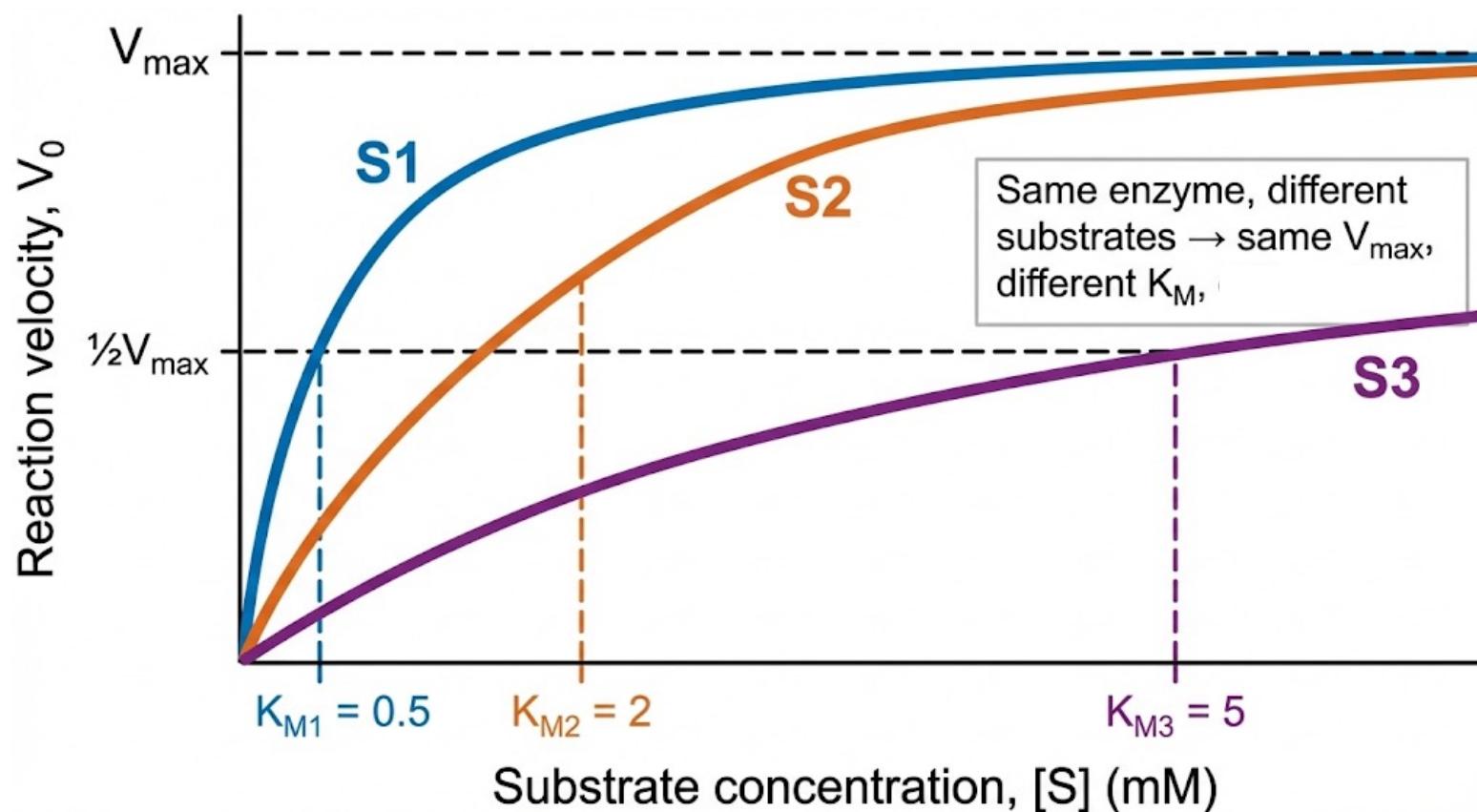


Graph of Michaelis–Menten Kinetics



The Brilliance of Km

- KM is also the substrate concentration at which the enzyme operates at one half of its maximum velocity
- Indicates how efficiently an enzyme selects its substrate and converts to product.
- So, if an enzyme has a **SMALL KM** they it achieves maximal catalytic efficiency (V_{max}) at a low substrate concentration!
- **KM** is unique for each enzyme/substrate pair
- For certain enzymes under certain conditions, KM can also be a measure of affinity between E and S – approximates the dissociation constant of the ES complex



Evidence suggests that the K_M value is approximately the substrate concentration of the enzyme *in vivo*.



QUICK QUIZ 1

What value of $[S]$, as a fraction of K_m , is required to obtain 80% V_{max} ?

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

$$V_0 = \frac{V_{max} [S]}{[S] + K_m}$$
$$0.8 V_{max} = \frac{V_{max} [S]}{[S] + K_m}$$
$$0.8 [S] + 0.8 K_m = [S]$$
$$0.8 K_m = -0.2 [S]$$
$$4 K_m = [S]$$

CLINICAL INSIGHT

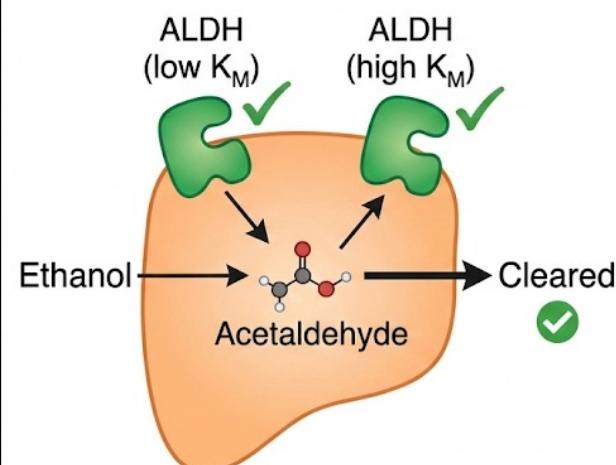


Variations in K_M Can Have Physiological Consequences

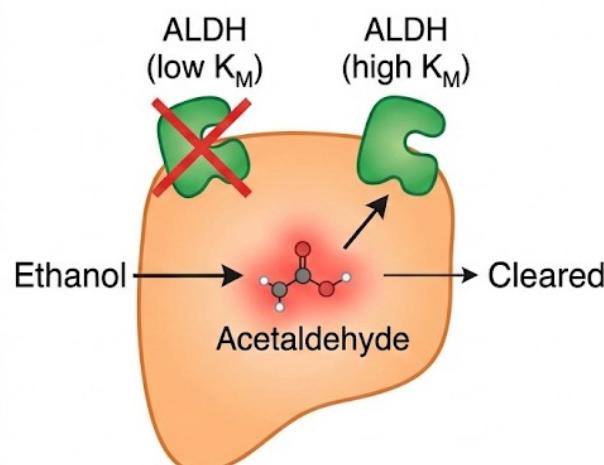
- Two enzymes play a key role in the metabolism of alcohol.



(1) Most people



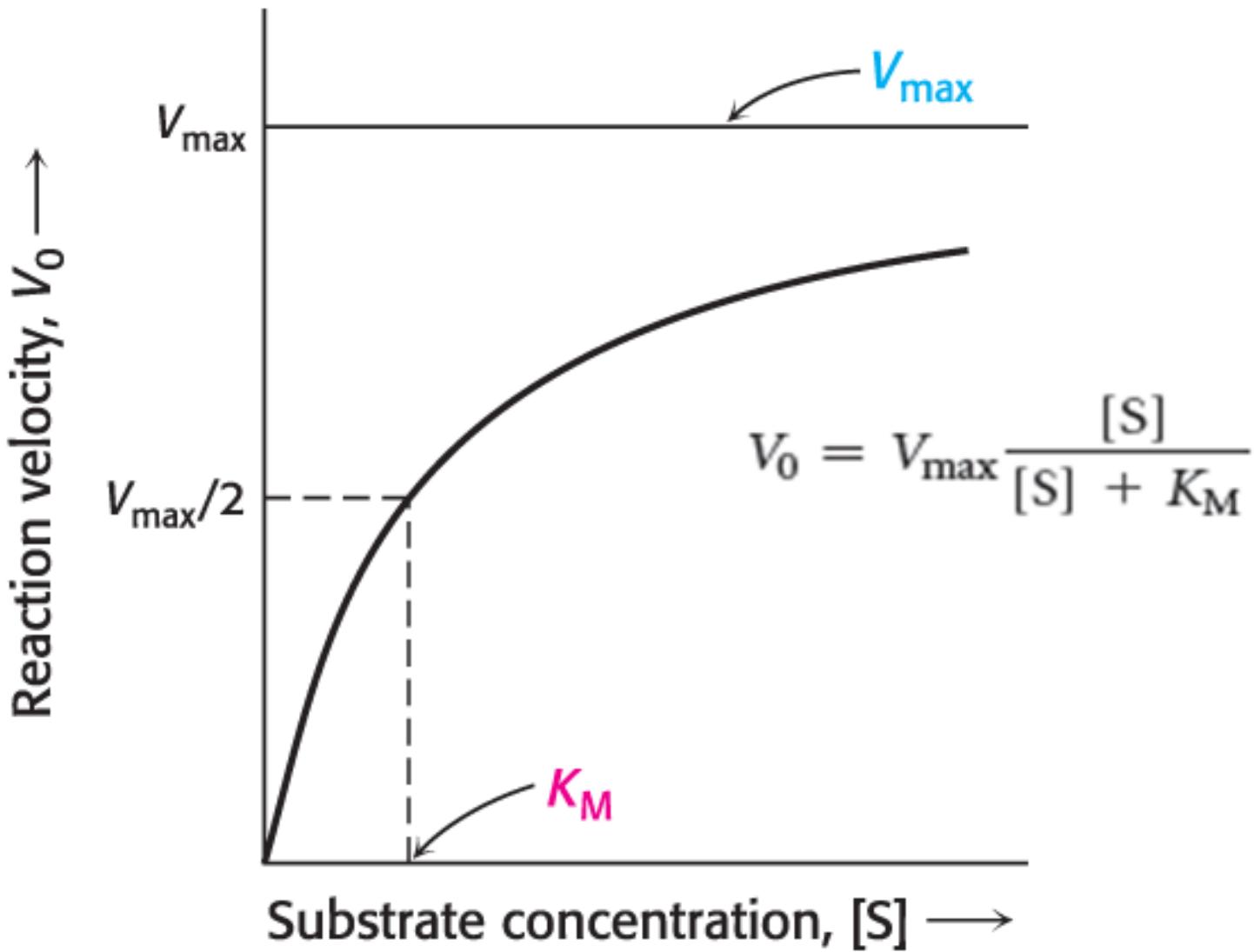
(2) Susceptible individuals



Efficient clearance.
No accumulation.

Acetaldehyde accumulating.
Flushing response.

The problem with hyperbolic curves: It's hard to estimate Vmax from a curve that never quite reaches it.



What if we could re-arrange it to make it more accurate in determining Vmax and Km?

K_M and V_{max} Values Can Be Determined by Several Means

- The Michaelis–Menten equation can be manipulated into one that yields a straight-line plot.

