Enzyme kinetics provide a *functional* description of a protein. It can yield tremendous insight, particularly when combined with structural data.

**Kinetics cannot prove a mechanism, though they can eliminate models that are inconsistent with the data.** Advanced kinetic tools, such as irreversible inhibition, pH rate profiles or kinetic isotope effects can strongly support a particular mechanism and can help identify key catalytic residues.

There is no escaping enzyme kinetics! It is better to understand it than to fear it.
Enzyme kinetics

The field of enzymology that deals with the factors that affect the rates of enzyme-catalyzed reactions.

Such factors can include pH, temperature, substrate concentration, inhibitor concentration and enzyme concentration on the rate of an enzyme-catalyzed reaction.

The kinetic treatment is also called “formal kinetics” since we make no hypothesis on the enzyme’s structure. As a consequence, we cannot obtain structural information on enzymes from the kinetic studies.
Why do we study enzyme kinetics?

• Can be used to characterize an enzyme’s substrate preference and cofactor requirements or to identify potent inhibitors of potential therapeutic value.

• Various modes of enzyme inhibition can provide insight into the mechanism of catalysis or the order of ligand binding.

• Advanced kinetic techniques can serve to exclude or identify an enzyme’s catalytic mechanism or to assist in the identification of key catalytic residues.
Why do we study enzyme kinetics?

Mitochondrial Creatine kinase structure

A full structural description of substrate binding to the active site

But.......
Why do we study enzyme kinetics?

"And for all the current and rather silly emphasis on structural biology, understanding enzymes means understanding catalysis and catalysis is concerned with kinetics, not structure: as Jeremy Knowles aptly remarked, studying the photograph of a racehorse cannot tell you how fast it can run."

Why bother with kinetics?

- The rates at which a reaction occurs, compared to other reactions in a pathway, will determine the rate limiting and controlling reaction.

  
  - A $\rightarrow$ B $\rightarrow$ C $\rightarrow$ D $\rightarrow$ E
    - if the reaction C$\rightarrow$D is the slowest then regulating the enzyme carrying out this reaction will control the amount of E made
    - [C] will accumulate
Enzymes are truly remarkable catalysts
They are usually remarkably specific (and, where they are not, this is a feature of interest).
Enzymes provide exceptional levels of catalysis.
There is an fascinating interplay between chemical restraints, metabolism, enzyme efficiency and evolution.
Imagine attempting to perform the chemical transformations common to biochemistry with an equivalent organic chemical reaction.
A general scheme for a simple enzyme-catalyzed reaction which converts a single substrate into a single product is:

\[ E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} EP \overset{k_3}{\underset{k_{-3}}{\rightleftharpoons}} E + P \]

This kinetic scheme is simplified significantly when the reaction proceeds at “initial velocity” i.e. at the onset of the reaction, [S] = 100% while [P] = 0%. While [P] remains very low, the back reaction is negligible and the above scheme:

\[ E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_{cat}}{\rightarrow} E + P \]

The assay of an enzyme under initial velocity conditions is, therefore, an important consideration in the practical design of enzyme assays.
Enzyme kinetics

\[
\begin{align*}
E + S & \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} ES & \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} EP & \underset{k_3}{\overset{k_{-3}}{\rightleftharpoons}} E + P
\end{align*}
\]

Initial rate:

\[
\begin{align*}
E + S & \overset{k_i}{\longrightarrow} ES & \overset{k_{cat}}{\longrightarrow} E + P
\end{align*}
\]

Practical considerations:
What this means « initial rate \([P] = \text{zero} »

As a approximate value \([P] < 10\% \text{ of } [S] \)

This depends
- on the equilibrium constant of the reaction
- on the enzyme inhibition by the \(P\)

Under the best conditions \(v_i\) is linear as a function of time (easy to measure)
\(Vi\) should be ALLWAYS proportional to \([E]_t\)
The rate of the reaction is measured by the appearance of product (or the disappearance of substrate). The overall rate of the reaction is governed by the rate of conversion of the final intermediate (in this case, ES) into free enzyme (E) and free product (P). Thus, the rate of the above reaction is given by:

\[ v_i = \frac{-d[S]}{dt} = \frac{d[P]}{dt} = k_{cat}[ES] \quad \text{[eq. 1]} \]

The preceding equation is not particularly useful. Since ES is an intermediate in the reaction, its concentration at any given time is unknown and it is not practical to directly follow its conversion into E + P. It would be much more useful to express the rate equation in terms of the total enzyme concentration ([E]_t) and the initial substrate concentration ([S]), both of which are known.
The Briggs-Haldane steady state approach

- The Henri-Michaelis-Menten equation was originally developed by Victor Henri (1903) and later confirmed by Leonor Michaelis and Maud Menten (1913).

\[
\begin{align*}
E + S & \rightleftharpoons_{K_{-1}}^k_1 \text{ES} \xrightarrow{k_{\text{cat}}} E + P
\end{align*}
\]

- The assumption of rapid equilibrium in the derivation of the Henri-Michaelis-Menten equation requires that the rate of dissociation of the ES complex \( (k_{-1}) \) far exceed the rate of conversion of the ES complex into E + P \( (k_{\text{cat}}) \). This assumption is invalid for many (if not most) enzymes and cannot be verified experimentally in an easy way. We will not talk about this situation later.

- In 1925, Briggs & Haldane developed an initial velocity rate equation that did not require the assumption of rapid equilibrium. Rather, the Briggs-Haldane approach was to assume a steady-state for the ES complex.
Steady state assumption:

- When $[S] \gg [E]$, the level of $[ES]$ stays constant after an initial burst phase.

$$[ES] = \text{const} \quad \text{or} \quad \frac{d[ES]}{dt} \approx 0$$

Note: The accompanying figure is somewhat deceptive. In fact, steady state is reached very quickly. The initial phase of an enzyme-catalyzed reaction, prior to the onset of steady state, can only be followed using specialized equipment in combination with rapid sample mixing techniques. The kinetics are much more complex but they can yield important information about the individual kinetic steps in an enzyme-catalyzed reaction. This type of kinetics is referred to as “pre-steady state”
• As before, this derivation deals only with initial velocity kinetics. We can treat the reverse reaction as negligible and simplify the scheme to:

\[
E + S \xleftrightarrow{k_1 \hspace{1cm} k_{-1}} ES \xrightarrow{k_2} E + P
\]

• The overall rate of production of ES is the sum of the elementary reaction rates leading to its appearance minus the sum of those leading to its disappearance.

\[
\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0
\]

Rearranging:

\[
[ES] = \frac{k_1[E][S]}{k_{-1} + k_2} \quad [eq. 6]
\]
As before,

\[ \frac{-d[S]}{dt} = \frac{d[P]}{dt} = k_2[ES] \]

and

\[ \frac{v_i}{[E]_t} = \frac{k_2[ES]}{[E] + [ES]} \]

Substituting in [eq. 6], we get:

\[ \frac{v_i}{[E]_t} = \frac{k_2k_1[E][S]/(k_1 + k_2)}{[E] + [E][S]k_1/(k_1 + k_2)} \]

\[ v_i = \frac{k_2[E]_t[S]}{\frac{k_1 + k_2}{k_1} + [S]} \] [eq. 7]
Derivation of the Briggs-Haldane equation

The Michaelis constant, $K_m$, has units of M and is defined as:

$$K_m = \left( \frac{k_1 + k_2}{k_1} \right)$$

Note that the $K_m$ definition change if the reaction mechanism is different!

and

$$(k_2 [E]_t) = V_{\text{max}}$$

Substituting these into [eq. 7] gives the final form of the Briggs-Haldane equation:

