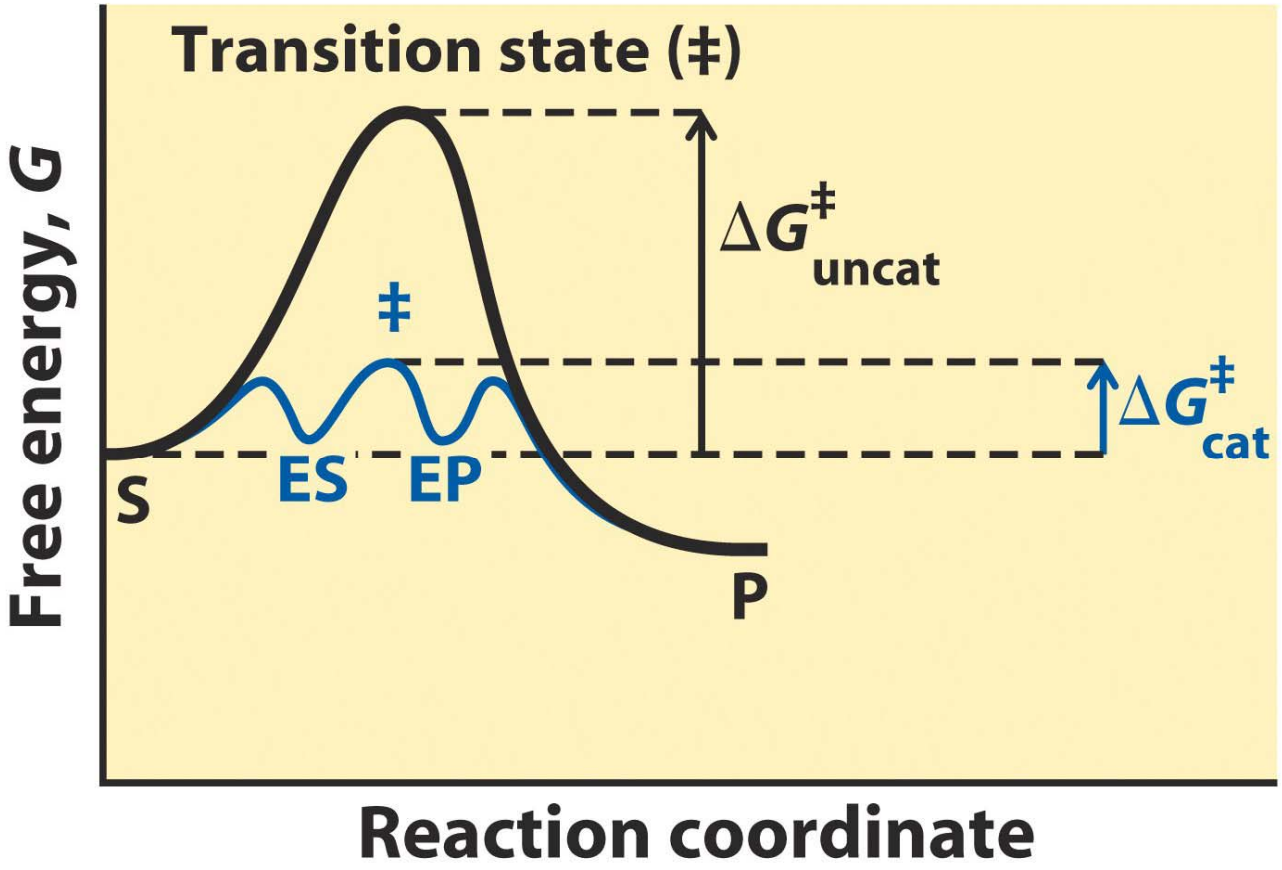
Overview of MM kinetics

- Prepared by Robert L Sinsabaugh and Marcy P Osgood in 2007.
- Includes assumptions and deriviation of original MM model.
- Includes limitations and implications of MM application to ecological systems.

Enzymes are organic catalysts.



- Enzymes accelerate thermodynamically favored reactions by lowering activation energy (ΔG++) by stabilizing the transition state through reversible bonds (H bonds, hydrophobic interactions) to the functional groups of amino acid side chains.

TABLE 6–4 Relationship between K'_{eq} and $\Delta G'^{\circ}$

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^{1}	−5.7
10^{2}	−11.4
10^{3}	−17.1

Note: The relationship is calculated from $\Delta G'^{\circ} = -RT \ln K'_{eq}$ (Eqn 6-3).