$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$

$$y = mx + b$$

slope y-intercept

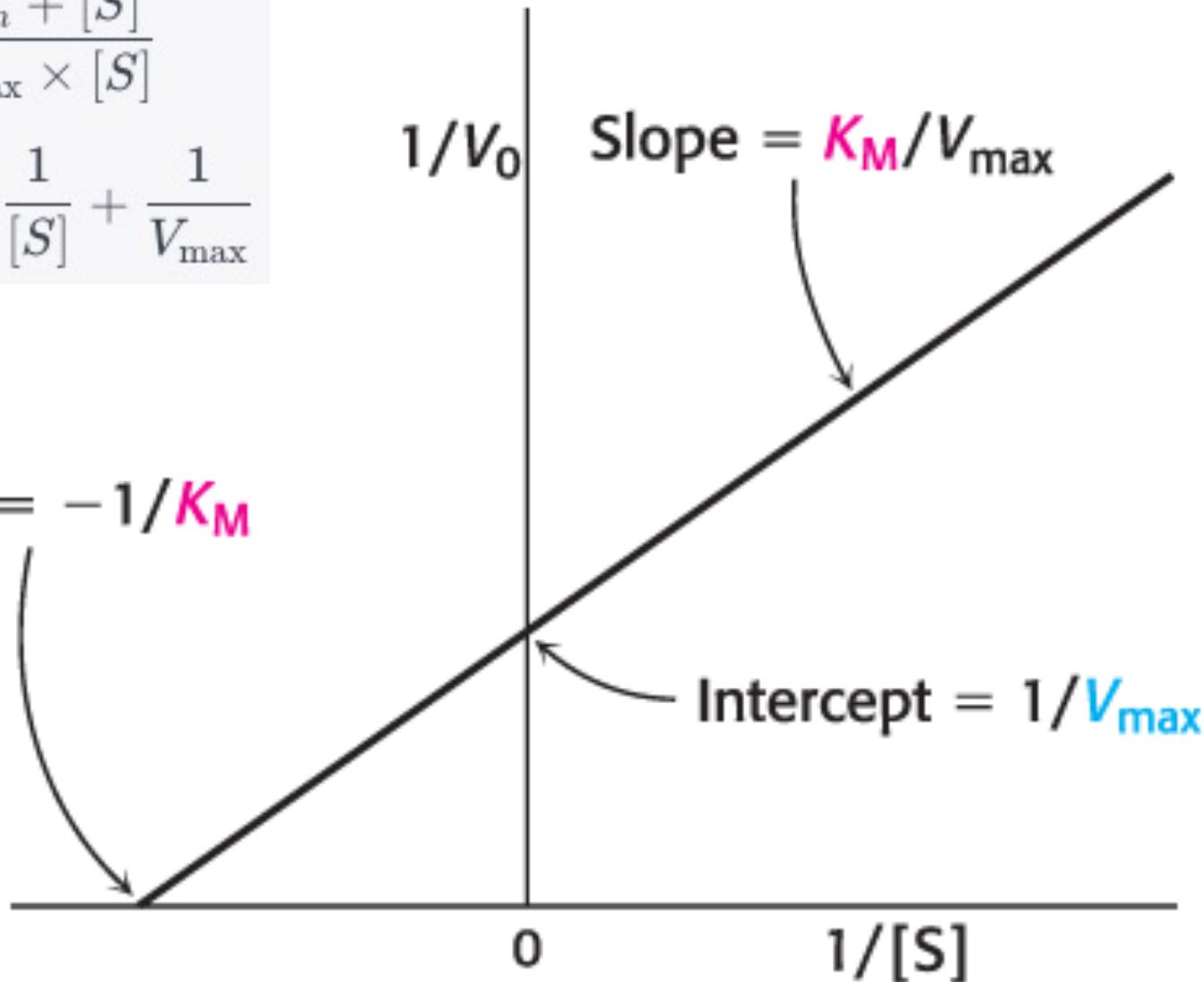
- This double-reciprocal equation is called the Lineweaver–Burk equation.

A Double-reciprocal, or Lineweaver–Burk, Plot

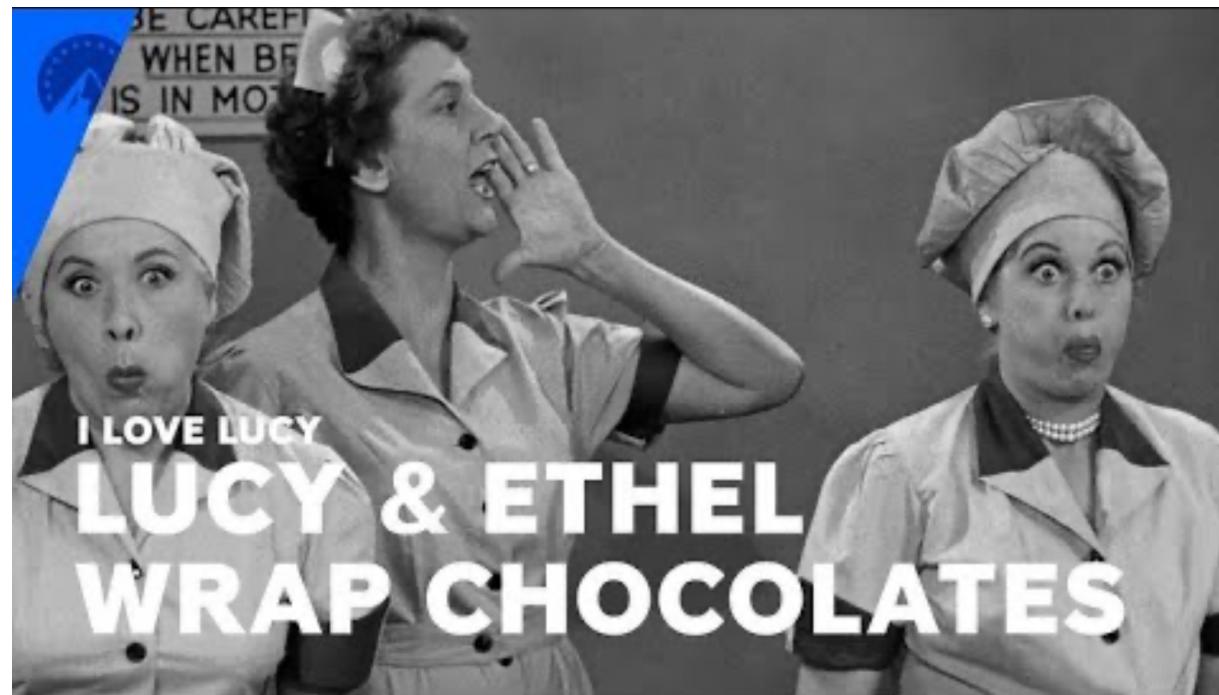
$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max} \times [S]}$$

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

Intercept = $-1/K_M$

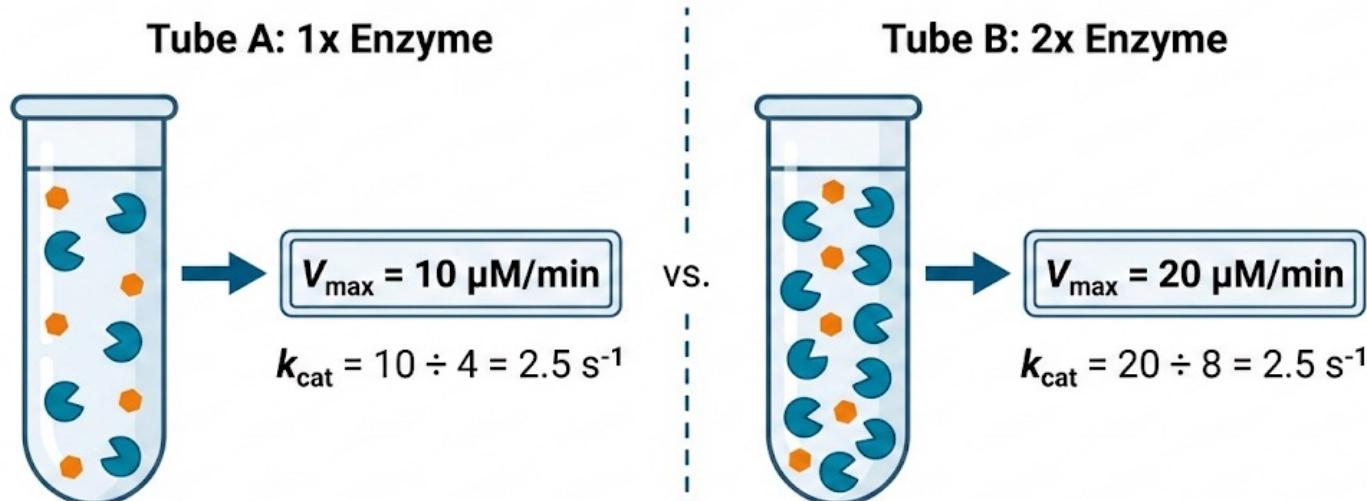


break



But Wait—Vmax Depends on How Much Enzyme We Added

Why Vmax Isn't Enough



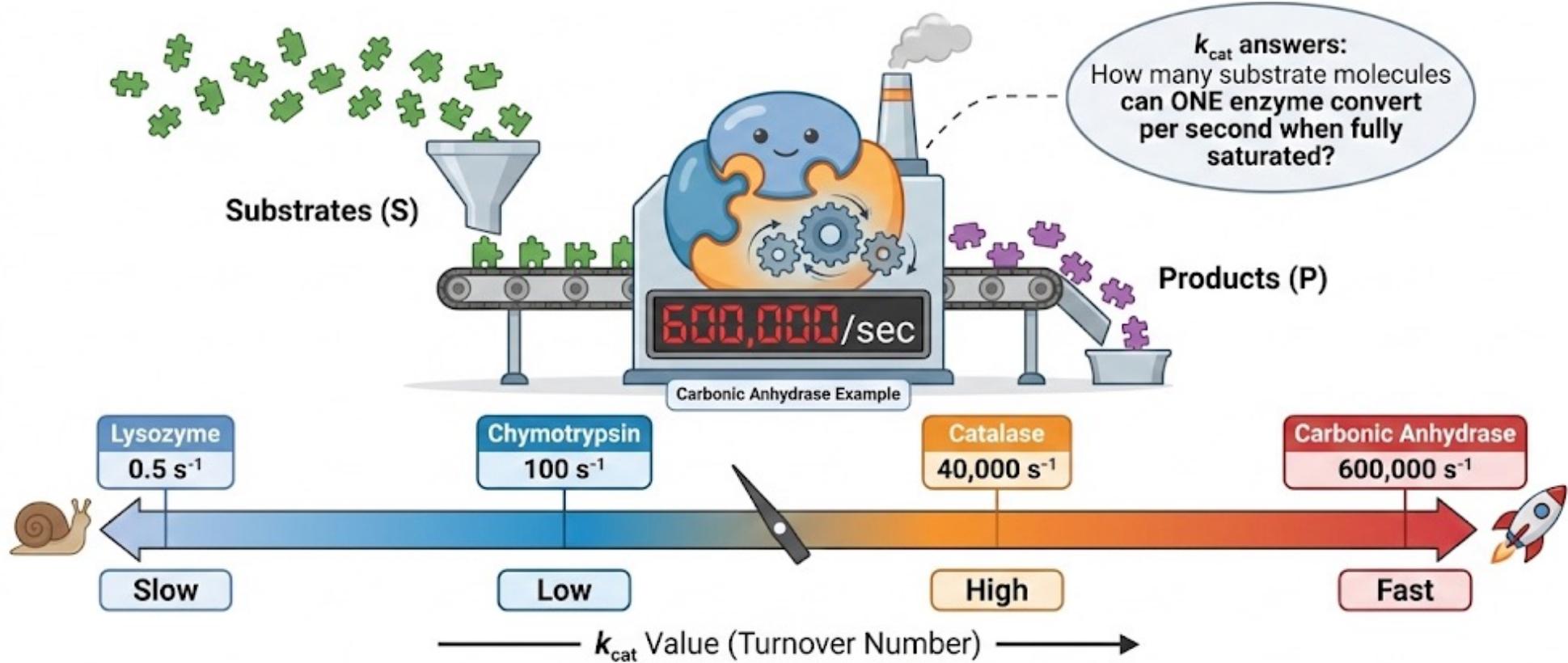
V_{max} changes with [E]. k_{cat} stays the same → k_{cat} is the enzyme's true speed.