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

Some special cases:

When $[S] >> K_m$, $v_i = V_{\text{max}}$
(i.e. velocity is independent of $[S]$; The enzyme is said to be “at saturation”)

When $[S] << K_m$, $v_i = (V_{\text{max}}/K_m)[S]$
(i.e. the velocity is linear with respect to $[S]$)
Derivation of the Briggs-Haldane equation

Question: is the steady-state assumption valid? If yes, under which time scale?
Complete equations for pre-steady-state condition are too complicated to obtain. A plot of the concentration in function of time can easily be obtained using simulation software, using reasonable rate constants.
There are two kind of simulation:

1. Numerical integration (remplace dt with finite Δt like 0.1 seconds)
2. Monte-Carlo – easier to work with
I used the following values:

\[
\begin{align*}
    k_1 &= 1\,000\,000\,\text{M}^{-1}\text{s}^{-1} \\
    k_{-1} &= 100\,\text{s}^{-1} \\
    k_2 &= 100\,\text{s}^{-1} \\
    [E]_t &= 0.1\,\mu\text{M} \\
    [S] &= 1\,\text{mM}
\end{align*}
\]

\[
K_m = \frac{(k_{-1} + k_2)}{k_1} = \frac{(100+100)}{1000000} = 0.0002\,\text{M} = 0.2\,\text{mM}
\]
Reaction progress is linear (steady-state condition)

Simulation of data using a Monte Carlo software
Steady-state kinetics
[ES] is constant for about 20 sec = steady-state

Pre-steady-state kinetics
The steady-state reached in a few milliseconds

Simulation of data using a Monte Carlo software

$k_1 = 1 \times 10^7 \text{ M}^{-1} \text{s}^{-1}$
$k_1 = 100 \text{ s}^{-1}$
$k_2 = 100 \text{ s}^{-1}$

$[E]_i = 0.1 \mu\text{M}$
$[S] = 1 \text{ mM}$
The Michaelis equation is still a differential equation. It may be integrated, but the procedure is complicated. In fact, the integrated form of Michaelis equation is not used often. 2 problems: the reverse reaction and the inhibition by the product!

\[ v_i = \frac{V_{\text{max}}[S]}{[S] + K_m} \quad \text{and} \quad [P] = [S_0] - [S] \]

The integral form of the Michaelis equation:

\[ V_{\text{max}} \times t = [P] - (K_m \times \ln(1 - [P]/[S_0])) \]
\( V_{\text{max}} \) is the maximal velocity of an enzyme-catalyzed reaction. The maximal velocity is reached when the substrate is saturating. \( V_{\text{max}} \) is dependent on \([E]_t\). Expressed in Units/ml or \(\mu\text{mol/min/mL}\).

\( k_{\text{cat}} \) (s\(^{-1}\)) is the catalytic constant, also called the “turnover number”. It is a pseudo-first order rate constant and is independent of the total enzyme concentration.

\[
(k_{\text{cat}} [E]_t) = V_{\text{max}}
\]

**Specific Activity** (U/mg total protein) is often used to characterize enzyme activity when the enzyme solution is impure. It is, most always, a quick but less accurate means of kinetically characterizing an enzyme.

1 International Unit (1 U) is the amount of enzyme which catalyzes the formation of 1 \(\mu\text{mole}\) of product per minute under defined conditions. i.e. 1 U = 1 \(\mu\text{mole/min}\).
The meaning of $K_m$

Substitution of $v=V_{\text{max}}/2$ into the Briggs-Haldane equation shows that:

$$v_0 = \frac{V_{\text{max}}}{2} = \frac{V_{\text{max}}[S]}{K_M + [S]}$$

$$\frac{1}{2} = \frac{[S]}{K_M + [S]}$$

rearranging gives

$$K_M = [S]$$

The value of $K_m$ does NOT give a measure of the affinity of the enzyme for the substrate. When $k_2$ is small relative to $k_1$, $K_m$ approximates to the dissociation constant of the ES complex, $K_S$. Since $K_S$ is a true dissociation constant, only $K_S$ gives a true measure of the enzyme-substrate binding affinity.

$$K_M = \frac{k_{-1}}{k_1} + \frac{k_2}{k_1} = K_S + \frac{k_2}{k_1}$$

$\therefore K_m \geq K_S$
$k_{\text{cat}}$, a pseudo-first order rate constant, includes the individual rate constants for all steps leading from the ES complex to product release. For example, in a more complex kinetic scheme such as:

$$
\text{E} + \text{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{EP} \overset{k_3}{\rightarrow} \text{E} + \text{P}
$$

It can be shown that $k_{\text{cat}}$ is comprised of all the individual rate constants between ES and E + P ($k_2$, $k_{-2}$ and $k_3$):

$$
k_{\text{cat}} = \frac{k_2 k_3}{(k_2 + k_{-2} + k_3)}
$$
A discussion of the transition state and of the free energy plots

\[ \text{E + S} \xrightleftharpoons[k_{-1}]{k_1} \text{ES} \xrightleftharpoons[k_2]{k_{-2}} \text{EP} \xrightleftharpoons[k_3]{k_{\text{cat}}} \text{E + P} \]

1. The enzymes cannot modify the equilibrium constant of the reactions

2. The free energy cannot be «measured»; it is calculated from the equilibrium (or rate) constant

\[ \Delta G^{\ddagger} = -RT \ln k_{\text{cat}} \]

\[ \Delta G^\circ = -RT \ln K \]

3. The «zero» (the reference state) is arbitrary. We can chose it at our convenience
A discussion of the transition state and of the free energy plots

**Transition state theory (TST)** was developed simultaneously by Henry Eyring, Meredith Gwynne Evans, and Michael Polanyi in 1935.

The transition state has a **MAXIMUM** of free energy.

Stable states correspond to a **MINIMUM** of free energy.

The abscissa is the « reaction coordinate » not « time » or « distance ».
A discussion of the transition state and of the free energy plots

$$k = A \exp\left(-\frac{E_a}{RT}\right)$$

Macroscopic rate constant

$$k = \kappa \left(\frac{k_B T}{h}\right) \exp\left(-\frac{\Delta G^\ddagger}{RT}\right)$$

Microscopic rate constant

---

A.

B.

TS

Unstable Intermediate

---

Svante A. Arrhenius
(1859 – 1927)
Winner of the Nobel Prize in Chemistry in 1903
Free energy of a reaction

The free energy change ($\Delta G$) of a reaction determines its spontaneity. A reaction is spontaneous if $\Delta G$ is negative (if the free energy of products is less than that of reactants).

For a reaction $E + S \leftrightarrow ES$

$$\Delta G = \Delta G^o' + RT \ln \left( \frac{[ES]}{[E][S]} \right)$$

$\Delta G^o'$ = standard free energy change (at pH 7, 1M reactants & products, if possible); $R$ = gas constant; $T$ = temp.

**Question:** how can you calculate the ln of the equilibrium constant, which has units ($M^{-1}$ here)?

**Answer:** you can not! Unless concentration is an approximation of activity, which is a dimensionless number. We can safely make this approximation in dilute solutions.
A discussion of the transition state and of the free energy plots

Attention! this is « G » and not « G° »; it depends on concentration

![Free Energy Profile Diagram](image)

**Fig. 3.** Free energy profile showing the influence of product concentration on the energy level of $E + P$. The concentration of $A$ is set equal to its dissociation constant from $EA$ and $K_{eq} = 0.1$.

The free energy profile depends on the concentration of the $S, P$
A parenthesis: discussion of the transition state and of the free energy plots

Fig. 4. Free energy profile showing the influence of substrate concentration on the energy level of E + A. $K_a$ is the dissociation constant of A from EA. The level of P is not specified in the profile.