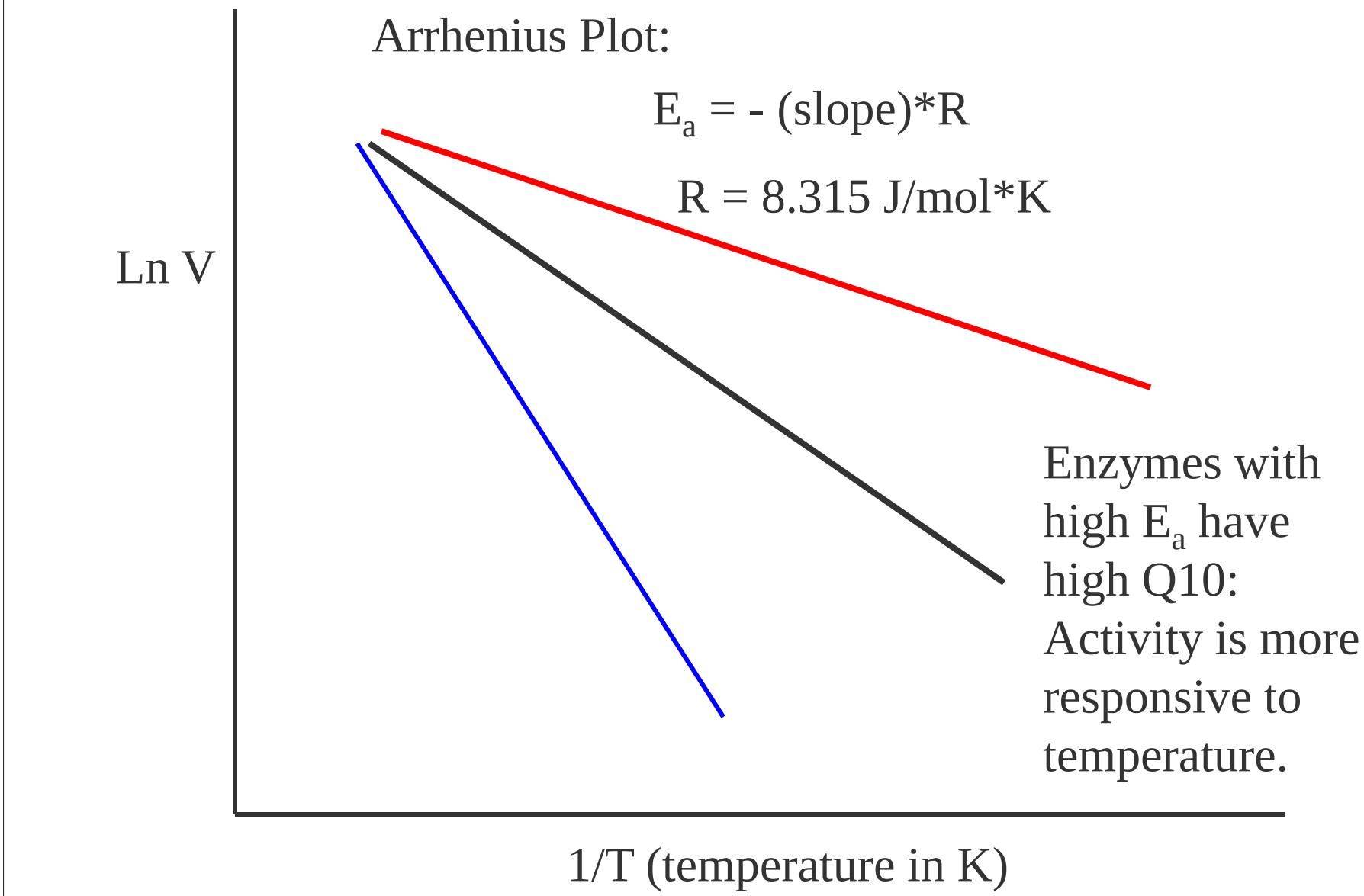
Effects of temperature on enzyme activity

- The activation energy E_a also determines how enzyme activity changes in response to temperature.

- The E_a of an enzymatic reaction is calcuated by measuring activity at several temperatures and plotting the data on an Arrhenius plot.