- V_{max} = maximum rate for *that tube* (not a property of the enzyme alone)
- If you double [E]total, you double V_{max}
- We need a property of *the enzyme itself*
- Enter: $k_{cat} = V_{max} / [E]_{\text{total}}$
- k_{cat} tells us: "How many reactions can ONE enzyme molecule do per second?"

Turnover Number: The Enzyme's Speed Limit

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

s^{-1} (reactions per second per enzyme)



k_{cat} tells us top speed, but it doesn't tell us how the enzyme performs when substrate is rare.

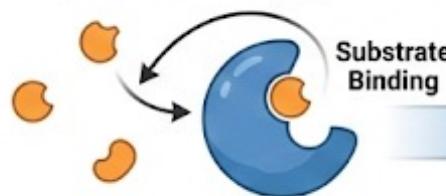
Catalytic Efficiency

$$V_0 = \frac{(k_{cat}/K_M)}{M^{-1}s^{-1}} \times [S] \times [E]_T$$

Valid when $[S] \ll K_M$
(unsaturated conditions)

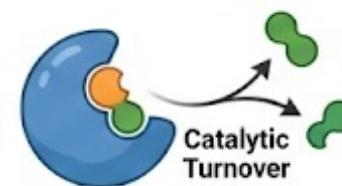
Units: $M^{-1}s^{-1}$

Finding Substrate (K_M Influence)



Measures affinity for substrate;
lower K_M means tighter binding, more efficient finding

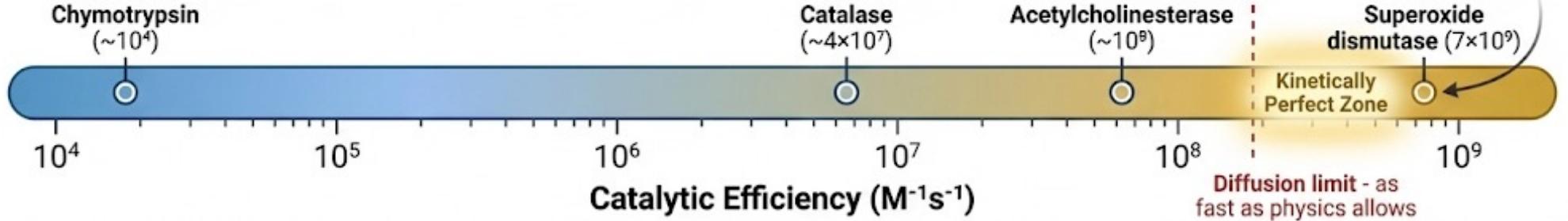
Converting Substrate (k_{cat} Influence)



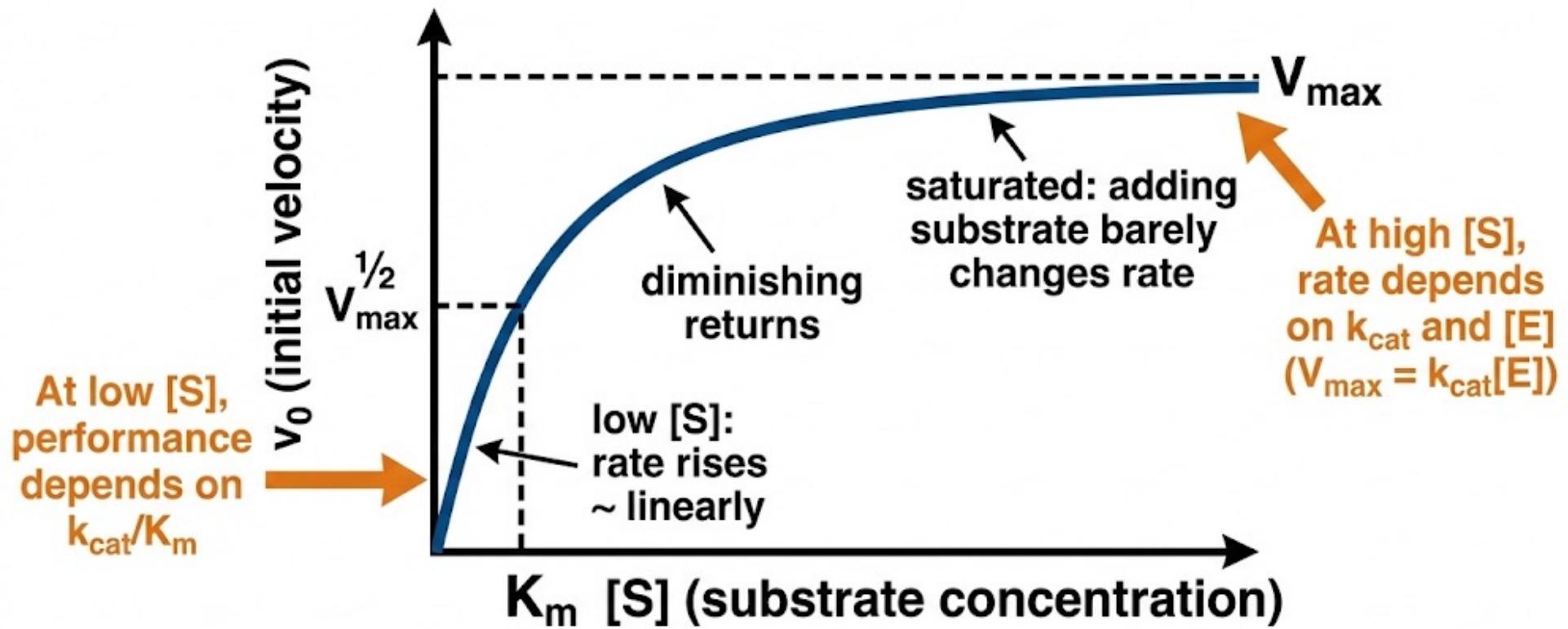
Measures rate of conversion to product;
higher k_{cat} means faster turnover, more efficient converting

Overall Efficiency

Combines both substrate finding and conversion capabilities



Reading the Michaelis–Menten curve

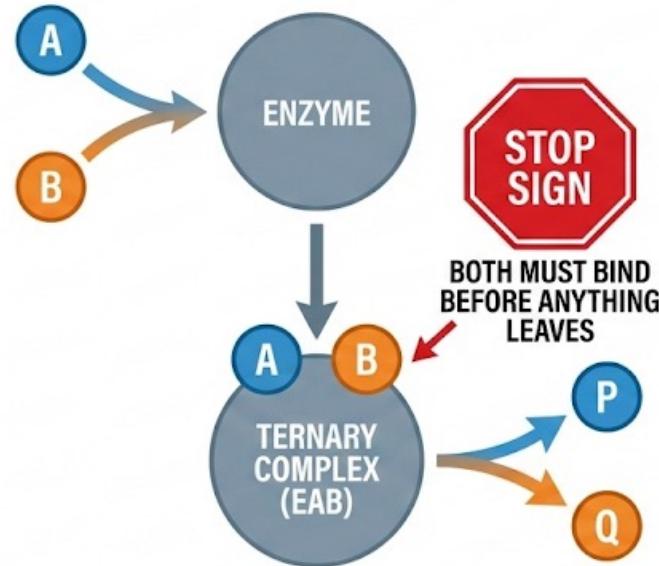


K_m is the midpoint of the curve, not automatically ‘affinity’.

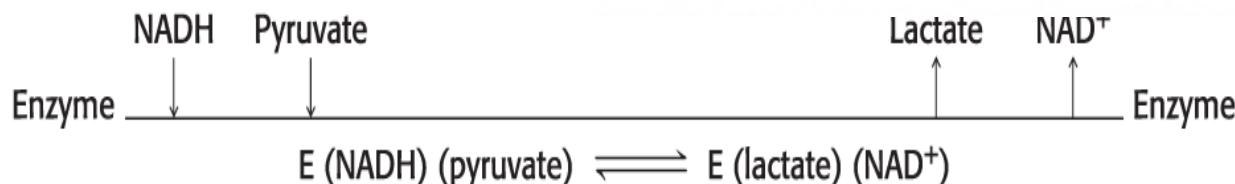
Most Biochemical Reactions Include Multiple Substrates-Cleland representation

Multiple substrate reactions can be divided into two groups.

Sequential reactions are characterized by formation of a ternary complex consisting of the two substrates and the enzyme.



TERNARY COMPLEX REQUIRED

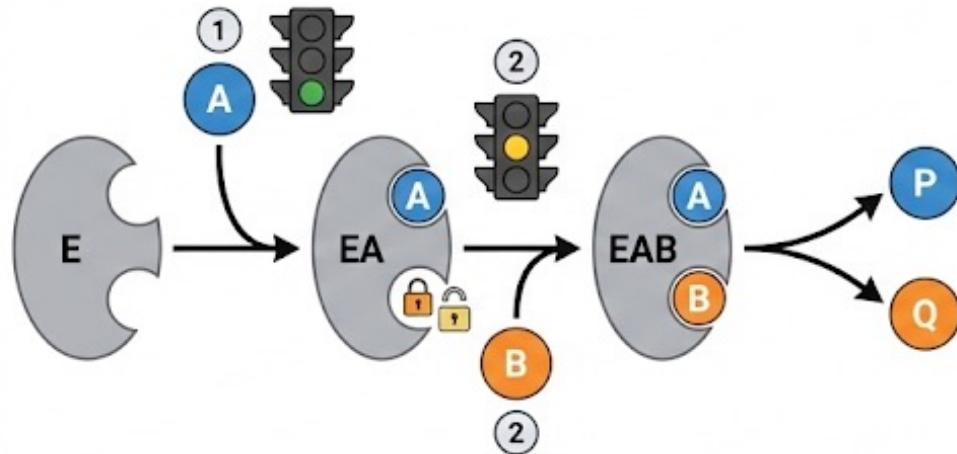


(A)