The free energy profile depends on the concentration of the S, P.
The free energy profile depends on the concentration of the S, P
How can we translate this in biochemical terms? If you have a high activity, the reaction will be under THERMODYNAMIC control.
How can we translate this in biochemical terms? If you have a high activity, the reaction will be under THERMODYNAMIC control.

Example: the nucleoside diphosphate kinase activity is high in cells

\[
\text{ATP} + \text{GDP} \leftrightarrow \text{ADP} + \text{GTP}
\]

The reaction is « at equilibrium » in vivo. In fact

\[
\frac{[\text{ATP}]}{[\text{ADP}]} = \frac{[\text{GTP}]}{[\text{GDP}]}
\]
Principle of microscopic reversibility: The sequence of transition states and reactive intermediates in the mechanism of a reversible reaction must be the same, but in reverse order, for the reverse reaction as for the forward reaction.

An interesting corollary: Catalysts activate both forward and reverse reactions.

Enzymes that catalyze protein cleavage... ...also catalyze protein synthesis!

So, how does our body selectively degrade the proteins it wants to eat, and synthesize the proteins it wants to use?
A discussion of the transition state and of the free energy plots.

**Principle of Microscopic Reversibility**

*Answer:* Isolate different reactions in different places.

*In the gut...*  
![Chemical reaction](image1)  
*subtilisin, trypsin, papain*  
*proteolysis is driven by water.*

*Inside cells...*  
![Chemical reaction](image2)  
*ribosome*  
*protein synthesis is driven by energy (ATP).*

Enzymes work both ways, but thermodynamics drives reactions in desired directions.
E. The principle of microscopic reversibility or detailed balance

The principle of microscopic reversibility or detailed balance is used in thermo-dynamics to place limitations on the nature of transitions between different quantum or other states. It applies also to chemical and enzymatic reactions: each chemical intermediate or conformation is considered as a "state." The principle requires that the transitions between any two states take place with equal frequency in either direction at equilibrium. That is, the process $A \rightarrow B$ is exactly balanced by $B \rightarrow A$, so equilibrium cannot be maintained by a cyclic process, with the reaction being $A \rightarrow B$ in one direction and $B \rightarrow C \rightarrow A$ in the opposite. A useful way of restating the principle for reaction kinetics is that the reaction pathway for the reverse of a reaction at equilibrium is the exact opposite of the pathway for the forward direction. In other words, the transition states for the forward and reverse reactions are identical. This also holds for (nonchain) reactions in the steady state, under a given set of reaction conditions.

The principle of microscopic reversibility is very useful for predicting the nature of a transition state from a knowledge of that for the reverse reaction. For example, as the attack of ethanol on acetic acid is general-base-catalyzed at low pH, the reverse reaction must involve the general-acid-catalyzed expulsion of ethoxide ion from the tetrahedral

![General-base catalysis](image1)

![General-acid catalysis](image2)
A discussion of the transition state and of the free energy plots

**Question**: How can we study the transition state structure?

**Answer**: we can not! Its life time is very short (1 ps = $10^{-12}$ s)
An important discussion on the transition state structure: The HAMMOND postulate (1955)

- Related species that are similar in energy are also similar in structure. The structure of a transition state resembles the structure of the closest stable species.
- Transition state structure for endothermic reactions resemble the product.
- Transition state structure for exothermic reactions resemble the reactants.
Hammond postulated that in highly exothermic reactions (left) the transition state (Ts) is structurally similar to the reactant (R), but that in highly endothermic reactions (right) the product (P) is a better model of the transition state. He cautioned against using the postulate in more thermoneutral reactions (center).
Question: How can we study the transition state structure?
Answer: we can not! Its life time is very short \((1 \text{ ps} = 10^{-12} \text{ s})\)
But…. We can use molecules which are similar to the postulated structure of the transition state. They are logically inhibitors rather than substrates. We will see that transition state analogs are GOOD inhibitors!

We will discuss this later.
The diagram illustrates the reaction coordinate for different conditions:

1. **k_{cat} condition**
   - \([s] = \text{or} > K_m\)
   - The reaction coordinate shows a higher energy barrier when \([s]\) is high or equal to \(K_m\).

2. **k_{cat}/K_m condition**
   - \([s] << K_m\)
   - The reaction coordinate shows a lower energy barrier when \([s]\) is much smaller than \(K_m\).

The energy change \(\Delta G\) is indicated on the diagram.
The kinetic significance of $k_{\text{cat}}/K_m$

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

When the substrate concentration is very low ($[S] << K_m$):

$$v_i = (V_{\text{max}}/K_m)[S]$$

Recall that $V_{\text{max}} = k_{\text{cat}}[E]_t$  
So,  
$$v_i = (k_{\text{cat}}/K_m)[S][E]_t$$

Also, when $[S]$ is very low, very little of the total enzyme species will be tied up in the ES complex (or any other intermediate complex).

$$[E]_t = [E] + [ES]$$
But at low substrate concentrations, $[E]_t \approx [E]$ (because $[ES] \approx 0$)

thus,

$$v_i = (k_{\text{cat}}/K_m)[S][E] \text{ when } [S] << K_m$$

$k_{\text{cat}}/K_m$ (catalytic efficiency) is a second-order rate constant that describes the conversion of free $E$ and free $S$ into $E + P$. The rate at low $[S]$ is directly proportional to the rate of enzyme-substrate encounter.
The kinetic significance of $k_{\text{cat}}/K_m$

$k_{\text{cat}}/K_m$ (catalytic efficiency) is a second-order rate constant that describes the conversion of free E and free S into E + P. The rate at low [S] is directly proportional to the rate of enzyme-substrate encounter.

If [S] << Km  What this means “<<“ (much smaller) ?
For practical means, a factor a 10 may be enough

All second-order reactions are limited by reactant diffusion. For physical reasons the second order rate constant $k_{II} < 10^8 \text{ M}^{-1}\text{s}^{-1}$, except:

1. Reactions involving proton transfer

2. Reactions where reagents encounter not just by diffusion, but also by electrostatics attraction
Signification du rapport \( \frac{k_{\text{cat}}}{K_m} \) : constante de vitesse d’ordre 2

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightleftharpoons[k_{-2}]{k_2} EP \xrightleftharpoons[k_{\text{cat}}/K_m]{k_3} E + P
\]

Conséquence: \( \frac{k_{\text{cat}}}{K_m} \) ne peut être supérieure à \( 10^9 \text{ M}^{-1} \text{ s}^{-1} \)

Réaction catalysée par la catalase

\[
\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}
\]

\( k_{\text{cat}} = 50,000 \text{ sec}^{-1} \),
\( K_m = 10^{-3} \text{ M} \),
\( k_{\text{cat}}/K_m = 5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1} \)

Réaction catalysée par la EcoR1

\[
\text{GAATTC} \xrightarrow{\text{CTTAAG}} 2 \text{H}_2\text{O} \xrightarrow{\text{G}_{\text{OH}} \text{pAATTC} \text{CTTAAp}} \text{HO}_{\text{G}}
\]

\( k_{\text{cat}} = 0.1 \text{sec}^{-1} \),
\( K_m = 10^{-9} \text{ M} \),
\( k_{\text{cat}}/K_m = 10^8 \text{ M}^{-1} \text{ s}^{-1} \)

a. Les deux enzymes sont efficaces
b. EcoR1 ne peut avoir une \( k_{\text{cat}} \) plus grande!
The kinetic significance of $k_{cat}/K_m$: The HALDANE relationship