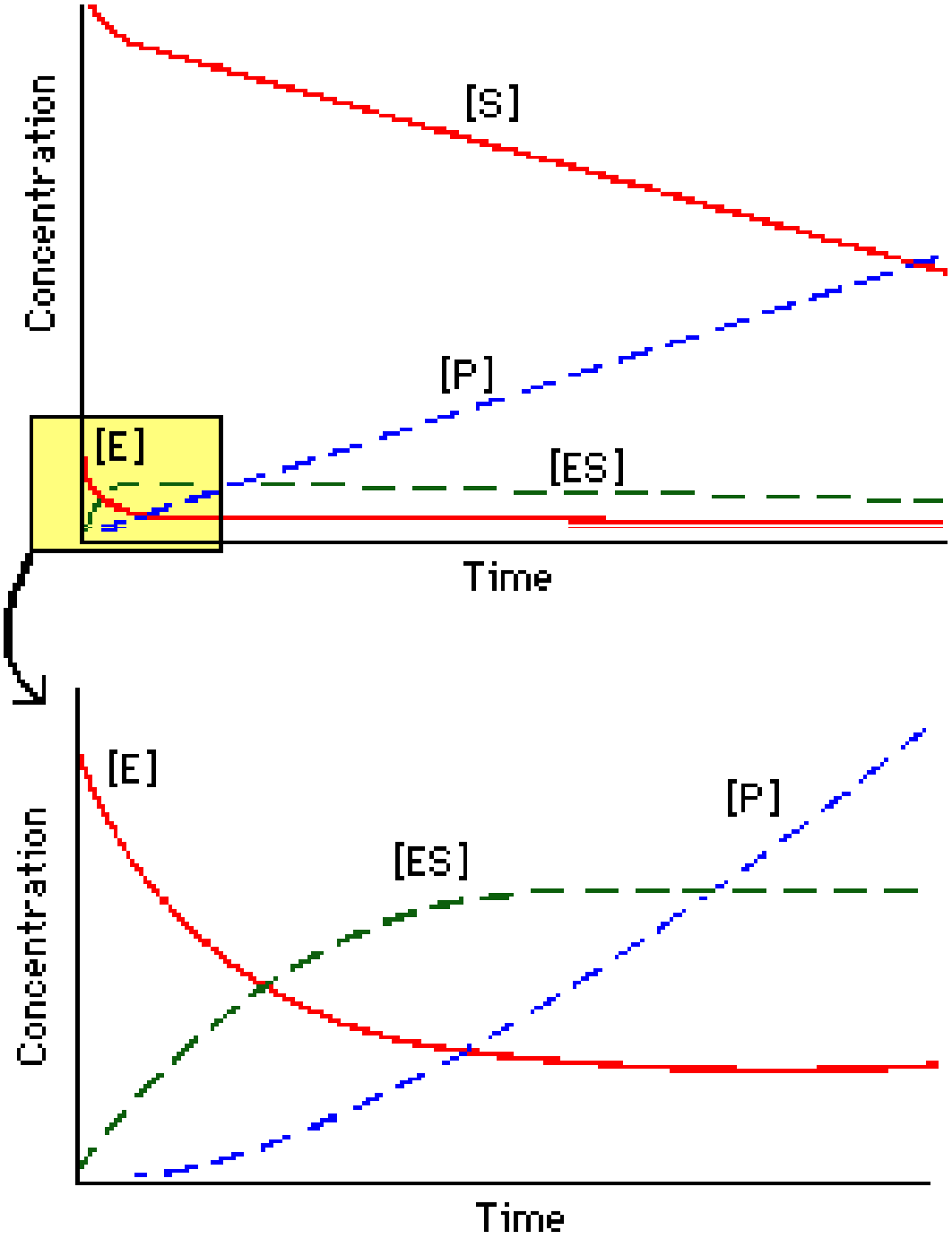
Arrhenius equation

- Used to empirically estimate the activation energy of an enzyme
- $\ln V = \ln A - (E_a/R)1/T$
  - V is the reaction rate or velocity
  - E_a is the activation energy
  - R is the universal gas constant
  - T is absolute temperature (K)
- $Q_{10} = k_{T+10}/k_T$
  - $Q_{10} = 2$ equivalent to $E_a = 48$ kJ/mol*K or 0.5 eV


Arrhenius Plot:
E_a = - (slope)*R
R = 8.315 J/mol*K
Ln V
Enzymes with high E_a have high Q10: Activity is more responsive to temperature.
1/T (temperature in K)

Enzyme kinetics: MM model

- $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$
- k_1 = rate of formation of ES
- k_{-1} = rate of disintegration of ES back to E + S
- k_2 = rate of formation of E + P from ES
- In early stages of reaction, when [P] is very small, can assume reverse reaction is negligible, and ignore k_{-2}


Concentration
[S]
[P]
[E]
[ES]
Time
[E]
[P]
[ES]
Concentration
Time

KINETICS

- k_1 k_2
- $E + S <====> ES <====> E + P$
- k_{-1}
- V_o = rate that product is formed = $k_2[ES]$
- Rate of formation of ES = $k_1[E][S]$
- Rate of disintegration of ES = $(k_{-1} + k_2)[ES]$
- At steady state $d[ES]/dt = 0$
- therefore $k_1[E][S] = (k_{-1} + k_2)[ES]$

KINETICS

- From steady state assumption:
- Define the Michaelis constant:
  $K_m = (k_2 + k_{-1})/k_1 = [E][S]/[ES]$
- Define $[E]_T = [E] + [ES]$ because the former is readily measurable.
- And add a simplifying assumption that [S]>>>[E] and do some algebraic manipulation:
- $V_0 = k_2[ES] = k_2[E]_T[S]/(K_m + [S])$

KINETICS

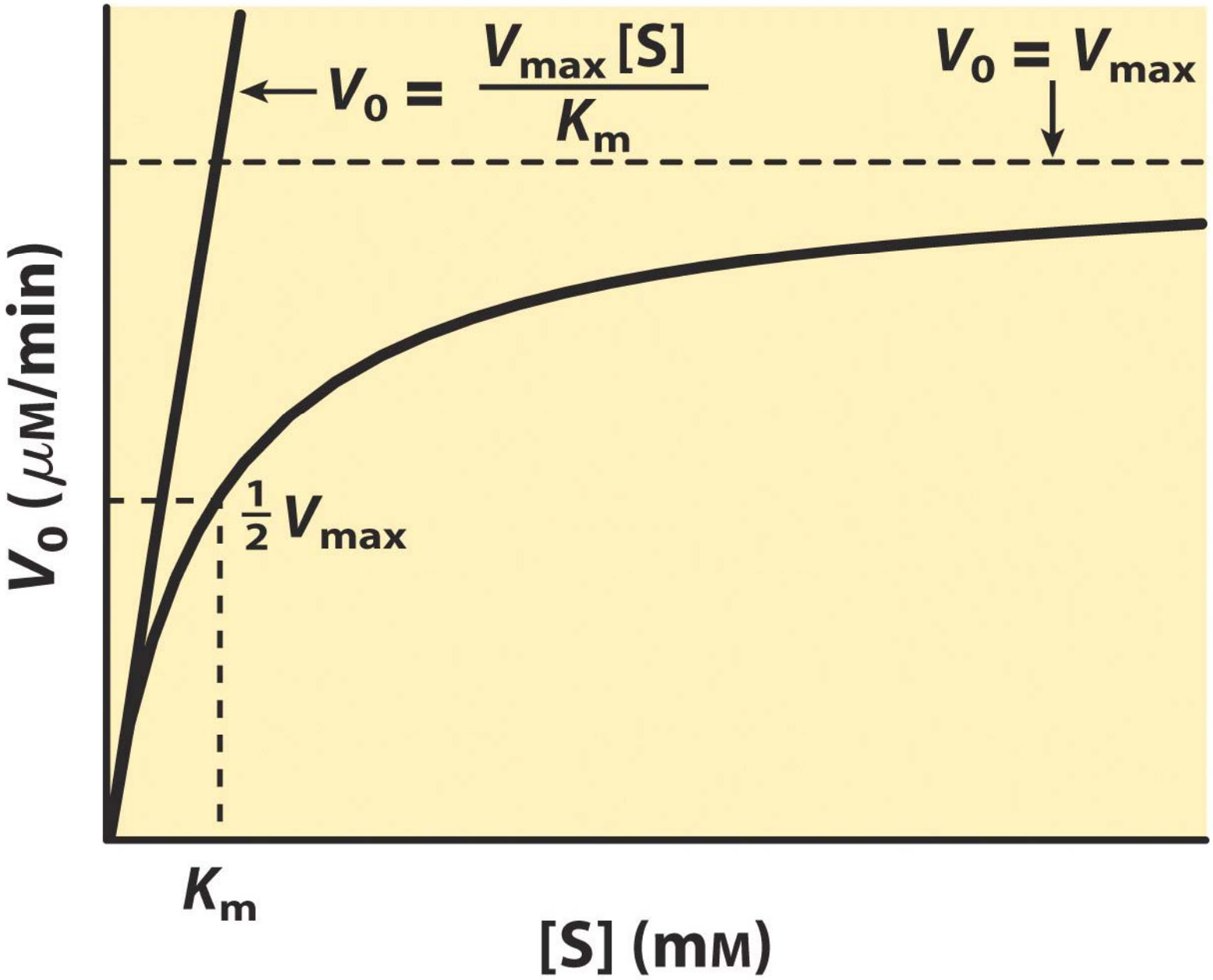
- Maximal rate of reaction is called V_{max};
- Attained when all enzyme active sites are saturated;
- $V_{max} = k_2[E]_T$
- Substituting into equation on previous slide:
- $V_0 = V_{max}\,[S]/(K_m + [S])$