Sequential reaction

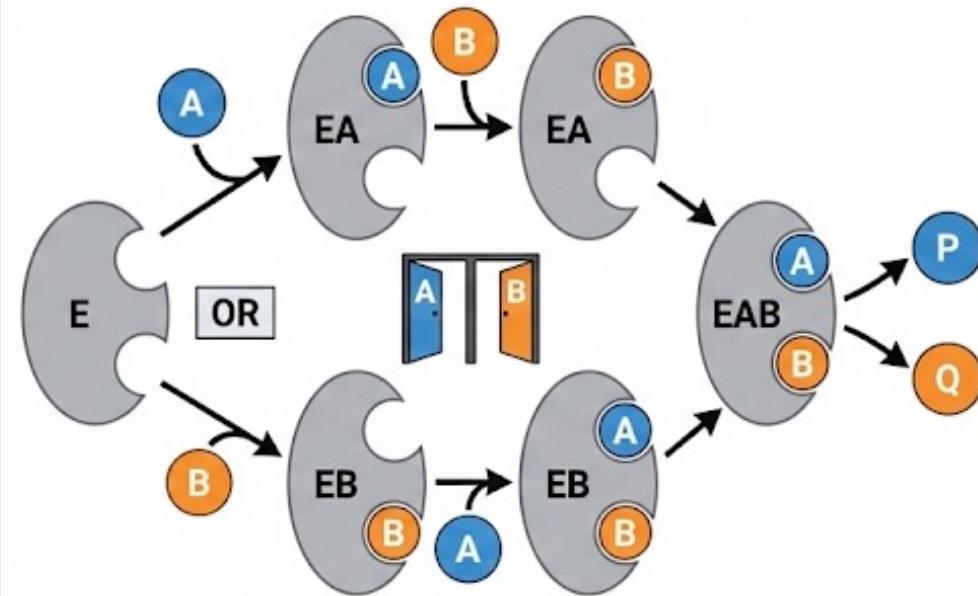
Two types of sequential reactions.

ORDERED SEQUENTIAL



e.g., Lactate dehydrogenase (NAD⁺ must bind before pyruvate)

RANDOM SEQUENTIAL

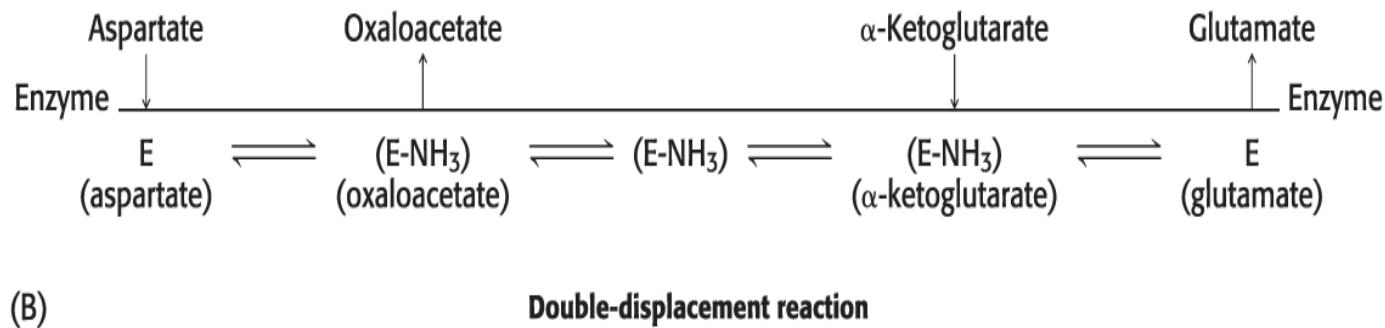


e.g., Creatine kinase (ATP or creatine can bind first)

COMPARISON

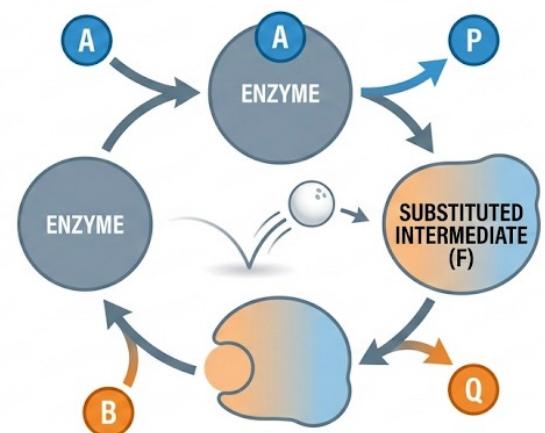
Feature	Ordered	Random
Binding sequence	Fixed (A then B)	Flexible (A or B first)
First substrate	Opens site for second	Both sites always accessible
Kinetic analysis	Simpler	More complex

Double displacement or ping-pong reactions.



Double-displacement reactions are characterized by the formation of a substituted enzyme intermediate. Double-displacement reactions are also called ping-pong reactions.

RIGHT PANEL: PING-PONG MECHANISM

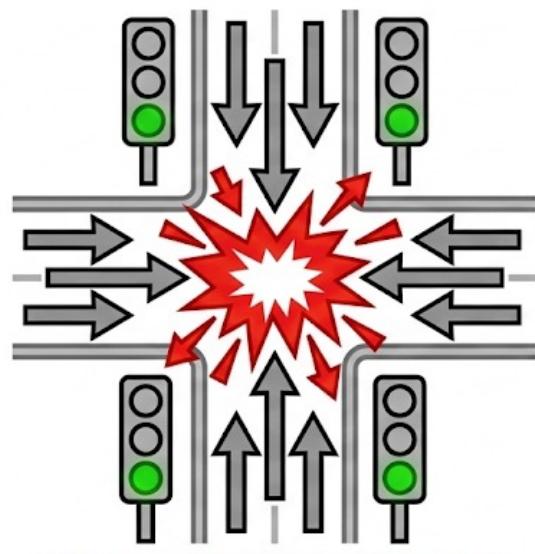


SUBSTITUTED ENZYME INTERMEDIATE

What if every enzyme ran at maximum speed?

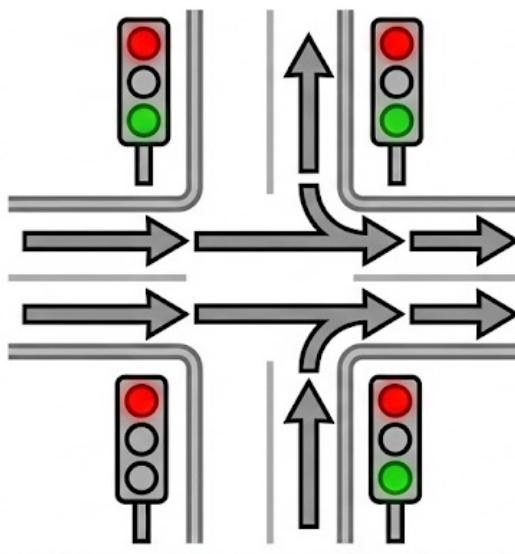
The problem with maximum speed

If Every Enzyme Ran at V_{max}...



Chaos: No Coordination

What Cells Actually Need...



Control: Smart Switches

?

What's missing?

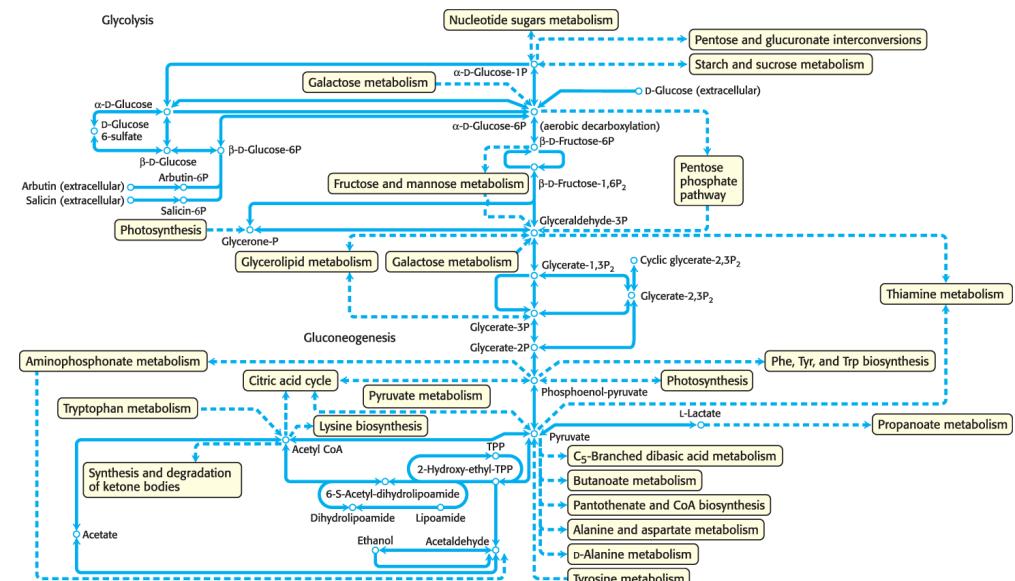
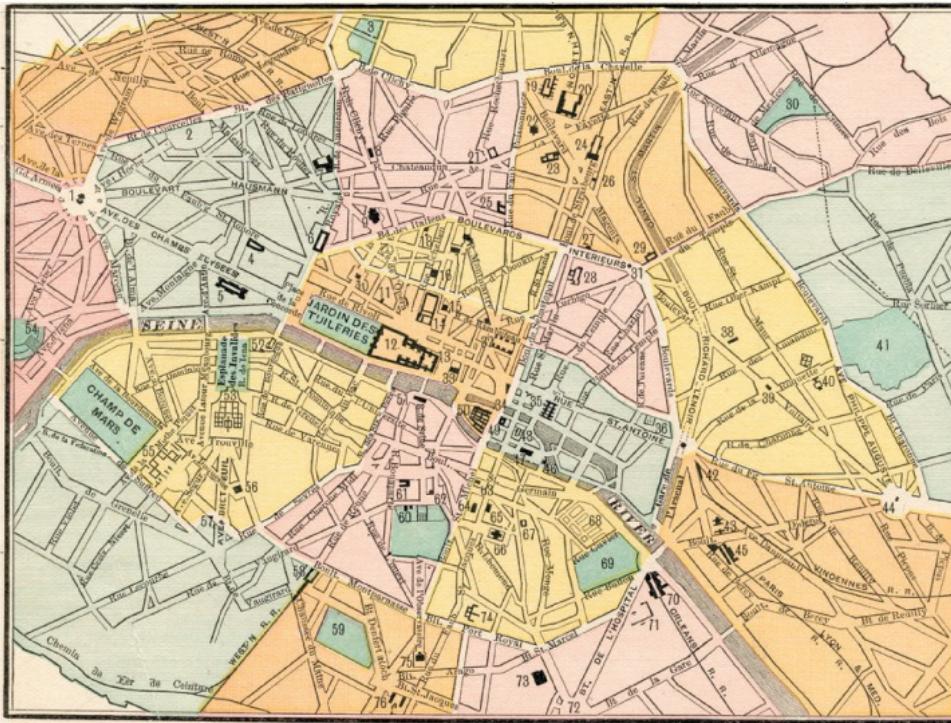
Allosteric enzymes are metabolism's traffic lights. ♦

- We've learned to measure how fast enzymes work
- Some enzymes approach the diffusion limit—as fast as physics allows
- But cells can't run every pathway at maximum speed simultaneously
- Resources are limited; pathways must be coordinated
- Question: How does a cell decide when to turn a pathway on or off?