The derivation of the irreversible Michaelis-Menten is an instructive exercise, however it is not a particularly realistic model for building certain kinds of models such as metabolic models. It is much better to consider the reversible Michaelis-Menten rate law. The derivation of the reversible form is very similar to the derivation of the irreversible rate law. The main difference is that the steady-state rate is given by an expression that incorporates both the forward and reverse rates for the product:

$$v = k_2 \ E \ S - k_{-2} \ E \ . \ P$$

The expression that describes the steady-state concentration of the enzyme substrate complex also has an additional term from the product binding $(k_2 EP)$. Taking these into consideration leads to the general reversible rate expression:

$$v = \frac{V_f \ S/K_S - V_r \ P/K_P}{1 + S/K_S + P/K_P}$$
At equilibrium the rate of the reversible reaction is zero. When positive, the reaction goes in the forward direction and in the reverse direction when negative. At equilibrium the equation reduces to

\[ 0 = V_f \frac{S_{eq}}{K_S} - V_r \frac{P_{eq}}{K_P} \]

where \( S_{eq} \) and \( P_{eq} \) represent the equilibrium concentrations for substrate and product. Rearrangement yields

\[ K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{V_f K_P}{V_r K_S} \]
The kinetic significance of $k_{\text{cat}}/K_m$: The HALDANE relationship

$$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{V_f K_P}{V_r K_S}$$

This expression is known as the **Haldane relationship** and shows that the four kinetic constants are not independent. The relationship can be used to eliminate one of the kinetic constants and substitute the equilibrium constants in its place. This is useful because equilibrium constants tend to be better known than kinetic constants.

A clever way to deduce the HALDANE relationship:

**for the S→P reaction**, the steady state will be

$$k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \quad [ES] = [E][S]/K_{m,f} \quad \text{and} \quad v_f = k_{2f} *[E][S]/K_{m,f}$$

**for the P→S reaction**, the steady state will be

$$k_f[E][P] - k_{-1}[EP] - k_2[EP] = 0 \quad [EP] = [E][P]/K_{m,r} \quad \text{and} \quad v_r = k_{2r} *[E][P]/K_{m,f}$$

At equilibrium $v_f = v_r$

$$k_{2f} *[E][S]/K_{m,f} = k_{2r} *[E][P]/K_{m,f}$$
The kinetic significance of $k_{\text{cat}}/K_m$ : alternate substrates

### Réactions parallèles

\[
\begin{align*}
\text{EA} & \rightarrow \text{E} + \text{P} \\
\text{EB} & \rightarrow \text{E} + \text{Q}
\end{align*}
\]

**Expression 1:**
\[
[\text{ES}] = \frac{k_1}{k_{-1} + k_2} [\text{E}][\text{S}]
\]

**Expression 2:**
\[
v_i = k_2 [\text{ES}]
\]

**Expression 3:**
\[
[\text{E}] \quad \text{La concentration d'enzyme libre}
\]

**Expression 4:**
\[
\begin{align*}
v_A &= \frac{k_{\text{cat}}^A}{K_m^A} [\text{E}][\text{A}] \\
v_B &= \frac{k_{\text{cat}}^B}{K_m^B} [\text{E}][\text{B}]
\end{align*}
\]

**Expression 5:**
\[
\begin{align*}
v_A &= \frac{k_{\text{cat}}^A}{K_m^A} [\text{A}] \\
v_B &= \frac{k_{\text{cat}}^B}{K_m^B} [\text{B}]
\end{align*}
\]

**Expression 6:**
\[
k_{\text{cat}}/K_m : \text{la constante de spécificité}
\]
The kinetic significance of $k_{\text{cat}}/K_m$: alternate substrates

Réactions parallèles

$E_A \rightarrow E + P$

$E_B \rightarrow E + Q$

Applications médicales

1. Traitement de intoxication avec le méthanol ou l’éthylene glycol: avec de l’éthanol!
2. Traitement du SIDA:
AZT => AZT-TP => incorporé dans l’ADN par la transcriptase inverse virale terminateur ce chaîn

L’AZT-TP n’est pas un substrat pour les polymérases cellulaires

[TTP] 10 µM, [AZT TP] 10 nM
The kinetic significance of \( k_{\text{cat}}/K_m \)

If \( k_{\text{cat}}/K_m \) is important, can we measure it DIRECTLY, without measuring \( k_{\text{cat}} \) and \( K_m \)?

The answer is YES, we can. There are 2 methods:

1. You measure the initial rate at low S, the plot of \( v \) versus \([S]\) is a straight line. Its slope is \( V_{\text{max}}/K_m \)

The systematic error may be estimated by simulation.
The kinetic significance of $k_{cat}/K_m$

2. The equation $v_i = (V_{max}/K_m)[S]$ (when $[S] << K_m$) can easily be integrated

\[ [S] = [S]_0 e^{-\frac{V_{max}}{K_m} \cdot t} \]

What are the units of $\frac{V_{max}}{K_m}$?

\[ \frac{\text{mole/m}\cdot\text{min}/\text{ml}}{\text{mole/m}^2} = \text{min}^{-1} \]

$t$ should be in \text{min}
The kinetic significance of $k_{\text{cat}}/K_m$

Tableau 1 : Paramètres $k_{\text{cat}}/K_m$ calculés à partir des courbes $[\text{TDP}]=f(t)$ avec $E_t=4.7\text{nM}$, $[\text{TDP}_0]=2$ à $200\mu\text{M}$ et $[\text{ATP}]=1\text{mM}$.

<table>
<thead>
<tr>
<th>TDP (µM)</th>
<th>$k_{\text{cat}}/K_m (10^8\text{M}^{-1}\cdot\text{s}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>20</td>
<td>6.9</td>
</tr>
<tr>
<td>200</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Figure 14: Cinétique d’ordre 1 de disparition du TDP (A) et de l’AZT (B)
Les cinétiques sont suivies à 340nm par le système couplé PK/LDH pour des concentrations en NDP kinase de *Dictyostelium* de 4.7 nM (A) et 1.8 µM (B). La constante de temps $k$ mesurée est de $7.10^6\text{M}^{-1}\cdot\text{s}^{-1}$ pour le TDP (A) et de $1.10^3\text{M}^{-1}\cdot\text{s}^{-1}$ pour l’AZT-DP (B).

Philippe Gonin, PhD thesis
Perfect Enzymes

• If design can be optimized, what is perfect?
• Catalytic definition
• Binding & Catalysis very fast
• Rate approaches diffusional limit of free E + S ≈ $10^8$M$^{-1}$s$^{-1}$.
  – Examine bimolecular rate: $k_{\text{cat}}/K_M$.
    Triose phosphate isomerase $\quad 3 \times 10^8$
    Acetylcholinesterase $\quad 1 \times 10^8$
  – “Perfect” conventionally means diffusion-limited
  – Natural selection can not improve $k_{\text{cat}}$
Perfect Enzymes

• Optimize binding to transition state
• May need to \textit{worsen} binding to substrate
  – When $[S] >> K_M$
  – To avoid increasing activation energy
• Generally expect high $K_M$
  – (bad substrate binding)
• All in name of improving rate.
How can an enzyme be “designed” to put binding energy best use?

• Intuitively, might seem that should
  – Bind substrate as tightly as possible
  – Reduce $G_{ES}$, i.e. stabilize ES
• Our intuition is **not correct**
• Following is a thought experiment
  – Near impossible to actually measure…
  – Will consider 2 limiting cases:
    • $[S] \gg K_M$ & $[S] \ll K_M$. 
Perfect Enzymes

[S]>>K_M: Design Optimal binding to transition state - reduces ΔG‡.