KINETICS

- $V_0 = V_{max}\,[S]/(K_m + [S])$
- Thus did Leonor Michaelis and Maude Menten describe the activity of simple enzymes in 1913.
- K_m = [S] when V= $V_{max}/2$

Leonor Michaelis
1875–1949

Maud Menten
1879–1960


$V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
V_0 (μM/min)
$\frac{1}{2} V_{max}$
K_m
[S] (mM)

MM KINETICS

- Review of assumptions:
- MM kinetics measure initial velocity V_0, before much [P] accumulates: k_{-2} is ignored.
- [S] >>>[E]
- Steady state assumption: [ES] remains constant
- E is not consumed but recycled.

MM Caveats

- MM model describes the reaction kinetics of a simple enzyme, i.e. an enzyme with only one active site and no regulatory sites, and a single substrate, i.e. no cofactors, no polymerizations, no condensations, etc.
- **V_{max} and K_m are independent parameters**:
  - V_{max} is a function of catalytic efficiency of the enzyme, i.e. how many substrate molecules can be converted per second (k_{cat}): A function of how many enzyme-substrate bonds need to be formed then broken over the course of a reaction.
  - K_m is a measure of the binding affinity of the substrate for the enzyme active site: A function of how well the functional groups of the substrate align with their complements on the enzyme, measured by the free energy change associated with enzyme-substrate binding.

TABLE 6–7 Turnover Numbers, k_{cat}, of Some Enzymes

Enzyme	*Substrate*	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Empirical estimation of MM parameters

- Measure V as a function of [S]:
- Lineweaver-Burke plot: linearized MM Eq.
  - $1/V_0 = K_m/V_{max} * (1/[S]) + 1/V_{max}$
- L-B weighs the velocities at lower [S] more heavily in determining the linear relationship.
- All points cluster because it is generally easier to measure V at high [S] than at low [S]
- K_m, determined from x-intercept, and V_{max}, from y-intercept, have high level of uncertainty.

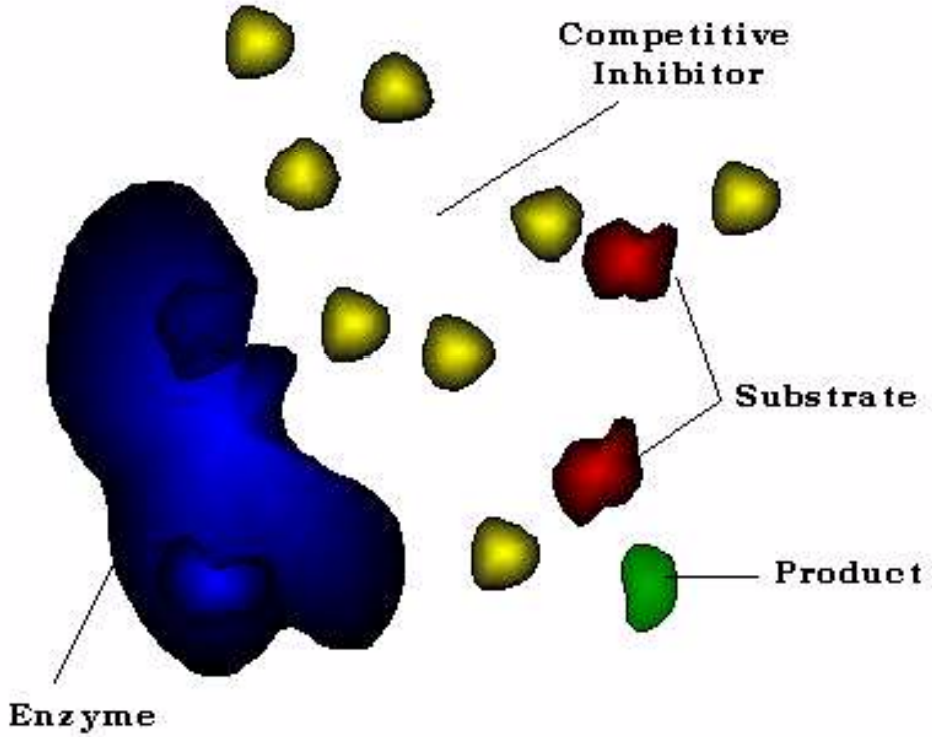
KINETICS…PLOTS

- Eadie-Hofstee plot: linearized MM Eq.
- $V_0 = V_{max} - K_m(V_0/[S])$
- Points are more spread out; better estimate of K_m (slope of line)
- V_{max} is y-intercept

MM Caveats

- V_{max} and K_m can be affected by environmental conditions, e.g. pH, ionic strength.
- V_{max} - but generally not K_m - is a function of temperature. Expressed as E_a.
- K_m - but generally not V_{max} - is a function of the concentration of competing substrates.