Section 7.3

Allosteric Enzymes Are Catalysts and Information Sensors

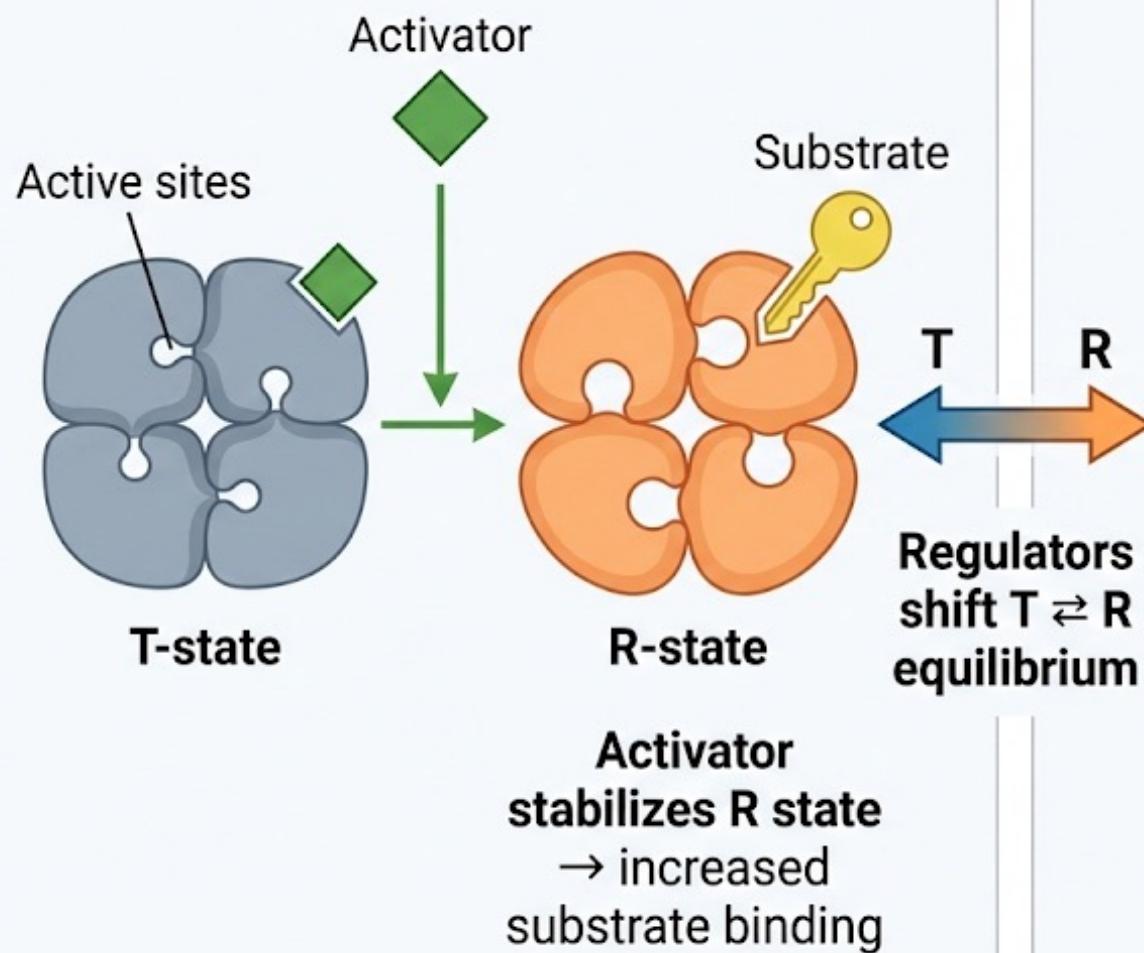
- Learning objective 5: Identify the key properties of allosteric proteins and describe the structural basis for these properties.
- Allosteric enzymes control the flux of biochemical reactions in metabolic pathways.
- Because of their regulatory properties, allosteric enzymes allow for the generation of complex metabolic pathways.



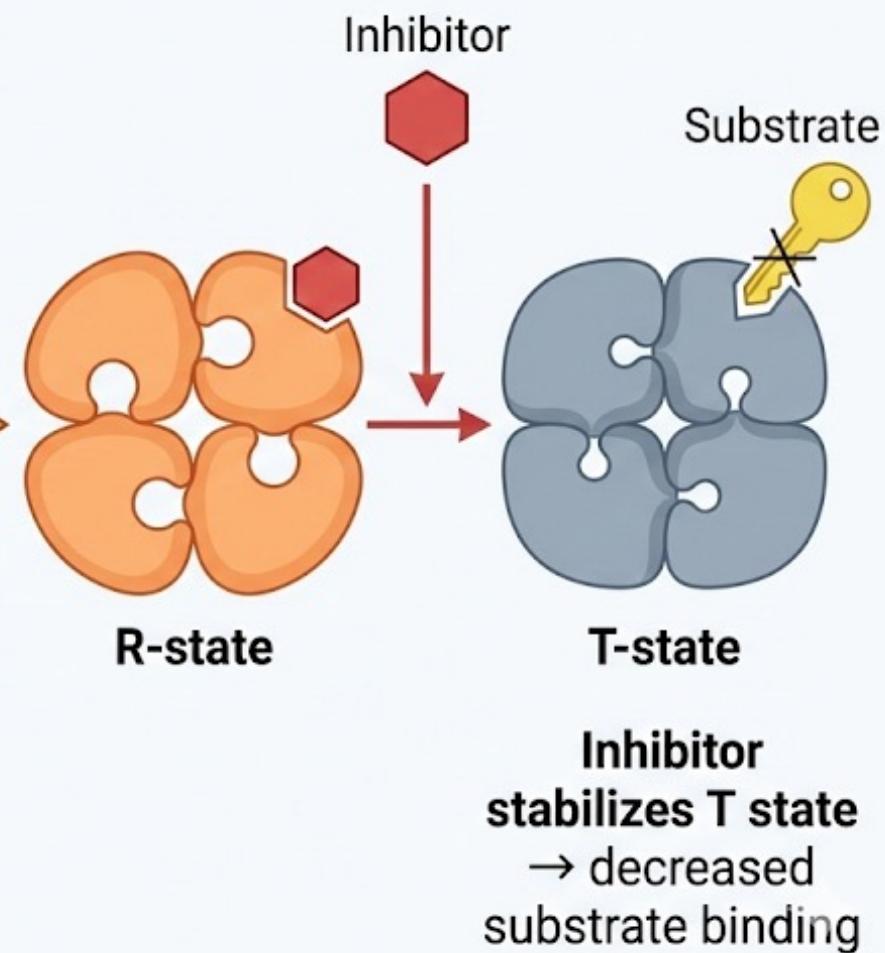
Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

Allosteric Activation and Inhibition

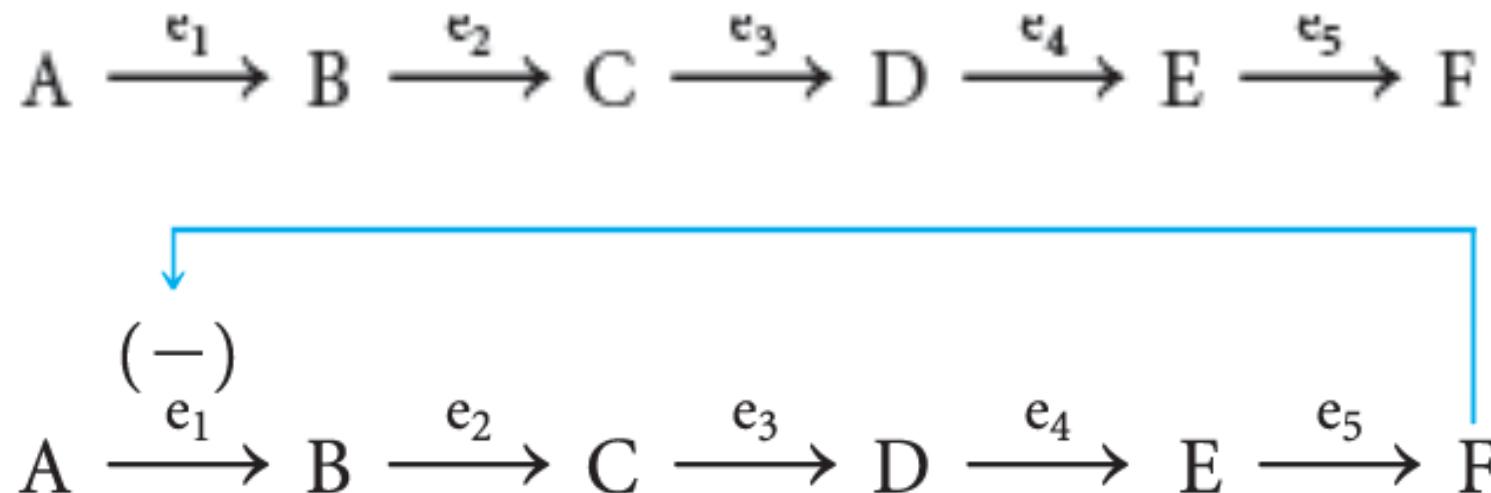
Allosteric Activation



Allosteric Inhibition



Allosteric Enzymes Are Regulated by Products of the Pathways Under Their Control



The conversion of A to B is the committed step because once this occurs, B is committed to being converted into F.