- [S]>>K_M means
  - Driven -> complex
  - ΔG_{binding} < 0 (i.e. favorable)
  - Michaelis-Menton formula reduces to \( v_o = k_{cat}[E_0] \)
- Optimal substrate-binding digs deeper hole, increasing activation barrier

\[ \Delta G \]

\[ \Delta G^\pm \]

\[ v_o = k_{cat}[E_0] \]

\[ \text{stabilize } ES \]

\[ \text{stabilize ES & ES} \]

\[ \text{stabilize ES} \]

\[ \Delta G^{(\text{increased})} \]

\[ \Delta G^{(\text{decreased})} \]

\[ \Delta G^{(\text{unchanged})} \]

\[ \Delta G^{(\text{unchanged rate})} \]
[S]««K_M: Design stabilized ES‡ or (ES + ES‡) - reduces ΔG‡.

- [S]««K_M means
  - ES complex formation requires energy
  - ΔG_{binding} > 0
  - Michaelis-Menton formula reduces to
    \[ v_o = \frac{k_{cat}}{K_M}[E_0][S] \]
- Substrate-binding has no effect on activation barrier
- Must change ES‡

Perfect Enzymes
Optimal design of Enzyme

- Strengthen Substrate-binding – No!:
  - At best - no effect
  - At worst - decreases rate
- Need to optimize binding to $\dagger$ to increase rate
  - Optimize interactions w/ strained state
  - Good enzymes will contort substrates towards transition state
  - Proposed by Pauling (1948)
Nature Sometimes Selects Imperfect Enzymes - Why?

- **Rate-limiting or regulatory**
  - \( v_0 \) independent of \([S]\) only w/ low \( K_M \)
  - \( v_0 = V_{\text{max}}[S]/(K_M + [S]) \)
  - Eg hexokinase: \( K_M = 0.1 \text{mM} \); \([\text{glucose}] = 5\text{mM}\)

- **When binding needs to drive essential conformational changes - Induced Fit**
  - Next topic

- **When specificity more important than catalysis**
  - Specificity not always mediated through \( K_M \)
Catalytic Perfection

\[ \frac{k_{cat}}{K_M} = \frac{k_2}{K_M} = \frac{k_1 k_2}{k_{-1} + k_2} = k_1 \text{ when } k_2 \gg k_{-1} \]

- How quickly can an enzyme convert substrate into product following enzyme-substrate encounter? This depends on the rates of the individual steps in the reaction. The rate is maximal when \( k_2 \gg k_{-1} \) which means that the reaction proceeds whenever a collision occurs.

- Many enzymes in metabolic pathways have evolved to function at substrate concentrations less than \( K_m \) to optimally and efficiently turnover metabolic intermediates.

- Some enzymes are so incredibly efficient that they instantaneously convert S into P following enzyme-substrate encounter. The reaction rate for these enzymes is limited only by the rate of diffusion (\( \sim 10^8 - 10^9 \text{ M}^{-1}\text{s}^{-1} \)). These enzymes are said to have reached evolutionary perfection.
Examples of rate constants

There is a wide variation in kinetic parameters reflecting the interplay between $K_M$ and $k_{cat}$. Because of the central role of the Enzyme·Substrate complex, there is also large variability depending on the nature of the substrate.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$K_M$ (M)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>$9.5 \times 10^{-5}$</td>
<td>$1.4 \times 10^4$</td>
<td>$1.5 \times 10^8$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>CO$_2$</td>
<td>$1.2 \times 10^{-2}$</td>
<td>$1.0 \times 10^6$</td>
<td>$8.3 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>HCO$_3^-$</td>
<td>$2.6 \times 10^{-2}$</td>
<td>$4.0 \times 10^3$</td>
<td>$1.5 \times 10^7$</td>
</tr>
<tr>
<td>Catalase</td>
<td>H$_2$O$_2$</td>
<td>$2.5 \times 10^{-2}$</td>
<td>$1.0 \times 10^7$</td>
<td>$4.0 \times 10^8$</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>$N$-Acetylglycine ethyl ester</td>
<td>$4.4 \times 10^{-1}$</td>
<td>$5.1 \times 10^{-2}$</td>
<td>$1.2 \times 10^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$N$-Acetylvaline ethyl ester</td>
<td>$8.8 \times 10^{-2}$</td>
<td>$1.7 \times 10^{-1}$</td>
<td>$1.9$</td>
</tr>
<tr>
<td></td>
<td>$N$-Acetytyrosine ethyl ester</td>
<td>$6.6 \times 10^{-4}$</td>
<td>$1.9 \times 10^2$</td>
<td>$2.9 \times 10^3$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>$5.0 \times 10^{-6}$</td>
<td>$8.0 \times 10^2$</td>
<td>$1.6 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>Malate</td>
<td>$2.5 \times 10^{-5}$</td>
<td>$9.0 \times 10^3$</td>
<td>$3.6 \times 10^7$</td>
</tr>
<tr>
<td>Urease</td>
<td>Urea</td>
<td>$2.5 \times 10^{-2}$</td>
<td>$1.0 \times 10^4$</td>
<td>$4.0 \times 10^5$</td>
</tr>
</tbody>
</table>
The enzymes can not modify the equilibrium constant of the reactions, but:

The equilibrium constant will change when substrates are bound to enzymes!

Comparing $A + B \rightleftharpoons C + D$

With

$EAB \rightleftharpoons ECD$
Rabbit muscle pyruvate kinase which catalyzes the reaction $\text{ATP + pyruvate} \rightleftharpoons \text{ADP + P-enolpyruvate}$ was studied by $^{31}$P NMR. At catalytic concentrations of the enzyme, pH 8.0 and $T = 15^\circ\text{C}$, the equilibrium constant

$$\frac{[\text{MgADP}][\text{P-enolpyruvate}]}{[\text{MgATP}][\text{pyruvate}]} \approx 3 \times 10^{-4}$$

whereas the equilibrium constant for enzyme-bound substrates and products,

$$K'_{eq} = \frac{[E \cdot P_1 \cdot P_2]}{[E \cdot S_1 \cdot S_2]} \approx 1$$
Equilibrium constants with enzyme-bound substrates

Pyruvate Kinase
\( T = 15^\circ, \text{pH 8.0} \)

Initial (no E)

Equilibrium Mixture (Catalytic E)

\[
\frac{[\text{Pyruvate}]}{[\text{ATP}]} \approx 15
\]
Fig. 2. $^{31}$P NMR spectra (at 40.3 MHz) of pyruvate kinase reaction at enzyme concentrations in excess of the substrates, pH = 8.0, $T = 15^\circ$C. Sample volumes $\sim 1.1$ ml with 10% D$_2$O. A, equilibrium mixture set up with enzyme (active sites) 2.8 mm; P-enolpyruvate ($PEP$), 2.4 mm; ADP, 2.4 mm; MgCl$_2$, 5.7 mm; potassium Hepes, 100 mm; KCl, 100 mm. B, spectrum after the addition of 50 μl of 400 mm EDTA (pH readjusted to 8.0) to the sample of spectrum A. NMR parameters: repetition time, 4.0 s; spectral width, 2 kHz; memory size, 8K; line broadening, 3Hz, A, 5500 scans; B, 3500 scans.
Equilibrium constants with enzyme-bound substrates

**Table III**

Equilibrium constants of kinase reactions

Equilibrium constants

\[ K_{eq} = \frac{[P_1][P_2]}{[S_1][S_2]} \]

(at catalytic enzyme concentrations)

and

\[ K'_{eq} = \frac{[E \cdot P_1 \cdot P_2]}{[E \cdot S_1 \cdot S_2]} \]