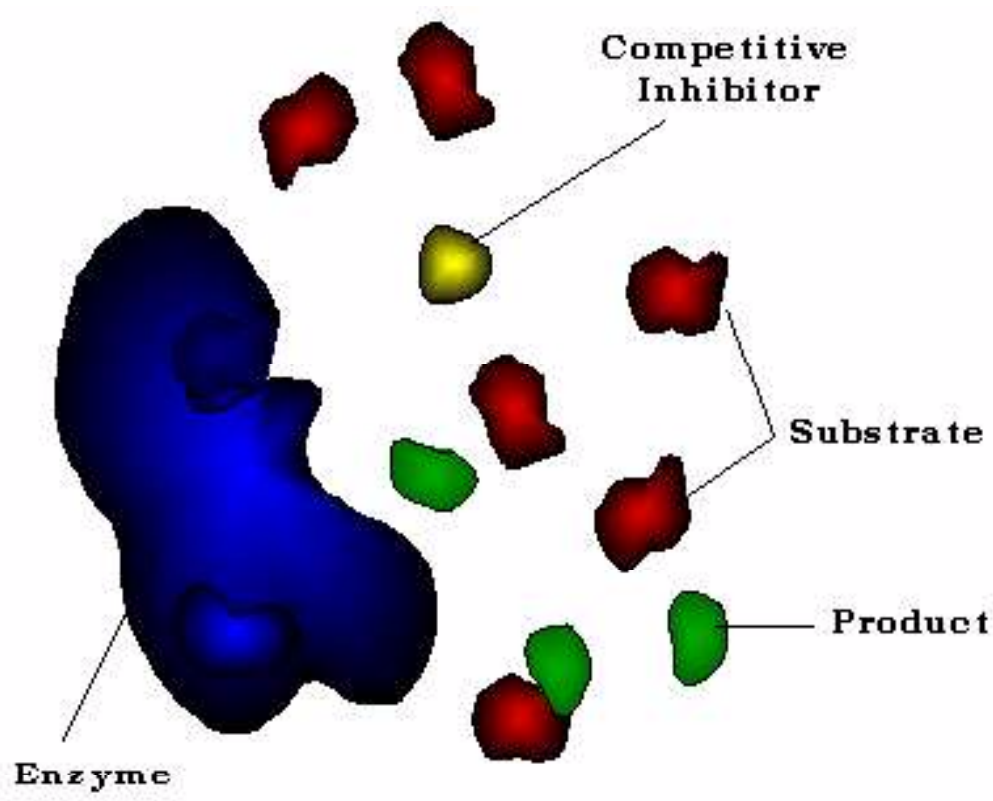
KINETICS: Inhibitors



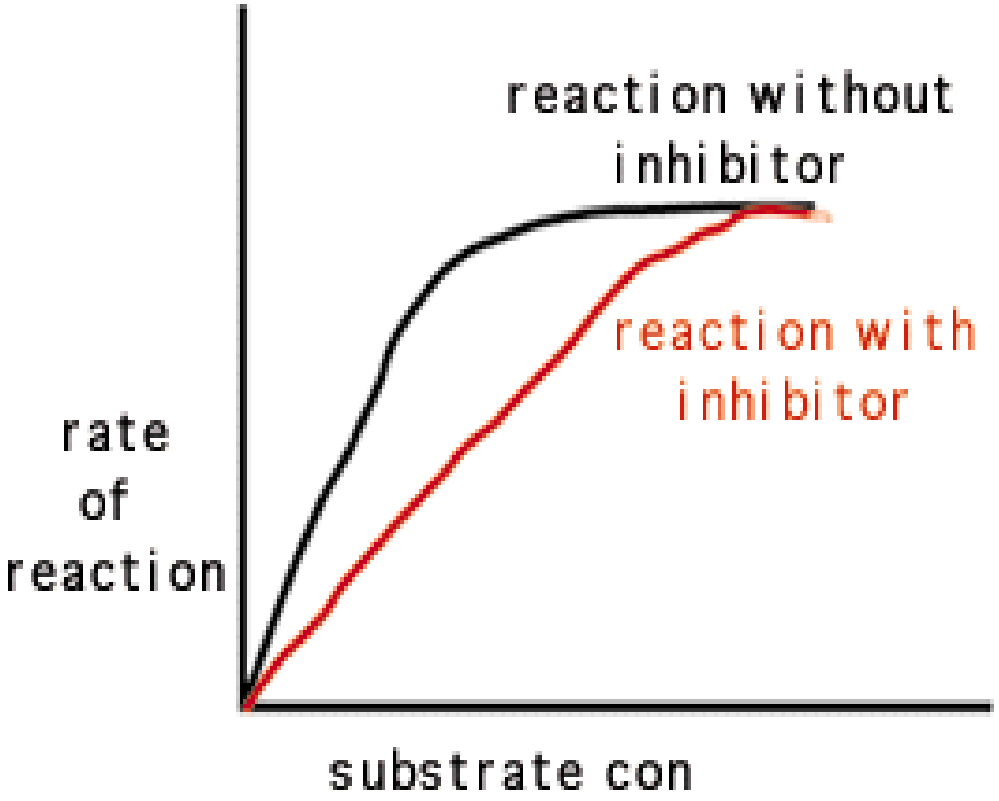
- Competitive inhibitors
- Compete with S for active site of enzyme.
- Often resemble S.
- K_m increases; it takes
- more S to reach $V_{max}/2$
- App. K_m (aka α) = K_m $(1+[I]/K_i)$
- $V = V_{max}\,[S]/(\alpha K_m + [S])$

KINETICS: Inhibitors

- Competitive inhibitors
- At very high [S], S "outcompetes" the inhibitor; V_{max} is the same