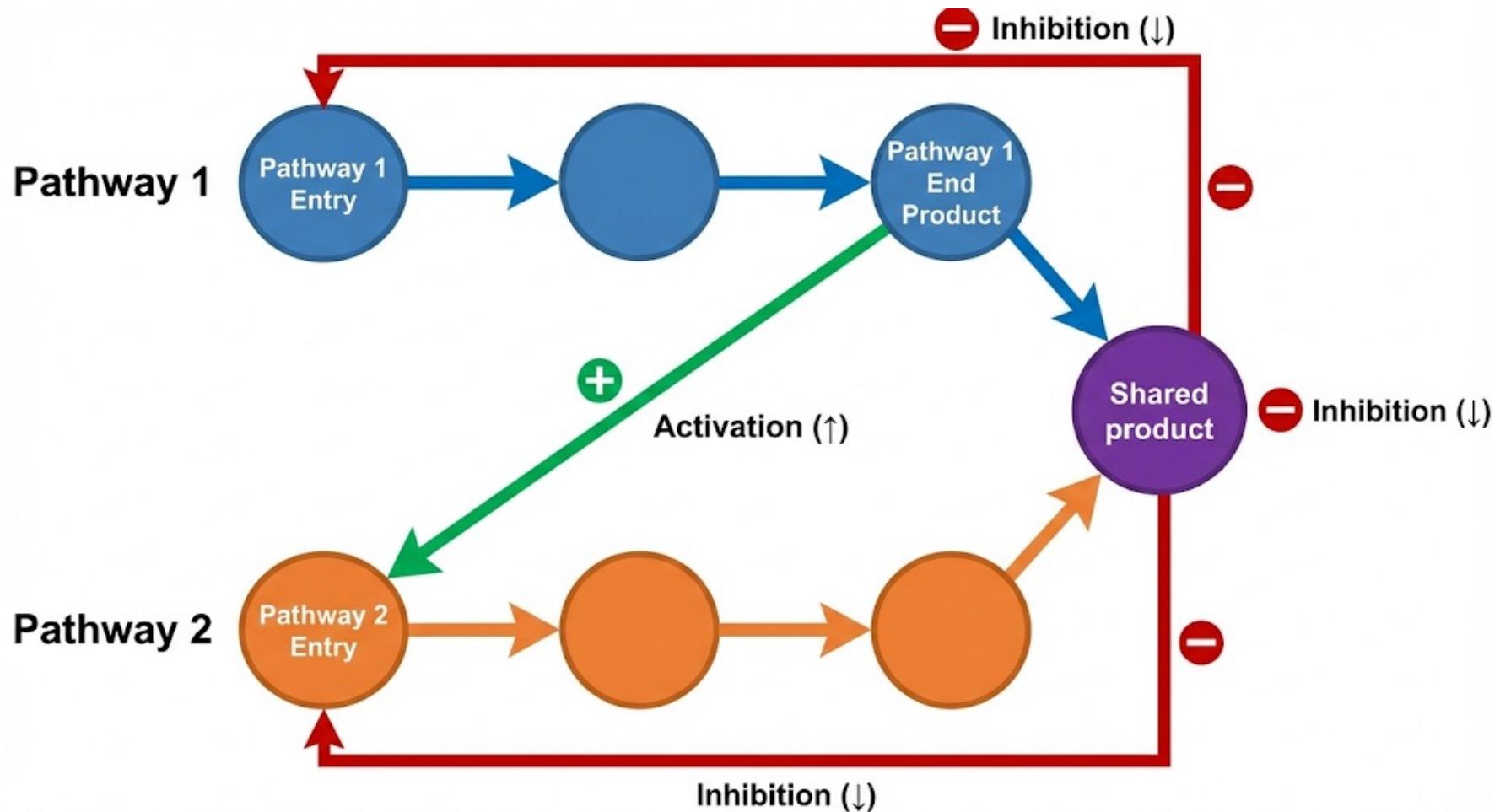
Allosteric enzymes catalyze the committed step of metabolic pathways. Michaelis–Menten enzymes facilitate the remaining steps.

The amount of F synthesized can be regulated by feedback inhibition.

The pathway product F inhibits enzyme e_1 by binding to a regulatory site on the enzyme that is distinct from the active site.

Allosteric Enzymes Are Regulated by Products of the Pathways Under Their Control (3/3)

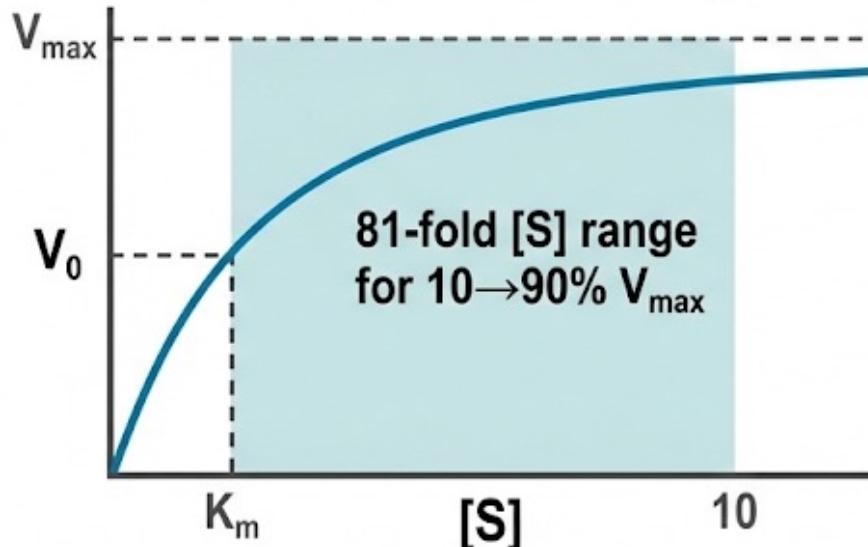
- The regulation of metabolic pathways can be quite complex.
- Allosteric enzymes may be inhibited or stimulated by several regulatory molecules.



The Signature of Cooperativity

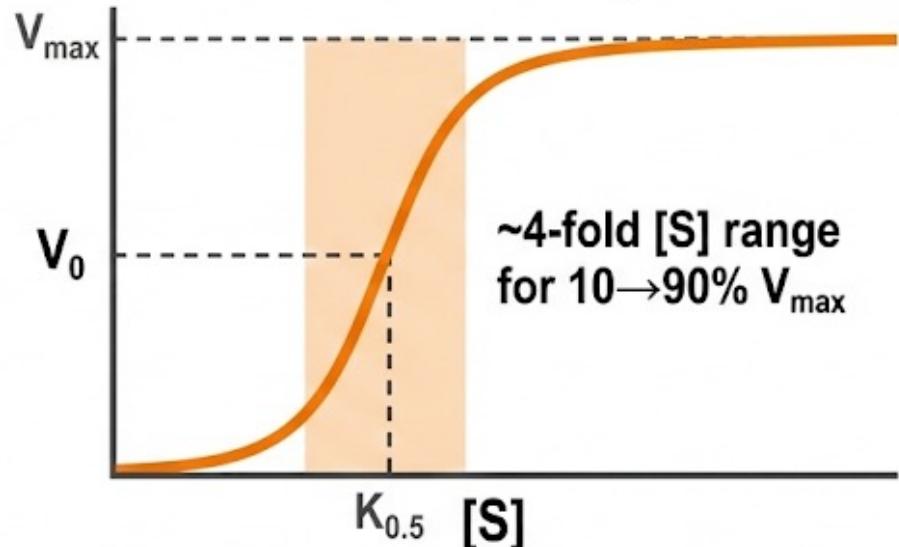
How to spot a 'smart switch' enzyme

Michaelis-Menten Enzyme



⌚️ **Gradual Response**

Allosteric Enzyme



ON OFF ⌗️ **Threshold Response**

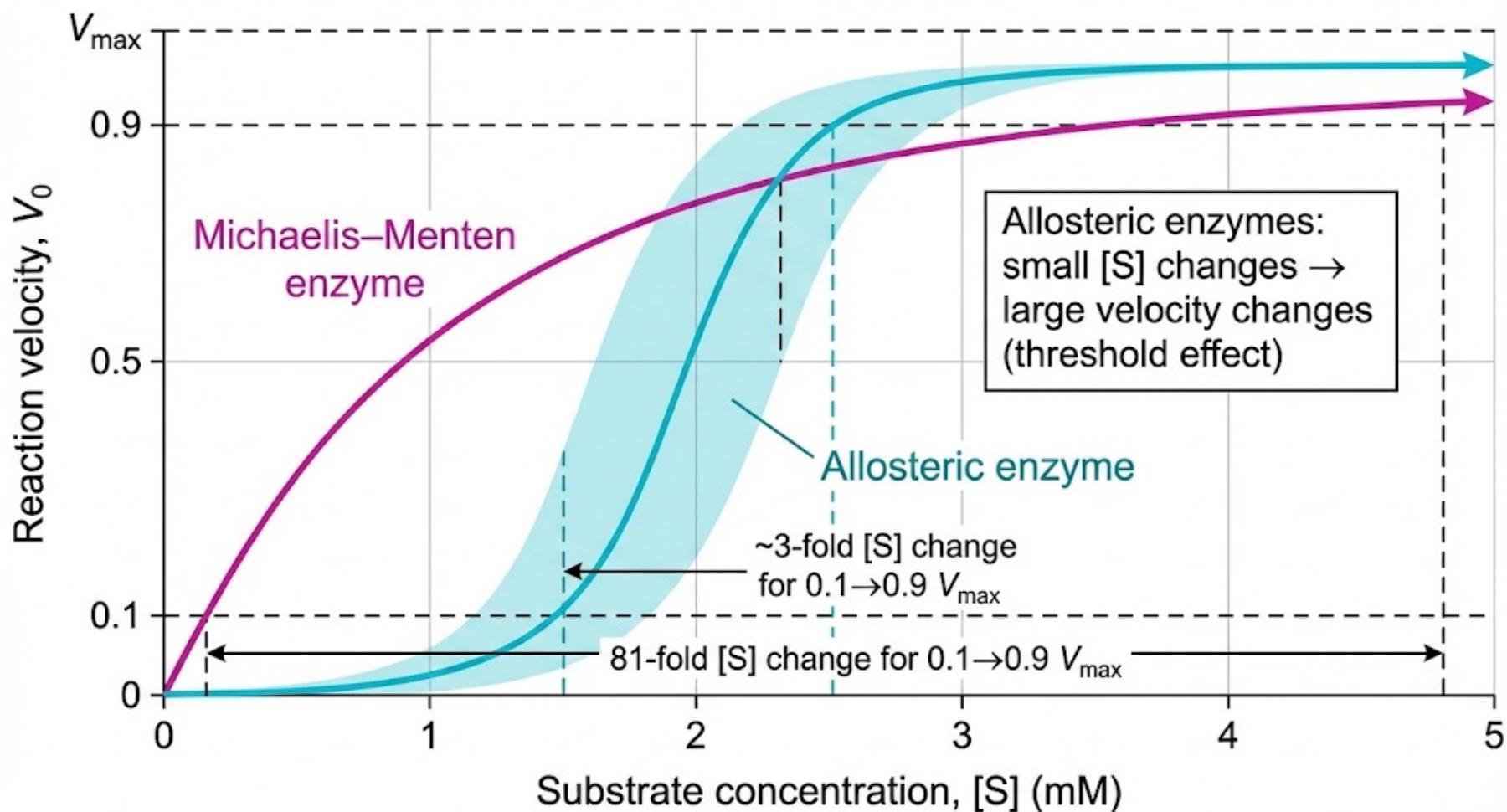
Hyperbolic = Gradual = Workhorse

Sigmoidal = Switch-like = Regulator

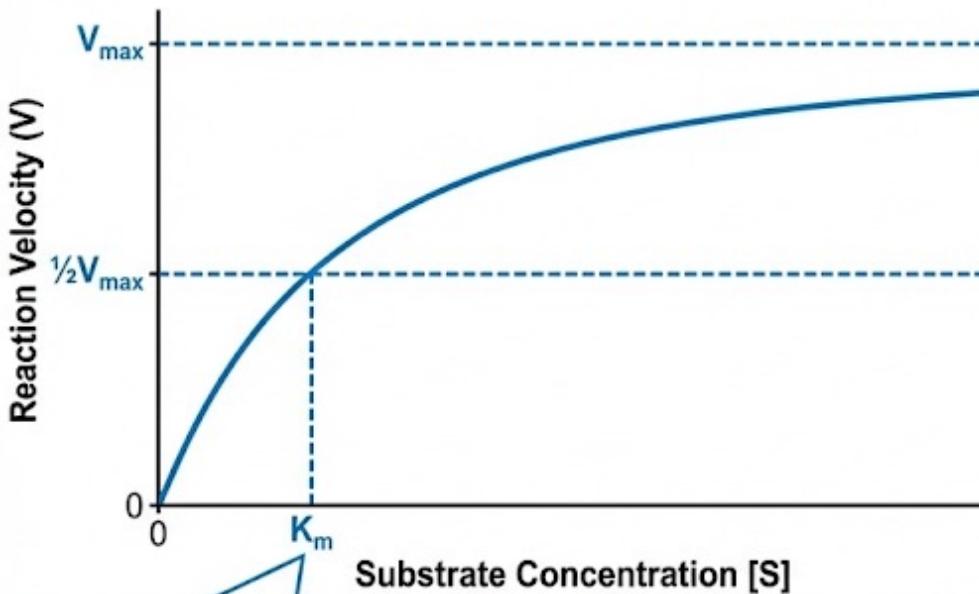
- Michaelis-Menten enzyme: Hyperbolic curve, gradual response
- Allosteric enzyme: Sigmoidal curve, threshold response
- Same inputs (substrate), dramatically different outputs
- The "S-shape" means: subunits are communicating

Allosterically Regulated Enzymes Do Not Conform to Michaelis–Menten Kinetics

- The reaction velocity of allosteric enzymes displays a sigmoidal relationship to substrate concentration.