(enzyme-bound substrates and products)

were determined by \(^{31}\)P NMR.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>pH</th>
<th>T</th>
<th>(K_{eq}) (Ref.)</th>
<th>(K'_{eq}) (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine kinase</td>
<td>7.25</td>
<td>12</td>
<td>0.1 (4)</td>
<td>1.2 (4, 5)</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>7.8</td>
<td>20</td>
<td>0.1(^a)</td>
<td>(~1.0)^(^a)</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>7.0</td>
<td>4</td>
<td>0.4 (6)</td>
<td>1.6 (6)</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>8.0</td>
<td>15</td>
<td>(3 \times 10^{-4})^(^b)</td>
<td>1.0-2.0(^b)</td>
</tr>
<tr>
<td>Pyruvate kinase (glycolate reaction)</td>
<td>8.0</td>
<td>20</td>
<td>(\geq50)^(^b)</td>
<td>1.0-3.0(^b)</td>
</tr>
<tr>
<td>3-P-glycerate kinase</td>
<td>7.0</td>
<td>1</td>
<td>(3 \times 10^{-4}) (26)</td>
<td>1.0 (3)</td>
</tr>
</tbody>
</table>

\(^a\) B. D. Nageswara, and M. Cohn, unpublished experiments.
Why studying enzyme kinetics with one substrate while most enzymes have 2 or more substrates?

\[
\frac{[E]}{v} = \frac{1}{k_3} + \frac{k_{-2} + k_3}{k_2 k_3} + \frac{k_2 k_3 + k_1(k_{-2} + k_3)}{k_1 k_2 k_3 [ATP]} + \frac{1}{k_6} + \frac{k_{-5} + k_6}{k_5 k_6} + \frac{k_5 k_6 + k_{-4}(k_{-5} + k_6)}{k_4 k_5 k_6 [NDP]}
\]

\[
V = \frac{V_{max}}{K_m^{[ATP]} + \frac{K_m^{[NDP]}}{[NDP]} + 1}
\]
Why studying enzyme kinetics with one substrate while most enzymes have 2 or more substrates?

\[
V = \frac{V_{\text{max}}}{\frac{K_{\text{m,ATP}}}{[\text{ATP}]} + \frac{K_{\text{m,NDP}}}{[\text{NDP}]} + 1}
\]

if \([\text{ATP}] = \text{const} \) and \([\text{NDP}] = \text{variable}\)

\[
V = \frac{V_{\text{app}} \cdot [\text{NDP}]}{K_{\text{m,app}} + [\text{NDP}]}
\]

\[
V = \frac{V_{\text{max}}}{\frac{K_{\text{m,NDP}}}{[\text{NDP}]} + \left(\frac{K_{\text{m,ATP}}}{[\text{ATP}]} + 1\right)}
\]

We call this \( W \)

\[
V = \frac{V_{\text{max}}}{\frac{K_{\text{m,app}}}{[\text{NDP}]} + 1}
\]

Same equation as for the reaction with one substrate
How to derive the Michaelis equation for more complicated systems?

2. Apply the same procedure as for the simple system, but with a longer algebra (you have more chance to make errors)
3. If you are good in computers, you may use a software for « formal calculations » like MAPLE or MATEMATICA. You just write the transformation rates for each species and the computer will obtain the Michaelis equation for you!
4. 2 tricks I will discuss
   4a. Use of transit times instead of rates
   4b. Use of the net rates method
Use of transit times instead of rate constants

The value of $V_{max}$, $k_{cal}$, or any rate constant for a series of sequential reactions may be derived by considering the *time* taken for each step, as follows. The dimensions of $v$ are moles per second. The dimensions of $1/v$ are seconds per mole, and $1/v$ is the time taken for 1 mol of reagents to give products. Similarly, the reciprocal of $k_{csx}$ (i.e., $[E]_0/V_{max}$) has the dimensions of seconds and is the time taken for one molecule of reagent to travel the whole reaction pathway in the steady state at saturating [S]. The reciprocal rate constant may be considered a *transit time*.

For a series of reactions as in equation 3.63, the reciprocal of the rate constant for any individual step is its transit time.

$$E\cdot P_1 \xrightarrow{k_1} E\cdot P_2 \xrightarrow{k_2} E\cdot P_3 \xrightarrow{k_3} E\cdot P_4 \xrightarrow{k_4} \cdots \xrightarrow{k_{n-1}} E\cdot P_n$$

The total time for one molecule to be converted from $P_i$ to $P_n$, $l/k$, is given by the sum of the transit times for each step. That is,

$$\frac{1}{k} = \frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3} + \frac{1}{k_4} + \cdots + \frac{1}{k_{n-1}}$$
Use of transit times instead of rate constants

where \( 1/k = 1/k_{\text{cat}} = [E]_0/V_{\text{max}} \) for saturating conditions. This is precisely the relationship derived earlier for \( f_{\text{cat}} \) in the acylenzyme mechanism for chymotrypsin (equation 3.23), and it gives the physical reason for the reciprocal relationship between \( k_{\text{cat}} \) and the first-order rate constants on the pathway: the reciprocals of the rate constants, i.e., the transit times, are additive, so that the time taken for a molecule to traverse the whole reaction pathway is the sum of the times taken for each step. For concentrations of S below saturating, the transit time for the whole reaction is \([E]_0/v\). This is the physical reason why the rate laws for steady state mechanisms are usually written in terms of \([E]_0/v\). As an example, we consider the Briggs-Haldane mechanism:

\[
\begin{align*}
E + A & \xrightleftharpoons[k_{-1}]{k_1} EA & \xrightarrow[k_2]{ } E + P
\end{align*}
\]
Use of transit times instead of rate constants

The binding step is reduced to the net rate constant

\[
\frac{k_1[A]k_2}{k_{-1} + k_2}
\]

The total transit time is

\[
\frac{[E]_0}{\nu} = \frac{k_{-1} + k_2}{k_1[A]k_2} + \frac{1}{k_2}
\]

Equation 3.66 is, in fact, the Lineweaver-Burk double-reciprocal plot (equation 3.28) in slight disguise: \([E]_0\) has been moved to the left-hand side, and from equation 3.14, \(K_M = (k_{-1} + k_2)k_x\).
The ping-pong mechanisms of equations 3.57 and 3.58, for example, may similarly be solved by inspection. Restating those equations as

\[
E + A \xrightleftharpoons[k_{-1}]{k_1} EA \xrightarrow{k_2} E - P \xrightarrow{k_3[B]} E + P - B
\]

The transit times are summed to give

\[
\frac{[E]_0}{v} = \frac{k_{-1} + k_2}{k_1[A]k_2} + \frac{1}{k_2} + \frac{1}{k_3[B]}
\]
It is possible to reduce the rate constants for a series of reactions as in equation 3.59 to a single net rate constant, or to a series of single rate constants, by just considering a net rate constant for the flux going through each step.

\[
\begin{align*}
A \rightleftharpoons & B \rightleftharpoons C \rightleftharpoons D \rightleftharpoons E \rightarrow F \\
& \hspace{1cm} \scriptstyle k_1 \quad k_2 \quad k_3 \quad k_4 \quad k_5
\end{align*}
\]

To illustrate this, we consider the simpler reaction

\[
\begin{align*}
X \rightleftharpoons & Y \rightarrow Z \\
& \hspace{1cm} \scriptstyle k_1 \quad k_2
\end{align*}
\]
The rate of X going to Z via Y is given by the rate of X going to Y (= ki[X]) times the probability of Y going to Z rather than reverting to X [i.e., \( k_2/(k_{-1} + k_2) \)]. The net rate constant for X \( \rightarrow \) Y, \( k_1' \), is thus given by

\[
k_1' = \frac{k_1k_2}{k_{-1} + k_2}
\]