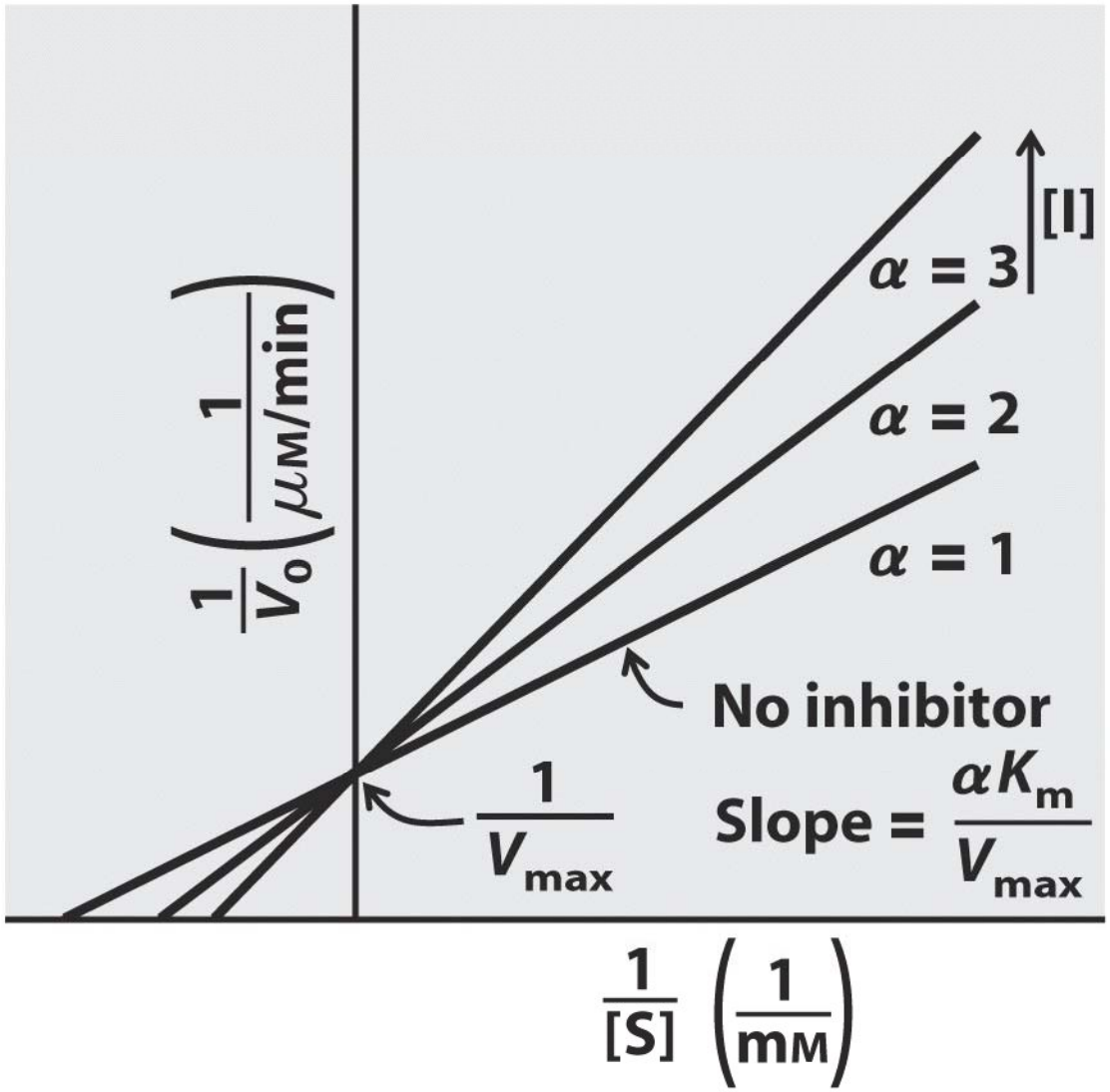


KINETICS: Inhibitors



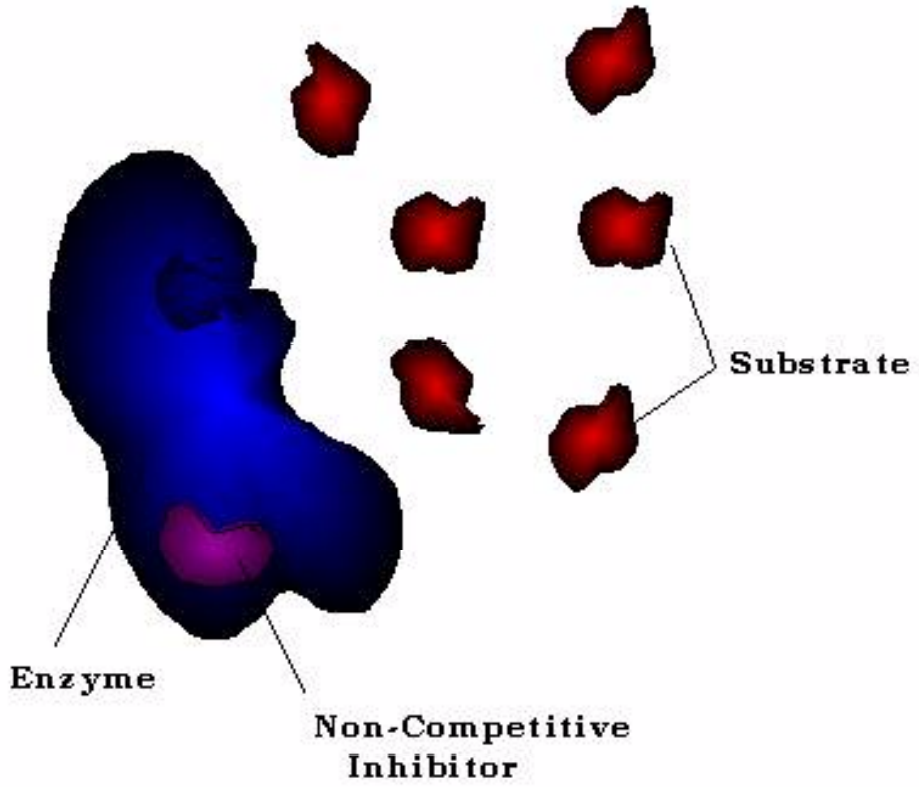
- **Competitive inhibition shown in a [S] *vs* Vo plot**

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



KINETICS: Inhibitors

- Noncompetitive inhibitors
- Bind to site distinct from active site of enzyme
- Do not resemble S
- K_m may change: binding compromises the capacity of the enzyme to make conformational changes associated with enzyme-substrate binding.