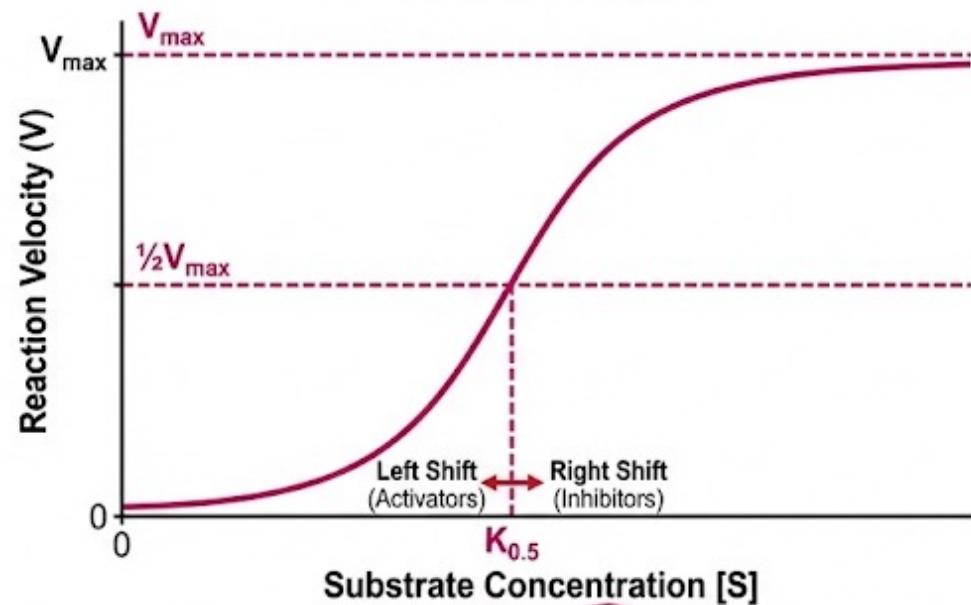
Michaelis-Menten Enzyme



K_m has mechanistic meaning — derived from rate constants

$$K_m = (k_{-1} + k_2)/k_1$$

Allosteric Enzyme



$K_{0.5} = [S]$ at half-maximal velocity

$K_{0.5}$ is purely operational — no simple mechanistic interpretation

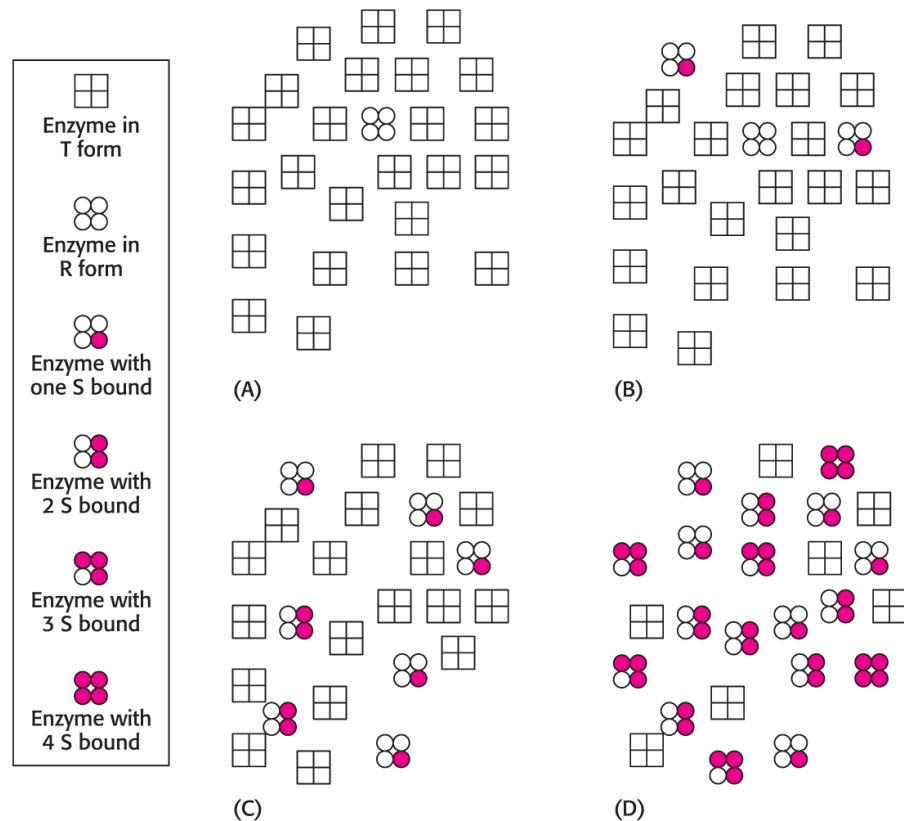
Parameter	Symbol	Curve Shape	Meaning
Michaelis constant	K_m	Hyperbolic	Related to rate constants; approximates affinity under certain conditions
Half-saturation constant	$K_{0.5}$	Sigmoidal	Substrate concentration at $\frac{1}{2}V_{max}$; affected by cooperativity and effectors

KEY INSIGHT: Both tell you the $[S]$ needed for half-maximal velocity, but only K_m has a simple relationship to binding affinity. $K_{0.5}$ reflects the COMBINED effects of substrate binding AND cooperative transitions.

ADDITIONAL NOTE: K_m is fixed for a given enzyme-substrate pair, while $K_{0.5}$ can SHIFT left or right with allosteric effectors.

Allosteric Enzymes Depend on Alterations in Quaternary Structure

- All allosteric enzymes display quaternary structure with multiple active sites and regulatory sites.
- One model that explains the behavior of allosteric enzymes is the **concerted model**.
- Features of the concerted model:
 - The enzyme exists in two different quaternary structures, designated T (tense) and R (relaxed).
 - T and R are in equilibrium, with T being the more stable state.
 - The R state is enzymatically more active than the T state.
 - T/R ratio = allostatic constant (L_0)
 - All active sites must be in the same state.
- The binding of substrate to one active site traps the other active sites in the R state and removes the substrate-bound enzyme from the $T \rightleftharpoons R$ equilibrium.
- This disruption of the $T \rightleftharpoons R$ equilibrium by the binding of substrate favors the conversion of more enzymes to the R state.



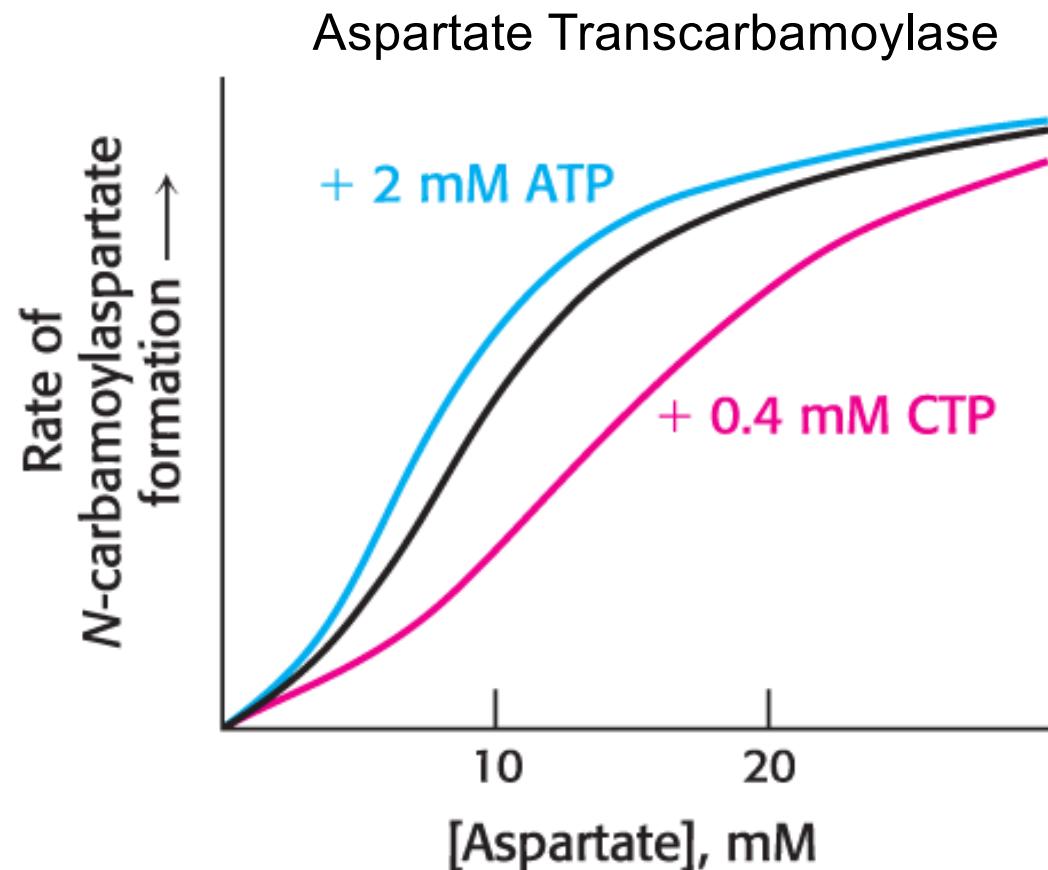
Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company



Heterotropic Effects – ATP and CTP on ATCase

Regulator Molecules Modulate the $T \rightleftharpoons R$ Equilibrium

- Allosteric regulators disrupt the $R \rightleftharpoons T$ equilibrium when they bind the enzyme.
- Inhibitors stabilize the T state, whereas activators stabilize the R state.
- The disruption of the $T \rightleftharpoons R$ equilibrium by substrates is called the homotropic effect.
- The disruption of the $T \rightleftharpoons R$ equilibrium by regulators is called the heterotropic effect.