The same treatment can be applied to equation 3.59, starting from the irreversible step on the right-hand side and progressively working to the left. For example, the net rate constant for D \( \rightarrow \) E, \( k_4' \), equals \( k_4k_5/(k_{-4} + k_5) \), as in equation 3.61. The net rate constant for C \( \rightarrow \) D, \( k_3' \), is calculated by analogy with equation 3.61 to be \( k_3k_4'/(k_{-3} + k_4) \). This is continued sequentially to give eventually the net rate constant for A \( \rightarrow \) B, i.e.,

\[
k_1' = k_1k_2'/(k_{-1} + k_2')
\]
4.2.1 Uni Uni Monosubstrate Mechanism

Let us start to illustrate the method with the simplest Uni Uni monosubstrate mechanism:

\[
E \xrightarrow{k_1} EA \xrightarrow{k_2} EP \xrightarrow{k_4} E + P
\]  

(4.8)

To start with, reaction (4.8) is first converted to a series of unidirectional rate constants (indicated by primes). The steady-state flux through each step is given by

\[
E \xrightarrow{k'_1} EA \xrightarrow{k'_3} EP \xrightarrow{k'_4} E + P
\]  

(4.9)

where each step has an associated net rate constant \(k'_i\). Since the mechanism is a linear series, the rate for the overall reaction is the sum of the inverse of the net rate constants through the individual steps.
Calculation of net rate constants

Thus, the rate equation is

\[
\frac{v_0}{E_0} = \frac{1}{\frac{1}{k'_1} + \frac{1}{k'_3} + \frac{1}{k'_4}}
\]  

(4.10)

First, one has to express the net rate constants in terms of the actual rate constants. For any irreversible step, the net rate constant and the actual rate constant is identical; thus,

\[ k'_3 = k_3 \quad \text{and} \quad k'_4 = k_4 \]  

(4.11)

Moving leftward, we express \( k'_1 \) as the real forward rate constant, \( k_1 \), multiplied by the factor that relates the fraction of EA reacting in the forward direction as opposed to the fraction that returns to E. Note that all bimolecular rate constants must be transformed into monomolecular, by multiplying by the concentration of substrate or product. Thus, we obtain

\[
k'_1 = \frac{k_1 A k_3}{k_2 + k_3}
\]  

(4.12)

Now, returning to Eq. (4.10) and substituting the \( k'_1 \) terms, we obtain

\[
\frac{v_0}{E_0} = \frac{k_1 k_3 k_4 A}{k_4 (k_2 + k_3) + k_1 A (k_3 + k_4)}
\]  

(4.13)

Equation (4.13) can be written in the Michaelis–Menten fashion as

\[
\frac{v}{v} = \frac{1}{k_{-k}}
\]
Equation (4.13) can be written in the Michaelis–Menten fashion as

\[
\frac{v_0}{E_0} = \frac{\left( \frac{k_3 k_4}{k_3 + k_4} \right) A}{k_4(k_2 + k_3) + A}
\]

Equation (4.14) shows the composition of kinetic constants:

\[
V_{\text{max}} = \frac{k_3 k_4 E_0}{k_3 + k_4} \quad \text{and} \quad K_A = \frac{k_4(k_2 + k_3)}{k_1(k_3 + k_4)}
\]

\[(4.15)\]

When the goal is to obtain \( V_{\text{max}} \) or \( V_{\text{max}}/K_A \), the following procedure avoids the need to derive the entire rate equation.

First. \( V_{\text{max}} \) is obtained at saturating \( A \) concentrations, such that \( k_1' \) will be infinite and the \( (1/k_1') \) term drops out, so that \( V_{\text{max}} \) becomes

\[
V_{\text{max}} = \frac{E_0}{\frac{1}{k_3'} + \frac{1}{k_4'}} = \frac{k_3 k_4 E_0}{k_3 + k_4}
\]

Second. \( V_{\text{max}}/K_A \) is obtained as \( A \) is extrapolated to near zero. In this case, the \( k_1' \) term becomes the smallest net rate constant, and

\[
\frac{V_{\text{max}}}{K_A} = k_1'E_0 = \frac{k_1 k_3 A E_0}{k_2 + k_3}
\]
4.2.2 Ordered Uni Bi Mechanism

The above process will be repeated with a more complex Ordered Uni Bi mechanism:

\[
\begin{align*}
    E & \xrightarrow{k_{i}A} EA \xrightarrow{k_{3}} EPQ \xrightarrow{k_{5}} EQ \xrightarrow{k_{7}} E + Q \\
    k_{3} & \quad k_{4} & \quad k_{5} & \quad k_{6} & \quad k_{7}
\end{align*}
\]  

(4.16)

First, reaction (4.16) is converted into a series of unidirectional or net rate constants. The steady-state flux through each step remains the same as above, and we obtain

\[
\begin{align*}
    E & \xrightarrow{k_{i}'} EA \xrightarrow{k_{3}'} EPQ \xrightarrow{k_{5}'} EQ \xrightarrow{k_{7}'} E + Q \\
    k_{3}' & \quad k_{4}' & \quad k_{5}' & \quad k_{6}' & \quad k_{7}'
\end{align*}
\]  

(4.17)

Because the mechanism is again a linear series, the rate for the overall reaction is the sum of the inverse of the net rate constants through the individual steps:

\[
\frac{v_{0}}{E_{0}} = \frac{1}{k_{i}'} + \frac{1}{k_{3}'} + \frac{1}{k_{5}'} + \frac{1}{k_{7}'}
\]  

(4.18)

where the net rate constants must now be expressed in terms of the actual rate constants, bearing in mind that all bimolecular rate constants must be transformed into the monomolecular rate constants by multiplying with the concentration of substrate or product.

We start with the only irreversible step in the mechanism in reaction (4.16); for that irreversible step, the net rate constant and the real rate constant are identical:

\[
k_{7}' = k_{7}
\]  

(4.19)

Moving from right to the left, we express \( k_{i}' \) as the real forward rate constant
Calculation of net rate constants

\[ k_7' = k_7 \]  \hspace{1cm} (4.19)

Moving from right to the left, we express \( k_7' \) as the real forward rate constant multiplied by a factor that relates the fraction of EQ reacting in the forward direction as opposed to the fraction that returns to EPQ. In this case, since \( k_7' = k_7 \), we obtain

\[ k_5' = k_5 \left( \frac{k_7}{k_7 + k_6 P} \right) \]  \hspace{1cm} (4.20)

Again, moving from right to the left, we obtain

\[ k_3' = k_3 \left( \frac{k_5'}{k_4 + k_5'} \right) \]  \hspace{1cm} (4.21)

Then, replacing \( k_5' \) from Eq. (4.20), we obtain
Calculation of net rate constants

\[
k'_3 = \frac{\left( \frac{k_5 k_6 k_7}{k_7 + k_6 P} \right)}{\left( k_4 + \frac{k_5 k_7}{k_7 + k_6 P} \right)} = \frac{k_3 k_5 k_7}{k_4 k_7 + k_5 k_7 + k_4 k_6 P} \tag{4.22}
\]

Also, again we deal with the leftwardmost term \( k'_i \):

\[
k'_i = \frac{k_1 k'_3}{k_2 + k'_3} \tag{4.23}
\]

which upon substituting \( k'_3 \) from Eq. (4.22), gives

\[
k'_1 = \frac{k_1 k_3 k_5 k_7 A}{k_2 k_4 k_7 + k_2 k_3 k_7 + k_3 k_5 k_7 + k_2 k_4 k_6 P} \tag{4.24}
\]