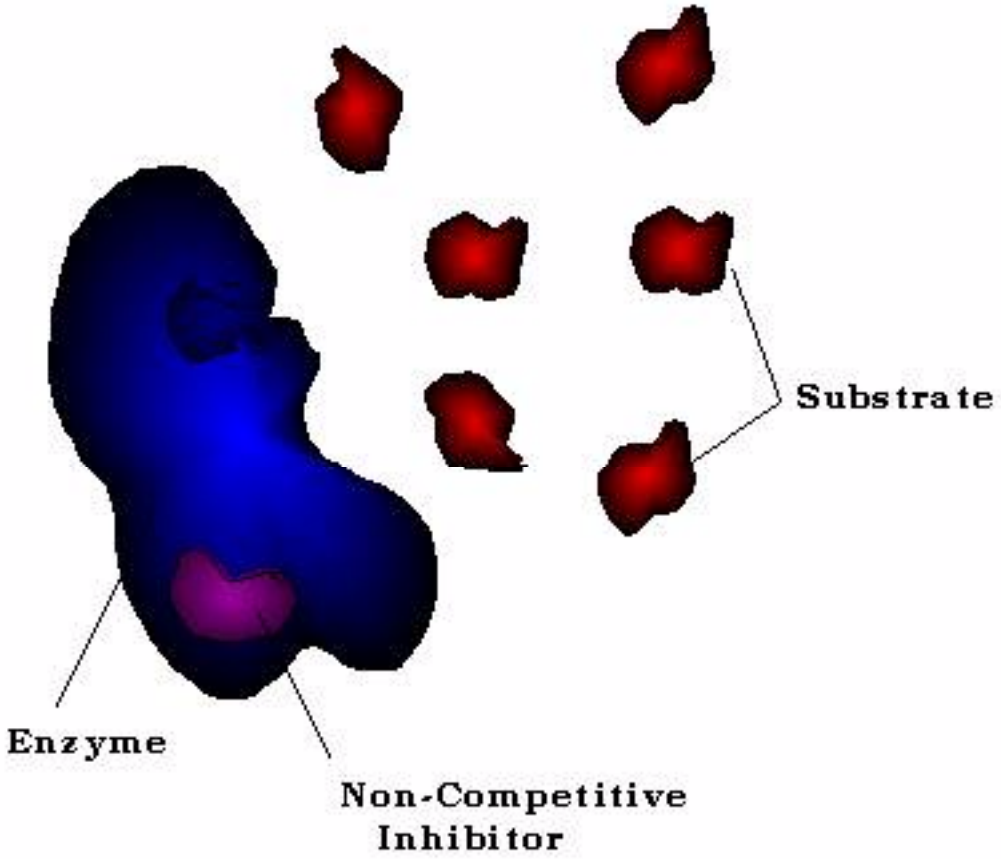


KINETICS: Inhibitors

Noncompetitive inhibitors

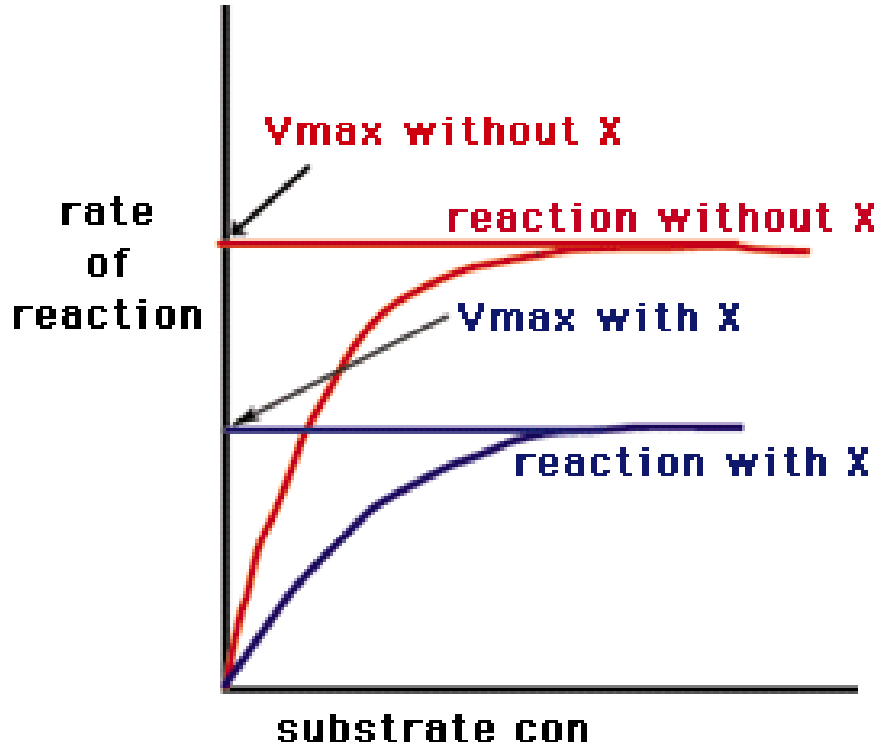
V_{max} is lowered

E_a increased



KINETICS: Inhibitors

- Noncompetitive inhibition shown in a [S] *vs* V_0 plot



"*Ecosystems .... are functionally a system of stored, immobilized enzymes.*"

-Robert G. Wetzel

- Nonetheless, for ecological systems MM is a metaphor, not a model.
- Literal application leads to simplistic or erroneous interpretation.