Quick Quiz 2



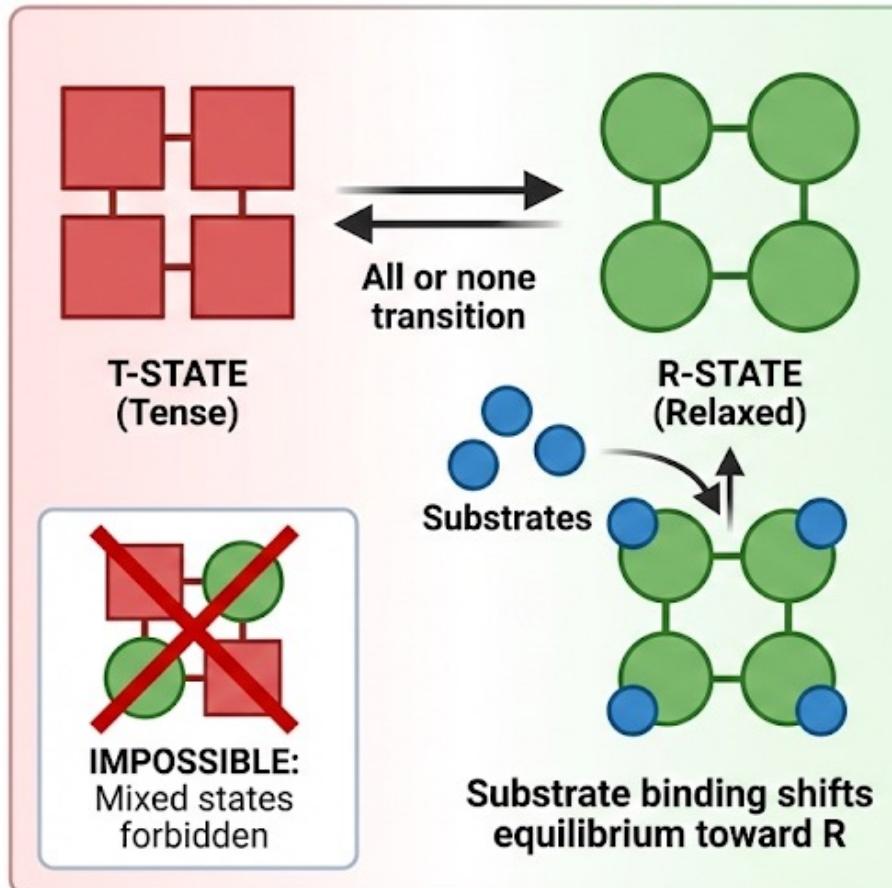
QUICK QUIZ 2

What would be the effect of a mutation in an allosteric enzyme that resulted in a T/R ratio of 0?

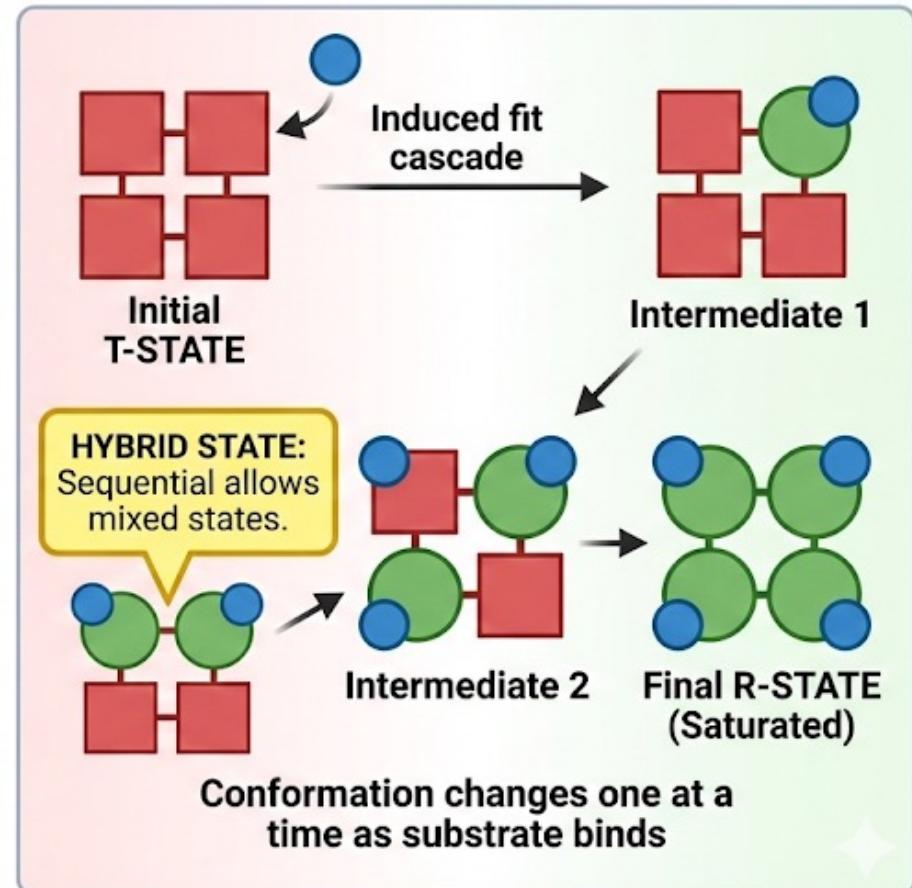
The Sequential Model Also Can Account for Allosteric Effects

- The sequential model for allosteric enzymes proposes that subunits undergo sequential changes in structure.

CONCERTED MODEL (MWC)



SEQUENTIAL MODEL (KNF)



CLINICAL INSIGHT

Loss of Allosteric Control May Result in Pathological Conditions

- Phosphoribosylpyrophosphate synthetase (PRS) is an allosteric enzyme in the purine nucleotide synthesis pathway.
- A mutation leading to the loss of regulatory control without an effect on catalytic activity leads to the overproduction of purine nucleotides.
- The overproduction results in the painful disease gout.



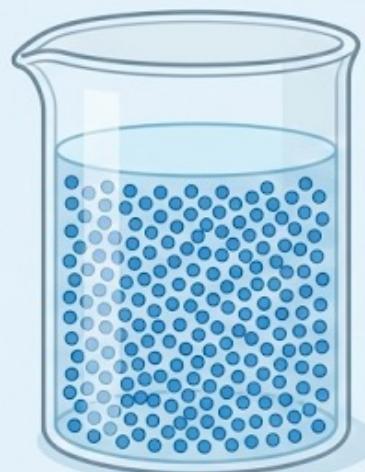
Mediscan/Alamy.

Section 8.4 Enzymes Can Be Studied One Molecule at a Time

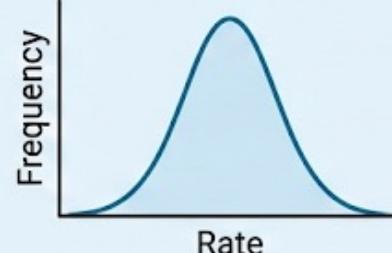
- Studies of individual enzyme molecules suggest that some enzymes may exist in multiple conformations that are in equilibrium.
- These different conformations may have different catalytic or regulatory properties.

SINGLE-MOLECULE VS. ENSEMBLE ENZYME STUDIES

ENSEMBLE MEASUREMENT



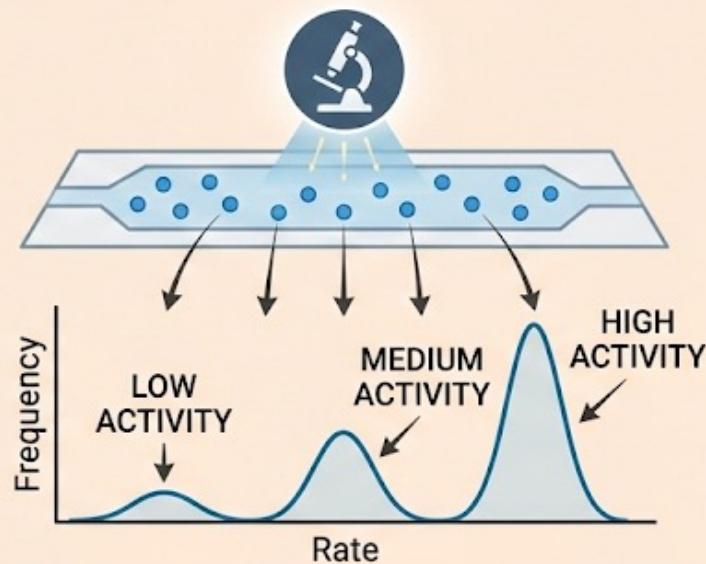
$$V_0 = 10.0 \mu\text{M/min}$$



Bulk Enzyme Solution

We measure the average rate of the entire population.

SINGLE-MOLECULE MEASUREMENT



Same average, different underlying populations!

Reveals heterogeneity & distinct conformations.

From Inference to Direct Observation

1965: MWC Model Proposed
(from kinetics only)



2012: First cryo-EM
allosteric structures

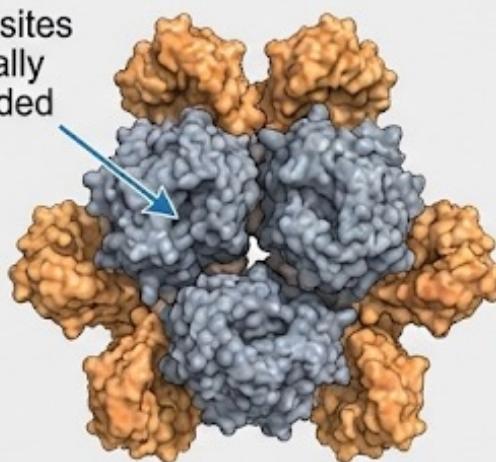


2020s: Multiple states
captured simultaneously



T State (Low activity)

Active sites
partially
occluded

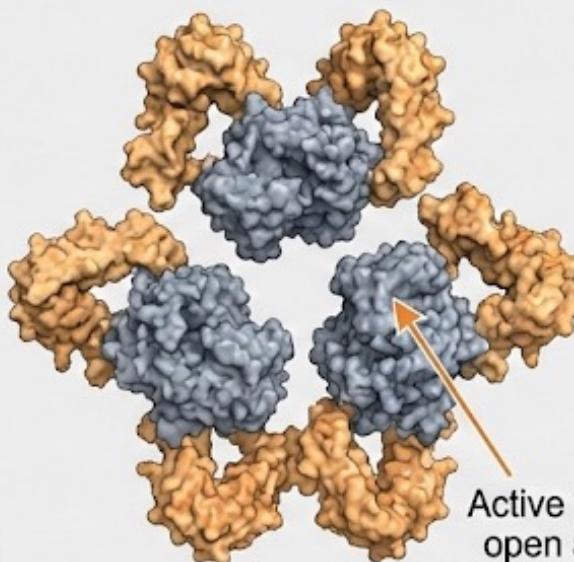


CTP
stabilizes T
↔
ATP
stabilizes R

~50 Å

R State (High activity)

Active sites
open and
accessible



Structural data based on PDB: 1RAI (T state), with R state derived for comparison.

Key Insight

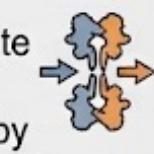
Cryo-EM can now capture proteins in multiple conformational states from a SINGLE sample - revealing the full $T \leftrightarrow R$ equilibrium in structural detail. The concerted model isn't just math – it's physical reality.

Why this matters

(1) Validates
60-year-old
kinetic models
(like MWC).



(2) Reveals
intermediate
states not
predicted by
simple models.



(3) Guides drug
design targeting
specific
conformations
(allosteric
modulators).