Now, we shall return to Eq. (4.18) and substitute all the \( k'_i \) terms:

\[
u_0 = \frac{E_0}{\frac{1}{k'_1} + \frac{1}{k'_3} + \frac{1}{k'_5} + \frac{1}{k'_7}}
\]

\[
u_0 = \frac{k_1 k_3 k_5 k_7 E_0 A}{k_7 (k_2 k_4 + k_2 k_5 + k_3 k_7) + k_2 k_4 k_6 P}
+ k_1 (k_3 k_7 + k_4 k_7 + k_5 k_7 + k_3 k_5) A + k_1 k_6 (k_3 + k_4) A P \tag{4.25}
\]
<table>
<thead>
<tr>
<th>Motion</th>
<th>Approx. Time Scale - log(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bond vibration</td>
<td>-14 to -13</td>
</tr>
<tr>
<td>proton transfer</td>
<td>-12</td>
</tr>
<tr>
<td>hydrogen bonding</td>
<td>-12 to -11</td>
</tr>
<tr>
<td>elastic vibration of globular region</td>
<td>-12 to -11</td>
</tr>
<tr>
<td>sugar repuckering</td>
<td>-12 to -9</td>
</tr>
<tr>
<td>rotation of side chains at surface</td>
<td>-11 to -10</td>
</tr>
<tr>
<td>torsional libration of buried group</td>
<td>-11 to -9</td>
</tr>
<tr>
<td>hinge bending at domain interfaces</td>
<td>-11 to -7</td>
</tr>
<tr>
<td>water structure reorganization</td>
<td>-8</td>
</tr>
<tr>
<td>helix breakdown/formation</td>
<td>-8 to -7</td>
</tr>
<tr>
<td>allosteric transitions</td>
<td>-5 to 0</td>
</tr>
<tr>
<td>local denaturation</td>
<td>-5 to 1</td>
</tr>
<tr>
<td>rotation of medium-sized interior sidechains</td>
<td>-4 to 0</td>
</tr>
</tbody>
</table>

Derivation of the Briggs-Haldane equation

• As before, this derivation deals only with initial velocity kinetics. We can treat the reverse reaction as negligible and simplify the scheme to:

\[
E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} E + P
\]

(Note that \(k_2\) is analogous to \(k_{\text{cat}}\))

• The overall rate of production of ES is the sum of the elementary reaction rates leading to its appearance minus the sum of those leading to its disappearance.

\[
\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0
\]

Rearranging:

\[
[ES] = \frac{k_1[E][S]}{k_{-1} + k_2}
\]  
[eq. 6]
Derivation of the Briggs-Haldane equation

• As before,

\[ v_i = \frac{-d[S]}{dt} = \frac{d[P]}{dt} = k_2[ES] \]

and

\[ \frac{v_i}{[E]_t} = \frac{k_2[ES]}{[E] + [ES]} \]

Substituting in \([eq. 6]\), we get:

\[ \frac{v_i}{[E]_t} = \frac{k_2k_1[E][S]/(k_1 + k_2)}{[E] + [E][S]k_1/(k_1 + k_2)} \]

\[ v_i = \frac{k_2[E]_t[S]}{(k_1 + k_2) + [S]} \]  \([eq. 7]\)
“Perfection” catalytique

\[
\frac{k_{cat}}{K_M} = \frac{k_2}{K_M} = \frac{k_1k_2}{k_{-1} + k_2} = k_1 \text{ quand } k_2 \gg k_{-1}
\]

- How quickly can an enzyme convert substrate into product following enzyme-substrate encounter? This depends on the rates of the individual steps in the reaction. The rate is maximal when \( k_2 \gg k_{-1} \) which means that the reaction proceeds whenever a collision occurs.

- Many enzymes in metabolic pathways have evolved to function at substrate concentrations less than \( K_m \) to optimally and efficiently turnover metabolic intermediates.

- Some enzymes are so incredibly efficient that they instantaneously convert S into P following enzyme-substrate encounter. The reaction rate for these enzymes is limited only by the rate of diffusion (\( \sim 10^8 - 10^9 \text{M}^{-1}\text{s}^{-1} \)). These enzymes are said to have reached evolutionary perfection.
Upper Limit for Catalytic Efficiency

Every $E + S$ encounter gives turnover to $E \cdot P$

$\Rightarrow$ catalysis limited by rate at which $E + S$ diffuse together

$E + S \rightarrow E \cdot S \rightarrow E \cdot P$

$\quad a \quad b$

$a$ – diffusion-limited

$b$ – zero activation energy

Diffusion upper limit $\approx 10^8 - 10^9$ M$^{-1}$ s$^{-1}$

$\Rightarrow k_{cat}/K_m \leq 10^8 - 10^9$ M$^{-1}$ s$^{-1}$

- Concept of “perfect” enzymes

- $k_{cat}$ and $K_m$-linked e.g. if $K_m = 10^9$ M, $k_{cat} \leq 1$ sec$^{-1}$ (EcoR1)

Tight binding $\rightarrow$ low $k_{cat}$
High $k_{cat}$ $\rightarrow$ weak binding
Steady-state Rate Law for a One-substrate, One-product Reaction with Two Reversible Steps

\[ A \xrightarrow{K_1} E \xrightleftharpoons{K_2}{K_3} E \cdot A \xrightarrow{K_4} E \cdot P \xrightarrow{K_5} P + E \]

Replacing every equilibrium rate constant by net rate constant:

Net rate constant:

\[ E_1 \xrightarrow{K_1} E_2 \xrightarrow{K_3} E_3 \xrightarrow{K_5} E_1 \]

steady state

each [E] depend on next net rate constant K magnitude

if \( K_3 \) large, [E_2] ↓

if \( K_3 \) small, [E_2] ↑

Therefore, \( E_1 \propto \frac{1}{K_1} \), \( \frac{E_1}{E_i} = \frac{1}{K_1} + \frac{1}{K_3} + \frac{1}{K_5} \)
Flux is constant at steady state:

\[
\text{rate } = E_1(K_1') = E_2(K_3') = E_3(K_5') \text{ at steady state}
\]

\[
\text{velocity } = E_1(K_1') = \frac{E_t}{\frac{1}{K_1'} + \frac{1}{K_3'} + \frac{1}{K_5'}}
\]

\[
\{\text{because } \frac{E_1}{E_t} = \frac{1}{K_1'} + \frac{1}{K_3'} + \frac{1}{K_5'} \}
\]

\[
\frac{v}{E_t} = \frac{1}{\sum_i \frac{1}{K_i'}}
\]

<Homework> Go back to derive an equation for a one-substrate, one-product reaction with one reversible steps
Free energy plots

\[ k_1 \quad E + S \quad k_{-1} \quad ES \quad k_2 \quad ES \quad k_2 \quad EP \quad E + P \]

\[ k_3 \]

\[ E + S \quad \xrightleftharpoons[k_{-1}]{k_1} \quad ES \quad \xrightarrow[k_2]{k_2} \quad EP \quad \xrightarrow[k_3]{k_3} \quad E + P \]

\[ ES \quad \xrightarrow[k_{cat}]{k_{cat}} \quad \dagger \]

\[ \Delta G^\ddagger = -RT \ln k_{cat} \]

\[ \Delta G = -RT \ln K \]

Diagram:
- Reaction coordinate: \( E + S \) to \( E + P \)
- \( \Delta G \)
- Transition state: \( \dagger \)
- Free energy plot with \( \Delta G^\ddagger \) at the transition state and \( \Delta G \) at the intermediate state (ES).