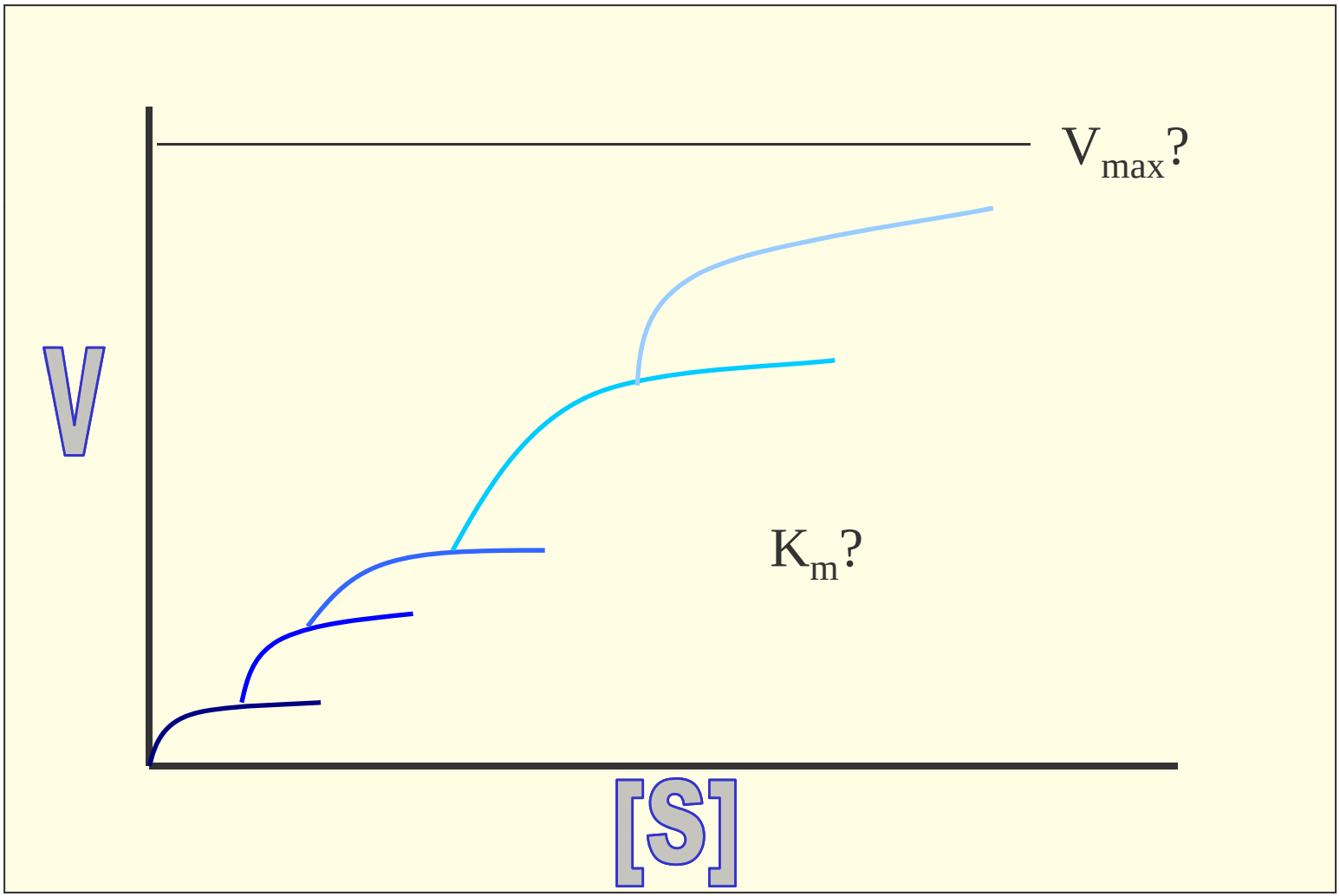
Ecosystems as enzyme systems

- 10^6 enzymes with varying E_a, V_{max}, K_m, pH optima, for a broad range of substrates.
- Few enzymatic reactions meet MM assumptions.
- Enzymes are distributed across many compartments, some biotic some not, that vary in redox, pH, water potential & substrate/inhibiter concentrations with boundary layers and diffusion effects.
- Kinetics and dynamics of extracellular enzymes are decoupled from enzyme producers.
- Enzyme expression controlled in myriad ways according to nutrient availability, substrate availability, stoichiometry, quorum signals, population replacement, etc.

MM metaphor

- "V_{max}" and "K_m" are statistical constructs.
  - Closer to maxima than means.
- Values are a function of method and conditions of measurement.
  - "V_{max}" and "K_m" decrease with disaggregation and mixing
  - Comparisons across studies difficult to make.
- "K_m" and "V_{max}" are not constants.
  - Often not independent
  - Interpretation of the metaphor is different from model.

Progressive saturation of compartmentalized enzyme reservoirs often means that $^{App}K_m$ estimates are a function of the range of [S] used to estimate it. $^{App}V_{max}$ is affected too: The high [S] needed to saturate some compartments will repress activity in others.



MM Metaphor vs. Model

- For ecological systems, use ${}^{App}V_{max}$ and ${}^{App}K_m$ to differentiate metaphor from model and reduce ambiguity.
- ${}^{App}V_{max}$ is a measure of effective enzyme concentration.
  - In MM model V_{max} is function of k_{cat} because [E] is fixed.
- ${}^{App}K_m$ is a measure of substrate concentration.
  - ${}^{App}K_m$ is measured against a background of natural substrates that compete for enzyme.
  - ${}^{App}K_m = K_m (1+[I]/K_I)$ where I is natural substrate pool.
  - ${}^{App}K_m$ often 1-3 orders of magnitude greater than K_m

MM metaphor

- Unlike the MM model, ${}^{App}V_{max}$ and ${}^{App}K_m$ are generally not independent.
- Optimal resource allocation (physiological controls on enzyme expression) and selection (community succession) act to scale substrate concentration and enzyme concentration.
- On small scales, relationships between substrate dynamics, enzyme dynamics, and cell dynamics are chaotic so ${}^{App}V_{max}$ and ${}^{App}K_m$ may be uncorrelated.

MM metaphor

- ${}^{App}E_a$, when calculated in relation to seasonal fluctuations in environmental temperature, reflect the dynamics of enzyme concentration, kinetic parameters, and substrate concentration, as well as thermodynamics.